Application of the Synthetic Aminosugars for Glycodiversification: Synthesis and Antimicrobial Studies of Pyranmycin

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Systematic Synthesis of Aminosugars and Their Stereoselective Glycosylation

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1. Introduction

Synthesis of oligosaccharides and glycoconjugates has become increasingly important for the elucidation of their biological functions. Aminosugar, as one of the main components of these diverse glycoconjugates, has attracted burgeoning interest. (*1*-*8*) Aminosugars are a group of structurally diverse unusual sugars bearing amino substitution on a normal sugar scaffold. Aminosugars can be found in bacteria, plant, and some mammalian cells, exerting unique but essential biological functions. (*2*-*8*) Therefore, if there is a library of aminosugars available, a modular approach can be readily employed

to construct a library of novel aminosugar-containing glycoconjugates for exploring important activities of interest in areas like biology, chemistry, and medicine. This strategy is termed glycodiversification or glycorandomization, a concept with fruitful applications (Figure 1).

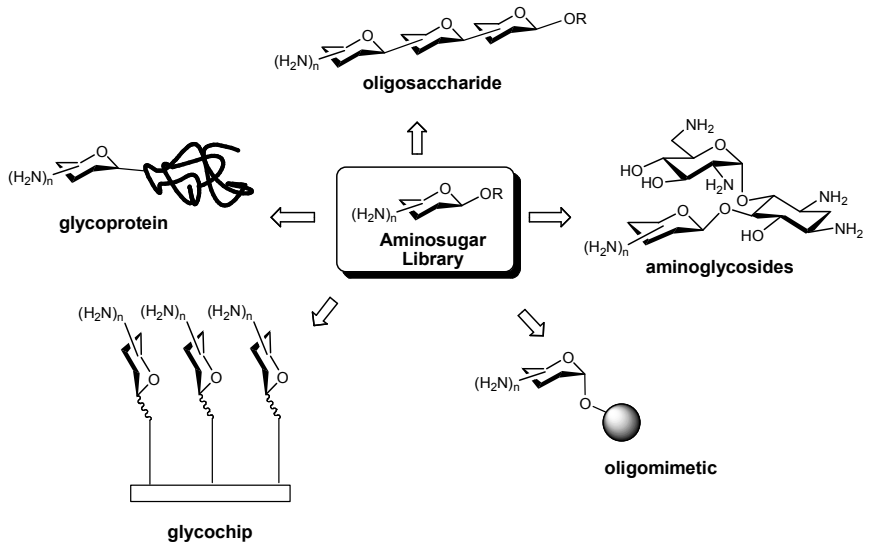
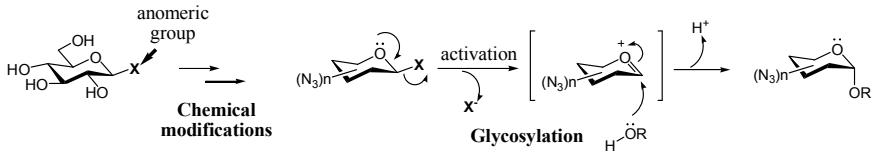


Figure 1. Prospective Glycoconjugates from Glycodiversification

In order to materialize the applications of aminosugar-containing glycoconjugates, numerous efforts have been devoted to the synthesis of aminosugars. (*9-11*) Normal sugars, such as glucose, galactose, and mannose, are commonly used as the starting material for the chemical synthesis of aminosugars due to their intrinsic chirality, availability in large quantity, and lower cost. For the synthesis and application of aminosugars, two major issues are typically needed to be resolved properly: protective group manipulation including both hydroxyl and amino groups, and the stereoselective glycosylation.

Protective group manipulations are necessary to achieve regiospecific reactions for the aminosugar synthesis. For example, the hydroxyl groups which are not involved in the glycosylation must be masked. Protective groups, especially at the C-2 position, will affect not only the reactivity of the glycosyl donor during glycosylation, but also the stereoselectivity. Amino groups often require different reagents for protection and deprotection as compared to hydroxyl groups, creating more synthetic complications. Meanwhile, the

protection of the anomeric position must be treated separately from the protection at other positions since it has to be stable enough to endure the reagents and conditions needed for the incorporation of amino groups. Nevertheless, the anomeric group is preferred to be labile so it can be activated at mild conditions for glycosylation. These two requirements regarding the anomeric groups work against each other (Figure 2). All these subtle demands make the preparation and utilization of aminosugars an extremely challenging and time-consuming task.



x group needs to be **stable** enough to endure the conditions for chemical modifications while being **labile** enough to be activated for glycosylation.

Figure 2. Challenges in Making the Aminosugar Donors

In addition, there are many reported methods for the preparation of specific aminosugars. (*9-11*) However, the general protocols or methods that will allow the synthesis of other aminosugars for different applications are not available. As a result, when initiating a synthesis for a desired aminosugar, one may still need to contribute a significant amount of effort in searching and optimizing the best approach among the vast number of documented prior reports. There may not be an ideal answer for this issue. Nevertheless, we wish to provide some solution to alleviate the synthetic burden through our efforts in this area. In this article, we will focus on the identification of general protocols that can be used for the systematic construction of two libraries of aminopyranoses, and provide a brief coverage of their use in the preparation of novel aminoglycosides, pyranmycins and kanamycin analogues.

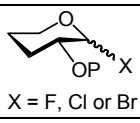
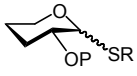
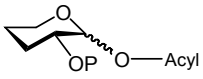
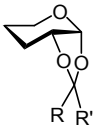
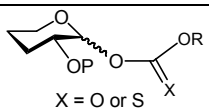
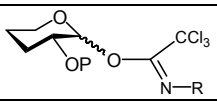
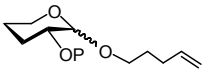
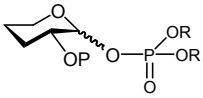
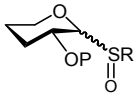
2. Synthesis of Aminosugars

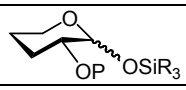
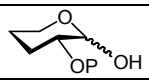
2.1. Choice of Starting Sugars

Based on a survey of current reviews, glycosyl donors can be generally classified into thirteen types based on the anomeric functional groups and their activating methods (Table I). (*12-16*) The predominantly used methods among

them include glycosyl halide, (*17*) thioglycoside, (*18*, *19*) trichloroimidate, (*15*, *20*) and 1-*O*-acyl sugar. (*21*)

Table I. Commonly Used Anomeric Functional Groups Employed in Glycosyl Donors

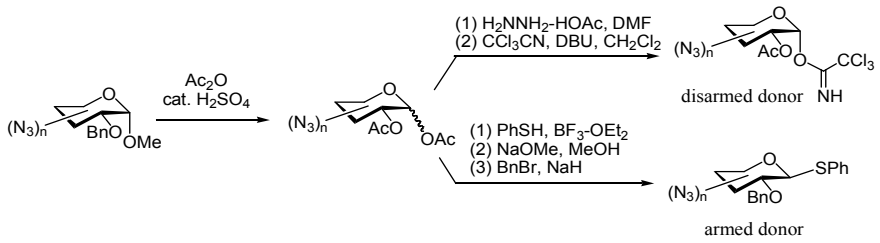
Name	Structures	Stability in epimerization conditions	Stability in hydride or radical-mediated deoxygenation	Stability in azido group substitution
Glycosyl Halide	 X = F, Cl or Br	Not stable	Not stable	Not stable
Thioglycoside		Stable	Not stable in radical-mediated deoxygenation	Stable
1-*O*-Acyl Sugar		Could be stable	Not stable in hydride-mediated deoxygenation	Could be stable
Ortho Ester		Stable	Stable	Stable
1-*O*- and *S*-Carbonate	 X = O or S	Not stable	Not stable	Not stable
Trichloroacetimidate		Not stable	Not stable	Not stable
4-Pentenyl Glycoside		Stable	Not stable in radical-mediated deoxygenation	Stable
Phosphate Derivatives		Not stable	Not stable	Not stable
Sulfoxide		Not stable in the presence of Tf_2O	Not Stable	Could be stable

1-*O*-Silylated Glycoside		Could be stable	Could be stable	Could be stable
1,2-Anhydro Sugar		Not stable	Not stable	Not stable
1-Hydroxyl Sugar		Not stable	Not stable	Not stable
Glycal		Stable	Not stable	Stable

However, when considering the systematic synthesis of aminosugars, and their glycosylation, not all these donors are suitable. As shown in Table I, most of the commonly employed glycosyl donors, such as glycosyl halides, glycosyl acetates, and glycosyl trichloroacetamidates, are not stable enough for the procedures of aminosugar synthesis, especially the incorporation of amino group and deoxygenation. 4-Pentenyl glycoside and glycal could be ideal options. Nevertheless, it is not very cost effective to use these molecules for the preparation of corresponding materials in large quantity.

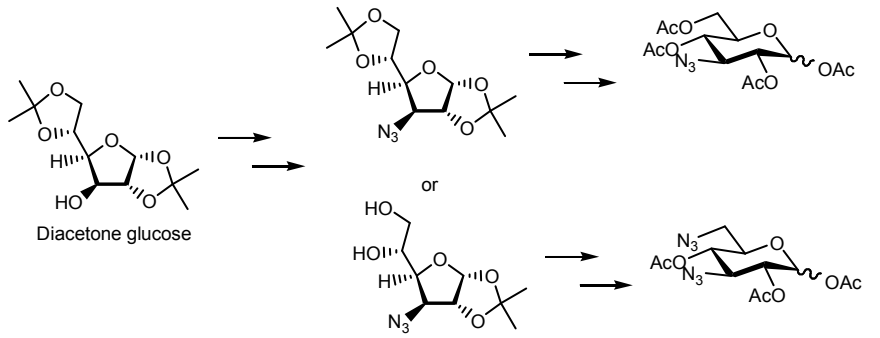
In addition, the "armed" and "disarmed" effects of protecting groups and azido groups on the reactivity of pyranose further limit the options for suitable donors. (*22*) The reactivity of glycosyl donors can be enhanced with an electron-donating protecting group, such as Bn, leading to the term: armed glycosyl donor. On the other hand, having an electron-withdrawing protecting group, such as Ac, Bz, or azido group, will decrease the reactivity of the glycosyl donor, which is termed as a disarmed donor.

One solution toward this problem is to have a rigid anomeric group that will enable the incorporation of an amino group and other modifications. After which, the anomeric group can be transformed into those functional groups that can serve as glycosyl donors. Following this strategy, methyl glucopyranosides that have a relatively stable anomeric methoxy group, and are available at lower cost, are commonly employed for synthesis of aminosugars. Combining with the concept of divergent synthesis, our group has examined a panel of reagents and completed the synthesis of 4- and/or 6-aminopyranoses. A general protocol with modest to excellent yields for converting diverse modified methyl glycosides into acetyl glycosides was also developed. Acetyl glycopyranosides can be transformed into two different glycosyl donors, glycosyl trichloroacetimidate and phenylthioglycosides, for "disarmed" and "armed" pyranoses following the reported procedures (Scheme 1). (*23*)



Scheme 1. General Procedure for the Synthesis of Glycosyl Donors

1,2:5,6-di-*O*-isopropylidine-D-glucofuranose is another important starting material since it gives immediate access to the modifications at the 3-OH group. Also, because it can be selectively hydrolyzed to 1,2-*O*-isopropylidine derivatives, further manipulation leading to the synthesis of 3,6-diaminopyranose can be readily achieved (Scheme 2). (*24*) Glucosamine is commonly used for the synthesis of 2-aminopyranoses since it has an amino group at the C-2 position and is of lower cost than galactosamine. (*25*)

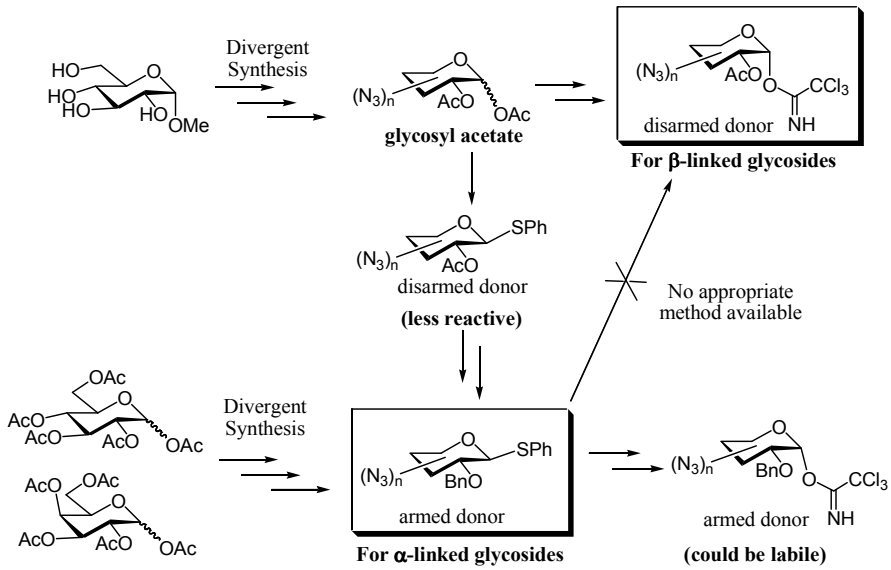


Scheme 2. Examples of Modification of 1,2:5,6-Di-O-isopropylidine-D-glucofuranose

Arylthio or alkylthio groups are resistant toward many organic operations used in aminosugar synthesis, and can be activated for glycosylation directly. Therefore, arylthio or alkylthioglycosides are often used for the synthesis of aminosugars and corresponding derivatives. (*19*) Although ethylthioglycopyranoside, tolylthioglycopyranoside or phenylthioglycopyranoside are expensive to purchase, these compounds can be prepared in large quantities from treating glycose pentaacetate with the

corresponding ethanethiol, thiophenol, or *p*-thiocresol under the catalysis of Lewis acids. (*26*) Our group favors the use of phenylthioglycopyranoside for two reasons. First, unlike ethylthioglycopyranoside, phenylthioglycopyranoside is easy to crystallize and thereby avoids the formidable task of column chromatography. Second, to ensure the completed conversion of glycosyl acetate to the corresponding thioglycoside, excess thiol is often required. (*27*) The excess thiophenol can be readily removed by co-evaporating with other organic solvents, and subsequently bleached for proper disposal. (*28*) However, *p*-thiocresol is a solid at room temperature, making its removal more challenging.

The overall strategy of constructing aminosugar libraries is summarized in Scheme 3. We prefer the use of disarmed trichloroacetimidate glycosyl donor for the formation of the β-glycosidic bond, and armed phenylthio glycosyl donor for the formation of the α-glycosidic bond. In general, trichloroacetimidate donors are more reactive than phenylthio glycosyl donors. Thus, the electron-withdrawing effect in the disarmed donors can be better countered by the higher reactivity of trichloroacetimidate glycosyl donor, while the electron-donating effect in the phenylthio glycosyl donors is advantageous for the less reactive disarmed donors.



Scheme 3. Overall Concept of Aminosugar Construction

Azido group incorporated glycosyl acetates that can be derived from methyl glucoside are better for the synthesis of disarmed donors bearing an acetyl protecting group at C-2 position, which is designed for the formation of β-linked glycosides. If necessary, these glycosyl acetates can also be converted into phenylthioglycosides. Although the disarmed donors like phenylthioglycosides can be less reactive and give rise to unsatisfactory yield in glycosylation, they can be converted to more reactive armed donors with Bn protecting groups favoring the formation of α-linked glycosides.

Based on our experience, however, it would be more convenient and cost effective to synthesize azido groups incorporated phenylthioglycosides from glucose pentaacetate or galacto pentaacetate. The disadvantage is that these glycosides can only lead to the synthesis of armed donors. To our knowledge and through our unsuccessful attempts, there is no simple method to convert armed donors into disarmed donors once the Bn groups have been incorporated. Incorporation of Bn groups prior to azido group incorporation is, however, necessary since acetyl groups are not stable enough under the chemical conditions employed. The reactivity of armed phenylthio glycosyl donors can be further enhanced by converting the phenylthio group into a trichloroacetimidate group. Nevertheless, such highly reactive armed donors could be too reactive to be properly purified in some of the aminosugar constructs.

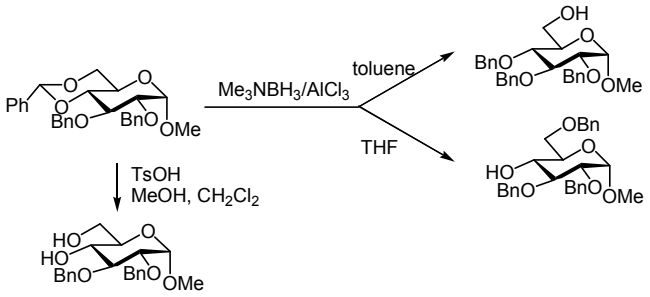
2.2. General Synthetic Protocols

To achieve divergent synthesis of aminosugar, four synthetic transformations for the development of general protocols should be considered in advance: regioselective protection and deprotection of hydroxyl groups (for example: cleavage of *O*-benzylidine acetale), epimerization of hydroxyl group, azido (amino) group incorporation, and regioselective deoxygenation. As long as the general protocols for the above-mentioned reactions can be established, a systematic approach for the synthesis designed aminosugars can be envisioned.

2.2.1. Synthesis and Regioselective Cleavage of *O*-benzylidine Acetales

There are many protecting groups for pyranoses reported in the literature. (*29*, *30*) We are especially interested in the benzylidene-type of protecting group because the 4,6-benzylidene pyranoses can lead to several regioselectively deprotected pyranoses, which is valuable in our divergent synthetic approach (Scheme 4). For example, by using appropriate solvent and reducing agent, the 4,6-benzylidene-protected pyranose can lead to the synthesis of derivatives with either 4-OH or 6-OH. (*31*) Alternatively, hydrolysis of benzylidene results in the expose of 4,6-dihydroxyl groups. (*32*) In our opinion, the selective protection

methods have been well developed for many pyranoses, such as glucose, galactose, and mannose, and hence will not be discussed in detail in this article.



Scheme 4. Sample of Regioselective Opening of 4,6-O-Benzelidene Acetals

2.2.2. Epimerization of Hydroxyl Group

Since the substitution of a hydroxyl group with a nucleophile is often carried out via S_N2 substitution, it is often necessary to stereoselectively epimerize a hydroxyl group on a pyranose scaffold that will allow the installation of the nucleophile with the desired stereochemistry. Four out of five hydroxyl groups in hexopyranose are chiral, therefore stereoselectively epimerizing a hydroxyl group on the glucopyranose and galactopyranose scaffold is essential to expand the possibilities for more sugar manipulation.

There are many well-established methods in the literature, which can be grouped into two types including oxidation-reduction and nucleophilic substitution processes. The former involves an oxidation of a hydroxyl group to a keto group followed by stereoselective hydride reduction. In this method, the vicinal protecting groups may influence the stereoselectivity. (*33*)

It is also worthy to point out that oxidation of a hydroxyl group using Swern oxidation and reduction of ketone using $NaBH_4$ are compatible to the presence of phenylthiol and azido groups, respectively.

The second method involves converting the secondary hydroxyl group into a leaving group using Tf_2O followed by S_N2 substitution using, for example, OAc^- or NO_2^-, as the nucleophiles. While Tf_2O may activate glycosyl sulfoxide, it is compatible to the presence of phenylthio group. Thus, both oxidation-reduction and nucleophilic substitution reactions can be applied in the synthesis of a glycosyl trichloroacetimidate library and phenyl thioglucopyranoside library. Three commonly used protocols in our laboratory are summarized in Table II.

Table II. Common Protocols for Epimerization of Hydroxyl Group

Types of Transformations	Examples of Reagents	Comments	References
Oxidation/reduction (Swern oxidation)	(1) $(COCl)_2$, DMSO, DIPEA (2) $NaBH_4$	Vicinal protecting groups (*i.e.* Bn) are essential for the selectivity. However, the selectivity may vary among different sugars. Others (*i.e.* Bz) may offer lower or no selectivity toward epimerization.	33
S_N2 substitution	(1) Tf_2O (2) $(n\text{-}Bu)_4N^+NO_2^-$	$(n\text{-}Bu)_4N^+NO_2^-$ can be soluble in CH_2Cl_2, providing better results than reagents like $NaNO_2$. In general, this method offers stereospecific epimerization.	34
S_N2 substitution	(1) Tf_2O (2) $(n\text{-}Bu)_4N^+OAc^-$ (3) hydrolysis of OAc	$(n\text{-}Bu)_4N^+OAc^-$ can be soluble in CH_2Cl_2, providing better result than reagent like KOAc or CsOAc. In general, this method offers stereospecific epimerization.	35

2.2.3. Azido Group Incorporation

The amino group needs to be protected in order to make the synthesis of the glycosyl donor and glycosylation feasible. We favor use of the azido group as an amino group surrogate for the synthesis of aminosugars (or azidosugars). There are several advantages that make the azido group a popular choice. First, the azido group is relatively stable to many reductive and oxidative conditions. Second, unlike the carbamate type protecting group for amines, azido compounds have good solubility in organic media, allowing an expedient chromatographic purification. Third, azido groups can be converted into amino groups conveniently by hydrogenation or the Staudinger reaction. More importantly, an azido group can be easily installed from an activated hydroxyl group via S_N2 substitution and, unlike amino group, no sequential protection is needed. Substitution of a hydroxyl group using the amino or carbamate-

protected amino groups as the nucleophiles can be relatively difficult since the protected amino group is not a good nucleophile, while a free amino group can act as a base and cause undesired elimination. Finally, the azido group can be applied to the "click" chemistry creating more diversification opportunities. (*36*)

Despite the advantage of easy manipulation, one-pot method for the incorporation of azido group is not recommended since it often generates a mixture that complicates the purification of the desired product. In the two-step method, selective tosylation of a primary hydroxyl group in the presence of secondary hydroxyl groups is one of the advantages of employing TsCl. The commonly used protocols are summarized in Table III. The azido group can be converted to amino group by methods, such as hydrogenation, Staudinger reaction, hydride-reducing agents *(37)*.

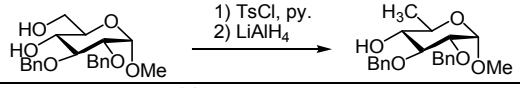
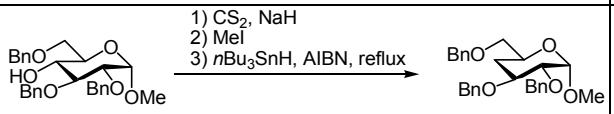
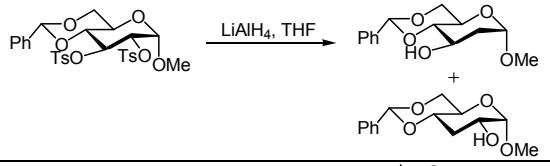
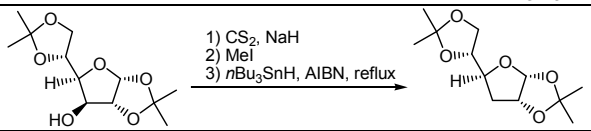
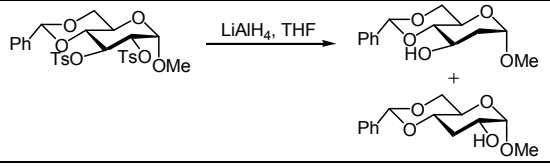
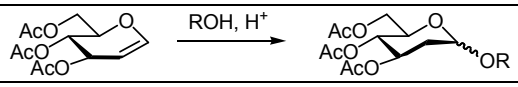
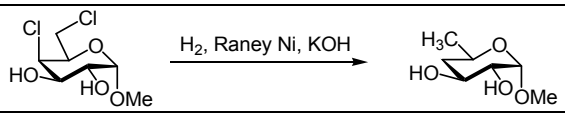
Table III. Common Protocols for Azide Substitution

Types of Transformations	Reagents	Typical Conditions	Types of Hydroxyl Groups	Notes	References
One-pot	DPPA(or HN_3), PPh_3 and DEAD	-40º to 0ºC, overnight	1º and 2º	Complex mixture may be obtained, difficult to purify	38
Two-step	(1) TsCl (2) NaN_3	(1) 0ºC to R.T., overnight (2) 80ºC, overnight	1º (2º tosylate is difficult to be replaced with N_3^-)	Can be used for selective azide substitution	39
Two-step	(1) MsCl (2) NaN_3	(1) 0ºC to R.T., couple hours (2) 120ºC, overnight	1º and 2º	MsCl is cheaper than Tf_2O	40
Two-step	(1) Tf_2O (2) NaN_3	(1) 0ºC, 0.5 hour (2) R.T., overnight	1º and 2º	Most expedient	39

2.2.4. Regioselective Deoxygenation

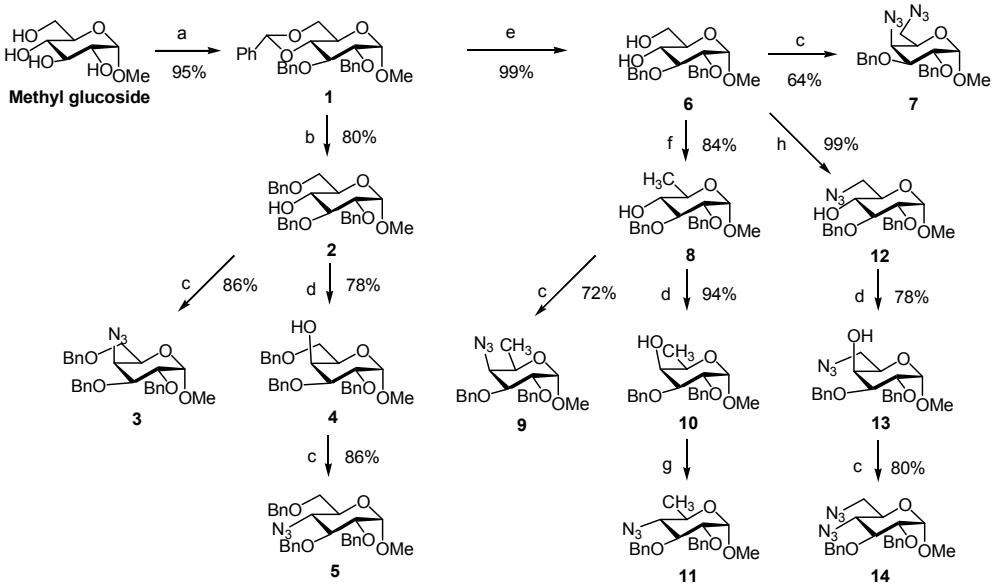
Many aminosugars contain the features of deoxygenation. Table IV summarizes the common protocols for regioselective deoxygenation in the literature. Since azido groups can be reduced under conditions of hydride reduction (e.g. $LiAlH_4$) or radical-initiated deoxygenation (e.g. Barton reduction or dehalogenation), (*41*, *44*) deoxygenation generally proceeds before the introduction of the azido group.

Table IV. Common Protocols for Regioselective Deoxygenation

Types of Transformations	Reactions	References
6-deoxy		39
4-deoxy		41
3-deoxy		42
3-deoxy		41
2-deoxy		42
2-deoxy		43
4,6-dideoxy		44

2.3. Divergent Synthesis of Glycosyl Trichloroacetimidate Library

The synthesis of our glycosyl trichloroacetimidate aminosugar library began with the commercially available methyl glucopyranoside (Scheme 5). Benzylidine protection, followed by benzylation of C-2 and C-3 hydroxyl groups, (*31*, *32*) yielded compound **1**. Compound **1** was treated with $NaBH_3CN/HCl$ in THF to selectively deprotect the C-4 hydroxyl group providing compound **2**. (*31*, *32*) Triflation followed by azide substitution of compound **2** generated azidosugar **3** in the *galacto*-configuration. Epimerization of the 4-OH of **2** was achieved with Swern oxidation and $NaBH_4$ reduction offering compound **4**, which allowed a S_N2 azide substitution that furnished azidosugar, **5** in the desired *gluco*-configuration.



Conditions: (a) (1) $PhCH(OMe)_2$, $TsOH-H_2O$, DMF, (2) BnBr, NaH, TBAI, THF; (b) BH_3-Me_3N, $AlCl_3$, THF; (c) (1) Tf_2O, py., CH_2Cl_2, (2) NaN_3, DMF; (d) $(COCl)_2$, DMSO, DIPEA, (2) $NaBH_4$, MeOH; (e) $TsOH-H_2O$, MeOH; (f) (1) TsCl, py, (2) $LiAlH_4$, THF; (g) (1) MsCl, Et_3N, CH_2Cl_2, (2) NaN_3, DMF; (h) (1) TsCl, py, (2) NaN_3, DMF.

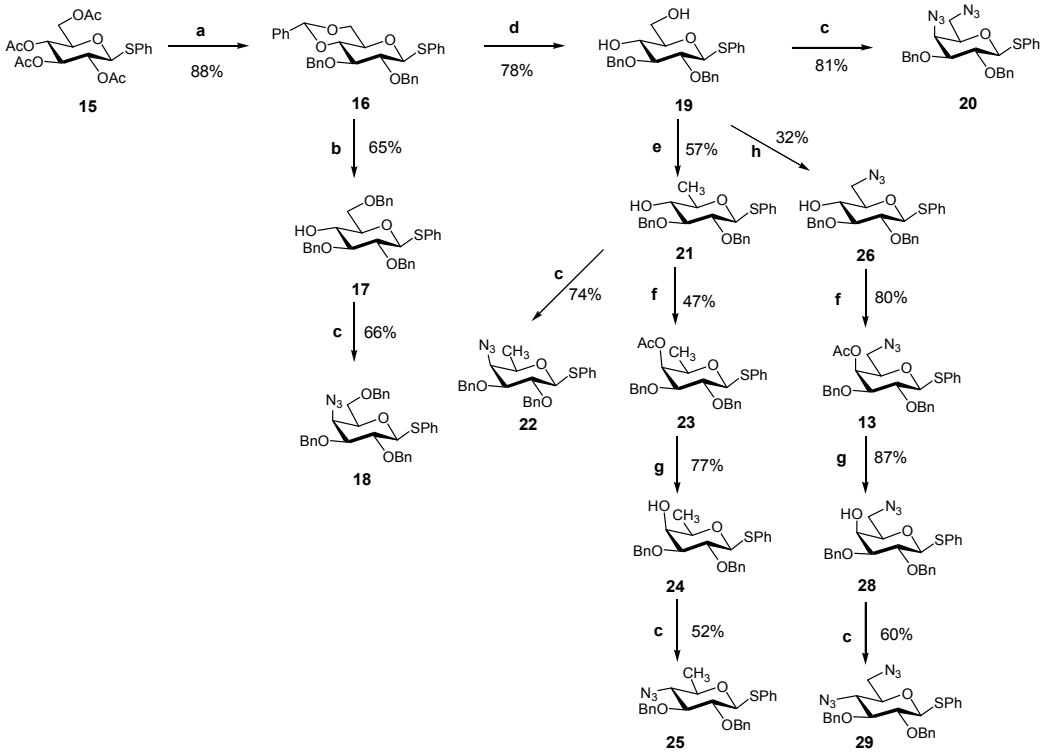
Scheme 5. Divergent Synthesis of Glycosyl Trichloroacetimidate Library

In another route, compound **1** was treated with TsOH to give compound **6** with free hydroxyl groups at the C-4 and C-6 positions, which branched into three distinct routes. In the first route, triflation followed by azide substitution of compound **6** provided azidosugar **7** with C-4 and C-6 diazido substituted. In the second route**,** compound **6** was selectively deoxygenated at the C-6 position by sequential tosylation and $LiAlH_4$ reduction. Compound **8** was subjected to azide substitution generating azidosugar, **9** with C-6 deoxygenation in the *galacto*-configuration. Alternatively, compound **8** underwent the Swern oxidation/$NaBH_4$ reduction protocol to invert the C-4 hydroxyl group that allowed the synthesis of azidosugar, **11** with C-6 deoxygenation in the *gluco*-configuration. In the last path, compound **6** was treated with TsCl, followed by azide substitution to selectively place an azido group on the C-6 position. The free C-4 hydroxyl group in the equatorial position of compound **12** was converted to the axial position yielding compound **13**, which enabled the synthesis of azidosugar **14** with C-4 and C-6 diazido in the *gluco*-configuration.

As described in the previous section, the anomeric methoxy group and all the benzyl groups of these azidosugars can be converted into acetyl groups using Ac_2O with a catalytic amount of H_2SO_4 (Scheme 1). The resulting acetyl glycosides can then be transformed into the glycosyl trichloroacetimidate as the glycosyl donor, (*23*) which could then be coupled to the acceptor of choice.

2.4. Divergent Synthesis of Phenylthioglucopyranoside Library

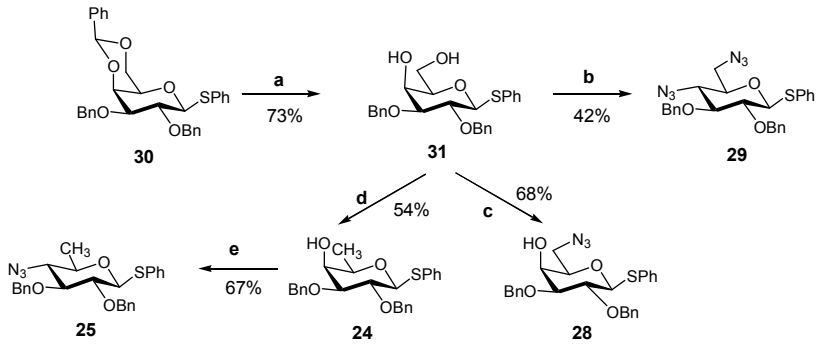
By employing the philosophy of divergent synthesis, and phenylthioglucopyranoside as the starting material, the other library, thioglycopyranoside-based aminosugar library can be constructed in a similar fashion (Scheme 6). We modify the literature procedure, which allows the purification of phenylthioglucopyranoside via recrystallization, and hence enables its large scale synthesis. (*26-28*)



Conditions: (a) (1) NaOMe, MeOH, (2) $PhCH(OMe)_2$, TsOH-H_2O, DMF, (3) BnBr, NaH, TBAI, THF; (b) BH_3-Me_3N, $AlCl_3$, THF; (c) (1) MsCl, Et_3N, DMAP, CH_2Cl_2, (2) NaN_3, DMF; (d) TsOH-H_2O, MeOH/CH_2Cl_2; (e) (1) TsCl, py, (2) $LiAlH_4$, THF; (f) (1) Tf_2O, py, CH_2Cl_2, (2) *n*-Bu_4NOAc, CH_2Cl_2; (g) K_2CO_3, MeOH; (h) (1) TsCl, py, (2) NaN_3, DMF.

Scheme 6. Divergent Synthesis of Phenylthioglucopyranoside Library

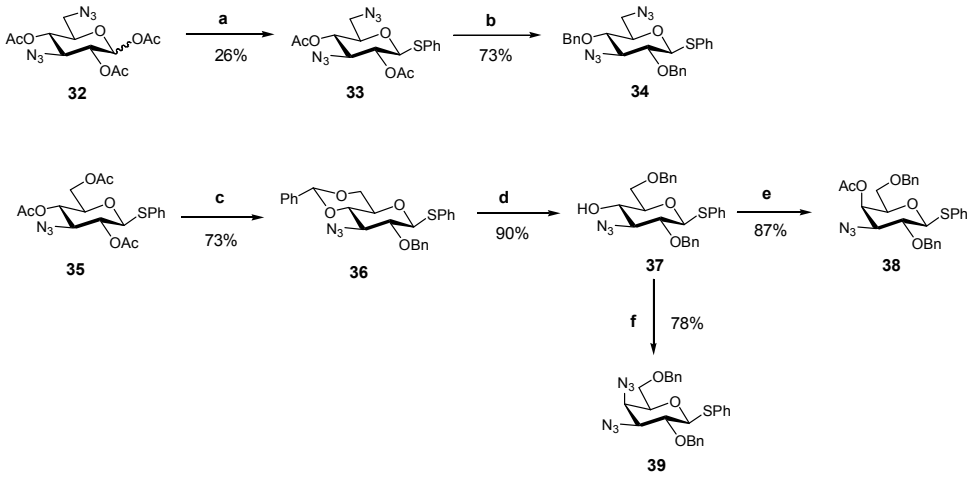
To avoid the cumbersome epimerization of C-4 free hydroxyl, an alternative route is to use the 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-phenylthio-β-D-galactopyranoside instead of glucopyranoside as starting material (Scheme 7).



Conditions: (a) TsOH-H_2O, MeOH/CH_2Cl_2; (b) (1) Tf_2O, py, CH_2Cl_2, (2) NaN_3, DMF; (c) (1) TsCl, py, (2) NaN_3, DMF; (d) (1) TsCl, py, (2) $LiAlH_4$, THF; (e) (1) MsCl, Et_3N, DMAP, CH_2Cl_2, (2) NaN_3, DMF.

Scheme 7. Alternative Synthesis Route

As mentioned previously, the C-3 azido group-incorporated sugars can be synthesized from 1,2:5,6-di-*O*-isopropylidine-D-glucofuranose. Further manipulations can be used to synthesize 3,6- or 3,4-diazidopyranoses (Scheme 8).



Conditions: (a) PhSH, BF_3-OEt_2, CH_2Cl_2; (b) (1) NaOMe, MeOH, (2) BnBr, NaH, TBAI, THF; (c) (1) NaOMe, MeOH, (2) $PhCH(OMe)_2$, TsOH-H_2O,

DMF, (3) BnBr, NaH, TBAI, THF; (d) BH_3-Me_3N, $AlCl_3$, THF; (e) (1) Tf_2O, py, CH_2Cl_2, (2) *n*-Bu_4NOAc, CH_2Cl_2; (f) (1) Tf_2O, py, CH_2Cl_2, (2) NaN_3, DMF.

Scheme 8. Synthesis of C3-azido Phenylthioglucopyranoside Compounds

3. Stereoselective Glycosylation

3.1. Background in Glycosylation

The development of methodologies for efficient glycosylation reaction, especially *O*-glycosylation reaction, has been a major issue for practical synthesis of oligosaccharides and glycoconjugates. As a result, intense research activities have been devoted to the study of stereoselective glycosylation. However, stereochemical problems in glycosidic bond formation have not been solved completely. (*12*, *14*, *45-47*)

Based on the general structure of pyranoses and the chirality of the anomeric center, four possible products can be formed (Figure 3). (*48*) The glycosylation, which involves α and β *manno*-type pyranose has been studied extensively by Crich. (*49*, *50*) Therefore, we will only discuss the α- and β-linkage of *gluco*-type.

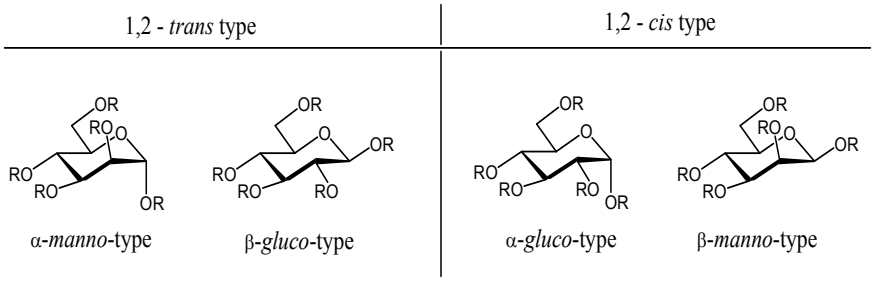


Figure 3. Four Possible Products of Glycosidation

3.1.1. Anomeric effect

In substituted cyclohexanes, the substituents usually prefer the equatorial position due to the steric effects (Figure 4). In rings containing oxygen atoms and with adjacent electronegative substituents there is a preference for this substituent to be *axial*. This is referred as the anomeric effect. (*51*) The

anomeric effect favors the formation of kinetic product, the α-anomer, for most glycosides.

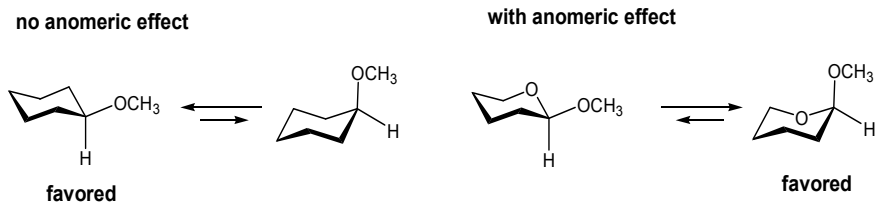


Figure 4. Anomeric Effect

3.1.2. Protecting Group on *O*-2 of the Glycosyl Donor

The stereospecific formation of a β-glycosidic bond can be achieved by the presence of an acyl protecting group at the *O*-2 position via neighboring group participation (Figure 5). The formation of an α-glycosidic bond is, however, more challenging, despite great advances. The stereocontrolled synthesis of α-glycosides can be affected by factors such as electronic effects, steric hindrance, solvent, and conformation. To our knowledge, there is no satisfactory general protocol for stereospecific glycosylation for formation of an α-glycosidic bond despite numerous efforts.

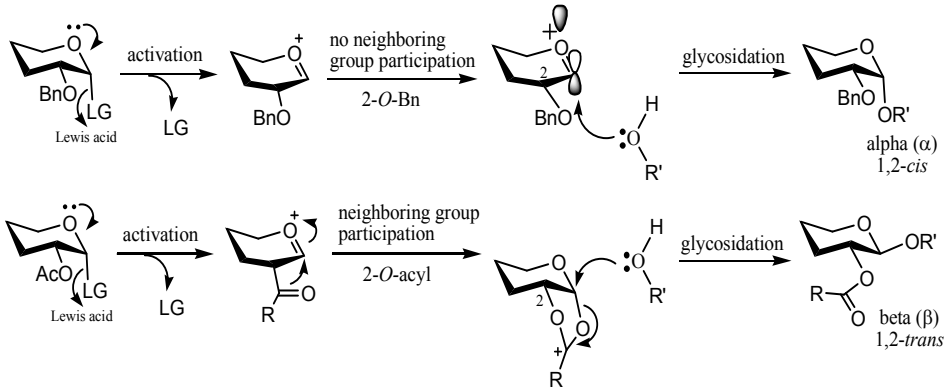


Figure 5. Common Mechanism for Glycosidation and the Associated Selectivity

3.2. Formation of β-Glycosidic Bond: Preparation of Pyranmycin Library

As mentioned previously, our group favors the use of glycosyl trichloroacetimidate library as the glycosyl donor for the formation of β-glycosidic bond leading to the development of the pyranmycin library, a library of neomycin B analogues (Figure 6).

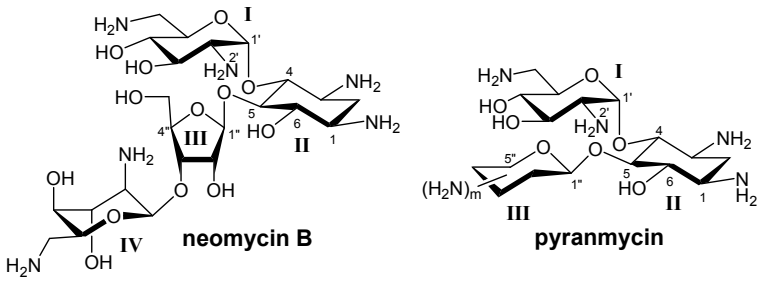


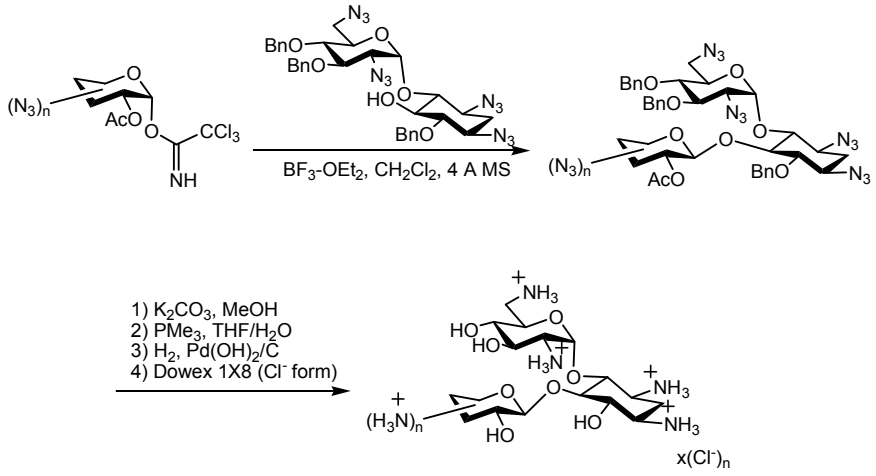
Figure 6. Structures of Neomycin B and Pyranmycin

Neomycin belongs to a group of aminoglycoside antibiotics containing a 4,5-disubstituted 2-deoxystreptamine core (ring II) and has been used against both gram-positive and gram-negative bacteria for more than fifty years. (*5*, *52*) The neomycin exerts its antibacterial activity by binding selectively to the A-site decoding region of the 16S ribosomal RNA of bacteria, and thereby disrupts the protein synthesis of these microorganisms.

The design of pyranmycin contains a ring III pyranose that is linked to the *O*-5 of neamine (rings I and II) via a β-glycosidic bond. It is known that neomycin is labile under acidic conditions due to the presence of a glycosidic bond from the ring III furanose. As a result, neomycin degrades readily into less active neamine (rings I and II) and inactive neobiosamine (rings III and IV). Since the corresponding glycosidic bond of pyranmycin is made from a pyranose, this gives pyranmycin better stability to acidic conditions. (*53*) Therefore, replacement of neobiosamine with a pyranose will yield a novel aminoglycoside with improved stability in acidic media.

Reported study has also showed that an intramolecular hydrogen bonding between the 2'-amino group of ring I and the *O*-4" atom of ring III helps to orient ring I for specific binding toward RNA. (*54*) Thus, the attachment of ring III via a β-glycosidic bond is crucial in offering the intramolecular hydrogen bonding similar to that in the neomycin. Therefore, our glycosyl trichloroacetimidate library is ideal for incorporation of the desired β-linked

pyranoses. With the appropriate neamine aglycon, we have prepared a library of pyranmycin that show comparable activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) (Scheme 9). (*23*, *39*, *55*)



Scheme 9. Synthesis of Pyranmycin Library

3.3. Formation of α-Glycosidic Bond: Preparation of Kanamycin Library

Kanamycin belongs to a group of aminoglycoside antibiotics with 4,6-disubstituted 2-deoxystreptamine (Figure 7). (*5*, *52*) Like neomycin, kanamycin also exerts prominent antibacterial activity against both gram positive and gram negative susceptible strains of bacteria. Nevertheless, kanamycin has become clinically obsolete due to the emergence of aminoglycoside resistant bacteria. (*56*) In order to revive the activity of kanamycin against drug resistant bacteria, numerous attempts have been devoted to the chemical modification of kanamycin. (*52*, *57*) Except for a few publications, (*58*) most works use various carbamates as protecting groups for kanamycin resulting in the production of kanamycin with polycarbamate groups. Two drawbacks were often encountered: the poor solubility of polycarbamates, which produce great difficulties in purification and characterization of these compounds, and the limited options for structural modifications imposed by the kanamycin scaffold.

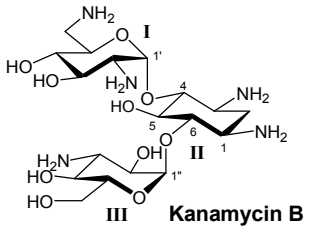
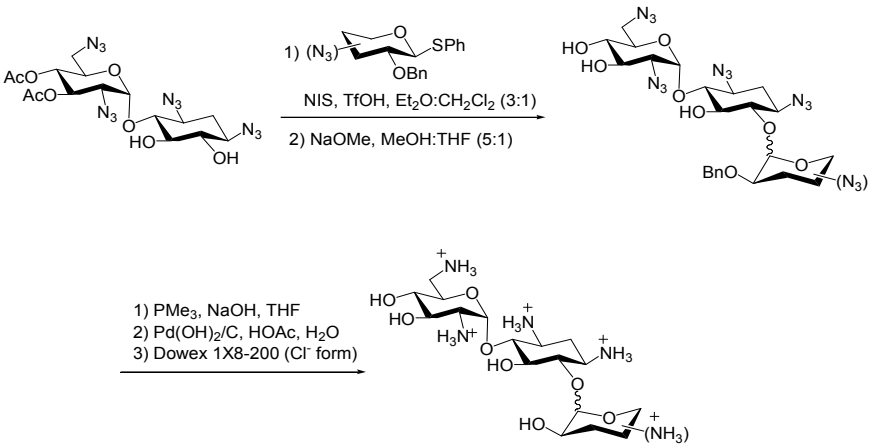


Figure 7. Structures of Kanamycin B

The α-glycosidic bond between rings II and III is important as the kanamycin analogous with a β-glycosidic bond manifests much weaker antibacterial activity. (*59*) Having a 2-*O*-Bn group, our phenylthioglycoside library is preferable for the formation of the needed α-glycosidic bond due to the anomeric effect. The neamine acceptor underwent regiospecific glycosylation at the *O*-6 position resulting in the desired 4,6-disubstituted 2-deoxystreptamine motif. The optimal stereoselectivity for the formation of the α-glycosidic bond can be accomplished by carrying out the reaction in a solution of Et_2O and CH_2Cl_2 in a 3 : 1 ratio. (*60*) The desired α-glycosylated compounds were often mixed with their inseparable β-epimer. After hydrolysis of the acetyl groups, the triols can be obtained in good purity and improved α/β ratio. The final products (in pure α form or α/β from 10/1 to 7/1) were synthesized as chloride salts using the Staudinger reaction followed by hydrogenation and ion-exchange (Scheme 10). (*61*) A library of kanamycin B has been prepared via the concept of glycodiversification. These compounds also show comparable activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).



Scheme 10. Synthesis of Kanamycin Analogue Library

4. Conclusion

Carbohydrate synthesis is one of the most formidable tasks in organic synthesis. The synthesis of aminosugar libraries for practical applications represents an even greater challenge. Nevertheless, through the use of standardized protocols and a divergent synthetic approach, systematic procedures have been developed. The advantage of employing two separate aminosugar libraries for stereoselective glycosylation has also been demonstrated in the library construction of pyranmycin and kanamycin B analogues. It is our hope that part of the problem associated with the synthesis and application of aminosugars can be resolved. However, there are several aspects that still require applicable solutions. For example, there is no convenient synthesis of 2-aminopyranose that will favor the stereospecific formation of α-glycosidic bond. The deoxygenation method that is compatible with the presence of an azido group, stereospecific glycosylation for the formation of α-glycosidic bond, and a convenient protocol for the stereoselective glycosylation of 2-deoxyglycopyranoses are several such examples that still need further perfection.

5. References

1. Dwek, R. A. *Chem. Rev*. **1996**, *96*, 683-720.

2. Kirschning, A.; Bechthold, A. F.-W.; Rohr, J. *Top. Curr. Chem.* **1997**, *188*, 1-84.
3. Johnson, D. A.; Liu, H.-w. *Curr. Opin. Chem. Biol.* **1998**, *2*, 642-649.
4. Weymouth-Wilson, A. C. *Nat. Prod. Rep.* **1997**, 99-110.
5. Hooper, I. R. *Aminoglycoside Antibiotics*; Springer-Verlag: New York, **1982**.
6. Priebe, W.; Van, N. T.; Burke, T. G.; Perez-Soler, R. *Anticancer Drugs* **1993**, *4*, 37-48.
7. Lothstein, L.; Sweatman, T, W.; Priebe, W. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1807-1812.
8. Kren, V.; Martinkova, L. *Curr. Med. Chem*. **2001**, *8*, 1313-1338.
9. Brimacombe, J. S. *Angew. Chem. Int. Ed*. **1971**, *10*, 236-248.
10. Umezawa, S.; Tsuchiya, T. in *Aminoglycoside Antibiotics*, Springer-Verlag **1982**, New York. p37-110.
11. Jurczak, J. in *Preparative Carbohydrate Chemistry*, Hanessian, S. Ed., Marcel Dekker, Inc. **1997**, NewYork, p595-614.
12. Toshima, K.; and Tatsuta, K. *Chem. Rev*. **1993,** *93*, 1503-1531.
13. Nicolaou, K. C.; Mitchell, H. J. *Angew. Chem. Int. Ed.* **2001**, *40*, 1576-1624.
14. Paulsen, H. *Angew. Chem. Int. Ed*. **1982**, *21*, 155-173.
15. Schmidt, R. R. *Angew. Chem. Int. Ed*. **1986**, *25*, 212-235.
16. Demchenko, A. V. *Synlett*. **2003**, *9*, 1225-1240.
17. For examples: (a) Lemieux, R. U.; Hendriks, K. S.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056-4062. (b) Paulsen, H. *Angew. Chem. Int. Ed*. **1982**, *21*, 184-201.
18. For examples: (a) Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P. *J. Am. Chem*. *Soc.* **1983**, *105*, 2430-2434. (b) Konradsaon, P.; Udodong, U. E.; Fraeer-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313-4316. (c) Vwneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331-1334. (d) Dasgupta, F.; Garegg, P. J. *Carbohydr. Res*. **1988**, *177*, c13-c17. (e) Fugedi, P.; Garegg, P. J. *Carbohydr. Res*. **1986**, *149*, c9-c12.
19. Garegg, P.J. *Adv. Carbohydr. Chem. Biochem.* **1997**, *52*, 179-205.
20. For examples: (a) Halcomb, R. L.; Boyer, S. H.; Danishefeky, S. J. *Angew. Chem. Int. Ed*. **1992**, *31*, 338-340. (b) Nicolaou, K. C.; Schreiner, E. P.; Iwabuchi, Y.; *Suzuki,* T. *Angew. Chem. Int. Ed*. **1992**, *31*, 340-342.
21. For examples: (a) Jutten, P.; Scharf, H. D.; Raabe, G.; *J. Org. Chem.* **1991**, *56*, 7144-7149. (b) Gurjar, M. K.; Viswanadham, G. *Tetrahedron Lett.* **1991**, *32*, 6191-6194.
22. (a) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H.; *J. Am. Chem. Soc*. **1999**, *121*, 734-753. (b) Ye, X.-S.; Wong, C.-H.; *J. Org. Chem.* **2000**, *65*, 2410-2431.
23. Chang, C.-W. T.; Hui, Y.; Elchert, B.; Wang, J.; Li, J.; Rai, R. *Org. Lett.* **2002**, *4*, 4603-4606.

24. Schmidt, O.T. *Methods Carbohydr. Chem.* **1963,** *2*, 318.
25. For example: Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett*. **1996**, *37*, 6029-6032.
26. For examples: (a) Tai, C.-A.; Kulkarni, S. S.; Hung, S.-C.; *J. Org. Chem*. **2003**, *68*, 8719-8722. (b) Benakli, K.; Zha, C.; Kerns, R. J.; *J. Am. Chem. Soc*. **2001**, *123*, 9461-9462.
27. A recent publication has used 60 mol% MeSSMe for the synthesis of methylthioglycoside (Mukhopadhyay, B.; Kartha, K. P. R.; Russell, D. A.; Field, R. A. *J. Org. Chem*. **2004**, *69*, 7758-7760). However, the maximum scale for the reported synthesis is 20g. In order to facilitate the library construction of phenylthioglycosides, it is preferable to start from 100 – 200g scale.
28. We have developed method for large-scale synthesis of phenylthioglycosides (100 – 200g scale), and the products can be purified via recrystallization (patenting).
29. Greene, T. W.; Wuts, P. G. M. *Protective groups in Organic Synthesis,* 3rd ed., John Wiley & Sons, New York, **1998**.
30. Kocienski, P. J. *Protective groups,* Georg Thieme Verlag, Stuttgart, New York, **1994**.
31. Garegg, P. J. in *Preparative Carbohydrate Chemistry*, Hanessian, S. Ed., Marcel Dekker, Inc. **1997**, New York, p53-67.
32. Garegg, P.J. *Pure Appl. Chem.* **1984**, *56*, 845-858, and references cited therein.
33. Chang, C.-W. T.; Hui, Y.; Elchert, Y. *Tetrahedron Letter*, **2001**, *42*, 7019-7023.
34. For examples: (a) Poirot, E.; Chang, A. H. C.; Horton, D.; Kovac, P. *Carbohydr. Res*. **2001**, *334*, 195-205. (b) Marcaurelle, L. A.; Bertozzi, C. R. *J. Am. Chem. Soc*. **2001**, *123*, 1587-1595.
35. For example: Dohi, H.; Nishida, Y.; Furuta, Y.; Uzawa, H.; Yokoyama, S.-I.; Ito, S.; Mori, H.; Kobayashi, K.; *Org. Lett*. **2002**, *4*, 355-357.
36. For examples: (a) Tornoe, C. W.; Christensen, C.; Meldal, M.; *J. Org. Chem.* **2002**, *67*, 3057-3064. (b) Kuijpers, B. H. M.; Groothuys, S.; Keereweer, A. R.; Quaedflieg, P. J. L. M.; Blaauw, R. H.; Delft, F. L.; Rutjes, F. P. J. T. *Org. Lett.* **2004**, *6*, 3123-3126. (c) Yang, J.; Hoffmeister, D.; Liu, L., Fu, X.; Thorson, J. S. *Bioorg. Med. Chem*. **2004**, *12*, 1577-1584. (d) Lin, H.; Walsh, C. T. *J. Am. Chem. Soc*. **2004**, *126*, 13998-14003. (e) Mocharla, V. P.; Colasson, C.; Lee, L. V.; Röper, S.; Sharpless, K. B.; Wong, C.-H.; Kolb, H. C. *Angew. Chem. Int. Ed*. **2005**, *44*, 116-120.
37. For reviewing: Scriven, E. F. N.; Turnbull, K. *Chem. Rev*. **1988**, *88*, 297-368.
38. For example: Tanaka, K. S. E.; Winters, G. C.; Batchelor, R. J.; Einstein, F. W. B.; Bennet, A. J. *J. Am. Chem. Soc*. **2001**, *123*, 998-999.

39. Elchert, B.; Li, J.; Wang, J.; Hui, Y.; Rai, R.; Ptak, R.; Ward, P.; Takemoto, J. Y.; Bensaci, M.; Chang, C.-W. T. *J. Org. Chem.* **2004**, *69*, 1513-1523.
40. For example: Takayanagi, M.; Flessner, T.; Wong, C.-H. *J. Org. Chem*. **2000**, *65*, 3811-3815.
41. (a) Barton, D.H.R.; McCombie, S.W. *J. Chem. Soc., Perkin Trans. I, **1975***, 1574-1585. (b) Robins, M.J.; Wilson, J.S.; Hansske, F. *J. Am. Chem. Soc., **1983**, 105*, 4059-4065. (c) Barton, D. H. R.; Ferreira, J. A.; Jaszberenyi, J. C. in *Preparative Carbohydrate Chemistry*, Hanessian, S. Ed., Marcel Dekker, Inc. **1997**, New York, p151-172, and references cited therein.
42. Chang, C.-W. T.; Clark, T.; Ngaara, M. *Tetrahedron Letter*, **2001**, *42*, 6797-6801.
43. Sabesan, S.; Neira, S.; *J. Org. Chem.* **1991**, *56*, 5468-5472.
44. Szarek, W. A.; Kong, X. in *Preparative Carbohydrate Chemistry*, Hanessian, S. Ed., Marcel Dekker, Inc. **1997**, New York, p105-125, and references cited therein.
45. Boons, G.J. *Tetrahedron.* **1996**, *52*, 1095-1121.
46. Jung, K.-H.; Muller, M.; Schmidt, R. R. *Chem. Rev*. **2000**, 100, 4423-4442.
47. Hanessian, S.; Lou, B. *Chem. Rev*. **2000**, 100, 4443-4463.
48. Schmidt, R. R. Jung, K.-H.; in *Preparative Carbohydrate Chemistry*, Hanessian, S. Ed., Marcel Dekker, Inc. **1997**, New York, p283-313, and references cited therein.
49. For examples: (a) Crich, D.; Sun, S. *J. Am. Chem. Soc*. **1998**, *120*, 435-436. (b) Crich, D.; Sun, S. *J. Org. Chem*. **1997**, *62*, 1198-1199. (c) Crich, D.; Smith, M. *Org. Lett*. **2000**, *2*, 4067-4069. (d) Crich, D.; Smith, M. *J. Am. Chem. Soc*. **2001**, *123*, 9015-9020.
50. Crich, D. in *Glycochemistry Principles, Synthesis, and Applications,* Wang, P.G. and Bertozzi, C. R. Ed. Marcel Dekker, Inc. **2001**; p53-75.
51. Lemieux, R.U. *Pure Appl. Chem.* **1971**, *25*, 527-548.
52. Haddad, J; Kotra, L. P.; Mobashery, S. in *Glycochemistry Principles, Synthesis, and Applications,* Wang, P.G. and Bertozzi, C. R. Ed. Marcel Dekker, Inc. **2001**; p307-351.
53. Bochkov, A. F.; Zaikov, G. E., *Chemistry of the O-Glycosidic Bond: Formation and Cleavage*, Pergamon Press, Elmsford, New York, **1979**.
54. Fourmy, D.; Recht, M. I.; Blanchard, S. C.; Puglisi, J. D. *Science*, **1996**, *274*, 1367-1371.
55. (a) Wang, J.; Li, J.; Tuttle, D.; Takemoto, J.; Chang, C.-W. T.; *Org. Lett.* **2002**, *4*, 3997-4000. (b) Li, J.; Wang, J.; Hui, Y.; Chang, C.-W. T.; *Org. Lett.* **2003**, *5*, 431-434. (c) Wang, J.; Li, J.; Czyryca, P. G.; Chang, H.; Kao, J.; Chang, C.-W. T.; *Bioorg. Med. Chem. Lett.* **2004**, *4*, 4389-4393.
56. For examples: (a) Mingeot-Leclercq, M.-P.; Glupczynski, Y.; Tulkens, P. M. *Antimicrob. Agents Chemother*. **1997**, *43*, 727-737. (b) Kotra, L. P.; Haddad, J.; Mobashery, S. *Antimicrob. Agents Chemother*. **2000**, *44*, 3249-

3256. (c) Cohen, M. L. *Science* **2002**, *257*, 1050-1055. (d) Neu, H. C. *Science* **2002**, *257*, 1064-1072.
57. For examples: (a) Tanaka, H.; Nishida, Y.; Furuta, Y.; Kobayashi, K. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 1723-1726, (b) Hanessian, S.; Tremblay, M.; Swayze, E. E. *Tetrahedron*, **2003**, *59*, 983-993, (c) Hanessian, S.; Kornienko, A.; Swayze, E. E. *Tetrahedron*, **2003**, *59*, 995-1007.
58. (a) Seeberger, P. H.; Baumann, M.; Zhang, G.; Kanemitsu, T.; Swayze, E. E.; Hofstadler, S. A.; Griffey, R. H. *Synlett*. **2003**, 1323-1326. (b) Chou, C.-H.; Wu, C.-S.; Chen, C.-H.; Lu, L.-D.; Kulkarni, S. S.; Wong, C.-H.; Hung, S.-C. *Org, Lett*. **2004**, *6*, 585-588.
59. Suami, T.; Nashiyama, S.; Ishikawa, Y.; Katsura, S. *Carbohydr. Res*. **1976**, *52*, 187-196.
60. Similar solvent effect in the selectivity of glycosylation can be found in: Greenberg, W. A,; Priestley, E. S.; Sears, P. S.; Alper, P. B.; Rosenbohm, C.; Hendrix, M.; Hung, S.-C.; Wong, C.-H. *J. Am. Chem. Soc*. **1999**, *121*, 6527-6541.
61. Li, J.; Wang, J.; Czyryca, P. G.; Chang, H.; Orsak, T.W.; Evanson, R.; Chang, C.-W. T. *Org. Lett.* **2004**, *6*, 1381-1384.