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Influence Of Conformational Restriction On The Antibacterial Activity And Ribosomal Selectivity Of Aminoglycoside Antibiotics

Michael Gabriel Pirrone
Wayne State University

INFLUENCE OF CONFORMATIONAL RESTRICTION ON THE ANTIBACTERIAL ACTIVITY AND RIBOSOMAL SELECTIVITY OF AMINOGLYCOSIDE ANTIBIOTICS

by

MICHAEL GABRIEL PIRRONE

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DEDICATION

I dedicate this work to my parents, Nina and Joe Pirrone, for their love, support, and encouragement throughout my Ph.D. I also dedicate this to my brother, Anthony, for always pushing me to better myself, and most of all to my brother, Joey, for being the most influential person in my life and leading me into science.

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LIST OF ABBREVIATIONS

µL	Microliters
2-DOS	2-deoxystreptamine
A	Adenine
AAC	Aminoglycoside acetyltransferase
Ac	Acetyl
AGA	Aminoglycoside antibiotic
AIBN	Azobisisobutyronitrile
AME	Aminoglycoside modifying enzyme
ANT	Aminoglycoside nucleotidyltransferase
APH	Aminoglycoside phosphotransferase
A-site	Aminoacyl site
ATP	Adenosine triphosphate
ax	axial
BAIB	bis(acetoxy)iodobenzene
Bn	Benzyl
Bu	Butyl
Bz	Benzoyl
C	Cytosine
c	Concentration
calcd.	Calculated

COSY	Correlation spectroscopy
CSA	Camphorsulphonic acid
DCC	*N,N*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DI	Deionized
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformaminde
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
EDPI	Energy-dependent phase I
EDPII	Energy-dependent phase II
eq	equatorial
E-site	Exit site
ESKAPE	Pathogenic *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa,* and *E. cloacae*
Et	Ethyl
G	Guanine
g	Gram
gg	Gauche, gauche
GNAT	GCN5-related N-acetyltransferase
gt	Gauche, trans
HMBC	Heteronuclear multiple bond correlation

HMDS	Hexamethyldisilazane
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum coherence
Hz	Hertz
hv	Light
IC50	Inhibitory concentration
IV	Intravenous
L	Liter
LCMS	Liquid chromatography mass spectrometry
L-HABA	4-Amino-2(*S*)-hydroxybutyryl
M	Molarity
Me	Methyl
mg	Milligram
MHz	Megahertz
MIC	Minimum inhibitory concentration
mL	Milliliter
mmol	millimole
mRNA	Messenger ribonucleic acid
MRSA	Methicillin-resistant *Staphylococcus aureus*
N_3	Azide
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	*N*-bromosuccinamide

NMR	Nuclear magnetic resonance
^{o}C	Degrees Celsius
Ph	Phenyl
PMB	*p*-Methoxybenzyl
ppm	Parts per million
Pr	*n*-Propyl
psi	Pounds per square inch
P-site	Peptidyl site
py	Pyridine
RNA	Ribonucleic acid
RND	Resistance nodulation division
ROE	Rotating-frame Overhauser effect
ROESY	Rotating-frame Overhauser effect spectroscopy
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
TB	*Mycobacterium tuberculosis*
TBAF	Tetrabutylammonium fluoride
TBAI	Tetrabutylammonium iodide
TEMPO	(2,2,6,6-Tetramethylpiperidin-1-yl)oxyl
TFA	Trifluoroacetic acid
tg	Trans, gauche
THF	Tetrahydrofuran

TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
TMS	Trimethylsilyl
TOCSY	Total correlations spectroscopy
tRNA	Transfer ribonucleic acid
Ts	Toluenesulfonyl
U	Uracil
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND AND SIGNIFICANCE

Antibiotic use in modern medicine began in 1941 when penicillin, discovered by Alexander Fleming,[1] was first administered to a patient infected with both staphylococci and streptococci bacteria.[2] Treatment of the first patient was an amazing success, however, even before the first human trials bacteria were already known to have developed a resistance to penicillin.[3] Since then infectious bacteria have managed to keep pace with our ability to fight them and have developed resistance mechanisms to nearly all of our current weapons. In particular, the NDM-1 enzyme presents a major threat as it confers resistance to nearly all antibiotics in clinical use.[4-5] It is estimated that over 2 million people in the United States alone suffer from antibiotic-resistant infections each year leading to the death of around 23,000 people per year.[3] Despite the rapid ability of bacteria to develop resistance to antibiotics, approvals for new antibiotics have rapidly decreased from 28 in the 1980s to just 7 in the 2000s. The main reasons for this decrease in development stem from the low profitability for antibiotics when compared to other drugs and from the smaller number of groups working on antibiotic projects in industry. The low profit margins are because antibiotics are generally given for 1 to 2 weeks to cure a patient from an infection, as opposed to drugs for chronic conditions which bring in revenue for the remainder of the patient's life.[6-7] Due to the low profitability and the merging of drug companies, antibiotic groups are frequently shut down or merged which reduces the number and diversity of projects.[6, 8] Although there has been something of a surge in antibiotic

research in the past decade resulting in the approval of several new drugs, there remains a need to increase the momentum in order to keep antibiotic resistant pathogens at bay.[6, 9]

Antibiotics are divided into four categories based on their mechanism of action. Inhibition of folic acid synthesis, as with sulfonamides, indirectly prevents DNA synthesis because folic acid derivatives are used in the synthesis of purine and pyrimidine bases needed to build DNA. Inhibition of enzymes involved in DNA replication as seen with quinolones and others. Cell wall synthesis inhibitors, such as penicillin, inhibit enzymes involved in the synthesis of the peptidoglycan which is used to make the bacterial cell wall. Finally, inhibitors of protein synthesis such as aminoglycosides interfere with ribosomal translation processes to slow the synthesis of proteins or reduce the fidelity of their synthesis.[2]

The first aminoglycoside, streptomycin **4**, was discovered by Selman Waksman in 1943 through isolation from the soil bacteria *Streptomyces griseus.*[10] This was the first antibiotic effective against *Mycobacterium tuberculosis* (TB), which had previously been a death sentence.[2, 11] Since the introduction of streptomycin many other aminoglycosides have been discovered and used as effective antibacterial agents for both Gram-positive and Gram-negative bacteria as well as mycobacteria.[11] Although aminoglycoside antibiotics (AGAs) are highly active against a broad spectrum of bacteria, the issues associated with their use including nephrotoxicity, ototoxicity, and resistance, have caused them to lose favor in the clinic. Recently, however, there has been a resurgence in the study of AGAs with a focus on chemical modification to circumvent resistance and increase selectivity especially in the ESKAPE pathogens.[12-15]

1.2 STRUCTURE OF AMINOGLYCOSIDE ANTIBIOTICS

Aminoglycosides are based on an aminocyclitol ring, usually a 2-deoxystreptamine **1** or streptidine **2** ring, substituted at various positions with amino sugars (Figure 1). The suffix of the aminoglycoside name indicates which genus of bacteria the drug was isolated from: AGAs isolated from *Streptomyces* end in *mycin*, and AGAs isolated from *Micromonospora* end in *micin*. Due to the relatively high number of amines and hydroxy groups AGAs are very polar and highly water soluble, which causes the oral bioavailability of the drug to be low making IV injection the preferred route of administration.[2, 16]

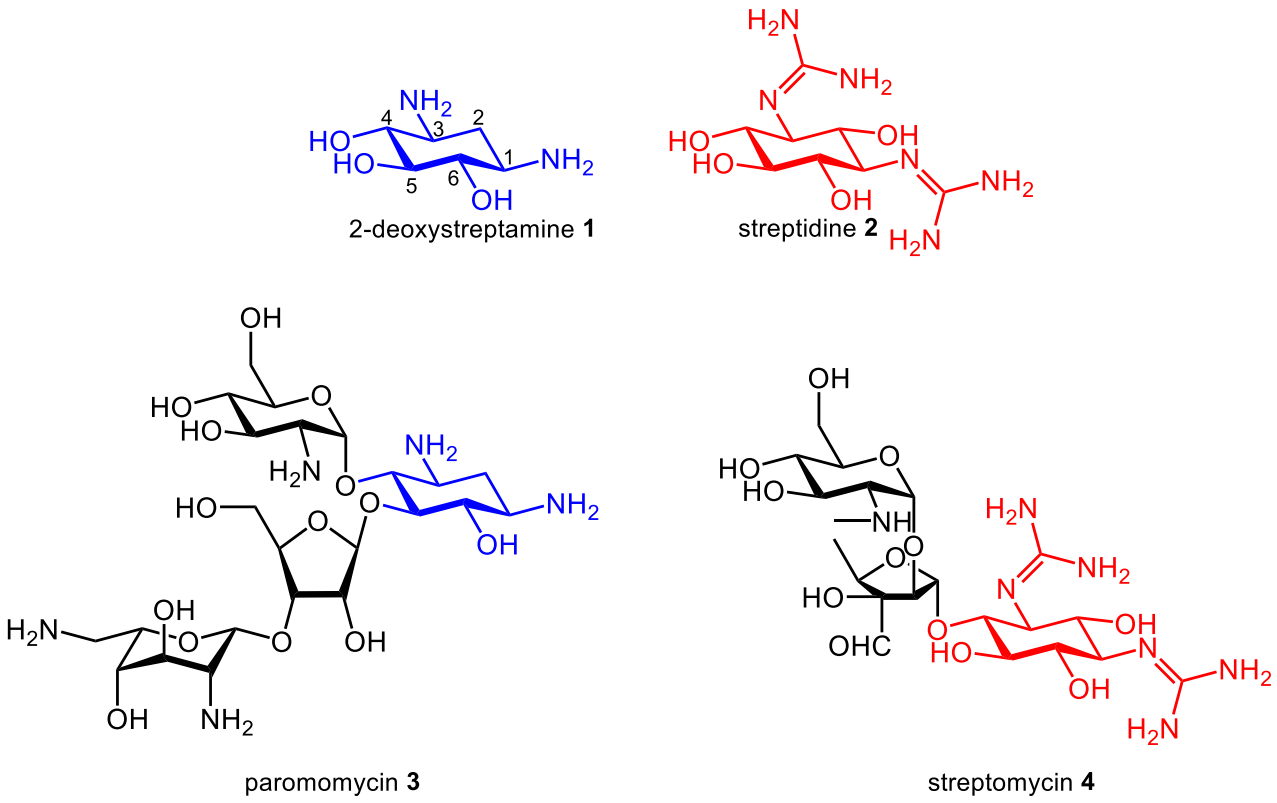


Figure 1: Structures of 2-Deoxystreptamine, Streptidine, Paromomycin, and Streptomycin

The 2-deoxystreptamine (2-DOS) AGAs are subdivided into 4,5-substituted and 4,6-substituted classes, although there are rare exceptions such as apramycin **5**, which is monosubstituted at the 4-position. The major examples of AGAs in the clinic, tobramycin **6** and gentamicin **7**, are members of the 4,6-series, however, there is growing interest in the 4,5-series as clinical candidates.[11-12, 17]

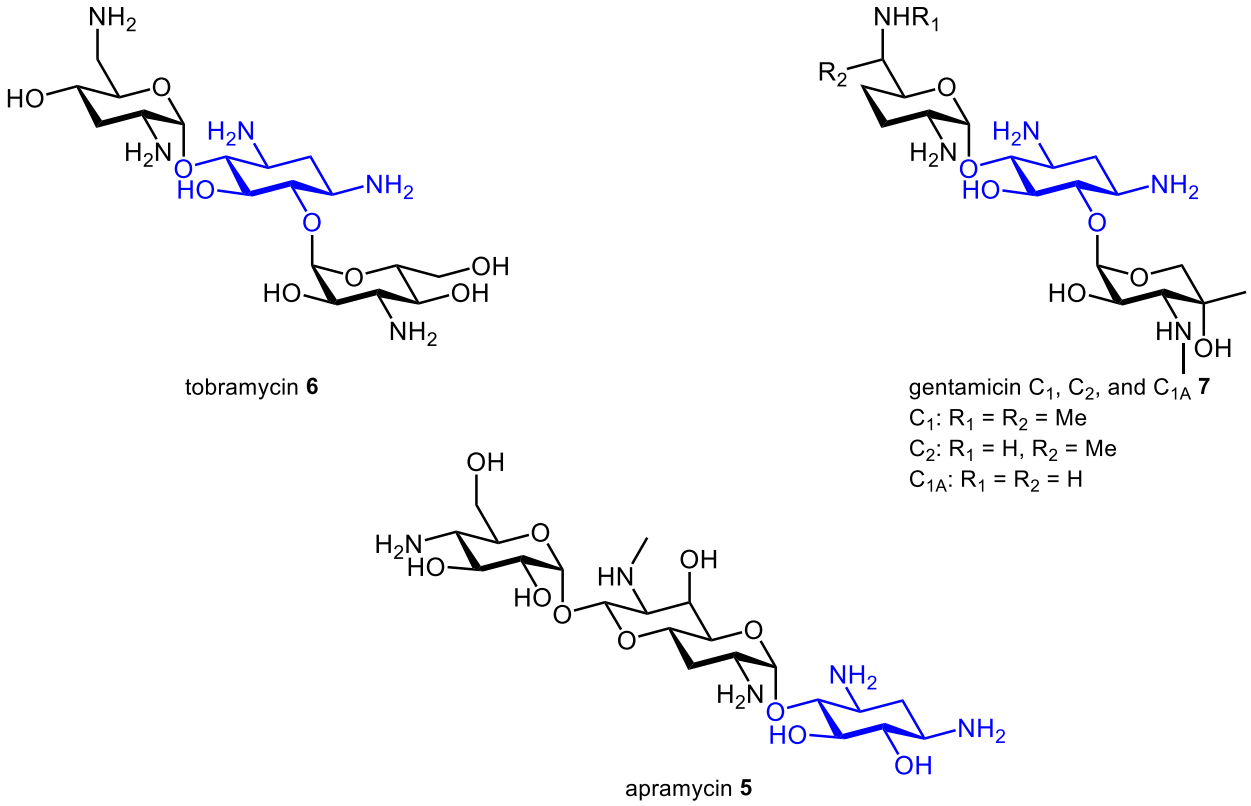


Figure 2: Structures of Apramycin, Tobramycin, and Gentamicin

1.3 AGA MECHANISM OF ACTION

The mechanism of action for aminoglycoside inhibition of protein synthesis is well studied.[18-20] Aminoglycosides inhibit protein synthesis in a concentration dependent manner as opposed to a time dependent one, therefore, concentrations in excess of the minimum inhibitory

concentration (MIC) for a short period of time are more effective than long term concentrations at the MIC.[2] In addition, aminoglycosides are able to kill bacterial cells as opposed to simply stopping their growth as with some antibiotics, making AGAs a better choice for immunocompromised patients. Although AGAs are effective against Gram-positive, Gram-negative, and mycobacteria, they remain ineffective against anerobic bacteria due to their uptake mechanism.

1.3.1 UPTAKE

There is some controversy as to whether AGAs diffuse through the cell membrane or pass through porin channels to enter bacterial cells.[21-22] Nevertheless it is known that the uptake of AGAs proceeds in three steps. First, due to the cationic nature of AGAs and the negative charge of the lipopolysaccharide outer membrane, the drug is held at the membrane electrostatically. Following this is an energy dependent phase I (EDPI) where the AGA passes through the cell membrane. This is tied to cellular respiration, which explains why AGAs are ineffective against anerobic bacteria. Finally, due to the buildup of faulty proteins essential for cell wall growth, energy dependent phase II begins (EDPII), where excess AGA may enter the cell.[2]

1.3.2 INHIBITION OF PROTEIN SYNTHESIS

Proteins are synthesized in the cell through translation of messenger RNA (mRNA) by ribosomal RNA (rRNA), which selects amino acid building blocks by pairing the codons in the mRNA to a specific set of anticodons in transfer RNA (tRNA).[23] Each tRNA has an amino acid which the rRNA stitches to the growing peptide chain in sequence to make the protein. AGAs inhibit protein synthesis by binding to the rRNA and interfering with translation.[11] Proteins are

synthesized in much the same way in both prokaryotic and eukaryotic cells, which means the AGAs must be selective for bacterial ribosomes although some activity against eukaryotic ribosomes is inevitable.

Ribosomes consist of two subunits, large and small, as well as several proteins. Prokaryotic ribosomes, as well as those found in mitochondria and chloroplasts, contain a 50S and a 30S subunit, while eukaryotic ribosomes contain a 60S and a 40S subunit. AGAs bind to helix 44 of the smaller subunit where the decoding A-site is located.[24-25]

There are three decoding sites in rRNA; the aminoacyl site (A-site), the peptidyl site (P-site), and the exit site (E-site). The A-site is where the mRNA initially binds to the ribosome and waits to be paired with the tRNA containing the correct anticodon. Binding of the tRNA causes a conformational change in the ribosome where rRNA bases A1492 and A1493 are flipped out of the helix causing the mRNA-tRNA pair to move to the P-site. In the P-site the peptide attached to the tRNA is transferred to the peptide chain being synthesized before the RNA passes to the E-site where it exits the ribosome (Figure 3).[2, 23]

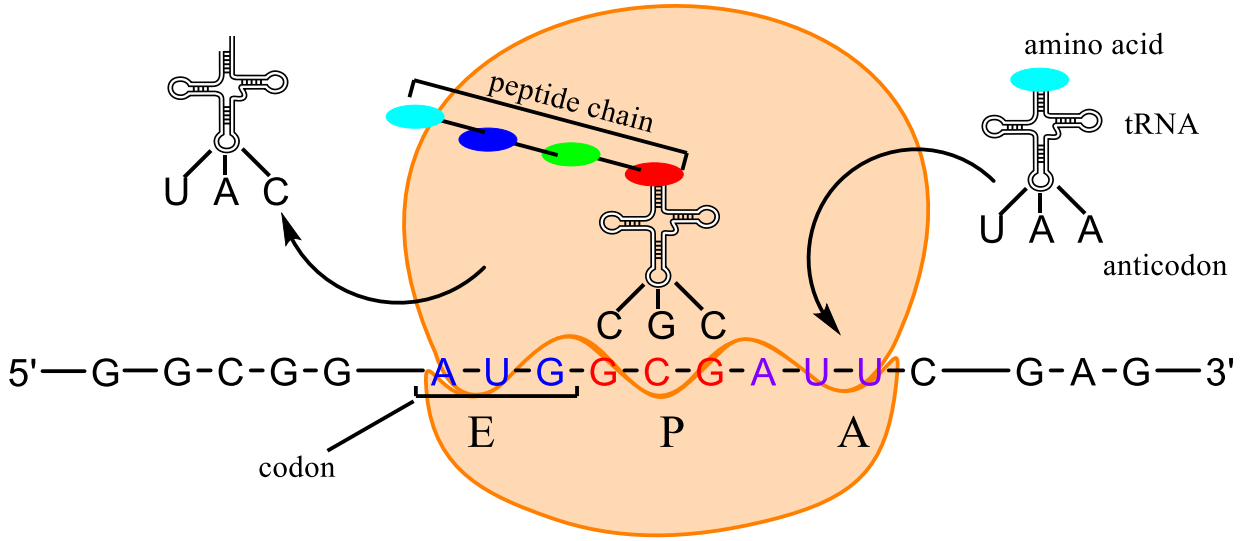


Figure 3: Translation of mRNA

AGAs bind to the A-site through two major interactions; their cationic nature causes an electrostatic interaction with the phosphate backbone of the rRNA as well as hydrogen bonding interactions with various bases in the A-site.[2, 11] Some of these hydrogen bonding interactions vary between AGAs, however, there are certain key interactions that are much more common. These include the pseudo base pair between the amine or hydroxy group at the 6'-positon and the ring oxygen of ring I with A1408 (Figure 4), as well as the 2-DOS hydrogen bonding network to A1406, UG1494, and U1495. The binding of the AGA in this way stabilizes the flipped-out conformation of A1492 and A1493 (Figure 5) which lowers the energy required for tRNA with incorrect anticodons to bind and reduces the fidelity of translation. Proteins with the incorrect amino acid sequence will not function properly and lead to cell death due to the buildup of reactive oxygen species[26-27] or production of free radicals due to oxidative stress.[28]

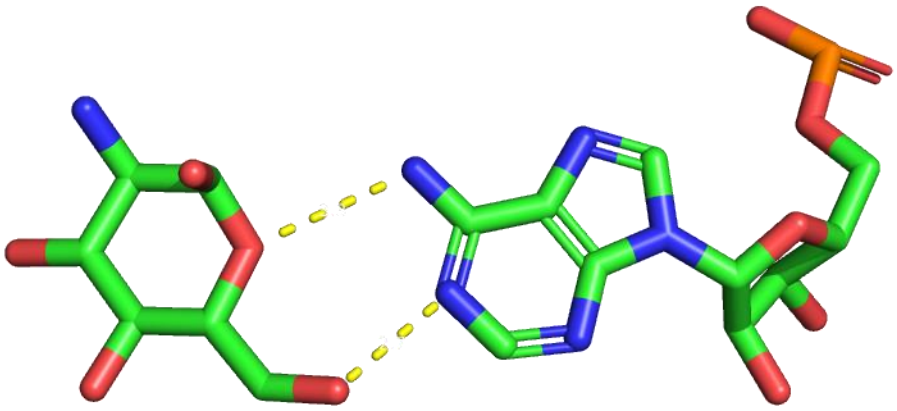


Figure 4: A Pseudo Base Pair Interaction

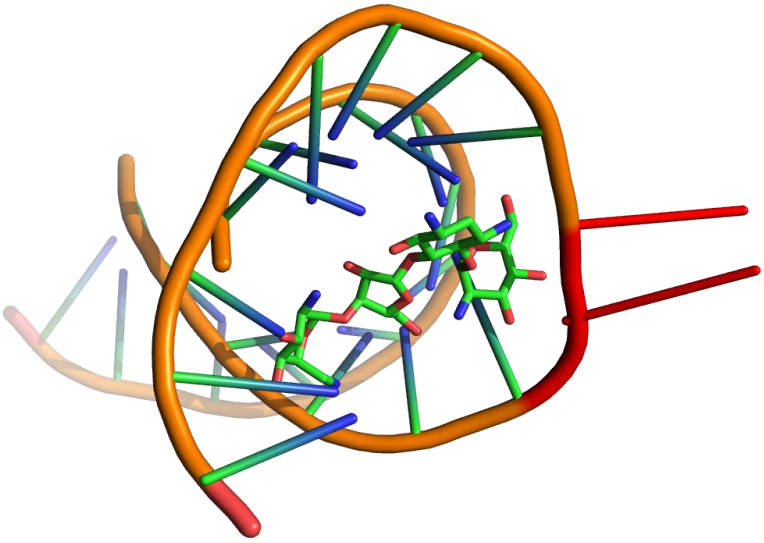


Figure 5: A1492 and A1493 in the Flipped-out Conformation in the Complex of Thermus Thermophilus 30S rRNA Subunit with Paromomycin PDBID:1FJG[29]

1.4 RESISTANCE AND TOXICITY

Although AGAs have many desirable properties as antibacterial drugs, there are a few key issues which have caused them to lose favor in the clinic. Thus, due to the similarity between bacterial and human decoding A-sites, AGAs can be toxic to humans.[30-31] Further, due to their initial widespread and improper use combined with the rapid evolution of bacteria, many species have developed AGA resistance. Although these problems may seem severe the source of these issues is well studied allowing medicinal chemists to overcome them via rational modification.

1.4.1 RESISTANCE

Bacterial resistance to AGAs stems mostly from three distinct mechanisms; target modification[32-34], altered transport[35-36], and substrate modification.[37-39] Target modification involves bacteria making changes to the A-site in the ribosome in order to prevent the AGAs from binding. Altered transport can cause reduced uptake, where the process of AGAs entering the cell is inhibited, as well as increased efflux, where the cell is able to remove AGAs after they have passed through the membrane. Finally, the most prevalent resistance mechanism is substrate modification using aminoglycoside modifying enzymes (AMEs), which modify functional groups on the AGA in order to prevent it from fitting into the binding site. Due to the nature of bacteria these resistance mechanisms are subject to horizontal gene transfer under the correct conditions allowing them to spread quickly between species if infections are not treated properly.

Bacteria which produce AGAs naturally must have resistance mechanisms to ensure that they are not killed by the compounds they produce. Although it is possible to modify AGAs to

circumvent resistance, the most important measure for managing AGA resistance is proper use of antibiotics.[9]

1.4.1.1 TARGET MODIFICATION

Target modification refers to alteration of the decoding A-site which can be done either through methylation during a post translational modification or through a point mutation where one RNA base is changed. These modifications are the least clinically relevant because they mostly occur in bacteria which produce AGAs. Cases of nucleotide mutation such as A1408G give high levels of resistance to 2-DOS AGAs and have been found in rare cases in *Mycobacterium tuberculosis*.[40-41]

Methylation of RNA bases is done by enzymes as a post translational modification in many AGA producing bacteria.[42] The most clinically relevant methylases are in the *arm* family[11] which have been found in *S. marcescens,*[33] *K. pneumoniae,*[43] and *E. coli* where they methylate G1405 (Figure 6).[44] These modifications greatly reduce the activity of 4,6-substituted 2-DOS aminoglycosides, but have little activity on the 4,5-series.[12]

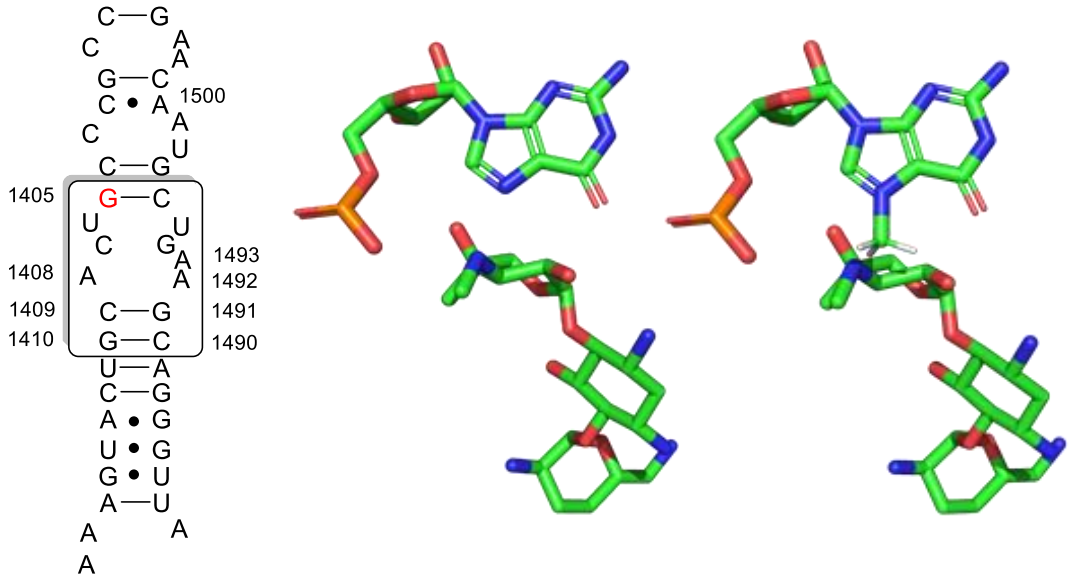


Figure 6: Gentamycin C1A Shown Bound to G1405 and G1405 Drawn with Methylation. PDBID 4LF9

1.4.1.2 ALTERED TRANSPORT

Altered transport refers to methods bacteria use to lower the concentration of AGA in the cell. Decreased uptake through the cell membrane can cause the internal concentration of AGAs to be much lower than expected relative to the extracellular concentration. Although there is controversy as to whether AGAs use porin channels to pass through the outer membrane, it is known that *P. aeruginosa* strains with inactive porin proteins are resistant to gentamicin.[11, 45] The genes controlling the porin proteins are also known to affect the modification of lipopolysaccharides, which can explain the difference in uptake if the AGAs do not pass through porins.

Bacterial cells can also have efflux systems, which lower the concentration of AGAs in the cell by pumping them out through the membrane. Strains of *P. aeruginosa* and *E. coli* are both

known to have efflux systems, of which the most common family is the resistance nodulation division (RND) which consists of an efflux pump paired with a periplasmic membrane fusion protein and an outer-membrane factor.[46] The levels of resistance conferred by different efflux pumps varies greatly. The MexAB-OprM pump found in *P. aeruginosa* is not very effective at removing a therapeutic dose of AGA, however, MexXY in the same species grants a broad range of AGA resistance. These efflux systems are only found in Gram-negative bacteria and are not restricted to efflux of AGAs but also can remove other antibiotics and dyes.[11]

1.4.1.3 AMINOGLYCOSIDE MODIFYING ENZYMES

The most prominent mechanism of AGA resistance in pathogenic bacteria is the aminoglycoside modifying enzymes (AMEs). These enzymes covalently modify AGAs, which prevents them from properly binding to the A-site due to steric constraints or blocking of key hydrogen bonding interactions. Most AMEs are encoded on plasmids, which facilitates rapid spread of resistance through horizontal gene transfer. There are three classes of AMEs determined by the type of group added during modification. Aminoglycoside acetyl transferases (AAC) acetylate amine groups, aminoglycoside phosphotransferases (APH) phosphorylate hydroxy groups, and aminoglycoside nucleotidyltransferase (ANT) adenylate hydroxy groups on the AGA.[47-48] AMEs are named based on the three-letter abbreviation of their class, the position they modify, their phenotype expressed as a Roman numeral, and finally a letter annotating the gene which encodes them.[49] For example, AAC(3)-Ia will acetylate N-3 of gentamicin and sisomicin, however, AAC(3)-VII will only acetylate gentamicin. Figure 5 shows some common AGAs and the different AMEs that can modify them.

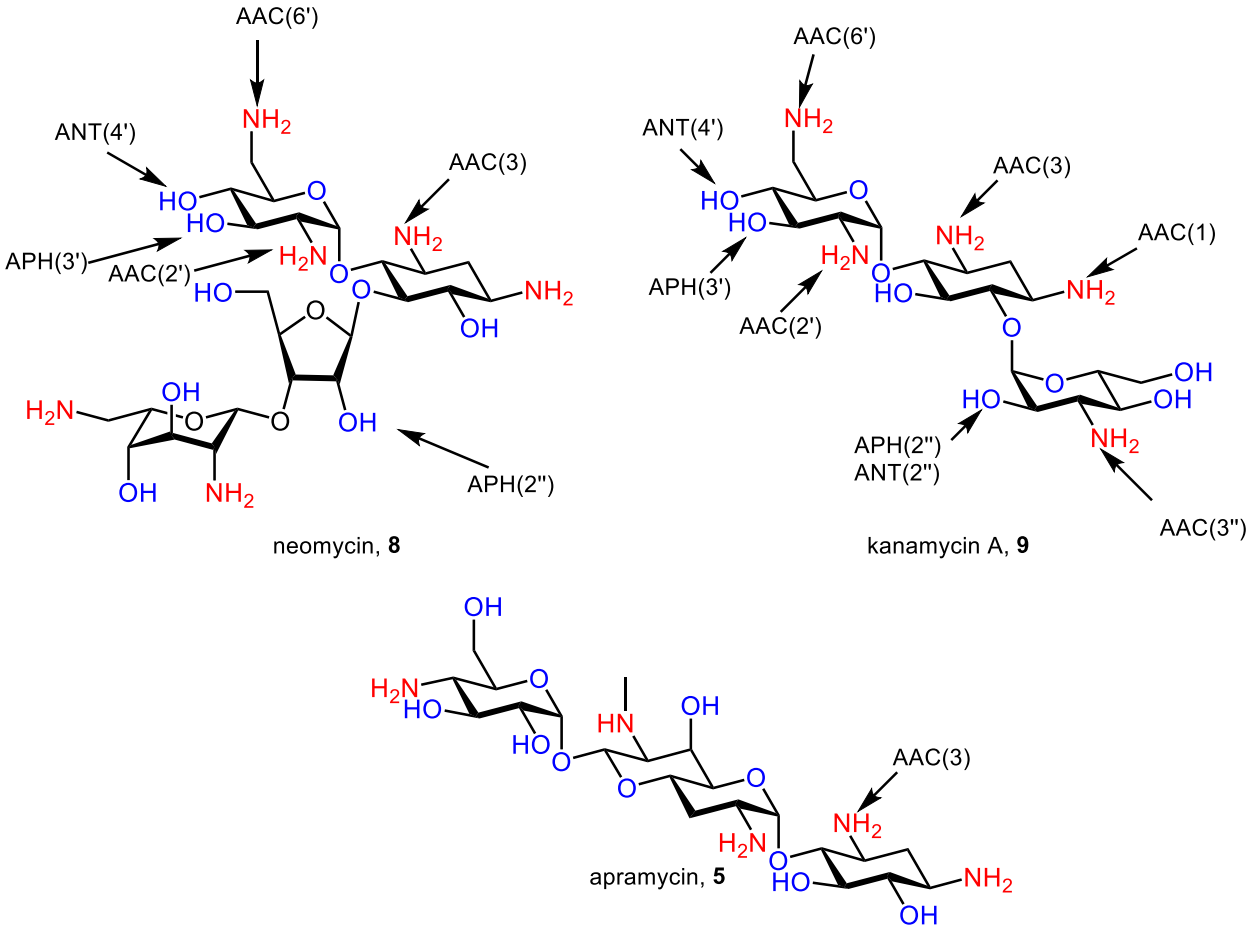


Figure 7: Aminoglycoside Modifying Enzyme Targets

The most common type of AMEs are the AACs which can be found in both Gram-positive and Gram-negative bacteria and cause resistance to a broad range of AGAs. There are four subclasses of AACs which act on amines at the 1, 3, 2', and 6'-positions common to most AGAs.[11] They are members of the GCN5-related N-acetyltransferase superfamily (GNAT), which notably share very little commonality in amino acid sequence but are characterized by the similarity in their folding pattern around their co-substrate, acetyl-CoA.[50] GNAT enzymes are generally promiscuous, and AACs have been found to act on different substrates in the cell indicating that they may have originally fulfilled a different purpose before evolving to modify AGAs.

The five members of the ANT enzyme family modify hydroxy groups at the 9, 3', 4', 6', and 2''-positions of various aminoglycosides using ATP as a co-substrate. The most clinically relevant member of this family is ANT(2'')-Ia having been found in many strains of Gram-negative bacteria and which causes high levels of gentamicin and tobramicin resistance in North America.[11] Nevertheless, ANT enzymes are the least prominent AMEs.

APHs use ATP as a co-substrate to phosphorylate hydroxy groups at the 4, 6, 9, 3', 2'', 3'', and 7''-positions of AGAs. The addition of a negatively charged phosphate group reduces binding due to both steric bulk and electrostatic repulsion with the negatively charged RNA backbone. The APH(3')-IIIa enzyme has been found in numerous Gram-positive bacteria and grants resistance to a broad range of AGAs including kanamycin **9**, paromomycin **3**, and neomycin **8**.

Resistance from AMEs can be overcome by either inhibiting the AMEs, or more commonly, synthetic modification of AGAs to block AME activity. Inspired by the natural AGA butirosin **10**, a semisynthetic derivative of kanamycin known as amikacin **11** was developed with a 4-amino-2-hydroxybutyramide group on *N*-1, resulting in a recovery of activity against bacterial strains with AAC(1) and AAC(3) enzymes. It has also been shown that alkylation of the 2'-amine of paromomycin and neomycin restores activity against bacteria with an AAC(2').[51]

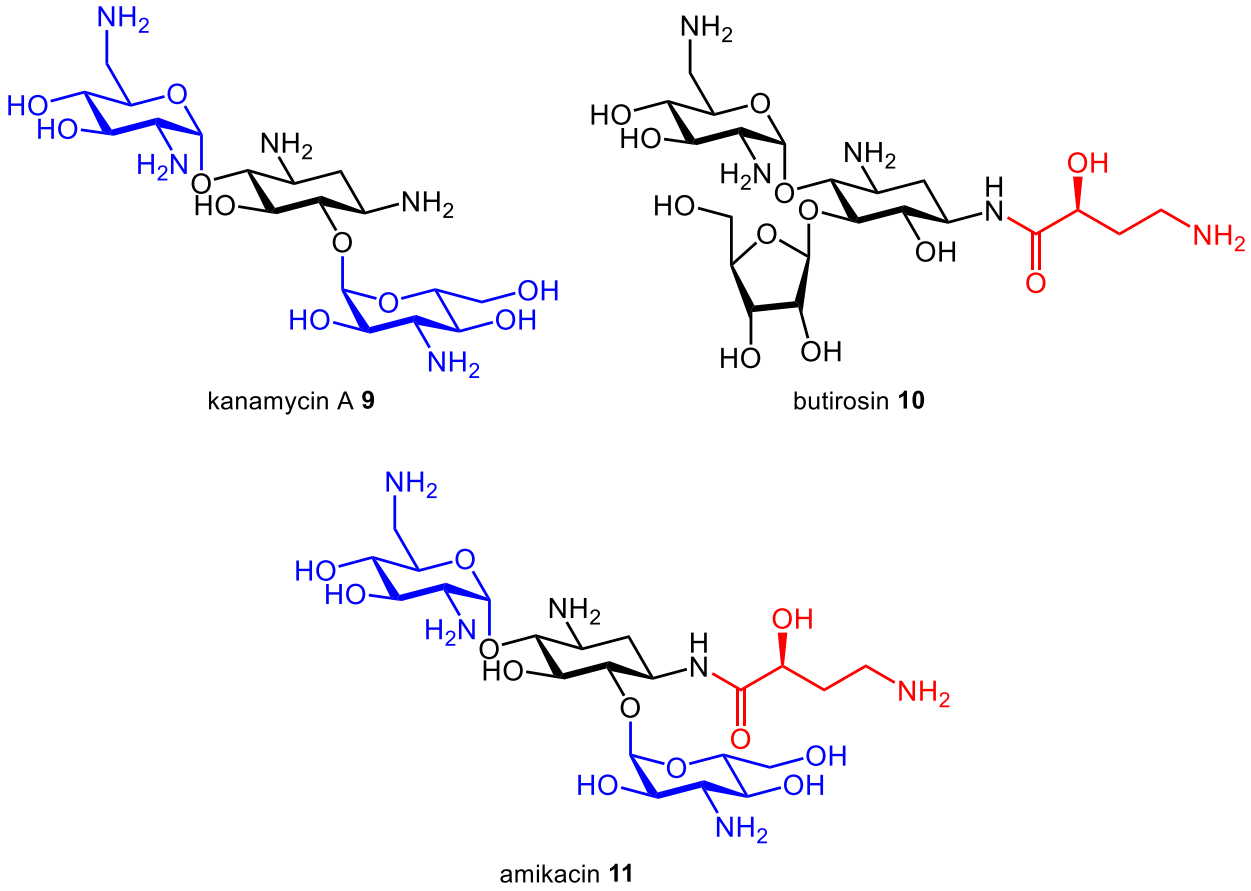


Figure 8: Amikacin, Inspired by Kanamycin A and Butirosin

1.4.2 TOXICITY

The major adverse effect of AGA treatment is toxicity to human cells, which mostly manifests as kidney damage through nephrotoxicity and hearing damage through ototoxicity. These side effects are expressed to different degrees based on the antibiotic and the individual being treated. In addition there seems to be no correlation between ototoxic potential and nephrotoxic potential for a given AGA.[11] The reason for this toxicity is due to the similar structure of human and bacterial ribosomes shown in Figure 7 with numbering to match the bacterial A-site. Paromomycin interacts through hydrogen bonding with 7 residues in the bacterial A-site

including G1405, A1408, C1490, G1491 A1493, G1494, and U1495. Of these 7 residues 5 are conserved between bacterial ribosomes and human mitochondrial ribosomes and 4 are conserved in human cytosolic ribosomes. Due to this similarity aminoglycosides can inhibit protein synthesis in human cells, albeit to a lesser extent. The mitochondrial ribosome with the A1555G mutation, (corresponding to 1490 in bacterial numbering), changes the interaction with C1410 from a non-canonical base pair to a Watson-Crick base pair, thus tightening up the binding site and increasing susceptibility to AGAs.[52-54] This mutation significantly increases the risk of hearing damage in patients treated with AGAs.

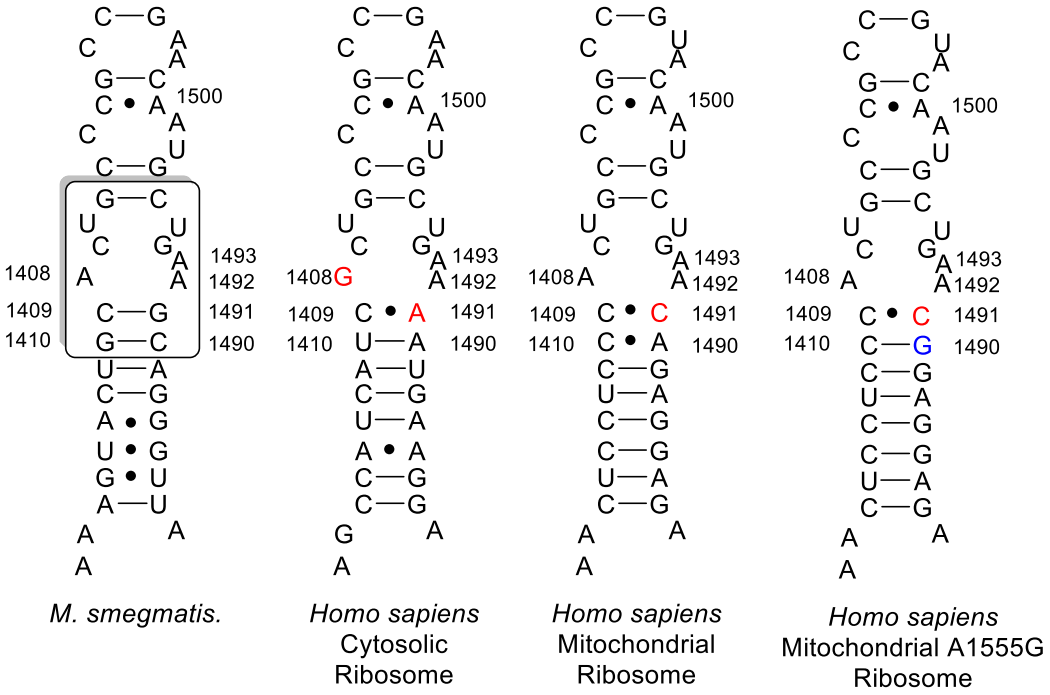


Figure 9:Ribosomal A-sites in Bacteria and Humans

1.4.2.1 NEPHROTOXICITY

Despite extensive study the mechanism of aminoglycoside nephrotoxicity is not completely understood.[2] The cationic nature of AGAs combined with IV administration results in about 90% of the dose being excreted through the kidneys within 24 hours.[55] Over the course of collection in the kidneys the proximal tubule can reabsorb a significant amount of the drug, causing kidney cells to maintain higher concentrations of the drug for longer than most other tissues. Once absorbed into the kidney cells AGAs, due to their polycationic nature, can bind to phospholipid membranes and inhibit lysosomal phospholipase activity.[56] There are two proposed mechanisms for the resulting kidney cell necrosis. Either the localization of the aminoglycosides by the lysosomes results in a concentration dependent toxicity, or the toxicity occurs after the AGAs are released from the lysosomes. In either case, aminoglycosides chelate with iron in the mitochondria to form reactive oxygen species (ROS).[57]

Clinically nephrotoxicity is the lesser of the two toxicity issues because it is reversible in most cases and more easily managed. Studies have shown that the best method of AGA administration to reduce nephrotoxicity is to use a once daily dose instead of a continuous dose because the kidney cells become saturated at a low concentration, preventing more of the drug from being absorbed as it is excreted.[58] Acylation of *N*-1 of the 2-DOS have also shown an increase in selectivity for bacteria over kidney cells by reducing the binding to phospholipids.[21] Additionally, hydration therapy has been reported to reduce the nephrotoxic effects of AGAs.[2]

1.4.2.2 OTOTOXICITY

Ototoxicity from aminoglycosides, which affects up to 20% of patients, is a more serious issue because it is difficult to monitor and results in permanent hearing damage. AGA ototoxicity affects both the vestibular system, which results in a loss of balance, and the cochlea, which results in a loss of hearing. Toxicity to the vestibular system and the cochlea vary randomly between AGAs. Neomycin, amikacin, and dihydrostreptomycin are more cochleatoxic, whereas streptomycin and gentamicin are more vestibulotoxic.[2, 11]

Uptake of AGAs in the ear occurs rapidly with toxicity setting in within four hours of the first dose in some patients.[59] It was previously thought that there was accumulation of AGAs in the inner ear although more recent studies show that concentrations do not even reach serum levels.[60] In some patients hearing loss does not occur until after treatment has finished because, although the half-life of AGAs is usually 3-5 hours, the inner ear retains AGAs much longer with a half-life of up to 30 days.[11]

The mechanism of ototoxicity is also not completely understood; however, it is known that the deafness occurs when cochlear hair cells die. The cochlear hair cells translate vibrations from sound into electrical impulses in the nerves. When these hair cells die, they do not regrow, which is why ototoxicity is permanent. The first hair cells to die are the basal cochlear cells which translate high frequency sound as the drug works its way to the apical cells which translate low frequency sounds.[61]

Cochlear cell death has been linked to the buildup of reactive oxygen species although the mechanism of their formation is uncertain.[62] It has been theorized that aminoglycosides form

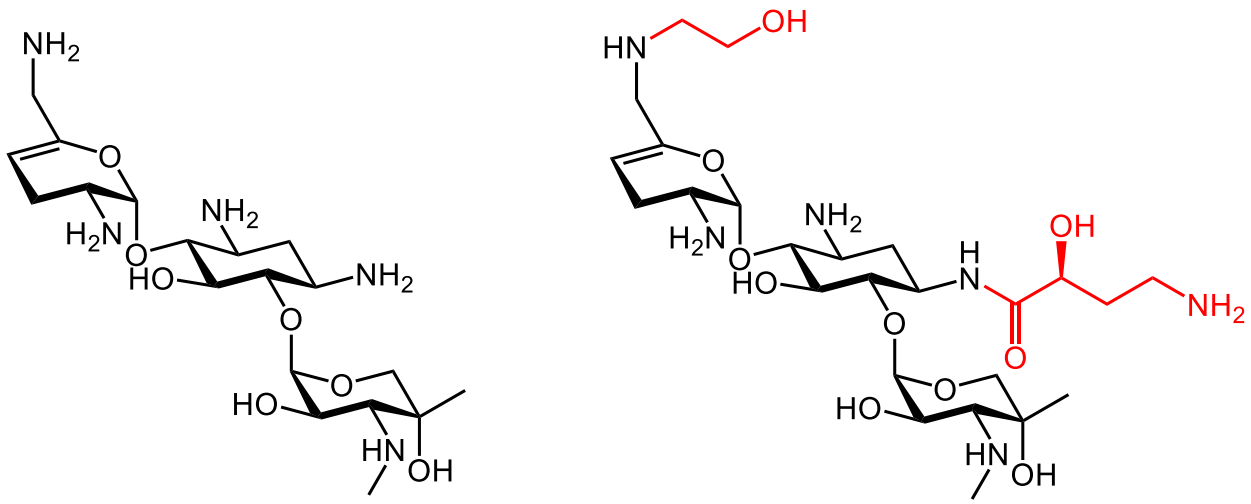
complexes with iron and arachidonic acid to produce ROS.[63] Another theory suggests that AGAs activate Rho-GTPase, which then activates the NADPH oxidase complex, in turn forming superoxide radicals.[64] Recently, however, it is thought that inhibition of protein synthesis in the mitochondrial ribosomes, which have a more similar A-site to bacteria, causes this buildup of reactive oxygen species.[65-67] This evidence is further supported by the fact that genetically susceptible individuals with an A1555G mutation in their mitochondrial RNA suffer a much greater risk of hearing damage when given aminoglycosides.[68] It has been shown that administration of Aspirin as a radical scavenger to neutralize ROS is effective at reducing ototoxicity.[69-70]

1.5 RECENT ADVANCES

One of the most important contributions to the search for better AGA derivatives is the suite of chemical biology tools developed by the Böttger group. Strains of *M. smegmatis* were developed with the A-sites of human cytosolic ribosomes, human mitochondrial ribosomes, and human mitochondrial ribosomes with the A1555G mutation.[71] This work has shown that helix 44 of the ribosome functions independently, and that by swapping the eukaryotic A-sites into strains of bacteria rapid preliminary screening of compounds for selectivity can be achieved.

The most recently approved AGA is plazomicin **13**, a sisomicin **12** derivative developed by Achaogen.[34, 72] Plazomicin was approved in 2018 for the treatment of drug resistant urinary tract infections. This AGA was designed with a 4-amino-2(*S*)-hydroxybutyryl (L-HABA) group on *N*-1 which protects from modification by AAC(1), AAC(3), APH(2''), and ANT(2'') enzymes in addition to reducing nephrotoxicity. The 2-hydroxyethyl group on *N*-6' protects from modification by

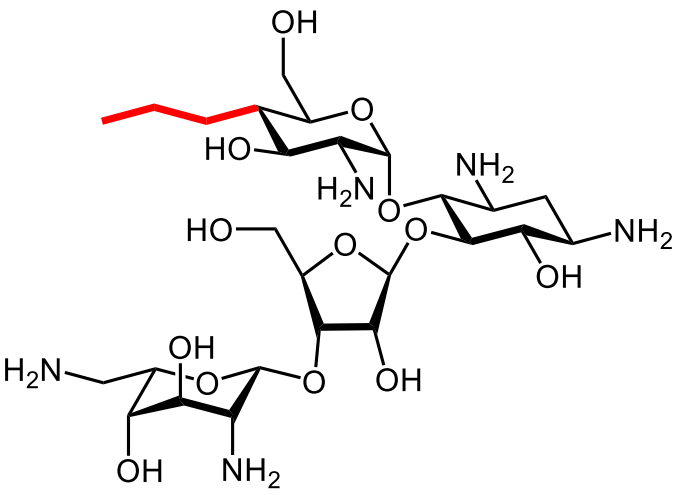
AAC(6'). This antibiotic is a step in the right direction, however, it still displays ototoxicity,[73] and as a member of the 4,6-substituted 2-DOS series, it loses activity in the presence of armA modification.



sisomicin **12** plazomicin **13**

Figure 10: Structures of Sisomicin and Plazomicin

Propylamycin **14**, 4'-deoxy-4'-*C*-propyl paromomycin, is a recently developed paromomycin derivative, which has been shown to have good activity against a wide range of Gram-positive and Gram-negative bacteria.[12] It is considered that replacement of *O*-4' with a methylene group causes *O*-5 to be more basic due to the increase in electron density, which in turn allows it to make a stronger hydrogen bond to A1408. It is also thought that there is a hydrophobic interaction with the propyl group further enhancing binding to the ribosome. The addition of the propyl group also protects from modifications by the ANT(4') and APH(3') enzymes leading to increased activity in the presence of resistance determinants.



propylamycin **14**

Figure 11: Structure of Propylamycin

Apramycin **5** is a unique aminoglycoside due to its bicyclic ring I and monosubstituted 2-DOS that has recently gained interest for clinical use. Although it is slightly less active than most AGAs that have been approved for clinical use, it has the best selectivity profile in recent studies.[74] The only known AME which affects apramycin is AAC(3)IV[75] making it an excellent candidate for multidrug resistant infectious diseases.[76-77] Phase 1 clinical trials for apramycin will begin in Germany in 2019.[78]

1.6 OVERALL GOALS

The goal of this project is to develop new AGAs, which are more selective for inhibition of protein synthesis in bacteria than in human cells. The interaction between the aminoglycoside ring 1 and A1408 is crucial for drug binding and selectivity suggesting that ring I is the area of interest for modification. Paromomycin is an interesting substrate for modification because it has relatively high selectivity, and as a member of the 4,5-disubstituted series is not affected by the

armA resistance mutation. X-ray structures show that the side chain of paromomycin is in a particular conformation when bound (Figure 10),[29] which suggests that modifications to the 6'-position which would encourage preorganization into this conformation would be ideal for increasing activity.

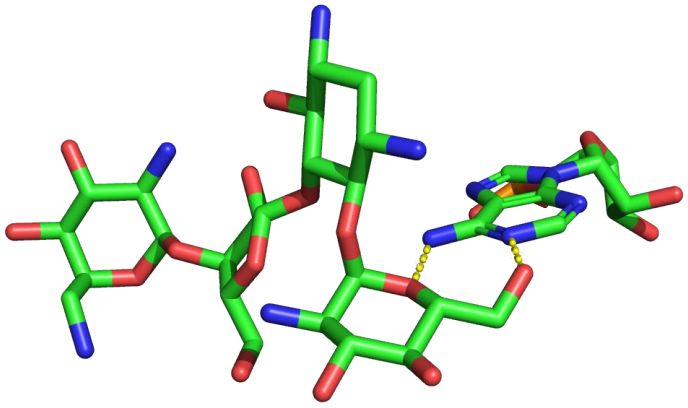


Figure 12: Pseudo Base Pair Interaction of Paromomycin Ring I with A1408[29]

In addition to the other benefits of the 4'-*C*-propyl group on propylamycin it is possible that the added steric bulk at the 4'-position causes the side chain to preorganize into the bound conformation. NMR studies of the side chain of propylamycin would shed new light on the function of this AGA, however, due to the complex NMR spectrum of this molecule a simpler substrate should be used. Synthesis of a model monosaccharide will be carried out in order to conduct an NMR study on the conformation of the side chain in solution.

CHAPTER 2: MODIFICATIONS TO THE 6'-POSITION OF PAROMOMYCIN AND NEOMYCIN

2.1 RIBOSOMAL INTERACTIONS WITH PAROMOMYCIN AND NEOMYCIN

Paromomycin and neomycin are members of the 4,5-series of 2-DOS aminoglycosides and differ only in the functional group at the 6'-position, a hydroxy group in paromomycin and an amine in neomycin. Despite the similarity these AGAs have very different selectivity profiles with neomycin being slightly more active in bacteria and significantly more active in human mitochondria.

The interactions between paromomycin and the bacterial ribosomal A-site are shown in Figure 11. The ring oxygen of ring I and the 6'-hydroxy group form a pseudo base pair interaction with A1408, while *H*-4' takes part in a CH-π interaction with G1491, and the 4'-hydroxy group forms a hydrogen bond to the phosphate of A1493.[29] On ring II the amine at the 1-position forms a hydrogen bond to U1495, and *N*-3 interacts with G1494 through hydrogen bonding. Only the 5''-hydroxy group of ring III interacts with the ribosome where a hydrogen bond to G1491 is stabilized by another hydrogen bond to *N*-2'. The 6'''-amine of ring IV forms a hydrogen bond to the backbone of C1490 and the 3'''-hydroxy group acts as a hydrogen bond donor to the phosphate of G1405.

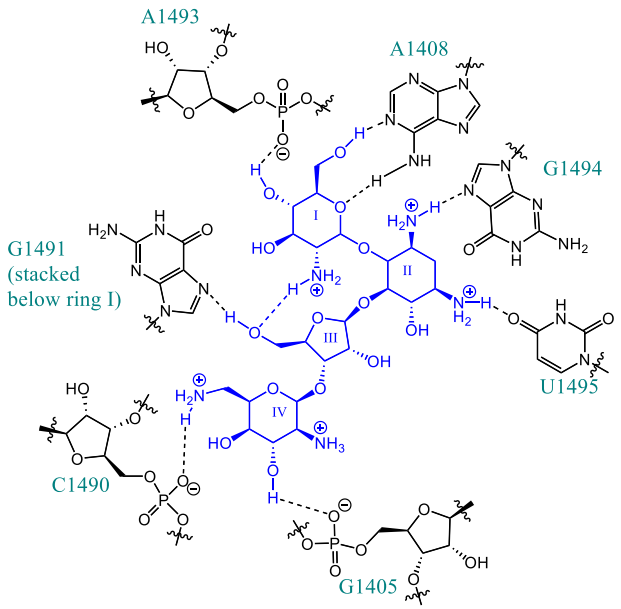


Figure 13: Interactions Between Paromomycin and the Bacterial Ribosome

Pyranose sugars have the three staggered conformations for the side chain shown in Figure 14.[79] In the gg conformation the C_6-X_6 bond is gauche to both the C_5-O_5 and the C_4-C_5 bonds, this conformation is the lowest in energy in a glucose system. The next most favorable conformation in glucose is gt, where the C_6-X_6 bond is gauche to the C_5-O_5 and trans to the C_4-C_5 bonds. The third conformation, known as tg, has the C_6-X_6 bond trans to the C_5-O_5 and gauche to the C_4-C_5 bonds.[80] The conformation adopted by paromomycin bound to the A-site is gt.[29, 81]

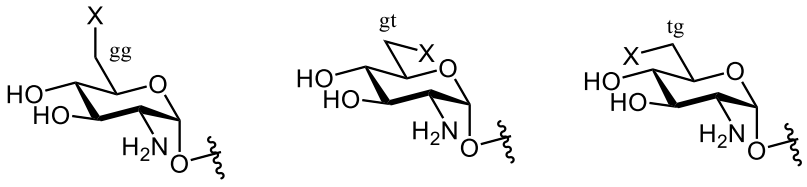


Figure 14: Pyranose Side Chain Conformations and Relative Populations in Free Solution[82]

2.2 RATIONAL

The interaction between the 6'-hydroxy group and A1408 is particularly interesting because crystal structures show that the side chain is in a particular conformation[29] (Figure 12) which is generally not the dominant conformation in a glucosamine system in free solution.[82] Due to the entropic penalty incurred by organizing the side chain into this conformation for binding, a hypothesis was formulated whereby activity may increase when the 6'-position is substituted in such a way that the bound conformation is more favorable. This prediction is backed up further by the structure of the naturally occurring aminoglycoside geneticin (G418) **15** which has a methyl group in the side chain with the 6'-(*R*) configuration, which should be preferred for this preorganization.

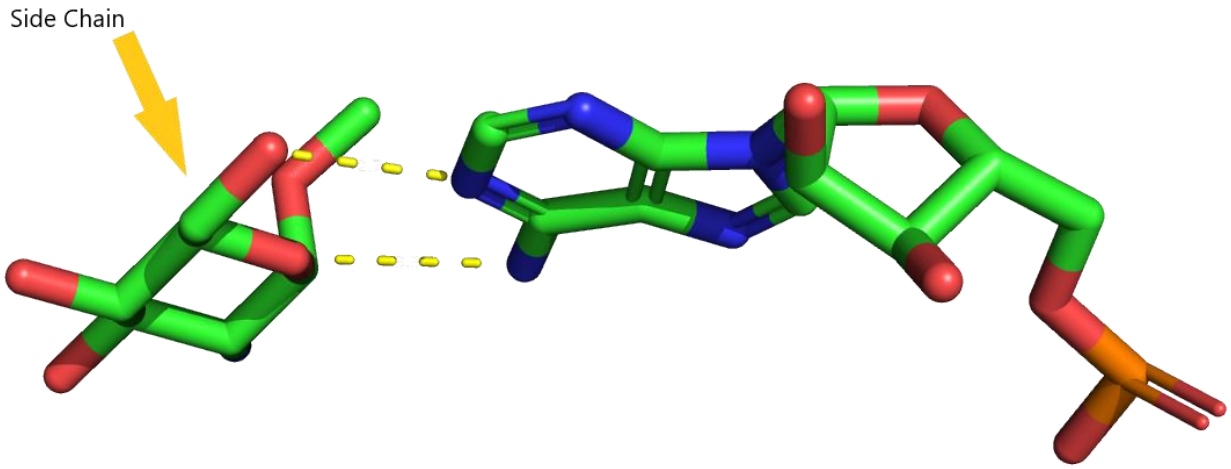
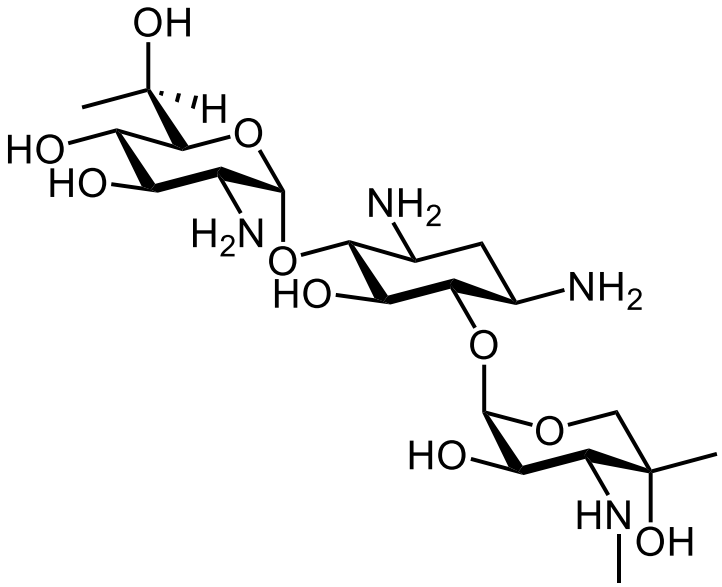


Figure 15: Paromomycin Ring I Bound to A1408 with the Ring I Side Chain in the gt Conformation[29]

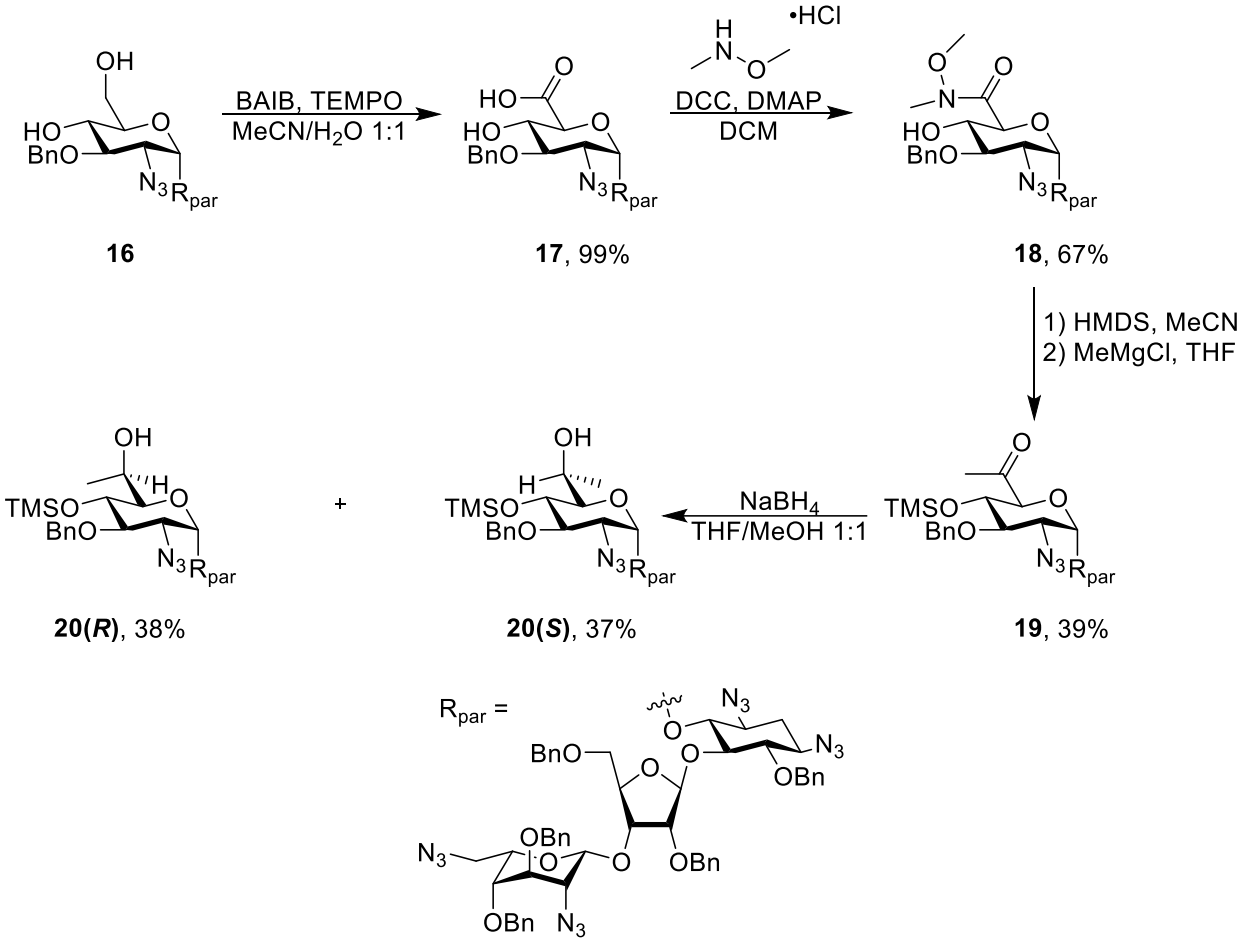


geneticin **15**

Figure 16: Structure of Geneticin

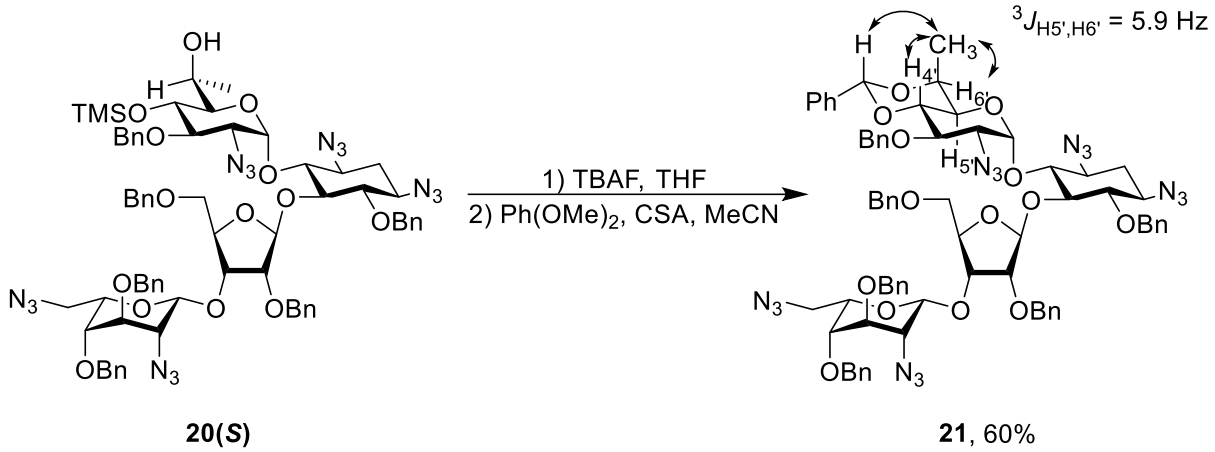
2.3 SYNTHESIS OF 6'-METHYL PAROMOMYCIN AND NEOMYCIN DERIVATIVES

Compounds **21(*R*)** and **21(*S*)** were made starting with diol **16** initially reported by the Vasella group.[83] Initial attempts to selectively oxidize the primary alcohol of **16** to the aldehyde followed by alkylation with Grignard reagents were met with low yields and difficult purification prompting a switch from the aldehyde to a Weinreb amide.[84] Selective oxidation of the 6'-hydroxy group with BAIB and TEMPO gave the carboxylic acid **17** in 99% yield.[85] Acid **17** was then coupled to Weinreb's amine using DCC and DMAP to give Weinreb amide **18** in 67% yield. The 4'-hydroxy group of **18** was then protected as a trimethylsilyl ether using hexamethyldisilazane in acetonitrile[86] followed by alkylation with the methyl Grignard reagent in THF to give ketone **19** in 39% yield. Reduction of ketone **19** with sodium borohydride resulted in a 1:1 mixture of diastereomers **20(*R*)** and **20(*S*)** in 75% yield.



Scheme 1: Synthesis of Intermediates 20(R) and 20(S)

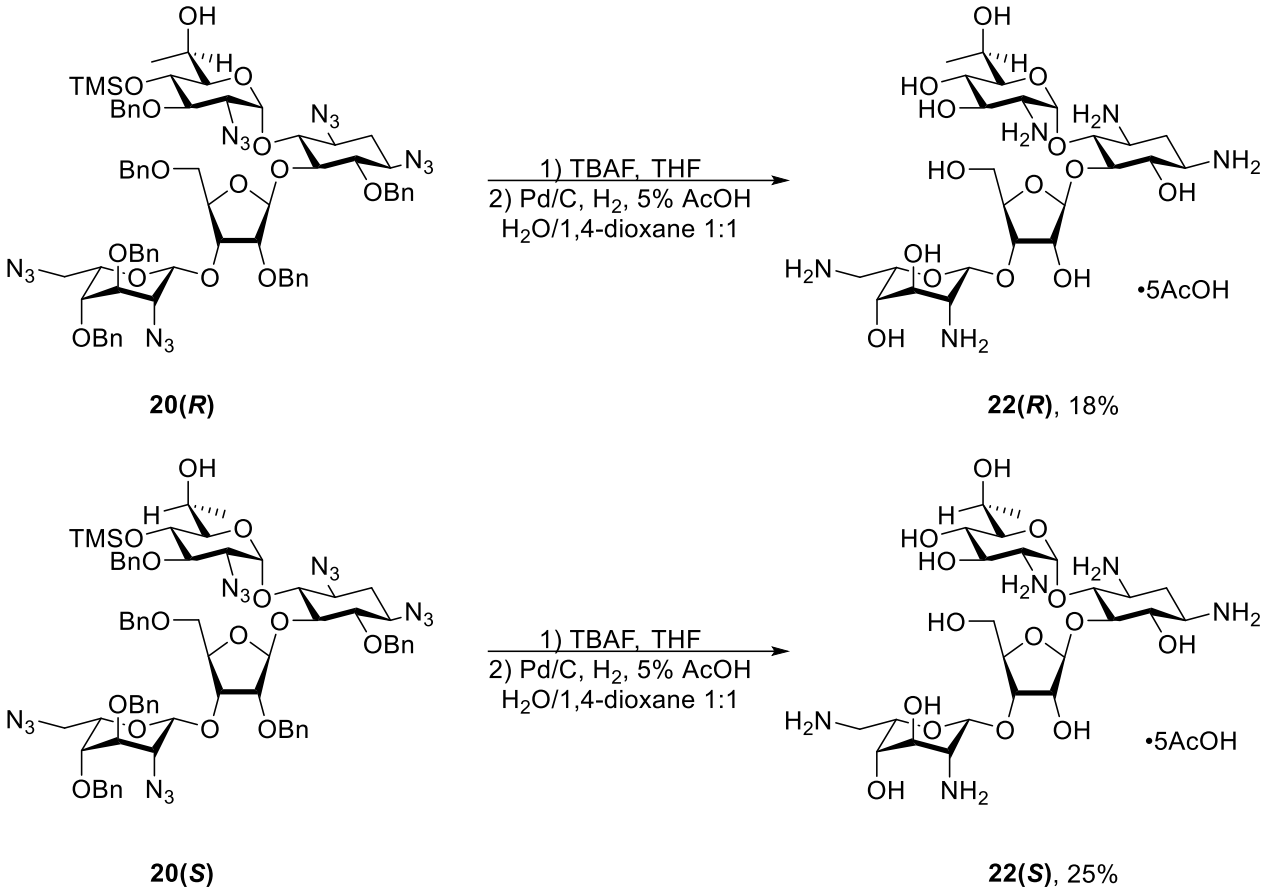
Determination of the configuration at the 6'-positions of alcohols **20(*R*)** and **20(*S*)** was done by deprotection of the trimethylsilyl ether of the less polar alcohol using tetrabutylammonium fluoride (TBAF), followed by formation of a benzylidene acetal to give compound **21** in 60% yield. The configuration of the 6'-position in this compound was determined through proton and ROESY NMR experiments. ROESY correlations between the methyl group, H-4', and the benzylidene proton, together with the coupling constant of 5.9 Hz between H-5' and H-6' indicate that the methyl group is axial, and the configuration of the 6' stereocenter is (*S*).



20(*S*) **21**, 60%

Scheme 2: Determination of Configuration at the 6'-Position

Compounds **20(*R*)** and **20(*S*)** were subjected to silyl ether deprotection using TBAF followed by global deprotection using palladium on carbon and acetic acid under 50 psi of hydrogen gas to give **22(*R*)** and **22(*S*)**. Purification over CM-Sephadex® 25 cation exchange resin followed by lyophilization with excess acetic acid afforded both 6'-methyl paromomycin derivatives as the pentaacetate salts in 18% and 25% yields for the **22(*R*)** and **22(*S*)** isomers, respectively.

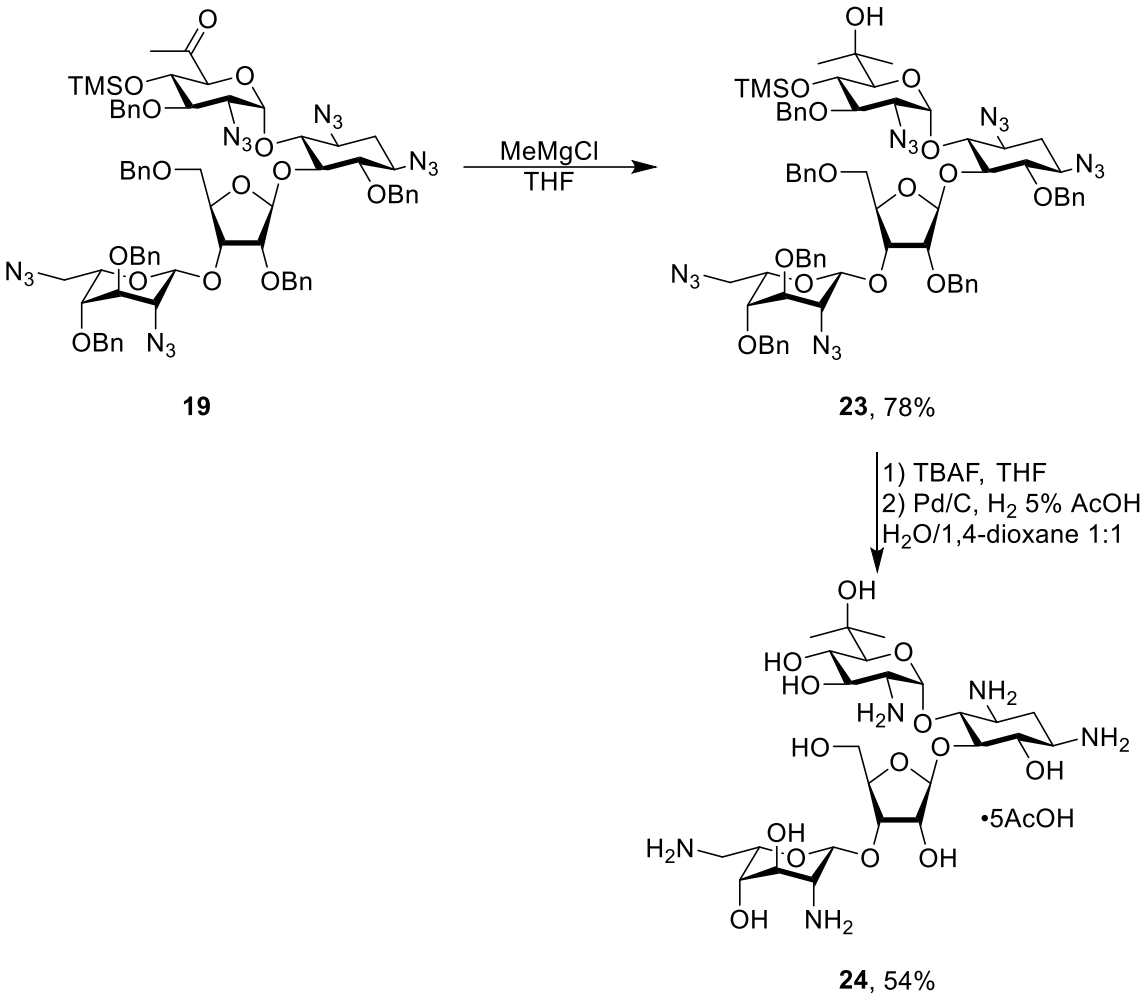


20(*R*) **22(*R*)**, 18%

20(*S*) **22(*S*)**, 25%

Scheme 3: Deprotection of 6'-Methyl paromomycin Derivatives

The 6',6'-dimethyl paromomycin **24** was accessed from methyl ketone **19**, which was further alkylated with methylmagnesium chloride to give the 6',6'-dimethyl alcohol **23** in 78% yield. Compound **23** was then subjected to TBAF for removal of the trimethylsilyl ether, followed by hydrogenolysis with palladium on carbon and acetic acid under 50 psi of hydrogen to give the 6',6'-dimethyl paromomycin **24** in 54% yield.



Scheme 4: Synthesis of 6',6'-Dimethyl paromomycin

2.4 SYNTHESIS OF 6'-ETHYL PAROMOMYCIN DERIVATIVES

Due to the modest yields in the formation of the 6'-methyl paromomycin derivatives, the 6'-ethyl paromomycin derivatives were made using an alternate route starting from diol **16**. The 6'-hydroxy group of **16** was protected as the triisopropylsilyl ether using TIPSOTf and 2,6-lutidine to give **25** in 82% yield. The 4'-hydroxy group of **25** was then converted to the 4-methoxybenzyl ether using 4-methoxybenzyl chloride and sodium hydride to give **26** in 89% yield. The silyl ether of **26** was then deprotected using TBAF to give the 6'-hydroxy compound **27** in 88% yield. After

oxidizing **27** to the aldehyde using Swern conditions[87] and alkylation with the ethyl Grignard reagent, an inseparable 3:1 mixture of diastereomers favoring the (S) isomer was obtained. This mixture was subjected to acid hydrolysis of the OPMB ether using trifluoroacetic acid which afforded **28(*R*)** and **28(*S*)** in 79% yield. Further purification using preparative HPLC gave **28(*R*)** and **28(*S*)** in 9% and 35% isolated yield respectively. The selectivity seen in this Grignard reaction agrees with the Cram chelation model[88] (Figure 17) where the nucleophile attacks from the less hindered side. Compounds **28(*R*)** and **28(*S*)** were deprotected using the standard hydrogenolysis conditions to give **29(*R*)** in 34% yield and **29(*S*)** in 35% yield.

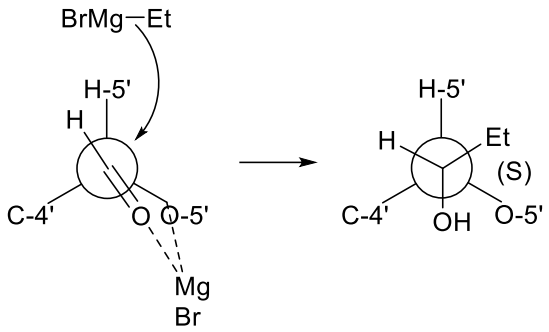
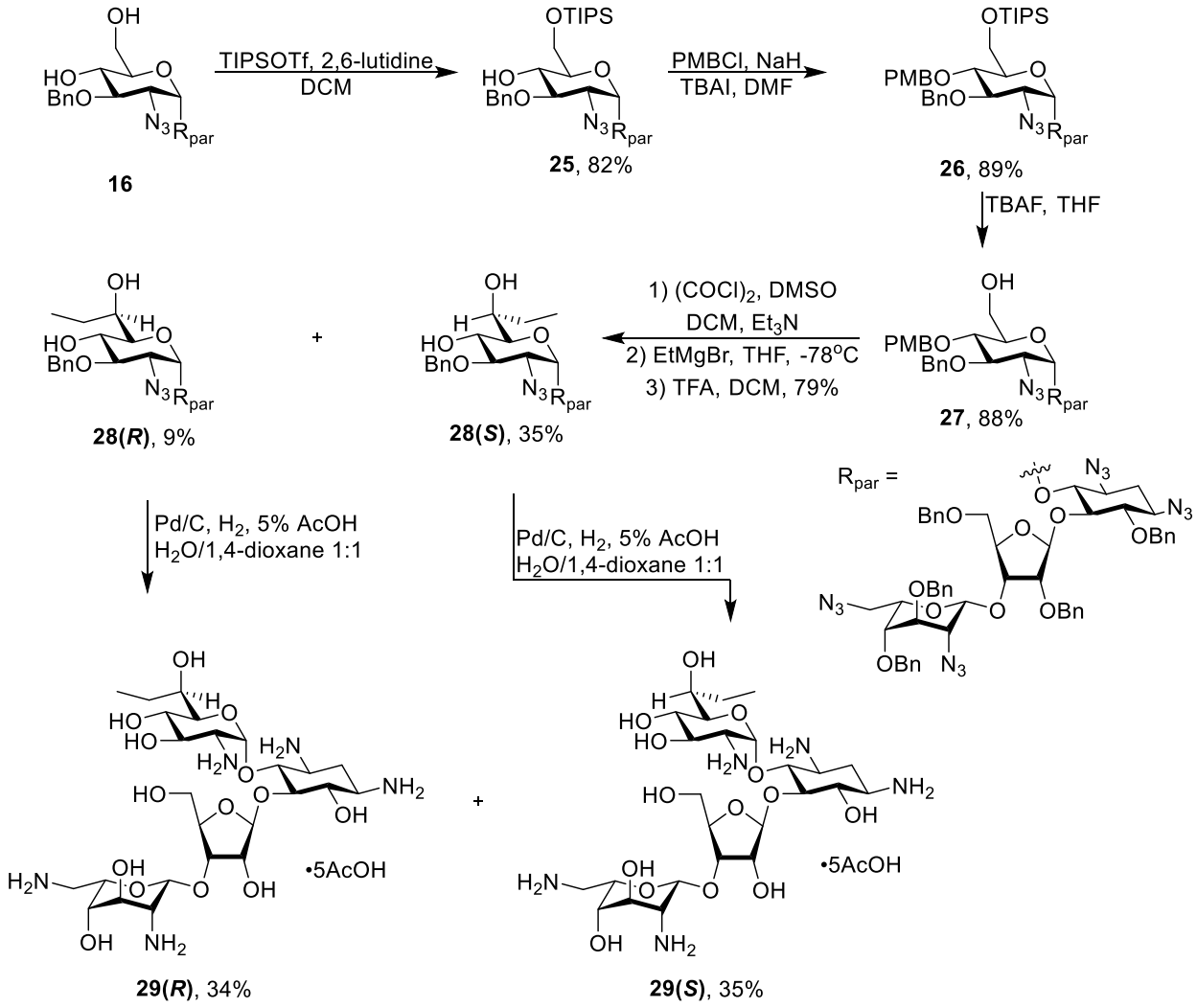


Figure 17: Cram Chelation Model of Grignard Reagent Attack



16 → **25**, 82% → **26**, 89% → **27**, 88%

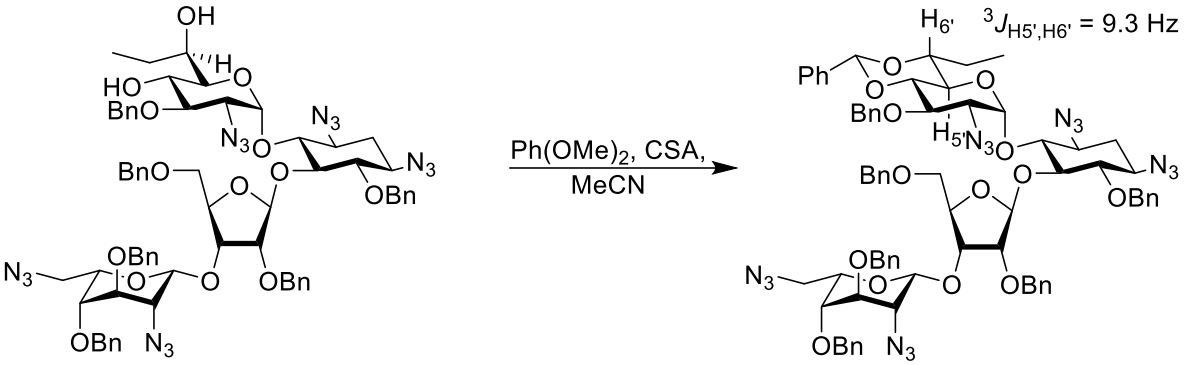
28(*R*), 9% + **28(*S*)**, 35%

29(*R*), 34% + **29(*S*)**, 35%

Scheme 5: Synthesis of 6'-Ethyl paromomycin Derivatives

The configurations of compounds **28(*R*)** and **29(*S*)** were determined in the same manner as the corresponding 6'-methyl compounds. Thus, the less polar isomer was converted to the benzylidene acetal using benzaldehyde dimethyl acetal and camphorsulfonic acid to give compound **30** in 47% yield. In the case of **30** the coupling constant of 9.3 Hz between H-5' and H-6' is sufficient to say that H-6' is axial and therefore the less polar compound is the (*R*) isomer.

This switch in the configuration/polarity relationship as compared to the 6'-methyl series is due to the absence of the 4'-OTMS group in the ethyl series.

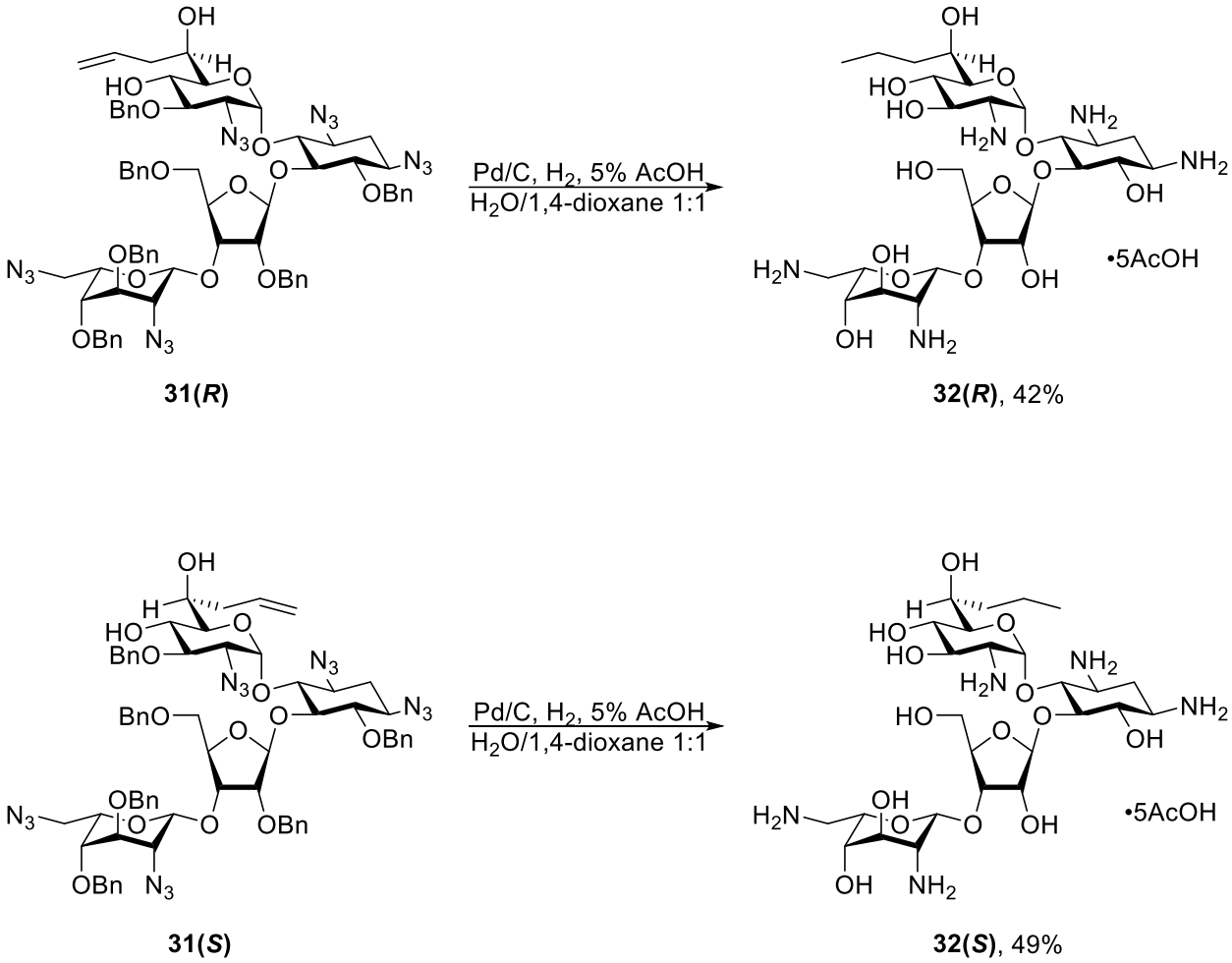


28(*R*) **30**, 47%

Scheme 6: Assignment of Configuration of 32(R)

2.5 SYNTHESIS OF 6'-PROPYL PAROMOMYCIN DERIVATIVES

The 6'-propyl paromomycin derivatives were synthesized starting from compounds **31(*R*)** and **31(*S*)** as previously reported by the Crich group.[89] The configuration of these compounds was proven in the same manner as in the methyl and ethyl series where the less polar isomer was determined to have the (*R*) configuration.[89] These compounds were simply deprotected using the standard hydrogenolysis conditions which also reduced the double bond to give **32(*R*)** in 42% yield, and **32(*S*)** in 49% yield.

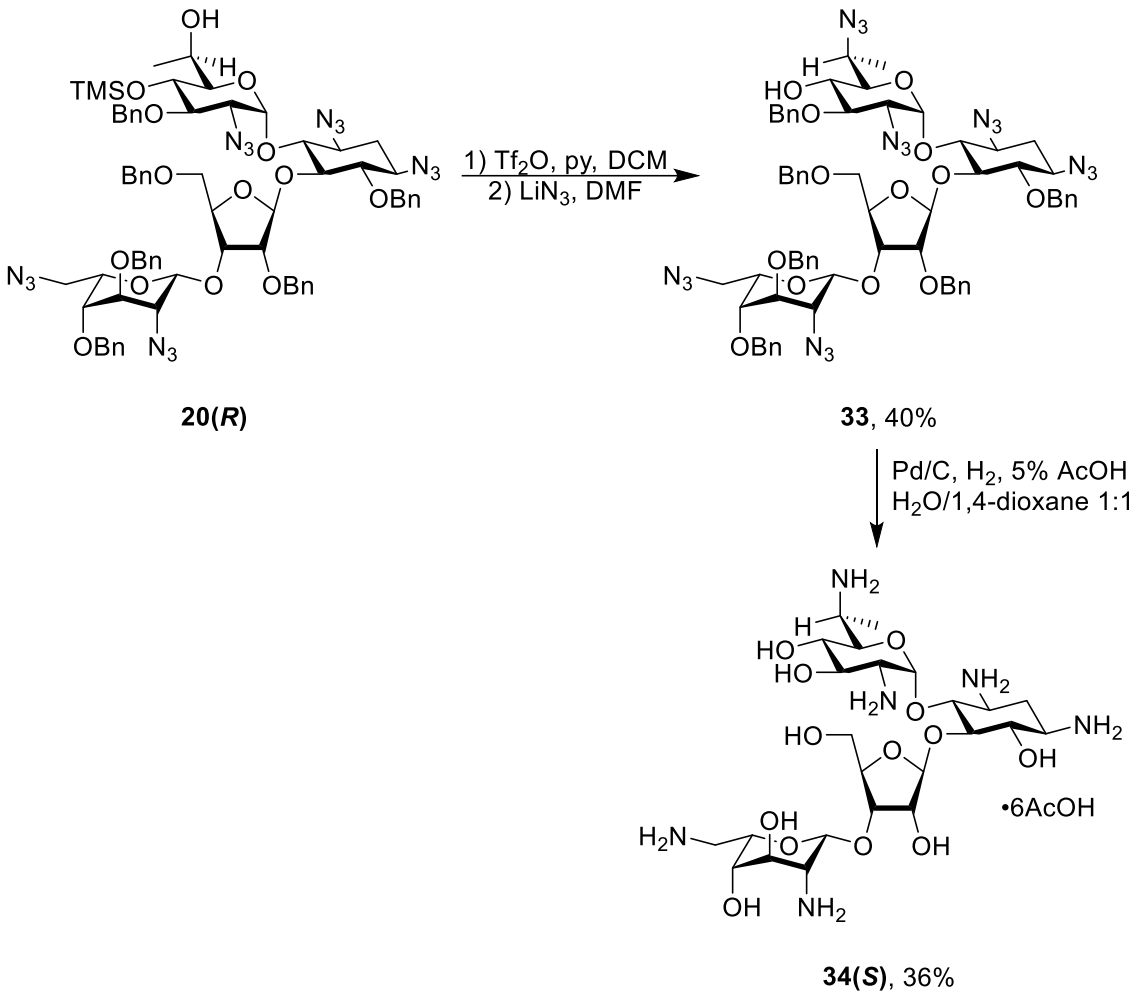


Scheme 7: Synthesis of 6'-Propyl paromomycin Derivatives

2.6 SYNTHESIS OF 6'-METHYL NEOMYCIN DERIVATIVES

The 6'-methyl methyl neomycin **34(*S*)** was first made from compound **20(*R*)** which was subjected to triflation using triflic anhydride and pyridine followed by displacement with lithium azide to give the 6'-azido derivative **33** in 40% yield as a single diastereomer. Due to the cleavage of the silyl ether in the triflation step, global deprotection was done under the hydrogenolysis conditions mentioned previously to give **34(*S*)** in 36% yield. After triflation the isomer **20(*S*)** was

much less stable and decomposed before the triflate could be displaced by azide forcing the development of a different route.

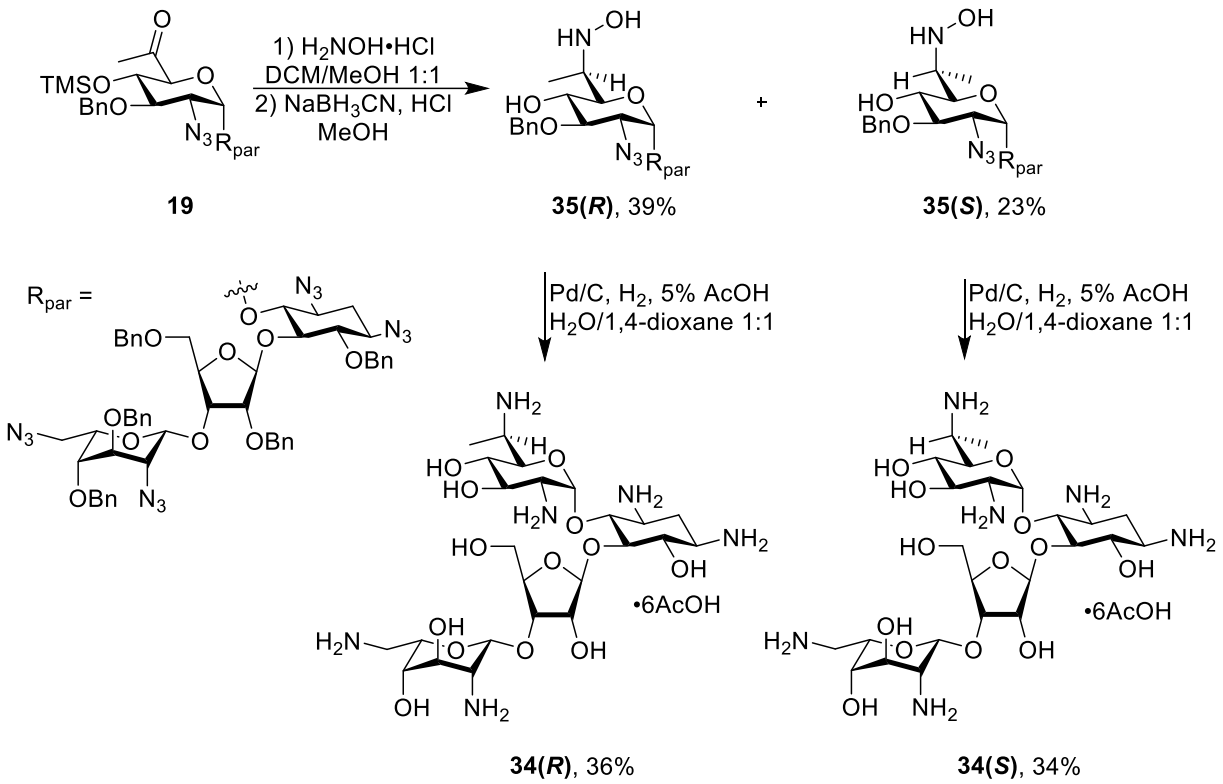


20(*R*) **33**, 40% **34(*S*)**, 36%

Scheme 8: Synthesis of 6'-(S)-Methyl neomycin

Accordingly, compound **19** was stirred with hydroxylamine hydrochloride to form an oxime followed by reduced using sodium cyanoborohydride in acidic methanol to give a mixture of hydroxylamines **35(*R*)** and **35(*S*)** in a 2:1 ratio of diastereomers favoring the (R) configuration as is predicted by the Cram chelation model. Compound **35(*R*)** was isolated in 39% yield and compound **35(*S*)** was isolated in 23% yield. Both compounds were subjected to standard

hydrogenolysis conditions to give **34(*R*)** in 36% yield and **34(*S*)** in 34% yield. The NMR spectra of **34(*S*)** matched those of the product from the previous route.



Scheme 9: Synthesis of 6'-Methyl neomycin Derivatives

2.7 NMR SPECTROSCOPIC ANALYSIS OF SIDE CHAIN CONFORMATION

NMR spectroscopic studies were conducted on compounds **22(*R*)** and **22(*S*)** to determine the conformation of the ring I side chain in solution (Figure 18). The methyl group of **22(*R*)** shows a strong ROE correlation to H-4' and weaker correlation to H-5'. This along with the $^{3}J_{H5',H6'}$ value for **22(*R*)** of 2.5 Hz indicates a large population of the gt conformation is present in solution.[90] The 6'-methyl group of **22(*S*)** shows a near equally strong correlation to H-4' and H-5', and has a $^{3}J_{H5',H6'}$ value of 1.6 Hz likely indicating similar populations of the gg and tg conformations.[90] This

NMR data strongly supports the theory that the methyl group causes preorganization into the gt conformation in the case of **22(*R*)**. The 6'-ethyl and propyl derivatives are considered to adopt comparable conformations of the side chain as the comparably configured 6'-methyl derivatives on the basis of their homologous structures and the similarity of the diagnostic coupling constants.

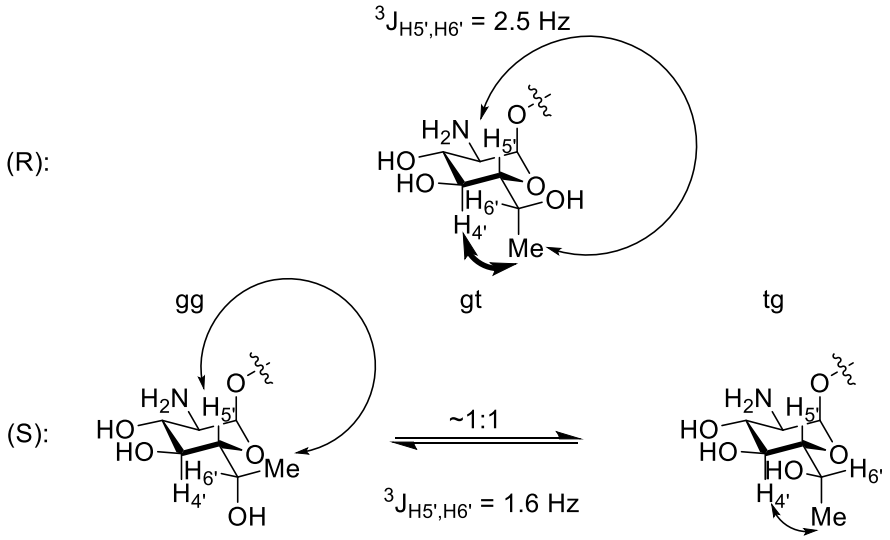


Figure 18: Coupling Constants and NOE Interactions Defining the Side Chain Conformations of the 6'-Methyl paromomycin Derivatives

The coupling constants between *H*-5' and *H*-6' in the case of the neomycin series were 2.9 Hz for **34(*R*)** and 3.0 Hz for **34(*S*)**. The (*R*) isomer is likely predominantly in the gt conformation as is the case with neomycin in solution. Based on the coupling constants it appears that the **34(*S*)** isomer is in the tg conformation which may be stabilized by a hydrogen bond from the protonated amine at the 6'-position to the 4'-hydroxy group (Figure 19).

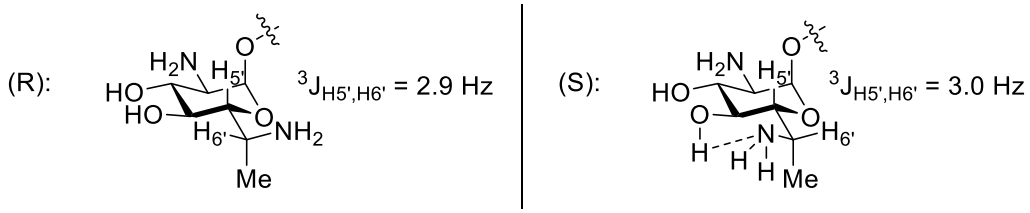


Figure 19: Coupling Constants Defining Side Chain Conformation in 6'-Methyl neomycin Derivatives

2.8 BIOLOGICAL DATA

All deprotected compounds were subjected to cell free ribosomal assays using the engineered *M. smegmatis* ribosomes developed by the Böttger group (Figure 9).[71] These ribosomal assays provide information about the activity and selectivity of each compound, and in combination with anti-bacterial data, determine which compounds are candidates for further screening. Bacterial strains tested include the Gram-positive MRSA as well as the Gram-negative *E. coli, P. aeruginosa, A. baumannii, K. pneumoniae, and E. cloacae.*

Table 1: Cell Free Ribosomal Assays for 6'-Alkylated Derivatives

		in vitro M. smegmatis IC_{50} µg/mL				Selectivity		
Line	Compound	Bacterial	Mit13	1490G*	Cyt14	Mit13	1490G*	Cyt14
1	Paromomycin	0.02	60	6	15	3000	300	750
2	**22(*R*)**	0.01	91	5.3	1.3	9100	530	130
3	**22(*S*)**	0.04	119	56	44	2975	1400	1100
4	**24**	0.04	211	56	30	5275	1400	750
5	**29(*R*)**	0.07	231	109	152	3300	1557	2171
6	**29(*S*)**	0.12	541	142	310	4508	1183	2583
7	**32(*R*)**	0.04	107	20	34	2675	500	850
8	**32(*S*)**	0.16	328	93	259	2050	581	1619
9	Neomycin	0.02	1.87	0.31	14	94	16	700
10	**34(*R*)**	0.01	7.9	0.72	64	790	72	6400
11	**34(*S*)**	0.01	4.2	0.62	12	420	62	1200

Cell free ribosomal assays were performed with four types of ribosomes; Bacterial, Mit13, 1490G, and Cyt14. The bacterial ribosomes have the standard *M. smegmatis* A-site, while Mit13

and Cyt14 have the human mitochondrial and human cytosolic A-sites, respectively. The 1490G ribosomes contain a human mitochondrial A-site with the A1555G deafness mutation known to cause higher susceptibility to ototoxicity (Figure 9). Selectivity factors, shown on the right of Table 1, are calculated by dividing the listed ribosome's IC_{50} by the IC_{50} of the bacterial ribosome and show the preference of the drug for bacterial over humanized ribosomes.

The cell free ribosomal data indicate that in paromomycin methylated at the 6'-position the (*R*) configuration results in four times the activity of the (*S*) isomer and twice the activity of the parent. Additionally, for the ethyl and propyl the (*R*) isomer is similarly 2-4 times more active than its (*S*) counterpart. The dimethyl compound **24** shows a decrease in activity over the parent. In the neomycin series both diastereomers of **34** showed an increase in activity over the parent as well as increases in selectivity, however, the (*R*) isomer had the greater improvement in selectivity. The general trend for selectivity is that alkylation at the 6'-position causes only minor changes in either the positive or negative direction, however, compound **22(*R*)** seems to be an outlier as the selectivity factor for Cyt14 decreased greatly. Although **22(*R*)** has great selectivity for bacteria over both mitoribosomes, the Cyt14 data is of concern for a drug candidate because it may lead to more widespread toxicity.

Table 2: MRSA MIC Assays for 6'-Alkylated Derivatives

Line	Bacteria / Resistance Mechanism / Compound	MRSA MIC (mg/L)			
		AG038	AG039	AG042	AG044
		-	ANT(4')-I AAC(6')-I	APH(2'') ANT(4')-I AAC(6')-I	-
1	Paromomycin	4	>256	>256	4-8
2	**22(*R*)**	4-8	>128	>128	2-4
3	**22(*S*)**	8	>64	>64	4
4	**24**	8-16	>128	>128	4-8
5	**29(*R*)**	4-8	>64	>64	-
6	**29(*S*)**	4-8	>32	>32	-
7	**32(*R*)**	4	>128	>128	2
8	**32(*S*)**	32-64	>64	>64	16
9	Neomycin	0.5-1	128	128	0.5-1
10	**34(*R*)**	-	128	128	2
11	**34(*S*)**	2	>128	>128	1

Table 3: *E. coli* MIC Assays for 6'-alkylated Derivatives: Wild Type

Line	Bacteria / Resistance Mechanism Compound	*E. coli* Wild Type MIC (mg/L)		
		AG001	AG055	AG006
		-	-	-
1	Paromomycin	2-4	2	1-2
2	**22(*R*)**	2-4	4	1
3	**22(*S*)**	8-16	8-16	2
4	**24**	8	8	2-4
5	**29(*R*)**	4-8	8	2-4
6	**29(*S*)**	4-8	8-16	2-4
7	**32(*R*)**	2	2	1
8	**32(*S*)**	32	32	4-8
9	Neomycin	1	1	0.25-0.5
10	**34(*R*)**	2	2	1-2
11	**34(*S*)**	2	2	0.5-1

Table 4: E. coli MIC Assays for 6'-alkylated Derivatives: Strains with Engineered Resistance

	Bacteria	*E. coli* with Engineered Resistance MIC (mg/L)						
		AG003	AG007	AG105	AG009	AG036	AG037	AG103
Line	Resistance Mechanism Compound	AAC(3)-IId	AAC(3)	AAC(2')	AAC(6')-Ib	ANT(4') ANT(4'')	APH(3'-5'')	armA
1	Paromomycin	4	2-4	1-2	2-4	64	>128	2
2	**22(*R*)**	4	2-4	2	2	32-64	>32	1
3	**22(*S*)**	8-16	4-8	4	4	>32	>32	8
4	**24**	8	4-8	2-4	4	16-32	>64	4-8
5	**29(*R*)**	4	4-8	4	8	-	-	-
6	**29(*S*)**	8	8-16	4-8	8	-	>32	8
7	**32(*R*)**	8	2-4	2	4	32-64	>32	1
8	**32(*S*)**	32	-	8	16	64	>128	16-32
9	Neomycin	1	1-2	0.5-1	4	4-8	>64	0.25-0.5
10	**34(*R*)**	2	2	1-2	2	16	>128	0.5
11	**34(*S*)**	2	-	-	0.5	16	>64	1

Table 5: Gram-negative ESKAPE Pathogen MIC Assays for 6'-alkylated Derivatives

	Bacteria	*P. aeruginosa* MIC (mg/L)				*A. baum.* MIC (mg/L)	*K. pneum.* MIC (mg/L)	*E. cloacae* MIC (mg/L)
		AG031	AG032	AG033	AG086	AG225	AG215	AG290
Line	Resistance Mechanism Compound	APH(3')-II	APH(3')-II	APH(3')-II AAC(6')-I	APH(3')-II	-	-	-
1	Paromomycin	>128	>128	>128	>128	2-4	1	2
2	**22(*R*)**	>32	>32	>32	-	2	1	1
3	**22(*S*)**	>32	>32	>32	-	4	2	2-4
4	**24**	>128	>128	>128	>128	4	2-4	2
5	**29(*R*)**	>32	>32	>32	-	8	2	2-4
6	**29(*S*)**	>64	>64	>64	-	4-8	2	2-4
7	**32(*R*)**	>128	>128	>128	>128	2	1	1
8	**32(*S*)**	>64	>64	>64	>64	8-16	4	4-8
9	Neomycin	32	32-64	>128	>128	1-2	0.25-0.5	1
10	**34(*R*)**	128	128	>128	-	2	1	1
11	**34(*S*)**	>128	128	>128	16	1	0.25-0.5	0.5

Minimum inhibitory concentration assays (MIC) with live clinical isolates of *E. coli* and ESKAPE pathogens are used to verify the results of the cell free ribosomal assays, as well as test compounds against known resistance determinants. ESKAPE pathogens include *E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa,* and *E. cloacae*, however, *E. faecium* is not

tested here because it is commonly found in the GI tract which AGAs are not typically used to treat. MIC data for the 6'-alkyl paromomycin derivatives with the (*R*) configuration agree with the cell free data where the methyl derivative is more active than the propyl one, which is more active than the ethyl one in cases where no resistance mechanisms are present. The MIC data for the 6'-alkyl series with the (*S*) configuration also agrees with the cell free translational assay trends where increasing the length of the chain decreases activity. In the neomycin series there is little difference in activity between the parent and either configuration of the methylated compounds.

For the MRSA strains AG039 and AG042, as well as *E. coli* strain AG036 where the ANT(4') resistance enzyme is present all compounds are inactive indicating that alkylation at the 6'-position does not block the ANT(4') AME that is present. Activity is also absent in the presence of the APH(3') AME found in all strains of *P. aeruginosa*. Lastly, the APH(3'-5'') AME found in *E. coli* strain AG037 also renders all of these compounds inactive.

2.9 CONCLUSIONS

Derivatives of paromomycin alkylated at the 6'-position cause a predictable change in the activity of the drug. Paromomycin derivatives with the (*R*) configuration at the 6'-position show activity 2-4 times greater than their (*S*) counterparts with overall activity decreasing as the length of the alkyl chain increases. The NMR analysis of the methyl derivatives shows the (*R*) configuration increases the population of the gt conformation in solution while the (*S*) configuration causes a lower population of the gt conformation. Based on the biological and NMR data, an increase in the population of the gt conformation causes an increase in activity for

paromomycin. The 6'-(*R*)-methyl paromomycin shows increased activity and selectivity for bacteria over mitoribosomes compared to the parent, however, the decrease in cytosolic ribosomal selectivity disqualifies it from being a drug candidate. The overall activity of the 6'-methyl neomycin derivatives is slightly lower than the parent in both cases, however, both diastereomers show a significant increase in selectivity with the (*R*) configuration being much more selective than (*S*).

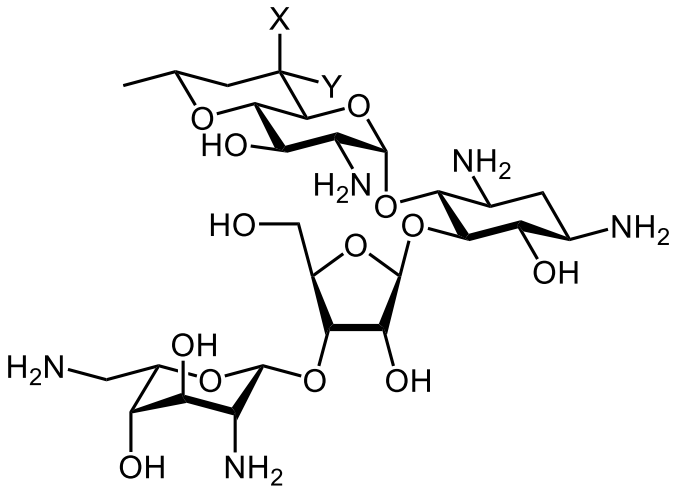
CHAPTER 3: BICYCLIC RING I DERIVATIVES OF PAROMOMYCIN

3.1 RATIONALE

Based on the data from the 6'-alkyl derivatives an increase in the population of the gt conformation of the ring I side chain causes an increase in activity in the paromomycin series. Therefore, complete organization of the side chain into the gt conformation should maximize this increase. By forming a bicyclic ring I, the conformation of the side chain can be locked such that there is no energy penalty for organizing into the bound conformation. Additionally, by varying the size of the conformation-locking appended ring, subtle changes in the angle of the side chain can be investigated to determine if a perfect gt conformation is ideal.

3.2 PREVIOUS WORK

Bicyclic ring I derivatives of paromomycin and neomycin have previously been synthesized in the Crich group where the 4'-hydroxyl group and the 6'-carbon were connected to form a 6 membered ring with an equatorial methyl group at the 8'-position as shown in Figure 20.[89] Compounds **36** and **38** where the hydrogen bond donating groups (OH or NH*2*) are equatorial, mimicking the gt conformation of the 5',6'-bond, showed significantly higher activity than their axial counterparts **37** and **39**, which mimic the gg conformation. These compounds also regained some activity in the presence of ANT(4') and APH(3') AMEs due to the absence of a 4'-hydroxyl group for derivatization.



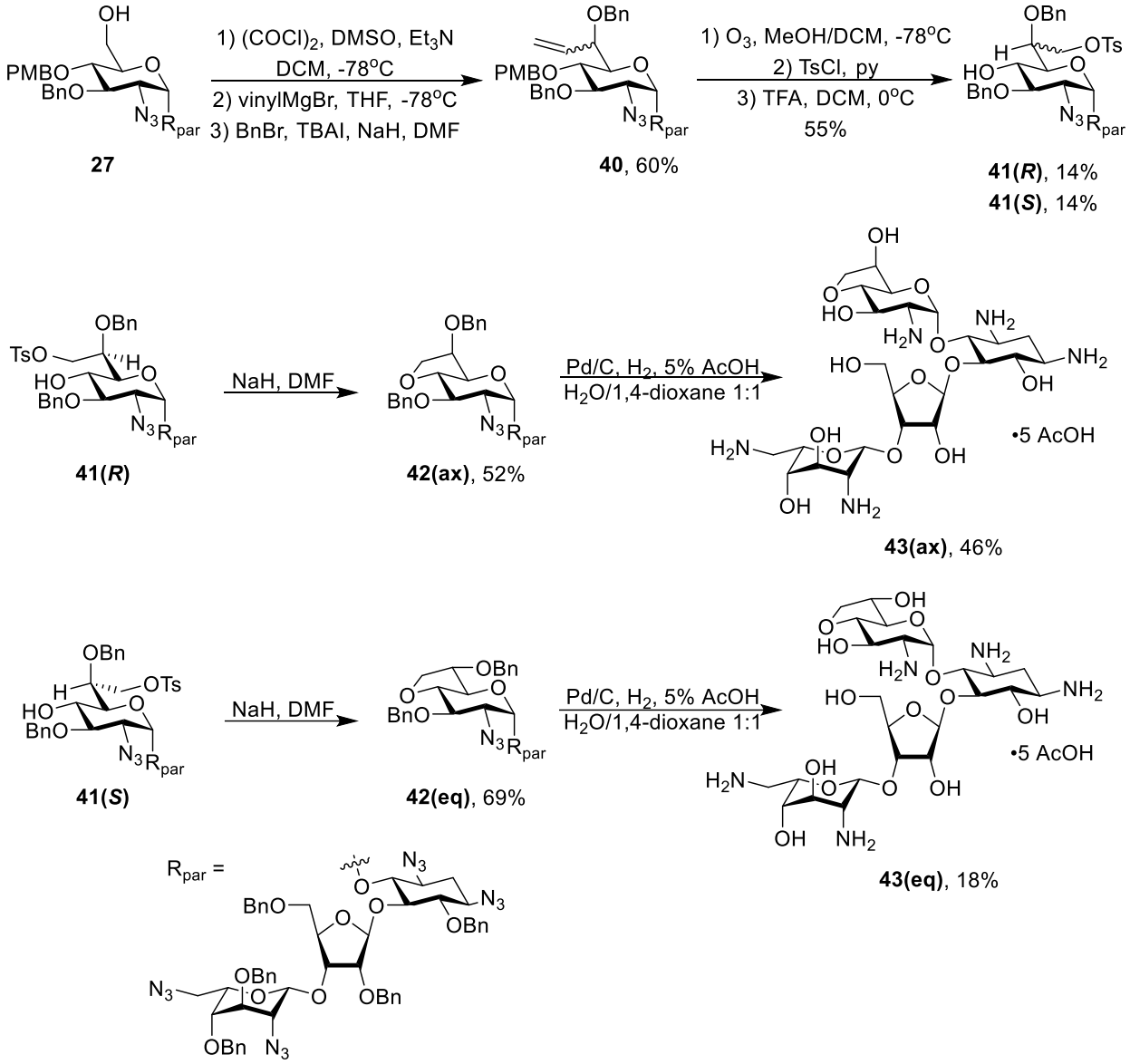
36: X = H, Y = OH
37: X = OH, Y = H
38: X = H, Y = NH_2
39: X = NH_2, Y = H

Figure 20: Previous Bicyclic Ring I Derivatives

3.3 SYNTHESIS OF 5-MEMBERED BICYCLIC DERIVATIVES

In order to further investigate the effect of locking the conformation of the ring I side chain, paromomycin derivatives with a five membered ring connecting *O*-4' and *C*-6' were synthesized starting with compound **27**, which was oxidized to the aldehyde using Swern conditions.[87] This was followed by alkylation with the vinyl Grignard reagent to give an inseparable 1:1 mixture of diastereomers. The mixture was then subjected to benzylation conditions using benzyl bromide and sodium hydride with TBAI gave an inseparable mixture of compounds **40** in 60% yield. Compounds **40** were then subjected to ozonolysis, followed by tosylation of the resulting 7'-hydroxyl group before removal of the PMB ether at the 4'-position using TFA to give diastereomers **41(*R*)** and **41(*S*)** each isolated in 14% yield after normal phase HPLC. Ring closing displacement of the 7'-*O*-tosylates using sodium hydride then gave

compounds **42(ax)** and **42(eq)** in 52% and 69% yields, respectively. The configuration at *C*-6' was assigned based on the $^{3}J_{H5',H6'}$ values of 4.3 Hz in **42(ax)** and 7.4 Hz in **42(eq)**. Finally, both **42(ax)** and **42(eq)** were subjected to the standard hydrogenolysis conditions to give **43(ax)** and **43(eq)** in 46% and 18% yield, respectively (Scheme 10). The assignment of configuration at *C*-6' in these compounds was verified during the conformational analysis of the deprotected compounds shown below.

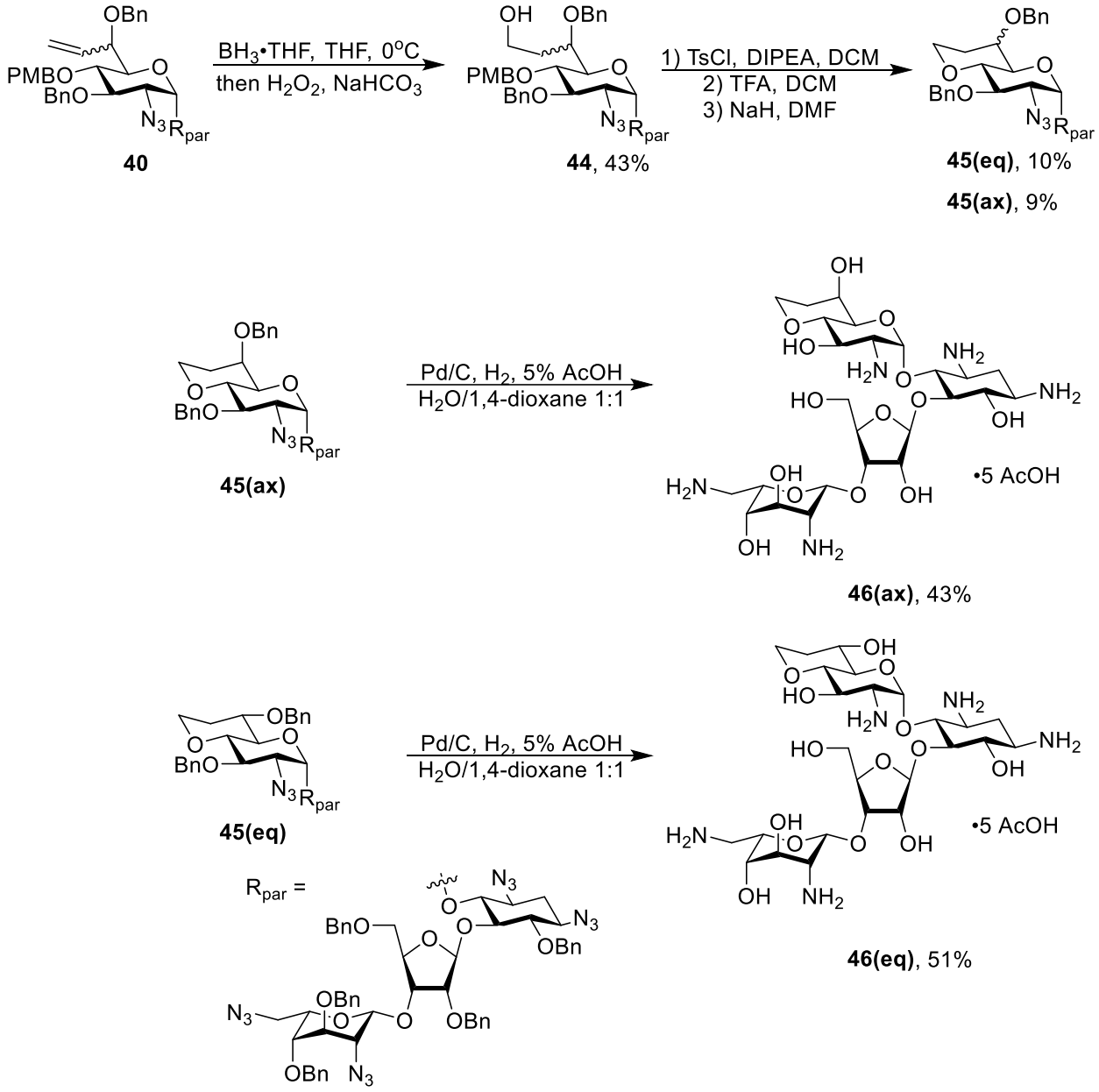


Scheme 10: Synthesis of Bicyclic Paromomycin Derivatives 43(ax) and 43(eq)

3.4 SYNTHESIS OF 6-MEMBERED BICYCLIC DERIVATIVES

In order to make a more direct comparison between ring sizes, 6-membered bicyclic derivatives related to the previous alcohols **36** and **37** were synthesized that lack the 8'-methyl group. To this end mixture **40** was subjected to hydroboration conditions using BH_3·THF followed by workup with hydrogen peroxide to give the 8'-hydroxy compounds **44** as an inseparable

mixture of diastereomers in 43% yield. Tosylation of the mixture of **44** with tosyl chloride and Hunig's base followed by OPMB removal using TFA, and ring closing displacement of the tosylates gave **45(eq)** and **45(ax)** in 10% and 9% yield, respectively. The configuration at *C*-6' of compounds **45(eq)** and **45(ax)** was assigned based on the $^{3}J_{H5',H6'}$ values of 9.4 Hz and 2.5 Hz, respectively. These assignments were further verified during the conformational analysis of the deprotected compounds below. Both isomers of **45** were subjected to hydrogenolysis conditions to give **46(eq)** and **46(ax)** in 51% and 43%, respectively (Scheme 11).

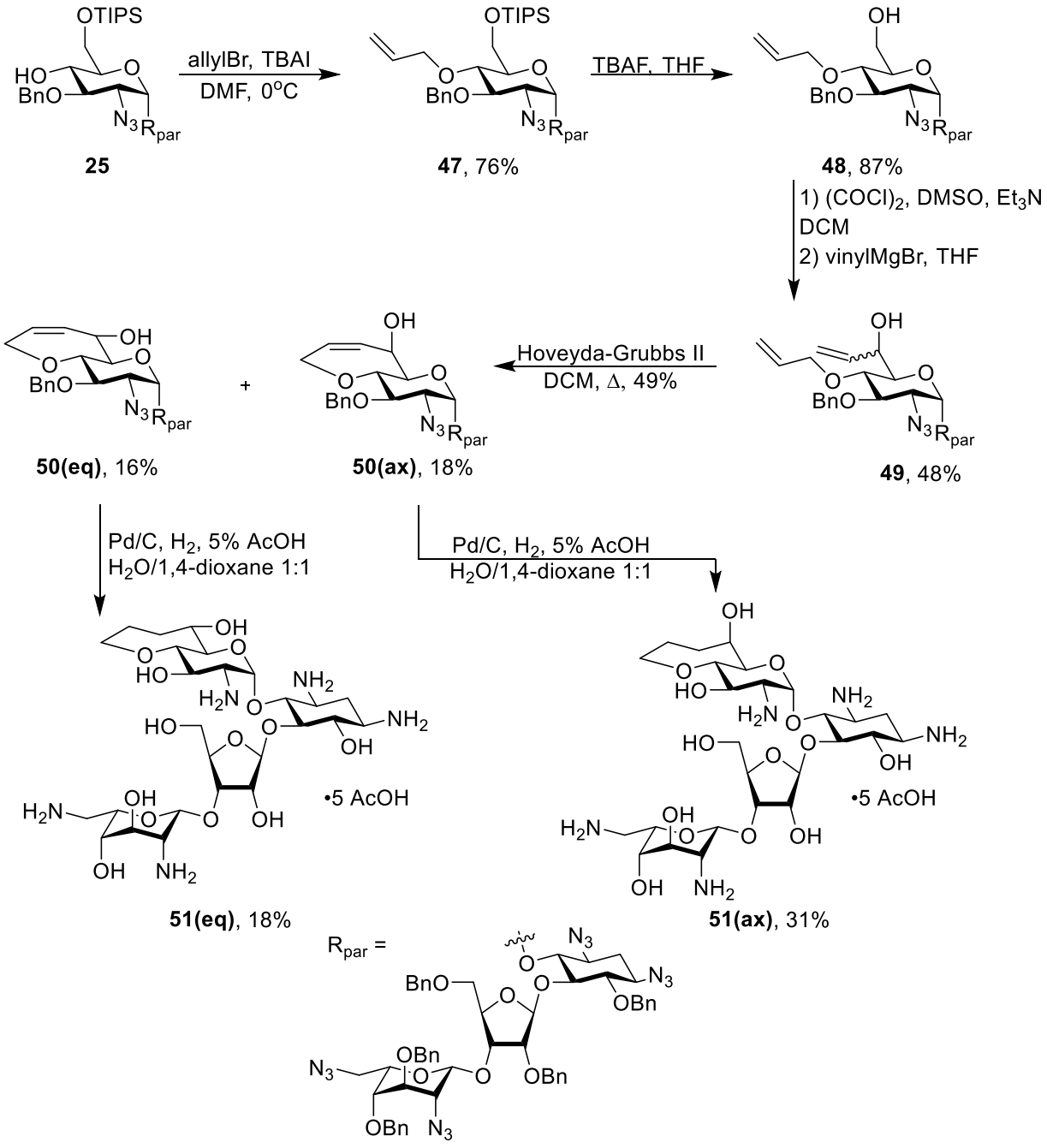


Scheme 11: Synthesis of Bicyclic 6-Membered Paromomycin Derivatives

3.5 SYNTHESIS OF 7-MEMBERED BICYCLIC DERIVATIVES

The bicyclic paromomycin derivatives with a 7-membered ring containing *O*-4' and *C*-6' were made starting with allylation of the 4'-hydroxyl group of **25** using allyl bromide, TBAI, and sodium hydride to give compound **47** in 76% yield. Compound **47** was then subjected to TBAF in

THF for removal of the 6'-OTIPS group to give **48** in 89% yield. Alcohol **48** was then oxidized using Swern conditions, followed by alkylation using the vinyl Grignard reagent to give compounds **49** as an inseparable mixture of diastereomers in 47% yield. Mixture **49** was subjected to ring closing metathesis using Hoveyda-Grubbs second generation catalyst,[91] which resulted in a mixture of diastereomers **50** in 49% yield, from which **50(ax)** and **50(eq)** were isolated in 18% and 16% yield, respectively. The configuration at *C*-6' in these compounds was assigned based on the $^{3}J_{5',6'}$ values of 2.4 Hz for **50(ax)** and 9.1 Hz for **50(eq)**. Compounds **50(ax)** and **50(eq)** were deprotected using standard hydrogenolysis conditions to give **51(ax)** and **51(eq)** in 31% and 18% yield, respectively (Scheme 12). The configuration at *C*-6' was further verified during conformational analysis of the deprotected compounds as described below.



Scheme 12: Synthesis of 7-Membered Bicyclic Paromomycin Derivatives

3.6 CONFORMATIONAL ANALYSIS OF BICYCLIC PAROMOMYCIN DERIVATIVES

Conformational analysis of the reported bicyclic paromomycin derivatives based on proton NMR coupling constants (Table 6) sheds light on the orientation of the bond between *C*-6' and *O*-6'. Both five and seven-membered rings are more conformationally labile than six-membered rings,[92] allowing the C5'-C6' bond access to a greater range of conformations and the 6'-*C*-O bond access to a correspondingly greater volume of chemical space. Based on deviations from limiting coupling constants obtained from the model compounds shown in Figure 21[90] variations in the conformation about the 5',6'-bond can be determined.

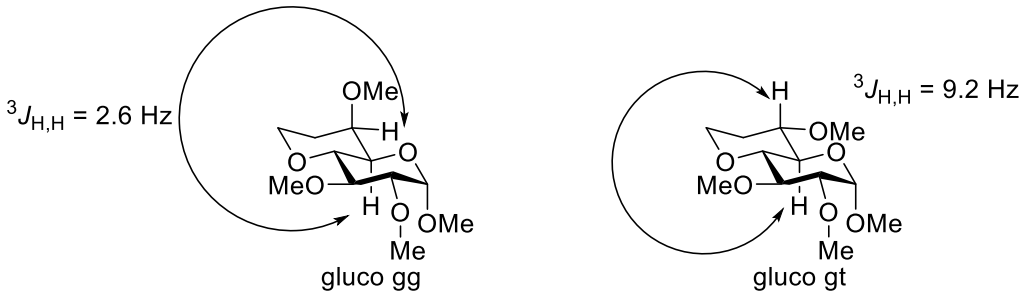


Figure 21: Model Compounds and Limiting Coupling Constants for the gg and gt Conformations[90]

The compounds with appended 6-membered rings in a trans-decalin system will have ideal chair conformations. The $^3J_{H5',H6'}$ values of 9.4 Hz for compounds **36**[89] and **46(eq)** indicate that the heteroatom attached to *C*-6' is equatorial and the side chain is in the gt conformation. Compounds **37** and **46(ax)** have $^3J_{H5',H6'}$ values of 2.8 Hz and 2.0 Hz, respectively, indicating they are ideal gg conformers and *O*-6' is axial.

Table 6: Essential Ring I Coupling Constants

		Hz				
Compound	Ring Size	$^3J_{H1,H2}$	$^3J_{H2,H3}$	$^3J_{H3,H4}$	$^3J_{H4,H5}$	$^3J_{H5,H6}$
36	6	4.1	9.9	9.5	9.5	9.4
37	6	4.1	-	-	10.0	2.8
43(eq)	5	4.3	9.8	9.8	10.0	7.9
43(ax)	5	4.2	9.9	9.9	9.9	4.5
46(eq)	6	4.1	10.0	10.0	9.5	9.4
46(ax)	6	4.1	10.2	10.2	9.4	2.0
51(eq)	7	3.8	10.8	9.1	9.4	10.0
51(ax)	7	3.9	10.5	9.5	9.5	3.5

Unlike 6-membered rings, saturated 5-membered rings prefer to adopt an envelope conformation, where four of the atoms are planar and the fifth, with the bulkiest substituent, extends out of the plane allowing the substituent to adopt a pseudo-equatorial orientation (Figure 22).[92] Additionally, in a trans-fused bicyclo[4.3.0]nonane system, the 6-membered ring can only adopt a proper chair conformation if one of the bridgehead atoms is out of the plane of the five membered ring, limiting the conformational space of the 5-membered ring as shown in Figure 21 with dashed lines along the fold of the envelope.

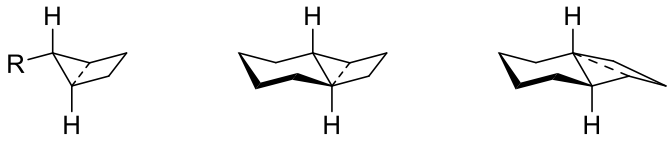


Figure 22: Conformations of Substituted 5-Membered Rings

In compound **43(ax)** the $^3J_{H2',H3'}$, $^3J_{H3',H4'}$, and $^3J_{H4',H5'}$ values of 9.9 Hz indicate that the six membered ring is in a chair conformation. In combination with the $^3J_{H5',H6'}$ value of 4.5 Hz confirming the dihedral angle between *H*-5' and *H*-6' is not zero, these coupling constants show

C-5’ is the out of plane atom in the 5-membered ring (Figure 23). Based on this analysis the conformation of the 5’,6’-bond approaches gg but is shifted minimally towards gt. Compound **43(eq)** has $^{3}J_{H2',H3'}$, $^{3}J_{H3',H4'}$, and $^{3}J_{H4',H5'}$ values of 9.8 Hz, 9.8 Hz, and 10 Hz, respectively, confirming that it also adopts a chair conformation of the 6-membered ring. The $^{3}J_{H5',H6'}$ value of 7.9 Hz shows these protons are not co-planar and that *C*-5’ is at the fold of the envelope in this case as well. In the case of **43(eq)** the conformation of the 5’,6’-bond is approximately gt with a slight distortion towards the tg conformation (Figure 23).

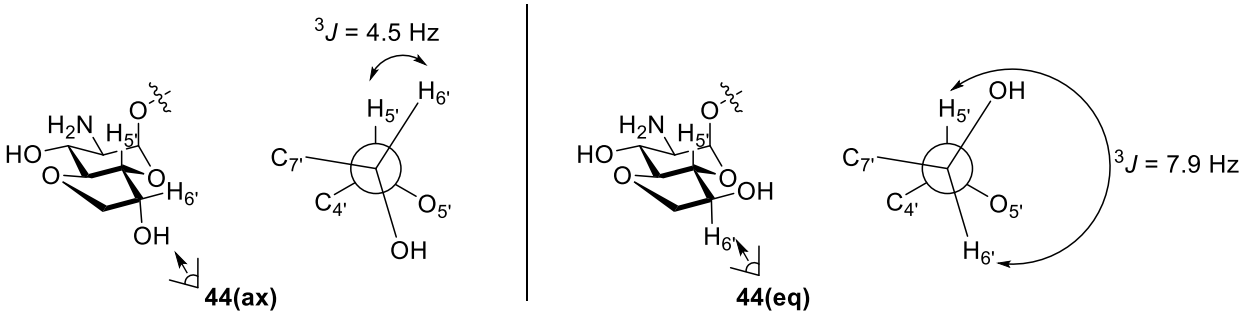


Figure 23: Conformational Analysis of 5-Membered Bicyclic Ring I Derivatives

Cycloheptane rings prefer to adopt a twist-chair conformation (Figure 24) where five atoms are in one plane with the remaining two atoms extending out of the plane in opposite directions.[92] Unlike cyclohexanes with their relatively high energy barriers between conformations, cycloheptanes[93] and oxepanes[94] have low energy barriers to inversion making them more conformationally labile.

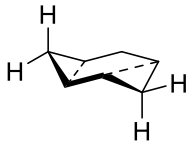


Figure 24: A 7-Membered Ring in the Twist-Chair Conformation

The bicyclic compound **51(ax)** has ^3J values between 9.5 and 11 Hz for protons at the 3', 4', and 5'-positions indicative of a chair conformation in the 6-membered ring. The $^3J_{H5',H6'}$ value is 3.5 Hz, suggesting that the 5',6'-bond is near the gg conformation, leaning slightly towards the gt conformation, but not quite as much as **43(ax)** (Figure 25). Bicyclic compound **51(eq)** also appears to adopt an ideal chair conformation in the 6-membered ring of the bicyclic system based on the $^3J_{H,H}$ values. With a $^3J_{H5',H6'}$ value of 10 Hz the side chain is locked into an almost perfect staggered gt conformation.

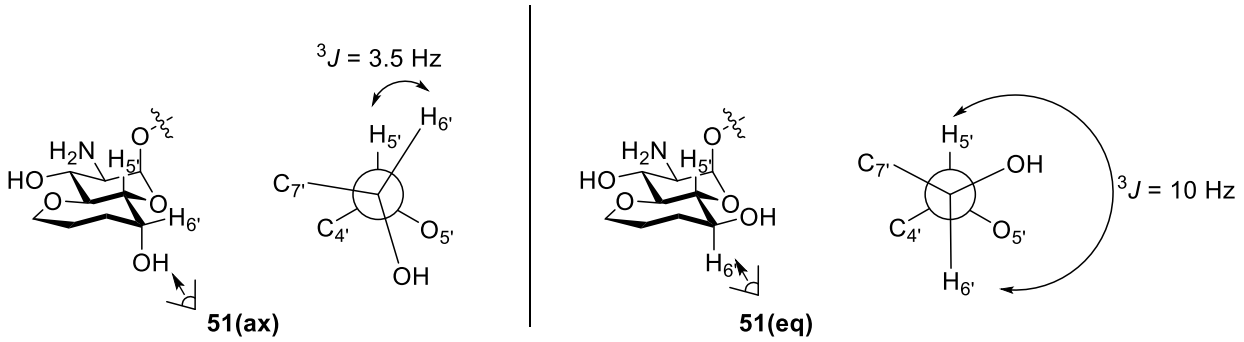


Figure 25: Conformational Analysis of 7-Membered Bicyclic Ring I Derivatives

Conformational analysis of these compounds suggests that the progression from the least gt-like conformation to most gt-like would be **46(ax)** = **37** < **51(ax)** < **43(ax)** < **43(eq)** < **51(eq)** = **46(eq)** = **36**.

3.7 BIOLOGICAL DATA

The work described in this chapter is based on the hypothesis that as the side chain approaches the ideal gt conformation, the activity should approach a maximum. Conversely, if the ideal bound conformation lies between the ideal gg and gt conformers the activity should not peak in the trans-decalinoid compounds. Therefore, Table 7 shows the cell free ribosomal

translation assay data for each of the bicyclic aminoglycoside derivatives discussed with compounds listed in order of increasing gt character. Consistent with the hypothesis, the data for the bacterial ribosome shows a trend of activity increasing as the conformation of the 5',6'-bond progresses towards the gt conformation. In the paromomycin series, ignoring the 8'-methyl compounds **36** and **37**, the bacterial IC_{50} trend shows that progression from least to most active is **46(ax)** < **51(ax)** < **43(ax)** < **43(eq)** < **51(eq)** < **46(eq)**, in near perfect agreement with the progression from the gg to the gt conformation. The exception to the trend is that based on conformational analysis **46(eq)** and **51(eq)** would be equal in activity, however, the difference in activity could simply be due to the added flexibility of the seven-membered ring or its greater steric bulk. Compounds **36** and **37** are more active than their counterparts **46(eq)** and **46(ax)**, lacking the 8'-methyl group, indicating that the appended methyl group positively influences binding.

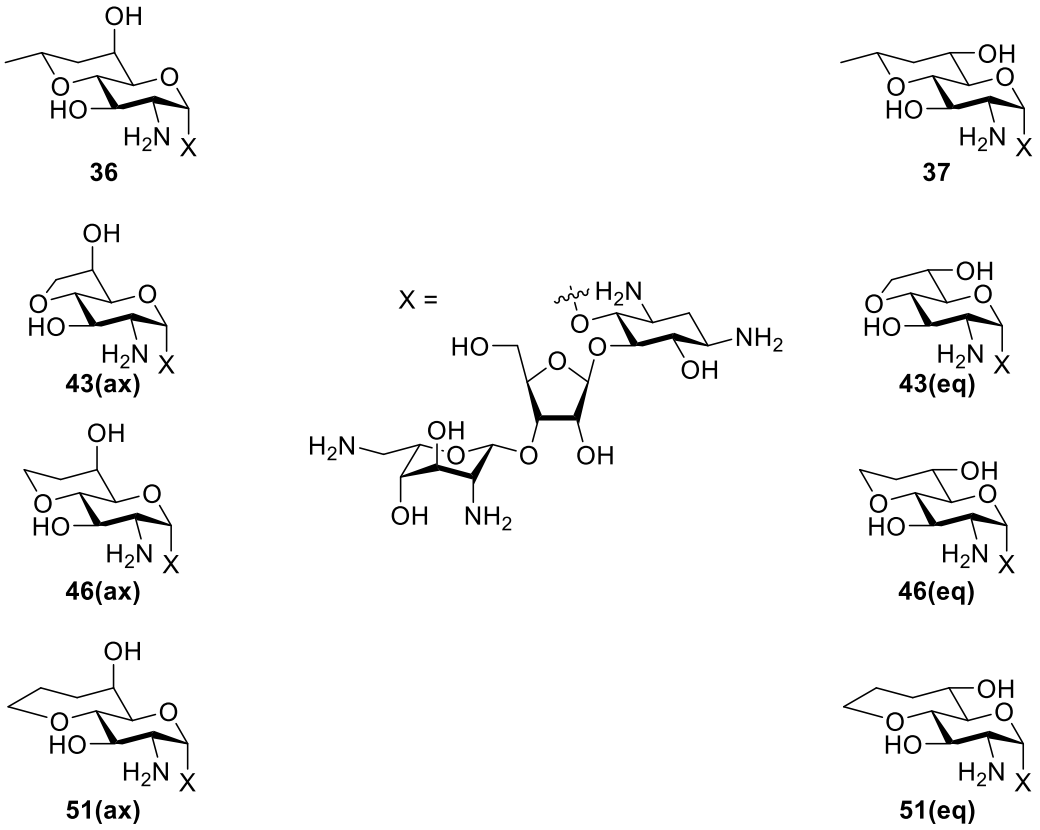


Figure 26: Bicyclic Paromomycin Derivatives

Analyzing the selectivity of these compounds (Figure 26) for the inhibition of bacterial over Mit13 mutant ribosomes shows there is also a trend of increasing selectivity as the conformation of the 5',6'-bond approaches the ideal gt conformation. The exception to this trend concerns **43(eq)** to **51(eq)** where the selectivity drops from 1889 to 1456 only to increase to 8825 in **46(eq)**. The selectivity for inhibition of bacterial over the A1490G mutant ribosome shows a similar pattern in which there is a dip in selectivity just before reaching an ideal gt conformation. Selectivity for bacterial inhibition over Cyt14 mutant ribosomes correlates the least with increasing gt-like conformation, peaking in **43(ax)** and showing the second most selectivity in **46(eq)**.

Table 7: Cell Free Ribosomal Assays for Bicyclic Derivatives

				in vitro M. smegmatis IC_{50} µg/mL				Selectivity		
Compound	Ring Size	$^3J_{H5,H6}$ (Hz)	Conformation	Bacterial	Mit13	1490G*	Cyt14	Mit13	1490G*	Cyt14
Paromomycin	-	-	-	0.02	60	6	15	3000	300	750
37	6	2.8	gg	0.47	193	213	169	411	453	360
46(ax)	6	2.0	gg	1.24	51	121	58	41	98	47
51(ax)	7	3.5	gg > gt	1.3	363	395	365	279	304	281
43(ax)	5	4.5	gg > gt	0.2	130	117	276	650	585	1380
43(eq)	5	7.9	gg < gt	0.09	170	5.9	38	1889	66	422
51(eq)	7	10.0	gt	0.09	131	63	58	1456	700	644
46(eq)	6	9.4	gt	0.04	329	44	50	8225	1100	1250
36	6	9.4	gt	0.02	232	12	15	11600	600	750

The general trend from the bacterial MIC assays stand with the cell-free ribosomal translation data however the difference between compounds is less pronounced. Corresponding 6 and 7-membered compounds show little difference in activity when tested against *E. coli* and ESKAPE strains which are not resistant to paromomycin. The 8'-methyl group on compounds **36** and **37** causes no significant improvement in activity over **46(eq)** and **46(ax)**.

All bicyclic paromomycin derivatives retained activity in strains of MRSA containing the ANT(4')-I AME, as well as in *E. coli* strain AG036 containing the ANT(4') AME, because the 4'-hydroxyl group has been converted to an ether. Compounds **46(eq)** and **36** also regained some activity in *P. aeruginosa* strains AG031 and AG032 known to have the APH(3')-II AME likely due to the added bulk on *O*-4' hindering the approach of the enzyme to the adjacent 3'-hydroxy group. All other strains resistant to paromomycin also show resistance to these bicyclic derivatives.

Table 8: MRSA MIC Assays for Bicyclic Compounds

			Bacteria	MRSA MIC (mg/L)			
				AG038	AG039	AG042	AG044
Compound	Ring Size	$^3J_{H5,H6}$ (Hz)	Resistance Mechanism Conformation	-	ANT(4')-I AAC(6')-I	APH(2'') ANT(4')-I AAC(6')-I	-
Paromomycin	-	-	-	4	>256	>256	4-8
37	6	2.8	gg	32	32-64	16-32	32
46(ax)	6	2.0	gg	32	32	32	32
51(ax)	7	3.5	gg > gt	32	64	64-128	128
43(ax)	5	4.5	gg > gt	8	4-8	4-8	8
43(eq)	5	7.9	gg < gt	16-32	16	8-16	16-32
51(eq)	7	10.0	gt	2	2-4	4	4
46(eq)	6	9.4	gt	4	2	2-4	4
36	6	9.4	gt	8-16	8	8	4

Table 9: E. coli MIC Assays for Bicyclic Compounds: Wild Type

			Bacteria	*E. coli* Wild Type MIC (mg/L)		
				AG001	AG055	AG006
Compound	Ring Size	$^3J_{H5,H6}$ (Hz)	Conformation	-	-	-
Paromomycin	-	-	-	2-4	2	1-2
37	6	2.8	gg	32	64-128	16-32
46(ax)	6	2.0	gg	32-64	>32	32
51(ax)	7	3.5	gg > gt	64	128	64
43(ax)	5	4.5	gg > gt	16	16	8
43(eq)	5	7.9	gg < gt	32	32	16
51(eq)	7	10.0	gt	4-8	4-8	4
46(eq)	6	9.4	gt	4-8	4-8	2
36	6	9.4	gt	8	8	2

Table 10: E. coli MIC Assays for Bicyclic Compounds: Strains with Engineered Resistance

			Bacteria	*E. coli* with Engineered Resistance MIC (mg/L)						
				AG003	AG007	AG105	AG009	AG036	AG037	AG103
Compound	Ring Size	$^3J_{H5,H6}$ (Hz)	Resistance Mechanism Conformation	AAC(3)-IId	AAC(3)	AAC(2')	AAC(6')-Ib	ANT(4') ANT(4'')	APH(3'-5'')	armA
Paromomycin	-	-	-	4	2-4	1-2	2-4	64	>128	2
37	6	2.8	gg	64-128	-	-	-	-	-	-
46(ax)	6	2.0	gg	>32	>32	32	>32	16	>32	>32
51(ax)	7	3.5	gg > gt	128	128	64	128	32	>128	128
43(ax)	5	4.5	gg > gt	16	8-16	8	16	2	>64	16
43(eq)	5	7.9	gg < gt	32-64	32-64	16	64	8	>128	16
51(eq)	7	10.0	gt	8	-	4	16	1	>128	-
46(eq)	6	9.4	gt	8	4-8	4	8	1	>128	2-4
36	6	9.4	gt	8	4-8	2-4	4	0.5	16	2

Table 11: Gram-negative ESKAPE Pathogen MIC Assays for Bicyclic Compounds

			Bacteria	*P. aeruginosa* MIC (mg/L)				*A. baum.* MIC (mg/L)	*K. pneum.* MIC (mg/L)	*E. cloacae* MIC (mg/L)
				AG031	AG032	AG033	AG086	AG225	AG215	AG290
Compound	Ring Size	$^3J_{H5,H6}$ (Hz)	Resistance Mechanism Conformation	APH(3')-II	APH(3')-II	APH(3')-II AAC(6')-I	APH(3')-II	-	-	-
Paromomycin	-	-	-	>128	>128	>128	>128	2-4	1	2
37	6	2.8	gg	>128	>128	>128	>128	-	-	-
46(ax)	6	2.0	gg	>64	>32	>32	-	32	>32	32
51(ax)	7	3.5	gg > gt	>128	>128	>128	-	64-128	64	32-64
43(ax)	5	4.5	gg > gt	>64	>64	>64	-	8-16	4	4-8
43(eq)	5	7.9	gg < gt	>128	>128	>128	-	32	32	16-32
51(eq)	7	10.0	gt	>128	>128	>128	-	4-8	2-4	2-4
46(eq)	6	9.4	gt	64	64	128	-	4-8	4	2
36	6	9.4	gt	32	16-32	>128	>128	2	2	2

3.8 CONCLUSION

Based on the results of the biological assays coupled with the conformational analysis of each bicyclic compound an ideal gt conformation is preferred when bound. There is an excellent correlation between the activity of these bicyclic compounds and the conformation of the 5',6'-bond, which shows that as the conformation approaches the gt conformation the activity

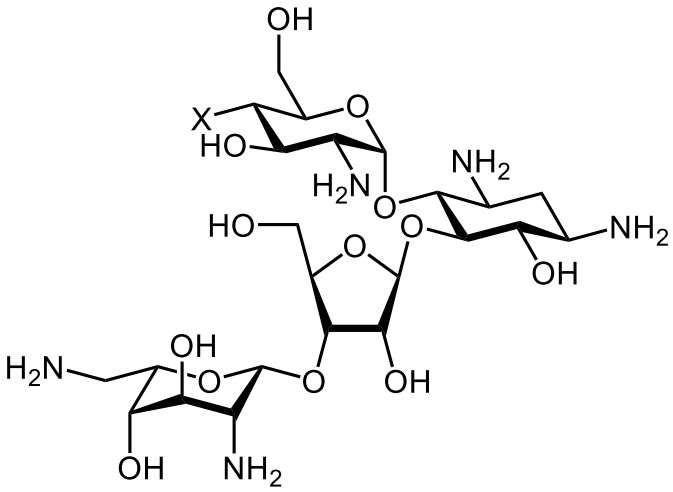
increases. The selectivity for inhibition of bacterial over Mit13 mutant ribosomes also has a direct correlation to the conformation of the side chain and increases as the conformation becomes more gt-like. The selectivity for inhibition of bacterial over A1490G and Cyt14 mutant ribosomes does not appear to show any correlation with the transition from the gg to the gt conformation but is near its maximum in compound **46(eq)**, which is near perfectly in the gt conformation. Although the assays on inhibition of live bacteria agree with the cell free ribosomal assay, the magnitude of the difference between compounds is smaller. The methyl group of compound **36**, which grants it higher activity than **46(eq)** in the cell-free translation assays does not have much effect in the bacterial assays, although it grants a significant advantage for inhibition of bacterial over Mit13 mutant ribosomes.

These bicyclic compounds also overcome resistance from ANT(4')-I in MRSA, as well as recovering some activity in the presence of APH(3')-II in *P. aeruginosa*. Conversion of the 4'-hydroxyl group to an ether prevents AMEs from modifying it, which grants these compounds immunity. In the case of **46(eq)** and **36** the protection from APH(3')-II likely comes from the added bulk of the bicyclic ring preventing approach of the AME to the 3'-position, further increasing the utility of this modification.

CHAPTER 4: EFFECTS OF SUBSTITUENTS AT THE 4'-POSITION ON THE RELATIVE POPULATIONS OF THE SIDE CHAIN CONFORMATIONS

4.1 RATIONALE

Propylamycin **14**, one of the lead compounds from the aminoglycoside project, was synthesized as part of a series of modifications to the 4'-position. Of all the 4'-modifications made,[12, 95-96] propylamycin showed the highest activity and the best selectivity profile, making it a good candidate for further study. There are several possible reasons for the increased activity of propylamycin including the increase in basicity of the ring oxygen (*O*-5') due to the deoxygenation of the 4'-position, causing less electron density to be pulled away from *O*-5' and therefore enhancing its ability to accept a hydrogen bond from A1408,[97-98] as well as possible hydrophobic interactions with the propyl group. In yet another hypothesis, because 4'-deoxy paromomycin **52** (Figure 27) suffers a reduction in activity,[12] a steric interaction between the propyl group and the side chain causes the latter to adopt a higher population of the gt conformation, and so increases affinity for the decoding A-site. Table 12 shows cell free ribosomal assay data for paromomycin, 4'-deoxy paromomycin, and propylamycin.



3: X = OH paromomycin
52: X = H 4'-deoxyparomomycin
14: X = nPr propylamycin

Figure 27: Structures of Paromomycin, 4'-Deoxyparomomycin, and Propylamycin

Table 12: Cell Free Ribosomal Assay Data for Paromomycin, 4'-Deoxy Paromomycin, and Propylamycin

		in vitro M. smegmatis IC_{50} µg/mL				Selectivity		
Line	Compound	Bacterial	Mit13	1490G*	Cyt14	Mit13	1490G*	Cyt14
1	Paromomycin	0.02	60	6	15	3000	300	750
2	4'-Deoxy Paromomycin	0.05	74	24	28	1480	480	560
3	Propylamycin	0.03	167	52	64	5567	1733	2133

In order to investigate the effects of 4'-substituents on the side chain conformation in solution, four model compounds (Figure 28) were synthesized for study by NMR spectroscopy. Because conformational analysis of the side chain by NMR spectroscopy requires unambiguous assignment of the diastereotopic hydrogens at the 6'-position,[80] methods were developed for the synthesis of model compounds with predictable stereoselective deuteration of one of these two protons.

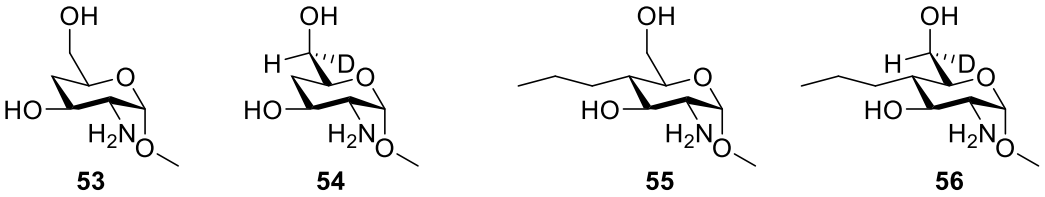
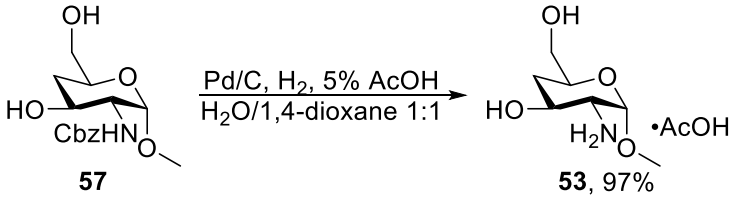


Figure 28: Ring I Models Designed for Conformational Analysis of the Side Chain

4.2 SYNTHESIS OF 4'-DEOXY RING I MODEL

Compound **53** was made simply by deprotecting compound **57**, which was prepared by the method reported by Mayer (Scheme 13).[99] Thus, compound **57** was subjected to hydrogenolysis conditions using palladium on carbon and acetic acid under 50 psi of H_2. The resulting compound **53** was obtained as the acetate salt in 97% yield.

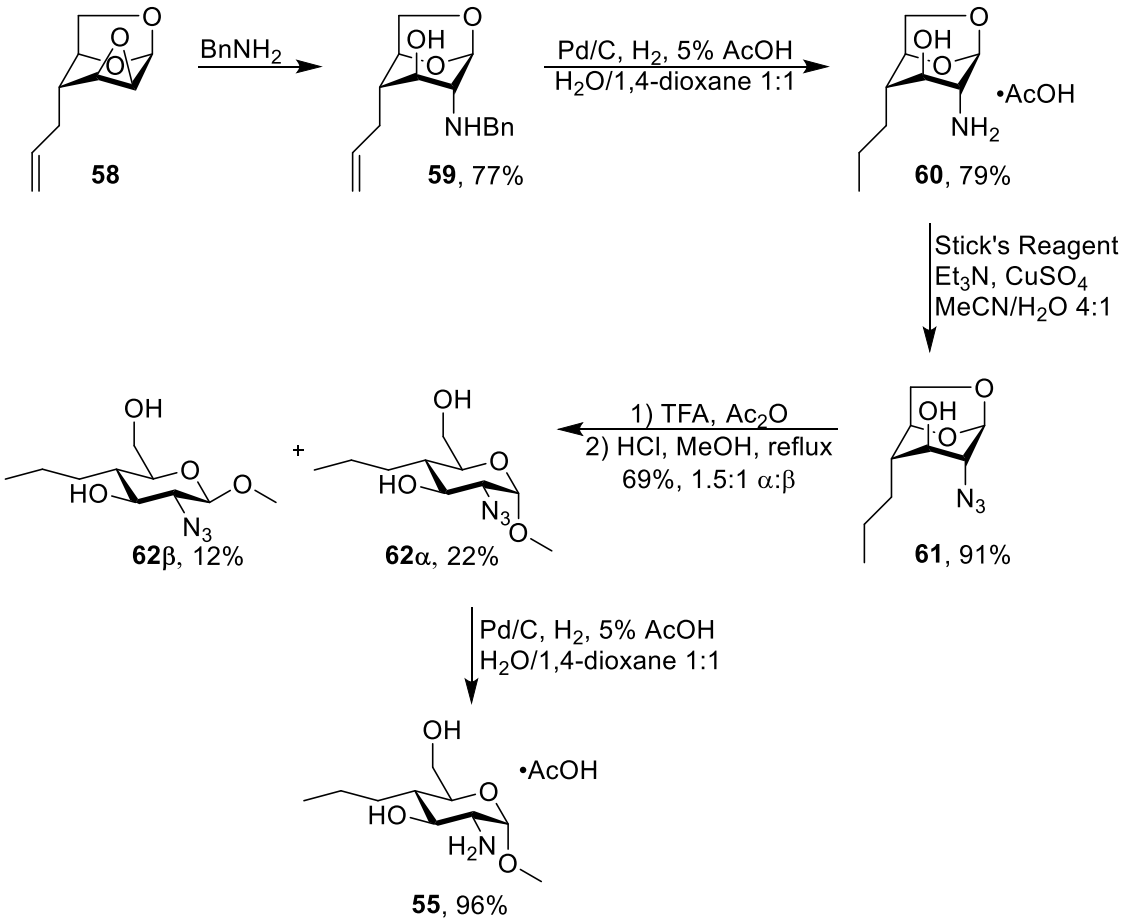


Scheme 13: Hydrogenolysis of 57

4.3 SYNTHESIS OF A 4'-DEOXY-4'-PROPYL RING I MODEL

The propylamycin ring I model was synthesized starting from compound **58** previously reported by Wakamatsu[100] and characterized by Shibasaki.[101] After failed attempts to open the epoxide with sodium azide, **58** was opened by heating in benzylamine to give the trans-diaxial Fürst-Plattner[102] product **59** in 77% yield (Scheme 14). Palladium catalyzed hydrogenolysis of the benzyl group and reduction of the allyl double bond under a hydrogen atmosphere gave the free amine **60** in 79% yield. Application of Stick's reagent[103] to amine **60** afforded the 2-azido

derivative **61** in 91% yield. Ring opening of the anhydro sugar **61** using TFA and acetic anhydride followed by Fischer glycosylation in methanolic HCl resulted in an anomeric mixture of **62** in 69% yield with an α/β ratio of 1.5:1. Compounds **62α** and **62β** were isolated in 22% and 12%, respectively, after silica gel flash column chromatography. Hydrogenolysis of **62α** gave the acetate salt **55** in 96% yield (Scheme 14). With ring I models **53** and **55** in hand a method to distinguish between 6-H_R and 6-H_S for the calculation of side chain populations was required.



Scheme 14: Synthesis of a 4'-Deoxy-4'-Propyl Ring I Model

4.4 SYNTHESIS OF A 4'-DEOXY-6'-(*S*)-DEUTERIO RING I MODEL

The method used to differentiate the protons at the 6-position was selective deuteration of the 6-H_S using the method previously developed by Meguro[104] and modified by Crich[82] based on selective functionalization of the exo-face of 1,6-anhydroglucose (Scheme 15). Regioselectivity in the initial bromination reaction, which was conducted in α,α,α-trifluorotoluene instead of the more common but environmentally unfriendly tetrachloromethane,[105] arises because the least electron deficient hydrogen atom of **63** is abstracted. Stereoselectivity in the quenching of the ensuing radical occurs because bromine approaches from the more accessible exo-face to give bromide **64**. Reduction of bromide **64** with Bu_3SnD results in retention of configuration, again due to quenching of the intermediate radical from the less hindered exo-face, to give the deuterated compound **65** with the (*S*) configuration at the 6-position. The proton NMR spectrum of **63** shows a doublet of doublets at δ 4.10 ppm corresponding to the endo H_{6R} with coupling constants of 7.7 and 1.1 Hz, as well as another doublet of doublets at δ 3.80 ppm corresponding to the exo H_{6S} with coupling constants of 7.7 and 5.7 Hz. After conversion to **64** the remaining H_6 becomes a singlet at δ 6.41 ppm due to the loss of the 7.7 Hz geminal coupling. Following deuteration the proton NMR spectrum of **65** matches that of **65** except for the absence of the peak at δ 3.8 ppm and a change in multiplicity of the peak at δ 4.1 ppm to a doublet with a 1.0 Hz coupling constant (Figure 29).

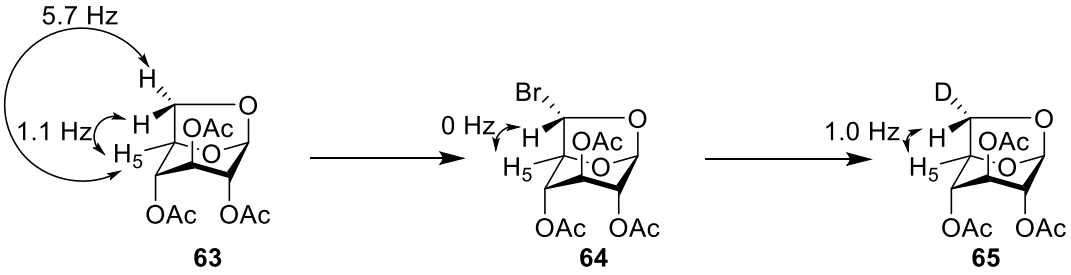
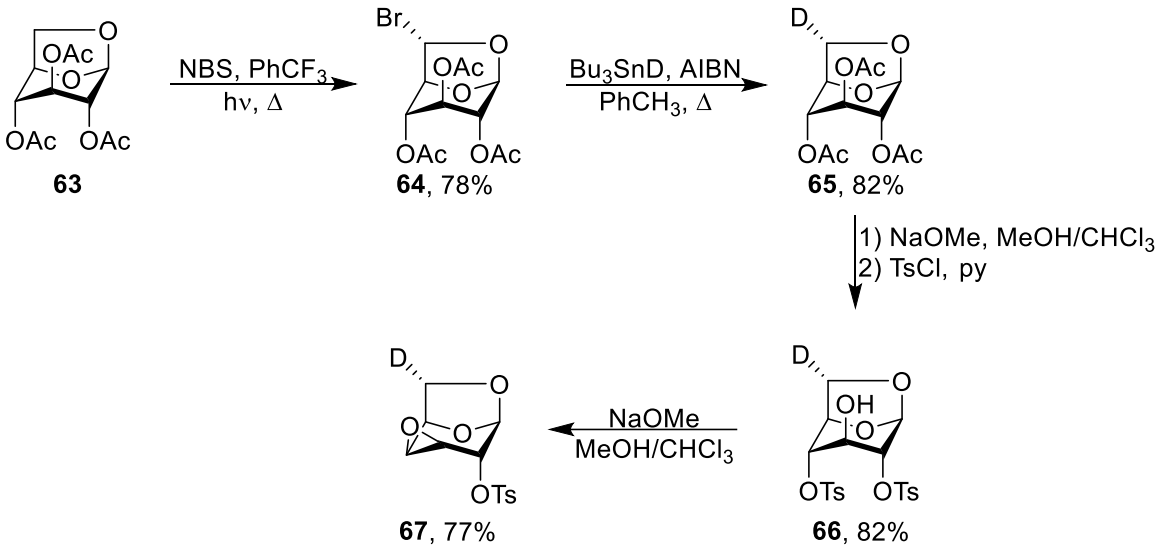


Figure 29: Regioselective Deuteration of H_6-exo

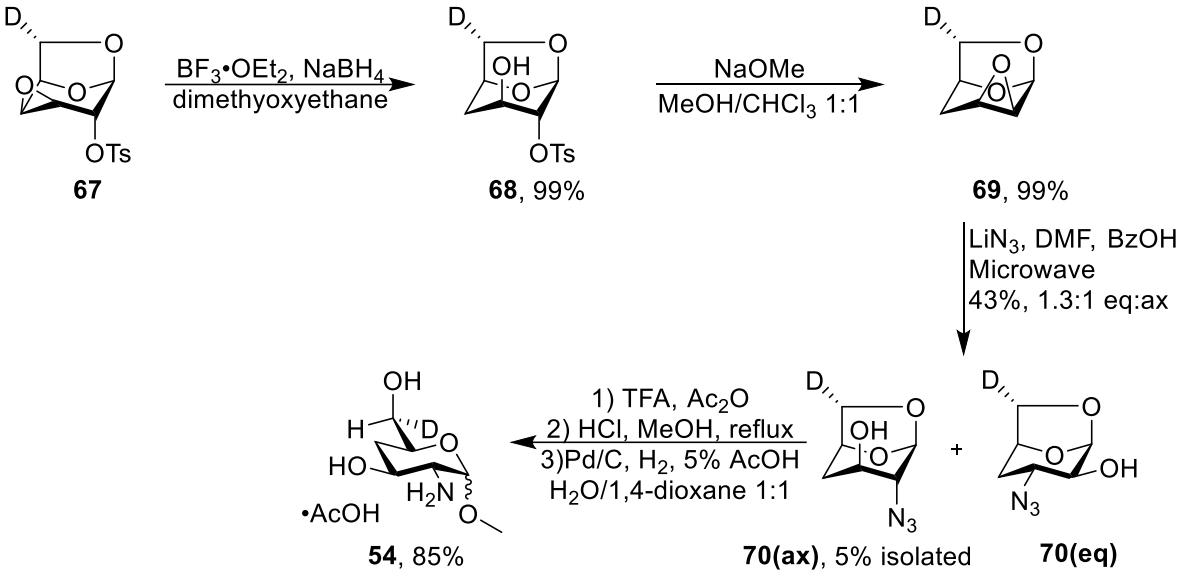
Following regioselective deuteration, removal of the acetates from **65** with NaOMe followed by tosylation gave di-tosylate **66**. Treatment of **66** with NaOMe results in selective displacement of the tosyl group at the 4-position to give the 3,4-epoxide **67**, which was used as the common intermediate for both deuterated ring I models.



Scheme 15: Synthesis of Labeled Intermediate 67

Epoxide **67** was opened using $BF_3{\cdot}OEt_2$ and $NaBH_4$ in dimethoxyethane to give **68** in 99% yield (Scheme 16). Treatment of alcohol **68** with sodium methoxide formed the 2,3-epoxide **69** in 99% yield. Epoxide **69** was subjected to microwave irradiation in DMF with lithium azide and

benzoic acid, which resulted in the formation of **70(ax)** as the minor isomer contrary to the Fürst-Plattner rule, as discussed below. HPLC purification resulted in isolation of 5% of the desired **70(ax)** which was subjected to ring opening using TFA and acetic anhydride followed by Fischer glycosylation in methanolic HCl. The resulting inseparable mixture of anomers was subjected to hydrogenolysis conditions which gave the inseparable mixture **54** in 85% yield. The NMR spectra of mixture **54** were well resolved and facilitated determination of 6-H_R and 6-H_S for the pure isotopomer **53,** due to the absence of a peak at δ 3.51 ppm, resulting from replacement of 6-H_S with deuterium.

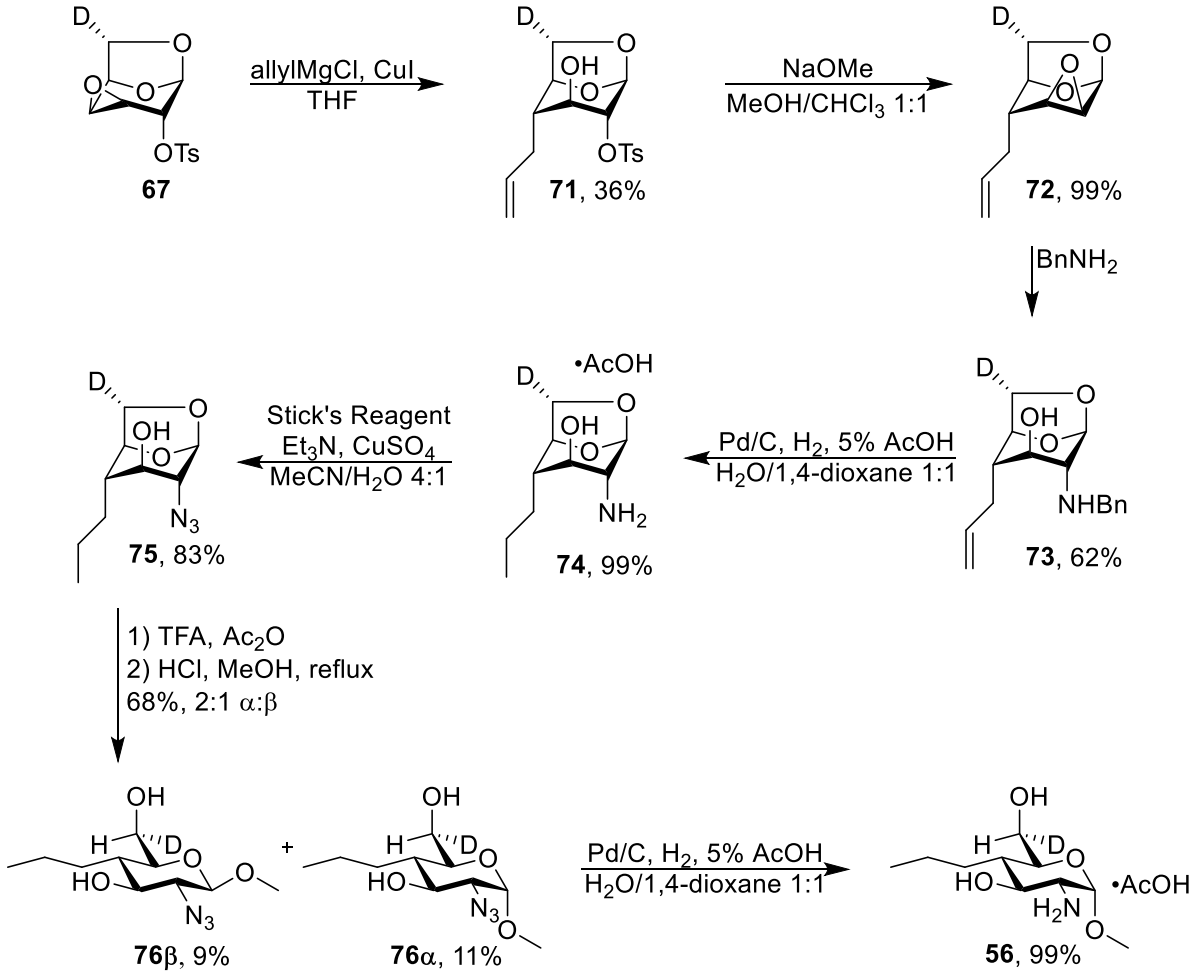


Scheme 16: Synthesis of a 4'-Deoxy-6'-(S)-deuterio Ring I Model

4.5 SYNTHESIS OF A 4'-DEOXY-4'-PROPYL-6'-(*S*)-DEUTERIO RING I MODEL

The deuterated propylamycin ring I model was made analogously to the non-deuterated isotopomer. Epoxide **67** was alkylated with allylmagnesium chloride and copper iodide to give **71** in 36% yield (Scheme 17). Alcohol **71** was treated with NaOMe to form the 2,3-epoxide **72** in 99%

yield, followed by selective opening with benzylamine to **73** in 62% yield. Compound **73** was subjected to hydrogenolysis conditions to give the amine **74**, with reduction of the allyl group at the 4-position, in 99% yield. Stick's reaction converted the amino group of **74** to the azide of **75** in 83% yield. Ring opening with TFA and acetic anhydride followed by Fischer glycosylation with methanolic HCl resulted in a 2:1 α/β mixture of **76** in 68% yield. Following silica gel flash column chromatography, **76α** and **76β** were isolated in 11% and 9% yield, respectively. The desired anomer, **76α**, was subjected to hydrogenolysis conditions to give the ring I model **56** with the deuterium at the 6-position with the (*S*) configuration. The NMR spectra of this compound matched those of the non-deuterated isotopomer, except for the absence of the 6-H_s signal at δ 3.66 ppm and the associated couplings, facilitating assignment of the protons at the 6-position.



Scheme 17: Synthesis of a 4'-Deoxy-4'-propyl-6'-(S)-deuterio Ring I Model

The change in regioselectivity in the ring openings of epoxides **69** and **72** with lithium azide and benzylamine respectively is noteworthy. The opening of **72** with benzylamine is consistent with the Fürst-Plattner rule and stereo-electronic control, despite suffering from a significant 1,3-diaxial interaction between the incoming nucleophile and the allyl group at the transition state.

In the opening of **69** with azide the minor regioisomer follows the Fürst-Plattner rule, affording the product in directly in a chair conformation with two axial substituents. The major

isomer on the other hand is necessarily initially formed in a twist-boat conformation, which then relaxes to a chair with two equatorial substituents. Presumably this diversion from the usual Fürst-Plattner and Bartonian prediciton[106] occurs because in the opening of the protonated epoxide there is considerable charge build up on carbon at the transition state. This partial positive charge on carbon is better accommodated on *C*-3 than on *C*-2 because of the absence of electron-withdrawing β-C-O bonds. Thus, the need to stabilize partial charge at the transition state overrides the stereo-electronic preferences of the Fürst-Plattner rule.

It can also be argued that ring opening of the protonated epoxide of **69** by azide proceeds with a loss of charge separation and so is highly exothermic, with a correspondingly early transition state that is not susceptible to stereo-electronic control. Opening of the neutral epoxide **72** by the neutral amine on the other hand proceeds with separation of charge and so is less exothermic, has a later transition state, and correspondingly obeys the dictates of stereo-electronic control. Finally, the selectivity of the opening of epoxide **72** is likely further aided by the presence of the allyl group at the 4-position which shields *C*-3 from nucleophilic attack, whereas *C*-3 is more accessible in **69** (Figure 30).

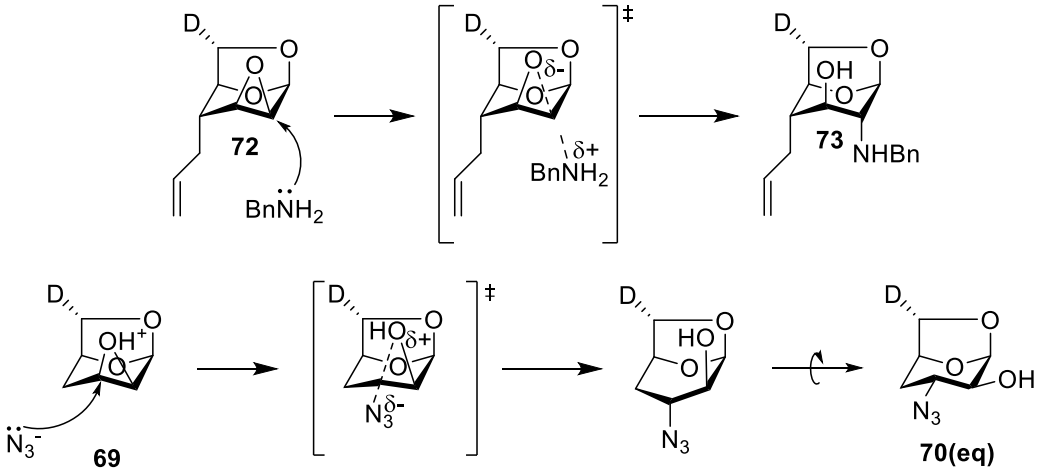


Figure 30: Mechanism of Epoxide Opening

4.6 ANALYSIS OF SIDE CHAIN POPULATIONS

Since the advent of the Karplus equation,[107] several methods have been devised to extract details about the conformation of bonds based on NMR coupling constants. The conformation of the side chain in carbohydrates has been a topic of study for many groups,[79-80, 108-109] due to its importance for carbohydrate-enzyme binding[29] and influence on selectivity of glycosylation reactions.[110-117] Rotation of the side chain is rapid enough that on the NMR time scale the individual rotamers are not visible and the observed ^{3}J values are a time weighted average based on the relative ratios of the populations of each side chain conformation and the limiting coupling constants for each rotamer as described by Equations 1 and 2 (Figure 31). Equation 3 simply states that the sum of the fractions of each population must total up to 1. With known limiting coupling constants, the fractions of each conformation of the side can be calculated from these equations.

$$(1)\quad {}^{3}J_{\mathrm{H5,H6R}} = {}^{3}J_{R,gg}f_{gg} + {}^{3}J_{R,gt}f_{gt} + {}^{3}J_{R,tg}f_{tg}$$

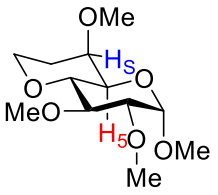
$$(2)\quad {}^{3}J_{\mathrm{H5,H6S}} = {}^{3}J_{S,gg}f_{gg} + {}^{3}J_{S,gt}f_{gt} + {}^{3}J_{S,tg}f_{tg}$$

$$(3)\quad 1 = f_{gg} + f_{gt} + f_{tg}$$

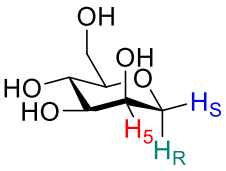
Figure 31: Equations for Determination of Side Chain Populations in Solution

Recently the Crich group described a study and evaluation of mimetics of each staggered side chain conformation, with both the gluco and galacto-configurations to determine better approximations of the limiting coupling constants for equations 1 and 2, allowing more accurate calculation of the population of side chain conformations in solution using experimental $^{3}J_{H5,H6}$ values.[90] The relevant models for the gluco series are shown in Figure 32 with the coupling constants for H_{6R} and H_{6S}. The averages of these coupling constants were used as more accurate limiting coupling constants, shown in Table 13, for calculation of side chain populations in the model compounds synthesized above. The digital resolution of the spectra from which these coupling constants are taken is 0.4 Hz indicating that the uncertainty in calculations based upon these coupling constants is about 5%.[118]

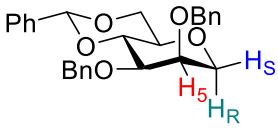
gg



$^3J_{H5,HS}$ = 2.1 (2.6)

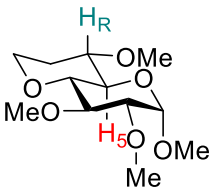


$^3J_{H5,HR}$ = 0.9
$^3J_{H5,HS}$ = 2.2

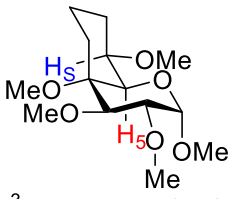


$^3J_{H5,HR}$ = 1.1
$^3J_{H5,HS}$ = 2.2

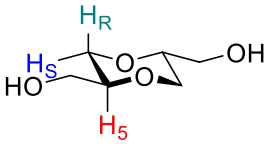
gt



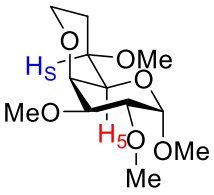
$^3J_{H5,HR}$ = 11.2 (9.2)



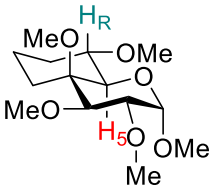
$^3J_{H5,HS}$ = 2.6 (3.1)



$^3J_{H5,HR}$ = 10.7
$^3J_{H5,HS}$ = 2.5

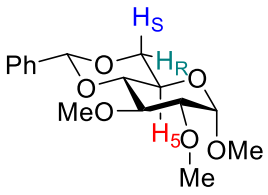


$^3J_{H5,HS}$ = 2.7 (3.2)

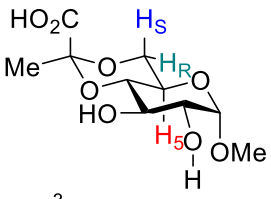


$^3J_{H5,HS}$ = 11.2 (9.2)

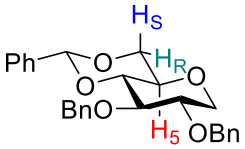
tg



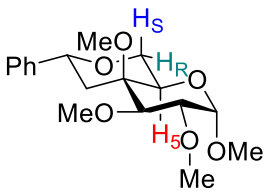
$^3J_{H5,HR}$ = 4.7
$^3J_{H5,HS}$ = 10.2



$^3J_{H5,HR}$ = 5.0
$^3J_{H5,HS}$ = 10.3



$^3J_{H5,HR}$ = 5.0
$^3J_{H5,HS}$ = 10.2



$^3J_{H5,HR}$ = 4.6
$^3J_{H5,HS}$ = 10.3

Figure 32: Models for Determination of Limiting Coupling Constants; Values in Hz, Measured Value in Parenthesis if Correction Factor Applied

Table 13: Limiting Coupling Constants

	gg	gt	tg
$^3J_{H5,H6R}$ Hz	1.0	11.0	4.8
$^3J_{H5,H6S}$ Hz	2.2	2.5	10.2

The experimental coupling constants from the α-methyl glycoside of glucosamine **76** (Figure 33) at pH 5 were determined previously as $^3J_{H5,H6R}$ = 4.9 Hz and $^3J_{H5,H6S}$ = 2.2 Hz,[82] from which it is calculated using the equations in Figure 31 and the limiting coupling constants from Table 13 that its side chain adopts a 62:40:-2 gg:gt:tg mixture of conformations. As the *α*-methyl glycoside of glucosamine serves as a model for ring I of paromomycin, the side chain of paromomycin ring I is likewise considered to take up a 62:40:-2 mixture of gg:gt:tg conformers (Table 14). Self-evidently a population of -2% is impossible. This is an artifact of the method and its errors, and is considered to be indistinguishable from a 0% population.

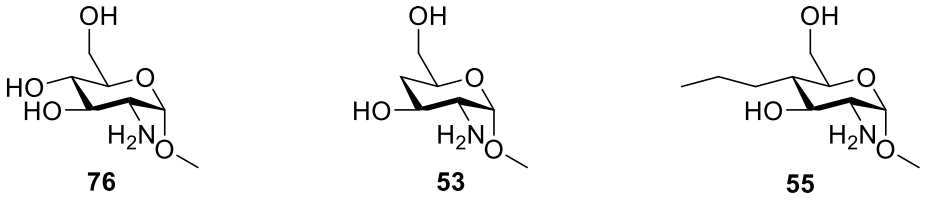


Figure 33: Ring I Models

The 4'-deoxy ring I model **53** displayed coupling constants of $^3J_{H5,H6R}$ = 5.8 Hz and $^3J_{H5,H6S}$ = 2.5 Hz revealing its side chain and by extrapolation that of the 4'-deoxy paromomycin adopt a 51:47:2 gg:gt:tg mixture of conformations. Finally, for the propylamycin ring I model **55** the coupling constants of 5.3 Hz for H_5-H_{6R} and 2.2 Hz for H_5-H_{6S} indicate that its side chain, and that of propylamycin, can be considered as a 58:44:-2 mixture of gg:gt:tg conformations.

Table 14: Side Chain Coupling Constants and Populations

Ring I Model	Aminoglycoside	$^3J_{H5,H6R}$	$^3J_{H5,H6S}$	f_{gg}	f_{gt}	f_{tg}
76	paromomycin	4.9	2.2	62	40	-2
53	4'-deoxy paromomycin	5.8	2.5	51	47	2
55	propylamycin	5.3	2.2	58	44	-2

4.7 DISCUSSION AND CONCLUSION

The calculations of side chain populations in ring I models indicate that there is a shift of about 4% from the gg to the gt conformation from paromomycin to propylamycin with no change in the population of the tg conformation. In the case of the 4-deoxy paromomycin ring I model, there is an 11% decrease in population of the gg conformation leading to an 7% increase in the population of the gt conformation, as well as a 5% increase in the population of the tg conformation. Considering the small difference in these values combined with the uncertainty of 5% in the calculations there appears to be no significant change in conformation of the ring I side chain when changing the substituent at the 4'-position. This leads to the conclusion that the gauche effect, which states that electronegative atoms on neighboring carbons cause a preference for a gauche conformation (Figure 34),[92] is the major factor in side chain populations and steric influence from the 4-position has a minor effect.

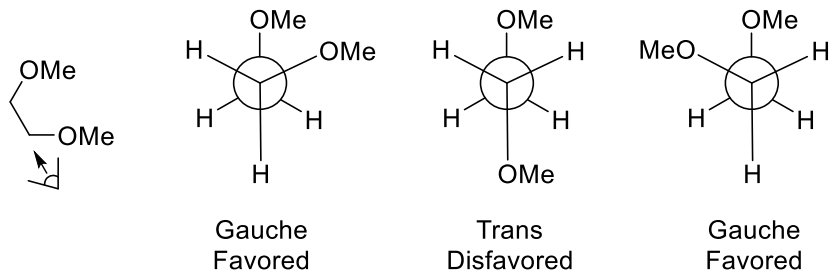


Figure 34: Newman Projections of 1,2-Dimethoxyethane

It is noteworthy that 4-deoxy glucose is also 4-deoxy galactose, however the populations of the side chain in **53** resemble glucose much more closely than galactose where the ratio of side chain conformations is 16:53:31 gg:gt:tg.[90] The gg conformation is disfavored in galactose and not in the 4-deoxy galactose system because of dipolar repulsion between the axial hydroxy group at the 4-position and the side chain hydroxy group when in the gg conformation (Figure 35).

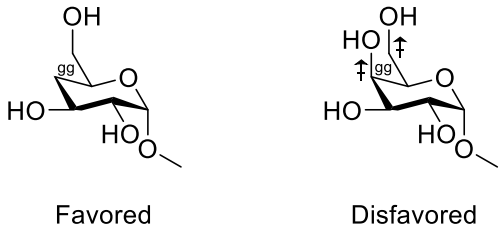


Figure 35: The gg Conformation is Disfavored in Galactose Due to Dipolar Repulsion, This Effect is Absent in 4-Deoxy Galactose

With the data suggesting that there is no significant change in the side chain conformation of ring I of propylamycin, 4'-deoxyparomomycin, and paromomycin, the increased antibacterioribosomal and antibacterial activity of propylamycin relative to the parent cannot be due to an increase in population of the gt conformation, and alternative explanations are therefore preferred. As noted above these include increased basicity of the ring oxygen, leading to an increase of strength of the critical hydrogen bond to A1408. The difference in antibacterioribosomal and antibacterial activity of propylamycin and 4'-deoxy paromomycin must be related to an interaction of the propyl group in propylamycin with the ribosome, possibly of a hydrophobic nature.

CHAPTER 5: OVERALL CONCLUSIONS

The conformation of the ring I side chain in paromomycin has a substantial effect on the activity and selectivity of the drug. By fusing ring I with a second ring connecting O-4' and C-6', it has been demonstrated that the ideal bound conformation for the ring I side chain is nearly a perfect gt conformation as evidenced by antibacterioribosomal and antibacterial activity reaching a maximum in compounds determined to have side chains in the ideal gt conformation. Small changes in the conformation of the side chain caused by increases or decreases in the size of the appended ring result in large changes in activity, further affirming the importance of effects of the ring I side chain conformation on the activity of AGAs.

It has been shown that alkylation at *C*-6' changes the relative conformational populations of the side chain such that the (*R*) configuration increases the relative population of the gt conformation resulting in an increase in activity. Alkylation at *C*-6' resulting in the (*S*) configuration causes a decrease in the relative population of the gt conformation, thus decreasing activity. Changes in the substituent at the 4'-position have a negligible effect on the relative populations of the side chain conformations and therefore any changes in activity of these AGAs are due to other factors.

The activity of AGAs with respect to the humanized ribosomes shows little correlation to the conformation of the side chain, such that selectivity for bacterial over humanized ribosomes reaches a maximum when the side chain is in the gt conformation. Based on these trends future AGAs designed so as to maximize the gt conformation of the side chain will benefit from increased activity and selectivity.

CHAPTER 6: EXPERIMENTAL SECTION

GENERAL EXPERIMENTAL

All reagents were purchased from commercial sources and used without further purification unless otherwise specifies. Thin-layer chromatography was performed on Sorbtech glass backed silica gel XHL plates with UV 254. Chromatographic purifications were carried out in Fisher silica gel 60 230-400 mesh unless otherwise specified. High resolution mass spectra were collected on a Waters LCT Premier XE ESI-TOF mass spectrometer. Optical rotations were measured using a Rudolph Research Autopol III polarimeter in a 1 dm cell. NMR spectra were collected on an Agilent 600 MHz DD2, Agilent 500 MHz VNMRS, or an Agilent 400-MR spectrometer as indicated. NMR spectra were assigned with the aid of advanced 1D and 2D techniques including COSY, HSQC, HMBC, TOCSY, and ROESY.

1,3,2',2''',6''',6'-Pentaazido-6,3',2'',5'',3''',4''',6'''-hexa-*O*-benzyl-6'-carboxyl-1,3,2',2''',6'''-pentadeaminoparomomycin (17). TEMPO (87.5 mg, 0.56 mmol) and BAIB (1.98 g, 6.16 mmol) were added to a stirred solution of **16** (3.60 g, 2.80 mmol) in 33 mL of 1:1 MeCN/H_2O. After 3.5 hours the MeCN was removed under vacuum and the aqueous solution was extracted with ethyl acetate. The organic layer was washed with 20% $Na_2S_2O_3$ solution, 1 N HCl, and brine followed by drying over sodium sulfate and silica gel column chromatography in 70% EtOAc in hexanes with 1% AcOH to give 3.60 g (2.77 mmol, 99%) of the orange foam **17.** $[\alpha]_D^{23}$ = 74.99 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CD_3OD/CDCl_3$ 4:1) δ 7.40 – 7.13 (m, 30H), 6.12 (d, *J* = 3.6 Hz, 1H, H-1'), 5.56 (d, *J* = 4.8 Hz, 1H, H-1''), 4.90 (d, *J* = 11.0 Hz, 1H, $PhCH_2O$), 4.87 (d, *J* = 10.8 Hz, 1H, $PhCH_2O$), 4.79 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.71 – 4.67 (m, 2H, $PhCH_2O$), 4.57 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.53

(d, *J* = 9.4 Hz, 1H, H-5'), 4.51 – 4.40 (m, 6H, $PhCH_2O$), 4.35 (d, *J* = 12.2 Hz, 1H, $PhCH_2O$), 4.33 (d, *J* = 12.2 Hz, 1H, $PhCH_2O$), 4.19 – 4.14 (m, 2H, H-3'', H-5''), 3.92 (t, *J* = 4.6 Hz, 1H, H-2''), 3.85 (dd, *J* = 9.9, 8.4 Hz, 1H, H-3'), 3.80 (t, *J* = 2.9 Hz, 1H, H-3'''), 3.79 – 3.71 (m, 4H, H-5, H-4', H-5'', H-5'''), 3.68 (t, *J* = 9.4 Hz, 1H, H-4), 3.55 – 3.50 (m, 2H, H-5'', H-6'''), 3.50 – 3.42 (m, 2H, H-1, H-3), 3.31 (t, *J* = 2.3 Hz, 1H, H-2'''), 3.29 – 3.23 (m, 2H, H-6, H-4'''), 3.11 (dd, *J* = 9.8, 3.6 Hz, 1H, H-2'), 3.03 (dd, *J* = 12.9, 4.1 Hz, 1H, H-6'''), 2.20 (dt, *J* = 12.8, 4.6 Hz, 1H, H-2eq), 1.39 (q, *J* = 12.6 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CD_3OD/CDCl_3$ 4:1) δ 171.9 (C-6'), 138.2, 138.1, 138.0, 137.6, 137.4, 137.3, 128.3, 128.2, 128.13, 128.10, 128.0, 127.94, 127.91, 127.9, 127.84, 127.82, 127.7, 127.58, 127.55, 127.4, 127.3, 127.2 (Ar), 106.7 (C-1''), 98.5 (C-1'''), 96.2 (C-1'), 83.9 (C-6), 82.0 (C-3''), 81.8 (C-5), 81.5 (C-2''), 78.7 (C-3'), 75.8 (C-4), 75.7 (C-4''), 74.8 ($PhCH_2O$), 74.6 (C-5''), 74.2 ($PhCH_2O$), 73.2 (C-3''), 73.1 ($PhCH_2O$), 72.9 ($PhCH_2O$), 72.3 ($PhCH_2O$), 71.9 (C-4'''), 71.8 (C-4'), 71.7 ($PhCH_2O$), 70.1 (C-5''), 62.1 (C-2'), 60.4 (C-1), 59.7 (C-3), 57.2 (C-2'''), 50.9 (C-6'''), 31.8 (C-2). ESI-HRMS: *m/z* calc for $C_{69}H_{69}N_{15}O_{15}Na$ $[M+Na]^+$ 1322.4995, found 1322.5044.

1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-(N-methyl-N-methoxy)amido-1,3,2',2''',6'''-pentadeaminoparomomycin (18). Compound **17** (4.64 g, 3.57 mmol), DMAP (0.0911 g, 0.716 mmol) DMAP, and N,O-dimethylhydroxylamine hydrochloride (0.5242 g, 5.37 mmol) were stirred under argon in 30 mL DCM followed by addition of DCC (1.1051 g, 5.356 mmol) in 5.7 mL of DCM. After two hours DCC (0.3684 g, 1.785 mmol) in 1 mL of DCM was added to the reaction mixture. After another hour no starting material was detected by TLC and the reaction mixture was concentrated under vacuum. The crude residue was dissolved in EtOAc and washed with 1N HCl and brine, dried with Na_2SO_4, filtered, and

concentrated. The crude residue was then subjected to flash column chromatography over silica gel with 40% EtOAc in hexanes. Following chromatography, the product still contained some dicyclohexyl urea biproduct which was removed by dissolving the residue in a minimal amount of toluene and filtering while cold. Concentration of the filtrate gave 3.22 g (2.40 mmol 67%) of **18** as a white foam. $[\alpha]_D^{23}$ = 89.10 (*c* = 1.0, $CHCl_3$) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 – 7.10 (m, 30H, Ar-H), 6.28 (d, *J* = 3.5 Hz, 1H, H-1'), 5.66 (d, *J* = 6.2 Hz, 1H, H-1''), 4.97 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.88 – 4.82 (m, 3H, H-1''', $PhCH_2O$), 4.79 (br d, *J* = 9.1 Hz, 1H, H-5'), 4.67 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.61 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.53 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.50 (d, *J* = 11.6 Hz, 1H, $PhCH_2O$), 4.44 – 4.38 (m, 3H, $PhCH_2O$), 4.30 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.27 – 4.23 (m, 2H, H-4''', $PhCH_2O$), 4.22 (dd, *J* = 5.0, 2.0 Hz, 1H, H-3''), 4.04 (t, *J* = 9.4 Hz, 1H, H-3'), 4.01 – 3.95 (m, 2H, H-5, H-4'), 3.89 (dd, *J* = 6.2, 5.0 Hz, 1H, H-2''), 3.78 – 3.73 (m, 4H, H-4, H-5'', H-3''', H-5'''), 3.72 (s, 3H, OCH_3), 3.60 (dd, *J* = 12.9, 8.4 Hz, 1H, H-6'''), 3.54 (dd, *J* = 10.4, 3.0 Hz, 1H, H-5''), 3.52 – 3.42 (m, 2H, H-1, H-3), 3.32 (t, *J* = 2.5 Hz, 1H, H-2'''), 3.28 (s, 3H, NCH_3), 3.24 (t, *J* = 9.3 Hz, 1H, H-6), 3.11 (d, *J* = 2.4 Hz, 1H, H-4'''), 3.03 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2'), 2.89 (dd, *J* = 13.0, 4.1 Hz, 1H, H-6'''), 2.24 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.33 (q, *J* = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 169.2 (C-6'), 138.2, 138.0, 137.8, 137.5, 137.0, 136.9, 128.7, 128.49, 128.47, 128.43, 128.40, 128.36, 128.3, 128.24, 128.17, 128.1, 127.8, 127.62, 127.61, 127.5 (Ar), 105.8 (C-1''), 98.8 (C-1'''), 96.3 (C-6), 84.5 (C-2''), 82.5 (C-4''), 82.2 (C-5), 81.8 (C-3'), 78.5 (C-3''), 75.6 ($PhCH_2O$), 75.1 ($PhCH_2O$), 74.6 (C-4), 74.3 (C-5'''), 73.4 ($PhCH_2O$), 73.3 ($PhCH_2O$), 72.9 (C-3'''), 72.4 ($PhCH_2O$), 71.9 (C-4'), 71.7 ($PhCH_2O$), 71.4 (C-4'''), 69.9 (C-5''), 68.5 (C-5'), 62.2 (C-2'), 62.1 (OCH_3), 60.4 (C-1), 60.3 (C-3), 57.2 (C-2'''), 51.0 (C-6'''), 32.8 (C-2), 32.4 (NCH_3). ESI-HRMS: *m/z* calc for $C_{67}H_{74}N_{16}O_{15}Na$ $[M+Na]^+$ 1365.5417, found 1365.5453.

1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-methyl-ketone-4'-*O*-trimethylsilyl-1,3,2',2''',6'''-pentadeaminoparomomycin (19). HMDS (0.55 mL, 2.6 mmol) was added to a stirred solution of compound **18** (1.17 g, 0.87 mmol) in 8.7 mL MeCN under argon. After three hours no starting material was detected by TLC. The reaction mixture was concentrated under vacuum and the white foam was used without further purification. ESI-HRMS: *m/z* calc for $C_{70}H_{82}N_{16}O_{15}SiNa$ $[M+Na]^+$ 1437.5813, found 1437.5868. 0.6 mL of 3M MeMgCl in THF were added to a stirred solution of amide in 8.8 mL of THF at -78°C. The reaction mixture was stirred for 5 minutes then transferred to an ice bath and stirred for another 10 minutes before quenching with 1 mL of NH_4Cl solution. The THF was then removed under vacuum, diluted with Et_2O, and washed with NH_4Cl solution and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified over silica gel with gradient elution of 0% ethyl acetate in hexanes to 80% to give 0.4673 g (0.3408 mmol, 39%) of ketone **19** as a white foam. $[\alpha]_D^{23}$ = 104.20 (*c* = 1.0, $CHCl_3$) ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.15 (m, 30H, Ar-H), 6.09 (d, *J* = 3.7 Hz, 1H, H-1'), 5.61 (d, *J* = 5.4 Hz, 1H, H-1''), 4.93 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.88 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.78 (s, 2H, $PhCH_2O$), 4.72 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.62 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.54 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.51 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.47 – 4.41 (m, 3H, $PhCH_2O$), 4.40 (d, *J* = 4.5 Hz, 1H, H-5'), 4.34 – 4.23 (m, 4H, H-3'', H-4'', $PhCH_2O$), 3.94 – 3.88 (m, 2H, H-5, H-2''), 3.82 – 3.76 (m, 3H, H-3', H-5'', H-5'''), 3.75 (t, *J* = 2.9 Hz, 1H, H-3'''), 3.63 (dd, *J* = 13.0, 8.5 Hz, 1H, H-6'''), 3.61 – 3.58 (m, 2H, H-4, H-4'), 3.56 (dd, *J* = 10.5, 3.4 Hz, 1H, H-5''), 3.48 – 3.39 (m, 2H, H-1, H-3), 3.35 (t, *J* = 2.7 Hz, 1H, H-2'''), 3.27 (t, *J* = 9.3 Hz, 1H, H-6), 3.13 (d, *J* = 2.6 Hz, 1H, H-4'''), 2.94 – 2.88 (m, 2H, H-2', H-6'''), 2.27 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 2.21 (s, 3H, CH_3), 1.42 (q, *J* = 12.7 Hz, 1H, H-2ax), 0.04 (s, 9H, $SiCH_3$). ^{13}C NMR (151

MHz, $CDCl_3$) δ 204.6 (C-6'), 138.3, 138.1, 137.8, 137.6, 137.0, 136.9, 128.7, 128.5, 128.4, 128.34, 128.32, 128.28, 128.23, 128.18, 127.82, 127.77, 127.74, 127.71, 127.54, 127.53, 127.49, 127.47 (Ar), 106.3 (C-1''), 98.5 (C-1'''), 96.3 (C-1'), 84.0 (C-6), 82.2 (C-2''), 82.0 (C-4''), 81.9 (C-5), 80.0 (C-3'), 76.1 (C-5'), 75.5 (C-4), 75.4 (C-3''), 75.2 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.3 (C-5'''), 73.3 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.9 (C-3'''), 72.5 (C-4'), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.2 (C-5''), 62.8 (C-2'), 60.3 (C-1), 59.9 (C-3), 57.3 (C-2'''), 51.1 (C-6'''), 32.4 (C-2), 28.6 (CH_3), 0.5 ($SiCH_3$). ESI-HRMS: *m/z* calc for $C_{69}H_{79}N_{15}O_{14}SiNa$ $[M+Na]^+$ 1392.5598, found 1392.5637.

1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-*C*-methyl-4'-*O*-trimethylsilyl-1,3,2',2''',6'''-pentadeaminoparomomycin (20(*R*) and 20(*S*)), $NaBH_4$ (0.0179 g, .4710 mmol) was added to a stirred solution of compound **19** (0.3228 g, .2355 mmol) in 2.4 mL of 1:1 THF/MeOH. The reaction mixture was stirred for 20 minutes then concentrated and the crude residue was dissolved in EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated to afford a 1:1 mixture of isomers of **20**. Silica gel chromatography eluting with 16% EtOAc in hexanes followed by 18% then 20% afforded the compounds **20(S)** (118.6 mg, 0.0864 mmol, 37%) and **20(R)** (123.0 mg, 0.0896 mmol, 38%) both as white foams. **20(R)** $[\alpha]_D^{23}$ = 97.00 (*c* = 1.0, $CHCl_3$), ^1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.15 (m, 30H, Ar-H), 6.09 (d, *J* = 3.8 Hz, 1H, H-1'), 5.63 (d, *J* = 5.4 Hz, 1H, H-1''), 4.95 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.91 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.82 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.75 – 4.70 (m, 2H, $PhCH_2O$), 4.62 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.56 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.52 – 4.39 (m, 4H, $PhCH_2O$), 4.33 – 4.30 (m, 2H, H-3'', $PhCH_2O$), 4.29 (q, *J* = 2.8 Hz, 1H, H-4''), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.02 – 3.97 (m, 1H, H-6'), 3.96 – 3.90 (m, 3H, H-5, H-5', H-2''), 3.83 – 3.75 (m, 4H, H-3', H-5'', H-3''', H-5'''), 3.70 – 3.63 (m, 2H, H-

4, H-6’’’), 3.57 (dd, *J* = 10.5, 3.4 Hz, 1H, H-5’’), 3.50 – 3.41 (m, 2H, H-1, H-3), 3.37 (t, *J* = 2.6 Hz, 1H, H-2’’’), 3.29 (t, *J* = 9.3 Hz, 1H, H-6), 3.21 (dd, *J* = 9.9, 8.5 Hz, 1H, H-4’), 3.13 (t, *J* = 2.6 Hz, 1H, H-4’’’), 2.88 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6’’’), 2.82 (dd, *J* = 10.2, 3.8 Hz, 1H, H-2’), 2.24 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.42 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.14 (d, *J* = 6.5 Hz, 3H, H-7’), 0.07 (s, 9H, $SiCH_3$). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.4, 138.2, 137.9, 137.6, 137.0, 136.9, 128.7, 128.5, 128.4, 128.35, 128.33, 128.28, 128.2, 127.82, 127.79, 127.75, 127.71, 127.5, 127.45, 127.38, 127.37, 127.2 (Ar), 106.3 (C-1’’), 98.5 (C-1’’’), 95.7 (C-1’), 84.1 (C-6), 82.2 (C-2’’), 81.99 (C-5), 81.96 (C-4’’), 80.3 (C-3’), 75.4 (C-3’’), 75.02 ($PhCH_2O$), 74.98 (C-4), 74.8 ($PhCH_2O$), 74.4 (C-5’’’), 74.3 (C-5’), 73.23 (C-4’), 73.21 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.9 (C-3’’’), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4’’’), 70.4 (C-5’’), 67.0 (C-6’), 63.3 (C-2’), 60.4 (C-1), 60.1 (C-3), 57.3 (C-2’’’), 51.2 (C-6’’’), 32.5 (C-2), 16.4 (C-7’), 0.7 ($SiCH_3$). ESI-HRMS: *m/z* calc for $C_{69}H_{81}N_{15}O_{14}SiNa$ $[M+Na]^+$ 1394.5754, found 1394.5784. **20(S)** $[\alpha]_D^{23}$ = 97.20 (*c* = 1.0, DCM), ^1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.16 (m, 30H, Ar-H), 6.11 (d, *J* = 3.8 Hz, 1H, H-1’), 5.68 (d, *J* = 5.7 Hz, 1H, H-1’’), 4.99 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.95 (d, *J* = 1.9 Hz, 1H, H-1’’’), 4.78 (s, 2H, $PhCH_2O$), 4.73 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.63 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.58 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.52 – 4.44 (m, 3H, $PhCH_2O$), 4.42 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.36 (dd, *J* = 4.8, 2.5 Hz, 1H, H-3’’), 4.32 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.30 (q, *J* = 2.5 Hz, 1H, H-4’’), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.00 (dd, *J* = 5.7, 4.8 Hz, 1H, H-2’’), 3.94 (t, *J* = 8.9 Hz, 1H, H-5), 3.93 – 3.88 (m, 1H, H-6’), 3.84 – 3.80 (m, 2H, H-5’’, H-5’’’), 3.79 – 3.75 (m, 2H, H-3’, H-3’’’), 3.68 (dd, *J* = 13.0, 8.7 Hz, 1H, H-6’’’), 3.61 (d, *J* = 9.7 Hz, 1H, H-5’), 3.58 (dd, *J* = 10.4, 2.9 Hz, 1H, H-5’’), 3.54 (dd, *J* = 9.8, 8.7 Hz, 1H, H-4), 3.47 – 3.41 (m, 3H, H-1, H-3, H-4’), 3.39 – 3.36 (m, 1H, H-2’’’), 3.28 (t, *J* = 9.3 Hz, 1H, H-6), 3.14 – 3.12 (m, 1H, H-4’’’), 2.88 (dd, *J* = 13.1, 3.7 Hz, 1H, H-6’’’), 2.76 (dd, *J* = 10.4, 3.8 Hz, 1H, H-2’), 2.24 (dt, *J* = 13.1, 4.5 Hz, 1H, H-2eq), 1.35

(q, *J* = 12.5 Hz, 1H, H-2ax), 1.27 (d, *J* = 6.5 Hz, 3H, CH_3), 0.10 (s, 9H, $SiCH_3$). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.4, 138.3, 137.9, 137.6, 137.0, 136.9, 128.7, 128.5, 128.41, 128.36, 128.32, 128.27, 128.19, 127.81, 127.79, 127.65, 127.58, 127.53, 127.51, 127.4, 127.1 (Ar), 106.2 (C-1''), 98.6 (C-1'''), 95.7 (C-1'), 84.4 (C-6), 82.4 (C-2''), 82.1 (C-4''), 82.0 (C-5), 80.1 (C-3'), 75.4 (C-3''), 75.1 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.8 (C-4), 74.6 (C-5'), 74.5 (C-5'''), 73.2 ($PhCH_2O$), 73.0 ($PhCH_2O$), 72.9 (C-3'''), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 71.3 (C-4'), 70.3 (C-5''), 64.3 (C-6'), 63.1 (C-2'), 60.4 (C-1), 60.1 (C-3), 57.3 (C-2'''), 51.2 (C-6'''), 32.7 (C-2), 20.7 (C-7'), 0.6 ($SiCH_3$). ESI-HRMS: *m/z* calc for $C_{69}H_{81}N_{15}O_{14}SiNa$ $[M+Na]^+$ 1394.5754, found 1394.5760.

6'-(*R*)-*C*-methyl-paromomycin pentaacetate salt (22(*R*)). 1M TBAF solution in THF (0.051 mL) was added dropwise to a stirred solution of compound **20(*R*)** (26.7 mg, 0.0171 mmol) in 1.7 mL of THF under argon. When the starting material was no longer visible by TLC, the reaction mixture was diluted with Et_2O and washed with of $NaHCO_3$ solution and brine. The organic layer was then dried with Na_2SO_4, filtered, and concentrated to give the intermediate alcohol which was used without further purification. The previous alcohol was stirred in 0.4 mL of 1:1 dioxane/10% AcOH in water with 58.0 mg of Pd/C under 50 psi of H_2 for 18 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified over a CM Sephadex C-25 column. The column was washed with 100 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization of the pure fractions with AcOH gave 2.6 mg (0.003 mmol) of the pentaacetate salt **22(*R*)** as a white solid in 18% yield. $[\alpha]_D^{23}$ = 41.27 (*c* = 0.6, H_2O), ^1H NMR (600 MHz, D_2O) δ 5.55 (d, *J* = 3.9 Hz, 1H,

H-1'), 5.31 (d, *J* = 2.8 Hz, 1H, H-1''), 5.19 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.44 (dd, *J* = 6.5, 5.0 Hz, 1H, H-3''), 4.28 (dd, *J* = 5.0, 2.8 Hz, 1H, H-2''), 4.25 – 4.21 (m, 1H, H-5'''), 4.16 – 4.11 (m, 3H, H-6', H-4'', H-3'''), 3.86 – 3.79 (m, 2H, H-5' [1dTOCSY 3.82 (dd, *J* = 10.1, 2.5 Hz)], H-5''), 3.77 – 3.72 (m, 3H, H-5, H-3', H-4'''), 3.70 (dd, *J* = 12.4, 4.9 Hz, 1H, H-5''), 3.61 (t, *J* = 9.4 Hz, 1H, H-4), 3.52 (dd, *J* = 10.4, 9.2 Hz, 1H, H-6), 3.48 – 3.43 (m, 1H, H-2'''), 3.38 – 3.32 (m, 2H, H-4', H-6'''), 3.29 (dd, *J* = 13.6, 3.9 Hz, 1H, H-6'''), 3.20 (dd, *J* = 10.7, 3.9 Hz, 1H, H-2'), 3.17 – 3.06 (m, 2H, H-1, H-3), 2.20 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2eq), 1.84 (s, 15H, AcOH), 1.51 (q, *J* = 12.6 Hz, 1H, H-2ax), 1.15 (d, *J* = 6.6 Hz, 3H, H-7'). ^{13}C NMR (151 MHz, D_2O) δ 181.4 (AcOH), 109.7 (C-1''), 96.9 (C-1'), 95.9 (C-1'''), 84.5 (C-6), 81.3 (C-4''), 81.0 (C-4), 75.3 (C-3''), 75.1 (C-5'), 73.5 (C-5), 73.4 (C-2''), 70.37 (C-4'), 70.35 (C-3'), 70.26 (C-5'''), 68.0 (C-3'''), 67.5 (C-4'''), 65.7 (C-6'), 60.3 (C-5''), 54.3 (C-2'), 51.0 (C-2'''), 50.2 (C-1), 49.3 (C-3), 40.4 (C-6'''), 31.1 (C-2), 23.3 (AcOH), 14.8 (C-7'). ESI-HRMS: *m/z* calc for $C_{24}H_{47}N_5O_{14}$ $[M+H]^+$ 630.3198, found 630.3212.

6'-(S)-*C*-methyl-paromomycin pentaacetate salt (22(*S*)). 1M TBAF solution in THF (0.075 mL) was added dropwise to a stirred solution of compound **20(*S*)** (32.8 mg, 0.024 mmol) in 2.3 mL of THF under argon. When the starting material was no longer visible by TLC, the reaction mixture was diluted with Et_2O and washed with of $NaHCO_3$ solution and brine. The organic layer was then dried with Na_2SO_4, filtered, and concentrated to give the intermediate alcohol which was used without further purification. The crude alcohol was stirred in 0.4 mL of 1:1 dioxane/10% AcOH in water with 58.0 mg of Pd/C under 50 psi of H_2 for 18 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified over a CM Sephadex C-25 column. The

column was washed with 100 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization of the pure fractions with AcOH gave 5.2 mg (0.006 mmol) of the pentaacetate salt **22(*S*)** as a white solid in 25% yield. $[\alpha]_D^{23}$ = 50.87 (*c* = 0.6, H_2O), ^{1}H NMR (600 MHz, D_2O) δ 5.61 (d, *J* = 3.8 Hz, 1H, H-1'), 5.31 (d, *J* = 2.5 Hz, 1H, H-1''), 5.20 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.46 (dd, *J* = 6.7, 4.9 Hz, 1H, H-3''), 4.29 (dd, *J* = 5.0, 2.5 Hz, 1H, H-2''), 4.26 – 4.21 (m, 1H, H-5'''), 4.17 – 4.10 (m, 3H, H-6' [1dTOCSY 4.13 (qd, *J* = 6.6, 1.6 Hz)], H-4'', H-3'''), 3.85 (dd, *J* = 12.4, 3.1 Hz, 1H, H-5''), 3.79 (dd, *J* = 10.8, 8.6 Hz, 1H, H-3'), 3.76 – 3.68 (m, 3H, H-5, H-5'', H-4'''), 3.59 – 3.50 (m, 4H, H-4, H-6, H-4', H-5'), 3.49 – 3.45 (m, 1H, H-2'''), 3.35 (dd, *J* = 13.7, 6.9 Hz, 1H, H-6'''), 3.29 (dd, *J* = 13.6, 3.9 Hz, 1H, H-6'''), 3.21 (dd, *J* = 10.8, 3.8 Hz, 1H, H-2'), 3.16 (ddd, *J* = 12.4, 10.4, 4.2 Hz, 1H, H-1), 2.96 (ddd, *J* = 12.1, 9.6, 4.3 Hz, 1H, H-3), 2.15 (dt, *J* = 12.9, 4.3 Hz, 1H, H-2eq), 1.84 (s, 15H, AcOH), 1.47 (q, *J* = 12.6 Hz, 1H, H-2ax), 1.21 (d, *J* = 6.6 Hz, 3H, H-7'). ^{13}C NMR (151 MHz, D_2O) δ 181.4 (AcOH), 109.8 (C-1''), 96.4 (C-1'), 95.8 (C-1'''), 84.8 (C-5), 81.8 (C-4), 81.2 (C-4''), 75.4 (C-5'), 75.2 (C-3''), 73.4 (C-2''), 73.2 (C-6), 70.3 (C-5'''), 69.7 (C-3'), 69.5 (C-4'), 68.0 (C-3'''), 67.4 (C-4'''), 64.1 (C-6'), 60.1 (C-5''), 54.3 (C-2'), 51.0 (C-2'''), 50.4 (C-1), 49.4 (C-3), 40.4 (C-6'''), 31.5 (C-2), 23.3 (AcOH), 18.9 (C-7'). ESI-HRMS: *m/z* calc for $C_{24}H_{47}N_5O_{14}$ $[M+H]^+$ 630.3198, found 630.3209.

1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-4',6'-*O*-benzylidene-6'-(*S*)-*C*-methyl-1,3,2',2''',6'''-pentadeaminoparomomycin (21). A 1 M TBAF solution in THF (0.38 mL) was added to a stirred solution of compound **20(*S*)** (0.1722 g, 0.126 mmol) in THF (4.6 mL) under Ar. After 2 hours the reaction mixture was diluted with Et_2O, washed with aqueous saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated. The resulting diol (0.1631 g,

0.125 mmol, 99 %) was used in the next step without purification. Benzaldehyde dimethyl acetal (23 µL, 0.15 mmol) was added to a stirred solution of diol (0.1631 g, 0.125 mmol) and CSA (3.2 mg, 14 µmol) in MeCN (3.3 mL). After 30 minutes CSA (2.2 mg, 9.5 µmol) and benzaldehyde dimethyl acetal (22 µL, 0.15 mmol) were added and the reaction mixture was stirred for an additional 30 minutes monitoring by LCMS and TLC until starting material was consumed. The reaction was quenched with Et_3N, diluted with Et_2O, and washed with aqueous saturated $NaHCO_3$ solution and brine. The organic layer was concentrated and the resulting residue was purified using silica gel column chromatography in 20 % EtOAc in hexanes to give the acetal **21** (0.1004 g, 0.0723 mmol) in 60 % yield as a white foam. $[\alpha]_D^{23}$ = 66.05 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, C_6D_6) δ 7.64 – 7.59 (m, 2H, Ar-H), 7.52 – 7.49 (m, 2H, Ar-H), 7.46 – 7.42 (m, 2H, Ar-H), 7.32 – 7.28 (m, 4H, Ar-H), 7.20 (t, *J* = 7.8 Hz, 2H, Ar-H), 7.18 – 6.96 (m, 23H, Ar-H), 6.41 (d, *J* = 3.8 Hz, 1H, H-1'), 5.98 (d, *J* = 5.5 Hz, 1H, H-1''), 5.71 (s, 1H, $PhCH(O)_2$), 5.06 (d, *J* = 11.5 Hz, 1H, $PhCH_2O$), 5.00 (d, *J* = 2.1 Hz, 1H, H-1'''), 4.96 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.90 (d, *J* = 11.5 Hz, 1H, $PhCH_2O$), 4.67 (dd, *J* = 10.3, 5.9 Hz, 1H, H-5'), 4.63 (q, *J* = 6.5 Hz, 1H, H-6'), 4.60 – 4.58 (m, 2H, H-4'', $PhCH_2O$), 4.50 (dd, *J* = 4.8, 2.7 Hz, 1H, H-3''), 4.45 – 4.37 (m, 4H, H-3', $PhCH_2O$), 4.31 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.29 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.14 (t, *J* = 5.2 Hz, 1H, H-2''), 4.08 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.00 – 3.97 (m, 2H, $PhCH_2O$), 3.95 (dd, *J* = 10.5, 2.3 Hz, 1H, H-5''), 3.83 – 3.77 (m, 2H, H-5, H-4'), 3.75 (ddd, *J* = 8.4, 4.1, 2.0 Hz, 1H, H-5'''), 3.67 (t, *J* = 3.1 Hz, 1H, H-3'''), 3.59 (dd, *J* = 10.5, 3.1 Hz, 1H, H-5''), 3.56 (t, *J* = 9.3 Hz, 1H, H-4), 3.43 (dd, *J* = 12.8, 8.4 Hz, 1H, H-6'''), 3.35 (t, *J* = 2.8 Hz, 1H, H-2'''), 3.13 (dd, *J* = 10.3, 3.8 Hz, 1H, H-2'), 2.96 (t, *J* = 2.6 Hz, 1H, H-4'''), 2.85 (t, *J* = 9.5 Hz, 1H, H-6), 2.78 – 2.68 (m, 2H, H-3, H-6'''), 2.54 (ddd, *J* = 12.3, 9.7, 4.5 Hz, 1H, H-1), 1.36 (dt, *J* = 12.9, 4.6 Hz, 1H, H-2eq), 1.26 (d, *J* = 6.8 Hz, 3H, H-7'), 0.82 (q, *J* = 12.6 Hz, 1H, H-2ax). ^{13}C NMR

(151 MHz, C_6D_6) δ 138.7, 138.6, 138.44, 138.35, 137.9, 137.4, 137.3, 128.44, 128.35, 128.33, 128.24, 128.22, 128.18, 128.17, 128.15, 128.01, 128.00, 127.98, 127.90, 127.88, 127.6, 127.3, 127.2, 126.4 (Ar), 106.3 (C-1''), 98.8 (C-1'''), 97.3 (C-1'), 94.0 ($PhCH(O)_2$), 83.9 (C-6), 82.6 (C-2''), 82.5 (C-4''), 81.8 (C-5), 76.3 (C-4'), 76.3 (C-3'), 75.9 (C-3''), 75.5 (C-4), 74.94 ($PhCH_2O$), 74.85 ($PhCH_2O$), 74.2 (C-5'''), 73.5 (C-3'''), 73.1 ($PhCH_2O$), 72.9 ($PhCH_2O$), 72.4 (C-4'''), 72.2 ($PhCH_2O$), 71.6 ($PhCH_2O$), 70.4 (C-5''), 70.3 (C-6'), 65.3 (C-5'), 62.8 (C-2'), 60.0 (C-1), 59.9 (C-3), 56.7 (C-2'''), 51.0 (C-6'''), 31.8 (C-2), 11.1 (C-7'). ESI-HRMS: *m/z* calc for $C_{73}H_{77}N_{15}O_{14}Na$ $[M+Na]^+$ 1410.5672, found 1410.5674.

1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-*C*-dimethyl-4'-*O*-trimethylsilyl-1,3,2',2''',6'''-pentadeaminoparomomycin (23). 0.1 mL of 3M MeMgCl in THF were added to a stirred solution of compound **19** (0.152 g, 0.111 mmol) in 1.1 mL of THF at -30°C. The reaction mixture was stirred for 30 minutes before quenching with 0.5 mL of NH_4Cl solution. The reaction mixture was then diluted with Et_2O then washed with NH_4Cl solution and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified over silica gel in 20% EtOAc in hexanes to give 0.120 g (0.087 mmol, 78%) of compound **23** as a white foam. $[\alpha]_D^{23}$ = 102.28 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.15 (m, 30H, Ar-H), 6.10 (d, *J* = 3.9 Hz, 1H, H-1'), 5.67 (d, *J* = 5.8 Hz, 1H, H-1''), 4.97 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.96 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.94 (d, *J* = 12.2 Hz, 1H, $PhCH_2O$), 4.75 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.64 (d, *J* = 11.5 Hz, 1H, $PhCH_2O$), 4.63 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.58 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.49 – 4.41 (m, 4H, $PhCH_2O$), 4.37 – 4.31 (m, 2H, H-3'', $PhCH_2O$), 4.28 (q, *J* = 2.7 Hz, 1H, H-4''), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 3.97 – 3.91 (m, 2H, H-5, H-2''), 3.85 – 3.76 (m, 4H, H-3',

H-3'', H-5'', H-5'''), 3.74 (d, *J* = 9.6 Hz, 1H, H-5'), 3.70 (dd, *J* = 13.0, 8.7 Hz, 1H, H-6'''), 3.64 (dd, *J* = 9.8, 8.9 Hz, 1H, H-4), 3.58 – 3.54 (m, 2H, H-5'', OH), 3.50 – 3.42 (m, 2H, H-1, H-3), 3.41 – 3.37 (m, 2H, H-4', H-2'''), 3.28 (t, *J* = 9.4 Hz, 1H, H-6), 3.14 – 3.11 (m, 1H, H-4'''), 2.86 (dd, *J* = 13.1, 3.6 Hz, 1H, H-6'''), 2.70 (dd, *J* = 10.0, 3.9 Hz, 1H, H-2'), 2.24 (dt, *J* = 13.3, 4.6 Hz, 1H, H-2eq), 1.38 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.26 (s, 3H, -CH_3), 1.19 (s, 3H, -CH_3), 0.08 (s, 9H, -OTMS). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.4, 138.3, 137.9, 137.6, 137.0, 136.9, 128.7, 128.5, 128.42, 128.38, 128.34, 128.31, 128.2, 128.14, 127.8, 127.68, 127.56, 127.45, 127.2, 127.1, 126.9 (Ar), 106.1 (C-1''), 98.6 (C-1'''), 95.6 (C-1'), 84.4 (C-6), 82.2 (C-2''), 82.0 (C-4''), 81.8 (C-5), 79.7 (C-3'), 75.3 (C-3''), 75.2 ($PhCH_2O$), 74.9 (C-4), 74.8 (C-5'), 74.6 (C-5'''), 73.6 ($PhCH_2O$), 73.4 (C-4'), 73.1 ($PhCH_2O$), 73.0 ($PhCH_2O$), 72.8 (C-3'''), 72.4 ($PhCH_2O$), 72.0 (C-6'), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.4 (C-5''), 63.5 (C-2'), 60.5 (C-1), 60.2 (C-3), 57.2 (C-2'''), 51.2 (C-6'''), 32.7 (C-2), 27.0 (-CH_3), 24.7 (-CH_3), 0.9 (-OTMS). ESI-HRMS: *m/z* calc for $C_{70}H_{83}N_{15}O_{14}SiNa$ $[M+Na]^+$ 1408.5911, found 1408.5900.

6',6'-*C*-dimethyl-paromomycin (24). A 1M TBAF solution in THF (0.13 mL) was added dropwise to a stirred solution of compound **23** (0.0564 mg, 0.0407 mmol) in 1.6 mL of THF under argon. When the starting material was no longer visible by TLC, the reaction mixture was diluted with Et_2O and washed with of $NaHCO_3$ solution and brine. The organic layer was then dried with Na_2SO_4, filtered, and concentrated to give the intermediate alcohol which was used without further purification. The crude alcohol was stirred in 0.6 mL of 1:1 dioxane/10% AcOH in water with 107.0 mg of Pd/C under 50 psi of H_2 for 21 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified over a CM Sephadex C-25 column. The column was washed

with 100 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization of the pure fractions with AcOH gave 20.4 mg (0.022 mmol) of the penta acetate salt **24** as a white solid in 54% yield. $[\alpha]_D^{23}$ = 35.00 (*c* = 1.0, H_2O), ^1H NMR (600 MHz, D_2O) δ 5.59 (d, *J* = 3.9 Hz, 1H, H-1'), 5.23 (d, *J* = 2.9 Hz, 1H, H-1''), 5.15 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.36 (t, *J* = 5.7 Hz, 1H, H-3''), 4.20 (dd, *J* = 5.1, 3.0 Hz, 1H, H-2''), 4.17 (td, *J* = 4.8, 3.9, 2.3 Hz, 1H, H-5'''), 4.09 (t, *J* = 3.2 Hz, 1H, H-3'''), 4.08 – 4.06 (m, 1H, H-4''), 3.77 (dd, *J* = 12.4, 3.1 Hz, 1H, H-5''), 3.74 – 3.65 (m, 4H, H-4, H-5, H-3', H-4'''), 3.62 (dd, *J* = 12.4, 4.9 Hz, 1H, H-5''), 3.54 – 3.49 (m, 2H, H-6, H-5'), 3.44 (br. s, 1H, H-2'''), 3.40 (t, *J* = 9.3 Hz, 1H, H-4'), 3.29 (dd, *J* = 13.7, 6.6 Hz, 1H, H-6'''), 3.26 – 3.21 (m, 2H, H-2', H-6'''), 3.21 – 3.12 (m, 2H, H-1, H-3), 2.21 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2eq), 1.77 (s, 15H, AcOH), 1.56 (q, *J* = 12.6 Hz, 1H, H-2ax), 1.20 (s, 3H, $-CH_3$), 1.14 (s, 3H, $-CH_3$). ^{13}C NMR (151 MHz, D_2O) δ 181.2 (AcOH), 109.8 (C-1''), 95.9 (C-1'), 95.5 (C-1'''), 84.4 (C-5), 81.4 (C-4''), 79.7 (C-4), 77.2 (C-5'), 75.4 (C-3''), 73.3 (C-2''), 72.6 (C-6), 72.3 (C-6'), 70.7 (C-4'), 70.2 (C-5'''), 69.7 (C-3'), 67.6 (C-3'''), 67.2 (C-4'''), 60.2 (C-5''), 53.9 (C-2'), 50.8 (C-2'''), 49.9 (C-1), 49.2 (C-3), 40.3 (C-6'''), 29.8 (C-2), 26.3 ($-CH_3$), 23.3 ($-CH_3$), 23.1 (AcOH). ESI-HRMS: *m/z* calc for $C_{25}H_{50}N_5O_{14}$ $[M+H]^+$ 644.3354, found 644.3358.

1,3,2',2''',6'''-pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-*O*-triisopropylsilyl-1,3,2',2''',6'''-pentadeaminoparomomycin (25). TIPSOTf (1.25 mL, 4.7 mmol) was added to a stirred solution of **16** (5.06 g, 3.93 mmol) and lutidine (2.3 mL, 19.7 mmol) in DCM (79 mL) under argon. The reaction mixture was stirred for 1 hour monitoring by TLC and LCMS then quenched with methanol and concentrated under vacuum. The crude residue was dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried with

Na_2SO_4, filtered, and concentrated. The crude product was purified over silica gel eluting with 18-20% EtOAc in hexanes to give **25** (4.64 g, 3.22 mmol) as a white foam in 82% yield. $[\alpha]_D^{23}$ = 68.1 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.11 (m, 30H, Ar-H), 6.14 (d, *J* = 3.7 Hz, 1H, H-1'), 5.67 (d, *J* = 5.8 Hz, 1H, H-1''), 4.97 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.87 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.85 (s, 2H, $PhCH_2O$), 4.69 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.62 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.59 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.52 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.45 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.43 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.40 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.30 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.29 – 4.27 (m, 1H, H-4''), 4.26 – 4.22 (m, 2H, H-3'', $PhCH_2O$), 4.01 (dt, *J* = 9.6, 5.7 Hz, 1H, H-5'), 3.98 – 3.91 (m, 4H, H-5, H-3', H-6', H-2''), 3.86 (dd, *J* = 10.1, 5.9 Hz, 1H, H-6'), 3.80 (dd, *J* = 10.4, 2.3 Hz, 1H, H-5''), 3.77 – 3.73 (m, 2H, H-3''', H-5'''), 3.70 (dd, *J* = 9.8, 8.9 Hz, 1H, H-4), 3.63 (dd, *J* = 13.0, 8.6 Hz, 1H, H-6'''), 3.56 (dd, *J* = 10.4, 3.2 Hz, 1H, H-5''), 3.50 – 3.40 (m, 3H, H-1, H-3, H-4'), 3.34 (t, *J* = 2.6 Hz, 1H, H-2'''), 3.26 (t, *J* = 9.4 Hz, 1H, H-6), 3.11 (t, *J* = 2.5 Hz, 1H, H-4'''), 3.03 (s, 1H, 4'-OH), 2.96 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2'), 2.87 (dd, *J* = 13.0, 3.9 Hz, 1H, H-6'''), 2.23 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.36 (q, *J* = 12.8 Hz, 1H, H-2ax), 1.19 – 1.12 (m, 3H, TIPS-CH), 1.12 – 1.08 (m, 18H, TIPS-CH_3). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.34, 138.29, 137.9, 137.6, 137.0, 136.9, 128.7, 128.50, 128.47, 128.42, 128.34, 128.32, 128.29, 128.24, 128.18, 127.83, 127.80, 127.77, 127.75, 127.5, 127.4, 127.3 (Ar), 106.0 (C-1''), 98.6 (C-1'''), 95.7 (C-1'), 84.3 (C-6), 82.5 (C-2''), 82.1 (C-4''), 81.9 (C-5), 79.4 (C-3'), 75.5 (C-3''), 75.0 ($PhCH_2O$), 74.6 (C-4), 74.3 (C-5'''), 74.2 (C-4'), 73.3 ($PhCH_2O$), 73.2 ($PhCH_2O$), 72.8 (C-3'''), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.8 (C-5'), 70.2 (C-5''), 65.6 (C-6'), 62.5 (C-2'), 60.3 (C-1), 60.1 (C-3), 57.3 (C-2'''), 51.1 (C-6'''), 32.6 (C-2), 18.0 (OTIPS-CH_3), 18.0 (OTIPS-CH_3), 11.8 (OTIPS-CH). ESI-HRMS: m/z calcd for $C_{74}H_{95}N_{16}O_{14}Si$ [M + NH_4]$^+$ 1459.6983, found 1459.7007.

1,3,2',2''',6''',-pentaazido-6,3',2'',5'',3''',4''',-hexa-*O*-benzyl-4'-*O*-(4-methoxybenzyl)-6'-*O*-triisopropylsilyl-1,3,2',2''',6''',-pentadeaminoparomomycin (26). NaH (0.2372g, 9.88 mmol) and TBAI (0.1468 g, .40 mmol) were added to a stirred solution of compound **25** (5.61 g, 3.89 mmol) in DMF (33 mL) at 0 ^{o}C. After 20 minutes PMBCl (1.6 mL, 11.70 mmol) was added and the reaction mixture was warmed to rt. After 1.5 hours the reaction was quenched with 2 mL of saturated NH_4Cl solution, diluted with EtOAc and washed with saturated NH_4Cl solution, water, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified over silica gel to give compound **26** (5.39g, 89%) as a white foam. $[\alpha]_D^{23}$ = 64.8 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.12 (m, 32H, Ar-H), 6.87 – 6.84 (m, 2H, Ar-H), 6.12 (d, *J* = 3.7 Hz, 1H, H-1'), 5.65 (d, *J* = 5.9 Hz, 1H, H-1''), 4.94 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.88 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.86 – 4.82 (m, 2H, $PhCH_2O$), 4.77 (d, *J* = 11.1 Hz, 1H, $PhCH_2O$), 4.66 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.63 – 4.60 (m, 2H, $PhCH_2O$), 4.58 – 4.54 (m, 2H, $PhCH_2O$), 4.46 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.44 – 4.39 (m, 2H, $PhCH_2O$), 4.31 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.26 (q, *J* = 2.8 Hz, 1H, H-4''), 4.25 – 4.21 (m, 2H, H-3'', $PhCH_2O$), 4.08 (dd, *J* = 10.4, 9.0 Hz, 1H, H-3'), 3.99 – 3.90 (m, 3H, H-5, H-5', H-2''), 3.86 (dd, *J* = 11.0, 1.7 Hz, 1H, H-6'), 3.80 (s, 3H, $-OCH_3$), 3.79 – 3.72 (m, 4H, H-4, H-5'', H-3''', H-5'''), 3.69 (dd, *J* = 10.9, 5.7 Hz, 1H, H-6'), 3.60 (dd, *J* = 12.9, 8.4 Hz, 1H, H-6'''), 3.55 (dd, *J* = 10.5, 3.3 Hz, 1H, H-5''), 3.48 – 3.37 (m, 2H, H-1, H-3), 3.37 – 3.32 (m, 2H, H-4', H-2'''), 3.24 (t, *J* = 9.3 Hz, 1H, H-6), 3.11 (t, *J* = 2.5 Hz, 1H, H-4'''), 3.05 (dd, *J* = 10.4, 3.7 Hz, 1H, H-2'), 2.88 (dd, *J* = 12.9, 4.2 Hz, 1H, H-6'''), 2.20 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.35 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.08 (d, *J* = 4.5 Hz, 21H, OTIPS). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.2, 138.3, 138.1, 137.9, 137.7, 137.03, 136.97, 130.5, 129.5, 128.7, 128.5, 128.41, 128.39, 128.35, 128.33, 128.31, 128.22, 128.20, 128.16, 127.82, 127.76, 127.73, 127.5, 127.4, 113.8 (Ar), 105.9 (C-1''), 98.6 (C-

1’’’), 95.5 (C-1’), 84.2 (C-6), 82.6 (C-2’’), 82.0 (C-4’’), 81.7 (C-5), 80.3 (C-3’), 77.9 (C-4’), 75.6 (C-3’’), 75.4 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.4 (C-5’’’), 74.3 (C-4), 74.2 ($PhCH_2O$), 73.3 ($PhCH_2O$), 73.2 ($PhCH_2O$), 72.9 (C-3’’’), 72.7 (C-5’), 72.3 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.4 (C-4’’’), 70.0 (C-5’’), 63.5 (C-2’), 63.0 (C-6’), 60.3 (C-1), 60.0 (C-3), 57.3 (C-2’’’), 55.2 (OCH_3), 51.0 (C-6’’’), 32.5 (C-2), 18.12 (OTIPS-CH_3), 18.10 (OTIPS-CH_3), 12.0 (OTIPS-CH). ESI-HRMS: m/z calcd for $C_{82}H_{99}N_{15}O_{15}SiNa$ $[M + Na]^+$ 1584.7112, found 1584.7095.

1,3,2’,2’’’,6’’’-pentaazido-6,3’,2’’,5’’,3’’’,4’’’-hexa-*O*-benzyl-4’-*O*-(4-methoxybenzyl)-1,3,2’,2’’’,6’’’-pentadeaminoparomomycin (27). A 1M solution of TBAF in THF (7.5 mL) was added to a stirred solution of **26** (3.92 g, 2.51 mmol) in THF (43 mL) and the reaction mixture was stirred under argon for 3 hours with monitoring by TLC. After completion, the reaction mixture was concentrated under vacuum and the residue was dissolved in ethyl acetate and washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under vacuum. Purification over silica gel eluting with 20-30% EtOAc in Hexanes gave the product **27** (3.1 g, 2.20 mmol) in 88% yield as a white foam. $[\alpha]_D^{23}$ = 79.10 (*c* = 1.0, $CHCl_3$), ^1H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.13 (m, 32H, Ar-H), 6.86 – 6.83 (m, 2H, Ar-H), 6.13 (d, *J* = 3.7 Hz, 1H, H-1’), 5.67 (d, *J* = 5.6 Hz, 1H, H-1’’), 4.97 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.89 (d, *J* = 1.9 Hz, 1H, H-1’’’), 4.83 (s, 2H, $PhCH_2O$), 4.75 (d, *J* = 10.9 Hz, 1H, $PhCH_2O$), 4.71 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.62 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.57 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.54 (d, *J* = 11.0 Hz, 1H, $PhCH_2O$), 4.51 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.49 – 4.43 (m, 2H, $PhCH_2O$), 4.40 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.34 – 4.29 (m, 3H, H-3’’, H-4’’, $PhCH_2O$), 4.24 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.02 (dd, *J* = 10.3, 9.0 Hz, 1H, H-3’), 3.99 (dd, *J* = 5.7, 4.5 Hz, 1H, H-2’’), 3.94 (t, *J* = 8.8 Hz, 1H, H-5), 3.90

(dt, *J* = 10.2, 3.1 Hz, 1H, H-5'), 3.82 (dd, *J* = 10.5, 2.1 Hz, 1H, H-5''), 3.80 – 3.77 (m, 4H, H-5''', -OCH_3), 3.76 (t, *J* = 2.8 Hz, 1H, H-3'''), 3.75 – 3.72 (m, 1H, H-6'), 3.66 (dd, *J* = 13.0, 8.6 Hz, 1H, H-6'''), 3.63 – 3.56 (m, 3H, H-4, H-6', H-5''), 3.46 – 3.39 (m, 2H, H-1, H-3), 3.37 – 3.33 (m, 2H, H-4', H-2'''), 3.29 (t, *J* = 9.3 Hz, 1H, H-6), 3.11 (t, *J* = 2.5 Hz, 1H, H-4'''), 2.94 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2'), 2.86 (dd, *J* = 13.0, 3.7 Hz, 1H, H-6'''), 2.22 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.39 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.2, 138.3, 138.0, 137.9, 137.5, 137.0, 136.9, 130.3, 129.4, 128.7, 128.5, 128.42, 128.40, 128.34, 128.29, 128.26, 128.20, 128.1, 127.83, 127.79, 127.75, 127.70, 127.6, 127.5, 127.1, 113.8 (Ar), 106.2 (C-1''), 98.6 (C-1'''), 95.7 (C-1'), 84.2 (C-6), 82.5 (C-2''), 82.1 (C-4''), 82.0 (C-5), 79.8 (C-3'), 77.4 (C-4'), 75.5 (C-3''), 75.3 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.9 (C-4), 74.4 (C-5'''), 73.2 ($PhCH_2O$), 72.8 (C-3'''), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.6 (C-5'), 71.4 (C-4'''), 70.3 (C-5''), 63.2 (C-2'), 61.6 (C-6'), 60.3 (C-1), 60.0 (C-3), 57.3 (C-2'''), 55.3 (-OCH_3), 51.1 (C-6'''), 32.4 (C-2). ESI-HRMS: m/z calcd for $C_{73}H_{79}N_{15}O_{15}Na$ $[M + Na]^+$ 1428.5778, found 1428.5724.

1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-*C*-ethyl-1,3,2',2''',6'''-pentadeaminoparomomycin (28(*R*) and 28(*S*)). Oxalyl chloride (0.125 mL, 1.42 mmol) was added to a stirred solution of DMSO (0.21 mL, 2.96 mmol) in DCM (7.1 mL) at -78°C under argon. After 15 minutes compound **27** (1.0011 g, 0.712 mmol) was dissolved in DCM (3 mL) and added to the cold reaction mixture dropwise. The vial containing **27** was rinsed twice with DCM (1.5 mL) to ensure complete transfer. After 1 hour triethylamine (0.44 mL, 3.16 mmol) was added and the reaction mixture was allowed to slowly warm to room temperature before dilution with ether and washing with saturated NH_4Cl solution, DI water, and brine. The organic layer was dried with

Na_2SO_4, filtered, and concentrated to give the intermediate aldehyde as a white foam (0.9823 g, 0.699 mmol) in 98% yield which was used in the next step without purification. Freshly prepared EtMgBr 1M solution (1.4 mL) was added to a stirred solution of aldehyde (0.470 g, 0.335 mmol) in THF (6.7 mL) at -78 °C. After 1 hour the reaction was quenched with 1 mL aqueous saturated NH_4Cl solution, diluted with Et_2O, washed with NH_4Cl solution and brine, dried with Na_2SO_4, and concentrated. The crude residue was then purified using silica gel column chromatography eluting with 25% EtOAc in Hexanes to give the intermediate alcohols (0.303 g, 0.211 mmol, 63%) as an inseparable mixture of diastereomers which were used without further purification. ESI-HRMS: m/z calcd for $C_{75}H_{83}N_{15}O_{14}Na$ $[M + Na]^+$ 1456.6091, found 1456.6062. TFA (0.33 mL) was added to a stirred solution of the alcohols (0.283g, 0.197 mmol) in DCM (3 mL) at 0 °C. After 1 hour the reaction mixture was diluted with Et_2O and washed with aqueous saturated $NaHCO_3$ solution and brine. The organic layer was dried with Na_2SO_4 and concentrated followed by purification using silica gel column chromatography eluting with 28% EtOAc in Hexanes to give **28(*R*)** (23.9 mg, 0.018) in 9% isolated yield, **28(*S*)** (90.3 mg, 0.069) in 35% isolated yield, as well as a mixture of **28(*R*)** and **28(*S*)** (91.5 mg, 0.070 mmol) in 35% yield. **28(*R*)** $[\alpha]_D^{23}$ = 88.00 (*c* = 0.5, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.12 (m, 30H, Ar-H), 6.14 (d, *J* = 3.7 Hz, 1H, H-1'), 5.67 (d, *J* = 5.7 Hz, 1H, H-1''), 4.97 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.91 – 4.87 (m, 2H, H-1''', $PhCH_2O$), 4.72 – 4.69 (m, 2H, $PhCH_2O$), 4.62 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.56 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.48 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.46 – 4.42 (m, 2H, $PhCH_2O$), 4.40 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.32 – 4.27 (m, 3H, H-3'', H-4'', $PhCH_2O$), 4.24 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 3.97 – 3.93 (m, 2H, H-5, H-2''), 3.88 (dd, *J* = 10.3, 8.6 Hz, 1H, H-3'), 3.81 (dd, *J* = 10.3, 1.9 Hz, 1H, H-5''), 3.79 – 3.74 (m, 3H, H-5', H-3''', H-5'''), 3.68 – 3.62 (m, 2H, H-6', H-6'''), 3.62 – 3.55 (m, 2H, H-4, H-5''), 3.48 – 3.41

(m, 3H, H-1, H-3, H-4'), 3.35 (t, *J* = 2.6 Hz, 1H, H-2'''), 3.28 (t, *J* = 9.3 Hz, 1H, H-6), 3.12 (t, *J* = 2.6 Hz, 1H, H-4'''), 2.89 – 2.85 (m, 2H, H-2', H-6'''), 2.24 (dt, *J* = 13.3, 4.6 Hz, 1H, H-2eq), 1.76 (dqd, *J* = 14.4, 7.5, 3.5 Hz, 1H, H-7'), 1.49 (ddd, *J* = 14.4, 8.6, 7.1 Hz, 1H, H-7'), 1.38 (q, *J* = 12.8 Hz, 1H, H-2ax), 1.02 (t, *J* = 7.4 Hz, 3H, H-8'). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 138.2, 138.1, 137.9, 137.5, 137.0, 136.9, 128.7, 128.6, 128.5, 128.4, 128.33, 128.32, 128.28, 128.26, 128.18, 128.15, 128.0, 127.80, 127.78, 127.76, 127.72, 127.5, 127.4, 127.2 (Ar), 106.1 (C-1''), 98.6 (C-1'''), 95.6 (C-1'), 84.3 (C-6), 82.4 (C-2''), 82.1 (C-4''), 81.9 (C-5), 79.6 (C-3'), 75.5 (C-3''), 75.4 (C-6'), 75.1 ($PhCH_2O$), 75.04 ($PhCH_2O$), 75.01 (C-4), 74.4 (C-5'''), 73.7 (C-4'), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.9 (C-3'''), 72.4 ($PhCH_2O$), 72.1 (C-5'), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.3 (C-5''), 62.5 (C-2'), 60.4 (C-1), 60.3 (C-3), 57.2 (C-2'''), 51.1 (C-6'''), 32.6 (C-2), 25.6 (C-7'), 9.8 (C-8'). ESI-HRMS: m/z calcd for $C_{67}H_{75}N_{15}O_{14}Na$ $[M + Na]^+$ 1336.5516, found 1336.5537. **28(*S*)** $[\alpha]_D^{23}$ = 74.30 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.15 (m, 30H, Ar-H), 6.17 (d, *J* = 3.6 Hz, 1H, H-1'), 5.70 (d, *J* = 5.9 Hz, 1H, H-1''), 5.01 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.94 – 4.90 (m, 2H, $PhCH_2O$), 4.72 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.68 (d, *J* = 11.4 Hz, 1H, $PhCH_2O$), 4.63 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.59 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.49 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.47 (s, 2H, $PhCH_2O$), 4.41 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.34 – 4.30 (m, 3H, H-3'', H-4'', $PhCH_2O$), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.00 (dd, *J* = 6.0, 4.6 Hz, 1H, H-2''), 3.96 (t, *J* = 8.9 Hz, 1H, H-5), 3.88 (dd, *J* = 10.3, 8.9 Hz, 1H, H-3'), 3.84 (dd, *J* = 10.4, 2.2 Hz, 1H, H-5''), 3.80 (ddd, *J* = 8.7, 3.8, 1.9 Hz, 1H, H-5'''), 3.77 (t, *J* = 2.9 Hz, 1H, H-3'''), 3.73 (dd, *J* = 9.8, 1.4 Hz, 1H, H-5'), 3.70 – 3.64 (m, 2H, H-6', H-6'''), 3.59 (dd, *J* = 10.4, 2.7 Hz, 1H, H-5''), 3.54 (t, *J* = 9.3 Hz, 1H, H-4), 3.52 – 3.49 (m, 1H, H-4'), 3.49 – 3.41 (m, 2H, H-1, H-3), 3.37 (t, *J* = 2.6 Hz, 1H, H-2'''), 3.29 (t, *J* = 9.4 Hz, 1H, H-6), 3.14 – 3.11 (m, 1H, H-4'''), 2.87 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6'''), 2.82 (dd, *J* = 10.3, 3.7 Hz, 1H, H-2'), 2.25 – 2.20 (m, 2H, H-2eq, -OH), 1.65 – 1.56

(m, 2H, H-7', -OH), 1.52 (dqd, *J* = 14.7, 7.5, 4.7 Hz, 1H, H-7'), 1.34 (q, *J* = 12.8 Hz, 1H, H-2ax), 1.02 (t, *J* = 7.4 Hz, 3H, H-8'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.3, 138.1, 137.9, 137.5, 137.0, 136.9, 128.7, 128.6, 128.5, 128.41, 128.36, 128.33, 128.26, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.0 (Ar), 106.1 (C-1''), 98.7 (C-1'''), 95.9 (C-1'), 84.4 (C-6), 82.5 (C-2''), 82.2 (C-4''), 82.0 (C-5), 79.8 (C-3'), 75.5 (C-3''), 75.1 ($PhCH_2O$), 74.9 ($PhCH_2O$), 74.8 (C-4), 74.5 (C-5'''), 73.1 ($PhCH_2O$), 72.9 (C-3'''), 72.7 (C-5'), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.7 (C-6'), 70.2 (C-5''), 70.0 (C-4'), 62.5 (C-2'), 60.4 (C-1), 60.3 (C-3), 57.2 (C-2'''), 51.2 (C-6'''), 32.7 (C-2), 27.0 (C-7'), 10.5 (C-8'). ESI-HRMS: m/z calcd for $C_{67}H_{75}N_{15}O_{14}Na$ $[M + Na]^+$ 1336.5516, found 1336.5549.

6'-(*R*)-*C*-ethyl-paromomycin (29(*R*)). Compound **28(*R*)** (18.8 mg, 0.0143 mmol) was added to a 16 mm test tube followed by 0.2 mL of dioxane and 0.2 mL of 10% AcOH in water. 38 mg of Pd/C were added to the tube and the reaction mixture was subjected to 48 psi H_2 for 48 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified using a CM Sephadex C-25 column. The column was washed with 50 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization with AcOH gave the acetate salt **29(*R*)** (2.3 mg, 0.0024 mmol) in 34% yield as a white powder. $[\alpha]_D^{23}$ = 57.61 (*c* = 0.1, H_2O), ^{1}H NMR (600 MHz, D_2O) δ 5.53 (d, *J* = 4.0 Hz, 1H, H-1'), 5.24 (d, *J* = 2.8 Hz, 1H, H-1''), 5.16 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.38 (t, 1H, H-3''), 4.22 (dd, *J* = 5.1, 2.9 Hz, 1H, H-2''), 4.20 – 4.16 (m, 1H, H-5'''), 4.10 (t, *J* = 3.1 Hz, 1H, H-3'''), 4.09 – 4.06 (m, 1H, H-4''), 3.81 – 3.68 (m, 7H, H-4, H-5, H-3', H-5' [$J_{5',6'}$ = 2.5 Hz extracted from HSQC], H-6', H-5'', H-4'''), 3.64 (dd, *J* = 12.4, 4.6 Hz, 1H, H-5''), 3.54 (t, *J* = 9.5 Hz, 1H, H-6), 3.46 (t, *J* = 2.3 Hz, 1H, H-2'''), 3.39 (t,

J = 9.5 Hz, 1H, H-4'), 3.36 – 3.15 (m, 5H, H-1, H-3, H-2', H-6''', H-6'''), 2.29 (dt, *J* = 13.2, 4.1 Hz, 1H, H-2eq), 1.80 (s, 15H, AcOH), 1.65 (q, *J* = 12.5 Hz, 1H, H-2ax), 1.54 – 1.44 (m, 1H, H-7'), 1.43 – 1.33 (m, 1H, H-7'), 0.84 (t, *J* = 7.4 Hz, 3H, H-8'). ^{13}C NMR (151 MHz, D_2O) δ 180.5 (AcOH), 109.7 (C-1''), 96.5 (C-1'), 95.4 (C-1'''), 84.1 (C-5), 81.3 (C-4''), 79.2 (C-4), 75.6 (C-5'), 75.2 (C-3''), 73.4 (C-2''), 72.5 (C-6), 71.1 (C-6'), 70.1 (C-5'''), 69.7 (C-4'), 69.5 (C-3'), 67.6 (C-3'''), 67.2 (C-4'''), 60.0 (C-5''), 53.8 (C-2'), 50.8 (C-2'''), 49.7 (C-1), 49.0 (C-3), 40.4 (C-6'''), 28.9 (C-2), 22.7 (AcOH), 22.2 (C-7'), 9.8 (C-8'). ESI-HRMS: m/z calcd for $C_{25}H_{49}N_5O_{14}$ $[M + H]^+$ 644.3354, found 644.3358.

6'-(*S*)-*C*-ethyl-paromomycin (29(*S*)). Compound **28(*S*)** (38.1 mg, 0.029 mmol) was added to a 16 mm test tube followed by 0.4 mL of dioxane and 0.4 mL of 10% AcOH in water. 77.5 mg of Pd/C were added to the tube and the reaction mixture was subjected to 50 psi H_2 for 48 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified using a CM Sephadex C-25 column. The column was washed with 250 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization with AcOH gave the acetate salt **29(*S*)** (9.5 mg, 0.010 mmol) in 35% yield as a white powder. $[\alpha]_D^{23}$ = 41.32 (*c* = 0.4, H_2O), ^{1}H NMR (600 MHz, D_2O) δ 5.65 (d, *J* = 3.8 Hz, 1H, H-1'), 5.26 (d, *J* = 2.6 Hz, 1H, H-1''), 5.16 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.39 (dd, *J* = 6.6, 5.0 Hz, 1H, H-3''), 4.23 (dd, *J* = 5.0, 2.6 Hz, 1H, H-2''), 4.20 – 4.16 (m, 1H, H-5'''), 4.10 (t, *J* = 3.1 Hz, 1H, H-3'''), 4.08 (ddd, *J* = 7.1, 4.5, 3.2 Hz, 1H, H-4''), 3.80 – 3.70 (m, 5H, H-4, H-5, H-3', H-6' [1dTOCSY 3.73, dd, *J* = 9.9, 3.9 Hz], H-5''), 3.69 – 3.68 (m, 1H, H-4'''), 3.64 (dd, *J* = 12.4, 4.7 Hz, 1H, H-5''), 3.57 – 3.50 (m, 3H, H-6, H-4', H-5'), 3.46 – 3.45 (m, 1H, H-2'''), 3.29 (dd, *J* = 13.7, 6.7 Hz, 1H, H-6'''), 3.26 – 3.15 (m, 4H, H-1, H-3, H-2', H-

6’’’), 2.25 (dt, *J* = 12.1, 3.5 Hz, 1H, H-2eq), 1.81 (s, 15H, AcOH), 1.62 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.52 (ddq, *J* = 14.6, 9.3, 7.3 Hz, 1H, H-7’), 1.38 (dqd, *J* = 14.6, 7.3, 3.7 Hz, 1H, H-7’), 0.83 (t, *J* = 7.5 Hz, 3H, H-8’). ^{13}C NMR (151 MHz, D_2O) δ 180.3 (AcOH), 109.7 (C-1’’), 95.8 (C-1’), 95.3 (C-1’’’), 84.2 (C-5), 81.3 (C-4’’), 78.8 (C-4), 75.23 (C-5’), 75.19 (C-3’’), 73.4 (C-2’’), 72.4 (C-6), 70.1 (C-5’’’), 69.7 (C-6’), 69.2 (C-4’), 68.9 (C-3’), 67.6 (C-3’’’), 67.2 (C-4’’’), 60.0 (C-5’’), 53.8 (C-2’), 50.8 (C-2’’’), 49.9 (C-1), 49.2 (C-3), 40.3 (C-6’’’), 29.1 (C-2), 26.1 (C-7’), 22.6 (AcOH), 9.9 (C-8’). ESI-HRMS: m/z calcd for $C_{25}H_{49}N_5O_{14}$ $[M + H]^+$ 644.3354, found 644.3369.

1,3,2’,2’’’,6’’’-Pentaazido-6,3’,2’’,5’’,3’’’,4’’’-hexa-*O*-benzyl-4’,6’-*O*-benzylidene-(R)-6’-*C*-ethyl-1,3,2’,2’’’,6’’’-pentadeaminoparomomycin (30). Benzaldehyde dimethyl acetal (1.0 µL, 67 µmol) was added to a stirred solution of **28(*R*)** (24.3 mg, 18.5 µmol) and CSA (3.0 mg, 13 µmol) in MeCN (0.5 mL) under argon. The reaction mixture was stirred for 1 hour monitoring by LCMS and TLC then quenched with Et_3N. The reaction mixture was diluted with Et_2O, washed with aqueous saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated. The crude residue was purified using silica gel column chromatography with 18 % EtOAc in hexanes to give acetal **30** (12.3 mg, 8.8 µmol) in 47 % yield as a white foam. $[\alpha]_D^{23}$ = 92.76 (*c* = 0.2, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.49 (d, *J* = 7.3 Hz, 2H, Ar-H), 7.40 – 7.13 (m, 33H, Ar-H), 6.19 (d, *J* = 3.9 Hz, 1H, H-1’), 5.66 (d, *J* = 5.6 Hz, 1H, H-1’’), 5.56 (s, 1H, $PhCH(O)_2$), 4.97 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.92 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.88 (d, *J* = 1.9 Hz, 1H, H-1’’’), 4.76 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.72 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.62 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.56 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.52 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.47 – 4.42 (m, 2H, $PhCH_2O$), 4.40 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.34 – 4.27 (m, 3H, H-3’’, H-4’’, $PhCH_2O$), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.09 (t, *J* =

9.6 Hz, 1H, H-3'), 4.00 – 3.92 (m, 2H, H-5, H-2''), 3.82 – 3.74 (m, 3H, H-5'', H-3''', H-5'''), 3.69 (t, *J* = 9.3 Hz, 1H, H-5'), 3.64 (dd, *J* = 12.9, 8.6 Hz, 1H, H-6'''), 3.60 (t, *J* = 9.2 Hz, 1H, H-4), 3.58 – 3.51 (m, 2H, H-6', H-5''), 3.49 – 3.40 (m, 3H, H-1, H-3, H-4'), 3.35 (t, *J* = 2.4 Hz, 1H, H-2'''), 3.29 (t, *J* = 9.3 Hz, 1H, H-6), 3.12 (br s, 1H, H-4'''), 3.06 (dd, *J* = 10.1, 3.9 Hz, 1H, H-2'), 2.88 (dd, *J* = 13.0, 3.9 Hz, 1H, H-6'''), 2.23 (dt, *J* = 13.4, 4.7 Hz, 1H, H-2eq), 1.96 (dtt, *J* = 15.3, 7.9, 4.6 Hz, 1H, H-7'), 1.62 (dp, *J* = 15.5, 7.7 Hz, 1H, H-7'), 1.37 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.10 (t, *J* = 7.4 Hz, 3H, H-8'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.3, 138.1, 137.9, 137.8, 137.6, 137.0, 136.9, 128.72, 128.66, 128.5, 128.4, 128.33, 128.31, 128.27, 128.21, 128.18, 128.14, 127.81, 127.77, 127.74, 127.73, 127.67, 127.5, 127.4, 127.3, 126.1 (Ar), 106.1 (C-1''), 100.8 ($PhCH(O)_2$), 98.6 (C-1'''), 96.1 (C-1'), 84.3 (C-6), 82.4 (C-2''), 82.1 (C-4''), 81.8 (C-5), 81.7 (C-4'), 80.5 (C-6'), 76.1 (C-3'), 75.5 (C-3''), 75.2 (C-4), 75.0 ($PhCH_2O$), 74.9 ($PhCH_2O$), 74.4 (C-5'''), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.9 (C-3'''), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.3 (C-5''), 67.2 (C-5'), 62.8 (C-2'), 60.4 (C-1), 60.0 (C-3), 57.3 (C-2'''), 51.1 (C-6'''), 32.6 (C-2), 24.7 (C-7'), 9.6 (C-8'). ESI-HRMS: m/z calcd for $C_{74}H_{79}N_{15}O_{14}Na$ $[M + Na]^+$ 1424.5829, found 1424.5809.

6'-(*R*)-*C*-propyl-paromomycin (32(*R*)). Compound **31(*R*)** (33.2 mg, 0.0251 mmol) was added to a 16 mm test tube followed by 0.4 mL of dioxane and 0.4 mL of 10% AcOH in water. 67.4 mg of Pd/C were added to the tube and the reaction mixture was subjected to 48 psi H_2 for 22 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified using a CM Sephadex C-25 column. The column was washed with 50 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%.

Lyophilization with AcOH gave the acetate salt **32(*R*)** (10.0 mg, 0.0104 mmol) in 42% yield as a white powder. $[\alpha]_D^{23}$ = 48.83 (*c* = 0.3, H_2O), ^{1}H NMR (600 MHz, D_2O) δ 5.52 (d, *J* = 4.0 Hz, 1H, H-1'), 5.24 (d, *J* = 2.9 Hz, 1H, H-1''), 5.16 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.38 (t, *J* = 5.7 Hz, 1H, H-3''), 4.21 (dd, *J* = 5.2, 2.9 Hz, 1H, H-2''), 4.20 – 4.15 (m, 1H, H-5'''), 4.10 (t, *J* = 3.2 Hz, 1H, H-3'''), 4.09 – 4.06 (m, 1H, H-4''), 3.92 – 3.88 (m, 1H, H-6'), 3.82 (t, *J* = 9.5 Hz, 1H, H-4), 3.79 – 3.68 (m, 5H, H-5, H-3', H-5' [$J_{5',6'}$ = 2.7 Hz extracted from HSQC trace], H-5'', H-4'''), 3.64 (dd, *J* = 12.4, 4.6 Hz, 1H, H-5''), 3.56 (t, *J* = 9.8 Hz, 1H, H-6), 3.46 (s, 1H, H-2'''), 3.42 – 3.36 (m, 2H, H-3, H-4'), 3.32 – 3.17 (m, 4H, H-1, H-2', H-6'''), 2.32 (dt, *J* = 11.4, 4.0 Hz, 1H, H-2eq), 1.82 (s, 15H, AcOH), 1.69 (q, *J* = 12.8 Hz, 1H, H-2ax), 1.44 – 1.32 (m, 3H, H-7', H-8'), 1.27 – 1.15 (m, 1H, H-8'), 0.78 (t, *J* = 7.0 Hz, 3H, H-9'). ^{13}C NMR (151 MHz, D_2O) δ 179.9 (AcOH), 109.7 (C-1''), 96.6 (C-1'), 95.5 (C-1'''), 84.0 (C-5), 81.4 (C-4''), 78.9 (C-4), 75.9 (C-5'), 75.2 (C-3''), 73.4 (C-2''), 72.3 (C-6), 70.1 (C-5'''), 69.7 (C-4'), 69.4 (C-3'), 69.0 (C-6'), 67.6 (C-3'''), 67.2 (C-4'''), 59.9 (C-5''), 53.8 (C-2'), 50.8 (C-2'''), 49.6 (C-1), 49.0 (C-3), 40.3 (C-6'''), 31.0 (C-7'), 28.4 (C-2), 22.5 (AcOH), 18.5 (C-8'), 13.0 (C-9'). ESI-HRMS: m/z calcd for $C_{26}H_{51}N_5O_{14}$ $[M + H]^+$ 658.3511, found 658.3529.

6'-(S)-*C*-propyl-paromomycin (32(*S*)). Compound **31(*S*)** (33.3 mg, 0.0251 mmol) was added to a 16 mm test tube followed by 0.4 mL of dioxane and 0.4 mL of 10% AcOH in water. 64.8 mg of Pd/C were added to the tube and the reaction mixture was subjected to 50 psi H_2 for 22 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified using a CM Sephadex C-25 column. The column was washed with 250 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%.

Lyophilization with AcOH gave the acetate salt **32(*S*)** (11.9 mg, 0.0124 mmol) in 49% yield as a white powder. $[\alpha]_D^{23}$ = 40.60 (*c* = 0.4, H_2O), ^1H NMR (600 MHz, D_2O) δ 5.65 (d, *J* = 3.9 Hz, 1H, H-1'), 5.26 (d, *J* = 2.5 Hz, 1H, H-1''), 5.16 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.39 (dd, *J* = 6.6, 4.9 Hz, 1H, H-3''), 4.23 (dd, *J* = 5.0, 2.5 Hz, 1H, H-2''), 4.17 (ddd, *J* = 6.4, 4.1, 1.5 Hz, 1H, H-5'''), 4.09 (t, *J* = 3.1 Hz, 1H, H-3'''), 4.07 (ddd, *J* = 7.0, 4.4, 3.0 Hz, 1H, H-4''), 3.85 (ddd, *J* = 9.8, 3.4, 1.7 Hz, 1H, H-6'), 3.79 – 3.74 (m, 2H, H-3', H-5''), 3.74 – 3.70 (m, 2H, H-4, H-5), 3.70 – 3.68 (m, 1H, H-4'''), 3.64 (dd, *J* = 12.4, 4.6 Hz, 1H, H-5''), 3.56 – 3.47 (m, 3H, H-6, H-4', H-5'), 3.45 (dt, *J* = 3.0, 1.4 Hz, 1H, H-2'''), 3.29 (dd, *J* = 13.7, 6.6 Hz, 1H, H-6'''), 3.25 – 3.15 (m, 4H, H-1, H-3, H-2', H-6'''), 2.25 (dt, *J* = 12.8, 4.3 Hz, 1H, H-2eq), 1.81 (s, 15H, AcOH), 1.63 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.53 (dtd, *J* = 13.7, 9.7, 9.1, 4.3 Hz, 1H, H-7'), 1.40 – 1.26 (m, 2H, H-7', H-8'), 1.26 – 1.19 (m, 1H, H-8'), 0.78 (t, *J* = 7.2 Hz, 3H, H-9'). ^{13}C NMR (151 MHz, D_2O) δ 180.3 (AcOH), 109.7 (C-1''), 95.8 (C-1'), 95.4 (C-1'''), 84.2 (C-5), 81.3 (C-4''), 78.9 (C-4), 75.6 (C-5'), 75.2 (C-3''), 73.4 (C-2''), 72.4 (C-6), 70.1 (C-5'''), 69.2 (C-4'), 68.9 (C-3'), 67.62 (C-3'''), 67.58 (C-6'), 67.2 (C-4'''), 60.0 (C-5''), 53.7 (C-2'), 50.8 (C-2'''), 49.9 (C-1), 49.1 (C-3), 40.3 (C-6'''), 34.9 (C-7'), 29.1 (C-2), 22.6 (AcOH), 18.6 (C-8'), 12.9 (C-9'). ESI-HRMS: m/z calcd for $C_{26}H_{51}N_5O_{14}$ $[M + H]^+$ 658.3511, found 658.3528.

1,3,2',6',2''',6'''-hexaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-1,3,2',6',2''',6'''-hexaadeaminoneomycin (33). Trifluoromethanesulfonic anhydride (35 µL, 0.24 mmol) was added to a stirred solution of **20(*R*)** (0.151 g, 0.110 mmol) and pyridine (0.09 mL, 1.1 mmol) in DCM (2.2 mL) at 0 °C. After 20 minutes the reaction was quenched with MeOH (0.02 mL, 0.49 mmol) and concentrated under vacuum. The resulting triflate was dissolved in DMF (1.1 mL) and LiN_3 (56.0 mg, 1.14 mmol) was added. After 1 h the reaction mixture was diluted with Et_2O,

washed with 1N HCl and brine, and concentrated. The crude residue was purified using silica gel column chromatography in 22% EtOAc in hexanes to give **33** (58.6 mg, 0.044 mmol) in 40% yield. $[\alpha]_D^{23}$ = 90.60 (*c* = 0.7, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.39 – 7.15 (m, 30H, Ar-H), 6.16 (d, *J* = 3.7 Hz, 1H, H-1'), 5.64 (d, *J* = 5.7 Hz, 1H, H-1''), 4.96 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.93 (d, *J* = 11.4 Hz, 1H, $PhCH_2O$), 4.87 (d, *J* = 2.0 Hz, 1H, H-1'''), 4.71 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.63 – 4.60 (m, 2H, $PhCH_2O$), 4.58 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.54 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.47 – 4.43 (m, 2H, $PhCH_2O$), 4.40 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.31 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.27 (q, *J* = 2.9 Hz, 1H, H-4''), 4.26 – 4.23 (m, 2H, H-3'', $PhCH_2O$), 3.97 – 3.92 (m, 2H, H-5, H-2''), 3.83 – 3.72 (m, 6H, H-3', H-5', H-6', H-5'', H-3''', H-5'''), 3.61 (dd, *J* = 13.0, 8.4 Hz, 1H, H-6'''), 3.59 – 3.54 (m, 2H, H-4, H-5''), 3.51 (td, *J* = 9.3, 2.8 Hz, 1H, H-4'), 3.49 – 3.40 (m, 2H, H-1, H-3), 3.34 (t, *J* = 2.6 Hz, 1H, H-2'''), 3.27 (t, *J* = 9.3 Hz, 1H, H-6), 3.13 (d, *J* = 2.6 Hz, 1H, H-4'''), 2.95 (dd, *J* = 10.3, 3.8 Hz, 1H, H-2'), 2.91 (dd, *J* = 12.9, 4.1 Hz, 1H, H-6'''), 2.24 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 2.07 (d, *J* = 3.1 Hz, 1H, 4'-OH), 1.44 (d, *J* = 6.9 Hz, 3H, H-7'), 1.35 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.2, 138.0, 137.8, 137.6, 137.0, 136.9, 128.70, 128.66, 128.5, 128.41, 128.39, 128.34, 128.31, 128.28, 128.16, 128.12, 128.08, 127.81, 127.80, 127.79, 127.77, 127.6, 127.5 (Ar), 106.1 (C-1''), 98.7 (C-1'''), 95.9 (C-1'), 84.3 (C-6), 82.3 (C-2''), 82.0 (C-4''), 81.7 (C-5), 80.0 (C-3'), 75.5 (C-3''), 75.1 (C-4), 75.1 ($PhCH_2O$), 74.8 ($PhCH_2O$), 74.3 (C-5'''), 73.6 (C-5'), 73.3 ($PhCH_2O$), 73.2 ($PhCH_2O$), 72.9 (C-3'''), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.4 (C-4'), 70.0 (C-5''), 62.4 (C-2'), 60.4 (C-1), 60.2 (C-3), 57.3 (C-2'''), 55.0 (C-6'), 51.0 (C-6'''), 32.7 (C-2), 15.3 (C-7'). ESI-HRMS: m/z calcd for $C_{66}H_{72}N_{18}O_{13}$ $[M + Na]^+$ 1347.5424, found 1347.5458.

1,3,2',2''',6''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-hydroxylamino-1,3,2',6',2''',6'''-hexaadeaminoneomycin (35(*R*) and 35(*S*)). Hydroxylamine hydrochloride (0.125 g, 1.80 mmol) was added to a stirred solution of **19** (0.507 g, 0.370 mmol) in 1:1 DCM/MeOH (7.4 mL). After 3 hours the reaction mixture was diluted with Et_2O, washed with 1 N HCl and brine, dried with Na_2SO_4, and concentrated. The resulting oxime was used in the next step without further purification. 10% HCl MeOH solution (0.5 mL) was added to a stirred solution of oxime and $NaBH_3CN$ (0.114 g, 1.81 mmol) in MeOH (7.4 mL) at 60 °C. HCl MeOH solution was added at 20 minutes (1 mL), 50 minutes (0.5 mL), and 1 hour (0.4 mL) to ensure reaction mixture was acidic. $NaBH_3CN$ (0.113 g, 1.80 mmol) was added at 30 minutes. After 1.5 hours the reaction mixture was diluted with Et_2O, washed with aqueous saturated $NaHCO_3$ and brine, dried with Na_2SO_4, and concentrated. The crude residue was purified using silica gel column chromatography in 40-60 % EtOAc in hexanes to give **35(*R*)** (0.192 g, 0.146 mmol) in 39% yield and **35(*S*)** (0.114 g, 0.087 mmol) in 23% yield. **35(*R*)** $[\alpha]_D^{23}$ = 83.24 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.13 (m, 30H, Ar-H), 6.15 (d, *J* = 3.7 Hz, 1H, H-1'), 5.68 (d, *J* = 5.9 Hz, 1H, H-1''), 4.99 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.900 (d, *J* = 11.1 Hz, 1H, $PhCH_2O$), 4.897 (d, 1.8 Hz, 1H, H-1'''), 4.75 – 4.69 (m, 2H, $PhCH_2O$), 4.63 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.58 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.50 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.48 – 4.39 (m, 3H, $PhCH_2O$), 4.33 – 4.28 (m, 3H, H-3'', H-4'', $PhCH_2O$), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.08 (dd, *J* = 10.0, 4.4 Hz, 1H, H-5'), 3.97 – 3.93 (m, 2H, H-5, H-2''), 3.90 (dd, *J* = 10.3, 8.7 Hz, 1H, H-3'), 3.83 (dd, *J* = 10.4, 2.2 Hz, 1H, H-5''), 3.80 – 3.75 (m, 2H, H-3''', H-5'''), 3.73 (t, *J* = 9.3 Hz, 1H, H-4), 3.67 (dd, *J* = 13.0, 8.6 Hz, 1H, H-6'''), 3.58 (dd, *J* = 10.4, 3.1 Hz, 1H, H-5''), 3.49 – 3.38 (m, 2H, H-1, H-3), 3.37 (t, *J* = 2.4 Hz, 1H, H-2'''), 3.35 – 3.28 (m, 3H, H-6, H-4', H-6'), 3.12 (t, *J* = 2.4 Hz, 1H, H-4'''), 2.91 – 2.84 (m, 2H, H-2', H-6'''),

2.20 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.40 (q, *J* = 12.8 Hz, 1H, H-2ax), 1.09 (d, *J* = 6.7 Hz, 3H, H-7'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.3, 138.05, 137.96, 137.5, 137.0, 136.9, 128.7, 128.6, 128.5, 128.43, 128.36, 128.34, 128.27, 128.22, 128.0, 127.83, 127.82, 127.79, 127.78, 127.5, 127.2 (Ar), 106.1 (C-1''), 98.6 (C-1'''), 95.7 (C-1'), 84.3 (C-6), 82.4 (C-2''), 82.1 (C-4''), 82.0 (C-5), 80.0 (C-3'), 75.5 (C-3''), 75.1 ($PhCH_2O$), 74.6 ($PhCH_2O$), 74.4 (C-5'''), 73.24 ($PhCH_2O$), 73.18 ($PhCH_2O$), 72.8 (C-3'''), 72.6 ($PhCH_2O$), 72.4 (C-4'), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.3 (C-5''), 69.1 (C-5'), 62.7 (C-2'), 60.4 (C-1), 60.3 (C-3), 58.3 (C-6'), 57.2 (C-2'''), 51.1 (C-6'''), 32.6 (C-2), 12.0 (C-7'). ESI-HRMS: m/z calcd for $C_{66}H_{75}N_{16}O_{14}$ $[M + H]^+$ 1315.5649, found 1315.5668. **35(*S*)** $[\alpha]_D^{23}$ = 82.66 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 – 7.12 (m, 30H, Ar-H), 6.09 (d, *J* = 3.8 Hz, 1H, H-1'), 5.67 (d, *J* = 5.6 Hz, 1H, H-1''), 4.97 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.88 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.86 (d, *J* = 11.1 Hz, 1H, $PhCH_2O$), 4.82 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.71 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.62 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.56 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.50 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.47 – 4.42 (m, 2H, $PhCH_2O$), 4.41 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.34 – 4.28 (m, 3H, H-3'', H-4'', $PhCH_2O$), 4.25 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.10 (dd, *J* = 10.1, 3.2 Hz, 1H, H-5'), 3.97 (dd, *J* = 5.7, 4.4 Hz, 1H, H-2''), 3.93 (t, *J* = 8.9 Hz, 1H, H-5), 3.88 (dd, *J* = 10.3, 8.7 Hz, 1H, H-3'), 3.82 (dd, *J* = 10.4, 2.0 Hz, 1H, H-5''), 3.79 – 3.74 (m, 2H, H-3''', H-5'''), 3.69 – 3.59 (m, 3H, H-4, H-4', H-6'''), 3.57 (dd, *J* = 10.7, 2.9 Hz, 1H, H-5''), 3.47 – 3.38 (m, 3H, H-1, H-3, H-6'), 3.35 (t, *J* = 2.4 Hz, 1H, H-2'''), 3.27 (t, *J* = 9.3 Hz, 1H, H-6), 3.12 (t, *J* = 2.3 Hz, 1H, H-4'''), 2.92 (dd, *J* = 10.3, 3.8 Hz, 1H, H-2'), 2.87 (dd, *J* = 13.0, 3.8 Hz, 1H, H-6'''), 2.21 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.39 (q, *J* = 12.8 Hz, 1H, H-2ax), 1.13 (d, *J* = 6.8 Hz, 3H, H-7'). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.3, 138.2, 137.9, 137.6, 137.0, 136.9, 128.7, 128.51, 128.48, 128.4, 128.34, 128.27, 128.19, 128.17, 127.83, 127.79, 127.75, 127.51, 127.50, 127.2 (Ar), 106.2 (C-1''), 98.6 (C-1'''), 96.0 (C-1'), 84.2 (C-6), 82.4 (C-2''), 82.1 (C-4''), 81.9

(C-5), 79.7 (C-3'), 75.5 (C-3''), 75.12 ($PhCH_2O$), 75.06 (C-4), 74.4 (C-5'''), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.8 (C-3'''), 72.4 ($PhCH_2O$), 71.8 (C-4'), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.3 (C-5''), 69.9 (C-5'), 62.6 (C-2'), 60.4 (C-1), 60.1 (C-3), 58.0 (C-6'), 57.3 (C-2'''), 51.1 (C-6'''), 32.5 (C-2), 13.0 (C-7'). ESI-HRMS: m/z calcd for $C_{66}H_{75}N_{16}O_{14}$ $[M + H]^+$ 1315.5649, found 1315.5677.

6'-(R)-*C*-methyl-neomycin (34(*R*)). Compound **35(*R*)** (43.7 mg, 0.0332 mmol) was stirred in 0.4 mL of 1:1 dioxane/10% AcOH in water with 79.0 mg of Pd/C under 50 psi of H_2 for 12 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified over a CM Sephadex C-25 column. The column was washed with 250 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization with AcOH gave the acetate salt **34(*R*)** (11.8 mg, 0.0119 mmol) as a white solid in 36% yield. $[\alpha]_D^{23}$ = 44.86 (*c* = 0.4, H_2O), ^1H NMR (600 MHz, D_2O) δ 5.92 (d, *J* = 3.9 Hz, 1H, H-1'), 5.29 (d, *J* = 2.8 Hz, 1H, H-1''), 5.16 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.34 (dd, *J* = 6.2, 5.0 Hz, 1H, H-3''), 4.24 (dd, *J* = 5.1, 2.8 Hz, 1H, H-2''), 4.18 (ddd, *J* = 6.0, 4.1, 1.5 Hz, 1H, H-5'''), 4.12 – 4.08 (m, 2H, H-4'', H-3'''), 3.88 – 3.81 (m, 2H, H-3', H-5'), 3.80 – 3.73 (m, 2H, H-5, H-5''), 3.71 – 3.66 (m, 3H, H-4, H-6', H-4'''), 3.60 (dd, *J* = 12.3, 5.4 Hz, 1H, H-5''), 3.51 (dd, *J* = 10.6, 9.0 Hz, 1H, H-6), 3.45 (t, *J* = 1.2 Hz, 1H, H-2'''), 3.38 (t, *J* = 9.5 Hz, 1H, H-4'), 3.32 – 3.21 (m, 3H, H-2', H-6'''), 3.20 – 3.12 (m, 2H, H-1, H-3), 2.20 (dt, *J* = 12.6, 4.3 Hz, 1H, H-2eq), 1.79 (s, 18H, AcOH), 1.58 (q, *J* = 12.6 Hz, 1H, H-2ax), 1.19 (d, *J* = 6.9 Hz, 3H, H-7'). ^{13}C NMR (151 MHz, D_2O) δ 180.6 (AcOH), 110.1 (C-1''), 95.5 (C-1'), 95.1 (C-1'''), 85.2 (C-5), 81.5 (C-4''), 77.1 (C-4), 75.5 (C-3''), 73.6 (C-2''), 72.8 (C-6), 71.4 (C-5'), 70.1 (C-5'''), 69.8 (C-4'), 68.5 (C-3'), 67.6 (C-3'''), 67.3 (C-4'''), 60.3 (C-5''), 53.6 (C-2'), 50.8 (C-2'''), 50.1 (C-1), 48.6 (C-3), 47.1 (C-

6’), 40.4 (C-6’’’), 29.9 (C-2), 22.8 (AcOH), 11.2 (C-7’). ESI-HRMS: m/z calcd for $C_{24}H_{48}N_6O_{13}$ $[M+H]^+$ 629.3358, found 629.3362.

6’-(S)-*C*-methyl-neomycin (34(*S*)). Compound **35(*S*)** (32.6 mg, 0.0248 mmol) was stirred in 0.4 mL of 1:1 dioxane/10% AcOH in water with 65.0 mg of Pd/C under 50 psi of H_2 for 12 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified over a CM Sephadex C-25 column. The column was washed with 250 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization with AcOH gave the acetate salt **34(*S*)** (8.3 mg, 0.0084 mmol) as a white solid in 34% yield. $[\alpha]_D^{23}$ = 40.36 (*c* = 0.3, H_2O), ^{1}H NMR (600 MHz, D_2O) δ 5.78 (d, *J* = 3.9 Hz, 1H, H-1’), 5.27 (d, *J* = 2.8 Hz, 1H, H-1’’), 5.16 (d, *J* = 1.8 Hz, 1H, H-1’’’), 4.36 (dd, *J* = 6.3, 5.0 Hz, 1H, H-3’’), 4.24 (dd, *J* = 5.0, 2.8 Hz, 1H, H-2’’), 4.18 (td, *J* = 5.1, 4.7, 2.3 Hz, 1H, H-5’’’), 4.12 – 4.07 (m, 2H, H-4’’, H-3’’’), 3.83 – 3.71 (m, 4H, H-5, H-3’, H-5’, H-5’’), 3.70 – 3.69 (m, 1H, H-4’’’), 3.69 – 3.64 (m, 2H, H-4, H-6’), 3.62 (dd, *J* = 12.4, 5.0 Hz, 1H, H-5’’), 3.52 (dd, *J* = 10.5, 9.1 Hz, 1H, H-6), 3.47 – 3.42 (m, 2H, H-4’, H-2’’’), 3.30 (dd, *J* = 13.7, 6.4 Hz, 1H, H-6’’’), 3.27 – 3.22 (m, 2H, H-2’, H-6’’’), 3.19 – 3.14 (m, 1H, H-1), 3.13 – 3.08 (m, 1H, H-3), 2.19 (dt, *J* = 12.9, 4.3 Hz, 1H, H-2eq), 1.80 (s, 18H, AcOH), 1.54 (q, *J* = 12.6 Hz, 1H, H-2ax), 1.29 (d, *J* = 6.9 Hz, 3H, H-7’). ^{13}C NMR (151 MHz, D_2O) δ 180.5 (AcOH), 109.9 (C-1’’), 95.6 (C-1’), 95.5 (C-1’’’), 84.9 (C-5), 81.5 (C-4’’), 79.0 (C-4), 75.4 (C-3’’), 73.5 (C-2’’), 72.8 (C-6), 72.0 (C-5’), 70.1 (C-5’’’), 69.9 (C-4’), 68.8 (C-3’), 67.6 (C-3’’’), 67.3 (C-4’’’), 60.2 (C-5’’), 53.5 (C-2’), 50.8 (C-2’’’), 50.1 (C-1), 48.8 (C-3), 47.3 (C-6’), 40.4 (C-6’’’), 30.1 (C-2), 22.8 (AcOH), 14.9 (C-7’). ESI-HRMS: m/z calcd for $C_{24}H_{48}N_6O_{13}$ $[M+H]^+$ 629.3358, found 629.3354.

Compound **35(*S*)** (0.039 mg, 0.030 mmol) was stirred in 0.8 mL of 1:1 dioxane/10% AcOH in water with 77.4 mg of Pd/C under 50 psi of H_2 for 72 hours. Once the reaction was determined to be complete by LCMS the reaction mixture was diluted with water and filtered through Celite. The resulting crude product was purified over a CM Sephadex C-25 column. The column was washed with 250 mL of DI water and eluted with NH_4OH in water starting at 0.1% and increasing stepwise by 0.1% every 20 mL to 0.8%. Lyophilization with AcOH gave the acetate salt **2** (10.5 mg, 0.0167 mmol) as a white solid in 56% yield.

1,3,2',2''',6''',6'-Pentaazido-6,3',6',2'',5'',3''',4''',6'-hepta-*O*-benzyl-6'-*C*-vinyl-1,3,2',2''',6''',6'-6'-paratoluenesulfonylmethyl-pentadeaminoparomomycin (41(*R*) and 41(*S*)). To a stirred solution of DMSO (0.22 mL 3.1 mmol) in 1 mL DCM at -78°C under argon was added oxalyl chloride (0.125 mL, 1.46 mmol). After stirring for 10 minutes compound **27** (1.00 g, 0.71 mmol) was dissolved in DCM (6 mL) and added dropwise. After an additional 45 minutes Et_3N (0.45 mL, 3.2 mmol) was added. The reaction mixture was stirred for an additional 2 hours then diluted with Et_2O, washed with aqueous NH_4Cl solution, DI water, and brine. The organic layer was concentrated under vacuum to give the intermediate aldehyde as a white foam which was used in the next step without further purification. ESI-HRMS: *m/z* calc for $C_{74}H_{79}N_{15}O_{14}Na$ $[M+Na]^+$ 1424.5829, found 1424.5815. To a stirred solution of aldehyde in THF (14.5 mL) at -78°C was added vinylMgBr solution (2.9 mL, 1 M in THF). After stirring for 45 minutes the reaction was quenched with aqueous saturated NH_4Cl solution, diluted with Et_2O, washed with aqueous saturated NH_4Cl solution, brine, dried with Na_2SO_4, and concentrated to give an inseparable mixture of diastereomers which were filtered through silica gel and used in the next step without

further purification. ESI-HRMS: *m/z* calc for $C_{74}H_{79}N_{15}O_{14}Na$ $[M+Na]^+$ 1424.5829, found 1424.5815. To a solution of alcohols in DMF (4.7 mL) at 0°C under argon was added NaH (60 % in mineral oil, 0.188 g, 7.78 mmol). After 15 minutes TBAI (0.118 g, 0.319 mmol) and BnBr (1.1 mL, 9.3 mmol) were added to the reaction mixture and stirring was continued for 40 minutes before quenching with aqueous saturated NH_4Cl solution. The reaction mixture was diluted with Et_2O, washed with aqueous saturated NH_4Cl solution followed by brine, dried with Na_2SO_4, and concentrated. The crude residue was purified over silica gel to give compounds **40** (0.650 g, 0.427 mmol, 60%) as an inseparable mixture of diastereomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 – 7.12 (m, 70H, Ar-H), 7.11 – 7.07 (m, 2H, Ar-H), 7.07 – 7.03 (m, 2H, Ar-H), 6.84 – 6.79 (m, 4H, Ar-H), 6.18 – 6.09 (m, 2H, H-1'a, H-7'a), 6.08 (d, *J* = 3.7 Hz, 1H, H-1'b), 5.95 (ddd, *J* = 17.4, 10.3, 8.7 Hz, 1H, H-7'b), 5.64 (d, *J* = 5.7 Hz, 1H, H-1''a), 5.61 (d, *J* = 5.7 Hz, 1H, H-1''b), 5.43 – 5.37 (m, 2H), 5.32 (dd, *J* = 10.3, 1.9 Hz, 1H), 5.10 (dd, *J* = 17.3, 1.9 Hz, 1H), 4.97 (d, *J* = 10.7 Hz, 1H), 4.94 (d, *J* = 10.7 Hz, 1H), 4.88 – 4.75 (m, 5H), 4.75 – 4.71 (m, 2H), 4.71 – 4.51 (m, 10H), 4.50 – 4.44 (m, 4H), 4.44 – 4.38 (m, 4H), 4.35 – 4.24 (m, 9H), 4.23 – 4.20 (m, 2H), 4.14 – 4.03 (m, 5H), 3.98 – 3.95 (m, 1H), 3.94 – 3.88 (m, 3H), 3.85 – 3.81 (m, 2H), 3.80 (s, 3H), 3.79 – 3.71 (m, 9H), 3.65 (dd, *J* = 12.9, 8.5 Hz, 1H), 3.63 – 3.51 (m, 4H), 3.50 – 3.37 (m, 4H), 3.35 (t, *J* = 2.5 Hz, 1H), 3.33 (t, *J* = 2.5 Hz, 1H), 3.26 (t, *J* = 9.2 Hz, 1H), 3.24 – 3.19 (m, 2H), 3.17 (dd, *J* = 10.4, 3.7 Hz, 1H), 3.13 (t, *J* = 2.7 Hz, 1H), 3.12 (t, *J* = 2.3 Hz, 1H), 2.97 – 2.93 (m, 2H), 2.89 (dd, *J* = 13.0, 4.0 Hz, 1H), 2.23 – 2.17 (m, 2H, H-2eqa, H-2eqb), 1.35 – 1.23 (m, 2H, H-2axa, H-2axb). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.09, 159.04, 138.7, 138.4, 138.3, 138.1, 138.0, 137.9, 137.62, 137.61, 137.1, 137.04, 137.00, 136.97 (Ar), 136.2 (C-7'a), 134.0 (C-7'b), 130.7, 130.5, 128.93, 128.88, 128.67, 128.66, 128.49, 128.48, 128.41, 128.38, 128.35, 128.32, 128.31, 128.25, 128.19, 128.17, 128.14, 128.11, 128.07, 128.03, 127.9,

127.83, 127.82, 127.78, 127.74, 127.72, 127.65, 127.61, 127.48, 127.45, 127.40, 127.37, 127.31, 120.26, 118.7, 113.74, 113.70 (Ar), 106.1 (C-1''a), 105.9 (C-1''b), 98.7 (C-1'''b), 98.6 (C-1'''a), 96.0 (C-1'b), 95.7 (C-1'b), 84.12, 84.05, 82.4, 82.1, 82.0, 81.93, 81.87, 81.6, 80.70, 80.67, 79.4, 78.2, 77.8, 77.7, 75.60, 75.59, 75.4, 74.99, 74.97, 74.8, 74.4, 74.3, 74.13, 74.07, 73.86, 73.84, 73.29, 73.28, 73.19, 73.11, 73.01, 72.95, 72.90, 72.39, 72.37, 71.8, 71.7, 71.5, 70.5, 70.3, 70.2, 69.8, 63.3, 63.2, 60.40, 60.39, 60.0, 59.7, 57.4, 57.3, 55.3, 55.2, 51.1, 50.9, 32.5 (C-2). ESI-HRMS: *m/z* calc for $C_{82}H_{87}N_{15}O_{15}Na$ $[M+Na]^+$ 1544.6404, found 1544.6403. Ozone gas was bubbled through a solution of mixture **40** (0.650 g, 0.427 mmol) in 23.2 mL 4:1 DCM/MeOH at -78°C. After 30 minutes the solution turned pale blue and the reaction mixture was sparged with argon followed by addition of $NaBH_4$ (59 mg, 1.6 mmol). After 1 hour the reaction was quenched with acetone and concentrated under vacuum. The crude residue was dissolved in Et_2O, washed with NH_4Cl solution followed by brine, dried with Na_2SO_4, and concentrated to give the alcohols as an inseparable mixture of diastereomers which were used without further purification. To a stirred solution of 7'-alcohols in pyridine (4.1 mL) was added TsCl (0.120 g, 0.629 mmol). After 19 hours TsCl (0.022 g, 0.115 mmol) and DMAP (5.9 mg 0.05 mmol) were added. After stirring for an additional 5 hours the reaction mixture was diluted with Et_2O, washed with 1 N HCl, saturated aqueous $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4 and concentrated to give the tosylates as an inseparable mixture of diastereomers which were used in the next step without further purification. TFA (0.89 mL) was added to a stirred solution of tosylates in DCM (8 mL) at 0°C. After 30 minutes the reaction mixture was diluted with Et_2O and washed with DI water, aqueous saturated $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4, concentrated, and purified over silica gel to give compounds **41(*R*)** and **41(*S*)** as a mixture of

diastereomers (0.367 g, 0.235 mmol, 55%). The mixture of diastereomers was then purified using silica gel HPLC to give **41(*R*)** (91.5 mg 0.059 mmol) in 14% isolated yield and **41(*S*)** (95.2 mg, 0.061 mmol) in 14% isolated yield. **41(*R*)** $[\alpha]_D^{23}$ = 60.29 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.3 Hz, 2H, ArH), 7.40 – 7.13 (m, 37H, ArH), 6.11 (d, *J* = 3.5 Hz, 1H, H-1'), 5.68 (d, *J* = 6.1 Hz, 1H, H-1''), 4.97 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.95 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.83 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.78 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.70 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.66 – 4.60 (m, 2H, $PhCH_2O$), 4.53 – 4.49 (m, 2H, $PhCH_2O$), 4.47 – 4.40 (m, 4H, $PhCH_2O$), 4.31 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.30 – 4.27 (m, 3H, H-7', H-3'', H-4''), 4.24 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.21 (dd, *J* = 11.1, 2.6 Hz, 1H, H-7'), 4.09 (dd, *J* = 9.7, 5.2 Hz, 1H, H-5'), 3.95 (dd, *J* = 6.1, 4.8 Hz, 1H, H-2''), 3.90 (m, 2H, H-5, H-3'), 3.80 (m, 2H, H-5'', H-5'), 3.76 (t, *J* = 2.8 Hz, 1H, H-3'''), 3.73 – 3.64 (m, 3H, H-4, H-6', H-6'''), 3.55 (dd, *J* = 10.4, 2.8 Hz, 1H, H-5''), 3.45 – 3.29 (m, 5H, H-1, H-3, H-6, H-4', H-2'''), 3.11 (t, *J* = 2.4 Hz, 1H, H-4'''), 2.85 (dd, *J* = 13.0, 3.7 Hz, 1H, H-6'''), 2.80 (dd, *J* = 10.4, 3.5 Hz, 1H, H-2'), 2.41 (s, 3H, Ar-CH_3), 2.14 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.37 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 144.9, 138.3, 138.2, 138.1, 137.7, 137.0, 136.95, 136.91, 133.1, 133.0, 129.9, 128.7, 128.6, 128.52, 128.50, 128.42, 128.35, 128.32, 128.28, 128.21, 128.18, 128.15, 128.11, 127.90, 127.86, 127.81, 127.75, 127.4, 127.1 (Ar), 106.0 (C-1''), 98.7 (C-1'''), 95.4 (C-1'), 84.1 (C-6), 82.6 (C-2''), 82.1 (C-4''), 81.9 (C-5), 79.3 (C-3'), 78.6 (C-6'), 75.5 (C-3''), 75.2 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.4 (C-5'''), 74.2 (C-4), 73.2 (C-4'), 73.1 ($PhCH_2O$), 72.8 (C-3'''), 72.3 ($PhCH_2O$), 72.2 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.2 (C-5''), 69.3 (C-5'), 67.9 (C-7'), 62.2 (C-2'), 60.45 (C-1), 60.41 (C-3), 57.2 (C-2'''), 51.1 (C-6'''), 32.2 (C-2), 21.6 (OTs-CH_3). ESI-HRMS: *m/z* calc for $C_{80}H_{85}N_{15}O_{17}SNa$ $[M+Na]^+$ 1582.5866, found 1582.5872. **41(*S*)** $[\alpha]_D^{23}$ = 58.69 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.77 (d, *J* = 8.3 Hz, 2H, ArH), 7.39 – 7.11 (m, 37H, ArH), 6.15

(d, *J* = 3.6 Hz, 1H, H-1'), 5.65 (d, *J* = 6.0 Hz, 1H, H-1''), 4.97 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$-), 4.89 – 4.86 (m, 2H, H-1''', $PhCH_2O$-), 4.70 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$-), 4.66 – 4.58 (m, 4H, $PhCH_2O$-), 4.54 – 4.47 (m, 3H, $PhCH_2O$-), 4.42 – 4.37 (m, 2H, $PhCH_2O$-), 4.34 (dd, *J* = 9.9, 6.2 Hz, 1H, H-7'), 4.30 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$-), 4.26 – 4.22 (m, 2H, H-4'', $PhCH_2O$-), 4.20 (dd, *J* = 5.0, 2.5 Hz, 1H, H-3''), 4.10 (dd, *J* = 10.0, 6.5 Hz, 1H, H-7'), 3.99 (dd, *J* = 9.9, 2.3 Hz, 1H, H-5'), 3.93 – 3.90 (m, 2H, H-5, H-6'), 3.88 (dd, *J* = 4.8, 1.2 Hz, 1H, H-2''), 3.86 (d, *J* = 9.6 Hz, 1H, H-3'), 3.76 – 3.71 (m, 3H, H-5'', H-3''', H-5'''), 3.62 (t, *J* = 9.4 Hz, 1H, H-4), 3.59 – 3.51 (m, 3H, H-4', H-5'', H-6'''), 3.48 – 3.37 (m, 2H, H-1, H-3), 3.35 (t, *J* = 9.3 Hz, 1H, H-6), 3.32 (t, *J* = 2.4 Hz, 1H, H-2'''), 3.11 (t, *J* = 2.5 Hz, 1H, H-4'''), 2.93 (dd, *J* = 10.3, 3.6 Hz, 1H, H-2'), 2.90 (dd, *J* = 12.9, 4.3 Hz, 1H, H-6'''), 2.43 (s, 3H, Ar-CH_3), 2.17 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.51 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 144.9, 138.2, 138.0, 137.6, 137.5, 137.04, 136.98, 129.9, 128.7, 128.6, 128.47, 128.45, 128.43, 128.39, 128.33, 128.29, 128.22, 128.14, 128.09, 127.96, 127.91, 127.80, 127.78, 127.73, 127.43, 127.40 (Ar), 105.9 (C-1''), 98.7 (C-1'''), 96.0 (C-1'), 84.0 (C-6), 82.2 (C-2''), 81.9 (C-4''), 81.7 (C-5), 79.9 (C-3'), 75.5 (C-3''), 75.1 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.4 (C-4), 74.2 (C-3'''), 74.0 ($PhCH_2O$), 73.6 (C-6'), 73.23 ($PhCH_2O$), 73.17 ($PhCH_2O$), 72.9 (C-5'''), 72.3 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.4 (C-4'''), 70.3 (C-5'), 69.9 (C-5''), 69.7 (C-4'), 68.6 (C-7'), 62.5 (C-2'), 60.5 (C-1), 60.4 (C-3), 57.2 (C-2'''), 50.9 (C-6'''), 32.2 (C-2), 21.7 (Ar-CH_3). ESI-HRMS: *m/z* calc for $C_{80}H_{85}N_{15}O_{17}SNa$ $[M+Na]^+$ 1582.5866, found 1582.5854.

4-*O*-(2-azido-3,6-di-*O*-benzyl-4,7-anhydro-2,7-dideoxy-D-glycero-α-D-gluco-heptapyranosyl)-5-*O*-[3-*O*-(2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-2,5-di-*O*-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-*O*-benzyl-2-deoxystreptamine (42(ax)). To a stirred

solution of compound **41(*R*)** (41.8 mg, 0.027 mmol) in DMF (1.1 mL) was added NaH (60% in mineral oil, 2.2 mg, 0.055 mmol). The reaction mixture was stirred for 1 hour, then quenched with aqueous saturated NH_4Cl solution, diluted with Et_2O, washed with brine, dried with Na_2SO_4, and concentrated under vacuum. The crude residue was purified using silica gel column chromatography (20 % EtOAc in hexanes) to obtain compound **42(ax)** (19.5 mg, 0.014 mmol) as a white foam in 52% yield. $[\alpha]_D^{23}$ = 72.00 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, C_6D_6) δ 7.54 – 7.46 (m, 4H, ArH), 7.33 – 7.29 (m, 2H, ArH), 7.28 – 7.22 (m, 4H, ArH), 7.18 – 6.94 (m, 25H, ArH), 6.46 (d, *J* = 3.8 Hz, 1H, H-1'), 5.98 (d, *J* = 5.4 Hz, 1H, H-1''), 5.14 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.99 (d, *J* = 2.1 Hz, 1H, H-1'''), 4.97 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.89 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.74 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.61 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.57 (q, *J* = 2.9 Hz, 1H, H-4''), 4.49 (dd, *J* = 4.9, 2.8 Hz, 1H, H-3''), 4.41 – 4.35 (m, 5H, $PhCH_2O$), 4.34 (d, *J* = 9.4 Hz, 1H, $PhCH_2O$), 4.30 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.25 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.21 (dd, *J* = 10.3, 4.3 Hz, 1H, H-5'), 4.12 (t, *J* = 5.2 Hz, 1H, H-2''), 4.11 – 4.05 (m, 2H, H-4', $PhCH_2O$), 4.00 – 3.88 (m, 5H, H-6', H-7', $PhCH_2O$), 3.86 (dd, *J* = 10.5, 2.5 Hz, 1H, H-5''), 3.81 (t, *J* = 8.9 Hz, 1H, H-5), 3.73 (ddd, *J* = 8.3, 4.6, 2.1 Hz, 1H, H-5'''), 3.69 – 3.64 (m, 2H, H-4, H-3'''), 3.56 (dd, *J* = 10.5, 3.2 Hz, 1H, H-5''), 3.39 (dd, *J* = 12.8, 8.2 Hz, 1H, H-6'''), 3.34 (t, *J* = 2.7 Hz, 1H, H-2'''), 3.18 (dd, *J* = 9.9, 3.9 Hz, 1H, H-2'), 2.97 – 2.95 (m, 1H, H-4'''), 2.88 (t, *J* = 9.4 Hz, 1H, H-6), 2.75 (dd, *J* = 12.9, 4.6 Hz, 1H, H-6'''), 2.71 (ddd, *J* = 12.8, 9.6, 4.6 Hz, 1H, H-3), 2.57 (ddd, *J* = 12.4, 9.6, 4.5 Hz, 1H, H-1), 1.41 (dt, *J* = 12.9, 4.6 Hz, 1H, H-2eq), 0.88 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, C_6D_6) δ 138.6, 138.4, 138.3, 138.0, 137.4, 137.3, 128.4, 128.35, 128.26, 128.22, 128.19, 128.00, 127.98, 127.94, 127.89, 127.6, 127.41, 127.37, 127.35 (Ar), 106.3 (C-1''), 98.8 (C-1', C-1'''), 83.9 (C-6), 82.4 (C-4''), 82.4 (C-2''), 81.6 (C-5), 79.3 (C-4'), 78.0 (C-3'), 75.9 (C-3''), 75.6 (C-4), 75.3 (C-6'), 75.0 ($PhCH_2O$), 74.1 (C-5', C-7', C-

5’’’), 73.6 (C-3’’’), 73.1 ($PhCH_2O$), 73.0 ($PhCH_2O$), 72.7 ($PhCH_2O$), 72.4 (C-4’’’), 72.2 ($PhCH_2O$), 71.9 ($PhCH_2O$), 71.6 ($PhCH_2O$), 70.0 (C-5’’), 62.5 (C-2’), 59.9 (C-1), 59.8 (C-3), 56.8 (C-2’’’), 50.9 (C-6’’’), 31.8 (C-2). ESI-HRMS: *m/z* calc for $C_{73}H_{77}N_{15}O_{14}Na$ $[M+Na]^+$ 1410.5672, found 1410.5699.

4-*O*-(2-Amino-4,7-anhydro-2,7-dideoxy-D-glycero-α-D-gluco-heptapyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine pentaacetate salt (43(ax)). To a solution of compound **42(ax)** (19.5 mg, 0.014 mmol) in 1:1 1,4-dioxane/10% aqueous AcOH (0.6 mL) was added Pd/C (10 wt%, 40.6 mg). The reaction mixture was stirred under 50 psi H_2 for 30 hours before filtration through Celtie and concentration. The crude residue was purified using CM Sephadex ion exchange column chromatography (0.1-0.8% aqueous NH_4OH) followed by lyophilization with acetic acid to give the pentaacetate salt **43(ax)** (5.9 mg, 0.0064 mmol) in 46% yield as a white powder. $[\alpha]_D^{23}$ = 38.64 (*c* = 0.2, water) ^{1}H NMR (600 MHz, D_2O) δ 5.67 (d, *J* = 4.2 Hz, 1H, H-1’), 5.22 (d, *J* = 1.9 Hz, 1H, H-1’’), 5.11 (d, *J* = 1.8 Hz, 1H, H-1’’’), 4.37 (dd, *J* = 7.2, 4.8 Hz, 1H, H-3’’), 4.33 (t, *J* = 4.5 Hz, 1H, H-6’), 4.25 (dd, *J* = 4.9, 2.0 Hz, 1H, H-2’’), 4.19 – 4.12 (m, 2H, H-7’, H-5’’’), 4.06 – 4.02 (m, 2H, H-4’’, H-3’’’), 3.91 (t, *J* = 9.9 Hz, 1H, H-3’), 3.77 – 3.70 (m, 3H, H-5’, H-7’, H-5’’), 3.67 (t, *J* = 9.1 Hz, 1H, H-5), 3.65 – 3.63 (m, 1H, H-4’’’), 3.62 – 3.56 (m, 2H, H-4, H-5’’), 3.51 (t, *J* = 9.9 Hz, 1H, H-4’), 3.44 (t, *J* = 9.8 Hz, 1H, H-6), 3.40 – 3.37 (m, 1H, H-2’’’), 3.25 (dd, *J* = 13.6, 6.8 Hz, 1H, H-6’’’), 3.19 (dd, *J* = 13.6, 3.8 Hz, 1H, H-6’’’), 3.13 (dd, *J* = 10.2, 4.2 Hz, 1H, H-2’), 3.10 – 3.05 (m, 1H, H-1), 3.04 – 2.98 (m, 1H, H-3), 2.12 (dt, *J* = 12.9, 4.4 Hz, 1H, H-2eq), 1.73 (d, *J* = 1.1 Hz, 15H, AcOH), 1.44 (q, *J* = 12.6 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, D_2O) δ 181.3 (AcOH), 110.0 (C-1’’), 98.3 (C-1’), 95.3 (C-1’’’), 85.1 (C-5), 81.0 (C-4’’), 79.3 (C-6), 77.0 (C-4’), 76.0 (C-4’), 74.8 (C-7’), 73.3 (C-3’’), 73.2 (C-2’’), 73.0 (C-4), 70.2 (C-5’),

69.9 (C-5’’’), 67.7 (C-3’), 67.6 (C-3’’’), 67.3 (C-6’), 59.8 (C-4’’’), 54.6 (C-5’’), 50.9 (C-2’), 50.2 (C-2’’’), 48.5 (C-1), 40.3 (C-3), 30.7 (C-2), 23.2 (AcOH). ESI-HRMS: *m/z* calc for $C_{24}H_{46}N_5O_{14}$ $[M+H]^+$ 628.3041, found 628.3060.

4-*O*-(2-azido-3,6-di-*O*-benzyl-4,7-anhydro-2,7-dideoxy-L-glycero-α-D-gluco-heptapyranosyl)-5-*O*-[3-*O*-(2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-2,5-di-*O*-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-*O*-benzyl-2-deoxystreptamine (42(eq)). To a stirred solution of compound **41(*S*)** (39.8 mg, 0.026 mmol) in DMF (1.0 mL) was added NaH (60% in mineral oil, 2.5 mg, 0.062 mmol). After stirring for 3 hours the reaction was not complete and NaH (2.5 mg, 0.062 mmol) was added. After an additional 30 minutes the reaction was quenched with aqueous saturated NH_4Cl solution, diluted with Et_2O, washed with brine, dried with Na_2SO_4, and concentrated under vacuum. The crude residue was purified using silica gel column chromatography (20 % EtOAc in hexanes) to obtain compound **42(eq)** (24.4 mg, 0.018 mmol) as a white foam in 69% yield. $[\alpha]_D^{23}$ = 76.40 (*c* = 1.0, $CHCl_3$) ^{1}H NMR (600 MHz, C_6D_6) δ 7.52 – 7.45 (m, 4H, ArH), 7.36 – 7.26 (m, 6H, ArH), 7.21 – 6.95 (m, 25H, ArH), 6.57 (d, *J* = 3.9 Hz, 1H, H-1’), 5.99 (d, *J* = 5.6 Hz, 1H, H-1’’), 5.08 (d, *J* = 11.6 Hz, 1H, $PhCH_2O$), 5.02 (d, *J* = 1.8 Hz, 1H, H-1’’’), 4.93 (d, *J* = 10.5 Hz, 1H, $PhCH_2O$), 4.87 (d, *J* = 11.6 Hz, 1H, $PhCH_2O$), 4.68 (d, *J* = 11.6 Hz, 1H, $PhCH_2O$), 4.58 – 4.55 (m, 2H, H-4’’, $PhCH_2O$), 4.54 – 4.45 (m, 3H, H-3’, H-5’, H-3’’), 4.41 – 4.36 (m, 2H, $PhCH_2O$), 4.36 – 4.32 (m, 2H, $PhCH_2O$), 4.31 – 4.26 (m, 2H, $PhCH_2O$), 4.13 (t, *J* = 5.2 Hz, 1H, H-2’’), 4.06 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.02 (td, *J* = 7.6, 5.3 Hz, 1H, H-6’), 3.99 – 3.95 (m, 2H, $PhCH_2O$), 3.94 – 3.89 (m, 2H, H-7’, H-5’’), 3.85 – 3.80 (m, 2H, H-4, H-7’), 3.77 – 3.70 (m, 2H, H-5, H-5’’’), 3.66 (t, *J* = 3.0 Hz, 1H, H-3’’’), 3.57 (dd, *J* = 10.5, 2.8 Hz, 1H, H-5’’), 3.42 (dd, *J* = 12.8, 8.4 Hz, 1H, H-6’’’),

3.36 – 3.33 (m, 1H, H-2’’’), 3.26 (t, *J* = 9.7 Hz, 1H, H-4’), 3.09 (dd, *J* = 9.8, 3.9 Hz, 1H, H-2’), 2.96 – 2.93 (m, 1H, H-4’’’), 2.86 (t, *J* = 9.4 Hz, 1H, H-6), 2.78 (ddd, *J* = 12.7, 9.8, 4.6 Hz, 1H, H-3), 2.71 (dd, *J* = 12.8, 4.2 Hz, 1H, H-6’’’), 2.56 (ddd, *J* = 12.5, 9.9, 4.4 Hz, 1H, H-1), 1.38 (dt, *J* = 12.7, 4.4 Hz, 1H, H-2eq), 0.86 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, C_6D_6) δ 138.6, 138.4, 138.34, 138.27, 138.0, 137.4, 137.3, 128.43, 128.35, 128.27, 128.22, 128.1, 128.00, 127.96, 127.94, 127.6, 127.31, 127.28 (Ar), 106.4 (C-1’’), 98.8 (C-1’’’), 98.3 (C-1’), 84.1 (C-6), 82.6 (C-2’’), 82.4 (C-4’’), 82.0 (C-4), 81.8 (C-4’), 78.9 (C-6’), 77.1 (C-5’), 77.0 (C-3’), 75.9 (C-3’’), 75.7 (C-5), 75.0 ($PhCH_2O$), 74.2 (C-5’’’), 73.5 (C-3’’’), 73.3 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.8 ($PhCH_2O$), 72.3 (C-7’), 72.2 (C-4’’’), 71.8 ($PhCH_2O$), 71.6 ($PhCH_2O$), 70.3 (C-5’’), 62.7 (C-2’), 60.0 (C-1), 59.9 (C-3), 56.7 (C-2’’’), 51.0 (C-6’’’), 32.0 (C-2). ESI-HRMS: *m/z* calc for $C_{73}H_{77}N_{15}O_{14}Na$ $[M+Na]^+$ 1410.5672, found 1410.5570.

4-*O*-(2-Amino-4,7-anhydro-2,7-dideoxy-L-glycero-α-D-gluco-heptapyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine pentaacetate salt (43(eq)). To a solution of compound **42(eq)** (24.4 mg, 0.018 mmol) in 1:1 1,4-dioxane/10% aqueous AcOH (0.6 mL) was added Pd/C (10 wt%, 47.8 mg). The reaction mixture was stirred under 50 psi H_2 for 30 hours before filtration through Celtie and concentration. The crude residue was purified using CM Sephadex ion exchange column chromatography (0.1-0.6% aqueous NH_4OH) followed by lyophilization with acetic acid to give the pentaacetate salt **43(eq)** (3.1 mg, 0.0033 mmol) in 18% yield as a white powder. $[\alpha]_D^{23}$ = 54.64 (*c* = 0.1, water) ^{1}H NMR (600 MHz, D_2O) δ 5.58 (d, *J* = 4.3 Hz, 1H, H-1’), 5.23 (d, *J* = 2.1 Hz, 1H, H-1’’), 5.11 (d, *J* = 1.8 Hz, 1H, H-1’’’), 4.42 (td, *J* = 8.0, 5.5 Hz, 1H, H-6’), 4.38 (dd, *J* = 7.0, 4.8 Hz, 1H, H-3’’), 4.24 (dd, *J* = 4.9, 2.1 Hz, 1H, H-2’’), 4.16 – 4.12 (m, 2H, H-7’, H-5’’’), 4.07 – 4.02 (m, 2H, H-4’’, H-3’’’), 3.94 (t, *J* = 9.8

Hz, 1H, H-3'), 3.83 (dd, *J* = 10.1, 7.9 Hz, 1H, H-5'), 3.75 (dd, *J* = 12.4, 3.1 Hz, 1H, H-5''), 3.68 (t, *J* = 9.2 Hz, 1H, H-5), 3.66 – 3.64 (m, 1H, H-4'''), 3.64 – 3.58 (m, 2H, H-7', H-5''), 3.56 (t, *J* = 9.4 Hz, 1H, H-4), 3.43 (t, *J* = 9.8 Hz, 1H, H-6), 3.39 – 3.34 (m, 2H, H-4', H-2'''), 3.26 (dd, *J* = 13.6, 6.8 Hz, 1H, H-6'''), 3.20 (dd, *J* = 13.6, 3.9 Hz, 1H, H-6'''), 3.10 – 3.03 (m, 2H, H-1, H-2'), 3.02 – 2.96 (m, 1H, H-3), 2.11 (dt, *J* = 12.9, 4.3 Hz, 1H, H-2eq), 1.75 (s, 13H, AcOH), 1.41 (q, *J* = 12.6 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, D_2O) δ 181.3 (AcOH), 109.8 (C-1''), 98.6 (C-1'), 95.6 (C-1'''), 84.9 (C-5), 81.1 (C-4''), 80.4 (C-4), 78.8 (C-4'), 77.1 (C-5'), 75.0 (C-3''), 73.5 (C-6), 73.4 (C-2''), 73.0 (C-7'), 70.8 (C-6'), 70.3 (C-5'''), 69.7 (C-3'), 67.9 (C-3'''), 67.4 (C-4'''), 59.9 (C-5''), 54.7 (C-2'), 51.0 (C-2'''), 50.3 (C-1), 48.8 (C-3), 40.4 (C-6'''), 31.2 (C-2), 23.2 (AcOH). ESI-HRMS: *m/z* calc for $C_{24}H_{46}N_5O_{14}$ $[M+H]^+$ 628.3041, found 628.3038.

4-*O*-(2-azido-3,6-di-*O*-benzyl-4,8-anhydro-2,7-dideoxy-D-glycero-α-D-gluco-octapyranosyl)-5-*O*-[3-*O*-(2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-2,5-di-*O*-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-*O*-benzyl-2-deoxystreptamine (45(ax)), and 4-*O*-(2-azido-3,6-di-*O*-benzyl-4,8-anhydro-2,7-dideoxy-L-glycero-α-D-gluco-octapyranosyl)-5-*O*-[3-*O*-(2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-2,5-di-*O*-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-*O*-benzyl-2-deoxystreptamine (45(eq)). 1 M BH_3 complex with THF (0.5 mL, 0.5 mmol) was added to a stirred solution of compounds **40** (0.69 g, 0.45 mmol) in THF (4.5 mL) at 0 °C. After 4 hours more BH_3 complex with THF (0.1 mL, 0.1 mmol) was added. After 2 more hours aqueous saturated $NaHCO_3$ solution (1.2 mL) and 30% hydrogen peroxide solution (0.46 mL) were added dropwise. After an additional hour the reaction mixture was diluted with Et_2O, washed with $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated. The crude

residue was purified using silica gel column chromatography in 25 to 35% EtOAc in hexanes to give compounds **44** (0.292 g, 0.190 mmol) in 42% yield as an inseparable mixture of diastereomers. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.09 (m, 74H, Ar-H), 6.84 – 6.80 (m, 4H, Ar-H), 6.19 (d, *J* = 3.7 Hz, 1H, H-1'a), 6.16 (d, *J* = 3.7 Hz, 1H, H-1'b), 5.65 (d, *J* = 6.6 Hz, 1H, H-1''a), 5.63 (d, *J* = 6.3 Hz, 1H, H-1''b), 4.98 – 4.94 (m, 2H), 4.89 – 4.72 (m, 10H), 4.67 (d, *J* = 10.7 Hz, 1H), 4.64 – 4.56 (m, 6H), 4.54 (d, *J* = 11.9 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 4.49 (d, *J* = 1.8 Hz, 1H), 4.47 (d, *J* = 1.6 Hz, 1H), 4.47 – 4.37 (m, 8H), 4.33 – 4.21 (m, 11H), 4.19 (dd, *J* = 5.0, 2.5 Hz, 1H), 4.11 (td, *J* = 10.6, 8.7 Hz, 2H), 4.06 – 4.03 (m, 1H), 4.02 – 4.00 (m, 1H), 3.97 – 3.88 (m, 4H), 3.87 – 3.81 (m, 2H), 3.80 (s, 3H), 3.77 (s, 3H), 3.73 (dt, *J* = 9.1, 2.8 Hz, 3H), 3.70 – 3.63 (m, 4H), 3.59 – 3.51 (m, 5H), 3.51 – 3.40 (m, 3H), 3.38 – 3.32 (m, 2H), 3.32 – 3.30 (m, 1H), 3.28 (d, *J* = 9.3 Hz, 1H), 3.17 (t, *J* = 9.5 Hz, 1H), 3.13 – 3.07 (m, 4H), 2.92 – 2.87 (m, 2H), 2.82 (dd, *J* = 13.0, 3.7 Hz, 1H), 2.23 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq a), 2.09 (dt, *J* = 13.5, 4.8 Hz, 1H, H-2eq b), 2.07 – 2.00 (m, 2H, H-7'a, H-7'b), 1.87 (m, 2H, H-7'a, H-7'b), 1.36 (q, *J* = 12.7 Hz, 1H, H-2ax a), 1.00 (q, *J* = 12.8 Hz, 1H, H-2ax b). ^{13}C NMR (151 MHz, $CDCl_3$) δ 159.3, 159.0, 138.3, 138.2, 138.14, 138.12, 138.0, 137.92, 137.87, 137.6, 137.5, 137.03, 137.00, 136.96, 136.94, 129.8, 128.8, 128.66, 128.65, 128.50, 128.47, 128.41, 128.39, 128.36, 128.34, 128.31, 128.22, 128.17, 128.16, 128.11, 127.90, 127.85, 127.81, 127.77, 127.74, 127.5, 127.2, 113.8, 113.7 (Ar), 106.0 (C-1'a), 105.9 (C-1'b), 98.67 (C-1'''a, C-1'''b), 95.84 (C-1'b), 95.43 (C-1'a), 84.14, 84.10, 82.59, 82.25, 82.10, 81.97, 81.70, 80.77, 80.49, 77.87, 77.52, 75.88, 75.62, 75.56, 75.39, 75.32, 75.03, 75.00, 74.92, 74.52, 74.37, 74.15, 73.97, 73.90, 73.85, 73.42, 73.28, 73.20, 72.93, 72.84, 72.60, 72.36, 72.34, 71.78, 71.73, 71.66, 71.44, 70.94, 70.34, 70.29, 69.93, 63.22, 63.06, 60.67, 60.42, 60.37, 60.29, 59.58, 57.27, 57.21, 55.28, 55.23, 51.08, 50.93, 32.95, 32.66, 32.47, 30.94, 21.02, 14.17. ESI-HRMS: m/z calcd for $C_{82}H_{89}N_{15}O_{16}Na$

$[M + Na]^+$ 1562.6509, found 1562.6514. TsCl (58.3 mg, 0.306 mmol) was added to a stirred solution of compounds **44** (0.292 g, 0.190 mmol) and Hunig's base (0.07 mL, 0.4 mmol) in DCM (1.9 mL). After 28 hours Hunig's base (0.12 mL, 0.67 mmol) and TsCl (0.117 g, 0.614 mmol) were added. After 24 additional hours TsCl (59.2 mg, 0.311 mmol) was added. After 28 additional hours TsCl (50.8 mg, 0.266 mmol) and Hunig's base (0.07 mL, 0.4 mmol) were added. After 18 additional hours the reaction mixture was diluted with Et_2O and washed with 1N HCl, $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was passed through silica gel and used in the next step without further purification. ESI-HRMS: m/z calcd for $C_{89}H_{95}N_{15}O_{18}SNa$ $[M + Na]^+$ 1717.6628, found 1717.6620. To a stirred solution of the 8'-OTs compounds in DCM (1.68 mL) at 0 °C was added TFA (0.19 mL). After 50 minutes the reaction mixture was diluted with Et_2O and washed with water, saturated $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated to give a mixture of diastereomers which were passed through silica gel and used in the next step without further purification. ESI-HRMS: m/z calcd for $C_{81}H_{87}N_{15}O_{17}SNa$ $[M + Na]^+$ 1596.6023, found 1596.6086. The crude residue was stirred in DMF at 0 °C followed by addition of 60% NaH in mineral oil (8.0 mg, 0.2 mmol). After 1.5 hours the reaction was quenched with NH_4Cl solution (1 mL), diluted with Et_2O, and washed with DI water and brine. The organic layer was dried with Na_2SO_4 and concentrated. The crude residue was purified using silica gel column chromatography in 12.5% EtOAc in hexanes to give **45(ax)** (22.6 mg, 0.0161 mmol) in 9% yield as the less polar diastereomer and **45(eq)** (26.4 mg, 0.0188 mmol) in 10% yield as the more polar diastereomer. **45(ax)** $[\alpha]_D^{23}$ = 71.46 (*c* = 1.0, DCM), ^1H NMR (600 MHz, $CDCl_3$) δ 7.42 – 7.10 (m, 35H, Ar-H), 6.03 (d, *J* = 3.8 Hz, 1H, H-1'), 5.63 (d, *J* = 5.2 Hz, 1H, H-1''), 4.95 (d, *J* = 9.5 Hz, 1H, $PhCH_2O$), 4.93 (d, *J* = 10.2 Hz, 1H,

$PhCH_2O$), 4.82 (d, *J* = 1.9 Hz, 1H, H-1’’’), 4.76 – 4.72 (m, 2H, $PhCH_2O$), 4.71 (d, *J* = 10.8 Hz, 1H, $PhCH_2O$), 4.63 (d, *J* = 12.2 Hz, 1H, $PhCH_2O$), 4.60 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.54 (d, *J* = 10.9 Hz, 1H, $PhCH_2O$), 4.52 (d, *J* = 10.4 Hz, 1H, $PhCH_2O$), 4.44 – 4.38 (m, 3H, $PhCH_2O$), 4.31 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.29 – 4.23 (m, 3H, H-3’’, H-4’’, $PhCH_2O$), 4.02 – 3.88 (m, 5H, H-5, H-3’, H-5’, H-6’, H-2’’), 3.85 – 3.80 (m, 2H, H-4’, H-8’), 3.78 – 3.71 (m, 4H, H-8’, H-5’’, H-3’’’, H-5’’’), 3.65 (dd, *J* = 9.8, 8.6 Hz, 1H, H-4), 3.60 – 3.53 (m, 2H, H-5’’, H-6’’’), 3.45 – 3.38 (m, 2H, H-1, H-3), 3.33 (t, *J* = 2.7 Hz, 1H, H-2’’’), 3.28 (t, *J* = 9.2 Hz, 1H, H-6), 3.14 (dd, *J* = 10.2, 3.8 Hz, 1H, H-2’), 3.12 (t, *J* = 2.8 Hz, 1H, H-4’’’), 2.92 (dd, *J* = 12.9, 4.3 Hz, 1H, H-6’’’), 2.23 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.93 – 1.88 (m, 1H, H-7’eq), 1.78 (tdd, *J* = 13.9, 5.5, 2.2 Hz, 1H, H-7’ax), 1.41 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 139.1, 138.5, 138.2, 137.8, 137.6, 137.03, 136.95, 128.7, 128.5, 128.4, 128.33, 128.30, 128.29, 128.25, 128.15, 128.0, 127.8, 127.7, 127.54, 127.48, 127.46, 127.41, 127.3, 127.1 (Ar), 106.2 (C-1’’), 98.6 (C-1’’’), 96.8 (C-1’), 83.8 (C-6), 82.1 (C-2’’), 82.0 (C-4’’), 81.5 (C-5), 77.7 (C-3’), 76.3 (C-4’), 75.5 (C-3’’), 75.4 (C-4), 75.0 ($PhCH_2O$), 74.8 ($PhCH_2O$), 74.2 (C-5’’’), 73.2 ($PhCH_2O$), 73.02 ($PhCH_2O$), 72.97 (C-3’’’), 72.4 ($PhCH_2O$), 72.3 (C-6’), 71.9 ($PhCH_2O$), 71.8 ($PhCH_2O$), 71.5 (C-4’’’), 70.6 (C-5’), 70.0 (C-5’’), 62.6 (C-2’), 62.3 (C-8’), 60.3 (C-1), 59.9 (C-3), 57.4 (C-2’’’), 51.0 (C-6’’’), 32.4 (C-2), 31.1 (C-7’). ESI-HRMS: m/z calcd for $C_{74}H_{83}N_{16}O_{14}$ $[M + NH_4]^+$ 1419.6275, found 1419.6295. **45(eq)** $[\alpha]_D^{23}$ = 79.48 (*c* = 1.0, DCM), ^1H NMR (600 MHz, $CDCl_3$) δ 7.44 – 7.05 (m, 35H, Ar-H), 6.25 (d, *J* = 3.8 Hz, 1H, H-1’), 5.68 (d, *J* = 6.2 Hz, 1H, H-1’’), 4.94 – 4.88 (m, 4H, H-1’’’, $PhCH_2O$), 4.77 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.71 (d, *J* = 11.1 Hz, 1H, $PhCH_2O$), 4.63 – 4.59 (m, 2H, $PhCH_2O$), 4.58 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.52 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.46 – 4.41 (m, 2H, $PhCH_2O$) 4.40 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.30 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.28 – 4.25 (m, 2H, H-3’’, H-4’’), 4.23 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.04 – 3.90 (m, 4H, H-5, H-3’, H-8’,

H-2''), 3.82 (t, *J* = 9.3 Hz, 1H, H-5'), 3.79 (dd, *J* = 10.4, 2.1 Hz, 1H, H-5''), 3.77 – 3.73 (m, 2H, H-3''', H-5'''), 3.72 (t, *J* = 9.4 Hz, 1H, H-4), 3.64 (dd, *J* = 13.0, 8.6 Hz, 1H, H-6'''), 3.56 (dd, *J* = 10.4, 2.8 Hz, 1H, H-5''), 3.51 (ddd, *J* = 11.1, 8.8, 5.2 Hz, 1H, H-6'), 3.44 (ddd, *J* = 12.6, 9.8, 4.5 Hz, 1H, H-3), 3.40 – 3.32 (m, 3H, H-1, H-8', H-2'''), 3.09 (t, *J* = 2.4 Hz, 1H, H-4'''), 3.03 – 2.99 (m, 2H, H-6, H-2'), 2.95 (t, *J* = 9.4 Hz, 1H, H-4'), 2.82 (dd, *J* = 13.0, 3.7 Hz, 1H, H-6'''), 2.14 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 2.07 – 2.03 (m, 1H, H-7'eq), 1.78 (tdd, *J* = 13.0, 11.1, 5.1 Hz, 1H, H-7'ax), 1.22 (q, *J* = 12.7 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 139.3, 138.4, 138.3, 138.0, 137.4, 137.0, 136.9, 128.7, 128.5, 128.43, 128.41, 128.32, 128.27, 128.22, 128.17, 128.0, 127.81, 127.77, 127.70, 127.6, 127.5, 127.4, 127.2, 127.1 (Ar), 105.8 (C-1''), 98.6 (C-1'''), 95.3 (C-1'), 84.3 (C-6), 82.8 (C-2''), 82.1 (C-4''), 81.6 (C-5), 80.8 (C-4'), 77.3 (C-3'), 76.8 (C-6'), 75.5 (C-3''), 75.00 ($PhCH_2O$), 74.96 ($PhCH_2O$), 74.4 (C-5'''), 74.1 (C-4), 73.4 ($PhCH_2O$), 73.2 ($PhCH_2O$), 73.0 (C-5'), 72.8 (C-3'''), 72.3 ($PhCH_2O$), 72.1 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.4 (C-4'''), 70.3 (C-5''), 66.1 (C-8'), 62.9 (C-2'), 60.3 (C-1), 60.0 (C-3), 57.2 (C-2'''), 51.1 (C-6'''), 32.5 (C-2), 32.1 (C-7'). ESI-HRMS: m/z calcd for $C_{74}H_{79}N_{15}O_{14}$ $[M + Na]^+$ 1424.5829, found 1424.5869.

4-*O*-(2-Amino-4,8-anhydro-2,7-dideoxy-D-glycero-α-D-gluco-octapyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine (46(ax)). To a solution of compound **45(ax)** (21.1 mg, 0.015 mmol) in 1:1 1,4-dioxane/10% aqueous AcOH (0.6 mL) was added Pd/C (10 wt%, 43.4 mg). The reaction mixture was stirred under 50 psi H_2 for 19 hours before filtration through Celtie and concentration. The crude residue was purified using CM Sephadex ion exchange column chromatography (0.1-0.8% aqueous NH_4OH) followed by lyophilization with excess acetic acid to give the pentaacetate salt **46(ax)** (6.0 mg, 0.0064 mmol)

in 43% yield as a white powder. $[\alpha]_D^{23}$ = 22.07 (*c* = 0.1, water), ^{1}H NMR (600 MHz, D_2O) δ 5.62 (d, *J* = 4.1 Hz, 1H, H-1'), 5.23 (d, *J* = 2.2 Hz, 1H, H-1''), 5.13 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.38 (dd, *J* = 7.0, 4.9 Hz, 1H, H-3''), 4.24 (dd, *J* = 4.8, 2.1 Hz, 1H, H-2''), 4.17 – 4.14 (m, 1H, H-5'''), 4.10 – 4.03 (m, 3H, H-6', H-4'', H-3'''), 3.81 (t, *J* = 10.1 Hz, 1H, H-3'), 3.77 (dd, *J* = 12.4, 2.9 Hz, 1H, H-5''), 3.72 – 3.65 (m, 3H, H-5, H-8', H-4'''), 3.65 – 3.58 (m, 3H, H-5', H-8', H-5''), 3.56 (t, *J* = 9.4 Hz, 1H, H-4), 3.48 – 3.38 (m, 3H, H-6, H-4', H-2'''), 3.27 (dd, *J* = 13.6, 6.8 Hz, 1H, H-6'''), 3.24 – 3.17 (m, 2H, H-2', H-6'''), 3.13 – 3.06 (m, 1H, H-1), 3.06 – 2.99 (m, 1H, H-3), 2.13 (dt, *J* = 12.8, 4.1 Hz, 1H, H-2eq), 1.76 (s, 16H, H-7'ax, AcOH), 1.71 – 1.66 (m, 1H, H-7'eq), 1.45 (q, *J* = 12.5 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, D_2O) δ 181.2 (AcOH), 109.9 (C-1''), 96.5 (C-1'), 95.4 (C-1'''), 85.0 (C-5), 81.0 (C-4''), 79.4 (C-4), 74.9 (C-3''), 73.8 (C-4'), 73.4 (C-2''), 73.1 (C-6), 70.2 (C-5', C-5'''), 67.7 (C-3'''), 67.6 (C-3'), 67.3 (C-4'''), 63.8 (C-6'), 62.3 (C-8'), 59.9 (C-5''), 54.5 (C-2'), 50.9 (C-2'''), 50.2 (C-1), 48.8 (C-3), 40.3 (C-6'''), 31.6 (C-7'), 30.7 (C-2), 23.1 (AcOH). ESI-HRMS: m/z calcd for $C_{25}H_{47}N_5O_{14}$ $[M + H]^+$ 642.3198, found 642.3193.

4-*O*-(2-Amino-4,8-anhydro-2,7-dideoxy-L-glycerol-α-D-gluco-octapyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine (46(eq)). To a solution of compound **45(eq)** (26.4 mg, 0.019 mmol) in 1:1 1,4-dioxane/10% aqueous AcOH (0.6 mL) was added Pd/C (10 wt%, 43.4 mg). The reaction mixture was stirred under 50 psi H_2 for 19 hours before filtration through Celtie and concentration. The crude residue was purified using CM Sephadex ion exchange column chromatography (0.1-0.8% aqueous NH_4OH) followed by lyophilization with excess acetic acid to give the pentaacetate salt **46(eq)** (9.1 mg, 0.0097 mmol) in 51% yield as a white powder. $[\alpha]_D^{23}$ = 3.70 (*c* = 0.1, water), ^{1}H NMR (600 MHz, D_2O) δ 5.53 (d,

J = 4.1 Hz, 1H, H-1'), 5.22 (d, *J* = 2.7 Hz, 1H, H-1''), 5.13 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.36 (dd, *J* = 6.6, 5.1 Hz, 1H, H-3''), 4.20 (dd, *J* = 5.0, 2.7 Hz, 1H, H-2''), 4.15 (ddd, *J* = 6.6, 4.1, 1.5 Hz, 1H, H-5'''), 4.07 (t, *J* = 3.1 Hz, 1H, H-3'''), 4.05 – 4.02 (m, 1H, H-4''), 3.89 – 3.85 (m, 1H, H-8'), 3.82 (t, *J* = 10.0 Hz, 1H, H-3'), 3.77 – 3.69 (m, 4H, H-4, H-5, H-6', H-5''), 3.66 (dt, *J* = 3.0, 1.4 Hz, 1H, H-4'''), 3.61 (dd, *J* = 12.4, 4.6 Hz, 1H, H-5''), 3.50 (dd, *J* = 10.4, 8.7 Hz, 1H, H-6), 3.44 – 3.37 (m, 3H, H-5', H-8', H-2'''), 3.29 – 3.17 (m, 4H, H-3, H-2', H-6''', H-6'''), 3.14 (td, *J* = 11.5, 10.7, 4.0 Hz, 1H, H-1), 3.06 (t, *J* = 9.5 Hz, 1H, H-4'), 2.24 (dt, *J* = 13.0, 4.3 Hz, 1H, H-2eq), 1.93 – 1.87 (m, 1H, H-7'eq), 1.75 (s, 15H, AcOH), 1.62 – 1.50 (m, 2H, H-2ax, H-7'ax). ^{13}C NMR (151 MHz, D_2O) δ 181.0 (AcOH), 109.7 (C-1''), 96.7 (C-1'), 95.4 (C-1'''), 84.2 (C-5), 81.3 (C-4''), 79.6 (C-4), 78.1 (C-4'), 75.1 (C-3''), 73.6 (C-5'), 73.3 (C-2''), 72.7 (C-6), 70.2 (C-5'''), 68.5 (C-6'), 67.6 (C-3'''), 67.4 (C-3'), 67.2 (C-4'''), 66.0 (C-8'), 59.9 (C-5''), 54.4 (C-2'), 50.8 (C-2'''), 49.8 (C-1), 49.0 (C-3), 40.3 (C-6'''), 33.1 (C-7'), 29.4 (C-2), 23.1 (AcOH). ESI-HRMS: m/z calcd for $C_{25}H_{47}N_5O_{14}$ $[M + H]^+$ 642.3198, found 642.3199.

4'-*O*-allyl-1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-6'-*O*-triisopropylsilyl-1,3,2',2''',6'''-pentadeaminoparomomycin (47). To a stirred solution of compound **25** (2.51 g 1.73 mmol) in DMF (34 mL) was added NaH (0.140 g, 3.50 mmol) and the reaction mixture was stirred for 20 minutes. TBAI (0.200 g, 0.541 mmol) and allylBr (0.30 mL, 3.5 mmol) were added and stirring was continued. After 3 hours the reaction was quenched with aqueous saturated NH_4Cl solution, diluted with Et_2O, washed with DI water and brine, dried with Na_2SO_4, and concentrated. The crude residue was purified using silica gel column chromatography (10% EtOAc in hexanes) to give compound **47** (1.94 g, 1.31 mmol) as a white foam in 76% yield. $[\alpha]_D^{23}$ = 68.80 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.38 (m, 2H,

ArH), 7.36 – 7.23 (m, 18H, ArH), 7.22 – 7.14 (m, 10H, ArH), 6.10 (d, *J* = 3.7 Hz, 1H, H-1'), 5.91 (ddt, *J* = 17.3, 10.7, 5.5 Hz, 1H, -CH_2-**CH**=CH_2), 5.65 (d, *J* = 5.9 Hz, 1H, H-1''), 5.25 (dq, *J* = 17.2, 1.7 Hz, 1H, -CH_2-CH=**CH_2**), 5.14 (dq, *J* = 10.4, 1.5 Hz, 1H, -CH_2-CH=**CH_2**), 4.94 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.87 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.83 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.80 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.67 (d, *J* = 10.7 Hz, 1H, $PhCH_2O$), 4.613 (d, *J* = 11.9, 1H, $PhCH_2O$), 4.610 (d, *J* = 12.1, 1H, $PhCH_2O$), 4.55 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.46 (d, *J* = 11.7 Hz, 1H, $PhCH_2O$), 4.409 (d, *J* = 12.0, 1H, $PhCH_2O$), 4.403 (d, *J* = 12.0, 1H, $PhCH_2O$), 4.33 – 4.21 (m, 5H, H-3'', H-4'', **-CH_2**-CH=CH_2, $PhCH_2O$), 4.13 (ddt, *J* = 12.7, 5.6, 1.5 Hz, 1H, **-CH_2**-CH=CH_2), 4.03 (dd, *J* = 10.4, 9.0 Hz, 1H, H-3'), 3.98 – 3.91 (m, 4H, H-5, H-5', H-6', H-2''), 3.84 (dd, *J* = 5.5, 11.1 Hz, 1H, H-6'), 3.76 – 3.71 (m, 4H, H-4, H-5'', H-3''', H-5'''), 3.59 (dd, *J* = 12.9, 8.4 Hz, 1H, H-6'''), 3.54 (dd, *J* = 10.4, 3.3 Hz, 1H, H-5''), 3.45 (ddd, *J* = 12.4, 9.7, 4.6 Hz, 1H, H-3), 3.45 (ddd, *J* = 12.4, 9.7, 4.6 Hz, 1H, H-1), 3.34 – 3.31 (m, 1H, H-2'''), 3.28 (t, *J* = 9.3 Hz, 1H, H-4'), 3.25 (t, *J* = 9.4 Hz, 1H, H-6), 3.12 – 3.10 (m, 1H, H-4'''), 3.04 (dd, *J* = 10.4, 3.7 Hz, 1H, H-2'), 2.89 (dd, *J* = 12.9, 4.2 Hz, 1H, H-6'''), 2.22 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.36 (q, *J* = 12.7 Hz, 1H, H-2ax), 1.17 – 1.06 (m, 21H, OTIPS). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.3, 138.1, 137.9, 137.7, 137.07, 136.93 (Ar), 134.9 (-CH_2-**CH**=CH_2), 128.7, 128.5, 128.41, 128.38, 128.35, 128.32, 128.31, 128.28, 128.23, 128.16, 127.82, 127.76, 127.73, 127.48, 127.46, 127.42 (Ar), 116.5 (-CH_2-CH=**CH_2**), 105.9 (C-1''), 98.6 (C-1'''), 95.6 (C-1'), 84.2 (C-6), 82.6 (C-2''), 82.0 (C-4''), 81.7 (C-5), 80.1 (C-3'), 78.1 (C-4'), 75.6 (C-3''), 75.4 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.5 (C-4), 74.2 (C-5'''), 73.5 (**-CH_2**-CH=CH_2), 73.3 ($PhCH_2O$), 73.2 ($PhCH_2O$), 72.9 (C-3'''), 72.7 (C-5'), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.4 (C-4'''), 69.9 (C-5''), 63.4 (C-2'), 62.9 (C-6'), 60.4 (C-1), 60.0 (C-3), 57.3 (C-2'''), 51.0 (C-6'''), 32.6 (C-2), 18.1 (^{i}Pr-CH_3), 18.1 (^{i}Pr-CH_3), 12.0 (^{i}Pr-CH-). ESI-HRMS: *m/z* calc for $C_{77}H_{95}N_{15}O_{14}SiNa$ $[M+Na]^+$ 1504.6856, found 1504.6855.

4'-*O*-allyl-1,3,2',2''',6'''-Pentaazido-6,3',2'',5'',3''',4'''-hexa-*O*-benzyl-1,3,2',2''',6'''-pentadeaminoparomomycin (48). To a stirred solution of **47** (2.60 g, 1.75 mmol) in THF (33 mL) was added TBAF solution (1 M in THF, 10.5 mL). The reaction mixture was stirred under argon for 1 hour with monitoring by TLC. After completion, the reaction mixture was concentrated under vacuum and the residue was dissolved in ethyl acetate and washed with saturated aqueous $NaHCO_3$ followed by brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated under vacuum. Purification using silica gel column chromatography (15-30% EtOAc in hexanes) gave the product **48** (2.03 g, 1.53 mmol) in 87% yield as a white foam. $[\alpha]_D^{23}$ = 85.20 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 – 7.38 (m, 2H, ArH), 7.36 – 7.24 (m, 19H, ArH), 7.22 – 7.13 (m, 9H, ArH), 6.13 (d, *J* = 3.7 Hz, 1H, H-1'), 5.89 (ddt, *J* = 17.2, 10.8, 5.5 Hz, 1H, -CH_2-**CH**=CH_2), 5.68 (d, *J* = 5.7 Hz, 1H, H-1''), 5.25 (dq, *J* = 17.2, 1.7 Hz, 1H, -CH_2-CH=**CH_2**), 5.14 (dq, *J* = 10.5, 1.4 Hz, 1H, -CH_2-CH=**CH_2**), 4.98 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.90 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.82 (d, *J* = 10.8 Hz, 1H, $PhCH_2O$), 4.79 (d, *J* = 10.8 Hz, 1H, $PhCH_2O$), 4.72 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.62 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.58 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.49 (d, *J* = 11.9 Hz, 1H, $PhCH_2O$), 4.464 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.460 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.40 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.34 – 4.29 (m, 3H, H-3'', H-4'', $PhCH_2O$), 4.28 – 4.22 (m, 2H, **-CH_2**-CH=CH_2, $PhCH_2O$), 4.11 (ddt, *J* = 12.7, 5.7, 1.5 Hz, 1H, **-CH_2**-CH=CH_2), 4.01 – 3.96 (m, 2H, H-3', H-2''), 3.95 (t, *J* = 9.0 Hz, 1H, H-5), 3.88 (dt, *J* = 10.0, 3.0 Hz, 1H, H-5'), 3.82 (dd, *J* = 10.4, 2.0 Hz, 1H, H-5''), 3.80 – 3.75 (m, 3H, H-6', H-3''', H-5'''), 3.69 – 3.64 (m, 2H, H-6', H-6'''), 3.63 – 3.57 (m, 2H, H-4, H-5''), 3.46-3.41 (m, 2H, H-1, H-3), 3.37 – 3.35 (m, 1H, H-2'''), 3.30 (t, *J* = 9.3 Hz, 1H, H-6), 3.25 (dd, *J* = 10.0, 9.0 Hz, 1H, H-4'), 3.13 – 3.10 (m, 1H, H-4'''), 2.90 (dd, *J* = 10.4, 3.7 Hz, 1H, H-2'), 2.86 (dd, *J* = 13.0, 3.7 Hz, 1H, H-6'''), 2.23 (dt, *J* = 13.1, 4.6 Hz, 1H, H-2eq), 1.40 (q, *J* = 13.1 Hz, 1H, H-2ax). ^{13}C NMR

(151 MHz, $CDCl_3$) δ 138.3, 138.0, 137.9, 137.5, 137.0, 136.9 (Ar), 134.7 (-CH_2-**CH**=CH_2), 128.7, 128.5, 128.42, 128.39, 128.34, 128.27, 128.26, 128.19, 127.83, 127.79, 127.75, 127.70, 127.55, 127.49, 127.1 (Ar), 116.8 (-CH_2-CH=**CH_2**), 106.2 (C-1''), 98.6 (C-1'''), 95.7 (C-1'), 84.2 (C-6), 82.5 (C-2''), 82.1 (C-3''), 82.0 (C-5), 79.5 (C-3'), 77.5 (C-4'), 75.5 (C-4''), 75.3 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.9 (C-4), 74.4 (C-5'''), 73.6 ($PhCH_2O$), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.8 (C-3'''), 72.4 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.6 (C-5'), 71.5 ($PhCH_2O$), 70.3 (C-5''), 63.1 (C-2'), 61.5 (C-6'), 60.3 (C-1), 60.1 (C-3), 57.3 (C-2'''), 51.1 (C-6'''), 32.5 (C-2). ESI-HRMS: *m/z* calc for $C_{68}H_{75}N_{15}O_{14}Na$ $[M+Na]^+$ 1348.5516, found 1348.5515.

4-*O*-(2-azido-3-*O*-benzyl-4,8-anhydro-2,7-dideoxy-D-glycero-α-D-gluco-nona-7-enopyranosyl)-5-*O*-[3-*O*-(2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-2,5-di-*O*-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-*O*-benzyl-2-deoxystreptamine (50(ax)), and 4-*O*-(2-azido-3-*O*-benzyl-4,8-anhydro-2,7-dideoxy-L-glycero-α-D-gluco-nona-7-enopyranosyl)-5-*O*-[3-*O*-(2,6-diazido-3,4-di-*O*-benzyl-2,6-dideoxy-β-L-idopyranosyl)-2,5-di-*O*-benzyl-β-D-ribofuranosyl]-1,3-diazido-6-*O*-benzyl-2-deoxystreptamine (50(eq)). To a stirred solution of DMSO (0.38 mL 5.4 mmol) in 2 mL DCM at -78°C under argon was added oxalyl chloride (0.22 mL, 2.6 mmol). After stirring for 10 minutes, compound **48** (1.70 g, 1.28 mmol) was dissolved in DCM (11 mL) and added dropwise. After an additional 3 hours Et_3N (0.75 mL, 5.4 mmol) was added. The reaction mixture was stirred for an additional 2 hours then diluted with Et_2O, washed with aqueous NH_4Cl solution, DI water, and brine. The organic layer was concentrated under vacuum to give the intermediate aldehyde as a white foam which was used in the next step without further purification. ESI-HRMS: *m/z* calc for $C_{69}H_{77}N_{15}O_{15}Na$ $[M+Na+MeOH]^+$ 1378.5621, found

1378.5647. To a stirred solution of aldehyde in THF (26 mL) at -78°C was added vinylMgBr solution (5.2 mL, 1 M in THF). After stirring for 1 hour the reaction was quenched with aqueous saturated NH_4Cl solution, diluted with Et_2O, washed with aqueous saturated NH_4Cl solution, brine, dried with Na_2SO_4, and concentrated. The crude residue was purified using silica gel column chromatography (15-25% EtOAc in hexanes) to give the inseparable mixture of diastereomers **49** (0.83 g, 0.614 mmol) in 48% yield. **49:** ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.45 – 7.17 (m, 60H, Ar-H), 6.13 – 6.10 (m, 2H, H-1'a, H-1'b), 6.03 – 5.84 (m, 4H, CH_2=**CH**-), 5.70 (d, *J* = 5.7 Hz, 1H, H-1'b), 5.68 (d, *J* = 5.5 Hz, 1H, H-1'a), 5.43 – 5.38 (m, 1H, **CH_2**=CH-CH_2), 5.36 (dt, *J* = 17.4, 1.6 Hz, 1H, **CH_2**=CH-CH_2), 5.31 – 5.22 (m, 5H), 5.19 – 5.14 (m, 2H), 5.03 – 4.98 (m, 2H), 4.96 – 4.91 (m, 2H), 4.86 – 4.82 (m, 3H), 4.79 (d, *J* = 10.7 Hz, 1H), 4.77 – 4.72 (m, 2H), 4.67 – 4.63 (m, 2H), 4.62 – 4.57 (m, 2H), 4.54 (d, *J* = 11.7 Hz, 1H), 4.52 – 4.47 (m, 4H), 4.47 – 4.45 (m, 1H), 4.45 – 4.42 (m, 2H), 4.39 – 4.25 (m, 13H), 4.22 – 4.16 (m, 1H), 4.12 (dd, *J* = 10.1, 3.2 Hz, 1H), 4.10 – 4.06 (m, 1H), 4.04 – 3.98 (m, 4H), 3.98 – 3.91 (m, 3H), 3.87 – 3.77 (m, 6H), 3.72 – 3.64 (m, 3H), 3.63 – 3.59 (m, 2H), 3.55 (t, *J* = 9.3 Hz, 1H), 3.50 – 3.42 (m, 4H), 3.39 (m, 2H, H-2'''a, H-2'''b), 3.37 – 3.27 (m, 3H), 3.21 – 3.16 (m, 1H), 3.15 (m, 2H, H-4'''a, H-4'''b), 2.96 (dd, *J* = 10.3, 3.7 Hz, 1H), 2.92 – 2.87 (m, 3H), 2.25 (m, 2H, H-2eqa, H-2eq b), 1.44 (q, *J* = 12.7 Hz, 1H, H-2ax a), 1.36 (q, *J* = 12.7 Hz, 1H, H-2axb). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.6, 138.4, 138.1, 138.0, 137.6, 137.02, 136.95, 136.0, 134.7, 134.5, 128.7, 128.54, 128.45, 128.43, 128.38, 128.30, 128.23, 128.19, 128.15, 127.86, 127.84, 127.83, 127.78, 127.71, 127.6, 127.5, 127.2, 127.1 (Ar), 117.0 (**CH_2**=CH-CH_2), 116.8 (**CH_2**=CH-CH_2), 116.6 (**CH_2**=CH-CH_2), 115.4 (**CH_2**=CH-CH_2), 106.3 (C-1''), 106.2 (C-1''), 98.7 (C-1'''), 98.6 (C-1'''), 95.73 (C-1'), 95.66 (C-1'), 84.3, 84.1, 82.4, 82.3, 82.2, 82.1, 82.08, 81.97, 80.2, 79.8, 78.8, 77.9, 75.6, 75.5, 75.3, 75.2, 75.07, 75.05, 74.9, 74.5, 74.4, 73.8, 73.4, 73.23, 73.16, 73.08,

72.96, 72.90, 72.42, 72.41, 71.7, 71.5, 70.33, 70.27, 70.0, 63.1 (C-2'), 63.0 (C-2'), 60.43 (C-1), 60.39 (C-1), 60.08 (C-3), 60.06 (C-3), 57.3 (C-2'''), 51.2 (C-6'''), 32.6 (C-2), 32.5 (C-2). ESI-HRMS: *m/z* calc for $C_{70}H_{77}N_{15}O_{14}Na$ $[M+Na]^+$ 1374.5672, found 1374.5682. To a stirred solution of compounds **49** (0.83 g 0.614 mmol) in DCM (6.1 mL) was added Hoveyda-Grubbs generation II catalyst (39.2 mg, 0.038 mmol). The reaction mixture was heated to reflux for 8 hours followed by addition of more catalyst (10.7 mg, 0.010 mmol). After an additional 30 minutes the reaction mixture was filtered through silica gel and concentrated. The crude residue was purified using silica gel column chromatography (25-27.5% EtOAc in hexanes) to give **50(ax)** (0.132 g, 0.100 mmol) in 16% isolated yield, **50(eq)** (0.150 g, 0.113 mmol) in 18% isolated yield, and a mixture of diastereomers (0.120 g, 0.091 mmol) in 15% yield. **50(ax)** $[\alpha]_D^{23}$ = 82.92 (*c* = 0.2, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 21H, ArH), 7.20 – 7.13 (m, 9H, ArH), 6.10 (d, *J* = 3.6 Hz, 1H, H-1'), 5.98 (ddd, *J* = 12.0, 5.7, 2.9 Hz, 1H, H-8'), 5.90 (ddd, *J* = 12.0, 7.2, 2.4 Hz, 1H, H-7'), 5.70 (d, *J* = 6.0 Hz, 1H, H-1''), 5.00 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.90 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.87 (d, *J* = 11.1 Hz, 1H, $PhCH_2O$), 4.78 (d, *J* = 11.2 Hz, 1H, $PhCH_2O$), 4.69 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.61 (t, *J* = 11.7 Hz, 2H, $PhCH_2O$), 4.49 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.47 (s, 2H, $PhCH_2O$), 4.39 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.35 (dt, *J* = 7.2, 2.4 Hz, 1H, H-6'), 4.34 – 4.28 (m, 3H, 3'', 4'', $PhCH_2O$), 4.23 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.20 (dd, *J* = 16.1, 5.7 Hz, 1H, H-9'), 4.08 (dt, *J* = 16.1, 2.8 Hz, 1H, H-9'), 4.04 – 3.99 (m, 3H, H-3', H-5', H-2''), 3.96 (t, *J* = 8.9 Hz, 1H, H-5), 3.85 (dd, *J* = 10.4, 2.0 Hz, 1H, H-5''), 3.78 (ddd, *J* = 8.7, 3.7, 1.9 Hz, 1H, H-5'''), 3.75 (t, *J* = 2.9 Hz, 1H, H-3'''), 3.73 (dd, *J* = 9.6, 8.6 Hz, 1H, H-4'), 3.66 (dd, *J* = 13.0, 8.7 Hz, 1H, H-6'''), 3.61 – 3.55 (m, 2H, 4, H-5''), 3.46 – 3.38 (m, 2H, H-1, H-3), 3.36 – 3.33 (m, 1H, H-2'''), 3.29 (t, *J* = 9.3 Hz, 1H, H-6), 3.11 – 3.08 (m, 1H, H-4'''), 2.90 (dd, *J* = 10.5, 3.6 Hz, 1H, H-2'), 2.84 (dd, *J* = 13.0, 3.7 Hz, 1H, H-6'''), 2.22 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.90 (d, *J*

= 2.4 Hz, 1H, 6'-OH), 1.38 (q, *J* = 12.8 Hz, 1H, H-2ax). ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.5, 138.3, 137.9, 137.5, 137.0, 136.9, 135.4 (C-8'), 128.7, 128.5, 128.4, 128.33, 128.30, 128.29, 128.23, 128.19, 128.0, 127.81, 127.76, 127.69, 127.63, 127.58, 127.46 (Ar), 127.0 (C-7'), 126.9 (Ar), 106.0 (C-1''), 98.7 (C-1'''), 95.7 (C-1'), 84.3 (C-6), 82.6 (C-2''), 82.3 (C-3''), 81.9 (C-3''), 79.4 (C-5), 77.8 (C-4'), 75.6 (C-4''), 75.4 ($PhCH_2O$), 75.0 ($PhCH_2O$), 74.8 (C-4), 74.4 (C-5'''), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.8 (C-3'''), 72.4 (C-5'), 72.3 ($PhCH_2O$), 71.7 ($PhCH_2O$), 71.4 (C-4'''), 70.3 (C-5''), 68.7 (C-6'), 67.5 (C-9'), 62.2 (C-2'), 60.3 (C-1), 60.2 (C-3), 57.2 (2'''), 51.1 (C-6'''), 32.6 (C-2). ESI-HRMS: *m/z* calc for $C_{68}H_{73}N_{15}O_{14}Na$ $[M+Na]^+$ 1346.5359, found 1346.5327. **50(eq)** $[\alpha]_D^{23}$ = 67.96 (*c* = 1.0, $CHCl_3$), ^{1}H NMR (600 MHz, $CHCl_3$) δ 7.39 – 7.23 (m, 21H, ArH), 7.22 – 7.15 (m, 9H, ArH), 6.18 (d, *J* = 3.6 Hz, 1H, H-1'), 5.85 (ddt, *J* = 12.2, 6.0, 3.0 Hz, 1H, H-8'), 5.79 (dt, *J* = 12.2, 2.2 Hz, 1H, H-7'), 5.68 (d, *J* = 5.7 Hz, 1H, H-1''), 4.98 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.90 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.87 (d, *J* = 11.0 Hz, 1H, $PhCH_2O$), 4.77 (d, *J* = 11.1 Hz, 1H, $PhCH_2O$), 4.73 (d, *J* = 10.6 Hz, 1H, $PhCH_2O$), 4.63 (d, *J* = 12.1 Hz, 1H, $PhCH_2O$), 4.56 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.49 (d, *J* = 11.8 Hz, 1H, $PhCH_2O$), 4.45 (d, *J* = 11.7 Hz, 2H, $PhCH_2O$), 4.41 (d, *J* = 12.0 Hz, 1H, $PhCH_2O$), 4.32 (dd, *J* = 7.6, 4.3 Hz, 3H, H-3'', H-4''), 4.28 – 4.23 (m, 2H, 9', $PhCH_2O$), 4.19 – 4.15 (m, 1H, H-6'), 4.01 – 3.93 (m, 3H, H-5, H-3', H-2''), 3.86 (dq, *J* = 15.7, 2.6 Hz, 1H, H-9'), 3.83 – 3.78 (m, 2H, H-5'', H-5'''), 3.77 – 3.72 (m, 2H, H-5', H-3'''), 3.67 (dd, *J* = 13.0, 8.7 Hz, 1H, H-6'''), 3.62 – 3.56 (m, 2H, H-4, H-5''), 3.48 – 3.40 (m, 2H, H-1, H-3), 3.38 – 3.36 (m, 1H, H-2'''), 3.30 (t, *J* = 9.3 Hz, 1H, H-6), 3.17 (dd, *J* = 9.6, 8.5 Hz, 1H, H-4'), 3.12 (t, *J* = 2.7 Hz, 1H, H-4'''), 3.00 (dd, *J* = 10.4, 3.6 Hz, 1H, H-2'), 2.86 (dd, *J* = 13.0, 3.7 Hz, 2H, H-6'''), 2.22 (dt, *J* = 13.2, 4.6 Hz, 1H, H-2eq), 1.42 (q, *J* = 12.7 Hz, 1H, H-2ax) ^{13}C NMR (151 MHz, $CDCl_3$) δ 138.4, 138.3, 137.9, 137.5, 137.0, 136.9, 132.7 (C-7'), 128.7 (Ar), 128.6 (C-8'), 128.5, 128.4, 128.35, 128.32, 128.27, 128.21, 128.1, 127.84, 127.82, 127.77, 127.74, 127.6,

127.51, 127.50, 127.2 (Ar), 106.1 (C-1''), 98.6 (C-1'''), 94.8 (C-1'), 84.2 (C-6), 83.8 (C-4'), 82.4 (C-2''), 82.1 (C-3''), 82.0 (C-5), 77.8 (C-3'), 75.6 ($PhCH_2O$), 75.4 (C-4''), 75.0 (C-4), 74.5 (C-5'''), 73.7 (C-6'), 73.2 ($PhCH_2O$), 73.1 ($PhCH_2O$), 72.8 (C-3'''), 72.4 ($PhCH_2O$), 72.0 (C-5'), 71.7 ($PhCH_2O$), 71.5 (C-4'''), 70.3 (C-5''), 67.7 (C-9'), 62.5 (C-2'), 60.3 (C-3), 60.2 (C-2), 57.2 (C-2'''), 51.1 (C-6'''), 32.4 (C-2). ESI-HRMS: *m/z* calc for $C_{68}H_{73}N_{15}O_{14}Na$ $[M+Na]^+$ 1346.5359, found 1346.5348.

4-*O*-(2-amino-4,9-anhydro-2,7,8-trideoxy-D-glycero-α-D-gluco-nonapyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine (51(ax)). To a solution of compound **50(ax)** (30.0 mg, 0.023 mmol) in 1:1 1,4-dioxane/10% aqueous AcOH (0.6 mL) was added Pd/C (10 wt%, 64.8 mg). The reaction mixture was stirred under 50 psi H_2 for 26 hours before filtration through Celtie and concentration. The crude residue was purified using CM Sephadex ion exchange column chromatography (0.1-0.7% aqueous NH_4OH) followed by lyophilization with acetic acid to give the pentaacetate salt **51(ax)** (6.8 mg, 0.0071 mmol) in 31% yield as a white powder. $[\alpha]_D^{23}$ = 13.33 (*c* = 0.03, water), ^{1}H NMR (600 MHz, D_2O) δ 5.64 (d, *J* = 3.9 Hz, 1H, H-1'), 5.22 (d, *J* = 2.3 Hz, 1H, H-1''), 5.12 (d, *J* = 1.8 Hz, 1H, H-1'''), 4.36 (dd, *J* = 6.9, 4.9 Hz, 1H, H-3''), 4.23 (dd, *J* = 4.9, 2.3 Hz, 1H, H-2''), 4.14 (ddd, *J* = 6.9, 4.0, 1.5 Hz, 1H, H-5'''), 4.05-4.08 (m, 3H, H-6', H-4'', H-3'''), 3.77 – 3.73 (m, 2H, H-3', H-5''), 3.72 – 3.67 (m, 2H, H-5, H-9'), 3.67 – 3.59 (m, 3H, H-4, H-5'', H-4'''), 3.50 (dd, *J* = 10.0, 3.4 Hz, 1H, H-5'), 3.47 (dd, *J* = 10.5, 9.1 Hz, 1H, H-6), 3.41 (dt, *J* = 3.0, 1.3 Hz, 1H, H-2'''), 3.36 (t, *J* = 9.4 Hz, 1H, H-4'), 3.26 (dd, *J* = 13.7, 6.7 Hz, 1H, H-6'''), 3.20 (dd, *J* = 13.7, 3.9 Hz, 1H, H-6'''), 3.16 (dd, *J* = 11.0, 3.9 Hz, 1H, H-2'), 3.11 (m, 2H, H-1, H-3), 2.17 (dt, *J* = 12.7, 4.3 Hz, 1H, H-2eq), 1.93 (m, 1H, H-8'), 1.84 – 1.77 (m, 1H, H-7'), 1.75 (s, 15H, AcOH), 1.58 – 1.46 (m, 3H, H-2ax, H-7', H-8'). ^{13}C NMR (151 MHz, D_2O)

δ 179.7 (AcOH), 108.6 (C-1''), 94.3 (C-1'), 94.0 (C-1'''), 83.6 (C-5), 79.7 (C-4''), 77.0 (C-4), 73.6 (C-3''), 73.4 (C-5'), 72.9 (C-4'), 72.0 (C-2''), 71.5 (C-6), 68.8 (C-5'''), 67.7 (C-9'), 66.5 (C-6'), 66.4 (C-3'), 66.3 (C-3'''), 65.9 (C-4'''), 58.5 (C-5''), 52.3 (C-2'), 49.4 (C-2'''), 48.7 (C-1), 47.4 (C-3), 39.0 (C-6'''), 28.6 (C-2), 24.5 (C-7'), 21.7 (AcOH), 17.7 (C-8'). ESI-HRMS: *m/z* calc for $C_{26}H_{50}N_5O_{14}$ $[M+H]^+$ 656.3354, found 656.3372.

4-*O*-(2-amino-4,9-anhydro-2,7,8-trideoxy-L-glycero-α-D-gluco-nonapyranosyl)-5-*O*-[3-*O*-(2,6-diamino-2,6-dideoxy-β-L-idopyranosyl)-β-D-ribofuranosyl]-2-deoxystreptamine (51(eq)). To a solution of compound **50(eq)** (22.8 mg, 0.017 mmol) in 1:1 1,4-dioxane/10% aqueous AcOH (0.6 mL) was added Pd/C (10 wt%, 48.2 mg). The reaction mixture was stirred under 50 psi H_2 for 23 hours before filtration through Celtie and concentration. The crude residue was purified using CM Sephadex ion exchange column chromatography (0.1-0.8% aqueous NH_4OH) followed by lyophilization with acetic acid to give the pentaacetate salt **51(eq)** (2.9 mg, 0.0030 mmol) in 18% yield as a white powder. $[\alpha]_D^{23}$ = 80.43 (*c* = 0.05, water), ^{1}H NMR (600 MHz, D_2O) δ 5.40 (d, *J* = 3.8 Hz, 1H, H-1'), 5.22 (d, *J* = 3.2 Hz, 1H, H-1''), 5.14 (d, *J* = 1.9 Hz, 1H, H-1'''), 4.36 (t, *J* = 5.7 Hz, 1H, H-3''), 4.19 – 4.15 (m, 2H, H-2'', H-5'''), 4.08 (t, *J* = 3.2 Hz, 1H, H-3'''), 4.07 – 4.04 (m, 1H, H-4''), 3.75 (dd, *J* = 12.4, 3.2 Hz, 1H, H-5''), 3.74 – 3.58 (m, 6H, H-4, H-5, H-3', H-9', H-5'', H-4'''), 3.53 – 3.39 (m, 4H, H-6, H-5', H-6', H2'''), 3.29 (dd, *J* = 13.6, 6.8 Hz, 1H, H-6'''), 3.25 – 3.18 (m, 2H, H-4', H-6'''), 3.15 (dd, *J* = 10.8, 3.8 Hz, 1H, H-2'), 3.13 – 3.07 (m, 2H, H-1, H-3), 2.16 (dt, *J* = 12.9, 4.3 Hz, 1H, H-2eq), 1.81 – 1.61 (m, 18H, H-7', H-8', AcOH), 1.57 – 1.44 (m, 2H, H-2ax, H-7'). ^{13}C NMR (151 MHz, D_2O) δ 181.3 (AcOH), 109.5 (C-1''), 96.8 (C-1'), 95.8 (C-1'''), 84.1 (C-5), 81.32 (C-4''), 81.28 (C-4), 76.6 (C-5'), 75.3 (C-3''), 74.8 (C-6'), 74.6 (C-4'), 73.3 (C-2''), 73.1 (C-6),

70.2 (C-5’’’), 68.9 (C-3’), 68.5 (C-9’), 67.8 (C-3’’’), 67.3 (C-4’’’), 60.1 (C-5’’), 54.1 (C-2’), 50.9 (C-2’’’), 50.0 (C-1), 49.3 (C-3), 40.4 (C-6’’’), 30.6 (C-2), 28.1 (C-7’), 23.1 (AcOH), 21.9 (C-8’). ESI-HRMS: *m/z* calc for $C_{26}H_{50}N_5O_{14}$ $[M+H]^+$ 656.3354, found 656.3359.

Methyl 2-amino-2,4-dideoxy-α-D-xylopyranoside (53). Compound **57** (93.8 mg, 0.30 mmol) was dissolved in 0.8 mL of a 1:1 mixture of 1,4-Dioxane and 10% aqueous AcOH followed by addition of Pd/C (20.8 mg). The reaction mixture was stirred under 50 psi H_2 for 5 hours followed by filtration over Celite® and lyophilization to obtain **53** as an off white solid (69.1 mg, 97%). $[\alpha]_D^{23}$ = 110.56 (*c* = 0.14, water), ^{1}H NMR (600 MHz, D_2O) δ 4.86 (d, *J* = 3.7 Hz, 1H, H1), 3.91 (td, *J* = 11.0, 5.1 Hz, 1H, H3), 3.79 (ddt, *J* = 11.8, 5.8, 2.5 Hz, 1H, H5), 3.51 (dd, *J* = 12.1, 3.1 Hz, 1H, H6), 3.43 (dd, *J* = 12.1, 6.2 Hz, 1H, H6’), 3.24 (s, 3H, OMe), 3.02 (dd, *J* = 10.5, 3.6 Hz, 1H, H2), 1.86 (ddd, *J* = 12.8, 5.1, 2.1 Hz, 1H, H4eq), 1.76 (s, 3H, AcOH), 1.33 (q, *J* = 12.0 Hz, 1H, H4ax). ^{13}C NMR (151 MHz, D_2O) δ 180.4 (AcOH), 96.6 (C1), 68.8 (C5), 64.3 (C3), 63.3 (C6), 55.2 (C2), 55.0 (OMe), 34.2 (C4), 22.6 (AcOH). ESI-HRMS: m/z calcd for $C_7H_{15}NO_4Na$ $[M + Na]^+$ 200.0899, found 200.0891.

4-*C*-Allyl-1,6-anhydro-2-*N*-benzyl-2,4-dideoxy-β-D-glucopyranose (59). A solution of **58** (0.3188 g, 1.90 mmol) in benzylamine (5.0 ml) was stirred for 3 days at 155°C. After concentration under reduced pressure the crude residue was purified by Flash column chromatography on silica gel eluting with 40% ethyl acetate in hexanes with 1% triethylamine added to afford **59** (0.4020 g, 77%). $[\alpha]_D^{23}$ = -49.4 (c = 1.00, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 – 7.23 (m, 5H: aromatic), 5.83 (ddq, *J* = 16.4, 14.4, 6.2, 5.3 Hz, 1H: CH_2CHCH_2-C4), 5.47 (s, 1H: H1), 5.21 – 5.10 (m, 2H: CH_2CHCH_2-C4), 4.40 (d, *J* = 5.0 Hz, 1H: H5), 4.06 (d, *J* = 7.0 Hz, 1H: H6a), 3.93 – 3.85 (m, 5H: $PhCH_2$), 3.80 – 3.72 (m, 1H, H6b), 3.65 (td, *J* = 2.9, 1.6 Hz, 1H: H3), 2.68 – 2.63 (m, 1H: H2), 2.49 – 2.33 (m,

2H: CH_2CHCH_2-C4), 1.77 (t, *J* = 7.2 Hz, 1H: H4). ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.9, 136.1 (CH_2CHCH_2-C4), 128.5, 128.1, 127.2, 117.5 (CH_2CHCH_2-C4), 102.6 (C1), 74.6 (C5), 70.3 (C3), 68.5 (C6), 62.2 (C2), 51.7 ($PhCH_2$), 44.6 (C4), 36.5 (CH_2CHCH_2-C4). ESI-HRMS: *m/z* calcd for $C_{16}H_{22}NO_3Na$ $[M+Na]^+$ 276.1600, found 276.1600.

2-amino-1,6-anhydro-2,4-dideoxy-4-*C*-propyl-β-D-glucopyranose acetate salt (60). Compound **59** (0.4020 g, 1.46 mmol) and Pd/C (0.1316 g) were stirred in a 1:2 mixture of 10% aqueous acetic acid and 1,4-dioxane (6 mL) under 40 psi H_2 for 7 hours. Pd/C (0.23 g) was added after 7 hours and the reaction mixture was stirred for an additional 14 hours before final addition of Pd/C (0.39 g). After an additional 28 hours the reaction mixture was filtered through Celite® and concentrated to give the product **60** (0.2858 g, 79%). $[\alpha]_D^{23}$ = -45.9 (*c* = 1.0, MeOH), ^1H NMR (600 MHz, CD_3OD) δ 5.32 (s, 1H, H-1), 4.41 (d, *J* = 5.2 Hz, 1H, H-5), 3.98 (d, *J* = 6.8 Hz, 1H, H-6a), 3.68 (dd, *J* = 6.9, 5.3 Hz, 1H, H-6b), 3.43 (t, *J* = 4.0 Hz, 1H, H-3), 2.82 (d, *J* = 4.0 Hz, 1H, H-2), 1.92 (s, 3H, AcOH), 1.64 – 1.47 (m, 4H, H-4, -CH_2CH_2-), 1.45 – 1.34 (m, 1H, -CH_2CH_2-), 0.97 (t, *J* = 7.3 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 104.9 (C-1), 79.1 (C-5), 74.6 (C-6), 72.6 (C-3), 60.7 (C-2), 48.8 (C-4), 37.9 (-**C**$H_2CH_2CH_3$), 25.4 (AcOH), 23.9 (-CH_2**C**H_2CH_3), 16.9 (-CH_2CH_2**C**H_3). ESI-HRMS: m/z calcd for $C_9H_{18}NO_3$ $[M + H]^+$ 188.1287, found 188.1286.

1,6-anhydro-2-azido-2,4-dideoxy-4-*C*-propyl-β-D-glucopyranose (61). Stick's reagent (0.4664 g, 2.23 mmol) was added to an ice cold stirred solution of $CuSO_4$ (0.0237 g, 0.148 mmol), triethylamine (0.62 mL, 4.5 mmol), and compound **60** (0.2778 g, 1.12 mmol) in 4:1 MeCN/water (14.8 mL). The reaction mixture was stirred for 1 hour before MeCN was removed under vacuum and the residue was diluted with EtOAc, washed with 1N HCl, saturated $NaHCO_3$ solution, and

brine. The organic layer was dried with Na_2SO_4 and concentrated to give **61** as a colorless gum (0.2186 g, 91%). $[\alpha]_D^{23}$ = -113.2 (*c* = 1.0, $CHCl_3$) ^{1}H NMR (499 MHz, $CDCl_3$) δ 5.46 (t, *J* = 1.4 Hz, 1H, H-1), 4.43 (d, *J* = 5.1 Hz, 1H, H-5), 4.10 (dd, *J* = 7.0, 0.8 Hz, 1H, H-6a), 3.78 (dd, *J* = 7.1, 5.1 Hz, 1H, H-6b), 3.65 (tt, *J* = 2.4, 1.2 Hz, 1H, H-3), 3.47 (t, *J* = 2.0 Hz, 1H, H-2), 1.72 – 1.56 (m, 3H, H-4, -**CH$_2$**CH_2CH_3), 1.55 – 1.45 (m, 1H, -CH_2**CH$_2$**CH_3), 1.44 – 1.34 (m, 1H, -CH_2**CH$_2$**CH_3), 0.97 (t, *J* = 7.2 Hz, 3H, -CH_2CH_2**CH$_3$**). ^{13}C NMR (126 MHz, $CDCl_3$) δ 100.6 (C-1), 75.0 (C-5), 71.2 (C-3), 68.6 (C-6), 63.6 (C-2), 44.3 (C-4), 33.3 (CH_3CH_2**CH$_2$**-), 20.5 (CH_3**CH$_2$**CH_2-), 14.0 (**CH$_3$**CH_2CH_2-). Compound not visible by ESIMS

Methyl 2-azido-2,4-dideoxy-4-*C*-propyl-D-glucopyranoside (62α and 62β). Compound **61** (0.219 g, 0.857 mmol) was dissolved in 8.6 mL Ac_2O followed by addition of 0.86 mL TFA and stirred under argon for 45 minutes. The reaction mixture was then diluted with Et_2O and washed with saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, filtered, and concentrated. The crude residue was then dissolved in 10% HCl MeOH solution (7 mL) and heated to reflux for 4.5 hours followed by concentration under vacuum. The resulting residue was subjected to flash column chromatography over silica gel in 45% to 50% ethyl acetate in hexanes which afforded 55 mg **62α** (22%) and 31 mg **62β** (12%). **62α**: $[\alpha]_D^{23}$ = 125.4 (*c* = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 4.76 (d, *J* = 3.5 Hz, 1H, H1), 3.78 (t, *J* = 10.2 Hz, 1H, H3), 3.74 – 3.68 (m, 1H, H6), 3.62 – 3.54 (m, 2H, H5, H6), 3.37 (s, 3H, OMe), 3.10 (dd, *J* = 10.0, 3.5 Hz, 1H, H2), 1.62 – 1.50 (m, 2H, H4, -CH_2CH_2-), 1.49 – 1.28 (m, 3H, -CH_2CH_2-), 0.91 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 99.2 (C1), 71.8 (C5), 68.5 (C3), 65.2 (C2), 61.8 (C6), 53.9 (OMe), 43.1 (C4), 28.8 (-CH_2CH_2-), 18.7 (-CH_2CH_2-), 13.7 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{19}N_3O_4Na$ $[M + Na]^+$ 268.1273, found

268.1273. **62β**: $[\alpha]_D^{23}$ = -31.8 (*c* = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 4.13 (d, *J* = 8.1 Hz, 1H, H1), 3.77 (dd, *J* = 12.1, 2.1 Hz, 1H, H6), 3.59 (dd, *J* = 12.1, 5.5 Hz, 1H, H6), 3.52 (s, 3H, OMe), 3.32 – 3.24 (m, 2H, H3, H5), 3.03 (dd, *J* = 9.5, 8.1 Hz, 1H, H2), 1.58 – 1.25 (m, 5H, H4, -CH_2CH_2-), 0.90 (t, *J* = 7.2 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 102.7 (C1), 76.2 (C5), 72.6 (C3), 68.6 (C2), 61.8 (C6), 55.6 (OMe), 42.6 (C4), 28.7 (-CH_2CH_2-), 18.6 (-CH_2CH_2-), 13.7 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{19}N_3O_4Na$ $[M + Na]^+$ 268.1273, found 268.1261.

Methyl 2-amino-2,4-dideoxy-4-*C*-propyl-α-D-glucopyranoside (55). Compound **62α** (13.6 mg, 0.30 mmol) was dissolved in 0.6 mL of a 1:1 mixture of 1,4-Dioxane and 10% aqueous AcOH followed by addition of Pd/C (2.9 mg). The reaction mixture was stirred under 50 psi H_2 for 1.5 hours followed by filtration over Celite® and lyophilization to obtain **55** as an off white solid (15.4 mg, 99%). $[\alpha]_D^{23}$ = 80.5 (*c* = 0.7, water), ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, *J* = 3.6 Hz, 1H, H1), 3.70 (t, *J* = 10.6 Hz, 1H, H3), 3.66 (dd, *J* = 12.3, 2.2 Hz, 1H, H6), 3.62 (ddd, *J* = 10.9, 5.3, 2.2 Hz, 1H, H5), 3.54 (dd, *J* = 12.3, 5.3 Hz, 1H, H6), 3.24 (s, 3H, OMe), 3.07 (dd, *J* = 10.3, 3.6 Hz, 1H, H2), 1.79 (s, 3H, AcOH), 1.49 (tt, *J* = 10.8, 4.0 Hz, 1H, H4), 1.41 – 1.32 (m, 1H, -CH_2CH_2-), 1.32 – 1.24 (m, 1H, -CH_2CH_2-), 1.23 – 1.06 (m, 2H, -CH_2CH_2-), 0.71 (t, *J* = 7.2 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, D_2O) δ 96.3 (C1), 71.6 (H5), 67.0 (C3), 61.1 (6), 55.3 (C2), 54.9 (OMe), 42.0 (C4), 27.7 (-CH_2CH_2-), 17.8 (-CH_2CH_2-), 13.8 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{22}NO_4$ $[M + H]^+$ 220.1549, found 220.1539.

1,6-Anhydro-4-deoxy-2-*O*-*p*-toluenesulfonyl-6-(*S*)-deuterio-β-D-glucopyranose (68). $NaBH_4$ (0.43 g, 11.4 mmol) and $BF_3{\cdot}OEt_2$ (0.85 mL, 6.9 mmol) were added to an ice cold solution of **67** (0.84 g, 2.8 mmol) in 1,2-dimethoxyethane. After 5 hours the reaction mixture was diluted

with Et_2O and washed with saturated $NaHCO_3$ solution and brine then dried with Na_2SO_4 and concentrated under vacuum to give **68** as a clear gum (0.85 g, 2.8 mmol 99%) which was used in the next step without purification. $[\alpha]_D^{23}$ = -27.02 (*c* = 1.0, $CHCl_3$), ^1H NMR (600 MHz, $CDCl_3$) δ 7.81 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.36 – 7.33 (m, 2H, Ar-H), 5.27 (d, *J* = 1.9 Hz, 1H, H-1), 4.52 (d, *J* = 4.1 Hz, 1H, H-5), 4.20 (q, *J* = 1.7 Hz, 1H, H-2), 4.09 (s, 1H, H-6), 3.92 (t, *J* = 6.3 Hz, 1H, H-3), 2.46 (d, *J* = 6.3 Hz, 1H, -OH), 2.44 (s, 3H, Ar-CH_3), 2.31 (ddd, *J* = 15.0, 5.7, 4.5 Hz, 1H, H-4ax), 1.70 (ddt, *J* = 15.0, 1.9, 1.0 Hz, 1H, H-4eq). ^{13}C NMR (151 MHz, $CDCl_3$) δ 145.3, 133.2, 130.1, 127.9 (Ar), 99.3 (C-1), 76.8 (C-2), 71.3 (C-5), 67.5 (t, *J* = 23.3 Hz, C-6), 66.5 (C-3), 32.5 (C-4), 21.7 (Ar-CH_3). ESI-HRMS: m/z calcd for $C_{13}H_{15}DO_6SNa$ $[M + Na]^+$ 324.0628, found 324.0627.

1,6;2,3-Bisanhydro-4-deoxy-6-(*S*)-deuterio-D-lyxopyranose (69). NaOMe (0.34 g, 6.3 mmol) was added to an ice cold stirred solution of **68** (0.824 g, 2.73 mmol) in 1:1 $CHCl_3$/MeOH (13.6 mL). After 4 hours the reaction mixture was diluted with DCM and washed with water. The aqueous phase was back extracted with DCM and the combined organic layers were washed with brine then dried with Na_2SO_4 and concentrated under vacuum to give **69** as a colorless oil (0.351 g, 99%). $[\alpha]_D^{23}$ = -28.60 (*c* = 1.0, DCM), ^1H NMR (400 MHz, $CDCl_3$) δ 5.64 (dd, *J* = 3.2, 1.0 Hz, 1H, H-1), 4.38 (br d, *J* = 6.0 Hz, 1H, H-5), 3.63 (d, *J* = 1.7 Hz, 1H, H-6), 3.33 (t, *J* = 3.6 Hz, 1H, H-2), 3.10 (dd, *J* = 3.3 Hz, 1H, H-3), 2.19 (ddd, *J* = 15.3, 5.9, 3.2 Hz, 1H, H-4ax), 1.94 (d, *J* = 15.3 Hz, 1H, H-4eq). ^{13}C NMR (101 MHz, $CDCl_3$) δ 98.0 (C-1), 68.0 (t, *J* = 23.1 Hz, C-6), 67.2 (C-5), 53.7 (C-2), 46.3 (C-3), 30.0 (C-4). ESI-HRMS: m/z calcd for $C_6H_7DO_3Na$ $[M + Na]^+$ 152.0434, found 152.0438.

1,6-Anhydro-2-azido-2,4-dideoxy-D-xylopyranose (70ax). Compound **69** (75.0 mg, 0.581 mmol), LiN_3 (145.1 mg, 2.964 mmol), benzoic acid (109.1 mg, 0.8934 mmol), and DMF (2.0

mL) were added to a microwave vial and irradiated while stirring in a Biotage® Initiator set to 110 ^{o}C for 75 minutes. The reaction mixture was then diluted with DCM and washed with aqueous saturated $NaHCO_3$ solution and brine. The organic layer was dried with Na_2SO_4 and filtered followed by concentration. The residue was subjected to silica gel preparative HPLC eluting with a gradient from 20 % to 60 % EtOAc in hexanes to give **70(ax)** (4.7 mg, 0.027 mmol) in 5 % isolated yield. $[\alpha]_D^{23}$ = 19.57 (*c* = 0.2, $CHCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.53 (t, *J* = 2.0 Hz, 1H, H-1), 4.59 (d, *J* = 4.4 Hz, 1H, H-5), 4.19 (s, 1H, H-6), 4.00 – 3.96 (m, 1H, H-3), 3.30 (d, *J* = 2.2 Hz, 1H, H-2), 2.55 (d, *J* = 7.7 Hz, 1H, -OH), 2.31 (dt, *J* = 15.2, 4.9 Hz, 1H, H-4ax), 1.84 (ddt, *J* = 15.3, 2.9, 1.6 Hz, 1H, H-4eq). ^{13}C NMR (151 MHz, $CDCl_3$) δ 100.8 (C-1), 72.0 (C-5), 67.6 (t, *J* = 23.3 Hz, C-6), 66.9 (C-3), 61.7 (C-2), 33.6 (C-4).

Methyl 2-amino-2,4-dideoxy-6-(*S*)-deuterio-D-xylopyranose acetate salt (54) TFA (0.05 mL) was added to a stirred solution of compound **70(ax)** (4.6 mg, 0.27 mmol) in Ac_2O (0.5 mL) at 0 ^{o}C and the reaction mixture was stirred for 3 hours monitoring by TLC. The reaction mixture was then diluted with Et_2O and washed with aqueous saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, and concentrated to give an inseparable mixture of anomers (8.3 mg, 0.026 mmol, 97 %) which were used in the next step without purification. ESI-HRMS: m/z calcd for $C_{12}H_{16}DN_3O_7Na$ $[M + Na]^+$ 339.1014, found 339.1027. The mixture of anomers from the previous step (8.3 mg, 0.026 mmol) were stirred in 10 % HCl methanol solution (0.6 mL) under reflux for 3 hours. After the reaction was complete by TLC and LCMS the reaction mixture was concentrated under vacuum and the resulting inseparable mixture of anomers of methyl glycosides was used in the next step without purification. ESI-HRMS: m/z calcd for $C_7H_{12}DN_3O_4Na$ $[M + Na]^+$ 227.0867,

found 227.0864. 10 wt% Pd/C (5.0 mg) was added to a solution of methyl glycosides (6.1 mg, 0.30 mmol) in 1:1 dioxane/10% aqueous acetic acid (0.4 mL) and the reaction mixture was stirred under 50 psi H_2 for 6 hours. The reaction mixture was then filtered through Celite® and concentrated under vacuum to give a mixture of anomers **54** (5.4 mg, 0.023 mmol, 76%) in a ratio of 2:1 α/β as the acetate salts. **54α:** ^{1}H NMR (600 MHz, D_2O) δ 4.87 (d, *J* = 3.6 Hz, 1H, H-1), 3.92 (td, *J* = 11.0, 5.0 Hz, 1H, H-3), 3.80 (ddd, *J* = 12.1, 6.3, 2.2 Hz, 1H, H-5), 3.42 (d, *J* = 6.0 Hz, 1H, H-6), 3.26 (s, 3H, -OCH_3), 3.03 (dd, *J* = 10.4, 3.7 Hz, 1H, H-2), 1.87 (ddd, *J* = 12.1, 5.0, 2.1 Hz, 1H, H-4eq), 1.78 (s, 3H, AcOH), 1.34 (q, *J* = 12.1 Hz, 1H, H-4ax). ^{13}C NMR (151 MHz, D_2O) δ 180.2 (AcOH), 96.6 (C-1), 68.7 (C-5), 64.3 (C-3), 63.2 – 62.8 (m, C-6), 55.2 (C-2), 55.0 (-OCH_3), 34.2 (C-4), 22.5 (AcOH). **54β:** ^{1}H NMR (600 MHz, D_2O) δ 4.37 (d, *J* = 8.4 Hz, 1H, H-1), 3.76 (dt, *J* = 10.9, 5.4 Hz, 1H, H-3), 3.57 (ddd, *J* = 11.8, 6.7, 2.1 Hz, 1H, H-5), 3.45 (d, *J* = 6.6 Hz, 1H, H-6), 3.41 (s, 3H, -OCH_3), 2.69 (dd, *J* = 10.3, 8.4 Hz, 1H, H-2), 1.91 (ddd, *J* = 12.9, 5.2, 2.0 Hz, 1H, H-4eq), 1.78 (s, 3H, AcOH), 1.31 (dt, *J* = 12.9, 11.5 Hz, 1H, H-4ax). ^{13}C NMR (151 MHz, D_2O) δ 180.2 (AcOH), 100.1 (C-1), 72.7 (C-5), 66.8 (C-3), 63.1 – 62.6 (m, C-6), 57.4 (C-2), 57.2 (-OCH_3), 34.4 (C-4), 22.5 (AcOH). ESI-HRMS: m/z calcd for $C_7H_{14}DNO_4Na$ $[M + Na]^+$ 201.1962, found 201.1956.

4-*C*-*A*llyl-1,6-Anhydro-6-(*S*)-deuterio-2,4-dideoxy-2-*O*-p-toluenesulfonyl-β-D-glucopyranose (71). Freshly prepared 0.5 M allylMgCl THF solution (16 mL) was added to an ice-cold stirred solution of epoxide **67** (0.590 g 1.97 mmol) and CuI (0.38 g, 0.20 mmol) in THF (20 mL). The reaction mixture was stirred for 11 hours followed by addition of more allylMgCl solution (8 mL). After another 17 hours the reaction mixture was concentrated under vacuum then diluted with Et_2O and washed with aqueous saturated NH_4Cl solution and brine. The organic

layer was dried with Na_2SO_4 and concentrated. The crude residue was then purified using silica gel flash column chromatography in 35 to 40 % EtOAc in hexanes to give **71** (0.24 g, 36 %). NMR spectra of **71** matched that of the non-deuterated isotopomer. $[\alpha]_D^{23}$ = -51.3 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 – 7.78 (m, 2H, Ar-H), 7.37 – 7.33 (m, 2H, Ar-H), 5.74 (ddt, *J* = 16.3, 10.5, 7.1 Hz, 1H, CH_2**CH**CH_2-), 5.27 (d, *J* = 1.5 Hz, 1H, H-1), 5.13 (dq, *J* = 6.1, 1.2 Hz, 1H, **CH$_2$**$CHCH_2$-), 5.10 (t, *J* = 1.3 Hz, 1H, **CH$_2$**$CHCH_2$-), 4.39 (s, 1H, H-5), 4.18 (dt, *J* = 2.5, 1.2 Hz, 1H, H-2), 3.99 (s, 1H, H-6), 3.69 (tt, *J* = 2.7, 1.2 Hz, 1H, H-3), 2.45 (s, 3H, Ar**CH$_3$**), 2.37 – 2.33 (m, 2H, CH_2CH**CH$_2$**-), 1.68 (t, *J* = 7.7 Hz, 1H, H-4). ^{13}C NMR (151 MHz, $CDCl_3$) δ 135.3 (CH_2**CH**CH_2-), 130.0 (Ar), 127.9 (Ar), 118.0 (**CH$_2$**$CHCH_2$-), 99.7 (C-1), 78.9 (C-2), 74.2 (C-5), 70.0 (C-3), 68.0 (t, *J* = 23.4 Hz, C-6), 43.0 (C-4), 35.4 (CH_2CH**CH$_2$**-), 21.7 (Ar**CH$_3$**). ESI-HRMS: m/z calcd for $C_{16}H_{19}DO_6Na$ $[M + Na]^+$ 364.0941, found 364.0936.

4-*C*-Allyl-1,6;2,3-bisanhydro-6-(*S*)-deuterio-4-deoxy-β-D-mannopyranose (72). NaOMe (0.088 g, 1.63 mmol) was added to an ice-cold stirred solution of compound **71** (0.240 g, 0.704 mmol) in 1:1 mixture of methanol and chloroform (3.5 mL) and the solution was allowed to warm to room temperature. After 2 hours the reaction mixture was diluted with Et_2O and washed with aqueous saturated NH_4Cl solution and brine. The organic layer was dried with Na_2SO_4 and concentrated to give **72** (0.118 g, 99 %) as a white waxy solid which was used without further purification. $[\alpha]_D^{23}$ = -15.2 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.82 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H, CH_2**CH**CH_2-), 5.65 (d, *J* = 3.2, 1H, H-1), 5.16 – 5.10 (m, 2H, **CH$_2$**$CHCH_2$-), 4.23 (s, 1H, H-5), 3.70 (d, *J* = 1.6 Hz, 1H, H-6), 3.34 (ddd, *J* = 3.8, 3.1, 0.7 Hz, 1H, H-2), 2.93 (dd, *J* = 4.0, 1.3 Hz, 1H, H-3), 2.32 (tt, *J* = 7.2, 1.3 Hz, 2H, CH_2CH**CH$_2$**-), 2.01 (t, *J* = 7.6 Hz, 1H, H-4). ^{13}C NMR (151 MHz,

$CDCl_3$) δ 135.2 (CH_2**CH**CH_2-), 117.8 (**CH_2**$CHCH_2$-), 98.0 (C-1), 70.9 (C-5), 68.2 (t, *J* = 23.0 Hz, C-6), 53.8 (C-2), 50.4 (C-3), 39.0 (C-4), 35.1 (CH_2CH**CH_2**-). ESI-HRMS: m/z calcd for $C_9H_{11}DO_3Na$ $[M + Na]^+$ 192.0747, found 192.0746.

4-*C*-allyl-1,6-anhydro-6-(*S*)-deuterio-2-*N*-benzyl-2,4-dideoxy-β-D-glucopyranose (73). A stirred solution of epoxide **72** (0.118 g, 0.427 mmol) in benzylamine (5.0 mL) was heated to 155°C for 1.5 days before concentration under vacuum. The crude residue was then purified using silica gel column chromatography in 40 % EtOAc in hexanes with 1% triethylamine added to give amine **73** (0.166 g, 86 %). $[\alpha]_D^{23}$ = -32.9 (*c* = 1.0, DCM), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 – 7.30 (m, 4H, Ar-H), 7.29 – 7.23 (m, 1H, Ar-H), 5.88 – 5.76 (m, 1H, CH_2**CH**CH_2-), 5.46 (s, 1H, H-1), 5.16 – 5.10 (m, 2H, **CH_2**$CHCH_2$-), 4.38 (s, 1H, H-5), 4.03 (s, 1H, H-6), 3.90 (d, *J* = 13.2 Hz, 1H, Ph**CH_2**N-), 3.86 (d, *J* = 13.2 Hz, 1H, Ph**CH_2**N-), 3.64 (dq, *J* = 3.0, 1.4 Hz, 1H, H-3), 2.64 (p, *J* = 1.2 Hz, 1H, H-2), 2.50 – 2.30 (m, 2H, CH_2CH**CH_2**-), 1.76 (t, *J* = 7.7 Hz, 1H, H-4). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.9 (Ar), 136.1 (CH_2**CH**CH_2-), 128.5 (Ar), 128.1 (Ar), 127.2 (Ar), 117.5 (**CH_2**$CHCH_2$-), 102.6 (C-1), 74.6 (C-5), 70.3 (C-3), 68.2 (t, *J* = 23.4 Hz, C-6), 62.2 (C-2), 51.7 (Ph**CH_2**N-), 44.6 (C-4), 36.5 (CH_2CH**CH_2**-). ESI-HRMS: m/z calcd for $C_{16}H_{21}DNO_3$ $[M + H]^+$ 277.1657, found 277.1664.

2-amino-1,6-anhydro-6-(*S*)-deuterio-2,4-dideoxy-4-*C*-propyl-β-D-glucopyranose acetate salt (74). Pd/C (10 % w/w, 27 mg) was added to a solution of **73** (0.137, 0.496 mmol) in a 1:1 mixture of 10 % aqueous acetic acid and 1,4-dioxane followed by pressurization to 40 psi of H_2. The reaction mixture was stirred vigorously for 9 hours before filtration through celite® and concentration under vacuum to give amine **74** (0.122 g, 99%) as the acetate salt which was used without further purification. $[\alpha]_D^{23}$ = -48.6 (*c* = 4.0, MeOH), ^{1}H NMR (600 MHz, D_2O) δ 5.41 (s, 1H,

H-1), 4.45 (s, 1H, H-5), 3.87 (s, 1H, H-6), 3.47 (t, *J* = 4.6 Hz, 1H, H-3), 3.01 (dd, *J* = 4.4, 1.0 Hz, 1H, H-2), 1.75 (s, 3H, AcOH), 1.53 (tdd, *J* = 6.8, 4.6, 1.5 Hz, 1H, H-4), 1.43 – 1.37 (m, 2H, CH_3CH_2**CH_2**-), 1.32 (dp, *J* = 13.3, 7.2 Hz, 1H, CH_3**CH_2**CH_2-), 1.21 (tdd, *J* = 15.1, 13.4, 7.1 Hz, 1H, CH_3**CH_2**CH_2-), 0.76 (t, *J* = 7.3 Hz, 3H, **CH_3**CH_2CH_2-). ^{13}C NMR (151 MHz, D_2O) δ 98.5 (C-1), 75.3 (C-5), 68.7 (t, *J* = 23.5 Hz, C-6), 68.3 (C-3), 55.6 (C-2), 43.6 (C-4), 33.0 (CH_3CH_2**CH_2**-), 23.0 (AcOH), 19.3 (CH_3**CH_2**CH_2-), 13.0 (**CH_3**CH_2CH_2-). ESI-HRMS: m/z calcd for $C_9H_{17}DNO_3$ $[M + H]^+$ 189.1349, found 189.1357.

1,6-anhydro-2-azido-6-(*S*)-deuterio-2,4-dideoxy-4-*C*-propyl-β-D-glucopyranose (75). Stick's reagent (0.209 g, 0.997 mmol) was added to an ice cold stirred solution of $CuSO_4$ (11 mg, 0.07 mmol), triethylamine (0.28 mL, 2.0 mmol), and compound **74** (0.123 g, 0.495 mmol) in 4:1 MeCN/water (6.6 mL). The reaction mixture was stirred for 9 hours before MeCN was removed under vacuum and the residue was diluted with EtOAc, washed with 1N HCl, saturated $NaHCO_3$ solution, and brine. The organic layer was dried with Na_2SO_4 and concentrated to give **75** as a colorless gum (0.1014 g, 96%). $[\alpha]_D^{23}$ = -40.5 (*c* = 1.0, DCM), ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.41 (s, 1H, H-1), 4.38 (s, 1H, H-5), 4.04 (s, 1H, H-6), 3.60 (s, 1H, H-3), 3.42 (s, 1H, H-2), 2.71 (br s, 1H, -OH), 1.66 – 1.51 (m, 3H, H-4, CH_3CH_2**CH_2**-), 1.50 – 1.41 (m, 1H, CH_3**CH_2**CH_2-), 1.40 – 1.30 (m, 1H, CH_3**CH_2**CH_2-), 0.93 (t, *J* = 7.3 Hz, 3H, **CH_3**CH_2CH_2-). ^{13}C NMR (151 MHz, $CDCl_3$) δ 100.5 (C-1), 74.9 (C-5), 71.1 (C-3), 68.2 (t, *J* = 23.2 Hz, C-6), 63.7 (C-2), 44.2 (C-4), 33.3 (CH_3CH_2**CH_2**-), 20.5 (CH_3**CH_2**CH_2-), 14.0 (**CH_3**CH_2CH_2-). Product was not visible on ESIMS

Methyl 2-azido-6-(*S*)-deuterio-2,4-dideoxy-4-*C*-propyl-D-glucopyranoside (76α and 76β). Compound **75** (0.091 g, 0.4248 mmol) was dissolved in 4.0 mL Ac_2O followed by addition of 0.40 mL TFA and stirred under argon for 2 hours. The reaction mixture was then diluted with

Et_2O and washed with saturated $NaHCO_3$ solution and brine, dried with Na_2SO_4, filtered, and concentrated. The crude residue was then dissolved in 10 % HCl MeOH solution (3.4 mL) and heated to reflux for 10 hours followed by concentration under vacuum. The resulting residue was subjected flash column chromatography over silica gel in 45 % to 50 % ethyl acetate in hexanes which afforded 11.7 mg **76α** (11 %), 9.5 mg **76β** (9 %), and 11.8 mg (11 %) of a mixture of anomers. **76α**: $[\alpha]_D^{23}$ = 98.75 (*c* = 1.0, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 4.76 (d, *J* = 3.5 Hz, 1H, H-1), 3.78 (t, *J* = 10.2 Hz, 1H, H-3), 3.58 (dd, *J* = 10.6, 5.4 Hz, 1H, H-5), 3.55 (d, *J* = 5.5 Hz, 1H, H-6), 3.37 (s, 3H, -OMe), 3.09 (dd, *J* = 10.1, 3.5 Hz, 1H, H-2), 1.61 – 1.49 (m, 2H, -CH_2CH_2-, H-4), 1.49 – 1.27 (m, 3H, -CH_2CH_2-), 0.91 (t, *J* = 7.2 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 99.2 (C-1), 71.8 (C-5), 68.5 (C-3), 65.2 (C-2), 61.5 (t, *J* = 21.3 Hz, C-6), 53.9 (-OMe), 43.1 (C-4), 28.8 (-CH_2CH_2-), 18.7 (-CH_2CH_2-), 13.6 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{18}DN_3O_4Na$ $[M + Na]^+$ 269.1336, found 269.1334. **76β**: $[\alpha]_D^{23}$ = -35.37 (*c* = 0.003, MeOH), ^{1}H NMR (600 MHz, CD_3OD) δ 4.13 (d, *J* = 8.1 Hz, 1H, H-1), 3.57 (d, *J* = 5.5 Hz, 1H, H-6), 3.52 (s, 3H, -OMe), 3.30 – 3.24 (m, 2H, H-3, H-5), 3.02 (dd, *J* = 9.5, 8.1 Hz, 1H, H-2), 1.58 – 1.24 (m, 5H, H4, -CH_2CH_2-), 0.90 (t, *J* = 7.1 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, CD_3OD) δ 102.7 (C-1), 76.1 (C-5), 72.6 (C-3), 68.6 (C-2), 61.5 (t, *J* = 21.9 Hz, C-6), 55.6 (-OMe), 42.6 (C-4), 28.6 (-CH_2CH_2-), 18.6 (-CH_2CH_2-), 13.6 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{18}DN_3O_4Na$ $[M + Na]^+$ 269.1336, found 269.1326.

Methyl 2-amino-6-(*S*)-deuterio-2,4-dideoxy-4-*C*-propyl-α-D-glucopyranoside (56). Compound **76α** (9.0 mg, 0.0365 mmol) was dissolved in 0.6 mL of a 1:1 mixture of 1,4-Dioxane and 10 % aqueous AcOH followed by addition of Pd/C (1.8 mg). The reaction mixture was stirred under 50 psi H_2 for 1 hour followed by filtration over Celite® and lyophilization to obtain **56** as an

off white solid (10.2 mg, 99 %). $[\alpha]_D^{23}$ = 56.40 (*c* = 0.5, water), ^{1}H NMR (600 MHz, D_2O) δ 4.84 (d, *J* = 3.6 Hz, 1H, H1), 3.71 (t, *J* = 10.5 Hz, 1H, H3), 3.62 (dd, *J* = 10.9, 5.4 Hz, 1H, H5), 3.53 (d, *J* = 5.5 Hz, 1H, H6), 3.25 (s, 3H, OMe), 3.08 (dd, *J* = 10.4, 3.6 Hz, 1H, H2), 1.80 (s, 3H, AcOH), 1.49 (tt, *J* = 10.8, 4.0 Hz, 1H, H4), 1.40 – 1.33 (m, 1H, -CH_2CH_2-), 1.32 – 1.24 (m, 1H, -CH_2CH_2-), 1.24 – 1.06 (m, 2H, -CH_2CH_2-), 0.72 (t, *J* = 7.2 Hz, 3H, -CH_3). ^{13}C NMR (151 MHz, D_2O) δ 179.9 (AcOH), 96.3 (C1), 71.6 (C5), 67.0 (C3), 60.99 (t, *J* = 21.0 Hz, C6), 55.3 (C2), 54.9 (OMe), 42.0 (C4), 27.7 (-CH_2CH_2-), 22.3 (AcOH), 17.8 (-CH_2CH_2-), 13.8 (-CH_3). ESI-HRMS: m/z calcd for $C_{10}H_{21}DNO_4$ $[M + H]^+$ 221.1612, found 221.1605.

REFERENCES

1. Fleming, A., On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. *Br J Exp Pathol* **1929,** *10*, 226-236.

2. Anderson, R. J.; Groundwater, P. W.; Todd, A.; Worsley, A. J., *Antibacterail Agents: Chemistry, Mode of Action, Mechanism of Resistance, and Clinical Applications*. Wiley: 2012.

3. CDC AR Threat Report. https://www.cdc.gov/drugresistance/biggest_threats.html (accessed September 2019).

4. Regional outbreak of New Delhi metallo-betalactamase-producing carbapenem-resistant *Enterobacteriaceae*, Italy, 2018–2019. ECDC: Stockholm, 2019.

5. Kumarasamy, K. K.; Toleman, M. A.; Walsh, T. R.; Bagaria, J.; Butt, F.; Balakrishnan, R.; Chaudhary, U.; Doumith, M.; Giske, C. G.; Irfan, S.; Krishnan, P.; Kumar, A. V.; Maharjan, S.; Mushtaq, S.; Noorie, T.; Paterson, D. L.; Pearson, A.; Perry, C.; Pike, R.; Rao, B.; Ray, U.; Sarma, J. B.; Sharma, M.; Sheridan, E.; Thirunarayan, M. A.; Turton, J.; Upadhyay, S.; Warner, M.; Welfare, W.; Livermore, D. M.; Woodford, N., Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* **2010,** *10*, 597-602.

6. Ventola, C. L., The antibiotic resistance crisis: part 1: causes and threats. *P T* **2015,** *40*, 277-283.

7. Bartlett, J. G.; Gilbert, D. N.; Spellberg, B., Seven ways to preserve the miracle of antibiotics. *Clin Infect Dis* **2013,** *56*, 1445-50.

8. Piddock, L. J. V., The crisis of no new antibiotics—what is the way forward? *Lancet Infect Dis* **2012,** *12*, 249-253.

9. Ventola, C. L., The antibiotic resistance crisis: part 2: management strategies and new agents. *P T* **2015,** *40*, 344-352.

10. Schatz, A.; Bugle, E.; Waksman, S. A., Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria.∗†. *SEBM* **1944,** *55*, 66-69.

11. Arya, D. P., *Aminoglycoside Antibiotics: from Chemical Biology to Drug Discovery*. Wiley Interscience: 2007.

12. Matsushita, T.; Sati, G. C.; Kondasinghe, N.; Pirrone, M. G.; Kato, T.; Waduge, P.; Kumar, H. S.; Sanchon, A. C.; Dobosz-Bartoszek, M.; Shcherbakov, D.; Juhas, M.; Hobbie, S. N.; Schrepfer, T.; Chow, C. S.; Polikanov, Y. S.; Schacht, J.; Vasella, A.; Böttger, E. C.; Crich, D., Design, Multigram Synthesis, and in Vitro and in Vivo Evaluation of Propylamycin: A Semisynthetic 4,5-Deoxystreptamine Class Aminoglycoside for the Treatment of Drug-Resistant Enterobacteriaceae and Other Gram-Negative Pathogens. *J. Am. Chem. Soc.* **2019,** *141*, 5051-5061.

13. Takahashi, Y.; Igarashi, M., Destination of Aminoglycoside Antibiotics in the 'Post-antibiotic era'. *J. Antibiot.* **2018,** *71*, 4-14.

14. Fosso, M. Y.; Li, Y.; Garneau-Tsodikova, S., New Trends in the use of Aminoglycosides. *MedChemComm* **2014,** *5*, 1075-1091.

15. Chandrika, N. T.; Garneau-Tsodikova, S., Comprehensive review of chemical strategies for the preparation of new aminoglycosides and their biological activities. *Chem. Soc. Rev.* **2018,** *47*, 1189-1249.

16. Forge, A.; Schacht, J., Aminoglycoside Antibiotics. *Audiol Neurotol* **2000,** *5*, 3-22.

17. Maarouf, M.; Lawrence, F.; Croft, S. L.; Robert-Gero, M., Ribosomes of Leishmania are a target for the aminoglycosides. *Parasitol. Res.* **1995,** *81*, 421-425.

18. Davies, J.; Gorni, L.; Davis, B. D., Misreading of RNA Codewords Induced by Aminoglycoside Antibiotics. *Mol. Pharmacol.* **1965,** *1*, 93-106.

19. Cabanas, M. J.; Vazquez, D.; Modolell, J., Inhibition of ribosomal translocation by aminoglycoside antibiotics. *Biochem. Biophys. Res. Commun.* **1978,** *83*, 991-7.

20. Bacot-Davis, V. R.; Bassenden, A. V.; Berghuis, A. M., Drug-target networks in aminoglycoside resistance: hierarchy of priority in structural drug design. *MedChemComm* **2016,** *7*, 103-113.

21. Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkens, P. M., Aminoglycosides: Activity and Resistance. *Antimicrob. Agents Chemother.* **1999,** *43*, 727-737.

22. Li, X. Z.; Plesiat, P.; Nikaido, H., The challenge of efflux-mediated antibiotic resistance in Gram-negative bacteria. *Clin Microbiol Rev* **2015,** *28*, 337-418.

23. Bolsover, S. R.; Shephard, E. A.; White, H. A.; Hyams, J. S., Manufacturing Protein. In *Cell Biology: A Short Cource*, 3 ed.; Wiley: New Jersey, 2011; pp 123-136.

24. Noeske, J.; Wasserman, M. R.; Terry, D. S.; Altman, R. B.; Blanchard, S. C.; Cate, J. H., High-resolution structure of the Escherichia coli ribosome. *Nat. Struct. Mol. Biol.* **2015,** *22*, 336-41.

25. Herzog, I. M.; Louzoun Zada, S.; Fridman, M., Effects of 5-O-Ribosylation of Aminoglycosides on Antimicrobial Activity and Selective Perturbation of Bacterial Translation. *J. Med. Chem.* **2016,** *59*, 8008-8018.

26. Bryan, L. E.; Kwan, S., Roles of ribosomal binding, membrane potential, and electron transport in bacterial uptake of streptomycin and gentamicin. *Antimicrob. Agents Chemother.* **1983,** *23*, 835-45.

27. Davis, B. D.; Chen, L. L.; Tai, P. C., Misread protein creates membrane channels: an essential step in the bactericidal action of aminoglycosides. *Proc Natl Acad Sci U S A* **1986,** *83*, 6164-8.

28. Kohanski, M. A.; Dwyer, D. J.; Hayete, B.; Lawrence, C. A.; Collins, J. J., A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **2007,** *130*, 797-810.

29. Carter, A. P.; Clemons, W. M.; Brodersen, D. E.; Morgan-Warren, R. J.; Wimberly, B. T.; Ramakrishnan, V., Functional insights from the structure of the 30S ribosomal subunit and its interactions with antibiotics. *Nature* **2000,** *407*, 340-348.

30. Bottger, E. C.; Schacht, J., The mitochondrion: a perpetrator of acquired hearing loss. *Hear Res* **2013,** *303*, 12-9.

31. Huth, M. E.; Ricci, A. J.; Cheng, A. G., Mechanisms of Aminoglycoside Ototoxicity and Targets of Hair Cell Protection. *Otolaryngol* **2011,** *2011*, 19.

32. Livermore, D. M.; Mushtaq, S.; Warner, M.; Zhang, J.-C.; Maharjan, S.; Doumith, M.; Woodford, N., Activity of aminoglycosides, including ACHN-490, against carbapenem-resistant Enterobacteriaceae isolates. *J. Antimicrob. Chemother.* **2010,** *66*, 48-53.

33. Doi, Y.; Yokoyama, K.; Yamane, K.; Wachino, J.; Shibata, N.; Yagi, T.; Shibayama, K.; Kato, H.; Arakawa, Y., Plasmid-Mediated 16S rRNA Methylase in *Serratia marcescens* Conferring High-Level Resistance to Aminoglycosides. *Antimicrob. Agents Chemother.* **2004,** *48*, 491-496.

34. Galani, I.; Souli, M.; Daikos, G. L.; Chrysouli, Z.; Poulakou, G.; Psichogiou, M.; Panagea, T.; Argyropoulou, A.; Stefanou, I.; Plakias, G.; Giamarellou, H.; Petrikkos, G., Activity of plazomicin (ACHN-490) against MDR clinical isolates of Klebsiella pneumoniae, Escherichia coli, and Enterobacter spp. from Athens, Greece. *J Chemother* **2012,** *24*, 191-194.

35. Moore, R. A.; DeShazer, D.; Reckseidler, S.; Weissman, A.; Woods, D. E., Efflux-mediated aminoglycoside and macrolide resistance in Burkholderia pseudomallei. *Antimicrob. Agents Chemother.* **1999,** *43*, 465-70.

36. Poole, K., Efflux-mediated multiresistance in Gram-negative bacteria. *Clin Microbiol Infect* **2004,** *10*, 12-26.

37. Garneau-Tsodikova, S.; Labby, K. J., Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. *MedChemComm* **2016,** *7*, 11-27.

38. Zárate, S. G.; De la Cruz Claure, M. L.; Benito-Arenas, R.; Revuelta, J.; Santana, A. G.; Bastida, A., Overcoming Aminoglycoside Enzymatic Resistance: Design of Novel Antibiotics and Inhibitors. *Molecules* **2018,** *23*, 284.

39. Llano-Sotelo, B.; Azucena, E. F., Jr.; Kotra, L. P.; Mobashery, S.; Chow, C. S., Aminoglycosides modified by resistance enzymes display diminished binding to the bacterial ribosomal aminoacyl-tRNA site. *Chem Biol* **2002,** *9*, 455-63.

40. Springer, B.; Kidan, Y. G.; Prammananan, T.; Ellrott, K.; Bottger, E. C.; Sander, P., Mechanisms of streptomycin resistance: selection of mutations in the 16S rRNA gene conferring resistance. *Antimicrob. Agents Chemother.* **2001,** *45*, 2877-84.

41. Basso, L. A.; Blanchard, J. S., Resistance to Antitubercular Drugs. In *Resolving the Antibiotic Paradox: Progress in Understanding Drug Resistance and Development of New Antibiotics*, Rosen, B. P.; Mobashery, S., Eds. Springer US: Boston, MA, 1998; pp 115-144.

42. Doi, Y.; Wachino, J. I.; Arakawa, Y., Aminoglycoside Resistance: The Emergence of Acquired 16S Ribosomal RNA Methyltransferases. *Infectious disease clinics of North America* **2016,** *30*, 523-537.

43. Galimand, M.; Courvalin, P.; Lambert, T., Plasmid-Mediated High-Level Resistance to Aminoglycosides in *Enterobacteriaceae* Due to 16S rRNA Methylation. *Antimicrob. Agents Chemother.* **2003,** *47*, 2565-2571.

44. Gonzalez-Zorn, B.; Teshager, T.; Casas, M.; Porrero, M. C.; Moreno, M. A.; Courvalin, P.; Dominguez, L., armA and aminoglycoside resistance in Escherichia coli. *Emerg Infect Dis* **2005,** *11*, 954-6.

45. Young, M. L.; Bains, M.; Bell, A.; Hancock, R. E., Role of Pseudomonas aeruginosa outer membrane protein OprH in polymyxin and gentamicin resistance: isolation of an OprH-deficient mutant by gene replacement techniques. *Antimicrob. Agents Chemother.* **1992,** *36*, 2566-2568.

46. Poole, K., Efflux-mediated antimicrobial resistance. *J. Antimicrob. Chemother.* **2005,** *56*, 20-51.

47. Wright, G. D., Aminoglycoside-modifying enzymes. *Curr Opin Microbiol* **1999,** *2*, 499-503.

48. Ramirez, M. S.; Tolmasky, M. E., Aminoglycoside modifying enzymes. *Drug Resist Update* **2010,** *13*, 151-171.

49. Vakulenko, S. B.; Mobashery, S., Versatility of aminoglycosides and prospects for their future. *Clin Microbiol Rev* **2003,** *16*, 430-450.

50. Vetting, M. W.; LP, S. d. C.; Yu, M.; Hegde, S. S.; Magnet, S.; Roderick, S. L.; Blanchard, J. S., Structure and functions of the GNAT superfamily of acetyltransferases. *Arch. Biochem. Biophys.* **2005,** *433*, 212-26.

51. Sati, G. C.; Sarpe, V. A.; Furukawa, T.; Mondal, S.; Mantovani, M.; Hobbie, S. N.; Vasella, A.; Bottger, E. C.; Crich, D., Modification at the 2'-Position of the 4,5-Series of 2-Deoxystreptamine Aminoglycoside Antibiotics To Resist Aminoglycoside Modifying Enzymes and Increase Ribosomal Target Selectivity. *ACS Infect Dis* **2019**.

52. Prezant, T. R.; Agapian, J. V.; Bohlman, M. C.; Bu, X. D.; Oztas, S.; Qiu, W. Q.; Arnos, K. S.; Cortopassi, G. A.; Jaber, L.; Rotter, J. I.; Shohat, M.; Fischelghodsian, N., Mitochondrial Ribosomal-Rna Mutation Associated with Both Antibiotic-Induced and Non-Syndromic Deafness. *Nat. Genet.* **1993,** *4*, 289-294.

53. Hobbie, S. N.; Akshay, S.; Kalapala, S. K.; Bruell, C. M.; Shcherbakov, D.; Bottger, E. C., Genetic analysis of interactions with eukaryotic rRNA identify the mitoribosome as target in aminoglycoside ototoxicity. *Proc Natl Acad Sci U S A* **2008,** *105*, 20888-93.

54. Hobbie, S. N.; Bruell, C. M.; Akshay, S.; Kalapala, S. K.; Shcherbakov, D.; Bottger, E. C., Mitochondrial deafness alleles confer misreading of the genetic code. *Proc Natl Acad Sci U S A* **2008,** *105*, 3244-9.

55. Contrepois, A.; Brion, N.; Garaud, J. J.; Faurisson, F.; Delatour, F.; Levy, J. C.; Deybach, J. C.; Carbon, C., Renal disposition of gentamicin, dibekacin, tobramycin, netilmicin, and amikacin in humans. *Antimicrob. Agents Chemother.* **1985,** *27*, 520-524.

56. Janknegt, R., Aminoglycoside monitoring in the once- or twice-daily era. *Pharm World Sci* **1993,** *15*, 151-155.

57. Priuska, E. M.; Schacht, J., Formation of free radicals by gentamicin and iron and evidence for an iron/gentamicin complex. *Biochem. Pharmacol.* **1995,** *50*, 1749-52.

58. Giuliano, R. A.; Paulus, G. J.; Verpooten, G. A.; Pattyn, V. M.; Pollet, D. E.; Nouwen, E. J.; Laurent, G.; Carlier, M.-B.; Maldague, P.; Tulkens, P. M.; De Broe, M. E., Recovery of cortical phospholipidosis and necrosis after acute gentamicin loading in rats. *Kidney Int* **1984,** *26*, 838-847.

59. Boettcher, F. A.; Henderson, D.; Gratton, M. A.; Danielson, R. W.; Byrne, C. D., Synergistic Interactions of Noise and Other Ototraumatic Agents. *Ear Hearing* **1987,** *8*, 192-212.

60. Henley, C. M.; Schacht, J., Pharmacokinetics of Aminoglycoside Antibiotics in Blood, Inner-Ear Fluids and Tissues and Their Relationship to Ototoxicity. *Audiology* **1988,** *27*, 137-146.

61. Fausti, S. A.; Rappaport, B. Z.; Schechter, M. A.; Frey, R. H.; Ward, T. T.; Brummett, R. E., Detection of aminoglycoside ototoxicity by high-frequency auditory evaluation: Selected case studies. *Am J Otolaryngol* **1984,** *5*, 177-182.

62. Mizuta, K.; Saito, A.; Watanabe, T.; Nagura, M.; Arakawa, M.; Shimizu, F.; Hoshino, T., Ultrastructural localization of megalin in the rat cochlear duct. *Hear. Res.* **1999,** *129*, 83-91.

63. Lesniak, W.; Pecoraro, V. L.; Schacht, J., Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. *Chem. Res. Toxicol.* **2005,** *18*, 357-64.

64. Jiang, H.; Sha, S. H.; Schacht, J., Rac/Rho pathway regulates actin depolymerization induced by aminoglycoside antibiotics. *J. Neurosci. Res.* **2006,** *83*, 1544-51.

65. Matt, T.; Akbergenov, R.; Shcherbakov, D.; Böttger, E. C., The Ribosomal A-site: Decoding, Drug Target, and Disease. *Isr. J. Chem.* **2010,** *50*, 60-70.

66. Pfister, P.; Hobbie, S.; Brull, C.; Corti, N.; Vasella, A.; Westhof, E.; Bottger, E. C., Mutagenesis of 16S rRNA C1409-G1491 base-pair differentiates between 6'OH and 6'NH3+ aminoglycosides. *J. Mol. Biol.* **2005,** *346*, 467-75.

67. Hobbie, S. N.; Bruell, C.; Kalapala, S.; Akshay, S.; Schmidt, S.; Pfister, P.; Böttger, E. C., A genetic model to investigate drug–target interactions at the ribosomal decoding site. *Biochimie* **2006,** *88*, 1033-1043.

68. Prezant, R. T.; Shohat, M.; Jaber, L.; Pressman, S.; Fischel-Ghodsian, N., Biochemical characterization of a pedigree with mitochondrially inherited deafness. *Am J Med Genet* **1992,** *44*, 465-72.

69. Song, B.-B.; Schacht, J., Variable efficacy of radical scavengers and iron chelators to attenuate gentamicin ototoxicity in guinea pig in vivo. *Hear. Res.* **1996,** *94*, 87-93.

70. Sha, S. H.; Schacht, J., Salicylate attenuates gentamicin-induced ototoxicity. *Lab. Invest.* **1999,** *79*, 807-13.

71. Hobbie, S. N.; Kalapala, S. K.; Akshay, S.; Bruell, C.; Schmidt, S.; Dabow, S.; Vasella, A.; Sander, P.; Bottger, E. C., Engineering the rRNA decoding site of eukaryotic cytosolic ribosomes in bacteria. *Nucleic Acids Res.* **2007,** *35*, 6086-6093.

72. Aggen, J.; Goldblum, A. A.; Linsell, M.; Dozzo, P.; Moser, H. E.; Hildebrandt, D.; Gliedt, M. Antibacterial Aminoglycoside Analogs. 2008.

73. Sonousi, A.; Sarpe, V. A.; Brilkova, M.; Schacht, J.; Vasella, A.; Böttger, E. C.; Crich, D., Effects of the 1-N-(4-Amino-2S-hydroxybutyryl) and 6’-N-(2-Hydroxyethyl) Substituents on

Ribosomal Selectivity, Cochleotoxicity, and Antibacterial Activity in the Sisomicin Class of Aminoglycoside Antibiotics. *ACS Infect. Dis.* **2018,** *4*, 1114-1120.

74. Matt, T.; Ng, C. L.; Lang, K.; Sha, S.-H.; Akbergenov, R.; Shcherbakov, D.; Meyer, M.; Duscha, S.; Xie, J.; Dubbaka, S. R.; Perez-Fernandez, D.; Vasella, A.; Ramakrishnan, V.; Schacht, J.; Böttger, E. C., Dissociation of antibacterial activity and aminoglycoside ototoxicity in the 4-monosubstituted 2-deoxystreptamine apramycin. *Proc. Natl. Acad. Sci* **2012,** *109*, 10984-10989.

75. Juhas, M.; Widlake, E.; Teo, J.; Huseby, D. L.; Tyrrell, J. M.; Polikanov, Y. S.; Ercan, O.; Petersson, A.; Cao, S.; Aboklaish, A. F.; Rominski, A.; Crich, D.; Böttger, E. C.; Walsh, T. R.; Hughes, D.; Hobbie, S. N., In vitro activity of apramycin against multidrug-, carbapenem- and aminoglycoside-resistant Enterobacteriaceae and Acinetobacter baumannii. *J. Antimicrob. Chemother.* **2019**, 944-952.

76. Smith, K. P.; Kirby, J. E., Evaluation of apramycin activity against carbapenem-resistant and -susceptible strains of Enterobacteriaceae. *Diagn Microbiol Infect Dis* **2016,** *86*, 439-441.

77. Kang, A. D.; Smith, K. P.; Eliopoulos, G. M.; Berg, A. H.; McCoy, C.; Kirby, J. E., Invitro Apramycin Activity against multidrug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa. *Diagn Microbiol Infect Dis* **2017,** *88*, 188-191.

78. First-In-Humans Study of Apramycin. https://clinicaltrials.gov/ct2/show/NCT04105205?term=apramycin&draw=2&rank=1.

79. Stenutz, R.; Carmichael, I.; Widmalm, G.; Serianni, A. S., Hydroxymethyl Group Conformation in Saccharides: Structural Dependencies of 2JHH, 3JHH, and 1JCH Spin−Spin Coupling Constants. *JOC* **2002,** *67*, 949-958.

80. Bock, K.; Duus, J. Ø., A Conformational Study of Hydroxymethyl Groups in Carbohydrates Investigated by 1H NMR Spectroscopy. *J. Carbohydr. Chem.* **1994,** *13*, 513-543.

81. Shalev, M.; Rozenberg, H.; Smolkin, B.; Nasereddin, A.; Kopelyanskiy, D.; Belakhov, V.; Schrepfer, T.; Schacht, J.; Jaffe, C. L.; Adir, N.; Baasov, T., Structural basis for selective targeting of leishmanial ribosomes: aminoglycoside derivatives as promising therapeutics. *Nucleic Acids Res.* **2015,** *43*, 8601-8613.

82. Kato, T.; Vasella, A.; Crich, D., Stereospecific synthesis of methyl 2-amino-2-deoxy-(6S)-deuterio-α,β-d-glucopyranoside and methyl 2,6-diamino-2,6-dideoxy-(6R)-deuterio-α,β-d-glucopyranoside: Side chain conformations of the 2-amino-2-deoxy and 2,6-diamino-2,6-dideoxyglucopyranosides. *Carbohydr. Res.* **2017,** *448*, 10-17.

83. Pathak, R.; Perez-Fernandez, D.; Nandurdikar, R.; Kalapala, S. K.; Böttger, E. C.; Vasella, A., Synthesis and Evaluation of Paromomycin Derivatives Modified at C(4'). *Helv. Chim. Acta* **2008,** *91*, 1533-1552.

84. Nahm, S.; Weinreb, S. M., N-methoxy-n-methylamides as effective acylating agents. *Tetrahedron Lett.* **1981,** *22*, 3815-3818.

85. Epp, J. B.; Widlanski, T. S., Facile Preparation of Nucleoside-5'-carboxylic Acids. *JOC* **1999,** *64*, 293-295.

86. Joseph, A. A.; Dhurandhare, V. M.; Chang, C.-W.; Verma, V. P.; Mishra, G. P.; Ku, C.-C.; Lin, C.-C.; Wang, C.-C., Chemoselective per-O-trimethylsilylation and homogeneous N-functionalisation of amino sugars. *Chem. Commun.* **2015,** *51*, 104-106.

87. Omura, K.; Swern, D., Oxidation of alcohols by "activated" dimethyl sulfoxide. a preparative, steric and mechanistic study. *Tetrahedron* **1978,** *34*, 1651-1660.

88. Cram, D. J.; Elhafez, F. A. A., Studies in Stereochemistry. X. The Rule of "Steric Control of Asymmetric Induction" in the Syntheses of Acyclic Systems. *J. Am. Chem. Soc.* **1952,** *74*, 5828-5835.

89. Mandhapati, A. R.; Yang, G.; Kato, T.; Shcherbakov, D.; Hobbie, S. N.; Vasella, A.; Böttger, E. C.; Crich, D., Structure-Based Design and Synthesis of Apramycin–Paromomycin Analogues: Importance of the Configuration at the 6′-Position and Differences between the 6′-Amino and Hydroxy Series. *J. Am. Chem. Soc.* **2017,** *139*, 14611-14619.

90. Amarasekara, H.; Dharuman, S.; Kato, T.; Crich, D., Synthesis of Conformationally-Locked cis- and trans-Bicyclo[4.4.0] Mono-, Di-, and Trioxadecane Modifications of Galacto- and Glucopyranose; Experimental Limiting 3JH,H Coupling Constants for the Estimation of Carbohydrate Side Chain Populations and Beyond. *JOC* **2018,** *83*, 881-897.

91. Garber, S. B.; Kingsbury, J. S.; Gray, B. L.; Hoveyda, A. H., Efficient and Recyclable Monomeric and Dendritic Ru-Based Metathesis Catalysts. *J. Am. Chem. Soc.* **2000,** *122*, 8168-8179.

92. Eliel, E. L.; Wilen, S. H., *Stereochemistry of Organic Compounds*. Wiley: New York, 1994.

93. Bocian, D. F.; Strauss, H. L., Vibrational spectra, conformations, and potential functions of cycloheptane and related oxepanes. *J. Am. Chem. Soc.* **1977,** *99*, 2866-2876.

94. Espinosa, A.; Gallo, M. A.; Entrena, A.; Gómez, J. A., Theoretical conformational analysis of seven-membered rings. V. MM2 and MM3 study of oxepane. *J. Mol. Struct.* **1994,** *323*, 247-256.

95. Matsushita, T.; Chen, W.; Juskeviciene, R.; Teo, Y.; Shcherbakov, D.; Vasella, A.; Böttger, E. C.; Crich, D., Influence of 4'-O-Glycoside Constitution and Configuration on Ribosomal Selectivity of Paromomycin. *J. Am. Chem. Soc.* **2015,** *137*, 7706-7717.

96. Duscha, S.; Boukari, H.; Shcherbakov, D.; Salian, S.; Silva, S.; Kendall, A.; Kato, T.; Akbergenov, R.; Perez-Fernandez, D.; Bernet, B.; Vaddi, S.; Thommes, P.; Schacht, J.; Crich, D.; Vasella, A.; Bottger, E. C., Identification and evaluation of improved 4'-O-(alkyl) 4,5-disubstituted 2-deoxystreptamines as next-generation aminoglycoside antibiotics. *MBio* **2014,** *5*, e01827-14.

97. Morgenthaler, M.; Schweizer, E.; Hoffmann-Röder, A.; Benini, F.; Martin, R. E.; Jaeschke, G.; Wagner, B.; Fischer, H.; Bendels, S.; Zimmerli, D.; Schneider, J.; Diederich, F.; Kansy, M.; Müller, K., Predicting and Tuning Physicochemical Properties in Lead Optimization: Amine Basicities. *ChemMedChem* **2007,** *2*, 1100-1115.

98. Pedersen, C. M.; Olsen, J.; Brka, A. B.; Bols, M., Quantifying the Electronic Effects of Carbohydrate Hydroxy Groups by Using Aminosugar Models. *Chem.: Eur. J.* **2011,** *17*, 7080-7086.

99. Lünse, C. E.; Schmidt, M. S.; Wittmann, V.; Mayer, G., Carba-sugars Activate the glmS-Riboswitch of Staphylococcus aureus. *ACS Chem. Biol* **2011,** *6*, 675-678.

100. Wakamatsu, T.; Nakamura, H.; Nishikimi, Y.; Yoshida, K.; Noda, T.; Taniguchi, M.; Ban, Y., The oxirane ring openings of the dianhydro sugar with high regioselectivity and its use in preparation of two chiral segments of 6-deoxyerythronolide B. *Tetrahedron Lett.* **1986,** *27*, 6071-6074.

101. Nakamura, H., Alkylated levoglucosan in organic synthesis. A formal total synthesis of elaiophylin. *Chem. Pharm. Bull.* **1990,** *38*, 2435-2441.

102. Fürst, A.; Plattner, P. A., Über Steroide und Sexualhormone. 160. Mitteilung. 2α, 3α- und 2β, 3β-Oxido-chlolestane; Konfiguration der 2-Oxy-cholestane. *Helv. Chim. Acta* **1949,** *32*, 275-283.

103. Goddard-Borger, E. D.; Stick, R. V., An Efficient, Inexpensive, and Shelf-Stable Diazotransfer Reagent: Imidazole-1-sulfonyl Azide Hydrochloride. *Org. Lett.* **2007,** *9*, 3797-3800.

104. Ohrui, H.; Horiki, H.; Kishi, H.; Meguro, H., Synthesis of (6*R*)- and (6*S*)-D-Glucose-62H through Stereospecific Photo-bromination of 1, 6-Anhydro-beta-D-glucopyranose Derivative. *Agric. Biol. Chem.* **1983,** *47*, 1101-1106.

105. Ogawa, A.; Curran, D. P., Benzotrifluoride: A Useful Alternative Solvent for Organic Reactions Currently Conducted in Dichloromethane and Related Solvents. *JOC* **1997,** *62*, 450-451.

106. Alt, G. H.; Barton, D. H. R., Some conformational aspects of neighbouring-group participation. *J. Chem. Soc.* **1954**, 4284-4294.

107. Karplus, M., Contact Electron-Spin Coupling of Nuclear Magnetic Moments. *J. Chem. Phys.* **1959,** *30*, 11-15.

108. Nishida, Y.; Hori, H.; Ohrui, H.; Meguro, H., 1H NMR Analyses of Rotameric Distribution of C5-C6 bonds of D-Glucopyranoses in Solution. *J. Carbohydr. Chem.* **1988,** *7*, 239-250.

109. Haasnoot, C. A. G.; de Leeuw, F. A. A. M.; Altona, C., The relationship between proton-proton NMR coupling constants and substituent electronegativities—I: An empirical generalization of the karplus equation. *Tetrahedron* **1980,** *36*, 2783-2792.

110. Moumé-Pymbock, M.; Furukawa, T.; Mondal, S.; Crich, D., Probing the Influence of a 4,6-O-Acetal on the Reactivity of Galactopyranosyl Donors: Verification of the Disarming Influence of the trans–gauche Conformation of C5–C6 Bonds. *J. Am. Chem. Soc.* **2013,** *135*, 14249-14255.

111. Kancharla, P. K.; Crich, D., Influence of Side Chain Conformation and Configuration on Glycosyl Donor Reactivity and Selectivity as Illustrated by Sialic Acid Donors Epimeric at the 7-Position. *J. Am. Chem. Soc.* **2013,** *135*, 18999-19007.

112. Jensen, H. H.; Nordstrøm, L. U.; Bols, M., The Disarming Effect of the 4,6-Acetal Group on Glycoside Reactivity: Torsional or Electronic? *J. Am. Chem. Soc.* **2004,** *126*, 9205-9213.

113. Morales, E. Q.; Padron, J. I.; Trujillo, M.; Vazquez, J. T., CD and 1H NMR Study of the Rotational Population Dependence of the Hydroxymethyl Group in .beta. Glucopyranosides on the Aglycon and Its Absolute Configuration. *JOC* **1995,** *60*, 2537-2548.

114. Padrón, J. I.; Morales, E. Q.; Vázquez, J. T., Alkyl Galactopyranosides: Rotational Population Dependence of the Hydroxymethyl Group on the Aglycon and Its Absolute Configuration and on the Anomeric Configuration. *JOC* **1998,** *63*, 8247-8258.

115. Roën, A.; Padrón, J. I.; Vázquez, J. T., Hydroxymethyl Rotamer Populations in Disaccharides. *JOC* **2003,** *68*, 4615-4630.

116. Nóbrega, C.; Vázquez, J. T., Conformational study of the hydroxymethyl group in α-d-mannose derivatives. *Tetrahedron: Asymmetry* **2003,** *14*, 2793-2801.

117. Dey, S.; Jayaraman, N., Glycosidic bond hydrolysis in septanosides: a comparison of mono-, di-, and 2-chloro-2-deoxy-septanosides. *Carbohydr. Res.* **2014,** *399*, 49-56.

118. Dharuman, S.; Amarasekara, H.; Crich, D., Interplay of Protecting Groups and Side Chain Conformation in Glycopyranosides. Modulation of the Influence of Remote Substituents on Glycosylation? *JOC* **2018,** *83*, 10334-10351.

ABSTRACT

INFLUENCE OF CONFORMATIONAL RESTRICTION ON THE ANTIBACTERIAL ACTIVITY AND RIBOSOMAL SELECTIVITY OF AMINOGLYCOSIDE ANTIBIOTICS

by

MICHAEL GABRIEL PIRRONE

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Advisor: Dr. David Crich

Major: Chemistry

Degree: Doctor of Philosophy

The ever-increasing threat posed by multidrug-resistant infectious bacteria necessitates the development of novel antibiotics. Aminoglycoside antibiotics are growing in interest due to their broad spectrum of activity, lack of known drug related allergies, low manufacturing cost, and their well-studied mechanism of action. The simplification of rational drug design due to the well-studied mechanism of action is the key to overcoming the issues presented by these drugs, namely ototoxicity and nephrotoxicity.

A study of the effect of the conformation of the aminoglycoside ring I side chain is described wherein it was discovered that an increase in a particular conformation augments the antibacterioribosomal and antibacterial activity of paromomycin, as well as decreasing the toxicity to human ribosomes.

Chapter one introduces the aminoglycoside antibiotics and describes advantages and disadvantages of their clinical use. Various resistance mechanisms arising from target

modification, altered transport, and aminoglycoside modifying enzymes are discussed as is the mechanism of bacterial inhibition and how this is related to toxicity in human cells.

Chapter two describes the synthesis of paromomycin and neomycin derivatives alkylated at *C*-6' with both the (*R*) and (*S*) configurations as well as NMR spectroscopic studies of the side chain conformation of these derivatives. These derivatives were subjected to cell free ribosomal translation assays using *M. smegmatis* ribosomes with decoding A-sites of the wild type, human mitochondrial, mutant human mitochondrial, and human cytosolic ribosomes, as well as to bacterial MIC assays using *E. coli* and ESKAPE pathogens. The (*R*) configuration results in a higher solution state population of the bound conformation resulting in higher activity than the equivalent modification with the (*S*) configuration, which reduces the population of the bound conformation in solution.

Chapter three describes the synthesis of paromomycin derivatives where ring I was fused to an additional ring by bridging *O*-4' and *C*-6', such that the conformation of the C_5-C_6 bond is locked. Conformational analysis by NMR spectroscopy shows a progression from the preferred solution state conformation of the ring I side chain to the ideal bound conformation as a function of the size of the fused ring. These derivatives were subjected to the cell free ribosomal assays and bacterial MIC assays. It was found that as the conformation of the locked side chain approached the ideal gauche, trans conformation the activity increased, leading to the conclusion that the gt conformation is the bound conformation.

Chapter four discusses the effect of the 4'-substituent on the population of ring I side chain conformers in an attempt to rationalize the differences in activity between paromomycin, 4'-deoxy paromomycin, and 4'-deoxy-4'-*C*-propyl paromomycin (propylamycin), a recently

published synthetic aminoglycoside demonstrating equal activity to paromomycin and reduced toxicity in an animal model. Models of ring I of 4'-deoxy paromomycin and propylamycin were synthesized with and without selective deuteration at the side chain carbon. NMR spectroscopic studies of the relative populations of conformers of the ring I side chain for these models were conducted leading to the conclusion that the substituent at the 4'-position has minimal effect on the relative populations of the side chain conformers of ring I: any differences in activity between these compounds are due to other factors.

AUTOBIOGRAPHICAL STATEMENT

Michael Gabriel Pirrone

EDUCATION AND PROFESSIONAL EXPERIENCE:

2014-present	Ph.D. in Organic Chemistry Wayne State University, Detroit, MI Advisor: Prof. David Crich
2012-2014	Co-Op Intern Henkel, Madison Heights, MI
2010-2014	Bachelor of Science, Chemistry with Honors Lawrence Technological University, Southfield, MI Advisor: Prof. Shannon Timmons

RESEARCH PUBLICATIONS AND PATENTS:

1. Newton, M. D.; Hartner, S. E.; Timmons, S.; Delaney, N. D.; **Pirrone, M. G.**; Baker, K. C.; Maerz, T., Contrast-Enhanced μCT of the Intervertebral Disc: A Comparison of Anionic and Cationic Contrast Agents for Biochemical and Morphological Characterization. *Journal of Orthopaedic Research* **2017,** *35*, 1067-1075.
2. Crich, D.; Sati, G. C.; Sonousi, A.; Yang, G.; Mandhapati, A. R.; **Pirrone, M. G**.; Kato, T.; Sarpe, V. A.; Vasella, A.; Bottger, E. C.; Hobbie, S. N. Neomycin and Paromomycin Derivatives. **2018**. Patent # WO2018187738
3. Matsushita, T.; Sati, G. C.; Kondasinghe, N.; **Pirrone, M. G.**; Kato, T.; Waduge, P.; Kumar, H. S.; Sanchon, A. C.; Dobosz-Bartoszek, M.; Shcherbakov, D.; Juhas, M.; Hobbie, S. N.; Schrepfer, T.; Chow, C. S.; Polikanov, Y. S.; Schacht, J.; Vasella, A.; Böttger, E. C.; Crich, D., Design, Multigram Synthesis, and in Vitro and in Vivo Evaluation of Propylamycin: A Semisynthetic 4,5-Deoxystreptamine Class Aminoglycoside for the Treatment of Drug-Resistant Enterobacteriaceae and Other Gram-Negative Pathogens. *J. Am. Chem. Soc.* **2019,** *141*, 5051-5061.
4. Sarpe, V. A.; **Pirrone, M. G.**; Haldimann, K.; Hobbie, S. N.; Vasella, A.; Crich, D., Synthesis of Saccharocin from Apramycin and Evaluation of Its Ribosomal Selectivity. *MedChemComm* **2019,** *10*, 554-558.

AWARDS:

1. Cal Stevens Memorial Scholarship **2017,** Wayne State University
2. Joseph Jasper Scholarship **2018,** Wayne State University
3. Rumble Fellowship **2018-2019,** Wayne State University
4. Schaap Endowed Distinguished Graduate Award **2018-2019,** Wayne State University

PRESENTATIONS:

1. Design, Synthesis, and Evaluation of Improved Aminoglycoside Antibiotics **2018**
Presented at the 29th International Carbohydrate Symposium; Lisbon, Portugal, July 14-18
2. Design, Synthesis, and Evaluation of Improved Aminoglycoside Antibiotics **2018**
Presented at the 256th American Chemical Society National Meeting; Boston, MA, Aug. 19-23