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Mapping Mechanisms in Glycosylation Reactions with Donor Reactivity: To Avoid Generation of Side Products

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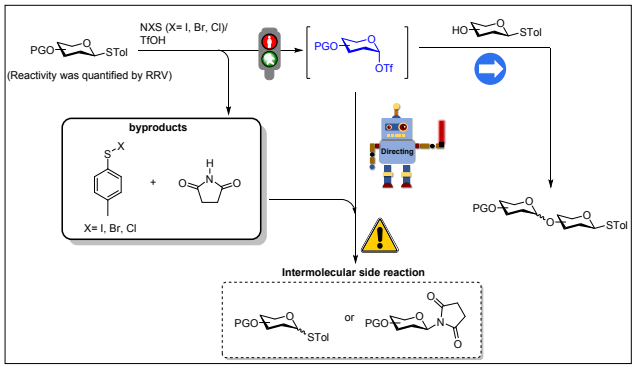


ABSTRACT

The glycosylation reaction, which is key for the studies on glycoscience, is challenging due to its complexity and intrinsic side reactions. Thioglycoside is one of the most widely used glycosyl donors in the synthesis of complex oligosaccharides. However, one of the challenges is its side reactions, which lowers its yield and limits its efficiency, thereby requiring considerable effort in the optimization process. Herein, we reported a multifaceted experimental approach that reveals the behaviors of side reactions, such as the intermolecular thioaglycon transformation and *N*-glycosyl succinimides, via the glycosyl intermediate. Our mechanistic proposal was supported by low temperature NMR studies that can further be mapped by utilizing relative reactivity values (RRVs). Accordingly, we also presented our findings to suppress the generation of side products in solving this particular problem for achieving high-yield glycosylation reactions.

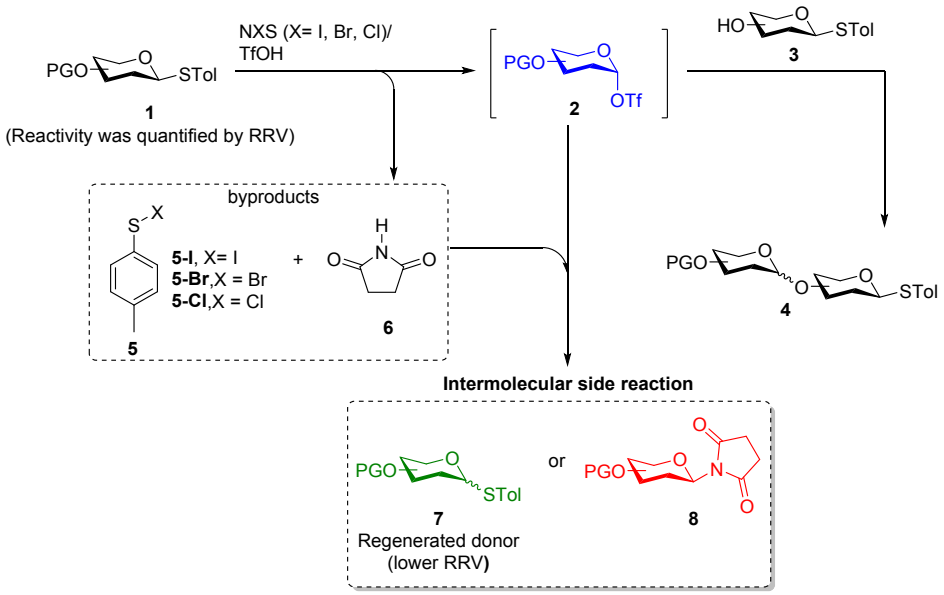
INTRODUCTION

In the past decades, chemical approaches have provided a significant advantage for accessing high-quality carbohydrate-based molecules in large quantities.[1-4] The creation and development of newly synthetic methodologies have given a direct approach to prepare and manipulate a vast variety of glycoconjugate molecules for glycoscience.[5-8] Glycans have been built sequentially by linking numerous saccharide building blocks to construct *O*-glycosidic linkages through chemical or enzymatical glycosylation reactions.[9-13] Chemical approaches show higher flexibility on the variety of sugar types and functional group modifications; however, the main challenges of chemical glycosylation between the glycosyl donor and glycosyl acceptor are to achieve high yield and stereoselectivity, which are known to be affected by numerous factors.[2-4,14-20]

Among the glycosyl donors reported, thioglycosides **1** are one of the most commonly used donors as they exhibit a high tolerance toward protecting group manipulations (Scheme 1).[21-24] Meanwhile, thioglycoside donors can be chemoselectively activated by various electrophilic promoters to give the desired product in good yield, such as *N*-halosuccinimide (NXS)/trifluoromethanesulfonic acid (TfOH), 1-benzenesulfinyl piperidine (BSP)/trifluoro-methanesulfonic anhydride (Tf_2O), and *p*-toluenesulfenyl chloride (TolSCl)/silver triflate (AgOTf).[23-25] The reactivity of thioglycosides **1** are highly associated with their protecting group patterns and conformation. Wong developed a relative reactivity values (RRVs) system to optimize the combination of building blocks for one-pot oligosaccharide synthesis.[26-30] Subsequently, Yeh, Huang and Yoshida developed a preactivation strategy that allows an iterative activation after the addition of **3** to give the product **4**.[31-40]

Despite the significant success of thioglycosides **1** documented in the literature,[41-44] several side reactions are frequently formed depending on various factors, such as the type of promotor, solvent, temperature, and particular donor/acceptor pair.[16,45-47] There are two major problems. First, intermolecular aglycon transfer **7** may be formed again from triflate intermediate **2** to significantly decrease the yield of the desired *O*-glycoside product and quench the reaction.[16,33,48-50] Second, an undesired side reaction of glycosyl triflate **2** to the corresponding stable *N*-glycosyl succinimide **8** has been noted upon the promotion using NXS/TfOH and requires a tedious separation process.[16,45-47]

Scheme 1. The suppression of side products for achieving high yield glycosylation reactions using RRV as an indicator.



Unfortunately, a suitable explanation that answers how these side reactions will happen is unclear, which can be attributed to the unclearness of the mechanism of the reaction itself. The central obstacle is that glycosyl triflate **2**, which serves as the main intermediate in TfOH-mediated thioglycoside reactions, is highly unstable to analyze.[46,51,52] By-products **5** and **6**, which are generated through the stoichiometric amount of NXS **5** required for anomeric leaving group activation,[25,53-55] induce the formation of side products **7**-**8** and lower the yield of desired **4**. Therefore, the lack of precise mechanistic study leads to indeterminacy, as the reaction yields are drastically fluctuant, even under slight changes of the protecting group, promotor or temperature. The unique glycosylation reactions must always be optimized individually, and the outcome is usually unpredictable.

Capitalizing on this viewpoint, our laboratory discovered that the RRVs of thioglycosides **1** provide a new angle to correlate the stereoselectivities and intermediate changes.[42] Herein, we further reported systematically mechanistic studies that connect the glycosyl triflate intermediate **2** to side products **7**-**8**. The detailed behavior of the side products can be successfully elucidated, as indicated by the RRVs of thioglycosides **1**. The generation of thioaglycon-transferred thioglycoside **7** is characterized through the intermolecular process between glycosyl triflate **2** and tolylsulfenyl halide (TolSX, **5)** in each NXS/TfOH system. The RRV platform can be

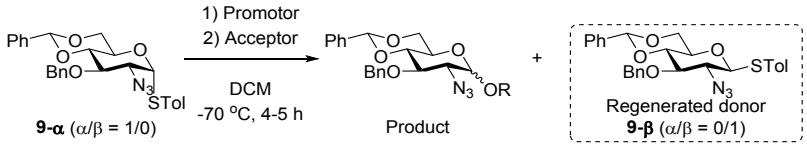
utilized for data organizations by a statistical approach. Additionally, it helps with the establishment of a general protocol to eliminate the formation of side products on numerous building blocks of thioglycosides to achieve a higher yielding glycosylation reaction of the desired product **4**.

RESULTS AND DISCUSSION

Since the presence of glucosamine derivative is essential to the activity of many natural products,[50,56] our work began by controlling preactivation-based glycosylation on thioglycoside donor **9-α** (α/β = 1/0) on the promotion of NIS/TfOH system.[40] We pre-mixed donor **9-α** with NIS (1.0 equiv.) and TfOH (1.0 equiv.) in DCM at -70 °C for 10 min (Table 1). The acceptor (MeOH, **10**) was then introduced in the next step and further stirred for 4-5 hours (Entry 1). However, the glycosylation reaction only furnished methyl glycoside **13** with a 31% yield and an α/β ratio of 1/9. Moreover, 44% of donor **9-β** (α/β = 0/1) was recovered with exclusive β-form even after a complete consumption of the donor **9-α** during preactivation. Similar situations also occurred in other activation systems, such as NBS/TfOH, NCS/TfOH, and TolSCl/AgOTf promotor systems, as the related glycosylation still recovered large amount of β-donor **9-β** in 30-40% yield (Entries 2-4). Even after excess amounts (2.0 equiv.) of TolSCl/AgOTf promotor was introduced, the regeneration of **9-β** increased to 42% (Entry 5). We also noticed that adding other acceptors still gave similar results, such as 6-OH glucoside **11** and 4-OH glucoside **12**. Apart from the corresponding product **14-15**, a considerable amount of **9-β** was observed in 43-45% yield.

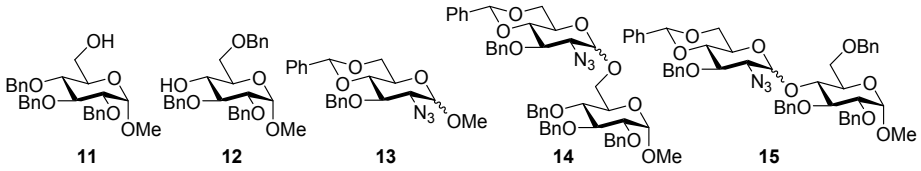
We initially assumed that generation of **9-β** was derived from thioglycoside epimerization or during aglycon transfer of thioglycosides.[16,33,48-50] However, we found that in a preactivation manner after complete consumption of the donor **9-α** the regeneration of **9-β** could still be observed after the introduction of the acceptor. The regeneration of **9-β** was highly associated with the presence of acceptor, because before the addition of acceptor only the corresponding glycosyl triflate could be observed in NMR. Therefore, it showed that generation of **9-β** underwent a different pathway rather than a simple thioglycoside epimerization or aglycon transfer of thioglycosides. This result raised our interest in understanding how the thioglycoside donor is regenerated and how this side product can be eliminated so as to achieve a high-yield glycosylation reaction.

Table 1. Preactivation-based glycosylation on donor 9-α.



Entry	Promotor (equiv.)	Acceptor	Product, yield[a](α/β)[b]	**9-β**, yield[a]
1	NIS (1), TfOH (1)	MeOH **10**	**13**, 31% (1/9.0)	44%
2	NBS (1), TfOH (1)	MeOH **10**	**13**, 33% (1/11)	40%
3	NCS (1), TfOH (1)	MeOH **10**	**13**, 34% (1/8.0)	36%
4	TolSCl (1), AgOTf (1)	MeOH **10**	**13**, 44% (1/8.1)	30%
5	TolSCl (2), AgOTf (2)	MeOH **10**	**13**, 33% (1/8.0)	42%
6	TolSCl (2), AgOTf (2)	6-OH Glc **11**	**14**, 30% (1/6.7)	45%
7	TolSCl (2), AgOTf (2)	4-OH Glc **12**	**15**, 30% (1/3.4)	43%

[a]Isolated yield. [b]Determined by HPLC



In light of donor regeneration, we further investigated the glycosylation reactions on more donors including **16-α** (α/β = 1/0), **17-α** (α/β = 1/0), **18-β** (α/β = 0/1), **19-β** (α/β = 0/1), **9-α** (α/β = 1/0) and **9-β** (α/β = 0/1) (Table 2). The main purpose of this study was to clarify the relationship between donor reactivity and the amount of regenerated donors and other side products. Therefore, we applied RRV of donor as the general parameter to outline the amount of regenerated donor produced on different donor building blocks. To simplify the analysis in this investigation, each donor was promoted under NIS/TfOH condition in DCM at -70 °C, and methyl alcohol (MeOH, **10**) was introduced as the modeling acceptor.

As our expectation, apart from the desired *O*-methyl glycosides **13, 20**-**23**, substantial amounts of donors **9-β**, **17-α**, **18-α**, **19-α** were regenerated back in 1 hour. The amount of regenerated donor was gradually eliminated as the reactivity of the donor kept on increasing (increasing RRVs of donors). It indicated that armed donor under the reaction conditions transfers faster into corresponding product than disarmed donor. Therefore, the side effect of donor regeneration was remarkably reduced. For example,

2-deoxy donors, **16-α** and **17-α** (Entries 2-3), which had the highest RRV of 1×10^6 and 5×10^5 respectively gave a minimal amount of regenerated donor **17-β** (0-8%) and the isolated yield of its corresponding product **20**-**21** was up to 83-84%. The activation of thioglucosides, **18-β** and **19-β** with a moderated RRV of 17000 and 2656 respectively turned out the desired *O*-methyl glucoside **22**-**23** in a moderate yield (55-64%) due to the generation of higher amount of regenerated donor **18-α** and **19-α** (25-36%) (Entries 3-4). With regard to 2-azido-2-deoxy glycosides, **9-α** and **9-β** (Entries 5-6), abundance of β-donor, **9-β** was recovered with a 42-44% yield and the lowest glycosylation yield of **13** was given with a 31-34% yield.

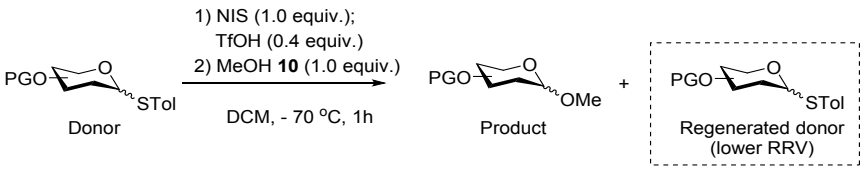
The anomeric configuration of the regenerated donor is associated with the RRV of both anomers. On 4,6-*O*-benzylidene donors (Entries 5-6), even when β-donor **9-β** (α/β = 0/1) or α-donor **9-α** (α/β = 1/0) could be activated individually in the beginning, a consistent result of β-counterpart **9-β** (α/β = 0/1) was regenerated. The RRV clearly showed that **9-β** (RRV = 5.4) was more stable than α-counterpart **9-α** (RRV = 314), suggesting that a regenerated donor towards β-anomer **9-β** was a relatively stable configuration.[57] The influence of benzylidene acetals on anomeric equilibria has been reported in the literature, but only of *O*-glycosides.[58] For the stability of thioglycosides, according to the RRV platform, the β-counterpart **9-β** is indeed more stable than its α-isomer **9-α**.

To further clarify the anomeric reactivity, Bols et al. have reported that β-anomer of **9-β** is more stable than the **9-α** in such conformationally fixed systems.[57] Upon activation of thioglycoside, the sulfonium ion was generated *in situ* via an intermolecular iodination reaction from iodonium ion (NIS). Since α-sulfonium ion performed an axial/pseudoaxial configuration at *C*1, the lone pairs of electrons present on *O*5 can easily participate to the *C*1 for the activation. The oxocarbenium ion was then formed together with TolSI via E1 elimination reaction for forming the oxocarbenium ion.[57] However, the activation of β-sulfonium ion was very limited. The β-sulfonium ion in turn showed an equatorial/pseudoequatorial configuration, which is not favorable for the E1 elimination.[57]

The story was completely different with regard to per-*O*-benzylated donor (conformationally flexible system) such as galactoside **18-β** (Entry 3). The anomeric configuration of the regenerated donor would turn into the thermodynamically favored α-form [**18-α** (α/β = 1/0), RRV = 3646) rather than the β-anomer [**18-β** (α/β = 0/1),

RRV = 17000). Similarly, α-thioglucoside [**19-α** (α/β = 1/0), RRV = 727] was more stable than its β-counterpart [**19-β** (α/β = 0/1), RRV = 2656] (Entry 4). This is in agreement with the recent work conducted by Zhu, Demchenko, Boons, and Jensen.[59-62] Their competitive study showed that α-thioglycoside demonstrates a noticeably low reactivity compared to β-thioglycoside on full benzylated donors. The electron density of *O*5 increased due to the electron donating effect of benzyl (Bn) functionality and it therefore enhances the nucleophilicity of thiotolyl group (-STol) of equatorial anomer (β-glycoside) to interact with the electrophilic promotor under NIS/TfOH condition.

Table 2. Organization of thioglycoside regeneration using RRV



Entry	Donor, (RRV)	Product, yield[a]	Regenerated donor, (RRV), yield[a]
1	α/β = 1/0 **16-α**, (1×10^6)	α/β = 1/1.5 **20**, 83%	-[b]
2	α/β = 1/0 **17-α**, (5×10^5)	α/β = 1/1.5 **21**, 84%	α/β = 0/1 **17-β**, (3×10^5), 8%
3	α/β = 0/1 **18-β**, (17000)	α/β = 1/2.0 **22**, 64%	α/β = 1/0 **18-α**, (3646), 25%
4	α/β = 0/1 **19-β**, (2656)	α/β = 1/3.0 **23**, 55%	α/β = 1/0 **19-α**, (727), 36%
5	α/β = 1/0 **9-α**, (314)	α/β = 1/5.7 **13**, 34%	α/β = 0/1 **9-β**, (5.4), 42%
6	α/β = 0/1 **9-β**, (5.4)	α/β = 1/5.7 **13**, 31%	α/β = 0/1 **9-β**, (5.4), 44%

Increasing yield

Increasing regenerated donor

[a]Isolated yield. [b]Not found.

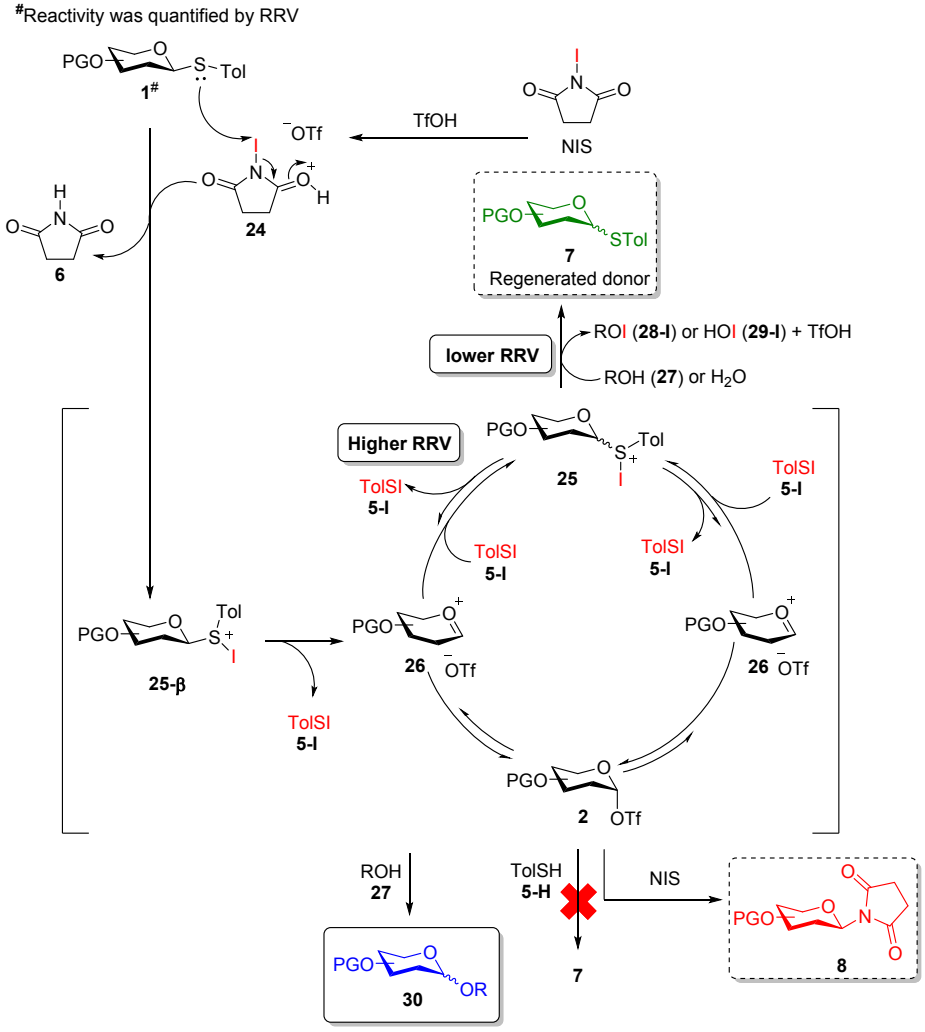
Considering the reaction mechanism, we proposed that the formation of regenerated donor **7** substantially undergoes an intermolecular process (Scheme 2). The NIS initiated thioglycoside donor **1** under TfOH catalyzation and then furnished the **β**-sulfonium ion **25-β**. The α-triflate intermediate **2** was then generated *in situ* as the most plausible intermediate with the departure of the TolSI **5-I** by-product.[46,51,52] Both glycosyl triflate **2** and oxocarbenium ion **26** determined the glycosidic bond formation with acceptor [ROH, (**27**)] to produce glycoside **30** through the nucleophilic substitution.[18,63-67]

In our present research, we also observed the regenerated donor **7** in the reaction mixture. Since glycosyl triflate **2** and the corresponding TolSI **5-I** byproduct accumulated using the NIS/TfOH promoter system under pre-mix conditions, we proposed that **7** was derived from glycosyl sulfonium ion **25**. After introducing protic reagent (either water or acceptor **27**), the protic reagent terminated **25** and expelled either hypoiodate [RO-I, (**28-I**)] or hypoiodous acid [HO-I (**29-I**)] to produce the thioaglycon-transferred side-product **7** (see Scheme 3 and Scheme 4). It was likely that the protic reagent (water or the acceptor **27**) reacted with TolSI **5-I** to give either hypoiodate [RO-I, (**28-I**)] or hypoiodous acid [HO-I (**29-I**)] and produced *p*-thiocresol [TolSH, (**5-H**)], which was the nucleophile; nevertheless, we found that **5-H** could not react with glycosyl triflate **2**. Therefore, the participation of **5-H** as the source of side product **7** was excluded (see Scheme 5).

Since the anomeric configuration of **7** was independent from that of **1**, an intermolecular reaction taking place between oxocarbenium ion **26** and TolSI **5-I** was presumably suggested. Interestingly, the regenerated **7** gave exclusive anomeric selectivities, and a more stable configuration of **7** (lower RRV) was eventually observed (as summarized in Table 1 and Table 2). However, despite the notable differences in the RRVs between the α- and β-thioglycoside, the complete selectivity far exceeded the ratio that would be expected from the thermodynamic control. Since the RRVs of the regenerated **7-α** and **7-β** could defer for an order, a plausible explanation was that, after the reaction between oxocarbenium ion **26** and TolSI **5-I**, upon preactivation condition, the reformed **25** that gave **7** anomer of higher RRV may equilibrate back to **26** faster; in contrast, the reformed **25** that resulted in **7** anomer of lower RRV equilibrated more slowly, of which the sulfonium salt was further quenched by protic reagent (water or the acceptor **27**) to give the regenerated **7** of lower RRV (as shown in Figure 1 and

Table 3). Therefore, the regenerated **7** performed an exclusive anomeric configuration in the form of lower RRV. The formation of the side product *N*-glycosyl succinimide **8** was also confirmed as a result of the side reaction of NIS and **2** (see Table 4 and Table 5).

Scheme 2. Proposed mechanism for the generation of side products on the promotion of NIS/TfOH.

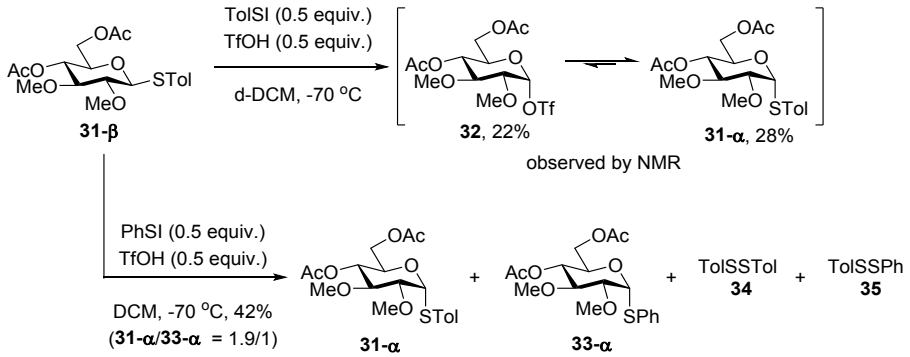


To further substantiate our results associated with the intermolecular reaction between glycosylation triflate and TolSI by-product, we further conducted cross-over experiments on the promotion of the tolylsulfenyl iodide (TolSI)/TfOH condition (Scheme 3).[68] The experimental evidence was supported by low-temperature nuclear magnetic resonance (NMR) experiments at -70 °C. When 0.5 equivalent of TolSI/TfOH was introduced in the reaction as the combined-promotor, our NMR spectrum showed that 50% of donor **31-β** still remained and a mixture of glycosyl triflate **32** and

thioaglycon-transferred thioglycoside **31-α** was detected. The NMR yield of triflate **32** was determined to be 22%, while **31-α** was 28%. The anomeric proton of glycosyl triflate **32** was detected at 6.18 ppm in ^{1}H NMR and the corresponding ^{13}C signal appeared at 104.8 ppm in ^{13}C NMR as demonstrated in previous literature.[42] Subsequently, a clear conversion from **32** to **31-α** was discovered when the temperature was warmed to 0 °C. This information suggested that TolSI would trap the triflate intermediate **32** to yield thioaglycon-transferred thioglycoside **31-α** under an intermolecular process (see spectra in supporting information). This result was similar to the works by Kartha and Field, in which intermolecular thioaglycon-transformation was observed as well on the activation of methyl thioglycosides via the participation of methylsulfenyl iodide.[68]

Moreover, using an alternative promotor of phenylsulfenyl iodide (PhSI)/TfOH turned out a mixture of thiotolyl-transferred thioglycoside **31-α** and thiophenyl-transferred thioglycoside **33-α** at a ratio of 1.9/1. The generation of **33-α** again indicated that reaction underwent a rapid transformation between glycosyl triflate intermediate and PhSI. We also observed the corresponding by-products tolyl disulfide (TolSSTol, **34**) and phenyl tolyl disulfide (TolSSPh, **35**), which indicated a spontaneous dimerization and disulfide exchange from PhSI and TolSI (see spectra in supporting information).[69]

Scheme 3. Cross-over experiments in support of intermolecular transformation.



A detailed investigation was further studied between glycosyl triflate **36D** and TolSCl **5-Cl** to precisely study the thioaglycon-transformation (Figure 1). The deuterium-labeled functionalizations were designed to remove peaks overlapping from the benzylic protons (4.4-5.0 ppm) in the ^{1}H NMR spectra to simplify the analysis. We

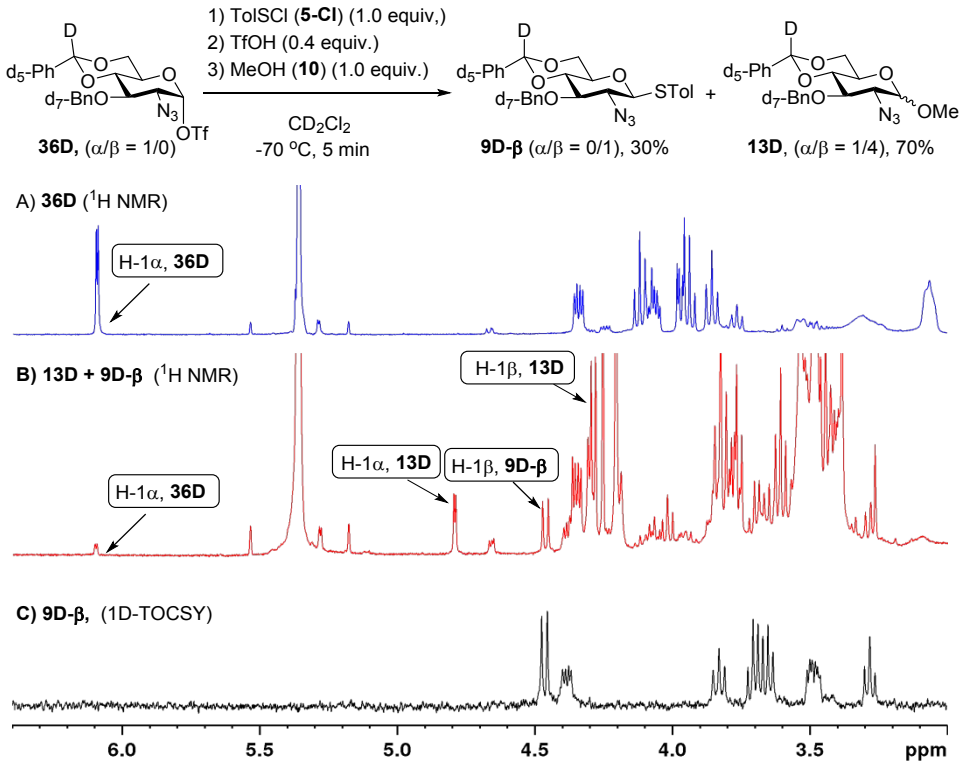
initially prepared pure α-glycosyl triflate **36D** (α/β = 1/0) on the promotion of BSP/Tf_2O at -70 °C as reported in the literatures (Figure 1A).[18,66,70,71] The observed chemical shift (δ) of the anomeric proton (H-1) signal was 6.05 ppm and the coupling constant value of 3.5 Hz suggested it to be an α-anomer. In ^{19}F NMR spectra, the characteristic peak of covalent anomeric triflates was -75.7 ppm and these results are in agreement with the NMR analysis observed in previous literature.[72]

Accompanied by the additional reagents of TolSCl **5-Cl** (1.0 equiv.) and TfOH (0.4 equiv.), as well as the corresponding acceptor (MeOH, **10**), a substantial amount of thioaglycon-transferred thioglycoside **9D-β** (α/β = 0/1) was regenerated with a decreasing amount of glycosyl triflate **36D** (Figure 1B). The NMR yield of thioaglycon-transferred thioglycoside **9D-β** was 30% in 5 mins, while the corresponding yield for methyl glycoside product **13D** (α/β = 1/3) was determined to be 69%. To specifically look for the characteristic peak on the sugar ring, a selective 1D-TOCSY spectrum of the peak at 4.46 ppm was observed (Figure 1C). The anomeric proton atom of **9D-β** was observed at 4.46 ppm with a $^3J_{H-H}$ value of 10.2 Hz, which was determined to be the β-anomer. The anomeric configuration of the transferred donor was consistent with observations of the NIS/TfOH activation system (Table 1). In addition, the identity of the thioaglycon-transferred thioglycoside **9D-β** could also be isolated and further confirmed by high-resolution mass spectrometry (HRMS). We noticed that the quantity of **9D-β** highly depended on the amount of TolSCl **5-Cl**. However, without adding the acceptor, glycosyl triflate **36D** and TolSCl **5-Cl** could not interact, thereby suggesting that acceptor provided an essential driving force for this intermolecular transformation between glycosyl triflate **36D** and TolSCl **5-Cl**.

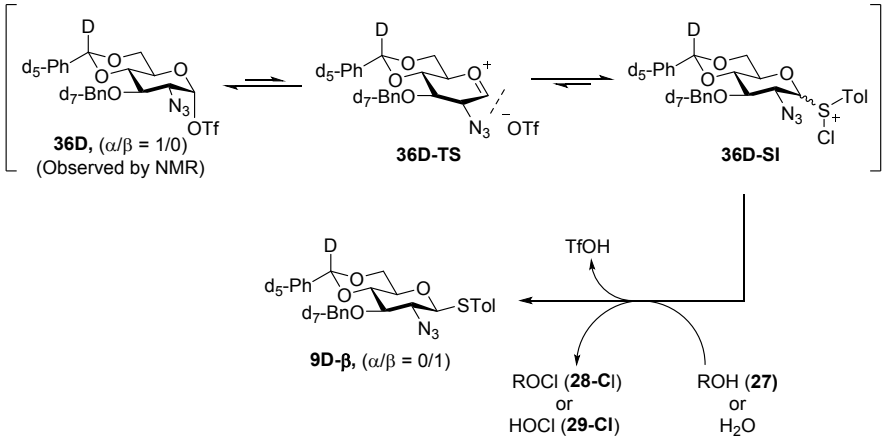
After introducing the acceptor (MeOH **10**), the glycosyl triflate **36D** was immediately converted into **9D-β**, which was accompanied with the decrease of TolSCl **5-Cl** at -70 °C in 5 mins. On the contrary, without adding the acceptor, the combination of glycosyl triflate, TfOH (0.2-1.0 equiv.), and excess TolSCl **5-Cl** (1.0-3.0 equiv.) could not result in **9D-β**, even after stirring for an additional 10-12 h and warming up to 0 °C. Therefore, it showed that an equimolar amount of acceptor was essential to promote this aglycon transformation under acidic conditions, as it quenched the glycosyl sulfonium ion to result in regenerated **9D-β**. Without adding the acceptor, the equilibration loop among glycosyl triflate **36D**, oxocarbenium ion **36D-TS**, and sulfonium ion **36D-SI** would continue (Scheme 4); therefore, only **36D** could be

observed in the NMR spectroscopy shown in Figure 1A, and the immediate formation of **9D-β** after the addition of MeOH implied that the regeneration of **9D-β** went through the sulfonium ion intermediate **36D-SI**.

Figure 1. Overall mechanistic studies of the intermolecular thioglycon transformation between triflate 36D and TolSCl: (A) ^{1}H-NMR of glycosyl triflate 36D on the promotion of BSP/Tf_2O system; (B) ^{1}H-NMR of thioaglycon-transferred thioglycoside 9D-β in the presence of TolSCl, TfOH, MeOH; (c) 1D-TOCSY spectrum of thioaglycon-transferred thioglycoside 9D-β.



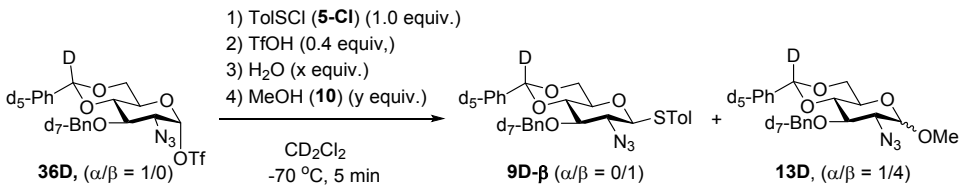
Scheme 4. The plausible mechanism of the aglycon transfer reaction on 9D-β.



To further confirm the role of protic reagents (acceptor or water), we extended our scope of the study to discuss the effect of solvent moisture (Table 3, Figure S1). Herein, the solvent moisture of DCM was dried to 5 ppm and different amount of H_2O was introduced in the reaction to precisely understand the effects of water. Indeed, we discovered that water did have an influence on the formation of thioaglycon-transferred thioglycoside **9D-β**, while the corresponding hydrolyzed side product could not be detected via low temperature NMR at -70 °C (Entries 1-5). As the quantity of **9D-β** accumulated, the yield of corresponding product **13D** gradually decreased. For acceptor dosage effect, we originally thought that generation of **9D-β** could be simply suppressed by using excess of acceptor, as high amount of acceptor may trap activated donor in order to improve the yield. Nonetheless, we noticed that the amount of **9D-β** rather increased with increasing acceptor dosage (Entries 6-11). Based on Table 3, all the above-mentioned reagents [TolSCl **5-Cl**, TfOH, protic reagent (MeOH **10**, H_2O)] were indispensable for the formation of **9D-β**. Without adding additional TfOH, the yield of **9D-β** was reduced to 13% (Entry 12).

With these experiments, we proposed that glycosyl sulfonium ion **36D-SI** could be the source of the regenerated donor **9D-β** (Scheme 4), which was formed *in situ* by mixing glycosyl triflate **36D** and TolSCl **5-Cl**. Next, protic reagents (H_2O or acceptor **27**) quenched the glycosyl sulfonium ion **36D-SI** by attacking the chloronium ion (Cl^+) and expelled the hypoiodate [RO-Cl (**28-Cl**)] or hypochlorous acid [HO-Cl (**29-Cl**)], resulting in a large quantity of thioaglycon-transferred side-product **9D-β**.

Table 3. The mechanism study to investigate the proton effect (H_2O, MeOH) in the formation of regenerated thioglycoside.



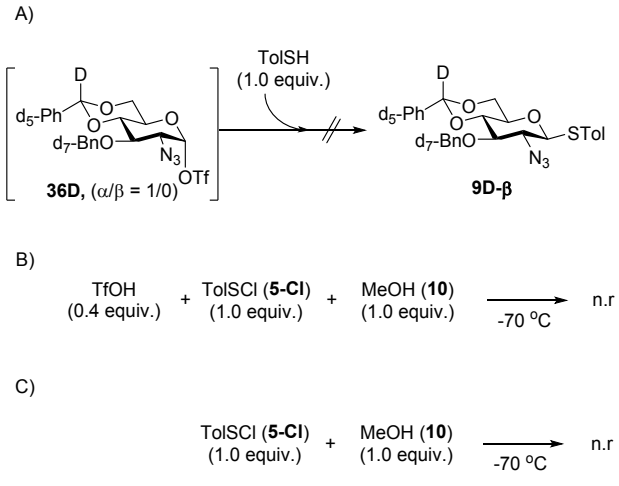
Entry	H_2O (x equiv.)	MeOH (**10**) (y equiv.)	**9D-β**, yield[a]	**13D**, yield[a]
1	-[b]	1.0	30%	70%
2	1.0	1.0	35%	51%
3	1.5	1.0	42%	45%
4	2.0	1.0	46%	44%
5	2.5	1.0	47%	45%

6	-[b]	0.5	10%	34%
7	-[b]	0.7	16%	45%
8	-[b]	1.0	30%	70%
9	-[b]	2.0	44%	41%
10	-[b]	3.0	44%	47%
11	-[b]	3.0	44%	47%
12[c]	2.5	1.0	13%	80%

[a]The yield was determined by NMR. [b]The solvent moisture was 5 ppm. [c]Without adding additional TfOH.

TolSH is obviously a possible nucleophile that leads to the occurrence of thioaglycon-transferred thioglycoside **9D-β**. However, in our modeling reaction (Scheme 5A), the addition of TolSH (1.0 equiv.) to glycosyl triflate **36D** did not transform **36D** into **9D-β**. Moreover, the combination of TolSCl **5-Cl** (1.0 equiv.) and MeOH **10** (1.0 equiv.) did not result in the corresponding TolSH at -70 °C, regardless if the TfOH (0.4 equiv.) was introduced or not (Scheme 5B, Scheme 5C). These results suggested that TolSH was not involved in aglycon transformation.

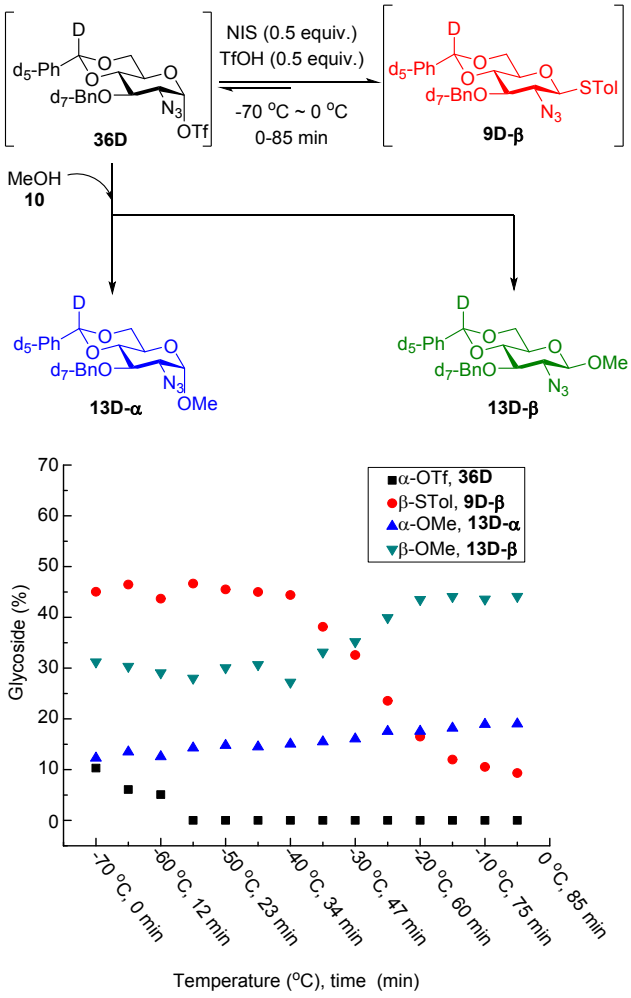
Scheme 5. The mechanism study to investigate aglycon transformation.



Having observed the thioaglycon-transformation, we attempted to determine whether the intermediate conversion could influence the stereoselectivity (Figure 2, Figure S2). The reaction was continuously monitored in the same reaction batch using variable-temperature (VT) NMR experiments from -70 °C to 0 °C in 85 mins. We noticed that the glycosyl triflate **36D** (black square) immediately converted into a mixture of methyl glycoside **13D-α** (blue triangle) and **13D-β** (green triangle) from -70 °C to -50 °C. However, the thioaglycon-transferred thioglycoside **9D-β** (red circle) was gradually consumed with the major increase of β-methyl glycoside product **13D-β** from

-40 °C to 0 °C after the combination of NIS (0.5 equiv.) and TfOH (0.5 equiv.) was added. This information indicated that both glycosyl triflate **36D** and thioaglycon-transferred thioglycoside **9D-β** were involved in controlling the rate of the glycosylation reactions. Once the thioaglycon-transferred thioglycoside **9D-β** was regenerated, **9D-β** would be further activated into α-triflate **36D**, which contributed selective β-glycosylation in the presence of a strong nucleophile such as MeOH.[63-65,71,73]

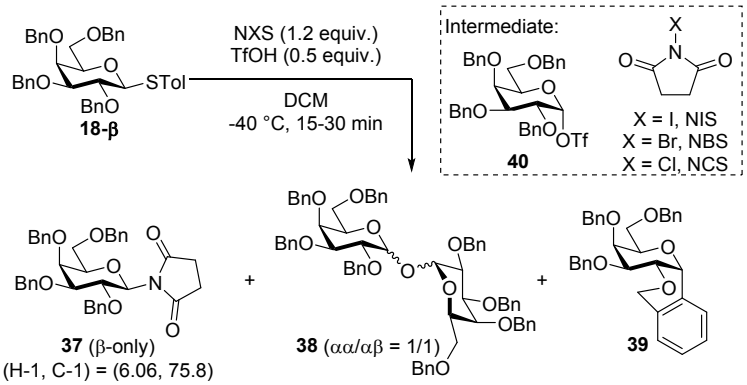
Figure 2. Transformation profile of glycosyl triflate and thioaglycon-transferred thioglycoside from -70 °C to 0 °C, in which all of the data points were continuously obtained in the same reaction batch.



Next, this study clarified the side reaction of *N*-glycosyl succinimide, as it is usually observed in NXS/TfOH-activated thioglycoside systems (Table 4).[45] Followed by the preactivation manner, we investigated the glycosylation reaction using **18-β** as

the donor in the absence of an acceptor at -40 °C on the promotion of NIS/TfOH, NBS/TfOH, and NCS/TfOH systems individually. The galactosyl donor **18-β** was preactivated completely in 15-30 mins to afford galactosyl succinimide **37** with a yield of 24-52%, digalactoside **38** with a yield of 11-23%, and cyclic galactoside **39** with a yield of 18-21%. During preactivation in the absence of acceptor, the galactosyl triflate intermediate **40**, which was generated in *in situ*, gradually decomposed without molecular sieve due to the detrimental effect of water from the organic solvent, and side products such as digalactoside **38** and cyclic galactoside **39** were further produced. The trehalose-type digalactoside **38** originated from the 1,1-glycosylayion due to the reaction occurring between hydrolyzed glycoside and remaining galactosyl donor **18-β**. The identity of **37** was confirmed by NMR. The observed chemical shift (δ) of the anomeric proton signal was 6.06 ppm, and corresponding anomeric ^{13}C signal was observed at 75.8 ppm as reported.[74] The coupling constant of anomeric proton (H-1) was determined to be 7.6 Hz, which referred to the β-anomer (see spectra in supporting information). We noticed that a substantial amount of *N*-galactosyl succinimide **37** (52%) was observed, especially in the NCS/TfOH system (Entry 1). However, the amount of **37** was gradually decreased to 24%-32% in the case of NBS/TfOH and NIS/TfOH, respectively (Entries 2-3). This result addressed that using a NIS/TfOH promotor system may slightly reduce the generation of *N*-galactosyl succinimide **37** compared to NBS/TfOH and NCS/TfOH systems.

Table 4. Study of *N*-galactosyl succinimide on the promotion of NXS/TfOH.



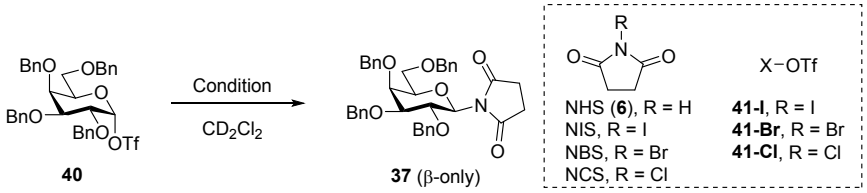
Entry	NXS	**37**, yield[a]	**38**, yield[a]	**39**, yield[a]
1	NCS	52%	-	-
2	NBS	32%	11%	21%
3	NIS	24%	23%	18%

[a]Isolated yield.

Since *N*-galactosyl succinimide **37** was obtained during preactivation, we proposed that glycosyl triflate **40**, which was *in situ* generated in the reaction, was the key intermediate (Table 5). To this point, we initially prepared exclusive galactosyl triflate **40** through the known procedure on the BSP/Tf_2O condition.[18,66,75] Subsequently, a 1.0 equivalent of NHS **6** (Entry 1) and 1.0 equivalent of NIS (Entry 2) were then introduced into the reaction individually. Interestingly, our result showed that galactosyl succinimide **37** was only observed in the presence of NIS (Entry 2), instead of NHS **6**. This result indicated that NHS **6** was not sufficiently reactive to displace the triflate of α-glycosyl triflate **40**. Consistent results of the intermediate change were also observed in other halide-containing promotor systems such as NBS (32%, Entry 3) and NCS (22%, Entry 4). Since succinimide **37** formation occurred in acidic condition, it was also reasonable that its transformation stopped when 1.0 equiv. of acid scavenger such as triethylamine (TEA) was added (Entries 5-7). Moreover, the condensation between glycosyl triflate **40** and NHS **6** was unsuccessful, even when iodine (I_2) or H_2O (Entries 8-9) were involved, confirming that *N*-halosuccinimide [NXS (X = I, Br, Cl)] was essential for the transformation from glycosyl triflate **40** into *N*-galactosyl succinimide **33**.

We proposed that the iodonium ions (I^+) from NIS could increase the reactivity of glycosyl triflate **40** with the release of iodine monotriflate (I-OTf, **41-I**) under acidic conditions.[76] Therefore, *N*-galactosyl succinimide **33** was afforded in 21%, and it was found that employing more NIS increased the amount of succinimide **37**. This agreed with the work of Madsen et al., in which introducing NIS significantly promoted glycosyl bromide by expelling iodine monobromide (I-Br) to result in a higher yield and faster conversion.[76] On the other hand, Cienfuegos et al. applied DFT calculations to investigate the mechanism of NIS-mediated nucleophilic additions to glycal, in which the reaction might not begin with the N-I bond cleavage due to a highly unfavorable charge separation. In contrast, a concerted I-OTf cleavage and succinimide addition without charge separation might be more favorable.[77] This could be the reason why NHS **6** could not directly displace triflate **40** to produce side product **37**, even in the presence of I_2 (Entry 8, Table 5), and the formation of β exclusive **37** could also be a result of a concerted reaction without charge separation between α-triflate **40** and NXS.

Table 5. Model studies to substantiate the formation of *N*-galactosyl succinimide.



Entry	Condition (equiv.)	Temperature (°C)	Time	**37**, yield
1	NHS (1.0)	-70 to rt	6 h	n.r.[b]
2	NIS (1.0)	-70	5 min	21%[a]
3	NBS (1.0)	-70	5 min	32%[a]
4	NCS (1.0)	-70	5 min	22%[a]
5	NIS (1.0), TEA (1.0)	-70 to rt	6 h	n.r.[b]
6	NBS (1.0), TEA (1.0)	-70 to rt	6 h	n.r.[b]
7	NCS (1.0), TEA (1.0)	-70 to rt	6 h	n.r.[b]
8	NHS (1.0), I_2 (1.0)	-70 to rt	6 h	n.r.[b]
9	NHS (1.0), H_2O (1.0)	-70 to rt	6 h	n.r.[b]

[a]NMR yield. [b]No reaction.

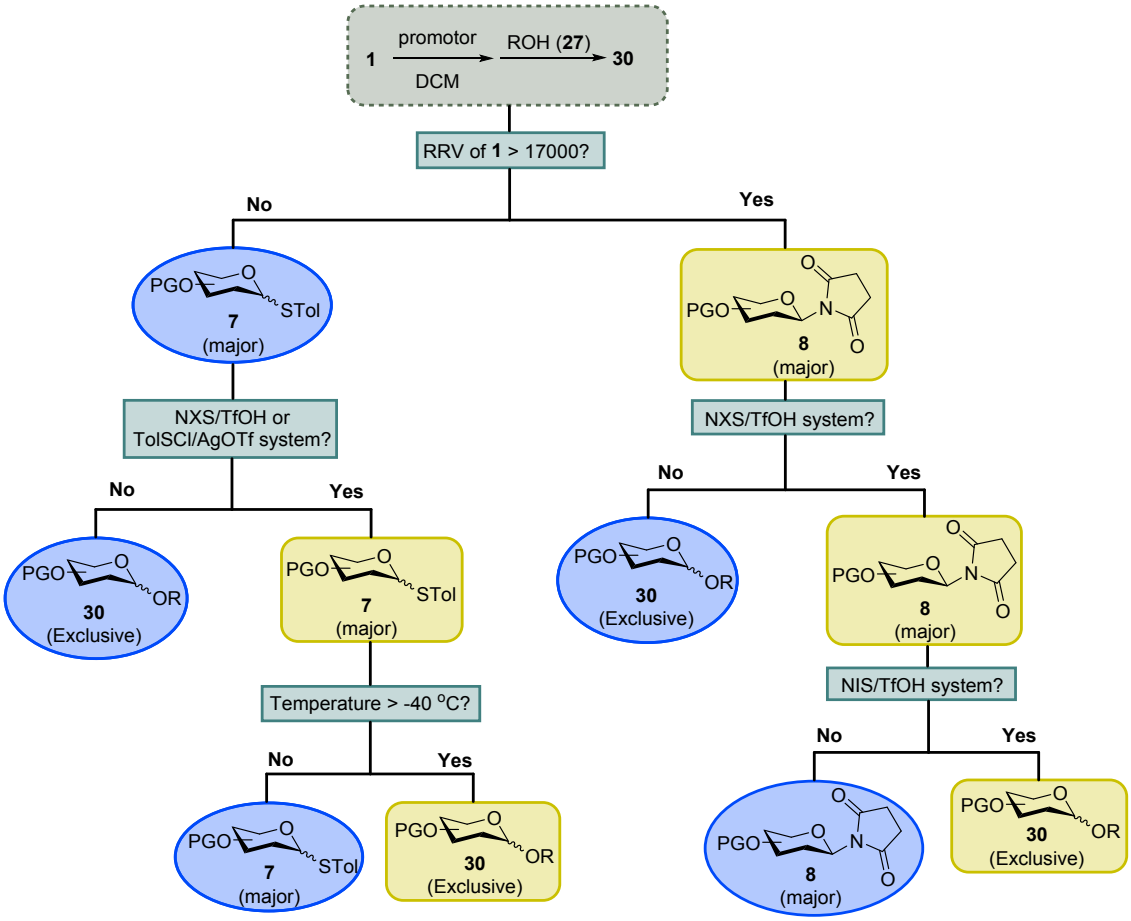
A suggested protocol was noted in this work to suppress the formation of side products **7**-**8** for achieving higher-yielding reactions of corresponding product **30** (Figure 3). We considered three major factors in chemical glycosylation, including donor reactivity (RRV), promotor system, and temperature. Frist, it was necessary to know the reactivity (RRV) of the glycosyl donor **1** as this would determine the side products, such as thioaglycon-transferred thioglycoside **7** and *N*-glycosyl succinimides **8**. The corresponding RRV of the thioglycoside **1** could be determined through the competition experiment or Auto-CHO software, as established by Wong and coworker.[26-30]

When the RRV of thioglycoside **1** is lower than 18.6, a high proportion of thioaglycon-transferred thioglycoside **7** may regenerate as the main side product, which significantly reduces the glycosylation yield and further limits one-pot glycosylation, especially in an NXS/TfOH or TolSCl/AgOTf system. Since the formation of **7** was driven by an intermolecular process from the TolSX by-product, using an alternative promotor system was a direct approach to solve this problem such as BSP/Tf_2O. Moreover, it was shown that **7** could also be fully activated by manipulating a higher temperature from -40 °C to 0 °C.

The highly active thioglycoside **1** (RRV more than 17000) turned out *N*-glycosyl succinimides **8** as the main side product. Our finding revealed that the NIS/TfOH promotor system could slightly regress the formation of *N*-glycosyl succinimides **8**

compared to an NBS/TfOH system or NCS/TfOH system. It is to be noted that usage of other promotor systems such as BSP/Tf_2O and TolSCl/AgOTf, can avoid **8**.

Figure 3. Suggested protocol for suppressing the formation of side products in chemical glycosylation.



CONCLUSIONS

The use of thioglycosides as glycosyl donors in the chemical synthesis of glycans and glycoconjugates has gained great popularity over the past years. Their distinct advantages are their high stability during building block manipulation and their ease of transformation into reactive intermediates using various promotors. Such features have facilitated the development of advanced methodologies such as one-pot protocols[47] and automated solid-phase synthesis.[16] However, side products are often created, which quench the reaction and increase the uncertainty in chemical glycosylation due to the requirement of stoichiometric or excess amounts of promoters. The unclear mechanism has

rendered it difficult to optimize the combination for achieving high-yield reactions. Herein, we established a series of mechanistic studies to rule out the generation of side products. Our low temperature NMR experiments revealed that the formation of thioaglycon-transferred thioglycoside and *N*-glycosyl succinimides follows an intermolecular process from glycosyl triflate. Therefore, the glycosylation reaction faces an unavoidable competition between side reactions and glycoside bond formation with the acceptors. Although both steric and electronic effects highly influence the glycosylation result, a thorough understanding to the mechanisms and the new factors discovered based on the mechanism studies could provide new ideas and solutions to increase the glycosylation yield. Eventually, a general guideline of how to suppress the side reactions was provided using the RRVs of the thioglycosides as an indicator under certain reaction conditions. Further studies of acceptor effects, solvent effect and other promotor systems are currently underway.

EXPERIMENTAL SECTION

General Methods

All reactions were conducted in flame-dried glassware, under nitrogen atmosphere. All solvents were purified and dried from a safe purification system containing activated Al_2O_3. All reagents obtained from commercial sources were used without purification, unless otherwise mentioned. Flash column chromatography was carried out by Silica Gel Geduran® Si 60 (0.040-0.063 mm, E. Merck). TLC was performed on pre-coated glass plates of Silica Gel 60 F254 (0.25 mm, E. Merck); detection was executed by spraying with a solution of $Ce(SO_4)_2$, $(NH_4)_2MoO_4$, and H_2SO_4 in water and subsequently heating on a hot plate. UV light for TLC analysis was UVGL-25 compact UV lamp (4 watt/ 254 nm), UVP. High performance liquid chromatography (HPLC) reactions were carried out by Agilent Technologies 1200 Serise. ^1H, ^{13}C NMR, DEPT and 2D-HSQC spectra were recorded by Bruker AV400, DRX500, AVIII 500, N600 MHz. Chemical shifts are in ppm from Me_4Si, generated from the $CDCl_3$ lock signal at δ 7.26 and CD_2Cl_2 lock signal at δ 5.32. Multiplicities are reports by using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, ABq = AB quartets, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets; *J* = coupling constant values in Hertz. Mass spectra were analyzed

by a Waters Premier XE instrument with ESI source. Structural assignments were made with additional information from selective 1D-TOCSY and 2D-HSQC experiments

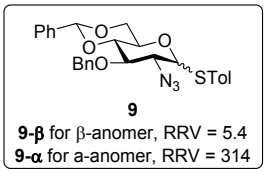
General Procedure for RRV Experiment of Thioglycosides

Two of thioglycoside donors (0.01 mmol) were mixed with molecular sieves in MeOH (0.05 mmol) and CH_2Cl_2 (1.0 ml). One of the thioglycoside performed known RRV, which was controlled as the reference donor, and the other one with unknown RRV. The reaction mixture was stirred at room temperature for 10 mins. The prepared mixture (30 µl) was through a filter, and 10-µl filtrate was then injected into an HPLC to determine the time absorbance $(A_x)_0$ and $(A_{ref})_0$ at 254 nm. Next, a solution of 0.5 M NIS in MeCN (20 ml, 0.01 mmol) was injected into the remained prepared mixture and then treated 0.1 M TfOH (10 ml, 0.001 mmol) for the thioglycoside activation. After that, the mixture was stirred at room temperature for 2 h. Next, the CH_2Cl_2 (2.0 ml) was added to diluted the reaction and then extract with saturated $Na_2S_2O_{3\ (aq)}$ containing 10 wt % $NaHCO_3$. After all of the organic layer was collected, the solution was dried over $MgSO_4$, and concentrated with rotary evaporator. Eventually, the residue dissolved in CH_2Cl_2 (1.0 ml) was measured absorbance at 254 nm to determine the ratio of the remaining unreacted donors $(A_x)t$ and $(A_{ref})t$ by HPLC with the same procedure as previously mentioned for $(A_x)_0$ and $(A_{ref})_0$. The ratio of RRVs, k_x/k_{ref}, was referred to the following equation: $\frac{k_x}{k_{ref}} = \frac{\ln (A_x)_t - \ln (A_x)_0}{\ln (A_{ref})_t - \ln (A_{ref})_0}$.[26,28-30]

Preactivation-based glycosylation in Table 1

Donor **9-α**[42,78] (100 mg, 0.204 mmol, α/β = 0/1, 1.0 equiv.) and molecular sieves (3Å, 100 mg) were dissolved in DCM (4 mL) to remove the solvent moisture at -70 °C under N_2 atmosphere for 10 mins. 1.0-2.0 equivalent of combined-promotor (NIS/TfOH, NBS/TfOH, NCS/TfOH, TolSCl/AgOTf) was then introduced individually in the reaction for the preactivation. After five minutes when TLC indicated that donor was completely activated, 1.0 equivalent of acceptor **10** (8 µL, 0.204 mmol, 1.0 equiv.), acceptor **11** (94 mg, 0.204 mmol, 1.0 equiv.), acceptor **12** (94 mg, 0.204 mmol, 1.0 equiv.) was added into the reaction mixture respectively at -70 °C and stirred for 4-5 hour. After completion of the reaction the solution was quenched by Et_3N (1 mL). The solution was filtered through celite and washed with DCM. The filtrate was evaporated in vacuo to furnish the crude oil, which was purified by flash column chromatography to give the corresponding product **13**[42] (25-36 mg, 31-44%, α/β = 1/8 ~ 1/11), **14**[71,79] (51 mg, 30%, α/β = 1/6.7), **15**[71,79] (51 mg, 30%, α/β = 1/3.4)

and thio-aglycon transferred thioglycoside **9-β**[42,78] (30-45 mg, 30-45%, α/β = 0/1).

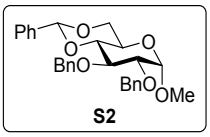


9
9-β for β-anomer, RRV = 5.4
9-α for a-anomer, RRV = 314

p-Tolyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-D-thioglucopyranoside (9).[42]

The preparation of **9** was followed by the general procedure as literature.[42] Compound *p*-tolyl 2-azido-2-deoxy-D-thioglucopyranoside[42,80] (100 mg, 0.321 mmol) was mixed with HMDS (140 μL, 0.642 mmol) in CH_2Cl_2 (1.0 mL) under N_2, and TMSOTf (6 μL, 0.032 mmol) was added at room temperature and further stirred for 3 h. Next, CH_2Cl_2 (1 mL), benzaldehyde (65 μL, 0.642 mmol) and TMSOTf (6 μL, 0.032 mmol) were added in the reaction, and the mixture was kept stirring at the same temperature for 4 h. Next, TBAF (385 mL, 0.385 mmol) was added, and the mixture was allowed to warm to room temperature and kept stirring for 1 h. Next, DMF (1 mL), BnBr (45 μL, 0.385 mmol), NaH (25.7 mg, 0.642 mmol) were added with stirring. The mixture was stirred at room temperature for 15 min. Eventually, the reaction solution was quenched with H_2O (10 mL). The aqueous layer was extracted with EtOAc (3 × 5 mL), dried with anhydrous $MgSO_4$, filtered, and concentrated *in vacuo*.[80,81] The mixture was purified by flash column chromatography (*n*-Hexane/EtOAc 15:1) on silica gel to furnish the α- and β- anomer of **8** individually (152 mg, 96%, α/β = 1/1.3). The preparation of **9-β** (**β**-anomer) was followed by the general procedure as **Preactivation-based glycosylation in Table 1**, and compound **9-β** (β-anomer) was obtained with the yield of 36% (36 mg). The RRV of β-anomer referring to previous report is 5.4.[42] The RRV of α-anomer followed **General Procedure for RRV Experiment of Thioglycosides** is 314. $[\alpha]^{25}_D$ -3.3 (*c* 0.8, $CHCl_3$); IR *v* 2106, 1492, 1453, 1274, 1089, 991, 809, 748, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.52-7.13 (m, 28H, Ar-H), 5.60 (s, 1H, CHPh), 5.56 (s, 1H, CHPh), 5.50 (d, *J* = 2.3 Hz, 1H, H-1α), 4.99-4.76 (m, 4H, CH_2Ph), 4.44 (td, *J* = 10.6, 4.8 Hz, 1H, H-5α), 4.43 (d, *J* = 10.0 Hz, 1H, H-1β), 4.38 (dd, *J* = 9.9, 4.6 Hz, 1H, H-6axα), 4.23 (dd, *J*= 9.9, 4.6 Hz, 1H, H-6eqα), 4.00-3.93 (m, 2H, H-2α, H-3α), 3.81-3.73 (m, 3H, H-4α, H-6axβ, H-6eqβ), 3.67-3.58 (m, 2H, H-3β, H-4β), 3.44 (td, *J* = 9.8, 5.0 Hz, 1H, H-5β), 3.32 (dd, *J* = 10.0, 8.4 Hz, 1H, H-2β), 2.36 (s, 3H, $ArCH_3$), 2.34 (s, 3H, $ArCH_3$); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 139.1 (C), 138.3 (C), 137.7 (C), 137.6 (C), 137.1 (C), 134.5 (CH), 133.1 (CH), 130.0 (CH), 129.9

(CH), 129.1 (CH), 128.4 (CH), 128.32 (CH), 128.29 (CH), 128.2 (CH), 128.0 (CH), 127.9 (CH), 126.0 (CH), 125.95 (CH), 101.5 (CH), 101.3 (CH), 88.2 (CH), 86.6 (CH), 82.8 (CH), 81.3 (CH), 81.0 (CH), 77.8 (CH), 75.21 (CH_2), 75.17 (CH_2), 70.46 (CH), 68.6 (CH_2), 68.5 (CH_2), 64.5 (CH), 63.7 (CH), 63.6 (CH), 21.2 (CH), 21.1 (CH); ^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.51-7.15 (m, 28H, Ar-H), 5.61 (s, 1H, CHPh), 5.58 (s, 1H, CHPh), 4.97-4.76 (m, 4H, CH_2Ph), 4.46 (d, *J* = 10.2 Hz, 1H, H-1β), 4.42 (td, *J* = 10.5, 5.0 Hz, 1H, H-5), 4.35 (dd, *J* = 10.4, 4.9 Hz, 1H, H-6axβ), 4.20 (dd, *J* = 9.9, 5.0 Hz, 1H, H-6eqα), 3.99-3.92 (m, 3H, H-2α, H-3α, H-6eqα), 3.79-3.76 (m, 3H, H-4α, H-6axβ, H-6eqβ), 3.66-3.63 (m, 2H, H-3β, H-4α), 3.44 (m, 1H, H-5β), 3.32 (dd, *J* = 10.2, 8.7 Hz, 1H, H-2β), 2.35 (s, 3H, $ArCH_3$), 2.34 (s, 3H, $ArCH_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 139.5 (C), 138.9 (C), 138.5 (C), 138.4 (C), 137.9 (C), 137.8 (C), 134.4 (CH), 133.6 (CH), 130.33 (CH), 130.25 (CH), 129.4 (CH), 128.7 (CH), 128.7 (CH), 128.6 (CH), 128.2 (CH), 126.5 (CH), 126.4 (CH), 101.9 (CH), 101.6 (CH), 88.7 (CH), 87.0 (CH), 83.1 (CH), 81.7 (CH), 81.4 (CH), 78.2 (CH), 75.3 (CH_2), 70.9 (CH), 69.0 (CH_2), 68.9 (CH_2), 65.2 (CH), 64.14 (CH), 64.09 (CH), 21.3 (CH_3), 21.2 (CH_3); HRMS (ESI) calcd for $C_{27}H_{27}N_3O_4NaS$ $[M+Na]^+$ 512.1620, found 512.1617.

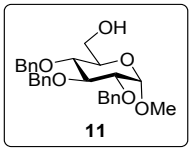


S2

Methyl 2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranoside (S2).[82]

To a solution of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside **S1** (1.5 g, 5.31 mmol) in DMF (20 mL) was added benzyl bromide (2.7 mL, 0.025 mol). The reaction was cooled in an ice bath, and sodium hydride (1.28 g, 0.032 mol) was added. The reaction stirred at room temperature overnight and quenched by water (5 mL) and extracted with EtOAc (20 mL ×2). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using EtOAc/ hexane 1/3 as the eluent to obtain **S2** as a colorless oil (2.1 g, 86%). $[\alpha]^{28}_D$ 4.6 (*c* 0.3, $CHCl_3$); IR *v* 2912, 1088, 1071, 1048, 1028, 736, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48-7.46 (m, 2H, Ph-H), 7.38-7.27 (m, 13H, Ph-H), 5.54 (s, 1H, CHPh), 4.90 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.84 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.82 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.70 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.59 (d, *J* = 3.6 Hz, 1H, H-1), 4.24 (dd, *J* = 10.1, 4.8 Hz, 1H, H-6_{eq}), 4.02 (t, *J* = 9.5 Hz, 1H, H-3), 3.81 (ddd, *J* = 10.1, 9.5, 4.8 Hz, 1H, H-5),

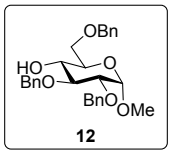
3.70 (t, J = 10.1, 4.8 Hz, 1H, H-6_{ax}), 3.58 (t, J = 9.5 Hz, 1H, H-4), 3.54 (dd, J = 9.5, 3.6 Hz, 1H, H-2), 3.39 (s, 3H, CH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.8 (C), 138.2 (C), 137.5 (C), 128.9 (CH), 128.8 (CH), 128.7 (CH), 128.6 (CH), 128.2 (CH), 128.0 (CH), 127.7 (CH), 127.4 (CH), 126.0 (CH), 101.3 (CH), 99.3 (CH), 82.2 (CH), 79.3 (CH), 78.6 (CH), 75.3 (CH_2), 73.8 (CH_2), 69.1 (CH_2), 62.4 (CH), 55.3 (CH), 29.7(CH_3); HRMS (ESI) calcd for $C_{28}H_{30}O_6Na$ $[M + Na]^+$ 485.1940, found 485.1937.



11

Methyl 2,3,4-tri-O-benzyl-α-D-glucopyranoside (11).[83]

To a solution of compound **S2** (0.3 g, 0.65 mmol) in BH_3/THF (0.47 mL, 4.86 mmol). The reaction was stirred for 10 min, and then TMSOTf (0.024 mL, 0.13 mmol) was added. The reaction stirred at 0 °C gradually to room temperature for 2 h, and quenched by MeOH (5 mL) and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using EtOAc/ hexane 1/2 as the eluent to obtain **11** as a colorless oil (0.316 g, 99%). $[\alpha]^{28}_D$ -204.2 (*c* 1.3, $CHCl_3$); IR *v* 2959, 1069, 1051, 737, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.33-7.26 (m, 15H, Ph-H), 4.98 (d, J = 10.9 Hz, 1H, CH_2Ph), 4.85 (d, J = 10.9 Hz, 1H, CH_2Ph), 4.82 (d, J = 12.2 Hz, 1H, CH_2Ph), 4.68 (d, J = 10.9 Hz, 1H, CH_2Ph), 4.66 (d, J = 11.0 Hz, 1H, CH_2Ph), 4.59 (d, J = 3.6 Hz, 1H, H-1), 4.03 (dd, J = 9.7, 9.3 Hz, 1H, H-3), 3.79 (dd, J = 11.7, 6.5 Hz, 1H, H-6a), 3.71 (dd, J = 11.7, 3.8 Hz, 1H, H-6b), 3.69 (t, J = 4.2 Hz, 1 H, H-5), 3.54 (dd, J = 9.3, 5.2 Hz, 1H, H-4), 3.52 (dd, J = 9.7, 3.6 Hz, 1H, H-2), 3.35 (s, 3 H, CH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.8 (C), 138.2 (C), 138.0 (C), 128.6 (CH), 128.5 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 127.4 (CH), 126.0 (CH), 98.2 (CH), 82.0 (CH), 80.0 (CH), 77.5 (CH), 75.7 (CH_2), 75.0 (CH_2), 73.4 (CH_2), 70.7 (CH), 61.9 (CH), 55.2 (CH_3); HRMS (ESI) calcd for $C_{28}H_{32}O_6Na$ $[M + Na]^+$ 487.2097, found 487.2049.

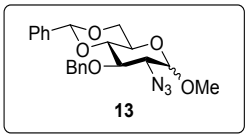


12

Methyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside (12).[83]

To a solution of compound **S2** (0.4 g, 0.865 mmol) in DCM was added Et_3SiH (1.24 mL, 7.78 mmol), TFA (0.5 mL, 6.48 mmol) and TFFA (0.025 mL, 0.173 mmol).

The reaction stirred at -40 °C for 6 h and quenched by Et_3N (5 mL). The mixture was extracted with EtOAc (20 mL) and $NaHCO_3$ (20 mL×2). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using EtOAc/ hexane 1/3 as the eluent to obtain **12** as a colorless oil (0.365 g, 91 %). $[\alpha]^{28}_D$ -290.6 (*c* 0.5, $CHCl_3$); IR *v* 2922, 1055, 1028, 736, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.28 (m, 15H, Ar-H), 4.98 (d, *J* =11.4 Hz, 1H, CH_2Ph), 4.78 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.75 (d, *J* = 11.4 Hz, 1H, CH_2Ph), 4.67 (d, *J* = 12.1 Hz, 1H, CH_2Ph), 4.64 (d, *J* = 3.6 Hz, 1H, H-1), 4.60 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 4.55 (d, *J* = 12.2 Hz, 1H, CH_2Ph), 3.79 (t, *J* = 9.1 Hz, 1H, H-3), 3.74-3.67 (m, 3H, H-5, H-6a, H-6b), 3.61 (td, *J* = 9.1, 2.1 Hz, 1H, H-4), 3.54 (dd, *J* = 9.1, 3.6 Hz, 1H, H-2), 3.39 (s, 3H, CH_3), 2.30 (d, *J* = 2.1 Hz, 1H, OH); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.8 (C), 138.2 (C), 138.0 (C), 128.6 (CH), 128.5 (CH), 128.3 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.7 (CH), 127.4 (CH), 126.0 (CH), 98.2 (CH), 81.4 (CH), 79.6 (CH), 75.4 (CH_2), 73.6 (CH_2), 73.2 (CH_2), 70.7 (CH), 69.9 (CH), 69.5 (CH), 55.2 (CH_3); HRMS (ESI) calcd for $C_{28}H_{32}O_6Na$ $[M + Na]^+$ 487.2097, found 487.2100.

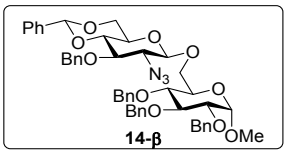


13

Methyl 2-azido-3-O-benzyl-2-deoxy-4,6-O-benzylidine-D-glucopyranoside (13).[42]

Synthesis procedure was shown as **Preactivation-based glycosylation in Table 1** to afford compound **13** (25 mg, 31%, α/β = 1/9.0) with NIS/TfOH; compound **13** (27 mg, 33%, α/β = 1/11) with NBS/TfOH; compound **13** (28 mg, 34%, α/β = 1/8.0) with NCS/TfOH; compound **13** (36 mg, 44%, α/β = 1/8.1) with TolSCl (1.0 equiv.)/AgOTf (1.0 equiv.); compound **13** (27 mg, 33%, α/β = 1/8.0) with TolSCl (2.0 equiv.)/AgOTf (2.0 equiv.). $[\alpha]^{25}_D$ -10.1 (*c* 1.4, $CHCl_3$); IR *v* 2109, 1093, 995, 749, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.50-4.26 (m, 20H, Ph-H), 5.59 (s, 1H, CHPh), 5.58 (s, 1H, CHPh), 4.99-4.91 (m, 2H, CH_2Ph), 4.82-4.78 (m, 3H, H-1α, CH_2Ph), 4.36 (dd, *J* = 10.5, 5.0 Hz, 1H, H-6aα), 4.30 (dd, *J* = 10.0, 4.7 Hz, 1H, H-6aβ), 4.07 (t, *J* = 9.0 Hz, 1H, H-3α), 3.87 (dt, *J* = 10.0, 4.8 Hz, 1H, H-5α), 3.83-3.74 (m, 2H, H-6bα, H-6bβ), 3.73-3.69 (m, 2H, H-4α, H-4β), 3.58 (t, *J* = 8.4 Hz, 1H, H-3β), 3.58 (s, 3H, OCH_3), 3.46-3.37 (m, H, H-2α, H-3β, H-5β, OCH_3); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 137.8 (C), 137.2 (C), 137.1 (C), 129.2 (C), 129.1 (CH), 128.40 (CH), 128.37 (CH), 128.3 (CH), 128.22 (CH),

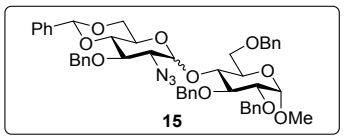
128.20 (CH), 128.1 (CH), 127.9 (CH), 126.0 (CH), 103.4 (CH), 101.5 (CH), 101.3 (CH), 99.4 (CH), 82.7 (CH), 81.6 (CH), 79.0 (CH), 76.3 (CH), 75.03 (CH_2), 75.93 (CH_2), 68.9 (CH_2), 68.6 (CH_2), 66.14 (CH), 66.11 (CH), 63.2 (CH), 62.6 (CH), 57.5 (CH_3), 55.4 (CH_3); HRMS (ESI) calcd for $C_{21}H_{23}N_3O_5Na$ $[M + Na]^+$ 420.1535, found 420.1527.



14-β

Methyl 2,3,4-tri-O-benzyl-6-O-(2-azido-3-O-benzyl-4,6-O-benzyli-dene-2-deoxy-β-D-glucopyranosyl)-α-D-glucopyranoside (14-β).[71,79]

Synthesis procedure was shown as **Preactivation-based glycosylation in Table 1** to afford compound **14** (51 mg, 30%, α/β = 1/6.7) with TolSCl (2.0 equiv.)/AgOTf (2.0 equiv.). $[\alpha]^{25}_D$ -29.5 (*c* 1.2, $CHCl_3$); IR *v* 2109, 1453, 1088, 735, 695 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.49-7.29 (m, 25H, Ph-H), 5.55 (s, 1H, CHPh), 5.01-4.91 (m, 3H, CH_2Ph), 4.85-4.78 (m, 3H, CH_2Ph), 4.67 (dd, *J* = 11.0, 9.3 Hz, 2H, CH_2Ph), 4.63 (d, *J* = 3.6 Hz, 1H, H-1), 4.31 (dd, *J* = 9.9, 5.0 Hz, 1H, H-6a'), 4.25 (d, *J* = 8.0 Hz, 1H, H-1'), 4.08 (dd, *J* = 10.5, 1.6 Hz, 1H, H-6a), 4.01 (t, *J* = 9.4 Hz, 1H, H-3), 3.80-3.68 (m, 4H, H-4', H-5, H-6b, H-6'b), 3.60 (t, 1H, *J* = 9.4 Hz, H-4), 3.59-3.48 (m, 3H, H-2, H-2', H-3'), 3.39 (s, 3H, OCH_3), 3.34 (dt, *J* = 9.9, 5.1 Hz, 1H, H-5'); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 138.7 (C), 138.4 (C), 138.1 (C), 137.7 (C), 137.1 (C), 129.0 (CH), 128.43 (CH), 128.36 (CH), 128.4 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.90 (CH), 127.86 (CH), 127.98 (CH), 127.72 (CH), 127.69 (CH), 127.6 (CH), 126.0 (CH), 102.4 (CH), 101.3 (CH), 98.2 (CH), 82.1 (CH), 81.4 (CH), 79.7 (CH), 79.2 (CH), 77.6 (CH), 75.7 (CH_2); 74.9 (CH_2), 74.8 (CH_2), 69.6 (CH), 68.7 (CH_2), 68.5 (CH_2), 66.2 (CH), 66.1 (CH), 55.2 (CH_3); HRMS (ESI) calcd for $C_{48}H_{51}N_3O_{10}Na$ $[M + Na]^+$ 852.3472, found 852.3452.



15

Methyl 2,3,4-tri-O-benzyl-4-O-(2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-D-glucopyranosyl)-α-D-glucopyranoside (15).[71,79]

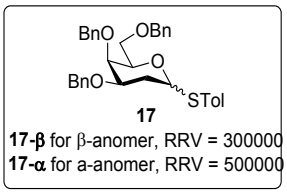
Synthesis procedure was shown as **Preactivation-based glycosylation in Table 1** to afford compound **15** (51 mg, 30%, α/β = 1/3.4) with TolSCl (2.0 equiv.)/AgOTf (2.0 equiv.). β-anomer (**15β**): $[\alpha]^{25}_D$ -0.9 (*c* 0.9, $CHCl_3$); IR *v* 2922, 2109, 1453, 1092,

771, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.48-7.26 (m, 25H, Ph-H), 5.47 (s, 1H, CHPh), 4.88 (t, *J* = 11.2 Hz, 2H, CH_2Ph), 4.82-4.70 (m, 4H, CH_2Ph), 4.64-4.61 (m, 2H, H-1, CH_2Ph), 4.42 (d, *J* = 12.0 Hz, 1H, CH_2Ph), 4.21 (d, *J* = 8.0 Hz, 1H, H-1'), 4.11 (dd, *J* = 8.8, 5.3 Hz, 1H, H-6'a), 3.98 (dd, *J* = 10.1, 3.0 Hz, 1H, H-6a), 3.94 (t, *J* = 10.1 Hz, 1H, H-4), 3.85 (t, *J* = 10.1 Hz, 1H, H-3), 3.76 (bd, *J* = 10.1 Hz, 1H, H-5), 3.70 (dd, *J* = 10.1, 3.0 Hz, 1H, H-6b), 3.65 (t, *J* = 8.8 Hz, 1H, H-4'), 3.52 (dd, *J* = 10.1, 4.0 Hz, 1H, H-2), 3.44-3.40 (m, 1H, H-6'b), 3.40 (s, 3H, OCH_3), 3.37-3.29 (m, 2H, H-2', H-3'), 3.01 (dt, *J* = 8.8, 5.0 Hz, 1H, H-5'); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ 139.3 (C), 138.4 (C), 137.9 (C), 137.8 (C), 137.3 (C), 129.0 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 128.04 (CH), 127.99 (CH), 127.9 (CH), 127.8 (CH), 127.5 (CH), 127.3 (CH), 126.0 (CH), 101.2 (CH), 101.2 (CH), 98.3 (CH), 81.7 (CH), 80.1 (CH), 79.2 (CH), 79.1 (CH), 77.0 (CH), 75.4 (CH_2); 74.7 (CH_2), 73.53 (CH_2), 73.50 (CH), 69.7 (CH), 68.5 (CH_2), 68.0 (CH_2), 66.6 (CH), 65.8 (CH), 55.3 (CH_3); HRMS (ESI) calcd for $C_{48}H_{51}N_3O_{10}Na$ $[M + Na]^+$ 852.3472, found 852.3490. α-anomer (**15α**): $[\alpha]^{25}_D$ 32.0 (*c* 0.5, $CHCl_3$); IR *v* 2923, 2107, 1453, 1094, 1050, 772, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.47-7.23 (m, 25H, Ph-H), 5.71 (d, *J* = 4.0 Hz, 1H, H-1), 5.54 (s, 1H, CHPh), 5.10 (d, *J* = 10.6 Hz, 1H, CH_2Ph), 4.95 (d, *J* = 10.9 Hz, 1H, CH_2Ph), 4.85 (d, *J* = 10.9 Hz, 1H, CH_2Ph), 4.77 (d, *J* = 10.3 Hz, 1H, CH_2Ph), 4.75 (d, *J* = 11.5 Hz, 1H, CH_2Ph), 4.65-4.55 (m, 4H, H-1', CH_2Ph), 4.11-4.05 (m, 2H, H-5, H-6a), 3.99 (t, *J* = 9.6 Hz, 1H, H-3), 3.96 (dd, *J* = 9.4, 7.1 Hz, 1H, H-4'), 3.85-3.78 (m, 3H, H-4, H-5', H-6'a), 3.70-3.63 (m, 2H, H-3', H-6'b), 3.60-3.56 (m, 2H, H-2', H-6b), 3.39 (s, 3H, OCH_3), 3.29 (dd, *J* = 10.1, 4.0 Hz, 1H, H-2); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.7 (C), 138.09 (C), 137.95 (C), 137.4 (C), 129.0 (CH), 128.5 (CH), 128.4 (CH), 128.3 (CH), 128.2 (CH), 128.1 (CH), 128.0 (CH), 127.9 (CH), 127.6 (CH), 127.5 (CH), 127.4 (CH), 126.0 (CH), 101.3 (CH), 98.1 (CH), 97.8 (CH), 76.2 (CH), 75.0 (CH_2), 73.5 (CH_2), 73.2 (CH_2), 69.4 (CH), 69.1 (CH_2), 68.7 (CH_2), 66.2 (CH), 63.4 (CH), 62.9 (CH), 55.3 (CH_3); HRMS (ESI) calcd for $C_{48}H_{51}N_3O_{10}Na$ $[M + Na]^+$ 852.3472, found 852.3463

Preactivation-based glycosylation in Table 2

To a suspension of the donor **9-α**,[78] **9-β**,[78] **16-α**,[41,42] **17-α**,[42] **18-β**,[42] **19-β**[42] (100 mg, 1.0 equiv.), molecular sieves (3Å, 100 mg) and NIS (1.0 equiv.) in CH_2Cl_2 (4 mL) was stirred at -70 °C under N_2 atmosphere for 1 h. TfOH (0.4 equiv.) was then added into the reaction mixture at -70 °C and stirred for 10 min at same temperature. After

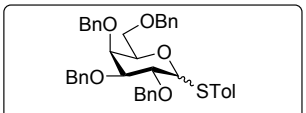
five minutes when TLC indicated that donor was completely activated, the methanol **10** (1.0 equiv.) was injected into the reaction mixture and further stirred for 1 h judged by TLC (Hexane/EtOAc 6:1). The solution was filtered through celite and washed with EtOAc. The filtrate was evaporated in vacuo to furnish the crude product, which was purified by flash chromatography to give the product **13**[42] (25-28 mg, 31-34%), **20**[41] (69 mg, 83%), **21**[42] (70 mg, 84%), **22**[42] (53 mg, 64%), **23**[42] (46 mg, 55%) and thio-aglycon transferred thioglycoside donor **9-β**[78] (42-44 mg, 42-44%), **17-β**[42] (8 mg, 8%), **18-α**[84] (25 mg, 25%), **19-α**[85,86] (36 mg, 36%).



p-Tolyl-3,4,6-tri-O-benzyl-2-deoxy-D-thiogalactopyranoside (17).

The preparation of **17** was followed by the general procedure as literature.[42] Compound *p*-Tolyl 3,4,6-tri-*O*-acetyl 2-deoxy-D-thiogalactopyranoside[87] (8.58 g, 0.0216 mol) and were dissolved in MeOH (100 mL), and the reaction mixture was stirred at room temperature for 1 h. After reaction was neutralized using acidic Amberlite resin IR-120, the mixture was filtered, and the solution was evaporated to give colorless oil. The colorless oil and BnBr (11 mL, 0.104 mol) were dissolved in DMF (60 mL), and NaH (5.2 g, 0.13 mol) was then added slowly at 0 °C. The reaction was stirred at room temperature overnight, and quenched by water (10 mL). The mixture was extracted with EtOAc (50 mL × 2). The organic layers were combined, dried over anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using EtOAc/*n*-Hexane 1/6 as the eluent to obtain **17** as a colorless oil (9.85 g, 85%, α/β = 1.8/1). The preparation of **17-β** (β-anomer) was followed by the general procedure as **Preactivation-based glycosylation in Table 2**, and compound **17-β** (β-anomer) was obtained with the yield of 36% (36 mg). The RRV of **17-β** (β-anomer) obtained by **General Procedure for RRV Experiment of Thioglycosides** is 300000.[42] The RRV of **17-α** (α-anomer) obtained by **General Procedure for RRV Experiment of Thioglycosides** is 500000. α-anomer (**17-α**):[88] $[\alpha]^{28}_D$ +128.7 (*c* 0.4, $CHCl_3$); IR *v* 2865, 1093, 1062, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.37-7.24 (m, 17H, Ar-H), 7.00 (d, *J* = 8.4 Hz, 2H, Ar-H), 5.65 (d, *J* = 5.3 Hz, 1H, H-1), 4.93 (d, *J* = 11.8 Hz, 1H,

CH_2Ph), 4.65-4.55 (m, 4H, CH_2Ph), 4.46-4.38 (m, 3H, H-5, CH_2Ph), 3.95 (d, *J* = 2.6 Hz, 1H, H-4), 3.91 (ddd, *J* = 13.0, 5.3, 2.6 Hz, 1H, H-3), 3.65-3.56 (m, 2H, H-6a, H-6b), 2.60 (td, *J* = 13.0, 5.3 Hz, 1H, H-2ax), 2.28 (s, 3H, CH_3), 2.15 (dd, *J* = 13.0, 5.3 Hz, 1H, H-2eq); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 136.8 (C), 138.2 (C), 138.1 (C), 131.8 (CH), 129.6 (CH), 129.4 (CH), 128.4 (CH), 128.1 (CH), 127.8 (CH), 127.6 (CH), 127.4 (CH), 84.7 (CH), 75.3 (CH), 74.3 (CH_2), 73.3 (CH), 72.1 (CH), 70.7 (CH), 70.5 (CH_2), 69.4 (CH_2), 31.8 (CH_2), 29.7 (CH_2), 21.0 (CH_3). β-anomer(**17-β**): $[\alpha]^{29}_D$ -20.7 (*c* 0.2, $CHCl_3$); IR *v* 2918, 1100, 1064, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 6.4 Hz, 2H, Ar-H), 7.35-7.24 (m, 15H, Ar-H), 7.02 (d, *J* = 8.0 Hz, 2H, Ph-H), 4.92 (d, *J* = 11.7 Hz, 2H, CH_2Ph), 4.67 (dd, *J* = 11.8, 2.2 Hz, 1H, H-1), 4.63-4.54 (m, 3H, CH_2Ph), 4.45 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.39 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 3.84 (t, *J* = 4.6 Hz, 1H, H-4), 3.67-3.62 (m, 2H, H-6a, H-6b), 3.57 (ddd, *J* = 11.8, 4.6, 2.2 Hz, 1H, H-3), 3.51 (t, *J*= 4.6 Hz, 1H, H-5), 2.16 (q, *J* = 11.8 Hz, 1H, H-2ax), 2.28 (s, 3H, CH_3), 2.13 (dd, *J* = 11.8, 2.2 Hz, 1H, H-2eq); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.3 (C), 138.1 (C), 137.6 (C), 131.9 (CH), 130.8 (CH), 130.2 (CH), 129.4 (CH), 128.4 (CH), 128.1 (CH), 127.8 (CH), 127.6 (CH), 127.4 (CH), 127.3 (CH), 83.2 (CH), 77.0 (CH), 76.7 (CH), 74.1 (CH_2), 73.5 (CH_2), 71.7 (CH), 70.2 (CH_2), 69.5 (CH_2), 32.4 (CH_2), 21.1 (CH_3); HRMS (ESI) calcd for $C_{34}H_{36}O_5NaS$ $[M + Na]^+$ 563.2232, found 563.2235.



18

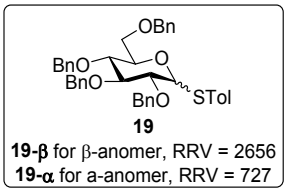
18-β for β-anomer, RRV = 17000

18-α for a-anomer, RRV = 3646

p-Tolyl 2,3,4,6-tetra-O-benzyl-1-thio-D-galactopyranoside (18).

The preparation of **18-β** was followed by the general procedure as literature.[42] 1,2,3,4,6-Penta-*O*-acetyl-thio-β-D-glucopyranose (5.0 g, 11.00 mmol) and NaOMe (59.4 mg, 1.10 mmol) were dissolved in MeOH (50 mL), and the reaction was processed at room temperature for 1 h. After that, amberlite 120 (H^+) was used to neutralize the reaction, and reaction was evaporated under high vacuum system to give white-solid compound. The white-solid compound was next mixed with BnBr (6.4 ml, 52.80 mmol) and stirred for 1 h at 0 °C in DMF (32 ml), and NaH (3.5 g, 88.00 mmol) was added into the mixture slowly at 0 °C. The reaction underwent at room temperature for 12 h. To workup the reaction, water was added into reaction in ice-bath, and aqueous

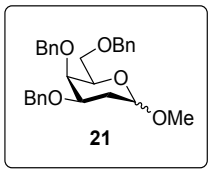
layer was extracted with EtOAc (3 × 5 mL), dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography (*n*-Hexane/EtOAc 6:1) on silica gel to give white-solid product **18-β** (6.0 g, 84%). The preparation of **18-α** (α-anomer) was followed by the general procedure as **Preactivation-based glycosylation in Table 2**, and compound **18-α** (α-anomer) was obtained with the yield of 25% (25 mg). The RRV of **18-β** (β-anomer) referring to previous report is 17000.[29,89] The RRV of **18-α** (α-anomer) obtained by **General Procedure for RRV Experiment of Thioglycosides** is 3646. β-anomer (**18-β**): $[\alpha]^{27}_D$ -1.5 (*c* 0.5, CH_2Cl_2); IR *v* 2862, 1089, 1028, 733, 696 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2) δ 7.44 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.38-7.27 (m, 24H, Ph-H), 7.03 (d, *J* = 7.9 Hz, 2H, Ar-H), 4.94, 4.57 (ABq, *J* = 11.2 Hz, 2H, CH_2Ph), 4.77, 4.74 (ABq, *J* = 10.3 Hz, 2H, CH_2Ph), 4.76, 4.71 (ABq, *J* = 11.5 Hz, 2H, CH_2Ph), 4.59 (d, *J* = 9.6 Hz, 1H, H-1), 4.49, 4.44 (ABq, *J* = 11.7 Hz, 2H, CH_2Ph), 3.98 (d, *J* = 2.6 Hz, 1H, H-4), 3.81 (t, *J* = 7.7 Hz, 1H, H-2), 3.65-3.59 (m, 4H, H-6a, H-6b, H-5, H-3); $^{13}C\{^1H\}$ NMR (125 MHz, CD_2Cl_2) δ 139.3 (C), 139.1 (C), 138.9 (C), 138.6 (C), 137.7 (C), 132.3 (CH), 130.8 (C), 129.9 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.2 (CH), 128.02 (CH), 127.96 (CH), 127.92 (CH), 127.86 (CH), 88.3 (CH), 84.5 (CH), 77.9 (CH), 77.6 (CH), 75.8 (CH_3), 75.0 (CH_3), 74.4 (CH), 73.8 (CH_3), 73.0 (CH_3), 69.4 (CH_3), 21.2 (CH_3); HRMS (ESI) calcd for $C_{41}H_{42}O_5NaS$ $[M + Na]^+$ 669.2651, found 669.2656. α-anomer (**18-α**):[84] White solid. ^1H NMR (600 MHz, $CDCl_3$) δ 7.40-7.22 (m, 22H, Ph-H), 7.00 (d, *J* = 7.9 Hz, 2H, Ph-H), 5.63 (d, *J* = 5.4 Hz, 1H, H-1), 4.94, 4.57 (ABq, *J* = 11.4 Hz, 2H, CH_2Ph), 4.86, 4.72 (ABq, *J* = 11.8 Hz, 2H, CH_2Ph), 4.77, 4.69 (ABq, *J* = 11.7 Hz, 2H, CH_2Ph), 4.47 (t, *J* = 6.4 Hz, 1H, H-5), 4.40, 4.36 (ABq, *J* = 11.7 Hz, 2H, CH_2Ph), 4.33 (dd, *J* = 10.1, 5.5 Hz, 1H, H-2), 3.98 (d, *J* = 1.8 Hz, 1H, H-4), 3.81 (dd, *J* = 10.0, 2.8 Hz, 1H, H-3), 9.54, 3.51 (ABq, *J* = 9.5 Hz, 1H, H-6a), 3.55, 3.50 (ABq, *J* = 9.6 Hz, 1H, H-6b), 2.27 (s, 3H, CH_3); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 138.8 (C), 138.7 (C), 138.1 (C), 137.1(C), 132.4 (CH), 130.7 (C), 129.6 (CH), 128.3 (CH), 128.22 (CH), 128.16 (CH), 128.0 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 88.0 (CH), 79.5 (CH), 76.6 (CH), 75.3 (CH), 74.8 (CH_2), 73.5 (CH_2), 73.4 (CH_2), 72.6 (CH_2), 70.3 (CH), 69.0 (CH_2), 21.1 (CH_3); HRMS (ESI) calcd for $C_{41}H_{42}O_5NaS$ $[M + Na]^+$ 669.2651, found 669.2656.



p-Tolyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (19).

The preparation of **19-β** was followed by the general procedure as literature.[42] 1,2,3,4,6-Penta-*O*-acetyl-thio-β-D-glucopyranose (5.0 g, 11.00 mmol) and NaOMe (59.4 mg, 1.10 mmol) were dissolved in MeOH (50 mL), and the reaction was processed at room temperature for 1 h. After that, amberlite 120 (H^+) was used to neutralize the reaction, and reaction was evaporated under high vacuum system to give white-solid compound. The white-solid compound was next mixed with BnBr (6.4 ml, 52.80 mmol) and stirred for 1 h at 0 °C in DMF (32 ml), and NaH (3.5 g, 88.00 mmol) was added into the mixture slowly at 0 °C. The reaction underwent at room temperature for 12 h. To workup the reaction, water was added into reaction in ice-bath, and aqueous layer was extracted with EtOAc (3 × 5 mL), dried with anhydrous $MgSO_4$, filtered and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography (*n*-Hexane/EtOAc 6:1) on silica gel to give white-solid product **19-β** (5.7 g, 80%). The preparation of **19-α** (α-anomer) was followed by the general procedure as **Preactivation-based glycosylation in Table 2**, and compound **19-α** (α-anomer) was obtained with the yield of 36% (36 mg). The RRV of **19-β** referring to previous report is 2656.[29] The RRV of **19-α** (α-anomer) obtained by **General Procedure for RRV Experiment of Thioglycosides** is 727. β-isomer (**19-β**): $[\alpha]^{27}_D$ -3.3 (*c* 0.8, CH_2Cl_2); IR *v* 2864, 1067, 735, 697 cm^{-1}; ^1H NMR (500 MHz, CD_2Cl_2) δ 7.47 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.41-7.24 (m, 18H, Ph-H), 7.21 (dd, *J* = 7.8, 2.0 Hz, 2H, Ar-H), 7.08 (d, *J* = 7.8 Hz, 2H, Ar-H), 4.89, 4.84 (ABq, *J* = 11.0 Hz, 2H, CH_2Ph), 4.89, 4.64 (ABq, *J* = 10.5 Hz, 2H, CH_2Ph), 4.82, 4.59 (ABq, *J* = 10.2 Hz, 2H, CH_2Ph), 4.63 (d, *J* = 9.8 Hz, 1H, H-1), 4.59, 4.53 (ABq, *J* = 11.9 Hz, 2H, CH_2Ph), 3.77 (dd, *J* = 10.8, 1.9 Hz, 1H, H-6a), 4.72 (dd, *J* = 10.8, 5.7 Hz, 1H, H-6b), 3.68 (t, *J* = 9.8 Hz, 1H, H-3), 3.61 (t, *J* = 9.8 Hz, 1H, H-4), 3.48 (ddd, *J* = 9.8, 5.7, 1.9 Hz, 1H, H-5), 3.46 (dd, *J* = 9.8, 5.7 Hz, 1H, H-2), 2.32 (s, 3H, CH_3); $^{13}C\{^1H\}$ NMR (125 MHz, CD_2Cl_2) δ 139.1 (C), 138.83 (C), 138.79 (C), 138.76 (C), 138.1 (C), 132.5 (CH), 130.5 (C), 130.0 (CH), 128.7 (CH), 128.6 (CH), 128.5 (CH), 128.3 (CH), 128.12 (CH), 128.10 (CH), 128.0 (CH), 127.91 (CH), 127.89 (CH), 88.1 (CH), 87.0 (CH), 81.3 (CH), 79.3 (CH), 78.2

(CH), 75.9 (CH_2), 75.5 (CH_2), 75.2 (CH_2), 73.7 (CH_2), 69.5 (CH_2), 21.2 (CH_3); HRMS (ESI) calcd for $C_{41}H_{42}O_5NaS$ $[M + Na]^+$ 669.2651, found 669.2648. α-isomer (**19-α**):[85,86] White solid. ^1H NMR (500 MHz, CD_2Cl_2) δ 7.41-7.39 (m, 4H, Ar-H), 7.36-7.26 (m, 16H, Ar-H), 7.23-7.21 (m, 2H, Ar-H), 7.08 (d, *J* = 7.8 Hz, 2H, Ph-H), 5.61 (d, *J* = 4.9 Hz, 1H, H-1), 4.95 (AB_q, *J* = 11.1 Hz, 1H, CH_2Ph), 4.84 (AB_q, *J* = 11.1 Hz, 1H, CH_2Ph), 4.78 (AB_q, *J* = 11.0 Hz, 1H, CH_2Ph), 4.77 (AB_q, *J* = 11.4 Hz, 1H, CH_2Ph), 4.64 (AB_q, *J* = 11.4 Hz, 1H, CH_2Ph), 4.55 (AB_q, *J* = 11.0 Hz, 1H, CH_2Ph), 4.50 (AB_q, *J* = 11.8 Hz, 1H, CH_2Ph), 4.40 (AB_q, *J* = 11.8 Hz, 1H, CH_2Ph), 4.34 (ddd, *J* = 10.0, 4.8, 2.3 Hz, 1H, H-5), 3.87 (dd, *J* = 9.5, 4.9 Hz, 1H, H-2), 3.84 (t, *J* = 9.5 Hz, 1H, H-3), 3.74 (dd, *J* = 10.7, 4.8 Hz, 1H, H-6a), 3.62 (dd, *J* = 10.7, 2.3 Hz, 1H, H-6b), 3.60 (t, *J* = 9.5 Hz, 1H, H-4), 2.31 (s, 3H, CH_3); $^{13}C\{^1H\}$ NMR (125 MHz, CD_2Cl_2) δ 139.4 (C), 139.0 (C), 138.7 (C), 138.4 (C), 138.1 (C), 133.1 (CH), 130.1 (CH), 128.8 (CH), 128.6 (CH), 128.5 (CH), 128.3 (CH), 128.21 (CH), 128.17 (CH), 128.0 (CH), 127.93 (CH), 127.87 (CH), 87.8 (CH), 82.7 (CH), 80.3 (CH), 78.0 (CH), 75.9 (CH_2), 75.3 (CH_2), 73.6 (CH_2), 72.6 (CH_2), 71.6 (CH), 69.4 (CH_2), 21.2 (CH_3); HRMS (ESI) calcd for $C_{41}H_{42}O_5NaS$ $[M + Na]^+$ 669.2651, found 669.2660.

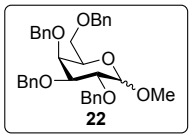


21

Methy 3,4,6-tri-O-benzyl-2-deoxy-D-galactopyranoside (21).

Synthesis procedure was shown as **Preactivation-based glycosylation in Table 2** to afford compound **21** (70 mg, 84%, α/β = 1/1.5). α-anomer (**21-α**): $[\alpha]^{27}_D$ 48.4 (*c* 0.7, $CHCl_3$); IR *v* 2909, 1375, 1094, 1050, 735, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.35-7.24 (m, 15H, Ar-H), 4.95 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.88 (d, *J* = 3.2 Hz, 1H, H-1), 4.63 (d, *J* = 11.6 Hz, 1H, CH_2Ph), 4.60 (s, 1H, CH_2Ph), 4.53 (d, *J* = 11.8, Hz, 1H, CH_2Ph), 4.43 (d, *J* = 11.8 Hz, 1H, CH_2Ph), 3.92-3.88 (m, 3H, H-3, H-4, H-5), 3.62-3.60 (m, 2H, H-6a, H-6b), 3.33 (s, 3H, OCH_3), 2.24 (td, *J* = 12.6, 3.2 Hz, 1H, H-2ax), 2.01 (dd, *J* = 12.6, 4.5 Hz, 1H, H-2eq); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.8 (C), 138.5 (C), 138.0 (C), 128.4 (CH), 128.2 (CH), 127.9 (CH), 127.7, (CH), 98.9 (CH), 74.7 (CH), 74.2 (CH_2), 73.4 (CH_2),73.1 (CH), 70.4 (CH_2), 69.8 (CH), 69.6 (CH_2), 54.8 (CH), 31.1 (CH_2); β-anomer (**21-β**): $[\alpha]^{27}_D$ -22.7 (*c* 2.2, $CHCl_3$); IR *v* 2859, 1496, 1097, 1061, 734, 696 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.31-7.24 (m, 15H, Ar-H), 4.91 (d, *J* = 11.8 Hz, 2H, CH_2Ph), 4.62 (d, *J* = 11.7 Hz, 1H, CH_2Ph), 4.57 (s, 1H, CH_2Ph), 4.43

(d, J = 11.7 Hz, 1H, CH_2Ph), 4.39 (d, J = 11.7 Hz, 1H, CH_2Ph), 4.32 (dd, J = 8.6, 3.7 Hz, 1H, H-1), 3.82 (t, J = 2.6 Hz, 1H, H-4), 3.66-3.59 (m, 2H, H-6a, H-6b), 3.52 (ddd, J = 12.0, 8.6, 2.6 Hz, 1H, H-3), 3.48-3.46 (m, 4H, H-5, OCH_3), 2.08-2.02 (m, 2H, H-2ax, H-2eq); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 138.9 (C), 138.3 (C), 138.1 (C), 128.4 (CH), 128.2 (CH), 127.9 (CH), 127.7 (CH), 101.4 (CH), 77.3 (CH_3), 74.2 (CH_2), 74.1 (CH_3), 73.6 (CH_2), 71.8 (CH), 70.3 (CH_2), 69.4 (CH_2), 56.4 (CH_3), 32.7 (CH_2), 30.9 (CH_3), 29.7 (CH_2); HRMS (ESI) calcd for $C_{28}H_{32}O_5Na$ $[M + Na]^+$ 471.2147, found 471.2150.

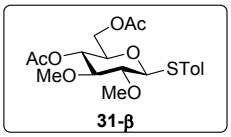


22

Methyl 2,3,4,6-tetra-O-benzyl-D-galactopyranoside (22).[42]

Synthesis procedure was shown as **Preactivation-based glycosylation in Table 2** to afford compound **22** (53 mg, 64%, α/β = 1/2.0). α-isomer (**22-α**): White-solid. $[\alpha]^{26}_D$ +100.7 (*c* 0.1, CH_2Cl_2); IR *v* 3054, 1422, 1264, 1050, 896, 730, 702 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.36-7.19 (m, 20H, Ph-H), 4.90, 4.52 (ABq, J = 11.4 Hz, 2H, CH_2Ph), 4.80, 4.69 (ABq, J = 11.8 Hz, 2H, CH_2Ph), 4.78, 4.65 (ABq, J = 12.1 Hz, 2H, CH_2Ph), 4.64 (d, J = 3.7 Hz, 1H, H-1), 4.43, 4.35 (ABq, J = 11.8 Hz, 2H, CH_2Ph), 3.99 (dd, J = 9.4, 3.0 Hz, 1H, H-2), 3.91-3.87 (m, 2H, H-3, H-4), 3.85 (t, J = 6.3 Hz, 1H, H-5), 3.48 (d, J = 6.4 Hz, 1H, H-6a, H-6b), 3.32 (s, 3H, OMe); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 138.84 (C), 138.66 (C), 138.53 (C), 137.99 (C), 128.36 (CH), 128.31 (CH), 128.23 (CH), 128.20 (CH), 128.09 (CH), 127.75 (CH), 127.69 (CH), 127.67 (CH), 127.55 (CH), 127.48 (CH), 98.81 (CH), 79.12 (CH), 77.20 (CH), 76.47 (CH), 75.20 (CH), 74.73 (CH_2), 73.56 (CH_2), 73.48 (CH_2), 73.29 (CH_2), 69.24 (CH), 60.09 (CH_2), 55.34 (CH_3); HRMS (ESI) calcd for $C_{35}H_{38}O_6Na$ $[M + Na]^+$ 577.2566, found 577.2560. β-isomer (**22-β**): White-solid. $[\alpha]^{27}_D$ +14.4 (*c* 0.3, CH_2Cl_2); IR *v* 3054, 2870, 1496, 1454, 1362, 1265, 1204, 1097, 1074, 1028, 896, 730, 697, 636 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.37-7.21 (m, 20H, Ph-H), 4.93, 4.60 (ABq, J = 11.6 Hz, 2H, CH_2Ph), 4.88, 4.74 (ABq, J = 11.1 Hz, 2H, CH_2Ph), 4.72, 4.70 (ABq, J = 11.8 Hz, 2H, CH_2Ph), 4.45, 4.40 (ABq, J = 11.8 Hz, 2H, CH_2Ph), 4.26 (d, J = 7.7 Hz, 1H, H-1), 3.88 (s, 1H, H-4), 3.79 (t, J = 8.7 Hz, 1H, H-2), 3.59 (d, J = 6.2 Hz, 2H, H-6a, H-6b), 3.55-3.52 (m, 4H, H-5, OMe), 3.51 (dd, J = 8.9, 2.7 Hz 1H, H-3); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$) δ 138.82 (C), 138.64 (C), 138.50 (C), 137.91 (C), 128.40 (CH), 128.32 (CH),

128.23 (CH), 128.12 (CH), 128.08 (CH), 127.86 (CH), 127.75 (CH), 127.67 (CH), 127.51 (CH), 127.47 (CH), 104.98 (CH), 82.15 (CH), 79.62 (CH), 75.11 (CH_2), 74.45 (CH_2), 73.54 (CH_2), 73.48 (CH), 73.37 (CH), 72.99 (CH_2), 68.85 (CH_2), 56.99 (CH_3); HRMS (ESI) calcd for $C_{35}H_{38}O_6Na$ $[M + Na]^+$ 577.2566, found 577.2568.



31-β

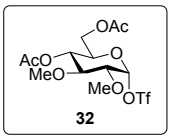
p-Tolyl 4,6-O-acetyl-2,3-di-O-methyl-1-thio-β-D-glucopyranoside (31-β).

Compound *p*-tolyl 4,6-*O*-benzylidene-2,3-di-*O*-methyl-1-thio-β-D-glucopyranoside[42] (1 g, 2.49 mmol) was first dissolved in the solvent mixture of AcOH, H_2O, and CH_2Cl_2 (4:1:1 v/v/v, 17 ml). The reaction mixture was heated for 1 h. The resulting solution was concentrated under reduced pressure to give the oil product (0.7 g). The oil product in pyridine (700 mL), Ac_2O (463 μL, 4.9 mmol) was injected into mixture and stirred for 2 h. The mixture was purified by flash column chromatography (*n*-Hexane/EtOAc 1:2) on silica gel to furnish the desired product **31-β** (0.7 g, 71%). The RRV obtained by following **General Procedure for RRV Experiment of Thioglycosides** is 5.0. $[\alpha]^{26}_D$ -42.5 (*c* 2.2, CH_2Cl_2); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 7.42 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.13 (d, *J* = 8.1 Hz, 2H, Ar-H), 4.82 (t, *J* = 9.8 Hz, 1H, H-4), 4.48 (d, *J* = 9.8 Hz, 1H, H-1), 4.14 (dd, *J* = 12.2, 6.0 Hz, 1H, H-6a), 4.06 (dd, *J* = 12.2, 3.6 Hz, 1H, H-6b), 3.58 (s, 3H, OCH_3), 3.54 (td, *J* = 9.8, 3.6 Hz, 1H, H-5), 3.52 (s, 3H, OCH_3), 3.30 (t, *J* = 9.8 Hz, 1H, H-3), 3.08 (t, *J* = 9.8 Hz, 1H, H-2), 2.33 (s, 3H, CH_3), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc); ^{13}C{^{1}H}NMR (150 MHz, CD_2Cl_2) δ 170.8 (C), 170.0 (C), 138.4 (C), 132.8 (CH), 130.0 (CH), 129.9 (CH), 87.7 (CH), 86.1 (CH), 82.4 (CH), 76.1 (CH), 70.1 (CH), 63.0 (CH_2), 61.1 (CH), 61.0 (CH), 21.2 (CH_3), 21.0 (CH_3), 20.9 (CH_3); HRMS (ESI) calcd for $C_{19}H_{26}O_7NaS$ $[M + Na]^+$ 421.1297, found 421.1295.

The procedure of cross-over experiments in Scheme 3.

Thioglucoside **31-β** (100 mg, 0.25 mmol, 1.0 equiv.) was initially dissolved in CD_2Cl_2 (3.0 mL) at -70 °C for 1h. In a separate flask, iodine (I_2, 7.9 mg, 0.0625 mmol, 0.25 equiv.) and *p*-tolyl disulfide [(TolSSTol), 15.4 mg, 0.0625 mmol, 0.25 equiv.] were added in to CD_2Cl_2 (0.5 mL) to prepare *p*-toluenesulfenyl iodide (TolSI).[90] After 5 minutes, the in situ prepared TolSI solution and TfOH (11 μL, 0.125 mmol, 0.5 equiv.) were injected slowly into the first flask (thioglucoside **31-β** in CD_2Cl_2) over 1 min and

the reaction was stirred for 4 hours at -70 °C. An aliquot of the reaction mixture was taken. The generation of **32** and **31-α** were further detected through low temperature NMR at -70 °C, and the NMR yield of triflate **32** was determined to be 22%, while **31-α** was 28%. Similarly, the further study using phenylsulfenyl iodide (PhSI) followed same procedure, except TolSSTol was changed into diphenyl disulfide [(PhSSPh), 13.7 mg, 0.0625 mmol, 0.25 equiv.], and the reaction mixture was stirred at -70 °C for 4 hours. After 1.0 equiv. of triethylamine was introduced to quench the reaction, the compound **31-α** and **33-α** were isolated in 42% by column chromatography, of which **31-α**/**33-α** ratio is 1.9/1.



32

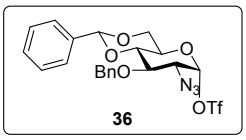
4,6-O-Acetyl-2,3-di-O-methyl-α-D-glucopyranosyl triflate (32).[42]

The preparation of **32** was followed by the procedure as **The procedure of cross-over experiments in Scheme 3**, and compound **32** was determined via low-temperature NMR at -70 °C. The corresponding spectra was same as report.[42] ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.18 (d, J = 2.9 Hz, 1H, H-1), 4.99 (t, J = 2.9 Hz, 1H, H-4), 4.22 (d, J = 12.7 Hz, 1H, H-6a), 4.06 (td, J = 12.7, 2.9 Hz, 1H, H-5), 4.00 (d, J = 12.7 Hz, 1H, H-6b), 3.55 (t, J = 2.9 Hz, 1H, H-3), 3.52 (t, J = 2.9 Hz, 1H, H-2) ^{13}C{^{1}H} NMR (125 MHz, CD_2Cl_2) δ 104.6 (CH, C-1), 78.5 (CH, C-2), 71.3 (CH, C-5), 66.6 (CH, C-3), 60.8 (CH_2, C-6).

The studies of the intermolecular thioaglycon transformation in Figure 1.

The suspension of the donor **9D** (25 mg, 0.051mmol, 1.0 equiv.), molecular sieves (4 Å, 25 mg) and 1-benzenesulfinyl piperidine (BSP) (11mg, 0.051 mmol, 1.0 equiv.) in CD_2Cl_2 (4 mL) was stirred at -70 °C under nitrogen atmosphere for 1 h. Tf_2O (9 μL, 0.051 mmol, 1.0 equiv.) was added into the reaction mixture at -70 °C to prepare glycosyl triflate intermediate **36D**. The identity of glycosyl triflate **36D** was detected by low temperature NMR experiment at -70 °C in 5 mins, of which chemical shift (δ) of the anomeric proton (H-1) signal was 6.05 ppm and the coupling constant value is 3.5 Hz.[42,71] After that, the mixture of TolSCl (8 μL, 0.051 mmol, 1.0 equiv.), TfOH (1.8 μL, 0.02 mmol, 0.4 equiv.) H_2O (0-2.3 μL, 0-0.128 mmol, 0-2.5 equiv.) and MeOH (1.0-6.1 μL, 0.026-0.153 mmol, 0.5-3.0 equiv.) was added in the reaction. As figure S1 was shown, the reaction gave thio-aglycon transferred thioglycoside **9D-β** and 1-methyl

glycoside **13D** in 5 min, which were detected by low temperature NMR at -70 °C after directly filtration. The water content of the solvents was determined using Karl Fischer titration. The intermediate transformation was monitored via low-temperature NMR experiment at temperature ranging -70 °C to 0 °C in 85 mins after the combination of NIS (6 mg, 0.026 mmol, 0.5 equiv.) and TfOH (2 μL, 0.026 mmol, 0.5 equiv.) was added. All of the data points in Figure S2 were continuously obtained in the same reaction batch. When temperature was increased from -40 °C to 0 °C, the thioaglycon-transferred thioglycoside **9D-β** was gradually consumed with the major increase of β-methyl glycoside product **13D-β**.



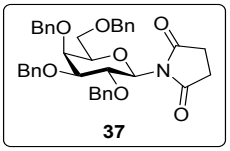
36

2-Azido-3-O-benzyl-O-benzylidene-2-deoxy-1-D-glucopyranosyl triflate (36).[42,71]

The preparation of **36** was followed by the procedure as **The studies of the intermolecular thioaglycon transformation in Figure 1**, and compound **36** was determined via low-temperature NMR at -70 °C. ^{1}H NMR (500 MHz, CD_2Cl_2) δ 6.05 (d, J = 3.0 Hz, 1H, H-1), 5.64 (s, 1H, PhCH), 4.96, 4.75 (ABq, J = 10.2 Hz, 2H, CH_2Ph) 4.30 (dd, J = 10.0, 4.5 Hz, 1H, H-6eq), 4.08 (t, J = 9.7 Hz, 1H, H-4), 4.02 (ddd, J = 10.0, 9.7, 4.5 Hz, 1H, H-5), 3.94 (dd, J = 9.7, 3.0 Hz, 1H, H-2), 3.90 (t, J = 9.7 Hz, 1H, H-3), 3.81 (t, J = 10.0 Hz, H-6ax); ^{13}C{^{1}H}NMR (125 MHz, CD_2Cl_2) δ 104.6 (CH), 100.6 (CH), 80.0 (CH), 76.0 (CH), 75.0 (CH_2), 67.1 (CH_2), 65.8 (CH), 60.6 (CH); ^{19}F NMR (470 MHz, CD_2Cl_2) δ -75.68 (s).

The protocol of NXS/TfOH preactivation-based reaction in Table 4

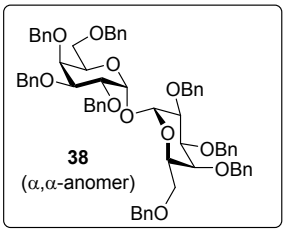
To a solution of thiogalactoside **18-β** (100 mg, 0.15 mmol) in CH_2Cl_2 (1.0 mL) was mixed with 3 Å molecular sieve (300 mg) and NXS (0.15 mmol; 20 mg for NCS; 33 mg for NBS; 35 mg for NIS) and stirred for 30-60 minutes at -40 °C. Later, TfOH (7 μL, 0.08 mmol) was injected into the solution and stirred at -40 °C for 15 minutes or longer time. The solution was filtered through celite and washed with DCM. The filtrate was evaporated in vacuo to furnish the crude oil, which was purified by flash column chromatography to give the corresponding product **37**[74], **38**[91] and **39**.



37

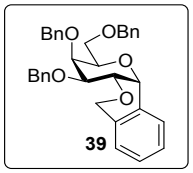
N-succinimidyl 2,3, 4, 6-tetra-O-benzyl-β-D-glucopyranoside (37)[74]

Compound **37** was obtained via the general procedure as **The protocol of NXS/TfOH preactivation-based reaction in Table 4**, and compound **37** was obtained in 52% (50 mg, α/β = 0/1) under NCS/TfOH condition, 32% (31 mg, α/β = 0/1) under NBS/TfOH condition and 24% (23 mg, α/β = 0/1) under NIS/TfOH. The corresponding spectra was same as report.[74]



1-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-2,3,4-O-benzyl-α-D-galactopyranoside (38).[91]

Compound **38** was obtained via the general procedure as **The protocol of NXS/TfOH preactivation-based reaction in Table 4**, and compound **38** was obtained in 11% (18 mg, αα/αβ = 1/1) under NBS/TfOH condition and 23% (37 mg, αα/αβ=1/1) under NIS/TfOH condition. α,α-isomer: White solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38-7.17 (m, 40H, Ph-H), 5.26 (d, *J* = 3.5 Hz, 2H, H-1, H-1'), 4.89, 4.52 (ABq, *J* = 11.4 Hz, 4H, CH_2Ph), 4.79, 4.73 (ABq, *J* = 11.7 Hz, 4H, CH_2Ph), 4.73, 4.63 (ABq, *J* = 12.2 Hz, 4H, CH_2Ph), 4.37, 4.29 (ABq, *J* = 11.8 Hz, 4H, CH_2Ph), 4.31 (t, *J* = 6.6 Hz, 2H, H-4, H-4') (ABq, *J* = 11.7 Hz, 2H, CH_2Ph), 4.07 (dd, *J* = 3.4, 9.6 Hz, 2H, H-2, H-2'), 4.02-3.97 (m, 4H, H-3, H-3', H-5, H-5'), 3.53-3.42 (m, 4H, H-6a, H-6a', H-6b, H-6b'); ^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$) δ 138.9 (C), 138.8 (C), 138.7 (C), 138.1 (C), 128.3 (CH), 128.2 (CH), 128.2 (CH), 128.1 (CH), 127.7 (CH), 127.6 (CH), 127.5 (CH), 127.3 (CH), 93.6 (CH), 78.7 (CH), 77.3 (CH), 77.1 (CH), 77.0 (CH), 76.9 (CH), 76.8 (CH), 76.6 (CH), 76.0 (CH), 75.1 (CH), 74.8 (CH_2), 73.4 (CH_2), 72.8 (CH_2), 69.7 (CH), 69.0 (CH_2); HRMS (ESI) calcd for $C_{68}H_{70}O_{11}Na$ $[M + Na]^+$ 1085.4816, found 1085.4822.



*(2R,3S,4R,4aS,10bR)-3,4-bis(benzyloxy)-2-((benzyloxy)methyl)-2,3,4,4a,6,10b-hexahydro-pyrano[3,2-c] isochromene (39)***.**

Compound **38** was obtained via the general procedure as **The protocol of NXS/TfOH preactivation-based reaction in Table 4**. Compound **38** was obtained in 21% (16 mg) under NBS/TfOH condition and 18% (14 mg) under NIS/TfOH condition. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.39 (m, 1H, Ph-H), 7.35-7.20 (m, 17H, Ph-H), 6.96-6.93 (m, 1H, Ph-H), 4.92 (d, *J* = 4 Hz, 1H, H-1), 4.79, 4.59 (ABq, *J* = 11.6 Hz, 2H, CH_2Ph), 4.74, 4.63 (ABq, *J* = 11.9 Hz, 2H, CH_2Ph), 4.66 (s, 2H, CH_2Ph), 4.57, 4.53 (ABq, *J* = 11.9 Hz, 2H, CH_2Ph), 4.22 (dd, *J* = 4.1, 6.9 Hz, 1H, H-2), 4.06 (t, *J* = 3.0 Hz, 1H, H-4), 3.99-3.92 (m, 2H, H-5, H-6a), 3.79 (dd, *J* = 2.7, 6.9 Hz, 1H, H-3), 3.78-3.74 (m, 1H, H-6b); ^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$) δ 138.5 (C), 138.4 (C), 138.3 (C), 135.2 (C), 131.8 (C), 128.6 (CH), 128.4 (CH), 128.3 (CH), 127.8 (CH), 127.7 (CH), 127.6 (CH), 127.1 (CH), 123.8 (CH), 76.3 (CH), 73.9 (CH), 73.7 (CH), 73.6 (CH_2), 73.3 (CH_2), 72.2 (CH_2), 67.7 (CH_2), 66.1 (CH), 65.2 (CH_2); HRMS (ESI) calcd for $C_{34}H_{34}O_5Na$ $[M + Na]^+$ 545.2304, found 545.2305.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxx

^{1}H, selective 1D-TOCSY, ^{13}C NMR, ^{19}F NMR, 2D-HSQC and HRMS spectra of all new compounds

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