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Concise synthesis and antitumor activities of trisaccharide steroidal saponins

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ABSTRACT

The naturally derived trisaccharide steroidal saponin **1** and structurally modified derivatives **2** and **3** bearing the same sarsasapogenin aglycon were synthesized concisely via a direct transglycosylation strategy. The antitumor activities of the synthetic trisaccharide saponins **1**–**3** and their corresponding α-isomers **1a**–**3a** were preliminarily evaluated against human gastric adenocarcinoma cell (MKN-45) and human epithelial cervical cancer cell (HeLa) by CCK-8 assay.

1. Introduction

In the past two decades, much attention has been focused on glycosylated saponins, naturally occurring metabolites from terrestrial plants and/or marine organisms, due to their excellent physiological and pharmacological activities.[1,2] Dioscin, the diosgenyl-3-*O*-β-chacotrioside isolated from oriental vegetables and traditional medicinal plants, displayed promising antitumor activities at the micromolar level in vitro.[3] Further investigations on structure–activity relationships of dioscin and its derivatives revealed that the trisaccharide moiety was essential for cytotoxic activity.[4] An in vitro assay indicated that OSW-1, one type of cholestane glycosides isolated from the bulbs of *Ornithogalum saundersiae*, exhibited exceptionally potent cytostatic activities against various malignant tumor cells while very little toxicity to normal cells. Thus, this compound was considered to be a novel therapeutic agent for treatment of diverse cancers.[5] Pharmaceutical Coramsine and Curaderm-BEC5 cream, whose primary integrants are solasodine glycoalkaloids (solamargine and solasonine), have been clinically applied for the treatment of various cancers.[6] The valuable pharmacological properties of natural glycosylated saponins make them attractive candidates for drug development. However, the limited accessibility of homogenous saponins from natural sources might have impeded research progress in this field. The chemical synthesis of the glycosylated saponins has been recently demonstrated to be a practical and efficacious tool to fulfill this endeavor.[7]

Smilax officinalis Kunth is an herbaceous plant used for the treatment of gout in Brazil.[8] In phytochemical investigations of the methanol extraction from the fresh roots of *Smilax officinalis* Kunth uncovered three new spirostan saponins that contain the same trisaccharide moiety but possess sarsasapogenin, neotigogenin, or tigogenin as the aglycon, respectively. The oligosaccharide chains linked to C-3 of the spirostan aglycons are quite rarely 4,6-dibranched oligosaccharide structure.[9] To best of our knowledge, there is neither a synthetic report nor biological evaluation done with these trisaccharide saponins. Being attracted by its peculiar oligosaccharide structure and curious of the structure–activity relationships (SAR), we herein present the first synthesis of trisaccharide spirostanic saponin **1** (Fig. 1), namely sarsasapogenin β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside, via a direct transglycosylation strategy. For the purpose of investigating the relationship between spirostan saponins and their biological activities, two trisaccharide saponin derivatives **2** and **3**, which contain a structurally modified trisaccharide unit on chemical structure, were synthesized. The inhibition effects of the synthetic trisaccharide saponins against the growth of two human cancer cell lines (MKN-45 and HeLa) were evaluated by anti-proliferative assay in vitro.

2. Results and discussion

To complete the synthesis of target molecules, we adopted a direct transglycosylation strategy using trisaccharide trichloroacetimidates as glycosyl donors. The synthesis started from the preparation of the 4,6-dibranched trisaccharide (Scheme 1). To this end, acidic cleavage of the benzylidene group of 4-methoxyphenyl

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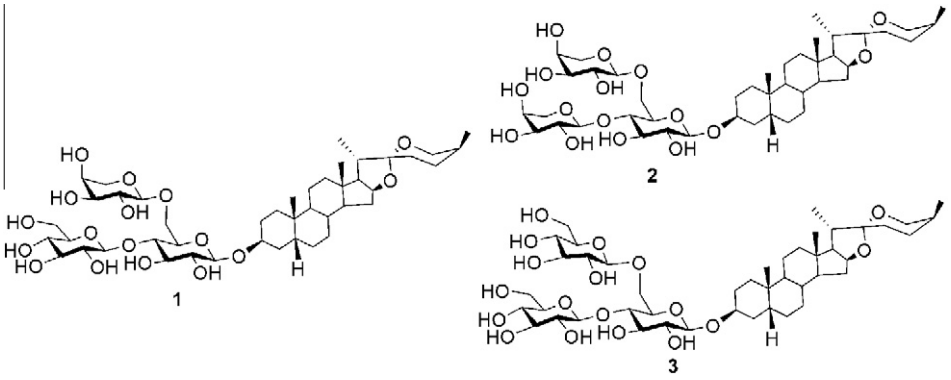
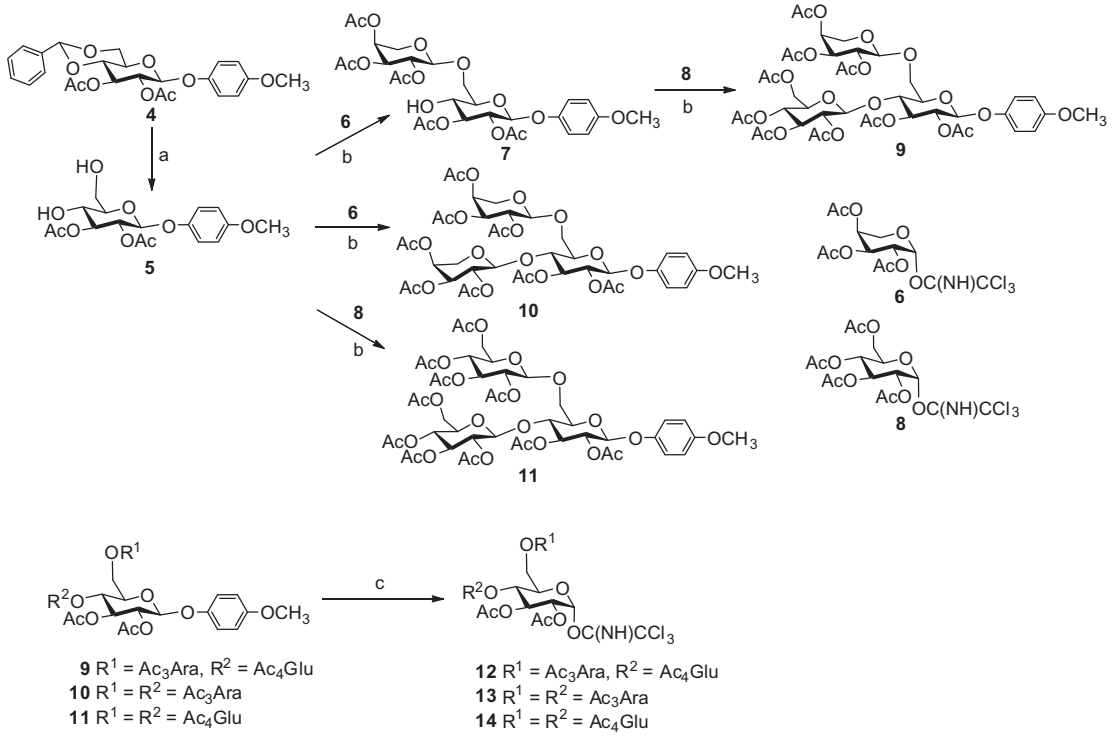


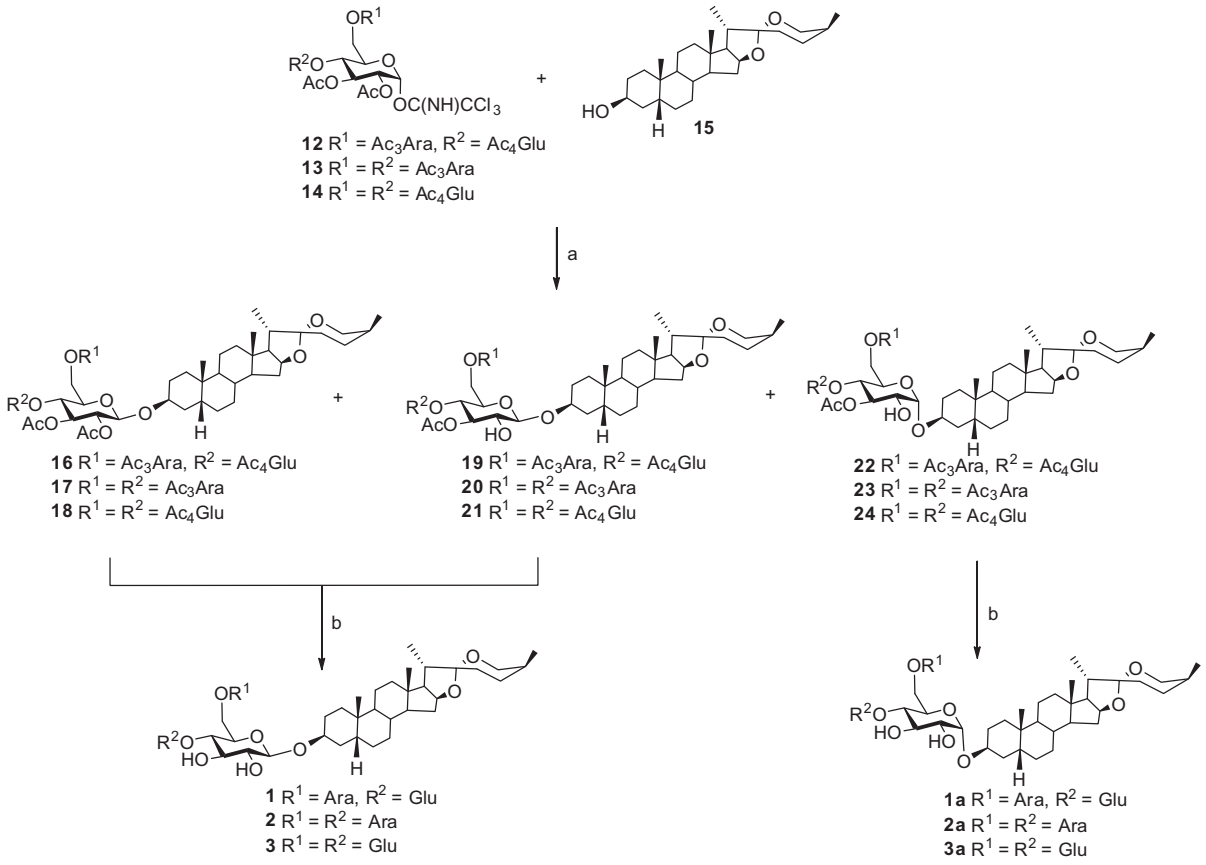
Figure 1. The chemical structures of trisaccharide spirostanic saponins **1**–**3**.



Scheme 1. Reagents and conditions (yields): (a) 80% HOAc, 70 °C (91% for **5**); (b) TMSOTf, CH_2Cl_2, 0 °C (78% for **7**, 66% for **9**, 71% for **10**, 62% for **11**); (c) CAN, MeCN–H_2O (4:1), 0 °C; then CCl_3CN, DBU, CH_2Cl_2, 0 °C (63% for **12**, 65% for **13**, 68% for **14**).

2,3-di-*O*-acetyl-4,6-di-*O*-benzylidene-β-D-glucopyranoside (**4**)[10] with 80% acetic acid at 70 °C afforded 4,6-diol **5** in 91% yield. Regioselective glycosylation of compound **5** and 1.1 equiv of 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl trichloroacetimidate **6** in anhydrous CH_2Cl_2 at 0 °C under the promotion of trimethylsilyl triflate (TMSOTf) gave the α-(1→6)-linked disaccharide **7** in 78% yield. The presence of the α-(1→6)-linkage in **7** was assigned from the chemical shift of H-1′ (δ: 4.55 ppm, $J_{1,2}$ 6.5 Hz) in the ^{1}H NMR spectrum and the downfield shift of glucosyl C-6 at 68.2 ppm in the ^{13}C NMR spectrum. Coupling of disaccharide **7** and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **8** in CH_2Cl_2 with TMSOTf as a catalyst furnished the desired trisaccharide **9** in a good yield. Direct condensation of compound **5** and 2.2 equiv of arabinosyl donor **6** in dry CH_2Cl_2 in the presence of TMSOTf gave the 4,6-diarabinosylated glucopyranoside **10** in 71% isolated yield. In the same way, the trisaccharide **11** was obtained in 62% yield from 4,6-glucosyaltion of **5** and 2.2 equiv of glucosyl donor **8** catalyzed by TMSOTf under standard glycosylation condition. Cerium ammonium nitrate (CAN)-promoted cleavage[11] of the anomeric 4-methoxyphenyl group of trisaccharides **9–11** in a 4:1 MeCN–H_2O solvent system, followed by trichloroacetimidate formation[12] with trichloroacetonitrile (Cl_3CCN) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), afforded the trisaccharide donors **12–14** in good yields for two steps.

Next, direct condensation of the trisaccharide donors **12–14** and sarsasapogenin aglycon **15** in the presence of TMSOTf in CH_2Cl_2 at 0 °C generated the fully protected trisaccharide saponins **16–18** (Scheme 2). Interestingly, the desired trisaccharide products **16–18** were only obtained in ~37% yields, together with C-2 deacetylated trisaccharide products **19–21** (~35%) and α-isomers of trisaccharide products **22–24** (~14%). The C-2 deacetylation of glucosyl residue in **19–21** was supported by analysis of ^{1}H NMR spectra. The chemical shifts of H-2′ were moved apparently to the upfield from ~4.86 ppm (in **16–18**) to ~3.45 ppm (in **19–21**). Moreover, the signals of H-1′ in **19–21** appeared at ~4.35 ppm ($J_{1,2}$ 7.8 Hz), which confirmed the β-linkages. The presence of the α-glycosyl bond



Scheme 2. Reagents and conditions (yields): (a) TMSOTf, CH_2Cl_2, 0 °C (37% for **16**, 35% for **17**, 39% for **18**, 34% for **19**, 35% for **20**, 36% for **21**, 15% for **22**, 13% for **23**, 14% for **24**); (b) 1 N NaOH, MeOH, rt (89% for **1**, 93% for **2**, 90% for **3**, 92% for **1a**, 90% for **2a**, 94% for **3a**).

formation between the glucosyl residue and the sarsasapogenin aglycon in **22–24** was identified from the ^{1}H NMR spectra giving the signals of H-1′ at ~4.92 ppm ($J_{1,2}$ ~3.6 Hz) and H-2′ at ~3.75 ppm. A detailed evaluation of this result suggested that the transglycosylation reaction proceeded through an orthoester formation–rearrangement pathway.[13]

Finally, deacetylation of the trisaccharide saponins **16–18** and **19–21** in methanol with 1 N aqueous sodium hydroxide smoothly generated the target compounds **1–3** in excellent yield. Similarly, the acetyl protecting groups of trisaccharide saponins **22–24** were removed by 1 N NaOH in methanol, giving the α-isomer products **1a–3a** in 90–94% isolated yield.

The anti-proliferative activities of trisaccharide saponins **1–3** and **1a–3a** against the two human tumor cell lines, MKN-45 and HeLa, were evaluated following the standard CCK-8 assay procedure.[14] The results are listed in Table 1. Compared with adriamycin, as a strong positive control, all the synthetic trisaccharide saponins showed moderate anti-proliferative activities ($IC_{50} \approx$ 11 μM) against the growth of MKN-45 and HeLa tumor cells. The structurally derived saponins **2** and **3** and their α-isomer saponins **1a–3a** exhibited similar IC_{50} values against both tumor cell lines as that of the natural saponin **1**, which indicated that the modification of sugar unit and the conversion of glycosyl bond between sugar chain and steroidal aglycon did not influence their bioactivities.

In conclusion, we have described the concise synthesis of the trisaccharide spirostanic saponins corresponding to *Smilax officinalis* Kunth via a direct transglycosylation way. All synthetic trisaccharide saponins showed the similar anti-proliferative activities toward MKN-45 and HeLa cells. This strategy also provided an entry to the preparation of the other similarly-structured oligosaccharides isolated from *Smilax* species.[8]

3. Experimental

3.1. General methods

Optical rotations were determined at 25 °C with a WZZ-2S automatic polarimeter. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H COSY, ^{1}H–^{13}C HMQC spectra were recorded with Bruker ARX 400 and 600 spectrometers for solutions in $CDCl_3$ and CD_3OD. Chemical shifts are given in ppm downfield from internal Me_4Si for $CDCl_3$. Mass spectra were measured on JEOL JMS-DX-303HF spectrometer or IT-TOF spectrometer using ESI technique to introduce the sample. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with

Table 1
Anti-proliferative activities of **1**–**3** and **1a**–**3a** against the tumor cells

Tumor cell	IC_{50} (μM) of compounds						
	1	**1a**	**2**	**2a**	**3**	**3a**	Adriamycin
MKN-45	11.6 ± 0.4	13.0 ± 1.3	11.5 ± 0.5	14.4 ± 0.1	11.1 ± 0.8	9.4 ± 0.6	0.1 ± 0.01
HeLa	11.9 ± 0.6	11.8 ± 0.3	10.5 ± 0.6	16.3 ± 0.3	9.8 ± 0.2	11.9 ± 1.3	0.08 ± 0.01

detection by charring with 30% (v/v) H_2SO_4 in MeOH. Column chromatography was conducted by elution of a silica gel column with EtOAc–petroleum ether (bp 60–90 °C) as the eluents. Solutions were concentrated at <60 °C under diminished pressure.

3.2. 4-Methoxyphenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside (5)

A solution of compound **4** (5.0 g, 10.9 mmol) in 80% HOAc (100 mL) was stirred at 70 °C for 2 h, at which time TLC (1:1 petroleum ether–EtOAc) indicated the complete disappearance of compound **4**. The reaction mixture was then co-evaporated with toluene for three times (3 × 50 mL). The residue was purified by silica gel column chromatography using 1:1 petroleum ether–EtOAc as the eluents to give diol **5** (3.66 g, 91%) as a white foam; $[\alpha]_D^{25}$ −16 (*c* 2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.07, 2.11 (2s, 2 × 3H, 2Ac), 3.53 (m, 1H, H-5), 3.77 (s, 3H, –OC*H*$_3$), 3.82–3.88 (m, 2H, H-4, H-6a), 3.95 (dd, 1H, *J* 3.2, 12.0 Hz, H-6b), 5.00 (d, 1H, *J* 7.6 Hz, H-1), 5.10 (t, 1H, *J* 9.6 Hz, H-3), 5.16 (dd, 1H, *J* 7.6, 9.6 Hz, H-2), 6.83, 6.92 (2d, 2 × 2H, *J* 9.1 Hz, *Ph*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.7, 20.9, 55.7, 62.0, 69.2, 71.3, 75.8, 75.9, 100.1, 114.7, 118.3, 150.9, 155.7, 169.6, 171.6; Anal. Calcd for $C_{17}H_{22}O_9$: C, 55.13; H, 5.99. Found: C, 55.36; H, 5.81.

3.3. 4-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)-2,3-di-*O*-acetyl-β-D-glucopyranoside (7)

To a solution of compound **5** (240 mg, 0.648 mmol) and arabinosyl imidate **6** (300 mg, 0.713 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TMSOTf (13 μL, 0.07 mmol) under a N_2 atmosphere at 0 °C. The mixture was stirred at these conditions for 1 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated the completion of the reaction. The reaction mixture was neutralized with Et_3N and concentrated. The residue was purified on a silica gel column using 3:2 petroleum ether–EtOAc as the eluents to yield syrupy **7** (318 mg, 78%); $[\alpha]_D^{25}$ −6 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.99, 2.04, 2.07, 2.11, 2.13 (5s, 5 × 3H, 5Ac), 3.62 (dd, 1H, *J* 2.0, 12.8 Hz, H-5a′), 3.66 (m, 1H, H-5), 3.75 (t, 1H, *J* 9.0 Hz, H-4), 3.78 (s, 3H, –OC*H*$_3$), 3.83 (dd, 1H, *J* 5.6, 10.8 Hz, H-6a), 4.02 (dd, 1H, *J* 4.0, 12.8 Hz, H-5b′), 4.15 (dd, 1H, *J* 3.6, 10.8 Hz, H-6b), 4.55 (d, 1H, *J* 6.5 Hz, H-1′), 4.92 (d, 1H, *J* 7.7 Hz, H-1), 5.06 (dd, 1H, *J* 3.5, 9.1 Hz, H-3′), 5.09 (t, 1H, *J* 9.0 Hz, H-3), 5.16 (dd, 1H, *J* 7.7, 9.0 Hz, H-2), 5.18 (dd, 1H, *J* 6.5, 9.1 Hz, H-2′), 5.25 (m, 1H, H-4′), 6.84, 6.92 (2d, 2 × 2H, *J* 9.1 Hz, *Ph*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8, 20.9, 55.6, 62.7, 67.3, 68.2, 69.0, 69.8, 69.9, 71.2, 74.9, 75.6, 100.2, 100.5, 114.7, 118.3, 151.1, 155.6, 169.5, 169.6, 170.1, 170.2, 171.3; Anal. Calcd for $C_{28}H_{36}O_{16}$: C, 53.50; H, 5.77. Found: C, 53.78; H, 5.84.

3.4. 4-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (9)

To a solution of compound **7** (200 mg, 0.318 mmol) and glucosyl imidate **8** (172 mg, 0.350 mmol) in anhydrous CH_2Cl_2 (4 mL) was added TMSOTf (6.5 μL, 0.035 mmol) under a N_2 atmosphere at 0 °C. The mixture was stirred for 1 h, then neutralized with Et_3N and concentrated. Purification of the residue on a silica gel column using 1:1 petroleum ether–EtOAc as the eluents gave syrupy **9** (201 mg, 66%); $[\alpha]_D^{25}$ −32 (*c* 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.97, 2.01, 2.02, 2.03, 2.04, 2.05, 2.07, 2.09, 2.13 (9s, 9 × 3H, 9Ac), 3.59–3.65 (m, 2H, H-5, H-5a′), 3.76 (dd, 1H, *J* 5.0, 11.4 Hz, H-6a), 3.78 (s, 3H, –OC*H*$_3$), 3.80 (m, 1H, H-5″), 3.85 (t, 1H, *J* 9.5 Hz, H-4), 4.01 (dd, 1H, *J* 4.7, 12.5 Hz, H-6a″), 4.06 (m, 2H, H-5b′, H-6b), 4.40 (dd, 1H, *J* 4.2, 12.5 Hz, H-6b″), 4.58 (d, 1H, *J* 8.0 Hz, H-1″), 4.60 (d, 1H, *J* 5.4 Hz, H-1′), 4.90 (d, 1H, *J* 7.8 Hz, H-1), 4.93 (dd, 1H, *J* 8.0, 9.2 Hz, H-2″), 5.03–5.14 (m, 4H, H-2, H-3, H-3′, H-4″), 5.16 (dd, 1H, *J* 5.4, 8.1 Hz, H-2′), 5.19–5.27 (m, 2H, H-3″, H-4′), 6.84, 6.92 (2d, 2 × 2H, *J* 9.1 Hz, *Ph*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.5, 20.6, 20.7, 20.8, 55.6, 61.6, 61.8, 66.1, 66.9, 67.8, 69.1, 69.5, 71.4, 71.7 (2C), 72.3, 72.8, 74.6, 75.9, 99.9, 100.2, 100.5, 114.7, 118.5, 151.0, 155.7, 168.9, 169.3, 169.5, 169.7, 169.8, 170.1, 170.2, 170.5; Anal. Calcd for $C_{42}H_{54}O_{25}$: C, 52.61; H, 5.68. Found: C, 52.46; H, 5.83.

3.5. 4-Methoxyphenyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (10)

To a solution of compound **5** (150 mg, 0.405 mmol) and arabinosyl imidate **6** (375 mg, 0.891 mmol) in anhydrous CH_2Cl_2 (6 mL) was added TMSOTf (16 μL, 0.09 mmol) under a N_2 atmosphere at 0 °C. The mixture was stirred for 2 h, then neutralized with Et_3N and concentrated. The residue was purified on a silica gel column using 1:1 petroleum ether–EtOAc as the eluents to afford syrupy **10** (255 mg, 71%); $[\alpha]_D^{25}$ −48 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.01, 2.02, 2.04, 2.06, 2.07, 2.08, 2.11, 2.12 (8s, 8 × 3H, 8Ac), 3.57–3.68 (m, 3H, H-5, H-5a′, H-5a″), 3.72 (dd, 1H, *J* 4.8, 11.0 Hz, H-6a),, 3.78 (s, 3H, –OC*H*$_3$), 3.86 (t, 1H, *J* 9.5 Hz, H-4), 3.93 (dd, 1H, *J* 5.0, 12.4 Hz, H-5b′), 4.01 (dd, 1H, *J* 4.9, 12.5 Hz, H-5b″), 4.08 (br d, 1H, *J* 11.0 Hz, H-6b), 4.54 (d, 1H, *J* 5.5 Hz, H-1′), 4.58 (d, 1H, *J* 5.4 Hz, H-1″), 4.92 (d, 1H, *J* 7.9 Hz, H-1), 4.93 (m, 5H, H-3, H-2′, H-2″, H-3′, H-3″), 5.19–5.27 (m, 3H, H-2, H-4′, H-4″), 6.82, 6.90 (2d, 2 × 2H, *J* 9.1 Hz, *Ph*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.6, 20.7, 20.8, 20.9, 55.6, 61.6, 61.7, 66.3, 66.9 (2C), 69.1, 69.4 (2C), 69.5, 71.5, 72.9, 74.5, 75.8, 99.6, 100.1 (2*C*), 114.6, 118.4, 151.0, 155.6, 169.3, 169.5, 169.6, 169.9, 170.0, 170.1, 170.2; Anal. Calcd for $C_{39}H_{50}O_{23}$: C, 52.82; H, 5.68. Found: C, 52.55; H, 5.76.

3.6. 4-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (11)

To a solution of compound **5** (150 mg, 0.405 mmol) and glucosyl imidate **8** (439 mg, 0.891 mmol) in anhydrous CH_2Cl_2 (6 mL) was added TMSOTf (24 μL, 0.133 mmol) under a N_2 atmosphere at 0 °C. The reaction mixture was stirred for 2 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated the disappearance of imidate donor **8**. The mixture was then neutralized with Et_3N and concentrated. Column chromatograph of the residue with 1:1 petroleum ether–EtOAc as the eluents furnished syrupy **11** (259 mg, 62%); $[\alpha]_D^{25}$ −35 (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.95, 1.98, 2.01, 2.02, 2.03, 2.05, 2.06, 2.07, 2.08, 2.09 (10s, 10 × 3H, 10Ac), 3.58 (m, 1H, H-5), 3.65 (m, 1H, H-5′), 3.72–3.78 (m, 2H, H-4, H-5″), 3.79 (s, 3H, –OCH_3), 3.85 (dd, 1H, *J* 6.2, 11.7 Hz, H-6a), 4.03–4.14 (m, 3H, 3 × H-6), 4.27 (dd, 1H, *J* 4.6, 12.4 Hz, H-6b′), 4.37 (dd, 1H, *J* 4.5, 12.4 Hz, H-6b″), 4.53 (d, 1H, *J* 8.0 Hz, H-1′), 4.66 (d, 1H, *J* 7.9 Hz, H-1″), 4.92 (d, 1H, *J* 7.9 Hz, H-1), 4.93 (dd, 1H, *J* 7.9, 9.2 Hz, H-2), 5.01 (dd, 1H, *J* 8.0, 9.4 Hz, H-2′), 5.03–5.26 (m, 6H, H-3, H-3′, H-4′, H-2″, H-3″, H-4″), 6.86, 6.92 (2d, 2 × 2H, *J* 9.2 Hz, *Ph*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 20.6, 20.7, 55.6, 61.6, 61.8, 67.3, 67.8, 68.0, 71.2, 71.3, 71.6, 71.8, 72.0, 72.2, 72.6, 72.7 (2C), 75.3, 76.3, 99.7, 100.6, 100.7, 114.7, 118.0, 150.8, 155.7, 169.0, 169.3, 169.4, 169.5, 169.8, 170.1, 170.2, 170.5, 170.6; Anal. Calcd for $C_{45}H_{58}O_{27}$: C, 52.43; H, 5.67. Found: C, 52.75; H, 5.80.

3.7. General procedure for preparing trisaccharide trichloroacetimidate donors 12–14

To a solution of trisaccharides **9–11** (0.2 mmol) in MeCN–H_2O (4:1, 30 mL) was added ceric ammonium nitrate (CAN; 274 mg,

0.5 mmol) and the mixture was stirred at rt for 40 min. An additional amount of CAN (55 mg, 0.1 mmol) was added, and the stirring was continued for another 1 h, at the end of which time TLC (2:3 petroleum ether–EtOAc) indicated the completion of the reaction. The resulting mixture was diluted with EtOAc (100 mL), washed successively with water, satd aq $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated, then subjected to a silica gel column chromatography (2:3 petroleum ether–EtOAc). The product generated above was then dissolved in anhydrous CH_2Cl_2 (5 mL). To the solution was added trichloroacetonitrile (80 μL, 0.8 mmol) and DBU (15 μL, 0.1 mmol) at 0 °C. The mixture was stirred for 1.5 h, concentrated, and the residue was subjected to a silica gel column (1:1 petroleum ether–EtOAc) to afford syrupy trisaccharide imidates **12–14** in good yield for two steps.

3.7.1. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (12)

From compound **9** (192 mg, 0.20 mmol) to yield **12** (126 mg, 63%) as a white foam; $[\alpha]_D^{25}$ +8 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.97, 1.99, 2.00, 2.01, 2.04, 2.08, 2.09, 2.14, 2.16 (9s, 9 × 3H, 9Ac), 3.59–3.71 (m, 2H, H-5, H-5a′), 3.77–3.88 (m, 2H, H-6a, H-5″), 3.84–3.89 (m, 2H, H-4, H-6a″), 4.03–4.08 (m, 2H, H-5b′, H-6b), 4.42 (dd, 1H, *J* 4.0, 12.6 Hz, H-6b″), 4.59 (d, 1H, *J* 5.0 Hz, H-1′), 4.66 (d, 1H, *J* 8.0 Hz, H-1″), 4.92 (dd, 1H, *J* 8.0, 9.2 Hz, H-2″), 5.02 (dd, 1H, *J* 3.7, 10.0 Hz, H-2), 5.04–5.15 (m, 3H, H-2′, H-3′, H-4″), 5.21–5.30 (m, 2H, H-4′, H-3″), 5.51 (t,1H, *J* 9.6 Hz, H-3), 6.51 (d, 1H, *J* 3.7 Hz, H-1), 8.62 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 20.6, 20.7, 20.9, 61.4, 61.7, 66.4, 66.7, 67.8, 69.3 (2C), 69.4, 70.1, 71.6, 72.1, 72.6, 73.2, 75.4, 90.8, 93.3, 100.1, 100.4, 161.2, 168.3, 169.4, 169.6, 169.9, 170.1, 170.2, 170.3, 170.5; Anal. Calcd for $C_{37}H_{48}Cl_3NO_{24}$: C, 44.57; H, 4.85; N, 1.40. Found: C, 44.78; H, 4.81; N, 1.53.

3.7.2. 2,3,4-Tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (13)

From compound **10** (178 mg, 0.20 mmol) to yield **13** (120 mg, 65%) as a white foam; $[\alpha]_D^{25}$ +20 (*c* 1.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 2.00, 2.04, 2.06, 2.07, 2.09, 2.10, 2.11, 2.12 (8s, 8 × 3H, 8Ac), 3.64 (dd, 1H, *J* 2.6, 12.3 Hz, H-5a′), 3.67 (dd, 1H, *J* 2.8, 11.8 Hz, H-5a″), 3.79 (dd, 1H, *J* 2.7, 11.4 Hz, H-6a), 3.91–4.06 (m, 5H, H-4, H-5, H-6b, H-5b′, H-5b″), 4.57 (d, 1H, *J* 5.4 Hz, H-1′), 4.61 (d, 1H, *J* 4.8 Hz, H-1″), 5.00 (dd, 1H, *J* 3.6, 10.0 Hz, H-2), 5.03–5.08 (m, 2H, H-3′, H-3″), 5.09–5.14 (m, 2H, H-2′, H-2″), 5.18–5.31 (m, 2H, H-4′, H-4″), 5.56 (dd, 1H, *J* 9.0, 10.0 Hz, H-3), 6.52 (d, 1H, *J* 3.6 Hz, H-1), 8.62 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.5, 20.6, 20.7, 20.8, 20.9, 21.0, 60.7, 61.6, 65.6, 66.0, 66.6, 67.0, 69.1, 69.2, 69.4, 69.5, 69.8, 70.3, 72.4, 74.8, 90.8, 93.3, 99.3, 99.5, 161.0, 169.4, 169.6, 169.7, 170.0, 170.2; Anal. Calcd for $C_{34}H_{44}Cl_3NO_{22}$: C, 44.14; H, 4.79; N, 1.51. Found: C, 43.92; H, 4.88; N, 1.63.

3.7.3. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranosyl trichloroacetimidate (14)

From compound **11** (206 mg, 0.20 mmol) to yield **14** (145 mg, 68%) as a white foam; $[\alpha]_D^{25}$ +17 (*c* 2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.98, 1.99, 2.00, 2.02, 2.04, 2.09, 2.11, 2.12 (8s, 10 × 3H, 10Ac), 3.72 (m, 1H, H-5), 3.81 (m, 1H, H-5′), 3.85–4.00 (m, 4H, H-4, H-5, H-6a,b), 4.05 (dd, 1H, *J* 2.0, 12.5 Hz, H-6a′), 4.18 (dd, 1H, *J* 2.3, 12.4 Hz, H-6a″), 4.31 (dd, 1H, *J* 4.9, 12.4 Hz, H-6b″), 4.42 (dd, 1H, *J* 4.2, 12.5 Hz, H-6b′), 4.62 (d, 1H, *J* 7.9 Hz, H-1′), 4.64 (d, 1H, *J* 8.2 Hz, H-1″), 4.92 (dd, 1H, *J* 7.9, 9.2 Hz, H-2′), 5.00–5.05 (m, 2H, H-2, H-2″), 5.07–5.14 (m, 2H, H-4′, H-4″), 5.19–5.31 (m, 2H, H-3′, H-3″), 5.51 (t, 1H, *J* 9.6 Hz, H-3), 6.48 (d, 1H, *J* 3.7 Hz, H-1), 8.62 (s, 1H, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 20.6, 20.7, 61.7, 61.9, 67.8, 67.9, 68.4, 69.2, 70.0, 71.4, 71.6, 72.0, 72.2, 72.6, 72.8, 73.2, 75.7, 90.8, 93.2, 100.4, 101.2, 161.2, 168.9, 169.3, 169.4, 169.6, 170.0, 170.2, 170.3, 170.5, 170.6; Anal. Calcd for $C_{40}H_{52}Cl_3NO_{26}$: C, 44.93; H, 4.90; N, 1.31. Found: C, 45.04; H, 4.79; N, 1.25.

3.8. General procedure for preparing the trisaccharide saponins 16–24

To a solution of tisaccharide imidate donors **12–14** (0.11 mmol) and sarsasapogenin aglycon **15** (0.10 mmol) in dried CH_2Cl_2 (5 mL) at 0 °C under N_2 protection was added catalytic amount of TMSOTf (3.6 μL, 0.02 mmol). The reaction mixture was stirred under these conditions for 40 min, at which time TLC (1:1 petroleum ether–EtOAc) indicated that the glycosyl imidate donors were completely consumed. The reaction mixture was then neutralized with Et_3N, concentrated under reduced pressure, and purified on a silica gel column with 2:3 petroleum ether–EtOAc as the eluents to give the desired trisaccharide saponins **16–18**, the 2-deacetyled trisaccharide saponins **19–21**, and the α-isomer of trisaccharide products **22–24**.

3.8.1. Sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (16)

From compound **12** (110 mg) and sarsasapogenin **15** (42 mg) to afford **16** (47 mg, 37%) as a white foam; $[\alpha]_D^{25}$ −35 (*c* 2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, 18-C*H*$_3$), 0.91 (s, 3H, 19-C*H*$_3$), 0.98 (d, 3H, *J* 6.7 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 1.97, 2.00, 2.01, 2.02, 2.08, 2.12, 2.13 (7s, 9 × 3H, 9Ac), 3.30 (d, 1H, *J* 11.0 Hz, H-26a), 3.47 (m, 1H, H-5′), 3.66 (dd, 1H, *J* 2.7, 12.3 Hz, H-5a″), 3.72 (dd, 1H, *J* 4.6, 11.0 Hz, H-6a′), 3.77 (t, 1H, *J* 9.8 Hz, H-4′), 3.79 (m, 1H, H-5‴), 3.92–4.07 (m, 5H, H-3, H-26b, H-6b′, H-6a‴, H-5b″), 4.35–4.42 (m, 2H, H-16, H-6b‴), 4.47 (d, 1H, *J* 8.0 Hz, H-1′), 4.57 (d, 1H, *J* 8.0 Hz, H-1‴), 4.61 (d, 1H, *J* 5.0 Hz, H-1″), 4.86 (dd, 1H, *J* 8.0, 9.8 Hz, H-2′), 4.91 (dd, 1H, *J* 8.0, 9.6 Hz, H-2‴), 5.03–5.09 (m, 2H, H-3″, H-4‴), 5.10–5.18 (m, 2H, H-3′, H-2″), 5.23 (t, 1H, *J* 9.6 Hz, H-3‴), 5.25 (m, 1H, H-4″); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.3, 16.0, 16.4, 20.5, 20.6, 20.7, 20.8, 20.9, 23.9, 25.7, 25.9, 26.5, 26.6, 27.1, 29.8, 30.2, 30.6, 31.8, 35.0, 35.3, 36.8, 40.1, 40.2, 40.6, 42.1, 56.4, 61.6, 62.1, 65.1, 65.5, 66.0, 66.8, 67.9, 69.3, 69.4, 71.6, 71.7, 71.8, 72.4, 72.9, 74.1, 74.2, 76.1, 81.0, 98.5, 99.8, 100.5, 109.7, 167.7, 168.9, 169.3, 169.4, 170.0, 170.2, 170.3, 170.5; ESI-HRMS calcd for $C_{62}H_{90}O_{26}Na$: 1273.5613 $(M+Na)^+$; found: 1273.5594; Anal. Calcd for $C_{62}H_{90}O_{26}$: C, 59.51; H, 7.25. Found: C, 59.73; H, 7.11.

3.8.2. Sarsasapogenyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (17)

From compound **13** (103 mg) and sarsasapogenin **15** (42 mg) to afford **17** (42 mg, 35%) as a white foam; $[\alpha]_D^{25}$ −43 (*c* 2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, 18-C*H*$_3$), 0.91 (s, 3H, 19-C*H*$_3$), 0.99 (d, 3H, *J* 6.7 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 2.00, 2.02, 2.03, 2.07, 2.08, 2.09, 2.10, 2.12 (8s, 8 × 3H, 8Ac), 3.30 (d, 1H, *J* 11.2 Hz, H-26a), 3.47 (m, 1H, H-5′), 3.63 (br d, 2H, *J* 12.2 Hz, H-5a″, H-5a‴), 3.69 (dd, 1H, *J* 4.5, 11.2 Hz, H-6a′), 3.79 (t, 1H, *J* 9.6 Hz, H-4′), 3.91 (dd, 1H, *J* 5.2, 12.2 Hz, H-5b″), 3.93–4.05 (m, 4H, H-3, H-26b, H-6b′, H-5b‴), 4.40 (m, 1H, H-16), 4.50 (d, 1H, *J* 8.0 Hz, H-1′), 4.54 (d, 1H, *J* 5.3 Hz, H-1″), 4.58 (d, 1H, *J* 4.8 Hz, H-1‴), 4.86 (dd, 1H, *J* 8.0, 9.6 Hz, H-2′), 5.00–5.15 (m, 4H, H-2″, H-2‴, H-3″, H-3‴), 5.15–5.25 (m, 3H, H-3′, H-4″, H-4‴); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 16.1, 16.5, 20.6, 20.7, 20.8, 20.9, 24.0, 25.8, 26.0, 26.6, 26.7, 27.1, 29.7, 30.3, 30.6, 31.8, 35.0, 35.3, 36.8,

40.1, 40.3, 40.7, 42.2, 56.4, 61.3 (2C), 62.2, 65.2, 65.6, 66.2, 66.9 (2C), 69.4 (2C), 69.5, 72.0, 73.2, 74.1, 74.2, 75.9, 81.0, 98.5, 99.5, 99.9, 109.7, 169.3, 169.4, 170.0, 170.1, 170.2; ESI-HRMS calcd for $C_{59}H_{86}O_{24}Na$: 1201.5401 $(M+Na)^+$; found: 1201.5366; Anal. Calcd for $C_{59}H_{86}O_{24}$: C, 60.09; H, 7.35. Found: C, 60.33; H, 7.18.

3.8.3. Sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-2,3-di-*O*-acetyl-β-D-glucopyranoside (18)

From compound **14** (118 mg) and sarsasapogenin **15** (42 mg) to afford **18** (52 mg, 39%) as a white foam; $[\alpha]_D^{25}$ −32 (*c* 3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, 18-CH_3), 0.91 (s, 3H, 19-CH_3), 0.98 (d, 3H, *J* 6.7 Hz, 21-CH_3), 1.08 (d, 3H, *J* 7.0 Hz, 27-CH_3), 1.98, 1.99, 2.01, 2.02, 2.03, 2.06, 2.08, 2.10 (8s, 10 × 3H, 10Ac), 3.30 (d, 1H, *J* 11.2 Hz, H-26a), 3.51 (m, 1H, H-5′), 3.68 (t, 1H, *J* 9.4 Hz, H-4′), 3.68–3.74 (m, 2H, H-5″, H-5‴), 3.80 (dd, 1H, *J* 6.2, 11.2 Hz, H-6a′), 3.95 (m, 2H, H-26b, H-6b′), 4.01–4.06 (m, 2H, H-3, H-6a″), 4.15 (dd, 1H, *J* 2.2, 12.4 Hz, H-6a‴), 4.31 (dd, 1H, *J* 4.5, 12.4 Hz, H-6b‴), 4.35 (dd, 1H, *J* 4.6, 12.5 Hz, H-6b″), 4.38 (m, 1H, H-16), 4.47 (d, 1H, *J* 8.2 Hz, H-1′), 4.50 (d, 1H, *J* 8.2 Hz, H-1″), 4.66 (d, 1H, *J* 7.8 Hz, H-1‴), 4.88 (dd, 1H, *J* 8.2, 9.8 Hz, H-2′), 4.92 (dd, 1H, *J* 8.2, 9.4 Hz, H-2″), 5.00 (dd, 1H, *J* 7.8, 9.4 Hz, H-2‴), 5.04 (t, 1H, *J* 9.6 Hz, H-4″), 5.09 (t, 1H, *J* 9.6 Hz, H-4‴), 5.13–5.20 (m, 3H, H-3′, H-3″, H-3‴); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 16.1, 16.5, 20.5, 20.6, 20.7, 20.9, 24.0, 25.8, 26.0, 26.5, 26.7, 27.1, 29.6, 30.3, 30.6, 31.8, 35.0, 35.3, 36.8, 40.1, 40.3, 40.7, 42.2, 56.4, 61.7, 61.9, 62.2, 65.2, 67.7, 68.0, 68.2, 71.4, 71.7 (2C), 71.8, 72.2, 72.5, 72.8, 74.1, 74.8, 76.3, 77.2, 81.0, 98.3, 100.4, 101.0, 109.7, 169.0, 169.2, 169.3, 169.4, 170.0, 170.2, 170.5, 170.6; ESI-HRMS calcd for $C_{65}H_{94}O_{28}Na$: 1345.5824 $(M+Na)^+$; found: 1345.5815; Anal. Calcd for $C_{65}H_{94}O_{28}$: C, 58.99; H, 7.16. Found: C, 58.74; H, 7.40.

3.8.4. Sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-3-*O*-acetyl-β-D-glucopyranoside (19)

From compound **12** (110 mg) and sarsasapogenin **15** (42 mg) to afford **19** (41 mg, 34%) as a white foam; $[\alpha]_D^{25}$ −26 (*c* 2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 0.99 (d, 3H, *J* 6.7 Hz, 21-CH_3), 1.08 (d, 3H, *J* 7.0 Hz, 27-CH_3), 1.97, 2.01, 2.02, 2.07, 2.08, 2.12 (6s, 8 × 3H, 8Ac), 3.30 (d, 1H, *J* 11.0 Hz, H-26a), 3.40–3.49 (m, 2H, H-2′, H-5′), 3.66 (dd, 1H, *J* 2.8, 12.3 Hz, H-5a″), 3.71 (dd, 1H, *J* 3.2, 11.0 Hz, H-6a′), 3.72 (t, 1H, *J* 9.6 Hz, H-4′), 3.79 (m, 1H, H-5‴), 3.93–4.09 (m, 5H, H-3, H-26b, H-5b″, H-6b′, H-6a‴), 4.34 (d, 1H, *J* 7.8 Hz, H-1′), 4.34–4.43 (m, 2H, H-16, H-6b‴), 4.57 (d, 1H, *J* 8.0 Hz, H-1‴), 4.61 (d, 1H, *J* 5.0 Hz, H-1″), 4.92 (dd, 1H, *J* 8.0, 9.4 Hz, H-2‴), 5.04–5.16 (m, 4H, H-3′, H-2″, H-3″, H-4‴), 5.22 (t, 1H, *J* 9.4 Hz, H-3‴), 5.26 (m, 1H, H-4″); ^{13}C NMR (150 MHz, $CDCl_3$): δ 14.3, 16.0, 16.5, 20.5, 20.6, 20.7, 20.8, 20.9, 23.9, 25.7, 25.9, 26.5, 26.6, 27.1, 29.7, 30.2, 30.5, 31.7, 35.0, 35.3, 37.1, 40.1, 40.2, 40.6, 42.1, 56.4, 61.7, 62.1, 65.1, 65.5, 66.2, 66.8, 67.9, 69.1, 69.4, 71.6, 71.8, 72.7, 72.9, 73.9, 74.3, 75.0, 75.9, 81.0, 99.8, 100.3, 110.0, 109.7, 167.7, 168.9, 169.3, 169.6, 170.1, 170.2, 170.5; ESI-HRMS calcd for $C_{60}H_{92}O_{25}N$: 1226.5953 $(M+NH_4)^+$; found: 1226.5945; Anal. Calcd for $C_{60}H_{88}O_{25}$: C, 59.59; H, 7.33. Found: C, 59.65; H, 7.56.

3.8.5. Sarsasapogenyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-3-*O*-acetyl-β-D-glucopyranoside (20)

From compound **13** (103 mg) and sarsasapogenin **15** (42 mg) to afford **20** (40 mg, 35%) as a white foam; $[\alpha]_D^{25}$ −28 (*c* 2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 0.99 (d, 3H, *J* 6.7 Hz, 21-CH_3), 1.08 (d, 3H, *J* 7.0 Hz, 27-CH_3), 2.03, 2.06, 2.07, 2.08, 2.09, 2.10, 2.11 (7s, 7 × 3H, 7Ac), 3.30 (d, 1H, *J* 11.0 Hz, H-26a), 3.38–3.50 (m, 2H, H-2′, H-5′), 3.63 (br d, 2H, *J* 12.2 Hz, H-5a″, H-5a‴), 3.69 (dd, 1H, *J* 4.2, 11.0 Hz, H-6a′), 3.74 (t, 1H, *J* 9.4 Hz, H-4′), 3.92–4.04 (m, 5H, H-3, H-26b, H-6b′, H-5b″, H-5b‴), 4.36 (d, 1H, *J* 7.8 Hz, H-1′), 4.40 (m, 1H, H-16), 4.55 (d, 1H, *J* 4.8 Hz, H-1″), 4.58 (d, 1H, *J* 5.4 Hz, H-1‴), 5.00–5.14 (m, 5H, H-3′, H-2″, H-2‴, H-3″, H-3‴), 5.16–5.23 (m, 2H, H-4″, H-4‴); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 16.1, 16.5, 20.6, 20.7, 20.8, 20.9, 21.1, 23.9, 25.8, 26.0, 26.5, 26.6, 27.1, 29.7, 30.5, 30.6, 31.8, 35.1, 35.3, 37.1, 40.2, 40.3, 40.7, 42.1, 56.4, 61.5, 62.2, 65.2, 65.6, 66.4, 66.6, 67.0, 69.1, 69.3, 69.5, 69.6, 72.8, 74.4, 74.7 (2C), 75.6, 81.0, 99.5, 99.6, 100.7, 109.7, 169.3, 169.4, 169.6, 170.1, 170.6; ESI-HRMS calcd for $C_{57}H_{88}O_{23}N$: 1154.5742 $(M+NH_4)^+$; found: 1154.5763; Anal. Calcd for $C_{57}H_{84}O_{23}$: C, 60.20; H, 7.44. Found: C, 60.41; H, 7.28.

3.8.6. Sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-β-D-glucopyranoside (21)

From compound **14** (118 mg) and sarsasapogenin **15** (42 mg) to afford **21** (46 mg, 36%) as a white foam; $[\alpha]_D^{25}$ −22 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H, 18-CH_3), 0.94 (s, 3H, 19-CH_3), 0.99 (d, 3H, *J* 6.7 Hz, 21-CH_3), 1.08 (d, 3H, *J* 7.0 Hz, 27-CH_3), 1.98, 2.01, 2.02, 2.03, 2.06, 2.08, 2.10 (7s, 9 × 3H, 9Ac), 3.30 (d, 1H, *J* 10.8 Hz, H-26a), 3.42–3.53 (m, 2H, H-2′, H-5′), 3.67 (t, 1H, *J* 9.5 Hz, H-4′), 3.67–3.74 (m, 2H, H-5″, H-5‴), 3.80 (dd, 1H, *J* 5.7, 11.2 Hz, H-6a′), 3.95 (dd, 1H, *J* 2.0, 10.8 Hz, H-26b), 4.00–4.08 (m, 3H, H-3, H-6b′, H-6a″), 4.15 (dd, 1H, *J* 2.0, 12.2 Hz, H-6a‴), 4.23–4.36 (m, 2H, H-6b″, H-6b‴), 4.34 (d, 1H, *J* 7.8 Hz, H-1′), 4.40 (m, 1H, H-16), 4.52 (d, 1H, *J* 8.0 Hz, H-1″), 4.65 (d, 1H, *J* 7.8 Hz, H-1‴), 4.92 (dd, 1H, *J* 8.0, 9.4 Hz, H-2″), 5.00 (dd, 1H, *J* 7.8, 9.4 Hz, H-2‴), 5.03–5.14 (m, 3H, H-3′, H-4″, H-4‴), 5.15–5.22 (m, 2H, H-3″, H-3‴); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.3, 16.1, 16.5, 20.5, 20.6, 20.7, 20.8, 20.9, 24.0, 25.8, 26.0, 26.5, 26.6, 27.1, 29.7, 30.0, 30.6, 31.8, 35.1, 35.3, 37.1, 40.2, 40.3, 40.7, 42.2, 56.4, 61.8, 62.0, 62.2, 65.2, 68.0, 68.1, 68.3, 71.5, 71.8 (2C), 72.2, 72.6, 72.8, 72.9, 73.9, 74.8, 75.0, 76.0, 81.0, 100.1, 100.8, 101.1, 109.7, 169.0, 169.2, 169.3, 169.4, 170.1, 170.2, 170.4, 170.5; ESI-HRMS calcd for $C_{63}H_{96}O_{27}N$: 1298.6164 $(M+NH_4)^+$; found: 1298.6183; Anal. Calcd for $C_{63}H_{92}O_{27}$: C, 59.05; H, 7.24. Found: C, 59.26; H, 7.06.

3.8.7. Sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-3-*O*-acetyl-α-D-glucopyranoside (22)

From compound **12** (110 mg) and sarsasapogenin **15** (42 mg) to afford **22** (18 mg, 15%) as a white foam; $[\alpha]_D^{25}$ −48 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, 18-CH_3), 0.97 (s, 3H, 19-CH_3), 0.99 (d, 3H, *J* 6.7 Hz, 21-CH_3), 1.08 (d, 3H, *J* 7.0 Hz, 27-CH_3), 1.98, 2.01, 2.02, 2.05, 2.08, 2.09, 2.11, 2.13 (8s, 8 × 3H, 8Ac), 3.30 (d, 1H, *J* 11.0 Hz, H-26a), 3.54 (m, 1H, H-5′), 3.64–3.82 (m, 5H, H-2′, H-4′, H-6a′, H-5a″, H-5‴), 3.90 (br d, 1H, *J* 11.0 Hz, H-26b), 3.92–3.98 (m, 2H, H-3, H-5b″), 4.01–4.07 (m, 2H, H-6b′, H-6a‴), 4.36–4.45 (m, 2H, H-16, H-6b‴), 4.56 (d, 1H, *J* 5.3 Hz, H-1″), 4.60 (d, 1H, *J* 8.0 Hz, H-1‴), 4.91 (d, 1H, *J* 3.6 Hz, H-1′), 4.92 (dd, 1H, *J* 8.0, 10.0 Hz, H-2‴), 5.03–5.10 (m, 2H, H-3″, H-4‴), 5.12–5.19 (m, 2H, H-3′, H-2″), 5.23–5.28 (m, 2H, H-4″, H-3‴); ESI-HRMS calcd for $C_{60}H_{88}O_{25}Na$: 1231.5507 $(M+Na)^+$; found: 1231.5489.

3.8.8. Sarsasapogenyl 2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→4)-[2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl-(1→6)]-3-*O*-acetyl-α-D-glucopyranoside (23)

From compound **13** (103 mg) and sarsasapogenin **15** (42 mg) to afford **23** (15 mg, 13%) as a white foam; $[\alpha]_D^{25}$ −54 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, 18-CH_3), 0.97 (s, 3H, 19-CH_3), 0.99 (d, 3H, *J* 6.7 Hz, 21-CH_3), 1.08 (d, 3H, *J* 7.0 Hz, 27-CH_3), 2.03, 2.07, 2.08, 2.09, 2.10, 2.12 (6s, 7 × 3H, 7Ac), 3.30 (d, 1H, *J* 11.0 Hz, H-26a), 3.51 (m, 1H, H-5′), 3.62–3.78 (m, 5H, H-2′, H-4′, H-6a′, H-5a″, H-5a‴), 3.93–4.06 (m, 5H, H-3, H-26b, H-6b′, H-5b″, H-5b‴), 4.40 (m, 1H, H-16), 4.54 (d, 1H, *J* 5.2 Hz, H-1″), 4.57 (d,

1H, *J* 4.8 Hz, H-1‴), 4.94 (d, 1H, *J* 4.0 Hz, H-1′), 5.01–5.13 (m, 4H, H-2″, H-2‴, H-3″, H-3‴), 5.16–5.23 (m, 3H, H-3′, H-4″, H-4‴); ESI-HRMS calcd for $C_{57}H_{84}O_{23}Na$: 1159.5296 $(M+Na)^+$; found: 1159.5314.

3.8.9. Sarsasapogenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)]-3-*O*-acetyl-α-D-glucopyranoside (24)

From compound **14** (118 mg) and sarsasapogenin **15** (42 mg) to afford **24** (18 mg, 14%) as a white foam; $[\alpha]_D^{25}$ −38 (*c* 1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H, 18-C*H*$_3$), 0.97 (s, 3H, 19-C*H*$_3$), 0.99 (d, 3H, *J* 6.7 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 1.98, 2.01, 2.02, 2.03, 2.08, 2.09 (6s, 9 × 3H, 9Ac), 3.30 (d, 1H, *J* 11.0 Hz, H-26a), 3.56 (m, 1H, H-5′), 3.67 (t, 1H, *J* 9.6 Hz, H-4′), 3.70–3.82 (m, 4H, H-2′, H-5″, H-5‴, H-6a′), 3.91–3.98 (m, 3H, H-3, H-26b, H-6b′), 4.05 (dd, 1H, *J* 2.0, 12.4 Hz, H-6a″), 4.16 (dd, 1H, *J* 2.4, 12.2 Hz, H-6a‴), 4.28 (dd, 1H, *J* 5.0, 12.4 Hz, H-6b″), 4.37 (dd, 1H, *J* 4.2, 12.2 Hz, H-6b‴), 4.40 (m, 1H, H-16), 4.57 (d, 1H, *J* 8.0 Hz, H-1″), 4.60 (d, 1H, *J* 7.6 Hz, H-1‴), 4.90 (d, 1H, *J* 3.6 Hz, H-1′), 4.92 (dd, 1H, *J* 8.0, 9.2 Hz, H-2″), 5.02 (dd, 1H, *J* 7.6, 9.4 Hz, H-2‴), 5.04–5.13 (m, 2H, H-4″, H-4‴), 5.15–5.26 (m, 3H, H-3′, H-3″, H-3‴); ESI-HRMS calcd for $C_{63}H_{92}O_{27}Na$: 1303.5718 $(M+Na)^+$; found: 1303.5690.

3.9. General procedure for preparing the trisaccharide saponins 1–3 and 1a–3a

To a solution of the protected trisaccharide saponins in MeOH (5 mL) was added 1 N aq NaOH solution until pH 10 was reached. The reaction mixture was stirred at rt for 3 h and then neutralized with Amberlite IR 120 (H^+). The solvent was filtered, and the filtrate was concentrated to dryness under diminished pressure, which was subjected to column chromatography using 5:2 CH_2Cl_2–MeOH as the eluents to afford the target trisaccharide saponins.

3.9.1. Sarsasapogenyl β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside (1)

From compound **16** (25 mg) and **19** (20 mg) to yield **1** (29 mg, 89%) as a white solid; $[\alpha]_D^{25}$ −46 (*c* 1, 3:1 $CHCl_3/CH_3OH$); ^1H NMR (400 MHz, CD_3OD): δ 0.78 (s, 3H, 18-C*H*$_3$), 0.97 (s, 3H, 19-C*H*$_3$), 0.98 (d, 3H, *J* 6.5 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 3.18–3.28 (m, 3H), 3.38 (t, 2H, *J* 9.0 Hz), 3.45–3.60 (m, 5H), 3.66 (dd, 1H, *J* 6.2, 12.0 Hz), 3.69 (t, 1H, *J* 9.1 Hz), 3.78–3.94 (m, 6H), 4.01 (br s, 1H, H-3), 4.11 (br d, 1H, *J* 10.0 Hz, H-26b), 4.32 (d, 1H, *J* 7.8 Hz, H-1′), 4.33 (d, 1H, *J* 7.0 Hz, H-1″), 4.39 (m, 1H, H-16), 4.54 (d, 1H, *J* 7.8 Hz, H-1‴); ^{13}C NMR (100 MHz, CD_3OD): δ 14.8, 16.4, 17.0, 22.1, 24.3, 26.8, 27.0, 27.5, 27.7, 27.8, 28.6, 31.3, 31.6, 32.7, 36.2, 36.8, 38.0, 41.5 (2C), 41.9, 43.5, 57.7, 62.5, 63.8, 66.2, 67.1, 68.5, 69.8, 71.6, 72.5, 74.5, 74.9, 75.1, 75.3, 76.1, 76.6, 77.9, 78.0, 80.7, 82.5, 103.0, 104.5, 105.3, 111.1; ESI-HRMS calcd for $C_{44}H_{72}O_{17}Na$: 895.4662 $(M+Na)^+$; found: 895.4657.

3.9.2. Sarsasapogenyl α-L-arabinopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside (2)

From compound **17** (30 mg) and **20** (20 mg) to yield **2** (34 mg, 93%) as a white solid; $[\alpha]_D^{25}$ −58 (*c* 1.5, 3:1 $CHCl_3/CH_3OH$); ^1H NMR (400 MHz, CD_3OD): δ 0.78 (s, 3H, 18-C*H*$_3$), 0.97 (s, 3H, 19-C*H*$_3$), 0.98 (d, 3H, *J* 6.5 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 3.22–3.35 (m, 2H), 3.44–3.60 (m, 7H), 3.65 (d, 1H, *J* 12.0 Hz), 3.71 (t, 1H, *J* 9.4 Hz), 3.78 (br s, 2H), 3.82–3.94 (m, 4H), 4.01 (br s, 1H, H-3), 4.10 (br d, 1H, *J* 11.2 Hz), 4.32 (d, 2H, *J* 7.4 Hz, H-1′, H-1″), 4.40 (m, 1H, H-16), 4.58 (d, 1H, *J* 7.8 Hz, H-1‴); ^{13}C NMR (100 MHz, CD_3OD): δ 14.8, 16.4, 17.0, 22.0, 24.3, 26.8, 27.0, 27.5, 27.7, 27.8, 28.6, 31.3, 31.6, 32.7, 36.2, 36.8, 38.0, 41.5 (2C), 41.9, 43.5, 57.7, 63.8, 66.2, 67.0, 67.8, 68.4, 69.8, 70.2, 72.5, 72.7, 74.5 (2C), 75.0, 75.2, 76.2, 76.4, 80.3, 82.5, 103.0, 105.3 (2C), 111.1; ESI-HRMS calcd for $C_{43}H_{70}O_{16}Na$: 865.4556 $(M+Na)^+$; found: 865.4559.

3.9.3. Sarsasapogenyl β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranoside (3)

From compound **18** (30 mg) and **21** (30 mg) to yield **3** (38 mg, 90%) as a white solid; $[\alpha]_D^{25}$ −42 (*c* 1, 3:1 $CHCl_3$/MeOH); ^1H NMR (400 MHz, CD_3OD): δ 0.78 (s, 3H, 18-C*H*$_3$), 0.98 (s, 3H, 19-C*H*$_3$), 0.99 (d, 3H, *J* 6.5 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.1 Hz, 27-C*H*$_3$), 3.17–3.42 (m, 10H), 3.47–3.55 (m, 2H), 3.62–3.74 (m, 3H), 3.84–3.97 (m, 4H), 4.04 (br s, 1H, H-3), 4.20 (br d, 1H, *J* 11.2 Hz), 4.34 (d, 1H, *J* 7.8 Hz, H-1′), 4.40 (m, 1H, H-16), 4.43 (d, 1H, *J* 7.8 Hz, H-1″), 4.56 (d, 1H, *J* 7.8 Hz, H-1‴); ^{13}C NMR (100 MHz, CD_3OD): δ 14.8, 16.4, 17.0, 22.1, 24.3, 26.8, 27.0, 27.5, 27.7, 27.8, 28.6, 31.1, 31.6, 32.7, 36.2, 36.8, 37.9, 41.5 (2C), 41.9, 43.5, 57.7, 62.5, 62.8, 63.8, 66.1, 68.7, 71.6, 71.7, 74.9, 75.1, 75.2, 75.4, 75.9, 76.6, 77.9, 78.0 (2C), 78.2, 80.8, 82.5, 102.8, 104.5, 104.8, 111.1; ESI-HRMS calcd for $C_{45}H_{78}O_{18}N$: 920.5213 $(M+NH_4)^+$; found: 920.5225.

3.9.4. Sarsasapogenyl β-D-glucopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)]-α-D-glucopyranoside (1a)

From compound **22** (12 mg) to yield **1a** (8 mg, 92%) as a white solid; $[\alpha]_D^{25}$ −34 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, CD_3OD): δ 0.78 (s, 3H, 18-C*H*$_3$), 0.98 (s, 3H, 19-C*H*$_3$), 0.99 (d, 3H, *J* 6.5 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 3.18–3.28 (m, 2H), 3.29–3.42 (m, 2H), 3.46 (dd, 1H, *J* 3.8, 9.4 Hz, H-2′), 3.40–3.64 (m, 5H), 3.65–3.71 (m, 1H), 3.72–3.82 (m, 3H), 3.85–3.98 (m, 4H), 4.03 (dd, 1H, *J* 1.6, 11.0 Hz, H-26b), 4.27–4.32 (m, 2H), 4.39 (m, 1H, H-16), 4.55 (d, 1H, *J* 7.8 Hz, H-1‴), 4.85 (d, 1H, *J* 3.8 Hz, H-1′); ESI-HRMS calcd for $C_{44}H_{76}O_{17}N$: 890.5108 $(M+NH_4)^+$; found: 890.5121.

3.9.5. Sarsasapogenyl α-L-arabinopyranosyl-(1→4)-[α-L-arabinopyranosyl-(1→6)]-α-D-glucopyranoside (2a)

From compound **23** (10 mg) to yield **2a** (6.8 mg, 90%) as a white solid; $[\alpha]_D^{25}$ −45 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, 3:1 CD_3OD/$CDCl_3$): δ 0.78 (s, 3H, 18-C*H*$_3$), 0.98 (s, 3H, 19-C*H*$_3$), 0.99 (d, 3H, *J* 6.5 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 3.29 (m, 1H), 3.47 (dd, 1H, *J* 3.8, 9.4 Hz, H-2′), 3.50–3.68 (m, 6H), 3.71 (t, 1H, *J* 9.8 Hz), 3.78 (t, 1H, *J* 9.2 Hz), 3.81 (br s, 2H), 3.89–3.98 (m, 4H), 4.02 (dd, 1H, *J* 1.6, 10.8 Hz), 4.15 (dd, 1H, *J* 1.6, 11.0 Hz, H-26b), 4.28–4.34 (m, 2H), 4.38–4.45 (m, 2H), 4.84 (d, 1H, *J* 3.8 Hz, H-1′); ESI-HRMS calcd for $C_{43}H_{74}O_{16}N$: 860.5002 $(M+NH_4)^+$; found: 860.5012.

3.9.6. Sarsasapogenyl β-D-glucopyranosyl-(1→4)-[β-D-glucopyranosyl-(1→6)]-α-D-glucopyranoside (3a)

From compound **24** (11 mg) to yield **3a** (7.3 mg, 94%) as a white solid; $[\alpha]_D^{25}$ −38 (*c* 0.1, CH_3OH); ^1H NMR (400 MHz, 3:1 CD_3OD/$CDCl_3$): δ 0.78 (s, 3H, 18-C*H*$_3$), 0.98 (s, 3H, 19-C*H*$_3$), 0.99 (d, 3H, *J* 6.5 Hz, 21-C*H*$_3$), 1.08 (d, 3H, *J* 7.0 Hz, 27-C*H*$_3$), 3.22–3.40 (m, 8H), 3.47 (dd, 1H, *J* 3.8, 9.4 Hz, H-2′), 3.64–3.69 (m, 2H), 3.72 (t, 1H, *J* 9.3 Hz), 3.79 (t, 1H, *J* 9.1 Hz), 3.85–3.99 (m, 5H), 4.14 (br d, 1H, *J* 9.6 Hz), 4.38 (d, 1H, *J* 7.7 Hz, H-1″), 4.40 (m, 1H, H-16), 4.47 (br s, 2H), 4.55 (d, 1H, *J* 7.9 Hz, H-1‴), 4.85 (d, 1H, *J* 3.8 Hz, H-1′); ESI-HRMS calcd for $C_{45}H_{78}O_{18}N$: 920.5213 $(M+NH_4)^+$; found: 920.5230.

3.10. Cell culture assay

Two different cell lines were used to investigate anti-proliferative activities of the synthetic trisaccharide saponins. Human gastric adenocarcinoma (MKN-45) and human epithelial cervical cancer cell (HeLa) were grown in GIBCO® RPMI Media 1640 supplemented with 10% fetal bovine serum. All cells were incubated at 37 °C with 5% CO_2. Anti-proliferative activities were measured using the Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories,

Kumamoto, Japan). The assay was performed according to the manufacturer's protocol. Briefly, 96-well plates were seeded at 1.5×10^4 cells/mL (100 μL per well) and incubated overnight to allow cells to attach to the surface. Cells were then exposed to varying concentrations (0–20 μM) of the synthetic compounds in a final volume of 100 μL for 48 h. The cultures in 96-well plates were placed in 100 μL of medium that contained CCK-8 and incubated for 2.5 h at 37 °C. The absorbance at 450 nm was determined by a microplate reader (Bio-Rad model 680, Hercules, CA, USA). Cell viability was expressed as a percentage of the control (untreated) cells. A dose–response curve was plotted for each compound (see Supplementary data, Figs. S2 and S3) and the half maximal (50%) inhibitory concentration (IC_{50}) was calculated. Each sample was tested in triplicate.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.08.026.

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