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Synthesis of cholestane saponins as mimics of OSW-1 and their cytotoxic activities

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ABSTRACT

To fulfill the structure–activity relationship (SAR) of OSW-1, and aim at finding the simplest structural part while maintaining most of the biological activities, six cholestane saponins were synthesized by introducing OSW-1 disaccharide (2-*O*-4-methoxybenzoyl-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl) and its 1→4-linked analogue to the 7-hydroxy or 16-hydroxy of steroidal sapogenins. Cytotoxic activities of the products were tested. Compounds **1** and **3** exhibited potent cytotoxicities against five types of human tumor cells, with minimum IC_{50} of 2.0 and 75 nM, respectively. And due to its high activity and easy accessibility compound **1** could be a potential candidate for new anti-tumor agents.

Steroidal saponins, a widespread secondary metabolites with a variety of bioactivities, are believed to be some of the principal constituents of many plant drugs and traditional medicines, and are described as being responsible for most of the observed biological effects. The discovery of OSW-1, a valuable cholestane saponin, attracted great attention to the cytotoxicity of this kind of compounds.

OSW-1 was isolated in the bulbs of *Ornithogalum saundersiae* in 1992.[1] Pharmacological studies showed that OSW-1 exhibited extremely potent cytotoxicity against various malignant tumor cells in vitro that is superior to the prominent cancer therapeutic agents presently in use, such as taxol.[2] Apart from excellent activity, OSW-1 also has high selectivity to tumor cells. Although a number of synthetic and pharmacologic studies of OSW-1 and its analogues have been reported,[3–14] the precise mechanism remains unclear and the SAR needs further investigation. For example, the necessity of C-22 carbonyl group is still controversial. Wojtkielewicz et al.[8] commented that the presence of a carbonyl group at C-22 was pharmacophore requirement, but Deng et al.[9] found that the 22-deoxy-OSW-1 was slightly more potent than OSW-1. The relationship between the position of sugar chain and its biological activity has been hardly reported. Ma et al.[3,4] has synthesized several steroidal saponins with the OSW-1 disaccharide attached at C-3β or C-16α hydroxyl group, but none of them was active. In addition, some naturally occurring 7-hydroxy saponins showed potent bioactivities.[15] Furthermore, Guan et al.[16] indicated that the xylose part and the C-7,15,16 in sapogenin of 23-Oxa-OSW-1 might be involved in the interaction with DNA.

Taking all the facts into consideration, to examine the importance of the link-position of sugar chain, fulfill the SAR of OSW-1, and aim at finding the simplest portion that maintains most of the biological activities, we designed and synthesized two 7-OH steroid aglycones: (25*R*)-3β,7β,26-trihydroxycholest-5-ene and (25*R*)-3β,7β,16β,26-tetrahydroxycholest-5-ene, plus one 16-OH aglycone, (25*R*)-3β,16β,26-trihydroxycholest-5-ene. By attaching these three aglycones with OSW-1 disaccharide (2-*O*-4-methoxybenzoyl-β-D-xylopyranosyl-(1→3)-2-*O*-acetyl-α-L-arabinopyranosyl) and its 1→4-linked analogue, respectively, six cholestane saponins **1–6** (Fig. 1) as mimics of OSW-1 were afforded. Their cytotoxic activities were evaluated in vitro using standard MTT method.

Inspired by the modified Clemmensen reduction reported by Williams et al.,[17] the sapogenin parts were synthesized in a facile way (Scheme 1). Diosgenin was treated with Zn and hydrochloric acid using Williams's method to afford (25*R*)-cholest-5-en-3β,16β,26-triol **7** in a yield of 76%. Selective protection of **7** with *tert*-butyldimethylsilyl chloride (TBSCl) gave the 3,26-bissilyloxy ether **8** in 95% yield. C16–OH was removed following Martin's method[18] by mesylation and reduction with lithium aluminum hydride (LAH). After screening several known methods for allylic oxidations at C-7 of steroids (Table 1), we chose Zheng and Li's

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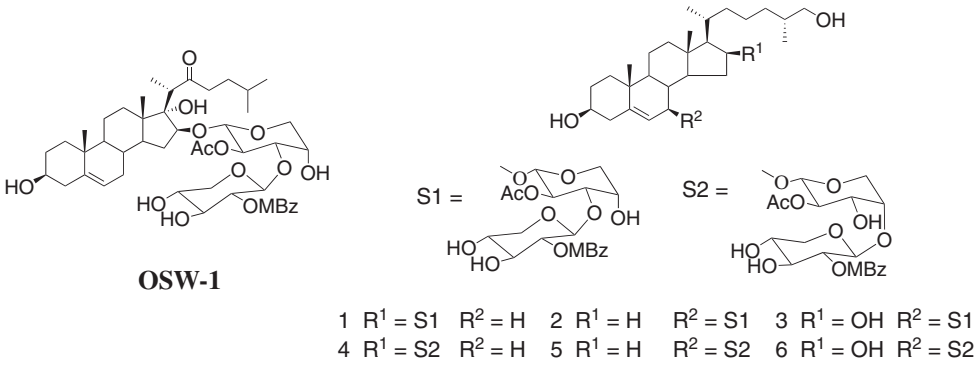
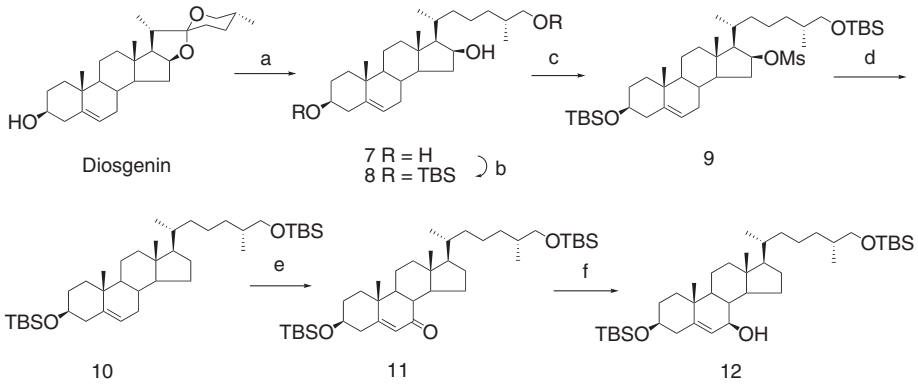


Figure 1. Structures of OSW-1 and synthetic analogues.



Scheme 1. Synthesis of aglycons **7** and **12**. Reagents and conditions: (a) Zn, HCl, EtOH, 50 °C, 76%; (b) TBSCl, imidazole, DMAP, DMF, rt, 95%; (c) MsCl, Py, 0 °C; (d) $LiAlH_4$, Et_2O, 0 °C to rt, 73% from **8**; (e) $Na_2Cr_2O_7{\cdot}2H_2O$, HOSu, acetone, 40 °C, 52%; (f) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF/MeOH, 93%.

Table 1
Allylic oxidation of **10** to the cholest-5-en-7-one **11**[a]

Reaction conditions	Yield (%)
PDC (25 equiv), 4 Å MS, pyridine, 100 °C, 3 h[20]	15
PDC (4 equiv), *t*-BuOOH (4 equiv), C_6H_6, 0 °C to rt 24 h[21]	18
CrO_3 (0.05 equiv), *t*-BuOOH (7 equiv), CH_2Cl_2, rt, 5 h[22]	27
CuI (0.6 equiv), *t*-BuOOH (10 equiv), TBAB (0.1 equiv), CH_2Cl_2, reflux, 6 h[23]	36
HOSu (4 equiv), $Na_2Cr_2O_7{\cdot}2H_2O$ (1.5 equiv), acetone, 40 °C, 48 h[19]	52
PDC (2 equiv), HOSu (4 equiv), acetone, 40 °C, 2 h	42

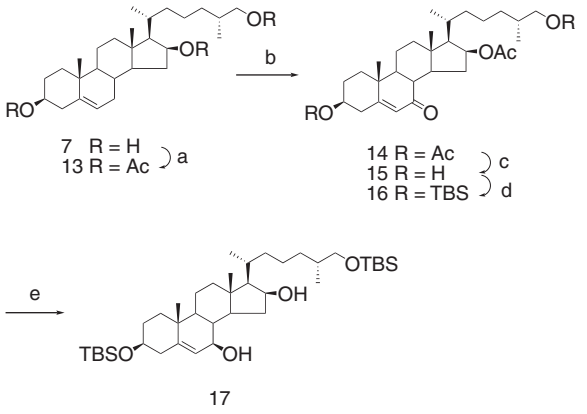
[a] PDC, pyridinium dichromate; HOSu, *N*-hydroxysuccinimide; TBAB, tetrabutyl ammonium bromide.

procedure[19] for our purpose, treating **10** with $Na_2Cr_2O_7{\cdot}2H_2O$ and *N*-hydroxysuccinimide (HOSu) to afford compound **11**, even though the yield was just moderate (52%). Compound **11** was then reduced by $NaBH_4/CeCl_3{\cdot}7H_2O$ to give compound **12** in a yield of 93% which can be used for the further conjugation. The stereochemistry of 7β-OH was confirmed by Pouzar's method[24] as the ^1H NMR data of compound **12** (H-6 δ 5.25, s, $J_{6,7}$ <1.0 Hz) are virtually the same as those of similar molecule reported in this reference. For its 7α-OH isomer, the H-6 in ^1H NMR should be doublet with a coupling constant about 5.0 Hz, and the chemical shift of H-6 should be low filed to about 5.60 ppm.

An optimized protecting strategy was used for better yield of allylic oxidation in the synthesis of compound **17**: C-3β, C-16β and C-26 hydroxy groups were masked by acetyl group (Ac) instead of TBS to afford compound **13** in 98% yield. Compound **13** was then oxidized by $Na_2Cr_2O_7{\cdot}2H_2O$ and HOSu in acetone to afford Δ^5-7-oxo compound **14** in a yield of 76%. The C-3 and C-26 Ac were selectively removed by MeONa meanwhile the C-16 Ac was untouched because of the steric hindrance. After masking C-3β and C-26 hydroxy group with TBS, the C-16 Ac was removed and carbonyl at C-7 was reduced by LAH in one operation to give compound **17** in a yield of 85% (Scheme 2).

The disaccharide moieties, OSW-1 disaccharide **18** and its 1→4 linked analogue **19**, was prepared by our previous method.[14] With the aglycones and the disaccharide donors in hand, the glycosylations were performed. Using Schmidt's glycosylation method, aglycones **8**, **12** and **17** were coupled with disaccharide trichloroacetimidate **18** under the promotion of trimethylsilyl triflate (TMSOTf), respectively, to give compounds **20**, **21** and **22**. The structure of compound **22** was determined by 2D NMR, for example, in HMBC spectrum, ara-H-1 (δ 4.45, d, J = 5.4 Hz, 1H) showed long range correlations with C-7 (δ 72.4), and the H-7 (δ 3.85, m, 1H) showed correlations with ara-C-1 (δ 100.1). Removal of all the protecting groups (two TBS, one TES and two PMB) by sequential treatment with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) in CH_2Cl_2/H_2O and bis (acetonitrile) palladium(II) chloride in acetone/H_2O in one operation furnished the analogues **1**, **2** and **3** (Scheme 3). 1→4 Linked analogue **19** was attached to aglycones **8**, **12** and **17** by the same method, respectively, then glycosides **4**, **5**, **6** were achieved (Scheme 4).

All the compounds were assayed for in vitro anti-tumor activities against a panel of five human caner cell lines including HCT-8, BEL-7402, BGC-823, A2780 and A-549 using taxol as positive control. The cells were allowed to proliferate in presence of samples for 48 h and the results were reported in IC_{50} values (Table 2). From the results, it was clear that compound **1,** with OSW-1 disaccharide linked at 16-OH of sapogenin, showed potent



Scheme 2. Synthesis of aglycone **17**. Reagents and conditions: (a) Ac_2O, pyridine, rt, 98%; (b) $Na_2Cr_2O_7{\cdot}2H_2O$, HOSu, acetone, 40 °C, 76%; (c) MeONa, MeOH, rt; (d) TBSCl, imidazole, DMAP, DMF, rt, 86% from **14**; (e) $LiAlH_4$, Et_2O, 0 °C to rt, 85%.

cytotoxicity with IC_{50} from 2.0 to 70 nM. For the compounds with OSW-1 disaccharide linked at 7-OH, compound **2,** a 16-deoxy analogue, was inactive (IC_{50} >10 μM) but its 16-OH derivative **3** exhibited significant activities with minimum IC_{50} 75 nM.

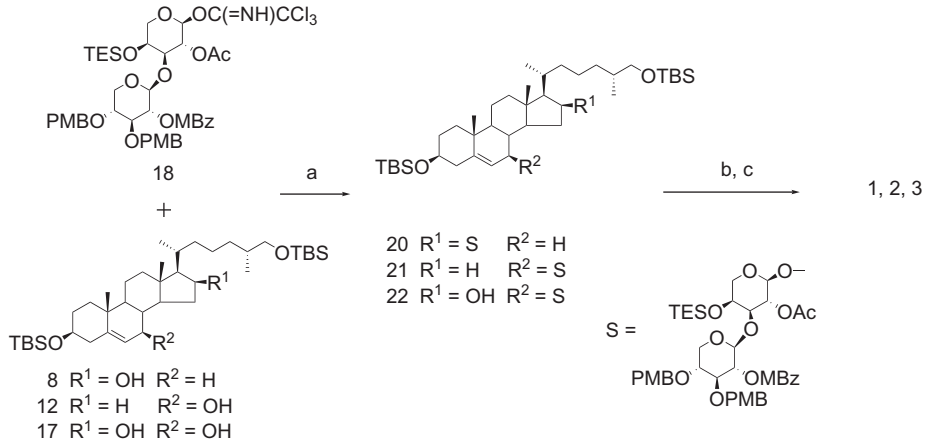
Compounds **4–6**, the saponins bearing 1→4 linked disaccharide, were inactive, which is in accordance with our previous finding.[14]

Comparing compound **1** with 22-deoxy-OSW-1,[9] since both compounds performed excellent anti-tumor activity, we could make a inference that 17-OH is not important, nor the introduction of one hydroxy group onto C-26. Compound **1** also showed comparable bioactivity with its 22-oxo analogue **26** (Fig. 2) we synthesized previously[14] which suggested that the C-22 carbonyl group could be truncated without significant loss of activity. In terms of connecting point of saccharides, even though in others' studies, altering link-position of saccharides always ended up with loss of anti-tumor properties,[3,4] compound **3** with disaccharide chain attached to 7-OH still exhibited relatively potent activity to tumor cell lines. This phenomenon indicated the linkage point of disaccharide is not restricted to 16-OH. However, compound **2,** the 16-deoxy derivative of **3** did not have any obvious activity which further revealed that oxygen-bearing substituent at C-16 is essential.

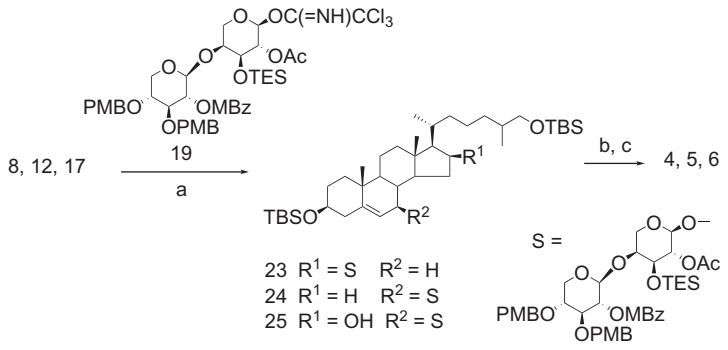
Table 2
Cytotoxic activities of compounds **1–6** against tumor cells[a]

Compounds	IC_{50} (M)				
	HCT-8	BEL-7402	BGC-823	A2780	A-549
1	7.0×10^{-8}	2.3×10^{-8}	2.0×10^{-9}	6.0×10^{-9}	9.3×10^{-9}
2	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
3	7.5×10^{-8}	5.0×10^{-7}	7.8×10^{-8}	1.9×10^{-6}	8.7×10^{-8}
4	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
5	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
6	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
26[14]	2.3×10^{-8}	3.9×10^{-8}	1.3×10^{-9}	7.3×10^{-8}	4.6×10^{-8}
Taxol	5.1×10^{-8}	6.0×10^{-9}	$<1.0 \times 10^{-9}$	$<1.0 \times 10^{-9}$	1.6×10^{-8}

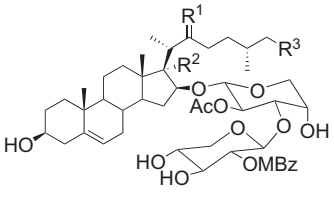
[a] The in vitro cytotoxicities against HCT-8 (colon carcinoma), BEL-7402 (liver cancer), BGC-823 (stomach carcinoma), A2780 (ovarian cancer) and A-549 (lung carcinoma) cell lines were evaluated by the standard MTT assay.



Scheme 3. Synthesis of saponins **1**, **2**, **3**. Reagents and conditions: (a) TMSOTf, 4 Å MS, CH_2Cl_2, −10 °C, 5 h, 62% for **20**, 56% for **21**, 30% for **22**; (b) DDQ, CH_2Cl_2/H_2O, rt, 3 h; (c) $Pd(CH_3CN)_2Cl_2$ acetone/H_2O, rt, 3 h, 52% for **1**, 67% for **2**, 57% for **3**.



Scheme 4. Synthesis of saponins **4**, **5**, **6**. Reagents and conditions: (a) TMSOTf, 4 Å MS, CH_2Cl_2, −10 °C, 5 h, 65% for **23**, 45% for **24**, 33% for **25**; (b) DDQ, CH_2Cl_2/H_2O, rt, 3 h; (c) $Pd(CH_3CN)_2Cl_2$ acetone/H_2O, rt, 3 h, 61% for **4**, 67% for **5**, 57% for **6**.



1	R^1 = H, H	R^2 = H	R^3 = OH
26	R^1 = O	R^2 = H	R^3 = OH
22-deoxy-OSW-1	R^1 = H, H	R^2 = OH	R^3 = H

Figure 2. Structures of synthesized analogues.

In summary, six cholestane saponins, as mimics of OSW-1, were synthesized in a facile way and their cytotoxicity was determined. Compounds **1** and **3** showed potent activity on various cancer cell lines. The results implied that the 22-one function was not necessary to the cytotoxicity. The disaccharide moiety could be linked to other position apart from 16-OH, but the oxygen-bearing substituent at C-16 might be required for the anti-tumor activity. To the best of our knowledge, compound **1** is the most simplified synthetic analogue which retains high potency and it could be a potential candidate for new anti-tumor agents.

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Supplementary data

Supplementary data (experimental procedures, analytical data for key compounds and cell proliferation assay) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.030.

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