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Convergent synthesis and cytotoxic activities of 26-thio- and selenodioscin

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ABSTRACT

Convergent block syntheses of 26-thio- and selenodioscin have been achieved by developing the highly stereoselective 1,2-*trans* glycosylations of chacotriosyl imidate without recourse to neighboring group assistance. Both thiodioscin and selenodioscin possess cytotoxic activities similar to dioscin, a natural spirostanol glycoside.

1. Introduction

Spirostanol glycosides, consisting of an aglycone of spirostan type with a sugar chain generally attached at position C-3, are abundant naturally occurring secondary metabolites [1]. Dioscin (Fig. 1), a representive of spirostan saponin, is composed by diosgenin and β-chacotriosyl moiety (α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamno-pyranosyl-(1 → 4)]-β-D-glucopyranoside), has been isolated from various plants [2–5]. It displays a broad spectrum of bioactivities such as antitumor [6–8], antiviral [9], antifungal [3,10], anti-inflammatory [11–13], and immunostimulant activities [14]. Additionally, dioscin is an active component of DI'AO XINXUEKANG, a clinic medicine for treatment of cardiovascular disease in China [15,16]. These properties make dioscin as an interesting lead for drug development. Various approaches to dioscin have been developed [17–23], and intensive efforts have been devoted to research on its structure–activity relationship including: (i) replacement of diosgenin with cholesterol [21,23], glycyrrhetic acid [22], hecogenin [24], or oleanic acid [25]; (ii) substitution of chacotriosyl group with other oligosaccharide moieties, or its decoration with functional groups at 4‴-OH and 6′-OH [26,27]; (iii) a change of original β-ether likage by α-glycosidic bond [21,23], or a triazole group [28]. Taken together, these results indicate that bioactivities of dioscin depend on both aglycone and sugar moiety. Recently, "key polar hydroxy groups" of dioscin against tumor cell lines have been determined based on syntheses of its eight monomethylated analogues [29]. In medicinal chemistry both divalent sulfur and selenium as bioisosteres [30] of oxygen are usually utilized in lead modifications. Organosulfides [31,32] and organoselenides [33] display a wide arrange of interesting bioactivities such as antioxidation, antivirus and antitumor. Consequently, we would like to substitute sulfur and selenium for oxygen at position 26 in dioscin skeleton to make 26-thio- and selenodioscin **1** and **2** (Fig. 1), and to evaluate their cytotoxicities. So far little information is available regarding the influence of spiroketal functionality of dioscin on its antitumor activities. Herein, we report our findings on 26-thio- and selenodioscin **1** and **2** (Fig. 1).

2. Experimental

2.1. General

Reagents were purchased and used without further purification. Dichloromethane (CH_2Cl_2), *N*, *N*–dimethylformamide (DMF) and pyridine were dried over calcium hydride. Methanol (MeOH) was distilled over magnesium. All reactions were carried out under argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Reactions were monitored with analytical TLC on silica gel 60-F254 precoated glass plates and visualized under UV (254 nm) and/or by staining with 8% H_2SO_4 in methanol. Column chromatography was carried out on 300–400 mesh of silica Gel 60. ^1H and ^{13}C NMR spectra were

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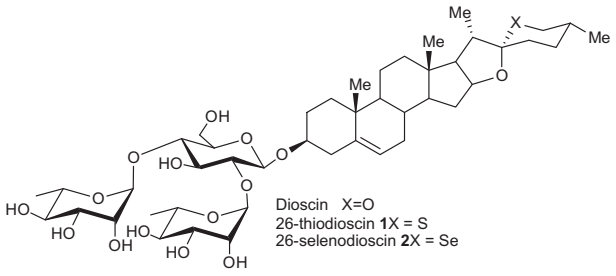


Fig. 1. Structures of dioscin, 26-thio- and selenodioscin **1** and **2**.

recorded on Jeol JNM-ECP 600 (600 MHz), and chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane as an internal standard. The peak patterns are shown as the following abbreviations: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. Mass spectra (MS) were measured by a Thermo Electron LCQ Classic or Micromass ZQ of Waters (ESI). High resolution mass spectra (HRMS) were recorded by a Micromass Q-Tof Ultima API mass spectrometer. Optical rotations were determined with a JASCOP-1020 polarimeter.

2.2. Synthesis

2.2.1. 26-Pseudodiosgenyl 4-methylbenzenesulfonate (**9**)

A solution of TsCl (1.73 g, 9.01 mmol) in dry $CHCl_3$ (15 mL) was slowly added to a chilled (−5 °C) solution of pseudodiosgenin **5** (2.51 g, 6.05 mmol), Et_3N (1.68 mL, 12.10 mmol) and $Me_3N{\cdot}HCl$ (58 mg, 0.61 mmol) in dry $CHCl_3$ (45 mL) over 2 h. After being stirred at −5 °C for 12 h, the mixture was successively washed with 0.5 M HCl (2 × 200 ml), saturated aqueous $NaHCO_3$ (2 × 200 mL) and brine (300 mL). The organic layers were dried over Na_2SO_4 and concentrated. The obtained residue was dissolved in acetone (42 mL) and H_2O (18 mL), and the resulting solution was stirred for 2 h at 60 °C, then the mixture was poured into ice-water (500 mL), the precipitate was collected by filtration and dissolved in CH_2Cl_2 (200 mL), the mixture was washed with brine (300 mL). The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2 = 5/1/1,) to yield **9** (3.05 g, 89%) as a white solid $[\alpha]_D^{20}$–27.7 (*c* 1.00, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.77 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 5.34 (d, *J* = 4.8 Hz, 1H), 4.71–4.66 (m, 1H), 3.88 (dd, *J* = 9.0, 4.8 Hz, 1H), 3.79 (dd, *J* = 9.6, 6.6 Hz, 1H), 3.51 (m, 1H), 2.44 (s, 3H), 1.52(s, 3H), 1.01 (s, 3H), 0.89 (d, *J* = 6.6 Hz, 3H), 0.64 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 150.8, 144.6, 140.8, 133.0, 129.77, 129.74, 127.9, 121.3, 104.1, 84.2, 74.9, 71.6, 64.1, 55.0, 50.0, 43.2, 42.2, 39.4, 37.2, 36.6, 34.0, 32.3, 32.1, 31.6, 31.2, 30.0, 22.9, 21.6, 20.9, 19.4, 16.4, 13.9, 11.6.

2.2.2. 26-pseudodiosgenyl thioacetate (**10**)

To a solution of **9** (369 mg, 0.65 mmol) in DMF (5 mL) was added $KSCOCH_3$ (222 mg, 1.95 mmol). The mixture was stirred for 10 h at room temperature, then the volatile was removed under reduced pressure. The resultant residue was diluted with CH_2Cl_2 (100 mL), and washed subsequently with H_2O (2 × 150 mL) and brine (300 mL). The organic layers were dried over Na_2SO_4 and concentrated to dryness. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 4/1,) to afford **10** (295 mg, 96%) as a white solid $[\alpha]_D^{20}$–22.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.32 (d, 1H, *J* = 5.4 Hz), 4.73–4.69 (m, 1H), 3.51–3.47 (m, 1H), 2.91 (dd, 1H, *J* = 13.2, 5.4 Hz), 2.75 (dd, 1H, *J* = 13.2, 7.2 Hz), 2.44 (d, 1H, *J* = 10.2 Hz), 2.30 (s, 3H), 1.56 (s, 3H), 1.00 (s, 3H), 0.93 (d, 3H, *J* = 6.6 Hz), 0.66 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 195.9, 151.3, 140.8, 121.3, 103.7, 84.2, 71.5, 64.1, 54.9, 50.0, 43.2, 42.2, 39.4, 37.2, 36.5, 35.6, 34.0, 33.2, 32.8, 32.1, 31.5, 31.2, 30.6, 23.2, 20.9, 19.3, 18.9, 13.9, 11.6; HRESIMS: $[M + H]^+$ cacld for $C_{29}H_{45}O_3S$: 473.3084; Found: 473.3090.

2.2.3. 26-thiodiosgenin (**3**)

To a solution of **10** (295 mg, 0.62 mmol) in methanol (20 mL) was added 5 mL of H_2O/MeOH (v/v = 1/9) containing KOH (70 mg, 1.25 mmol). After stirring for 15 min. at room temperature, 6 M HCl (320 μL, 1.87 mmol) was added and the resultant mixture was stirred for another 30 min at room temperature, then kept for 12 h at 0 °C. The solid was collected by filtration and dried *in vacuo* to afford **10a** in quantitative yield. A solution of **10a** (239 mg, 0.55 mmol) in 20 ml of aqueous ethanolic HCl (0.3 M, v/v = 1/19) was refluxed for 5 h, then cooled to room temperature, diluted with water (200 mL) to result in the formation of a precipitate, which was collected by filtration, washed with water, died *in vacuo* followed by silica gel column chromatography (petroleum ether/EtOAc = 5/1) to give **3** (209 mg, 87% over two steps) as a white solid $[\alpha]_D^{20}$–162.7 (*c* 1.10, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.34 (d, 1H, *J* = 4,8 Hz), 4.62 (dd, 1H, *J* = 15.0, 7.2 Hz), 3.55–3.48 (m, 1H), 2.52 (t, 1H, *J* = 13.2 Hz), 2.28 (d, 2H, *J* = 13.2 Hz), 2.22 (t, 1H, *J* = 12.6 Hz), 1.01 (s, 3H), 1.00 (d, 3H, *J* = 8.4 Hz), 0.92 (d, 3H, *J* = 6.6 Hz), 0.80 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 140.8, 121.4, 97.5, 81.6, 71.7, 62.8, 56.6, 50.0, 44.4, 42.2, 40.3, 39.7, 38.5, 37.2, 36.6, 33.3, 32.1, 32.0, 31.7, 31.6, 31.41, 31.38, 22.4, 20.8, 19.4, 16.5, 16.2; HRESIMS: cacld for $C_{27}H_{43}O_2S$, $[M + H^+]$, 431.2978. Found: 431.2983.

2.2.4. 26, 26′-(Bispseudodiosgenyl) diselenide (**11**)

To a suspension of $CsOH{\cdot}H_2O$ (75.6 mg, 0.45 mmol) and activated selenium power (23.7 mg, 0.30 mmol) in DMF (2 mL) was added $N_2H_4{\cdot}H_2O$ (55 μL, 0.90 mmol) under N_2 atmosphere. After being stirred for 2 h at r.t., **9** (170.6 mg, 0.30 mmol) was added. The mixture was continued to stir for another 4 h at 60 °C, then evaporated under reduced pressure to give a residue, which was taken into CH_2Cl_2 (100 mL). The mixture was washed successively with H_2O (2 × 150 mL), brine (300 mL). The collected organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 3/1) to give **11** (131.9 mg, 92%) as a yellow solid $[\alpha]_D^{20}$ 34.2 (*c* 1.21, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.34 (d, 2H, *J* = 4.8 Hz), 4.75–4.70 (m, 2H), 3.53–3.49 (m, 2H), 3.02 (dd, 2H, *J* = 12.0, 5.4 Hz), 2.82 (dd, 2H, *J* = 12.0, 7.8 Hz), 2.45 (d, 2H, *J* = 10.2 Hz), 2.30 (m, 4H), 2.22 (t, 4H, *J* = 10.8 Hz) 1.58 (s, 6H), 1.01 (s, 6H), 0.99 (d, 6H, *J* = 6.6 Hz), 0.68 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 151.5, 140.8, 121.3, 103.7, 84.3, 71.6, 64.2, 55.0, 50.0, 43.2, 42.2, 39.5, 39.1, 37.2, 36.6, 34.1, 33.9, 33.8, 32.2, 31.6, 31.2, 23.4, 21.0, 19.5, 19.4, 14.0, 11.7.

2.2.5. 26-selenodiosgenin (**4**)

To a solution of **11** (286 mg, 0.30 mmol) in acetic acid (30 mL) was added zinc powder (59 mg, 0.90 mmol). After refluxing for 24 h at 150 °C, the mixture was cooled to room temperature and the solid was filtered off. The filtrate was removed under reduced pressure to give a residue, which was dissolved in dioxane (15 mL), then 20 mL of 10% KOH in EtOH/H_2O (v/v = 1/1) was added. After being stirred for 2 h at room temperature, the solution was poured into ice-water (300 mL) and extracted with dichloromethane (2 × 100 mL). The organic layers were washed with brine, dried over Na_2SO_4 and concentrated. The residue was applied to silica gel column chromatography eluting with petroleum ether and EtOAc (v/v = 6/1) to afford **4** (250 mg, 92%) as a white solid $[\alpha]_D^{20}$–192.8 (*c* 1.00, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 5.34 (d, 1H, *J* = 4.8 Hz), 4.64 (dd, 1H, *J* = 15.6, 7.8 Hz), 3.53–3.50 (m, 1H), 2.58 (t, 1H, *J* = 12.0 Hz), 2.37–2.34 (m, 1H), 2.31–2.28 (m, 1H),

2.25–2.20 (m, 2H), 2.04–1.97 (m, 2H), 1.02 (d, 3H, *J* = 7.2 Hz), 1.01 (s, 3H), 0.96 (d, 3H, *J* = 6.6 Hz), 0.80 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 140.8, 121.4, 97.9, 82.7, 71.7, 62.9, 56.6, 50.0, 45.4, 42.2, 40.4, 40.1, 39.7, 37.2, 36.6, 34.0, 32.6, 32.0, 31.6, 31.4, 31.1, 24.8, 23.7, 20.8, 19.4, 17.7, 16.6.

*2.2.6. 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-benzoyl-D-glucopyranoside (**13**)*

A solution of thioglycoside **7** (1.73 g, 3.50 mmol) in dry CH_2Cl_2 (30 mL) was stirred for 30 min at −30 °C in the presence of freshly activated 4 Å molecular sieves under argon atmosphere. At this point TMSOTf (126 μL, 0.70 mmol) was added followed by slow addition of rhamnosyl imidate **8** (4.56 g, 10.49 mmol). After further stirring for 2 h at −30 °C, the reaction was quenched by Et_3N. The solid was filtered off, and the filtrate was concentrated to result in a residue, which was purified by silicagel column chromatography (petroleum ether/EtOAc = 2/1,) to give a mixture of **12** contaminated by rhamnosyl hemiacetal **14**, which was dissolved in 30 mL of acetone/H_2O (9/1) in an ice-water bath, and treated with NBS (1.16 g, 6.5 mmol). After being stirred for 2 h, more NBS (580 mg, 3.25 mmol) was added to drive the reaction to completion. After another 2 h, the reaction was diluted with CH_2Cl_2 (200 mL) and washed with saturated aqueous $NaHCO_3$ (2 × 200 mL) and brine (300 mL). The organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 2/1,) to give **13** (2.34 g, 72% over two steps) as a white foam, which was directly taken into next step.

*2.2.7. 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**6**)*

To a solution of **13** (1.92 g, 2.06 mmol) and DBU (125 μL, 0.82 mmol) in dry CH_2Cl_2 (25 mL) in an ice-water bath was added Cl_3CCN (2.06 mL, 20.58 mmol). After being stirred for 2 h, the volatile was removed under reduced pressure and the residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 2/1) to afford **6** (2.02 g, 91%) as a white solid $[\alpha]_D^{20}$ 103.2 (*c* 1.05, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.74 (s, 1H), 8.05 (m, 4H), 7.59–7.54 (m, 2H), 7.47–7.43 (m, 4H), 6.48 (d, 1H, *J* = 3.6 Hz), 5.89 (t, 1H, *J* = 10.2 Hz), 5.20 (dd, 1H, *J* = 9.6, 3.0 Hz), 5.15 (dd, 1H, *J* = 3.6, 1.8 Hz), 5.11 (dd, 1H, *J* = 9.6, 3.6 Hz), 4.93–4.87 (m, 3H), 4.83–4.80 (m, 2H), 4.79 (d, 1H, *J* = 1.2 Hz), 4.51 (dd, 1H, *J* = 12.6, 3.6 Hz), 4.33–4.30 (m, 1H), 4.10 (t, 1H, *J* = 9.6 Hz), 4.05 (dd, 1H, *J* = 10.2, 4.2 Hz), 3.90–3.87 (m, 1H), 3.78–3.75 (m, 1H), 2.00 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.86 (s, 3H), 1.85 (s, 3H), 1.13 (d, 3H, *J* = 6.6 Hz), 0.69 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.92, 169.88, 169.86, 169.82, 169.2, 169.0, 165.7, 165.1, 161.2, 133.2, 133.1, 129.9, 129.7, 129.6, 129.2, 128.3, 99.4, 99.1, 94.1, 90.6, 76.3, 72.3, 71.5, 70.8, 70.5, 69.9, 69.3, 68.4, 68.1, 67.5, 67.2, 62.0, 60.3, 20.65, 20.61, 20.45, 20.38, 17.2, 16.8, 14.1; HRESIMS: cacld for $C_{46}H_{52}NO_{22}Cl_2^{37}$-Cl, 1077.2012. Found: 1077.1991.

*2.2.8. 26-thiodiosgenyl 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-benzoyl-α-D-glucopyranoside (**15α**) and 26-thiodiosgenyl 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-benzoyl-β-D-glucopyranoside (**15β**).*

2.2.8.1. Procedure A. A solution of 26-thiodiosgenin **3** (86 mg, 0.20 mmol) in dry CH_2Cl_2 (4 mL) was stirred for 30 min at −30 °C in the presence of freshly activated 4 Å molecular sieves under argon atmosphere. TMSOTf (7.2 μL, 0.034 mmol) was added followed by slow addition of α-chacotriosyl imidate **6** (280 mg, 0.26 mmol). After further being stirred for 4 h at −30 °C, the reaction was quenched by Et_3N. The solid was filtered off, and the filtrate was concentrated to give a residue, which was purified by silica gel column chromatography (petroleum ether/EtOAc = 3/1) to give a mixture of **15α** and **15β**, which was further separated by silica gel column chromatography (CH_2Cl_2/EtOAc = 20/1) to afford **15α** (125 mg, 46%) and **15β** (72 mg, 27%). **15α**: $[\alpha]_D^{20}$ 76.6 (*c* 0.80, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.05–8.03 (m, 4H), 7.56–7.51 (m, 2H), 7.43–7.39 (m, 4H), 5.77 (t, 1H, *J* = 9.6 Hz), 5.25 (d, 1H, *J* = 4.2 Hz), 5.22 (dd, 1H, *J* = 10.2, 3.6 Hz), 5.17 (dd, 1H, *J* = 10.2, 3.6 Hz), 5.15–5.11 (m, 1H), 5.05 (d, 1H, *J* = 3.6 Hz), 4.90 (t, 1H, *J* = 9.6 Hz), 4.89 (t, 1H, *J* = 10.2 Hz), 4.85 (d, 1H, *J* = 1.8 Hz), 4.77–4.72 (m, 3H), 4.63 (dd, 1H, *J* = 15.6, 7.2 Hz), 4.52 (dd, 1H, *J* = 12.6, 5.4 Hz), 4.29–4.26 (m, 1H), 3.91–3.86 (m, 2H), 3.80–3.75 (m, 1H), 3.72 (dd, 1H, *J* = 9.6, 3.0 Hz), 3.48–3.42 (m, 1H), 2.53 (t, 1H, *J* = 13.2 Hz), 2.47 (d, 2H, *J* = 7.8 Hz), 2.28 (d, 1H, *J* = 12.6 Hz), 2.01 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.87 (s, 3H), 1.85 (s, 3H), 1.12 (d, 3H, *J* = 6.0 Hz), 1.05 (s, 3H), 1.00 (d, 3H, *J* = 7.2 Hz), 0.92 (d, 3H, *J* = 6.6 Hz), 0.80 (s, 3H), 0.69 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.0, 169.90, 169.87, 169.2, 165.9, 165.2, 140.1, 132.99, 132.96, 129.83, 129.77, 129.71, 129.6, 128.3, 128.2, 121.9, 99.4, 99.0, 97.5, 96.2, 81.6, 79.1, 78.7, 77.8, 72.4, 71.1, 70.6, 70.1, 69.5, 68.8, 68.5, 68.3, 67.4, 66.8, 62.8, 56.6, 49.9, 44.3, 40.3, 40.0, 39.7, 38.4, 37.0, 36.7, 33.2, 32.0, 31.7, 31.4, 31.3, 27.8, 22.4, 20.73, 20.67, 20.5, 19.3, 17.4, 16.8, 16.5, 16.2; HRESIMS: cacld for $C_{71}H_{92}O_{23}SNa$, $[M + Na]^+$, 1367.5642. Found: 1367.5691. **15β**: $[\alpha]_D^{20}$ 34.6 (*c* 0.80, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.04–8.01 (m, 4H), 7.55–7.52 (m, 2H), 7.44–7.38 (m, 4H), 5.60 (t, 1H, *J* = 9.4 Hz), 5.33 (d, 1H, *J* = 5.4 Hz), 5.14–5.11 (m, 2H), 5.09 (m, 1H), 4.95–4.93 (m, 1H), 4.89 (t, 1H, *J* = 10.4 Hz), 4.85 (t, 1H, *J* = 10.2 Hz), 4.83 (brs, 1H), 4.77 (d, 1H, J = 11.52 Hz), 4.74 (brs, 1H), 4.65 (d, 1H, *J* = 7.68 Hz), 4.64–4.60 (m, 1H), 4.49 (dd, 1H, *J* = 12.0, 5.4 Hz), 4.35–4.30 (m, 1H), 3.94 (t, 1H, *J* = 9.6 Hz), 3.85–3.81 (m, 1H), 3.77 (t, 1H, *J* = 7.2 Hz), 3.70–3.66 (m, 1H), 3.58–3.53 (m, 1H), 2.52 (t, 1H, *J* = 11.4 Hz), 2.38 (d, 1H, *J* = 10.8 Hz), 2.28 (d, 1H, *J* = 11.4 Hz), 2.23 (t, 1H, *J* = 12.6 Hz), 1.96 (s, 6H), 1.92 (s, 3H), 1.90 (s, 3H), 1.86 (s, 3H), 1.72 (s, 3H), 1.13 (d, 3H, *J* = 6.0 Hz), 1.00 (d, 3H, *J* = 6.6 Hz), 0.93 (s, 3H), 0.90 (d, 3H, *J* = 6.0 Hz), 0.78 (s, 3H), 0.66 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.93, 169.88, 169.80, 169.6, 168.8, 165.7, 164.9, 140.0, 133.2, 132.9, 132.3, 130.8, 130.0, 129.8, 129.7, 129.0, 128.8, 128.34, 128.31, 121.9, 99.4, 98.9, 98.0, 97.4, 81.5, 79.4, 77.3, 76.1, 75.9, 72.9, 71.7, 71.0, 70.4, 70.0, 69.1, 68.7, 68.4, 67.5, 66.4, 62.7, 56.5, 49.8, 44.3, 40.2, 39.6, 38.45, 38.36, 36.8, 36.7, 33.2, 32.02, 31.99, 31.7, 31.3, 29.6, 27.6, 22.4, 20.73, 20.68, 20.65, 20.62, 20.5, 20.2, 19.1, 17.1, 16.8, 16.5, 16.1; HRESIMS: cacld for $C_{71}H_{92}O_{23}SNa$, $[M + Na]^+$, 1367.5642. Found: 1367.5696.

2.2.8.2. Procedure B. A solution of 26-thiodiosgenin **3** (10 mg, 0.023 mmol) and imidate **6** (33 mg, 0.030 mmol, 1.3 equiv.) in a mixed solvent of CH_2Cl_2/^{t}BuCN/$PhCF_3$ (2.1 mL, v/v/v = 1.3/1/5) was stirred for 30 min at −20 °C in the presence of freshly activated 5 Å molecular sieves under argon atmosphere to remove a trace amount of water. Then a solution of $HB(C_6F_5)_4$ in CH_2Cl_2 (0.1 mL, 0.023 mmol/mL, 0.1 equiv.) was added. After the resulting mixture was stirred for 2 h at −20 °C, the reaction was quenched by addition of Et_3N. The solid was filtered off, and the filtrate was concentrated to give a residue, which was purified by silica gel column chromatography (petroleum ether/EtOAc = 2/1) to afford a mixture of **15β** and **15α** (31 mg, 99%), the ratio of which determined by ^{1}H NMR was 10.9/1.

*2.2.9. 26-selenodiosgenyl 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-benzoyl-α-D-glucopyranoside (**16α**) and 26-selenodiosgenyl 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-benzoyl-β-D-glucopyranoside (**16β**).*

2.2.9.1. Procedure C. Following the procedure A for the syntheses of **15α** and **15β**, condensation of 26-selenodiosgenin **4** (81 mg, 0.17 mmol) with imidate **6** (238 mg, 0.22 mmol) in dry CH_2Cl_2 (4 mL) in the presence of TMSOTf (6.2 μL, 0.034 mmol) at −78 °C

afforded **16α** (76 mg, 32%) and **16β** (110 mg, 46%). **16α**: $[\alpha]_D^{20}$ −1.9 (*c* 1.40, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 8.06 (d, 2H, *J* = 4.2 Hz), 8.04 (d, 2H, *J* = 4.2 Hz), 7.57–7.52 (m, 2H), 7.44–7.40 (dd, 4H, *J* = 13.8, 7.8 Hz), 5.78 (t, 1H, *J* = 9.6 Hz), 5.26 (d, 1H, *J* = 4.2 Hz), 5.23 (dd, 1H, *J* = 10.2, 3.0 Hz), 5.18 (dd, 1H, *J* = 9.6, 3.6 Hz), 5.15–5.12 (m, 1H), 5.06 (d, 1H, *J* = 3.6 Hz), 4.93–4.88 (m, 2H), 4.86 (brs, 1H), 4.78–4.76 (m, 1H), 4.74 (d, 1H, *J* = 13.2 Hz), 4.66 (dd, 1H, *J* = 15.6, 7.2 Hz), 4.53 (dd, 1H, *J* = 12.0, 4.8 Hz), 4.32–4.26 (m, 1H), 3.91–3.89 (m, 2H), 3.80–3.77 (m, 1H), 3.73 (dd, 1H, *J* = 9.6, 3.6 Hz), 3.48–3.43 (m, 1H), 2.59 (t, 1H, *J* = 12.0 Hz), 2.48 (d, 2H, *J* = 7.8 Hz), 2.37 (d, 1H, *J* = 11.4 Hz), 2.26–2.20 (m, 1H), 2.02 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.88 (s, 3H), 1.86 (s, 3H), 1.13 (d, 3H, *J* = 6.0 Hz), 1.06 (s, 3H), 1.02 (d, 3H, *J* = 7.2 Hz), 0.96 (d, 3H, *J* = 6.6 Hz), 0.81 (s, 3H), 0.69 (d, 3H, *J* = 6.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.05, 169.93, 169.90, 169.2, 166.0, 165.2, 140.1, 133.02, 132.99, 129.88, 129.80, 129.76, 129.6, 128.34, 128.28, 121.9, 99.4, 99.0, 97.9, 96.2, 82.7, 79.2, 78.7, 77.8, 72.5, 71.1, 70.7, 70.2, 69.5, 68.9, 68.6, 68.4, 67.4, 66.9, 62.9, 56.6, 49.9, 45.4, 40.4, 40.1, 39.7, 37.0, 36.7, 34.0, 32.6, 32.0, 31.3, 31.1, 27.9, 24.8, 23.7, 20.8, 20.5, 19.3, 17.7, 17.4, 16.8, 16.6; HRESIMS: $[M + Na]^+$ cacld for $C_{71}H_{92}O_{23}SeNa$: 1415.5087; Found: 1415.5119. **16β**: $[\alpha]_D^{20}$ −44.5 (*c* 1.70, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): δ 8.03 (d, 2H, *J* = 8.4 Hz), 8.01 (d, 2H, *J* = 8.4 Hz), 7.57–7.52 (m, 2H), 7.45–7.39 (m, 4H), 5.60 (t, 1H, *J* = 9.0 Hz), 5.33 (d, 1H, *J* = 5.4 Hz), 5.15–5.12 (m, 2H), 5.10–5.09 (m, 1H), 4.95 (dd, 1H, *J* = 3.6, 1.8 Hz), 4.89 (t, 1H, *J* = 9.6 Hz), 4.85 (t, 1H, *J* = 9.6 Hz), 4.83 (d, 1H, *J* = 1.8 Hz), 4.77 (dd, 1H, *J* = 12.0, 1.8 Hz), 4.74 (d, 1H, *J* = 12 Hz), 4.65 (d, 1H, *J* = 7.8 Hz) 4.64 (dd, 1H, *J* = 12.4, 7.8 Hz), 4.49 (dd, 1H, *J* = 12.6, 5.4 Hz), 4.34–4.31 (m, 1H), 3.95 (t, 1H, *J* = 9.6 Hz), 3.88–3.82 (m, 1H), 3.77 (t, 1H, *J* = 8.4 Hz), 3.71–3.67 (m, 1H), 3.57–3.53 (m, 1H), 2.57 (t, 1H, *J* = 12.0 Hz), 2.39–2.34 (m, 2H), 2.24–2.18 (m, 2H), 1.96 (s, 6H), 1.93 (s, 3H), 1.90 (m, 3H), 1.86 (s, 3H), 1.72 (s, 3H), 1.13 (d, 3H, *J* = 6.6 Hz), 1.01 (d, 3H, *J* = 6.6 Hz), 0.95 (d, 3H, *J* = 7.8 Hz) 0.94 (s, 3H), 0.78 (s, 3H), 0.66 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (150 MHz, $CDCl_3$): δ 169.93, 169.87, 169.80, 169.5, 168.8, 165.7, 164.9, 140.0, 133.2, 132.9, 130.0, 129.8, 129.7, 129.1, 128.34, 128.29, 121.9, 99.4, 98.9, 98.0, 97.8, 82.6, 79.4, 77.3, 76.1, 75.9, 72.9, 71.0, 70.4, 70.0, 69.1, 68.7, 68.4, 67.5, 66.4, 62.8, 62.7, 56.4, 49.8, 45.4, 40.3, 40.1, 39.6, 38.4, 36.8, 36.7, 33.9, 32.6, 32.0, 31.3, 31.1, 29.6, 24.8, 23.6, 20.73, 20.67, 20.62, 20.5, 20.2, 19.1, 17.6, 17.1, 16.8, 16.5; HRESIMS: $[M + Na]^+$ cacld for $C_{71}H_{92}O_{23}SeNa$: 1415.5087; Found: 1415.5144.

2.2.9.2. Procedure D. Following the procedure B for the syntheses of **15α** and **15β**, the coupling of 26-selenodiosgenin **4** (10 mg, 0.021 mmol) with imidate **6** (29 mg, 0.027 mmol, 1.3 equiv) in a mixed solvent of CH_2Cl_2/^{t}BuCN/$PhCF_3$ (2.6 mL, v/v/v = 3/1/5) by the promotion of $HB(C_6F_5)_4$ at −20 °C furnished a mixture of **16β** and **16α** (21 mg, 72%) at the ratio of 13.1/1.

*2.2.10. 26-thiodiosgenyl 2,4-di-O-α-L-rhamnopyranosyl-β-D-glucopyranoside (**1**)*

To a solution of **15β** (40 mg, 0.030 mmol) in dry CH_2Cl_2 (1 mL) and MeOH (1 mL) was added a solution of methanolic sodium methoxide (1.89 mmol/mL, 230 μL, 0.44 mmol). The reaction mixture was stirred at room temperature for 24 h, and neutralized to pH = 6 with resin (H^+). The solid was filtered off, and the filtrate was concentrated. The resultant residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH containing 8% H_2O = 7/1) to give **1** (24 mg, 95%) as a white solid. $[\alpha]_D^{12}$ −97.4 (*c* 0.85, $CHCl_3$/MeOH containing 8% H_2O = 1/1); ^1H NMR (600 MHz, pyridine-d_5): δ 6.36 (s, 1H), 5.82 (s, 1H), 5.27 (s, 1H), 4.93–4.86 (m, 3H), 4.79–4.75 (m, 2H), 4.64 (brs, 1H), 4.59–4.56 (m, 1H), 4.51–4.48 (m, 1H), 4.35–4.28 (m, 3H), 4.19–4.17 (m, 3H), 4.05 (d, 1H, *J* = 12.6 Hz), 3.86–3.80 (m, 1H), 3.60 (d, 1H, *J* = 7.8 Hz), 2.74 (d, 1H, *J* = 12.0 Hz), 2.68 (t, 1H, *J* = 12.0 Hz), 2.60 (t, 1H, *J* = 12.6 Hz), 2.30 (d, 1H, *J* = 12.0 Hz), 1.72 (d, 3H, *J* = 6.0 Hz), 1.58 (d, 3H, *J* = 6.0 Hz), 1.07 (d, 3H, *J* = 7.2 Hz), 1.01 (s, 3H), 0.79 (m, 6H); ^{13}C NMR (150 MHz, pyridine-d_5): δ 140.5, 121.5, 102.6, 101.8, 100.0, 97.5, 81.7, 78.2, 77.8, 77.7, 77.5, 76.7, 73.8, 73.6, 72.6, 72.4, 72.2, 70.1, 69.2, 63.1, 61.0, 56.4, 50.0, 44.4, 40.2, 40.2, 39.5, 38.7, 37.2, 36.8, 33.3, 32.1, 32.0, 31.9, 31.45, 31.39, 29.9, 22.4, 20.8, 19.1, 18.4, 18.2, 16.3; HRESIMS: $[M + Na]^+$ cacld for $C_{45}H_{72}O_{15}SNa$: 907.4484; Found: 907.4501.

*2.2.11. 26-thiodiosgenyl 2,4-di-O-α-L-rhamnopyranosyl-α-D-glucopyranoside (**1α**)*

Following the protocol for **1**, **15α** (40 mg, 0.030 mmol) was treated with NaOMe in MeOH and CH_2Cl_2 for 48 h to afford **1α** (26 mg, 99%) as a white solid. $[\alpha]_D^{12}$ −46.0 (*c* 0.09, $CHCl_3$/MeOH containing 8% H_2O = 1/1); ^1H NMR (600 MHz, pyridine-d_5): δ 5.89 (s, 1H), 5.81 (s, 1H), 5.46 (d, 1H, *J* = 3.0 Hz), 5.27 (d, 1H, *J* = 3.6 Hz), 4.89–4.83 (m, 1H), 4.81–4.76 (m, 2H), 4.69–4.65 (m, 1H), 4.62–4.55 (m, 2H), 4.52–4.48 (m, 1H), 4.42–4.37 (m, 2H), 4.33–4.28 (m, 2H), 4.26 (d, 1H, *J* = 10.2 Hz), 4.21 (d, 1H, *J* = 12.0 Hz), 4.14–4.11 (m, 2H), 3.73–3.68 (m, 1H), 2.64–2.55 (m, 2H), 2.49–2.42 (m, 1H), 2.29 (d, 1H, *J* = 10.8 Hz), 1.66 (d, 3H, *J* = 6.0 Hz), 1.64 (d, 3H, *J* = 6.0 Hz), 1.07 (d, 3H, *J* = 6.0 Hz), 0.82 (m, 6H), 0.78 (s, 3H); ^{13}C NMR (150 MHz, pyridine-d_5) δ 140.8, 121.3, 103.9, 102.7, 97.9, 97.5, 81.7, 79.3, 78.9, 78.4, 73.6, 72.6, 72.45, 72.40, 72.3, 72.0, 70.2, 70.0, 63.1, 61.2, 56.4, 49.9, 44.4, 40.5, 40.2, 39.5, 38.7, 37.0, 36.6, 33.3, 32.1, 32.0, 31.9, 31.4, 31.3, 28.4, 22.4, 20.8, 19.0, 18.4, 18.3, 16.3; HRESIMS: $[M + Na]^+$ cacld for $C_{45}H_{72}O_{15}SNa$: 907.4484; Found: 907.4505.

*2.2.12. 26-selenodiosgenyl 2,4-di-O-α-L-rhamnopyranosyl-β-D-glucopyranoside (**2**)*

Following the protocol for **1**, **16β** (40 mg, 0.029 mmol) was treated with NaOMe in MeOH and CH_2Cl_2 for 48 h to afford **2** (21 mg, 78%) as a white solid. $[\alpha]_D^{12}$ −148.7 (*c* 0.80, $CHCl_3$: MeOH containing 4% H_2O = 1/1); ^1H NMR (600 MHz, pyridine-d_5) δ 6.40 (s, 1H), 5.86 (s, 1H), 5.28 (d, 1H, *J* = 5.4 Hz), 4.97–4.92 (m, 3H), 4.83–4.78 (m, 2H), 4.67 (brs, 1H), 4.62 (dd, 1H, *J* = 9.0, 3.6 Hz), 4.53 (dd, 1H, *J* = 9.6, 3.6 Hz), 4.41–4.30 (m, 3H), 4.22–4.18 (m, 3H), 4.07 (dd, 1H, *J* = 12.6, 3.6 Hz), 3.88–3.83 (m, 1H), 3.62 (d, 1H, *J* = 9.6 Hz), 2.80–2.75 (m, 1H), 2.72–2.69 (d, 1H, *J* = 11.4 Hz), 2.66 (t, 1H, *J* = 10.8 Hz), 2.37 (d, 1H, *J* = 11.4 Hz), 2.27–2.23 (m, 1H), 2.04–2.00 (m, 2H), 1.75 (d, 3H, *J* = 6.0 Hz), 1.62 (d, 3H, *J* = 6.6 Hz), 1.07 (d, 3H, *J* = 6.6 Hz), 1.02 (s, 3H), 0.83 (d, 3H, *J* = 6.6 Hz), 0.79 (s, 3H); ^{13}C NMR (150 MHz, pyridine-d_5) δ 140.5, 121.5, 102.6, 101.8, 100.0, 97.8, 82.8, 78.2, 77.7, 77.6, 77.5, 76.6, 73.8, 73.6, 72.5, 72.4, 72.2, 70.1, 69.2, 63.2, 61.0, 56.3, 50.0, 45.4, 40.3, 39.4, 38.6, 37.2, 36.8, 34.0, 32.8, 32.0, 31.3, 31.2, 29.9, 24.7, 23.6, 20.8, 19.1, 18.4, 18.2, 17.7, 16.4; HRESIMS: $[M + H^+]$ cacld for $C_{45}H_{73}O_{15}Se$: 933.4109; Found: 933.4137.

*2.2.13. 26-selenodiosgenyl 2,4-di-O-α-L-rhamnopyranosyl-α-D-glucopyranoside (**2α**)*

Following the protocol for **1**, **16α** (40 mg, 0.029 mmol) was treated with NaOMe (10 mg, 0.18 mmol) in MeOH and CH_2Cl_2 for 48 h to afford **2α** (24 mg, 90%) as a white solid. $[\alpha]_D^{12}$ −87.4 (*c* 0.85, $CHCl_3$/MeOH containing 8% H_2O = 1/1); ^1H NMR (600 MHz, pyridine-d_5) δ 5.92 (s, 1H), 5.83 (s, 1H), 5.48 (d, 1H, *J* = 3.0 Hz), 5.27 (d, 1H, *J* = 3.6 Hz), 4.91–4.85 (m, 1H), 4.81–4.76 (m, 2H), 4.68–4.65 (m, 1H), 4.62–4.57 (m, 2H), 4.52 (dd, 1H, *J* = 9.0, 3.0 Hz), 4.42–4.37 (m, 2H), 4.33–4.28 (m, 2H), 4.26 (d, 1H, *J* = 10.2 Hz), 4.21 (d, 1H, *J* = 12.0 Hz), 4.14–4.11 (m, 2H), 3.73–3.68 (m, 1H), 2.66–2.60 (m, 2H), 2.48 (t, 1H, *J* = 11.4 Hz), 2.35 (d, 1H, *J* = 12.0 Hz), 2.26–2.22 (m, 1H), 2.14 (d, 1H, *J* = 12.0 Hz), 2.00–1.97 (m, 1H), 1.93–1.89 (m, 2H), 1.66 (d, 3H, *J* = 6.0 Hz), 1.64 (d, 3H, *J* = 6.0 Hz), 1.07 (d, 3H, *J* = 6.0 Hz), 0.82 (m, 6H), 0.78 (s, 3H); ^{13}C NMR (150 MHz, Pyridine-d_5): δ 140.8, 121.3, 103.9, 102.7, 98.0,

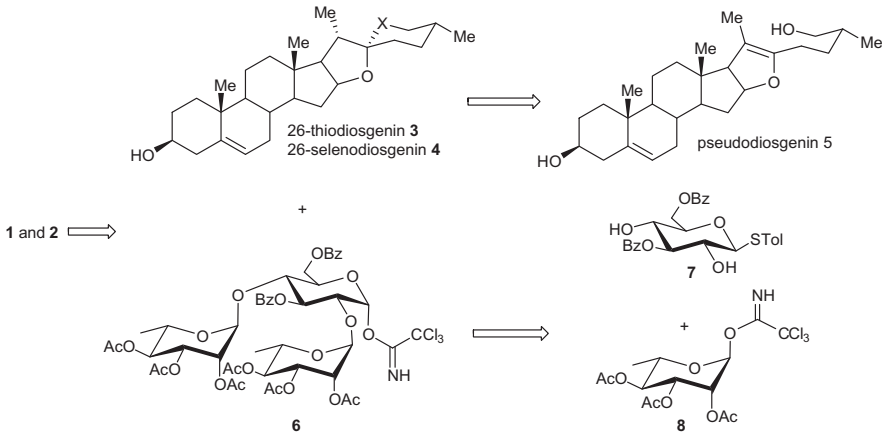
97.9, 82.8, 79.3, 79.0, 78.4, 73.6, 72.6, 72.5, 72.4, 72.3, 72.0, 70.2, 70.1, 63.2, 61.2, 56.4, 49.9, 45.4, 40.5, 40.3, 39.5, 37.0, 36.7, 34.0, 32.8, 32.0, 31.3, 31.1, 28.4, 24.7, 23.6, 20.8, 19.0, 18.4, 18.3, 17.7, 16.4; HRESIMS: $[M + Na]^+$ cacld for $C_{45}H_{72}O_{15}SeNa$: 955.3929; Found: 955.3950.

3. Results and discussion

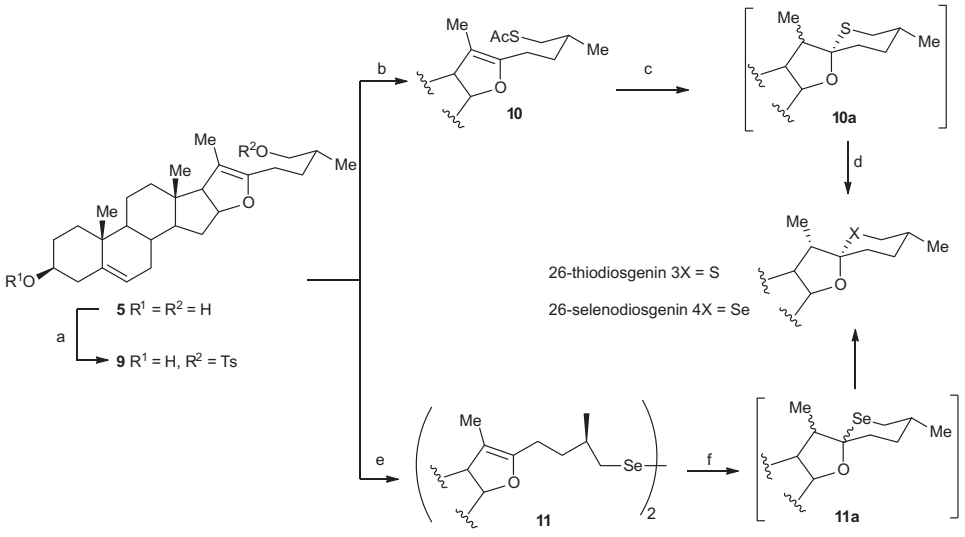
In carbohydrate chemistry, convergent block synthesis has some advantages over linear stepwise synthesis due to its reduced overall number of steps and more time efficiency when the stereochemistry of the desired glycosidic bonds could be warranted [34]. Therefore, we decided to construct 26-thio- and selenodioscin **1** and **2** by convergent strategy involving condensations of chacotriosyl donor **6** and 26-thio- and selenodiosgenin **3** and **4** [35] (Scheme 1). **6** could stem from the coupling of glucosyl thioglycoside **7** [25] with L-rhamnosyl trichloroacetimidate **8** [36]. **3** and **4** could be prepared from well-known pseudodiosgenin **5** [37–39]. It is envisioned that owing to triosyl donor **6** bearing a (1 → 2)-linked glycosidic bond at the reducing end and devoid of neighboring-group participation, its 1,2-*trans* glycosylations with aglycones would be challenging.

To prepare thio- and selenodiosgenin **3** and **4**, transformation of pseudodiosgenin **5** into its 26-tosylate was first performed. Based on the precedent protocols for **9** [37–39], a modified procedure consisting of treatment of **5** with tosyl chloride (1.5 equiv.) in $CHCl_3$ in the presence of $Me_3N{\cdot}HCl$ (0.1 equiv) followed by refluxing in acetone/water furnished **9** in excellent 89% yield over two steps. Adopting Uhle's method [40], tosylate **9** was converted into 26-thiodiosgenin **3** in overall 74% yield by a three-step sequence including displacement of tosylate with thioacetate, hydrolysis of thioester in aqueous methanolic potassium hydroxide leading to **10a** and its subsequent isomerization in acidic conditions (Scheme 2). We observed that **10a** occurred as a mixture of epimers at C-20 [40] due to H-16 displaying two sets of multiplets at 4.62 and 4.53 ppm in its ^1H NMR spectrum.

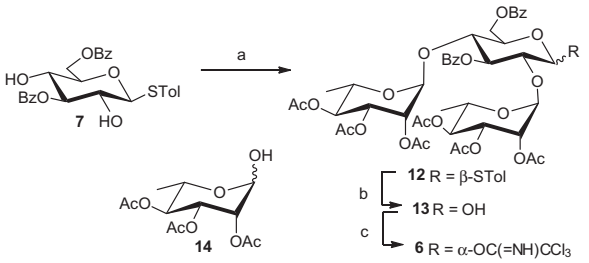
Subjection of **9** to cesium diselenide [41] in DMF resulted in diselenide **11** in 92% yield [35], exposure of which to Zn/HOAc at 150 °C for 24 h stereoselectively afforded selenodiosgenin **4** in 87% yield via reductive cleavage of diselenide accompanied by concomitant ring F formation (Scheme 2). It should be noted that the reaction at lower temperature (e.g. 118 °C) produced a mixture **11a** composed of four stereoisomers arising from stereogenic centers of C-20 and C-22, ^1H NMR spectrum of which displays four sets of



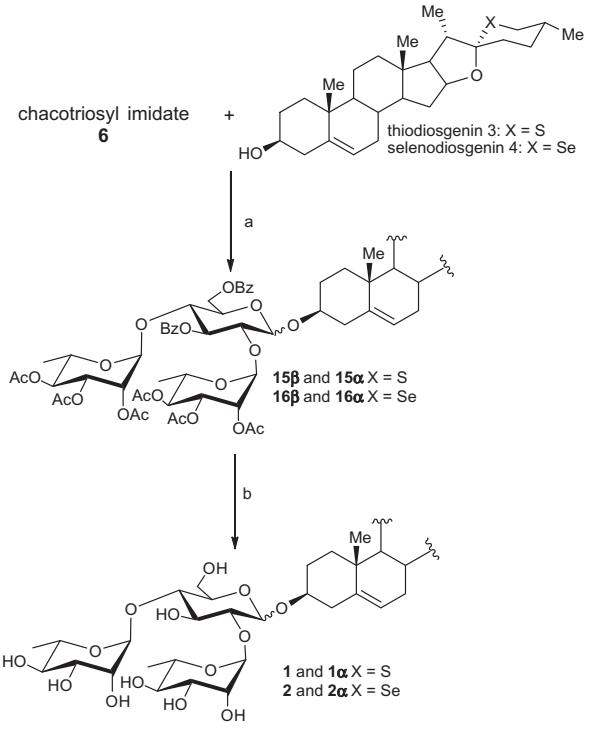
Scheme 1. Retrosynthetic analysis of 26-thiodioscin and selenodioscin **1** and **2**.



Scheme 2. Synthesis of 26-thiodiosgenin and selenodiosgenin **3** and **4**. (a) (i) Et_3N, $Me_3N{\cdot}HCl$, TsCl, $CHCl_3$, −5 °C; (ii) acetone/H_2O = 7/3, 60 °C, 89% over two steps; (b) $KSCOCH_3$, DMF, 60 °C, 96%; (c) KOH, MeOH, H_2O; (d) 0.3 M HCl, EtOH, H_2O, reflux, 87%; (e) $CsOH{\cdot}H_2O$, Se, $N_2H_4{\cdot}H_2O$, DMF, 60 °C, 92%; (f) (i) Zn, CH_3COOH, 150 °C; (ii) KOH, H_2O, EtOH, dioxane, 87% over two steps.



Scheme 3. Synthesis of chacotriosyl imidate 6. (a) 8, TMSOTf, CH_2Cl_2, −30 °C; (b) NBS, acetone, H_2O, 72% over two steps; (c) Cl_3CCN, DBU, CH_2Cl_2, 91%.



Scheme 4. Syntheses of 26-thiodioscin and selenodioscin **1** and **2** and their α-anomers. (a) Table 1, Entries 1–8; (b) CH_3ONa, CH_3OH/CH_2Cl_2 = 1/1, r.t., 95% for **1**; 78% for **2**; 99% for **1α**; 90% for **2α**.

multiplets ranging from 4.84 to 4.50 ppm assigned to H-16. However, our efforts were unrewarded to convert **9** to 26-thiodiosgenin **3** via disulfide in analogy to preparation of selenodiosgenin **4**. The spectra data of ^1H and ^{13}C for **3** and **4** are fully identical with the reported [35].

Donor **6** was prepared from thioglycoside **7** [25]. Thus, **7** smoothly reacted with rhamosyl imidate **8** [36] to furnish trisaccharide **12** by means of an inverse procedure, i.e. adding a solution of donor to a premixed solution of acceptor and catalytic amounts of TMSOTf [42]. However, we had difficulty in obtaining pure **12** by silica gel chromatography due to its contamination by rhamnosyl hemiacetal **14** arising from hydrolysis of imidate **8** during the reaction. Moreover, taking into account that activation of thioglycoside by an electrophilic reagent would likely damage functionalitiy of *O*, *S*-ketal or *O*, *Se*-ketal in thio- or selenodiosgenin **3** or **4** to cause undesirable reactions during the glycosylation, the crude thioglycoside was hydrolyzed with NBS in acetone/water to afford 72% yield of hemiacetal **13** over two steps, which was subsequently converted into α-chacotriosyl imidate **6** in a 91% yield (Scheme 3), whose activation in the presence of a catalytic amount of acid is compatible with the occurrence of *O*, *S*-ketal or *O*, *Se*-ketal in the aglycone.

Chacotriosyl donor and its congeners have been utilized to synthesize saponins by a convergent strategy; however, moderate stereoselectivity was obtained in most cases [20,22,23,43–45]. With the chacotriosyl donor and aglycones in hand, we set out to explore their highly stereoselective couplings to efficiently obtain saponins **1** and **2** (Scheme 4). At first, glycosylation of thiodiosgenin **3** with donor **6** was performed promoted by TMSOTf at −30 °C to provide **15β** (27%) along with **15α** (46%) at a ratio of α/β = 1.7/1 (Table 1, Entry 1). The ratio of the glycosylation was increased to 1/2.4 (α/β) with β-anomer as major product when carrying out the reaction at −78 °C (Table 1, Entry 2). After extensive screening of reaction conditions including solvents and promoters, the combination of a mixture of $PhCF_3$/*t*-BuCN/CH_2Cl_2 as solvent and $HB(C_6F_5)_4$ as promoter [46] emerged as the reagent system of choice, which significantly improved the sterochemistry of the reaction generating glycosides **15** at the ratio of α/β = 1/10.9 in excellent 99% yield (Table 1, Entry 6). The stereochemistry of the glycosylation was contributed to the cooperation of the solvent effect with counter anion of the catalyst [46]. The established protocol also allowed for the condensation of selenodiosgenin **4** with imidate **6** to afford the corresponding glycoside **16** in 72% yield with the β anomer as the predominant product (α/β = 1/13.1) (Table 1, Entry 7), which showed a considerable improvement as compared with the ratio (α/β = 1/1.4) obtained under the conventional conditions (Table 1, Entry 8).

Each protected saponin **15β**, **16β**, **15α** and **16α** was deprotected under Zemplén conditions to give **1**, and **2** as well as their corresponding α isomers **1α** and **2α** in 95%, 78%, 99%, 90% yield, respectively (Scheme 4).

The cytotoxicities (IC_{50}) of **1**, **2**, **1α**, **2α** and dioscin [17–23] against non-small lung cancer cell line A-549 and chronic myelogenous leukemia cell line K562 as well as normal human live cell line L-02 were summarized in Table 2, which were determined by the standard MTT assay [47] using adriamycin as a positive

Table 1
Glycosylations of chacotriosyl imidate **6** with thio- and selenodiosgenin **3** and **4**.

Entry	Acceptor	Solvent	Promoter	*T*/°C	Yield[a] /%	α/β
1	**3**	CH_2Cl_2	TMSOTf (0.2equiv)	−30	73	1.7/1[b]
2	**3**	CH_2Cl_2	TMSOTf (0.2equiv)	−78	77	1/2.4[c]
3	**3**	CH_2Cl_2/^{t}BuCN (40/1, v/v)	TMSOTf (0.2equiv)	−78	44	1/3.2[c]
4	**3**	CH_2Cl_2/^{t}BuCN (8/1, v/v)	TMSOTf (0.2equiv)	−78	44	1/3.4[c]
5	**3**	CH_2Cl_2/^{t}BuCN (5/1, v/v)	$HB(C_6F_5)_4$(0.1equiv.)	−20	64	1/9.3[c]
6	**3**	CH_2Cl_2/^{t}BuCN/$PhCF_3$ (1.3/1/5, v/v/v)	$HB(C_6F_5)_4$(0.1equiv.)	−20	99	1/10.9[c]
7	**4**	CH_2Cl_2/^{t}BuCN/$PhCF_3$ (3/1/5, v/v/v)	$HB(C_6F_5)_4$(0.1equiv.)	−20	72	1/13.1[c]
8	**4**	DCM	TMSOTf (0.2equiv)	−78	78	1/1.4[b]

[a] Yield after purification by chromatography.
[b] Ratio calculated by separating α- and β-isomer.
[c] Ratio determined by ^1H NMR.

Table 2
Cytotoxicities (IC_{50}/μM) of dioscin, 26-thio- and selenodioscin **1** and **2** and their α-anomers **1α** and **2α**.

Comd.	IC_{50}/μM[a]		
	A-549	K562	L-02
Dioscin	4.02 ± 0.16	5.12 ± 0.13	6.71 ± 0.07
1	3.72 ± 0.17	4.10 ± 0.15	7.62 ± 0.08
2	5.00 ± 0.16	4.96 ± 0.15	7.35 ± 0.14
1α	>10	>10	>10
2α	>10	>10	>10
ADM[b]	0.25 ± 0.02	0.16 ± 0.01	0.18 ± 0.01

[a] Data are represented the mean value ± SD of three experiments performed in triplicate.
[b] Abbreviation for adriamycin.

control. Both **1α** and **2α** with α-glycosidic linkages between chacotriosyl moiety and aglycones had IC_{50} greater than 10 μM in all three cell lines tested, whereas the **1** and **2** with β glycosidic linkages were much more cytotoxic to them, which were comparable to those of dioscin. The results indicated that β configurations of glycosides play a crucial role in the cytotoxicity of **1** and **2** and substitution of oxygen at position 26 in dioscin with sulfur or selenium atom had no apparent influence on the observed cytotoxicities. Additionally, ^{1}H and ^{13}C NMR spectra of thiodioscin **1**, selenodioscin **2** appeared to be quite similar to those of dioscin with the exception of atoms located close to C-22 and C-26 due to the replacement of oxygen with sulfur and selenium thereby highlighting their similar three-dimensional structures which suggest that appropriate conformation have a significant influence on the cytotoxicities of discin [28].

Dioscin-induced apoptosis in K562 and A549 cells has been ascribed to mitotic arrest of cell cycle and caspase-3 and caspase-9 activation, respectively [48,49]. The similar three dimensional structures and comparable cytotoxicities among thiodioscin, selenodioscin, and dioscin imply that the similar molecular mechanisms might contribute to the cytotoxicities exibited by thiodioscin and selenodioscin.

4. Conclusions

In summary, highly stereoselective 1,2-*trans* glycosylations of chacotriosyl donor have been achieved and thio- and selenodioscin have been successfully constructed by a convergent strategy and their cytotoxicities have been evaluated. The fact that 1,2-*trans* glycosidic bonds between oligosaccharide moieties and aglycones are common in natural saponins implies that the developed convergent stereoselective glycosylation might find more applications in the syntheses of saponins. The low cytotoxicities of **1**, **2** and comparable cytotoxicities among thiodioscin **1**, selenodioscin **2**, and dioscin in the three cell lines tested provide information to the field of anticancer spirostan saponins in that three dimensional structures of dioscin and its derivatives might be the essential to their cytotoxicities. Further investigations on thiodioscin and selenodioscin are ongoing in our laboratory in that saponins have versatile bioactivities and organosulsur/organoselenium compounds possess unique properties such as antioxidation and quench of redicals.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2013.05.018.

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