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Amphiphilic-Modulation of Glycosylated Antitumor Ether Lipids Results in a Potent Triamino Scaffold against Epithelial Cancer Cell Lines and BT474 Cancer Stem Cells

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ABSTRACT

The problems of resistance to apoptosis-inducing drugs, recurrence, and metastases that have bedeviled cancer treatment have been attributed to the presence of cancer stem cells (CSCs) in tumors, and there is currently no clinically indicated drug for their eradication. We previously reported that glycosylated antitumor ether lipids (GAELs) display potent activity against CSCs. Here, we show that by carefully modulating the amphiphilic nature of a monoamine-based GAEL, we can generate a potent triamino scaffold that is active against a panel of hard-to-kill epithelial cancer cell lines (including triple-negative) and BT474 CSCs. The most active compound of this set, which acts via a non-membranolytic, non-apoptotic caspase-independent mechanism, is more effective than cisplatin and doxorubicin against these cell lines, and more potent than salinomycin against BT474 CSCs. Understanding the combination of factors crucial for the enhanced cytotoxicity of GAELs opens new avenues to develop potent compounds against drug-resistant cancer cells and CSCs.

INTRODUCTION

The development of resistance to currently used chemotherapeutic drugs, which are mostly pro-apoptotic, is a major impediment to the treatment of cancer.[1–3] Also, the presence of cancer stem cells (CSCs) in the bulk tumor has been implicated in tumor relapse that usually occurs after the initial treatment with existing chemotherapeutic agents.[4–6] These two factors account for the major reason a cure for cancer has remained elusive. Glycosylated antitumor ether lipids (GAELs), a subclass of antitumor ether lipids typified by glycolipid **1** (Figure 1), kill cancer cells via a non-apoptotic cell death mechanism,[7,8] which bears close resemblance to methuosis.[9,10] Methuosis is a non-apoptotic cell death that is characterized by vacuolization of macropinosomes which leads to cell rupture.[10,11] GAELs have also demonstrated the ability to kill CSCs,[12] a characteristic possessed by very few compounds. The non-apoptotic mechanism of action of this class of drugs provides new opportunities to manipulate cell death in a therapeutic context and to eliminate apoptosis-resistant cancer cells.

Structure-activity studies of GAELs have revealed the importance of the *C*-2 amino group as it affects cytotoxicity.[13,14] Other cationic amphiphilic drugs (CADs) have also been shown to induce non-apoptotic cell death pathways implicating lysosomes in the cell-death processes.[15] Most CADs are easily protonated at normal body pH, leaving them with a net positive charge. Meanwhile, cancer cell membranes typically carry a net negative charge relative to normal cell membranes due to an elevated expression of anionic molecules such as phosphatidylserine,[16,17] *O*-glycosylated mucins,[18,19] sialilated gangliosides,[20] and heparan sulfates.[21] The role of cationicity in anticancer agents may be to enhance the affinity of CADs for the cancer cells.[22] Upon binding to cell membrane, hydrophobicity of molecules becomes crucial in determining how well they permeate such membrane.[23–25] Membrane fluidity is typically increased in cancer cells relative to normal cells,[26,27] which may facilitate cancer cell

membrane destabilization by membrane-bound CADs. Consequently, the intrinsic properties of classical cationic amphiphiles may allow strong binding to cancer cells and the ability to cross the negatively charged hydrophobic membrane of cancer cells.

Previous studies showed that GAELs without the amino group were up to 10-fold less active relative to the monoamine-based compound **1**.[28] On the other hand, there was a significant loss of activity in studies with diglycosylated GAEL **2** (Figure 1) relative to **1**.[29] This diglycosylated analog was prepared to impart two amino functionalities (on different sugars) into a single molecule. In contrast, GAELs with two amino substituents on a single sugar molecule **3** (α- and β-) exhibited better cytotoxic properties than **1** and **2** (Figure 1).[30] The fact that analogs with two amino groups on a single sugar molecule display better activities than analogs with two amino groups on separate sugars suggests that the presence of a polar head group with a compensating long lipid (hydrophobic) tail are structural requirements for active analogs. Hence, we were curious about how the activity would be affected by three amino groups with an appropriately compensating hydrophobic moiety. We have previously demonstrated that fusing other domains with **1** did not significantly alter cytotoxicity.[31] This study provided insights on possible modifications that could be made to GAELs. More recently, it was demonstrated that the L-enantiomers of this class of experimental drugs retained the non-apoptotic mechanism of action and anti-CSC activity, and that introduction of a second amine group enhanced potency.[32] Amphiphilicity and cationicity have often been employed to overcome the problems of electrostatic interactions and transmembrane navigation in cancer cells,[15] and bacteria.[33] We therefore envisioned that conjoining myristylamine-like amphiphiles to GAELs would modulate the overall amphiphilic nature of these agents, and amplify their antitumor activities due to the presence of an additional amino group. In addition, these compounds would traverse the negatively charged hydrophobic membrane of cancer cells more easily. Myristylamine **(4)** (Figure 1) is an amphiphilic

cationic lipid that exhibits little activity against epithelial cancer cells relative to GAELs.[14] We decided to investigate the triamino concept by imparting a third amino functionality to β-diamino **3** using classical amphiphiles with different hydrophobic lengths and a polar (amino) group. Whereas the α-anomer of **3** was observed to be slightly more potent than its β-anomer,[30] further studies with the benzylated α-, and β- analogs showed similar potency between both configurations.[32] We therefore synthesized six β-triamino GAELs **5** - **10** (Figure 2) because β-configurations could be easily established by chemical methods. Herein, we investigated the effects of a third amino group, the hydrophobic aliphatic carbon chain lengths, and the point of covalent attachment on the cytotoxicity of GAEL using a variety of cancer cell lines, including drug-resistant human epithelial cancer cells. We also probed the role of the glycerol backbone on the cytotoxicity of the triamino GAELs. The effect on the viability of BT474 CSCs was investigated, as well as mechanistic studies were performed to gain insights into the mode of action of the triamino GAEL derivatives.

RESULTS AND DISCUSSION

Chemistry

In designing the triamino molecules, we wanted to preserve all the functional groups and key features of the lead mono- (**1**) and diamino (**3**) analogs. To ensure physiological protonation of **3**, the *C*-2 and *C*-6 amino groups were alkylated with laurylamine via reductive amination to afford compounds **5** and **7** respectively (Figure 2). The difference in the points of covalent attachment between these compounds was meant to probe the position of the primary amine of GAELs. The length of the laurylamine domain was also investigated by keeping the polar head constant while varying the length of carbon chain to give **6** and **8**, which were prepared similarly as **5** and **7**. A C12 aliphatic hydrocarbon chain is often regarded as a safe limit for positive charge and hydrophobic compromise, beyond which hemolytic activity dramatically increases with no significant change in efficacy.[34] To determine the role of the third amino group in **7** we prepared **9** by coupling chlorambucil to the amino moiety of laurylamine (Figure 2). This transformation converts the terminal primary amine into an amide bond that cannot be protonated at physiological pH, while retaining the C12 tether and diamino nature of the parent compound **3**. The chlorambucil moiety was used to neutralize the third cationic charge because it has been shown to be less cytotoxic to the epithelial cancer cells used in this study,[31] and it imparts additional lipophilicity to the molecule. To contextualize the exact role of the amines, the triamino scaffold was explored without its glycerol backbone (compound **10**) to determine whether cytotoxicity was solely based on the triamino groups or on the entire molecule. The glycerolipid backbone of GAELs has been reported to be essential for cytotoxicity.[14,29]

Chemical Synthesis

Synthesis of the triamino GAELs (**5 – 10**) commenced with the preparation of thioglycoside donor **12** from commercial glucosamine hydrochloride (**11)** as earlier reported (Scheme 1).[31,32] Donor **12** was then glycosylated with 12-azidododecanol under *N*-iodosuccinimide/silver triflate-promoted conditions to give β-glucopyranoside **13**, which was subsequently deprotected in a single step with ethylenediamine, followed by a palladium-catalyzed hydrogenation to afford 1-(12-aminododecyl)-2,6-diamino-2,6-dideoxy β-glucopyranoside (**10**) (Scheme 1). *n*-Azidoalkanals **16a-b** were prepared by S_N2 displacement of bromine from commercially available *n*-bromoalcohols **14a-b** with sodium azide to give *n*-azidoalcohols **15a-b**, and a successive oxidation of the primary alcohols to aldehydes by pyridinium chlorochromate (PCC) (Scheme 2). Thus, prepared compounds **16a-b** were immediately reacted with the glycerolipids **19** and **21**, via reductive amination to give protected derivatives **20a-b** and **22a-b** respectively (Scheme 3). A palladium-catalyzed hydrogenation of **20a-b** afforded the desired compounds **5** and **6** in good yields. In a similar manner, **7** and **8** were prepared by deprotecting **22a-b** with ethylenediamine, followed by palladium-catalyzed hydrogenation (Scheme 3).

Chlorambucil-linked glucopyranoside **9** was prepared by deprotecting **22a** with ethylenediamine and reprotecting the primary and secondary amines with di-*tert*-butyl dicarbonate to give a *Boc*-protected glucopyranoside-*sn*-glycerol **23** (Scheme 4). Catalytic hydrogenation and amide coupling with a pre-activated chlorambucil gave chlorambucil-linked *Boc*-protected β-glucopyranoside-*sn*-glycerol **24** in good yield, and was finally deprotected with trifluoroacetic acid to afford the chlorambucil-linked β-glucopyranoside-*sn*-glycerol compound **9**.

***In vitro* Screening against Epithelial Cancer Cell Lines**

To determine the cytotoxic effects of the newly synthesized triamino compounds **5 – 10** on the viability of human epithelial cancer cell lines, exponentially-growing cancer cell lines were incubated with varying concentrations of each compound (0 – 20 μM) for 48 h followed by viability assay using the MTS reagent. The parent compounds **1, 3** and **4**, as well as the clinically-used drugs cisplatin and chlorambucil served as reference compounds for the studies.

The results of the study revealed that of the six triamino compounds synthesized (Figure 2), compound **7** displayed the most potent activity against breast (JIMT1, MDA-MB-231, BT474), pancreas (MiaPaCa2), and prostate (DU145, PC3) cancer cell lines (Figures 3A-C). The CC_{50} values ranged from 1.5 μM – 4.0 μM, a consistent 3- to 7-fold increase in activity (depending on the cell line) when compared to reference **1** (Table 1). Cisplatin and chlorambucil were also less potent than **7** against these cell lines (Figure 3G). Although doxorubicin was more active than **7** based on CC_{50} values, it could not achieve a 90 % reduction in cell viability at the highest concentration tested (Figure S1) whereas **7** reduced cell viability to zero across the panel at concentrations between of 4.0 – 10.0 μM (Figure 4). Compound **5** was the next active triamino GAEL after **7**, and its activity was similar to or slightly less than the activity of derivative **1**. Compound **5** was less potent than reference **1** against BT474 cells, and was only slightly better against prostate cancer cells (DU145, PC3) with a barely 2-fold increase (Table 1). The shorter chain triamino compounds **6** and **8**, as well as the chlorambucil-linked analog **9** displayed comparably low activities relative to **5** and **7** across all cell lines (CC_{50} values ranged from 10.5 μM to > 20 μM, Table 1 and Figure 3A-C). Compound **10** was not active at the highest concentration tested (20 μM). Thus, while increasing the number of amino moieties does lead to enhanced potency, it is clear that other structural features play a role in influencing the overall activity of the compounds.

To investigate SAR of GAELs, we compared the activity of compounds designed to explore the position of the covalent linkage, hydrophobicity, and the role of the glycerol moiety using compound **1** as the reference compound. Compounds **5** and **7** were designed to explore the effect of fusing a laurylamine moiety at different positions on the sugar of the GDG on the activity of the compounds. In **5**, the substitution was at the *C*-2 position while in **7** it was at the *C*-6 position. As mentioned above, **7** was extremely active and is in fact the most active GAEL synthesized to date. The activity of **5** was 2- to 7-fold less than **7** depending on the cell line, thus the positioning of the moiety has a significant impact on activity. This view is confirmed by comparing the results of the activities obtained with **6** and **8**, which have shorter hydrophobic moieties that are linked at the *C*-2 and *C*-6 positions respectively in a manner analogous to **5** and **7**. Compound **8** with the substituent at *C*-6 position of the sugar displayed slightly better activity than **6** that was substituted at *C*-2 position with the majority of the cell lines, except for MiaPaCa2 cells. Although the differences in potency between **6** and **8** were not as pronounced as between **5** and **7**, the phenomenon seems to suggest that the point of covalent attachment (or position of primary amine) plays a crucial role in the potency of the triamino scaffold. This observation suggests a requirement for primary amines at *C*-2 position (substitutions at *C*-6) of the glucosamine moiety that preserves the exact form of the lead monoamino analog **1** (Figure 1).

As hydrophobicity is expected to play a major role in amphiphilic-modulation of the compounds, the effect of the carbon chain length of the hydrophobic moiety on activity was probed by comparing the activities of **5** with **6** and **7** with **8**. The results revealed that **5** and **7** were more active than **6** and **8** respectively. The longer carbon chain length, as presented by **5** and **7**, is expected to impart a compensating hydrophobicity to the terminal amino group while the shorter length in **6** and **8** will result in molecules that are less hydrophobic (Figure 2). Hydrophobicity plays a vital role in the movement of amphiphilic molecules across cell membranes or insertion of molecules into membranes. While the

positively charged amino groups on the compounds (protonated at physiologic pH) electrostatically localize them to the abundantly expressed anionic charges on cancer cell membranes, their hydrophobic nature will facilitate their insertion or uptake across the richly lipophilic membranes. Compounds **5** and **7** probably are inserted or transverse the cell membrane more easily than **6** and **8** and could thus explain the distinct difference in activity.

Compound **7** was linked to chlorambucil to generate compound **9** because chlorambucil has been previously reported to exhibit very low activity (CC_{50} > 150 μM) against the epithelial cancer cell lines used in this study.[31] However, it modulates the activities of GAELs by imparting lipophilicity.[31] The chlorambucil-linked analog **9** was synthesized to neutralize the cationic effect of the terminal amino group through the formation of an amide bond that removes the possibility of protonation. The use of a N-acetyl derivative to neutralize this cationic charge was undesirable as NHAc groups alter the activities of GAELs against prostate cancer cell lines,[28] whereas chlorambucil does not. Thus, compound **9** is less cationic than **7**, but is more lipophilic. Compound **9** displayed low activity similar to **6** and **8**. The loss of activity of **9** relative to **7** confirms that a certain threshold of hydrophobicity-charge ratio must be maintained for optimal activity. The TFA salt of compound **9** was stable at 30 μM concentration in a pH 2-3 in aqueous DMSO solution for at least one week. The results of studies with compound **10** supports this hydrophobicity-charge balance postulate. Compound **10** that has all the three amino groups but lacks the hydrophobic domain (glycerol backbone and the long lipid tail) of GAELs (Figure 2) was the least active of all the analogs tested against all cell lines (Figures 3A–C and Table 1). These results leave us with two conclusions: 1) a glycerol backbone and long lipid tail is essential for the activity of all GAEL analogs, as previously reported.[14] 2) the cationic charges as presented by amino groups are necessary, but insufficient for the activity of this scaffold. Rather, a balance of charge(s) and an appropriately compensating hydrophobicity is required.

The BT474 cell line appeared to be unusually resistant to being killed by the triamino GAELs (Figure 3A), the only exception being compound **7**, but even this compound did not achieve 100 % cell kill at 20 μM after 48 h incubation. Compound **6** enhances the growth of BT474 cells up to a concentration of 15 μM. BT474 cells are characterized by the over-expression of human epidermal growth receptor 2 (HER-2) and estrogen receptor (ER), and are resistant to ER antagonists such as tamoxifen.[35] The overexpression of these receptors could be a reason why the cells resist these compounds. Triple negative breast cancer (TNBC) cell lines lack HER-2 and ER, as well as the progesterone receptor (PR). They are very resistant to conventional chemotherapeutic agents and prognosis for patients with TNBC is worse than patients with the receptors.[36] We therefore assessed the effect of **7**, the most potent triamino GAEL, against a panel of TNBC cell lines: MDA-MB-453, MDA-MB-468, MDA-MB-231, BT549 and Hs578t. The results displayed in Figure 3F show that **7** was able to achieve > 95 % reduction of cell viability at about 6.0 μM, a > 3-fold increase in activity compared to **1**.

We also investigated the effect of **7** on cells derived from other hard-to-treat cancers; brain (U87, U251) and ovarian (A2780s, A2780cp). A2780cp is a cisplatin-resistant isogenic cell line of the drug sensitive A2780s line. Compound **7** displayed CC_{50} values of < 4.0 μM against the brain and ovarian cancer cell lines (Figures 3D and E, Table 2) and reduced viability to almost zero at 4.0 - 8.0 μM. Thus, **7** was effective against epithelial cancer cell lines representing hard-to-treat cancers including TNBC, androgen-resistant prostate cancer, drug resistant ovarian cancer and brain cancer.

Effect of GAELs on BT474 Cancer Stem Cell Spheroid Viability and Integrity

The inability to eliminate CSCs is one of the major impediments to finding a cure for cancer.[6] The propensity of CSCs to evade apoptosis leads to recurrence subsequent to treatment, and CSCs are thought to be also responsible for metastases.[6] There are only a few compounds that are currently known

to kill CSCs,[37] and it was previously reported that mono and diamino GAELs have the ability to kill these cells.[12,30,32] We therefore evaluated whether the newly synthesized triamino molecules are also able to kill CSCs. CSCs were isolated from BT474 cells by sorting for cells with high levels of aldehyde dehydrogenase 1 (ALDH 1), a biomarker for breast CSCs.[38–40] The isolated cells were grown in low adhesion plates to form tumorspheres and were subsequently incubated for 3 days with the GAELs. The viability of the CSCs was determined with the MTS assay.

The results of these studies showed that the triamino GAELs were effective in inhibiting the viability of the CSCs. The order of potency of these compounds against the BT474 CSCs was similar to what was previously observed with the unsorted cells; **7** > > **5** > **8**, **6** > > > **10** (Figure 5A). Compound **10** and myristylamine **4** had no effect on the viability of the CSC spheroids at the concentrations examined. It is worth noting that in spite of differences in the potency of triamino compounds **5** – **8**, they all ultimately reduced the viability of the CSCs to near zero at the highest concentration examined (20 μM).

The activity of the most potent analog **7** was then compared with doxorubicin, salinomycin, and cisplatin. The results showed that while doxorubicin affected the viability of the CSCs, this compound was unable to reduce the viability below 20 % despite increase in concentration, whereas the viability of spheroids incubated with **7** reached almost 2 % viability at about 10 μM (Figure 5B). Cisplatin had very little effect on the viability of the CSCs and viability was reduced by only 20 % at the highest concentration tested (20 μM). Its inability to substantially reduce the viability of BT474 CSCs is consistent with the characteristic resistance of CSCs to apoptotic cell death,[41] since cisplatin induces apoptosis[42] in cells. Compound **7** also displayed significantly better activity than salinomycin (Figure 5B), a well-documented anti-CSC experimental agent.[43] Salinomycin has been reported to reduce the proportion of CSCs by >100-fold relative to paclitaxel, a commonly used breast cancer chemotherapeutic drug.[44] The effects of the lead monoamino GAEL **1**, diamino **3**, and triamino **7** against

BT474 CSCs show an increasingly better and enhanced cytotoxicity, relative to salinomycin (Figure 5C).

The effect of these compounds on the integrity of BT474 CSCs spheroids was also examined. The results show that after 3 days, the tumor spheres incubated with 7.5 μM of **7** had completely disintegrated into small amorphous structures while partial disintegration was observed for **5**, **6**, and **8** at the same concentrations (Figure 5D). However, at a concentration of 20 μM, where all the compounds reduced the viability of the CSCs to near zero (Figure 5A), disintegration was complete with **5** and **7** with no difference between the two, while **6** and **8** still showed fairly large amorphous structures (Figure 5D). At this high concentration, **5** and **7** have a similar effect on the integrity of the spheroids. The presence of the laurylamine in **5** and **7** may explain their superior disintegrative properties compared to **6** and **8** with shorter hydrophobic chains. In contrast, tumor spheres incubated with 7.5 μM of doxorubicin and salinomycin were still intact, while a higher concentration (20 μM) of salinomycin resulted in jagged edges, suggesting the onset of disintegration. Spheroids incubated with **4** and **10** were as intact as the control, although tumor spheres with 20 μM of **4** was not as compact. These results show that the triamino GAELs retain the intrinsic ability to disintegrate BT474 tumor spheres while compounds **4, 10**, and doxorubicin could not achieve this feat at the highest concentration tested.

Mechanism of Action Studies

Our previous studies showed that mono and diamino GAELs do not induce the activation of caspases and kill cells by an apoptosis-independent pathway.[14,30] We investigated whether triamino GAELs also act via an apoptosis-independent pathway by assessing the role of caspases in **7**-induced cell death using the cell permeable pan-caspase inhibitor *N*-(2-quinolyl)-L-valyl-L-aspartyl-(2,6-difluorophenoxy) methylketone **25** (Q-VD-OPh) (Figure S2).[45] When DU145 and JIMT1 cell lines were incubated with

varying concentrations of doxorubicin or **7**, with or without 40 μM of **25**, the cytotoxicity of **7** was not significantly altered (Figure 6A). In contrast, the cytotoxicity of doxorubicin, which induces caspases-dependent apoptosis,[46–48] was attenuated in the presence of **25**. These results suggest the mechanism of cell death initiated by **7** is caspase-independent and likely similar to that of the mono or diamino GAELs in being apoptosis-independent.

The membranolytic effect of **7** was also investigated to exclude the possibility that cell death was due to the effects of **7** on the integrity of the cell membrane. The ability of **7** to perturb the membrane integrity of DU145 and JIMT1 cells was therefore investigated using membrane-impermeable ethidium homodimer-1 dye. The dye emits red fluorescence when it binds to nuclei acids.[49] Incubation of cells with 6 μM of **7** for 4 h showed noticeable changes in the morphology of the cells under bright field. However, the cells did not stain red indicating that the membrane was intact. In contrast, cells incubated with 0.01 % triton X-100 for 10 min were all rounded up, and stained red (Figure 6B). Compound **7** also displayed less than 20 % hemolysis of ovine erythrocytes at a concentration that was about fifteen times the CC_{50} value against BT474 cells *in vitro* (Figure S3). Taken together, these results indicate that **7** is not lytic and cell death is not due to necrosis.

CONCLUSIONS

Despite the huge investment in cancer research, several cancers such as brain, ovarian, pancreatic, and triple negative breast cancer do not have any effective treatments. In addition, the problem of drug resistance which leads to recurrence of tumors after initial treatments has limited the effectiveness of current anticancer therapies, leading to growing morbidity and mortality. CSCs, which are refractory to most current chemotherapeutic agents,[50] are believed to drive tumor growth[51] and generate drug-resistant variants. The elimination of CSCs is key in the fight to find a cure for cancer. GAELs are novel agents that kill cells by a non-apoptotic mechanism, and are also able to kill CSCs.[12,30,32] Our previous studies that showed a correspondence between amphiphilicity and cytotoxicity of GAELs led to the postulation that manipulating the balance between these two parameters could enhance the potency of this class of drugs without changing the mechanism of action.

We conducted SAR studies to explore the effect of an extra amino group with hydrophobic moiety of different chain lengths attached at different positions of glucosamine-derived GAEL **3**. The results of this study show that changes to the amino groups (cationic charge) alone or hydrophobicity alone is insufficient for optimum activity, but a combination of both, maintained at a certain threshold is needed. Compound **7** with an additional amino group attached to a *C12* moiety at position 6 of the sugar was identified as the most active GAEL synthesized to date. GAEL **7** showed significant activity against a range of drug-resistant cancer cell lines derived from several tissues, as well as BT474 breast CSCs. The activity against TNBC lines ($\geq$ 95 % cell kill at 6 μM) is noteworthy as there are no drugs currently available to treat TNBC. With respect to CSCs, compound **7** was more potent than doxorubicin, cisplatin and the well-studied anti-CSC agent salinomycin. The mode of action of GAEL **7** was independent of caspase activation, and it was not due to necrosis.

The validation of GAELs as a potent agent against TNBC cells and one of the very few agents capable of killing CSCs, perhaps due to their apoptosis-independent mechanism of action, provides a promising avenue to develop novel therapeutics that can prevent tumor relapse as well as circumvent resistance to pro-apoptotic drugs. The potential of these compounds to overcome the twin-problem of resistance and tumor relapse represent a viable alternative to expand the chemotherapeutic space in cancer management.

EXPERIMENTAL SECTION

Chemistry. All chemicals and reagents were purchased from Sigma-Aldrich (Oakville, ON, Canada), except 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol **(17)** that was purchased from Chem-Implex Inc. (Wood Dale, IL, USA). The chemicals were all used without further purification. Air and moisture-sensitive reactions were performed under a nitrogen atmosphere with dry solvents. Thin-layer chromatography (TLC) was carried out on aluminum-backed silica gel 60 F_{254} GF plates (Merck KGaA, Germany) with the indicated solvents, and visualized under ultraviolet light and/or charring with 10 % H_2SO_4 in methanol. Compounds were purified by flash chromatography on silica gel 60 (230-400 ASTM mesh) or reverse-phase C18 silica gel (Silicyle, USA). Yields refer to chromatography-purified homogenous materials, except otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AMX-300 and AMX-500 spectrometers (Germany) as solutions, and reported in the order of chemical shifts (δ) in ppm relative to the indicated solvent, multiplicity (s, singlet; d, doublet; t, triplet and m, multiplet), number of protons, and coupling constants (*J*) in hertz (Hz). ^{1}H and ^{13}C of compounds were assigned based on Proton, COSY, Carbon-13, DEPT-135 and HSQC experiments. Electrospray ionization mass spectrometry (ESI-MS) on a Varian 500-MS Ion Trap spectrometer (USA) was obtained for all intermediates while matrix assisted laser desorption ionization (MALDI) coupled with time of flight (ToF) mass analyzer was performed on a Bruker Daltonics Ultraflex MALDI TOF/TOF mass spectrometer to characterize the molecular weight of final compounds. Optical rotations of final compounds were measured on an Autopol® IV automatic digital polarimeter (Rudolph Research Analytical, USA). The purity of final compounds as determined by elemental analysis was > 95 %.

*Representative Procedure A: Catalytic Hydrogenation for Preparation of Compounds **5** and **6**.*

1-O-Hexadecyl-2-O-methyl-3-O-[2'-N-(12-aminododecyl)-6'-amino-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol **(5):** A solution of **20a** (0.20 g, 0.28 mmol) in methanol (5.0 ml) was treated with a catalytic amount of Pd/C catalyst (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 using a gradient elution of water and methanol to give **5** (0.14 g, 76 %). $[\alpha]_D^{25}$ = -5.2° (*c* = 0.1, methanol); ^{1}H NMR (500 MHz, CD_3OD) δ 4.30 (d, *J* = 8.1 Hz, 1H, H-1), 3.91 (dd, *J* = 10.5, 4.7 Hz, 1H), 3.63 (dd, *J* = 10.5, 4.5 Hz, 1H), 3.70 – 3.50 (m, 2H), 3.50 – 3.40 (m, 6H), 3.34 – 3.32 (m, 1H), 3.26 – 3.23 (m, 1H, H-3), 3.20 – 3.12 (m, 1H), 3.03 (dd, *J* = 13.5, 2.7 Hz, 1H), 2.96 – 2.88 (m, 1H), 2.76 – 2.65 (m, 3H), 2.34 (dd, *J* = 9.8, 8.1 Hz, 1H, H-2) 1.60 – 1.45 (m, 6H), 1.38 – 1.22 (m, 43H), 0.88 (t, *J* = 6.9 Hz, 3H, terminal C*H*$_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 104.0 (C-1), 79.2, 76.3, 75.0, 72.2 (C-3), 71.2, 70.2, 68.1, 63.6 (C-2), 56.7, 49.0, 42.5, 40.5, 31.7, 30.6, 29.6, 29.4, 29.38, 29.36, 29.34, 29.33, 29.31, 29.28, 29.18, 29.12, 29.06, 27.0, 26.5, 25.8, 22.3, 13.1 (terminal *C*H$_3$). MALDI TOF-MS *m/e* calc'd for $C_{38}H_{79}N_3O_6K$: 712.5606, measured *m/e*: 712.5598 $[M + K]^+$

1-O-Hexadecyl-2-O-methyl-3-O-[2'-N-(3-aminopropyl)-6'-amino-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol **(6):** A solution of **20b** (0.11 g, 0.18 mmol) was reduced via catalytic hydrogenation and purified by reverse-phase C18 silica gel to give **6** (0.072 g, 72 %). $[\alpha]_D^{25}$ = -3.6° (*c* = 0.1, methanol); ^{1}H NMR (300 MHz, CD_3OD) 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, 12H), 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, 27H), 0.91 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD) δ 104.1 (C-1), 80.6, 78.2, 75.7, 72.7, 72.0, 71.5, 70.0, 64.4, 62.7, 58.2, 33.1, 30.80, 30.77, 30.6, 30.5, 29.4, 27.3, 23.8, 14.5. MALDI TOF-MS *m/e* calc'd for $C_{29}H_{61}N_3O_6Na$: 570.4458, measured *m/e*: 570.4461 $[M + Na]^+$

*Representative Procedure B: Deprotection of Amine (removal of phthalimido group) for Preparation of Compounds **7** and **8**.*

1-O-Hexadecyl-2-O-methyl-3-O-[2'-amino-6'-N-(12-aminododecyl)-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol **(7):** A solution of **22a** (0.45 g, 0.53 mmol) in 1-butanol (5.0 ml) was treated with ethylenediamine (5.0 ml) and stirred for 3 h at 90 °C. The mixture was concentrated under high *vacuo* and purified by flash chromatography (dichloromethane/methanol, 3:1, v/v). The resulting compound was then reduced by catalytic hydrogenation (following representative procedure A) and purified by reverse-phase C18 silica gel to afford **7** (0.198g, 55 %). $[\alpha]_D^{25}$ = -5.9° (*c* = 0.1, methanol); ^{1}H NMR (500 MHz, CD_3OD) δ 4.48 (d, *J* = 8.1 Hz, 1H, H-1), 3.94 (dd, *J* = 10.5, 6.7 Hz, 1H), 3.68 (dd, *J* = 10.5, 6.7 Hz, 1H), 3.57 – 3.52 (m, 2H), 3.51 (m, 1H), 3.49 – 3.40 (m, 6H), 3.37 (dd, *J* = 10.2, 8.8 Hz, 1H, H-3), 3.31 – 3.28 (m, 2H), 3.28 – 3.24 (m, 2H), 3.08 – 3.00 (m, 1H), 2.88 – 2.82 (m, 1H), 2.58 (dd, *J* = 10.2, 8.1 Hz, 1H, H-2), 1.62 – 1.52 (m, 6H), 1.41 – 1.24 (m, 43H), 0.89 (t, *J* = 6.8 Hz, 3H, terminal C*H*$_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 101.9 (C-1), 79.0, 75.9, 73.5 (C-3), 71.4, 71.3, 70.1, 67.8, 62.7 (C-2), 56.7, 51.2, 51.0, 31.69, 31.67, 29.44, 29.39, 29.36, 29.35, 29.33, 29.29, 29.28, 29.25, 29.20, 29.14, 29.08, 28.89, 28.5, 28.3, 26.7, 26.4, 25.8, 22.3, 13.1 (terminal *C*H$_3$). MALDI TOF-MS *m/e* calc'd for $C_{38}H_{79}N_3O_6Na$: 696.5867, measured *m/e*: 696.5871 $[M + Na]^+$

*1-O-Hexadecyl-2-O-methyl-3-O-[2'-amino-6'-N-(3-aminopropyl)-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol **(8)***: Compound **22b** (0.17 g, 0.24 mmol) was treated with ethylenediamine and purified by flash chromatography (dichloromethane/methanol, 3:1, v/v). The resulting compound was subsequently reduced by catalytic hydrogenation and purified by reverse-phase C18 silica gel to give **8** (0.064g, 45.5 %). $[\alpha]_D^{25}$ = -2.8° (*c* = 0.1, methanol); ^{1}H NMR (500 MHz, CD_3OD) 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 – 3.50 (m, 2H), 3.50 – 3.42 (m,

4H), 3.40 – 3.38 (m, 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, 4H), 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, CD_3OD) δ 102.5 (C-1), 79.0, 75.7, 74.5, 72.6, 71.2, 69.8, 68.4, 56.7, 54.7, 51.4, 49.1, 31.7, 29.4, 29.34, 29.26, 29.19, 29.06, 26.3, 25.8, 22.3, 13.0. MALDI TOF-MS *m/e* calc'd for $C_{29}H_{62}N_3O_6$: 548.4697, measured *m/e*: 548.4701 $[M + H]^+$

*1-O-Hexadecyl-2-O-methyl-3-O-[6'-N-(12-N-chlorambucil dodecyl)-2',6'-diamino-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol.2TFA **(9)***: A solution of **24** (0.10 g, 0.086 mmol) in DCM (5.0 ml) was treated with trifluoroacetic acid (2.0 ml) and stirred for 1 h. The reaction was then concentrated in *vacuo* and purified by flash chromatography (dichloromethane/methanol, 5:1, v/v) to give compound **9** (0.07g, 85 %). $[\alpha]_D^{25}$ = 7.3° (*c* = 0.1, methanol); ^{1}H NMR (500 MHz, CD_3OD) δ 7.09 – 7.01 (m, 2H), 6.71 – 6.64 (m, 2H), 4.66 (d, *J* = 8.4 Hz, 1H, H-1), 4.01 (dd, *J* = 10.6, 4.9 Hz, 1H), 3.77 (dd, *J* = 10.7, 3.0 Hz, 1H), 3.74 – 3.44 (m, 18H), 3.27 – 3.11 (m, 4H), 3.09 – 3.04 (m, 2H), 2.99 – 2.85 (m, 1H), 2.51 (m, 2H), 2.16 (t, *J* = 7.6 Hz, 2H), 1.90 – 1.80 (m, 2H), 1.74 – 1.65 (m, 2H), 1.56 (m, 2H), 1.48 (t, *J* = 6.8 Hz, 2H), 1.29 (m, 42H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.5 (C-amide), 144.6, 130.4, 129.2, 112.1, 99.0 (C-1), 78.8, 72.2, 72.1, 71.9, 71.4, 69.4, 69.3, 57.0, 55.8, 53.2, 40.3, 38.9, 35.1, 33.8, 31.6, 29.35, 29.34, 29.31, 29.24, 29.22, 29.19, 29.17, 29.06, 29.04, 28.99, 28.95, 28.8, 27.6, 26.6, 26.2, 25.8, 25.7, 22.3, 13.0. MALDI TOF-MS *m/e* calc'd for $C_{52}H_{96}Cl_2N_4O_7Na$: 981.6554, measured *m/e*: 981.6559 $[M + Na]^+$

*1-(12-aminododecyl)-2,6-diamino-2,6-dideoxy β-D-glucopyranoside (**10**):* Compound **13** (0.11 g, 0.18 mmol) was deprotected following representative procedure B, and the crude was purified by flash chromatography (dichloromethane/ methanol, 7:1, v/v) to afford 1-(12-azidododecyl)-2,6-diamino-2,6-dideoxy glucopyranoside (0.049 g, 67 %). This compound was then treated with catalytic amount of

Pd/C, following representative procedure A, and filtered from the Pd catalyst. The crude was concentrated in *vacuo* and purified by reverse-phase C18 silica gel to give compound **10** (0.032 g, 80 %). $[\alpha]_D^{25}$ = 36.6° (*c* = 0.1, methanol); ^{1}H NMR (300 MHz, CD_3OD) δ 4.28 (d, *J* = 7.9 Hz, 1H, H-1), 3.96 – 3.85 (m, 1H), 3.61 – 3.50 (m, 1H), 3.50 – 3.40 (m, 3H), 3.36 -3.20 (m, 4H), 2.70 – 2.55 (m, 1H, H-2), 1.73 – 1.52 (m, 4H), 1.48 – 1.28 (m, 16H); ^{13}C NMR (75 MHz, CD_3OD) δ 104.4 (C-1), 77.4, 77.2, 72.8, 70.8, 58.4, 52.8, 52.5, 30.8, 30.7, 30.66, 30.65, 30.5, 30.3, 30.0, 27.9, 27.2. MALDI TOF-MS *m/e* calc'd for $C_{18}H_{39}N_3O_4Na$: 400.2578, measured *m/e*: 400.2581 $[M + Na]^+$

*Phenyl 3,4-di-O-acetoxy-6-deoxy-6-azido-2-deoxy-2-N-phthalimido-1-thio-β-D-glucopyranoside (**12**):* Compound **12** was prepared from a commercially available glucosamine hydrochloride (**11**) as previously reported and NMR data were consistent with earlier reports.[31,32]

Representative Procedure C. Glycosylation Reaction.

1-(12-azidododecyl)-6-azido-6-deoxy-3,4-di-O-acetoxy-2-deoxy-2-N-phthalimido β-D-glucopyranoside (**13**): A solution of the thioglycoside donor **12** (0.15 g, 0.29 mmol), 12-azidododecanol (glycoside acceptor **15a)** (0.08 g, 0.35 mmol) and *N*-iodosuccinimide (NIS) (0.10g, 0.44 mmol) in dry CH_2Cl_2 (15.0 ml) were treated with AgOTf (0.011 g, 0.044 mmol) and stirred for 3 h under nitrogen gas. The insoluble NIS was filtered using celite and the filtrate washed with $Na_2S_2O_3$ (×2), $NaHCO_3$ (×3), H_2O (×1) and saturated brine (×1) successively. The organic layer was dried over anhydrous Na_2SO_4, concentrated in *vacuo* and purified by flash chromatography (hexanes/ ethyl acetate, 3:1, v/v) to give (**13**) (0.12 g, 66 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.65 (m, 4H), 5.77 (dd, *J* = 10.8, 9.0 Hz, 1H, H-3), 5.37 (d, *J* = 8.5 Hz, 1H, H-1), 5.02 (dd, *J* = 10.1, 9.0 Hz, 1H, H-4), 4.29 (dd, *J* = 10.8, 8.5 Hz, 1H, H-2), 4.15 – 3.96 (m, 2H), 3.90 – 3.77 (m, 2H), 3.50 – 3.38 (m, 1H), 3.26 – 3.15 (m, 2H), 2.01 (s, 6H), 1.63 – 1.50 (m, 4H), 1.48 – 1.28 (m, 16H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 169.6, 134.3, 131.4,

123.5, 97.9 (C-1), 73.6, 70.6, 70.4, 70.0, 64.6, 54.7, 51.4, 51.2, 29.5, 29.4, 29.3, 29.1, 28.8, 26.7, 25.9, 25.7, 21.0, 20.4, 14.2. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{30}H_{41}N_7O_8Na^+$: 650.80, found: 650.8

N-Azidoalkanals **(16a** and **16b):** Commercially available 12-bromo-1-dodecanol **14a** (0.20 g, 0.75 mmol) was dissolved in dry DMF (5.0 ml) and treated with NaN_3 (0.49 g, 7.54 mmol) at 70 °C for 3 h. The crude product was concentrated under *vacuo*, worked up with H_2O (×2) and brine (×1) successively, and re-concentrated to give 12-azido-1-dodecanol **15a** in excellent yield. This compound was subsequently dissolved in dry DCM (5.0 ml) and treated with pyridinium chlorochromate PCC (0.48 g, 2.25 mmol) for 2 h. The reaction was monitored with TLC using $KMnO_4$ stain. The mixture was filtered through a pad of silica and concentrated under low *vacuo* to give 12-azidododecanal **16a**. Compound **16b** was also prepared from 3-bromo-1-propanol **14b** following the same procedure. The resulting compounds were used immediately without further purification.

*1-O-Hexadecyl-2-O-methyl-3-O-(6'-azido-6'-deoxy-3',4'-di-O-acetoxy-2'-deoxy-2'-N-phthalimido-β-D-glucopyranoside)-sn-glycerol (**18**):* Thioglycoside donor **12** (0.58 g, 1.14 mmol) was glycosylated with 1-*O*-hexadecyl-2-*O*-methyl-*sn*-glycerol **(17**) (0.45 g, 1.36 mmol) as described in representative procedure C, and purified by flash chromatography (hexanes/ethyl acetate, 3:2, v/v) to afford **18** (0.72 g, 70 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.70 (m, 4H), 5.85 (dd, *J* = 10.8, 8.9 Hz, 1H, H-3), 5.39 (d, *J* = 8.4 Hz, 1H, H-1), 5.06 (dd, *J* = 9.5 Hz, 1H, H-4), 4.33 (dd, *J* = 10.8, 8.4 Hz, 1H, H-2), 3.96 – 3.82 (m, 1H), 3.62 (dd, *J* = 10.7, 5.0 Hz, 1H), 3.57 – 3.40 (m, 4H), 3.37 – 3.05 (m, 7H), 2.04 (s, 3H, -COC***H₃***), 1.87 (s, 3H, -COC***H₃***), 1.39 (m, 2H), 1.26 (s, 26H), 0.88 (t, *J* = 6.9 Hz, 3H, CH_2C***H₃***); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 170.1, 134.2, 130.9, 123.5, 98.5 (C-1), 79.9, 78.6, 73.6, 71.9, 71.6, 70.6, 70.4, 69.8, 68.7, 62.7, 57.6, 54.6, 51.2, 31.9, 29.7, 29.65, 29.63, 29.60, 29.5, 29.4, 26.1, 26.0, 22.7, 20.6, 20.5, 14.1. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{38}H_{58}N_4O_{10}Na^+$: 753.41, found: 753.4.

*1-O-Hexadecyl-2-O-methyl-3-O-(2'-amino-6'-azido-2',6'-dideoxy-β-D-glucopyranoside)-sn-glycerol (**19**):* Compound **18** (0.070 g, 0.096 mmol) was deprotected in a single step following representative procedure B, and purified by flash chromatography (dichloromethane/methanol, 4:1, v/v) to give **19** (0.036 g, 72.8 %). ^{1}H NMR (300 MHz, CD_3OD) δ 4.26 (d, *J* = 8.0 Hz, 1H, H-1), 3.91 (dd, *J* = 9.1, 4.5 Hz, 1H), 3.68 (dd, *J* = 10.6, 4.5 Hz, 1H), 3.61 – 3.51 (m, 3H), 3.51 – 3.37 (m, 8H), 3.30 – 3.21 (m, 2H), 2.60 (dd, *J* = 8.0, 3.8 Hz, 1H, H-2), 1.60 1.55 (m, 2H), 1.30 – 1.27 (m, 26H), 0.89 (t, *J* = 6.7 Hz, 3H, -$CH_2C\boldsymbol{H_3}$); ^{13}C NMR (75 MHz, CD_3OD) δ 103.2 (C-1), 79.0, 76.0, 75.8, 71.2, 70.0, 68.0, 56.9, 56.6, 51.3, 31.7, 29.4, 29.3, 29.2, 29.1, 25.8, 22.3, 13.0. ESI-MS: *m/z* $[M + H]^+$ calc'd for $C_{26}H_{53}N_4O_6^+$: 516.39, found: 516.4.

Representative Procedure D. Reductive Amination.

*1-O-Hexadecyl-2-O-methyl-3-O-[2'-N-(12-azidododecyl)-6'-azido-2'6'-dideoxy-β-D-glucopyranoside]-sn-glycerol (**20a**):* A solution of **19** (0.20 g, 0.39 mmol) in dry DCE was treated with 12-azidododecanal (**16a**) (0.088 g, 0.39 mmol) and two drops of acetic acid. The reaction was stirred at RT for 6 h under nitrogen gas. Sodium borohydride, $NaBH_4$ (0.045 g, 1.16 mmol) was then added to the mixture and stirred overnight at 0 °C to RT. The resulting mixture was quenched with saturated sodium bicarbonate, extracted with DCM (×3), concentrated in *vacuo,* and purified by flash chromatography (hexanes/ethyl acetate, 2:1 to 100% ethyl acetate, then dichloromethane/methanol, 20:1, v/v) to afford **20a** (0.20 g, 69 %). ^{1}H NMR (300 MHz, CD_3OD) 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); ^{13}C NMR (75 MHz, CD_3OD) δ 102.7 (C-1), 80.4, 77.4, 74.6, 72.9, 72.8, 71.5, 69.4, 64.0, 58.2,

52.7, 52.5, 33.2, 30.9, 30.86, 30.76, 30.71, 30.6, 30.4, 30.0, 29.3, 28.1, 27.9, 27.3, 23.8, 14.6. ESI-MS: *m/z* $[M + H]^+$ calc'd for $C_{38}H_{75}N_7O_6H^+$: 726.58, found: 726.7

*1-O-Hexadecyl-2-O-methyl-3-O-[2'-N-(3-azidopropyl)-6'-azido-2',6'-dideoxy-glucopyranoside]-sn-glycerol (**20b**):* A solution of **19** (0.15 g, 0.29 mmol) was treated with 3-azidopropanal (**16b**) (0.03 g, 0.30 mmol) via reductive amination (representative procedure D) and purified by flash chromatography (dichloromethane/methanol, 7:1, v/v) to afford **20b** (0.11 g, 64 %). ^{1}H NMR (300 MHz, CD_3OD) 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); ^{13}C NMR (75 MHz, CD_3OD) δ 104.1 (C-1), 80.6, 78.2, 75.7, 72.7, 72.0, 71.5, 69.7, 64.4, 62.7, 58.2, 33.1, 30.80, 30.77, 30.6, 30.5, 29.4, 27.3, 23.8, 14.5. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{29}H_{57}N_7O_6Na^+$: 622.43, found: 622.5

1-O-hexadecyl-2-O-methyl-3-O-(2'-N-phthalimido-6'-amino-2',6'-dideoxy-β-D-glucopyranoside)-sn-glycerol (21): Compound **18** (0.62 g, 0.85 mmol) was dissolved in a solution of sodium methoxide (0.05 g, 0.93 mmol) in methanol (20.0 ml) and stirred for 25 mins. The reaction was monitored with TLC and quenched with a catalytic amount of Dowex 50WX2 (50-100 mesh) ion exchange resin. The resulting mixture was filtered by suction, concentrated in *vacuo* and purified by flash chromatography (hexanes/ethyl acetate, 1:1, v/v). This was then reduced by catalytic hydrogenation (following representative procedure A) and purified by flash chromatography (dichloromethane/methanol, 6:1, v/v) to yield **21** (0.282 g, 55 %). ^{1}H NMR (500 MHz, CD_3OD) δ 7.95 – 7.68 (m, 4H), 5.25 (d, *J* = 8.5 Hz, 1H, H-1), 4.30 (dd, *J* = 10.4, 9.5 Hz, 1H) 4.04 – 3.83 (m, 3H), 3.59 – 3.29 (m, 4H), 3.28 – 3.09 (m, 8H), 1.45 – 1.35 (m, 2H), 1.35 – 1.15 (m, 26H), 0.90 (t, *J* = 6.9 Hz, 3H, -$CH_2C\boldsymbol{H}_3$); ^{13}C NMR (126 MHz, CD_3OD) δ 170.1, 134.1, 122.8, 99.0 (C-1), 78.8, 76.3, 72.8, 71.1, 70.8, 69.4, 67.9, 57.2, 56.4, 42.3, 31.7,

29.4, 29.3, 29.12, 29.10, 29.05, 25.7, 22.3, 13.0. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{34}H_{56}N_2O_8Na^+$: 643.39, found: 644.4.

*1-O-Hexadecyl-2-O-methyl-3-O-[2'-N-pthalimindo-6'-N-(12-azidododecyl)-2',6'-β-D-dideoxy-glucopyranoside]-sn-glycerol (**22a**):* A solution of compound **21** (0.45 g, 0.73 mmol) in DCM was treated with 12-azidododecanal (**16a**) (0.17 g, 0.73 mmol) via reductive amination (representative procedure D) and purified by flash chromatography (dichloromethane/methanol, 9:1, v/v) to afford **22a** (0.46 g, 73 %). ^{1}H NMR (500 MHz, CD_3OD) 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); ^{13}C NMR (126 MHz, CD_3OD) δ 134.1, 134.0, 122.7, 98.8 (C-1), 78.6, 73.2, 71.4, 71.1, 70.6, 69.0, 68.5, 56.7, 56.5, 54.4, 53.4, 33.0, 32.6, 31.7, 29.38, 29.35, 29.33, 29.30, 29.22, 29.20, 29.18, 29.17, 29.15, 29.10, 29.06, 29.05, 28.98, 28.4, 27.8, 26.5, 25.8, 24.4, 22.3, 13.0. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{46}H_{79}N_5O_6Na^+$: 853.09, found: 853.1

*1-O-Hexadecyl-2-O-methyl-3-O-[2'-N-pthalimido-6'-N-(3-azidopropyl)-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol (**22b**):* A solution of **21** (0.21 g, 0.34 mmol) in DCM was treated with 3-azidopropanal (**16b**) (0.035 g, 0.34 mmol) via reductive amination (representative procedure D) and purified by flash chromatography (dichloromethane/methanol, 10:1, v/v) to afford **22b** (0.17 g, 70 %). ^{1}H NMR (500 MHz, CD_3OD) δ 7.92 – 7.78 (m, 4H), 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); ^{13}C NMR (126 MHz, CD_3OD) δ 134.42, 133.93, 122.0, 97.8 (C-1), 77.0, 73.4, 70.6, 70.4, 70.2, 69.3, 68.0, 55.9, 56.4, 54.4,

53.4, 31.5, 30.4, 29.25, 29.23, 29.19, 29.17, 29.15, 29.10, 29.06, 29.05, 28.98, 28.4, 27.8, 26.5, 25.7, 24.5, 22.3, 13.0. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{37}H_{61}N_5O_8Na^+$: 726.44, found: 726.5

*1-O-Hexadecyl-2-O-methyl-3-O-[6'-N-Boc-(12-azidododecyl)-2'-N-boc-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol **(23)**:* Compound **22a** (0.45 g, 0.53 mmol) was deprotected following representative procedure B and purified by flash chromatography (dichloromethane/methanol, 3:1, v/v). This compound was then treated with Boc_2O (0.23 g, 1.05 mmol) and Et_3N (0.2 ml, 1.44 mmol) in methanol, and stirred at 50 °C for 5 h. The resulting mixture was concentrated in *vacuo* and purified by flash chromatography (hexanes/ethyl acetate, 1:1, v/v) to afford **23** (0.21 g, 51.2 %). ^{1}H NMR (500 MHz, CD_3OD) δ 4.28 (d, *J* = 8.1 Hz, 1H, H-1), 3.81 (dd, *J* = 10.5, 4.6 Hz, 1H), 3.64 – 3.52 (m, 3H), 3.50 – 3.40 (m, 8H), 3.39 – 3.32 (m, 5H), 3.29 – 3.25 (m, 2H), 3.18 – 3.08 (m, 1H), 1.63 – 1.51 (m, 6H), 1.45 (s, 18H, *boc*), 1.41 – 1.24 (m, 42H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 157.0, 156.8, 128.5, 102.1 (C-1), 79.8, 79.5, 79.1, 78.57, 78.53, 75.3, 73.8, 72.2, 71.3, 70.1, 68.0, 56.9, 51.1, 36.2, 31.7, 29.41, 29.38, 29.31, 29.29, 29.27, 29.23, 29.1, 28.9, 28.5, 27.5, 27.4, 26.6, 26.5, 25.9, 24.2, 22.3, 13.1. ESI-MS: *m/z* $[M + Na]^+$ calc'd for $C_{48}H_{93}N_5O_{10}Na^+$: 922.68, found: 922.8

*1-O-Hexadecyl-2-O-methyl-3-O-[6'-N-Boc-(12-N-chlorambucil dodecyl)-2'-N-boc-2',6'-dideoxy-β-D-glucopyranoside]-sn-glycerol **(24)**:* A solution of chlorambucil (0.067 g, 0.22 mmol) and diisopropylethylamine (0.2 ml) in dry DMF (5.0 ml) was pre-activated with TBTU (0.07 g, 0.22 mmol). In a separate flask, compound **23** (0.21 g, 0.23 mmol) was reduced by catalytic hydrogenation following representative procedure A, concentrated, and added to the solution containing the pre-activated chlorambucil. This was stirred for 5 h and purified by flash chromatography (hexanes/ethyl acetate, 1:1, v/v) to yield **24** (0.15 g, 55.6 %). ^{1}H NMR (500 MHz, CD_3OD) δ 7.09 – 7.01 (m, 2H), 6.71 – 6.64 (m,

2H), 4.66 (d, *J* = 8.4 Hz, 1H, H-1), 4.01 (dd, *J* = 10.6, 4.9 Hz, 1H), 3.77 (dd, *J* = 10.7, 3.0 Hz, 1H), 3.74 – 3.44 (m, 18H), 3.27 – 3.11 (m, 4H), 3.09 – 3.04 (m, 2H), 2.99 – 2.85 (m, 1H), 2.51 (t, *J* = 7.6 Hz, 1H), 2.16 (t, *J* = 7.6 Hz, 2H), 1.90 – 1.80 (m, 2H), 1.74 – 1.65 (m, 2H), 1.56 (t, *J* = 6.8 Hz, 2H), 1.48 (t, *J* = 6.8 Hz, 2H), 1.45 (s, 18H, *Boc*), 1.29 (m, 44H), 0.89 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OD) δ 174.5 (C-amide), 157.0, 156.8, 144.6, 130.4, 129.2, 128.5, 112.1, 98.8 (C-1), 78.6, 72.1, 72.0, 71.8, 71.3, 69.4, 69.3, 57.0, 55.8, 53.2, 40.3, 38.9, 35.1, 33.8, 31.6, 29.35, 29.34, 29.31, 29.24, 29.22, 29.19, 29.17, 29.06, 29.04, 28.99, 28.95, 28.8, 27.6, 26.6, 26.2, 25.8, 25.7, 22.3, 13.0. ESI-MS: *m/z* $[M + H]^+$ calc'd for $C_{62}H_{112}Cl_2N_4O_{11}Na^+$: 1181.76, found: 1181.8

Biology. The cytotoxic effects of compounds **1, 5 – 10** against a panel of human epithelial cell lines were assessed using the MTS assay.[32] The cell lines were derived from cancers of the breast (JIMT1, BT474, MDA-MB-231, MDA-MB-453, MDA-MB-468, BT549, Hs578t), pancreas (MiaPaCa2), prostate (DU145, PC3), brain (U87, U251) and ovaries (A2780s, A2780cp). Exponentially growing cells were treated with test compounds and then incubated for 48 h. All compounds were tested up to 20 μM and **7** re-tested at much lower concentrations (Figure 4). The results for compounds **1, 5 – 10** are shown in figure 3 and the CC_{50} values are displayed in Tables 1 and 2, along with the values obtained for **1** and **4** from previous studies.[14]

Cell Culture

MDA-MB-231, BT474, MiaPaCa2, DU145 and PC3 cell lines were grown from frozen stocks of cell lines that were originally obtained from ATCC (Manassas, VA, USA). JIMT1 cells were grown from frozen stocks of cells obtained from DSMZ (Braunschweig, Germany). MDA-MB-231, JIMT1 and DU145 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), PC3 cells grown in F12K medium while BT474 cells were grown in Hybricare medium (ATCC). MiaPaCa2 was grown in DMEM supplemented with FBS to a concentration of 10 % and horse serum to a final concentration of 2.5 %. The cells were grown in media supplemented with 10 % fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (0.1 mg/ml) in a humidified 5 % atmospheric incubator at 37 ºC.

Isolation of ALDH-positive breast cancer cell population

The ALDEFLUOR assay kit (STEMCELL Technologies, Vancouver, BC, Canada) was used to isolate a CSC enriched cell population from BT474 cells according manufacturer's instructions, with non-stained cells and cells stained in the presence of ALDH1 inhibitor (diethylaminobenzaldehyde, DEAB) as controls for the sorting. The optimum time for staining at 37 °C was determined to be 45 mins for

BT474 cells. Following the staining, ALDH1-stained cells were sorted from the bulk population by flow cytometery on a 4-laser MoFloXPP high speed/pressure cell sorter. The sorted cells were pelleted by centrifugation, resuspended in supplemented Mammocult medium (STEMCELL Technologies) and dispersed into ultra-low adhesive 6-well plates or 35 mm dishes for 7 days to allow the formation of spheroids (mammospheres). After 7 days, the spheres were separated from the media and single cells by sieving the contents of the well through a 40 μm nylon cell strainer (BD Falcon). The spheres retained in the sieve were washed with Hanks buffer and subsequently trypsinised to obtain a single cell suspension of CSCs. The cell numbers were determined with a Coulter ZM counter and the cells were seeded into the appropriate low adhesion tissue culture ware for various studies.

Cytotoxicity Assay

Cell viability was determined with the CellTitre 96 Aqueous One solution (MTS assay kit; Promega). Equal numbers of cancer cells (7500 – 9000) in media (100 μL) were dispersed into 96-well plates. As blanks, media without cells (100 μL) were also placed in some wells and treated similarly to the cell-containing wells. After an incubation period of 24 h, a solution of test compound (100 μL) in medium at twice the desired concentration was added to each well. The treated cells were then incubated further for 48 h, after which 20 % v/v MTS reagent, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium was added to each well. The plates were then incubated for 1 – 4 h on a Nutator mixer in a 5 % CO_2 incubator. The optical density (OD) was read at 490 nm on a SpectraMax M2 plate reader (Molecular Devices Corp., Sunnyvale, CA, USA). The values of blank were subtracted from each value and the viability values of the treated samples relative to the controls with vehicle were calculated. The values for the plots are the means ± standard deviation. Values of zero indicate there are no viable cells in the wells.

The caspase inhibition assay was performed in similar fashion to MTS assay with the addition of 40 μM pan-caspase inhibitor **25** after the first 24 h of cell incubation.

Membrane Permeability Assay

JIMT1 and DU145 were grown in DMEM medium supplemented with 10 % FBS and antibiotics (penicillin and streptomycin). Equal numbers of the cells were dispersed into 96-well plates. After 24 h, the cells were incubated with varying concentrations of compound **7** (4 - 6 μM) for 5 - 6 h. Subsequently, the cell membrane impermeant DNA-staining dye, ethidium homodimer-1 (EthD-1, Molecular Probes) at a final concentration of 2 μM was added and the cells analyzed by fluorescence microscopy. EthD-1 staining was compared to negative controls with no treatment and positive control treated with 0.01% Triton X-100 for 10 mins.

Hemolytic Assay

The hemolytic activity of **7** was determined using ovine red blood cell of a one-year old lamb, following a modified protocol.[52] The erythrocytes were washed three times in a buffered saline (10 mM Tris, 150 mM NaCl, pH 7.4) just prior to the assay. The final cell concentration used was 2.5×10^8 cells/mL. The cell suspension (350 μL) and varying amounts of buffer and test compound stock solution were pipetted into Eppendorf tubes to give a final volume of 1500 μL. The suspensions were then incubated for 30 minutes with gentle shaking and subsequently cooled in ice water. This was then centrifuged at 2000g at 4 °C for 5 minutes and 200 μL of the resulting supernatant mixed with 1800 μL of 0.5 % NH_4OH. The optical density (OD_{540}) was then determined at 540 nm. The blank (0 % hemolysis) and positive (100 % hemolysis) control were then determined similarly using the supernatants obtained after centrifugation of 350 μL erythrocyte stock suspension diluted and incubated in 1150 μL of buffer and 1.0 % NH_4OH

respectively. % hemolysis was calculated as: [% hemolysis = (X – 0%)/ (100% - 0%)], where X is the optical density (OD) values of compound at varying concentrations.

Statistical Analysis

The results of the study represent the mean ± standard deviation of multiple independent determinations, as described in the experimental section. Preliminary statistical analysis was performed using a two-tailed paired student's test to evaluate the null hypothesis, and further subjected to one-way analysis of variance (ANOVA). The cut-off level for statistical significance was set to 5 %, i.e. $p < 0.05$, when compared to vehicle-treated cells (blank) under same conditions. A p-value ≥ 0.05 was considered statistically insignificant.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org. Purity analysis, ^{1}H and ^{13}C NMR spectral of compounds **5 – 10**, molecular formula strings.

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Notes

The authors declare that a patent WO2015/179983A1 has been filed on the contents of this paper

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ABBREVIATIONS USED

CADs, cationic amphiphilic drugs; CSCs, cancer stem cells; ER, estrogen receptor; GAELs, glycosylated antitumor ether lipids; GDG, glucosamine-derived glycerolipid; HER, hormonal epidermal receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer.

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negative bacteria to rifampicin and minocycline. *J. Med. Chem.* **2017**, *60*, 3684–3702.

Table 1

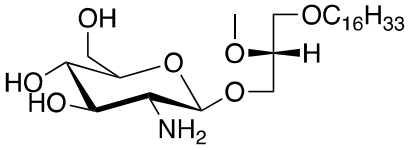
Compd	JIMT1	MDA-MB-231	BT474	MiaPaCa2	DU145	PC3
1[b]	9.0	7.1	8.0	9.0	10.0	13.5
4[b]	27.0	NT	NT	18.0	16.5	24.0
5	8.0	5.5	13.5	8.5	7.5	8.5
6	12.5	17.5	>20	14.5	18.5	14.5
7	3.4	1.5	1.6	4.0	3.8	2.0
8	13.5	13.5	>20	18.5	16.5	12.5
9	16.0	10.5	13.0	>20	>20	>20
10	>20	>20	>20	>20	>20	>20

[a]Breast (JIMT1, MB-MDA-231, BT474), pancreas (MiaPaCa2) and prostate (DU145, PC3) cancer cell lines. The CC_{50} value is defined as the concentration required to decrease cell viability by 50% relative to the untreated control. Values were determined by MTS assay. The CC_{50} values were obtained by estimating the drug concentration at 50% viability on the *y-axis* of the plots for each cell line. NT: Not Tested. [b]*(Previously published)*.[14]

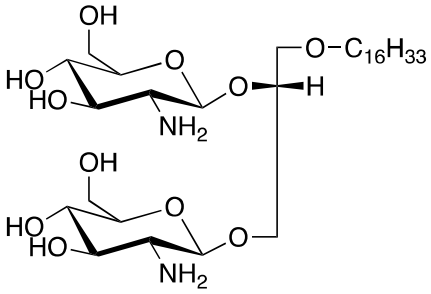
Table 2

Classification	Cell lines	CC_{50} (μM)
Breast (Triple-negative)	MDA-MB-453	4.0
	MDA-MB-468	2.0
	BT549	2.5
	Hs578t	1.8
Brain	U87	2.5
	U251	2.6
Ovarian	A2780s	2.4
	A2780cp	3.8

Figure 1



1



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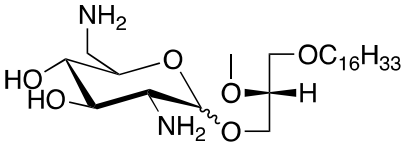

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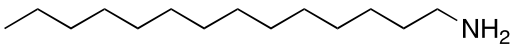

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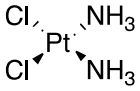
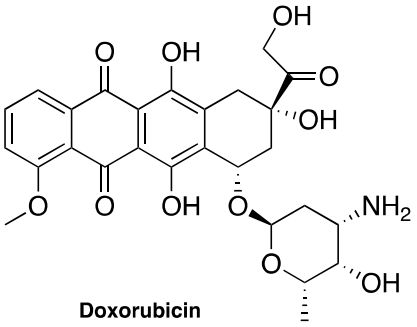
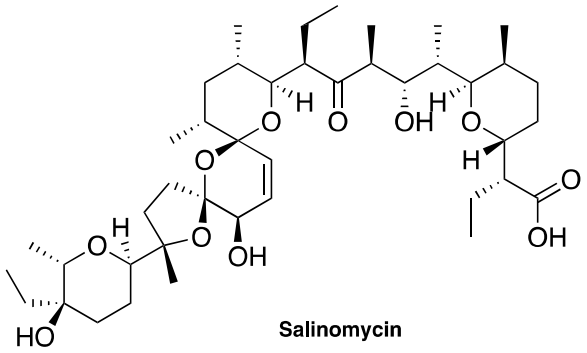


Cisplatin

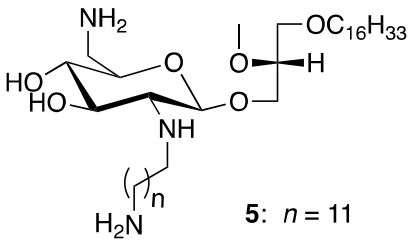


Doxorubicin



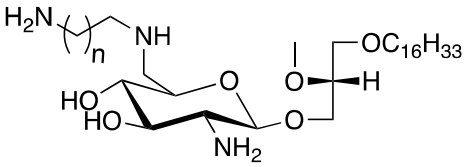
Salinomycin

Figure 2



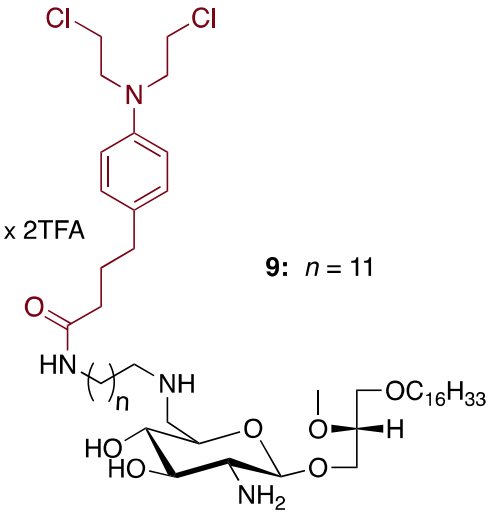
5: $n = 11$

6: $n = 2$

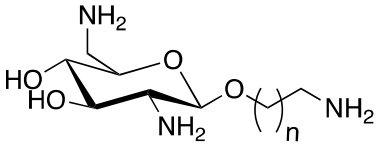


7: $n = 11$

8: $n = 2$



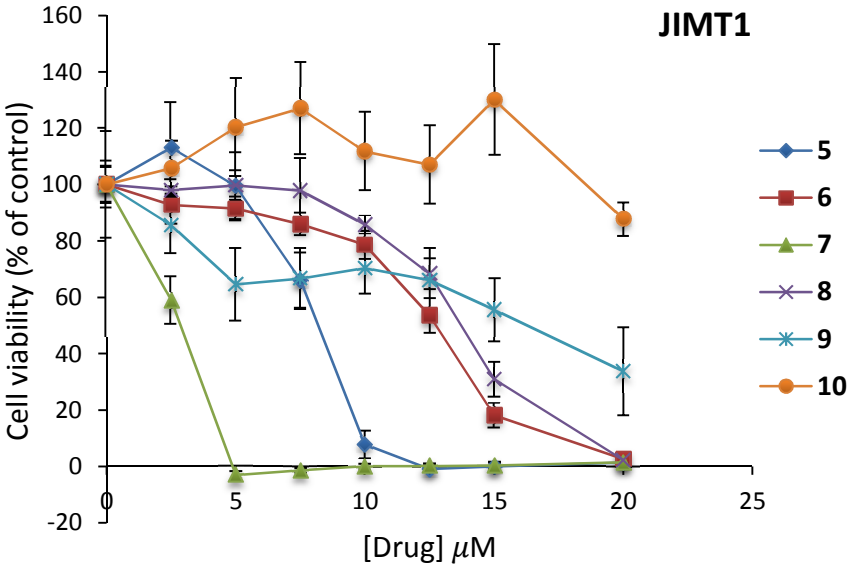
9: $n = 11$

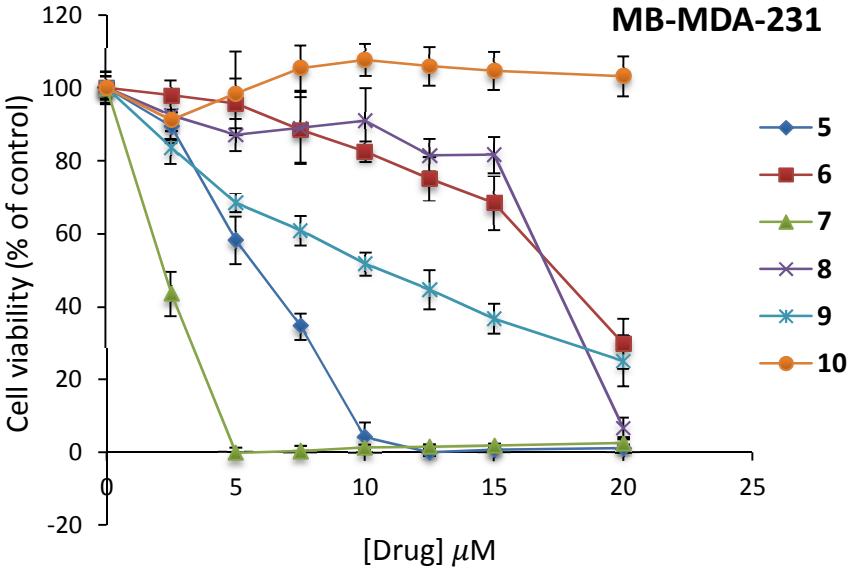


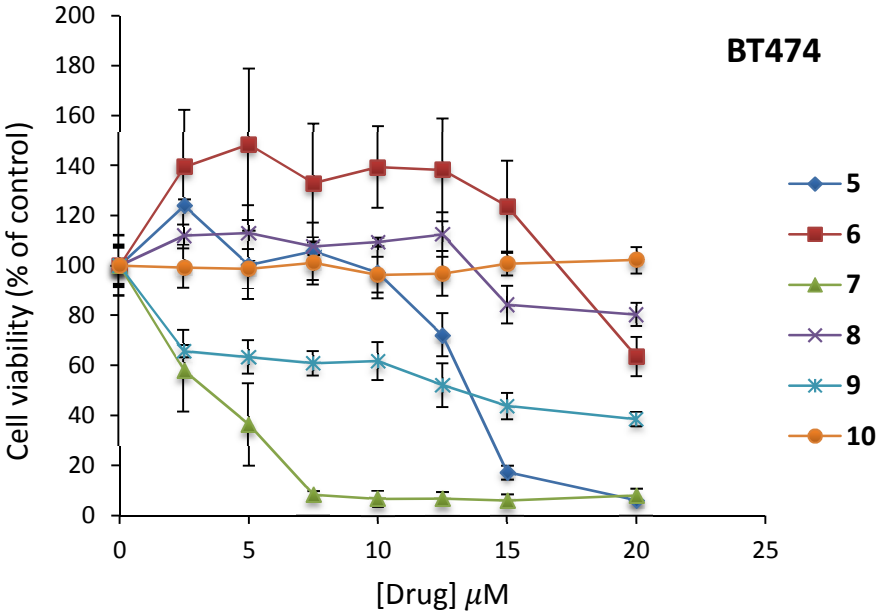
10: $n = 11$

Figure 3

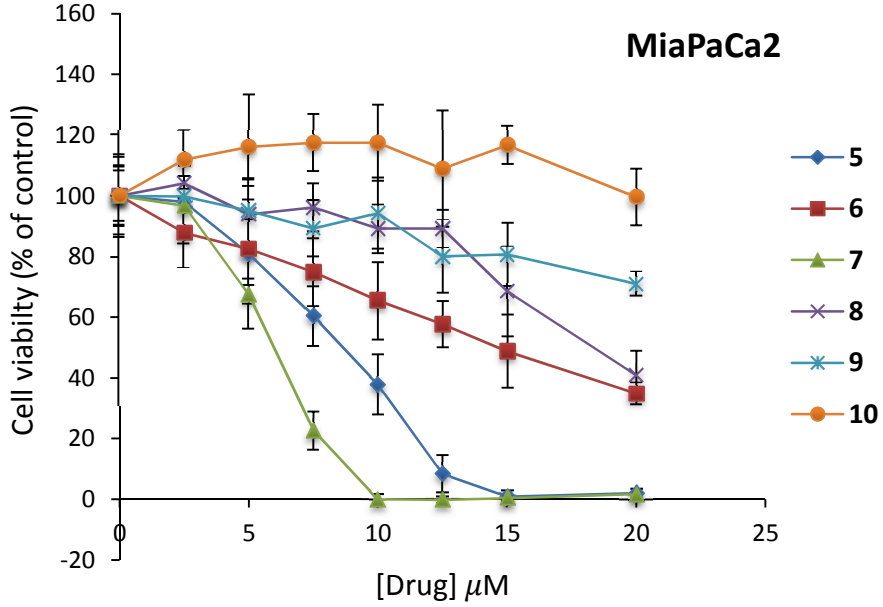
A. Breast (JIMT1, MDA-MB-231, BT474)



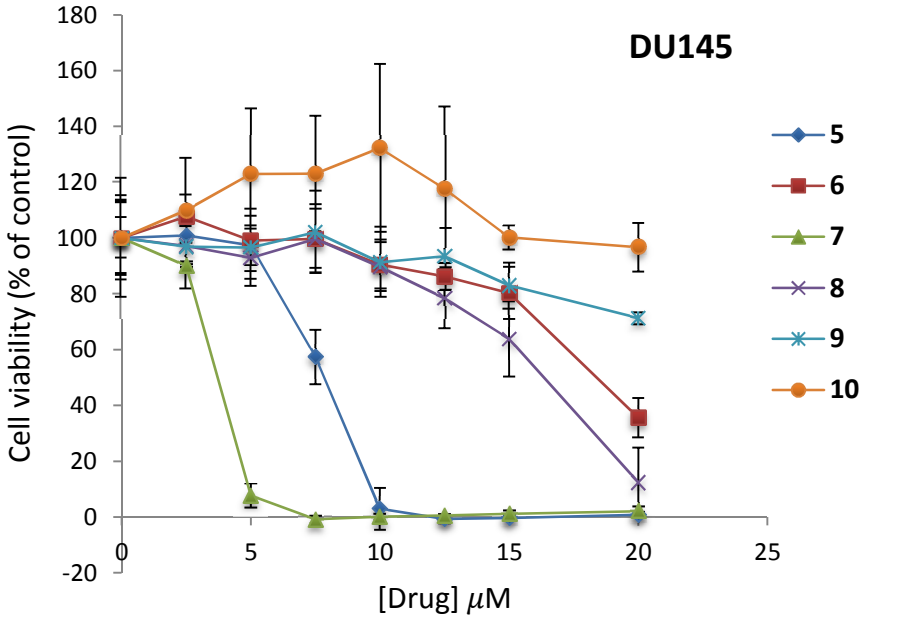


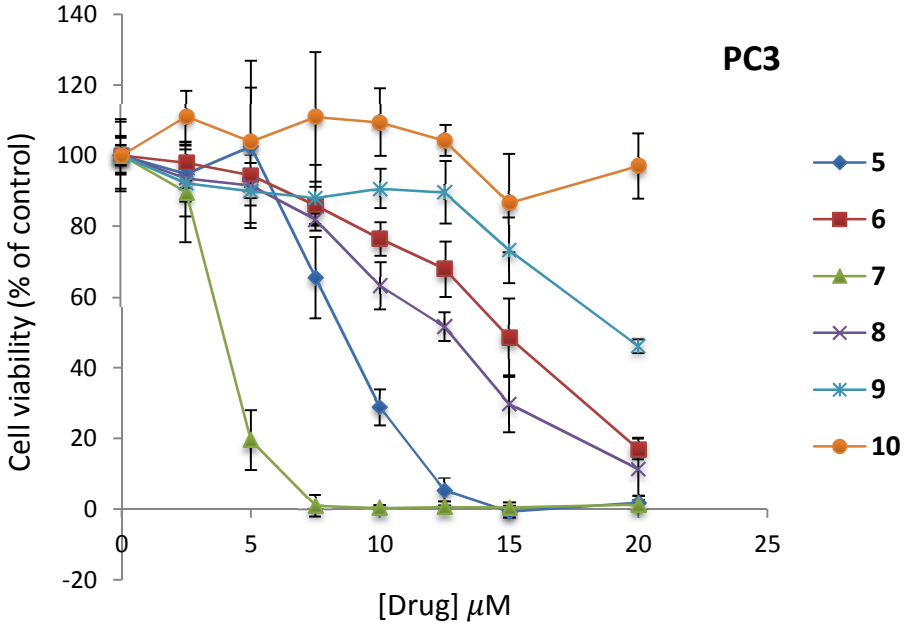


B. Pancreas (MiaPaCa2)

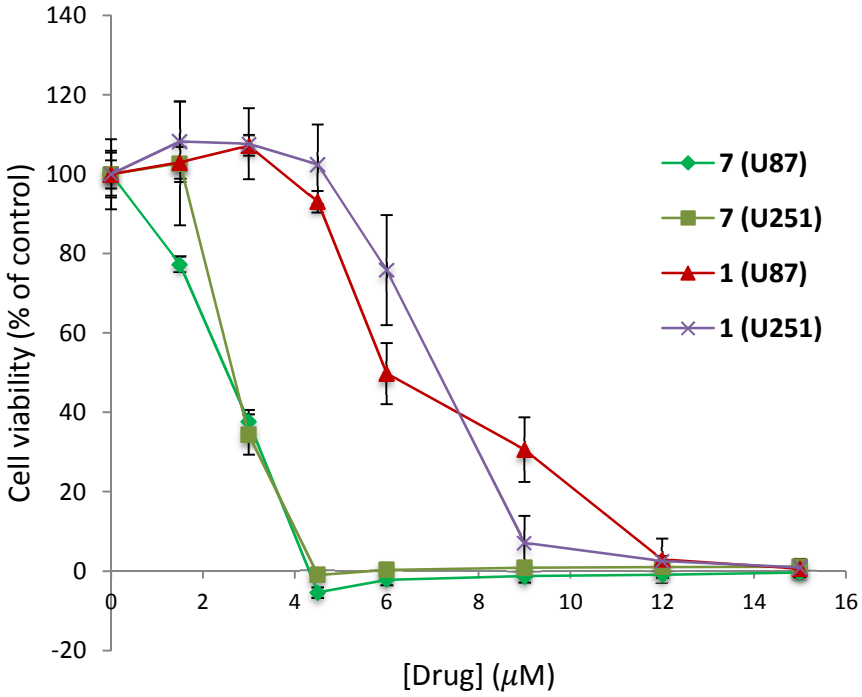


C. Prostate (DU145, PC3)

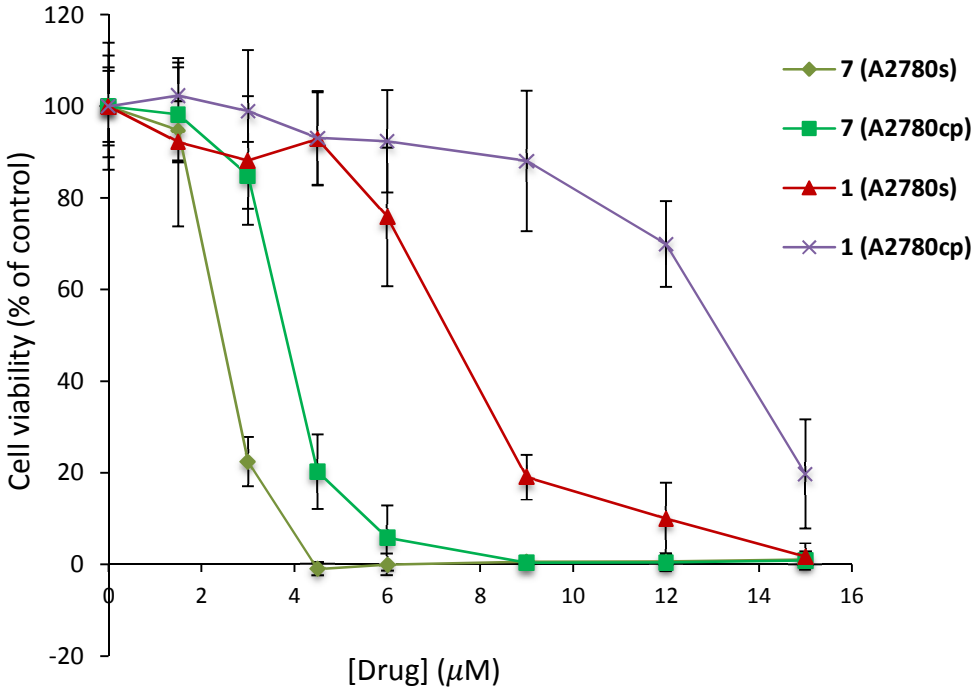




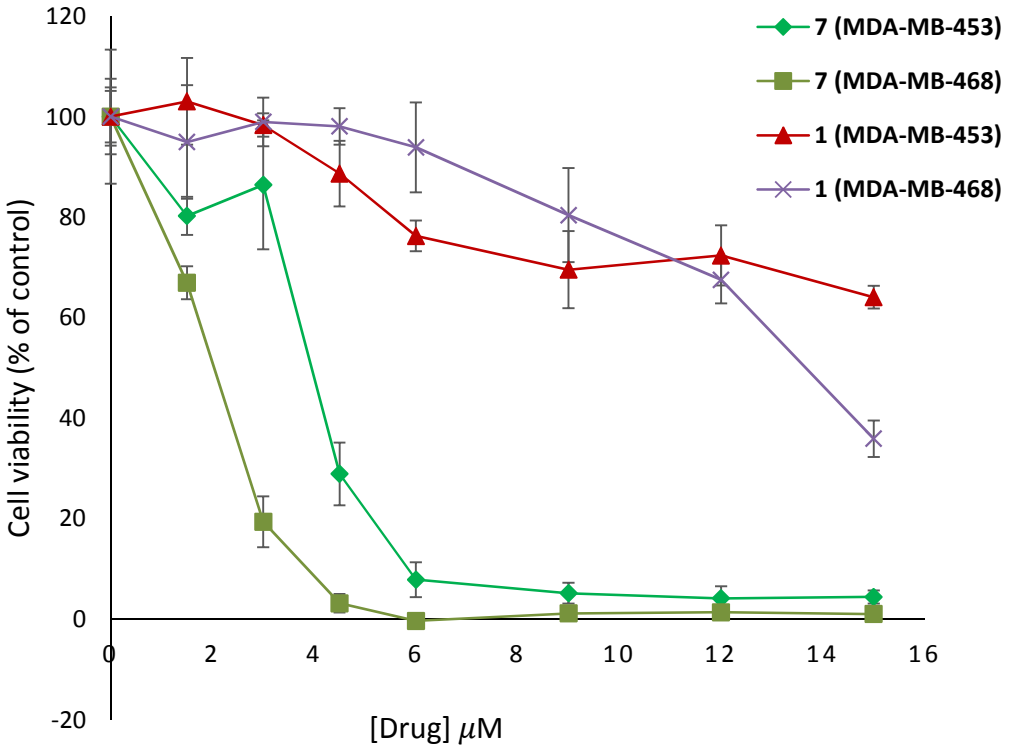
D. Brain (U87, U251)

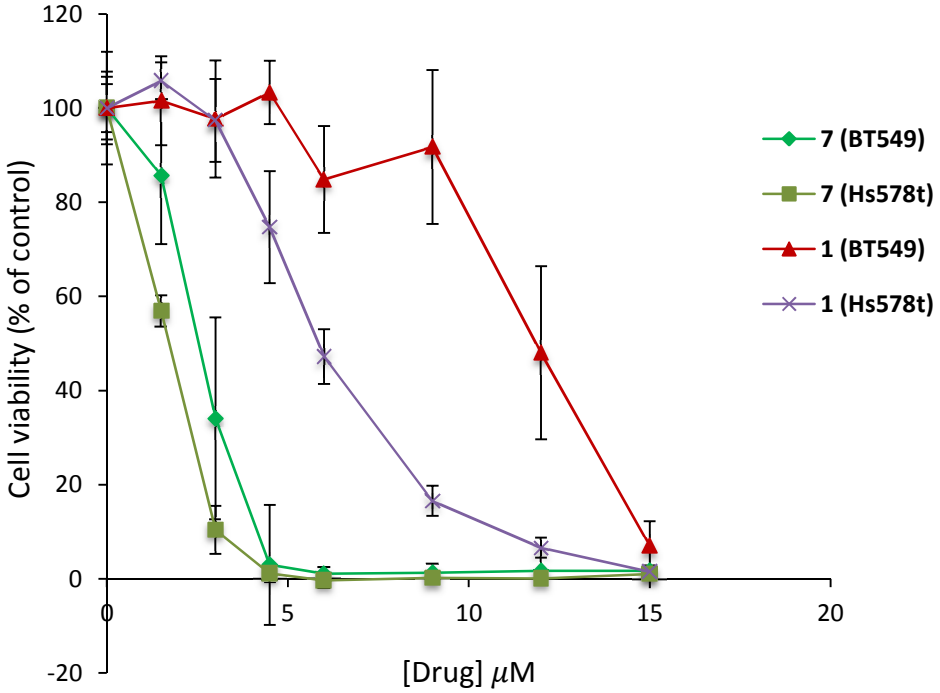


E. Ovarian (A2780s, A2780cp)

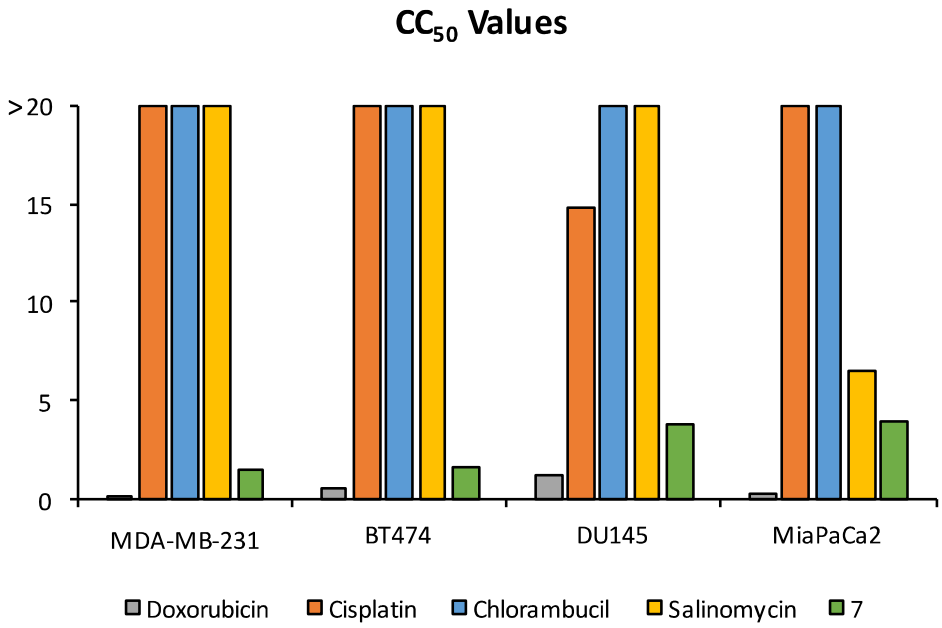


F. Triple negative breast (MDA-MB-453, MDA-MB-468, BT549, Hs578t)



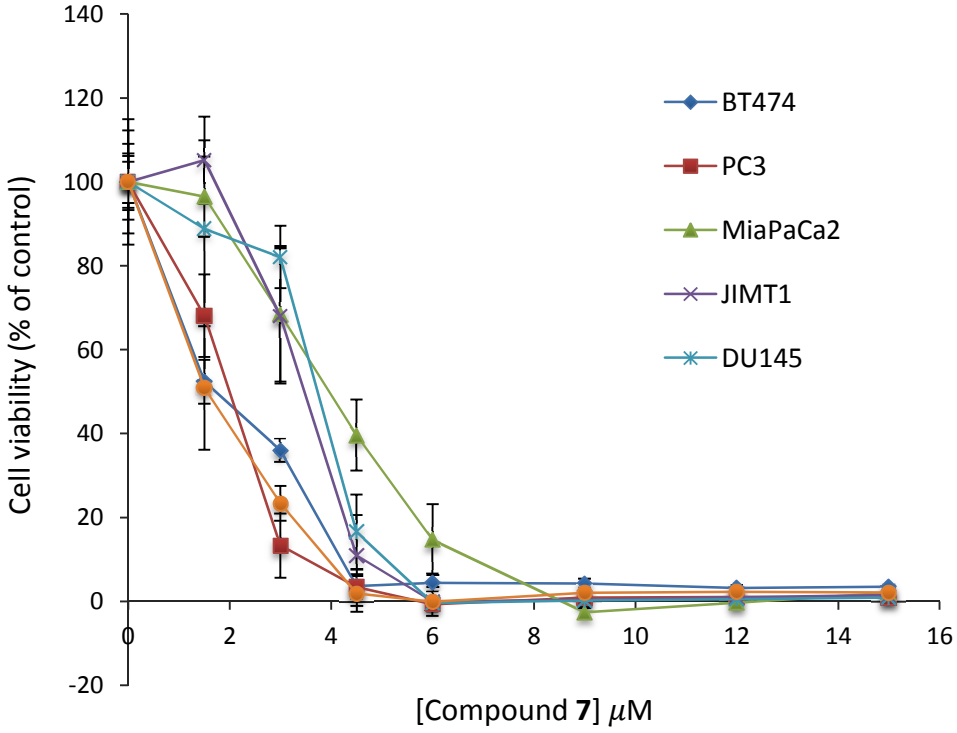


G.



[a]The cancer cell lines were grown in 96-well plates and incubated with varying concentrations (0 – 20 μM) of the test compounds for 48 h. MTS reagent was added at the end of the incubation and the plates were incubated further for 1 – 4 h. The OD_{490} was read in a plate reader. Wells with media but no cells were treated in similar fashion and the values utilized as blanks. The results represent the mean ± SD of six independent determinations.

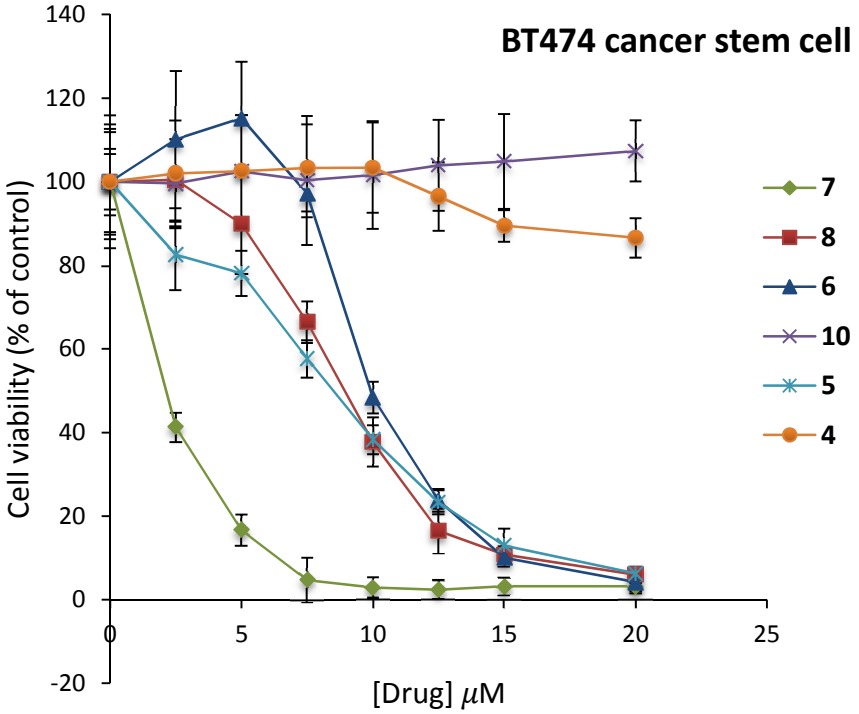
Figure 4



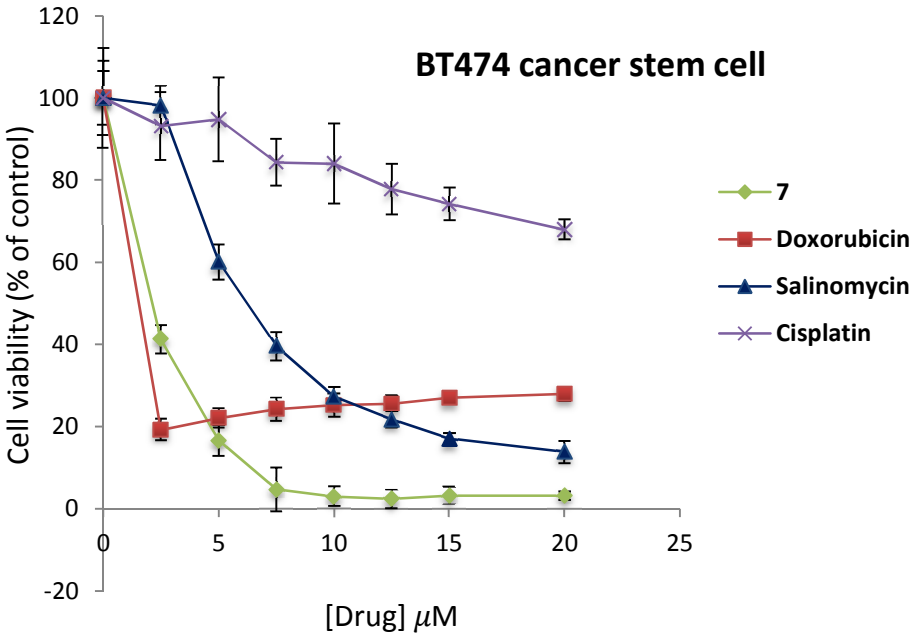
[a]Error bars indicate standard deviations from six representative experiments (n = 6)

Figure 5

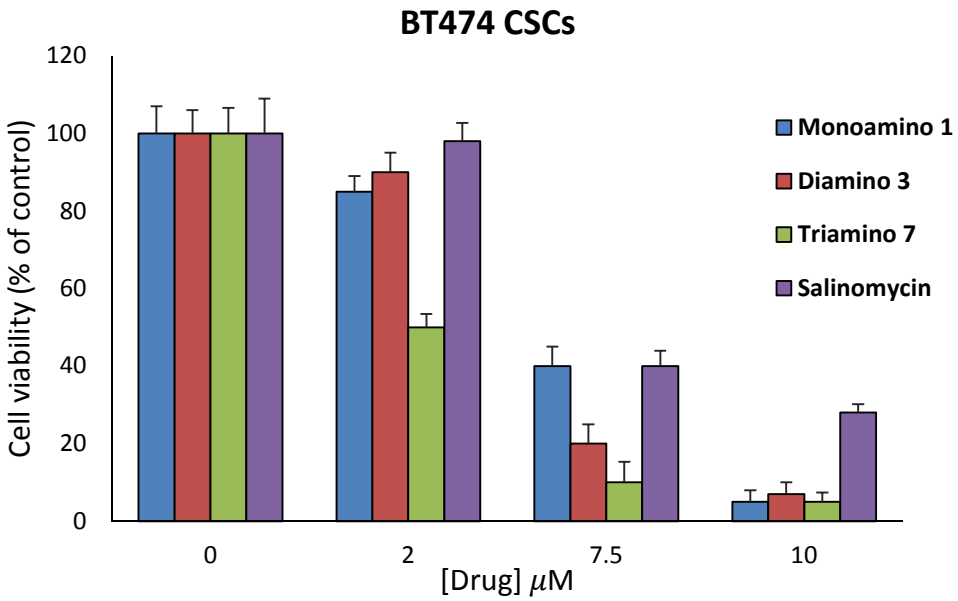
A.


BT474 cancer stem cell
Cell viability (% of control)
[Drug] μM
7
8
6
10
5
4

B.

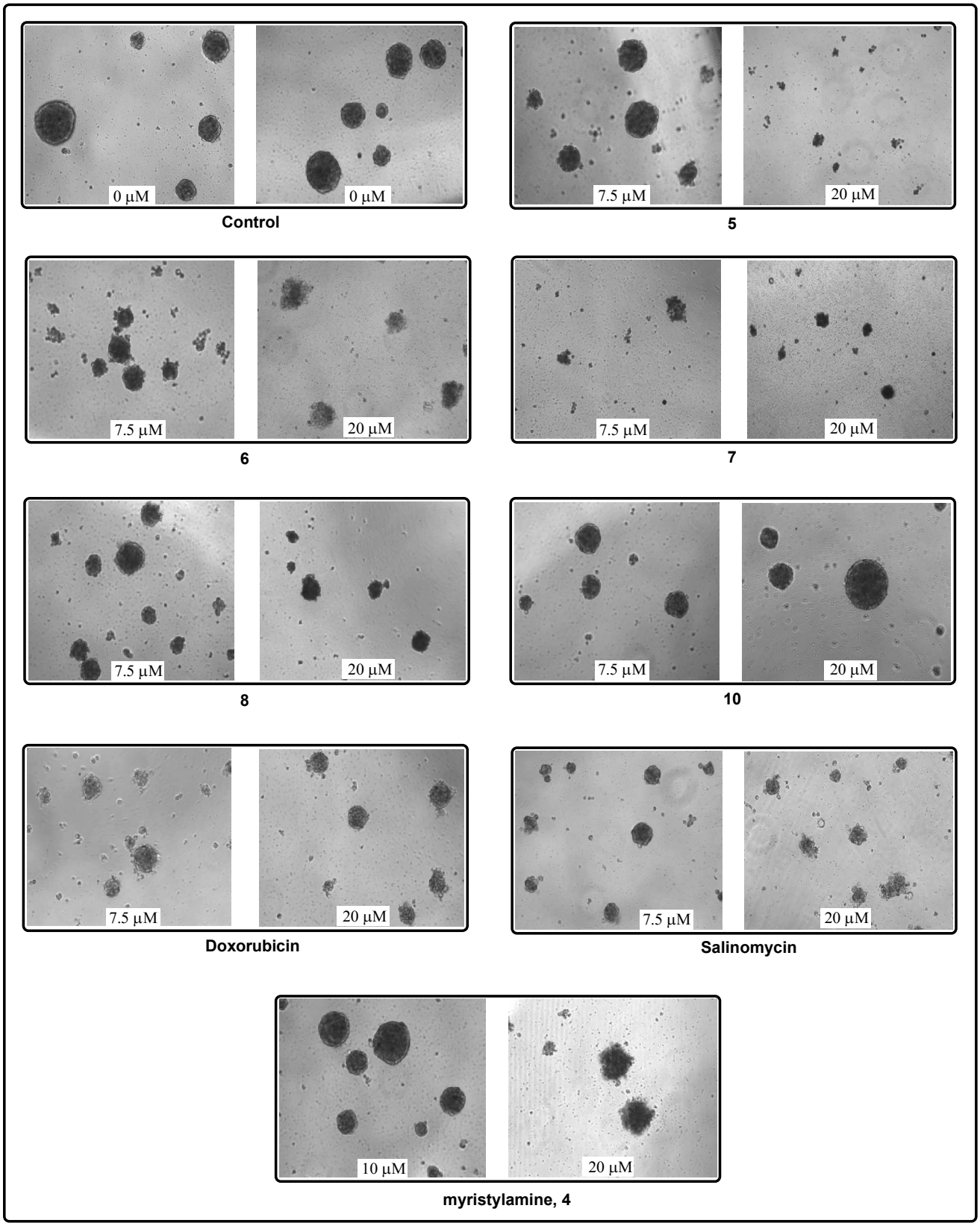

BT474 cancer stem cell
Cell viability (% of control)
[Drug] μM
7
Doxorubicin
Salinomycin
Cisplatin

C.



[a]α-diamino **3** was used for comparison as it was slightly more potent than its β-anomer

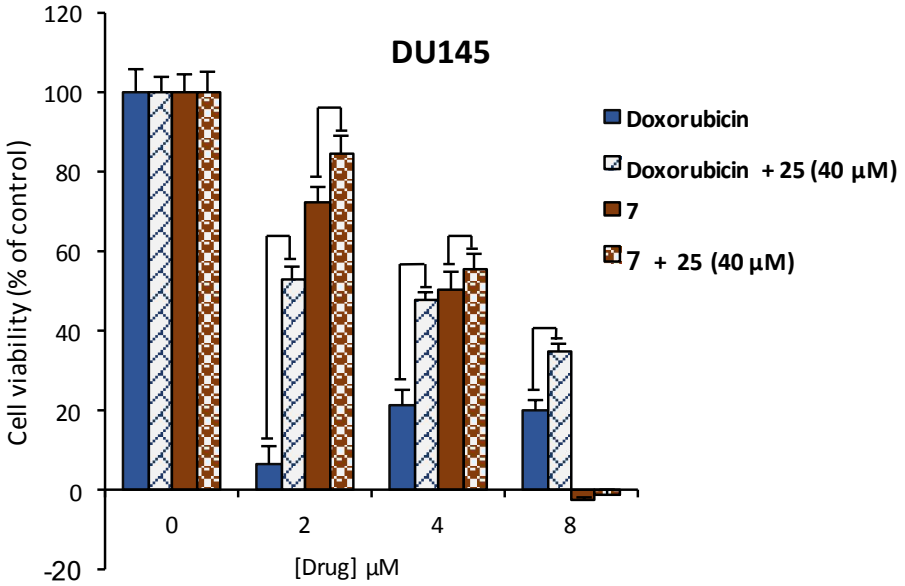
D.

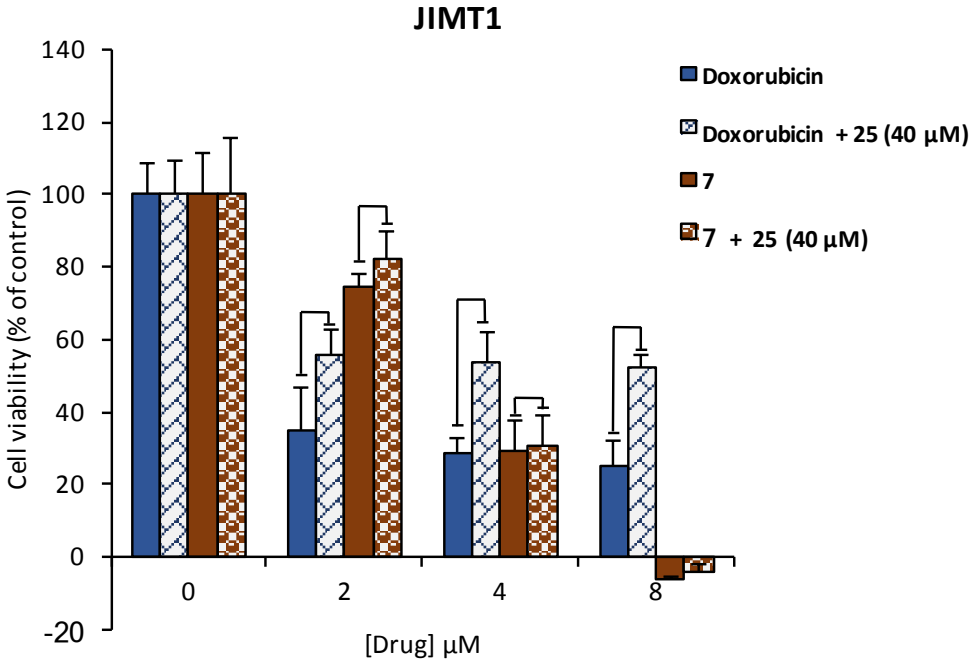

0 μM
0 μM
Control
7.5 μM
20 μM
5
7.5 μM
20 μM
6
7.5 μM
20 μM
7
7.5 μM
20 μM
8
7.5 μM
20 μM
10
7.5 μM
20 μM
Doxorubicin
7.5 μM
20 μM
Salinomycin
10 μM
20 μM
myristylamine, 4

[a]Equal numbers of BT474 cancer stem cells were dispersed into ultra-low adhesion 48-well plates in mammocult medium and incubated for 6 days to form spheroids. Compounds **4** – **8**, **10**, doxorubicin, salinomycin, and cisplatin (0, 7.5, 20 μM) were added to the spheres and incubated for 3 days. At the end of the incubation, images were taken with an Olympus IX70 microscope (magnification ×10) to assess the integrity (D). Subsequently, the viability (A – C) of the cells in the wells was determined by adding MTS reagent and incubating in a 5 % CO_2 incubator for 5 h. Wells with only medium but no cells were treated in an identical manner to serve as blanks for viability (A – C) studies while wells with both medium and cells but no drugs were treated in similar manner to serve as control for integrity (D) studies. The results are the means ± standard deviation of 6 independent wells.

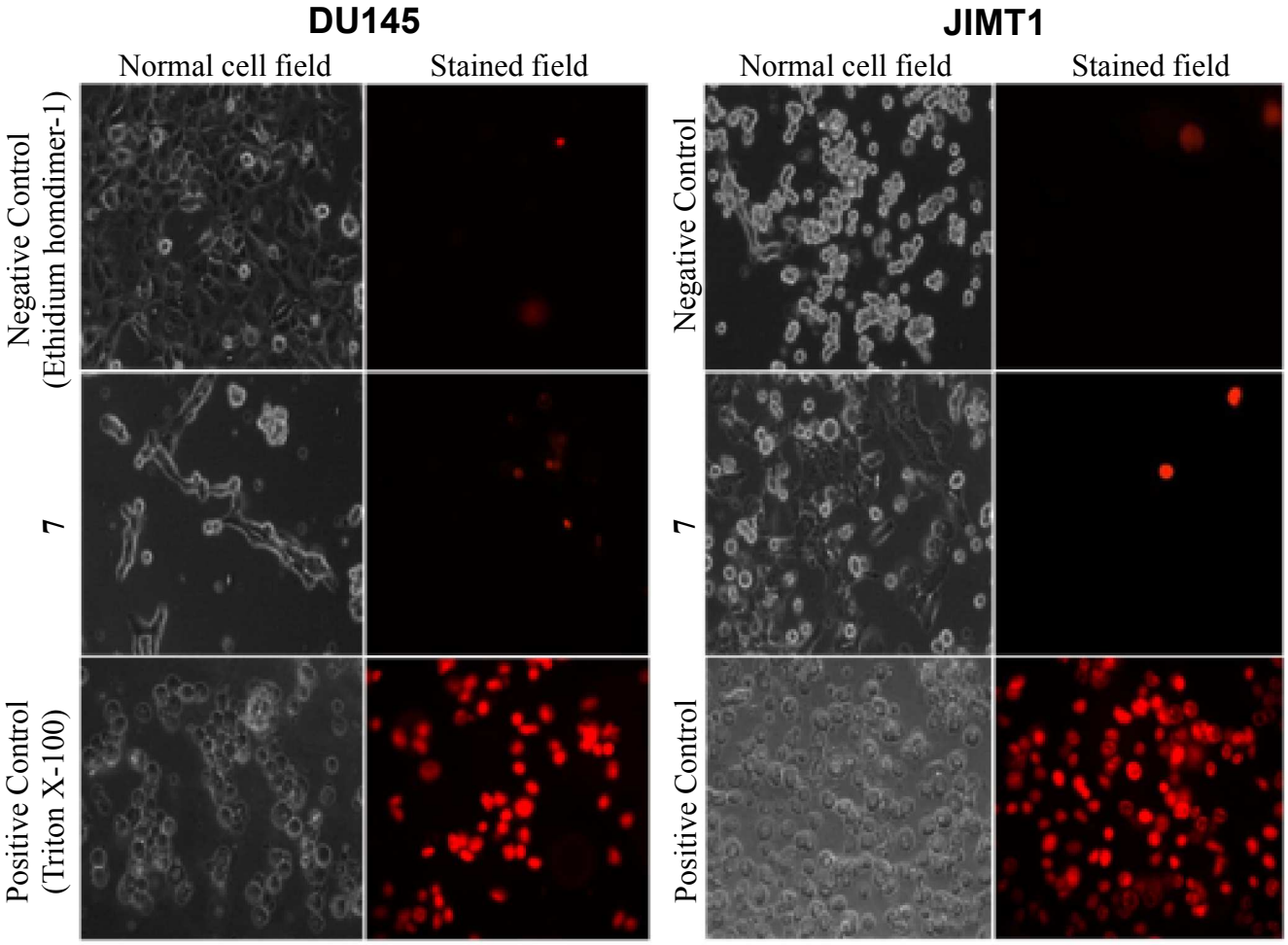
Figure 6

A.


DU145
Cell viability (% of control)
120
100
80
60
40
20
0
-20
0
2
4
8
[Drug] µM
Doxorubicin
Doxorubicin + 25 (40 µM)
7
7 + 25 (40 µM)

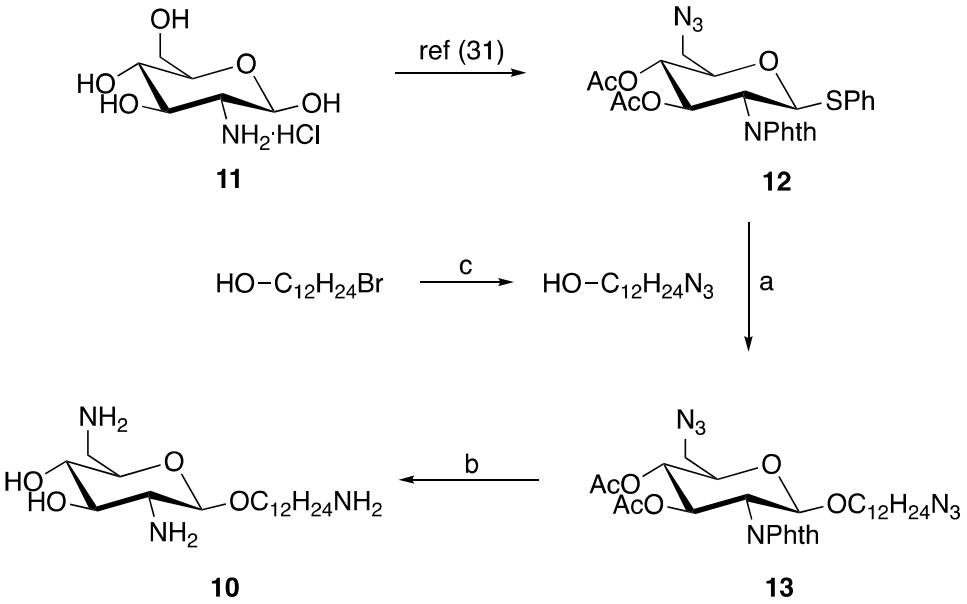

JIMT1
Cell viability (% of control)
140
120
100
80
60
40
20
0
-20
0
2
4
8
[Drug] µM
Doxorubicin
Doxorubicin + 25 (40 µM)
7
7 + 25 (40 µM)

B.



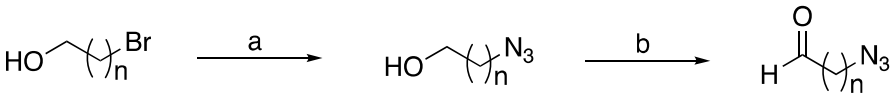
[a]Pan-caspase inhibition of DU145 and JIMT1 cells when treated with varying concentrations of doxorubicin and compound **7**, with and without 40 μM of inhibitor **25** (A); and micrographs of DU145 and JIMT1 cells taken 4 h after treatment with ethidium homodimer-1 dye alone (Negative control), compound **7**, and 1 μL of 0.01 % Triton X-100 (Positive control). Magnification ×20. Red color indicates nuclear DNA stained with dye.

Scheme 1



[a]Reagents and conditions: a) AgOTf, NIS, CH_2Cl_2 (66 %); b) i) ethylenediamine, 1-butanol, 90 °C (71 %), ii) Pd/C, H_2, MeOH (75 %); c) NaN_3, DMF, 70 °C (80 %);

Scheme 2



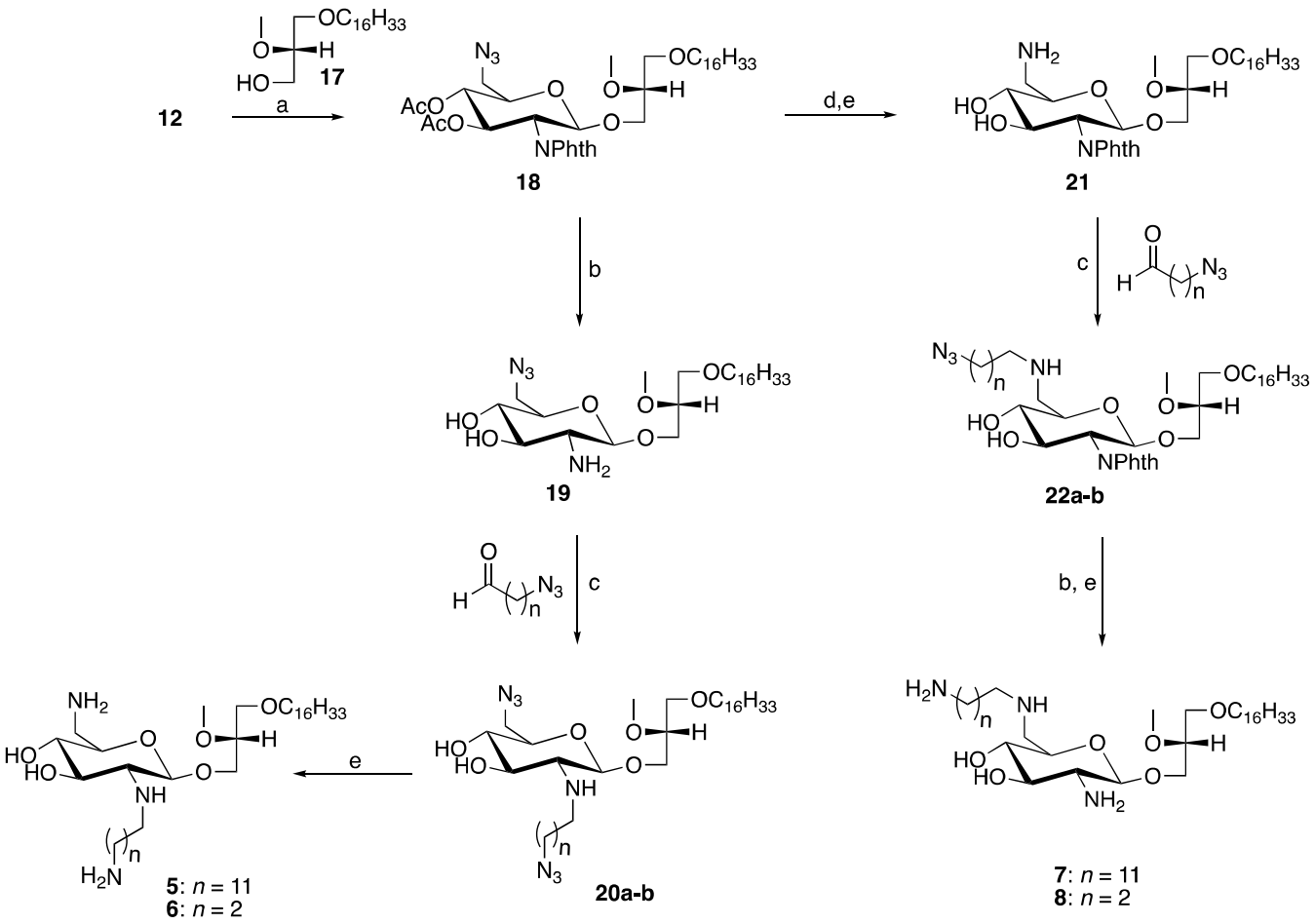
14a: $n = 11$
14b: $n = 2$

15a: $n = 11$
15b: $n = 2$

16a: $n = 11$
16b: $n = 2$

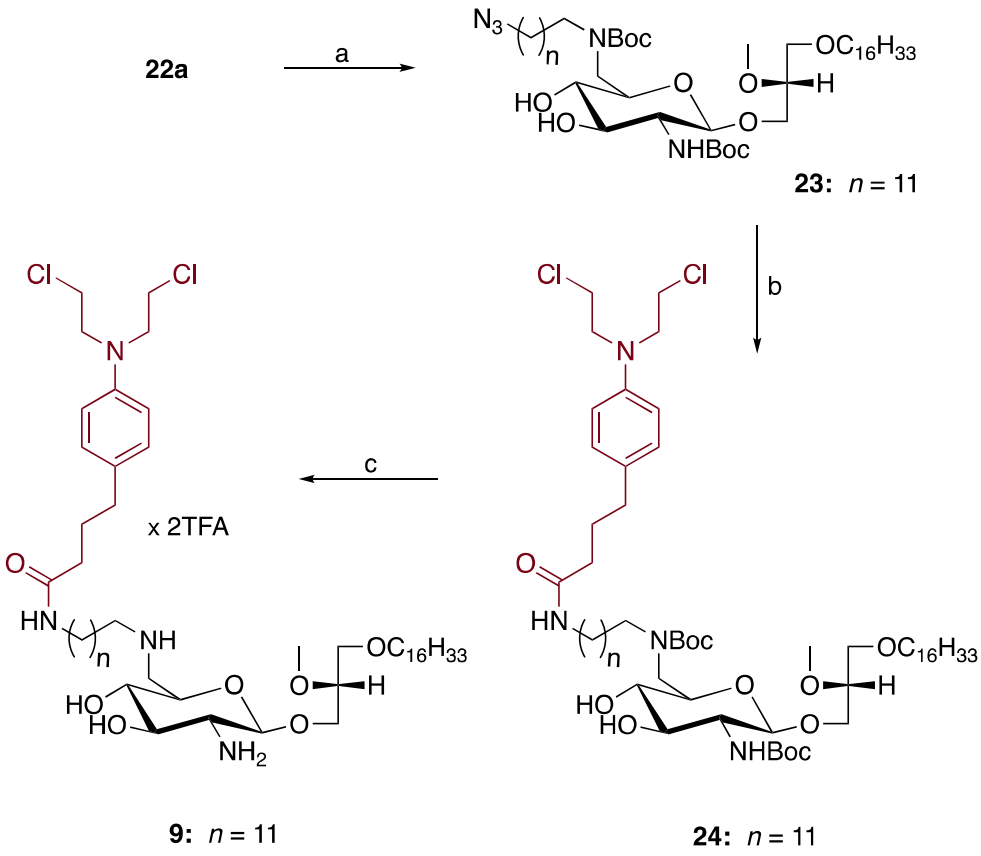
[a]Reagents and conditions: a) NaN_3, DMF, 70 °C (93 %); b) PCC, CH_2Cl_2

Scheme 3



[a]Reagents and conditions: a) AgOTf, NIS, CH_2Cl_2 (69 %); b) ethylenediamine, 1-butanol, 90 °C (62 – 68 %); c) i) DCE, acetic acid, 6 h ii) $NaBH_4$, 0 °C to RT (64 – 70 %); d) NaOMe, MeOH, 25 mins, DOWEX (74 %); e) Pd/C, H_2, MeOH (75 – 82 %)

Scheme 4



[a]Reagents and conditions: a) i) ethylenediamine, 1-butanol, 90 °C (65 %), ii) Boc_2O, MeOH, 55 °C (90 %); b) i) Pd/C, H_2, MeOH (78 %), ii) chlorambucil, DIPEA, TBTU, DMF (73 %); c) trifluoroacetic acid, CH_2Cl_2 (85 %)

Legends

Table 1. Cytotoxicity of compounds **1, 4 - 10** on human epithelial cancer cell lines (CC_{50} values in μM)[a]

Table 2. Cytotoxicity of compound **7** on triple-negative breast, brain, and ovarian cancer cell lines

Figure 1. Structures of some previously synthesized GAELs and some reference anticancer drugs

Figure 2. Structures of newly synthesized triamino compounds **5 - 10**

Figure 3. Effects of compounds **1, 5 - 10** on the viability of a panel of human epithelial cancer cells, and comparism of **7** with clinically used anticancer drugs.[a]

Figure 4. Effects of triamino GAEL **7** on the viability of BT474, PC3, MiaPaCa2, JIMT1, DU145 and MDA-MB-231 cells.[a]

Figure 5. Effects of compounds **4 - 10**, doxorubicin, salinomycin and cisplatin on the viability (A-C)[a] and integrity (D)[a] of BT474 cancer stem cell spheroids

Figure 6. Pan-caspase inhibition (A) and cell membrane permeabilization (B)[a]

Scheme 1. Synthesis of triamino glucopyranoside glycolipid **10**[a]

Scheme 2. Synthesis of *n*-azidoalkanal (**16a:** $n = 11$; **16b:** $n = 2$)[a]

Scheme 3. Synthesis of triamino glucopyranoside-*sn*-glycerol compounds **5 – 8**[a]

Scheme 4. Synthesis of chlorambucil-linked glucopyranoside-*sn*-glycerol **9**[a]

Table of contents graphic