Synthesis of the Cytotoxic Gitogenin 3β-*O*-[2-*O*-(α-L-Rhamnopyranosyl)-β-D-galactopyranoside] and its Congeners

Yingxia Li,*[a] Yichun Zhang,[a] Tiantian Guo,[a] Huashi Guan,[a] Yan Hao,[b] Biao Yu*[b]

[a] Key Laboratory of Marine Drugs, The Ministry of Education of China, Marine Drug and Food Institute, Ocean University of China, Qingdao 266003, P. R. of China
E-mail: liyx417@ouc.edu.cn; E-mail: byu@mail.sioc.ac.cn

[b] State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, P. R. of China

Received 20 July 2005; revised 3 October 2005

Abstract: (25*R*)-5a-Spirostan-2a,3b-diol (gitogenin) 3β-*O*-[2-*O*-(α-L-rhamnopyranosyl)-β-D-galactopyranoside] (**1**), a cytotoxic spirostan saponin isolated from the underground parts of *Hosta longipes* (Liliaceae), was concisely synthesized. In this context, its congeners **2**–**4** were also prepared. All four compounds showed comparable potency to dioscin in inhibition against the growth of tumor cells.

Key words: gitogenin 3β-*O*-[2-*O*-(α-L-rhamnopyranosyl)-β-D-galactopyranoside], saponin, chacotrioside, synthesis, antitumor

(25*R*)-5α-Spirostan-2α,3β-diol (gitogenin) 3β-*O*-[2-*O*-(α-L-rhamnopyranosyl)-β-D-galactopyranoside] (**1**) was isolated by Mimaki et al. from the underground parts of *Hosta longipes* and *H. sieboldii* (Liliaceae),[1,2] and showed remarkable inhibition activity against the growth of human promyelocytic leukaemia HL-60 cells with an IC_{50} value of 3.0 μg/mL.[2] In continuation of our research on antitumor saponins,[3,4] here we chose saponin **1** as a target to develop an approach to the first synthesis of gitogenin glycosides, which constitute a rare group of steroidal saponins in nature. In this context, gitogenin 3β-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-galactopyranoside] (**2**), gitogenin 3β-*O*-[2,4-di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (chacotrioside) (**3**) (namely introduction of the β-chacotriosyl sugar residue of the most active spirostan saponins[5] onto the 3-OH of gitogenin), and tigogenin 3β-*O*-chacotrioside (**4**), were also prepared. This enabled us to compare the antitumor activities of these congeners with diosgenin 3β-*O*-chacotrioside (dioscin), which represents one of the most potent antitumor spirostan saponins.[4,5]

The rare steroid gitogenin has been prepared from the abundant diosgenin by Sondheimer et al. using a five-step sequence as depicted in Scheme 1.[6] We first attempted to follow this synthetic approach. But the low yield in the transformation of the bromide **6** to the 2-acetoxy-3-one **7** (ca. 20%, the literature yield was 25%) and the difficulty associated with the purification of **6** and **7** prompted us to seek for a new procedure for the preparation of gitogenin.

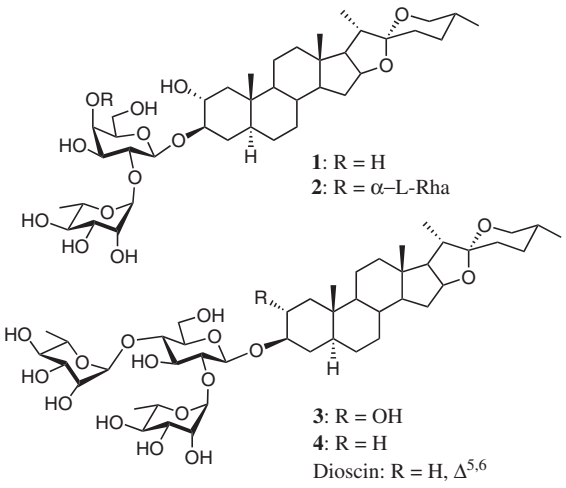
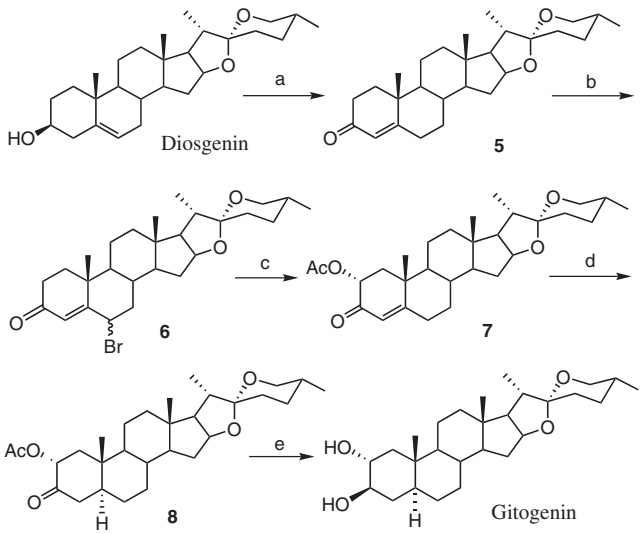


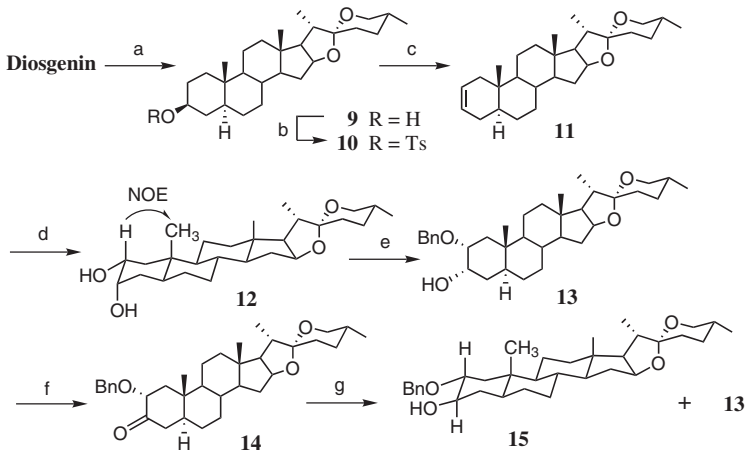
Figure 1 Saponins **1**–**4**

To convert diosgenin into gitogenin, a 2α-hydroxyl group needs to be introduced and the C-5,6 double bond to be stereoselectively saturated. Thus, hydrogenation of diosgenin in the presence of Pd/C provided tigogenin **9** stereoselectively in 93% yield[7] (Scheme 2). Tosylation of **9** with *p*-toluenesulfonyl chloride in pyridine at room tem-



Scheme 1 *Reagents*: a) cyclohexanone, Al(*i*-OPr)$_3$; b) NBS, CCl_4; c) KOAc, AcOH; d) Pd/C, H_2; e) $LiAlH_4$.

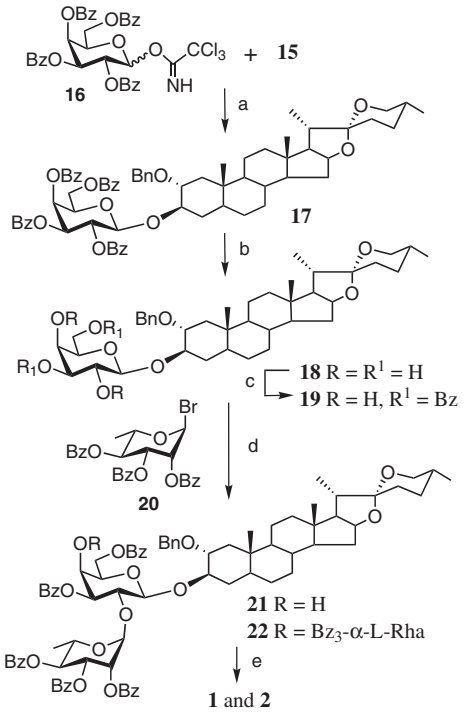
SYNTHESIS 2006, No. 5, pp 0775–0782
Advanced online publication: 07.02.2006
DOI: 10.1055/s-2006-926316; Art ID: F12205SS



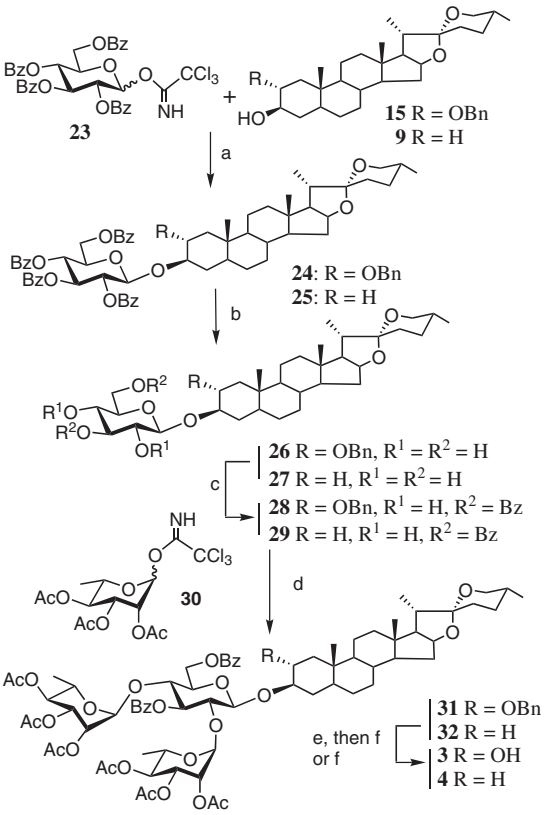
Scheme 2 *Reagents and conditions*: a) H_2, Pd/C, CH_2Cl_2–MeOH, r.t., 93%; b) TsCl, pyridine, 0 °C then r.t.; c) silica gel, EtOAc–PE, r.t., 73% (for two steps); d) OsO_4, NMO, *t*-BuOH–THF–H_2O (10:8:1), r.t., 77; e) Bu_2SnO, toluene, reflux, 6 h; then BnBr, $Bu_4N^+Br^-$, reflux, 2 h, 98%; f) Dess–Martin periodinane, CH_2Cl_2, r.t.; g) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, MeOH, –40 °C, 55% (two steps for **15**), 28% (two steps for **13**).

perature led to a single product, conceivably the corresponding 3-OTs derivative **10**, as detected in TLC. Surprisingly, a less polar compound **11** was obtained from silica gel column chromatography of the crude **10**. In fact, elution after loading the crude **10** onto silica gel for 48 hours provided **11** in a good 73% yield (from **9**). Stirring the crude **10** overnight in the presence of silica gel in CH_2Cl_2 at r.t. converted it completely into **11**. The structure of compound **11** was confirmed by 2D NMR analysis, i.e., COSY, HMQC, HMBC, and DEPT spectra, to be the desired $\Delta^{2,3}$ intermediate. Elimination of the 3-*O*-tosyl ester under such mild conditions was unexpected.[8] Oxidation of olefin **11** with a catalytic amount of OsO_4 in the presence of NMO afford the 2α,3α-diol **12** in 77% yield.[9] The stereochemistry of the nascent 2-OH in **12** was confirmed by the NOE correlation observed between H-2 and H-19 and the chemical shifts of 2-axial proton (δ = 3.76, br d, *J* = 11.3 Hz) and 3-equatorial proton (δ = 3.96, br s). Selective protection of the equatorial 2α-OH of diol **12** was examined via the organotin-mediated regioselective benzylation.[9] Thus, treatment of the 2,3-diol **12** with Bu_2SnO in anhydrous toluene under azeotropic conditions followed with BnBr and $Bu_4N^+I^-$ at reflux led to the desired 2-O-benzylated product **13** in an excellent 98% yield. ^1H NMR spectra of **13** showed that the chemical shift of 2-H moved upfield (δ = 3.63, ddd, *J* = 12.1, 2.9, 1.8 Hz). The final inversion of the 3α-OH of **13** into the β-configuration was attempted by an oxidation-reduction sequence. Treatment of **13** with Dess–Martin periodinane[10] gave the 3-ketone **14**, which was directly subjected to reduction with $NaBH_4$ in the presence of $CeCl_3{\cdot}7H_2O$ at –40 °C.[11] The desired 3β-OH product **15** was obtained as the major product in 55% yield (over two steps), meanwhile, the 3α-OH epimer **13** was also isolated in 28%. The chemical shift and peak shape of the 3-H (δ = 3.94–3.89, m) for **15** on the ^1H NMR spectra, strikingly different from those of the 3-equatorial proton (δ = 4.41 ppm, br s) for **13**, confirmed the β-configuration of 3-OH. Thus, 2-*O*-benzylgitogenin **15** was conveniently synthesized from diosgenin in total seven steps and in a remarkable 28% overall yield.

We then focused our attention on the incorporation of the 2-*O*-benzylgitogenin **15** with sugar residues. To this end, glycosylation of 2-*O*-benzylgitogenin **15** with 2,3,4,6-tetra-*O*-benzoyl-D-galactopyranosyl trichloroacetimidate (**16**) under the action of a catalytic amount of TMSOTf afforded the 3-*O*-β-galactopyranoside **17** in quantitative yield[12] (Scheme 3). Removal of the benzoyl groups with NaOMe in MeOH readily gave **18**, which was subjected to 1-BBTZ [1-(benzoyloxy)benzotriazole] in the presence of Et_3N in CH_2Cl_2 to selectively protect the 3,6-OHs on the β-galactopyranosyl residue, leading to 2,4-diol **19** in a good 68% yield.[13] Regioselective glycosylation of diol **19** has been investigated using two rhamnopyranosyl donors. Coupling of **19** with 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetimidate (**30**, 1.1 → 3.0 equiv) under the promotion of a catalytic amount of TMSOTf or $BF_3{\cdot}OEt_2$ gave poor regioselectivity and low yield of the desired 2-O-glycosylated product **21**. The reaction results were very different from those for diosgenin.[14] Apparently, the poor reactive selectivity of 2′-OH in **19** resulted from its diminished reactivity due to the presence of 2α-OBn in the sapogenin. To address this problem, a larger and lower reactive glycosyl donor should be adopted. Fortunately, by using the 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (**20**, 1.1 equiv) as the donor, selective glycosylation of 2′-OH in **19** was carried out smoothly under the action of AgOTf to obtain the desired product **21** in a satisfactory 62% yield; in the mean time, the 2,4-di-O-glycosylated product **22** was isolated in 17% yield. This protocol has previously been used for the selective rhamnosylation of the 2-OH of a 3,6-di-*O*-pivaloyl-β-D-glucopyranoside.[15] Removal of the 2-*O*-benzyl group on **21** by hydrogenolysis in the presence of Pd/C, followed by removal of the benzoyl groups on the sugar residue with NaOMe in MeOH afforded the desired natural saponin **1** in good yield (76% for two steps). The analytical data for the synthetic compound **1** were in well agreement with



Scheme 3 *Reagents and conditions*: a) **16** (1.3 equiv), TMSOTf (0.1 equiv), CH_2Cl_2, 0 °C, quant.; b) NaOMe, MeOH, r.t., 99%; c) BBTZ, Et_3N, CH_2Cl_2, r.t., 68%; d) **20** (1.1 equiv), AgOTf (1.2 equiv), CH_2Cl_2, –20 °C, 62% (for **21**), 17% (for **22**); e) H_2, 10% Pd/C, CH_2Cl_2–MeOH, r.t., overnight; then NaOMe, MeOH–CH_2Cl_2, r.t., overnight, 76% (two steps for **1**), 83% (two steps for **2**).



Scheme 4 *Reagents and conditions*: a) **23** (1.3 equiv), TMSOTf (0.1 equiv), CH_2Cl_2, 0 °C, quant. (for **24**); 97% (for **25**); b) NaOMe, MeOH, r.t., 85% (for **26**); 91% (for **27**); c) BBTZ, Et_3N, CH_2Cl_2, r.t., 55% (for **28**); 40% (for **29**); d) **30** (5 equiv), TMSOTf (0.2 equiv), CH_2Cl_2, –20 °C; e) H_2, 10% Pd/C, CH_2Cl_2–MeOH, r.t.; f) NaOMe, MeOH–CH_2Cl_2, r.t., 61% (three steps for **3**); 52% (two steps for **4**).

those reported for the natural product.[1] Employing the similar deprotection procedure, gitogenin trisaccharide **2** was obtained in 83% from **22**.

The next task was to introduce the chacotriosyl residue onto gitogenin **15** and tigogenin **9** to prepare saponins **3** and **4**. The procedure was straightforward as shown in Scheme 4, adopting modification of the previous procedure for the preparation of dioscin and its derivatives.[4] Thus, glycosylation of **15** and **9** with 2,3,4,6-tetra-*O*-benzoyl-D-glucopyranosyl trichloroacetimidate **23** under the action of a catalytic amount of TMSOTf afforded the 3-*O*-β-glucopyranosides **24** and **25**, respectively, in excellent yields. Removal of the benzoyl groups from the β-glucopyranosyl residue with NaOMe in methanol, followed by selective protection of the 3,6-OHs with BBTZ in the presence of Et_3N, provided the corresponding 3,6-di-*O*-benzoyl derivatives **28** and **29** in moderate yields. Glycosylation of the diols **28** and **29** with 2,3,4-tri-*O*-acetyl-L-rhamnopyranosyl trichloroacetimidate (**30**) under the promotion of TMSOTf afforded the desired trisaccharides **31** and **32**, respectively, which were directly subjected to deprotection to provide the desired chacotriosyl saponins **3** and **4** in good yields. The ^{13}C NMR data for the synthetic compound **4** were in well agreement with those reported for the metabolic product.[16]

The inhibition activities of saponins **1**–**4** against the growth of three tumor cell lines, i.e., A549 (human lung carcinoma cell), BGC-823 (human gastric cancer cell), and HGC-27 (human gastric carcinoma cell), were evaluated following a standard MTT assay with dioscin as a positive control.[17] The IC_{50} values are listed in Table 1. The tigogenin 3-*O*-chacotrioside **4** showed similar potency as dioscin against the three tumor cell lines; while the gitogenin glycosides **1**–**3** were slightly less potent.

Table 1 Inhibition Activities of Compounds **1**–**4** against the Growth of Tumor Cells

Tumor cells	IC_{50} (μM)				
	1	**2**	**3**	**4**	Dioscin
A549	2.5	3.9	2.3	0.81	0.52
BGC-823	2.6	2.2	4.0	1.2	2.0
HGC-27	2.1	4.2	2.7	2.2	1.4

Solvents were purified in the usual way. Petroleum ether (PE) refers to the fraction boiling in the range 60–90 °C. TLC was performed on precoated Merck silica gel 60 F_{254} plates. Flash column chroma-

tography was performed on silica gel (200–300 mesh, Qingdao, China). Optical rotations were determined with a JASCO P-1020 polarimeter. Melting points were determined with a Yanaco apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were taken on a Jeol JNM-ECP 600 MHz spectrometer with TMS as an internal standard, and chemical shifts recorded in δ values. Mass spectra were obtained on a Q-TOF GlOBAL mass spectrometer.

(25*R*)-5α-Spirostan-3β-ol (9)

A suspension of diosgenin (2.0 g, 4.8 mmol) and Pd/C (10%, cat.) in CH_2Cl_2–EtOH (1:1, 60 mL) in the presence of AcOH (2 drops) was stirred under 1 atm H_2 for 12 h, and then filtered and concentrated. The residue was purified by recrystallization from hexane to give **9** (1.87 g, 93%) as a white solid; mp 189.0–194.0 °C; $[\alpha]_D^{20}$ –67.9 (*c* = 0.20, $CHCl_3$); R_f 0.23 ($CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 4.39 (q, *J* = 7.3 Hz, 1 H, H-16), 3.59 (m, 1 H), 3.47 (m, 1 H), 3.37 (t, *J* = 11.0 Hz, 1 H,), 1.98 (m, 1 H), 0.96 (d, *J* = 7.3 Hz, 3 H), 0.82 (s, 3 H), 0.79 (d, *J* = 6.4 Hz, 3 H), 0.76 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 109.3, 80.6, 71.3, 66.8, 62.2, 56.3, 54.4, 44.9, 41.6, 40.6, 40.1, 38.2, 37.0, 35.6, 35.1, 32.3, 31.8, 31.5, 31.4, 30.3, 28.8, 28.6, 21.1, 17.1, 16.5, 14.5, 12.4.

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{27}H_{45}O_3$: 417.3369; found: 417.3387.

(25*R*)-5α-Spirostan-2-ene (11)

An ice-cold solution of **9** (10.2 g, 24.5 mmol) in anhyd pyridine (90 mL) was treated in portions with *p*-toluenesulfonyl chloride (14.0 g, 73.5 mmol) and then permitted to stir overnight at r.t. The solution was concentrated to afford a residue, which was dissolved in EtOAc (350 mL) and then washed successively with 5% HCl (3 × 70 mL), aq sat. $NaHCO_3$ solution (3 × 70 mL) and brine (70 mL). The organic layer was dried (Na_2SO_4) and then filtered and concentrated. The residue was loaded onto a silica gel column with $CHCl_3$–PE (1:5), and was eluted after 48 h (1:5 to 1:3 $CHCl_3$–PE) to afford **11** as a white solid (7.12 g, 73%); mp 171.0–173.0 °C; R_f 0.56 (1:20 EtOAc–PE); $[\alpha]_D^{20}$ –32.2 (*c* = 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 5.59 (m, 2 H, H-2, H-3), 4.39 (q, *J* = 7.4 Hz, 1 H, H-16), 3.47 (ddd, *J* = 1.9, 4.0, 10.6 Hz, 1 H, H-26), 3.38 (t, *J* = 10.6 Hz, 1 H, H-26), 2.01–0.69 (m, 26 H), 0.96 (d, *J* = 7.0 Hz, 3 H, H-21), 0.80–0.77 (m, 9 H, H-18, H-19, H-27).

^{13}C NMR ($CDCl_3$): δ = 125.8 (2 C, C-2, C-3), 109.2 (C-22), 80.8 (C-16), 66.8 (C-26), 62.2 (C-17), 56.3 (C-14), 54.0 (C-9), 41.6 (C-20), 41.4 (C-5), 40.4 (C-13), 40.0 (C-12), 39.7 (C-1), 35.2 (C-8), 34.7 (C-10), 32.0, 31.7, 31.4, 30.3 (2 C, C-4, C-25), 28.8, 28.6, 20.7 (C-11), 17.1 (C-27), 16.4 (C-18), 14.5 (C-21), 11.7 (C-19).

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{27}H_{43}O_2$: 399.3263; found: 399.3265.

(25*R*)-5α-Spirostan-2α,3α-diol (12)

A solution of **11** (10.5 g, 26.0 mmol) in THF (20 mL) was added dropwise to a solution of OsO_4 (catalyst) in *tert*-butyl alcohol–THF–H_2O (10:8:1, 190 mL) containing NMO (*N*-methylmorpholine *N*-oxide, 10.67 g, 79.0 mmol). After stirring the mixture at r.t. for 2 h, aq sat. NaHS solution (60 mL) was added, and stirring continued for 30 min. The resulting mixture was extracted with CH_2Cl_2 (3 × 250 mL). The organic layer was washed with water (3 × 100 mL) and brine (100 mL), and dried (Na_2SO_4) and then filtered and concentrated. The residue was applied to a silica gel column chromatography (1:3 EtOAc–PE) to afford **12** (8.37 g, 74%) as a white solid; R_f 0.23 (1:2 EtOAc–PE); $[\alpha]_D^{20}$ –61.2 (*c* = 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 4.39 (q, *J* = 7.7 Hz, 1 H, H-16), 3.96 (br s, 1 H, H-3), 3.76 (br d, *J* = 11.3 Hz, 1 H, H-2), 3.47 (ddd, *J* = 1.5, 3.7, 11.0 Hz, 1 H, H-26), 3.37 (t, *J* = 11.0 Hz, 1 H, H-26), 0.96 (d, *J* = 7.0 Hz, 3 H), 0.81 (s, 3 H), 0.79 (d, *J* = 6.6 Hz, 3 H), 0.76 (s, 3 H).

^{13}C NMR (pyridine-d_5): δ = 109.2, 81.1, 69.8, 69.1, 66.8, 63.0, 56.5, 54.6, 42.0, 41.9, 40.8, 40.1, 38.7, 37.2, 35.6, 34.7, 32.5, 32.1, 31.8, 30.6, 29.2, 28.2, 21.1, 17.3, 16.7, 15.0, 12.8.

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{27}H_{45}O_4$: 433.3318; found: 433.3306.

(25*R*)-5α-Spirostan-2α-*O*-benzyloxy-3α-ol (13)

A mixture of **12** (6.50 g, 15.0 mmol) and Bu_2SnO (6.73 g, 27.0 mmol) in anhyd toluene (300 mL) was stirred at reflux for 6 h with the azeotropic removal of H_2O (Dean–Stark trap). The resulting solution was cooled to r.t., and then BnBr (5.35 mL, 45.1 mmol) and $Bu_4N^+I^-$ (6.09 g, 16.5 mmol) were added. The mixture was refluxed for 2.5 h. TLC indicated that the reaction was complete. The mixture was evaporated to a syrup which was dissolved in CH_2Cl_2 (350 mL), then washed successively with 5% $Na_2S_2O_3$ (3 × 80 mL), H_2O (2 × 80 mL), and brine (80 mL). The organic layer was dried (Na_2SO_4) and then filtered and concentrated. The residue was purified by silica gel column chromatography (1:15 EtOAc–PE) to afford **13** as a white solid (9.86 g, 98%); mp 166.5–169 °C; R_f 0.29 (1:6 EtOAc–PE); $[\alpha]_D^{20}$ –67.8 (*c* = 0.20, $CHCl_3$).

^{1}H NMR (pyridine-d_5): δ = 7.52–7.29 (m, 5 H, ArH), 4.73 (d, 1 H, *J* = 11.7 Hz, CH_2Ph), 4.64 (d, *J* = 11.8 Hz, 1 H, CH_2Ph), 4.53 (q, *J* = 6.6 Hz, 1 H, H-16), 4.41 (br s, 1 H, H-3), 3.63 (ddd, *J* = 1.8, 2.9, 12.1 Hz, 1 H, H-2), 3.57 (br d, *J* = 12.8 Hz, 1 H, H-26), 3.49 (t, *J* = 10.3 Hz, 1 H, H-26), 1.12 (d, *J* = 7.0 Hz, 3 H), 0.84 (s, 3 H), 0.79 (s, 3 H), 0.67 (d, *J* = 4.4 Hz, 3 H).

^{13}C NMR (pyridine-d_5): δ = 140.1, 128.6 (2 C), 128.0 (2 C), 127.7, 109.2, 81.1, 77.8, 70.1, 66.8, 66.3, 63.0, 56.4, 54.6, 42.0, 40.8, 40.1, 38.8, 38.6, 37.2, 35.3, 34.7, 32.4, 32.1, 31.8, 30.6, 29.3, 28.1, 21.1, 17.3, 16.7, 15.0, 12.9.

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{34}H_{51}O_4$: 523.3787; found: 523.3787.

(25*R*)-5α-Spirostan-2α-*O*-benzyloxy-3β-ol (15)

A solution of **13** (8.46 g, 15.3 mmol) in CH_2Cl_2 (140 mL) was added to Dess–Martin periodinane reagent (24.0 g, 56.6 mmol). After stirring at r.t. for 12 h, the solution was washed with aq $NaHCO_3$–10% $Na_2S_2O_3$ (3 × 30 mL), 5% HCl (80 mL), and brine (80 mL). The organic layer was dried (Na_2SO_4), filtered and concentrated to afford the corresponding crude 3-ketone **14**, which was directly subjected to the next reaction. Part of the crude product was purified by a silica gel column chromatography (1:10 EtOAc–PE) to afford pure **14** for analysis.

14

R_f 0.29 (1:6 EtOAc–PE); $[\alpha]_D^{20}$ –9.1 (*c* = 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 7.38–7.26 (m, 5 H, ArH), 4.88 (d, *J* = 11.5 Hz, 1 H, CH_2Ph), 4.48 (d, *J* = 11.9 Hz, 1 H, CH_2Ph), 4.39 (q, *J* = 7.4 Hz, 1 H, H-16), 4.03 (dd, *J* = 6.8, 12.4 Hz, 1 H, H-2), 3.47 (ddd, 1 H, *J* = 2.3, 4.1, 10.6 Hz, H-26), 3.36 (t, *J* = 11.0 Hz, 1 H, H-26), 2.37–2.30 (m, 2 H), 2.16 (dd, *J* = 3.2, 13.7 Hz, 1 H), 1.04 (s, 3 H), 0.96 (d, *J* = 6.8 Hz, 3 H), 0.79 (d, *J* = 6.4 Hz, 3 H), 0.78 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 209.0 (C=O), 138.0, 128.4 (2 C), 127.7 (3 C), 109.2, 80.7, 79.2, 72.1, 66.8, 62.1, 55.8, 53.8, 48.1, 46.7, 44.1, 41.6, 40.6, 39.7, 37.2, 34.3, 31.7, 31.3, 30.3, 28.8, 28.3, 21.4, 17.1, 16.4, 14.5, 13.0.

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{34}H_{49}O_4$: 521.3631; found: 521.3616.

To a solution of the crude **14** in THF–MeOH (10:4, 140 mL) containing $CeCl_3 \cdot 7H_2O$ (2.0 g) at –40 °C was added a solution of $NaBH_4$ (2.0 g) in MeOH (10 mL) dropwise. After stirring at –40 °C for 2 h, the resulting mixture was concentrated and then dissolved

in CH_2Cl_2 (350 mL). The solution was then washed with 5% HCl (2 × 70 mL), aq sat $NaHCO_3$ solution (70 mL), and brine (70 mL). The organic layer was dried (Na_2SO_4) then filtered and concentrated. The residue was applied to a silica gel column chromatography (1:12 to 1:5 EtOAc–PE) to afford **15** (4.61 g, 55%) and **13** (2.37 g, 28%) as white solids.

15

Carbonization point 290 °C; R_f 0.19 (1:6 EtOAc–PE); $[\alpha]_D^{20}$ –96.0 (c = 0.40, $CHCl_3$).

^{1}H NMR (pyridine-d_5): δ = 7.58–7.28 (m, 5 H, ArH), 6.22 (br s, 1 H), 4.92 (d, J = 11.8 Hz, 1 H, CH_2Ph), 4.90 (d, J = 11.8 Hz, 1 H, CH_2Ph), 4.56 (q, J = 7.7 Hz, 1 H, H-16), 3.94–3.89 (m, 1 H, H-3), 3.72–3.68 (m, 1 H, H-2), 3.58 (br d, 1 H, J = 10.3 Hz, H-26), 3.49 (t, J = 10.6 Hz, 1 H, H-26), 1.12 (d, J = 6.9 Hz, 3 H), 0.84 (s, 3 H), 0.82 (s, 3 H), 0.68 (d, J = 5.5 Hz, 3 H).

^{13}C NMR (pyridine-d_5): δ = 140.5, 128.6 (2 C), 128.1 (2 C), 127.5, 109.2, 81.7, 81.1, 75.0, 72.0, 66.9, 63.0, 56.4, 54.5, 44.8, 43.0, 42.0, 40.8, 40.2, 37.4 (2 C), 34.7, 32.4, 32.2, 31.8, 30.6, 29.3, 28.3, 21.5, 17.3, 16.7, 15.0, 13.5.

ESI-HRMS: m/z calcd for $[M + Na]^+$ $C_{34}H_{50}O_4Na$: 545.3607; found: 545.3610.

Gitotenin 2α-*O*-Benzyl-3β-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranoside) (17)

To a mixture of **15** (1.80 g, 3.44 mmol), **16** (3.82 g, 5.16 mmol) and powdered 4Å molecular sieves in anhyd CH_2Cl_2 (70 mL) at 0 °C was added TMSOTf (59 μL, 0.34 mmol). After stirring at 0 °C for 0.5 h and then at r.t. for 0.5 h, the reaction was quenched with Et_3N. The solid was then filtered off. The filtrate was concentrated and purified by silica gel column chromatography (1:10:2 EtOAc–PE–$CHCl_3$) to give **17** (quant.) as a white solid; mp 211–212.5 °C; R_f 0.21 (1:5 EtOAc–PE); $[\alpha]_D^{20}$ +11.8 (c = 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 8.04–7.19 (m, 25 H, ArH), 5.97 (d, J = 2.3 Hz, 1 H, H-4′), 5.79 (dd, J = 8.2, 10.5 Hz, 1 H, H-2′), 5.58 (dd, J = 3.7, 10.6 Hz, 1 H, H-3′), 5.06 (d, 1 H, J = 8.2 Hz, H-1′), 4.93 (d, J = 11.5 Hz, 1 H, CH_2Ph), 4.67 (d, J = 11.5 Hz, 1 H, CH_2Ph), 4.57 (dd, J = 6.0, 11.0 Hz, 1 H, H-6′), 4.39–4.34 (m, 2 H, H-16, H-6′), 4.22–4.20 (m, 1 H, H-5′), 3.76–3.80 (m, 1 H, H-3), 3.53–3.56 (m, 1 H, H-2), 3.46 (ddd, J = 1.9, 3.7, 10.6 Hz, 1 H, H-26), 3.36 (t, J = 11.0 Hz, 1 H, H-26), 0.95 (d, J = 6.8 Hz, 3 H), 0.78 (d, J = 6.4 Hz, 3 H), 0.72 (s, 3 H), 0.70 (s, 3 H).

ESI-HRMS: m/z calcd for $[M + H]^+$ $C_{68}H_{77}O_{13}$: 1101.5364; found: 1101.5378.

Gitotenin 2α-*O*-Benzyl-3β-*O*-(3,6-di-*O*-benzoyl-β-D-galactopyranoside) (19)

Compound **17** was dissolved in MeOH–$CHCl_3$ (1:1, 80 mL), and then NaOMe (catalyst) was added. After stirring at r.t. for 2 h, the resulting solution was neutralized with ion-exchange resin (H^+), and then filtered and concentrated. The residue was purified by silica gel column chromatography (30:1 to 6:1 $CHCl_3$–MeOH) to afford the corresponding gitotenin 2α-*O*-benzyl-3β-*O*-(β-D-galactopyranoside) (**18**) as a white solid (2.34 g, 99%); R_f 0.38 (10:1 $CHCl_3$–MeOH). To a stirred solution of **18** (2.00 g, 2.92 mmol) and 1-BBTZ (2.80 g, 11.68 mmol) in CH_2Cl_2 (60 mL) was added Et_3N (2.42 mL, 17.25 mmol). The mixture was stirred at r.t. for 12 h. Removal of the solvent afforded a residue, which was subjected to silica gel column chromatography (1:5:2 EtOAc–PE–$CHCl_3$) to give **19** (1.78 g, 68%) as a white solid; mp 162.0–163.0 °C; R_f 0.23 (1:3 EtOAc–PE); $[\alpha]_D^{20}$ –47.1 (c = 0.36, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 8.09–7.22 (m, 15 H, ArH), 5.12 (dd, J = 3.3, 9.9 Hz,1 H, H-3′), 4.69–4.63 (m, 3 H, CH_2Ph, H-6′), 4.62 (d, J = 7.7 Hz, 1 H, H-1′), 4.45 (dd, J = 6.2, 11.3 Hz, 1 H, H-6′), 4.40 (q, J = 7.0 Hz, 1 H, H-16), 4.20 (d, J = 3.0 Hz, 1 H, H-4′), 4.13 (dd, J = 8.4, 9.9 Hz, 1 H, H-2′), 3.91 (t, J = 7.0 Hz, 1 H, H-5′), 3.85 (m, 1 H), 3.56 (m, 1 H), 3.48 (m, 1 H), 3.37 (t, J = 11.0 Hz, 1 H, H-26), 3.06 (br s, 1 H, OH), 2.48 (br s, 1 H, OH), 0.97 (d, J = 7.0 Hz, 3 H), 0.81 (s, 3 H), 0.79 (d, J = 6.2 Hz, 3 H), 0.76 (s, 3 H).

ESI-HRMS: m/z calcd for $[M + Na]^+$ $C_{54}H_{68}O_{11}Na$: 915.4659; found: 915.4654.

Gitotenin 2α-*O*-Benzyl-3β-*O*-[2-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-3,6-di -*O*-benzoyl-β-D-galactopyranoside] (21) and Gitotenin 2α-*O*-Benzyl-3β-*O*-[2,4 -di-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl)-3,6-di-*O*-benzoyl-β-D-galactopyranoside] (22)

To a mixture of **19** (1.22 g, 1.37 mmol), **20** (813 mg, 1.51 mmol), and powdered 4Å molecular sieves in anhyd CH_2Cl_2 (60 mL) at –40 °C was added a solution of AgOTf (422 mg, 1.64 mmol) in toluene (5 mL). After stirring at –40 °C for 0.5 h and then at 0 °C for 1 h, the reaction was quenched with Et_3N. The solid was then filtered off, and the filtrate was concentrated under vacuum to give a yellow oil which was subjected to silica gel column chromatography (1:20 EtOAc–toluene) to give **21** (1.14 g, 62%) and **22** (0.41 g, 17%) as white solids.

21

Mp 183–185.5 °C; R_f 0.24 (1:20 EtOAc–toluene); $[\alpha]_D^{20}$ +41.5 (c = 0.40, $CHCl_3$).

^{1}H NMR($CDCl_3$): δ = 8.04–7.05 (m, 30 H, ArH), 5.76 (dd, J = 3.7, 10.3 Hz, 1 H, Rha-3), 5.60 (t, J = 10.3 Hz, 1 H, Rha-4), 5.48 (dd, J = 1.4, 3.3 Hz, 1 H, Rha-2), 5.32 (d, J = 1.4 Hz, 1 H, Rha-1), 5.30 (dd, J = 3.3, 9.9 Hz, 1 H, H-3′), 4.93 (d, J = 11.0 Hz, 1 H, CH_2Ph), 4.83 (d, 1 H, J = 7.3 Hz, H-1′), 4.70–4.63 (m, 3 H, Rha-5, H-6′, CH_2Ph), 4.45 (dd, J = 5.5, 11.4 Hz, 1 H, H-6′), 4.41 (q, J = 7.0 Hz, 1 H, H-16), 4.29 (dd, J = 7.7, 9.9 Hz, 1 H, H-2′), 4.25 (d, J = 2.9 Hz, 1 H, H-4′), 3.96 (ddd, J = 5.9, 9.2, 14.7 Hz, 1 H), 3.93 (t, J = 6.6 Hz, 1 H), 3.56 (ddd, 1 H, J = 5.2, 9.2, 13.9 Hz), 3.48 (ddd, J = 2.0, 4.2, 10.6 Hz, 1 H, H-26), 3.38 (t, J = 11.0 Hz, 1 H, H-26), 1.34 (d, J = 6.2 Hz, 3 H, Rha-CH_3), 0.96 (d, J = 7.0 Hz, 3 H), 0.79 (d, J = 6.2 Hz, 3 H), 0.73 (s, 3 H), 0.70 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 166.5. 165.8, 165.6, 165.2, 164.8, 139.3, 133.3–133.0, 129.8–127.3, 109.2, 98.7 (C-1′), 98.1 (Rha-1), 81.1, 80.8, 78.0, 76.7 (C-3′), 73.9 (C-2′), 72.7, 71.9, 71.7 (C-5′, Rha-4), 70.9 (Rha-2), 69.5 (Rha-3), 67.0, 66.8 (C-4′, C-26), 62.1 (2 C), 56.0, 54.1, 43.9, 43.5, 41.6, 40.5, 39.8, 36.7, 34.4, 32.8, 32.0, 31.9, 31.8, 31.4, 30.3, 29.7, 29.4, 28.8, 28.0, 22.7, 21.1, 19.2, 17.6, 17.1, 16.4, 14.5, 14.1, 13.1.

22

Mp 106.0–108.0 °C; R_f 0.43 (1:20 EtOAc–toluene); $[\alpha]_D^{20}$ +68.7 (c = 0.32, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 8.05–7.04 (m, 45 H, ArH), 5.89 (dd, J = 1.1, 2.9 Hz, 1 H, H-3′), 5.81 (dd, J = 9.9, 12.3 Hz, 1 H, Rha-3), 5.79 (dd, J = 3.3, 10.3 Hz, 1 H, Rha-3), 5.60 (t, J = 10.3 Hz, 1 H, Rha-4), 5.55 (t, J = 9.8 Hz, 1 H, Rha-4), 5.49 (m, 2 H, 2 × Rha-2), 5.39 (d, 1 H, J = <1 Hz, Rha-1), 5.18 (d, 1 H, J = <1 Hz, Rha-1), 5.07 (d, J = 11.7 Hz, 1 H, CH_2Ph), 4.92 (d, J = 7.7 Hz, 1 H, H-1′), 4.80 (d, J = 11.7 Hz, 1 H, CH_2Ph), 4.73 (m, 1 H, Rha-5), 4.63 (dd, J = 5.5, 11.0 Hz, 1 H), 4.53 (dd, J = 8.4, 11.0 Hz, 1 H), 4.49 (d, J = 2.6 Hz, 1 H), 4.46 (dd, J = 2.6, 10.3 Hz, 1 H), 4.42 (q, J = 7.3 Hz, 1 H), 4.24 (m, 1 H, Rha-5), 4.02 (m, 2 H), 3.64 (ddd, J = 5.1, 8.8, 13.6 Hz, 1 H), 3.49 (m, 1 H, H-26), 3.39 (t, J = 11.0 Hz, 1 H, H-26), 1.38 (d, J = 6.2 Hz, 3 H), 1.17 (d, J = 6.2 Hz, 3 H), 0.97 (d, J = 7.0 Hz, 3 H), 0.80 (d, J = 6.6 H z, 3 H), 0.74 (s, 3 H), 0.71 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 166.0, 165.8 (2 C), 165.7, 165.3, 165.0, 164.9, 164.6, 139.5, 133.2–132.8, 130.9, 129.8–128.1, 127.4, 127.1, 109.2, 99.5, 99.3, 98.3, 81.7, 80.8, 77.7, 76.6, 74.8, 74.0, 72.6, 72.1, 71.8, 71.5, 71.3, 70.6, 70.4, 69.9, 69.7, 68.1, 67.0, 66.9,

62.4, 62.2, 56.0, 54.1, 44.0, 43.7, 41.6, 40.6, 39.8, 36.7, 34.5, 33.0, 32.0, 31.9, 31.8, 31.4, 30.3, 29.7, 29.6, 29.4, 28.8, 28.0, 27.7, 22.7, 21.1, 19.1, 17.5, 17.1, 16.4, 14.5, 14.1, 13.1.

Gitogenin 3β-*O*-[2-*O*-(α-L-Rhamnopyranosyl)-β-D-galactopyranoside] (1)

A suspension of **21** (120 mg, 0.089 mmol) and Pd/C (10%, catalyst) in CH_2Cl_2–MeOH (1:1, 16 mL) in the presence of AcOH (2 drops) was stirred under 1 atm of H_2 for 12 h, then filtered and concentrated. The residue was subjected to silica gel column chromatography (1:4 to 1:2, EtOAc–PE) to give a white solid, which was dissolved in MeOH–$CHCl_3$ (1:1, 16 mL), and then NaOMe (100 mg) was added. After stirring at r.t. for 12 h, the solution was neutralized with ion-exchange resin (H^+), then filtered and concentrated. The residue was purified by silica gel column chromatography (6:1 to 4:1 $CHCl_3$–MeOH) to afford **1** as a white solid (50.2 mg, 76% for two steps); mp 235.0–237.0 °C; R_f 0.35 (2:1 $CHCl_3$–MeOH); $[\alpha]_D^{20}$ –67.2 (*c* = 0.10, 1:1 $CHCl_3$–MeOH) {Lit.[1] $[\alpha]_D$ –70, (*c* = 0.10, 1:1 $CHCl_3$–MeOH)}.

^{1}H NMR (pyridine-d_5): δ = 6.32 (s, 1 H, Rha-1), 5.03 (d, *J* = 7.8 Hz, 1 H, H-1′), 4.87 (m, 1 H), 4.83 (dd, *J* = 1.9, 3.7 Hz, 1 H), 4.68 (dd, *J* = 7.8, 9.6 Hz, 1 H), 4.63 (dd, *J* = 3.7, 9.6 Hz, 1 H), 4.53 (q, *J* = 7.8 Hz, 1 H), 4.47 (m, 2 H), 4.39 (dd, *J* = 5.5, 11.0 Hz, 1 H), 4.29 (m, 2 H), 4.12 (m, 2 H), 3.90 (m, 1 H), 3.56 (dd, *J* = 11.0 Hz, 1 H, H-26), 3.48 (t, *J* = 10.6 Hz, 1 H, H-26), 1.59 (d, *J* = 6.4 Hz, 3 H), 1.11 (d, *J* = 6.9 Hz, 3 H), 0.88 (s, 3 H), 0.78 (s, 3 H), 0.67 (d, *J* = 5.5 Hz, 3 H).

^{13}C NMR (pyridine-d_5): δ = 109.2, 102.1, 101.6, 85.4, 81.1, 77.0, 76.6, 76.1, 74.1, 72.8, 72.5, 70.8, 70.6, 69.4, 66.8, 63.0, 62.2, 56.3, 54.3, 45.8, 44.6, 41.9, 40.7, 40.0, 36.8, 34.6, 33.6, 32.2, 32.1, 31.8, 30.6, 29.2, 28.1, 21.4, 18.5, 17.3, 16.6, 15.0, 13.5.

ESI-HRMS: *m*/*z* calcd for $[M + K]^+$ $C_{39}H_{64}O_{13}K$: 779.3984; found: 779.3989.

Gitogenin 3β-*O*-[2,4-Di-*O*-(α-L-rhamnopyranosyl)-β-D-galactopyranoside] (2)

A procedure similar to that described for the preparation of **1** from **21** was employed for the preparation of **2** from **22** (230 mg); yield: 94 mg (83% for two steps); white solid; mp 191.0–194.0 °C; R_f 0.28 (2:1 $CHCl_3$–MeOH); $[\alpha]_D^{20}$ –80.6 (*c* = 0.20, 1:1 $CHCl_3$–MeOH).

^{1}H NMR (CD_3OD): δ = 5.25 (d, *J* = 1.8 Hz, 1 H, Rha-1), 5.15 (d, *J* = 1.9 Hz, 1 H, Rha-1), 4.43 (d, *J* = 7.3 Hz, 1 H, H-1′), 4.37 (q, *J* = 7.3 Hz, 1 H, H-16), 4.11 (m, 1 H), 4.01 (dd, *J* = 1.4, 3.2 Hz, 1 H), 3.90 (m, 2 H), 3.77 (dd, *J* = 7.8, 11.0 Hz, 1 H), 3.75 (dd, *J* = 2.8, 9.6 Hz, 1 H), 3.69–3.57 (m, 6 H), 3.50 (ddd, *J* = 5.5, 9.2, 14.2 Hz, 1 H), 3.43 (ddd, *J* = 2.3, 4.1, 11.0 Hz, 1 H, H-26), 3.37 (td, *J* = 5.5, 9.6 Hz, 2 H), 3.34 (s, 1 H), 1.25 (d, *J* = 6.4 Hz, 3 H), 1.22 (d, *J* = 5.9 Hz, 3 H), 0.94 (d, *J* = 7.3 Hz, 3 H), 0.88 (s, 3 H), 0.78 (d, *J* = 6.4 Hz, 3 H), 0.78 (s, 3 H).

^{13}C NMR (CD_3OD): δ = 110.5, 103.3, 102.6, 101.5, 85.0, 82.2, 77.2, 77.1, 76.8, 76.6, 73.9, 73.8, 72.4, 72.3, 72.2, 72.1, 71.6, 70.6, 69.8, 67.8, 63.8, 63.1, 57.4, 55.7, 46.1, 45.8, 42.9, 41.7, 41.1, 37.9, 35.8, 33.9, 33.3, 32.6, 32.4, 31.4, 29.9, 29.0, 22.3, 18.0, 17.9, 17.5, 17.0, 14.9, 13.7.

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{45}H_{75}O_{17}$: 887.5004; found: 887.5017.

Gitogenin 2α-*O*-Benzyl-3β-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside) (24)

A procedure similar to that described for the preparation of **17** was employed for the preparation of **24** from 2α-*O*-benzylgitotenin **15** (600 mg, 1.15 mmol) and 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate **23** (1.28 g, 1.725 mmol); yield: 1.27 g (ca. 100%); white solid: R_f 0.21 (1: 5 EtOAc–PE); $[\alpha]_D^{20}$ –14.6 (*c* = 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 7.99–7.16 (m, 25 H, ArH), 5.87 (t, *J* = 9.7 Hz, 1 H, H-3′), 5.71 (t, *J* = 9.6 Hz, 1 H, H-4′), 5.54 (dd, *J* = 7.8, 9.7 Hz, 1 H, H-2′), 5.05 (d, *J* = 7.8 Hz, 1 H, H-1′), 4.88 (d, *J* = 11.5 Hz, 1 H, C*H*$_2$Ph), 4.60 (d, *J* = 11.5 Hz, 1 H, C*H*$_2$Ph), 4.53 (dd, *J* = 3.2, 12.4 Hz, 1 H, H-6′), 4.42 (dd, *J* = 4.6, 12.4 Hz, 1 H, H-6′), 4.37 (q, *J* = 7.8 Hz, 1 H, H-16), 4.04 (m, 1 H), 3.73 (m, 1 H), 3.48 (m, 2 H), 3.36 (t, *J* = 11.0 Hz, 1 H, H-26), 0.95 (d, *J* = 6.9 Hz, 3 H), 0.79 (d, *J* = 6.4 Hz, 3 H), 0.71 (s, 3 H), 0.66 (s, 3 H).

ESI-HRMS: *m*/*z* calcd for $[M + Na]^+$ $C_{68}H_{76}O_{13}Na$: 1123.5184; found: 1123.5205.

Tigogenin 3β-*O*-(2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranoside) (25)

A procedure similar to that described for the preparation of **17** was employed for the preparation of **25** from tigogenin **9** (1.00 g, 2.4 mmol) and 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate **23** (2.31 g, 3.12 mmol); yield: 2.32 g (97%); white solid; R_f 0.32 (1:4 EtOAc–PE); $[\alpha]_D^{20}$ +43.0 (*c* = 0.20, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 8.02–7.26 (m, 20 H, ArH), 5.89 (t, *J* = 9.5 Hz, 1 H), 5.62 (t, 1 H, *J* = 9.5 Hz), 5.49 (dd, 1 H, *J* = 7.7, 9.5 Hz), 4.94 (d, 1 H, *J* = 7.7 Hz, H-1′), 4.60 (dd, *J* = 3.3, 12.1 Hz, 1 H), 4.52 (dd, *J* = 6.2, 12.1 Hz, 1 H), 4.38 (q, *J* = 7.0 Hz, 1 H, H-16), 4.16 (m, 1 H), 3.59 (m, 1 H, H-3), 3.47 (ddd, *J* = 1.8, 4.1, 10.6 Hz, 1 H, H-26), 3.37 (t, *J* = 11.0 Hz, 1 H, H-26), 0.96 (d, *J* = 7.0 Hz, 3 H), 0.79 (d, *J* = 6.2 Hz, 3 H), 0.73 (s, 3 H), 0.69 (s, 3 H).

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{61}H_{71}O_{12}$: 995.4946; found: 995.4985.

Tigogenin 3β-*O*-(β-D-Glucopyranoside) (27)

A procedure similar to that for the preparation of **18** was used for the preparation of **27** from **25** (1.67 g); yield: 879 mg (91%); white solid; R_f 0.26 (8:1 $CHCl_3$–MeOH); $[\alpha]_D^{20}$ –70.1 (*c* = 0.28, 1:1 $CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6): δ = 4.90 (d, *J* = 4.7 Hz, 1 H, OH), 4.87 (d, *J* = 5.1 Hz, 1 H, OH), 4.85 (d, *J* = 4.7 Hz, 1 H, OH), 4.44 (t, *J* = 5.9 Hz, 1 H, 6′-OH), 4.26 (q, *J* = 7.3 Hz, 1 H, H-16), 4.21 (d, *J* = 8.0 Hz, 1 H, H-1′), 3.64 (m, 1 H), 3.56 (m, 1 H), 3.42 (m, 2 H), 3.20 (t, *J* = 11.0 Hz, 1 H), 3.11 (td, *J* = 4.4, 8.8 Hz, 1 H), 3.07–2.99 (m, 2 H), 2.88 (td, *J* = 4.8, 8.0 Hz, 1 H), 0.89 (d, *J* = 7.0 Hz, 3 H), 0.77 (s, 3 H), 0.73 (d, *J* = 6.2 Hz, 3 H), 0.71 (s, 3 H).

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{33}H_{55}O_8$: 579.3897; found: 579.3889.

Gitogenin 2α-*O*-Benzyl-3β-*O*-(3,6-di-*O*-benzoyl-β-D-glucopyranoside) (28)

Compound **26** (780 mg, 85%) was synthesized from **24** (1.48 g) by a procedure similar to that used for **18**.

26

Yield: 780 mg (85%); white solid; R_f 0.51 (8:1 $CHCl_3$–MeOH); $[\alpha]_D^{20}$ –64.1 (*c* = 0.20, 1:1 $CHCl_3$–MeOH).

ESI-HRMS: *m*/*z* calcd for $[M + H]^+$ $C_{40}H_{61}O_9$: 685.4316; found: 685.4320.

Then a procedure similar to that used in the preparation of **19** was employed for the preparation of **28** from **26** (780 mg, 1.14 mmol).

28

Yield: 556 mg (55%); white solid; R_f 0.20 (1:3 EtOAc–PE); $[\alpha]_D^{20}$ –38.1 (*c* = 0.19, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ = 8.08–7.20 (m, 15 H, ArH), 5.19 (t, *J* = 9.5 Hz, 1 H, H-3′), 4.72 (dd, *J* = 5.9, 12.1 Hz, 1 H, H-6′), 4.71 (d, *J* = 11.3 Hz, 1 H, C*H*$_2$Ph), 4.65 (d, *J* = 8.1 Hz, 1 H, H-1′), 4.62 (d, *J* = 11.3 Hz, 1 H, C*H*$_2$Ph), 4.60 (dd, *J* = 2.2, 11.3 Hz, 1 H, H-6′), 4.40 (q, *J* = 7.7 Hz, 1 H, H-16), 3.78 (m, 1 H), 3.74 (m, 2 H, H-2′,

H-4′), 3.66 (ddd, *J* = 2.2, 4.0, 9.5 Hz, 1 H, H-5′), 3.56 (m, 1 H), 3.48 (m, 1 H, H-26), 3.37 (t, *J* = 11.0 Hz, 1 H, H-26), 0.96 (d, *J* = 7.0 Hz, 3 H), 0.80 (s, 3 H), 0.80 (d, *J* = 6.6 Hz, 3 H), 0.75 (s, 3 H).

ESI-HRMS: *m/z* calcd for $[M + H]^+$ $C_{54}H_{69}O_{11}$: 893.4840; found: 893.4810.

Tigogenin 3β-*O*-(3,6-Di-*O*-benzoyl-β-D-glucopyranoside) (29)

Compound **29** was synthesized as a white solid from **27** (860 mg, 1.09 mmol) by a procedure similar to that used for **19**; yield: 472 mg (40%); R_f 0.14 (1:3 EtOAc–PE); $[\alpha]_D^{20}$ –32.3 (*c* = 0.2, $CHCl_3$).

^{1}H NMR (DMSO-d_6): δ = 8.01–7.52 (m, 10 H, ArH), 5.58 (d, *J* = 6.2 Hz, 1 H, OH), 5.34 (d, *J* = 5.5 Hz, 1 H, OH), 5.08 (t, *J* = 9.5 Hz, 1 H, H-3′), 4.52 (dd, *J* = 2.2, 11.7 Hz, 1 H, H-6′), 4.50 (d, *J* = 8.1 Hz, 1 H, H-1′), 4.43 (dd, *J* = 7.3, 11.7 Hz, 1 H, H-6′), 4.27 (q, *J* = 7.0 Hz, 1 H, H-16), 3.72 (m, 1 H), 3.57–3.40 (m, 3 H), 3.31 (m, 1 H), 3.20 (t, *J* = 11.0 Hz, 1 H), 0.90 (d, *J* = 7.0 Hz, 3 H), 0.73 (d, *J* = 6.5 Hz, 3 H), 0.70 (s, 6 H).

ESI-HRMS: *m/z* calcd for $[M + H]^+$ $C_{47}H_{63}O_{10}$: 787.4421; found: 787.4427.

Gitogenin 3β-*O*-[2,4-Di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (3)

To a mixture of **28** (0.558 g, 0.625 mmol) and powdered 4Å molecular sieves in anhyd CH_2Cl_2 (25 mL) at –30 °C was added $BF_3{\cdot}OEt_2$ (97 mL, 0.747 mmol), followed by a solution of **30** (1.36 g, 3.12 mmol) in CH_2Cl_2 (5 mL). After stirring at –30 °C for 0.5 h and then at 0 °C for 1 h, the reaction was quenched with Et_3N. The solid was filtered off, and the filtrate was concentrated under vacuum to give a yellow oil. The oil was subjected to silica gel column chromatography (1:3 to 1:2 EtOAc–PE) to afford the crude **31**, which was directly subjected to the next reaction; *R* 0.26 (2:3 EtOAc–PE). A suspension of the crude **31** and 10% Pd/C (catalyst) in CH_2Cl_2–MeOH (1:1, 30 mL) was stirred in the presence of AcOH (2 drops) under 1 atm of H_2 for 12 h, then filtered and concentrated. The residue was subjected to silica gel column chromatography (1:10 EtOAc–$CHCl_3$ to 1:7 EtOAc–$CHCl_3$) to give gitogenin 3β-*O*-[2,4-di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,6-di-*O*-benzoyl-β-D-glucopyranoside]; yield: 0.556 g (66%); white solid; R_f 0.12 (1:8 EtOAc–$CHCl_3$); $[\alpha]_D^{20}$ –50.4 (*c* = 0.30, $CHCl_3$).

Gitogenin 3β-*O*-[2,4-Di-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-3,6-di-*O*-benzoyl-β-D-glucopyranoside]

^{1}H NMR ($CDCl_3$): δ = 8.11–7.42 (m, 10 H, ArH), 5.62 (t, *J* = 9.2 Hz, 1 H, H-3′), 5.16 (m, 3 H, 2 × Rha-3, Rha-2), 4.98 (m, 2 H, H-6′, Rha-2), 4.92–4.88 (m, 3 H, Rha-1, 2 × Rha-4), 4.78 (d, *J* = 1.1 Hz, 1 H, Rha-1), 4.64 (d, *J* = 7.7 Hz, 1 H, H-1′), 4.44 (dd, *J* = 4.0, 12.4 Hz, 1 H, H-6′), 4.40 (q, *J* = 7.3 Hz, 1 H, H-16), 4.32 (m, 1 H, Rha-5), 3.99 (t, *J* = 9.5 Hz, 1 H, H-4′), 3.87 (ddd, *J* = 2.2, 4.0, 9.8 Hz, 1 H, H-5′), 3.79 (t, *J* = 8.8 Hz, 1 H, H-2′), 3.72 (m, 1 H, Rha-5), 3.62 (m, 1 H), 3.53 (br s, 1 H, OH), 3.47 (m, 2 H), 3.37 (t, *J* = 11.0 Hz, 1 H, H-26), 2.03, 1.98, 1.96, 1.92, 1.88, 1.75 (s, 3 H each, 6 × $OCOCH_3$), 1.14 (d, *J* = 6.2 Hz, 3 H), 0.97 (d, *J* = 6.9 Hz, 3 H), 0.81 (s, 3 H), 0.79 (d, *J* = 6.6 Hz, 3 H), 0.75 (s, 3 H), 0.69 (d, *J* = 6.2 Hz, 3 H).

^{13}C NMR ($CDCl_3$): δ = 170.1, 169.9 (3 C), 169.6, 169.0, 165.8, 165.0, 133.4, 133.2, 130.1 (2 C), 130.0 (2 C), 129.6, 129.0, 128.4 (4 C), 109.3, 100.6, 99.1, 97.9, 86.9, 80.8, 76.7, 75.6, 73.5, 71.0, 70.5, 70.1, 70.0, 69.1, 68.6, 68.5, 67.6, 66.8, 66.5, 62.1, 62.0, 56.0, 54.1, 44.5, 44.3, 41.6, 40.6, 39.9, 36.6, 34.4, 33.4, 32.0, 31.7, 31.3, 30.3, 28.8, 27.8, 21.1, 20.8, 20.7, 20.6, 20.3, 17.1, 17.0, 16.9, 16.5, 14.5, 13.2.

ESI-HRMS: *m/z* $[M + H]^+$ $C_{71}H_{95}O_{25}$: 1347.6162; found: 1347.6199.

The above product (440 mg, 0.327 mmol) was dissolved in MeOH–$CHCl_3$ (1:1, 20 mL), and NaOMe (200 mg) was added. After stirring at r.t. for 20 h, the solution was neutralized with ion-exchange resin (H^+), then filtered and concentrated. The residue was purified by silica gel column chromatography (6:1 to 4:1 $CHCl_3$–MeOH) to afford **3**.

3

Yield: 267 mg (92%); white solid; mp 232.4–232.5 °C; R_f 0.38 (3:1 $CHCl_3$–MeOH); $[\alpha]_D^{20}$ –84.9 (*c* = 0.20, 1:1 $CHCl_3$–MeOH).

^{1}H NMR (DMSO-d_6): δ = 5.02 (br s, 1 H, Rha-1), 4.98 (d, *J* = 7.0 Hz, 1 H, 3′-OH), 4.78 (t, *J* = 5.5 Hz, 1 H, 6′-OH), 4.73 (d, *J* = 4.4 Hz, 1 H, Rha-2-O*H*), 4.68 (d, *J* = 5.1 Hz, 1 H, Rha-4-O*H*), 4.66 (d, *J* <1 Hz, 1 H, Rha-1), 4.64 (d, 1 H, *J* = 4.4 Hz, Rha-4-O*H*), 4.59 (d, 1 H, *J* = 4.4 Hz, Rha-2-O*H*), 4.52 (d, *J* = 5.8 Hz, 1 H, Rha-3-O*H*), 4.48 (d, *J* = 5.9 Hz, 1 H, Rha-3-O*H*), 4.40 (d, 1 H, *J* = 8.1 Hz, H-1′), 4.26 (q, 1 H, *J* = 7.0 Hz, H-16), 4.22 (d, 1 H, *J* = 3.3 Hz, 2-OH), 3.92–3.83 (m, 2 H, 2 × Rha-5), 3.69 (m, 1 H, Rha-2), 3.59 (m, 2 H, Rha-2, H-6′), 3.44–3.16 (m, 13 H), 1.10 (d, *J* = 6.2 Hz, 3 H), 1.08 (d, *J* = 6.2 Hz, 3 H), 0.89 (d, *J* = 7.0 Hz, 3 H), 0.77 (s, 3 H), 0.73 (d, *J* = 6.6 Hz, 3 H), 0.71 (s, 3 H).

^{13}C NMR (DMSO-d_6): δ = 108.4, 100.5 (2 C), 99.0, 83.1, 80.2, 77.3, 76.7, 75.9, 75.3, 71.9 (2 C), 70.7, 70.6 (2 C), 70.4, 69.3, 68.7, 68.0, 65.9, 61.9, 60.1, 55.5, 53.6, 54.3, 45.3, 43.8, 41.1, 40.1, 36.2, 33.9, 32.3, 31.7, 31.4, 30.9, 29.8, 28.5, 27.5, 20.7, 17.8, 17.7, 17.1, 16.2, 14.6, 12.9.

ESI-HRMS: *m/z* calcd for $[M + K]^+$ $C_{45}H_{74}O_{17}K$: 925.4563; found: 925.4532.

Tigogenin 3β-*O*-[2,4-Di-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (4)

To a mixture of **29** (0.452 g, 0.574 mmol) and powdered 4Å molecular sieves in anhyd CH_2Cl_2 (25 mL) at –30 °C was added TMSOTf (23 mL, 0.132 mmol), followed by a solution of **30** (1.25 g, 2.87 mmol) in CH_2Cl_2 (5 mL). After stirring at –30 °C for 0.5 h and then at 0 °C for 1 h, the reaction was quenched with Et_3N. The solid was filtered off, and the filtrate was concentrated under vacuum to give a yellow oil. The oil was subjected to silica gel column chromatography (1:3 to 1:2 EtOAc–PE) to afford the crude **32**, which was directly subjected to the next reaction; R_f 0.26 (1:2 EtOAc–PE). Compound **4** was synthesized as a white solid from **32** using a procedure similar to that used for **3**. Compound **4**; yield: 52% (for two steps); mp 228.3–229.4 °C; R_f 0.21 (8:1 $CHCl_3$–MeOH); $[\alpha]_D^{20}$ –88.4 (*c* = 0.20, 1:1 $CHCl_3$–MeOH);

^{1}H NMR (DMSO-d_6): δ = 4.99 (s, 1 H, Rha-1), 4.92 (d, *J* = 6.6 Hz, 1 H, OH), 4.72 (t, *J* = 6.2 Hz, 1 H, 6′-OH), 4.71 (d, *J* = 4.4 Hz, 1 H, OH), 4.68 (s, 1 H, Rha-1), 4.66 (d, *J* = 5.2 Hz, 1 H), 4.64 (d, *J* = 4.4 Hz, 1 H), 4.58 (d, *J* = 4.4 Hz, 1 H), 4.51 (d, *J* = 5.9 Hz, 1 H), 4.48 (d, *J* = 5.9 Hz, 1 H), 4.39 (d, *J* = 7.7 Hz, 1 H, H-1′), 4.26 (q, *J* = 7.0 Hz, 1 H, H-16), 3.96–3.16 (m, 17 H), 1.10 (d, *J* = 6.2 Hz, 3 H), 1.07 (d, *J* = 6.2 Hz, 3 H), 0.90 (d, *J* = 7.0 Hz, 3 H), 0.76 (s, 3 H), 0.73 (d, *J* = 6.6 Hz, 3 H), 0.71 (s, 3 H).

^{13}C NMR (pyridine-d_5): δ = 109.2, 102.9, 102.1, 99.8, 81.1, 78.6, 78.0, 77.9, 77.1, 77.0, 74.1, 73.9, 72.8, 72.7, 72.5 (2 C), 70.4, 69.5, 66.8, 63.0, 61.4, 56.4, 54.4, 44.5, 42.0, 40.8, 40.1, 37.2, 35.9, 35.2, 34.4, 32.4, 32.1, 31.8, 30.6, 29.9, 29.2, 28.9, 21.2, 18.6, 18.5, 17.3, 16.6, 15.0, 12.4.

ESI-HRMS: *m/z* $[M + H]^+$ $C_{45}H_{75}O_{16}$: 871.5055; found: 871.5028.

Acknowledgment

This work was supported by the National Basic Research Program of China (2003CB716400) and the Chinese Academy of Sciences (KGCX2-SW-213-05).

References

(1) Mimaki, Y.; Kanmoto, T.; Kuroda, M.; Sashida, Y.; Satomi, Y.; Nishino, A. *Phytochemistry* **1996**, *42*, 1065.
(2) Mimaki, Y.; Kuroda, M.; Kameyama, A.; Yokosuka, A.; Sashida, Y. *Phytochemistry* **1998**, *48*, 1361.
(3) Yu, B.; Hui, Y. *Chemical Synthesis of Bioactive Steroidal Saponins,* In *Glycochemistry: Principles, Synthesis, and Application*; Wang, P. G.; Bertozzi, C. R., Eds.; Marcel Dekker Inc: New York, **2001**, 163–174.
(4) (a) Li, M.; Han, X.; Yu, B. *Carbohydr. Res.* **2003**, *338*, 117. (b) Zhang, Y.; Li, Y.; Zhu, S.; Guan, H.; Lin, F.; Yu, B. *Carbohydr. Res.* **2004**, *339*, 1753. (c) Zhang, Y.; Li, Y.; Guo, T.; Guan, H.; Shi, J.; Yu, Q.; Yu, B. *Carbohydr. Res.* **2005**, *340*, 1453.
(5) (a) Nakamura, T.; Komori, C.; Lee, Y. Y.; Hashimoto, F.; Yahara, S.; Nohara, T.; Ejima, A. *Biol. Pharm. Bull.* **1996**, *19*, 564. (b) Liu, M. J.; Wang, Z.; Ju, Y.; Zhou, J. B.; Wang, Y.; Wong, R. N. S. *Biol. Pharm. Bull.* **2004**, *27*, 1059.
(6) Herran, J.; Rosenkranz, G.; Sondheimer, F. *J. Am. Chem. Soc.* **1954**, *76*, 5531.
(7) Williams, J. R.; Gong, H.; Hoff, N.; Olubodun, O. I.; Carroll, P. J. *Org. Lett.* **2004**, *6*, 269.
(8) (a) Schröder, M. *Chem. Rev.* **1980**, *80*, 187. (b) Kametani, T.; Katoh, T.; Fujio, J.; Nogiwa, I.; Tsubuki, M.; Honda, T. *J. Org. Chem.* **1988**, *53*, 1982. (c) Kametani, T.; Katoh, T.; Tsubuki, M.; Honda, T. *J. Am. Chem. Soc.* **1986**, *108*, 7055. (d) Khripach, V. A.; Zhabinskii, V. N.; Antonchick, A. P.; Konstantinova, O. V.; Schneider, B. *Steroids* **2002**, *67*, 1101.
(9) (a) David, S.; Hanessian, S. *Tetrahedron* **1985**, *41*, 643. (b) Simas, A. B. C.; Pais, K. C.; Desilva, A. A. T. *J. Org. Chem.* **2003**, *68*, 5426.
(10) (a) Ireland, R. E.; Liu, L. *J. Org. Chem.* **1993**, *58*, 2899. (b) Boeckman, J. R. K.; Shao, P.; Mulins, J. J. *Org. Synth.* **1999**, *77*, 141.
(11) Molander, G. A. *Chem. Rev.* **1992**, *92*, 29.
(12) Deng, S.; Yu, B.; Xie, J.; Hui, Y. *J. Org. Chem.* **1999**, *64*, 7265.
(13) (a) Lu, J.; Chan, T. H. *J. Org. Chem.* **1998**, *63*, 6035. (b) Kim, S.; Chang, H.; Kim, W. J. *J. Org. Chem.* **1985**, *50*, 1751.
(14) Du, Y.; Gu, G.; Wei, G.; Hua, Y.; Linhard, R. J. *Org. Lett.* **2003**, *5*, 3627.
(15) Li, B.; Yu, B.; Hui, Y.; Li, M.; Han, X.; Fung, K. P. *Carbohydr. Res.* **2001**, *331*, 1.
(16) Ikeda, T.; Tsumagari, H.; Okawa, M.; Nohara, T. *Chem. Pharm. Bull.* **2004**, *52*, 142.
(17) Liu, X.; Cui, Y.; Yu, Q.; Yu, B. *Phytochemistry* **2005**, *66*, 1671.