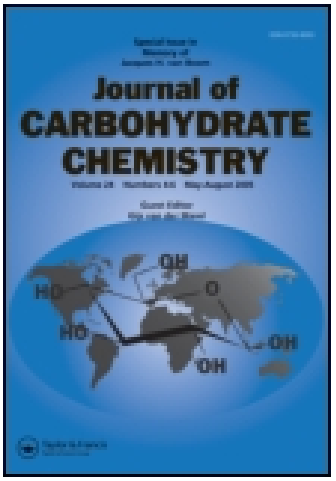


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

Efficient Synthesis and Antitumor Activities of Indioside E Analogs

Yisheng Zhao[ab], Lian An[bc], Guofeng Gu[ab] & Zhongwu Guo[b]

[a] School of Pharmaceutical Science, Shandong University, Jinan 25012, PR China

[b] National Glycoengineering Research Center, Shandong University, Jinan 250010, PR China

[c] School of Life Science, Shandong University, Jinan 250100, PR China

Published online: 03 Apr 2014.

To cite this article: Yisheng Zhao, Lian An, Guofeng Gu & Zhongwu Guo (2014) Efficient Synthesis and Antitumor Activities of Indioside E Analogs, Journal of Carbohydrate Chemistry, 33:3, 152-168, DOI: 10.1080/07328303.2014.900564

To link to this article: http://dx.doi.org/10.1080/07328303.2014.900564

Journal of Carbohydrate Chemistry, 33:152–168, 2014

ISSN: 0732-8303 print / 1532-2327 online
DOI: 10.1080/07328303.2014.900564

Efficient Synthesis and Antitumor Activities of Indioside E Analogs

Yisheng Zhao,[1,2] Lian An,[2,3] Guofeng Gu,[1,2] and Zhongwu Guo[2]

[1]School of Pharmaceutical Science, Shandong University, Jinan 25012, PR China
[2]National Glycoengineering Research Center, Shandong University, Jinan 250010, PR China
[3]School of Life Science, Shandong University, Jinan 250100, PR China

An efficient synthesis of seven analogs of a naturally occurring saponin, indioside E, is described. The synthesis used a 3,4,6-*O*-protected D-galactopyranosyl thioglycoside as the key intermediate to enable regioselective glycosylation and one-pot multistep reactions. Antitumor activities of the synthetic saponins against a human hepatocellular carcinoma cell line BEL-7402 and a human breast adenocarcinoma cell line MCF-7 were evaluated by means of the CCK-8 assay. Analogs carrying trisaccharides with the D-xylopyranose in indioside E substituted with a D-ribopyranose and an L-arabinopyranose or with its diosgenin aglycon replaced with tigogenin exhibited similar antitumor activities as indioside E, but not other analogs.

Keywords Saponin; Indioside E; Thioglycoside; One-pot synthesis; Antitumor

INTRODUCTION

Indioside E (**1**) (Fig. 1), a diosgenyl triglycoside extracted from the *Solanum indicum* L. plant that has long been used as a traditional anti-inflammatory medicine in China,[1] has displayed promising cytotoxic activity toward human hepatocellular carcinoma BEL-7402.[2] Further investigation on the structure-activity relationships of indioside E and its derivatives containing various aglycons indicated that the spirotane type of aglycon was essential for its anticancer activity.[3] For example, neosaponin **2** (Fig. 1), an analog of **1** that had the diosgenin glycon replaced with tigogenin, exhibited very good cytostatic activities against various tumor cells while showing low toxicity to normal hepatocyte cell HL7702.[3] Time-lapse microscopic, immunocytochemical,

Received January 14, 2014; accepted February 28, 2014.
Address correspondence to Guofeng Gu, School of Pharmaceutical Science, Shandong University, Jinan 25012, PR China; and National Glycoengineering Research Center, Shandong University, Jinan 250010, PR China. E-mail: guofenggu@sdu.edu.cn

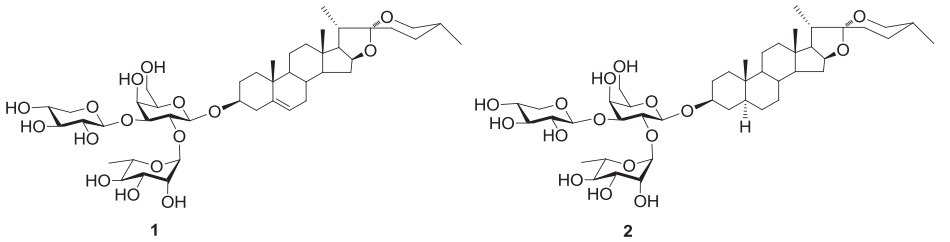


Figure 1: The structures of indioside E (**1**) and neosaponin (**2**).

and many other studies have demonstrated that the cytostatic activity of **2** was due to plasma membrane perturbation and cytoskeleton destruction.[3]

In addition to aglycon, the carbohydrate moiety of saponins may also play an important role in their biological activities. For instance, structure-activity relationship studies on dioscin (diosgenin 3-*O*-chacotrioside) and its derivatives revealed that its trisaccharide moiety was essential for the cytotoxic activity.[4–6] The cell apoptosis activity of solasodine glycoalkaloids was mainly due to the rhamnose residue in their sugar chains.[7] Thus, the influence of the glycan structure in indioside E on its bioactivity is worth investigating. In this regard, we designed and synthesized herein a series of diosgenyl and tigogenyl saponins **3a–c** and **4a–c** (Fig. 2), in which the D-xylopyranose residue on the galactose 3-*O*-position of the glycan in **1** and **2** was substituted with D-lyxopyranose, D-ribopyranose, and L-arabinopyranose, while the rhanmose residue remained unchanged, considering the important impact of rhanmose on the biological activities of other saponins.[7,8] The antitumor activities of the synthetic saponins **2–4** against two human cancer cell lines, BEL-7402 and MCF-7—a human breast adenocarcinoma cell—were evaluated by in vitro antiproliferative assays.

RESULTS AND DISCUSSION

In the past few decades, many practical and efficient synthetic methods have been developed for saponins.[9–13] Recently, we prepared several saponins that

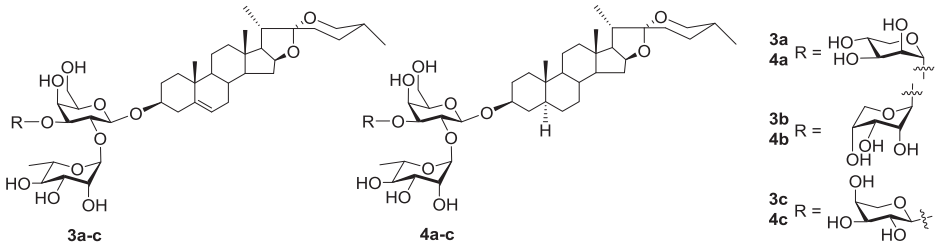
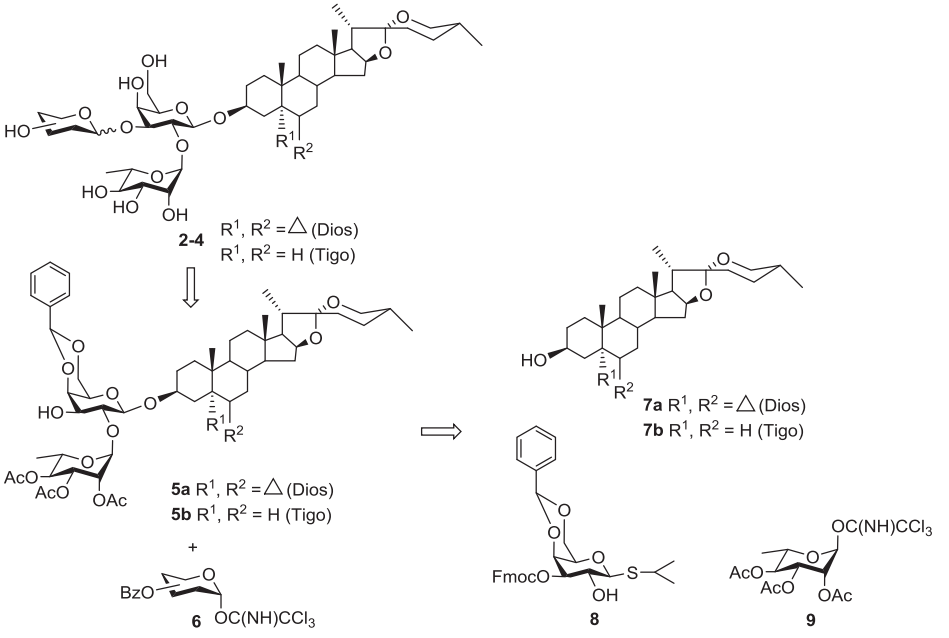


Figure 2: Designed indioside E derivatives, **3a–c** and **4a–c**, to be studied in this research.

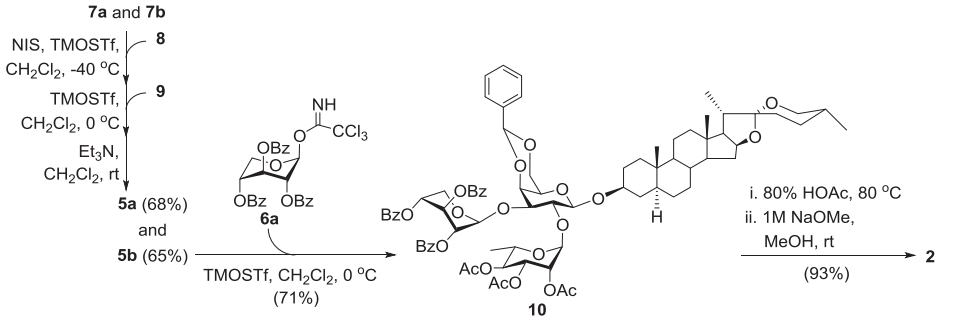
contained 2-*O*- and 2,4-*O*-branched oligosaccharides by a one-pot multistep glycosylation strategy, which used a partially protected thiogalactoside as the key building block.[8,14] This efficient protocol was then explored in the synthesis of target molecules **2–4**. As outlined in Scheme 1, disconnecting the glycosidic linkage at the galactose 3-*O*-postion would afford two common intermediates **5a** and **5b** containing the same disaccharide moiety, which could be readily prepared in a one-pot three-step manner upon chemoselective galactosylation of aglycons **7a** and **7b** with partially protected thiogalactoside **8**, 2-*O*-rhamnosyaltion of the resultant intermediate with trichloroacetimidate **9**, and then removal of the 9-fluorenylmethyloxycarbonyl (Fmoc) group with triethylamine (TEA). The use of **8** to facilitate a one-pot three-step assembly protocol would significantly simplify this synthesis by eliminating a deprotection step and multiple steps of column purification.



Scheme 1: Retrosynthetic plan for target molecules **2–4.**

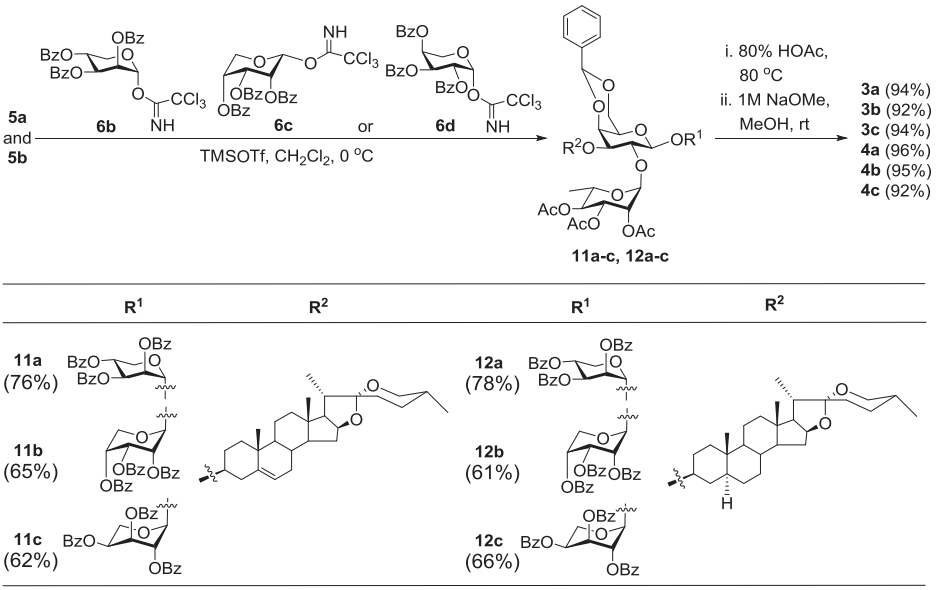
The synthesis of neosaponin **2** is shown in Scheme 2. First, disaccharide glycosides **5a**[2] and **5b** were prepared by a one-pot multistep protocol in very good overall yields, 68% and 65%, respectively. This involved direct glycosylation of diosgenin **7a** and tigogenin **7b** with **8**[15] by the treatment of *N*-iodosuccinimide (NIS) and trimethylsilyl triflate (TMSOTf) at –40°C, followed by rhamnosylation of the resulting saponin intermediate with **9** in the presence of TMSOTf at 0°C and TEA-promoted removal of the Fmoc group on the galactose 3-*O*-position. The coupling constants of the galactosyl H-1 signal

($J_{1,2}$ = 7.2 Hz) at δ 4.48 ppm and the rhamnosyl H-1 signal ($J_{1,2}$ = 1.6 Hz) at δ ~5.32 ppm in the ^{1}H NMR spectra of **5a** and **5b** confirmed their correct glycosidic configurations. No trace of self-condensed products was detected in our experiments. Moreover, such high glycosylation efficiency in condensation of steroidal aglycone with a free 2-OH galactosyl thioglycoside could be rationalized as formation of 1,2-anhydrosugar intermediate presumed by Linhardt et al.[15] Next, glycosylation of **5b** with trichloroacetimidate **6a** in the presence of TMSOTf furnished the fully protected trisaccharyl saponin **10**[3] in a very good yield (71%). Finally, global deprotection of **10** was accomplished in two steps, including removal of the benzylidene group using 80% HOAc and saponification of the resulting intermediate with NaOMe in methanol, which afforded the target molecule **2** in a 93% yield. It is worth mentioning that the small proton coupling constants ($J_{1,2}$ < 1.0, $J_{2,3} = J_{3,4}$ = 3.2, $J_{4,5a}$ = 1.8 Hz) of xylosyl residue in **10** revealed that the benzoylated D-xylopyranose residue in **10** adopted a $^1C_4$ conformation, whereas after complete deprotection the xylopyranose residue in **2** resumed the $^4C_1$ conformation (H-1^{Xyl}: δ 4.40 ppm, $J_{1,2}$ = 6.6 Hz), which was consistent with previously reported data.[2,3]



Scheme 2: Synthesis of neosaponin **2**.

Saponins **3a–c** and **4a–c** were prepared by a similar strategy (Sch. 3). Glycosylation of **5a** with imidates **6b–d** in the presence of a catalytic amount of TMSOTf at 0°C afforded fully protected trisaccharyl saponins **11a–c** in good yields (62%–76%). Likewise, glycosylation **5b** with **6b–d** gave **12a–c** (61%–78%). Subsequently, **11a–c** and **12a–c** were globally deprotected in two steps according to the protocol described above to furnish target molecules **3a–c** and **4a–c** (92%–96%). The NMR spectra of **11** and **12** suggested that the benzoylated ribopyranose and arabinopyranose residues in **11b,c** and **12b,c** adopted a $^1C_4$ conformation. Interestingly, after global deprotection, while the arabinose residue in **3c** and **4c** was converted to the $^4C_1$ conformation (H-1^{Ara}: δ 4.40 ppm, $J_{1,2}$ = 7.0 Hz), the ribose residue in **3b** and **4b** kept the $^1C_4$ conformation (H-1^{Rib}: δ 4.92 ppm, $J_{1,2}$ = 3.9 Hz).



Scheme 3: Synthesis of target molecules **3a–c** and **4a–c.**

The antiproliferative activities of synthetic saponins **2–4** against two human tumor cell lines, BEL-7402 and MCF-7, were assessed according to the standard protocol of the CCK-8 assay[16] with natural indioside E (**1**) as a positive control. The results are shown in Table 1. As reported,[2,3] **1** exhibited strong antiproliferative activities against both tumor cell lines with half-maximal (50%) inhibitory concentrations (IC_{50}) of 3.76 μM for BEL-7402 and 2.93 μM for MCF-7. The antitumor activities of **2,** the ribosyl derivative **3b**,

Table 1: Antiproliferative activities of **1–4** against tumor cell lines BEL-7402 and MCF-7

	IC_{50} (μM)[a]	
Compound	BEL-7402	MCF-7
Indioside E (**1**)	3.76 ± 0.59	2.93 ± 0.77
2	4.29 ± 0.61	3.20 ± 0.06
3a	10.61 ± 1.65	9.33 ± 0.94
3b	3.37 ± 0.42	3.22 ± 0.56
3c	4.67 ± 0.63	3.99 ± 0.37
4a	6.35 ± 0.51	7.06 ± 0.55
4b	5.42 ± 0.33	4.94 ± 0.73
4c	7.89 ± 0.76	9.00 ± 0.86

[a] IC_{50} values are the mean results of three independent experiments.

and the arabinosyl derivative **3c** were comparable to that of **1**, while other synthetic saponins exhibited a two- to three-fold decrease in antiproliferative activity against both tumor cell lines. These results indicate that the xylose residue of trisaccharide moiety in **1** plays a critical role in its antitumor activity. In particular, when the D-xylose residue was replaced with D-lyxose, the antitumor activities of the resulting **3a** and **4a** were significantly reduced. It seemed that, although the spirotane type of aglycon in **1** was critical for its anticancer activity,[3] some small change in the aglycon structure had little influence on its antitumor activity, because **2** had similar activity as **1**. On the other hand, comparing the activities of **3b** to **4b** and **3c** to **4c**, which were only different in their aglycon structures, led us to suggest that after the oligosaccharide in **1** was changed, the aglycon structure did have some impact on the biological activities. Evidently, depending on specific situations, both the glycan and the aglycon may affect the function of a saponin.

In summary, seven analogs of naturally occurring indioside E bearing different aglycons and glycans were synthesized by a highly efficient strategy based on one-pot multistep glycosylation. In vitro biological assays of the synthetic analogs against tumor cell lines BEL-7402 and MCF-7 revealed that the glycan in indioside E had a big impact on its antitumor activity, although some small change in the aglycon structure (e.g., **1** vs. **2**) had little influence on the antitumor activity. However, after the glycan was changed, the aglycon structure did matter significantly. Combined with previous observations,[3] the results have demonstrated that both the carbohydrate moiety and the aglycon play an important role in the biological functions of saponins, whereas the action mechanisms of these saponins and the exact roles that the carbohydrate and the aglycon play in the function are interesting questions that are worth further investigations.

EXPERIMENTAL SECTION

General Methods

Optical rotations were determined at 20°C with a Rudolph Autopol I automatic polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker 400 or a Varian 600 MHz spectrometer for solutions in $CDCl_3$ or CD_3OD. Chemical shifts (δ) are given in ppm downfield from internal Me_4Si. Positive-mode electrospray ionization (ESI) high-resolution mass spectroscopy (HRMS) was recorded on a JEOL JMS-DX-303HF spectrometer. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} plates with detection by charring with 30% (v/v) H_2SO_4 in MeOH or by a UV detector. Column chromatography was conducted by elution of a silica gel column with mixtures of ethyl acetate and

petroleum ether (b.p. 60–90°C) as the eluents. Solutions were concentrated at <60°C under diminished pressure.

General Procedure for One-Pot Synthesis of Disaccharide Saponins 5a and 5b

To a solution of steroidal aglycon **7a** or **7b** (1.0 mmol) and thioglycoside **8** (1.2 mmol) in anhydrous CH_2Cl_2 (10 mL) were added NIS (320 mg, 1.42 mmol) and TMSOTf (27 μL, 0.15 mmol) at –40°C under an N_2 atmosphere. The reaction mixture was stirred under this condition for 30 min, when TLC (3:1 petroleum ether-EtOAc) indicated the complete consumption of **7**. The reaction mixture was warmed to 0°C, and then a solution of trichloroacetimidate **9** (1.5 mmol) in anhydrous CH_2Cl_2 (3 mL) was quickly injected into the reaction mixture, which was followed by the addition of TMSOTf (9 μL, 0.05 mmol). The resulting mixture was stirred for another 1 h, at which time TLC (2:1 petroleum ether-EtOAc) indicated the completion of reaction. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and then quenched with triethylamine (3.0 mL, 21 mmol). The resulting mixture was stirred at rt for 1.5 h and then concentrated to dryness under reduced pressure. Purification of the residue on a silica gel column with petroleum ether-EtOAc (3:2) as the eluent yielded disaccharide saponins **5a** and **5b**.

*Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-benzylidene-β-D-galactopyranoside (5a)*[2]

This was prepared according to the above procedure from 660 mg of **7**, 415 mg of diosgenin **8a,** and 650 mg of **9** to get 638 mg of **5a** (68%) as a white foamy solid. $[\alpha]_D^{20}$ –97.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.34 (m, 5 H), 5.54 (s, 1H, PhC*H*), 5.39 (d, 1H, J = 4.6 Hz, H-6), 5.35 (dd, 1H, J = 3.4, 1.6 Hz, H-2^{Rha}), 5.32 (d, 1H, J = 1.6 Hz, H-1^{Rha}), 5.28 (dd, 1H, J = 10.0, 3.4 Hz, H-3^{Rha}), 5.07 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.50 (m, 1H, H-5^{Rha}), 4.48 (d, 1H, J = 7.2 Hz, H-1^{Gal}), 4.41 (q, 1H, J = 7.4 Hz, H-16), 4.31 (d, 1H, J = 11.9 Hz, H-6a^{Gal}), 4.15 (d, 1H, J = 3.5 Hz, H-4^{Gal}), 4.07 (d, 1H, J = 11.9 Hz, H-6b^{Gal}), 3.82 (dd, 1H, J = 9.6, 3.5 Hz, H-3^{Gal}), 3.77 (dd, 1H, J = 9.6, 7.2 Hz, H-2^{Gal}), 3.63 (m, 1H, H-3), 3.42–3.51 (m, 2H, H-26a, H-5^{Gal}), 3.37 (t, 1H, J = 10.9 Hz, H-26b), 2.12, 2.02, 1.98 (3s, 3 × 3 H, 3Ac), 1.21 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 1.03 (s, 3H, CH_3-19), 0.97 (d, 3H, J = 6.9 Hz, CH_3-21), 0.79 (d, 3H, J = 6.0 Hz, CH_3-27), 0.78 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.08, 170.06, 170.02, 140.4, 121.8, 109.3, 101.4, 98.9, 97.3, 80.8, 78.3, 75.7, 74.5, 74.2, 71.3, 69.6, 69.3, 69.2, 66.8, 66.4, 66.1, 62.1, 56.5, 50.1, 41.6, 40.3, 39.8, 38.4, 37.2, 36.9, 32.1, 31.8, 31.42, 31.39, 30.3, 29.5, 28.8, 20.95, 20.88, 20.85, 20.80, 19.3, 17.2, 17.1, 16.3, 14.5. ESI-HRMS (positive ion): Calcd for ($C_{52}H_{72}O_{15}$+NH_4^+): 954.5209; found m/z: 954.5222.

*Tigogenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-4,6-di-*O*-benzylidene-β-D-galactopyranoside (5b)*

This was prepared according to the above procedure from 660 mg of **7**, 418 mg of diosgenin **8b,** and 650 mg of **9** to get 610 mg of **5b** (65%) as a white foamy solid. $[\alpha]_D^{20}$ –84.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.34 (m, 5 H), 5.54 (s, 1H, PhC*H*), 5.38–5.27 (m, 3H, H-1,2,3^{Rha}), 5.07 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.52 (m, 1H, H-5^{Rha}), 4.48 (d, 1H, J = 7.2 Hz, H-1^{Gal}), 4.39 (q, 1H, J = 7.4 Hz, H-16), 4.31 (d, 1H, J = 11.9 Hz, H-6a^{Gal}), 4.14 (d, 1H, J = 3.5 Hz, H-4^{Gal}), 4.07 (d, 1H, J = 11.9 Hz, H-6b^{Gal}), 3.86–3.74 (m, 2H, H-2,3^{Gal}), 3.63 (m, 1H, H-3), 3.48 (dd, 1H, J = 10.9, 3.2 Hz, H-26a), 3.45 (s, 1H, H-5^{Gal}), 3.38 (t, 1H, J = 10.9 Hz, H-26b), 2.13, 2.04, 1.98 (3s, 3 × 3 H, 3Ac), 1.20 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 0.97 (d, 3H, J = 6.9 Hz, CH_3-21), 0.83 (s, 3H, CH_3-19), 0.79 (d, 3H, J = 6.2 Hz, CH_3-27), 0.76 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.03, 170.00, 169.97, 109.2, 101.3, 98.8, 97.2, 80.8, 77.9, 75.8, 74.2 (2C), 71.3, 69.7, 69.3, 69.1, 66.8, 66.4, 66.1, 62.2, 56.3, 54.3, 44.6, 41.6, 40.6, 40.0, 36.9, 35.8, 35.1, 34.2, 32.3, 31.8, 31.4, 30.3, 29.3, 28.8, 28.7, 21.0, 20.9, 20.8, 20.7, 17.2, 17.1, 16.5, 14.5, 12.2. ESI-HRMS (positive ion): Calcd for ($C_{52}H_{74}O_{15}$+NH_4^+): 956.5366; found *m*/*z*: 956.5380.

General Procedure for Preparation of Fully Protected Saponins 10, 11a–c, and 12a-c

To a solution of saponins **5a** or **5b** (0.15 mmol) and imidates **6a–d** (0.30 mmol) in anhydrous CH_2Cl_2 (5 mL) was added TMSOTf (9 μL, 0.05 mmol) at 0°C under an N_2 atmosphere. The reaction mixture was stirred under this condition for 1 h, neutralized with Et_3N, and concentrated to dryness. The residue was subjected to flash chromatograph with petroleum ether and EtOAc (3:1) as the eluent to give **10, 11a–c,** and **12a–c**.

*Tigogenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-β-D-xylopyranosyl-(1→3)]-4,6-di-*O*-benzylidene-β-D-galactopyranoside (10)[3]*

Prepared from **5b** (140 mg) and **6a** (182 mg) to yield **10** (147 mg, 71%) as a white foamy solid. $[\alpha]_D^{20}$ –73.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–7.06 (m, 20H, *Ph*), 5.65 (t, 1H, J = 3.2 Hz, H-3^{Xyl}), 5.54 (s, 1H, PhC*H*), 5.34–5.27 (m, 2H, H-3^{Rha}, H-4^{Xyl}), 5.26–5.20 (m, 3H, H-1,2^{Rha}, H-1^{Xyl}), 5.08 (d, 1H, J = 3.2 Hz, H-2^{Xyl}), 4.97 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.71 (dd, 1H, J = 12.9, 1.8 Hz, H-5a^{Xyl}), 4.57 (d, 1H, J = 7.8 Hz, H-1^{Gal}), 4.51 (m, 1H, H-5^{Rha}), 4.46 (d, 1H, J = 3.5 Hz, H-4^{Gal}), 4.39 (q, 1H, J = 7.4 Hz, H-16), 4.33 (d, 1H, J = 12.0 Hz, H-6a^{Gal}), 4.15–4.05 (m, 2H, H-2,6b^{Gal}), 3.89 (dd, 1H, J = 9.6, 3.5 Hz, H-3^{Gal}), 3.85 (d, 1H, J = 12.9 Hz, H-5b^{Xyl}), 3.71 (m, 1H, H-3), 3.52–3.42 (m, 2H, H-5^{Gal}, H-26a), 3.37 (t, 1H, J = 10.9 Hz, H-26b), 2.00, 1.95, 1.88 (3s, 3 × 3H, 3Ac), 1.09 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 0.96 (d, 3H, J = 6.9 Hz, CH_3-21),

0.83 (s, 3H, CH_3-19), 0.79 (d, 3H, J = 6.1 Hz, CH_3-27), 0.76 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 165.5, 165.1, 164.6, 109.3, 100.8, 100.0, 98.9, 97.0, 83.8, 80.8, 77.7, 76.0, 71.5, 71.5, 70.1, 69.3, 68.8, 68.3, 67.0, 66.8, 66.2 (2C), 65.9, 62.2, 58.7, 56.3, 54.3, 44.6, 41.6, 40.6, 40.0, 37.0, 35.8, 35.1, 34.0, 32.3, 31.8, 31.4, 30.3, 29.2, 28.8, 28.7, 21.0, 20.9, 20.8, 20.6, 17.2 (2C), 16.5, 14.5, 12.3. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{94}O_{22}$+Na^+): 1405.6192; found m/z: 1405.6137.

Diosgenyl 2,3,4-tri-O-*acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri*-O-*benzoyl-α-D-lyxopyranosyl-(1→3)]-4,6-di*-O-*benzylidene-β-D-galactopyranoside (11a)*

This was prepared from **5a** (140 mg) and **6b** (182 mg) to yield **11a** (158 mg, 76%) as a white foamy solid. $[\alpha]_D^{20}$ –69.6 (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13–7.26 (m, 20H, *Ph*), 5.75–5.66 (m, 3H, H-2,3,4^{Lyx}), 5.49 (dd, 1H, J = 3.2, 1.6 Hz, H-2^{Rha}), 5.44 (s, 1H, PhC*H*), 5.38 (d, 1H, J = 5.0 Hz, H-6), 5.36 (d, 1H, J = 1.2 Hz, H-1^{Lyx}), 5.28–5.24 (m, 2H, H-1,3^{Rha}), 5.12 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.64 (m, 1H, H-5^{Rha}), 4.56 (d, 1H, J = 7.8 Hz, H-1^{Gal}), 4.41 (q, 1H, J = 7.2 Hz, H-16), 4.37 (d, 1H, J = 3.2 Hz, H-4^{Gal}), 4.32 (d, 1H, J = 11.6 Hz, H-6a^{Gal}), 4.22–4.11 (m, 2H, H-2^{Gal}, H-5a^{Lyx}), 4.06 (d, 1H, J = 11.6 Hz, H-6b^{Gal}), 3.99–3.88 (m, 2H, H-3^{Gal}, H-5b^{Lyx}), 3.67 (m, 1H, H-3), 3.52–3.43 (m, 2H, H-5^{Gal}, H-26a), 3.37 (t, 1H, J = 10.9 Hz, H-26b), 2.02, 1.95, 1.94 (3s, 3 × 3H, 3Ac), 1.22 (d, 3H, J = 6.1 Hz, H-6^{Rha}), 1.03 (s, 3H, CH_3-19), 0.97 (d, 3H, J = 6.9 Hz, CH_3-21), 0.79 (d, 3H, J = 6.0 Hz, CH_3-27), 0.78 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 165.9, 165.1, 164.9, 140.5, 121.8, 109.3, 100.4, 98.7, 98.0, 95.0, 80.8, 77.6, 77.2, 72.1, 71.9, 71.4, 70.3, 69.7, 69.4, 69.3, 69.1, 67.2, 66.9, 66.2 (2C), 62.1, 61.5, 56.5, 50.1, 41.6, 40.3, 39.8, 38.2, 37.2, 36.9, 32.1, 31.9, 31.45, 31.40, 30.3, 29.4, 28.8, 20.9, 20.85, 20.82, 20.6, 19.3, 17.2, 17.1, 16.3, 14.5. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{92}O_{22}$+NH_4^+): 1398.6419; found m/z: 1398.6448.

Diosgenyl 2,3,4-tri-O-*acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri*-O-*benzoyl-β-D-ribopyranosyl-(1→3)]-4,6-di*-O-*benzylidene-β-D-galactopyranoside (11b)*

Prepared from **5a** (140 mg) and **6c** (182 mg) to yield **11b** (135 mg, 65%) as a white foamy solid. $[\alpha]_D^{20}$ –65.1 (c 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–7.21 (m, 20H, *Ph*), 5.70 (d, 1H, J = 3.4 Hz, H-2^{Rib}), 5.58 (t, 1H, J = 3.4 Hz, H-3^{Rib}), 5.54 (m, 1H, H-4^{Rib}), 5.51 (s, 1H, PhC*H*), 5.39 (d, 1H, J = 4.9 Hz, H-6), 5.34 (s, 1H, H-1^{Rib}), 5.32 (dd, 1H, J = 10.0, 3.2 Hz, H-3^{Rha}), 5.25 (s, 1H, H-1^{Rha}), 5.21 (dd, 1H, J = 3.2, 1.6 Hz, H-2^{Rha}), 5.11 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.59 (m, 1H, H-5^{Rha}), 4.58 (d, 1H, J = 7.7 Hz, H-1^{Gal}), 4.48 (d, 1H, J = 12.4 Hz, H-5a^{Rib}), 4.43 (d, 1H,

$J = 3.4$ Hz, H-4^{Gal}), 4.41 (q, 1H, $J = 7.4$ Hz, H-16), 4.29 (d, 1H, $J = 11.6$ Hz, H-6a^{Gal}), 4.12 (dd, 1H, $J = 9.6$, 7.7 Hz, H-2^{Gal}), 4.06 (d, 1H, $J = 11.6$ Hz, H-6b^{Gal}), 3.91 (dd, 1H, $J = 9.6$, 3.4 Hz, H-3^{Gal}), 3.86 (d, 1H, $J = 12.4$ Hz, H-5b^{Rib}), 3.67 (m, 1H, H-3), 3.47 (dd, 1H, $J = 10.9$, 4.7 Hz, H-26a), 3.43 (s, 1H, H-5^{Gal}), 3.38 (t, 1H, $J = 10.9$ Hz, H-26b), 2.02, 1.95, 1.87 (3s, 3 × 3H, 3Ac), 1.24 (d, 3H, $J = 6.1$ Hz, H-6^{Rha}), 1.04 (s, 3H, CH_3-19), 0.98 (d, 3H, $J = 6.9$ Hz, CH_3-21), 0.79 (d, 3H, $J = 5.8$ Hz, CH_3-27), 0.79 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.0, 169.9, 166.2, 166.0, 165.1, 140.4, 121.9, 109.3, 101.3, 100.5, 98.8, 97.4, 84.1, 80.8, 77.8, 77.2, 75.3, 72.2, 71.6, 70.2, 69.3, 69.1, 68.9, 67.9, 66.8, 66.1, 65.7, 62.2, 62.1, 56.5, 50.1, 41.6, 40.3, 39.7, 38.2, 37.2, 36.9, 32.1, 31.8, 31.44, 31.39, 30.3, 29.4, 28.8, 20.87, 20.85, 20.81, 20.6, 19.3, 17.2, 17.1, 16.3, 14.5. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{92}O_{22}$+NH_4^+): 1398.6419; found m/z: 1398.6445.

*Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl-(1→3)]-4,6-di-*O*-benzylidene-β-D-galactopyranoside (11c)*

This was prepared from **5a** (140 mg) and **6d** (182 mg) to yield **11c** (128 mg, 62%) as a white foamy solid. $[\alpha]_D^{20}$ –49.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.00 (m, 20H, *Ph*), 5.85 (t, 1H, $J = 3.4$ Hz, H-2^{Ara}), 5.65 (m, 1H, H-4^{Ara}), 5.59–5.53 (br s, 2H, H-3^{Ara}, PhC*H*), 5.38 (d, 1H, $J = 4.0$ Hz, H-6), 5.28 (dd, 1H, $J = 10.0$, 3.4 Hz, H-3^{Rha}), 5.23 (dd, 1H, $J = 3.4$, 1.0 Hz, H-2^{Rha}), 5.17 (s, 1H, H-1^{Rha}), 5.10 (s, 1H, H-1^{Ara}), 4.95 (t, 1H, $J = 10.0$ Hz, H-4^{Rha}), 4.64 (t, 1H, $J = 10.6$ Hz, H-5a^{Ara}), 4.56 (d, 1H, $J = 7.7$ Hz, H-1^{Gal}), 4.51 (d, 1H, $J = 3.2$ Hz, H-4^{Gal}), 4.46 (m, 1H, H-5^{Rha}), 4.41 (q, 1H, $J = 7.4$ Hz, H-16), 4.33 (d, 1H, $J = 12.2$ Hz, H-6a^{Gal}), 4.15–4.02 (m, 2H, H-2,6b^{Gal}), 3.88 (dd, 1H, $J = 9.6$, 3.2 Hz, H-3^{Gal}), 3.84 (dd, 1H, $J = 10.6$, 4.8 Hz, H-5b^{Ara}), 3.66 (m, 1H, H-3), 3.51–3.43 (m, 2H, H-5^{Gal}, H-26a), 3.38 (t, 1H, $J = 10.9$ Hz, H-26b), 1.99, 1.97, 1.94 (3s, 3 × 3H, 3Ac), 1.04 (d, 3H, $J = 6.2$ Hz, H-6^{Rha}), 1.03 (s, 3H, CH_3-19), 0.97 (d, 3H, $J = 7.0$ Hz, CH_3-21), 0.79 (d, 3H, $J = 5.0$ Hz, CH_3-27), 0.78 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.03 (2C), 169.99, 165.3, 165.2, 164.8, 140.4, 121.8, 109.3, 100.8, 99.7, 99.0, 97.1, 80.8, 78.0, 77.2, 76.1, 71.9, 71.5, 70.0, 69.9, 69.3, 68.9, 67.2, 66.8, 66.3, 65.9, 65.8, 65.6, 62.1, 56.5, 50.1, 41.6, 40.3, 39.8, 38.3, 37.2, 36.9, 32.1, 31.8, 31.4, 30.3, 29.7, 29.4, 28.8, 20.8, 20.6, 19.3, 17.14, 17.09, 16.3, 14.5. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{92}O_{22}$+Na^+): 1403.5972; found m/z: 1403.5985.

*Tigogenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-D-lyxopyranosyl-(1→3)]-4,6-di-*O*-benzylidene-β-D-galactopyranoside (12a)*

This was prepared from **5b** (140 mg) and **6b** (182 mg) to yield **12a** (162 mg, 78%) as a white foamy solid. $[\alpha]_D^{20}$ –54.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz,

$CDCl_3$): δ 8.14–7.25 (m, 20H, *Ph*), 5.78–5.63 (m, 3H, H-2,3^{Xyl}, PhC*H*), 5.47 (br d, 1H, H-4^{Lyx}), 5.44 (s, 1H, H-1^{Lyx}), 5.36 (s, 1H, H-1^{Rha}), 5.29 (dd, 1H, H-3^{Rha}), 5.27 (br s, 1H, H-2^{Rha}), 5.08 (d, 1H, J = 2.6 Hz, H-2^{Xyl}), 5.11 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.63 (m, 1H, H-5^{Rha}), 4.56 (d, 1H, J = 7.7 Hz, H-1^{Gal}), 4.38 (q, 1H, J = 7.4 Hz, H-16), 4.37 (d, 1H, J = 3.6 Hz, H-4^{Gal}), 4.33 (d, 1H, J = 12.3 Hz, H-6a^{Gal}), 4.23–4.10 (m, 2H, H-2^{Gal}, H-5a^{Lyx}), 4.06 (d, 1H, J = 12.0 Hz, H-6b^{Gal}), 3.98–3.88 (m, 2H, H-3^{Gal}, H-5b^{Xyl}), 3.71 (m, 1H, H-3), 3.50–3.41 (m, 2H, H-5^{Gal}, H-26a), 3.37 (t, 1H, J = 10.9 Hz, H-26b), 2.03, 1.95, 1.94 (3s, 3 × 3H, 3Ac), 1.21 (d, 3H, J = 6.1 Hz, H-6^{Rha}), 0.96 (d, 3H, J = 6.9 Hz, CH_3-21), 0.83 (s, 3H, CH_3-19), 0.798 (d, 3H, J = 6.2 Hz, CH_3-27), 0.76 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 165.9, 165.1, 164.9, 109.3, 100.4, 98.7, 97.9, 94.9, 80.8, 77.3, 71.9 (2C), 71.4, 70.3, 69.6, 69.4, 69.3, 69.2, 68.2, 67.2, 66.8, 66.21, 66.16, 62.2, 61.5, 56.3, 54.3, 41.6, 40.6, 40.0, 37.0, 35.8, 35.1, 33.9, 32.3, 31.8, 31.4, 30.3, 29.2, 28.9, 28.8, 21.0, 20.9, 20.8, 20.7, 17.1 (2C), 16.5, 14.5, 12.3. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{94}O_{22}$+NH_4^+): 1400.6575; found m/z: 1400.6590.

*Tigogenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-β-D-ribopyranosyl-(1→3)]-4,6-di-*O*-benzylidene-β-D-galactopyranoside (12b)*

This was prepared from **5b** (140 mg) and **6c** (182 mg) to yield **12b** (126 mg, 61%) as a white foamy solid. $[\alpha]_D^{20}$ –59.6 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.22 (m, 20H, *Ph*), 5.70 (d, 1H, J = 3.5 Hz, H-2^{Rib}), 5.58 (t, 1H, J = 3.5 Hz, H-3^{Rib}), 5.53 (m, 1H, H-4^{Rib}), 5.51 (s, 1H, PhC*H*), 5.35 (dd, 1H, J = 10.0, 3.4 Hz, H-3^{Rha}), 5.34 (s, 1H, H-1^{Rib}), 5.25 (d, 1H, J = 1.5 Hz, H-1^{Rha}), 5.20 (dd, 1H, J = 3.4, 1.5 Hz, H-2^{Rha}), 5.10 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.61 (m, 1H, H-5^{Rha}), 4.57 (d, 1H, J = 7.6 Hz, H-1^{Gal}), 4.48 (d, 1H, J = 12.2 Hz, H-5a^{Rib}), 4.42 (d, 1H, J = 3.0 Hz, H-4^{Gal}), 4.39 (q, 1H, J = 7.4 Hz, H-16), 4.31 (d, 1H, J = 11.4 Hz, H-6a^{Gal}), 4.11 (dd, 1H, J = 9.6, 7.6 Hz, H-2^{Gal}), 4.06 (d, 1H, J = 11.4 Hz, H-6b^{Gal}), 3.98 (dd, 1H, J = 9.6, 3.0 Hz, H-3^{Gal}), 3.87 (d, 1H, J = 12.2 Hz, H-5b^{Rib}), 3.73 (m, 1H, H-3), 3.46 (dd, 1H, J = 10.9, 5.6 Hz, H-26a), 3.42 (s, 1H, H-5^{Gal}), 3.37 (t, 1H, J = 10.9 Hz, H-26b), 2.03, 1.96, 1.87 (3s, 3 × 3H, 3Ac), 1.22 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 0.96 (d, 3H, J = 6.9 Hz, CH_3-21), 0.84 (s, 3H, CH_3-19), 0.79 (d, 3H, J = 6.2 Hz, CH_3-27), 0.76 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3, 170.0, 169.9, 166.2, 166.0, 165.1, 109.2, 101.3, 100.5, 98.8, 97.3, 84.2, 80.8, 77.5, 75.3, 72.0, 71.7, 70.3, 69.3, 69.1, 68.8, 67.9, 66.8, 66.2, 66.1, 65.7, 62.3, 62.2, 56.3, 54.4, 44.6, 41.6, 40.6, 40.1, 37.0, 35.8, 35.1, 33.9, 32.3, 31.8, 31.4, 30.3, 29.3, 28.8, 28.8, 21.0, 20.9, 20.8, 20.6, 17.1, 16.5, 14.5, 12.3. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{94}O_{22}$+NH_4^+): 1400.6575; found m/z: 1400.6603.

*Tigogenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-α-L-arabinopyranosyl-(1→3)]-4,6-di-*O*-benzylidene-β-D-galactopyranoside (12c)*

This was prepared from **5b** (140 mg) and **6d** (182 mg) to yield **12c** (137 mg, 66%) as a white foamy solid. $[\alpha]_D^{20}$ –31.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–6.98 (m, 20H, *Ph*), 5.85 (t, 1H, J = 3.5 Hz, H-2^{Ara}), 5.64 (m, 1H, H-4^{Ara}), 5.58–5.53 (m, 2H, H-3^{Ara}, PhC*H*), 5.30 (dd, 1H, J = 10.0, 3.4 Hz, H-3^{Rha}), 5.22 (dd, 1H, J = 3.4, 1.0 Hz, H-2^{Rha}), 5.18 (s, 1H, H-1^{Rha}), 5.10 (s, 1H, H-1^{Ara}), 4.94 (t, 1H, J = 10.0 Hz, H-4^{Rha}), 4.65 (t, 1H, J = 10.5 Hz, H-5a^{Ara}), 4.56 (d, 1H, J = 7.8 Hz, H-1^{Gal}), 4.51 (d, 1H, J = 3.5 Hz, H-4^{Gal}), 4.46 (m, 1H, H-5^{Rha}), 4.39 (q, 1H, J = 7.4 Hz, H-16), 4.34 (d, 1H, J = 13.1 Hz, H-6a^{Gal}), 4.13–4.04 (m, 2H, H-2,6b^{Gal}), 3.86 (dd, 1H, J = 9.6, 3.5 Hz, H-3^{Gal}), 3.85 (dd, 1H, J = 10.5, 4.6 Hz, H-5b^{Ara}), 3.71 (m, 1H, H-3), 3.51–3.43 (m, 2H, H-5^{Gal}, H-26a), 3.37 (t, 1H, J = 10.9 Hz, H-26b), 1.99, 1.97, 1.94 (3s, 3 × 3H, 3Ac), 1.02 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 0.96 (d, 3H, J = 6.8 Hz, CH_3-21), 0.82 (s, 3H, CH_3-19), 0.79 (d, 3H, J = 6.2 Hz, CH_3-27), 0.76 (s, 3H, CH_3-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0, 169.9, 165.3, 165.2, 164.8, 109.3, 100.8, 99.7, 99.0, 96.9, 80.8, 77.8, 77.3, 76.2, 71.6, 70.1, 69.9, 69.3, 68.8, 67.1, 66.8, 66.3, 65.9, 65.8, 62.2, 56.3, 54.3, 44.6, 41.6, 40.6, 40.0, 37.0, 35.8, 35.1, 34.0, 32.3, 31.8, 31.4, 30.3, 29.2, 28.8, 28.7, 21.0, 20.9, 20.8, 20.7, 17.2, 17.1, 16.5, 14.5, 12.3. ESI-HRMS (positive ion): Calcd for ($C_{78}H_{94}O_{22}$+NH_4^+): 1400.6575; found *m*/*z*: 1400.6603.

General Procedure for Preparation of Target Molecules 2–4

A solution of fully protected saponins **10, 11a–c,** or **12a–c** (0.05 mmol) in 80% aq. AcOH (10 mL) was stirred at 70°C for 3 h, when TLC (1:1 petroleum ether-EtOAc) showed the completion of the reaction. The solvent was coevaporated with toluene (3 × 15 mL) under reduced pressure to give a residue, which was dissolved in methanol (10 mL). To this solution was added 1 M MeONa in methanol until the pH value reached 10. The reaction mixture was stirred at rt for 3 h and then neutralized with Amberlite IR 120 (H^+). The mixture was filtered off through filter paper, and the filtrate was combined and concentrated under diminished pressure. The residue was then subjected to column chromatography with CH_2Cl_2-MeOH (3:1) as the eluent to afford the desired saponins **2–4**.

Tigogenyl α-L-rhamnopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-galactopyranoside (2)[3]

This was prepared from **10** (70 mg) to yield **2** (40.3 mg, 93%) as a white foamy solid. $[\alpha]_D^{20}$ –74.0 (*c* 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, 2:1 $CDCl_3/CD_3OD$): δ 5.16 (s, 1H, H-1^{Rha}), 4.46 (d, 1H, J = 7.8 Hz, H-1^{Gal}), 4.43–4.37 (m, 2H, H-1^{Xyl}, H-16), 4.10 (m, 1H, H-5^{Rha}), 4.09 (s, 1H), 4.03 (d, 1H, J = 2.4 Hz), 3.95 (br s, 1H), 3.91 (dd, 1H, J = 11.5, 5.1 Hz, H-5a^{Xyl}), 3.81–3.74

(m, 3H), 3.73–3.61 (m, 3H), 3.55 (m 1H), 3.51–3.44 (m, 2H), 3.39 (t, 1H, J = 9.5 Hz), 3.36 (m, 1H), 3.31 (dd, 1H, J = 8.5, 7.6 Hz), 3.23 (t, 1H, J = 10.7 Hz, H-26b), 1.26 (d, 3H, J = 6.1 Hz, H-6^{Rha}), 0.97 (d, 3H, J = 6.9 Hz, CH_3-21), 0.83 (s, 3H, CH_3-19), 0.80 (d, 3H, J = 6.2 Hz, CH_3-27), 0.77 (s, 3H, CH_3-18). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 109.5, 104.7 (C-1^{Xyl}), 100.8 (C-1^{Rha}), 99.1 (C-1^{Gal}), 83.5, 80.9, 76.0, 74.5, 73.9, 73.0, 72.6, 71.0, 70.4, 69.4, 69.1, 68.3, 66.7, 65.4, 61.9, 61.1, 56.2, 54.3, 44.5, 41.5, 40.5, 39.9, 36.9, 35.6, 34.9, 33.6, 32.2, 31.4, 31.1, 30.1, 29.1, 28.6, 28.5, 20.9, 17.1, 16.8, 16.3, 14.1, 11.9. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{72}O_{16}$+NH_4^+): 874.5159; found m/z: 874.5165.

Diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-D-lyxopyranosyl-(1→3)]-β-D-galactopyranoside (3a)

This was prepared from **11a** (70 mg) to yield **3a** (40.7 mg, 94%) as a white foamy solid. $[\alpha]_D^{20}$ –43.0 (c 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, CD_3OD): δ 5.37 (d, 1H, J = 4.3 Hz, H-6), 5.10 (s, 1H, H-1^{Rha}), 4.76 (d, 1H, H-1^{Lyx}, overlapped by CD_3OD), 4.48 (t, 1H, J = 3.6 Hz), 4.38 (q, 1H, J = 7.2 Hz, H-16), 4.15 (m, 1H, H-5^{Rha}), 4.05 (s, 1H), 3.94 (br s, 1H), 3.80–3.73 (m, 6H), 3.73–3.69 (m, 2H), 3.64 (dd, 1H, J = 9.6, 3.6 Hz), 3.61 (m 1H, H-3), 3.57 (dd 1H, J = 10.2, 4.2 Hz), 3.47 (t, 1H, J = 6.2 Hz), 3.43 (dd, 1H, J = 10.9, 2.7 Hz, H-26a), 3.37 (t, 1H, J = 9.5 Hz), 3.31 (t, 1H, J = 10.9 Hz, H-26b), 1.22 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 1.03 (s, 3H, CH_3-19), 0.95 (d, 3H, J = 7.0 Hz, CH_3-21), 0.80 (s, 3H, CH_3-18), 0.78 (d, 3H, J = 5.4 Hz, CH_3-27). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 140.4, 121.5, 109.6, 100.8 (C-1^{Rha}), 99.5 (C-1^{Gal}), 97.1 (C-1^{Lyx}), 80.9, 78.7, 78.1, 74.0, 73.2, 72.6, 71.0 (2C), 70.1, 69.2, 68.4, 68.1, 66.8, 64.9, 64.1, 61.8, 61.1, 56.4, 50.0, 41.5, 40.2, 39.6, 38.2, 37.1, 36.7, 31.9, 31.5, 31.3, 31.1, 30.1, 29.4, 28.5, 20.7, 19.0, 17.0, 16.8, 16.1, 14.2. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{70}O_{16}$+NH_4^+): 872.5002; found m/z: 872.5012.

Diosgenyl α-L-rhamnopyranosyl-(1→2)-[β-D-ribopyranosyl-(1→3)]-β-D-galactopyranoside (3b)

Prepared from **11b** (70 mg) to yield **3b** (39.8 mg, 92%) as a white foamy solid. $[\alpha]_D^{20}$ –113.0 (c 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, 2:1 $CDCl_3/CD_3OD$): δ 5.37 (d, 1H, J = 4.6 Hz, H-6), 5.07 (s, 1H, H-1^{Rha}), 4.92 (d, 1H, J = 3.9 Hz, H-1^{Rib}), 4.47 (d, 1H, J = 7.7 Hz, H-1^{Gal}), 4.42 (q, 1H, J = 7.2 Hz, H-16), 4.11 (m, 1H, H-5^{Rha}), 4.02 (d, 1H, J = 2.8 Hz), 3.98–3.92 (m, 2H), 3.89 (br s, 1H), 3.83–3.74 (m, 4H), 3.71 (dd, 1H, J = 11.7, 5.3 Hz), 3.69–3.61 (m, 4H), 3.52–3.45 (m, 2H), 3.61 (m, 1H, H-3), 3.40 (t, 1H, J = 9.6 Hz), 3.36 (t, 1H, J = 11.4 Hz, H-26b), 1.27 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 1.04 (s, 3H, CH_3-19), 0.98 (d, 3H, J = 7.0 Hz, CH_3-21), 0.81 (s, 3H, CH_3-18), 0.80 (d, 3H, J = 6.5 Hz, CH_3-27). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 140.4, 121.6, 109.6, 102.8 (C-1^{Rib}), 100.9 (C-1^{Rha}), 99.6 (C-1^{Gal}), 82.8, 80.9, 78.0, 74.9, 74.1, 72.5, 71.0, 70.8, 70.4, 69.0, 68.7, 68.4, 67.0, 66.8, 64.0, 61.8, 61.1, 56.4, 50.0, 41.5, 40.2, 39.6, 38.1, 37.1, 36.7, 31.9, 31.5, 31.3, 31.1, 30.1, 29.4, 28.5, 20.7, 19.0, 17.1, 16.8, 16.1,

14.2. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{70}O_{16}$+NH_4^+): 872.5002; found *m*/*z*: 872.5016.

Diosgenyl α-L-rhamnopyranosyl-[(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-galactopyranoside (3c)

This was prepared from **11c** (70 mg) to yield **3c** (40.7 mg, 94%) as a white foamy solid. $[\alpha]_D^{20}$ –109.0 (*c* 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, CD_3OD): δ 5.38 (d, 1H, J = 4.8 Hz, H-6), 5.18 (s, 1H, H-1^{Rha}), 4.47 (d, 1H, J = 7.8 Hz, H-1^{Gal}), 4.40 (d, 1H, J = 7.0 Hz, H-1^{Ara}), 4.39 (q, 1H, J = 7.2 Hz, H-16), 4.13 (m, 1H, H-5^{Rha}), 4.03 (d, 1H, J = 2.9 Hz), 3.91 (br s, 1H), 3.84 (dd, 1H, J = 12.4, 2.8 Hz), 3.82–3.79 (m, 1H), 3.76 (dd, 1H, J = 9.4, 7.8 Hz, H-2^{Gal}), 3.71–3.66 (m, 4H), 3.63 (dd, 1H, J = 9.4, 3.4 Hz), 3.61 (m, 1H, H-3), 3.60 (dd, 1H, J = 9.0, 7.0 Hz, H-2^{Ara}), 3.54 (d,1H, J = 11.9 Hz), 3.52–3.47 (m, 2H), 3.43 (m, 1H), 3.37 (t, 1H, J = 9.5 Hz), 3.31 (t, 1H, J = 10.9 Hz, H-26b), 1.22 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 1.03 (s, 3H, CH_3-19), 0.95 (d, 3H, J = 7.0 Hz, CH_3-21), 0.79 (s, 3H, CH_3-18), 0.78 (d, 3H, J = 6.4 Hz, CH_3-27). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 140.4, 121.5, 109.6, 104.8 (C-1^{Ara}), 100.6 (C-1^{Rha}), 99.4 (C-1^{Gal}), 83.7, 80.9, 77.9, 74.4, 73.9, 71.1, 71.0, 70.4, 68.9, 68.3, 68.1, 66.8, 65.7, 61.8, 61.1, 56.4, 50.0, 41.5, 40.2, 39.6, 38.1, 37.1, 36.8, 31.9, 31.5, 31.3, 31.1, 30.1, 29.5, 28.5, 20.7, 19.0, 17.1, 16.8, 16.1, 14.2. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{70}O_{16}$+NH_4^+): 872.5002; found *m*/*z*: 872.5012.

Tigogenyl α-L-rhamnopyranosyl-(1→2)-[α-D-lyxopyranosyl-(1→3)]-β-D-galactopyranoside (4a)

This was prepared from **12a** (70 mg) to yield **4a** (41.6 mg, 96%) as a white foamy solid. $[\alpha]_D^{20}$ –43.0 (*c* 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, CD_3OD): δ 5.08 (s, 1H, H-1^{Rha}), 4.78 (d, 1H, H-1^{Lyx}, overlapped by CD_3OD), 4.48 (d, 1H, J = 6.0 Hz, H-1^{Gal}), 4.36 (q, 1H, J = 7.0 Hz, H-16), 4.13 (m, 1H, H-5^{Rha}), 4.05 (s, 1H), 3.94 (br s, 1H), 3.80–3.67 (m, 8H), 3.63 (dd, 1H, J = 9.0, 3.0 Hz), 3.57 (dd, 1H, J = 9.6, 3.0 Hz), 3.46 (t, 1H, J = 6.0 Hz), 3.43 (dd, 1H, J = 10.9, 3.6 Hz, H-26a), 3.37 (t, 1H, J = 9.5 Hz), 3.30 (t, 1H, J = 10.9 Hz, H-26b), 1.20 (d, 3H, J = 6.1 Hz, H-6^{Rha}), 0.93 (d, 3H, J = 7.0 Hz, CH_3-21), 0.84 (s, 3H, CH_3-19), 0.77 (d, 3H, J = 6.7 Hz, CH_3-27), 0.76 (s, 3H, CH_3-18). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 109.5, 100.9 (C-1^{Rha}), 99.3 (C-1^{Gal}), 97.1 (C-1^{Lyx}), 80.9, 78.8, 73.9, 73.3, 72.6, 71.0 (2C), 70.1, 69.2, 68.3, 68.1, 66.8, 65.0, 64.2, 61.9, 61.1, 56.2, 54.3, 44.5, 41.5, 40.5, 39.9, 36.9, 35.6, 34.9, 33.7, 32.1, 31.5, 31.1, 30.1, 29.1, 28.5 (2C), 20.9, 17.0, 16.8, 16.3, 14.1, 11.9. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{72}O_{16}$+NH_4^+): 874.5159; found *m*/*z*: 874.5168.

Tigogenyl α-L-rhamnopyranosyl-(1→2)-[β-D-ribopyranosyl-(1→3)]-β-D-galactopyranoside (4b)

This was prepared from **12b** (70 mg) to yield **4b** (41.2 mg, 94%) as a white foamy solid. $[\alpha]_D^{20}$ –92.0 (*c* 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, 3:1

$CDCl_3/CD_3OD$): δ 5.05 (s, 1H, H-1^{Rha}), 4.92 (d, 1H, J = 3.9 Hz, H-1^{Rib}), 4.48 (d, 1H, J = 7.7 Hz, H-1^{Gal}), 4.39 (q, 1H, J = 7.4 Hz, H-16), 4.10 (m, 1H, H-5^{Rha}), 4.01 (d, 1H, J = 2.9 Hz), 3.98–3.92 (m, 2H), 3.88 (dd, 1H, J = 3.0, 1.4 Hz), 3.83–3.72 (m, 5H), 3.70 (dd, 1H, J = 11.7, 5.2 Hz), 3.67–3.62 (m, 3H), 3.52–3.45 (m, 2H), 3.39 (t, 1H, J = 9.6 Hz), 3.35 (t, 1H, J = 11.4 Hz, H-26b), 1.27 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 1.04 (s, 3H, CH_3-19), 0.98 (d, 3H, J = 7.0 Hz, CH_3-21), 0.81 (s, 3H, CH_3-18), 0.81 (d, 3H, J = 6.6 Hz, CH_3-27). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 109.5, 102.9 (C-1^{Rib}), 100.9 (C-1^{Rha}), 99.2 (C-1^{Gal}), 82.8, 80.9, 74.9, 74.1, 72.6, 71.1, 70.8, 70.4, 69.1, 68.8, 68.4, 67.0, 66.8, 64.0, 61.9, 61.2, 56.2, 54.3, 44.5, 41.5, 40.5, 39.9, 36.9, 35.6, 34.9, 33.6, 32.2, 31.5, 31.1, 30.1, 29.1, 28.6, 28.5, 20.9, 17.1, 16.8, 16.3, 14.1, 11.9. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{72}O_{16}$+NH_4^+): 874.5159; found m/z: 874.5169.

Tigogenyl α-L-rhamnopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-galacto pyranoside (4c)

Prepared from **12c** (70 mg) to yield **4c** (39.9 mg, 92%) as a white foamy solid. $[\alpha]_D^{20}$ –69.0 (c 0.1, 2:3 $CHCl_3/CH_3OH$); ^{1}H NMR (600 MHz, 3:1 $CDCl_3/CD_3OD$): δ 5.17 (s, 1H, H-1^{Rha}), 4.47 (d, 1H, J = 7.8 Hz, H-1^{Gal}), 4.40 (d, 1H, J = 7.0 Hz, H-1^{Ara}), 4.39 (q, 1H, J = 7.2 Hz, H-16), 4.12 (m, 1H, H-5^{Rha}), 4.06 (d, 1H, J = 2.9 Hz), 3.91 (dd, 1H, J = 3.2, 1.6 Hz), 3.88 (dd, 1H, J = 12.4, 2.7 Hz), 3.85 (m, 1H), 3.78 (dd, 1H, J = 9.4, 7.8 Hz, H-2^{Gal}), 3.76–3.69 (m, 4H), 3.68–3.63 (m, 2H), 3.61 (dd, 1H, J = 9.0, 7.0 Hz, H-2^{Ara}), 3.55 (d, 1H, J = 11.9 Hz), 3.53 (dd, 1H, J = 9.1, 3.3 Hz), 3.49 (t, 1H, J = 6.0 Hz), 3.46 (m, 1H, H-26a), 3.40 (t, 1H, J = 9.6 Hz), 3.33 (t, 1H, J = 10.9 Hz, H-26b), 1.24 (d, 3H, J = 6.2 Hz, H-6^{Rha}), 0.96 (d, 3H, J = 7.0 Hz, CH_3-21), 0.85 (s, 3H, CH_3-19), 0.80 (d, 3H, J = 6.3 Hz, CH_3-27), 0.78 (s, 3H, CH_3-18). ^{13}C NMR (150 MHz, 2:1 $CDCl_3/CD_3OD$): δ 109.5, 104.8 (C-1^{Ara}), 100.7 (C-1^{Rha}), 99.1 (C-1^{Gal}), 83.7, 80.9, 74.5, 73.9, 72.7, 72.6, 71.1, 71.0, 70.4, 69.0, 68.3, 68.1, 66.8, 65.7, 61.9, 61.2, 56.2, 54.3, 44.5, 41.5, 40.5, 39.9, 36.9, 35.6, 34.9, 33.6, 32.2, 31.4, 31.1, 30.1, 29.1, 28.6, 28.5, 20.9, 17.0, 16.8, 16.3, 14.2, 11.9. ESI-HRMS (positive ion): Calcd for ($C_{44}H_{72}O_{16}$+NH_4^+): 874.5159; found m/z: 874.5176.

Cell Culture Assays

BEL-7402 and MCF-7 cells were grown in GIBCO RPMI Media 1640 supplemented with 10% fetal bovine serum. All cells were incubated at 37°C under 5% CO_2. The antiproliferative activities of saponins **1**–**4** were assessed by means of the Cell Counting Kit-8 (CCK-8) assay (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's protocol. Briefly, 96-well plates were seeded with 2 × 10^4 cells/mL and incubated overnight to allow cells to attach to the plate surface. Cells were then exposed to serially diluted solutions (0–40 μM) of **1**–**4** (to get a final volume of 100 μL) for 48 h. Then, 10 μL of CCK-8 was added into each well. After incubation at 37°C for 3 h, the

absorbance value (OD value) at 450 nm was determined by a PE EnSpire 2300 multimode reader. The rate of cell growth inhibition was calculated according to the following equation:

$$\text{Cell growth inhibition rate} = [1 - (OD_{\text{treated}} - OD_{\text{blank}})/ (OD_{\text{untreated}} - OD_{\text{blank}})] \times 100\% \quad (1)$$

The IC_{50} value was determined by a modified Kou-type method:[17]

$$\lg IC_{50} = X_m - I[P - (3 - P_m - Pn)/4],$$

where X_m is the logarithmic maximum concentration, I is the logarithmic maximum concentration/adjacent concentration, P is the sum of the positive response rate, P_m is the largest positive response rate, and P_n is the smallest positive response rate. Each sample was tested in triplicate.

FUNDING

This work was supported by grants from the Major State Basic Research Development Program of China (973 Program, No. 2012CB822102), the National Major Scientific and Technological Special Project for Significant New Drugs Development (No. 2012ZX09502001-005), and the Shandong Provincial Natural Science Foundation (No. ZR2010BQ004).

SUPPLEMENTARY DATA

Supplementary data including ^1H NMR, ^{13}C NMR, and ESI-MS spectra of **5, 10–12,** and **2–4** are available from the corresponding author.

REFERENCES

1. Yahara, S.; Nakamura, T.; Someya, Y.; Matsumoto, T., Yamashita, T.; Nohara, T. Steroidal glycoside, indioside A–E, from *Solanum indicum*. *Phytochemistry* **1996**, *43*, 1319–1323.

2. Gu, G.; Du, Y.; Linhardt, R.J. Facile synthesis of saponins containing 2,3-branched oligosaccharides by using partially protected glycosyl donors. *J. Org. Chem.* **2004**, *69*, 5497–5500.

3. Gao, J.; Li, X.; Gu, G.; Sun, B.; Cui, M.; Ji, M.; Lou, H.-X. Efficient synthesis of trisaccharide saponins and their tumor cell killing effects through oncotic necrosis. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 622–627.

4. Mimaki, Y.; Yokosuka, A.; Kuroda, M.; Sashida, Y. Cytotoxic activities and structure-cytotoxic relationship of steroidal saponins. *Biol. Pharm. Bull.* **2001**, *24*, 1286–1289.

5. Li, M.; Han, X.; Yu, B. Synthesis of monomethylated dioscin derivatives and their antitumor activities. *Carbohydr. Res.* **2003**, *338*, 117–121.

6. Zhu, S.; Zhang, Y.; Li, M.; Yu, J.; Zhang, L.; Li, Y.; Yu, B. Synthesis and cytotoxicities of dioscin derivatives with decorated chacotriosyl residues. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5629–5632.

7. Chang, L.C.; Tsai, T.R.; Wang, J.J.; Lin, C.N.; Kuo, K.W. The rhamnose moiety of solamargine plays a crucial role in triggering cell death by apoptosis. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 21.

8. Fang, M.; Gu, L.; Gu, G.; Fang, J. Facile synthesis and antitumor activities of timosaponin AIII and its analogs. *J. Carbohydr. Chem.* **2012**, *31*, 187–202.

9. Pellissier, H. The glycosylation of steroids. *Tetrahedron* **2004**, *60*, 5123–5162.

10. Yu, B.; Zhang, Y.; Tang, P. Carbohydrate chemistry in the total synthesis of saponins. *Eur. J. Org. Chem.* **2007**, 5145–5161.

11. Li, Y.; Yang, X.; Liu, Y.; Zhu, C.; Yang, Y.; Yu, B. Gold(I)-catalyzed glycosylation with glycosyl ortho-alkynylbenzoates as donors: general scope and application in the synthesis of a cyclic triterpene saponin. *Chem. Eur. J.* **2010**, *16*, 1871–1882.

12. Xiao, G.; Yu, B. Total synthesis of starfish saponin Goniopectenoside B. *Chem. Eur. J.* **2013**, *19*, 7708–7712.

13. Adak, S.; Emmadi, M.; Kulkarni, S.S. Synthesis of the trisaccharide moiety and a cholesteryl analog of phyteumosides. *RSC Adv.* **2014**, *4*, 7611–7616.

14. Gu, G.; An, L.; Fang, M.; Guo, Z. Efficient one-pot synthesis of tigogenin saponins and their antitumor activities. *Carbohydr. Res.* **2014**, *383*, 21–26.

15. Du, Y.; Gu, G.; Wei, G.; Hua, Y.; Linhardt, R.J. Synthesis of saponins using partially protected glycosyl donors. *Org. Lett.* **2003**, *5*, 3627–3630.

16. Shi, C.-S.; Kehrl, J.H. Pyk2 amplifies epidermal growth factor and c-Src-induced Stat3 activation. *J. Biol. Chem.* **2004**, *279*, 17224–17231.

17. Peng, Z.R.; Zhong, W.H.; Liu, J.; Xiao, P.T. Effects of the combination of hyperbaric oxygen and 5-fluorouracil on proliferation and metastasis of human nasopharyngeal carcinoma CNE-2Z cells. *Undersea Hyperb. Med.* **2010**, *37*, 141–150.