

Article

Design and Synthesis of a Novel Ganglioside Ligand for Influenza A Viruses [†]

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Abstract: A novel ganglioside bearing Neu α 2-3Gal and Neu α 2-6Gal structures as distal sequences was designed as a ligand for influenza A viruses. The efficient synthesis of the designed ganglioside was accomplished by employing the cassette coupling approach as a key reaction, which was executed between the non-reducing end of the oligosaccharide and the cyclic glucosylceramide moiety. Examination of its binding activity to influenza A viruses revealed that the new ligand is recognized by Neu α 2-3 and 2-6 type viruses.

Keywords: ganglioside; sialic acid; total synthesis; glycosylation; influenza virus

1. Introduction

Influenza viruses cause a substantial number of deaths during annual epidemics and occasional pandemics [1,2]. Based on the antigenicity of their internal proteins the viruses are divided into three types, A, B, and C, of which either type A or B viruses cause seasonal influenza in humans. When the viruses bind to the host cell, hemagglutinin (HA) on their cell surface plays a significant role in the infection process. The HA protein recognizes sialoglycoconjugates expressed on the plasma membrane of the host cell, for example, sialoglycoproteins and gangliosides (sialoglycosphingolipids), as cellular ligands. Furthermore, HA can also recognize specific linkages between sialic acid (Neu5Ac/Gc) and lactosamine (LacNAc: Gal β 1-4GlcNAc) residues, which are found at the terminal end of glycoconjugates [3,4]. The structure and distribution of sialoglycans are crucial for viruses to determine their host animals, and two major linkage types, that is, Neu5Ac α 2-3LacNAc and Neu5Ac α 2-6LacNAc, are essential for viral transmission. Human and swine viruses predominantly recognize the Neu5Ac α 2-6LacNAc sequence, while avian and equine viruses bind preferentially to the Neu5Ac α 2-3Gal (including Neu5Ac α 2-3LacNAc) moiety. Swine are considered as intermediate hosts between humans and birds since they possess an abundance of both Neu5Ac α 2-3LacNAc and Neu5Ac α 2-6LacNAc structures as receptor carbohydrate determinants. The simultaneous infection of an intermediate host, such as swine, with avian and human viruses could lead to genetic recombination between the viruses, resulting in the generation of a new pathogenic virus that could potentially cause severe pandemics. However, the exact natural ligand for influenza A viruses in an intermediate host, such as pigs, remains unclear. Therefore, in this study, we focused on identifying a new carbohydrate ligand that was not only highly recognized by influenza A viruses but also functions as a natural receptor for viral HA. For this purpose, a ganglioside bearing both the Neu5Ac α 2-3 and Neu5Ac α 2-6LacNAc sequences was designed (Figure 1). It was hypothesized that the designed ganglioside **1** could be recognized by human- and avian-derived viruses because it contains two types of sialoglycan in a single molecule. We report the chemical synthesis of ganglioside **1** and its binding activity to influenza A viruses.

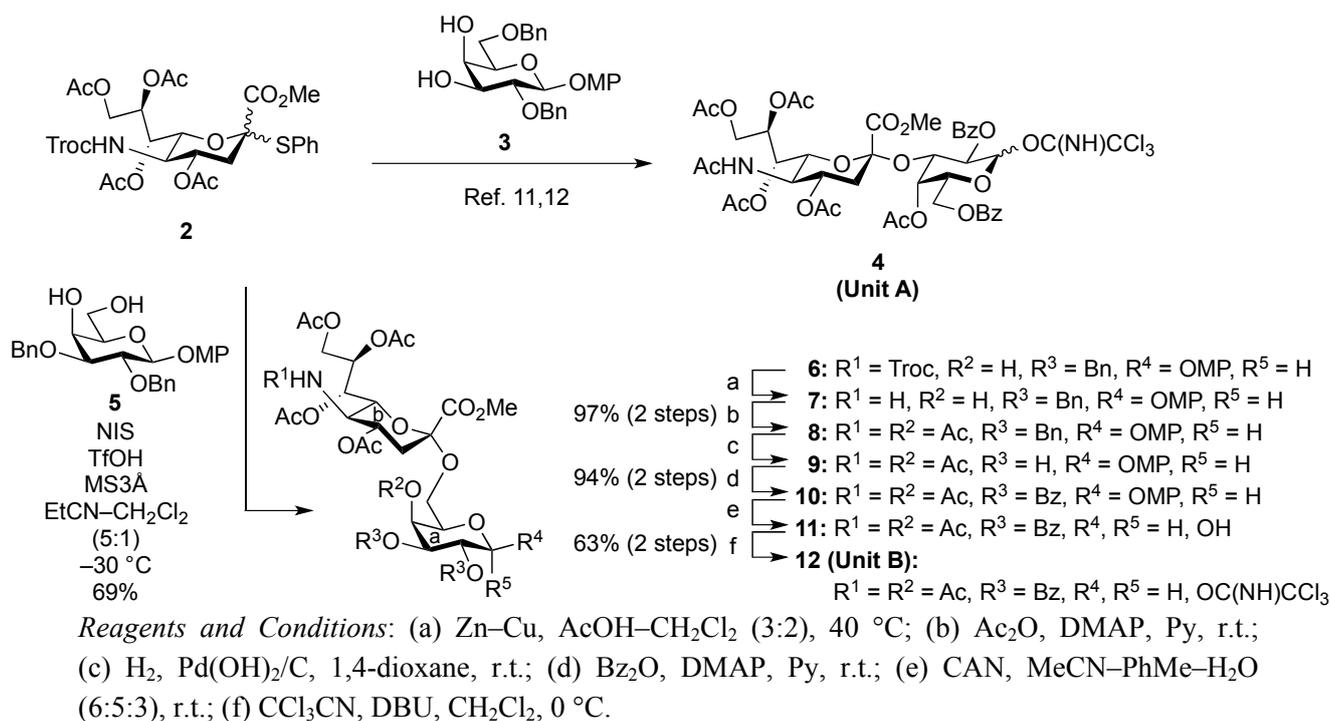
2. Results and Discussion

2.1. Chemical Synthesis

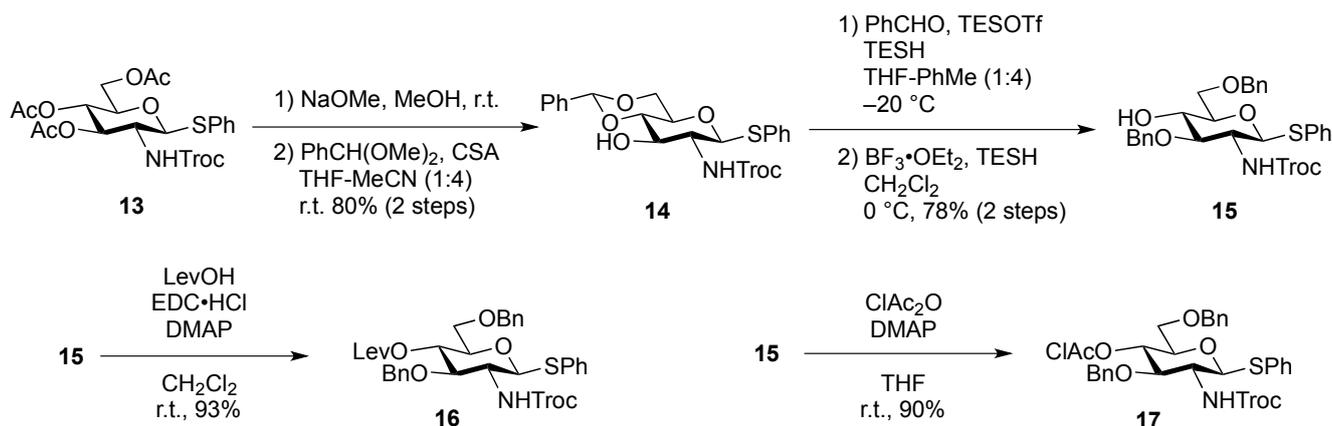
It was envisaged that the efficient synthesis of **1** could be achieved using the cassette approach between the non-reducing end of the oligosaccharide and the glucosylceramide, which was recently developed by our group [5–10]. Furthermore, it was thought that the construction of the non-reducing end of the heptasaccharide moiety, which includes two types of sialoside, Neu5Ac α 2-3/2-6Gal, should be executed through a convergent synthetic approach. For the convergent synthesis of a relatively large oligosaccharide such as **1**, the design of the building blocks often affects the efficiency of the total synthesis as well as the overall yield. Our preliminary experiment on the synthesis of a ganglioside similar to **1** gave a significant finding that monosaccharyl (GlcN) units are more useful as glycosyl donors than oligosaccharyl (Neu5Ac α 2-3/2-6Gal β 1-4GlcN) donors for the formation of branched structure at the 3- and 6-positions of the inner galactose residue (data not shown). Therefore, target **1**

and the hydroxyl group at the C-4 position of the galactose residue afforded compound **8** in excellent yield. Hydrogenolysis of the benzyl groups over Pearlman's catalyst in 1,4-dioxane followed by benzylation generated compound **10** in 94% yield over two steps. The exposure of **10** to CAN and H₂O [18] (giving **11**) and the subsequent introduction of a trichloroacetimidate group [19] afforded the Neu5Ac α 2-6Gal donor **12** (**Unit B**) in 63% yield over two steps.

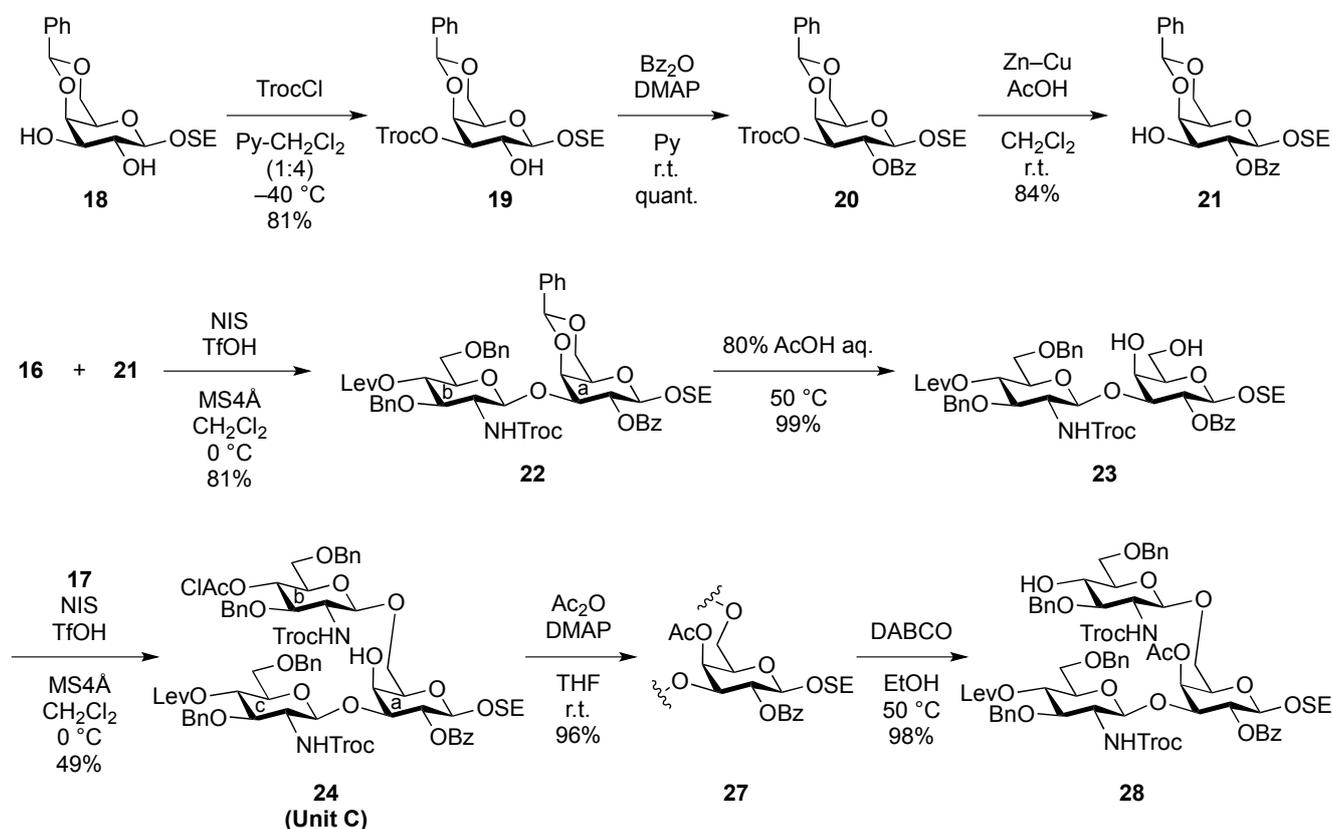
Scheme 1. Efficient synthesis of **Units A** and **B** from the *N*-Troc-protected sialyl donor **2**.



The inner core trisaccharide structure, **Unit C**, was prepared starting from the 2-*N*-Troc protected glucosamine derivative **13** (Schemes 2 and 3). First, the glucosaminyl donors **16** and **17** were prepared as shown in Scheme 2. Removal of the acetyl groups from **13** and the subsequent formation of cyclic benzylidene acetal between O-4 and O-6 afforded **14** in good yield. The following benzylation step in the presence of a Troc group was conducted under reductive conditions. Optimization of this reductive benzylation with benzaldehyde, TESOTf, and triethylsilane [20] revealed that the use of toluene as a solvent could increase the yield. The successive reductive opening of the benzylidene group by treatment with BF₃ etherate and triethylsilane [21] gave **15** in 78% yield over two steps from **14**. The obtained alcohol **15** was transformed into two types of glucosaminyl donors, namely, **16** and **17**, via the introduction of a levulinoyl (Lev) and monochloroacetyl (ClAc) group to the hydroxyl group at C-4, respectively.

Scheme 2. Preparation of glucosaminy donors **16** and **17** towards Unit C.

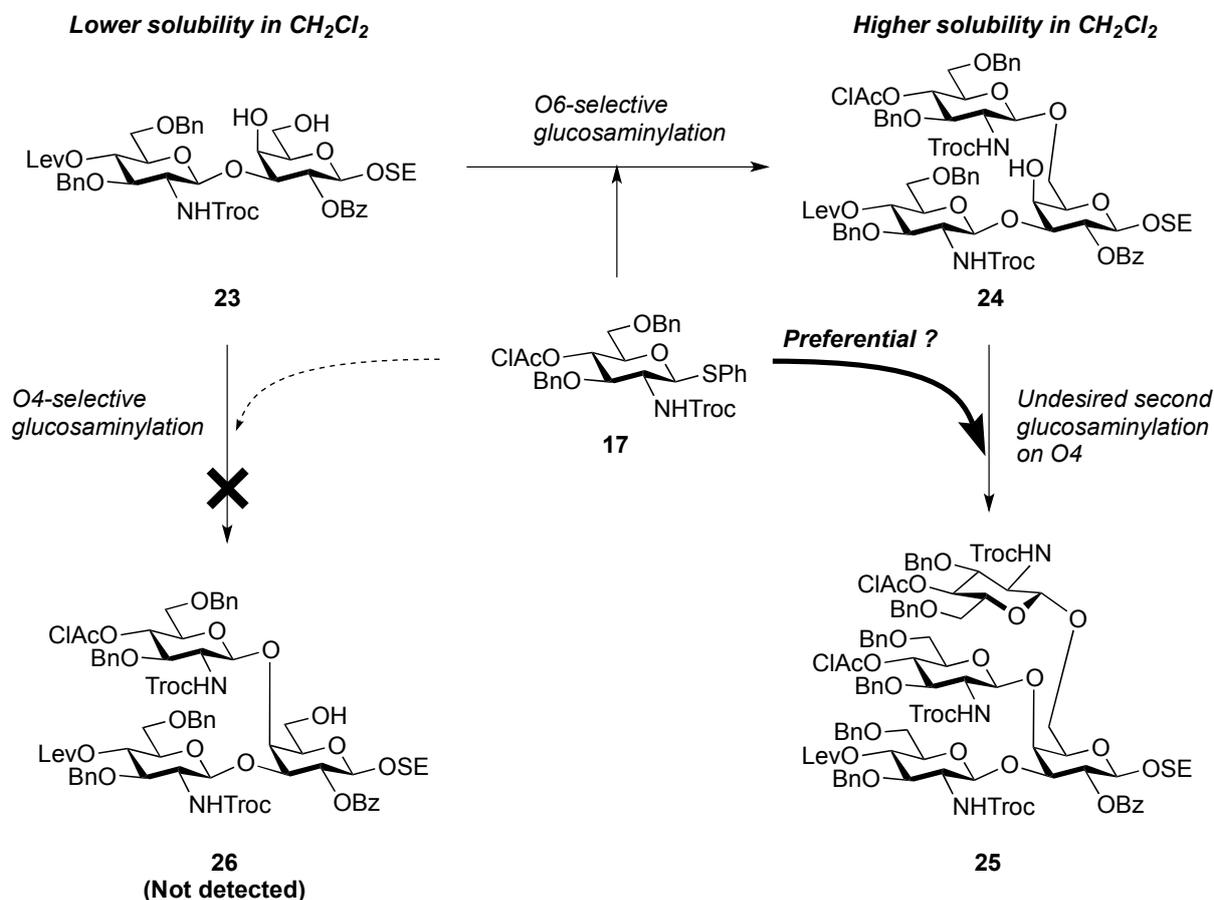
Scheme 3. Assembly of the inner core fragment, Unit C.



The glucosaminy donors **16** and **17** were then incorporated into the galactosyl acceptor **21**, which was prepared readily from the known galactose derivative **18** [22] via three steps. Selective protection of the hydroxyl group at C-3 by the Troc group was carried out under basic conditions with TrocCl at a low temperature (-40 °C) to afford **19** in 81% yield. Benzoylation under standard conditions (giving **20**) and the selective removal of the Troc group with Zn-Cu furnished the galactosyl acceptor **21** in good yield. The obtained **21** was then subjected to glycosylation with donor **16** in the presence of NIS and TfOH in CH₂Cl₂ at 0 °C, affording the disaccharide **22** in 81% yield. The stereochemical assignment was confirmed by ¹H-NMR, where the *J*_{1,2} value of 7.6 Hz for H-1 indicated the β-configuration of the glucosamine residue. Next, hydrolysis of the benzylidene group with 80% AcOH aq at 50 °C afforded

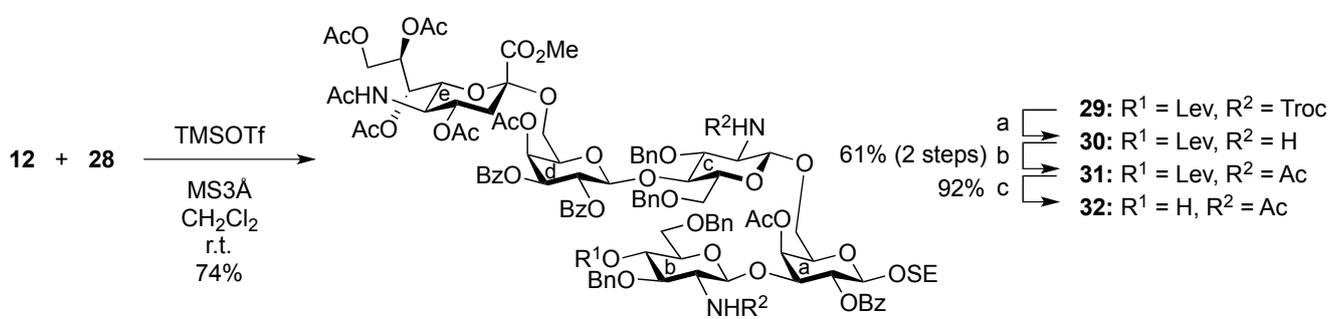
the diol **23** at an almost quantitative yield. A second round of glucosaminidation was conducted between **17** and **23** under the same conditions as those of the initial glucosaminidation between **16** and **21**. As a result, the desired trisaccharide **24** was obtained as **Unit C** in a moderate yield of 49%. In this reaction, a non-negligible amount of the tetrasaccharide **25**, in which both hydroxyl groups were glucosaminylated, was observed as a byproduct (Figure 2). An attempt at using a lower temperature to increase the selectivity failed due to the poor solubility of acceptor **23** in CH_2Cl_2 . Furthermore, changing the other factors for glycosylation, for example, the leaving group (using trichloroacetimidate) and how the donor was added, did not improve the yield of **24**. It is of importance that the generation of the tetrasaccharide **25** during the reaction was faster than the complete consumption of the acceptor **23**. In addition, the trisaccharide **26**, which was glucosaminylated at C-4 of the galactose residue, was not detected among the by-products. These findings suggested that the newly formed trisaccharyl alcohol **24** was preferred to the disaccharyl acceptor **23** as a glycosyl acceptor. This phenomenon might be explained by the poor solubility of the disaccharyl alcohol **23** in CH_2Cl_2 compared with the trisaccharyl alcohol **24** (Figure 2). Next, the acetylation of **24** with acetic anhydride and DMAP in THF [23] was carried out to protect the free hydroxyl group, affording **27** in 96% yield (Scheme 3). The monochloroacetyl group on **27** was then unblocked using DABCO in ethanol [24] with an excellent yield, providing the inner core trisaccharide acceptor **28**, which was ready for the next glycosylation step.

Figure 2. Explanation for the poor regioselectivity observed during glucosaminylation.



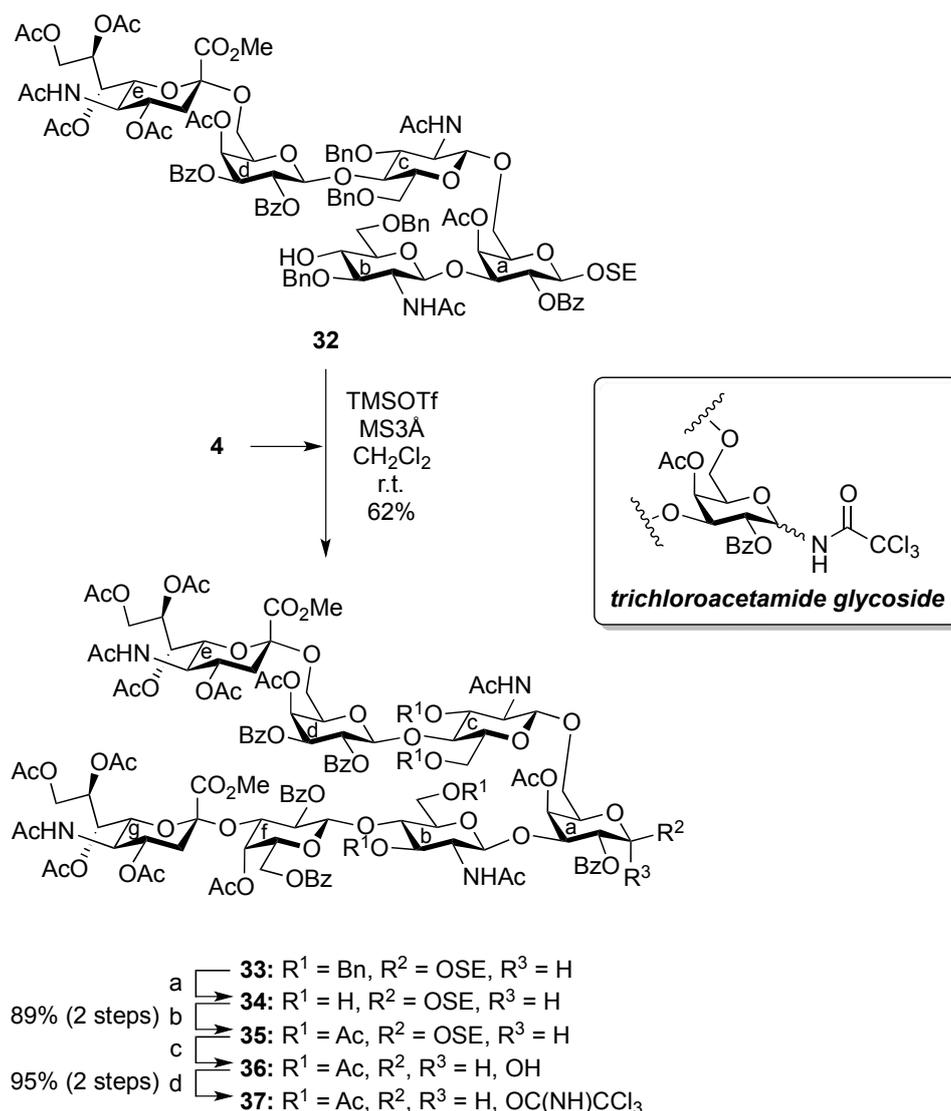
As depicted in Scheme 4, the coupling of the trisaccharide acceptor **28** with the Neu5Ac α 2-6Gal donor **12** promoted by TMSOTf was conducted in CH₂Cl₂ at room temperature, affording the pentasaccharide **29** in 74% yield. During this glycosylation step, the generation of several by-products containing trichloroacetamide glycoside, which is occasionally formed as a by-product during glycosylation using trichloroacetimidate donors, made the purification process an arduous task. Column chromatography on silica gel followed by gel filtration was found to be useful for purification. Next, the conversion of the Troc carbamate at C-2 of both glucosamine residues into acetamide was achieved by treatment of alloyed zinc with copper in AcOH and followed by acetylation, giving the acetamide compound **31** in 61% yield over two steps. Finally, cleavage of the levulinoyl group by using hydrazine monoacetate in THF [25] released the 4-OH to provide the pentasaccharide acceptor **32** in 92% yield.

Scheme 4. Coupling of Units A and C followed by transformation of the corresponding acceptor.



Reagents and Conditions: (a) Zn–Cu, AcOH–(CH₂Cl₂)₂ (3:2), 50 °C; (b) Ac₂O, DMAP, Py, r.t.; (c) NH₂NH₂·AcOH, THF, r.t.

Scheme 5 shows the assembly of the non-reducing end heptasaccharide moiety. The Neu5Ac α 2-3Gal donor **4** was coupled with **32** in the presence of TMSOTf in CH₂Cl₂ at room temperature to provide the heptasaccharide **33** in 62% yield. During this glycosylation step, the generation of the trichloroacetamide glycoside and the dimer of donor **4**, which was formed by the nucleophilic attack of the hydrolyzed donor on the oxocarbenium species derived from the donor, as by-products, made the purification of the desired product **33** laborious. The structure of isolated **33** was elucidated based on its MS, ¹H, and ¹³C-NMR spectra. For instance, the β -configuration of the newly formed glycosidic linkage was evident from the coupling constant of the anomeric proton at δ 5.01 ($J_{1,2} = 7.5$ Hz). Next, cleavage of the benzyl groups by hydrogenolysis (giving **34**) followed by acetylation with conventional conditions afforded **35** in 89% yield over two steps. Selective exposure of the anomeric hydroxyl group was easily achieved by treatment with trifluoroacetic acid in CH₂Cl₂ to yield **36**. This was then converted to the corresponding trichloroacetimidate donor **37** in 95% yield over two steps from **35**, which was then ready for cassette coupling with the glucosylceramide block **38** (Unit D).

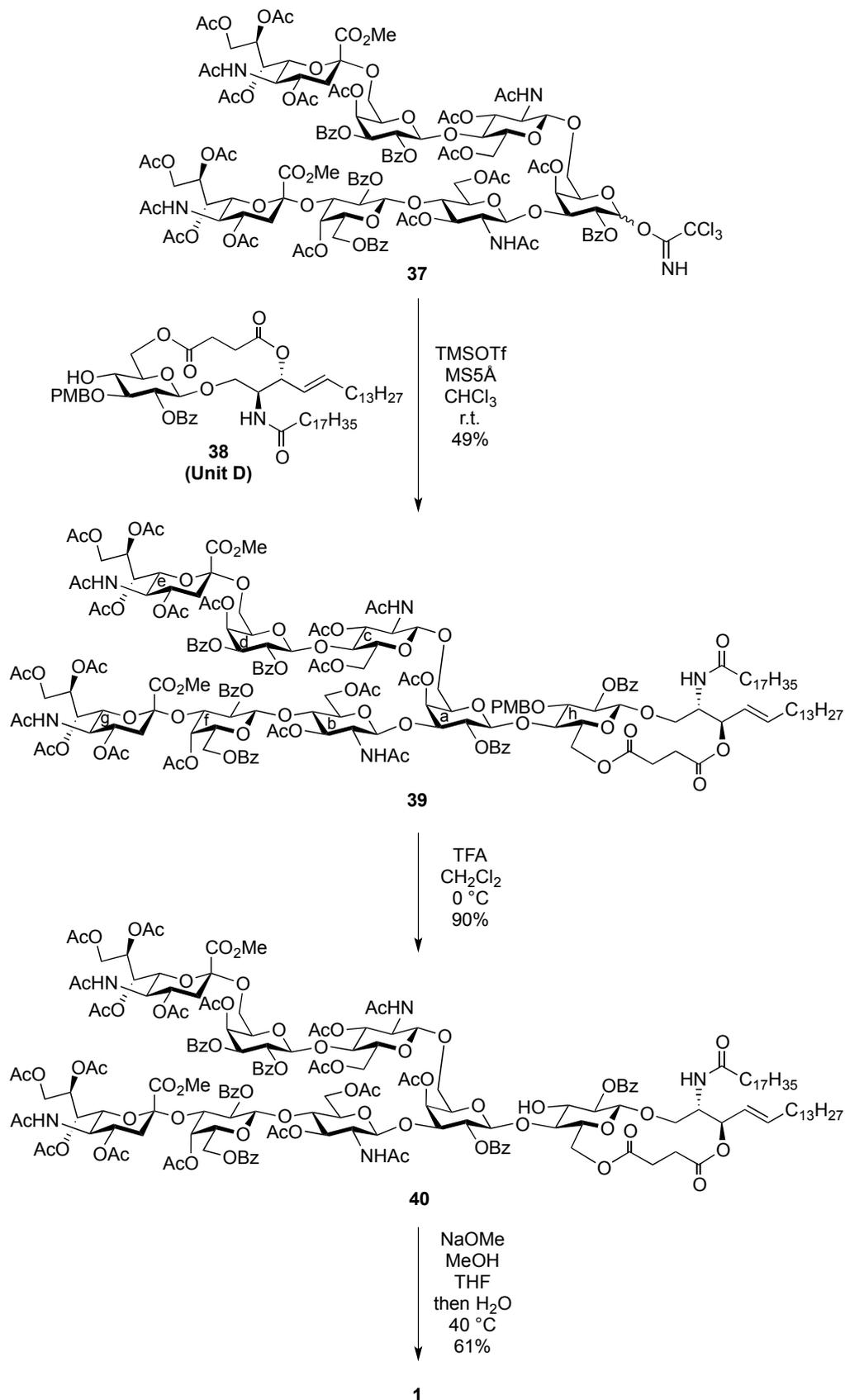
Scheme 5. Synthesis of the non-reducing end heptasaccharide part.

Reagents and Conditions: (a) H₂, Pd(OH)₂/C, 1,4-dioxane, r.t.; (b) Ac₂O, DMAP, Py, r.t.; (c) TFA, CH₂Cl₂, 0 °C; (d) CCl₃CN, DBU, CH₂Cl₂, 0 °C.

We previously addressed the development of the cassette coupling approach between a non-reducing end oligosaccharide and a glucosylceramide (GlcCer) moiety for the synthesis of various glycolipids, particularly gangliosides [5–10]. This approach resulted in a solution to the inevitable low yield of sugar and ceramide fragments. Hitherto, we developed two types of GlcCer units: one is a cyclic type GlcCer tethered by succinic ester between the sugar and lipid portions [5,7,8], while the other is an acyclic type GlcCer [6,9,10]. In this study, we chose the cyclic type due to its ease of preparation. The reported cyclic GlcCer acceptor **38** [7] was subjected to glycosylation with the oligosaccharide donor **37** in the presence of TMSOTf in CHCl₃ at room temperature, affording the fully protected ganglioside **39** in a moderate yield of 49%. In this reaction, chloroform was employed as solvent instead of the conventional dichloromethane because of the somewhat poor solubility of **38** in CH₂Cl₂. Following the same protocol as that used for the other glycosylation reactions, the structure of **39** was elucidated. Next, cleavage of the *p*-methoxybenzyl (PMB) group by TFA in CH₂Cl₂ at 0 °C provided **40** in 90%

yield. Finally, global deprotection under Zemplén conditions followed by saponification generated the target ganglioside **1** in good yield (Scheme 6).

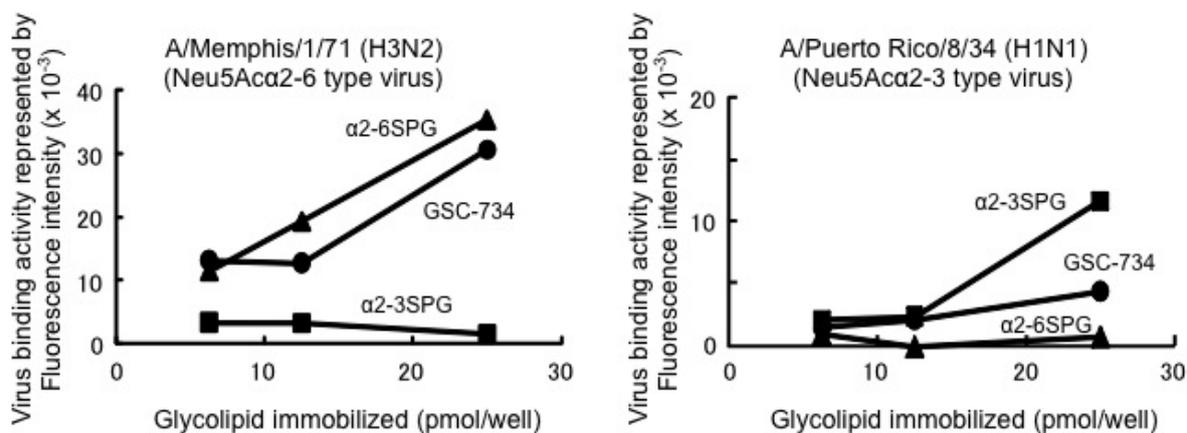
Scheme 6. Final coupling by the cassette approach followed by global deprotection.



2.2. Binding Assay

The synthesized ganglioside **1** (termed **GSC-734**) was then assessed for its binding activity to influenza viruses (Figure 3). The binding assay showed that GSC-734 was recognized by Neu5Ac α 2-3 and 2-6 type viruses. Moreover, the binding activity of the Neu5Ac α 2-6 type virus (H3N2) was almost identical to that of the previously reported α 2-6 sialylparagloboside [26]. This observation suggests that the branched structure of the sugar part does not potently influence its binding activity to the viruses.

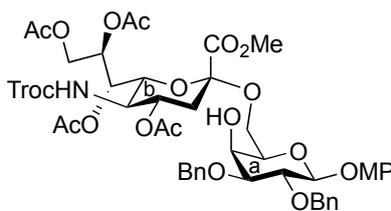
Figure 3. Binding activity of the synthetic ligand (**GSC-734**) for influenza A viruses.



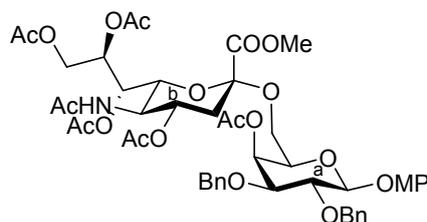
3. Experimental

3.1. General Methods for Chemical Synthesis

All reactions were carried out under a positive pressure of argon, unless otherwise noted. All chemicals were purchased from commercial suppliers and used without further purification, unless otherwise noted. Molecular sieves were purchased from Wako Chemicals Inc. (Osaka, Japan) and dried at 300 °C for 2 h in a 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 60F254 on glass plate, Darmstadt, Germany). Compound detection was either by exposure to UV light (2536 Å) or by soak in a solution of 10% H₂SO₄ in ethanol followed by heating. Silica gel (80 mesh and 300 mesh) manufactured by Fuji Silysia Co. (Kasugai, Japan) was used for flash column chromatography. Quantity of silica gel was usually estimated as 100 to 150-fold weight of sample to be charged. Solvent systems in chromatography were specified in *v/v*. Evaporation and concentration were carried out *in vacuo*. ¹H-NMR and ¹³C-NMR spectra were recorded with JEOL ECA 400/500/600 spectrometers. Chemical shifts in ¹H-NMR spectra are expressed in ppm (δ) relative to the signal of Me₄Si, adjusted to δ 0.00 ppm. Data are presented as follow: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = double of doublet, dt = double of triplet, m = multiplet and/or multiple resonances), integration, coupling constant in Hertz (Hz), position of the corresponding proton. COSY methods were used to confirm the NMR peak assignments. MALDI-TOF mass spectra were run in a Bruker Autoflex (Billerica, MA, USA) and CHCA was used as the matrix. High-resolution mass (ESI-TOF MS) spectra were run in a Bruker micrOTOF. Optical rotations were measured with a 'Horiba SEPA-300' high-sensitive polarimeter (Kyoto, Japan).

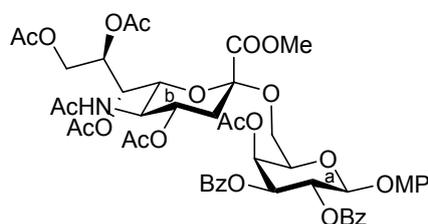


4-Methoxyphenyl (methyl 4,7,8,9-tetra-O-acetyl-3,5-dideoxy-5-(2,2,2-trichloroethoxycarbonyl)-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-2,3-di-O-benzyl- β -D-galactopyranoside (6). To a mixture of **2** (691 mg, 0.964 mmol) and **5** (300 mg, 0.643 mmol) in EtCN/CH₂Cl₂ (5:1, 9.6 mL) was added 3 Å molecular sieves (991 mg) at r.t. After stirring for 1 h and then cooling to -30 °C, NIS (324 mg, 1.44 mmol) and TfOH (12.7 μ L, 0.144 mmol) were added to the mixture. After stirring for 45 min at the same temperature as the reaction was monitored by TLC (1:3 EtOAc–toluene, twice development), the reaction was quenched by the addition of triethylamine. The precipitate was filtered through Celite. The filtrate was evaporated to remove EtCN and then diluted with CHCl₃, washed with satd aq Na₂S₂O₃ and brine. The organic layer was subsequently dried over Na₂SO₄, concentrated and the residue was purified by silica gel column chromatography (1:5 EtOAc–toluene) to give **6** (473 mg, 69%) along with its β -isomer (96 mg, 14%). $[\alpha]_D -13.9^\circ$ (c 0.4, CHCl₃); ¹H-NMR (500 MHz, CDCl₃) δ 7.38–6.80 (m, 14 H, Ar), 5.39 (m, 1 H, H-8b), 5.36 (dd, 1 H, $J_{6,7} = 1.7$ Hz, H-7b), 5.00 (d, 1 H, $J_{gem} = 10.2$ Hz, OCH₂), 4.99 (m, 1 H, H-4b), 4.89 (d, 1 H, $J_{gem} = 12.2$ Hz, OCH₂), 4.86 (d, 1 H, $J_{5,NH} = 9.7$ Hz, NH), 4.84 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1a), 4.83 (d, 1 H, $J_{gem} = 10.2$ Hz, OCH₂), 4.76 (2 d, 2 H, $J_{gem} = 12.0$ Hz, OCH₂), 4.47 (d, 1 H, $J_{gem} = 12.2$ Hz, OCH₂), 4.32 (dd, 1 H, H-9b), 4.19 (dd, 1 H, $J_{6,7} = 1.7$ Hz, H-6b), 4.10 (dd, 1 H, H-9'b), 4.07 (near d, 1 H, H-4a), 3.94–3.89 (m, 2 H, H-2a, H-5a), 3.81–3.75 (m, 7 H, H-6a, 2 OMe), 3.65–3.61 (m, 2 H, H-6'a, H-5b), 3.57 (dd, 1 H, H-3a), 2.67–2.63 (m, 2 H, H-3beq, OH), 2.12–1.99 (m, 12 H, 4 Ac), 1.91 (t, 1 H, H-3bax); ¹³C-NMR (100 MHz, CDCl₃) δ 193.2, 191.4, 170.7, 170.3, 170.1, 169.9, 167.9, 155.2, 154.0, 151.7, 138.4, 137.9, 129.0, 128.4, 128.3, 128.2, 128.1, 127.8, 127.7, 127.6, 125.3, 118.6, 114.4, 102.9, 98.7, 95.3, 80.6, 78.7, 77.2, 75.3, 74.5, 72.6, 72.3, 72.2, 68.6, 68.4, 67.5, 66.0, 63.1, 62.3, 55.6, 53.0, 51.6, 37.5, 29.7, 29.5, 21.4, 21.0, 20.8, 20.7. m/z (MALDI): found $[M+Na]^+$ 1094.32, C₄₈H₅₆Cl₃NO₂₀ calcd for $[M+Na]^+$ 1094.32.

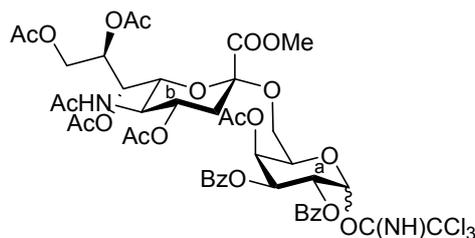


4-Methoxyphenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzyl- β -D-galactopyranoside (8). To a solution of **6** (3.77 g, 3.51 μ mol) in AcOH/CH₂Cl₂ (3:2, 70 mL) was added Zn/Cu couple (18.9 g) at r.t. The reaction mixture was heated to 40 °C and was stirred for 45 min at the same temperature as the reaction was monitored by TLC (4:1 toluene–EtOAc). The precipitate was filtered through Celite and the filtrate was co-evaporated with toluene. The obtained residue was exposed to high vacuum for 6 h. The crude residue was dissolved in pyridine (35 mL) and acetic anhydride (1.32 μ L, 14.0 mmol), DMAP (4.3 mg, 35.1 μ mol) were then added to the mixture at 0 °C. After stirring for 3 d at r.t as the reaction was monitored by TLC (2:1 toluene–EtOAc), the reaction mixture was evaporated. The

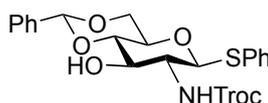
residue was diluted with CHCl_3 , washed with 2 M HCl, H_2O , satd aq NaHCO_3 and brine, dried over Na_2SO_4 , and concentrated. The obtained residue was purified by silica gel column chromatography (4:1 toluene–EtOAc) to give **8** (3.35 g, 97%). $[\alpha]_{\text{D}} -11.3^\circ$ (c 1.0, CHCl_3); $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 7.37–7.24 (m, 10 H, 2 Ph), 6.97 (2 d, 4 H, Ar), 5.95 (d, 1 H, $J_{\text{NH},5} = 6.3$ Hz, NH), 5.62 (d, 1 H, $J_{3,4} = 2.3$ Hz, H-4a), 5.41 (m, 1 H, H-8b), 5.32 (d, 1 H, H-7b), 4.96–4.81 (m, 5 H, H-1a, H-4b, 3OCH₂), 4.68 (d, 1 H, OCH₂), 4.34 (d, 1 H, H-9b), 4.14–4.05 (m, 3 H, H-5b, H-6b, H-9'b), 3.90–3.69 (m, 11 H, H-2a, H-3a, H-5a, H-6a, H-6'a, 2 OMe), 2.62 (m, 1 H, $J_{\text{gem}} = 11.5$ Hz, H-3beq), 2.16–1.87 (m, 19 H, 6 Ac, H-3bax); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 170.4, 170.2, 170.0, 169.8, 169.7, 169.5, 167.7, 154.9, 151.2, 138.2, 137.5, 128.7, 128.0, 127.9, 127.7, 127.3, 127.2, 118.0, 114.1, 102.2, 98.4, 79.1, 78.3, 75.0, 72.4, 71.8, 71.3, 68.7, 68.3, 67.0, 65.9, 62.7, 62.3, 55.2, 52.5, 48.6, 37.5, 22.7, 20.7, 20.5, 20.4, 20.4, 20.3. m/z (MALDI): found $[\text{M}+\text{Na}]^+$ 1004.17, $\text{C}_{49}\text{H}_{59}\text{NO}_{20}$ calcd for $[\text{M}+\text{Na}]^+$ 1004.35.



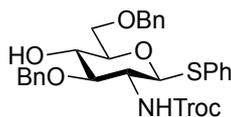
4-Methoxyphenyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl- β -D-galactopyranoside (10). To a solution of **8** (3.34 g, 3.41 μmol) in 1,4-dioxane (34 mL) was added $\text{Pd}(\text{OH})_2/\text{C}$ (3.34 g). After stirring for 45 min at r.t. under a hydrogen atmosphere as the reaction was monitored by TLC (15:1 CHCl_3 –MeOH), the mixture was filtered through Celite. The filtrate was concentrated and the obtained crude residue was roughly purified by silica gel column chromatography. The obtained product was exposed to high vacuum for 24 h. The residue was then dissolved in pyridine (34 mL). Benzoic anhydride (3.09 g, 13.6 mmol) and DMAP (20.8 mg, 0.171 μmol) were added to the mixture at 0 °C. After stirring for 9 h at r.t. as the reaction was monitored by TLC (15:1 CHCl_3 –MeOH), the reaction was quenched by the addition of MeOH at 0 °C. The mixture was co-evaporated with toluene and the residue was then diluted with CHCl_3 , and washed with 2 M HCl, H_2O , satd aq NaHCO_3 and brine. The organic layer was subsequently dried over Na_2SO_4 , and concentrated. The resulting residue was purified by silica gel column chromatography (1:1 toluene–EtOAc) to give **10** (3.22 g, 94%). $[\alpha]_{\text{D}} +40.3^\circ$ (c 1.0, CHCl_3); $^1\text{H-NMR}$ (500 MHz, CDCl_3) δ 8.00–7.37 (m, 10 H, 2 Ph), 7.06–6.76 (2 d, Ar), 5.91 (near t, 1 H, $J_{1,2} = 7.8$ Hz, $J_{2,3} = 10.6$ Hz, H-2a), 5.78 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4a), 5.60 (dd, 1 H, H-3a), 5.50 (m, 1 H, $J_{7,8} = 6.8$ Hz, H-8b), 5.33–5.27 (m, 2 H, H-1a, H-7b), 5.15 (d, 1 H, NH), 4.87 (m, 1 H, $J_{3\text{eq},4} = 4.6$ Hz, H-4b), 4.41 (dd, 1 H, $J_{\text{gem}} = 12.4$ Hz, H-9), 4.30 (near t, 1 H, $J_{5,6} = 6.9$ Hz, H-5a), 4.17–4.02 (m, 3 H, H-5b, H-6b, H-9'b), 3.89–3.73 (m, 7 H, 2 OMe, H-6a), 3.58 (near t, 1 H, $J_{\text{gem}} = 10.1$ Hz, H-6'a), 2.55 (dd, 1 H, $J_{\text{gem}} = 12.4$ Hz, H-3beq), 2.25–1.89 (m, 19 H, 6 Ac, H-3bax); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 170.5, 170.4, 170.0, 169.7, 169.5, 167.7, 165.1, 165.0, 155.1, 151.0, 133.0, 129.4, 129.3, 129.1, 128.8, 128.1, 128.1, 118.2, 114.1, 100.1, 98.9, 72.5, 71.7, 71.6, 69.4, 68.6, 67.9, 67.0, 62.9, 55.2, 52.7, 48.7, 37.6, 22.8, 20.8, 20.5, 20.4, 20.3. m/z (MALDI): found $[\text{M}+\text{Na}]^+$ 1032.21, $\text{C}_{49}\text{H}_{55}\text{NO}_{22}$ calcd for $[\text{M}+\text{Na}]^+$ 1032.31.



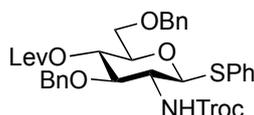
(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl-D-galactopyranosyl trichloroacetimidate (**12**). To a solution of **10** (3.22 g, 3.19 mmol) in MeCN/PhMe/H₂O (32 mL, 6:5:3) was added diammonium cerium (IV) nitrate (CAN; 17.5 g, 31.9 mmol) at r.t. The mixture was stirred for 2 h at r.t., as the proceeding of the reaction was monitored by TLC (10:1 CHCl₃–MeOH). The reaction mixture was diluted with CHCl₃ and washed with H₂O, satd aq NaHCO₃ and brine. The organic layer was subsequently dried over Na₂SO₄ and concentrated. The resulting residue was purified by silica gel column chromatography (70:1 CHCl₃–MeOH) to give **11**. The obtained hemiacetal compound **11** (2.27 g, 2.51 mmol) was dissolved in CH₂Cl₂ (50 mL). To the mixture was added CCl₃CN (2.5 mL, 25.1 mmol), DBU (449 μ L, 3.01 mmol) at 0 °C. After stirring for 6 h at 0 °C as the reaction was monitored by TLC (15:1 CHCl₃–MeOH), the reaction mixture was evaporated. The crude residue was purified by silica gel column chromatography (70:1 CHCl₃–MeOH) to give **12** (2.10 g, 63% over 2 steps). **12** α : ¹H-NMR (500 MHz, CDCl₃) δ 8.61 (s, 1 H, C=NH), 7.95–7.28 (m, 10 H, 2 Ph), 6.80 (d, 1 H, $J_{1,2}$ = 3.4 Hz, H-1a), 5.90–5.82 (m, 2 H, H-2a, H-3a), 5.79 (m, 1 H, $J_{3,4}$ = 2.8 Hz, H-4a), 5.38–5.30 (m, 3 H, H-7b, H-8b, NHb), 4.87–4.86 (m, 1 H, H-4b), 4.51 (br t, 1 H, H-5a), 4.28 (dd, 1 H, J_{gem} = 12.6 Hz, $J_{8,9}$ = 2.9 Hz, H-9b), 4.11 (dd, 1 H, $J_{8,9'}$ = 5.9 Hz, H-9'b), 4.06–3.97 (m, 3 H, H-6a, H-5b, H-6b), 3.78 (s, 3 H, COOMe), 3.42 (dd, 1 H, $J_{5,6'}$ = 2.6 Hz, J_{gem} = 10.6 Hz, H-6'a), 3.55 (dd, 1 H, J_{gem} = 11.6 Hz, $J_{3eq,4}$ = 3.6 Hz, H-3beq), 2.35–1.87 (m, 19 H, 6 Ac, H-3bax).



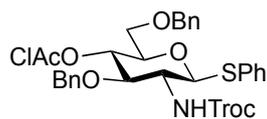
Phenyl 4,6-O-benzylidene-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl)- β -D-glucopyranoside (**14**). To a solution of **13** (8.43 g, 14.7 mmol) in MeOH (84 mL) was added NaOMe (28% solution in MeOH, 39.7 mg, 0.735 mmol) at 0 °C. After stirring for 2.5 h at room temperature as the reaction was monitored by TLC (10:1 CHCl₃–MeOH), the reaction was neutralized with Dowex (H⁺) resin. The resin was filtered through cotton and the filtrate was then evaporated. The residue was exposed to high vacuum for 12 h. The obtained crude mixture was then dissolved in THF/MeCN (1:4, 135 mL). To the mixture were added benzaldehyde dimethyl acetal (4.39 mL, 29.4 mmol) and (\pm)-camphor-10-sulfonic acid (CSA) (512 mg, 2.21 mmol) at 0 °C. After stirring for 1 h at room temperature as the reaction was monitored by TLC (20:1 CHCl₃–MeOH), the reaction was quenched by the addition of triethylamine. The reaction mixture was concentrated and then subjected to crystallization from hot acetone/*n*-hexane to give **14** (6.27 g, 80%) as a white crystal. The physical data of **14** was identical to those reported in the literature [27].



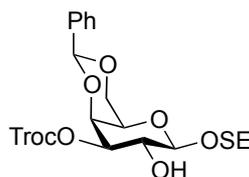
Phenyl 3,6-di-O-benzyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbamoyl)-β-D-glucopyranoside (15). To a solution of **14** (4.89 g, 9.15 mmol) in THF/toluene (1:4, 9.0 mL) was added TESOTf (4.1 mL, 18.3 mmol) at $-20\text{ }^{\circ}\text{C}$. After stirring for 45 min at $-20\text{ }^{\circ}\text{C}$, benzaldehyde (4.7 mL, 45.7 mmol) and triethylsilane (2.2 mL, 13.7 mmol) were added to the mixture. After stirring for 2 h at $-20\text{ }^{\circ}\text{C}$ as the reaction was monitored by TLC (1:4 EtOAc–toluene), the reaction was quenched by satd aq Na_2CO_3 . Dilution of the mixture with EtOAc provided a solution, which was then washed with satd aq Na_2CO_3 and brine. The organic layer was subsequently dried over Na_2SO_4 and concentrated. The obtained residue was exposed to high vacuum for 24 h. The resulting residue was dissolved in CH_2Cl_2 (183 mL) and cooled to $0\text{ }^{\circ}\text{C}$. $\text{BF}_3\cdot\text{OEt}_2$ (4.7 mL, 18.3 mmol) and triethylsilane (14.6 mL, 91.5 mmol) were added to the solution at $0\text{ }^{\circ}\text{C}$ and the mixture was then stirred for 1 h at $0\text{ }^{\circ}\text{C}$ as the reaction was monitored by TLC (1:4 EtOAc–toluene). The reaction was quenched by the addition of satd aq Na_2CO_3 at $0\text{ }^{\circ}\text{C}$ and then diluted with CHCl_3 , and washed with satd aq Na_2CO_3 and brine. The organic layer was subsequently dried over Na_2SO_4 and concentrated. The resulting residue was purified by silica gel column chromatography (200:1 CHCl_3 –MeOH) to give **15** (4.50 g, 78%). $[\alpha]_{\text{D}} -24.2^{\circ}$ (c 0.3, CHCl_3); $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.49–7.21 (m, 15 H, 3 Ph), 5.17 (d, 1 H, $J_{2,\text{NH}} = 8.2\text{ Hz}$, NH), 4.91 (d, 1 H, $J_{1,2} = 10.1\text{ Hz}$, H-1), 4.75 (s, 4 H, 2 OCH_2), 4.56 (2 d, 2 H, OCH_2), 3.77–3.67 (m, 4 H, H-3, H-5, H-6, H-6'), 3.52–3.42 (m, 2 H, H-2, H-4), 2.85 (s, 1 H, OH); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 153.8, 137.9, 137.7, 132.7, 132.3, 128.9, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 95.4, 86.0, 81.9, 78.0, 74.5, 74.4, 73.7, 72.6, 70.4, 56.0. m/z (MALDI): found $[\text{M}+\text{Na}]^+$ 648.05, $\text{C}_{29}\text{H}_{30}\text{Cl}_3\text{NO}_6\text{S}$ calcd for $[\text{M}+\text{Na}]^+$ 648.08.



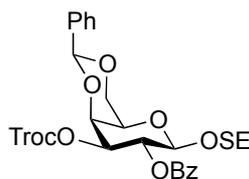
Phenyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl-1-thio-2-(2,2,2-trichloroethoxycarbamoyl)-β-D-glucopyranoside (16). To a solution of **15** (200 mg, 0.314 mmol) in CH_2Cl_2 (3.1 mL) were added levulinic acid (55 mg, 0.472 mmol), EDC·HCl (90 mg, 0.472 mmol), and DMAP (4.2 mg, 3.44 μmol). After stirring for 4 h at r.t. as the reaction was monitored by TLC (2:3 EtOAc–*n*-hexane), the reaction mixture was diluted with CHCl_3 , and washed with 2 M HCl and brine. The organic layer was subsequently dried over Na_2SO_4 , and concentrated, and the obtained residue was purified by silica gel column chromatography (1:4 EtOAc–*n*-hexane) to give **16** (215 mg, 93%). $[\alpha]_{\text{D}} +3.7^{\circ}$ (c 0.9, CHCl_3); $^1\text{H-NMR}$ (600 MHz, acetone- d_6) δ 7.53–7.24 m, 16 H, 3 Ph, NH, 5.06 (d, 1 H, $J_{1,2} = 10.3\text{ Hz}$, H-1), 5.03 (t, 1 H, $J_{3,4} = 9.6\text{ Hz}$, $J_{4,5} = 9.7\text{ Hz}$, H-4), 4.83 (2 d, 2 H, $J_{\text{gem}} = 12.4\text{ Hz}$, OCH_2), 4.72 (2 d, 2 H, $J_{\text{gem}} = 11.0\text{ Hz}$, OCH_2), 4.52 (2 d, 2 H, $J_{\text{gem}} = 11.7\text{ Hz}$, OCH_2), 3.99 (t, 1 H, $J_{2,3} = 9.7\text{ Hz}$, H-3), 3.79–3.75 (m, 2 H, H-2, H-5), 3.65 (dd, 1 H, $J_{\text{gem}} = 11.0\text{ Hz}$, $J_{5,6} = 2.8\text{ Hz}$, H-6), 3.65 (dd, 1 H, $J_{5,6'} = 6.9\text{ Hz}$, H-6'), 2.73–2.40 (m, 4 H, $\text{CH}_2\text{CH}_2\text{C}(=\text{O})\text{O}$), 2.09 (s, 3 H, $\text{C}(=\text{O})\text{CH}_3$); $^{13}\text{C-NMR}$ (150 MHz, CDCl_3) δ 166.1, 153.7, 137.6, 137.5, 132.6, 132.1, 129.0, 128.5, 128.3, 128.0, 128.0, 127.9, 127.7, 95.4, 85.2, 79.0, 76.8, 74.5, 74.4, 73.6, 73.2, 69.5, 56.7, 40.5. m/z (MALDI): found $[\text{M}+\text{Na}]^+$ 746.09, $\text{C}_{34}\text{H}_{36}\text{Cl}_3\text{NO}_8\text{S}$ calcd for $[\text{M}+\text{Na}]^+$ 746.11.



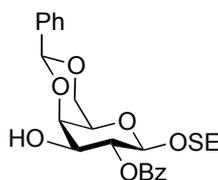
Phenyl 3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-1-thio-2-(2,2,2-trichloroethoxycarbonyl)- β -D-glucopyranoside (**17**). To a solution of **15** (200 mg, 0.314 mmol) in THF (3.1 mL) were added monochloroacetic anhydride (81 mg, 0.472 mmol) and DMAP (0.5 mg, 4.09 μ mol) at 0 °C. After stirring for 7 h at r.t. as the reaction was monitored by TLC (2:3 EtOAc-*n*-hexane), THF was evaporated and the residue was then diluted with CHCl₃, and washed with 2 M HCl, H₂O, satd aq NaHCO₃, and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The residue was purified by silica gel column chromatography (1:8 EtOAc-*n*-hexane) to give **17** (200 mg, 90%). $[\alpha]_D -2.6^\circ$ (c 1.0, CHCl₃); ¹H-NMR (600 MHz, CDCl₃) δ 7.51–7.21 (m, 15 H, 3 Ph), 5.37 (d, 1 H, $J_{NH,2} = 8.2$ Hz, NH), 5.12 (d, 1 H, $J_{1,2} = 10.3$ Hz, H-1), 5.07 (near t, 1 H, $J_{3,4} = 8.9$ Hz, $J_{4,5} = 9.6$ Hz, H-4), 4.75 (2 d, 2 H, $J_{gem} = 11.8$ Hz, OCH₂), 4.61 (2 d, 2 H, $J_{gem} = 11.7$ Hz, OCH₂), 4.48 (m, 2 H, $J_{gem} = 11.7$ Hz, OCH₂), 4.11 (near t, 1 H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 8.9$ Hz, H-3), 3.68–3.55 (m, 5 H, H-5, H-6, H-6', CH₂Cl), 3.36 (br dd, 1 H, $J_{2,3} = 9.6$ Hz, H-2); ¹³C-NMR (125 MHz, CDCl₃) δ 166.1, 153.7, 137.7, 137.6, 132.8, 132.0, 129.1, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 95.4, 85.2, 79.0, 77.6, 74.7, 74.5, 73.7, 73.4, 69.7, 56.9, 40.5. *m/z* (MALDI): found $[M+Na]^+$ 724.05, C₃₁H₃₁Cl₄NO₇S calcd for $[M+Na]^+$ 724.05.



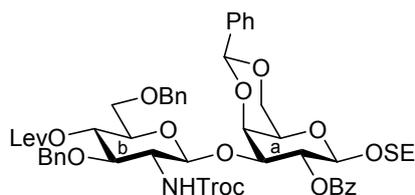
2-(Trimethylsilyl)ethyl 4,6-O-benzylidene-3-O-(2,2,2-trichloroethoxycarbonyl)- β -D-galactopyranoside (**19**). To a solution of **18** (49.5 mg, 0.134 mmol) in pyridine/CH₂Cl₂ (1:4, 1.3 mL) was added trichloroethyl chloroformate (20 μ L, 0.148 mmol) at -40 °C. After stirring for 3 h at the same temperature as the reaction was monitored by TLC (30:1 CHCl₃-MeOH), solvents were removed by co-evaporation with toluene, and then the residue was diluted with CHCl₃, washed with 2 M HCl, H₂O, satd aq NaHCO₃, and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The resulting residue was purified by silica gel column chromatography (1:5 EtOAc-*n*-hexane) to give **19** (59.1 mg, 81%). $[\alpha]_D +41.7^\circ$ (c 1.0, CHCl₃); ¹H-NMR (400 MHz, CDCl₃) δ 7.48–7.30 (m, 5 H, Ph), 5.49 (s, 1 H, PhCH<), 4.82–4.72 (m, 3 H, H-3, OCH₂CCL₃), 4.45 (d, 1 H, $J_{3,4} = 3.7$ Hz, H-4), 4.34 (d, 1 H, $J_{1,2} = 7.7$ Hz, H-1), 4.33 (d, 1 H, $J_{gem} = 13.8$ Hz, H-6), 4.08–4.01 (m, 3 H, H-2, H-6', OCH₂CH₂SiMe₃), 3.60–3.53 (m, 1 H, OCH₂CH₂SiMe₃), 3.48 (s, 1 H, H-5), 2.51 (br s, 1 H, OH), 1.03–0.97 (m, 2 H, OCH₂CH₂SiMe₃), 0.00 (s, 9 H, OCH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, CDCl₃) δ 153.6, 137.3, 129.0, 128.1, 126.2, 102.3, 100.9, 94.2, 78.2, 77.2, 76.9, 73.0, 69.0, 68.5, 67.5, 66.2, 18.1, -1.4. *m/z* (MALDI): found $[M+Na]^+$ 565.11, C₁₆H₁₆Cl₃O₇ calcd for $[M+Na]^+$ 565.06.



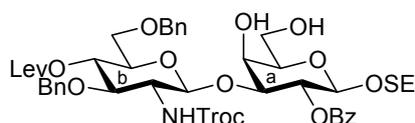
2-(Trimethylsilyl)ethyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,2,2-trichloroethoxycarbonyl)- β -D-galactopyranoside (**20**). To a solution of **19** (2.39 g, 4.39 mmol) in THF (1.3 mL) were added benzoic anhydride (1.49 g, 6.59 mmol) and DMAP (268 mg, 2.20 μ mol) at 0 °C. After stirring for 8.5 h at r.t. as the reaction was monitored by TLC (30:1 CHCl₃–MeOH), THF was evaporated and the residue was then diluted with CHCl₃, washed with 2 M HCl, H₂O, satd aq NaHCO₃, and brine. The organic layer was subsequently dried over Na₂SO₄, concentrated. The residue was purified by silica gel column chromatography (20:1 toluene–EtOAc) to give **20** (2.90 g, quant.). $[\alpha]_D^{+38.8^\circ}$ (c 1.2, CHCl₃); ¹H-NMR (400 MHz, CDCl₃) δ 8.11–7.43 (m, 10 H, 2 Ph), 5.79–7.75 (near t, 1 H, $J_{2,3} = 10.6$ Hz, $J_{1,2} = 7.8$ Hz, H-2), 5.64 (s, 1 H, PhCH<), 5.22 (dd, 1 H, $J_{3,4} = 3.7$ Hz, H-3), 4.81–4.78 (2 d, 2 H, OCH₂CCl₃, H-1), 4.68 (d, 1 H, $J_{\text{gem}} = 11.9$ Hz, OCH₂CCl₃), 4.58 (d, 1 H, H-4), 4.67–4.50 (dd, 1 H, $J_{\text{gem}} = 12.4$ Hz, $J_{5,6} = 1.8$ Hz, H-6), 4.23–4.19 (dd, 1 H, $J_{5,6'} = 1.8$ Hz, H-6'), 4.15–4.08 (m, 1 H, OCH₂CH₂SiMe₃), 3.68–3.62 (m, 2 H, H-5, OCH₂CH₂SiMe₃), 1.03–0.91 (m, 2 H, OCH₂CH₂SiMe₃), 0.00 (s, 9 H, OCH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, CDCl₃) δ 164.8, 153.7, 137.2, 133.1, 129.8, 129.7, 129.1, 128.3, 128.2, 126.4, 101.1, 100.5, 94.1, 76.8, 76.6, 73.4, 69.2, 68.9, 67.0, 66.2, 17.9, –1.5. *m/z* (MALDI): found $[M+Na]^+$ 669.09, C₂₈H₃₃Cl₃O₉Si calcd for $[M+Na]^+$ 669.09.



2-(Trimethylsilyl)ethyl 2-O-benzoyl-4,6-O-benzylidene- β -D-galactopyranoside (**21**). To a solution of **20** (2.18 g, 3.36 μ mol) in AcOH/CH₂Cl₂ (3:2, 33.6 mL) was added Zn/Cu couple (6.00 g) at r.t. The reaction mixture was stirred for 1 h at r.t. as the reaction was monitored by TLC (30:1 CHCl₃–MeOH). The precipitate was filtered through Celite and the filtrate was co-evaporated with toluene. The residue was diluted with CHCl₃ and washed with satd aq NaHCO₃ and brine, dried over Na₂SO₄, and concentrated. The obtained residue was purified by silica gel column chromatography (4:1 EtOAc–*n*-hexane) to give **21** (1.33 g, 84%). $[\alpha]_D^{-3.8^\circ}$ (c 1.1, CHCl₃); ¹H-NMR (400 MHz, CDCl₃) δ 8.15–7.45 (m, 10 H, 2 Ph), 5.65 (s, 1 H, PhCH<), 5.43 (near t, 1 H, $J_{2,3} = 10.3$ Hz, $J_{1,2} = 8.3$ Hz, H-2), 4.68 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1), 4.45 (d, 1 H, $J_{\text{gem}} = 12.3$ Hz, H-6), 4.33 (d, 1 H, $J_{3,4} = 4.2$ Hz, H-4), 4.18 (dd, 1 H, $J_{\text{gem}} = 12.4$ Hz, H-6'), 4.12 (m, 1 H, OCH₂CH₂SiMe₃), 3.97 (m, 1 H, $J_{3,\text{OH}} = 11.0$ Hz, $J_{2,3} = 10.3$ Hz, H-3), 3.68–3.60 (m, 2 H, H-5, OCH₂CH₂SiMe₃), 2.72 (d, 1 H, OH), 0.98 (m, 2 H, CH₂CH₂SiMe₃), 0.00 (s, 3 H, CH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, CDCl₃) δ 166.2, 137.4, 132.9, 130.0, 129.8, 129.2, 128.2, 126.4, 101.4, 100.3, 75.6, 72.9, 71.9, 69.0, 67.0, 66.5, 17.9, –1.5. *m/z* (MALDI): found $[M+Na]^+$ 495.12, C₂₅H₃₂O₇Si calcd for $[M+Na]^+$ 495.18.

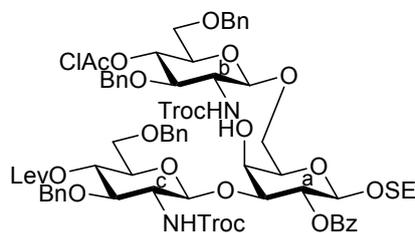


2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl-2-(2,2,2-trichloroethoxycarbamoyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-2-O-benzoyl-4,6-O-benzylidene- β -D-galactopyranoside (**22**). To a mixture of **16** (230 mg, 0.317 mmol) and **21** (100 mg, 0.212 mmol) in CH₂Cl₂ (3.2 mL) was added 4 Å molecular sieves AW-300 (400 mg) at r.t. After stirring for 1 h, the mixture was cooled to 0 °C. NIS (143 mg, 0.636 mmol) and TfOH (2.8 μ L, 31.7 μ mol) were then added to the mixture at 0 °C. After stirring for 2 h at the same temperature as the reaction was monitored by TLC (1:4 EtOAc–toluene), the reaction was quenched by the addition of triethylamine. The solution was diluted with CHCl₃ and filtered through Celite. The filtrate was then washed with satd aq Na₂S₂O₃ and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The resulting residue was purified by silica gel column chromatography (1:4 EtOAc–toluene, then 200:1 CHCl₃–MeOH) to give **22** (186 mg, 81%). $[\alpha]_D^{25} +17.4^\circ$ (c 0.8, CHCl₃); ¹H-NMR (600 MHz, CDCl₃) δ 8.07–7.13 (m, 20 H, 4 Ph), 5.55 (near t, 1 H, $J_{2,3} = 10.3$ Hz, $J_{1,2} = 7.6$ Hz, H-2a), 5.49 (s, 1 H, PhCH<), 5.26 (d, 1 H, $J_{NH,2} = 6.2$ Hz, NH), 5.08 (d, 1 H, $J_{1,2} = 7.6$ Hz, H-1b), 4.92 (near t, 1 H, $J_{3,4} = 9.9$ Hz, $J_{4,5} = 9.4$ Hz, H-4b), 4.53 (d, 1 H, H-1a), 4.55–4.51 (m, 2 H, 2 OCH₂), 4.46–4.41 (m, 3 H, 3 OCH₂), 4.40 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4a), 4.26 (d, 1 H, $J_{gem} = 12.4$ Hz, H-6'a), 4.15 (t, 1 H, $J_{3,4} = 9.9$ Hz, H-3b), 4.00–3.95 (m, 2 H, H-3a, OCH₂CH₂SiMe₃), 3.83 (d, 1 H, $J_{gem} = 12.4$ Hz, H-6a), 3.66 (br m, 1 H, H-5b), 3.55 (m, 3 H, H-6b, H-6'b, OCH₂CH₂SiMe₃), 3.35 (s, 1 H, H-5a), 3.28 (br d, 1 H, OCH₂), 3.17 (br dd, 1 H, H-2b), 2.61–2.36 (m, 4 H, CH₂CH₂C(=O)O), 2.10 (s, 3 H, C(=O)CH₃), 0.86–0.76 (m, 2 H, CH₂CH₂SiMe₃), –0.12 (m, 9 H, CH₂CH₂SiMe₃); ¹³C-NMR (125 MHz, CDCl₃) δ 206.2, 171.6, 164.9, 153.4, 138.1, 138.0, 137.8, 133.0, 130.2, 129.9, 128.9, 128.4, 128.3, 128.3, 128.1, 127.7, 127.6, 127.6, 127.5, 126.5, 101.1, 100.8, 100.2, 95.4, 78.7, 77.5, 76.2, 74.2, 73.4, 73.3, 73.2, 71.6, 70.5, 69.9, 68.8, 66.7, 66.6, 58.2, 37.7, 29.7, 27.8, 17.9, –1.6. *m/z* (MALDI): found $[M+Na]^+$ 1108.51, C₅₃H₆₂Cl₃NO₁₅Si calcd for $[M+Na]^+$ 1108.29.

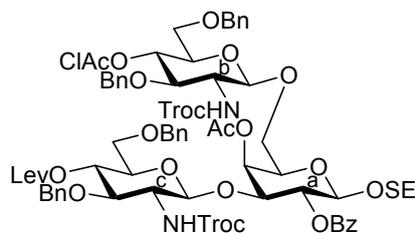


2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl-2-(2,2,2-trichloroethoxycarbamoyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-2-O-benzoyl- β -D-galactopyranoside (**23**). Compound **22** (42 mg, 38.3 μ mol) was dissolved in 80% AcOH aq (1.5 mL) and the solution was stirred for 4 h at 50 °C as the reaction was monitored by TLC (15:1 CHCl₃–MeOH). After the reaction mixture was co-evaporated with toluene, the crude residue was purified by silica gel column chromatography (50:1 CHCl₃–MeOH) to give **23** (38 mg, 99%). $[\alpha]_D^{25} -1.43^\circ$ (c 0.7, CHCl₃); ¹H-NMR (400 MHz, acetone-*d*₆) δ 8.34–7.32 (m, 15 H, 3 Ph), 7.03 (d, 1 H, $J_{NH,2} = 9.2$ Hz, NH), 5.60 (near t, 1 H, $J_{2,3} = 9.7$ Hz, $J_{1,2} = 8.0$ Hz, H-2a), 5.13 (d, 1 H, $J_{1,2} = 8.6$ Hz, H-1b), 5.09 (near t, $J_{3,4} = 9.9$ Hz, $J_{4,5} = 8.3$ Hz, H-4b), 4.77–4.64 (m, 5 H, H-1a, 4 OCH₂), 4.48 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4a), 4.51 (d, 1 H, OCH₂), 4.23 (dd, 1 H, H-3a), 4.13–4.08 (m, 2 H, H-3b, OCH₂), 3.96–3.63 (m, 9 H, 2 OCH₂, H-2b, H-5b, H-6b, H-6'b, H-5a, H-6a, H-6'a), 2.98 (s, 2 H, 2 OH), 2.80–2.49 (m, 4 H, CH₂CH₂C(=O)O), 2.20 (s, 3 H, C(=O)CH₃), 1.01–0.83 (m, 2 H, CH₂CH₂SiMe₃), 0.00 (s, 9 H, CH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, acetone-*d*₆) δ 206.5, 172.5, 165.6,

154.8, 139.5, 133.7, 131.7, 130.7, 129.2, 129.0, 128.8, 128.4, 128.3, 128.2, 128.0, 102.7, 101.7, 96.8, 82.4, 80.2, 75.9, 74.6, 74.1, 74.0, 73.9, 72.1, 72.0, 70.4, 69.5, 67.0, 62.6, 58.3, 38.1, 29.6, 28.6, 18.6, -1.4. m/z (MALDI): found $[M+Na]^+$ 1020.26, $C_{46}H_{58}Cl_3NO_{15}Si$ calcd for $[M+Na]^+$ 1020.25.

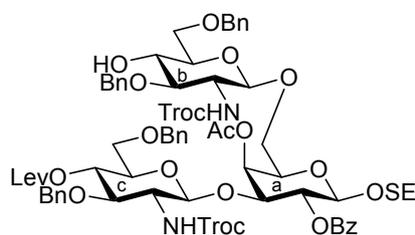


2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl-2-(2,2,2-trichloroethoxycarbonyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-[3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)- β -D-glucopyranosyl-(1 \rightarrow 6)]-2-O-benzoyl- β -D-galactopyranoside (**24**). To a mixture of **17** (694 mg, 0.987 mmol) and **23** (658 mg, 0.658 mmol) in CH_2Cl_2 (6.6 mL) was added 4 Å molecular sieves (1.40 g) at r.t. After stirring for 1 h, the mixture was cooled to 0 °C. NIS (296 mg, 1.32 mmol) and TfOH (8.7 μ L, 98.7 μ mol) were then added to the mixture at 0 °C. After stirring for 2 h at r.t. as the reaction was monitored by TLC (30:1 $CHCl_3$ -MeOH), the reaction was quenched by the addition of triethylamine. The solution was diluted with $CHCl_3$ and filtered through Celite. The filtrate was then washed with satd aq $Na_2S_2O_3$ and brine. The organic layer was subsequently dried over Na_2SO_4 , and concentrated. The residue was purified by silica gel column chromatography (100:1 $CHCl_3$ -MeOH) to give **24** (511 mg, 49%). $[\alpha]_D -19.4^\circ$ (c 1.8, $CHCl_3$); 1H -NMR (400 MHz, $DMSO-d_6$) δ 8.20–7.32 (m, 27 H, 5 Ph, NHb, NHc), 5.38 (t, 1 H, $J_{2,3} = 8.3$ Hz, $J_{1,2} = 8.7$ Hz, H-2a), 5.14 (t, 1 H, $J_{4,5} = 9.6$ Hz, H-4c), 5.06 (d, 1 H, $J_{gem} = 12.4$ Hz, OCH_2), 5.01 (t, 1 H, $J_{3,4} = 9.6$ Hz, $J_{4,5} = 9.1$ Hz, H-4b), 4.90 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1c), 4.75 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1b), 4.66 (d, 1 H, H-1a), 4.91–4.61 (m, 10 H, OH, 9 OCH_2), 4.49 (2 d, 2 H, OCH_2), 4.24 (br s, 1 H, H-4a), 4.04–3.52 (m, 18 H, H-3a, H-5a, H-6a, H-6'a, H-2b, H-3b, H-5b, H-6b, H-6'b, H-2c, H-3c, H-5c, H-6c, H-6'c, $OCH_2CH_2SiMe_3$, CH_2Cl), 2.83–2.80 (m, 2 H, $CH_2CH_2C(=O)O$), 2.56 (m, 2 H, $CH_2CH_2C(=O)O$), 2.26 (s, 3 H, $C(=O)CH_3$), 0.93–0.78 (m, 2 H, $CH_2CH_2SiMe_3$), 0.00 (s, 9 H, $CH_2CH_2SiMe_3$); ^{13}C -NMR (100 MHz, $DMSO-d_6$) δ 206.8, 171.5, 166.5, 164.7, 154.4, 153.8, 138.4, 138.3, 138.2, 133.2, 130.3, 129.6, 128.6, 128.4, 128.3, 128.2, 127.7, 127.7, 127.6, 101.9, 101.1, 100.2, 96.4, 96.0, 80.7, 79.4, 78.9, 73.7, 73.6, 73.3, 72.8, 72.7, 72.6, 72.5, 72.2, 72.0, 70.9, 70.7, 69.6, 69.0, 68.7, 68.6, 66.1, 57.0, 41.1, 37.4, 29.7, 27.8, 17.6, -1.3. m/z (MALDI): found $[M+Na]^+$ 1611.27, $C_{71}H_{83}Cl_7N_2O_{22}Si$ calcd for $[M+Na]^+$ 1611.29.

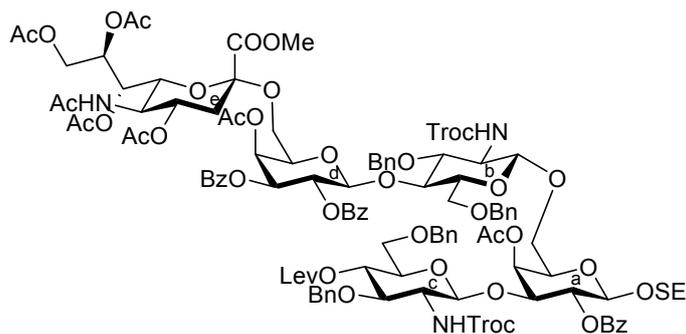


2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl-2-(2,2,2-trichloroethoxycarbonyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-[3,6-di-O-benzyl-4-O-chloroacetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)- β -D-glucopyranosyl-(1 \rightarrow 6)]-4-O-acetyl-2-O-benzoyl- β -D-galactopyranoside (**27**). To a solution of **24** (759 mg, 0.476 mmol) in THF (4.8 mL) were added acetic anhydride (94 μ L, 0.956 mmol) and DMAP (5.8 mg, 47.6 μ mol) at 0 °C. The reaction mixture was stirred for 1.5 h at r.t. as the reaction was

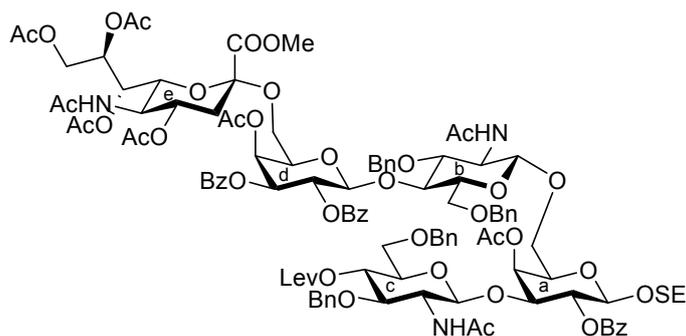
monitored by TLC (15:1 CHCl₃–MeOH). After THF was evaporated, the obtained crude residue was purified by silica gel column chromatography (100:1 CHCl₃–MeOH) to give **27** (745 mg, 96%). [α]_D –5.5° (c 1.8, CHCl₃); ¹H-NMR (400 MHz, DMSO-*d*₆) δ 8.20–7.32 (m, 27 H, 5 Ph, NHb, NHc), 5.58 (br s, 1 H, H-4a), 5.34 (near t, 1 H, $J_{2,3} = 9.7$ Hz, $J_{1,2} = 8.2$ Hz, H-2a), 5.16–5.07 (m, 3 H, H-4c, H-4b, OCH₂), 4.86–4.61 (m, 10 H, H-1b, H-1c, H-1a, 7 OCH₂), 4.58–4.39 (m, 3 H, 3 OCH₂), 4.31 (br dd, 1 H, H-3a), 4.18 (br dd, 1 H, H-6a), 4.06–3.48 (m, 17 H, H-5a, H-6'a, H-2b, H-3b, H-5b, H-6b, H-6'b, H-2c, H-3c, H-5c, H-6c, H-6'c, OCH₂, OCH₂CH₂SiMe₃, CH₂Cl), 2.80 (m, 2 H, CH₂CH₂C(=O)O), 2.54 (m, 2 H, CH₂CH₂C(=O)O), 2.25–2.22 (2 s, 6 H, 2 C(=O)CH₃), 0.96–0.79 (m, 2 H, CH₂CH₂SiMe₃), 0.00 (s, 9 H, CH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, DMSO-*d*₆) δ 206.7, 179.5, 171.3, 170.0, 166.5, 164.5, 154.4, 153.8, 138.5, 138.3, 138.3, 138.2, 133.3, 130.1, 129.6, 128.7, 128.4, 128.3, 128.3, 128.1, 127.7, 127.6, 127.6, 127.5, 127.2, 101.3, 100.9, 100.0, 96.3, 96.0, 79.3, 79.0, 77.6, 73.6, 73.1, 72.7, 72.4, 72.3, 72.0, 71.1, 70.3, 70.2, 69.0, 68.8, 68.4, 66.4, 57.0, 41.1, 37.4, 29.7, 27.8, 20.8, 17.6, –1.3. *m/z* (MALDI): found [M+Na]⁺ 1653.57, C₇₃H₈₅Cl₇N₂O₂₃Si calcd for [M+Na]⁺ 1653.30.



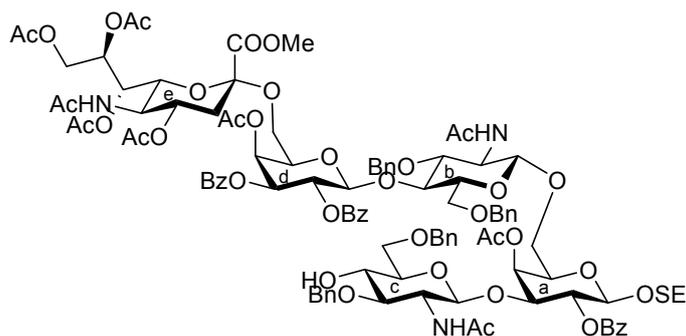
2-(Trimethylsilyl)ethyl 3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl-2-(2,2,2-trichloroethoxycarbamoyl)- β -D-glucopyranosyl-(1 \rightarrow 3)-[3,6-di-O-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbamoyl)- β -D-galactopyranosyl-(1 \rightarrow 6)]-4-O-acetyl-2-O-benzoyl- β -D-galactopyranoside (**28**). To a solution of **27** (712 mg, 0.435 mmol) in EtOH (4.4 mL) was added DABCO (732 mg, 6.52 mmol) at 0 °C. After stirring for 1 h at 50 °C as the reaction was monitored by TLC (30:1 CHCl₃–MeOH), EtOH was evaporated. The obtained crude residue was purified by silica gel column chromatography (100:1 CHCl₃–MeOH) to give **28** (665 mg, 98%). [α]_D –1.8° (c 1.5, CHCl₃); ¹H-NMR (500 MHz, CDCl₃) δ 8.19–7.27 (m, 25 H, 5 Ph), 5.63 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4a), 5.52 (near t, 1 H, $J_{2,3} = 10.3$ Hz, $J_{1,2} = 8.0$ Hz, H-2a), 5.32 (br d, 1 H, NHb), 5.18 (d, 1 H, $J_{NH,2} = 6.9$ Hz, NHc), 5.07 (d, 1 H, $J_{1,2} = 9.2$ Hz, H-1c), 5.05 (near t, 1 H, $J_{3,4} = 9.2$ Hz, H-4c), 4.85 (d, 1 H, $J_{1,2} = 8.6$ Hz, H-1b), 4.91–4.80 (m, 3 H, 3 OCH₂), 4.71 (d, 1 H, OCH₂), 4.63 (d, 1 H, H-1a), 4.69–4.59 (m, 6 H, 6 OCH₂), 4.50 (d, 1 H, OCH₂), 4.16 (br t, 1 H, $J_{2,3} = 10.3$ Hz, H-3c), 4.09 (m, 1 H, OCH₂CH₂SiMe₃), 4.07 (dd, 1 H, H-3a), 4.01 (dd, 1 H, $J_{5,6} = 4.1$ Hz, $J_{gem} = 12.8$ Hz, H-6a), 3.89–3.56 (m, 11 H, H-5a, H-6'a, H-3b, H-5b, H-6b, H-6'b, H-5c, H-6c, H-6'c, OCH₂, OCH₂CH₂SiMe₃), 3.44 (br dd, 1 H, H-2b), 3.23 (br dd, 1 H, H-2c), 2.96 (s, 1 H, OH), 2.72–2.37 (m, 4 H, CH₂CH₂C(=O)O), 2.22 (m, 6 H, 2 C(=O)CH₃), 1.01–0.86 (m, 2 H, CH₂CH₂SiMe₃), 0.00 (s, 9 H, CH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, CDCl₃) δ 206.1, 171.4, 169.9, 164.8, 154.0, 153.3, 138.2, 138.1, 137.7, 137.5, 133.2, 129.9, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 100.7, 100.2, 95.6, 95.4, 80.5, 77.5, 74.3, 74.3, 73.9, 73.7, 73.4, 73.4, 73.2, 73.1, 71.5, 71.4, 70.5, 70.1, 69.6, 67.4, 57.9, 57.3, 37.6, 29.6, 27.8, 20.8, 17.9, –1.5. *m/z* (MALDI): found [M+Na]⁺ 1577.28, C₇₁H₈₄Cl₆N₂O₂₂Si calcd for [M+Na]⁺ 1577.33.



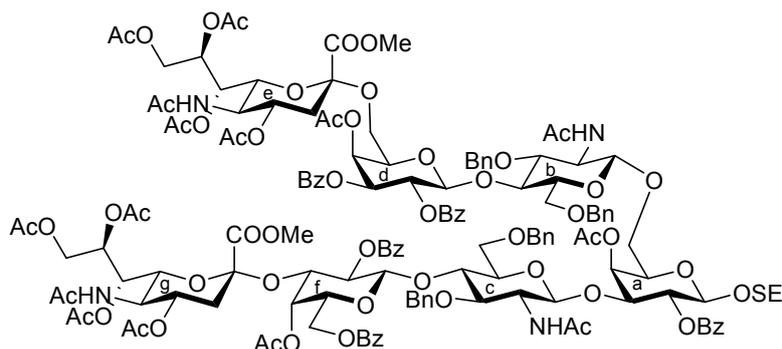
2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-*O*-acetyl-2,3-di-*O*-benzoyl- β -*D*-galactopyranosyl-(1 \rightarrow 4)-3,6-di-*O*-benzyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonyl)- β -*D*-glucopyranosyl-(1 \rightarrow 6)-[3,6-di-*O*-benzyl-2-deoxy-4-*O*-levulinoyl-2-(2,2,2-trichloroethoxycarbonyl)- β -*D*-glucopyranosyl-(1 \rightarrow 3)]-4-*O*-acetyl-2-*O*-benzoyl- β -*D*-galactopyranoside (**29**). To a mixture of **12** (80 mg, 76.5 μ mol) and **28** (70 mg, 45.0 μ mol) in CH₂Cl₂ (0.9 mL) was added 3 Å molecular sieves (250 mg) at r.t. After stirring for 1 h, the mixture was cooled to 0 °C. TMSOTf (1.4 μ L, 7.65 μ mol) was then added to the mixture at 0 °C. After stirring for 25 h at r.t., TMSOTf (1.4 μ L, 7.65 μ mol) was added to the mixture. After the stirring was continued for 3 h at r.t. as the reaction was monitored by TLC (30:1 CHCl₃–MeOH), the reaction was quenched by the addition of triethylamine. The mixture was diluted with CHCl₃ and filtered through Celite. The filtrate was then washed with satd aq NaHCO₃ and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The resulting residue was purified by silica gel column chromatography (90:1 CHCl₃–MeOH) followed by gel filtration column chromatography (LH-20) using MeOH as eluent, giving **29** (81 mg, 74%). [α]_D –2.9° (c 1.4, CHCl₃); ¹H-NMR (600 MHz, CDCl₃) δ 8.05–7.14 (m, 35 H, 7 Ph), 5.61 (d, 1 H, $J_{3,4}$ = 3.3 Hz, H-4d), 5.59 (near t, 1 H, $J_{1,2}$ = 8.3 Hz, H-2d), 5.45 (d, 1 H, $J_{3,4}$ = 3.4 Hz, H-4a), 5.40–5.36 (m, 1 H, H-8e), 5.36 (t, 1 H, $J_{1,2}$ = 8.2 Hz, H-2a), 5.34 (m, 1 H, H-7e), 5.30 (dd, 1 H, $J_{2,3}$ = 10.2 Hz, H-3d), 5.23 (2 br d, 2 H, NHc, NHb), 5.02 (d, 1 H, $J_{2,NH}$ = 6.9 Hz, NHc), 5.12 (d, 1 H, J_{gem} = 11.0 Hz, OCH₂), 4.94 (d, 1 H, H-1d), 4.94–4.86 (m, 2 H, H-1c, H-4c), 4.84 (m, 1 H, $J_{3eq,4}$ = 4.2 Hz, H-4e), 4.73–4.68 (m, 3 H, 3 OCH₂), 4.58–4.30 (m, 10 H, H-1a, H-1b, H-9e, 7 OCH₂), 4.14–3.71 (m, 15 H, H-3a, H-6'a, H-3b, H-4b, H-3c, H-4c, H-5d, H-6d, H-6'd, H-5e, H-6e, OCH₂, OCH₃), 3.58–3.37 (m, 10 H, H-6a, H-2b, H-5b, H-6b, H-6'b, H-5c, H-6c, H-6'c, 2 OCH₂), 3.28–3.26 (m, 1 H, H-5a), 3.08 (br dd, 1 H, H-2c), 2.60–2.51 (m, 3 H, H-3eeq, CH₂CH₂C(=O)O), 2.38–2.24 (m, 2 H, CH₂CH₂C(=O)O), 2.16–1.91 (m, 25 H, 8 C(=O)CH₃, H-3eax), 0.85–0.69 (m, 2 H, CH₂CH₂SiMe₃), –0.14 (s, 9 H, CH₂CH₂SiMe₃); ¹³C-NMR (100 MHz, CDCl₃) δ 206.0, 171.4, 170.9, 170.7, 170.3, 170.1, 169.8, 169.6, 167.8, 165.3, 165.2, 164.8, 153.9, 153.3, 138.6, 138.1, 138.0, 137.7, 133.3, 133.1, 129.8, 129.6, 129.5, 129.1, 128.5, 128.4, 128.4, 128.3, 128.3, 128.2, 127.9, 127.8, 127.7, 127.6, 127.6, 127.4, 100.6, 100.1, 99.8, 99.0, 95.4, 77.4, 76.8, 76.1, 74.4, 74.3, 73.9, 73.7, 73.3, 73.3, 73.1, 72.8, 71.7, 71.5, 71.4, 70.2, 70.1, 69.6, 68.7, 68.4, 68.1, 67.3, 62.4, 57.9, 57.0, 52.9, 49.4, 37.7, 37.6, 29.6, 27.8, 23.1, 21.0, 20.8, 20.5, 17.8, –1.5. *m/z* (MALDI): found [M+Na]⁺ 2463.09, C₁₁₃H₁₃₁Cl₆N₃O₄₂Si calcd for [M+Na]⁺ 2462.60.



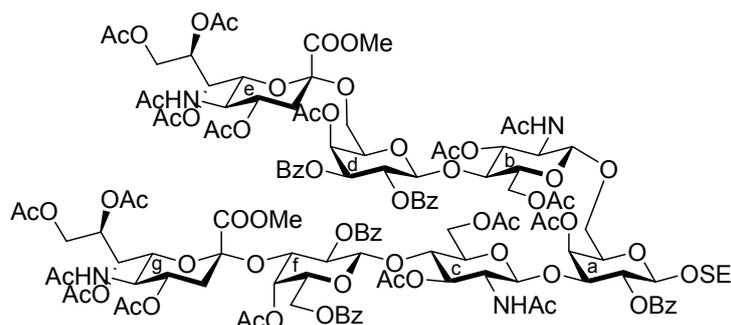
2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)-[2-acetamido-3,6-di-O-benzyl-2-deoxy-4-O-levulinoyl- β -D-glucopyranosyl-(1 \rightarrow 3)]-4-O-acetyl-2-O-benzoyl- β -D-galactopyranoside (**31**). To a solution of **29** (220 mg, 89.9 μ mol) in CH₂Cl₂/AcOH (2:3, 3.5 mL) was added Zn/Cu couple (2.20 g) at r.t. The reaction mixture was heated to 50 °C and was stirred for 2.5 h at the same temperature as the reaction was monitored by TLC (15:1 CHCl₃-MeOH). The precipitate was filtered through Celite and the filtrate was co-evaporated with toluene. The obtained residue was exposed to high vacuum for 6 h. The crude residue was dissolved in pyridine (3.6 mL) and acetic anhydride (68 μ L, 0.719 mmol), DMAP (2.5 mg, 47.6 μ mol) were then added to the mixture at 0 °C. After stirring for 12 h at r.t as the reaction was monitored by TLC (15:1 CHCl₃-MeOH), the reaction mixture was evaporated. The obtained residue was diluted with CHCl₃ and washed with 2 M HCl, satd aq NaHCO₃, and brine, dried over Na₂SO₄, and concentrated. The resulting residue was purified by silica gel column chromatography (40:1 CHCl₃-MeOH) to give **31** (120 mg, 61%). $[\alpha]_D -1.9^\circ$ (c 0.5, CHCl₃); ¹H-NMR (600 MHz, CDCl₃) δ 8.02–7.13 (m, 35 H, 7 Ph), 5.93 (d, 1 H, $J_{2,NH} = 8.3$ Hz, NHb), 5.63 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4d), 5.56 (near t, 1 H, $J_{1,2} = 7.5$ Hz, $J_{2,3} = 8.2$ Hz, H-2d), 5.41–5.31 (m, 5 H, H-2a, H-4a, H-3d, H-7e, H-8e), 5.26 (d, 1 H, $J_{2,NH} = 7.6$ Hz, NHc), 5.13 (d, 1 H, $J_{5,NH} = 7.5$ Hz, NHe), 4.95 (d, 1 H, $J_{1,2} = 8.2$ Hz, H-1c), 4.90–4.83 (m, 4 H, H-4c, H-1d, H-4e, OCH₂), 4.70 (d, 1 H, $J_{gem} = 11.7$ Hz, OCH₂), 4.58 (d, 1 H, $J_{1,2} = 6.9$ Hz, H-1c), 4.44 (d, 1 H, $J_{1,2} = 8.3$ Hz, H-1a), 4.54–4.31 (m, 7 H, H-9e, 3 OCH₂), 4.13 (t, 1 H, $J_{2,3} = 9.6$ Hz, H-3c), 4.10–4.02 (m, 4 H, H-6a, H-4b, H-5e, H-9e), 3.97–3.75 (m, 9 H, H-3a, H-6'a, H-3b, H-6d, H-6e, OCH₂CH₂SiMe₃, OCH₃), 3.67–3.35 (m, 11 H, H-5a, H-2b, H-5b, H-6b, H-6'b, H-5c, H-6c, H-6'c, H-5d, H-6'd, OCH₂CH₂SiMe₃), 3.07 (dd, 1 H, H-2c), 2.54–2.51 (m, 3 H, H-3eeq, CH₂CH₂C(=O)O), 2.35–2.27 (m, 2 H, CH₂CH₂C(=O)O), 2.15–1.89 (m, 28 H, 9 C(=O)CH₃, H-3eax), 0.88–0.69 (m, 2 H, OCH₂CH₂SiMe₃), -0.16 (s, 9 H, OCH₂CH₂SiMe₃); ¹³C (125 MHz, CDCl₃) δ 206.1, 171.5, 170.9, 170.7, 170.5, 170.3, 170.1, 169.9, 169.7, 167.8, 165.3, 165.1, 138.9, 138.2, 138.1, 133.4, 133.3, 133.2, 129.8, 129.7, 129.6, 129.1, 128.6, 128.5, 128.5, 128.4, 128.3, 127.9, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 100.6, 100.2, 99.9, 99.7, 99.0, 78.0, 77.4, 74.6, 73.6, 73.4, 73.3, 73.2, 73.1, 72.7, 71.8, 71.8, 71.6, 71.5, 70.2, 70.1, 69.7, 68.8, 68.8, 68.1, 67.6, 67.4, 67.2, 62.7, 62.5, 57.6, 52.9, 49.5, 37.8, 37.7, 29.7, 27.9, 23.4, 23.2, 22.7, 21.0, 20.8, 20.7, 20.6, 17.8, -1.5. *m/z* (MALDI): found $[M+Na]^+$ 2198.83, C₁₁₁H₁₃₃N₃O₄₀Si calcd for $[M+Na]^+$ 2198.81.



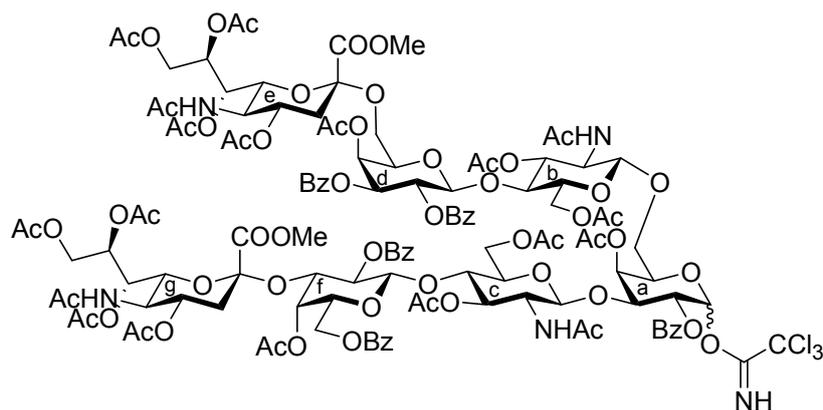
2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)-[2-acetamido-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)]-4-O-acetyl-2-O-benzoyl- β -D-galactopyranoside (**32**). To a solution of **31** (119 mg, 54.7 μ mol) in THF (2.0 mL) was added hydrazine monoacetate (4.9 mg, 54.7 μ mol) at 0 $^{\circ}$ C. After stirring for 1 h at r.t. as the reaction was monitored by TLC (30:1 CHCl₃–MeOH), THF was evaporated. The residue was diluted with CHCl₃ and washed with 2 M HCl, satd aq NaHCO₃, and brine, dried over Na₂SO₄, and concentrated. The obtained residue was purified by silica gel column chromatography (40:1 CHCl₃–MeOH) to give **32** (104 mg, 92%). $[\alpha]_D -5.2^{\circ}$ (c 1.9, CHCl₃); ¹H-NMR (500 MHz, CDCl₃) δ 8.02–7.20 (m, 35 H, 7 Ph), 6.01 (d, 1 H, $J_{\text{NH},2} = 8.6$ Hz, NHb), 5.64 (d, 1 H, $J_{3,4} = 3.5$ Hz, H-4d), 5.58 (near t, 1 H, $J_{1,2} = 8.0$ Hz, $J_{2,3} = 10.3$ Hz, H-2d), 5.43–5.32 (m, 6 H, H-2a, H-4a, H-3d, H-7e, H-8e, NHc), 5.21 (d, 1 H, $J_{\text{NH},5} = 7.4$ Hz, NHe), 4.89–4.81 (m, 3 H, H-1d, H-4e, OCH₂), 4.82 (d, 1 H, $J_{1,2} = 9.7$ Hz, H-1c), 4.72–4.65 (2 d, 2 H, $J_{\text{gem}} = 11.5$ Hz, 2 OCH₂), 4.60–4.45 (m, 5 H, H-1b, 4 OCH₂), 4.44 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1a), 4.37–4.32 (m, 2 H, OCH₂, H-9e), 4.12–4.04 (m, 4 H, H-6a, H-6d, H-6e, H-9'e), 3.98–3.62 (m, 14 H, H-3a, H-5a, H-6'a, H-2b, H-3b, H-6b, H-6'b, H-3c, H-5d, H-6'd, OCH₃, OCH₂CH₂SiMe₃), 3.54 (t, 1 H, $J_{3,4} = J_{4,5} = 12.0$ Hz, H-4c), 3.49–3.36 (m, 6 H, H-5c, H-6c, H-6'c, H-4b, H-5b, OCH₂CH₂SiMe₃), 3.16 (dd, 1 H, H-2c), 2.96 (s, 1 H, OH), 2.53 (dd, 1 H, $J_{\text{gem}} = 12.4$ Hz, $J_{3\text{eq},4} = 4.6$ Hz, H-3eeq), 2.18–1.87 (m, 25 H, 8 C(=O)CH₃, H-3eax), 0.88–0.69 (m, 2 H, OCH₂CH₂SiMe₃), -0.16 (s, 9 H, OCH₂CH₂SiMe₃); ¹³C-NMR (125 MHz, CDCl₃) δ 170.8, 170.6, 170.4, 170.2, 170.1, 169.9, 169.6, 169.5, 167.7, 165.6, 165.2, 165.0, 138.7, 138.4, 138.0, 137.7, 133.4, 133.2, 133.1, 129.7, 129.7, 129.6, 129.5, 129.0, 128.4, 128.4, 128.3, 128.3, 128.2, 127.8, 127.8, 127.7, 127.6, 127.5, 127.3, 100.4, 100.2, 100.1, 99.6, 98.9, 79.8, 77.9, 75.2, 74.5, 73.8, 73.5, 73.5, 73.2, 73.1, 73.0, 72.8, 72.6, 71.7, 71.6, 71.4, 70.7, 70.1, 70.0, 68.7, 68.1, 67.5, 67.3, 67.2, 62.5, 62.4, 56.4, 54.0, 52.8, 49.2, 37.7, 29.6, 23.3, 23.1, 22.8, 20.9, 20.7, 20.6, 20.5, 17.7, -1.6 . *m/z* (MALDI): found $[\text{M}+\text{Na}]^+$ 2100.96, C₁₀₆H₁₂₇N₃O₃₈Si calcd for $[\text{M}+\text{Na}]^+$ 2100.78.



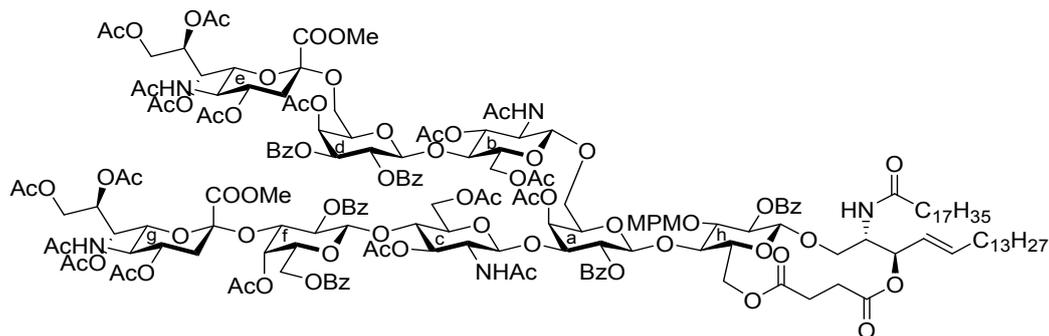
2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 3)-4-O-acetyl-2,6-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)]-4-O-acetyl-2-O-benzoyl- β -D-galactopyranoside (**33**). To a mixture of **4** (103 mg, 98.7 μ mol) and **32** (93 mg, 44.5 μ mol) in CH₂Cl₂ (1.8 mL) was added 3 Å molecular sieves (300 mg) at r.t. After stirring for 1 h, the mixture was cooled to 0 °C. TMSOTf (1.8 μ L, 9.94 μ mol) was then added to the mixture at 0 °C. The reaction was stirred for 2 h at r.t. as the reaction was monitored by TLC (15:1:1 CHCl₃–MeOH–EtOAc). Another portion of TMSOTf (1.8 μ L, 9.94 μ mol) was added to the mixture at 0 °C. After the stirring was continued for 4 h at r.t, the reaction was quenched by the addition of triethylamine. The reaction mixture was diluted with CHCl₃ and filtered through Celite. The filtrate was then washed with satd aq NaHCO₃ and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The resulting residue was purified by silica gel column chromatography (40:1 CHCl₃–MeOH) to give **33** (82 mg, 62%). $[\alpha]_D$ -5.1° (c 0.8, CHCl₃); ¹H-NMR (600 MHz, CDCl₃) δ 8.22–7.12 (m, 45 H, 9 Ph), 5.92 (d, 1 H, NHb), 5.65 (m, 1 H, H-4g), 5.63 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4d), 5.55 (near t, 1 H, $J_{1,2} = 8.2$ Hz, $J_{2,3} = 10.2$ Hz, H-2d), 5.40 (m, 1 H, H-8e), 5.37 (dd, 1 H, H-3d), 5.33–5.13 (m, 7 H, H-2a, H-4a, H-7e, H-2f, H-7g, NHc, NHe), 5.06 (d, 1 H, $J_{3,4} = 3.4$ Hz, H-4f), 5.01 (d, 1 H, $J_{1,2} = 7.5$ Hz, H-1f), 5.00 (d, 1 H, $J_{5,NH} = 7.5$ Hz, NHg), 4.87–4.82 (m, 5 H, H-1d, H-4e, H-4g, 2 OCH₂), 4.75 (dd, 1 H, $J_{2,3} = 10.2$ Hz, H-3f), 4.71 (d, 1 H, $J_{gem} = 11.6$ Hz, OCH₂), 4.68 (d, 1 H, $J_{1,2} = 7.5$ Hz, H-1c), 4.68–4.47 (m, 4 H, H-1b, 3 OCH₂), 4.42 (d, 1 H, $J_{1,2} = 10.7$ Hz, H-1a), 4.28 (m, 4 H, H-9e, H-9g, 2 OCH₂), 4.11–4.00 (m, 7 H, H-4b, H-4c, H-6c, H-6'c, H-5e, H-6e, H-9'e), 3.97–3.55 (m, 23 H, H-3a, H-5a, H-6a, H-6'a, H-2b, H-3b, H-6b, H-6'b, H-3c, H-6d, H-6'd, H-6f, H-6'f, H-5g, H-6g, H-9'g, 2 OCH₃, OCH₂CH₂SiMe₃), 3.49–3.40 (m, 3 H, H-5b, H-5c, OCH₂CH₂SiMe₃), 3.31–3.28 (m, 2 H, H-5c, H-5f), 3.14 (br dd, 1 H, H-2c), 2.53 (dd, 1 H, $J_{gem} = 11.3$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3geq), 2.49 (m, 1 H, $J_{gem} = 11.7$ Hz, $J_{3eq,4} = 4.8$ Hz, H-3eeq), 2.15–1.74 (m, 43 H, 14 Ac, H-3eax), 1.70 (t, 1 H, H-3gax), 1.51 (s, 3 H, Ac), 0.88–0.68 (m, 2 H, OCH₂CH₂SiMe₃), -0.17 (s, 9 H, OCH₂CH₂SiMe₃); ¹³C-NMR (150 MHz, CDCl₃) δ 170.9, 170.7, 170.7, 170.3, 170.2, 170.1, 170.1, 170.0, 169.8, 169.7, 169.6, 168.0, 167.8, 165.7, 165.5, 165.3, 165.2, 164.9, 138.8, 138.8, 138.7, 138.1, 133.4, 133.3, 133.2, 133.1, 133.0, 130.3, 129.9, 129.8, 129.7, 129.7, 129.6, 129.5, 129.0, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 129.6, 129.5, 129.0, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.5, 127.4, 127.2, 127.2, 127.0, 100.5, 100.3, 99.6, 99.5, 99.0, 96.8, 78.1, 77.6, 77.5, 75.2, 75.0, 74.6, 74.5, 73.6, 73.3, 73.0, 72.7, 72.6, 71.8, 71.7, 71.4, 71.3, 70.4, 70.2, 70.1, 69.4, 68.7, 68.1, 68.0, 67.5, 67.3, 67.2, 67.1, 66.6, 62.4, 61.3, 53.0, 52.9, 49.4, 48.8, 37.8, 37.3, 29.7, 23.3, 23.2, 23.1, 22.6, 21.3, 21.0, 20.8, 20.8, 20.7, 20.6, 20.6, 20.3, 17.7, -1.5 . m/z (MALDI): found $[M+Na]^+$ 2986.28, C₁₄₈H₁₇₄N₄O₅₈Si calcd for $[M+Na]^+$ 2986.05.



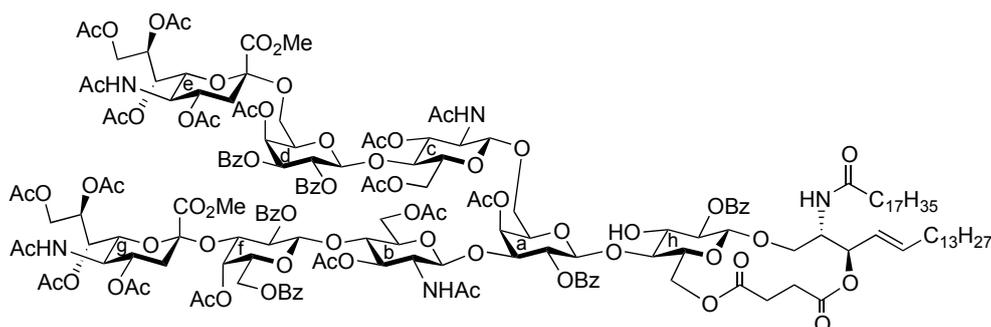
2-(Trimethylsilyl)ethyl (methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylate)-(2 \rightarrow 3)-4-O-acetyl-2,6-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)]-4-O-acetyl-2-O-benzoyl- β -D-galactopyranoside (**35**). To a solution of **33** (42.0 mg, 14.2 μ mol) in 1,4-dioxane (0.6 mL) was added Pd(OH)₂/C (210 mg). After stirring for 4 h at r.t. under a hydrogen atmosphere as the reaction was monitored by TLC (10:1 CHCl₃-MeOH), the mixture was filtered through Celite. The filtrate was concentrated and the obtained crude residue was roughly purified by silica gel column chromatography (10:1 CHCl₃-MeOH). The obtained product was exposed to high vacuum for 24 h. The residue was then dissolved in pyridine (1.4 mL). Acetic anhydride (11 μ L, 0.114 mmol) and DMAP (1.0 mg, 8.18 μ mol) were added to the mixture at 0 °C. After stirring for 72 h at r.t. as the reaction was monitored by TLC (10:1 CHCl₃-MeOH), the reaction was quenched by the addition of MeOH at 0 °C. The mixture was co-evaporated with toluene and the residue was then diluted with CHCl₃, and washed with 2 M HCl, H₂O, satd aq NaHCO₃ and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The resulting residue was purified by silica gel column chromatography (40:1 CHCl₃-MeOH) to give **35** (35 mg, 89%). [α]_D +5.1° (c 0.7, CHCl₃); ¹H-NMR (500 MHz, CDCl₃) δ 8.17–7.27 (m, 25 H, 5 Ph), 5.92 (d, 1 H, $J_{\text{NH},2}$ = 9.2 Hz, NHb), 5.68 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4d), 5.77 (m, 1 H, H-8g), 5.55 (near t, 2 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.3 Hz, H-2d), 5.44 (m, 1 H, H-3d), 5.38 (m, 1 H, H-8e), 5.32–5.16 (m, 7 H, H-2a, H-3a, H-3b, H-7e, H-2f, H-7g, NHe), 5.09 (d, 1 H, $J_{3,4}$ = 2.8 Hz, H-4f), 4.96 (d, 1 H, $J_{\text{NH},5}$ = 9.8 Hz, NHg), 4.91–4.80 (m, 6 H, NHc, H-3c, H-1d, H-4e, H-1f, H-4g), 4.74–4.71 (dd, 1 H, $J_{2,3}$ = 9.7 Hz, H-3f), 4.47 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1b), 4.43 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1a), 4.41–4.28 (m, 6 H, H-6a, H-1c, H-6d, H-9e, H-6f, H-9g), 4.17–3.93 (m, 10 H, H-6'a, H-6b, H-6'b, H-6'd, H-5e, H-6e, H-9'e, H-6'f, H-9'g, OCH₂CH₂SiMe₃), 3.88–3.68 (m, 15 H, H-3a, H-2b, H-4b, H-5b, H-2c, H-4c, H-6c, H-6'c, H-5g, 2 OCH₃), 3.55–3.53 (m, 2 H, H-5d, H-6g), 3.48–3.42 (m, 3 H, H-5a, H-5f, OCH₂CH₂SiMe₃), 3.27–3.25 (m, 1 H, $J_{5,4}$ = 9.7 Hz, H-5c), 2.55 (dd, 1 H, J_{gem} = 12.6 Hz, $J_{3\text{eq},4}$ = 4.6 Hz, H-3geq), 2.51 (dd, 1 H, J_{gem} = 12.6 Hz, $J_{3\text{eq},4}$ = 4.6 Hz, H-3eeq), 2.20–1.78 (m, 52 H, 17 Ac, H-3eax), 1.62 (t, 1 H, H-3gax), 1.54 (s, 6 H, 2 Ac), 0.88–0.72 (m, 2 H, OCH₂CH₂SiMe₃), –0.15 (s, 9 H, OCH₂CH₂SiMe₃); ¹³C-NMR (150 MHz, CDCl₃) δ 170.9, 170.7, 170.7, 170.5, 170.4, 170.4, 170.2, 170.1, 170.1, 170.0, 169.8, 169.8, 169.7, 168.0, 167.7, 165.6, 165.3, 165.0, 164.9, 164.8, 133.5, 133.3, 133.2, 130.3, 129.8, 129.7, 129.5, 129.0, 128.9, 128.7, 128.5, 128.5, 128.4, 128.4, 101.2, 100.7, 100.6, 100.5, 100.4, 99.2, 96.8, 75.6, 74.6, 72.9, 72.7, 72.5, 72.4, 71.9, 71.7, 71.1, 70.9, 70.0, 69.8, 69.6, 69.4, 68.6, 67.8, 67.4, 67.1, 66.2, 62.9, 62.4, 62.2, 62.0, 61.0, 60.9, 53.9, 53.0, 49.4, 48.8, 37.8, 37.2, 23.2, 23.1, 22.7, 21.3, 21.0, 20.9, 20.8, 20.7, 20.6, 20.5, 20.4, 17.8, –1.5. *m/z* (MALDI): found [M+Na]⁺ 2793.65, C₁₂₈H₁₅₈N₄O₆₂Si calcd for [M+Na]⁺ 2793.90.



(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 3)-4-O-acetyl-2,6-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 3)-[(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-O-acetyl-2,3-di-O-benzoyl- β -D-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-O-acetyl-2-deoxy- β -D-glucopyranosyl-(1 \rightarrow 6)]-4-O-acetyl-2-O-benzoyl-D-galactopyranosyl trichloroacetimidate (**37**). To a solution of **35** (28 mg, 10.2 μ mol) in CH₂Cl₂ (0.75 mL) was added TFA (0.25 mL) at 0 °C. After stirring for 2 h at 0 °C as the reaction was monitored by TLC (15:1:1 CHCl₃–MeOH–EtOAc), the reaction mixture was co-evaporated with toluene and then roughly purified by silica gel column chromatography (20:1 CHCl₃–MeOH). The obtained product was exposed to high vacuum for 24 h and then dissolved in CH₂Cl₂ (1.0 mL). CCl₃CN (10.2 μ L, 0.102 mmol) and DBU (1.8 μ L, 12.2 μ mol) were added to the mixture at 0 °C. After stirring for 45 min at 0 °C as the reaction was monitored by TLC (10:1 CHCl₃–MeOH), the reaction mixture was evaporated. The obtained crude residue was purified by silica gel column chromatography (30:1 CHCl₃–MeOH) to give **37** (27 mg, 95%, α : β = 3:1). **37 α** : ¹H-NMR (600 MHz, CDCl₃) δ 8.60 (s, 1 H, C(=NH)), 8.19–7.27 (m, 25 H, 5 Ph), 6.48 (d, 1 H, $J_{1,2}$ = 4.1 Hz, H-1a), 5.81 (d, 1 H, $J_{NH,2}$ = 9.0 Hz, NHc), 5.68 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4d), 5.60 (m, 1 H, H-8g), 5.55 (near t, 1 H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 10.3 Hz, H-2d), 5.48 (dd, 1 H, $J_{2,3}$ = 10.3 Hz, H-2a), 5.43–5.42 (m, 2 H, H-3a, H-4a), 5.38 (m, 1 H, H-8e), 5.32 (dd, 1 H, H-7e), 5.24 (m, 2 H, H-3a, H-7g), 5.20 (near t, 1 H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 9.6 Hz, H-2f), 5.16 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, H-3b), 5.13 (d, 1 H, $J_{5,NH}$ = 9.6 Hz, NHe), 4.92 (d, 1 H, $J_{3,4}$ = 2.3 Hz, H-4f), 4.90–4.80 (m, 8 H, H-3c, H-1d, H-4e, H-1f, H-3d, H-4g, NHc, NHg), 4.73 (dd, 1 H, H-3f), 4.55 (d, 1 H, $J_{1,2}$ = 7.6 Hz, H-1b), 4.45 (d, 1 H, $J_{1,2}$ = 7.4 Hz, H-1c), 4.41–4.01 (m, 13 H, H-3a, H-6a, H-6'a, H-5e, H-6e, H-9e, H-9'e, H-6d, H-6'd, H-6f, H-6'f, H-9g, H-9'g), 3.88–3.70 (m, 15 H, H-2b, H-4b, H-6b, H-6'b, H-2c, H-4c, H-6c, H-6'c, H-5e, 2 OCH₃), 3.55–3.36 (m, 5 H, H-5a, H-5b, H-5c, H-5d, H-5f), 2.55 (dd, 1 H, J_{gem} = 13.1 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3_{eeq}), 2.51 (dd, 1 H, J_{gem} = 11.6 Hz, $J_{3eq,4}$ = 4.8 Hz, H-3_{geq}), 2.20–1.78 (m, 52 H, 17 Ac, H-3_{eax}), 1.63–1.39 (m, 7 H, 2 Ac, H-3_{gax}).

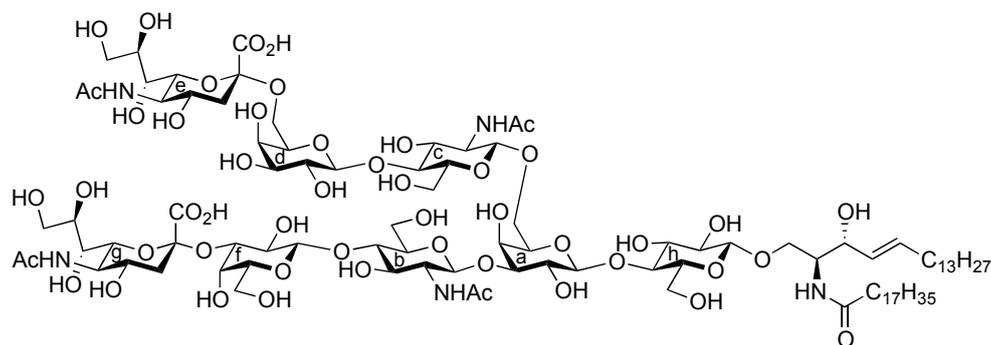


(2*S*,3*R*,4*E*)-1-*O*-((Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonate)-(2 \rightarrow 3)-4-*O*-acetyl-2,6-di-*O*-benzoyl- β -*D*-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyl-(1 \rightarrow 3))-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-4-*O*-acetyl-2,3-di-*O*-benzoyl- β -*D*-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyl-(1 \rightarrow 6)]-4-*O*-acetyl-2-*O*-benzoyl- β -*D*-galactopyranosyl)-(1 \rightarrow 4)-2-*O*-benzoyl-3-*O*-*p*-methoxybenzyl- β -*D*-glucopyranosyl)-2-octadecanamido-3,6'-succinyl-octadec-4-ene-1,3-diol (**39**). To a mixture of **37** (38 mg, 13.4 μ mol) and **38** (14 mg, 13.4 μ mol) in CH₂Cl₂ (1.3 mL) was added 5 Å molecular sieves (100 mg) at r.t. After stirring for 1 h, the mixture was cooled to 0 °C. TMSOTf (0.3 μ L, 1.34 μ mol) was then added to the mixture at 0 °C. After stirring for 1 h at r.t. as the reaction was monitored by TLC (15:1:5 CHCl₃-MeOH-EtOAc), the reaction was quenched by the addition of triethylamine. The reaction mixture was diluted with CHCl₃ and filtered through Celite. The filtrate was then washed with satd aq NaHCO₃ and brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The residue was purified by silica gel column chromatography (40:1:5 CHCl₃-MeOH-EtOAc) to give **39** (24 mg, 49%). [α]_D -10.3° (c 2.0, CHCl₃); ¹H-NMR (500 MHz, CD₃CN) δ 8.13–7.39 (m, 30 H, 6 Ph), 7.04 (d, 2 H, Ar), 6.66 (d, 2 H, Ar), 6.14 (d, 1 H, $J_{5,NH}$ = 9.7 Hz, NHc), 6.09 (d, 1 H, $J_{2,NH}$ = 9.2 Hz, NH^{Cer}), 6.00 (d, 1 H, $J_{5,NH}$ = 9.7 Hz, NHg), 5.95 (d, 1 H, $J_{2,NH}$ = 9.1 Hz, NHb), 5.79 (d, 1 H, $J_{2,NH}$ = 9.8 Hz, NHc), 5.63 (dt, 1 H, $J_{5,6}$ = 6.9 Hz, J = 14.9 Hz, J = 6.9 Hz, H-5^{Cer}), 5.54 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4d), 5.47 (m, 3 H, H-3d, H-7e, H-8e), 5.37 (near t, 1 H, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10.3 Hz, H-2d), 5.36 (m, 1 H, H-8g), 5.27 (d, 1 H, $J_{3,4}$ = 3.5 Hz, H-4a), 5.25–5.17 (m, 4 H, H-2a, H-4f, H-7g, H-4^{Cer}), 5.11 (br t, 1 H, $J_{3,4}$ = $J_{2,3}$ = 7.5 Hz, H-3^{Cer}), 5.03 (near t, 1 H, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.3 Hz, H-2f), 4.94 (t, 1 H, $J_{1,2}$ = $J_{2,3}$ = 8.3 Hz, H-2h), 4.88 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.3 Hz, H-3b), 4.76 (m, 8 H, H-1c, H-3c, H-1d, H-4e, H-1f, H-3f, H-4g, ArCH₂), 4.68 (d, 1 H, $J_{1,2}$ = 8.1 Hz, H-1a), 4.54 (d, 1 H, $J_{1,2}$ = 8.5 Hz, H-1b), 4.47 (d, 1 H, H-1h), 4.46 (d, 1 H, J_{gem} = 10.9 Hz, ArCH₂), 4.35 (dd, 1 H, $J_{8,9}$ = 2.9 Hz, J_{gem} = 12.6 Hz, H-9e), 4.30–4.21 (m, 4 H, H-6b, H-6'b, H-6d, H-9g), 4.11–3.89 (m, 13 H, H-3a, H-6'a, H-6c, H-6'c, H-6'd, H-5e, H-6e, H-9'e, H-6f, H-9g, H-6h, H-6'h, H-2^{Cer}), 3.78–3.53 (m, 21 H, H-5a, H-4b, H-2c, H-4c, H-5d, H-6'a, H-6'f, H-5g, H-6g, H-3h, H-4h, H-1^{Cer}, 3 OCH₃), 3.51 (m, 1 H, H-2b), 3.40–3.32 (m, 3 H, H-5b, H-5h, H-1^{Cer}), 3.18–3.12 (m, 2 H, H-5c, H-5f), 2.56 (dd, 1 H, $J_{3eq,4}$ = 4.6 Hz, J_{gem} = 12.6 Hz, H-3eq), 2.48–2.34 (m, 5 H, H-3geq, 2 C(=O)CH₂), 2.21–1.61 (m, 62 H, H-3eax, H-6^{Cer}, H-6^{Cer}, C(=O)CH₂^{Cer}, 19 Ac), 1.55–1.08 (m, 53 H, H-3gax, 26 -CH₂-), 0.87 (t, 6 H, 2 -CH₃^{Cer}); ¹³C-NMR (150 MHz, CDCl₃) δ 172.6, 171.0, 170.9, 170.8, 170.7, 170.6, 170.5, 170.4, 170.3, 170.2, 170.1, 170.0, 169.7, 169.7, 169.5, 168.0, 167.7, 165.6, 165.2, 165.2, 165.1, 165.0, 164.5, 159.1, 138.6, 133.8, 133.3, 133.1, 130.7, 130.3, 129.8, 129.7, 129.6, 129.5, 129.5, 129.5, 129.0, 128.9, 128.8, 128.6, 128.6, 128.5, 128.4, 128.3, 124.8, 113.9, 101.6, 101.3, 100.8, 100.6, 100.3, 99.7, 99.3, 96.8, 91.8, 80.8, 79.7, 76.3, 75.9, 74.7, 74.3, 73.2, 72.9, 72.7, 72.6, 72.5, 71.9, 71.8, 71.7, 71.7, 71.0, 70.9, 70.1, 69.8, 69.7, 69.4, 68.6, 67.8, 67.4, 67.1, 66.3, 63.2, 63.0, 62.6, 62.1, 61.9, 61.1, 60.9, 55.1, 53.6, 53.1, 53.0, 49.9, 49.4, 48.8, 37.9, 37.2, 37.1, 36.6, 32.7, 32.2, 31.9, 30.0, 29.7, 29.6, 29.5, 29.5, 29.3, 29.3, 29.2, 28.8, 27.1, 25.5, 23.6, 23.2, 23.1, 22.7, 21.3, 21.0, 20.9, 20.8, 20.7, 20.7, 20.6, 20.5, 20.5, 19.7, 14.1. *m/z* (MALDI): found [M+Na]⁺ 3709.15, C₁₈₄H₂₃₉N₅O₇₃ calcd for [M+Na]⁺ 3709.50.



(2*S*,3*R*,4*E*)-1-*O*-({(Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylate)-(2 \rightarrow 3)-4-*O*-acetyl-2,6-di-*O*-benzoyl- β -*D*-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyl-(1 \rightarrow 3)-[(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylate)-(2 \rightarrow 6)-4-*O*-acetyl-2,3-di-*O*-benzoyl- β -*D*-galactopyranosyl-(1 \rightarrow 4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy- β -*D*-glucopyranosyl-(1 \rightarrow 6)]-4-*O*-acetyl-2-*O*-benzoyl- β -*D*-galactopyranosyl)-(1 \rightarrow 4)-2-*O*-benzoyl- β -*D*-glucopyranosyl)-2-octadecanamido-3,6'-succinyl-octadec-4-ene-1,3-diol (**40**). To a solution of **39** (9.0 mg, 2.47 μ mol) in CH₂Cl₂ (0.60 mL) was added TFA (0.30 mL) at 0 °C. After stirring for 30 min at 0 °C as the reaction was monitored by TLC (10:1 CHCl₃–MeOH), the reaction was quenched by the addition of satd aq NaHCO₃. The mixture was diluted with CHCl₃ and washed with brine. The organic layer was subsequently dried over Na₂SO₄, and concentrated. The residue was purified by silica gel column chromatography (20:1 CHCl₃–MeOH) to give **40** (8.0 mg, 91%). [α]_D –6.3° (c 0.8, CHCl₃); ¹H-NMR (600 MHz, CDCl₃) δ 8.17–7.27 (m, 30 H, 6 Ph), 6.68 (d, 1 H, $J_{2,\text{NH}}$ = 8.2 Hz, NH^{Cer}), 6.21 (d, 1 H, $J_{2,\text{NH}}$ = 9.0 Hz, NHb), 5.85 (near dt, 1 H, $J_{5,6}$ = 6.9 Hz, J = 14.4 Hz, J = 7.5 Hz, H-5^{Cer}), 5.70 (d, 1 H, $J_{3,4}$ = 3.4 Hz, H-4d), 5.59 (m, 1 H, H-8g), 5.57 (near t, 1 H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 10.3 Hz, H-2d), 5.45 (dd, 1 H, H-3d), 5.41–5.38 (m, 1 H, H-8e), 5.35–5.32 (m, 2 H, H-2a, H-7e), 5.27–5.17 (m, 7 H, H-4a, H-3b, H-2f, H-7g, NHc, H-3^{Cer}, H-4^{Cer}), 5.09 (d, 1 H, $J_{3,4}$ = 2.7 Hz, H-4f), 5.06 (near t, 1 H, $J_{1,2}$ = 4.8 Hz, $J_{2,3}$ = 5.5 Hz, H-2h), 4.94 (d, 1 H, $J_{5,\text{NH}}$ = 10.3 Hz, NHg), 4.91–4.81 (m, 5 H, H-3c, H-1d, H-4e, H-4g, NHc), 4.74–4.70 (m, 2 H, H-1a, H-3f), 4.62 (d, 1 H, H-1h), 4.45–4.34 (m, 6 H, H-1b, H-1c, H-9e, H-6f, H-9g, H-6h), 4.27–4.23 (m, 3 H, H-2^{Cer}, H-6a, H-6d), 4.17–3.98 (m, 11 H, H-6'a, H-6c, H-6'c, H-6d, H-5e, H-6e, H-9'e, H-9'g, H-5h, H-6h, H-6'h), 3.88–3.65 (m, 17 H, H-3a, H-2b, H-4b, H-6b, H-6'b, H-2c, H-4c, H-5g, H-3h, H-4h, H-1^{Cer}, 2 OCH₃), 3.60–3.53 (m, 3 H, H-5d, H-6g, H-1^{Cer}), 3.50–3.44 (m, 2 H, H-5b, H-5f), 3.29–3.23 (m, 2 H, H-5a, H-5c), 2.56–2.39 (m, 6 H, H-3eeq, H-3geq, 2 C(=O)CH₂), 2.20–1.78 (m, 56 H, 17 Ac, H-3eax, H-6^{Cer}, H-6'^{Cer}, C(=O)CH₂^{Cer}), 1.62 (t, 1 H, $J_{\text{gem}} = J_{3\text{ax},4} = 12.6$ Hz, H-3gax), 1.54 (s, 3 H, Ac), 1.51–1.46 (m, 2 H, C(=O)CH₂CH₂), 1.42 (s, 3 H, Ac), 1.38–1.20 (m, 50 H, 25 –CH₂–), 0.88 (m, 6 H, 2 –CH₃^{Cer}); ¹³C-NMR (150 MHz, CDCl₃) δ 172.9, 171.0, 170.9, 170.8, 170.8, 170.7, 170.6, 170.5, 170.5, 170.3, 170.3, 170.3, 170.1, 170.1, 170.0, 169.9, 169.8, 169.7, 169.7, 168.0, 167.8, 165.7, 165.4, 165.3, 165.2, 165.0, 164.7, 138.5, 134.0, 133.5, 133.3, 133.2, 130.3, 129.8, 129.7, 129.7, 129.6, 129.5, 129.4, 129.0, 129.0, 128.7, 128.6, 128.5, 128.4, 128.4, 128.4, 125.0, 101.7, 101.2, 100.7, 99.8, 99.6, 99.3, 98.5, 96.8, 82.0, 77.5, 75.2, 74.5, 74.2, 73.8, 73.5, 72.9, 72.7, 72.6, 72.5, 72.0, 71.9, 71.7, 71.2, 71.0, 70.9, 70.7, 70.1, 69.9, 69.6, 69.4, 68.6, 67.8, 67.6, 67.4, 67.2, 67.1, 66.6, 66.3, 62.9, 69.4, 68.6, 67.8, 67.6, 67.4, 67.2, 67.1, 66.6, 66.3, 62.9, 62.5, 62.4, 62.0, 61.1, 60.9, 54.2, 53.9, 53.0, 53.0, 50.1, 49.5, 48.8, 37.8, 37.2, 36.4, 32.2, 31.9, 30.4, 29.7, 29.7, 29.7, 29.6, 29.5, 29.5, 29.3, 29.2, 28.8, 25.6, 23.6, 23.2, 23.1, 22.7, 22.5, 21.4, 21.1, 20.8, 20.8, 20.7, 20.6, 20.5, 20.4, 14.1. *m/z* (MALDI): found

$[M+Na]^+$ 3589.60, $C_{176}H_{231}N_5O_{72}$ calcd for $[M+Na]^+$ 3589.45. HRMS (ESI): found $[1/2M+Na]^+$ 1806.2182, $C_{176}H_{231}N_5O_{72}$ calcd for $[1/2M+Na]^+$ 1806.2181.



{3-O-[(5-Acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)- β -D-galactopyranosyl-(1 \rightarrow 4)]-2-acetamido-2-deoxy- β -D-glucopyranosyl]-6-O-[(5-acetamido-3,5-dideoxy-D-glycero- α -D-galacto-2-nonulopyranosylonic acid)- β -D-galactopyranosyl-(1 \rightarrow 4)]-2-acetamido-2-deoxy- β -D-glucopyranosyl]- β -D-galactopyranosyl}-(1 \rightarrow 4)- β -D-glucopyranosyl-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (**1**). To a solution of **40** (8.0 mg, 2.24 μ mol) in MeOH/THF (1:1, 1.0 mL) was added NaOMe (28% solution in MeOH, 12.1 μ g, 0.672 μ mol) at 0 $^{\circ}$ C. After stirring for 7 d at 40 $^{\circ}$ C as the reaction was monitored by TLC (6:4:1 $CHCl_3$ -MeOH-0.2% aq $CaCl_2$) and MALDI-TOF MS, water (0.1 mL) was added to the reaction mixture. After stirring for 2 d at 40 $^{\circ}$ C, the reaction was neutralized with Dowex (H^+) resin. The resin was filtered through cotton and the filtrate was then evaporated. The residue was purified by gel filtration column chromatography (LH-20) using MeOH as eluent followed by silica gel column chromatography (6:4:1 $CHCl_3$ -MeOH- H_2O) to give **1** (3.0 mg, 61%). $[\alpha]_D -78.8^{\circ}$ (c 0.4, MeOH); 1H -NMR (600 MHz, 1:1 CD_3OD - D_2O) δ 5.73 (m, 1 H, H-5^{Cer}), 5.46 (m, 1 H, H-4^{Cer}), 2.86 (dd, 1 H, H-3^{geq}), 2.78 (dd, 1 H, H-3^{eeq}), 2.20 (t, 2 H, $NHCOCH_2$), 2.04 (s, 14 H, H-6^{Cer}, H-6^{CCer}, 4 NAc), 1.73 (m, 2 H, H-3^{gax}, H-3^{eax}), 1.58 (m, 2 H, $NHCOCH_2CH_2$), 1.30 (m, 50 H, 25 $-CH_2-$), 0.92 (t, 6 H, 2 $-CH_3$); ^{13}C -NMR (125 MHz, 5:6:0.5 CD_3OD - D_2O - $CDCl_3$) δ 174.2, 174.1, 173.7, 173.3, 134.1, 128.6, 102.9, 102.3, 102.0, 101.8, 99.9, 79.9, 79.0, 77.8, 76.5, 75.1, 74.6, 74.1, 74.0, 73.5, 73.1, 72.5, 72.2, 72.0, 71.7, 71.6, 71.1, 70.9, 70.6, 70.2, 69.3, 68.7, 68.2, 67.9, 67.8, 67.5, 67.3, 66.8, 62.4, 62.2, 60.4, 59.7, 54.6, 54.5, 52.4, 51.6, 51.5, 48.1, 46.7, 39.7, 39.4, 35.6, 31.6, 31.1, 31.0, 29.0, 28.8, 28.7, 28.6, 28.6, 28.5, 28.4, 25.3, 21.8, 21.6, 21.4, 21.2, 21.1, 12.9. HRMS (ESI): found $[M+Na]^-$ 2225.0943, $C_{98}H_{171}N_5O_{49}$ calcd for $[M+Na]^-$ 2225.0940.

3.2. Materials and Methods for Binding Assay

3.2.1. Virus Preparation

A/Memphis/1/71 (H3N2) and A/Puerto Rico/8/34 (H1N1) were used in this study. The virus was propagated in 11 days old embryonated hen's eggs. The virus was purified by ultra-centrifugation and stored at -80° C before use.

3.2.2. Solid-Phase Binding Assay

Virus binding to sialylglycolipids was determined according to a method described previously [28]. Synthetic and authentic gangliosides were 2-fold serially diluted in 100% ethanol from 0.625 to

2.5 pmol/ μ L. Ten μ L of each diluted ganglioside was placed into a well of a polystyrene Universal-BINDTM microplates (flat-bottom, Product# 2503, Corning, Tokyo, Japan) and incubated for approximately 1 h at 37 °C until the ethanol had completely evaporated. gangliosides were covalently immobilized on the surface of plates by exposure for 1 min under ultraviolet irradiation (254 nm) according to the manufacture's instruction. Each well was blocked for 1 h at room temperature with PBS containing 2% bovine serum albumin. After 3 washes with PBS, the plates were incubated in solutions containing viruses in PBS (128 HA unit/50 μ L/well) overnight at 4 °C. After 5 washes with ice-cold PBS, the plates were incubated in a substrate solution containing 40 μ M 2'-(4-methylumbelliferyl)- α -D-N-acetylneuraminic acid in PBS (50 μ L/well) at 37 °C for 60 min to detect virus neuraminidase (NA) activity associated with bound viruses. The reactions were terminated by addition of 500 mM carbonate buffer (pH 10.2) (50 μ L/well). Fluorescence intensity of the 4-methylumbelliferone released by viral NAs was measured at 355 nm (excitation) and 460 nm (emission). Triplicate measurements were performed in each assay. The direct virus binding activity was calculated as follows: Virus-binding score = [(average of NA activity of triplicate ganglioside-immobilized wells) – (average of NA activity of triplicate ethanol-treated wells)]/[NA activity of applied virus (128 HA units/50 μ L)]. The virus-binding score was expressed as mean score \pm SD.

4. Conclusions

We efficiently synthesized a novel ganglioside designed as a ligand for influenza A viruses employing the cassette coupling approach between the heptasaccharyl sugar part and the cyclic glucosylceramide moiety. The present study revealed that the cassette approach can be applied to the synthesis of lacto-series gangliosides as well as ganglio-series gangliosides. This success will expand the applicability of the cassette approach to the synthesis of other glycolipids. In addition, we examined the binding activity of the synthesized ganglioside ligand to influenza A viruses. It was found that the synthetic ligand is recognized by Neu α 2-3 and 2-6 type viruses, suggesting that a glycan structure containing both the Neu α 2-3Gal and Neu α 2-6Gal sequences in a single molecule could exist as a natural ligand for influenza A viruses. To identify an actual natural ligand for influenza viruses, the synthesis of a series of gangliosides with both the Neu α 2-3Gal and Neu α 2-6Gal sequences in a single molecule is currently underway.

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