Bioorganic & Medicinal Chemistry 20 (2012) 2690–2700

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

New insights into the structure–cytotoxicity relationship of spirostan saponins and related glycosides

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

[a] Instituto de Productos Naturales y Agrobiología-C.S.I.C., Av. Astrofísico Francisco Sánchez 3, 38206 La Laguna, Tenerife, Spain
[b] Institute of Pharmacy and Food, University of Havana, San Lázaro y L, 10400 La Habana, Cuba
[c] Department of Biochemistry, Molecular Biology and Physiology, University of Las Palmas de Gran Canaria, Unidad Asociada C.S.I.C., Instituto Canario de Investigación del Cáncer (ICIC), 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 2 December 2011
Revised 3 February 2012
Accepted 8 February 2012
Available online 15 February 2012

Keywords:
Steroids
Saponins
Structure–activity relationship
Cytotoxicity
HL-60 cell

ABSTRACT

A variety of spirostan saponins and related glycosides were synthesized and evaluated for their cytotoxicity against the human myeloid leukemia cell line (HL-60). A linear glycosylation strategy allowed for accessing a variety of functionalization patterns at both the spirostanic and the saccharide moieties, which provides new information regarding the structure–cytotoxicity relationship of this family of steroidal glycosides. Intriguing results were achieved with respect to hecogenyl and 5α-hydroxy-laxogenyl β-chacotriosides, turning out to be the former very cytotoxic and the latter no cytotoxic at all. Importantly, the partially pivaloylated β-D-glucosides of 5α-hydroxy-laxogenin were the most potent cytotoxic compounds among all tested glycosides. This comprises the first report on acylated spirostanyl glucosides displaying significant cytotoxicity, and therefore, it opens up new opportunities toward the development of saponin analogues as anticancer agents.

1. Introduction

Saponins[1] are triterpene and steroid glycosides of important biological and medicinal applications.[1,2] Spirostan saponins are the most abundant steroidal saponins, which bear the oligosaccharide moiety directly attached to the C-3 hydroxyl group. Several of these saponins have exhibited potent cytotoxic,[3] antifungal,[4] antiinflammatory,[5] and antiviral activities,[6] which makes them important leads for drug development. A very promising property of spirostan saponins is their anticancer activity, exhibiting dioscin (**1**, Fig. 1) the most potent anticancer activity among the members of this family.[3f–h,7]

Dioscin (**1**) is a diosgenyl glycoside endowed with a trisaccharide moiety—namely chacotrioside—including two α-L-rhamnosyl residues attached at positions 2 and 4 of the inner D-glucose. Previously, structure–activity relationship (SAR) studies based on dioscin (**1**) have focused either on the variations of the oligosaccharide moiety,[8,6a,3a,b] the replacement of the glycosidic linkage by an heterocyclic ring,[9] or the substitution of the spirostanic skeleton by other steroidal or triterpenic aglycones.[6a,10] However, little information is available regarding the effect of the functionalization pattern of this steroidal nucleus on the cytotoxicity of the resulting spirostanyl glycosides. In addition, there are certain gaps on the SAR studies concerning either other types of branching or the presence of acylated functionalities in the saccharide portion.

Herein we report on the synthesis of novel spirostanic saponins as well as on the new insights in the SAR studies derived from the cytotoxicity evaluation of these compounds. The synthetic strategy is directed toward the production of spirostanyl glycosides bearing varied oxygenated functionalities on the steroidal aglycone and different sugar moieties, including tri, di and monosaccharides inspired in the oligosaccharide portions of natural saponins. The cytotoxic saponins dioscin (**1**) and filiasparoside A[3d] (**2**) were chosen as models for the design of the new analogs. Figure 1 depicts the structural features of the saponins chosen to be synthesized and biologically tested for their cytotoxicity against human leukemia cell line (HL-60). It has been proposed that the presence of L-rhamnose units facilitates the cellular uptake of saponins, probably due to a better interaction with lectins.[3a] Accordingly, we were prompted to accomplish the synthesis of B and C-ring functionalized spirostanyl glycosides not only including the 2,4-branched trisaccharide moiety of dioscin (i.e., chacotrioside) but also β-D-glucopyranosides branched with α-L-rhamnopyranosyl residues either at position 2 or at positions 4 and 6. The interest on the α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside saponins derives from previous SAR studies, which have shown that the

* Corresponding authors. Tel.: +53 7 8702102; fax: +53 7 8733502 (D.G.R.).
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.
doi:10.1016/j.bmc.2012.02.026

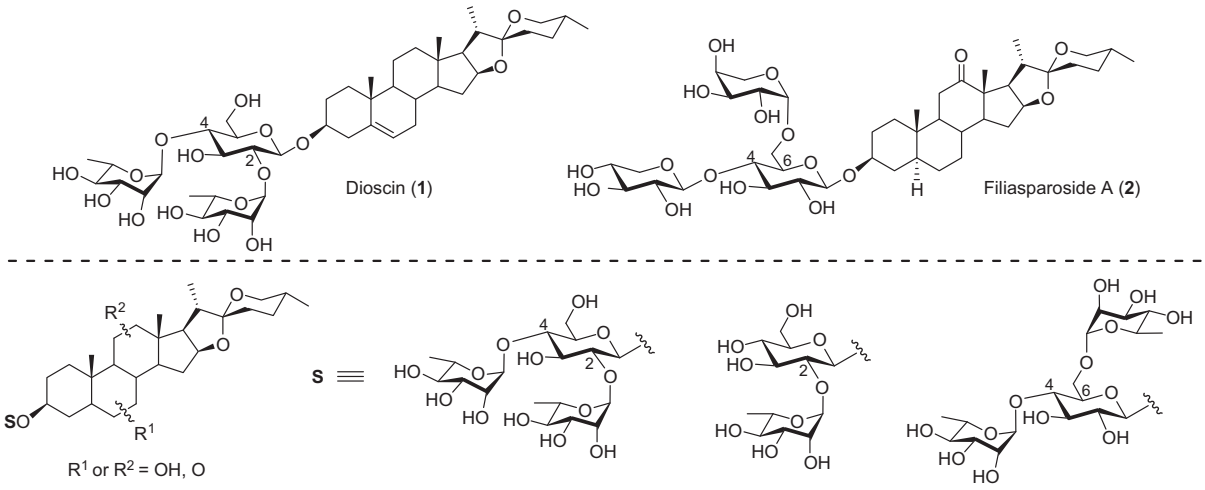


Figure 1. Natural spirostan saponins and their synthetic analogs.

corresponding diosgenyl disaccharide displays cytotoxic activity against HL-60 as high as that of dioscin.[8b,g] On the other hand, the interest on the α-L-rhamnopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside scaffold is inspired on filiasparoside A, which possess the same type of trisaccharide connectivity but including β-D-xylopyranosyl and α-D-arabinopyranosyl units instead of the desired α-L-rhamnosyl.

2. Results and discussion

2.1. Chemical synthesis

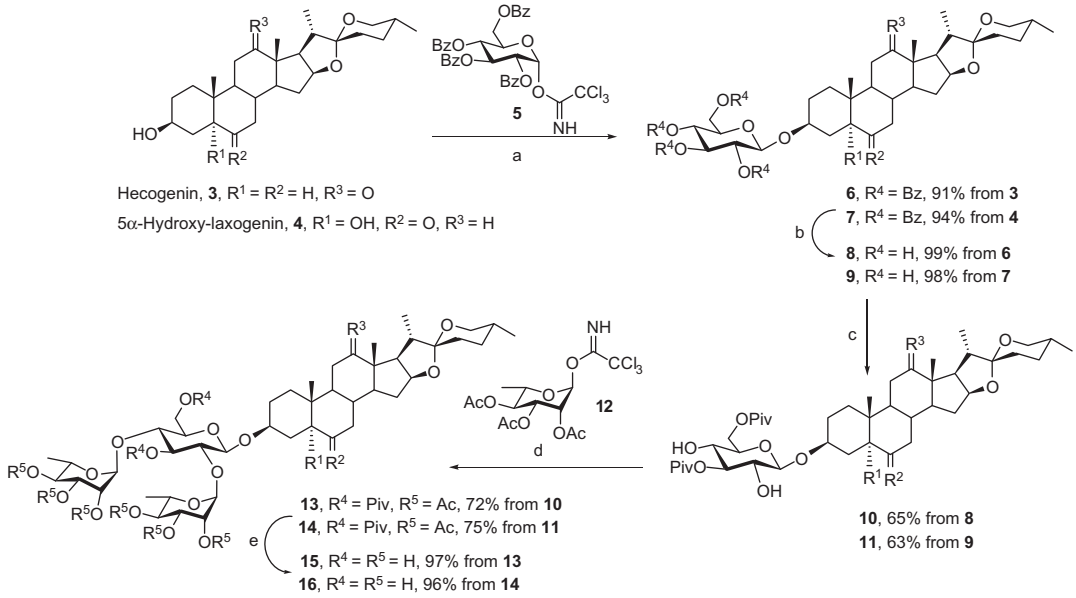
Taking dioscin (**1**) and filiasparoside A (**2**) as lead structures, we first decided to evaluate the influence of the functionalization at the steroidal aglycone in the cytotoxicity of the resulting spirostan saponins. Interestingly, despite the importance that has been attributed to the spirostanic nature of the steroidal aglycone,[10a,d] detailed SAR studies focused on assessing the effect of oxygenated functionalities at different positions of such a skeleton have remained elusive so far. For this, the available spirostan sapogenins hecogenin (**3**) and 5α-hydroxy-laxogenin (**4**) were selected for the introduction of the β-chacotrioside moiety, thus producing C and B-ring functionalized analogs of the naturally occurring dioscin (**1**). Hecogenin is the aglycone of the cytotoxic filiasparoside A and possesses a carbonyl functionality at C-12, whilst 5α-hydroxy-laxogenin is a synthetic derivative of diosgenin having a ketol functionality on ring B.[11] Both sapogenins were subjected to a linear glycosylation strategy in order to produce exclusively the 1,2-*trans*-glycosidic linkage between the glucose and the steroid.[12]

As shown in Scheme 1, glycosylation of sapogenins **3** and **4** with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**5**)[13] in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf)[14] provided the desired 3-*O*-β-glucopyranosides **6** and **7** in 91% and 94%, respectively. Remotion of the benzoyl groups under typical Zemplen conditions (NaOMe/MeOH) afforded the deprotected β-D-glucosides **8** and **9**. These intermediates were then subjected to selective pivaloylation at OH-3 and OH-6 according to a reported procedure[15] to furnish compounds **10** and **11** in 65% and 63%, respectively. The 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**12**)[16] was next employed as donor for the double glycosylation step following the Schmidt's inverse procedure,[17] which produced the protected spirostanyl trisaccharide **13** and **14** in 72% and 75% yields, respectively. Global deprotection furnished the hecogenyl and 5α-hydroxy-laxogenyl saponins **15** and **16** in 41% and 43% overall yields. Importantly, the overall yields of these β-chacotriosides were higher than the 32% overall yield reported for the synthesis of dioscin.[18]

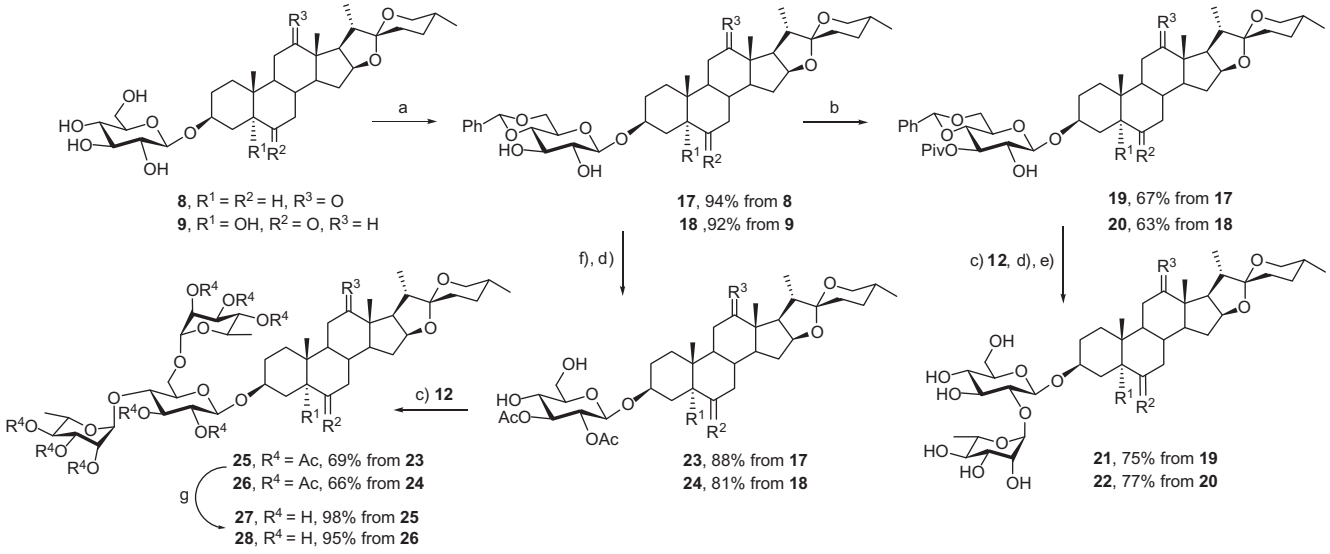
Additionally, we turned to the synthesis of further saponins with the same hecogenyl and 5α-hydroxy-laxogenyl aglycones but including structural variations at the oligosaccharide moiety. Two main structural aspects were chosen for this study: (i) the number and (ii) position of the L-rhamnosyl units attached to the inner D-glucose. In an important SAR study on diosgenyl saponins, Mimaki and co-workers reported that while diosgenyl β-D-glucoside shows no cytotoxic activity against HL-60 cells, the incorporation of α-L-rhamnosyl at C-2 of the glucosyl unit leads to a disaccharide saponin with potent cytotoxicity. In contrast, when L-rhamnose is attached to C-3 or C-4 of D-glucose, the resulting diosgenyl saponins do not exhibit significant cytotoxicity.[8g] In consequence, we decided to obtain spirostan saponins with the L-rhamnose joined to C-2 of the β-D-glucoside. Alternatively, we were also interested on the production of novel spirostan saponins with L-rhamnosyl including 4,6-branched trisaccharide moieties, which have been neither synthesized nor evaluated for their cytotoxic activity.

As shown in Scheme 2, the access to the mentioned saponins required of more elaborated routes including several protection/deprotection procedures. Thus, the β-D-glucosides **8** and **9** were capped at positions 4 and 6 as benzylidene acetals and next subjected to selective pivaloylation of the OH-3 groups to afford compounds **19** and **20** in 63% and 58% yields, respectively, over the two steps. Glycosylation of acceptors **19** and **20** with donor **12** according to the Schmidt's inverse procedure[17] (i.e., the promotor and the acceptor are first mixed and then added to the trichloroacetimidate donor) led to the fully protected α-(1→2)-linked spirostan disaccharide only in moderate yields (i.e., about 50%), as a result of the partial cleavage of the benzylidene acetal. Attempts to improve the glycosylation efficiency with the use of the Schmidt's glycosylation procedure[19] (i.e., the promotor is added to the mixture of the acceptor and the trichloroacetimidate donor) were successful, with increments in the reaction yields up to about 80%. Finally, a two-step deprotection protocol afforded saponins **21** and **22** in 75% and 77% yields, respectively, over the three steps.

Alternatively, synthesis of the 4,6-branched trisaccharide saponins encompassed acetylation of the 4,6-*O*-benzylidene-β-D-glucosides **17** and **18**, followed by cleavage of the acetal group to afford acceptors **23** and **24** in good yields. Subsequent glycosylation with



Scheme 1. Synthesis of β-chacotrioside-based spirostan saponins functionalized on rings B and C. 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 2. Synthesis of disaccharide and trisaccharide spirostan saponins including L-rhamnosyl units at different positions. Reagents and conditions: (a) $PhCH(OMe)_2$, *p*-TsOH, DMF, 50 °C; (b) PivCl, Py, −15 °C→rt; (c) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 4 Å MS, −78 °C→rt; (d) *p*-TsOH, THF/MeOH (1:1, v/v); (e) aq NaOH, THF/MeOH, 50 °C; (f) Ac_2O, pyridine, 0 °C→rt; (g) NaOMe, MeOH.

donor **12** gave the fully protected spirostan saponins **25** and **26** in 69% and 66% yields, respectively. Global deprotection with NaOMe in MeOH furnished the final saponins **27** and **28** in 56% and 47% overall yields, respectively.

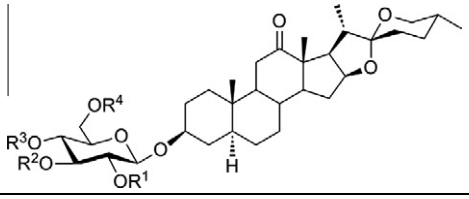
2.2. Biological studies

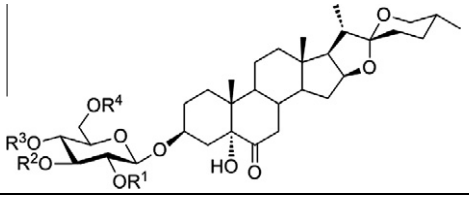
Table 1 shows the results of the in vitro cytotoxic activities of the spirostanyl glycosides against the human myeloid leukemia HL-60 cell line, as determined by the 3-[4,5-dimethylthiazol-2-yl-]-2,5-diphenyl tetrazolium bromide (MTT) dye-reduction assay. Compounds **11**, **15** and **20** were the most cytotoxic ones, with IC_{50} values in the order of that of dioscin (**1,** IC_{50} 3.8 μM).[8g]

As previously reported for diosgenyl β-D-glucoside,[8g] their analogous hecogenyl β-D-glucoside **8** and 5α-hydroxy-laxogenyl β-D-glucoside **9** showed no significant cytotoxic activity (IC_{50} >100 μM). However, further incorporation of pivaloyl groups at OH-3 and OH-6 of the D-glucose unit of both compounds led to the appearance of cytotoxicity in glucosides **10** and **11**, being this latter compound the most potent one among all tested glycosides.

Intriguingly, the partially protected hecogenyl β-D-glucoside **19** exhibited low activity whilst the 5α-hydroxy-laxogenyl β-D-glucoside **20** was very cytotoxic against HL-60 cells, displaying the same behaviour that their congeners **10** and **11**, respectively. We may reason about the effect of such acyl and acetal groups on the bioactivity of these compounds, since this is the first report in the

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





	R^1	R^2	R^3	R^4	IC_{50} (μM)		R^1	R^2	R^3	R^4	IC_{50} (μM)
8	H	H	H	H	>100	**9**	H	H	H	H	>100
10	H	Piv	H	Piv	12 ± 3.5	**11**	H	Piv	H	Piv	2.1 ± 0.5
15	Rha	H	Rha	H	4.3 ± 1.0	**16**	Rha	H	Rha	H	>100
19	H	Piv	4,6-*O*-Benzylidene		45 ± 8.5	**20**	H	Piv	4,6-*O*-Benzylidene		3.3 ± 2.2
21	Rha	H	H	H	>100	**22**	Rha	H	H	H	86.7 ± 10.4
27	H	H	Rha	Rha	>100	**28**	H	H	Rha	Rha	>100

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

literature regarding the cytotoxic activity of pivaloylated (or any other type of acylated) and benzylidene-protected spirostanyl glucosides. Indeed, the lipophilicity of the sugar moiety with some hydroxyl groups capped as esters and acetal is significantly increased, but the more or less hydrophobic nature of the steroidal aglycone must be also considered. It turns out that there are varied issues influencing this type of bioactivity: (i) the nature and (ii) position of the capping functionalities in the inner D-glucose, and (iii) the functionalization level of the spirostanic skeleton. Such structural factors might be evaluated in further SAR studies focused exclusively on spirostanyl glucosides with dissimilar protecting groups at the sugar moiety and varied functionalization patterns in the spirostanic aglycone.

Analysis of Table 1 also provides important information regarding the effect of the number and position of the L-rhamnose units as well as the B and C-ring functionalization on the cytotoxicity of the spirostan saponins. Hecogenyl chacotrioside **15**, which is an hydrid saponin analogue integrating structural elements from both dioscin (**1**) and filiasparoside A (**2**),[3d] proved to be as cytotoxic as those natural saponins. Contrarily, 5α-hydroxy-laxogenyl chacotrioside **16**, which is a B-ring functionalized dioscin analogue, showed no cytotoxicity against HL-60 cells. Although further SAR studies must be carry out to achieve general conclusions regarding the effect of further functionalizations on the cytotoxicity of chacotrioside saponins, it seems that the incorporation of polar oxygenated groups into ring B leads to a significant reduction of the cytotoxic activity of spirostanyl chacotriosides. These results are intriguing when compared with the cytotoxicity of the pivaloylated glucosides of the same aglycones, which exhibit exactly the opposite biological behavior, that is, being the B-ring functionalized spirostanyl glucoside more cytotoxic than the C-ring functionalized one.

It is also interesting to note that neither the disaccharide saponins **21** and **22** nor the trisaccharide saponins **27** and **28** showed significant cytotoxic activity. For the case of the disaccharide saponins, the lack of cytotoxicity against HL-60 cells contrasts with the considerable cytotoxicity (IC_{50} 2.5 μM) of the diosgenyl saponin having the same disaccharide portion,[8g] that is, α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside. As shown before, this latter information suggests that the cytotoxicity of spirostanyl glycosides depends not only of isolated structural factors but of combination of properties at both the saccharide and the steroidal moieties. Indeed, much needs to be done to achieve concluding evidences regarding the structural elements influencing the cytotoxic activity of spirostanyl glycosides.

In order to get deeper insights into the cytotoxic effect of the pivaloylated spirostanyl glycosides, the most active compound (i.e., **11**) was chosen to explore its mechanism of action and to assess whether it is similar to that reported for other spirostan saponins.[8b] Figure 2 shows the effect of compound **11** on human HL-60 cell viability, as illustrated by a representative IC_{50} plot in HL-60 cell (Fig. 2A). It also reveals that this glucoside induces important morphological changes and inhibits cellular growth, as visualized by phase contrast microscopy (Fig. 2B).

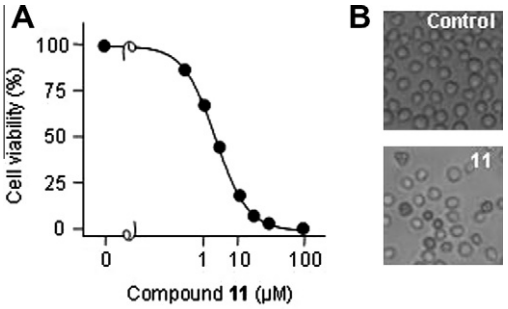


Figure 2. (A) Effect of compound **11** on human HL-60 cell viability. Cells were cultured in the presence of the indicated concentrations of **11** for 72 h, and thereafter cell viability was determined by the MTT dye-reduction assay. The results of a representative experiment are shown. Each point represents the mean of triplicate determinations. (B) Photomicrographs of representative fields of HL-60 cells in culture using an inverted phase contrast microscope after treatment with a 10 μM solution of compound **11** for 24 h.

In order to evaluate whether compound **11** decreases cell viability through activation of apoptosis, morphological changes characteristic of apoptotic cells (condensed and fragmented chromatin) were analysed by fluorescent microscopy. As shown in Figure 3A, compound **11** induces the appearance of condensed and fragmented chromatin, while control HL-60 cells appeared normal with the nuclei round and homogeneous. Moreover, the percentage of hypodiploid cells (i.e., sub-G_1 fraction) increased about sevenfold in compound **11**-treated HL-60 compared with control cells after 24 h exposure at a concentration of 10 μM (Fig. 3B).

Apoptosis can occur with or without the activation of caspases, a family of cysteine proteases which are constitutively expressed as zymogens. To determine whether caspases were involved in the response of the cells to compound **11**, we examined whether this compound induces caspases activation. To this end, HL-60 cells were treated with increasing concentrations of compound **11** and cell lysates were assayed for cleavage of the tetrapeptide substrates DEVD-*p*NA, IETD-*p*NA and LEHD-*p*NA as specific substrates for caspase-3/7, caspase-8 and caspase-9, respectively. As shown in Figure 4, induction of both initiator caspases (caspases-8 and -9)

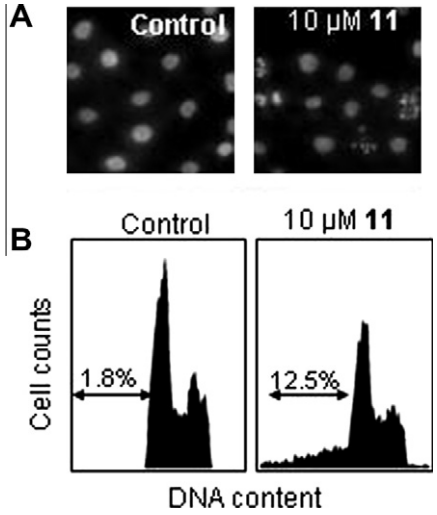


Figure 3. (A) Photomicrographs of representative fields of HL-60 cell stained with Hoechst 33258 to evaluate nuclear chromatin condensation. (B) Cells were incubated with a 10 μM solution of compound **11** for 24 h and subjected to flow cytometry using propidium iodide labelling. Hypodiploid cells (apoptotic cells) are shown in region marked with an arrow.

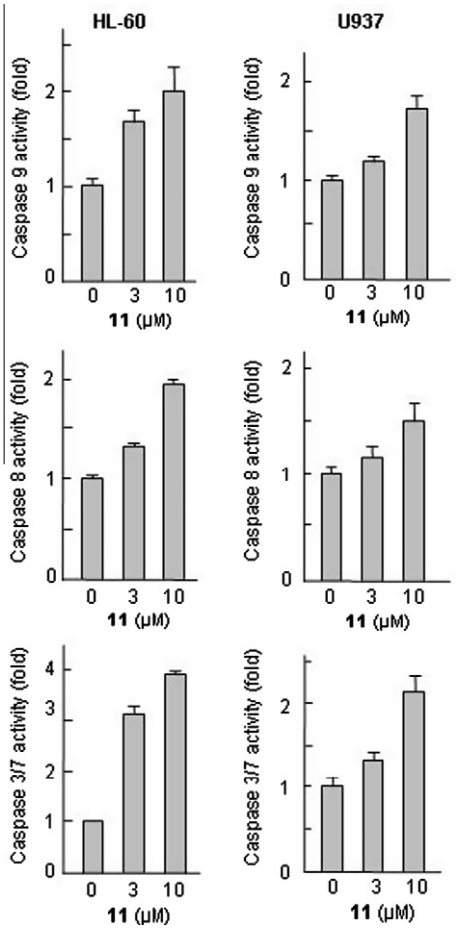


Figure 4. Activation of caspase-9, -8 and 3/7 in response to compound **11**. HL-60 and U937 cells were treated with the indicated concentrations of compound **11** and harvested at 24 h. Cell lysates were assayed for caspase-9, -8 and -3/7 activities using the LEHD-*p*NA, IETD-*p*NA and DEVD-*p*NA colorimetric substrates, respectively. Results are expressed as fold increase in caspase activity relative to control. Values represent the mean ± SE; this histogram is representative of two independent experiments, each one performed in duplicate.

and the executioner caspase-3/7 activities was significantly detectable after 24 h of treatment.

3. Conclusions

We have implemented a linear glycosylation strategy to produce novel spirostan saponins with structural variations at both the saccharidic and steroidal moieties. The cytotoxicity evaluation of the final saponins and the partially protected spirostanyl glucosides provides important information regarding some of the structural elements required for the cytotoxic activity against HL-60 cells. Such a biological assessment showed that the synthetic hecogenyl saponin including the β-chacotrioside residue was as potent as the natural ones dioscin and filiasparoside A. However, the β-chacotrioside saponin derived from 5α-hydroxy-laxogenin was not cytotoxic; suggesting that the more hydrophilic nature of this aglycone compared that of dioscin (i.e., diosgenin) leads to a significant drop in the bioactivity. Interestingly, the pivaloylated β-D-glucosides of 5α-hydroxy-laxogenin showed very high cytotoxicity, being the first report describing potent cytotoxic activity for any type of partially or fully acylated spirostanyl glucosides. Detailed antiproliferative studies with the more active pivaloylated β-D-glucoside proved that this type of compound exerts cell growth inhibition effect similar to that of previously reported diosgenyl saponins. We believe that these results are of outmost importance for a better understanding of the cytotoxic activity of spirostan saponins and related glycosides, as they provide new information on the structure–activity relationship of these amphipathic molecules.

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–^{1}H 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. All commercially available chemicals were used without further purification. Hecogenin (**3**) is a commercially available spirostan sapogenin and 5α-hydroxy-laxogenin (**4**) was obtained as described in Ref. 11δ

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

The glucosyl donor **5** (1.29 g, 1.75 mmol), hecogenin **3** (579 mg, 1.34 mmol) and 4 Å molecular sieves were suspended in dry CH_2Cl_2 (25 mL) and the mixture was stirred at room temperature under nitrogen atmosphere for 15 min. A solution of TMSOTf (12 μL, 0.067 mmol) in CH_2Cl_2 (2.4 mL) was added and the reaction mixture was stirred for 1 h. The reaction was quenched by addition of Et_3N and then filtered. The filtrate was evaporated to dryness and the crude product was purified by flash column chromatography (*n*-hexane/EtOAc 4:1) to afford **6** (1.23 g, 91%) as a white solid. R_f = 0.30 (*n*-hexane/EtOAc 3:1). Mp: 144–146 °C. $[\alpha]_D^{20}$ +26.1 (*c* 1.7, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2928, 2871, 2377, 1729, 1266, 1109, 1097, 1069, 1027. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.77 (s, 3H,

CH_3); 0.78 (d, 3H, J = 7.0 Hz, CH_3); 1.02 (s, 3H, CH_3); 1.06 (d, 3H, J = 6.4 Hz, CH_3); 2.34 (t, 1H, J = 13.7 Hz); 2.51 (dd, 1H, J = 6.8/8.5 Hz); 3.34 (t, 1H, J = 11.0 Hz, H-26ax); 3.48 (m, 1H, H-26eq); 3.57 (m, 1H, H-3α); 4.13–4.18 (m, 1H, H-5 Glc); 4.30–4.36 (m, 1H, H-16α); 4.54 (dd, 1H, J = 6.1/12.1 Hz, H-6a Glc); 4.59 (dd, 1H, J = 3.5/12.0 Hz, H-6b Glc); 4.92 (d, 1H, J = 7.8 Hz, H-1 Glc); 5.48 (dd, 1H, J = 7.9/9.7 Hz, H-2 Glc); 5.61 (t, 1H, J = 9.7 Hz, H-4 Glc); 5.89 (t, 1H, J = 9.7 Hz, H-3 Glc); 7.27–7.44 (m, 9H, Ar-*H*); 7.47–7.57 (m, 3H, Ar-*H*); 7.83 (dd, 2H, J = 1.2/8.3 Hz, Ar-*H*); 7.90 (dd, 2H, J = 1.1/8.4 Hz, Ar-*H*); 7.95 (dd, 2H, J = 1.2/8.4 Hz, Ar-*H*); 8.00 (dd, 2H, J = 1.2/8.4 Hz, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.8, 13.2, 15.9, 17.1 (CH_3); 28.2, 28.8, 29.1 (CH_2); 30.2 (*C*H); 31.1, 31.4, 31.5 (CH_2); 34.3 (*C*H); 34.4 (CH_2); 36.1 (*C*); 36.3, 37.7 (CH_2); 42.2, 44.5, 53.6 (*C*H); 55.1 (*C*); 55.5, 55.8 (*C*H); 63.4, 66.9 (CH_2); 70.2, 72.1, 72.2, 73.1, 79.2, 79.8, 100.3 (*C*H); 109.3 (*C*); 128.2, 128.3, 128.4, 128.5 (*C*H); 128.8, 128.9, 129.4, 129.5 (*C*); 129.6, 129.7, 129.8, 129.9, 133.0, 133.1, 133.4 (*C*H); 165.0, 165.3, 165.8, 166.0, 213.4 (*C*=O).HRMS (ESI-FT-ICR) m/z: 1031.4542 $[M+Na]^+$ (calcd for $C_{61}H_{68}O_{13}Na$: 1031.4558).

4.1.2. 5α-Hydroxy-laxogenyl 2,3,4,6-tetra-O-benzoyl-β-D-glucopyranoside(7)

The glucosyl donor **5** (1.29 g, 1.75 mmol), 5α-hydroxy-laxogenyl **4** (600 mg, 1.34 mmol) and TMSOTf (12 μL, 0.067 mmol) were reacted in dry CH_2Cl_2 (25 mL) in a similar way as described in the synthesis of **6**. Flash column chromatography purification (*n*-hexane/EtOAc 5:2) afforded **7** (1.29 g, 94%) as a white solid. R_f = 0.25 (*n*-hexane/EtOAc 5:2). Mp: 153–155 °C. $[\alpha]_D^{20}$ −38 (*c* 2.0, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3503, 2947, 2871, 2372, 2346, 1725, 1266, 1105, 1093, 1069, 1026. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.70 (s, 3H, CH_3); 0.73 (s, 3H, CH_3); 0.78 (d, 3H, J = 6.4 Hz, CH_3); 0.97 (d, 3H, J = 6.8 Hz, CH_3); 2.10 (dd, 1H, J = 4.2/13.1 Hz); 2.65 (t, 1H, J = 12.5 Hz); 3.35 (t, 1H, J = 10.9 Hz, H-26ax); 3.47 (m, 1H, H-26eq); 3.99 (m, 1H, H-3α); 4.10–4.16 (m, 1H, H-5 Glc); 4.36–4.42 (m, 1H, H-16α); 4.53 (dd, 1H, J = 5.7/12.0 Hz, H-6a Glc); 4.60 (dd, 1H, J = 3.4/12.0 Hz, H-6b Glc); 4.95 (d, 1H, J = 7.8 Hz, H-1 Glc); 5.49 (dd, 1H, J = 7.8/9.8 Hz, H-2 Glc); 5.63 (t, 1H, J = 9.7 Hz, H-4 Glc); 5.87 (t, 1H, J = 9.6 Hz, H-3 Glc); 7.25–7.44 (m, 9H, Ar-*H*); 7.47–7.56 (m, 3H, Ar-*H*); 7.82 (dd, 2H, J = 1.3/8.5 Hz, Ar-*H*); 7.90 (dd, 2H, J = 1.2/8.4 Hz, Ar-*H*); 7.95 (dd, 2H, J = 1.3/8.4 Hz, Ar-*H*); 8.00 (dd, 2H, J = 1.3/8.4 Hz, Ar-*H*). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 14.4, 16.3, 17.1 (CH_3); 21.2, 28.3, 28.7, 29.5 (CH_2); 30.2 (*C*H); 31.3, 31.5, 33.1 (CH_2); 36.6 (*C*H); 39.6 (CH_2); 41.0 (*C*); 41.6 (*C*H); 41.7 (CH_2); 42.3 (*C*); 44.5, 56.1, 62.0 (*C*H); 63.3, 66.8 (CH_2); 70.0, 72.0, 72.1, 73.0, 75.8 (*C*H); 80.1 (*C*); 80.4, 99.9 (*C*H); 109.2 (*C*); 128.2, 128.4 (*C*H); 128.7, 128.8, 129.3, 129.6 (*C*); 129.7, 129.8, 129.9, 133.1, 133.2, 133.4 (*C*H); 165.1, 165.2, 165.8, 166.1, 211.0 (*C*=O). HRMS (ESI-FT-ICR) m/z: 1047.4504 $[M+Na]^+$ (calcd for $C_{61}H_{68}O_{14}Na$: 1047.4507).

4.1.3. Hecogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside(10)

NaOMe was added to a solution of compound **6** (850 mg, 0.84 mmol) in CH_2Cl_2/MeOH (40 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 **8** (506 mg, 99%). This product was dissolved in anhydrous pyridine (25 mL) and the solution was cooled to −15 °C under nitrogen atmosphere. Pivaloyl chloride (0.52 mL, 4.2 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 (50 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 2:1) to afford diol **10** (416 mg, 65%) as a white solid. R_f = 0.30 (*n*-hexane/EtOAc 2:1). Mp: 185–187 °C. $[\alpha]_D^{20}$ −5.0 (*c* 1.4, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3447, 2958, 2930, 2873, 2373, 2346, 1717, 1458, 1285, 1161, 1076, 1040. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.77 (d, 3H, J = 6.3 Hz, CH_3); 0.88 (s, 3H, CH_3); 1.03 (s, 3H, CH_3); 1.04 (d, 3H, J = 6.9 Hz, CH_3); 1.19, 1.22 (2 × s, 2 × 9H, 2 × $(CH_3)_3C$); 2.19 (dd, 1H, J = 5.0/14.2 Hz); 2.38 (t, 1H, J = 13.9 Hz); 2.49 (dd, 1H, J = 6.6/8.8 Hz); 3.33 (t, 1H, J = 10.9 Hz, H-26ax); 3.41–3.46 (m, 3H, H-2 Glc, H-4 Glc, H-26eq); 3.54–3.63 (m, 2H, H-3α, H-5 Glc); 4.22 (dd, 1H, J = 6.9/11.7 Hz, H-6a Glc); 4.30–4.34 (m, 1H, H-16α); 4.40–4.43 (m, 2H, H-6b Glc, H-1 Glc); 4.86 (t, 1H, J = 9.3 Hz, H-3 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.9, 13.2, 15.9, 17.1, 27.0, 27.1 (CH_3); 28.3, 28.7, 29.2 (CH_2); 30.1 (*C*H); 31.1, 31.4, 31.5 (CH_2); 34.3 (*C*H); 36.1 (*C*); 36.4, 37.7 (CH_2); 38.8, 38.9 (*C*); 42.2, 44.6, 53.5 (*C*H); 55.1 (*C*); 55.4, 55.7 (*C*H); 63.7, 66.8 (CH_2); 70.0, 72.1, 74.2, 77.8, 78.7, 79.1, 101.1 (*C*H); 109.2 (*C*); 178.5, 180.1, 213.4 (*C*=O). HRMS (ESI-FT-ICR) m/z: 783.4655 $[M+Na]^+$ (calcd for $C_{43}H_{68}O_{11}Na$: 783.4659).

4.1.4. 5α-Hydroxy-laxogenyl 3,6-di-*O*-pivaloyl-β-D-glucopyranoside (11)

Glucoside **7** (773 mg, 0.75 mmol) was deprotected with NaOMe in CH_2Cl_2/MeOH (36 mL, 1:1, v/v) in a similar way as described in the synthesis of **8** to furnish **9** (435 mg, 98%). This compound was dissolved in anhydrous pyridine (25 mL) and the solution was cooled to −15 °C under nitrogen atmosphere. Pivaloyl chloride (0.46 mL, 3.75 mmol) was added dropwise and the reaction course was monitored by TLC. The stirring was continued at room temperature until disappearance of the intermediate. The mixture was then diluted with EtOAc (50 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 3:2) to afford diol **11** (367 mg, 63%) as a white solid. R_f = 0.23 (*n*-hexane/EtOAc 3:2). Mp: 199–201 °C. $[\alpha]_D^{20}$ −82.8 (*c* 1.8, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3447, 2954, 2932, 2911, 2873, 2371, 2346, 1716, 1458, 1288, 1166, 1076, 1051, 1035. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (s, 3H, CH_3); 0.78 (d, 3H, J = 6.6 Hz, CH_3); 0.79 (s, 3H, CH_3); 0.96 (d, 3H, J = 6.9 Hz, CH_3); 1.20, 1.23 (2 × s, 2 × 9H, 2 × $(CH_3)_3C$); 2.73 (dd, 1H, J = 12.0/12.8 Hz); 3.00, 3.09, 3.24 (3 × s, 3 × 1H, 3 × O*H*); 3.35 (t, 1H, J = 10.9 Hz, H-26ax); 3.41–3.48 (m, 3H, H-26eq, H-2 Glc, H-4 Glc); 3.56–3.61 (m, 1H, H-5 Glc); 3.98 (m, 1H, H-3α); 4.21 (dd, 1H, J = 6.7/11.8 Hz, H-6a Glc); 4.37–4.46 (m, 3H, H-16α, H-6b Glc, H-1 Glc); 4.88 (t, 1H, J = 9.3 Hz, H-3 Glc). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 13.9, 14.4, 16.4, 17.1 (CH_3); 21.2 (CH_2); 27.0, 27.1 (CH_3); 28.6, 28.8, 29.6 (CH_2); 30.3 (*C*H); 31.4, 31.5, 33.3 (CH_2); 36.7 (*C*H); 38.9, 39.0 (*C*); 39.6 (CH_2); 41.1 (*C*); 41.6 (*C*H); 41.8 (CH_2); 42.5 (*C*); 44.4, 56.1, 62.1 (*C*H); 63.7, 66.9 (CH_2); 70.1, 72.2, 74.1, 76.5, 77.8 (*C*H); 80.2 (*C*); 80.5, 102.2 (*C*H); 109.2 (*C*); 178.7, 180.2, 212.7 (*C*=O). HRMS (ESI-FT-ICR) m/z: 799.4595 $[M+Na]^+$ (calcd for $C_{43}H_{68}O_{12}Na$: 799.4608).

4.1.5. Hecogenyl 2,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,6-di-*O*-pivaloyl-β-D-glucopyranoside (13)

$BF_3{\cdot}Et_2O$ (150 μL, 1.195 mmol) was added under nitrogen atmosphere to a stirring suspension of acceptor **10** (206.7 mg, 0.272 mmol) and 4 Å molecular sieves in dry CH_2Cl_2 (40 mL) at −80 °C. After stirring for 1 h at this temperature, a solution of rhamnopyranosyl trichloroacetimidate **12** (366 mg, 0.842 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 **13** (255.7 mg, 72%) as a white solid. R_f = 0.24 (*n*-hexane/EtOAc 2:1). Mp: 134–135 °C. $[\alpha]_D^{20}$ −45.0 (*c* 1.4, $CDCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2959, 2934, 2874, 2371, 2346, 1749, 1458, 1374, 1369, 1244, 1223, 1147, 1076, 1041. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.77 (d, 3H, *J* = 6.3 Hz, C*H*$_3$); 0.87 (s, 3H, C*H*$_3$); 1.03 (s, 3H, C*H*$_3$); 1.05 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.13 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.15 (s, 9H, (C*H*$_3$)$_3$C); 1.16 (d, 3H, *J* = 6.2 Hz, C*H*$_3$ Rha′); 1.20 (s, 9H, (C*H*$_3$)$_3$C); 1.93, 1.95, 2.00, 2.02, 2.08, 2.10 (6 × s, 6 × 3H, 6 × C*H*$_3$CO); 2.21 (dd, 1H, *J* = 4.9/14.3 Hz, H-11β); 2.37 (t, 1H, *J* = 14.1 Hz, H-11α); 2.49 (dd, 1H, *J* = 6.8/8.7 Hz); 3.33 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.46–3.49 (m, 1H, H-26eq); 3.59–3.70 (m, 3H, H-2 Glc, H-4 Glc, H-3α); 3.74–3.78 (m, 1H, H-5 Glc); 3.89 (m, 1H, H-5 Rha); 4.19 (dd, 1H, *J* = 6.1/11.8 Hz, H-6a Glc); 4.30–4.38 (m, 2H, H-5 Rha′, H-16α); 4.40 (dd, 1H, *J* = 2.2/12.0 Hz, H-6b Glc); 4.56 (d, 1H, *J* = 7.3 Hz, H-1 Glc); 4.81 (d, 1H, *J* = 1.6 Hz, H-1 Rha); 4.87 (d, 1H, *J* = 1.9 Hz, H-1 Rha′); 4.97–5.05 (m, 3H, H-2 Rha′, H-4 Rha, H-4 Rha′); 5.13–5.16 (m, 2H, H-2 Rha, H-3 Rha′); 5.19 (dd, 1H, *J* = 3.5/9.8 Hz, H-3 Rha); 5.27 (t, 1H, *J* = 8.7 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.8, 13.2, 15.9, 17.0, 17.1, 17.2, 20.61, 20.69, 20.74, 20.76, 26.8, 27.1 (*C*H$_3$); 28.4, 28.7, 29.1 (*C*H$_2$); 30.1 (*C*H); 31.1, 31.4, 31.5, 33.8 (*C*H$_2$); 34.3 (*C*H); 36.2 (*C*); 36.5, 37.7 (*C*H$_2$); 38.8 (*C*); 42.2, 44.4, 53.5 (*C*H); 55.1 (*C*); 55.4, 55.7 (*C*H); 63.1 (*C*H$_2$); 66.4 (*C*H); 66.9 (*C*H$_2$); 67.9, 68.7, 68.9, 69.5, 69.9, 70.5, 71.2, 72.3, 75.1, 75.8, 76.9, 77.9, 79.1, 97.0, 97.2, 98.7 (*C*H); 109.2 (*C*); 169.5, 169.6, 169.7, 169.8, 169.9, 170.0, 176.5, 177.8, 213.3 (*C*═O). HRMS (ESI-FT-ICR) *m*/*z*: 1327.6415 $[M+Na]^+$ (calcd for $C_{67}H_{100}O_{25}Na$: 1327.6451).

4.1.6. 5α-Hydroxy-laxogenyl 2,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,6-di-*O*-pivaloyl-β-D-glucopyranoside(14)

Acceptor **11** (206.5 mg, 0.266 mmol) and donor **12** (358.1 mg, 0.824 mmol) in dry CH_2Cl_2 (40 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (148 μL, 1.17 mmol) in a similar way as described in the synthesis of **13**. Flash column chromatography purification (*n*-hexane/EtOAc 3:2) afforded **14** (351.5 mg, 75%) as a white solid. R_f = 0.28 (*n*-hexane/EtOAc 3:2). Mp: 129–131 °C. $[\alpha]_D^{20}$ −98.2 (*c* 0.9, $CDCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3503, 2956, 2871, 2372, 2346, 1749, 1374, 1246, 1225, 1146, 1053. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.73 (s, 3H, C*H*$_3$); 0.76 (d, 3H, *J* = 6.6 Hz, C*H*$_3$); 0.77 (s, 3H, C*H*$_3$); 0.94 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.13 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.15 (s, 9H, (C*H*$_3$)$_3$C); 1.17 (d, 3H, *J* = 6.1 Hz, C*H*$_3$ Rha′); 1.19 (s, 9H, (C*H*$_3$)$_3$C); 1.93, 1.94, 2.01, 2.04, 2.08, 2.09 (6 × s, 6 × 3H, 6 × C*H*$_3$CO); 2.77 (t, 1H, *J* = 12.6 Hz); 3.33 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.43–3.46 (m, 1H, H-26eq); 3.62 (t, 1H, *J* = 7.3 Hz, H-2 Glc); 3.69 (t, 1H, *J* = 8.2 Hz, H-4 Glc); 3.83 (m, 1H, H-5 Glc); 3.87–3.93 (m, 1H, H-5 Rha); 3.96–4.02 (m, 1H, H-3α); 4.20 (dd, 1H, *J* = 6.5/11.8 Hz, H-6a Glc); 4.23–4.29 (m, 1H, H-5 Rha); 4.35–4.40 (m, 2H, H-6b Glc, H-16α); 4.57 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 4.86 (m, 2H, H-1 Rha, H-1 Rha′); 4.98 (t, 1H, *J* = 10.1 Hz, H-4 Rha); 5.01 (t, 1H, *J* = 9.9 Hz, H-4 Rha′); 5.05 (dd, 1H, *J* = 1.9/3.2 Hz, H-2 Rha); 5.11 (dd, 1H, *J* = 1.7/3.3 Hz, H-2 Rha′); 5.14 (dd, 1H, *J* = 3.2/10.1 Hz, H-3 Rha); 5.22 (dd, 1H, *J* = 7.3/7.9 Hz, H-3 Glc); 5.28 (dd, 1H, *J* = 3.3/10.2 Hz, H-3 Rha′). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 13.9, 14.4, 16.4, 17.0, 17.1, 17.2, 20.63, 20.65, 20.69, 20.71, 20.75 (*C*H$_3$); 21.2 (*C*H$_2$); 26.8, 27.1 (*C*H$_3$); 28.5, 28.7, 29.7 (*C*H$_2$); 30.2 (*C*H); 31.3, 31.5, 32.8 (*C*H$_2$); 36.7 (*C*H); 38.7, 38.8 (*C*); 39.6 (*C*H$_2$); 41.0 (*C*); 41.6 (*C*H); 41.8 (*C*H$_2$); 42.4 (*C*); 44.3, 56.0, 62.0 (*C*H); 63.3 (*C*H$_2$); 66.8 (*C*H); 66.9 (*C*H$_2$); 68.0, 68.7, 68.9, 69.8, 69.9, 70.3, 70.4, 71.0, 71.2, 72.8, 74.0, 74.9, 76.8, 77.7 (*C*H); 80.1 (*C*); 80.5, 95.7, 97.1, 100.4 (*C*H); 109.2 (*C*); 169.6, 169.8, 169.9, 170.1, 170.2, 170.4, 176.7, 177.9, 211.4 (*C*═O). HRMS (ESI-FT-ICR) *m*/*z*: 1343.6378 $[M+Na]^+$ (calcd for $C_{67}H_{100}O_{26}Na$: 1343.6401).

4.1.7. Hecogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside(15)

An aqueous solution of NaOH (1 M, 1 mL) was added to a solution of compound **13** (198 mg, 0.152 mmol) in THF/MeOH (4 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 **15** (130 mg, 97%) as a white solid. R_f = 0.29 ($CHCl_3$/MeOH 5:1). Mp: 278–279 °C. $[\alpha]_D^{20}$ −91.4 (*c* 1.4, C_5H_5N). IR (KBr, cm^{-1}) ν_{max}: 3420, 2928, 2864, 2371, 2345, 1701, 1654, 1560, 1050. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.71 (d, 3H, *J* = 6.0 Hz, C*H*$_3$); 0.90 (s, 3H, C*H*$_3$); 1.10 (s, 3H, C*H*$_3$); 1.37 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.64 (d, 3H, *J* = 6.0 Hz, C*H*$_3$ Rha); 1.76 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 2.13 (m, 1H); 2.28 (dd, 1H, *J* = 5.0/14.2 Hz); 2.44 (t, 1H, *J* = 13.7 Hz); 2.78 (dd, 1H, *J* = 6.7/8.6 Hz); 3.50 (t, 1H, *J* = 10.7 Hz, H-26ax); 3.60 (m, 1H, H-26eq); 3.72 (m, 1H, H-5 Glc); 3.89 (m, 1H, H-3α); 4.11–4.14 (m, 1H, 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′); 4.47–4.52 (m, 1H, H-16α); 4.55 (dd, 1H, *J* = 3.2/9.1 Hz, H-3 Rha); 4.61 (dd, 1H, *J* = 3.5/9.5 Hz, H-3 Rha′); 4.70 (m, 1H, H-2 Rha); 4.84 (m, 1H, H-2 Rha′); 4.89–4.95 (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* = 1.3 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.3, 14.3, 16.5, 17.7, 18.9, 19.1 (*C*H$_3$); 29.1, 29.7, 30.1 (*C*H$_2$); 31.0 (*C*H); 31.9, 32.2, 32.3, 34.8 (*C*H$_2$); 34.9 (*C*H); 36.9 (*C*); 37.1, 38.5 (*C*H$_2$); 43.1, 44.9, 54.8 (*C*H); 55.8 (*C*); 56.1, 56.4 (*C*H); 61.9, 67.4 (*C*H$_2$); 69.9, 70.9, 72.8, 72.9, 73.2, 73.3, 74.3, 74.6, 76.9, 77.0, 78.2, 78.5, 79.4, 80.1, 100.3, 102.5, 103.4 (*C*H); 109.8 (*C*); 213.2 (*C*═O).HRMS (ESI-FT-ICR) *m*/*z*: 907.4676 $[M+Na]^+$ (calcd for $C_{45}H_{72}O_{17}Na$: 907.4667).

4.1.8. 5α-Hydroxy-laxogenyl 2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (16)

Compound **14** (210 mg, 0.159 mmol) was deprotected with NaOH (1 M, 1.5 mL) in THF/MeOH (6 mL, 1:1, v/v) in a similar was as described in the synthesis of **15**. Flash column chromatography purification ($CHCl_3$/MeOH 4:1) afforded **16** (138 mg, 96%) as a white solid. R_f = 0.28 ($CHCl_3$/MeOH 4:1). Mp: 274–276 °C. $[\alpha]_D^{20}$ −138.6 (*c* 1.4, C_5H_5N). IR (KBr, cm^{-1}) ν_{max}: 3368, 2929, 2871, 2372, 2346, 1702, 1654, 1560, 1131, 1043. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.70 (d, 3H, *J* = 5.8 Hz, C*H*$_3$); 0.83 (s, 3H, C*H*$_3$); 0.98 (s, 3H, C*H*$_3$); 1.15 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.62 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 1.79 (d, 3H, *J* = 6.1 Hz, C*H*$_3$ Rha′); 2.36 (dd, 1H, *J* = 11.6/13.4 Hz); 2.79 (dd, 1H, *J* = 3.8/13.9 Hz); 3.07 (t, 1H, *J* = 12.6 Hz); 3.40–3.44 (m, 1H, H-5 Glc); 3.49 (t, 1H, *J* = 10.7 Hz, H-26ax); 3.57–3.60 (m, 1H, H-26eq); 4.02–4.13 (m, 3H, H-6a Glc, H-6b Glc, H-3 Glc); 4.21 (dd, 1H, *J* = 7.7/9.1 Hz, H-2 Glc); 4.30–4.37 (m, 3H, H-4 Glc, H-4 Rha, H-4 Rha′); 4.52 (dd, 1H, *J* = 3.4/9.3 Hz, H-3 Rha); 4.56 (m, 1H, H-16α); 4.63 (dd, 1H, *J* = 3.4/9.4 Hz, H-3 Rha′); 4.67 (dd, 1H, *J* = 1.3/3.3 Hz, H-2 Rha); 4.74 (m, 1H, H-3α); 4.76 (dd, 1H, *J* = 1.4/3.4 Hz, H-2 Rha′); 4.84 (d, 1H, *J* = 7.7 Hz, H-1 Glc); 4.89–4.93 (m, 2H, H-5 Rha, H-5 Rha′); 5.83 (d, 1H, *J* = 1.3 Hz, H-1 Rha); 6.36 (d, 1H, *J* = 1.4 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 14.6, 15.4, 16.9, 17.7, 18.9, 19.1 (*C*H$_3$); 22.0, 29.5, 29.7, 30.7 (*C*H$_2$); 31.0 (*C*H); 32.3, 32.4, 33.4 (*C*H$_2$); 37.4 (*C*H); 40.4 (*C*H$_2$); 41.7 (*C*); 42.4 (*C*H); 42.6 (*C*H$_2$); 43.3 (*C*); 45.2, 56.8 (*C*H); 61.8 (*C*H$_2$); 63.4 (*C*H); 67.3 (*C*H$_2$); 69.9, 70.9, 72.8, 72.9, 73.1, 74.3, 74.5, 74.6, 77.3, 78.2, 78.4, 79.1 (*C*H); 80.3 (*C*); 81.4, 100.1, 102.4, 103.3 (*C*H); 109.7 (*C*); 212.7 (*C*═O). HRMS (ESI-FT-ICR) *m*/*z*: 923.4603 $[M+Na]^+$ (calcd for $C_{45}H_{72}O_{18}Na$: 923.4616).

4.1.9. Hecogenyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-β-D-glucopyranoside (19)

Benzaldehyde dimethyl acetal (1.8 mL, 11.7 mmol) was added to a solution of compound **8** (960 mg, 1.623 mmol) in anhydrous

DMF (30 mL). The pH was adjusted to 3 by addition of *p*-TsOH·H_2O and the mixture was rotated in the rotavapor under reduced pressure at 50 °C for 3 h. The reaction was quenched by addition of Et_3N and the volatiles were removed under reduced pressure. Flash column chromatography (*n*-hexane/EtOAc 2:3) afforded compound **17** (1.04 g, 94%). A solution of **17** (519 mg, 0.763 mmol) in anhydrous pyridine (15 mL) was cooled to −15 °C and pivaloyl chloride (0.24 mL, 1.91 mmol) was added dropwise under nitrogen atmosphere. The reaction course was monitored by TLC and the stirring was continued at room temperature until disappearance of the intermediate. The reaction was quenched with MeOH and concentrated to dryness. The resulting crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:1) to furnish**19** (390 mg, 67%) as a white solid. R_f = 0.29 (*n*-hexane/EtOAc 3:1). Mp: 171–173 °C. $[\alpha]_D^{20}$ −29.0 (*c* 1.0, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3449, 2932, 2874, 2371, 1712, 1178, 1156, 1076, 1047. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.78 (d, 3H, *J* = 6.3 Hz, C*H*$_3$); 0.90 (s, 3H, C*H*$_3$); 1.04 (s, 3H, C*H*$_3$); 1.06 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.22 (s, 9H, (C*H*$_3$)$_3$C); 2.21 (dd, 1H, *J* = 4.9/14.3 Hz); 2.40 (t, 1H, *J* = 13.9 Hz); 2.51 (dd, 1H, *J* = 6.7/8.6 Hz); 3.34 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.47–3.68 (m, 5H, H-26eq, H-2 Glc, H-4 Glc, H-5 Glc, H-3α); 3.79 (t, 1H, *J* = 10.2 Hz, H-6a Glc); 4.32–4.38 (m, 2H, H-6b Glc, H-16α); 4.56 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 5.17 (t, 1H, *J* = 9.5 Hz, H-3 Glc); 5.50 (s, 1H, C*H*Ph); 7.32–7.34 (m, 3H, Ph); 7.41–7.43 (m, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.9, 13.2, 16.0, 17.1, 27.0 (*C*H$_3$); 28.3, 28.8, 29.1 (*C*H$_2$); 30.2 (*C*H); 31.1, 31.4, 31.5 (*C*H$_2$); 34.3 (*C*H); 36.2 (*C*); 36.5, 37.8 (*C*H$_2$); 38.9 (*C*); 42.2, 44.4, 53.5 (*C*H); 55.1 (*C*); 55.5, 55.7, 66.4 (*C*H); 66.9, 68.7 (*C*H$_2$); 73.5, 73.6, 78.4, 78.5, 79.2, 101.1, 101.8 (*C*H); 109.2 (*C*); 125.9, 128.2, 128.9 (*C*H); 137.0 (*C*); 178.7, 213.5 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 787.4386 $[M+Na]^+$ (calcd for $C_{45}H_{64}O_{10}Na$: 787.4397).

4.1.10. 5α-Hydroxy-laxogenyl 4,6-*O*-benzylidene-3-*O*-pivaloyl-β-D-glucopyranoside (20)

Compound **9** (490 mg, 0.807 mmol) and benzaldehyde dimethyl acetal (0.9 mL, 5.81 mmol) in anhydrous DMF (20 mL) were reacted in the presence of *p*-TsOH·H_2O in a similar was as described in the synthesis of **17**. Flash column chromatography (*n*-hexane/EtOAc 1:5) afforded **18** (517 mg, 92%) as a pure compound. A solution of **18** (93.3 mg, 0.134 mmol) in anhydrous pyridine (5 mL) was cooled to −15 °C under nitrogen atmosphere and pivaloyl chloride (41 μL, 0.335 mmol) was added dropwise. The reaction was monitored by TLC and the stirring was continued at room temperature until disappearance of the intermediate. The reaction was quenched with MeOH and concentrated to dryness. The resulting crude product was purified by flash column chromatography (*n*-hexane/EtOAc 2:1) to furnish **20** (66 mg, 63%) as a white solid. R_f = 0.37 (*n*-hexane/EtOAc 2:1). Mp: 183–185 °C. $[\alpha]_D^{20}$ −105.2 (*c* 1.0, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3404, 2953, 2877, 1706, 1180, 1162, 1080, 1054, 1036, 984. ^1H 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*$_3$); 0.96 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.21 (s, 9H, (C*H*$_3$)$_3$C); 2.11 (dd, 1H, *J* = 3.9/12.0 Hz); 2.72 (t, 1H, *J* = 12.9 Hz); 3.36 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.45–3.51 (m, 2H, H-26eq, H-5 Glc); 3.52 (dd, 1H, *J* = 7.6/9.1 Hz, H-2 Glc); 3.63 (t, 1H, *J* = 9.5 Hz, H-4 Glc); 3.77 (t, 1H, *J* = 10.2 Hz, H-6a Glc); 4.01 (m, 1H, H-3α); 4.33 (dd, 1H, *J* = 4.8/10.5 Hz, H-6b Glc); 4.40 (m, 1H, H-16α); 4.55 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 5.15 (t, 1H, *J* = 9.5 Hz, H-3 Glc); 5.49 (s, 1H, C*H*Ph); 7.32–7.33 (m, 3H, Ph); 7.40–7.42 (m, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.0, 14.4, 16.4, 17.1 (*C*H$_3$); 21.2 (*C*H$_2$); 27.1 (*C*H$_3$); 28.5, 28.7, 29.7 (*C*H$_2$); 30.2 (*C*H); 31.3, 31.5, 33.2 (*C*H$_2$); 36.7 (*C*H); 38.9 (*C*); 39.6 (*C*H$_2$); 41.1 (*C*); 41.6 (*C*H); 41.8 (*C*H$_2$); 42.5 (*C*); 44.4, 56.1, 62.0, 66.3 (*C*H); 66.9, 68.7 (*C*H$_2$); 73.4, 73.5, 76.4, 78.6 (*C*H); 80.4 (*C*); 80.5, 101.1, 102.8 (*C*H); 109.3 (*C*); 125.9, 128.2, 128.9 (*C*H); 137.0 (*C*); 178.6, 212.2 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 803.4299 $[M+Na]^+$ (calcd for $C_{45}H_{64}O_{11}Na$: 803.4346).

4.1.11. Hecogenyl 2-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (21)

$BF_3{\cdot}Et_2O$ (32 μL, 0.254 mmol) was added to a stirring suspension of **19** (161.8 mg, 0.212 mmol), rhamnopyranosyl trichloroacetimidate **12** (138.2 mg, 0.318 mmol) and 4 Å molecular sieves in CH_2Cl_2 (10 mL) at −80 °C under nitrogen atmosphere. The reaction mixture was stirred for 2 h at room temperature and then quenched with Et_3N, filtered and concentrated to dryness. The resulting crude product was purified by flash column chromatography (*n*-hexane/EtOAc 3:1) to furnish the corresponding glycoside (184 mg, 87%) as a white amorphous solid. R_f = 0.27(*n*-hexane/EtOAc 3:1). HRMS (ESI-FT-ICR) *m*/*z*: 1059.5396 $[M+Na]^+$ (calcd for $C_{57}H_{80}O_{17}Na$: 1059.5293). This compound (89 mg, 0.089 mmol) was dissolved in THF/MeOH (2 mL, 1:1, v/v) and *p*-TsOH·H_2O (33.9 mg, 0.178 mmol) was added. The reaction mixture was stirred for 3 h, quenched with Et_3N and the volatiles were removed under reduced pressure. An aqueous solution of NaOH (1 mol/L, 0.2 mL) was added to a solution of the crude product in THF/MeOH (2 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 **21** (57 mg, 75% from **19**) as a white solid. R_f = 0.24($CHCl_3$/MeOH 5:1). Mp: 199–201 °C. $[\alpha]_D^{20}$ −77.4 (*c* 1.2, C_5H_5N). IR (KBr, cm^{-1}) ν_{max}: 3368, 2937, 2870, 2368, 1709, 1564, 1053. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.71 (d, 3H, *J* = 5.7 Hz, C*H*$_3$); 0.91 (s, 3H, C*H*$_3$); 1.10 (s, 3H, C*H*$_3$); 1.37 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.77 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha); 2.28 (dd, 1H, *J* = 5.0/13.9 Hz); 2.44 (t, 1H, *J* = 13.7 Hz); 2.78 (dd, 1H, *J* = 6.6/8.5 Hz); 3.50 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.60 (dd, 1H, *J* = 3.3/11.0 Hz, H-26eq); 3.89–3.99 (m, 2H, H-3α, H-5 Glc); 4.17 (dd, 1H, *J* = 8.8/9.5 Hz, H-4 Glc); 4.26–4.40 (m, 4H, H-2 Glc, H-3 Glc, H-6a Glc, H-4 Rha); 4.48–4.54 (m, 1H, H-16α); 4.58 (dd, 1H, *J* = 2.4/11.7 Hz, H-6b Glc); 4.62 (dd, 1H, *J* = 3.5/9.1 Hz, H-3 Rha); 4.82 (dd, 1H, *J* = 1.3/3.5 Hz, H-2 Rha); 5.07 (d, 1H, *J* = 7.6 Hz, H-1 Glc); 6.38 (d, 1H, *J* = 1.4 Hz, H-1 Rha). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.3, 14.3, 16.5, 17.7, 19.1 (*C*H$_3$); 29.0, 29.6, 30.1 (*C*H$_2$); 31.0 (*C*H); 31.9, 32.1, 32.2, 34.7 (*C*H$_2$); 34.8 (*C*H); 36.8 (*C*); 37.1, 38.4 (*C*H$_2$); 43.1, 44.8, 54.7(*C*H); 55.8 (*C*); 56.0, 56.3 (*C*H); 63.2, 67.4 (*C*H$_2$); 69.9, 72.4, 72.9, 73.3, 74.6, 77.0, 78.5, 78.8, 80.1, 100.3, 102.6 (*C*H); 109.7 (*C*); 213.3 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 761.4067 $[M+Na]^+$ (calcd for $C_{39}H_{62}O_{13}Na$: 761.4088).

4.1.12. 5α-Hydroxy-laxogenyl 2-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside(22)

Acceptor **20** (198 mg, 0.254 mmol) and donor **12** (165.6 mg, 0.381 mmol) in dry CH_2Cl_2 (5 mL) were reacted in the presence of $BF_3{\cdot}Et_2O$ (39 μL, 0.305 mmol) in a similar way as described in the synthesis of **21**. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 2:1) to afford the corresponding glycoside (225 mg, 84%) as a white solid. R_f = 0.26 (EtOAc/*n*-hexane 3:2). Mp: 194–196 °C. HRMS (ESI-FT-ICR) *m*/*z*: 1075.5198 $[M+Na]^+$ (calcd for $C_{57}H_{80}O_{18}Na$: 1075.5242). This compound (92 mg, 0.087 mmol) was deprotected according to the procedure described in the synthesis of **21**. The resulting crude product was purified by flash column chromatography ($CHCl_3$/MeOH 5:1) to afford **22** (60 mg, 77% from **20**) as a white solid. R_f = 0.31($CHCl_3$/MeOH 4:1). Mp: 209–211 °C. $[\alpha]_D^{20}$ −124.2 (*c* 1.0, C_5H_5N). IR (KBr, cm^{-1}) ν_{max}: 3390, 2937, 2375, 1720, 1564, 1056. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.70 (d, 3H, *J* = 5.4 Hz, C*H*$_3$); 0.83 (s, 3H, C*H*$_3$); 1.01 (s, 3H, C*H*$_3$); 1.15 (d, 3H, *J* = 6.9 Hz, C*H*$_3$); 1.81 (d, 3H, *J* = 6.0 Hz, C*H*$_3$ Rha); 2.37 (dd, 1H, *J* = 11.4/13.3 Hz);

2.82 (dd, 1H, *J* = 3.8/12.6 Hz); 3.09 (t, 1H, *J* = 12.6 Hz); 3.50 (t, 1H, *J* = 10.7 Hz, H-26ax); 3.58 (dd, 1H, *J* = 3.3/10.8 Hz, H-26eq); 3.64 (m, 1H, H-5 Glc); 4.09–4.16 (m, 2H, H-3 Glc, H-4 Glc); 4.26 (dd, 1H, *J* = 7.9/8.5 Hz, H-2 Glc); 4.30–4.36 (m, 2H, H-6a Glc, H-4 Rha); 4.46 (dd, 1H, *J* = 2.4/11.8 Hz, H-6b Glc); 4.57 (m, 1H, H-16α); 4.64 (dd, 1H, *J* = 3.3/9.3 Hz, H-3 Rha); 4.74 (dd, 1H, *J* = 1.4/3.3 Hz, H-2 Rha); 4.82 (m, 1H, H-3α); 4.91 (d, 1H, *J* = 7.9 Hz, H-1 Glc); 6.35 (d, 1H, *J* = 1.5 Hz, H-1 Rha). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 14.6, 15.4, 16.9, 17.7, 19.1 (*C*H$_3$); 22.0, 29.5, 29.7, 30.8 (*C*H$_2$); 31.0 (*C*H); 32.2, 32.4, 33.2 (*C*H$_2$); 37.4 (*C*H); 40.4 (*C*H$_2$); 41.7 (*C*); 42.4 (*C*H); 42.6 (*C*H$_2$); 43.3 (*C*); 45.2, 56.8 (*C*H); 63.1 (*C*H$_2$); 63.3 (*C*H); 67.3 (*C*H$_2$); 69.9, 72.2, 72.9, 73.2, 74.0, 74.6, 78.4, 78.6, 79.9 (*C*H); 80.2 (*C*); 81.4, 99.9, 102.5 (*C*H); 109.7 (*C*); 212.9 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 777.4040 [M+Na]$^+$ (calcd for $C_{39}H_{62}O_{14}Na$: 777.4037).

4.1.13. Hecogenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside(23)

Ac_2O (3 mL) was added at room temperature to solution of **17** (346 mg, 0.508 mmol) in anhydrous pyridine (3 mL). The resulting reaction mixture was stirred overnight and then poured into water and extracted with EtOAc (30 mL). The organic layer was washed with dilute aq HCl and brine, then dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was dissolved in THF/MeOH (10 mL, 1:1, v/v) and treated with *p*-TsOH·H_2O (106.3 mg, 0.559 mmol). The reaction mixture was stirred for 3 h, then quenched with Et_3N and concentrated reduced pressure. The crude product was purified by flash column chromatography (*n*-hexane/EtOAc 1:3) to afford diol **23** (302 mg, 88% from **17**) as a white solid. R_f = 0.33 (EtOAc/*n*-hexane 3:1). Mp: 228–229 °C. $[\alpha]_D^{20}$ −18.0 (*c* 1.5, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3421, 2929, 2873, 2377, 2346, 1748, 1710, 1457, 1374, 1242, 1159, 1076, 1040. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.77 (d, 3H, *J* = 6.3 Hz, C*H*$_3$); 0.86 (s, 3H, C*H*$_3$); 1.03 (s, 3H, C*H*$_3$); 1.04 (d, 3H, *J* = 7.3 Hz, C*H*$_3$); 2.03, 2.07 (2 × s, 2 × 3H, 2 × C*H*$_3$CO); 2.19 (dd, 1H, *J* = 4.9/14.3 Hz); 2.38 (t, 1H, *J* = 14.2 Hz); 2.49 (dd, 1H, *J* = 6.8/8.7 Hz); 3.18 (m, 1H, O*H*); 3.33 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.37–3.41 (m, 1H, H-5 Glc); 3.46–3.48 (m, 1H, H-26eq); 3.53 (m, 1H, H-3α); 3.73 (t, 1H, *J* = 9.3 Hz, H-4 Glc); 3.81 (dd, 1H, *J* = 4.3/11.8 Hz, H-6a Glc); 3.89 (dd, 1H, *J* = 3.2/12.0 Hz, H-6b Glc); 4.30–4.36 (m, 1H, H-16α); 4.58 (d, 1H, *J* = 8.0 Hz, H-1 Glc); 4.84 (dd, 1H, *J* = 8.0/9.6 Hz, H-2 Glc); 5.01 (t, 1H, *J* = 9.5 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 11.9, 13.2, 15.9, 17.1, 20.7, 20.8 (*C*H$_3$); 28.3, 28.7, 29.1 (*C*H$_2$); 30.2 (*C*H); 31.1, 31.4, 31.5 (*C*H$_2$); 34.3 (*C*H); 34.4 (*C*H$_2$); 36.1 (*C*); 36.4, 37.7 (*C*H$_2$); 44.2, 44.5, 53.5 (*C*H); 55.1 (*C*); 55.5, 55.7 (*C*H); 62.2, 66.9 (*C*H$_2$); 69.4, 71.6, 75.4, 75.9, 79.2, 99.6 (*C*H); 109.2 (*C*); 169.5, 171.5, 213.5 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 699.3712 [M+Na]$^+$ (calcd for $C_{37}H_{56}O_{11}Na$: 699.3720).

4.1.14. 5α-Hydroxy-laxogenyl 2,3-di-*O*-acetyl-β-D-glucopyranoside (24)

Compound **18** (411 mg, 0.590 mmol) was subjected to acetylation and benzylidene deprotection in a similar was as described in the synthesis of **23**. Flash column chromatography purification (*n*-hexane/EtOAc 1:4) afforded **24** (331 mg, 81% from **18**) as a white solid. R_f = 0.36(EtOAc/*n*-hexane 4:1). Mp: 233–235 °C. $[\alpha]_D^{20}$ −116 (*c* 1.4, $CHCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3421, 2951, 2872, 2371, 2345, 1735, 1724, 1449, 1374, 1242, 1165, 1076, 1052. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.75 (s, 3H, C*H*$_3$); 0.78 (d, 3H, *J* = 6.6 Hz, C*H*$_3$); 0.79 (s, 3H, C*H*$_3$); 0.96 (d, 3H, *J* = 6.8 Hz, C*H*$_3$); 2.07, 2.08 (2 × s, 2 × 3H, 2 × C*H*$_3$CO); 2.72 (t, 1H, *J* = 12.1 Hz); 3.10 (s, 1H); 3.35 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.42–3.50 (m, 2H, H-26eq, H-5 Glc); 3.68 (t, 1H, *J* = 9.2 Hz, H-4 Glc); 3.79 (dd, 1H, *J* = 4.9/12.0 Hz, H-6a Glc); 3.91 (dd, 1H, *J* = 2.8/11.7 Hz, H-6b Glc); 3.99 (m, 1H, H-3α); 4.40 (m, 1H, H-16α); 4.64 (d, 1H, *J* = 7.9 Hz, H-1 Glc); 4.83 (dd, 1H, *J* = 8.0/9.5 Hz, H-2 Glc); 5.04 (t, 1H, *J* = 9.4 Hz, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.0, 14.4, 16.4, 17.1, 20.8, 20.9 (*C*H$_3$); 21.2, 28.1, 28.8, 29.7 (*C*H$_2$); 30.2 (*C*H); 31.3, 31.5, 32.8 (*C*H$_2$); 36.7 (*C*H); 39.6 (*C*H$_2$); 41.1 (*C*); 41.6 (*C*H); 41.8 (*C*H$_2$); 42.4 (*C*); 44.5, 56.1, 62.1 (*C*H); 62.2, 66.9 (*C*H$_2$); 69.5, 71.6, 75.1, 75.7, 75.9 (*C*H); 80.2 (*C*); 80.5, 99.0 (*C*H); 109.3 (*C*); 169.9, 171.8, 212.1 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 715.3671 [M+Na]$^+$ (calcd for $C_{37}H_{56}O_{12}Na$: 715.3669).

4.1.15. Hecogenyl 4,6-di-O-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,3-di-*O*-acetyl-β-D-glucopyranoside (25)

Acceptor **23** (93 mg, 0.137 mmol) and donor **12** (185 mg, 0.425 mmol) in dry CH_2Cl_2 (10 mL) were reacted in the presence of BF_3·Et_2O (76 μL, 0.603 mmol) in a similar way as described in the synthesis of **13**. Flash column chromatography purification (*n*-hexane/EtOAc 2:1) afforded **25** (115 mg, 69%) as a white solid. R_f = 0.45 (*n*-hexane/EtOAc 1:1). Mp: 137–139 °C. $[\alpha]_D^{20}$ −59.4 (*c* 1.6, $CDCl_3$). IR (KBr, cm^{-1}) ν_{max}: 2933, 2873, 2375, 2346, 1751, 1439, 1374, 1369, 1243, 1223, 1138, 1076, 1043. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.77 (d, 3H, *J* = 6.3 Hz, C*H*$_3$); 0.86 (s, 3H, C*H*$_3$); 1.02 (s, 3H, C*H*$_3$); 1.04 (d, 3H, *J* = 7.0 Hz, C*H*$_3$); 1.14 (d, 3H, *J* = 6.1 Hz, C*H*$_3$ Rha); 1.17 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 1.95, 1.96, 2.02, 2.03 (4 × s, 4 × 3H, 4 × C*H*$_3$CO); 2.06 (s, 6H, 2 × C*H*$_3$CO); 2.09, 2.11 (2 × s, 2 × 3H, 2 × C*H*$_3$CO); 2.18 (dd, 1H, *J* = 4.6/14.1 Hz, H-11β); 2.37 (t, 1H, *J* = 13.9 Hz, H-11α); 2.49 (dd, 1H, *J* = 6.7/8.6 Hz); 3.33 (t, 1H, *J* = 11.0 Hz, H-26ax); 3.46–3.51 (m, 3H, H-5 Glc, H-3α, H-26eq); 3.78–3.85 (m, 3H, H-5 Rha, H-4 Glc, H-6a Glc); 3.94–3.99 (m, 2H, H-5 Rha′, H-6b Glc); 4.29–4.35 (m, 1H, H-16α); 4.54 (d, 1H, *J* = 8.0 Hz, H-1 Glc); 4.79–4.83 (m, 3H, H-2 Glc, H-1 Rha, H-1 Rha′); 5.00–5.08 (m, 3H, H-2 Rha, H-3 Rha, H-4 Rha); 5.13 (dd, 1H, *J* = 3.4/9.9 Hz, H-3 Rha′); 5.16–5.22 (m, 4H, H-2 Rha′, H-3 Rha′, H-4 Rha′, H-3 Glc). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.3, 13.6, 16.4, 17.5, 17.7, 21.0, 21.1, 21.2, 21.5 (*C*H$_3$); 28.7, 29.2, 29.6 (*C*H$_2$); 30.6 (*C*H); 31.5, 31.8, 31.9 (*C*H$_2$); 34.7 (*C*H); 34.9 (*C*H$_2$); 36.5 (*C*); 36.8, 38.1 (*C*H$_2$); 42.6, 44.9, 53.9 (*C*H); 55.5 (*C*); 55.9, 56.2 (*C*H); 65.9 (*C*H$_2$); 66.8 (*C*H); 67.3 (*C*H$_2$); 68.2, 69.3, 69.6, 69.9, 70.0, 70.9, 71.3, 72.7, 73.8, 74.4, 77.6, 79.6, 97.8, 100.1, 100.2 (*C*H); 109.6 (*C*); 169.9, 170.1, 170.2, 170.3, 170.5, 170.6, 170.7, 170.8, 213.8 (*C*=O). HRMS (ESI-FT-ICR) *m*/*z*: 1243.5492 [M+Na]$^+$ (calcd for $C_{61}H_{88}O_{25}Na$: 1243.5512).

4.1.16. 5α-Hydroxy-laxogenyl 4,6-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,3-di-*O*-acetyl-β-D-glucopyranoside(26)

Acceptor **24** (62 mg, 0.090 mmol) and donor **12** (120.6 mg, 0.277 mmol) in dry CH_2Cl_2 (10 mL) were reacted in the presence of BF_3·Et_2O (50 μL, 0.394 mmol) in a similar way as described in the synthesis of **13**. Flash column chromatography purification (*n*-hexane/EtOAc 1:1) afforded **26** (73 mg, 66%) as a white amorphous solid. R_f = 0.28 (*n*-hexane/EtOAc 1:1). $[\alpha]_D^{20}$ −112.9 (*c* 0.9, $CDCl_3$). IR (KBr, cm^{-1}) ν_{max}: 3506, 2934, 2872, 1753, 1368, 1245, 1136, 1076, 1043. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.74 (s, 3H, C*H*$_3$); 0.78 (d, 3H, *J* = 7.0 Hz, C*H*$_3$); 0.79 (s, 3H, C*H*$_3$); 0.96 (d, 3H, *J* = 6.8 Hz, C*H*$_3$); 1.14 (d, 3H, *J* = 6.1 Hz, C*H*$_3$ Rha); 1.20 (d, 3H, *J* = 6.3 Hz, C*H*$_3$ Rha′); 1.96, 1.97, 2.03, 2.05, 2.06, 2.07, 2.11, 2.12 (8 × s, 8 × 3H, 8 × C*H*$_3$CO); 2.72 (t, 1H, *J* = 12.2 Hz, H-7α); 3.35 (t, 1H, *J* = 10.9 Hz, H-26ax); 3.45–3.48 (m, 1H, H-26eq); 3.55 (m, 1H, H-5 Glc); 3.73–3.83 (m, 3H, H-4 Glc, H-6a Glc, H-5 Rha); 3.91–3.99 (m, 3H, H-6b Glc, H-5 Rha′, H-3α); 4.39 (m, 1H, H-16α); 4.58 (d, 1H, *J* = 8.0 Hz, H-1 Glc); 4.82 (m, 3H, H-2 Glc, H-1 Rha, H-1 Rha′); 5.01–5.08 (m, 3H, H-2 Rha, H-3 Rha, H-4 Rha); 5.12–5.23 (m, 4H, H-3 Glc, H-2 Rha′, H-3 Rha′,H-4 Rha′). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 14.1, 14.4, 16.4, 17.1, 17.2, 17.3, 20.6, 20.7, 20.8, 20.9, 21.0 (*C*H$_3$); 21.2, 28.3, 28.7, 29.6 (*C*H$_2$); 30.2 (*C*H); 31.3, 31.5, 33.0 (*C*H$_2$); 36.7 (*C*H); 39.6 (*C*H$_2$); 41.1 (*C*); 41.6 (*C*H); 41.8 (*C*H$_2$); 42.5 (*C*); 44.7, 56.1, 62.1 (*C*H); 65.9 (*C*H$_2$); 66.6 (*C*H); 66.9 (*C*H$_2$); 67.8, 68.8, 69.4, 69.6, 69.7, 70.5, 70.9, 72.2, 73.6, 74.1, 75.1, 77.4, 80.4 (*C*H); 80.5(*C*); 97.4, 99.3, 99.6 (*C*H); 109.3 (*C*); 169.7, 169.8, 169.9, 170.1, 170.2, 170.3, 170.4, 211.2 (*C*=O).HRMS

(ESI-FT-ICR) m/z: 1259.5439 $[M+Na]^+$ (calcd for $C_{61}H_{88}O_{26}Na$: 1259.5462).

4.1.17. Hecogenyl 4,6-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (27)

NaOMe was added to a solution of **25** (87 mg, 0.071 mmol) in THF/MeOH (4 mL, 1:1, v/v) until pH was set to 9–10. The reaction mixture was stirred at room temperature for 2 h, then neutralized with acid resin Dowex-50 (H^+) and filtered. The filtrate was concentrated under reduced pressure to dryness and the resulting crude product was purified by flash column chromatography ($CHCl_3$/MeOH 4:1) to afford **27** (62 mg, 98%) as a white amorphous solid. R_f = 0.31 ($CHCl_3$/MeOH 3:1). $[\alpha]_D^{20}$ +39.2 (*c* 1.1, C_5H_5N). IR (KBr, cm^{-1}) ν_{max}: 3367, 2929, 2873, 1701, 1654, 1455, 1375, 1099, 1059, 1040. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.69 (s, 3H, CH_3); 0.71 (d, 3H, J = 6.0 Hz, CH_3); 1.09 (s, 3H, CH_3); 1.37 (d, 3H, J = 6.9 Hz, CH_3); 1.66 (d, 3H, J = 6.0 Hz, CH_3 Rha); 1.73 (d, 3H, J = 6.3 Hz, CH_3 Rha′); 2.36 (t, 1H, J = 13.8 Hz); 2.77 (dd, 1H, J = 6.6/8.8 Hz); 3.51 (t, 1H, J = 10.9 Hz, H-26ax); 3.61 (dd, 1H, J = 3.8/11.0 Hz, H-26eq); 3.84–4.03 (m, 4H, H-2 Glc, H-4 Glc, H-6a Glc, H-3α); 4.12 (t, 1H, J = 9.1 Hz, H-3 Glc); 4.21–4.26 (m, 2H, H-5 Glc, H-4 Rha′); 4.33–4.41 (m, 3H, H-4 Rha, H-5 Rha, H-6b Glc); 4.48–4.52 (m, 2H, H-3 Rha′, H-16α); 4.56 (dd, 1H, J = 3.2/9.1 Hz, H-3 Rha); 4.60 (m, 1H, H-2 Rha); 4.68 (m, 1H, H-2 Rha′); 4.92 (d, 1H, J = 7.9 Hz, H-1 Glc); 4.94 (m, 1H, H-5 Rha′); 5.40 (d, 1H, J = 1.6 Hz, H-1 Rha); 5.66 (d, 1H, J = 1.6 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 12.1, 14.3, 16.5, 17.7, 18.9, 19.0 (*C*H_3); 29.1, 29.7, 30.2 (*C*H_2); 31.0 (*C*H); 31.9, 32.1, 32.2 (*C*H_2); 34.8 (*C*H); 35.2 (*C*H_2); 36.7 (*C*); 37.0, 38.4 (*C*H_2); 43.1, 44.8, 54.8 (*C*H); 55.8 (*C*); 55.9, 56.3 (*C*H); 67.4, 67.8 (*C*H_2); 70.2, 71.1, 72.7, 73.0, 73.2, 74.3, 74.4, 75.8, 75.9, 77.2, 77.9, 80.1, 80.2, 102.5, 102.6, 103.3 (*C*H); 109.7 (*C*); 213.2 (*C*=O). HRMS (ESI-FT-ICR) m/z: 907.4668 $[M+Na]^+$ (calcd for $C_{45}H_{72}O_{17}Na$: 907.4667).

4.1.18. 5α-Hydroxy-laxogenyl 4,6-di-O-(α-L-rhamnopyranosyl)-β-D-glucopyranoside(28)

NaOMe was added to a solution of **26** (58 mg, 0.047 mmol) in THF/MeOH (4 mL, 1:1, v/v) until pH was set to 9–10. The reaction mixture was stirred at room temperature for 2 h, then neutralized with acid resin Dowex-50 (H^+) and filtered. The filtrate was concentrated under reduced pressure to dryness and the resulting crude product was purified by flash column chromatography ($CHCl_3$/MeOH 4:1) to afford **28** (40 mg, 95%)as a white amorphous solid. R_f = 0.26 ($CHCl_3$/MeOH 3:1). $[\alpha]_D^{20}$ −162.5 (*c* 0.4, C_5H_5N). IR (KBr, cm^{-1}) ν_{max}: 3368, 2929, 2873, 2371, 2345, 1703, 1654, 1457, 1375, 1097, 1059, 1040. ^1H NMR (500 MHz, pyridine-d_5): δ = 0.69 (d, 3H, J = 6.0 Hz, CH_3); 0.78 (s, 3H, CH_3); 0.81 (s, 3H, CH_3); 1.14 (d, 3H, J = 6.9 Hz, CH_3); 1.66 (d, 3H, J = 6.0 Hz, CH_3 Rha); 1.72 (d, 3H, J = 6.3 Hz, CH_3 Rha′); 2.66 (m, 1H); 3.00 (t, 1H, J = 12.6 Hz); 3.49 (t, 1H, J = 10.9 Hz, H-26ax); 3.59 (dd, 1H, J = 3.6/10.9 Hz, H-26eq); 3.78–3.80 (m, 1H, H-5 Glc); 3.97–4.02 (m, 2H, H-2 Glc, H-6a Glc); 4.09–4.13 (m, 2H, H-3 Glc, H-4 Glc); 4.23–4.40 (m, 4H, H-4 Rha, H-4 Rha′, H-5 Rha, H-6b Glc); 4.49 (dd, 1H, J = 3.5/9.3 Hz, H-3 Rha′); 4.52–4.57 (m, 2H, H-3 Rha, H-16α); 4.58 (m, 1H, H-2 Rha); 4.66 (m, 1H, H-2 Rha′); 4.76 (m, 1H, H-3α); 4.87 (d, 1H, J = 7.9 Hz, H-1 Glc); 4.92 (m, 1H, H-5 Rha′); 5.36 (d, 1H, J = 1.3 Hz, H-1 Rha); 5.64 (d, 1H, J = 1.6 Hz, H-1 Rha′). ^{13}C NMR (125 MHz, pyridine-d_5): δ = 14.4, 15.4, 16.9, 17.7, 18.8, 18.9 (*C*H_3); 22.0, 29.7, 29.8, 30.7 (*C*H_2); 31.0 (*C*H); 32.2, 32.3, 34.0 (*C*H_2); 37.5 (*C*H); 40.4 (*C*H_2); 41.7 (*C*); 42.4 (*C*H); 42.6 (*C*H_2); 43.3 (*C*); 45.2, 56.8, 63.4 (*C*H); 67.3, 68.0 (*C*H_2); 70.0, 71.0, 72.7, 72.9, 73.0, 73.1, 74.3, 74.4, 74.9, 75.7, 75.8, 77.1, 79.8 (*C*H); 80.4 (*C*); 81.3, 102.7, 102.8, 103.2 (*C*H); 109.7 (*C*); 212.9 (*C*=O). HRMS (ESI-FT-ICR) m/z: 923.4608 $[M+Na]^+$ (calcd for $C_{45}H_{72}O_{18}Na$: 923.4616).

4.2. Biological activity

4.2.1. Cell culture

Human HL-60 myeloid leukemia cells were grown in RPMI 1640 (Sigma) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Sigma) and 100 U/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.

4.2.2. Assay for growth inhibition and cell viability

The cytotoxicity of spirostanyl glycosides was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.[20] Briefly, 1×10^4 exponentially growing cells were seeded in 96-well microculture plates with various spirostanyl glycoside concentrations (0.3–100 μM) in a volume of 200 μL. DMSO concentration was the same in all the treatments and did not exceed 0.1% (v/v). After 72 h, surviving cells were detected based on their ability to metabolize 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (Sigma) into formazan crystals. Optical density was read with an ELISA reader at a wavelength of 570 nm and was used as a measure of cell viability. The MTT dye-reduction assay measures mitochondrial respiratory function and can detect the onset of cell death earlier than dye-exclusion methods. Cell survival was calculated as the fraction of cells alive relative to control for each point: cell survival (%) = mean absorbance in treated cells/mean absorbance in control wells × 100. Concentrations inducing a 50% inhibition of cell growth (IC_{50}) were determined graphically for each experiment. Parameters describing the concentration–response curves (IC_{50}) were determined using the curve fitting routine of the computer software Prism™ (GraphPad) and the equation derived by DeLean et al.[21]

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 F.E.). K. Pérez-Labrada gratefully acknowledges to MAEC-AECID for a doctoral scholarship.

References and notes

1. Hostettmann, K.; Marston, A. *Saponins*; Cambridge University Press: Cambridge, UK, 1995.
2. (a) Augustin, J. M.; Kuzina, V.; Andersen, S. B.; Bak, S. *Phytochemistry* **2011**, *72*, 435; (b) Sun, H.-X.; Xie, Y.; Ye, Y.-P. *Vaccine* **2009**, *27*, 1787; (c) Vincken, J.-P.; Heng, L.; de Groot, A.; Gruppen, H. *Phytochemistry* **2007**, *68*, 275; (d) Sparg, S. G.; Light, M. E.; van Staden, J. *J. Ethnopharmacol.* **2004**, *94*, 219; (e) Lacaille-Dubois, M. A.; Wagner, H. *Stud. Nat. Prod. Chem.* **2000**, *21*, 633; (f) Attele, A. S.; Wu, J. A.; Yuan, C. S. *Biochem. Pharmacol.* **1999**, *58*, 1685; (g) Walt, J. M.; Breyer-Brandwijk, M. G. *The Medicinal and Poisonous Plants of Southern and Eastern Africa*; E. and S. Livingstane Ltd: London, 1962.
3. (a) Podolak, I.; Galanty, A.; Sobolewska, D. *Phytochem. Rev.* **2010**, *9*, 425; (b) Man, S.; Gao, W.; Zhang, Y.; Huang, L.; Liu, C. *Fitoterapia* **2010**, *81*, 703; (c) Yokosuka, A.; Jitsuno, M.; Yui, S.; Yamazaki, M.; Mimaki, Y. *J. Nat. Prod.* **2009**, *72*, 1399; (d) Zhou, L.-B.; Chen, T.-H.; Bastow, K. F.; Shibano, M.; Lee, K.-H.; Chen, D.-F. *J. Nat. Prod.* **2007**, *70*, 1263; (e) Hernández, J. C.; León, F.; Estévez, F.; Quintana, J.; Bermejo, J. *Bioorg. Med. Chem.* **2004**, *12*, 4423; (f) Watanabe, K.; Mimaki, Y.; Sakagami, H.; Sashida, Y. *J. Nat. Prod.* **2003**, *66*, 236; (g) González, A. G.; Hernández, J. C.; León, F.; Padrón, J. I.; Estévez, F.; Quintana, J.; Bermejo, J. *J. Nat. Prod.* **2003**, *66*, 793; (h) Hu, K.; Dong, A. J.; Yao, X. S.; Kobayashi, H.; Iwasaki, S. *Planta Med.* **1996**, *62*, 573; (i) Nakamura, T.; Komori, C.; Lee, Y.-Y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564; (j) Chiang, H. C.; Tseng, T. H.; Wang, C. J.; Chen, C. F.; Kan, W. S. *Anticancer Res.* **1991**, *11*, 1911.
4. (a) Takechi, M.; Shimada, S.; Tanaka, Y. *Phytochemistry* **1991**, *30*, 3943; (b) Hufford, C. D.; Liu, S.; Clark, A. M. *J. Nat. Prod.* **1988**, *51*, 94.

5. (a) Kim, S. Y.; Son, K. H.; Chang, H. W.; Kang, S. S.; Kim, H. P. *Arch. Pharm. Res.* **1999**, *22*, 313; (b) Mimaki, Y.; Nakamura, O.; Sashida, Y.; Nikaido, T.; Ohmoto, T. *Phytochemistry* **1995**, *38*, 1279; (c) Baek, S. H.; Kim, S. H.; Son, K. H.; Chung, K. C.; Chang, H. W. *Arch. Pharm. Res.* **1994**, *17*, 218.
6. (a) Song, G.; Yang, S.; Zhang, W.; Cao, Y.; Wang, P.; Ding, N.; Zhang, Z.; Guo, Y.; Li, Y. *J. Med. Chem.* **2009**, *52*, 7368; (b) Ikeda, T.; Ando, J.; Miyazono, A.; Zhu, X.-H.; Tsumagari, H.; Nohara, T.; Yokomizo, K.; Uyeda, M. *Biol. Pharm. Bull.* **2000**, *23*, 363.
7. (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) Wang, Z.; Zhou, J.; Ju, Y.; Zhang, H.; Liu, M.; Li, X. *Biol. Pharm. Bull.* **2001**, *24*, 159.
8. (a) Kaskiw, M. J.; Tassotto, M. L.; Mok, M.; Tokar, S. L.; Pycko, R.; Tĥng, J.; Jiang, Z.-H. *Bioorg. Med. Chem.* **2009**, *17*, 7670; (b) Hernández, J. C.; León, F.; Brouard, I.; Torres, F.; Rubio, S.; Quintana, J.; Estévez, F.; Bermejo, J. *Bioorg. Med. Chem.* **2008**, *16*, 2063; (c) Wang, Y.; Zhang, Y.; Shu, Z.; Zhu, S.; Li, Y.; Li, M.; Yu, B. *Bioorg. Med. Chem.* **2007**, *15*, 2528; (d) Wang, Y.; Zhang, Y.; Yu, B. *ChemMedChem* **2007**, *2*, 288; (e) Li, W.; Qiu, Z.; Wang, Y.; Zhaug, Y.; Li, M.; Yu, J.; Zhang, L.; Zhu, Z.; Yu, B. *Carbohydr. Res.* **2007**, *342*, 2705; (f) Zhu, S.; Zhang, Y.; Li, M.; Yu, J.; Zhang, L.; Li, Y.; Yu, B. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5629; (g) Mimaki, Y.; Yokosuka, A.; Kuroda, M.; Sashida, Y. *Biol. Pharm. Bull.* **2001**, *24*, 1286.
9. Pérez-Labrada, K.; Brouard, I.; Morera, C.; Estévez, F.; Bermejo, J.; Rivera, D. G. *Tetrahedron* **2011**, *67*, 7713.
10. (a) Gao, J.; Li, X.; Gu, G.; Sun, B.; Cui, M.; Ji, M.; Lou, H.-X. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 622; (b) Gauthier, C.; Legault, J.; Piochon, M.; Lavoie, S.; Tremblay, S.; Pichette, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2310; (c) Miyashita, H.; Koi, Y.; Nohara, T.; Ikeda, T. *Carbohydr. Res.* **2008**, *343*, 1309; (d) Miyashita, H.; Ikeda, T.; Nohara, T. *Carbohydr. Res.* **2007**, *342*, 2182.
11. Rivera, D. G.; León, F.; Coll, F.; Davison, G. P. *Steroids* **2006**, *71*, 1.
12. Yu, B.; Zhang, Y.; Tang, P. *Eur. J. Org. Chem.* **2007**, *31*, 5145.
13. Fukase, K.; Winarno, H.; Kusumoto, S. *Chem. Express* **1993**, *8*, 409.
14. Deng, S.; Yu, B.; Xie, J.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 7265.
15. Jiang, L.; Chan, T.-H. *J. Org. Chem.* **1998**, *63*, 6035.
16. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
17. Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353.
18. Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099.
19. (a) Deng, S.; Yu, B.; Hui, Y.; Yu, H.; Han, X. *Carbohydr. Res.* **1999**, *317*, 53; (b) Deng, S.; Yu, B.; Hui, Y. *Tetrahedron Lett.* **1998**, *39*, 6511.
20. Rivero, A.; Quintana, J.; Eiroa, J. L.; López, M.; Triana, J.; Bermejo, J.; Estévez, F. *Eur. J. Pharmacol.* **2003**, *482*, 77.
21. DeLean, A.; Munson, P. J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.