

Effect of Fluorous Tags on Glycosylation of Saccharide Primers in Animal Cells

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A series of fluorous-tagged saccharide primers with different contents of fluorous atoms was synthesized and introduced into mouse melanoma B16 cells. The primers did not affect cell morphology and viability at a concentration of 50 μM. The numerous fluorine atoms did not pose a steric barrier to primer assimilation into cells and did not affect cellular-enzyme-catalyzed glycosylation. The lactoside primers were sialylated to afford GM3-type oligosaccharide. On the other hand, the GlcNAc primers were galactosylated to afford a lactosamine derivative that was further sialylated by cellular enzymes to afford a sialylated lactosamine.

Glycolipids are ubiquitous in membranes of biological systems and play essential roles in physiological processes. However, their ready availability is hampered by synthetic means that remain to be daunting. Recently, the saccharide primer method[1] has been reported as a promising approach for the production of glycolipids. The strategy is easy and convenient. Significantly, the use of saccharide primers and cells simplifies the rather tedious conventional method that requires several chemical synthetic steps. The cells used already provide the glycosyl donor and enzyme required in this strategy. Moreover, glycosylation is regio- and stereospecific. Among the requirements of the saccharide primer method include (a) amphiphilic saccharide primer that is synthetically accessible in only a few steps; (b) assimilation of the primer without damage to cells;[2] and (c) recognition of saccharides by cellular enzymes in the Golgi,[3] the site of glycosylation. Since glycosylation of the primer is parallel to glycosylation of natural precursors in the biosynthetic pathway, the kind of cell used determines the products. The length of the aglycon unit[4] is crucial to ensure that the primers interact with the lipid bilayer and reach the Golgi where elongation takes place. The hydrophobic (aglycon moiety) and hydrophilic (saccharide moiety) balance also plays an important role not only in cellular uptake but more importantly, in the subsequent release of the glycosylation products to the culture medium.

Saccharide primers with fluorine atoms in the saccharide or aglycon moieties have been reported as building blocks for the synthesis of gangliosides. When administered to mouse melanoma B16 cells, the primers were sialylated by cellular enzymes and the elongated products were released to the culture medium. A series of fluorinated galactosides,[5] dodecyl 2-deoxy-2-fluoro-β-D-galactopyranoside (2F Gal), dodecyl 4-deoxy-4-fluoro-β-D-galactopyranoside (4F Gal), and dodecyl 6-deoxy-6-fluoro-β-D-galactopyranoside (6F Gal), which contain a fluorine atom at different positions of the galactose residue was chemically synthesized and introduced to B16 cells. Although the primers were taken up by cells, only 2F Gal primer was sialylated by B16 cells to give a ganglioside GM4 analog. Saccharide primers with numerous fluorine atoms in the aglycon unit were likewise used. Cellular uptake of a fluorous-tagged lactoside primer, 6-(perfluorohexyl)hexyl 4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranoside[6] resulted to sialylation of the terminal galactose residue to afford a GM3-type oligosaccharide. Significantly, the presence of many fluorine atoms in the aglycon unit did not have any adverse effect to the cells nor inhibit cellular enzyme-catalyzed glycosylation. The fluorous tag was employed to facilitate the separation of the products from the culture medium by simple extraction with a fluorous solvent.[7]

In this research, several saccharide primers (lactose and GlcNAc derivatives) with different lengths of fluorous tags were chemically synthesized and introduced to mouse melanoma B16 cells to establish the effect of numerous fluorine atoms in the aglycon unit on the production of glycolipids via saccharide primer strategy. The lactoside primers and GlcNAc primers were prepared by conventional chemical synthetic methods and introduced to mouse melanoma B16 cells.

Results and Discussion

The lactoside primers [Lac H8F4 (each number indicates the number of methylene and difluoromethylene, respectively), Lac H6F6, Lac H4F8, Lac H3F8, Lac H2F8, and Lac H2F10] and GlcNAc primers [GN H8F4, GN H6F6, GN H4F8, GN H3F8, and GN H2F8] (Chart 1) were synthetically accessible in a few steps as shown in Scheme 1. The saccharide primers were prepared with conventional methods of glycosylation of the partially fluorinated alcohol with either the lactose or GlcNAc derivative.[8] After the glycosylation, deacylation was accomplished under Zemplen conditions.

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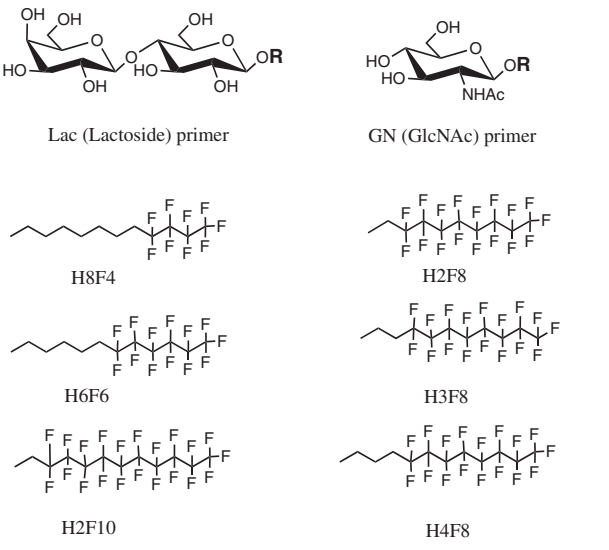
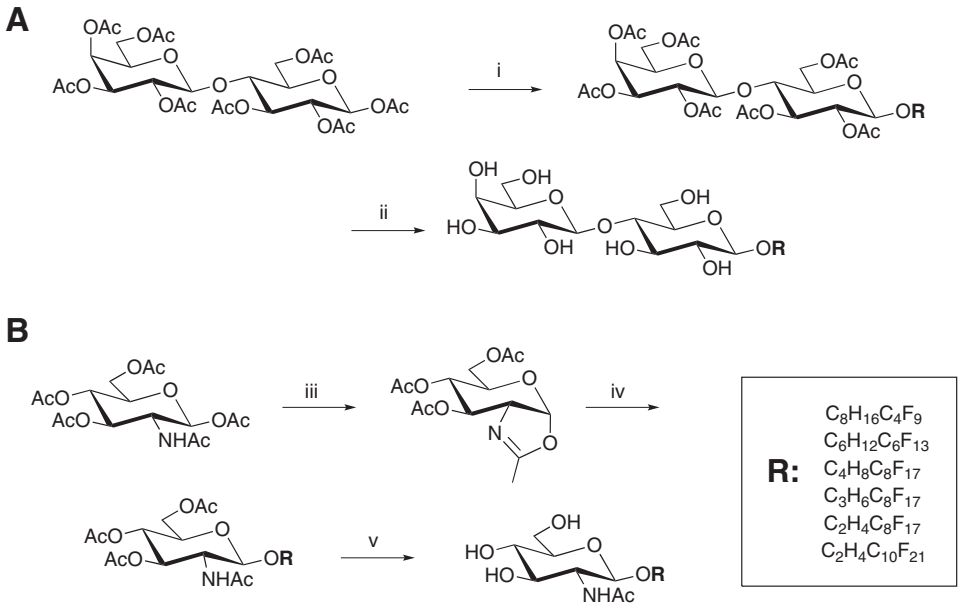
The primers were administered to the culture medium of mouse melanoma B16 cells and the effect on cellular enzyme-mediated glycosylation by the number of fluorine atoms in the aglycon unit was determined. All the primers did not exhibit any adverse effects on B16 cell morphology and viability as shown in Figure 1. After 48-h incubation of cells (seeded cell number: 2×10^6 cells per 10 cm dish; 7 mL DMEM-F12) in the presence of the primers (50 μM), the cell and culture medium fractions were collected and the lipids were extracted. Separation of the lipids and analysis by HPTLC gave the following pattern of results for both fluorous-tagged primers with Lac (Figures 2A and 2B) and GlcNAc (Figures 2C and 2D): (1) HPTLC of the lipids from the cell fraction showed that the primers were taken in by the cells, (2) the bands on the cell fraction corresponding to primers with more fluorine atoms are more intense, (3) except for Lac H4F8 and GN H4F8 having the same kind of fluorous tag, HPTLC of the culture medium fraction showed a new band corresponding to a glycosylated primer, and (4) most of the products of glycosylation are found in the culture medium. The products of glycosylation were separated using fluorous solid phase extraction cartridges and the mass spectra of the products of glycosylation were taken. Results suggested sialylation of the fluorous-tagged lactoside primers to afford the following GM3-type oligosaccharides and the respective yield: sialylated (SA) Lac H8F4 (17.8%), SALac H6F6 (14.1%), SALac H3F8 (1.8%), SALac H2F8 (3.7%), and SALac H2F10 (1.2%). On the other hand, the fluorous-tagged GlcNAc primers were initially galactosylated to give a lactosamine derivative that was further sialylated. From the HPTLC results of the culture medium fraction, the absence of a band corresponding to the lactosamine product suggests that after galactosylation of the GlcNAc primers, sialylation follows almost instantaneously and the product subsequently released to the culture medium. Incorporation of GlcNAc primers to B16 cells afforded the sialylated lactosamines and the respective yield: SAGalGN H8F4 (2.2%), SAGalGN H6F6 (1.7%), SAGalGN H3F8 (0.8%), and SAGalGN H2F8 (12.8%).

Sialylation of the primers was anticipated since B16 cells produce GM3 as a major ganglioside. The sialyl transferases of B16 cells recognized and elongated the primers to give a sialylated dodecyl lactoside derivative (GM3-type oligosaccharide) and a sialylated dodecyl lactosamine derivative. The monosialylated primers were confirmed to be α-(2→3)-linked[6] except for monosialylated GN H2F8 which may possibly be a



H8F4 = 9,9,10,10,11,11,12,12,12-nonafluorododecyl
H6F6 = 7,7,8,8,9,9,10,10,11,11,12,12,12-tridecafluorododecyl
H4F8 = 5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12- heptadecafluorododecyl
H2F10 = 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-henicosafluorododecyl
H2F8 = 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluorodecyl
H3F8 = 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11- heptadecafluoroundecyl

Chart 1.



Scheme 1. Synthesis of fluorous-tagged (**A**) lactoside primers: (i) **R**OH, $BF_3 \cdot OEt_2$, CH_2Cl_2, rt; or HBr–AcOH, CH_2Cl_2, rt then **R**OH, Ag_2CO_3, $AgClO_4$, CH_2Cl_2, MS4A, rt; (ii) NaOMe, MeOH and (**B**) GlcNAc primers: (iii) TMSOTf, 1,2-dichloroethane; (iv) **R**OH, CSA, 1,2-dichloroethane, 90 °C, 2 h; (v) NaOMe, MeOH.

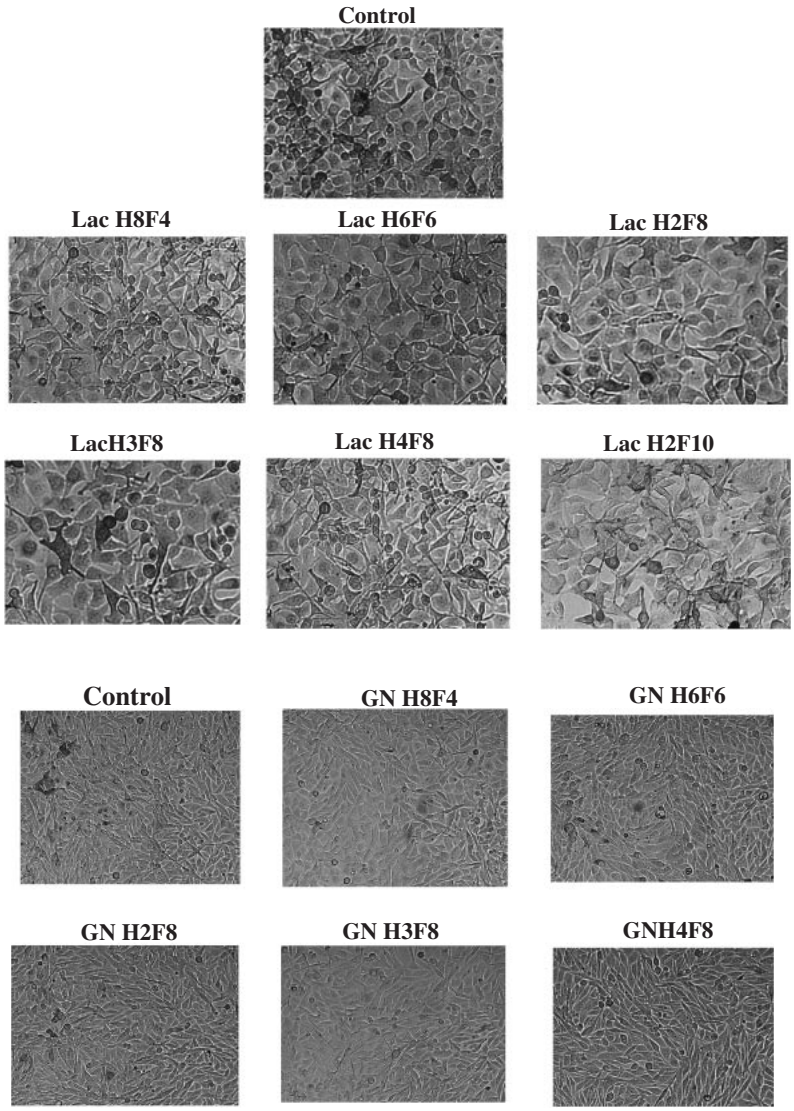


Figure 1. Effect of fluorous-tagged primers on B16 cells after incubation for 48 h. B16 melanoma cells (2×10^6, 100 mm dish) were incubated for 48 h in serum free 1:1 DMEM–F12 (7 mL) supplemented with transferrin and insulin (TI/DF) in the absence or presence of 50 μM primer.

mixture of α-(2→3) and α-(2→6)-linked as evidenced by the presence of two bands as shown in the HPTLC results in Figure 2D.

Interestingly, the GlcNAc primers were galactosylated initially to afford a lactosamine that was further elongated by sialyl transferases. It has been reported that a terminal β-galactoside is a prerequisite for cellular mediated sialylation.[9] Hence, galactosylation of the GlcNAc primers to generate a lactosamine prior to sialylation must have taken place. This is supported by the electrospray ionization mass spectral results (negative ion mode). For example, fragmentation of sialylated GalGN H6F6 (m/z 1075.2) gave a pattern that clearly shows a peak at m/z 784.1 corresponding to the galactosylated GN H6F6 primer (lactosamine derivative) as shown in Figure 3.

Surprisingly, among the primers, both Lac H4F8 and GN H4F8 having the same fluorous tag hardly gave any products. Although HPTLC results showed that these primers are present in the cell fraction, a band corresponding to a glycosylated primer is either in trace amounts only or is totally absent from the HPTLC of the culture medium fraction. One of the requirements to be satisfied for cellular glycosylation is that primers should reach the Golgi, the site of glycosylation. The presence of these primers in the cell fraction based on HPTLC results could be inferred by the following possibilities: (1) both Lac H4F8 and GN H4F8 primers did not reach the Golgi because the cells preferentially remain at the membrane due to strong interaction between the fluorous tag and the membrane components and (2) the Lac H4F8 and GN H4F8 primers reached the glycosylation site but were not recognized by the glycosyl transferases. The presence of a larger number of fluorine atoms could not readily account for the absence of products because Lac H2F10 that has more fluorine atoms than Lac H4F8 was sialylated. Whether this phenomenon is specific only for B16 cells or not, Lac H4F8 primer was also introduced to African green monkey Vero cells. As shown in Figure 4, Lac H4F8 primer likewise did not give any glycosylation product.

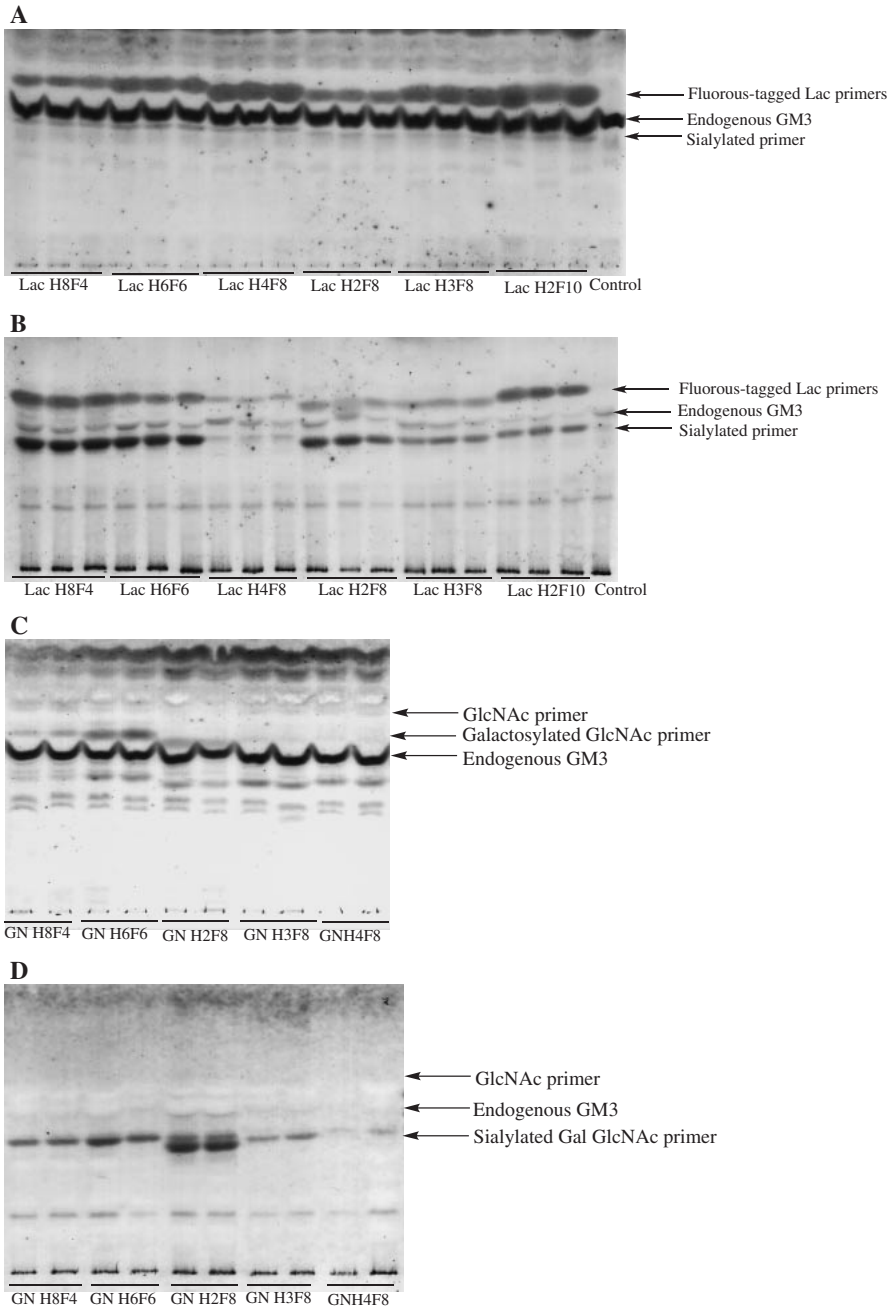


Figure 2. HPTLC profile of lipids (A and C, cell fraction; B and D, culture medium fraction) obtained after incubation of B16 cells with fluorous-tagged lactoside and GlcNAc primers.

Further investigation is currently undertaken to explain these results.

Compounds with numerous fluorine atoms in the aglycon unit did not pose any steric barrier to primer assimilation into cells. The more the fluorine atoms present, the more likely that they remain in the cells. Based on HPTLC results of the cell fraction, the band corresponding to primers Lac H2F10 and Lac H4F8, or Lac H3F8, are more intense than primers Lac H6F6 or Lac H8F4 suggesting that the presence of more fluorine atoms enhances primer retention in the cell. Results also confirmed that the carbon–fluorine bond is stable.[10] The fluorous-tagged primers diffused in cells, were elongated by cellular enzymes and then released to the culture medium without any structural modification except glycosylation. The fluorous tag did not affect in any way the regio- and stereospecific glycosylation of primers by cellular enzymes. The glycosylation products have the same glycan structures as those biosynthesized by the cells used. This is particularly true with the lactoside primers that gave analogs of GM3, the major ganglioside produced by B16 cells.

Among the many advantages of the incorporation of fluorine atoms in the saccharide primer structure include the ease of separation of the glycosylated primer. Separation of the glycosylated Lac H2F8 from the rest of the components of the culture medium was accomplished by gradient elution with methanol using fluorous solid phase extraction cartridges. As

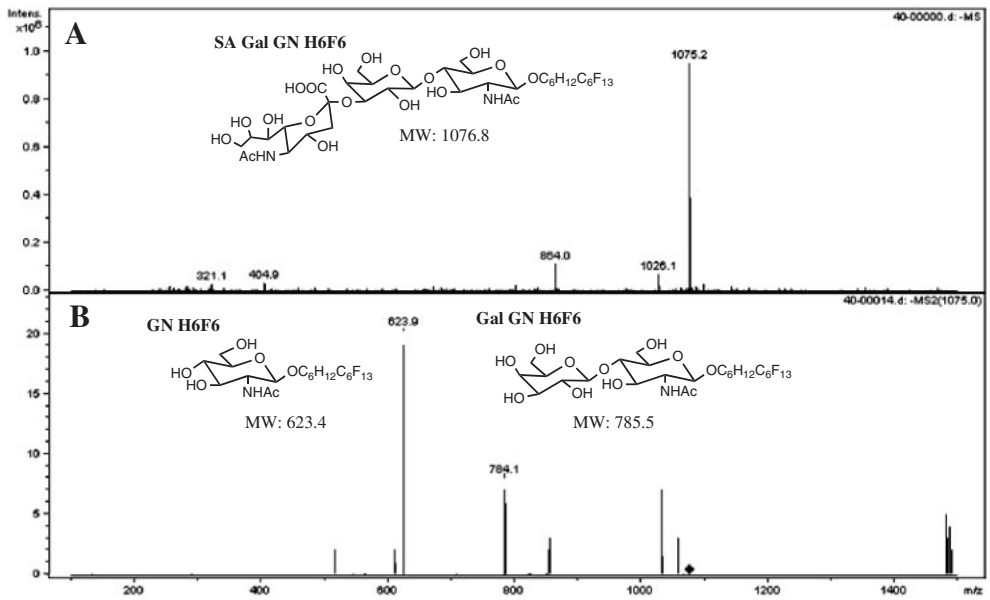


Figure 3. Electrospray ionization mass spectrum (negative ion mode) of (A) SAGalGN H6F6 obtained after incubation of B16 cells with GNH6F6 and (B) fragmentation of SAGalGN H6F6 gave 2 major peaks corresponding to GalGN H6F6 primer and GN H6F6.

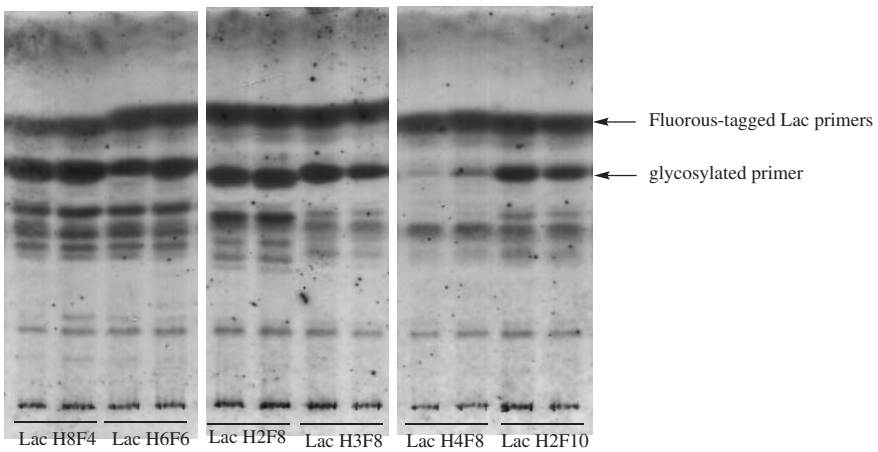


Figure 4. HPTLC profile of lipids from the culture medium fraction obtained after incubation of Vero cells with fluorous-tagged lactoside primers. Vero cells (2×10^6, 100 mm dish) were incubated for 48 h in serum free 1:1 DMEM–F12 (7 mL) supplemented with transferrin and insulin (TI/DF) in the absence or presence of 50 μM primer.

shown in Figure 5, the glycosylated primers could be effectively separated using 40% MeOH in water. Similarly, the rest of the glycosylation products could be separated by gradient elution using fluorous solid phase extraction cartridges.

The presence of fluorine atom enhances primer hydrophobicity. However, the number of fluorine is a significant consideration in the preparation of oligosaccharides by the saccharide primer strategy. Cellular uptake and glycosylation are affected by varying the number of fluorine atoms in the aglycon unit. Incorporation of fluorine atom in the saccharide primers does not only facilitate purification methods but more importantly, may shed light on the glycosylation requirements and processes in biological systems.

Conclusion

The number of fluorine atoms in the aglycon unit of the saccharide primers is an essential consideration in the preparation of oligosaccharides by the saccharide primer strategy. The presence of numerous fluorine atoms did not affect the regio- and stereospecific glycosylation by cells. However, the uptake, release and the amount of glycosylated products vary depending on the number of fluorine atoms present. Generally, the more the fluorine atoms present, the more likely the primers remained in the cells and the smaller the amount of product released into the culture medium.

Experimental

General methods: ^{1}H and ^{13}C NMR spectra were recorded at a 600 MHz JEOL spectrometer in Me_2SO or in MeOH-d_4 or in $CDCl_3$ using tetramethylsilane as internal reference. High-resolution mass spectra of the fluorous-tagged primers were recorded with a JEOL JMS-700AM spectrometer. All chemical synthetic reactions were monitored by thin layer chromatography on Silica Gel 60 F-254 (Merck), with detection by UV light or by

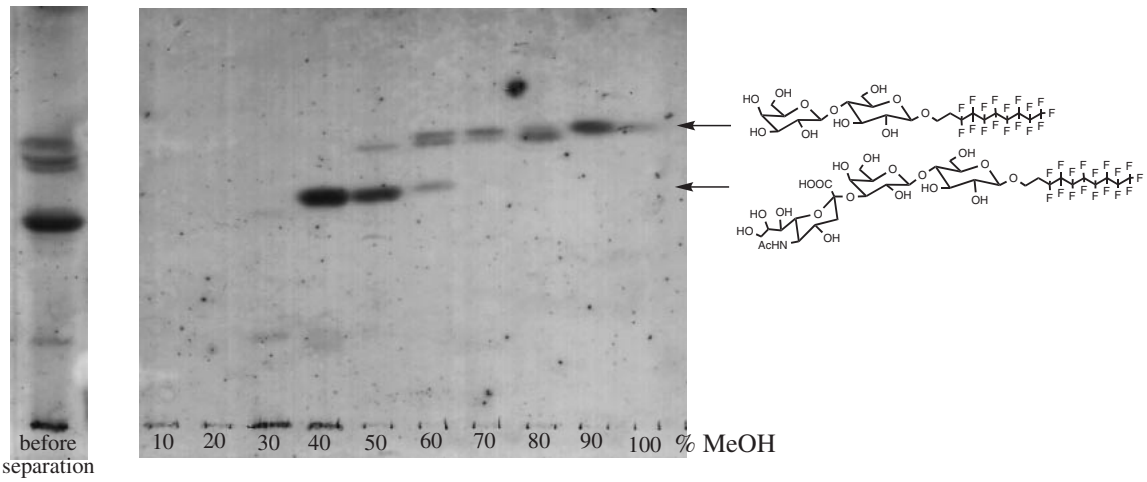


Figure 5. HPTLC of lipids obtained after separation by gradient elution using fluorous solid phase extraction (FSPE) cartridges. 2×10^6 B16 cells were seeded into 10 cm dish containing 7 mL of 10% FBS DMEM/F12. Then, 50 μM Lac H2F8 primer in 7 mL ITS-X DMEM/F12 were administered into cells and incubated for 48 h. The culture medium was collected and the lipids were extracted. Separation was carried out as follows: pre-treatment of the FSPE cartridges (2 g) with THF (4 mL) followed by water (4 mL), then sample loading and washing with water (5 mL), and finally gradient elution with 5 mL MeOH/water.

visualizing by spraying with anisaldehyde–H_2SO_4 and heating. Lipids from the cell homogenate and culture medium fractions were analyzed by HPTLC with $CHCl_3$:MeOH:0.25%KCl(aq) = 5:4:1 (v/v/v) as developing solvent. HPTLC plates were sprayed with resorcinol and heated to detect the separated glycolipids that were quantified by densitometry. Column chromatography was performed on Silica Gel 60 (70–230 mesh, E. Merck, Darmstadt). The mass spectrum of the products of cellular-enzyme-catalyzed glycosylation was recorded on a Bruker Esquire HCT Ultra ESI LC MS using MeOH:acetonitrile (1:1, v/v). Solid phase extraction cartridges were by Fluorous Technologies Inc.

Chemical Synthesis of Saccharide Primers. General Procedure: Glycosylation of the perfluoroalcohols was carried out at room temperature using peracetyl lactose[5,6] (for the preparation of Lac H2F10 and Lac H2F8) or peracetyl lactosyl bromide (for the preparation of Lac H6F6, Lac H3F8, Lac H4F8, and Lac H8F4) in dichloromethane with Lewis acid or silver carbonate and silver perchlorate as promoter, respectively. Usual work-up procedures followed by silica gel column chromatographic separation afforded the desired products of glycosylation: AceLac H8F4 (36%), AceLac H6F6 (24%), AceLac H4F8 (16%), AceLac H2F8 (75%), and AceLac H2F10 (71%). On the other hand, all the GlcNAc primers were prepared via glycosylation using the oxazoline[8] derivative and camphor sulfonic acid (CSA) in dichloroethane at 90 °C to afford AceGN H8F4 (17%), AceGN H6F6 (49%), AceGN H4F8 (60%), AceGN H3F8 (42%), and AceGN H2F8 (44%).

Deacylation was carried out using methanol and sodium methoxide. Stirring at room temperature followed by neutralization with cation-exchange resin afforded the fluorous-tagged saccharide primers and the respective yields: Lac H8F4 (68%), Lac H6F6 (98%), Lac H4F8 (47%), Lac H2F8 (96%), Lac H2F10 (quant) and GN H8F4 (97%), GN H6F6 (88%), GN H4F8 (92%), GN H3F8 (93%), GN H2F8 (85%).

2-(Perfluorodecyl)ethyl 4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (Lac H2F10); ^{1}H NMR (CD_3OD, 600 MHz): δ 2.15–2.25 (m, 2H), 3.28 (t, $J = 8.3$ Hz, 1H), 3.38–3.46 (m, 1H), 3.49 (dd, $J = 9.6$ Hz, 3.4 Hz, 1H), 3.52–3.63 (m, 4H), 3.71 (dd, $J = 11.3$ Hz, 4.5 Hz, 1H), 3.76–3.98 (m, 6H), 4.30 (d, $J = 7.6$ Hz, 1H), 4.36 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 32.46 (t, $J = 21.7$ Hz), 61.80, 62.38, 62.56, 70.16, 72.41, 74.43, 74.71, 76.24, 76.40, 76.95, 80.50, 104.30, 104.96, 108.00–121.87 (m); HRMS (FAB) m/z calcd for $C_{24}H_{26}F_{21}O_{11}$ ($[M + H]^+$): 889.1140, found 889.1184.

2-(Perfluorooctyl)ethyl 4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (Lac H2F8); ^{1}H NMR (CD_3OD, 600 MHz): δ 2.55–2.63 (m, 2H), 3.25 (t, $J = 8.6$ Hz, 1H), 3.40–3.45 (m, 1H), 3.47 (dd, $J = 9.6$ Hz, 3.4 Hz, 1H), 3.50–3.59 (m, 3H), 3.53 (dd, $J = 9.6$ Hz, 2.1 Hz, 1H), 3.68 (dd, $J = 11.3$ Hz, 4.5 Hz, 1H), 3.77 (dd, $J = 11.7$ Hz, 7.6 Hz, 1H), 3.78–3.93 (m, 4H), 4.17 (dt, $J = 10.3$ Hz, 6.9 Hz, 1H), 4.32 (d, $J = 7.6$ Hz, 1H), 4.34 (d, $J = 8.2$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 32.52 (t, $J = 21.7$ Hz), 61.85, 62.46, 62.62, 70.26, 72.51, 74.55, 74.80, 76.34, 76.51, 77.04, 80.53, 104.40, 105.05, 108.89–119.93 (m); HRMS (FAB) m/z calcd for $C_{22}H_{26}F_{17}O_{11}$ ($[M + H]^+$): 789.1204 found 789.1179.

3-(Perfluorooctyl)propyl 4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (Lac H3F8); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.87–1.95 (m, 2H), 2.25–2.40 (m, 2H), 3.28 (dd, $J = 8.9$ Hz, 7.6 Hz, 1H), 3.40–3.43 (m, 1H), 3.48 (dd, $J = 9.6$ Hz, 3.4 Hz, 1H), 3.53–3.59 (m, 4H), 3.66 (dt, $J = 9.6$ Hz, 5.5 Hz, 1H), 3.70 (dd, $J = 11.3$ Hz, 5.0 Hz, 1H), 3.76–3.87 (m, 3H), 3.90 (dd, $J = 12.4$ Hz, 5.0 Hz, 1H), 3.98 (dt, $J = 10.3$ Hz, 5.0 Hz, 1H), 4.30 (d, $J = 8.2$ Hz, 1H), 4.36 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 21.84, 28.85 (t, $J = 21.7$ Hz), 61.92, 62.40, 69.16, 70.14, 72.42, 74.49, 74.71, 76.29, 76.94, 80.70, 104.02, 104.96, 109.26–119.93 (m); HRMS (FAB) m/z calcd for $C_{23}H_{28}F_{17}O_{11}$ ($[M + H]^+$): 803.1360, found 803.1309.

4-(Perfluorooctyl)butyl 4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (Lac H4F8); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.70–1.77 (m, 4H), 2.15–2.23 (m, 2H), 3.24–3.30 (m, 1H), 3.25 (t, $J = 8.6$ Hz, 1H), 3.30–3.42 (m, 4H), 3.53 (t, $J = 8.9$ Hz, 1H), 3.55 (dd, $J = 15.8$ Hz, 8.9 Hz, 1H), 3.58–3.63 (m, 1H), 3.68 (dd, $J = 11.7$ Hz, 5.5 Hz, 1H), 3.85–3.92 (m, 3H), 3.92–3.97 (m, 1H), 4.30 (d, $J = 7.6$ Hz, 1H), 4.41 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 16.93, 28.80, 30.25 (t, $J = 22.4$ Hz), 60.73, 61.19, 68.82, 68.97, 71.24, 73.38, 73.51, 75.12, 75.16, 75.76, 79.54, 102.89, 103.78, 106.26–121.59 (m); HRMS

(FAB) m/z calcd for $C_{24}H_{30}F_{17}O_{11}$ ($[M + H]^+$): 817.1517, found 817.1497.

6-(Perfluorohexyll)hexyl 4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (Lac H6F6); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.40–1.48 (m, 4H), 1.58–1.70 (m, 4H), 2.08–2.19 (m, 2H), 3.27 (t, $J = 8.6$ Hz, 1H), 3.35–3.42 (m, 1H), 3.49 (dd, $J = 9.6$ Hz, 3.4 Hz, 1H), 3.51–3.60 (m, 5H), 3.71 (dd, $J = 11.3$ Hz, 4.5 Hz, 1H), 3.76–3.95 (m, 5H), 4.29 (d, $J = 7.6$ Hz, 1H), 4.36 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 21.09, 26.61, 29.76, 30.35, 31.62 (t, $J = 22.4$ Hz), 61.90, 62.37, 70.13, 70.62, 72.41, 74.56, 74.68, 76.24, 76.31, 76.92, 80.70, 104.06, 104.96, 108.05–120.20 (m); HRMS (FAB) m/z calcd for $C_{24}H_{34}F_{13}O_{11}$ ($[M + H]^+$): 745.1894, found 745.1880.

8-(Perfluorobutyl)octyl 4-*O*-(β-D-Galactopyranosyl)-β-D-glucopyranoside (Lac H8F4); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.30–1.48 (m, 8H), 1.55–1.68 (m, 4H), 2.05–2.15 (m, 2H), 3.28 (t, $J = 8.6$ Hz, 1H), 3.40 (dt, $J = 6.9$ Hz, 2.7 Hz, 1H), 3.49 (dd, $J = 9.6$ Hz, 3.4 Hz, 1H), 3.51–3.61 (m, 5H), 3.71 (dd, $J = 11.7$ Hz, 4.1 Hz, 1H), 3.81 (dd, $J = 11.7$ Hz, 7.6 Hz, 1H), 3.81–3.92 (m, 4H), 4.29 (d, $J = 7.6$ Hz, 1H), 4.36 (d, $J = 7.6$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 20.98, 26.72, 29.84, 30.06, 30.47, 31.50 (t, $J = 22.4$ Hz), 61.85, 62.27, 70.01, 70.78, 72.29, 74.43, 74.57, 76.10, 76.20, 76.79, 80.69, 103.95, 104.84, 107.61–121.87 (m); HRMS (FAB) m/z calcd for $C_{24}H_{38}F_9O_{11}$ ($[M + H]^+$): 673.2270, found 673.2304.

4-(Perfluorooctyl)butyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (GN H4F8); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.62–1.70 (m, 4H), 1.96 (s, 3H), 2.13–2.21 (m, 2H), 3.24–3.29 (m, 1H), 3.30–3.37 (m, 1H), 3.44 (dd, $J = 10.3$ Hz, 8.9 Hz, 1H), 3.48–3.53 (m, 1H), 3.62–3.72 (m, 2H), 3.88 (dd, $J = 11.7$ Hz, 2.1 Hz, 1H), 3.90–3.98 (m, 1H), 4.40 (d, $J = 8.2$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 18.19, 22.86, 29.96, 31.50 (t, $J = 22.4$ Hz), 57.40, 62.86, 69.60, 72.23, 76.09, 78.02, 102.64, 105.53–119.05 (m), 173.65; HRMS (FAB) m/z calcd for $C_{20}H_{23}F_{17}NO_6$ ($[M + H]^+$): 696.1254, found 696.1278.

3-(Perfluorooctyl)propyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (GN H3F8); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.81–1.87 (m, 2H), 1.96 (s, 3H), 2.00–2.32 (m, 2H), 3.22–3.29 (m, 1H), 3.29–3.35 (m, 1H), 3.44 (dd, $J = 10.7$ Hz, 8.6 Hz, 1H), 3.55 (dt, $J = 9.6$ Hz, 6.2 Hz, 1H), 3.64 (dd, $J = 10.3$ Hz, 8.2 Hz, 1H), 3.68 (dd, $J = 11.7$ Hz, 5.5 Hz, 1H), 3.88 (dd, $J = 11.7$ Hz, 2.1 Hz, 1H), 3.98 (dt, $J = 10.3$ Hz, 5.5 Hz, 1H), 4.39 (d, $J = 8.3$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 21.86, 22.83, 28.78 (t, $J = 21.7$ Hz), 57.33, 62.83, 68.75, 72.16, 75.94, 78.03, 102.70, 106.66–122.55 (m), 173.60; HRMS (FAB) m/z calcd for $C_{19}H_{21}F_{17}NO_6$ ($[M + H]^+$): 682.1097, found 682.1122.

2-(Perfluorooctyl)ethyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (GN H2F8); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.93 (s, 3H), 2.35–2.53 (m, 2H), 3.26–3.30 (m, 2H), 3.42 (dd, $J = 10.7$ Hz, 8.6 Hz, 1H), 3.60–3.64 (m, 1H), 3.66 (dd, $J = 12.1$ Hz, 6.2 Hz, 1H), 3.79 (dt, $J = 10.3$ Hz, 5.9 Hz, 1H), 3.86 (dd, $J = 11.7$ Hz, 2.1 Hz, 1H), 4.13 (dt, $J = 10.3$ Hz, 6.2 Hz, 1H), 4.42 (d, $J = 8.3$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 22.83, 32.33 (t, $J = 20.1$ Hz), 57.21, 62.30, 62.79, 72.13, 75.91, 78.11, 102.63, 108.02–120.05 (m), 173.75; HRMS (FAB) m/z calcd for $C_{18}H_{19}F_{17}NO_6$ ($[M + H]^+$): 668.0941, found 668.0969.

6-(Perfluorohexyl)hexyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (GN H6F6); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.25–1.35 (m, 4H), 1.45–1.56 (m, 4H), 1.87 (s, 3H), 2.00–2.13 (m, 2H), 3.13–3.17 (m, 1H), 3.18–3.23 (m, 1H), 3.31–3.40 (m, 2H), 3.53 (dd, $J = 10.3$ Hz, 8.3 Hz, 1H), 3.58 (dd, $J = 12.0$ Hz, 5.8 Hz, 1H), 3.78 (dd, $J = 11.7$ Hz, 2.1 Hz, 1H), 3.80 (dt, $J = 9.6$ Hz, 6.2 Hz, 1H), 4.28 (d, $J = 8.2$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 18.19, 22.86, 29.96, 31.49 (t, $J = 21.7$ Hz), 57.40, 62.86, 69.60, 72.22, 76.09, 78.02, 102.64, 110.02–123.55 (m), 173.65; HRMS (FAB) m/z calcd for $C_{20}H_{27}F_{13}NO_6$ ($[M + H]^+$): 624.1631, found 624.1600.

8-(Perfluorobutyl)octyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (GN H8F4); ^{1}H NMR (CD_3OD, 600 MHz): δ 1.30–1.42 (m, 8H), 1.48–1.61 (m, 4H), 1.95 (s, 3H), 2.08–2.20 (m, 2H), 3.21–3.24 (m, 1H), 3.26–3.31 (m, 1H), 3.39–3.46 (m, 2H), 3.60 (dd, $J = 10.3$ Hz, 8.3 Hz, 1H), 3.65 (dd, $J = 12.1$ Hz, 5.9 Hz, 1H), 3.83–3.88 (m, 2H), 4.36 (d, $J = 8.3$ Hz, 1H); ^{13}C NMR (CD_3OD, 125 MHz): δ 21.25, 22.98, 27.04, 30.06, 30.24, 30.38, 30.63, 31.63 (t, $J = 22.4$ Hz), 57.47, 62.85, 70.55, 72.21, 76.10, 77.97, 102.74, 107.02–120.05 (m), 173.62; HRMS (FAB) m/z calcd for $C_{20}H_{30}F_9NO_6Na$ ($[M + Na]^+$): 574.1827, found 574.1847.

Cellular Uptake of Glycoside Primers. Cell culture, incubation of cells with primer and identification of glycosylated product were carried out according to the literature cited in the text.[1,6]

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