

# Regioselective One-pot Protection and Protection-glycosylation of Carbohydrates

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**Abstract:** Deciphering the roles and structure–activity relationships of carbohydrates in biological processes requires access to sugar molecules of confirmed structure and high purity. Chemical synthesis is one of the best ways to obtain such access. However, the synthesis of carbohydrates has long been impeded by two major challenges – the regioselective protection of the polyol moiety of each monosaccharide building block and the stereoselective glycosylation to produce oligosaccharides of desired length. Here, we review the development of the first regioselective protection-glycosylation and a revolutionary regioselective combinatorial one-pot protection of monosaccharides that can be used to differentiate the various hydroxy groups of monosaccharides with a vast array of orthogonal protective groups in one-pot procedures.

**Keywords:** Carbohydrate chemistry · Glycosylation · One-pot reactions · Protective groups · Regioselective etherification

## 1. Introduction

Carbohydrates play essential roles in biological processes<sup>[1]</sup> but their interactions with other biological agents at the molecular level are still unclear. In order to elucidate their physiological functions and structure–activity relationships, homogeneous and well-defined materials are indispensable. However, the isolation and purification of oligosaccharides and glycoconjugates from natural sources in adequate amount and purity are often impractical owing to their structural microheterogeneity. Chemical synthesis is therefore one of the most convenient ways to generate these biologically important compounds. The structural diversity that enables their complex functions in biological processes has retarded advances in their syntheses. The level as yet attained is still far behind the standardized acquisition protocols available for peptides and nucleotides.

For the syntheses of oligosaccharides,<sup>[2–4]</sup> the regio- and stereoselectivity as well as the eventual functional group modification of the target molecules have

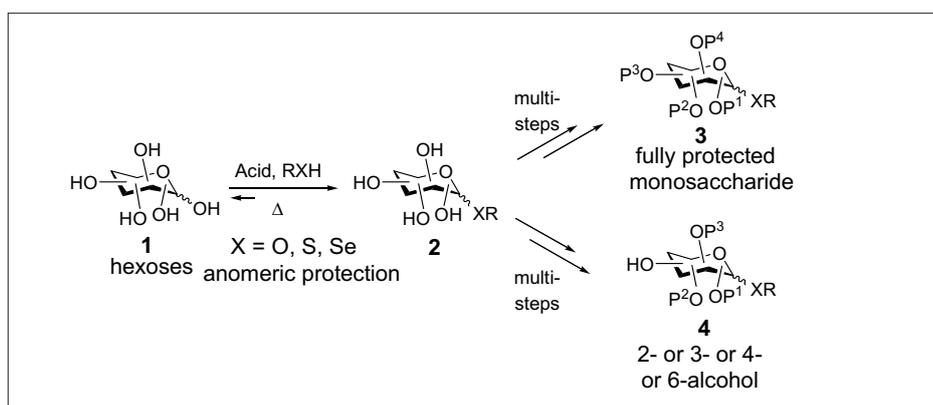


Fig. 1. Traditional method in monosaccharide protection.

to be taken into account. Therefore, the efficient preparation of various monosaccharide building blocks that comply with the target molecules and their rapid stereocontrolled assembly into long heterooligomers<sup>[5–17]</sup> are the major challenges in oligosaccharide synthesis. Typical building block synthesis still entails laborious multistep protocols and requires tedious purifications of intermediates.

Two types of monosaccharide building blocks are necessary. One has a strategically exposed free hydroxyl group to serve as nucleophilic acceptor; the other contains a labile leaving group at the anomeric carbon to act as glycosyl donor in the ensuing glycosylation. Generally, the anomeric position of hexose **1** can be selectively protected by alcohols, thiols or selenols in an acid-catalyzed reaction to afford the corresponding hexopyranoside **2** (Fig. 1).<sup>[2,18]</sup> The remaining hydroxyls, on the other hand, have nearly similar reactivities and further differentiations usually generate mixtures of regioisomers. Thus,

the transformations of tetraols **2** into fully protected monosaccharides of type **3** or alcohols of type **4** with a free hydroxyl at either C(2), C(3), C(4), or C(6) are said to be tricky and require multiple protection–deprotection sequences.

## 2. One-pot Protection–Glycosylation of Monosaccharides

In 2002, we found that the Et<sub>3</sub>SiH-reductive etherification<sup>[19]</sup> of glucose trimethylsilyl ether **5a** or **5b** with various aldehydes catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf) at –78 °C successfully distinguished C(2) and C(3) hydroxyl groups. Here, a single O(3) ether was obtained without hydrolyzing or opening the benzylidene acetal at O(4), O(6). After treatment with tetra-*n*-butylammonium fluoride (TBAF) to remove the trimethylsilyl (TMS) group at O(2), the corresponding 2-alcohols **6a1–6a5** and **6b1** were obtained in high yields, as sum-

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Table 1. TMSOTf-activated  $\text{Et}_3\text{SiH}$ -reductive O(3)-etherification of **5a** and **5b** with a series of aldehydes.

entry	S. M.	R	product	yield [%]
1	<b>5a</b>	Ph	<b>6a1</b>	94
2	<b>5a</b>	4-(MeO) $\text{C}_6\text{H}_4$	<b>6a2</b>	91
3	<b>5a</b>	3,4-(MeO) $\text{C}_6\text{H}_3$	<b>6a3</b>	87
4	<b>5a</b>	2-naphthyl	<b>6a4</b>	81
5	<b>5a</b>	4-(Cl) $\text{C}_6\text{H}_4$	<b>6a5</b>	77
6	<b>5a</b>	( <i>E</i> )-MeHC=CH	<b>6a6</b>	68 <sup>a</sup>
7	<b>5a</b>	( <i>E</i> )-PhHC=CH	<b>6a7</b>	87 <sup>a</sup>
8	<b>5b</b>	Ph	<b>6b1</b>	86

<sup>a</sup>The reaction was carried out at  $-86\text{ }^\circ\text{C}$ .

marized in Table 1.<sup>[20]</sup> The lower reactivity of the C(2) versus C(3) hydroxyl group is believed to arise from the inductive effect of the two adjacent anomeric oxygen atoms (field effect) and the steric hindrance provided by the anomeric methoxy or *p*-methylphenyl thio group (toluonyl, Tol) and the O(3)-TMS etheral moiety. The regioselective etherifications of a series of *O*-trimethylsilylated pyranosides with different aldehydes were studied and are listed in Table 2. The transformations of 4,6-*O*-isopropylidene ketal **7**,  $\alpha$ -allyl pyranoside **9** and  $\alpha,\alpha'$ -trehalose derivative **11** similarly gave the corresponding O(3)-Bn products **8**, **10** and **12** in good yields and with complete regioselectivity (Table 2, entries 1–3). The challenging O(6) benzylation of  $\beta$ -cyclodextrin<sup>[21]</sup> was achieved from trimethylsilylated  $\beta$ -cyclodextrin **13** furnishing the corresponding O(6)-Bn compound **14** in 62% yield (Table 2, entry 4). Differentiation of O(2) and O(6) hydroxyl groups of the galactopyranosides **15** and **19** using the same conditions was also effective, and gave the corresponding O(6)-etherified compounds **16–18** and **20** in excellent yields and selectivity (Table 2, entries 5–8), while standard Williamson's condition (sodium hydride and benzyl bromide) gave the corresponding O(2)-benzylated compounds as major products.<sup>[22]</sup>

Since coupling reactions of sugars often employ TMSOTf as promoter, we inferred that further O(2) glycosylation reaction to prepare 1 $\rightarrow$ 2 linked disaccharides can be continued after the reductive etherification in the same pot. This was indeed the case for perbenzylated D-galacto- (**21**), D-manno- (**22**), L-fuco- (**25**) or D-glucopyranosyl trichloroacetimidate (**26**) illustrated in Schemes 1 and 2. As ex-

pected, we successfully obtained the corresponding disaccharide  $\alpha$ -D-Gal(1 $\rightarrow$ 2)D-Glc (**23**, 61%),  $\alpha$ -D-Man(1 $\rightarrow$ 2)D-Glc (**24**, 74%),  $\alpha$ -L-Fuc(1 $\rightarrow$ 2)D-Gal (**27**, 56%),  $\alpha$ -D-Gal(1 $\rightarrow$ 2)D-Gal (**28**, 55%) and  $\alpha$ -D-Glc(1 $\rightarrow$ 2)D-Gal (**29**, 51%). To the best of our knowledge, this is the first regioselective one-pot protection-glycosylation reaction ever reported.

### 3. Regioselective One-pot Protection of Monosaccharides

Monosaccharide derivatives can be functionalized with orthogonal protective groups by successively adding various reagents in the same pot. For success of such

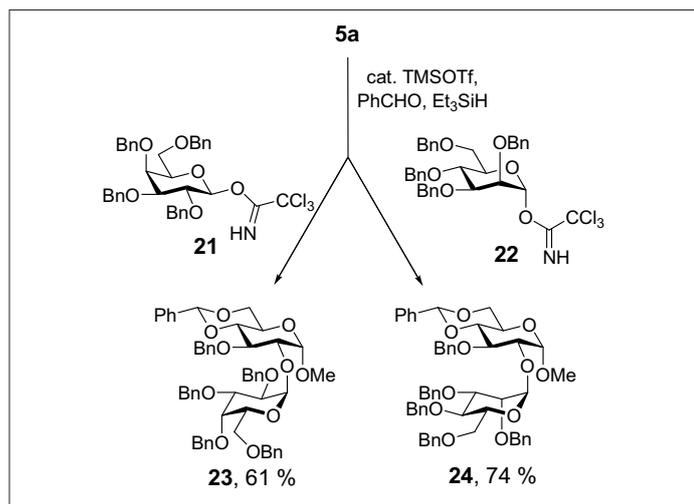
Table 2. TMSOTf-activated  $\text{Et}_3\text{SiH}$ -reductive etherification of various *O*-trimethylsilylated sugars at  $-78\text{ }^\circ\text{C}$ .

entry	Silylated sugar	Product	yield (%)
1			75
2			87
3			87
4			62
5			96
6			95
7			92
8			91

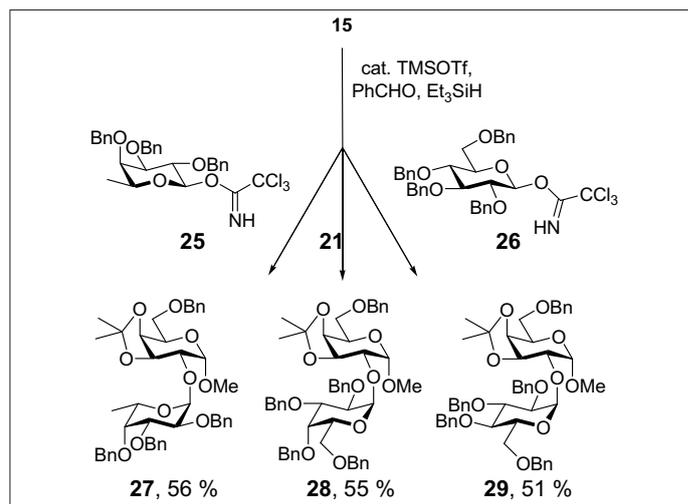
PMB = para-methoxybenzyl; 2NAP = 2-naphthylmethyl

a strategy, judicious selection of protective groups and catalysts must be realized. Benzyl-type ethers offer a wider diversity than other protective groups and can be removed under unique conditions.<sup>[23–25]</sup> Regarding the catalyst, copper triflate  $[\text{Cu}(\text{OTf})_2]$  exhibited the best reactivity among other metal triflates in reductive etherification and was therefore applied in the regioselective benzylation of the bis-TMS compound **30**.<sup>[26]</sup> Unfortunately, the reaction leads to only 72% yield for the desired O(3)-benzylated product **31** along with the diol by-product **32** (19%) (Scheme 3). Using TMSOTf suppressed this problem.

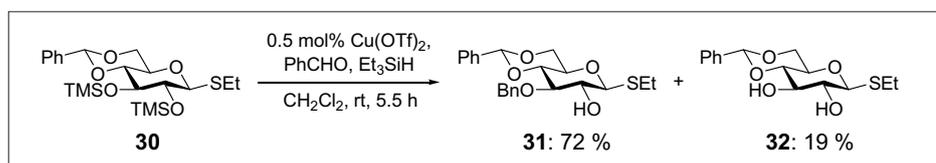
The low solubility of unprotected sugars in common organic solvents, such as



Scheme 1. One-pot regioselective protection and  $O(2)$   $\alpha$ -galactosidation and  $\alpha$ -mannosidation.



Scheme 2. One-pot regioselective  $O(2)$   $\alpha$ -glycosidations.



Scheme 3. Regioselective  $O(3)$  benzylation.

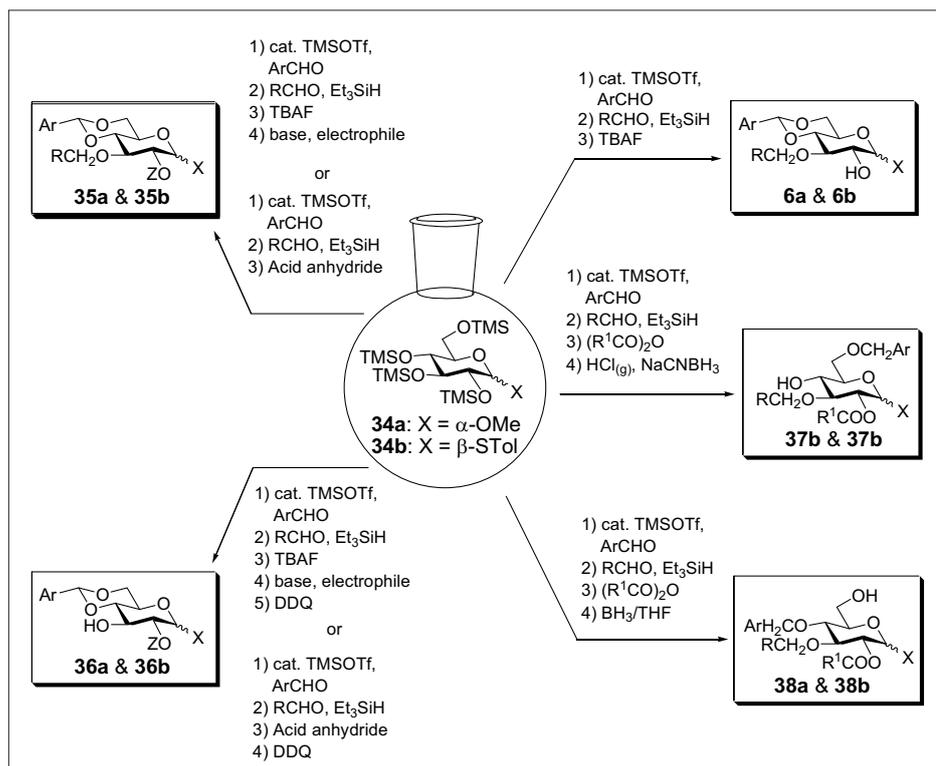
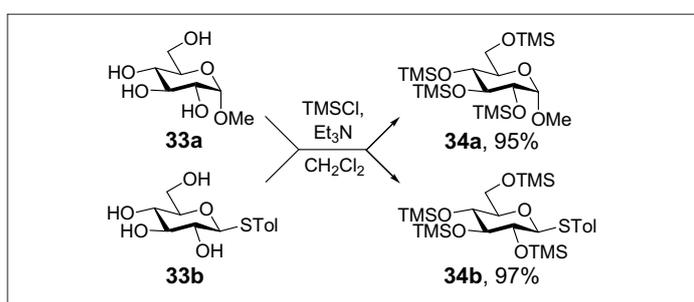


Fig. 2. Combinatorial and regioselective one-pot strategies for the protection and semi-protection of  $D$ -glucopyranosides.



Scheme 4. Persilylation of glucopyranosides.

$\text{CH}_2\text{Cl}_2$  and toluene, hampered the regioselective modification of the hydroxyl groups. Their transformation into the per- $O$ -TMS derivatives not only provided the functional groups required for the subsequent one-pot protection strategy but also greatly enhanced solubility in organic solvents. Therefore, methyl  $\alpha$ - $D$ -glucopyranoside **33a** and  $p$ -methylphenyl  $\beta$ - $D$ -thioglucopyranoside **33b** were treated with TMSCl and  $\text{Et}_3\text{N}$  to generate the corresponding per- $O$ -TMS ethers **34a** and **34b** in nearly quantitative yields, respectively (Scheme 4).<sup>[27]</sup> Our strategy, summarized in Fig. 2, is to regioselectively install combinatorial and orthogonal protective groups throughout the sugar molecule by simply adding various reagents in succession in the same pot. Thus, the per- $O$ -silylated sugars bearing an anomeric group [XR,  $X = \text{O}$  or S, where  $X = \text{O}$  can serve as the terminal end of the oligosaccharide, and  $X = \text{S}$  as the elongation unit] can be manipulated to produce either the fully protected monosaccharides **35** or the 2-, 3-, 4-, 6- alcohols **6**, **36**, **37**, **38**.<sup>[27,28]</sup> The novelty of this approach lies in the tuning of the reaction conditions to generate a single regioisomer at each stage using the same catalyst that allows sequential addition of reagents in the same vessel.

### 3.1 One-pot Synthesis of 2-Alcohols

The silylated sugars **34a** and **34b** were first treated with 1 equivalent of ArCHO in  $\text{CH}_2\text{Cl}_2$  in the presence of TMSOTf as catalyst at  $0^\circ\text{C}$  forming the corresponding arylidene acetal at  $O(4)$ ,  $O(6)$ . Then,  $\text{Et}_3\text{SiH}$  and 1 equivalent of the same or another aryl aldehyde (see Table 1) were added to the mixture cooled to  $-86^\circ\text{C}$  giving the desired  $O(3)$ -ethers. Quenching with stoichiometric amounts of TBAF removed the TMS group at  $O(2)$  giving **6a** and **6b**.

### 3.2 One-pot Synthesis of Fully Protected Glycosides

The first three steps used in the preparations of **6a** and **6b** were repeated, followed by the addition of appropriate electrophiles under basic conditions. This produced the corresponding **35a** and **35b** in good yields. Accordingly, allyl and various benzyl ethers, as well as esters were introduced at O(2). For the O(2)-etherifications, allyl bromide, benzyl bromide, 4-methoxybenzyl chloride, 2-naphthylmethyl bromide, 4-chlorobenzyl chloride and 4-bromobenzyl bromide were used as electrophiles and NaH was employed as base in DMF. For the O(2) acylations, Ac<sub>2</sub>O, chloroacetic anhydride and BzCl were used as electrophiles and Et<sub>3</sub>N as base.

Alternative O(2)-acylations could be performed under acidic conditions using TMSOTf.<sup>[29,30]</sup> Employing carboxylic anhydrides (Bz<sub>2</sub>O or Ac<sub>2</sub>O), the O(2)-acylated compounds produced under these conditions were identical to those obtained under basic conditions. However, O(3)-PMB ethers could not be made with the latter method as these ethers were cleaved by the anhydrides.

### 3.3 One-pot Synthesis of 3-Alcohols

In the preparation of the fully protected glycosides, PMB or 2-naphthylmethyl (2-NAP) could be used as temporary protective groups of O(3). Thus, the same operations as described for the fully protected glycosides were carried out using anisaldehyde or 2-naphthaldehyde as the second step aldehyde. The subsequent addition of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) provided the corresponding 3-alcohols **36a** and **36b** that bear alkyl or acyl functionalities at O(2). It is worth noting that unlike PMB, 2-NAP survived the O(2) acidic esterification conditions.

### 3.4 One-pot Synthesis of 4-Alcohols

After 4,6-arylideneation, O(3)-arylmethylation, and acidic O(2) acylation of **34a** and **34b**, regioselective O(4) opening of arylidene acetals in the same pot using HCl<sub>(g)</sub>/NaBH<sub>3</sub>CN<sup>[31]</sup> successfully delivered the 4-alcohols **37a** and **37b**, respectively in moderate to good yields. The O(2)-acyl groups remain intact in the presence of NaBH<sub>3</sub>CN and HCl; the yields, however, were only moderate because of partial hydrolysis of the benzylidene acetal brought about by unavoidable reagent moisture. Alternative conditions using Me<sub>2</sub>EtSiH/TMSOTf or Cu(OTf)<sub>2</sub> are being explored in our group.<sup>[32,33]</sup>

### 3.5 One-pot Synthesis of 6-Alcohols

After 4,6-arylideneation, O(3)-arylmethylation, and acidic O(2)-acylation of **34a** and **34b**, regioselective O(6) opening of arylidene acetals in the same vessel us-

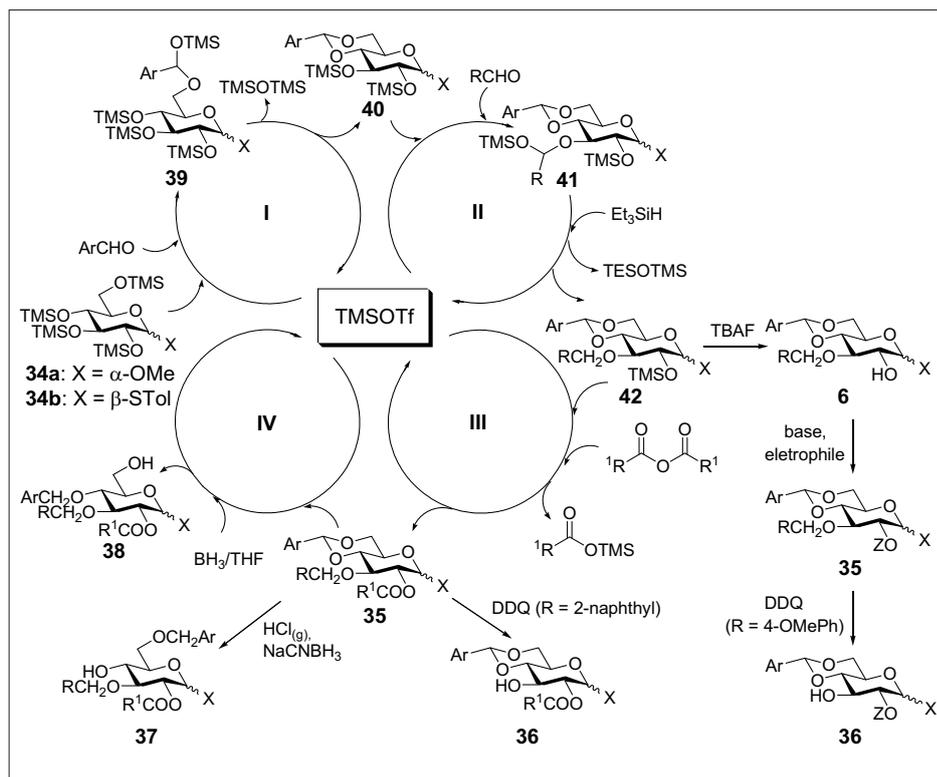


Fig. 3. Proposed mechanism showing the roles of TMSOTf in one-pot protection of D-glucopyranosides.

ing BH<sub>3</sub>/THF as reducing agent catalyzed by TMSOTf<sup>[34]</sup> at 0 °C produced 6-alcohols **38a** and **38b**, respectively.

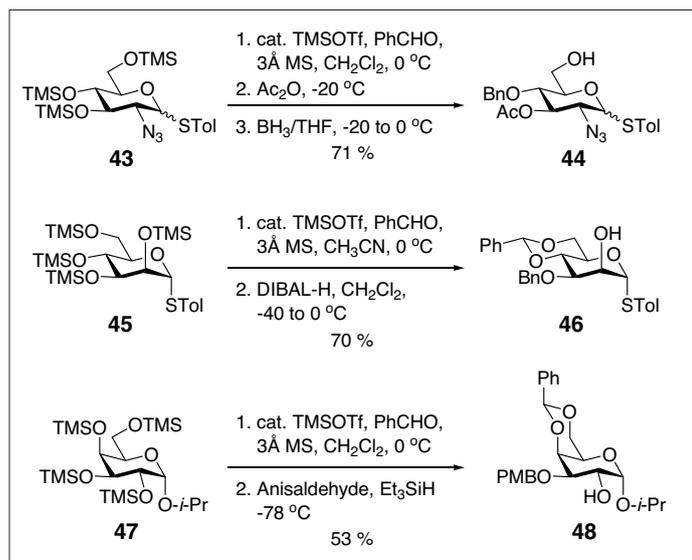
### 3.6 Mechanism

TMSOTf plays a versatile role as component of multiple catalytic cycles in our one-pot methodology (Fig. 3). In the first catalytic cycle, the reactions of **34a** and **34b** with aryl aldehyde form TMS-acetals at the most accessible C(6)-hydroxyl groups. Due to the proximity of the O(4)-TMS group intermediates, compounds **39** are transformed into the corresponding arylidene acetals **40** with the release of TMSOTMS (driving force for the reaction). TMSOTf then catalyzes the Et<sub>3</sub>SiH-reductive O(3)-etherifications of **40** with a second aryl aldehyde to furnish the corresponding O(2)-TMS ethers **41** via the intermediacy of TMS-acetals **41** in the second catalytic cycle. Compounds **42** were quenched with TBAF to provide the 2-alcohols **6**, and the exposed hydroxyl can be further modified under basic conditions to yield the fully protected compounds **35**. When R is PMB, further addition of DDQ led to the corresponding 3-alcohols **36**. In catalytic cycle III, TMSOTf concomitantly catalyzes the acylation of **42** with acid anhydride to give the fully protected esters **35**. After acidic esterification, regioselective O(3)-deprotection (when R = 2-NAP) using DDQ also afforded the related 3-alcohols **36**. Similarly, after completion of catalytic cycle

III, the O(4) opening of the arylidene by HCl<sub>(g)</sub>/NaCNBH<sub>3</sub> in the same pot gave 4-alcohols **37**. The 4,6-O-arylidene acetal can then be selectively opened at O(6) by further treatment of esters **35** with BH<sub>3</sub>/THF, in the same vessel to afford the corresponding 6-alcohol **38** (catalytic cycle IV).

### 3.7 One-pot Protection of Other Monosaccharides

Although the conditions and strategies here were optimized for D-glucopyranosides, similar approaches can also be applied for the protection of other monosaccharides. Our preliminary studies on the one-pot protections of 2-azido-2-deoxy-D-glucopyranoside, D-mannopyranoside and D-galactopyranoside are depicted in Scheme 5. The silylated 2-azido-2-deoxy-D-glucopyranoside **43** was benzylideneated using benzaldehyde and catalytic TMSOTf and acylated by acetic anhydride. The concomitant opening of the benzylidene group using BH<sub>3</sub> catalyzed by TMSOTf furnished the 6-alcohol **44** in 71% yield. The 2,3- and 4,6-di-benzylideneation of the silylated mannopyranoside **45** at 0 °C in CH<sub>3</sub>CN followed by the selective opening of the 2,3-benzylidene using DIBAL-H gave the 2-alcohol **46** in 70% yield. The 4,6-benzylideneation and regioselective 4-methoxybenzylation of persilylated isopropyl α-D-galactopyranoside **47** afforded 2-alcohol **48** in 53% yield.



Scheme 5. One-pot preparation of semi-protected pyranosides.

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#### 4. Conclusion

We have successfully developed a highly regioselective reductive benzylation of hexopyranosides and demonstrated the first examples of a one-pot sequential protection-glycosylation procedure leading to biologically active  $\alpha$ 1 $\rightarrow$ 2 linked disaccharides. These results led us to develop a highly efficient and effective methodology to prepare monosaccharide building blocks with combinatorial and orthogonal protective groups in one-pot. Per-*O*-trimethylsilylated glucosides were transformed into the fully protected monosaccharides or the 2-, 3-, 4-, 6-alcohols having benzyl-type ethers with additional options for ester or ether type protective groups at the O(2) position, a key factor for the stereocontrol of the ensuing glycosylation step. *Via* the TMSOTf-catalyzed reactions, the desired building blocks with various protective groups can be easily produced after a single workup and purification procedure. We, therefore, believe that such a method will greatly expedite and improve the overall synthetic processes of oligosaccharide synthesis.

Received: September 17, 2010

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