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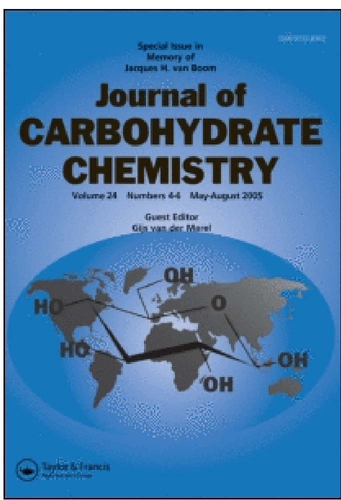

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Effect of Anomeric Linkage on the Sialylation of Glycosides by Cells

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Effect of Anomeric Linkage on the Sialylation of Glycosides by Cells

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The synthesis of sialylated glycosides using saccharide primers and cells was investigated. α· and β· Saccharide primers were chemically synthesized and introduced into B16 melanoma cells to prime oligosaccharide synthesis. Incorporation of α- and β-dodecyl lactosides into B16 cells resulted in the sialylation of the galactose residue to give GM3-type oligosaccharides. The β-dodecyl galactoside primer was sialylated but the α-dodecyl galactoside primer was not. Both the α- and β-dodecyl glucoside primers were not elongated. In the glycosylation of primers by cells, this research confirmed that sialyl transferases tolerate acceptor modifications and are permissive to primer elongation regardless of the α- or β-linkage to the aglycon unit. However, the

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presence of the terminal galactose residue that is β-linked to the adjacent saccharide or aglycon unit is essential for sialylation by cellular enzymes to occur.

Keywords Sialylation, Glycoside primer, Oligosaccharide, Ganglioside, α,β Anomer

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INTRODUCTION

Sialic acid, located at the nonreducing end of carbohydrate chains of glycoconjugates, plays an important role in a number of biochemical and immunochemical events.[1–3] Sialylated oligosaccharides have useful applications to study protein and carbohydrate interactions, for drug delivery, and as potential inhibitors.[4,5] Sialic acid-containing gangliosides are located at the outer cell surface of plasma membranes and serve as binding sites for enzymes, hormones, toxins, lectins, and viruses.[6]

Considering the roles played by sialic acid-containing glycoconjugates in biochemical and cellular processes, development of methods for the rapid and efficient synthesis of sialylated oligosaccharides is necessary to clarify their vital functions.[7] Several strategies involve a chemical or an enzymatic synthetic approach.[8] However, the stereoselective glycosylation of sialic acid by the creation of α-glycosidic linkage is a daunting task.[9,10] Aside from poor stereoselectivity, low yield is also one among the many shortcomings.[11] The carboxyl group attached to the anomeric position reduces reactivity in glycosylation. Although organic synthesis yields a reasonable amount of sialylated oligosaccharide with high purity, the approach requires a multistep operation involving tedious protection and deprotection schemes.

New methods of glycosylation are undertaken to afford sialylated oligosaccharides with high stereoselectivity and yield.[12] Recently, a biocombinatorial method of preparing GM3-type oligosaccharides (sialylated lactosides) has been reported.[13,14] This approach involves the use of saccharide primers (simple amphiphilic building blocks) and cells. Elongation of the saccharide primers is cellular enzyme-mediated. Depending on the type of cell used, a single building block could yield a number of oligosaccharides that are released to the culture medium.[15] As part of our continued interest in the synthesis of oligosaccharides using this strategy, we embarked on the fast and easy preparation of sialylated oligosaccharides by introducing chemically synthesized lactoside, galactoside, and glucoside primers into B16 melanoma cells. To establish the significance of the glycosidic linkage in priming oligosaccharide synthesis, primers with α- and β-linked aglycon units were used. The efficiency of glycosylation is dependent not only on the saccharide unit but also on the aglycon structure. The alkyl chain length of the aglycon plays an important role to allow the incorporation of primers into the cell and the subsequent release of the elongated products to the culture medium. The alkyl chain length

of dodecyl has been reported to be the most appropriate and thus, dodecyl was used as aglycon for all the primers in this research. [15]

RESULTS AND DISCUSSION

Chemical Synthesis of Saccharide Primers

The preparation of the saccharide primers is shown in Scheme 1. Generally, the saccharide primers were prepared in two steps: glycosylation followed by deacylation. Glycosylation of lactose peracetate (**1**) with dodecanol in the presence of a Lewis acid afforded α,β mixture of the dodecyl lactoside derivatives that was separated by column chromatography. Usual *O*-deacylation with NaOMe-MeOH gave the α- and β-linked dodecyl D-lactoside primers. The structures of the desired compounds were confirmed by both ^{1}H NMR and ^{13}C NMR results. The anomeric configuration of the α- and β-lactoside was confirmed by the chemical shift for H-1 and the $J_{1,2}$ coupling constant. Resonances at 4.60 ($J = 3.84$ Hz) and at 4.15 ($J = 7.68$ Hz) in the ^{1}H NMR spectra confirmed the formed α- and β-anomeric linkage, respectively.

The reaction of 2,3,4,6-tetra-*O*-benzylgalactosyl chloride (**3**) with dodecanol in the presence of AgOTf gave a mixture of α- and β-glycosylation products that were separated by column chromatography. Subsequent deprotection with Pd/C afforded n-dodecyl α-D-galactopyranoside and n-dodecyl β-D-galactopyranoside. Resonances at 4.58 ($J = 3.84$ Hz) and 4.22 ($J = 7.83$ Hz) in the ^{1}H NMR spectra confirmed the α- and β-anomeric linkage, respectively.

Reaction of 2,3,4,6-tetra-*O*-benzylglucosyl imidate (**5**) with dodecanol in the presence of TBDMSOTf gave a mixture of α- and β-glycosylation products. Separation of glycosylation products by column chromatography followed by deprotection with Pd/C afforded n-dodecyl α-D-glucopyranoside and n-dodecyl β-D-glucopyranoside. Resonances at 4.59 ($J = 3.84$ Hz) and at 4.08 ($J = 7.74$ Hz) in the ^{1}H NMR spectra confirmed the α- and β-anomeric linkage, respectively.

Effect of Saccharide Primer on Cell Growth and Cellular Glycosylation of the Saccharide Primers

Fifty μM of each of the saccharide primers were administered to mouse B16 melanoma cells for their feasibility as substrate for GM3 oligosaccharide biosynthesis. β-glucoside primer exhibited cytotoxicity. The α-glucoside primer and α-galactoside primer significantly reduced the number of cells by 50% and 70%, respectively, relative to control. The rest of the primers did not exhibit adverse effects to cell morphology and viability as shown in Table 1.

After incubation for 48 hr, lipids from the culture media were collected by using SepPak C18 column, while lipids from the cell homogenates were

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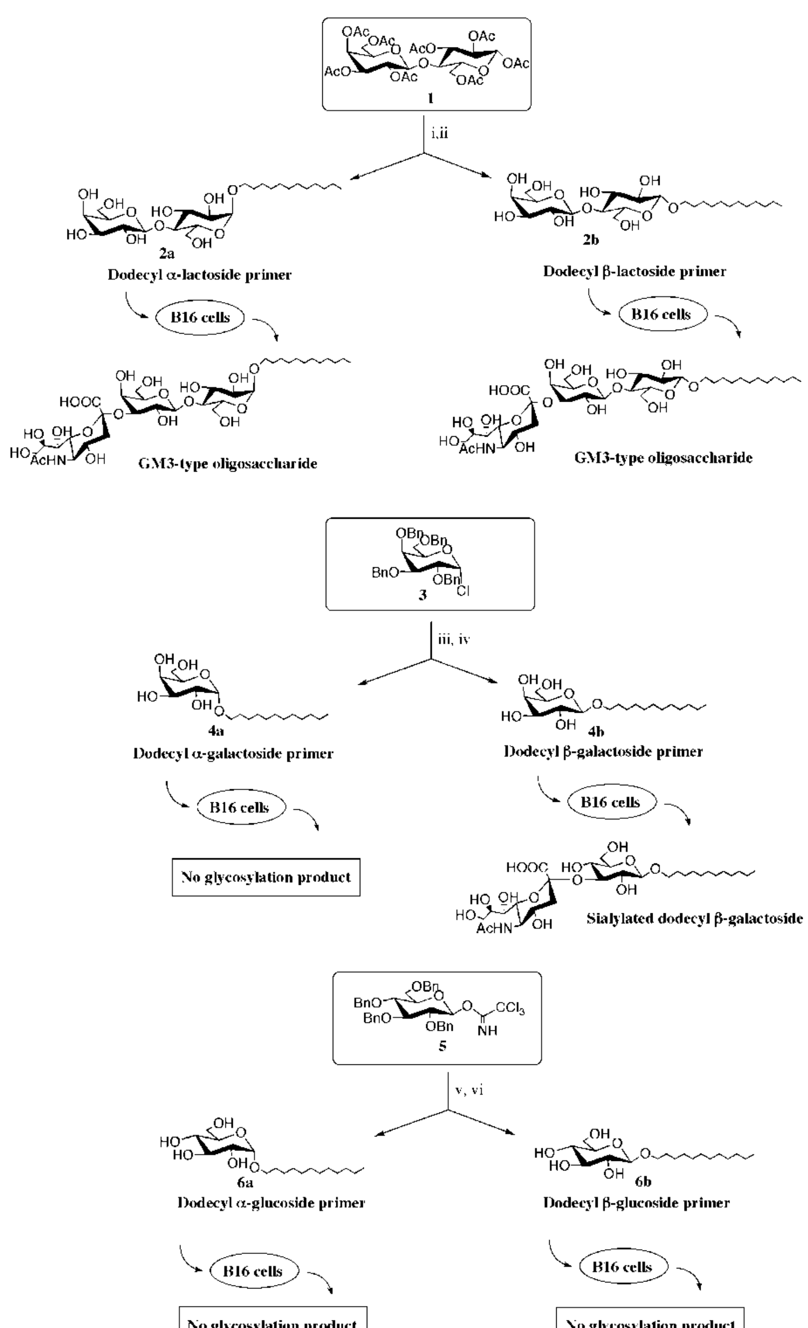

Scheme 1: Chemical synthesis of glycoside primers and subsequent uptake by B16 cells to prime oligosaccharide synthesis. Reagents and conditions: i. $CH_3(CH_2)_{11}OH$, $BF_3 \cdot OEt_2$, $ClCH_2$, CH_2Cl, 20 hr, 50°C; ii. NaOMe, MeOH, 12 hr, rt iii. $CH_3(CH_2)_{11}OH$, AgOTf, toluene, 12 hr–15°C; iv. 5% Pd/C, EtOH, 3 days, rt; v. $CH_3(CH_2)_{11}OH$, diethyl ether, TBDMSOTf, 6 hr, rt; vi. 5% Pd/C, EtOH, 6 days, rt.

Table 1: Cell number after 48-hr incubation of cells with the saccharide primer.

Saccharide Primer	Cell Number[a]
	9.1×10^6
	7.6×10^6
	6.4×10^6
	3.5×10^6
	Cells died
	5.1×10^6

[a]Control: 10.8×10^6.

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collected by extraction with chloroform:methanol and then with chloroform:2-propanol:water. HPTLC results of the lipid extracts from the culture media showed new bands corresponding to glycosylated products from the α-lactoside primer, the β-lactoside primer, and the β-galactoside primer. The HPTLC results of the lipid extracts from the cell fraction show the presence of the band corresponding to the α-galactoside primer and the α-glucoside primer, indicating that both primers were taken up by cells. However, neither the α-galactoside primer nor the α-glucoside primer gave any glycosylation product.

Identification of the Glycosylated Lactoside

To elucidate the glycan structure modified in the cells, the glycosylated products were scraped from the HPTLC plate, extracted with methanol, and dried. Since the glycosylated products were obtained in minute amounts, analysis by NMR was not possible and thus, identification was carried out by mass spectral analysis and the use of appropriate enzymes to confirm the structure. MALDI TOF mass spectral results obtained for each of the glycosylated primers revealed peaks at m/z 823.84, at m/z 823.62, and at m/z 662.22 that correspond to a monosialylated α-lactoside, a monosialylated β-lactoside, and a monosialylated β-galactoside, respectively. Treatment of glycosylated

products with α-2,3-sialidase confirmed that the glycosylation products are GM3-type oligosaccharides (sialylated dodecyl α-lactoside and sialylated dodecyl β-lactoside) and sialylated dodecyl β-galactoside. Thus, uptake of either the α- or the β-lactoside primers by B16 cells resulted in the sialylation of the galactose residue to give a glycosylated product having the same glycan structure as ganglioside GM3. Similarly, uptake of the β-galactoside primer resulted in sialylation of the galactose residue.

Sialyltransferases reside in the Golgi compartment and transfer a sialic acid residue from CMP-sialic acid to the C-6 or C-3 hydroxyl groups at the nonreducing Gal-, Gal NAc-, or GlcNAc residue. For glycosylation by cells to occur, the primers must diffuse through the plasma membrane and enter the Golgi. Results revealed that cells are not selective to the type of anomeric linkage of the primers they take in. Both the α- and β-lactoside primers could pass through the plasma membrane and function as acceptors for GM3 synthase, α2-3 sialyl transferase. It is noteworthy that sialyltranferases used the α-lactoside primer as substrate even if the anomeric linkage to the aglycon is different from lactosyl ceramide, the common natural intermediate in glycosphingolipid biosynthesis. Although the α-galactoside and the α-glucoside primers were not glycosylated, HPTLC results showing the presence of these primers in the cell fraction confirmed that these primers have been taken up by the cell. Thus, the anomeric linkage of the aglycon unit seems irrelevant for primer incorporation into cells and elongation. However, the anomeric linkage of the terminal galactoside residue that is the site for sialylation of the primers plays a significant role.

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Cells take up saccharide primers that act as substrates to give sialylated oligosaccharides. In the biosynthetic pathway, cellular enzymes recognize and sialylate the terminal galactose residue of lactosyl ceramide. Results showed that sialylation occurred only with the α- and β-lactoside primers and with the β-galactoside primer. These primers have a terminal galactose residue that is β-linked. On the other hand, the α-galactoside primer and the α- and β-glucoside primers that do not have a terminal β-linked galactose moiety were not recognized as acceptors by α-(2 $\rightarrow$ 3)-sialyl transferase and were not sialylated. Thus, a terminal galactose moiety that is β-linked to the adjacent saccharide or aglycon unit is essential for the efficient saccharide sialylation by cellular enzymes.

EXPERIMENTAL

General Methods

Specific rotations were determined with a JASCO DIP 1000 N digital polarimeter at 28.5°C, and ^{1}H NMR spectra were recorded at a 600 MHz

JEOL spectrometer in Me_2SO-d_6, MeOH-d_3, or $CDCl_3$ using Me_4Si as internal reference. All reactions were monitored by thin layer chromatography (TLC) on Silica Gel 60 F-254 (E. Merck), with detection by UV light or by visualizing by spraying with anisaldehyde-H_2SO_4 and heating. Column chromatography was performed on Silica Gel 60 (70–230 mesh, E. Merck, Darmstadt). The MALDI TOF mass spectrum was recorded on a Bruker MALDI TOF mass spectrometer with a 2,5-dihydrobenzoic acid (DHB) matrix.

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Chemical Synthesis of Glycoside Primers

*n-Dodecyl α-D-lactoside and n-dodecyl β-D-lactoside (**2a** and **2b**).* To a suspension of molecular sieves in 100 mL 1,2-dichloroethane was added 20.0 g (29.5 mmol) of peracetyllactose (**1**) and dodecanol (27.5 g, 147 mmol) and stirred at 50°C for 2 hr under a stream of nitrogen. $BF_3 \cdot Et_2O$ (18.5 ml, 147 mmol) was added and the mixture was stirred at 50°C for 20 hr. The reaction was stopped by the addition of saturated sodium bicarbonate and filtered through a bed of celite. Chloroform was added and the organic layer was washed successively with water and with satd. sodium chloride. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to afford the $\alpha:\beta$ mixture of glycosylation product (7.48 g, 31.4%), which was separated by silica gel column chromatography (ethyl acetate:hexane, 1:2). When a sufficient amount of products necessary for cellular experiments was obtained in pure form (i.e., α-glycosylation product and β-glycosylation product), separation of the rest of the glycosylation product was stopped and thus, the rest of the glycosylation product remained as mixture. Deacylation was carried out by dissolving the α-glycosylation product (1.94 g, 2.41 mmol) in methanol (24.5 mL) and to the solution was added a catalytic amount of sodium methoxide. The mixture was stirred for 12 hr at rt. Deacylation of the β-glycosylation product (330 mg, 0.410 mmol) in methanol (4 mL) was likewise carried out. After completion of the reaction, the respective mixture was neutralized with cation-exchange resin (DIAION SK1B H^+ form), filtered, and evaporated to afford n-dodecyl α-D-lactoside **(2a)** and n-dodecyl β-D-lactoside (**2b**), both as an amorphous mass in 86% and 49% yield, respectively.

n-Dodecyl 4-*O*-(2′,3′,4,′6′-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-α-D-glucopyranoside: $[\alpha]_D$ +2.2° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 5.47 (t, 1H, $J = 9.36$ Hz, H-3), 5.35 (s, 1H, H-4′), 5.12 (t, 1H, H-2′), 4.97 (s, 1H, H-1), 4.96 (s, 1H, H-3′), 4.77 (dd, 1H, H-2), 4.50 (d, 1H, $J = 8.22$ Hz, H-1′), 4.43 (d, 1H, H-6), 4.15–4.09 (m, 3H, H-6′a,b, H-6), 3.73 (t, 1H, $J = 9.90$ Hz, H-4), 3.66, 3.38 (q, 2H, OCH_2), 2.150–1.96 (s, 21H, O$\underline{Ac}$), 1.58 (m, 2H, $OCH_2C\underline{H}_2$), 1.36–1.26 (m, 18H, $C\underline{H}_2$) 0.88 (t, 3H, CH_3). Anal. calcd for $C_{38}H_{60}0_{18}$ (804.9): C, 56.71%; H, 7.51%; found: C, 56.58%; H, 7.85%. n-dodecyl α-D-lactoside **(2a)**: ^{1}H NMR (Me_2SO-d_6): δ 4.16 (d, 1H, $J = 7.2$ Hz,

H-1′), 4.60 (d, 1H, $J = 3.84$ Hz, H-1), 1.51 (m, 2H, $OCH_2C\underline{H}_2$), 1.23 (m, 18H, $C\underline{H}_2$), 0.85 (t, 3H, CH_3). MALDI TOF MS: calcd for $C_{24}H_{46}O_{11}$ $(M+Na)^+$, 533.3; found: $(M+Na)^+$, 532.98.

n-dodecyl 4-*O*-(2′,3′,4,′6′-tetra-*O*-acetyl-*β*-D-galactopyranosyl)-(1 → 4)-2,3,6-tri-*O*-acetyl-*β*-D-glucopyranoside: $[\alpha]_D$ $-0.93°$ (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 5.26 (s, 1H, H-4′), 5.11 (t, 1H, $J = 9.36$ Hz H-3), 5.02 (t, 1H, $J = 8.22$ Hz, H-2′), 4.87 (dd, 1H, $J = 10.44$ Hz, H-3′), 4.79 (t, 1H, $J = 8.22$ Hz, H-2), 4.44 (d, 1H, $J = 8.28$ Hz, H-1′), 4.37 (d, 1H, $J = 8.28$ Hz, H-1), 4.05–4.01 (m, 3H, H-6, 6′a,b), 3.80 (t, 1H, $J = 7.14$ Hz, H-5′), 3.57–3.69 (m, 2H, H-4, OCH_2), 3.52 (m, 1H, H-5), 3.36 (q, 1H, OCH_2), 2.04–1.94 (s, 21H, O$\underline{Ac}$), 1.45 (m, 2H, $OCH_2C\underline{H}_2$), 1.71–1.64 (m, 18H, $C\underline{H}_2$), 0.79 (t, 3 H, CH_3). Anal. calcd for $C_{38}H_{60}0_{18}$ (804.9): C, 56.71%; H, 7.51%; found: C, 56.45%; H, 7.43%. n-dodecyl *β*-D-lactoside **(2b)**: ^{1}H NMR (Me_2SO-d_6): δ 4.18 (d, 1H, $J = 6.6$ Hz, H-1′), 4.15 (d, 1H, $J = 7.68$ Hz, H-1), 2.98 (t, 2H, $OC\underline{H}_2CH_2$), 1.50 (m, 2H, $OCH_2C\underline{H}_2$), 1.23 (m, 18H, $C\underline{H}_2$), 0.85 (t, 3H, CH_3). MALDI TOF MS: calcd for $C_{24}H_{46}O_{11}$ $(M+Na)^+$, 533.3; found: $(M+Na)^+$, 532.98.

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*n-Dodecyl α-D-galactopyranoside and n-dodecyl β-D-galactopyranoside **4a** and **4b**).* 2,3,4,6-Tetra-*O*-benzylgalactosyl chloride (0.80 g, 1.43 mmol) **(3)** and dodecanol (1.33 g, 7.15 mmol) were added to a suspension of molecular sieves and 15.0 mL toluene, and the resulting mixture was stirred at −15°C under a stream of nitrogen. Silver triflate (1.10 g, 4.29 mmol) was added and the mixture was stirred for 12 hr. The reaction was stopped by the addition of saturated sodium bicarbonate and filtered. Chloroform was added to the filtrate and washed with sodium bicarbonate, water, and saturated sodium chloride, dried with anhydrous sodium sulfate, filtered, and evaporated to dryness. The $\alpha:\beta$ mixture of the glycosylation product was separated by silica gel column chromatography (ethyl acetate:hexane, 1:4) to afford 217 mg (21.4%) of dodecyl 2,3,4,6-tetra-*O*-benzyl-*α*-D-galactopyranoside the *α*-glycosylation product and 290 mg (29%) of the *β*-glycosylation product. The *α*-glycosylation product (200 mg, 0.282 mmol) in ethanol (30 mL) and the *β*-glycosylation product (250 mg, 0.353 mmol) in ethanol (30 mL) were hydrogenolyzed respectively over 5% Pd/C for 3 days at rt. After completion of the reaction, the mixture was filtered through a bed of celite and concentrated to afford the syrupy n-dodecyl *α*-D-galactopyranoside **(4a)** and n-dodecyl *β*-D-galactopyranoside **(4b)** primers in 78% and 70% yield, respectively.

n-dodecyl 2,3,4,6-tetra-*O*-benzyl-*α*-D-galactopyranoside: ^{1}H NMR ($CDCl_3$):δ 7.28–7.24 (m, 20H, 4Bn), 4.82 (s, 1H, H-1), 3.59 (q, 1H, OCH_2), 3.40 (q, 1H, OCH_2), 1.59 (m, 2H, $OCH_2C\underline{H}_2$), 1.35–1.20 (m, 18H, $C\underline{H}_2$), 0.85 (t, 3H, CH_3). Anal. calcd for $C_{46}H_{60}0_6$ (708.97): C, 77.93%; H, 8.53%; found: C, 77.98%; H, 8.32%. n-dodecyl *α*-D-galactopyranoside **(4a)**: ^{1}H NMR (Me_2SO-d_6) δ 4.58 (d, 1H, $J = 3.84$ Hz, H-1), 1.45 (m, 2H, $OCH_2C\underline{H}_2$), 1.30–1.10 (m, 18H, $C\underline{H}_2$), 0.80 (t, 3H, $CH_2C\underline{H}_3$). MALDI TOF MS: calcd for $C_{18}H_{36}O_6$ $(M+Na)^+$, 371.25; found: $(M+Na)^+$, 371.19.

n-dodecyl 2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranoside: ^{1}H NMR ($CDCl_3$): δ 7.32–7.26 (m, 20H, 4Bn), 4.33 (d, 1H, $J = 7.74$ Hz, H-1), 3.92 (q, 1H, Hz, OCH_2), 3.88 (s, 1H, H-2), 3.808 (t, 1H, $J = 8.76$ Hz, H-6), 3.5 (d, 1H, $J = 6.0$ Hz, H-3), 1.62–1.58 (m, 2H, $OCH_2C\underline{H}_2$), 1.26–1.24 (m, 18H, $C\underline{H}_2$), 0.88 (t, 3H, CH_3). Anal. calcd for $C_{46}H_{60}0_6$ (708.97): C, 77.93%; H, 8.53%; found: C, 77.88%; H, 8.62%. n-dodecyl β-D-galactopyranoside **(4b)**: ^{1}H NMR (Me_2SO-d_6) δ 4.22 (d, 1H, $J = 7.83$ Hz, H-1), 1.59 (m, 2H, $OCH_2C\underline{H}_2$), 1.21–1.28 (m, 18H, $C\underline{H}_2$), 0.80 (t, 3H, $CH_2C\underline{H}_3$). MALDI TOF MS: calcd for $C_{18}H_{36}O_6$ $(M + Na)^+$, 371.25; found: $(M + Na)^+$, 371.11.

*n-Dodecyl α-D-glucopyranoside and n-dodecyl β-D-glucopyranoside (**6a** and **6b**).* 2,3,4,6-Tetra-*O*-benzylglucosyl imidate (1.50 g, 2.19 mmol) (**5**) and dodecanol (1.63 mL, 8.75 mmol) were added to a suspension of molecular sieves and 40.0 mL diethyl ether and stirred under a stream of nitrogen. TBDMSOTf (0.80 mL, 1.00 mmol) was added and the mixture was stirred at rt for 5 hr. The reaction was stopped by the addition of sodium bicarbonate and ether was added. The organic layer was washed successively with saturated sodium bicarbonate, water, and saturated sodium chloride, dried with anhydrous sodium sulfate, filtered, and evaporated to dryness. The α:β mixture of glycosylation product was separated by silica gel column chromatography (ethyl acetate:hexane, 1:9) to afford 420 mg (27%) of the α-glycosylation product and 450 mg (29%) of the β-glycosylation product. The α-glycosylation product (302 mg, 0.427 mmol) in ethanol (50 mL) and the β-glycosylation product (226 mg, 0.319 mmol) in ethanol (30 mL) were hydrogenolyzed respectively over 5% Pd/C for 6 days at rt. After completion of the reaction, the mixtures were filtered through a bed of celite and concentrated to afford the syrupy n-dodecyl α-D-glucopyranoside **(6a)** and n-dodecyl β-D-glucopyranoside primers **(6b)** in 77% and 68% yield, respectively.

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n-dodecyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside: $[\alpha]_D$ +0.94° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.35–7.26 (m, 20H, 4Bn), 4.78 (s, 1H, H-1), 3.77 (d, 1H, $J = 9.9$ Hz, H-6), 3.56 (d, 1H, $J = 9.9$ Hz, H-6), 3.41 (q, 1H, OCH_2), 1.62 (m, 2H, $OCH_2C\underline{H}_2$), 1.35–1.25 (m, 18H, $C\underline{H}_2$), 0.88 (t, 3 H, CH_3). Anal. calcd for $C_{46}H_{60}0_6$ (708.97): C, 77.93%; H, 8.53%; found: C, 77.83%; H, 8.52%. n-Dodecyl α-D-glucopyranoside primers **(6a)**: ^{1}H NMR (MeOH-d_3) δ 4.59 (d, 1H, $J = 3.84$ Hz, H-1), 3.57 (m, 2H, OCH_2, H-5), 3.48–3.38 (m, 2H, H-2, H-4), 3.34–3.26 (m, 2H, H-3, OCH_2), 3.16 (dd, 1H, $J = 9.36$ Hz, H-6), 3.04 (t, 1H, $J = 9.36$ Hz, H-6), 1.501 (m, 2H, $OCH_2C\underline{H}_2$), 1.30–1.23 (m, 18H, $C\underline{H}_2$), 0.85 (t, 3H, $CH_2C\underline{H}_3$). MALDI TOF MS: calcd for $C_{18}H_{36}O_6$ $(M + Na)^+$, 371.25; found: $(M + Na)^+$, 371.31.

n-Dodecyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside: $[\alpha]_D - 0.32°$ (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$): δ 7.34–7.26 (m, 20H, 4Bn), 4.39 (d, 1H, $J = 7.68$ Hz, H-1), 3.97 (q, 1H, OCH_2), 3.65 (t, 1H, $J = 9.36$ Hz, H-6), 3.58 (t, 1H, $J = 9.36$ Hz, H-6), 3.52 (q, 1H, OCH_2), 1.66 (m, 2H, $OCH_2C\underline{H}_2$), 1.41–1.25 (m, 18H, $C\underline{H}_2$), 0.89 (t, 3 H, CH_3). Anal. calcd for $C_{46}H_{60}0_6$ (708.97): C,

77.93%; H, 8.53%; found: C, 77.91%; H, 8.64%. n-Dodecyl β-D-glucopyranoside primers **(6b)**: ^{1}H NMR (MeOH-d_3) δ 4.08 (d, 1H, $J = 7.74$ Hz, H-1), 3.73 (q, 1H, OCH_2), 3.63 (d, 1H, $J = 10.98$ Hz H-5), 3.44–3.41 (m, 2H, H-4, OCH_2), 3.12 (t, 1H, $J = 8.28$ Hz, H-6), 3.03 (s, 2H, H-2, H-3), 2.91 (t, 1H, $J = 8.22$ Hz, H-6), 1.49 (m, 2H, $OCH_2C\underline{H}_2$), 1.32–1.18 (m, 18H, $C\underline{H}_2$), 0.84 (t, 3H, $CH_2C\underline{H}_3$). MALDI TOF MS: calcd for $C_{18}H_{36}O_6$ $(M + Na)^+$, 371.25; found: $(M + Na)^+$, 371.40.

Cellular Uptake of Glycoside Primers

Cell culture and incubation of cells with lactoside primer were carried out according to the literature.[13]

Identification of Glycosylated Product

The glycosylated products were extracted according to the literature and the MALDI TOF mass spectra were taken. Treatment of the glycosylated products with α2,3-sialidase (cloned from *Salmonella typhimurium* LT2 and expressed in *Escherichia coli*) was carried out to confirm their structure.[16]

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