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Synthesis of diosgenyl α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside (gracillin) [1] and related saponins

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Abstract

Diosgenyl α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside (gracillin), a monodesmosidic saponin isolated from *paris*, *dioscorea*, and *costacea* species with promising cardiovascular and antitumor activities, was synthesized by stepwise glycosylation.

Keywords: Diosgenyl α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside (gracillin); Diosgenyl α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside; Diosgenyl β-D-glucopyranoside (trillin); Saponin; Synthesis

1. Introduction

Saponins constitute a structurally and biologically diverse class of molecules that are widely distributed in terrestrial plant and some marine organisms [1]. The extreme difficulties associated with the purification of closely related saponins from a natural source provides synthesis with a realistic opportunity to contribute to the availability of homogeneous saponins. Herein, we report the syntheses of a structurally typical diosgenyl glycoside, diosgenyl α-L-rhamnopyranosyl-(1 → 2)-[β-D-glucopyranosyl-(1 → 3)]-β-D-glucopyranoside (gracillin). Gracillin was isolated from *paris* [2,3], *dioscorea* [4–6], and *costacea* species [7], the former two species are widely used in China as folk drugs [8], while the later species have various pharmacological usages in India [7]. The pharmacological activity of gracillin itself was also determined to have promising cardiovascular and antitumor activities [2–4].

2. Results and discussion

There are two basic strategies to construct a monodesmosidic saponin. One is to connect the first

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[1] The chemical abstracts index name for gracillin [19083-00-2] is as follows: (3β, 25*R*)-spirost-5-en-3-yl 6-deoxyl-α-L-mannopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranoside

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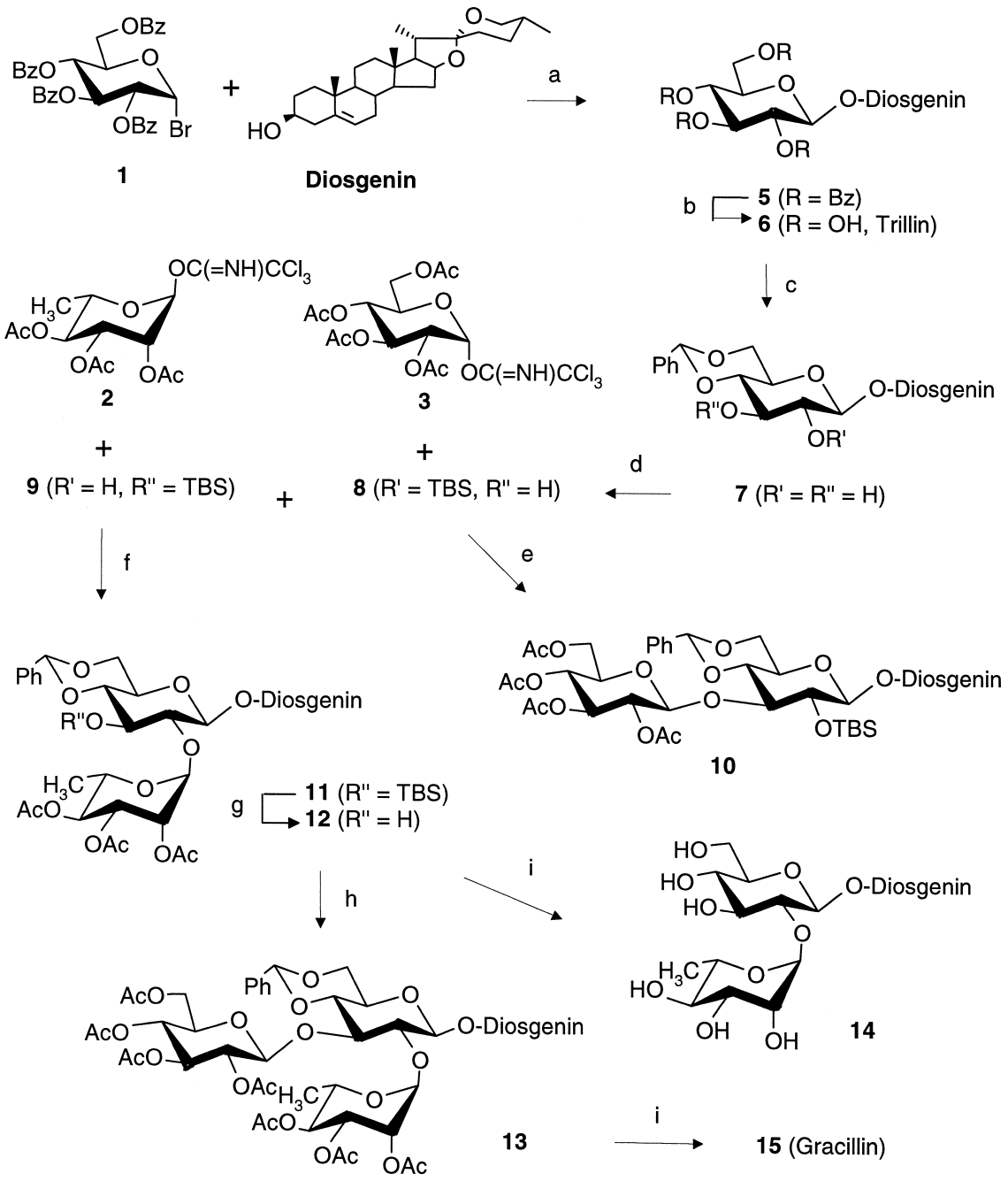
monosaccharide unit to the aglycone first, then manipulate the protecting group on the sugar moiety and extend the sugar chain sequentially. The second strategy is to prepare a suitably protected and activated oligosaccharide donor first, then attach it to the aglycone. Employing the first strategy, the formation of



4. Reagents and conditions

(a) AgOTf (1.1 equiv.), CH_2Cl_2, −20 °C → r.t., 2 h, 73%; (b) NaOMe, MeOH, CH_2Cl_2, overnight, 88%; (c) $PhCH(OMe)_2$, CSA, DMF, 50 °C, 2 h, 89%; (d) TBDMSCl (1.5 equiv.), imidazole (2.3 equiv.), 40–50 °C, 3 h, **8** (34.8%), **9** (52.2%); (e) **3** (2.3 equiv.), borontrifluoride ether complex (1.1 equiv.), 4 Å MS, CH_2Cl_2, −78 °C → r.t., 37%; (f) **2** (2.8 equiv.), borontrifluoride ether complex (1.9 equiv.), 4 Å MS, CH_2Cl_2, −78 °C → r.t., 100%; (g) TBAF (0.5 M), THF, r.t., 5 h, 84%; (h) **3** (3.5 equiv.), borontrifluoride ether complex (2.3 equiv.), 4 Å MS, CH_2Cl_2, −78 °C → r.t., 84%; (i) HOAc (80%), 70 °C, 2 h; NaOMe, MeOH, CH_2Cl_2, r.t., overnight, **14** (77%), **15** (79%).

Scheme 1.

the glycosidic bond between the sugar and aglycone can be sought to be stereospecific and in high yield, and many derivatives with the same aglycone and different sugar chains can be obtained by the way to the final target. Herein, gracillin was synthesized as shown in Scheme 1.

Diosgenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranoside (**5**) was prepared stereospecifically and in good yield by using 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**1**) [9] as the glycosyl donor. In the glycosylation of a steroid, the protecting group on the glycosyl donor is essential. Glucosylation of diosgenin using 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide as the glycosyl donor resulted in very low yield of glycosylation product, owing to the two main side reactions: acetyl transfer and orthoester formation [10,11], both of which have been reported to be suppressed by replacing the acetyl protecting group with the bulky pivaloyl group [12,13]. Herein, the benzoyl group was successfully used to serve the same purpose. On the other hand, promotion of the reaction is another important fact in the above glycosylation reaction [10,11]. In our case, using AgOTf as the promoter gave **5** in good yield (73%), and only trace of orthoester **4** (α-D-glucopyranosyl 3,4,6-tri-*O*-benzoyl-1,2-diosgenyl orthophenanoate) was detected. On the contrary, a trace of **5** and 80% of orthoester **4** were produced using AgOTf–collidine as the promoter. By using silver carbonate as the promoter, a 48% yield of **5** and a 12% yield of orthoester **4** were furnished. Treatment of **5** with NaOMe in MeOH gave trillin (**6**) [14], which was converted to diol **7** by protection of the 4′,6′-OH groups with an *O*-benzylidene group. The regioselective protection of the 2′-OH and 3′-OH of **7** with a *tert*-butyldimethylsilyl (TBDMS) group was found to be very difficult, although Nicolaou et al. [15] achieved a 88% yield in putting a TBDMS group on the 3-OH of ethyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside. Under the similar conditions [(*t*-$BuMe_2SiCl$ (1.3 equiv), imidazole (1.6 equiv), DMF, 0 °C, 12 h)], only half of **7** was detected to be consumed to produce a mixture of **8** and **9** in the ratio of 1:2. Therefore, the reaction temperature was raised to 40–50 °C, so that the reaction was completed in 3 h, and a 97% yield of the monosilylated products **8** and **9** was obtained in the ratio of 1:1.5. Compound **9** was found to be easy to precipitate from the mixture of **8** and **9** in petroleum ether, and the remaining mixture was further separated by careful chromatography on a silica gel column. The ^1H–^1H COSY spectrum of the acetylated compound of **9** (diosgenyl 2-*O*-acetyl-4,6-*O*-benzylidene-3-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside) showed a downfield signal for H-2′ (4.93 ppm, t, *J* 8.6 Hz). Both **8** and **9** were used in the following reactions. Compound **9** having its 2′-OH free was glycosylated by 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate (**2**) [16] in the presence of borontrifluoride ether complex to quantitatively provide **11**. Desilylation of **11** under tetrabutylammonium fluoride (TBAF) gave **12** (84%) with 3′-OH free, which was treated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **3** [17] in the presence of borontrifluoride ether–complex to furnish the diosgenyl trisaccharide **13** in 84% yield. The three anomeric configurations of **13** were confirmed by the ^1H–^1H COSY spectrum. On the other hand, treatment of 3′-OH free **8** with the glucosyl imidate **3** under borontrifluoride ether complex gave the expected glycosylation product **10** only in 37% yield, together with 19% of the acetyl-transfer product (diosgenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside) and 14% of recovered **8**, providing the stereoelectronic effect of the substrate **8** [18]. Finally, debenzylidenation of **12** and **13** under 80% HOAc, followed by deacetylation under NaOMe in MeOH, furnished the naturally existing saponin **14** [19] and **15** (gracillin). The physical data for both compounds were in accord with those reported.

3. Experimental

General methods.—Solvents were purified in the usual way, and melting points uncorrected. Optical rotations were determined with a Perkin-Elmer Model 241 MC polarimeter at ambient temperature. TLCs were performed on precoated plates of Silica Gel HF_{254} (0.5 mm, Qingdao, China). Flash column chromatography was performed on Silica Gel H (10–40 μ, Qingdao, China). ^1H NMR spectra were recorded on a Bruker AM 300, AM 400 or AM 600 spectrometer with Me_4Si as the internal standard. IR spectra were recorded with a FTS-185 spectrometer. Mass spectra were obtained on a HP5989A or a VG Quattro mass spectrometer. Elemental analyses were performed on a Perkin-Elmer Model 2400 instrument.

α-D-glucopyranosyl 3,4,6-tri-O-benzoyl-1,2-diosgenyl orthophenanoate (**4**).—To a mixture of **1** (1.0 g, 1.52 mmol), diosgenin (0.67 g, 1.62 mmol), and 4 Å molecular sieves (MS, 2.0 g) in anhydrous CH_2Cl_2 (15 mL) at −20 °C under N_2, was added

2,4,6-collidine (0.22 mL, 1.65 mmol), then AgOTf (0.43 g, 1.67 mmol) in dry toluene (5 mL). The resulting solution, kept at room temperature overnight, was diluted with CH_2Cl_2 (30 mL), and filtered. The filtrates, which were washed with brine, saturated $NaHCO_4$, and brine, were dried with anhydrous $MgSO_4$ and concentrated. Chromatography of the residue on a silica gel column (10:1 petroleum ether–EtOAc) afforded **4** as a white solid (1.2 g, 80%): mp 120–122 °C; $[\alpha]_D^{18}$ −48.8° (*c* 1.0, $CHCl_3$); R_f 0.47 (5:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11–7.23 (m, 20 H), 6.05 (d, 1 H, $J_{1',2'}$ 5.2 Hz, H-1′), 5.78 (d, 1 H, $J_{2',3'}$ 2.2 Hz, H-3′), 5.49 (d, 1 H, $J_{4',5'}$ 8.8 Hz, H-4′), 5.26 (d, 1 H, *J* 4.8 Hz, H-6), 4.79 (dd, 1 H, H-2′), 4.50 (dd, 1 H, $J_{5',6'a}$ 2.9, $J_{6'a,6'b}$ 12.2 Hz, H-6′a), 4.42–4.31 (m, 2 H, H-6′b, H-16), 4.09 (m, 1 H, H-5′), 3.48–3.26 (m, 3 H, H-3, H-26), 0.93 (s, 3 H), 0.85 (d, 3 H, *J* 4.7 Hz), 0.78 (d, 3 H, *J* 6.5 Hz), 0.75 (s, 3 H); EIMS (*m/z* %): 993 (M^+, 0.02), 579 (28.9), 397 (6.1), 335 (8.4), 282 (9.9), 253 (5.8), 231 (51.2), 139 (23.3), 105 (100.0), 77 (18.2); Anal. Calcd. for $C_{61}H_{68}O_{12}$: C, 73.77; H, 6.90. Found: C, 73.74; H, 7.02.

Diosgenyl 2, 3, 4, 6 - tetra - O - benzoyl - β - D - glucopyranoside (**5**).—To a mixture of **1** (7.8 g, 11.8 mmol), diosgenin (5.2 g, 12.5 mmol), and 4 Å MS (15 g) in anhydrous CH_2Cl_2 (150 mL) at −20 °C under N_2, was added AgOTf (3.35 g, 13.0 mmol) in dry toluene (30 mL). The resulting solution, kept at this temperature for 4 h, was diluted with CH_2Cl_2 (300 mL), and filtered. The filtrates, which were washed with brine, saturated $NaHCO_4$, and brine, were dried over anhydrous $MgSO_4$ and concentrated. Chromatography of the residue on a silica gel column (20:1 petroleum ether–EtOAc, then 20:1 toluene–EtOAc) gave **5** as a white solid (8.6 g, 73%): mp 195–196 °C; $[\alpha]_D^{24}$ −20.3° (*c* 1.0, $CHCl_3$); R_f 0.38 (5:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02–7.26 (m, 20 H), 5.90 (t, 1 H, $J_{3',4'}$ 9.6 Hz, H-3′), 5.63 (t, 1 H, $J_{4',5'}$ 9.7 Hz, H-4′), 5.50 (dd, 1 H, $J_{2',3'}$ 9.7 Hz, H-2′), 5.22 (d, 1 H, *J* 4.9 Hz, H-6), 4.94 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.63–4.49 (m, 2 H, H-6′), 4.40 (m, 1 H, H-16), 4.18–4.12 (m, 1 H, H-5′), 3.56–3.33 (m, 3 H, H-26, H-3), 2.15 (m, 2 H), 0.97 (d, 3 H, *J* 6.79 Hz), 0.90 (s, 3 H), 0.79 (d, 3 H, *J* 6.3 Hz), 0.76 (s, 3 H); EIMS (*m/z* %): 579 (1.7), 396 (4.9), 282 (9.1), 253 (5.6), 231 (5.8), 139 (12.9), 106 (9.1), 105 (100.0), 77 (13.5); Anal. Calcd. for $C_{61}H_{68}O_{12}$: C, 73.77; H, 6.90. Found: C, 73.34; H, 6.94.

*Diosgenyl β-D-glucopyranoside (**6**, trillin).*—To a solution of **5** (8.6 g, 8.66 mmol) in 1:1 MeOH–CH_2Cl_2 (100 mL), was added NaOMe–MeOH (0.1 N, 8 mL). The solution, after refluxing for 6 h, was cooled to room temperature, was then neutralized with Dowex-50 (H^+) resin. The mixture was filtered and concentrated to give a residue, which was washed with EtOAc to give trillin as a white solid (4.28 g, 86%): mp > 210 °C, lit. 263–264 °C [14]; $[\alpha]_D^{24}$ −83.8° (*c* 1.0, pyridine), lit. −94.3° (*c* 1.5, $CHCl_3$) [14]; R_f 0.27 (10:1 CH_2Cl_2–MeOH); IR (KBr); ν 3600–3200, 983, 962, 921 < 900 cm^{-1}, ^{1}H NMR (300 MHz, pyridine-d_5): δ 5.38 (d, 1 H, *J* 4.8 Hz, H-6), 5.14 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.68 (m, 2 H, H-2′, H-3′), 4.51 (dd, 1 H, $J_{6'a,5'}$ 5.1 Hz, $J_{6'a,6'b}$ 11.7 Hz, H-6′a), 4.43–4.35 (m, 2 H, H-6′b, H-16), 4.16 (t, 1 H, $J_{4',5'}$ 7.9 Hz, H-4′), 4.07–3.99 (m, 2 H, H-5′, H-3), 3.68–3.57 (m, 2 H, H-26), 2.80 (m, 1 H), 2.52 (m, 1 H), 1.21 (d, *J* 6.9 Hz, 3 H), 0.97 (s, 3 H), 0.90 (s, 3 H), 0.76 (d, 3 H, *J* 5.3 Hz).

Diosgenyl 4,6-O-benzylidene-β-D-glucopyranoside (**7**).—To a solution of **6** (1.0 g, 1.7 mmol) and $PhCH(OMe)_2$ (0.3 mL, 2.0 mmol) in dry DMF (30 mL), was added camphorsulfonic acid (CSA, until the pH of the solution reached pH 2–3). The resulting mixture, stirred at 40 °C under reduced pressure for 3 h, was neutralized by Et_3N (1.0 mL), diluted with EtOAc (50 mL), then washed with brine, dried over anhydrous $MgSO_4$, and concentrated. Chromatography of the residue on a silica gel column (3:1 toluene–EtOAc) afforded **7** as a white solid (1.02 g, 89%): mp 192–193 °C; $[\alpha]_D^{18}$ −99.8° (*c* 1.0, $CHCl_3$); R_f 0.25 (2:1 toluene–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51–7.36 (m, 5 H), 5.53 (s, 1 H, PhCH), 5.38 (d, 1 H, *J* 5.1 Hz, H-6), 4.51 (d, 1 H, $J_{1',2'}$ 7.7 Hz, H-1′), 4.41 (m, 1 H, H-16), 4.32 (dd, 1 H, $J_{6'a,5'}$ 3.9 Hz, $J_{6'a,6'b}$ 10.5 Hz, H-6′a), 3.83 (t, 1 H, $J_{3',4'}$ 9.0 Hz, H-3′), 3.79 (t, 1 H, $J_{6'b,5'}$ 10.3 Hz, H-6′b), 3.63–3.34 (m, 6 H, H-2′, H-4′, H-5′, H-26, H-3), 1.02 (s, 3 H), 0.97 (d, 3 H, *J* 7.0 Hz), 0.79 (d, 3 H, *J* 6.1 Hz), 0.79 (s, 3 H); EIMS (*m/z* %): 664 (M^+, 0.3), 397 (26.3), 283 (34.6), 282 (65.7), 253 (30.0), 139 (100.0), 91 (20.5), 69 (26.7); Anal. Calcd. for $C_{40}H_{56}O_8$: C, 72.26; H, 8.49. Found: C, 71.97; H, 8.91.

Diosgenyl 4,6-O-benzylidene-2-O-tert-butyldimethylsilyl-β-D-glucopyranoside (**8**) *and diosgenyl 4,6-O-benzylidene - 3 - O - tert - butyldimethylsilyl - β - D - glucopyranoside* (**9**).—To a solution of **7** (1.12 g, 1.68 mmol) in dry DMF (40 mL) was added imidazole (260 mg, 3.86 mmol) and *tert*-butylchlorodimethylsilane (TBDMSCl 380 mg, 2.52 mmol). The mixture, stirred at 40–50 °C under N_2 for 3 h, was then diluted with EtOAc, washed with brine, dried

over $MgSO_4$, and concentrated. Chromatography of the residue on a silica gel column (20:1 petroleum ether–EtOAc) afforded **8** and **9** as a white solid (1.27 g, 97%, **8**:**9** 1:1.5). The solid was dissolved in petroleum ether, and after most of **9** was precipitated, the solution was concentrated and chromatographed on a silica gel column (30:1 petroleum ether–EtOAc). **8**: mp 138–140 °C; $[\alpha]_D^{18}$ −84.6° (*c* 1.0, $CHCl_3$); R_f 0.40 (8:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.35 (m, 5 H), 5.52 (s, 1 H, PhCH), 5.37 (d, 1 H, *J* 4.8 Hz, H-6), 4.50 (d, 1 H, $J_{1',2'}$ 7.5 Hz, H-1′), 4.41 (m, 1 H, H-16), 4.32 (dd, 1 H, $J_{6'a,5'}$ 4.8 Hz, $J_{6'a,6'b}$ 10.3 Hz, H-6′a), 3.77 (t, 1 H, H-6′b), 3.74 (t, 1 H, $J_{3',4'}$ 8.9 Hz, H-3′), 3.58–3.37 (m, 6 H, H-2′, H-4′, H-5′, H-26, H-3), 1.02 (s, 3 H), 0.97 (d, 3 H, *J* 6.9 Hz), 0.91 (s, 9 H), 0.79 (d, 3 H, *J* 3.3 Hz), 0.79 (s, 3 H), 0.15 (s, 3 H), 0.14 (s, 3 H); EIMS (*m/z* %): 778 (M − 1, 0.15), 722 (0.3), 397 (100), 379 (21.1), 283 (37.5), 271 (22.0), 253 (59.2), 215 (10.5), 161 (25.9), 149 (41.4), 139 (29.1), 121 (15.2), 105 (14.8), 69 (19.4); Anal. Calcd. for $C_{46}H_{70}O_8Si$: C, 70.91; H, 9.06. Found: C, 70.62; H, 9.05. **9**: mp 123–125 °C; $[\alpha]_D^{20}$ −81.7° (*c* 1.0, $CHCl_3$); R_f 0.44 (8:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49–7.33 (m, 5 H), 5.49 (s, 1 H, PhCH), 5.38 (d, 1 H, H-6), 4.49 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.40 (m, 1 H, H-16), 4.30 (dd, 1 H, $J_{6'a,5'}$ 4.8 Hz, $J_{6'a,6'b}$ 10.5 Hz, H-6′a), 3.80–3.70 (m, 2 H, H-6′b, H-3′), 3.64–3.33 (m, 6 H, H-2′, H-4′, H-5′, H-26, H-3), 1.02 (s, 3 H), 0.96 (d, 3 H, *J* 6.9 Hz), 0.86 (s, 9 H), 0.78 (d, 3 H, *J* 3.8 Hz), 0.78 (s, 3 H), 0.09 (s, 3 H), 0.02 (s, 3 H); EIMS (*m/z* %): 779 (M^+, 0.15), 722 (1.1), 397 (100), 379 (16.6), 283 (37.6), 271(21.6), 253 (49.5), 149 (19.4), 139 (28.4), 105 (12.7), 69 (17.1); Anal. Calcd. for $C_{46}H_{70}O_8Si$: C, 70.91; H, 9.06. Found: C, 70.45; H, 8.99.

*Diosgenyl 2, 3, 4, 6 - tetra - O - acetyl - β - D - glucopyranosyl-(1 → 3)-4,6-*O*-benzylidene-2-*O*-tert-butyldimethylsilyl-β-D-glucopyranoside* (**10**).—To a solution of **8** (360 mg, 0.462 mmol) and 4 Å MS (0.6 g) in dry CH_2Cl_2 (15 mL) at −78 °C under Ar, was added borontrifluoride–ether complex (0.07 mL, 0.57 mmol), followed by a solution of **3** (520 mg, 1.06 mmol) in CH_2Cl_2 (4 mL). The mixture, warmed up naturally to room temperature and allowed to stir for another 7 h, was then neutralized with Et_3N (0.1 mL), filtered, and concentrated. The residue was chromatographed on a silica gel column (30:1 → 4:1 petroleum ether–EtOAc) to afford **10** as a white solid (190 mg, 37%), together with diosgenyl 3-*O*-acetyl-4,6-*O*-benzylidene-2-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranoside (70 mg, 19%), and recovered **8** (52 mg, 14%). **10**: mp 126–128 °C; $[\alpha]_D^{23}$ −70.0° (*c* 1.0, $CHCl_3$); R_f 0.27 (4:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.47–7.39 (m, 5 H), 5.53 (s, 1 H, PhCH), 5.35 (d, 1 H, *J* 4.3 Hz, H-6), 5.07–5.01 (m, 3 H, H-3″, H-4″, H-1″), 4.89 (t, 1 H, $J_{1'',2''}$ 9.2 Hz, $J_{2'',3''}$ 9.2 Hz, H-2″), 4.47 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.41 (m, 1 H, H-16), 4.31 (dd, 1 H, $J_{6'a,5'}$ 4.8 Hz, $J_{6'a,6'b}$ 10.5 Hz, H-6′a), 4.05 (dd, 1 H, $J_{6''a,5''}$ 4.4 Hz, $J_{6''a,6''b}$ 12.4 Hz, H-6″a), 3.96–3.90 (m, 2 H, H-3′, H-6″b), 3.82–3.74 (m, 2 H, H-5″, H-6′b), 3.62–3.54 (m, 2 H, H-2′, H-4′), 3.48–3.28 (m, 4 H, H-5′, H-26, H-3), 2.05, 1.98, 1.97, 1.95 (4 s, 12 H), 1.01 (s, 3 H), 0.97 (d, 3 H, *J* 6.9 Hz), 0.89 (s, 9 H), 0.79 (d, 3 H, *J* 3.6 Hz), 0.79 (s, 3 H), 0.12 (s, 3 H), 0.09 (s, 3 H); EIMS (*m/z* %): 712 (2.0), 397 (72.1), 331 (53.9), 283 (20.5), 253 (38.1), 169 (100.0), 109 (30.8); Anal. Calcd. for $C_{60}H_{88}O_{17}Si$: C, 64.96; H, 8.00. Found: C, 64.83; H, 8.18.

*Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4,6-*O*-benzylidene-3-*O*-tert-butyldimethylsilyl-β-D-glucopyranoside* (**11**).—To a solution of **9** (1.33 g, 1.71 mmol) and 4 Å MS (2.0 g) in dry CH_2Cl_2 (30 mL) at −78 °C under Ar, was added borontrifluoride–ether complex (0.4 mL, 3.25 mmol), then a solution of **2** (2.1 g, 4.83 mmol) in CH_2Cl_2 (10 mL). The mixture, warmed up naturally to room temperature and allowed to stir for another 3 h, was then neutralized with Et_3N (0.5 mL), filtered, and concentrated. The residue was chromatographed on a silica gel column (10:1 → 8:1 petroleum ether–EtOAc) to afford **11** as a white solid (1.8 g, 100%): mp > 210 °C; $[\alpha]_D^{22}$ −87.6° (*c* 1.0, $CHCl_3$); R_f 0.22 (8:1 petroleum ether–EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.55–7.44 (m, 5 H), 5.49 (s, 1 H, PhCH), 5.49 (m, 2 H, H-6, H-2′), 5.39 (d, 1 H, $J_{1'',2''}$ 0.9 Hz, H-1″), 5.29 (dd, 1 H, $J_{3'',2''}$ 3.3 Hz, $J_{3'',4''}$ 10.1 Hz, H-3″), 5.15 (t, 1 H, H-4″), 4.72–4.68 (m, 2 H, $J_{1',2'}$ 8.1 Hz, H-5″, H-1′), 4.50 (m, 1 H, H-16), 4.38 (dd, 1 H, $J_{6'a,5'}$ 4.0 Hz, $J_{6'a,6'b}$ 10.7 Hz, H-6′a), 4.05 (t, 1 H, $J_{3',2'}$ 8.7 Hz, H-3′), 3.82–3.73 (m, 3 H, H-2′, H-6′b, H-3), 3.55–3.46 (m, 4 H, H-4′, H-5′, H-26), 2.20, 2.11, 2.05 (3 s, 9 H), 1.30 (d, *J* 6.2 Hz, CH_3-6″), 1.12 (s, 3 H), 1.06 (d, 3 H, *J* 6.9 Hz), 0.88 (s, 3 H), 0.88 (d, 3 H, *J* 4.2 Hz), 0.82 (s, 9 H), 0.02 (s, 3 H), 0.00 (s, 3 H); FABMS (*m/z* %): 1051 (M^+, 3.5), 1049 (M − 1, 3.5), 637 (3.5), 531 (7.5), 397 (100.0), 273 (70.0), 171 (29.0), 139 (50.0), 110 (73.5); Anal. Calcd. for $C_{58}H_{86}O_{15}Si$: C, 66.26; H, 8.25. Found: C, 66.24; H, 8.40.

*Diosgenyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4,6-*O*-benzylidene-β-D-glucopyranoside* (**12**).—To a solution of **11** (1.79 g, 1.7 mmol) in dry THF

(10 mL) was added a solution of BU_4NF in THF (1.4 g, 5.35 mmol, 0.5 M). The mixture, which was stirred at room temperature for 5.5 h, was diluted with EtOAc, washed with brine, dried over anhydrous $MgSO_4$, and concentrated. The residue was chromatographed on a silica gel column (5:1 → 3:1 petroleum ether–EtOAc) to give **12** as a white solid (1.34 g, 84%): mp 169–171 °C; $[\alpha]_D^{22}$ −94.8° (*c* 1.0, $CHCl_3$); R_f 0.21 (3:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.37 (m, 5 H), 5.50 (s, 1 H, PhCH), 5.41 (d, 1 H, *J* 4.9 Hz, H-6), 5.35 (d, 1 H, $J_{2'',1''}$ 1.7 Hz, H-1″), 5.34 (dd, 1 H, $J_{2'',3''}$ 4.8 Hz, H-2″), 5.27 (dd, 1 H, $J_{3'',4''}$ 10.1 Hz, H-3″), 5.08 (t, 1 H, H-4″), 4.61 (d, 1 H, $J_{1',2'}$ 7.6 Hz, H-1′), 4.48–4.37 (m, 2 H, H-5″, H-16), 4.32 (dd, 1 H, $J_{6'a,5'}$ 4.7 Hz, $J_{6'a,6'b}$ 10.1 Hz, H-6′a), 3.93 (t, 1 H, $J_{3',2'}$ 8.9 Hz, H-3′), 3.75 (t, 1 H, H-6′b), 3.67 (t, 1 H, $J_{2',3'}$ 8.3 Hz, H-2′), 3.72–3.56 (m, 1 H, H-3), 3.52–3.33 (m, 4 H, H-4′, H-5′, H-26), 2.13, 2.03, 1.98 (3 s, 9 H), 1.21 (d, *J* 6.3 Hz, CH_3-6″), 1.02 (s, 3 H), 0.97 (d, 3 H, *J* 6.8 Hz), 0.79 (d, 3 H, *J* 4.0 Hz), 0.78 (s, 3 H); FABMS (*m/z* %): 938 (M + 1, 3.5), 936 (M − 1, 3.5), 523 (3.5), 397 (57.0), 273 (48.0), 253 (29.0), 171 (30.0), 153 (66.0), 111 (100.0); Anal. Calcd. for $C_{52}H_{72}O_{15}$: C, 66.65; H, 7.74. Found: C, 66.80; H, 8.12.

Diosgenyl 2,3,4-tri-O-*acetyl*-α-L-*rhamnopyranosyl*-*(1 → 2)*-*[(2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl)*-*(1 → 3)]*-*4,6*-O-*benzylidene*-β-D-*glucopyranoside* (**13**). —To a solution of **12** (232 mg, 0.248 mmol) and 4 Å MS (0.5 g) in dry CH_2Cl_2 (10 mL) at −78 °C under Ar, was added borontrifluoride–ether complex (0.07 mL, 0.57 mmol), then a solution of **3** (370 mg, 0.751 mmol) in CH_2Cl_2 (3.7 mL). The mixture, warmed up naturally to room temperature and allowed to stir for another 7 h, was then neutralized with Et_3N (0.25 mL), filtered, and concentrated. The residue was chromatographed on a silica gel column (3:1 → 2:1 petroleum ether–EtOAc) to afford **13** as a white solid (264 mg, 84%): mp 136–138 °C; $[\alpha]_D^{22}$ −73.9° (*c* 1.0, $CHCl_3$); R_f 0.26 (2:1 petroleum ether–EtOAc); ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.47–7.38 (m, 5 H), 5.50 (s, 1 H, PhCH), 5.42 (d, 1 H, H-6), 5.24 (bs, 2 H, H-1″, H-2″), 5.20 (dd, 1 H, $J_{3'',2''}$ 3.0 Hz, $J_{3'',4''}$ 10.2 Hz, H-3″), 5.14 (t, 1 H, $J_{3''',2'''}$ 9.6 Hz, H-3‴), 5.09 (t, 1 H, H-4″), 5.02 (t, 1 H, H-4‴), 4.93 (t, 1 H, H-2‴), 4.82 (d, 1 H, $J_{1''',2'''}$ 8.4 Hz, H-1‴), 4.60 (d, 1 H, $J_{1',2'}$ 7.8 Hz, H-1′), 4.55–4.52 (m, 1 H, H-5″), 4.42 (m, 1 H, H-16), 4.30 (dd, 1 H, $J_{6'a,5'}$ 4.8 Hz, $J_{6'a,6'b}$ 10.2 Hz, H-6′a), 4.09–4.06 (m, 2 H, H-3′, H-6‴a), 3.96 (dd, 1 H, H-6‴b), 3.80–3.76 (m, 2 H, H-2′, H-6′b), 3.69 (t, 1 H, $J_{4',3'}$ 9.3 Hz, H-4′), 3.67–3.61 (m, 1 H, H-3), 3.49–3.36 (m, 4 H, H-5′, H-5‴, H-26), 2.20, 2.03, 2.02, 2.00, 1.99, 1.97, 1.95 (7 s, 21 H), 1.19 (d, *J* 6.3 Hz, CH_3-6″), 1.03 (s, 3 H), 0.98 (d, 3 H, *J* 7.2 Hz), 0.80 (d, 3 H, *J* 6.0 Hz), 0.79 (s, 3 H); FABMS (*m/z* %): 1267 (M^+, 11.5), 1265 (5.5), 1147 (2.5), 853 (6.5), 397 (52.0), 331 (28.5), 273 (55.0), 253 (45.5), 171 (51.0), 169 (81.0), 153 (100.0); Anal. Calcd. for $C_{66}H_{90}O_{24}$: C, 62.55; H, 7.16. Found: C, 62.65; H, 7.39.

Diosgenyl α-L-*rhamnopyranosyl*-*(1 → 2)*-β-D-*glucopyranoside* (**14**).—A solution of **12** (118 mg, 0.126 mmol) in 80% HOAc (10 mL) was stirred at 70 °C for 2 h. The solvent was removed under vacuum to give a residue, which was dissolved in a solution of 1:1 MeOH–CH_2Cl_2 (4 mL) containing NaOMe (0.01 N). The solution, kept overnight at room temperature, was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated. Chromatography of the residue on a silica gel column (10:1 CH_2Cl_2–MeOH) gave **14** as a white solid (70 mg, 77%): mp 211–213 °C; $[\alpha]_D^{24}$ −95.6° (*c* 1.0, pyridine), lit. −94.3° (*c* 0.72) [19]; R_f 0.30 (6:1 CH_2Cl_2–MeOH); ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.49 (s, 1 H, H-1″), 5.38 (d, 1 H, *J* 4.6 Hz, H-6), 5.15 (d, 1 H, $J_{1',2'}$ 7.2 Hz, H-1′), 5.12 (m, 1 H, H-3″), 4.90 (dd, 1 H, *J* 1.8, 3.2 Hz, H-2″), 4.74 (dd, 1 H, *J* 3.4, 9.4 Hz, H-6′a), 4.63 (m, 2 H, H-4″, H-6′b), 4.48–4.37 (m, 4 H, H-5″, H-2′, H-16, H-3′), 4.27 (t, 1 H, $J_{4',5'}$ 9.1 Hz, H-4′), 4.03 (m, 2 H, H-3, H-5′), 3.62 (m, 2 H, H-26), 2.85 (m, 2 H), 1.87 (d, 3 H, $J_{6'',5''}$ 6.1 Hz, H-6″), 1.21 (d, 3 H, *J* 6.9 Hz), 1.13 (s, 3 H), 0.90 (s, 3 H), 0.76 (d, 3 H, *J* 5.3 Hz); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.4, 122.3, 109.8, 102.6, 100.9, 81.6, 80.2, 78.8, 78.5, 78.4, 74.7, 73.4, 73.1, 72.4, 70.0, 67.4, 63.4, 63.2, 57.2, 50.8, 42.5, 41.0, 40.4, 39.5, 38.0, 37.7, 32.8, 32.4, 32.2, 31.1, 30.7, 29.8, 21.6, 19.9, 19.2, 17.8, 16.8, 15.5.

Diosgenyl α-L-*rhamnopyranosyl*-*(1 → 2)*-*[(*β-D-*glucopyranosyl)*-*(1 → 3)]*-β-D-*glucopyranoside* (**15**, gracillin).—A solution of **13** (190 mg, 0.15 mmol) in 80% HOAc (15 mL) was stirred at 70 °C for 2 h. The solvent was removed under vacuum to give a residue, which was dissolved in a solution of 1:1 MeOH–CH_2Cl_2 (8 mL) containing NaOMe (0.01 N). The solution, kept overnight at room temperature, was neutralized with Dowex-50 (H^+) resin, filtered, and concentrated. Chromatography of the residue on a silica gel column (10:1 CH_2Cl_2–MeOH) gave **15** (gracillin) as a white solid (105 mg, 79%): mp > 210 °C, lit. 298–302 °C [7]; $[\alpha]_D^{24}$ −80.6° (*c* 1.0, pyridine), lit. −86.2° (*c* 0.12, DMF) [7]; R_f 0.47 (5:1 CH_2Cl_2–MeOH); IR (KBr); ν 3419, 981, 963, 920

< 900, 867, 838, 813; ^{1}H NMR (300 MHz, pyridine-d_5): δ 6.49 (s, 1 H, H″), 5.40 (d, 1 H, J 4.6 Hz, H-6), 5.20 (d, 1 H, $J_{1‴,2‴}$ 7.8 Hz, H-1‴), 5.09–4.99 (m, 4 H, H-1′, H-2″, H-3″, H-4″), 4.71–4.01 (m, 14 H), 3.92 (m, 1 H, H-5′), 3.60 (m, 2 H, H-26), 2.80 (m, 2 H), 1.83 (d, 3 H, J 5.3, H-6″), 1.21 (d, 3 H, J 6.9 Hz), 1.13 (s, 3 H), 0.90 (s, 3 H), 0.76 (d, 3 H, J 5.1 Hz); ^{13}C NMR (75 MHz, pyridine-d_5): δ 141.3, 122.3, 109.7, 105.0, 102.7, 100.5, 90.0, 81.6, 79.2, 79.0, 78.4, 78.2, 77.5, 75.5, 74.6, 73.3, 73.0, 72.0, 70.1, 67.4, 63.4, 62.9, 57.2, 50.8, 42.5, 41.0, 40.4, 39.2, 38.0, 37.7, 32.8, 32.7, 32.3, 32.2, 31.1, 30.6, 29.8, 21.9, 19.9, 19.2, 17.8, 16.8, 15.5.

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