European Journal of Medicinal Chemistry 75 (2014) 247–257

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and cytotoxicity assay of four ganglioside GM3 analogues

Huanhuan Qu [a,b], Jian-Miao Liu [c], Joanna Wdzieczak-Bakala [c], Dan Lu [a], Xianran He [d], Wenji Sun [e], Matthieu Sollogoub [a], Yongmin Zhang [a,d,e,*]

[a] Sorbonne Universités, UPMC Univ Paris 06, LabEx Michem, CNRS, UMR 8232, IPCM, F-75005 Paris, France
[b] Glycochemistry & Glycobiology Lab, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Pudong, Shanghai 201203, China
[c] Institut de Chimie des Substances Naturelles, UPR 2301, CNRS, avenue de la Terrasse, F-91198 Gif sur Yvette, France
[d] Institute for Interdisciplinary Research, Jianghan University, Wuhan Economic and Technological Development Zone, Wuhan 430056, China
[e] Biomedicine Key Laboratory of Shaanxi Province, Northwest University, Xi'an 710069, China

ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form
21 January 2014
Accepted 24 January 2014
Available online 28 January 2014

Dedicated to Professor Max Malacria on the occasion of his 65th birthday.

Keywords:
Synthesis
Sialylation
Cytotoxicity
Cancer
Ganglioside GM3
Sialic acid

ABSTRACT

A concise and efficient synthetic route for preparation of four ganglioside GM3 analogues was described. The key step is a highly regioselective and stereoselective α-sialylation from a suitably protected glycoside acceptor with a sialyl xanthate to provide the sialo-oligosaccharide in good yield. The cytotoxic properties of the synthetic gangliosides were evaluated against normal human keratinocytes and human HCT116 and K562 cancer cells. Two of them exhibited good antiproliferative activity and displayed a better cytotoxicity against cancer cell than HaCaT normal cell.

1. Introduction

Glycosphingolipids (GSLs) are components of all animal cell membranes and are involved in many cellular functions including proliferation, adhesion, motility, and differentiation [1]. Ganglioside GM3 (NeuAcα3Galβ4Glcβ1Cer), the first and simplest member in the metabolic series of a GSLs family containing sialic acids (*N*-acetyl- and *N*-glycolyl-neuraminic acids and their *O*-acyl derivatives), is known as one of the most abundant tumor-associated carbohydrate antigens on several types of tumors [2]. Glycosphingolipid structures, and their changes associated with biological functions, have been the central focus of our studies, since structural change is the starting point for understanding biological significance, and enzymatic/genetic mechanisms [3].

Our previous researches cooperated with Hakomori's group indicate that clustered GSLs associated with c-Src, small G-protein (Rho A), and focal adhesion kinase (FAK) are involved in GSL-dependent cell adhesion coupled with activation of these signal transducers [4]. A typical example is GM3 ganglioside clustering at the cell surface of mouse melanoma B16, forming a "glycosignaling domain" (GSD) separable from cholesterol- and caveolin-enriched membrane fractions derived from caveolae [5]. GM3 in B16 cells is also recognized as a melanoma-associated antigen, and may have a role in initiating adhesion of melanoma to endothelial cells, the first step in metastasis [6]. Antigenicity, mediation of adhesion, and initiation of signaling through GM3 ganglioside at the B16 cell surface are thought to be maintained by GM3 clustering in GSD. If GM3 clustering in GSD is inhibited, antigenicity, adhesion, and signaling through GM3 could be blocked. This concept suggests that compounds having structural features analogous to those of GM3, may destroy or reduce clustering of GM3 in GSD, and inhibit GM3-dependent adhesion and signaling. So the purpose of this work was to search for ganglioside GM3 analogues which could disrupt the structure and function of GSD in cancer cells.

* Corresponding author. Université Pierre et Marie Curie-Paris 6, Institut Parisien de Chimie Moléculaire, CNRS UMR 8232, 4 place Jussieu, 75005 Paris, France.
E-mail address: yongmin.zhang@upmc.fr (Y. Zhang).

0223-5234/$ – see front matter © 2014 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2014.01.054

Studies utilizing synthetic molecules that resemble natural GSLs provide important information that is not available from studies utilizing molecules from natural sources. Recently, the development of synthetic gangliosides has attracted much attention. Glycosylations to form anomeric linkages of sialic acid (sialylations) are often complicated by the intrinsic structural features of sialic acid, resulting in poor yields and/or stereoselectivities [7]. Although notable progress has been made in this research field in the last decade, good yields along with complete control of stereoselectivities for the synthesis of sialosides are still a goal to be reached.

Herein we would like to report the preparation of four GM3 analogues **1**–**4** (Fig. 1) based on a facile protocol that can provide a series of gangliosides. As a part of a parallel investigation, *in vitro* cytotoxicity of these compounds was determined against the human keratinocyte and human HCT116 and K562 cancer cells. We believe this work will facilitate construction of various GM3 analogues with analogous or even improved biological properties compared to those of the natural GSLs, and provide meaningful and useful references for exploring new carbohydrate anticancer agents.

2. Results and discussion

Sialylation is the key step of the total synthesis of our target glycosides. In spite of extensive efforts and notable progress, the chemical synthesis of sialosides in high yield with complete stereoselectivity remains a significant challenge [7]. The presence of a destabilizing electron-withdrawing carboxylic group along with a tertiary anomeric center and the lack of a participating auxiliary often drive glycosylation reactions toward competitive elimination reactions resulting in poor stereoselectivity (β-anomer) and in the formation of a 2,3-dehydro derivative [8]. To overcome these problems, different approaches have emerged. A variety of leaving groups and activation conditions has been developed. The original purpose of our study was to synthesize α(2 → 3) sialosides (NeuAcα3Gal) and α(2 → 4) sialosides (NeuAcα4Glc), which removed respectively a monosaccharide from natural ganglioside GM3. But we failed in the preparation of α(2 → 4) sialosides (NeuAcα4Glc). Next, we tried the sialylations of glucose at position C-3, but the yields were less than 6%. Fortunately, the yield of α-sialylation of glucose at position C-6 was high. In this paper, we describe in complete detail the total synthesis of our target GSLs.

As illustrated in Table 1, we compared three sialic acid donors **5** [9], **5a** [10] and **5b** [10] to optimize the α-sialylation of galactoside acceptors with the sialic acid donors [11–13]. Considering the synthetic strategy after the sialylation, we gave priority to use previously reported diol **6a** [14] as the acceptor. Unfortunately, we didn't obtain our desired product (entry 1–4). Along with the results reported in Ref. [15], using acetyl groups as the protecting groups of acceptor was inappropriate (entry 5), the yield of sialylation was low. Entry 6–9, galactoside acceptor **6** containing benzyl as protective groups was used. The reaction in the presence of benzenesulfenyl chloride (PhSCl), Silver trifluoromethanesulfonate (AgOTf) and di-tert-butylpyridine (DTBP) as promoters in a 2:1 mixture of $MeCN/CH_2Cl_2$ at low temperature (entry 6) afforded α-sialoside in significantly higher yield than the other three entries. So for the sialylation of glucoside, we directly selected this Martichonok and Whitesides' method [9]. Similarly, considering the synthetic strategy, the previously reported 2-(trimethylsilyl)ethyl glycoside **7** [16] was our first choice to be used as the glucoside acceptor. Not only because it was convenient to prepare **7**, but also because using acetyl groups as the protecting groups simplified the synthesis after the sialylation. The sialylation of glucoside acceptor **7** with sialic acid donors **5** afforded α-sialoside in 82% yield. This yield is regarded as satisfactory, so there is no need to try other protecting groups. Based on these observations, for synthesis of our target gangliosides, compounds **5**, **6** [17], **7** and **8** [18] were chosen as building blocks (Fig. 2).

Sialylation of acceptor **6** occurred at the more reactive 3-OH position and gave rise predominantly to the α-product **9** since the proton signal H-3eq for sialic acid residue appeared at $\delta = 2.53$ ppm (β-product $\delta = 2.41$ ppm). Catalytic hydrogenolysis of **9** provided the disaccharide **10**. Acetylation of **10** for protection of the

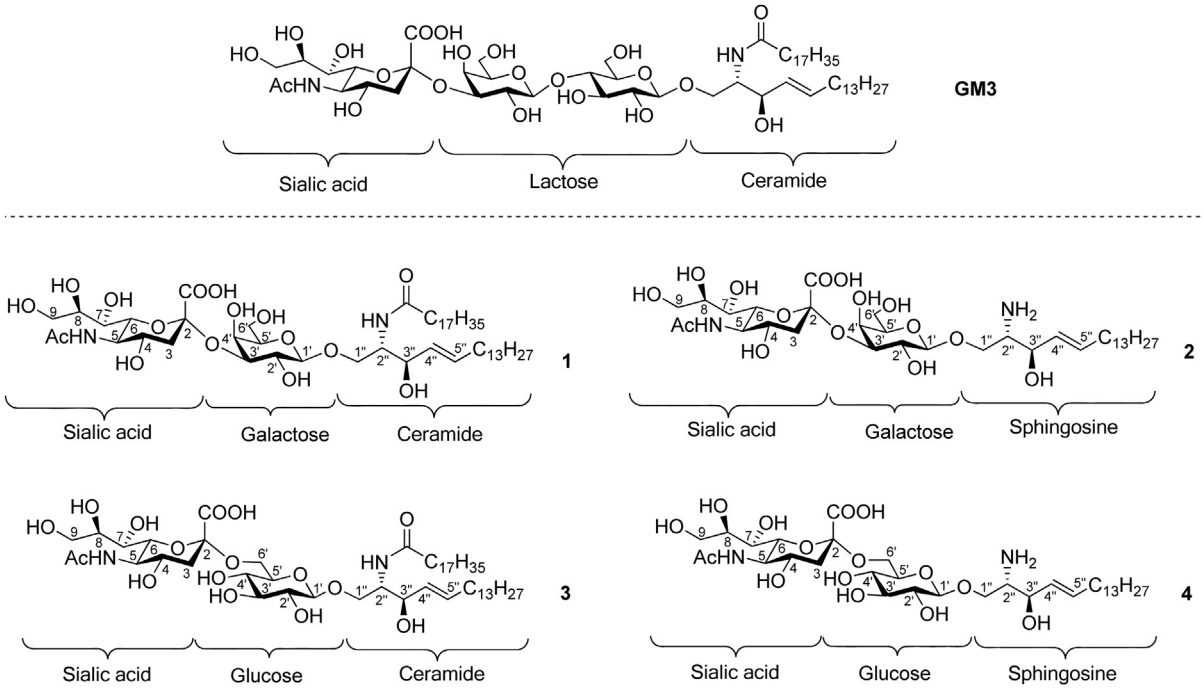
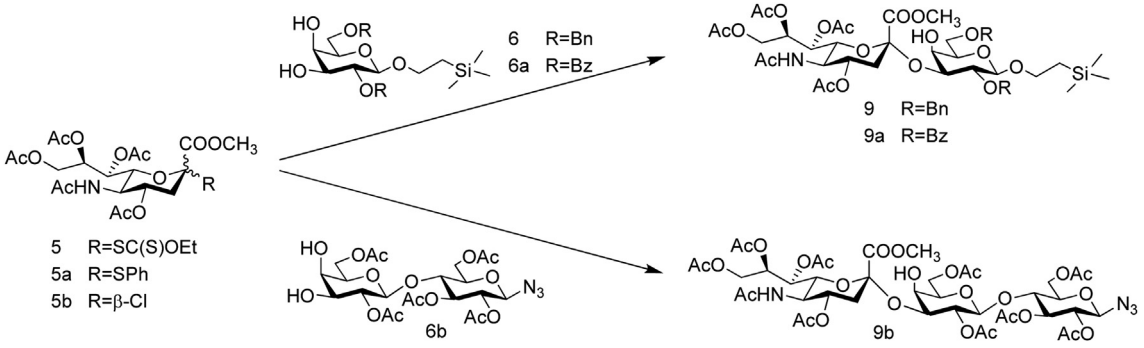


Fig. 1. Structures of ganglioside GM3 and the target GM3 analogues.

Table 1
Optimization of the α-sialylation.



Entry	Acceptor [equiv]	Donor [equiv]	Promoter [equiv]	Conditions	Solvent	Product	Yield [%][d]	Reference
1	**6a** (1)	**5** (1.5)	PhSCl/AgOTf/DTBP (2/2/2.1)	−68 °C/3h	MeCN/CH_2Cl_2[b]	**9a**	Nd[e]	[9]
2	**6a** (1.5)	**5a** (1)	NIS/TfOH (1.5/0.4)	−45 °C/3h	MeCN	**9a**	Nd	[11]
3	**6a** (3)	**5b** (1)	Ag_2CO_3 (3.5)	−45 °C → r.t./2 days	CH_2Cl_2	**9a**	Nd	[12]
4	**6a** (1)	**5b** (1)	$Hg(CN)_2/HgBr_2$ (1.4/0.5)	r.t./2 days	CH_2Cl_2	**9a**	Nd	[13]
5	**6b** (Np[a])	**5a** (Np)	NIS/TfOH (Np)	−35 °C/2 days	MeCN	**9b**[c]	26[c]	[15]
6	**6** (1)	**5** (1.5)	PhSCl/AgOTf/DTBP (2/2/2.1)	−68 °C/3h	MeCN/CH_2Cl_2[b]	**9**	75	[9]
7	**6** (1.5)	**5a** (1)	NIS/TfOH (1.5/0.4)	−45 °C/3h	MeCN	**9**	39	[11]
8	**6** (3)	**5b** (1)	Ag_2CO_3 (3.5)	−45 °C → r.t./2 days	CH_2Cl_2	**9**	5	[12]
9	**6** (1)	**5b** (1)	$Hg(CN)_2/HgBr_2$ (1.4/0.5)	r.t./2 days	CH_2Cl_2	**9**	8	[13]

[a] Not reported.
[b] Solvent ratio of 2:1.
[c] This experiment was described in Ref. [15].
[d] Based on the stating material (acceptor or donor) present in the smallest amount.
[e] Not detected.

remaining hydroxyl groups yielded the octaacetylated derivative **11** quantitatively. Acid catalyzed cleavage of the 2-(trimethylsilyl)ethyl glycoside **11** was performed in CH_2Cl_2 using trifluoroacetic acid to afford hemiacetal **12** as a mixture of α/β isomers. This hemiacetal was then treated with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) to give the trichloroacetimidate **13** in 88% yield for two steps (Scheme 1). The ^{1}H NMR spectrum showed that α-trichloroacetimidate is the predominant product ($\alpha/\beta = 94/6$) of this reaction on the basis of the H-1′ and H-2′ coupling constant ($J_{1',2'} = 3.9$ Hz) of the galactose residue. This is because an axial trichloroacetimidate is the thermodynamically more stable isomer [19].

Condensation of trichloroacetimidate **13** with azidosphingosine derivative **8** was performed using $BF_3 \cdot Et_2O$ as promoter to provide the desired glycolipid **14** in 83% yield, as shown in Scheme 2. The β configuration of the newly introduced glycosidic linkage was confirmed from the ^{1}H NMR spectrum ($J_{1',2'} = 8.0$ Hz). The azide group of **14** was reduced by triphenylphosphine in a mixture of toluene and water at 45 °C for 12 h to give an amino derivative **15** in 76% yield. Then **15** was condensed with stearic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in dry CH_2Cl_2 to give the glycosyl ceramide **16**. The acyl groups of compound **16** on hydroxyls were subsequently removed by reaction with NaOMe/MeOH. After adding several drops of water, ganglioside **1** was obtained in 81% yield for two steps. On the other hand, all the acyl groups of compound **14** on hydroxyls were removed by reaction with NaOMe/MeOH. After adding several drops of water, compound **17** was obtained quantitatively. Then azide **17** was reduced with propanedithiol/triethylamine [20] to afford the new ganglioside **2** in 93% yield.

As shown in Schemes 3 and 4, the synthetic route of gangliosides **3** and **4** is similar to that described above.

In vitro cytotoxicity of the synthetic gangliosides **1**–**4** was determined against the human keratinocyte and human HCT116 and K562 cancer cells. The IC_{50} (μM) values obtained with tested cell lines are summarized in Table 2. Results demonstrate that compound **3** and **4** have a similar antiproliferative activity and display a better cytotoxicity against cancer cell than HaCaT normal cell. Compound **2** had no effect on cell proliferation as compared to compound **1**. The effect of selected compounds **3** and **4** on the cell morphology was then investigated at 100 μM. As shown in Fig. 3, after 3 days of treatment, changes can be clearly observed.

3. Conclusions

In this study, we accomplished the synthesis of four GM3 analogues from simple and commercially available substrates and reagents. The key step is a highly regioselective and stereoselective α-sialylation from a suitably protected glycoside acceptor with a sialyl xanthate to provide the sialo-oligosaccharide in good yield. Two glucose-containing GM3 analogues, in which sialic acids are linked by (α2 → 6)-glycosidic linkage to glucosides, exhibited good

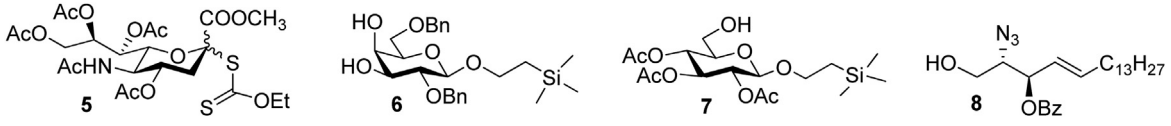
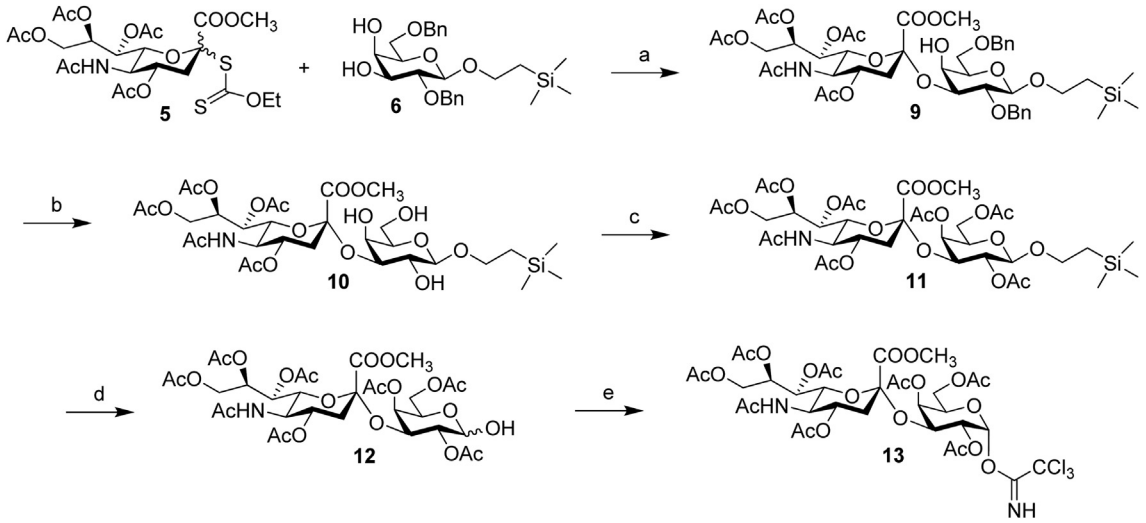


Fig. 2. Key building blocks for synthesis of the target gangliosides.



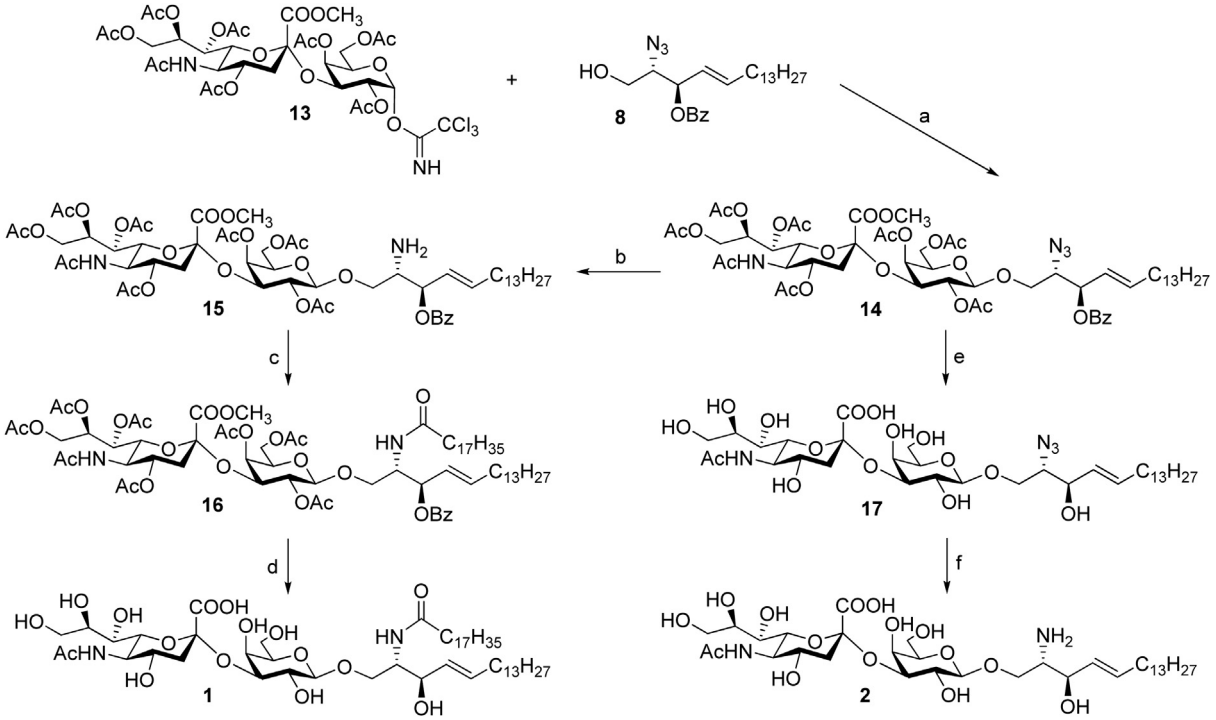
Scheme 1. Reagents and conditions: (a) MeCN, CH_2Cl_2, 4 Å powdered molecular sieves, 1 h, AgOTf, DTBP, −68 °C, PhSCl, 3 h, 75%; (b) Pd/C, MeOH, H_2, 40 °C, 5 h, 98%; (c) Pyr., Ac_2O, r.t., 18 h, quant.; (d) CF_3COOH, CH_2Cl_2, 0 °C, 1 h, r.t., 1 h; (e) CCl_3CN, DBU, CH_2Cl_2, −5 °C, 3 h, 88% (two steps from 11).

antiproliferative activity and displayed a better cytotoxicity against cancer cell than HaCaT normal cell, indicating that a (α2 → 6)-glycosidic linkage is more important than a (α2 → 3) one in GM3 molecule for such a biological activity. Based on this observation, we are planning to design and synthesize galactose-containing GM3 analogues having a structure of NeuAcα2 → 6Gal to analyze their *in vitro* cytotoxicity in future. The cytotoxicity of these derivatives is possibly caused by their inhibition on activity of various growth factor receptor (GFR)-associated tyrosine kinases, as it was reported that exogenous addition of GM3 inhibited BHK cell growth induced by fibroblast growth factor [21] and the phosphorylation of platelet-derived GFR [22] and epidermal GFR (EGFR) [23]. Being GM3 analogues, it is logical that these compounds demonstrated a similar biological activity. Further study is needed to understand the mechanism.

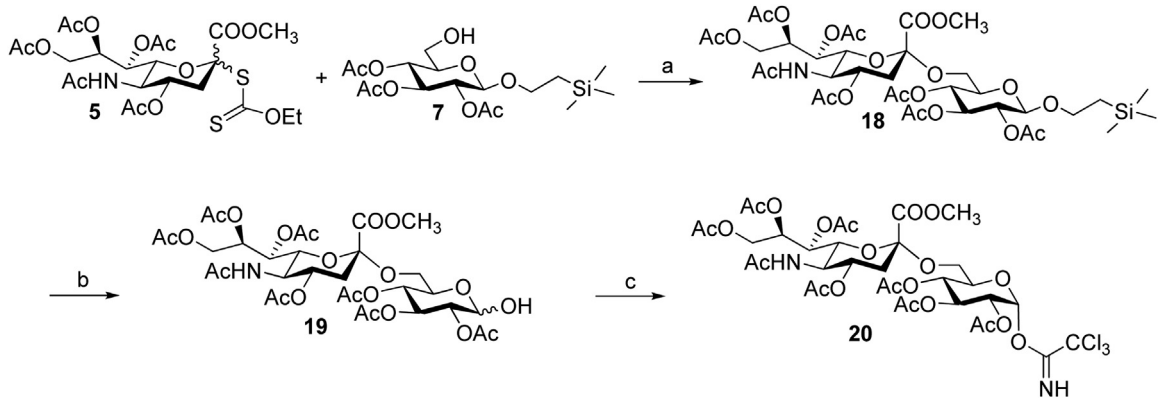
4. Experimental section

4.1. General chemical methods

All chemicals were purchased as reagent grade and used without further purification. PhSCl was prepared as previously reported [9]. CH_2Cl_2 was freshly distilled from P_2O_5. All reactions were carried out under anhydrous conditions with freshly distilled



Scheme 2. Reagents and conditions: (a) $BF_3.Et_2O$, CH_2Cl_2, 4 Å powdered molecular sieves, −15 °C, 2.5 h, 83%; (b) PPh_3, toluene, H_2O, 45 °C, 12 h, 76%; (c) Stearic acid, EDC, CH_2Cl_2, r.t., 20 h; (d) NaOMe, MeOH, r.t., 14 h; H_2O, 0 °C, 1 h, 81% (two steps from 15); (e) NaOMe, MeOH, r.t., 14 h; H_2O, 0 °C, 1 h, 98%; (f) $HS(CH_2)_3SH$, Et_3N, MeOH, r.t., 4 days, 93%.



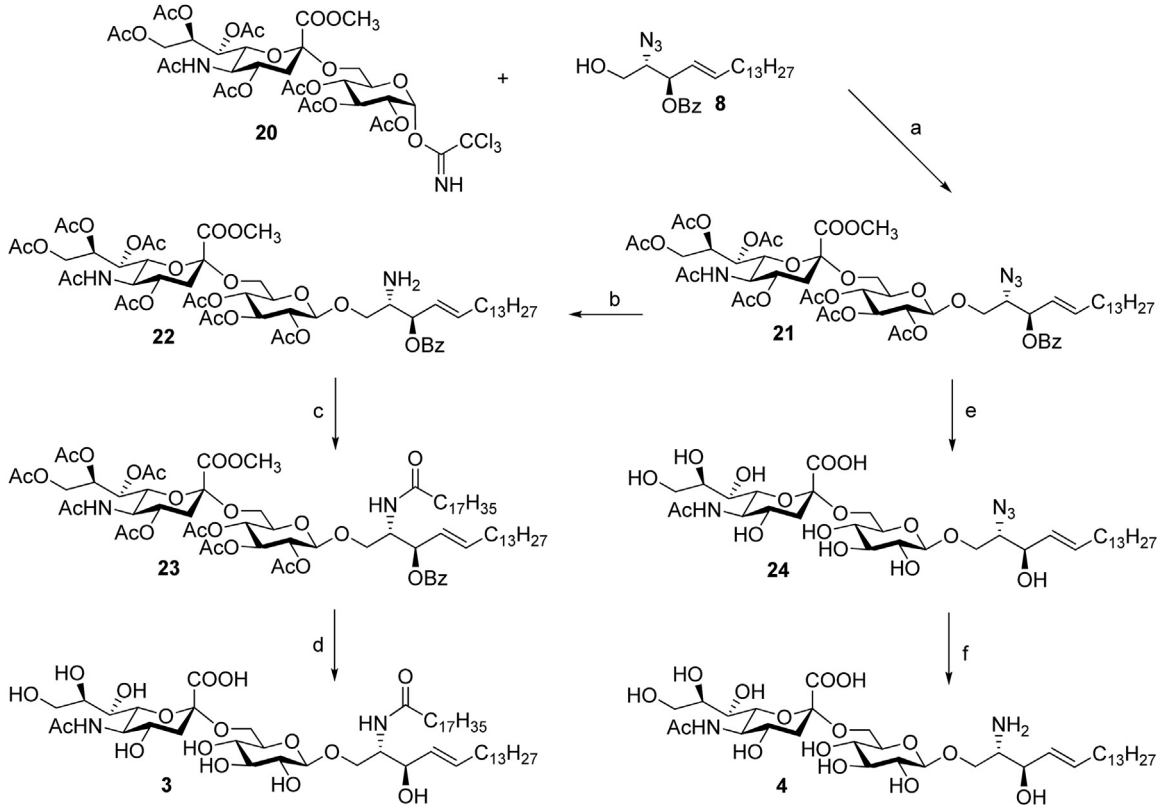
Scheme 3. Reagents and conditions: (a) MeCN, CH_2Cl_2, 4 Å powdered molecular sieves, 1 h, AgOTf, DTBP, −68 °C, PhSCl, 3 h, 82%; (b) CF_3COOH, CH_2Cl_2, 0 °C, 1 h, r.t., 1 h; (c) CCl_3CN, DBU, CH_2Cl_2, −5 °C, 3 h, 85% (two steps from 18).

solvents, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of silica gel 60 F_{254} (Merck) and detection by staining with sulfuric acid or acidic ceric ammonium molybdate. Solvents were evaporated under reduced pressure and below 40 °C (bath). Flash column chromatography was performed on silica gel 60 (230–400 mesh, Merck). Proton nuclear magnetic resonance (^{1}H NMR) and Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded at 400 (100.6) and 300 (75.5) MHz with Bruker AVANCE DRX 400 and 300 spectrometers. The chemical shifts were referenced to the solvent peak, $\delta = 7.26$ ppm (^{1}H) and $\delta = 77.16$ ppm (^{13}C) for $CDCl_3$, $\delta = 3.31$ ppm (^{1}H) and $\delta = 49.00$ ppm (^{13}C) for CD_3OD, $\delta = 2.50$ ppm (^{1}H) and $\delta = 39.52$ ppm (^{13}C) for DMSO, at 25 °C, and coupling constants were given in Hz. High-resolution mass spectra (HRMS) were recorded with a Bruker micrOTOF spectrometer in electrospray ionization (ESI) mode, using Tuning-Mix as reference. Optical rotations were measured at 589 nm (Na line) at 20 °C with a Perkin–Elmer Model 343 digital polarimeter, using a 10 cm, 1 mL cell.

4.2. 2-(Trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-2,6-di-O-benzyl-β-D-galactopyranoside (**9**)

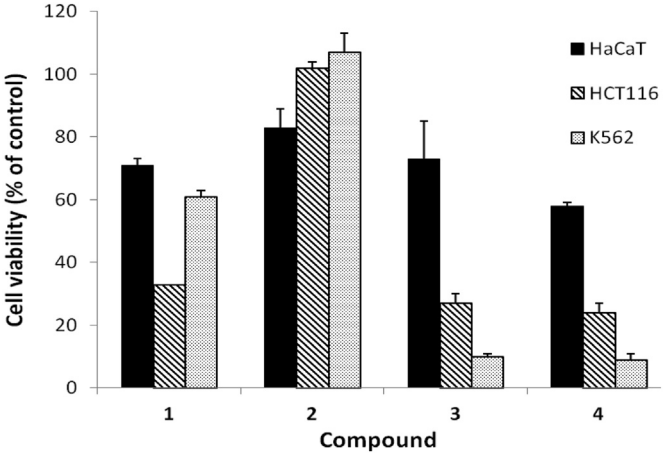
4.2.1. Method A (**5** + **6**)

A mixture of compound **5** (1.06 g, 1.78 mmol) and **6** (548.0 mg, 1.19 mmol), 4 Å powdered molecular sieves (2.50 g), dry CH_3CN (20 mL), and dry CH_2Cl_2 (10 mL) was stirred under nitrogen for 1 h. AgOTf (611.5 mg, 2.38 mmol) and DTBP (0.56 mL, 2.50 mmol) were



Scheme 4. Reagents and conditions: (a) $BF_3.Et_2O$, CH_2Cl_2, 4 Å powdered molecular sieves, −15 °C, 2.5 h, 77%; (b) PPh_3, toluene, H_2O, 45 °C, 12 h, 82%; (c) Stearic acid, EDC, CH_2Cl_2, r.t., 20 h; (d) NaOMe, MeOH, r.t., 14 h; H_2O, 0 °C, 1 h, 79% (two steps from 22); (e) NaOMe, MeOH, r.t., 14 h; H_2O, 0 °C, 1 h; (f) $HS(CH_2)_3SH$, Et_3N, MeOH, r.t., 4 days, 90% (two steps from 21).

Table 2
Cytotoxicity (IC_{50}[a]) of synthetic compounds against different human cell lines.



Human cell lines	Compounds			
	1	2	3	4
HaCaT (human HaCaT keratinocyte line)	nd[b]	nd[b]	nd[b]	nd[b]
HCT116 (human colorectal carcinoma HCT116 cells)	540	nd[b]	480	430
K562 (human leukemia K562 cells)	nd[b]	nd[b]	270	320

[a] IC_{50}(μM): A sample's concentration which produces a 50% reduction cell growth. Each drug concentration was tested in triplicate.
[b] nd: Not-detected.

added, and the mixture was cooled to −68 °C and kept protected from light. PhSCl (0.28 mL, 2.42 mmol) in dry CH_2Cl_2 (1 mL) was added by running the solution down the cold wall of the reaction flask, and the stirring was continued for 3 h at −68 °C. The mixture was diluted with a suspension of silica gel (5 g) in EtOAc (30 mL), filtered (Celite), washed (saturated aqueous $NaHCO_3$ and water), dried ($MgSO_4$), and concentrated. The residue was chromatographed (Cy-EtOAc 1:3) to give 9 as a white amorphous solid (833.6 mg, 75%).

4.2.2. Method B (**5a** + **6**)

To a mixture of compound **5a** (39.1 mg, 0.07 mmol) and **6** (50.5 mg, 0.11 mmol), 4 Å powdered molecular sieves (150 mg) was added MeCN (10 mL), and the mixture was stirred at room temperature for 1 h. A solution of *N*-iodosuccinimide (24.7 mg, 0.11 mmol) in MeCN (0.3 mL) was added under nitrogen, and the temperature was lowered to −45 °C. Trifluoromethanesulfonic acid (4 μL, 0.05 mmol) was added, and the mixture was stirred for 3 h at −45 °C. iPr_2NH (50 μL) was added, and the stirring was continued for 5 min. The mixture was filtered (Celite), washed (saturated aqueous $NaHCO_3$ and water), dried ($MgSO_4$), and concentrated. The residue was chromatographed (Cy-EtOAc 1:3) to give 9 as a white amorphous solid (25.5 mg, 39%).

4.2.3. Method C (**5b** + **6**)

To a stirred mixture of **6** (262.6 mg, 0.57 mmol), Ag_2CO_3 (183.4 mg, 0.67 mmol), and 482.1 mg of drierite was added CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 1 h, then the mixture was cooled to −45 °C. A solution of **5b** (96.9 mg, 0.19 mmol) in 2 mL of CH_2Cl_2 was added dropwise. After stirring at −45 °C for 20 h, then at room temperature for 1 day, the mixture was filtered. The filtrate was washed with water, dried ($MgSO_4$), and concentrated. The residue was chromatographed (Cy-EtOAc 1:3) to give 9 as a white amorphous solid (9.1 mg, 5%).

4.2.4. Method D (**5b** + **6**)

To a stirred mixture of **6** (180.7 mg, 0.39 mmol), $HgBr_2$ (194.6 mg, 0.54 mmol), $Hg(CN)_2$ (48.0 mg, 0.19 mmol), and 4 Å powdered molecular sieves (300 mg) in dry CH_2Cl_2 (15 mL) was added **5b** (200.2 mg, 0.39 mmol) in CH_2Cl_2 (2 mL). The mixture was stirred at room temperature for 2 days and then diluted with EtOAc (20 mL), and the solution was washed with 30% KI aqueous solution (20 mL × 3), dried over $MgSO_4$, and concentrated. The residual

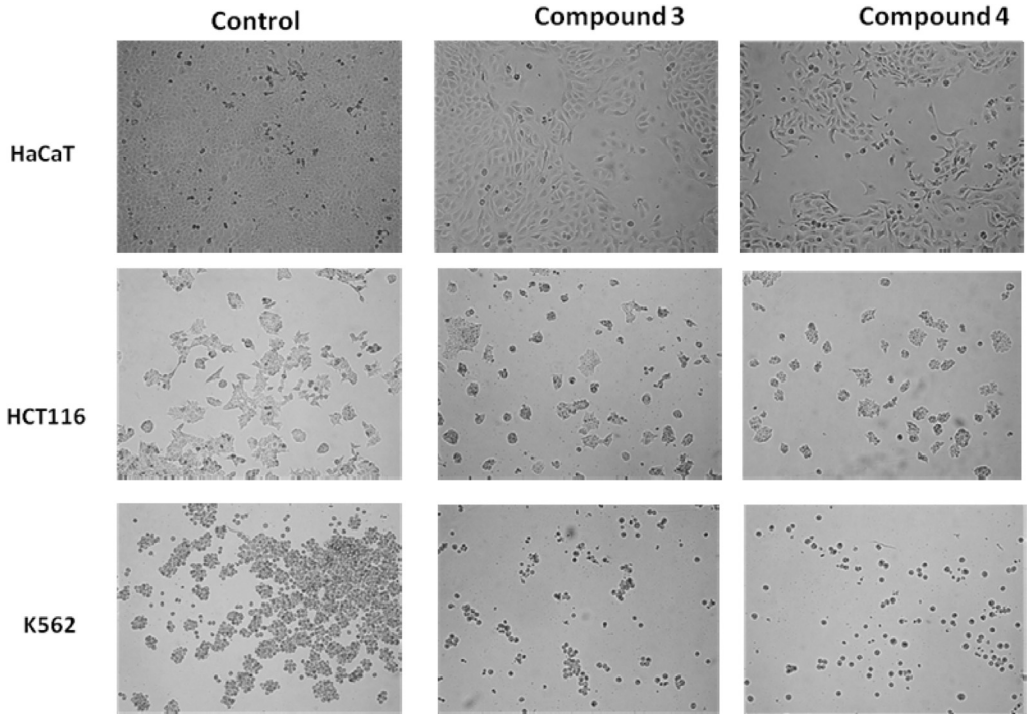


Fig. 3. The effect of selected compounds **3** and **4** on the cell morphology.

syrup was chromatographed (Cy-EtOAc 1:3) to give **9** as a white amorphous solid (29.1 mg, 8%). R_f = 0.38 (Cy-EtOAc 1:5). $[\alpha]_D^{20}$ = −12.0 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.25 (m, 10H, Ar–H), 5.46–5.35 (m, 1H, H-8), 5.30 (dd, *J* = 8.1, 2.1 Hz, 1H, H-7), 5.19 (d, *J* = 9.6 Hz, 1H, NH), 4.92–4.80 (m, 2H, H-4, OC*H*$_2$Ph), 4.71 (d, *J* = 11.8 Hz, 1H, OC*H*$_2$Ph), 4.58 (s, 2H, OC*H*$_2$Ph), 4.43 (d, *J* = 7.7 Hz, 1H, H-1′), 4.31 (dd, *J* = 12.5, 2.6 Hz, 1H, Ha-9), 4.14 (dd, *J* = 9.6, 3.3 Hz, 1H, H-3′), 4.11–3.94 (m, 4H, H-5, H-6, OC*H*$_a$CH$_2$Si, Hb-9), 3.83–3.70 (m, 6H, H-4′, H$_2$-6′, $COOCH_3$), 3.67–3.59 (m, 2H, H-5′, OC*H*$_b$CH$_2$Si), 3.51 (dd, *J* = 9.5, 7.8 Hz, 1H, H-2′), 2.53 (dd, *J* = 13.0, 4.7 Hz, 1H, H-3eq), 2.09 (s, 3H, OAc), 2.07–2.03 (m, 1H, H-3ax), 2.01 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.86 (s, 3H, NAc), 1.02 (dd, *J* = 9.3, 8.0 Hz, 2H, OCH$_2$*CH*$_2$Si), 0.01 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.01 (C═O), 170.71 (C═O), 170.39 (C═O), 170.21 (C═O), 170.11 (C═O), 168.79 (C═O), 139.31 (C aromatic), 138.37 (C aromatic), 128.47 (2C, CH aromatic), 128.22 (2C, CH aromatic), 127.84 (2C, CH aromatic), 127.74 (C, CH aromatic), 127.69 (2C, CH aromatic), 127.41 (C, CH aromatic), 103.31 (C-1′), 98.12 (C-2), 77.85 (C-2′), 75.85 (C-3′), 74.95 (OCH$_2$Ph), 73.64 (OCH$_2$Ph), 72.86, 72.79 (C-5′, C-6), 69.47 (C-6′), 69.20 (C-4), 68.93 (C-8), 68.37 (C-4′), 67.48 (OCH$_2$CH$_2$Si), 67.40 (C-7), 62.46 (C-9), 53.13 (COO*CH*$_3$), 49.41 (C-5), 37.01 (C-3), 23.28 (CH_3, NAc), 21.26 (CH_3, OAc), 20.94 (CH_3, OAc), 20.89 (CH_3, OAc), 20.76 (CH_3, OAc), 18.59 (OCH$_2$CH$_2$Si), −1.31 (3C, Si(CH$_3$)$_3$). ESI-HRMS (*m*/*z*) calcd for $C_{45}H_{63}NO_{18}SiNa$ $[M + Na]^+$: 956.3707, found: 956.3714.

4.3. 2-(Trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-β-D-galactopyranoside (**10**)

A solution of **9** (524.2 mg, 0.561 mmol) in methanol (15 mL) was treated with Pd/C (10%, 200 mg) under H_2 (160 kPa) for 5 h at 40 °C, then filtered and evaporated. The residue was purified by flash column chromatography (CH_2Cl_2–MeOH 15:1). Compound **10** was obtained (414.6 mg, 98%) as a white amorphous solid. R_f = 0.39 (CH_2Cl_2–MeOH 15:1). $[\alpha]_D^{20}$ = −10.2 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.48–5.36 (m, 2H, H-8, NH), 5.31–5.25 (m, 1H, H-7), 4.99–4.87 (m, 1H, H-4), 4.39 (d, *J* = 7.7 Hz, 1H, H-1′), 4.29 (dd, *J* = 12.4, 2.4 Hz, 1H, Ha-9), 3.99 (ddd, *J* = 13.6, 9.9, 4.6 Hz, 5H, H-6, H-3′, Hb-9, H-5, OC*H*$_a$CH$_2$Si), 3.84 (t, *J* = 5.7 Hz, 2H, H$_2$-6′), 3.80 (s, 3H, $COOCH_3$), 3.73 (s, 1H, H-4′), 3.68–3.58 (m, 2H, H-2′, OC*H*$_b$CH$_2$Si), 3.52 (t, *J* = 5.5 Hz, 1H, H-5′), 2.83 (s, 1H, OH), 2.74–2.64 (m, 3H, OH, H-3eq, OH), 2.10 (d, *J* = 2.3 Hz, 6H, 2 × OAc), 2.07–2.04 (m, 1H, H-3ax), 2.01 (s, 6H, 2 × OAc), 1.86 (s, 3H, NAc), 1.11–0.94 (m, 2H, OCH$_2$*CH*$_2$Si), −0.01 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.99 (C═O), 170.83 (C═O), 170.48 (C═O), 170.30 (C═O), 170.15 (C═O), 168.36 (C═O), 102.64 (C-1′), 97.84 (C-2), 76.90 (C-3′), 73.73 (C-5′), 72.76 (C-6), 69.37 (C-2′), 68.72 (C-4), 68.62 (C-4′), 68.38 (C-8), 67.26 (OCH$_2$CH$_2$Si), 67.16 (C-7), 62.63 (C-9), 62.19 (C-6′), 53.34 (COO*CH*$_3$), 49.53 (C-5), 37.49 (C-3), 23.21 (CH_3, NAc), 21.29 (CH_3, OAc), 20.91 (CH_3, OAc), 20.85 (CH_3, OAc), 20.84 (CH_3, OAc), 18.28 (OCH$_2$*CH*$_2$Si), −1.32 (3C, Si(CH$_3$)$_3$). ESI-HRMS (*m*/*z*) calcd for $C_{31}H_{51}NO_{18}SiNa$ $[M + Na]^+$: 776.2768, found: 776.2804.

4.4. 2-(Trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-2,4,6-tri-O-acetyl-β-D-galactopyranoside (**11**)

A solution of **5** (350.2 mg, 0.465 mmol) in 8 mL of pyridine and 4 mL of acetic anhydride was stirred at room temperature for 8 h and then concentrated, co-evaporated with toluene. The resulting residue was purified by flash column chromatography (Cy-EtOAc 1:5). Compound **7** was obtained (408.8 mg, quantitative) as a white amorphous solid. R_f = 0.34 (EtOAc). $[\alpha]_D^{20}$ = −9.2 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.60–5.49 (m, 1H, H-8), 5.35 (dd, *J* = 9.1, 2.4 Hz, 1H, H-7), 5.16 (d, *J* = 10.0 Hz, 1H, NH), 4.99 (dd, *J* = 9.9, 8.2 Hz, 1H, H-2′), 4.89 (d, *J* = 2.6 Hz, 1H, H-4′), 4.84 (dd, *J* = 10.7, 4.5 Hz, 1H, H-4), 4.56 (d, *J* = 8.0 Hz, 1H, H-1′), 4.51 (dd, *J* = 10.1, 3.3 Hz, 1H, H-3′), 4.34 (dd, *J* = 12.4, 2.5 Hz, 1H, Ha-9), 4.08–3.91 (m, 5H, H$_2$-6′, H-5, Hb-9, OC*H*$_a$CH$_2$Si), 3.86–3.79 (m, 4H, H-5′, $COOCH_3$), 3.63 (dd, *J* = 10.8, 2.6 Hz, 1H, H-6), 3.57 (dt, *J* = 9.8, 5.1 Hz, 1H, OC*H*$_b$CH$_2$Si), 2.56 (dd, *J* = 12.7, 4.6 Hz, 1H, H-3eq), 2.18 (s, 3H, OAc), 2.16 (s, 3H, OAc), 2.06 (d, *J* = 1.4 Hz, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.98 (s, 3H, OAc), 1.83 (s, 3H, NAc), 1.69 (t, *J* = 12.4 Hz, 1H, H-3ax), 1.03–0.85 (m, 2H, OCH$_2$*CH*$_2$Si), −0.01 (s, 9H, Si(CH$_3$)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.96 (C═O), 170.66 (C═O), 170.59 (C═O), 170.46 (C═O), 170.45 (2 × C═O), 169.74 (2 × C═O), 168.11 (C═O), 100.59 (C-1′), 96.89 (C-2), 72.14 (C-6), 71.69 (C-3′), 70.54 (C-5′), 69.96 (C-2′), 69.45 (C-4), 68.00 (C-8), 67.82 (C-4′), 67.46 (OCH$_2$CH$_2$Si), 67.16 (C-7), 62.51 (C-9), 62.15 (C-6′), 53.23 (COO*CH*$_3$), 49.21 (C-5), 37.64 (C-3), 23.25 (CH_3, NAc), 21.55 (CH_3, OAc), 21.15 (CH_3, OAc), 20.86 (3 × CH_3, OAc), 20.82 (CH_3, OAc), 20.75 (CH_3, OAc), 18.05 (OCH$_2$*CH*$_2$Si), −1.31 (3C, Si(CH$_3$)$_3$). ESI-HRMS (*m*/*z*) calcd for $C_{37}H_{57}NO_{21}SiNa$ $[M + Na]^+$: 902.3085, found: 902.3117.

4.5. O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-2,4,6-tri-O-acetyl-β-D-galactopyranosyl trichloroacetimidate (**13**)

To a solution of compound **11** (151.8 mg, 0.173 mmol) in 2 mL of CH_2Cl_2 was added 4 mL of trifluoroacetic acid dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. Then the mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and then with brine, dried over $MgSO_4$. After concentration, the residue was purified by flash column chromatography (CH_2Cl_2–MeOH 25:1). The crude intermediate **12** obtained in α/β isomers, R_f = 0.25 (CH_2Cl_2–MeOH 25:1), were dissolved in 4 mL of dry CH_2Cl_2, 0.4 mL of trichloroacetonitrile was added to the solution and then 34 μL of DBU was added dropwise at −5 °C under nitrogen. The mixture was stirred at −5 °C for 3 h. After concentration, the residue was purified by flash column chromatography (CH_2Cl_2–MeOH 25:1) to afford compound **13** as white foam (140.7 mg, 88% for two steps). R_f = 0.31 (CH_2Cl_2–MeOH 25:1). $[\alpha]_D^{20}$ = +7.9 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.64 (d, *J* = 9.8 Hz, 1H, C═NH), 6.49 (d, *J* = 3.9 Hz, 1H, H-1′), 5.52 (dd, *J* = 5.6, 3.1 Hz, 1H, H-8), 5.39–5.20 (m, 3H, H-7, NH, H-2′), 4.96 (d, *J* = 3.5 Hz, 1H, H-4′), 4.86 (dd, *J* = 4.5, 1.6 Hz, 1H, H-4), 4.71 (dd, *J* = 10.1, 3.5 Hz, 1H, H-3′), 4.39 (dd, *J* = 12.4, 2.4 Hz, 1H, Ha-9), 4.09–3.89 (m, 5H, H$_2$-6′, H-5′, H-5, Hb-9), 3.83 (s, 3H, $COOCH_3$), 3.64 (dd, *J* = 10.7, 2.7 Hz, 1H, H-6), 2.57 (dd, *J* = 12.6, 4.6 Hz, 1H, H-3eq), 2.15 (s, 3H, OAc), 2.13 (d, *J* = 5.7 Hz, 3H, OAc), 2.09 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.81 (s, 3H, NAc), 1.69 (t, *J* = 12.4 Hz, 1H, H-3ax). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.89 (C═O), 170.67 (2 × C═O), 170.45 (C═O), 170.42 (C═O), 170.30 (C═O), 169.72 (C═O), 169.45 (C═O), 168.00 (C═O), 161.17 (C═NH), 96.68 (C-2), 94.11 (C-1′), 90.72 (CCl_3), 72.30 (C-6), 71.67 (C-5′), 71.17 (C-3′), 69.40 (C-4), 68.80 (C-2′), 68.31 (C-8), 67.46 (C-4′), 67.29 (C-7), 62.62 (C-9), 61.64 (C-6′), 53.26 (COO*CH*$_3$), 49.09 (C-5), 37.56 (C-3), 23.19 (CH_3, NAc), 21.53 (CH_3, OAc), 20.83 (4 × CH_3, OAc), 20.76 (CH_3, OAc), 20.73 (CH_3, OAc). ESI-HRMS (*m*/*z*) calcd for $C_{34}H_{45}Cl_3N_2O_{21}Na$ $[M + Na]^+$: 945.1473, found: 945.1437.

4.6. O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-l,3-diol (**14**)

A solution of **13** (138.1 mg, 0.149 mmol) and 3-*O*-benzoyl-azidosphingosine **6** (115.6 mg, 0.269 mmol) in 5 mL of dry CH_2Cl_2 was

stirred with 4 Å powdered molecular sieves (300 mg) under nitrogen. The mixture was cooled to −15 °C, and $BF_3{\cdot}Et_2O$ (94 μL, 0.74 mmol) was added dropwise, stirred for 2.5 h at −15 °C and then filtered through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ and then with water, dried over $MgSO_4$ and concentrated. The residue was applied to a flash chromatography eluted with CH_2Cl_2–MeOH 25:1 to give the product **14** (147.3 mg, 83%) as an amorphous solid. R_f = 0.38 (CH_2Cl_2–MeOH 25:1). $[\alpha]_D^{20} = -14.5$ (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–7.97 (m, 2H, Ar–H), 7.62–7.38 (m, 3H, Ar–H), 5.92 (dd, *J* = 14.2, 7.3 Hz, 1H, H-5″), 5.65–5.45 (m, 3H, H-3″, H-4″, H-8), 5.38 (dd, *J* = 9.0, 2.7 Hz, 1H, H-7), 5.16 (d, *J* = 10.2 Hz, 1H, NH), 5.05 (dd, *J* = 10.1, 8.0 Hz, 1H, H-2′), 4.88 (ddd, *J* = 12.1, 9.1, 3.6 Hz, 2H, H-4, H-4′), 4.64 (d, *J* = 8.0 Hz, 1H, H-1′), 4.55 (dd, *J* = 10.1, 3.4 Hz, 1H, H-3′), 4.35 (dd, *J* = 12.4, 2.7 Hz, 1H, Ha-9), 4.09–3.93 (m, 5H, H-5, H_2-6′, H-2″, Hb-9), 3.89–3.79 (m, 5H, Ha-1″, H-5′, $COOCH_3$), 3.71–3.60 (m, 2H, Hb-1″, H-6), 2.57 (dd, *J* = 12.7, 4.6 Hz, 1H, H-3eq), 2.26–2.20 (m, 3H, OAc), 2.16 (d, *J* = 2.2 Hz, 3H, OAc), 2.08 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.05 (m, 2H, H_2-6″), 2.02 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.97 (s, 3H, OAc), 1.84 (s, 3H, NAc), 1.70 (td, *J* = 12.5, 4.1 Hz, 1H, H-3ax), 1.36 (m, 2H, H_2-7″), 1.24 (s, 20H, 10 × CH_2), 0.86 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.99 (C=O), 170.72 (C=O), 170.59 (C=O), 170.48 (C=O), 170.43 (C=O), 170.41 (C=O), 169.75 (C=O), 169.74 (C=O), 168.11 (C=O), 165.21 (PhC = O), 138.88 (C-5″), 133.25 (C, CH aromatic), 130.15 (C aromatic), 129.87 (2C, CH aromatic), 128.54 (2C, CH aromatic), 122.92 (C-4″), 100.71 (C-1′), 96.91 (C-2), 74.92 (C-3″), 72.21 (C-6), 71.50 (C-3′), 70.88 (C-5′), 69.51 (C-4′), 69.43 (C-2′), 68.04 (2C, C-8, C-1″), 67.71 (C-4), 67.19 (C-7), 63.80 (C-2″), 62.40 (C-9), 62.08 (C-6′), 53.27 ($COOCH_3$), 49.26 (C-5), 37.62 (C-3), 32.48 (CH_2, C-6″), 32.02, 29.78, 29.75, 29.69, 29.52, 29.45, 29.28, 28.80, 22.78 (11 × CH_2), 23.27 (CH_3, NAc), 21.57 (CH_3, OAc), 21.08 (CH_3, OAc), 20.87 (3 × CH_3, OAc), 20.81 (CH_3, OAc), 20.71 (CH_3, OAc), 14.21 (CH_3). ESI-HRMS (*m*/*z*) calcd for $C_{57}H_{82}N_4O_{23}Na$ $[M + Na]^+$: 1213.5262, found: 1213.5272.

4.7. *O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 3)-(2,4,6-tri-O-acetyl-β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-2-amino-3-O-benzoyl-4-octadecene-l,3-diol (**15**)*

To a solution of compound **14** (58.3 mg, 0.049 mmol) in 5 mL of toluene and 0.2 mL of water was added 32.1 mg of triphenylphosphine. The mixture was stirred at 45 °C for 12 h. After concentration, the residue obtained was flash-chromatographed, eluting with CH_2Cl_2–MeOH 20:1 to afford **15** (43.4 mg, 76%) as an amorphous solid. R_f = 0.32 (CH_2Cl_2–MeOH 20:1). $[\alpha]_D^{20} = +1.0$ (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13–7.72 (m, 2H, Ar–H), 7.57–7.34 (m, 3H, Ar–H), 5.93–5.68 (m, 1H, H-5″), 5.52 (ddd, *J* = 12.0, 7.3, 2.0 Hz, 2H, H-4″, H-8), 5.44–5.30 (m, 2H, H-3″, H-7), 5.26–5.21 (m, 1H, NH), 5.05–4.93 (m, 1H, H-2′), 4.87 (dt, *J* = 12.1, 4.6 Hz, 2H, H-4′, H-4), 4.59 (d, *J* = 7.9 Hz, 1H, H-1′), 4.57–4.50 (m, 1H, H-3′), 4.34 (dd, *J* = 12.3, 2.6 Hz, 1H, Ha-9), 4.06–3.91 (m, 4H, H-5, H_2-6′, Hb-9), 3.87–3.76 (m, 5H, H-5′, $COOCH_3$, Ha-1″), 3.76–3.69 (m, 1H, Hb-1″), 3.63 (dt, *J* = 10.5, 3.3 Hz, 1H, H-6), 3.30 (dd, *J* = 10.1, 6.5 Hz, 1H, H-2″), 3.09 (s, 2H, NH_2), 2.56 (dd, *J* = 12.7, 4.6 Hz, 1H, H-3eq), 2.19 (s, 3H, OAc), 2.14 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.02 (d, *J* = 5.3 Hz, 2H, H_2-6″), 2.00 (s, 3H, OAc), 1.97 (d, *J* = 7.5 Hz, 6H, 2 × OAc), 1.83 (s, 3H, NAc), 1.68 (t, *J* = 12.4 Hz, 1H, H-3ax), 1.39–1.31 (m, 2H, H_2-7″), 1.24–1.17 (m, 20H, 10 × CH_2), 0.85 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.96 (C=O), 170.66 (C=O), 170.59 (C=O), 170.50 (C=O), 170.39 (2 × C=O), 169.85 (C=O), 169.74 (C=O), 168.06 (C=O), 165.39 (PhC = O), 137.78 (C-5″), 133.03 (C, CH aromatic), 130.55 (C aromatic), 129.70 (2C, CH aromatic), 128.45 (2C, CH aromatic), 124.43 (C-4″), 100.88 (C-1′), 96.88 (C-2), 76.26 (C-3″), 72.16 (C-6), 71.45 (C-3′), 70.70 (C-5′), 70.66 (C-1″), 69.82 (C-2′), 69.43 (C-4′), 67.99 (C-8), 67.71 (C-4), 67.19 (C-7), 62.48 (C-9), 62.02 (C-6′), 53.69 (C-2″), 53.22 ($COOCH_3$), 49.19 (C-5), 37.62 (C-3), 32.50 (CH_2, C-6″), 31.99, 29.75, 29.73, 29.67, 29.52, 29.42, 29.31, 29.00, 22.76 (11 × CH_2), 23.22 (CH_3, NAc), 21.53 (CH_3, OAc), 21.05 (CH_3, OAc), 20.84 (3 × CH_3, OAc), 20.78 (CH_3, OAc), 20.69 (CH_3, OAc), 14.19 (CH_3). ESI-HRMS (*m*/*z*) calcd for $C_{57}H_{85}N_2O_{23}$ $[M + H]^+$: 1165.5538, found: 1165.5532.

4.8. *O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulo pyranosylonic acid)-(2 → 3)-(β-D-galactopyranosyl)-(1→1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-l,3-diol (**1**)*

The mixture of **15** (19.9 mg, 0.017 mmol), stearic acid (16.8 mg, 0.059 mmol), EDC (16 μL, 0.090 mmol) in 4 mL of CH_2Cl_2 was stirred at room temperature for 20 h. Then the mixture was washed with water, dried over $MgSO_4$ and concentrated. The resulting residue was purified by flash column chromatography (Cy-EtOAc 1:3) to afford crude intermediate **16** as an amorphous solid. R_f = 0.23 (Cy-EtOAc 1:3). A solution of this crude product in 5 mL of NaOMe/MeOH (0.04 M) was stirred at room temperature for 14 h. A few drops of water were added at 0 °C. After stirring at room temperature for 1 h, the mixture was neutralized by Amberlite IR 120/H^+ ion exchange resin. After filtration and concentration, the residue obtained was flash-chromatographed, eluting with $CHCl_3$–MeOH 3:1 to afford **1** (14.0 mg, 81% for two steps) as a white amorphous solid. R_f = 0.35 (EtOAc-iPrOH-H_2O 3:2:1). The NMR spectral data were in good agreement with those reported in literature [24]. ESI-HRMS (*m*/*z*) calcd for $C_{53}H_{97}N_2O_{16}$ $[M - H]^-$: 1017.6833, found: 1017.6865.

4.9. *O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulo pyranosylonic acid)-(2 → 3)-(β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-2-azido-4-octadecene-l,3-diol (**17**)*

A solution of compound **14** (66.9 mg, 0.056 mmol) in 5 mL of NaOMe/MeOH (0.04 M) was stirred at room temperature for 14 h. A few drops of water were added at 0 °C. After stirring at room temperature for 1 h, the mixture was neutralized by Amberlite IR 120/H^+ ion exchange resin. After filtration and concentration, the residue obtained was flash-chromatographed, eluting with $CHCl_3$–MeOH 3:1 to afford **17** (42.5 mg, 98%) as an amorphous solid. R_f = 0.33 (EtOAc-iPrOH-H_2O 3:2:1). $[\alpha]_D^{20} = -5.5$ (*c* 1.0 in $CHCl_3$–MeOH 1:1). ^{1}H NMR (400 MHz, CD_3OD): δ 5.76 (m, 1H, H-5″), 5.58–5.44 (m, 1H, H-4″), 4.30 (d, *J* = 7.8 Hz, 1H, H-1′), 4.22–4.16 (m, 1H, H-3″), 4.03 (dd, *J* = 9.6, 3.1 Hz, 1H, H-3′), 3.95–3.91 (m, 1H, H-4′), 3.88 (dd, *J* = 10.1, 2.9 Hz, 1H, Ha-1″), 3.86–3.83 (m, 1H, H-8), 3.82–3.78 (m, 1H, Ha-9), 3.72 (ddt, *J* = 8.1, 6.7, 4.2 Hz, 5H, Ha-6′, H-4, H-5, Hb-1″, Hb-6′), 3.67–3.59 (m, 4H, H-2″, Hb-9, H-6, NH), 3.59–3.55 (m, 1H, H-2′), 3.51 (dd, *J* = 10.6, 8.9 Hz, 2H, H-7, H-5′), 2.89–2.81 (m, 1H, H-3eq), 2.07 (dd, *J* = 13.3, 6.5 Hz, 2H, H_2-6″), 2.01 (s, 3H, NAc), 1.80–1.72 (m, 1H, H-3ax), 1.40 (m, 2H, H_2-7″), 1.29 (s, 20H, 10 × CH_2), 0.90 (t, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 175.43 (C=O), 135.77 (C-5″), 129.63 (C-4″), 104.96 (C-1′), 77.83 (C-3′), 76.61 (C-5′), 74.96 (C-6), 73.48 (C-3″), 72.84 (C-8), 70.66 (C-2′), 69.98 (C-1″), 69.94 (C-7), 69.28 (C-4), 68.98 (C-4′), 67.32 (C-2″), 64.44 (C-9), 62.66 (C-6′), 53.92 (C-5), 42.03 (C-3), 33.40 (CH_2, C-6″), 33.05, 30.77, 30.73, 30.59, 30.45, 30.25, 30.19, 23.71 (11 × CH_2), 22.62 (CH_3, NAc), 14.42 (CH_3). ESI-HRMS (*m*/*z*) calcd for $C_{35}H_{61}N_4O_{15}$ $[M - H]^-$: 777.4139, found: 777.4109.

4.10. *O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulo pyranosylonic acid)-(2 → 3)-(β-D-galactopyranosyl)-(1 → 1)-(2S,3R,4E)-2-amino-4-octadecene-l,3-diol (**2**)*

To a solution of **17** (27.8 mg, 0.036 mmol) in dry MeOH (3 mL) were added under nitrogen propane-1,3-dithiol (0.3 mL) and triethylamine (0.3 mL), and the mixture was stirred at room

temperature for 4 days. A white precipitate was formed. After filtration, and washing with MeOH, the filtrate was concentrated. The residue obtained was flash-chromatographed, eluting with $CHCl_3$–MeOH 3:1 to afford **2** (25.2 mg, 93%) as a white amorphous solid. R_f = 0.28 (EtOAc-iPrOH-H_2O 3:2:1). $[\alpha]_D^{20}$ = −2.3 (*c* 1.0 in $CHCl_3$–MeOH 1:1). ^{1}H NMR (400 MHz, CD_3OD): δ 5.93–5.81 (m, 1H, H-5″), 5.54–5.44 (m, 1H, H-4″), 4.35 (d, *J* = 7.8 Hz, 1H, H-1′), 4.33–4.29 (m, 1H, H-3″), 4.04 (dd, *J* = 9.7, 3.1 Hz, 1H, H-3′), 3.99–3.93 (m, 2H, Ha-1″, H-4′), 3.91 (d, *J* = 2.7 Hz, 1H, Hb-1″), 3.87–3.69 (m, 6H, H-8, Ha-9, Ha-6′, H-4, H-5, Hb-6′), 3.64–3.48 (m, 5H, Hb-9, H-6, H-2′, H-5′, H-7), 3.42–3.37 (m, 1H, H-2′), 3.35 (s, 1H, NH), 2.87 (dd, *J* = 12.5, 3.9 Hz, 1H, H-3eq), 2.10 (dd, *J* = 13.8, 6.7 Hz, 2H, H_2-6″), 2.02 (d, *J* = 1.7 Hz, 3H, NAc), 1.70 (d, *J* = 11.3 Hz, 1H, H-3ax), 1.42 (dd, *J* = 13.9, 6.7 Hz, 2H, H_2-7″), 1.29 (s, 20H, 10 × CH_2), 0.90 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, CD_3OD): δ 175.58 (C═O), 174.78 (C═O), 136.74 (C-5″), 128.29 (C-4″), 104.21 (C-1′), 101.00 (C-2), 77.60 (C-3′), 76.95 (C-5′), 74.99 (C-6), 72.92 (C-8), 70.86 (C-3″), 70.75 (C-2′), 70.01 (C-7), 69.20 (C-4), 68.94 (C-4′), 66.84 (C-1″), 64.56 (C-9), 62.76 (C-6′), 56.79 (C-2″), 53.99 (C-5), 42.19 (C-3), 33.37 (CH_2, C-6″), 33.05, 30.77, 30.73, 30.72, 30.62, 30.45, 30.40, 30.16, 23.71 (11 × CH_2), 22.60 (CH_3, NAc), 14.42 (CH_3). ESI-HRMS (*m*/*z*) calcd for $C_{35}H_{63}N_2O_{15}$ $[M - H]^-$: 751.4234, found: 751.4265.

4.11. 2-(Trimethylsilyl)ethyl O-(methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)-2,3,4-tri-O-acetyl-β-D-glucopyranoside (**18**)

A mixture of compound **5** (740.0 mg, 1.24 mmol) and **7** (336 mg, 0.83 mmol), 4 Å powdered molecular sieves (2.0 g), dry CH_3CN (20 mL), and dry CH_2Cl_2 (10 mL) was stirred under nitrogen for 1 h. AgOTf (427 mg, 1.66 mmol) and DTBP (0.39 mL, 1.74 mmol) were added, and the mixture was cooled to −68 °C and kept protected from light. PhSCl (0.20 mL, 1.74 mmol) in dry CH_2Cl_2 (1 mL) was added by running the solution down the cold wall of the reaction flask, and the stirring was continued for 3 h at −68 °C. The mixture was diluted with a suspension of silica gel (5 g) in EtOAc (30 mL), filtered (Celite), washed (saturated aqueous $NaHCO_3$ and water), dried ($MgSO_4$), and concentrated. The residue was chromatographed (Cy-EtOAc 1:3) to give **18** as a white amorphous solid (598.9 mg, 82%). R_f = 0.55 (Cy-EtOAc 1:3, 2 times). $[\alpha]_D^{20}$ = −0.6 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37–5.26 (m, 2H, H-8, H-7), 5.18–5.06 (m, 3H, H-5′, H-3′, NH), 4.98–4.79 (m, 2H, H-2′, H-4), 4.44 (d, *J* = 8.0 Hz, 1H, H-1′), 4.27 (dd, *J* = 12.4, 2.5 Hz, 1H, Ha-9), 4.13–3.86 (m, 5H, Hb-9, H-5, H-6, O*CH*$_a$$CH_2$Si, Ha-6′), 3.78 (s, 3H, $COOCH_3$), 3.63–3.48 (m, 3H, H-4′, Hb-6′, O*CH*$_b$$CH_2$Si), 2.61 (dd, *J* = 12.8, 4.6 Hz, 1H, H-3eq), 2.13 (d, *J* = 2.8 Hz, 6H, 2 × OAc), 2.05 (s, 3H, OAc), 2.02 (d, *J* = 3.0 Hz, 9H, 3 × OAc), 1.99 (s, 3H, OAc), 1.96–1.88 (m, 1H, H-3ax), 1.86 (s, 3H, NAc), 0.98–0.80 (m, 2H, OCH_2*CH*$_2$Si), −0.01 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.18 (C═O), 170.75 (C═O), 170.64 (C═O), 170.33 (C═O), 170.16 (2 × C═O), 169.45 (C═O), 169.19 (C═O), 167.65 (C═O), 100.38 (C-1′), 98.60 (C-2), 73.54 (C-3′), 72.44 (C-6), 72.38 (C-4′), 71.58 (C-2′), 69.17 (C-4), 68.45 (C-5′), 68.17 (C-8), 67.47 (OCH_2CH_2Si), 67.29 (C-7), 62.89 (C-6′), 62.49 (C-9), 52.82 ($COOCH_3$), 49.47 (C-5), 37.76 (C-3), 23.34 (CH_3, NAc), 21.31 (CH_3, OAc), 21.00 (CH_3, OAc), 20.98 (CH_3, OAc), 20.89 (2 × CH_3, OAc), 20.83 (CH_3, OAc), 20.81 (CH_3, OAc), 17.97 (OCH_2CH_2Si), −1.29 (3C, $Si(CH_3)_3$). ESI-HRMS (*m*/*z*) calcd for $C_{37}H_{57}NO_{21}SiNa$ $[M + Na]^+$: 902.3085, found: 902.3124.

4.12. O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 6)-2,3,4-tri-O-acetyl-β-D-glucopyranosyl trichloroacetimidate (**20**)

To a solution of compound **18** (361 mg, 0.41 mmol) in 3 mL of CH_2Cl_2 was added 6 mL of trifluoroacetic acid dropwise at 0 °C. The mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h. Then the mixture was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and then with brine, dried over $MgSO_4$. After concentration, the residue was purified by flash column chromatography (Cy-EtOAc 1:3). The crude intermediate **19** obtained in α/β isomers, R_f = 0.33 (EtOAc), were dissolved in 12 mL of dry CH_2Cl_2, 1.2 mL of trichloroacetonitrile was added to the solution and then 93 μL of DBU was added dropwise at −5 °C under nitrogen. The mixture was stirred at −5 °C for 3 h. After concentration, the residue was purified by flash column chromatography (Cy-EtOAc 1:4) to afford compound **20** as white foam (322.0 mg, 85% for two steps). R_f = 0.43 (EtOAc). $[\alpha]_D^{20}$ = +3.8 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (s, 1H, NH), 6.53 (d, *J* = 3.6 Hz, 1H, H-1′), 5.51 (t, *J* = 9.8 Hz, 1H, H-3′), 5.39 (m, 1H, H-8), 5.30–5.27 (m, 2H, H-5′, H-7), 5.09 (dd, *J* = 10.2, 3.6 Hz, 1H, H-2′), 4.86–4.80 (m, 1H, H-4), 4.23 (d, *J* = 11.3 Hz, 1H, Ha-9), 4.03 (m, 5H, H-4′, Hb-9, H-5, Ha-6′, H-6), 3.78 (s, 3H, $COOCH_3$), 3.40 (d, *J* = 11.1 Hz, 1H, Hb-6′), 2.59 (dd, *J* = 12.7, 4.4 Hz, 1H, H-3eq), 2.13 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.07 (s, 3H, OAc), 2.01 (s, 9H, 3 × OAc), 1.98 (s, 3H, OAc), 1.92 (m, 1H, H-3ax), 1.85 (s, 3H, NAc). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.16 (C═O), 170.72 (C═O), 170.32 (2 × C═O), 170.19 (C═O), 170.12 (C═O), 170.00 (C═O), 169.12 (C═O), 167.70 (C═O), 161.03 (C═NH), 98.37 (C-2), 93.26 (C-1′), 90.84 (CCl_3), 72.41, 70.66, 70.38, 69.92, 69.09, 68.03, 67.54, 67.25 (C-6, C-4′, C-3′, C-2′, C-4, C-8, C-5′, C-7), 62.48 (C-9), 61.96 (C-6′), 53.03 ($COOCH_3$), 49.43 (C-5), 37.79 (C-3), 23.31 (CH_3, NAc), 21.24 (CH_3, OAc), 20.97 (CH_3, OAc), 20.93 (CH_3, OAc), 20.85 (CH_3, OAc), 20.73 (2 × CH_3, OAc), 20.57 (CH_3, OAc). ESI-HRMS (*m*/*z*) calcd for $C_{34}H_{45}Cl_3N_2O_{21}Na$ $[M + Na]^+$: 945.1473, found: 945.1431.

4.13. O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 6)-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 1)-(2S,3R,4E)-2-azido-3-O-benzoyl-4-octadecene-l,3-diol (**21**)

A solution of **20** (114.6 mg, 0.124 mmol) and 3-*O*-benzoyl-azidosphingosine **6** (90.2 mg, 0.21 mmol) in 6 mL of dry CH_2Cl_2 was stirred with 4 Å powdered molecular sieves (500 mg) under nitrogen. The mixture was cooled to −15 °C, and $BF_3 \cdot Et_2O$ (78 μL, 0.62 mmol) was added dropwise, stirred for 2.5 h at −15 °C and then filtered through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ and then with water, dried over $MgSO_4$ and concentrated. The residue was applied to a flash chromatography eluted with Cy-EtOAc 1:2 to give the product **21** (113.7 mg, 77%) as an amorphous solid. R_f = 0.48 (EtOAc). $[\alpha]_D^{20}$ = −39.1 (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–8.01 (m, 2H, Ar–H), 7.59–7.53 (m, 1H, Ar–H), 7.48–7.41 (m, 2H, Ar–H), 5.95–5.87 (m, 1H, H-5″), 5.59–5.49 (m, 2H, H-3″, H-4″), 5.36–5.27 (m, 2H, H-8, H-7), 5.16 (ddd, *J* = 19.1, 8.1, 5.2 Hz, 3H, H-5′, H-3′, NH), 5.03–4.95 (m, 1H, H-2′), 4.85 (ddd, *J* = 12.3, 9.7, 4.7 Hz, 1H, H-4), 4.47 (d, *J* = 7.9 Hz, 1H, H-1′), 4.26 (dd, *J* = 12.4, 2.6 Hz, 1H, Ha-9), 4.02 (ddd, *J* = 11.2, 9.7, 3.2 Hz, 3H, Hb-9, H-5, H-6), 3.96–3.85 (m, 3H, H-2″, Ha-6′, Ha-1″), 3.75 (s, 3H, $COOCH_3$), 3.62 (dd, *J* = 5.7, 2.5 Hz, 1H, H-4′), 3.57–3.50 (m, 2H, Hb-1″, Hb-6′), 2.59 (dd, *J* = 12.8, 4.6 Hz, 1H, H-3eq), 2.12 (d, *J* = 1.5 Hz, 6H, 2 × OAc), 2.08 (s, 3H, OAc), 2.06 (d, *J* = 2.1 Hz, 2H, H_2-6″), 2.05 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.02 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.96–1.90 (m, 1H, H-3ax), 1.86 (s, 3H, NAc), 1.37 (d, *J* = 6.9 Hz, 2H, H_2-7″), 1.24 (s, 20H, 10 × CH_2), 0.87 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.12 (C═O), 170.71 (C═O), 170.55 (C═O), 170.32 (C═O), 170.17 (C═O), 170.11 (C═O), 169.40 (C═O), 169.13 (C═O), 167.75 (C═O), 165.21 (PhC═O), 139.22 (C-5″), 133.33 (C, CH aromatic), 130.08 (C aromatic), 129.89 (2C, CH aromatic), 128.59 (2C, CH aromatic), 122.73 (C-4″), 100.91 (C-1′), 98.61 (C-2), 75.02 (C-3″), 73.26 (C-3′), 72.67 (C-4′), 72.52 (C-6), 71.28 (C-2′), 69.12 (C-4), 68.41 (C-5′), 68.35 (C-1″), 68.17 (C-8), 67.35 (C-7), 63.71

(C-2″), 62.91 (C-6′), 62.55 (C-9), 52.85 (COO*CH*$_3$), 49.52 (C-5), 37.78 (C-3), 32.53 (CH$_2$, C-6″), 32.05, 29.81, 29.78, 29.73, 29.54, 29.48, 29.31, 28.90, 22.81 (11 × CH$_2$), 23.32 (CH$_3$, NAc), 21.26 (CH$_3$, OAc), 20.96 (CH$_3$, OAc), 20.95 (CH$_3$, OAc), 20.85 (CH$_3$, OAc), 20.79 (CH$_3$, OAc), 20.78 (CH$_3$, OAc), 20.76 (CH$_3$, OAc), 14.24 (CH$_3$). ESI-HRMS (*m/z*) calcd for $C_{57}H_{82}N_4O_{23}Na$ [M + Na]$^+$: 1213.5262, found: 1213.5274.

4.14. *O-(Methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2 → 6)-(2,3,4-tri-O-acetyl-β-D-glucopyranosyl)-(1 → 1)-(2S,3R,4E)-2-amino-3-O-benzoyl-4-octadecene-l,3-diol (**22**)*

To a solution of compound **21** (51.2 mg, 0.043 mmol) in 5 mL of toluene and 0.2 mL of water was added 28.2 mg of triphenylphosphine. The mixture was stirred at 45 °C for 12 h. After concentration, the residue obtained was flash-chromatographed, eluting with CH_2Cl_2–MeOH 30:1 to afford **22** (41.1 mg, 82%) as an amorphous solid. $R_f = 0.24$ (CH_2Cl_2–MeOH 30:1). $[\alpha]_D^{20} = +0.4$ (*c* 1.0 in $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–7.96 (m, 2H, Ar–H), 7.59–7.41 (m, 3H, Ar–H), 5.93–5.84 (m, 1H, H-5″), 5.51 (dd, *J* = 15.4, 7.7 Hz, 1H, H-4″), 5.40–5.28 (m, 4H, H-8, H-3″, NH, H-7), 5.18–5.12 (m, 2H, H-5′, H-3′), 5.00–4.94 (m, 1H, H-2′), 4.94–4.87 (m, 1H, H-4), 4.46 (d, *J* = 8.0 Hz, 1H, H-1′), 4.27 (dd, *J* = 12.4, 2.5 Hz, 1H, Ha-9), 4.03 (td, *J* = 11.7, 7.4 Hz, 3H, Hb-9, H-6, H-5), 3.90 (dd, *J* = 11.3, 4.5 Hz, 1H, Ha-6′), 3.82–3.78 (m, 1H, Ha-1″), 3.75 (s, 3H, COOCH$_3$), 3.61 (dd, *J* = 9.8, 4.4 Hz, 2H, H-4′, Hb-1″), 3.50 (dd, *J* = 11.3, 2.2 Hz, 1H, Hb-6′), 3.28 (dd, *J* = 11.0, 6.4 Hz, 1H, H-2″), 2.58 (dd, *J* = 13.1, 4.8 Hz, 1H, H-3eq), 2.13 (s, 3H, OAc), 2.11 (s, 3H, OAc), 2.10–2.06 (m, 2H, H$_2$-6″), 2.05 (s, 6H, 2 × OAc), 2.02 (s, 3H, OAc), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.94 (dd, *J* = 9.3, 3.5 Hz, 1H, H-3ax), 1.86 (s, 3H, NAc), 1.40–1.33 (m, 2H, H$_2$-7″), 1.26–1.21 (m, 20H, 10 × CH$_2$), 0.87 (t, *J* = 6.9 Hz, 3H, CH$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.09 (C=O), 170.73 (C=O), 170.55 (C=O), 170.40 (C=O), 170.17 (C=O), 170.07 (C=O), 169.46 (C=O), 169.20 (C=O), 167.82 (C=O), 165.43 (PhC = O), 138.05 (C-5″), 133.16 (C, CH aromatic), 130.53 (C aromatic), 129.71 (2C, CH aromatic), 128.56 (2C, CH aromatic), 124.31 (C-4″), 101.32 (C-1′), 98.50 (C-2), 76.43 (C-3″), 73.31 (C-3′), 72.67 (C-4′), 72.28 (C-6), 71.92 (C-1″), 71.52 (C-2′), 69.18 (C-4), 68.50 (C-5′), 68.30 (C-8), 67.39 (C-7), 62.78 (C-6′), 62.50 (C-9), 53.83 (C-2″), 52.90 (COO*CH*$_3$), 49.63 (C-5), 37.60 (C-3), 32.57 (CH$_2$, C-6″), 32.06, 29.79, 29.74, 29.58, 29.49, 29.36, 29.09, 22.83 (11 × CH$_2$), 23.34 (CH$_3$, NAc), 21.25 (CH$_3$, OAc), 21.02 (CH$_3$, OAc), 21.01 (CH$_3$, OAc), 20.87 (CH$_3$, OAc), 20.83 (CH$_3$, OAc), 20.80 (CH$_3$, OAc), 20.77 (CH$_3$, OAc), 14.25 (CH$_3$). ESI-HRMS (*m/z*) calcd for $C_{57}H_{85}N_2O_{23}$ [M + H]$^+$: 1165.5538, found: 1165.5527.

4.15. *O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2 → 6)-(β-D-glucopyranosyl)-(1 → 1)-(2S,3R,4E)-2-octadecanamido-4-octadecene-l,3-diol (**3**)*

The mixture of **22** (16.3 mg, 0.014 mmol), stearic acid (13.9 mg, 0.049 mmol), EDC (13 μL, 0.075 mmol) in 4 mL of CH_2Cl_2 was stirred at room temperature for 20 h. Then the mixture was washed with water, dried over $MgSO_4$ and concentrated. The resulting residue was purified by flash column chromatography (Cy-EtOAc 1:3) to afford crude intermediate **23** as an amorphous solid. $R_f = 0.41$ (Cy-EtOAc 1:3). A solution of this crude product in 5 mL of NaOMe/MeOH (0.04 M) was stirred at room temperature for 14 h. A few drops of water were added at 0 °C. After stirring at room temperature for 1 h, the mixture was neutralized by Amberlite IR 120/H$^+$ ion exchange resin. After filtration and concentration, the residue obtained was flash-chromatographed, eluting with $CHCl_3$–MeOH 3:1 to afford **3** (11.3 mg, 79% for two steps) as a white amorphous solid. R_f = 0.35 (EtOAc-iPrOH-H_2O 3:2:1). The NMR spectral data were in good agreement with those reported in literature [25]. ESI-HRMS (*m/z*) calcd for $C_{53}H_{97}N_2O_{16}$ [M – H]$^-$: 1017.6844, found: 1017.6864.

4.16. *O-(5-Acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonic acid)-(2 → 6)-(β-D-glucopyranosyl)-(1 → 1)-(2S,3R,4E)-2-amino-4-octadecene-l,3-diol (**4**)*

A solution of compound **21** (29.8 mg, 0.025 mmol) in 5 mL of NaOMe/MeOH (0.04 M) was stirred at room temperature for 14 h. A few drops of water were added at 0 °C. After stirring at room temperature for 1 h, the mixture was neutralized by Amberlite IR 120/H$^+$ ion exchange resin. After filtration and concentration, the residue was dried *in vacuo* to afford crude intermediate **24**, $R_f = 0.32$ (EtOAc-iPrOH-H_2O 3:2:1). To a solution of this crude product in dry MeOH (3 mL) were added under nitrogen propane-1,3-dithiol (0.3 mL) and triethylamine (0.3 mL), and the mixture was stirred at room temperature for 4 days. A white precipitate was formed. After filtration, and washing with MeOH, the filtrate was concentrated. The residue obtained was flash-chromatographed, eluting with $CHCl_3$–MeOH 3:1 to afford **4** (16.9 mg, 90% for two steps) as a white amorphous solid. R_f = 0.28 (EtOAc-iPrOH-H_2O 3:2:1). $[\alpha]_D^{20} = +1.4$ (*c* 1.0 in $CHCl_3$–MeOH 1:1). ^{1}H NMR (400 MHz, DMSO-d6): δ 8.41 (d, *J* = 50.0 Hz, 1H, NH), 5.74–5.60 (m, 1H, H-5″), 5.45 (dd, *J* = 15.4, 6.0 Hz, 1H, H-4″), 4.17 (d, *J* = 7.4 Hz, 2H, H-3″, H-1′), 3.83 (d, *J* = 9.1 Hz, 1H, Ha-1″), 3.69 (dt, *J* = 10.5, 9.1 Hz, 3H, Ha-9, Hb-1″, Hb-9), 3.57 (d, *J* = 9.2 Hz, 3H, Ha-6′, H-4, H-4′), 3.39–3.30 (m, 3H, H-3′, H-5, Hb-6′), 3.22 (d, *J* = 8.6 Hz, 2H, H-8, H-5′), 3.17–3.08 (m, 2H, H-6, H-2″), 3.02 (dd, *J* = 16.4, 8.5 Hz, 2H, H-2′, H-7), 2.63 (d, *J* = 7.4 Hz, 1H, H-3eq), 2.07–1.92 (m, 2H, H$_2$-6″), 1.89 (s, 3H, NAc), 1.34 (m, 3H, H-3ax, H$_2$-7″), 1.24 (s, 20H, 10 × CH$_2$), 0.85 (t, *J* = 6.8 Hz, 3H, CH$_3$). ^{13}C NMR (100 MHz, DMSO-d6): δ 172.45 (C=O), 170.72 (C=O), 132.91 (C-5″), 128.58 (C-4″), 103.24 (C-1′), 100.08 (C-2), 76.31 (C-6), 74.88 (C-8), 73.14 (C-7), 72.74 (C-3′), 71.35 (C-4), 70.15 (C-2′), 69.58 (C-3″), 69.05 (C-5′), 67.67 (C-1″), 67.05 (C-4′), 63.33 (C-9), 63.28 (C-6′), 55.72 (C-2″), 53.06 (C-5), 41.65 (C-3), 31.67 (CH$_2$, C-6″), 31.31, 29.08, 29.03, 28.96, 28.72, 28.66, 22.11 (11 × CH$_2$), 22.50 (CH$_3$, NAc), 13.96 (CH$_3$). ESI-HRMS (*m/z*) calcd for $C_{35}H_{63}N_2O_{15}$ [M – H]$^-$: 751.4234, found: 751.4263.

4.17. *Cell culture*

Human leukemia K562 and colorectal carcinoma HCT116 cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and were grown in RPMI 1640 containing 10% fetal calf serum (FCS) and 1% glutamine. Human HaCaT keratinocyte line was purchased from Cell Lines Service GmbH (CLS, Eppelheim, Germany) and cultured in DMEM medium (high glucose) supplemented with 2 mM L-glutamine and 10% FCS. All cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO_2.

5. Cytotoxicity assay

In order to evaluate the cytotoxicity of the studied compounds, we used three different human cell lines: 2 types of cancer cell and a normal cell line. Stock solution of each compound was prepared in DMSO at a concentration of 0.02 M and then diluted to their final concentration in triplicate with medium before use. 0.1% DMSO has been used as the vehicle control for all the experiments. Cell viability was assessed using the Promega CellTiter-Blue reagent (Promega, WI, USA) according to the manufacturer's instructions. Briefly, the cells were seeded in 96-well plates (5000 cell/well) containing 50 μL of growth medium. After 24 h of culture, the cells were supplemented with 50 μL of the studied compound dissolved in DMSO (less than 0.1% in each preparation). After 72 h of

incubation, 20 μL of resazurin were added and after 2 h the fluorescence was recorded (560 nm Ex/590 nm Em) using a Victor microtiter plate fluorimeter (Perkin–Elmer, USA). The IC_{50} value corresponds to the concentration of the compounds that caused a decrease of 50% in fluorescence of drug-treated cells relative to control cells. After treatment, cells were analyzed by inverted light microscope (TE 2000E, Nikon, Champigny-sur-Marne, France).

Acknowledgments

We thank the China Scholarship Council for a Ph.D. fellowship to Huanhuan Qu. Financial supports from the Centre National de la Recherche Scientifique (CNRS) and the Université Pierre et Marie Curie (LIA Programme) are gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.01.054.

References

[1] (a) S. Hakomori, Annual Review of Biochemistry 50 (1981) 733–764;
(b) S. Roseman, Journal of Biological Chemistry 276 (2001) 41527–41542;
(c) T. Yamakawa, Glycoconjugate Journal 13 (1996) 123–126.
[2] (a) R.D. Astronomo, D.R. Burton, Nature Reviews Drug Discovery 9 (2010) 308–324;
(b) Z. Guo, Q. Wang, Current Opinion in Chemical Biology 13 (2009) 608–617;
(c) J. Zhu, J.D. Warren, S.J. Danishefsky, Expert Review of Vaccines 8 (2009) 1399–1413;
(d) P. Bruni, C. Donati, Cellular and Molecular Life Sciences 65 (2008) 3725–3736;
(e) S.I. Hakomori, FEBS Letters 584 (2010) 1901–1906;
(f) J. Inokuchi, Proceedings of the Japan Academy Series B 87 (2011) 179–198;
(g) N.V. Prokazova, N.N. Samovilova, E.V. Gracheva, N.K. Golovanova, Biochemistry (Moscow) 74 (2009) 235–249;
(h) A.R. Todeschini, S.I. Hakomori, Biochimica et Biophysica Acta 1780 (2008) 421–433;
(i) T.W. Tillack, M. Allietta, R.E. Moran, W.W. Young, Journal of Biochimica and Biophysica Acta 733 (1983) 15–24;
(j) S. Hakomori, Journal of Biological Chemistry 265 (1990) 18713–18716.
[3] H.J. Jennings, R. Roy, A. Gamian, Journal of Immunology 137 (1986) 1708–1713.
[4] (a) S. Yamamura, K. Handa, S. Hakomori, Biochemical and Biophysical Research Communications 236 (1997) 218–222;
(b) K. Iwabuchi, S. Yamamura, A. Prinetti, K. Handa, S. Hakomori, Journal of Biological Chemistry 273 (1998) 9130–9138;
(c) K. Iwabuchi, Y. Zhang, K. Handa, D.A. Withers, P. Sinaÿ, S. Hakomori, Journal of Biological Chemistry 275 (2000) 15174–15181.
[5] K. Iwabuchi, K. Handa, S. Hakomori, Journal of Biological Chemistry 273 (1998) 33766–33773.
[6] (a) Y. Hirabayashi, A. Hanaoka, M. Matsumoto, T. Matsubara, M. Tagawa, S. Wakabayashi, M. Taniguchi, Journal of Biological Chemistry 260 (1985) 13328–13333;
(b) G.A. Nores, T. Dohi, M. Taniguchi, S. Hakomori, Journal of Immunology 139 (1987) 3171–3176;
(c) N. Kojima, M. Shiota, Y. Sadahira, K. Handa, S. Hakomori, Journal of Biological Chemistry 267 (1992) 17264–17270.
[7] (a) G.J. Boons, A.V. Demchenko, Chemical Reviews 100 (2000) 4539–4565;
(b) M. Kiso, H. Ishida, H. Ito, Special problems in glycosylation reactions: Sialidations, in: B. Ernst, G.W. Hart, P. Sinaÿ (Eds.), Carbohydrates in Chemistry and Biology, vol. 1, Wiley-VCH, Weinheim, New York, 2000, pp. 345–366;
(c) M. von Itzstein, R.J. Thomson, Topics in Current Chemistry 186 (1997) 119–170;
(d) D.K. Ress, R.J. Linhardt, Current Organic Synthesis 1 (2004) 31–46.
[8] C. De Meo, G.-J. Boons, A.V. Demchenko, Synthesis of glycosides of sialic acid, in: J.P. Kamerling (Ed.), Comprehensive Glycoscience, vol. 1, Elsevier Ltd., 2007, pp. 583–604.
[9] V. Martichonok, G.M. Whitesides, Journal of Organic Chemistry 61 (1996) 1702–1706.
[10] A. Marra, P. Sinaÿ, Carbohydrate Research 187 (1989) 35–42.
[11] (a) A.J. Ratcliffe, B. Fraser-Reid, Journal of the Chemical Society Perkin Transactions 1 (1990) 747–750;
(b) I. Braccini, C. Derouet, C. Esnault, H. du Penhoat, J.-M. Mallet, V. Michon, P. Sinaÿ, Carbohydrate Research 246 (1993) 23–41;
(c) A. Hasegawa, T. Nagahama, H. Ohki, M. Kiso, Journal of Carbohydrate Chemistry 11 (1992) 699–714;
(d) A. Hasegawa, H. Ogawa, M. Kiso, Carbohydrate Research 224 (1992) 185–192;
(e) M. Kiso, H. Katagiri, H. Furui, K. Ando, H. Ishida, A. Hasegawa, Journal of Carbohydrate Chemistry 13 (1994) 163–174;
(f) Y. Nakahara, S. Shibayama, Y. Nakahara, T. Ogawa, Carbohydrate Research 280 (1996) 67–84;
(g) J. Seifert, T. Ogawa, Y. Ito, Tetrahedron Letters 40 (1999) 6803–6807;
(h) R. Roy, F.O. Andersson, M. Letellier, Tetrahedron Letters 33 (1992) 6053–6056;
(i) S. Cao, R. Roy, Tetrahedron Letters 37 (1996) 3421–3424.
[12] (a) H. Kunz, H. Waldmann, U. Klinkhammer, Helvetica Chimica Acta 71 (1988) 1868–1874;
(b) G. Pazynina, A. Tuzikov, A. Chinarev, P. Obukhova, N. Bovin, Tetrahedron Letters 43 (2002) 8011–8013;
(c) H. Paulsen, H. Tietz, Carbohydrate Research 125 (1984) 47–64;
(d) S. Sato, S. Fujita, K. Furuhata, H. Ogura, S. Yoshimura, M. Itoh, Y. Shitori, Chemical and Pharmaceutical Bulletin 35 (1987) 4043–4048;
(e) A. Hasegawa, Y. Ito, H. Ishida, M. Kiso, Journal of Carbohydrate Chemistry 8 (1989) 125–133.
[13] (a) H. Paulsen, H. Tietz, Angewandte Chemie International Edition 21 (1982) 927–928;
(b) H. Iijima, T. Ogawa, Carbohydrate Research 186 (1989) 95–106;
(c) C. Shimizu, K. Achiwa, Carbohydrate Research 166 (1987) 314–316;
(d) C. Shimizu, N. Ohta, K. Achiwa, Chemical and Pharmaceutical Bulletin 38 (1990) 3347–3354;
(e) K. Suzuki, R. Kobayashi, K. Furuhata, H. Ogura, Chemical and Pharmaceutical Bulletin 38 (1990) 2083–2087;
(f) R. Roy, M. Letellier, D. Fenske, H.C. Jarrell, Journal of Chemical Society Chemical Communications (1990) 378–380;
(g) H. Ohrui, Y. Nishida, H. Itoh, H. Meguro, Journal of Organic Chemistry 56 (1991) 1726–1731.
[14] (a) H. Maeda, H. Ishida, M. Kiso, A. Hasegawa, Journal of Carbohydrate Chemistry 14 (1995) 369–385;
(b) A.J. Janczuk, W. Zhang, P.R. Andreana, J. Warrick, P.G. Wang, Carbohydrate Research 337 (2002) 1247–1259.
[15] J. Xue, Y. Pan, Z. Guo, Tetrahedron Letters 43 (2002) 1599–1602.
[16] (a) K.P.R. Kartha, R.A. Field, Synlett 3 (1999) 311–312;
(b) L. Fan, O. Hindsgaul, Organic Letters 4 (2002) 4503–4506;
(c) S. Malik, K.P.R. Kartha, Synlett 11 (2009) 1809–1811.
[17] (a) P.I. Kitov, C. Railton, D.R. Bundle, Carbohydrate Research 307 (1998) 361–369;
(b) R. Autar, S. Khan, M. Schad, J. Hacker, M.J. Liskamp, R.J. Pieters, ChemBioChem 4 (2003) 1317–1325;
(c) K. Jansson, S. Ahlfors, T. Frejd, J. Kihlberg, G. Magnusson, J. Dahmen, G. Noori, K. Stenvall, Journal of Organic Chemistry 53 (1988) 5629–5647.
[18] (a) R.R. Schmidt, P. Zimmermann, Tetrahedron Letters 27 (1986) 481–484;
(b) K. Ohashi, S. Kosai, M. Arizuka, T. Watanabe, Y. Yamagiwa, T. Kamikawa, M. Kates, Tetrahedron 45 (1989) 2557–2570;
(c) F. Compostella, L. Franchini, G.B. Giovenzana, L. Panza, D. Prosperi, F. Ronchetti, Tetrahedron: Asymmetry 13 (2002) 867–872;
(d) J. Ohlsson, G. Magnusson, Carbohydrate Research 331 (2001) 91–94.
[19] R.R. Schmidt, P. Zimmermann, Angewandte Chemie International Edition English 25 (1986) 725–726.
[20] Y. Zhang, K. Iwabuchi, S. Nunomura, S. Hakomori, Biochemistry 39 (2000) 2459–2468.
[21] E.G. Bremer, S. Hakomori, Biochemical and Biophysical Research Communications 106 (1982) 711–718.
[22] E.G. Bremer, S. Hakomori, D.F. Bowen-Pope, E. Raines, E.R. Ross, Journal of Biological Chemistry 259 (1984) 6818–6825.
[23] E.G. Bremer, J. Schlessinger, S. Hakomori, Journal of Biological Chemistry 261 (1986) 2434–2440.
[24] (a) T. Murase, A. Kameyama, K.P.R. Kartha, H. Ishida, M. Kiso, A. Hasegawa, Journal of Carbohydrate Chemistry 8 (1989) 265–283;
(b) E. Klenk, L. Georgias, Zeitschrift für Physikalische Chemie 348 (1967) 1261–1267;
(c) A. Hasegawa, M. Kiso, Japanese Kokai Tokkyo Koho (1990). JP 02049795 A 19900220.
[25] A. Hasegawa, Y. Kojima, M. Ogawa, M. Kiso, Journal of Carbohydrate Chemistry 11 (1992) 343–357.