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Synthesis and enzymatic susceptibility of a series of novel GM2 analogs*

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Abstract A series of GM2 analogs in which GM2 epitope was coupled to a variety of glycosyl lipids were designed and synthesized to investigate the mechanism of enzymatic hydrolysis of GM2 ganglioside. The coupling of *N*-Troc-protected sialic acid and *p*-methoxyphenyl galactoside acceptor gave the crystalline disaccharide, which was further coupled with galactosamine donor to give the desired GM2 epitope trisaccharide. After conversion into the corresponding glycosyl donor, the trisaccharide was coupled with galactose, glucose and artificial ceramide (B30) to give the final compounds. The result on hydrolysis of GM2 analogs indicates that GM2 activator protein requires one spacer sugar between GM2 epitope and the lipid moiety to assist the hydrolysis of the terminal GalNAc residue.

Keywords GM2 ganglioside · GM2 epitope · GM2 activator · Artificial ceramide

*Synthetic studies on sialoglycoconjugates, Part 140. For part 139, see Ref [1].

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Introduction

It has been widely recognized that complex carbohydrates are information-rich molecules. Unlike the proteins and nucleic acids, unlimited structural variations of complex carbohydrate chains can be formed through the changes in i) the sequential arrangement of the monosaccharide units, ii) the anomeric configuration of each sugar residue, and iii) the linkage between the two neighboring sugar residues. Although complex carbohydrate chains in glycoconjugates have been shown to play diverse biological functions, it is still not possible to directly decipher the structure and function of a complex carbohydrate chain.

While studying the catabolism of GM2, GalNAcβ(1-4)[NeuAcα2-3]Galβ1-4GlcCer, we found that the hydrolysis of GalNAc in this ganglioside requires both β-hexosaminidase A and a protein cofactor, GM2-activator protein [2]. We have subsequently shown that in addition to the GalNAc, the enzymatic hydrolysis of the Neu5Ac in GM2 also requires GM2 activator protein [3]. In GM2, both the GalNAc and the Neu5Ac are linked to the penultimate Gal to form a branched trisaccharide (GalNAcβ1-4[Neu5Acα2-3]Galβ-). We hypothesized that the catabolism of GM2 must be controlled by this specific branched trisaccharide-epitope (GM2-epitope). We have used the synthetic approach [4] to decipher the effect of mutating the linkages of the GalNAc- and the Neu5Ac in GM2 on its susceptibility to enzymatic hydrolysis to support our

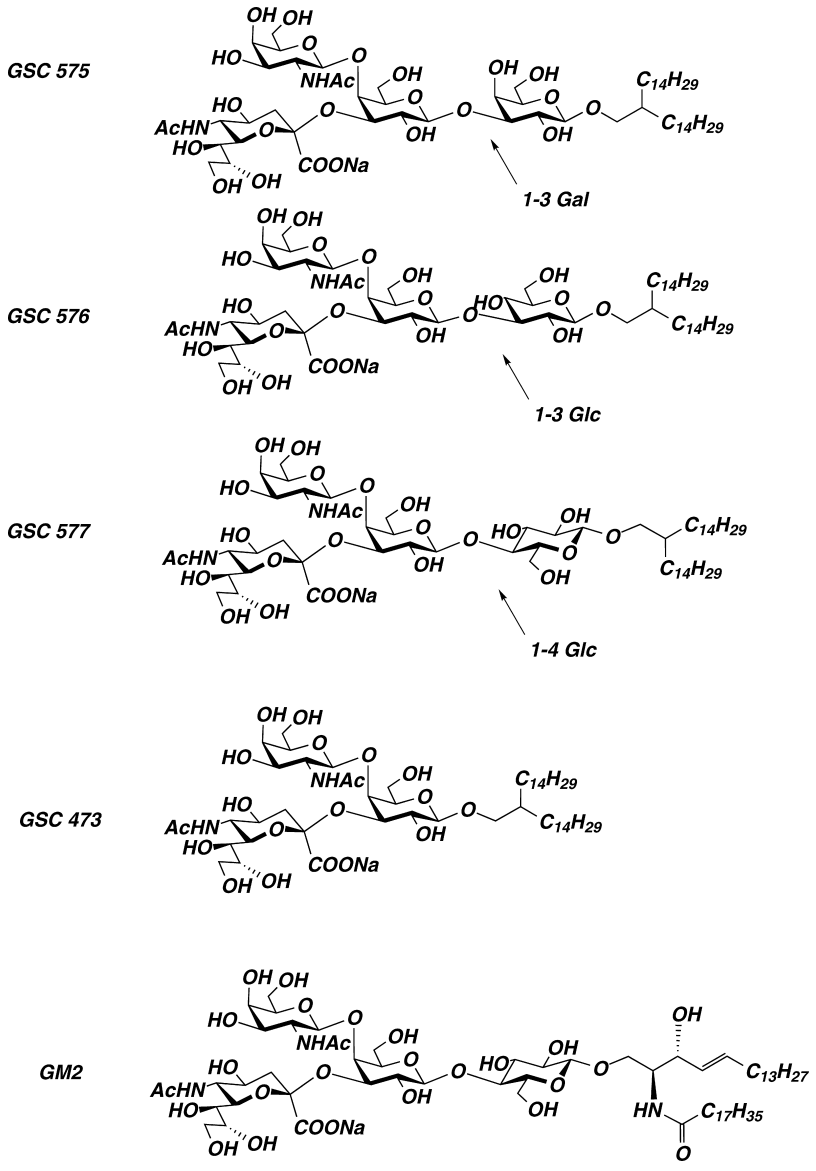


Fig. 1 Structures of GM2 and its homologues.

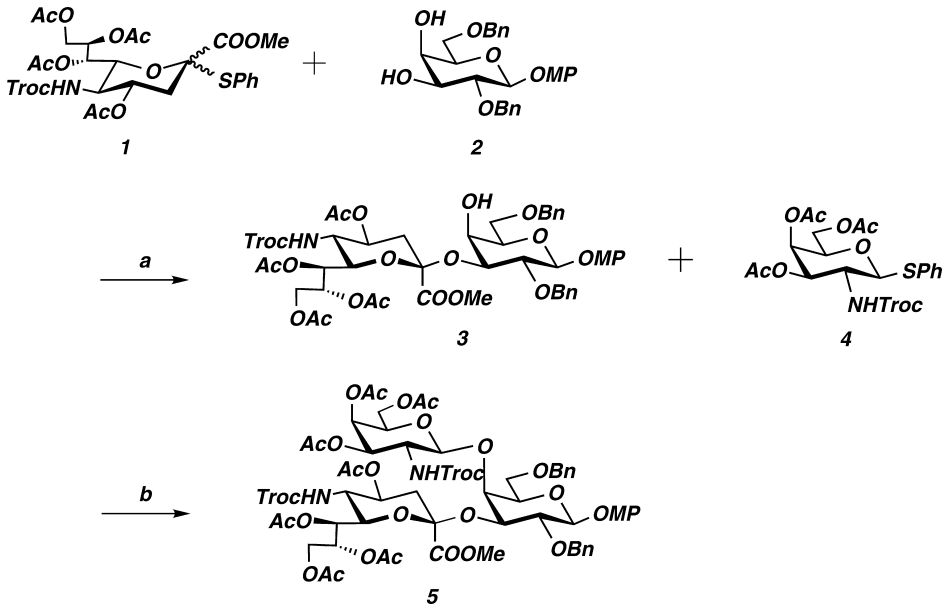
hypothesis [5]. We here report the design, synthesis and enzymatic hydrolysis of novel GM2 analogs (Fig. 1), in which glucosyl ceramide of the original GM2 ganglioside was replaced with a variety of glycosyl lipids, in order to investigate the mechanism of enzymatic hydrolysis of GM2 ganglioside in more detail.

Results and discussion

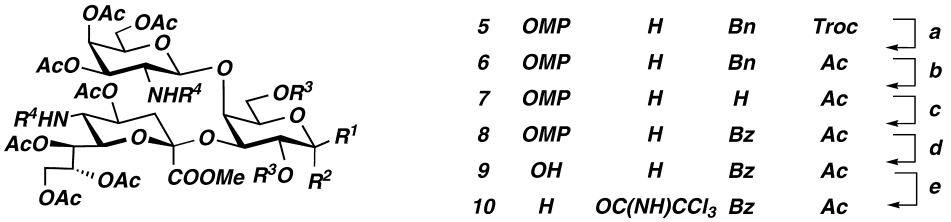
Chemical synthesis

The most important aspect in the systematic synthesis of a series of the desired target compounds is the development of an efficient procedure to construct the GM2 epitope trisaccharide, GalNAcβ1-4(NeuAcα2-3)Gal, because the target compounds contain this trisaccharide as a common component. This problem was solved by employing suitably protected building blocks, *N*-Troc-protected sialic acid donor **1**, *p*-methoxyphenyl galactoside acceptor **2** and *N*-Troc-protected galactose donor **4** (Scheme 1). In particular, *N*-Troc-protected sialic acid donor, equipped with phenylsulfenyl functionality as a leaving group [7], has been proven to be highly reactive [6]. The NIS-TfOH promoted glycosylation of **2** [6] with **1** in propionitrile at -50°C gave the desired disaccharide **3** as crystalls (52%). It should be pointed out that the disaccharide **3** is the first crystalline building block of sialyl α(2-3)galactose to our best knowledge. The disaccharide **3** obtained was directly applied for the further glycosylation with the donor **4**, which was prepared in 70% yield in 3 steps from the galactosamine hydrochloride [8], to give the desired trisaccharide **5** in 70% yield.

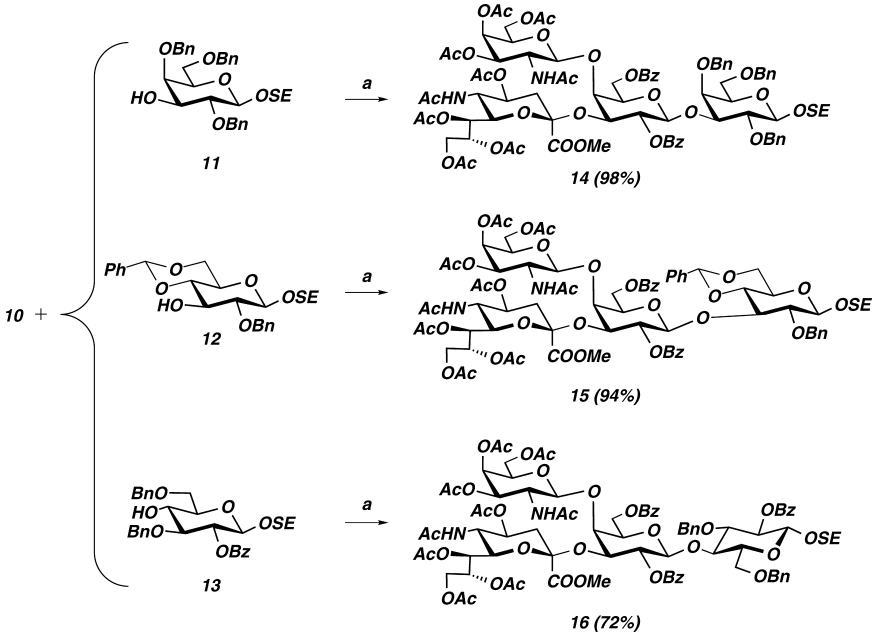
Scheme 1 (a) NIS, TfOH, MS3Å, EtCN, −50°C, 52%; (b) NIS, TfOH, MS4Å, 0°C, 70%.



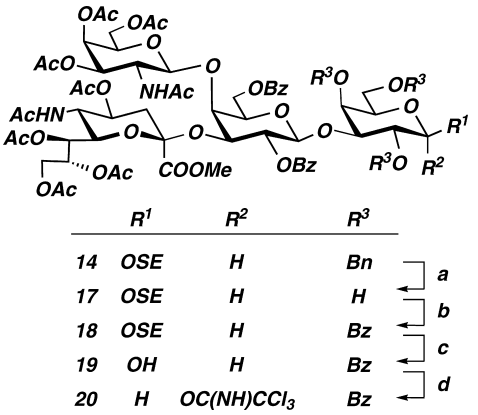
Scheme 2 (a) Cd-Pb, AcOH, DMF, 40°C, 84%; (b) H_2, $Pd(OH)_2$, EtOH, 40°C, 90%; (c)Bz_2O, DMAP, pyridine, 40°C, 90%; (d) CAN, toluene, MeCN, H_2O, r.t., 76%; (e) CCl_3 CN, DBU, CH_2 Cl_2 r.t., 94%.



Scheme 3 (a)TMSOTf, MS4Å, CH_2 Cl_2, 0°C.



The trisaccharide **5** obtained above was converted into the corresponding glucosyl donor **10** as shown in Scheme 2. The selective deblocking of the Troc group of **5** with Cd/Pd [9] in acetic acid/DMF proceed smoothly to give a free amino derivative, which, on successive treatment with acetic anhydride in pyridine afforded the corresponding *N*-acetyl derivative **7**. It is noteworthy that the conventional condition employing zinc in acetic acid was not sufficient to remove the



	R^1	R^2	R^3	
14	OSE	H	Bn	a
17	OSE	H	H	b
18	OSE	H	Bz	c
19	OH	H	Bz	d
20	H	$OC(NH)CCl_3$	Bz	

Scheme 4 (a) H_2, $Pd(OH)_2$, EtOH, 40°C, 97%; (b) Bz_2O, DMAP, pyridin, 40°C, 93%; (c) TFA, CH_2 Cl_2, r.t., 91%; (d) CCl_3CN, DBU, CH_2Cl_2, r.t., 94%.

coupled Troc group completely. Hydrogenolytic removal of the benzyl (Bn) groups in **6** and the following benzoylation gave **8**. Compound **8** was then transformed into the corresponding trichloroacetimidate **10** by selective removal of the *p*-methoxyphenyl (MP) group with ceric ammonium nitrate (CAN) and subsequent imidate formation.

The donor **10** was coupled with a series of glycosyl acceptors **11**[10], **12**[11], **13**[11] by a treatment with TMSOTf to afford the corresponding tetrasaccharides **14**–**16** (72–98%), respectively, as shown in Scheme 3. The tetrasaccharide **14** was converted into the corresponding donor **20**, as shown in Scheme 4, by essentially the same procedure employed for the conversion of **6** into **10** except for the selective removal of the 2-(trimethylsilyl)ethyl (SE) group with trifluoroacetic acid (TFA).

The imidate **20** was coupled with 2-tetradecylhexadecanol **21**, synthetic lipid, and the resulting **22** was treated under alkaline condition to give the desired GM2 ganglioside analog (**GSC-575**) in high yield (Scheme 5). The other analogs, **GSC-576** and **GSC-577**, were respectively synthesized from the corresponding tetrasaccharide **15** and **16** by the same procedure described for the synthesis of GSC-575, as shown in Scheme 6 and 7. **GSC-473**, in which GM2 epitope trisaccharide was directly tethered to a lipid, was efficiently synthesized by coupling the trisaccharide donor **10** with lipid **21**, followed by an alkaline treatment to remove all the acyl groups.

Enzymatic hydrolysis

Figure 2 shows the hydrolysis GM2 analogs by Hex A isolated from Human liver. GM2 isolated from a Tay-Sachs brain and GSC-577 which has the synthetic lipid (B-30) in stead of ceramide moiety were practically hydrolyzed by Hex A in the presence of GM2 activator protein after 24 h incubation. Similarly, GSC-576 and GSC-575, in which GM2 epitope was linked to 3-OH of the spacer Glc or Gal, were hydrolyzed. However, we could not observe any hydrolysis product of GSC-473, in which GM2 epitope was directly linked to the synthetic lipid, with or without GM2 activator protein. These results indicate that GM2 activator protein requires one spacer sugar between GM2 epitope and the lipid moiety to carry out the hydrolysis of the

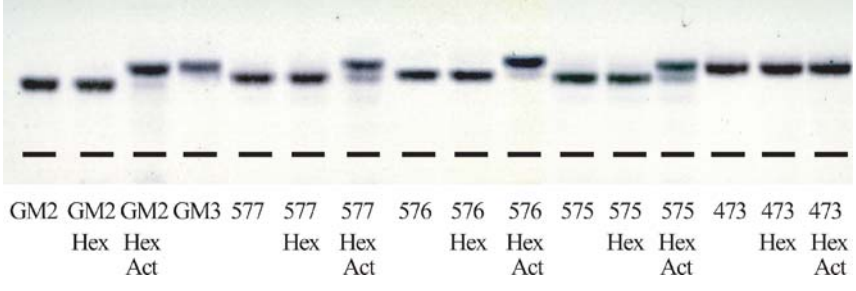
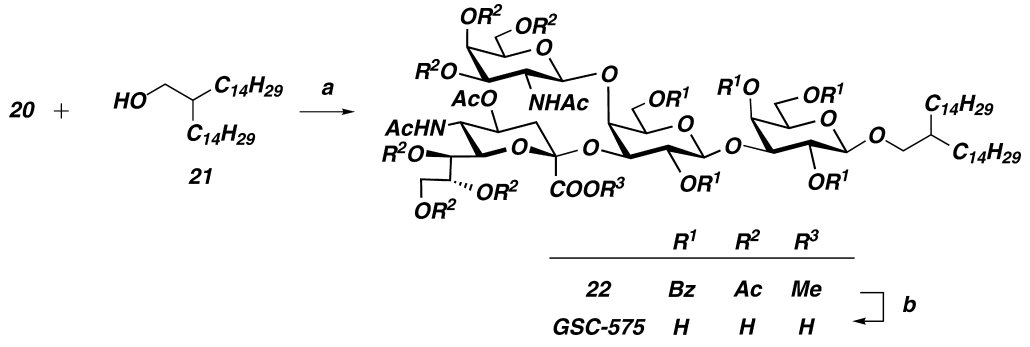
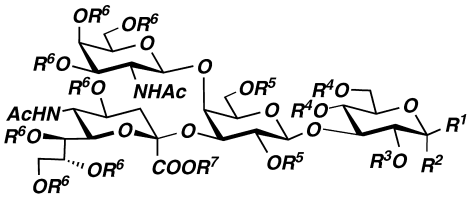


Fig. 2 TLC analysis showing the hydrolysis of the GalNAc in GM2 and GSC samples by 0.1 U of Hex A isolated from Human liver with or without GM2 activator protein after 24 h incubation at 37°C. *Hex*: Hex A, *act:* GM2 activator protein.



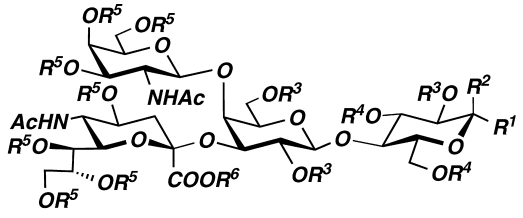
	R^1	R^2	R^3	
22	Bz	Ac	Me	b
GSC-575	H	H	H	

Scheme 5 (a) TMSOTf, MS4Å, CH_2Cl_2, 0°C, 84%; (b) NaOMe, MeOH, then, H_2O, 40°C, 92%.



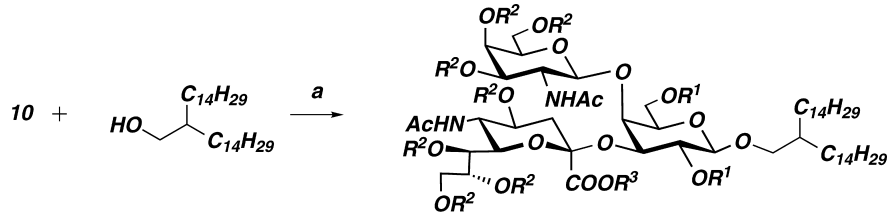
	R^1	R^2	R^3	R^4	R^5	R^6	R^7	
15	OSE	H	Bn	BzId	Bz	Ac	Me	a
23	OSE	H	Bn	H	Bz	Ac	Me	b
24	OSE	H	Bn	Ac	Bz	Ac	Me	c
25	OSE	H	H	Ac	Bz	Ac	Me	d
26	OSE	H	Bz	Ac	Bz	Ac	Me	e
27	OH, H		Bz	Ac	Bz	Ac	Me	f
28	H	$OC(NH)CCl_3$	Bz	Ac	Bz	Ac	Me	g
29	OB30	H	Bz	Ac	Bz	Ac	Me	h
GSC-576	OB30	H	H	H	H	H	H	

Scheme 6 (a) AcOHaq., r.t., 96%; (b) Ac_2O, pyridine, r.t., 98%; (c) H_2, $Pd(OH)_2$, EtOH, 40°C quant.; (d) Bz_2O, DMAP, pyridine, 40°C, 86%; (e) TFA, CH_2 Cl_2, r.t., 87%; (f) CCl_3CN, DBU, CH_2Cl_2, r.t., quant; (g) B30-OH, TMSOTf, MS4Å, CH_2Cl_2, 0°C, 22%; (h) NaOMe, MeOH, then, H_2O, 40°C, 91%.



	R^1	R^2	R^3	R^4	R^5	R^6	
16	OSE	H	Bz	Bn	Ac	Me	a
30	OSE	H	Bz	H	Ac	Me	b
31	OSE	H	Bz	Ac	Ac	Me	c
32	OH, H		Bz	Ac	Ac	Me	d
33	H	$OC(NH)CCl_3$	Bz	Ac	Ac	Me	e
34	OB30	H	Bz	Ac	Ac	Me	f
GSC-577	OB30	H	H	H	H	H	

Scheme 7 (a) H_2, $Pd(OH)_2$, EtOH, 40°C, quant.; (b) Ac_2O, pyridine, r.t., quant (c) TFA, CH_2Cl_2, r.t., quant.; (d) CCl_3CN, DBU, CH_2Cl_2, r.t., 94%; (e) B30-OH, TMSOTf, MS4Å, CH_2Cl_2, 0°C, 18%; (f) NaOMe, MeOH, then, H_2O, 40°C, 91%.



	R^1	R^2	R^3	
35	Bz	Ac	Me	b
GSC-473	H	H	H	

Scheme 8 (a) TMSOTf, MS4Å, CH_2Cl_2, 0°C, 92%; (b) NaOMe, MeOH, then H_2O, 40°C, 91%.

terminal GalNAc residue at least and that GM2 activator protein may not recognize the linkage and the nature of spacer sugar.

Experimental section

Chemical synthesis

General methods

^{1}H- and ^{13}C-NMR spectra were taken by Varian INOVA 400 and 500. Chemical shifts are expressed in ppm (δ) relative to the signal of either $CHCl_3$ or Me_4Si, adjusted to 7.26 or 0.00 ppm, respectively. MALDI-TOF MS spectra were recorded in positive ion mode on a Bruker Autoflex with the use of α-cyano-4-hydroxy-cinnamic acid (CHCA) as a matrix. Molecular sieves were purchased from Wako Chemicals Inc. and dried at 300°C for 2 h in muffle furnace prior to use. Drierite was powdered and dried at 300°C for 6 h in muffle furnace prior to use. Solvents as reaction media were dried over molecular sieves and used without purification. TLC analysis was performed on Merck TLC (silica gel $60F_{254}$ on glass plate). Silica gel (80 mesh and 300 mesh) manufactured by Fuji Silysia Co. was used for flash column chromatography. Solvent systems in chromatography were specified in v/v. Evaporation and concentration were carried out in vacuo.

4-Methoxyphenyl [methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-(2,2,2- trichloroethoxycarbonylamino)-D-*glycero-α*-D- *galacto -2-nonuropyranosylonate-(2→3)]-2,6-di-O-benzyl-β*-D-*galactopyranoside (3)*

To a solution of **1** [6] (4.00 g, 5.58 mmol) and **2** [6] (1.74 g, 3.72 mmol) in propionitrile (47 mL) was added molecular sieves 3 Å(1.80 g), and the mixture was stirred for 1 h at room temperature under argon atmosphere, then cooled to −50°C. *N*-Iodosuccinimide (NIS; 1.88 g, 8.37 mmol) and trifluoromethanesulfonic acid (TfOH; 74 μL, 0.84 mmol) were added to the mixture and this was stirred for 30 min at −50°C, neutralized with sodium hydrogen carbonate ($NaHCO_3$). The insoluble materials were filtered off and washed with ethyl acetate. The filtrate and washings were combined, successively washed with saturated sodium thiosulfate and brine, dried (Na_2SO_4) and concentrated. Silica gel column chromatography (1:4 ethyl acetate-toluene) of the residue and crystallization from ethyl acetate-hexane gave **3** (2.05 g, 52%) as crystals; mp 162–164°C, $[\alpha]_D = -6.33°$ ($c = 0.49$, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta = 1.94$, 1.99, 2.01, 2.10 (4 s, 12H, 4AcO), 2.64 (dd, 1H, H-3b$_{eq}$), 2.70 (d, 1H, OH), 3.68 (q, 1H, H-5b), 3.77–3.86 (m, 10H, MeO, COOMe, H-2a, H-4a, H-6a, H-6′a), 3.99 (dd, 1H, H-9b), 4.11 (d, 1H, $J_{6,7}$ 1.8 Hz, H-6b), 4.23–4.29 (m, 2H, H-3a, H-9′b), 4.48 (d, 1H, CH_2Ph), 4.57 (s, 1H, CH_2Ph), 4.81–4.99 (m, 6H, H-1a, H-4b, NH, CH_2Ph, 2COCH_2CCl$_3$), 5.37 (dd, 1H, $J_{6,7}$ 1.8 Hz, H-7b), 5.41–5.46 (m, 1H, H-8b), 6.78–7.05 (m, 4H, Ph), 7.26–7.42 (m, 5H, Ph).

^{13}C-NMR (100 MHz, $CDCl_3$): $\delta = -0.026$, 20.57, 20.72, 20.78, 21.10, 37.06, 51.47, 53.09, 55.63, 62.25, 67.40, 68.17, 68.40, 68.48, 69.35, 72.31, 73.07, 73.60, 74.54, 74.96, 75.70, 76.68, 95.32, 97.79 (anomer C), 102.80 (anomer C), 114.45, 118.52, 127.45, 127.58, 127.64, 127.64, 127.86, 128.15, 128.33, 138.15, 138.86, 151.61, 154.10, 155.20, 168.46, 169.93, 170.26, 170.57.

Anal. Calcd for $C_{49}H_{58}Cl_3NO_{20}$ (1087.35): C 54.13, H 5.38, N 1.29; found C 53.89, H 5.20, N 1.01.

4-Methoxyphenyl [3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-*galactopyranosyl]-(1→4)-[methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonylamino)*-D-*glycero-α*-D-*galacto-2-nonuropyranosylonate-(2→3)]-2,6-di-O-benzyl-β*-D-*galactopyranoside (5)*

To a mixture of **3** (300 mg, 280 mmol) and **4** [8] (321 mg, 560 μmol) in dichloromethane (17 mL) was added molecular sieves 4 Å(621 mg), and the mixture was stirred for 1 h at room temperature under argon atmosphere, then cooled to 0°C. NIS (251 mg, 1.12 mmol) and TfOH (10 μL, 112 μmol) were added, and this was stirred for 30 min at 0°C. The insoluble materials were filtered off and washed with chloroform. The filtrate and washings were combined, successively washed with saturated sodium carbonate, saturated sodium thiosulfate and brine, dried (Na_2SO_4) and concentrated. Silica gel column chromatography (130:1 chloroform-methanol) of the concentrate gave **5** (300 mg, 70%) with a trace amount of impurity; ^{1}H-NMR (400 MHz, $CDCl_3$): $\delta =$ 1.65, 1.85, 1.98, 1.99, 2.00, 2.13, 2.18 (7s, 21H, 7AcO), 3.55 (q, 1H, H-5b), 3.65 (t, 1H, H-5a), 3.75–3.84 (m, 5H, H-2a, H-6a, MeO), 3.90 (q, 1H, H-6′a), 3.96 (s, 3H, COOMe), 4.00–4.11 (m, 4H, H-3a, H-6b, H-9b, H-2c, H-5c), 4.14–4.215 (m, 2H, H-6b, H-9′b), 4.31–4.34 (m, 2H, H-6′c, CH_2Ph), 4.47–4.54 (m, 4H, CH_2Ph, 3COCH_2CCl$_3$), 4.87–4.99 (m, 5H, H-1a, H-1c, NHb, CH_2Ph, COCH_2CCl$_3$), 5.13 (d, 1H, CH_2Ph), 5.20–5.44 (m, 5H, H-4b, H-7b, H-8b, H-3c, H-4c), 6.19 (d, 1H, NHc), 6.81–7.07 (m, 4H, Ph), 7.26–7.34 (m, 10H, 2Ph).

4-Methoxyphenyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-*galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy*-D-*glycero-α*-D-*galacto-2-nonuropyranosylonate-(2→3)]-2,6-di-O-benzyl-β*-D-*galactopyranoside (6)*

To a solution of **5** (30 mg, 0.02 mmol) in DMF (0.5 mL) were added acetic acid (0.5 mL) and cadmium/lead (Cd/Pb)

complex (220 mg), and the mixture was stirred for 30 min at 40°C. The insoluble materials were filtered off and washed with chloroform. The filtrate and washings were combined, successively washed with water, saturated sodium carbonate and brine, dried (Na_2SO_4) and concentrated. The residue was treated with pyridine (0.5 mL) and acetic anhydride (0.5 mL) for 1 h, then cooled to 0°C. Methanol was added and the mixture was concentrated, and the residue was diluted with ethyl acetate, successively washed with cold 2 M hydrochloric acid, water, saturated sodium carbonate and brine, dried (Na_2SO_4) and concentrated. Silica gel column chromatography (50:1 chloroform-methanol) of the residue gave **6** (21 mg, 84%); $[\alpha]_D = -21.5°$ ($c = 0.82$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.90–2.18 (m, 27H, 2AcN, 7AcO), 2.21–2.32 (m, 2H, H-3b$_{ax}$, H-3b$_{eq}$), 3.64 (t, 1H), 3.73–3.80 (m, 5H, H-2a, OMe), 3.88 (q, 1H), 3.91–4.10 (m, 10H, H-3a, H-4a, H-5b, H-6b, H-9b, H-9′b, COOMe), 4.18 (q, 1H), 4.25 (t, 1H), 4.38 (m, 1H, H-2c), 4.51–4.57 (m, 3H, Ph*CH*$_2$), 4.88 (d, 1H, H-1a), 4.90 (d, 1H, H-1c), 5.02 (d, 1H, PH*CH*$_2$), 5.13–5.18 (m, 2H, H-4b, H-3c), 5.24 (m, 2H, H-7b, H-8b), 5.34 (d, 1H, H-4c), 6.39 (d, 1H, NHc), 6.79–7.33 (m, 14H, 3Ph).

^{13}C-NMR (100MHz, $CDCl_3$): δ = 20.48, 20.61, 20.73, 20.80, 20.84, 20.99, 23.15, 35.39, 49.12, 50.76, 53,32, 55.58, 61.26, 62.08, 66.71, 66.98, 67.61, 68.98, 69.53, 69.93, 71.61, 72.22, 73.61, 73.77, 75.26, 75.53, 77.47, 78.43, 99.29 (anomer C), 102.27 (anomer C), 103.05 (anomer C), 114.46, 118.63, 127.57, 127.62, 127.69, 128.16, 128.31, 138.07, 138.24, 151.23, 155.28, 158.49, 168.55, 169.42, 169.53, 169.64, 170.16, 170.27, 170.50, 170.63, 170.71, 173.02, 183.37.

MALDI-TOF MS
Calcd for $C_{61}H_{76}N_2O_{27}$
$[M+Na]^+$: 1291.45 $[M+K]^+$: 1307.43
Found : 1291.40, 1307.36

*4-Methoxyphenyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-β-*D*-galactopyranoside (**7**)*

A solution of **6** (1.40 g, 1.10 mmol) in ethanol (22.0 mL) was vigorously stirred with palladium hydroxide [$Pd(OH)_2$; 200 mg] for 22 h at 40°C under hydrogen atmosphere. The catalyst was collected and washed with chloroform. The combined filtrate and washings was concentrated and the residue was chromatographed on a silica gel column (30:1 chloroform-methanol) to give **7** (1.08 g, 90%); $[\alpha]_D = -9.1°$ ($c = 1.00$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.91–2.16 (9 s, 27H, 2AcN, 7AcO), 2.28 (t, 1H, J_{gem} 13.9 Hz, H-3b$_{ax}$), 2.56 (dd, 1H, J_{gem} = 13.9 Hz, H-3b$_{eq}$), 3.10 (t, 1H, H-5a), 3.57–3.66 (m, 2H, H-6a), 3.77 (s, 3H, OMe), 3.83–3.91 (m, 2H, H-2a, H-6′a), 3.93 (s, 3H, COOMe), 4.02–4.28 (m, 9H, H-3a, H-5b, H-9b, H-2c), 4.30 (q, 1H, H-9′b), 4.81 (d, 1H, H-1a), 5.00 (d, 1H, H-1c), 5.21–5.38 (m, 5H, H-4b, H-7b, NHb, H-3c, H-4c), 5.47 (br, 1H, H-8b), 6,24 (d, 1H, PH*CH*$_2$), 6.82–7.02 (m, 4H, Ph)

^{13}C-NMR (100 MHz, $CDCl_3$)

20.65, 20.76, 20.92, 21.06, 21.27, 23.37, 35.83, 49.61, 51.21, 53.48, 55.82, 59.44, 62.33, 62.86, 66.81, 67.94, 68.91, 69.16, 69.40, 70.69, 70.90, 72.60, 73.80, 74.66, 75.81, 76.79, 99.50 (anomer C), 102.59 (anomer C), 102.86 (anomer C), 114.72, 119.05, 151.22, 155.72, 168.79, 169.86, 169.91, 170.54, 170.61, 170.65, 170.70, 171.21

MALDI-TOF MS
Calcd for $C_{47}H_{64}N_2O_{27}$
$[M+Na]^+$: 1111.36 $[M+K]^+$: 1127.33
Found : 1111.36

*4-Methoxyphenyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-2,6-di-O-benzoyl-β-*D*-galactopyranoside (8)*

To a solution of **7** (75 mg, 0.062 mmol) in pyridine (1.2 mL) were added 4-dimethylaminopyridine (3 mg) and benzoic anhydride (57 mg, 0.76 mmol), and the mixture was stirred for 2 h at room temperature under argon atmosphere, then cooled to 0°C. Methanol was added and the mixture was concentrated to a residue, which was extracted with chloroform, successively washed with 1 M hydrochloric acid, water, saturated sodium carbonate and brine, dried (Na_2SO_4) and concentrated. Silica gel column chromatography (50:1 chloroform-methanol) of the residue afforded **8** (78 mg, 90%); $[\alpha]_D = -13.1°$ ($c = 0.95$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.70–2.17 (m, 28H, 2AcN, 7AcO, H-3b$_{ax}$), 2.49 (dd, 1H, H-3b$_{eq}$), 3.70 (s, 3H, OMe), 3.82–4.10 (m, 12H, H-4a, H-5a, H-5b, H-6b, H-9b, H-5c, H-6c, H-6′c, COOMe), 4.18 (dd, 1H, H-9′b), 4.44 (dd, 1H, H-3a), 4.52 (q, 1H, H-6a), 4.73 (dd, 1H, H-6′a), 4.94 (m, 1H, H-4b), 5.11 (d, 1H, H-1a), 5,16 (d, 1H, H-1c), 5.24 (m, 2H, H-7b, NHb), 5.37 (d, 1H, H-4c), 5.46 (m, 1H, H-8b), 5.62 (m, 2H, H-2a, H-3c), 6.15 (d, 1H, NHc), 6.63–8.09 (m, 14H, 3Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 20.30, 20.47, 20.57, 20.67, 20.70, 20.72, 21.18, 23.07, 23.41, 36.29, 49.09, 52.02, 53.05, 55.49, 61.52, 62.26, 63.73, 66.45, 67.00, 67.35, 68.80, 69.94, 70.15, 72.05, 72.15, 73.67, 75.19, 76.75, 98.22 (anomer C), 100.58 (anomer C), 114.28 (anomer C), 118.96, 128.36, 128.45, 129.57, 129.71, 129.92, 129.96, 133.14, 133.21, 151.24, 155.44, 164.76, 166.03, 168.14, 169.81, 169.95, 170.26, 170.30, 170.37, 170.46, 170.56, 171.23.

MALDI-TOF MS
Calcd for $C_{61}H_{72}N_2O_{29}$
$[M+Na]^+$: 1319.41 $[M+K]^+$: 1335.39
Found : 1319.27, 1335.23.

*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-non-uropyranosylonate-(2→3)]-2,6-di-O-benzoyl-β-*D*-galacto-pyranose (9)*

To a solution of **8** (2.52 g, 1.95 mmol) in a mixture of toluene (8 mL), acetonitrile (11 mL) and water (5 mL) was added ceric ammonium nitrate (CAN; 10.7 g, 19.5 mmol), and the mixture was stirred for 10 min at room temperature and extracted with ethyl acetate. The extract was successively washed with water, saturated sodium hydrogen carbonate and water, dried (Na_2SO_4) and concentrated. Silica gel column chromatography (30:1 chloroform-methanol) of the residue afforded **9** (1.77 g, 76%) as an anomeric mixture.

*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-2,6-di-O-benzoyl-β-*D*-galactopyranosyl trichloroacetimidate (10)*

To a solution of **9** (385 mg, 0.323 mmol) in dichloromethane (6.5 mL) were added trichloroacetonitrile (0.65 mL, 6.46 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 0.06 mL, 0.388 mmol) at 0°C under argon atmosphere, and the mixture was stirred for 1.5 h at room temperature and concentrated. Silica gel column chromatography (30:1 chloroform-methanol) of the residue gave **10** (404 mg, 94%) as an anomeric mixture.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-O-benzyl-β-*D*-galactopyranoside (14)*

To a solution of **10** (506 mg, 0.379 mmol) and **11** [10] in dichloromethane (11.4 mL) was added molecular sieves 4 Å(AW-300; 600 mg), and the mixture was stirred for 1 h at room temperature, then cooled to 0°C. Trimethylsilyl trifluoromethanesulfonate (TMSOTf; 1.4 μL, 7.6 μmol) was added to the mixture and this was stirred for 1.5 h at 0°C. Insoluble materials were filtered off and washed with chloroform. The combined filtrate and washings was successively washed with water, saturated sodium carbonate and brine, dried (Na_2SO_4) and concentrated. Silica gel column chromatography (40:1 chloroform-methanol) of the concentrate afforded **14** (326 mg, 98%); $[\alpha]_D = -15.4^\circ$ ($c = 0.36$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.90$–1.04 (m, 2H, $Me_3SiCH_2CH_2$), 1.99–2.25 (m, 28H, 2AcN, 7AcO, H-$3b_{ax}$), 3.00 (t, 1H, H-$3b_{eq}$), 3.47 (m, 1H, $Me_3SiCH_2CH_2$), 3.53–3.56 (m, 2H, H-5d, H-6d), 3.64-3.69 (m, 2H, H-2d, H-6′d), 3.88–4.01 (m, 7H, H-5a, H-6b, H-3d, $Me_3SiCH_2CH_2$, COOMe), 4.02 (d, 1H, H-4a), 4.07–4.20 (m, 6H, H-5b, H-9b, H-5c, H-6c, H-6′c, H-4d), 4.25-4.33 (m, 5H, H-3a, H-9′b, H-2c, H-1d), 4.36 (d, 1H, J_{gem} 11.7 Hz, $PhCH_2$), 4.50 (q, 2H, J_{gem} 11.7 Hz, $PhCH_2$), 4.56 (m, 1H, J_{gem} 11.7 Hz, H-6a), 4.75 (dd, 2H, J_{gem} 11.7 Hz, $PhCH_2$), 4.86 (dd, 1H, J_{gem} 11.7 Hz, H-6′a), 5.05 (d, 1H, J_{gem} 11.7 Hz, $PhCH_2$), 5.12 (d, 1H, H-1c), 5.16 (m, 1H, H-4b), 5.19 (d, 1H, H-1a), 5.23 (d, 1H, NHb), 5.34 (dd, 1H, H-7b), 5.38–5.44 (m, 3H, H-8b, H-3c, H-4c), 5.58 (t, 1H, H-2a), 6.04 (d, 1H, NHc), 7.23–8.10 (m, 25H, 5Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = -0.02$, 18.34, 20.47, 20.52, 20.59, 20.71, 20.81, 21.08, 23.12, 23.18, 29.69, 35.73, 49.30, 51.18, 53.25, 59.30, 61.61, 62.06, 64.01, 66.42, 67.15, 67.21, 67.45, 68.08, 69.58, 70.12, 70.55, 70.85, 72.20, 72.26, 73.48, 73.70, 73.86, 74.13, 74.36, 75.12, 76.53, 77.48, 79.77, 79.96, 98.81 (anomer C), 101.28 (anomer C), 101.52 (anomer C), 103.16 (anomer C), 126.97, 127.22, 127.47, 127.61, 127.76, 127.93, 128.14, 128.24, 128.36, 128.54, 129.04, 129.50, 129.56, 129.77, 129.85, 133.03, 133.28, 138.14, 138.98, 139.12, 166.15, 168.00, 169.59, 169.68, 170.34, 170.46, 170.56.

MALDI-TOF MS
Calcd for $C_{86}H_{106}N_2O_{33}Si$
$[M+Na]^+$: 1745.63 $[M+K]^+$: 1761.61
Found : 1745.49, 1761.46.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2-O-benzyl-4,6-O-benzylidene-β-*D*-glucopyranoside (15)*

The GM2 epitope donor **10** (205 mg, 0.153 mmol) and the glucose acceptor **12**[11] (140 mg, 0.306 mmol) were coupled according to the procedure as described for **14**, to give the tetrasaccharide **15** (235 mg, 94%), $[\alpha]_D = -22.2^\circ$ ($c = 1.00$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.80$–0.95 (m, 2H, $Me_3SiCH_2CH_2$), 1.78–2.22 (m, 28H, 2AcN, 7AcO, H-$3b_{ax}$), 2.35 (dd, 1H, H-$3b_{eq}$), 3.39–3.49 (m, 3H, H-2d, H-5d, $Me_3SiCH_2CH_2$), 3.70 (t, 1H, H-5a), 3.76–3.92 (m, 7H, H-6b, H-4d, H-6d, $Me_3SiCH_2CH_2$, COOMe), 3.96 (d, 1H, H-4a), 3.99–4.13 (m, 4H, H-5b, H-9b, H-2c, H-6d), 4.17 (dd, 1H, H-9′b), 4.22 (dd, 1H, H-6a), 4.32–4.37 (m, 2H, H-3a, H-3d),

4.39 (d, 1H, H-1d), 4.59 (s, 2H, PhCH_2), 4.77 (q, 1H, H-6′a), 5.02 (m, 1H, H-4b), 5.12 (d, 1H, $J_{1,2}$ 8.06 Hz, H-1a), 5.14 (d, 1H, H-1c), 5.19 (d, 1H, NHb), 5.27 (dd, 1H, H-7b), 5.37 (d, 1H, H-4c), 5.45 (m, 1H, H-8b), 5.54 (q, 1H, $J_{1,2}$ 8.06 Hz, H-2a), 5.56–5.60 (m, 2H, H-3c, PhC*H*), 6.16 (d, 1H, NHc), 7.13–8.01 (m, 20H, 4Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = -1.61$, 18.23, 20.29, 20.39, 20.53, 20.65, 20.71, 21.11, 23.05, 23.33, 29.59, 36.00, 49.01, 51.79, 52.96, 61.36, 62.10, 63.20, 65.81, 66.37, 66.99, 67.19, 67.75, 68.72, 68.78, 69.93, 70.37, 70.73, 71.81, 71.96, 73.96, 74.80, 75.73, 79.99, 80.85, 81.51, 98.22, 100.67, 100.86, 100.97, 103.15 (4anomer C, PhCH), 126.04, 127.05, 127.17, 127.88, 127.95, 128.09, 128.32, 128.56, 129.38, 129.63, 129.76, 129.91, 132.74, 133.09, 137.23, 138.76, 164.46, 165.58, 168.02, 169.46, 169.67, 170.09, 170.32, 170.43, 171.09.

MALDI-TOF MS
Calcd for $C_{111}H_{148}N_2O_{36}$
$[M+Na]^+$: 2109.34 $[M+K]^+$: 2125.45
Found : 2109.37.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→4)-2-O-benzoyl-3,6-di-O-benzyl-β-*D*-glucopyranoside (16)*

The GM2 epitope donor **10** (200 mg, 0.150 mmol) and the glucose acceptor **13** [11] (169 mg, 0.300 mmol) were coupled according to the procedure as described for **14**, to give the tetrasaccharide **16** (213 mg, 72%). The physicochemical and spectral data were identical with the data previously reported [12].

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-O-β-*D*-galactopyranoside (17)*

A solution of **14** (261 mg, 0.160 mmol) in ethanol (5.0 mL) was vigorously stirred with $Pd(OH)_2$ (260 mg) for 4.5 h at 40°C under hydrogen atmosphere. The usual work-up as described for **7** gave **17** (227 mg, 97%), which was used for the next reaction without further purification.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-O-benzoyl-β-*D*-galactopyranoside (18)*

To a solution of **17** (199 mg, 0.137 mmol) in pyridine (0.7 mL) were added 4-dimethylaminopyridine (5 mg) and benzoic anhydride (186 mg, 0.822 mmol), and the mixture was stirred for 12 h at room temperature under argon atmosphere. The usual work-up as described for the synthesis of **8** and silica gel column chromatography (40:1 chloroform-methanol) afforded **18** (226 mg, 93%); $[\alpha]_D = 2.0°$ (c = 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.87$–0.98 (m, 2H, $Me_3SiCH_2CH_2$), 1.68–2.36 (9s, 27H, 2AcN, 7AcO), 1.86 (t, 1H, H-3b$_{ax}$), 2.61 (dd, 1H, H-3b$_{eq}$), 3.61-3.69 (m, 2H, H-2c, $Me_3SiCH_2CH_2$), 3.83–3.97 (m, 7H, H-4a, H-5a, H-5b, H-6b, COOMe), 4.01–4.06 (m, 2H, H-9b, $Me_3SiCH_2CH_2$), 4.12–4.29 (m, 4H, H-9′b, H-5c, H-6c, H-6′c), 4.31 (t, 1H, H-5d), 4.36 (dd, 1H, H-3a), 4.48 (m, 1H, H-6a), 4.54 (dd, 1H, H-2d), 4.62–4.72 (m, 4H, H-6′a, H-1d, H-6d, H-6′d), 4.94 (m, 1H, H-4b), 5.11 (d, 1H, H-1a), 5.17 (d, 1H, NHb), 5.28 (d, 1H, H-1c), 5.31 (dd, 1H, H-7b), 5.36 (t, 1H, H-2a), 5.47 (m, 1H, H-8b), 5.51 (d, 1H, H-4c), 5.68 (t, 1H, H-2d), 5.93–5.98 (m, 2H, H-3c, NHc), 6.01 (d, 1H, H-4d), 7.31–8.23 (m, 25H, 5Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = -1.70$, -0.07, 17.71, 20.20, 20.41, 20.65, 20.68, 20.73, 21.02, 23.04, 23.42, 36.28, 48.95, 52.64, 52.79, 61.38, 61.78, 63.08, 63.24, 66.01, 66.88, 67.09, 67.15, 68.71, 68.94, 69.86, 70.14, 70.38, 71.50, 71.71, 71.75, 71.91, 73.38, 73.45, 74.74, 97.76 (anomer C), 99.49 (anomer C), 99.86 (anomer C), 100.61 (anomer C), 127.93, 128.14, 128.21, 128.30, 128.47, 129.46, 129.64, 129.68, 129.79, 129.92, 130.02, 132.43, 132.70, 132.83, 132.99, 133.19, 164.17, 164.64, 165.75, 166.08, 168.07, 169.64, 169.70, 170.00, 170.19, 170.26, 170.32, 170.54, 170.58, 171.47.

MALDI-TOF MS
Calcd for $C_{86}H_{100}N_2O_{36}Si$
$[M+Na]^+$: 1787.57 $[M+K]^+$: 1803.55
Found : 1787.51, 1803.50.

*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-O-benzoyl-*D*-galactopyranose (19)*

The 2-(trimethylsilyl)ethyl group of **18** (37 mg, 20.9 μmol) was removed by treatment with trifluoroacetic acid (1.0 mL) in dichloromethane (2.0 mL) for 1.5 h

at room temperature. Toluene was added and the mixture was concentrated. Silica gel column chromatography (30:1 chloroform-methanol) of the residue on silica gel gave **19** (32 mg, 91%) as an anomeric mixture.

*(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-O-benzoyl-*D*-galactopyranosyl trichloroacetimidate (20)*

Compound **19** (153 mg, 91.8 μmol) was treated with trichloroacetonitrile (0.18 mL, 1.84 mmol) and DBU (15 μL, 0.11 mmol) in dichloromethane (1.8 mL) for 1.5 h at room temperature. The mixture was concentrated and the residue was chromatographed (40:1 chloroform-methanol) on a silica gel column to afford **20** (156 mg, 94%) as an anomeric mixture.

*2-(Tetradecyl)hexadecyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2,4,6-tri-O-benzoyl-β-*D*-glucopyranoside (22)*

To a solution of **20** (100 mg, 55 μmol) and 2-(tetradecyl)hexadecanol (**21**; 48 mg, 110 μmol) in dry dichloromethane (1.7 mL) was added molecular sieves 4 Å (AW-300; 150 mg), and the mixture was stirred for 1 h at room temperature, then cooled to 0°C. TMSOTf (0.2 μL, 1.1 μmol) was added to the mixture and this was stirred for 30 min at 0°C. The usual work-up as described for **14** and silica gel column chromatography (50:1 chloroform-methanol) gave **22** (97 mg, 84%); $[\alpha]_D = 1.9°$ ($c = 1.00$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H, CH_2CH_3), 1.26 (s, 52H, $26CH_2$), 1.49–2.19 (m, 28H, 2AcN, 7AcO, H-3b$_{ax}$), 2.46 (dd, 1H, H-3b$_{eq}$), 3.15 (t, 1H, CH_2CH), 3.45 (m, 1H, H-2c), 3.67–3.85 (m, 10H, H-4a, H-5a, H-5b, H-6b, H-9b, CH_2CH, COOMe), 3.96 (m, 4H, H-9′b, H-5c, H-6c, H-6′c), 4.08 (t, 1H, H-5d), 4.19 (dd, 1H, H-3a), 4.35–4.39 (m, 2H, H-6a, H-3d), 4.44–4.48 (m, 3H, H-1d, H-6d, H-6′d), 4.56 (m, 1H, H-6′a), 4.68 (m, 1H, 4b), 4.94 (d, 1H, H-1a), 4.99 (d, 1H, NHb), 5.10–5.18 (m, 3H, H-2a, H-7b, H-1c), 5.29 (m, 1H, H-8b), 5.34 (d, 1H, H-4c), 5.52 (t, 1H, H-2d), 5.78–5.86 (m, 3H, H-3c, H-4d, NHc), 7.12–8.07 (m, 25H, 5Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 14.08$, 20.20, 20.43, 20.69, 21.02, 22.64, 23.05, 23.42, 26.38, 26.70, 29.32, 29.57, 29.62, 29.66, 29.77, 29.79, 30.61, 30.82, 31.88, 36.35, 37.79, 49.00, 52.80, 61.42, 61.75, 62.85, 63.31, 66.02, 66.92, 67.11, 68.74, 68.90, 69.89, 70.26, 70.62, 71.54, 71.72, 71.97, 72.81, 73.30, 73.39, 74.71, 97.76 (anomer C), 99.43 (anomer C), 99.90 (anomer C), 101.82 (anomer C), 127.93, 128.16, 128.25, 128.31, 128.51, 129.50, 129.60, 129.65, 129.74, 129.83, 129.89, 130.04, 132.46, 132.69, 132.84, 133.01, 133.22, 164.57, 165.80, 165.88, 166.11, 168.11, 169.65, 169.69, 169.99, 170.22, 170.27, 170.35, 170.54, 170.63, 171.51.

*2-(Tetradecyl)hexadecyl (2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-β-*D*-galactopyranosyl-(1→3)-β-*D*-galactopyranoside (GSC-575)*

To a solution of **22** (80 mg, 38.3 μmol) in methanol (3.0 mL) was added 28% sodium methoxide in methanol (74 μL, 383 μmol), and the mixture was stirred for 12 h at room temperature under argon atmosphere. Water (1.0 mL) was added and the mixture was stirred for 12 h at room temperature, and then neutralized with Dowex (HCR-W2-H). After filtration, the filtrate was concentrated. Sephadex LH-20 column chromatography (methanol) of the residue gave the title compound (GSC-575; 44 mg, 98%); $[\alpha]_D = 85.3°$ ($c = 0.22$, CH_3OH)

MALDI-TOF MS
Calcd for $C_{61}H_{112}N_2O_{24}$
$[M+Na]^+$: 1279.75 $[M+K]^+$: 1295.70
Found : 1279.75, 1295.70.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-2-O-benzyl-β-*D*-glucopyranoside (23)*

Compound **15** (50 mg, 30.6 μmol) was treated with 80% acetic acid (1.5 mL) for 12 h at room temperature. After concentration, the residue was purified by a silica gel column (40:1 chloroform-methanol) to give **23** (45 mg, 96%); $[\alpha]_D = -0.18°$ ($c = 0.92$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.78$–0.97 (m, 2H, $Me_3SiCH_2CH_2$), 1.99–2.28 (m, 29H, 2AcN, 7AcO, H-3b$_{ax}$, H-3b$_{eq}$), 2.38 (m, 2H, 2OH), 3.27 (t, 1H, H-2d), 3.37 (m, 1H, H-5d), 3.46 (m, 1H, $Me_3SiCH_2CH_2$), 3.66 (t, 1H, H-4d), 3.71 (t, 1H, H-3d), 3.85 (m, 1H, H-6d), 3.88–4.23 (m, 15H, H-4a, H-5b, H-6b, H-9b, H-9′b, H-5c, H-6c, H-6′c, H-6′d, $Me_3SiCH_2CH_2$, COOMe), 4.28–4.34 (m, 3H, H-3a, H-2c, $PhCH_2$), 4.38 (d, 1H, H-1d), 4.53 (q, 1H, J_{gem} 12 Hz, H-6a), 4.62 (d, 1H, $PhCH_2$), 4.89 (dd, 1H, J_{gem} 12 Hz, H-6′a), 5.02 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1a), 5.09 (d, 1H, H-1c), 5.10–5.16 (m, 1H, H-4b), 5.35–5.48 (m, 5H, H-7b, H-8b, NHb, H-3c, H-4c), 5.57 (q, 1H, $J_{1,2}$ 8.0 Hz, H-2a), 6.28 (d, 1H, NHc), 7.13–8.20 (m, 15H, 3Ph).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 18.24, 20.48, 20.52, 20.57, 20.68, 20.79, 21.10, 23.14, 23.20, 29.64, 35.71, 49.12, 51.07, 53.27, 61.61, 62.02, 63.43, 64.02, 66.33, 67.03, 67.30, 67.62, 68.76, 69.99, 70.11, 70.24, 70.69, 72.16, 72.40, 73.82, 74.75, 76.68, 80.62, 85.70, 98.73 (anomer C), 101.38 (anomer C), 101.44 (anomer C), 103.03 (anomer C), 126.43, 127.10, 127.99, 128.20, 128.41, 129.12, 129.57, 129.75, 129.78, 133.08, 133.33, 138.89, 164.31, 166.24, 167.88, 169.66, 170.34, 170.38, 170.41, 170.44, 170.51, 170.57, 171.10.

MALDI-TOF MS
Calcd for $C_{72}H_{94}N_2O_{33}Si$
$[M+Na]^+$: 1565.54 $[M+K]^+$: 1581.51
Found : 1565.44, 1581.43.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-4,6-di-O-acetyl-2-O-benzyl-β-*D*-glucopyranoside (24)*

Compound **23** (298 mg, 0.193 mmol) was treated with acetic anhydride (1.0 mL, 10.6 mmol) and pyridine (1.0 mL, 12.4 mmol) for 12 h at room temperature under argon atmosphere. After usual work-up as described for **8** and silica gel column chromatography (30:1 chloroform-methanol) gave **24** (307 mg, 98%); $[\alpha]_D = -0.11^\circ$ (c = 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.85–0.94 (m, 2H, $Me_3SiCH_2CH_2$), 1.90–2.23 (m, 34H, 2AcN, 9AcO, H-3b$_{ax}$), 2.55 (dd, 1H, H-3b$_{eq}$), 3.28 (t, 1H, $J_{1,2}$ 7.8 Hz, H-2d), 3.45–3.50 (m, 1H, $Me_3SiCH_2CH_2$), 3.56–3.60 (m, 1H, H-5d), 3.67–3.72 (m, 1H, H-2c), 3.82–4.03 (m, 13H, H-4a, H-5a, H-5b, H-6b, H-9b, H-5c, H-6c, H-6′c, H-3d, $Me_3SiCH_2CH_2$, COOMe), 4.07–4.17 (m, 2H, H-9′b, H-4d), 4.29 (t, 1H, H-6d), 4.32 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1d), 4.40 (dd, 1H, H-3a), 4.44 (d, 1H, H-1d, J_{gem} 12 Hz, $PhCH_2$), 4.48 (t, 1H, J_{gem} 11 Hz, H-6a), 4.69 (t, 1H, J_{gem} 11 Hz, H-6′a), 4.71 (d, 1H, J_{gem} 12 Hz, $PhCH_2$), 5.13 (d, 1H, NHb), 5.16 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1a), 5.25 (d, 1H, H-1c), 5.30 (dd, 1H, H-7b), 5.38 (dd, 1H, $J_{1,2}$ 7.8 Hz, H-2a), 5.41 (d, 1H, H-4c), 5.44–5.47 (m, 1H, H-8b), 5.83 (dd, 1H, H-3c), 6.78 (d, 1H, NHc), 7.27–8.14 (m, 15H, 3Ph).

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 18.23, 20.33, 20.40, 20.62, 20.72, 20.74, 20.80, 21.07, 21.20, 23.13, 23.38, 36.36, 49.24, 52.59, 52.96, 61.48, 61.78, 62.48, 63.02, 66.19, 67.06, 67.14, 67.58, 68.74, 68.82, 69.33, 70.01, 71.06, 71.32, 71.97, 73.65, 74.16, 74.28, 76.68, 78.33, 81.70, 98.06 (anomer C), 99.80 (anomer C), 100.29 (anomer C), 102.86 (anomer C), 126.85, 127.58, 128.16, 128.33, 128.50, 129.00, 129.47, 129.56, 129.82, 130.00, 133.06, 133.33, 138.69, 148.46, 164.60, 165.83, 168.14, 169.63, 169.80, 170.15, 170.18, 170.22, 170.24, 170.32, 170.53, 170.66, 170.80, 171.50.

MALDI-TOF MS
Calcd for $C_{76}H_{98}N_2O_{35}Si$
$[M+Na]^+$: 1649.56 $[M+K]^+$: 1665.54
Found : 1649.49, 1665.47.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-4,6-di-O-acetyl-β-*D*-glucopyranoside (25)*

A solution of **24** (201 mg, 123 μmol) in ethanol (15.0 mL) was vigorously stirred with $Pd(OH)_2$ (200 mg) for 1 h at 40°C under hydrogen atmosphere. The usual work-up as described for the synthesis of **6** and silica gel column chromatography (30:1 chloroform-methanol) gave **25** (200 mg, quantitative); $[\alpha]_D = -13.3^\circ$ (c = 1.00, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.88–1.00 (m, 2H, $Me_3SiCH_2CH_2$), 1.62–2.17 (m, 34H, 2AcN, 9AcO, H-3b$_{ax}$), 2.50 (dd, 1H, H-3b$_{eq}$), 2.80 (s, 1H, OH), 3.32 (t, 1H, H-2d), 3.55 (m, 2H, H-5d, $Me_3SiCH_2CH_2$), 3.79–4.16 (m, 15H, H-4a, H-5a, H-5b, H-6b, H-9b, H-2c, H-5c, H-6c, H-6′c, H-3d, H-6d, $Me_3SiCH_2CH_2$, COOMe), 4.18 (d, 1H, H-6′d), 4.29 (d, 1H, H-9′b), 4.38 (m, 2H, H-3a, H-6a), 4.65 (m, 1H, H-6′a), 4.82–4.92 (m, 3H, H-4b, H-4d, NHb), 5.08 (d, 1H, H-1a), 5.22 (t, 2H, H-7b, H-1c), 5.30–5.35 (m, 2H, H-2a, H-4a), 5.52 (m, 1H, H-8b), 5.64 (dd, 1H, H-3c), 6.28 (s, 1H, NHc), 7.44–8.15 (m, 10H, 2Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): δ = 17.81, 20.06, 20.23, 20.48, 20.57, 21.00, 22.85, 23.20, 29.44, 36.30, 48.69, 51.95, 52.79, 61.23, 62.44, 62.54, 63.21, 66.66, 66.76, 67.02, 67.27, 68.48, 68.67, 69.56, 69.92, 70.15, 71.53, 71.74, 73.60, 73.76, 74.07, 80.11, 97.77 (anomer C), 99.90 (anomer C), 100.78 (anomer C), 101.94 (anomer C), 128.19, 128.32, 129.35, 129.70, 129.75, 129.90, 132.90, 133.12, 164.67, 165.74, 168.06, 169.71, 169.85, 170.03, 170.07, 170.17, 170.23, 170.34, 170.58, 170.74, 171.19.

MALDI-TOF MS
Calcd for $C_{69}H_{92}N_2O_{35}Si$
$[M+Na]^+$: 1559.51 $[M+K]^+$: 1575.49
Found : 1559.49, 1575.43.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-O-benzoyl-β-*D*-glucopyranoside (26)*

To a solution of **25** (197 mg, 137 μmol) in pyridine (2.6 mL) were added 4-dimethylaminopyridine (3 mg) and benzoic anhydride (58 mg, 256 μmol), and the mixture was

stirred for 3 h at room temperature under argon atmosphere. The usual work-up as described for the synthesis of **8** and silica gel column chromatography (40:1 chloroform-methanol) afforded **25** (180 mg, 86%); $[\alpha]_D = -10.5^\circ$ ($c = 0.95$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.86$–1.05 (m, 2H, $Me_3Si\mathit{CH_2}CH_2$), 1.71–2.27 (m, 34H, 2AcN, 11AcO, H-3b$_{ax}$), 2.76 (dd, 1H, H-3b$_{eq}$), 3.58 (m, 2H, H-5d, $Me_3SiCH_2\mathit{CH_2}$), 3.63 (m, 1H, H-2c), 3.91 (m, 4H, H-6b, COOMe), 3.95–4.01 (m, 3H, H-5a, H-5b, $Me_3SiCH_2\mathit{CH_2}$), 4.03–4.13 (m, 4H, H-4d, H-5c, H-6c, H-6′c), 4.21 (dd, 1H, H-9b), 4.26 (dd, 1H, J_{gem} 12.2 Hz, H-6d), 4.34 (dd, 1H, J_{gem} 12.2 Hz, H-6′d), 4.42–4.47 (m, 2H, H-6a, H-9′b), 4.54 (dd, 1H, H-3a), 4.62 (d, 1H, H-1d), 4.78 (q, 1H, H-6′a), 4.88 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1a), 4.97 (m, 1H, H-4b), 5.12 (d, 1H, NHb), 5.29 (t, 1H, H-2d), 5.34 (d, 1H, H-1c), 5.38 (dd, 1H, H-7b), 5.42–5.48 (m, 3H, H-2a, H-4c, H-3d), 5.66 (m, 1H, H-8b), 5.95 (dd, 1H, H-3c), 6.52 (d, 1H, NHc), 7.48–8.25 (m, 15H, 3Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = -1.65, -1.50, -0.11$, 17.73, 20.22, 20.30, 20.55, 20.60, 20.65, 20.72, 21.21, 23.01, 23.37, 29.57, 36.49, 49.01, 52.79, 53.15, 61.40, 61.84, 61.96, 62.79, 66.15, 66.88, 67.27, 67.40, 68.70, 68.87, 69.99, 70.01, 71.51, 71.72, 71.80, 72.51, 72.88, 73.28, 73.40, 74.73, 97.71 (anomer C), 99.42 (anomer C), 100.15 (anomer C), 100.24 (anomer C), 128.14, 128.28, 128.43 128.58, 129.20, 129.40, 129.52, 129.66, 129.71, 129.91, 130.16, 133.05, 133.26, 133.37, 164.61, 165.05, 165.79, 168.20, 169.70, 169.98, 170.04, 170.11, 170.33, 170.38, 170.60, 171.43.

MALDI-TOF MS
Calcd for $C_{70}H_{96}N_2O_{36}Si$
$[M+Na]^+$: 1663.54 $[M+K]^+$: 1679.51
Found : 1663.41, 1679.42.

*2-(Tetradecyl)hexadecyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→3)-4,6-di-O-acetyl-2-O-benzoyl-β-*D*-glucopyranoside (29)*

Compound **15** was converted into the title compound by benzoylation (**26**; 86%), removal of 2-(trimethylsilyl)ethyl group (**27**; 87%), activation as trichloroacetimidate (**28**; quantitative), and introduction of B30 (**29**; 22%), as described for the synthesis of **22** from **17**; $[\alpha]_D = -4.3^\circ$ ($c = 0.68$, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): $\delta = 0.88$ (t, 6H, $2CH_2\mathit{CH_3}$), 1.26 (s, 52H, $26CH_2$), 1.69 (t, 1H, H-3b$_{ax}$), 1.54–2.19 (11s, 33H, 2AcN, 9AcO), 2.50 (dd, 1s, H-3b$_{eq}$), 3.10 (t, 1s, $CH_2\mathit{CH}$), 3.55–3.63 (m, 2H, H-2c, H-5d), 3.65–3.84 (m, 10H, H-4a, H-5a, H-5c, H-6b, H-6c, H-6′c, H-9b, H-9′b, $\mathit{CH_2}$CH), 3.96 (d, 3H, COOMe), 4.14–4.23 (m, 3H, H-3a, H-3d, H-6d), 4.27 (dd, 1H, H-6′d), 4.31 (d, 1H, H-1d), 4.49 (dd, 1H, H-6a), 4.62 (t, 1H, H-6′a), 4.80 (m, 1H, H-4b), 4.88 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1a), 4.93 (d, 1H, NHb), 5.02 (t, 1H, H-4d), 5.12 (t, 1H, H-2d), 5.18 (m, 3H, H-1c, H-7b, H-8b), 5.22 (t, 1H, $J_{1,2}$ 7.6 Hz, H-2a), 5.35 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4c), 5.78 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3c), 6.59 (d, 1H, NHc), 7.27–8.10 (m, 15H, 3Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = 20.28$, 20.36, 20.41, 20.63, 20.68, 20.76, 20.96, 21.13, 22.65, 23.08, 23.37, 26.42, 26.74, 27.04, 29.33, 29.67, 29.78, 30.00, 30.59, 30.77, 30.89, 31.03, 31.88, 36.35, 37.05, 37.81, 49.11, 52.82, 61.25, 61.43, 62.27, 62.64, 65.76, 67.01, 67.07, 68.71, 68.80, 68.97, 70.04 70.14, 70.70, 71.21, 71.75, 72.03, 72.62, 73.09, 73.43, 73.55, 76.55, 77.42, 97.80 (anomer C), 99.57 (anomer C), 99.91 (anomer C), 101.63 (anomer C), 128.12, 128.36, 128.43, 128.56, 129.46, 129.53, 129.62, 129.68, 129.85, 129.96, 132.86, 133.20, 133.41, 133.35, 164.13, 164.85, 165.74, 168.22, 169.51, 169.70, 169.80, 170.00, 170.08, 170.13, 170.18, 170.30, 170.39, 170.49, 170.64, 170.73, 170.80, 171.62.

MALDI-TOF MS
Calcd for $C_{101}H_{144}N_2O_{36}$
$[M+Na]^+$: 1983.94 $[M+K]^+$: 1999.91
Found : 1983.53.

*2-(Tetradecyl)hexadecyl(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-β-*D*-galactopyranosyl-(1→3)-β-*D*-glucopyranoside (GSC-576)*

To a solution of **29** (34 mg, 17.3 μmol) in methanol (2.0 mL) was added 28% sodium methoxide in methanol (catalytic amount), and the mixture was stirred for 12 h at room temperature under argon atmosphere. The work-up as described for **GSC-575** gave **GSC-576** (20 mg, 91%); $[\alpha]_D = 9.7^\circ$ ($c = 0.16$, CH_3OH).

MALDI-TOF MS
Calcd for $C_{61}H_{112}N_2O_{24}$
$[M+Na]^+$: 1279.75 $[M+K]^+$: 1295.72
Found : 1279.73, 1295.70.

*2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-*D*-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-β-*D*-glucopyranoside (30)*

A solution of **16** (209 mg, 120 μmol) in ethanol (12.0 mL) was vigorously stirred with $Pd(OH)_2$ (210 mg) for 1 h at 40°C under hydrogen atmosphere. The usual work-up as described for compound **6** and column chromatography (30:1 chloroform-methanol) gave **30** (186 mg, quantitative).

2-(Trimethylsilyl)ethyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-(1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-O-benzoyl-β-D-glucopyranoside (31)

Compound **30** (162 mg, 0.104 mmol) was treated with acetic anhydride (2.0 mL, 5.3 mmol) and pyridine (1.0 mL, 6.2 mmol) for 4 h at room temperature under argon atmosphere. After usual work-up as described for **8** and silica gel column chromatography (35:1 chloroform-methanol) gave **31** (170 mg, quantitative); $[\alpha]_D = -10.5^\circ$ (c = 0.95, $CHCl_3$): ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.86–1.05 (m, 2H, $Me_3Si\mathit{CH_2}CH_2$), 1.71–2.27 (m, 34H, 2AcN, 11AcO, H-3b$_{ax}$), 2.76 (dd, 1H, H-3b$_{eq}$), 3.58 (m, 2H, H-5d, $Me_3SiCH_2\mathit{CH_2}$), 3.63 (m, 1H, H-2c), 3.91 (m, 4H, H-6b, COOMe), 3.95–4.01 (m, 3H, H-5a, H-5b, $Me_3SiCH_2\mathit{CH_2}$), 4.03–4.13 (m, 4H, H-5c, H-6c, H-6′c, H-4d), 4.21 (dd, 1H, H-9b), 4.26 (dd, 1H, J_{gem} = 12.2 Hz, H-6d), 4.34 (dd, 1H, J_{gem} = 12.2 Hz, H-6′d), 4.42–4.47 (m, 2H, H-6a, H-9′b), 4.54 (dd, 1H, H-3a), 4.62 (d, 1H, H-1d), 4.78 (q, 1H, H-6′a), 4.88 (d, 1H, H-1a), 4.97 (m, 1H, H-4b), 5.12 (d, 1H, NHb), 5.29 (t, 1H, H-2d), 5.34 (d, 1H, H-1c), 5.38 (dd, 1H, H-7b), 5.36 (t, 1H, H-2a), 5.42–5.48 (m, 3H, H-2a, H-4c, H-3d), 5.66 (m, 1H, H-8b), 5.95 (dd, 1H, H-3c), 6.52 (d, 1H, NHc), 7.48–8.25 (m, 15H, 3Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = -1.65, -1.50, -0.11$, 17.73, 20.22, 20.30, 20.55, 20.60, 20.65, 20.72, 21.21, 23.01, 23.37, 29.57, 36.49, 49.01, 52.79, 53.15, 61.40, 61.84, 61.96, 62.79, 66.15, 66.88, 67.27, 67.40, 68.70, 68.87, 69.99, 70.01, 71.51, 71.72, 71.80, 72.51, 72.88, 73.28, 73.40, 74.73, 97.71 (anomer C), 99.42 (anomer C), 100.15 (anomer C), 100.24 (anomer C), 128.14, 128.28, 128.43 128.58, 129.20, 129.40, 129.52, 129.66, 129.71, 129.91, 130.16, 133.05, 133.26, 133.37, 164.61, 165.05, 165.79, 168.20, 169.70, 169.98, 170.04, 170.11, 170.33, 170.38, 170.60, 171.43.

MALDI-TOF MS
Calcd for $C_{76}H_{96}N_2O_{36}Si$
$[M+Na]^+$: 1663.54 $[M+K]^+$: 1679.51
Found : 1663.41, 1679.51.

2-(Tetradecyl)hexadecyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonuropyranosylonate-(2→3)]-(2,6-di-O-benzoyl-β-D-galactopyranosyl)-(1→4)-3,6-di-O-acetyl-2-O-benzoyl-β-D-glucopyranoside (34)

Compound **31** was converted into the title compound by removal of 2-(trimethylsilyl)ethyl group (**32**; quantitative), activation as trichloroacetimidate (**33**; 94%), and introduction of B30 (**34**; 18%), as described for the synthesis of **22** from **18**; $[\alpha]_D = -5.9^\circ$ (c = 0.36, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.88 (t, 6H, $2CH_2\mathit{CH_3}$), 1.26 (s, 52H, $26CH_2$), 1.81–2.15 (m, 34H, 2AcN, 9AcO, H-3b$_{ax}$), 2.64 (dd, 1H, H-3b$_{eq}$), 3.14 (t, 1H, $CH_2\mathit{CH}$), 3.47 (m, 1H, H-5d), 3.53 (m, 1H, H-2c), 3.69–3.88 (m, 9H, H-4a, H-5a, H-5b, H-6b, $\mathit{CH_2}$CH, COOMe), 3.91–4.00 (m, 4H, H-5c, H-6c, H-6′c, H-4d), 4.05–4.16 (m, 2H, H-9b, H-6d), 4.22 (d, 1H, H-6′d), 4.33 (m, 2H, H-6a, H-9′b), 4.41 (m, 2H, H-3a, H-1d), 4.66 (m, 1H, H-6′a), 4.75 (d, 1H, H-1a), 4.84 (m, 1H, H-4b), 4.99 (d, 1H, NHb), 5.16–5.36 (m, 5H, H-2a, H-7b, H-1c, H-4c, H-2d, H-3d), 5.53 (m, 1H, H-8b), 5.83 (dd, 1H, H-3c), 6.42 (d, 1H, NHc), 7.35–8.13 (m, 15H, 3Ph).

^{13}C-NMR (125 MHz, $CDCl_3$): $\delta = -0.02$, 14.09, 19.71, 20.30, 20.41, 20.64, 20.69, 20.74, 20.87, 21.32, 22.68, 23.13, 23.48, 26.46, 26.77, 27.10, 29.35, 29.58, 29.65, 29.69, 29.87, 30.03, 30.69, 30.93, 31.91, 32.75, 34.41, 36.61, 37.08, 37.87, 49.19, 52.87, 52.99, 61.47, 61.90, 62.01, 62.86, 66.20, 66.97, 67.34, 68.78, 70.08, 70.73, 71.58, 71.64, 71.90, 72.61, 72.79, 73.18, 73.35, 73.40, 74.77, 97.78 (anomer C), 99.43 (anomer C), 100.19 (anomer C), 101.41 (anomer C), 128.36, 128.53, 128.67, 129.30, 129.51, 129.75, 130.27, 133.12, 133.35, 133.45, 164.77, 165.04, 165.88, 168.30, 169.82, 170.03, 170.14, 170.23, 170.43, 170.58, 170.74, 171.59.

MALDI-TOF MS
Calcd for $C_{101}H_{144}N_2O_{36}$
$[M+Na]^+$: 1983.94 $[M+K]^+$: 1999.91
Found : 1983.88.

2-(Tetradecyl)hexadecyl (2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)- (1→4)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonuropyranosylonate-(2→3)]-2,6-di-O-benzoyl-β-D-galactopyranoside (35)

The GM2 epitope donor **10** (30 mg, 22.4 μmol) and B30 (**21**; 20 mg, 44.8 μmol) was coupled according to the procedure as described for **14**, to give **35** (33 mg, 92%) after silica gel column chromatography (50:1 chloroform-methanol); $[\alpha]_D = -9.4^\circ$ (c = 0.74, $CHCl_3$); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 0.89 (t, 6H, $2CH_2\mathit{CH_3}$), 1.26 (s, 52H, $26CH_2$), 1.45 (br, 1H), 1.74–2.16 (m, 28H, 2AcN, 7AcO, H-3b$_{ax}$), 2.38 (dd, 1H, H-3b$_{eq}$), 3.26 (t, 1H), 3.81–3.87 (m, 6H, H-5b, H-6b, COOMe), 3.94 (d, 1H, H-4a), 3.95–4.06 (m, 5H, H-2c, H-5a, H-6c, H-6′c, H-9b), 4.11 (t, 1H, H-5c), 4.16 (dd, 1H, H-9′b), 4.31 (dd, 1H, $J_{2,3}$ 10.3 Hz, H-3a), 4.47 (q, 1H, J_{gem} 11.5 Hz, H-6a), 4.58 (d, 1H, H-1a), 4.73 (q, 1H, J_{gem} 11.5 Hz, H-6′a), 4.98 (m, 1H, H-4b), 5.11 (d, 1H, H-1e), 5.13 (d, 1H, HHb), 5.24 (dd, 1H, $J_{7,8}$ 10.0 Hz, H-7b), 5.34 (d, 1H, $J_{3,4}$ 3.2 Hz, H-4c), 5.38 (t, 1H, $J_{2,3}$ 10.3 Hz, H-2a), 5.42 (m, 1H, $J_{7,8}$ 10.0 Hz, H-8b), 5.56 (dd, 1H, $J_{3,4}$ 3.2 Hz, H-3c), 6.09 (d, 1H, NHc), 7.39–8.07 (m, 10H, 2Ph).

^{13}C-NMR (100 MHz, $CDCl_3$): $\delta = -0.04$, 20.29, 20.48, 20.64, 20.71, 20.75, 20.78, 21.16, 22.66, 23.13, 23.37, 26.52, 26.80, 29.34, 29.60, 29.66, 29.69, 29.81, 29.87, 30.71, 31.00, 31.90, 36.12, 37.87, 49.11, 51.85, 53.06, 61.48, 62.12, 63.76, 66.32, 67.04, 67.36, 68.88, 70.03, 70.23, 71.98, 72.03, 73.23, 73.82, 75.88, 98.28 (anomer C), 100.75 (anomer C), 101.69 (anomer C), 128.33, 128.47, 129.50, 129.82, 129.91, 129.95, 132.99, 133.20, 164.51, 166.06, 168.14, 169.76, 169.81, 170.28, 170.40, 170.42, 170.46, 170.61, 171.15.

MALDI-TOF MS
Calcd for $C_{84}H_{126}N_2O_{28}$
$[M+Na]^+$: 1633.84 $[M+K]^+$: 1649.81
Found : 1633.82, 1649.81.

*2-(Tetradecyl)hexadecyl(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-β-*D*-galactopyranosyl-(1→4)- β-*D*-glucopyranoside (GSC-577)*

To a solution of **34** (10 mg, 9.2 μmol) in methanol (1.4 mL) was added 28% sodium methoxide in methanol (catalytic amount), and the mixture was stirred for 12 h at room temperature under argon atmosphere. The work-up and purification as described for **GSC-575** gave **GSC-577** (12 mg, quantitative); $[\alpha]_D = 9.2^\circ$ (*c* 0.5, CH_3OH).

MALDI-TOF MS
Calcd for $C_{55}H_{102}N_2O_{19}$
$[M+Na]^+$: 1139.67 $[M+K]^+$: 1155.65
Found : 1139.66, 1155.67.

*2-(Tetradecyl)hexadecyl(2-acetamido-2-deoxy-β-*D*-galactopyranosyl)-(1→4)-[5-acetamido-3,5-dideoxy-*D*-glycero-α-*D*-galacto-2-nonuropyranosylonate-(2→3)]-β-*D*-galactopyranoside (GSC-473)*

To a solution of **35** (33 mg, 20.4 μmol) in methanol (2.0 mL) was added 28% sodium methoxide in methanol (catalytic amount), and the mixture was stirred for 12 h at room temperature under argon atmosphere. The work-up and purification as described for **GSC-575** afforded **GSC-473** (22 mg, quantitative); $[\alpha]_D = 9.16^\circ$ (*c*, CH_3OH).

MALDI-TOF MS
Calcd for $C_{61}H_{112}N_2O_{24}$
$[M+Na]^+$: 1279.75 $[M+K]^+$: 1295.72
Found : 1279.23, 1295.18.

Enzymatic hydrolysis

Materials

GM2 was isolated from the brain of a Tay-Sachs patient according the published method [13]. GSC samples were chemically synthesized (see the synthesis section). Hex A from human liver was prepared according to our previous report [2, 3]. The following were obtained from commercial sources: precoated silica gel-60 TLC plate, Merck (Darmstadt, Germany); GM3, Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of the highest grade from commercial sources.

Enzymatic hydrolysis

For enzymatic hydrolysis of the GalNAc from GSC or GM2, each reaction mixture contained 2.5 nmol of the substrate, 0.1 U of Hex A and 1 μg of GM2 activator protein in 25 μl of 10 mM sodium acertate buffer, pH 4.6. Incubations were carried out at 37°C for 24 h.

TLC Analysis

After incubation, each reaction mixture was evaporated to dryness and analyzed by thin layer chromatography. The solvent system used for developing GM2 and GSC was chroloform/methanol/12 mM $MgCl_2$ (5/4/1). The plate was sprayed with the diphenylamine-aniline-phosphoric acid (DPA) reagent as previously described [14]. The quantitative estimation of ganglioside bands on a TLC plate was carried out using an EPSON Perfection 3170 photo scanner and NIH 1.63 program.

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