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An Alternative and Facile Synthetic Approach for the Precursors of 3- and 6-Aminosugar Donors and Study of One-Pot Glycosyltrasferation

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[1]An Alternative and Facile Synthetic Approach for the Precursors of 3- and 6-Aminosugar Donors and Study of One-Pot Glycosyltrasferation

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3- and 6-aminosugars are common motifs in bioactive compounds playing pivotal roles in the bioactivity to which the core molecules they are attached. However, *de novo* synthesis of 3- and 6-aminosugars and their corresponding glycosyl donors can be time and resource consuming. The reported acid-catalyzed hydrolysis of kanamycin derivatives offers an alternative route to the synthesis of these molecules. Not only can the 3- and 6-aminosugars be provided, but the direct chemical glycosyltransferation of these aminosugars is proven feasible.

Keywords: Kanamycin-hydrolysis, Aminosugars, Glycosyltransferation

Aminosugars, such as 3- and 6-aminosugars are prevalent in diverse naturally occurring or synthetic bioactive compounds.[1,2] For example, 3-aminosugar can be found in antibacterial erythromycin and azithromycin, anticancer daunorubicin, and antifungal amphotericin B (Figure 1). A synthetic aminoglycoside, **TC007**,[3] which has been reported for its uses as the potential treatment of spinal muscular atrophy (SMA), also contains a 3-aminosugar. The glycolipid bearing 6-aminosugar has been investigated for its antiviral activity.[4] In addition, many bioactive glycosides often employ glycodiversification approach to incorporate various aminosugars for revealing useful structure-activity relationship.[5-7] All these point to the essential need to prepare corresponding aminosugar-based glycosyl donors.

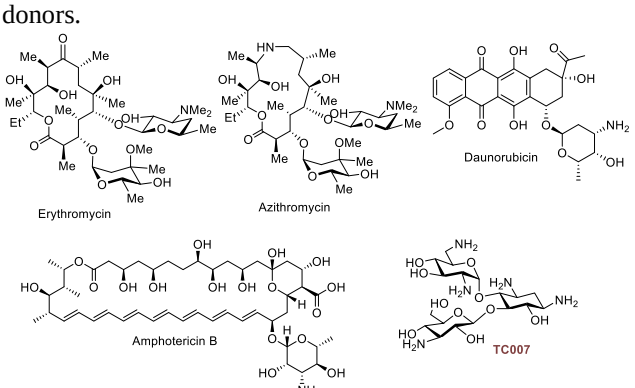
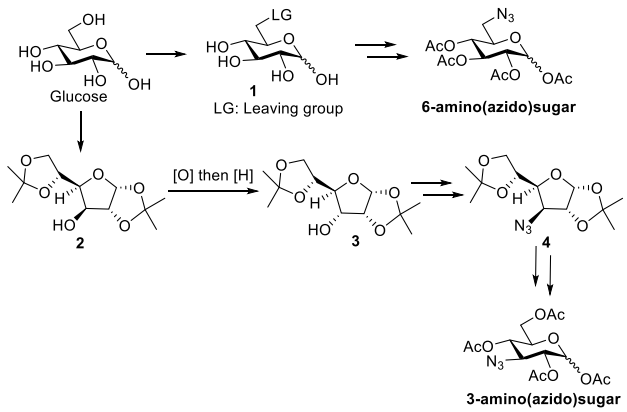


Figure 1. Bioactive compounds bearing 3- and 6-aminosugars

Most of the reported chemical synthesis of glycosyl donors carrying 3- and 6-aminosugar scaffolds start with cost-effective glucose.[8] However, the overall transformation can be labor-intensive, which involves many protections and deprotection steps, stereoselective and regioselective modification of hydroxyl groups, and amino group manipulation. For example, regioselective conversion of 6-OH of glucose to 6-NH_2 takes 4-6 steps depending on the availability of the starting material (Scheme 1).[9] The stereoselective synthesis of 3-aminosugar usually started with commercially available diacetone-D-glucose. Multiple synthetic steps and at least 2-3 column chromatography purification were needed to offer the precursor of glycosyl door, **4** making the overall process time consuming and expensive.[10] In addition, hazardous chromium-based oxidants were employed in several reported syntheses.[10,11] Therefore, there is a need for an alternative route for 3-and 6-aminoglucopyransyl donors. Herein, an alternative route involving the hydrolysis of azidokanamycin and cbz-kanamycin is developed for accessing these glycosyl donors in fewer synthesis steps. In addition, the feasibility of one-pot glycosyltransferation during the hydrolysis of acid-catalyzed hydrolysis of kanamycin A derivatives is also demonstrated.

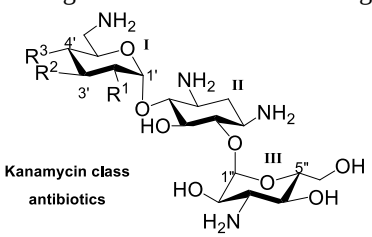


Scheme 1. Reported syntheses of aminosugars from glucose.

Naturally occurring aminoglycosides, such as kanamycin class of antibiotic, consist of various aminosugars that are linked together via glycosidic bond (Figure 2).[12-14] Thus, an alternative approach for the synthesis of glycosyl donors of 3- and 6-aminosugars is to conduct acid-catalyzed hydrolysis of aminoglycosides and yield the desired aminosugars. Considering the synthetic efficiency and cost, we selected kanamycin A, which has 6-aminosugar as the ring I and 3-aminosugar as ring III, as the starting material for the initial study.

Acid-catalyzed hydrolysis of kanamycin offers two aminosugars (rings I and III), which are difficult to

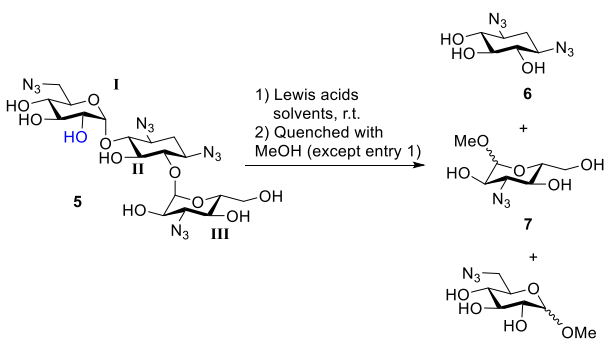
separate, especially the aminosugars from rings I and III come in as a mixture of anomers.[15,16] In addition, the reported methods, commonly using hydrochloric acids and sulfuric acids, cause the formation of brownish by-products in the viscous form which makes the purification of the desired products even more challenging. Thus, we decided to employ azidokanamycin[16] as the starting material since the resulting azidosugars can be purified with traditional silica gel-based column chromatography.



	R^1	R^2	R^3
Kanamycin A	OH	OH	OH
Kanamycin B	NH_2	NH_2	OH
Tobramycin	NH_2	H	OH

Figure 2. Structures of kanamycin class antibiotics.

Various solvents and acids were examined for the hydrolysis of "tetraazidokanamycin (5)" (Scheme 2), and the results are summarized in Table 1. For purification, all of the reactions were quenched with methanol to yield methyl glycosides except when methanol was used as a solvent (Entry 1). From the results, HCl generated from mixing AcCl with MeOH provided the best outcome to obtain 3- and 6 amino sugar precursors, yielding compounds **6** (34%), **7** (11%), and **8** (2%). The isolated azidosugars, **7** and **8**, can be converted into the corresponding glycosyl donors using reported protocol.[17] All other conditions generated either no hydrolysis or a mixture of products that could not be separated. This might be because of the solubility issue of starting material in the solvent used or the acid not being strong enough to hydrolyze the glycosidic bond.

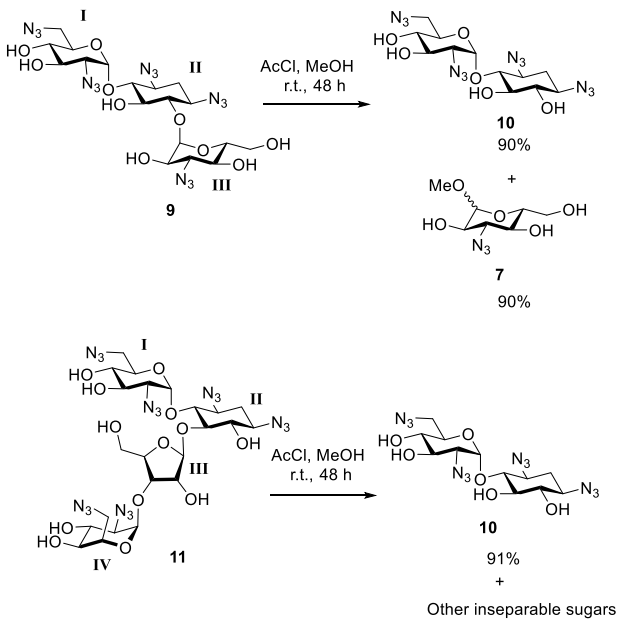


Scheme 2. Hydrolysis of tetraazidokanamycin A

Table 1. Different conditions for the hydrolysis of tetraazidokanamycin A

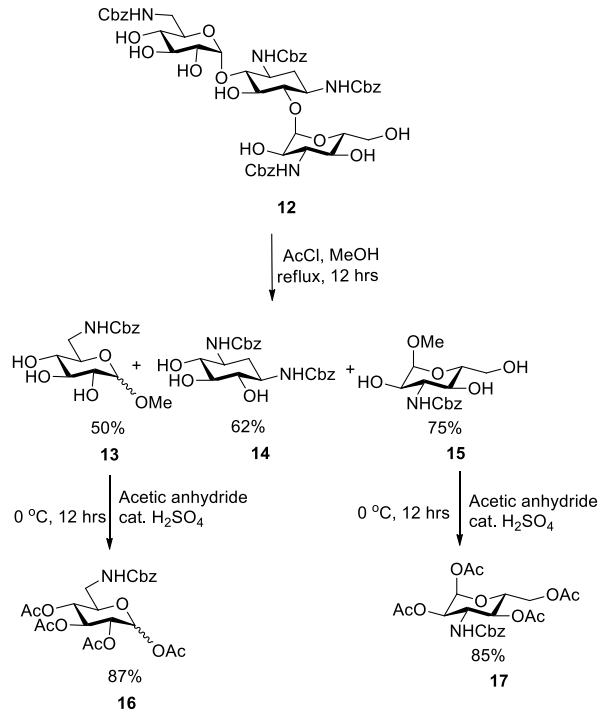
Entry	Solvents	Acid	Product (% yield)
1	MeOH	HCl (MeOH and AcCl)	6 (34) + 7 (11) +8 (2)
2	DCM	TMSOTf	No Hydrolysis
3	DCM	BF_3-OEt_2	No Hydrolysis
4	1,4-Dioxane	TMSOTf	6 (90) +Inseparable Mixture
5	Acetonitrile	TMSOTf	6 (90) + Inseparable Mixture

With the establishment of optimal conditions, we also examined the acid-catalyzed hydrolysis using pentaazidokanamycin B and hexaazidoneomycin B (Scheme 3). Excellent yields were observed from the hydrolysis of both aminoglycoside derivatives using the AcCl/MeOH condition. Interestingly, the yields were not changed, even after changing the condition to TMSOTf/MeCN. For "pentaazidokanamycin B (9)", only the glycosidic bond between rings II and III was cleaved, resulting the formation of tetraazidoneamine and the corresponding 2-azidosugar (ring II). For the hydrolysis of "hexaazidoneomycin B (11)", there was also no hydrolysis of the glycosidic bond between rings I and II leading to the formation of tetraazidoneamine and mixture probably produced from breaking of glycosidic bonds between rings II and III, and III and IV.



Scheme 3. Hydrolysis of different aminoglycosides

In azidokanamycin A, which has a hydroxyl group (-OH) at 2′ position, resulted in the breaking of the glycosidic bond between ring I and II. The presence of 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 (N_3) in **9** 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.[18]



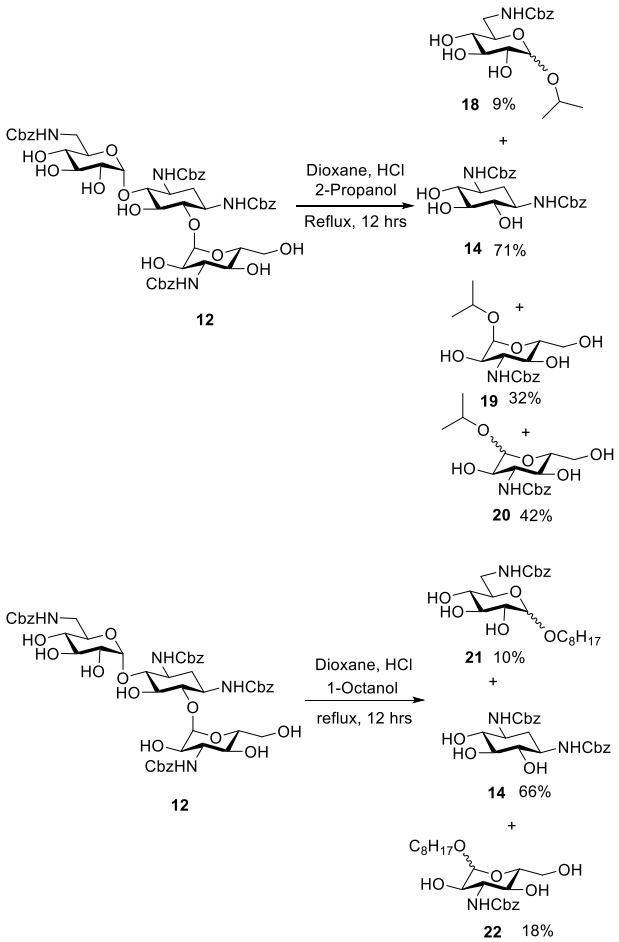
Scheme 4. Synthesis of 3- and 6-amino sugar donors.

Although acid-catalyzed hydrolysis of tetraazidokanamycin A furnished the desired aminosugars in just 2 synthetic steps compared to the previously reported method[17] which required 5 steps to achieve the products , the cost of synthesis was high with poor yield. Therefore, we also examined the use of tetracarbobenzoxy (Cbz)-protected kanamycin A, **12**[19] to provide aminosugars.

The synthesis of compound **12** from kanamycin A was much cheaper, resulted in higher yield, and involved no safety issues compared to the synthesis of azidokanamycin A. Compound **12** was subjected to hydrolysis using MeOH/AcCl and various Lewis acids. To our delight, not only the Cbz-protected aminosugars can be obtained by using MeOH/AcCl, but the yields are better than hydrolysis of tetraazidokanamycin A (Scheme 4). The subsequent acetylation of compound **13** and **15** resulted in the synthesis of amino sugar donor's precursors, compound **16** and **17** in an excellent yield. Interestingly, no hydrolysis was observed when using other acids like TsOH, TMSOTf, and BF_3-OEt_2.

Glycosyltransferation is common in an enzymatic process catalyzed by glycosyltransferases. However, it is rather unusual in chemical synthesis. To expand the applications of the developed protocol, we attempted to explore the possibility of utilizing alcohols other than MeOH for the hydrolysis, so the methodology can be feasible for possible chemical glycosyltransferation. 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, isopropanol and 1-octanol under 4 N HCl in 1,4-dioxane provided the hydrolyzed adducts (Scheme 5). Although the only modest yields were obtained, the outcome proved that it is possible to conduct hydrolysis of **12** and use the hydrolyzed aminosugars for chemical glycosyltransferation.



Scheme 5. One pot glycosyltransferation.

In conclusion, we have developed convenient protocols to furnish the protected 3- and 6-aminosugars. These protocols utilizing the hydrolysis of aminoglycosides can shorten the synthetic steps as compared to other methods using unmodified pyranoses as the starting material. In addition, our results suggest that the presence of hydrogen bonding at the 2′ position can direct the composition of products from acid hydrolysis of aminoglycosides. Finally, we have proved that it is possible to conduct glycosyltransferation in one-pot.

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Supporting Information is available on http://dx.doi.org/10.1246/cl.******.

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