Bioorganic & Medicinal Chemistry 20 (2012) 4522–4531

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Effect of C-ring modifications on the cytotoxicity of spirostan saponins and related glycosides

Karell Pérez-Labrada [a,b], Ignacio Brouard [b,*], Sara Estévez [c], María Teresa Marrero [c], Francisco Estévez [c], Daniel G. Rivera [d,*]

[a] Institute of Pharmacy and Food, University of Havana, San Lázaro y L, 10400 La Habana, Cuba
[b] Instituto de Productos Naturales y Agrobiología-C.S.I.C., Av. Astrofísico F. Sánchez 3, 38206 La Laguna, Tenerife, Spain
[c] Department of Biochemistry, Molecular Biology and Physiology, University of Las Palmas de Gran Canaria, Instituto Canario de Investigación del Cáncer, Plaza Dr. Pasteur s/n, 35016 Las Palmas de Gran Canaria, Spain
[d] Center for Natural Products Study, Faculty of Chemistry, University of Havana, Zapata y G, 10400 La Habana, Cuba

ARTICLE INFO

Article history:
Received 20 February 2012
Revised 3 May 2012
Accepted 9 May 2012
Available online 17 May 2012

Keywords:
Steroids
Saponins
Glycosides
Cytotoxicity
HL-60 cells

ABSTRACT

Twelve C-ring modified spirostanyl glycosides were synthesized and evaluated for their cytotoxicity against the human myeloid leukemia cell line (HL-60). With the aim of assessing the influence of the hydrophobic character, the conformational flexibility and the stereochemistry of the C-ring functionalities on the cytotoxic activity, a variety of spirostanic aglycones incorporating methylene, methoxyl, α,β-unsaturated ketone and lactone groups were subjected to a linear glycosylation strategy leading to glycosides derived from the 3,6-dipivaloylated β-D-glucoside and the β-chacotrioside moieties. The 3,6-dipivaloylated spirostanyl β-D-glucosides showed moderate to good cytotoxic activity against HL-60, but no significant cytotoxicity against benign blood cells. However, the cytotoxicity of spirostanyl β-chacotriosides was highly dependent on the nature of the C-ring functional groups of the steroidal aglycones. Actually, the chacotrioside-based saponins either with no functionality or bearing a hydrophobic methylene group at C-12 were the most cytotoxic ones against both HL-60 and benign blood cells. On the other hand, the incorporation of very polar functionalities and the opening of the ring C with the consequent loss of rigidity led to a significant drop in the cytotoxicity against HL-60. These results confirm that spirostanyl β-chacotriosides including very lipophilic aglycones are the most cytotoxic ones among their congeners.

1. Introduction

Dioscin (**1**)[1] and filiasparoside A (**2**)[2] are two important members of the family of spirostan saponins, which show promise as anticancer agents owing to their high cytotoxicity against a variety of cancer cells. In a previous report,[3] we have described the synthesis and cytotoxicity evaluation of a new spirostan saponin incorporating the trisaccharide portion of dioscin—namely β-chacotriose—and the spirostanic aglycone of filiasparoside A, that is, hecogenin. As shown in Figure 1, the new saponin (**3**) comprises a hybrid structure integrating structural elements of the two naturally occurring spirostan saponins. Importantly, we found that this hecogenyl β-chacotrioside **3** exhibits cytotoxic activity as high as that of the natural products utilized as models. It was also found that hecogenyl saponins including other oligosaccharides—like the 2-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside and the 4,6-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside—showed no cytotoxicity at all, thus proving the importance of the α-L-rhamnosyl residues attached at positions 2 and 4 of the inner D-glucose. Interestingly, we discovered that an intermediate of the synthetic route, that is, the dipivaloylated hecogenyl β-glucoside **4**,[3] exhibits very good cytotoxic activity against HL-60 cells, thus providing a new lead compound for further structural optimization with related pivaloylated spirostanyl glucosides.

Herein we report on the synthesis and cytotoxicity evaluation of new spirostan saponins having a variety of structural modifications on ring C of the steroidal skeletons. Accordingly, the focus is posed on assessing the influence of the hydrophobic character, conformational flexibility and stereochemistry of the C-ring functionalities on the cytotoxic activity against HL-60 cells. Additionally, we are interested on determining whether novel pivaloylated spirostanyl glucosides—analogous to compound **4** but with such a new type of functionalities on ring C—show cytotoxicity against such cancer cells.

2. Results and discussion

2.1. Chemical synthesis

Considering that synthetic hecogenyl glycosides including only the β-chacotrioside and the 3,6-dipivaloylated β-glucoside moieties

* Corresponding authors. Tel.: +53 7 8702102; fax: +53 7 8733502.
E-mail addresses: ibrouard@ipna.csic.es (I. Brouard), dgr@fq.uh.cu (D.G. Rivera).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.05.018

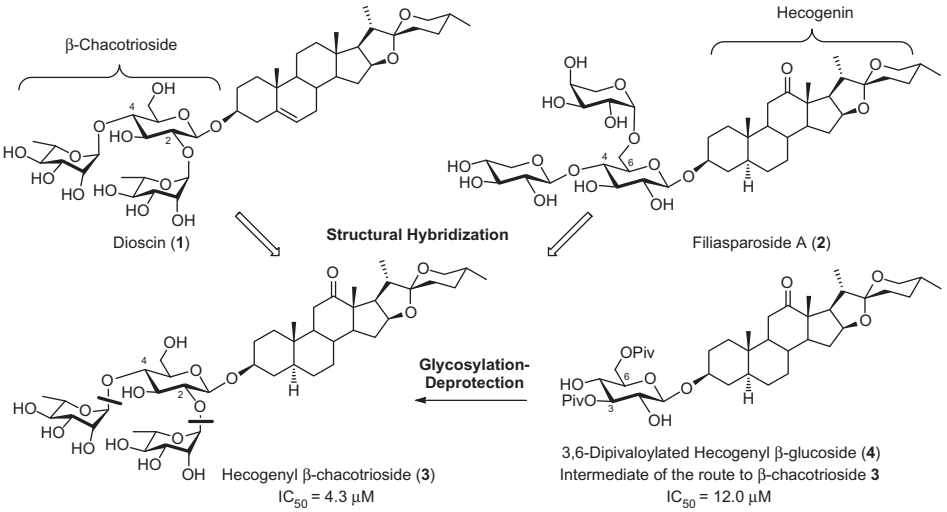
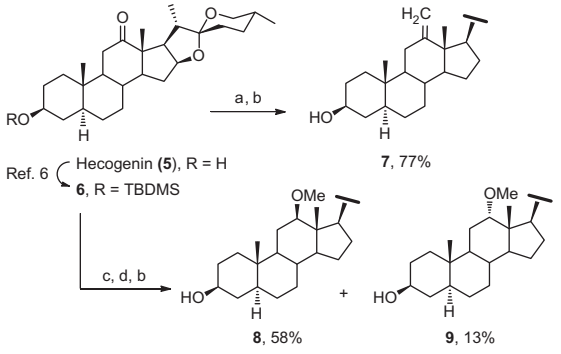


Figure 1. New hecogenyl glycosides with cytotoxic activity against HL-60.

have exhibited cytotoxicity,[3,4] we implemented a linear glycosylation strategy capable to ensure the 1,2-*trans*-glycosidic linkage in all glycosides along the synthetic route.[5] Such a strategy firstly requires the preparation of the individual spirostanic aglycones bearing the desired functionalities on ring C, followed by the stepwise construction of the oligosaccharidic moiety. Importantly, this type of approach also allows accessing the 3,6-dipivaloylated spirostanyl β-glucosides, which are advanced intermediates in the route toward the β-chacotriosides.

As depicted in Scheme 1, hecogenin (**5**) was initially subjected to silylation of its 3β-OH group according to a reported procedure.[6] The resulting TBDMS-hecogenyl derivative **6** was next submitted to a series of transformations aiming to incorporate more or less polar functionalities at position 12. Accordingly, Wittig olefination of **6** with methyltriphenylphosphonium bromide and BuLi as base followed by TBAF-mediated 3β-OH deprotection afforded the methylene-spirostane **7** in 77% yield over two steps. Alternatively, ketone **6** was reduced with $NaBH_4$ in $MeOH/CH_2Cl_2$ to yield a mixture of the 12α- and 12β-spirostanols, which was subjected to methylation without previous separation of their components. The epimeric mixture was then purified by flash column chromatography and the resulting pure compounds were deprotected upon treatment with TBAF in THF to afford the 12β- and 12α-methoxy spirostanes **8** and **9** in 58% and 13% overall yields, respectively.

To assess the influence on the cytotoxicity of the more or less polar nature of the C-ring functionalities of the target glycosides,



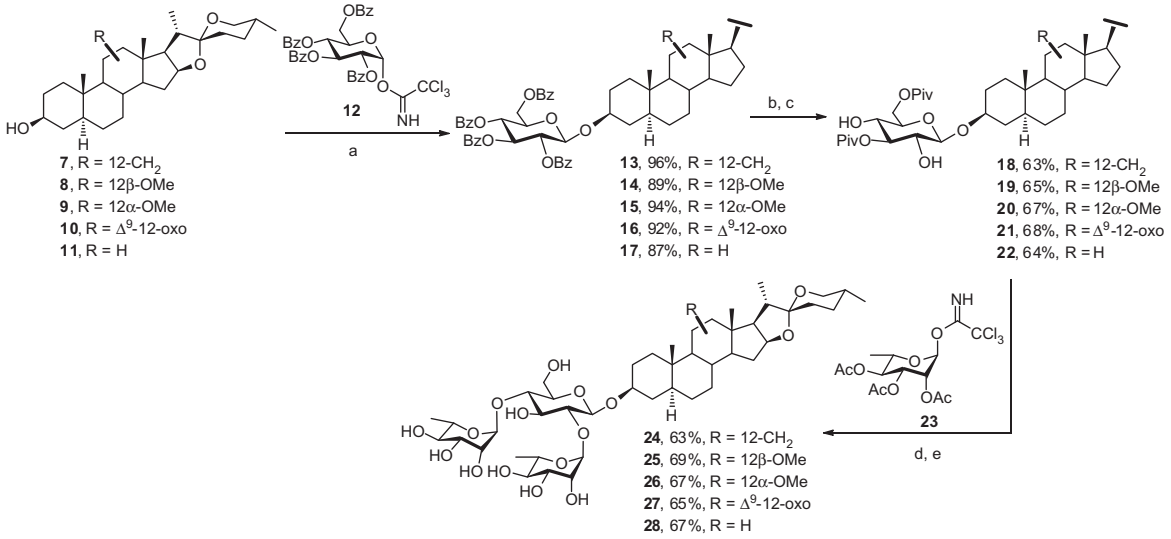
Scheme 1. Synthesis of spirostanic aglycones functionalized on the ring C. Reagents and conditions: (a) $Ph_3CH_3P^+Br$, BuLi, THF; (b) TBAF, THF, 65 °C; (c) $NaBH_4$, CH_2Cl_2/MeOH; (d) MeI, NaH, DMF.

we decided to include in the glycosylation strategies not only the synthetic spirostanes **7**, **8** and **9**, but also the natural sapogenins Δ^9-hecogenin (**10**) and tigogenin (**11**). As shown in Scheme 2, the five spirostanols were subjected to glycosylation with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**12**)[7] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to furnish the 3-*O*-β-glucosides **13**–**17** in 87–96% yield. Global deprotection of the spirostanyl β-glucosides under typical Zemplen conditions (NaOMe/MeOH) followed by selective pivaloylation[8] gave rise to the 3,6-dipivaloylated β-glucosides **18**–**22** in 63–68% yield over two steps. These five glucosides are not only key intermediates of the final saponins, but also important targets for comparison of their cytotoxicity with that of the previously reported[3] 3,6-dipivaloylated hecogenyl glucoside **4**.

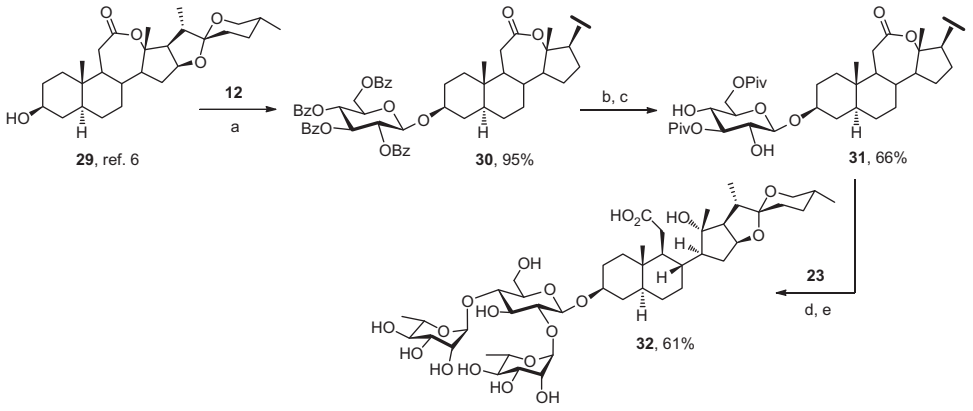
Finally, glucosides **18**–**22** were subjected to double glycosylation with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**23**)[9] according to the Schmidt's inverse procedure,[10] followed by deprotection to give the spirostanyl β-chacotriosides **24**–**28** in 63–69% yield over two steps. As noticed, the overall yield of this synthetic planning was kept over 35% in all cases, similar to those reported for other spirostan saponins.[3,11]

With the five 3,6-dipivaloylated glucosides **13**–**17** and the five spirostanyl saponins **24**–**28** in hands, it is possible to evaluate a set of compounds endowed either with dissimilar functionalities at position 12 (i.e., alkene, methoxyl and α,β-unsaturated ketone) or with no functionality at all (i.e., saponin **28** derived from tigogenin[12]). Such a set of glycosides are well suited for comparison of their cytotoxicity against HL-60 with that of the previously obtained hecogenyl glycosides **3** and **4**. Nevertheless, we were also interested in obtaining spirostanyl glycosides with an opened ring C, thus assessing the influence of such a lack of C-ring rigidity on the cytotoxicity of the resulting seco-compounds. To this end, we planned a route toward a new chacotrioside-based saponin incorporating a seco-spirostanic aglycone arising from the ring opening of a C-ring lactone functionality.

As depicted in Scheme 3, lactone **29**—produced by the Baeyer–Villiger oxidation of the 12-oxo function of hecogenin (**5**) as previously described[6]—was subjected to the linear glycosylation strategy developed for the previous spirostanyl glycosides. Thus, glycosylation of **29** with donor **12** leading to the β-glucoside **30** was followed by deprotection and selective pivaloylation to furnish the key intermediate **31** in 66% over three steps. Subsequent glycosylation with donor **23** followed by simultaneous deprotection and lactone-ring opening led to the



Scheme 2. Synthesis of C-ring modified spirostanyl β-chacotriosides. Reagents and conditions: (a) TMSOTf, 4 Å MS, CH_2Cl_2, rt; (b) NaOMe, MeOH; (c) PivCl, Py, −15 °C→rt; (d) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, −78 °C→rt; (e) aq NaOH, THF/MeOH, 50 °C.



Scheme 3. Synthesis of a seco-spirostanyl β-chacotrioside. Reagents and conditions: (a) TMSOTf, 4 Å MS, CH_2Cl_2, rt; (b) NaOMe, MeOH; (c) PivCl, Py, −15 °C→rt; (d) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, −78 °C→rt; (e) aq NaOH, THF/MeOH, 50 °C, then H^+ to pH 5.

seco-spirostanyl β-chacotrioside **32** in 61% yield. With this final saponin in hand, we can next assess the influence of such a greater conformational flexibility and polarity of ring C on the cytotoxic activity against HL-60 cells, thus having a more detailed picture of the structural factors influencing the bioactivity of this family of compounds.

2.2. Biological studies

Table 1 shows the results of the in vitro cytotoxic activities of 12 spirostanyl glycosides (i.e., six 3,6-dipivaloylated β-D-glucosides and six β-chacotriosides) against the human myeloid leukemia HL-60 cell line, as determined by the 3-[4,5-dimethylthiazol-2-yl-]-2,5-diphenyl tetrazolium bromide (MTT) dye-reduction assay. Accordingly, it was shown that human myeloid leukemia HL-60 cells were highly sensitive to the anti-proliferative effect of all glycosides, with the exception of saponin **32.** It seems obvious that the lower rigidity and lipophilicity of the seco-spirostanic aglycone of **32** compared to those of the traditional (natural or synthetic) spirostan saponins leads to the complete loss of cytotoxicity.

Analysis of Table 1 shows that the 3,6-dipivaloylated spirostanyl glucosides exhibited moderate to good cytotoxic activity, being the Δ^9-hecogenyl β-glucoside **21** the most potent among all analogous glucosides herein produced. Interestingly, the spirostanyl glucoside **31** bearing the lactone functionality in ring C resulted as cytotoxic as glucoside **22**, which is derived from the more lipophilic tigogenin.

Table 1
Cytotoxicity of spirostanyl glycosides against HL-60 cells[a]

3,6-Dipivaloylated glucosides		Chacotrioside-based saponins	
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
18	15.7 ± 1.2	**24**	9.7 ± 2.4
19	19.2 ± 6.2	**25**	21.8 ± 3.9
20	21.5 ± 4.7	**26**	31.5 ± 2.9
21	5.9 ± 0.9	**27**	30.4 ± 10.4
22	8.9 ± 0.7	**28**	2.6 ± 2.1
31	9.9 ± 1.8	**32**	>100

[a] Cells were cultured for 72 h and the IC_{50} values were calculated as described in Section 4.

Saponin **28** displayed the greatest cytotoxicity of all spirostanyl β-chacotriosides, a result that is in agreement with previous reports on the cytotoxic activity of this compound against other cancer cell lines.[12] It must be noticed that the cytotoxicity of the hecogenyl β-chacotrioside **3**—used as model compound—is in the same order of magnitude that those of saponins **28** and **24**, in

which the 12-oxo functionality has been either removed (in **28**) or replaced by a methylene group (in **24**). On the other hand, substitution of the 12-oxo group by other functionalities, like 12-methoxy (**25** and **26**) and Δ^9-12-oxo (**27**), led to five to sixfold decrease in the cytotoxicity against HL-60. In agreement with our previous report,[3] we might conclude that a greater lipophilic character of the aglycone leads to a more potent cytotoxicity of chacotrioside-based spirostan saponins. Of course, this cannot be strictly applied to the case of the 3,6-dipivaloylated spirostanyl glucosides, as we have seen that the cytotoxicity depends not only on the structural features of the aglycone but of the glycoside as a whole. Actually, analysis of Table 1 demonstrates that the effect of the structural variations in ring C on the cytotoxicity is much less pronounced in the case of the 3,6-dipivaloylated spirostanyl glucosides **18**–**22** than in the β-chacotriosides **24**–**28**. This is supported as well by our previous results,[3] in which the 3,6-dipivaloylated glucoside of the less lipophilic 5α-hydroxy-laxogenin turned out to be the most cytotoxic (i.e., IC_{50} = 2.1 ± 0.5 μM) among all tested spirostanyl glycosides. Whereas we have proven that the cytotoxicity mechanism in HL-60 of the 3,6-dipivaloylated glucosides[3] is alike to that of β-chacotrioside saponins, the current results corroborate that the structural factors influencing the cytotoxicity of both types of glycosides are not strictly the same.

In order to assess whether the obtained spirostanyl glycosides were also cytotoxic against non-transformed cell, some of the active compounds were assayed for their cytotoxicity against human peripheral blood mononuclear cells (PBMCs). A comparative study was performed to determine the effect of the 3,6-dipivaloylated glucosides **18**, **19**, **22** and **31** as well as β-chacotriosides **24** and **28** on the viability of human HL-60 cells and PBMCs. As shown in Figure 2, PBMCs were more resistant than HL-60 cells for the 3,6-dipivaloylated glucosides **18**, **19, 22** and **31**. However, it turned out that β-chacotriosides **24** and **28** were also cytotoxic against PBMCs, although the sensitivity of HL-60 cells was higher than that of PBMCs. Thus, the percentage of cell viability was 23 ± 3.8% and 64.2 ± 10% in HL-60 cells and PBMCs, respectively, upon treatment with 30 μM of saponin **24**. A similar trend was observed after treatment of both cells with 10 μM of saponin **28**, as the percentage of cell viability was 17 ± 4.8% and 45 ± 10% in HL-60 cells and PBMCs, respectively.

An important conclusion arises from this study, that is, the 3,6-dipivaloylated spirostanyl glucosides are much more cytotoxic for HL-60 cells than for PBMCs, while the selectivity of cytotoxic spirostanyl β-chacotriosides between blood malign and benign cells is very low. Whereas this result fully agrees with previous reports stating that spirostanyl monosaccharides are less hemolytic than trisaccharides, it is known that those spirostanyl monosaccharides show no cytotoxicity either against cancer cells.[12a] Accordingly, this family of 3,6-dipivaloylated glucosides are the first spirostanyl glycosides exhibiting good cytotoxicity against cancer cells while no significant cytotoxicity against benign blood cells. Indeed, this feature show great promise for the development of cytotoxic agents based on spirostanyl glucoside scaffolds.

3. Conclusions

We have produced a series of 12 C-ring modified spirostanyl glycosides incorporating either the 3,6-dipivaloylated β-glucoside or the β-chacotrioside moiety. The six 3,6-dipivaloylated spirostanyl glucosides showed moderate to good cytotoxic activity, regardless the more or less lipophilic nature of the C-ring functionalities. Some of these spirostanyl glucosides exhibited good selectivity be-

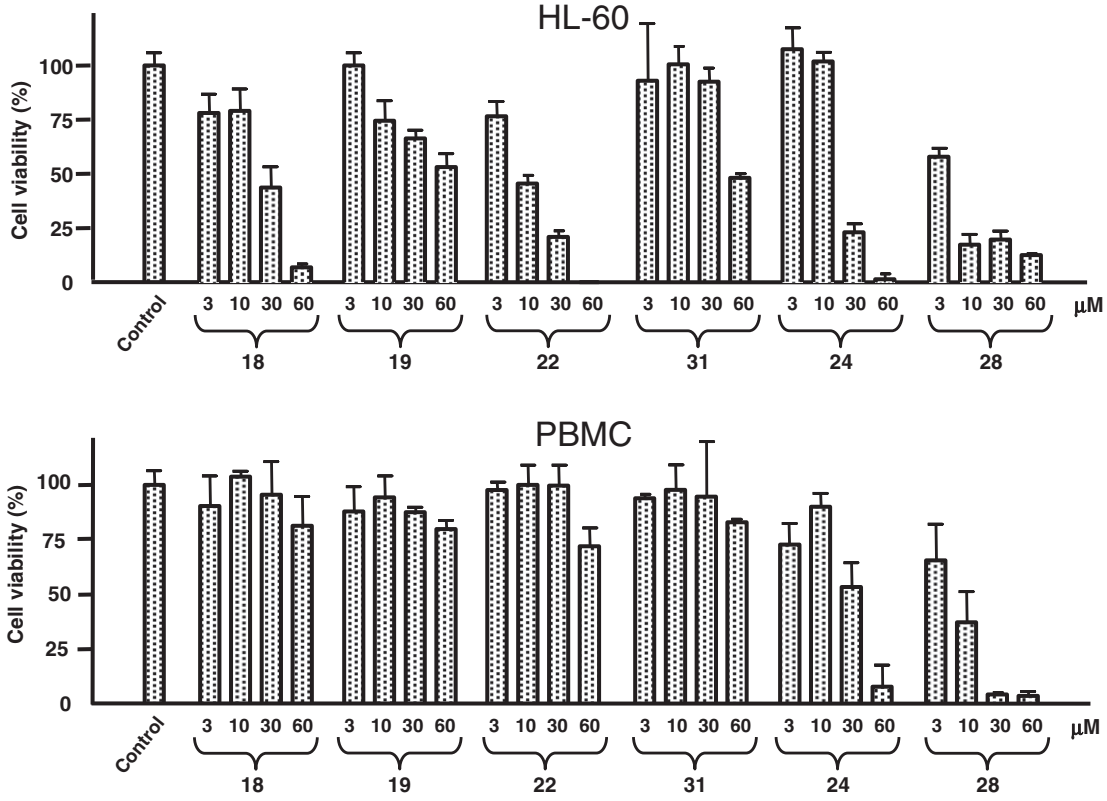


Figure 2. Differential effect of selected saponins (**18**, **19**, **22**, **31**, **24** and **28**) on proliferation of normal PBMCs versus HL-60 cells. Proliferation of HL-60 cells and quiescent PBMCs cultured in presence of the indicated concentrations of the selected saponins for 24 h. Values represent means ± SEs of two independent experiments each performed in triplicate.

tween malign and benign blood cells, which along with their great synthetic availability supports our proposal[3] of considering this family of spirostanyl glycosides as a new type of cytotoxic lead compound. The biological evaluation of the spirostanyl β-chacotriosides confirms that those saponins incorporating very lipophilic aglycones are the most cytotoxic ones among their congeners, although they were also toxic against benign blood cells. In contrast, either the incorporation of more polar functionalities or a lack of rigidity derived from the opening of the fused-ring system leads to a significant drop in the cytotoxicity against HL-60. These results support previous information from our and other groups as well as provide new insights into the structure–cytotoxicity relationship of spirostan saponins and related glycosides.

4. Experimental

4.1. General

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 500 MHz for ^{1}H, 100 and 125 MHz for ^{13}C, respectively. Chemical shifts (δ) are reported in parts per million relative to the residual solvent signals, and coupling constants (J) are reported in hertz. NMR peak assignments were accomplished by analysis of the ^{1}H–1H COSY and HSQC data. High resolution ESI mass spectra were obtained from a Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer, an RF-only hexapole ion guide and an external electrospray ion source. Flash column chromatography was carried out using Silica Gel 60 (230–400 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel aluminum sheets. Hecogenin (**5**), Δ^9-hecogenin (**10**) and tigogenin (**11**) are naturally occurring, commercially available spirostan sapogenins. Compound **6** was synthesized according to a reported procedure.[6] All commercially available chemicals were used without further purification.

4.1.1. 12-Methylene tigogenin (7)

BuLi (2.2 mL, 2.5 M in *n*-hexane) was added to a suspension of the phosphonium salt $CH_3Ph_3P^+Br$ (1.97 g, 5.52 mmol) in THF (40 mL) under nitrogen atmosphere at 0 °C. The mixture was stirred for 1 h at this temperature and treated with a solution of compound **6** (1.0 g, 1.84 mmol) in THF (40 mL). The reaction mixture was stirred for 3 h and then concentrated under reduced pressure to dryness. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with dilute aq HCl (3 × 30 mL), satd aq $NaHCO_3$ (3 × 30 mL) and brine (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 50:1) to afford corresponding methylene–spirostane as a white solid. This pure compound was dissolved in anhydrous THF (30 mL) and treated with tetra-*n*-butyl ammonium fluoride (TBAF, 690 mg, 2.64 mmol). The reaction mixture was stirred at 65 °C for 8 h and then evaporated under reduced pressure to dryness. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with dilute aq HCl (3 × 15 mL), satd aq $NaHCO_3$ (3 × 15 mL) and brine (30 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:1) to afford compound **7** (521 mg, 77%) as a white solid. R_f = 0.31 (*n*-hexane/EtOAc 3:1). Mp: 232–234 °C. $[\alpha]_D^{20}$ −29.3 (*c* 1.4, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3234, 2933, 2885, 1048. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.78 (d, 3H, J = 6.3 Hz, CH_3); 0.85 (s, 3H, CH_3); 0.89 (s, 3H, CH_3); 1.07 (d, 3H, J = 6.9 Hz, CH_3); 2.32 (dd, 1H, J = 8.8/6.6 Hz); 3.37 (t, 1H, J = 10.9 Hz, H-26ax); 3.47 (ddd, 1H, J = 10.9/4.4/2.2 Hz, H-26eq); 3.58 (m, 1H, H-3α); 4.40 (m, 1H, H-16α); 4.55 (s, 1H, C=CH_{2a}); 4.58 (s, 1H, C=CH_{2b}). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.1, 14.1, 17.1, 17.4 (CH_3); 28.5, 28.8 (CH_2); 30.3 (CH); 31.2, 31.3, 31.4, 31.8, 31.9 (CH_2); 34.9 (CH); 35.7 (C); 36.8, 38.0 (CH_2); 41.8, 44.8 (CH); 47.8 (C); 55.6, 56.7, 56.9 (CH); 66.8 (CH_2); 71.1, 80.4 (CH); 103.9 (CH_2); 109.3, 156.6 (C). HRMS (ESI-FT-ICR) m/z: 451.3197 [M+Na]$^+$ (calcd for $C_{28}H_{44}O_3Na$: 451.3188).

4.1.2. 12β-Methoxy tigogenin (8) and 12α-methoxy tigogenin (9)

$NaBH_4$ (503 mg, 13.3 mmol) was added to a solution of compound **6** (4.84 g, 8.89 mmol) in CH_2Cl_2/MeOH (200 mL, 1:1, v/v) at −10 °C. The reaction mixture was stirred at this temperature for 30 min and then treated with acetic acid (500 μL). The volatiles were evaporated under reduced pressure to dryness and the resulting crude product was dissolved in the solvent mixture of dry DMF/THF (15 mL, 2:1, v/v). The stirring solution was cooled to 0 °C and treated with NaH (60% in mineral oil, 355 mg, 8.89 mmol) under nitrogen atmosphere. The suspension was stirred for 10 min under nitrogen atmosphere, treated with CH_3I (0.7 mL, 10.7 mmol) and then stirred at room temperature for additional 10 h. The reaction mixture was poured into 100 mL of cold water and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with aq 10% HCl (3 × 40 mL), satd aq $NaHCO_3$ (3 × 40 mL), brine (50 mL) and then dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 50:1) to furnish the 12β-OMe isomer (3.09 g, 62% yield after two steps) and the 12α-OMe isomer (698 mg, 14% yield).

4.1.3. 12β-Methoxy tigogenin (8)

The 12β-OMe isomer (1.55 g, 2.76 mmol) was deprotected with TBAF (1.44 g, 5.52 mmol) in anhydrous THF (60 mL) in a similar way as described in Section 4.1.1. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded **8** (1.15 g, 93%) as a white solid. R_f = 0.22 (*n*-hexane/EtOAc 3:1). Mp: 193–194 °C. $[\alpha]_D^{20}$ −115.9 (*c* 1.5, $CHCl_3$). IR (KBr, cm^{-1}) ν: 3361, 2933, 2877, 1055. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.73 (s, 3H, CH_3); 0.78 (d, 3H, J = 6.3 Hz, CH_3); 0.82 (s, 3H, CH_3); 0.99 (d, 3H, J = 6.6 Hz, CH_3); 2.75 (dd, 1H, J = 11.0/4.4 Hz, H-12α); 3.31 (s, 3H, OCH_3); 3.36 (t, 1H, J = 11.0 Hz, H-26ax); 3.46 (ddd, 1H, J = 11.0/4.4/2.3 Hz, H-26eq); 3.58 (m, 1H, H-3α); 4.37 (m, 1H, H-16α). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.3, 12.2, 13.4, 17.1 (CH_3); 25.3, 28.5, 28.8 (CH_2); 30.2 (CH); 31.1, 31.3, 31.4, 31.9 (CH_2); 34.2 (CH); 35.7 (C); 36.9, 38.0 (CH_2); 42.4, 44.8 (CH); 45.6 (C); 53.1, 54.9 (CH); 56.8 (CH_3); 62.0 (CH); 66.8 (CH_2); 71.1, 80.6, 89.5 (CH); 109.5 (C). HRMS (ESI-FT-ICR) m/z: 469.3289 [M+Na]$^+$ (calcd for $C_{28}H_{46}O_4Na$: 469.3294).

4.1.4. 12α-Methoxy tigogenin (9)

The 12α-OMe isomer (349 mg, 0.622 mmol) was deprotected with TBAF (324 mg, 1.24 mmol) in anhydrous THF (15 mL) in a similar way as described in Section 4.1.1. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded **9** (253 mg, 91%) as a white solid. R_f = 0.26 (*n*-hexane/EtOAc 3:1). Mp: 191–193 °C. $[\alpha]_D^{20}$ −51.8 (*c* 0.3, $CHCl_3$). IR (KBr, cm^{-1}) ν: 3364, 2933, 2879, 1055. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.77 (s, 3H, CH_3); 0.78 (d, 3H, J = 6.3 Hz, CH_3); 0.81 (s, 3H, CH_3); 0.96 (d, 3H, J = 7.3 Hz, CH_3); 2.51 (dd, 1H, J = 8.8/6.9 Hz); 3.04 (t, 1H, J = 2.6 Hz, H-12β); 3.30 (s, 3H, OCH_3); 3.37 (t, 1H, J = 10.9 Hz, H-26ax); 3.47 (ddd, 1H, J = 10.9/4.2/2.0 Hz, H-26eq); 3.58 (m, 1H, H-3α); 4.36 (m, 1H, H-16α). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.2, 14.5, 17.0, 17.1 (CH_3); 23.5, 28.6, 28.8 (CH_2); 30.3 (CH); 31.4, 31.5, 31.6, 31.9 (CH_2); 35.1 (CH); 35.2 (C); 36.9, 38.2 (CH_2); 41.6 (CH); 44.5 (C); 44.7, 47.7, 48.9, 52.9 (CH); 57.4 (CH_3); 66.8 (CH_2); 71.2, 80.4, 82.2 (CH); 109.2 (C). HRMS (ESI-FT-ICR) m/z: 469.3297 [M+Na]$^+$ (calcd for $C_{28}H_{46}O_4Na$: 469.3294).

4.1.5. 12-Methylene-tigogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (13)

The glucosyl donor **12** (177 mg, 0.239 mmol) and the spirostanic acceptor **7** (78.7 mg, 0.184 mmol) were suspended in dry CH_2Cl_2 (4 mL) in the presence of 4 Å molecular sieves. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 15 min and treated with a solution of TMSOTf (1.8 μL, 0.01 mmol) in CH_2Cl_2 (0.35 mL). The stirring was continued for 1 h and then the reaction was quenched by addition of Et_3N and filtered. The filtrate was evaporated to dryness and the crude product was purified by flash column chromatography (*n*-hexane/EtOAc 6:1) to afford **13** (177.9 mg, 96%) as a white solid. R_f = 0.30 (*n*-hexane/EtOAc 5:1). Mp: 182–183 °C. $[\alpha]_D^{20}$ −6.7 (*c* 1.4, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 2955, 2930, 2874, 1735, 1269, 1096, 1070. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.64 (s, 3H, CH_3); 0.72 (s, 3H, CH_3); 0.81 (d, 3H, *J* = 6.3 Hz, CH_3); 1.10 (d, 3H, *J* = 6.8 Hz, CH_3); 3.38 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.45–3.50 (m, 1H, H-26eq); 3.55–3.64 (m, 1H, H-3α); 4.11–4.17 (m, 1H, H-5 Glc); 4.38–4.46 (m, 1H, H-16α); 4.53 (dd, 1H, *J* = 12.1/5.9 Hz, H-6a Glc); 4.60 (dd, 1H, *J* = 12.1/3.9 Hz, H-6b Glc); 4.77 (d, 1H, *J* = 2.0 Hz, C=CH_{2a}); 4.84 (d, 1H, *J* = 2.1 Hz, C=CH_{2b}); 4.95 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 5.49 (dd, 1H, *J* = 9.8/7.8 Hz, H-2 Glc); 5.63 (t, 1H, *J* = 9.8/9.5 Hz, H-4 Glc); 5.89 (t, 1H, *J* = 9.6 Hz, H-3 Glc); 7.28–7.56 (m, 12H, Ar-*H*); 7.83, 7.90, 7.95, 8.01 (4 × m, 4 × 2H, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.1, 14.1, 17.1, 17.4 (*C*H_3); 28.5, 28.8, 29.7 (*C*H_2); 30.3 (*C*H); 31.2, 31.4, 31.7, 32.2 (*C*H_2); 34.9 (*C*H); 35.7 (*C*); 36.8, 38.0 (*C*H_2); 40.6 (*C*); 41.8, 44.7, 62.2 (*C*H); 63.5, 66.8 (*C*H_2); 70.6, 71.1, 72.5, 72.6, 80.4, 81.2, 100.1 (*C*H); 104.3 (*C*H_2); 109.6 (*C*); 128.6, 128.7, 128.8, 128.9 (*C*H); 129.2, 129.3, 129.9 (*C*); 130.1, 130.2, 133.4, 133.5, 133.6, 133.8 (*C*H); 156.6 (*C*); 165.5, 165.7, 166.3, 166.5 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1029.4763 $[M+Na]^+$ (calcd for $C_{62}H_{70}O_{12}Na$: 1029.4765).

4.1.6. 12β-Methoxy tigogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (14)

The glucosyl donor **12** (192 mg, 0.259 mmol), the spirostanic acceptor **8** (89 mg, 0.199 mmol) and TMSOTf (1.8 μL, 0.01 mmol) were reacted in dry CH_2Cl_2 (4 mL) in a similar way as described in Section 4.1.3. Flash column chromatography purification (*n*-hexane/EtOAc 5:1) afforded **14** (181.6 mg, 89%) as a white solid. R_f = 0.25 (*n*-hexane/EtOAc 5:1). Mp: 158–160 °C. $[\alpha]_D^{20}$ +24.5 (*c* 1.1, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 2930, 2877, 2379, 1730, 1271, 1094, 1071. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.70, 0.71 (2 × s, 2 × 3H, 2 × CH_3); 0.78 (d, 3H, *J* = 6.4 Hz, CH_3); 1.00 (d, 3H, *J* = 6.4 Hz, CH_3); 2.74 (dd, 1H, *J* = 10.8/4.4 Hz, H-12α); 3.33 (s, 3H, OCH_3); 3.37 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.45–3.49 (m, 1H, H-26eq); 3.60 (m, 1H, H-3α); 4.13–4.18 (m, 1H, H-5 Glc); 4.38 (m, 1H, H-16α); 4.53 (dd, 1H, *J* = 11.9/5.8 Hz, H-6a Glc); 4.60 (dd, 1H, *J* = 12.0/3.4 Hz, H-6b Glc); 4.94 (d, 1H, *J* = 7.8 Hz, H-1 Glc); 5.48 (dd, 1H, *J* = 9.7/7.8 Hz, H-2 Glc); 5.63 (t, 1H, *J* = 9.7 Hz, H-4 Glc); 5.90 (t, 1H, *J* = 9.7 Hz, H-3 Glc); 7.25–7.55 (m, 12H, Ar-*H*); 7.83, 7.90, 7.96, 8.02 (4 × m, 4 × 2H, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.3, 12.1, 13.4, 17.1 (*C*H_3); 25.2, 28.4, 28.8, 29.3 (*C*H_2); 30.3 (*C*H); 31.1, 31.4, 31.9 (*C*H_2); 34.2 (*C*H); 34.4 (*C*H_2); 35.7 (*C*); 36.8 (*C*H_2); 42.4, 44.7 (*C*H); 45.6 (*C*); 53.1, 55.0 (*C*H); 56.7 (*C*H_3); 62.1 (*C*H); 63.4, 66.8 (*C*H_2); 70.2, 72.1, 72.2, 73.1, 79.7, 80.5, 89.4, 99.9 (*C*H); 109.5 (*C*); 128.2, 128.3, 128.4 (*C*H); 128.8, 128.9, 129.5 (*C*); 129.7, 129.8, 132.9, 133.0, 133.1, 133.4 (*C*H); 165.0, 165.2, 165.8, 166.0 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1047.4860 $[M+Na]^+$ (calcd for $C_{62}H_{72}O_{13}Na$: 1047.4871).

4.1.7. 12α-Methoxy-tigogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (15)

The glucsosyl donor **12** (375.7 mg, 0.507 mmol), the spirostanic acceptor **9** (174 mg, 0.390 mmol) and TMSOTf (3.5 μL, 0.02 mmol) were reacted in dry CH_2Cl_2 (10 mL) in a similar way as described in Section 4.1.3. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded **15** (375.8 mg, 94%) as a white solid. R_f = 0.37 (*n*-hexane/EtOAc 3:1). Mp: 176–178 °C. $[\alpha]_D^{20}$ +8.2 (*c* 1.1, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 2950, 2929, 2870, 2372, 1736, 1316, 1266, 1178, 1096, 1071. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3H, CH_3); 0.75 (s, 3H, CH_3); 0.78 (d, 3H, *J* = 6.6 Hz, CH_3); 0.97 (d, 3H, *J* = 6.9 Hz, CH_3); 2.51 (dd, 1H, *J* = 8.8/6.9 Hz); 3.03 (t, 1H, *J* = 2.5 Hz, H-12β); 3.33 (s, 3H, OCH_3); 3.37 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.48 (m, 1H, H-26eq); 3.58 (m, 1H, H-3α); 4.16 (m, 1H, H-5 Glc); 4.36 (m, 1H, H-16α); 4.53 (dd, 1H, *J* = 12.0/6.0 Hz, H-6a Glc); 4.60 (dd, 1H, *J* = 11.9/3.4 Hz, H-6b Glc); 4.94 (d, 1H, *J* = 7.9 Hz, H-1 Glc); 5.49 (dd, 1H, *J* = 9.8/7.9 Hz, H-2 Glc); 5.63 (t, 1H, *J* = 9.8 Hz, H-4 Glc); 5.90 (t, 1H, *J* = 9.8 Hz, H-3 Glc); 7.25–7.43 (m, 9H, Ar-*H*); 7.47–7.55 (m, 3H, Ar-*H*); 7.83, 7.90, 7.95, 8.01 (4 × m, 4 × 2H, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.0, 14.5, 17.0, 17.1 (*C*H_3); 23.4, 28.5, 28.8, 29.3 (*C*H_2); 30.3 (*C*H); 31.4, 31.5, 31.7, 34.6 (*C*H_2); 35.0 (*C*H); 35.2 (*C*); 36.6 (*C*H_2); 41.6, 44.4 (*C*H); 44.5 (*C*); 47.6, 48.8, 53.0 (*C*H); 57.3 (*C*H_3); 63.4, 66.8 (*C*H_2); 70.2, 72.1, 72.2, 73.1, 79.9, 80.4, 82.2, 100.1 (*C*H); 109.1 (*C*); 128.1, 128.2, 128.3, 128.4 (*C*H); 128.8, 128.9, 129.5 (*C*); 129.7, 129.8, 132.9, 133.0, 133.1, 133.3 (*C*H); 165.0, 165.2, 165.8, 166.0 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1047.4863 $[M+Na]^+$ (calcd for $C_{62}H_{72}O_{13}Na$: 1047.4871).

4.1.8. Δ⁹-Hecogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (16)

The glucosyl donor **12** (189.7 mg, 0.256 mmol), the spirostanic acceptor **10** (84.4 mg, 0.197 mmol) and TMSOTf (1.8 μL, 0.01 mmol) were reacted in dry CH_2Cl_2 (4 mL) in a similar way as described in Section 4.1.3. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded **16** (182.5 mg, 92%) as a white solid. R_f = 0.27 (*n*-hexane/EtOAc 3:1). Mp: 195–197 °C. $[\alpha]_D^{20}$ +24.8 (*c* 1.5, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 2930, 2870, 2371, 1736, 1266, 1096, 1071. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.79 (d, 3H, *J* = 6.3 Hz, CH_3); 0.90 (s, 3H, CH_3); 0.95 (s, 3H, CH_3); 1.10 (d, 3H, *J* = 6.9 Hz, CH_3); 2.16 (m, 1H); 2.38 (dd, 1H, *J* = 8.8/7.3 Hz); 2.42–2.47 (m, 1H); 3.35 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.47–3.50 (m, 1H, H-26eq); 3.55 (m, 1H, H-3α); 4.17 (m, 1H, H-5 Glc); 4.38 (m, 1H, H-16α); 4.55 (dd, 1H, *J* = 12.0/6.0 Hz, H-6a Glc); 4.59 (dd, 1H, *J* = 12.0/3.5 Hz, H-6b Glc); 4.92 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 5.48 (dd, 1H, *J* = 9.8/7.9 Hz, H-2 Glc); 5.61 (m, 2H, H-4 Glc, H-11); 5.89 (t, 1H, *J* = 9.7 Hz, H-3 Glc); 7.27–7.44 (m, 9H, Ar-*H*); 7.48–7.58 (m, 3H, Ar-*H*); 7.82 (dd, 2H, *J* = 8.5/1.3 Hz, Ar-*H*); 7.90 (dd, 2H, *J* = 8.3/1.1 Hz, Ar-*H*); 7.95 (dd, 2H, *J* = 8.2/1.3 Hz, Ar-*H*); 8.01 (dd, 2H, *J* = 8.5/1.3 Hz, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.1, 15.0, 17.1, 18.3 (*C*H_3); 27.5, 28.7, 29.1 (*C*H_2); 30.2 (*C*H); 31.3, 31.4, 32.4, 34.4, 34.5 (*C*H_2); 36.7 (*C*H); 39.2 (*C*); 42.3, 42.4 (*C*H); 51.0 (*C*); 52.4, 53.7 (*C*H); 63.3, 66.9 (*C*H_2); 70.0, 72.1, 72.9, 79.4, 79.7, 100.4 (*C*H); 109.4 (*C*); 119.9, 128.2, 128.3, 128.4 (*C*H); 128.7, 128.8, 129.4, 129.5 (*C*); 129.6, 129.7, 129.8, 133.1, 133.2, 133.3, 133.4 (*C*H); 165.0, 165.2, 165.8, 166.0 (*C*=O); 170.8 (*C*); 204.9 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1029.4384 $[M+Na]^+$ (calcd for $C_{61}H_{66}O_{13}Na$: 1029.4401).

4.1.9. Tigogenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (17)

The glucosyl donor **12** (1.04 g, 1.40 mmol), the spirostanic acceptor **11** (420 mg, 1.01 mmol) and TMSOTf (9.1 μL, 0.051 mmol) were reacted in dry CH_2Cl_2 (20 mL) in a similar way as described in Section 4.1.3. Flash column chromatography purification (*n*-hexane/EtOAc 4:1→2:1) afforded **17** (874.5 mg, 87%) as a white solid. R_f = 0.29 (*n*-hexane/EtOAc 4:1). Mp: 143–145 °C. $[\alpha]_D^{20}$ +13.6 (*c* 1.1, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 2930, 2874, 1736, 1269, 1095, 1071. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.69 (s, 3H, CH_3); 0.73 (s, 3H, CH_3); 0.78 (d, 3H, *J* = 6.3 Hz, CH_3); 0.95 (d, 3H, *J* = 6.9 Hz, CH_3); 3.37 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.47 (m, 1H, H-26eq); 3.59 (m, 1H, H-3α); 4.15 (m, 1H, H-5 Glc); 4.38 (m, 1H, H-16α); 4.52 (dd, 1H, *J* = 12.0/6.0 Hz, H-6a Glc); 4.60 (dd, 1H, *J* = 12.0/3.5 Hz, H-6b Glc);

4.94 (d, 1H, J = 7.6 Hz, H-1 Glc); 5.48 (dd, 1H, J = 9.6/7.9 Hz, H-2 Glc); 5.62 (t, 1H, J = 9.8 Hz, H-4 Glc); 5.89 (t, 1H, J = 9.6 Hz, H-3 Glc); 7.27–7.44 (m, 9H, Ar-*H*); 7.48–7.55 (m, 3H, Ar-*H*); 7.83, 7.90, 7.95, 8.01 (4 × m, 4 × 2H, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.2, 14.5, 16.4, 17.1 (CH_3); 21.0, 28.5, 28.8, 29.3 (CH_2); 30.3 (*C*H); 31.4, 31.7, 32.2, 34.6 (CH_2); 35.0 (*C*H); 35.6 (*C*); 36.8, 40.1 (CH_2); 40.6 (*C*); 41.6, 44.7, 62.2 (*C*H); 63.5, 66.8 (CH_2); 70.2, 72.1, 72.2, 73.1, 80.0, 80.8, 100.0 (*C*H); 109.2 (*C*); 128.1, 128.2, 128.3, 128.4 (*C*H); 128.8, 128.9, 129.5 (*C*); 129.7, 129.8, 133.0, 133.1, 133.2, 133.4 (*C*H); 165.0, 165.3, 165.8, 166.1 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1017.4747 $[M+Na]^+$ (calcd for $C_{61}H_{70}O_{12}Na$: 1017.4765).

4.1.10. 12-Methylene-tigogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (18)

NaOMe was added to a solution of compound **13** (157.9 mg, 0.157 mmol) in CH_2Cl_2/MeOH (10 mL, 1:1, v/v) until pH was set to 9–10. The reaction mixture was stirred overnight at room temperature, and then neutralized with acid resin Dowex-50 (H^+) and filtered. The filtrate was concentrated under reduced pressure and the residue was washed with Et_2O (5 × 10 mL) and dried in vacuo to furnish the corresponding β-D-glucoside. This product was dissolved in anhydrous pyridine (5 mL) and the solution was cooled to −15 °C under nitrogen atmosphere. Pivaloyl chloride (97 μL, 0.785 mmol) was added dropwise and the reaction course was monitored by TLC. The stirring was continued at room temperature until the disappearance of the intermediate. The mixture was then diluted with EtOAc (25 mL) and washed with dilute aq HCl, satd aq $NaHCO_3$ and brine. The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 5:1) to afford **18** (75.1 mg, 63%) as a white solid. R_f = 0.26 (*n*-hexane/EtOAc 5:1). Mp: 115–116 °C. $[\alpha]_D^{20}$ −33.1 (*c* 1.3, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3450, 2955, 2933, 2875, 1721, 1287, 1165, 1074, 1050. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.76 (s, 3H, CH_3); 0.80 (d, 3H, J = 6.3 Hz, CH_3); 1.07 (s, 3H, CH_3); 1.10 (d, 3H, J = 6.6 Hz, CH_3); 1.21, 1.22 (2 × s, 2 × 9H, 2 × (CH_3)$_3$C); 3.37 (t, 1H, J = 11.0 Hz, H-26ax); 3.43–3.52 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.54–3.59 (m, 1H, H-5 Glc); 3.62–3.67 (m, 1H, H-3α); 4.25 (dd, 1H, J = 11.7/6.6 Hz, H-6a Glc); 4.40–4.46 (m, 3H, H-16α, H-6b Glc, H-1 Glc); 4.60 (d, 1H, J = 1.6 Hz, C=CH_{2a}); 4.77 (d, 1H, J = 1.9 Hz, C=CH_{2b}); 4.85 (t, 1H, J = 9.4 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.4, 12.1, 12.8, 13.9, 27.0, 27.1 (CH_3); 28.6, 28.8, 29.3 (CH_2); 30.3, 30.4 (*C*H); 31.1, 31.4, 31.6 (CH_2); 32.2 (*C*H); 33.8, 36.7, 37.8 (CH_2); 38.8, 39.0 (*C*); 41.8, 44.6 (*C*H); 46.6 (*C*); 53.5, 53.8, 56.8, 58.2 (*C*H); 63.6, 66.8 (CH_2); 70.1, 72.0, 74.2, 76.1, 78.0, 79.4, 81.1, 101.1 (*C*H); 104.9 (CH_2); 109.2, 155.7 (*C*); 178.6, 180.3 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 781.4872 $[M+Na]^+$ (calcd for $C_{44}H_{70}O_{10}Na$: 781.4867).

4.1.11. 12β-Methoxy-tigogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (19)

Glucoside **14** (158.6 mg, 0.155 mmol) was deprotected with NaOMe in CH_2Cl_2/MeOH (10 mL, 1:1, v/v) in a similar way as described in Section 4.1.8. The resulting product was selectively protected with pivaloyl chloride (95 μL, 0.775 mmol) in anhydrous pyridine (5 mL) according to the procedure described in Section 4.1.8. Flash column chromatography purification (*n*-hexane/EtOAc 4:1) afforded **19** (78.3 mg, 65%) as a white solid. R_f = 0.29 (*n*-hexane/EtOAc 4:1). Mp: 142–144 °C. $[\alpha]_D^{20}$ −73.6 (*c* 1.4, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3454, 2932, 2874, 2379, 1722, 1164, 1083, 1049. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.73 (s, 3H, CH_3); 0.78 (d, 3H, J = 6.6 Hz, CH_3); 0.82 (s, 3H, CH_3); 1.00 (d, 3H, J = 6.6 Hz, CH_3); 1.21, 1.24 (2 × s, 2 × 9H, 2 × (CH_3)$_3$C); 2.76 (dd, 1H, J = 11.0/4.4 Hz, H-12α); 3.32 (s, 3H, OCH_3); 3.36 (t, 1H, J = 11.0 Hz, H-26ax); 3.44–3.47 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.56 (m, 1H, H-5 Glc); 3.62 (m, 1H, H-3α); 4.25 (dd, 1H, J = 12.0/6.6 Hz, H-6a Glc); 4.39 (m, 1H, H-16α); 4.43 (dd, 1H, J = 11.7/2.5 Hz, H-6b Glc); 4.44 (d, 1H, J = 7.6 Hz, H-1 Glc); 4.85 (t, 1H, J = 9.1 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.3, 12.2, 13.4, 17.1 (CH_3); 25.3 (CH_2); 27.0, 27.1 (CH_3); 28.6, 28.8, 29.3 (CH_2); 30.3 (*C*H); 31.1, 31.3, 31.9 (CH_2); 34.2 (*C*H); 34.3 (CH_2); 35.9 (*C*); 36.9 (CH_2); 38.8, 39.0 (*C*); 42.4, 44.8 (*C*H); 45.6 (*C*); 53.1, 54.9 (*C*H); 56.8 (CH_3); 62.0 (*C*H); 63.5, 66.8 (CH_2); 70.1, 72.1, 74.2, 78.0, 78.7, 80.6, 89.4, 100.8 (*C*H); 109.5 (*C*); 178.6, 180.2 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 799.4968 $[M+Na]^+$ (calcd for $C_{44}H_{72}O_{11}Na$: 799.4972).

4.1.12. 12α-Methoxy-tigogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (20)

Glucoside **15** (342.3 mg, 0.334 mmol) was deprotected with NaOMe in CH_2Cl_2/MeOH (20 mL, 1:1, v/v) in a similar way as described in Section 4.1.8. The resulting product was selectively protected with pivaloyl chloride (205 μL, 1.67 mmol) in anhydrous pyridine (8 mL) according to the procedure described in Section 4.1.8. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded **20** (173.9 mg, 67%) as a white solid. R_f = 0.32 (*n*-hexane/EtOAc 3:1). Mp: 167–169 °C. $[\alpha]_D^{20}$ −26.7 (*c* 0.5, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3454, 2931, 2873, 2379, 1724, 1164, 1083, 1050. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.77 (s, 3H, CH_3); 0.78 (d, 3H, J = 6.3 Hz, CH_3); 0.81 (s, 3H, CH_3); 0.97 (d, 3H, J = 6.9 Hz, CH_3); 1.21, 1.24 (2 × s, 2 × 9H, 2 × (CH_3)$_3$C); 2.51 (dd, 1H, J = 8.9/6.7 Hz); 3.04 (t, 1H, J = 2.6 Hz, H-12β); 3.30 (s, 3H, OCH_3); 3.37 (t, 1H, J = 10.9 Hz, H-26ax); 3.44–3.49 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.55 (m, 1H, H-5 Glc); 3.61 (m, 1H, H-3α); 4.27 (dd, 1H, J = 12.0/6.6 Hz, H-6a Glc); 4.36 (m, 1H, H-16α); 4.40–4.44 (m, 2H, H-6b Glc, H-1 Glc); 4.84 (t, 1H, J = 9.1 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.2, 14.5, 17.0, 17.1 (CH_3); 23.5 (CH_2); 27.0, 27.1 (CH_3); 28.6, 28.8, 29.3 (CH_2); 30.3 (*C*H); 31.4, 31.5, 31.8, 34.5 (CH_2); 35.1 (*C*H); 35.3 (*C*); 36.7 (CH_2); 38.8, 38.9 (*C*); 41.6, 44.5 (*C*H); 44.6 (*C*); 47.6, 48.9, 52.9 (*C*H); 57.4 (CH_3); 63.5, 66.8 (CH_2); 70.1, 72.0, 74.3, 78.1, 78.8, 80.4, 82.2, 100.8 (*C*H); 109.2 (*C*); 178.6, 180.3 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 799.4970 $[M+Na]^+$ (calcd for $C_{44}H_{72}O_{11}Na$: 799.4972).

4.1.13. Δ^9-Hecogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (21)

Glucoside **16** (165.5 mg, 0.164 mmol) was deprotected with NaOMe in CH_2Cl_2/MeOH (10 mL, 1:1, v/v) in a similar way as described in Section 4.1.8. The resulting product was selectively protected with pivaloyl chloride (100 μL, 0.82 mmol) in anhydrous pyridine (5 mL) according to the procedure described in Section 4.1.8. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) afforded **21** (79.5 mg, 68%) as a white solid. R_f = 0.33 (*n*-hexane/EtOAc 2:1). Mp: 139–140 °C. $[\alpha]_D^{20}$ −8.0 (*c* 1.3, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3452, 2959, 2933, 2877, 1707, 1285, 1167, 1082, 1052. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.79 (d, 3H, J = 6.3 Hz, CH_3); 0.92 (s, 3H, CH_3); 1.07 (s, 3H, CH_3); 1.10 (d, 3H, J = 6.9 Hz, CH_3); 1.21, 1.24 (2 × s, 2 × 9H, 2 × (CH_3)$_3$C); 2.04 (m, 2H); 2.19 (m, 1H); 2.38 (dd, 1H, J = 8.8/6.9 Hz); 2.41 (d, 1H, J = 2.5 Hz); 2.50 (m, 1H); 3.04 (d, 1H, J = 4.7 Hz); 3.35 (t, 1H, J = 10.9 Hz, H-26ax); 3.40–3.43 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.56 (m, 1H, H-5 Glc); 3.61 (m, 1H, H-3α); 4.25 (dd, 1H, J = 12.0/6.6 Hz, H-6a Glc); 4.38 (m, 1H, H-16α); 4.42 (dd, 1H, J = 12.0/2.2 Hz, H-6b Glc); 4.43 (d, 1H, J = 7.6 Hz, H-1 Glc); 4.85 (t, 1H, J = 9.3 Hz, H-3 Glc), 5.68 (d, 1H, J = 1.9 Hz, H-11). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.1, 15.0, 17.1, 18.4, 27.0, 27.1 (CH_3); 27.7, 28.7, 29.2 (CH_2); 30.2 (*C*H); 31.3, 31.5, 32.4, 34.4, 34.7 (CH_2); 36.8 (*C*H); 38.8, 39.0, 39.3 (*C*); 42.4, 42.5 (*C*H); 51.0 (*C*); 52.4, 53.7 (*C*H); 63.6, 66.9 (CH_2); 70.0, 72.1, 74.2, 77.9, 78.3, 79.7, 101.3 (*C*H); 109.4 (*C*); 120.0 (*C*H); 170.7 (*C*); 178.6, 180.2, 204.9 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 781.4500 $[M+Na]^+$ (calcd for $C_{43}H_{66}O_{11}Na$: 781.4503).

4.1.14. Tigogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (22)

Glucoside **17** (758.4 mg, 0.762 mmol) was deprotected with NaOMe in CH_2Cl_2/MeOH (60 mL, 1:1, v/v) in a similar way as

described in Section 4.1.8. The resulting product was selectively protected with pivaloyl chloride (470 μL, 3.81 mmol) in anhydrous pyridine (15 mL) according to the procedure described in Section 4.1.8. Flash column chromatography purification (*n*-hexane/EtOAc 3:1) afforded **22** (364.3 mg, 64%) as a white solid. R_f = 0.29 (*n*-hexane/EtOAc 3:1). Mp: 196–197 °C. $[\alpha]_D^{20}$ −49.5 (*c* 0.95, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3451, 2932, 2873, 1721, 1287, 1241, 1164, 1086, 1051. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.75 (s, 3H, C*H*$_3$); 0.78 (d, 3H, *J* = 6.3 Hz, C*H*$_3$); 0.81 (s, 3H, C*H*$_3$); 0.95 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.21, 1.24 (2 × s, 2 × 9H, 2 × (C*H*$_3$)$_3$C); 3.37 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.44–3.48 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.56 (m, 1H, H-5 Glc); 3.61 (m, 1H, H-3α); 4.25 (dd, 1H, *J* = 12.0/6.6 Hz, H-6a Glc); 4.38 (m, 1H, H-16α); 4.42 (dd, 1H, *J* = 12.0/2.5 Hz, H-6b Glc); 4.44 (d, 1H, *J* = 7.9 Hz, H-1 Glc); 4.85 (t, 1H, *J* = 9.1 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.3, 14.5, 16.5, 17.1 (*C*H$_3$); 21.0 (*C*H$_2$); 27.0, 27.1 (*C*H$_3$); 28.6, 28.8, 29.4 (*C*H$_2$); 30.3 (*C*H); 31.4, 31.7, 32.2, 34.5 (*C*H$_2$); 35.1 (*C*H); 35.7 (*C*); 36.9 (*C*H$_2$); 38.8, 39.0 (*C*); 40.0 (*C*H$_2$); 40.5 (*C*); 41.6, 44.8, 54.3, 56.2, 62.2 (*C*H); 63.6, 66.8 (*C*H$_2$); 70.1, 72.0, 74.2, 78.0, 79.0, 80.8, 100.9 (*C*H); 109.2 (*C*); 178.6, 180.3 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 769.4874 $[M+Na]^+$ (calcd for $C_{43}H_{70}O_{10}Na$: 769.4867).

4.1.15. 12-Methylene-tigogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl) -β-D-glucopyranoside (24)

$BF_3{\cdot}Et_2O$ (130 μL, 1.034 mmol) was added under nitrogen atmosphere to a stirring suspension of acceptor **18** (178.7 mg, 0.235 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (10 mL) at −80 °C. After stirring for 1 h at this temperature, a solution of rhamnopyranosyl trichloroacetimidate **23** (316.9 mg, 0.729 mmol) in CH_2Cl_2 (10 mL) was added and the stirring was continued for 5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (30 mL) and filtered through a pad of Celite. The filtrate was washed with satd aq $NaHCO_3$ (2 × 15 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 2:1) to afford the protected saponin (302 mg, partially unpurified with donor **23**). An aqueous solution of NaOH (1.0 M, 3 mL) was added to a solution of this crude product in THF/MeOH (15 mL, 1:1, v/v) and the reaction mixture was stirred at 50 °C overnight. The solution was neutralized with acid resin Dowex-50 (H^+) and then filtered to remove the resin. The filtrate was concentrated under reduced pressure and the resulting crude product was purified by flash column chromatography ($CHCl_3$/MeOH 5:1) to afford the pure glycoside **24** (130 mg, 63%) as a white solid. R_f = 0.32 ($CHCl_3$/MeOH 5:1). Mp: 159–161 °C. $[\alpha]_D^{20}$ −87.6 (*c* 1.50, C_5H_5N). IR (KBr, cm^{-1}) *v*: 3390, 2929, 1572, 1050. ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.74 (m, 3H, C*H*$_3$); 0.86 (m, 3H, C*H*$_3$); 1.04 (m, 3H, C*H*$_3$); 1.26 (m, 3H, C*H*$_3$); 1.64 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.77 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 3.50–3.75 (m, 3H, H-26ax, H-26eq, H-5 Glc); 3.89–4.03 (m, 1H, H-3α); 4.13 (dd, 1H, *J* = 12.0/3.9 Hz, H-6a Glc); 4.23–4.41 (m, 6H, H-2 Glc, H-3 Glc, H-4 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.55 (dd, 1H, *J* = 3.2/9.5 Hz, H-3 Rha); 4.61 (dd, 1H, *J* = 2.9/9.3 Hz, H-3 Rha′); 4.68–4.72 (m, 2H, H-16α, H-2 Rha); 4.84 (m, 1H, H-2 Rha′); 4.92–5.02 (m, 5H, H-5 Rha, H-5 Rha′, H-1 Glc, C=C*H*$_2$); 5.86 (s, 1H, H-1 Rha); 6.38 (s, 1H, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.0, 14.7, 17.7, 17.8, 18.9, 19.1 (*C*H$_3$); 29.6, 29.8, 30.0, 30.2 (*C*H$_2$); 31.1, 31.2 (*C*H); 32.0, 32.1, 32.4, 32.5 (*C*H$_2$); 32.7, 34.1 (*C*H); 34.2, 34.9 (*C*H$_2$); 35.7 (*C*H); 35.8, 36.9, 37.6, 38.6 (*C*H$_2$); 42.7, 42.9, 44.9, 45.0, 45.1, 46.3 (*C*H); 47.4 (*C*); 48.0, 48.7, 51.6, 54.4, 54.6, 57.6, 57.7, 58.2, 59.5 (*C*H); 62.0, 67.4, 67.7 (*C*H$_2$); 69.6, 69.9, 70.9, 72.8, 72.9, 73.2, 73.3, 74.0, 74.4, 74.6, 74.9 (*C*H); 76.9 (*C*); 77.4, 77.7, 78.1, 78.4, 78.6, 79.5, 81.2, 81.9, 96.4, 100.3, 100.4, 100.5, 102.6, 103.4 (*C*H); 105.6, 106.9 (*C*H$_2$); 109.7, 109.8 (*C*); 121.6 (*C*H); 143.9 (*C*); 156.9 (*C*H). HRMS (ESI-FT-ICR) *m/z*: 905.4871 $[M+Na]^+$ (calcd for $C_{46}H_{74}O_{16}Na$: 905.4875).

4.1.16. 12β-Methoxy-tigogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (25)

Acceptor **19** (176.9 mg, 0.228 mmol) and donor **23** (307.3 mg, 0.707 mmol) in dry CH_2Cl_2 (30 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (127 μL, 1.003 mmol) in a similar way as described in Section 4.1.13. The resulting crude product was purified by flash column chromatography (*n*-hexane/EtOAc 2:1) to afford the protected saponin (431 mg), which was deprotected with NaOH (1.0 M, 5 mL) in THF/MeOH (20 mL, 1:1, v/v) in a similar way as described in Section 4.1.13. Flash column chromatography purification ($CHCl_3$/MeOH 4:1) afforded **25** (141.8 mg, 69%) as a white solid. R_f = 0.35 ($CHCl_3$/MeOH 4:1). Mp: 168–170 °C. $[\alpha]_D^{20}$ −128.9 (*c* 1.4, C_5H_5N). IR (KBr, cm^{-1}) *v*: 3396, 2930, 2373, 1625, 1396, 1044. ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.71 (d, 3H, *J* = 5.7 Hz, C*H*$_3$); 0.87 (s, 3H, C*H*$_3$); 0.94 (s, 3H, C*H*$_3$); 1.27 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.64 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.76 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 2.77 (dd, 1H, *J* = 11.0/4.4 Hz, H-12α); 3.38 (s, 3H, OC*H*$_3$); 3.54 (t, 1H, *J* = 10.6 Hz, H-26ax); 3.60–3.63 (m, 1H, H-26eq); 3.72 (m, 1H, H-5 Glc); 3.94 (m, 1H, H-3α); 4.12 (dd, 1H, *J* = 12.1/3.5 Hz, H-6a Glc); 4.23–4.41 (m, 6H, H-2 Glc, H-3 Glc, H-4 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.54–4.63 (m, 3H, H-3 Rha, H-3 Rha′, H-16α); 4.70 (dd, 1H, *J* = 3.2/1.4 Hz, H-2 Rha); 4.84 (dd, 1H, *J* = 3.3/1.7 Hz, H-2 Rha′); 4.90–4.96 (m, 2H, H-5 Rha, H-5 Rha′); 4.98 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 5.86 (d, 1H, *J* = 1.7 Hz, H-1 Rha); 6.38 (d, 1H, *J* = 1.5 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.0, 12.7, 14.6, 17.7, 18.9, 19.0 (*C*H$_3$); 29.3, 29.7, 30.3 (*C*H$_2$); 31.0 (*C*H); 31.9, 32.2, 32.5, 34.7 (*C*H$_2$); 34.9 (*C*H); 36.4 (*C*); 37.5 (*C*H$_2$); 43.4, 45.0 (*C*H); 46.3 (*C*); 53.5, 55.5 (*C*H); 56.8 (*C*H$_3$); 61.8 (*C*H$_2$); 63.3 (*C*H); 67.2 (*C*H$_2$); 69.9, 70.8, 72.8, 72.9, 73.1, 73.2, 74.3, 74.5, 79.1, 81.4, 89.7, 100.2, 102.5, 103.3 (*C*H); 109.8 (*C*). HRMS (ESI-FT-ICR) *m/z*: 923.4972 $[M+Na]^+$ (calcd for $C_{46}H_{76}O_{17}Na$: 923.4980).

4.1.17. 12α-Methoxy-tigogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl) -β-D-glucopyranoside (26)

Acceptor **20** (120.6 mg, 0.155 mmol) and donor **23** (209 mg, 0.481 mmol) in dry CH_2Cl_2 (20 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (86 μL, 0.682 mmol) in a similar way as described in Section 4.1.13. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 2:1) to afford the protected saponin (298 mg). This product was deprotected with NaOH (1.0 M, 4 mL) in THF/MeOH (16 mL, 1:1, v/v) in a similar way as described in Section 4.1.13. Flash column chromatography purification ($CHCl_3$/MeOH 5:1→4:1) afforded **26** (93.6 mg, 67%) as a white solid. R_f = 0.42 ($CHCl_3$/MeOH 4:1). Mp: 173–175 °C. $[\alpha]_D^{20}$ −46.3 (*c* 0.8, C_5H_5N). IR (KBr, cm^{-1}) *v*: 3401, 2931, 1640, 1403, 1046. ^{1}H NMR (500 MHz, pyridine-d_5): δ = 0.70 (d, 3H, *J* = 5.4 Hz, C*H*$_3$); 0.87 (s, 6H, 2 × C*H*$_3$); 1.18 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.64 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.76 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 2.77 (dd, 1H, *J* = 8.6/6.7 Hz); 3.06 (t, 1H, *J* = 2.3 Hz, H-12β); 3.34 (s, 3H, OC*H*$_3$); 3.51 (t, 1H, *J* = 10.6 Hz, H-26ax); 3.59 (m, 1H, H-26eq); 3.72 (m, 1H, H-5 Glc); 3.90 (m, 1H, H-3α); 4.13 (dd, 1H, *J* = 12.1/3.5 Hz, H-6a Glc); 4.20–4.40 (m, 6H, H-2 Glc, H-3 Glc, H-4 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.54–4.62 (m, 3H, H-3 Rha, H-3 Rha′, H-16α); 4.69 (dd, 1H, *J* = 3.2/1.6 Hz, H-2 Rha); 4.84 (dd, 1H, *J* = 3.5/1.3 Hz, H-2 Rha′); 4.90–4.96 (m, 2H, H-5 Rha, H-5 Rha′); 4.97 (d, 1H, *J* = 7.3 Hz, H-1 Glc); 5.86 (d, 1H, *J* = 1.6 Hz, H-1 Rha); 6.38 (d, 1H, *J* = 1.8 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.7, 15.4, 17.6, 17.7, 18.9, 19.0 (*C*H$_3$); 23.8, 29.3, 29.7, 30.3 (*C*H$_2$); 31.0 (*C*H); 32.2, 32.3, 32.7, 34.9 (*C*H$_2$); 35.7 (*C*H); 35.9 (*C*); 37.4 (*C*H$_2$); 42.4, 45.0 (*C*H); 45.2 (*C*); 48.3, 49.7, 54.0 (*C*H); 57.3 (*C*H$_3$); 61.8, 67.2 (*C*H$_2$); 69.9, 70.8, 72.8, 72.9, 73.1, 73.2, 74.3, 74.5, 77.3, 77.6, 78.3, 78.5, 79.1, 81.2, 82.3, 100.3, 102.5, 103.3 (*C*H); 109.6 (*C*). HRMS (ESI-FT-ICR) *m/z*: 923.4980 $[M+Na]^+$ (calcd for $C_{46}H_{76}O_{17}Na$: 923.4980).

4.1.18. Δ^9-Hecogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (27)

Acceptor **21** (216.8 mg, 0.286 mmol) and donor **23** (385.5 mg, 0.887 mmol) in dry CH_2Cl_2 (40 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (160 μL, 1.258 mmol) in a similar way as described in Section 4.1.13. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:2) to afford the protected saponin (512 mg). This product was deprotected with NaOH (1.0 M, 6 mL) in THF/MeOH (24 mL, 1:1, v/v) in a similar way as described in Section 4.1.13. Flash column chromatography purification ($CHCl_3$/MeOH 4:1) afforded **27** (164.2 mg, 65%) as a white solid. R_f = 0.33 ($CHCl_3$/MeOH 4:1). Mp: 165–167 °C. $[\alpha]_D^{20}$ −81.6 (*c* 1.3, C_5H_5N). IR (KBr, cm^{-1}) *ν*: 3387, 2937, 2877, 2405, 1670, 1380, 1052. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.80 (d, 3H, *J* = 5.7 Hz, CH_3); 1.06 (s, 3H, CH_3); 1.11 (s, 3H, CH_3); 1.42 (d, 3H, *J* = 6.9 Hz, CH_3); 1.62 (d, 3H, *J* = 6.3 Hz, CH_3 Rha); 1.73 (d, 3H, *J* = 6.3 Hz, CH_3 Rha′); 2.65 (dd, 1H, *J* = 8.6/7.1 Hz); 3.54 (t, 1H, *J* = 10.7 Hz, H-26ax); 3.64 (m, 1H, H-26eq); 3.69 (m, 1H, H-5 Glc); 3.88 (m, 1H, H-3α); 4.08 (dd, 1H, *J* = 12.0/3.5 Hz, H-6a Glc); 4.11–4.17 (m, 2H, H-2 Glc, H-3 Glc); 4.22–4.28 (m, 4H, H-4 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.46 (dd, 1H, *J* = 9.2/3.3 Hz, H-3 Rha); 4.51 (dd, 1H, *J* = 9.3/3.2 Hz, H-3 Rha′); 4.57 (m, 1H, H-16α); 4.61 (dd, 1H, *J* = 3.2/1.6 Hz, H-2 Rha); 4.74 (dd, 1H, *J* = 3.2/1.3 Hz, H-2 Rha′); 4.78 (m, 1H, H-5 Rha); 4.82 (m, 1H, H-5 Rha′); 4.90 (d, 1H, *J* = 7.3 Hz, H-1 Glc); 5.70 (s, 1H, H-1 Rha); 5.83 (s, 1H, H-11); 6.24 (s, 1H, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 14.0, 15.6, 17.7, 18.7, 18.8, 18.9 (*C*H_3); 28.2, 29.5, 29.9 (*C*H_2); 30.8 (*C*H); 32.1, 33.0, 34.5 (*C*H_2); 35.3 (*C*H); 37.3 (*C*); 39.3, 42.8 (*C*H_2); 43.2, 51.0, 53.0 (*C*H); 54.7 (*C*); 61.7, 67.3 (*C*H_2); 69.6, 70.7, 72.5, 72.6, 72.8, 72.9, 73.9, 74.2, 76.9, 77.1, 78.1, 78.2, 79.5, 80.5, 100.2, 102.2, 103.2 (*C*H); 109.4 (*C*); 120.2 (*C*H); 172.1 (*C*); 205.6 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 905.4497 $[M+Na]^+$ (calcd for $C_{45}H_{70}O_{17}Na$: 905.4497).

4.1.19. Tigogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (28)

Acceptor **22** (100 mg, 0.134 mmol) and donor **23** (180.4 mg, 0.415 mmol) in dry CH_2Cl_2 (20 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (75 μL, 0.590 mmol) in a similar way as described in Section 4.1.13. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 2:1) to afford the protected saponin (241 mg). This product was deprotected with NaOH (1.0 M, 4 mL) in THF/MeOH (16 mL, 1:1, v/v) in a similar way as described in Section 4.1.13. Flash column chromatography purification ($CHCl_3$/MeOH 5:1) afforded **28** (78.2 mg, 67%) as a white solid. R_f = 0.29 ($CHCl_3$/MeOH 5:1). Mp: 164–166 °C. $[\alpha]_D^{20}$ −105.6 (*c* 0.9, C_5H_5N). IR (KBr, cm^{-1}) *ν*: 3327, 2933, 1622, 1403, 1044, 978. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.71 (d, 3H, *J* = 5.4 Hz, CH_3); 0.84 (s, 3H, CH_3); 0.88 (s, 3H, CH_3); 1.15 (d, 3H, *J* = 6.9 Hz, CH_3); 1.65 (d, 3H, *J* = 6.3 Hz, CH_3 Rha); 1.77 (d, 3H, *J* = 6.3 Hz, CH_3 Rha′); 3.52 (t, 1H, *J* = 10.7 Hz, H-26ax); 3.61 (m, 1H, H-26eq); 3.73 (m, 1H, H-5 Glc); 3.94 (m, 1H, H-3α); 4.13 (dd, 1H, *J* = 3.5/12.3 Hz, H-6a Glc); 4.23–4.42 (m, 6H, H-2 Glc, H-3 Glc, H-4 Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.54–4.58 (m, 1H, H-16α); 4.62 (dd, 1H, *J* = 9.1/3.5 Hz, H-3 Rha); 4.70 (m, 1H, H-2 Rha); 4.76 (dd, 1H, *J* = 9.1/3.5 Hz, H-3 Rha′); 4.84 (m, 1H, H-2 Rha′); 4.91–4.97 (m, 3H, H-5 Rha, H-5 Rha′, H-1 Glc); 5.87 (d, 1H, *J* = 1.6 Hz, H-1 Rha); 6.39 (d, 1H, *J* = 1.3 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.9, 15.5, 17.0, 17.8, 18.9, 19.1 (*C*H_3); 21.7, 29.4, 29.7, 30.4 (*C*H_2); 31.0 (*C*H); 32.3, 32.6, 32.9, 34.9 (*C*H_2); 35.7 (*C*H); 36.4 (*C*); 37.7 (*C*H_2); 41.2 (*C*); 42.4, 45.1, 54.9, 56.9 (*C*H); 61.9 (*C*H_2); 63.5 (*C*H); 67.3 (*C*H_2); 69.7, 69.9, 70.9, 72.9, 73.0, 73.2, 73.3, 74.0, 74.4, 74.6, 74.9, 81.6, 100.3, 102.6, 103.4 (*C*H); 109.7 (*C*). HRMS (ESI-FT-ICR) *m/z*: 893.4876 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{16}Na$: 893.4875).

4.1.20. (25*R*)-C-Homo-12a-oxa-3β-hydroxy-5α-spirostan-12-one (29)

A solution of *m*-CPBA (2.2 g, 12.8 mmol) in CH_2Cl_2 (100 mL) was added to a stirred solution of hecogenin **5** (2.2 g, 5.12 mmol) in CH_2Cl_2 (100 mL) at 0 °C. The reaction was allowed to reach room temperature and stirred for 5 days in the dark. The mixture was diluted with $CHCl_3$ (150 mL) and washed with solutions of 10% Na_2SO_3 (2 × 100 mL) and 10% $NaHCO_3$ (2 × 100 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The resulting crude product was purified by flash column chromatography (*n*-hexane/EtOAc 1:1) to yield the lactone **29** (1.58 g, 69%). R_f = 0.38 (*n*-hexane/EtOAc 2:3). Mp: 215–217 °C. $[\alpha]_D^{20}$ −93.0 (*c* 1.2, $CHCl_3$). IR (KBr, cm^{-1}) *ν*: 3424, 2937, 2870, 1700, 1052. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.77 (s, 3H, CH_3); 0.78 (d, 3H, *J* = 6.3 Hz, CH_3); 1.03 (d, 3H, *J* = 7.3 Hz, CH_3); 1.44 (s, 3H, CH_3); 2.01 (m, 1H); 2.31 (m, 1H); 2.47 (dd, 1H, *J* = 13.7/12.0 Hz); 2.55 (dd, 1H, *J* = 9.3/6.4 Hz); 2.74 (d, 1H, *J* = 12.9 Hz); 3.31 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.47 (ddd, 1H, *J* = 11.0/4.4/2.2 Hz, H-26eq); 3.60 (m, 1H, H-3α); 4.36 (m, 1H, H-16α). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.6, 13.9, 17.1, 18.5 (*C*H_3); 28.2, 28.7 (*C*H_2); 30.1 (*C*H); 31.3, 31.4, 32.2, 34.4, 36.5, 36.6 (*C*H_2); 36.8 (*C*); 37.9 (*C*H_2); 38.2, 42.6, 43.7, 50.3, 56.7, 62.9 (*C*H); 66.9 (*C*H_2); 70.7, 75.6 (*C*H); 86.3, 108.7 (*C*); 174.7 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 469.2927 $[M+Na]^+$ (calcd for $C_{27}H_{42}O_5Na$: 469.2930).

4.1.21. (25*R*)-C-Homo-12a-oxa-12-oxo-5α-spirostan-3β-yl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (30)

The glucosyl donor **12** (216 mg, 0.291 mmol), the spirostanic acceptor **29** (100 mg, 0.224 mmol) and TMSOTf (2 μL, 0.0112 mmol) were reacted in dry CH_2Cl_2 (4.5 mL) in a similar way as described in Section 4.1.3. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) afforded **30** (218.3 mg, 95%) as a white solid. R_f = 0.26 (*n*-hexane/EtOAc 2:1). Mp: 211–213 °C. $[\alpha]_D^{20}$ −79.5 (*c* 1.1, $CHCl_3$). IR (KBr, cm^{-1}) *ν*: 2933, 2870, 1734, 1271, 1095, 1071. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.63 (s, 3H, CH_3); 0.76 (d, 3H, *J* = 6.3 Hz, CH_3); 1.03 (d, 3H, *J* = 6.9 Hz, CH_3); 1.41 (s, 3H, CH_3); 2.00 (m, 1H); 2.27 (m, 1H); 2.41 (dd, 1H, *J* = 13.6/12.3 Hz); 2.53 (dd, 1H, *J* = 9.3/6.4 Hz); 2.63 (dd, 1H, *J* = 13.6/0.9 Hz); 3.30 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.46 (ddd, 1H, *J* = 11.0/4.1/2.2 Hz, H-26eq); 3.55 (m, 1H, H-3α); 4.16 (m, 1H, H-5 Glc); 4.35 (m, 1H, H-16α); 4.53 (dd, 1H, *J* = 12.0/6.0 Hz, H-6a Glc); 4.58 (dd, 1H, *J* = 12.0/3.5 Hz, H-6b Glc); 4.91 (d, 1H, *J* = 7.9 Hz, H-1 Glc); 5.46 (dd, 1H, *J* = 9.8/7.9 Hz, H-2 Glc); 5.59 (t, 1H, *J* = 9.8/9.5 Hz, H-4 Glc); 5.88 (t, 1H, *J* = 9.8/9.5 Hz, H-3 Glc); 7.24–7.28 (m, 2H, Ar-*H*); 7.31–7.42 (m, 7H, Ar-*H*); 7.46–7.51 (m, 2H, Ar-*H*); 7.54–7.57 (m, 1H, Ar-*H*); 7.81 (dd, 2H, *J* = 8.3/1.1 Hz, Ar-*H*); 7.89 (dd, 2H, *J* = 8.2/1.3 Hz, Ar-*H*); 7.94 (dd, 2H, *J* = 8.4/1.4 Hz, Ar-*H*); 7.99 (dd, 2H, *J* = 8.5/1.3 Hz, Ar-*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.4, 13.9, 17.0, 18.5 (*C*H_3); 28.1, 28.7, 29.2 (*C*H_2); 30.1 (*C*H); 31.4, 32.1, 34.3, 34.5 (*C*H_2); 36.4 (*C*); 36.5, 36.6 (*C*H_2); 38.0, 42.5, 43.5, 50.2, 56.7, 63.0 (*C*H); 63.3, 66.9 (*C*H_2); 70.1, 72.0, 72.1, 73.0, 75.6, 79.6 (*C*H); 86.2 (*C*); 100.3 (*C*H); 108.7 (*C*); 128.2, 128.3, 128.4, 128.5 (*C*H); 128.7, 128.8, 129.4, 129.5 (*C*); 129.6, 129.7, 129.8, 129.9 (*C*H); 164.9, 165.2, 165.8, 166.0, 174.5 (*C*=O). HRMS (ESI-FT-ICR) *m/z*: 1047.4508 $[M+Na]^+$ (calcd for $C_{61}H_{68}O_{14}Na$: 1047.4507).

4.1.22. (25*R*)-C-Homo-12a-oxa-12-oxo-5α-spirostan-3β-yl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (31)

Glucoside **30** (201.2 mg, 0.196 mmol) was deprotected with NaOMe in CH_2Cl_2/MeOH (10 mL, 1:1, v/v) in a similar way as described in Section 4.1.8. The resulting product was selectively protected with pivaloyl chloride (120 μL, 0.98 mmol) in anhydrous pyridine (6 mL) according to the procedure described in Section 4.1.8. Flash column chromatography purification (*n*-hexane/EtOAc

3:2) afforded **31** (100.5 mg, 66%) as a white solid. R_f = 0.23 (*n*-hexane/EtOAc 3:2). Mp: 254–256 °C. $[\alpha]_D^{20}$ −53.3 (*c* 1.4, $CHCl_3$). IR (KBr, cm^{-1}) *v*: 3461, 2933, 2874, 2372, 1718, 1160, 1080, 1047. ^1H NMR (500 MHz, $CDCl_3$): δ = 0.77 (s, 3H, C*H*$_3$); 0.78 (d, 3H, *J* = 6.3 Hz, C*H*$_3$); 1.04 (d, 3H, *J* = 7.3 Hz, C*H*$_3$); 1.21, 1.23 (2 × s, 2 × 9H, 2 × (C*H*$_3$)$_3$C); 1.44 (s, 3H, C*H*$_3$); 2.01 (t, 1H, *J* = 6.7 Hz); 2.31 (m, 1H); 2.39 (s, 1H); 2.47 (dd, 1H, *J* = 13.6/12.3 Hz); 2.55 (dd, 1H, *J* = 9.3/6.5 Hz); 2.73 (d, 1H, *J* = 13.6 Hz); 3.03 (d, 1H, *J* = 4.7 Hz); 3.31 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.42–3.49 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.56 (m, 1H, H-5 Glc); 3.63 (m, 1H, H-3α); 4.24 (dd, 1H, *J* = 12.0/6.6 Hz, H-6a Glc); 4.37 (m, 1H, H-16α); 4.41 (dd, 1H, *J* = 12.0/2.3 Hz, H-6b Glc); 4.43 (d, 1H, *J* = 7.9 Hz, H-1 Glc); 4.85 (t, 1H, *J* = 9.1 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.5, 13.9, 17.1, 18.5, 27.0, 27.1 (*C*H$_3$); 28.3, 28.7, 29.2 (*C*H$_2$); 30.1 (*C*H); 31.4, 32.2, 34.4, 36.6, 36.7 (*C*H$_2$); 38.1 (*C*H); 38.8, 39.0 (*C*); 42.6, 43.6, 50.2, 56.6, 63.0 (*C*H); 63.6, 67.0 (*C*H$_2$); 70.0, 72.1, 74.2, 75.6, 77.9, 78.4 (*C*H); 86.2 (*C*); 101.1 (*C*H); 108.7 (*C*); 174.7, 178.6, 180.2 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 799.4608 $[M+Na]^+$ (calcd for $C_{43}H_{68}O_{12}Na$: 799.4608).

4.1.23. (25*R*)-3β-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl)]-12,13-seco-5α-spirostan-12-oic acid (32)

Acceptor **31** (166.2 mg, 0.214 mmol) and donor **23** (288 mg, 0.663 mmol) in dry CH_2Cl_2 (30 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (120 μL, 0.942 mmol) in a similar way as described in Section 4.1.13. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:2) to afford the protected saponin (397 mg). This product was deprotected with NaOH (1 M, 5 mL) in THF/MeOH (20 mL, 1:1, v/v) in a similar way as described in Section 4.1.13. Flash column chromatography purification ($CHCl_3$/MeOH 3:1) afforded **32** (120 mg, 61%) as a white solid. R_f = 0.33 ($CHCl_3$/MeOH 3:1). Mp: 189–191 °C. $[\alpha]_D^{20}$ −105.6 (*c* 1.3, C_5H_5N). IR (KBr, cm^{-1}) *v*: 3390, 2932, 2874, 1675, 1399, 1044. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.72 (d, 3H, *J* = 5.2 Hz, C*H*$_3$); 0.82 (s, 3H, C*H*$_3$); 1.24 (d, 3H, *J* = 5.7 Hz, C*H*$_3$); 1.40 (s, 3H, C*H*$_3$); 1.64 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.73 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 2.08 (m, 1H); 2.37 (m, 1H); 2.62 (m, 1H); 2.76 (m, 1H); 3.45 (t, 1H, *J* = 10.7 Hz, H-26ax); 3.54 (m, 1H, H-26eq); 3.73 (m, 1H, H-5 Glc); 3.87 (m, 1H, H-3α); 4.12–4.39 (m, 7H, H-2 Glc, H-3 Glc, H-4 Glc, H-6a Glc, H-6b Glc, H-4 Rha, H-4 Rha′); 4.55 (dd, 1H, *J* = 9.1/3.2 Hz, H-3 Rha); 4.60 (dd, 1H, *J* = 9.1/3.2 Hz, H-3 Rha′); 4.70 (m, 2H, H-2 Rha, H-16α); 4.83 (m, 1H, H-2 Rha′); 4.92 (m, 2H, H-5 Rha, H-5 Rha′); 4.96 (d, 1H, *J* = 7.3 Hz, H-1 Glc); 5.85 (d, 1H, *J* = 1.5 Hz, H-1 Rha); 6.36 (d, 1H, *J* = 1.3 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.6, 15.0, 17.7, 18.9, 19.0 (*C*H$_3$); 29.4, 29.6, 30.2 (*C*H$_2$); 30.6 (*C*H$_3$); 31.1 (*C*H); 31.7, 32.3, 34.9 (*C*H$_2$); 36.1 (*C*H); 38.2 (*C*); 38.3, 44.1, 44.8, 49.6, 52.9 (*C*H); 62.0 (*C*H$_2$); 62.2 (*C*H); 67.5 (*C*H$_2$); 69.9, 70.9, 72.8, 72.9, 73.1, 73.2, 74.3, 74.6, 77.3, 77.8, 78.4, 78.7, 79.3, 79.5, 100.5, 102.6, 103.4 (*C*H); 109.1 (*C*); 175.2 (*C*═O). HRMS (ESI-FT-ICR) *m/z*: 941.4708 $[M+Na]^+$ and 963.4525 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{19}Na$: 941.4722 and $C_{45}H_{73}O_{19}Na_2$: 963.4541, respectively).

4.2. Biological activity

4.2.1. Cell culture

HL-60 cells (German Collection of Microorganisms and Cell Cultures, Braunnschweig, Germany) were cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2. The cell numbers were counted by a hematocytometer, and the viability was always greater than 95% in all experiments as assayed by the 0.025% Trypan blue exclusion method. Stock solutions of 100 mM spirostanyl glycosides were made in dimethylsulfoxide (DMSO), and aliquots were frozen at −20 °C. Human peripheral blood mononuclear cells were isolated from heparin-anticoagulated blood of healthy volunteers by centrifugation with Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden), as previously described.[13]

4.2.2. Cytotoxicity assay

The cytotoxicity of compounds was evaluated by colorimetric 3-(4,5-dimethyl-2-thyazolyl-)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described.[14] Concentrations inducing a 50% inhibition of cell growth (IC_{50}) were determined graphically using the curve-fitting algorithm of the computer software Prism 4.0 (GraphPad, La Jolla, CA). Values are means ± SEs from three independent experiments, each performed in triplicate.

Acknowledgements

This work was partially supported by CSIC (Proyecto Intramural de Incorporación - 2007022), the Ministry of Science and Innovation of Spain and the European Regional Development Fund (SAF2010-21380 to FE). K. Pérez-Labrada gratefully acknowledges to MAEC-AECID for a doctoral scholarship. S.E. and M.T.M. thank the Spanish Ministry of Education and University of Las Palmas de Gran Canaria, respectively, for financial support.

References and notes

1. (a) Wang, Y.; Che, C.-M.; Chiu, J.-F.; He, Q.-Y. *J. Proteome Res.* **2007**, *6*, 4703; (b) Wang, Y.; Cheung, Y. H.; Yang, Z.; Chiu, J.-F.; Che, C.-M.; He, Q.-Y. *Proteomics* **2006**, *6*, 2422; (c) Cheung, J. Y.-N.; Ong, R. C.-Y.; Suen, Y.-K.; Ooi, V.; Wong, H. N.-C.; Mak, T. C.-W.; Fung, K.-P.; Yu, B.; Kong, S.-K. *Cancer Lett.* **2005**, *217*, 203; (d) Liu, M. J.; Wang, Z.; Ju, Y.; Zhou, J. B.; Wang, Y.; Wong, R. N.-S. *Biol. Pharm. Bull.* **2004**, *27*, 1059; (e) Watanabe, K.; Mimaki, Y.; Sakagami, H.; Sashida, Y. *J. Nat. Prod.* **2003**, *66*, 236; (f) Wang, Z.; Zhou, J.; Ju, Y.; Zhang, H.; Liu, M.; Li, X. *Biol. Pharm. Bull.* **2001**, *24*, 159.
2. Zhou, L.-B.; Chen, T.-H.; Bastow, K. F.; Shibano, M.; Lee, K.-H.; Chen, D.-F. *J. Nat. Prod.* **2007**, *70*, 1263.
3. Pérez-Labrada, K.; Brouard, I.; Estévez, S.; Marrero, M. T.; Estévez, F.; Bermejo, J.; Rivera, D. G. *Bioorg. Med. Chem.* **2012**, *20*, 2690.
4. Pérez-Labrada, K.; Brouard, I.; Morera, C.; Estévez, F.; Bermejo, J.; Rivera, D. G. *Tetrahedron* **2011**, *67*, 7713.
5. Yu, B.; Zhang, Y.; Tang, P. *Eur. J. Org. Chem.* **2007**, *31*, 5145.
6. Rivera, D. G.; Pando, O.; Coll, F. *Tetrahedron* **2006**, *62*, 8327.
7. Fukase, K.; Winarno, H.; Kusumoto, S. *Chem. Express* **1993**, *8*, 409.
8. Jiang, L.; Chan, T.-H. *J. Org. Chem.* **1998**, *63*, 6035.
9. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
10. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353.
11. Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099.
12. Whereas saponin **28** has been previously reported, its cytotoxicity against HL-60 has not been evaluated so far. Accordingly, its synthesis was accomplished in this work for a comparison of the cytotoxicity with the other C-ring modified glycosides. For a report of a previous synthesis and cytotoxicity against the A-549 cell line, see: (a) Wang, Y.; Zhang, Y.; Zhu, Z.; Zhu, S.; Li, Y.; Li, M.; Yu, B. *Bioorg. Med. Chem.* **2007**, *15*, 2528; (b) Li, Y.; Zhang, Y.; Guo, T.; Guan, H.; Hao, Y.; Yu, B. *Synthesis* **2006**, 775.
13. Rubio, S.; Quintana, J.; Eiroa, J. L.; Triana, J.; Estévez, F. *Carcinogenesis* **2007**, *28*, 2105.
14. Burmistrova, O.; Quintana, J.; Díaz, J. G.; Estévez, F. *Cancer Lett.* **2011**, *309*, 71.