

## Formation of the steroidal 3 $\beta$ -hydroxy-6-oxo-moiety. Synthesis and cytotoxicity of glucolaxogenin

María A. Fernández-Herrera,<sup>a</sup> Jesús Sandoval-Ramírez,<sup>\*a</sup> Hugo López-Muñoz,<sup>b</sup> and Luis Sánchez-Sánchez<sup>b</sup>

<sup>a</sup> *Facultad de Ciencias Químicas, Benemérita Universidad Autónoma de Puebla. Ciudad Universitaria, Blvd. 14 Sur y Av. Sn. Claudio, San Manuel, 72570 Puebla, Pue., México*

<sup>b</sup> *Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México Batalla 5 de Mayo esq. Fuerte de Loreto Col. Ejército de Oriente, 09230 México D.F., México*  
E-mail: [jsandova@siu.buap.mx](mailto:jsandova@siu.buap.mx)

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### Abstract

An efficient alternative route to the synthesis of the steroidal 3 $\beta$ -hydroxy-6-oxo moiety starting from diosgenin and cholesterol is described. The sequential tosylation, oxidative hydroboration, and selective reduction steps provided the target 3 $\beta$ -hydroxy-6-oxo moiety, yielding laxogenin **1** in 82% and 3 $\beta$ -hydroxycholestan-6-one **8** in 83% overall yield. The cleavage of the S-O bond of 3 $\beta$ -tosylate-6-oxo intermediates was succeeded by means of sodium naphthalenide at -80 °C; when the reduction was explored at room temperature the cleavage of the C-O bond was favored and the corresponding *i*-steroids were observed. Glucolaxogenin **15** was synthesized in 68% from **1**, and its antiproliferative activity was evaluated in cervical cancer cells HeLa, CaSki and ViBo. The effect on peripheral blood lymphocytes was assessed founding that the cell growth was unaffected showing therefore high selectivity.

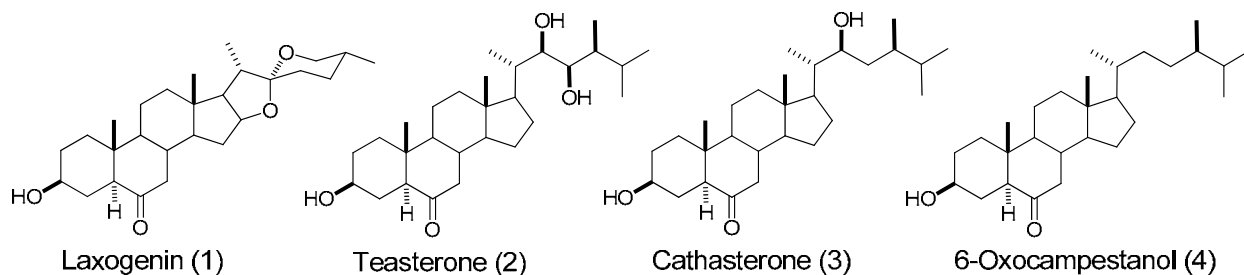
**Keywords:** 3 $\beta$ -Hydroxy-6-oxo moiety, sodium naphthalenide, cervical cancer cells, glucolaxogenin, lymphocytes

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### Introduction

The 3 $\beta$ -hydroxy-6-oxo moiety has been found in several naturally occurring steroids such as laxogenin **1** and several brassinosteroids (growth phytohormones) i.e. teasterone **2**, cathasterone **3** and 6-oxocampestanol (**4**, Figure 1).<sup>1</sup> In the general brassinosteroid biosynthetic pathway, the 3 $\beta$ -hydroxy-6-oxo moiety is early introduced in 6-oxocampestanol **4**,<sup>2</sup> inducing enzymes to hydroxylate the campestanic side chain to afford teasterone **2**, one of the most distributed brassinosteroids in plant kingdom.<sup>3</sup> Laxogenin is a naturally occurring compound which was

isolated by Okanishi *et al.* and by Son *et al.* from *Smilax sieboldi* (a climbing shrub of the Liliaceae family native to Japan, Korea, and China), and later by Okuyama *et al.* from *Allium chinense* G. Don (Liliaceae family as well).<sup>4</sup> Laxogenin became important due to its growth promoting activity in plants,<sup>5,3b</sup> as an analogue of brassinosteroids, and recently because of its cytotoxic activity.<sup>6</sup> Several pathways for the synthesis of laxogenin have been reported, in moderate to good yields. Iglesias-Arteaga *et al.* reported the synthesis of **1** by means of a selective oxidation of 6 $\beta$ -hydroxytigogenin ( $\beta$ -chlorogenin);<sup>3b</sup> Yu *et al.* reported a protocol based on the hydroboration of benzylated diosgenin<sup>7</sup> and more recently, our group reported a route supported on the transformation of the 5 $\beta$ ,6 $\beta$ -epoxide of the diosgenin acetate.<sup>8</sup> Since some brassinosteroids have recently shown antiproliferative activity;<sup>9</sup> herein we report an alternative route for the synthesis of laxogenin glucoside and the determination of its antiproliferative activity.



**Figure 1.** Examples of naturally occurring steroids bearing the 3 $\beta$ -hydroxy-6-oxo-moiety.

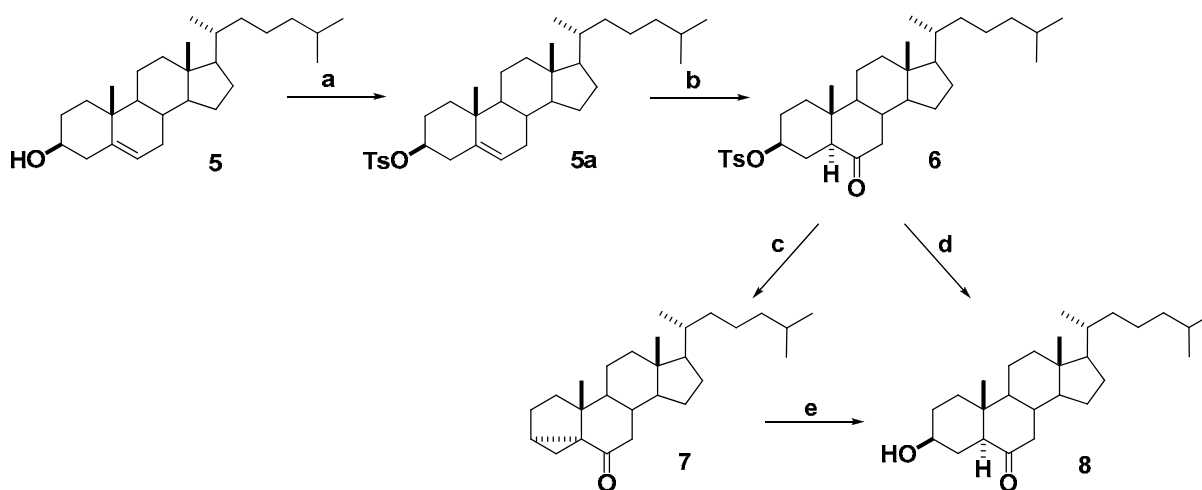
## Results and Discussion

In order to insert an electron-withdrawing protecting group at C-3 (instead of electron-donating substituents such as ethers), cholesterol **5** was transformed into cholesteryl tosylate **5a**.<sup>10</sup> In our experience using tosylates in the hydroboration-oxidation reaction, better yields have been obtained. The 6-oxo functionality was inserted following the Brown's oxidative hydroboration procedure:<sup>11,3b,7</sup> the double bond of **5a** was treated by diborane (prepared *in situ* by means of NaBH<sub>4</sub> and BF<sub>3</sub>·OEt<sub>2</sub>), and the corresponding steroidal boranes were oxidized with 35% H<sub>2</sub>O<sub>2</sub> in the presence of methanolic KOH. Subsequent PDC oxidation of the introduced hydroxyl groups at C-6 provided the ketone **6** in good yield. All these steps have been commonly followed by other authors and in our case, they were achieved successfully.

To recover the hydroxyl group at C-3, we envisaged the reductive cleavage of tosylates by means of sodium naphthalenide. The radical anion sodium naphthalenide (SN) is a one electron donor, easy to prepare.<sup>12</sup> The SN promotes reactions as the reductive cyclization of steroidal acetylenic ketones,<sup>13</sup> coupling of ketones<sup>14</sup> and thiocarbonyl functionalities;<sup>15</sup> converts vicinal cyanohydrins and geminal dihalides into olefins,<sup>16</sup> and removes *p*-toluensulfonamide, mesylate and tosylate protecting groups among others.<sup>17</sup> The reaction conditions in the reductive cleavage

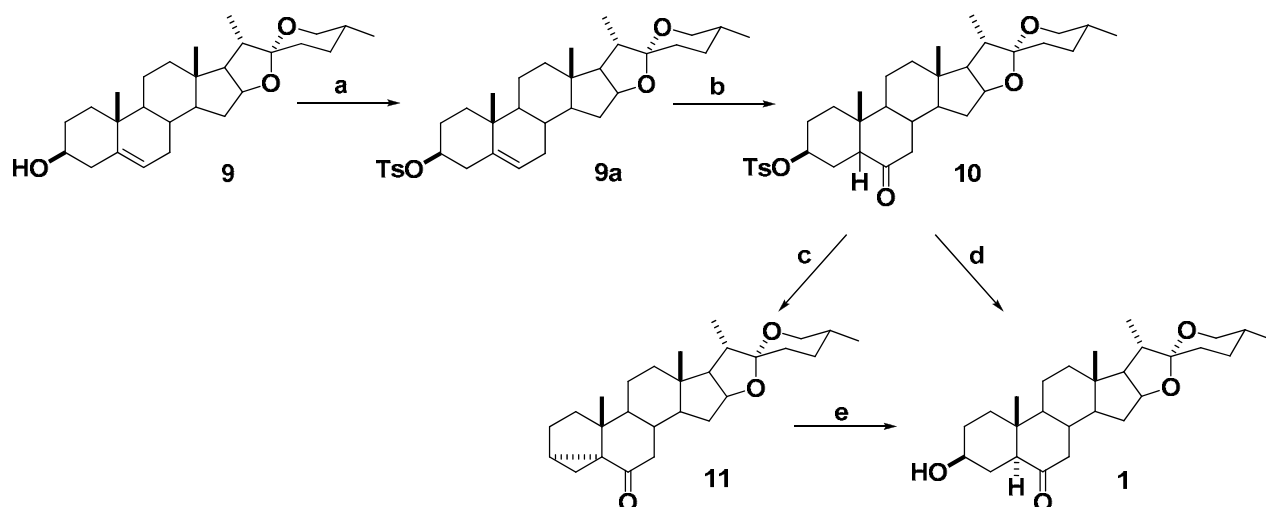
of tosylates by means of SN have been widely studied as aforementioned, finding that vicinal groups have influence in the reaction pathway. In this report we determined the reaction conditions for the reduction of steroidal tosylates located at C-3, bearing at C-6 a carbonyl function.

The reductive cleavage of a great variety of simple tosylates using SN has been previously reported at room temperature as described by Closson.<sup>17e</sup> However, under similar conditions, the reduction of **6** with SN afforded the 3 $\alpha$ ,5-cyclo-5 $\alpha$ -steroid **7** instead of the desired 3 $\beta$ -hydroxy-6-oxo-5 $\alpha$ -steroid **8** (Scheme 1). The synthesis of the A-cyclosteroid **7** starting from **6** has been previously reported by means of KOH/MeOH, under reflux, in 85% yield.<sup>18</sup> The SN treatment of **6** directed to **7** instantaneously, at room temperature in a better yield (95%). When the SN treatment was carried out at 0 °C compound **8** was disclosed, and a practically quantitative yield of **8** was obtained working at -80 °C. In this manner, compound **8** was obtained in 83% overall yield from **5**; the 5 $\alpha$  stereochemistry was confirmed by <sup>1</sup>H-NMR.



**Scheme 1.** *Reagents and conditions:* **a:** *p*-TsCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, quantitative. **b:** NaBH<sub>4</sub>, BF<sub>3</sub>·OEt<sub>2</sub>, THF, rt; then 2% KOH/MeOH, 35% H<sub>2</sub>O<sub>2</sub>, rt; then PDC, CH<sub>2</sub>Cl<sub>2</sub>, rt, 90%. **c:** SN, THF, rt, 95%. **d:** same reagents as **c** but at -80 °C, 92%. **e:** AcOH/H<sub>2</sub>SO<sub>4</sub> aq, 72%.

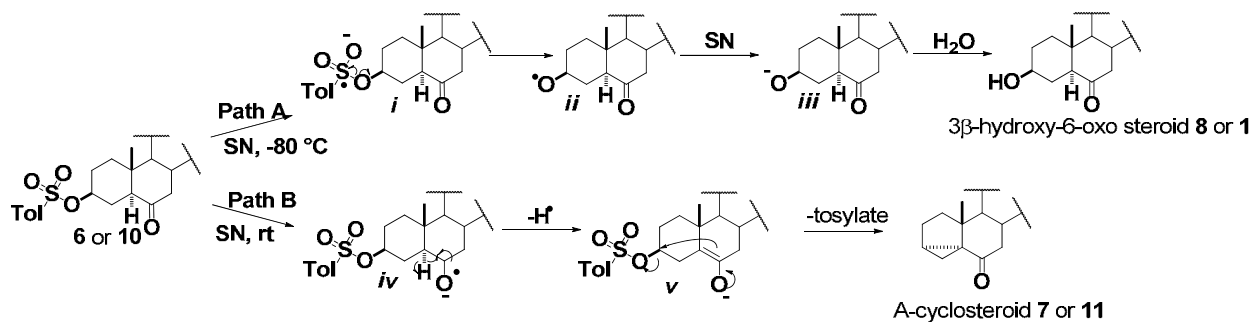
The same sequence of reactions was applied to diosgenin **9** to yield the corresponding laxogenin **1** in 82% overall yield (Scheme 2). It is important to note that the labile spiroketal side chain of diosgenin resulted unchanged either under borane or SN reductive reaction conditions.



**Scheme 2.** *Reagents and conditions:* **a:** *p*-TsCl, pyridine, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, quantitative. **b:** NaBH<sub>4</sub>, BF<sub>3</sub>·OEt<sub>2</sub>, THF, rt; then 2% KOH/MeOH, 35% H<sub>2</sub>O<sub>2</sub>, rt; then PDC, CH<sub>2</sub>Cl<sub>2</sub>, rt, 92%. **c:** SN, THF, rt, 91%. **d:** same reagents as **c** but at -80 °C, 89%. **e:** AcOH/H<sub>2</sub>SO<sub>4</sub> aq, 60%.

On the other hand, 3 $\alpha$ ,5-cyclo-5 $\alpha$ -steroids such as **7** and **11**, were transformed into the 3 $\beta$ -hydroxy-6-oxosteroids (**8** and **1** respectively) by acidic hydrolysis as reported.<sup>18,19</sup>

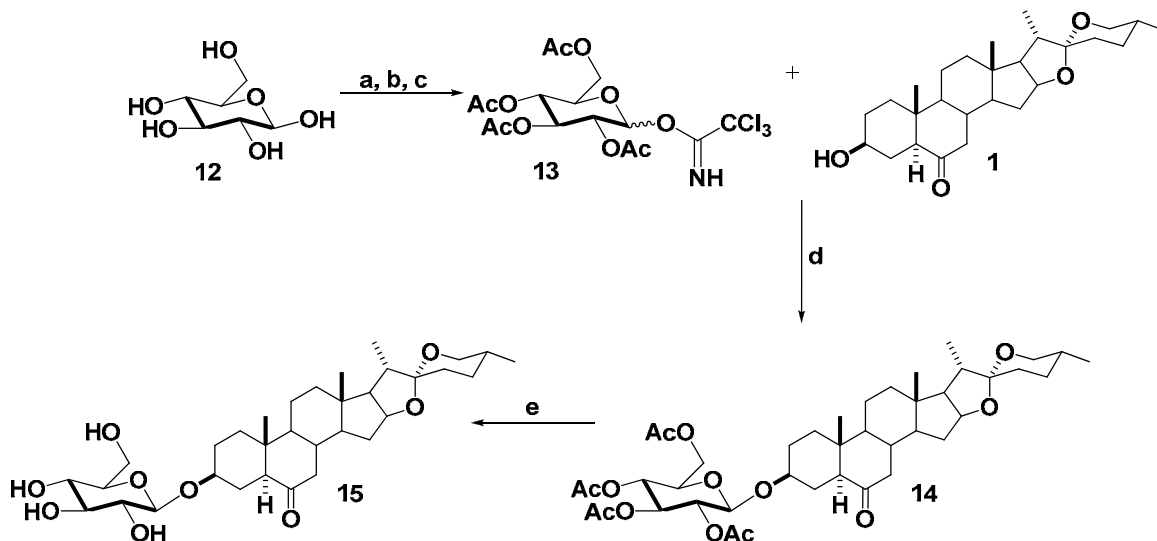
A plausible reaction mechanism is proposed (Scheme 3) to explain the results under the action of SN: in *Path A*, one electron from SN promotes the formation of the radical anion *i* that loses a sulfinate moiety through the homolytic cleavage of the S-O bond. One more electron from SN transforms the steroidal radical *ii*, into the alkoxide *iii* which traps a proton during the quenching to yield the 3 $\beta$ -hydroxy-6-oxo moiety (compounds **8** and **1**). In *Path B* the electron from SN is trapped by the carbonyl group obtaining the radical anion *iv* which through the elimination of the hydrogen atom at C-5 generates the enolate *v*; the latter undergoes the elimination of a tosylate group (C-O bond cleavage)<sup>17c</sup> affording the A-cyclosteroid **7** or **11**.



**Scheme 3.** Plausible mechanisms for SN reduction of steroidal 6-oxotoluenesulfonates.

Laxogenin **1** showed to be poorly soluble in EtOH, EtOAc or DMSO; therefore, in order to evaluate **1** in cell cultures, to determine its antiproliferative effect we proceeded to obtain its

glycosylated derivative. The glucolaxogenin was prepared under the standard glycosylation procedure (Scheme 4). The glucosyl donor was prepared from commercially available glucose **12**, which was firstly peracetylated and then selectively deprotected at the anomeric center by means of hydrazine acetate. Finally, the formation of the corresponding 2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl trichloroacetimidate **13** was performed by the treatment with trichloroacetonitrile. The glucosyl donor was obtained in 86% overall yield. Glycosylation of the 3 $\beta$ -OH group of **1** with trichloroacetimidate **13**, promoted by TMSOTf, provided the desired  $\beta$ -glycoside **14** in 68% isolated yield. The presence of the 2-*O*-acetyl group on donor **13** ensured the exclusive formation of the  $\beta$ -anomer. Final removal of the acetyl groups on the sugar residue of **14** with NaOMe in MeOH furnished glucolaxogenin **15**.

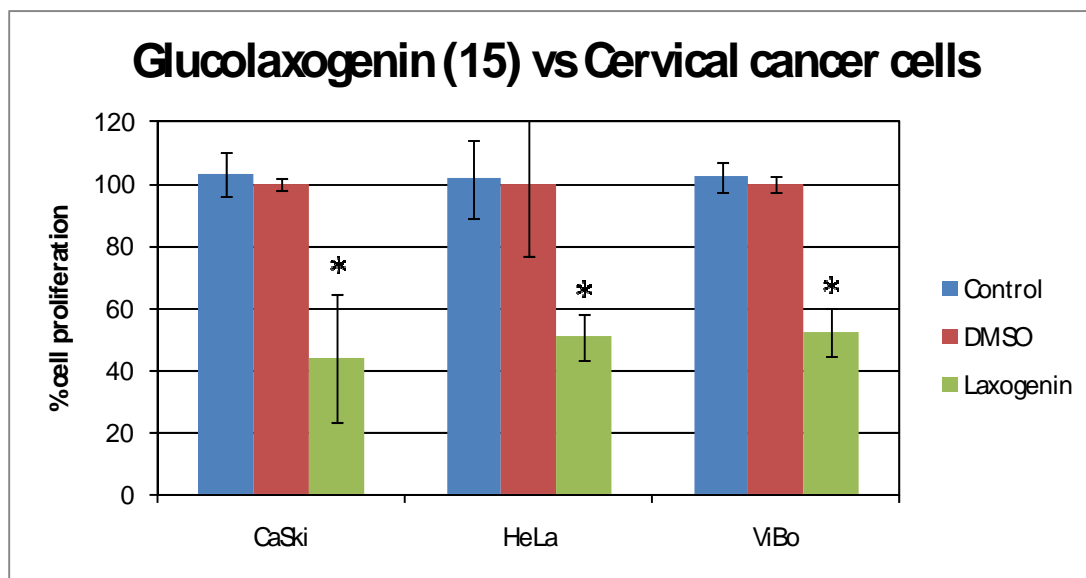


**Scheme 4.** Reagents and conditions: **a**: Ac<sub>2</sub>O, I<sub>2</sub>, rt, quantitative. **b**: Hydrazine acetate, DMF, rt, 88%. **c**: CCl<sub>3</sub>CN, DBU, CH<sub>2</sub>Cl<sub>2</sub>, rt, 90%. **d**: TMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, -20 °C, 68%. **e**: NaOMe, MeOH, rt, quantitative.

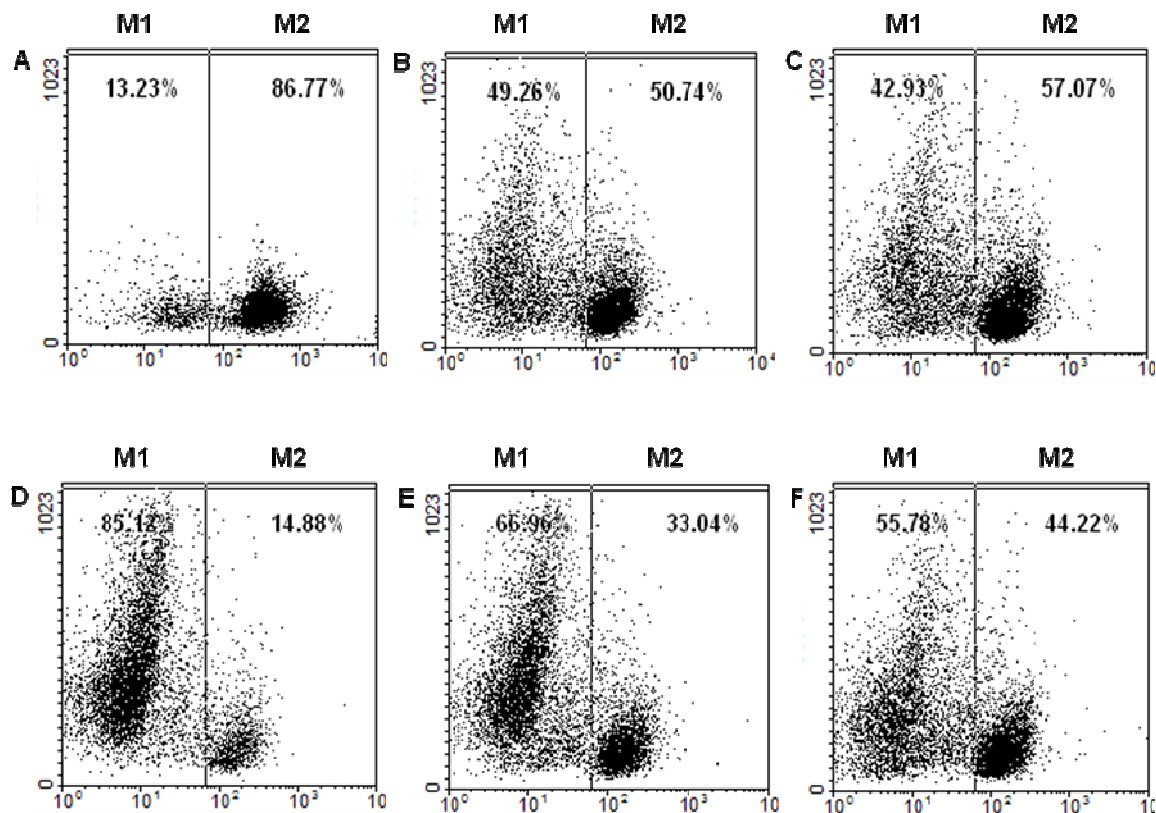
The inhibitory effect of compound **15** (Table 1, Figure 2) on the proliferation of cervical cancer cells HeLa, CaSki and ViBo was determined. Cells were cultured in 96 well-plates and treated with **15**. Antiproliferative activity (IC<sub>50</sub>) was determined after 24 hours by staining with crystal violet dye. It is well known that some compounds used in chemotherapy such as cisplatin present antiproliferative activity and apoptosis in cervical cancer cells like HeLa and Caski in a nM range (around 40).<sup>20</sup>

**Table 1.** *In vitro* antiproliferative activity (IC<sub>50</sub>, mM) of glucolaxogenin.

Cell lines	Glucolaxogenin (15)
HeLa	0.094
CaSki	0.120
ViBo	0.169

**Figure 2.** Experimental data is presented as the mean  $\pm$  S.D. of three independent experiments with six repetitions. \* $p < 0.05$  vs 0  $\mu\text{g/mL}$  ("Student's" t- test).

However, major compounds used currently in chemotherapy present problems for selective activity towards malignant cells and produce undesirable secondary effects, and therefore immune system is usually affected. For this reason, glucolaxogenin effect on the proliferation of enriched lymphocyte population (ELP) was assessed. ELPs from normal blood donor were labeled with 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE), stimulated with phytohemagglutinin (PHA), or treated with glucolaxogenin **15**, and cultured for 72 hours. Cells were harvested and analyzed by flow cytometry; data were processed through CellQuest software. The effect of **15** on proliferative potential of ELPs is shown in Figure 3, expressing that in normal conditions, proliferating cells were 49.26% (Figure 3-B). When lymphocytes were treated with the higher concentration of **15** (0.169 mM for ViBo), proliferating cells were 55.78% (Figure 3-F). At lower concentrations (0.094 mM for HeLa and 0.120 mM for CaSki) cells were surprisingly induced to proliferate, reaching 85.17 and 66.96% (Figures 3-D and 3-E respectively).



**Figure 3.** M1 is the proliferating cells region, and M2 is the non proliferating cells region. A) ELPs untreated, B) ELPs in the presence of PHA stimulation, C) ELPs treated with 10  $\mu$ L of DMSO, D) ELPs treated with 0.094 mM of **15**, E) ELPs treated with 0.120 mM of **15**, F) ELPs treated with 0.169 mM of **15**

## Conclusions

The 3 $\beta$ -hydroxy-6-oxo steroidal moiety was obtained starting from diosgenin and cholesterol, using a three-step procedure in high overall yield. The reported conditions for the oxidative-hydroboration and the subsequent oxidation of the resulting alcohols were optimized and the reduction of tosylates was afforded by sodium naphthalenide. We achieved the selective fission of either the S-O or C-O bond in dependence of the temperature. A likely reaction mechanism to explain the generation of all products is proposed. When this procedure was applied to diosgenin **9**, the naturally occurring laxogenin **1** was obtained in 82% overall yield. Glucolaxogenin **15** was synthesized in order to evaluate its antiproliferative activity in cervical cancer cell lines HeLa, CaSki and ViBo and in non tumoral cells (lymphocytes). Remarkably, while **15** affected the proliferation of these cell cultures, normal cells were induced to proliferate at the same concentrations. This selective action gives to glucolaxogenin a high potential for therapeutic use.

## Experimental Section

**General.** Melting points were obtained on a Melt-temp apparatus and were not corrected.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded at 400 and 100 MHz respectively on a Varian Mercury spectrometer. The spectra were registered in  $\text{CDCl}_3$  and referenced to TMS. The chemical shift values are reported as ppm units and coupling constants are expressed in Hertz (Hz). All assignments were confirmed with the aid of two dimensional experiments (COSY, HSQC and HMBC). HRMS data were taken on a JEOL The MStation spectrometer. Optical rotations were measured in a Perkin Elmer 241 polarimeter.

### Antiproliferative activity

Three different cervical cancer cell lines were used: HeLa, CaSki and ViBo. Cells were obtained from American Type Culture Collection (ATCC Rockville, MD). The cervical cancer cell lines were cultured in RPMI-1640 medium containing 5% NCS (Newborn Calf Serum). Assays were performed by seeding 7500 cells in 96-well tissue culture plates in a volume of 100  $\mu\text{L}$  of RPMI-1640 medium supplemented with 5% NCS per well. The antiproliferative effect was evaluated 24 hours after the addition of compound **15** by crystal violet staining.

### CSFE labeling assay

Heparinized blood samples were obtained from healthy human volunteers. Peripheral blood mononuclear cells (PBMCs) were isolated using standard Hypaque (Sigma-aldrich USA) density gradient centrifugation. PBMCs were washed twice with RPMI 1640 (GIBCO USA) medium containing 10% fetal bovine serum (FBS; from GIBCO, USA), penicillin (100 U/mL) and streptomycin (100 U/mL). The lymphocyte population was further enriched (ELP) by the elimination of the adherent cells (cells were incubated at 37  $^\circ\text{C}$ , 5%  $\text{CO}_2$  for 1 hour, and no adherent cells were harvested). ELPs were re-suspended into RPMI-1640 medium at a concentration of  $1 \times 10^6$  cells/mL. CFSE (from Sigma-Aldrich, USA) was added to the cell suspension at the final concentration of 12  $\mu\text{M}$  and incubated for 15 min at room temperature in the darkness. Labeling was completed by adding the same volume of FBS to quench the free CFSE during 5 min at room temperature. Labeled cells were washed 5 times with sterile phosphate buffer solution (PBS) containing 10% FBS, counted and re-suspended into RPMI-1640 medium at  $1 \times 10^6$  cells/mL. Unstimulated, PHA-stimulated or treated cells were plated at  $2 \times 10^5$  cells/well in 96-well flat-bottomed cell culture plates, and five replicate samples for each treated amount were prepared. The cells were incubated in a 5%  $\text{CO}_2$  incubator at 37  $^\circ\text{C}$  for 72 hours. Cultured cells were then harvested, washed twice with PBS and fixed with 1% formaldehyde, then analyzed using flow cytometry, acquiring a minimal of 20,000 events from each sample; data analysis was performed using CellQuest (Becton-Dickinson) software.

**Cholesteryl tosylate 5a.** Pyridine (6.3 mL, 77.6 mmol) was added dropwise to a solution of **5**, (3 g, 7.8 mmol), and *p*-TsCl (14.8 g, 77.6 mmol) in  $\text{CH}_2\text{Cl}_2$  (30 mL). The reaction mixture was maintained in the darkness under stirring for a period of 3 h. The organic phase was washed with



an ice cooled solution of 5% HCl (5x50 mL) and H<sub>2</sub>O (2x50 mL); dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to afford 4.2 g (quantitative yield) of the desired tosylate **5a**. This compound was found to quickly decompose and therefore it was immediately used in the next step without further purification.

**6-Oxo-5a-cholestan-3β-yl tosylate 6.** To a solution of **5a** (2 g, 3.7 mmol) in THF (30 mL) NaBH<sub>4</sub> was added (0.8 g, 21.2 mmol). The system was sealed under argon atmosphere, and maintained at room temperature; then, BF<sub>3</sub>·OEt<sub>2</sub> (1.4 mL, 11.2 mmol) was added dropwise and the reaction mixture was maintained for 2 h. The mixture was concentrated under vacuum and then dissolved in a solution of KOH/MeOH (2%, 100 mL); then, 10 mL of 35% H<sub>2</sub>O<sub>2</sub> were added dropwise and the reaction mixture was stirred for 1 h. The addition of water produced a precipitate which was filtered off and dissolved in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). To the intermediate alcohol, PDC (1.1 eq) was added and the reaction was stirred at room temperature for 4 h. The crude was filtered off through a pad of celite and eluted with 50 mL of CH<sub>2</sub>Cl<sub>2</sub>, concentrated under vacuum and purified by column chromatography with hexanes:EtOAc (9:1) to afford 1.9 g (90%) of compound **6**; colorless solid; mp 171-172 °C (dec); [ $\alpha$ ]<sub>D</sub> = -5.2 (c = 1.3, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub> δ): 7.76 (2H, m, H-ortho), 7.30 (2H, m, H-meta), 4.39 (1H, m, H-3), 2.43 (3H, s, CH<sub>3</sub>-Ts), 2.28 (1H, dd,  $J_{\text{gem}}$  = 13.1 Hz,  $J_{8,7eq}$  = 4.4 Hz, H-7eq), 2.13 (1H, dd,  $J_{4eq,5}$  = 4.0 Hz,  $J_{4ax,5}$  = 12.0 Hz, H-5), 2.02 (1H, ddd,  $J_{\text{gem}}$  = 12.0 Hz,  $J_{11ax,12eq}$  =  $J_{11eq,12eq}$  = 4 Hz, H-12eq), 1.89 (1H, dd,  $J_{\text{gem}}$  = 13.1 Hz,  $J_{8,7ax}$  = 13.1 Hz, H-7ax), 0.90 (3H, d,  $J_{20,21}$  = 6.5 Hz, CH<sub>3</sub>-21), 0.87 (3H, d,  $J_{25,27}$  = 1.9 Hz, CH<sub>3</sub>-27), 0.85 (3H, d,  $J_{25,26}$  = 1.8 Hz, CH<sub>3</sub>-26), 0.72 (3H, s, CH<sub>3</sub>-19), 0.64 (3H, s, CH<sub>3</sub>-18). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>, δ): 36.5 (C-1), 28.1 (C-2), 81.5 (C-3), 27.0 (C-4), 56.4 (C-5), 209.2 (C-6), 46.6 (C-7), 37.9 (C-8), 53.7 (C-9), 40.7 (C-10), 21.8 (C-11), 39.5 (C-12), 43.0 (C-13), 56.1 (C-14), 24.0 (C-15), 29.8 (C-16), 56.7 (C-17), 12.3 (C-18), 13.1 (C-19), 35.7 (C-20), 18.7 (C-21), 36.1 (C-22), 23.9 (C-23), 39.4 (C-24), 28.1 (C-25), 22.7 (C-26), 22.9 (C-27), 144.3 (C-*ipso*), 127.4 (C-ortho), 129.7 (C-meta), 134.3 (C-para), 21.5 (CH<sub>3</sub>-Ts). HRMS for C<sub>34</sub>H<sub>52</sub>O<sub>4</sub>S calcd: 557.3620 [M+H]<sup>+</sup>, found: 557.3616.

**3a,5-Cyclo-5a-cholestan-6-one 7.** A solution of the radical anion sodium naphthalenide was prepared dissolving naphthalene (158 mg, 1.23 mmol) and sodium (24 mg, 1.03 mmol), in dry THF (5.1 mL) under argon atmosphere, at room temperature. After stirring for 1 h, a deep green color appeared. A solution of **6** (100 mg, 0.18 mmol) in dry THF (0.3 mL) was added to the green solution which faded immediately; the mixture was stirred at room temperature for 10 min more and quenched with H<sub>2</sub>O (5 mL). The reaction mixture was washed with brine (3x30 mL) and H<sub>2</sub>O (3x30 mL) and extracted with EtOAc, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated; chromatographed with hexanes: EtOAc (98:2) to yield 66 mg (95%) of compound **7**; colorless solid; mp 96-97 °C; [ $\alpha$ ]<sub>D</sub> = 45 (c = 1.0, CHCl<sub>3</sub>). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub> δ): 2.43 (1H, d,  $J_{\text{gem}}$  = 12.0 Hz, H-7eq), 2.05 (1H, ddd,  $J_{\text{gem}}$  = 12.4 Hz,  $J_{11ax,12ax}$  = 9.6 Hz,  $J_{11eq,12ax}$  = 3.2 Hz, H-12ax), 1.00 (3H, s, CH<sub>3</sub>-19), 0.92 (3H, d,  $J_{20,21}$  = 6.8 Hz, CH<sub>3</sub>-21), 0.87 (3H, d,  $J_{25,27}$  = 2.0 Hz, CH<sub>3</sub>-27), 0.86 (3H, d,  $J_{25,26}$  = 2.4 Hz, CH<sub>3</sub>-26), 0.71 (3H, s, CH<sub>3</sub>-18), 0.71 (1H, dd,  $J_{\text{gem}}$  =  $J_{3,4\text{pseudo-ax}}$  = 5.2 Hz, H-4pseudo-ax). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub> δ): 33.5 (C-1), 26.0 (C-2), 35.4 (C-3), 11.8 (C-4), 46.8 (C-5), 209.4 (C-6), 44.9 (C-7), 34.9 (C-8), 46.1 (C-9), 42.8 (C-10), 22.7 (C-11), 39.8 (C-

12), 46.4 (C-13), 56.1 (C-14), 24.1 (C-15), 28.2 (C-16), 57.0 (C-17), 12.2 (C-18), 19.8 (C-19), 35.8 (C-20), 18.8 (C-21), 36.2 (C-22), 23.9 (C-23), 39.5 (C-24), 28.1 (C-25), 22.9 (C-26), 23.0 (C-27). HRMS for  $C_{27}H_{44}O$  calcd: 385.3426  $[M+H]^+$ , found: 385.3421.

**3 $\beta$ -Hydroxy-5 $\alpha$ -cholestan-6-one 8. Starting from 6.** A solution of the radical anion sodium naphthalenide was prepared as described above, and cooled down to -80 °C. Then compound 7 (100 mg, 0.18 mmol) in dry THF (0.3 mL) was introduced. The mixture was stirred for 10 min more before quenching with  $H_2O$  (5 mL). The reaction mixture was washed with brine (3x30 mL),  $H_2O$  (3x30 mL) and extracted with EtOAc, dried over anhydrous  $Na_2SO_4$  and evaporated; chromatographed with hexanes:EtOAc (9:1) to yield 67 mg (92%) of compound 8.

**Starting from 7.** To 100 mg (0.26 mmol) of 7 in 5 mL of acetic acid was added 1 mL of 5 N sulfuric acid solution and the mixture was refluxed for 2 h. The solution was cooled and diluted with crushed ice on water, and the precipitate was collected and dried. The precipitate was suspended in 10 mL of 4% potassium carbonate in methanol and 3 mL of water, and heated under reflux conditions for 3 h. Methanol was distilled off until the compound began to precipitate from solution. The solution was cooled, and diluted with water and ice, and the precipitate was collected and dried under vacuum. Chromatography was performed with hexanes:EtOAc (9:1) to yield 58 mg (55%) of compound 8; colorless solid; mp 140-142 °C;  $[\alpha]_D = -5.6$  ( $c = 1.2$ ,  $CHCl_3$ ).  $^1H$  NMR (400 MHz,  $CDCl_3$   $\delta$ ): 3.56 (1H, m, H-3), 2.31 (1H, dd,  $J_{gem} = 12.8$  Hz,  $J_{8,7eq} = 4.8$  Hz, H-7eq), 2.19 (1H, dd,  $J_{4ax,5} = 12.8$  Hz,  $J_{4eq,5} = 2.8$  Hz, H-5), 0.91 (3H, d,  $J_{20,21} = 6.8$  Hz,  $CH_3$ -21), 0.87 (3H, d,  $J_{25,27} = 1.6$  Hz,  $CH_3$ -27), 0.85 (3H, d,  $J_{25,26} = 2.0$  Hz,  $CH_3$ -26), 0.75 (3H, s,  $CH_3$ -19), 0.66 (3H, s,  $CH_3$ -18).  $^{13}C$  NMR (100 MHz,  $CDCl_3$   $\delta$ ): 36.7 (C-1), 30.7 (C-2), 70.6 (C-3), 30.1 (C-4), 56.1 (C-5), 210.7 (C-6), 46.8 (C-7), 38.0 (C-8), 53.9 (C-9), 41.0 (C-10), 21.6 (C-11), 39.5 (C-12), 43.0 (C-13), 56.7 (C-14), 24.1 (C-15), 28.1 (C-16), 56.8 (C-17), 12.1 (C-18), 13.2 (C-19), 35.7 (C-20), 18.7 (C-21), 36.1 (C-22), 23.9 (C-23), 39.5 (C-24), 28.1 (C-25), 22.6 (C-26), 22.9 (C-27). HRMS for  $C_{27}H_{46}O_2$  calcd: 403.3531  $[M+H]^+$ , found: 403.3524.

**Diosgenin tosylate 9a.** Pyridine (5.83 mL, 72.40 mmol) was added dropwise to a solution of 9, (3 g, 7.24 mmol), and *p*-TsCl (13.80 g, 72.40 mmol) in  $CH_2Cl_2$  (30 mL). The reaction mixture was maintained in the darkness under stirring for a period of 3 h. The organic phase was washed with an ice cooled solution of 5% HCl (5x50 mL) and  $H_2O$  (2x50 mL); dried over anhydrous  $Na_2SO_4$  and concentrated under vacuum to afford 4.12 g (quantitative yield) of the desired tosylate 9a. This compound was found to quickly decompose and therefore was immediately used in the next step without further purification.

**(25R)-6-Oxo-5 $\alpha$ -spirostan-3 $\beta$ -yl tosylate 10.** To a solution of 9a (2 g, 3.52 mmol) in THF (30 mL)  $NaBH_4$  was added (0.8 g, 21.2 mmol). The system was sealed under argon atmosphere and maintained at room temperature; then,  $BF_3 \cdot OEt_2$  (1.4 mL, 11.2 mmol) was added dropwise and the reaction mixture was maintained for 2 h. The mixture was concentrated under vacuum and then dissolved in a solution of KOH/MeOH (2%, 100 mL); then, 10 mL of 35%  $H_2O_2$  were added dropwise and the reaction mixture was stirred for 1 h. The addition of water produced a precipitate which was filtered off and dissolved in  $CH_2Cl_2$  (20 mL). To the intermediate alcohol,

PDC (1.1 eq) was added and the reaction was stirred at room temperature for 4 h. The crude was filtered off through a pad of celite and eluted with 50 mL of  $\text{CH}_2\text{Cl}_2$ , concentrated under vacuum and purified by column chromatography with hexanes:EtOAc (85:15) to afford 1.9 g (92%) of compound **10**; colorless needles; mp 153-155 °C;  $[\alpha]_D = -60.3$  ( $c = 1.1$ ,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$   $\delta$ ): 7.75 (2H, m, H-*ortho*), 7.30 (2H, m, H-*meta*), 4.38 (1H, m, H-16), 4.38 (1H, m, H-3), 3.45 (1H, ddd,  $J_{25,26eq} = 4.4$  Hz,  $J_{gem} = 10.8$  Hz,  $J_{24,26eq} = 1.6$  Hz, H-26eq), 3.34 (1H, dd,  $J_{25,26ax} = J_{gem} = 10.8$  Hz, H-26ax), 2.31 (1H, d,  $J_{7ax,7eq} = 8.8$  Hz, H-7eq), 2.14 (1H, dd,  $J_{4eq,5} = 3.2$  Hz,  $J_{4ax,5} = 12.4$  Hz, H-5), 2.43 (3H, s,  $\text{CH}_3$ -Ts), 0.96 (3H, d,  $J_{20,21} = 6.8$  Hz,  $\text{CH}_3$ -21), 0.78 (3H, d,  $J_{25,27} = 6.4$  Hz,  $\text{CH}_3$ -27), 0.75 (3H, s,  $\text{CH}_3$ -19), 0.74 (3H, s,  $\text{CH}_3$ -18).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$   $\delta$ ): 36.3 (C-1), 28.0 (C-2), 81.2 (C-3), 26.9 (C-4), 56.3 (C-5), 208.7 (C-6), 46.6 (C-7), 37.3 (C-8), 53.6 (C-9), 40.6 (C-10), 21.3 (C-11), 39.4 (C-12), 40.9 (C-13), 56.3 (C-14), 31.6 (C-15), 80.3 (C-16), 61.9 (C-17), 13.1 (C-18), 16.4 (C-19), 41.6 (C-20), 14.5 (C-21), 109.1 (C-22), 31.3 (C-23), 28.8 (C-24), 30.3 (C-25), 66.8 (C-26), 17.2 (C-27), 144.3 (C-*ipso*), 127.4 (C-*ortho*), 129.6 (C-*meta*), 134.2 (C-*para*), 21.7 ( $\text{CH}_3$ -Ts). HRMS for  $\text{C}_{34}\text{H}_{48}\text{O}_6\text{S}$  calcd: 585.3205  $[\text{M}+\text{H}]^+$ , found: 585.3199.

**(25R)-3 $\alpha$ ,5-Cyclo-5 $\alpha$ -spirostan-6-one 11.** A solution of the radical anion sodium naphthalenide was prepared as described above. Then a solution of **10** (100 mg, 0.17 mmol) in dry THF (0.3 mL) was introduced to the complex and the mixture was stirred at room temperature for 10 min more before quenching with  $\text{H}_2\text{O}$  (5 mL). The reaction mixture was washed with brine (3x30 mL) and  $\text{H}_2\text{O}$  (3x30 mL) and extracted with EtOAc, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated; chromatographed with hexanes:EtOAc (95:5) to yield 65 mg (91%) of compound **11**; colorless solid; mp 183-184 °C;  $[\alpha]_D = -49$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$   $\delta$ ): 4.41 (1H, m, H-16), 3.47 (1H, ddd,  $J_{24,26eq} = 2$  Hz,  $J_{25,26eq} = 4.4$  Hz,  $J_{gem} = 10.8$  Hz, H-26eq), 3.36 (1H, dd,  $J_{25,26ax} = J_{gem} = 10.8$  Hz, H-26ax), 2.46 (1H, dd,  $J_{gem} = 16.4$  Hz,  $J_{8,7eq} = 4.4$  Hz, H-7eq), 2.11 (1H, m, H-8), 1.02 (3H, s,  $\text{CH}_3$ -19), 0.98 (3H, d,  $J_{20,21} = 6.8$  Hz,  $\text{CH}_3$ -21), 0.83 (3H, s,  $\text{CH}_3$ -18), 0.79 (3H, d,  $J_{25,27} = 6.4$  Hz,  $\text{CH}_3$ -27), 0.73 (1H, dd,  $J_{gem} = J_{3,4pseudo-ax} = 4.8$  Hz, H-4pseudo-ax).  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$   $\delta$ ): 33.5 (C-1), 25.9 (C-2), 35.5 (C-3), 11.8 (C-4), 46.8 (C-5), 208.9 (C-6), 44.9 (C-7), 34.3 (C-8), 46.1 (C-9), 40.7 (C-10), 22.7 (C-11), 39.8 (C-12), 46.3 (C-13), 56.7 (C-14), 31.6 (C-15), 80.5 (C-16), 62.0 (C-17), 16.6 (C-18), 19.9 (C-19), 41.7 (C-20), 14.6 (C-21), 109.2 (C-22), 31.4 (C-23), 28.8 (C-24), 30.3 (C-25), 66.8 (C-26), 17.2 (C-27). HRMS for  $\text{C}_{27}\text{H}_{40}\text{O}_3$  calcd: 413.3011  $[\text{M}+\text{H}]^+$ , found: 413.3005.

**(25R)-3 $\beta$ -Hydroxy-5 $\alpha$ -spirostan-6-one (1, Laxogenin). Starting from 10.** A solution of the radical anion sodium naphthalenide was prepared as described above, and cooled down to -80 °C. Then compound **10** (100 mg, 0.17 mmol) in dry THF (0.3 mL) was introduced. The mixture was stirred for 10 min more before quenching with  $\text{H}_2\text{O}$  (5 mL). The crude was washed with brine (3x30 mL),  $\text{H}_2\text{O}$  (3x30 mL) and extracted with EtOAc, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and evaporated; chromatographed with hexanes:EtOAc (7:3) to yield 66 mg (89%) of laxogenin **1**.

**Starting from 11.** The same procedure described above for the synthesis of **8** starting from **7** was followed to obtain 50 mg (48%) of compound **1** after chromatography with hexanes:EtOAc (7:3); colorless solid; mp 210-212 °C;  $[\alpha]_D = -84$  ( $c = 1.0$ ,  $\text{CHCl}_3$ ).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$   $\delta$ ):

4.40 (1H, m, H-16), 3.56 (1H, m, H-3), 3.47 (1H, ddd,  $J_{24,26eq} = 2.0$  Hz,  $J_{25,26eq} = 4.4$  Hz,  $J_{gem} = 11.2$  Hz, H-26eq), 3.35 (1H, dd,  $J_{25,26ax} = J_{gem} = 11.2$  Hz, H-26ax), 2.34 (1H, d,  $J_{gem} = 11.6$  Hz, H-7eq), 2.20 (1H, dd,  $J_{4ax,5} = 12.4$  Hz,  $J_{4eq,5} = 2.8$  Hz, H-5), 0.97 (3H, d,  $J_{20,21} = 6.8$  Hz, CH<sub>3</sub>-21), 0.79 (3H, d,  $J_{25,27} = 6.8$  Hz, CH<sub>3</sub>-27), 0.77 (3H, s, CH<sub>3</sub>-19), 0.77 (3H, s, CH<sub>3</sub>-18). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub> δ): 36.7 (C-1), 30.7 (C-2), 70.5 (C-3), 30.1 (C-4), 56.8 (C-5), 210.3 (C-6), 46.8 (C-7), 37.4 (C-8), 53.9 (C-9), 41.0 (C-10), 21.4 (C-11), 39.5 (C-12), 41.0 (C-13), 56.5 (C-14), 31.6 (C-15), 80.4 (C-16), 62.0 (C-17), 16.5 (C-18), 13.3 (C-19), 41.6 (C-20), 14.6 (C-21), 109.2 (C-22), 31.4 (C-23), 28.8 (C-24), 30.3 (C-25), 66.9 (C-26), 17.2 (C-27). HRMS for C<sub>27</sub>H<sub>42</sub>O<sub>4</sub> calcd: 431.3117 [M+H]<sup>+</sup>, found: 431.3112. The spectroscopic data are in accordance with those in the literature.<sup>3b,4,8</sup>

**2,3,4,6-Tetra-*O*-Acetyl-D-glucopyranosyl trichloroacetimidate 13.** Glucose **12** (2 g) was suspended in Ac<sub>2</sub>O (10 mL) and stirred. Iodine (100 mg) was added and stirring was continued for 5 minutes until TLC showed the reaction was completed. The reaction mixture was poured into ice-cold diluted sodium thiosulphate solution (50 mL). The organic phase was extracted with EtOAc and washed with saturated aqueous NaHCO<sub>3</sub> (2x50 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and evaporated affording quantitatively 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose. The latter crude product and hydrazine acetate (1.45 g, 15.71 mmol) in DMF (20 mL) were stirred at room temperature for 2 h, the mixture was diluted with EtOAc, washed with water (3x60 mL) and brine (3x60 mL), dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum to afford 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (3.1 g, 88%). This compound was immediately used in the next step without further purification. 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose, CCl<sub>3</sub>CN (5.4 mL, 54.0 mmol) and DBU (catalytic) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) were stirred at room temperature for 3 h, the solution was concentrated under vacuum and the resulting residue was purified by flash column chromatography (hexanes:EtOAc 8:2 with 1% of triethylamine,) to give trichloroacetimidate **13** (4.0 g, 90 %) as a syrup. This compound was found to quickly decompose in the NMR tube and therefore it was immediately used in the next step without further purification.

**(25R)-3β-(2,3,4,6-Tetra-*O*-Acetyl-β-D-glucopyranosyloxy)-5α-spirostan-6-one 14.** A solution of donor **13** (1.34 g, 2.79 mmol), laxogenin (**1**) (1 g, 2.32 mmol) and 4 Å molecular sieves (100 mg) in dry CH<sub>2</sub>Cl<sub>2</sub> (20 mL) was stirred at room temperature for 15 min and then cooled down to -20 °C. A solution of TMSOTf (50.4 μL, 0.28 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (1 mL) was slowly added to the reaction. After being stirred for another 1 h, the reaction was quenched with triethylamine (40 μL) and filtered off. The filtrates were concentrated under vacuum to give a residue, which was purified by flash column chromatography (hexanes:EtOAc, 6:4) to afford **14** (1.2 g, 68%) as a white powder;  $[\alpha]_D -32.4$  (*c* 1.0, CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub> δ): 5.12 (1H, m, H-4'), 5.01 (1H, m, H-3'), 4.87 (1H, m, H-2'), 4.55 (1H, d,  $J_{2',1'} = 7.8$  Hz, H-1'), 4.32 (1H, m, H-16), 4.18 (1H, dd,  $J_{gem} = 12.2$  Hz,  $J_{5'a,6'a} = 1.7$  Hz, H-6'a), 4.03 (1H, dd,  $J_{gem} = 12.2$  Hz,  $J_{5',6'b} = 5.4$  Hz, H-6'b), 3.61 (1H, m, H-5'), 3.51 (1H, m, H-3), 3.42 (1H, ddd,  $J_{24,26eq} = 1.8$  Hz,  $J_{25,26eq} = 4.5$  Hz,  $J_{gem} = 10.8$  Hz, H-26eq), 3.29 (1H, dd,  $J_{25,26ax} = J_{gem} = 10.8$  Hz, H-26ax), 2.01-1.92 (12H, Me-OAc), 0.90 (3H, d,  $J_{20,21} = 7.0$  Hz, CH<sub>3</sub>-21), 0.75 (3H, d,  $J_{25,27} = 6.5$  Hz, CH<sub>3</sub>-27), 0.72 (3H, s, CH<sub>3</sub>-18), 0.70 (3H, s, CH<sub>3</sub>-19). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub> δ): 36.7 (C-1), 30.2 (C-2), 78.2

(C-3), 32.8 (C-4), 56.2 (C-5), 210.1 (C-6), 46.5 (C-7), 39.1 (C-8), 53.0 (C-9), 41.7 (C-10), 21.3 (C-11), 41.1 (C-12), 42.3 (C-13), 55.0 (C-14), 32.3 (C-15), 80.1 (C-16), 62.9 (C-17), 16.8 (C-18), 12.9 (C-19), 46.1 (C-20), 14.9 (C-21), 108.9 (C-22), 30.1 (C-23), 27.2 (C-24), 31.4 (C-25), 66.3 (C-26), 17.0 (C-27), 98.6 (C-1'), 71.5 (C-2'), 68.2 (C-3'), 73.4 (C-4'), 71.3 (C-5'), 62.1 (C-6'), 170.3-169.5 (C=O-OAc), 20.1-21.7 (CH<sub>3</sub>-OAc). HRMS for C<sub>41</sub>H<sub>60</sub>O<sub>13</sub> calcd: 761.4068 [M+H]<sup>+</sup>, found: 761.4059.

**(25R)-3β-(β-D-Glucopyranosyloxy)-5α-spirostan-6-one (15, Glucolaxogenin).** Compound **14** (1 g, 1.32 mmol) was dissolved in MeOH (5 mL) and NaOMe was added dropwise (1 mL, 1 M in MeOH). The mixture was stirred 15 min at rt, neutralized with Dowex-50 (H<sup>+</sup>) resin and filtered off, and the filtrates were concentrated. Purification of the product by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>:MeOH, 95:5) gave **15** (934 mg, quantitative) as a white solid; [α]<sub>D</sub> -67.8 (c 1.0, CH<sub>3</sub>OH); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD δ): 4.39 (1H, m, H-16), 4.37 (1H, d, *J*<sub>2',1'</sub> = 7.8 Hz, H-1'), 3.82 (1H, dd, *J*<sub>gem</sub> = 12.0 Hz, *J*<sub>5'a,6'a</sub> = 1.8 Hz, H-6'a), 3.68 (1H, m, H-3), 3.61 (1H, dd, *J*<sub>gem</sub> = 12.0 Hz, *J*<sub>5',6'b</sub> = 5.6 Hz, H-6'b), 3.42 (1H, ddd, *J*<sub>24,26eq</sub> = 2.0 Hz, *J*<sub>25,26eq</sub> = 4.4 Hz, *J*<sub>gem</sub> = 10.8 Hz, H-26eq), 3.32 (1H, m, H-4'), 3.29 (1H, m, H-26ax), 3.24 (1H, m, H-3'), 3.22 (1H, m, H-5'), 3.12 (1H, dd, *J*<sub>1',2'</sub> = 7.8 Hz, *J*<sub>3',2'</sub> = 9.1 Hz, H-2'), 2.32 (1H, dd, *J*<sub>4eq,5</sub> = 2.4 Hz, *J*<sub>4ax,5</sub> = 12.4 Hz, H-5), 2.21 (1H, dd, *J*<sub>gem</sub> = 13.0 Hz, *J*<sub>8,7eq</sub> = 4.6 Hz, H-7eq), 2.09 (1H, dd, *J*<sub>gem</sub> = *J*<sub>8,7ax</sub> = 13.0 Hz, H-7ax), 1.89 (1H, m, H-20), 0.94 (3H, d, *J*<sub>20,21</sub> = 7.0 Hz, CH<sub>3</sub>-21), 0.78 (3H, s, CH<sub>3</sub>-18), 0.77 (3H, d, *J*<sub>25,27</sub> = 6.5 Hz, CH<sub>3</sub>-27), 0.75 (3H, s, CH<sub>3</sub>-19). <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD δ): 36.9 (C-1), 30.0 (C-2), 78.6 (C-3), 32.6 (C-4), 57.6 (C-5), 213.4 (C-6), 47.6 (C-7), 39.0 (C-8), 57.6 (C-9), 42.3 (C-10), 22.6 (C-11), 40.8 (C-12), 42.3 (C-13), 55.0 (C-14), 32.6 (C-15), 82.1 (C-16), 63.8 (C-17), 17.0 (C-18), 13.6 (C-19), 43.0 (C-20), 15.0 (C-21), 110.7 (C-22), 30.1 (C-23), 27.5 (C-24), 31.6 (C-25), 68.0 (C-26), 17.7 (C-27), 102.4 (C-1'), 75.3 (C-2'), 71.8 (C-3'), 78.2 (C-4'), 74.0 (C-5'), 62.9 (C-6'). HRMS for C<sub>33</sub>H<sub>52</sub>O<sub>9</sub> calcd: 593.3645 [M+H]<sup>+</sup>, found: 593.3634.

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## References

1. (a) Cutler, H. G.; Yokota, T.; Adam, G. *Brassinosteroids. Chemistry, Bioactivity, and Applications*. American Chemical Society: Washington. 1991. (b) Khripach, V. A.; Zhabinskii, V. N.; de Groot, A. E. *Brassinosteroids, A New Class of Plant Hormones*. Academic Press: San Diego. 1999. (c) Sakurai, A.; Yokota, T.; Clouse, S. D. (Eds). *Brassinosteroids. Steroidal Plant Hormones*. Springer-Verlag: Tokyo. 1999. (d) Hayat, S.;

- Ahmad, A. *Brassinosteroids. Bioactivity and Crop Productivity*. Kluwer Academic Publ: Dordrecht. 2003. (e) Adam, G.; Marquardt, V. *Phytochemistry* **1986**, 25, 1787.
2. (a) Winter, J. *Stud. Nat. Prod. Chem.* **2001**, 25, 413. (b) Fujioka, S.; Takatsuto, S.; Yoshida, S. *Plant. Physiol.* **2002**, 130, 930. (c) Nakajima, N.; Fujioka, S.; Tanaka, T.; Takatsuto, S.; Yoshida, S. *Phytochemistry* **2002**, 60, 275.
  3. (a) Takatsuto, S. *J. Chem. Soc., Perkin Trans. I* **1986**, 1833. (b) Iglesias-Arteaga, M. A.; Pérez-Gil, R.; Pérez-Martínez, C. S.; Coll-Manchado, F. *J. Chem. Soc., Perkin Trans. I* **2001**, 261. (c) Ramírez, J. A.; Mancusso, R.; Sarno, S.; Galagovsky, L. R. *Molecules* **2000**, 5, 367.
  4. (a) Okanishi, T.; Akahori, A.; Yasuda, F. *Chem. Pharm. Bull.* **1965**, 13, 545. (b) Woo, M. H.; Do, J. C.; Son, K. H. *J. Nat. Prod.* **1992**, 55, 1129. (c) Baba, M.; Ohmura, M.; Kishi, N.; Okada, Y.; Shibata, S.; Peng, J.; Yao, S. S.; Nishino, H.; Okuyama, T. *Biol. Pharm. Bull.* **2000**, 23, 660.
  5. Robaina-Rodríguez, C. M.; Teixeira-Zullo, M. A.; Müller-Queiróz, H.; de Burgos, M.; Alonso-Becerra, E.; Coll-Manchado, F. *Polish J. Chem.* **2006**, 80, 637.
  6. (a) Nishino, H.; Nishino, A.; Satomi, Y.; Takayasu, J.; Hasegawa, T.; Tokuda, H.; Fukuda, T.; Tanaka, H.; Shibata, S.; Fujita, K.; Okuyama, T. *J. Kyoto Pref. Univ. Med.* **1990**, 99, 1159. (b) Okuyama, T.; Matsuda, M.; Masuda, Y.; Baba, M.; Masubuchi, H.; Adachi, M.; Okada, Y.; Hashimoto, T.; Zou, L.; Nishiro, H. *Chin. Pharm. J.* **1995**, 47, 421. (c) He, X.; Wang, N.; Qiu, F.; Yao, X. *Acta Pharm. Sin.* **2003**, 38, 433.
  7. Yu, B.; Tao, H. *J. Org. Chem.* **2002**, 67, 9099.
  8. Iglesias-Arteaga, M. A.; Símuta-López, E. M.; Xochihua-Moreno, S.; Viñas-Bravo, O.; Montiel-Smith, S.; Meza-Reyes, S.; Sandoval-Ramírez, J. *J. Braz. Chem. Soc.* **2005**, 16, 381.
  9. Malíková, J.; Swaczynová, J.; Kolář, Z.; Strnad, M. *Phytochemistry* **2008**, 69, 418.
  10. Wuts, P. G. M.; Greene, T. W. *Greene's Protective Groups in Organic Synthesis*. John Wiley & Sons: Hoboken. 2007; pp 273.
  11. Brown, H. C. *Organic Synthesis via Boranes*. John Wiley & Sons: New York. 1975.
  12. Scott, N. D.; Walker, J. F.; Hansley, V. L. *J. Am. Chem. Soc.* **1936**, 58, 2442.
  13. Pradhan, S. K.; Radhakrishnan, T. V.; Subramanian, R. *J. Org. Chem.* **1976**, 41, 1943.
  14. Clive, D. L. J.; Keshava Murthy, K. S.; Zhang, C.; Hayward, W. D.; Daigneault, S. *J. Chem. Soc., Chem. Commun.* **1990**, 509.
  15. Nicolaou, K. C.; Hwang, C. K.; Duggan, M. E.; Reddy, K. B.; Marron, B. E.; McGarry, D. G. *J. Am. Chem. Soc.* **1986**, 108, 6800.
  16. (a) Marshall, J. A.; Karas, L. J. *J. Am. Chem. Soc.* **1978**, 100, 3615. (b) Oku, A.; Yagi, K. *J. Am. Chem. Soc.* **1974**, 96, 1966. (c) Sargent, G. D.; Tatum Jr., C. M.; Kastner, S. M. *J. Am. Chem. Soc.* **1972**, 94, 7174.
  17. (a) Ji, S.; Gortler, L. B.; Waring, A.; Battisti, A. J.; Bank, S.; Closson, W. D.; Wriede, P. A. *J. Am. Chem. Soc.* **1967**, 89, 5311. (b) Carnahan Jr., J. C.; Closson, W. D. *Tetrahedron Lett.* **1972**, 13, 3447. (c) Ganson, J. R.; Schulenberg, S.; Closson, W. D. *Tetrahedron Lett.* **1970**, 11, 4397. (d) Jarrell, H. C.; Ritchie, R. G. S.; Szarek, W. A.; Jones, J. K. N. *Can. J. Chem.* **1973**, 51, 1767. (e) Closson, W. D.; Wriede, P.; Bank, S. *J. Am. Chem. Soc.* **1966**, 88, 1581.

18. Dodson, R. M.; Riegel, B. *J. Org. Chem.* **1948**, *13*, 424.
19. Thompson, M. J.; Mandava, N. B.; Meudt, W. J.; Lusby, W. R.; Spaulding, D. W. *Steroids* **1981**, *38*, 567.
20. Girón, R. A.; Montaña, L. F.; Escobar, M. L.; López-Marure, R. *FEBS* **2009**, *276*, 5598.