

***Development of the “Arming Participating Group” and its Application to Stereoselective Oligosaccharide Assembly***

***Dissertation Proposal by James T. Smoot  
Advisor: Alexei V. Demchenko***

**Department of Chemistry and Biochemistry  
University of Missouri-St. Louis**



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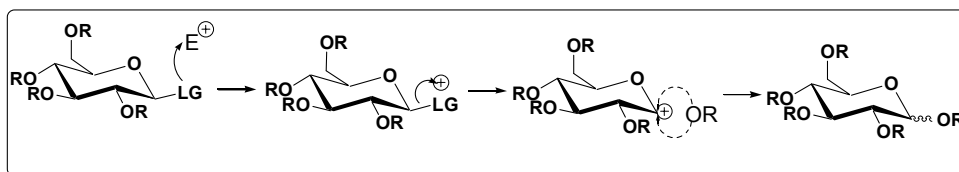
## Introduction

Carbohydrates are ubiquitous in nature. They are present as glycosides, glycoconjugates (glycoproteins or glycolipids), oligosaccharides, and polysaccharides in which monosaccharide residues are connected via 1,2-*cis* or 1,2-*trans* glycosidic bonds.<sup>1</sup> They are important for many biological processes, such as Metabolism, cell-cell recognition, cell adhesion, viral and bacterial infection (for cell membrane penetration), and immune response.<sup>2</sup> They have also shown promise as antibiotic and anti-cancer drugs.<sup>3</sup> Biological studies require significant quantities of chirally pure compounds. Isolation of pure material from natural sources, however, remains a daunting task. One approach to overcome this is to chemically synthesize the required compounds. In line with recent developments in the area of synthetic chemistry, there is a demand to synthesize these compounds in the most straightforward way.<sup>4</sup> This project proposes a conceptually novel glycosylation approach that minimizes protective group manipulations between glycosylation steps, by utilizing a new class of protective group, the so-called “arming participating substituent”.

## Background Information

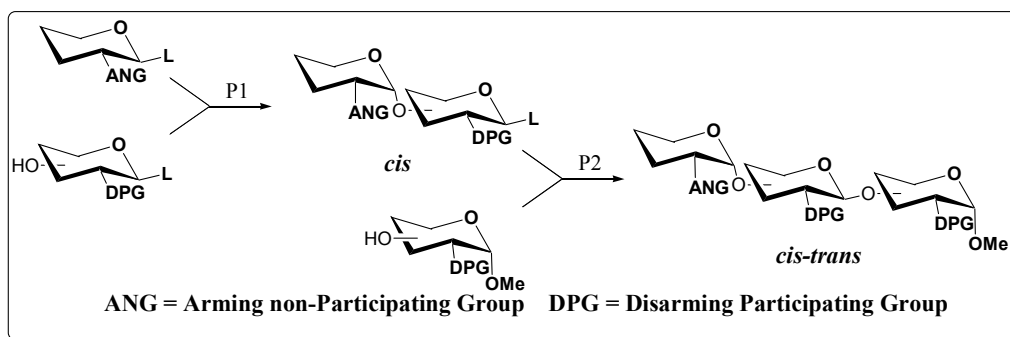
The majority of modern glycosylation reactions are performed by introducing a leaving group at the anomeric center, which can be selectively activated, usually with an electrophile, to undergo the glycosylation reaction with the hydroxyl group of a glycosyl acceptor (*Scheme 1*).<sup>4</sup> In the past few decades, many developments have been made with respect to the anomeric leaving groups, leading to glycosyl donors that are stable toward protective group manipulations and possess orthogonality of activation.<sup>4</sup> For example, some leaving groups can be selectively

## Scheme 1. Glycosylation Mechanism



activated over other classes of leaving groups or chemoselectively activated over the same type of leaving group that has been electronically deactivated. The “Armed-Disarmed strategy” that was developed in 1988 by Fraser-Reid, allows for a glycosyl donor bearing an arming (ether) protective group at C-2 to be activated over a glycosyl acceptor, the leaving group of which has been electronically deactivated by a disarming (ester) group at C-2 (Scheme 2).<sup>5</sup> The chemoselective activation is achieved by the addition of a mild promoter (P1) leading to the predominant formation of the 1,2-*cis* glycosidic linkage. It has been proposed that the electron withdrawing group decreases the nucleophilicity of the leaving group and also destabilizes the carbocation intermediate during glycosylation (Scheme 3).<sup>6</sup> Following this step, a more powerful promoter (P2) is added to activate the disarmed moiety to give the 1,2-*trans* linkage.

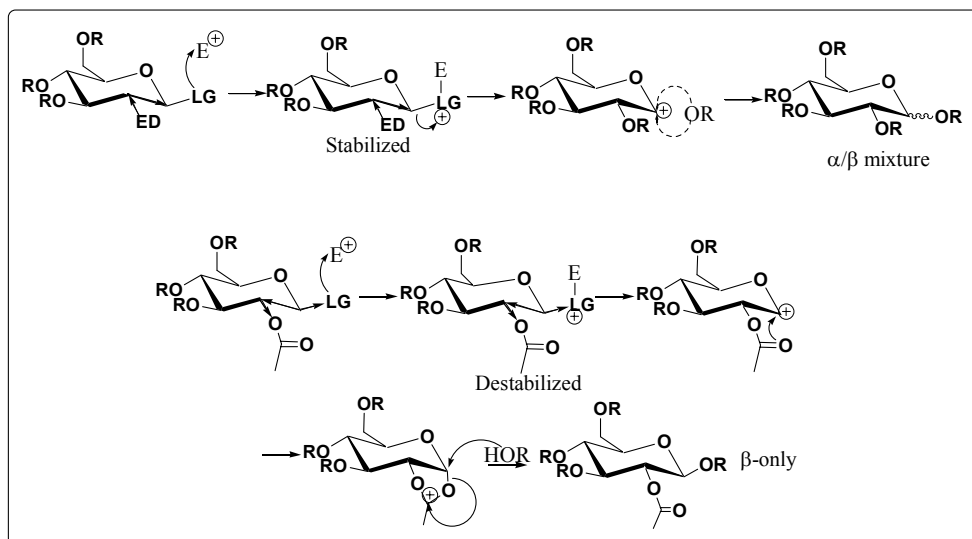
## Scheme 2. “Armed-Disarmed” Glycosylation Strategy



The drawbacks of this method are: the necessity to re-protect the oxygen at C-2 with a non-participating group, if a *cis-cis* linked trisaccharide is desired. Also, this method does not permit the formation of *trans-trans* or *trans-cis* glycosylation patterns, as it would be difficult to select

one disarming substituent over another.

### Scheme 3. “Armed-Disarmed” Glycosylation Mechanism

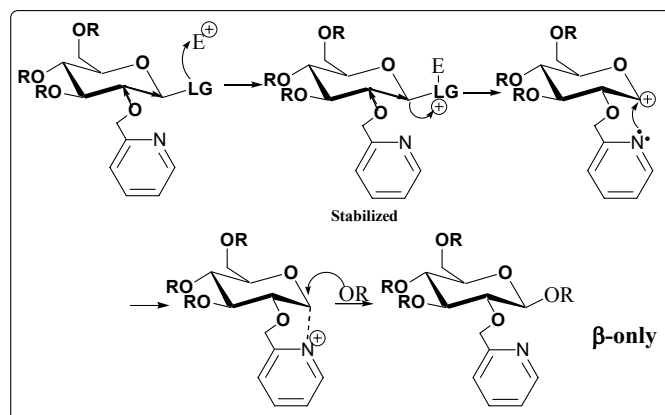


### Preliminary Results

In attempts to address the drawbacks inherent to this approach, it would be beneficial to develop a new glycosylation strategy for the convenient preparation of *trans-trans* and *trans-cis* glycosylation patterns without protective group manipulations between glycosylation steps. This would require investigation of new moieties that could be inserted to the O-2 position as an ether (arming) participating (*trans*-directing) group. These would contain a heteroatom (such as oxygen, nitrogen or sulfur) that would be able to donate an electron pair to coordinate the carbocation during the activation and would “tie-up” the  $\alpha$ -face of the molecule, like an ester group, so that only the *1,2-trans* anomer is formed (Scheme 4).<sup>7</sup>

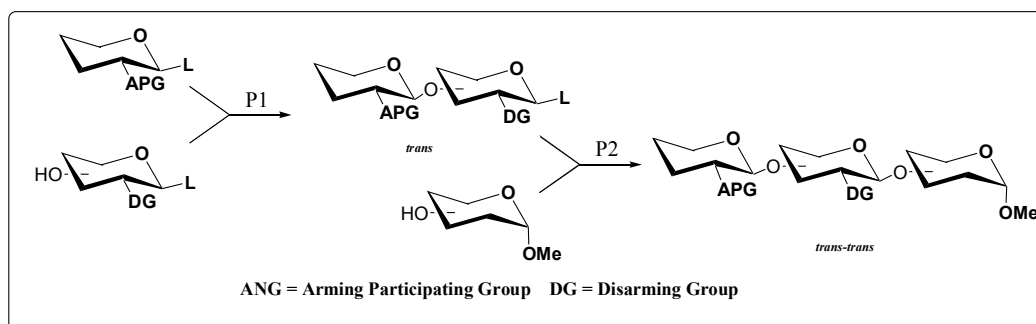
The advantage of this procedure would be its ability to form a *trans-trans* glycosylation pattern without protective group manipulations between the glycosylation steps (Scheme 5). Another major benefit to having this type of group, not considering the armed-disarmed strategy, is the

#### Scheme 4. “Inverse Armed-Disarmed” Glycosylation Mechanism



ability to form 1,2-*trans* linkages with an ether group present. At the present time, the methods for synthesizing heparin derivatives use an ether group at O-2 in the formation of some of the glycosidic linkages.<sup>8</sup> The ether is necessary for orthogonal deprotection of esters for their selective sulfonation. As a result, researchers are forced to utilize other effects (such as temperature and solvent) to try to enrich the *trans/cis* ratio. This results in lower yields and difficulties associated with separation of the anomers.

#### Scheme 5. “Inverse Armed-Disarmed” Glycosylation Strategy

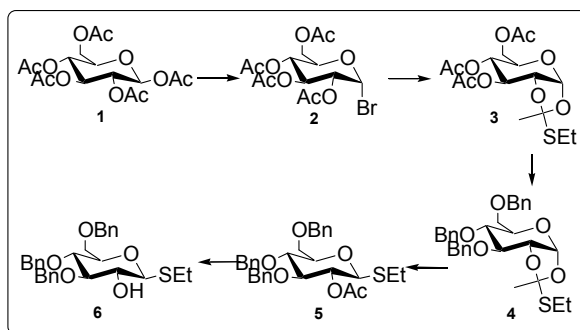


#### Preliminary Screening of Potential Arming Protective Groups

For the novel group screenings and glycosylation studies, we prepared the 2-OH intermediate **6** from 2-O-Ac precursor **5** (Scheme 6). Compound **5** was prepared from the commercially

available glucose pentaacetate by known methods.<sup>9</sup> With the key intermediate **6** in hand, many potential protective groups were screened to evaluate their application as a participating protective group. The results for 2-OH protection are given below (Table 1). The result of "no" implies "no expected product formation" or "no reaction". For these reactions a variety of conditions were employed (i.e. temperature variation, catalyst addition {NIS, NBS, copper bromide}, base and solvent). We believe the C-2 position to be a very hindered position. This arises from comparative studies with the less hindered ethyl 3,4-O-isopropylidene-thio- $\beta$ -D-galactose, a common building block in our laboratory. Neither iodothiophene nor nitrobenzyl bromide reacted with the primary alcohol of the isopropylidene derivative. All of the chemicals in Table 1 are commercially available except for the dimethoxy-benzyl bromide (**7c**), which was synthesized by radical bromination of dimethoxy-toluene with NBS in carbon tetrachloride. The dichlorodiphenylsilane was used both "as-is" and as the monochloro silyl ether after reaction with methanol or t-butanol. Once the 2-OH was protected, the donors were then tested in glycosylation reactions. For comparison, ethyl 2,3,4,6-O-benzyl-thio- $\alpha$ -D-glucose (**7l**) was prepared.

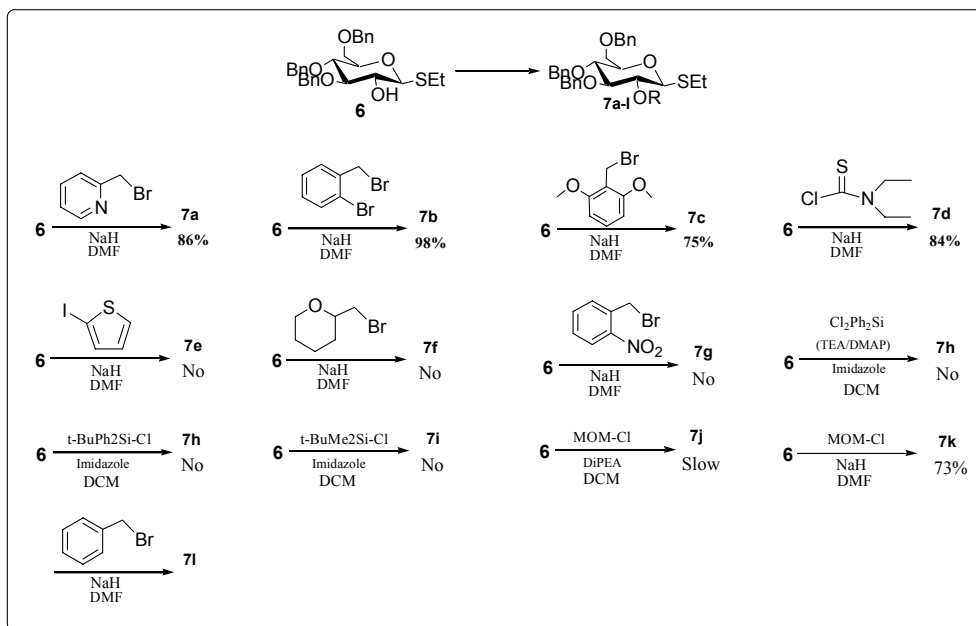
**Scheme 6. Synthesis of the key intermediate **6** (2-OH, 1-SEt)**



The following glycosylation trials were performed in dry dichloroethane (to avoid solvent effects) at room temperature (unless otherwise specified) under an argon blanket and in the

presence of molecular sieves. The results are summarized in Table 2.

**Table 1. 2-O-Protection of the key intermediate 6**



**Table 2. Glycosylation Results for Diacetone Glucose 12 and 2-O-Protected-SEt**

Entry	Donor	Promoter	Temperature	Yield %	$\alpha/\beta$ ratio
1	7a	IDCP	RT	*	---
2	7a	MeOTf	RT	*	---
3	7a	DMTST	RT	*	---
4	7b	IDCP	RT	58	1.8/1
5	7b	IDCP	-35->RT	87	2.3/1
6	7c	IDCP	RT	78	1.8/1
7	7d	IDCP	RT	*	---
8	7d	IDCP	0 -> RT	*	---
9	7d	DMTST	RT	*	---
10	7d	NIS/TMSOTf	0 -> RT	*	---
11	7d	CuBr <sub>2</sub> /TBAB	RT	*	---
12	7k	DMTST	RT	**	---
13	7l	IDCP	RT	62	2.7/1

IDCP = Iodonium dicollidine perchlorate, TBAB = tetrabutyl ammonium bromide,

DMTST = dimethyl-thiomethyl sulfonium triflate, NIS = N-iodosuccinimide

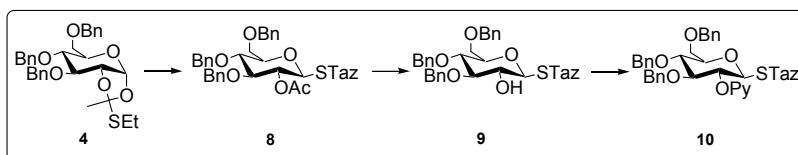
\* = Un-reactive salt formation from electrophilic attack of promoter on nitrogen

\*\* = Mixture of products

Of the five compounds that were studied in the glycosylation, only two allowed efficient glycosylations. The 2-bromobenzyl (**7b**) and the di-methoxybenzyl (**7c**) both gave good yields and showed an increase in the  $\beta/\alpha$  ratio compared to the “regular” 2-O-benzyl group (donor **7l**). Unfortunately the N-containing groups, picolyl-bromide (**7a**-pyridine derivative) and the diethylthiocarbamoyl-chloride (**7d**), gave poor results. We believe that this is due to an unreactive salt formation with the electrophilic promoters for thioglycoside activation. The 2-O-MOM (donor **7k**) gave a number of byproducts and was not further pursued.

In order to avoid the use of an electrophilic promoter in the glycosylation reactions, we decided to investigate other leaving groups. We chose the S-thiazolyl (STaz) group, which was already being studied in our lab.<sup>10</sup> The STaz could be activated by many promoters, including the less electrophilic heavy metal salt-based systems AgOTf and CuOTf<sub>2</sub>. The synthesis of the STaz donor follows the same general scheme as the SEt donor up through intermediate **4**. The attempts of using an STaz as the orthoester did not give favorable results when compared to the yields obtained with the SEt orthoester. We chose, therefore, to use the SEt as the orthoester protective group for the early steps and use then an excess of HSTaz for the orthoester (**4**) opening. This method resulted in the successful anomeric STaz insertion to afford **8** in 85% yield. The de-acetylation step was performed with NaOMe/MeOH and afforded **9** in 92% yield, which was then reacted with NaH and bromomethyl pyridine hydrobromide (Scheme 7).<sup>11</sup> For this reaction, equimolar amounts of NaH and the hydrobromide must be combined and cooled to  $-20^{\circ}\text{C}$ . The sugar is added to this cold solution and stirred for 5 minutes, followed by addition of the excess NaH, for the alkoxide formation. This pre-neutralization of the hydrobromide salt is done in order to avoid significant loss of product due to hydrolysis of the STaz group.

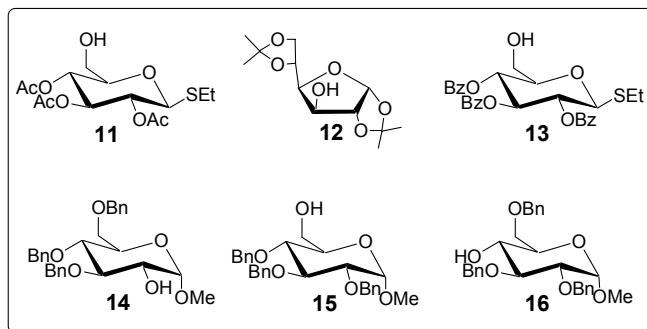
### Scheme 7. Synthesis of 2-OPy-STaz



The hydrolyzed product is then consumed by the bromomethylpyridine, leading to unwanted disubstituted by-products. After synthesis of the 2-O-picolyl donor (**10**), it was glycosidated with different acceptors (**11-16**, Figure 1) in the presence of different promoters as summarized in Table 3. For comparative studies the standard tetra-benzyl-STaz donor (**17**) was reacted with diacetone glucose. This afforded the requisite disaccharide in an 83% yield as a mixture of anomers ( $\alpha/\beta$  2/1). All glycosylations were performed in dichloroethane in the presence of molecular sieves and under argon. Upon completion, the reactions were quenched with excess triethylamine, filtered, and extracted with dichloromethane/water. The disaccharides were then purified by column chromatography on silica gel or size exclusion chromatography on Sephadex LH-20.

To summarize these results, the initial studies with the 2-O-picolyl group and the STaz as the leaving group, showed promising results when promoted with metal triflates. All glycosylations proceeded smoothly and afforded the desired disaccharides as pure 1,2-*trans* diastereomers. As seen in Table 3, the CuOTf<sub>2</sub>/TfOH system gave the best results (The discovery of the yield improvement due to TfOH addition came after the ester protected thioglycoside work had been done and thus have yet to be performed.). This is of great benefit as CuOTf<sub>2</sub> has been shown to act as a chemoselective promoter for armed S-Taz-glycosides. No traces of 1,2-*cis*-linked disaccharides were detected by <sup>1</sup>H NMR spectroscopy.

**Figure 1 Glycosyl Acceptors used for Glycosylations with Donor 10**



**Table 3. Glycosidation of 2-O-Pic-STaz (10) with Acceptors 11-16**

Entry	Acceptor	Promoter	Yield, %	$\alpha/\beta$ Ratio
1	11	CuOTf <sub>2</sub>	52	$\beta$ only
2	12	CuOTf <sub>2</sub>	28	$\beta$ only
3	13	CuOTf <sub>2</sub>	77	$\beta$ only
4	13	AgOTf	71	$\beta$ only
5	14	CuOTf <sub>2</sub>	59	$\beta$ only
6	14	AgOTf	53	$\beta$ only
7	14	CuOTf <sub>2</sub> /TfOH	80	$\beta$ only
8	15	CuOTf <sub>2</sub>	63	$\beta$ only
9	16	CuOTf <sub>2</sub>	65	$\beta$ only
10	16	CuOTf <sub>2</sub> /TfOH	78	$\beta$ only
11*	12	AgOTf	83	2/1

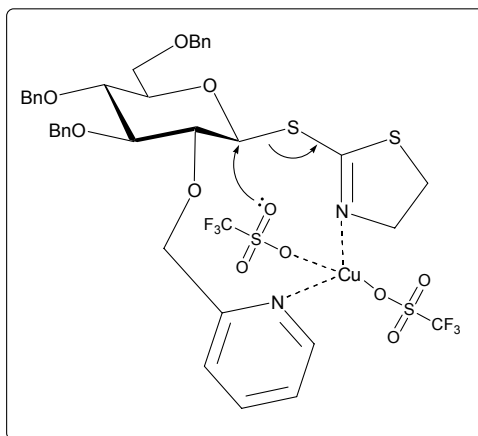
\* = comparison reaction involving tetra-benzyl-Staz donor

### Future Work

**Optimization of the Reaction Conditions:** With the exception of entry 2 (Table 3), which had significant yield loss due to the acceptor hydrolysis, the yields were moderate to high for glycosylation with picolyl present at O-2. We plan to investigate other promoter systems, the amount of triflic acid required and reaction temperature. Work-up procedure and separation techniques still have yet to be optimized.

**Mechanistic Studies:** It is possible that the reaction proceeds via a concerted STaz departure-triflate coordination (Figure 2). To identify the pathway, studies need to be conducted on the intermediate such as low temperature NMR (to observe anomeric triflates), MALDI, or attempt to grow a crystal for X-Ray diffraction, if sufficiently stable.

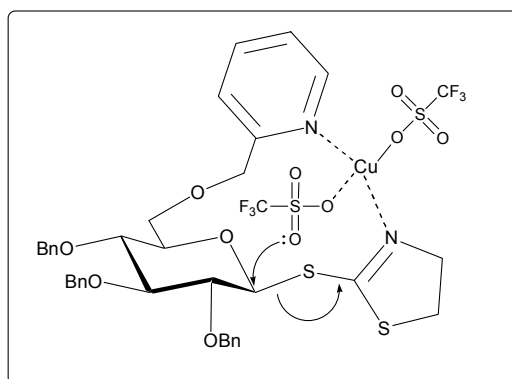
**Figure 2: Plausible Glycosylation Pathway**



**Investigation of “Inverse Armed-Disarmed Strategy”:** After having demonstrated that the picolyl group can be used as an effective participating group and can be promoted by copper triflate, we intend to determine its effectiveness for use in the “Inverse Armed-Disarmed Strategy”. We are hopeful that this method will be successful, as studies performed in our lab have shown that armed STaz glycosyl donors can be activated over disarmed STaz acceptors in the presence of copper triflate. The disarmed STaz moiety would then be activated with a stronger promoter such as silver triflate. A disarming effect could be achieved with the use of either partial ester (O-Ac or O-Bz) or non-partial ester functionality (halogeno-ester) to allow *trans-trans* or *trans-cis* glycosylation patterns respectively.

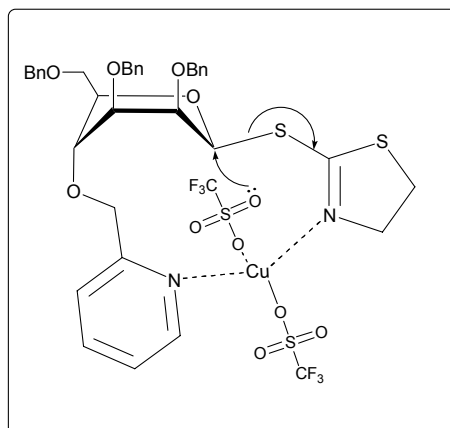
**Investigation of Application to 1,2-*cis* Glycosylation:** With the complete stereoselectivity attained by neighboring group participation, we intend to investigate the ability of the picolyl group to direct stereochemistry via remote participation for synthesis of  $\alpha$ -glucosides and  $\beta$ -mannosides. The synthesis of 6-O-Pic-1-STaz glucose will be performed to attempt to form the 1,2-*cis* linkage of the  $\alpha$ -glucoside. The anticipated intermediate is shown in (Figure 3).

**Figure 3 Participation of 6-O-Pic for the Synthesis of  $\alpha$ -Glucosides**



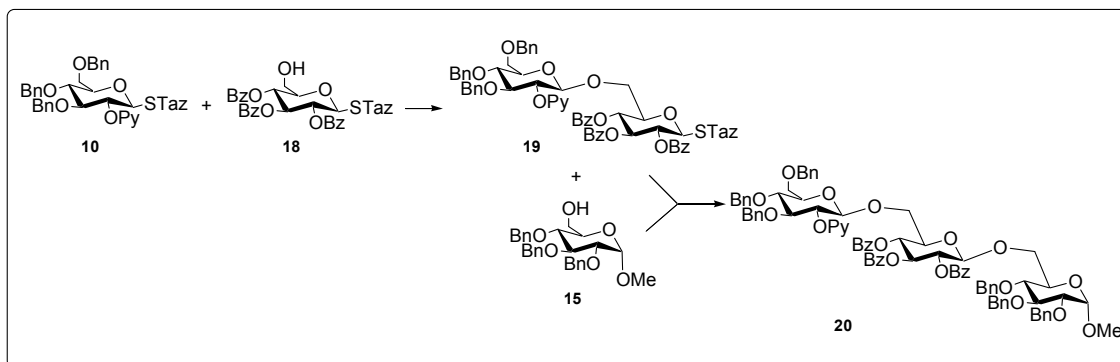
If the  $\alpha$ -glucoside synthesis is successful, this procedure will then be expanded to include attempts to synthesize  $\beta$ -mannosides. For the synthesis of  $\beta$ -mannoside, the picolyl group would be positioned on O-4. This would allow coordination of the bottom ( $\alpha$ ) face of the monosaccharide allowing, predominantly, formation of the  $\beta$ -conformation. If  $\beta$ -mannosides can be synthesized efficiently by this method, it would represent a major advance in the field of carbohydrate chemistry.  $\beta$ -Mannosides are difficult to synthesize due to the strong anomeric effect.<sup>12</sup> This leads to low  $\alpha/\beta$  selectivity. The structure of the anticipated intermediate is shown in Figure 4.

**Figure 4: Participation of 6-O-Pic for the synthesis of  $\beta$ -Mannosides**



**Application to Target Synthesis:** Utilizing the “Inverse Armed-Disarmed Strategy”, we plan to investigate the application our methodology for the synthesis of a *trans-trans* trisaccharide (Scheme 8). The disaccharide **19** will be synthesized by coupling our picolyl donor (**10**) with the deactivated tribenzoyl S-Taz acceptor (**18**) in the presence of  $\text{CuOTf}_2$ . This disaccharide will then be coupled with acceptor (**15**) in the presence of  $\text{AgOTf}$  to give the desired *trans-trans* trisaccharide.

**Scheme 8. “Inverse Armed-Disarmed” for *trans-trans* Trisaccharide**

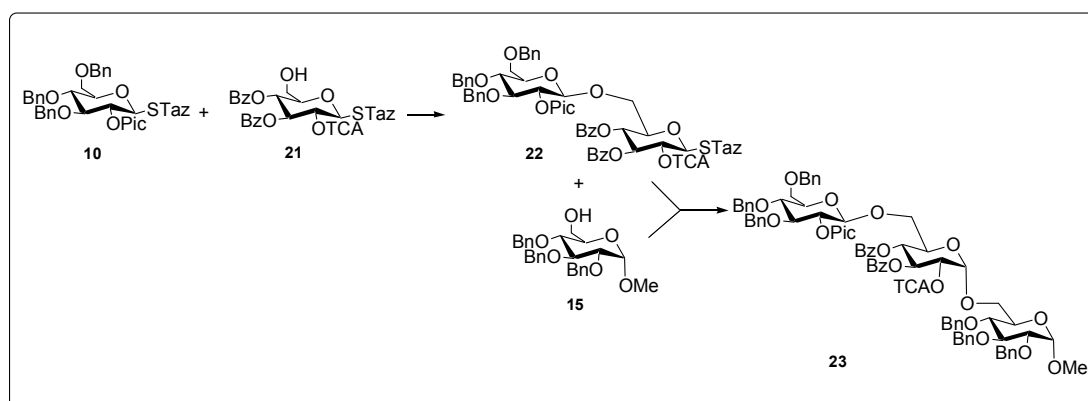


We also intend to investigate the application of the picolyl donor for the formation of *trans-cis* trisaccharides (Scheme 9). For this scheme we plan to couple our donor (**10**) with the

2-O-trichloroacetate or 2-O-chloroacetate protected acceptor (**21**) to yield disaccharide (**22**). Chloroacetate protected saccharides are deactivated 1,2-*cis* directing compounds, which when promoted with AgOTf in the presence of acceptor (**15**), should yield the desired *trans-cis* trisaccharide (**23**).

In the event that unexpected difficulties arise, we plan to investigate the application to other heavy metal triflate activated donors, such as S-benzoxazolyl (S-Box) glycosides.

### Scheme 9. “Inverse Armed-Disarmed” for *trans-cis* Trisaccharide



### Conclusion

To overcome the drawbacks of the “Armed-Disarmed Strategy”, we are proposing a complimentary “Inverse Armed-Disarmed Strategy” based upon a novel arming-participating protective group that can be introduced with the commercially available picolyl bromide. When combined with the newly developed method, utilizing S-Taz glycosides to participate in an “Armed-Disarmed” fashion, will permit formation of: *cis-cis*, *cis-trans*, *trans-trans*, and *trans-cis* glycosylation patterns the latter two of which are not possible with S-Et donors. A broader impact of this project may result in the development of general heavy metal salt-mediated methodology for the stereoselective construction of both 1,2-*cis* and 1,2-*trans* glycosidic bonds.

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