(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 December 2015 (03.12.2015)

WIPO | PCT

(10) International Publication Number
WO 2015/179983 A1

(51) International Patent Classification:
A61K 31/7028 (2006.01) *A61P 35/00* (2006.01)
A61K 31/70 (2006.01)

(21) International Application Number:
PCT/CA2015/050490

(22) International Filing Date:
29 May 2015 (29.05.2015)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/005,063 30 May 2014 (30.05.2014) US

(71) Applicant: **THE UNIVERSITY OF MANITOBA** [CA/CA]; 631 Drake Building, 181 Freedman Cresent, Winnipeg, Manitoba R3T 5V4 (CA).

(72) Inventors: **OGUNSINA, Makanjuola**; c/o 631 Drake Building, 181 Freedman Cresent, Winnipeg, Manitoba R3T 5V4 (CA). **SAMADDER, Pranati**; c/o 631 Drake Building, 181 Freedman Crescent, Winnipeg, Manitoba R3T 5V4 (CA). **SCHWEIZER, Frank**; c/o 631 Drake Building, 181 Freedman Crescent, Winnipeg, Manitoba R3T 5V4 (CA). **ARTHUR, Gilbert**; c/o 631 Drake Building, 181 Freedman Crescent, Winnipeg, Manitoba R3T 5V4 (CA). **IDOWU, Temilolu**; 321 Bairdmore Boulevard, Winninpeg, Manitoba R3T 6A3 (CA).

(74) Agent: **ADE & COMPANY INC.**; 2157 Henderson Hwy, Winnipeg, Manitoba R2G 1P9 (CA).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: DI- AND TRI-CATIONIC GLYCOSYLATED ANTITUMOR ETHER LIPIDS, L-GUCOSYLATED GAELS AND RHAMNOSE-LINKED GAELS AS CYTOTOXIC AGENTS AGAINST EPITHELIAL CANCER CELLS AND CANCER STEM CELLS

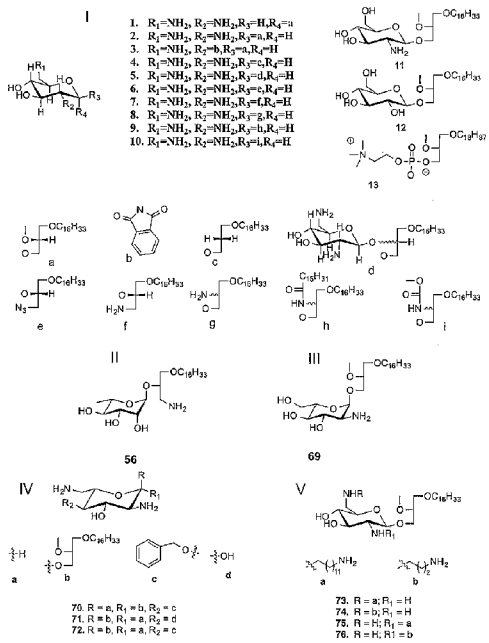


Figure 1-1

(57) Abstract: Glycosylated Antitumor Ether Lipids (GAELs) kill cancer cells by a nonapoptotic pathway which is an attractive strategy to avoid resistance. To further optimize the antitumor effect, we prepared various analogs of di-, and tri-cationic GAEL analogs differing in the nature of the sugar (D-giucose or L-glucose), the anomeric linkage as well as position of the glycerolipid moiety. The di- and tri-cationic GAELs were synthesized and their in vitro anticancer properties were evaluated against drug resistant and aggressively growing cancer cell lines derived from human breast, prostate, pancreatic and ovarian cancers. The most potent dicationic GAEL analogs were also studied against cancer stem cells obtained from breast BT 474, prostate DU145 and ovarian A2780cp cell lines. Our results indicate that the number of positive charges, the position of the amino substituents and the nature of the sugar have significant effects on the anticancer activities of these compounds. The most active analog kill 50% of the cells at concentration range of 0.5-5μM and 90% of the cells at the concentration of 1-10μM depending on type of cancer cells.

WO 2015/179983 A1

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

DI- AND TRI-CATIONIC GLYCOSYLATED ANTITUMOR ETHER LIPIDS, L-GUCOSYLATED GAELS AND RHAMNOSE-LINKED GAELS AS CYTOTOXIC AGENTS AGAINST EPITHELIAL CANCER CELLS AND CANCER STEM CELLS.

PRIOR APPLICATION INFORMATION

The instant application claims the benefit of US Provisional Patent Application, filed May 30, 2014, under serial number 62/005,063, entitled 'Dicationic Glycosylated Antitumor Ether Lipids, L-gucosylated GAELs and Rhamnose-linked GAELs as cytotoxic agents against epithelial cancer cells and cancer stem cells', the contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

Despite the huge investment into finding effective treatments for cancer, it is still one of the major health problems in developed and developing countries. The UN in February 2014 estimated new cancer cases worldwide to rise from 14 million to 22 million per year within the next two decades, and annual cancer deaths rising from 8.2 million to 13 million. Cancer has therefore been described by the UN as a major obstacle to human development and well-being worldwide.

The major problems impeding the development of cures to cancer are drug resistance, radiotherapy resistance and metastases. Many of the existing anticancer drugs act by disrupting cell DNA, preventing DNA synthesis and targeting microtubules. Radiotherapy kills cells by damaging DNA. This perturbation in cell physiology induced by these drugs or radiation induces cell apoptosis to kill cancer cells. While many drugs are initially successful in killing the cancer cells, resulting in tumor shrinkage, there is invariably a relapse and the tumor reappears with cells that resist chemotherapeutic agents. (Tan, D. S. et al., *J. Natl. Cancer Inst.* **2008** *100*, 672–679; Tanner, M. et al., J. *Mol. Cancer Ther.* **2004**, *3*, 1585–1592; Ajani, J. A. et al., *Journal of Clinical Oncology* **2009,** *27.* 162-163). This resistance to chemotherapeutic agents results in tumors that are refractory to treatment, leading to metastases and death. There are also very few drugs, if any, for treatment of cancers

that have metastasized. There is increasing evidence that the relapse of tumors and development of drug and radiation resistant tumors as well as progression to metastases may be due to the presence of a small population of cells in the tumor called cancer stem cells (CSC) or tumor initiating cells. CSCs are distinct from the cells of the bulk tumor in having the capacity for self-renewal, asymmetric division and differentiation. These cells resist apoptotic cell death induced by chemotherapy and radiotherapy, and may ultimately generate drug resistant differentiated cells that make up the bulk of tumor that recurs. CSCs have been identified and isolated from virtually all solid and haematological tumors (Garvalov, B. K.. and Acker, T. *J. Mol. Med.* **2011**, *89*, 95–107; Zobalova, R.; Stantic, M.; Stapelberg, M.; Prokopova, K.; Dong, J.; Truksa, J. et al, Drugs that Kill Cancer Stem-like Cells. In: Shostak S, editor. Cancer Stem Cells Theories and Practice, ISBN: 978-953-307-225-8, **2011**). In light of the potential role these cells play in tumor relapse, drug resistance and metastases, effective cancer treatment will require targeting the CSCs along with the bulk differentiated cells of the tumor.

Only a few compounds have been identified that kill CSCs. They include parthenolide, salinomycin, metformin, lapatinib, and mitoVES. The mechanism of cell death induction is via apoptosis, generation of reactive oxygen species and inhibition of proinflammatory cytokines NFκB. The significant toxicity of salinomycin may impact the further development of this compound which has been in use for agricultural purposes for decades (Zobalova, R.; Stantic, M.; Stapelberg, M.; Prokopova, K.; Dong, J.; Truksa, J. et al, Drugs that Kill Cancer Stem-like Cells. In: Shostak S, editor. Cancer Stem Cells Theories and Practice, ISBN: 978-953-307-225-8, **2011**).

We have been working on a class of antitumor ether lipids (AEL) called glycosylated antitumor ether lipids (GAELs). The cytotoxic properties of GAELs, prototypified by compound **11**, have been established to be superior to the most studied analog of AELs, edelfosine. Unlike edelfosine, which kills cells by apoptosis, GAELs kill cell by an apoptotic independent mechanism. The mechanism of action involves the perturbation of endocytosis pathway to generate large acidic vacuoles that ultimately leads to the release of acid hydrolases to induce cell death (Erukulla,

R. V. et al., *J. Med. Chem.* **1996**, *39*, 1545–1548; Samadder, P. et al., *Biochem. Cell Biol.* **2009**, *87*, 401–414; Samadder, P. et al., G. *Anticancer Res.* **2011**, *31*, 3809–3818; Samadder, P. et al., G. *Anticancer Res.* **1998**, *18*, 465–470).

This ability to kill cells by an apoptosis-independent pathway led us to postulate that GAELs will have the ability to kill CSCs as they are unaffected by the apoptosis-inhibiting mechanisms or strategies the cells use to prevent death by apoptosis. Recently, we validated our hypothesis by demonstrating the cytotoxic activity of GAELs against CSCs isolated from BT474 cell lines (Samadder P. et al., Eur J Med Chem 2014, 78,225-235).

Our ongoing structure activity studies on GAELs have led to the observation that the potency of anticancer activities of GAELs is intimately linked with their cationic nature. Also because the *O*-glycosidic bond in compounds may be susceptible to hydrolysis by glycosidases, development of non-hydrolysable analogs will enhance the stability of the compounds *in vivo*.

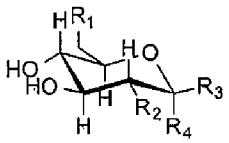
SUMMARY OF THE INVENTION

According to an aspect of the invention, there is provided a method of treating cancer in an individual in need of such treatment comprising administering to said individual an effective amount of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V'), as set forth below.

According to another aspect of the invention, there is provided use of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V') as described herein for treating cancer

According to yet another aspect of the invention, there is provided use of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V') in the manufacture of a medicament for treating cancer.

I



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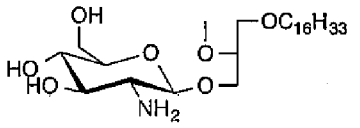
2. R_1=NH_2, R_2=NH_2,R_3=a,R_4=H
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H



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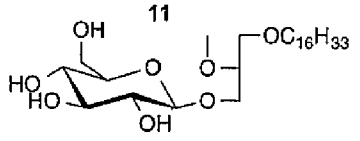

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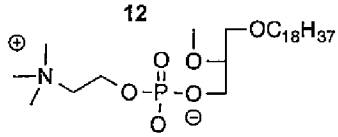

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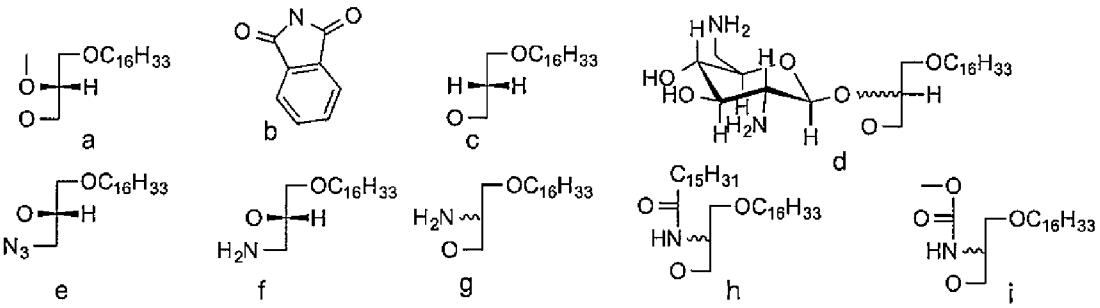

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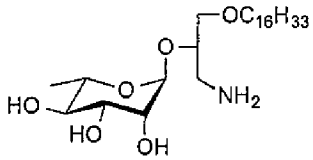

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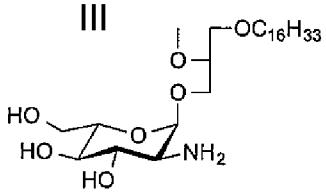
III



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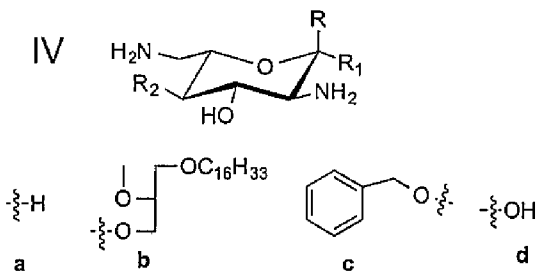
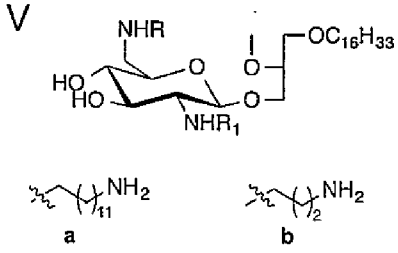
IV



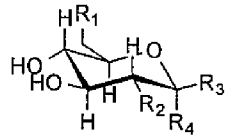
70. R = a, R_1 = b, R_2 = c
71. R = b, R_1 = a, R_2 = d
72. R = b, R_1 = a, R_2 = c

V

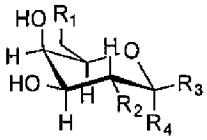


73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

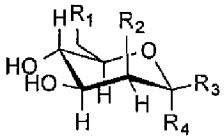
I'



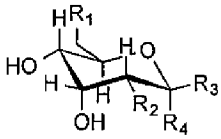
D-glucose



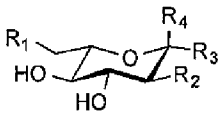
D-galactose



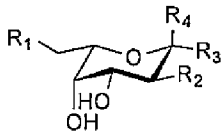
D-mannose



D-allose

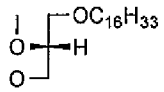


L-glucose

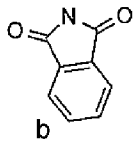


L-galactose

1. R_1=NH_2, R_2=NH_2,R_3=H,R_4=a
2. R_1=NH_2, R_2=NH_2,R_3=a,R_4=H
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H



a



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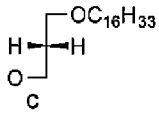

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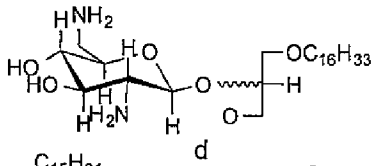

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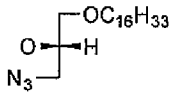

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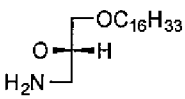

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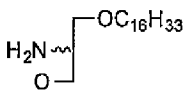

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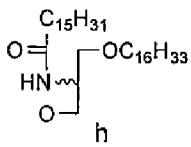

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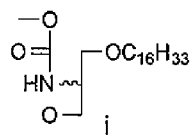
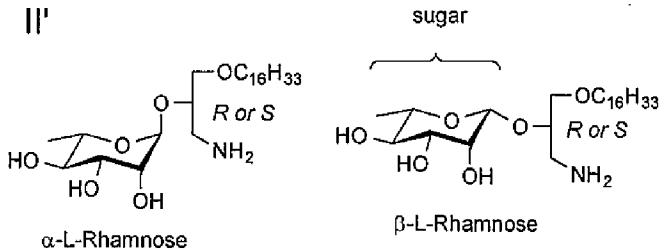


i

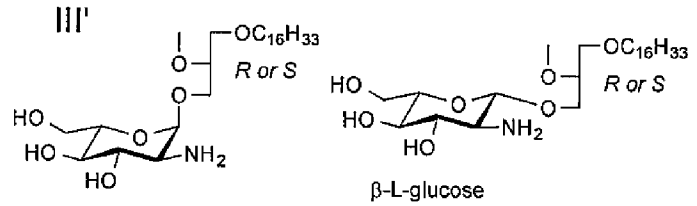
II'



56

sugar = 6-deoxy-α-D-galactose, 6-deoxy-β-D-galactose, 6-deoxy-α-L-galactose, 6-deoxy-β-L-galactose, 6-deoxy-α-D-glucose, 6-deoxy-β-D-glucose, 6-deoxy-α-L-glucose-based, 6-deoxy-β-L-glucose, 6-deoxy-α-D-mannose, 6-deoxy-β-D-mannose, 6-deoxy-α-L-mannose, or 6-deoxy-β-L-mannose

III'



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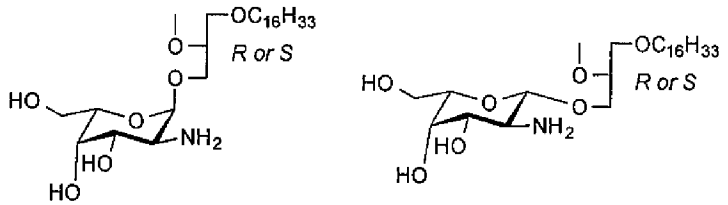
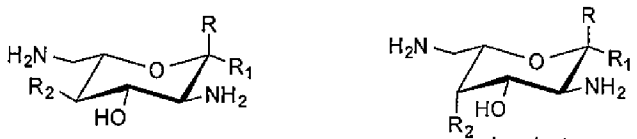
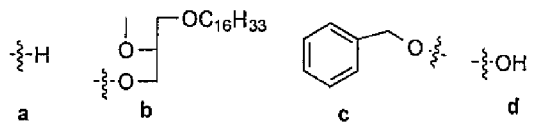


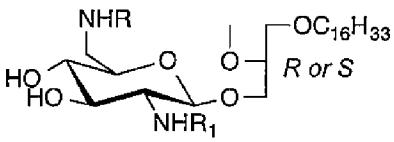
IV'



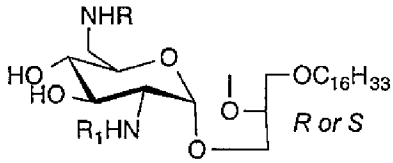


70. R = a, R_1 = b, R_2 = c
71. R = b, R_1 = a, R_2 = d
72. R = b, R_1 = a, R_2 = c

V'



β-D-glucose



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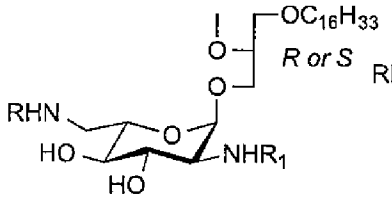
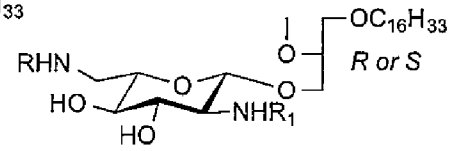


α-L-glucose



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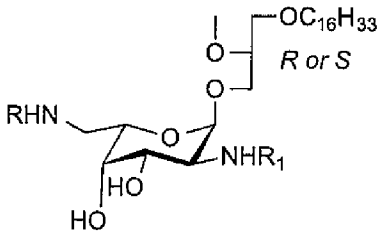
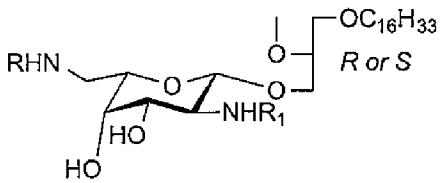


α-L-galactose



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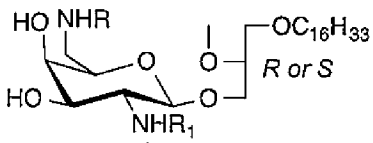

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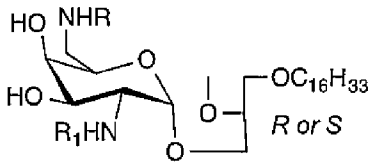

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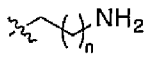

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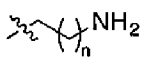


b

n = 1,2,3,....., 16

73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Structures of the synthesized glycolipids evaluated for anticancer properties (Formulas (I), (II), (III), (IV), (V)) and structures of related compounds in formulas (I'), (II'), (III'), (IV'), (V)') which differ from formula (I), (II), (III), (IV), (V)) by variations of the sugar moiety. Compounds **11**, **12** and **13** are reference compounds and not covered in this patent

Figure 2A. Effects of compounds **1 – 11** on the viability of MDA-MB231 cells. MDA-MB-231 cells were cultured in DMEM medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1-11** (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2B. Effects of compounds **1 – 11** on the viability of JIMT-1 cells. JIMT-1 cells were cultured in DMEM medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1-11** (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2C. Effects of compounds **1 – 11** on the viability of BT-474 cells. BT-474 cells were cultured in DMEM/F12 medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1-11** (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2D. Effects of compounds **1 – 11** on the viability of DU145 cells. DU145 cells were cultured in DMEM medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1-11** (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2E. Effects of compounds **1 – 11** on the viability of PC3 cells. PC3 cells were cultured in F12K medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1-11** (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2F. Effects of compounds **1 – 11** on the viability of MiaPaCa2 cells. MiaPaCa2 cells were cultured in DMEM medium supplemented with 10% FBS and 2.5% horse serum. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1-11** (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD490 was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2G. Effect of compounds **1**, **11**, **56**, **69 or 73** on the viability of A2780s, A2780cp, U87 and U251 cells. A2780s and A2780cp cells were cultured in DMEM/F12 medium supplemented with 10% FBS. U251 and U87 cells were cultured in DMEM supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with the GAELs compounds **1**, **11**, **56**, **69 or 73** for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added

and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with no cells were treated in a similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2H. Effect of compounds **1, 69 - 72** on the viability of DU145 cells. DU145 cells were cultured in DMEM medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1, 69 - 72** for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2I. Effects of compounds **1, 69 - 72** on the viability of MiaPaCa2 cells. MiaPaCa2 cells were cultured in DMEM medium supplemented with 10% FBS and 2.5% horse serum. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1, 69 - 72** for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2J. Effects of compounds **1, 69 – 72** on the viability of JIMT-1 cells. JIMT-1 cells were cultured in DMEM medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compounds **1, 69 – 72** for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2K. Effects of compounds **1, 69-72** on the viability of MDA-MB-231 cells. MDA-MB-231 cells were cultured in DMEM medium supplemented with 10% FBS. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were

incubated with compounds **1, 69-72** for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2L to 2N. Effects of compounds **1**, **56** or **69** on the viability of primary epithelial ovarian cancer cells grown as adherent and spheroid cultures. EOC cells were isolated from the ascites of ovarian cancer patients. Cells were seeded in regular 96-well plates and grown as adherent cultures. 24 h after seeding the cells were incubated with compounds **1**, **56**, or **69** for 48 h and the viability of the cells were determined with the MTS assay. Spheroid cultures were obtained by seeding the cells in ultra-low adhesion 96-well plates for 3 days. The spheroids were incubated with compounds **1**, **56** or **69** for 3 days. At the end of the incubations, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 2O. Effect of compound 73 on the viability of epithelial cancer cell line. BT474, PC3, MiaPaCa2, JIMT1, DU145 and MDA-MB-231 cells were grown in their respective growth medium described in the methods section. Equal numbers were dispersed into 96-well plates. After 24 h, the cells were incubated with compound 73 for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in a similar fashion and the values utilized as blanks. The results represent the mean ± standard deviation of 6 independent determinations.

Figure 3A. Effects of compounds **1**, **2**, **4**, **8, 56** and **69** on the viability of cancer stems cells isolated form BT-474 breast cancer cell lines. BT474 cancer stem cells were obtained by staining for ALDH1 and sorting the cells by flow cytometry. The spheroids were grown in ultra low adhesion plates in mammocult medium for 6 days. The spheroids formed were harvested and trypsinised and equal numbers were

seeded in 48-well low adhesion plates for 5-6 days to allow formation of spheroids. The spheroids were incubated with varying concentrations of compounds **1**, **2**, **4** or **8** (0-30 μM) for 6 days. At the end of the incubation the MTS reagent was added to each well and the plates were incubated in a 5% CO_2 incubator for 4 h. The absorbance was read at 490 nm in a plate reader. The results are the means ± standard deviation for 4 independent determinations.

Figure 3B. Effects of compounds **1**, **2**, **4**, **8** and **56** on the viability of cancer stems cells isolated form DU145 prostate cancer cell lines. DU145 cancer stem cells were obtained by staining for ALDH1 and sorting the cells by flow cytometry. The spheroids were grown in ultra low adhesion plates in prostatosphere growth medium for 6 days. The spheroids formed were harvested and trypsinised and equal numbers were seeded in 48-well low adhesion plates for 5-6 days to allow formation of spheroids. The spheroids were incubated with varying concentrations of compounds **1**, **2**, **4**, **8** or **56** (0-30 μM) for 6 days. At the end of the incubation the MTS reagent was added to each well and the plates were incubated in a 5% CO_2 incubator for 4 h. The absorbance was read at 490 nm in a plate reader. The results are the means ± standard deviation for 4 independent determinations.

Figure 3C. Effect of **1**, **56**, **69** or **73** on viability of cancer stem cells isolated from A2780cp ovarian cancer cell line. A2780 cancer stem cells were obtained by staining A2780cp cells for ALDH1 and sorting the cells by flow cytometry. The spheroids/aggregates were grown in ultra low adhesion plates in TPM medium for 6 days. The spheroids formed were harvested and trypsinised and equal numbers were seeded in 48-well low adhesion plates for 3 days followed by incubation with varying concentrations of compounds **1**, **56, 69** or **73** for 3 days. At the end of the incubation the MTS reagent was added to each well and the plates were incubated in a 5% CO_2 incubator for 4 h. The absorbance was read at 490 nm in a plate reader. The results are the means ± standard deviation for 4 independent determinations.

Figure 4A. Effect of GAEL compounds **1**, **2**, **4**, and **8** on the integrity of BT474 breast cancer stem cell spheroids. Equal numbers of BT474 cancer stem cells were seeded into ultra low adhesion 48-well plates and grown for 5 days to allow for

spheroid formation. The spheroids were incubated with or without 10 μM GAELs for up to 6 days. The images were taken after 4 days of incubation with an Olympus IX70 microscope at a magnification of x10.

Figure 4B. Effect of GAEL compounds **56** and **69** on the integrity of BT474 breast cancer stem cell spheroids. Equal numbers of BT474 cancer stem cells were seeded into ultra low adhesion 48-well plates and grown for 5 days to allow for spheroid formation. The spheroids were incubated with or without 10 μM GAELs for up to 6 days. The images were taken after 4 days of incubation with an Olympus IX70 microscope at a magnification of x10.

Figure 4C. Effect of GAEL compound **1** on the integrity of EOC 258 ovarian cancer cell spheroids. Equal numbers of EOC 258 cells were seeded into ultra low adhesion 96-well plates and grown for 3 days to allow for spheroid formation. The spheroids were incubated with or without **1** (0-10 μM) for 3 days. The images were taken with an Olympus IX70 microscope at a magnification of x10.

Figure 5A. Effect of GAEL compounds **1**, **2**, **4**, and **8** on the integrity of DU145 prostate cancer stem cell spheroids. Equal numbers of DU145 cancer stem cells were seeded into ultra low adhesion 48-well plates and grown for 5 days to allow for spheroid formation. The spheroids were incubated with or without 10 μM GAELs for up to 6 days. The images were taken after 4 days of incubation with an Olympus IX70 microscope at a magnification of x10.

Figure 5B. Effect of GAEL compound **56** on the integrity of DU145 prostate cancer stem cell spheroids. Equal numbers of DU145 cancer stem cells were seeded into ultra low adhesion 48-well plates and grown for 5 days to allow for spheroid formation. The spheroids were incubated with or without 10 μM GAELs for up to 6 days. The images were taken after 4 days of incubation with an Olympus IX70 microscope at a magnification of x10.

Figure 5C. Effect of GAEL compound **56** on the integrity of A2780 ovarian cancer stem cells. Equal numbers of A2780 cancer stem cells were seeded into ultra low adhesion 48-well plates and grown for 4 days to allow for spheroid/aggregate formation. The spheroids were incubated with or without 10 μM GAELs for up to 6

days. The images were taken after 4 days of incubation with an Olympus IX70 microscope at a magnification of x10

Figure 6. Tolerability of female Rag2M mice to compound **56**. Female Rag2 mice were individually weighed and administered compound **56** intravenously or orally according to individual body weight. The mice were monitored for behavioural changes, body weight for 14 days. All mice were sacrificed on day 15 and necropsy was performed to compound **56**. The figure shows the body weight of the mice following treatment for 14 days. The weights are the average of 3 mice..

DESCRIPTION OF THE PREFERRED EMBODIMENTS

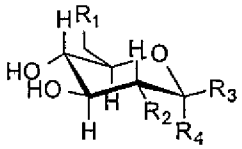
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned hereunder are incorporated herein by reference.

Glycosylated Antitumor Ether Lipids (GAELs) kill cancer cells by a non-apoptotic pathway, which is an attractive strategy to avoid resistance. To further optimize the antitumor effect, we prepared various analogs of dicationic GAEL analogs, differing in the nature of the sugar, the anomeric linkage as well as position of the glycerolipid moiety. As discussed below, fifteen dicationic GAELs and four tricationic GAELs were synthesized and their in vitro anticancer properties were evaluated against drug resistant and aggressively growing cancer cell lines derived from human breast, prostate and pancreatic cancers. The most potent dicationic and tricationic GAEL analogs were also studied against cancer stem cells obtained from breast BT474 cell line and prostate DU 145 cells and A2780cp. The anticancer activities of the dicationic GAELs were compared to their mono-cationic analogs which have previously been studied in our group. Our results indicate that the number of positive charges, the position of the amino substituents and the nature of the sugar have significant effects on the anticancer activities of these compounds. The most

active analogs kill 50% of the cells at concentration range of 0.5-5 μM and 90% of the cells at the concentration of 1-7 μM, depending on type of cancer cells, as discussed below. Replacement of the sugar with L-glucosamine showed activity comparable with analogs with D-glucosamine. The mono cationic L-rhamnose derived analog has CC_{50} and CC_{90} values in the range of 2 -11 μM and 6.5 to 14 μM respectively against the cell lines tested. Our results also show that primary ovarian cancer cells derived from the ascites of ovarian cancer patients are particularly susceptible to GAELs with CC_{50}'s in the nM to low μM range. The sensitivity of these cells to GAELs were observed whether the cells were grown as adherent (2D) cultures or as spheroidal (3D) cultures

In one embodiment of the invention, there is provided a method of treating cancer in an individual in need of such treatment comprising administering to said individual an effective amount of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V')

I



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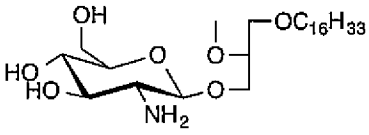
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H



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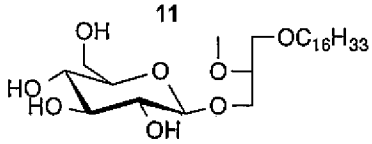

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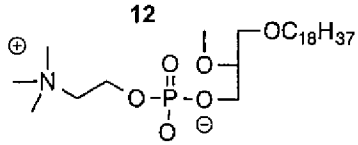

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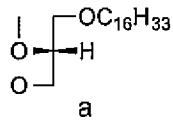

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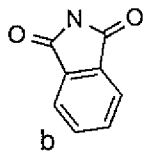

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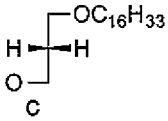

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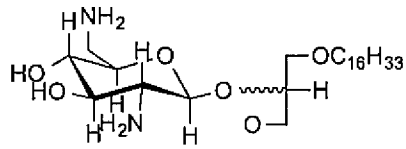

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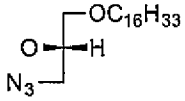

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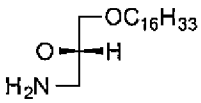

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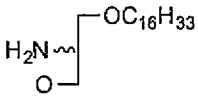

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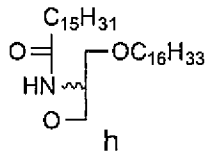

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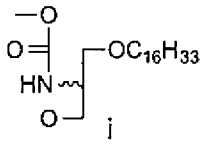
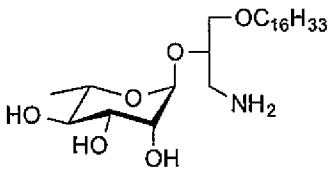


i

II



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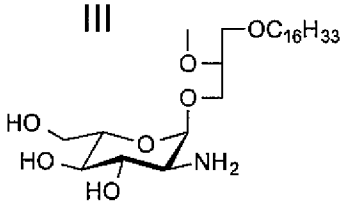
III



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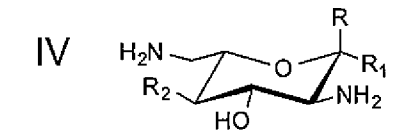
IV



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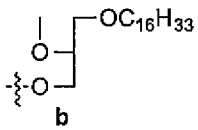
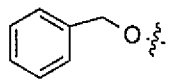


b

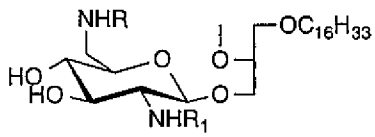


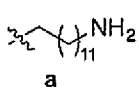
c

d

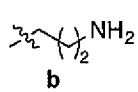
70. R = a, R_1 = b, R_2 = c
71. R = b, R_1 = a, R_2 = d
72. R = b, R_1 = a, R_2 = c

V





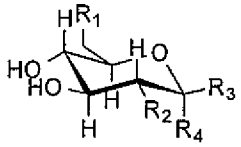
a



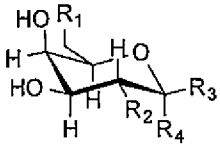
b

73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

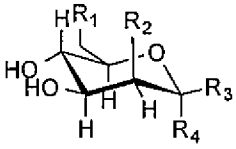
I'



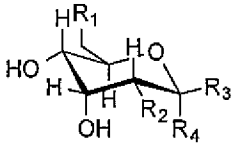
D-glucose



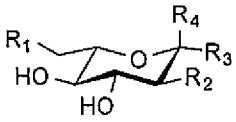
D-galactose



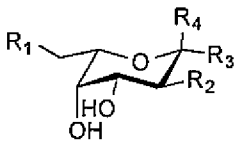
D-mannose



D-allose

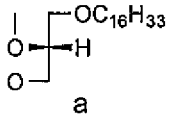


L-glucose

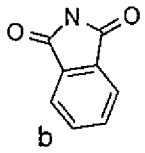


L-galactose

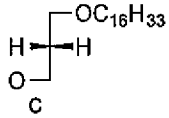
1. R_1=NH_2, R_2=NH_2,R_3=H,R_4=a
2. R_1=NH_2, R_2=NH_2,R_3=a,R_4=H
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H



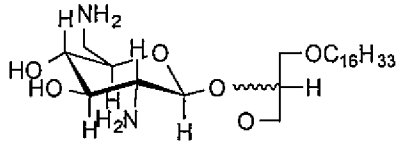
a



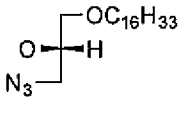
b



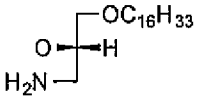
c



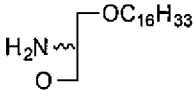
d



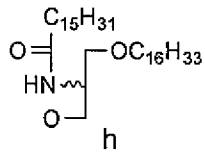
e



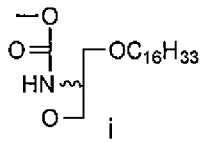
f



g

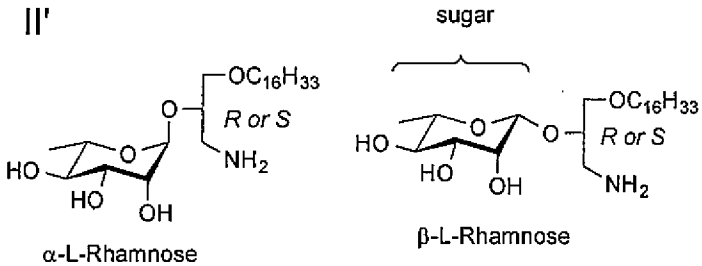


h



i

II'



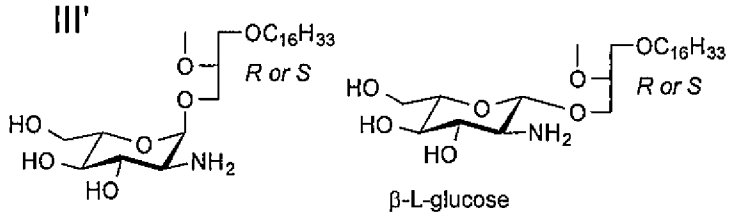
α-L-Rhamnose

β-L-Rhamnose

56

sugar = 6-deoxy-α-D-galactose, 6-deoxy-β-D-galactose, 6-deoxy-α-L-galactose, 6-deoxy-β-L-galactose, 6-deoxy-α-D-glucose, 6-deoxy-β-D-glucose, 6-deoxy-α-L-glucose-based, 6-deoxy-β-L-glucose, 6-deoxy-α-D-mannose, 6-deoxy-β-D-mannose, 6-deoxy-α-L-mannose, or 6-deoxy-β-L-mannose

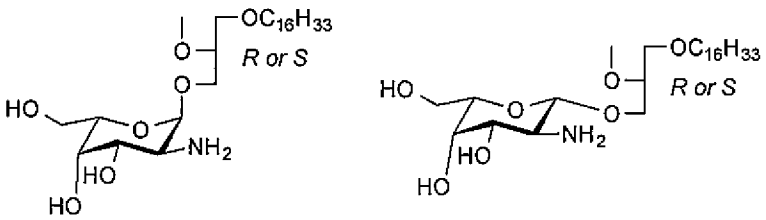
III'



α-L-glucose

β-L-glucose

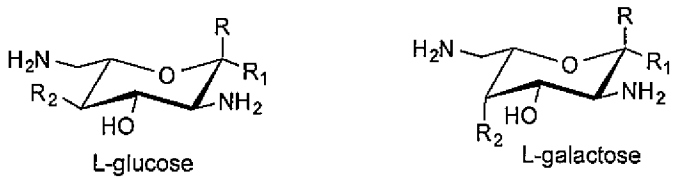
69



α-L-galactose

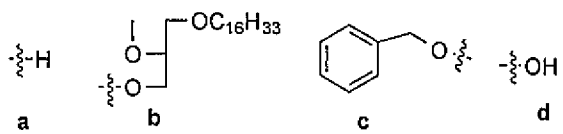
β-L-galactose

IV'



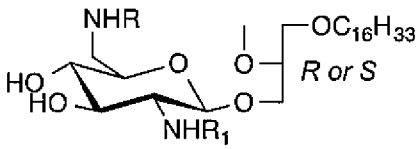
L-glucose

L-galactose

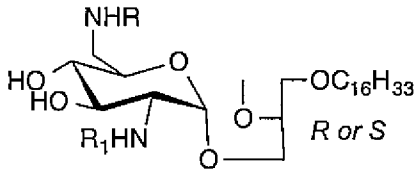


70. R = a, R_1 = b, R_2 = c
71. R = b, R_1 = a, R_2 = d
72. R = b, R_1 = a, R_2 = c

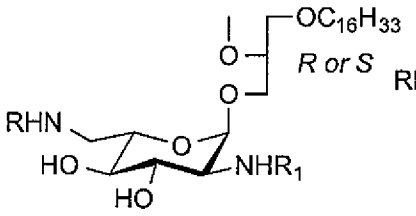
V'



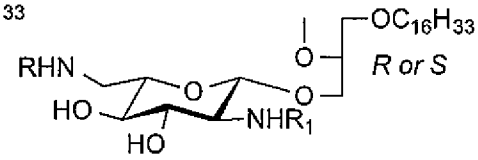
β-D-glucose



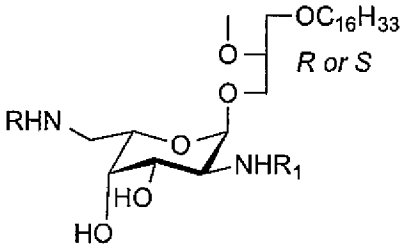
α-D-glucose



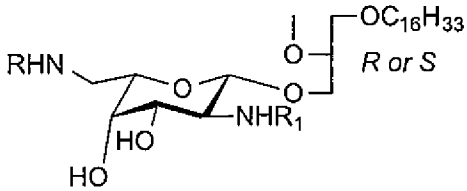
α-L-glucose



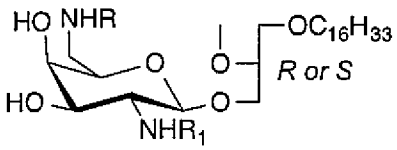
β-L-glucose



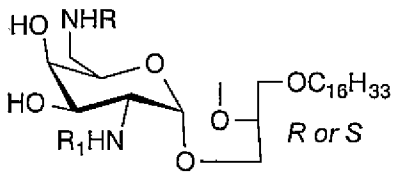
α-L-galactose



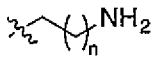
β-L-galactose



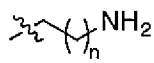
β-D-galactose



α-D-galactose



a



b

n = 1,2,3,....., 16

73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

In another embodiment of the invention, the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol.

As will be appreciated by one of skill in the art, the compounds of formula (I) are D-glucose based. In alternative embodiments, the compounds may be D-galactose-based, D-mannose-based, D-allose, L-glucose or L-galactose-based as shown in formula (I'). The stereochemistry of the glycerolipid may be R or S as shown in formula (I').

Similarly, the compound of formula (II) is α-L-rhamnose based but in alternative embodiments may be α-L-rhamnose, 6-deoxy-α-D-galactose, 6-deoxy-β-D-galactose 6-deoxy-α-L-galactose, 6-deoxy-β-L-galactose, 6-deoxy-α-D-glucose, 6-deoxy-β-D-glucose, 6-deoxy-α-L-glucose-based, 6-deoxy-β-L-glucose-based, 6-deoxy-α-D-mannose, 6-deoxy-β-D-mannose, 6-deoxy-α-L-mannose-based or 6-deoxy-β-L-mannose-based. The stereochemistry of the glycerolipid may be R or S as shown in formula (II')

The compound of formula (III) is α-L-glucose based but in other embodiments

may be β-L-glucose, α-L-galactose or β-L-galactose-based as shown in formula (III') The stereochemistry of the glycerolipid may be R or S.

In yet other embodiments, where appropriate, O-glycoside may be replaced with C-glycoside.

The compound of formula (IV) are α-L-gluco-based diamino glycolipids or β-L-gluco diamino glycolipids that can contain one or more benzylether groups at the sugar portion but in alternative embodiments may be β-L-galacto or α-L-galacto-based as shown in formula (IV'). The stereochemistry of the glycerolipid may be R or S.

The compounds of formula (V) are tricationic β-D-gluco-based glycolipids with alkylamino substituents at the 2- or 6-position of D-glucose but in alternative embodiments may be α-D-gluco-based, α-L-gluco-based, β-L-gluco-based, α-D-galacto-based, β-D-galacto-based, α-L-galacto-based or β-L-galacto-based as shown in formula (V'). The stereochemistry of the glycerolipid may be R or S.

As will be apparent to one of skill in the art, other suitable substitutions and alterations can be determined through routine experimentation, based on the guidance on the relationship between structure and activity of the compounds provided herein.

According to another aspect of the invention, there is provided a compound of formula (I), formula (II), formula (III), formula (IV) or formula (V).

In a preferred embodiment of the invention, the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-

Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol.

According to another aspect of the invention, there is provided use of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V') for treating cancer.

In a preferred embodiment of the invention, the compound is selected from the group consisting of: In another embodiment of the invention, the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol.

According to another aspect of the invention, there is provided use of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula

(III'), formula (IV), formula (IV'), formula (V) or formula (V') in the manufacture of a medicament for treating cancer.

In a preferred embodiment of the invention, the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol.

According to another aspect of the invention, there is provided a method for manufacture of a medicament for treating cancer comprising admixing a compound of formula (I), formula (II), formula (III), formula (IV) or formula (V) with a suitable excipient. The selected compound may be in an effective amount or therapeutic amount, that is, an amount that is suitable for the reduction of size of a tumor and/or for killing cancerous cells, as described herein.

In a preferred embodiment of the invention, the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-

amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol.

As will be appreciated by one of skill in the art, an "effective amount" is the amount required to kill a desired percentage of the cancerous cells. This percentage may be for example 40%, 50%, 60%, 70%, 80% or 90% or any other amount suitable for reducing the size of a cancerous tumor. The effective amount for a given individual or patient will of course depend on many factors, including the age, weight and general condition of the individual as well as the type and stage of the cancer. For illustrative purposes, a suitable dosage may be 0.1 mg/Kg body weight to 5 mg/Kg body weight, although other suitable dosages may be determined by one of skill in the art using the methods described herein.

While not wishing to be bound to a particular theory or hypothesis, it is believed that GAELS with more than one cationic group will be more potent than the monocationic lead compounds in killing cancer cells and cancer stem cells. We also hypothesize that GAELs with sugars that are not found in mammals will show cytotoxic activity against epithelial cancer cells and cancer stem cells and will have the advantage of presenting non-hydrolysable glycosidic bonds that will increase the half-life of the compounds in vivo.

According to a first aspect of the invention, there are provided methods to synthesize dicationic and tricationic GAEL analogs, as discussed herein.

In another embodiment there are provided methods to synthesize GAELs bearing a rhamnose sugar or monocationic and dicationic L-sugars as discussed herein.

There is also provided a method of killing cancer stem cells and cancer stem cell spheroids by administering effective amount of a compound of formula (I), formula (I'), formula (II), formula (II') formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V').

In an alternative embodiment, there is provided a method of treating a cancer that is refractory to treatment with existing apoptosis-inducing agents comprising administering to an individual in need of such treatment an effective amount of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V').

It is of note that monocationic GAEL, **11** has previously been demonstrated to be active against ovarian (OVCAR 3), colon (T84) brain (U251) and lung (A549 and A427) cell lines. Consequently, the more active analogues **1**, **56** and **69** will be even more active against these cell lines. Secondly the mechanism of action of the compound is independent of the tissue type so the compounds are expected to be effective against cancer stem cells derived from different cancers, as discussed herein.

In a preferred embodiment the cancer is selected from a group consisting of pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, small cell lung cancer, colon cancer, liver cancer, skin cancer (melanoma) and brain cancer. Ovarian cancer is expected to be particularly susceptible to the GAEL compounds

Examples of other suitable cancers include but are by no means limited to: drug resistant cancers (cancers that initially respond and then develop resistance to apoptosis-inducing drugs); recurring cancers (cancers that respond to treatment (surgery/chemotherapy/radiation therapy) and after a while recur), and metastasized or advanced stage cancers (which usually receive palliative care).

As will be well known to one of skill in the art, "recurring" refers to cancers that initially respond positively to treatment such that the tumor disappears to the point

where it is undetectable and the patient is said to be in remission. The period of remission is patient and cancer dependent. When the tumor subsequently reappears in such a patient, the cancer is said to have recurred.

A tumor is "resistant" to a chemotherapeutic agent when the initial treatment with the agent results in tumor shrinkage but subsequently the tumor starts to grow and increase in size despite being given the maximum tolerable levels of the agent.

Thus, as discussed above, there is provided a method of treating a resistant cancer or a recurring cancer comprising administering to an individual in need of such treatment an effective amount of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V').

In a preferred embodiment of the invention, the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol.

Although there are a large number of chemotherapeutic agents in clinical use for cancer treatment, they have proved to have limited efficacy in the overall treatment

of the disease. There is still no cure for the disease and mortality rates are still unacceptably high for most solid tumors. Evidence is accumulating that a major obstacle to preventing the recurrence of the cancer may be due to the role played by CSCs (Garvalov, B. K. and Acker, T., J. Mol. Med. 2011, 89, 95–107; Zobalova, R.; Stantic, M.; Stapelberg, M.; Prokopova, K.; Dong, J.; Truksa, J. et al, Drugs that Kill Cancer Stem-like Cells. In: Shostak S, editor. Cancer Stem Cells Theories and Practice, ISBN: 978-953-307-225-8, 2011; Samadder P. et al., Eur J Med Chem2014, 78,225-235,). These cells have been implicated in tumor progression, drug resistance and metastases and eliminating or blunting the activity of CSCs is increasingly recognized to be essential towards discovering a cure for the disease (Zobalova, R.; Stantic, M.; Stapelberg, M.; Prokopova, K.; Dong, J.; Truksa, J. et al, Drugs that Kill Cancer Stem-like Cells. In: Shostak S, editor. Cancer Stem Cells Theories and Practice, ISBN: 978-953-307-225-8, 2011). Several approaches to curtail the activity of CSCs in tumors have been suggested (Zobalova, R.; Stantic, M.; Stapelberg, M.; Prokopova, K.; Dong, J.; Truksa, J. et al, Drugs that Kill Cancer Stem-like Cells. In: Shostak S, editor. Cancer Stem Cells Theories and Practice, ISBN: 978-953-307-225-8, 2011). They include direct elimination of the CSCs, targeting the CSC niche to challenge their survival or reducing the aggressive behaviour of the cells by targeting the cellular machinery responsible. The ability of CSCs to resist apoptotic cell death is one of the major reasons for the lack of efficacy of conventional drugs because these drugs invariably kill cells by apoptosis.

An effective way to eliminate CSCs will be to develop compounds that kill cells by non-apoptotic mechanism. Such compounds will by-pass the variety of strategies used by CSCs to evade cell death by apoptosis. Since we have previously demonstrated that GAELs kill cells by a non-apoptotic mechanism that involves generation of acidic vacuoles (Samadder, P. et al., Anticancer Res. 2011, 31, 3809–3818; Samadder, P. et al., Anticancer Res. 1998, 18, 465–470; Xu, Y.et al., Chem Med Chem 2013, 8, 511–520; Arthur, G. and Bittman, R. Anticancer Agents Med Chem. 2014, 14, 592-606; Jahreiss, L. et al., Autophagy 2009, 5, 835–846.), we postulated that GAELs could potentially be toxic against CSCs.

GAELs containing amino substituent at C_2 position of the sugar moiety have been reported to show higher toxicity to cancer cells compared to an analog without a cationic moiety like compound **12** (Erukulla, R. V. et al., J. Med. Chem. 1996, 39, 1545–1548.). To synthesize novel analogs more active than reference compound **11**, we designed and synthesized compounds **1-10**, as shown in Figure 1. As discussed herein, compounds **1**, **2**, **4** and **8** with free diamino substituents were significantly more active than our reference compound Gln **11**. This shows that dicationic GAEL analogs with the sugar moiety at the sn3 position of the glycerolipid are more active.

The lack of activity of compound **3** in comparison to Gln **11** despite the free amine at the C_6 position of the sugar shows that the amino substituent at the C_2-position of the sugar is very significant for anticancer activity.

Comparison of activity of compounds **6** and **7** with that of compound **8** showed that the position of the sugar and the second cationic substituent on the glycerolipid is important for their anticancer activity.

The significantly higher activity of compound **2** when compared to that of compound **8** shows that the presence of two amino substituents on the sugar moiety is optimal for anticancer activity. Additional substitution of compound **2** with a second diamino sugar moiety as in compound **5** or acylation of the exocyclic amino group in **8** by a fatty acid as in compound **9** greatly reduce anticancer activity. This may be due to effects of these substitutions on physical properties of these compounds which significantly may affect their pharmacokinetic properties especially absorption. Unlike edelfosine, replacement of the methoxy group at sn2-position of the glycerolipid with methyl carbamate significantly reduced anticancer activity in GAEL analog and it did not enhance selectivity toward prostate cancer.

Tricationic GAELs as well as GAELs with L sugars also possess the ability to cause the disintegration of CSCs spheroids/aggregates and kill the cells.

The ability of the dicationic GAELs to cause the disintegration of cancer stem cell spheroids derived from breast and prostate cell lines is an indication that the structural changes that have been made, relative to the prototypic Gln, have not nullified the mechanism of action of the compound. The ability to disrupt CSC

spheroids and kill the cells is an indication that they share the common mechanism of killing the CSCs via an apoptosis-independent mechanism.

In summary, our SAR studies show that the nature of the sugar greatly affects the cytotoxicity against cancer cell lines. For instance, we demonstrated that monocationic 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2'-amino-2'-deoxy–α-D-galactopyranosyl)-sn-glycerol is more active than 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2'-amino-2'-deoxy–α-D-glucopyranosyl)-sn-glycerol against various cancer cell lines (Samadder P et al. Eur J Med Chem. **2014**, *78*, 225-35). As a result, we can infer that the dicationic and tricationic D-galactose-based analogs would be as active or more active than the di- and tricationic D-glucose analogs. Moreover, as we have demonstrated the dicationic L-glucose-based GAEL 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-L-glucopyranosyl)-sn-glycerol **69** to be as active as the dicationic D-glucose version 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol **2** we can infer that the dicationic L-galactose analogs will also be active and even more so than the glucose-versions.

The invention will now be further elucidated by way of examples; however, the invention is not necessarily limited to the examples.

2. Results

2.1.1. Synthesis of Dicationic GAELs analogs **1-10.**

It is of note that the synthesis protocols outlined below illustrate one method for the synthesis of the compounds described herein. Modifications to these methods will be apparent to one of skill in the art and/or can be determined by routine experimentation and are within the scope of the invention.

To synthesize dicationic GAEL analogs that are potentially more active than the lead compound, monocationic Gln **11**, an amino substituent was introduced to the C_6-position of the sugar to give compounds **1-4**. The rationale behind introduction of extra amino substituent at this position is because previous reports showed that the non-cationic analog compound **12** was less potent than edelfosine **13** which in turn was less potent than Gln **11** (Erukulla, R. V. et al., *J. Med. Chem.* **1996**, *39*, 1545–

1548; Samadder, P. et al., *Anticancer Res.* **1998**, *18*, 465–470; Xu, Y. et al., *ChemMedChem* **2013,** 8, 511–520). Evidently, the amino substituent on C_2 position of the sugar in Gln **11** increased activity, so we hypothesized that an additional amino substituent in **11** may increase the anticancer activity of the compound.

Compounds **1** and **2** were synthesized to evaluate the effect of the configuration of the glycosidic linkage on activity, though a previous report showed that the α-anomer of Gln **11** was more active than the β-anomer.

Compound **3**, with a phthalimido at the C2-position of the sugar, was synthesized to study how the absence of cationic group at C-2 affects the activity in this compound series. The reason behind this is that previous studies showed that when the free amine at the C_2-position of the sugar was modified with various substituents such as azide, guanidyl, and benzyl amine derivatives, the anticancer activity were significantly reduced, up to or greater than 3 fold (Samadder, P. et al., *Anticancer Res.* **2011**, *31*, 3809–3818; Xu, Y. et al., Chem Med Chem 2013, 8, 511–520; Jahreiss, L. et al., *Autophagy* 2009, *5,* 835–846).

Compounds **4** and **5** were synthesized to explore the effect of the methoxy substituent at the *sn*2 position of the glycerol moiety. Another reason for synthesizing **5** was to investigate if an additional diamino substituted sugar moiety will increase activity compared to a methoxy substituent at *sn*2 position of the glycerol backbone.

Compounds **6-8** were synthesized to explore how the position of the second amino group in the glycerolipid affected the antitumor properties.

Compound **9** was synthesized to explore how the presence of two lipid tails and modification of the free amino substituent at *sn*-2 position affect the biological properties in compound **8**.

We also synthesized selected carbamate **10** to explore whether the presence of a methoxycarbamate substituent at the *sn*-2-position of the glycerolipid will promote selectivity for prostate cancer as a previous study has shown that edelfosine analogs bearing a methoxycarbamate functionality at that position was selective for prostate cancer (Byun, H-S. et al., Chem Med Chem, 2010, 5, 1045-1052).

Compounds **5**, **8**, **9**, and **10** are diastereomeric mixtures of two compounds

based on the stereochemistry at the sn-2- position of the glycerolipid. We have previously reported that the stereochemistry at position 2 has little or no effect on anticancer activity and edelfosine, the most studied antitumor ether lipid, is usually used as a racemic mixture (Ogunsina, M. et al., Molecules, 2013, 18, 15288-15304).

Compound **1** was synthesized by coupling of glycoside donor **20** to the commercially available lipid alcohol **21** to give a mixture of α- and β- glycolipid **22** and **23**, respectively (4:1) (see Scheme 1). The glycoside donor **20** was synthesized from glucosamine hydrochloride **14** in 7 steps. The amino substituent of glucosamine **14** was converted to azide as previously reported (Xu, Y. et al., *Chem Med Chem* **2013,** 8, 511–520) in the presence of cupric sulphatepentahydrate, water and triethylamine at room temperature overnight. This was followed by acetate protection of the hydroxyl group using acetic anhydride in pyridine in the presence of DMAP at room temperature overnight to give compound **15**. The thiophenyl glycoside **16** was synthesized from **15** using thiophenol and boron trifluoride diethyl etherate complex in dichloromethane at room temperature for 18 hrs. To install the azido function at the C_6-position, the acetate protection was removed using sodium methoxide in methanol to give compound **17** which was subsequently converted to sulphonate ester **18** using toluenesulfonyl chloride in pyridine with DMAP as the catalyst at room temperature. Nucleophilic displacement of sodium azide in DMF produced 2,6-diazido analog **19** which was subsequently protected using acetic anhydride in pyridine with DMAP as the catalyst to give the glycoside donor **20**.The α-glycolipid **22** was isolated in pure form and then deprotected to remove the acetate group to afford the 2,6-diazido compound **24** which was subsequently reduced with trimethylphosphine in THF to give the α-configured diamino compound **1**.

To synthesize compounds **2-5**, the glycoside donor **30** was glycosylated to the lipid alcohol **21**, **34** and **37** to produce the protected glycolipid analogs **31, 34** and **38** (see Scheme 2). We have previously reported the synthesis of lipid alcohol **34** (Xu, Y. et al., *Chem Med Chem* **2013,** 8, 511–520), and lipid alcohol **37** is commercially available. The glycoside donor **30** was synthesized from the glucosamine hydrochloride **14** in seven steps. Glucosamine hydrochloride **14** was dissolved in

aqueous solution of sodium hydroxide to which phthalic anhydride was added to protect the amino substituent and the reaction mixture was left overnight at room temperature. The water was removed and the residue dispersed in pyridine followed by addition of acetic anhydride for acetate protection of the hydroxyl group to give compound **25**.The procedures used to convert compound **15** to glycoside donor **20** described above were repeated to convert **25** to the glycoside donor **30**. Removal of the acetate and phthalimido protecting group were done in one reaction using ethylenediamine in butanol (1:1) at 90°C for 2 h which converted compounds **31**, **35** and **38** to their 6-azido glycolipid analogs **36** and **39**, respectively. The acetate protective groups of compound **31** were selectively removed using a catalytic amount of sodium methoxide for 20 minutes to give 6-azido-2-phthalimido glycolipid analog **33**. Reductionof the azido function in compounds **32**, **33**, **36** and **39** was achieved by using trimethylphosphine in THF and water (9:1) to produce the desired target compounds **2 – 5** respectively.

To synthesize compounds **6** and **7**, the azido lipid alcohol **42** was synthesized from the commercially available lipid diol **40** in two synthetic steps. Compound **40** was converted to sulphonate ester **41** using toluene sulfonyl chloride in pyridine and DMAP as catalyst. The azide **42** was synthesized from **41** by nucleophilic substitution reaction using sodium azide in DMF. The glycoside donor **26** was glycosylated with **42** to afford the protected glycolipid **43**. The acetate and phthalimido protective groups of **43** were removed with ethylenediamine in butanol (1:1) at 90°C for 2 hrs to give the desired compound **6.** The azido substituent of **6** was reduced as described above to give the diamino analog **7**.

To synthesize compound **8**, the glycoside donor **26** was glycosylated to the lipid alcohol **44**, which was synthesized from the commercially available lipid alcohol as previously reported (Byun, H-S. et al., Chem Med Chem, 2010, 5, 1045-1052). The glycosylation reaction gave the protected glycolipid compound **45**. Reduction of the azido substituent at position *sn*2 of the glycerolipid gave compound **46** which was subsequently deprotected using ethylenediamine in butanol (1:1) at 90°C for 2 hrs to give the desired compound **8**.

To synthesize compound **9**, the amine **46** was coupled using TBTU to the palmitic acid 47 (Bera, S. et al., Molecules 2012, 17, 9129–9141) to give compound **48** which was subsequently deprotected using ethylenediamine in butanol (1:1) at 90°C for 2 hrs to produce the compound of choice **9**.

Compound **10** was synthesized by reacting the methyl chloroformate **49** with amine **46** to give the carbamate **50** which was subsequently deprotected using ethylenediamine in butanol (1:1) at 90°C for 2 hrs to afford the carbamate analog **10** as the desired compound (Byun, H-S. et al., Chem Med Chem, 2010, 5, 1045-1052).

2.1.2. Synthesis of L-Rhamnose Derived GAEL **56** and L-Glucosamine Derived GAEL **69**

To enhance metabolic stability, we synthesized L-rhamnose derived GAEL analog **56** and L-glucosamine derived GAEL **69,** where we employed L- sugars that are not naturally present in humans because they are expected to resist glycosidases breakdown in human. To synthesize compound **56**, we employed the chemistry described above for the synthesis of compound **7** (see scheme 5). For the synthesis of compound **69,** we started with the commercially available L-mannose **57** which was converted to protected L-glucosamine analog **65** in eight synthetic steps (see Scheme 6). L-mannose **57** was acetylated to give the pentaacetate **58** which was converted to the bromide **59** using HBr in acetic acid (33%$^{w}/_{w}$). The bromide **59** was subjected to phase transfer catalyzed anomeric S_N2 nucleophilic substitution reaction using tetrabutyl ammonium hydrogen sulphate and sodium carbonate in an immiscible mixture of ethyl acetate and water to give the phenyl β-L- thiomanopyranoside **60** which was subsequently deprotected to give **61**. The hydroxyl group at C_3 position was selectively protected by benzyl ester to give compound **62**. The hydroxyl functional groups at the C_4 and C_6 position were protected together using benzylidienediacetal to give compound **68.** We tried to employ triflic anhydride to activate the –OH substituent at C_2 position for subsequent conversion using S_N2 nucleophilic substitution reaction to give the L- glucosamine derivative **65,** but this approach was not successful. So we used the mesylate **64** which worked. As

described above, the protected glucosamine derivative **65** was glycosylated to lipid **21** to give the protected glycolipid **66** as mainly α anomer (90%). The benzylidenediacetal protecting group was removed using an acetic acid water mixture (80/20) at 60°C for 3hrs to give **67**. The benzoate ester was subsequently removed using excess sodium methoxide in methanol for 2hrs to give the azide **68** which was subsequently reduced as described above to give the target compound **69.**

2.1.3. Synthesis of L-gluco-based glycolipids 70-72.

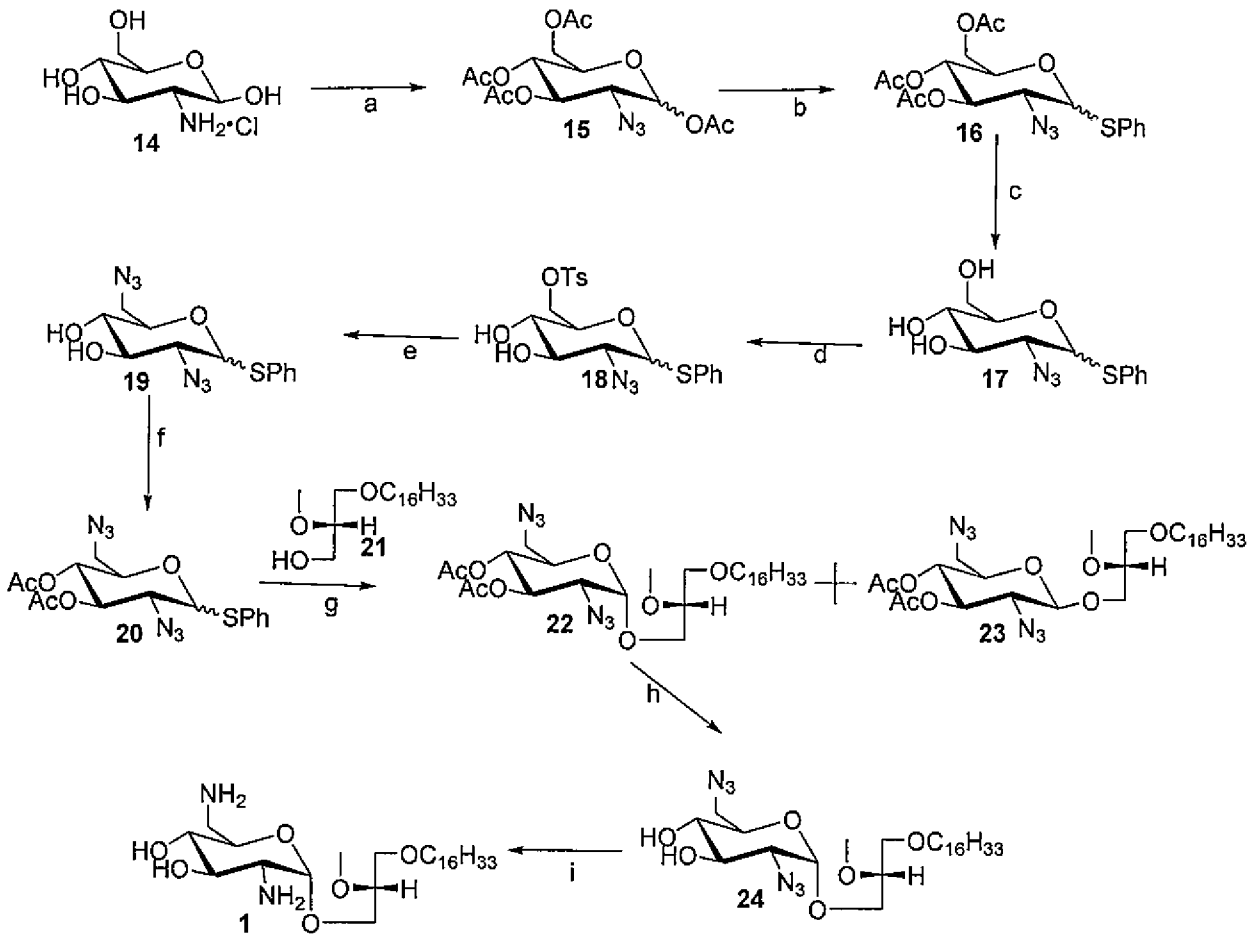
The synthesis of compound **70** started from compound **63** as previously outlined herein. Regioselective ring opening of the benzylidine ring under reductive diborane conditions afforded diol **77**. Both hydroxyl groups in **77** were activated as sulfonate esters and nucleophilic displacement of the sufonate esters with sodium azide in DMF at elevated temperature produced diazido-thioglycoside **79**. NIS activated glycosylation of thioglycoside donor **79** with commercially available lipid alcohol **21** produced an anomeric mixture of L-gluco-based β-glycoside **80** and L-gluco-based α-glycoside **81**. Saponification of the ester functionality in **80** and **81** using basic sodium methoxide in methanol produced alcohols **82** and **83**, respectively. Reduction of both azide groups in glycolipid **82** using trimethylphosphine in aqueous THF produced target compound **70**. Using the same conditions to diazido-based α-glycolipid **83** gave target compound **71**. Finally exposure of **71** to catalytic hydrogenation using palladium on charcoal produced unprotected target compound 72.

2.1.4. Synthesis of D-gluco-based tricationic glycolipids 73-76.

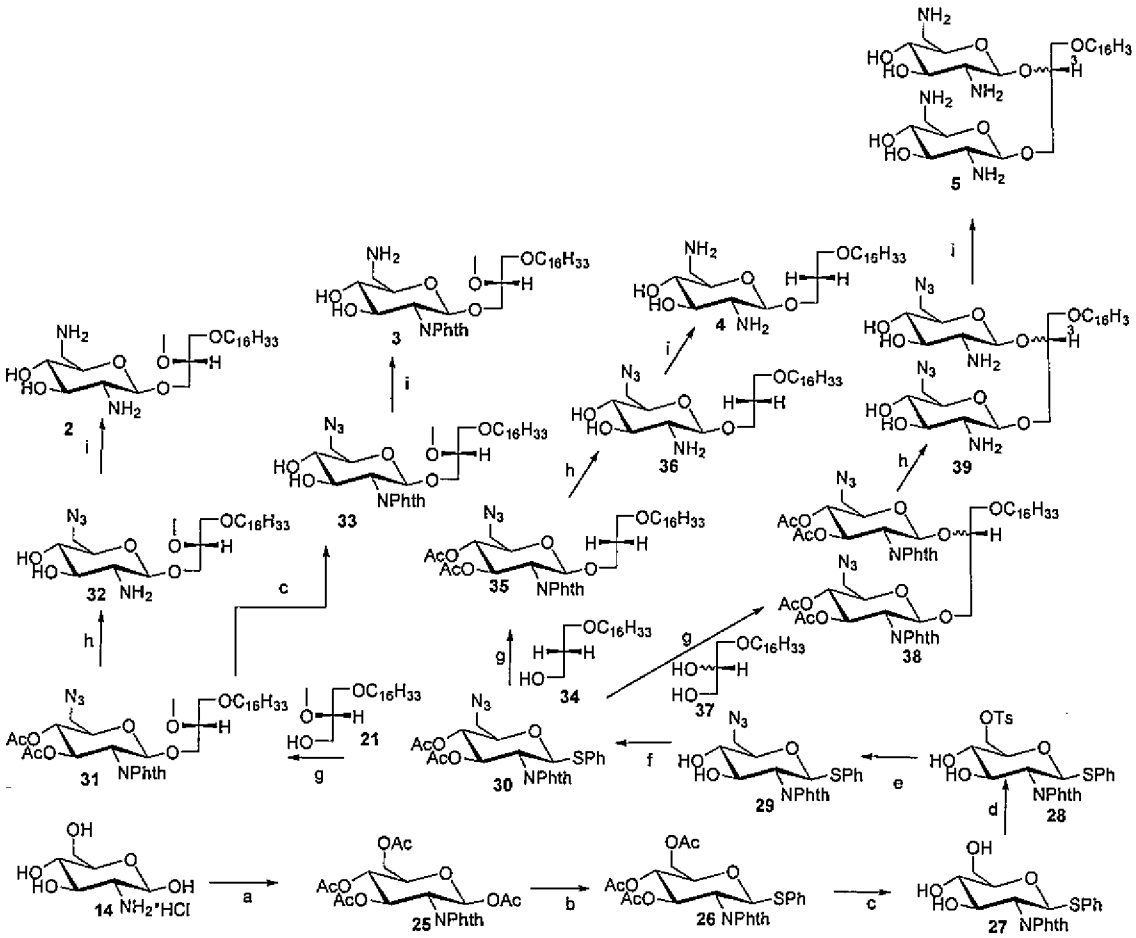
Target compounds **73** and **74** were prepared from previously prepared glycolipid **33**. At first the 6-azido group in glycolipid **33** was reduced by catalytic hydrogenation to produce 6-amino-based glycolipod **3**. Reductive amination of the amino function in **3** with various azido aldehydes **89** generated phthalimido-protected glycolipid 84. Deprotetction of the phthalimido group using ethylendiamine in butanol

at elevated temperature produced azido-based glycolipid **85** which was exposed to catalytic hydrogenation to produce target compounds **73** and **74**.

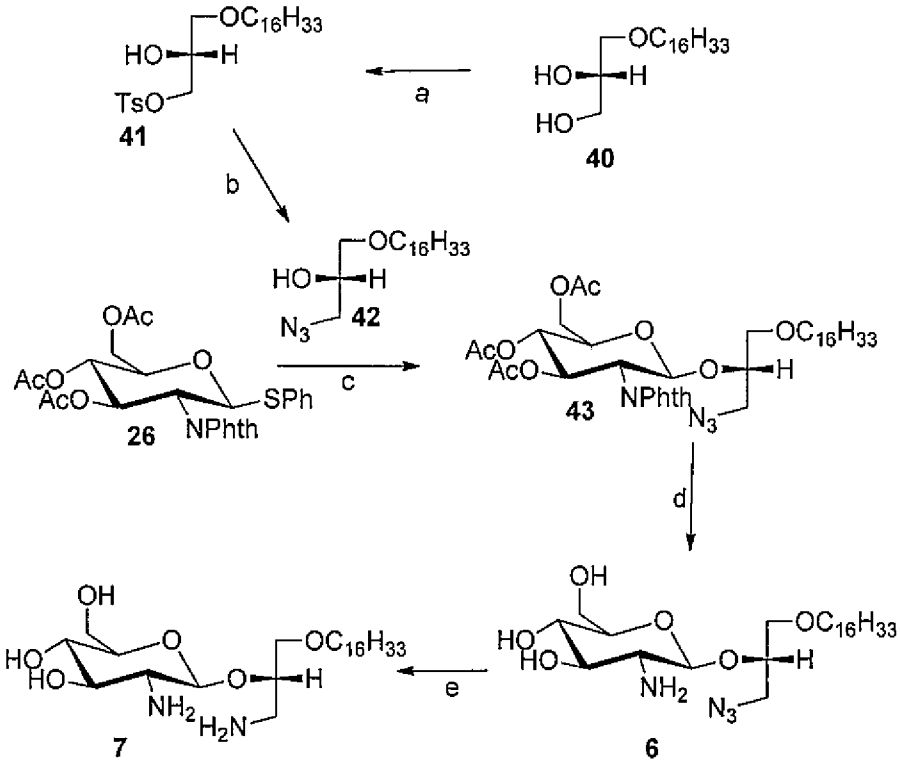
Target compounds **75** and **76** were prepared from preciously described phthalimido-protected glycolipid 33. Removal of the phthalimido group using using ethylendiamine in butanol at elevated temperature generated amine **32** which reacted with various azido aldehydes **89** using reductive amination conditions to afford diazido analogs **88**. Catalytic hydrogenation of diazido-based glycolipids using catalytic hydrogenation gave the desired target tricationic glycolipids **75** and **76**.



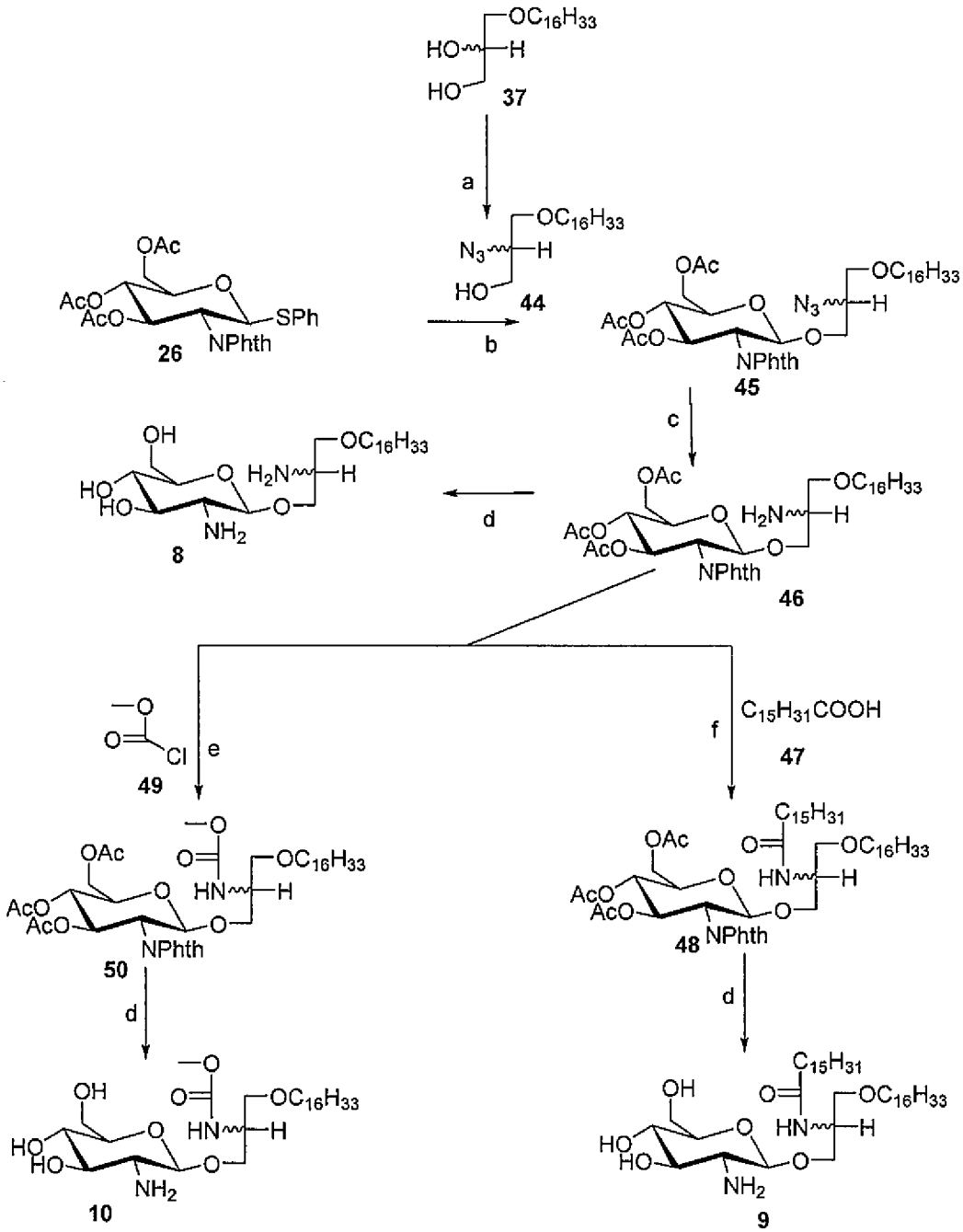
Scheme 1. Synthesis of compound **1.** *Reagents and conditions*: (a) 1. TfN_3, $CuSO_4$, ETN_3, H_2O, rt; 2. Ac_2O, DMAP, Pyridine, 18 hrs, rt (b) PhSH, $BF_3.Et_2O$, DMAP, DCM,18, hrs, rt (c) MeONa, MeOH,1 hr (d) TsCl, Pyridine, DMAP, 0°C - rt, 18 hrs (e) DMF, NaN_3, 70°C (f) Ac_2O, DMAP, Pyridine, 18 hrs, rt (g) AgOTf, NIS, DCM, 3 hrs, rt (h)MeONa, MeOH, 30 minutes (i) $P(CH_3)_3$, THF, H_2O, 2 hrs, rt. Abbreviations: 4-Dimethyl amino pyridine (DMAP), Dichloromethane (DCM), 2,2,*N*,*N*-dimethylformamide (DMF), room temperature (rt), *N*-iodosuccinimide (NIS)



Scheme 2. Synthesis of compounds **2-5**. Reagents and conditions: (a) 1. Phthalic anhydride, NaOH, H_2O, 18 hrs, rt; 2. Ac_2O, DMAP, Pyridine, 18 hrs, rt (b) PhSH, $BF_3.Et_2O$, DMAP, DCM,18, hrs, rt (c) MeONa, MeOH, 20 minutes (d) TsCl, Pyridine, DMAP, 0°C - rt, 18 hrs (e) DMF, NaN_3, 70°C (f) Ac_2O, DMAP, Pyridine, 18 hrs, rt (g) AgOTf, NIS, DCM, 3 hrs, rt (h) Ethylinediamine/butanol (1:1), 90°C 2 hrs, rt (i) $P(CH_3)_3$, THF, H_2O, 2 hrs, rt. Abbreviations: 4-Dimethyl amino pyridine (DMAP), Dichloromethane (DCM), 2,2,*N*,*N*-dimethylformamide (DMF), room temperature (rt), *N*-iodosuccinimide (NIS)

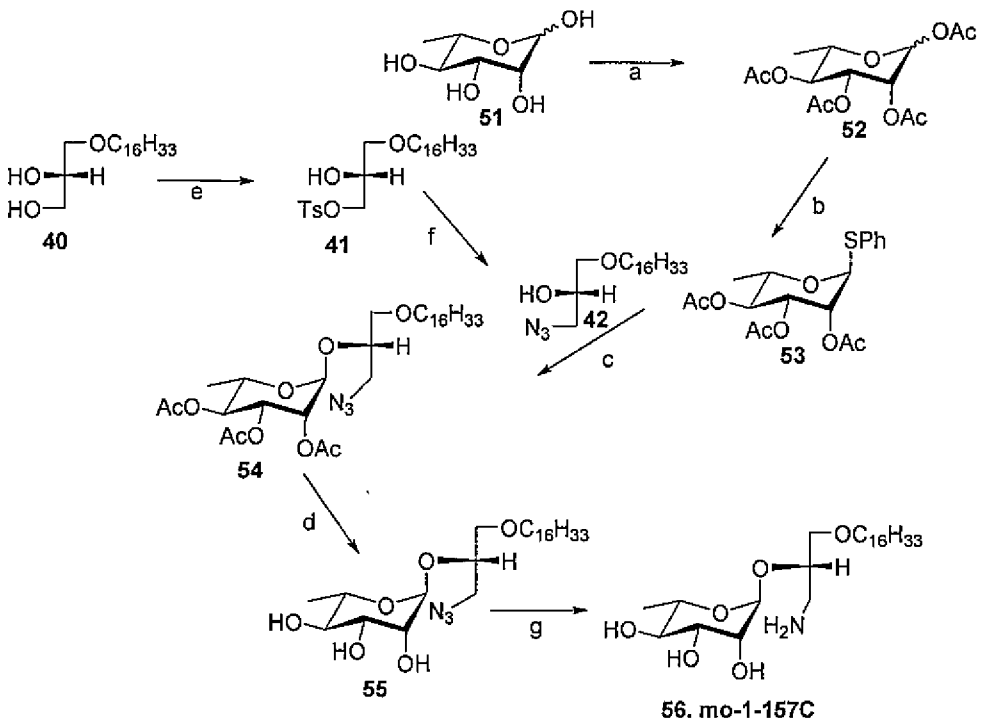


Scheme 3. Synthesis of compounds **6** and **7**. Reagents and conditions: (a) TsCl, Pyridine, DMAP, 0°C - rt, 18 hrs (b) DMF, NaN_3, 70°C (c) AgOTf, NIS, DCM, 3 hrs, rt(d) Ethylinediamine/butanol (1:1), 90°C 2 hrs, rt(e) $P(CH_3)_3$, THF, H_2O, 2 hrs, rt. Abbreviations: 4-Dimethyl amino pyridine (DMAP), Dichloromethane (DCM), 2,2,*N*,*N*-dimethylformamide (DMF), room temperature (rt), *N*-iodosuccinimide (NIS)

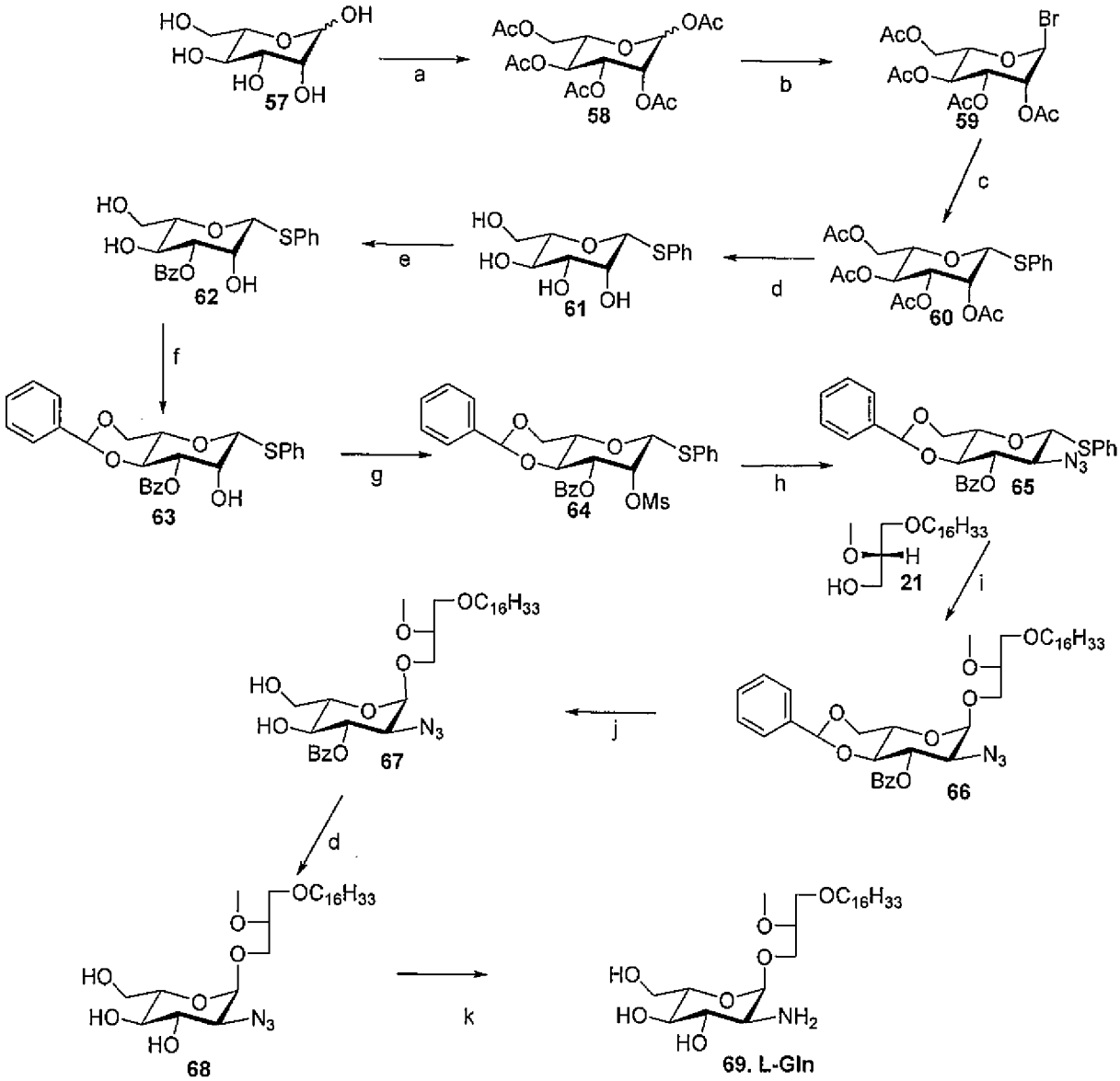


Scheme 4. Synthesis of compounds **8-10**. Reagents and conditions: (a) DIAD, Ph_3P, Me_3SiN_3, DCM, (b) AgOTf, NIS, DCM, 3 hrs, rt (c) $P(CH_3)_3$, THF, H_2O, 2 hrs, rt (d) Ethylinediamine/butanol (1:1), 90°C 2 hrs, rt. (e) Et_3N, $ClCO_2Me$, DCM, (f)TBTU, DIPEA, DMF, 3 hrs, rt. Abbreviations: 4-Dimethyl amino pyridine (DMAP), Dichloromethane (DCM), 2,2,*N*,*N*-dimethylformamide (DMF), room temperature (rt),

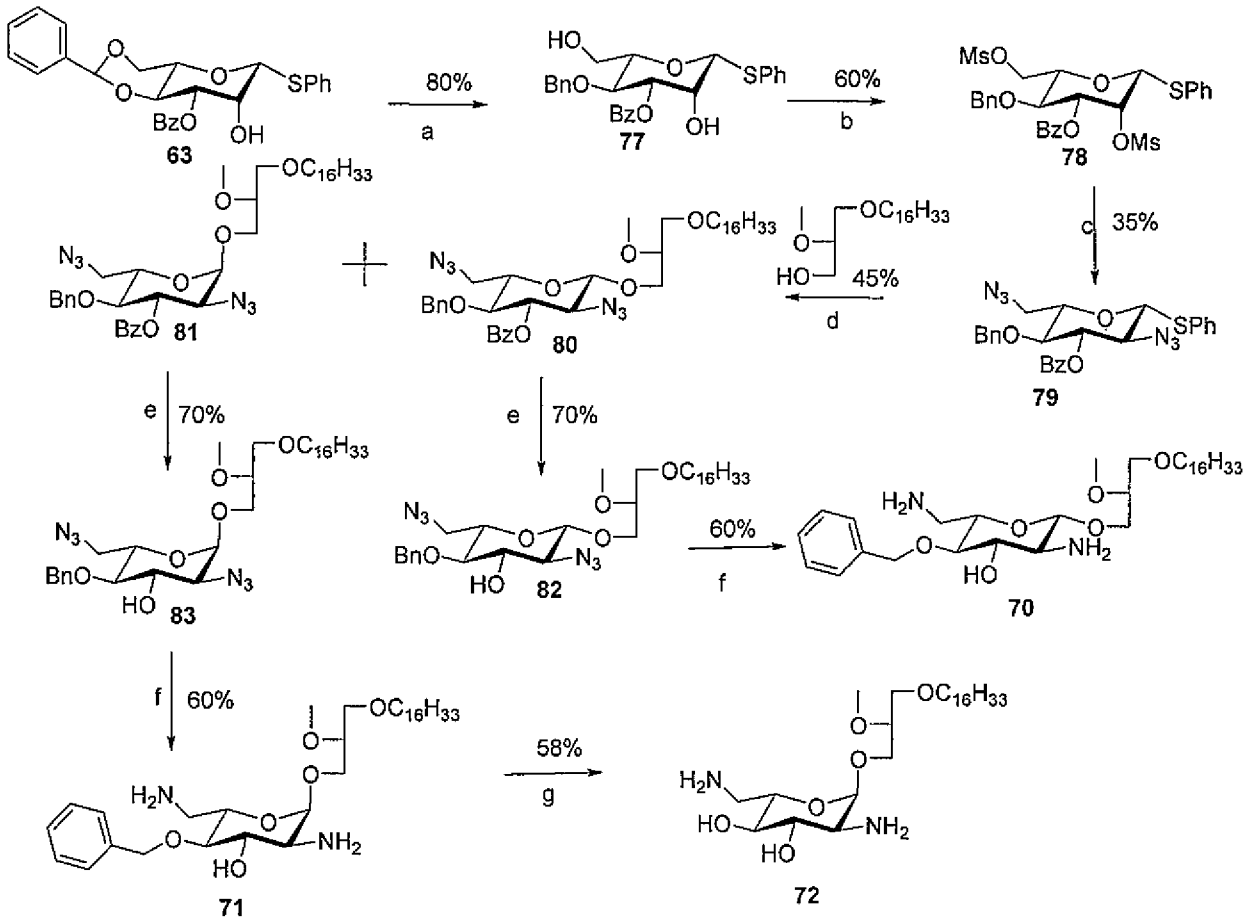
N-iodosuccinimide (NIS), Diisopropylethylamine (DIPEA), Diisopropylazodicarboxylate (DIAD).



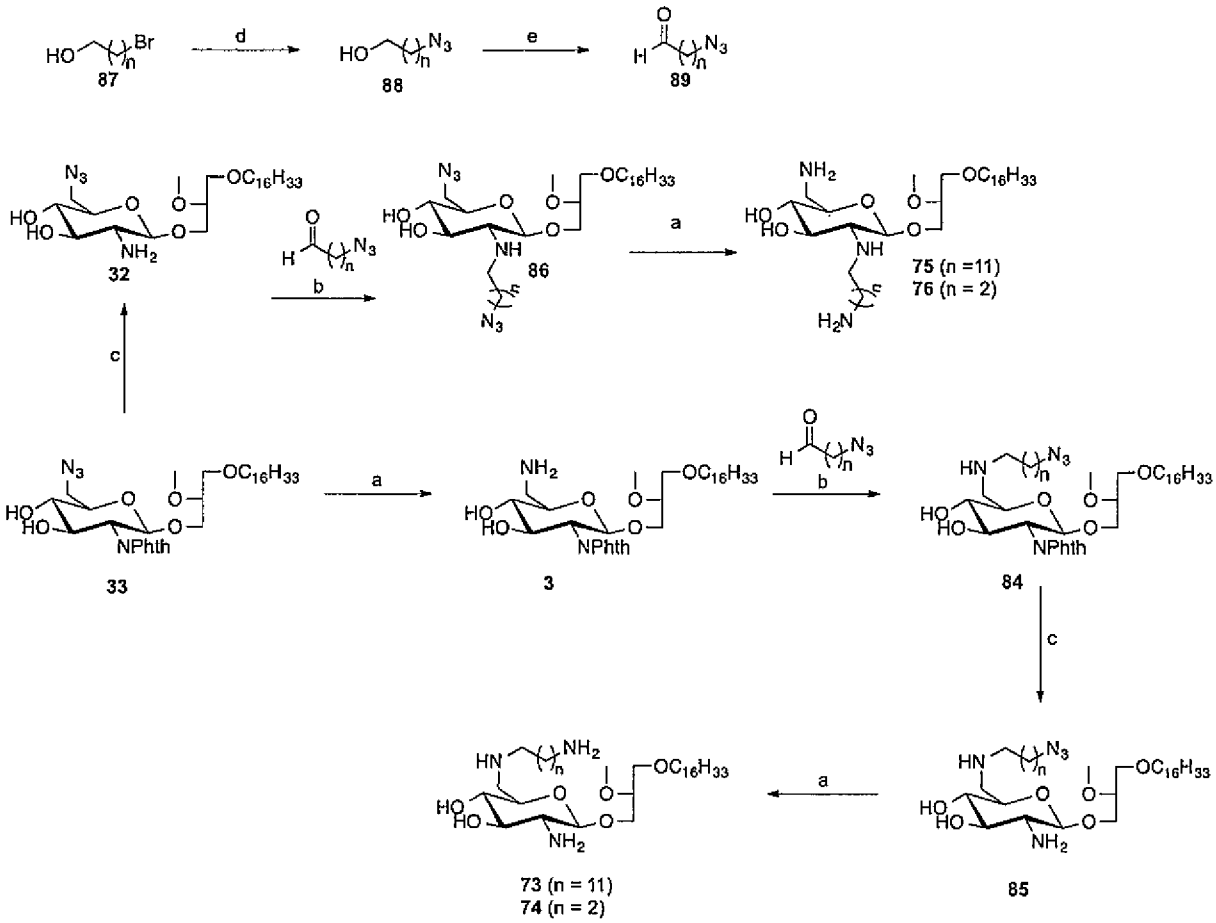
Scheme 5. Synthesis of compound **56** (mo-1-157C). *Reagents and conditions*: (a) Ac_2O, DMAP, Pyridine, 18 hrs, rt (b) PhSH, $BF_3.Et_2O$, DCM,18, hrs, rt (c) AgOTf, NIS, DCM, 3 hrs, rt (d) MeONa, MeOH, 1hr minutes (e) TsCl, Pyridine, DMAP, 0°C - rt, 18 hrs (f) DMF, NaN_3, 70°C (g) $P(CH_3)_3$, THF, H_2O, 2 hrs, rt. Abbreviations: 4-Dimethyl amino pyridine (DMAP), Dichloromethane (DCM), 2,2,*N*,*N*-dimethylformamide (DMF), room temperature (rt), *N*-iodosuccinimide (NIS)



Scheme 6. Synthesis of compounds of L-Gln. *Reagents and conditions*: (a) Ac_2O, DMAP, Pyridine, 18 hrs, rt (b)HBr in AcOH (33%), DCM, 0°C, 2 hrs (c) PhSH, EtOAc, H_2O, Na_2CO_3, TBAHS, rt, 6 hrs (d) MeONa, MeOH, 1hr (e) Me_2SnCl_2, BzCl, DIPEA, THF/H_2O (19:1), rt, 16 hrs (f) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 4 hrs (g) MsCl, Pyridine, DMAP, rt, 18 hrs (h) DMF, NaN_3, 120°C (i)AgOTf, NIS, DCM, 3 hrs, rt (j) AcOH, H_2O, 60°C, 5 hrs(K) $P(CH_3)_3$, THF, H_2O, 2 hrs, rt



Scheme 7. Synthesis of Compounds **70-72**. Reactions and Conditions; (a) BH_3.THF, TMSOTf, DCM, 4 hrs (b) MsCl, DMAP, Pyridine (c) DMF, NaN_3, 140^{O}C (d) AgOTf, NIS, DCM (e) NaOMe, MeOH, 5hrs (f) $P(CH_3)_3$, THF, H_2O, 2 hrs (g) H_2, Pd, MeOH, 5 hrs



Scheme 8. Synthesis of compounds **73 – 76**

Reactions and conditions: a) $Pd(OH)_2/C$, H_2, 2 h; b) i) DCM, 0 °C to RT, overnight, ii) $NaBH_4$, CH_3COOH, 2 h; c) ethylenediamine, butanol, 90 °C, 3 h; d) NaN_3, DMF, 70 °C, 3 h; e) PCC, DCM, 2 h

2.3. In vitro screening against epithelial cancer cell lines

2.3.1 Effect of dicationic GAELs (**1-10**).

To determine the effect of compounds **1-10** on the viability of the epithelial cancer cell lines, exponentially growing cells, BT-474, JIMT-1, MDA-MB-231 (breast), DU145, PC3 (prostate), MiaPaCa2 (pancreas) were incubated with varying concentrations of **1-8** (0-30 μM) for 48 h followed by viability assays with the MTS reagent. The results of the viability studies are shown in Figs 2A to 2E.

Gln**11**, a monocationic and the most studied GAEL was selected as the reference compound for comparison with the new dicationicglycolipids.

The CC_{50} values for all the compounds are summarized in Table 1 and the CC90 values are summarized in Table 2.

The most potent of the compounds tested against all six cell lines is α-dicationic glycolipid **1** with CC_{50} values of 3.0 to 7.5 μM depending on the cell line. 90% loss of cell viability was observed at a concentration range 4.5 – 9.5 μM depending on cell lines.

Comparison of **1** with the β- dicationic analog **2** with CC_{50} values in the range of 4.2 – 11.5μM, showed that the α-analog **1** is consistently more active against all the cell lines (see figure 2). This shows that as previously reported for the monocationic analog **11** (Samadder P. et al., Eur J Med Chem2014, 78, 225-235), the α- analogs of this class of compounds are more active.

Comparison of the β- dicationic analog **2** with β-monocationic analog **11,** CC_{50} value in the range of 8 – 13.5 μM showed that **2** is significantly more active across all the six cell lines except BT 474 cell lines.

Compound **3** with phthalimido substituent at C_2 position of the sugar and free amine at C_6 position of the sugar has CC_{50} values in the range of 15 - > 30 μM and it is significantly less active than the reference monocationic analog **11** against all cell lines. This showed that unmodified amino substituent at the C_2 position is essential for activity.

Compound **4**, an analog of **2** without a methoxy substituent at the position *sn* 2 of the glycero moiety has activity comparable to that of **2** and showed a CC_{50} value in the range of 4.0 – 8.5 μM. In fact, there was no statiscally significant difference between the activity of **2** and **4** for all the cell lines except BT 474. This suggests that the methoxy substituent may have no significant role on activity.

The diastereomeric mixture and diglycosylated analog **5** was not able to reduce viability of cells by 50% at the highest dose tested, 30 μM. Thus, replacing the methoxy substituent at *sn2* position of the glycerol moiety with another dicationic sugar reduced activity significantly. This may be due to increased hydrophilicity

leading to reduced cellular absorption.

Compounds **6 – 8** were synthesized primarily to determine the effect of the position of the sugar on the glycerolipid.

The azide compound **6** has CC_{50} values in the range of 6 – 22 μM. It was significantly more sensitive to PC-3 cell lines than Gln, 6.0 μM compared to 13.5 μM and least sensitive to BT 474 cell lines when compared to Gln, 22 μM compared to 8 μM. For other cell lines the activities are comparable.

The dicationic analog **7** is significantly less active than **6** across all cell lines. This may be attributed to possible physical interaction between the primary amino substituent on *sn3* of the lipid and the amino substituent at the C_2 position of the sugar which may alter the conformation of the compound.

Compound **8** with the sugar moiety on *sn 3*-position of the glycerolipid, when compared to compounds **6** and **7** with the lipid position on *sn 2* position, was significantly more active than Gln **11** except against the BT 474 cell line. This result shows that activity of this class of compound is better when the sugar moiety is on *sn3* position of the glycerolipid. The increased potency of compound **8** compared to Gln **11** against most of the cell lines also shows that replacement of the methoxy group at the *sn* 2-position of the glycerolipid with an amino substituent significantly increased the activity.

Also, comparison of activity of compounds **7** and **8** with that of **1**, **2**, and **4** demonstrates better activities can be achieved when the two amino groups are located on the sugar as compared to when the sugar has one and the other is on the lipid.

Compound **9**, with a second hydrophobic C_{16} moiety attached to the *sn 2*-position of the glycerolipid, was not active at the highest concentration tested, 30 μM. At this dose, there was no significant difference in viability compared to the control across all the cell lines tested. Lack of activity may be due to increased lipophillicity which might have decreased the cellular absorption of the drug.

Compound **10**, with a methycarbamate substituent at the sn 2-position of the glycerolipid, was synthesized to promote selectivity against prostate cancer cell lines.

The rationale is because an edelfosine analog substituted at this position has selectivity against prostate cancer cell lines. The CC_{50} values of compound **10**, which is in the range of 14 – 23 μM, is significantly less active than Gln**11**. This shows that carbamate substituent neither increased activity nor promoted selectivity for prostrate cell line in the GAEL series compared to the edelfosine series. This also confirms difference in anticancer properties of GAELs and that of other AELs.

Effect of Tricationic GAELs

Tricationic GAELs, compound **73** and **75** were cytotoxic against all cell lines examined (Figure 2O, Table 6). Compound **73** had CC_{50}'s of 1.5 – 4 μM.2.3.2.Effect of GAELs with L sugars (**56, 69,70, 71, 72**).

The rhamnose derived GAEL compound **56** killed 50% of the cancer cells in the range of 4.8 -11.0 μM across all the cell lines tested and the CC_{90} values were in the range 6.5 to 14 μM. These values are significantly lower than the corresponding values for the reference drug Gln **11** (see Table 2).

For the L-Gln **69**, we observed activity and CC_{50} in the range of 6.5 to12.5 μM. Except for DU -145, compound **69** appears to be significantly more active than Gln 11 against other cell lines tested (see Table 2). This is really expected because the sample we tested is about 90% α- anomer because previous studies showed that for Gln **11** with the D- sugar, the α- anomer was about 1.5 to 2 times more active than corresponding β-anomers. GAELs bearing dicationic L sugars were synthesized and shown to be as active as D-sugar GAELs (Table 5). The most active compounds were those bearing a phenyl group (Figs 2H-2K). No differences were observed with the alpha or beta anomeric forms of these compounds.

The activities observed with these L-sugar derived GAELs confirmed our hypothesis that use of a sugar unnatural to humans will show activity, as discussed above.

2.4 Effect **1-10** and **56** and **69** on the integrity and viability of cancer stem spheroids

The results of our studies revealed that the dicationic and L-sugar derived

GAELs effectively caused the disintegration of BT474, DU145 and A2780 cancer stem cell spheroids (Fig 3). Analysis of the viability of the cells at the end of the incubation period revealed that incubating with between 5-10 μM was sufficient to completely inhibit the viability of the CSCs (Figs 3A – 3C).

2,5. Tolerability /toxicity of **56** (Rhamnose –GAEL).

The toxicity of 56 administered intravenously or orally to female Rag2M mice was established. Toxicity of the mice was assessed by monitoring the behaviour, body weight and assessement of the major tissues following necropsy. No toxicity was observed in mice administered 300 mg/kg of 56 orally (the highest concentration tested). Intavenous delivery of up to 50 mg/kg of **56** did not result in any observed abnormalities in behaviour or loss of body weight. Necropsy did not reveal any abnormalities in the tissues.

4.1. Materials and methods: Synthesis of GAELs

Solvents were dried over CaH_2. ^1H, ^{13}C NMR spectra were recorded with a JMN A500 FT NMR spectrometer at 500 or 300 MHz and at 126 or 75 MHz, respectively, and chemical shifts were reported in parts per million (ppm). Optical rotations were measured with a Jasco P-1020 digital polarimeter. Thin-layer chromatography (TLC) was carried out on aluminum-backed silica gel GF plates (250 mm thickness) and plates were visualized by charring with 10% H_2SO_4 in EtOH and/or short wavelength UV light. Compounds were purified by flash chromatography on silica gel 60 (230-400 ASTM mesh). Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)-MS was recorded on a Persptive Voyager RP mass spectrometer. High-resolution (HR) mass spectra were recorded on a JEOL JMS700 under FAB conditions. Purity of compounds **1-10** was assessed by elemental analysis of elements (C, H, N) and were within ± 0.5 % of the theoretical values.

4.2. Chemistry : general methods

General method for acetylation

Acetylation reactions were carried out in pyridine with dimethyl amino pyridine (DMAP), 0.2 equivalent, as the catalyst using acetic anhydride (2 equivalents). After stirring for 18 hrs at room temperature, the reaction was stopped by addition of methanol (10 ml), and then concentrated to dryness. The resulting residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate (3 times) and distilled water (2 times). The resulting organic layer was dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography.

General method for glycosylation reaction

The glycoside donor and 1.1 equivalent of the lipid alcohol, the glycoside acceptor, were dissolved in anhydrous dichloromethane (DCM) under argon atmosphere. N-Iodosuccinimide (NIS), 1.5 equivalents of the glycoside donor and silver triflate AgOTf), 0.2 equivalent were simultaneously added. The reaction mixture was left under vigorous stirring for 3 hrs. At the completion of reaction (TLC monitoring), the reaction mixture was diluted by DCM (20 ml) and then filtered over Celite. The resulting organic layer was washed with saturated sodium thiosulphate solution (2 times), saturated sodium bicarbonate (3 times) and water (2 times). The organic layer was the dried over anhydrous Na_2SO_4 and then concentrated under vacuum to give a brownish gel residue. The residue was then purified by flash chromatography (Hexane/Ethyl acetate, 4:6).

General method for conversion of primary hydroxyl group to azide

Triisopropylbenzylsulphonylchloride (TIBS) or p-toluenesulphonyl chloride were used to activate the hydroxyl group using DMAP as catalyst in anhydrous pyridine under argon or Nitrogen atmosphere. The reaction was stirred vigorously at room temperature for 12 - 24 hrs after which it was stopped by addition of methanol (10 ml) and then stirred vigorously for 10 minutes. The methanol and pyridine were removed under high vacuum. The crude mixture was dissolved in EtOAc and washed with 5% HCl (2 times), then saturated sodium bicarbonate solution (2 times) and water (once) to give a dark brown organic layer. The organic solvent was removed under vacuum

and crude residue was purified by flash chromatography EtOAc/Hexane, (4:6) or 100% ethyl acetate depending on the compound. The sulphonate ester was replaced with azide in a nucleophilic substitution reaction using sodium azide (10 equivalents) in anhydrous DMF at 70 – 90°C under argon or nitrogen atmosphere for 12 – 24 hrs. At the end of the reaction, the solvent DMF was removed under high vacuum. The residue was resuspended in ethyl acetate and then filtered to remove excess sodium azide. The solvent was removed under vacuum and the residue was purified using flash chromatography.

General method for deprotection of acetate group

The acetate protected compound was dispersed in methanol followed by addition of NaOMe (0.5 equivalent). The mixture was vigorously stirred for 1 hr and at the completion of the reaction was added ion exchange resin (H^+). When the reaction mixture was clear, the resin was filtered and then concentrated under vacuum. The residue was purified by flash chromatography (100% ethyl acetate). For selective deprotection of acetate in the presence of phthalimido protective group, only catalytic amount of sodium methoxide was used and the solution was stirred for 20 - 40 minutes.

General method for simultaneous deprotection of acetate and phthalimido group

To simultaneously remove acetate and phthalimido protecting group, the protected compound is dissolved in a mixture of ethylenediamine/n-butanol (1:1), the the solution is heated to 90°C, under vigorous stirring for 2 hrs. Then the reaction mixture is concentrated under high vacuum and then purified using C_{18} coated silica gel column by gradient elution (100% water/0% methanol to 0% water/100% methanol).

General method for reduction of azide

To reduce the azido protecting group free amine, the azide was suspended in a

mixture of THF/water (9:1), then trimethyl phosphine in THF (1M) was added (5 to 10 equivalent of trimethyl phosphine). The reaction mixture was stirred at room temperature for 2 hrs, then it was concentrated under vacuum. The residues especially in case of final compounds were purified using C_{18} coated silica gel column chromatography by gradient elution (100% water/0% methanol to 0% water/100% methanol). For lipid molecule with azido group the purification were carried using normal phase flash chromatography by hexane/ethylacetate mixture (9:1) and 100% ethyl acetate for protected glycolipid.

1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-*α/β*-D-glucopyranose (15):

D-glucosamine hydrochloride **14** (3g, 13.92mmol) was dissolved in water (15). To the solution was added Et_3N (2.8g, 27.8mmol) and $CUSO_4.5H_2O$ (36 mg, 0.015mmol). Triflicazide (16mmol) prepared as previously described by Xu, Y. et al., Chem Med Chem 2013, 8, 511–520) was then added to the reaction mixture. The blue mixture was stirred vigorously for 18 hrs and then concentrated under vacuum at room temperature. The residue was dissolved in pyridine (30 ml) and DMAP (150 mg, 1.2 mmol) was added followed by addition of acetic anhydride (9 ml, 96 mmol). After stirring for 18 hrs at room temperature, the reaction was stopped by addition of methanol (10 ml), and then concentrated to dryness. The resulting residue was dissolved in ethyl acetate (120 ml) and washed with saturated sodium bicarbonate (3 times) and distilled water (2 times). The resulting organic layer was dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography (Hexane:EtOAc, 6:4) to yield **15** as an off white solid (4.5g, 85%). The NMR data was in agreement with previously reported data (Xu, Y. et al., 2013). ES-MS: calcd $C_{14}H_{19}N_3O_9Na^+$ m/z: 396.1, found: $[M+Na]^+$m/z: 396.2.

Phenyl 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-*α/β*-D-glucopyranose (16)

To a solution of **15** (4.5 g, 12.1 mmol) in of DCM (60 ml) at room temperature was added thiophenol (2.4 ml 24 mmol) and $BF_3.Et_2O$ (3 ml, 24 mmol). After stirring overnight at room temperature the reaction was stopped with saturated sodium

bicarbonate solution, the separated organic layer was then washed with saturated sodium bicarbonate solution (3 times), distilled water (2 times) and the organic layer was dried over Na_2SO_4 and then concentrated to dryness The residue was purified by flash chromatography (Hexane:EtOAc, 6:4) to afford **16** as a brownish white solid. *α/β* (4/1)mixture (3.9 g, 76%). The NMR data was in agreement with previously reported data (Xu, Y. et al., Chem Med Chem 2013, 8, 511–520). ES-MS: calcd $C_{18}H_{21}N_3O_7SNa^+$m/z: 446.1, found $[M+Na]^+$m/z: 446.3.

Phenyl -2-azido-2-deoxy-1-thio-*α/β*-D-glucopyranose (17)

To a dispersion of 16 (3.9 g) in methanol was added NaOMe (1 g). The mixture was vigorously stirred for 1 hr and at the completion of the reaction was added about 1 g of ion exchange resin (H^+). When the reaction mixture was clear, the resin was filtered and then concentrated under vacuum. The residue was purified by flash chromatography (100% ethyl acetate) to give 17 as an off-white solid. *α/β* (3/1) mixture (2 g, %). Characteristic proton NMR data: ^{1}H NMR (300 MHz, Chloroform-d) δ 5.53 (d, J = 4.4 Hz, 0.75H, *α* H-1), 4.54 (d, J = 10.2 Hz, 0.25H, *β* H-1).ES-MS: calcd $C_{12}H_{15}N_3O_4SNa^+$ m/z: 320.1, found $[M+Na]^+$ m/z: 320.3.

Phenyl-2-azido-2-deoxy-1-thio-6-*O*-(-2,4,6-triisopropylbenzylsulphonyl-*α/β*-D-glucopyranose (18)

Compound **17** (3.2 g, 10.76 mmole), triisopropylbenzylsulphonylchloride (TIBS)and DMAP were added together in a 100ml flask cooled to 0°C under vacuum for 20-30 minutes, after which the vacuum atmosphere was replaced with nitrogen atmosphere. 50 ml of dry pyridine was added via septum to ensure dry condition. The reaction was stirred vigorously at room temperature for 24 hrs after which it was stopped by addition of methanol (10 ml) and then stirred vigorously for 10 minutes. The methanol and pyridine were removed under high vacuum. The crude mixture was dissolved in EtOAc and washed with 5% HCl (2 times,) then saturated sodium bicarbonate solution (2 times) and water (once) to give a dark brown organic layer. The organic solvent was removed under vacuum and crude residue was purified by

flash chromatography (EtOAc/Hexane, 4:6) to give **18** as brown foam (2.5 g, 4.43 mmol).Yield 41%). Characteristic proton NMR data: ^{1}H NMR (300 MHz, Chloroform-d) δ 7.60 – 7.37 (m, 2H, TIBS aromatic proton), 7.32 – 7.16 (m, 5H, thiophenyl proton), 5.53 (d, J = 4.3 Hz, 0.48H, *α* H-1), 4.44 (d, J = 9.1 Hz, 0.52H, *β* H-1), 1.37 – 1.14 (m, 18H, TIBS Isopropyl –CH_3). ES-MS: calcd $C_{27}H_{37}N_3O_6S_2Na^+$ m/z: 586.2, found $[M+Na]^+$ m/z: 586.4.

Phenyl -2, 6-diazido-2, 6-dideoxy-1-thio-*α/β*- D-glucopyranose (19)

Compound **18** (2.5 g, 4.43 mmol) was dissolved in anhydrous DMF (25 ml) under Nitrogen gas atmosphere, then NaN_3 (2.3 g, 35.44 mmol) was added and the reaction mixture was heated to 70°C with vigorous stirring overnight (18 hrs). The DMF was removed under high vacuum and the residue was suspended in ethyl acetate and then filtered to remove excess sodium azide. The organic layer was then concentrated under vacuum and the purified by flash chromatography (100% EtOAc) to give **19** as a brownish gel (1.36 g, 4.4 mmol). Yield 99%. Characteristic proton NMR data: ^{1}H NMR (300 MHz, Chloroform-d) δ 7.67 – 7.19 (m, 5H, thiopenyl aromatic protons), 5.64 (d, J = 4.8 Hz, 0.55H, αH-1), 4.51 (d, J = 9.9 Hz, 0.45H, βH-1). ES-MS: calcd $C_{12}H_{14}N_6O_3SNa^+$m/z: 345.1, found $[M+Na]^+$ m/z: 345.5.

Phenyl – 3,4-diacetyl-2, 6-diazido-2, 6-dideoxy-1-thio-*α/β*- D-glucopyranose (20)

To a solution of compound **19** (1.36 g, 4.4 mmol) in pyridine (25 ml) was added a catalytic amount of DMAP (100 mg, mmol) and acetic anhydride (5 ml). The solution was stirred vigorously overnight at room temperature. At the completion of the reaction, excess acetic anhydride was quenched by addition of methanol (5 ml). The solvents, methanol and pyridine were removed under high vacuum to give a brownish residue. The residue was dissolved in DCM and then washed with 5% HCl solution (2 times), saturated sodium bicarbonate (3 times) and distilled water (2 times). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated under vacuum to a give a brown gel residue. The residue was the purified by flash chromatography (Hexane/Ethyl acetate, 4:6) to give compound **20** as a brown gel (1.5 g, 4.40 mmol),

yield 100%. Characteristic proton NMR data:^{1}H NMR (300 MHz, Chloroform-d) δ 7.65 – 7.24 (m, 15H, thiophenyl aromatic protons), 5.66 (d, J = 5.6 Hz, 0.46H, αH-1), 4.51 (d, J = 10.2 Hz, 0.54H), 2.13 – 1.99 (m, 6H, acetate -CH_3).ES-MS: calcd $C_{16}H_{18}N_6O_5SNa^+$m/z: 429.10found $[M+Na]^+$ m/z: 429.1.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(3', 4'-O-diacetyl-2', 6'-diazido-2', 6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol (22)

Compound **20** (161 mg, 0.4mmol) and compound **21** (168 mg, 0.48mmol) were dissolved in anhydrous DCM (10 ml) under argon atmosphere. NIS (180 mg, 0.8 mmol) and silver triflate (20 mg, 0.08 mmol) were added. The reaction mixture was left under vigorous stirring for 3 hrs. At the completion of reaction (TLC monitoring), the reaction mixture was diluted by DCM (20 ml) and then filtered over Celite. The resulting organic layer was washed with saturated sodium thiosulphate solution (2 times), saturated sodium bicarbonate (3 times) and water (2 times). The organic layer was then dried over anhydrous Na_2SO_4 and then concentrated under vacuum to give a brownish gel residue. The residue was then purified by flash chromatography ((Hexane/Ethyl acetate, 4:6)) to isolate compound **22** as a brown gel from the mixture of compounds **22** and **23.** NMR data for compound **22**: ^{1}H NMR (300 MHz, Chloroform-d) δ 5.52 – 5.43 (m, 1H, H-3), 5.06 (d, J = 3.5 Hz, 1H, αH-1), 5.00 (dd, J = 10.2, 9.1 Hz, 1H, H-4), 4.07 (dt, J = 10.2, 4.4 Hz, 1H), 3.91 (dd, J = 9.7, 2.5 Hz, 1H), 3.69 – 3.51 (m, 5H), 3.48 (s, 3H, -OCH_3), 3.47 – 3.35 (m, 1H), 3.29 (m, 3H, H-2, H-6), 2.09 (s, 3H, Acetate CH_3), 2.05 (s, 3H, Acetate CH_3), 1.58 (m, 2H), 1.26 (s, 26H, Lipid tail), 0.89 (t, J = 6.6 Hz, 3H, lipid terminal –CH_3).^{13}C NMR (75 MHz, CDCl3) δ 170.13, 170. 01, 98.04, 79.11, 71.86, 70.10, 69.72, 69.46, 68.90, 67.91, 60.90, 57.96, 50.96, 31.93, 29.70, 29.36, 26.11, 22.69, 20.62, 14.11.ES-MS: calcd $C_{30}H_{54}N_6O_8Na^+$m/z: 649.4, found $[M+Na]^+$ m/z: 649.4.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diazido-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol (24)

^{1}H NMR (300 MHz, Chloroform-d) δ 4.98 (d, J = 3.5 Hz, 1H, H-1), 4.00 (dd, J =

10.4, 8.6 Hz, 1H, H-3), 3.94 – 3.78 (m, 3H), 3.64 – 3.53 (m, 5H), 3.52 – 3.41 (m, 8H), 3.18 (dd, J = 10.3, 3.5 Hz, 1H, H-2), 1.58 (m, 2H), 1.27 (s, 26H, lipid tail), 0.89 (t, J = 6.6 Hz, 3H, lipid terminal $-CH_3$).^{13}C NMR (75 MHz, CDCl3) δ 98.21, 79.24, 71.90, 71.72, 71.39, 70.90, 69.67, 67.18, 62.82, 57.91, 51.36, 31.94, 29.72, 29.67, 29.64, 29.62, 29.52, 29.37, 26.10, 22.70, 14.12.ES-MS: calcd $C_{26}H_{50}N_6O_6Na^+$m/z: 565.4found $[M+Na]^+$ m/z: 565.3.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol (1)

^{1}H NMR (300 MHz, Methanol-d4) δ 4.8 (d, J = 3.6 Hz, 1H, H-1), 3.85 (m, 1H), 3.66 – 3.40 (m, 11H), 3.18 (dd, J = 9.3 Hz, 1H, H-4), 3.07 – 2.95 (m, 1H, H-6a), 2.77 (dd, J = 13.4, 6.9 Hz, 1H, H-6b) 2.60 (dd, J = 10.0, 3.6 Hz, 1H, H-2), 1.58 (q, J = 7.0 Hz, 2H), 1.32 (s, 26H), 0.93 (t, J = 6.5 Hz, 3H).^{13}C NMR (75 MHz, MeOD) δ 100.82, 80.67, 76.13, 73.90, 73.50, 72.73, 71.24, 68.25, 58.21, 57.36, 43.78, 33.12, 30.83, 30.80, 30.64, 30.52, 27.30, 23.78, 14.50.MALDI-HRMS: calcd $C_{26}H_{54}N_2O_6Na^+$m/z: 513. 3880, found $[M+Na]^+$ m/z: 513.3956.

1, 3, 4, 6-tetra-*O*-acetyl-2-deoxy-2-*N*-phthalimido-D-glucopyranoside (25)

Glucosamine hydrochloride**14** (3.016 g, 14 mmol) and NaOH (28 mmol) were dissolved in 50 ml of water. The resulting mixture was stirred at room temperature for 30 minutes. Phthalic anhydride (2.34 g, 157 mmol) was added to the solution. The mixture was stirred vigorously at room temperature for 18 hours. The mixture was concentrated and dried using rotary evaporator. The residue was dissolved in pyridine (30 mL), and then Ac_2O (19.8 mL) was added to the solution. The resulting solution was allowed to stir vigorously overnight. The reaction was checked by the TLC. Methanol (6 mL) was used to quench the excess of Ac_2O, and then excess pyridine was removed under high vacuum. The remaining solid was dissolved in CH_2Cl_2 (40 mL), and then the solution was washed with 10% HCl (40 ml×1), saturated $NaHCO_3$ solution (40 ml×3), H2O (40 ml x1) and brine (40 mL×1) and dried over anhydrous $MgSO_4$. The final solution was concentrated under reduced pressure, and the

obtained product **25** (3.3g, 49.4%) was dried overnight. NMR data were consistent with data in the literature (Xu, Y. et al., Chem Med Chem 2013, 8, 511–520).

Phenyl 3,4,6-tri-*O*-acetyl-2-*N*-phthalimido-2-deoxy-1-thio-β-D-glucopyranose (26)

To a solution of **25** (1.5 g, 3.16mmol) in DCM (20 ml) at room temperature was added thiophenol (1.2 ml, 9.48 mmol) and $BF_3.Et_2O$ (0.94 ml, 9.48 mmol). After stirring overnight at room temperature the reaction was stopped with saturated sodium bicarbonate solution, the separated organic layer was then washed with saturated sodium bicarbonate solution (3 times), distilled water (2 times) and the organic layer was dried over Na_2SO_4 and then concentrated to dryness. The residue was purified by flash chromatography (Hexane:EtOAc, 6:4) to afford **26** as a brownish white solid (1.3gyield 77%). The NMR data was in agreement with previously reported data (Xu, Y. et al., Chem Med Chem 2013, 8, 511–520).

Phenyl -2-phthalimido-2-deoxy-1-thio-β-D-glucopyranose (27)

To a dispersion of **26** (1.3 g, 2.85 umol) in methanol was added NaOMe (150 mg). The mixture was vigorously stirred until complete dissolution of **26** (about 15 to 30 minutes) and at the completion of the reaction was added about 1 g of ion exchange resin (H^+). When the reaction mixture was clear, the resin was filtered and then concentrated under vacuum. The residue was purified by flash chromatography (100% ethyl acetate) to give **27** as a white solid, yield 80%. NMR data for compound **27:** ^{1}H NMR (300 MHz, Methanol-d4) δ = 8.04 – 7.74 (m, 4H, , phthalimido aromatic protons)), 7.49 – 7.17 (m, 5H, thiophenyl aromatic protons), 5.61 (d, J=10.4, 1H, H-1), 4.28 (dd, J=10.2, 7.8, 1H, H-3), 4.08 (dd, J=10.4, 2H), 3.97 (dd, J=12.0, 2.0, 1H), 3.78 (dd, J=12.1, 5.1, 1H), 3.59 – 3.41 (m, 2H).^{13}C NMR (75 MHz, MeOD) δ = 135.67, 134.47, 132.84, 130.00, 128.71, 124.49, 124.20, 85.49, 82.69, 73.87, 72.28, 62.86, 57.82. ES-MS: calcd $C_{20}H_{19}NO_6SNa^+$ m/z: 424.1, found $[M+Na]^+$ m/z: 424.1.

Phenyl-2-N-phthalimido-2-deoxy-6-(*O*-toluenesulphonyl)-1-thio-β-D-glucopyranose (28)

To a solution of compound **27** (700 mg, 1.743 mmol) in anhydrous pyridine (15 ml) at 0°C was added p-toluenesulphonyl chloride (398 mg, 2.092 mmol) and DMAP (50 mg) under nitrogen atmosphere. The reaction was warmed up to room temperature and left overnight, after which it was stopped by addition of methanol (5 ml). The solvent was then removed under high vacuum and the residue was purified by flash chromatography (Hexane/EtOAc, 9:1) to give **28** as a white foam (722 mg, 1.3mmol), yield 74%. NMR data for compound **28:** ^{1}H NMR (300 MHz, Chloroform-d) δ = 7.93 – 7.68 (m, 6H, aromatic protons), 7.44 – 7.13 (m, 7H, aromatic protons), 5.53 (d, J=10.3, 1H, H-1), 4.45 – 4.25 (m, 3H, H-3), 4.19 – 4.02 (m, 2H, H-2), 3.78 – 3.46 (m, 2H), 3.31 (br s, 1H, OH), 3.05 (br s, 1H, OH), 2.45 (s, 3H, toluene CH_3).^{13}C NMR (DEPT135) (75 MHz, CDCl3) δ = 134.32, 132.61, 129.96, 128.87, 128.07, 83.51, 77.24, 72.62, 70.95, 68.58, 55.23, 21.69. ES-MS: calcd $C_{27}H_{25}NO_8S_2Na^+$ m/z: 578.1, found $[M+Na]^+$ m/z: 578.2.

Phenyl -2-N-phthalimido-2-deoxy-6-azido-6-deoxy-1-thio-β-D-glucopyranose (29)

Compound **28** (2.8 g, 5.19 mmol) was dissolved in anhydrous DMF (25 ml) under nitrogen gas atmosphere, then NaN_3 (2.7 g, 41.53 mmol) was added and the reaction mixture was heated to 70°C with vigorous stirring overnight (18 hrs). The DMF was removed under high vacuum and the residue was suspended in ethyl acetate and then filtered to remove excess sodium azide. The organic layer was then concentrated under vacuum and the residue was partially purified by flash chromatography (100% EtOAc) to give **29** as a brownish gel (1.99 g, 4.67 mmol yield 90%). Compound **29** was not characterized using NMR spectroscopy. ES-MS: calcd $C_{20}H_{18}N_4O_5SNa^+$ m/z: 449.1, found $[M+Na]^+$ m/z: 449.1.

Phenyl 3,4-diacetyl-2-N-phthalimido-2-deoxy-6-azido-6-deoxy-1-thio-β-D-glucopyranose (30)

To a solution of compound **29** (1.5 g, 3.66 mmol) in pyridine (25 ml) was added a catalytic amount of DMAP (150 mg, mmol) and acetic anhydride (3 ml). The solution was stirred vigorously overnight at room temperature. At the completion of the

reaction, excess acetic anhydride was quenched by addition of methanol (5 ml). The solvents, methanol and pyridine were removed under high vacuum to give a brownish residue. The residue was dissolved in DCM and then washed with 5% HCl solution (2 times), saturated sodium bicarbonate (3 times) and distilled water (2 times). The organic layer was dried over anhydrous Na_2SO_4 and the concentrated under vacuum to a give a brown gel residue. The residue was the purified by flash chromatography (Hexane/Ethyl acetate, 4:6) to give compound **30** as a light yellow solid (1.63 g, 3.2mMol), yield 87.4%. Characteristic proton NMR data: ^{1}H NMR (300 MHz, Chloroform-d) δ = 7.80 (m, 4H, phthalimido aromatic protons), 7.49 – 7.18 (m, 5H, thiophenyl aromatic protons), 5.57 (d, J=10.2, 1H, H-1), 4.27 (ddd, J=10.0, 8.3, 5.8, 1H), 4.14 (dd, J=10.2,1H, H-2), 3.66 – 3.55 (m, 2H, H-6a), 3.55 – 3.42 (m, 2H, H-6b), 3.37 (d, J=4.0, 1H), 3.30 (d, J=5.9, 1H). ^{13}C NMR (75 MHz, CDCl3) δ = δ 170.13, 170. 01, 134.41, 133.28, 128.95, 128.31, 123.54, 83.67, 78.59, 73.04, 72.23, 55.57, 51.59, 21.46, 20.87.Chemical Formula: ES-MS: calcd $C_{24}H_{22}N_4O_7SNa^+$533.1, found $[M+Na]^+$ m/z:: 533.1.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(3',4'-O-diacetyl-2'-N-phthalimido-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (31)

^{1}H NMR (300 MHz, Chloroform-d) δ = 7.81 (m, 4H, phthalimido aromatic protons), 5.86 (dd, J=10.8, 9.0, 1H, H-3), 5.40 (d, J=8.5, 1H, H-1), 5.06 (dd, J=10.1, 9.0, 1H,H-4), 4.33 (dd, J=10.8, 8.4, 1H, H-2), 3.98 – 3.83 (m, 2H), 3.63 (dd, J=10.7, 4.9, 1H), 3.52 – 3.41 (m, 2H), 3.38 – 3.08 (m, 8H), 2.06 (s, 3H, Acetate CH_3), 1.88 (s, 3H, Acetate CH_3), 1.66 – 1.58 (m, 2H), 1.27 (s, 26H, Lipid tail), 0.89 (t, J = 6.6 Hz, 3H, lipid terminal –CH_3). ^{13}C NMR (75 MHz, CDCl3) δ = 170.11, 169.63, 134.25, 123.54, 98.47, 78.60, 73.61, 71.66, 70.41, 70.38, 69.82, 68.71, 57.59, 54.64, 51.23, 31.94, 29.71, 29.67, 29.61, 29.48, 29.37, 26.01, 22.70, 20.66, 20.48, 14.12. ES-MS: calcd$C_{38}H_{58}N_4O_{10}Na^+$ m/z: 753.4, found $[M+Na]^+$ m/z: 753.5

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(-2'-amino-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (32)

^{1}H NMR (300 MHz, Methanol-d4) δ = 4.29 (d, J=8.1, 1H,H-1), 3.95 (dd, J=10.5, 4.3, 1H), 3.71 (dd, J=10.5, 4.2, 1H), 3.64 – 3.51 (m, 4H), 3.51 – 3.38 (m, 7H), 3.29 – 3.20 (m, 2H,H-3), 2.70 – 2.55 (dd, J= 8.1,1H, H-2), 1.59-1.52 (m 2H), 1.32 (s, 26H, Lipid tail), 0.88 (t, J = 6.6 Hz, 3H, lipid terminal –CH_3).^{13}C NMR (75 MHz, MeOD) δ = 104.73, 80.48, 79.10, 77.41, 77.31, 72.65, 71.46, 69.44, 58.33, 58.04, 52.79, 33.10, 30.80, 30.61, 30.50, 27.25, 23.76, 14.47. ES-MS: calcd$C_{26}H_{52}N_4O_6Na^+$ m/z:539.4, found $[M+Na]^+$ m/z: 539.4

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(-2',6'-diamino-2',6'-dideoxy-β-D-glucopyranosyl)-sn-glycerol (2)

^{1}H NMR (300 MHz, Methanol-d4) δ = 4.26 (d, J=8.0, 1H,H-1), 3.95 (dd, J=10.8, 4.6, 1H), 3.70 (dd, J=10.6, 4.2, 1H), 3.66 – 3.45 (m, 8H), 3.33 – 3.14 (m, 3H), 3.06 (dd, J=13.4, 2.7, 1H), 2.61 (dd, J=9.6, 8.0, 1H,H-2), 1..66 -1.54 (m, 2H), 1.32 (s, 26H, lipid tail), 0.87 (t, *J* = 6.6 Hz, lipid terminal –CH_3).^{13}C NMR (75 MHz, MeOD) δ = 104.98, 80.58, 78.08, 77.51, 73.51, 72.68, 71.41, 69.74, 58.45, 58.17, 43.99, 33.12, 30.83, 30.79, 30.64, 30.52, 27.28, 23.78.MALDI-HRMS: calcd $C_{26}H_{54}N_2O_6Na^+$m/z: 513. 3880, found $[M+Na]^+$ m/z: 513.3612.

1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(-2'-N-phthalimido-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (33)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.89 – 7.77 (m, 4H, phthalimido aromatic protons), 5.20 (d, *J* = 8.3 Hz, 1H, H-1), 4.34 (dd, *J* = 10.9, 8.5 Hz, 1H, H-3), 4.13 (dd, *J* = 10.9, 8.3 Hz, 1H, H-2), 3.87 (dd, *J* = 10.7, 4.6 Hz, 1H, H-6a), 3.76-3.66 (m, 1H), 3.62 – 3.49 (m, 4H, H-4, H-5, H-6b), 3.49 – 3.39 (m, 2H), 3.33 (dq, *J* = 9.9, 4.6, 4.2 Hz, 2H), 3.26 (d, *J* = 4.0 Hz, 1H), 3.23 – 3.07 (m, 5H), 1.45 – 1.36 (m, 2H), 1.26 (s, 26H, lipid tail), 0.90 (t, *J* = 6.6 Hz, 3H lipid terminal –CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 168.40, 134.18, 131.70, 123.42, 98.68, 78.67, 77.46, 75.23, 72.78, 71.69, 69.95, 68.32, 57.56, 56.56, 51.48, 31.94, 29.72, 29.67, 29.62, 29.47, 29.38, 26.00, 22.70, 14.13.ES-MS: calcd: $C_{34}H_{54}N_4O_8Na^+$ m/z: 669.4, found $[M+Na]^+$ m/z: 669.4.

1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-(-2'-N-phthalimido-6'-amino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (3)

^{1}H NMR (300 MHz, Methanol-d_4) δ 8.03 – 7.61 (m, 4H), 5.20 (d, *J* = 8.5 Hz, 1H, H-1), 4.33 (dd, *J* = 10.7, 8.6 Hz, 1H, H-3), 4.00 (dd, *J* = 10.7, 8.5 Hz, 1H, H-2), 3.87 (dd, *J* = 11.0, 4.2 Hz, 1H), 3.72 – 3.55 (m, 2H), 3.43 – 3.26 (m, 17H), 3.26 – 3.07 (m, 6H, H-6a), 2.88 (dd, *J* = 13.5, 7.5 Hz, 1H, H-6b),), 1.58 – 1.46 (m, 2H), 1.32 (s, 26H), 0.83 (t, *J* = 6.8 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 168.40, 134.18, 131.70, 123.42, 98.68, 78.67, 77.46, 75.23, 72.78, 71.69, 69.95, 57.56, 56.56, 52.45, 51.48, 31.94, 29.72, 29.67, 29.62, 29.47, 29.38, 26.00, 22.70, 14.13.MALDI-HRMS: calcd: $C_{34}H_{56}N_2O_8Na^+$ m/z: 643.3934, found $[M+Na]^+$ m/z:643.3857.

1-*O*-Hexadecyl-2-deoxy-3-O-(3',4'-O-diacetyl-2'-N-phthalimido-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (35)

Compound **30** (0.4 mmol) and the previously reported lipid compound **34** (168 mg, 0.48mmol) were dissolved in anhydrous DCM (10 ml) under argon atmosphere. NIS (180 mg, 0.8 mmol) and silver triflate (20 mg, 0.08 mmol) were added. The reaction mixture was left under vigorous stirring for 3 hrs. At the completion of reaction (TLC monitoring), the reaction mixture was diluted by DCM (20 ml) and then filtered over Celite. The resulting organic layer was washed with saturated sodium thiosulphate solution (2 times), saturated sodium bicarbonate (3 times) and water (2 times). The organic layer was then dried over anhydrous Na_2SO_4 and then concentrated under vacuum to give a brownish gel residue. The residue was then purified by flash chromatography (Hexane/Ethyl acetate, 4:6) to give compound **35** as a white solid. Yield 51%.

^{1}H NMR (300 MHz, Chloroform-d) δ = 7.85 (dd, J=5.5, 3.1, 2H, phthalimido aromatic protons), 7.73 (dd, J=5.5, 3.1, 2H, phthalimido aromatic protons), 5.79 (dd, J=10.8, 9.0, 1H, H-3), 5.38 (d, J=8.5, 1H, H-1), 5.05 (dd, J=10.1, 9.0, 1H, H-4), 4.30 (dd, J=10.8, 8.5, 1H, H-2), 3.96 – 3.81 (m, 2H), 3.63 – 3.52 (m, 1H), 3.43 (dt, J=13.6, 6.9, 1H), 3.28 – 3.16 (m, 3H), 3.15 – 2.99 (m, 2H), 2.03 (s, 3H, Acetate CH_3), 1.85 (s, 3H, Acetate CH_3), 1.76 – 1.58 (m, 2H), 1.26 (s, 26H, Lipid tail), 0.89 (t, J = 6.6 Hz, 3H,

lipid terminal –CH_3).^{13}C NMR (75 MHz, CDCl3) δ = 170.12, 169.62, 134.27, 123.57, 97.99, 73.60, 71.82, 71.00, 70.53, 70.37, 67.04, 66.96, 54.68, 51.24, 31.91, 29.73, 29.68, 29.59, 29.48, 29.34, 26.07, 22.67, 14.10.ES-MS: calcd: $C_{37}H_{56}N_4O_9Na^+$ m/z: 723.4, found $[M+Na]^+$ m/z: 723.5.

1-*O*-Hexadecyl-2-deoxy-3-O-(2'-amino-6'-azido-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (36)

^{1}H NMR (300 MHz, Methanol-d4) δ = 4.28 (d, J=7.9, 1H,H-1), 3.98 (dd, J=9.6, 6.3, 1H), 3.67 (dt, J=9.4, 6.4, 1H), 3.55 (td, J=6.4, 3.0, 2H), 3.51 – 3.41 (m, 5H,H-6), 3.30 – 3.21 (m, 2H, H-3), 2.63 (dd, J=9.8,7.9, 1H), 1.96 – 1.82 (m, 2H, -OCH_2-**CH_2**-CH_2O-), 1.66 - 1.57 (m, 2H), 1.32 (s, 26H, Lipid tail), 0.86 (t, J = 6.7 Hz, 3H, lipid terminal –CH_3).^{13}C NMR (75 MHz, MeOD) δ = 104.54, 77.34, 72.72, 72.10, 68.77, 67.91, 58.37, 52.81, 33.12, 31.16, 30.82, 30.65, 30.52, 27.31, 23.78, 14.50.ES-MS: calcd: $C_{33}H_{52}N_4O_7Na^+$ m/z: 639.4, found $[M+Na]^+$ m/z: 639.4.

1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol (4)

^{1}H NMR (300 MHz, Methanol-d4) δ = 4.25 (d, J=8.0, 1H,H-1), 3.99 – 3.61 (m, 2H), 3.50-3.60 (m, J=6.3, 3.8, 3H), 3.45 (t, J=6.5, 3H), 3.31 – 3.14 (m, 2H, H-3), 3.06 (dd, J=13.4, 2.8, 1H,H-6a), 2.76 (dd, J=13.4, 7.0, 1H,H-6b), 2.59 (dd, J=9.5, 8.0, 1H, H-2), 1.96 – 1.82 (m, 2H, -OCH_2-**CH_2**-CH_2O-), 1.64 - 1.55 (m, 2H), 1.32 (s, 26H, Lipid tail), 0.85 (t, J = 6.7 Hz, 3H, lipid terminal –CH_3). ^{13}C NMR (75 MHz, MeOD) δ = 104.78, 78.00, 77.55, 73.57, 72.10, 68.70, 67.89, 58.50, 44.00, 33.11, 31.21, 30.82, 30.66, 30.51, 27.32, 23.77, 14.50. MALDI-HRMS: calcd $C_{25}H_{52}N_2O_5Na^+$m/z: 483.3774, found $[M+Na]^+$ m/z:483.3781.

1-*O*-Hexadecyloxy-,2S/R,3-di (-3,4-diacetyl-6'azido-2-*N*-phthalimido-2',6'-dideoxy- β-D- glucopyranosyl)-glycerol (38)

^{1}H NMR (300 MHz, Chloroform-d) δ 7.95 – 7.65 (m, 8H, phthalimido aromatic protons), 5.71 (td, J = 10.4, 9.1 Hz, 2H, H-3a, H-3b), 5.53 (d, J = 8.5 Hz, 1H, H-1a),

5.20 (d, J = 8.4 Hz, 1H, H-1b), 5.02 (dd, J = 10.1, 8.9 Hz, 1H), 4.94 – 4.81 (m, 1H), 4.28-4.15 m, 2H, H-2a), 3.97 – 3.67 (m, 4H), 3.67 – 3.28 (m, 6H, H-2b), 3.26 – 2.98 (m, 3H), 2.06 (s, 6H, Acetate CH_3), 1.86 (s, 3H, Acetate CH_3), 1.64 (s, 3H, Acetate CH_3)1.66– 1.57 (m, 2H), 1.28 (s, 26H, Lipid tail), 0.89 (t, J = 6.7 Hz, 3H, lipid terminal –CH_3. ^{13}C NMR DEPT (75 MHz, $CDCl_3$) δ δ 134.30, 134.19, 123.81, 123.52, 96.74, 96.69, 76.57, 73.25, 72.90, , 71.53,70.40, 70.39, 70.36, 70.15, 70.14, 70.12, 69.77, 69.76,54.68, 54.41, 51.22, 51.23, 51.09, 31.89, 31.85, 29.65, 25.97, 25.87, 21.71, 22.65, 21.47, 20.68, 20.43, 14.13.ES-MS: calcd: $C_{55}H_{72}N_8O_{17}Na^+$m/z: 1139.5, found $[M+Na]^+$ m/z: 1139.4.

1-*O*-Hexadecyloxy-2S/R,3-di(-2'-amino-6'azido-2',6'-dideoxy-β-D-glucopyranosyl)-glycerol (39)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.49 (d, *J* = 8.0Hz, 1H, H-1a), 4.33 (dd, *J* = 8.4, 1H, H-1b), 4.16 – 3.97 (m, 2H), 3.78 (dd, *J* = 10.7, 5.5 Hz, 1H), 3.67 (dd, *J* = 5.1, 2.7 Hz, 2H), 3.59 – 3.39 (m, 2H), 3.36 – 3.22 (m, 7H, H-3a, H3b), 2.75 – 2.54 (m, 2H, H-2a, H-2b), 1.60 m, 2H), 1.31 (s, 26H, Lipid tail), 0.93 (t, *J* = 4.5Hz, 3H, lipid terminal –CH_3).^{13}C NMR (75 MHz, MeOD) δ 104.87, 104.28, 78.63, 77.23, 77.05, 72.64, 72.60, 72.52, 71.57, 70.72, 58.48, 58.35, 52.79, 33.10, 30.81, 30.65, 30.49, 27.31, 23.76, 14.47.ES-MS: calcd: $C_{31}H_{60}N_8O_9Na^+$ m/z: 711.4, found $[M+Na]^+$ m/z: 711.4.

1-*O*-Hexadecyloxy-2S/R, 3-di(-2',6'-diamino-2',6'-dideoxy-β-D-glucopyranosyl)-glycerol (5)

^{1}H NMR (300 MHz, Methanol-d4) δ 4.44 (d, J = 8.3 Hz, 1H, H-1a), 4.29 (d, J = 8.4 Hz, 1H, H-1b), 4.03 (d, J = 14.5 Hz, 2H), 3.8 - 3.59 (m, 3H), 3.56 – 3.35 (m, 5H), 3.28 – 3.17 (m, 4H, H-3a, H-3b), 3.15 – 3.01 (m, 2H, H-6a'', H-6b''), 2.81 – 2.69 (m, 2H, H-6a', H-6b'), 2.64 – 2.58 (m, 2H, H-2a, H-2b), 1.60 (s, 2H), 1.32 (s, 26H,Lipid tail), 0.92 (t, J = 6.8 Hz, 3H, lipid terminal –CH_3).13C NMR (75 MHz, MeOD) δ 103.58, 103.27, 77.43, 76.41, 76.38, 72.11, 72.03, 70.19, 70.13, 69.47, 69.45, 57.17, 57.14, 48.04, 42.55, 42.47, 42.44, 31.61, 29.42, 29.31 , 29.19, 25.77, 19.91, 23.67, 21.21, 11.80. MALDI-HRMS: calcd: $C_{31}H_{64}N_4O_9Na^+$ m/z: 659.4571, found $[M+Na]^+$

m/z:659.2064.

p-Toluene, 3-Hexadecyloxy-2R-hdroxyl propyl-1-sulphonate (41)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.78 (d, *J* = 8.2 Hz, 2H, aromatic protons), 7.33 (d, *J* = 8.1 Hz, 2H, aromatic protons), 4.11 – 4.00 (m, 2H, MsO-CH_2), 3.99 – 3.89 (m, 1H, HO-CH), 3.46 – 3.31 (m, 4H), 2.80 (d, *J* = 5.4 Hz, 1H, OH), 2.42 (s, 3H, mesylate –CH_3), 1.55 – 1.41 (m, 2H), 1.25 (s, 26H, Lipid tail), 0.87 (t, *J* = 6.4 Hz, 3H, lipid terminal –CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 144.90, 132.77, 129.88, 127.99, 71.73, 70.77, 70.56, 68.25, 31.93, 29.71, 29.68, 29.64, 29.61, 29.48, 29.37, 26.01, 22.68, 21.58, 14.11.

3-Hexadecyloxy-2R-hdroxyl propyl-1-azide(42)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 3.88 (p, *J* = 5.4 Hz, 1H, HO-CH), 3.48 – 3.34 (m, 4H), 3.31 (dd, *J* = 5.5, 2.9 Hz, 2H, -CH_2N_3), 3.17 (s, 1H, OH),1.55 – 1.41 (m, 2H, 1.25 (s, , 26H, Lipid tail), 0.85 (t, *J* = 6.6 Hz, 3H, terminal lipid -CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 71.92, 71.71, 69.59, 53.54, 31.93, 29.71, 29.67, 29.61, 29.52, 29.47, 29.37, 26.05, 22.67, 14.03.ES-MS: calcd: $C_{19}H_{39}N_3O_2Na^+$m/z: 364.3, found $[M+Na]^+$ m/z: 364.5.

1-*O*-Hexadecyloxy-2R-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy- β-D-glucopyranosyl)-3-azido glycerol(43)

^{1}H NMR (300 MHz, Chloroform-d) δ 7.97 – 7.63 (m, 4H, phthalimido aromatic protons), 5.80 (dd, J = 10.7, 9.1 Hz, 1H, H-3), 5.53 (d, J = 8.5 Hz, 1H, H-1), 5.16 (dd, J = 10.7 Hz, 1H, H-4), 4.32 (ddd, J = 12.1, 8.5, 5.4 Hz, 2H, H-2), 4.19 (dd, J = 12.2, 2.5 Hz, 1H), 3.96 – 3.73 (m, 2H, H-5), 3.60 (dt, J = 10.0, 4.8 Hz, 1H), 3.48 – 3.30 (m, 4H), 3.27 – 3.16 (m, 1H), 2.12(s, 3H, acetate –CH_3), 2.04 (s, 3H, acetate –CH_3), 1.88 (s, 3H, acetate –CH_3), 1.48 (m, 2H), 1.09 (s, 26H, Lipid tail), 0.88 (t, J = 6.6 Hz, 3H, Lipid terminal –CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.58, 170.11, 169.47, 134.21, 131.56, 123.50, 98.61, 78.66, 71.96, 71.79, 70.74, 70.24, 69.03, 62.16, 54.64, 52.46, 31.93, 29.69, 29.66, 29.62, 29.58, 29.45, 29.36, 26.06, 22.69, 20.75, 20.63, 20.44,

14.11.ES-MS: calcd:$C_{39}H_{58}N_4O_{11}Na^+$m/z: 781.4, found $[M+Na]^+$ m/z: 781.4.

1-*O*-Hexadecyloxy-2R-(2'-amino-2'-deoxy- β-D- glucopyranosyl)-3-azido glycerol(6)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.45 (d, J = 8.0 Hz, 1H, H-1), 4.02 (ddt, J = 9.1, 6.3, 3.0 Hz, 1H), 3.69 (ddd, J = 16.6, 7.3, 3.6 Hz, 3H), 3.63 – 3.54 (m, 2H), 3.48 (dt, J = 12.5, 6.3 Hz, 4H), 3.32 -3.27 (m, J = 8.4 Hz, 2H, H-3), 2.66 (dd, J = 8.0, 6.8, 1H, H-2), 1.64 – 1.49 (m, 2H), 1.32 (s, 26H), 0.93 (t, J = 7.1, Hz, 3H Lipid terminal – CH_3). ^{13}C NMR (75 MHz, MeOD) δ 104.02, 78.30, 78.13, 77.23, 72.70, 71.78, 71.70, 62.78, 61.56, 58.30, 53.17, 33.15, 30.87, 30.83, 30.76, 30.70, 30.66, 30.55, 27.30, 23.81, 20.97, 14.57.MALDI-HRMS: calcd: $C_{25}H_{50}N_4O_6Na^+$ m/z: 525.3628, found $[M+Na]^+$ m/z: 525.3064.

1-*O*-Hexadecyloxy-2R-(2'-amino-2'-deoxy- β-D- glucopyranosyl)-3-amino glycerol(7)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.40 (d, J = 8.1 Hz, 1H, H-1), 3.93 – 3.82 (m, 1H, -O-CH), 3.70 (dt, J= 15.3, 4.6 Hz, 3H), 3.60 – 3.41 (m, 4H), 3.36 – 3.22 (m, 2H, H-3), 2.97 – 2.71 (m, 2H, $-CH_2NH_2$), 2.63 (t, J = 8.4 Hz, 1H, H-2), 1.58 (m, 2H), 1.32 (s, 26H), 0.87 (t, J = 6.9 Hz, 3H).^{13}C NMR (75 MHz, MeOD) δ 104.46, 80.02, 78.22, 77.78, 72.70, 72.67, 71.78, 62.81, 58.45, 43.77, 33.11, 30.80, 30.66, 30.51, 27.31, 23.77, 14.49.MALDI-HRMS: calcd: $C_{25}H_{52}N_2O_6Na^+$m/z: 499.3723, found $[M+Na]^+$ m/z:499.2997.

2(R/S)-Azido-3-hexadecyloxy-1-propanol (44)

Diisopropylazodicarboxylate (DIAD; 3.2 ml, 15 mmol) was added to a solution of racemic mixture of 3-*O*- hexadecyl-sn-glycerol **37** (3.42 g, 13 mmol) in 180 ml of DCM at 0°C. After the mixture was stirred for 3h under Nitrogen gas, Me_2SiN_3 was added. The mixture was stirred at the same temperature for 3 h, then at room temperature until glycerol **37** had reacted completely. The reaction mixture was concentrated to give a yellow residue which was dissolved in a minimal amount of

DCM and passed through a pad of silica gel in a sintered glass funnel. The pad was rinsed with hexane/EtOAc (50:1) until the excess yellow DIAD began to elute. After concentration of the eluted silyloxyazide, the residue was dissolved in 30 ml THF and treated with a solution of $(nBu)_4NF$ (1 M, 25 ml) in THF. The mixture was stirred for 3 hrs at room temperature and then was diluted with 250 ml Et_2O and washed with water (2 times) and brine (2 times). The organic layer was separated, dried over Na_2SO_4 and concentrated. The crude product was purified by column on silica gel with hexane/EtOAc (4:1) to give compound **44** as a colorless gel. The NMR data corresponds to what has been previously reported (Byun, H-S. et al., Chem Med Chem, 2010, 5, 1045-1052)

1-*O*-Hexadecyloxy-2S/R-azido,3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D-glucopyranosyl)-glycerol (45)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.85 – 7.69 (m, 4H, phthalimido aromatic protons), 5.79 (ddd, *J* = 10.7, 9.1, 4.7 Hz, 1H, H-3), 5.39 (dd, *J* = 10.9, 8.5 Hz, 1H, H-1), 5.16 (ddd, *J* = 10.2, 9.1, 3.7 Hz, 1H, H-4), 4.39 – 4.24 (m, 2H, H-2), 4.20 – 4.05 (m, 2H), 4.01 – 3.81 (m, 2H, H-5), 3.65 – 3.55 (m, 1H), 3.55 – 3.45 (m, 1H), 3.44 – 3.32 (m, 1H), 3.31 – 3.10 (m, 2H),2.09 (s, 3H), 2.01 (s, 3H), 1.84 (s, 3H), 1.46 - 138 (m, 2H), 1.30 (s, 26H, Lipid tail), 0.85 (t,*J* = 6.6 Hz, 3H, Terminal Lipid CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.60, 170.06, 169.42, 134.27, 134.23, 131.47, 123.54, 98.56, 98.46, 71.96, 71.68, 71.61, 70.65, 70.59, 70.25, 69.94, 69.05, 68.91, 68.90, 61.93, 61.90, 60.54, 60.32, 59.90, 54.49, 31.90, 29.67, 29.63, 29.59, 29.55, 29.46, 29.39, 29.33, 25.90, 22.66, 20.98, 20.71, 20.58, 20.41, 14.18, 14.09.ES-MS: calcd: $C_{39}H_{58}N_4O_{11}Na^+$m/z: 781.4, found $[M+Na]^+$ m/z: 781.4.

1-*O*-Hexadecyloxy-2S/R-amino-3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D- glucopyranosyl)-glycerol (46)

^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.97 – 7.63 (m, 4H, phthalimido aromatic protons), 5.79 (t, *J* = 9.9 Hz, 1H,H-3), 5.35 (d, *J* = 8.4 Hz, 1H, H-1), 5.16 (t, *J* = 9.6 Hz, 1H, H-4), 4.37 – 4.29 (m, 1H, H-2), 4.22 – 4.08 (m, 1H), 3.92 – 3.77 (m, 2H, H-5),

3.75 – 3.59 (m, 1H), 3.48 (dd, *J* = 9.6, 5.3 Hz, 1H), 3.40 (dd, *J* = 9.6, 6.8 Hz, 1H), 3.26 – 3.06 (m, 3H), 3.06 - 2.94 (m, 1H, -$CHNH_2$), 2.25 (broad s, 2H, Amino protons), 2.10 (s, 3H), 2.02 (s, 3H), 1.85 (s, 3H), 1.49 – 1.34 (m, 2H), 1.23 (s, 26H, Lipid tail), 0.86 (t, *J* = 6.4 Hz, 3H, Lipid terminal -CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.67, 170.12, 169.47, 134.34, 131.37, 123.62, 98.58, 98.37, 72.64, 72.48, 72.32, 71.88, 71.42, 70.71, 69.01, 62.02, 58.10, 54.65, 50.66, 50.61, 31.90, 29.68, 29.64, 29.58, 29.52, 29.45, 29.34, 26.05, 22.67, 20.74, 20.61, 20.43, 18.40, 14.10.ES-MS: calcd:$C_{39}H_{60}N_2O_{11}Na^+$m/z: 755.4, found $[M+Na]^+$ m/z: 755.4.

1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol (8)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.70 (dd, *J* = 8.3, 3.1 Hz, 1H, H-1), 4.19 – 4.02 (m, 1H), 4.01 – 3.87 (m, 2H), 3.79 – 3.73 (m, 1H), 3.72 – 3.61 (m, 3H), 3.65 – 3.57 (m, 1H, H-3), 3.57 – 3.48 (m, 2H), 3.49 -3.32 (m, 2H, -$CHNH_2$), 2.96 (ddd, *J* = 10.3, 8.4, 1.7 Hz, 1H, H-2), 1.71 – 1.55 (m, 2H), 1.33 (s, 26H, lipid tail), 0.92 (t, *J* = 6.4 Hz, 3H, lipid terminal –CH_3).^{13}C NMR (75 MHz, MeOD) δ 100.58, 100.18, 78.63, 73.85, 72.93, 71.74, 71.65, 68.58, 68.43, 62.15, 62.10, 52.72, 52.69, 33.09, 30.80, 30.77, 30.64, 30.53, 30.48, 27.13, 23.75.MALDI-HRMS: calcd: $C_{25}H_{52}N_2O_6Na^+$ m/z: 499.3723, found $[M+Na]^+$ m/z: 499.3450.

1-*O*-Hexadecyloxy-2S/R-*N*-hexadecylacyl-3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D- glucopyranosyl)-glycerol (48)

Compound **46** (0.18 mmol, 128 mg) was dissolved in 10 ml of anhydrous DMF, palmitic acid **47** (0.21 mmol, 54 mg) and the coupling agent TBTU (0.25 mmol, 81 mg) were simultaneously added under argon atmosphere. The reaction mixture was left to stir for 5 hrs at room temperature. After complete disappearance of **46**, the reaction mixture was concentrated under vacuum and the residue obtained was purified by flash chromatography using hexane/EtOAc (4:1) to give compound **48** as a white compound (yield 60%). ES-MS: calcd: $C_{55}H_{90}N_2O_{12}Na^+$m/z: 993.6, found $[M+Na]^+$ m/z: 993.4.

1-*O*-Hexadecyloxy-2S/R-*N*-hexadecylacyl-3-(-2'-amino-2'-deoxy-β-D-glucopyranosyl)-glycerol (9)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.59 (dd, J = 8.3, 6.3 Hz, 1H, H-1R/S), 4.42 – 4.20 (m, 1H, H-4 R/S), 3.92 (dd, J = 12.1, 4.3 Hz, 2H, H-6a R/S), 3.83 – 3.63 (m, 3H, H-6b R/S), 3.66 – 3.44 (m, 6H, H-3 R/S), 2.86 (ddd, J = 12.7, 10.5, 8.3 Hz, 1H, H-2 R/S), 2.29 – 2.17 (m, 2H, -NHCO-CH_2), 1.71 - 1.51 (m, 4H), 1.33 (s, 50H, two lipid tails), 0.93 (t, J = 6.2 Hz, 6H, terminal –CH_3 of the two lipid tails).^{13}C NMR (75 MHz, MeOD) δ 176.79, 100.75, 100.45, 78.56, 74.02, 72.49, 72.45, 71.81, 71.02, 70.71, 70.53, 62.32, 57.54, 55.16, 50.52, 50.39, 49.88, 37.29, 37.19, 33.11, 30.85, 30.81, 30.74, 30.59, 30.52, 30.30, 27.33, 27.13, 23.77, 14.48.MALDI-HRMS: calcd: $C_{41}H_{82}N_2O_7Na^+$ m/z: 737.6020, found $[M+Na]^+$ m/z:737.3607s

1-*O*-Hexadecyloxy-2S/R-*N*-methylcarbamoyl-3-(-3',4',6'-triacetyl-2-*N*-phthalimido-2'-deoxy-β-D- glucopyranosyl)-glycerol (50)

To a solution of compound **46** (0.16 mmol, 114, mg) in DCM was added methylchloroformate **49** (0.311 mmol, 29.4 mg) and Et_3N (0.35 mmol, 35.5 mg) at 0°C. The mixture was stirred overnight and then concentrated under vacuum to give residue which was purified with by flash chromatography using hexane/EtOAc (3:2) to give the carbamate glycolipid **50** (80% yield) as a white solid (Byun et al 2010).

^{1}H NMR (300 MHz, Chloroform-d) δ 7.92 – 7.68 (m, 4H, phthalimido aromatic protons), 5.79 (dd, J = 10.7, 9.1 Hz, 1H, H-3), 5.35 (d, J = 8.4 Hz, 1H, H-1), 4.83 (d, J = 7.4 Hz, 1H, Carbamate -NH), 4.42 – 4.23 (m, 2H), 4.17 (ddd, J = 12.3, 3.9, 2.4 Hz, 1H), 3.85 (dddd, J = 17.5, 13.1, 6.7, 3.2 Hz, 4H), 3.53 (s, 3H, Carbamate -CH_3), 3.30 (ddd, J = 18.5, 8.1, 3.9 Hz, 2H), 3.11 (ddt, J = 29.1, 9.3, 6.7 Hz, 2H), 2.11 (s, 3H), 2.02 (s, 3H), 1.85 (s, 3H), 1.45 – 1.34 (m, 2H), 1.27 (s, 26H, Lipid tail), 0.88 (t, J = 6.4 Hz, 3H, Lipid terminal -CH_3).^{13}C NMR (75 MHz, $CDCl_3$) δ 170.69, 170.11, 169.47, 134.32, 134.28, 131.42, 123.62, 98.49, 98.39, 71.89, 71.39, 71.32, 70.67, 70.63, 68.95, 68.91, 68.76, 68.66, 61.98, 54.59, 31.91, 29.69, 29.64, 29.58, 29.45, 29.34, 26.00, 25.97, 22.67, 20.73, 20.61, 20.43, 14.11.ES-MS: calcd:$C_{41}H_{62}N_2O_{13}Na^+$m/z:

813.4, found $[M+Na]^+$ m/z: 813.3.

1-*O*-Hexadecyloxy-2S/R-*N*-methylcarbamoyl-(-2'-amino-2'-deoxy-β-D-glucopyranosyl)-glycerol (10)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.26 (dd, J = 8.1, 2.4 Hz, 1H, H-1), 4.00 – 3.89 (m, 2H), 3.87 (d, J = 1.5 Hz, 1H), 3.71 (ddd, J = 8.6, 4.8, 2.0 Hz, 2H), 3.66 (s, 3H, carbamate $-CH_3$), 5.55 - 3.44 (m 4H, H-3), 3.38 – 3.22 (m, 3H), 265 – 2.58 (m, 1H, H-2) 1.60 – 1.55 (m, 2H), 1.31 (s, 26H, lipid tail), 0.92 (t, J = 6.8 Hz, 3H, lipid terminal $-CH_3$).^{13}C NMR (75 MHz, MeOD) δ 105.05, 104.71, 78.23, 77.57, 77.51, 72.44, 71.84, 71.74, 70.97, 70.60, 70.28, 62.80, 62.69, 58.36, 58.34, 52.56, 52.23, 33.11, 30.82, 30.79, 30.65, 30.59; 30.50, 27.27, 23.77, 14.49.MALDI-HRMS: calcd: $C_{27}H_{54}N_2O_8Na^+$ m/z: 557.3778, found $[M+Na]^+$ m/z: 557.3364.

1-*O*-Hexadecyloxy-2R-(–α-L-rhamnopyranosyl)-3-amino glycerol (56)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.65 (d, J = 1.3 1H, H-1) 3.65 (dd, J = 1.3,2.3 Hz, 1H), 3.54 (dp, J = 10.5, 5.6, 5.0 Hz, 2H), 3.45 (dd, J = 9.5, 3.4 Hz, 1H), 3.34 (h, J = 4.7 Hz, 1H), 3.29 – 3.19 (m, 2H), 3.14 (d, J = 21.0 Hz, 2H), 2.57 (qd, J = 13.5, 5.4 Hz, 2H), 1.40 -1.34m, 2H), 1.08 (s, 29H, H-6, lipid tail), 0.69 (t, J = 6.4 Hz, 3H, lipid terminal $-CH_3$).^{13}C NMR (75 MHz, MeOD) δ 101.91, 79.55, 73.98, 72.69, 72.66, 72.45, 72.39, 70.10, 43.50, 33.10, 30.81, 30.78, 30.66, 30.50, 27.33, 23.76, 18.08, 14.47.MALDI-HRMS: calcd: $C_{25}H_{51}NO_6Na^+$ m/z:484.6738, found $[M+Na]^+$ m/z:484.6365.

1-*O*-Hexadecyloxy-2R-*O*-methyl(2'amino-2'deoxy–α-L-glucopyranosyl)-3-amino glycerol (69)

^{1}H NMR (300 MHz, Methanol-d_4) δ 4.71 (d, J = 3.6 Hz, 1H, H-1), 3.76 – 3.66 (m, 1H), 3.62 (dd, J = 11.8, 5.3 Hz, 1H), 3.47 (dd, J = 9.8, 6.9 Hz, 4H), 3.42 – 3.29 (m, 3H, H-3), 3.26 – 3.13 (m, 2H), 2.50 (dd, J = 9.8, 3.5 Hz, 1H, H-2), 1.50 - 146 (m, 2H), 1.22 (s, 26H, lipid tail), 0.83 (t, J = 6.5 Hz, 3H, lipid terminal CH_3).^{13}C NMR (75 MHz, MeOD) δ 100.59, 80.50, 76.34, 74.22, 72.70, 71.88, 71.41, 67.92, 62.68, 58.26,

57.28, 33.11, 30.82, 30.65, 30.51, 27.29, 23.77, 14.49.MALDI-HRMS: calcd: $C_{26}H_{53}NO_7Na^+$ m/z:514.6998, found $[M+Na]^+$ m/z: 484.3300

Compound 89: A solution of **87** in dry DMF was treated with NaN_3 at 90 °C for 3 h. The mixture was then concentrated under *vacuo*, worked up with H_2O (×2) and brine (×1) successively and re-concentrated to give **88** in excellent yield. **88** was subsequently dissolved in dry DCM, treated with PCC (3 equiv.) and stirred at RT for 2 h. The reaction was monitored with TLC using $KMnO_4$ stain. The resulting mixture was filtered through a pad of silica and concentrated under low *vacuo* to give **89**. The resulting compound was used immediately without further purification

Compound 84a (n = 11)**:** A solution of **3** (0.45 g, 0.73 mmol) in dry DCM was treated with **89a** (n = 11) (0.17 g, 0.73 mmol) and stirred overnight at 0 °C to RT. Two drops of acetic acid and sodium borohydride, $NaBH_4$ (0.099 g, 2.19 mmol) in methanol were then added to the mixture and stirred further for 2 h at RT. The resulting mixture was concentrated in *vacuo*, extracted with ethyl acetate and purified by flash chromatography (dichloromethane/ methanol, 10:1, v/v) to afford **84a** (0.46 g, 73 %). ^{1}H NMR (500 MHz, MeOD): δ = 7.92 – 7.78 (m, 4H), 5.21 (d, J = 8.5 Hz, 1H, H-1), 4.32 (dd, J = 10.8, 8.6 Hz, 1H, H-3), 4.00 (dd, J = 10.8, 8.5 Hz, 1H, H-2), 3.84 – 3.72 (m, 1H), 3.63 – 3.53 (m, 1H), 3.50 – 3.38 (m, 3H), 3.30 – 3.23 (m, 5H), 3.23 – 3.09 (m, 6H), 3.07 – 3.01 (m, 2H), 1.87 – 1.80 (m, 2H), 1.73 – 1.66 (m, 2H), 1.50 – 1.17 (m, 44H), 0.89 (t, J = 6.9 Hz, 3H). ESI-MS: m/z $[M + Na]^+$ calc'd for $C_{46}H_{79}N_5O_6Na^+$: 853.09, found: 853.1

Compound 84b (n = 2)**:** A solution of **3** (0.21 g, 0.34 mmol) in dry DCM was treated with **89b** (n = 2) (0.035 g, 0.34 mmol) and stirred overnight at 0 °C to RT. Two drops of acetic acid and sodium borohydride, $NaBH_4$ (0.005 g, 0.10 mmol) in methanol were then added to the mixture and stirred further for 2 h at RT. The resulting mixture was concentrated in vacuo, extracted with ethyl acetate and purified by flash chromatography (dichloromethane/ methanol, 10:1, v/v) to afford **84b** (0.17 g, 70 %).

1H NMR (500 MHz, MeOD): δ = 7.92 – 7.78 (m, 4H), δ 5.17 (d, J = 8.0 Hz, 1H, H-1), 4.33 (dd, J = 10.5, 4.9 Hz, 1H), 3.90 (dd, J = 10.5, 3.9 Hz, 1H), 3.61 – 3.42 (m, 7H), 3.38 (t, J = 6.7 Hz, 4H), 3.26 (dd, J = 10.0, 8.7 Hz, 1H), 3.15 – 3.09 (m, 1H), 2.94 (dd, J = 14.2, 2.4 Hz, 1H), 2.68 – 2.55 (m, 5H), 1.83 – 1.68 (m, 3H), 1.61 – 1.51 (m, 2H), 1.39 – 1.27 (m, 26H), 0.89 (t, J = 6.8 Hz, 3H). ESI-MS: m/z $[M + Na]^+$ calc'd for $C_{37}H_{61}N_5O_8Na^+$: 726.44, found: 726.5

Compound 85a (n = 11): A solution of **84a** (0.45 g, 0.53 mmol) in butanol (6.0 ml) was treated with ethylenediamine (6.0 ml) and stirred at 90 °C for 3 h. The mixture was concentrated under high vacuo and purified by flash chromatography (dichloromethane/ methanol, 3:1, v/v) to give **85a** (0.26 g, 68%). ^{1}H NMR (500 MHz, MeOD): δ = 4.35 (d, J = 8.0 Hz, 1H, H-1), 3.93 (dd, J = 10.6, 5.0 Hz, 1H), 3.75 (dd, 1H), 3.63 – 3.47 (m, 4H), 3.48 – 3.43 (m, 4H), 3.48 – 3.24 (m, 7H), 3.23 – 3.11 (m, 1H), 3.06 (dd, J = 13.1, 9.0 Hz, 1H), 3.01 – 2.91 (m, 1H), 2.63 (dd, J = 10.1, 8.0 Hz, 1H), 1.73 – 1.63 (m, 1H), 1.63 – 1.51 (m, 4H), 1.42 – 1.26 (m, 42H), 0.89 (t, J = 6.8 Hz, 3H). ESI-MS: m/z $[M + H]^+$ calc'd for $C_{38}H_{77}N_5O_6H^+$: 700.59, found: 700.5

Compound 85b (n = 2): A solution of **84b** (0.17 g, 0.24 mmol) in butanol (4.0 ml) was treated with ethylenediamine (4.0 ml) and stirred at 90 °C for 3 h. The mixture was concentrated under high vacuo and purified by flash chromatography (dichloromethane/ methanol, 3:1, v/v) to give **85b** (0.089 g, 65%). 1H NMR (500 MHz, MeOD): δ = 4.23 (d, J = 8.0 Hz, 1H, H-1), 3.90 (dd, J = 10.5, 4.9 Hz, 1H), 3.66 (dd, J = 10.5, 3.9 Hz, 1H), 3.61 – 3.42 (m, 7H), 3.38 (t, J = 6.7 Hz, 4H), 3.26 (dd, J = 10.0, 8.7 Hz, 1H), 3.15 – 3.09 (m, 1H), 2.94 (dd, J = 14.2, 2.4 Hz, 1H), 2.68 – 2.55 (m, 5H), 1.83 – 1.68 (m, 3H), 1.61 – 1.51 (m, 2H), 1.39 – 1.27 (m, 26H), 0.89 (t, J = 6.8 Hz, 3H). ESI-MS: m/z $[M + Na]^+$ calc'd for $C_{29}H_{59}N_5O_6Na^+$: 596.44, found: 596.5

Compound 73: A solution of **85a** (0.07 g, 0.096 mmol) in methanol (4.0 ml) was treated with a catalytic amount of $Pd(OH)_2/C$ (10 % wt.) and stirred under H_2 gas atmosphere for 1 h. The resulting solution was filtered, concentrated in vacuo and

purified by reverse-phase C18 silica gel to give **73** (0.048g, 72 %). ^{1}H NMR (500 MHz, MeOD): δ = 4.35 (d, J = 7.1 Hz, 1H, H-1), 3.93 (dd, J = 10.5, 6.7 Hz, 1H), 3.75 – 3.64 (m, 1H), 3.63 – 3.47 (m, 4H), 3.47 – 3.42 (m, 4H), 3.41 – 3.23 (m, 7H), 3.23 – 3.11 (m, 2H), 3.06 (dd, J = 13.1, 9.0 Hz, 1H), 3.01 – 2.91 (m, 1H), 2.65 (dd, J = 9.6, 7.1 Hz, 1H, H-2), 1.73 – 1.63 (m, 1H), 1.63 – 1.51 (m, 4H), 1.42 – 1.26 (m, 41H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, MeOD): δ = 103.11 (C-1), 78.97, 75.98, 73.75, 72.95, 71.21, 69.81, 68.27, 56.79, 56.67, 55.66, 54.70, 53.39, 40.24, 38.62, 35.53, 31.68, 30.23, 29.80, 29.39, 29.36, 29.33, 29.25, 29.22, 29.08, 29.06, 27.26, 26.53, 26.38, 25.88, 22.34, 13.07. HRMS: m/z $[M + Na]^+$ calc'd for $C_{38}H_{79}N_3O_6Na^+$: 696.5867, found: 696.580

Compound 74: A solution of **85b** (0.082 g, 0.14 mmol) in methanol (4.0 ml) was treated with a catalytic amount of $Pd(OH)_2/C$ (10 % wt.) and stirred under H_2 gas atmosphere for 1 h. The resulting solution was filtered, concentrated in *vacuo* and purified by reverse-phase C18 silica gel to give **74** (0.054g, 69 %). ^{1}H NMR (500 MHz, MetOD): δ = 4.23 (d, *J* = 8.0 Hz, 1H, H-1), 3.90 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.66 (dd, *J* = 10.5, 3.9 Hz, 1H), 3.61 – 3.42 (m, 7H), 3.38 (t, *J* = 6.7 Hz, 4H), 3.26 (dd, *J* = 10.0, 8.7 Hz, 1H), 3.15 – 3.09 (m, 1H), 2.94 (dd, *J* = 14.2, 2.4 Hz, 1H), 2.68 – 2.55 (m, 5H), 1.83 – 1.68 (m, 3H), 1.61 – 1.51 (m, 2H), 1.39 – 1.27 (m, 26H), 0.89 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (126 MHz, MeOD): δ = 102.52, 78.97, 75.66, 74.45, 72.63, 71.24, 69.83, 68.35, 56.70, 54.70, 51.44, 49.10, 31.66, 29.37, 29.34, 29.26, 29.19, 29.06, 26.26, 25.83, 22.32, 13.04. HRMS: *m/z* $[M + K]^+$ calc'd for $C_{29}H_{61}N_3O_6Na^+$: 571.4458, found: 571.42

Compound 86a (n = 11)**:** A solution of **32** (0.20 g, 0.39 mmol) in dry DCM was treated with **89a** (n = 11) (0.088 g, 0.39 mmol) and stirred overnight at 0 °C to RT. Two drops of acetic acid and sodium borohydride, $NaBH_4$ (0.045 g, 1.161 mmol) in methanol were then added to the mixture and stirred further for 2 h at RT. The resulting mixture was concentrated in vacuo, extracted with ethyl acetate and purified by flash chromatography (dichloromethane/ methanol, 7:1, v/v) to afford **86a** (0.20 g,

69 %). ^{1}H NMR (300 MHz, MeOD): δ = 4.60 (d, J = 8.1 Hz, 1H, H-1), 3.98 (dd, J = 8.9, 4.2 Hz, 1H), 3.74 (dd, J = 10.5, 3.8 Hz, 1H), 3.64 – 3.38 (m, 11H), 3.36 – 3.24 (m, 4H), 3.18 – 3.04 (m, 1H), 3.02 – 2.90 (m, 1H), 2.69 (dd, J = 10.2, 8.1 Hz, 1H, H-2), 1.72 – 1.50 (m, 6H), 1.48 – 1.28 (m, 42H), 0.88 (t, J = 6.9 Hz, 3H). ESM-MS: m/z [M + H]$^+$ calc'd for $C_{38}H_{75}N_7O_6H^+$: 726.58, found: 726.7

Compound 86b (n =11)**:** A solution of **32** (0.15 g, 0.29 mmol) in dry DCM was treated with **89b** (n = 2) (0.03 g, 0.30 mmol) and stirred overnight at 0 °C to RT. Two drops of acetic acid and sodium borohydride, $NaBH_4$ (0.041 g, 0.91 mmol) in methanol were then added to the mixture and stirred further for 2 h at RT. The resulting mixture was concentrated in vacuo, extracted with ethyl acetate and purified by flash chromatography (dichloromethane/ methanol, 7:1, v/v) to afford **86b** (0.11 g, 64 %). ^{1}H NMR (300 MHz, MeOD): δ = 4.43 (d, J = 8.1 Hz, 1H, H-1), 3.99 (dd, J = 10.6, 4.2 Hz, 1H), 3.89 (dd, J = 11.9, 2.0 Hz, 1H), 3.77 – 3.65 (m, 2H), 3.65 – 3.23 (m, 13H), 3.22 – 3.06 (m, 1H), 3.02 – 2.89 (m, 1H), 2.55 (dd, J = 10.4, 8.1 Hz, 1H, H-2) 1.90 – 1.77 (m, 2H), 1.65 – 1.52 (m, 2H), 1.45 – 1.23 (m, 26H), 0.91 (t, J = 6.9 Hz, 3H). ESI-MS: m/z [M + Na]$^+$ calc'd for $C_{29}H_{57}N_7O_6Na^+$: 622.43, found: 622.5

Compound 75: A solution of **86a** (0.20 g, 0.28 mmol) in methanol (5.0 ml) was treated with a catalytic amount of $Pd(OH)_2/C$ (10 % wt.) and stirred under H_2 gas atmosphere for 1 h. The resulting solution was filtered, concentrated in vacuo and purified by reverse-phase C18 silica gel to give **75** (0.14 g, 76 %). ^{1}H NMR (300 MHz, MeOD): δ = 4.60 (d, J = 8.1 Hz, 1H, H-1), 3.98 (dd, J = 8.9, 4.2 Hz, 1H), 3.74 (dd, J = 10.5, 3.8 Hz, 1H), 3.64 – 3.38 (m, 11H), 3.36 – 3.24 (m, 4H), 3.18 – 3.04 (m, 1H), 3.02 – 2.90 (m, 1H), 2.69 (dd, J = 10.2, 8.1 Hz, 1H, H-2), 1.72 – 1.50 (m, 6H), 1.48 – 1.28 (m, 42H), 0.88 (t, J = 6.9 Hz, 3H); 13C NMR (75 MHz, MeOD) δ = 102.71 (C-1), 80.40, 77.38, 74.55, 72.88, 72.76, 71.54, 69.35, 63.97, 58.21, 52.67, 52.52, 33.16, 30.90, 30.86, 30.76, 30.71, 30.57, 30.38, 30.02, 29.26, 28.10, 27.93, 27.33, 23.83, 14.60. HRMS: m/z [M + K]$^+$ calc'd for $C_{38}H_{79}N_3O_6K^+$: 712.5606, found: 712.559

Compound 76: A solution of **86b** (0.11 g, 0.18 mmol) in methanol (5.0 ml) was treated with a catalytic amount of $Pd(OH)_2/C$ (10 % wt.) and stirred under H_2 gas atmosphere for 1 h. The resulting solution was filtered, concentrated in vacuo and purified by reverse-phase C18 silica gel to give **76** (0.072 g, 72 %). 1H NMR (300 MHz, MeOD): δ = 4.43 (d, J = 8.1 Hz, 1H, H-1), 3.99 (dd, J = 10.6, 4.2 Hz, 1H), 3.89 (dd, J = 11.9, 2.0 Hz, 1H), 3.77 – 3.65 (m, 2H), 3.65 – 3.23 (m, 13H), 3.22 – 3.06 (m, 1H), 3.02 – 2.89 (m, 1H), 2.55 (dd, J = 10.4, 8.1 Hz, 1H, H-2) 1.90 – 1.77 (m, 2H), 1.65 – 1.52 (m, 2H), 1.45 – 1.23 (m, 26H), 0.91 (t, J = 6.9 Hz, 3H); 13C NMR (75 MHz, MeOD): δ = 104.10 (C-1), 80.58, 78.18, 75.72, 72.70, 72.00, 71.52, 69.65, 64.36, 62.66, 58.18, 33.10, 30.80, 30.77, 30.62, 30.49, 29.35, 27.26, 23.76, 14.47. HRMS: m/z $[M + Na]^+$ calc'd for $C_{29}H_{61}N_3O_6Na^+$: 571.4458, found: 571.442

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol (70)

^1H NMR (500 MHz, Methanol-d_4) δ 7.43 – 7.20 (m, 5H, aromatic proton), 4.78 (dd, J = 11.2 Hz, 2H, benzyl CH_2), 4.23 (d, J = 7.9 Hz, 1H, β- H_1), 3.93 – 3.98 (m, 1H, -**CH**-O-CH_3), 3.67 – 3.45 (m, 10H, -OCH_3, H_5), 3.36 – 3.11 (m, 2H), 2.95 (dd, J = 13.4, 2.8 Hz, 1H, H_{6b}), 2.72 – 2.55 (m, 2H, H_2, H_{6a}), 1.58 – 1.54 (m, 2H, -OCH_2**CH_2**-), 1.28 (broad s, 26H, lipid tail), 0.89 (t, J = 6.8 Hz, 3H, terminal lipid CH_3).^{13}C NMR (126 MHz, MeOD) δ 138.43, 127.95, 127.38, 103.65, 79.45, 79.33, 76.61, 75.73, 74.24, 71.24, 69.66, 68.81, 57.37, 56.69, 42.42, 31.65, 29.36, 29.33, 29.15, 29.05, 25.80, 22.31, 13.01.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-L-glucopyranosyl)-sn-glycerol (71)

^1H NMR (500 MHz, Methanol-d_4) δ 4.77 (d, J = 3.7 Hz, 1H, α-H_1), 3.83 – 3.73 (m, 1H, -**CH**-O-CH_3), 3.61 – 3.39 (m, 11H), 3.14 (dd, J = 9.8, 8.8 Hz, 1H, H_3), 2.97 (dd, J = 13.4, 3.1 Hz, 1H, H_{6a}), 2.71 (dd, J = 13.4, 7.1 Hz, 1H, H_{6b}), 2.56 (dd, J = 9.9, 3.7 Hz,

1H, H_2), 1.63 – 1.49 (m, 2H, -OCH_2**CH_2**-), 1.29 (broad s, 26H, lipid tail), 0.89 (t, *J* = 6.9 Hz, 3H, terminal lipid CH_3).^{13}C NMR (126 MHz, MeOD) δ 99.09, 79.03, 74.76, 72.54, 72.06, 71.22, 69.85, 66.54, 56.82, 55.91, 42.38, 31.64, 29.34, 29.32, 29.03, 25.83, 22.30, 12.99.

1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol (72)

^{1}H NMR (300 MHz, Methanol-d_4) δ 7.41 – 7.07 (m, 5H, aromatic proton), 4.85 (d, *J* = 11.3, 1H, benzyl CH_2), 4.68 (d, *J* = 3.6, 1H, α-H_1), 4.55 (dd, *J* = 11.3, 1H, benzyl CH_2), 3.72 – 3.60 (m, 1H, -**CH**-O-CH_3), 3.58 – 3.28 (m, 12H), 3.07 (dd, *J* = 10.0, 1.5 Hz, 1H, H_{6b}), 2.59 – 2.46 (m, 2H, , H_2, H_{6a}), 1.55 – 1.36 (m, 2H, -OCH_2**CH_2**), 1.19 (broad s, , 26H), 0.80 (t, *J* = 6.7, 3H, terminal lipid CH_3). ^{13}C NMR (75 MHz, MeOD) δ 139.99, 129.31, 128.82, 100.36, 80.83, 80.40, 76.80, 75.66, 73.08, 72.71, 71.14, 67.78, 58.27, 57.68, 43.72, 33.12, 30.83, 30.63, 30.52, 27.29, 23.78, 14.51.

4.3. Biological methods

4.3.1. Effect of GAELs on viability of epithelial cancer cell lines

The cell lines were cultured from frozen stocks originally obtained from ATCC. MDA-MB-231, JIMT-1, DU145, U81, U251 cells were grown in DMEM medium. BT474, A2780s, A2780cp cells were grown in DMEM/F12 medium supplemented with 10% FBS. MiaPaCa2 was cultured in DMEM supplemented with 10% FBS and 2.5% horse serum. PC3 cells were cultured in F12K medium supplemented with 10% FBS. All the media were supplemented with penicillin/streptomycin.

The effects of the GAELs on the viability of the various epithelial cancer cell lines was determined as previously described. Briefly, equal numbers of the cells were dispersed into 96-well plates. After 24 h, the cells were incubated with the compounds (0-30 μM) for 48 h. At the end of the incubation, MTS reagent (20% vol/vol) was added and the plates were incubated for 1-4 h. The OD_{490} was read with a plate reader. Wells with media but no cells were treated in similar fashion and the

values utilized as blank. The results represent the mean ± standard deviation of 6 independent determinations.

4.3.1.2. **Isolation of primary epithelial ovarian cancer cells (EOC) from ascites fluid of ovarian cancer patients.** The isolation of EOC cells from the ascites fluid of ovarian cancer patients was performed as described by Shepherd et al 2007, Nature protocols 1, 2643-2649). The cells were grown in DMEM/F12 supplemented with 10% FBS medium.

4.3.2. Isolation of breast cancer stem cells from BT-474, prostate cancer stem cells from DU145, and ovarian cancer stem cells from A2780cp cell lines and determination of the effect of GAELs on the viability of the cancer stem cells.

A population enriched in BT474 breast cancer stem cells or DU145 prostate cancer stem cells or A2780 ovarian cancer stem cells was obtained by staining the cells for aldehyde dehydrogenase using the Aldefluor assay kit from Stem Cell Technologies (Vancouver, BC, Canada) according to the instruction of the manufacturer with the appropriate controls. The stained cells were sorted from the bulk population by flow cytometry on a 4 laser MoFloXPP high speed/pressure cell sorter. The cells were pelleted by centrifugation. BT474 cells were resuspended into ultra-low adhesion plates in mammocult medium. The DU145 stem cells were resuspended in their growth medium (DMEM/F12 medium supplemented with 20 ng/ml EGF, and 10 ng/ml basic FGF, 5 µg/ml insulin, 0.4% BSA, with 1% antibiotics; (Salvatori et al 2012, PLos One 7(2) e31467.doi:10.1371/journal.pone.0031476), The dishes were incubated at 37 C in a CO_2 incubator for 4-6 days for spheroid formation.

The spheres are separated from single cells with a 40 µm nylon cell strainer. The spheres retained in the strainer were washed with PBS and trypsinised to obtain single cells. The cell numbers were counted with a Coulter ZM counter and the cells were dispersed into 48-well low adhesion plates (Grenier) in a volume of 500 ul. The cells were incubated for 4-6 days to allow for formation of spheroids. Subsequently, the stock GAELs in ethanol were diluted to twice the final concentration in the media

and a volume of 500 µl was added to the wells. Wells with growth medium but no cells were treated as the wells with cells. After 6 days incubation, MTS reagent, (2% vol/vol) was added to each well and the plates were incubated for 1-4 hrs for formation of colour. The OD_{490} were read in a Molecular Device absorbance plate reader using the SpectroMax software.

4.3.3 Tolerability Studies

A total of 30 female Rag2 mice were individually weighed and administered compound **56** intravenously or orally according to individual body weight. The mice was monitored for behavioural changes, body weight for 14 days. All mice were sacrificed on day 15 and necropsy was performed.

The scope of the claims should not be limited by the preferred embodiments set forth in the examples, but should be given the broadest interpretation consistent with the description as a whole.

TABLE 1

Table 1. Cytotoxicity of compounds **1-10** and Gln **11** on a panel of human epithelial cancer cell lines: breast (BT474, JIMT1, MDA-MB-231), pancreas (MiaPaCa2) and prostrate (DU145, PC3). The CC_{50} value is defined as the concentration required to decrease cell viability by 50% relative to the untreated control, while the CC_{90} values is defined as the concentration required to decrease cell viability by 90% relative to untreated control. The values were obtained by estimating the drug concentration at 50% and 10% viability on the y-axis using line plots. NT – Not tested

A.

	CC_{50} values (μM)					
Drugs	MD 231	DU 145	JIMT1	MiaPaCa2	PC3	BT474
1	3.0	5.2	3.5	3.5	3.5	7.5
2	5.5	6.0	4.2	7.0	11	11.5
3	20.0	>30	>30	>30	15.0	NT
4	4.5	6.0	4.0	6.5	8	8.5
5	>30	>30	>30	>30	>30	>30
6	11.0	12.5	9.5	11.5	6.0	22
7	12.0	17.5	14.0	15.0	9.5	25
8	6.0	6.0	5.5	8.5	9	15.5
9	>30	>30	>30	>30	>30	>30
10	16	14	12.5	18	20	23
11 (Gln)	NT	10	9	9	13.5	8

B

	CC_{90} values (μM)					
Drugs	MD 231	DU 145	JIMT1	MiaPaCa2	PC3	BT474
1	4.5	7.4	4.9	6.5	6.0	9.5
2	7.0	8.5	6.5	12	14	14.0
3	>30	>30	>30	>30	30.0	NT
4	7.0	8.5	6.0	13	14	17.5
5	>30	>30	>30	>30	>30	>30
6	15.0	18	13.5	18.5	15.0	28
7	17.5	25.0	19.0	28.0	17.0	>30
8	9	9	7.5	14	14	28
9	>30	>30	>30	>30	>30	>30
10	19	19	15	29	29	29
11 (Gln)	NT	15	16	18	28	13

Table 2. Cytotoxicity of compounds **56**, L-Gln **69** and Gln **11** on a panel of human epithelial cancer cell lines: breast (BT474, JIMT1, MDA-MB-231), pancreas (MiaPaCa2) and prostrate (DU145, PC3). The CC_{50} value is defined as the concentration required to decrease cell viability by 50% relative to the untreated control, while the CC_{90} values is defined as the concentration required to decrease cell viability by 90% relative to untreated control. The values were obtained by estimating the drug concentration at 50% and 10% viability on the y-axis using line plots. NT – Not tested.

A

	CC_{50} values (μM)					
	MDA-MB-231	DU 145	JIMT1	MiaPaCa2	PC3	BT 474
56.	4.8	8.2	5.5	8.5	11.0	
69	11.0	12.5	6.5	7.5	12.5	
11	NT	10	9	9	13.5	8

B

	CC_{90} values (μM)					
	MDA-MB- 231	DU 145	JIMT1	MiaPaCa2	PC3	BT 474
56	6.5	12.5	6	12.5	14.0	
69	17	16	11	18	16	
11	NT	15	16	18	28	13

Table 3 . Clinical parameters of EOC samples from patient ascites.

Patient Ascites sample	Diagnosis	FIGO Stage	Treatment prior to ascites collection	Platinum resistance status at time Ascites sample collected
EOC216B	Adenocarcinoma	Not indicated	No surgery. No chemotherapy	Not clinically resistant
EOC216H	Adenocarcinoma	Not indicated	Carboplatin/Paclitaxel (3 cycles) Caelyx (1 cycle)	Clinically resistant to platinum-base chemotherapy
EOC258	High grade Serous	Not indicated	No surgery. No chemotherapy	Not clinically resistant

Table 4 . CC_{50} values for GAELs against primary ovarian cancer cells. Cytotoxicity of compounds **1, 56** and **69** on primary ovarian cancer cells isolated from ascites of ovarian cancer patients. The CC_{50} value is defined as the concentration required to decrease cell viability by 50% relative to the untreated control, while the CC_{90} values is defined as the concentration required to decrease cell viability by 90% relative to untreated control. The values were obtained by estimating the drug concentration at 50% and 10% viability on the y-axis using line plots. NT – Not tested

	CC_{50} (μM)						
	EOC 216H		EOC216B		EOC258		EOC260
	Adherent	Spheroid	Adherent	Spheroid	Adherent	Spheroid	Adherent
1	0.15	0.21	0.24	0.6	0.3	0.6	0.2
56	1.1	1.0	0.5	1.2	0.55	1.65	0.6
69	1.02	1.5	0.56	1.25	1.4	1.2	0.3

Table 5. Cytotoxicity of compounds **1, 56, 69, 70 - 72** on a panel of human epithelial cancer cell lines: breast (BT474, JIMT1, MDA-MB-231), pancreas (MiaPaCa2) and prostrate (DU145, PC3). The CC_{50} value is defined as the concentration required to decrease cell viability by 50% relative to the untreated control, while the CC_{90} values is defined as the concentration required to decrease cell viability by 90% relative to untreated control. The values were obtained by estimating the drug concentration at 50% and 10% viability on the y-axis using line plots. NT – Not tested

	JIMT1	MDA-MB-231	DU-145	Mlapaca2	PC3	BT474
			CC_{50}			
1	3.5	3.0	5.2	3.5	3.5	7.5
56	5.5	4.8	8.2	8.5	11.0	NT
69	6.5	11.0	12.5	7.5	12.5	NT
70	2.0	4.0	3.6	5.0	NT	NT
72	2.0	4.0	3.6	4.5	NT	NT
71	4.0	5.5	6.0	6.0	NT	NT
			CC_{90}			
1	4.9	4.5	7.4	6.5	6.0	NT
56	6	6.5	12.5	12.5	14	NT
69	11	17.0	16	18	16	NT
70	3.5	4.9	4.9	7.1	NT	NT
72	3.5	4.9	4.9	7.0	NT	NT
71	6.5	7.5	8.0	15.0	NT	NT

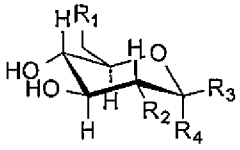
Table 6. Cytotoxicity of compounds 73-76 on a panel of human epithelial cancer cell lines: breast (BT474, JIMT1, MDA-MB-231), pancreas (MiaPaCa2) and prostrate (DU145, PC3). The CC50 value is defined as the concentration required to decrease cell viability by 50% relative to the untreated control, while the CC90 values is defined as the concentration required to decrease cell viability by 90% relative to untreated control. The values were obtained by estimating the drug concentration at 50% and 10% viability on the y-axis using line plots. NT – Not tested

	DU145		MB-MDA-231		JIMT1		MiaPaCa2		BT474		PC3	
CODE	***CC_{50}***	***CC_{90}***	***CC_{50}***	***CC_{90}***	***CC_{50}***	***CC_{90}***	***CC_{50}***	***CC_{90}***	***CC_{50}***	***CC_{90}***	***CC_{50}***	***CC_{90}***
73	**3.8**	**4.8**	**1.5**	**3.8**	**3.4**	**4.6**	**4.0**	**6.6**	**1.6**	**4.2**	**2.0**	**3.2**
74	**16.5**	**20.0**	**13.5**	**19.5**	**13.5**	**18.5**	**18.5**	**>20**	**>20**	**>20**	**12.5**	**20.0**
76	**18.5**	**>20**	**17.5**	**>20**	**12.5**	**16.5**	**14.5**	**>20**	**>20**	**>20**	**14.5**	**>20**
75	**7.5**	**9.5**	**5.5**	**9.5**	**8.0**	**9.5**	**8.5**	**12**	**13.5**	**16.5**	**8.5**	**11.5**

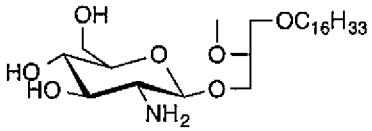
CLAIMS

1. A method of killing cancer stem cells and cancer stem cell spheroids or aggregates by administering an effective amount of a compound selected from the group of compounds of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) and formula (V')

I



1. R_1=NH_2, R_2=NH_2,R_3=H,R_4=a
2. R_1=NH_2, R_2=NH_2,R_3=a,R_4=H
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H



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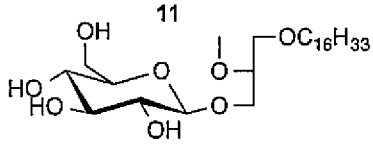

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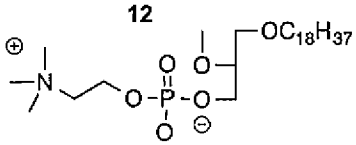

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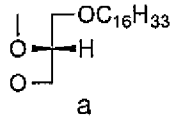

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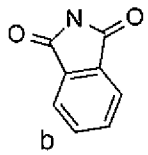

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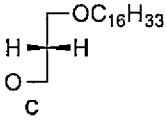

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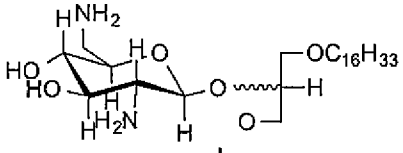

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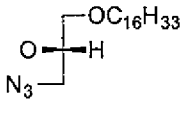

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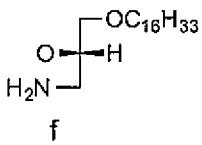

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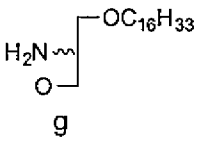

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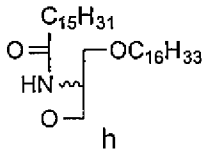

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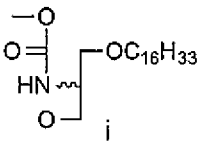
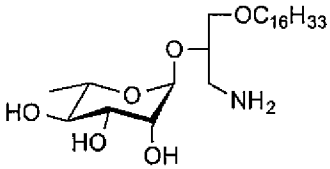


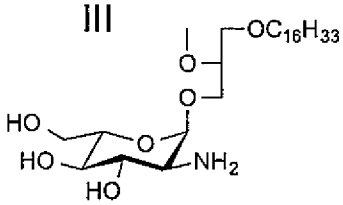
i

II



56

III



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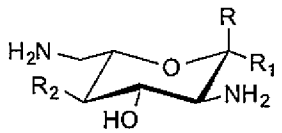
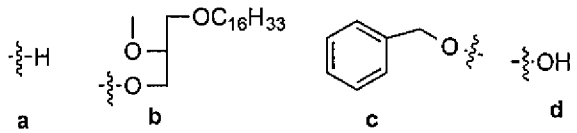
IV

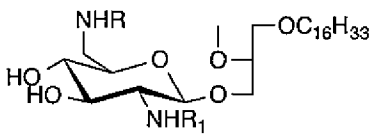




a b c d

70. R = a, R_1 = b, R_2 = c
71. R = b, R_1 = a, R_2 = d
72. R = b, R_1 = a, R_2 = c

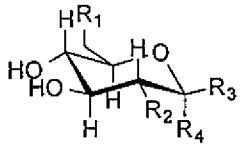
V



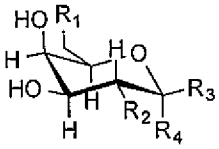
a b

73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

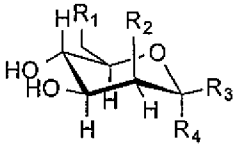
I'



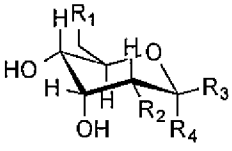
D-glucose



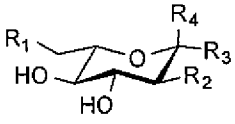
D-galactose



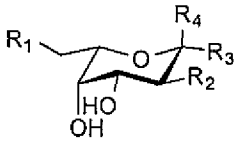
D-mannose



D-allose



L-glucose



L-galactose

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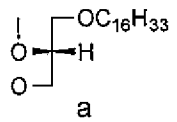
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H



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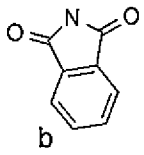

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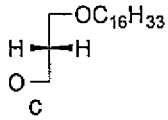

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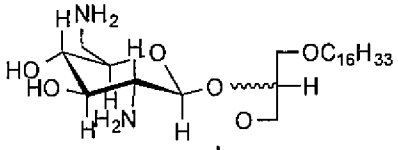

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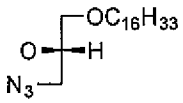

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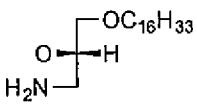

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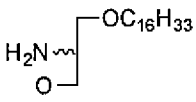

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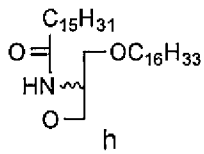

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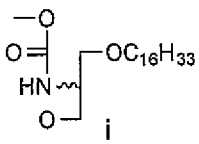
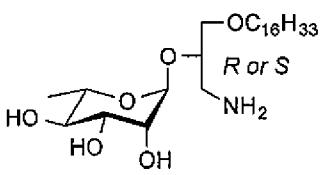


i

5

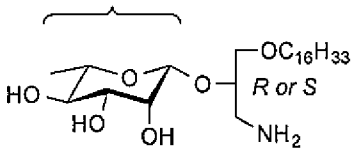
II'



α-L-Rhamnose

56

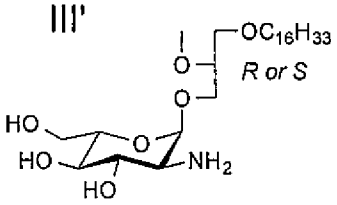
sugar



β-L-Rhamnose

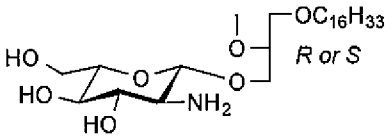
sugar = 6-deoxy-α-D-galactose, 6-deoxy-β-D-galactose, 6-deoxy-α-L-galactose, 6-deoxy-β-L-galactose, 6-deoxy-α-D-glucose, 6-deoxy-β-D-glucose, 6-deoxy-α-L-glucose-based, 6-deoxy-β-L-glucose, 6-deoxy-α-D-mannose, 6-deoxy-β-D-mannose, 6-deoxy-α-L-mannose, or 6-deoxy-β-L-mannose

III'

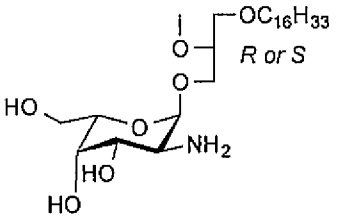


α-L-glucose

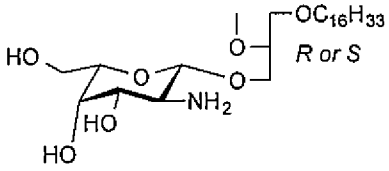
69



β-L-glucose

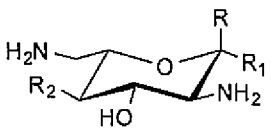


α-L-galactose

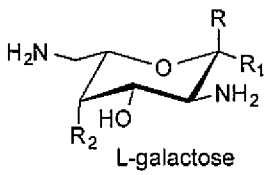


β-L-galactose

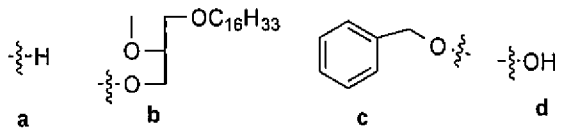
IV'



L-glucose



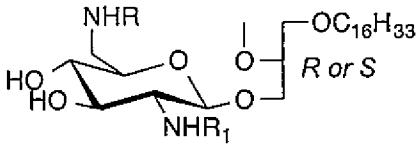
L-galactose



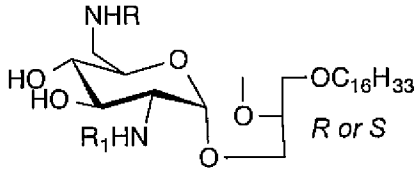
a **b** **c** **d**

70. R = a, R_1 = b, R_2 = c
71. R = b, R_1 = a, R_2 = d
72. R = b, R_1 = a, R_2 = c

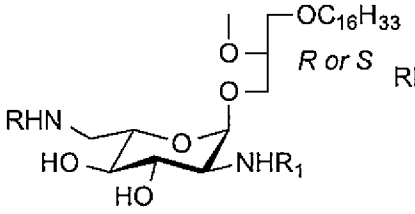
V'



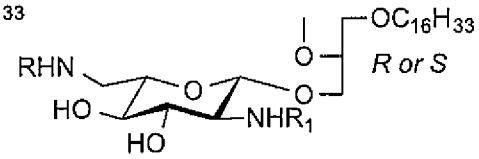
β-D-glucose



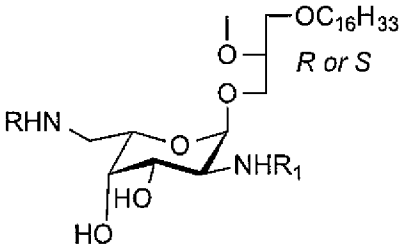
α-D-glucose



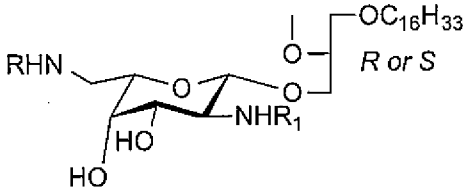
α-L-glucose



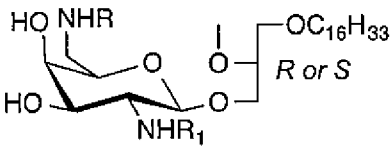
β-L-glucose



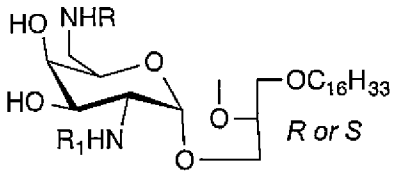
α-L-galactose



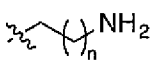
β-L-galactose



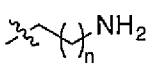
β-D-galactose



α-D-galactose



a



b

n = 1,2,3,....., 16

73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

2. A method of killing non-stem cancer cells by administering an effective amount of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V') as recited in claim 1.

3. A method of treating cancer in an individual in need of such treatment comprising administering to said individual an effective amount of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III') formula (IV), formula (IV'), formula (V) or formula (V') as recited in claim 1.

4. The method according to claim 3 wherein the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and the corresponding D-galactose-, D-allose, D-mannose analogs of formula (I'), the corresponding β-L-rhamnose analog of formula (II") the corresponding β-L-glucose, α-L-galactose or β-L-galactose analog of formula (III'), the corresponding L-galactose analog of formula (IV') or the corresponding α-D-glucose, α-L-glucose, β-L-glucose, α-L-galactose, β-L-galactose, β-D-galactose or α-D-galactose analog of formula (V').

5. The method according to claim 3 wherein the cancer is selected from the group consisting of pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, small cell lung cancer, liver cancer, colon cancer, and brain cancer.

6. The method according to claim 3 wherein the cancer is a recurring cancer or a resistant cancer.

7. The method according to claim 3 wherein the cancer is metastasized or advanced stage cancer.

8. The method according to claim 3 wherein the effective amount is between about 0.1 mg/Kg body weight to 15 mg/Kg body weight is delivered by the appropriate means into the patient (intravenously or orally or peritoneally or topically or a combination thereof).

9. Use of a compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V') in the manufacture of a medicament for treating cancer.

10. The use according to claim 9 wherein the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-O-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol, 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-

2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and the corresponding D-galactose-, D-allose, D-mannose analogs of formula (I'), the corresponding β-L-rhamnose analog of formula (II") the corresponding β-L-glucose, α-L-galactose or β-L-galactose analog of formula (III'), the corresponding L-galactose analog of formula (IV') or the corresponding α-D-glucose, α-L-glucose, β-L-glucose, α-L-galactose, β-L-galactose, β-D-galactose or α-D-galactose analog of formula (V').

11. The use according to claim 9 wherein the cancer is selected from the group consisting of pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, small cell lung cancer, liver cancer, colon cancer, skin cancer (melanoma) and brain cancer.

12. The use according to claim 9 wherein the cancer is a resistant cancer or a recurring cancer.

13. The use according to claim 9 wherein the cancer is metastasized or advanced stage cancer.

14. Use of an effective amount of compound of formula (I), formula (I'), formula (II), formula (II'), formula (III), formula (III'), formula (IV), formula (IV'), formula (V) or formula (V') as recited in claim 1, which kill cancer cells by a non-apoptotic mechanism in combination with a second agent having anticancer properties.

15. The use according to claim 14 wherein the second agent is selected from the group consisting of: a biological therapeutic; radiotherapy; hormonal therapy; a chemotherapeutic agent; an alkylating agent; an antibiotic; an antimetabolite; and a plant product.

16. The use according to claim 14 wherein the compound is selected from the group consisting of: 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–α-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-deoxy-3-O-(2'6'-diamino-2',6'-dideoxy–β-D-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyloxy-2S/R-amino-3-(-2'-amino-2'-deoxy-β-D- glucopyranosyl)-glycerol ;1-*O*-Hexadecyloxy-2R-(-α-L-rhamnopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyloxy-2R-*O*-methyl-(2'amino-2'deoxy-α-L-glucopyranosyl)-3-amino glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-

benzyl-2',6'-diamino-2',6'-dideoxy–β-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(2',6'-diamino-2',6'-dideoxy-α-L-glucopyranosyl)-sn-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-O-(4'-benzyl-2',6'-diamino-2',6'-dideoxy– α-L-glucopyranosyl)-sn-glycerol;1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[6'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol; 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(12-aminododecyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol, 1-*O*-Hexadecyl-2-*O*-methyl-3-*O*-[2'-*N*-(3-aminopropyl)-2',6'-diamino-2',6'-dideoxy-glucopyranoside]-*sn*-glycerol and the corresponding D-galactose-, D-allose, D-mannose analogs of formula (I'), the corresponding β-L-rhamnose analog of formula (II") the corresponding β-L-glucose, α-L-galactose or β-L-galactose analog of formula (III'), the corresponding L-galactose analog of formula (IV') or the corresponding α-D-glucose, α-L-glucose, β-L-glucose, α-L-galactose, β-L-galactose, β-D-galactose or α-D-galactose analog of formula (V').

17. The use according to claim 14 wherein the cancer is selected from the group consisting of pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, small cell lung cancer, liver cancer, colon cancer, liver cancer, skin cancer, melanoma and brain cancer.

18. The use according to claim 14 wherein the cancer is a drug-resistant or therapy-resistant cancer or a recurring cancer.

19. The method according to claim 14 wherein the cancer is metastasized or advanced stage cancer.

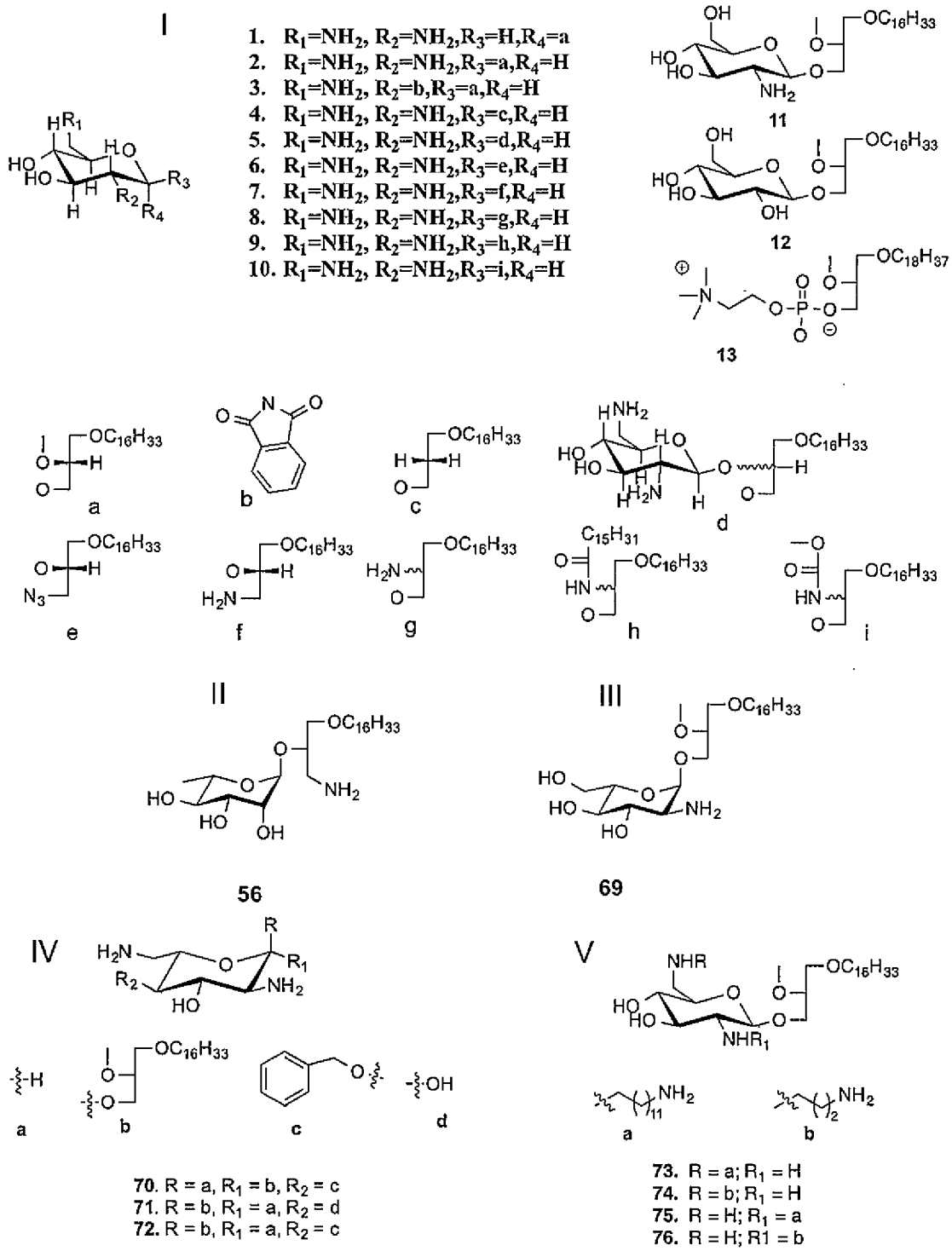
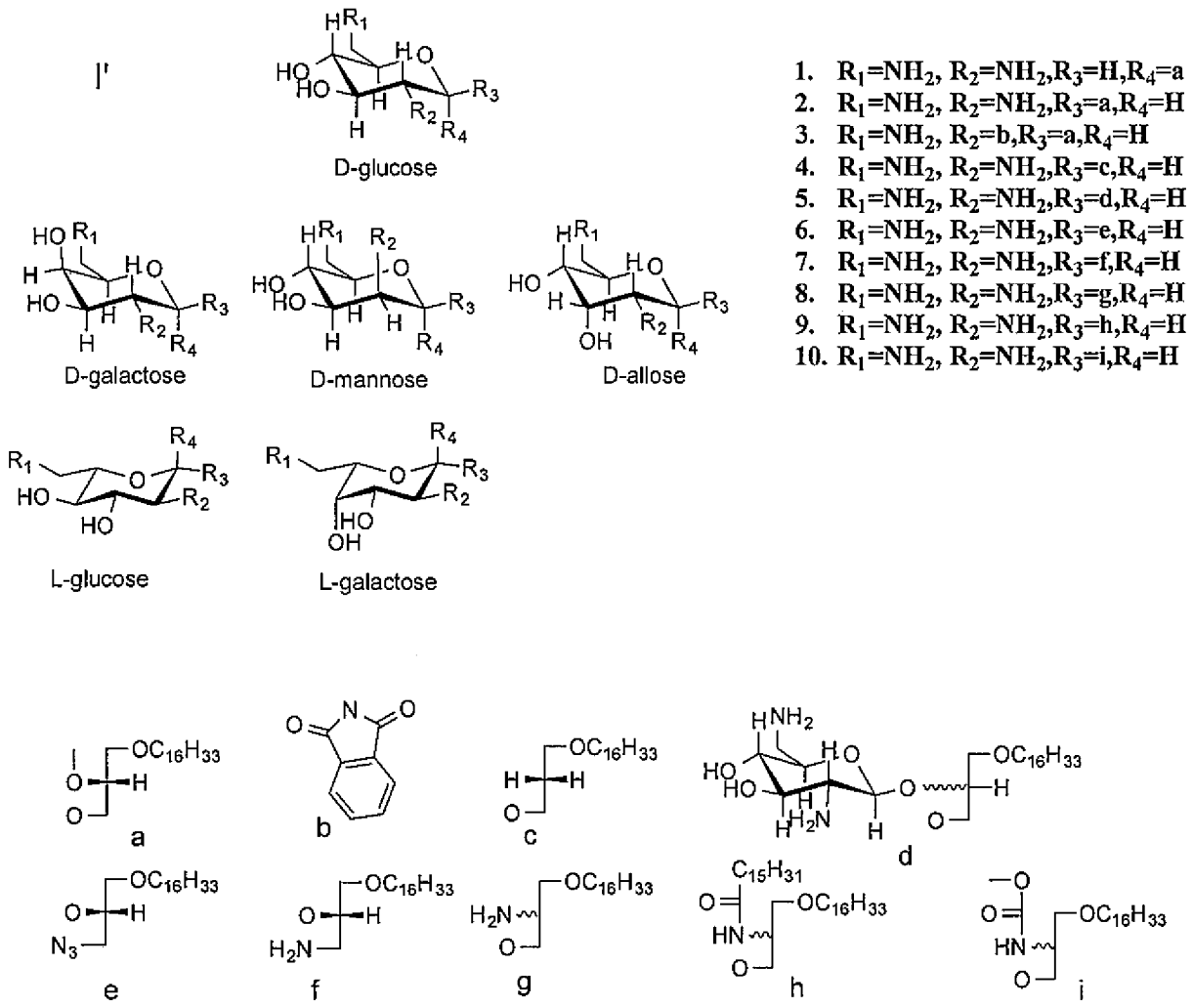

I
1. R1=NH2, R2=NH2,R3=H,R4=a
2. R1=NH2, R2=NH2,R3=a,R4=H
3. R1=NH2, R2=b,R3=a,R4=H
4. R1=NH2, R2=NH2,R3=c,R4=H
5. R1=NH2, R2=NH2,R3=d,R4=H
6. R1=NH2, R2=NH2,R3=e,R4=H
7. R1=NH2, R2=NH2,R3=f,R4=H
8. R1=NH2, R2=NH2,R3=g,R4=H
9. R1=NH2, R2=NH2,R3=h,R4=H
10. R1=NH2, R2=NH2,R3=i,R4=H
11
12
13
a
b
c
d
e
f
g
h
i
II
III
56
69
IV
V
70. R = a, R1 = b, R2 = c
71. R = b, R1 = a, R2 = d
72. R = b, R1 = a, R2 = c
73. R = a; R1 = H
74. R = b; R1 = H
75. R = H; R1 = a
76. R = H; R1 = b

Figure 1-1



1. R_1=NH_2, R_2=NH_2,R_3=H,R_4=a
2. R_1=NH_2, R_2=NH_2,R_3=a,R_4=H
3. R_1=NH_2, R_2=b,R_3=a,R_4=H
4. R_1=NH_2, R_2=NH_2,R_3=c,R_4=H
5. R_1=NH_2, R_2=NH_2,R_3=d,R_4=H
6. R_1=NH_2, R_2=NH_2,R_3=e,R_4=H
7. R_1=NH_2, R_2=NH_2,R_3=f,R_4=H
8. R_1=NH_2, R_2=NH_2,R_3=g,R_4=H
9. R_1=NH_2, R_2=NH_2,R_3=h,R_4=H
10. R_1=NH_2, R_2=NH_2,R_3=i,R_4=H

Figure 1-2

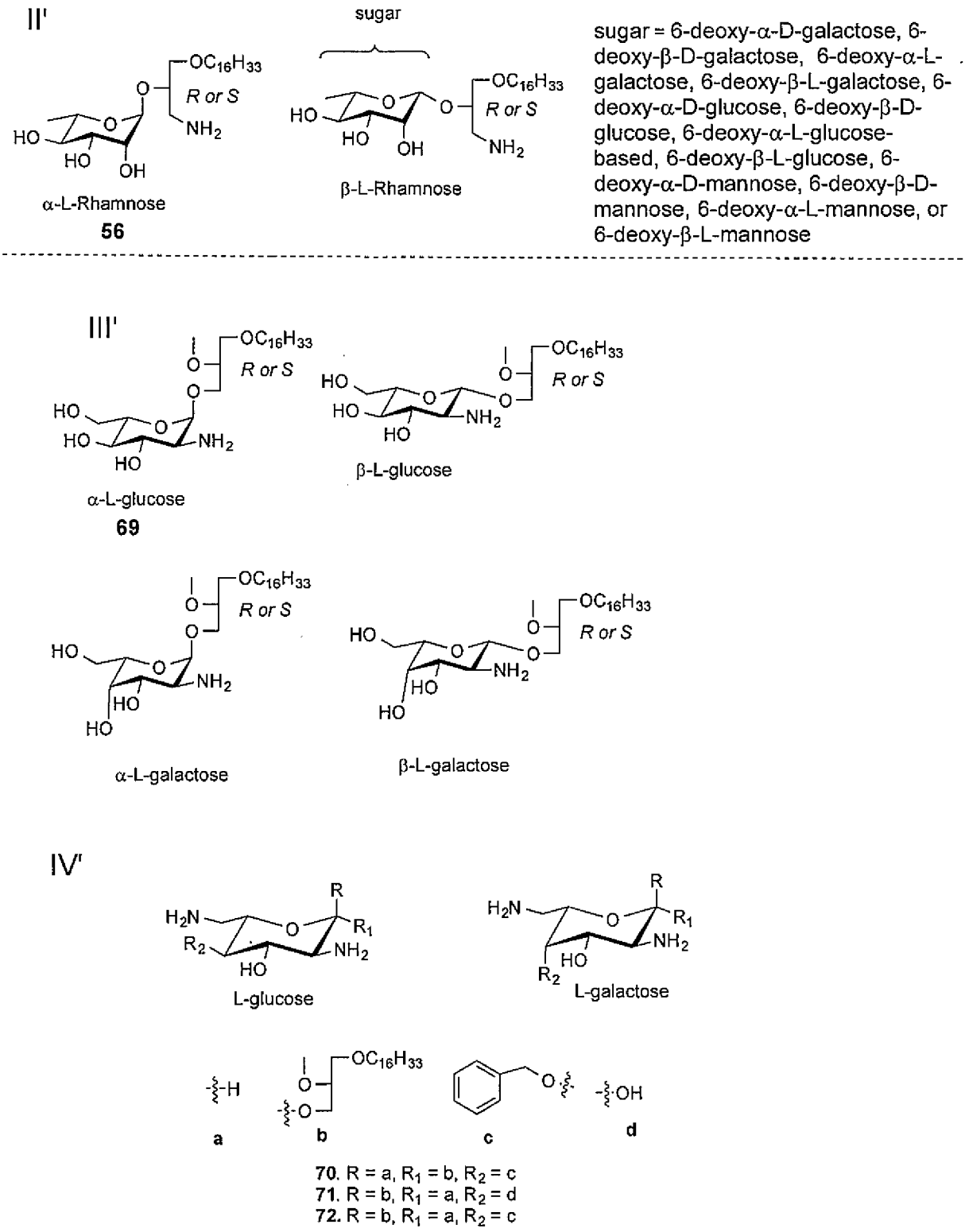
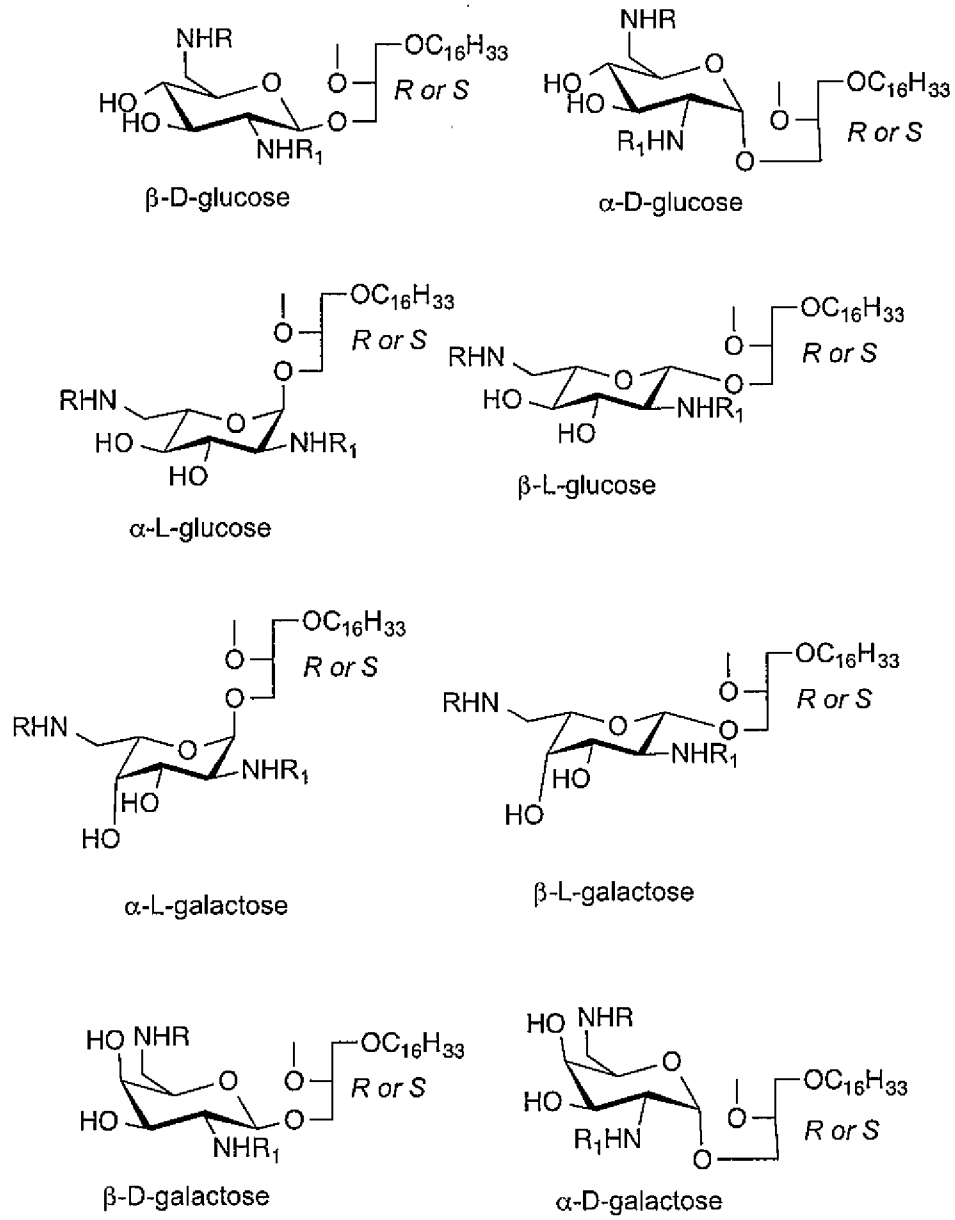

II'
sugar
α-L-Rhamnose
56
β-L-Rhamnose
sugar = 6-deoxy-α-D-galactose, 6-deoxy-β-D-galactose, 6-deoxy-α-L-galactose, 6-deoxy-β-L-galactose, 6-deoxy-α-D-glucose, 6-deoxy-β-D-glucose, 6-deoxy-α-L-glucose-based, 6-deoxy-β-L-glucose, 6-deoxy-α-D-mannose, 6-deoxy-β-D-mannose, 6-deoxy-α-L-mannose, or 6-deoxy-β-L-mannose
III'
α-L-glucose
69
β-L-glucose
α-L-galactose
β-L-galactose
IV'
L-glucose
L-galactose
a
b
c
d
70. R = a, R1 = b, R2 = c
71. R = b, R1 = a, R2 = d
72. R = b, R1 = a, R2 = c

Figure 1-3

V'



73. R = a; R_1 = H
74. R = b; R_1 = H
75. R = H; R_1 = a
76. R = H; R1 = b

Figure 1-4

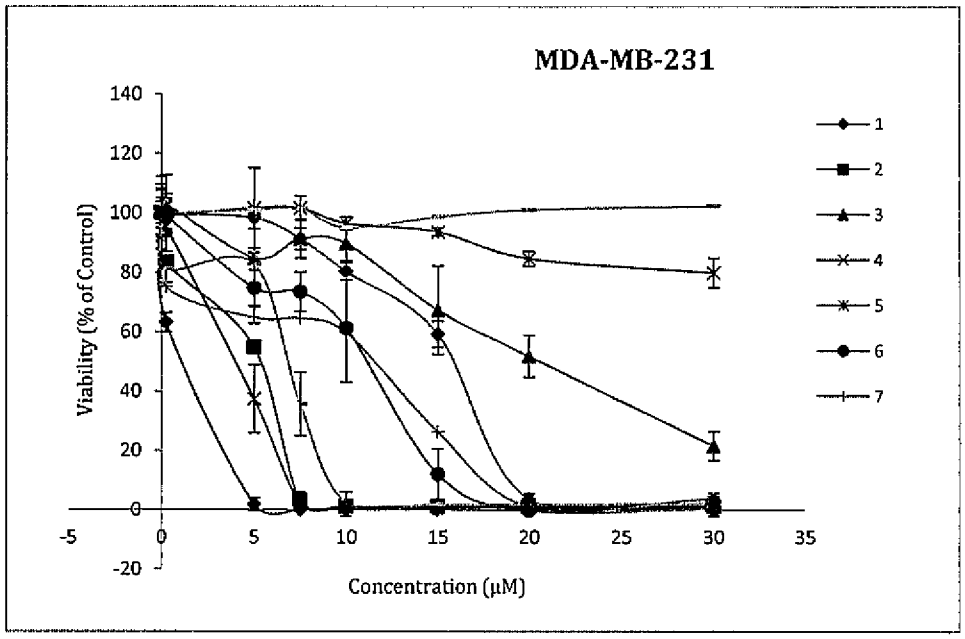


Figure 2A

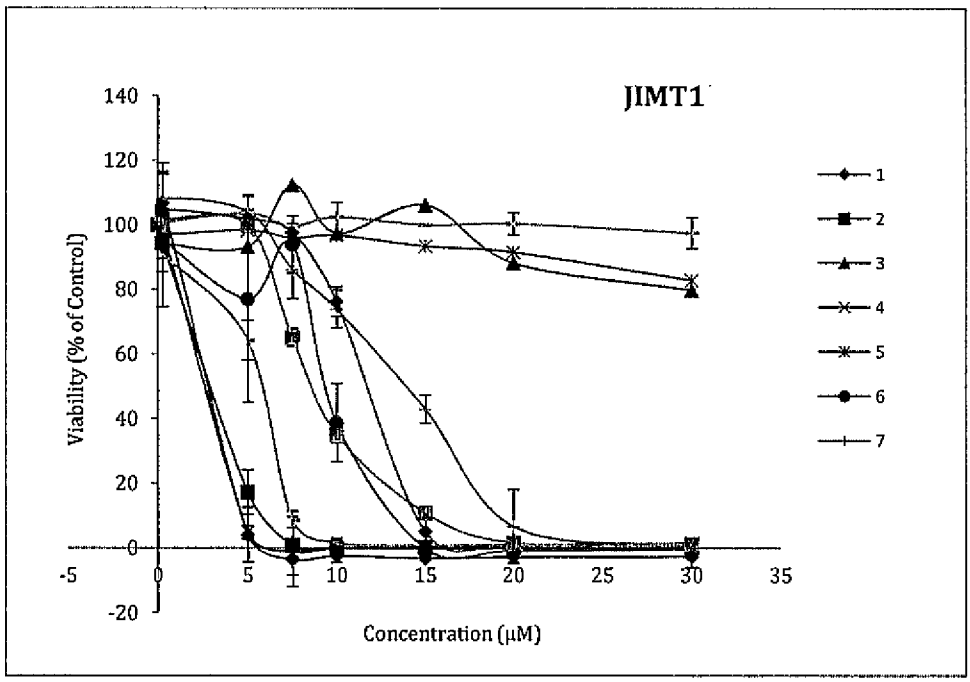


Figure 2B

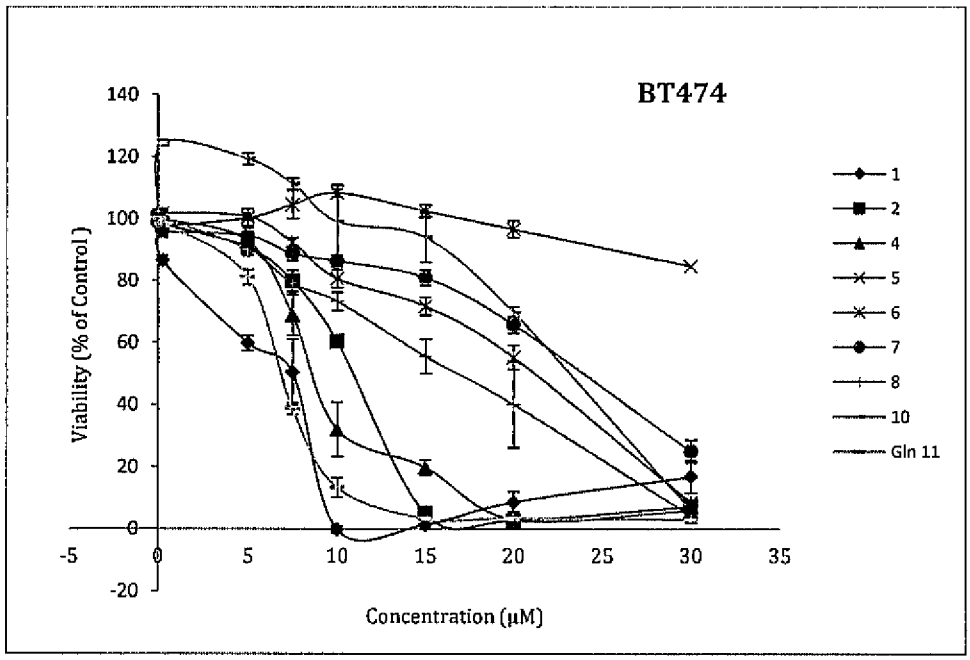


Figure 2C

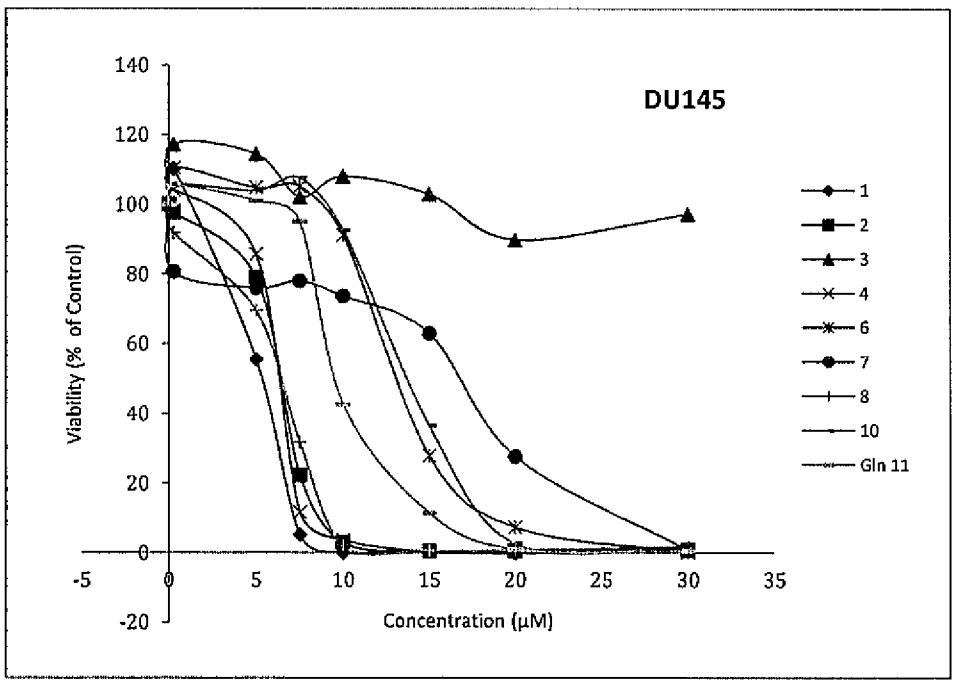


Figure 2D

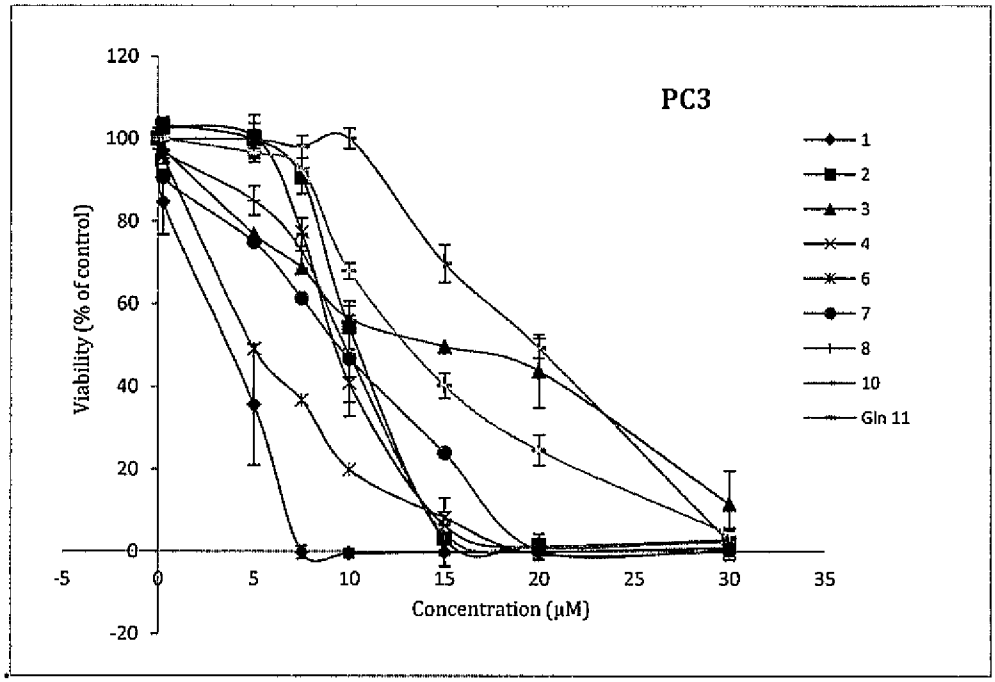


Figure 2E

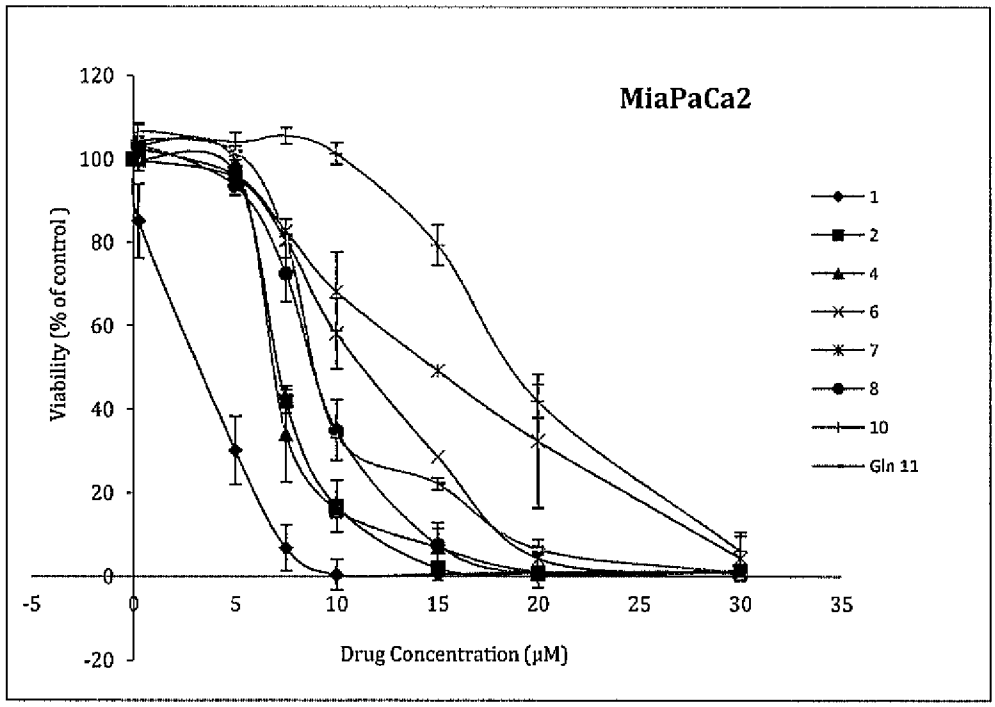


Figure 2F

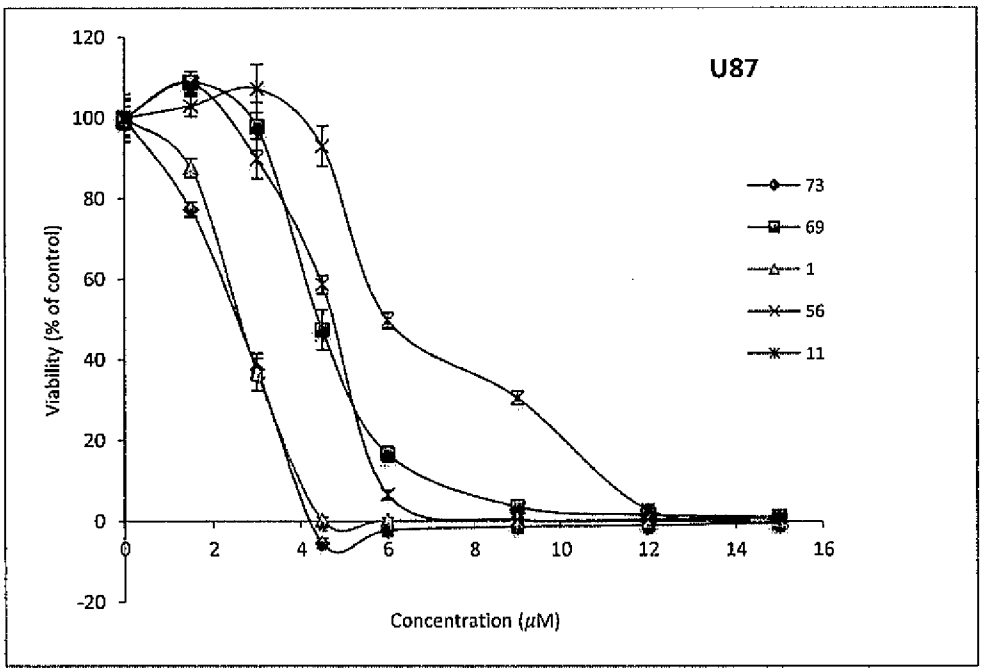


Figure 2G(i)

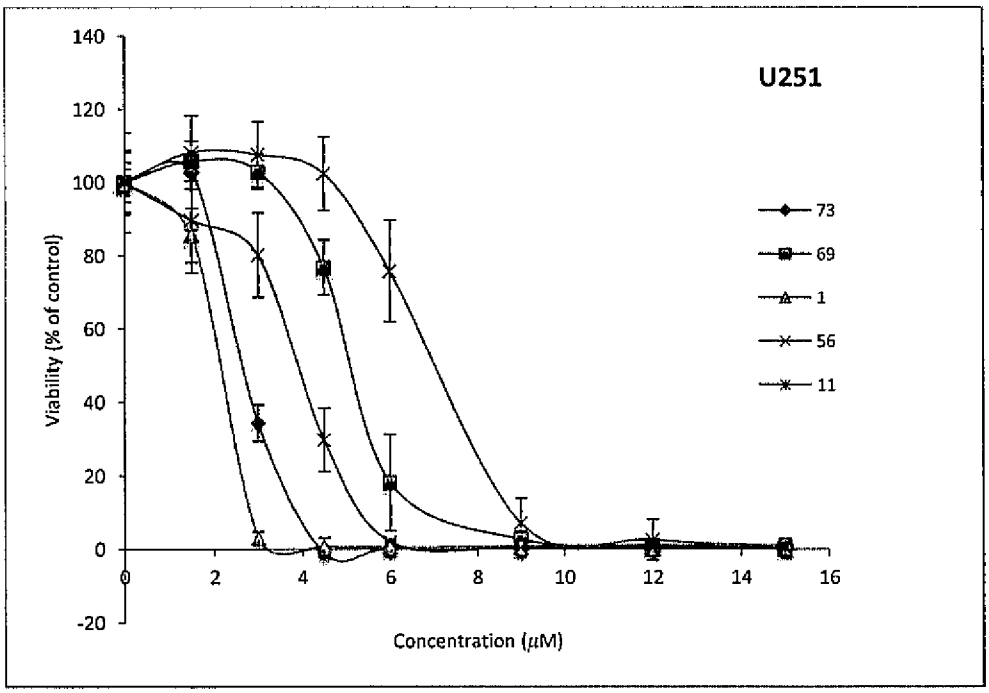


Figure 2G(ii)

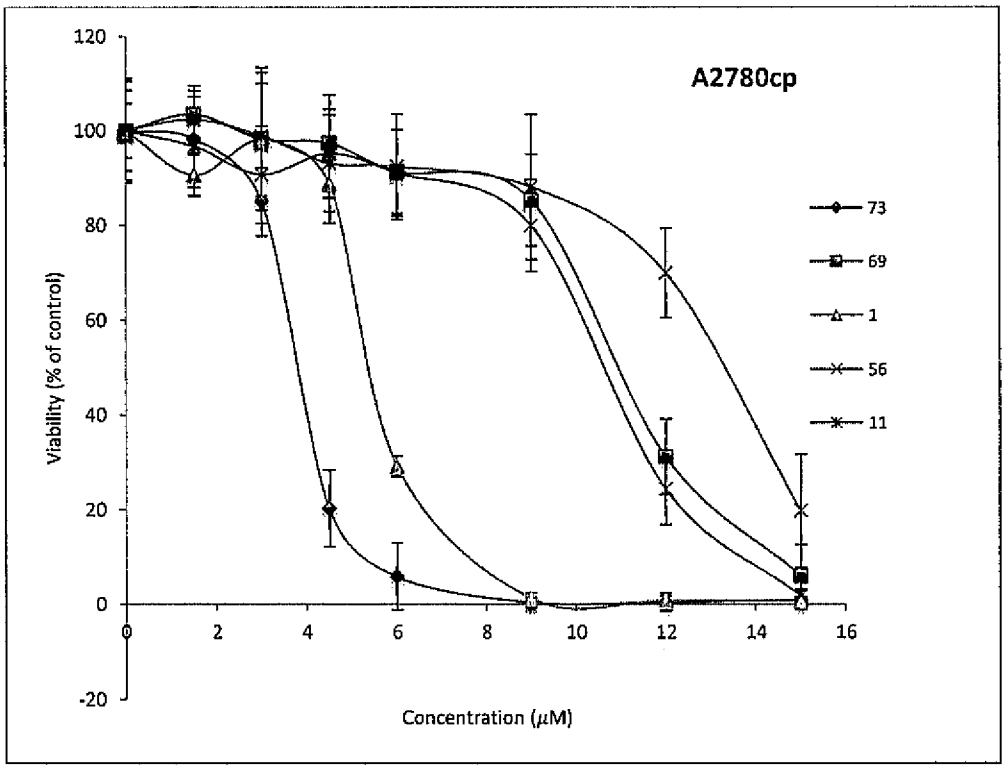


Figure 2G(iii)

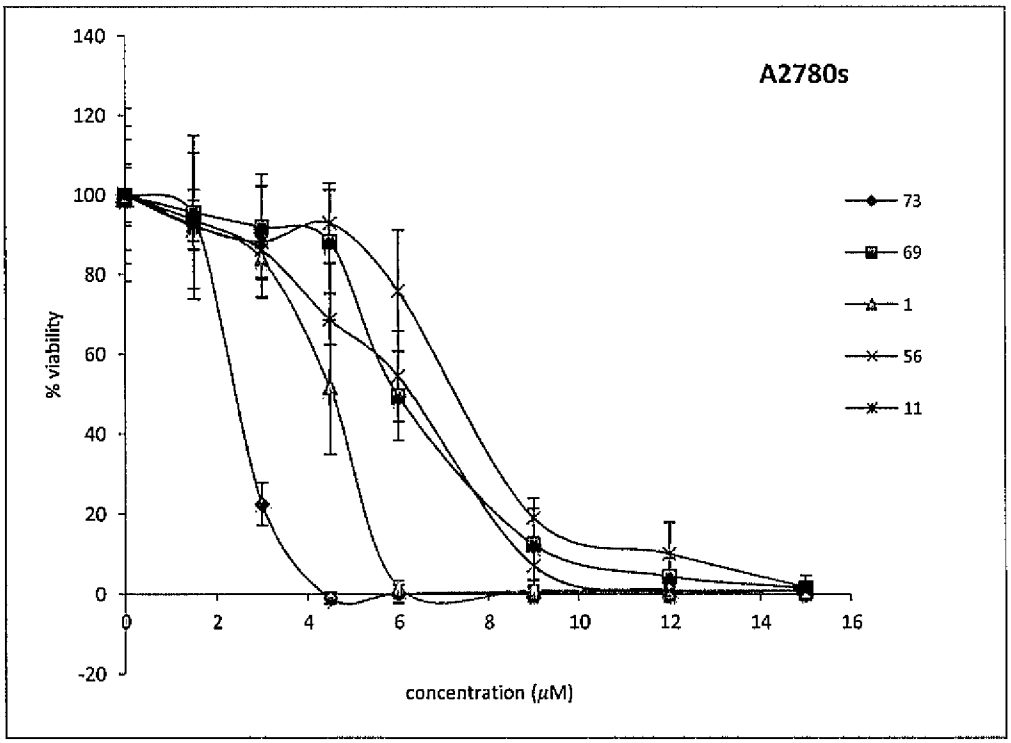


Figure 2G(iv)

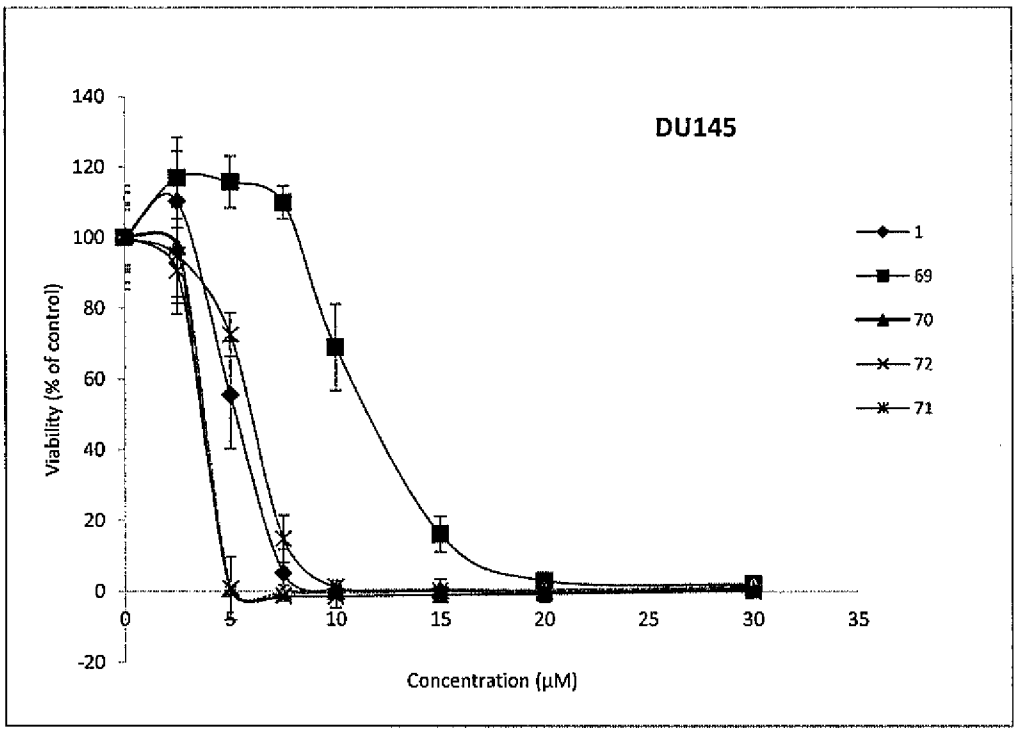


Figure 2H

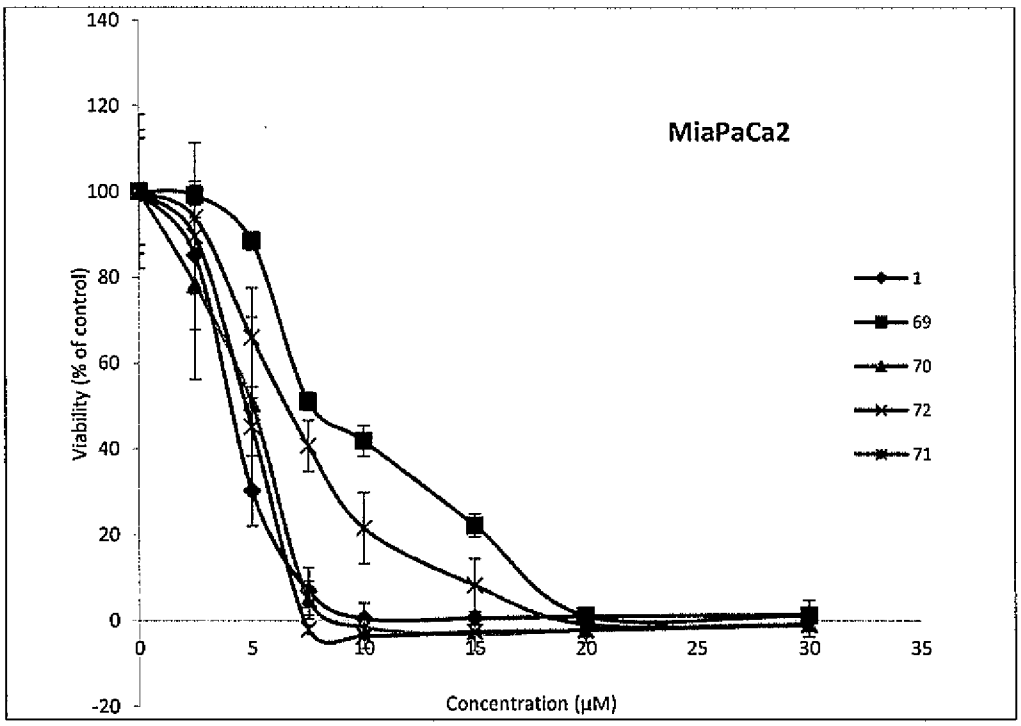


Figure 2I

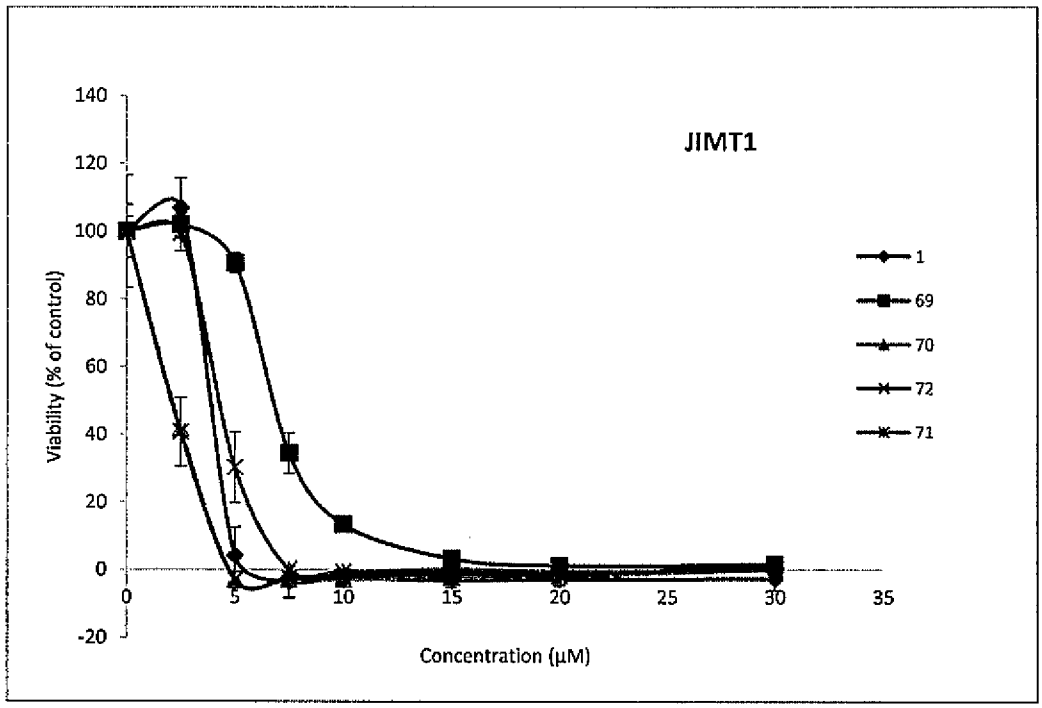


Figure 2J

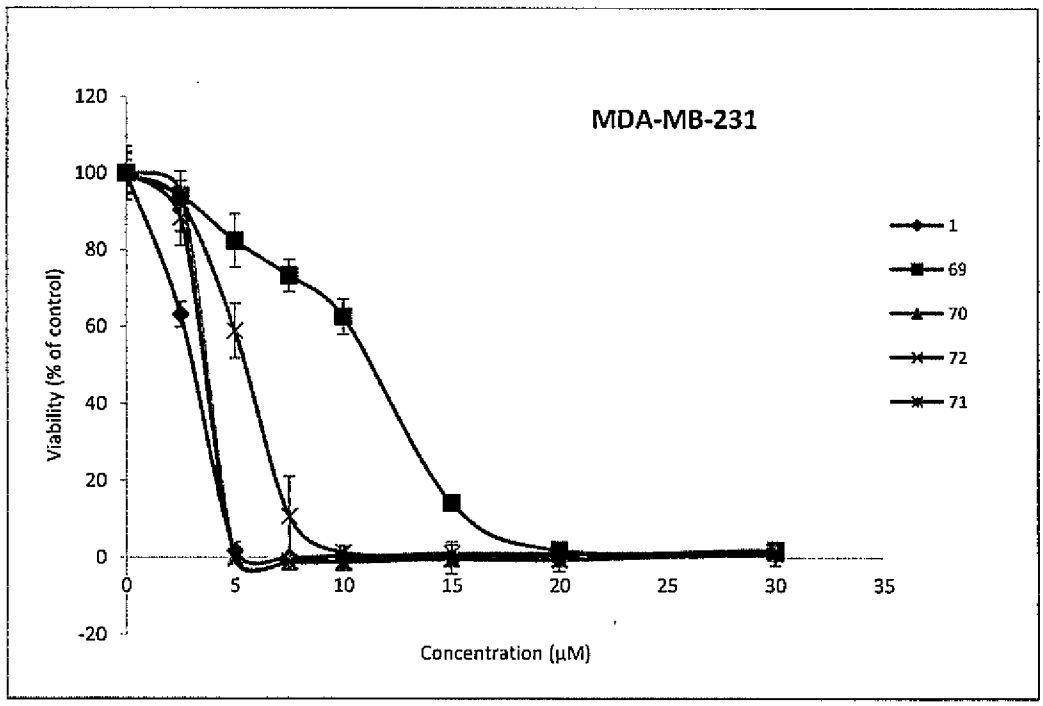


Figure 2K

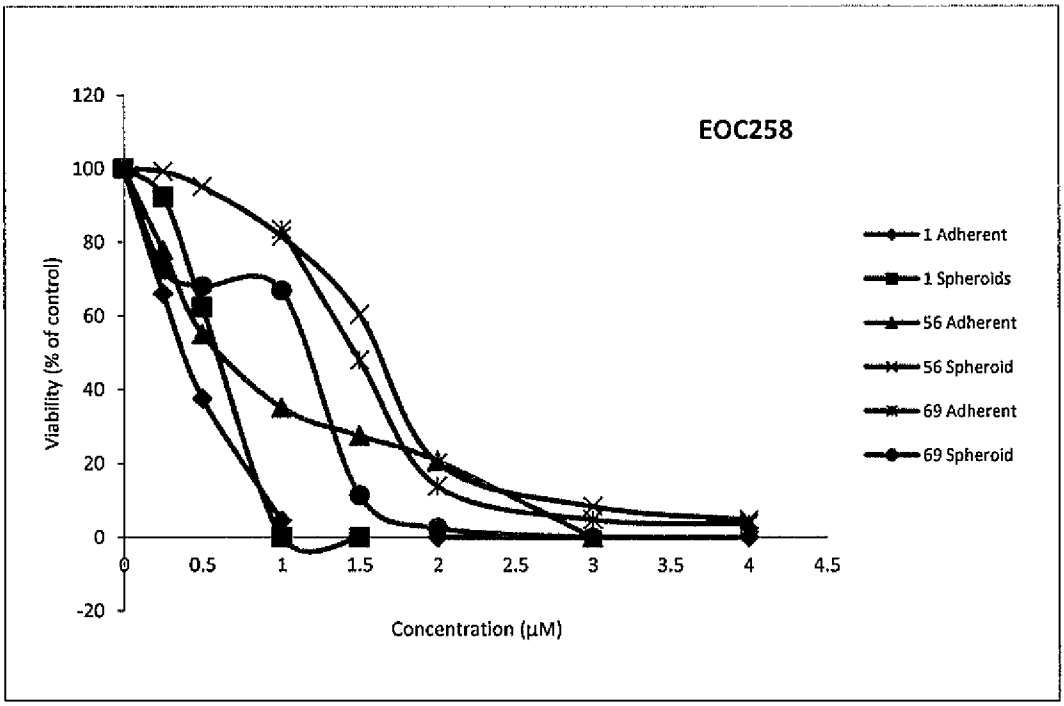


Figure 2L

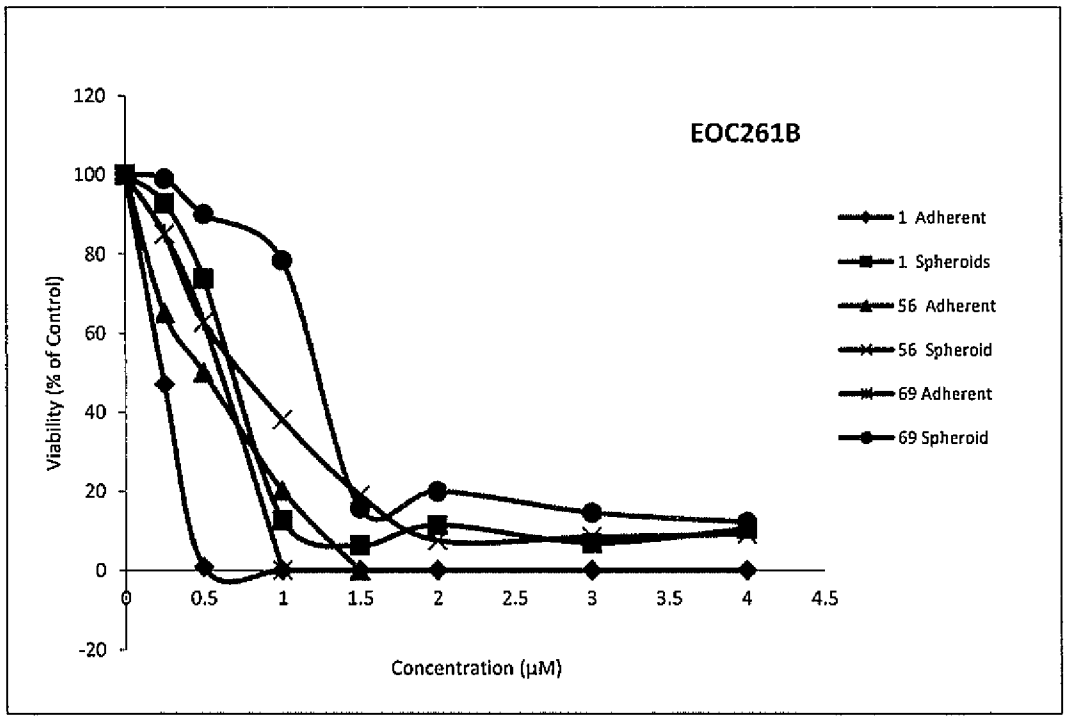


Figure 2M

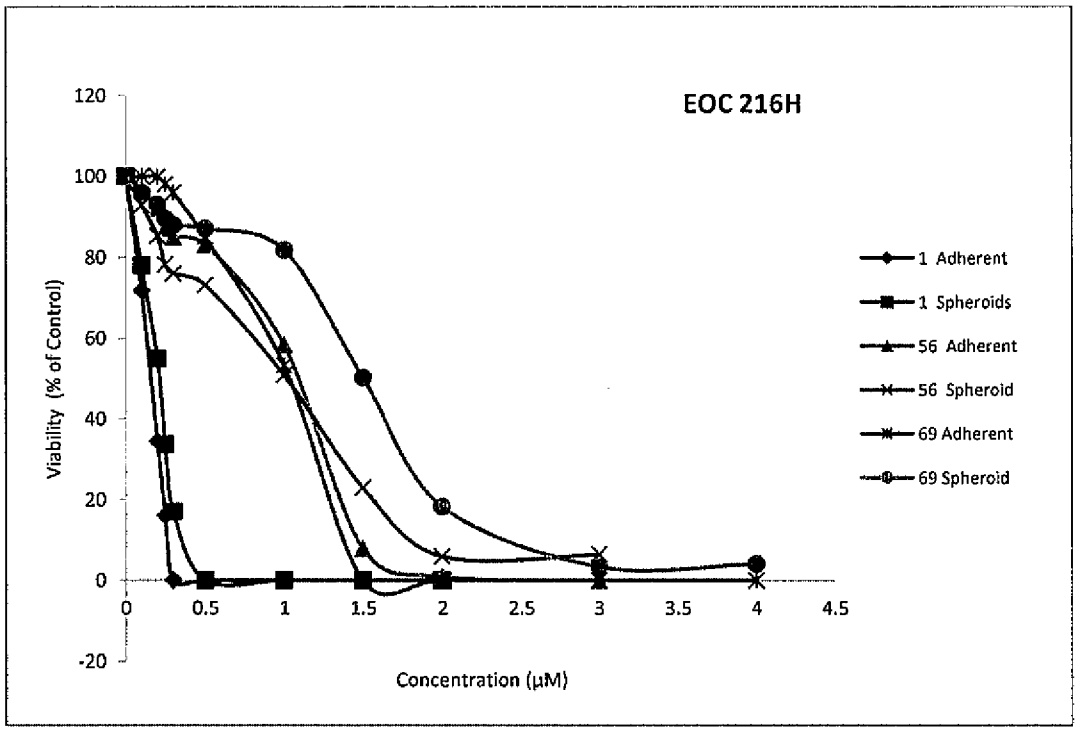


Figure 2N

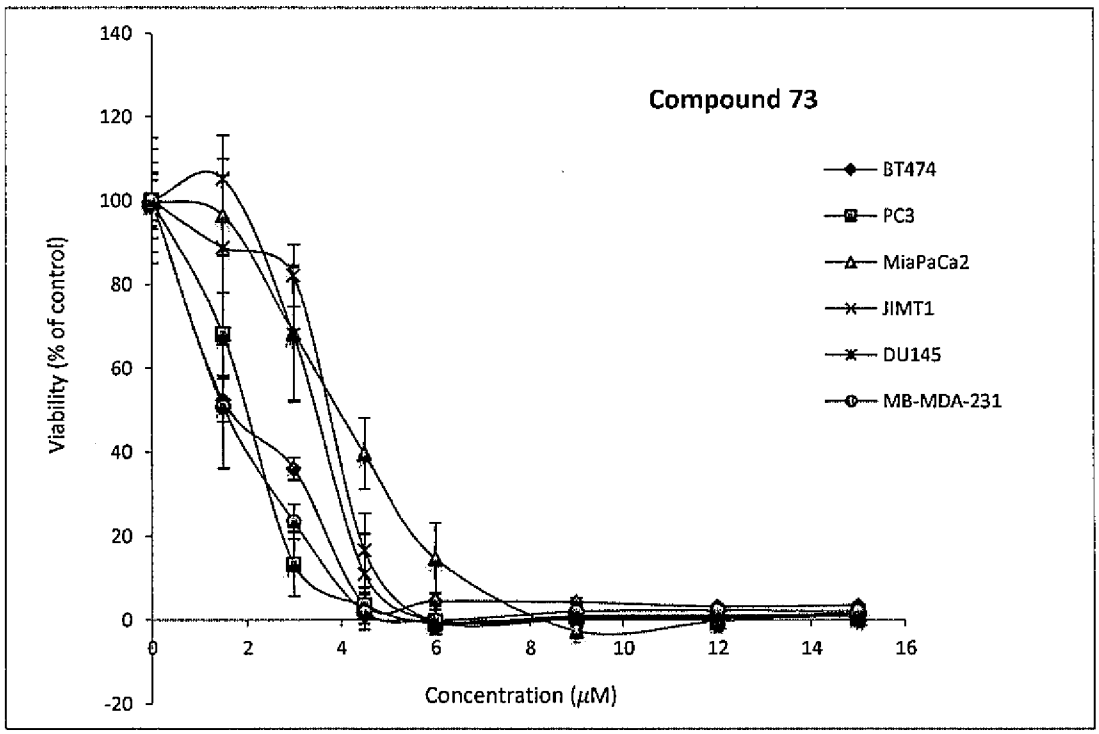


Figure 2O

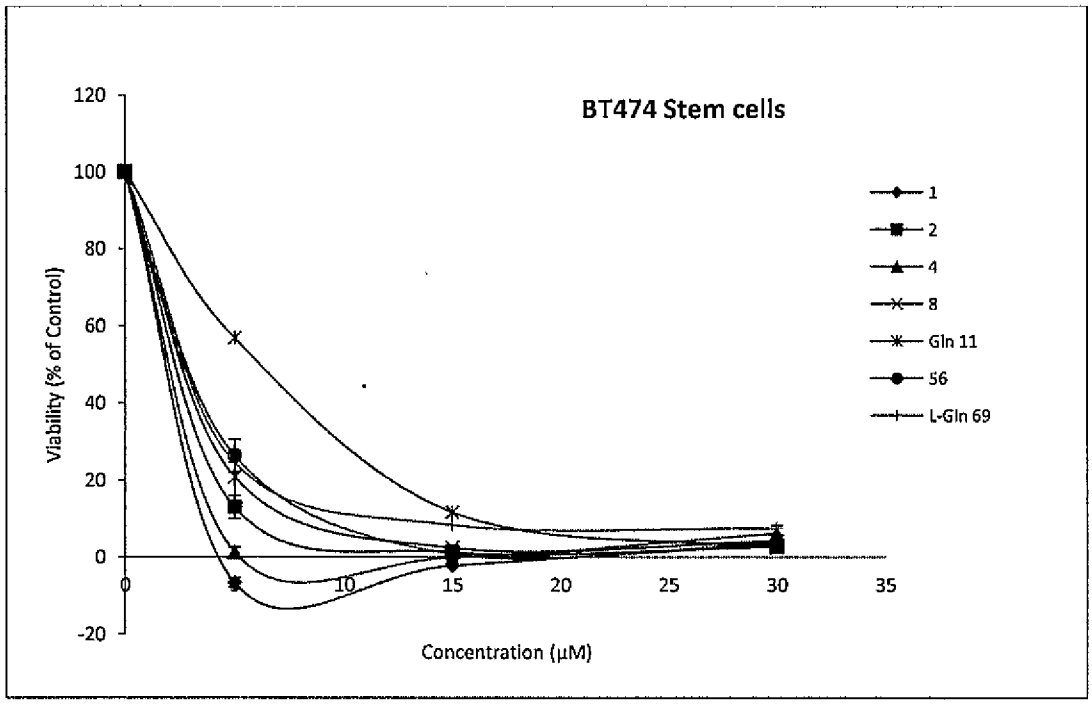


Figure 3A

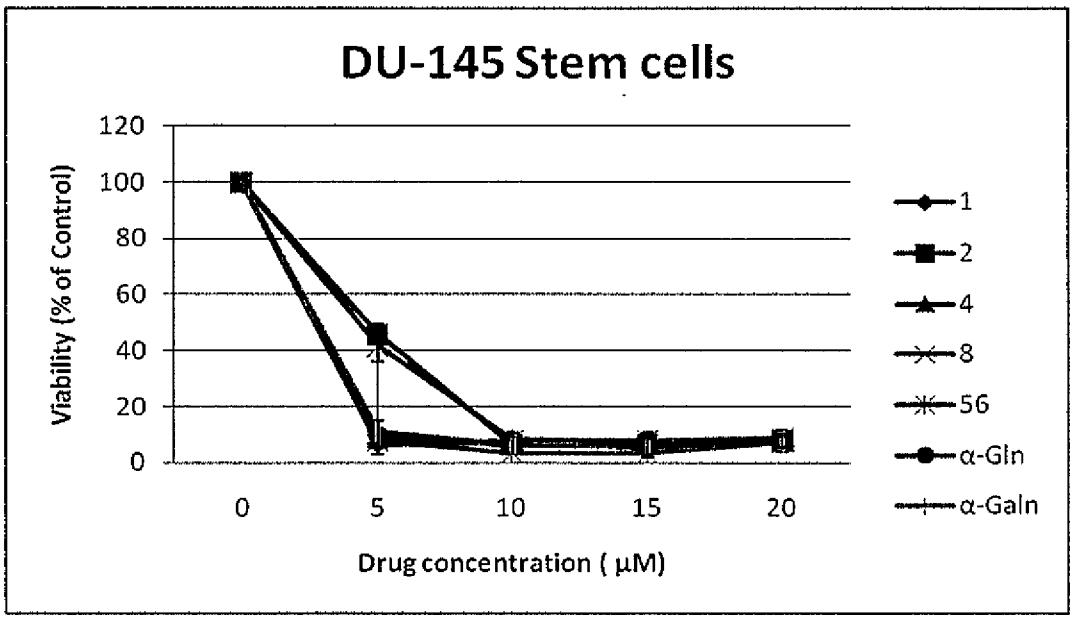


Figure 3B

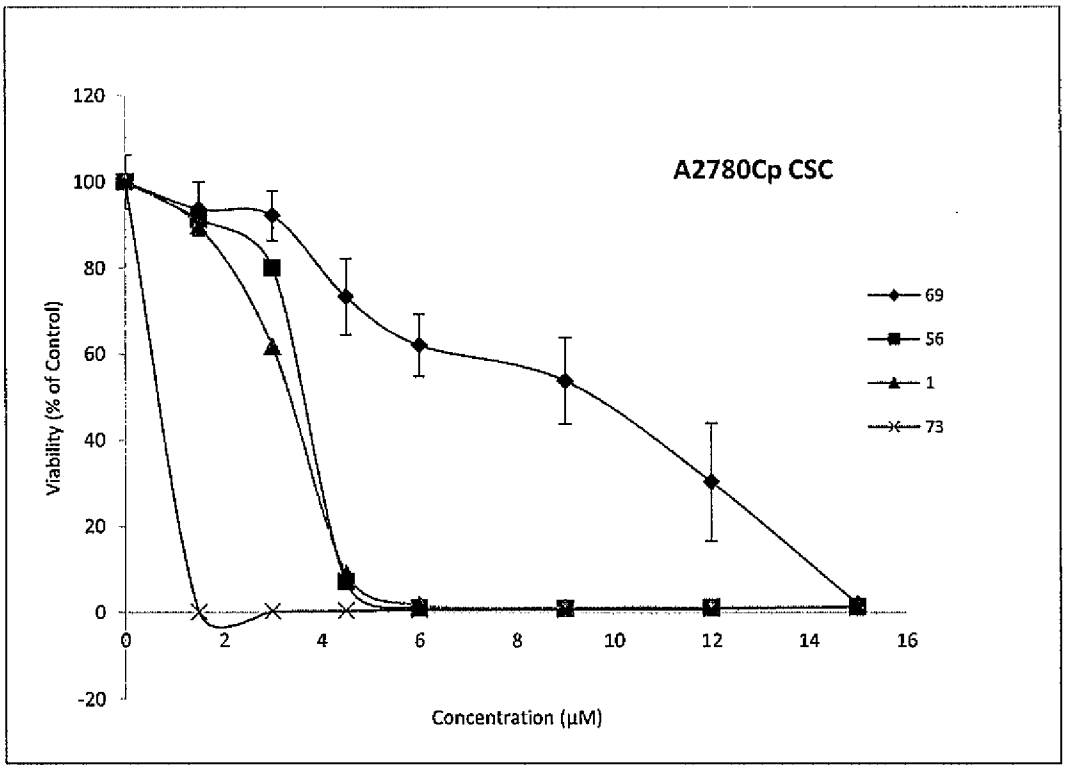

A2780Cp CSC
Viability (% of Control)
Concentration (μM)
120
100
80
60
40
20
0
-20
0
2
4
6
8
10
12
14
16
69
56
1
73

Figure 3C

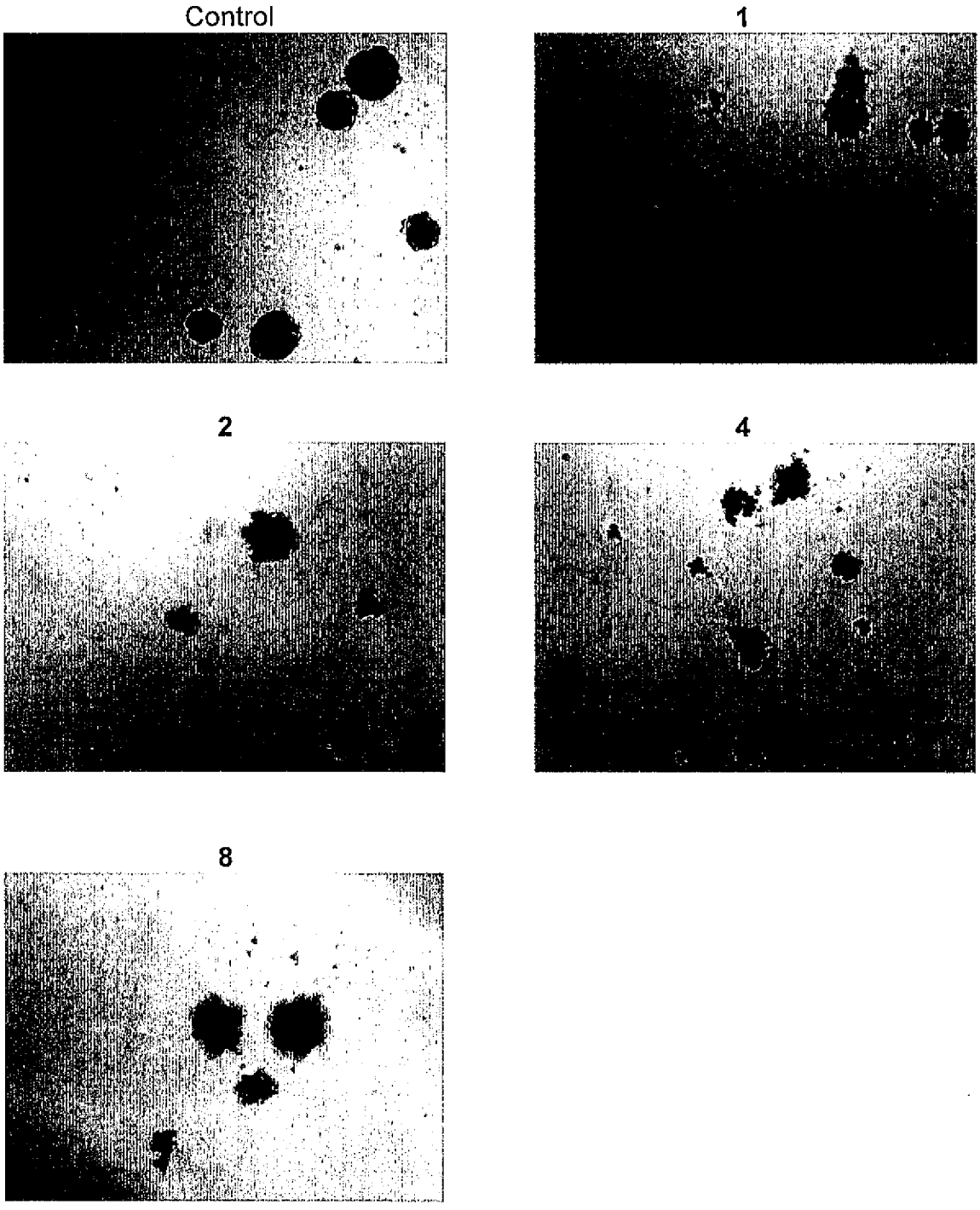


Figure 4A

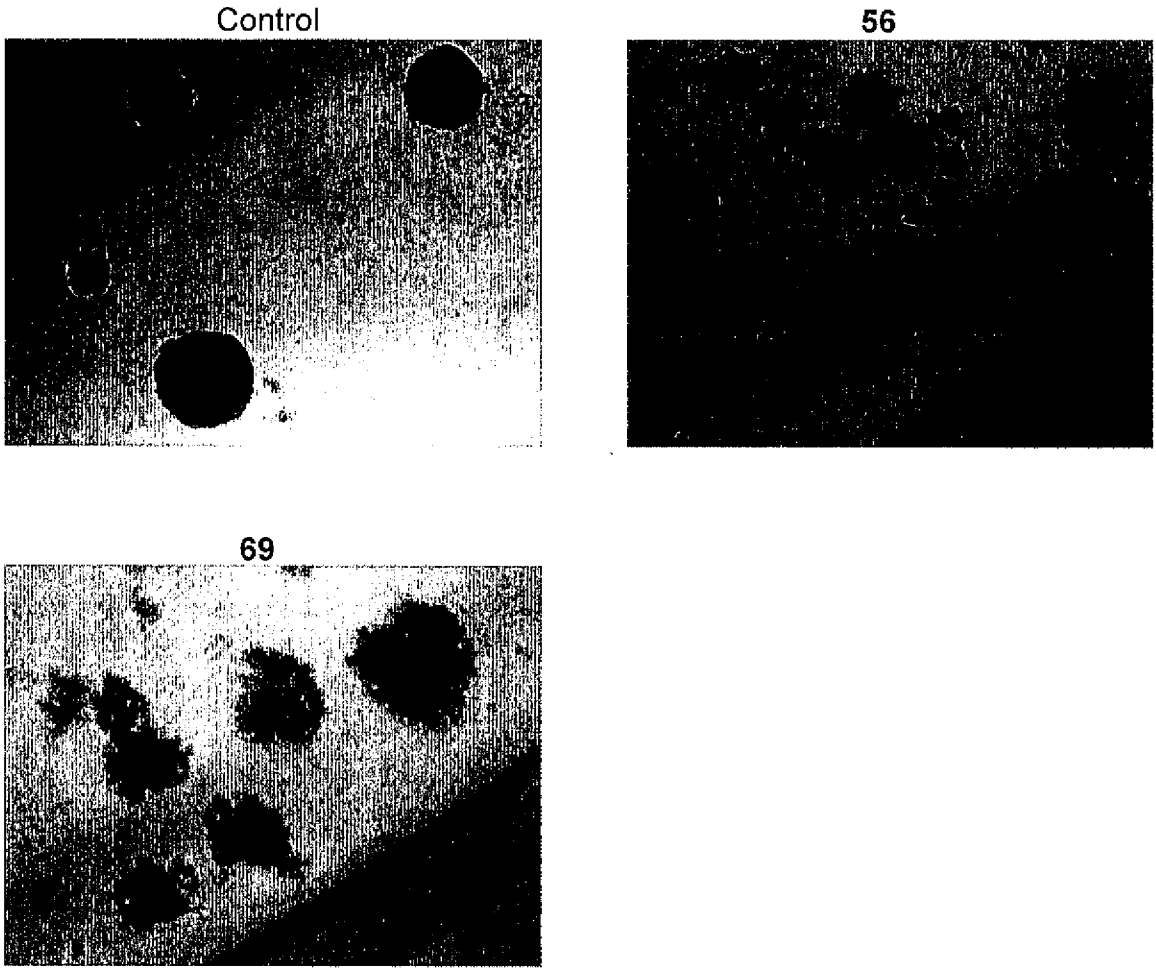


Figure 4B

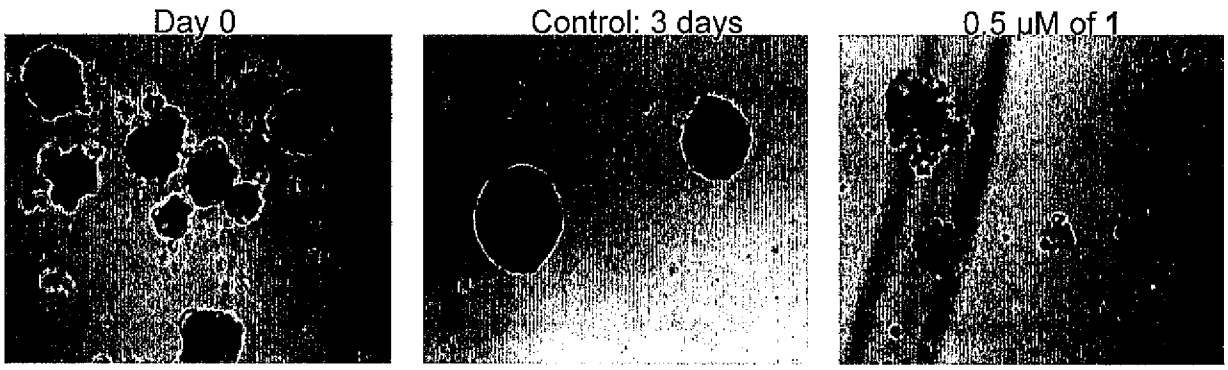


Figure 4C

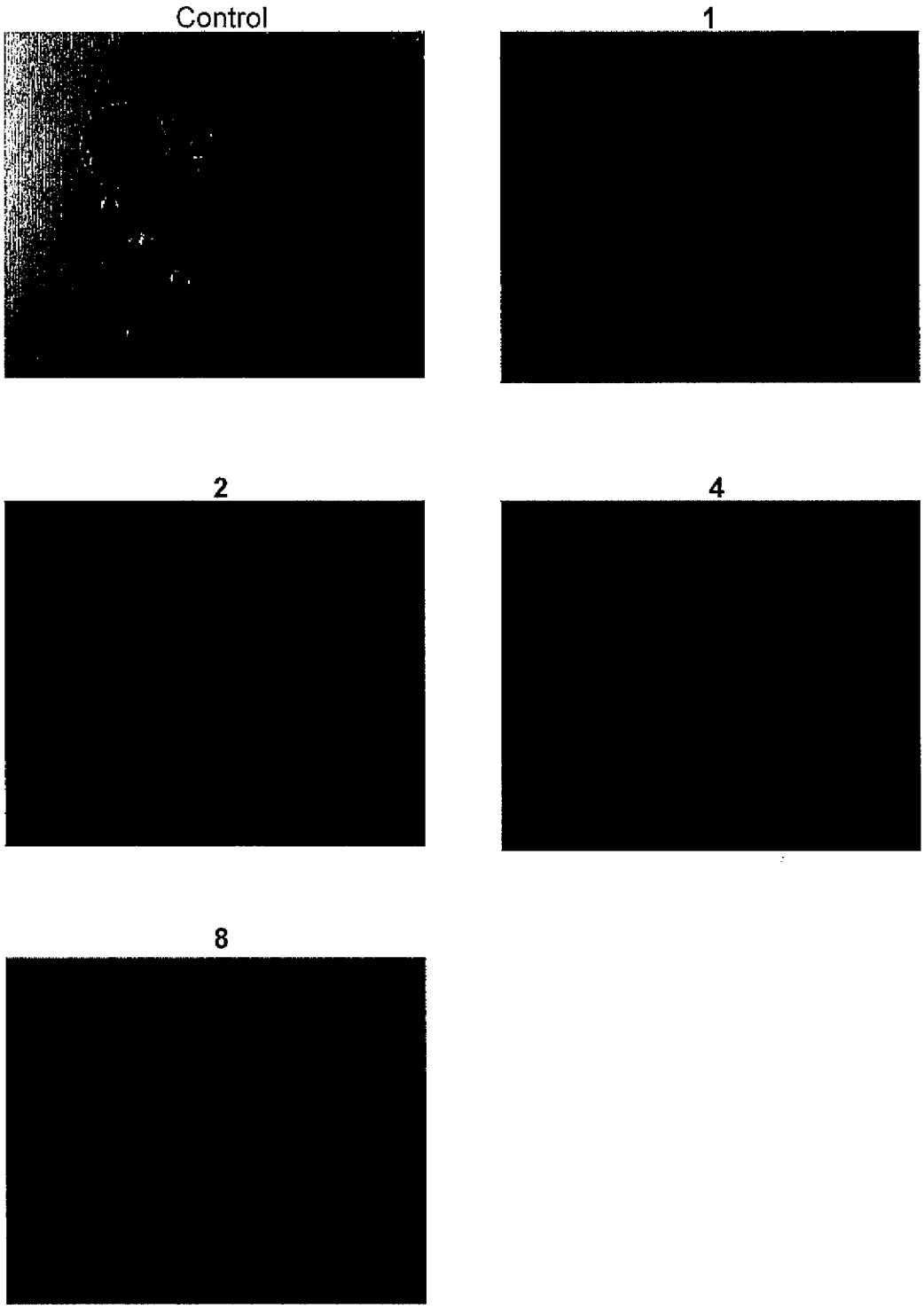


Figure 5A

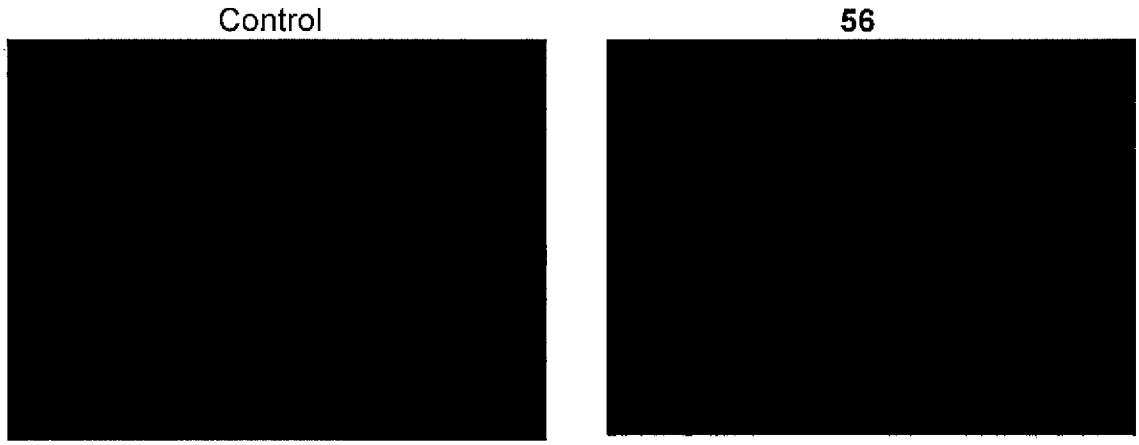


Figure 5B

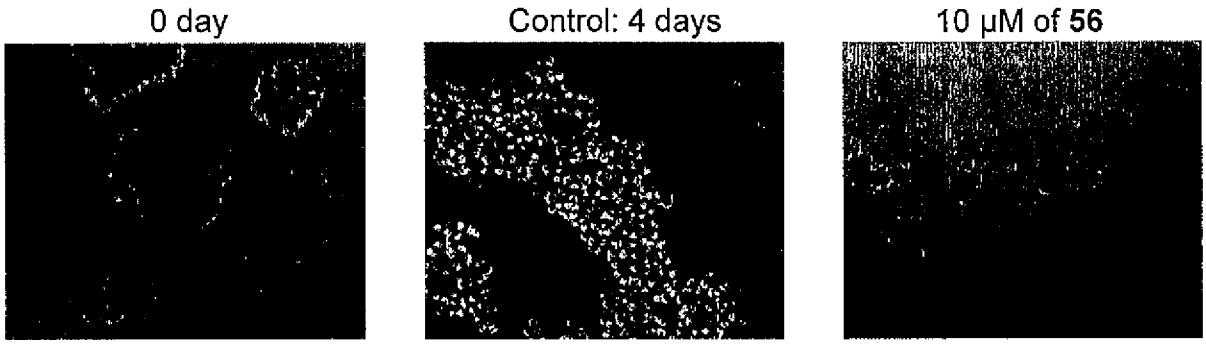


Figure 5C

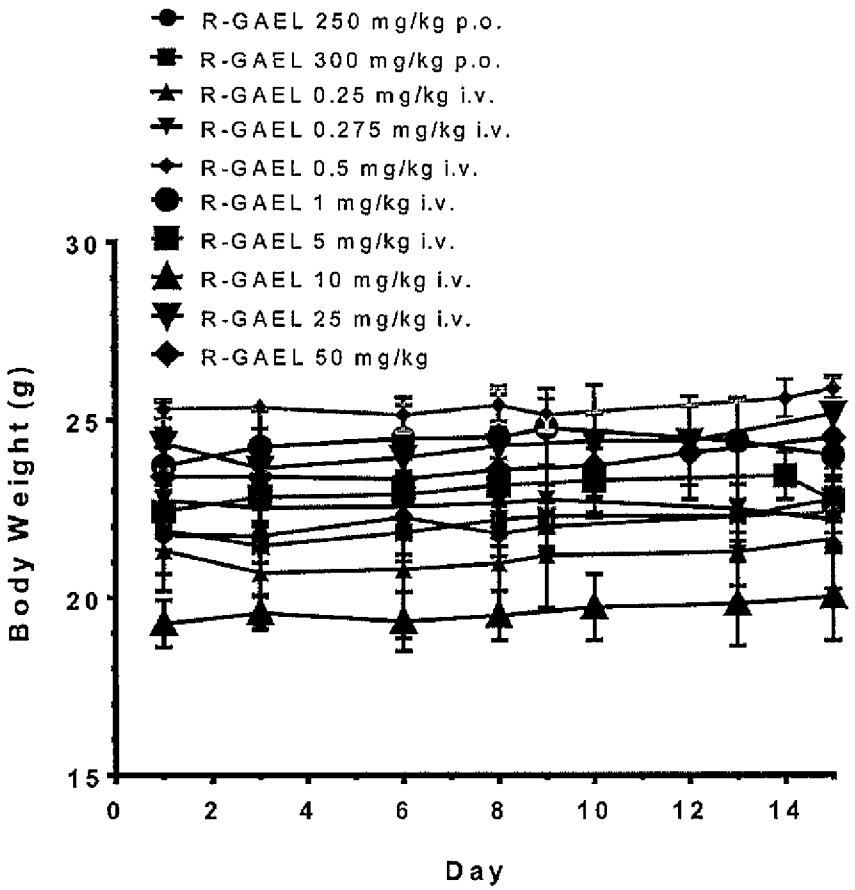

R-GAEL 250 mg/kg p.o.
R-GAEL 300 mg/kg p.o.
R-GAEL 0.25 mg/kg i.v.
R-GAEL 0.275 mg/kg i.v.
R-GAEL 0.5 mg/kg i.v.
R-GAEL 1 mg/kg i.v.
R-GAEL 5 mg/kg i.v.
R-GAEL 10 mg/kg i.v.
R-GAEL 25 mg/kg i.v.
R-GAEL 50 mg/kg
Body Weight (g)
30
25
20
15
0
2
4
6
8
10
12
14
Day

Figure 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2015/050490

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 31/7028*** (2006.01) , ***A61K 31/70*** (2006.01) , ***A61P 35/00*** (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K 31/7028 (2006.01), ***A61K 31/70*** (2006.01), ***A61P 35/00*** (2006.01).

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
QUESTEL (FAMPAT), PubMed, STN. Glycosylated antitumor?r ether lipids/ GAEL; cancer/tumo?r.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2013/116949A1 ARTHUR *et al.*, 15 AUGUST 2013 (15-08-2015) Glycosylated antitumor ether lipids as novel cancer stem cell cytotoxic agents. **D1**: See entire document, especially page 6, lines 12-page 8, line18; page 16, line 29- page 18, line 26; claim 1.	9-18
A	FR2967068 A1 DECOUT et al. 11 May 2012 (11-05-2012) [Derivatives of 6-amino-6-deoxyglucosamine and use of same as antibacterial agents] See page 12, lines 15-18.	
A	WO2011/089561 A1 SPARSO et al. 28 July 2011 (28-07-2011) Methods for producing amino-substituted glycolipid compounds See figures.	

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search 25 September 2015 (25-09-2015)	Date of mailing of the international search report 14 October 2015 (14-10-2015)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer C. Bourque (819) 576-2951

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ARTHUR *et al.*, Anticancer Agents Med. Chem., 14 (4), pp. 592-606, May 2014 (05-2014) "Glycosylated Antitumor Ether Lipids: Activity and Mechanism of Action." [ISSN:1871-5206(Print)/1875-5992(Electronic)] [doi:10.2174/1871520614666140309231144] http://www.eurekaselect.com/120832/article See entire article, especially sections 8 and 9.	
A	JAHREISS *et al.*, Autophagy, 5(6), pp 835-846. 16 August 2009 (16-08-2009) "1-O-Hexadecyl-2-O-methyl-3-O-(2'-acetamido-2'-deoxy-∃-D-glucopyranosyl)-sn-glycerol (Gln) induces cell death with more autophagosomes which is autophagy-independent" [ISSN: 1554-8627 (Print)/ 1554-8635 (Electronic)] [doi: 10.4161/auto.9120] http://www.tandfonline.com/doi/abs/10.4161/auto.9120 See entire article, especially Results section.	
A	SAMADDER *et al.*, Eur. J. Med. Chem., 78, pp. 225-235. 6May 2014 (06-05-2014) "Cytotoxic properties of D-gluco-, D-galacto- and D-manno-configured 2-amino-2-deoxy-glycerolipids against epithelial cancer cell lines and BT-474 breast cancer stem cells." [ISSN: 0223-5234 (Print)/ 1768-3254 (Electronic)] [doi: 10.1016/j.ejmech.2014.03.057] http://www.sciencedirect.com/science/article/pii/S0223523414002712 See entire article, especially sections 2 and 3.	
A	XU *et al.* Chem. Med. Chem., 8 (3), pp. 511-520, March 2013 (03-2013), "Structure-activity relationships of glucosamine-derived glycerolipids: the role of the anomeric linkage, the cationic charge and the glycero moiety on the antitumor activity." [ISSN: 1860-7179 (Print)/ 1860-7187 (Electronic)] [doi: 10.1002/cmdc.201200489] http://onlinelibrary.wiley.com/doi/10.1002/cmdc.201200489/pdf See entire article, especially Results and Discussion section.	
A	SAMADDER *et al.,* Anticancer Research, 31, pp. 3809-3818, November 2011 (11-2011) "An Active Endocytosis Pathway Is Required for the Cytotoxic Effects of Glycosylated Antitumor Ether Lipids." [ISSN: 0250-7005 (Print)/ 1791-7530 (Electronic)] http://ar.iiarjournals.org/content/31/11/3809.long See entire article, especially Results and Discussion section.	
A	SAMADDER *et al.*, Biochem. Cell. Biol., 87(2), pp. 401-414. 9 April 2009 (09-04-2009) "A glycosylated antitumor ether lipid kills cells via paraptosis-like cell death." [ISSN: 0829-8211 (Print)/ 1208-6002 (Electronic)] [doi: 10.1139/o08-147] http://www.nrcresearchpress.com/doi/full/10.1139/O08-147#.Vg1rR5eVTOQ See entire article, especially Results section.	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2015/050490

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☑ Claim Nos.: 1-8 and 19
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-8 and 19 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under PCT Rule 39.1(iv). However, this Authority has carried out a search based on the alleged effect or purpose/use of the product defined in claims 1-8 and 19.

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

The claims are directed to a plurality of inventive concepts and thus do not comply with Rule 13 of the PCT.

See extra sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Group A - Claims 1-19 (in part) which are directed to the use of 2,6-diamino-2,6-dideoxy-D-glucose glycerol lipids (e.g. compounds 1-10 and 73-76) in the treatment of cancer.

Remark on Protest

☐ The additional search fees were accompanied by the applicant=s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Box No. III Observations where unity of invention is lacking (continued …)

The claims are directed to a plurality of inventive concepts as follows:

(i) **Group A** - Claims 1-19 (in part) are directed to the use of 2,6-diamino-2,6-dideoxy-D-glucose glycerol lipid ethers (e.g. compounds 1-10 and 73-76) or 2,6-diamino-2,6-dideoxy-L-glucose glycerol lipid ethers (e.g. compounds 70-72) in the treatment of cancer;

Group B - Claims 1-19 (in part) are directed to the use of 2-amino-2-D-glucose glycerol lipid ethers (e.g. compound 11) or 2-amino-2-deoxy-L-glucose glycerol lipid ethers (e.g. compound 69) in the treatment of cancer;

Group C - Claims 1-3 (in part), 5-9 (in part), 11-15 (in part), 17-19 (in part) are directed to the use of D-glucose glycerol lipid ethers (e.g. compound 12) in the treatment of cancer;

Group D - Claims 1-3 (in part), 5-9 (in part), 11-15 (in part), 17-19 (in part) are directed to the use of a glycerol lipid ether phosphate (e.g. compound 13) in the treatment of cancer;

Group E - Claims 1-19 (in part) are directed to the use of rhamnose glycerol lipid ethers (e.g. compound 56) in the treatment of cancer;

Group F - Claims 1-19 (in part) are directed to the use of 2,6-diamino-2,6-dideoxy-galactose glycerol lipid ethers and 2-amino-2-deoxygalactose in the treatment of cancer;

Group G - Claims 1-19 (in part) are directed to the use of 2,6-diamino-2,6-dideoxy-D-mannose glycerol lipid ethers in the treatment of cancer; and

Group H - Claims 1-19 (in part) are directed to the use of 2,6-diamino-2,6-dideoxy-D-allose glycerol lipid ethers in the treatment of cancer.

(ii) The claims are directed to the use of numerous sugar-glycerol-lipid ethers or a glycerol-lipid ether in the treatment of cancer. The common feature among **Groups A-H** is the use of a glycerol-lipid moiety in the treatment of cancer. However, this feature is not novel, nor inventive. Each of WO2013/116949A1; SAMADDER et al. (Eur. J. Med. Chem., 78, pp.225-235); JAHREISS et al. (Autophagy, 5(6), pp. 835-846); and XU et al. (Chem. Med. Chem., 8 (3), pp. 511-520); disclose the use of sugar-glycerol-lipids and amino-sugar-glycerol lipids in the treatment of cancer. Accordingly, there is no unity between the use of the sugar based glycerol lipids of **Groups A-C** and **E-H** and the non-sugar glycerol lipid of **Group D**. Furthermore, the attachment of a sugar moiety (such as a non-amino sugar [e.g. **Group C** or **Group E**], an aminosugar [e.g. **Group B**, or **Group F** (in part)], or a diaminosugar [e.g. **Group A**, **Group F** (in part), **Group G**, or **Group H**] to the glycerol lipid ether in said cancer treatment does not present any single general inventive concept which links all the **Groups A, B, C, E, F, G** and **H.** Additionally, there is no unity between the groups having different base sugars (e.g. **Group B** and **Group F** (in part); or **Group C** and **Group E**) since the substitution of one sugar (e.g. glucosamine) for another (e.g. galactosamine) is not inventive. Accordingly, there is no unity among **Groups A-J** as mentioned above.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

(iii) The examiner has carried out a partial international search on those parts of the international application which relate to the invention first mentioned in **Group A.** Claims 1-19 (in part) are directed to the use of 2,6-diamino-2,6-dideoxy-D-glucose glycerol lipid ethers (e.g. compounds 1-10 and 73-76) or 2,6-diamino-2,6-dideoxy-L-glucose glycerol lipid ethers (e.g. compounds 70-72) in the treatment of cancer.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2015/050490

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2013116949A1	15 August 2013 (15-08-2013)	WO2013116949A1	15 August 2013 (15-08-2013)
		CA2869495A1	15 August 2013 (15-08-2013)
		US2015011486A1	08 January 2015 (08-01-2015)
FR2967068A1	11 May 2012 (11-05-2012)	FR2967068A1	11 May 2012 (11-05-2012)
		WO2012062996A1	18 May 2012 (18-05-2012)
WO2011089561A1	28 July 2011 (28-07-2011)	WO2011089561A1	28 July 2011 (28-07-2011)
		AU2011208429A1	12 July 2012 (12-07-2012)
		AU2011208429B2	22 May 2014 (22-05-2014)
		CA2785624A1	28 July 2011 (28-07-2011)
		CN102844324A	26 December 2012 (26-12-2012)
		EP2526110A1	28 November 2012 (28-11-2012)
		JP2013517762A	20 May 2013 (20-05-2013)
		MX2012008389A	15 August 2012 (15-08-2012)
		NZ600764A	29 November 2013 (29-11-2013)
		RU2012136133A	27 February 2014 (27-02-2014)
		US2012315373A1	13 December 2012 (13-12-2012)
		US9045514B2	02 June 2015 (02-06-2015)