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The synthesis of cholestane and furostan saponin analogues and the determination of sapogenin's absolute configuration at C-22

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ABSTRACT

A facile and efficient way for the synthesis of cholestane and furostan saponin analogues was established and adopted for the first time. Following this strategy, starting from diosgenin, three novel cholestane saponin analogues: (22S,25R)-3β,22,26-trihydroxy-cholest-5-ene-16-one 22-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside] **11**, (25R)-3β,16β,26-trihydroxy-cholest-5-ene-22-one 16-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranoside] **14** and (25R)-3β,16β,26-trihydroxy-cholest-5-ene-22-one 16-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside] **17**, three novel furostan saponin analogues: (22S,25R)-furost-5-ene-3β,22,26-triol 22-*O*-(α-D-glucopyranoside) **23**, (22R,25R)-furost-5-ene-3β,22,26-triol 22-*O*-(α-D-glucopyranoside) **24** and (22S,25R)-furost-5-ene-3β,22,26-triol 22-*O*-[*O*-α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranoside] **26**, were synthesized ultimately. The structures of all the synthesized analogues were confirmed by spectroscopic methods. The S-chirality at C-22 of cholestane was confirmed by Mosher's method. The absolute configuration at C-22 of furostan saponin analogues was distinguished by conformational analysis combined with the NMR spectroscopy. The cytotoxicities of the synthetic analogues toward four types of tumor cells were shown also.

1. Introduction

The family of steroid saponins is an excellent source of diverse chemical structures with a broad range of promising pharmaceutical properties (e.g. antiviral, antibacterial, antifungal, anti-inflammatory and antitumor) [1–6], which has been reported in literature. According to the structure of sapogenin, steroid saponins were divided into spirostan saponins, cholestane saponins and furostan saponins. In 1992, the discovery of OSW-1 [7], a valuable cholestane saponin, attracted attention to the cytotoxicity of this kind of compounds. Along with the development of phytochemistry**,** many novel structured furostan saponins, just like icogenin [8] and protodioscin [9] which showed good cytotoxic activities, were separated and distinguished from nature plants.

Biological activity and bioavailability of steroid saponins did not only depend on the structure of sapogenin, but also the saccharide part, which decreased hydrophobic property of the steroidal sapogenin and made it more available for biochemical processes in the organism.

The research results concerning the synthesis and cytotoxicity estimation of icogenin analogues were published recently by our research group [10]. For further study, more cholestane and furostan saponin analogues were designed and synthesized. One of the intermediates, (25R)-26-acetyloxy-3β-*tert*-butyldimethylsilyloxycholest-5-ene-16,22-dione **1** [10], was prepared using the most common steroid sapogenin diosgenin as raw material. This compound was taken as the starting material for the synthesis of target saponin analogues.

2. Experimental

2.1. General

Optical rotatings were recorded in a Perkin-Elmer 341 LC polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Unity Inova 300, 400, 500 and 600 MHz spectrometers. The chemical shifts are reported in ppm using TMS as an internal standard. Mass spectra were obtained on a VGZAB-2F mass spectrometer for ESI-MS. HRMS was recorded on an Aglient 1100 series LC/MSD TOF. Analytical thin layer chromatography (TLC) was carried out on TLC plates silica gel 60 F254 percolated by Branch of Qingdao Haiyang Chemical Plant. Chromatography was performed with silica gel H (HG/T2354-92) and sephadex LH-20 (GE Healthcare).

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2.2. Synthesis

2.2.1. (22R,25R)-26-Acetyloxy-3β-tert-butyldimethylsilyloxy-furost-5-ene-22-ol (compound **2**) and (22S,25R)-26-acetyloxy-3β-tert-butyldimethylsilyloxy-cholest-5-ene-16β,22-diol (compound **3**)

To a solution of compound **1** (2.50 g, 4.26 mmol) in dry THF (60 ml) under argon cooled to −15 °C, $NaBH_4$ (1.78 g, 46.86 mmol) and $CeCl_3 \cdot 7H_2O$ (2.22 g, 5.96 mmol) were added. The reaction mixture was stirred for 1 h, then cooled to −40 °C and quenched with methyl alcohol. The suspension was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ and brine respectively, and then dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column to afford compound **2** (899 mg, 36%, white solid) and compound **3** (1.01 g, 42%, white solid).

Compound **2**: m.p.: 104.2–106.2 °C. $[\alpha]_D^{20}$ −51.5° (*c* = 0.66, CH_2Cl_2). ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 5.39 (d, 1H, *J* = 4.8 Hz, H-6), 4.59 (q, 1H, *J* = 6.9, 14.4 Hz, H-16), 3.81–4.01 (m, 2H, H-26), 3.47 (m, 1H, H-3), 2.12–2.30 (m, 2H), 2.06 (s, 3H, Ac), 0.05 (s, 6H). ^{13}C NMR (δ, d-pyridine, 100 MHz): 170.75 (Ac), 141.50 (C-5), 121.43 (C-6), 110.47 (C-22), 81.15 (C-16), 72.82 (C-3), 69.37 (C-26), 63.81, 56.68, 50.41, 43.34, 40.85, 40.68, 40.01, 37.49, 37.06, 36.92, 33.30, 32.56, 32.47, 32.38, 31.74, 28.21, 26.09 (t-Bu), 21.19, 20.74, 19.50, 18.32, 17.03, 16.51, 16.42, −4.35 ($(CH_3)_2$-Si). LC-HRESIMS *m*/*z* 589.4108 $[M+H]^+$ (calcd for $C_{35}H_{61}O_5Si$, 589.4163).

Compound **3**: m.p.: 86.6–88.6 °C. ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 5.31 (d, 1H, *J* = 4.2 Hz, H-6), 4.33 (dd, 1H, *J* = 3.9, 11.1 Hz, H-16), 3.83–4.00 (m, 2H), 3.61 (d, 1H, *J* = 9.3 Hz, H-22), 3.43–3.50 (m, 1H, H-3), 2.05 (s, 3H, Ac), 0.04 (s, 6H, Me_2Si). ^{13}C NMR (δ, $CDCl_3$, 100 MHz): 171.40 (Ac), 141.59 (C-5), 120.93 (C-6), 77.46 (C-22), 72.56 (C-3), 71.91 (C-16), 69.12 (C-26), 57.17 (C-17), 54.65 (C-14), 50.15 (C-9), 42.74, 42.47, 39.99, 37.30, 36.55, 35.95, 34.90, 32.65, 32.02, 31.80, 31.52, 30.71, 29.61, 25.91 (3C), 20.96, 20.71, 19.39, 18.22, 17.07, 16.01, 12.96, −4.61 (2C). LC-HRESIMS *m*/*z* 591.4394 $[M+Na]^+$ (calcd for $C_{35}H_{63}O_5Si$ 591.4439).

2.2.2. (S)-MTPA ester **3a**

To a mixture of compound **3** (20 mg, 0.034 mmol), DMAP (8.3 mg, 0.068 mmol) and triethylamine (7 μl, 0.051 mmol) in dry CH_2Cl_2 (2 ml) was added (R) – MTPACl (13 μl, 0.068 mmol). The reaction mixture was stirred at room temperature for 1 h, concentrated and the residue was purified by column chromatography to yield the (S)-MTPA ester (**3a**) (20 mg, 74%).

^{1}H NMR (δ, $CDCl_3$, 300 MHz): 7.558–7.583 (m, 2H, Ph), 7.393–7.410 (m, 3H, Ph), 5.457 (t, 1H, *J* = 6.9 Hz, H-22), 5.316 (d, 1H, *J* = 4.5 Hz, H-6), 4.390 (m, 1H, H-16), 3.713–3.971 (m, 2H, H-26), 3.509 (s, 3H, MeO), 3.470 (m, H-3), 2.029 (s, 3H, Ac), 0.997 (s, 3H, H-19), 0.937 (d, 3H, *J* = 7.2 Hz, H-21), 0.909 (s, 3H, H-18), 0.860 (s, 3H, H-26), 0.060 (s, 6H, Me_2Si).

2.2.3. (R)-MTPA ester **3b**

Following the same procedure as **3a**, compound **3** (20 mg, 0.034 mmol) was converted to (R)-MTPA ester (**3b**) (18 mg, 67%).

^{1}H NMR (δ, $CDCl_3$, 300 MHz): 7.567–7.591 (m, 2H, Ph), 7.383–7.420 (m, 3H, Ph), 5.427 (t, 1H, *J* = 6.9 Hz, H-22), 5.315 (d, 1H, *J* = 4.5 Hz, H-6), 4.319 (m, 1H, H-16), 3.747–3.977 (m, 2H, H-26), 3.570 (s, 3H, MeO), 3.456 (m, H-3), 2.030 (s, 3H, Ac), 0.984 (s, 3H, H-19), 0.892 (s, 3H, H-26), 0.864 (d, 3H, *J* = 6.6 Hz, H-21), 0.853 (s, 3H, H-18), 0.054 (s, 6H, Me_2Si).

2.2.4. 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (compound **5**)

To a solution of carefully dried **4** (1.10 g, 1.77 mmol) in dry CH_2Cl_2 (20 ml) under argon was added NIS (600 mg, 2.65 mmol) at −15 °C for 15 min, then AgOTf (680 mg, 2.65 mmol) was added. The reaction was stirred for 10 min avoiding the light, then 2.5 ml distilled water was added and warmed up to room temperature. After the reaction was stirred for 1 h, saturated aqueous $NaHCO_3$ was added. The organic layer was separated and the water layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with brine and dried with anhydrous $NaSO_4$, and then filtered. The filtrate was concentrated in vacuo to give a residue (1.09 g). The residue was carefully dried, dissolved in dry CH_2Cl_2 (50 ml) under argon at 0 °C (ice bath), trichloroacetonitrile (0.56 ml, 5.65 mmol) and DBU (84 μl, 0.56 mmol) were added. The reaction was stirred for 6 h, concentrated and the residue was purified by column chromatography to yield compound **5** (860 mg, 67%).

^{1}H NMR (δ, $CDCl_3$, 300 MHz): 6.45 (d, 1H, *J* = 3.6 Hz, H-1), 5.51 (t, 1H, *J* = 9.6 Hz, H-4), 5.19 (dd, 1H, *J* = 3.3, 9.9 Hz, H-3′), 5.11 (t, 1H, *J* = 9.9 Hz, H-4′), 5.01 (dd, 1H, *J* = 1.8, 3.3 Hz, H-2′), 4.90 (d, 1H, *J* = 1.5 Hz, H-1′), 4.29 (dd, 1H, *J* = 4.2, 12.3 Hz, H-6a), 4.15–4.19 (m, 1H, H-5), 4.07–4.12 (m, 1H, H-6b), 3.98 (dd, 1H, *J* = 3.6, 9.9 Hz, H-2), 3.83–3.88 (m, 1H, H-5′), 2.13 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.00 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.16 (d, 3H, *J* = 6.3 Hz, H-6′). ^{13}C NMR (δ, $CDCl_3$, 125 MHz): 170.47, 170.03 (2C), 169.90, 169.63, 169.51, 161.19 (C = NH), 94.16, 90.64, 76.11, 71.31, 70.86, 70.03 (3C), 68.05, 67.71, 67.28, 61.39, 20.83, 20.71, 20.62 (2C), 20.60, 20.52, 17.27. LC-HRESIMS *m*/*z* 744.0854 $[M+Na]^+$ (calcd for $C_{26}H_{34}O_{16}NNaCl_3$ 744.0835).

2.2.5. (22S,25R)-26-Acetyloxy-3β-tert-butyldimethylsilyloxycholest-5-ene-16β,22-diol 22-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-β-D-glucopyranoside] (compound **6**), (22S,25R)-26-acetyloxy-3β-tert-butyldimethylsilyloxycholest-5-ene-16β,22-diol 16-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-glucopyranoside] (compound **7**) and (22S,25R)-26-acetyloxy-3β-tert-butyldimethylsilyloxycholest-5-ene-16β,22-diol 16-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-β-D-glucopyranoside] (compound **8**)

A solution of carefully dried cholestane sapogenin **3** (1.23 g, 1.70 mmol) and carefully dried disaccharide trichloroacetimidate **5** (2.01 g, 3.40 mmol) in dry CH_2Cl_2 (80 ml) was stirred with 4 Å molecular sieves (1.5 g) under argon at room temperature for 15 min, and then TMSOTf (31 μl, 0.17 mmol) was slowly added. The reaction mixture was stirred for an additional 30 min, quenched with triethylamine, diluted with CH_2Cl_2. The molecular sieves were filtered out. The filtrate was washed with saturated $NaHCO_3$ and brine respectively, and then dried over anhydrous $NaSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column to afford three products: compound **6** (190 mg, 10%), compound **7** (901 mg, 46%), compound **8** (233 mg, 13%).

Compound **6**: ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 5.22–5.29 (m, 3H, H-6, 3′, 3″), 5.07 (d, 1H, *J* = 1.5 Hz, H-1″), 4.91–5.06 (m, 3H), 4.66 (d, 1H, *J* = 7.8 Hz, H-1′), 4.26–4.31 (m, 2H, H-16, 5″), 3.99–4.16 (m, 3H), 3.84–3.94 (m, 2H, H-26), 3.65–3.75 (m, 2H), 3.42–3.52 (m, 1H, H-3), 2.10 (s, 3H), 2.06 (s, 3H), 2.03 (s, 6H), 2.01 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 0.04 (s, 6H, Me_2Si). LC-HRESIMS *m*/*z* 1173.5968 $[M+Na]^+$ (calcd for $C_{59}H_{94}O_{20}NaSi$ 1173.6005).

Compound **7**: ^{1}H NMR (δ, $CDCl_3$, 300 MHz): 5.32 (t, 1H, *J* = 9.9 Hz, H-3′), 5.25 (d, 1H, *J* = 6.0 Hz, H-6), 5.17 (dd, 1H, *J* = 3.3, 10.2 Hz, H-3″), 5.06 (t, 1H, *J* = 9.9 Hz, H-4′), 5.04–5.06 (m, 1H, H-2″), 4.98 (d, 1H, *J* = 3.0 Hz, H-1′), 4.95 (t, 1H, *J* = 9.9 Hz, H-4″), 4.91 (d, 1H, *J* = 1.2 Hz, H = 1″), 4.42–4.48 (m, 1H, H-16), 4.28 (dd, 1H, *J* = 3.9, 12.3 Hz, H-6a′), 4.01–4.05 (m, 1H, H-6b′), 3.80–3.97 (m, 5H, H-26, 22, 2′, 5′),

3.64–3.73 (m, 1H, H-5″), 3.41–3.52 (m, 1H, H-3), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 0.04 (s, 6H, Me_2Si). ^{13}C NMR (δ, $CDCl_3$, 150 MHz): 171.34, 170.64, 170.11, 169.98, 169.90, 169.71, 169.27, 141.81 (C-5), 120.77 (C-6), 98.91 (C-1′), 94.39 (C-1″), 75.95 (C-2′), 75.14 (C-22), 73.46 (C-16), 72.44 (C-3), 71.79 (C-3′), 70.87 (C-4′), 69.95 (C-2″), 69.21 (C-26), 68.84 (C-5″), 68.52 (C-4″), 68.39 (C-3″), 67.19 (C-5′), 61.92 (C-6′), 56.47, 54.58, 50.09, 42.68, 42.17, 39.72, 37.26, 36.49, 35.04, 33.32, 33.20, 33.04, 31.98, 31.61, 31.57, 30.40, 25.90 (3C), 20.95, 20.81, 20.77, 20.71, 20.70, 20.57 (2C), 20.48, 19.14, 18.22, 17.19, 17.15, 12.85, 11.16, −4.61 (2C). LC-HRESIMS *m/z* 1151.6271 $[M+H]^+$ (calcd for $C_{59}H_{95}O_{20}Si$ 1151.6186).

Compound **8**: ^1H NMR (δ, $CDCl_3$, 300 MHz): 5.30 (d, 1H, *J* = 3.9 Hz, H-6), 5.22 (t, 1H, *J* = 9.3 Hz, H-3′), 5.13–5.01 (m, 4H, H-1″, 2″, 3″, 4″), 4.94 (t, 1H, *J* = 9.3 Hz, H-4′), 4.48 (d, 1H, *J* = 7.8 Hz, H-1′), 4.16–4.30 (m, 2H, H-16, 5′), 3.72–4.09 (m, 5H, H-22, 26, 6a′, 6b′), 3.68–3.70 (m, 1H, H-2′), 3.55–3.62 (m, 1H, H-5″), 3.43–3.48 (m, 1H, H-3), 2.12 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 0.04 (s, 6H, Me_2Si). LC-HRESIMS *m/z* 1151.6274 $[M+H]^+$ (calcd for $C_{59}H_{95}O_{20}Si$ 1151.6186).

2.2.6. (22S,25R)-26-Acetyloxy-3β-tert-butyldimethylsilyloxy-22-hydroxycholest-5-ene-16-one 22-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-β-D-glucopyranoside] (compound **9**)

To a solution of compound **6** (20 mg, 0.017 mmol) in CH_2Cl_2 (2 ml) was added pyridinium dichromate (13 mg, 0.035 mmol). The reaction was stirred at room temperature for 48 h and was quenched with saturated $NaSO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with saturated $NaHCO_3$ and brine, dried with anhydrous $NaSO_4$, and then filtered. The filtrate was concentrated in vacuo and purified by column chromatography to yield compound **9** (15 mg, 67%).

^1H NMR (δ, $CDCl_3$, 300 MHz): 5.33 (d, 1H, *J* = 3.9 Hz, H-6), 5.22 (t, 1H, *J* = 9.3 Hz, H-3′), 5.15–5.19 (m, 1H, H-3″), 5.03–5.09 (m, 3H, H-1″,2″,4″), 4.94 (t, 1H, *J* = 9.3 Hz, H-4′), 4.45 (d, 1H, *J* = 7.8 Hz, H-1′), 4.07–4.31 (m, 4H, H-22, 5′, 6a′, 6b′), 3.85–3.97 (m, 2H, H-26), 3.66–3.71 (m, 1H, H-2′), 3.61–3.64 (m, 1H, H-5″), 3.45–3.58 (m, 1H, H-3), 2.12 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 0.06 (s, 6H, Me_2Si). ^{13}C NMR (δ, $CDCl_3$, 150 MHz): 219.92 (C-16), 171.22, 170.64, 170.42, 169.98, 169.88, 169.71, 169.32, 141.45 (C-5), 120.64 (C-6), 101.72 (C-1′), 96.87 (C-1″), 79.13 (C-2′), 74.98, 74.65, 72.37, 71.66, 70.95, 69.52, 69.31, 69.11, 68.66, 66.67, 62.83, 62.13, 49.88, 49.15, 43.01, 42.71, 39.19, 38.90, 36.97, 36.61, 34.66, 32.40, 31.97, 31.58, 31.02, 30.82, 29.23, 25.92 (3C), 20.97, 20.85, 20.77, 20.73, 20.70, 20.64, 20.61, 19.40, 18.24, 17.16, 16.65, 14.11, 12.89, 11.35, −4.58 (2C). LC-HRESIMS *m/z* 1149.6113 $[M+H]^+$ (calcd for $C_{59}H_{93}O_{20}Si$ 1149.6024).

2.2.7. (22S,25R)-3β,22,26-Trihydroxycholest-5-ene-16-one 22-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside] (compound **11**)

To a solution of compound **9** (26 mg, 0.023 mmol) in MeOH–CH_2Cl_2 (4:1, 2 ml) was added *p*-toluenesulfonic acid monohydrate (0.4 mg, 0.0023 mmol). The reaction was stirred at room temperature for 6 h and was quenched with saturated $NaHCO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with saturated $NaHCO_3$ and brine, dried with anhydrous $NaSO_4$, and then filtered. The filtrate was concentrated in vacuo to give a residue and purified by silica gel column chromatography to afford compound **10** (22 mg, 94%), which was dissolved in MeOH–CH_2Cl_2 (1:1, 2 ml), and then catalytic amount of NaOMe/MeOH solution (1 M) was added. The reaction was refluxed at 80 °C for 24 h, neutralized to neutral with Amberlite® IR-120 (H+), and filtered. The filtrate was concentrated in vacuo. The residue was purified with sephadex LH-20 column chromatography (MeOH) to afford compound **11** (15 mg, 88% two steps).

^1H NMR (δ, $CDCl_3$, 600 MHz): 6.40 (s, 1H, H-1″), 5.32 (d, 1H, *J* = 4.2 Hz, H-6), 4.77–4.81 (m, 1H, H-2″), 4.73 (dd, 1H, *J* = 3.0, 9.6 Hz, H-3″), 4.13–4.48 (m, 8H, H-22, 1′, 2′, 3′, 4′, 6a′, 6b′, 4″), 3.70–3.82 (m, 5H, H-3, 26, 5′, 5″), 1.35 (d, 3H, *J* = 6.6 Hz, H-6″), 1.10 (d, 3H, *J* = 6.6 Hz, H-27), 1.02 (s, 3H), 0.80 (s, 3H). LC-HRESIMS *m/z* 763.4229 $[M+Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$ 763.4244).

2.2.8. (25R)-26-Acetyloxy-3β-tert-butyldimethylsilyloxy-16β-hydroxycholest-5-ene-22-one 16-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-glucopyranoside] (compound **12**)

Following the same procedure as compound **9**, compound **7** (60 mg, 0.051 mmol) was converted to compound **12** (50 mg, 83%).

^1H NMR (δ, $CDCl_3$, 600 MHz): 5.29 (d, 1H, *J* = 3.0 Hz, H-6), 5.22 (t, 1H, *J* = 9.6, H-3′), 5.21 (dd, 1H, *J* = 3.6, 10.2 Hz, H-3″), 5.03 (t, 1H, *J* = 9.6 Hz, H-4′), 4.99 (dd, 1H, *J* = 1.2 Hz, H-2″), 4.93 (t, 1H, *J* = 10.2 Hz, H-4″), 4.91 (d, 1H, *J* = 3.6 Hz, H-1′), 4.83 (s, 1H, H-1″), 4.39 (q, 1H, *J* = 5.4, 13.2 Hz, H-16), 4.28 (dd, 1H, *J* = 3.6, 12.6 Hz, H-6a′), 4.04 (d, 1H, *J* = 12.0 Hz, H-6b′), 3.93 (m, 2H, H-26), 3.87 (m, 1H, H-5′), 3.74 (dd, 1H, *J* = 3.6, 9.6 Hz, H-2′), 3.55 (d, 1H, *J* = 9.6 Hz, H-5″), 3.46 (m, 1H, H-3), 3.04–3.10 (m, 1H), 2.93–2.98 (m, 1H), 2.54–2.61 (m, 1H), 2.12 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 0.04 (s, 6H, Me_2Si). ^{13}C NMR (δ, $CDCl_3$, 150 MHz): 213.65 (C-22), 171.18, 170.60, 170.31, 169.99, 169.79, 169.59, 169.51, 141.63 (C-5), 120.69 (C-6), 98.97 (C-1″), 93.31 (C-1′), 75.84 (C-2′), 74.24 (C-16), 72.43 (C-3), 71.80 (C-3′), 71.07 (C-4′), 70.04 (C-2″), 69.15 (C-26), 68.21 (C-3″), 68.14 (C-4″), 67.92 (C-5″), 66.96 (C-5′), 61.43 (C-6′), 54.98, 53.40, 49.86, 43.41, 42.70, 41.63, 39.88, 37.74, 37.22, 36.46, 31.99, 31.97, 31.84, 31.65, 31.60, 26.94, 25.90 (3C), 20.93, 20.84, 20.81, 20.72, 20.68, 20.62, 20.58, 20.50, 19.35, 18.23, 17.06, 17.08, 16.75, 13.50, −4.62 (2C). LC-HRESIMS *m/z* 1171.5868 $[M+Na]^+$ (calcd for $C_{59}H_{92}O_{20}NaSi$ 1171.5843).

2.2.9. (25R)-26-Acetyloxy-3β,16β-dihydroxycholest-5-ene-22-one 16-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-α-D-glucopyranoside] (compound **13**)

Following the same procedure as compound **10**, compound **12** (53 mg, 0.046 mmol) was converted to compound **13** (45 mg, 92%).

^1H NMR (δ, $CDCl_3$, 600 MHz): 5.32 (d, 1H, *J* = 4.8 Hz, H-6), 5.21 (m, 2H, H-3′, 3″), 5.02 (t, 1H, *J* = 10.2 Hz, H-4′), 4.98 (dd, 1H, *J* = 1.2, 3.0 Hz, H-2″), 4.92 (t, 1H, *J* = 10.2 Hz, H-4″), 4.90 (d, 1H, *J* = 3.6 Hz, H-1′), 4.82 (s, 1H, H-1″), 4.38 (q, 1H, *J* = 4.8, 12.6 Hz, H-16), 4.27 (dd, 1H, *J* = 3.2, 12.6 Hz, H-6a′), 4.03 (d, *J* = 12.0 Hz, H-6b′), 3.91–3.92 (m, 2H, H-26), 3.85–3.86 (m, 1H, H-5′), 3.73 (dd, 1H, *J* = 3.6, 10.8 Hz, H-2′), 3.54 (d, 1H, *J* = 10.2 Hz, H-5″), 3.48–3.53 (m, 1H, H-5), 3.04–3.09 (m, 1H), 2.92–2.98 (m, 1H), 2.54–2.59 (m, 1H), 2.27 (dd, 1H, *J* = 3.6, 13.2 Hz), 2.11 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.04 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H). ^{13}C NMR (δ, $CDCl_3$, 150 MHz): 213.64 (C-22), 171.18, 170.58, 170.29, 169.98, 169.75, 169.57, 169.50, 140.86 (C-5), 121.17 (C-6), 98.96 (C-1″), 93.28 (C-1′), 75.87, 74.18, 71.75, 71.52, 71.09, 70.02, 69.13, 68.18, 68.11, 67.90, 66.93, 61.40, 54.95, 53.32, 49.74, 43.37, 42.07, 41.59, 39.82, 37.72, 37.07, 36.36, 31.97, 31.79, 31.59, 31.57, 31.48, 26.91, 20.90, 20.81 (2C), 20.70, 20.66, 20.61, 20.56, 20.48, 19.30, 17.03 (2C), 16.72, 13.47. LC-HRESIMS *m/z* 1057.5116 $[M+Na]^+$ (calcd for $C_{53}H_{78}O_{20}Na$ 1057.49841).

*2.2.10. (25R)-3β,16β,26-Trihydroxy-cholest-5-ene-22-one 16-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranoside] (compound **14**)*

Following the same procedure as compound **11** from **10**, compound **13** (45 mg, 0.043 mmol) was converted to compound **14** (30 mg, 94%).

m.p.: 173.0–175.0 °C. ^{1}H NMR (δ, C_5D_5N, 600 MHz): 5.89 (s, 1H, H-1″), 5.39 (d, 1H, *J* = 4.8 Hz, H-6), 5.37 (d, 1H, *J* = 3.6 Hz, H-1′), 4.81 (m, 1H, H-2″), 4.74 (q, 1H, *J* = 5.4, 12.6 Hz, H-16), 4.51 (dd, 1H, *J* = 3.6, 9.6 Hz, H-6a′), 4.37–4.42 (m, 3H, H-5′, 6b′, 3″), 4.23–4.27 (m, 2H, H-4′, 4″), 4.13 (dd, 1H, *J* = 3.6, 9.6 Hz, H-2′), 3.90–3.93 (m, 1H, H-5″), 3.82–3.87 (m, 1H, H-3), 3.65–3.76 (m, 2H, H-26), 1.59 (d, 3H, *J* = 6.0 Hz), 1.23 (s, 3H), 1.11 (d, 3H, *J* = 3.6 Hz), 1.07 (s, 3H). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 213.39 (C-22), 141.88 (C-5), 121.37 (C-6), 104.40, 95.02, 79.74, 74.40, 74.14, 73.92, 73.87, 72.67, 72.22, 71.78, 71.30, 70.02, 67.36, 62.42, 55.85, 54.26, 50.54, 43.79, 43.62, 42.10, 40.46, 38.47, 37.84, 36.98, 36.71, 32.70, 32.16, 31.80, 30.01, 27.61, 21.26, 19.61, 18.37, 17.31, 17.21, 14.19. LC-HRESIMS *m*/*z* 763.4333 $[M+Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$ 763.4244).

*2.2.11. (25R)-26-Acetyloxy-3β-tert-butyldimethylsilyloxy-16β-hydroxycholest-5-ene-22-one 16-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-β-D-glucopyranoside] (compound **15**)*

Following the same procedure as compound **9**, compound **8** (13 mg, 0.0113 mmol) was converted to compound **15** (10 mg, 77%).

^{1}H NMR (δ, $CDCl_3$, 600 MHz): 5.38 (dd, 1H, *J* = 3.6, 9.6 Hz, H-3″), 5.30 (d, 1H, *J* = 5.4 Hz, H-6), 5.14 (t, 1H, *J* = 9.6 Hz, H-3′), 5.09 (s, H-1″), 5.07 (t, 1H, *J* = 10.2 Hz, H-4′), 5.01 (m, 1H, H-2″), 4.92 (t, 1H, *J* = 9.6 Hz, H-4″), 4.23 (dd, 1H, *J* = 5.4, 7.2 Hz, H-6a′), 4.02–4.06 (m, 2H, H-1′, 6b′), 3.92–3.93 (m, 2H, H-26), 3.85–3.89 (m, 1H, H-5′), 3.73–3.76 (m, 1H, H-2′), 3.62–3.64 (m, 1H, H-5″), 3.45–3.49 (m, 1H, H-3), 2.12 (s, 3H), 2.07 (s, 3H), 2.05 (s, 6H), 2.00 (s, 6H), 1.98 (s, 3H), 0.05 (s, 6H, Me_2Si). ^{13}C NMR (δ, $CDCl_3$, 150 MHz): 214.68 (C-22), 171.22, 170.58, 169.93, 169.87, 169.71 (2C), 169.65, 141.46 (C-5), 120.84 (C-6), 101.55 (C-1′), 96.85 (C-1″), 80.22 (C-2′), 74.94, 73.90, 72.61, 72.10, 71.55, 69.76, 69.41, 69.16, 68.33, 66.40, 62.19, 55.91, 54.34, 50.07, 43.95, 42.75, 41.61, 39.29, 38.40, 37.29, 36.51, 32.02, 31.87, 31.66, 31.31, 29.68, 26.77, 25.92 (3C), 20.97 (2C), 20.84 (2C), 20.75 (2C), 20.68 (2C), 19.48, 18.24, 17.65, 16.62, 16.15, 13.43, −4.60 (2C). LC-HRESIMS *m*/*z* 1149.6122 $[M+H]^+$ (calcd for $C_{59}H_{93}O_{20}Si$ 1149.6024).

*2.2.12. (25R)-3β,16β,26-Trihydroxy-cholest-5-ene-22-one 16-O-[O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside] (compound **17**)*

Following the same procedure as compound **11**, compound **15** (26 mg, 0.023 mmol) was converted to compound **17** (16 mg, 94% two steps).

^{1}H NMR (δ, C_5D_5N, 600 MHz): 6.64 (s, 1H, H-1″), 5.33 (d, 1H, *J* = 4.8 Hz, H-6), 4.84 (m, 1H, H-2″), 4.76–4.78 (m, 2H, H-16, 3″), 4.56 (d, 1H, *J* = 7.8 Hz, H-1′), 4.12–4.49 (m, 7H, H-2′, 3′, 4′, 5′, 6a′, 6b′, 4″), 3.80–3,85 (m, 1H, H-3), 1.75 (d, 1H, *J* = 6.0 Hz), 1.18 (s, 3H), 1.08 (d, 3H, *J* = 6.6 Hz), 1.00 (s, 3H). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 214.95 (C-22), 141.92 (C-5), 121.26 (C-6), 103.39, 101.93, 79.91, 19.70, 78.69, 77.06, 74.18, 72.73, 72.38, 72.27, 71.27, 69.62, 67.60, 62.72, 57.09, 54.86, 50.55, 43.93, 43.53, 42.09, 39.84, 39.69, 37.76, 37.64, 36.92, 36.39, 32.66, 32.08, 31.74, 27.85, 21.10, 19.66, 19.07, 17.40, 16.71, 14.03. LC-HRESIMS *m*/*z* 763.4309 $[M+Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$ 763.4329).

*2.2.13. (25R)-26-acetyloxy-3β-benzoyloxycholest-5-ene-16,22-dione (compound **18**)*

To a solution of compound **1** (5.6 g, 9.55 mmol) in CH_2Cl_2–MeOH (1:3, 80 ml) was added catalytic amount of *p*-toluenesulfonic acid monohydrate. The reaction was stirred at room temperature for 6 h and was quenched with saturated $NaHCO_3$. The organic layer was separated and the water layer was extracted with CH_2Cl_2 three times. The combined organic layer was washed with saturated $NaHCO_3$ and brine, dried with anhydrous $NaSO_4$, and then filtered. The filtrate was concentrated in vacuo to give a residue. The residue was dissolved in pyridine (60 ml) at 0 °C (ice bath), benzoyl chloride (1.66 ml, 14.32 mmol) was added. The reaction was stirred for 24 h and concentrated in vacuo to give a residue. The residue was purified by column chromatography to yield compound **18** (5.12 g, 93%).

^{1}H NMR (δ, $CDCl_3$, 300 MHz): 8.04 (d, 2H, *J* = 7.6 Hz, Ph), 7.55 (t, 1H, *J* = 7.6 Hz, Ph), 7.43 (t, 2H, *J* = 7.6 Hz, Ph), 5.42 (d, 1H, *J* = 5.1 Hz, H-6), 4.82–4.92 (m, 1H, H-3), 3.93–3.96 (m, 2H, H-26), 2.17 (s, 3H), 2.05 (s, 3H), 1.10 (s, 3H), 0.96 (d, 3H, *J* = 6.6 Hz), 0.81 (s, 3H). ^{13}C NMR (δ, $CDCl_3$, 100 MHz): 218.02 (C-16), 213.33 (C-22), 171.37 (Bz), 165.93 (Ac), 139.81 (C-5), 133.63, 132.73, 130.11, 129.47, 128.40, 128.22, 121.88 (C-6), 74.26 (C-3), 69.01 (C-26), 66.10 (C-17), 51.09, 49.53, 43.31, 41.61, 39.63, 38.49, 38.06, 37.13, 36.67, 36.64, 32.00, 31.68, 30.87, 27.71, 26.64, 20.91, 20.45, 19.32, 16.76, 15.33, 12.93. LC-HRESIMS *m*/*z* 577.3532 $[M+H]^+$ (calcd for $C_{36}H_{49}O_6$ 577.3532).

*2.2.14. (22R,25R)-26-Acetyloxy-3β-benzoyloxyfurost-5-ene-22-ol (compound **19**)*

To a solution of compound **18** (1.6 g, 2.77 mmol) in dry THF (50 ml) under argon cooled to −15 °C, $NaBH_4$ (1.16 g, 30.5 mmol) and $CeCl_3{\cdot}7H_2O$ (1.45 g, 3.88 mmol) were added. The reaction mixture was stirred for 16 h, then cooled to −40 °C and quenched with methyl alcohol. The suspension was diluted with CH_2Cl_2. The organic layer was washed with saturated $NaHCO_3$ and brine respectively, and then dried over anhydrous $NaSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column to afford furostan sapogenin **19** (1.1 g, 69%, white solid).

^{1}H NMR (δ, C_5D_5N, 300 MHz): 8.25 (d, 2H, *J* = 7.2 Hz, Ph), 7.44–7.55 (m, 3H, Ph), 5.37 (s, 1H, H-6), 4.98–5.04 (m, 2H, H-3, 16), 3.95–4.12 (m, 2H, H-26), 1.96 (s, 3H), 1.34 (d, 3H, *J* = 6.6 Hz), 1.02 (s, 3H), 0.92 (s, 3H), 0.91 (d, 3H, *J* = 6.6 Hz). ^{13}C NMR (δ, C_5D_5N, 100 MHz): 170.77 (Ac), 165.98 (Bz), 139.90 (C-5), 133.28, 131.39, 129.92 (2C), 128.86 (2C), 122.83 (C-6), 110.49 (C-22), 81.15 (C-16), 74.84 (C-3), 69.38 (C-26), 63.78 (C-17), 56.54 (C-14), 50.17 (C-9), 40.82, 40.70, 39.91, 38.52, 37.19, 37.07, 36.98, 33.31, 32.45, 32.28, 31.63, 28.22, 28.14, 21.12, 20.75, 19.38, 17.04, 16.49, 16.41. LC-HRESIMS *m*/*z* 601.3582 $[M+Na]^+$ (calcd for $C_{36}H_{50}O_6Na$ 601.3505).

*2.2.15. (22S,25R)-26-Acetyloxy-3β-benzoyloxyfurost-5-ene-22-ol 22-O-(3,4,6-tetra-O-acetyl-α-D-glucopyranoside) (compound **21**) and (22R,25R)-26-acetyloxy-3β-benzoyloxyfurost-5-ene-22-ol 22-O-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside) (compound **22**)*

A solution of carefully dried furostan sapogenin 19 (128 mg, 0.218 mmol) and carefully dried monosaccharide trichloroacetimidate 20 (107 mg, 0.218 mmol) in dry CH_2Cl_2 (6 ml) was stirred with 4 Å molecular sieves (100 mg) under argon at −20 °C for 15 min, and then TMSOTf (3.9 μl, 0.0218 mmol) was added. The reaction mixture was stirred for an additional 1 h, quenched with triethylamine, diluted with CH_2Cl_2. The molecular sieves were filtered out. The filtrate was washed with saturated $NaHCO_3$ and brine respectively, then dried over anhydrous $NaSO_4$, and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column to afford two products: compound **21** (55 mg, 28%), compound **22** (58 mg, 29%).

Compound **21**: m.p.: 65.8–67.8 °C. ^{1}H NMR (δ, C_5D_5N, 600 MHz): 8.24 (d, 2H, *J* = 7.2 Hz, Ph), 7.57 (t, 1H, *J* = 7.6 Hz, Ph), 7.46 (t, 2H, *J* = 7.6 Hz, Ph), 6.04 (t, 1H, *J* = 9.6 Hz, H-3′), 5.87 (d, 1H, *J* = 3.6 Hz, H-1′), 5.50 (t, 1H, *J* = 9.6 Hz, H-4′), 5.36–5.38 (m, 2H, H-6, 2′), 5.00–5.05

(m, 1H, H-16), 4.91–4.99 (m, 1H, H-3), 4.61–4.64 (m, 1H, H-5′), 4.46–4.56 (m, 2H, H-6a′, 6b′), 4.01–4.13 (m, 2H, H-26), 2.15 (s, 3H, Ac), 2.13 (s, 3H, Ac), 2.08 (s, 6H, Ac), 2.04 (s, 3H, Ac), 1.20 (d, 3H, J = 6.6 Hz), 1.04 (s, 3H), 0.98 (d, 3H, J = 6.6 Hz), 0.85 (s, 3H). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 170.82, 170.47 (2C), 170.41, 170.03, 166.01, 140.03 (C-5), 133.32, 131.38, 129.94 (2C), 128.89 (2C), 122.69 (C-6), 115.45 (C-22), 89.36 (C-1′), 83.05, 74.78, 71.58, 70.86, 69.89, 69.08, 68.71, 63.67, 62.29, 56.44, 50.21, 41.24, 39.80, 38.52, 37.16, 37.01, 32.26, 32.45, 32.33, 32.29, 31.43, 28.14 (2C), 21.07, 20.78 (3C), 20.61, 20.56, 20.55, 19.39, 16.84, 16.48, 15.40. LC-HRESIMS m/z 931.4524 $[M+Na]^+$ (calcd for $C_{50}H_{68}O_{15}Na$ 931.4455).

Compound **22**: m.p.: 47.6–49.6 °C. ^{1}H NMR (δ, C_5D_5N, 600 MHz): 8.24 (d, 2H, J = 7.2 Hz, Ph), 7.54 (t, 1H, J = 7.6 Hz, Ph), 7.46 (t, 2H, J = 7.6 Hz, Ph), 6.85 (d, 1H, J = 3.6 Hz, H-1′), 5.98 (t, 1H, J = 10.2 Hz, H-3′), 5.61 (t, 1H, J = 10.2 Hz, H-4′), 5.36 (d, 1H, J = 4.8 Hz, H-6), 4.99–5.05 (m, 1H, H-3), 4.73 (q, 1H, J = 7.8, 15.0 Hz, H-16), 4.28–4.54 (m, 3H, H-2′, 6a′, 6b′), 4.00–4.13 (m, 2H, H-26), 2.25 (s, 3H, Ac), 2.20 (s, 3H, Ac), 2.09 (s, 3H, Ac), 2.07 (s, 3H, Ac), 1.96 (s, 3H, Ac), 1.15 (d, 3H, J = 6.6 Hz), 1.00 (s, 3H), 0.95 (d, 3H, J = 6.6 Hz), 0.80 (s, 3H). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 170.85, 170.49, 170.08, 169.88, 169.77, 166.01, 139.89 (C-5), 133.31, 131.40, 129.95 (2C), 128.89 (2C), 122.76 (C-6), 114.82 (C-22), 91.58 (C-1′), 82.71, 74.85, 71.42, 69.85, 69.35, 69.01, 69.03, 63.07, 62.30, 56.20, 50.01, 41.07, 40.55, 39.50, 38.50, 37.16, 36.96, 33.13, 32.30, 32.15, 31.42, 31.32, 28.14, 27.86, 20.99, 20.91, 20.85 (2C), 20.79, 20.56, 19.35, 16.83, 16.10, 14.26. LC-HRESIMS m/z 931.4432 $[M+Na]^+$ (calcd for $C_{50}H_{68}O_{15}Na$ 931.4450).

2.2.16. (22S,25R)-Furost-5-ene-3β,22,26-triol 22-O-(α-D-glucopyranoside) (compound **23**)

To a solution of compound **21** (39 mg, 0.043 mmol) in MeOH–CH_2Cl_2 (1:1, 3 ml) was added 0.26 ml NaOMe/MeOH solution (1 M). The reaction was refluxed at 80 °C for 48 h, and then concentrated in vacuo. The residue was purified with sephadex LH-20 column chromatography (MeOH) to afford compound **23** (23 mg, 92%).

^{1}H NMR (δ, C_5D_5N, 600 MHz): 5.85 (d, 1H, J = 3.6 Hz, H-1′), 5.37 (q, 1H, J = 7.8, 15.0 Hz, H-16), 5.30 (d, 1H, J = 4.8 Hz, H-6), 4.55–4.62 (m, 2H), 4.37–4.41 (m, 1H), 4.11–4.16 (m, 2H), 3.71–3.83 (m, 4H, H-3, 26, 5′). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 141.95 (C-5), 121.08 (C-6), 114.54 (C-22), 93.08 (C-1′), 82.55 (C-16), 75.56, 75.00, 73.71, 72.62, 71.27, 67.59, 63.33, 56.34, 50.36, 43.53, 41.13, 40.92, 39.80, 37.83, 37.18, 37.04, 34.06, 32.65, 32.43, 32.28, 31.63, 30.02, 28.14, 21.17, 19.60, 17.22, 16.49, 16.17. LC-HRESIMS m/z 617.3701 $[M+Na]^+$ (calcd for $C_{33}H_{54}O_9Na$ 617.3665).

2.2.17. (22R,25R)-Furost-5-ene-3β,22,26-triol 22-O-(α-D-glucopyranoside) (compound **24**)

Following the same procedure as compound **23**, compound **22** (42 mg, 0.046 mmol) was converted to compound **24** (16 mg, 93%).

^{1}H NMR (δ, C_5D_5N, 600 MHz): 6.06 (d, 1H, J = 3.6 Hz, H-1′), 5.38 (q, 1H, J = 7.8, 15.0 Hz, H-16), 5.36 (d, 1H, J = 4.8 Hz, H-6), 4.12–4.78 (m, 5H, H-2′, 3′, 4′, 6a′, 6b′), 3.70–3.86 (m, 4H, H-3, 26, 5′). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 142.01 (C-5), 121.05 (C-6), 114.80 (C-22), 110.80 (C-1′), 81.93 (C-16), 75.56, 75.01, 73.72, 72.63, 71.28, 67.55, 63.57, 56.34, 50.48, 43.55, 40.87, 40.52, 39.87, 37.83, 37.61, 37.06, 34.07, 32.66, 32.35, 32.24, 31.78, 29.29, 28.46, 21.23, 19.62, 17.21, 16.49, 16.36. LC-HRESIMS m/z 617.3654 $[M+Na]^+$ (calcd for $C_{33}H_{54}O_9Na$ 617.3660).

2.2.18. (22R,25R)-26-Acetyloxy-3β-benzoyloxyfurost-5-ene-22-ol 22-O-[O-2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3,4,6-tri-O-acetyl-β-D-glucopyranoside] (compound **25**)

A solution of carefully dried furostan sapogenin **19** (208 mg, 0.36 mmol) and carefully dried disaccharide trichloroacetimidate **5** (130 mg, 0.18 mmol) in dry CH_2Cl_2 (6 ml) was stirred with 4 Å molecular sieves (100 mg) under argon at −20 °C for 15 min, and then TMSOTf (3.3 μl, 0.018 mmol) was added. The reaction mixture was stirred for 2 h, quenched with triethylamine, diluted with CH_2Cl_2. The molecular sieves were filtered out. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column to afford compound **25** (64 mg, 32%).

^{1}H NMR (δ, C_5D_5N, 300 MHz): 8.26 (d, 2H, J = 7.8 Hz, Ph), 7.54 (t, 1H, J = 7.6 Hz, Ph), 7.46 (t, 2H, J = 7.8 Hz, Ph), 6.12 (t, 1H, J = 9.6 Hz, H-3′), 5.78 (d, 1H, J = 3.6 Hz, H-1′), 5.48–5.65 (m, 4H, H-4′, 2″, 3″, 4″), 5.35 (m, 2H, H-6, 1″), 4.87–5.13 (m, 2H, H-3, 16), 4.76 (dd, 1H, J = 4.8, 12.3 Hz, H-6a′), 4.05–4.59 (m, 6H, H-26, 2′, 5′, 6b′, 5″), 2.21 (s, 3H, Ac), 2.19 (s, 3H, Ac), 2.10 (s, 3H, Ac), 2.05 (s, 6H, Ac), 2.04 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.39 (d, 3H, J = 6.0 Hz), 1.34 (d, 3H, J = 6.3 Hz), 1.06 (d, 3H, J = 6.9 Hz), 1.03 (s, 3H), 0.86 (s, 3H). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 170.91, 170.61, 170.32, 170.18, 170.04, 169.85, 166.03, 164.54, 140.15 (C-5), 133.33, 131.39, 129.95 (2C), 128.90 (2C), 123.16 (C-6), 119.68 (C-22), 99.18, 92.20, 90.51, 75.40, 74.87, 72.77, 72.57, 71.42, 70.47, 70.00, 69.30, 69.18, 68.54, 67.64, 63.01, 56.70, 49.88, 40.60, 38.81, 38.56, 37.20, 36.96, 36.67, 36.01, 33.57, 32.30, 31.35, 31.18, 29.22, 28.17, 20.82 (3C), 20.78 (2C), 20.59, 20.51 (2C), 20.44, 19.38, 17.55, 17.34, 15.34. LC-HRESIMS m/z 1161.4956 $[M+Na]^+$ (calcd for $C_{60}H_{82}O_{21}Na$ 1161.52408).

2.2.19. (22S,25R)-Furost-5-ene-3β,22,26-triol 22-O-[O-α-L-rhamnopyranosyl-(1 → 2)-α-D-glucopyranoside] (compound **26**)

Following the same procedure as compound **23**, compound **25** (32 mg, 0.028 mmol) was converted to compound **26** (18 mg, 86%).

^{1}H NMR (δ, C_5D_5N, 600 MHz): 6.27 (s, 1H, H-1″), 5.97 (d, J = 3.0 Hz, 1H, H-1′), 5.29 (d, J = 4.2 Hz, 1H, H-6), 4.77 (m, 1H, H-2″), 4.17–4.56 (m, 10H, H-16, 2′, 3′, 4′, 5′, 6a′, 6b′, 3″, 4″, 5″), 3.78–3.93 (m, 3H, H-3, 26), 1.17 (s, 3H, H-6″), 0.99 (s, 3H, H-19), 0.94 (d, 3H, J = 7.2 Hz, H-19), 0.92 (d, 3H, J = 7.2 Hz, H-27), 0.85 (s, 3H, H-18). ^{13}C NMR (δ, C_5D_5N, 150 MHz): 141.66 (C-5), 121.34 (C-6), 118.89 (C-22), 103.25 (C-1″), 93.19 (C-1′), 90.37 (C-16), 76.96, 75.28, 74.49, 74.08, 72.90, 72.67, 72.59, 72.21, 71.32, 69.75, 67.34, 62.87, 56.87, 50.23, 43.60, 40.35, 39.31, 37.77, 37.40, 37.21, 36.96, 32.64, 32.23, 31.90, 31.64, 29.79, 20.95, 20.52, 19.58, 18.65, 17.35, 15.40, 12.24. LC-HRESIMS m/z 763.4235 $[M+Na]^+$ (calcd for $C_{39}H_{64}O_{13}Na$ 763.4239).

2.3. Conformational analysis

2.3.1. Conformational analysis of compounds **21** and **22**

Molecular structures of the 22S isomer of **21** and 22R isomer of **22** were built using Sybyl 7.3 version Molecular Modeling Software. Structural energy minimization was performed using the standard Tripos molecular mechanics force field and Gasteiger–Hückel charge in the software, with a 0.001 kcal/mol energy gradient convergence criterion on the Silicon Graphics IRIS Octane computer system. Structure manipulations and analysis were carried out with the set of tools provided with the SYBYL package. Molecular dynamics simulations and the minimizations of structures over Cartesian coordinates employed the Tripos force field.

2.3.2. Details of the simulation procedure

First a very fast and flexible systematic search was introduced, which could guarantee a completion of the task: to enumerate all of the different torsional rotational settings. Molecular structures of the 22S isomer of **21** and 22R isomer of **22** were searched systematically by changing the value of two specified torsions between oxygen atom at C-16 and anomeric proton, each conformers were minimized the strain energy and the results were analyzed using the Molecular Spreadsheet. The conformer of the minimal energy

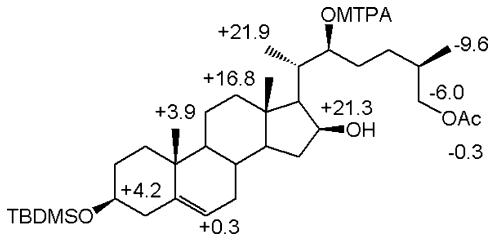
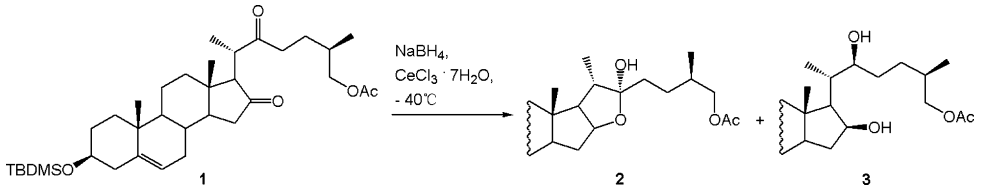
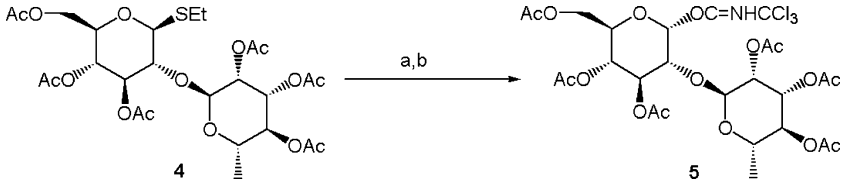


Fig. 1. Chemical shift differences between **3a** (S)-MTPA ester and **3b** (R)-MTPA ester $\Delta\delta$ (Hz) = $\delta_{(S)\text{-MTPA}} - \delta_{(R)\text{-MTPA}}$.

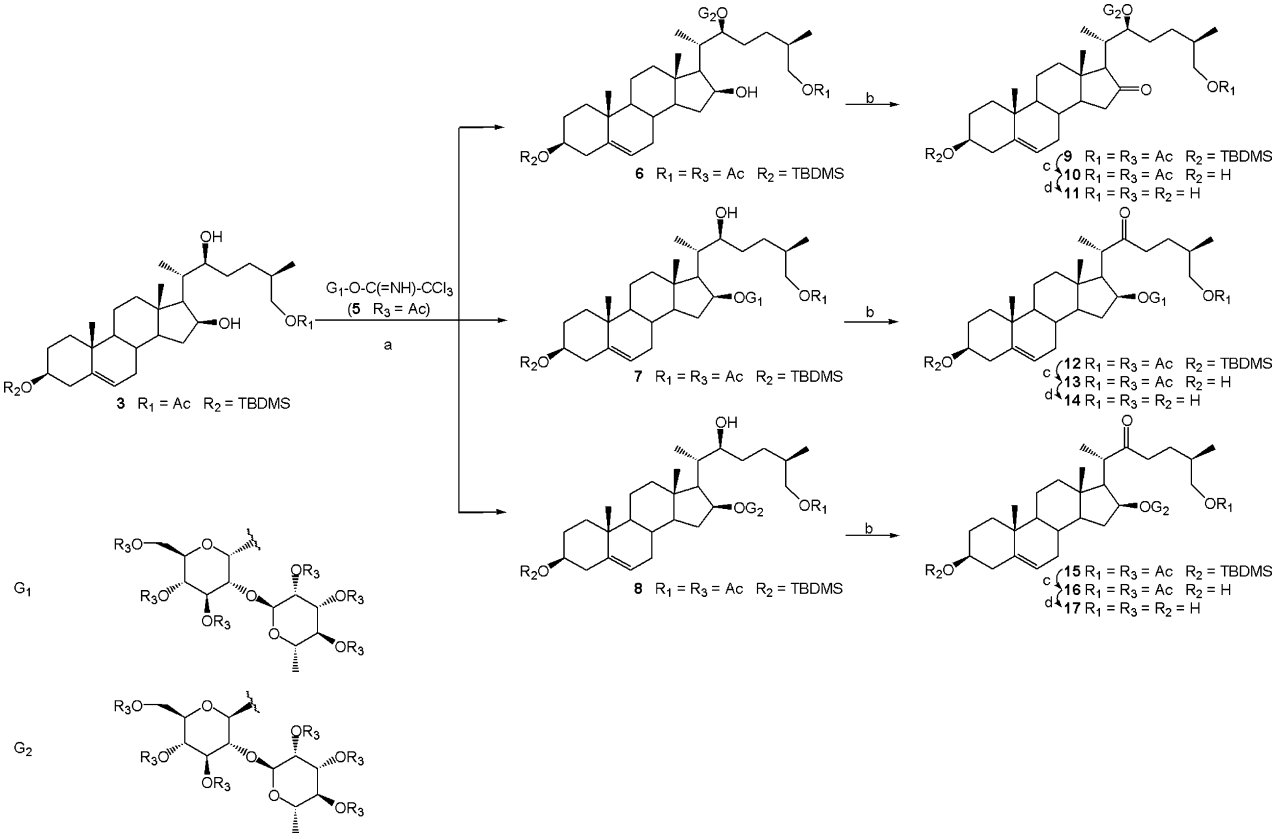
which obtained by systematically exploring different sets of starting coordinates was regard as the global minimum. Molecular dynamics (MD) simulation started from this conformer to maintain constant temperature (1000 K) during the simulation. A single molecular dynamics trajectory at constant temperature was generated. The molecular dynamics trajectory was updated using a modified Beeman method to integrate the Newtonian equations of motion. The time interval for the dynamics steps was set to 1 fs, and the total length of the simulated trajectory reached 1 ns. From the generated trajectory, 500 structures, equally spaced in time, were collected, with the interval between snapshots set to 2 ps. Each structure from the generated ensemble was then subjected to molecular dynamics simulated annealing computation. During a 2 ps simulation, the temperature changed between the starting



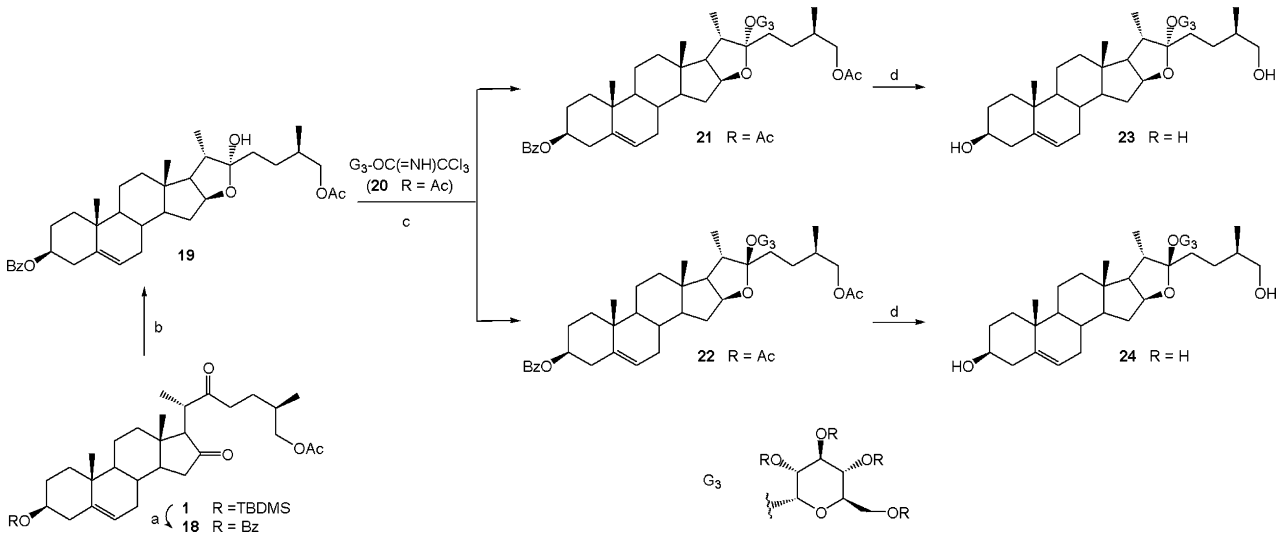
Scheme 1. The synthesis of cholestane and furostan sapogenins.



Scheme 2. (a) NIS, AgOTf, CH_2Cl_2, −15 °C → r.t. 1 h, 88% and (b) CCl_3CN, DBU, CH_2Cl_2, 0 °C, 6 h, 80%.



Scheme 3. (a) TMSOTf, 4 Å MS, dry CH_2Cl_2, r.t.; (b) PDC, CH_2Cl_2, r.t.; (c). *p*-TsOH·H_2O, MeOH/CH_2Cl_2, r.t. and (d) MeONa, MeOH/CH_2Cl_2, r.t.



Scheme 4. (a) *p*-TsOH·H_2O, MeOH/CH_2Cl_2, r.t., 6 h, then benzoyl chloride, pyridine, 0 °C, 24h, 93% two steps; (b) $NaBH_4$/$CeCl_3$·$7H_2O$, dry THF, −15 °C, 16 h, 69%; (c) TMSOTf, 4 Å MS, dry CH_2Cl_2, −20 °C, 1 h and (d) MeONa, MeOH/CH_2Cl_2, 80 °C, 48 h.

value of 1000 K and the final value of 100 K. The linear scaling protocol was used. Finally, all structures were minimized in Cartesian coordinate space with the value of the rms termination criterion set to 0.01 kcal/mol/Å. The value of the dielectric constant in all calculations was 1.0; the default value for the Tripos force field. The final set of 500 minimized structures was then further analyzed.

3. Results and discussion

At the beginning, intermediate (25R)-26-acetyloxy-3β-*tert*-butyldimethylsilyloxycholest-5-ene-16,22-dione **1** was reduced by $NaBH_4$/$CeCl_3$·$7H_2O$ in THF under controlled conditions (Scheme 1). In previous reports [11], it had been well documented that 16-ketone was more reactive than 22-ketone, which predicated the

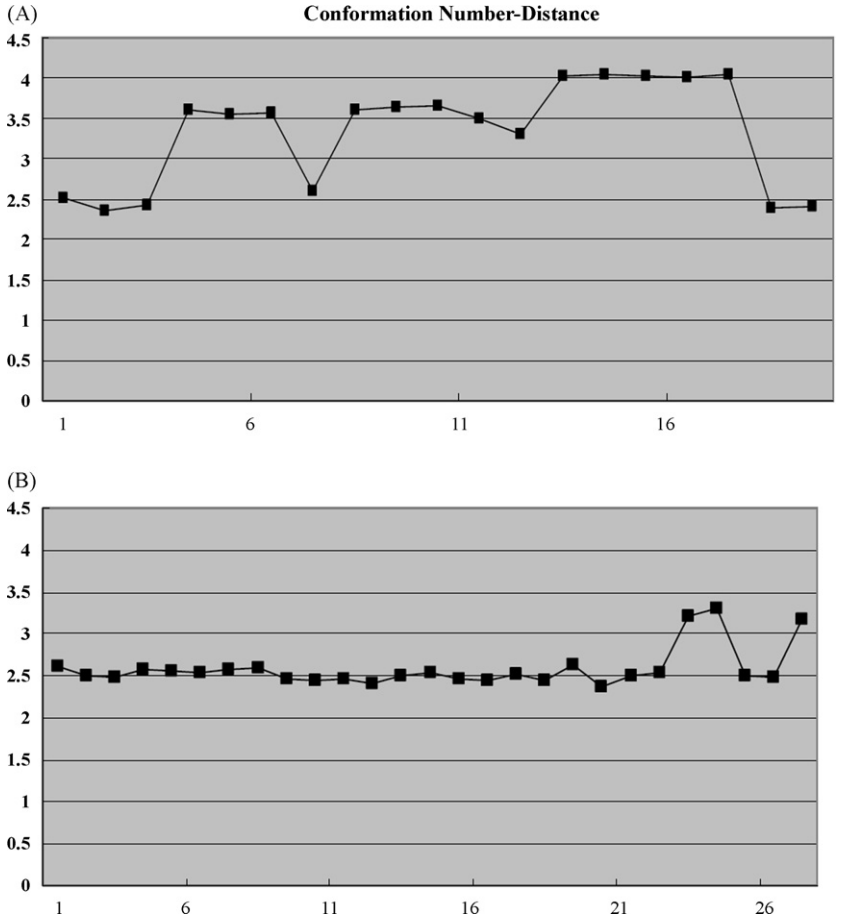


Fig. 2. Comparison of distances between oxygen atom at C-16 and anomeric proton in conformers of two epimers (**21** and **22**): (A) epimer **21** and (B) epimer **22**.

reduction of 16-ketone was easier than 22-ketone. Eventually, the reduction of compound **1** afforded two products: (22R,25R)-26-acetyloxy-3β-*tert*-butyldimethylsilyloxyfurost-5-ene-22-ol **2** and (22S,25R)-26-acetyloxy-3β-*tert*-butyldimethylsilyloxycholest-5-ene-16β,22-diol **3**. The relatively steady compound **3** was glycosylated firstly.

Before the glycosylation occurred, the absolute configuration of C-22 hydroxy in compound **3** was ascertained above all. 16β,22-Dihydroxy compound **3** was converted to 22-(S)- and 22-(R)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) derivatives (**3a** and **3b**) following the application of classical Mosher's method [12], which allowed us to assign the absolute configuration of C-22 as S (Fig. 1). The steric hindrance around the hydroxyl group of C-16 was considered to prevent bulky MTPA group from being introduced in.

Thioglycoside [α-L-Rh*ap*-(1-2)-β-D-Gl*cp*] **4**, which was prepared in accordance with the procedure described in previous literature [10], was transformed into trichloroacetimidate **5** in Scheme 2. Treatment of the hydrolysate of thioglycoside **4** with trichloroacetonitrile and 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) afforded disaccharide trichloroacetimidate **5** finally.

With compound **3** and disaccharide trichloroacetimidate **5** in hand, glycosylation in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) was carried out (Scheme 3) [13]. Apart from a pair of epimeric 16β-*O*-glycosides **7** and **8**, 22-*O*-glycoside **6** was formed also. During the glycosylation, disaccharide donor may be attacked by either the oxygen atom at C-16 (for 16β-*O*-glycoside) or that at C-22 (for 22-*O*-glycoside). The proportion of three products (**6**:**7**:**8** = 3:14:3) was determined by the relative rate of the competing reactions.

The 16β-*O*-glycosides (**7** and **8**) and 22-*O*-glycoside (**6**) were readily distinguished by analysis of their ^{1}H NMR spectra. The most characteristic ^{1}H signal of the 16β-*O*-glycoside **7** was a doublet at 4.98 ppm (d, J = 3.0 Hz) corresponding to the anomeric proton. For compound **8**, anomeric proton signal shifted upfield to 4.48 ppm (d, J = 7.8 Hz). For 22-*O*-glycoside **6**, anomeric proton appeared at 4.66 ppm (d, J = 7.8 Hz).

All of the glycosidic products (**6**, **7**and **8**) were subjected to the oxidation with pyridinium dichromate (PDC). The generated ketones showed different chemical shifts in ^{13}C NMR spectra. The ketone carbon C-22 appeared at 213.65 ppm for compound **12** and 214.68 ppm for compound **15**, while compound **9** showed a characteristic ^{13}C signal of ketone carbon C-16 at 219.92 ppm.

Desilylation with *p*-toluenesulfonic acid monohydrate (*p*-TsOH·H_2O) afforded 3-*t*-butyldimethylsilyl (TBDMS) deprotection compounds **10**, **13** and **16**. A final step of the synthesis was the deprotection of acetyl groups, which was achieved by treatment with MeONa in MeOH/CH_2Cl_2. All novel cholestane saponin analogues (**11**, **14** and **17**) were obtained and fully characterized by spectral analysis (^{1}H, ^{13}C NMR and HRMS).

Subsequently, glycosylation of furostan was executed in Scheme 4. In previous research [10,14], 22-hemiketal or ketal in furostan was found to be very unstable in acid condition. To avoid this side reaction of furostan glycosylation products in final deprotection of 3β-TBDMS group with acid reagents, 3β-TBDMS group of (25R)-26-acetyloxy-3β-*tert*-butyldimethylsilyloxycholest-5-ene-16,22-dione **1** was substituted with benzoyl group firstly. Removal of 3β-TBDMS group with *p*-TsOH·H_2O, succeeding protection of exposed 3β-hydroxy

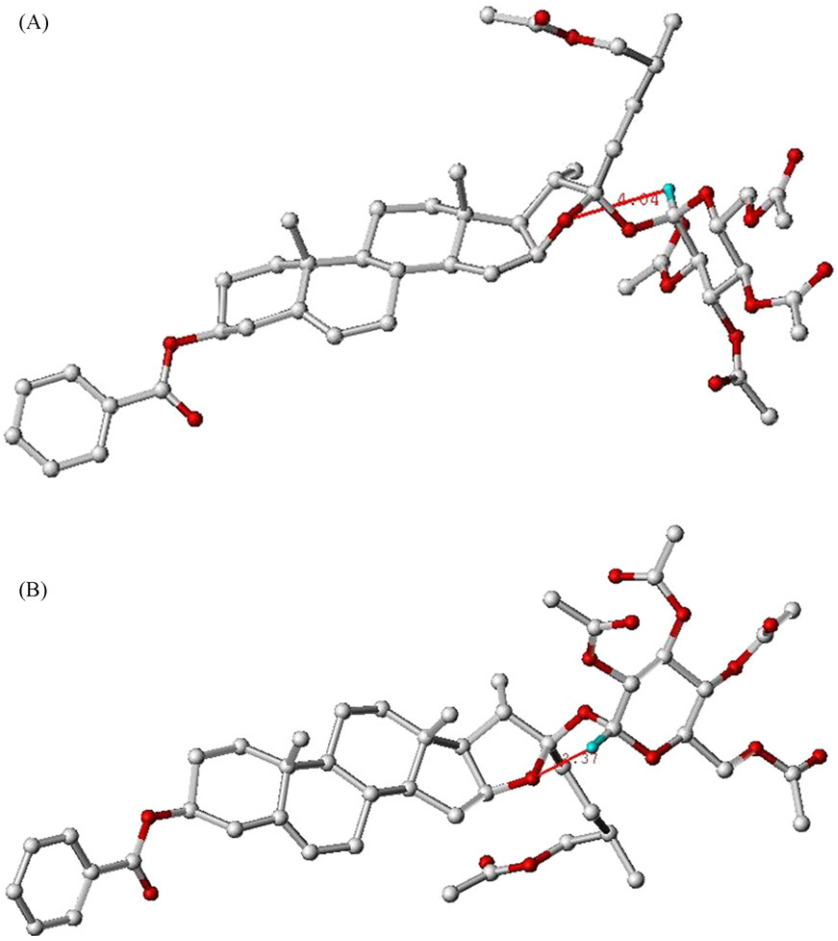
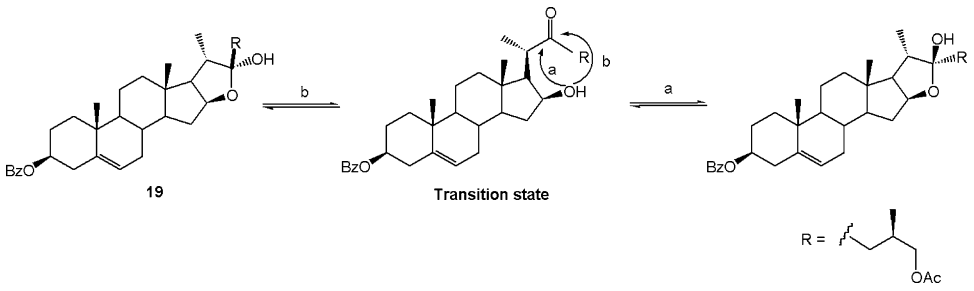
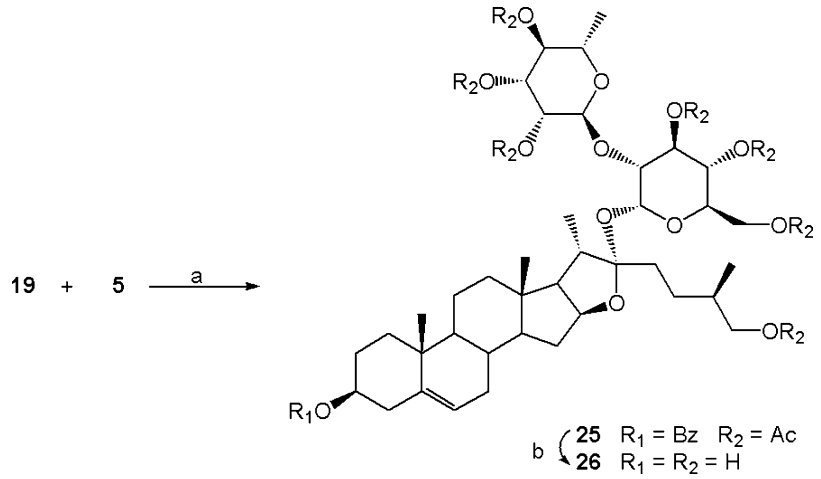


Fig. 3. The conformers of two epimers (**21** and **22**): (A) the biggest distance conformer of epimer **21** and (B) the smallest distance conformer of epimer **22**.



Scheme 5. The formation of two configurations at C-22.



Scheme 6. (a) TMSOTf, 4 °C MS, dry CH_2Cl_2, −20 °C, 2 h, 32% and (b) MeONa, MeOH/CH_2Cl_2, 80 °C, 48 h, 86%.

with benzoyl chloride, afforded compound **18**. Reduction with $NaBH_4/CeCl_3{\cdot}7H_2O$ in THF, compound **19** was obtained in a satisfactory yield in the end. Then compound **19** was glycosylated with D-glucose trichloroacetimidate **20** in the presence of TMSOTf [13], the total conversion was about 60% and a pair of epimeric 22-*O*-glycosides **21** and **22** was gained in almost equal amounts. The glycosidic products were distinguished by NMR spectra. The obvious evidence was the carbon signal of C-22 (it appeared at 110.49 ppm in **19**) in ^{13}C NMR spectra, which appeared at 115.45 ppm in compound **21** and 114.82 ppm in compound **22**. Another characteristic was doublet of anomeric proton in ^1H NMR spectra, which appeared at 5.87 ppm (J = 3.6 Hz) in compound **21** and 6.85 ppm (J = 3.6 Hz) in compound **22** (Scheme 4).

The anomeric proton signal of compound **22** shifted to a very low field at 6.85 ppm. To explain this phenomenon, exhaustive conformational analysis [13] for two epimers (**21** and **22**) was carried out. As some of the conformers were very similar to each other, to simplify analysis, the values of dihedral angles between oxygen atom at C-16 and anomeric proton measured in generated structures were chosen as a criterion describing their similarity. Two conformers were assumed identical if the largest difference observed between the values of all of their matching dihedral angles was less than 2° and only one of them was further considered. Then after applying a cutoff of 5 kcal/mol, 19 conformers of compound **21** and 27 conformers of compound **22** were obtained at last (Fig. 2).

Inspection of Figs. 2 and 3 indicates that there was a distinctive difference in conformational freedom between the two epimers. The figure of compound **21** showed that quite substantial proportions of generated conformers had the distance of about ~3.5 Å which is similar to the conformer of compound **21** in Fig. 3. While the figure of compound **22** showed that quite substantial proportions of generated conformers had the distance of about ~2.5 Å, which is similar to the conformer of compound **22** in Fig. 3. These figures suggested that the distance between oxygen atom at C-16 and anomeric proton of compound **22** was quite closer than that of compound **21**. The electrophilic deshielding effect of oxygen atom at C-16 induced the remarkable downfield shift of anomeric proton in compound **22**.

The sapogenins of compounds **21** and **22** displayed two configurations (22S and 22R) at C-22. The epimerization of C-22 can be explained as the same process of the mutarotation of aldoses and ketoses [15,16] following acid catalysis (Scheme 5). The mutarotation of C-22 occurred in the glycosylation of sapogenin **19** catalyzed by Lewis acid TMSOTf.

Table 1
The in vitro cytotoxicities (IC_{50}, μM) of synthetic cholestane and furostan saponin analogues.[a]

Cell lines	Dioscin	Cholestane saponin analogues **11**	Cholestane saponin analogues **14**	Cholestane saponin analogues **17**	Furostan saponin analogues **23**	Furostan saponin analogues **24**	Furostan saponin analogues **26**
BxPC3	5.53	>100	>100	>100	>100	50.3	>100
U251	2.79	>100	15.8	>100	>100	47.7	>100
HEPG2	4.15	>100	16.8	>100	>100	45.9	>100
MCF-7	3.35	>100	>100	>100	>100	>100	>100

[a] The in vitro cytotoxic activities against BxPC3 (pancreatic cancer), U251 (glioblastoma), HEPG2 (hepatocellular cancer) and MCF-7 (breast cancer) cell lines were evaluated by the standard MTT assay using dioscin, a natural product, as a positive control.

The epimers were respectively subjected to deprotection of acetyl groups and benzoyl group with MeONa to afford furostan saponin analogues **23** and **24** ultimately.

Subsequently, glycosylation of compound **19** and disaccharide trichloroacetimidate **5** was executed (Scheme 6) [13], and the 22-*O*-glycoside **25** was gained eventually. The structure of glycosylation product **25** was validated by NMR spectra. In ^{1}H NMR spectra, characteristic was doublet of anomeric proton at 5.78 ppm (J = 3.6 Hz). In ^{13}C NMR spectra, the carbon signal of C-22 was deshielded to 119.68 ppm under the influence of glycosidic bond (in **19** it appeared at 110.49 ppm). The protecting groups were removed in the same way as described above (**23** and **24**) and a novel furostan saponin analogue **26** was obtained.

The in vitro cytotoxicities of the synthetic cholestane and furostan saponin analogues (**11**, **14**, **17**, **23**, **24**, **26**) against BxPC3 (pancreatic cancer cells), U251 (glioblastoma cells), HEPG2 (hepatocellular cancer cells) and MCF-7 (breast cancer cells) were evaluated by the standard MTT assay using dioscin as a positive control. The results are listed in Table 1. Among the tested compounds, only compounds **14** and **24** showed weak cytotoxicities to U251 and HEPG2 cell lines, and compound **24** showed similar cytotoxicities to BxPC3 cell line at concentrations of 1–100 μM, which were about 10–50 times less active than dioscin.

Acknowledgements

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