Effect of Aglycon Structure on Saccharide Elongation by Cells

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Alkyl *N*-acetyl-*β*-D-glucosaminide (GlcNAc primers) with different aglycon moieties were synthesized and used to determine the effect of the aglycon structure on cellular saccharide elongation. Dodecyl *N*-acetyl-*β*-D-glucosaminide (GlcNAc-C12), tridecan-7-yl *N*-acetyl-*β*-D-glucosaminide (GlcNAc-2C6), and pentacosan-13-yl *N*-acetyl-*β*-D-glucosaminide (GlcNAc-2C12) primers were synthesized by glycosylation of dodecan-1-ol, tridecan-7-ol, and pentacosan-13-ol, respectively, with peracetylglucosamine. These primers were introduced to mouse B16 melanoma cells to prepare glycolipids. After 48 h incubation, results showed that GlcNAc-C12 was elongated to give NeuAc-Gal-GlcNAc-C12. GlcNAc-2C6 was also elongated to afford Gal-GlcNAc-2C6 and NeuAc-Gal-GlcNAc-2C6. On the other hand, GlcNAc-2C12 primer was not elongated. Significantly, the results demonstrated that the amount of glycosylated product increased 1.5-times by modifying the aglycon structure of GlcNAc from C_{12} to 2 C_6 despite having almost the same number of C-units.

Introduction. – Carbohydrates are known as biomakers. For example, carbohydrates play significant roles in generation of cells, canceration, immunity, cell adhesion, and fecundation [1–5]. We can obtain important information on some vital biological phenomena by investigating the carbohydrates involved. However, the preparation of carbohydrates is not simple. The number of OH groups that are found in the structure makes the carbohydrate synthesis difficult. During carbohydrate synthesis, repeated introduction and removal of the protecting groups is required. So, we developed an approach that combines chemical synthesis and biosynthesis for the facile preparation of carbohydrates.

The strategy combining chemical synthesis and biosynthesis to prepare a saccharide by using a saccharide primer is called biocombinatorial synthesis [6]. In this method, various kinds of carbohydrates can be synthesized from one kind of alkyl glycoside (primer) and a combination of different cell types. For example, GM3 analog can be obtained from B16 cells using a lactoside primer. On the other hand, GM3, GM2, and GM1 can be obtained from the same primer by using MDCK (*Madin–Darby* canine kidney) cells.

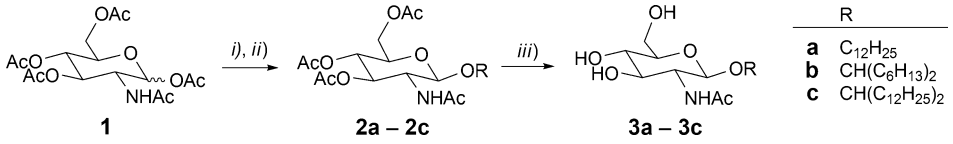
The mechanism of primer elongation involves endocytosis, followed by transport of the primer to the endoplasmic reticulum (ER) and/or the *Golgi* where the primer is elongated by glycosyltransferase. Finally, the product is released from the cells to the culture medium. Previously, it was reported that a dodecyl aglycon unit was best suited to obtain elongated products from B16 cells [7]. However, only minute amounts of the

desired carbohydrate could be obtained. In this work, the structure of aglycon was modified to determine the effect of the aglycon structure on cellular elongation.

Results and Discussion. – *Synthesis of the Primers.* For the synthesis of primers, the aglycon moieties were prepared by *Grignard* reaction using HCOOEt and various alkyl bromides [8]. Glycosylation of the long-chain alkanols (aglycon moieties) with 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (**1**) gave the desired products, **2a**–**2c**, as outlined in *Scheme* [9]. Finally, the protecting group was removed under *Zemplen* condition to afford GlcNAc-C12 (**3a**), GlcNAc-2C6 (**3b**), and GlcNAc-2C12 (**3c**). The structures of the synthesized compounds were confirmed by the ^{1}H- and ^{13}C-NMR data.

Effect of Aglycon Structure. Each primer was administered to mouse melanoma B16 cells [10]. The cells were incubated for 48 h in the absence (control) and presence of each primer (*Fig. 1*). The primers did not affect cell morphology. As shown in *Fig. 1*, there is no significant difference between the cells cultured in the absence and presence of primers. Moreover, the primers were not cytotoxic at 50 μM concentration. After 48 h, the medium and cell fractions were collected, and the glycolipids were extracted using *SepPak C18* column and by sonication, respectively, and were monitored on a

Scheme. *Chemical Synthesis of GlcNAc-C12* (**3a**), *GlcNAc-2C6* (**3b**), *and GlcNAc-2C12* (**3c**) *Primers*



1 **2a – 2c** **3a – 3c**

i) Trimethylsilyl trifluoromethanesulfonate (TMSOTf), $ClCH_2CH_2Cl$. *ii*) Long-chain alkanol, camphorsulfonic acid (CSA), $ClCH_2CH_2Cl$. *iii*) MeONa, MeOH.

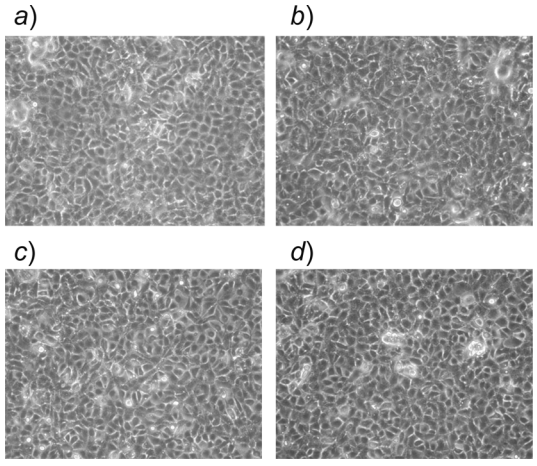


Fig. 1. *Effect of aglycon structure on B16 cells after incubation for 48 h in the absence* (*a*), *or in the presence of GlcNAc-C12* (*b*), *GlcNAc-2C6* (*c*), *and GlcNAc-2C12* (*d*)

HPTLC plate. The HPTLC results showed the absence of band corresponding to GlcNAc-2C12, suggesting that most of the primer was taken up and remained in the cells (*Fig. 2*,*a*). Moreover, HPTLC results confirmed that GlcNAc-2C12 was not elongated.

On the other hand, HPTLC results in *Fig. 2*,*b*, indicated that GlcNAc-2C6 primer was elongated and afforded two kinds of products that were analyzed by ESI-MS. Quantification using a densitometer revealed that the amount of the product obtained from GlcNAc-2C6 primer was larger than that obtained from GlcNAc-C12 primer. Results evidenced that GlcNAc-2C6 primer was elongated by the addition of a galactose unit, or by galactose and sialic acid units. Treatment with enzyme confirmed

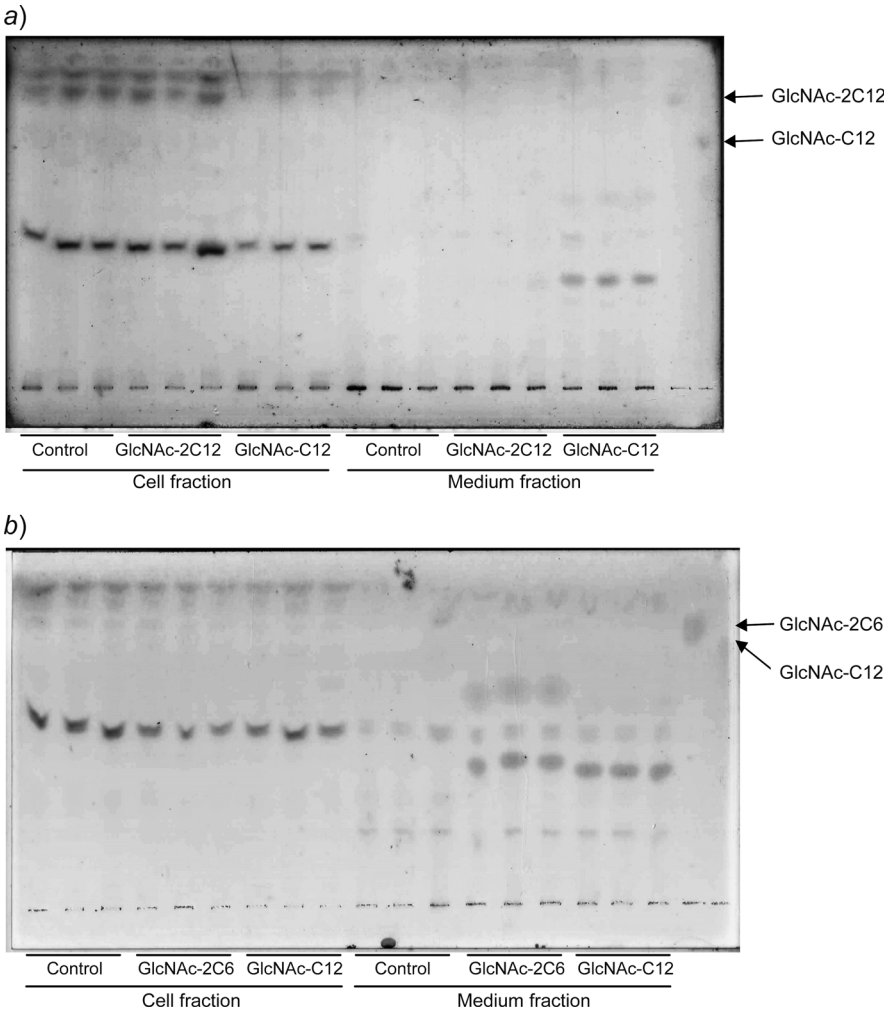


Fig. 2. *HPTLC Profile of lipids obtained after incubation of B16 cells with* a) *GlcNAc-2C12 or GlcNAc-C12 and* b) *GlcNAc-2C6 or GlcNAc-C12*

that the sialic acid residue of GlcNAc-2C6 is α2,3-linked to the galactose unit as shown in *Fig. 3*. This result agreed with previous reports that B16 cells add an α2,3-linked sialic acid unit to the galactose residue of primers [11]. Comparing GlcNAc-C12 with GlcNAc-2C6, only GlcNAc-2C6 primer afforded galactosylated GlcNAc-2C6, suggesting that the galactosyl GlcNAc-2C6 is stable.

Administration of a mixture of GlcNAc-2C6 (50 μM) and GlcNAc-C12 primers (50 μM) was also studied to determine which primer could easily be glycosylated. As shown in *Fig. 4*, only the GlcNAc-2C6 primer was elongated. The amount of products obtained from the mixture of GlcNAc-2C6 and GlcNAc-C12 was less than that when the primers were administered separately. This may be due to the cytotoxic effect of the mixture of GlcNAc-2C6 and GlcNAc-C12 (*cf. Fig. 5*).

The cells treated with the mixture of GlcNAc-2C6 and GlcNAc-C12 primers were dead and detached from the substrate after 48 h.

On the other hand, separate administration up to a concentration of 100 μM did not exhibit cytotoxicity. No significant difference could be observed before and after treatment of up to 100 μM as shown in *Fig. 6*, *a* and *b*. However, a morphological change was observed after 48 h treatment with 150 μM GlcNAc-C12 or GlcNAc-2C6. Moreover, the mixture of primers gave mostly Gal-GlcNAc-2C6 and NeuAc-Gal-GlcNAc-2C6. Based on these results, GlcNAc-2C6 primer is elongated more than GlcNAc-C12 primer, when a mixture is administered.

Cellular saccharide elongation occurs *via* endocytosis, transport to the ER and/or the *Golgi*, enzyme reaction, and release of elongated primers from the cells. GlcNAc-

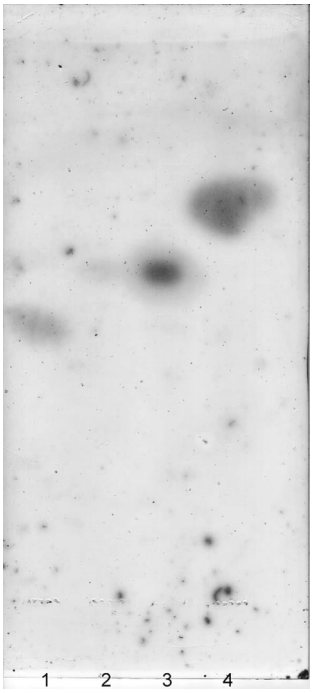


Fig. 3. *HPTLC Profile before and after treatment with α2,3-sialidase. Lane 1:* before treatment of NeuAc-Gal-GlcNAc-2C6. *Lane 2:* after treatment of NeuAc-Gal-GlcNAc-2C6. *Lane 3:* Gal-GlcNAc-2C6; *Lane 4:* GlcNAc-2C6.

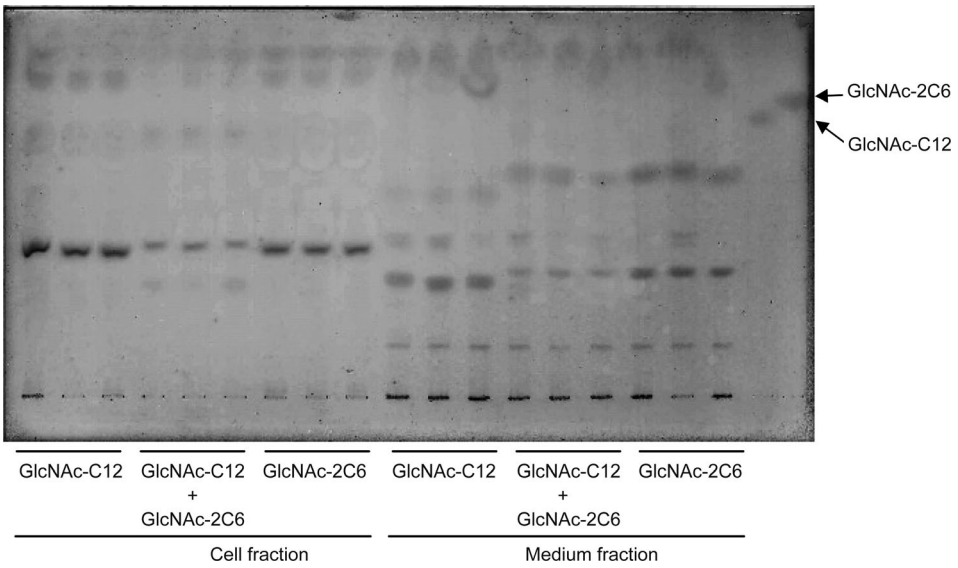


Fig. 4. *HPTLC Profile of lipids obtained after incubation of B16 cells with GlcNAc-2C6, GlcNAc-C12, and a mixture of GlcNAc-2C6 and GlcNAc-C12*

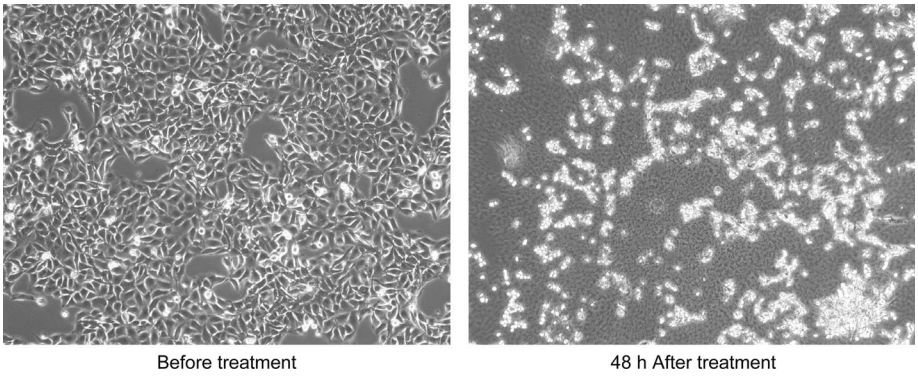


Fig. 5. *Effect of the mixture of GlcNAc-C12* (50 μM) *and GlcNAc-2C6* (50 μM) *on B16 cells*

2C6 gave 1.5-times more product than GlcNAc-C12, despite the fact that both GlcNAc-C12 and GlcNAc-2C6 were taken up by B16 cells. Although the aglycon moieties have almost the same number of C-units, the structure of the aglycon may have a different effect on transport to ER and/or the *Golgi*, enzyme reaction, and release of elongated primers from the cells.

Conclusions. – In this study, biocombinatorial synthesis using saccharide primers and animal cells was carried out to produce the glycolipid GM3. GlcNAc-C12, GlcNAc-2C6 and GlcNAc-2C12 primers were chemically synthesized, and were

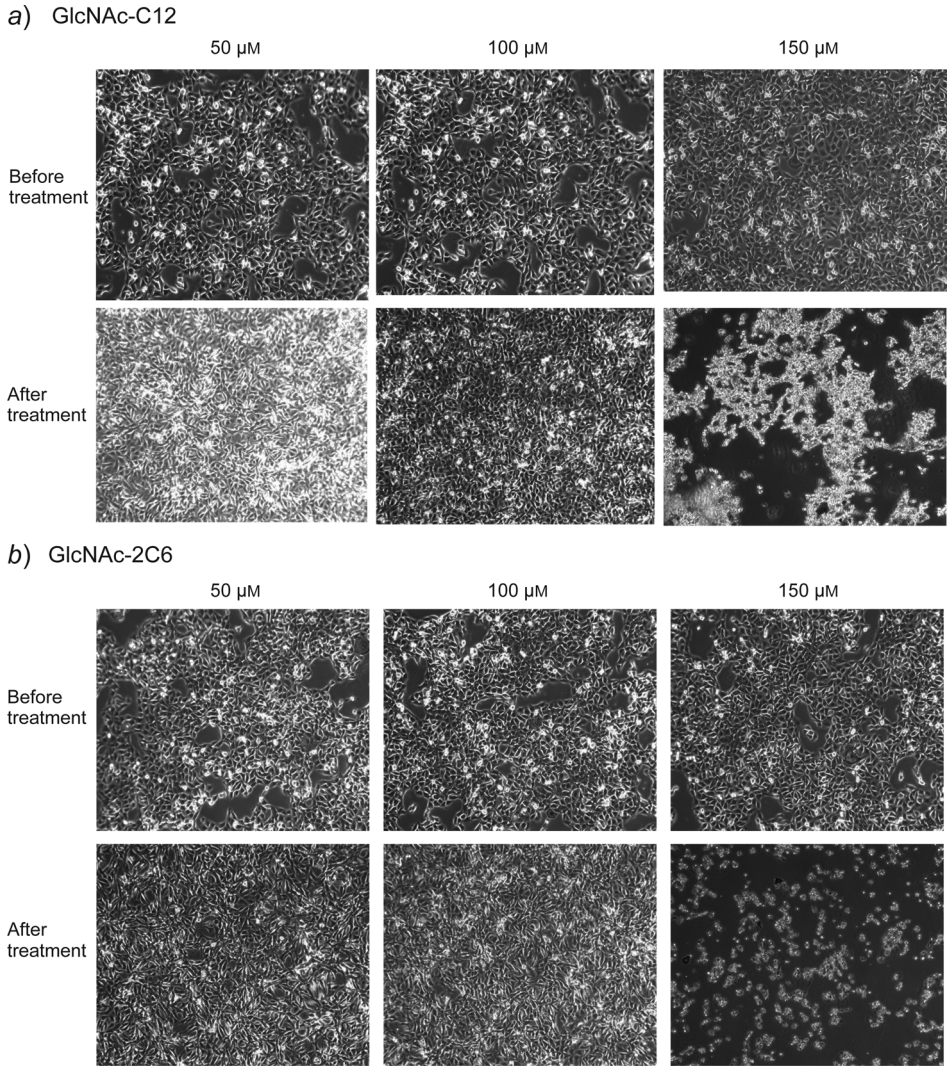


Fig. 6. *The treatment of different concentrations of* a) *GlcNAc-C12 and* b) *GlcNAc-2C6*

introduced to mouse melanoma B16 cells to serve as substrate for glycosylation by cellular enzymes. The primer with dialkyl chain afforded 1.5-times more product than the primer with a single alkyl chain.

By improving the method for obtaining GM3, the relation between GM3, which is expressed in nerve cells, and the brain disease such as *Alzheimer* may be clarified. Moreover, since GM3 is involved in the infection of the influenza virus, development of strategy for diagnosis can be achieved when sufficient amount of GM3 is readily available.

Experimental Part

General. All reactions were monitored by TLC. TLC: Silica gel *60 F-254* (*E. Merck*, DE-Darmstadt); visualization by spraying with anisaldehyde/H_2SO_4 and heating. Column chromatography (CC): silica gel *60* (SiO_2, 230–400 mesh; *E. Merck*, DE-Darmstadt). ^{1}H-NMR Spectra: *JEOL* spectrometer at 600 MHz (*JEOL*, Tokyo, Japan).

Chemical Synthesis of Alcohols (aglycon moiety) [12]. Mg (120 mmol) in a flask was dried overnight under N_2. THF (50 ml), alkyl bromide (90.2 mmol), HCOOEt (41 mmol) were added, and the mixture was stirred under reflux for 10 min. After cooling to r.t., to the resulting slurry were added H_2O and 1M H_2SO_4. The mixture was filtered using *Celite*. The desired product was recrystallized from MeCN, tridecan-7-ol (72.1%), pentacosan-13-ol (88.6%).

Tridecan-7-ol. ^{1}H-NMR (600 MHz, (D_6)DMSO): 0.85 (*t*, *J* = 6.6, 6 H); 1.20–1.30 (br., 20 H); 3.32–3.37 (br., 1 H); 4.10 (*d*, *J* = 5.5, 1 H). ^{13}C-NMR (150 MHz, CD_3OD): 14.07; 22.61; 25.61; 29.38; 31.84; 37.49; 72.02.

Pentacosan-13-ol. ^{1}H-NMR (600 MHz, (D_6)DMSO): 0.86 (*t*, *J* = 7.1, 6 H); 1.22–1.30 (br., 44 H); 3.33–3.38 (br., 1 H).

Chemical Synthesis of Saccharide Primers [13]. 2-Acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (**1**; 1.5 mmol) was mixed with trimethylsilyl trifluoromethylsulfonate (TMSOTf; 1.9 mmol) in $ClCH_2CH_2Cl$ under N_2 at 0° for 30 min. The soln. was refluxed with stirring overnight. The mixture was neutralized with Et_3N, the product was washed with sat. $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. To the mixture was added the long-chain alkyl alcohol (1.5 mmol) and (+)-10-camphorsulfonic acid (CSA; 2.2 mmol) in $ClCH_2CH_2Cl$ (10 ml), and the resulting mixture was stirred at 90° for 4 h. After completion of the reaction, $CHCl_3$ was added, and the mixture was washed with sat. $NaHCO_3$, H_2O, and brine, dried (Na_2SO_4), and concentrated *in vacuo*. The peracetylated glycosides **2a**–**2c** were purified by CC (toluene/AcOEt 1:1).

Deacylation was carried out in the presence of MeONa (0.22 mmol) in MeOH (5 ml) for 3 h. After treatment with cation-exchange resin, concentration *in vacuo* afforded GlcNAc-C12 (**3a**; 16.5%), GlcNAc-2C6 (**3b**; 17.8%), and GlcNAc-2C12 (**3c**; 10.8%).

*Dodecyl 3,4,6-Tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-*D*-glucopyranoside* (**2a**). ^{1}H-NMR ($CDCl_3$, 600 MHz): 0.83 (*t*, *J* = 6.6, 3 H); 1.20–1.25 (br., 18 H); 1.47–1.55 (*m*, 2 H); 1.90 (*s*, 3 H); 1.98 (*s*, 3 H); 1.99 (*s*, 3 H); 2.04 (*s*, 3 H); 3.42 (*q*, *J* = 7.1, 7.1, 1 H); 3.66–3.67 (*m*, 1 H); 3.75–3.83 (*m*, 2 H); 4.08 (*d*, *J* = 12.1, 1 H); 4.22 (*dd*, *J* = 4.4, 12.1 H); 4.65 (*d*, *J* = 8.2, 1 H); 5.02 (*t*, *J* = 9.6, 1 H); 5.28 (*t*, *J* = 9.9, 1 H); 5.66 (*d*, *J* = 8.8, 1 H). ^{13}C-NMR (150 MHz, $CDCl_3$): 14.06; 20.60; 20.67; 20.71; 22.62; 23.26; 25.81; 29.28; 29.31; 29.37; 29.57; 29.60; 31.84; 54.79; 62.14; 68.68; 69.92; 71.64; 72.31; 100.62; 169.39; 170.13; 170.72; 170.83.

*Dodecyl 2-(Acetylamino)-2-deoxy-β-*D*-glucopyranoside* (= GlcNAc-C12; **3a**). ^{1}H-NMR (600 MHz, CD_3OD): 0.90 (*t*, *J* = 7.2, 3 H); 1.29–1.35 (br., 18 H); 1.52–1.55 (*m*, 2 H); 1.97 (*s*, 3 H); 3.25–3.32 (*m*, 3 H); 3.42–3.46 (*m*, 2 H); 3.61–3.69 (*m*, 2 H); 3.86–3.89 (*m*, 2 H); 4.38 (*d*, *J* = 8.8, 1 H). ^{13}C-NMR (150 MHz, CD_3OD): 14.45; 22.99; 23.74; 27.16; 30.50; 30.56; 30.68; 30.77; 30.82; 30.85; 33.08; 57.42; 62.80; 70.58; 72.13; 76.07; 77.95; 102.72; 173.61.

*Tridecan-7-yl 3,4,6-Tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-*D*-glucopyranoside* (**2b**). ^{1}H-NMR ($CDCl_3$, 600 MHz): 0.84 (*t*, *J* = 7.1, 6 H); 1.21–1.40 (br., 20 H); 1.89 (*s*, 3 H); 1.98 (*s*, 3 H); 1.99 (*s*, 1 H); 2.03 (*s*, 3 H); 3.50 (*t*, *J* = 5.8, 1 H); 3.64–3.72 (*m*, 2 H); 4.08 (*dd*, *J* = 1.4, 11.3, 1 H); 4.17 (*dd*, *J* = 4.9, 12.1, 1 H); 4.71 (*d*, *J* = 8.3, 1 H); 4.99 (*t*, *J* = 9.6, 1 H); 5.32 (*t*, *J* = 9.9, 1 H); 5.57 (*d*, *J* = 8.8, 1 H). ^{13}C-NMR (150 MHz, $CDCl_3$): 14.02; 14.04; 20.61; 20.67; 22.56; 22.60; 23.26; 25.01; 25.06; 29.28; 29.51; 31.78; 31.86; 34.00; 34.68; 55.47; 62.46; 69.00; 71.41; 72.35; 81.11; 100.20; 169.44; 169.95; 170.63; 170.80.

*Tridecan-7-yl 2-(Acetylamino)-2-deoxy-β-*D*-glucopyranoside* (= GlcNAc-2C6; **3b**). ^{1}H-NMR (600 MHz, CD_3OD): 0.90 (*t*, *J* = 7.2, 6 H); 1.23–1.40 (br., 16 H); 1.41–1.63 (br., 4 H); 3.20–3.26 (*m*, 1 H); 3.28–3.36 (*m*, 2 H); 3.48 (*t*, *J* = 9.6, 1 H); 3.53–3.63 (*m*, 2 H); 3.69 (*dd*, *J* = 5.5, 11.6, 1 H); 3.85 (*d*, *J* = 11.5, 1 H); 4.46 (*d*, *J* = 8.2, 1 H). ^{13}C-NMR (150 MHz, CD_3OD): 14.47; 23.13; 23.75; 23.78; 25.94; 26.21; 30.61; 30.84; 33.04; 33.14; 34.84; 35.80; 57.91; 62.89; 72.14; 75.89; 77.66; 81.48; 102.20; 173.41.

*Pentacosan-13-yl 3,4,6-Tri-*O*-acetyl-2-(acetylamino)-2-deoxy-β-*D*-glucopyranoside* (**2c**). ^{1}H-NMR ($CDCl_3$, 600 MHz): 0.84 (*t*, *J* = 7.1, 6 H); 1.14–1.32 (br., 44 H); 1.89 (*s*, 3 H); 1.98 (*s*, 3 H); 1.99 (*s*, 1 H); 2.03 (*s*, 3 H); 3.49 (*t*, *J* = 2.7, 1 H); 3.63–3.73 (*m*, 2 H); 4.08 (*dd*, *J* = 2.8, 12.1, 1 H); 4.17 (*dd*, *J* = 4.9,

12.1, 1 H); 4.71 (*d*, *J* = 8.3, 1 H); 4.99 (*t*, *J* = 9.6, 1 H); 5.32 (*t*, *J* = 9.9, 1 H); 5.58 (*d*, *J* = 8.8, 1 H). ^{13}C-NMR (150 MHz, $CDCl_3$): 14.08; 20.62; 20.68; 22.64; 23.28; 25.09; 29.31; 29.63; 29.64; 29.68; 29.70; 29.88; 31.86; 33.95; 34.68; 55.44; 62.43; 68.96; 71.38; 72.31; 81.17; 100.20; 169.45; 169.96; 170.64; 170.81.

*Pentacosan-13-yl 2-(Acetylamino)-2-deoxy-β-*D*-glucopyranoside* (= GlcNAc-2C6; **3c**). ^{1}H-NMR (600 MHz, CD_3OD): 0.90 (*t*, *J* = 6.9, 6 H); 1.22–1.40 (br., 40 H); 1.42–1.63 (br., 4 H); 3.22–3.25 (*m*, 1 H); 3.30–3.35 (*m*, 2 H); 3.48 (*t*, *J* = 9.6, 1 H); 3.55–3.63 (*m*, 2 H); 3.69 (*dd*, *J* = 5.5, 12.0, 1 H); 3.85 (*dd*, *J* = 2.2, 12.1, 1 H); 4.46 (*d*, *J* = 8.3, 1 H). ^{13}C-NMR (150 MHz, CD_3OD): 14.49; 23.17; 23.77; 25.90; 26.22; 30.52; 30.75; 30.82; 30.90; 31.14; 33.11; 34.75; 35.73; 57.91; 62.89; 72.14; 75.89; 77.67; 81.44; 102.20; 173.40.

General Method for Incubation of B16 Melanoma Cells with GlcNAc Primers. B16 Cells were obtained from *Riken* Cell Bank (Tsukuba, Japan). *Dulbecco*'s Modified *Eagles*'s Medium/*Ham*'s F-12 (D-MEM/F-12) and Insulin-Transferrin-Selenium-X (ITS-X) were from *Life Technologies* (Carlsbad, CA, USA). Fetal bovine serum (FBS) was from *JRH Biosciences* (Lenexa, KS, USA). Antibiotic-antimycotic soln. (100 ×) was from *Nacalai Tesque* (Kyoto, Japan). *SepPak C18* Cartridges were from *Waters* (Boston, MA, USA). High-performance thin layer chromatography (HPTLC; SiO_2 *60 F-254*) was from *E. Merck* (DE-Darmstadt). The GlcNAc primers were dissolved in sterilized DMSO to an initial concentration of 50 mM.

Cell Culture. Mouse B16 melanoma cells were cultured using D-MEM/F-12 supplemented with 10% FBS and 1% antibiotic-antimycotic solution in humidified atmosphere of 5% CO_2 air at 37°. Cells were detached by application of 0.25% trypsin/ethylenediaminetetraacetate (EDTA) and passaged every 3 d.

Incubation of Cells with GlcNAc Primers. Cells (2.0×10^6) were seeded into 100-mm culture dishes containing 7 ml of medium and incubated for 48 h. Then, the cells were washed with TI-DF (D-MEM/F-12 containing 1% ITS-X without phenol red) to remove the serum, and were incubated with 50 μM of each primer for 48 h at 37°. After incubation, culture media were collected, and the cells were washed with phosphate-buffered saline (PBS), harvested with 0.25% EDTA in PBS, and centrifuged at 1000 rpm for 5 min.

The lipids were extracted from the cell pellet with $CHCl_3$/MeOH 2 : 1, then with $CHCl_3$/^{i}PrOH/H_2O 7 : 11 : 2 in a sonicated bath. Lipids from the culture media were purified using a *SepPak C18* column. Lipids from the cell homogenate and culture medium fractions were analyzed by HPTLC with $CHCl_3$/MeOH/0.25% aq. KCl soln. 5 : 4 : 1 as developing solvent. HPTLC Plates were sprayed with resorcinol, and then with orcinol–H_2SO_4 reagent, and heated to detect the separated glycolipids, which were later quantified using *CS-9300PC* dual-wavelength flying spot scanning densitometer (*Shimadzu*, Kyoto, Japan).

Mass Spectrometry. The structure analyses of glycosylated products were carried out with an ESI mass spectrometer (*Bruker Daltonics*, Billerica, MA, USA). The primers and glycosylated products were dissolved in MeOH and analyzed using 10% MeCN soln.

Digestion of Elongated Products by Enzymes. Enzymatic digestion of elongated products was carried out in 100 μl of AcONa buffer (pH 5.5) containing 5 mU of α2,3-sialidase from *salmonella typhimurium LC2* (*TAKARA BIO INC.*, Shiga, Japan). The reaction was carried out overnight at 37°. The products were extracted using *SepPak C18* column and separated on an HPTLC plate with $CHCl_3$/MeOH/0.25% aq. KCl soln. 5 : 4 : 1 as developing solvent. HPTLC Plates were sprayed with resorcinol, and with orcinol–H_2SO_4 reagent, and heated to detect the glycolipids.

REFERENCES

[1] A. Varki, *Glycobiology* **1993**, *3*, 97.
[2] S. Hakomori, *Annu. Rev. Biochem.* **1981**, *50*, 733.
[3] Y. C. Lee, *Trends Glycosci. Glycotechnol*. **2010**, *22*, 95.
[4] S. Hakomori, *Glycoconj. J.* **2000**, *17*, 143.
[5] D. J. Miller, R. L. Ax, *Mol. Reprod. Dev.* **1990**, *26*, 184.
[6] T. Sato, K. Hatanaka, H. Hashimoto, T. Yamamoto, *Trends Glycosci. Glycotechnol.* **2007**, *19*, 1.
[7] H. Nakajima, Y. Miura, T. Yamagata, *J. Biochem*. **1998**,*124*, 148.
[8] W. Yue, Y. Zhao, S. Shao, H. Tian, Z. Xie, Y. Geng, F. Wang, *J. Mater. Chem.* **2009**, *19*, 2199.

[9] S. Nishimura, K. Matsuoka, T. Furuike, S. Ishii, K. Kurita, *Macromolecules* **1991**, *24*, 4236.
[10] Y. Miura, T. Yamagata, *Biochem. Biophys. Res. Commun.* **1997**, *241*, 698.
[11] Y. Hirabayashi, A. Hamaoka, M. Matsumoto, T. Matsubara, M. Tagawa, S. Wakabayashi, M. Taniguchi, *J. Biol. Chem*. **1985**, *260*, 13328.
[12] A. K. Boal, K. Das, M. Gray, V. Rotello, *Chem. Mater*. **2002**, *14*, 2628.
[13] T. Sato, M. Takashiba, R. Hayashi, X. Zhu, T. Yamagata, *Carbohydr. Res.* **2008**, *343*, 831.

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