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Two-Step Functionalization of Oligosaccharides Using Glycosyl Iodide and Trimethylene Oxide and Its Applications to Multivalent Glycoconjugates

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Abstract

Oligosaccharide conjugates, such as glycoproteins and glycolipids, are potential chemotherapeutics and also serve as useful tools for understanding the biological roles of carbohydrates. With many modern isolation and synthetic technologies providing access to a wide variety of free sugars, there is increasing need for general methodologies for carbohydrate functionalization. Herein, we report a two-step methodology for the conjugation of per-*O*-acetylated oligosaccharides to functionalized linkers that can be used for various displays. Oligosaccharides obtained from both synthetic and commercial sources were converted to glycosyl iodides and activated with I_2 to form reactive donors that were subsequently trapped with trimethylene oxide to form iodopropyl conjugates in a single step. The terminal iodide served as a chemical handle for further modification. Conversion into the corresponding azide followed by copper-catalyzed azide-alkyne cycloaddition afforded multivalent glycoconjugates of Gb3 for further investigation as anti-cancer therapeutics.

Keywords

carbohydrates; functionalization; glycosylation; glycosyl iodides; multivalent glycoconjugates

Introduction

Glycosylation is one of the most abundant post-translational modifications in eukaryotic cells, leading to structurally diverse biomolecules that mediate cellular interactions in processes ranging from cancer progression to immune response.^[1] With no corresponding genetic code and a plethora of possible structures,^[2] understanding the functions of complex oligosaccharides, as well as isolating them from natural resources, is challenging. Obtaining sufficient amounts of pure and homogenous oligosaccharides^[3] and further modifying isolated oligosaccharides typically requires multiple synthetic steps and purifications. These

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difficulties have directed research efforts toward the chemical synthesis of oligosaccharides, with a focus on developing methodologies that reduce the number of protecting-group manipulations, control stereochemistry of the glycosidic bonds, and eliminate labor-intensive purifications.

Contemporary advances in synthesis emphasize the efficiency of preparing complex oligosaccharides by using automated synthesis,^[4] one-pot procedures,^[5] and chemoenzymatic processes.^[6] One of the most exciting developments is exemplified in the multi-gram production of iGb3, which was obtained as the free trisaccharide by using *E. coli*.^[7] This approach holds enormous potential and provides impetus for the concomitant development of generalized methods for conjugating free sugars. Indeed, Castillon et al. recently showed that commercially available iGb3 could be per-*O*-acetylated (per-*O*-Ac) and converted into a glycosyl iodide, which underwent glycosylation with the tin acetal of ceramide (Scheme 1).^[8] This finding encouraged us to explore a similar strategy to introduce functionalized linkers that could be useful tools for multivalent display of carbohydrate head groups.^[9] We imagined a conjugation methodology that would begin with readily available per-*O*-acetylated oligosaccharides derived from either synthetic or natural sources. Activation of the anomeric acetate, and subsequent trapping with a suitable acceptor, would afford the corresponding glycoconjugate after removal of the acetate protecting groups. In this manner, various sugars could be functionalized for the preparation of sugar arrays for high-throughput screening^[10] and applications in chemotherapeutics development.^[11] We were especially interested in controlling the stereochemistry at the anomeric position because the chiral center may affect bioassay results.^[12]

Based upon earlier results from our lab, our efforts focused on the idea of using trimethylene oxide (TMO) as an acceptor. We had reported the unique reactivity of armed ether-protected glycosyl iodides with oxa- and thiocycloalkane acceptors (Scheme 2)^[13] to yield iodoalkyl conjugates with high stereo-control. The S_N2-like displacement provided a convenient way to functionalize fully protected sugars in two consecutive steps.

Preparing benzyl-protected monosaccharides with an anomeric acetate is relatively straightforward, but requires several steps and is not well suited for oligosaccharides. Per-*O*-silylation of oligosaccharides can be achieved in a single step, but conversion of the anomeric silyl ether into the corresponding glycosyl iodide is often complicated by competing cleavage of internal glycosidic linkages, resulting in a complex mixture of degradation products. In contrast, acetylation is a commonly used protecting group for carbohydrate isolation and purification.^[14] The electron-withdrawing nature of acetyl protecting groups stabilizes glycosidic linkages and provides well-resolved proton NMR spectra, facilitating quality control and compound characterization. However, a major challenge of utilizing ester-protected glycosyl iodides is their lack of reactivity. Per-*O*-acetylated glycosyl iodides have been shown to survive aqueous extractions, column chromatography, and are even so stable that X-ray crystal structures have been obtained.^[15] The “disarmed”^[16] nature of ester-protected glycosyl iodides necessitates using a promoter (Lewis acids or metals) or heat to afford reasonable yields of the glycosylation products. In addition, C-2 ester-protected glycosyl donors often form orthoesters as a side reaction.^[17] For example, in the case of iGb3 noted above, stannyl alkoxides were needed for efficient

addition and a Lewis acid was required to avoid orthoester formation.^[8, 18] Other groups have successfully prepared β -linked glycosides from ester-protected glycosyl iodides by using various activating reagents, including NIS/I₂/TMSOTf (NIS=*N*-iodosuccinimide, Tf=triflate),^[17] I₂/DDQ (DDQ=2,3-dichloro-5,6-dicyano-1,4-benzoquinone),^[19] and NBS/Zn (NBS=*N*-bromosuccinimide) salts.^[20] These methods all have their merits, but the substrates were limited to monosaccharides and, in most cases, long reaction times were required. These findings suggested to us that a rapid and generalized conjugation methodology for ester-protected glycosyl iodides is still needed.

Herein, we report an anomeric-functionalization methodology that combines per-*O*-Ac glycosyl iodide formation and I₂-promoted TMO addition under microwave irradiation (Figure 1). The formed β -glycosides were functionalized with an iodopropyl linker at the anomeric position, serving as a useful chemical handle for further manipulations. With the functionalized glycosides in hand, multivalent glycoconjugates and glycopeptides were constructed to demonstrate the utility of this methodology.

Results and Discussion

Previous reports from our lab showed per-*O*-TMS glycosyl iodides to be highly reactive donors, which when coupled with various acceptors form glycoconjugates with exquisite stereo-control.^[21] Although the methodology worked well with monosaccharide-derived iodides, TMSI-promoted glycosylation of oligosaccharides was complicated and consistently led to a complex reaction mixture in a short period of time (<30 min). Crude MS analysis revealed bond cleavage, trans-silylation, and desilylation products.^[21a, 22] Mindful of the fact that glycosyl iodide reactivity is highly attenuated by acetate protecting groups, we prepared C-6,6' acetate analogues **1** and **2** by using regioselective silyl exchange technology (ReSET) of per-*O*-silyl lactose.^[22,23] We hoped that incorporation of the acetates would protect the inter-glycosidic linkage from cleavage and that the presence of the remaining silyl ethers would sufficiently activate the iodide. Remarkably, when compound **1** or **2** was treated with TMSI, the corresponding iodide (**3**) was obtained without internal-bond cleavage or silyl migration.^[22] Trimethylene oxide (TMO) was then introduced to the iodide and the reaction mixture was kept in the dark for 36 h. After acidic methanolysis to remove the silyl protecting groups and reacetylation for isolation and characterization purposes, the β -TMO adduct (**4**) was obtained in 56% yield over four steps (Scheme 3). The glycosylation required no promoter or heat, indicating that iodide **3** is a highly reactive donor.

Encouraged by these results, we turned our attention to per-*O*-acetylated oligosaccharides. Investigations began with the study of β -lactose octaacetate (**5 β**), which was treated with TMSI and monitored by NMR spectroscopy to observe iodide formation. Unlike benzylated or silylated analogues, which formed the corresponding α -iodides within minutes, **5 β** first formed **6 β** and then gradually anomerized to the α -iodide (**6 α**) over six to eight hours.^[24] When treating the β -iodide (**6 β**) with 1.5 equivalents of TMO at room temperature, the corresponding orthoester (**7**) was obtained in 88% yield after eight hours. In contrast, the α -iodide (**6 α**) was unreactive at room temperature; even after five days at 40°C only a low yield of the desired glycoconjugate (**4**) was produced, along with unreacted **6 α** (Scheme 4).

These results led us to conclude that a suitable promoter that avoids orthoester formation is necessary to optimize the production of **4**.

Several groups have observed orthoester formation during glycosylation studies.^[15b, 19b, 20a, 25] Common methods to circumvent this side reaction include introducing a Lewis acid or applying heat to the reaction.^[20b, 26] Initially, we followed the method reported by Stachulsky et al.,^[15b] utilizing NIS and triflic acid, but only 20–30% yield of **4** was obtained. We then explored I₂-promoted glycosidation^[19] in combination with microwave irradiation to heat the reaction more efficiently.^[27] Having observed significant reactivity differences between the lactosyl iodide anomers, our study evaluated I₂ activation of **6α** and **6β** independently (Scheme 5). In each case, commercially available per-*O*-acetylated lactose was treated with TMSI to generate the lactosyl iodide, which was monitored by real-time NMR spectroscopy^[24] or TLC analysis; the two lactosyl iodide anomers are distinguishable by TLC analysis (see the Supporting Information, Figure S1). Once each iodide was in hand, TMO and I₂ were added to the flask and the reaction was subjected to microwave irradiation (Scheme 5). We hypothesized that the anomers might have different reactivity and glycosylation profiles, but the yields were similar, being only slightly higher for the β-anomer. Reactions of **6α** and **6β** with TMO gave target molecule **4** in 51–57% yield, di-TMO adduct **8** in 12–15% yield, and per-*O*-Ac lactose **5** in 9–13% yield, suggesting that both anomers proceed through a common reaction intermediate (Scheme 5).

Based upon studies by Field et al.,^[19b] Murakami et al.,^[20a] and Demchenko et al.,^[28] a proposed mechanism of I₂-promoted TMO addition is shown in Figure 2. Both α-iodide and β-iodide can be activated by I₂, forming a better leaving group at the anomeric position. The C-2 acetate is positioned to displace the activated β-triiodide intramolecularly, forming the α-face-blocking oxonium ion. Direct attack by TMO at the anomeric position affords the β-glycoside after iodide opening of the activated oxetane. The small amount of α-TMO-adduct formation may result from TMO attack on the α-face, but this occurs to a lesser extent because neighboring-group participation predominates.

The di-TMO adduct (**8**) had a similar *R_f* value as the target mono-TMO adduct (**4**), making isolation difficult and lowering the yield. Different reaction temperatures (0–70°C) were screened, but compound **8** was observed in all instances. We reasoned that the side product could be forming by means of a radical side reaction and decided to introduce butylated hydroxytoluene (BHT), a common radical scavenger,^[29] to suppress possible radical formation (Table 1). When using CH₂Cl₂ as solvent, compound **4** was obtained in 61% yield, but trace amounts of **8** were still observed (Table 1, entry 1). When changing the solvent to CHCl₃, compound **4** was isolated in 51% yield and compound **8** did not form, as evidenced by TLC and crude MS analysis. However, the reaction in CHCl₃ also gave the α-TMO adduct in trace amounts. This adduct usually co-eluted with per-*O*-Ac lactose (Table 1, entry 2). Using benzene as the solvent led to target molecule **4** in 54% yield, but it took longer to generate the iodide and the amount of α-TMO-adduct side product slightly increased (Table 1, entry 3).

Taking purification and reaction time into consideration, we decided to use 1.2 equivalents of TMSI in CHCl₃ for iodide formation, 1.5 equivalents of TMO, 1.0 equivalent of I₂, and 50 mol% of BHT, under microwave irradiation at 70°C for 20 min (Table 1 entry 2), as the standard conditions to explore the methodology with different per-*O*-Ac sugars (Table 2). Commercially available per-*O*-Ac monosaccharides (**9β**, **10β**, **10α**, and **11α**) were first subjected to the glycosyl iodide/TMO addition reaction. Iodide formation time was established at the time the per-*O*-acetylated starting material had been completely consumed. In general, the β-acetates (**9β** and **10β**) reacted faster than the α-acetates (**10α** and **11α**) in TMSI-promoted iodide formation,^[30] and led to slightly better yields of the corresponding TMO adduct (Table 2, entries 1 and 2 versus Table 2 entries 3 and 4). β-Glucose pentaacetate (**9β**) and β-galactose pentaacetate (**10β**) reacted to form the corresponding iodides in less than one hour, and afforded the corresponding TMO adducts (**13** and **14**) in 84 and 87% yields, respectively (Table 1, entries 1 and 2). Galactose was slightly more reactive than glucose in both iodide formation and TMO glycosidation. In contrast, **10α** required a longer reaction time (2.5 h) to be completely consumed in the iodide formation step. After I₂-promoted TMO glycosylation, the reaction afforded **14** in a slightly lower yield of 79% (Table 1, entry 3). We attributed the increased reactivity of the β-anomers to neighboring-group participation. Nevertheless, using either α- or β-acetates gave rise to the same β-TMO adduct (**14**), providing further evidence of common reaction intermediates. The methodology was extended to include α-mannose pentaacetate (**11α**). Iodide formation of **11α** required 4.5 h to complete, and the corresponding α-TMO adduct (**15**) was obtained in 71% yield (Table 2, entry 4).

Not only could we apply the methodology to the monosaccharides and per-*O*-Ac lactose (**5β**), but also the per-*O*-Ac globotriaose (**12**), which was obtained from ReSET modules.^[22] Compound **12** was a mixture of anomeric acetates in a ratio of α/β=1:5. The iodide formation was completed within one hour and led to the corresponding β-TMO adduct (**16**) in 72% yield. It is worth noting that the per-*O*-Ac globotriaose (α/β=1:1) starting material could be isolated in the same reaction. Based on the recovery of starting material, the yield of the desired β-TMO adduct (**16**) was 81% (Table 2, entry 6). These encouraging results illustrate the broad potential for modification of other per-*O*-acetylated oligosaccharides, whether obtained commercially or isolated from a natural source.

With the iodopropyl-containing oligosaccharides in hand, we started exploring further applications of the functionalized oligosaccharides. Both compounds **4** and **16** were transformed to azide compounds **17** and **19** in over 90% yields. After global deacetylation by using NaOMe/MeOH, compounds **18** and **20** were obtained in quantitative yields and contained useful “chemical handles” for further modifications (Scheme 6).

Cu-catalyzed azide–alkyne cycloaddition (CuAAC)^[31] allowed us to construct multivalent glycoconjugates. Tetraalkyne **21**^[32] was chosen as the core structure for the display. When azido lactose **17** was stirred with **21** in DMF/H₂O with CuSO₄·5H₂O, sodium ascorbate and tris-(benzyltriazolylmethyl)amine (TBTA),^[33] the protected lactose tetramer was obtained after 20 h. The protected lactose tetramer was treated with NaOMe/MeOH for one hour to afford the fully deprotected lactose tetramer (**22**) in 78% yield over two steps. When using

azido triglobotriaose **19** as the reagent, globotriaose tetramer **23** was obtained in 60% yield after the two-step CuAAC-deprotection procedure (Scheme 7). Both compounds **22** and **23** were water soluble, and served as important substrates for ongoing biological studies in our laboratory.

Previously, our lab demonstrated using copper-catalyzed azide-alkyne cycloaddition to construct a synthetic trivalent mimotope of HIV gp120 conjugated to pan allelic HLA DR binding epitope (PADRE), which displayed enhanced binding affinity to HIV-1-neutralizing monoclonal antibody, MAb b12.^[32a, 34] PADRE,^[35] a known artificial T-helper (T_H) epitope containing thirteen amino acids, has not only been used in HIV research, but also in cancer-vaccine development as an immunogenic carrier to stimulate the immune response. The combination of tumor-associated carbohydrate antigens (TACAs) and PADRE has been demonstrated by Dumy et al. as a new family of immunostimulants.^[36] This new class of multivalent glycoconjugates could also provide promising antitumor-vaccine candidates in the future.^[11c, 37] Applying the TMO-addition concept and click chemistry, trimeric globotriaose-PADRE conjugate **25** was constructed as a potential cancer-vaccine candidate (Scheme 8). Following the published protocol,^[32, 38] compounds **20** and **24**^[32] were reacted with sodium ascorbate, aminoguanidine, CuSO₄·5H₂O, and tris(3-hydroxypropyltriazolylmethyl)amine ligand (THPTA) in PBS buffer pH 7.4 containing 5% DMSO, under O₂-free conditions overnight. After desalting, purification, and lyophilization procedures, the trimeric globotriaose-PADRE conjugate (**25**) was obtained as a fluffy white solid in 75% yield, and was analyzed by MALDI-TOF MS and analytical HPLC (see the Supporting Information, Figure S2 and S3). The biological activity of this construct is currently under investigation.

Conclusion

A rapid, step-economical, one-pot methodology for the functionalization of per-*O*-Ac oligosaccharides has been developed. The methodology integrates in situ iodide formation by using TMSI and I₂-promoted TMO addition to form iodopropyl-linked per-*O*-Ac β-glycosides. A proposed mechanism for the I₂-promoted TMO addition by using per-*O*-Ac iodide highlights neighboring-group participation at the C-2 position. The mechanism explains the observed stereochemistry, and microwave irradiation hastens the process allowing efficient conjugation within 20 min, compared to hours or days in previously reported methods.

The iodopropyl linker serves as a useful chemical handle for further manipulation. One application is to transform the terminal iodide into the corresponding azide for click chemistry. With the proper alkyne cores, multivalent glycoconjugates were readily prepared in respectable yields. These multivalent glycoconjugates (**23** and **25**) contain tumor-associated carbohydrate antigens (TACAs) and immuno-stimulating peptides (PADRE), presenting potential applications as cancer-vaccine or adjuvant candidates.

Experimental Section

3-Iodopropyl (2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl- β -D-glucopyranoside (**4**)

Method A—In an oven-dried NMR tube, compound **2** (66 mg, 0.08 mmol) was dissolved in dry $[D_6]$ benzene (0.8 mL). After TMSI (13 μ L, 0.10 mmol) was introduced to the reaction vessel, the reaction was kept at 10°C and gradually warmed to room temperature over 4–5 h. The reaction was monitored by proton NMR spectroscopy until the corresponding iodide (**6**) formed in situ. Next, trimethylene oxide (TMO, 16 μ L, 0.24 mmol) was added to the reaction vessel and the reaction was allowed to react in the dark for 36 h. The reaction mixture was then azeotroped with dry benzene (5 mL \times 3) to remove excess reagent and solvent. The resulting residue was dissolved in MeOH (5 mL) and stirred with the Dowex acidic resin (pH \approx 3) at room temperature. After 2 h, the resin was filtered and the solvent was evaporated under reduced pressure to afford a viscous orange oil. The resulting oil was then dissolved in CH_2Cl_2 (1 mL) under Ar. Ac_2O (1.0 mL), NEt_3 (1.5 mL), and catalytic 4-dimethylaminopyridine (DMAP, \approx 2 mg) were then added to the solution, and the reaction mixture was stirred at room temperature overnight. Next, the reaction mixture was concentrated under reduced pressure, and the resulting oil was purified by using flash column chromatography (ethyl acetate/*n*-hexane=50:50) to afford compound **4** (36 mg, 56% yield) as a white foam (Scheme 2).

Method B—To a 10 mL microwave reaction vessel, compound **5b** (150 mg, 0.22 mmol) was dissolved in anhydrous CH_2Cl_2 (2 mL) and TMSI (39 μ L, 0.27 mmol) was added under Ar. After 50 min, TLC analysis revealed the complete consumption of starting material. TMO (22 μ L, 0.33 mmol) and I_2 (56 mg, 0.22 mmol) were then added to the reaction mixture. The reaction vessel was sealed and subjected to microwave irradiation for 20 min (standard mode, 70°C, \approx 40 psi, \approx 70 W). The reaction mixture was then diluted with ethyl acetate (30 mL), and washed with saturated $Na_2S_2O_3$ (aq.) (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 , concentrated under reduced pressure, and purified by gradient flash column chromatography (ethyl acetate/*n*-hexane = 40:60 to 50:50) to obtain compound **4** (99 mg, 57% yield), compound **8** (25 mg, 13% yield), and **5** (13 mg, 9% yield) as white foams (Scheme 4). Data for compound **4**: R_f =0.43 (ethyl acetate/*n*-hexane=1:1).

$[\alpha]_D^{20}=+1.1$ (c0.47, $CHCl_3$); 1H NMR (600 MHz, $CDCl_3$): δ = 5.35 (d, J = 3.5 Hz, 1H, H-4'), 5.20 (appt. t, J = 9.5 Hz, 1H, H-3), 5.11 (dd, J =10.4, 7.9 Hz, 1H, H-2'), 4.95 (dd, J =10.4, 3.5 Hz, 1H, H-3'), 4.88 (dd, J = 9.5, 8.0 Hz, 1H, H-2), 4.15–4.47 (m, 3H, H-1, H-1', H-6a), 4.19–4.05 (m, 3H, H-6ab, H-6'b), 3.92–3.86 (m, 2H, OCH, H-5), 3.79 (appt. t, J = 9.5 Hz, 1H, H-4), 3.62–3.56 (m, 2H, CH_2I), 2.15 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 6H), 1.97 ppm (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 170.4, 170.3, 170.2, 170.1, 169.8, 169.7, 169.1, 101.1, 100.7, 76.3, 72.7, 72.6, 71.6, 71.0, 70.7, 69.2, 69.1, 66.6, 61.9, 60.8, 32.8, 20.9, 20.85, 20.82, 20.67, 20.66, 20.65, 20.5, 3.0 ppm; HRMS (ESI-ion trap): m/z calcd for $C_{29}H_{41}IO_{18} + NH_4^+$: 822.1676 [$M + NH_4^+$]; found: 822.1692.

α -(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl- β -D-glucopyranosyl iodide (6 α)

In an oven-dried NMR tube, compound **5 β** (100 mg, 0.15 mmol) was dissolved in dry CDCl_3 (0.5 mL). After TMSI (23 μL , 0.16 mmol) was introduced at room temperature, the reaction was monitored by proton NMR spectroscopy until the corresponding α -iodide (**6 α**) formed in situ (≈ 6 h). $R_f=0.15$ (ethyl acetate/*n*-hexane = 2:3); In situ ^1H NMR (600 MHz, CDCl_3): δ = 6.92 (d, J = 4.3 Hz, 1H, H-1), 5.47 (appt. t, J = 9.5 Hz, 1H, H-3), 5.36 (d, J = 3.4 Hz, 1H, H-4'), 5.13 (dd, J = 10.4, 7.9 Hz, 1H, H-2'), 4.97 (dd, J = 10.4, 3.5 Hz, 1H, H-3'), 4.57–4.44 (m, 2H, H-1', H-6a), 4.24–4.04 (m, 5H, H-2, H-5', H-6b, H-6'a), 3.97 (dd, J = 10.1, 2.2 Hz, 1H, H-5), 3.90–3.87 (m, 2H, H-4, H-6'b), 2.16 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H), 2.07–2.06 (m, 9H), 1.97 ppm (s, 3H); In situ ^{13}C NMR (150 MHz, CDCl_3): δ = 170.3, 170.2, 170.1, 169.8, 169.2, 168.9, 164.8, 100.8, 75.7, 74.7, 72.6, 71.1, 71.0, 70.8, 70.6, 69.1, 66.6, 60.9, 60.8, 29.7, 29.3, 20.8, 20.79, 20.68, 20.67, 20.6, 20.5 ppm; HRMS (ESI-ion trap): m/z calcd for $\text{C}_{26}\text{H}_{35}\text{IO}_{17} + \text{NH}_4^+$: 764.1257 [$M + \text{NH}_4^+$]; found: 764.1279.

β -(2,3,4,6-Tetra-O-acetyl- β -D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl- β -D-glucopyranosyl iodide (6 β)

In an oven-dried NMR tube, compound **5 β** (100 mg, 0.15 mmol) was dissolved in dry CDCl_3 (0.5 mL). After TMSI (23 μL , 0.16 mmol) was introduced at room temperature, the reaction was monitored by proton NMR spectroscopy until the corresponding β -iodide (**6 β**) formed in situ (≈ 40 min). $R_f=0.50$ (ethyl acetate/*n*-hexane = 2:3); In situ ^1H NMR (400 MHz, CDCl_3): δ = 5.73 (d, J = 8.8 Hz, 1H, H-1), 5.29 (d, J = 2.9 Hz, 1H, H-4'), 5.18 (appt. t, J = 8.8 Hz, 1H, H-2), 5.10–5.01 (m, 2H, H-3, H-2'), 4.91 (dd, J = 10.4, 3.3 Hz, 1H, H-3'), 4.52–4.34 (m, 2H, H-1', H-6a), 4.13–3.99 (m, 3H, H-6b, H-6'ab), 3.93 (appt. t, J = 9.5 Hz, 1H, H-4), 3.85 (appt. t, J = 6.7 Hz, 1H, H-5'), 3.64 (dd, J = 10.0, 3.3 Hz, 1H, H-5), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.91 ppm (s, 3H); In situ ^{13}C NMR (100 MHz, CDCl_3): δ = 170.2, 170.1, 170.0, 169.9, 168.5, 169.0, 168.9, 100.9, 78.1, 75.3, 75.2, 72.6, 70.8, 70.5, 68.9, 66.5, 61.7, 60.7, 56.6, 20.7, 20.65, 20.61, 20.51, 20.50, 20.46, 20.3 ppm; HRMS (ESI-ion trap): m/z calcd for $\text{C}_{26}\text{H}_{35}\text{IO}_{17} + \text{NH}_4^+$: 764.1257 [$M + \text{NH}_4^+$]; found: 764.1264.

Per-O-Ac lactose TMO orthoester (7)

In an oven-dried NMR tube, compound **5 β** (50 mg, 0.07 mmol) was dissolved in dry CHCl_3 (0.5 mL). After TMSI (11 μL , 0.08 mmol) was introduced, the reaction was monitored by proton NMR spectroscopy until the starting material was completely consumed. Once the β -iodide (**6 β**) formed, TMO (16 μL , 0.24 mmol) was added to the reaction vessel and the reaction was allowed to react in the dark for 8 h. The reaction mixture was then azeotroped with dry benzene to remove the excess solvent and reagents. The resulting residue was purified by using flash column chromatography (ethyl acetate/*n*-hexane = 60:40) to afford compound **7** (52 mg, 88% yield) as a white foam. $R_f=0.46$ (ethyl acetate/*n*-hexane = 3:2); ^1H NMR (600 MHz, CDCl_3): δ = 5.67 (d, J = 5.1 Hz, 1H, H-1), 5.55–5.53 (m, 1H, H-3), 5.38 (d, J = 2.8 Hz, 1H, H-4'), 5.18 (dd, J = 10.4, 8.1 Hz, 1H, H-2'), 5.00 (dd, J = 10.4, 3.5 Hz, 1H, H-3'), 4.61 (d, J = 8.0 Hz, 1H, H-1'), 4.32 (dd, J = 4.6, 2.2 Hz, 1H, H-2), 4.25 (dd, J = 12.0, 2.3 Hz, 1H, H-6a), 4.15–4.08 (m, 3H, H-6b, H-6'ab), 3.94 (appt. t, J = 6.8 Hz, 1H,

H-5'), 3.86–3.82 (m, 1H, H-5), 3.64 (d, $J = 9.6$ Hz, 1H, H-4), 3.60–3.52 (m, 2H, OCH₂), 3.24 (appt. t, $J = 6.6$ Hz, 2H, CH₂I), 2.17 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.97 (s, 3H), 1.73 ppm (s, 3H); ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.7, 170.3, 170.2, 170.1, 169.4, 169.1, 121.4, 102.4, 96.8, 72.8, 70.9, 70.8, 69.9, 68.8, 66.9, 66.8, 63.4, 62.9, 60.9, 32.9, 20.9, 20.8, 20.7, 20.68, 20.64, 20.5, 20.3, 3.0$ ppm; HRMS (ESI-ion trap): m/z calcd for C₂₉H₄₁IO₁₈ + NH₄⁺: 822.1676 [$M + \text{NH}_4^+$]; found: 822.1696.

3-(3-Iodopropoxy)propyl (2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl- β -D-glucopyranoside (8)

Compound **8** (25 mg, 13%) was obtained as a white foam (see compound **4**, method B for reaction and purification conditions). $R_f = 0.41$ (ethyl acetate/*n*-hexane = 1:1); ¹H NMR (600 MHz, CDCl₃): $\delta = 5.35$ (d, $J = 2.5$ Hz, 1H, H-4'), 5.22–5.17 (m, 1H, H-3), 5.11 (dd, $J = 10.3, 8.0$ Hz, 1H, H-2'), 4.95 (dd, $J = 10.3, 3.4$ Hz, 1H, H-3'), 4.91–4.86 (m, 1H, H-2), 4.48 (dd, $J = 14.9, 6.7$ Hz, 3H, H-1, H-1', H-6a), 4.20–4.04 (m, 3H, H-6b, H-6'ab), 3.95–3.85 (m, 2H, H-5', OCH), 3.79 (appt. t, $J = 9.4$ Hz, 1H, H-4), 3.68–3.54 (m, 2H, H-5, OCH), 3.49–3.44 (m, 4H, OCH₂×2), 3.26 (appt. t, $J = 6.7$ Hz, 2H, CH₂I), 2.15 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.04 (s, 6H), 1.96 (s, 3H), 1.88–1.78 ppm (m, 2H); ¹³C NMR (150 MHz, CDCl₃): $\delta = 170.4, 170.3, 170.1, 170.0, 169.8, 169.6, 169.1, 101.1, 100.7, 76.3, 72.8, 72.6, 71.7, 70.9, 70.7, 70.0, 69.0, 67.2, 67.0, 66.6, 62.0, 60.8, 33.3, 29.8, 20.9, 20.8, 20.7, 20.64, 20.63, 20.5, 3.5$ ppm; HRMS (ESI-ion trap): m/z calcd for C₃₂H₄₇IO₁₉ + NH₄⁺: 880.6519 [$M + \text{NH}_4^+$]; found: 880.6500.

General procedure for optimized TMO-addition reaction (Table 2)

The per-*O*-Ac sugar (β -D-glucose pentaacetate (**9 β**), β -D-galactose pentaacetate (**10 β**), α -D-galactose pentaacetate (**10 α**), α -D-mannose pentaacetate (**11 α**), β -D-lactose octaacetate (**5 β**), and per-*O*-Ac globotriaose(**12**)) was placed in 10 mL microwave reaction vessels with a stir bar in a vacuum oven at 80°C overnight before the reaction was carried out. After the reaction vessel was cooled to room temperature in an Ar-filled desiccator, the reaction vessel was sealed with a Teflon cap. Anhydrous CHCl₃ (2 mL) was added to the reaction vessel to dissolve the per-*O*-Ac sugar (0.25 mmol, 1.0 equiv), followed by TMSI (0.3 mmol, 1.2 equiv) at room temperature. After the appropriate reaction time interval (Table 2), TLC analysis revealed the complete consumption of starting material. Next, butylated hydroxytoluene (BHT, 0.13 mmol, 0.5 equiv), TMO (0.38 mmol, 1.5 equiv), and I₂ (0.25 mmol, 1.0 equiv) were added to the reaction mixture. The reaction vessel was then subjected to microwave irradiation for 20 min (standard mode, 70°C, ≈ 40 psi, ≈ 70 W). The reaction mixture was diluted with ethyl acetate (30 mL), and washed with saturated Na₂S₂O₃ (aq.) (20 mL) and brine (20 mL). The organic layer was dried over Na₂SO₄, concentrated under reduced pressure, and the resulting residue was purified by using gradient flash column chromatography (ethyl acetate/*n*-hexane) to obtain the corresponding per-*O*-Ac-TMO adduct (**4**, **13**, **14**, **15**, and **16**) as a white foam.

3-Iodopropyl 2,3,4,6-tetra-O-acetyl- β -D-glucopyranoside (13)—Following the general procedure for optimized TMO addition, β -D-glucose pentaacetate (**9 β**) (100 mg, 0.26 mmol) was dissolved in dry CHCl₃ (2 mL) and treated with TMSI (44 μ L, 0.31 mmol). After the starting material was completely consumed, BHT (28 mg, 0.13 mmol), TMO (26

μL , 0.29 mmol), and I_2 (66 mg, 0.26 mmol) were added to the reaction mixture, followed by microwave irradiation for 20 min (standard mode, 70°C , ≈ 40 psi, ≈ 70 W). After workup, the resulting residue was purified by gradient flash column chromatography (ethyl acetate/*n*-hexane = 40:60 to 50:50) to obtain **13** (113 mg, 84% yield) as a white foam (Table 2, entry

1). $R_f = 0.45$ (ethyl acetate/*n*-hexane = 2:3). $[\alpha]_{\text{D}}^{25} = +2.9$ (c0.84, CHCl_3); ^1H NMR (800 MHz, CDCl_3): $\delta = 5.21$ (appt. t, $J = 9.6$ Hz, 1H, H-3), 5.08 (t, $J = 9.7$ Hz, 1H, H-4), 4.98 (dd, $J = 9.6, 8.0$ Hz, 1H, H-2), 4.52 (d, $J = 8.0$ Hz, 1H, H-1), 4.27 (dd, $J = 12.3, 4.8$ Hz, 1H, H-6a), 4.15 (dd, $J = 12.3, 2.3$ Hz, 1H, H-6b), 3.94 (dt, $J = 9.9, 5.0$ Hz, 1H, OCH), 3.71 (ddd, $J = 9.9, 4.7, 2.4$ Hz, 1H, H-5), 3.62–3.57 (m, 1H, OCH), 3.28–3.19 (m, 2H, RCH_2I), 2.09 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 2.07–1.95 ppm (m, 2H, CH_2); ^{13}C NMR (200 MHz, CDCl_3): $\delta = 170.7, 170.3, 169.4, 169.3, 101.0, 72.7, 71.8, 71.2, 69.2, 68.4, 61.9, 32.7, 20.8, 20.7, 20.6, 20.5, 3.09$ ppm; HRMS (ESI-ion trap): m/z calcd for $\text{C}_{17}\text{H}_{25}\text{IO}_{10} + \text{NH}_4^+$: 534.0831 [$M + \text{NH}_4^+$]; found: 534.0831.

3-Iodopropyl 2,3,4,6-tetra-O-acetyl- β -D-galactopyranoside (14)—Following the general procedure for optimized TMO addition, β -D-galactose pentaacetate (**10 β**) (100 mg, 0.26 mmol) was dissolved in dry CHCl_3 (2 mL) and was treated with TMSI (44 μL , 0.31 mmol). After the starting material was completely consumed, BHT (28 mg, 0.13 mmol), TMO (26 μL , 0.29 mmol), and I_2 (66 mg, 0.26 mmol) were added to the reaction mixture, followed by microwave irradiation for 20 min (standard mode, 70°C , ≈ 40 psi, ≈ 70 W). After workup, the resulting residue was purified by gradient flash column chromatography (ethyl acetate/*n*-hexane = 40:60 to 50:50) to obtain **14** (117 mg, 87% yield) as a white foam (Table 2, entry 2). $R_f = 0.38$ (ethyl acetate/*n*-hexane = 2:3). $[\alpha]_{\text{D}}^{25} = +9.1$ (c1.19, CHCl_3); ^1H NMR (800 MHz, CDCl_3): $\delta = 5.40$ (d, $J = 3.5$ Hz, 1H, H-4), 5.19 (dd, $J = 10.5, 8.0$ Hz, 1H, H-2), 5.03 (dd, $J = 10.5, 3.5$ Hz, 1H, H-3), 4.49 (d, $J = 8.0$ Hz, 1H, H-1), 4.19 (dd, $J = 11.3, 6.8$ Hz, 1H, H-6a), 4.13 (dt, $J = 6.8, 5.4$ Hz, 1H, H-6b), 3.96 (dt, $J = 9.8, 4.9$ Hz, 1H, OCH), 3.92 (appt. t, $J = 6.8$ Hz, 1H, H-5), 3.63–3.57 (m, 1H, OCH), 3.29–3.21 (m, 2H, RCH_2I), 2.15 (s, 3H), 2.10 (s, 3H), 2.06 (d, 3H), 1.99 (s, 3H), 2.11–1.95 ppm (m, 2H, CH_2); ^{13}C NMR (200 MHz, CDCl_3): $\delta = 170.4, 170.3, 170.2, 169.5, 101.5, 70.8, 70.7, 69.2, 68.8, 67.0, 61.3, 32.7, 20.9, 20.7, 20.66, 20.6, 3.2$ ppm; HRMS (ESI-ion trap): m/z calcd for $\text{C}_{17}\text{H}_{25}\text{IO}_{10} + \text{NH}_4^+$: 534.0831 [$M + \text{NH}_4^+$]; found: 534.0830.

3-Iodopropyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranoside (15)—Following the general procedure for optimized TMO addition, α -D-mannose pentaacetate (**11 α**) (100 mg, 0.26 mmol) was dissolved in dry CHCl_3 (2 mL) and was treated with TMSI (44 μL , 0.31 mmol). After the starting material was completely consumed, BHT (28 mg, 0.13 mmol), TMO (26 μL , 0.29 mmol), and I_2 (66 mg, 0.26 mmol) were added to the reaction mixture, followed by microwave irradiation for 20 min (standard mode, 70°C , ≈ 40 psi, ≈ 70 W). After workup, the resulting residue was purified by gradient flash column chromatography (ethyl acetate/*n*-hexane = 40:60 to 50:50) to obtain **15** (113 mg, 84% yield) as a white foam (Table 2, entry 4). $R_f = 0.55$ (ethyl acetate/*n*-hexane = 2:3); $[\alpha]_{\text{D}}^{25} = +43.3$ (c0.30, CHCl_3); ^1H NMR (800 MHz, CDCl_3): $\delta = 5.31$ –5.27 (m, 2H, H-2, H-4), 5.24 (dd, $J = 3.0, 1.6$ Hz, 1H, H-2), 4.83 (d, $J = 1.6$ Hz, 1H, H-1), 4.29 (dt, $J = 13.8, 6.9$ Hz, 1H, H-6a), 4.16–4.13 (m, 1H, H-6b), 4.04–4.00 (m, 1H, H-5), 3.84–3.81 (m, 1H, OCH), 3.55–3.50 (m, 1H, OCH), 3.33–

3.25 (m, 2H, CH₂I), 2.16 (s, 3H), 2.13–2.04 (m, 2H, CH₂) 2.11 (s, 3H), 2.05 (s, 3H), 2.00 ppm (s, 3H); ¹³C NMR (200 MHz, CDCl₃): δ = 170.7, 170.1, 169.9, 169.7, 97.7, 69.5, 69.1, 68.7, 67.3, 66.0, 62.5, 32.6, 20.9, 20.8, 20.7, 20.6, 2.8 ppm; HRMS (ESI-ion trap): *m/z* calcd for C₁₇H₂₅IO₁₀ + NH₄⁺: 534.0831 [*M* + NH₄⁺]; found: 534.0836.

3-Iodopropyl (2,3,4,6-Tetra-*O*-acetyl-α-*D*-galactopyranosyl)-(1-4)-*O*-(2,3,6-tri-*O*-acetyl-β-*D*-galactopyranosyl)-(1-4)-*O*-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside (16)

—Following the general procedure for optimized TMO addition, per-*O*-Ac globotriaose (12) (210 mg, 0.22 mmol) was dissolved in dry CHCl₃ (2 mL) and treated with TMSI (48 μL, 0.33 mmol). After the starting material was completely consumed, BHT (22 mg, 0.11 mmol), TMO (22 μL, 0.29 mmol), and I₂ (56 mg, 0.26 mmol) were added to the reaction mixture, followed by microwave irradiation for 20 min (standard mode, 70°C, ≈40 psi, ≈70 W). After workup, the resulting residue was purified by gradient flash column chromatography (ethyl acetate/*n*-hexane=50:50 to 60:40) to obtain 16 (170 mg, 72% yield) as a white foam (Table 2, entry 6). *R*_f=0.38 (ethyl acetate/*n*-hexane = 2:1); ¹H NMR (800 MHz, CDCl₃): δ = 5.59 (d, *J* = 2.6 Hz, 1H, H-4'), 5.39 (dd, *J*=11.0, 3.4 Hz, 1H, H-3''), 5.21 (appt. t, *J* = 8.7 Hz, 1H, H-3), 5.18 (dd, *J* = 3.4 Hz, 1H, H-2''), 5.10 (dd, *J*=10.8, 7.8 Hz, 1H, H-2'), 4.98 (d, *J* = 3.6 Hz, 1H, H-1''), 4.88 (dd, *J* = 9.4, 8.1 Hz, 1H, H-2), 4.73 (dd, *J* = 10.8, 2.6 Hz, 1H, H-3'), 4.51 (d, *J* = 7.7 Hz, 1H, H-1'), 4.49–4.47 (m, 2H, H-1, H-6a), 4.43 (dd, *J*=11.1, 6.2 Hz, 1H, H-6'a), 4.18–4.09 (m, 5H, H-5'', H-6''ab, H-6b, H-6'b), 4.01 (d, *J* = 2.1 Hz, 1H, H-4'), 3.91–3.88 (m, 1H, OCH), 3.79 (appt. t, *J* = 9.4 Hz, 1H, H-4), 3.75 (dd, *J* = 12.3 Hz, 1H, H-5'), 3.64 (ddd, *J* = 9.8, 5.2, 1.9 Hz, 1H, H-5), 3.59 (ddd, *J* = 9.8, 8.2, 4.5 Hz, 1H, OCH), 3.28–3.18 (m, 2H, CH₂I), 2.13 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.066 (s, 6H), 2.063 (s, 3H), 2.056 (s, 3H), 2.04 (s, 3H), 1.98 ppm (s, 3H); ¹³C NMR (200 MHz, CDCl₃): δ = 170.7, 170.5, 170.46, 170.44, 170.1, 169.8, 169.6, 169.5, 168.9, 101.1, 100.7, 99.6, 76.9, 76.4, 73.0, 72.8, 72.6, 71.8, 71.7, 69.2, 68.9, 68.8, 67.9, 67.1, 67.0, 62.1, 61.3, 60.3, 32.8, 20.94, 20.91, 20.85, 20.74, 20.72, 20.7, 20.68, 20.65, 20.60, 20.5, 3.0 ppm; HRMS (ESI-ion trap): *m/z* calcd for C₄₁H₅₇IO₂₆ + NH₄⁺: 1110.2526 [*M* + NH₄⁺]; found: 1110.2550.

3-Azidopropyl (2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1-4)-*O*-2,3,6-tri-*O*-acetyl-β-*D*-glucopyranoside (17)

NaN₃ (43 mg, 0.66 mmol) was added to a dry DMF (5 mL) solution of compound 4 (180 mg, 0.22 mmol). The reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was then diluted with ethyl acetate, and washed with saturated Na₂S₂O₃ (aq.) and brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The resulting residue was purified by using flash column chromatography (ethyl acetate/*n*-hexane = 50:50) to afford compound 17 (153 mg, 97% yield) as a white foam. *R*_f=0.25 (ethyl acetate/*n*-hexane = 1:1); ¹H NMR (600 MHz, CDCl₃): δ = 5.35 (d, *J* = 3.0 Hz, 1H, H-4'), 5.20 (appt. t, *J* = 9.3 Hz, 1H, H-3), 5.11 (dd, *J*=10.3, 8.0 Hz, 1H, H-2'), 4.95 (dd, *J* = 10.4, 3.3 Hz, 1H, H-3'), 4.89 (appt. t, *J* = 8.8 Hz, 1H, H-2), 4.54–4.43 (m, 3H, H-1, H-1', H-6a), 4.14–4.04 (m, 3H, H-6b, H-6'ab), 3.91 (dd, *J*=10.0, 5.3 Hz, 1H, OCH), 3.87 (appt. t, *J* = 6.8 Hz, 1H, H-5'), 3.79 (appt. t, *J* = 9.5 Hz, 1H, H-4), 3.66–3.52 (m, 2H, H-5, OCH), 3.35 (dt, *J*=11.2, 6.3 Hz, 2H, CH₂N₃), 2.16 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H), 2.05 (s, 9H), 1.97 (s, 3H), 1.88–1.78 ppm (m, 2H); ¹³C NMR (150 MHz, CDCl₃): δ = 170.4, 170.1, 170.0,

169.7, 169.6, 169.1, 101.1, 100.6, 76.2, 72.7, 72.66, 72.63, 70.9, 70.7, 69.0, 66.6, 66.5, 61.9, 60.8, 47.9, 28.9, 20.8, 20.7, 20.69, 20.64, 20.62, 20.5 ppm; HRMS (ESI-ion trap): m/z calcd for $C_{29}H_{41}N_3O_{16} + NH_4^+$: 737.2723 [$M + NH_4^+$]; found: 737.2740.

3-Azidopropyl (β -O- α -galactopyranosyl)-(1-4)-O- β -D-glucopyranoside (**18**)

Compound **17** (140 mg, 0.19 mmol) was dissolved in dry MeOH (5 mL) and treated with 25% NaOMe in MeOH solution (60 μ L). After 10 min, H₂O (1 mL) was added to dissolve the forming white precipitate. The reaction mixture was stirred for another 1 h and was acidified to pH 6 by adding Dowex H⁺ resin. The solution was filtered through a plug of C₁₈ reverse-phase silica gel and washed with MeOH and water to remove the resin and desalt. The filtrate was concentrated and lyophilized to afford compound **18** (81 mg, quantitative) as a white fluffy foam. R_f = 0.67 (ethyl acetate/MeOH/H₂O=5:4:1); ¹H NMR (800 MHz, MeOD): δ =4.36 (d, J =7.7 Hz, 1H, H-1'), 4.29 (d, J =7.8 Hz, 1H, H-1), 3.96 (dt, J =10.1, 6.1 Hz, 1H, OCH), 3.90 (dd, J =12.1, 2.4 Hz, 1H, H-6a), 3.86–3.83 (m, 1H, H-6b), 3.81 (d, J =4.9 Hz, 1H, H-4'), 3.77 (dt, J =14.0, 7.0 Hz, 1H, H-6'a), 3.72–3.67 (m, 1H, H-6'b), 3.64 (dt, J =10.1, 6.1 Hz, 1H, OCH), 3.61–3.47 (m, 5H, H-3, H-4, H-2', H-3', H-5'), 3.47–3.43 (m, 2H, CH₂N₃), 3.41 (ddd, J =9.7, 4.1, 2.5 Hz, 1H, H-5), 3.25 (dd, J =9.2, 7.9 Hz, 1H, H-2), 1.90–1.84 ppm (m, 2H); ¹³C NMR (200 MHz, CDCl₃): δ =105.1, 104.3, 80.6, 77.0, 76.4, 76.3, 74.8, 74.7, 72.6, 70.3, 70.2, 67.6, 62.5, 61.9, 30.2 ppm; HRMS (ESI-ion trap): m/z calcd for $C_{15}H_{27}N_3O_{11} + Na^+$: 448.1538 [$M+Na^+$]; found: 448.1531.

3-Azidopropyl (2,3,4,6-Tetra-O-acetyl- α -D-galactopyranosyl)-(1-4)-O-(2,3,6-tri-O-acetyl- β -D-galactopyranosyl)-(1-4)-O-2,3,6-tri-O-acetyl- β -D-glucopyranoside (**19**)

NaN₃ (59 mg, 0.9 mmol) was added to a dry DMF (5 mL) solution of compound **16** (197 mg, 0.18 mmol). The reaction mixture was allowed to stir at room temperature for 2 h. The reaction mixture was then diluted with ethyl acetate, and washed with saturated Na₂S₂O₃ (aq.) and brine. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The resulting residue was purified by using flash column chromatography (ethyl acetate/*n*-hexane=60:40) to afford compound **19** (167 mg, 92% yield) as a white foam. R_f = 0.42 (ethyl acetate/*n*-hexane=2:1); ¹H NMR (600 MHz, CDCl₃): δ =5.53 (d, J =3.1 Hz, 1H, H-4''), 5.33 (dd, J = 11.0, 3.3 Hz, 1H, H-3''), 5.18–5.10 (m, 2H, H-3, H-2''), 5.05 (dd, J = 7.8 Hz, 1H, H-2''), 4.93 (d, J =3.6 Hz, 1H, H-1''), 4.83 (appt. t, J =8.7 Hz, 1H, H-2), 4.67 (d, J =10.8 Hz, 1H, H-3'), 4.49–4.33 (m, 5H, H-1, H-1', H-6a, H-6'a), 4.16–3.98 (m, 5H, H-5'', H-6''ab, H-6b, H-6'b), 3.95 (s, 1H, H-4'), 3.87–3.84 (m, 1H, OCH), 3.75–3.69 (m, 2H, H-4, H-5'), 3.63–3.50 (m, 2H, H-5, OCH), 3.35–3.23 (m, 2H, CH₂N₃), 2.07 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.007 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.81–1.73 ppm (m, 2H); ¹³C NMR (150 MHz, CDCl₃): δ =170.7, 170.5, 170.48, 170.45, 170.1, 169.8, 169.7, 169.6, 168.9, 101.1, 100.6, 99.7, 76.5, 73.1, 72.8, 72.6, 71.8, 71.7, 69.0, 68.9, 67.9, 67.2, 67.1, 66.5, 62.2, 61.8, 60.3, 48.0, 29.0, 20.9, 20.8, 20.73, 20.70, 20.66, 20.62, 20.5 ppm; HRMS (ESI-ion trap): m/z calcd for $C_{41}H_{57}N_3O_{26} + NH_4^+$: 1025.3569 [$M+NH_4^+$]; found: 1025.3591.

3-Azidopropyl α -D-galactopyranosyl)-(1-4)-O- β -D-galactopyranosyl)-(1-4)-O- β -D-glucopyranoside (20)

Compound **19** (30 mg, 0.03 mmol) was dissolved in dry MeOH (1 mL) and treated with 25% NaOMe in MeOH solution (13 μ L). After 10 min, H₂O (1 mL) was added to dissolve the forming white precipitate. The reaction mixture was stirred for another 1 h and was acidified to pH 6 by adding Dowex H⁺ resin. The solution was filtered through a plug of C₁₈ reverse-phase silica gel and washed with MeOH and water to remove the resin and desalt. The filtrate was concentrated and lyophilized to afford compound **20** (18 mg, quantitative yield) as a white fluffy foam. *R*_f = 0.40 (ethyl acetate/MeOH/H₂O = 5:4:1); ¹H NMR (600 MHz, D₂O): δ = 4.75 (d, *J* = 4.0 Hz, 1H), 4.31 (d, *J* = 7.8 Hz, 1H), 4.29 (d, *J* = 8.0 Hz, 1H), 4.16 (appt. t, *J* = 6.4 Hz, 1H), 3.87–3.82 (m, 3H), 3.82 (s, 2H), 3.76–3.67 (m, 3H), 3.67–3.61 (m, 4H), 3.61–3.47 (m, 7H), 3.47 (s, 3H), 3.42–3.34 (m, 2H), 3.26 (appt. t, *J* = 6.7 Hz, 2H), 3.15 (s, 1H), 3.11 (appt. t, *J* = 8.6 Hz, 1H), 1.77–1.71 ppm (m, 2H); ¹³C NMR (150 Hz, D₂O): δ = 103.2, 102.0, 100.3, 78.5, 77.3, 75.4, 74.8, 74.4, 72.8, 72.1, 70.8, 70.7, 69.1, 68.9, 68.5, 67.3, 60.4, 60.3, 59.9, 47.8, 28.2, 23.2 ppm; HRMS (ESI-ion trap): *m/z* calcd for C₂₁H₃₇N₃O₁₆ + Na⁺: 610.2066 [*M* + Na⁺]; found: 610.2061.

Lactose click tetramer (22)

Tetraalkyne **21** (17 mg, 0.06 mmol), compound **17** (220 mg, 0.31 mmol), and TBTA ligand (25 mg, 0.05 mmol) were added to a 25 mL round-bottomed flask. The compounds were dissolved in dry DMF (1 mL), followed by the addition of an aqueous solution (0.3 mL) of CuSO₄·5H₂O (9 mg, 0.04 mmol) and sodium ascorbate (10 mg, 0.05 mmol). The reaction mixture was allowed to stir at room temperature in the dark for 20 h. After TLC analysis revealed the complete consumption of compound **21**, the solvent was evaporated, and the resulting residue was purified by using flash column chromatography (ethyl acetate/MeOH = 15:1) to afford the protected click product. The product was then dissolved in dry MeOH (2 mL) and treated with 25% NaOMe in MeOH solution (40 μ L). After 1 h, the reaction mixture was concentrated under reduced pressure, and the resulting residue was purified by using C₁₈ reverse-phase column chromatography (MeOH/H₂O = 20:80) to afford compound **22** (94 mg, 78% yield) as a white powder. C₁₈ reverse phase *R*_f = 0.18 (MeOH/H₂O = 1:5); ¹H NMR (800 MHz, D₂O): δ = 8.00–7.90 (m, 4H), 4.53–4.48 (m, 16H), 4.45 (d, *J* = 7.8 Hz, 4H, H-1'), 4.43 (d, *J* = 8.0 Hz, 4H, H-1), 3.94 (dd, *J* = 19.5, 7.3 Hz, 4H, H-6a), 3.90–3.83 (m, 4H, H-4'), 3.83–3.70 (m, 16H, H-5, H-6b, H-6'ab), 3.68–3.62 (m, 12H, H-3, H-3', H-5'), 3.62–3.58 (m, 4H, OCH), 3.56–3.53 (m, 8H, H-2', H-4), 3.38 (s, 8H), 3.36–3.29 (m, 4H, H-2), 2.28–2.09 ppm (m, 8H); ¹³C NMR (200 MHz, D₂O): δ = 143.9, 124.9, 102.8, 101.9, 78.3, 75.2, 74.6, 74.2, 72.6, 72.4, 70.8, 68.4, 67.7, 66.2, 63.3, 60.9, 59.9, 46.9, 29.4 ppm; HRMS (ESI-ion trap): *m/z* calcd for C₇₉H₁₃₀N₁₂O₄₉ + 2H⁺: 995.4045 [*M* + 2H⁺]; found: 995.4119.

Globotriaose click tetramer (23)

Tetraalkyne **21** (12 mg, 0.04 mmol), compound **19** (180 mg, 0.18 mmol), and TBTA ligand (17 mg, 0.03 mmol) were added to a 25 mL round-bottomed flask. The compounds were dissolved in dry DMF (1 mL), followed by the addition of an aqueous solution (0.3 mL) of CuSO₄·5H₂O (6 mg, 0.02 mmol) and sodium ascorbate (6 mg, 0.03 mmol). The reaction

mixture was allowed to stir at room temperature in the dark for 16 h. After TLC analysis revealed the complete consumption of compound **21**, the solvent was evaporated, and the resulting residue was purified by using gradient flash column chromatography (ethyl acetate/MeOH = 15:1 to 10:1) to afford the protected click product. The product was then dissolved in dry MeOH (1.5 mL) and treated with 25% NaOMe in MeOH (25 μ L). After 10 min, H₂O (1 mL) was added to dissolve the forming white precipitate and the reaction mixture was allowed to stir for 1 h. Next, the reaction mixture was concentrated under reduced pressure, and the resulting residue was purified by using C₁₈ reverse-phase column chromatography (MeOH/H₂O = 1:5) to afford compound **23** (53 mg, 60% yield) as a white powder. C₁₈ reverse phase R_f = 0.29 (MeOH/H₂O = 1:5); ¹H NMR (800 MHz, D₂O): δ = 7.91 (s, 4H), 4.90 (d, J = 3.9 Hz, 4H, H-1''), 4.50–4.43 (m, 20 H, H-1', CH₂ × 8), 4.38 (d, J = 8.0 Hz, 4H, H-1), 4.31 (appt. t, J = 6.4 Hz, 4H), 3.99 (d, J = 2.8 Hz, 4H, H-4''), 3.98 (d, J = 3.0 Hz, 4H, H-4'), 3.93–3.68 (m, 40 H, H-6ab, H-6'ab, H-3', H-3'', H-2'', H-5', H-5'', OCH), 3.68–3.63 (m, 8 H, H-5', H-5''), 3.62–3.57 (m, 8 H, H-6''ab), 3.56–3.48 (m, 12 H, H-2', H-4, OCH), 3.32 (s, 8H, CH₂ × 4), 3.27 (appt. t, J = 8.4 Hz, 4H, H-2), 2.18–2.09 ppm (m, 8H, CH₂ × 4). ¹³C NMR (200 MHz, D₂O): δ = 143.9, 124.9, 103.1, 101.9, 100.1, 78.4, 77.2, 75.3, 74.6, 74.2, 72.7, 71.9, 70.7, 70.6, 68.9, 68.7, 68.4, 67.6, 66.2, 63.2, 60.3, 60.2, 59.8, 46.9, 29.4, 23.1 ppm; HRMS (ESI-ion trap): m/z calcd for C₁₆₃H₁₇₀N₁₂O₆₉ + 2H⁺: 1319.5101 [M + 2H⁺]; found: 1319.5159.

Trivalent-globobotriaose/PADRE click conjugate (**25**)

The procedure for synthesizing compound **25** was modified from previous publications.^[32,38] To a 10 mL flame-dried round-bottomed flask was added 500 μ L of compound **24** (1.4 mg) and 1.5 mL compound **20** (4.2 mg) solution. Both compounds were dissolved in degassed 100 mm PBS buffer at pH 7.4. A pre-mixed solution of CuSO₄·5H₂O (20 μ L of 20 mm stock solution in degassed HPLC-grade H₂O) and THPTA (42 μ L of 50 mm stock solution in degassed HPLC-grade H₂O) was added, and the reaction mixture was allowed to stir under Ar. A solution of aminoguanidine (200 μ L of 100 mm stock solution in degassed HPLC-grade H₂O) was also added followed by the addition of a freshly prepared solution of sodium ascorbate (200 μ L of 100 mm stock solution in degassed HPLC-grade H₂O). The composition of the reaction mixture is summarized in Table S1 (see the Supporting Information). The reaction mixture was allowed to stir at room temperature for 18 h. Work-up involved removal of salts by passing the mixture through Bio-gel P-10 gel (90–180 μ m, exclusion limit: 1.5 kDa to 20 kDa) in 50 mm EDTA, at pH 7.4, PBS buffer. Fractions were collected and the buffer was exchanged by using C₁₈ Sep-PakTM in 1:1 MeCN/0.1 % aqueous formic acid (FA). The white fluffy solid **25** (4.2 mg, 75% yield) was obtained after lyophilization and was analyzed by HPLC (see the Supporting Information, Figure S2) and MALDI-HRMS (see the Supporting Information, Figure S3). HRMS (MALDI-TOF): m/z calcd for C₁₅₂H₂₅₀N₂₈O₇₀ + Na⁺: 3610.6756 [M + Na⁺]; found: 3610.6914.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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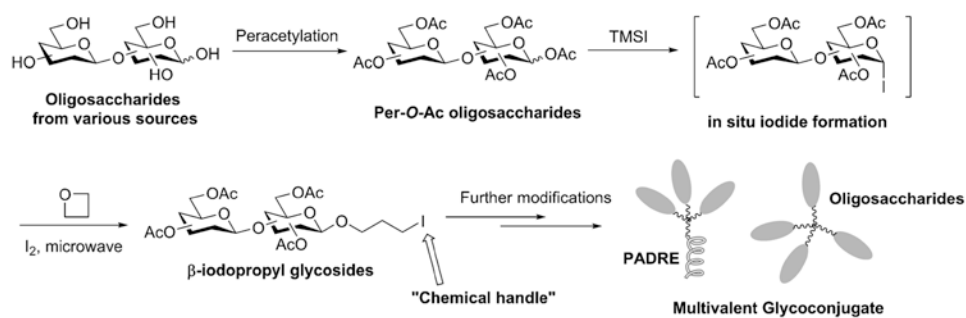


Figure 1.
A universal functionalization method for per-*O*-Ac oligosaccharides.

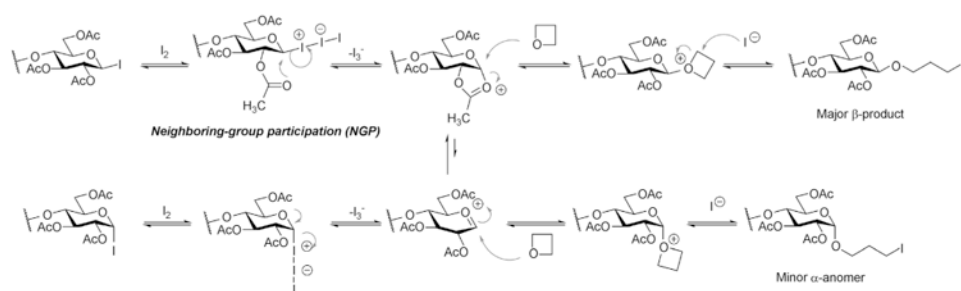
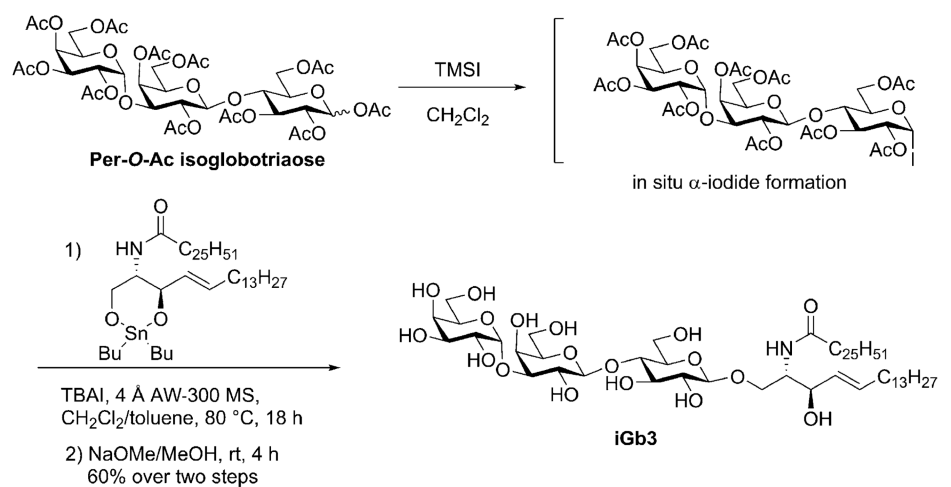
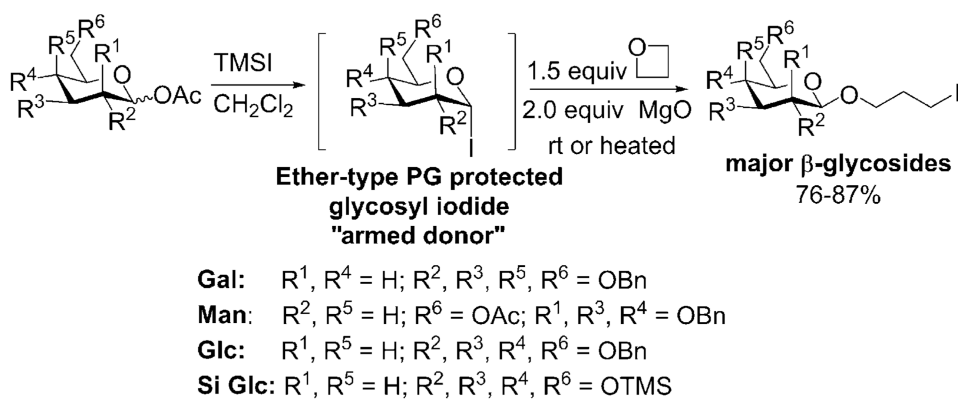


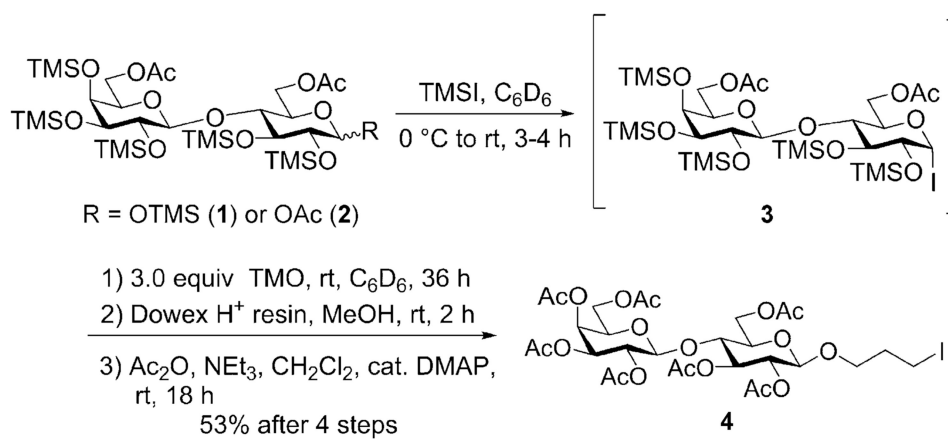
Figure 2.
Proposed mechanism for I_2 -promoted TMO addition.



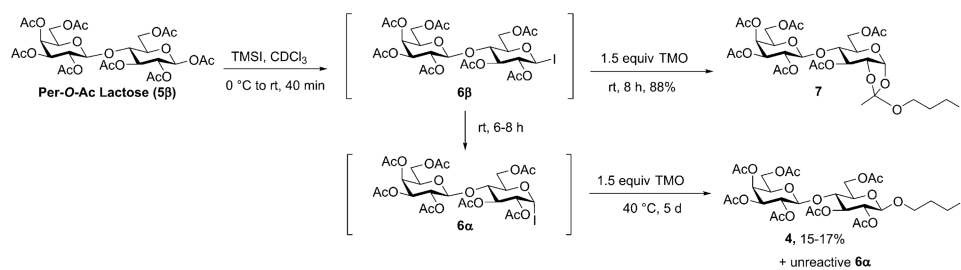
Chemistry. Author manuscript; available in PMC 2015 July 09.

**Scheme 2.**

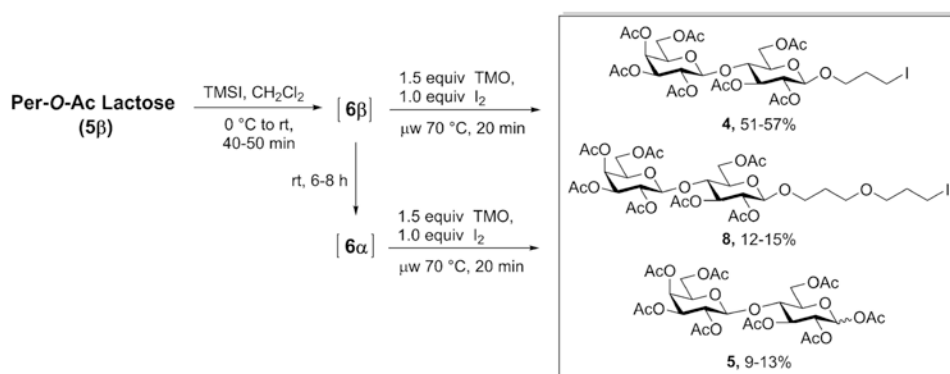
TMO addition to ether-protected glycosyl iodides affords β -glycosides (Bn=benzyl).

**Scheme 3.**

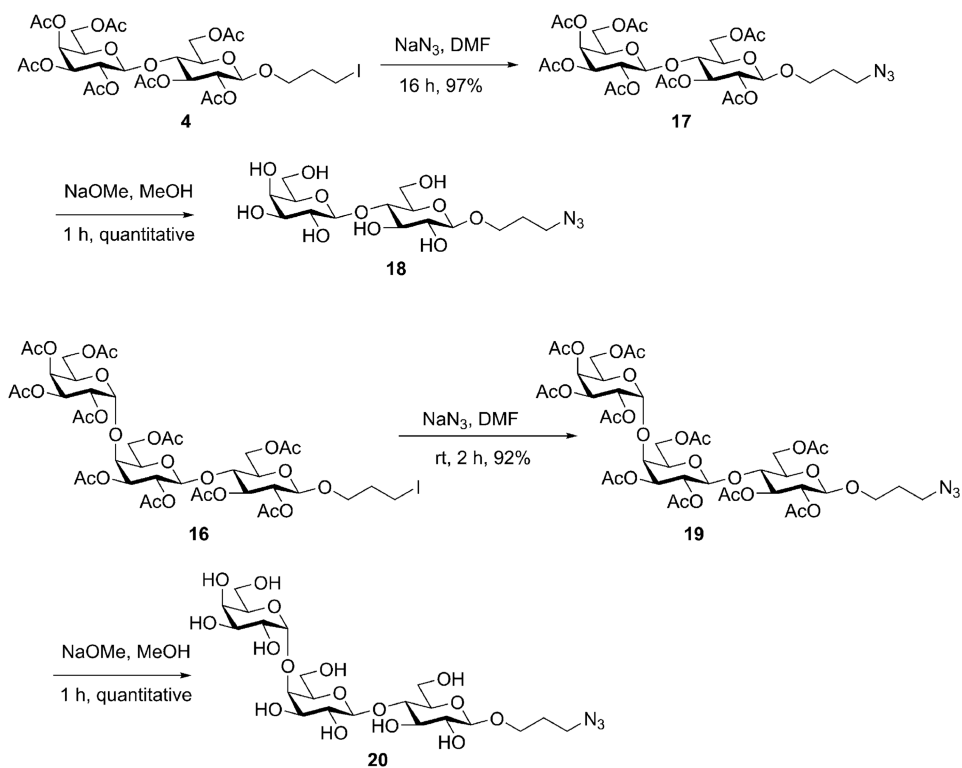
6,6'-Di-*O*-acetylated lactosyl iodide reacts with TMO.

**Scheme 4.**

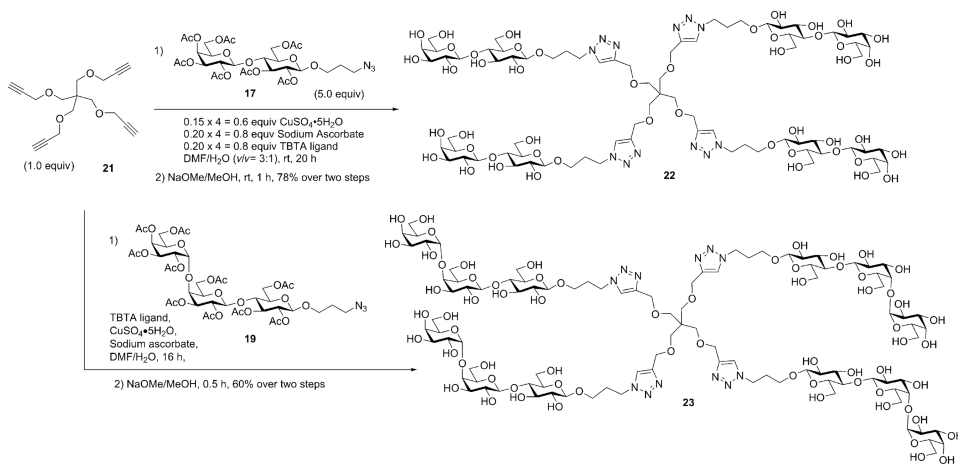
Results of TMO-addition reaction to “disarmed” per-*O*-Ac lactosyl iodide.

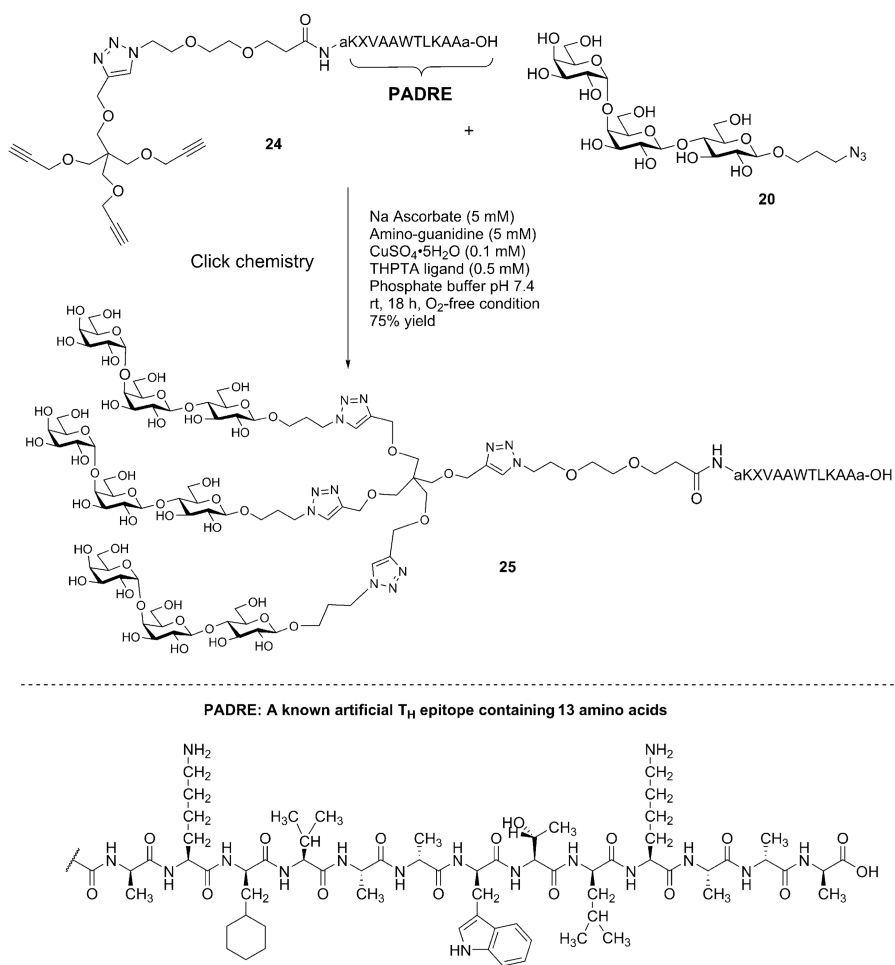
**Scheme 5.**

I₂-promoted TMO addition under microwave irradiation.

**Scheme 6.**

Synthesis of functionalized lactose **18** and globotriaose **20**.

**Scheme 7.**Construction of lactose and globotriaose tetramers **22** and **23**.



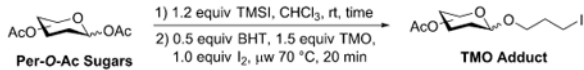
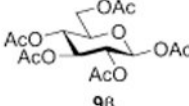
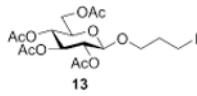
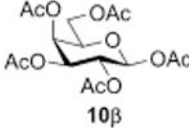
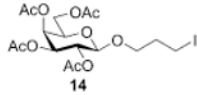
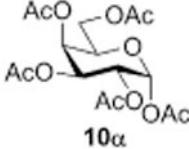
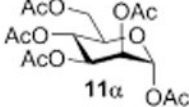
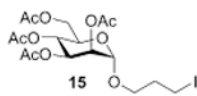
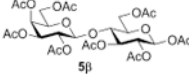
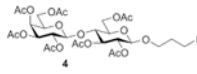
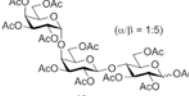
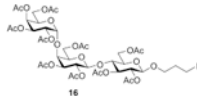
Scheme 8.
Synthesis of globotriaose–PADRE construct as a cancer-vaccine candidate.

Table 1

Optimization of TMO addition by introducing BHT and different solvents.

| <p>Per-O-Ac Lactose (5)</p> <p>1) 1.2 equiv TMSI, solvent, time</p> <p>2) 1.5 equiv TMO, 1.0 equiv I₂, 50 mol% BHT, μw 70 °C, 20 min</p> <p>4</p> <p>+ Byproducts</p> | | | | |
|--|---------------------------------|---------------------------------|--------------------------------|--|
| Entry | Solvent | Iodide formation <i>t</i> [min] | Isolated yield of 4 [%] | Byproducts (≈20–30%) |
| 1 | CH ₂ Cl ₂ | 40 | 61 | di-TMO adduct (8), per- <i>O</i> -Ac lactose (5) |
| 2 | CHCl ₃ | 50 | 51 | alpha anomer, per- <i>O</i> -Ac lactose (5) |
| 3 | PhH | 90 | 54 | alpha anomer, per- <i>O</i> -Ac lactose (5) |

Table 2TMO-addition results for per-*O*-Ac sugars.

| <div style="display: flex; align-items: center; justify-content: space-between;"><div style="text-align: center;"><p>Per-<i>O</i>-Ac Sugars</p></div><div style="text-align: center;"><p>TMO Adduct</p></div></div> | | | | |
|--|---|------------------|--|----|
| Entry | Starting material | Iodide formation | Product (isolated yield [%]) | |
| 1 |  <p>9β</p> | 50 min |  <p>13</p> | 84 |
| 2 |  <p>10β</p> | 40 min |  <p>14</p> | 87 |
| 3 |  <p>10α</p> | 2.5 h | 14 | 79 |
| 4 |  <p>11α</p> | 4.5 h |  <p>15</p> | 71 |
| 5 |  <p>5β</p> | 50 min |  <p>4</p> | 51 |
| 6 |  <p>12</p> <p>(α/β = 1:5)</p> | 1 h |  <p>16</p> | 72 |