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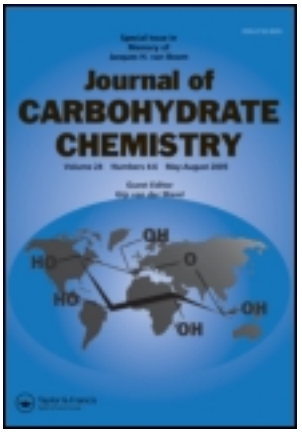

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

Rapid Chemo-Enzymatic Synthesis of Peracetylated GlcNAcβ3Galβ-Aglycones

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Version of record first published: 09 Nov 2011.

To cite this article: N. Merbouh , J. R. Brown , F. K. Wallner , M. Morton , J. D. Esko & P. H. Seeberger (2011): Rapid Chemo-Enzymatic Synthesis of Peracetylated GlcNAcβ3Galβ-Aglycones, Journal of Carbohydrate Chemistry, 30:4-6, 373-390

To link to this article: http://dx.doi.org/10.1080/07328303.2011.617023

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Journal of Carbohydrate Chemistry, 30:373–390, 2011
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ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328303.2011.617023

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Rapid Chemo-Enzymatic Synthesis of Peracetylated GlcNAcβ3Galβ-Aglycones

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The inhibition of sialyl Lewis X can prevent unwanted cellular extravasation that is associated with inflammatory diseases and tumor metastasis. Described is an efficient methodology to prepare peracetylated GlcNAcβ3Gal-aglycone disaccharides subsequently used as metabolic decoys to inhibit sialyl Lewis X expression. Four glycosides were synthesized from one single parent disaccharide that in turn was prepared by large-scale enzymatic synthesis. The procedure avoids lengthy and inefficient synthetic steps to afford the desired disaccharides quickly with good overall yields.

Keywords Disaccharides; Decoy; Sialyl Lewis X; Glycosyltransferase

INTRODUCTION

Both the inflammatory response and circulating tumor cells are known to use a similar mode of action at the molecular level by binding cell surface glycoproteins containing sialyl Lewis X (sLex) to selectin receptors present on the surface of leukocytes and the blood vessel.[1] During an inflammatory response, vascular leukocytes are recruited by interactions between sialyl Lewis X (sLex)-containing glycoproteins on the leukocyte surface and selectin receptors on endothelial cells. Similarly, metastasizing tumor cells use comparable ligand-receptor interactions to bind to platelets as

Received May 29, 2011; accepted August 20, 2011.
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well as endothelial cells.[2,3] Great efforts have been directed at the design of therapeutics that will inhibit metastasis and inflammation by interfering with selectin-dependent adhesion using carbohydrate-based competitive ligands,[4] or inhibit/decrease expression of sialyl Lewis X on the cell surface by blocking the assembly of specific oligosaccharides. Exogenous decoy acceptors such as peracetylated sugars were shown to efficiently cross the hydrophobic barriers of the cell and Golgi membranes and to down-regulate the expression of sLex ligands, resulting in suppression of intercellular adhesion in HL-60 leukemia cells.[5–9] The decoys act as a substrate for the fucosyltransferase, thereby interrupting the formation of the natural ligand.

Benzyl-*N*-acetylgalactosamine glycoside (GalNAcα-*O*-Bn) was used to decrease cell surface expression of Lewis blood group antigens, thus successfully inhibiting adhesion of the treated cells to activated endothelial cells (Fig. 1).[10] This simple benzyl glycoside interfered with a very early step of glycoprotein synthesis and consequently affected all cellular *O*-linked oligosaccharide biosynthesis.[5,10,11] GalNAcα-*O*-Bn was found to prime the production of *O*-linked oligosaccharides, similar to those typically found on mucins, interrupting the incorporation of endogenous acceptors necessary for the synthesis of the natural ligand. Interestingly, the nature of the aglycone was found to greatly influence the amount and the structure of the oligosaccharide produced.[12–14]

In addition to monosaccharides, disaccharides can also act as primers for *O*-linked oligosaccharides, since they closely resemble natural glycan intermediates and can better target specific enzymes in biosynthetic pathways.[7] However, due to their hydrophilic nature, disaccharides and larger oligosaccharides are often poorly taken up by cells. The use of short-chain acyl or acetoxymethyl esters to block the hydroxyl groups in a biologically reversible manner is often necessary to overcome the delivery problem.[15,16] Intracellular carboxylesterases provide access to the deblocked nonacetylated

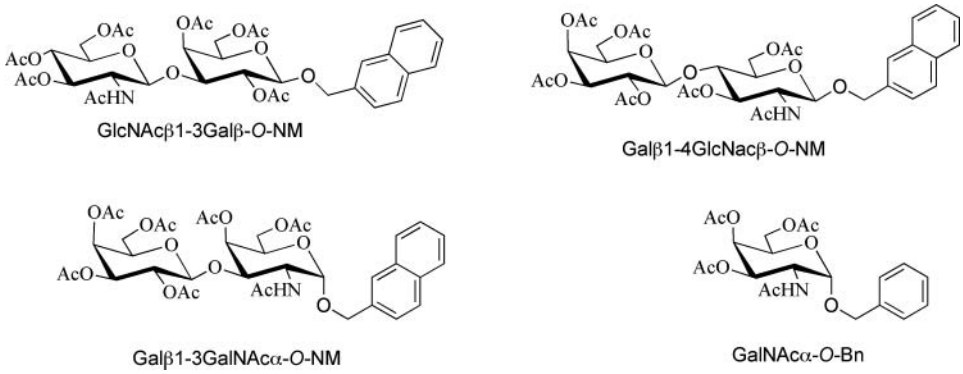

Figure 1: Examples of exogenous primers for *O*-linked Oligosaccharides production.

molecules, which in turn are used in the biosynthetic Golgi apparatus, enabling peracetylated disaccharides such as Galβ1-4GlcNAcβ-*O*-naphthalenemethanol (Galβ1-4GlcNAcβ-*O*-NM), GlcNAcβ1-3Galβ-*O*-NM, and Galβ1-3GalNAcα-*O*-NM (Fig. 1) to prime oligosaccharide synthesis and to generate mucin-like molecules.[8,17] As a result, these metabolic decoys, much like GalNAcα-*O*-Bn, divert the assembly of the *O*-linked chains from endogenous glycoproteins and inhibit the expression of the cell surface Lewis determinants, which are recognized by selectins.[7,17–20] A much lower dose of disaccharides ($\sim$25 μM) can be used to effect the same degree of Lewis X inhibition compared with the monosaccharide primers (1 to 2 mM).

Despite the promise of this strategy, a full exploration of its therapeutic potential is impeded by the serious hurdle of synthesizing a large collection of decoys. Access to disaccharides such as Galβ1-4GlcNAcβ-*O*-NM or GlcNAcβ1-3Galβ-*O*-NM requires several lengthy steps. Synthetic schemes that are neither modular nor flexible leave room for improvement. Here we describe a shorter and more flexible method to generate GlcNAcβ1-3Galβ-*O*-aglycone derivatives. The new synthetic pathway will facilitate the synthesis of more diverse collections of decoys.

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RESULTS AND DISCUSSION

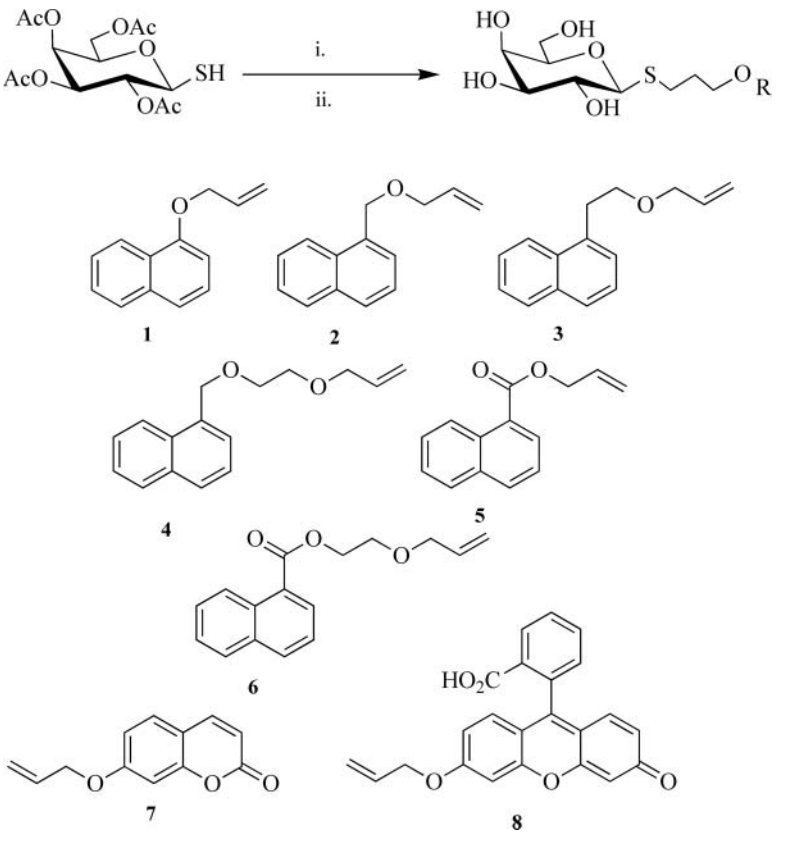
Generally, disaccharides such as Galβ1-4GlcNAcβ-*O*-NM or GlcNAcβ1-3Galβ-*O*-NM are chemically synthesized[7,17,20] using protocols requiring multiple protecting group manipulations. Chemo-enzymatic syntheses, utilizing native or engineered glycosyltransferases, represent an attractive alternative. Nowadays, purified bacterial glycosyltransferases are a reliable source of stable and catalytically efficient enzymes that serve for the facile preparation of synthetic derivatives.[21–23] Enzymatic glycosylations are highly stereo- and regioselective[24] and provide the opportunity to generate saccharides coupled to different aglycones when combined with the use of a traceless linker (3-mercaptopropanol).[25] Here, we describe a combined enzymatic and chemical approach to synthesize peracetylated GlcNAcβ1,3Galβ-aglycone decoys. The method relies on a three-step protocol: (a) selection of an appropriate linker group that is compatible with the enzymatic coupling step and allows for (b) enzymatic coupling to produce 250 to 500 mg of a parent GlcNAcβ1,3Galβ-linker disaccharide and (c) rapid chemical coupling of the parent disaccharide with different aglycones.

Enzymatic Activity/Evaluation and Synthesis

Initially, several galactose derivatives were evaluated to determine whether they were suitable for enzymatic glycosylation. Using aglycones **1–8** (Sch. 1), a variety of galactose derivatives carrying a 3-mercaptopropanol

linker (**12–20**) were photochemically synthesized starting from tetraacetyl-β-1-thio-galactopyranose (Sch. 2).[25] All the aglycones were chosen for their strong UV or fluorescence activity to facilitate the subsequent reactions and purifications. The photochemical reaction was followed by the removal of the acetyl groups using catalytic sodium methoxide in methanol, and the subsequent continuous extraction of the resulting sugar using dichloromethane from a water/methanol mixture. The resulting sugars were purified by chromatography using silica gel and lyophilized as a precaution against sulfur oxidation, prior to their use.

The resulting galactose derivatives (**12–20**) were subsequently screened as acceptor substrates using bacterial β3-*N*-acetylglucosaminyltransferase (β3GlcNAcT) to select the best candidate for a large-scale enzymatic synthesis. β3GlcNAcT utilizes UDP-GlcNAc to transfer GlcNAc to galactose-containing substrates. A crude bacterial lysate from *Escherichia coli* expressing the fusion protein was used since the recombinant form of the enzyme



Scheme 1: (i) **1–8**, MeOH, hν; (ii) MeONa/MeOH.

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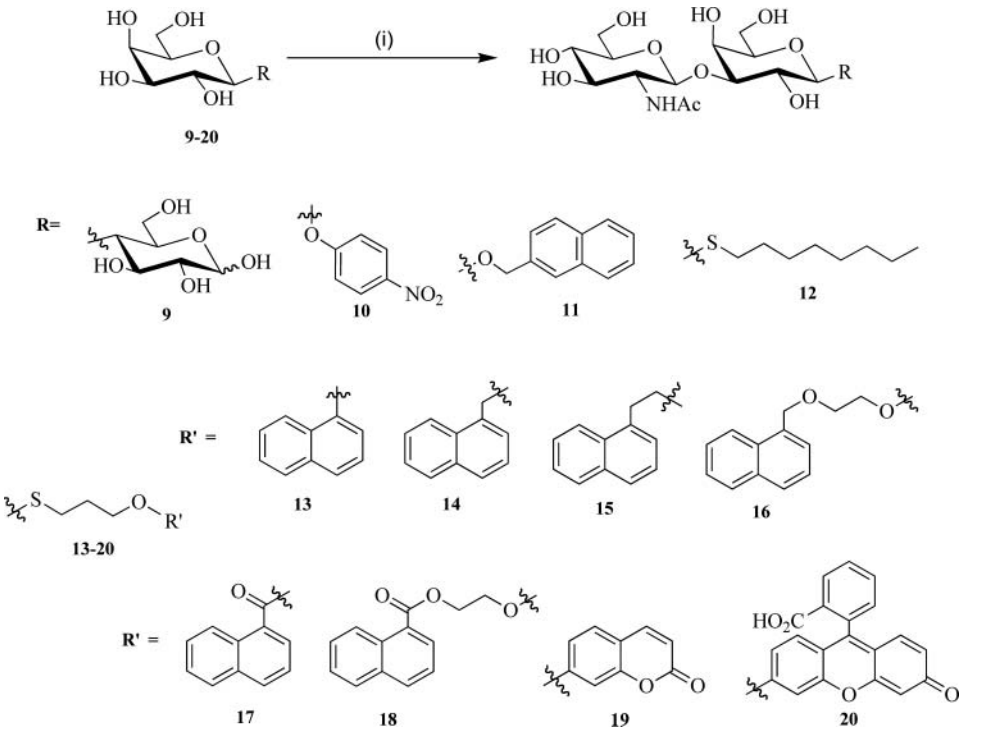

Scheme 2: (i) β3GlcNAcT, UDP-(^{3}H)GlcNAc, H_2O, 37°C.

is not available.[26] A single colony from an agar plate containing lgtA β3-*N*-acetylglucosaminyltransferase (pWc:lgtA/AD202)[31] was inoculated into cultures containing Y2T medium and the cell lysate was prepared as described by Blixt et al.[21] The amount of glycosylated product formed with each galactose acceptor was compared to the amount of product formed when either lactose **9** (10.97 nmol product), Galβ-*p*-nitrophenol **10** (7.86 nmol product), or Gal-β-naphthalenemethanol **11** (4.33 nmol product) was used since these sugars are all known acceptors for this enzyme.[26] Importantly, the crude cell lysate sufficed for efficient production of these glycosides, thus obviating the need to utilize the purified enzyme. These assays revealed that galactose derivative **19** (9.27 nmol product) acted as an excellent acceptor (Fig. 2). Compound **19** was an ideal acceptor with good affinity for the enzyme and with good solubility in aqueous and organic media, and it carried a coumarin chromophore, to aid the separation and purification from the aqueous reaction mixture.

In order to produce a large amount of starting material **21**, a large-scale enzymatic reaction was required. The large-scale enzymatic glycosylation reaction with **19** to produce GlcNAcβ3Galβ-*S*-coumarin **21** relied on the fusion protein. Disaccharide product **21** was readily purified from the enzyme mixture by

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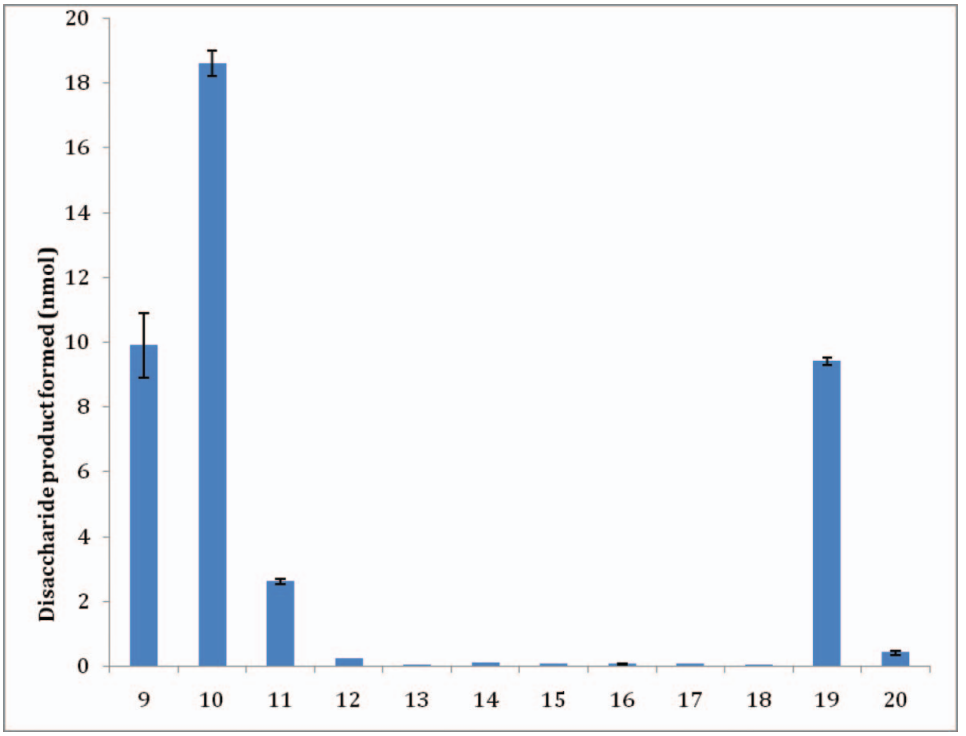

Figure 2: Screening aglycone/linkers using a radioactive enzyme assay with β3GlcNAcT and UDP-(^{3}H)GlcNAc. The amount of radiolabeled disaccharide product formed (^{3}H)GlcNAcβ3Gal-*O*-R was measured (color figure available online).

centrifugation followed by C18 Sep Pak chromatography. Subsequently, disaccharide **21** was chemically peracetylated using acetic anhydride in pyridine to produce peracetylated disaccharide **22** in 80% yield after purification.[27] The configuration of the C3-C1′ newly formed bond was assessed using COSY and HETCOR 2D-NMR to confirm the β-configuration with a coupling constant $J_{H1'\text{-}H2'}$ of 7.9 Hz (Fig. 3).

Glycosylations

Compound **22** served as a glycosylating agent (*parent molecule*) to produce four GlcNAcβ3Gal-aglycone disaccharides. Activation of the peractetylated thioglycoside **22** using *N*-iodosuccinimide and silver triflate allowed for coupling to benzyl alcohol, 1-naphthalenemethanol, 2-naphthaleneethanol, and 2-naphthalenemethanol, yielding compounds **23, 24, 25,** and **26**, respectively (Sch. 3). Compound **26** was identical to a sample prepared according to the literature procedure and was used to prove the efficiency of the method for the rapid synthesis of the decoys. [7]

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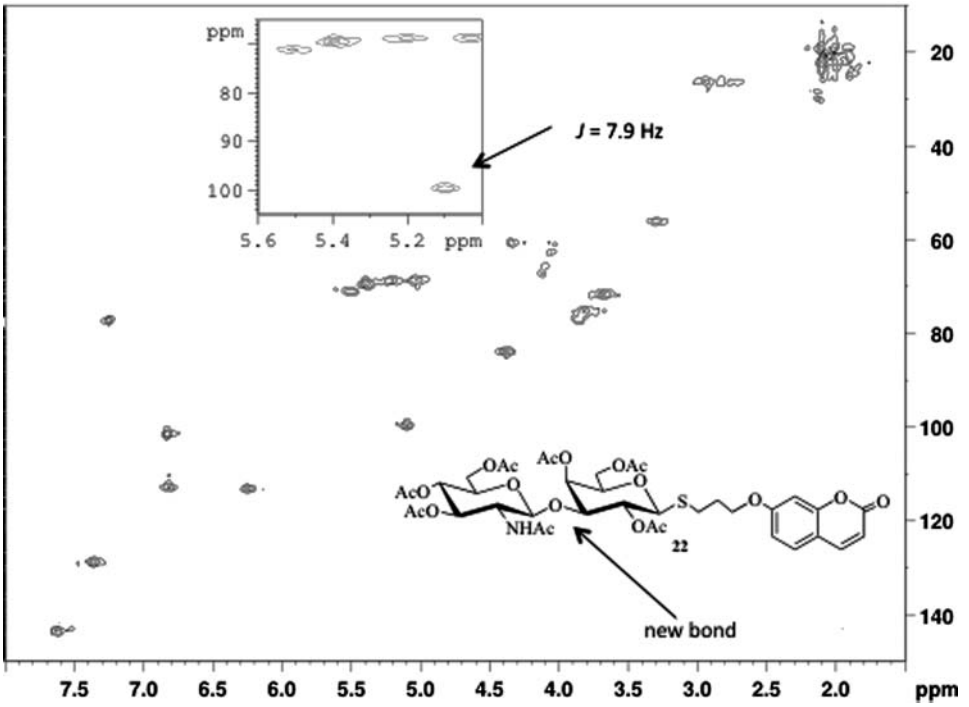

Figure 3: Partial HETCOR of compound **22** used to determine the stereochemistry of the newly formed glycosidic bond.

CONCLUSION

In conclusion, we have devised a method for the rapid production of peracetylated GlcNAcβ1,3Galβ-aglycone derivatives. A variety of derivatives can be synthesized from one single parent molecule with good overall yields. The synthesis combined a large-scale enzymatic glycosylation of a monosaccharide carrying a traceless linker, followed by acetylation of the product. Glycosylation of the desired aglycone afforded the "decoy" ready for use. This method uses just a single acetyl-protecting group in an overall sequence that requires three steps and three purifications to afford the final compounds in short time and rather high yield starting from readily available tetra-acetyl-β-1-thio-galactopyranose and allyloxycoumarin.

EXPERIMENTAL

All solvents and reagents used were reagent grade or better and were used as received. Analytical thin-layer chromatography was performed on Merck silica gel 60 F_{254} plates (glass backed, 0.25 mm). Compounds were visualized by dipping the plates in a cerium sulfate-ammonium molybdate solution or

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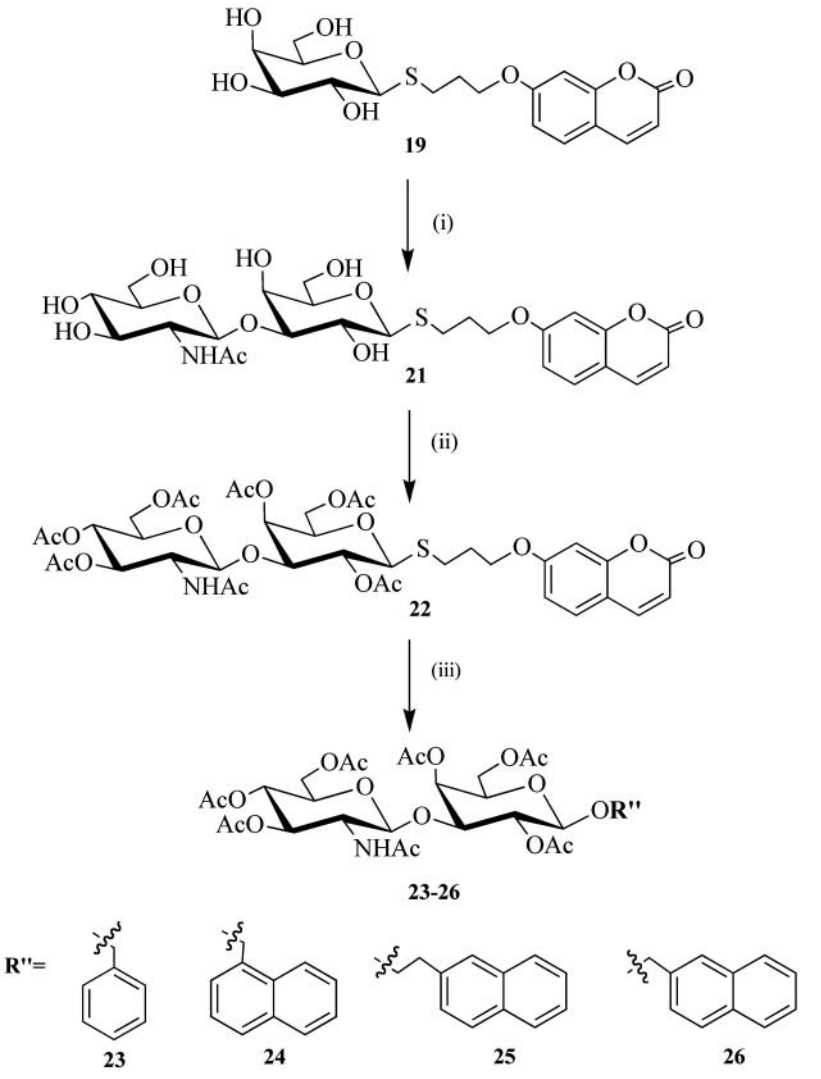

Scheme 3: (i) β3GlcNAcT, UDP-GlcNAc, H_2O, 37°C; (ii) Ac_2O, pyridine, rt; (iii) alcohol, NIS, AgOTf, DCM, 4 Å sieves, 0°C.

10% H_2SO_4 in MeOH, followed by heating. Liquid column chromatography was performed using forced nitrogen flow (unless stated otherwise) on silica gel (standard grade, 60 Å, 32–63 μm) provided by Fluka. ^{1}H NMR and ^{13}C NMR were recorded on a Varian Inova 300, a Bruker AC300F, or a Bruker Avance 500, and were referenced to residual solvent peaks. High-resolution FAB mass spectra were provided by the Mass Spectrometry Facility, Department of Chemistry and Biochemistry, University of Notre Dame. The ESI/MS

spectra were recorded on a Waters Micromass ZQ. The microscale photochemical reactor used was purchased from Ace Glass and equipped with a quartz well. The mercury lamp (254 nm) with its power supply was purchased from UVP.

Compounds **1**, **9**, and **10** were commercially available, while compounds **4**,[25] **5**,[25] **7**,[28] **8**,[29] **11**,[7] and **12**[30] were prepared according to published procedures. 2-Naphthylmethyl 2-acetamido-3,4-6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1,3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranoside **26** was used as the control disaccharide in all biological assays and was identical to a sample prepared according to literature procedures.[7]

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Allyl Ethers and Allyl Esters Preparation

1-(Allyloxymethyl)naphthalene (2)

To a solution of 1-naphthalenemethanol (1.58 g, 10 mmol) in DMF (30 mL) was added NaH (0.5 g, 60% dispersion in paraffin, washed with hexane) at rt, over 30 min. To the mixture, allylbromide (1.30 g, 10.7 mmol) was added, and the mixture further stirred for 1 h. When TLC showed almost quantitative conversion of the alcohol to the corresponding allyl ether, the reaction was quenched by addition of water in small portions, and the solvent was removed under vacuum. The resulting oil was partitioned between water (50 mL) and DCM (50 mL), and the aqueous phase was further extracted with DCM (2 × 50 mL). The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The resulting allylether was isolated by chromatography on silica gel (hexanes/EtOAc, 4/1 as eluent) as an oil in about 60% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.12 (dd, 1H, $J = 1.5$, 8.55 Hz), 7.90–7.79 (m, 2H), 7.56–7.40 (m, 4H), 6.05–5.90 (m, 1H), 5.33 (dq, 1H, $J = 1.2$, 10.3 Hz), 5.22 (dq, 1H, $J = 1.5$, 17.2 Hz), 4.96 (s, 2H), 4.10 (dt, 2H, $J = 1.5$, 5.7 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.0, 134.0, 132.0, 128.8, 128.7, 126.6, 126.4, 126.0, 125.4, 124.4, 117.5, 71.5, 70.8, 30.0 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{14}H_{14}O$: 198.1045, found: 198.1044.

1-(2-(Allyloxy)ethyl)naphthalene (3)

To a solution of 1-naphthaleneethanol (1.72 g, 10 mmol) in DMF (30 mL) was added NaH (0.5 g, 60% dispersion in paraffin, previously washed with hexane) at rt, over 30 min. To the mixture, allylbromide (1.30 g, 10.7 mmol) was added, and the mixture further stirred for 1 h. After 1 h, TLC showed almost quantitative conversion of the alcohol to the corresponding allyl ether. The reaction was quenched by addition of water in small portion, and the solvent was removed under vacuum. The resulting oil was partitioned between water (50 mL) and DCM (50 mL), and the aqueous phase further extracted with DCM

(2 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent removed under vacuum. The resulting allylether was isolated by chromatography on silica gel (hexanes/EtOAc, 4/1 as eluent) as an oil with a 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.07 (d, 1H, J = 8.1 Hz), 7.87–7.71 (m, 2H), 7.55–7.35 (m, 4H), 6.00–5.86 (m, 1H), 5.33 (dq, 1H, J = 1.8, 17.1 Hz), 5.22 (dq, 1H, J = 1.8, 10.2 Hz), 4.01 (dt, 2H, J = 1.5, 5.7 Hz), 3.78 (t, 2H, J = 7.2 Hz), 3.39 (t, 2H, J = 7.3 Hz) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 135.0, 134.0, 132.3, 129.0, 127.3, 127.0, 126.1, 125.8, 125.7, 124.0, 117.2, 72.2, 70.9, 33.6, 29.9 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{15}H_{16}O$: 212.1201, found: 212.1201.

2-(Allyloxy)ethyl 1-naphthoate (6)

To a solution of allyloxyethanol (2.04 g, 20 mmol) and pyridine (1.6 mL) in DCM (50 mL), a solution of 1-naphthoyl chloride (1.90 g, 10 mmol) in DCM (50 mL) was added dropwise, at rt, over 30 min. The mixture was stirred for an additional hour until the TLC showed almost quantitative conversion of the acyl chloride to the corresponding allyl ester. The reaction was quenched by addition of water in small portions, and the solvent was removed under vacuum. The resulting oil was partitioned between water (50 mL) and DCM (50 mL), and the aqueous phase further extracted with DCM (2 × 50 mL). The organic phase was dried (Na_2SO_4) and the solvent removed under vacuum. The resulting allyl ester was isolated by chromatography on silica gel (hexanes/EtOAc, 3/2 as eluent) as an oil with a yield of 45%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.92 (dd, 1H, J = 1.2, 8.7 Hz), 8.21 (dd, 1H, J = 1.3, 8.2 Hz), 7.99 (d, 1H, J = 8.4 Hz), 7.86 (dd, 1H, J = 1.2, 8.4 Hz), 7.63–7.44 (m, 3H), 6.00–5.86 (m, 1H), 5.32 (dq, 1H, J = 1.5, 17.1 Hz), 5.20 (dq, 1H, J = 1.2, 10.6 Hz), 4.71 (m, 2H), 4.08 (dt, 2H, J = 1.5, 5.4 Hz), 3.82 (m, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.8, 134.7, 134.0, 133.6, 131.6, 130.6, 128.7, 128.0, 127.3, 126.4, 126.0, 124.7, 117.5, 72.3, 68.2, 64.4 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{16}H_{17}O_3$: 257.1178, found: 257.1187.

Preparation of Galactose Derivatives 13–20

To a solution of tetra-acetyl-β-1-thio-galactopyranose (0.5 g, 1.38 mmol) in MeOH (15 mL) was added the allylester or allylether derivative (1.2–2 equiv.), and the solution was transferred into a photochemical cell. The solution was flushed with N_2 for 15 min and exposed to UV light (mercury lamp 254 nm) through a quartz well. The solution was irradiated until the TLC (silica gel, hexanes/EtOAc: 3/2) showed no remaining sugar; most reactions were complete after 4 to 6 h, and longer irradiations only resulted in small yield changes. The solution was then transferred into an Erlenmeyer flask (50 mL) and a freshly prepared solution of sodium methoxide in MeOH was added to reach pH 11. The solution was stirred until the TLC showed complete deprotection of the

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acetyl groups: only one spot on TLC (silica gel, hexanes/EtOAc: 3/2), with an Rf of zero (for the sugar part); the deprotected compound can be visualized by TLC (silica gel, EtOAc/MeOH: 8/1). The methanolic solution was then filtered through a short plug of silica gel, and the solvent removed under vacuum. The resulting solid/oil was dissolved in a minimum amount of water (8 mL max. containing 1 to 2 mL of methanol) and charged onto a heavy solvent extractor, and continuously extracted with DCM overnight. The DCM (containing at this point only the desired sugar and the excess ether/ester) was subsequently removed under vacuum and the residue purified by chromatography (silica gel, EtOAc/MeOH: 8/1) to yield the desired compound as either a solid or oil. The compounds were found to be stable, and were freeze-dried as a precaution. All reported yields are based on the tetra-acetyl-β-1-thio-D-galactopyranose amount used in the photochemical reaction, with only the pure fractions used for the enzymatic assays.

3-(Naphthalen-1-yloxy)propyl-1-thio-β-D-galactopyranoside (13)

Isolated in 42% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 8.19 (d, 1H, J = 6.9 Hz) 7.87 (d, 1H, J = 2.1 Hz) 7.63 (m, 4H) 6.97 (d, 1H, J = 7.3 Hz) 4.97 (d, 1H, J = 5.6 Hz) 4.79 (d, 1H, J = 5.5 Hz) 4.57 (t, 1H, J = 5.5 Hz) 4.41 (d, 1H, J = 4.4 Hz) 4.26 (m, 3H) 3.69 (s, 1H) 3.41 (m, 5H) 2.89 (m, 2H) 2.16 (t, 2H, J = 6.5 Hz) ppm. ^{13}C NMR (75 MHz, DMSO d_6) δ: 154.46, 134.48, 127.89, 126.88, 126.71, 125.74, 125.42, 122.03, 120.31, 105.62, 86.21, 79.61, 75.18, 70.26, 68.83, 66.92, 60.98, 29.89, 26.37 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{19}H_{24}O_6S$: 380.1294, found: 380.1311.

3-(Naphthalen-1-ylmethoxy)propyl-1-thio-β-D-galactopyranoside (14)

Isolated in 47% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 8.07 (d, 1H, J = 8.5 Hz), 7.93 (d, 1H, J = 8.0 Hz), 7.87 (d, 1H, J = 8.5 Hz), 7.56–7.44 (m, 4H), 4.93 (d, 1H, J = 6.0 Hz), 4.898 (s, 2H), 4.81 (d, 1H, J = 5.5 Hz), 4.60 (t, 1H, J = 5.5 Hz), 4.42 (d, 1H, J = 4.5 Hz), 4.19 (d, 1H, J = 9.5 Hz), 3.68 (d, 1H, J = 3.5 Hz), 3.59 (t, 2H, J = 6.0 Hz), 3.51–3.43 (m, 2H), 3.36–3.33 (m. 2H), 3.28–3.25 (m, 2H), 2.76–2.59 (m, 2H), 1.83 (quintet, 2H, J = 7.0 Hz) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 140.1, 139.5, 138.0, 135.6, 135.1, 134.3, 133.2, 132.8, 132.1, 130.5, 92.6, 85.2, 80.2, 77.3, 76.1, 75.2, 75.1, 67.4, 36.1, 33.4 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{20}H_{27}O_6S$: 395.1528, found: 395.1526.

3-(2-(Naphthalen-1-yl)ethoxy)propyl-1-thio-β-D-galactopyranoside (15)

Isolated in 39% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 8.02 (d, 1H, J = 8.5 Hz), 7.90 (d, 1H, J = 7.5 Hz), 7.77 (d, 1H, J = 7.5 Hz), 7.55–7.48 (m, 2H), 7.44–7.39 (m, 2H), 4.93 (d, 1H, J = 6.0 Hz), 4.79 (d, 1H, J = 5.5 Hz), 4.59 (t, 1H, J = 5.5 Hz), 4.40 (d, 1H, J = 4.5 Hz), 4.17 (d, 1H, J = 9.5 Hz), 3.67 (t, 2H, J = 7.0 Hz), 3.49–3.35 (m, 6H, under solvent peak), 3.34–3.36 (m. 4H), 2.66–2.54 (m, 2H), 1.74 (quintet, 2H, J = 7.0 Hz) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 135.0,

133.6, 131.6, 128.7, 127.2, 127.0, 126.2, 125.8 (2C), 123.8, 86.5, 78.7, 73.9, 70.4, 69.7, 68.8, 68.7, 60.9, 32.3, 29.5, 27.1 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{21}H_{29}O_6S$: 409.1685, found: 409.1663.

3-(2-(2-(Naphthalen-1-yl)ethoxy)ethoxy)propyl-1-thio-β-D-galactopyranoside (16)

Isolated in 45% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 7.89 (m, 4H), 7.50 (m, 3H), 4.92 (d, 1H, $J = 5.4$ Hz), 4.77 (d, 1H, $J = 5.4$ Hz), 4.67 (s, 2H), 4.55 (t, 1H, $J = 5.5$ Hz), 4.39 (d, 1H, $J = 4.3$ Hz), 4.20 (d, 1H, $J = 9.2$ Hz), 3.47 (m, 12H), 2.66 (m, 2H), 1.79 (m, 2H) ppm. ^{13}C NMR (75 MHz, DMSO d_6) δ: 136.63, 133.27, 132.88, 128.29, 128.18, 128.02, 126.63, 126.29, 86.26, 79.59, 75.17, 72.55, 70.25, 69.96, 69.64, 69.48, 68.83, 60.99, 40.83, 39.16, 30.26, 26.38 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{22}H_{31}O_7S$: 439.1791, found: 439.1768.

3-Propyl-1-naphtoate-1-thio-β-D-galactopyranoside (17)

Isolated in 50% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 8.76 (d, 1H, $J = 8.4$ Hz), 8.18 (t, 2H, $J = 8.5$ Hz), 8.04 (d, 1H, $J = 7.9$ Hz), 7.64 (m, 3H), 4.99 (d, 1H, $J = 5.5$ Hz), 4.82 (d, 1H, $J = 5.3$ Hz), 4.57 (t, 1H, $J = 5.5$ Hz), 4.45 (dd, 3H, $J = 12.2, 6.2$ Hz), 4.28 (d, 1H, $J = 9.3$ Hz), 3.71 (t, 1H, $J = 3.6$ Hz), 3.42 (m, 5H), 2.82 (m, 2H), 2.08 (t, 2H, $J = 6.7$ Hz) ppm. ^{13}C NMR (75 MHz, DMSO d_6) δ: 166.85, 133.43, 133.30, 129.96, 128.73, 127.88, 126.83, 126.39, 125.09, 124.97, 85.73, 79.17, 74.72, 69.78, 68.39, 63.78, 60.54, 28.82, 25.82 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{20}H_{25}O_7S$: 409.1321, found: 409.1332.

3-(2-Propoxyethyl)-1-naphthoate-1-thio-β-D-galactopyranoside (18)

Isolated in 40% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 8.73 (d, 1H, $J = 8.5$ Hz), 8.18 (d, 1H, $J = 8.5$ Hz), 8.12 (d, 1H, $J = 6.0$ Hz), 8.03 (d, 1H, $J = 8.0$ Hz), 7.67–7.58 (m, 2H), 4.93 (d, 1H, $J = 5.5$ Hz), 4.80 (d, 1H, $J = 5.5$ Hz), 4.54 (t, 1H, $J = 5.5$ Hz), 4.48 (m, 2H), 4.39 (d, 1H, $J = 4.5$ Hz), 4.18 (d, 1H, $J = 9.5$ Hz), 3.75 (m, 2H), 3.66 (t, 1H, $J = 3.5$ Hz) 3.56 (t. 2H, $J = 6.5$ Hz), 3.50–3.40 (m, 2H), 3.38–3.314 (m, 2H), 3.27–3.23 (m, 1H), 2.72–2.59 (m, 2H), 1.81 (quintet, 2H, $J = 6.5$ Hz) ppm. ^{13}C NMR (75 MHz, D_2O) δ: 169.8, 134.9 (2C), 132.1, 131.8, 130.0, 129.3, 128.0, 127.7, 126.7, 126.1, 87.8, 80.2, 75.6, 71.3, 70.9, 70.2, 69.7, 65.7, 62.4, 31.0, 28.5 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{22}H_{29}O_8S$: 453.1583, found: 453.1568.

3-(7-Propoxy)-2H-chromen-2-one-1-thio-β-D-galactopyranoside (19)

Isolated in 40% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 7.99 (d, 1H, $J = 9.5$ Hz), 7.63 (d, 1H, $J = 8.4$ Hz), 6.97 (m, 2H), 6.29 (d, 1H, $J = 9.5$ Hz), 4.96 (d, 2H, $J = 5.5$ Hz), 4.79 (d, 1H, $J = 5.4$ Hz), 4.56 (t, 1H, $J = 5.5$ Hz), 4.40 (d, 1H, $J = 4.4$ Hz), 4.23 (d, 1H, $J = 9.2$ Hz), 4.17 (t, 2H, $J = 6.2$ Hz), 3.68 (m, 1H), 3.46 (m, 1H), 3.29 (m, 3H), 2.77 (m, 2H), 2.03 (m, 2H) ppm. ^{13}C NMR (75 MHz,

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DMSO d_6) δ: 162.22, 160.78, 155.87, 144.82, 129.98, 113.15, 112.92, 112.81, 101.71, 86.22, 79.64, 75.15, 70.21, 68.84, 67.46, 61.00, 29.56, 26.05 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{18}H_{23}O_8S$: 399.1114, found: 399.1111.

3-(2-(3-Oxo-6-propoxy-3H-xanthen-9-yl)benzoic acid-1-thio-β-D-galactopyranoside (20)

Isolated in 22% yield, ^{1}H NMR (300 MHz, DMSO d_6) δ: 10.13 (broad s, 1H), 8.01 (d, 1H, $J = 7.5$ Hz), 7.76 (m, 2H), 7.28 (d, 1H, $J = 7.8$ Hz), 6.93 (d, 1H, $J = 2.4$ Hz), 6.66 (m, 4H), 4.95 (d, 1H, $J = 5.7$ Hz), 4.79 (d, 1H, $J = 5.4$ Hz), 4.56 (t, 1H, $J = 5.4$ Hz), 4.39 (d, 1H, $J = 4.5$ Hz), 4.22 (d, 1H, $J = 9.3$ Hz), 4.13 (t, 2H, $J = 6.0$ Hz), 3.67 (m, 1H), 3.47 (m, 1H), 3.32 (m, 5H), 2.74 (m, 2H), 2.03 (t, 2H, $J = 6.6$ Hz) ppm. ^{13}C NMR (75 MHz, DMSO d_6) δ: 168.6, 160.3, 152.5, 151.8, 151.7, 135.6, 130.1, 129.1, 128.9, 126.0, 124.6, 124.0, 112.2, 110.9, 101.3, 85.7, 79.1, 74.6, 69.7, 68.3, 66.7, 60.5, 29.1, 25.6 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{29}H_{29}O_{10}S$: 569.1481, found: 569.1469.

Radioactive *N*-Acetylglucosaminyltransferase Assays to Screen the Galactose Derivatives

The assays were conducted in a final volume of 100 μL at 37°C for 1 h, containing the different acceptor substrates **9–20** (10 mM) and donor substrate UDP[6-^{3}H]GlcNAc (2 mM, 1086 cpm/nmol, NEN Life Science Products) in sodium cacodylate (50 mM, pH 7.5), $MnCl_2$ (10 mM), and bovine serum albumin (1%). Reactions were initiated by adding crude lysate (10 μL, ~0.5 mU) diluted in buffer containing bovine serum albumin (1%). Transfer of GlcNAc to the various galactose acceptor substrates using *N*-acetylglucosaminyltranferase is expressed in nmol of disaccharide product formed. Each reaction product was diluted with 0.5 mL of 0.5M NaCl and applied to a Sep-Pak C18 column (100 mg, Waters). After washing with 25 mL of water, the disaccharide product is eluted with 50% methanol, dried, and counted by scintillation counting.

Preparation of Compound 21

Scale-up of the enzymatic reaction was carried out in several steps and any solubility problems were resolved by adding 10% ethanol. The enzyme reactions were performed first on a small scale (~10 mg) and then on a larger scale (~1 g). Reactions contained the acceptor substrate (**19**) (20 mmol, 40 mM) and UDP-GlcNAc (20 mmol, 40 mM) in sodium cacodylate buffer (125 mL, 100 mM, pH 7.5) with $MnCl_2$ (20 mM) and bovine serum albumin (0.1%). The reactions were initiated by adding the crude enzyme lysate (10 U, based on the *N*-acetylglucosaminyltranferase activity). The reaction was stirred at rt for 3 to 5 days. The reactions were monitored by TLC using EtOAC/MeOH: 8/1 as

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eluent, as well as by mass spectrometry. At the end of the reaction, the disaccharide was centrifuged, the supernatant was applied to a C18 Sep Pak (20g, prepacked), the column was washed with several portions of water, and the desired disaccharide was eluted using pure methanol. The elution of the compound can readily be followed by the use of a UV lamp (long wave), as the coumarin moiety tends to fluoresce. This enzymatic step typically gave an overall yield of $\geq$45% (based on the monosaccharide starting material). Although on large-scale reactions it is known that UDP inhibits the enzyme-catalyzed reactions,[26] it was difficult to circumvent. Finally, the disaccharide was peracetylated and purified to afford compound **22** in 82% yield, ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.64 (d, 1H, J = 9.1 Hz), 7.37 (d, 1H, J = 7.8 Hz), 6.84 (s, 1H), 6.83 (d, 1H, J = 7.8), 6.26 (d, 1H, J = 9.1 Hz), 5.54 (d, 1H, J = 4.6 Hz), 5.41 (d, 1H, J = 3.2 Hz), 5.21 (t, 1H, J = 9.3 Hz), 5.11 (d, 1H, J = 7.9 Hz), 5.04 (t, 1H, J = 9.5 Hz), 4.39 (dd, 2H, J = 17.2, 10.2 Hz), 4.34 (dd, 1H, J = 12.0, 2.3 Hz), 4.13–4.01 (m, 7H), 3.86 (dd, 1H, J = 9.3, 3.2 Hz), 3.83 (t, 1H, J = 6.5 Hz), 3.68 (dt, 1H, J = 9.3, 3.2 Hz), 3.31 (dt, 1H, J = 0.2, 8.3 Hz), 2.93 (m, 1H), 2.80 (m, 1H), 2.15 (s, 3H), 2.10 (s, 6H), 2.05 (s, 3H), 2.02 (s, 6H), 1.9 (s, 3H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.8, 170.6, 170.5, 169.8, 162.1, 161.2, 155.9, 143.5, 128.8, 113.2, 112.8, 112.6, 101.5, 99.7, 83.9, 75.2, 71.6, 71.1, 69.4, 68.8, 68.7, 66.7, 62.2, 61.3, 56.3, 29.2, 26.4, 23.3, 21.1, 20.8, 20.8, 20.7 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{38}H_{48}O_{19}NS$: 854.2541, found: 854.2546.

General Procedure for the Glycosylation of Compound 22

The glycosylation agent and the nucleophile were dried by coevaporation with toluene (3 $\times$ 5 mL) and then kept under vacuum for 2 h. A solution of alcohol (1 equiv.), thioglycoside (1.2 equiv.), and powdered molecular sieves (4 Å, 50 to 100 mg) in anhydrous DCM (5 to 10 mL) was cooled to 0°C and then *N*-iodosuccinimide (3 equiv.) and silver triflate (0.3 equiv.) were added. After stirring for 15 to 20 min at 0°C, Et_3N (0.05 mL) was added and the reaction mixture was diluted with DCM (10 mL) and filtered through Celite. The filtrate was washed successively with a saturated aqueous solution of $Na_2S_2O_3$, water, and brine. After drying (Na_2SO_4), the organic phase was filtered and concentrated and the residue was purified by chromatography (hexanes/EtOAc, 1:1$\rightarrow$1:2).

Benzyl 3-(3,4,6-O-acetyl-2-acetamide-2-deoxy-β-glucosyl)-2,4,6-O-acetyl-β-galactoside (23)

Compound **14** (45.1 mg, 52.8 mmol) was dried under vacuum for 3 h. Activated molecular sieves (MS, 4Å, powdered, 44 mg) were added, followed by CH_2Cl_2 (5 mL) and benzyl alcohol (4.6 mL, 45 mmol). The resulting mixture was cooled to 0°C and *N*-iodosuccinimide (NIS, 31.6 mg, 0.14 mmol) and silver(I) trifluoromethanesulphonate (AgOTf, 5.1 mg, 20 mmol) were added.

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Within 20 min, the mixture turned red. The mixture was stirred at 0°C for 1 h and the reaction was quenched with triethylamine (50 mL). The mixture was diluted with CH_2Cl_2 and filtered through celite, which was subsequently washed with CH_2Cl_2. The combined filtrates were washed with sat. $Na_2S_2O_3$, water, and brine (20 mL each); dried; and concentrated to give 41 mg crude product. Flash chromatography (hexanes/EtOAc 1:3) yielded compound **15** (7.3 mg, 23%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.87–7.28 (m, 5H), 5.52 (dd, 1H, J = 10.4, 9.0 Hz), 5.46 (d, 1H, J = 7.7 Hz), 5.39 (d, 1H, J = 3.5 Hz), 5.21 (dd, 1H, J = 9.8, 8.0 Hz), 5.07–5.02 (2H, m), 4.90 (d, 1H, J = 12.4 Hz), 4.64 (d, 1H, J = 12.4 Hz), 4.42 (d, 1H, J = 8.0 Hz), 4.32 (dd, 1H, J = 12.2, 2.5 Hz), 4.20–4.08 (m, 3H), 3.84–3.79 (m, 2H), 3.66 (dt, 1H, J = 10.0, 3.3 Hz), 3.32 (dt, 1H, J = 10.5, 7.9 Hz), 2.15 (s, 3H), 2.12 (s, 6H), 2.07 (s, 3H), 2.03 (s, 3H), 1.93 (s, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.9, 170.8, 170.7, 170.6, 170.0, 169.8, 169.7, 137.0, 128.6, 128.2, 128.0, 99.8, 99.6, 76.3, 71.8, 71.5, 71.3, 71.0, 70.6, 69.4, 69.0, 62.3, 61.5, 56.4, 23.5, 21.2, 21.0, 20.9, 20.9 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{33}H_{44}O_{17}N$: 726.2609, found: 726.2592.

1-Naphtalenemethyl 3-(3,4,6-O-acetyl-2-acetamide-2-deoxy-β-glucosyl)-2,4,6-O-acetyl-β-galactoside (24)

Compound **14** (51.5 mg, 60.3 mmol) and 1-naphtalenemethanol (19.1 mg, 121 mmol) were evaporated from toluene thrice, NIS (44.7 mg, 0.2 mmol) was added, and the residue was protected from light, dried under vacuum overnight, and dissolved in CH_2Cl_2 (5 mL). MS (4Å, powdered) was added and the mixture was cooled to 0°C. AgOTf (36 mL, 0.51 M in toluene, 18 mmol) was added and the reaction was stirred at 0°C to 12°C for 1.5 h. Triethylamine (50 mL) was added and the mixture was diluted with CH_2Cl_2 (10 mL) and filtered through celite, which was subsequently washed with CH_2Cl_2 (10 mL). The combined filtrates were washed with sat. $Na_2S_2O_3$, water, and brine (25 mL each); dried; and concentrated to give 76 mg crude product. Flash chromatography (hexanes/EtOAc 1:3 to 0:1) yielded the title compound **16** (17 mg, 36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.07 (dd, 1H, J = 9.0, 1.9 Hz), 7.86 (m, 2H), 7.51 (m, 2H), 7.42 (dd, 1H, J = 7.8, 4.1 Hz), 7.41 (s, 1H), 5.45–5.33 (m, 3H), 5.14 (dd, 1H, J = 10.1, 8.1 Hz), 4.98 (m, 1H), 4.92 (d, 1H, J = 8.1 Hz), 4.34 (d, 1H, J = 8.0 Hz), 4.26 (dd, 1H, J = 12.2, 2.6 Hz), 4.18, (m, 2H), 4.03 (dd, 1H, J = 12.2, 3.9 Hz), 3.75 (t, 1H, J = 6.4 Hz), 3.70 (dd, 1H, J = 9.9, 3.5 Hz), 3.57 (dt, 1H, J = 6.4, 2.9 Hz), 3.26 (dt, 1H, J = 10.4, 7.9 Hz), 2.13–1.95 (m, 13H), 1.84 (m, 2H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.9, 170.8, 170.6, 170.0, 169.7, 169.6, 134.0, 132.1, 132.1, 129.5, 128.8, 127.8, 126.6, 126.2, 125.2, 124.4, 99.8, 98.6, 76.2, 71.8, 71.5, 71.3, 70.9, 69.5, 62.2, 61.5, 56.3, 21.0, 20.9, 20.9, 20.8 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{37}H_{46}O_{17}N$: 776.2766, found: 776.2781.

2-Naphtaleneethyl 3-(3,4,6-O-acetyl-2-acetamide-2-deoxy-β-glucosyl)-2,4,6-O-acetyl-β-galactoside (25)

Compound **14** (51.3 mg, 60.1 mmol) and 2-naphthaleneethanol (20.9 mg, 121 mmol) were evaporated from toluene thrice. NIS (43.9 mg, 0.2 mmol) was added and the residue was protected from light, dried under vacuum overnight, and dissolved in anhydrous CH_2Cl_2 (5 mL). Powdered molecular sieves (4 Å) were added and the mixture was cooled to 0°C. AgOTf (36 mL, 0.51 M in toluene, 18 mmol) was added and the reaction was stirred at 0°C to 12°C for 1.5 h. Triethylamine (50 mL) was added and the mixture was diluted with CH_2Cl_2 (10 mL) and filtered through celite, and was subsequently washed with CH_2Cl_2 (10 mL). The combined filtrates were washed with sat. $Na_2S_2O_3$, water, and brine (25 mL each); dried; and concentrated to give 79 mg crude product. Flash chromatography (hexanes/EtOAc 1:3) yielded **17** (23 mg, 48%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.81 (t, 2H, $J = 7.4$ Hz), 7.78 (d, 1H, $J = 8.5$ Hz), 7.65 (s, 1H), 7.45 (ddd, 2H, $J = 8.0$, 6.5, 1.9 Hz), 5.48 (dd, 1H, $J = 10.5$, 9.3 Hz), 5.44 (d, 1H, $J = 7.8$ Hz), 5.38 (d, 1H, $J = 3.3$ Hz), 5.15 (dd, 1H, $J = 10.0$, 8.1 Hz), 5.04 (t, 1H, $J = 9.7$ Hz), 5.02 (d, 1H, $J = 8.1$ Hz), 4.38 (d, 1H, $J = 8.1$ Hz), 4.32 (dd, 1H, $J = 12.2$, 2.5 Hz), 4.24 (dt, 1H, $J = 9.7$, 5.3 Hz), 4.18–4.08 (m, 3H), 3.83–3.79 (m, 2H), 3.75 (dd, 1H, $J = 9.3$, 7.3 Hz), 3.66 (dt, 1H, $J = 9.2$, 3.6 Hz), 3.32 (dt, 1H, $J = 10.5$, 7.7 Hz), 3.12–3.02 (m, 2H), 2.14 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.02 (s, 6H), 1.90 (s, 3H), 1.73 (s, 3H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.9, 170.8, 170.6, 170.6, 170.0, 169.7, 169.7, 136.3, 133.7, 132.3, 128.0, 127.7, 127.7, 127.7, 127.5, 126.2, 125.6, 101.3, 99.8, 76.3, 71.8, 71.4, 71.4, 70.9, 70.6, 69.4, 69.0, 62.3, 61.5, 56.3, 36.3, 23.4, 21.0, 20.9, 20.8 ppm. HR-MS (FAB+ of M+H, PEG): m/z calcd for $C_{38}H_{48}O_{17}N$: 790.2922, found: 790.2949.

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ACKNOWLEDGEMENTS

P.H.S. gratefully acknowledges financial support from NIH (5 R01 HL64799-06 and 5 P01 CA071932-10). J.D.E. gratefully acknowledges financial support from NIH (CA112278). We thank Dr. V. Mountain for critically editing this manuscript. We would like to thank Dr. Warren Wakarchuk and Dr James Paulson for the kind gift of lgtA β-3-*N*-acetylglucosaminyltransferase.

N. Merbouh and J. R. Brown contributed equally to the work and should be considered as first coauthors.

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