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First total synthesis of natural pulsatilla saponin D via highly stereospecific glycosylation

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ABSTRACT

The first total synthesis of pulsatilla saponin D, a potent anti-lung cancer natural product was accomplished in eight steps from L-arabinopyranoside in high overall yield via a highly stereospecific glycosylation featuring an oxocarbenium mechanism between the trisaccharide derivative and aglycone as the key step.

1. Introduction

Triterpene glycosides (saponins) are widely distributed in plants and animals.[1,2] Saponins are found in many well known traditional medicines such as ginseng, licorice, horse chestnut, red clover, and primula.[2,3] *Pulsatilla* genus saponins have been reported mainly as two types, oleanane and lupane-type saponins.[4] Oleanane-type saponins have shown good in vitro cytotoxic activity against several human cancer cell lines. Among the oleanane-type saponins, pulsatilla saponin D **1** (hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-α-L-arabinopyranoside) was isolated from Pulsatilla japonica in low yield.[5,6] It has exhibited very potent in vivo antitumor activity, even greater than taxol and doxorubicin, in mice bearing Lewis lung carcinoma.[7]

Despite its high clinical activity[7] and reported total syntheses of some members of the saponin family,[8,9] the total synthesis of pulsatilla saponin D **1** has not been reported to date. This is due to its complex structure and difficulty of stereospecific glycosylation at C-1 of the trisaccharide. In this paper, we present the first total and practical synthesis of pulsatilla saponin D **1** via a highly selective glycosylation that could be used to supply significant quantities of this scarcely available drug candidate.

2. Results and discussion

For the synthesis of pulsatilla saponin D **1**, a retrosynthetic analysis was proposed as shown in Scheme 1. The convergent synthesis utilizes a highly stereospecific and selective β-glycosylation of aglycone **2** with the trisaccharide moiety **10** as the key step.

Initially, an efficient synthesis of the trisaccharide moiety **10**, that is, responsible for the antitumor activity,[10] was accomplished from protected L-arabinose **4** through the stepwise stereospecific α-glycosylation of protected L-rhamnose **5** and subsequent β-glycosylation of the protected D-glucose **3** as key steps described in Scheme 2.[2] Thus, selective protection of the C-1 hydroxyl group of L-arabinose according to modified literature conditions[11] followed by formation of the acetonide at C-3,4 gave compound **4**. Glycosylation of acceptor **4** with the donor, trichloroacetimidate **5** (R=Bz), was achieved in the presence of TMSOTf[12] in CH_2Cl_2 at −78 °C to give disaccharide **6** in 93% yield with exclusively the natural α-configuration at C-1′.[13] The high stereoselectivity achieved in the glycosylation using TMSOTf was attributed to a neighboring group effect of the C-2 substituent of compound **5** via formation of an acyloxonium ion with concomitant stabilization of the positive charge at C-1.[14] The relative configuration at H-1′ and H-2′ of the L-rhamnopyranoside of the protected disaccharide **6** was assigned as diequatorial with α-configuration at the anomeric C-1′ carbon due to lack of a vicinal coupling constant at δ 6.25 between the anomeric H-1′ and H-2′. After deprotection of the isopropylidene moiety of compound **6** with *p*-TsOH to give

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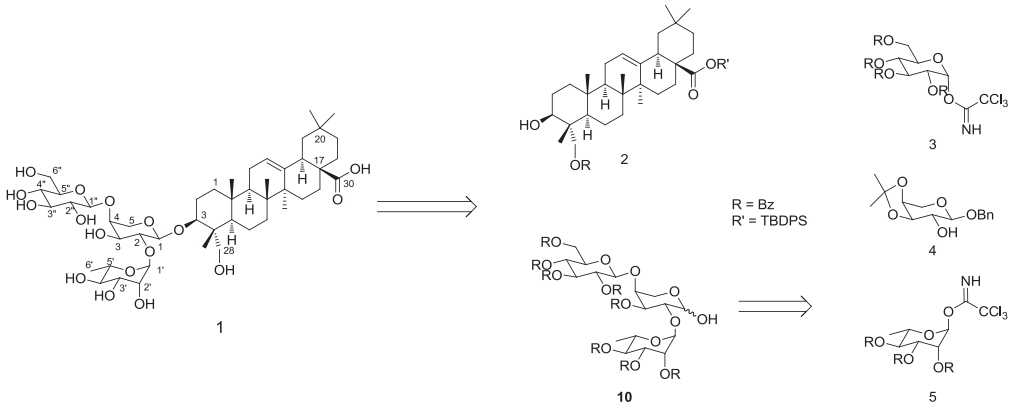
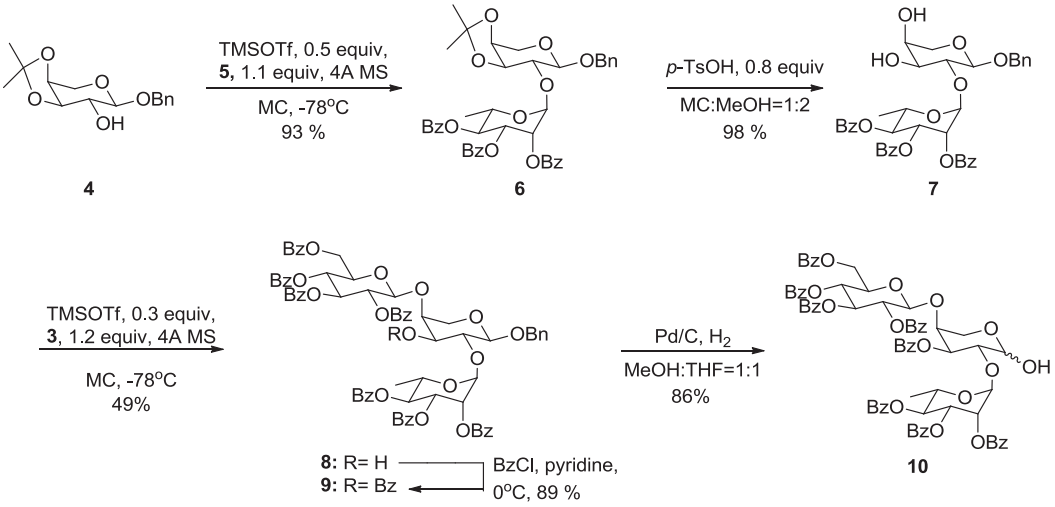


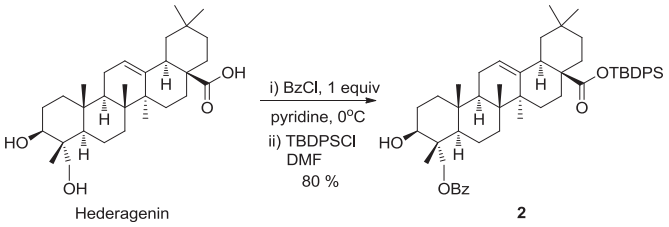
Scheme 1. Restrosynthetic analysis of pulsatilla saponin D.



Scheme 2. Synthesis of trisaccharide.

compound **7** in 98% yield, subsequent β-selective glycosylation at C-1″ of the perbenzoylated D-glucose trichloroacetimidate **3** (R=Bz)[10,15] with the more nucleophilic hydroxyl at C-4 of disaccharide **7** using TMSOTf as a promoter at −78 °C afforded trisaccharide **8** (Scheme 2). Benzoylation of the last C-3 hydroxyl group of compound **8** with benzoyl chloride in the presence of imidazole cleanly afforded compound **9**[16] in 89% yield. Similarly, the relative configuration at H-1″ and H-2″ of the D-glucopyranoside of the protected trisaccharide **9** was assigned as diaxial with β-configuration at the anomeric C-1″ carbon due to the large vicinal coupling constant ($J_{1'',2''}$=7.53 Hz) at δ 4.90 and 5.42 between the anomeric H-1″ and H-2″. Specific β-glycosylation could be rationalized using a similar mechanism as for formation of disaccharide **6**. The benzyl group at the C-1 position of trisaccharide **9** was selectively deprotected with Pd/C catalyst under H_2 to give compound **10** (α/β=1:2 at C-1, yield 86%).

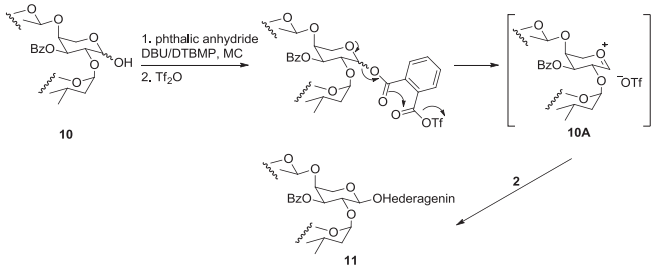
After successful synthesis of the trisaccharide moiety **10**, we turned our attention to protection of the aglycone, commercially available hederagenin, at the C-28 hydroxyl and C-17 acid groups. Initial regioselective benzoylation of the primary C-28 hydroxyl group[11] with benzoyl chloride in the presence of pyridine, and in situ silylation of the C-17 acid with TBDPSCl in DMF afforded the diprotected compound **2** in 80% overall yield (Scheme 3). Under these conditions, the secondary hydroxyl group at C-3 of hederagenin remained unprotected.



Scheme 3. Protection of hederagenin.

For the key stereospecific acetalization at the C-1 position of the trisaccharide moiety **10** and the C-3 position of the hederagenin aglycone **2**, a highly β-selective glycosylation method must be applied. Typical glycosylation methods such as the Koenigs–Knorr[17] and trichloroacetimidate reactions[18] afforded α/β mixtures by a non-participation mechanism. The formation of anomeric bonds has been controllable using solvents such as acetonitrile or ether,[19,20] but in our case, these methods afforded α/β mixtures. Kim et al. reported a one-pot, highly selective β-glycosylation method under non-participation conditions via a phthalic anhydride-mediated intermediate and Tf_2O as an activating agent.[21] A mechanism was proposed for the selective β-glycosylation with trisaccharide **10** via an oxocarbenium ion in equilibrium with the

β-triflate. Subsequent reaction of the equilibrium mixture with protected hederagenin **2** as the donor would provide the desired β-anomer as shown in Scheme 4. In accordance with the proposed mechanism for stereospecific β-glycosylation, treatment of the trisaccharide **10** with phthalic anhydride in the presence of DBU, followed by DTBMP and Tf_2O as activating agents at −78 °C, and finally



Scheme 4. Proposed mechanism[21] of the α-glycosylation with trisaccharide **12** employing phthalic anhydride and Tf_2O.

addition of the protected hederagenin **2** afforded the fully protected pulsatilla saponin D **11** in 70% yield (Scheme 5). ^{13}C NMR chemical shifts of each sugar component with the correct configuration for the protected compound **11** were observed at δ 97.88 ppm (Rha), δ 100.16 ppm (Ara), and δ 100.86 ppm (Glu), respectively. This glycosylation method was β-stereospecific as was confirmed for the deprotected pulsatilla saponin D **1**. The potential α-isomer side product was not detected in the NMR spectrum (<3%). The TBDPS protecting group of compound **11** was removed with TBAF, and all of the benzoyl groups were subsequently deprotected by potassium *tert*-butoxide in THF in a one-pot reaction to give pulsatilla saponin D **1** in 84% yield for the two steps. The anomeric ^{13}C NMR chemical shifts of each sugar component (arabinose δ 104.30 ppm, rhamnose δ 101.56 ppm, glucose δ 106.64 ppm) of compound **1** were again observed to be the same as those of the natural product.[5,6,22] The absolute configuration of C1−H and C2−H of the arabinopyranoside of pulsatilla saponin D was assigned as trans diaxial as shown in structures **11** and **1**, respectively, in Scheme 5, due to the large vicinal coupling constant ($J_{1,2}$=7.03 Hz) at δ 4.97 between the anomeric H-1 and H-2. The anomeric configuration at C-1 of the arabinopyranoside of structures **11** and **1** was determined on the basis of the $^{13}C-^{1}H$ coupling constants, 158.9 Hz (β-glucopyranoside), 163.2 Hz (α-arabinopyranoside), and 173.7 Hz (α-rhamnopyranoside). The anomer that has an equatorially disposed hydrogen at C-1 has a larger coupling constant with the $J_{C\text{-}1,H\text{-}1}$ value of such pyranosides in general ~170 Hz, whereas pyranosides with an axially oriented hydrogen at C-1 have a $J_{C\text{-}1,H\text{-}1}$ coupling of ~160 Hz.[23] Thus, the C−H coupling constant of 163.2 Hz at 104.30 ppm unequivocally established the β-configuration for C-1 (Ara).

The synthesized pulsatilla saponin D had identical spectral and physical properties to the compound isolated from natural sources. Therefore, the first total synthesis of pulsatilla saponin D **1** using a β-selective glycosylation between the trisaccharide and aglycone was successfully completed in a high overall yield of 17.4% from L-arabinose in eight steps.

Furthermore, the synthesized pulsatilla saponin D was tested on a human lung cancer cell line (A-549) to confirm its authenticity. The in vitro anticancer activity of totally synthesized pulsatilla saponin D against the lung cancer cell line (A-549) had a similar IC_{50} value (IC_{50}=5.8 μM) with that of isolated pulsatilla saponin D (IC_{50}=6.3 μM). This result revealed that pulsatilla saponin D showed five-fold more potent in vitro anti-lung cancer activity than the clinically useful drug Iressa® (IC_{50}=26.08 μM).

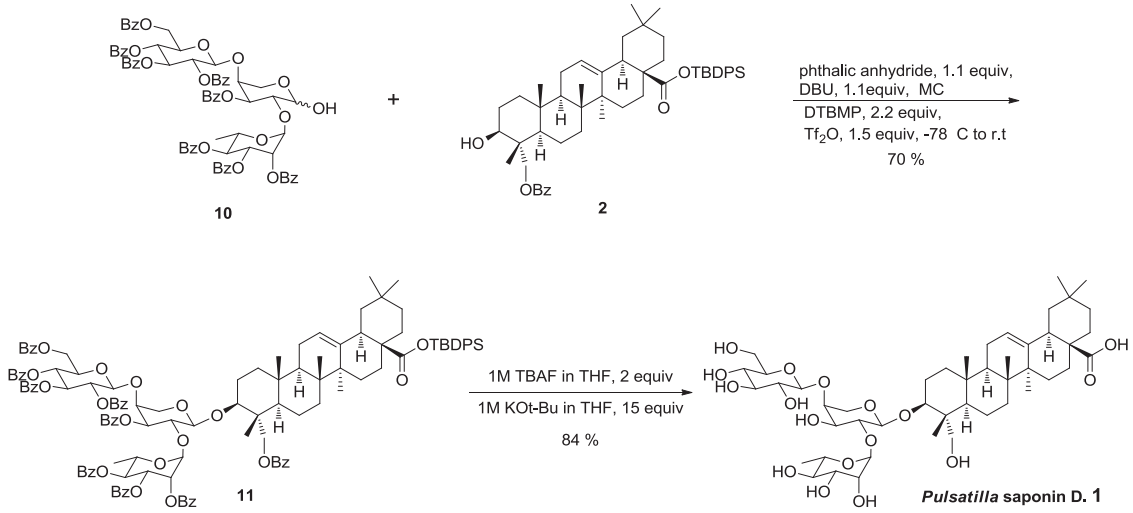
3. Conclusion

In conclusion, the first total and practical synthesis of pulsatilla saponin D in 17.4% overall yield from L-arabinose in eight steps was accomplished via a phthalic anhydride-mediated β-stereospecific glycosylation between trisaccharide and aglycone precursors. The potential α-anomer side product was not detected by NMR (<3%). The synthesized pulsatilla saponin D and isolated pulsatilla saponin D had similar in vitro cytotoxic activity against a human lung cancer cell line (A-549). Further derivatization of pulsatilla saponin D and structure−activity relationship (SAR) testing for the development of anti-lung cancer drugs is ongoing, and the results of this investigation will be reported in due course.

4. Experimental section

4.1. General procedures

All commercial reagents and solvents were used as received without further purification unless specified. Reaction solvents were distilled from calcium hydride for dichloromethane and from sodium metal and benzophenone for tetrahydrofuran. The reactions were monitored and the R_f values determined using analytical thin layer chromatography (TLC) with Merck silica gel 60 and F-254 precoated plates (0.25-mm thickness). Spots on the TLC



Scheme 5. Synthesis of pulsatilla saponin D.

plates were visualized using ultraviolet light (254 nm) and a basic potassium permanganate solution or cerium sulfate/ammonium dimolybdate/sulfuric acid solution followed by heating on a hot plate. Flash column chromatography was performed with Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded on Bruker DPX-250, 400 or Varian Unity-Inova 500 Spectrometer. Proton chemical shifts are reported in parts per million (ppm (δ)) relative to internal tetramethylsilane (TMS, δ 0.00) or with the solvent reference relative to TMS employed as the internal standard ($CDCl_3$, δ 7.26 ppm; CD_3OD-d_4, δ 3.31 ppm, DMSO-d_6, δ 2.50 ppm). Data are reported as follows: chemical shift {multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), and multiplet (m)], coupling constants [Hz], integration}. ^{13}C NMR spectra were recorded on Bruker DPX-250 (63 MHz), 400 (100 MHz) or Varian Unity-Inova 500 (125 MHz) spectrometer with complete proton decoupling. Carbon chemical shifts are reported in parts per million (ppm (δ)) relative to TMS with the respective solvent resonance as the internal standard ($CDCl_3$, δ 77.0 ppm; CD_3OD-d_4, δ 49.0 ppm, DMSO-d_6, δ 39.5 ppm). Infrared (IR) spectra were recorded on a Nicolet Model Impact FT-IR 400 spectrometer. Data are reported in wave numbers (cm^{-1}). MALDI-TOF masses were recorded on an Applied Biosystems 4700 proteomics analyzer spectrometer.

4.2. Benzyl-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-3,4-*O*-isopropylidene-α-L-arabinopyranoside (6)

A suspension of isopropylidene **4** (630 mg, 2.25 mmol, 1 equiv), trichloroacetimidate **5** (1.54 mg, 2.48 mmol, 1.1 equiv), and 4 Å molecular sieves (1000 mg) in dry CH_2Cl_2 (40 mL) was stirred for 30 min at rt and then cooled to −78 °C under the N_2 atmosphere. A CH_2Cl_2 solution of TMSOTf (203 μL, 1.125 mmol, 0.5 equiv) was added dropwise. After stirring for 2 h at this temperature, the mixture was neutralized with triethylamine (Et_3N), filtered with Celite, and then concentrated under reduced pressure. The residue was purified by a silica gel column chromatography using the 2:1 hexanes/ethyl acetate to give compound **4** as white amorphous solids (1.546 g, 93%). Mp 64–67 °C; $[\alpha]_D$ +81.7 (*c* 0.1, $CHCl_3$); R_f=0.46 (hexanes/ethyl acetate, 2:1, v/v); IR ν_{max} (KBr, cm^{-1}) 2932, 1731, 1602, 1452, 1384, 1315, 1263, 1118; ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm 1.18 (d, *J*=6.16 Hz, 3H), 1.35 (s, 3H), 1.54 (s, 3H), 3.86 (dd, *J*=12.79, 2.84 Hz, 1H), 3.96 (dt, *J*=7.23, 3.57 Hz, 1H), 4.20 (dd, *J*=12.95, 2.53 Hz, 1H), 4.25–4.34 (m, 2H), 4.42–4.53 (m, 1H), 4.56 (d, *J*=7.42 Hz, 1H), 4.66 (d, *J*=11.69 Hz, 1H), 4.97 (d, *J*=11.69 Hz, 1H), 5.49 (s, 1H), 5.62 (t, *J*=9.87 Hz, 1H), 5.76–5.79 (m, 1H), 5.79–5.86 (m, 1H), 7.17–8.13 (m, 20H); ^{13}C NMR (63 MHz, $CDCl_3$) δ ppm 17.44, 26.07, 27.92, 62.98, 66.74, 70.22, 70.51, 70.59, 71.93, 73.22, 76.14, 78.96, 96.34, 99.77, 110.57, 127.94, 128.13, 128.34, 128.45, 128.57, 128.62, 129.34, 129.43, 129.52, 129.77, 130.04, 133.15, 133.32, 133.50, 137.33, 165.57, 165.67, 165.81; MALDI-TOF *m*/*z* 761.2669 (obsd $[M+Na]^+$), 738.2676 (calcd for $C_{42}H_{42}O_{12}$).

4.3. Benzyl-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside (7)

p-TsOH (285 mg, 1.67 mmol, 0.8 equiv) was added to a solution of **6** (1.55 g, 2.09 mmol, 1 equiv) in CH_2Cl_2/MeOH (1:2, 60 mL) and the solution was stirred at rt. When TLC (1:1, hexanes/ethyl acetate) showed that starting material had completely disappeared, Et_3N (1.50 mL) was added and the mixture was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography using the 1:1 hexanes/ethyl acetate to give compound (**7**) as white amorphous solids (1433 mg 98%). Mp 85–88 °C; $[\alpha]_D$ +85.3 (*c* 0.1, $CHCl_3$); R_f=0.12 (hexanes/ethyl acetate, 1:1, v/v); IR ν_{max} (KBr, cm^{-1}) 3454, 2925, 1727, 1602, 1451, 1384, 1315, 1264, 1175, 1096; ^{1}H NMR (250 MHz $CDCl_3$) δ ppm 1.09 (d, *J*=6.32 Hz, 3H), 3.22 (d, *J*=5.05 Hz, 1H), 3.60 (d, *J*=10.27 Hz, 1H), 3.71 (d, *J*=6.95 Hz, 1H), 3.89–4.02 (m, 4H), 4.36–4.50 (m, 1H), 4.55–4.66 (m, 2H), 4.93 (d, *J*=11.53 Hz, 1H), 5.47 (s, 1H), 5.62 (t, *J*=9.79 Hz, 1H), 5.75–5.85 (m, 2H), 7.15–8.08 (m, 20H); ^{13}C NMR (63 MHz, $CDCl_3$) δ ppm 17.27, 64.41, 66.94, 67.95, 70.24, 70.57, 70.82, 71.75, 72.99, 76.36, 98.09, 99.89, 128.04, 128.36, 128.44, 128.53, 128.58, 129.18, 129.31, 129.36, 129.74, 129.96, 133.19, 133.33, 133.51, 136.88, 165.80, 165.82; MALDI-TOF *m*/*z* 721.2385 (obsd $[M+Na]^+$), 698.2363 (calcd for $C_{39}H_{38}O_{12}$).

4.4. Benzyl-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-3-hydroxyl-α-L-arabinopyranoside (8)

A suspension of **7** (150 mg, 0.215 mmol, 1 equiv), trichloroacetimidate **3** (175 mg, 0.236 mmol, 1.1 equiv), and 4 Å molecular sieves (100 mg) in dry CH_2Cl_2 (10 mL) was treated with TMSOTf (19 μL, 0.107 mmol, 0.5 equiv) in the same manner as that described for compound **6**. The product was purified by a silica gel column chromatography using the 2:1 hexanes/ethyl acetate to give compound (**8**) as white amorphous solids (191 mg, 71%). Mp 117–121 °C; $[\alpha]_D$ +53.0 (*c* 0.1, $CHCl_3$); R_f=0.07 (hexanes/ethyl acetate, 2:1, v/v); IR ν_{max} (KBr, cm^{-1}) 3431.71, 2920.6, 1729.83, 1602.56, 1451.17, 1383.68, 1265.07, 1105.01; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.06 (d, *J*=5.87 Hz, 3H, H-6′), 2.52 (br s, 1H, OH), 3.61 (d, *J*=10.27 Hz, 1H, H-5$_a$), 3.76–3.86 (m, 2H, H-2, H-3), 4.03 (br s, 1H, H-4), 4.15–4.26 (m, 2H, H-5$_b$, H-5″), 4.32 (dd, *J*=9.54, 5.87 Hz, 1H, H-5′), 4.49–4.61 (m, 3H, H-1, H − 6″$_a$, $-CH_2Ph_a$), 4.70 (dd, *J*=12.10, 3.30 Hz, 1H, H − 6″$_b$), 4.90 (d, *J*=11.74 Hz, 1H, $-CH_2Ph_b$), 5.01 (s, 1H, H-1′), 5.13 (d, *J*=7.34 Hz, 1H, H-1″), 5.54 (t, *J*=9.90 Hz, 1H, H-4′), 5.58–5.64 (m, 2H, H-2″, H-2′), 5.67–5.76 (m, 2H, H-4″, H-3′), 5.95 (t, *J*=9.90 Hz, 1H, H-3″), 7.13–7.65 (m, 26H), 7.78–7.96 (m, 10H), 8.04 (d, *J*=7.34 Hz, 2H), 8.07 (d, *J*=7.34 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 17.33 (C-6′), 63.14 (C-6″), 63.41 (C-5), 66.90 (C-5′), 69.76 (C-4″), 70.16 (C-3′), 70.27 ($-CH_2Ph$), 70.76 (C-2′), 71.84 (C-4′), 72.29 (C-2″), 72.40 (C-3), 72.49 (C-5″), 72.83 (C-3″), 76.18 (C-2), 77.53 (C-4), 97.85 (C-1′), 99.54 (C-1), 102.48 (C-1″), 127.98, 128.37, 128.41, 128.46, 128.50, 128.55, 126.67, 128.86, 128.95, 129.35, 129.47, 129.61, 129.67, 129.77, 129.81, 129.87, 129.96, 130.02, 133.16, 133.27, 133.36, 133.57, 137.03, 165.26, 165.42, 165.71, 165.86, 166.18; MALDI-TOF *m*/*z* 1300.2690 (obsd $[M+Na]^+$), 1276.2640 (calcd for $C_{73}H_{64}O_{21}$).

4.5. Benzyl-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-3-*O*-benzoyl-α-L-arabinopyranoside (9)

To a solution of **8** (120 mg, 0.0939 mmol, 1 equiv) in dry pyridine (5 mL), BzCl (21.8 μL, 0.188 mmol, 2 equiv) was added slowly at 0 °C. After addition was complete, the reaction mixture was warmed up to rt and stirred overnight. Water (5 mL) was added to quench the reaction and mixture was neutralized with 10% HCl (20 mL×3)to eliminate pyridine, extracted with CH_2Cl_2 (60 mL), and washed with brine (25 mL). The organic layer was dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. The product was purified by a silica gel column chromatography using the 2:1 hexanes/ethyl acetate to give compound (**9**) as white amorphous solids (116 mg, 89%). Mp 113–116 °C; $[\alpha]_D$ +52.8 (*c* 0.1, $CHCl_3$); R_f=0.21 (hexanes/ethyl acetate, 2:1, v/v); IR ν_{max} (KBr, cm^{-1}) 2932, 1734, 1602, 1451, 1385, 1265, 1093; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.06 (d, *J*=6.53 Hz, 3H, H-6′), 3.75 (d, *J*=11.54 Hz, 1H, H-5$_a$), 4.10–4.16 (m, 1H, H-5″), 4.18 (dd, *J*=8.03, 6.02 Hz, 1H, H-2), 4.28 (dd, *J*=12.30, 4.27 Hz, 1H, H-5$_b$), 4.35–4.43 (m, 2H, H-5′, H-4), 4.50 (dd, 1H, H − 6″$_a$), 4.60 (d, *J*=11.04 Hz, 1H, $-CH_2Ph_a$), 4.64–4.71 (m, 2H, H-1, H − 6″$_b$), 4.90 (d, *J*=7.53, 1H, H-1′), 4.95–5.00 (m, 2H, H-1″, $-CH_2Ph_b$), 5.23 (dd, *J*=8.53, 3.01 Hz, 1H, H-3), 5.42 (d, *J*=7.53, 1H, H-2′), 5.53 (t, *J*=10.04 Hz, 1H, H-4′), 5.58–5.65 (m, 1H, H, H-2″),

5.67–5.74 (m, 2H, H-3′, H-4″), 5.86 (t, J=9.54 Hz, 1H, H-3″), 7.02–8.06 (m, 45H); ^{13}C NMR (63 MHz, $CDCl_3$) δ ppm 17.21 (C-6′), 62.82 (C-6″), 64.17 (C-5), 67.08 (C-5′), 69.52 (C-4″), 69.80 (C-3′), 70.39 (C-2′), 70.51 (−CH_2Ph), 71.84 (C-4′), 72.02 (C-2″), 72.23 (C-5″), 72.67 (C-3″), 73.75 (C-2), 73.74 (C-3), 74.21 (C-4), 98.04 (C-1′), 100.04 (C-1), 101.73 (C-1″), 127.90, 128.30, 128.34, 128.45, 128.77, 128.81, 129.23, 129.40, 129.59, 129.67, 129.75, 129.83, 132.81, 133.05, 133.30, 133.50, 137.06, 164.61, 165.14, 165.48, 165.81, 166.02, 166.12; MALDI-TOF m/z 1403.4294 (obsd [M+Na]$^+$), 1380.4202 (calcd for $C_{80}H_{68}O_{22}$).

4.6. 1-Hydroxy-2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-3-*O*-benzoyl-α-L-arabinopyranoside (10)

A suspension of the fully protected disaccharide **9** (140 mg, 0.101 mmol, 1 equiv), and palladium 10 wt. % on carbon (650 mg) in MeOH/THF (1:1, 40 mL) was stirred for 3 h at rt under the H_2 atmosphere. The reaction mixture was filtered with Celite and the filtrate was concentrated under reduced pressure. The product was purified by a silica gel column chromatography using the 2:1 hexanes/ethyl acetate to give compound (**10**) as white amorphous solids (α/β mixture; α/β=1:2) (112 mg, 86%); R_f=0.14 (hexanes/ethyl acetate, 2:1, v/v). MALDI-TOF m/z 1336.6494 (obsd [M+Na]$^+$), 1313.6417 (calcd for $C_{73}H_{62}O_{22}$).

4.7. *tert*-Butyl diphenyl silyl 23-*O*-benzoylhederagenate (2)

To a solution of hederagenin (70 mg, 0.148 mmol, 1 equiv) in pyridine (2 mL) was added BzCl (20.6 μL, 0.178 mmol, 1.2 equiv) at 0 °C. After addition completed, the reaction mixture was warmed up to rt. Water (0.5 mL) was added to quench the reaction and mixture was neutralized with 10% HCl (5 mL×3) to eliminate pyridine, extracted with CH_2Cl_2 (10 mL), and washed with brine (5 mL) and dried over $MgSO_4$. The mixture was filtered and the filtrate was concentrated under reduced pressure to give crude. To a solution of crude, imidazole (30 mg, 0.444 mmol 3 equiv) in DMF was added TBDPSCl (46 μL, 1.78 mmol, 1.2 equiv) at 80 °C. When reaction was finished, cooled to rt and the reaction mixture was quenched with slow addition of water (1 mL), extracted with ethyl acetate (10 mL), and washed with brine (5 mL). The organic layer was dried over $MgSO_4$, filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography using the 2:1 hexanes/ethyl acetate to give compound (**2**) as white amorphous solids (96.6 mg, 80%). Mp 154–157 °C; $[\alpha]_D$ +26.2 (c 0.1, $CHCl_3$); R_f=0.5 (hexanes/ethyl acetate, 2:1, v/v); IR ν_{max} (KBr, cm^{-1}) 3449, 2930, 2860, 1717, 1654, 1459, 1385, 1271, 1116; ^{1}H NMR (250 MHz, $CDCl_3$) δ ppm 0.40 (s, 3H), 0.83 (s, 3H), 0.91 (s, 6H), 0.94 (s, 3H), 1.08 (s, 3H), 1.13 (s, 9H), 1.15–2.19 (m, 22H), 2.88 (dd, J=13.90, 4.26 Hz, 1H), 3.42–3.52 (m, 1H), 3.98 (d, J=11.37 Hz, 1H), 4.50 (d, J=11.53 Hz, 1H), 5.26 (br s, 1H), 7.29–8.07 (m, 15H); ^{13}C NMR (63 MHz, $CDCl_3$) δ ppm 12.20, 16.00, 17.17, 18.33, 19.48, 23.52, 23.65, 25.49, 26.29, 27.24, 27.61, 30.88, 32.51, 32.78, 33.23, 34.15, 36.92, 38.62, 39.39, 41.87, 42.00, 42.64, 46.41, 48.08, 48.40, 66.91, 72.53, 122.48, 127.63, 127.68, 128.62, 129.68, 130.05, 130.33, 132.09, 132.21, 133.24, 135.89, 135.93, 143.68, 166.91, 176.70; MALDI-TOF m/z 837.4901 (obsd [M+Na]$^+$), 814.4993 (calcd for $C_{53}H_{70}O_5Si$).

4.8. *tert*-Butyl diphenyl silyl 3-*O*-(2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→4)]-3-*O*-benzoyl-α-L-arabinopyranoyl)-23-*O*-benzoylhederagenate (11)

A suspension of compound **10** (144 mg, 0.11 mmol, 1 equiv), phthalic anhydride (18.2 mg 0.12 mmol, 1.1 equiv), 4 Å molecular sieves (100 mg), and DBU (18.3 μL, 0.12 mmol, 1.1 equiv) in CH_2Cl_2 (5 mL) was stirred for 15 min at rt under the N_2 atmosphere, and then to the reaction mixture were added in sequence DTBMP (50.4 mg 0.25 mmol, 2.2 equiv) and Tf_2O (28 μL, 0.17 mmol, 1.5 equiv) at −78 °C and stirred another 15 min. And then reaction mixture was slowly added to the protected hederagenin **2** (100 mg 0.12 mmol, 1.1 equiv) at −78 °C, stirred briefly at this temperature, and warmed up to 0 °C for 1 h. The mixture was neutralized with $NaHCO_3$ (5 mL), extracted with ethyl acetate (10 mL), washed with brine (5 mL), and dried over $MgSO_4$. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography using the 2:1 hexanes/ethyl acetate to give compound (**11**) as white amorphous solids (163 mg, 70%). Mp 97–100 °C; $[\alpha]_D$ +71.0 (c 0.1, $CHCl_3$); R_f=0.35 (hexanes/ethyl acetate, 2:1, v/v); IR ν_{max} (KBr, cm^{-1}) 2932, 2863, 1727, 1602, 1451, 1384, 1264, 1094; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.36 (s, 3H), 0.41 (s, 3H), 0.83 (s, 3H), 0.90 (s, 3H), 0.94 (s, 3H), 1.02 (s, 3H), 1.12 (s, 9H), 1.16 (d, J=6.02 Hz, 3H, H′-6), 1.18–1.97 (m, 22H), 2.88 (dd, J=13.05, 3.51 Hz, 1H, H-18), 3.48 (dd, J=11.29, 4.27 Hz, 1H, H-3), 3.79 (dd, J=10.54, 4.02 Hz, 1H, H-5$_a$), 3.91 (d, J=11.54 Hz, 1H, H-28$_a$), 4.10 (d, J=11.54 Hz, 1H, H-28$_b$), 4.22–4.32 (m, 4H, H-2′, H-5′, H-5″, H-5), 4.49 (dd, J=8.53, 4.52 Hz, 1H, H-4′), 4.54 (dd, J=12.55, 4.02 Hz, 1H, H − 6$''_a$), 4.76 (dd, J=12.30, 3.26 Hz, 1H, H − 6$''_b$), 4.85 (s, 1H, H-1′), 5.15 (d, J=8.03 Hz, 1H, H-1″), 5.27 (m, 2H, H-1, H-12), 5.34 (br s, 1H, H-3′), 5.54 (dd, J=10.04, 8.03 Hz, 1H, H-2″), 5.60 (t, J=10.04 Hz, 1H, H-4), 5.70 (br s, 1H, H-2), 5.71–5.77 (m, 1H, H-4″), 5.77–5.81 (m, 1H, H-3), 5.90 (t, J=9.54 Hz, 1H, H-3″), 7.08–8.11 (m, 55H); ^{13}C NMR (100 MHz, $CDCl_3$) δ ppm 12.66, 15.80, 17.10, 17.55, 18.10, 19.42, 23.48, 23.62, 25.28, 25.32, 27.20, 27.52 (C-6′), 30.82, 32.47, 32.61, 33.19, 34.12, 36.50, 38.55, 39.31, 41.83, 41.92, 42.35, 46.36, 48.03, 48.08, 48.13, 62.91 (C-6″, C-28), 65.49 (C-5), 67.55 (C-5″), 68.48 (C-3′), 69.75 (C-4″), 69.89 (C-3), 70.86 (C-2, C-4′), 71.68 (C-2″), 71.78 (C-4), 72.26 (C-5′), 73.03 (C-3″), 73.96 (C-2′), 82.09 (C-3), 97.91 (C-1′),100.17 (C-1), 100.86 (C-1″), 122.47, 127.59, 127.63, 127.99, 128.17, 128.30, 128.39, 128.45, 128.47, 128.56, 128.63, 128.88, 128.97, 128.99, 129.32, 129.41, 129.52, 129.80, 129.85, 129.88, 129.91, 130.01, 130.09, 130.43, 132.17, 132.92, 133.04, 133.18, 133.31, 133.55, 133.85, 135.88, 143.61, 165.03, 165.19, 165.51, 165.72, 165.85, 165.88, 166.11, 176.63; MALDI-TOF m/z 2109.8608 (obsd [M+Na]$^+$), 2086.8620 (calcd for $C_{126}H_{130}O_{26}Si$).

4.9. Pulsatilla saponin D (1)

To a solution of the fully protected compound **11** (65 mg, 0.031 mmol, 1 equiv) in THF (5 mL) was added TBAF in THF (1.0 mol/L, 62 μL) at rt. When starting material was completely disappeared on TLC, potassium *tert*-butoxide in THF solution (1.0 mol/L, 467 μL) was added and the mixture was stirred overnight. The reaction mixture was quenched with Amberlite IR-120H resin to pH 7 and filtered and the filtrate was concentrated under reduced pressure. The residue was purified by a silica gel column chromatography using the 20:10:1 chloroform/methanol/water to give pulsatilla saponin D (**1**) as white solids (22.4 mg, 84%). Mp 239–242 °C; $[\alpha]_D$ +17.0 (c 0.2, CH_3OH) [lit.[5] mp 239–242; $[\alpha]_D$ +14.9]; R_f=0.29 (chloroform/methanol/water, 20:10:1, v/v); IR ν_{max} (KBr, cm^{-1}) 3434, 2931, 1702, 1637, 1460, 1385, 1264, 1075; ^{1}H NMR (400 MHz, Pyr-d_5) δ ppm 0.93 (s, 3H), 0.99 (s, 3H), 1.01 (s, 3H), 1.08 (s, 3H), 1.18–2.21 (m, 22H), 1.23 (s, 3H), 1.64 (d, J=6.53 Hz, 3H, H-6′), 3.28 (d, J=11.04 Hz, 1H, H-18), 3.64 (d, J=12.05 Hz, 1H, H-5$_a$), 3.73 (d, J=10.54 Hz, 1H, H-28$_a$), 3.85–3.91 (m, 1H, H-5″), 3.98–4.07 (m, 2H, H-3, H-2″), 4.12–4.25 (m, 5H, H-4, H-3″, H-4″, H-3, H-28$_b$), 4.25–4.32 (m, 1H, H-4′), 4.36–4.41 (m, 2H, H-6″), 4.45–4.54 (m, 2H, H-2, H-5$_b$), 4.62 (dd, J=3.03, 6.01 Hz, 1H, H-3′), 4.67–4.75 (m, 2H, H-5′, H-2′), 4.97 (d, J=7.03 Hz, 1H, H-1), 5.11 (d, J=7.53 Hz, 1H, H-1″), 5.46 (br s, 1H, H-12), 6.25 (s, 1H, H-1′); ^{13}C NMR (100 MHz, Pyr-d_5) δ ppm 13.94, 15.97, 17.34, 18.01, 18.52 (C-6′), 23.57, 23.67, 23.73, 26.06, 26.20, 28.24, 30.84, 32.75, 33.11, 33.16, 34.10, 36.78,

38.87, 39.63, 41.85, 42.04, 43.39, 46.31, 46.53, 47.66, 48.05, 62.37 (C-6″), 63.77 (C-28), 65.34 (C-5), 69.52 (C-5′), 71.10 (C-4″), 72.16 (C-2′), 72.34 (C-3′), 74.00 (C-4′), 74.95 (C-3), 75.37 (C-2″), 76.13 (C-2), 78.42 (C-3″), 78.67 (C-5″), 80.36 (C-4), 80.92 (C-3), 101.57 (C-1′), 104.30 (C-1), 106.65 (C-1″), 122.47, 144.72, 180.14; MALDI-TOF m/z 935.5026 (obsd $[M+Na]^+$), 912.5083 (calcd for $C_{47}H_{76}O_{17}$).

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Supplementary data

A supplementary data file (^1H and ^{13}C NMR) of the natural and synthesized pulsatilla saponin D (**1**). Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2013.04.095.

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