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Design, synthesis and biological evaluation of new ganglioside GM3 analogues as potential agents for cancer therapy

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Abstract

Ganglioside GM3 is well known as a tumor-associated carbohydrate antigen on several types of tumor. Many studies have demonstrated that GM3 plays roles in cells proliferation, adhesion, motility and differentiation, which is involved in the process of cancer development. In the present study, we developed methods to synthesize GM3 analogues conveniently. By enzymatic hydrolysis and chemical procedures, two novel analogues and two known analogues were synthesized, containing lactose and

glucosamine. Then anti-proliferation and anti-migration activities were evaluated by cytotoxicity assays and wound healing tests, and the data demonstrated that these analogues exhibited anticancer activities. Based on our previous studies, the structure-activity relationships were discussed. This study could provide valuable sight to find new antitumor agents for cancer therapy.

Keywords: GM3 analogues; synthesis; cancer; antitumor; anti-proliferation; anti-migration.

1.Introduction

Glycosphingolipids (GSLs) are ubiquitous components on animal cell membranes, and exposed on the outer surface. Various studies have demonstrated that they play crucial roles in cells proliferation, adhesion, motility and differentiation [1,2,3]. Ganglioside GM3, the first and simplest member in the metabolic series of a GSLs family, contains a single terminal sialic acid, lactose and ceramide (**Figure 1**). It has been revealed that GM3 is implicated in the human cancer development [4,5,6].

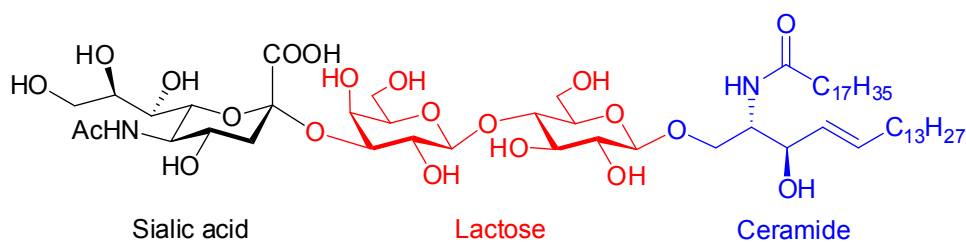


Figure 1. Structure of ganglioside GM3.

Ganglioside GM3 is over expressed on some cancers, and it's well known as a tumor-associated carbohydrate antigen (TACA) on several types of tumor. Studies have already showed that GM3 is recognized as an important melanoma-associated antigen, and may have a role in metastasis [7]. GM3 can have effects on the tumor-associated receptors including epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor-2 (VEGFR-2), tumor cell migration and glycolipid-enriched membrane microdomain [8-12].

Sialic acid is a nine-carbon monosaccharide involved in a variety of biological processes. For example, being at the terminus of natural cell-surface glycoconjugates, sialic acid is ideally positioned to mediate carbohydrate-protein interactions in cell-cell recognition process [13]. Sialylated oligosaccharides are considered as important TACAs. In particular, sialylated TACAs with a core of *N*-acetyl glucosamine or galactosamine, are over expressed in many types of human cancers [14].

From the previous studies in our laboratory, some GM3 analogues with anticancer activities have been synthesized [15,16]. In the present study, we developed methods to synthesize GM3 analogues from commercially available substrates and reagents. Then two known analogues **C1** [17] and **C3** [18] (**Figure 2**) and two novel analogues **C2** and **C4** (**Figure 2**) were synthesized by using a chemoenzymatic approach. At last, by cytotoxicity assays and wound healing tests, results showed that these analogues can inhibit cancer cell proliferation and migration.

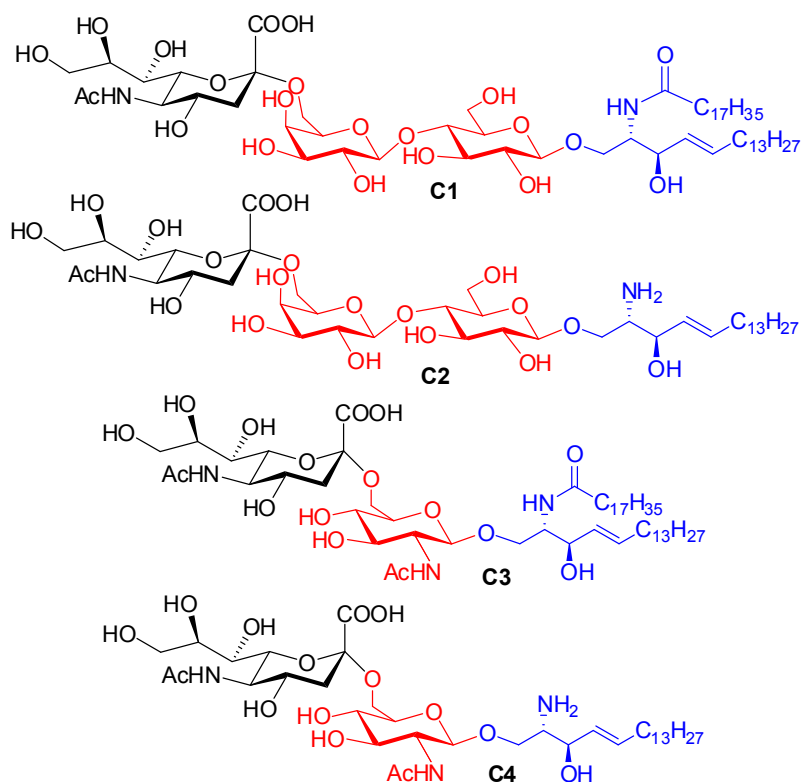


Figure 2. Structures of four synthesized GM3 analogues: **C1**, **C2**, **C3**, **C4**.

2. Results and discussion

2.1. Synthesis of lactose-containing GM3 analogues

For synthesizing lactose-containing GM3 analogues **C1** and **C2**, according to the retrosynthetic analysis, three key building blocks are needed (**Figure 3**). Firstly, the sialic acid was activated as sialyl xanthate form based on the previous report [19]. Then lactal residue **L1** bearing a free 6-OH at the galactose was obtained by enzymatic hydrolysis according to the reported method. In this study, we introduced enzymatic hydrolysis to prepare GM3 building block **L1**, which can avoid multiple chemical procedures. The enzyme acetyl xylan esterase from *Bacillus pumilus* (AXE) can remove the acetyl group at C-6 position of galactose from per-acetylated lactal [20]. Finally, the 3-*O*-benzoyl azidosphingosine block was synthesized from commercially available D-(+)-galactose as previous reports with some modifications [21,22].

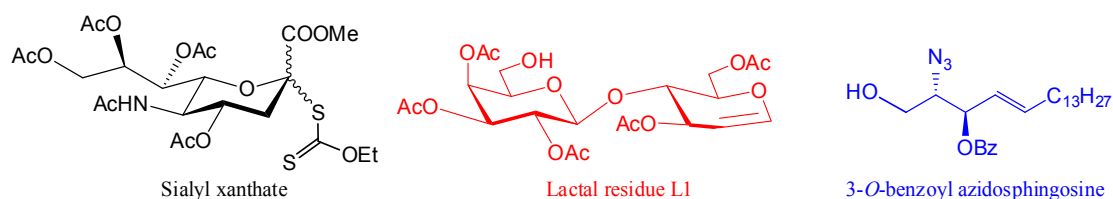
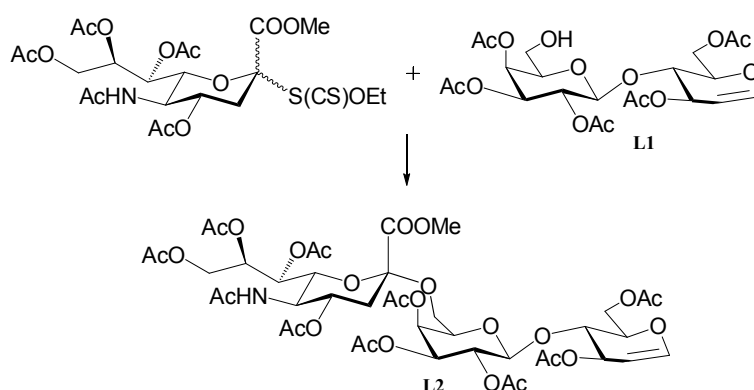


Figure 3. Three key building blocks for synthesizing analogues **C1** and **C2**: sialyl xanthate block, lactal residue **L1** block and 3-*O*-benzoyl azidosphingosine block.

Next, the key step was stereoselective α -sialylation of sialyl xanthate to free 6-OH of per-acetylated lactal residue **L1**. And for this step, Martichonok and Whitesides method was used. The sialyl xanthate reacting with PhSOTf forms the oxonium cations. Then oxonium cation is stabilized by acetonitrile to form nitrilium cations. Nitrilium cations are involved in the α -sialylation selectivity in acetonitrile solvent [19]. As shown in **Scheme 1**, the α -sialylation reaction was carried out at $-68\text{ }^\circ\text{C}$ with benzenesulfonyl chloride (PhSCl) and silver trifluoromethanesulfonate (AgOTf) as promoters in the mixture of $\text{CH}_2\text{Cl}_2:\text{CH}_3\text{CN}$ (1:2) for 3 h. PhSCl was freshly prepared each time. At last, the sialylated trisaccharide **L2** was obtained as α isomer in the yield of **65%**. The stereoselectivity of this glycosylation was confirmed by ^1H NMR, for H-3eq of sialic acid residue, the chemical shift was smaller for β -glycosides than for α -glycosides. In this step, it needs the careful chromatography to purify the crude product.

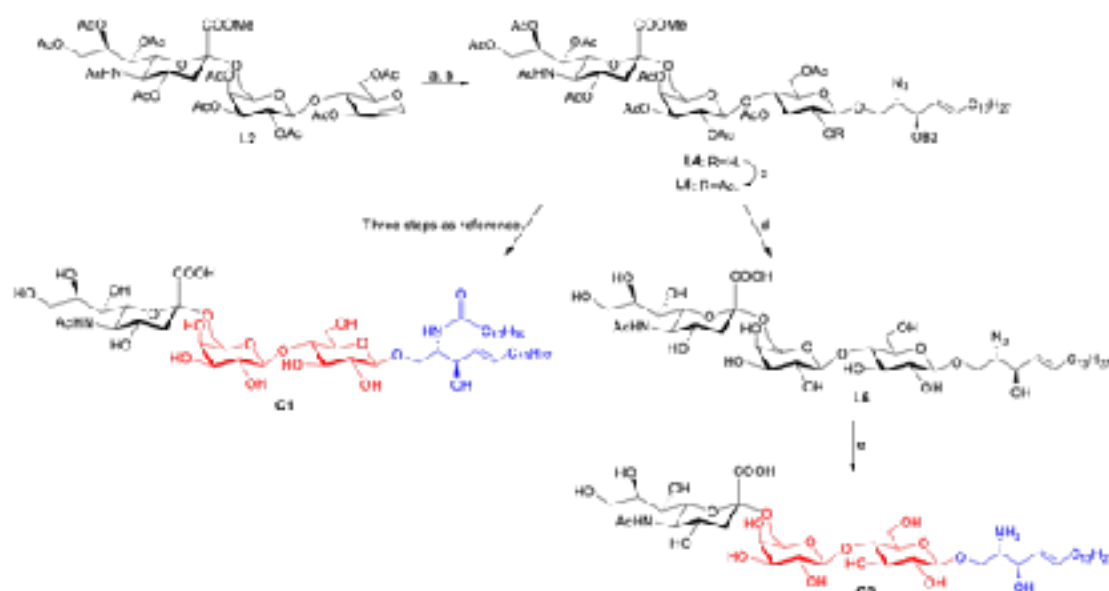


Scheme 1. Reagents and conditions: CH_3CN , CH_2Cl_2 , molecular sieves, r.t., 1 h, AgOTf, DTBP, $-68\text{ }^\circ\text{C}$, PhSCl, 3 h, **65%**.

Then the double bond in **L2** was oxidized to form the epoxide mixture **L3**. Due to low stability of the epoxide, the crude product was not separated by column and directly used in the next step. The epoxide ring was further opened and conjugated

with the lipid precursor 3-*O*-benzoyl azidosphingosine using ZnCl₂ at low temperature in dry THF, resulting in the formation of glycolipid intermediate **L4**, as shown in **Scheme 2**. The free C-2 OH in **L4** was protected by acetyl group using Ac₂O and pyridine, forming **L5**. In fact, although the mixture **L3** was used for conjugating with lipid precursor, in this step, after careful purification, pure **L5** could be obtained. Next, as the literature reported [17], the azido group of **L5** was reduced to free amino group, further reacted with stearic acid to give the glycosylated ceramide, and removed all the protecting groups. At last, after three steps, compound **C1** was obtained. For synthesizing analogue **C1**, compared with the synthetic route in the literature, ours is different, which is much shorter, more convenient and economical, avoiding redundant procedures.

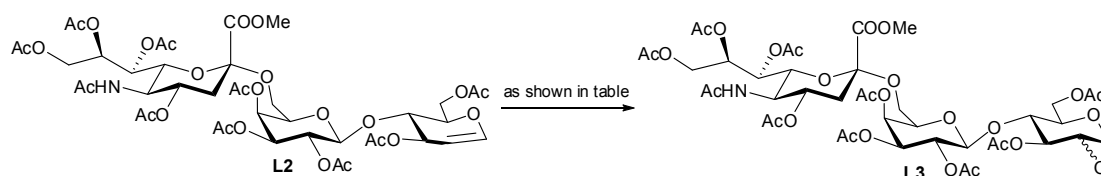
On the other hand, the protected groups of compound **L5** were removed by Zemplén transesterification (NaOMe/MeOH). After adding several drops of water, deprotected product **L6** was obtained. Subsequently, without further purification, the azido group in **L6** was reduced with propanedithiol/triethylamine to afford the new analogue **C2** in 78% yield.



Scheme 2. Reagents and conditions: (a) oxone, H₂O, tetrabutylammonium hydrogen sulfate, NaHCO₃, acetone, CH₂Cl₂, 0 °C - r.t., 2.5 h; (b) 3-*O*-benzoyl azidosphingosine, ZnCl₂, THF, -45 °C - r.t., 3 h; (c) Ac₂O, pyridine, r.t., 24 h, 32% (three steps from **L2**); (d) NaOMe, MeOH, r.t., 14 h; H₂O, 0 °C, 2 h; (e) HS(CH₂)₃SH, Et₃N, MeOH, r.t., 4 days, 78% (two steps from **L5**).

Epoxides are important intermediates to synthesize carbohydrate derivatives. It has been reported that the epoxides of glycals were used to prepare ganglioside GM4 [23]. Actually, we tried to optimize the conditions for forming epoxide, and the results are shown in **Table 1**. At first, we used *m*-CPBA as the oxidant, but the yield was not good (**entry 1**). Then we increased the equivalent of *m*-CPBA, and the yield was improved (**entry 2**). Further, we tried to reduce the reaction temperature, and a decent yield was obtained (**entry 3**). The high reactivity of formed oxirane can be rapidly opened by the acids during the course of the epoxidation. Next, we selected oxone/acetone under the biphasic solution of H₂O and CH₂Cl₂, and decent yield was also obtained (**entry 4**). In this case, dimethyldioxirane (DMDO) generated *in situ* from oxone/acetone under biphasic solution [24]. Finally, adding the phase transfer catalyst tetrabutylammonium hydrogen sulfate (TBAHS), good yield of **L3** was obtained (**entry 5**).

Table 1. Optimized conditions for forming epoxides.



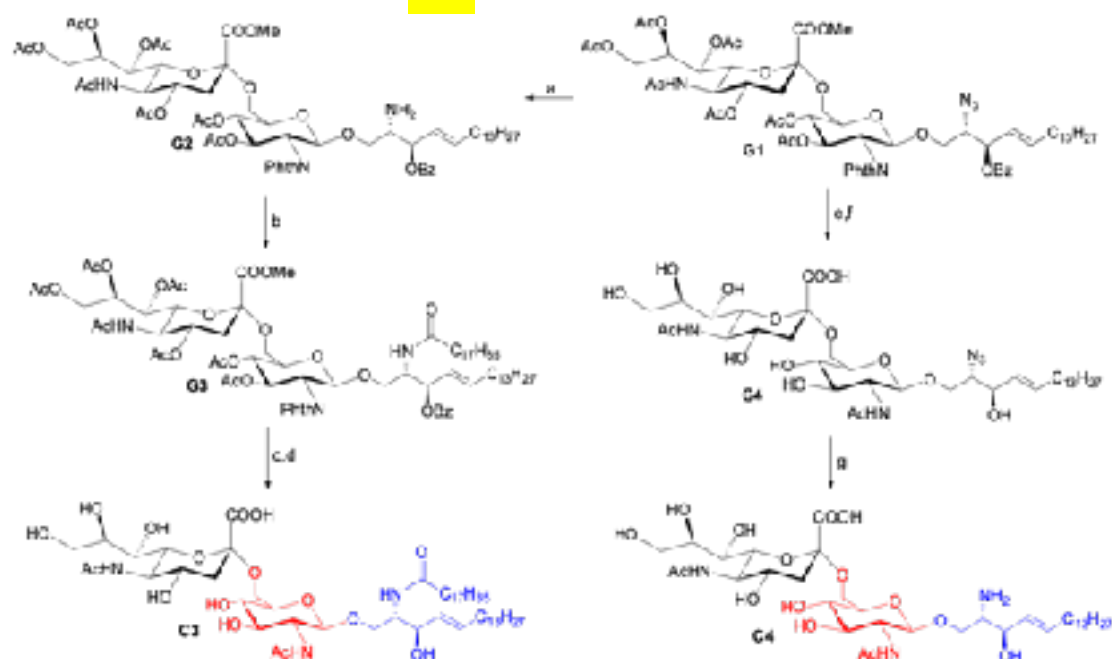
Entry	Conditions	Yield (%)	Gluco: Manno
1	1.5 eq <i>m</i> -CPBA, CH ₂ Cl ₂ , r.t., 5 h.	20	9: 1
2	2.5 eq <i>m</i> -CPBA, CH ₂ Cl ₂ , r.t., 2 h.	40	8:1
3	2.5 eq <i>m</i> -CPBA, CH ₂ Cl ₂ , 0 °C - r.t., 3 h.	49	7:3
4	2 eq oxone, H ₂ O, NaHCO ₃ , acetone, CH ₂ Cl ₂ , 0 °C - r.t., 3 h.	50	4:1
5	2 eq oxone, H ₂ O, NaHCO ₃ , TBAHS, acetone, CH ₂ Cl ₂ , 0 °C - r.t., 2.5 h.	75	9:1

The ratio of gluco and manno is from ¹H NMR.

2.2.Synthesis of glucosamine-containing GM3 analogues

For synthesizing glucosamine-containing analogues **C3** and **C4**, we started with glycolipid intermediate **G1** prepared previously based on the literature [25]. Then, the azido group of **G1** was reduced by triphenylphosphine in a mixture of toluene and water at 50 °C for 24 h to give an amino free derivative **G2**. This intermediate was not purified and directly reacted with stearic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) in the dry CH₂Cl₂ to give the glycosylated ceramide **G3** in 65% yield for two steps. At last, deprotection of compound **G3**, acetyl, benzoyl and phthalic groups were removed by MeNHNH₂. Further adding NaOH solution, methyl ester was hydrolyzed. For the obtained NH₂ group, it was selectively acetylated using Ac₂O in CH₂Cl₂ and MeOH, and the known analogue **C3** was obtained in 68% yield as shown in Scheme 3.

Similarly, all the protected groups of compound **G1** were removed, and for the free NH₂ group, it was also selectively acetylated using Ac₂O in CH₂Cl₂ and MeOH. Subsequently, the azido group in **G4** was reduced with propanedithiol/triethylamine to afford the new analogue **C4** in 68% yield.



Scheme 3. Reagents and conditions: (a) PPh₃, toluene, H₂O, 50 °C, 24 h; (b) Stearic acid, EDC, CH₂Cl₂, r.t., 24 h, 65% (two steps from **G1**); (c) MeNHNH₂, EtOH, 80 °C, 18 h; NaOH, H₂O, r.t., 1h; (d) Ac₂O, CH₂Cl₂, MeOH, r.t., overnight, 68% (two steps from **G3**); (e) MeNHNH₂, EtOH, 80 °C, 18 h; NaOH, H₂O, r.t., 1h; (f) Ac₂O, CH₂Cl₂, MeOH, r.t., overnight; (g) HS(CH₂)₃SH, Et₃N, MeOH, r.t., 4 days, 68% (three steps from **G1**).

2.3. Biological evaluation

2.3.1. Anti-proliferation activities

The effect of GM3 analogues **C1**, **C2**, **C3** and **C4** on proliferation of human colon cancer HCT116 cells and chronic myeloid leukemia K562 cells was evaluated by MTT assay. The results revealed that these analogues can inhibit the proliferation of these two cancer cells. The IC₅₀ values are shown in **Table 2**.

Table 2. IC₅₀ (μM) values of synthesized GM3 analogues.

Cell lines	C1	C2	C3	C4
HCT116	200.9±40.6	150.2±35.9	186.5±34.9	131.3±12.9
K562	201.4±45.8	149.4±38.7	95.3±20.7	81.15±21.5

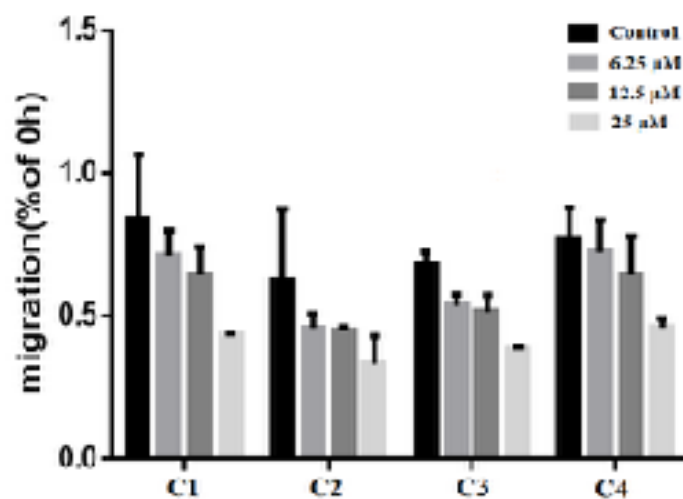
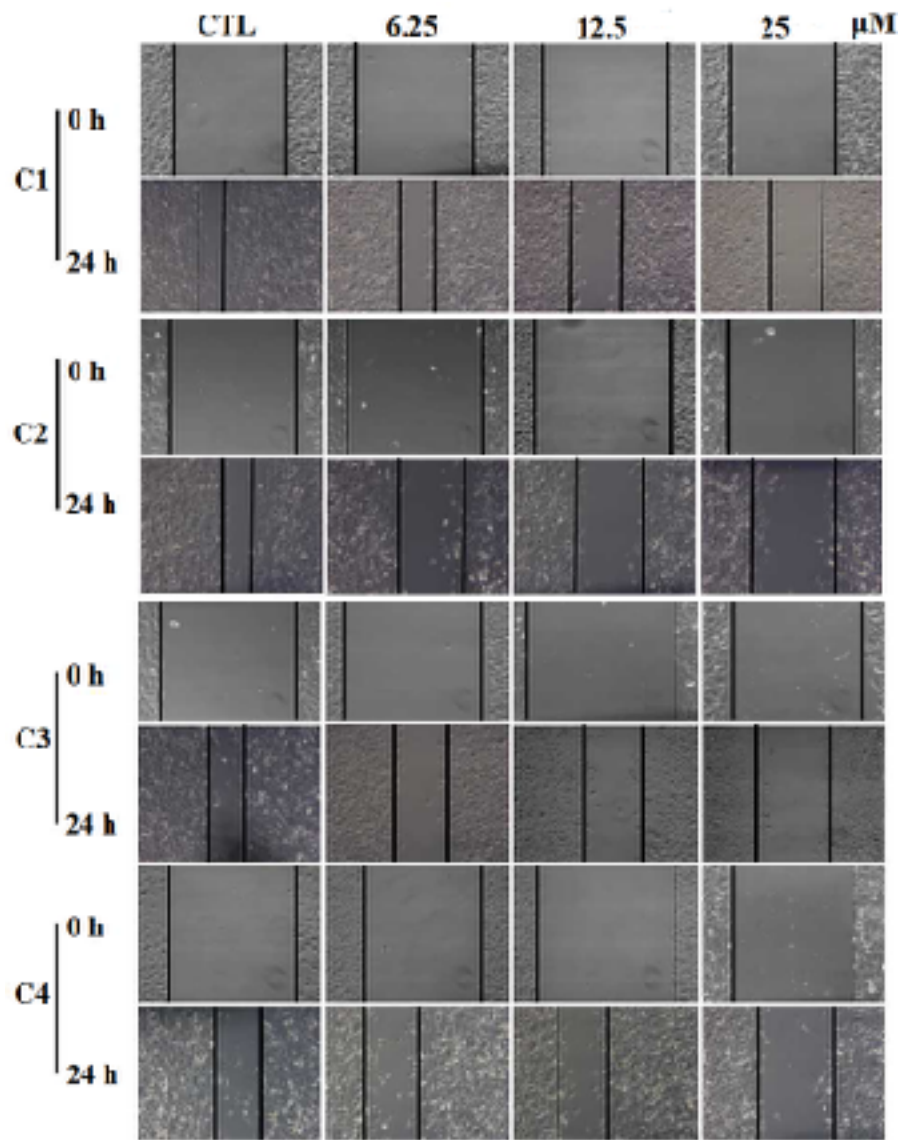


Figure 4. GM3 analogues inhibited melanoma B16 cell migration after 24 h of exposure. Inhibition in migration of cancer cells was observed upon treatment with GM3 analogues. Values were calculated as a percentage of the 0 hour (wound width at exposure time point/wound width at 0 h %).

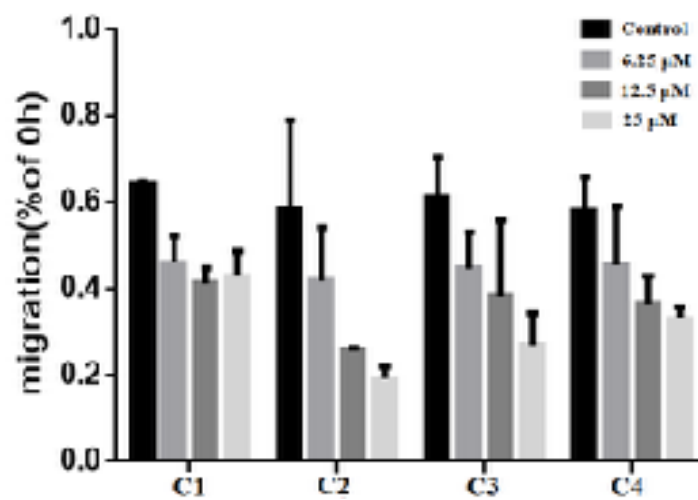
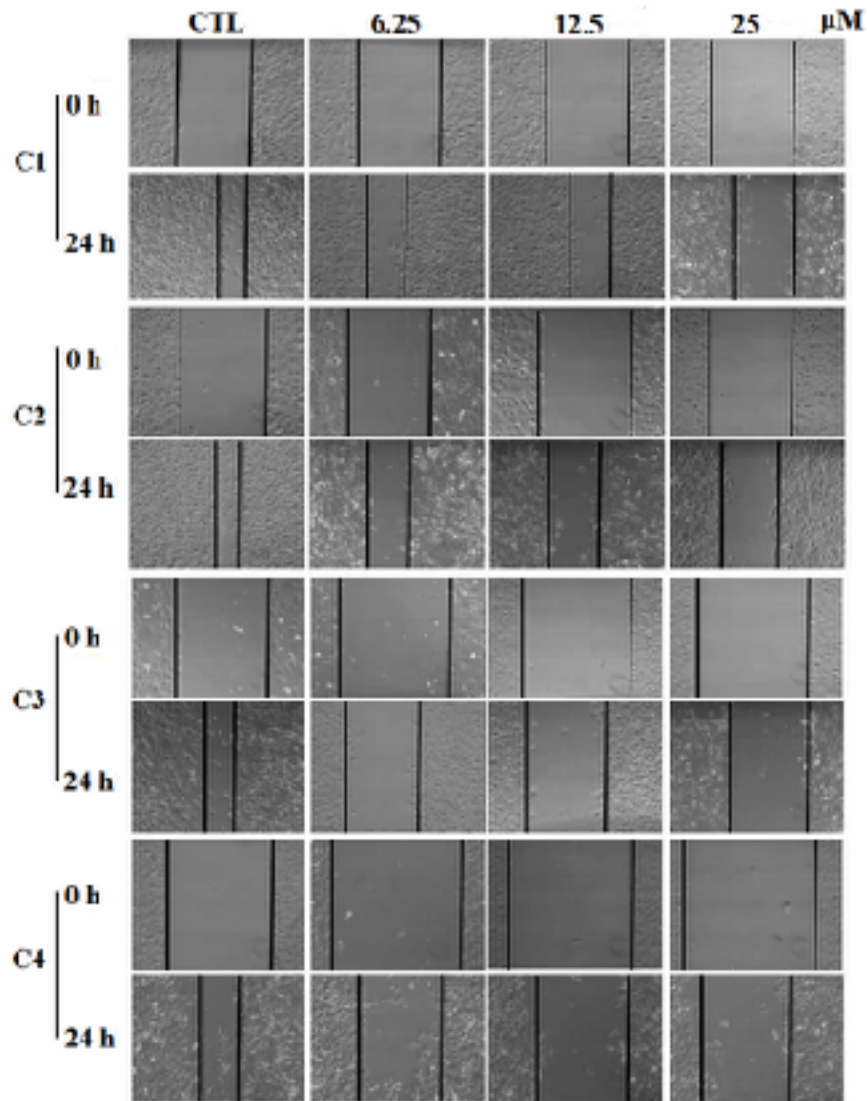


Figure 5. GM3 analogues inhibited melanoma B16-F10 cell migration after 24 h of exposure. Inhibition in migration of cancer cells was observed upon treatment with GM3 analogues. Values were calculated as a percentage of the 0 hour (wound width at exposure time point/wound width at 0 h %).

2.3.2. Anti-migration activities

Cell migration was investigated by wound healing assays *in vitro*, and the effect of GM3 analogues on tumor cell migration was studied. We also found that synthesized GM3 analogues could inhibit the migration of melanoma B16 (**Figure 4**) and highly metastatic melanoma B16-F10 cells (**Figure 5**). From these results, it demonstrated that these four analogues can inhibit tumor cell migration.

Compared with GM3 having ceramide, lyso-GM3 contains sphingosine in which the fatty acid chain is removed from ceramide. In most natural or synthesized glycosphingolipids, the lipid moiety is ceramide. Limited studies have demonstrated that GM3 and lyso-GM3 have different effects on tumor EGFR, adhesion and signaling [26-28]. In this study, two new analogues C2 and C4 with sphingosine were synthesized for biological studies along with two known analogues C1 and C3 with ceramide.

2.4. Structure-activity relationships discussion

Together with some other analogues which were synthesized previously in our laboratory [15,16], for a series of GM3 analogues containing different sugars with α -2,6 sialosides, the structure-activity relationships were discussed. We found that both different glycosyl residues and ceramide or sphingosine moiety could influence anti-proliferation and anti-migration activities.

Firstly, for anti-proliferation ability of analogues with ceramide in HCT116 cell line, we found that: mannose-containing analogue (64 μM) > galactose-containing analogue (86 μM) > glucosamine-containing analogue (187 μM) > lactose-containing analogue (201 μM) > glucose-containing analogue (480 μM). Then in the K562 cell line, it was: glucosamine-containing analogue (95 μM) > mannose-containing analogue (160 μM) > lactose-containing analogue (201 μM) > glucose-containing analogue (270 μM) > galactose-containing analogue (396 μM). Furthermore, for the analogues with sphingosine in HCT116 cell line, it was: galactose- (130 μM), glucosamine- (131 μM) containing analogues > lactose-containing analogue (150 μM) > mannose-containing analogue (182 μM) > glucose-containing analogue (430 μM), and in K562 cell line, it was: glucosamine-containing analogue (81 μM) > mannose-

(148 μM), lactose- (149 μM) containing analogues > galactose-containing analogue (206 μM) > glucose-containing analogue (320 μM). In addition, our data showed that in HCT116 cells, except for mannose- and galactose-containing analogues, for glucosamine-, lactose- and glucose-containing analogues, the molecules with sphingosine showed much better inhibitory effect than corresponding analogues with ceramide. Then in K562 cells, except for glucose-containing analogues, for mannose-, galactose-, lactose- and glucosamine-containing analogues, the molecules with sphingosine were also more potent than the corresponding analogues with ceramide moiety.

Secondly, the anti-migration ability was also summarized. At first, for analogues with ceramide in B16 cells, glucosamine-, lactose- and galactose-containing analogues exhibited similar inhibitory effects, and there was no inhibitory effect about mannose-containing analogue. However, in B16-F10 cells, it seemed that glucosamine-, lactose-, mannose- and galactose-containing analogues were similar. Next for analogues with sphingosine in B16 and B16-F10 cells, it was shown that: mannose-containing analogue > galactose-containing analogue > lactose-containing analogue > glucosamine-containing analogue. Moreover, we found that in B16 cells, for mannose- and galactose-containing analogues, molecules with sphingosine were much better than the corresponding analogues with ceramide, but for lactose- and glucosamine-containing analogues, there was no significant difference. Furthermore, in B16-F10 cells, except for glucosamine-containing analogues, molecules with sphingosine also exhibited more potency than the corresponding analogues with ceramide.

These are preliminary results from our study, and we think that more compounds are needed for better understanding the structure-activity relationships.

3. Conclusion

In this study, we successfully synthesized two novel and two known GM3 analogues, which contain lactose and glucosamine residue, by developed methods from commercially available substrates and reagents. Then their effects against cancer cell proliferation and migration were studied. The results demonstrated that our

synthesized GM3 analogues displayed anti-proliferation and anti-migration effects on cancers. These synthesized GM3 analogues exhibited antitumor activities, which can provide valuable sights to search new antitumor agents for cancer therapy.

4. Experimental section

4.1. General methods

All chemicals were purchased as reagent grade and used without further purification. All reactions were carried out under N₂ atmosphere and anhydrous conditions with freshly distilled solvents, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) on a pre-coated plate of silica gel 60 F254 (Merck) and detection by charring with sulfuric acid. Solvents were evaporated under reduced pressure and below 40 °C (water bath). Column chromatography was performed on silica gel 60 (230-400 mesh, Merck). All the new compounds were fully characterized by ¹H and ¹³C NMR, as well as HRMS. ¹H NMR and ¹³C NMR spectra were recorded at 400 MHz with Bruker AVANCE DRX 400 spectrometer. The chemical shifts were referenced to the solvent peak, 7.26 ppm (¹H) and 77.16 ppm (¹³C) for CDCl₃, 3.31 ppm (¹H) and 49.00 ppm (¹³C) for CD₃OD at 25 °C, and coupling constants were given in Hz. High-resolution mass spectra (HRMS) were recorded with a Bruker Micro-TOF 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. *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galactopyranosylate)-(2 \rightarrow 6)-2,3,4-tri-*O*-acetyl- β -*D*-galactopyranoside-(1 \rightarrow 4)-3,6-di-*O*-acetyl-*D*-glucal (L2)

A mixture of sialyl xanthate (483 mg, 0.81 mmol) and acceptor **L1** (230 mg, 0.44 mmol) with 4Å powdered molecular sieves (1.5 g) in the dry CH₃CN (7 mL) and CH₂Cl₂ (3.5 mL) were stirred at the room temperature for 1 h. Then AgOTf (208 mg, 0.81 mmol) and DTBP (184 μ L, 0.82 mmol) were added, and the mixture was cooled to -68 °C and kept protected from light. PhSCl (97 μ L, 0.84 mmol) in dry CH₂Cl₂

(0.51 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 (3.5 g) in EtOAc (21 mL), filtered through celite, washed with saturated aqueous NaHCO₃ and water, dried over MgSO₄ and concentrated under reduced pressure. The residue was chromatographed (Cy-EtOAc 1:3) to give **L2** as a white foam (286 mg, 65%). R_f = 0.35 (Cy-EtOAc 1:3, twice). [α]²⁰_D = -19.7 (c 1.0 in CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 6.38 (d, *J* = 6.2 Hz, 1H, H-1''), 5.43 (d, *J* = 3.4 Hz, 1H), 5.37-5.25 (m, 5H), 5.18-5.13 (m, 3H), 5.03 (dd, *J* = 9.7, 4.0 Hz, 1H), 4.85-4.83 (m, 2H), 4.70 (d, *J* = 5.9 Hz, 1H, H-1'), 4.33-4.21 (2m, 2H), 4.10-4.04 (m, 4H), 3.78 (s, 3H, CH₃), 3.38 (dd, *J* = 7.3, 4.0 Hz, 1H), 2.51 (dd, *J* = 12.6, 4.2 Hz, 1H, H-3eq), 2.17 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.10 (2s, 6H, 2 × OAc), 2.06 (s, 3H, OAc), 2.05 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.01 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.89 (m, 1H, H-3ax), 1.87 (s, 3H, NAc). ¹³C NMR (100 MHz, CDCl₃): δ 170.91 (C=O), 170.65 (C=O), 170.47 (C=O), 170.34 (C=O), 170.15 (C=O), 169.94 (C=O), 169.89 (C=O), 169.68 (C=O), 169.39 (C=O), 167.87 (C=O), 145.21 (C-1''), 100.45 (C-1'), 99.09 (C-2''), 99.09 (C-2), 74.11, 72.59, 71.79, 71.05, 69.09, 68.64, 68.42, 68.04, 67.10, 67.07, 62.94, 62.51, 61.97, 52.92 (COOCH₃), 49.46 (C-5), 37.89 (C-3), 23.19 (CH₃, NAc), 21.08 (CH₃, OAc), 20.99 (CH₃, OAc), 20.82 (CH₃, OAc), 20.76 (CH₃, OAc), 20.72 (CH₃, OAc), 20.70 (CH₃, OAc), 20.66 (CH₃, OAc), 20.56 (CH₃, OAc). ESI-HRMS (m/z) calcd for C₄₂H₅₇NNaO₂₆ [M+Na]⁺: 1014.3067, found: 1014.3052.

4.3. *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-2,3,4-tri-*O*-acetyl- β -*D*-galactopyranoside - (1 \rightarrow 4)-3,6-di-*O*-acetyl- α -*D*-glucopyranoside/ β -*D*-mannopyranoside (**L3**)

Method (A): The **L2** (30 mg, 0.03 mmol) was dissolved in the dry CH₂Cl₂ (1 mL), then cooled to 0 °C. *m*-CPBA (17 mg, 0.075 mmol) was added, and gradually warmed to the room temperature. Finally, the mixture was stirred at room temperature for 3 h. After that, the reaction was quenched by adding Na₂S₂O₃ solution, further washed by saturated aqueous NaHCO₃ and water, dried over MgSO₄, and concentrated under reduced pressure, to obtain the **L3** mixture (15 mg, 49%) as a

white foam.

Method (B): A solution of oxone (246 mg, 0.40 mmol) in water (1 mL) was added dropwise at 0 °C for 15 min to a vigorously stirred biphasic mixture of **L2** (200 mg, 0.20 mmol), tetrabutylammonium hydrogen sulfate (23 mg, 0.07 mmol), acetone (83 μ L), CH₂Cl₂ (1.7 mL) and saturated NaHCO₃ solution (2.8 mL). Stirring was maintained for 30 min at 0 °C, and 2.5 h at room temperature. The aqueous phase was extracted with CH₂Cl₂ and the combined organic phases were washed with water and dried over MgSO₄, and concentrated under reduced pressure, to obtain the **L3** mixture (152 mg, 75%) as a white foam. R_f = 0.30 (CH₂Cl₂-MeOH 10:1, twice). Due to 1,2-anhydro sugar was unstable, and the mixture was directly used for the next step without further purification.

4.4. *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-*O*-(2,3,4-tri-*O*-acetyl- β -*D*-galactopyranosyl)-(1 \rightarrow 4)-*O*-(2,3,6-tri-*O*-acetyl- β -*D*-glucopyranosyl)-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-azido-3-*O*-benzoyl-4-octadecene-1,3-diol (**L5**)

The crude mixture **L3** (90 mg, 0.09 mmol) and 3-*O*-benzoyl azidosphingosine (116 mg, 0.27 mmol) were dissolved in 0.45 mL of dry THF. The solution was cooled to -45 °C and ZnCl₂ (45 μ L of a 1 M solution in diethyl ether, 0.045 mmol) was added dropwise. The reaction was allowed to warm to room temperature and was stirred for 3 h. The reaction was quenched by filtration through celite, concentrated, and subjected to the silica gel column using cyclohexane-acetone 1:1 as eluent, to obtain the crude **L4**, R_f = 0.40 (EtOAc). Next, the crude **L4** (60 mg) was dissolved in the pyridine (5 mL), adding 0.5 mL Ac₂O, and then stirred at room temperature for 24 h. After concentration, the mixture was subjected to the silica gel column using CH₂Cl₂-MeOH 20:1 as eluent to afford the known compound **L5** [17] (57 mg, 43% for two steps from **L3**) as a white solid. R_f = 0.45 (EtOAc).

4.5. *O*-(5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonic acid)-(2 \rightarrow 6)-*O*-(β -*D*-galactopyranosyl)-(1 \rightarrow 4)-*O*-(β -*D*-glucopyranosyl)-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (**C1**)

The synthesis of known analogue **C1** started from compound **L5** for three steps as literature reported [17]. ¹H NMR (400 MHz, CD₃OD): δ 5.88-5.81 (m, 1H, H-5cer), 5.51-5.32 (m, 1H, H-4cer), 4.36-4.28 (m, 2H), 3.92-3.46 (m, 23H), 2.80 (dd, *J* = 12.3, 4.3 Hz, 1H, H-3eq), 2.18-2.01 (m, 3H, H-3ax, CH₂), 1.98 (s, 3H, NAc), 1.42-1.37 (m, 4H), 1.26 (s, 50H, 25 × CH₂), 0.87 (t, *J* = 6.9 Hz, 6H, 2 × CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 167.75 (C=O), 167.71 (C=O), 133.37 (5cer), 128.15 (4cer), 99.93 (C-1'), 98.84 (C-1''), 98.71 (C-2), 75.71, 72.48, 72.43, 71.57, 71.45, 71.04, 69.41, 69.31, 68.70, 68.24, 67.73, 66.94, 62.79, 62.72, 62.52, 62.45, 60.41 (2cer), 49.39 (C-5), 37.92 (C-3), 30.91 (CH₂), 29.09 (CH₂), 23.05 (CH₃, NAc), 14.18 (CH₃).

4.6. *O*-(5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonic acid)-(2 \rightarrow 6)-*O*-(β -*D*-galactopyranosyl)-(1 \rightarrow 4)-*O*-(β -*D*-glucopyranosyl)-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-amino-4-octadecene-1,3-diol (**C2**)

The compound **L5** (25 mg, 0.017 mmol) in 3.3 mL of NaOMe/MeOH (0.04 M) was stirred at room temperature for 14 h. Then a few drops of water were added at 0 °C. After stirring at room temperature for another 2 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 **L6**. The crude intermediate in the anhydrous MeOH (2.1 mL) were added propane-1,3-dithiol (0.16 mL) and triethylamine (0.16 mL), and the mixture was stirred at ambient temperature for 4 days. A white precipitate was formed. After filtration and washing with MeOH, the filtrate was concentrated under reduced pressure. The residue obtained was flash-chromatographed, eluted with CHCl₃-MeOH 3:1 to yield the analogue **C2** (12 mg, 78% for two steps from **L5**) as a white amorphous solid. *R*_f = 0.35 (EtOAc-*i*PrOH-H₂O 3:2:1). [α]_D²⁰ = +12.1 (*c* 1.0 in 1:1 CHCl₃-MeOH). ¹H NMR (400 MHz, CD₃OD): δ 5.92-5.86 (m, 1H, H-5cer), 5.50-5.34 (m, 1H, H-4cer), 4.35-4.30 (m, 2H), 3.91-3.67 (m, 21H), 3.35-3.23 (m, 2H), 2.80 (dd, *J* = 12.5, 4.3 Hz, 1H, H-3eq), 2.03 (s, 3H, NAc), 1.71-1.65 (m, 2H, H₂-6cer), 1.47-1.42 (m, 3H, H-3ax, H₂-7cer), 1.31 (s, 20H, 10 × CH₂), 0.92 (t, *J* = 6.8 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 167.87 (C=O), 167.00 (C=O), 136.82 (5cer), 128.73 (4cer), 100.45 (C-1'), 99.15

(C-1''), 99.09 (C-2), 74.10, 72.59, 71.80, 71.05, 70.76, 69.35, 69.10, 68.65, 68.42, 68.02, 67.78, 67.07, 62.95, 62.65, 62.50, 61.97, 57.58 (2cer), 51.69 (C-5), 37.89 (C-3), 31.27 (CH₂), 30.13 (CH₂), 29.63 (CH₂), 23.19 (CH₃, NAc), 14.92 (CH₃). ESI-HRMS (m/z) calcd for C₄₁H₇₃N₂O₂₀ [M-H]⁻: 913.4762, found: 913.4750.

4.7. *O*-(methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonate)-(2 \rightarrow 6)-3,4-di-*O*-acetyl-2-phthalimido-2-deoxy- β -*D*-glucopyranoside-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-octadecanamido-3-*O*-benzoyl-4-octadecene-1,3-diol (*G3*)

The compound **G1** (30 mg, 0.023 mmol) in 3 mL of toluene and 0.12 mL of water was added 17 mg of triphenylphosphine. The mixture was stirred at 50 °C for 24 h. After concentration, the crude residue **G2** was directly used for the next step. The intermediate, stearic acid (19 mg, 0.069 mmol), EDC (7 mg, 0.046 mmol) in 6 mL of CH₂Cl₂ were stirred at room temperature for 24 h. Then the mixture was washed with water, dried over MgSO₄ and concentrated under reduced pressure. The resulting residue was purified by silica gel chromatography (Cy-EtOAc 1:3) to afford compound **G3** (23 mg, 65% for two steps from **G1**) as an amorphous solid. R_f = 0.39 (Cy-EtOAc 1:3). [α]_D²⁰ = -20.7 (c 1.0 in CHCl₃). ¹H NMR (400 MHz, CDCl₃): δ 7.97-7.95 (m, 1H, Ar-H), 7.83 (m, 2H, Ar-H), 7.75-7.65 (m, 6H, Ar-H), 5.95-5.91 (m, 1H, H-5''), 5.66-5.61 (m, 1H, H-4''), 5.37-5.15 (m, 5H), 4.85 (m, 1H, H-4), 4.28-4.25 (m, 2H), 4.04-4.01 (m, 4H), 3.82-3.78 (m, 3H), 3.76 (s, 3H, COOCH₃), 3.64 (dd, *J* = 10.7, 4.6 Hz, 1H), 3.54 (d, *J* = 11.9 Hz, 1H), 2.59 (dd, *J* = 12.4, 4.5 Hz, 1H, H-3eq), 2.14 (s, 3H, OAc), 2.12 (s, 3H, OAc), 2.09 (m, 2H, CH₂CO), 2.07 (s, 3H, OAc), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.94 (m, 2H, H₂-6''), 1.87 (s, 3H, OAc), 1.86 (s, 3H, NAc), 1.65-1.59 (m, 3H, H-3ax, H₂-7''), 1.26 (s, 50H, 25 \times CH₂), 0.88 (t, *J* = 6.8 Hz, 6H, 2 \times CH₃). ¹³C NMR (100 MHz, CDCl₃): δ 171.02, 170.65, 170.57, 170.35, 170.28, 170.17, 170.15, 170.03, 168.55, 167.70, 164.87 (C=O), 138.92 (C-5''), 134.22, 133.12, 131.45, 129.70, 128.40 (C, CH aromatic), 123.61 (C-4''), 98.76 (C-1'), 98.49 (C-2), 74.78, 72.59, 72.45, 71.00, 69.15, 69.00, 68.58, 68.11, 67.37, 63.35 (C-2''), 62.38 (C-6'), 60.79 (C-9), 54.46 (C-2'), 52.64 (COOCH₃), 49.49 (C-5), 38.18 (C-3), 31.91, 29.67, 29.65, 29.63, 29.58, 29.40, 29.33, 29.14, 28.68 (CH₂),

23.19 (CH₃, NAc), 21.07, 20.85, 20.84, 20.82, 20.78, 20.76, 20.70 (CH₃, OAc), 15.22 (CH₃). ESI-HRMS (m/z) calcd for C₈₁H₁₁₉N₃NaO₂₄ [M+Na]⁺: 1540.8081, found: 1540.8068.

4.8. *O*-(5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonic acid)-(2 \rightarrow 6)-2-acetamido-2-deoxy- β -*D*-glucopyranoside-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-octadecanamido-4-octadecene-1,3-diol (**C3**)

The compound **G3** (19 mg, 0.013 mmol) was dissolved in the dry EtOH (9 mL), and MeHNNH₂ (4.5 mL) was added. The mixture was refluxed at 80 °C for 18 h. After concentration, the residue was purified by column Sephadex HL-20, eluted with MeOH-CH₂Cl₂ 1:1, to obtain the intermediate. Then it was dissolved in 2 ml MeOH, and a few drops of 0.5 M NaOH aqueous solution were added. When the hydrolysis was completed after 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. The intermediate was dissolved in the MeOH (1.5 mL) and CH₂Cl₂ (1.5 mL), to which 0.3 mL of acetic anhydride was introduced. The mixture was stirred at room temperature overnight. After concentration, the crude product was flash-chromatographed, eluted with EtOAc-*i*PrOH-H₂O 2:2:1 to yield the old analogue **C3** [18] (9 mg, 68% from **G3**) as a white amorphous solid. R_f = 0.35 (EtOAc-*i*PrOH-H₂O 2:2:1). ¹H NMR (400 MHz, CD₃OD): δ 5.82-5.74 (m, 1H, H-5''), 5.57-5.45 (m, 1H, H-4''), 3.92-3.76 (m, 18H), 2.78 (dd, *J* = 12.4, 4.2 Hz, 1H, H-3_{eq}), 2.23 (m, 2H), 2.18 (s, 6H, 2 \times NAc), 1.82-1.79 (m, 3H), 1.31 (s, 52H, 26 \times CH₂), 0.90 (t, *J* = 6.8 Hz, 6H, 2 \times CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 170.74 (C=O), 170.36 (C=O), 134.73 (C-5''), 128.51 (C-4''), 99.90 (C-1'), 99.24 (C-2), 75.29, 74.06, 71.15, 71.03, 68.42, 68.32 66.84 66.81, 63.31, 63.27, 58.85 (C-2''), 56.76 (C-2'), 52.50 (C-5), 38.30 (C-3), 31.99 (CH₂), 31.66 (CH₂), 29.37 (CH₂), 29.20 (CH₂), 29.05 (CH₂), 22.32 (CH₃, 2 \times NAc), 14.39 (CH₃).

4.9. *O*-(5-acetamido-3,5-dideoxy-*D*-glycero- α -*D*-galacto-2-nonulopyranosylonic acid)-(2 \rightarrow 6)-2-acetamido-2-deoxy- β -*D*-glucopyranoside-(1 \rightarrow 1)-(2*S*,3*R*,4*E*)-2-amino-4-octadecene-1,3-diol (**C4**)

The compound **G1** (38 mg, 0.03 mmol) was dissolved in the dry EtOH (26 mL), and MeHNNH₂ (13 mL) was added. The mixture was refluxed at 80 °C for 18 h. After concentration, the residue was purified by column Sephadex HL-20, eluted with MeOH-CH₂Cl₂ 1:1, to obtain the intermediate. Then it was dissolved in 4 ml MeOH, and several drops of 0.5 M NaOH aqueous solution were added. After 1 h, the mixture was neutralized by Amberlite IR 120/H⁺ ion exchange resin. Further, filtration and concentration, the residue was dried in vacuo to afford crude intermediate. Then it was dissolved in the MeOH and CH₂Cl₂ 7 mL (1:1), to which 0.5 mL of acetic anhydride was introduced. The mixture was stirred at room temperature overnight. After concentration, the crude product was purified by column Sephadex HL-20, eluted with MeOH-CH₂Cl₂ 1:1, to obtain **G4**, R_f = 0.32 (EtOAc-*i*PrOH-H₂O 2:2:1). **G4** dissolved in the anhydrous MeOH (2.1 mL) were added propane-1,3-dithiol (0.16 mL) and triethylamine (0.16 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 under reduced pressure. The residue obtained was flash-chromatographed, eluted with EtOAc-*i*PrOH-H₂O 2:2:1 to yield the analogue **C4** (16 mg, 68% from **G1**) as a white amorphous solid. R_f = 0.25 (EtOAc-*i*PrOH-H₂O 2:2:1). [α]²⁰_D = -26.4 (*c* 1.0 in 1:1 CHCl₃-MeOH). ¹H NMR (400 MHz, CD₃OD): δ 5.90-5.87 (m, 1H, H-5''), 5.54-5.48 (m, 1H, H-4''), 4.01-3.61 (m, 18H), 2.60 (dd, *J* = 12.4, 4.6 Hz, 1H, H-3eq), 2.13-2.11 (m, 2H, H₂-6''), 2.04 (s, 3H, NAc), 2.03 (s, 3H, NAc), 1.44-1.40 (m, 3H, H-3ax, H₂-7''), 1.31 (s, 20H, 10 × CH₂), 0.90 (t, *J* = 6.8 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CD₃OD): δ 175.59 (C=O), 175.55 (C=O), 175.53 (C=O), 136.33 (C-5''), 128.69 (C-4''), 102.61 (C-1'), 101.46 (C-2), 73.48, 73.00, 71.66, 71.35, 71.20, 70.30, 69.45, 68.82, 64.74, 64.50, 58.45 (C-2''), 56.48 (C-2'), 54.20 (C-5), 42.45 (C-3), 33.02 (CH₂), 30.81 (CH₂), 30.79 (CH₂), 30.45 (CH₂), 30.43 (CH₂), 23.70 (CH₃, NAc), 22.65 (CH₃, NAc), 14.42 (CH₃). ESI-HRMS (*m/z*) calcd for C₃₇H₆₆N₃O₁₅ [M-H]⁻: 792.4499, found: 792.4486.

4.10. Biological evaluation

4.10.1. Cell culture

Human colon cancer HCT116 cells, human leukemia K562 cells, murine

melanoma B16 cells and highly metastatic murine melanoma B16-F10 cells were originally obtained from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (China), and cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS, Hyclone), as well as penicillin and streptomycin (100 mg/ml each), at 37 °C in a humidified atmosphere of 5% CO₂.

4.10.2. Cytotoxicity assay

Cell viability was assessed using MTT method [29]. Briefly, the cells (8×10^3 /well) were seeded into 96-well plates, and were treated with GM3 analogues (C1, C2, C3, C4) at 20, 200, 400, 1000 μ M or vehicle DMSO for 48 h. At the end of the incubation period, 20 μ l (5 mg/mL) MTT solution was added into each well of the 96-well plate. Following 4 h of incubation at 37 °C and 5% CO₂, absorbance was detected at a wavelength of 570 nm. The results are presented as means \pm SD from the three independent experiments.

4.10.3. Wound healing test

Wound healing test was used to evaluate the effect of GM3 analogues on tumor cell motility by B16 and B16-F10 tumor cell lines [30]. The cells (1.5×10^5 cells/24 wells) were cultured into 24-well plates and grown in RPMI-1640 medium containing 10% FBS to nearly confluent cell monolayer. A 10 μ L plastic pipette tip was used to draw a linear “wound” in the cell monolayer of each well. The monolayer was then washed twice with serum free RPMI-1640 medium to remove debris or detached cells, and GM3 analogues were added at different concentrations in fresh medium with FBS, RPMI-1640 medium containing DMSO was added to the control well as the solvent control, and subsequently cultured for 24 h. The wound healing of the scratched cells was photographed under a DMIL LED AE2000 inverted microscope (Leica, Wetzlar, Germany) and the effect of GM3 analogues on tumor cell motility was expressed as migration % of 0 hour (wound width at exposure time point/wound width at 0 hour). The experiments were performed in triplicate.

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Supporting information

Supplementary data of NMR spectra associated with this article can be found in the online version.

References

- 1.S. Hakomori, Glycosphingolipids in cellular interaction, differentiation, and oncogenesis, *Annu. Rev. Biochem.* 50 (1981) 733-764.
- 2.T. Yamakawa, A reflection on the early history of glycosphingolipids, *Glycoconj. J.* 13 (1996) 123-126.
- 3.S. Roseman, Reflections on glycobiology, *J. Biol. Chem.* 276 (2001) 41527-41542.
- 4.S. Hakomori, K. Handa, GM3 and cancer, *Glycoconj. J.* 32 (2015) 1-8.
- 5.M. Ono, K. Handa, S. Sonnino, D.A. Withers, H. Nagai, S. Hakomori, GM3 ganglioside inhibits CD9-facilitated haptotactic cell motility: coexpression of GM3 and CD9 is essential in the downregulation of tumor cell motility and malignancy, *Biochem.* 40 (2001) 6414-6421.
- 6.C. Zheng, M. Terreni, M. Sollogoub, Y. Zhang, Ganglioside GM3 and its role in cancer, *Curr. Med. Chem.* 26 (2019) 2933-2947.
- 7.Y. Zhang, K. Iwabuchi, S. Nunomura, S. Hakomori, Effect of synthetic sialyl 2-->1 sphingosine and other glycosylsphingosines on the structure and function of the "glycosphingolipid signaling domain (GSD)" in mouse melanoma B16 cells, *Biochem.* 39 (2000) 2459-2468.
- 8.E.G. Bremer, J. Schlessinger, S. Hakomori, Ganglioside-mediated modulation of cell growth. Specific effects of GM3 on tyrosine phosphorylation of the epidermal growth factor receptor, *J. Biol. Chem.* 261 (1986) 2434-2440.
- 9.T.W. Chung, S.J. Kim, H.J. Choi, K.J. Kim, S.H. Kim, H.J. Lee, J.H. Ko, Y.C. Lee, A. Suzuki, C.H. Kim, Ganglioside GM3 inhibits VEGF/VEGFR-2-mediated

- angiogenesis: direct interaction of GM3 with VEGFR-2, *Glycobiol.* 19 (2009) 229-239.
- 10.S. Yamamura, K. Handa, S. Hakomori, A close association of GM3 with c-Src and Rho in GM3-enriched microdomains at the B16 melanoma cell surface membrane: a preliminary note, *Biochem. Biophys. Res. Commun.* 236 (1997) 218-222.
- 11.N. Kojima, S. Hakomori, Cell adhesion, spreading, and motility of GM3-expressing cells based on glycolipid-glycolipid interaction, *J. Biol. Chem.* 266 (1991) 17552-17558.
- 12.Y. Li, X. Huang, W. Zhong, J. Zhang, K. Ma, Ganglioside GM3 promotes HGF-stimulated motility of murine hepatoma cell through enhanced phosphorylation of cMet at specific tyrosine sites and PI3K/Akt-mediated migration signaling, *Mol. Cell. Biochem.* 382 (2013) 83-92.
- 13.A. Varki, Biological roles of oligosaccharides: all of the theories are correct, *Glycobiology* 3 (1993) 97-130.
- 14.Z. Yin, X. Huang, Recent development in carbohydrate based anti-cancer vaccines, *J. Carbohydr. Chem.* 31 (2012) 143-186.
- 15.H. Qu, J. Liu, J. Wdzieczak-Bakala, D. Lu, X. He, W. Sun, M. Sollogoub, Y. Zhang, Synthesis and cytotoxicity assay of four ganglioside GM3 analogues, *Eur. J. Med. Chem.* 75 (2014) 247-257.
- 16.C. Zheng, H. Guan, Y. Liu, Z. Li, T. Bavaro, M. Terreni, M. Sollougoub, J. Xu, Y. Zhang, Chemoenzymatically synthesized ganglioside GM3 analogues with inhibitory effects on tumor cell growth and migration, *Eur. J. Med. Chem.* 165 (2019) 107-114.
- 17.A. Hasegawa, M. Ogawa, M. Ki, Synthesis of a ganglioside GM3 position isomer, N-acetylneuraminosyl-(α (2 \rightarrow 6)-lactosyl- β (1 \rightarrow 1)-ceramide, *Biosci. Biotechnol. Biochem.* 56 (1992) 535-536.
- 18.A. Hasegawa, Y. Kojima, M. Ogawa, M. Kiso, Synthetic studies on sialoglycoconjugates 37: Synthesis of sialyl- α (2 \rightarrow 6)-D-glucopyranosyl, 2-acetamido-2-deoxyhexopyranosyl, and sialyl- α (2 \rightarrow 3)-2-acetamido-2-deoxy-Dglucopyranosyl ceramide or the analogs at the lipophilic part, *J. Carbohydr. Chem.*

- 11 (1992) 343-357.
- 19.V. Martichonok, G.M. Whitesides, Stereoselective α -sialylation with sialyl xanthate and phenylsulfenyl triflate as a promotor, *J. Org. Chem.* 61 (1996) 1702-1706.
- 20.T. Bavaro, M. Filice, P. Bonomi, Q. Abu alassal, G. Speranza, J. Guisan, M. Terreni, Regioselective deprotection of peracetylated disaccharides at the primary position catalyzed by immobilized acetyl xylan esterase from *Bacillus pumilus*, *Eur. J. Org. Chem.* (2011) 6181-6185.
- 21.R.R. Schmidt, P. Zimmermann, Synthesis of D-erythro sphingosines, *Tetrahedron Lett.* 27 (1986) 481-484.
- 22.Y. Gao, X. He, F. Ding, Y. Zhang, Recent progress in chemical syntheses of sphingosines and phytosphingosines, *Synthesis* 48 (2016) 4017-4037.
- 23.J. Gervay, J.M. Peterson, T. Oriyama, S.J. Danishefsky, An unexpected sialylation: Total syntheses of GM4 and a Positional Isomer, *J. Org. Chem.* 58 (1993) 5465-5468.
- 24.D. Lafont, J. D'Attoma, R. Gomez, P.G. Goekjian, Epoxidation of glycals with oxone–acetone–tetrabutylammonium hydrogen sulfate: a convenient access to simple β -D-glycosides and to α -D-mannosamine and D-talosamine donors, *Tetrahedron: Asymmetry* 22 (2011) 1197–1204.
- 25.C. Zheng, T. Bavaro, S. Tengattini, A. G. Mascherpa, M. Sollogoub, Y. Zhang, M. Terreni, Chemoenzymatic synthesis of glycoconjugates mediated by regioselective enzymatic hydrolysis of acetylated 2-amino pyranose derivatives, *Eur. J. Org. Chem.* 22 (2019) 3622-3631.
- 26.Y. Zhang, K. Iwabuchi, S. Nunomura, S. Hakomori, Effect of synthetic sialyl 2→1 sphingosine and other glycosylsphingosines on the structure and function of the “glycosphingolipid signaling domain (GSD)” in mouse melanoma B16 cells, *Biochem.* 39 (2000) 2459-2468.
- 27.K. Iwabuchi, Y. Zhang, K. Handa, D.A. Withers, P. Sinaÿ, S. Hakomori, Reconstitution of membranes simulating “glycosignaling domain” and their susceptibility to lyso-GM3, *J. Bio. Chem.* 275 (2000) 15174-15181.
- 28.Y. Murozuka, N. Watanabe, K. Hatanaka, S. Hakomori, Lyso-GM3, its dimer, and

multimer: their synthesis, and their effect on epidermal growth factor-induced receptor tyrosine kinase, *Glycoconj. J.* 24 (2007) 551-563.

29.T. Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays, *J. Immunol. Methods* 65 (1983) 55-63.

30.C. Tabolacci, A. Lentini, P. Mattioli, B. Provenzano, S. Oliverio, F. Carlomosti, S. Beninati, Antitumor properties of aloe-emodin and induction of transglutaminase 2 activity in B16-F10 melanoma cells, *Life Sciences* 87 (2010) 316-324.