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# The synthesis of pennogenin 3-O-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside

Xin-Feng Luo[a], Fan Lei[a], Yi He[a], Shu-Chen Pei[a], Li Hai[a], Shan Qian[b] and Yong Wu[a]*

[a]*Key Laboratory of Drug Targeting and Drug Delivery Systems of the Education Ministry, West China School of Pharmacy, Sichuan University, Chengdu 610041, China;*
[b]*Bioengineering College, Xihua University, Chengdu 610039, China*

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Pennogenin 3-O-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, a monodesmosidic saponin isolated from *Paris polyphylla* Smith var. *yunnanensis* with promised antitumor activities, was firstly synthesized from glucoside thiol via nine steps and with 27% overall yield.

**Keywords:** pennogenin; α-L-rhamnopyranoside; β-D-glucopyranoside; antitumor activity; synthesis

## 1. Introduction

Spirostanol glycosides constitute a large group of steroidal saponins and have a broad range of interesting bioactivities [1]. Among the spirostanol glycosides, saponin **1** (pennogenin 3-O-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside; Figure 1) was isolated firstly from the dried rhizome of *Paris axialis* H. Li, and it also exists widely in nature, in plants used in traditional Chinese herbal medicine, such as Rhizoma Paridis ('Chonglou' in Chinese) [2]. This saponin exhibits potent antitumor activity with the $IC_{50}$ values ranging from 0.5 to 5.1 μg/ml against human promyelocytic leukemia HL-60 cells [2a]. On the other hand, cytometric analysis indicated that saponin **1** caused a concentration- and time-dependent apoptosis of L1210 cell ($EC_{50} = 5$ μM) [2b]. Recently, we have also isolated it from *P. polyphylla* var. *yunnanensis*, which shows strong antitumor activity against human HepG 2 cells with $IC_{50}$ value of 2.25 μg/ml, using hydroxycamptothecin as positive control ($IC_{50} = 2.15$ μg/ml). In order to further study the anticancer activity of saponin **1**, saponin **1** was first synthesized.

## 2. Results and discussion

This monodesmosidic saponin **1** bears three 1,2-trans-pyranosidic linkages, which could be constructed stereo-selectively by stepwise glycosylation with a sugar donor equipped with a participating group at its 2-OH. However, employing this strategy, the aglycone participating in reactions continuously must be consumed heavily unavoidably. Herein, the aglycone (pennogenin) is very precious, which was isolated from the rhizome of *Paris polyphylla* Smith *var. yunnanensis* (Pyunnanensis) [3]. So we select to prepare a suitably protected and activated oligosaccharide donor first, and then attach it to the aglycone. Compound **1** was synthesized as shown in Schemes 1 and 2.

*Corresponding author. Email: wyong@scu.edu.cn

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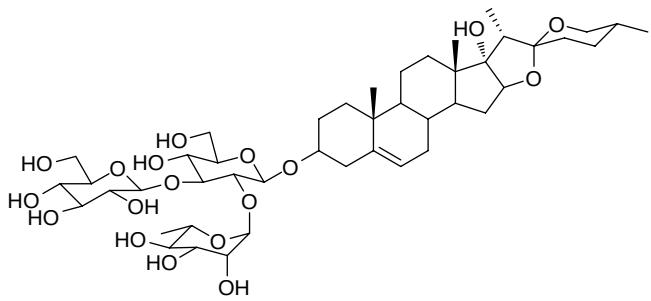
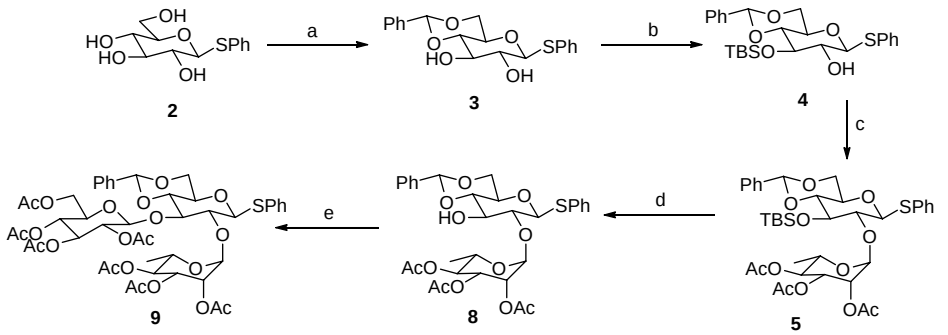


Figure 1. Structure of compound **1**.

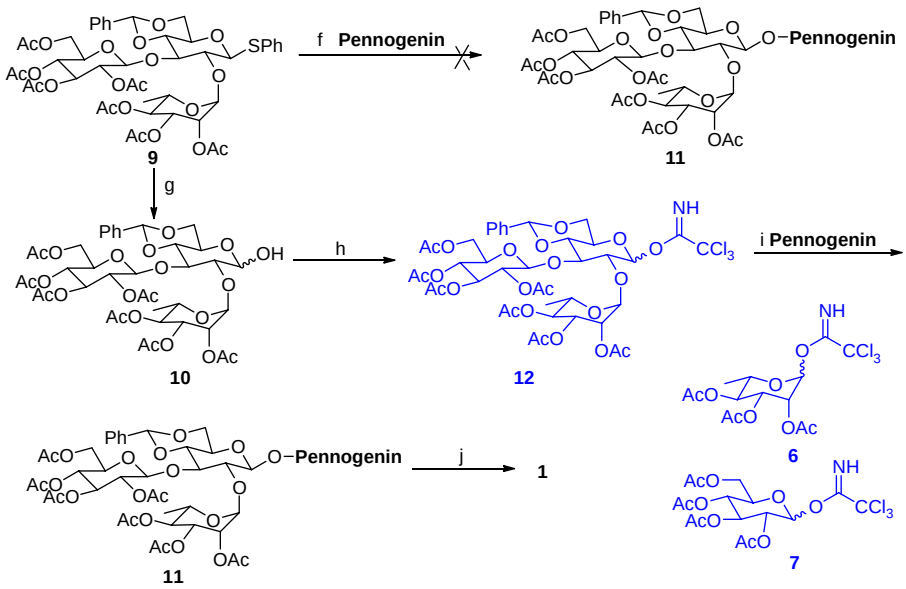


Scheme 1. Synthesis of intermediate **9**. Reagents and conditions: (a) $PhCH(OMe)_2$, TsOH, DMF, 60°C, 2 h, 100%; (b) TBDMSCl, imidazole, $CH_2Cl_2$, 40°C, 4 h, 95%; (c) **6**, $BF_3{\cdot}Et_2O$, 4 Å MS, $CH_2Cl_2$, −78°C, 3 h, 89%; (d) TBAF, THF, r.t., 6 h, 80%; (e) **7**, $BF_3{\cdot}Et_2O$, 4 Å MS $CH_2Cl_2$, −78°C, 5 h, 64%.

Compound **2** was afforded through three steps from D-glucose [4], which was converted to diol **3** by protection of the 4′,6′-OH with an O-benzylidene group [5]. Reaction of **3** with *tert*-butyldimethylsilyl (TBDMS) chloride in the presence of imidazole resulted in a totally regioselective silylation at C-3, affording compound **4** in high yield [6]. Compound **4**, having its 2′-OH free, was glycosylated by 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl trichloroacetimidate **6** [7]. In the presence of borontrifluoride ether complex, compound **5** was provided [8,9]. Desilylation of **5** under tetrabutylammonium fluoride (TBAF) gave **8** (80% yield) with 3′-OH free [10,11], which was treated with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate **7** [12] in the presence of borontrifluoride ether complex to furnish triosaccharide **9** in 64% yield.

Subsequently, thiosaccharide **9** was used as a glycosyl donor to transfer to aglycone by standard glycosylation. Unfortunately, direct glycosylation of pennogenin with **9** under the promotion of N-iodosuccinimide (NIS)–TfOH was troublesome [13]. The reaction resulted in a complex mixture of products and the desired protected glycoside resulted in a poor yield. A detailed evaluation of the result suggested that thioglycoside **9** was not an active glycosyl donor matching 3-OH of pennogenin aglycone in this glycosylation.

To circumvent this problem, compound **9** was converted into more efficient trichloroacetimidate **12** in 86% yield in




Scheme 2. Synthesis of saponin **1**. Reagents and conditions: (f) NIS, TfOH, 4 Å MS, $CH_2Cl_2$; (g) NBS, acetone–$H_2O$, 0°C → r.t., 2 h, 93%; (h) $CNCCl_3$, DBU, $CH_2Cl_2$, 0°C → r.t., 3 h, 92%; (i) TMSOTf, 4 Å MS, $CH_2Cl_2$, 0°C → r.t., 87%; (j) 80% HOAc, 3 h, 70°C; NaOMe/MeOH, r.t., overnight, 84% (two steps).

two steps as follows: (i) N-bromosuccinimide (NBS) promoted chemoselective hydrolysis of thioglycoside **9** in acetone–$H_2O$ co-solvent system to get compound **10** [14]; (ii) trichloroacetimidate activation of anomeric carbon using trichloroacetonitrile ($CCl_3CN$) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane. As expected, trisaccharide imidate **12** was successfully and efficiently transformed to the aglycone in the presence of TMSOTf at 0°C to afford the corresponding protected glycose **11** in 87% yield [15–17].

The aglycone with trisaccharide imidate will always provide the α and β glycoside; interestingly, the stereo-selectivity of this glycosylation was predominantly β stero-controlled without the neighboring group participation effect of donor **12**, which was adjusted by the coupling constant 7.8 Hz of the glucopyranosyl H-1 in $^1$H NMR spectrum and in $^{13}$C NMR spectrum the chemical shift of C-1 at δ 99.9. Debenzylidenation of **11** under 80% HOAc, followed by deacetylation under NaOMe in MeOH, furnished the naturally existing saponin **1** [10,18].

## 3. Experimental

### *3.1 General experimental procedures*

Melting points were observed in an open capillary tube and are uncorrected. Elemental analyses were carried out by Atlantic Microlab (Atlanta, GA, USA). $^1$H and $^{13}$C NMR spectra were acquired on a Bruker AC-E 200 ($^{13}$C NMR) or a Varian INOVA-400/54 ($^1$H NMR) spectrometer in $CDCl_3$, with TMS as an internal standard. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument. All starting materials were commercially available and of analytical grade. All reactions were carried out under inert gas (argon). Reaction progress was monitored by TLC, performing on precoated silica gel $GFA_{254}$ plates (0.2 mm; Chemical Industry Institute, Yantai, China). Column chromatography was performed using

silica gel (10–40 μm; Qingdao Sea Chemical Factory, Qingdao, China).

### 3.2 Preparation of key intermediates and compound 1

#### 3.2.1 4,6-O-benzylidene-1-thio-β-D-glucopyranoside (3)

To a solution of **2** (1.69 g, 6.2 mmol) and $PhCH(OMe)_2$ (2 ml, 13.3 mmol) in dry DMF (30 ml) was added *p*-TsOH (until the pH of the solution reached 2–3). The resulting mixture, stirred at 60°C under reduced pressure for 3 h, was neutralized by $Et_3N$ (3.0 ml), diluted with EtOAc (50 ml), then washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated. Chromatography of the residue on a silica gel column (20:1, petroleum ether–EtOAc) afforded **3** as a white solid (2.22 g, 100%); $R_f$ 0.15 ($CH_2Cl_2$/MeOH: 50/1); mp: 172–174°C; $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.54–7.52 (m, 2H, Ar), 7.48–7.46 (m, 2H, Ar), 7.34–7.32 (m, 6H, Ar), 5.50 (s, 1H, PhCH), 4.59 (d, 1H, $J_{1,2} = 10.0$ Hz, H-1), 4.35 (dd, $J_{4,5} = 4.4$ Hz, $J_{3,4} = 10.8$ Hz, H-4), 3.81–3.71 (m, 2H, $H_2$-6), 3.51–3.41 (m, 3H, H-2, H-3, H-5); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 136.7, 132.9, 131.2, 129.1, 128.9, 128.1, 128.0, 126.1, 101.5, 88.4, 80.0, 74.4, 72.4, 70.5, 66.4; elemental analysis: Found: C, 63.32%, H, 5.59%; calcd for $C_{19}H_{20}O_5S$: C, 62.25%, H, 5.50%; ESI-MS: *m/z* 361 $[M + H]^+$.

#### 3.2.2 3-O-butyldimethysilyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (4)

To a solution of **3** (1.0 g, 2.78 mmol) in dry $CH_2Cl_2$ (20 ml), TBDMS chloride (0.617 g, 4.17 mmol) and imidazole (0.378 g, 5.56 mmol) were added under an argon atmosphere and the mixture was refluxed overnight. After dilution with EtOAc (50 ml), the crude was submitted to standard work-up and purified by silica gel column (35:1, petroleum ether–EtOAc) to afford **4** as a colorless oil (1.25 g, 95%); $R_f$ 0.7 (8:1, petroleum ether–EtOAc); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.60–7.58 (m, 2H, Ar), 7.48–7.46 (m, 2H, Ar), 7.41–7.35 (m, 6H, Ar), 5.52 (s, 1H, PhCH), 4.66 (d, 1H, $J_{1,2} = 9.6$ Hz, H-1), 4.37 (dd, 1H, $J_{4,5} = 4.0$ Hz, $J_{3,4} = 10.4$ Hz, H-4), 3.80–3.76 (m, 2H, $H_2$-6), 3.50–3.41 (m, 3H, H-2, H-3, H-5), 1.27 (s, 6H, $2 \times CH_3$), 0.87 (s, 9H, $3 \times CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 137.3, 133.5, 131.8, 129.6, 129.3, 128.6, 128.5, 126.6, 101.9, 89.0, 80.6, 74.9, 73.0, 71.1, 67.0, 31.1, 26.3, −4.85, −4.21; elemental analysis: Found: C, 63.26%, H, 7.22%; calcd for $C_{25}H_{34}O_5SSi$: C, 63.20%, H, 7.19%; ESI-MS: *m/z* 475 $[M + H]^+$.

#### 3.2.3 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-3-O-butyldimethysilyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (5)

To a solution of **4** (1.28 g, 2.7 mmol) and 4 Å MS (2 g) in dry $CH_2Cl_2$ (30 ml) at −78°C under Ar was added borontrifluoride–ether complex (0.65 ml, 5.28 mmol), then a solution of **6** (3.6 g, 8.3 mmol) in $CH_2Cl_2$ (10 ml). The mixture, warmed up naturally to room temperature (r.t.) and allowed to stir for another 3 h, was then neutralized with $Et_3N$ (1 ml), filtered, and concentrated. The residue was chromatographed on a silica gel column (25:1, petroleum ether–EtOAc) to afford **5** as a white solid (1.79 g, 89%); $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.57–7.54 (m, 2H, Ar), 7.47–7.44 (m, 2H, Ar), 7.40–7.31 (m, 6H, Ar), 5.52 (s, 1H, PhCH), 5.40–5.30 (m, 3H, H-1′, H-2′, H-3′), 5.14 (t, $J = 9.6$ Hz, 1H, H-4′), 4.71 (d, 1H, $J = 10$ Hz, H-1), 4.52–4.41 (m, 1H, H-5′), 4.35 (dd, 1H, $J_{6a,5} = 4.4$ Hz, $J_{6a,6b} = 10.6$ Hz, H-6a), 3.93 (t, $J = 8.8$ Hz, 1H, H-3), 3.79–3.71 (m, 1H, H-6b), 3.66 (t, $J = 8.4$ Hz, 1H, H-2), 3.48–3.41 (m, 2H, H-4, H-5), 2.15 (s, 3H), 2.07 (s, 3H), 2.01 (s, 3H), 1.29 (d, 3H, $J = 6.8$ Hz), 0.89 (s, 9H), 0.11 (s, 3H), 0.04 (s, 3H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 170.4, 137.5, 133.7, 131.9, 129.8, 129.5, 128.8, 128.6, 126.7, 106.1, 101.9, 91.1,

86.3, 82.4, 79.1, 78.7, 71.1, 71.1, 70.9, 69.0, 68.2, 31.0, 26.1, 21.1, 17.0, −2.1; elemental analysis: Found: C, 59.50%, H, 6.75%; calcd for $C_{37}H_{50}O_{12}SSi$: C, 59.48%, H, 6.71%; ESI-MS: *m/z* 748 $[M + H]^+$.

### *3.2.4 2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1 → 2)-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**8**)*

To a solution of **5** (0.67 g, 0.90 mmol) in dry THF (10 ml) was added a solution of $BU_4NF$ in THF (0.7 g, 2.7 mmol, 0.5 M). The mixture, which was stirred at r.t. for 5 h, was diluted with EtOAc, washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated. The residue was chromatographed on silica gel column (6:1, petroleum ether–EtOAc) to give **8** as a colorless oil (0.45 g, 80%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.57–7.54 (m, 2H, Ar), 7.47–7.44 (m, 2H, Ar), 7.40–7.31 (m, 6H, Ar), 5.49 (s, 1H, PhCH), 5.37–5.30 (m, 3H, H-1′, H-2′, H-3′), 5.12 (t, $J = 9.6$ Hz, 1H, H-4′), 4.70 (d, 1H, $J = 10.0$ Hz, H-1), 4.47–4.40 (m, 1H, H-5′), 4.33 (dd, 1H, $J_{6a,5} = 4.4$ Hz, $J_{6a,6b} = 10.6$ Hz, H-6a), 3.90 (t, $J = 8.4$ Hz, 1H, H-3), 3.75–3.70 (m, 1H, H-6b), 3.65 (t, $J = 8.8$ Hz, 1H, H-2), 3.46–3.42 (m, 2H, H-4, H-5), 2.14 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H), 1.27 (d, 3H, $J = 8.0$ Hz); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 170.3, 137.4, 133.6, 131.8, 129.7, 129.4, 128.7, 128.5, 126.6, 106.0, 101.8, 91.0, 86.2, 82.3, 79.0, 78.6, 71.0, 71.0, 70.8, 68.9, 68.1, 30.9, 21.1, 16.9; elemental analysis: Found: C, 58.85%, H, 5.74%; calcd for $C_{31}H_{36}O_{12}S$: C, 58.70%, H, 5.66%; ESI-MS: *m/z* 633 $[M + H]^+$.

### *3.2.5 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-1-thio-β-D-glucopyranoside (**9**)*

To a solution of **8** (0.36 g, 0.58 mmol) and 4 Å MS (1.2 g) in dry $CH_2Cl_2$ (10 ml) at −78°C under Ar was added borontrifluoride–ether complex (0.15 ml, 1.17 mmol), then a solution of **7** (0.76 g, 1.75 mmol) in $CH_2Cl_2$ (5 ml). The mixture, warmed up naturally to r.t. and allowed to stir for another 8 h, was then neutralized with $Et_3N$ (1 ml), filtered, and concentrated. The residue was chromatographed on a silica gel column (25:1, petroleum ether–EtOAc) to afford **9** as a colorless oil (0.36 g, 64%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.53–7.33 (m, 10H, 2 × Ph), 5.50 (s, 1H, PhCH), 5.40–5.39 (m, 1H), 5.36 (s, 1H), 5.28 (dd, 1H, $J = 4.4$, 10.6 Hz), 5.20 (t, 1 H, $J = 9.6$ Hz), 5.14 (t, 1H, $J = 9.2$ Hz), 5.05–4.98 (m, 2H), 4.85 (d, 1H, $J = 8.0$ Hz), 4.72 (d, 1H, $J = 6.0$ Hz), 4.59–4.52 (m, 1H), 4.18–4.11 (m, 2H), 3.97 (dd, 1H, $J = 4.8$, 10.0 Hz), 3.85 (t, 1H, $J = 8.8$ Hz), 3.74 (t, 1H, $J = 9.6$ Hz), 3.64–3.59 (m, 2H), 3.47–3.41 (m, 1H), 2.24 (s, 3H), 2.06 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.95 (s, 3H), 1.23 (d, $J = 6.3$ Hz, $CH_3$-6′); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 170.1, 137.5, 133.5, 131.7, 129.9, 129.0, 128.8, 128.5, 126.6, 106.7, 106.0, 101.8, 91.0, 86.2, 82.3, 79.0, 78.6, 74.8, 72.6, 71.0, 71.0, 70.8, 69.3, 68.9, 68.1, 62.7, 30.6, 21.0, 16.7; elemental analysis: Found: C, 56.13%, H, 5.65%; calcd for $C_{45}H_{54}O_{21}S$: C, 56.02%, H, 5.56%; ESI-MS: *m/z* 963 $[M + H]^+$.

### *3.2.6 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 (**10**)*

A solution of **9** (100 mg, 0.1 mmol) in 50:1 acetone–$H_2O$ (15 ml) was added to NBS (140 mg, 0.8 mmol) at 0°C. The mixture, warmed up naturally to r.t. and allowed to stir for another 1 h, was then neutralized with $Na_2S_2O_3$ (100 mg). The solvent was removed under vacuum to give a residue, which was dissolved in $CH_2Cl_2$ (20 ml), washed with brine and water, dried over

anhydrous $Na_2SO_4$, and concentrated. The residue was chromatographed on silica gel column (4:1, petroleum ether–EtOAc) to give **10** as a colorless oil (84 mg, 93%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.51–7.48 (m, 2H, Ar), 7.37–7.29 (m, 3H, Ar), 5.38 (s, 1H, PhCH), 5.33 (s, 1H), 5.30–5.23 (m, 2H), 5.15–5.06 (m, 2H), 5.03–4.97 (m, 1H), 4.89 (d, 1H, $J = 7.6$ Hz), 4.66 (d, 1H, $J = 7.2$ Hz), 4.52–4.48 (m, 1H), 4.27–4.22 (m, 1H), 4.18–4.14 (m, 1H), 3.92–3.88 (m, 2H), 3.83–3.80 (m, 2H), 3.73–3.69 (m, 1H), 3.56 (s, 1H), 3.47 (t, 1H, $J = 8.0$ Hz), 3.36–3.31 (m, 1H), 2.24 (s, 3H, $CH_3CO$), 2.09 (s, 3H, $CH_3CO$), 2.07 (s, 3H, $CH_3CO$), 2.07 (s, 3H, $CH_3CO$), 2.04 (s, 3H, $CH_3CO$), 2.03 (s, 3H, $CH_3CO$), 1.98 (s, 3H, $CH_3CO$), 1.21 (d, $J = 6.0$ Hz, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 170.7, 137.7, 129.5, 128.5, 126.4, 106.5, 106.1, 101.9, 97.8, 86.3, 82.4, 79.1, 78.7, 74.9, 72.7, 71.1, 71.1, 70.9, 69.4, 68.9, 68.2, 62.8, 30.7, 21.1, 16.6; elemental analysis: Found: C, 53.79%, H, 5.79%; calcd for $C_{39}H_{50}O_{22}$: C, 53.59%, H, 5.81%; ESI-MS: *m/z* 871 $[M + H]^+$.

### *3.2.7 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 -α-D–glucopyranosyl trichloroacetimidate (**12**)*

Trichloroacetonitrile (0.035 ml, 0.34 mmol) was added to a solution of trisaccharide **10** (100 mg, 0.11 mmol) in $CH_2Cl_2$ (3 ml) at 0°C under Ar. After stirring for 30 min, DBU (0.015 ml, 0.1 mmol) was added to the mixture and stirred for 3 h. The mixture was concentrated under diminished pressure, and the residue was purified by silica gel column chromatography (4:1, petroleum ether–EtOAc) to give **12** as a colorless oil (107 mg, 92%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.55–7.53 (m, 2H, Ar), 7.33–7.28 (m, 3H, Ar), 5.38 (s, 1H, PhCH), 5.32–5.25 (m, 3H), 5.16–5.08 (m, 2H), 5.02 (t, 1H, $J = 8.8$ Hz), 4.81 (d, 1H, $J = 8.0$ Hz), 4.71–4.65 (m, 2H), 4.54–4.50 (m, 1H), 4.38–4.33 (m, 1H), 4.28–4.23 (m, 1H), 4.19–4.15 (m, 1H), 3.89–3.78 (m, 3H), 3.65 (t, 1H, $J = 8.4$ Hz), 3.58 (s, 1H), 3.50 (t, 1H, $J = 8.2$ Hz), 2.24 (s, 3H, $CH_3CO$), 2.10 (s, 3H, $CH_3CO$), 2.08 (s, 3H, $CH_3CO$), 2.07 (s, 3H, $CH_3CO$), 2.05 (s, 3H, $CH_3CO$), 2.04 (s, 3H, $CH_3CO$), 1.98 (s, 3H, $CH_3CO$), 1.21 (d, 3H, $J = 6.0$ Hz, $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 170.5, 161.8, 137.5, 129.4, 128.7, 126.3, 106.3, 106.0, 101.7, 92.7, 86.3, 84.3, 82.2, 79.0, 78.6, 74.8, 72.6, 71.0, 71.1, 70.7, 69.3, 68.7, 68.0, 62.6, 30.5, 21.0, 16.7; elemental analysis: Found: C, 48.51%, H, 4.96%, Cl, 10.48%, N, 1.38%; calcd for $C_{41}H_{50}Cl_3NO_{22}$: C, 48.47%, H, 4.81%, Cl, 10.50%, N, 1.41%; ESI-MS: *m/z* 1014 $[M + H]^+$.

### *3.2.8 Pennogenin 3-O-[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-D-glucopyranoside (**11**)*

To a mixture of compound pennogenin (100 mg, 0.23 mmol), **12** (280 mg, 0.28 mmol), and powered 4 Å molecular sieves in dried $CH_2Cl_2$ (10 ml) at 0°C was added TMSOTf (4 μl, 0.023 mmol). After stirring at 0°C and then at r.t. for 1 h, the reaction was quenched with $Et_3N$. The solid was then filtered off. The filtrate was concentrated under vacuum to give colorless oil that was purified by column chromatography (3:1, petroleum ether–EtOAc) to give **11** as a white solid (258 mg, 87%); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$: 7.47–7.37 (m, 5H, Ar), 5.50 (s, 1H, PhCH), 5.40 (d, 1H, H-6), 5.26–5.23 (m, 2H, H-1″, H-2″), 5.20 (dd, $J_{3'',2''} = 3.0$ Hz, $J_{3'',4''} = 10.2$ Hz, H-3″), 5.15 (t, $J_{3''',2'''} = 9.2$ Hz, H-3‴), 5.10 (t, $J = 9.2$ Hz, 1H, H-4″), 5.03 (t, $J = 9.8$ Hz, 1H, H-4‴), 4.94 (t, $J = 9.6$ Hz, 1H, H-2‴), 4.84 (d, 1H, $J_{1''',2'''} = 8.0$ Hz, H-1‴), 4.61 (d, 1H, $J_{1',2'} = 7.8$ Hz, H-1′), 4.55–4.52 (m, 1H, H-5″), 4.31 (dd, 1H, $J_{6'a,5'} = 4.8$ Hz,

$J_{6'a,6'b} = 10.2$ Hz, H-6′a), 4.10–4.07 (m, 2H, H-3′, H-6‴a), 3.98 (t, 1H, $J = 7.5$ Hz, H-16), 3.96 (dd, 1H, H-6‴b), 3.81–3.76 (m, 2H, H-2′, H-6′b), 3.70 (t, 1H, $J_{4',3'} = 9.2$ Hz, H-4′), 3.68–3.62 (m, 1H, H-3), 3.49–3.36 (m, 4H, H-5′, H-5‴, H-26), 2.22, 2.03, 2.02, 2.00, 1.99, 1.97, 1.95 (7s, 21H, $CH_3CO$), 1.20 (d, 3H, $J_{6'',5''} = 6.0$ Hz, $CH_3$-6″), 1.04 (s, 3H, $CH_3$), 0.99 (d, $J = 7.2$ Hz, $CH_3$), 0.80 (s, 3H, $CH_3$), 0.79 (s, 3H, $CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$: 170.1, 140.8, 137.5, 133.5, 131.7, 129.9, 129.0, 128.8, 128.5, 126.6, 121.4, 110.1, 107.8, 106.7, 106.0, 101.8, 90.9, 90.1, 86.2, 82.3, 79.0, 78.6, 74.8, 72.6, 71.7, 71.0, 71.0, 70.8, 69.3, 68.9, 68.1, 66.8, 62.7, 52.8, 49.8, 44.6, 43.9, 42.5, 37.1, 36.6, 32.0, 31.6, 31.6, 31.2, 30.8, 30.1, 29.7, 28.1, 21.5, 21.0, 20.7,19.4, 17.1, 17.0, 16.7, 8.1; elemental analysis: Found: C, 61.77%, H, 7.07%; calcd for $C_{66}H_{90}O_{25}$: C, 61.55%, H, 6.94%; ESI-MS: *m/z* 1284 $[M + H]^+$.

### *3.2.9 Pennogenin 3-O-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**1**)*

A solution of **11** (130 mg, 0.1 mmol) in 80% HOAc (20 ml) was stirred at 70°C for 3 h. The solvent was removed under vacuum to give a residue, which was dissolved in the solution of NaOMe/MeOH (0.4 M, 10 ml), and the mixture was stirred overnight. The solution was neutralized with ion-exchange resin ($H^+$), filtered, and concentrated. The residue was purified by column chromatography to afford **1** as a white solid (76 mg, 84%); mp: 300–302°C; $^1$H NMR (400 MHz, $C_5D_5N$) $\delta$: 6.28 (d, 1H, $J_{1'',2''} = 10.8$ Hz, H-1″), 5.40 (d, 1H, $J = 4.0$ Hz, H-6), 5.11 (d, 1H, $J = 7.8$ Hz, H-1‴), 4.94–4.91 (m, 3H, H-2″, H-3″, H-4″), 4.62–4.54 (m, 3H), 4.46–4.35 (m, 2H), 4.27–4.17 (m, 5H), 4.08–3.90 (m, 6H), 3.61–3.54 (m, 4H), 2.80–2.75 (m, 2H), 2.34–2.28 (m, 2H), 2.17–2.11 (m, 3H), 1.98–1.89 (m, 2H), 1.78–1.73 (m, 6H), 1.61–1.48 (m, 8H), 1.30 (d, 3H, $J = 7.2$ Hz), 1.11 (d, 3H, $J = 4.4$ Hz), 0.99–0.87 (m, 4H), 0.72 (d, 3H, $J = 4.4$ Hz); $^{13}$C NMR (100 MHz, $C_5D_5N$) $\delta$: 140.7, 121.8, 109.8, 104.5, 102.1, 99.9, 90.1, 89.9, 89.5, 78.6, 78.4, 77.8, 77.8, 77.6, 77.0, 75.0, 72.4, 72.4, 71.4, 69.5, 69.5, 66.6, 62.4, 62.4, 53.0, 50.2, 45.1, 44.7, 38.6, 37.5, 37.1, 32.3, 32.0, 32.0, 32.0, 31.8, 30.4, 30.0, 28.8, 20.9, 19.4, 18.6, 17.2, 17.1, 9.7; elemental analysis: Found: C, 59.98%, H, 8.05%; calcd for $C_{45}H_{72}O_{18}$: C, 59.95%, H, 31.98%; ESI-MS: *m/z* 901 $[M + H]^+$.

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