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An improved synthesis of the saponin, polyphyllin D

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Abstract

Polyphyllin D, namely diosgenyl α-L-rhamnopyranosyl-(1→2)-[(α-L-arabinofuranosyl)-(1→4)]-β-D-glucopyranoside, was synthesized from diosgenyl-β-D-glucopyranoside in four steps and in 30% overall yield, taking advantage of regioselective pivaloylation and α-L-rhamnopyranosylation reactions.

Keywords: Diosgenyl α-L-rhamnopyranosyl-(1→2)-[(α-L-arabinofuranosyl)-(1→4)]-β-D-glucopyranoside; Polyphyllin D; Glycosylation; Regioselective; Synthesis

1. Introduction

Polyphyllin D, namely diosgenyl α-L-rhamnopyranosyl-(1→2)-[(α-L-arabinofuranosyl)-(1→4)]-β-D-glucopyranoside, has been isolated from various *Paris* species that have a history of medicinal properties.[1–5] This saponin showed cytotoxic effects against tumor cells (e.g., $ED_{50} = 0.94$ μg/mL against P-388),[2] stimulant effects for cell beating and calcium uptake by the myocardial cells,[3] as well as haemostatic[4] and immunomodulating effects.[5] The sugar structure of polyphyllin D represents a 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 sugar or sugar chain at the 4-position. We have reported a general approach to synthesizing this type of saponin.[6] Herein we report an improved method.

2. Results and discussion

In our previous synthetic route to polyphyllin D, seven steps were used starting from diosgenyl β-D-glucopyranoside (trillin, **1**).[6,7] In the present approach, four steps were required (Scheme 1). Diosgenyl β-D-glucopyranoside (**1**) was readily prepared in quantitative yield and in large amounts (~ 35-g scale) by glycosylation of diosgenin with 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate, followed by removal of the benzoyl groups.[8] Treatment of **1** with pivaloyl chloride afforded the 3,6-di-*O*-Piv product **2** in 60% yield. (Chan and Jiang have recently reported the regioselective acylation of hexopyranosides with pivaloyl chloride.[9]) Glycosylation of diol

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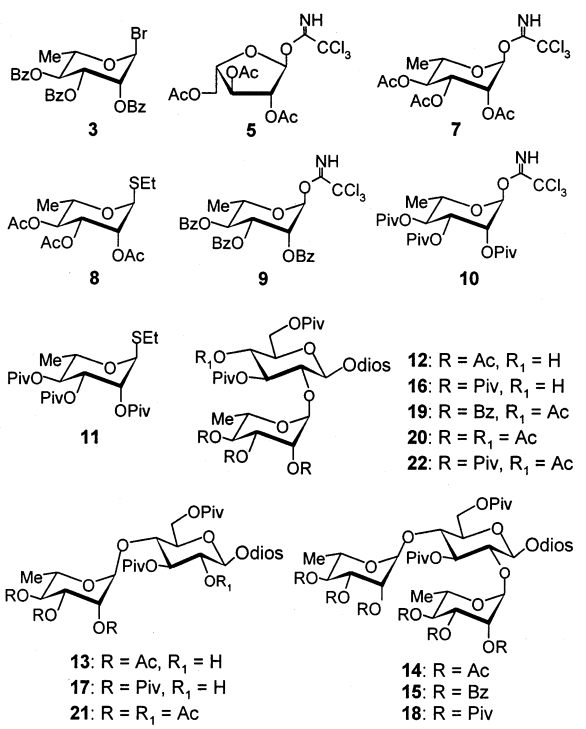
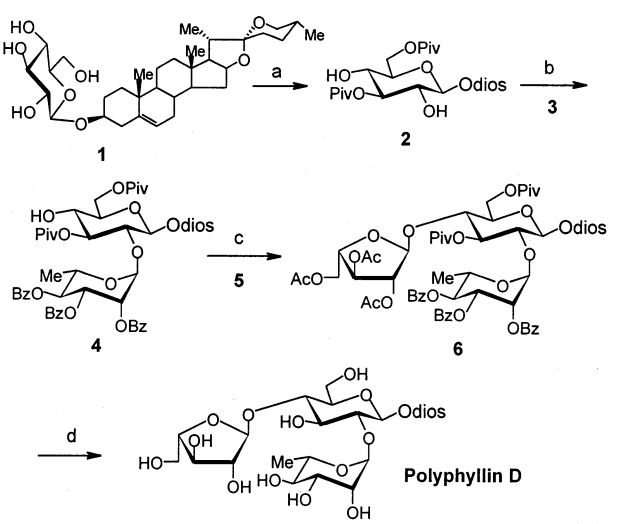
2 with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl bromide (**3**)[10] under the promotion of AgOTf mainly provided the desired 2-O-glycosylated product **4** (60%). The 2,4-di-O-glycosylated product **15** was obtained in 8% yield, along with recovered **2** in 24% yield; no 4-O-glycosylated product was isolated (Table 1, entry 8). Coupling of **4** with 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl trichloroacetimidate (**5**)[6] under the action of $BF_3{\cdot}OEt_2$ afforded **6** in 86% yield. Polyphyllin D was then readily obtained by removal of the acyl protecting groups (Ac, Bz, and Piv) by NaOH (91%).



Regioselective coupling of diol **2** with an α-L-rhamnopyranosyl donor has been investigated using several rhamnopyranosyl donors and conditions (Scheme 2). The results were listed in Table 1. Coupling of **2** with tri-*O*-acetyl-, benzoyl-, or pivaloyl-protected rhamnopyranosyl trichloroacetimidates (**7**,[11] **9**,[12] and **10**) under the promotion of $BF_3{\cdot}OEt_2$ gave poor regioselectivity and low yields of desired 2-O-glycosylated products (entries 1, 2, 5, 6, 9). Glycosylation of **2** with tri-*O*-benzoyl thiorhamnopyranoside **8**[13] under the action of NIS–AgOTf gave 2,4-di-O-glycosylated product **14** in considerable amount (34%, when **8**:**2** = 1.2:1, entry 3) or as the major product (72%, when **8**:**2** = 2.1:1, entry 4). However, under similar conditions, glycosylation of **2** with tri-*O*-pivaloylthiorhamnopyranoside **11** gave the 2-O-glycoylated product **16** in 62% yield (entry 11). These results demonstrated that enlarging the bulkiness of the glycosyl donors comparably increased the regioselectivity of the glycosylation (cf. donors **8** and **11**). However, this steric effect was shown to be trivial for trichloroacetimidate donors (cf. donors **7**, **9** and **10**).

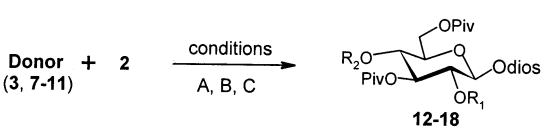
The glycosylation positions of the monoglycosylated products were determined by comparison of the 'acylation shift' with their acetylated counterparts. Treatment of monorhamnosylated products **4**, **12**, **13**, and **16** with Ac_2O in pyridine gave the corresponding acetylated products **19**–**22**, respectively. For the 2-O-rhamnosylated products **19**, **20**, and **22** (from **4**, **12**, and **16**, respectively), the chemical shift of the Glc-4-H were found to be downshifted to 4.99 ppm (t, *J* 9.7 Hz), 4.93 ppm (t, *J* 9.8 Hz), and 4.92 ppm (t, *J* 9.7 Hz), respectively. For the 4-O-rhamnosylated product **21** (from **13**), the chemical shift of Glc-2-H was found to be downshifted to 4.81 ppm (t, *J* 9.2 Hz) ppm. The differentiation of signals for Glc-2-H with Glc-4-H in compounds **19**–**22** was confirmed by ^1H–^1H COSY measurements.



Scheme 1. Reagents and conditions: (a) PivCl, pyridine, 0 °C, 60%; (b) **3**, AgOTf, 4 Å MS, CH_2Cl_2, −16 °C, 64%; (c) **5**, $BF_3{\cdot}OEt_2$, 4 Å MS, CH_2Cl_2, rt, 86%; (d) NaOH, 1:1:1 MeOH–H_2O–THF, 40 °C, 91%.

Table 1
Regioselective glycosylation of diol **2**

Entry	Donor	Conditions	Products (yields %)
1	**7**	A	**12** (23)
		(**7**:**2** = 1.2:1)	**13** (12)
			14 (3)
2		A	**12** (16)
		(**7**:**2** = 2.1:1)	**13** (10)
			14 (37)
3	**8**	B	**12** (34)
		(**8**:**2** = 1.2:1)	**13** (11)
			14 (34)
			2 (13)
4		B	**12** (14)
		(**8**:**2** = 2.1:1)	**13** (2)
			14 (72)
			2 (3)
5	**9**	A	**15** (25)
		(**9**:**2** = 1.2:1)	**2** (54)
6		A	**15** (52)
		(**9**:**2** = 2.1:1)	**2** (27)
7	**3**	C	**15** (3)
		(**3**:**2** = 1.2:1)	**4** (40)
			2 (48)
8		C	**15** (8)
		(**3**:**2** = 2.1:1)	**4** (60)
			2 (24)
9	**10**	A	**16** (12)
		(**10**:**2** = 1.2:1)	**17** (20)
			18 (24)
			2 (33)
10	**11**	B	**16** (62)
		(**11**:**2** = 1.2:1)	**17** (9)
			18 (2)
			2 (24)



Scheme 2. Conditions A: $BF_3{\cdot}OEt_2$, 4 Å MS, CH_2Cl_2, −78 °C. Conditions B: NIS, AgOTf, 4 Å MS, CH_2Cl_2, −20 °C. Conditions C: AgOTf, 4 Å MS, CH_2Cl_2, −20 °C.

3. Experimental

General methods.—See Ref. 7.

*Diosgenyl 3,6-di-*O*-pivaloyl-β-*D*-glucopyranoside* (**2**).—To a solution of diosgenyl β-D-glucopyranoside (**1**) (0.59 g, 1.00 mmol) in pyridine (15 mL) at 0 °C was slowly added pivaloyl chloride (< 0.64 mL, 5.1 mmol). The reaction was monitored by TLC, and the addition of pivaloyl chloride was stopped when the starting material disappeared. The reaction mixture was diluted with ethyl acetate and then washed with dilute HCl solution, satd aq $NaHCO_3$, and brine, respectively. The organic layer was dried over anhyd Na_2SO_4 and evaporated to dryness. The residue was subjected to flash column chromatography (8:1 petroleum ether–EtOAc) to give **2** (0.46 g, 60%) as a white foam: $[\alpha]_D^{18}$ 32.3° (*c* 0.87 $CHCl_3$); R_f 0.65 (3:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.35 (d, 1 H, *J* 5.2 Hz, H-6), 4.85 (t, 1 H, *J* 9.1 Hz), 4.44–4.36 (m, 3 H), 4.25 (dd, 1 H, *J* 11.8, 6.3 Hz), 3.58–3.32 (m, 6 H); EIMS (*m*/*z*): 744, 397, 282 (base), 139. Anal. Calcd for $C_{43}H_{68}O_{10}{\cdot}0.5\ H_2O$: C, 68.50; H, 9.22. Found: C, 68.60; H, 9.30.

*Diosgenyl 2-*O*-(2,3,4-tri-*O*-benzoyl-α-*L*-rhamnopyranosyl)-3,6-di-*O*-pivaloyl-β-*D*-glucopyranoside* (**4**) *and diosgenyl 2,4-di-*O*-(2,3,4-tri-*O*-benzoyl-α-*L*-rhamnopyranosyl)-3,6-di-*O*-pivaloyl-β-*D*-glucopyranoside* (**15**).—To a suspension of diol **2** (87 mg, 0.12 mmol), glycosyl bromide **3** (132 mg, 0.26 mmol), and 4 Å MS (90 mg) in dry CH_2Cl_2 (2 mL) at −16 °C, was added a solution of AgOTf (66 mg, 0.26 mmol) in toluene (1 mL). After being stirred for 2 h, the reaction was quenched with Et_3N (0.5 mL) and then filtered and concentrated. Chromatography of the residue on a silica gel column (30:1 toluene–EtOAc) gave **4** (85 mg, 60%) and **15** (15 mg, 8%) as white foams. **4**: R_f 0.41 (10:1 toluene–EtOAc); $[\alpha]_D^{18}$ 43.3° (*c* 1.14, $CHCl_3$); ^{1}H NMR (300 MHz, [b1]$CDCl_3$): δ 8.06–7.23 (m, 15 H), 5.77 (d, 1 H, *J* 3.3 Hz), 5.67–5.60 (m, 2 H), 5.44 (brs, 1 H), 5.24 (s, 1 H), 5.16 (t, 1 H, *J* 9.2 Hz), 4.82–4.77 (m, 1 H), 4.68 (d, 1 H, *J* 7.7 Hz), 4.44–4.41 (m, 2 H), 4.28 (dd, 1 H, *J* 11.9, 6.6 Hz), 4.14–4.11 (m, 1 H), 3.85 (dd, 1 H, *J* 9.0, 8.0 Hz), 3.67–3.59 (m, 2 H), 3.50–3.39 (m, 2 H); ESI-MS (*m*/*z*): 1226 (M + Na). Anal. Calcd for $C_{70}H_{90}O_{17}$: C, 69.86; H, 7.54. Found: C, 69.64; H, 7.84. **15**: R_f 0.50 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ 53.1° (*c* 1.30, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07–7.19 (m, 30 H), 5.77–5.44 (m, 8 H), 5.22 (s, 1 H), 5.16 (s, 1 H), 4.80 (d, 1 H, *J* 7.3 Hz), 4.75–4.71 (m, 1 H), 4.63 (dd, 1 H, *J* 11.9, 2.2 Hz), 4.44–4.37 (m, 2 H), 4.32–4.29 (m, 1 H), 3.97–3.92 (m, 2 H), 3.80 (dd, 1 H, *J* 8.5, 7.5 Hz), 3.94 (m, 1 H), 3.48–3.36 (m, 2 H); ESIMS (*m*/*z*): 1684

(M + Na). Anal. Calcd for $C_{97}H_{104}O_{24}$: C, 70.10; H, 6.79; Found: C, 69.87; H, 6.53.

Diosgenyl 4-O-(2,4,5-tri-O-acetyl-α-L-arabinofuranosyl)-2-O-(2,3,4-tri-O-benzoyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**6**).—To a suspension of 2,3,5-tri-*O*-acetyl-α-L-arabinofuranosyl trichloroacetimidate (**5**) (282 mg, 0.67 mmol), **4** (139 mg, 0.11 mmol) and 4 Å MS (100 mg) in dry CH_2Cl_2 (2 mL) at 0 °C, was slowly added $BF_3{\cdot}Et_2O$ (0.04 mL, 0.32 mmol). After being stirred for 2 h, the reaction was quenched with Et_3N (0.5 mL) and then filtered and concentrated. Chromatography of the residue on a silica gel column (5:1 petroleum ether–EtOAc) gave **6** (145 mg, 86%) as a white amorphous solid: $[\alpha]_D^{14}$ 11.7° (*c* 1.17, $CHCl_3$); R_f 0.56 (2:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05–7.77 (m, 15 H), 5.76 (dd, 1 H, *J* 3.4, 3.2 Hz), 5.63–5.60 (m, 2 H), 5.03 (s, 1 H), 4.97 (dd, 1 H, *J* 1.6, 1.5 Hz), 4.70–4.68 (m, 2 H), 4.54 (dd, 1 H, *J* 11.9, 1.8 Hz), 4.69–4.40 (m, 1 H), 4.34–4.29 (m, 2 H), 4.23–4.11 (m, 3 H), 3.85–3.79 (m, 2 H); ESIMS (*m*/*z*): 1484 (M + Na). Anal. Calcd for $C_{81}H_{104}O_{24}$: C, 66.56; H, 7.17. C, 66.58; H, 7.23.

Diosgenyl 4-O-α-L-arabinofuranosyl-2-O-α-L-rhamnopyranosyl-β-D-glucopyranoside (polyphyllin D).—A solution of **6** (92 mg, 0.06 mmol) and NaOH (50 mg, 1.25 mmol) in water (1 mL), MeOH (1 mL), and THF (1 mL) was stirred at 40 °C overnight. The mixture was neutralized with Dowex-50 (H^+ form) resin, and then filtered and concentrated. Chromatography of the residue on a silica gel column (5:1 CH_2Cl_2–MeOH) gave polyphyllin D (49 mg, 91%) as a white solid: $[\alpha]_D^{21}$ −113.6° (*c* 0.53, MeOH), Lit. −113° (*c* 0.53, MeOH),[1] Lit. −116.3° (*c* 0.52, MeOH).[6b]

2,3,4-Tri-O-pivaloyl-α-L-rhamnopyranosyl trichloroacetimidate (**10**).—To a solution of L-rhamnose monohydrate (1.61 g, 8.83 mmol) and DMAP (380 mg, 3.12 mmol) in pyridine (20 mL) was added pivaloyl chloride (10 mL, 81.69 mmol) dropwise at 0 °C. After being stirred at 70 °C for 24 h, and then cooled to 50 °C, MeOH (10 mL) was added. The resulting mixture was kept for another 1 h and then concentrated under reduced pressure and diluted with EtOAc. The solution was washed with 1 N HCl, satd aq $NaHCO_3$, and water, respectively. The organic layer was dried over $MgSO_4$, and then filtered and concentrated. The residue was purified by a silica gel column chromatography (25:1 petroleum ether–EtOAc) to give 1,2,3,4-tetra-*O*-pivaloyl-α/β-L-rhamnopyranoside (4.21 g, 95%) as a white foam, which was directly used in the next step.

To a solution of 1,2,3,4-tetra-*O*-pivaloyl-α/β-L-rhamnopyranoside (2.00 g, 4.00 mmol) in dry CH_2Cl_2 (10 mL), was added at 0 °C to a solution of HOAc (9 mL) containing HBr (33%). After being stirred at rt for 23 h, the mixture was coevaporated with toluene under reduced pressure. The residue was diluted with CH_2Cl_2 (20 mL) and then washed with satd aq $NaHCO_3$ and water, respectively. The organic layer was dried over $MgSO_4$ and then filtered and concentrated. The residue was purified by a silica gel column chromatography (8:1 petroleum ether–EtOAc) to give 2,3,4-tri-*O*-pivaloyl-α/β-L-rhamnopyranose (1.56 g, 94%) as a white foam: ^{1}H NMR (300 MHz, $CDCl_3$) (α anomer): 5.43 (dd, 1 H, *J* 10.1, 2.1 Hz), 5.24 (m, 1 H), 5.14 (m, 2 H), 4.16 (m, 1 H), 1.05 (m, 30 H). EIMS (*m*/*z*): 399 (M^+ − water), 85, 57. Anal. Calcd for $C_{21}H_{36}O_8$: C, 60.55; H, 8.71. Found: C, 60.79; H, 8.78.

To a solution of 2,3,4-tri-*O*-pivaloyl-α/β-L-rhamnopyranose (800 mg, 1.92 mmol) in dry CH_2Cl_2 (5 mL), was added Cl_3CCN (0.78 mL, 7.79 mmol) and a catalytic amount of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU). The mixture was stirred at rt for 12 h, and then concentrated under reduced pressure. The residue was purified by a silica gel column chromatography (35:1 petroleum ether–EtOAc, containing 1% NEt_3) to give **10** (956 mg, 89%) as a white foam: R_f 0.43 (25:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −35.8° (*c* 0.63, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.74 (s, 1 H), 6.16 (s, 1 H), 5.42 (m, 2 H), 5.26 (t, 1 H, *J* 9.8 Hz), 4.10 (m, 1 H), 1.29–1.24 (m, 12 H), 1.14 (s, 9 H), 1.08 (s, 1 H); EIMS (*m*/*z*): 399, 85, 57 (base). Anal. Calcd for $C_{23}H_{36}Cl_3NO_8$: C, 49.25; H, 6.47; N, 2.50. Found: C, 49.61; H, 6.55; N 2.60.

Ethyl 2,3,4-tri-O-pivaloyl-1-thio-α-L-rhamnopyranoside (**11**).—To a solution of ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside (490 mg, 1.47 mmol)[14] in MeOH (10 mL) was added NaOMe (50 mg). After being stirred at rt overnight, the solution was neutralized with Dowex-50 (H^+) resin and then filtered and concentrated. The residue was dissolved in dry pyridine (5 mL) and then treated with DMAP (60 mg, 0.49 mmol) and pivaloyl chloride (3 mL, 24.36 mmol) for 15 h at 70 °C. The mixture was evaporated to dryness under reduced pressure. The residue was diluted with EtOAc and then washed with 1 N HCl, satd aq $NaHCO_3$, and water, respectively. The organic layer was dried over $MgSO_4$ and then filtered and concentrated. The residue was applied to silica gel column chromatography (20:1 petroleum ether–EtOAc) to give **11** (526 mg, 78%) as a white amorphous solid: R_f 0.25 (35:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ 55.9° (*c* 0.63, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.52 (t, 1 H, *J* 1.4 Hz), 5.11 (m, 2 H), 4.78 (s, 1 H), 3.68 (m, 1 H), 2.72 (dd, 1 H, *J* 14.8, 7.4 Hz), 1.30 (m, 15 H), 1.17 (s, 9 H), 1.12 (s, 9 H); EIMS (*m*/*z*): 399 (M − SEt), 85, 57 (base). Anal. Calcd for $C_{23}H_{40}O_7S$: C, 59.97; H, 8.75. Found: C, 60.07; H, 8.58.

Glycosylation of diol **2** *with rhamnopyranosyl donors (***7***–***11***) (Scheme 2, Table 1)*

Condition A. To a suspension of diol **2** (∼150 mg, 1.0 equiv), donor (**7**, **9**, **10**) (1.2 or 2.1 equiv), and 4 Å MS (∼100 mg) in dry CH_2Cl_2 (5 mL) at −78 °C, was added $BF_3{\cdot}Et_2O$ (0.025 mL, 1.0 equiv). After being stirred for 2.5 h, the reaction was quenched with Et_3N (0.5 mL), and then filtered and concentrated. The products were isolated by silica gel column chromatography (6:1–4:1 petroleum ether–EtOAc).

Condition B. To a suspension of diol **2** (∼150 mg, 1.0 equiv), donor (**8**, **11**) (1.2 or 2.1 equiv), and 4 Å MS (∼100 mg) in dry CH_2Cl_2 (5 mL) at −20 °C, was added NIS (1.0 equiv), followed by AgOTf (0.5 equiv) in toluene. After being stirred for 2.5 h, the reaction was quenched with Et_3N (0.5 mL) and then filtered and concentrated. The products were isolated by silica gel column chromatography (6:1–4:1 petroleum ether–EtOAc).

Diosgenyl 2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**12**).—R_f 0.37 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −66.7° (*c* 1.30, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.38 (d, 1 H, *J* 4.6 Hz), 5.24–5.20 (m, 2 H), 5.09–5.12 (m, 2 H), 4.97 (s, 1 H), 4.58 (d, 1 H, *J* 7.7 Hz), 4.50–4.37 (m, 3 H), 4.29–4.25 (m, 1 H), 3.72 (dd, 1 H, *J* 9.0, 8.0 Hz), 3.64–3.34 (m, 5 H); ESIMS (*m*/*z*): 1039 (M + Na). Anal. Calcd for $C_{55}H_{84}O_{17}{\cdot}H_2O$: C, 63.81; H, 8.37. Found: C, 63.66; H, 7.99.

Diosgenyl 4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**13**).—R_f 0.30 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −59.3 ° (*c* 0.34, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.35 (d, 1 H, *J* 4.1 Hz), 5.22 (dd, 1 H, *J* 3.2, 3.1 Hz), 5.15 (t, 1 H, *J* 8.6 Hz), 4.86 (s, 1 H), 4.52–4.38 (m, 3 H), 4.26–4.20 (m, 1 H), 3.94–3.89 (m, 1 H), 3.74–3.68 (m, 2 H), 3.57–3.34 (m, 4 H); ESIMS (*m*/*z*): 1039 (M + Na). Anal. Calcd for $C_{55}H_{84}O_{17}{\cdot}H_2O$: C, 63.81; H, 8.37. Found: C, 63.72; H, 8.68.

Diosgenyl 2,4-di-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**14**).—R_f 0.25 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −59.3° (*c* 0.34, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 5.37 (m, 1 H), 5.31 (t, 1 H, *J* 8.2 Hz), 5.22–5.14 (m, 3 H), 5.06–4.99 (m, 3 H), 4.90 (s, 1 H), 4.83 (s, 1 H), 4.60 (d, 1 H, *J* 7.5 Hz), 4.46–4.36 (m, 3 H), 4.03–4.18 (m, 2 H), 3.91–3.38 (m, 6 H), 2.12, 2.10, 2.04, 2.02, 1.97, 1.95 (s each, 3 H each); ESIMS (*m*/*z*): 1312 (M + Na). Anal. Calcd for $C_{67}H_{100}O_{24}{\cdot}2.5\ H_2O$: C, 60.30; H, 7.93. Found: C, 60.21; H, 7.73.

Diosgenyl 2-O-(2,3,4-tri-O-pivaloyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**16**).—A white amorphous solid: R_f 0.15 (6:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −59.1° (*c* 0.98, $CHCl_3$), ^1H NMR (300 MHz, $CDCl_3$): 5.38 (d, 1 H, *J* 4.1 Hz), 5.26 (m, 2 H), 5.12 (t, 1 H, *J* 10.2 Hz), 5.04 (t, 1 H, *J* 9.3 Hz), 4.94 (s, 1 H), 4.57 (d, 1 H, *J* 8.0 Hz), 4.50–4.39 (m, 3 H), 4.24 (dd, 1 H, *J* 11.8, 6.6 Hz), 3.75 (t, 1 H, *J* 8.0), 3.60–3.46 (m, 3 H), 3.43–3.39 (m, 2 H); ESIMS (*m*/*z*): 1144 (M + 1). Anal. Calcd for $C_{64}H_{102}O_{17}{\cdot}H_2O$: C, 66.18; H, 9.03; Found: C, 66.36; H, 8.78.

Diosgenyl 4-O-(2,3,4-tri-O-pivaloyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**17**).—A white amorphous solid: R_f 0.30 (6:1 petroleum ether–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): 5.36 (d, 1 H, *J* 4.9 Hz), 5.25 (m, 1 H), 5.16 (t, 1 H, *J* 10.2 Hz), 5.01 (dd, 1 H, *J* 3.0, 1.9 Hz), 4.86 (d, 1 H, *J* 1.9 Hz), 4.44 (m, 3 H), 4.17 (dd, 1 H, *J* 9.9, 6.2 Hz), 3.93 (dd, 1 H, *J* 9.3, 6.0 Hz), 3.73 (t, 1 H, *J* 6.9 Hz), 3.51 (m, 3 H), 3.38 (m, 2 H); ESIMS (*m*/*z*): 1165 ($M + Na^+$), 1144 (M + 1).

Diosgenyl 2,4-di-O-(2,3,4-tri-O-pivaloyl-α-L-rhamnopyranosyl)-3,6-di-O-pivaloyl-β-D-glucopyranoside (**18**).—A white amorphous solid: R_f 0.27 (8:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −50.0° (*c* 0.79, $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): 5.37–5.29 (m, 3 H), 5.25–5.20 (m, 2 H), 5.14–5.06 (m, 2 H), 5.00 (t, 1 H, *J* 1.0 Hz), 4.88 (s, 1 H), 4.77 (s, 1 H), 4.60 (d, 1 H, *J* 7.7 Hz), 4.51–4.40 (m, 3 H), 4.26 (dd, 1 H, *J* 12.1, 5.8 Hz), 3.94 (dd, 1 H, *J* 9.3, 6.0 Hz), 3.76–3.55 (m, 4 H), 3.49 (d, 1 H, *J* 6.2 Hz), 3.39 (t, 1 H, *J* 10.7 Hz); ESIMS (*m*/*z*): 1541 (M^+). Anal. Calcd for $C_{85}H_{136}O_{24}$: C, 66.20; H, 8.89; Found: C, 65.90; H, 8.72.

Diosgenyl 2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-4-O-acetyl-3,6-di-pivaloyl-β-D-glucopyranoside (**20**).—A solution of **12** (22 mg, 0.022 mmol) in pyridine (0.7 mL) and Ac_2O (0.3 mL) was stirred at rt overnight, and then poured into water and extracted with EtOAc. The organic layer was washed with dilute aq HCl solution and brine, respectively, and then was dried over anhyd Na_2SO_4 and evaporated to dryness. The residue was applied to a silica gel column (5:1 petroleum ether–EtOAc) to give **20** (15 mg, 65%) as a white foam: R_f 0.51 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −71.7° (*c* 0.14, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.38 (d, 1 H, *J* 3.9 Hz), 5.30 (t, 1 H, *J* 9.4 Hz), 5.22 (dd, 1 H, *J* 3.2, 9.7 Hz), 5.16 (s, 1 H), 5.03 (t, 1 H, *J* 9.9 Hz), 4.93 (t, 1 H, *J* 9.8 Hz), 4.86 (s, 1 H), 4.61 (d, 1 H, *J* 7.7 Hz), 4.48–4.39 (m, 2 H), 4.15–4.07 (m, 2 H), 3.75 (t, 1 H, *J* 8.6 Hz), 3.71–3.19 (m, 1 H), 3.67–3.57 (m, 1 H), 3.48–3.35 (m, 2 H), 2.10, 2.02, 1.99, 1.96 (s each, 3 H each), 1.20, 1.11 (s each, 9 H, each); ESIMS: 1082 (M + Na). Anal. Calcd for $C_{57}H_{86}O_{18}\cdot H_2O$: C, 63.55; H, 8.23. Found: C, 63.55; H, 7.99.

Diosgenyl 4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-2-O-acetyl-3,6-di-O-pivaloyl-β-D-glucopyranoside (**21**).—A procedure similar to that for the preparation of **20** was employed. A solution of **13** (21 mg, 0.022 mmol) with pyridine (0.7 mL) and Ac_2O (0.3 mL) was stirred at rt overnight to give **21** (17 mg, 74%) as a white foam: R_f 0.49 (3:1 petroleum ether–EtOAc); $[\alpha]_D^{20}$ −67.0° (*c* 0.38, $CHCl_3$), ^{1}H NMR (300 MHz, $CDCl_3$): 5.34 (d, 1 H, *J* 4.5 Hz), 5.26 (dd, 1 H, *J* 10.1, 3.1 Hz), 5.06–4.99 (m, 2 H), 4.85 (s, 1 H), 4.81 (dd, 1 H, *J* 9.2, 8.2 Hz), 4.58 (d, 1 H, *J* 8.0 Hz), 4.51 (d, 1 H, *J* 11.9 Hz), 4.44–4.38 (m, 1 H), 4.24 (dd, 1 H, *J* 12.0, 5.3 Hz), 3.92–3.88 (m, 1 H), 3.76 (t, 1 H, *J* 9.0 Hz), 3.69–3.65 (m, 1 H), 3.48–3.35 (m, 3 H), 2.11, 2.03, 2.01, 1.98 (s each, 3 H each), 1.22, 1.16 (s each, 9 H each); ESIMS (*m*/*z*): 1082 (M + Na). Anal. Calcd for $C_{57}H_{86}O_{18}\cdot H_2O$: C, 63.55; H, 8.33; Found: C, 63.48; H, 8.11.

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