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Taming the Reactivity of Glycosyl Iodides To Achieve Stereoselective Glycosidation

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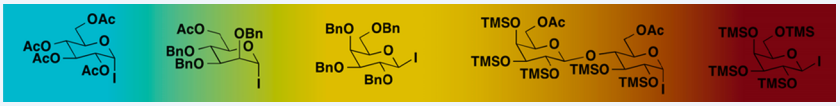

CONSPECTUS: Although glycosyl iodides have been known for more than 100 years, it was not until the 21st century that their full potential began to be harnessed for complex glycoconjugate synthesis. Mechanistic studies in the late 1990s probed glycosyl iodide formation by NMR spectroscopy and revealed important reactivity features embedded in protecting-group stereoelectronics. Differentially protected sugars having an anomeric acetate were reacted with trimethylsilyl iodide (TMSI) to generate the glycosyl iodides. In the absence of C-2 participation, generation of the glycosyl iodide proceeded by inversion of the starting anomeric acetate stereochemistry. Once formed, the glycosyl iodide readily underwent in situ anomerization, and in the presence of excess iodide, equilibrium concentrations of α- and β-iodides were established. Reactivity profiles depended upon the identity of the sugar and the protecting groups adorning it. Consistent with the modern idea of disarmed versus armed sugars, ester protecting groups diminished the reactivity of glycosyl iodides and ether protecting groups enhanced the reactivity. Thus, acetylated sugars were slower to form the iodide and anomerize than their benzylated analogues, and these disarmed glycosyl iodides could be isolated and purified, whereas armed ether-protected iodides could only be generated and reacted in situ. All other things being equal, the β-iodide was orders of magnitude more reactive than the thermodynamically more stable α-iodide, consistent with the idea of in situ anomerization introduced by Lemieux in the mid-20th century.

Glycosyl iodides are far more reactive than the corresponding bromides, and with the increased reactivity comes increased stereocontrol, particularly when forming α-linked linear and branched oligosaccharides. Reactions with per-*O*-silylated glycosyl iodides are especially useful for the synthesis of α-linked glycoconjugates. Silyl ether protecting groups make the glycosyl iodide so reactive that even highly functionalized aglycon acceptors add. Following the coupling event, the TMS ethers are readily removed by methanolysis, and since all of the byproducts are volatile, multiple reactions can be performed in a single reaction vessel without isolation of intermediates. In this fashion, per-*O*-TMS monosaccharides can be converted to biologically relevant α-linked glycolipids in one pot. The stereochemical outcome of these reactions can also be switched to β-glycoside formation by addition of silver to chelate the iodide, thus favoring S_N2 displacement of the α-iodide. While iodides derived from benzyl and silyl ether-protected oligosaccharides are susceptible to interglycosidic bond cleavage when treated with TMSI, the introduction of a single acetate protecting group prevents this unwanted side reaction. Partial acetylation of armed glycosyl iodides also attenuates HI elimination side reactions. Conversely, fully acetylated glycosyl iodides are deactivated and require metal catalysis in order for glycosidation to occur. Recent findings indicate that I_2 activation of per-*O*-acetylated mono-, di-, and trisaccharides promotes glycosidation of cyclic ethers to give β-linked iodoalkyl glycoconjugates in one step. Products of these reactions have been converted into multivalent carbohydrate displays. With these synthetic pathways elucidated, chemical reactivity can be exquisitely controlled by the judicious selection of protecting groups to achieve high stereocontrol in step-economical processes.

INTRODUCTION

The day we first encountered glycosyl iodides can be recalled with uncommon clarity. Michael Hadd, then a graduate student at the University of Arizona, decided to react per-*O*-acetylglucose (**1α**) with trimethylsilyl iodide (TMSI) in the presence of an alcohol to generate the glycoside (Figure 1). It seemed like a reasonable proposition given the propensity of carbohydrate-derived acetals to undergo Lewis acid activation to give glycosides. However, the ^{1}H NMR spectrum of the major purified compound (**2α**) looked nothing like the spectrum of the anticipated product. There was no evidence of glycoside formation, and the anomeric proton resonance appeared near 7.0 ppm, which was further downfield than we had ever observed by ^{1}H NMR analysis. At the same time, the ^{13}C NMR spectrum showed no anomeric carbon resonance in the usual region (~90 ppm); instead, it was shifted upfield by approximately 20 ppm. The real surprise came when the mass spectrum indicated that iodide had displaced the anomeric acetate. Looking back today, it may seem ridiculous that when these results were shared there was widespread disbelief. Most thought that the data were incorrectly analyzed—after all, everybody knew that glycosyl iodides were too reactive to be synthetically useful. It seemed implausible that a glycosyl iodide could be isolated from the reaction after column chromatography. Nevertheless, the experimental evidence was indisputable. Although we did not know it at the time, that day marked a

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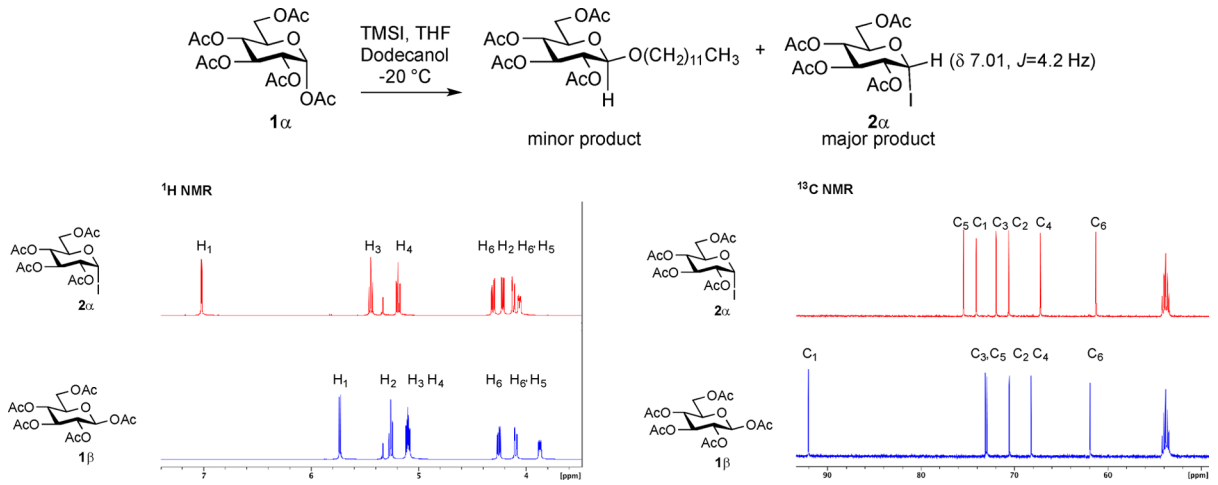

Figure 1. The reaction that led to decades of glycosyl iodide exploratory chemistry.

sea change in our research program: there was no doubt that 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl iodide (**2*α***) was the major product of the reaction. But how could it be that a glycosyl iodide could survive column chromatography?

This curiosity sparked an extensive literature review. SciFinder had just become widely accessible, and using this tool we found that indeed, leaders in the carbohydrate synthesis field had routinely dismissed glycosyl iodides as too reactive to be useful.[1] The search also revealed that there had been some dabbling with glycosyl iodides in the 1970s, but no follow-up studies were reported.[2] Fortunately, the Beilstein database was also available, leading us to a rich carbohydrate literature going back to the early 1900s and the laboratories of Emil Fischer. Notably, Fischer had been experimenting with reacting per-*O*-acetylated sugars with hydrogen halides,[3] and in 1910 his lab published an especially relevant paper describing the reaction of per-*O*-acetylated glucose with HI in acetic acid to give a 54% yield of 2,3,4,6-tetra-*O*-acetyl-*α*-D-glucopyranosyl iodide (Figure 2).[4] The glycosyl iodide was reported to be an unstable crystalline material that reacted with silver carbonate in methanol to afford a methyl glycoside. Thus, it seemed that Fischer and co-workers had achieved this simple reaction about 85 years before Hadd's inspiration—one important difference being the addition of silver carbonate to activate the iodide.

A few other papers from the early 20th century reported variations on the theme of acetylated glycosyl iodide/metal activation in much the same way that bromides and chlorides were treated.[5] Although the increased reactivity of glycosyl iodides relative to bromides was noted, it seems that this attribute was not enough to justify the experimental difficulties associated with their preparation, and the literature fell silent for several decades. Then in 1974, Kronzer and Schuerch reported a reaction in which sodium iodide was added to the reaction of 2,3,4,6-tetra-*O*-benzyl-*α*-D-glucopyranosyl bromide to promote glycoside formation (Figure 3).[2] Their results showed remarkable acceleration of the reaction, which was presumed to proceed through glycosyl iodide formation, and the reactions occurred under metal-free conditions. They remarked that this example "reemphasizes the significant control possible on the glycoside-forming reaction by judicious choice of leaving group." Although they concluded that glycosyl iodides should be further investigated as substrates for oligosaccharide synthesis, such studies do not appear to have been pursued.

Shortly thereafter, Thiem and Meyer reported the use of TMSI to generate glycosyl iodides from a host of precursors, including anhydrosugars, methyl glycosides, and pentaacetylated hexoses.[6] The confluence of these two papers in the context of the early work of Fischer shaped our research vision and set us on a voyage of chemical discovery that continues to this day. Important lessons were to be learned by the rediscovery of science in a time when new ideas change the interpretation of previous results, allowing innovations to emerge.

STEROELECTRONIC EFFECTS AND GLYCOSYL IODIDE REACTIVITY

The concept of "armed" and "disarmed" glycosyl donors has come of age in the 21st century.[7] Generally, electron-withdrawing groups such as acetates stabilize the anomeric C–L bond of the donor (Figure 4). As noted above, Fischer's peracetylated glycosyl iodides were isolable and even crystalline. Being "disarmed", these donors required silver catalysis for glycosidation to proceed. In contrast, glycosyl iodides armed with benzyl ether protecting groups proved to be so reactive that they were only generated and reacted in situ (Figure 3). More recently, per-*O*-trimethylsilyl ether-protected glycosyl iodides have been shown to be even more reactive than their benzyl ether counterparts and are therefore described as "super armed". While Bols and co-workers have shown that glycosyl donors having bulky *tert*-butyldimethylsilyl ethers undergo conformational interconversion, making them super armed,[8] trimethylsilyl ether-protected donors do not appear to undergo this conformational chair flip; instead, their hyper-reactivity is derived from inductive effects (vide infra).

It was not until Nguyen and Hadd began mechanistic studies in the late 1990s that armed glycosyl iodides were fully characterized, and even then, the reactions had to be performed at −100 °C in an NMR spectrometer.[9] Mechanistic studies commenced by reacting the disarmed *α*-anomer of per-*O*-acetylated glucose (**1*α***) with TMSI at −20 °C (Figure 5). As the reaction proceeded, evidence of the starting material and two new products was observed in the NMR spectrum. The anomeric proton of the major product appeared as a doublet at 5.82 ppm (J = 9.3 Hz),

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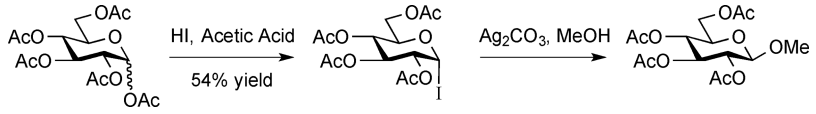

Figure 2. Fischer's first report of glycosyl iodide reactions from 1910.

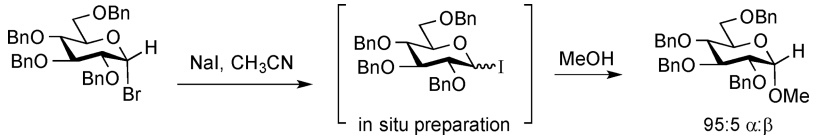

Figure 3. Kronzer and Schuerch reaction to generate glycosyl iodide in situ.

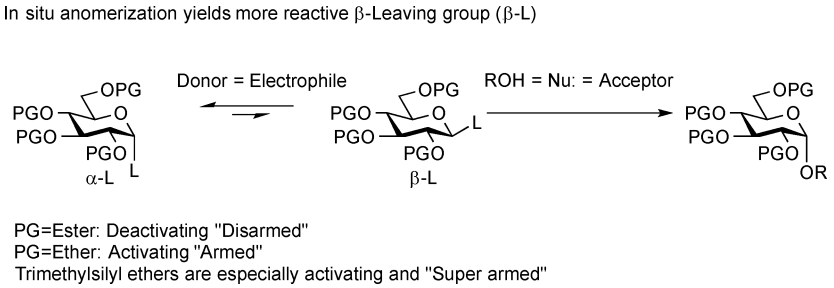

Figure 4. The armed vs disarmed nature of the protecting groups and the anomeric configuration of the iodide are two factors governing reactivity.

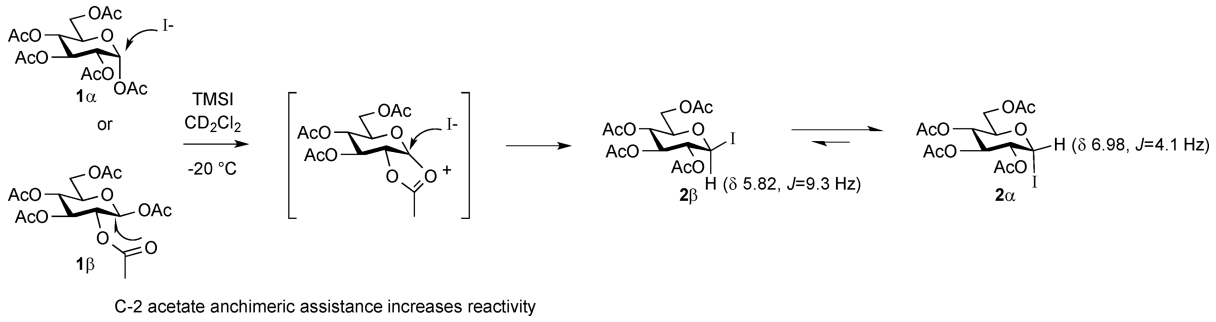

Figure 5. Anomeric acetates of ester-protected (disarmed) sugars initially convert to the β-iodide regardless of starting configuration. The kinetic product (**2β**) anomerizes to the thermodynamic product (**2α**) over time.

while that of the minor product appeared at ~6.98 ppm (J = 4.1 Hz) and matched the data that had been reported by Thiem[6] for 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl iodide (**2α**). The major product was determined to be 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl iodide (**2β**), and over time it equilibrated to **2α**, presumably via S_N2 displacement by iodide. The β-anomer of per-*O*-acetylated glucose (**1β**) reacted similarly, albeit an order of magnitude faster than the α-acetate. The increased activity of the β-acetate was attributed to anchimeric assistance from the C-2 acetate with the so-formed cyclic acetoxonium intermediate subject to S_N2-like addition of iodide. The data from these NMR experiments were consistent with Fischer's observations. From optical rotation experiments, he noted that both forms of a per-*O*-acetylated sugar merged to the same glycosyl iodide when treated with HI in acetic acid—another humbling reminder of Fischer's extraordinary chemical intuition even before the birth of conformational analysis.

The story was all together different when armed per-*O*-benzyl sugars were engaged (Figure 6). The reactions had to be performed at a much lower temperature (−100 °C) in order to detect the β-iodides. Upon activation with TMSI

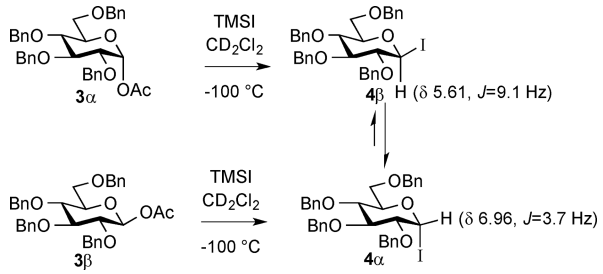

Figure 6. Anomeric acetates of ether-protected (armed) sugars are more reactive than ester-protected analogues.

and in the absence of C-2 participation, the α-acetate most readily underwent S_N2-like displacement, and anomerization of the β-iodide to the thermodynamically more stable α-anomer occurred rapidly. These combined mechanistic studies made clear three conceptual ideas: reactions of glycosyl iodides proceed through S_N2-like displacement of activated anomeric groups; the β-glycosyl iodide is often the kinetic product and over time anomerizes to the thermodynamically more stable α-anomer; and the reactivity

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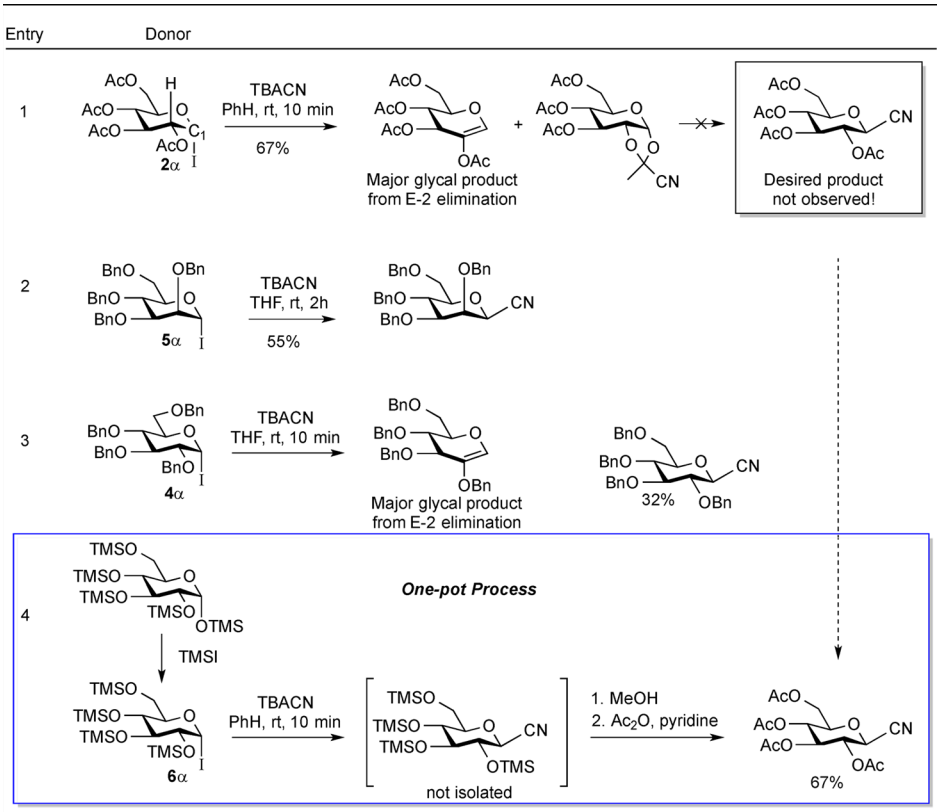
Table 1. Anionic Addition of Cyanide Requires Armed and Super Armed Donors



of the glycosyl iodide depends upon the identity of the sugar and the protecting groups adorning it. Most importantly, "seeing was believing": the NMR experiments allowed us to literally watch glycosyl iodides being formed and transformed, and through that process we began to understand how they had been mischaracterized as too reactive to be useful. We became convinced that if we could control the stereochemistry of the iodide, we could control the stereoselectivity of glycosidation. In the simplest terms, reactions of α-glycosyl iodides would afford β-glycosides and vice versa. The studies that followed focused on establishing the boundaries of this simple process and were inspired by the earlier work of Lemieux and co-workers that exploited the exceptional reactivity of β-halides generated in situ.[10]

ANIONIC ADDITIONS TO GLYCOSYL IODIDES: PREPARATION OF *C*- AND *O*-GLYCOSIDES

The synthesis of C-linked sugars has been an active area of research for decades. Conjugation through carbon rather than oxygen affords enzyme-resistant analogues that can be incorporated into glycopeptide and glycolipid constructs.[11] Examples of *C*-glycoside synthesis emanating from our laboratories capture many of the salient features of glycosyl iodide chemistry. Early on we noted that per-*O*-acetylated iodides did not efficiently react with anionic acceptors such as tetrabutylammonium cyanide (TBACN), $TBAN_3$, or TBAOAc under neutral conditions. Either E2 elimination occurred or cyanoethylidene derivatives formed, and attempts at rearrangement to the β-glycoside were generally not successful (Table 1, entry 1). To increase the reactivity of the C_1–I bond relative to the C_2–H bond, the sugars were armed with benzyl ether protecting groups. While the reaction of TBACN with per-*O*-benzylated mannosyl iodide (**5*α***) readily afforded the β-cyanoglycoside, the glucosyl corollary was plagued by competing E2 elimination. It would be another 5 years before Bhat et al.[12] recognized that glucosyl donors behave best when super armed with silyl ether protecting groups (Table 1, entry 4). The glucosyl iodide (**6*α***) could be generated directly from per-*O*-silylated glucose upon treatment with TMSI. The reaction was complete within 15 min at 0 °C, which was faster than conversion of the anomeric acetate of the benzyl-protected analogue (**3*α***). Subsequent reaction with TBACN was also faster, and high yields of β-cyanoglucoside were obtained (Table 1, entry 4). This reaction also revealed an appealing transient quality of TMS ethers, which are readily removed upon methanolysis, and since all of the byproducts are volatile, multiple reactions can be performed in a single reaction vessel without isolation of intermediates. In this fashion, per-*O*-TMS-glucose could be converted to per-*O*-acetylated β-cyanoglucose in a one-pot, four-step process affording the product originally sought in the failed experiment shown in Table 1, entry 1.

Later, Kulkarni et al.[13] showed that Grignard reagents just as easily displace armed glycosyl iodides. These reactions were especially well suited for per-*O*-benzyl-protected galactosyl iodide (**7*α***), which is more reactive than the corresponding glucosyl C-4 epimer and did not require the hyper-reactivity afforded by TMS protecting groups (Figure 7). Reaction of **7*α*** with allylmagnesium chloride resulted in an 87% yield of the β-*C*-allyl glycoside, which was subsequently transformed into a C-linked sugar amino acid.[14] The principle of tuning the reactivity of α- versus β-iodides also played out well in the synthesis of a C

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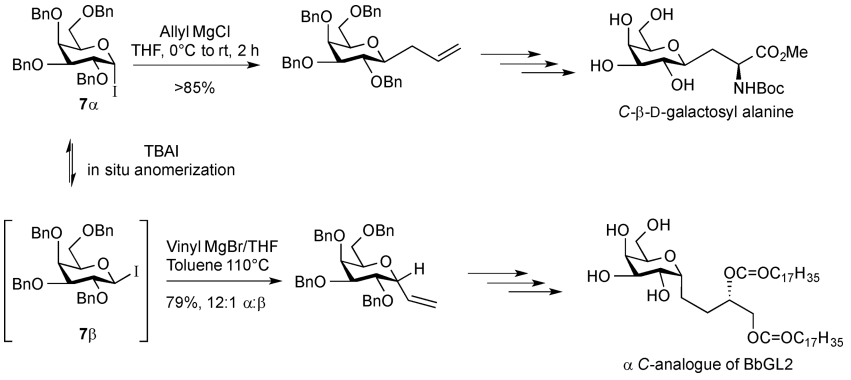
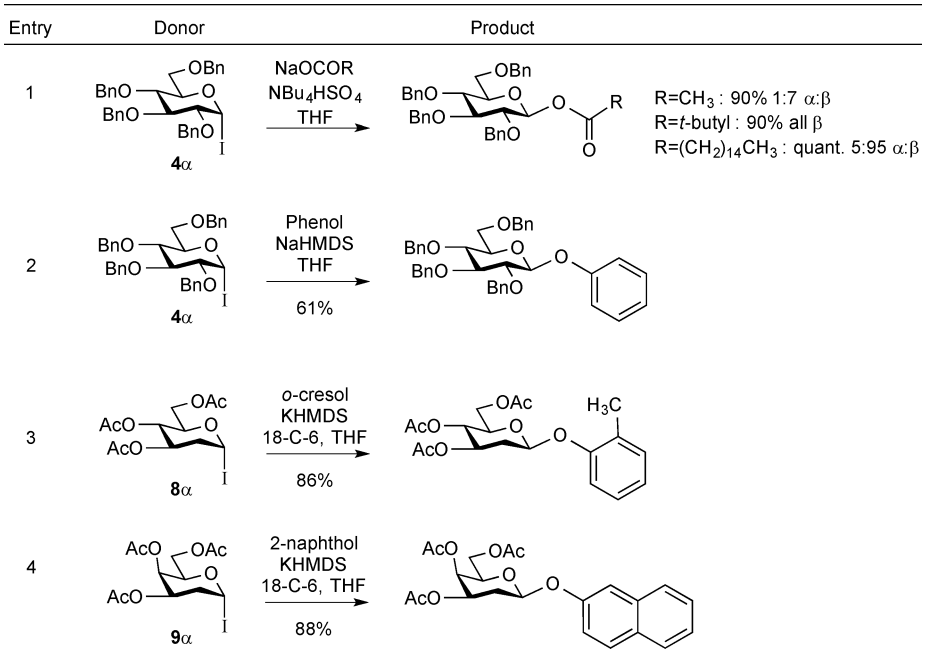


Figure 7. Grignard additions to armed galactosyl iodides proceed with inversion of stereochemistry.

Table 2. Anionic Additions to Glycosyl Iodides



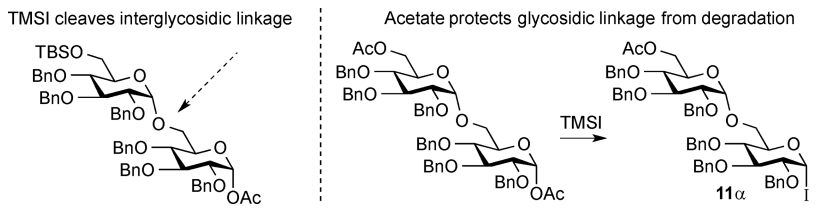


Figure 8. Interglycosidic linkage of oligosaccharides is susceptible to TMSI cleavage unless a disarming group is incorporated at C-6.

analogue of BbGL2, an immunogenic glycolipid isolated from *Borrelia burgdorferi* and the etiological agent of Lyme disease. In this case, the α-linked *C*-galactoside was desired, and the synthesis was achieved by subjecting **7*α*** to in situ anomerization with the addition of TBAI at elevated temperature, effectively increasing the concentration of the more reactive β-glycosyl iodide (**7*β***). Nucleophilic displacement of the β-iodide with vinylmagnesium bromide led to inversion of the stereochemistry to give the α-*C*-glycoside in high yield. Subsequent modifications afforded the *C*-glycoside of this important bioactive glycoconjugate.[13]

The *C*-glycoside story is mirrored in anionic *O*-glycoside reactions. Addition of alkoxides to glycosyl iodides generally results in E2 elimination. However, carboxylate anions and stabilized phenoxides do add to benzyl-protected glycosyl iodides via S_N2 displacement.[15] Remarkably, 2-deoxyglycosyl iodides also undergo nucleophilic attack with aryl alkoxides even if they are disarmed with acetate protecting groups, and in these cases there is little difference in reactivity between the 2-deoxygalactose and 2-deoxyglucose analogues (Table 2).[16]

■ OLIGOSACCHARIDE SYNTHESES

Although glycosyl halides have been utilized in synthesis for more than a century, they have not been widely employed in oligosaccharide syntheses. Interestingly, in 1972 Fréchet and

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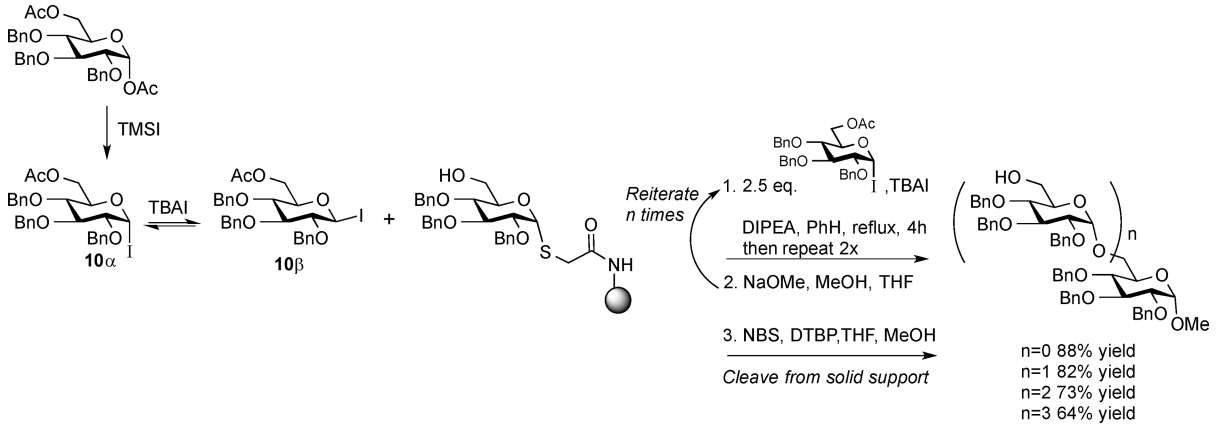

Figure 9. Solid-phase oligosaccharide synthesis.

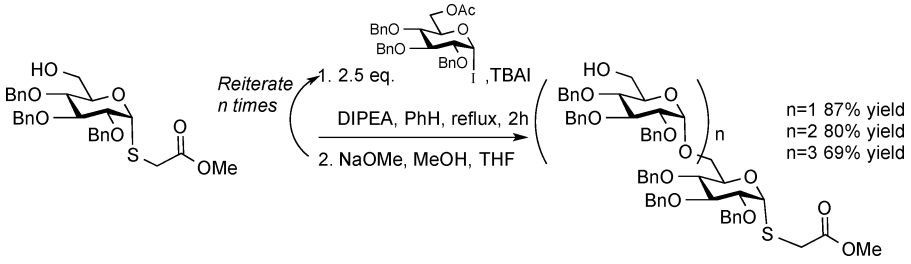

Figure 10. Solution-phase [1 + 1] oligosaccharide synthesis is time-efficient.

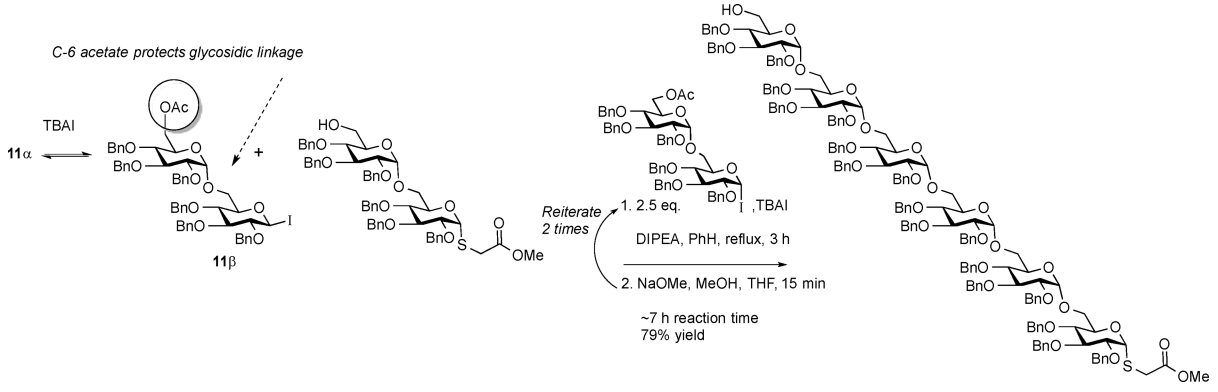

Figure 11. Solution-phase [2 + 2] and [2 + 4] additions proved most efficient.

Schuerch used glycosyl bromides in what appears to be the first solid-phase oligosaccharide syntheses appearing in the literature.[17] The reactions took days to complete for each sugar added, and the stereochemical outcome was not well controlled or characterized. One cannot help but wonder whether Schuerch ever considered using glycosyl iodides instead, since after all he was one of the first to convert glycosyl bromides to iodides in situ (vide supra).

It was not until the syntheses of 1,6-linked glucose oligomers by Lam et al.[18] that the advantages of using glycosyl iodides rather than glycosyl bromides were cemented. In the course of these investigations, we also discovered that interglycosidic bonds are susceptible to cleavage by TMSI, making it difficult to prepare armed oligosaccharide glycosyl iodide donors (Figure 8). Initially, this limitation was addressed strategically by adding glucosyl iodide monomers to the growing oligosaccharide chain. Later it was discovered that a single acetate protecting group at C-6 (**11*α***) was enough to attenuate the reactivity of the interglycosidic linkage, allowing the addition of armed disaccharide iodide donors to [2 + 2] glycosidation strategies.

Solid-phase oligosaccharide syntheses occurred under neutral conditions without metal catalysis (Figure 9). Glucosyl iodide (**10*α***) was generated from the 1,6-diacetate precursor and then treated with TBAI to generate the reactive *β*-iodide (**10*β***), which underwent nucleophilic attack to give the *α*-glycoside. Each monomer addition was reacted for 4 h, and as is commonly done in solid-phase reactions, each addition was repeated twice. Therefore, the total reaction time for addition of a monomer unit was reduced by 75% (12 h) compared with the Fréchet methodology (48 h per glycosyl bromide monomer). Moreover, only *α*-glycosides formed according to NMR detection limits.

Encouraged by these results, Lam et al.[19] went on to study solution-phase reactions, which turned out to be far more efficient. The reactions were nearly quantitative and complete within 2−3 h per monomer unit. Because the solution-phase

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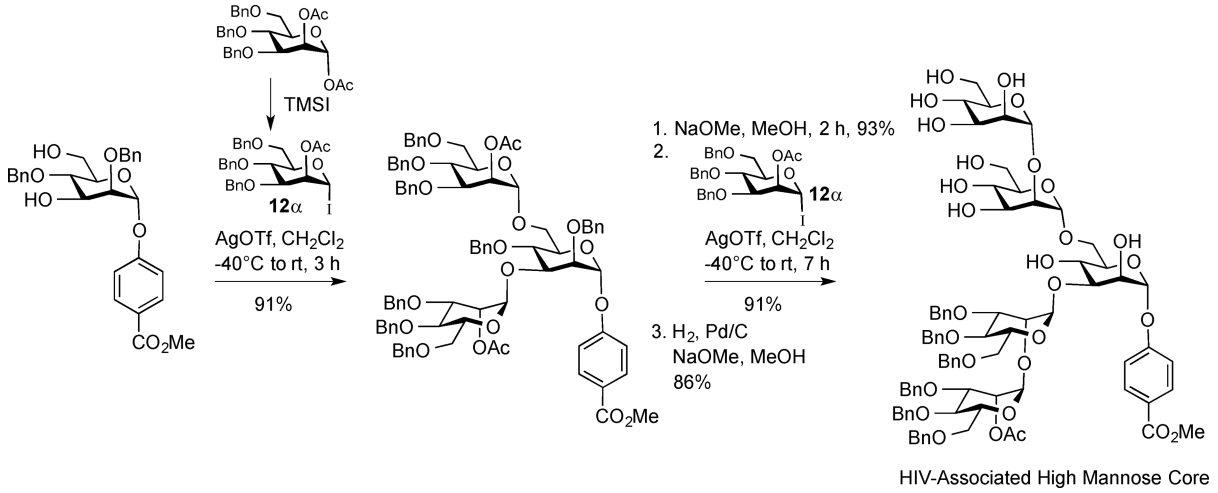

Figure 12. Silver triflate-catalyzed α-mannosylation at low temperature.

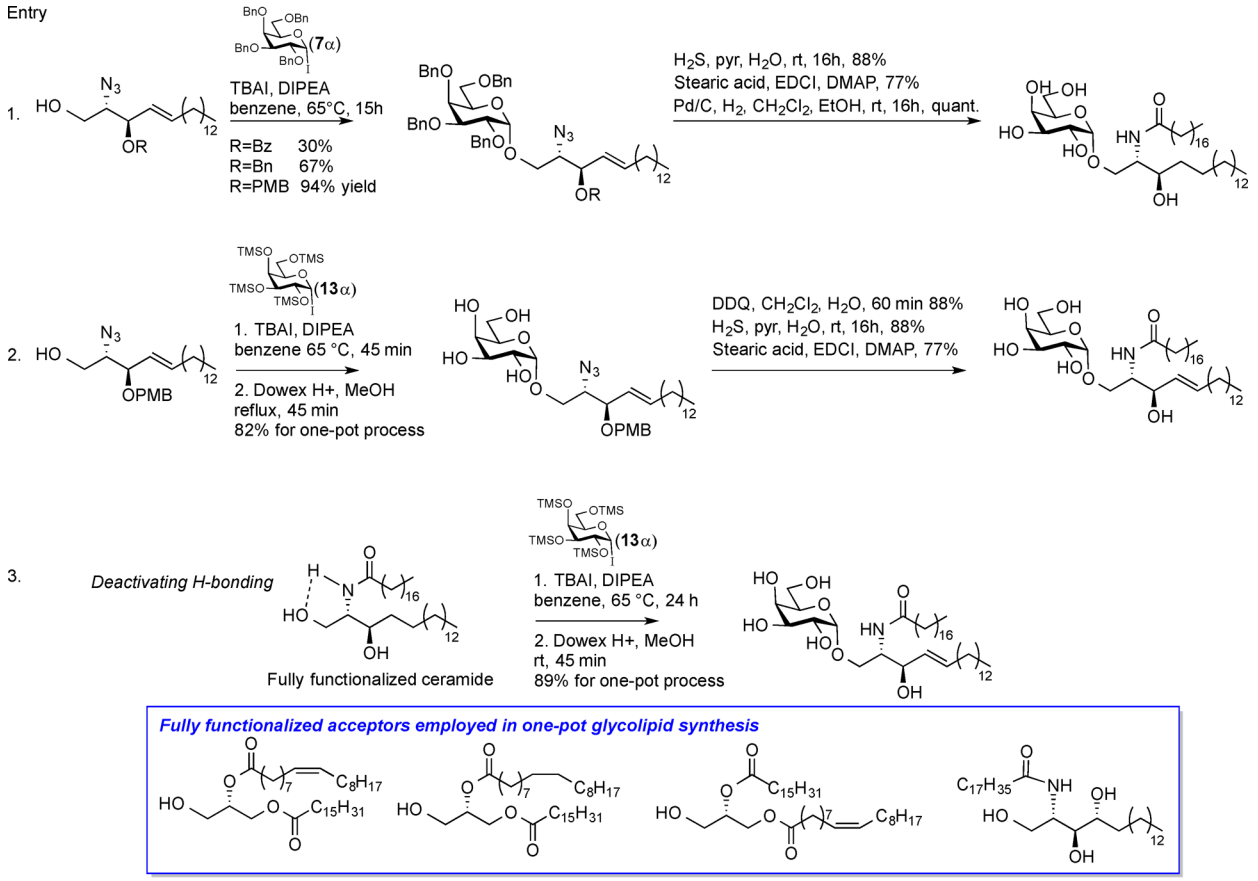

Figure 13. One-pot synthesis of α-linked glycolipids.

reactions were so clean, purification of the products was facile (Figure 10).

Solution-phase reactions using a [2 + 2] addition strategy were most efficient, and a hexamer could be prepared and purified in less time than it took for one iteration in the solid-phase reaction (Figure 11). These studies taught us important lessons regarding the utility of glycosyl iodides in oligosaccharide synthesis. Perhaps most importantly, we found that the C-6 acetate in the dimer donor (**11*β***) protected the inter-residue linkage from cleavage by TMSI. Analogues having a silyl protecting group at C-6 were readily cleaved, so silyl groups not only activate the iodide toward attack but also activate the glycosidic linkage toward cleavage. These combined studies indicated that there was little advantage to solid-phase strategies, and subsequent studies directed toward making branched oligosaccharides were conducted in the solution phase.

BRANCHED OLIGOSACCARIDE SYNTHESES

During oligosaccharide syntheses, the most precious components are the increasingly complex acceptors, and to ensure efficient coupling, excess donor is added. Since in situ

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Figure 14. Limiting TBAI to 1.5 equiv minimizes trans-silylation side reactions.

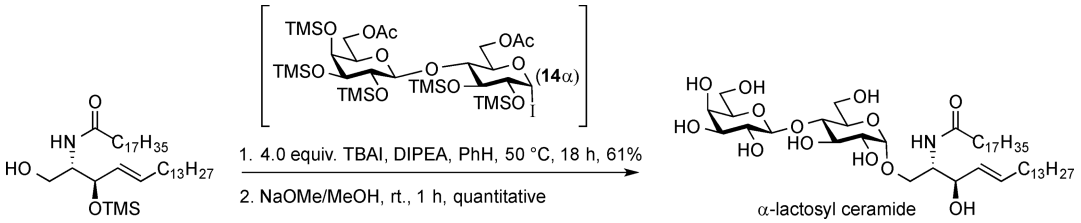

Figure 15. One-pot synthesis of α-lactosyl ceramide.

anomerization reactions require reflux, the excess donor usually undergoes elimination during the course of the reaction, forming the glycal in addition to the desired product.[20] When targeting the high-mannose oligosaccharide core associated with HIV envelope protein, Lam et al.[21] employed silver catalysis to ameliorate glycal formation (Figure 12). The combination of having a C-2 acetate and conducting the reaction at low temperature led to the efficient synthesis of the α-linked high-mannose core associated with HIV envelope protein. The symmetrical nature of the core was well-suited for a double addition of mannosyl donors (**12α**). These reactions reinforced the benefits of using glycosyl iodides over other halides. Whereas similar reactions with glycosyl chlorides required 2 days for completion of the reaction, the glycosyl iodide reactions were finished within 3 h, and the overall yields were slightly improved as well (91% for the iodide and 79% for the chloride).

DEVELOPMENT OF ONE-POT GLYCOSIDATIONS

For the past two decades, α-linked glycosyl ceramides have been of high interest in the biological field. In the early 1990s, researchers in Japan isolated bioactive glycolipids from the marine sponge *Agelas mauritianus* off the coast of Okinawa.[22,23] Subsequent structure−activity relationship studies revealed that α-linked glycosyl ceramides are potent immune-modulating compounds.[24−26] During fruitful discussions with Randy Brutkiewicz, we became increasingly interested in α-linked glycolipids as synthetic targets. Previously, we had used trichloroacetimidate chemistry to make β-GalCer,[27] and we were especially hopeful that using armed glycosyl iodides would alleviate the need for metal catalysis. The ability to achieve neutral glycosidations could have advantages in terms of drug development protocols. Ultimately, we sought to add fully functionalized lipids (Figure 13) to the glycosyl iodide in a one-step fashion.

It had been well-documented that fully functionalized ceramide is a poor nucleophile because of intramolecular hydrogen bonding that attenuates the primary hydroxyl reactivity (Figure 13, entry 3). A reasonable solution to this problem entails installing the amide linkage after the glycosylation event. Using an azide functionality as a placeholder for the amide has proven successful, since it is not capable of the same hydrogen-bonding deactivation as the amide hydrogen. Du et al.[28] engaged azidosphingosine in a reaction with per-*O*-benzylgalactosyl iodide (**7α**) and immediately noticed a unique reactivity profile of the acceptor depending upon the protecting group at the secondary alcohol (Figure 13, entry 1). Electron-withdrawing groups on the secondary hydroxyl such as benzoyl gave only a 30% yield of the desired product. Switching to a benzyl protecting group increased the yield to 63%, and *p*-methoxybenzyl protection proved to be even better, giving a 90% yield of the desired glycoside. These results sensitized us to the fact that electron-donating groups also increase hydroxyl nucleophilicity. In this manner, both the glycosyl iodide donor and the azidosphingosine acceptor were armed for maximal reactivity. In subsequent studies, Du and co-workers were able to achieve addition of fully functionalized ceramide acceptors to per-*O*-silylated galactosyl iodides (**13α**).[29] In a one-pot, three-step protocol, per-*O*-silylated galactose was reacted with ceramide for 24 h in benzene to afford α-GalCer in 89% yield. Schombs and co-workers expanded the repertoire of accessible glycolipids to include glucosyl analogues.[30] While the one-pot protocol also worked well with per-*O*-silylglucosyl donors (**6α**), the reactions were slower and the yields slightly lower. Occasionally in reactions employing per-*O*-silylated donors, the presence of silylated acceptor was observed. This side reaction presumably arises from in situ generation of TMSI promoted by iodide attack on the sugar C-6 trimethylsilyl ether (Figure 14). Importantly, Schombs and co-workers determined that limiting TBAI to 1.5 equiv could avert this side reaction, and efficient syntheses of glycolipids were obtained.

In an alternative strategy, Hsieh and co-workers found that C-6-acetylated TMS-protected donors efficiently undergo one-pot glycosidation to give high yields of α-lactosyl ceramide (Figure 15).[31] The lactosyl iodide donor **14α** having only two different protecting groups (acetate and TMS) can be prepared in three steps and 73% overall yield starting from lactose.[32] The presence of acetate groups at both C-6 positions protects the disaccharide from inter-residue cleavage by the action of TMSI, yet this donor is reactive enough to undergo nucleophilic attack by fully functionalized ceramide acceptors. Moreover, excess TBAI can be used since there is no chance of transilylation at C-6. Accordingly, only the α-linked product is observed. Taken together, these results show that this methodology nicely complements the β-glycosyl ceramide syntheses developed by Castillon.[33]

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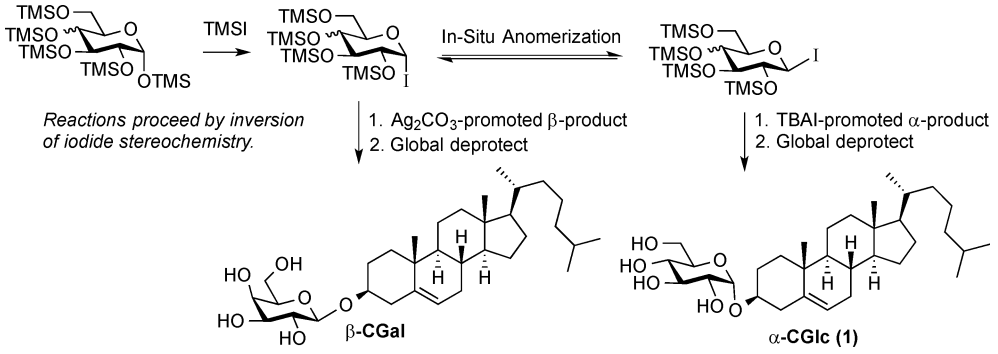

Figure 16. Generalized one-pot strategy for the stereoselective synthesis of either α- or β-linked steryl glycosides.

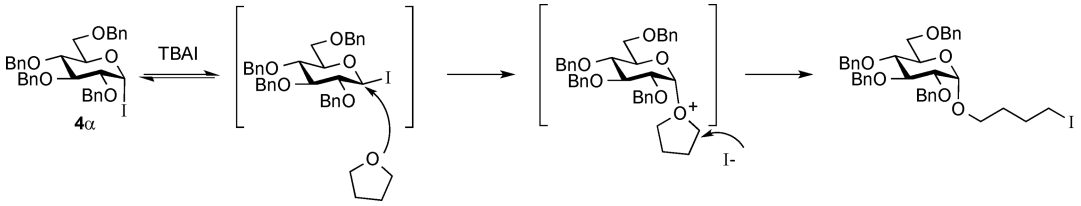

Figure 17. Addition of THF to glycosyl iodides and subsequent ring opening by iodide gives functionalized iodoalkyl glycosides in one step.

Table 3. Cyclic Ether Addition to Mannosyl Iodides Affords β-Selective Glycosidation

Entry			
1	15α	1.5 eq.  , MgO, CH_2Cl_2, reflux, 24 h	1:4 α:β
		85% yield	
2	C-6 acetate mitigates HI elimination	1.5 eq.  , MgO, CH_2Cl_2, rt, 18 h	1:6 α:β
		83% yield	
3		1.5 eq.  , MgO, CH_2Cl_2, rt, 30 min	1:3 α:β, 1:1 regioisomers
		90% yield	
4		1.5 eq.  , MgO, CH_2Cl_2, reflux, 6 h	1:30 α:β
		89% yield	
5		1.5 eq.  , MgO, CH_2Cl_2, reflux, 18 h	only β
		81% yield	
6	16α	1.5 eq.  , MgO, CH_2Cl_2, rt, 2 days	only β
		92% yield	

The glycosyl iodide synthetic platform has proven to be equally effective in targeting steryl glycosides. By the end of the 20th century, the prevalence of steryl glycosides in pathogenic bacteria was revealed. β-Linked cholesteryl galactosides were found in *Borrelia burgdorferi*, the causal agent of Lyme disease, and α-linked cholesteryl glucosides were isolated from *Helicobacter pylori*, a coevolutionary human pathogen known to cause gastric ulcers and cancer.[34] In targeting these compounds, we found that per-*O*-silylglycosyl iodides could be used to achieve either α- or β-glycosidation depending upon the reaction conditions (Figure 16). Direct displacement of the α-iodide by

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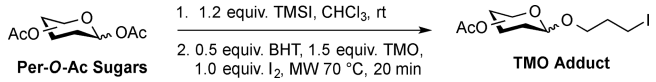
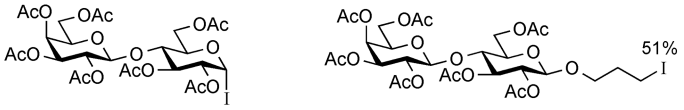
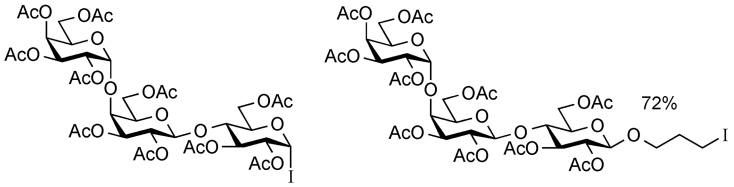
Table 4. Reactions of Disarmed Glycosyl Iodides under Microwave Conditions



Entry	Iodide	Product and isolated yield from per-*O*-acetylated starting material	
1	**2**α		84%
2	**17**α		87%
3	**18**α		71%
4	**19**α		51%
5	**20**α		72%

Gb3 trisaccharide

cholesterol gives the β-product.[35] However, some α-product is observed in these reactions, presumably as a result of in situ anomerization from residual iodide generated during the reaction. To minimize this competitive reaction, Ag_2CO_3 is added to chelate the iodide. In this manner, respectable yields of β-steryl glycosides are obtained.[36] Alternatively, the β-iodide can be generated in situ by the addition of TBAI. The reactivity of the β-iodide is orders of magnitude greater than that of the α-linked anomer, and cholesterol addition to the β-iodide drives the equilibration process yielding the α-product. Reactions to obtain α-linked steryl glycosides proceed with high stereochemical integrity to the extent that only the α-anomer is observed.[35,36]

■ GLYCOSYL IODIDES A CENTURY LATER

Much of the utility of glycosyl iodides has been highlighted in the examples described above. Suffice it to say that glycosyl iodides are more reactive than most donors, which has profound effects upon the stereochemical outcome and reaction time. With appropriate protecting group selections, fully functionalized acceptors can be incorporated in one-pot glycosidations that are step-economical and highly efficient. But are glycosyl iodides inherently unique? Studies with cyclic ether acceptors suggest that indeed they are. Early in our investigations, tetrahydrofuran (THF) adducts were observed when TBAI was used to catalyze in situ anomerization reactions (Figure 17). Simply switching the solvent to benzene or dichloromethane circumvented this annoying side reaction. Years later, however, when targeting glycosides with functional handles for multivalent display, we realized that the products of cyclic ether addition could be beneficial, since in just one step iodoalkyl glycosides could be prepared. This reaction pathway becomes possible because armed glycosyl iodides are so reactive that they do not require metal catalysis, thus leaving iodide available for other reaction pathways such as in situ anomerization or, in this case, ring opening of the activated cyclic ether.

Dabideen et al.[37] initially focused on the reactions of armed glycosyl donors, which undergo cyclic ether attack without activation. The reactivities of various acceptors were correlated with the strain energies of the ethers: THF was less reactive than trimethylene oxide, which in turn was less reactive than propylene oxide. In the course of these studies, we discovered that addition of MgO increased the yields and alleviated the need to remove the TMSOAc in vacuo, making the reactions easier to run. We also found that lower temperatures (−60 °C) improved the β-stereoselectivity, which ranged from 50:1 to 10:1 (β:α) for armed galactosyl, glucosyl, and mannosyl iodides, respectively.[38]

Cyclic ether additions to mannosyl iodides were especially impressive (Table 3). It is often difficult to achieve β-mannosidation because anchimeric assistance and the anomeric effect both favor α-glycosidation. E1 elimination of HI is another complicating factor when armed mannosyl iodides are heated. To mitigate glycal formation, a C-6 acetate was incorporated into donor **15α**. At the same time, a nonparticipating protecting group was required at C-2 to prevent anchimeric assistance. With donor **15α** in hand, a series of investigations was performed. In the case of thiirane addition (Table 3, entry 5), only the β-product was obtained,

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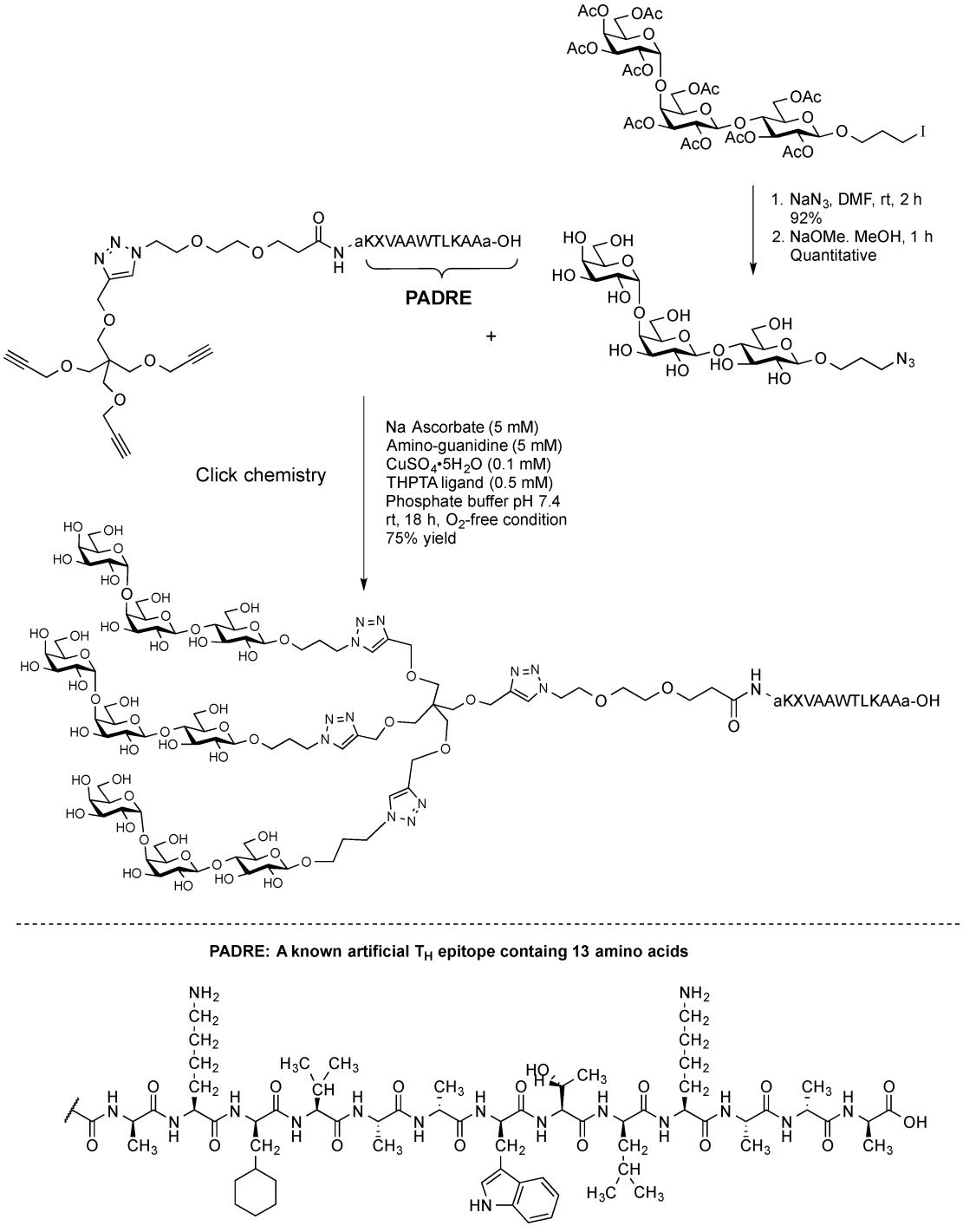

Figure 18. Synthesis of multivalent iGb3 as a potential vaccine candidate.

and the β:α ratio was as high as 30:1 for thiitane (entry 4). While the oxygen analogue (trimethylene oxide; entry 2) gave a 10:1 ratio, using the conformationally biased trans-fused cyclic acetal protecting group developed by the Crich group[39] led to exclusive formation of the β-product (entry 6). Computational studies indicated that these two compounds (**15α** and **16α**) have different transition state structures,[40] but nevertheless, functionally they are both predispositioned for β-mannoside formation.

Conditions have also been established for cyclic ether addition to disarmed glycosyl donors, in which case I_2 activation and microwave conditions are required (Table 4).[41] This protocol is especially useful because it works well for mono-, di-, and trisaccharides and the resulting iodoalkyl glycoconjugate can be easily substituted with other functional groups.

Hsieh and co-workers reported that Gb3 could be incorporated onto peptide scaffolds using this highly efficient process. In the reaction, per-*O*-acetylated Gb3 was first converted to the glycosyl iodide by the action of TMSI, and then trimethylene oxide (TMO), I_2, and butylhydroxytoluene (BHT) (to quench radical side reactions) were added. After 20 min of microwave irradiation, the iodoalkyl conjugate was obtained in 72% yield (Table 4, entry 5). The iodide was

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converted to the azide upon reaction with NaN_3, and the acetates were quantitatively removed using sodium methoxide in methanol. Subsequent cycloaddition with the trivalent alkyne bearing an immunogenic peptide afforded multivalent constructs of Gb3 as potential cancer vaccine candidates (Figure 18).

This sequence of reactions brings us full circle to where we started decades ago with reactions of per-*O*-acetylated glycosyl iodides, yet we have learned to harness the potential of these compounds to achieve complex syntheses in a step-efficient manner, even under metal-free conditions.

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Notes

The authors declare no competing financial interest.

Biography

Jacquelyn Gervay-Hague earned B.S. (1985) and Ph.D. (1990) degrees from The University of California, Los Angeles, working under the direction of Professor Michael E. Jung. She then moved to Yale University as a National Institutes of Health Postdoctoral Fellow to work with Professor Samuel J. Danishefsky. In 1992, Professor Gervay-Hague began her independent academic career in the Department of Chemistry at the University of Arizona. She was promoted to Associate Professor in 1998. Professor Gervay-Hague spent a sabbatical year as an on-site consultant at Roche Bioscience in Palo Alto, CA, in 2000. During that time, she was recruited to the University of California, Davis, where she was appointed Professor of Chemistry in 2001. Professor Gervay-Hague is a synthetic organic chemist. Her research program focuses on developing efficient methods to make pure glycoconjugates in order to understand the chemistry and physics of their interactions in biology.

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J.G.-H. acknowledges the many contributions of her co-workers to the studies described herein. The inventive mind of Michael J. Hadd coupled with the strong work ethic and creativity of Truc Nguyen, Abhijit Bhat, Darren Dabideen, and Mohamed El-Badry established that armed glycosyl iodides provide a solid chemical platform for organic synthesis. Son Lam led investigations into the challenging area of oligosaccharide syntheses showing that strategically placed acetate groups protect the glycosidic linkage. Fortuitously, Eric (Wenjun) Du, Suvarn Kulkarni, and Matthew Schombs happened to be in the lab at the same time, and together they exploited the full potential of armed glycosyl iodides. Their one-pot glycolipid syntheses paved the way for Ryan Davis, Hsiao-Wu Hsieh, and Huy Nguyen to break new ground in stereoselective glycosidations of cholesterol and ceramides, even with disarmed glycosyl iodides. Much of the scientific work was made possible by funding from the National Institutes of Health (R01GM-090262). J.G.-H. thanks Mr. Simon Park for assistance with graphics.

REFERENCES

(1) Schmidt, R. R. New Methods for the Synthesis of Glycosides and Oligosaccharides - Are There Alternatives to the Koenigs-Knorr Method. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212−235.

(2) Kronzer, F. J.; Schuerch, C. Use of 2,3,4,6-Tetra-Ortho-Benzyl-Alpha-D-Glycopyranosyl Iodides in Alpha-Glycoside Synthesis. *Carbohydr. Res.* **1974**, *34*, 71−78.

(3) Fischer, E.; Armstrong, E. F. Ueber die isomeren Acetohalogen-Derivate des Traubenzuckers und die Synthese der Glucoside. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 2885−2900.

(4) Fischer, E.; Fischer, H. Über einige Derivate des Milchzuckers und der Maltose und über zwei neue Glucoside. *Ber. Dtsch. Chem. Ges.* **1910**, *43*, 2521−2536.

(5) Hudson, C. S.; Kunz, A. Relations between rotatory power and structure in the sugar group. X.(2) The chloro-, bromo- and iodo-acetyl derivatives of lactose. *J. Am. Chem. Soc.* **1925**, *47*, 2052−2055.

(6) Thiem, J.; Meyer, B. Synthesen mit Iod- und Bromtrimethylsilan in der Saccharidchemie. *Chem. Ber.* **1980**, *113*, 3075−3085.

(7) *Reactivity Tuning in Oligosaccharide Assembly*; Fraser-Reid, B., López, J. C., Eds.; Topics in Current Chemistry, Vol. *301*; Springer Science and Business Media: New York, 2011.

(8) Pedersen, C. M.; Nordstrøm, L. U.; Bols, M. "Super armed" glycosyl donors: Conformational arming of thioglycosides by silylation. *J. Am. Chem. Soc.* **2007**, *129*, 9222−9235.

(9) Gervay, J.; Nguyen, T. N.; Hadd, M. J. Mechanistic studies on the stereoselective formation of glycosyl iodides: First characterization of beta-D-glycosyl iodides. *Carbohydr. Res.* **1997**, *300*, 119−125.

(10) Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. Halide Ion Catalyzed Glycosidation Reactions Syntheses of Alpha-Linked Disaccharides. *J. Am. Chem. Soc.* **1975**, *97*, 4056−4062.

(11) Franck, R. W.; Tsuji, M. alpha-C-galactosylceramides: Synthesis and immunology. *Acc. Chem. Res.* **2006**, *39*, 692−701.

(12) Bhat, A. S.; Gervay-Hague, J. Efficient syntheses of beta-cyanosugars using glycosyl iodides derived from per-O-silylated mono- and disaccharides. *Org. Lett.* **2001**, *3*, 2081−2084.

(13) Kulkarni, S. S.; Gervay-Hague, J. Efficient synthesis of a C-analogue of the immunogenic bacterial glycolipid BbGL2. *Org. Lett.* **2006**, *8*, 5765−5768.

(14) Thota, V. N.; Gervay-Hague, J.; Kulkarni, S. S. Synthesis of beta-C-galactosyl D- and L-alanines. *Org. Biomol. Chem.* **2012**, *10*, 8132−8139.

(15) Gervay, J.; Hadd, M. J. Anionic additions to glycosyl iodides: Highly stereoselective syntheses of beta-, C-, N-, and O-glycosides. *J. Org. Chem.* **1997**, *62*, 6961−6967.

(16) Lam, S. N.; Gervay-Hague, J. Efficient route to 2-deoxy beta-O-aryl-D-glycosides via direct displacement of glycosyl iodides. *Org. Lett.* **2003**, *5*, 4219−4222.

(17) Fréchet, J. M.; Schuerch, C. Solid-Phase Synthesis of Oligosaccharides 0.2. Steric Control by C-6 Substituents in Glucoside Syntheses. *J. Am. Chem. Soc.* **1972**, *94*, 604−609.

(18) Lam, S. N.; Gervay-Hague, J. Solution-phase hexasaccharide synthesis using glucosyl iodides. *Org. Lett.* **2002**, *4*, 2039−2042.

(19) Lam, S. N.; Gervay-Hague, J. Solution- and solid-phase oligosaccharide synthesis using glucosyl iodides: a comparative study. *Carbohydr. Res.* **2002**, *337*, 1953−1965.

(20) Lam, S. N.; Gervay-Hague, J. Glycal scavenging in the synthesis of disaccharides using mannosyl iodide donors. *J. Org. Chem.* **2005**, *70*, 2387−2390.

(21) Lam, S. N.; Gervay-Hague, J. Efficient synthesis of Man(2), Man(3), and Man(5) oligosaccharides, using mannosyl iodide donors. *J. Org. Chem.* **2005**, *70*, 8772−8779.

(22) Natori, T.; Koezuka, Y.; Higa, T. Agelasphins, novel α-galactosylceramides from the marine sponge Agelas mauritianus. *Tetrahedron Lett.* **1993**, *34*, 5591−5592.

(23) Natori, T.; Morita, M.; Akimoto, K.; Koezuka, Y. Agelasphins, novel antitumor and immunostimulatory cerebrosides from the marine sponge Agelas mauritianus. *Tetrahedron* **1994**, *50*, 2771−2784.

(24) Kawano, T.; Cui, J.; Koezuka, Y.; Toura, I.; Kaneko, Y.; Motoki, K.; Ueno, H.; Nakagawa, R.; Sato, H.; Kondo, E.; Koseki, H.; Taniguchi, M. CD1d-Restricted and TCR-Mediated Activation

DOI: 10.1021/acs.accounts.5b00357
Acc. Chem. Res. 2016, 49, 35−47

of Vα14 NKT Cells by Glycosylceramides. *Science* **1997**, *278*, 1626−1629.
(25) Broxmeyer, H. E.; Dent, A.; Cooper, S.; Hangoc, G.; Wang, Z. Y.; Du, W.; Gervay-Hague, J.; Sriram, V.; Renukaradhya, G. J.; Brutkiewicz, R. R. A role for natural killer T cells and CD1d molecules in counteracting suppression of hematopoiesis in mice induced by infection with murine cytomegalovirus. *Exp. Hematol.* **2007**, *35*, 87−93.
(26) Brutkiewicz, R. R.; Willard, C. A.; Gillett-Heacock, K. K.; Pawlak, M. R.; Bailey, J. C.; Khan, M. A.; Nagala, M.; Du, W.; Gervay-Hague, J.; Renukaradhya, G. J. Protein kinase C delta is a critical regulator of CD1d-mediated antigen presentation. *Eur. J. Immunol.* **2007**, *37*, 2390−2395.
(27) McReynolds, K. D.; Hadd, M. J.; Gervay-Hague, J. Synthesis of biotinylated glycoconjugates and their use in a novel ELISA for direct comparison of HIV-1 gp120 recognition of GalCer and related carbohydrate analogues. *Bioconjugate Chem.* **1999**, *10*, 1021−1031.
(28) Du, W. J.; Gervay-Hague, J. Efficient synthesis of alpha-galactosyl ceramide analogues using glycosyl iodide donors. *Org. Lett.* **2005**, *7*, 2063−2065.
(29) Du, W.; Kulkarni, S. S.; Gervay-Hague, J. Efficient, one-pot syntheses of biologically active alpha-linked glycolipids. *Chem. Commun.* **2007**, 2336−2338.
(30) Schombs, M.; Park, F. E.; Du, W. J.; Kulkarni, S. S.; Gervay-Hague, J. One-Pot Syntheses of Immunostimulatory Glyeolipids. *J. Org. Chem.* **2010**, *75*, 4891−4898.
(31) Hsieh, H. W.; Schombs, M. W.; Gervay-Hague, J. Integrating ReSET with Glycosyl Iodide Glycosylation in Step-Economy Syntheses of Tumor-Associated Carbohydrate Antigens and Immunogenic Glycolipids. *J. Org. Chem.* **2014**, *79*, 1736−1748.
(32) Hsieh, H. W.; Schombs, M. W.; Witschi, M. A.; Gervay-Hague, J. Regioselective Silyl/Acetate Exchange of Disaccharides Yields Advanced Glycosyl Donor and Acceptor Precursors. *J. Org. Chem.* **2013**, *78*, 9677−9688.
(33) Morales-Serna, J. A.; Diaz, Y.; Matheu, I.; Castillon, S. Efficient Synthesis of beta-Glycosphingolipids by Reaction of Stannylceramides with Glycosyl Iodides Promoted by TBAI/AW 300 Molecular Sieves. *Eur. J. Org. Chem.* **2009**, *2009*, 3849−3852.
(34) Shimamura, M. Immunological Functions of Steryl Glycosides. *Arch. Immunol. Ther. Exp.* **2012**, *60*, 351−359.
(35) Davis, R. A.; Fettinger, J. C.; Gervay-Hague, J. Tandem Glycosyl Iodide Glycosylation and Regioselective Enzymatic Acylation Affords 6-*O*-Tetradecanoyl-alpha-D-cholesterylglycosides. *J. Org. Chem.* **2014**, *79*, 8447−8452.
(36) Kulkarni, S. S.; Gervay-Hague, J. Two-Step Synthesis of the Immunogenic Bacterial Glycolipid BbGL1. *Org. Lett.* **2008**, *10*, 4739−4742.
(37) Dabideen, D. R.; Gervay-Hague, J. Unique reactions of glycosyl iodides with oxa- and thiocycloalkane acceptors. *Org. Lett.* **2004**, *6*, 973−975.
(38) El-Badry, M. H.; Gervay-Hague, J. Thermal effect in beta-selective glycosylation reactions using glycosyl iodides. *Tetrahedron Lett.* **2005**, *46*, 6727−6728.
(39) Crich, D. Mechanism of a Chemical Glycosylation Reaction. *Acc. Chem. Res.* **2010**, *43*, 1144−1153.
(40) El-Badri, M. H.; Willenbring, D.; Tantillo, D. J.; Gervay-Hague, J. Mechanistic studies on the stereoselective formation of beta-mannosides from mannosyl iodides using alpha-deuterium kinetic isotope effects. *J. Org. Chem.* **2007**, *72*, 4663−4672.
(41) Hsieh, H. W.; Davis, R. A.; Hoch, J. A.; Gervay-Hague, J. Two-Step Functionalization of Oligosaccharides Using Glycosyl Iodide and Trimethylene Oxide and Its Applications to Multivalent Glycoconjugates. *Chem. - Eur. J.* **2014**, *20*, 6444−6454.

DOI: 10.1021/acs.accounts.5b00357
Acc. Chem. Res. 2016, 49, 35−47