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The synthesis of gracillin and dioscin: two typical representatives of spirostanol glycosides

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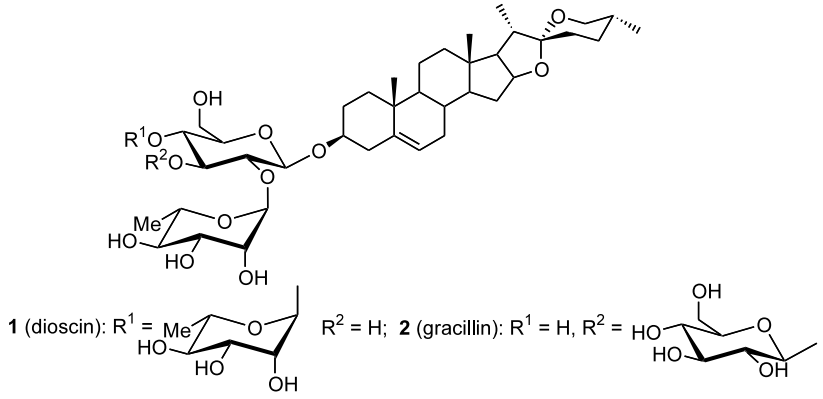
Abstract

Two representative spirostanol saponins that have the typical structure for the sugar moiety, diosgenyl α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (gracillin) and diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (dioscin), were easily synthesized by a general approach. A procedure using guanidine for the selective deblocking of acetyl while retaining benzoyl protecting groups is described. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords: Spirostanol glycosides; Glycosylation; Gracillin; Dioscin; Guanidine

1. Introduction

Spirostanol glycosides constitute a large group of steroidal saponins. They exist extensively in nature and have a broad range of interesting bioactivities.[1] Dioscin (**1**) and gracillin (**2**) are two typical representatives of spirostanol saponins. They exist widely in the natural plants used in traditional Chinese herbal medicine, such as *Dioscorea*, *Paris* and *Costacea* species, which exhibit cardiovascular and antitumor activities.[2] Their sugar moiety shows the typical structural pattern of diosgenyl saponins, with a β-D-glucopyranoside as the first sugar attached to diosgenin, which in turn has an α-L-rhamnopyranose substituted at the 2-position and another terminal sugar or sugar chain at the 3- (such as gracillin) or 4-position (such as dioscin). Using diosgenyl 4′,6′-*O*-benzylidene-β-D-glucopyranoside as the key intermediate, dioscin and gracillin had been synthesized, respectively through two approaches.[3,4] In these approaches, the 2′-OH and 3′-OH of diosgenyl 4′,6′-*O*-benzylidene-β-D-glucopyranoside were difficult to selectively protect. Herein, we report another synthetic approach in which selective protection is avoided in the preparation of these two saponins.



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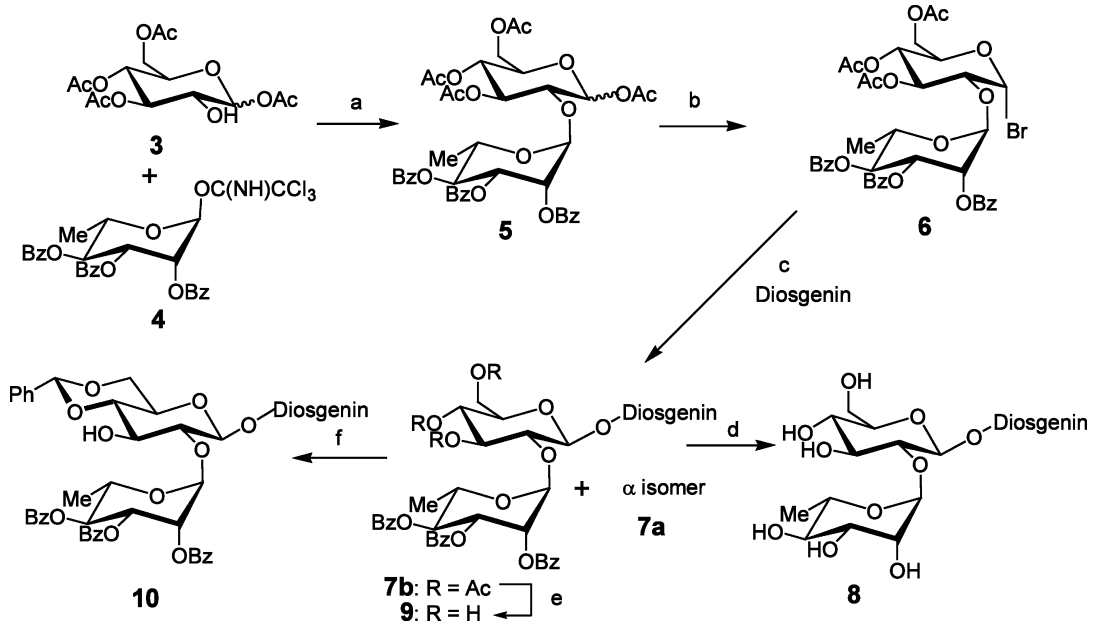
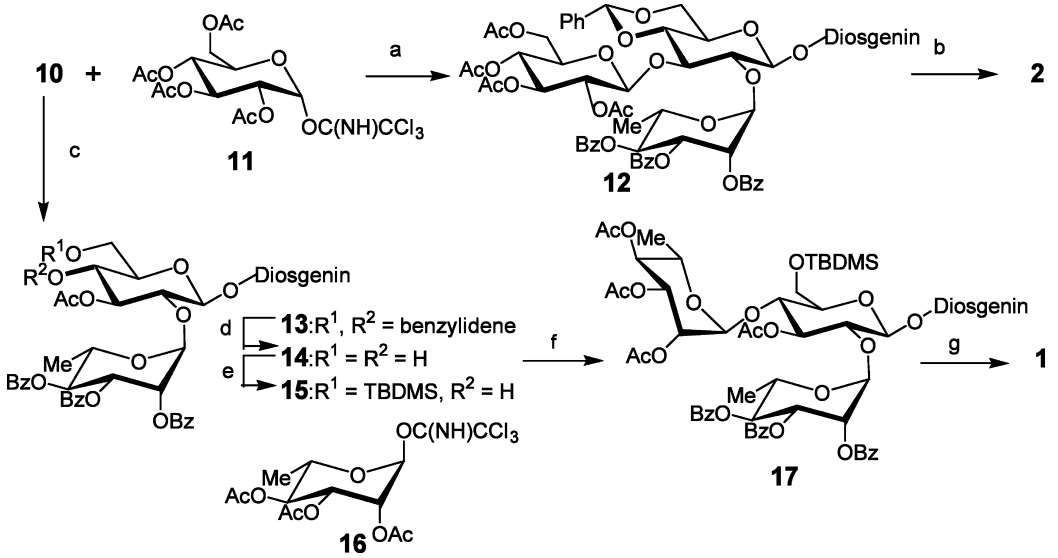
2. Results and discussion

In previous approaches, the regioselective protection of diosgenyl 4′,6′-*O*-benzylidene-β-D-glucopyranoside was very difficult. Now we have designed a strategy to avoid this problem. In the structures of dioscin and gracillin, there is a mutually shared sugar fragment, neohesperidose [α-L-Rha*p*-(1→2)-D-Glc*p*]. So we fabricated a protected neohesperidose first and attached it to diosgenin, then selectively removed the protective groups on glucose and extended the sugar chain to produce the target compounds. As shown in Scheme 1, the disaccharide **5** was prepared by glycosylation of 1,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose (**3**)[5] with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroimidate (**4**).[6] Compound **5** was then treated with 33% HBr–HOAc in CH_2Cl_2 to form the disacchride donor **6**. In our previous work, we found that $CdCO_3$ is an effective catalyst in glycosylating spirostanol sapogenins with the glycosyl bromide as the donor and acetonitrile as the



Scheme 1. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, 4 Å MS, CH_2Cl_2, −78 °C→rt, 3 h, 85%; (b) 33% HBr–HOAc, CH_2Cl_2, 0 °C→rt, 3 h, 95%; (c) $CdCO_3$, CH_3CN, 65 °C, 2 h, 68% for **7b** and 9% for **7a**; (d) MeONa, 1:1 MeOH–CH_2Cl_2, rt, overnight, 100%; (e) guanidine, 1:1 EtOH–CH_2Cl_2, −10 °C, 5 h, 71%; (f) $PhCH(OMe)_2$, DMF, CSA, 60 °C, 2 h, 91%.



Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, 4 Å MS, CH_2Cl_2, −78 °C→rt, 4 h, 83%; (b) 80% HOAc, 2 h, 70 °C and then MeONa, 1:1 MeOH–CH_2Cl_2, rt, 81%; (c) Ac_2O–Py, 98%; (d) 80% HOAc, 3 h, 70 °C 85%; (e) TBDMSCl, imidazole, DMAP, 40 °C, 95%; (f) $BF_3{\cdot}Et_2O$, 4 Å MS, CH_2Cl_2, −78 °C→rt, 3 h, 86%; (g) TBAF, THF, rt, 6 h and then MeONa, 1:1 MeOH–CH_2Cl_2, rt, overnight, 83%.

solvent. When we used **6** as the donor to glycosylate diosgenin by this method, a protected diosdenyl disaccharide **7b** was obtained in 68%, along with its α isomer **7a** in 9% yield. Although there was no aryl neighboring group participation, we still predominantly obtained the β-type product because of the influence of the solvent on glycosylation.[7] Treatment of **7b** with NaOMe gave a naturally existing saponin **8**, ophiopogonin C′.[8] Selective deprotection of the acetyl groups in **7b** with 0.25 equiv of guanidine in 1:1 MeOH–CH_2Cl_2 at −10 °C efficiently provided the key intermediate **9** in 71% yield.[9] Benzylidenation of **9** afforded the receptor intermediate **10** in 89% yield, from which the target saponins were readily synthesized by general methods.

As shown in Scheme 2, receptor intermediate **10** was glycosylated with donor 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl trichloroimidate (**11**)[10] to give the protected diosgenyl trisaccharides **12**. Deprotection of **12** with 80% HOAc and then MeONa afforded the target saponin **2**. Treatment of **10** with Ac_2O–pyridine, deprotection of the benzylidene group and then protection of the 6′-OH with TBDMS gave another receptor intermediate **15**. Glycosylation of **15** with donor 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroimidate (**16**)[11] provided the protected diosgenyl trisaccharide **17**. Deprotection of TBDMS with TBAF and then treatment with MeONa afforded target saponin **1**.

In conclusion, a general synthetic approach was devised to prepare in satisfactory yields the spirostan saponins (dioscin and gracillin) that have the typical structure of the branched sugar moieties.

3. Experimental

3.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer model 241MC automatic polarimeter in $CHCl_3$, MeOH or Py. Melting points were determined with a 'Yanaco' apparatus. ^1H and ^{13}C NMR spectra were recorded with Mercury 300 or Inova 500 spectrometers for solutions in $CDCl_3$ or Py-d_5. Chemical shifts were given in ppm downfield from internal Me_4Si. Mass spectra were recorded with a VG AutoSpec Ultima-TOF mass spectrometer using the FAB technique to introduce the sample. Thin-layer chromatography (TLC) was performed on Silica Gel HF_{254} (Qingdao) with detection by charring with 5% (v/v) H_2SO_4 in EtOH, or in some cases by a UV lamp. Column chromatography was conducted by elution of a column of silica gel (100–200 or 140–180 mesh) (Qingdao) with EtOAc–petroleum ether (bp 60–90 °C) or CH_2Cl_2–MeOH as the eluent. Solutions were concentrated at <60 °C under diminished pressure.

3.2. 2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-1,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose (5)

To a solution of 1,3,4,6-tetra-*O*-acetyl-α,β-D-glucopyranose (**3**) (6.0 g, 17.24 mmol) and 4 Å molecular sieves (10 g) in dry CH_2Cl_2 (120 mL) under Ar at −78 °C, was added $BF_3{\cdot}Et_2O$ (0.5 mL, 4.06 mmol), followed by a solution of 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroimidate (**4**) (14.80 g, 23.68 mmol) in dry CH_2Cl_2 (30 mL). The mixture was warmed to rt and stirred for 3 h, neutralized with Et_3N and filtered, and the filtrate was then concentrated. Purification of the product by column chromatography (6:1→4:1 petroleum ether–EtOAc) gave **5** (11.80 g, 85%) as a yellow syrup: ^1H NMR (300 MHz, $CDCl_3$): δ 8.10–7.23 (m, 15 H, H–OBz), 6.42 (d, 0.5 H, 3.9 Hz, H-1α), 6.79 (d, 0.5 H, 8.4 Hz, H-1β), 5.71–5.39 (m, 4 H, H-2′, H-3′, H-4′, H-3), 5.20 (brs, 1 H, H-1′), 5.12 (t, 0.5 H, 9.6 Hz, H-4α), 5.09 (t, 0.5 H, 9.6 Hz, H-4β), 4.36–3.87 (m, 5 H, H-2, H-5, H-6a,b), 2.34, 2.19 (s each, 1.5 H each, H–OAc for α isomer), 2.22, 2.18 (s each, 1.5 H each, H–OAc for β isomer), 2.09, 2.06 (s each, 3 H each, H–OAc), 1.33 (d, 1.5 H, 6.0 Hz, H-6′α), 1.32 (d, 1.5 H, 6.0 Hz, H-6′β); FABMS (*m*/*z*): 806.3 [M^+], 747.2, 459.2, 215.1; Anal. Calcd for $C_{41}H_{42}O_{17}$: C, 61.03; H, 5.25. Found: C, 61.24; H, 5.31.

3.3. 2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide (6)

To a solution of **5** (9.8 g, 12.16 mmol) in dry CH_2Cl_2 (50 mL) under an Ar atmosphere at 0 °C, was added HBr–HOAc (33%, 10 mL). After being stirred at 0 °C for 2 h and then at rt for 1 h, the mixture was diluted with CH_2Cl_2 (100 mL) and then poured into satd $NaHCO_3$ with ice. The organic layer was washed with satd $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated to gave **6** (9.57 g, 95%) as a white solid: mp 82–84 °C; $[\alpha]_D^{25}$ +164.2° (*c* 0.72, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.10–7.23 (m, 15 H, H–OBz), 6.51 (d, 1 H, 3.9 Hz, H-1), 5.76–5.71 (m, 2 H, H-3′, H-4′), 5.61 (t, 1 H, 9.6 Hz, H-3), 5.51 (m, 1 H, H-2′), 5.19–5.12 (m, 2 H, H-1′, H-4), 4.42–4.32 (m, 3 H, H-5′, H-5, H-6a), 4.14 (m, 1 H, H-6b), 3.87 (dd, 1 H, 9.6, 3.9 Hz, H-2), 2.12, 2.10, 2.06 (s each, 3 H each, H–OAc), 1.35 (d, 3 H, 6.0 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.7, 170.4, 169.9, 166.1, 165.8, 165.4, 133.9, 133.7, 133.4, 130.1 (2C), 130.0 (2C), 129.9 (2C), 129.3 (overlap, 2C), 129.2, 128.9 (2C), 128.7 (2C), 128.5 (2C), 100.2, 88.9, 78.9, 72.5, 71.7, 71.5, 71.0, 69.6, 68.7, 67.3, 61.3, 20.9, 20.8 (2C), 17.8.

3.4. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-glucopyranoside (7a) and diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (7b)

A solution of diosgenin (1.2 g, 2.90 mmol) and $CdCO_3$ (0.80 g, 4.65 mmol) in CH_3CN (50 mL) was refluxed under Ar and evaporated to about one-half of the solvent volume to render the system anhydrous. After the flask was cooled to rt, **6** (3.7 g, 4.47 mmol) was added, and the mixture was stirred at 65 °C for 2 h. The mixture was filtered, and the filtrate was then concentrated. Purification of the product by column chromatography (8:1→6:1 petroleum ether–EtOAc) gave **7a** (305 mg, 9%) and **7b** (2.29 g, 68%) as white solids: for **7a**: mp 130–132 °C; $[\alpha]_D^{25}$ +88.5° (*c* 1.03, $CHCl_3$): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10–7.23 (m, 15 H, H–OBz), 5.79 (dd, 1 H, 3.3, 9.9 Hz, H-3″), 5.65 (t, 1 H, 9.9 Hz, H-4″), 5.55 (1 H, 9.6 Hz, H-3′), 5.49 (dd, 1 H, 3.3, 1.8 Hz, H-2″), 5.38 (d, 1 H, 4.5 Hz, H-6), 5.14–5.13 (m, 2 H, H-1′, H-1″), 5.02 (t, 1 H, 9.6 Hz, H-4′), 4.41 (m, 1 H, H-16), 4.32–4.08 (m, 4 H, H-5′, H-6′a,b, H-5″), 3.81 (d, 1 H, 3.6, 9.6 Hz, H-2′), 3.57–3.33 (m, 3 H, H-3, H-26a,b), 2.16, 2.06, 2.03, (s each, 3 H each, H–OAc), 1.31 (d, 3 H, 6.0 Hz, H-6″), 1.07 (s, 3 H), 0.97 (3 H, 6.9 Hz), 0.80 (s, 3 H), 0.78 (d, 3 H, 6.0 Hz); Anal. Calcd for $C_{66}H_{80}O_{18}$: C, 68.24; H, 6.95. Found: C, 68.48; H, 6.79; for **7b**: mp 131–133 °C; $[\alpha]_D^{25}$ +54.4° (*c* 1.07, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08–7.25 (m, 15 H, H–OBz), 5.78 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.69 (t, 1 H, 10.0 Hz, H-4″), 5.44 (d, 1 H, 4.5 Hz, H-6), 5.41 (dd, 1 H, 1.5, 3.5 Hz, H-2″), 5.36 (t, 1 H, 9.5 Hz, H-3′), 5.26 (d, 1 H, 1.5 Hz, H-1″), 5.01 (t, 1 H, 9.5 Hz, H-4′), 4.72 (m, 1 H, H-5″), 4.68 (d, 1 H, 8.0 Hz, H-1′), 4.41 (m, 1 H, H-16), 4.31–4.09 (m, 2 H, H-6′a,b), 3.84 (d, 1 H, 8.0, 9.5 Hz, H-2′), 3.74 (m, 1 H, H-5′), 3.66 (m, 1 H, H-3), 3.47 (m, 1 H, H-26a), 3.38 (t, 1 H, H-26b), 2.15, 2.08, 2.03 (s each, 3 H each, H–OAc), 1.32 (d, 3 H, 6.0 Hz, H-6″), 0.95 (d, 3 H, 7.0 Hz), 0.88 (s, 3 H), 0.77 (d, 3 H, 6.0 Hz), 0.74 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 170.4, 169.7, 165.7, 165.5, 165.4, 140.1, 133.5, 133.2, 133.1, 129.9 (2C), 129.8 (2C), 129.7 (2C), 129.3 (overlap, 2C), 129.2, 128.6 (2C), 128.4 (2C), 128.3 (2C), 122.2, 109.3, 100.1, 97.3, 80.8, 79.8, 75.3, 75.0, 71.9, 71.6, 71.2, 69.5, 68.9, 66.8, 66.7, 62.2, 62.1, 56.4, 50.0, 41.6, 40.2, 39.7, 38.7, 37.1, 36.8, 32.1, 31.9, 31.5, 31.4, 30.3, 29.8, 28.8, 20.8 (2C), 20.7, 20.6, 19.2, 17.4, 17.1, 16.2, 14.5; FABMS (*m*/*z*): 1161.2 [M + 1], 747.2, 459.2; Anal. Calcd for $C_{66}H_{80}O_{18}$: C, 68.24; H, 6.95. Found: C, 68.52; H, 6.94.

3.5. Preparation of diosgenyl α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (ophiopogonin C′, 8)

To a solution of **7b** (256 mg, 0.22 mmol) in 1:1 MeOH–CH_2Cl_2 (5 mL) was added MeONa (0.05 mL, 1 M in MeOH). The mixture was stirred overnight at rt, neutralized with Dowex-50 (H^+) resin and filtered, and then the filtrate was concentrated. Purification of the product by column chromatography (10:1→8:1 CH_2Cl_2–MeOH) gave **8** (158 mg, 100%) as a white solid: mp 212–214 °C, lit.[8] 211–213 °C; $[\alpha]_D^{25}$ +93.5° (*c* 1.0, Py), lit.[8] +94.3° (*c* 0.72, Py); ^{13}C NMR (75 MHz, Py-d_5): δ 140.7, 121.6, 109.1, 101.9, 100.2, 81.0, 79.4, 78.1, 77.8 (overlap, 2C), 74.0, 72.7, 72.4, 71.7, 69.3, 66.7, 62.7, 62.5, 56.5, 49.1, 41.8, 40.3, 39.7, 38.8, 37.3, 37.0, 32.1, 32.0, 31.7, 31.5, 30.4, 30.0, 29.1, 20.9, 19.3, 18.5, 17.2, 16.2, 14.9.

3.6. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (9)

To a solution of **7b** (796 mg, 0.69 mmol) in 1:1 CH_2Cl_2–EtOH (15 mL) was added guanidine (10 mg, 0.17 mmol) under Ar at −10 °C. The mixture was stirred for 5 h, neutralized with satd NH_4Cl and diluted with CH_2Cl_2 (50 mL). The organic layer was washed with satd NH_4Cl and brine, dried over Na_2SO_4, and concentrated. Purification of the product by column chromatography (30:1→8:1 CH_2Cl_2–MeOH) gave **9** (507 mg, 71%) as a white solid; mp 155–157 °C; $[\alpha]_D^{25}$ +32.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.08–7.24 (m, 15 H, H–OBz), 5.79 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.75 (dd, 1 H, 1.5, 3.5 Hz, H-2″), 5.68 (t, 1 H, 10.0 Hz, H-4″), 5.56 (brs, 1 H, H-1″), 5.44 (d, 1 H, 4.5 Hz, H-6), 4.72 (m, 1 H, H-5″), 4.63 (d, 1 H, 8.0 Hz, H-1′), 4.41 (m, 1 H, H-16), 3.92–3.76 (m, 3 H, H-2′, H-3′, H-4′), 3.66–3.57 (m, 3 H, H-5′, H-6′a,b), 3.48 (t, 1 H, H-26a), 3.40–3.36 (m, 2 H, H-3, H-26b), 1.35 (d, 3 H, 6.0 Hz, H-6″), 0.98 (d, 3 H, 7.0 Hz), 0.94 (s, 3 H), 0.79 (d, 3 H, 6.0 Hz), 0.73 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.0, 165.9, 165.7, 140.2, 133.5, 133.2, 133.1, 130.0 (2C), 129.8 (2C), 129.7 (2C), 129.4, 129.3, 129.1, 128.6 (2C), 128.4 (2C), 128.3 (2C), 122.0, 109.3, 100.1, 97.5, 80.8, 79.5, 77.8, 77.3, 75.1, 71.8, 70.8 (overlap, 2C), 70.3, 66.8, 66.6, 62.5, 62.1, 56.4, 50.0, 41.6, 40.3, 39.7, 38.9, 37.1, 36.8, 32.1, 31.9, 31.5, 31.4, 30.3, 29.9, 28.8, 20.8, 19.3, 17.4, 17.1, 16.3, 14.5; FABMS (*m*/*z*): 1035.5 [M + 1], 459.1, 397.3; Anal. Calcd for $C_{60}H_{74}O_{15}$: C, 69.61; H, 7.21. Found: C, 69.47; H, 7.32.

3.7. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-4,6-*O*-benzylidene-β-D-glucopyranoside (10)

To a solution of **9** (256 mg, 0.25 mmol) and Ph-$CH(OMe)_2$ (0.1 mL, 0.67 mmol) in dry DMF (8 mL) was added camphorsulfonic acid (CSA, 40 mg). The mixture was stirred at 60 °C under reduced pressure for 2 h, neutralized with Et_3N, diluted with EtOAc (30 mL), then washed with brine, dried over Na_2SO_4, and concentrated. Purification of the product by column

chromatography (10:1 → 5:1 petroleum ether–EtOAc) gave **10** (252 mg, 91%) as a white solid: mp 156–158 °C; $[\alpha]_D^{25}$ +19.2° (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 8.09–7.24 (m, 20 H, H–OBz and H–Ph), 5.83 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.76 (dd, 1 H, 2.0, 3.5 Hz, H-2″), 5.68 (t, 1 H, 10.0 Hz, H-4″), 5.64 (d, 1 H, 2.0 Hz, H-1″), 5.53 (s, 1 H, H–C*H*–Ph), 5.49 (d, 1 H, 4.5 Hz, H-6), 4.74 (m, 1 H, H-5″), 4.72 (d, 1 H, 8.0 Hz, H-1′), 4.43 (m, 1 H, H-16), 4.35 (dd, 8.0, 9.5 Hz, H-2′), 4.05 (t, 1 H, H-3′), 3.82–3.76 (m, 2 H, H-6′a,b), 3.67 (m, 1 H, H-3), 3.56–3.36 (m, 4 H, H-4′, H-5′, H-26a,b), 1.37 (d, 3 H, 6.5 Hz, H-6″), 0.99 (d, 3 H, 7.0 Hz), 0.95 (s, 3 H), 0.80 (d, 3 H, 6.0 Hz), 0.78 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.0, 165.9, 165.8, 140.3, 137.2, 133.6, 133.5, 133.3, 130.2 (2C), 130.0 (2C), 129.9 (2C), 129.7, 129.6, 129.5, 129.4, 128.8 (2C), 128.6 (2C), 128.5 (2C), 128.4 (2C), 126.5 (2C), 122.4, 109.5, 102.1, 100.8, 97.7, 81.0, 80.8, 79.9, 77.5, 75.4, 72.2, 70.8, 70.3, 68.9, 66.9, 66.1, 62.3, 56.7, 50.2, 41.8, 40.5, 39.9, 39.1, 37.4, 37.1, 32.4, 32.1, 31.7, 31.6, 30.5, 29.9, 29.0, 21.0, 19.5, 17.7, 17.4, 16.5, 14.8; FABMS (*m*/*z*): 1123.4 [M + 1], 1035.6, 459.1, 397.2; Anal. Calcd for $C_{67}H_{78}O_{15}$: C, 71.63; H, 7.00. Found: C, 71.34; H, 7.19.

3.8. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1 → 2)-[2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)]-4,6-*O*-benzylidene-β-D-glucopyranoside (12)

To a solution of **10** (42 mg, 37 μmol) and 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (5 mL) under Ar at −78 °C, was added $BF_3{\cdot}Et_2O$ (0.02 mL, 170 μmol), then a solution of **11** (55 mg, 111 μmol) in dry CH_2Cl_2 (1 mL). The mixture was naturally warmed to rt and stirred for 4 h, neutralized with Et_3N and filtered, and then the filtrate was concentrated. Purification of the product by column chromatography (5:1 → 3:1 petroleum ether–EtOAc) gave **12** (45 mg, 83%) as a white solid: mp 196–198 °C; $[\alpha]_D^{25}$ +5.2° (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.15–7.24 (m, 20 H, H–OBz and H-Ph), 5.77 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.72 (dd, 1 H, 1.5, 3.5 Hz, H-2″), 5.69 (t, 1 H, 10.0 Hz, H-4″), 5.52 (brs, 2 H, overlap, H–C*H*–Ph, H-1″), 5.48 (d, 1 H, 4.5 Hz, H-6), 5.30 (t, 1 H, 9.5 Hz, H-3‴), 5.02–5.49 (m, 3 H, H-1‴, H-2‴, H-4‴), 4.82 (m, 1 H, H-5″), 4.68 (d, 1 H, 8.0 Hz, H-1′), 4.42 (m, 1 H, H-16), 4.33 (m, 1 H, H-5‴), 4.20–4.09 (m, 2 H, H-6‴a,b), 4.02 (m, 1 H, 10.0 Hz, H-3′), 3.88 (m, 1 H, H-4′), 3.79 (m, 1 H, H-2′), 3.68–3.64 (m, 3 H, H-3, H-6′a,b), 3.40–3.36 (m, 3 H, H-5′, H-26a,b), 1.99, 1.98, 1.87, 1.73 (s each, 3 H each, H–OAc), 1.35 (d, 3 H, 6.0 Hz, H-6″), 0.98 (d, 3 H, 7.0 Hz), 0.93 (s, 3 H), 0.79 (d, 3 H, 6.0 Hz), 0.78 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.7, 169.9, 169.6, 169.2, 165.8, 165.7, 165.4, 140.0, 137.2, 133.4, 133.2, 133.1, 130.1 (2C), 129.8 (2C), 129.7 (2C), 129.5, 129.3, 129.2, 129.1, 128.5 (2C), 128.4 (2C), 128.3 (2C), 128.2 (2C), 126.1 (2C), 122.3, 109.3, 100.4, 100.3, 99.1, 97.2, 80.9, 80.8, 79.3, 78.8, 77.3, 76.0, 73.0, 71.7, 71.6, 71.5, 70.3, 70.1, 68.8, 68.7, 66.8, 66.7, 66.2, 62.1 (2C), 56.4, 50.0, 41.6, 40.3, 39.7, 38.7, 37.2, 36.8, 32.2, 31.9, 31.5, 31.4, 30.3, 29.9, 28.8, 20.8, 20.7, 20.6, 20.5, 19.3, 17.3, 17.1, 16.3, 14.5; FABMS (*m*/*z*): 1475.8 [M + Na], 514.0, 459.1, 397.3; Anal. Calcd for $C_{81}H_{96}O_{24}$: C, 66.92; H, 6.66. Found: C, 66.65; H, 6.45.

3.9. Preparation of diosgenyl α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside (gracillin, 2)

A solution of **12** (145 mg, 100 μmol) in 80% HOAc (10 mL) was stirred at 70 °C for 2 h, then concentrated to dryness under reduced pressure. The residue was redissolved in 1:1 MeOH–CH_2Cl_2 (5 mL) at rt, and MeONa was then added (0.05 mL 1 M in MeOH). The mixture was stirred overnight, neutralized with Dowex-50 (H^+) resin and filtered, and the filtrate was then concentrated. Purification of the product by column chromatography (10:1 → 8:1 CH_2Cl_2–MeOH) gave **2** (70 mg, 81%) as a white solid: mp >250 °C, lit.[2] 298–302 °C; $[\alpha]_D^{25}$ −83.4° (*c* 1.0, Py), lit.[2] −86.2° (*c* 0.12, DMF); ^{13}C NMR (125 MHz, Py-d_5): δ 140.8, 121.8, 109.3, 104.5, 102.2, 100.0, 89.5, 81.1, 78.7, 78.5, 77.9, 77.8, 77.1, 75.0, 74.1, 72.8, 72.5, 71.5, 69.6, 69.5, 66.9, 62.9, 62.5 (overlap, 2C), 56.7, 50.3, 42.0, 40.5, 39.9, 38.7, 37.5, 37.2, 32.3, 32.2, 31.9, 31.7, 30.6, 30.1, 29.3, 21.1, 19.4, 18.7, 17.3, 16.3, 14.5; FABMS (*m*/*z*): 885 [M + 1], 723, 631, 577, 415, 397.

3.10. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1 → 2)-3-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranoside (13)

To a solution of **10** (99 mg, 88 μmol) in dry Py (2 mL) was added Ac_2O (1 mL) at rt. The mixture was stirred for overnight and concentrated to dryness. Purification of the product by column chromatography (8:1 → 6:1 petroleum ether–EtOAc) gave **13** (102 mg, 98%) as a white solid: mp 213–215 °C; $[\alpha]_D^{25}$ +29.6° (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 8.10–7.25 (m, 20 H, H–OBz and H–Ph), 5.81 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.70 (dd, 1 H, 2.0, 3.5 Hz, H-4″), 5.52 (t, 1 H, 9.5 Hz, H-3′), 5.50–5.45 (m, overlap, 3 H, 10.0 Hz, H-6, H-1″, H-2″), 5.34 (s, 1 H, H–C*H*–Ph), 4.79 (d, 1 H, 8.0 Hz, H-1′), 4.74 (m, 1 H, H-5″), 4.43 (m, 1 H, H-16), 4.35 (dd, 8.0, 9.5 Hz, H-2′), 3.88–3.67 (m, 3 H, H-3, H-26a,b), 3.62–3.54 (m, 2 H, H-6′a,b), 3.48 (m, 1 H, H-5′), 3.39 (t, 1 H, 10.0 Hz, H-4′), 2.19 (s, 3 H, H–OAc), 1.34 (d, 3 H, 6.5 Hz, H-6″), 0.97 (d, 3 H, 7.0 Hz), 0.90 (s, 3 H), 0.79 (d, 3 H, 6.5 Hz), 0.77 (s, 3 H); Anal. Calcd for $C_{69}H_{80}O_{16}$: C, 71.11; H, 6.92. Found: C, 71.36; H, 6.65.

3.11. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-acetyl-β-D-glucopyranoside (14)

A solution of **13** (95 mg, 88 μmol) in 80% HOAc (80 mL) at 70 °C. The mixture was stirred for 3 h, then concentrated to dryness under reduced pressure. Purification of the product by column chromatography (2:1→2:3 petroleum ether–EtOAc) gave **14** as a white solid (75 mg, 85%): mp 138–140 °C; $[\alpha]_D^{25}$ +51.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.25 (m, 15 H, H–OBz), 5.77 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.71 (dd, 1 H, 2.0, 3.5 Hz, H-4″), 5.48 (dd, 1 H, 3.5, 1.5 Hz, H-2″), 5.46 (d, 1 H, 4.5 Hz, H-6), 5.35 (d, 1 H, 1.5 Hz, H-1″), 5.12 (t, 1 H, 9.5 Hz, H-3′), 4.76 (m, 1 H, H-5″), 4.70 (d, 1 H, 8.0 Hz, H-1′), 4.43 (m, 1 H, H-16), 3.94 (m, 1 H, H-4′), 3.82 (m, 1 H, H-5′), 3.79 (dd, 8.0, 9.5 Hz, H-2′), 3.72–3.38 (m, 5 H, H-3, H-26a,b, H-6′a,b), 2.27 (s, 3 H, H–OAc), 1.34 (d, 3 H, 6.5 Hz, H-6″), 0.97 (d, 3 H, 7.0 Hz), 0.92 (s, 3 H), 0.79 (d, 3 H, 6.5 Hz), 0.78 (s, 3 H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3, 165.7, 165.6, 165.5, 140.0, 133.5, 133.3, 133.2, 129.9 (2C), 129.8 (2C), 129.7 (2C), 129.3, 129.2, 129.1, 128.6 (2C), 128.4 (2C), 128.3 (2C), 122.3, 109.3, 99.7, 97.4, 80.8, 79.5, 79.3, 77.2, 75.2, 71.8, 71.1, 70.5, 69.7, 66.8, 66.7, 62.5, 62.1, 56.4, 50.0, 41.6, 40.3, 39.7, 38.7, 37.1, 36.8, 32.1, 31.9, 31.5, 31.4, 30.3, 29.9, 28.8, 20.8, 19.3, 17.4, 17.1, 16.3, 14.5; Anal. Calcd for $C_{62}H_{76}O_{16}$: C, 69.12; H, 7.11. Found: C, 69.21; H, 7.35.

3.12. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (15)

To a solution of **14** (68 mg, 63 μmol), imidazole (25 mg), and DMAP (5 mg) in dry DMF (5 mL) under Ar at 40 °C, was added *tert*-butylchlorodimethylsilane (TBDMSCl, 15 mg, 95 μmol). The mixture was stirred for 4 h, diluted with EtOAc (20 mL) and washed with brine, dried over Na_2SO_4, and concentrated. Purification of the product by column chromatography (5:1 petroleum ether–EtOAc) gave **15** (72 mg, 95%) as a white solid: mp 124–126 °C; $[\alpha]_D^{25}$ +42.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.11–7.25 (m, 15 H, H–OBz), 5.79 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.69 (dd, 1 H, 2.0, 3.5 Hz, H-4″), 5.47–5.45 (m, 2 H, H-6, H-2″), 5.31 (d, 1 H, 1.5 Hz, H-1″), 5.19 (t, 1 H, 9.5 Hz, H-3′), 4.79 (m, 1 H, H-5″), 4.65 (d, 1 H, 8.0 Hz, H-1′), 4.43 (m, 1 H, H-16), 3.92–3.60 (m, 5 H, H-3, H-2′, H-4′, H-6′a,b), 3.49–3.38 (m, 3 H, H-26a,b, H-5′), 2.26 (s, 3 H, H–OAc), 1.33 (d, 3 H, 6.0 Hz, H-6″), 0.98 (d, 3 H, 7.0 Hz), 0.92 (s, 3 H), 0.91 (s, 9 H), 0.79 (d, 3 H, 6.0 Hz), 0.78 (s, 3 H), 0.10, 0.09 (s each, 3 H each); FABMS (*m*/*z*): 1191.5 [M + 1], 459.2, 397.3; Anal. Calcd for $C_{68}H_{90}O_{16}Si$: C, 68.54; H, 7.61. Found: C, 68.86; H, 7.94.

3.13. Diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→4)]-3-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (17)

To a solution of **15** (64 mg, 37 μmol) and 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (5 mL) under Ar at −78 °C, was added $BF_3{\cdot}Et_2O$ (0.02 mL, 170 μmol), then a solution of **11** (100 mg, 111 μmol) in dry CH_2Cl_2 (1 mL). The mixture was warmed naturally to rt and stirred for 3 h, neutralized with Et_3N and filtered, and then the filtrate was concentrated. Purification of the product by column chromatography (5:1 petroleum ether–EtOAc) gave **17** (68 mg, 86%) as a white solid: mp 105–107 °C; $[\alpha]_D^{25}$ +16.3° (*c* 0.71, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.10–7.24 (m, 15 H, H–OBz), 5.77 (dd, 1 H, 3.5, 10.0 Hz, H-3″), 5.67 (t, 1 H, 10.0 Hz, H-4″), 5.56, 5.44 (d, 1 H, 4.5 Hz, H-6), 5.42 (dd, 1 H, 1.5, 3.5 Hz, H-2″), 5.39 (t, 1 H, 9.5 Hz, H-4‴), 5.21 (dd, 1 H, 1.5, 3.5 Hz, H-2‴), 5.19 (d, 1 H, 1.5 Hz, H-1″), 5.18 (dd, 1 H, 3.5, 10.0 Hz, H-3‴), 5.04 (t, 1 H, 10.0 Hz, H-3′), 4.87 (d, 1 H, 1.5 Hz, H-1‴), 4.73 (m, 1 H, H-5‴), 4.63 (d, 1 H, 7.5 Hz, H-1′), 4.42 (m, 1 H, H-16), 3.93–3.86 (m, 3 H, H-6′a,b, H-5″), 3.80 (t, 1 H, 9.5 Hz, H-4′), 3.65 (m, 1 H, H-3), 3.62 (dd, 1 H, 9.5, 7.5 Hz, H-2′), 3.47 (m, 1 H, H-5′), 3.42–3.36 (m, 2 H, H-26a,b), 2.24, 2.11, 2.04, 1.97 (s each, 3 H each, H–OAc), 1.32 (d, 3 H, 6.0 Hz, H-6″), 1.18 (d, 3 H, 6.5 Hz, H-6‴), 0.97 (d, 3 H, 7.0 Hz), 0.89 (s, 3 H), 0.86 (s, 9 H, H–*t*-Bu), 0.79 (d, 3 H, 6.0 Hz), 0.77 (s, 3 H), 0.08, 0.05 (s each, 3 H each, H–Si–CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.4, 170.0, 169.9, 169.8, 165.7, 165.4, 165.3, 140.3, 133.4, 133.2, 133.0, 129.9 (2C), 129.8 (2C), 129.7 (2C), 129.4, 129.3, 129.2, 128.6 (2C), 128.3 (2C), 128.2 (2C), 121.9, 109.3, 99.6, 99.4, 99.1, 97.4, 91.9, 80.8, 78.6, 75.4, 75.0, 73.0, 71.9, 71.2, 70.7, 69.8, 69.7, 69.0, 67.5, 66.8, 66.6, 62.1, 61.7, 56.4, 50.0, 41.6, 40.2, 39.7, 38.6, 37.2, 36.8, 32.1, 31.8, 31.5, 31.4, 30.3, 29.8, 29.7, 25.8 (3C), 21.4, 20.9, 20.8, 20.7, 20.6, 20.5, 19.2, 18.3, 17.4, 17.3, 17.1, 16.2, 14.5, −5.2, −5.6; FABMS (*m*/*z*): 1485.5 [M + Na]; Anal. Calcd for $C_{80}H_{106}O_{23}Si$: C, 65.64; H, 7.30. Found: C, 65.31; H, 7.10.

3.14. Preparation of diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside (dioscin, 1)

To a solution of **17** (450 mg, 31 μmol) in THF (10 mL) was added tetrabutylammonium flouride (TBAF, 300 mg, 11 mmol) at rt. The mixture was stirred for 6 h, neutralized with Et_3N and filtered, and then the filtrate was concentrated. Purification of the product by column chromatography (3:1 petroleum ether–EtOAc) gave diosgenyl 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1

→4)]-3-*O*-acetyl-β-D-glucopyranoside as a white solid. This product was redissolved in 1:1 MeOH–CH_2Cl_2 (5 mL) at rt, and MeONa (0.05 mL 1 M in MeOH) was then added. The mixture was stirred overnight, neutralized with Dowex-50 (H^+) resin and filtered, and the filtrate was then concentrated. Purification of the product by column chromatography (10:1→8:1 CH_2Cl_2–MeOH) afforded **1** (228 mg, 83%) as a white solid: mp >250 °C, lit.[2] 275–277 °C; $[\alpha]_D^{25}$ −116.5° (*c* 1.0, MeOH), lit.[2] −121° (*c* 1.0, MeOH); ^{13}C NMR (125 MHz, Py-d_5): δ 140.8, 121.8, 109.3, 102.9, 102.0, 100.3, 81.1, 78.6, 78.1, 78.0, 77.8, 77.0, 74.1, 73.9, 72.9, 72.8, 72.6, 70.4, 69.5, 66.9, 62.9, 61.5, 61.3, 50.3, 42.0, 40.5, 39.9, 39.0, 37.5, 37.2, 32.3, 32.2, 31.8, 31.7, 30.6, 30.2, 30.0, 29.3, 21.1, 19.4, 18.7, 18.5, 17.3, 16.4, 15.0. FABMS (*m*/*z*): 869 [M + 1], 723, 631, 415, 397.

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