AN ALTERNATE AND FACILE METHOD FOR THE SYNTHESIS OF
PRECURSORS OF 3- AND 6- AMINOSUGAR DONORS AND
A ONE-POT GLYCOSYLATION APPROACH

by

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ABSTRACT

An alternate and facile method for the synthesis of precursors of 3- and 6- aminosugar donors and a one-pot glycosylation approach

by

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Aminoglycosides have long been used for their broad-spectrum activity against gram-positive and gram-negative bacteria. However, the continuous incidence of bacterial resistance has forced researchers to explore additional applications, including potential treatments for genetic neural disorders, diseases associated with mutations of gap junction proteins, and fungal infection. For these novel applications of aminoglycosides, chemical modification of naturally occurring aminoglycosides is often necessary. One such modified aminoglycoside, TC007, has been identified as a potent lead in the treatment of spinal muscular atrophy (SMA). Even though our previous lab members had already synthesized TC007, there has been a high demand for this compound for testing against SMA and characterizing its molecular interactions in the cell. However, de novo synthesis of 3- and 6-aminosugars and their corresponding glycosyl donors can be time- and resource-consuming and challenging to master. For example, the synthesis of the 3-aminoglucopyranosyl donor, essential for the synthesis of this compound, involves a metal-based reagent and is challenging as it requires more than
ten synthetic steps with a meager overall yield. An alternative approach is to utilize natural products containing 3-and 6-aminopyranose. Many naturally occurring or synthetic bioactive compounds contain 3-and 6-aminoglucopyranosyl moieties. The reported acid-catalyzed hydrolysis of azidokanamycin A offered an alternative route to the synthesis of these molecules. The 3-and 6 aminosugar donor was synthesized in just two steps; however, the yield was very poor. An excellent yield was achieved with the hydrolysis of azidokanamycin B, but the cost of starting material was very high. Nevertheless, the synthesis of donors from carboxy benzyl (Cbz or Z) protected kanamycin A (Z4K) was found to be the best route with excellent yield, short synthetic steps, and it was very cost-effective. The acidic hydrolysis of these aminoglycosides not only provided the 3- and 6-aminosugars but also proved the direct chemical glycosylation of these aminosugars was feasible using isopropanol and octanol as the acceptor.

(76 pages)
PUBLIC ABSTRACT

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Uddav Pandey, Master of Science

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The synthesis of 3- and 6- aminosugars from the old route requires many synthetic steps and is challenging. An alternative approach is to utilize acid-catalyzed hydrolysis of kanamycin derivatives. The 3-and 6 aminosugar donor was synthesized in just two steps with excellent yield and cost-effective. The acidic hydrolysis of these aminoglycosides provided not only the 3- and 6-aminosugars, but the direct chemical glycosylation of these aminosugars was proven feasible using isopropanol and octanol as the acceptor.
DEDICATION

I want to dedicate my work to my family for their endless love and support.
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LIST OF ABBREVIATION

Ac₂O: Acetic anhydride
AcCl: Acetyl Chloride
AcOH: Acetic acid
AG: Aminoglycoside
CBz: Benzyloxycarbonyl
DBU: 1,8-Diazabicyclo [5.4.0] undec-7-ene
DCM: Dichloromethane
DMF: Dimethylformamide
DMSO: Dimethyl sulfoxide
DNA: Deoxy Ribonucleic Acid
Eq: Equivalent
Et₃N: Triethylamine
FDA: Food and Drug Administration
HRMS: High-Resolution Mass Spectrometry
Me: Methyl
MeOH: Methanol
NMR: Nuclear Magnetic Resonance
PMe₃: Trimethylphosphine
R.T: Room temperature
rRNA: Ribosomal Ribonucleic Acid
SMA: Spinal Muscular Atrophy
SMN: Survival Motor Neuron
SN2: Nucleophilic substitution

Tf$_2$O: Trifluoromethanesulfonic acid anhydride

THF: Tetrahydrofuran

TLC: Thin Layer Chromatography

Z4K: Tetrakis(berzyloxy carbonyl)kanamycin A
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CHAPTER 1

INTRODUCTION

1-1 Aminoglycosides

The discovery of kanamycin in 1957 by Hamao Umezawa\textsuperscript{1} was a breakthrough in the area of antibiotics due to its antibacterial activity.\textsuperscript{2} Kanamycin belongs to a group of chemicals known as aminoglycosides (AGs). AGs are broad-spectrum antibiotics that have been in use for over 70 years.\textsuperscript{3-4} Structurally, these compounds are made up of one or more amino saccharides and a 2-deoxystreptamine connected through a glycosidic linkage. AGs are classified based on the substitution position of 2-deoxystreptamine. The neomycin class of AGs contains a 4,5 disubstituted 2-deoxystreptamine, kanamycins contain a 4,6-disubstituted 2-deoxystreptamine, and a third-class includes a monosubstituted 2-deoxystreptamine, e.g., streptomycin (figure 1-1).

Despite the potential for renal toxicity and ototoxicity, kanamycin and other AGs remained essential for the treatment of infectious diseases for several decades.\textsuperscript{5} However, years of continual usage have caused bacteria to develop resistance which has compromised the clinical use of AGs.\textsuperscript{6-7}

1-2 Repurposing the application of aminoglycosides

Reviving the antibacterial activity of AGs and searching for different applications has been the primary focus for many researchers around the globe.\textsuperscript{8-9} Beyond the traditional antibacterial uses, potential applications of aminoglycosides include treatments for genetic disorders,\textsuperscript{10} diseases associated with mutations of gap junction
proteins, and fungal infections. For these novel applications of aminoglycosides, chemical modifications of naturally occurring aminoglycosides are often necessary.

**Figure 1-1.** Structure of streptomycin, 2-deoxystreptamine, kanamycin, and neomycin classes
1-3 Aminoglycosides in the treatment of genetic disorders

The critical interaction of drugs with nucleotides of the ribosomal RNA (rRNA) are different in bacteria and humans.\(^{16}\) The selective binding of AGs to the bacterial ribosome results in the antibacterial activity. The A1408 and G1491 of the bacterial ribosome small subunit decoding site likely determines the selectivity of aminoglycosides.\(^{17-18}\) The guanine residue at position 1408 and an adenine residue at position 1491 in eukaryotic 18S rRNA reduces interaction with aminoglycosides, though conserved nucleotides suggest the binding of certain aminoglycosides to the small subunit of both bacterial and eukaryotic ribosomes.\(^{10}\) This finding was a turning point to the utilization of AGs in the treatment of genetic diseases, especially those due to nonsense mutations. Nonsense mutations result from a change in a single nucleotide in a DNA sequence that converts the codon of an amino acid into a termination codon.\(^{19}\) The consequence of a nonsense mutation is the premature termination of protein translation, causing the production of non-functional proteins.\(^{20-21}\) Burke and Mogg\(^{22}\) demonstrated that aminoglycosides could suppress the effect of a nonsense mutation by binding to the decoding site. The impact of this binding is the reduction of the translational fidelity by AG, allowing a random amino acid to incorporate at a premature termination codon in mammalian cells. Hence, the protein translation proceeds through to the natural stop codon.\(^{23}\) This concept introduced a broad area of novel research in the field of aminoglycosides as a stop codon read-through inducer and as a therapeutic agent for human genetic diseases, including spinal muscular atrophy.
1-4 Spinal Muscular Atrophy

Abnormalities caused by a defect in an individual’s genome are genetic disorders. These disorders are inheritable or could result from a mutation in the DNA. Spinal muscular atrophy (SMA) is a neurodegenerative disorder caused by the homozygous loss of the Survival Motor Neuron 1 (SMN1) gene. It is a leading cause of infant mortality from genetic disorders and affects one in every 8,000 – 10,000 births worldwide. SMA is characterized by the death of motor neurons present in the anterior horn of the spinal cord. It causes muscle weakness and wasting of skeletal muscles. There are five types of SMA based on disease severity and the age of onset. SMA type 0 is the rarest, yet the most severe form of SMA, and it manifests itself before childbirth. It can be fatal inside the womb and mostly fatal during the early months after birth. Type I SMA is the most common and severe form that occurs in the first months of life (0-6 months), while Type II is an intermediate form that manifests between 6 and 18 months of life. Type III shows after 18 months, whereas Type IV SMA appears after 35 years of age. Numerous research has been carried out over the past several decades to develop new and improved drugs to treat SMA. Several drugs are in the clinical trial, and a couple were approved by the Food and Drug Administration (FDA) in 2017.

Through collaboration with Dr. Christian Lorson and co-workers (Department of Veterinary Pathobiology, Bond Life Sciences Center, University of Missouri), our libraries of previously synthesized aminoglycosides were screened to identify the modified AG TC007 (figure 1-2) as the lead in the treatment of SMA.

The interaction of TC007 with the eukaryotic ribosome is not fully explored yet. However, recent studies revealed the specific binding of TC007 in the decoding site of
the eukaryotic ribosome which triggers the insertion of an amino acid such as tyrosine in the premature stop codon. This binding is so specific that it only affects the mutant stop codon and not the natural stop codon. This process allows the ribosome to read-through until it finds a natural stop codon. Hence, TC007 became a potential candidate to be used as a therapeutic agent against SMA. 35-36

TC007 is produced using a glycosylation strategy,34 not a direct modification strategy.37 The direct-modification approach commonly employs a naturally occurring aminoglycoside as the starting material and provides a cost-effective synthesis of desired products, albeit lacking flexibility in structural variation.38 The glycosylation strategy allows more structural flexibility but requires the synthesis of designed glycosyl donors, which can be both time- and resource-consuming.39-40 Therefore, my goal was to prepare more TC007 by synthesizing the glycosyl donor through an alternative route.

1-5 3-and 6-aminosugars

The incorporation of amino sugars in different chemical entities has been prevalent in drug design for decades.41 3-aminoglucopyranose and 6-aminoglucopyranose has received significant attention due to their considerable biological and pharmacological importance. Aminosugars, such as 3- and 6-aminosugars, are prevalent in diverse naturally occurring as well as synthetic bioactive compounds.42-43 For example, antibacterial erythromycin and azithromycin, anticancer daunorubicin, and antifungal amphotericin B contain 3-aminosugar (figure 1-2). A 3-aminosugar is also present in the synthetic AG TC007, and the 6-aminosugars are present in antiviral glycolipid derivatives.44 Also, workers often employ a glycodiversification approach to incorporate various aminosugars to reveal useful structure-activity relationships of
bioactive glycosides. All these points to the essential need to prepare aminosugar-based glycosyl donors.

Most of the reported chemical synthesis of glycosyl donors carrying 3- and 6-aminosugar scaffolds start with cost-effective glucose. However, the overall transformation can be labor-intensive, which involves many protections and deprotection steps, stereoselective and regioselective modifications of hydroxyl groups, and amino group manipulation. For example, regioselective conversion of the 6-OH of glucose to 6-NH₂ takes 4-6 synthetic steps, depending on the starting material (scheme 1-1). The stereoselective synthesis of 3-aminosugar usually starts with commercially available glucose. Six synthetic steps and at least 2-3 column chromatography purifications are needed to produce the precursor of the glycosyl donor, 9, making the overall process hard to master. Also, several reported syntheses employ hazardous chromium-based oxidants. Therefore, an alternative route is sought for the synthesis of 3- and 6-aminoglucopyranosyl donors.

Scheme 1-1. Reported syntheses of 6-aminosugar from glucose.
1-6 Synthesis of TC007 via old route

The synthesis of TC007 through the old route followed the protocol, as described by Dr. Ravi Rai. The synthesis of the acceptor started from the commercially available neomycin B. The amino groups were converted into azido groups and then benzylated to produce compound 2 (scheme 1-2). The glycosidic bond between rings II and III was cleaved by refluxing 3 in the presence of copper (II) chloride, and this gave the known neamine derivative 4, the glycosyl acceptor.
The synthesis of the 3- amino sugar donor precursor 9 started from the commercially available glucose 5. Swern oxidation of the diacetone-D-glucose and reduction of the corresponding ketone with NaBH₄ gave the epimer alcohol 7. Triflation, SₐN₂ azido substitution, and acid-catalyzed hydrolysis resulted in 3-azido sugar 9 (scheme 1-3). The acetylation of 3- azido sugar 9, provided the tetraacetyl pyranose 10. Treatment with hydrazine acetate selectively hydrolyzed the acetyl group at the anomeric position to give 11, whose free hydroxyl group was then activated in the presence of trichloroacetonitrile to afford the glycosyl donor 12 (scheme 1-4).

Glycosylation of acceptor 4 and donor 12 in the presence of the Lewis acid BF₃-OEt₂ provided compound 16 (scheme 1-5). BF₃-OEt₂ activated the donor 12 and formed an oxocarbenium intermediate, which in the presence of a 2-O-acyl group gave an acyloxonium intermediate. Thus, the attack by an acceptor (a nucleophile) was only possible from the open-top face, controlling the stereochemistry in the formation of the β-anomer. The deacetylation using sodium methoxide in methanol, Staudinger reduction of the azide, hydrogenolysis, and ion-exchange, provided TC007 as a chloride salt.
Scheme 1-2. Synthesis of Glycosyl acceptor

Scheme 1-3. Synthesis of 3-amino sugar by old route
**Scheme 1-4. Synthesis of Glycosyl donor by old route**

![Chemical reaction diagram for Scheme 1-4]

**Scheme 1-5. Glycosylation**

![Chemical reaction diagram for Scheme 1-5]
1-7 Hydrolysis of Aminoglycosides

Leach and Teeters\textsuperscript{56} were pioneers in the hydrolysis of neomycin. Cron \textit{et al.}\textsuperscript{57} studied the hydrolysis of kanamycin and the antimicrobial activity of its hydrolyzed products. The hydrolysis of tobramycin to synthesize more active antibiotics also increased the importance of research in the field of aminoglycoside hydrolysis.\textsuperscript{58}

Naturally occurring aminoglycosides, such as the kanamycin class of antibiotic, consist of various aminosugars that are linked together via glycosidic bonds (figure 1-1).\textsuperscript{53-55} Thus, an alternative approach for the synthesis of glycosyl donors of 3- and 6-aminosugars is to conduct acid-catalyzed hydrolysis of aminoglycosides and recover the desired aminosugars.

Acid-catalyzed hydrolysis of kanamycin offers two aminosugars (rings I and III), which are difficult to separate. The separation gets more difficult due to the fact that the aminosugars occur as a mixture of anomers.\textsuperscript{35,51} The reported methods also commonly use hydrochloric and sulfuric acids which cause the formation of viscous, brownish by-products. This makes purification of the desired products even more challenging. Nevertheless, very little work has been reported on the use of hydrolysis of aminoglycosides to synthesize different amino sugars, and a detailed study of breaking the glycosidic bond is still in progress.

Herein, we develop an alternative approach involving the hydrolysis of azidokanamycin, and cbz-kanamycin for obtaining the precursor of glycosyl donors in fewer synthesis steps, increasing the yield and making it safer and more economical. We will also demonstrate the feasibility of one-pot glycosylation during the acid-catalyzed hydrolysis of kanamycin A derivatives.
CHAPTER 2
MATERIALS AND METHODS

2-1 Experimental Reagents and Instruments

All chemicals were purchased through commercially available sources without any further purification. Mass was measured by high-resolution mass spectrometry (HRMS). Bruker Ascend (500 MHz) NMR instrument was used for the $^1$H and $^{13}$C nuclei. CDCl$_3$, CD$_3$OD, and DMSO-d$_6$ were used as solvents. Parts per million (ppm) were used to express the chemical shifts on d scale. The peaks splitting pattern were expressed as (s: for the singlet), (d: doublet), (t: triplet), (q: quadrature), (m: multiplet), and (ddd: doublet of doublets of doublets). Coupling constants $J$ were measured in Hertz (Hz).

2-2 Experimental Procedures

2-2.1 General procedure for the hydrolysis of azido-compounds

Method 1:

Hydrolysis of tetraazidokanamycin A

To 25 mL of methanol cooled to 0 °C, 8.33 mL of acetyl chloride (excess) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then 0.5g of tetraazidokanamycin A $^{59}$ (0.85mmol, 1 equivalent) was added to the reaction mixture. After 48 hours of the reaction at room temperature, the formation of the product was confirmed by TLC (Dichloromethane (DCM): Methanol = 90:10, Retention Factor (R$_f$) = 0.4 to 0.8). The crude reaction mixture after concentrated under reduced pressure and
loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford **18-20**.

**Hydrolysis of pentaazidokanamycin B**

To 25 mL of methanol cooled to 0 °C, 8.33 mL of acetyl chloride (excess) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then, 0.5 g of pentaazidokanamycin B 59 (0.81mmol, 1 equivalent) was added to the reaction mixture. After 48 hours of the reaction at room temperature, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, Rf = 0.6 to 0.8). The crude reaction mixture was concentrated under reduced pressure and loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford **19 and 22**.

**Hydrolysis of hexaazidoneomycin B**

To 25 mL of methanol cooled to 0 °C, 12.5 mL of acetyl chloride (excess) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then, 0.5 g hexaazidoneomycin B 60 (0.64mmol, 1 equivalent) was added to the reaction mixture. After 48 hours of the reaction at room temperature, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, Rf = 0.8). The crude reaction mixture was concentrated under reduced pressure and loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford **22**.
Method 2

Hydrolysis of tetraazidokanamycin A

To 25 mL of acetonitrile cooled to 0 °C, 0.33 mL of TMSOTf (1.87 mmol, 2.2 equivalent) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then, 0.5 g of azidokanamycin (0.85 mmol, 1 equivalent) was added to the reaction mixture. After 48 hours of the reaction at room temperature, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, Rf = 0.4 to 0.8). The reaction solution was quenched with methanol, concentrated under reduced pressure, and the crude reaction mixture was loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford 18.

Hydrolysis of pentaazidokanamycin B

To 25 mL of acetonitrile cooled to 0 °C, 0.33 mL of TMSOTf (1.87 mmol, 2.2 equivalent) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then 0.5 g of pentaazidokanamycin B (0.81 mmol, 1 equivalent) was added to the reaction mixture. After 48 hours of the reaction at room temperature, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, Rf = 0.6 to 0.8). The reaction solution was quenched with methanol, concentrated under reduced pressure, and the crude reaction mixture was loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford 19 and 22.
Hydrolysis of hexazidoneomycin B

To 25 mL of acetonitrile cooled to 0 °C, 0.5 mL of TMSOTf (2.816 mmol, 4.4 equivalent) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then 0.5 g of hexazidoneomycin B (0.64 mmol, 1 equivalent) was added to the reaction mixture. After 48 hours of the reaction at room temperature, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, Rf = 0.8). The reaction solution was quenched with methanol, concentrated under reduced pressure, and the crude reaction mixture was loaded to silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford 22.

2-2.2 Hydrolysis of Tetrakis (bezyloxy carbonyl) kanamycin A (Z4K) in methanol, isopropanol, and octanol

Z4K in methanol

To 25 mL of methanol cooled to 0 °C, 8.33 mL of acetyl chloride (excess) was added dropwise, keeping the flask in the ice bath and stirred for 5 minutes. Then 1.0 g of Z4K (0.97 mmol, 1 equivalent) was added to the reaction mixture. After refluxing the reaction mixture for 12 hours, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, Rf = 0.1 to 0.4). The crude reaction mixture after concentrated under reduced pressure and loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford 25-27.
Z4K in isopropanol

To a solution of 1 g of Z4K (0.97 mmol, 1 equivalent) in 10 mL of anhydrous dioxane, 10 mL of anhydrous dioxane-HCl (4M) was added, followed by the addition of isopropanol (11.76 mmol, 12 equivalent) and stirred for 5 minutes. After refluxing the reaction mixture for 12 hours, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, R\textsubscript{f} = 0.1 to 0.6). The crude reaction mixture after concentrated under reduced pressure and loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (95:5) to afford 26 and 30-32.

Z4K in octanol

To a solution of 1 gram of Z4K (0.97 mmol, 1 equivalent) in 10 mL of anhydrous dioxane, 10 mL of anhydrous dioxane-HCl (4M) was added followed by the addition of 1-octanol (11.76 mmol, 12 equivalent) and stirred for 5 minutes. After refluxing the reaction mixture for 12 hours, the formation of the product was confirmed by TLC (DCM: Methanol = 90:10, R\textsubscript{f} = 0.1 to 0.7). The crude reaction mixture after concentrated under reduced pressure and loaded to a silica gel column. The column was eluted using DCM and methanol solvent mixture with the proportion from (100:0) to (97:3) to afford 26 and 32-34.

2.2.3 General procedure for acetylation

To a solution (0.1 g, 0.305 mmol) of glucopyranose in acetic anhydride at 0°C, 6-7 drops of concentrated H\textsubscript{2}SO\textsubscript{4} were added slowly and stirred overnight at room temperature. The saturated NaHCO\textsubscript{3} (aq) and ethyl acetate (50 mL) were added to the reaction mixture after the confirmation of reaction completion by TLC (Hexane: Ethyl acetate= 50:50, R\textsubscript{f} =
0.7 for 28 and 0.4 for 29). After stirring for an hour, the organic layer was separated, washed with water and brine, and dried over Na₂SO₄. The products (28-29) obtained were yellowish oils containing only α anomers as the product.
CHAPTER 3

RESULTS

3-1 Synthesis of 3- and 6- aminosugar via the new route

Various solvents and acids were examined for the hydrolysis of tetraazidokanamycin A and these results are summarized in Table 3-1. For purification, all the reactions were quenched with methanol to yield methyl glycosides except when using methanol as a solvent (Entry 1). From the results, HCl generated from mixing AcCl with MeOH provided the best outcome to obtain 3- and 6-aminosugar precursors, yielding compounds 18 (34%), 19 (11%), and 20 (2%). The isolated azidosugars, 19 and 20, can be converted into the corresponding glycosyl donors using reported protocol. All other conditions generated either no hydrolysis or a mixture of products that could not be separated. The reason might be an issue of the solubility of the starting material in the solvent used or the acid not being strong enough to hydrolyze the glycosidic bond.

With the establishment of optimal conditions, we also examined the acid-catalyzed hydrolysis using pentaazidokanamycin B (21) and hexaazidoneomycin B (23) (scheme 3-1). Two conditions were examined: AcCl/MeOH and TMSOTf/MeCN. Interestingly, for pentaazidokanamycin B, only the glycosidic bond between rings II and III was cleaved, resulting in the formation of tetraazidoneamine and the corresponding 3-azidosugar (ring III). For the hydrolysis of hexaazidoneomycin B, there was also no hydrolysis of the glycosidic bond between rings I and II, leading to the formation of tetraazidoneamine and a mixture probably produced from breaking of the glycosidic bonds between rings II and III and between rings III and IV. Hydrolysis of
azidokanamycin A, which has a hydroxyl group (-OH) at a 2′ position, resulted in the breaking of the glycosidic bond between ring I and II. The presence of the hydroxyl group could have eased the intramolecular hydrogen bonding to favor the hydrolysis of the glycosidic bond. However, the absence of intramolecular hydrogen bonding in the presence of the azido group (N₃) in 21 and 23 could have prohibited the breaking of the bond between ring I and II in the aminoglycosides subjected to hydrolysis. This result is consistent with the hydrolysis of azidotobramycin that has been reported previously.⁵⁸

Table 3-1. Hydrolysis of tetraazidokanamycin A

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvents</th>
<th>Acid</th>
<th>Product (% yield)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MeOH</td>
<td>HCl (MeOH and AcCl)</td>
<td>18 (34) + 19 (11) + 20 (2)</td>
</tr>
<tr>
<td>2</td>
<td>DCM</td>
<td>TMSOTf</td>
<td>No Hydrolysis</td>
</tr>
<tr>
<td>3</td>
<td>DCM</td>
<td>BF₃-OEt₂</td>
<td>No Hydrolysis</td>
</tr>
<tr>
<td>4</td>
<td>1,4-dioxane</td>
<td>TMSOTf</td>
<td>18 (90) + inseparable mixture</td>
</tr>
<tr>
<td>5</td>
<td>Acetonitrile</td>
<td>TMSOTf</td>
<td>18 (90) + inseparable mixture</td>
</tr>
</tbody>
</table>
Although acid-catalyzed hydrolysis of tetraazidokanamycin A furnished desired aminosugars, the cost of synthesis was high and the yield poor. Therefore, we also examined the use of tetracarbobenzoxy (Cbz)-protected kanamycin A \(24\) \(^{62}\) to provide aminosugars. The synthesis of \(24\) from kanamycin A was much cheaper, resulting in a higher yield and involving no safety issues compared to the synthesis of azidokanamycin A. Compound \(24\) was subjected to hydrolysis using MeOH/\(\text{AcCl}\) and various Lewis acids. To our delight, not only could the Cbz-protected aminosugars be obtained by using MeOH/\(\text{AcCl}\) (Method 1), but the yields were better than with hydrolysis of
tetraazidokanamycin A (Scheme 3-2). The subsequent acetylation of compound 25 and 27 resulted in the synthesis of amino sugar donor precursors, compounds 28 and 29, in excellent yield. Interestingly, there was no hydrolysis when using other acids like TsOH, TMSOTf, and BF$_3$-OEt$_2$.

**Scheme 3-2.** Synthesis of 3- and 6-amino sugar donors.

3-2 One-pot glycosylation

Glycosylation is a natural enzymatic process catalyzed by glycosyltransferases. However, it is somewhat unusual in chemical synthesis. To expand the applications of the developed protocol, we attempted to explore the possibility of utilizing alcohols other than methanol for the hydrolysis so that the methodology can be feasible for possible chemical glycosylation. In this design, the aminosugars on aminoglycosides will undergo
cleavage of glycosidic bonds and subsequent glycosylation with provided alcohol in one-pot. Among the alcohols and acids examined, both isopropanol and 1-octanol under 4 N HCl in 1,4-dioxane produced the hydrolyzed adducts (Scheme 3-3). Although only modest yields were obtained, the outcome proved that it is possible to conduct hydrolysis of 24 and use the hydrolyzed aminosugars for chemical glycosylation.

Scheme 3-3. One-pot glycosylation.
3-3 Characterization of $^1$H-NMR and $^{13}$C-NMR

1,3-diazido-2-deoxystreptamine (18)

Yield: 34%; white solid; $^1$H NMR (CD$_3$OD, 500 MHz): δ 3.38 (m, 2H), 3.18 - 3.27 (m, 3H), 2.09 (dt, $J_1$ = 4.4 Hz, 1H), 1.25 (q, $J_2$ = 12.6 Hz, 1H).

Methyl 3-Azido-3-deoxy-D-glucopyranoside (19)

Yield: 11%; light white oil (α: β anomers = 3:2); α anomer: $^1$H NMR (CD$_3$OD, 500 MHz): δ 4.85 (d, $J_1$ = 3.6 Hz, 1H), 3.91 (dd, $J_2$ = 2.1, 12.2 Hz, 1H), 3.83 - 3.47 (m, 5H), 3.62 (s, 3H).

Methyl 6-Azido-6-deoxy-D-glucopyranoside (20)

Yield: 2%; light white oil (α: β anomers = 1:1); α anomer: $^1$H NMR (CD$_3$OD, 500 MHz): δ 4.70 (d, $J_1$ = 3.6 Hz, 1H), 3.61 (dd, $J_2$ = 2.1, 12.2 Hz, 1H), 3.54 -3.37 (m, 4H), 3.62 (s, 3H), 3.26 (dd, $J_3$ = 9.02, 9.0 Hz, 1H).
1,3,2′,6′-Tetraazidoneamine (22)\textsuperscript{64}

Yield: 90%; white solid; \textsuperscript{1}H NMR (CD\textsubscript{3}OD, 500 MHz): δ 5.63 (d, \( J = 3.7 \) Hz, 1H), 4.16 (ddd, \( J = 10.4, 5.6, 2.8 \) Hz, 1H), 3.85 (dd, \( J = 10.4, 8.7 \) Hz, 1H), 3.55 - 3.33 (m, 6H), 3.31 - 3.21 (m, 2H), 3.12 (dd, \( J = 10.5, 3.8 \) Hz, 1H), 2.24 (dt, \( J = 13.1, 4.2 \) Hz, 1H), 1.89 (q, \( J = 12.5 \) Hz, 1H).

Methyl 6-Benzyloxy carbonylamino-6-deoxy-D-glucopyranoside (25)\textsuperscript{65}

Yield: 50%; white solid (α: β anomers = 2:1); α anomer: \textsuperscript{1}H NMR (DMSO-\textsubscript{d}6, 500 MHz) δ 7.65 – 7.09 (m, 5H), 5.10 (s, 2H), 4.65 (d, \( J = 3.8 \) Hz, 1H), 3.61 – 3.56 (m, 1H), 3.56 – 3.54 (m, 4H), 3.54 (d, \( J = 2.9 \) Hz, 1H), 3.49 (s, 1H), 3.39 (dd, \( J = 9.6, 3.8 \) Hz, 1H), 3.15 (dd, \( J = 18.1, 8.8 \) Hz, 1H).

\(N,N\)-bis(benzyloxy carbonyl)-2-deoxystreptamine (26)\textsuperscript{66}

Yield: 62%; white solid; \textsuperscript{1}H NMR (DMSO-\textsubscript{d}6, 500 MHz) δ 7.46 – 7.32 (m, 10H), 7.31 (d, \( J = 6.7 \) Hz, 1H), 7.10 (d, \( J = 8.0 \) Hz, 1H), 5.00 (q, \( J = 12.7 \) Hz, 4H), 4.88 (s, 1H),
4.71 (s, 1H), 4.13 (s, 1H), 3.24 (d, \( J = 8.9 \) Hz, 2H), 3.09 – 2.97 (m, 3H), 1.81 (d, \( J = 12.0 \) Hz, 1H), 1.26 – 1.16 (m, 1H).

\[
\begin{align*}
\text{Methyl 3-Benzylxocarbonylamino-3-deoxy-D-glucopyranoside (27)}
\end{align*}
\]

Yield: 75%; white solid; \( \alpha \) anomer: \(^1\)H NMR (CD\(_3\)OD, 500 MHz) \( \delta \) 7.54 – 7.16 (m, 5H), 5.11 (s, 2H), 4.71 (d, \( J = 3.6 \) Hz, 1H), 3.87 – 3.75 (m, 2H), 3.69 (dd, \( J = 11.8 \), 5.5 Hz, 1H), 3.64 – 3.56 (m, 1H), 3.50 (dd, \( J = 11.1 \), 2.5 Hz, 1H), 3.46 (s, 3H), 3.37 (t, \( J = 9.8 \) Hz, 1H); \(^{13}\)C NMR (CD\(_3\)OD, 500 MHz) \( \delta \) 158.27, 136.99, 128.02(3C), 127.48(2C), 99.46, 72.69, 70.33, 68.61, 66.08, 61.29, 56.59, 54.10; ESI/APCI calculated for C\(_{15}\)H\(_{2}\)NO\(_{7}\)Na ([M + Na]+) m/z 350.1216, measured m/z 350.1208.

\[
\begin{align*}
\text{Acetyl 3-Benzyloxycarbonylamino-2,4,6-tri-O-actyl-3-deoxy-D-glucopyranoside (29)}^{67}
\end{align*}
\]

Yield: 85%; yellowish oil; \( \alpha \) anomer: \(^1\)H NMR (CDCl\(_3\), 500 MHz) \( \delta \) 7.43 – 7.27 (m, 5H), 6.35 (d, \( J = 3.8 \) Hz, 1H), 5.49 (t, \( J = 9.8 \) Hz, 1H), 5.22 – 5.08 (m, 3H), 4.33 – 4.27 (m, 1H), 4.27 – 4.20 (m, 2H), 4.15 (dd, \( J = 8.5 \), 5.3 Hz, 1H), 2.36 – 1.78 (m, 12H); \(^{13}\)C NMR (CDCl\(_3\), 500 MHz) \( \delta \) 170.64, 169.65, 169.39, 168.75, 167.75, 132.45, 130.87, 128.80, 128.57, 128.25, 89.07, 69.89, 69.83, 69.19, 68.16, 67.89, 66.32, 61.46, 20.88, 20.70, 20.67, 20.57, 20.45.
Acetyl 6-Benzyloxy carbamylamino-2,3,4-tri-O-actyl-6-deoxy-D-glucopyranoside (28)  

Yield: 87%; yellowish oil; α anomer: \(^1\)H NMR (CDCl\(_3\), 500 MHz) δ 7.47 – 7.13 (m, 5H), 6.32 (d, \(J = 3.7\) Hz, 1H), 5.57 – 5.46 (m, 1H), 5.26 – 5.19 (m, 1H), 5.19 – 5.07 (m, 2H), 4.34 (dd, \(J = 12.7, 3.8\) Hz, 1H), 4.31 – 4.23 (m, 1H), 4.04 – 3.84 (m, 1H), 3.32 – 3.15 (m, 1H), 2.13 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H); \(^1^3\)C NMR (CDCl\(_3\), 500 MHz) δ 170.31, 170.14, 169.77, 168.99, 167.75, 132.43, 130.87, 128.78 (3C), 88.97, 70.57, 69.68, 69.36, 68.67, 68.14, 53.43, 20.87, 20.65, 20.63, 20.44.

Isopropyl 6-Benzyloxy carbamylamino-6-deoxy-D-glucopyranoside (30)  

Yield: 9%; white solid (α: β anomers = 1:1); β anomer: \(^1\)H NMR (CD\(_3\)OD, 500 MHz) δ 7.48 – 7.28 (m, 5H), 5.10 (d, \(J = 4.1\) Hz, 2H), 4.31 (d, \(J = 7.6\) Hz, 1H), 3.93 – 3.85 (m, 1H), 3.68 (ddd, \(J = 11.6, 7.3, 3.7\) Hz, 1H), 3.59 (ddd, \(J = 16.5, 13.3, 7.5\) Hz, 1H), 3.18 – 3.10 (m, 2H), 1.24 – 1.14 (m, 6H); \(^1^3\)C NMR (CD\(_3\)OD, 500 MHz) δ 157.76, 135.40, 128.08 (2C), 127.47 (3C), 101.34, 76.34, 74.53, 72.09 , 70.57, 69.71, 66.05 , 41.79, 20.76, 20.26.
Isopropyl 3-Benzylxycarbonylamino-3-deoxy-α-D-glucopyranoside (31)

Yield: 32%; white solid; α anomer: $^1$H NMR (CD$_3$OD, 500 MHz) $\delta$ 7.42 – 7.27 (m, 5H), 5.11 (s, 2H), 4.92 (d, $J = 3.7$ Hz, 1H), 4.00 (dt, $J = 12.3$, 6.2 Hz, 1H), 3.79 (t, $J = 10.0$ Hz, 2H), 3.75 – 3.65 (m, 2H), 3.45 (dd, $J = 10.6$, 3.6 Hz, 1H), 3.39 – 3.34 (m, 1H), 1.29 (d, $J = 6.2$ Hz, 3H), 1.22 (t, $J = 8.2$ Hz, 3H); $^{13}$C NMR (CD$_3$OD, 500 MHz) $\delta$ 157.72, 137.01, 127.98 (2C), 127.46 (3C), 96.42, 72.76, 70.29, 69.56, 68.80, 66.05, 61.31, 56.55, 22.27, 20.23.

Octyl 6-Benzyloxycarbonylamino-6-deoxy-D-glucopyranoside (33)
Yield: 10%; white solid (α: β anomers = 1:1); α anomer: $^1$H NMR (CD$_3$OD, 500 MHz) δ 7.41 – 7.29 (m, 5H), 5.10 (s, 2H), 4.75 (d, $J = 3.8$ Hz, 1H), 3.85 (dt, $J = 9.5$, 6.8 Hz, 1H), 3.68 – 3.57 (m, 3H), 3.46 – 3.35 (m, 2H), 3.21 – 3.12 (m, 2H), 1.67 – 1.55 (m, 2H), 1.36 – 1.27 (m, 10H), 0.91 (t, $J = 6.9$ Hz, 3H); $^{13}$C NMR (CD$_3$OD, 500 MHz) δ 157.72, 136.98, 128.15 (3C), 127.39(2C), 98.56, 76.34, 74.62, 72.21, 71.68, 69.52, 67.77, 66.49, 31.45, 29.43, 29.34, 25.91, 25.72, 22.32, 13.05; ESI/APCI calculated for C$_{22}$H$_{35}$NO$_7$Na ([M + Na]$^+$) m/z 448.2311, measured m/z 448.2314.

Octyl 3-Benzyloxy carbonylamino-3-deoxy-D-glucopyranoside (34)

Yield: 18%; white solid (α: β anomers = 4:1); α anomer: $^1$H NMR (CD$_3$OD, 500 MHz) δ 7.66 – 7.02 (m, 5H), 5.11 (s, 2H), 4.80 (d, $J = 3.6$ Hz, 1H), 3.96 – 3.85 (m, 1H), 3.84 – 3.74 (m, 2H), 3.73 – 3.58 (m, 2H), 3.60 – 3.45 (m, 2H), 3.36 (d, $J = 9.9$ Hz, 1H), 1.76 – 1.51 (m, 2H), 1.48 – 1.27 (m, 10H), 0.92 (td, $J = 6.8$, 2.3 Hz, 3H); $^{13}$C NMR (CD$_3$OD, 500 MHz) δ 158.33, 136.97, 128.01(3C), 127.46(2C), 98.29, 77.67, 71.92, 70.42, 69.48, 68.71, 67.77, 66.10, 61.34, 56.63, 31.64, 29.04, 25.74, 22.35, 13.07; ESI/APCI calculated for C$_{22}$H$_{35}$NO$_7$Na ([M + Na]$^+$) m/z 448.2311, measured m/z 448.2314.
CHAPTER 4

CONCLUSIONS AND SIGNIFICANCE

Our laboratory has previously synthesized TC007 and identified it as a potent lead in the treatment of spinal muscular atrophy. By modifying the old route, the synthetic steps were shortened, and yield increased.

The acid-catalyzed hydrolysis of tetraazidokanamycin A, pentaazidokanamycin B, and Z4K were analyzed. The synthesis of 3- and 6- amino sugars from the hydrolysis of tetraazidokanamycin A was a turning point in shortening the synthetic steps involved. The cost of amino sugar preparation was low; however, the yields were 11% for 3-aminosugar and 2% for 6-aminosugar. Utilizing the same established protocol, hydrolysis of pentaazidokanamycin B resulted 90% yield of 3-amino sugar. The cost of azidokanamycin B, being 25 times more, could not override the shortcomings of utilizing azidokanamycin A in the hydrolysis. Last but not the least, we synthesized, z4k, a very cheap starting material compared to azidokanamycin A and B. The production of 3- and 6- amino sugars from the hydrolysis of z4k were in excellent yield compared to the hydrolysis of tetraazidokanamycin A and was cost-effective compared to both azidokanamycin A and B. Finally, we have developed convenient protocols to furnish the protected 3- and 6-aminosugars. These protocols utilizing the hydrolysis of aminoglycosides are cost-effective, result in higher yields, and can shorten the synthetic steps as compared to other methods using unmodified pyranoses as the starting material.

In addition, our results from the hydrolysis of different aminoglycosides suggest that the presence of hydrogen bonding at a 2′ position can direct the composition of products from acid hydrolysis of aminoglycosides.
Finally, we have proven that it is possible to conduct glycosylation in one-pot.
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APPENDIX

$^1\text{H}$ and $^{13}\text{C}$ spectrum of newly synthesized compounds
13C-NMR (500 MHz, CD3OD) of Methyl 3-Benzylxycarbonylamino-3-deoxy-D-glucopyranoside (27)
$^1$H NMR (500 MHz, CD$_3$OD) of Isopropyl 6-Benzyloxy carbonylamino-6-deoxy-D-glucopyranoside (28)
$^{13}$C-NMR (500 MHz, CD$_3$OD) of isopropyl 3-Benzoylcarbonylamino-3-deoxy-α-D-glucopyranoside (29)
$^1$H-NMR (500 MHz, CD$_3$OD) of Isopropyl 3-Benzoxycarbonylamino-3-deoxy-D-glucopyranoside (30)
$^{13}$C-NMR (500 MHz, CD$_3$OD) of Octyl 3-Benzylxycarbonylamino-3-deoxy-D-glucopyranoside (32)
$^{13}$C-NMR (500 MHz, CD$_3$OD) of Octyl-6-deoxy-6-(benzyloxycarbonylamino)-D-glucopyranoside (33)
$^{1}H$-NMR (500 MHz, CD$_3$OD) of Octyl 3-Benzoylcarnbonylamino-3-deoxy-D-glucopyranoside (34)
$^{13}$C-NMR (500 MHz, CD$_3$OD) of Octyl 3-Benzoxycarbonylamino-3-deoxy-D-glucopyranoside (34)
CURRICULUM VITAE

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CAREER OBJECTIVE
To obtain a research position in a competitive institution that will allow me to apply my extensive knowledge in multi-step synthesis of bioactive molecules. Research interests include organic synthesis, drug discovery, library synthesis, and methodology development.

EDUCATION
B.S., Biochemistry, Pokhara University, Kathmandu, Nepal, 2010
M.S., Radiochemistry, Norwegian University of Life Sciences, Aas, Norway, 2013
M.S., Student, Utah State University, Logan, USA, 2016-present.

RESEARCH EXPERIENCE
Graduate Assistant, Utah State University, August 2016- Present
- Synthesized a complex sugar molecule, TC007, a potent compound in the treatment of spinal muscular atrophy.
- Synthesized imines from aldehydes and amino acids extracted from waste.
- Bioassay on lignin degradation by fungi, a green approach using fluorescent lignin probes.

Graduate Researcher, Norwegian University of Life Sciences, August 2010-December 2013
- Assessed the effects of gamma and radon in the indoor environment of a mining site.
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Fall 2016, CHEM 1225, Laboratory instructor, General chemistry laboratory II,
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**Publications**

Pandey, U.; Subedi, Y. P.; Alfindee, M. N.; Shepherd, T.; Chang, C.-W T. Acid-catalyzed hydrolysis of kanamycin derivatives for improving the synthesis of 3- and 6-aminosugar donors. (Submitted to Chemistry letter).

**Poster Presentation:**
258th ACS National Meeting, San Diego, August 2019, “Revised synthesis of TC007, aminoglycosides with potent lead for SMA treatment”.

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