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Synthesis and cytotoxic activity of diosgenyl saponin analogues

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Abstract—Diosgenyl saponins are steroidal glycosides that are often found as major components in many traditional oriental medicines. Recently, a number of naturally occurring diosgenyl saponins have been shown to exert cytotoxic activity against several strains of human cancer cells. Use of these saponin compounds for cancer treatment is hampered due to the lack of understanding of their action mechanism as well as limited access to such structurally complicated molecules. In the present paper, we have prepared a group of diosgenyl saponin analogues which contain a β-D-2-amino-2-deoxy-glucopyranose residue having different substituents at the amino group. Moderate cytotoxic activity is found for most analogues against neuroblastoma (SK-N-SH) cells, breast cancer (MCF-7) cells, and cervical cancer (HeLa) cells. The analogue **13** that contains an α-lipoic acid residue exhibits the highest potency against all three cancer cell lines with IC_{50} ranging from 4.8 μM in SK-N-SH cells to 7.3 μM in HeLa cells. Preliminary mechanistic investigation with one saponin analogue (**10**) shows that the compound induces cell cycle arrest at G_1 phase in SK-N-SH cells, but the same compound induces cell cycle arrest at G_2 phase in MCF-7 cells. This result suggests that the cytotoxic activity of these saponin analogues may involve different action mechanisms in cell lines derived from different cancer sites.

1. Introduction

Saponins are steroid or triterpenoid glycosides that are widely distributed in plants and some marine organisms. Saponins exhibit a broad spectrum of biological activities and have attracted much attention in recent years.[1] Diosgenyl saponins bear the diosgenin as the aglycone, and they are often found as major components in many traditional Chinese medicines. Various biological activities have been observed for this group of saponins, including antibacterial,[2] antiviral,[3] antifungal,[4] and anti-inflammatory activities.[5] Recently, a number of diosgenyl saponins were found to exhibit cytotoxic properties against several strains of human cancer cells,[6–8] and these saponins exerted their antitumor effect by inducing apoptosis in cancer cells.[9–12] Among a great number of naturally occurring diosgenyl saponins, dioscin[9,12,13] is best known to have these properties (Fig. 1).

The structural diversity of naturally occurring diosgenyl saponins lies mainly in the sugar moiety. The first sugar attached to the 3-hydroxyl group of the aglycone is usually β-D-glucopyranose which in turn often has an α-L-rhamnopyranose residue attached to the 2-*O*-position and another sugar or sugar chain to the 3-*O*- or 4-*O*-position.[1] Diosgenyl saponins have great potential to be explored for cancer treatment. However, due to the complexity of their structures and thus limited accessibility, as well as a poor understanding of the underlying mechanism of their antitumor activity, no diosgenyl saponin molecule has yet been clinically used as an anticancer agent. To date, limited information on the structure activity relationship (SAR) of diosgenyl saponins and their cytotoxicity is available.[8] Although both the aglycone and the carbohydrate moieties are required for the exhibition of their antitumor activity, the exact role of the carbohydrate moiety remains unclear. Recently, Myszka et al.[14] reported the apoptosis-inducing property in B cell chronic leukemia cells by synthetic diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and one urea derivative in combination with cladribine (2-CdA). Their finding indicates that diosgenyl saponin analogues carrying only one sugar residue might show good antitumor potential. We initiated our research on diosgenyl saponins in order to discover simpler structural analogues with potent anticancer activity. Toward that end, we have designed and

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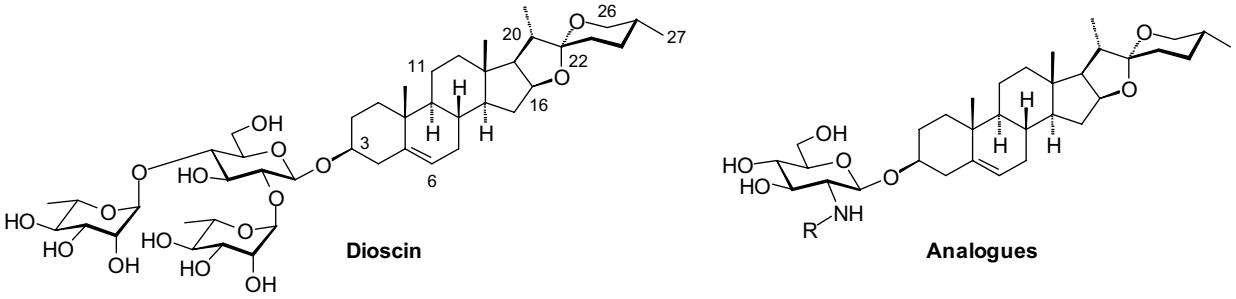


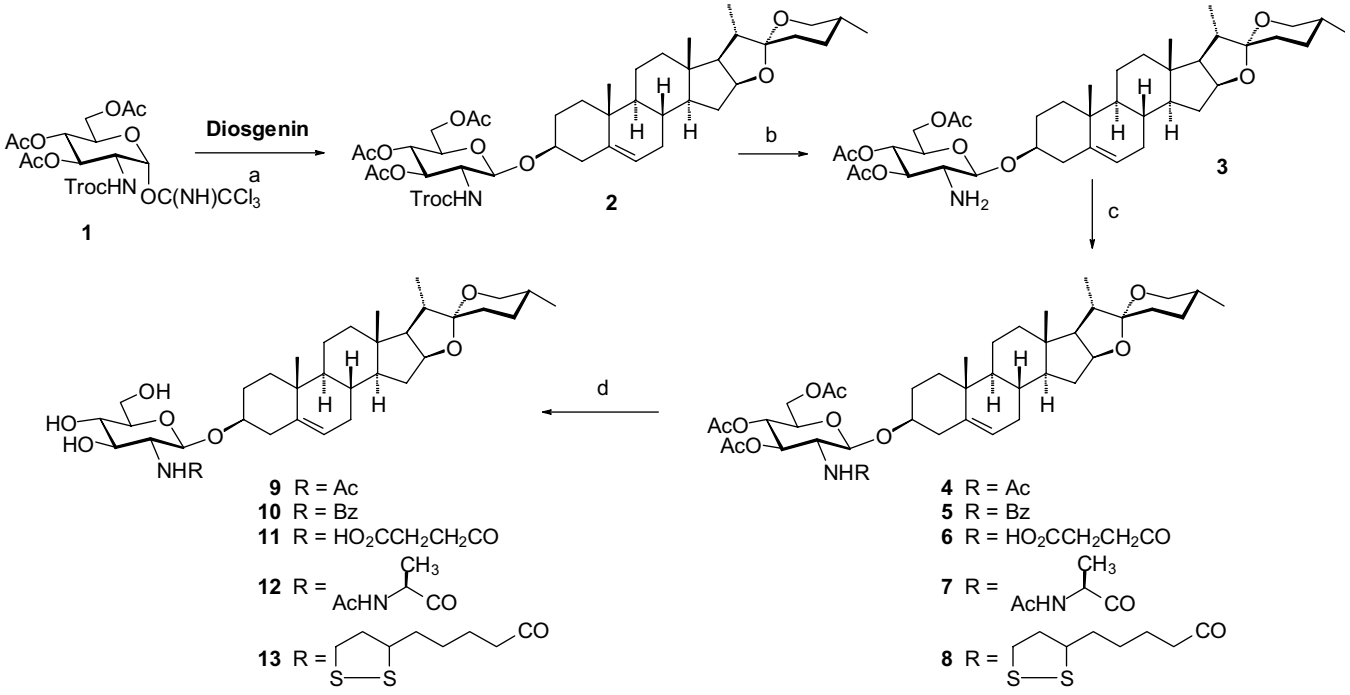
Figure 1. The structures of diosgenyl saponin dioscin and designed analogues.

synthesized a group of analogues having a β-D-glucosamine residue attached to the aglycone (Fig. 1). The presence of the NH_2 group allows the straightforward preparation of structural analogues with different substituents at the amino group. In this paper, we are describing the synthesis and cytotoxic activity of these saponin analogues against a number of human cancer cells. Also presented are the structural features in relation to their activity and the preliminary result of the saponin effects on cell cycle arrest.

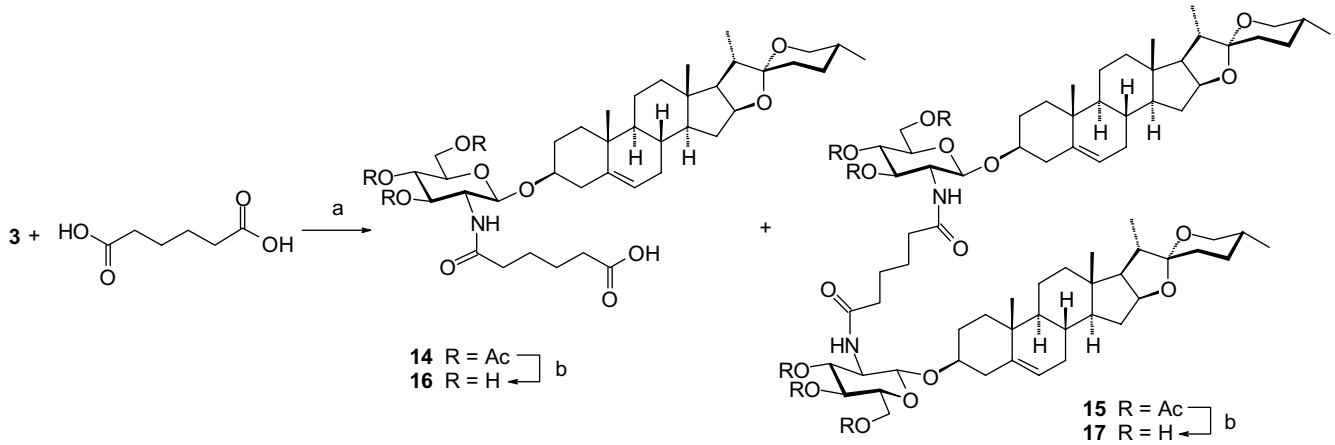
2. Results and discussion

2.1. Chemical synthesis

A number of diosgenyl saponins and derivatives have been synthesized in recent years,[15,16] most notably by the group of Yu.[17–19] The 2,2,2-trichloroethoxycarbonyl (Troc) group is an efficient amine-protection group which can be cleaved under reductive β-elimination.[20] Due to its neighboring group participating capacity, the Troc group has been widely employed in the syntheses of β-glycosides of glucosamine derivatives.[21,22] For our present targets, we choose the readily available glycosyl imidate **1**[22] as the glycosylation donor (Scheme 1) to construct the β-linked glycosides. Thus, the glycosylation reaction between diosgenin and **1** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the catalyst affords the expected β-glycoside **2** in 98% yield. The β-linkage in **2** is confirmed by the coupling constant of the anomeric proton of its ^{1}H NMR spectral data (δ 4.80, d, J = 8.5 Hz).[22] Compound **2** is then treated with zinc dust in acetic acid to provide free amine **3** in good yield. Treatment of **3** with various acylating reagents gives compounds **4**–**8** in good yield (73–92%). The coupling reaction between **3** and *N*-acetyl-L-alanine results in almost complete racemization of the alanine residue when *N*,*N*′-diisopropylcarbodiimide (DIC) is used as the condensation agent. The so-formed diastereomeric



Scheme 1. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 30 min, 98%; (b) Zn dust, HOAc, rt, 16 h, 96%; (c) for **4**, Ac_2O, pyridine, rt, 4 h, 92%; for **5**, BzCl, pyridine, rt, 4 h, 84%; for **6**, succinic anhydride, pyridine, rt, 4 h, 73%; for **7**, AcHN-L-Ala-OH, HBTU, DIPEA, DMF, rt, 16 h, 78%; for **8**, (±)-α-lipoic acid, HBTU, DIPEA, DMF, rt, 16 h, 83%; (d) $NaOCH_3$, CH_3OH–CH_2Cl_2, rt, 4 h, 95–100%.



Scheme 2. Reagents and conditions: (a) HBTU, DIPEA, DMF, rt, 16 h, 21% for **14** and 56% for **15**; (b) CH_3ONa, CH_3OH/CH_2Cl_2 (1:1), rt, 4 h, 98–100%.

mixture of **7** is chromatographically inseparable, but readily recognized on its ^1H NMR spectrum (500 MHz in $CDCl_3$: δ 4.84, d, $J = 8.5$ Hz for the anomeric proton of the sugar residue in L-alanine-bearing isomer; δ 4.90, d, $J = 8.5$ Hz for the anomeric proton of the sugar residue in D-alanine-bearing isomer). This racemization can be largely suppressed (<5%) when 2-(1*H*-benzotriazole-1-yl)-1,1,3,3,-tetramethylaminium hexafluorophosphate (HBTU)[23] is employed as the activating agent. The final removal of the acetyl groups in **4**–**8** by treatment with sodium methoxide in dichloromethane–methanol affords the corresponding diosgenyl saponin analogues **9**–**13**, respectively. The structures of compounds **9**–**13** have been confirmed by ^1H NMR, ^{13}C NMR, and MS spectral data.

Many biological systems interact through multiple simultaneous molecular contacts, and in recent years the design of multivalent ligands and inhibitors has become a focus of inquiry in molecular biochemistry and drug discovery.[24] In the present study, we have prepared one dimeric diosgenyl saponin analogue (Scheme 2), hoping to improve the antitumor activity of the monomeric analogue. Thus, the reaction of two equivalents of amine **3** with one equivalent of dicarboxylic acid, adipic acid, in the presence of HBTU as the activating agent, gives the desired dimer **15** in 56% yield, together with 21% of the monomeric product **14**. Compounds **14** and **15** are treated with sodium methoxide to give saponin analogues **16** and **17**, respectively, in nearly quantitative yield. Their structures have been confirmed by spectroscopic methods.

2.2. Biological evaluation

Each of these saponin analogues is new compound, with the exception of compound **9**. The synthesis of compound **9** was reported earlier,[14] but no biological activity has yet been described for this compound. We investigated the cytotoxic activity of each of these compounds against several human cancer cell lines including SK-N-SH (neuroblastoma), MCF-7 (breast cancer), and HeLa (cervical cancer). The rapid assay employed was the MTT method that measures cell viability, according to the method described by Carmichael et al.,[25] and the results are listed in Table 1. The results are presented as the inhibitory concentration (IC_{50}) that inhibits the growth of cells by 50% when compared with untreated cells. Moderate inhibitory activity was observed for most compounds against all three cancer cell lines, with IC_{50} values in the μM range, varying from 4.8 μM (for compound **13** in SK-N-SH cells) to greater than 20 μM (for compounds **16** and **17** in HeLa cells). When comparing SK-N-SH and HeLa cells, the cervical cancer cell line (HeLa) is slightly more resistant to the inhibitory effects of all compounds, with the exception of compound **11**. At concentrations greater than the IC_{50} values, the MTT absorbance readings for each of compounds **9**–**13** against all three cancer cell lines approached zero, indicating that the compounds were cytotoxic at those concentrations and that cell death had occurred. These observations, in addition to the loss of cell viability shown by the MTT assay, indicate that the saponin analogues tested have both growth inhibitory and cytotoxic properties.

Table 1. The in vitro cytotoxic activity (IC_{50}, μM) of synthetic saponin analogues[a,b,c]

Compound	Cancer cell line		
	SK-N-SH	MCF-7	HeLa
9	11.6 ± 0.88	18.3 ± 0.63	21.5 ± 1.06
10	14.9 ± 1.62	19.4 ± 0.35	18.0 ± 1.41
11	14.1 ± 1.37	22.0 ± 1.41	13.9 ± 0.88
12	15.3 ± 0.42	11.0 ± 0.35	20.0 ± 1.27
13	4.8 ± 0.28	6.9 ± 0.60	7.3 ± 0.56
16	16.3 ± 0.56	11.3 ± 0.42	>20.0
17	19.5 ± 0.98	19.2 ± 0.56	>20.0
Doxorubicin		0.06	

[a] The inhibitory concentration (IC_{50}) is the concentration in μM that inhibits cell growth by 50% in three days compared to cells that remained untreated.

[b] IC_{50} results are based on the average absorbance readings from eight replicate wells per concentration of compound tested, and represent the average $IC_{50} \pm SD$ value obtained from a minimum of $n = 2$ independent MTT assays for each compound.

[c] MCF-7 cells were treated with the chemotherapeutic agent Doxorubicin as a positive control for the MTT assay.

In regard to the structure–activity relationship (SAR) of these saponin compounds, the structural modification at the 2-position of the sugar residue appears to affect the potency and selectivity of their anticancer activity. Most notably, compound **13** which has an α-lipoic acid residue is the most active one against all three cancer cell lines. α-Lipoic acid is a biogenic antioxidant that physiologically acts as a coenzyme in the process of oxidative decarboxylation of α-keto acids. In cells, the disulfide bond of α-lipoic acid can readily be reduced to produce its open chain form as dihydrolipoic acid.[26] α-Lipoic acid has been reported to inhibit the proliferation of WM35 and A350 melanoma cells at different concentrations,[27] although the exact mechanism is unclear. Other disulfide organic sulfur compounds (OSC) are also known for their antiproliferative actions on human cancers.[28] Generally, disulfides can act as oxidants as well as reductants in biological systems, but their oxidizing behavior is more pronounced.[29] Whether or not the increased cytotoxicity of compound **13** might be related to the redox property of the α-lipoic acid residue attached at the 2-*N*-position of the sugar is worth further investigation. Among all the analogues, compounds **11** and **16** are perhaps structurally most close: compound **11** bears a succinic acid residue, while **16** an adipic acid residue which has two more CH_2 units. Compound **11** (IC_{50} 22.0 μM) is less active than **16** (IC_{50} 11.3 μM) against MCF-7 cells, but their relative activity is reversed against HeLa cells (IC_{50} 13.9 μM for **11** and $IC_{50} > 20.0$ μM for **16**). Selective activity against different cancer cells is also observed for compounds **12** (with the L-alanine residue) and **16**. Both compounds are more active against MCF-7 cells than against the other two cell types. The dimeric analogue **17** shows, however, lower activity than the monomeric compound **16** against all three cancer cell lines tested. This may indicate that divalent structures do not result in increased anticancer activity for diosgenyl saponins.

The action mechanism of the anticancer activity of diosgenyl saponins is still poorly understood. Recently, the cytotoxicity of these saponins has been shown to correlate with their propensity for cellular internalization.[30] These compounds have also been reported to induce apoptosis in cancer cells.[10–12,14] Dioscin[9] and its structural analogue, methyl protodioscin,[31,32] were shown to induce G_2/M cell cycle arrest in HepG2 liver cancer cells and human leukemia K562 cells. Since the MTT assay of the present study measures the activity of reductases in the mitochondria of viable cells, it is a relative measure of the number of cells that continue to undergo active metabolism, and a reduction in the MTT absorbance reading relative to untreated cells does not directly measure cell death. We have since tested the synthesized saponin compounds against these and other cancer cell lines from different tissue origins and found variations in cellular responses, including the mechanisms of cell death (manuscript in preparation). Our preliminary investigation shows that in neuroblastoma (SK-N-SH) cells, compound **10** induces cell cycle arrest at G_1 phase while in breast cancer (MCF-7) cells, it induces cell cycle arrest at G_2 phase (data not shown). The finding that compound **10** induces cell cycle arrest at either G_1 or G_2 phase in different cancer cells suggests that the cytotoxic effect of diosgenyl saponins may involve different action mechanisms in different cancer cells.

3. Conclusion

We have prepared a group of diosgenyl saponin analogues bearing a β-D-2-amino-2-deoxy-glucopyranose residue with different substituents at the amino group. Moderate cytotoxic activity is found for most analogues against SK-N-SH neuroblastoma cells, MCF-7 breast cancer cells, and HeLa cervical cancer cells. The analogue **13** that contains an α-lipoic acid residue exhibits the highest activity against all three cell lines with IC_{50} value in the μM range. Further research is focused on the design and synthesis of more structural analogues with increased cytotoxicity and selectivity, as well as more detailed investigation of their action mechanisms.

4. Experimental

4.1. General method

All air and moisture sensitive reactions were performed under nitrogen atmosphere. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Aldrich, and other dry solvents were prepared in usual way. ACS grade solvents were purchased from Fisher and used for chromatography without distillation. TLC plates (silica gel $60F_{254}$, thickness 0.25 mm) and silica gel 60 (40–63 μm) for flash column chromatography were purchased from SILICYCLE Inc., Canada. ^1H and ^{13}C NMR spectra were recorded on Varian Unity Inova 500 MHz spectrometer. Tetramethylsilane (TMS, δ 0.00 ppm) or solvent peaks were used as internal standards for ^1H and ^{13}C spectra. Multiplicity of proton signals is indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and approx (approximate). For spectroscopic assignments, sugar carbon atoms are designated with 1, 2, 3..., and diosgenyl carbon atoms are designated with 1′, 2′, 3′..., etc. Optical rotations were measured on a Perkin-Elmer 241 Polarimeter at room temperature (20–22 °C). Low resolution electrospray ionization (ESI) mass spectra were obtained from a Biflex-IV MALDI linear/reflector instrument at the University of Manitoba, Canada, and high resolution (HR) ESI mass spectra were measured on the Applied Biosystems Mariner Biospectrometry Workstation at the University of Alberta, Canada.

4.2. Diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (2)

A suspension of **1** (5.48 g, 8.77 mmol), diosgenin (3.63 g, 8.64 mmol), and activated molecular sieves (4 Å, 2.0 g) in anhydrous CH_2Cl_2 (50 mL) was stirred at room temperature for 15 min and then cooled to 0 °C under N_2 atmosphere. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 8.0 mL, 0.02 M in CH_2Cl_2)

was added dropwise. The mixture was stirred for 30 min, quenched by Et_3N (0.1 mL), and then filtered through Celite. The filtrate was concentrated in vacuo with the resulting residue purified by flash column chromatography (CH_2Cl_2/MeOH, 100:1) to give compound **2** (7.21 g, 94%) as a white foam. R_f 0.36 (hexane/acetone, 1.5:1); $[\alpha]_D^{22}$ −34.72 (*c* 0.71, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.80 (d, 3H, J = 6.0 Hz, CH_3), 0.98 (d, 3H, J = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.03 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.08 (s, 3 H, CH_3CO), 3.37 (dd, 1H, J = 11.0, 11.0 Hz, H-26′a), 3.46–3.55 (m, 3H, H-26′b, H-3′, H-2), 3.72 (m, 1H, H-5), 4.09–4.15 (m, 1H, H-6b), 4.28 (dd, 1H, J = 12.0, 4.5 Hz, H-6a), 4.41 (approx q, 1H, $J \approx$ 7.5 Hz, H-16′), 4.68 (d, 1H, J = 11.5 Hz, Troc-H), 4.79 (d, 1H, J = 11.5 Hz, Troc-H), 4.82 (d, 1H, J = 8.5 Hz, H-1), 5.05 (dd, 1H, J = 9.5, 10.0 Hz, H-4), 5.31 (m, 1H, H-6′), 5.38 (dd, 1H, J = 9.5, 10.5 Hz, H-3), 5.48 (d, 1H, J = 9.0 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.35, 14.68, 16.42, 17.29, 19.51, 20.81, 20.87, 20.95, 28.91, 29.54, 30.41, 31.49, 31.96, 32.18, 36.96, 37.24, 38.86, 39.86, 40.38, 41.72, 50.14, 56.60, 60.60, 62.18, 62.38, 66.96, 69.05, 71.70, 71.92, 74.50, 79.96, 80.93, 95.65, 99.35, 109.45, 122.02, 140.39, 154.17, 169.71, 170.84, 170.94; HRESI-MS Calcd for $C_{42}H_{60}NO_{12}Cl_3Na$ $[M+Na]^+$: 898.3073, found (positive mode): 898.3072.

4.3. Diosgenyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (4)

To a solution of **2** (1.65 g, 1.88 mmol) in acetic acid (40 ml), zinc dust (4.0 g) was added and the mixture was stirred at room temperature for 16 h. The mixture was filtered and the solid was thoroughly washed with CH_2Cl_2 (100 mL). The filtrate was concentrated in vacuo and the residue was re-dissolved with CH_2Cl_2 (400 mL). The solution was washed with saturated $NaHCO_3$ solution (50 mL), dried with Na_2SO_4, and concentrated under vacuum to give a white solid **3** (1.27 g, 96%) which was used without further purification in the next step.

To a cooled solution (ice water bath) of **3** (97 mg, 0.14 mmol) in pyridine (3.2 mL), acetic anhydride (2.5 mL) was added. The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (100 mL) and washed successively with cold 4 N HCl solution (100 mL) and saturated $NaHCO_3$ solution (50 mL × 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane/CH_2Cl_2/ethyl acetate, 3:3:7) to give the pure compound **4** (94 mg, 92%) as a white solid. R_f 0.40 (CH_2Cl_2/MeOH, 16:1); $[\alpha]_D^{22}$ −61.0 (*c* 0.36, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79 (s, 3H, CH_3), 0.79 (d, 3H, J = 6.5 Hz, CH_3), 0.87 (d, 3H, J = 7.0 Hz, CH_3), 0.89 (s, 3H, CH_3), 1.96 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 3.38 (dd, 1H, J = 11.0, 11.0 Hz, H-26′a), 3.48 (dd, 1H, J = 11.0, 4.5 Hz, H-26′b), 3.50 (m, 1H), 3.69–3.78 (m, 2 H), 4.10 (dd, 1 H, J = 12.0, 2.0 Hz, H-6b), 4.26 (dd, 1H, J = 12.0, 5.0 Hz, H-6a), 4.40 (approx q, 1H, $J \approx$ 8.0 Hz, H-16′), 4.85 (d, 1H, J = 8.0 Hz, H-1), 5.05 (dd, 1H, J = 9.5, 9.5 Hz, H-4), 5.34 (m, 1H, H-6′), 5.38 (dd, 1H, J = 9.5. 9.5 Hz, H-3), 5.46 (d, 1H, J = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.34, 14.74, 16.49, 17.35, 19.59, 21.02, 22.87, 23.64, 29.00, 29.64, 29.92, 30.50, 31.59, 31.80, 32.04, 32.23, 37.06, 37.35, 39.04, 39.94, 40.47, 41.81, 50.23, 55.71, 56.68, 62.27, 62.48, 67.07, 69.02, 71.84, 72.35, 79.81, 81.02, 99.45, 109.53, 122.10, 140.57, 169.10, 170.50, 170.80, 171.10; HRESI-MS Calcd for $C_{41}H_{61}NO_{11}Na$ $[M+Na]^+$: 766.4137, found (positive mode): 766.4140.

4.4. Diosgenyl 3,4,6-tri-*O*-acetyl-2-benzamido-2-deoxy-β-D-glucopyranoside (5)

In a similar way as described for the preparation of **4**, compound **3** (160 mg, 0.228 mmol) was treated with benzoyl chloride (0.03 mL, 0.26 mmol) in pyridine (2.0 mL) to give **5** (155 mg, 84%) as a white solid. R_f 0.56 (CH_2Cl_2/MeOH, 25:1); $[\alpha]_D^{22}$ −34.7 (*c* 0.36, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.76 (s, 3H, CH_3), 0.78 (d, 3H, J = 5.5 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.96 (d, 3H, J = 7.5 Hz, CH_3), 1.99 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.09 (s, 3 H, CH_3CO), 3.37 (dd, 1H, J = 11.0, 11.0 Hz, H-26′a), 3.46 (dd, 1H, J = 11.0, 3.5 Hz, H-26′b), 3.50 (m, 1H, H-2), 3.76–3.78 (m, 1H, H-5), 3.98 (m, 1H, H-3′), 4.11 (d, 1H, J = 11.5 Hz, H-6b), 4.30 (dd, 1H, J = 12.0, 4.7 Hz, H-6a), 4.39 (approx q, 1H, $J \approx$ 7.5 Hz, H-16′), 4.95 (d, 1H, J = 8.0 Hz, H-1), 5.12 (dd, 1H, J = 9.5, 9.5 Hz, H-4), 5.25 (m, 1H, H-6′), 5.52 (dd, 1H, J = 9.5, 9.5 Hz, H-3), 6.23 (d, 1H, J = 9.0 Hz, NH); ^{13}C NMR (125 MHz, CDCl3): δ 14.71, 16.45, 17.33, 19.53, 20.91, 21.01, 28.97, 29.62, 30.47, 31.55, 32.00, 32.22, 37.00, 37.34, 38.99, 39.91, 40.43, 41.77, 50.19, 55.84, 56.64, 62.24, 62.53, 67.03, 68.69, 71.94, 72.43, 79.95, 80.98, 99.75, 109.49, 121.96, 127.02, 128.90, 131.90, 134.54, 140.56, 167.91, 169.69, 170.98, 171.16; HRESI-MS Calcd for $C_{46}H_{63}NO_{11}Na$ $[M+Na]^+$: 828.4293, found (positive mode): 828.4289.

4.5. Diosgenyl 3,4,6-tri-*O*-acetyl-2-(3-carboxylpropanamido)-2-deoxy-β-D-glucopyranoside (6)

In a similar way as described for the preparation of **4**, compound **3** (193 mg, 0.27 mmol) was treated with succinic anhydride (33 mg, 0.33 mmol) in pyridine (3.0 mL) to give compound **6** (141 mg, 73%) as a white solid. R_f 0.43 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ −58.8 (*c* 0.11, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, J = 7.0 Hz, CH_3), 0.95 (d, 3H, J = 7.5 Hz, CH_3), 1.00 (s, 3H, CH_3), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.07 (s, 3 H, CH_3CO), 2.46 (t, 2H, J = 7.5 Hz, CH_2CO), 2.70 (m, 2H, CH_2CO), 3.37 (dd, 1H, J = 11.0, 11.0 Hz, H-26′a), 3.45 (dd, 1H, J = 11.0, 5.0 Hz, H-26′b), 3.49 (m, 1H, H-2), 3.70–3.77 (m, 2H, H-5, H-3′), 4.10 (dd, 1H, J = 12.5, 2.5 Hz H-6a), 4.26 (dd, 1H, J = 12.5, 5.5 Hz, H-6b), 4.39 (approx q, 1H, $J \approx$ 8.0 Hz, H-16′), 4.85 (d, 1H, J = 8.0 Hz, H-1), 5.02 (dd, 1 H, J = 10.0, 10.0 Hz, H-4), 5.32 (m, 1H, H-6′), 5.37 (dd, 1H, J = 10.0, 10.0 Hz, H-3), 6.03 (d, 1H, J = 8.5 Hz, NH);HRESI-MS Calcd for $C_{43}H_{63}NO_{13}Na$ $[M+Na]^+$: 824.4192, found (positive mode): 824.4193.

4.6. Diosgenyl 3,4,6-tri-*O*-acetyl-2-[(*S*)-2-acetamido-propanamido]-2-deoxy-β-D-glucopyranoside (7)

To a solution of **3** (300 mg, 0.43 mmol) in dry DMF (5.0 mL), *N*-acetyl-L-alanine (56 mg, 0.43 mmol), diisopropylethylamine (DIPEA, 0.15 mL, 0.86 mmol), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU, 163 mg, 0.43 mmol) were added and the mixture was stirred for 16 h. The solvent was removed in vacuo and the residue was dissolved with CH_2Cl_2 (150 mL) and washed with H_2O (10 mL × 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was subjected to flash column chromatography (CH_2Cl_2/MeOH, 25:1) to give compound **7** (272 mg, 78%) as a white solid. R_f 0.36 (CH_2Cl_2/MeOH, 25:1); $[\alpha]_D^{22}$ −53.2 (*c* 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (s, 3H, CH_3), 0.82 (d, 3H, *J* = 6.0 Hz, CH_3), 0.98 (d, 3H, *J* = 7.0 Hz, CH_3), 1.02 (s, 3H, CH_3), 1.99 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 3.37 (m, 1H, H-26′a), 3.48–3.51 (m, 2H, H-26′b, H-2), 3.74–3.83 (m, 2H, H-3′, H-5), 4.12 (dd, 1H, *J* = 12.0, 2.0 Hz H-6b), 4.29 (dd, 1H, *J* = 12.0, 4.5 Hz, H-6a), 4.29 (m, 1H, C*H*NHAc), 4.43 (approx q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.80 (d, 1H, *J* = 8.5 Hz, H-1), 4.91 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.20 (dd, 1H, *J* = 10.5, 10.5 Hz, H-3), 5.26 (m, 1H, H-6′), 7.62 (d, 1H, *J* = 7.5 Hz, NH), 7.87 (d, 1H, *J* = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.74, 16.50, 17.35, 18.27, 19.59, 20.90, 21.01, 23.40, 29.01, 29.67, 30.51, 31.60, 32.05, 32.27, 37.04, 37.34, 39.03, 39.95, 40.47, 41.82, 49.15, 50.26, 55.32, 56.69, 62.30, 62.52, 67.07, 69.10, 71.81, 72.31, 79.88, 81.00, 98.15, 108.16, 120.68, 139.30, 168.34, 169.00, 169.60, 169.61, 171.54; HRESI-MS Calcd for $C_{44}H_{66}N_2O_{12}Na$ $[M+Na]^+$: 837.4508, found (positive mode): 837.4506.

4.7. Diosgenyl 3,4,6-tri-*O*-acetyl-2-{5-[(*R*/*S*)-1,2-dithiolan-3-yl]-pentanamido}-2-deoxy-β-D-glucopyranoside (8)

In a similar way as described for the preparation of **7**, compound **3** (150 mg, 0.21 mmol) was treated with (±)-α-lipoic acid (44 mg, 0.21 mmol), DIPEA (0.075 mL, 0.43 mmol), and HBTU (82 mg, 0.21 mmol) in dry DMF (5.0 mL) to give **8** (158 mg, 83%) as a white solid. R_f 0.32 (hexane/EtOAc/MeOH, 1:1:0.025); $[\alpha]_D^{22}$ −175.0 (*c* 0.03, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.96 (d, 3H, *J* = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.23 (m, 1H, CH*H*CO), 2.45 (m, 1H, C*H*HCO), 3.08–3.20 (m, 2H, CH_2S), 3.17 (m, 1 H), 3.37 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26′a), 3.45–3.56 (m, 3H, H-26′b, H-2, CHS), 3.65 (m, 1H, H-3′), 3.70 (m, 1H, H-5), 4.09 (dd, 1H, *J* = 12.0, 2.5 Hz, H-6b), 4.26 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6a), 4.40 (approx q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.89 (two sets of d, each 0.5H, *J* = 8.0 Hz, H-1), 5.03 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.33 (m, 1H, H-6′), 5.41 (two sets of dd, each 0.5H, *J* = 10.0, 10.0 Hz, H-3), 5.48 (two sets of d, each 0.5H, *J* = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.72, 16.49, 17.34, 19.59, 20.88, 20.96, 25.42, 25.47, 28.98, 29.64, 30.48, 31.59, 32.04, 32.28, 34.78, 34.83, 36.67, 37.04, 37.32, 38.65, 39.11, 39.92, 40.45, 41.79, 50.21, 55.73, 56.07, 56.58, 56.66, 62.26, 62.49, 67.04, 69.08, 71.81, 72.27, 79.72, 79.95, 80.99, 99.32, 109.50, 122.11, 140.54, 169.71, 170.98, 170.99, 172.98; HRESI-MS Calcd for $C_{47}H_{71}NO_{11}S_2Na$ $[M+Na]^+$: 912.4360, found (positive mode): 912.4358.

4.8. Diosgenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (9)

Compound **4** (100 mg, 0.13 mmol) was dissolved in MeOH/CH_2Cl_2 (2:1, 9 mL) and then NaOMe solution in methanol (0.6 mL) was added until pH 10. After stirring for 4 h at room temperature, the mixture was neutralized by adding weak acidic ion-exchange resin (Amberlite IRC-50, H^+ form). The resin was filtered and the filtrate concentrated in vacuo. The residue was applied to a Sephadex LH-20 gel filtration column (MeOH/CH_2Cl_2, 2:1) to yield compound **9** (81 mg, 98%) as a white solid. R_f 0.21 (CH_2Cl_2/MeOH, 2:1); $[\alpha]_D^{22}$ −66.6 (*c* 0.26, $CH_3OH/CHCl_3$, 1:1); lit.[14] $[\alpha]_D^{22}$ −75 (*c* 0.5, $CH_3OH/CHCl_3$, 1:1); ^{1}H NMR (500 MHz, C_5D_5N plus two drops of CD_3OD): δ 0.73 (d, 3H, *J* = 6.0 Hz, CH_3), 0.84 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.18 (d, 3H, *J* = 7.0 Hz, CH_3), 2.18 (s, 3H, CH_3CO), 3.50 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26′a), 3.60 (dd, 1H, *J* = 10.5, 3.5 Hz, H-26′b), 3.83–3.88 (m, 1H, H-3′), 3.91–3.94 (m, 1H, H-5), 4.14 (m, 1 H, H-2), 4.30 (dd, 1H, *J* = 12.0, 6.0 Hz, H-6a), 4.37–4.44 (m, 2H, H-3, H-4), 4.50 (dd, 1H, *J* = 10.0, 2.0 Hz, H-6b), 4.55 (approx q, 1H, *J* = 7.0 Hz, H-16′), 5.21 (d, 1H, *J* = 7.5 Hz, H-1), 5.35–5.36 (m, 1H, H-6′), 8.89 (d, 1H, *J* = 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N plus two drops of CD_3OD): δ 15.54, 16.86, 17.82, 19.92, 21.57, 24.08, 29.73, 30.64, 31.07, 32.12, 32.28, 32.66, 32.74, 37.50, 37.91, 40.00, 40.34, 40.93, 42.43, 50.71, 57.13, 58.49, 63.13, 63.34, 67.33, 72.81, 76.76, 78.98, 78.99, 81.57, 101.27, 109.74, 122.20, 141.44, 171.01; ESI-MS Calcd for $C_{35}H_{55}NO_8$ $[M]^+$: 617.39, found (positive mode): 618.64 $[M+H]^+$. The NMR spectral data are in general agreement with the reported data[14] which were measured in DMSO-d_6.

4.9. Diosgenyl 2-benzamido-2-deoxy-β-D-glucopyranoside (10)

In a similar way as described for the preparation of **9**, compound **5** (71 mg, 0.09 mmol) was treated with sodium methoxide to give **10** (55 mg, 92%) as a white solid. R_f 0.18 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ −28.0 (*c* 0.16, $CHCl_3/CH_3OH$, 1:1); ^{1}H NMR (500 MHz, C_5D_5N): δ 0.69 (d, 3H, *J* = 5.5 Hz, CH_3), 0.82 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 1.14 (d, 3H, *J* = 6.0 Hz, CH_3), 2.43 (m, 1 H), 2.69 (m, 1H), 3.50 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26′a), 3.59 (dd, 1H, *J* = 10.5, 3.0 Hz, H-26′b), 3.90 (m, 1H, H-3′), 4.06 (m, 1H, H-5), 4.32 (m, 1H, H-6a), 4.43 (m, 1H, H-2), 4.54 (approx q, 1H, *J* = 7.5 Hz, H-16′), 4.60 (br d, 1H, *J* = 11.0 Hz, H-6b), 4.68–4.80 (m, 2H, H-3, H-4), 5.22 (m, 1H, H-6′), 5.56 (d, 1H, *J* = 8.5 Hz, H-1), 6.58 (s, 1H, OH), 7.37–7.44 (m, 5H, 3 Ar–H, 2 OH), 8.39 (d, 2 H, *J* = 6.5 Hz, 2 Ar–H), 9.44 (d, 1H, *J* = 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.51, 16.80, 17.80, 19.80, 21.51, 29.71, 30.47, 30.64, 31.05, 32.04, 32.25, 32.68, 37.43, 37.87, 40.09, 40.28,

40.89, 42.40, 50.64, 57.07, 59.47, 63.31, 67.31, 73.10, 76.30, 79.10, 79.17, 81.54, 101.16, 109.71, 122.19, 128.51, 129.06, 131.67, 137.32, 141.26, 141.28, 168.94; ESI-MS Calcd for $C_{40}H_{57}NO_8$ $[M]^+$: 679.41, found (positive mode): 680.69 $[M+H]^+$.

4.10. Diosgenyl 2-(3-carboxylpropanamido)-2-deoxy-β-D-glucopyranoside (11)

In a similar way as described for the preparation of **9**, compound **6** (66 mg, 0.08 mmol) was treated with sodium methoxide to give **11** (55 mg, quantitatively) as a white solid. R_f 0.28 ($CHCl_3$/MeOH/H_2O, 6:4:0.5); $[\alpha]_D^{22}$ −29.4 (*c* 0.14, $CHCl_3$/CH_3OH, 1:1); ^1H NMR (500 MHz, CD_3OD): δ 0.69 (s, 3H, CH_3), 0.70 (d, 3H, J = 5.5 Hz, CH_3), 0.86 (d, 3H, J = 7.0 Hz, CH_3), 0.93 (s, 3H, CH_3), 2.40 (m, 4 H), 3.14–3.26 (m, 3 H), 3.32–3.50 (m, 3 H), 3.54–3.60 (m, 1 H), 3.74–3.82 (m, 1 H), 4.29 (approx q, 1H, J = 7.5 Hz, H-16′), 4.46 (d, 1H, J = 8.0 Hz, H-1), 4.63–4.68 (m, 1 H), 5.27 (m, 1H, H-6′), 8.89 (d, 1H, J = 9.5, NH); ^{13}C NMR (125 MHz, CD_3OD): δ 15.90, 16.96, 17.69, 20.04, 22.16, 30.07, 30.83, 31.63, 32.61, 32.96, 32.97, 33.10, 33.36, 33.58, 38.15, 38.68, 40.18, 41.10, 41.60, 43.08, 51.84, 57.97, 57.98, 63.00, 63.92, 68.03, 72.15, 76.20, 78.05, 80.42, 82.38, 101.28, 110.74, 122.80, 142.09, 175.93, 178.86; ESI-MS Calcd for $C_{37}H_{57}NO_{10}$ $[M]^+$: 675.40, found (positive mode): 676.38 $[M+H]^+$, 698.86 $[M+Na]^+$.

4.11. Diosgenyl 2-[(*S*)-2-acetamido-propanamido]-2-deoxy-β-D-glucopyranoside (12)

In a similar way as described for the preparation of **9**, compound **7** (168 mg, 0.21 mmol) was treated with sodium methoxide to give **12** (141 mg, quantitatively) as a white solid. R_f 0.21 (CH_2Cl_2/MeOH, 8:1); $[\alpha]_D^{22}$ −44.8 (*c* 0.36, $CHCl_3$/CH_3OH, 1:1); ^1H NMR (500 MHz, $CDCl_3$/CD_3OD, 1:1): δ 0.71 (s, 3H, CH_3), 0.72 (d, 3H, J = 5.0 Hz, CH_3), 0.88 (d, 3H, J = 7.0 Hz, CH_3), 0.92 (s, 3H, CH_3), 1.29 (d, 3H, J = 7.0 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 3.21 (m, 1 H), 3.26 (m, 1 H), 3.30 (m, 1 H), 3.38 (m, 1 H), 3.40–3.51 (m, 3 H), 3.64 (dd, 1H, J = 12.0, 5.0 Hz, H-6a), 3.78 (dd, 1H, J = 12.0, 2.5 Hz, H-6b), 4.24 (q, 1H, J = 7.0 Hz, C*H*NHAc), 4.33 (approx q, 1H, J = 7.5 Hz, H-16′), 4.47 (d, 1H, J = 8.0 Hz, H-1), 5.25 (m, 1H, H-6′); ^{13}C NMR (125 MHz, $CDCl_3$/CD_3OD, 1:1): δ 15.63, 17.56, 18.27, 18.95, 20.59, 22.29, 23.66, 30.10, 30.89, 31.65, 32.71, 32.92, 33.10, 33.50, 38.27, 38.65, 40.21, 41.17, 41.75, 43.11, 50.73, 51.63, 57.98, 58.58, 63.22, 63.53, 68.33, 72.33, 75.40, 77.57, 80.53, 82.53, 100.65, 111.14, 123.11, 141.95, 173.20, 175.20. ESI-MS Calcd for $C_{38}H_{60}N_2O_9$ $[M]^+$: 688.43, found (positive mode): 689.61 $[M+H]^+$, 711.57 $[M+Na]^+$.

4.12. Diosgenyl 2-deoxy-2-{5-[(*R*/*S*)-(1,2-dithiolan-3-yl)]-pentamido}-β-D-glucopyranoside (13)

In a similar way as described for the preparation of **9**, compound **8** (139 mg, 0.16 mmol) was converted to **13** (119 mg, quantitatively) as a white solid. R_f 0.24 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ −44.8 (*c* 0.21, $CHCl_3$/CH_3OH, 1:1); ^1H NMR (500 MHz, C_5D_5N): δ 0.70 (d, 3H, J = 5.5 Hz, CH_3), 0.85 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.15 (d, 3H, J = 7.0 Hz, CH_3), 2.22 (m, 1 H), 2.48 (t, 2H, J = 7.0 Hz), 2.54 (m, 1 H), 2.72 (m, 1 H), 2.97–3.08 (m, 2H, CH_2S), 3.47–3.53 (m, 2H, H-26′a, CHS), 3.60 (dd, 1H, J = 12.0, 3.5 Hz, H-26′b), 3.87–3.94 (m, 1H, H-3′), 4.00 (m, 1H, H-5), 4.25 (dd, 1H, J = 9.0, 9.0 Hz, H-4), 4.40 (dd, 1H, J = 12.0, 5.5 Hz, H-6a), 4.50–4.59 (m, 4H, H-2, H-3, H-6b, H-16′), 5.32 (two sets of d, each 0.5H, J = 8.0 Hz, H-1), 5.36 (m, 1H, H-6′), 8.82 (d, 1H, J = 7.5 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.51, 16.83, 17.79, 19.99, 21.57, 26.54, 29.74, 30.68, 31.06, 32.14, 32.26, 32.66, 32.76, 35.46, 37.28, 37.52, 37.89, 39.12, 40.14, 40.32, 40.86, 40.92, 42.42, 50.70, 57.12, 57.28, 58.45, 63.20, 63.33, 67.31, 72.99, 76.79, 78.89, 79.02, 81.54, 101.22, 109.72, 122.25, 141.45, 173.74; ESI-MS Calcd for $C_{41}H_{65}NO_8S_2$ $[M]^+$: 763.42, found (positive mode): 764.70 $[M+H]^+$.

4.13. Diosgenyl 3,4,6-tri-*O*-acetyl-2-(5-carboxylpentamido)-2-deoxy-β-D-glucopyranoside (14) and *N*,*N*′-bis(diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosid-2-yl) hexandiamide (15)

In a similar way as described for the preparation of **7**, compound **3** (130 mg, 0.19 mmol) was treated with adipic acid (12 mg, 0.085 mmol), DIPEA (0.064 mL, 0.37 mmol), and HBTU (70 mg, 0.19 mmol) in dry DMF (5.0 mL) to give the monomeric product **14** (15 mg, 21%) and the dimeric product **15** (71 mg, 56%). For **14**: R_f 0.24 (hexane/EtOAc/MeOH, 1:1:0.1); $[\alpha]_D^{22}$ −36.8 (*c* 0.25, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, J = 7.0 Hz, CH_3), 0.96 (d, 3H, J = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.01 (s, 6H, 2 CH_3CO), 2.07 (s, 3H, CH_3CO), 2.29 (m, 2 H), 2.34 (m, 2 H), 3.37 (dd, 1H, J = 11.0, 11.0 Hz, H-26′a), 3.46–3.52 (m, 2H, H-26′b, H-2), 3.72–3.77 (m, 2H, H-5, H-3′), 4.10 (dd, 1H, J = 12.0, 1.5 Hz, H-6b), 4.28 (dd, 1H, J = 12.0, 4.5 Hz, H-6a), 4.41 (approx q, 1H, $J \approx 7.5$ Hz, H-16′), 4.85 (d, 1H, J = 8.0 Hz, H-1), 5.03 (dd, 1H, J = 9.5, 10.0 Hz, H-4), 5.31 (m, 1H, H-6′), 5.38 (dd, 1H, J = 9.5, 10.5 Hz, H-3), 5.86 (d, 1H, J = 9.0 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.73, 16.50, 17.35, 19.58, 20.88, 20.96, 21.02, 21.03, 24.30, 24.99, 28.98, 29.63, 29.91, 30.47, 31.56, 31.57, 32.03, 32.26, 36.36, 37.03, 37.33, 39.05, 39.93, 40.45, 41.79, 50.21, 55.37, 56.67, 62.24, 62.53, 67.05, 69.14, 71.75, 72.50, 79.76, 81.00, 99.44, 109.54, 122.03, 140.57, 169.73, 171.00, 171.22, 173.12, 178.16; ESI-MS Calcd for $C_{45}H_{67}NO_{13}$ $[M]^+$: 829.46, found (positive mode): 830.71 $[M+H]^+$. For **15**: R_f 0.29 (hexane/EtOAc/MeOH, 1:1:0.1); $[\alpha]_D^{22}$ −61.7 (*c* 0.29, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.80 (d, 3H, J = 7.0 Hz, CH_3), 0.97 (d, 3H, J = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.02 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.19 (m, 2 H), 3.37 (dd, 1H, J = 11.0, 11.0 Hz, H-26′a), 3.48 (dd, 1H, J = 11.0, 4.0 Hz, H-26′b), 3.50 (m, 1H, H-2), 3.73–3.81 (m, 2H, H-5, H-3′), 4.09–4.12 (dd, 1H, J = 12.0, 2.5 Hz H-6b), 4.28 (dd, 1H, J = 12.0, 5.0 Hz, H-6a), 4.41 (approx q, 1H, $J \approx 7.5$ Hz, H-16′), 4.90 (d, 1H, J = 8.5 Hz, H-1), 5.06 (dd, 1H, J = 9.5, 10.0 Hz, H-4), 5.29 (m, 1H, H-6′), 5.45 (dd, 1H, J = 9.5, 10.5 Hz, H-3), 6.13 (d, 1H, J = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.74, 16.51, 17.36, 19.61, 20.91, 21.03, 21.10, 24.31, 28.99, 29.61, 29.91, 30.50, 31.58, 31.59, 32.04,

32.28, 36.06, 37.06, 37.35, 39.05, 39.92, 40.46, 41.80, 50.23, 55.34, 56.67, 62.26, 62.49, 67.06, 69.25, 71.72, 72.76, 79.59, 80.98, 99.33, 109.52, 121.97, 140.69, 169.70, 170.98, 171.17, 173.01; HRESI-MS Calcd for $C_{84}H_{124}N_2O_{22}Na$ $[M+Na]^+$: 1535.8538; found (positive mode): 1535.8533.

4.14. Diosgenyl 2-(5-carboxylpentamido)-2-deoxy-β-D-glucopyranoside (16)

In a similar way as described for the preparation of **9**, compound **14** (26 mg, 0.03 mmol) was converted to **16** (22 mg, quantitatively) as a white solid. R_f 0.20 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ −14.0 (*c* 0.14, $CHCl_3$/CH_3OH, 1:1); ^1H NMR (500 MHz, C_5D_5N): δ 0.68 (d, 3 H, J = 5.0 Hz, CH_3), 0.81 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.13 (d, 3H, J = 7.0 Hz, CH_3), 2.52 (m, 5 H), 2.70 (m, 1 H), 3.50 (dd, 1H, J = 10.5, 10.5 Hz, H-26′a), 3.58 (dd, 1H, J = 10.5, 3.5 Hz, H-26′b), 3.88 (m, 1H, H-3′), 3.97 (m, 1H, H-5), 4.21 (dd, 1H, J = 9.0, 9.0 Hz, H-4), 4.36 (dd, 1H, J = 12.0, 5.5 Hz, H-6a), 4.43–4.58 (m, 4H, H-2, H-3, H-6b, H-16′), 5.25 (d, 1H, J = 7.5 Hz, H-1), 5.33 (m, 1H, H-6′), 8.82 (m, 1H, J = 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.30, 16.62, 17.59, 19.73, 21.34, 25.60, 26.39, 29.51, 30.49, 30.85, 31.87, 32.05, 32.43, 32.50, 34.92, 37.18, 37.28, 37.69, 39.80, 40.11, 40.70, 42.31, 50.49, 56.89, 58.28, 63.11, 64.74, 67.11, 72.83, 76.70, 78.74, 78.83, 81.34, 101.04, 109.52, 121.94, 141.29, 173.64, 176.06; ESI-MS calcd for $C_{39}H_{61}NO_{10}$ $[M]^+$: 703.43, found (positive mode): 704.71 $[M+H]^+$.

4.15. *N*,*N*′-Bis(diosgenyl 2-deoxy-β-D-glucopyranosid-2-yl) hexandiamide (17)

In a similar way as described for the preparation of **9**, compound **15** (27 mg, 0.02 mmol) was converted to **17** (22.5 mg, quantitatively) as a white solid. R_f 0.35 (CH_2Cl_2/MeOH, 5:1); $[\alpha]_D^{22}$ −39.2 (*c* 0.14, $CHCl_3$/CH_3OH, 1:1); ^1H NMR (500 MHz, C_5D_5N): δ 0.68 (d, 3H, J = 5.5 Hz, CH_3), 0.86 (s, 3 H, CH_3), 0.95 (s, 3H, CH_3), 1.15 (d, 3 H, J = 7.0 Hz, CH_3), 2.49 (m, 1 H), 2.55 (m, 2 H), 2.68 (m, 1 H), 3.50 (dd, 1H, J = 10.5, 10.5 Hz, H-26′a), 3.59 (dd, 1H, J = 10.5, 2.5 Hz, H-26′b), 3.89 (m, 1H, H-3′), 3.95 (m, 1H, H-5), 4.21 (dd, 1H, J = 9.0, 9.0 Hz, H-4), 4.36 (dd, 1H, J = 12.0, 5.5 Hz, H-6a), 4.45 (dd, 1H, J = 10.0, 9.0 Hz, H-3), 4.52–4.58 (m, 3H, H-2, H-6b, H-16′), 5.25 (d, 1H, J = 8.5 Hz, H-1), 5.32 (m, 1H, H-6′), 8.75 (m, 1H, J = 9.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.52, 16.89, 17.80, 19.96, 21.58, 26.20, 29.73, 30.69, 31.06, 32.12, 32.28, 32.69, 32.76, 37.20, 37.51, 37.89, 39.92, 40.33, 40.94, 42.44, 50.71, 57.12, 58.36, 63.28, 63.34, 67.32, 72.93, 76.95, 78.80, 79.01, 81.56, 101.16, 109.73, 122.21, 141.41, 174.09; HRESI-MS Calcd for $C_{72}H_{112}N_2O_{16}Na$ $[M+Na]^+$: 1283.7904, found (positive mode): 1283.7903.

4.16. Cell lines, cell culture, and medium

Cell lines used in this study include SK-N-SH (neuroblastoma), MCF-7 (breast cancer), and HeLa (cervical cancer). SK-N-SH and MCF-7 cell lines were supplied by the American Type Culture Collection (Manassas, Virginia), and HeLa cells were obtained from the Lady Davis Institute for Medical Research (Montreal, Quebec). All cell lines were grown in Dulbecco's modified Eagle's medium (Sigma) containing 10% fetal bovine serum (PAA Laboratories) at 37 °C in a 5% CO_2 humidified atmosphere.

4.17. Cell proliferation assay

Measurement of cell proliferation was performed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Carmichael et al.[25] Briefly, 2000 cells were plated out into each well of a 96-wellplate and allowed to adhere. Saponin analogues (dissolved in DMSO (Sigma) and diluted with tissue culture medium) were added at increasing concentrations (0–30 μM, 8 wells per concentration). The cells were incubated in the presence of each saponin analogue for 72 h. MTTreagent (Sigma) dissolved in phosphate-buffered saline was added to each well at a concentrationof 0.5 mg/mL, and the cells were incubated for four additional hours. Following this time, the medium containing the MTT reagent was aspirated, and DMSO (100 μL) was added to each well. The absorbance of each well was measured in a microplate reader (PowerWave XS, Bio-Tek) at a wavelength of 490 nm. The IC_{50} value for each compound tested was determined by plotting concentration vs. percent absorbance obtained in the MTT assay. The IC_{50} results listed in Table 1 represent the average IC_{50} value obtained from multiple MTT assays for each compound.

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