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Structural analogues of diosgenyl saponins: Synthesis and anticancer activity

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ABSTRACT

Saponins display various biological activities including anti-tumor activity. Recently intensive research has been focused on developing saponins for tumor therapies. The diosgenyl saponin dioscin is one of the most common steroidal saponins and exhibits potent anticancer activity in several human cancer cells through apoptosis-inducing pathways. In this paper, we describe the synthesis of several diosgenyl saponin analogues containing either a 2-amino-2-deoxy-β-D-glucopyranosyl residue or an α-L-rhamnopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl residue with different acyl substituents on the amino group. The cytotoxic activity of these compounds was evaluated in MCF-7 breast cancer cells and HeLa cervical cancer cells. Structure–activity relationship studies show that the disaccharide saponin analogues are in general less active than their corresponding monosaccharide analogues. The incorporation of an aromatic nitro functionality into these saponin analogues does not exhibit significant effect on their cytotoxic activity.

1. Introduction

Saponins are glycosides of steroids and triterpenoids that are widely distributed in terrestrial plants and in some marine organisms. In recent years, saponins have received considerable attention because of their various biological activities. In particular, their favorable anti-tumorigenic properties have attracted intensive research in developing saponins for tumor therapies. Diosgenyl saponins are the most abundant steroidal saponins and many of them display potent anticancer activity. The diosgenyl saponin dioscin (Chart 1) is one of the most common steroidal saponins found in plants and exhibits cytotoxicity in several cancer cells with an IC_{50} value typically in μM.[1–6] Dioscin has also been shown to induce apoptosis in HL-60 (human leukemia) cells[7] and HeLa (cervical cancer) cells[6] through activation of caspase-9 and -3, together with down-regulation of anti-apoptotic Bcl-2 protein. Recently, Wang et al.[8] reported that mitochondria was the major cellular target of dioscin in its induction of apoptosis in HL-60 cells and that dioscin exerted its cytotoxicity through multiple apoptosis-inducing pathways. Apoptosis permits the removal of damaged, senescent or unwanted cells in multi-cellular organisms without damaging the cellular microenvironment. Since cancer cells have gained the ability to evade apoptosis, re-introducing apoptosis into cancer cells is considered to be the most suitable method of anticancer therapy.[9] Because of their unique apoptosis-inducing properties in cancer cells, dioscin and other diosgenyl saponins are currently being explored for tumor therapy.[10–12]

In an effort to study the structure–activity relationship and develop more potent anticancer agents, a number of dioscin derivatives and analogues have been synthesized.[2,13–16] Previously, we reported a group of diosgenyl saponin analogues containing a 2-amino-2-deoxy-β-D-glucopyranose residue with different acyl substituents at the amino group (structure **I**, Chart 1).[17] Moderate cytotoxic activity was found for most analogues against neuroblastoma (SK-N-SH) cells, breast cancer (MCF-7) cells, and cervical cancer (HeLa) cells. In the present paper, we describe the synthesis and anticancer property of new saponin analogues containing either a monosaccharide residue (structure **I**, Chart 1) or a disaccharide residue (structure **II**, Chart 1) with an acyl substituent at the amino group. In particular, some analogues contain an aromatic nitro functionality which is known to be a common functionality performing electron transfer. Electron transfer and oxidative stress have been increasingly implicated in the actions of anticancer drugs.[18,19] Electron transfer pathways can endogenously lead to the formation of reactive oxygen species, which cause oxidative stress in cells and lead to the induction of apoptosis. We hypothesize that the incorporated aromatic nitro group will exert oxidative stress in cancer cells and thus improve the cytotoxic activity of these saponin analogues.

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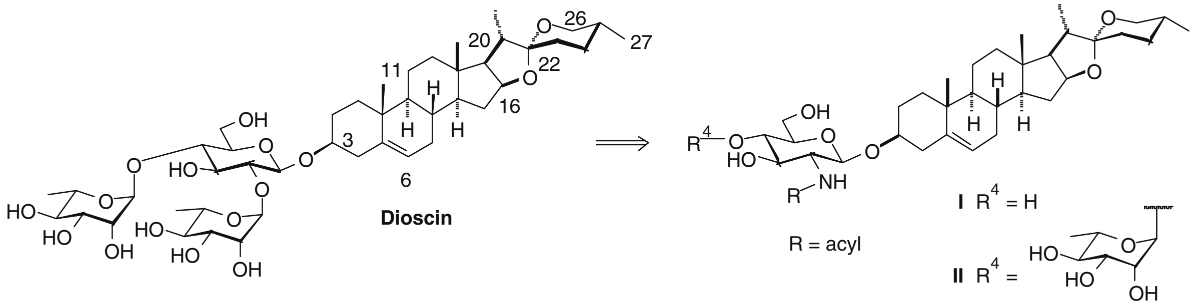


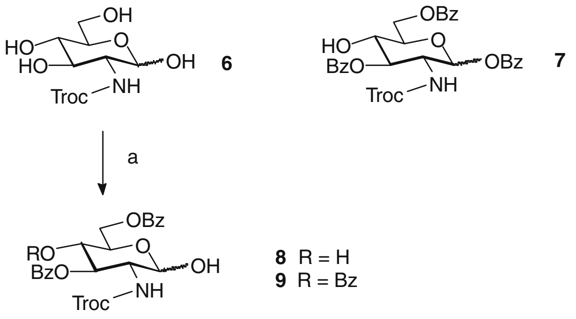
Chart 1. Diosgenyl saponin dioscin and structural analogues **I** and **II**.

2. Results and discussion

2.1. Chemical synthesis

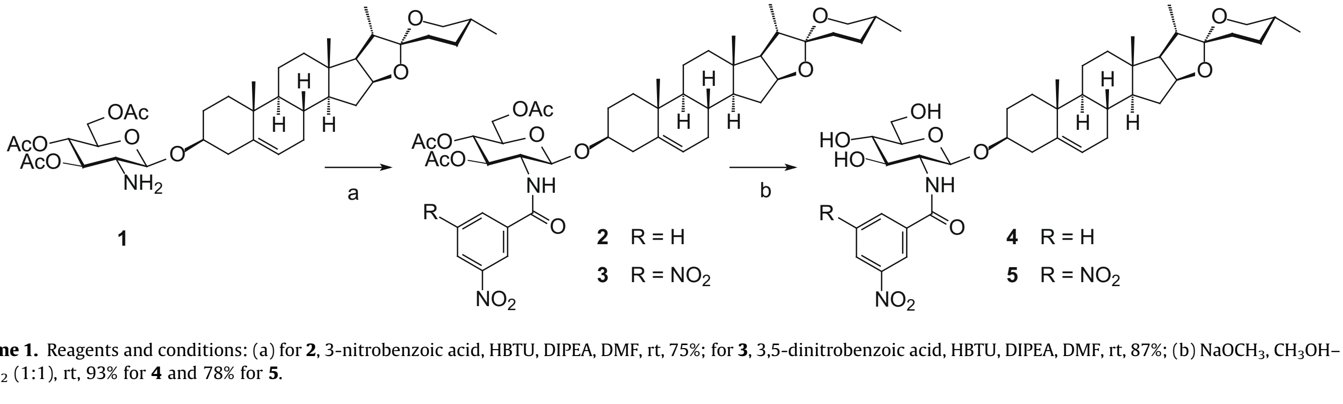
Two diosgenyl saponin analogues incorporating an aromatic nitro group at the 2-amino group of the D-glucosamine residue (**4** and **5**, Scheme 1) were synthesized. Starting from the previously reported amine **1**[17], 3-nitrobenzoic acid and 3,5-dinitrobenzoic acid were coupled to the amine in the presence of 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) and diisopropylethylamine (DIPEA) to give **2** and **3**, respectively, in good yield. Compounds **2** and **3** were then treated with sodium methoxide to provide saponin analogues **4** and **5**.

For the synthesis of saponin analogues containing a disaccharide residue, we first tried the convergent strategy by preparing the disaccharide donor, which will then be coupled to the diosgenyl aglycone. Since the 4-OH group in the D-glucosamine derivatives is known to be substantially less reactive than the 3-OH group,[20] we attempted to prepare 1,3,6-tri-*O*-benzoylated **7** as the potential glycosylation acceptor to build the disaccharide from the readily available trichloroethoxycarbonyl (Troc) protected glucosamine derivative **6** (Scheme 2). When **6** was treated with 3.1 equiv of benzoyl chloride in the presence of 4-dimethylaminopyridine (DMAP) at −40 °C, compound **7** was not formed in any detectable amount. Instead, the 3,6-di-*O*-benzoylated **8** and the 3,4,6-tri-*O*-benzoylated product **9** were obtained in 23% and 27%, respectively, together with an 8% of a less polar compound which was structurally not confirmed but likely to be the 1,3,4,6-tetra-*O*-benzoylated product according to its ^{1}H NMR spectral data. This result indicated that the anomeric hydroxyl group was the least reactive among the four hydroxyl groups present in **6**. Thus, we decided to prepare 3,-6-di-*O*-benzoylated derivative **8** which would allow regio-selective glycosylation at 4-OH over the anomeric-OH if **8** were used as a glycosylation acceptor (Scheme 2). When compound **6** was treated with 2.1 equiv of benzoyl chloride at −40 °C, compound **8** was obtained in 65% yield, together with 14% of **9**. The structure of **8** was confirmed through extensive 2D NMR spectral analysis (including ^{1}H–^{1}H COSY and ^{1}H–^{13}C HSQC).



Scheme 2. Reagents and conditions: (a) BzCl (2.1 equiv), DMAP, pyridine–CH_2Cl_2, −40 °C, 65% for **8** and 14% for **9**.

Glycosylation of **8** with L-rhamnopyranosyl imidate **10** in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as the catalyst at −20 °C afforded disaccharide **11** in 71% yield (Scheme 3). The formation of the di-rhamnopyranosylated trisaccharide in ~15% yield was observed from its crude ^{1}H NMR spectrum but an analytically pure product was not obtained. The glycosidic linkage at 4-*O*-position in **11** was confirmed by converting **11** into its acetylated derivative **12**. The ^{1}H NMR spectrum of **12** showed an additional acetyl group signal at δ 2.28 ppm and a new signal at δ 6.24 ppm (d, J = 3.5 Hz, H-1^{1}), indicating the presence of an acetyl group at the anomeric position, which in turn confirmed that compound **11** had a free anomeric hydroxyl group. Compound **11** was then treated with trichloroacetonitrile and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) to give imidate **13** in 80% yield. Glycosylation of donor **13** with diosgenin in the presence of TMSOTf as the catalyst provided product **14** in 80% yield. The newly formed



Scheme 1. Reagents and conditions: (a) for **2**, 3-nitrobenzoic acid, HBTU, DIPEA, DMF, rt, 75%; for **3**, 3,5-dinitrobenzoic acid, HBTU, DIPEA, DMF, rt, 87%; (b) $NaOCH_3$, CH_3OH–CH_2Cl_2 (1:1), rt, 93% for **4** and 78% for **5**.

β-glycosidic linkage and the previously formed α-rhamnopyranosyl linkage in **14** were confirmed by the anomeric signals in its ^{1}H and ^{13}C NMR spectra: δ 4.77 ppm (d, $J = 8.0$ Hz, H-1$^{\mathrm{I}}$) and 4.87 ppm (br s, H-1$^{\mathrm{II}}$); δ 98.89 and 99.88 ppm (C-1$^{\mathrm{I}}$ and C-1$^{\mathrm{II}}$).

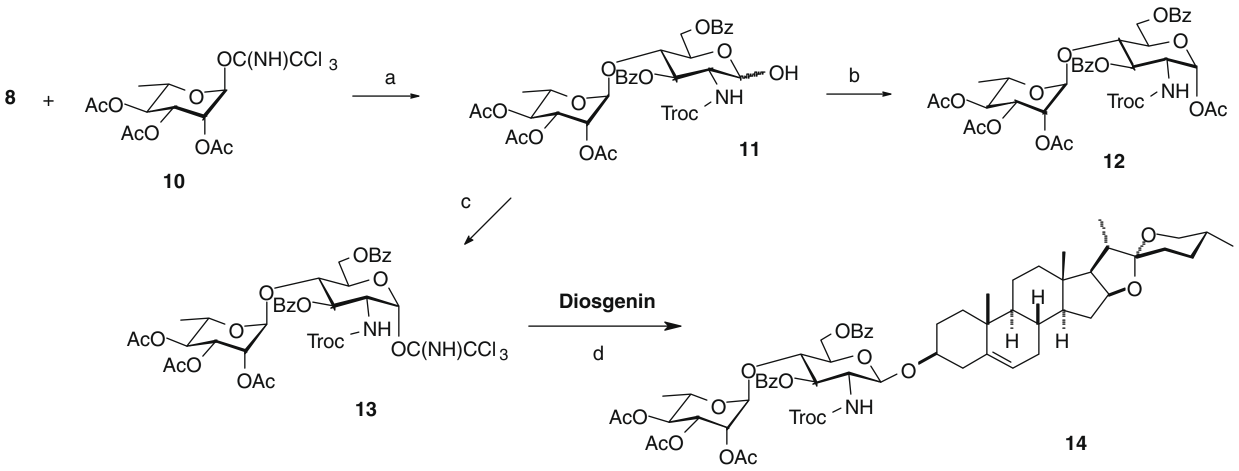
Although the yield was good for the convergent synthesis of **14** according to Schemes 2 and 3, both compounds **8** and **11** existed as α/β mixtures and therefore presented challenges in chromatographic separation from unwanted products which also existed as α/β mixtures. Thus, we investigated an alternative route to access **14** starting from the previously reported intermediate **15**[17] (Scheme 4). Compound **15** was treated with guanidinium nitrate–sodium methoxide at room temperature to give the *O*-deacetylated product **16** in 90% yield leaving the *N*-Troc group intact.[21] Selective benzoylation of **16** with 2.1 equiv of benzoyl chloride in the presence of DMAP at −40 °C gave 3,6-di-*O*-benzoylated product **17** in 69% yield. The structure of **17** was confirmed by ^{1}H–^{1}H COSY 2D NMR spectroscopy. Reaction of **17** with L-rhamnopyranosyl donor **10** using TMSOTf as the catalyst afforded **14** in 87% yield, which was identical, as indicated by ^{1}H and ^{13}C NMR spectra and TLC, with compound **14** prepared through Scheme 3.

The final steps for the preparation of disaccharide saponin analogues (**23**–**26**, Scheme 5) followed the same approach as that used for the synthesis of monosaccharide analogues (**4** and **5**, Scheme 1). Thus, the *N*-Troc protecting group in **14** was removed by treating with zinc dust in acetic acid, affording amine **18** in 95%. Amine **18** was then treated with benzoyl chloride, (±)-α-lipoic acid, 3-nitrobenzoic acid, and 3,5-dinitrobenzoic acid to give *N*-acylated products **19**–**22**, respectively, in 76–83% yield. Finally the *O*-acetyl and *O*-benzoyl groups in **19**–**22** were removed by treating with sodium methoxide to provide saponin analogues **23**–**26**, respectively, in very good yield. The structures of the synthesized saponin analogues were confirmed by ^{1}H NMR, ^{13}C NMR, and mass spectroscopic data.

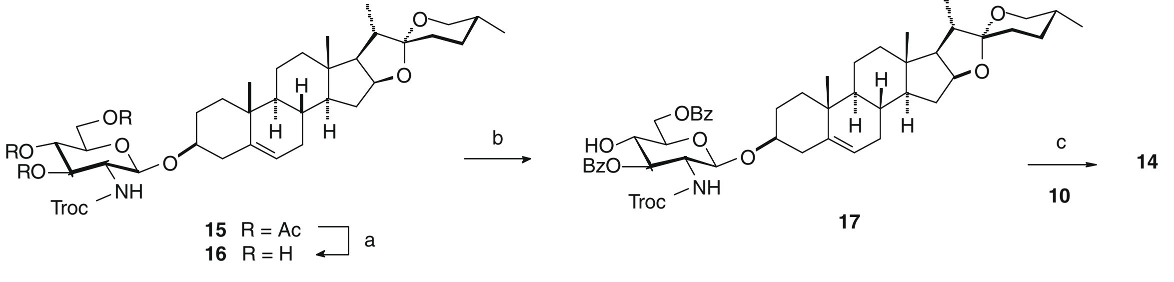
2.2. Biological studies

Previously, we reported the synthesis of several monosaccharide diosgenyl saponin analogues and their moderate cytotoxic activity against neuroblastoma (SK-N-SH) cells, breast cancer (MCF-7) cells, and cervical cancer (HeLa) cells.[17] The analogue containing 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. The role of α-lipoic acid in biological systems is very complex. Its disulfide bond allows the molecule to act as an oxidant as well as a reductant; however, based on its redox potential, its oxidizing behavior is more pronounced in biological systems.[22] Wenzel et al.[23] showed that α-lipoic acid induced apoptosis in human colon cancer cells by increasing superoxide free radical ($O_2^{-\bullet}$)-generation in mitochondrial. In recent years, extensive evidence supports the involvement of reactive oxygen species (ROS) and oxidative stress (OS) in the mechanism of many anticancer drugs.[18] Several saponin analogues synthesized here (**4**, **5**, **25**, and **26**) contain an aromatic nitro functionality which is anticipated to exert oxidative stress in cells and thus improve their anticancer potency. In addition, the preparation of disaccharide saponin analogues (**23**–**26**) would allow us to study the effect of the α-L-rhamnosyl moiety at 4-*O*-position of the inner sugar residue of these saponin analogues.

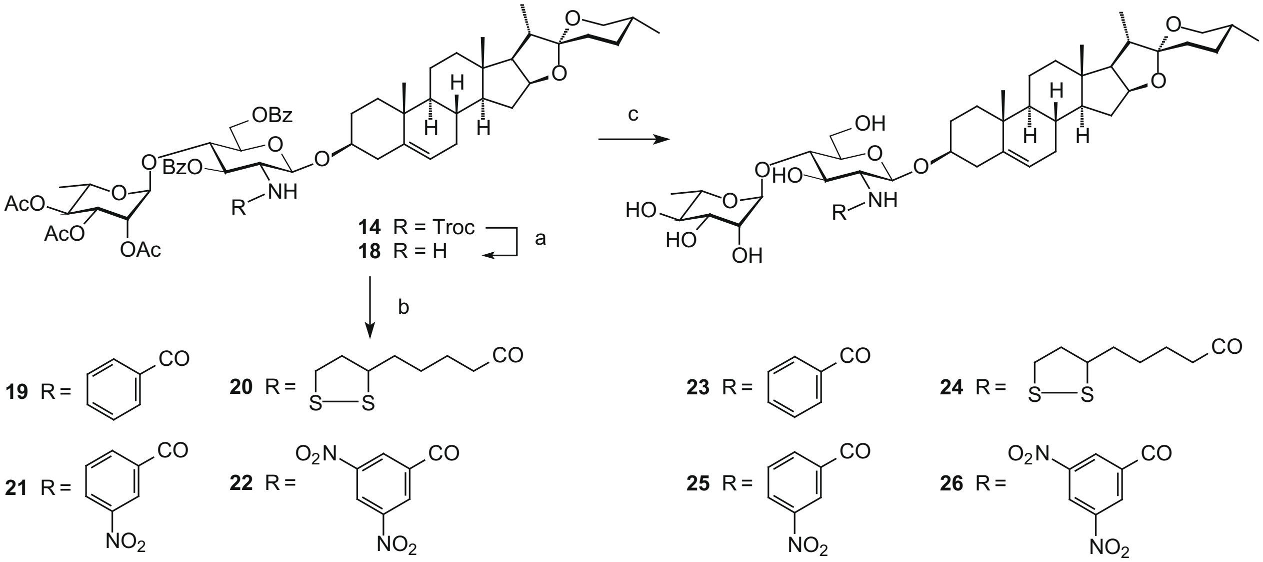
The cytotoxic activity of the synthesized saponin analogues was measured through MTT assay[24] using breast cancer (MCF-7) and cervical cancer (HeLa) cells. The anticancer drug Doxorubicin was also included in the study as a comparison. The data presented in



Scheme 3. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C, 71%; (b) Ac_2O, pyridine, rt, 86%; (c) CCl_3CN, DBU, rt, 80%; (d) TMSOTf, CH_2Cl_2, rt, 80%.



Scheme 4. Reagents and conditions: (a) guanidinium nitrate, $NaOCH_3$, CH_3OH–CH_2Cl_2 (9:1), rt, 90%; (b) BzCl (2.1 equiv), DMAP, pyridine–CH_2Cl_2, −40 °C, 69%; (c) TMSOTf, CH_2Cl_2, rt, 87%.



Scheme 5. Reagents and conditions: (a) Zn dust, HOAc, rt, 95%; (b) for **19**, BzCl, pyridine, rt, 80%; for **20**, (±)-α-lipoic acid, HBTU, DIPEA, DMF, rt, 83%; for **21**, 3-nitrobenzoic acid, HBTU, DIPEA, DMF, rt, 76%; for **22**, 3,5-dinitrobenzoic acid, HBTU, DIPEA, DMF, rt, 83%; (c) NaOCH$_3$, CH$_3$OH–CH$_2$Cl$_2$, rt, 91–96%.

Table 1 show that these analogues are significantly less active than Doxorubicin. Comparing compounds with the same N-substitution, all disaccharide saponin analogues (**23**–**26**) show lower cytotoxicity by a magnitude of 2–6-fold in both MCF-7 cells and HeLa cells than their corresponding monosaccharide analogues. The largest decrease in potency is observed for analogues containing the α-lipoic acid residue (**24** vs monosaccharide analogue **I** when R = α-lipoyl). For example, compound **24** has the IC$_{50}$ value of 39.9 μM against HeLa cells while the monosaccharide analogue **I** (R = α-lipoyl) has the IC$_{50}$ of 7.3 μM against HeLa cells. With respect to the effect of aromatic nitro functionality on the cytotoxicity, the *N*-(3-nitrobenzoyl)-substituted analogues are more active than the *N*-benzoyl-substituted analogues in both MCF-7 and HeLa cells (**4** vs structure **I** when R = Bz, and **25** vs **23**), which indicates that the addition of the nitro group on the aromatic ring increases the cytotoxicity of these compounds. However, this enhancement seems to be diminished when a second nitro group is present on the aromatic ring as indicated by the similar IC$_{50}$ values between *N*-(3,5-dinitrobenzoyl)-substituted analogues and *N*-benzoyl-substituted analogues (**5** vs **I** when R = Bz, and **26** vs **23**). These data suggest that the aromatic nitro functionality exhibits only marginal effect on the cytotoxicity of these saponin analogues when compared to simple *N*-benzoyl substitution.

Table 1
The in vitro cytotoxic activity (IC$_{50}$, μM) of diosgenyl saponin analogues[a,b]

Compound	R[c]	Cancer cell line	
		MCF-7	HeLa
Monosaccharide analogue ***I***			
I[d]	Bz	19.4	18.0
I[d]	α-Lipoyl	6.9	7.3
4	3-NO$_2$-Bz	13.6	16.1
5	3,5-(NO$_2$)$_2$-Bz	18.7	21.0
Disaccharide analogue ***II***			
23	Bz	>50[e]	[f]
24	α-Lipoyl	18.8	39.9
25	3-NO$_2$-Bz	22.8	39.4
26	3,5-(NO$_2$)$_2$-Bz	39.5	[f]
Doxorubicin		0.73	1.03

[a] The inhibitory concentration (IC$_{50}$) is the concentration in μM that inhibits cell growth by 50% in 3 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.

[c] R group refers to the substituent at the amino group; see general structure in Chart 1.

[d] Data from Ref. 17.

[e] Tested to 50 μM and the absorbance decreased to 68% at 50 μM.

[f] Tested to 50 μM, no change of absorbance.

3. Conclusion

We have synthesized several diosgenyl saponin analogues containing either a 2-amino-2-deoxy-β-D-glucopyranosyl residue or an α-L-rhamnopyranosyl-(1→4)-2-amino-2-deoxy-β-D-glucopyranosyl residue with different acyl substituents on the amino group. The cytotoxic activity of these compounds was evaluated in MCF-7 breast cancer cells and HeLa cervical cancer cells. Structure–activity relationship analysis indicated that the addition of an α-L-rhamnopyranosyl residue at 4-*O*-position of the inner β-D-glucosamine residue resulted in decrease in the cytotoxicity of these saponin analogues. The incorporation of an aromatic nitro functionality onto the amino group seems to exert only marginal effect on their anticancer activity.

4. Experimental

4.1. General methods

All air and moisture sensitive reactions were performed under nitrogen atmosphere. All commercial reagents were used as supplied. Anhydrous pyridine was distilled over potassium hydroxide, dichloromethane over calcium hydride, and methanol from magnesium turnings and a catalytic amount of iodine. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Aldrich. ACS grade solvents were purchased from Fisher Scientific and used for chromatography without distillation. TLC plates (Silica Gel 60 F$_{254}$, thickness 0.25 mm) and Silica Gel 60 (40–63 μm) for flash column chromatography were purchased from SILICYCLE INC., Canada. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer. Tetramethylsilane (TMS, δ 0.00 ppm) or solvent peaks were used as internal standards for ^{1}H and ^{13}C NMR spectra. The chemical shifts were given in ppm and coupling constants in Hz indicated to a resolution of ±0.5 Hz. Multiplicity of proton signals is indicated as follows: s (singlet), d (doublet), dd (double 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 with 1′, 2′, 3′ ..., etc. In the case of disaccharide residue, sugar units are designated as **I**, **II**, beginning at the reducing end of the molecule, according to the Whelan system (Rule 2-Carb-37.2).[25] MALDI mass spectra were obtained from Bruker Daltonics Apex-Qe FTICR MS mass spectrometer and ESI mass spectra were measured on the Applied Biosystems Mariner Biospectrometry Workstation, both at the University of Alberta, Canada. Optical rotations were measured with Perkin–Elmer 343 Polarimeter at 22 °C. Elemental analyses were carried out on a CEC (SCP) 240-XA Analyzer instrument by Lakehead University Instrumentation Laboratory (LUIL).

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

A mixture of compound **1**[17] (380 mg, 0.54 mmol), 3-nitrobenzoic acid (135 mg, 0.81 mmol), 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU, 307 mg, 0.81 mmol), diisopropylethylamine (DIPEA, 209 mg, 0.28 mL, 1.62 mmol) in dry dimethyl formamide (5 mL) was stirred at room temperature for 16 h. The solvent was then removed under high vacum and the residue dissolved in dichloromethane (100 mL). The organic layer was washed with sodium bicarbonate solution (20 mL) and dried with sodium sulfate and concentrated. The residue was purified by repeated flash chromatography (hexane–ethyl acetate, 1:1; then dichloromethane–methanol, 100:2) to give **2** (345 mg, 75%). R_f 0.35 (hexane–ethyl acetate, 1:1); $[\alpha]_{\mathrm{D}}^{22}$ −20.8 (*c* 1.0, chloroform–methanol, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 0.76 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.0 Hz, CH_3), 0.93 (s, 3H, CH_3), 0.96 (d, 3H, *J* 7.0 Hz, CH_3), 2.04 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 3.36 (dd, 1H, *J* 11.0, 11.0 Hz, H-26′a), 3.47 (dd, 1H, *J* 11.0, 4.5 Hz, H-26′b), 3.50 (m, 1H, H-3′), 3.85 (ddd, 1H, *J* 10.0, 5.0, 2.5 Hz, H-5), 4.15–4.20 (m, 2H, H-2, H-6a), 4.33 (dd, 1H, *J* 12.0, 5.0 Hz, H-6b), 4.39 (approx. q, 1H, *J* ≈ 8.0 Hz, H-16′), 4.96 (d, 1H, *J* 8.5 Hz, H-1), 5.19 (dd, 1H, *J* 10.0, 9.5 Hz, H-4), 5.21 (m, 1H, H-6′), 5.56 (dd, 1H, *J* 10.0, 10.0 Hz, H-3), 7.01 (d, 1H, *J* 8.5 Hz, NH), 7.61 (t, 1H, *J* 8.0 Hz, Ar-H), 8.16 (d, 1H, *J* 8.0 Hz, Ar-H), 8.29 (br d, 1H, *J* 8.0 Hz, Ar-H), 8.49 (t, 1H, *J* 1.5 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 14.67, 16.40, 17.30, 19.48, 20.79, 20.97, 28.93, 29.54, 29.84, 30.42, 31.49, 31.50, 31.94, 32.18, 36.96, 37.28, 38.97, 40.37, 41.72, 50.12, 55.61, 56.58, 62.20, 62.59, 66.98, 69.06, 71.93, 72.88, 79.77, 80.90, 99.44 (C-1), 109.44 (C-22′), 121.01 (C-6′), 126.38, 130.12, 133.73, 135.77, 140.41 (C-5′), 148.12, 165.26 (CO), 169.49 (CO), 170.92 (CO), 171.77 (CO); LRESIMS (*m*/*z*): 873 (M+Na$^+$); HRMALDIMS calcd for $C_{46}H_{63}N_2O_{13}$ (M+H$^+$): 851.43247, found: 851.43213.

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

In a similar way as described for the preparation of **2**, compound **1** (200 mg, 0.28 mmol) was treated with 3,5-dinitrobenzoic acid (91 mg, 0.43 mmol) in the presence of HBTU (162 mg, 0.43 mmol), DIPEA (110 mg, 0.15 mL, 0.85 mmol) in dry dimethylformamide (5 mL) to give **3** (223 mg, 87%) after chromatographic purification (hexane–ethyl acetate, 1.75:1). R_f 0.50 (hexane–ethyl acetate, 1:1); $[\alpha]_{\mathrm{D}}^{22}$ −14.3 (*c* 1.0, chloroform–methanol, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 0.76 (s, 3H, CH_3), 0.78 (d, 3H, *J* 6.5 Hz, CH_3), 0.94 (s, 3H, CH_3), 0.96 (d, 3H, *J* 7.0 Hz, CH_3), 2.02 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 3.36 (dd, 1H, *J* 11.0, 11.0 Hz, H-26′a), 3.46 (dd, 1H, *J* 11.0, 4.5 Hz, H-26′b), 3.52 (m, 1H, H-3′), 3.83 (dd, 1H, *J* 9.5, 5.0, 2.5 Hz, H-5), 4.10–4.20 (m, 2H, H-2, H-6a), 4.32 (dd, 1H, *J* 12.0, 5.0 Hz, H-6b), 4.40 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.93 (d, 1H, *J* 8.5 Hz, H-1), 5.19 (dd, 1H, *J* 9.5, 9.5 Hz, H-4), 5.23 (m, 1H, H-6′), 5.52 (dd, 1H, *J* 10.0, 10.0 Hz, H-3), 7.04 (d, 1H, *J* 8.5 Hz, NH), 8.94 (d, 2H, *J* 2.0 Hz, Ar-H), 9.14 (t, 1H, *J* 2.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): *δ* 14.72, 16.46, 17.34, 19.53, 20.84, 21.02, 28.97, 29.59, 39.91, 30.47, 31.51, 31.55, 32.00, 32.20, 37.01, 37.29, 39.09, 39.89, 40.42, 41.77, 50.17, 56.02, 56.62, 62.23, 62.53, 67.04, 68.90, 72.05, 72.82, 79.82, 80.95, 99.32 (C-1), 109.51 (C-22′), 121.51 (C-6′), 122.27, 127.45, 137.59, 140.25 (C-5′), 148.87, 162.97 (CO), 169.52 (CO), 171.01 (CO), 171.99 (CO); LRESIMS (*m*/*z*): 918 (M+Na$^+$); HRMALDIMS calcd for $C_{46}H_{62}N_3O_{15}$ (M+H$^+$): 896.41754, found: 896.41724.

4.4. Diosgenyl 2-deoxy-2-(3-nitrobenzamido)-β-D-glucopyranoside (4)

Compound **2** (220 mg, 0.258 mmol) was dissolved in dry dichloromethane–methanol (1:1, 8 mL) and treated with catalytical amount of sodium methoxide at room temperature for 16 h. The solution was then neutralized with weakly acidic resin (IRC-50, H$^+$ form) and concentrated in vacuo. The residue was purified through Sephadex LH-20 gel filtration chromatography (dichloromethane–methanol, 1:1) to give **4** (173 mg, 93%). R_f 0.34 (hexane–ethyl acetate–methanol, 1:1:0.2); $[\alpha]_{\mathrm{D}}^{22}$ −36.9 (*c* 1.0, chloroform–methanol, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD, 1:1): *δ* 0.77 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.5 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.96 (d, 3H, *J* 6.5 Hz, CH_3), 2.08 (m, 1H), 2.28 (m, 1H), 3.35 (m, 2H, H-26′a, H-26′b), 3.46 (m, 2H), 3.54 (m, 1H, H-3′), 3.71 (dd, 1H, *J* 10.0, 9.0 Hz), 3.77 (dd, 1H, *J* 12.0, 5.0 Hz, H-6a), 3.85 (dd, 1H, *J* 10.0, 8.5 Hz, H-2), 3.90 (dd, 1H, *J* 12.0, 2.5 Hz, H-6b), 4.40 (approx. q, 1H, *J* 8.5 Hz, H-16′), 4.75 (d, 1H, *J* 8.5 Hz, H-1), 5.27 (m, 1H, H-6′), 7.71 (dd, 1H, *J* 8.0, 8.0 Hz, Ar-H), 8.23 (br d, 1H, *J* 8.0 Hz, Ar-H), 8.38 (br d, 1H, *J* 8.0 Hz, Ar-H), 8.74 (dd, 1H, *J* 2.0, 2.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$–CD_3OD, 1:1): *δ* 14.88, 16.80, 17.51, 19.84, 21.55, 29.39, 30.21, 30.94, 31.99, 32.19, 32.37, 32.77, 37.54, 37.97, 39.75, 40.46, 41.03, 42.41, 50.92, 57.27, 57.92, 62.42, 62.86, 67.58, 71.71, 75.02, 77.06, 80.12, 81.80, 100.59 (C-1), 110.39 (C-22′), 122.41 (C-6′), 123.09, 126.71, 130.58, 134.19, 137.36, 141.15 (C-5′), 149.04, 167.79 (CO); LRESIMS (*m*/*z*): 759.4 (M+Cl$^-$); HRESIMS calcd for $C_{40}H_{56}N_2O_{10}Cl$ (M+Cl$^-$): 759.36290, found (negative mode): 759.36311.

4.5. Diosgenyl 2-deoxy-2-(3,5-dinitrobenzamido)-β-D-glucopyranoside (5)

In a similar way as described for the synthesis of **4**, compound **3** (120 mg, 0.134 mmol) was treated with sodium methoxide to provide **5** (80 mg, 78%). R_f 0.30 (dichloromethane–methanol, 100:8); $[\alpha]_{\mathrm{D}}^{22}$ −2.2 (*c* 0.3, chloroform–methanol, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$–CD_3OD, 1:1): *δ* 0.77 (s, 3H, CH_3), 0.80 (d, 3H, *J* 6.0 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.97 (d, 3H, *J* 6.5 Hz, CH_3), 2.10 (m, 1H), 2.27 (m, 1H), 3.37 (m, 2H), 3.45–3.57 (m, 3H), 3.73 (dd, 1H, *J* 10.0, 9.0 Hz), 3.80 (dd, 1H, *J* 12.0, 4.5 Hz, H-6a), 3.85 (dd, 1H, *J* 10.5, 8.5 Hz, H-2), 3.90 (dd, 1H, *J* 12.0, 2.5 Hz, H-6b), 4.41 (m, 1H, H-16′), 4.78 (d, 1H, *J* 8.5 Hz, H-1), 5.28 (m, 1H, H-6′), 9.13 (d, 2H, *J* 2.0 Hz, Ar-H), 9.16 (t, 1H, *J* 2.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$–CD_3OD, 1:1): *δ* 13.70, 15.62, 16.34, 18.67, 20.38, 28.20, 29.04, 29.77, 30.81, 31.00, 31.19, 31.59, 36.37, 36.77, 38.61, 39.28, 39.85, 41.23, 49.73, 56.09, 56.92, 61.25, 61.67, 66.42, 70.49, 73.78, 75.93, 78.85, 80.63, 99.25 (C-1), 109.22 (C-22′), 120.48 (C-6′), 121.32, 127.24, 137.96, 139.89 (C-5′), 148.29, 164.04 (CO); LRESIMS (*m*/*z*): 804.4 (M+Cl$^-$); HRESIMS calcd for $C_{40}H_{55}N_3O_{12}Cl$ (M+Cl$^-$): 804.34798, found (negative mode): 804.34718.

4.6. 3,6-Di-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose (8) and 3,4,6-tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose (9)

To a solution of **6** (3.0 g, 8.46 mmol) and 4-dimethylaminopyridine (DMAP, 103 mg, 0.10 mmol) in anhydrous pyridine (9 mL) and dry CH_2Cl_2 (6 mL), benzoyl chloride (2.06 mL, 17.77 mmol) in dry CH_2Cl_2 (8 mL) was added drop-wise under N_2 atmosphere at −40 °C. The reaction was carefully monitored by TLC every 20 min. After being stirred at room temperature for 2 h, the reaction was quenched with methanol (1.0 mL) and the mixture further stirred for 30 min. The solution was then diluted with CH_2Cl_2 (200 mL) and washed with cold HCl solution (4 N, 50 mL) and saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was subjected to flash chromatography (hexane–ethyl acetate, 1.5:1) to yield **8** (3.10 g, 65%) and **9** (666 mg, 14%), both as anomeric mixtures (α/β, 8:1). For **8**: R_f 0.26 (hexane–ethyl acetate, 1.5:1); $[\alpha]_D^{22}$ +0.05 (*c* 0.5, $CHCl_3$) for the anomeric mixture (α/β = 8:1); ^{1}H NMR (500 MHz, $CDCl_3$) for the α-isomer: δ 3.40 (br s, 1H, OH), 3.69 (br s, 1H, OH), 3.87 (dd, 1H, *J* 10.0, 9.5 Hz, H-4), 4.21 (ddd, 1H, *J* 10.5, 9.0, 3.5 Hz, H-2), 4.34 (m, 1H, H-5), 4.41 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.66 (dd, 1H, *J* 12.0, 2.0 Hz, H-6a), 4.74 (dd, 1H, *J* 12.0, 4.0 Hz, H-6b), 4.76 (d, 1H, *J* 12.0 Hz, Troc-Hb), 5.40 (d, 1H, *J* 3.5 Hz, H-1), 5.65 (dd, 1H, *J* 10.5, 9.5 Hz, H-3), 5.81 (d, 1H, *J* 9.0 Hz, NH), 7.44 (m, 4H, Ar-H), 7.57 (m, 2H, Ar-H), 8.04 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) for the α-isomer: δ 54.06 (C-2), 63.58 (C-6), 69.63 (C-4), 70.80 (C-5), 74.44 (Troc-CH_2), 74.68 (C-3), 92.44 (Troc-CCl_3), 95.45(C-1), 128.68, 129.13, 129.80, 130.08, 130.31, 133.57, 133.91, 154.43 (Troc-CO), 167.23 (CO), 168.07 (CO); Anal. Calcd for $C_{23}H_{22}Cl_3NO_9$: C, 49.09; H, 3.94; N, 2.49. Found: C, 49.08; H, 3.84; N, 2.23. For **9**: R_f 0.48 (hexane–ethyl acetate, 1.5:1); $[\alpha]_D^{22}$ +26.4 (*c* 0.5, $CHCl_3$) for the anomeric mixture (α/β = 8:1); ^{1}H NMR (500 MHz, $CDCl_3$) for the α-isomer: δ 3.92 (br s, 1H, OH), 4.33 (ddd, 1H, *J* 10.0, 3.5 Hz, H-2), 4.41 (dd, 1H, *J* 12.5, 4.0 Hz, H-6a), 4.47 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.63–4.69 (m, 2H, H-5, H-6b), 4.70 (d, 1H, *J* 12.0 Hz, Troc-Hb), 5.47 (br s, 1H, H-1), 5.70 (d, 1H, *J* 10.0 Hz, NH), 5.72 (dd, 1H, *J* 9.5, 10.0 Hz), 5.90 (dd, 1H, *J* 10.5, 10.0 Hz), 7.26–7.57 (m, 10H, Ar-H), 7.92 (m, 3H, Ar-H), 8.05 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$) for the α-isomer: δ 54.73 (C-2), 63.00 (C-6), 68.40, 69.49, 71.41, 74.54, 92.31 (C-1), 95.37 (Troc-CCl_3), 128.60, 128.63, 128.97, 129.03, 129.71, 130.01, 130.15, 133.43, 133.64, 154.43 (Troc-CO), 165.42 (CO), 166.63 (CO), 166.87 (CO); Anal. Calcd for $C_{30}H_{26}Cl_3NO_{10}$: C, 54.03; H, 3.93; N, 2.10. Found: C, 54.03; H, 3.92; N, 1.89.

4.7. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose (11)

A suspension of **8** (5.0 g, 8.88 mmol), **10** (3.88 g, 8.88 mmol), and activated molecular sieves (4 Å, 3.0 g) in anhydrous CH_2Cl_2 (15 mL) was stirred under N_2 atmosphere at room temperature for 30 min and then cooled to −20 °C. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 5.5 mL, 0.02 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 30 min, quenched by saturated $NaHCO_3$ solution (10 mL), extracted with CH_2Cl_2 (3 × 20 mL) and dried over Na_2SO_4. The filtrate was concentrated in vacuo with the resulting residue purified by flash chromatography (hexane–ethyl acetate, 1.5:1) to give **11** (5.26 g, 71%) as an anomeric mixture (α/β = 10:1). R_f 0.29 (hexane–ethyl acetate, 1.5:1); $[\alpha]_D^{22}$ +19.8 (*c* 0.5, $CHCl_3$) for the anomeric mixture (α/β = 10:1); ^{1}H NMR (500 MHz, $CDCl_3$) for the α-isomer: δ 0.68 (d, 3H, *J* 6.0 Hz, CH_3), 1.89 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.70–3.74 (m, 2H, OH, H-5^{II}), 4.15 (dd, 1H, *J* 9.5, 9.5 Hz, H-4^{I}), 4.19 (ddd, 1H, *J* 10.0, 9.5, 3.0 Hz, H-2^{I}), 4.40 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.41 (m, 1H), 4.48 (dd, 1H, *J* 12.5, 3.0 Hz, H-6^{I}a), 4.65 (d, 1H, *J* 12.0 Hz, Troc-Hb), 4.87–4.94 (m, 3H), 5.13–5.18 (m, 2H), 5.36 (br s, 1H, H-1^{I}), 5.74 (d, 1H, *J* 10.0 Hz, NH), 5.77 (dd, 1H, *J* 11.0, 10.5 Hz, H-3^{I}), 7.40–7.62 (m, 6H, Ar-H), 8.08 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$) for the α-isomer: δ 17.04, 20.99, 54.91, 62.74, 67.60, 68.95, 69.41, 70.16, 70.52, 72.12, 74.47, 75.91, 92.10 (C-1^{I}), 95.31 (Troc-CCl_3), 98.36 (C-1^{II}), 128.54, 128.70, 129.58, 130.03, 130.35, 133.46, 133.63, 154.70 (Troc-CO), 166.29 (CO), 166.59 (CO), 170.05 (CO), 170.32 (CO), 170.58 (CO); Anal. Calcd for $C_{35}H_{38}Cl_3NO_{16}$: C, 50.34; H, 4.59; N, 1.68. Found: C, 50.31; H, 4.55; N, 1.52.

For structure confirmation that the glycosylation occurred at 4-OH in **8**, disaccharide **11** was converted into its acetylated derivative **12**. To a cooled solution (ice water bath) of **11** (78 mg, 0.09 mmol) in pyridine (2.1 mL), acetic anhydride (1.7 mL) was added. The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (150 mL) and washed successively with cold HCl solution (4 N, 20 mL), and saturated $NaHCO_3$ solution (20 mL × 2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane–ethyl acetate, 1.5:1) to give **12** (70 mg, 86%). R_f 0.73 (CH_2Cl_2–MeOH, 25:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.67 (d, 3H, *J* 6.0 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.28 (s, 3H, CH_3CO), 3.70 (m, 1H, H-5^{II}), 4.17 (m, 2H), 4.33 (ddd, 1H, *J* 10.5, 10.0, 3.5 Hz, H-2^{I}), 4.39 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.51 (dd, 1H, *J* 12.5, 2.5 Hz, H-6^{I}a), 4.67 (d, 1H, *J* 12.0 Hz, Troc-Hb), 4.75 (m, 1H, H-6^{I}b), 4.89 (dd, 1H, *J* 10.0, 9.5 Hz, H-4^{II}), 4.90 (d, 1H, *J* 1.5 Hz, H-1^{II}), 5.15 (m, 3H, NH, H-2^{II}, H-3^{II}) 5.65 (dd, 1H, *J* 11.0, 10.5 Hz, H-3^{I}), 6.24 (d, 1H, *J* 3.5 Hz, H-1^{I}), 7.42–7.50 (m, 4H, Ar-H), 7.59 (m, 2H, Ar-H), 8.05 (m, 4H, Ar-H).

4.8. 2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate (13)

To a solution of **11** (500 mg, 0.58 mmol) in anhydrous CH_2Cl_2 (3 mL), trichloroacetonitrile (CCl_3CN, 1.0 mL, 9.97 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU, 88 mg, 87 μL, 0.58 mmol) were added and stirred at room temperature for 20 min. The solid was filtered through celite and the filtrate was concentrated in vacuo. The residue was purified with flash chromatography (hexane–ethyl acetate, 1.5:1 with 0.5% triethylamine) to afford **13** (466 mg, 80%) as a syrup. R_f 0.29 (hexane–ethyl acetate, 1.5:1); $[\alpha]_D^{22}$ +26.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.68 (d, 3H, *J* 6.0 Hz, CH_3), 1.90 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 3.71 (m, 1H, H-5^{II}), 4.22 (dd, 1H, *J* 9.5, 9.5 Hz, H-4^{I}), 4.30 (m, 1H, H-5^{I}), 4.42 (ddd, 1H, *J* 10.5, 10.0, 3.5 Hz, H-2^{I}), 4.48 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.53 (dd, 1H, *J* 12.5, 3.5 Hz, H-6^{I}a), 4.63 (d, 1H, *J* 12.0 Hz, Troc-Hb), 4.79 (dd, 1H, *J* 12.5, 1.5 Hz, H-6^{I}b), 4.88 (dd, 1H, *J* 10.0, 10.0 Hz, H-4^{II}), 4.91 (br s, 1H, H-1^{II}), 5.15 (m, 1H, H-2^{II}), 5.17 (dd, 1H, *J* 10.0, 3.5 Hz, H-3^{II}), 5.22 (d, 1H, *J* 10.0 Hz, NH), 5.71 (dd, 1H, *J* 10.5, 9.5 Hz, H-3^{I}), 6.42 (d, 1H, *J* 3.5 Hz, H-1^{I}), 7.47 (m, 4H, Ar-H), 7.60 (m, 2H, Ar-H), 8.06 (m, 4H, Ar-H), 8.81 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.06, 20.88, 20.92, 20.96, 54.46, 62.20, 67.68, 68.58, 70.11, 70.74, 71.66, 71.85, 74.61, 75.89, 90.83, 94.87 (C-1^{I}), 95.09 (Troc-CCl_3), 99.03 (C-1^{II}), 128.65, 128.69, 129.19, 129.81, 129.99, 130.25, 133.43, 133.92, 154.44 (Troc-CO), 160.70 (CO), 165.94 (CO), 166.73 (CO), 170.01 (CO), 170.20 (CO); Anal. Calcd for $C_{37}H_{38}Cl_6N_2O_{16}$: C, 45.37; H, 3.91; N, 2.86. Found: C, 45.12; H, 3.62; N, 2.63.

4.9. Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (14)

4.9.1. Method A

A suspension of **13** (230 mg, 0.23 mmol), diosgenin (95 mg, 0.23 mmol), and activated molecular sieves (4 Å, 2.0 g) in anhydrous CH_2Cl_2 (3 mL) was stirred at room temperature for 15 min under N_2 atmosphere. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.45 mL, 0.01 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 15 min, quenched by saturated $NaHCO_3$ solution (10 mL), extracted with CH_2Cl_2 (3 × 10 mL) and dried over Na_2SO_4. The filtrate was concentrated in vacuo with the resulting residue purified by flash chromatography (hexane–ethyl acetate–methanol, 5:2:0.05) to give compound **14** (221 mg, 80%) as white foam.

4.9.2. Method B

A suspension of **17** (230 mg, 0.24 mmol), **10** (191 mg, 0.44 mmol), and activated molecular sieves (4 Å, 2.0 g) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 15 min under N_2 atmosphere. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.44 mL, 0.01 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 30 min, quenched by saturated $NaHCO_3$ solution (5 mL), extracted with CH_2Cl_2 (3 × 20 mL) and dried over Na_2SO_4. The filtrate was concentrated in vacuo with the resulting residue purified by flash chromatography (hexane–ethyl acetate–methanol, 5:2:0.05) to give compound **14** (262 mg, 87%) as white foam.

R_f 0.17 (hexane–ethyl acetate–methanol, 6:2:0.05); $[\alpha]_D^{22}$ −52.5 (*c* 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.67 (d, 3H, *J* 6.0 Hz, CH_3), 0.77 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.0 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.98 (d, 3H, *J* 6.5 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 3.38 (dd, 1H, *J* 11.5, 10.5 Hz, H-26′a), 3.48 (m, 2H, H-26′b, H-3′), 3.69 (m, 1H, H-5^{II}), 3.75 (m, 1H, H-2^{I}), 3.86 (m, 1H, H-5^{I}), 4.04 (dd, 1H, *J* 9.0, 9.5 Hz, H-4^{I})), 4.41 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.52 (dd, 1H, *J* 12.0, 5.0 Hz, H-6^{I}a), 4.54 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.71 (d, 1H, *J* 12.0 Hz, Troc-Hb), 4.77 (d, 1H, *J* 8.0 Hz, H-1^{I}), 4.80 (dd, 1H, *J* 12.5, 2.5 Hz, H-6^{I}b), 4.87 (br s, 1H, H-1^{II}), 4.88 (dd, 1H, *J* 10.0, 9.5 Hz, H-4^{II}), 5.12 (m, 1H, H-2^{II}), 5.15 (dd, 1H, *J* 10.0, 3.5 Hz, H-3^{II}), 5.29 (m, 1H, H-6′), 5.60 (dd, 1H, *J* 10.5, 9.0 Hz, H-3^{I}), 7.45 (m, 4H, Ar-H), 7.58 (m, 2H, Ar-H), 8.05 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.74, 16.48, 17.02, 17.35, 19.53, 20.91, 28.98, 29.71, 30.49, 31.56, 32.03, 32.25, 36.96, 37.18, 39.01, 39.95, 40.45, 41.78, 50.16, 56.67, 57.17, 62.25, 62.99, 67.05, 67.66, 68.65, 70.19, 70.73, 73.25, 73.45, 74.49, 80.17, 81.01, 95.50 (Troc-*C*Cl_3), 98.89 (C-1^{II}), 99.88 (C-1^{I}), 109.51 (C-22′), 121.99 (C-6′), 128.60, 128.67, 129.52, 130.02, 130.22, 133.28, 133.72, 140.49 (C-5′), 154.35 (CO), 166.06 (CO), 166.39 (CO), 170.05 (CO), 170.16 (CO), 170.26 (CO); Anal. Calcd for $C_{62}H_{78}Cl_3NO_{18}$: C, 60.46; H, 6.38; N, 1.14. Found: C, 60.14; H, 6.48; N, 0.89.

4.10. Diosgenyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (16)

Guanidinium nitrate (3.72 g, 30.4 mmol) was dissolved in MeOH–CH_2Cl_2 (9:1, 300 mL) and sodium methoxide in methanol solution (1.0 M, 6 mL) was added. To the above solution, compound **15**[17] (2.88 g, 3.29 mmol) was added and the mixture was stirred at room temperature for 20 h. The mixture was then 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 subjected to flash chromatography (CH_2Cl_2–MeOH, 12.5:1) to give **16** (2.21 g, 90%). The compound was freeze-dried from dioxane to ensure removal of residual methanol which interferes with the subsequent reaction. R_f 0.19 (CH_2Cl_2–MeOH, 14.3:1); $[\alpha]_D^{22}$ −45.0 (*c* 0.5, MeOH); ^1H NMR (500 MHz, C_5D_5N): δ 0.70 (d, 3H, *J* 5.5 Hz, CH_3), 0.84 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.15 (d, 3H, *J* 7.0 Hz, CH_3), 2.49 (m, 1H), 2.62 (m, 1H), 3.51 (dd, 1H, *J* 11.0, 10.0 Hz, H-26′a), 3.60 (dd, 1H, *J* 10.0, 3.5 Hz, H-26′b), 3.85 (m, 1H, H-3′), 4.05 (m, 1H, H-5), 4.30 (dd, 1H, *J* 9.0, 9.0 Hz), 4.33 (dd, 1H, *J* 10.0, 9.0 Hz), 4.42–4.49 (m, 2H, H-2, H-6a), 4.55 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.61 (br d, 1H, *J* 11.5 Hz, H-6b), 4.95 (d, 1H, *J* 12.0 Hz, Troc-Ha), 5.17 (d, 1H, *J* 12.0 Hz, Troc-Hb), 5.18 (m, 1H, H-6′), 5.22 (d, 1H, *J* 8.0 Hz, H-1), 6.61 (br s, 1H, OH), 7.52 (br s, 1H, OH), 7.59 (br s, 1H, OH), 9.26 (d, 1H, *J* 9.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.39, 16.69, 17.68, 19.79, 21.42, 29.60, 30.44, 30.94, 31.94, 32.14, 32.53, 37.35, 37.70, 39.79, 40.18, 40.78, 42.30, 50.54, 56.95, 60.17, 63.10, 63.21, 67.20, 67.54, 72.64, 74.94, 76.60, 79.04, 81.42, 97.50 (Troc-*C*Cl_3), 101.47 (C-1), 109.61 (C-22′), 121.98 (C-6′), 141.22 (C-5′), 156.20 (CO); Anal. Calcd for $C_{36}H_{54}Cl_3NO_9$: C, 57.56; H, 7.25; N, 1.86. Found: C, 57.61; H, 7.41; N, 1.67.

4.11. Diosgenyl 3,6-di-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (17)

To a solution of **16** (386 mg, 0.51 mmol) and 4-dimethylaminopyridine (DMAP, 6 mg, 0.05 mmol) in anhydrous pyridine (4 mL) and CH_2Cl_2 (2 mL), benzoyl chloride (0.12 mL, 1.03 mmol) in dry CH_2Cl_2 (2 mL) was added drop-wise under N_2 atmosphere at −40 °C. After being stirred at room temperature for 2 h, the reaction was quenched with MeOH (1.0 mL) and the mixture stirred for 30 min. The solution was then diluted with CH_2Cl_2 (150 mL) and washed with cold HCl solution (4 N, 30 mL) and saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was subjected to flash chromatography (toluene–acetone, 11:1) to give compound **17** (340 mg, 69%). R_f 0.24 (toluene–acetone, 11:1); $[\alpha]_D^{22}$ −33.5 (*c* 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.5 Hz, CH_3), 0.97 (s, 3H, CH_3), 0.98 (d, 3H, *J* 8.0 Hz, CH_3), 3.38 (dd, 1H, *J* 11.0, 11.0 Hz, H-26′a), 3.40 (br s, 1H, OH), 3.48 (dd, 1H, *J* 11.5, 3.5 Hz, H-26′b), 3.53 (m, 1H, H-3′), 3.75–3.81 (m, 3H, H-2, H-4, H-5), 4.41 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.57 (d, 1H, *J* 12.0 Hz, Troc-Ha), 4.63 (d, 1H, *J* 12.5, 1.5 Hz, H-6a), 4.71 (d, 1H, *J* 12.0 Hz, Troc-Hb), 4.72 (m, 1H, H-6b), 4.83 (d, 1H, *J* 8.5 Hz, H-1), 5.27 (m, 1H, H-6′), 5.40 (d, 1H, *J* 9.0 Hz, NH), 5.50 (dd, 1H, *J* 10.0, 8.5 Hz, H-3), 7.38–7.45 (m, 4H, Ar-H), 7.56 (m, 2H, Ar-H), 8.02 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.75, 16.49, 17.35, 19.56, 21.00, 28.98, 29.72, 30.49, 31.56, 32.03, 32.25, 36.99, 37.25, 39.03, 39.97, 40.46, 41.78, 50.21, 56.56, 56.68, 62.26, 63.99, 67.05, 70.02, 74.23, 74.48, 75.81, 80.10, 81.00, 95.61 (Troc-*C*Cl_3), 99.90 (C-1), 109.53 (C-22′), 121.99 (C-6′), 128.62, 128.68, 129.17, 129.80, 130.10, 130.25, 133.50, 133.85, 140.51 (C-5′), 154.46 (CO), 167.21 (CO), 167.51 (CO); Anal. Calcd for $C_{50}H_{62}Cl_3NO_{11}$: C, 62.60; H, 6.51; N, 1.46. Found: C, 63.00; H, 6.32; N, 1.10.

4.12. Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-2-benzamido-3,6-di-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (19)

4.12.1. Preparation of amine 18

To a solution of **14** (217 mg, 0.17 mmol) in acetic acid (10 mL), zinc dust (2.0 g) was added and the mixture was stirred at room temperature for 20 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 in CH_2Cl_2 (200 mL). The solution was washed with saturated $NaHCO_3$ solution (20 mL), dried with Na_2SO_4, and concentrated under vacuum to give a white solid **18** (180 mg, 96%) which was used without further purification in the next step.

To a cooled solution (ice bath) of **18** (85 mg, 0.08 mmol) in pyridine (2.0 mL), benzoyl chloride (14.0 µL, 0.12 mmol) was added. The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (150 mL), washed with cold HCl solution (4 N, 20 mL) and saturated $NaHCO_3$ solution (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was subjected to flash chromatography (toluene–acetone, 9:1) to give **19** (92 mg, 80%). R_f 0.25 (toluene–acetone, 9:1); $[\alpha]_D^{22}$ −33.9 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.69 (d, 3H, *J* 6.0 Hz, CH_3), 0.76 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.5 Hz, CH_3), 0.92 (s, 3H, CH_3), 0.97 (d, 3H, *J* 7.0 Hz, CH_3), 1.90 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* 10.5, 11.5 Hz, H-26′a), 3.49 (m, 2H, H-26′b, H-3′), 3.72 (m, 1H, H-5^{II}), 3.93 (m, 1H, H-5^{I}), 4.14 (dd, 1H, *J* 9.0, 9.5 Hz, H-4^{I}), 4.27 (m, 1H, H-26′), 4.40 (approx. q, 1H, *J* ≈ 7.0 Hz, H-16′), 4.55 (dd, 1H, *J* 12.0, 5.0 Hz, H-6^{I}a), 4.83 (dd, 1H, *J* 12.0, 2.0 Hz, H-6^{I}b), 4.88 (dd, 1H, *J* 10.0, 10.0 Hz, H-4^{II}), 4.91 (d, 1H, *J* 8.5 Hz, H-1^{I}), 4.92 (br s, 1H, H-1^{II}), 5.16 (m, 1H, H-2^{II}), 5.17 (m, 1H, H-3^{II}), 5.21 (m, 1H, H-6′), 5.71 (dd, 1H, *J* 9.0, 9.0 Hz, H-3^{I}), 6.24 (d, 1H, *J* 9.0 Hz, NH), 7.28–7.60 (m, 11H, Ar-H), 8.00 (m, 2H, Ar-H), 8.08 (m, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.73, 16.46, 17.02, 17.34, 19.50, 20.91, 28.98, 29.71, 30.48, 31.53, 32.02, 32.22, 36.93, 37.21, 39.14, 39.93, 40.43, 41.77, 50.16, 55.51, 56.65, 62.24, 63.11, 67.03, 67.61, 68.73, 70.17, 70.76, 73.27, 74.02, 76.81, 79.94, 80.99, 98.67 (C-1^{II}), 100.22 (C-1^{I}), 109.49 (C-22′), 121.61 (C-6′), 126.96, 128.61, 128.73, 129.53, 130.04, 130.15, 131.65, 133.26, 133.60, 134.65, 140.64 (C-5′), 166.12 (CO), 166.92 (CO), 168.06 (CO), 170.04 (CO), 170.18 (CO), 170.23 (CO); LRESIMS (*m/z*): 1182 (M+Na$^+$); HRESIMS calcd for $C_{66}H_{81}NO_{17}Na$ (M+Na$^+$): 1182.5396, found: 1182.5394.

4.13. Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-{5-[(*R*/*S*)-1,2-dithiolan-3-yl]-pentanamido}-β-D-glucopyranoside (20)

To a solution of **18** (95 mg, 0.09 mmol) in dry DMF (5.0 mL), (±)-α-lipoic acid (18 mg, 0.09 mmol), DIPEA (0.03 mL, 0.17 mmol), and HBTU (33 mg, 0.09 mmol) were added and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo and the residue was dissolved in 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 chromatography (toluene–acetone, 9:1) to give **20** (92 mg, 83%) as an inseparable diastereomeric mixture (in 1:1 ratio). R_f 0.29 (toluene–acetone, 9:1); $[\alpha]_D^{22}$ −50.7 (*c* 0.33, $CHCl_3$) for the diastereomeric mixture (1:1); ^1H NMR (500 MHz, $CDCl_3$): δ 0.68 (d, 3H, *J* 6.0 Hz, CH_3), 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.5 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.98 (d, 3H, *J* 7.0 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 3.03–3.15 (m, 2H, CH_2S), 3.31 (m, 1H), 3.37 (dd, 1H, *J* 11.0, 11.0 Hz, H-26′a), 3.46 (m, 2H, H-3′, H-26′b), 3.69 (m, 1H, H-5^{II}), 3.86 (m, 1H, H-5^{I}), 4.04 (m, 1H, H-2^{I}), 4.05 (dd, 1H, *J* 9.0, 9.5 Hz, H-4^{I}), 4.42 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.51 (dd, 1H, *J* 12.0, 5.0 Hz, H-6^{I}a), 4.78 (m, 2H, H-1^{I}, H-6^{I}b), 4.87 (br s, 1H, H-1^{II}), 4.88 (dd, 1H, *J* 9.5, 9.5 Hz, H-4^{II}), 5.14 (m, 1H, H-2^{II}), 5.15 (m, 1H, H-3^{II}), 5.28 (m, 1H, H-6′), 5.54 (d, 1H, *J* 9.0 Hz, NH), 5.56 (m, 1H, H-3^{I}), 7.45 (m, 4H, Ar-H), 7.58 (m, 2H, Ar-H), 8.05 (m, 4H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.72, 16.47, 17.01, 17.33, 19.53, 20.90, 20.95, 25.35, 28.76, 28.98, 29.71, 30.48, 31.56, 32.03, 32.26, 34.66, 36.64, 36.95, 37.20, 38.57, 39.22, 39.94, 40.29, 40.44, 41.78, 50.17, 54.98, 56.27, 56.67, 62.26, 63.07, 67.04, 67.59, 68.72, 70.15, 70.74, 73.17, 73.89, 76.80, 79.72, 80.99, 98.62 (C-1^{II}), 99.90 (C-1^{I}), 109.49 (C-22′), 121.92 (C-6′), 128.58, 128.72, 129.61, 130.01, 130.18, 133.25, 133.67, 140.58 (C-5′), 166.08 (CO), 166.68 (CO), 170.02 (CO), 170.16 (CO), 170.22 (CO), 172.96 (CO); LRESIMS (*m/z*): 1267 (M+Na$^+$); HRESIMS calcd for $C_{67}H_{89}NO_{17}S_2Na$ (M+Na$^+$): 1266.5469, found: 1266.5463.

4.14. Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-(3-nitrobenzamido)-β-D-glucopyranoside (21)

In a similar way as described for the preparation of **2**, compound **18** (150 mg, 0.14 mmol) was coupled with 3-nitrobenzoic acid (28 mg, 0.16 mmol) in the presence of HBTU (53 mg, 0.14 mmol) and DIPEA (0.05 mL, 0.28 mmol) in dry dimethylformamide (5.0 mL) to give **21** (143 mg, 83%). R_f 0.28 (toluene–acetone, 11:1); $[\alpha]_D^{22}$ −29.8 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.72 (d, 3H, *J* 6.5 Hz, CH_3), 0.76 (s, 3H, CH_3), 0.79 (d, 3H, *J* 6.5 Hz, CH_3), 0.91 (s, 3H, CH_3), 0.97 (d, 3H, *J* 7.0 Hz, CH_3), 1.90 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* 11.0, 11.0 Hz, H-26′a), 3.47 (m, 1H, H-26′b), 3.53 (m, 1H, H-3′), 3.73 (m, 1H, H-5^{II}), 3.98 (m, 1H, H-5^{I}), 4.19 (dd, 1H, *J* 9.0, 9.0 Hz, H-4^{I}), 4.36 (dd, 1H, *J* 10.0, 8.0 Hz, H-2^{I}), 4.40 (approx. q, 1H, *J* ≈ 7.0 Hz, H-16′), 4.55 (dd, 1H, *J* 12.5, 5.0 Hz, H-6^{I}a), 4.88 (m, 1H, H-6^{I}b), 4.90 (dd, 1H, *J* 9.5, 10.0 Hz, H-4^{II}), 4.95 (br s, 1H, H-1^{II}), 4.96 (d, 1H, *J* 8.0 Hz, H-1^{I}), 5.15 (m, 2H, H-2^{II}, H-3^{II}), 5.19 (m, 1H, H-6′), 5.71 (dd, 1H, *J* = 9.0, 9.0 Hz, H-3^{I}), 6.69 (d, 1H, *J* 9.0 Hz, NH), 7.39 (m, 1H, Ar-H), 7.46–7.61 (m, 6H, Ar-H), 7.94 (m, 1H, Ar-H), 8.02 (m, 2H, Ar-H), 8.08 (m, 2H, Ar-H), 8.25 (m, 1H, Ar-H), 8.42 (m, 1H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.72, 16.46, 17.05, 17.34, 19.49, 20.92, 20.97, 28.98, 29.75, 30.48, 31.51, 31.56, 32.03, 32.22, 36.90, 37.19, 39.34, 39.93, 40.42, 41.77, 50.16, 55.56, 56.64, 62.23, 63.00, 67.02, 67.64, 68.82, 70.09, 70.66, 73.24, 74.18, 76.32, 79.71, 80.97, 98.53 (C-1^{II}), 99.99 (C-1^{I}), 109.47 (C-22′), 121.92 (C-6′), 122.04, 126.24, 128.64, 128.69, 129.28, 129.98, 130.06, 130.18, 133.15, 133.35, 133.82, 136.13, 140.44 (C-5′), 148.27, 165.63 (CO), 166.19 (CO), 167.13 (CO), 170.01 (CO), 170.26 (CO), 170.35 (CO); LRESIMS (*m/z*): 1228 (M+Na$^+$); HRESIMS calcd for $C_{66}H_{80}N_2O_{19}Na$ (M+Na$^+$): 1227.5252, found: 1227.5240.

4.15. Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-deoxy-2-(3,5-dinitrobenzamido)-β-D-glucopyranoside (22)

In a similar way as described for the preparation of **2**, compound **18** (150 mg, 0.14 mmol) was treated with 3,5-dinitrobenzoic acid (34 mg, 0.16 mmol) in the presence of HBTU (53 mg, 0.14 mmol), DIPEA (0.05 mL, 0.28 mmol) in dry DMF (5.0 mL) to give **22** (143 mg, 83%). R_f 0.32 (toluene–acetone, 11:1); $[\alpha]_D^{22}$ −25.9 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.73 (d, 3H, *J* 6.0 Hz, CH_3), 0.76 (s, 3H, CH_3), 0.78 (d, 3H, *J* 6.0 Hz, CH_3), 0.91 (s, 3H, CH_3), 0.96 (d, 3H, *J* 6.5 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* 11.0, 11.0 Hz, H-26′a), 3.47 (m, 1H, H-26′b), 3.54 (m, 1H, H-3′), 3.73 (m, 1H, H-5^{II}), 4.01 (m, 1H, H-5^{I}), 4.20 (dd, 1H, *J* 9.5, 8.5 Hz, H-4^{I}), 4.40 (approx. q, 1H, *J* ≈ 7.0 Hz, H-16′), 4.41 (m, 1H, H-2^{I}), 4.55 (dd, 1H, *J* 12.5, 5.0 Hz, H-6^{I}a), 4.90 (dd, 1H, *J* 9.5, 10.0 Hz, H-4^{II}), 4.92 (m, 1H, H-6^{I}b), 4.96 (br s, 1H, H-1^{II}), 4.97 (d, 1H, *J* 8.0 Hz, H-1^{I}), 5.13 (m, 1H, H-2^{II}), 5.14 (dd, 1H, *J* 10.0 Hz, 3.0 Hz, H-3^{II}), 5.19 (m, 1H, H-6′), 5.67 (dd, 1H, *J* 9.0, 8.5 Hz, H-3^{I}), 7.01 (d, 1H, *J* 9.0 Hz, NH), 7.38–7.62 (m, 6H, Ar-H), 8.01–8.10 (m, 4H, Ar-H), 8.80 (d, 2H, *J* 2.0 Hz, Ar-H), 9.08 (t, 1H, *J* 2.0 Hz, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.73, 16.48, 17.08, 17.36, 19.49, 20.90, 20.95, 28.99, 29.79, 30.49, 31.51, 31.57, 32.05, 32.23, 36.90, 37.18, 39.45, 39.93, 40.44, 41.78, 50.17, 55.66, 56.65, 62.25, 62.92, 67.04, 67.71, 68.86, 70.05, 70.60, 73.22, 74.19, 75.99, 79.60, 80.98, 98.44 (C-1^{II}), 99.74 (C-1^{I}), 109.50 (C-22′), 121.32 (C-6′), 122.10, 127.43, 128.70, 128.80, 129.03, 129.88, 130.09, 130.20, 133.46, 134.07, 137.89, 140.27 (C-5′), 148.78, 163.40 (CO), 166.29 (CO), 167.27 (CO), 170.01 (CO), 170.35 (CO), 170.55 (CO); LRESIMS (*m/z*): 1273 (M+Na$^+$); HRESIMS calcd for $C_{66}H_{79}N_3O_{21}Na$ (M+Na$^+$): 1272.5103, found: 1272.5095.

4.16. Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-benzamido-2-deoxy-β-D-glucopyranoside (23)

In a similar way as described for the preparation of **4**, compound **19** (50 mg, 0.04 mmol) was treated with sodium methoxide to yield **23** (33 mg, 95%). R_f 0.47 (CH_2Cl_2–MeOH, 5:1); $[\alpha]_D^{22}$ −55.2 (*c* 0.33, MeOH); ^{1}H NMR (500 MHz, C_5D_5N plus two drops of D_2O): δ 0.68 (d, 3H, *J* 5.0 Hz, CH_3), 0.80 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 1.13 (d, 3H, *J* 7.0 Hz, CH_3), 1.68 (d, 3H, *J* 5.5 Hz, CH_3), 2.39 (m, 1H), 2.68 (m, 1H), 3.49 (dd, 1H, *J* 10.5, 10.5 Hz, H-26′a), 3.58 (m, 1H, H-26′b), 3.78 (m, 1H, H-5$^{\text{I}}$), 3.83 (m, 1H, H-3′), 4.13 (dd, 1H, *J* 12.0, 4.5 Hz, H-6$^{\text{I}}$a), 4.26 (br d, 1H, *J* 11.0 Hz, H-6$^{\text{I}}$b), 4.37 (dd, 1H, *J* 9.5, 9.5 Hz), 4.52 (m, 2H), 4.57 (dd, 1H, *J* 9.0, 3.0 Hz, H-3$^{\text{II}}$), 4.63 (dd, 1H, *J* 9.0 Hz, 9.5 Hz), 4.72 (br s, 1H, H-2$^{\text{II}}$), 4.76 (dd, 1H, *J* 9.5 Hz, 9.5 Hz), 5.02 (m, 1H), 5.54 (d, 1H, *J* 8.5 Hz, H-1$^{\text{I}}$), 5.71 (m, 1H, H-6′), 5.93 (br s, 1H, H-1$^{\text{II}}$), 7.40 (m, 3H, Ar-H), 8.40 (m, 2H, Ar-H), 9.57 (d, 1H, *J* 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 13.38, 16.67, 17.66, 18.83, 19.66, 21.39, 29.57, 30.47, 30.92, 31.90, 32.12, 32.54, 37.28, 37.71, 39.87, 40.14, 40.75, 42.27, 50.52, 55.42, 56.92, 61.75, 63.17, 67.17, 70.74, 72.97, 73.17, 74.07, 74.35, 77.67, 79.05, 79.49, 81.41, 100.65 (C-1$^{\text{II}}$), 103.24 (C-1$^{\text{I}}$), 109.59 (C-22′), 122.11 (C-6′), 128.44, 129.00, 131.64, 137.14, 141.03 (C-5′), 168.84 (CO); LRESIMS (*m*/*z*): 848 (M+Na$^+$); HRESIMS calcd for $C_{46}H_{67}NO_{12}Na$ (M+Na$^+$): 848.4560, found: 848.4552.

4.17. Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-deoxy-2-{5-[(*R*/*S*)-1,2-dithiolan-3-yl]-pentanamido}-β-D-glucopyranoside (24)

In a similar way as described for the preparation of **4**, compound **20** (50 mg, 0.04 mmol) was converted to **24** (46 mg, 96%) as a diastereomeric mixture in 1:1 ratio. R_f 0.53 (CH_2Cl_2–MeOH, 5:1); $[\alpha]_D^{22}$ −78.0 (*c* 0.1, MeOH) for the diastereomeric mixture (1:1); ^{1}H NMR (500 MHz, CD_3OD): δ 0.76 (d, 3H, *J* 6.5 Hz, CH_3), 0.77 (s, 3H, CH_3), 0.92 (d, 3H, *J* 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 1.20 (d, 3H, *J* 6.5 Hz, CH_3), 2.21 (m, 2H), 2.30 (m, 1H), 2.41 (m, 1H), 3.05 (m, 1H), 3.13 (m, 1H), 3.25–3.31 (m, 2H), 3.36 (dd, 1H, *J* 10.5, 10.5 Hz, 1H, H-26′), 3.41 (m, 1H), 3.46 (m, 1H), 3.49–3.64 (m, 5H), 3.75–3.79 (m, 2H), 3.90 (m, 1H), 4.35 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.53 (d, 0.5H, *J* 8.0 Hz, H-1$^{\text{I}}$), 4.54 (d, 0.5H, *J* 8.0 Hz, H-1$^{\text{I}}$), 4.82 (d, 1H, *J* 1.5 Hz, H-1$^{\text{II}}$), 5.33 (m, 1H, H-6′); ^{13}C NMR (125 MHz, CD_3OD): δ 15.03, 16.92, 17.65, 17.99, 20.09, 22.11, 27.02, 30.02, 30.13, 30.78, 31.58, 32.54, 32.88, 33.32, 40.39, 41.04, 41.46, 41.56, 43.04, 51.74, 57.69, 57.91, 70.76, 72.34, 72.57, 73.85, 74.58, 76.99, 80.21, 82.35, 101.02 ((C-1$^{\text{II}}$), 103.10 (C-1$^{\text{I}}$), 110.73 (C-22′), 122.91 (C-6′), 141.98 (C-5′), 176.47 (CO); LRESIMS (*m*/*z*): 932 (M+Na$^+$); HRESIMS calcd for $C_{47}H_{75}NO_{19}S_2Na$ (M+Na$^+$): 932.4628, found: 932.4619.

4.18. Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-deoxy-2-(3-nitrobenzamido)-β-D-glucopyranoside (25)

In a similar way as described for the preparation of **4**, compound **21** (50 mg, 0.04 mmol) was converted to **25** (32 mg, 92%). R_f 0.50 (CH_2Cl_2–MeOH, 5:1); $[\alpha]_D^{22}$ −41.8 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.75 (m, 6H, 2CH_3), 0.91 (d, 3H, *J* 7.0 Hz, CH_3), 0.92 (s, 3H, CH_3), 1.21 (d, 3H, *J* 6.5 Hz, CH_3), 3.27 (dd, 1H, *J* 10.5, 11.5 Hz, H-26′a), 3.28 (m, 1H, H-26′b)), 3.38 (dd, 1H, *J* 10.0, 9.5 Hz), 3.39 (m, 1H), 3.49 (m, 1H, H-3′), 3.57–3.60 (m, 2H), 3.68 (dd, 1H, *J* 12.0, 4.0 Hz, H-6$^{\text{I}}$b), 3.75–3.87 (m, 4H), 3.93 (m, 1H, H-5$^{\text{II}}$), 4.34 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.70 (d, 1H, *J* 8.5 Hz, H-1$^{\text{I}}$), 5.24 (m, 1H, H-6′), 7.72 (t, 1H, *J* 8.0 Hz, Ar-H), 8.20 (m, 1H, Ar-H), 8.38 (m, 1H, Ar-H), 8.69 (m, 1H, Ar-H); The signal for the L-rhamnopyranosyl anomeric proton (H-1$^{\text{II}}$) was not observed; it was likely buried under the large solvent peak at δ 4.87 ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 15.04, 16.91, 17.65, 17.97, 19.92, 22.10, 30.02, 30.80, 31.58, 32.54, 32.86, 33.25, 38.07, 38.57, 40.35, 41.02, 41.54, 43.02, 51.74, 57.90, 58.80, 62.12, 63.86, 67.99, 70.77, 72.36, 72.58, 73.87, 74.46, 77.15, 80.13, 80.63, 82.32, 101.26 (C-1$^{\text{II}}$), 103.13 (C-1$^{\text{I}}$), 110.71 (C-22′), 122.85 (C-6′), 123.42, 127.18, 131.26, 134.56, 138.14, 141.84 (C-5′), 149.79, 168.49 (CO); LRESIMS (*m*/*z*): 893 (M+Na$^+$); HRESIMS calcd for $C_{46}H_{66}N_2O_{14}Na$ (M+Na$^+$): 893.4411, found: 893.4403.

4.19. Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-deoxy-2-(3,5-dinitrobenzamido)-β-D-glucopyranoside (26)

In a similar way as described for the preparation of **4**, compound **22** (62 mg, 0.05 mmol) was converted to **26** (42 mg, 91%). R_f 0.55 (CH_2Cl_2–MeOH, 5:1); $[\alpha]_D^{22}$ −58.0 (*c* 0.1, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.74 (s, 3H, CH_3), 0.75 (d, 3H, *J* 6.0 Hz, CH_3), 0.90 (d, 3H, *J* 7.0 Hz, CH_3), 0.92 (s, 3H, CH_3), 1.20 (d, 3H, *J* 6.0 Hz, CH_3), 2.03 (m, 1H), 2.26 (m, 1H), 3.27 (m, 2H, 2 H-26′), 3.37 (dd, 1H, *J* 10.0, 9.5 Hz), 3.38 (m, 1H), 3.50 (m, 1H, H-3′), 3.59 (dd, 1H, *J* 9.5, 3.5 Hz, H-3$^{\text{II}}$), 3.61 (dd, 1H, *J* 9.5, 9.5 Hz), 3.68 (dd, 1H, *J* 12.0, 4.0 Hz), 3.77 (dd, 1H, *J* 10.5, 9.0 Hz), 3.82 (m, 2H), 3.85 (dd, 1H, *J* 10.5, 8.5 Hz), 3.92 (m, 1H, H-5$^{\text{II}}$), 4.33 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16′), 4.70 (d, 1H, *J* 8.0 Hz, H-1$^{\text{I}}$), 5.25 (m, 1H, H-6′), 9.04 (d, *J* 2.0 Hz, Ar-H), 9.11 (t, 1H, *J* 2.0 Hz, Ar-H); The signal for the L-rhamnopyranosyl anomeric proton (H-1$^{\text{II}}$) was not observed; it was likely buried under the large solvent peak at δ 4.87 ppm. ^{13}C NMR (125 MHz, CD_3OD): δ 15.03, 16.89, 17.65, 17.96, 19.91, 22.09, 30.01, 30.80, 31.58, 32.54, 32.86, 33.25, 38.07, 38.56, 40.34, 41.02, 41.53, 43.02, 51.74, 57.89, 59.02, 62.09, 63.85, 67.98, 70.77, 72.36, 72.57, 73.85, 74.47, 77.20, 80.03, 80.51, 82.32, 101.04 (C-1$^{\text{II}}$), 103.17 (C-1$^{\text{I}}$), 110.71 (C-22′), 122.19 (C-6′), 122.91, 128.59, 139.53, 141.79 (C-5′), 150.25, 166.09 (CO); LRESIMS (*m*/*z*): 938 (M+Na$^+$); HRESIMS calcd for $C_{46}H_{65}N_3O_{16}Na$ (M+Na$^+$): 938.4262, found: 938.4256.

4.20. Cell lines, cell culture, and medium

Cell lines used in this study include MCF-7 (breast cancer) and HeLa (cervical cancer). MCF-7 cell line was supplied by the American Type Culture Collection (Manassas, Virginia), and HeLa cell line was 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.

4.21. Cell proliferation assay

Measurement of cell proliferation was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Carmichael et al.[24] Briefly, 2000 cells were plated out into each well of a 96-well plate and allowed to adhere. Saponin analogues, dissolved in dimethylsulfoxide (DMSO, Sigma) and diluted with tissue culture medium, were then added at increasing concentrations (0–50 μM, eight wells per concentration). The cells were incubated in the presence of each saponin analogue for 72 h. MTT reagent (Sigma) dissolved in phosphate buffered saline was added to each well at a concentration of 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 versus 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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