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Article

Synthesis of 5,6-Dihydro-OSW-1 and Its Antitumor Activities†

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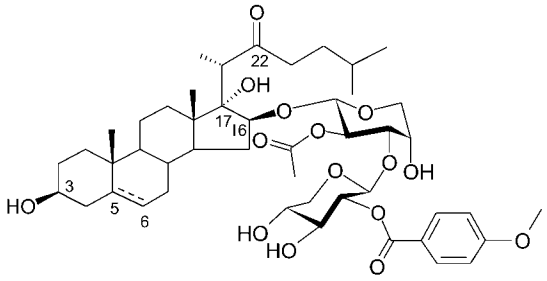
5,6-Dihydro-OSW-1 (**1**) was synthesized following our previous procedure for the total synthesis of OSW-1. This compound demonstrated slightly stronger potency than that of OSW-1 against the growth of cancer cells.

Keywords 5,6-dihydro-OSW-1, analog, synthesis, anti-tumor

Introduction

A group of cholestane saponins featuring a novel 3*β*,16*β*,17*α*-trihydroxycholest-5-en-22-one aglycone with a sugar residue at the 16-OH has been disclosed by Sashida, Mimaki and coworkers, from the bulbs of *Ornithogalum saudersiae* and taxonomically related plants.[1-5] Those saponins have attracted great attention due to their potent anti-tumor activities.[2-5] OSW-1, the major and representative member, was tested against the NCI (the US. National Cancer Institute) 60 cell lines, showing 10—100 times more intense potency than those of the clinically applied anticancer agents, *e.g.*, cisplatin, as positive controls.[2] Requirement of the 16-*O*-disccharide moiety of OSW-1 for its significant cytotoxic activity was clearly concluded, and those with modification on the sugar residue showed much weaker activities.[2,4,5] Inversion of the C-16 configuration, where the disaccharide is attached, was also not allowed to retain the significant cytotoxic activity of OSW-1.[6] However, substitution with a glucose on the 3-OH, a site remote to the 16-*O*-disaccharide, did not affect apparently the cytotoxic activity.[2] Thus, we anticipated that saturation of the 5,6-double bond, a position distant from the disaccharide residue, would not alter significantly the cytotoxicity of OSW-1, and the resulting 5,6-dihydro-OSW-1 (**1**) would be a more convenient target than OSW-1 for chemical synthesis. Here we report the synthesis of **1** and the test of its anti-tumor activity.

Although 5,6-dihydro-OSW-1 (**1**) could be prepared by direct hydrogenolysis of OSW-1, the synthesis was carried out employing a similar route as that we developed previously for the synthesis of OSW-1 to examine whether the yields of some steps could be improved



OSW-1: $\Delta^{5,6}$; **1**: $C^5H(\alpha)$-C^6H_2

with substrates in the absence of the 5,6-double bond[7] (Scheme 1). Thus, hydrogenolysis of 3*β*-hydroxyandrost-5-en-17-one **2** under forced conditions (2.0×10^6 Pa of H_2, 10% Pd/C, 50 ℃, 24 h) afforded **3** in good yield (80%).[8] Wittig olefination of ketone **3** gave diene **4** stereoselectively,[9] which was subjected to TBDPS (*tert*-butyldiphenylsilyl) protection to provide the 3-*O*-TBDPS ether **5**.[10] Ene reaction of **5** with paraformaldehyde in the presence of catalytic BF_3-OEt_2 generated the desired homoallylic alcohol **6** stereoselectively. Oxidation of alcohol **6** with Dess-Martin periodinane provided aldehyde **7**. Grignard addition of aldehyde **7** with 3-methylbutylmagnesium bromide led to the 22*α*-ol **8**, which was oxidized with PDC (pyridium dichromate) to afford C-22-keto **9**. Then, the C-22 carbonyl of **9** was masked as an ethylene glycol ketal under mild conditions (catalytic TsOH, $HC(OEt)_3$, r.t.), providing **10** slowly (6 d). After transformation of the 3-*O*-TBDPS ether into the 3-*O*-TBS ether (two steps), the resulting 16(17)-ene **11** was subjected to OsO_4 (1.2 equiv.) to furnish the desired 16*α*,17*α*-diol **12** in good yield (77%). The 16*α*-OH configuration was then inverted via an oxidation-reduction sequence: TPAP/NMO (tetrapropylammounium perruthenate/4-morpholin-*N*-

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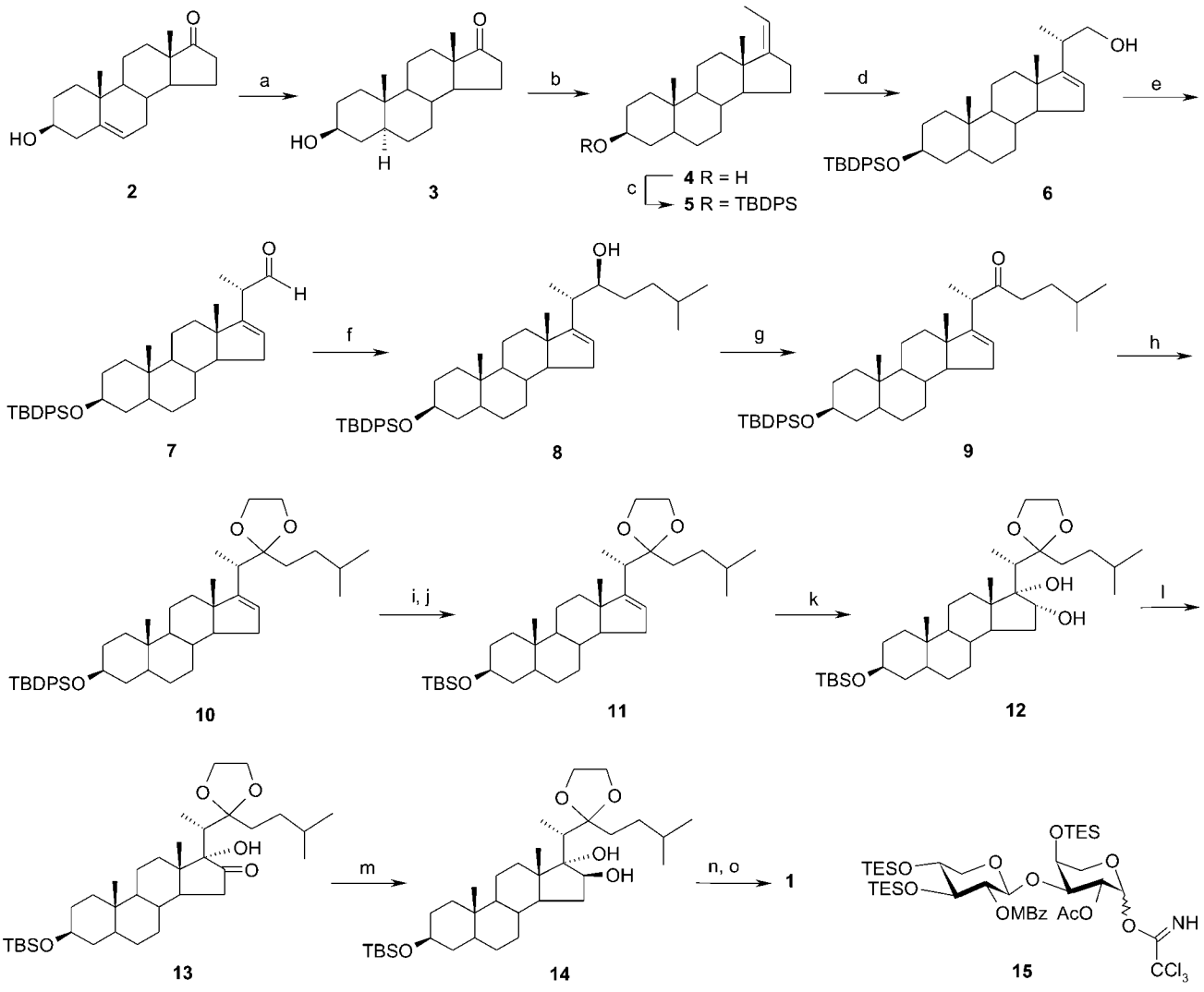
oxide) oxidation[11] of the 16α-OH gave 16-ketone **13**, which was reduced with $NaBH_4/CeCl_3$ to provide the 16β-ol **14** stereoselectively. Finally, coupling of diol **14** with disaccharide trichloroacetimidate **15**[7] provided the corresponding glycoside, which was subjected to deprotection with $Pd(MeCN)_2Cl_2$, furnishing the desired target **1**.

Comparing these transformations (**3**→**1**) to those performed in our previous synthesis of OSW-1 (**2**→OSW-1)[7] under similar conditions, the considerable difference in terms of the outcome of yield was observed in the dihydroxylation of the 16,17-ene (**11**→**12**, 77%). In the course of the 16-OH oxidation (**12**→**13**), it was found that when substrate **12** was undertaken in Swern oxidation conditions, the 22-cycloethyleneketal protecting group was likely to be wiped off. To avoid this side-reaction, several methods were tried, and finally TPAP/NMO oxidation was proved to be the most efficient and convenient method, giving the corresponding ketone in an excellent yield of 91%. In our previous synthesis, the 5,6-double bond competed with the 16,17-double bond for dihydroxylation (with OsO_4) even at low temperature (−20 ℃), leading to the corresponding 16α,17α-diol in 41% yield. An improved 57% yield for this step was reported recently by performing the dihydroxylation from −78 ℃ to r.t.[12]

The *in vitro* anti-tumor activities of the synthetic 5,6-dihydro-OSW-1 (**1**) and OSW-1 against AGS (stomach cancer cells), 7404 (liver carcinoma cells), and MCF-7 (breast cancer cells) were evaluated by the standard MTT assay using cisplatin as a positive control.[13-15] The results are listed in Table 1. In fact, the

Scheme 1



Reagents and conditions: (a) H_2 (2.0×10^6 Pa), 10% Pd/C, EtOH, 50 ℃, 24 h, 80%. (b) Ph_3PEtBr, *t*-BuOK, THF, reflux, 98%. (c) TBDPSCl, imidazole, DMF, r.t., 90%. (d) $(CH_2O)_n$, BF_3•Et_2O, CH_2Cl_2, r.t., 73%. (e) Dess-Martin periodinane, CH_2Cl_2, r.t., 84%. (f) 3-methylbutyl magnesium bromide, Et_2O, r.t., 64%. (g) PDC, 4Å MS, DMF-CH_2Cl_2, r.t., 86%. (h) $HOCH_2CH_2OH$, $CH(OEt)_3$, *p*-TsOH•H_2O, CH_2Cl_2, r.t., 6 d, 90%. (i) TBAF, THF, r.t., 93%; (j) TBSCl, imidazole, DMF, r.t., 95%. (k) OsO_4, pyridine-THF, r.t.; then H_2S (g), 77%. (l) TPAP/NMO, 4Å MS, CH_2Cl_2, r.t., 91%. (m) $NaBH_4$, $CeCl_3$•$7H_2O$, THF, 0 ℃, 91%. (n) **15**, TMSOTf (0.05 equiv.), 4Å MS, CH_2Cl_2, −20 ℃, 60%. (o) $Pd(CN)_2Cl_2$, acetone-water (*V*/*V*, 20/1), r.t., 52%.

anti-tumor potency of **1** was slightly higher than that of OSW-1.

Table 1 Cytotoxic activities of the 5,6-dihydro-OSW-1 (**1**), OSW-1, and cisplatin against tumor cells[a]

Tumor cell[b]	$IC_{50}/(\mu mol \cdot L^{-1})$		
	1	OSW-1	Cisplatin
AGS	0.71	1.42	24.1
7404	0.025	0.10	8.37
MCF-7	0.029	0.27	18.7

[a] The standard MTT assay was followed. The IC_{50} values of cisplatin against these three cell lines used in our assays are consistent with those determined by others.[13-15] [b]AGS: human stomach cancer cell line. 7404: human liver carcinoma cell line. MCF-7: human breast cancer cell line.

Experimental

3*β*-*O*-(*Tert*-butyldiphenylsilyl)-5*α*-23,24-bisnorchol-16-en-22-ol (6)

In Ar atmosphere, to a stirred anhydrous CH_2Cl_2 (80 mL) solution of **5** (11.3 g, 20.9 mmol) and paraformaldhyde (3.14 g, 104 mmol) was added BF_3-OEt_2 solution in CH_2Cl_2 (14.8 mL, 10 mg/mL, 1.04 mmol) slowly at room temperature. After stirring for other 10 min, the reaction was quenched by Et_3N (0.60 mL), then filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc＝50/1 to 20/1, *V*/*V*) to yield **6** (8.77 g, 73%) as a white solid. $[\alpha]_D^{25}$ −9.2 (*c* 1.18, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.70—7.30 (m, 10H), 5.40 (brs, 1H), 3.65—3.48 (m, 3H), 2.37 (q, *J*＝6.9 Hz, 1H), 1.05 (s, 9H), 1.02 (d, *J*＝6.9 Hz, 3H), 0.84 (s, 3H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 157.68, 135.75, 134.90, 129.35, 127.37, 122.85, 72.78, 66.51, 57.27, 54.95, 47.16, 45.02, 38.38, 36.87, 35.63, 35.32, 34.90, 34.16, 31.89, 31.70, 31.09, 28.62, 26.99, 21.03, 19.12, 18.04, 16.32, 12.30; EI/MS *m/z*: 570 (M^+), 513 (91.98, M^+ − Bu-*t*), 199 (67.35).

3*β*-*O*-(*Tert*-butyldiphenylsilyl)-5*α*-22-oxo-23,24-bisnorchol-16-ene (7)

An anhydrous CH_2Cl_2 (100 mL) solution of **6** (7.42 g, 13.0 mmol) and Dess-Martin periodinane (16.5 g, 39.0 mmol) was stirred overnight at room temperature. The reaction was quenched by addition of saturated $NaHCO_3$ and saturated $Na_2S_2O_3$ solutions, the mixture was then extracted with CH_2Cl_2, the organic layer was washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc＝50/1, *V*/*V*) to yield **7** (6.19 g, 84%) as a white solid. $[\alpha]_D^{25}$ +15.7 (*c* 0.71, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 9.43 (d, *J*＝2.4 Hz, 1H, 22-H), 7.70—7.30 (m, 10H), 5.43 (brs, 1H), 3.65—3.55 (m, 1H), 3.00 (q, *J*＝6.8 Hz, 1H), 1.15 (d, *J*＝6.9 Hz, 3H), 1.04 (s, 9H), 0.82 (s, 3H), 0.74 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 201.10, 152.10, 135.78, 134.89, 129.38, 127.40, 126.95, 72.75, 56.92, 54.86, 47.25, 46.01, 45.01, 38.36, 36.86, 35.64, 34.60, 34.13, 31.85, 31.70, 31.93, 28.59, 26.99, 20.94, 19.13, 16.11, 14.32, 12.31; HRMS (ESI) *m/z*: calcd for $C_{38}H_{51}O_2Si$ ($M-H^+$) 567.3664, found 567.3677.

3*β*-*O*-(*Tert*-butyldiphenylsilyl)-5*α*-cholestan-16-en-22*α*-ol (8)

In Ar atmosphere, to a stirred anhydrous Et_2O (7.0 mL) solution of magnesium (171 mg, 7.03 mmol) and catalytic I_2, 3-methyl-1-bromo-butane (1.1 mL, 8.74 mmol) was added, and the solution was heated slightly to initiate the reaction. After 15 min vigorous reaction, magnesium vanished, yielding a clear solution, namely the Grignard reagent solvent of 3-methylbutyl magnesium bromide.

In Ar atmosphere, the obtained Grignard reagent solution was added slowly to a stirred anhydrous Et_2O (40 mL) solution of **7** (1.96 g, 3.45 mmol). After 10 min reaction, saturated NH_4Cl solution was added to quench the reaction. The reaction mixture was extracted with Et_2O. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc＝50/1, *V*/*V*) to yield **8** (1.40g, 64%) as a colorless foam-like solid. $[\alpha]_D^{25}$ −8.2 (*c* 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.70—7.30 (m, 10H), 5.46 (brs, 1H), 3.65—3.55 (m, 2H), 2.30—2.20 (m, 1H), 1.05 (s, 9H), 1.00 (d, *J*＝6.9 Hz, 3H), 0.89 (d, *J*＝6.6, 3H), 0.88 (d, *J*＝6.6, 3H), 0.84 (s, 3H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 158.79, 135.77, 134.96, 134.90, 129.36, 127.40, 124.12, 73.08, 72.80, 57.80, 54.90, 47.03, 45.04, 38.39, 37.74, 36.87, 35.63, 35.51, 34.79, 34.02, 32.44, 31.88, 31.73, 31.11, 28.65, 28.10, 27.01, 22.62, 22.56, 20.98, 19.13, 16.82, 14.18, 12.33; EI/MS *m/z*: 583 (74.43, M^+ − Bu-*t*), 199 (100).

3*β*-*O*-(*Tert*-butyldiphenylsilyl)-5*α*-22-oxo-cholestan-16-ene (9)

To a stirred solution of CH_2Cl_2 (20 mL) and DMF (4.0 mL) were added **8** (2.16 g, 3.38 mmol), PDC (1.91 g, 5.08 mmol) and 4Å MS (1.80 g). The reaction mixture was stirred for 8 h at room temperature, and then filtered. The filtrate was washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc＝50/1, *V*/*V*) to yield **9** (1.86 g, 86%) as a colorless foam-like solid. $[\alpha]_D^{25}$ +46.8 (*c* 0.98, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.70—7.30 (m, 10H), 5.32 (brs, 1H), 3.65—3.53 (m, 1H), 3.15 (q, *J*＝6.8 Hz, 1H), 2.53—2.26 (m, 2H), 1.12 (d, *J*＝6.9 Hz, 3H), 1.04 (s, 9H), 0.86 (d, *J*＝6.3 Hz, 3H), 0.84 (d, *J*＝6.3 Hz, 3H), 0.82 (s, 3H), 0.79 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 211.16, 154.39, 135.67, 134.84, 134.79, 129.33, 127.36, 125.00, 72.71, 57.01, 54.81, 47.47, 45.60, 44.98, 38.35, 38.18, 36.83, 35.57, 34.69, 34.14, 33.00, 31.80, 31.68, 31.08, 28.57,

27.52, 26.98, 22.44, 22.18, 20.97, 19.07, 19.07, 16.81, 16.38, 12.27; HRMS (ESI) *m/z*: calcd for $C_{43}H_{63}O_2Si$ ($M+H^+$) 639.4592, found 639.4579.

3*β*-*O*-(*Tert*-butyldiphenylsilyl)-5*α*-22,22-ethylenedioxy-cholestan-16-ene (10)

In Ar atmosphere, an anhydrous CH_2Cl_2 (20 mL) solution of **9** (1.78 g, 2.78 mmol), $(CH_2OH)_2$ (1.55 mL, 27.8 mmol), $HC(OEt)_3$ (2.30 mL, 17.6 mmol) and *p*-TsOH-H_2O (27 mg, 0.142 mmol) was stirred for 6 d at room temperature, and then Et_3N (0.30 mL) was added to quench the reaction. The reaction mixture was extracted with CH_2Cl_2, washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=100/1, *V*/*V*) to yield **10** (1.72 g, 90%) as a colorless foam-like solid. $[\alpha]_D^{25}$ −3.4 (*c* 0.88, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 7.70—7.30 (m, 10H), 5.62 (d, *J*=2.2 Hz, 1H), 3.96—3.90 (m, 4H), 3.65—3.53 (m, 1H), 2.46—2.36 (q, *J*=6.9 Hz, 1H), 1.04 (s, 9H), 1.00 (d, *J*=6.9 Hz, 3H), 0.86 (d, *J*=6.3 Hz, 3H), 0.84 (d, *J*=6.3 Hz, 3H), 0.82 (s, 3H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 156.68, 135.77, 134.96, 134.89, 129.36, 127.40, 123.91, 113.83, 72.81, 65.82, 65.22, 57.23, 54.95, 47.68, 45.05, 39.13, 38.41, 36.87, 35.63, 34.95, 33.91, 32.41, 31.96, 31.74, 31.29, 28.69, 28.36, 27.01, 22.64, 21.07, 19.13, 17.37, 15.79, 12.32; EI/MS *m/z*: 625 (6.98, M^+−Bu-*t*), 199 (29.63), 143(100).

3*β*-*O*-(*Tert*-butyldimethylsilyl)-5*α*-22,22-ethylenedioxy-cholestan-16-ene (11)

To a stirred anhydrous THF (7.0 mL) solution of **10** (1.73 g, 2.53 mmol) was added TBAF (4.0 mL, 1 mol/L in THF, 4.0 mmol), the mixture was stirred overnight at room temperature, and then extracted with Et_2O. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=10/1, *V*/*V*) to yield 3-ol (1.05 g, 93%) as a colorless foam-like solid, with recovery of **10** (76 mg, 4%).

The obtained 3-ol (1.05 g, 2.36 mmol) was dissolved in DMF (10 mL), to which TBSCl (473 mg, 3.14 mmol) and imidazole (409 mg, 6.00 mmol) were added. The resulting mixture was stirred at room temperature for 7 h, and then was extracted with Et_2O. The organic layer was washed with brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=100/1, *V*/*V*) to yield **11** (1.25 g, 95%) as a colorless foam-like solid. $[\alpha]_D^{25}$ +4.5 (*c* 0.99, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 5.65 (brs, 1H), 3.95 (d, *J*=1.1 Hz, 4H), 3.60—3.50 (m, 1H), 2.48—2.38 (q, *J*=6.9 Hz, 1H), 1.03 (d, *J*=7.1 Hz, 3H), 0.89 (s, 9H), 0.87 (d, *J*=7.1 Hz, 3H), 0.85 (d, *J*=7.1 Hz, 3H), 0.81 (s, 3H), 0.78 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 156.66, 123.90, 113.82, 72.13, 65.80, 65.21, 57.24, 55.09, 47.68, 45.30, 39.13, 38.69, 37.02, 35.72, 34.98, 34.29, 33.13, 32.40, 32.03, 31.94, 31.29, 28.75, 28.34, 25.93, 22.62, 21.10, 18.22, 17.37, 15.78, 12.33, −4.58; HRMS (ESI) *m/z*: calcd for $C_{35}H_{63}O_3Si$ ($M+H^+$) 559.4541, found 559.4529.

3*β*-*O*-(*Tert*-butyldimethylsilyl)-5*α*-22,22-ethylenedioxy-cholestane-16*α*,17*α*-diol (12)

11 (916 mg, 1.64 mmol) was dissolved in a solution of anhydrous THF (20 mL)/pyridine (1.0 mL), OsO_4 (500 mg, 1.97 mmol) was added carefully at room temperature, and 4 h later H_2S (g) was bubbled through overnight. The reaction mixture was filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=20/1, *V*/*V*) to yield **12** (749 mg, 77%) as a white solid, with recovery of **11** (28 mg, 3%). $[\alpha]_D^{25}$ −18.9 (*c* 1.18, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 4.27—4.23 (m, 1H), 4.03 (d, *J*=4.1 Hz, 1H), 4.00—3.90 (m, 4H), 3.60—3.50 (m, 1H), 3.09 (s, 1H), 1.11 (d, *J*=7.1 Hz, 3H), 0.89 (s, 9H), 0.88 (d, *J*=7.1 Hz, 3H), 0.87 (d, *J*=7.1 Hz, 3H), 0.78 (s, 3H), 0.75 (s, 3H), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 115.69, 82.37, 75.88, 72.11, 65.77, 64.37, 53.75, 50.20, 47.72, 45.31, 44.89, 38.63, 37.01, 35.34, 33.18, 33.05, 32.74, 32.06, 31.89, 31.29, 28.71, 28.10, 25.93, 22.73, 20.60, 18.24, 14.50, 13.03, 12.30, −4.59; HRMS (ESI) *m/z*: calcd for $C_{35}H_{64}O_5SiNa$ ($M+Na^+$) 615.4415, found 615.4418.

3*β*-*O*-(*Tert*-butyldimethylsilyl)-5*α*-22,22-ethylenedioxy-16-oxocholestane-17*α*-ol (13)

In Ar atmosphere, to a stirred anhydrous CH_2Cl_2 (5.0 mL) solution of **12** (160 mg, 0.270 mmol) were added TPAP (23 mg, 65.4 μmol), NMO (95 mg, 0.811 mmol) and 4Å MS (200 mg). The reaction mixture was stirred overnight at room temperature, and then was filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=10/1, *V*/*V*) to yield **13** (145 mg, 91%) as a white solid. $[\alpha]_D^{25}$ −117.8 (*c* 0.89, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) *δ*: 4.75 (s, 1H), 4.06—3.94 (m, 4H), 3.60—3.50 (m, 1H), 2.77—2.70 (q, *J*=7.4 Hz, 1H), 1.02 (d, *J*=7.4 Hz, 3H), 0.92—0.86 (br, 18H), 0.83 (s, 3H), 0.06 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) *δ*: 215.69, 115.40, 85.47, 72.02, 63.40, 63.22, 53.69, 47.27, 45.14, 44.87, 41.11, 38.56, 37.19, 36.89, 35.55, 34.13, 32.74, 32.26, 32.15, 31.88, 30.45, 28.60, 28.28, 25.93, 22.68, 22.42, 20.30, 18.22, 15.17, 14.26, 12.33, −4.58; HRMS (ESI) *m/z*: calcd for $C_{35}H_{62}O_5SiNa$ ($M+Na^+$) 613.4259, found 613.4280.

3*β*-*O*-(*Tert*-butyldimethylsilyl)-5*α*-22,22-ethylenedioxy-cholestane-16*β*,17*α*-diol (14)

In Ar atmosphere, **13** (46 mg, 77.8 μmol), $NaBH_4$ (43 mg, 1.14 mmol) and $CeCl_3$•$7H_2O$ (87 mg, 0.234 mmol) were dissolved in anhydrous THF (2.5 mL). The mixture was stirred 3.5 h in 0 ℃, and then quenched by addition of methanol. The reaction mixture was extracted with Et_2O. The organic layer was washed with

brine, dried ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=5/1, *V*/*V*) to yield **14** (42 mg, 91%) as a white solid. $[\alpha]_D^{25}$ −3.3 (*c* 1.10, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 4.16—3.97 (m, 6H), 3.94—3.86 (m, 1H), 2.62—2.54 (q, *J*=7.2 Hz, 1H), 1.18 (d, *J*=7.2 Hz, 3H), 0.94—0.82 (br, 18H), 0.80 (s, 3H, 19-CH_3), 0.05 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 116.51, 86.76, 81.54, 72.16, 64.08, 62.84, 53.89, 48.16, 47.51, 44.92, 38.63, 37.10, 35.92, 35.38, 33.76, 33.41, 32.82, 32.74, 32.03, 31.92, 28.75, 25.95, 22.71, 22.26, 20.88, 18.25, 12.77, 12.35, 11.89, −4.58; HRMS (ESI) *m/z*: calcd for $C_{35}H_{64}O_5SiNa$ ($M+Na^+$) 615.4415, found 615.4435.

1-*O*-(17α-Hydroxy-5α-cholestan-16β-yl)-2-*O*-acetyl-3-*O*-[2-*O*-(4-methoxybenzoyl)-β-*D*-xylo-pyranosyl]-β-*L*-arabinopyranoside (5,6-Dihydro-OSW-1, 1)

In Ar atmosphere, **14** (50 mg, 84.3 μmol), disaccharide trichloroacetimidate **15** (162 mg, 171 μmol) and 4Å MS (260 mg) were dissolved in anhydrous CH_2Cl_2 (2.50 mL). The resulting mixture was stirred at room temperature for 30 min, and then cooled to −20 ℃. TMS-OTf (1.2 mL, 0.0045 mol/L in CH_2Cl_2) was added to the reaction mixture, and the reaction was continued for 30 min. Et_3N (0.10 mL) was added to quench the reaction, and the reaction mixture was filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc=10/1, *V*/*V*) to yield glycosylation product (70 mg, 60%) as a white solid.

The obtained white solid (70 mg, 50.9 μmol) was dissolved in acetone-water (4 mL, 20∶1, *V*∶*V*), to which $Pd(MeCN)_2Cl_2$ (*ca.* 4 mg) was added. After stirring at room temperature for 4 h, the mixture was evaporated under reduced pressure to give a residue, which was purified by silica gel column chromatography (CH_2Cl_2/CH_3OH=15/1, *V*/*V*) to yield **1** (23 mg, 52%) as a white solid. $[\alpha]_D^{25}$ −12.6 (*c* 0.80, CH_3OH); ^{1}H NMR (300 MHz, C_5D_5N) δ: 8.30 (dd, *J*=8.8, 1.9 Hz, 2H), 7.07 (dd, *J*=8.8, 1.6 Hz, 2H), 5.65 (t, *J*=8.0 Hz, 1H), 5.53 (t-like, *J*=6.3, 7.7 Hz, 1H), 5.10 (d, *J*=7.7 Hz, 1H), 4.76 (s, 1H), 4.56 (d, *J*=6.0 Hz, 1H), 4.37 (brs, 1H), 4.31—4.10 (m, 6H), 3.73 (s, 3H), 5.15 (q, *J*=7.7 Hz, 1H), 1.95 (s, 1H), 1.25 (d, *J*=7.4 Hz, 3H), 0.86 (d, *J*=6.5 Hz, 3H), 0.83 (d, *J*=6.5 Hz, 3H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, C_5D_5N) δ: 218.93, 169.24, 165.41, 163.87, 132.41, 114.10, 103.17, 100.81, 88.38, 85.64, 80.97, 76.34, 75.11, 72.03, 70.71, 67.80, 65.54, 55.47, 54.17, 48.13, 46.82, 46.31, 45.15, 39.25, 37.44, 35.76, 35.50, 34.53, 32.94, 32.68, 32.46, 29.95, 29.08, 27.70, 22.80, 22.46, 21.10, 20.87, 13.89, 12.48, 11.81; HRMS (ESI) *m/z*: calcd for $C_{47}H_{70}O_{15}Na$ ($M+Na^+$) 897.4607, found 897.4620.

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