

*Development of Novel Glycosylation  
Methodology Based on S-Thiazolinyl  
Glycosides*

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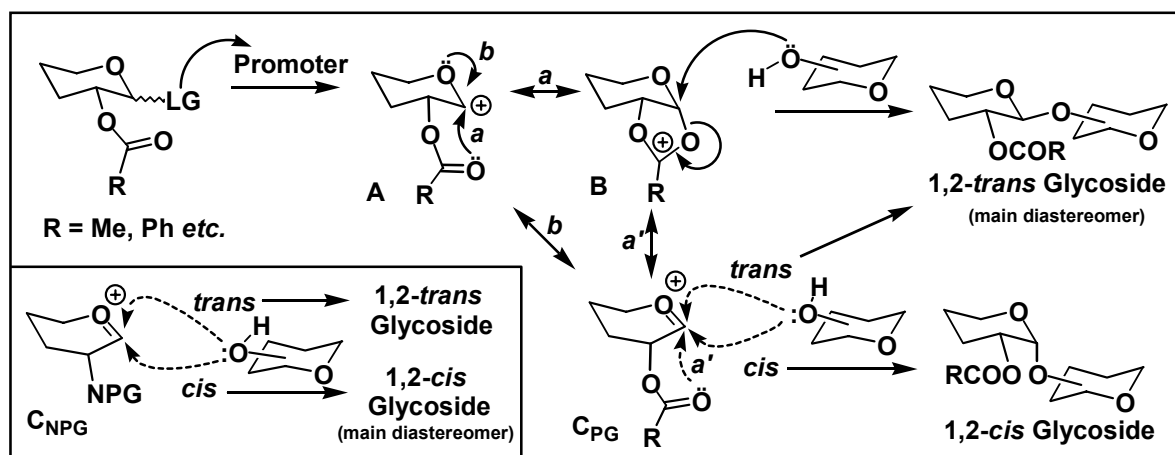
## Introduction

The development of new synthetic methods and strategies for the chemical synthesis of biologically important glycostructures and glycomimetics is the central theme in our research. The basis of this dissertation proposal is the elaboration of a new method for convergent oligosaccharide synthesis and its application to the efficient synthesis of complex structures of biological importance. Specific aims are as follows:

- A Development of the STaz methodology: invention of a new versatile glycosylation procedure.
- B Strategy design based on STaz glycosides: applications of the STaz glycosyl donors to convergent oligosaccharide synthesis; selective and orthogonal activation concept.
- C Application to the synthesis of  $\alpha$ -(1 $\rightarrow$ 3)-glucan of *Cryptococcus neoformans* and *Streptococcus pneumoniae* type 6A oligosaccharides.

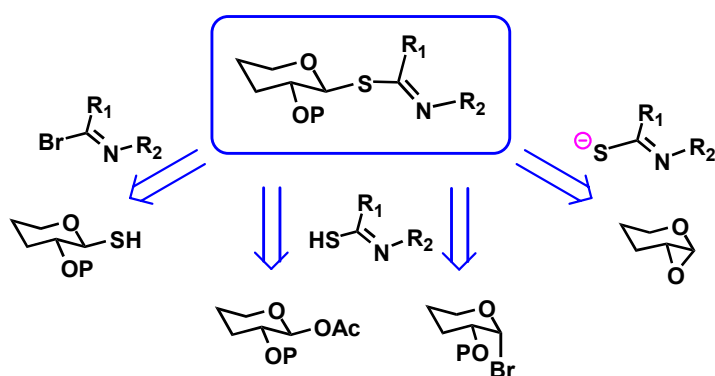
**Background Information:** The majority of carbohydrates found in nature exist as polysaccharides, glycoconjugates or glycosides in which monosaccharide residues are joined together via O-glycosidic bonds.<sup>1</sup> The necessity to form either a 1,2-*cis* or a 1,2-*trans* glycosidic bond with complete stereoselectivity is the main reason chemical O-glycosylation is placed among the most challenging problems of modern synthetic chemistry. To address these challenges many new glycosyl donors have been developed. It is well established that 1,2-*trans* glycosides { $\beta$ -glycosides for D-glucose, D-galactose, L-fucose, or  $\alpha$ -glycosides for D-mannose, L-arabinose, *etc.*} can be prepared with the assistance of a neighboring participating group. These glycosylations proceed primarily via a bicyclic intermediate, the acyloxonium cation **B**, formed as a result of the intramolecular stabilization of the carbocation **A** (Scheme 1, pathway a). In this case attack of a nucleophile (alcohol, glycosyl acceptor) is only possible from the top face of the ring, allowing, therefore, stereoselective formation of the 1,2-*trans* glycoside. Indeed, traditional glycosyl donors such as halides, thioglycosides or imidates provide excellent stereoselectivity and high yields. The 1,2-*cis* Glycosides can be synthesized with a glycosyl donor bearing a non-participating moiety at C-2 (*O*-benzyl, *O*-methyl, azido, *etc.*).<sup>2</sup>

**Scheme 1. Synthesis of 1,2-*trans* and 1,2-*cis* Glycosides**



Recently we became interested in a class of glycosyl donors with a generic thioimidoyl leaving group ( $\text{SCR}_1=\text{NR}_2$ ) taking into consideration the following advantageous features of the substituted thioimidates. First, these compounds can be synthesized via a number of well-established reaction pathways starting from anomeric halides, acetates, 1,2-anhydro or 1-thiosugars, and therefore are easily accessible. Second, we assumed that the glycosyl donor reactivity could be adjusted by varying the  $\text{R}_1$  and  $\text{R}_2$  substituents. Third, the overall size of the anomeric substituent may also influence the reactivity/stability of these derivatives to allow the application of (chemo)selective glycosylation approaches.

**Scheme 2: Synthesis of a new class of glycosyl donors.**

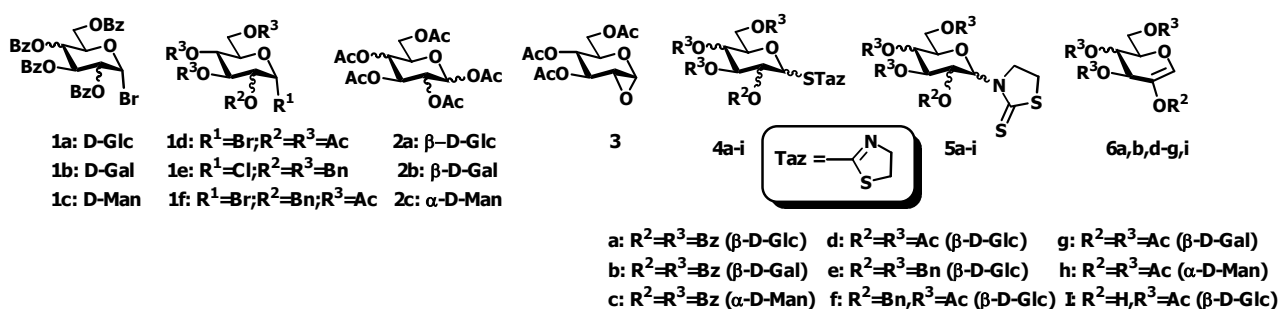


Typically, the lower stability of these derivatives, compared to the “stable” glycosyl donors, e.g. S-alkyl/aryl glycosides, toward protecting group manipulations is the major drawback of thioimidates, which limited their application in convergent oligosaccharide synthesis. We have already reported the synthesis of S-benzoxazolyl (SBox) glycosides and their evaluation in stereoselective 1,2-cis and 1,2-trans glycosylations.<sup>3,4</sup> We demonstrated that the SBox glycosides provide high stereoselectivity and remarkably high yields. Being very stable, they survive a range of reaction conditions associated with major protecting group manipulations. However, lower stability of the SBox glycosides toward harsh reaction conditions (TfOH or NaH) in comparison to the corresponding S-alkyl/aryl glycosides stimulated us to continue the search for suitable leaving groups of this class.

**Preliminary results :** Herein the discovery of S-thiazolinyl (STaz) glycosides and their application in stereoselective glycosylations is described.<sup>5</sup> We established that anomeric halides (1), acetates (2), or 1,2-anhydro sugars (3) serve as suitable precursors for the synthesis of STaz glycosides (4). 2-Mercaptothiazoline (HSTaz), an odorless solid, was used as the aglycone source. Conversion of

1-3 allowed formation of the desired S-linked derivatives with complete stereoselectivity and in moderate to high yields (50-90%). Significant amounts of by-products, N-linked (5) and/or 1,2-anhydro derivative (6), were also detected in some experiments due to ambient reactivity of HSTaz and its relatively high basicity (pKa=13.01).

**Figure 1**

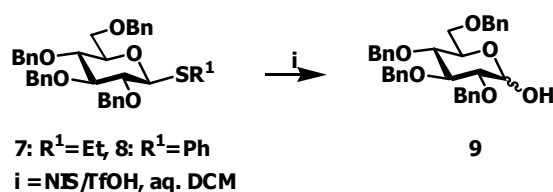


**Table 1 : Synthesis of STaz glycosides**

SM	Conditions	Products	Yield,%
1a	HSTaz, NaH, MeCN, rt	4a-5a-6a	49-0-41
1a	NaSTaz, 15-cr-5, MeCN, rt	4a-5a-6a	50-0-41
1a	KSTaz, 18-cr-6, MeCN, rt	4a-5a-6a	41-0-39
1a	NaSTaz, 15-cr-5, acetone, rt	4a-5a-6a	60-0-20
1b	KSTaz, 18-cr-6, MeCN, rt	4b-5b-6b	90-0-0
1c	KSTaz, 18-cr-6, MeCN, rt	4c-5c-6a	70-0-0
1d	NaSTaz, MeCN, rt	4d-5d-6d	53-11-13
1e	NaSTaz, 15-cr-5, acetone, rt	4e-5e-6e	55-38-0
1f	NaSTaz, 15-cr-5, MeCN, rt	4f-5f-6f	46-28-21
2a	HSTaz, BF <sub>3</sub> -Et <sub>2</sub> O, MS3A <sup>o</sup> , DCM, 45 °C	4d-5d-6d	91-0-0
2b	HSTaz, BF <sub>3</sub> -Et <sub>2</sub> O, MS3A <sup>o</sup> , DCM, 45 °C	4g-5g-6g	85-0-0
2c	HSTaz, BF <sub>3</sub> -Et <sub>2</sub> O, MS3A <sup>o</sup> , DCM, 45 °C	4h-5h-6a	70-0-0
3	HSTaz, ZnCl <sub>2</sub> , DCM, rt	4i-5i-6i	78-0-0

The STaz moiety stability toward acid hydrolysis was also investigated. In these studies **4e** was treated under standard conditions for the thioglycoside hydrolysis<sup>6</sup> to afford hemiacetal (**9**) (Scheme 3). Remarkably, **4e** appears to be much more stable than either **7**<sup>7</sup> or **8**.<sup>8</sup> For example, treatment of **4e** with NIS/TfOH in wet CH<sub>2</sub>Cl<sub>2</sub> resulted in trace formation of **9** (<5% in 1 h), while hydrolysis of either **7** or **8** was complete in less than 5 min.

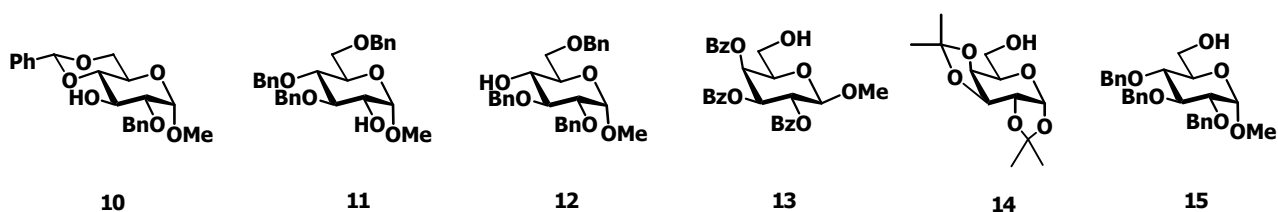
**Scheme 3: Hydrolytic stability study of the STaz moiety**



Moreover, the STaz moiety was found to be stable toward many common protective group manipulations such as typical acetylation (Ac<sub>2</sub>O/pyridine), deacetylation (NaOMe/MeOH), benzylation (BnBr/NaH, DMF), triphenylmethylation (TrCl/pyridine), as well as benzylidene acetal formation (PhCH(OMe)<sub>2</sub>, CSA) and cleavage (3%TFA, aq.CH<sub>2</sub>Cl<sub>2</sub>). Consequently, we turned our attention to the investigation of the STaz derivatives as glycosyl donors.

A number of coupling experiments were performed to find the most suitable promoter for the activation of the STaz moiety. Many different promoters such as AgOTf, MeOTf, NIS/TfOH, Cu(OTf)<sub>2</sub>, NBS, BF<sub>3</sub>-OEt<sub>2</sub>, CSA, or AgCO<sub>3</sub>/TMSOTf were used. We discovered that MeOTf, AgOTf, or Cu(OTf)<sub>2</sub> seem to be the most suitable activators. Importantly, no reaction took place in the presence of NIS/catalytic TfOH, a common promoter for thioglycoside glycosidation, whereas the reaction was completed in minutes in the presence of stoichiometric TfOH. Perbenzoyl derivatives of glucose, galactose and mannose (**4a-c**) were used for the synthesis of 1,2-trans-linked disaccharides. Differently protected glycosyl acceptors **10-15** were selected for this purpose (Figure 2).<sup>9</sup> The corresponding disaccharides were obtained in very high yields in the presence of each promoter; complete stereoselectivity was achieved reliably with the assistance of a participating substituent at C-2.<sup>10</sup>

Figure 2



We also demonstrated that 1,2-cis glycosides could be obtained from benzylated STaz glycosides **4e** or **4f**. Most reactions gave high yields in the presence of AgOTf, however, the stereoselectivity is average in DCE ( $\alpha/\beta$  3-5/1); still, it appears to be significantly higher than that achieved with S-alkyl/aryl glycosides ( $\alpha/\beta$  1-1.5/1). Also, these results could be improved (up to  $\alpha/\beta$  19/1) by varying solvent, promoter, protecting group pattern, etc.<sup>2</sup> Some of these results are summarized in Table 2 and 3.

Table 2: Synthesis of 1,2-trans glycosides with STaz glycosyl donors.

Entry	Donor	Acceptor	Promoter	Yield
1	<b>4a</b>	<b>10</b>	AgOTf	91%
2	<b>4a</b>	<b>11</b>	MeOTf	99%
3	<b>4a</b>	<b>12</b>	NIS/TfOH	93%
4	<b>4a</b>	<b>13</b>	AgOTf	90%
5	<b>4a</b>	<b>14</b>	MeOTf	82%
6	<b>4a</b>	<b>15</b>	Cu(OTf) <sub>2</sub>	99%
7	<b>4a</b>	<b>12</b>	AgOTf	92%
8	<b>4a</b>	<b>15</b>	AgOTf	84%
9	<b>4a</b>	<b>12</b>	AgOTf	85%
10	<b>4a</b>	<b>15</b>	AgOTf	87%
11	<b>4b</b>	<b>12</b>	AgOTf	92%
12	<b>4c</b>	<b>12</b>	AgOTf	85%

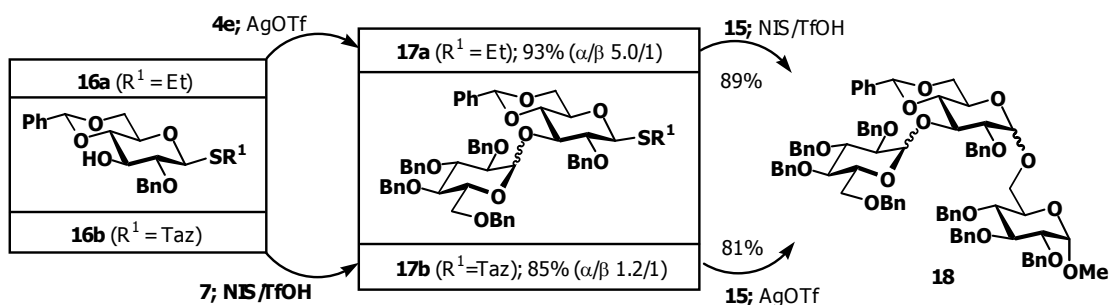
**Table 3: Synthesis of 1,2-cis glycosides with STaz glycosyl donors.**

Entry	Donor	Acceptor	Solvent	Yield	$\alpha/\beta$
1	<b>4e</b>	<b>10</b>	DCE	96%	2.9/1
2	<b>4e</b>	<b>10</b>	T/D*	85%	7.7/1
3	<b>4e</b>	<b>11</b>	DCE	88%	1.5/1
4	<b>4e</b>	<b>11</b>	T/D	97%	4.5/1
5	<b>4e</b>	<b>12</b>	T/D	95%	3.5/1
6	<b>4e</b>	<b>13</b>	T/D	82%	4.1/1
7	<b>4e</b>	<b>14</b>	T/D	76%	4.0/1
8	<b>4e</b>	<b>15</b>	T/D	85%	2.7/1
9	<b>4f</b>	<b>12</b>	DCE	78%	1.5/1
10	<b>4f</b>	<b>11</b>	DCE	88%	9.3/1
11	<b>4f</b>	<b>12</b>	DCE	89%	>19/1
12	<b>4f</b>	<b>13</b>	DCE	74%	4.8/1

\* toluene:dioxane (1/3 v/v)

Additionally, we decided to evaluate the applicability of the STaz technique in convergent glycosylation strategies.<sup>11</sup> We assumed that STaz glycosides could be activated over thioglycosides (AgOTf). Conversely, it should be possible to activate S-alkyl/phenyl glycosyl donors over STaz glycosides (NIS/cat. TfOH). To prove this hypothesis we synthesized a trisaccharide **18** via two conceptually different pathways: STaz→SEt→OMe and SEt→STaz→OMe activation sequence (Scheme 4). As a result, **18** was isolated in 83% and 69% overall yields respectively.

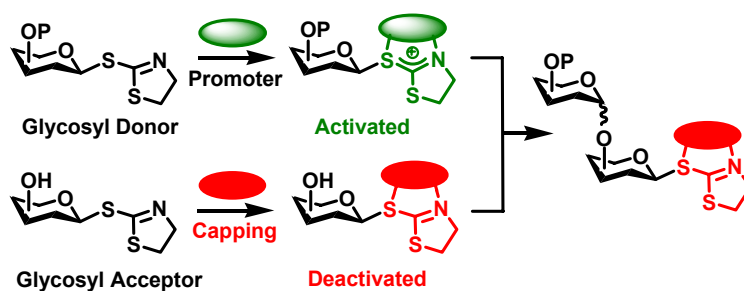
**Scheme 4 : Orthogonality of STaz and SEt glycosides**



We also discovered a new glycosylation strategy that allows chemoselective activation of STaz or other glycosyl donors over a glycosyl acceptor bearing a deactivated anomeric STaz moiety. This

temporary immobilization is achieved by engaging the glycosyl acceptor unit into a palladium(II) complex. Overall, this approach allowed chemoselective activation of a “free” STaz leaving group (glycosyl donor) over the deactivated (complexed, capped) STaz moiety (glycosyl acceptor) as illustrated in Scheme 5.<sup>12</sup> Thus, the chemoselective glycosylation could be achieved without the necessity to activate or deactivate the anomeric moiety by changing the electronic environment around the anomeric center, as it was previously explored in armed-disarmed<sup>13</sup> glycosylation strategies. We believe that this method of general character; similar strategies may be executed with other glycosyl donors, as well as employed in temporary masking of certain functionalities of complex molecules.

**Scheme 5.** Outline of the temporary deactivation concept



## Proposed Research:

### A. Development of STaz glycosidation methodology

**Synthesis of STaz glycosides:** only moderate yields were achieved due to competing elimination and/or *N*-substitution processes. We plan to investigate whether this could be addressed by varying the reaction conditions, such as the counter ion ( $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ) or reaction solvent (acetone, MeCN, THF, DMSO, DMF, dioxane). In less polar solvents the dissociation is suppressed and, as a result, salts of HSTaz exist as close ion pairs, which would lead to the glycosylation of the softer center, the sulfur atom, at a higher rate. In more polar solvents the salts are dissociated, and this creates a favorable environment for free ion glycosylation of two competing centers simultaneously.

**Stability of the STaz moiety:** the kinetics of the acid hydrolysis will be studied in the presence of TfOH or NBS, monitored by GC (per-acetylated derivatives), HPLC, MALDI-TOF, and/or NMR techniques. Similarly, stability studies toward various reaction conditions (NaH, MeONa, CSA, a range of the pH values) will be conducted.

**Glycosidation of STaz, mechanistic and other studies:** departed aglycone isolation and characterization, low temperature NMR studies of heavy metal complexation, and further search for suitable activation conditions and pathways.

## B. Strategy design

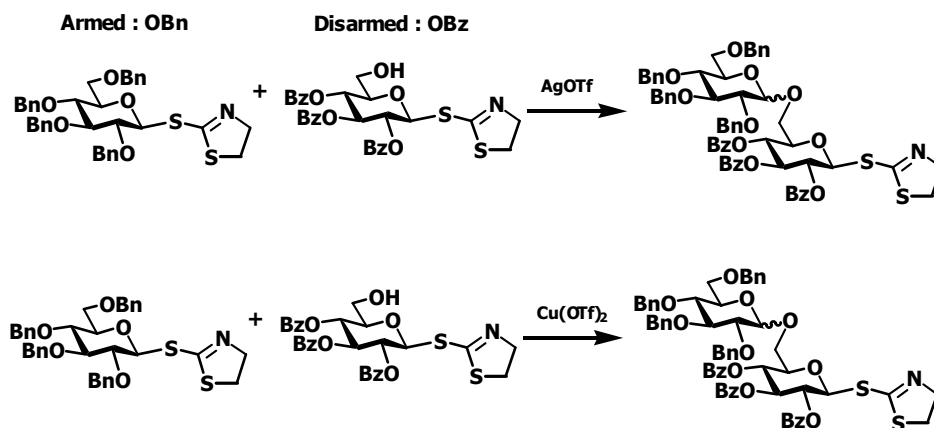
**STaz as glycosyl donors:** Further importance of thioimidates derives from evaluation of their applicability to convergent oligosaccharide synthesis. So far we demonstrated that STaz derivatives can be readily activated in the presence of S-Ethyl using AgOTf as a promoter. In order to achieve even more flexibility, we would like to investigate whether the STaz moiety could be activated in the presence of O-pentenyl (AgOTf or MeOTf), fluoro (MeOTf), S-phenyl (AgoTf) and SBox derivatives {Cu(OTf)<sub>2</sub>}.

**STaz as glycosyl acceptors:** A principal motivation for these investigations derives from the fact that prior to our studies, thioimidates had been considered too labile to be selectively protected and glycosidated. So far, we established that the STaz glycosides withstand SEt (NIS/TfOH) and SBox {Cu(OTf)<sub>2</sub>} activation conditions. Other glycosyl donors will be investigated as follows: SPh glycosides (NBS), armed O-pentenyl glycosides (IDCP), bromide {Ag<sub>2</sub>CO<sub>3</sub> or HgO/Hg(CN)<sub>2</sub>}, per-benzyl STaz (IDCP), trichloroacetimidates (TMSOTf). Glycosyl fluorides can be activated in the presence of a range of promoters, many of which might be found to be suitable for selective activation: e.g. SnCl<sub>2</sub>/AgClO<sub>4</sub>, Cp<sub>2</sub>ZrCl<sub>2</sub>/AgClO<sub>4</sub>, SiF<sub>4</sub>, La(ClO<sub>4</sub>)<sub>3</sub>, Yb(OTf)<sub>3</sub>, SO<sub>4</sub>/ZrO<sub>2</sub>, TfOH, HClO<sub>4</sub>. The STaz moiety-containing glycosyl acceptors will be used for these studies.

**Chemoselective activation (armed-disarmed strategy) :** We would like to investigate whether armed STaz glycoside (donor) could be activated over electronically disarmed STaz glycosyl

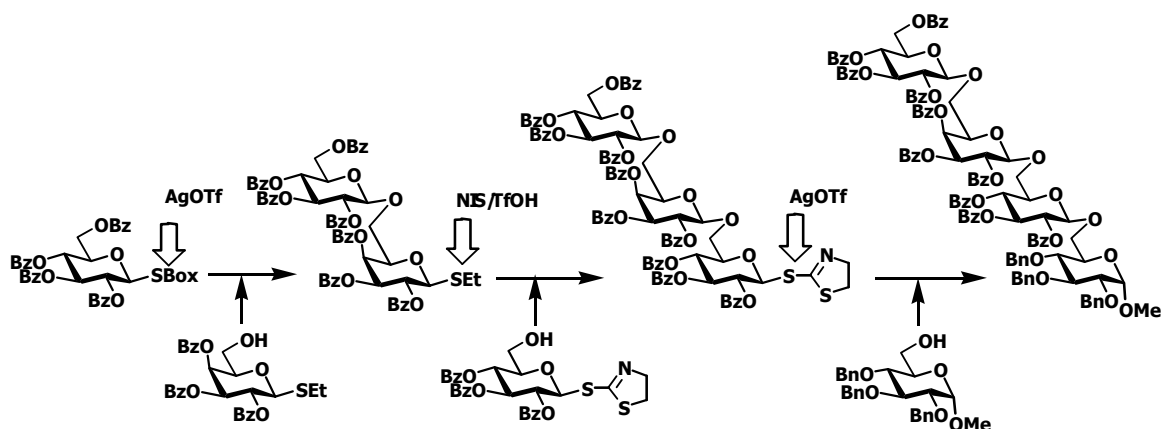
acceptor in the presence of AgOTf or Cu(OTf)<sub>2</sub> (Scheme 6) This approach is correlated to classic armed-diarmed glycosylation concept.<sup>13</sup> Interestingly, this concept does not seem to be applicable to SBox glycosides.

**Scheme 6 : Chemoselective activation of STaz glycosides.**



**Thioimidates in one-pot glycosylation strategies:** Further improvement may emerge with examination of a highly efficient one-pot, *in-situ* procedure.<sup>14</sup> One of the feasible pathways is shown in Scheme 7. Initially, the SBox moiety will be activated with AgOTf over the S-ethyl moiety of the glycosyl acceptor. Subsequently, S-ethyl moiety will be activated over S-thiazolyl acceptor upon addition of NIS/TfOH. Next, the STaz moiety will be activated by addition of AgOTf. This approach would allow to obtain a tetrasaccharide derivative in three synthetic steps with no requirement for intermediate protecting group manipulations or purification of intermediates.

### Scheme 7: One-pot oligosaccharide synthesis



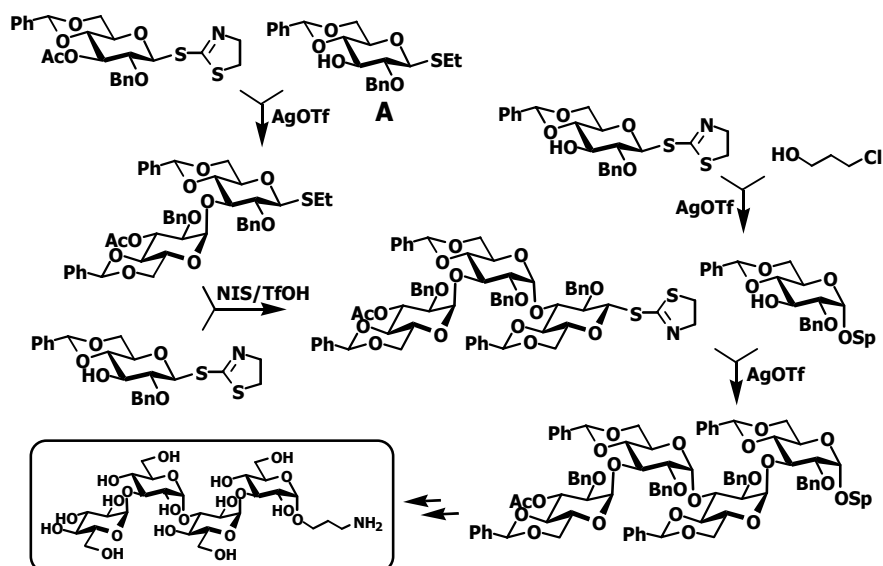
#### C. Application to Target Synthesis

##### **Synthesis of $\alpha$ -(1 $\rightarrow$ 3)-Glucan of *Cryptococcus neoformans*:**

One of the major drawbacks in studying the biological functions of glycoconjugates and oligosaccharides is the low availability of pure samples from natural sources. In principle, chemical synthesis would allow access to considerably larger quantities of chirally pure oligosaccharides. Even though some efficient approaches are available, every synthetic target containing one or more “difficult” (e.g. 1,2-cis-linked) residues still requires a careful selection of techniques.<sup>2,15</sup>

As a possible application of the developed methodology, we will obtain  $\alpha$ -glucans structurally similar to those expressed on the cell surface of *C. neoformans*, a fungal pathogen that has emerged as an important cause of mortality in immunocompromised patients, especially those with AIDS.<sup>16</sup> The advantage of the STaz-based strategy is its simplicity, as all components for the synthesis are obtained from the common building block A (Scheme 8). It is probable that the synthetic  $\alpha$ -glucans will be used as immobilized ‘bait’ on a matrix in order to purify potential binding partners (proteins). The spacer unit can be used for immobilization, a possible extension of the proposed approach would be direct synthesis and deprotection on polymer support.

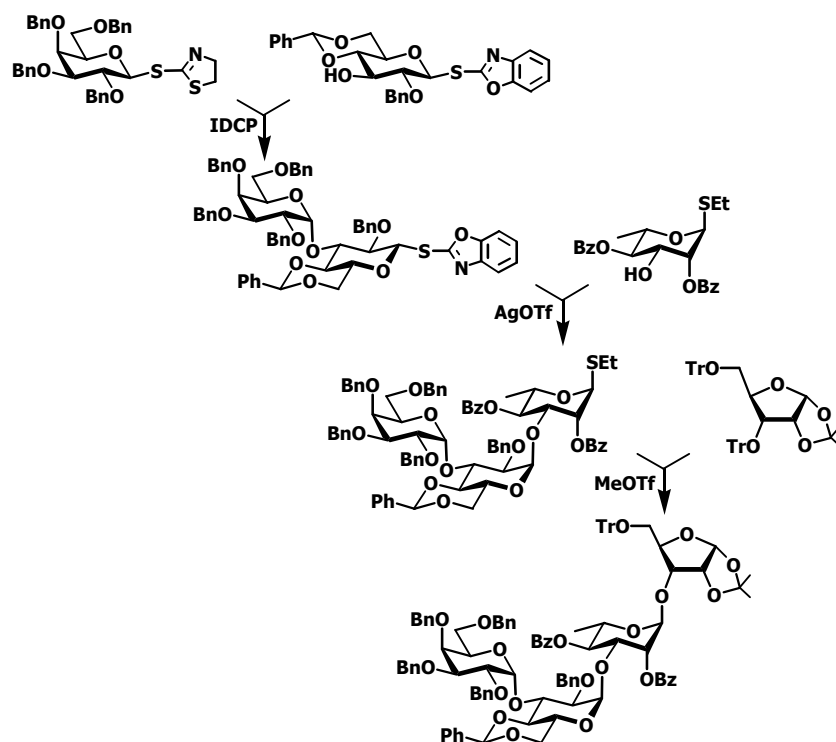
##### **Scheme 8: Synthesis of $\alpha$ -(1 $\rightarrow$ 3)-Glucan of *C. neoformans***



### Synthesis of Oligosaccharides of *S. pneumoniae* Type 6A (SPn 6A):

*S. pneumoniae* (SPn) serogroups 6A and 6B are equally important causes of respiratory tract infections, yet only 6B is included in current vaccines. However, recent observations disproved this hypothesis: it has been shown that 6B-based vaccines produce mainly 6B-specific antibodies that cross-react with 6A at a much lower rate. Therefore, the importance of including the serotype 6A carbohydrate conjugates for future generations of anti-SPn vaccines has been proposed. However, the achievement of this is challenging for a number of reasons, major of which is lower hydrolytic stability and therefore accessibility from the natural sources of serogroup 6A specific oligosaccharide. We propose the chemical synthesis of the repeating oligosaccharide unit of SPn group 6A and derivatives thereof. The synthesis of the repeating unit and its mimetics will be carried out with the use of the thioimidoyl glycosylation methodology as illustrated in Scheme 9.

**Scheme 9** Synthesis of oligosaccharides of SPn 6A



### Conclusions:

To address the current challenges associated with stereoselective glycosylation a novel class of glycosyl donors will be developed. The STaz glycosides fulfill many requirements for modern glycosyl donors: accessibility, odorless preparation, mild activation conditions, stability toward major protecting group manipulations, orthogonality toward other glycosyl donors and excellent stereoselectivity in the nucleophilic displacement reactions. Here we propose optimization of the reaction conditions, investigation of the reaction mechanism and metal ion complexation.

Upon demonstrating the versatility of the proposed synthetic methodology, our attention will focus on development of convergent strategies for rapid oligosaccharide assembly. Nearly quantitative yields have been achieved with these glycosyl donors and their remarkable reactivity will become specifically important for glycosylations on a polymer support, or via highly convergent multi step one pot oligosaccharide syntheses.

Many biologically important and therapeutically active compounds can be assembled by this methodology. We propose the synthesis of saccharides of high potential biological activity, structurally derived from  $\alpha$ -glucan of *Cryptococcus neoformans* and *Streptococcus pneumoniae* 6A. We believe that the proposed developments in both basic and applied research will lead to discovery of novel synthetic methodologies that could be applied to the synthesis of excellent carbohydrate-based therapeutics.

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