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Synthesis of cholestane glycosides bearing OSW-1 disaccharide or its 1→4-linked analogue and their antitumor activities

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ABSTRACT

For further structure–activity relationship (SAR) research of OSW saponins, a cholestane glycoside, namely 3β, 16β, 26-trihydroxycholest-5-en-22-one 16-*O*-(2-*O*-4-methoxybenzoyl-β-D-xylopyranosyl)-(1→3)-2-*O*-acetyl-α-L-arabinopyranoside (**1**) together with two 1→4-linked disaccharide analogues (**2** and **3**) were synthesized. Their cytotoxic activities were evaluated by the standard MTT assay. Compound **1** showed potent cytotoxicity against five types of human tumor cells, with IC_{50} ranging between 1.3 and 73 nM.

OSW-1 belongs to a family of saponins isolated from the bulbs of *Ornitnogalum saundersiae* by Sashida et al.[1] It was found that OSW-1 exhibited exceptionally potent cytotoxic activities on various malignant tumor cells in vitro,[2] with IC_{50} values ranging between 0.1 and 0.7 nM, which are around 10–100 times more potent than clinically applied anticancer agents, such as mitomycin C, adriamycin, cisplatin, camptothecin, and taxol. OSW-1 was also found to be cytostatic in the US National Cancer Institute 60-cell in vitro screen, with a mean IC_{50} of 0.78 nM and a mean IC_{100} of 58 nM. Because of its high potentiality, approaches toward the synthesis of the aglycone[3] and the whole structure[4–8] of OSW-1 have been developed by several groups. Various analogues[9–19] have also been prepared and tested for cytotoxicity in recent years. According to the previous SAR studies, it is known that: (1) the steroidal C17-side chain could tolerate certain modifications without significant loss of activity; (2) the disaccharide moiety is of importance to cytotoxicity, removal of the acetyl (Ac) and the 4-methoxybenzoyl (MBz) groups on the disaccharide moiety decreased the activity about 1000 times; (3) it is also necessary to have the oligosaccharide chain attached to 16-β-OH, other substitute positions (e.g., 3-OH, 16-α-OH) led to deactivation. However, much remains to be discovered about the structural elements of OSW-1 that are associated with its high activity.

In the synthesis of aglycone part of OSW-1, the introduction of 17-OH involves a series of tedious processes and expensive, high toxic reagents such as OsO_4. But no study has reported the necessity of 17-OH to its activity. In modification of disaccharide, Ma et al.[9] synthesized OSW-1 analogues bearing 1→3 and 1→4 linked OSW-1 disaccharide attached at 3-OH for the first time, but both of them were inactive. Their work could not demonstrate which kind of linkage would contribute to the activity as the substitute position of disaccharide at 3-OH might be inappropriate. Therefore, for further SAR research of OSW saponins and simplifying the synthesis procedure, we designed and synthesized 3β, 16β, 26-trihydroxycholest-5-en-22-one (17-deoxy-26-hydroxyl OSW-1 aglycone) from diosgenin, which was then attached with OSW-1 disaccharide and its 1→4 linked analogue at 16-β-OH, affording three OSW-1 analogues **1**, **2**, **3**, whose antitumor activities were tested (Fig. 1).

The sapogenin **9** was prepared from diosgenin according to the method reported by several groups previously.[20,21] Diosgenin was protected with *t*-butyldimethylsilyl group at 3-OH to provide **4**, and then oxyfunctionalized of C-16 and 5(6)-double bond with Oxone in the presence of $NaHCO_3$ to afford the mixture of epoxide diastereomers **5a** and **5b**, which could be distinguished by their ^1H NMR spectra.[21] The mixture of **5a** and **5b** was treated with Zn/KI in Ac_2O/HOAc to give 16, 22-dione **6**. Removal of the 26-*O*-acetyl group in **6** by treating with CH_3ONa/CH_3OH provided **7a**

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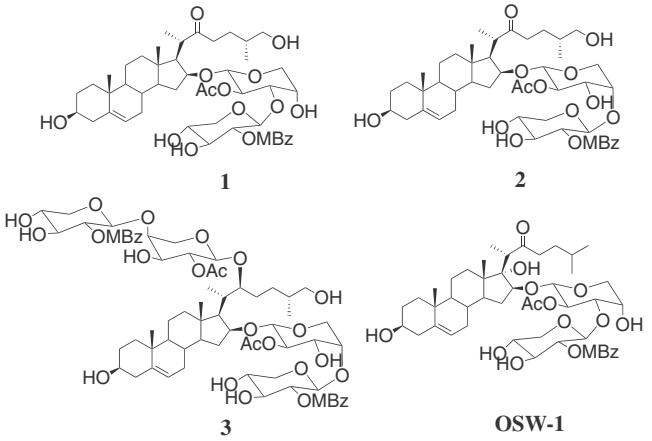
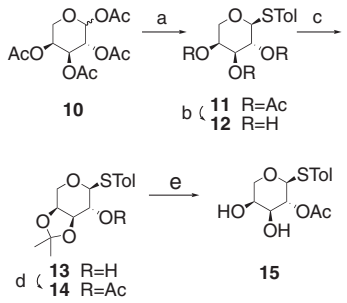


Figure 1. Structures of OSW-1 and synthesized OSW-1 analogues.



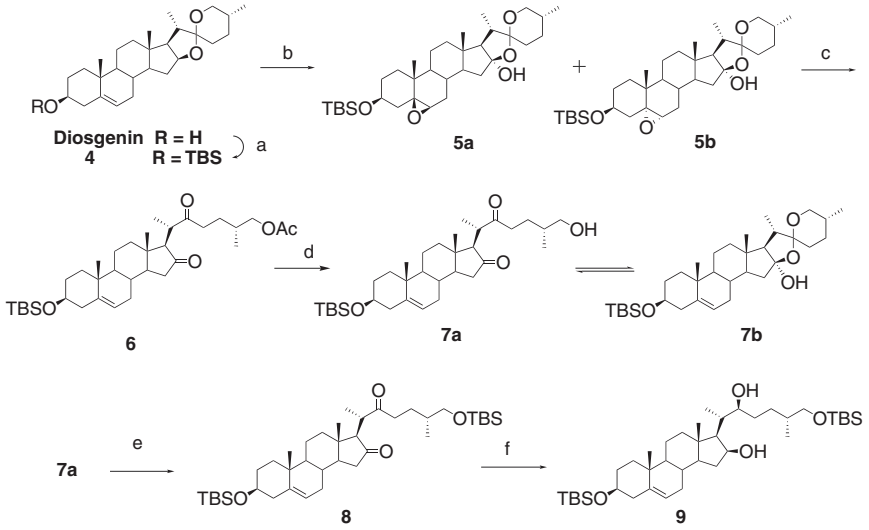
Scheme 2. Reagents and conditions: (a) *p*-Thiocresol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to rt., 8 h, 85%; (b) CH_3ONa, CH_3OH, rt, 2 h, 90%; (c) $(CH_3)_2C(OCH_3)_2$, CSA, DMF, under vacuum, 5 h, 95%; (d) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 1.5 h, 90%; (e) Amberlite IR-120 (H^+), CH_3OH, 50 °C, 4 h, 81%.

and **7b** in 64% yield, which then underwent ring-chain tautomerization (Scheme 1). When the mixture of **7a** and **7b** were exposed to silylation using TBDMSCl in pyridine, only **7a** could be silylated to afford **8**, and **7b** was still untouched. Dione **8** was then reduced under $NaBH_4/CeCl_3$ to provide 16β, 22β-dihydroxyl aglycone **9** in a yield of 73%. The absolute configuration of 22(*S*) was determined by Mosher's method in our lab.

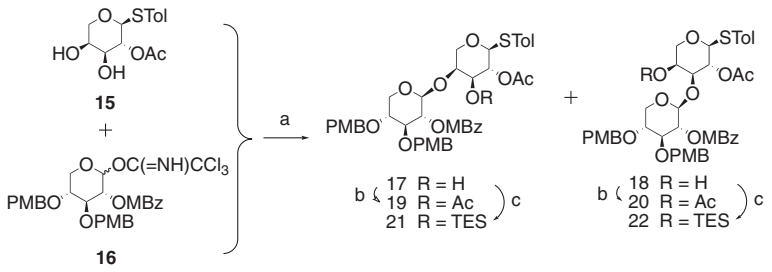
The synthesis of saccharide moiety was referred to Yu and Jin's route.[4,5,9] Analyzing their methods, it is found that the anomeric substitute of acceptor plays an important role in the regioselective glycosylation with the xylose donor: when 1-OH was α-form and substituted with thioether, due to the stereoelectronic effect, 4-OH is more active than 3-OH. So for regioselective synthesis of 1→4-linked disaccharide, we synthesized *p*-methylphenyl 2-*O*-acetyl-1-thio-α-L-arabinopyranoside **15** as an acceptor. The acceptor **15** was readily prepared from L-arabinose in six steps as illustrated in Scheme 2. Tetraacetyl-L-arabinose **10** underwent thioglycosidation followed by deacetylation to give **12** in excellent yield. Compound **12** was then isopropylidenated to form 3, 4-*O*-isopropylidene-α-L-arabinopyranoside **13** in the yield of 95%. After acetylation to 2-OH of **13**, isopropylidene group was removed to give diol acceptor **15** in 81% yield. The xylosyl donor **16** was smoothly prepared by the approach of Jin[5] with modified workup procedures.

Glycosylation of diol acceptor **15** with xylosyl donor **16** in the presence of $BF_3{\cdot}Et_2O$ afforded the desired 1→4-linked disaccharide **17** as the major product in 61% yield, which was separated from its 1→3-linked isomer **18** (in 18% yield) through silica gel column chromatography. Their structures were determined by 2D NMR. The classification of the glycosylation position was also confirmed by comparison of the 'acylation shift'[4] of **17** with its acetylation product **19**, or **18** with its acetylation product **20**. The chemical shift of ara-H-3 was found to be downshifted to 5.05 ppm from 3.70 to 3.66 ppm after acetylation; the chemical shift of ara-H-4 for **18** was 3.87–3.86 ppm, while in **20** it was 5.18–5.14 ppm. After the silylation of **17** and **18** separately, **21** and **22** were afforded, which were subsequently converted to the corresponding trichloroacetimidate **23** and **24** in satisfactory yield (Scheme 3). The arabinosyl moiety of **21** is conformationally preferred the $^1C_4$ form and the xylosyl unit was still $^4C_1$, which was deduced from the coupling constants between trans H-1 and H-2 of both sugars, that is, ara-H-1 was broad single, and $J_{1,2}$ (xyl) = 6.0 Hz.

Under the promotion of trimethylsilyl triflate (TMSOTf), coupling of 16β, 22β-diol aglycone **9** with disaccharide imidate **24** gave 16-glycosidation saponin **25** in 60% yield after chromatography on silica gel which indicated the 16-OH in **9** was more active than 22-OH under the condition of Schmidt glycosylation. The saponin **25** was exposed to oxidation with pyridinium dichromate (PDC) to afford 22-one **26**. Removal of all protecting groups by sequential treatment with 2,3-dichloro-5,6-dicyanobenzoquinone



Scheme 1. Reagents and conditions: (a) TBDMSCl, DMAP, imidazole, DMF, 50 °C, 30 min, 98%; (b) Oxone, $NaHCO_3$, acetone/H_2O/CH_2Cl_2, rt, 48 h, 85%; (c) Zn powder, KI, HOAc/Ac_2O, rt, 24 h, 86%; (d) CH_3ONa, CH_3OH/CH_2Cl_2, 64%; (e) TBDMSCl, DMAP, pyridine, 79%; (f) $NaBH_4/CeCl_3{\cdot}7H_2O$, THF, 73%.



Scheme 3. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, −60 to −40 °C, 4 Å MS, CH_2Cl_2, 61% for **17**, 18% for **18**; (b) Ac_2O/pyridine, DMAP, 60% for **19**, 55% for **20**; (c) TESCl, pyridine, DMAP, 70% for **21**, 80% for **22**.

(DDQ) in CH_2Cl_2/H_2O and bis (acetonitrile) palladium (II) chloride in acetone/H_2O in one operation[5] furnished the analogue **1** in the yield of 65% (Scheme 4).

Coupling of **9** with disaccharide imidate **23** using the same method, 16-glycosidation saponin **27** was formed in 42% yield, and, unexpectedly, 16, 22-bidesmosidic steroid glycoside **28** was also achieved in a yield of 25%.Their structures were determined by 2D NMR, for example, in HMBC spectrum of **28**, two overlapped ara-H-1 (δ 4.36 br s, 2H) show long range correlations with C-16 (δ 72.49) and C-22 (δ 77.00), respectively. The saponin **27** was exposed to oxidation with PDC to afford 22-one **29**. Removal of all protecting groups of **28** and **29** by the same method used in the synthesis of **1**, furnished the analogues **2**, **3** in the yield of 75% and 59%, respectively (Scheme 4). The arabinosyl residues of **27** and **28** were still in $^1C_4$ form, but after deprotection, changed to the $^4C_1$ form according to their ^1H NMR spectra.

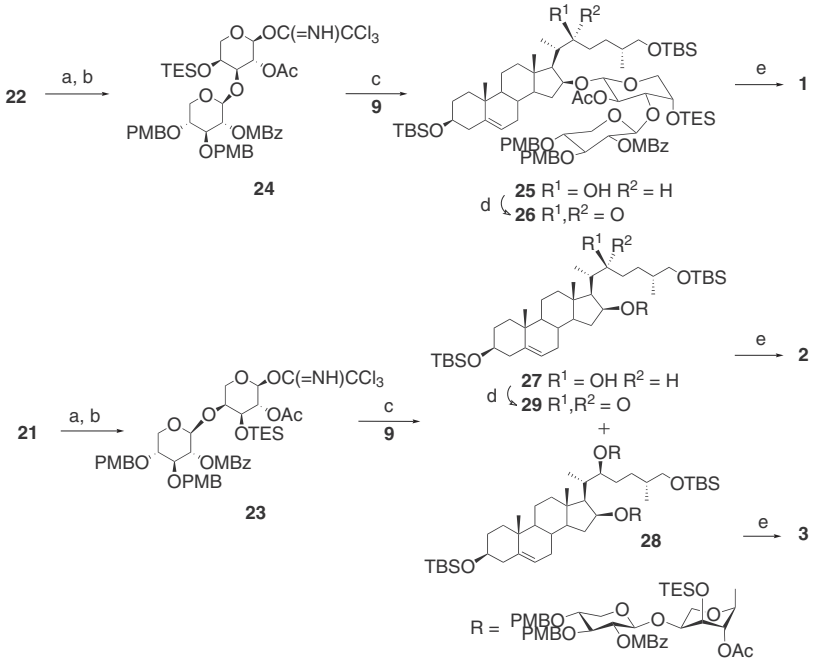
The in vitro antitumor activities of the synthetic glycosides **1**, **2**, and **3** against HCT-8, BEL-7402, BGC-823, A2780 and A-549 were evaluated by the standard MTT assay using taxol as positive control. The results are listed in Table 1. It was shown that compound **1**, 17-deoxy-26-hydroxyl OSW-1, exhibited potent cytotoxic activity on various cancer cell lines especially BGC-832 (IC_{50} 1.3 nM), which is comparable to that of OSW-1. This result indicated the 17-OH could be truncated without great loss of activity. On the other hand, compound **2**, the 1→4-linked disaccharide isomer of **1**, was inactive in a concentration of 10^{-5} μM, neither of compound **3**. The results implied that the 1→3 glycosidic linkage in disaccharide is critical to the cytotoxicities.

Table 1
Cytotoxic activities of compounds **1**–**3** against tumor cells[a]

Tumor cells	IC_{50} (M)			
	1	**2**	**3**	Taxol
HCT-8	2.3×10^{-8}	$>10^{-5}$	$>10^{-5}$	5.1×10^{-8}
BEL-7402	3.9×10^{-8}	$>10^{-5}$	$>10^{-5}$	6.0×10^{-9}
BGC-823	1.3×10^{-9}	$>10^{-5}$	$>10^{-5}$	$<1.0 \times 10^{-9}$
A2780	7.3×10^{-8}	$>10^{-5}$	$>10^{-5}$	$<1.0 \times 10^{-9}$
A-549	4.6×10^{-8}	$>10^{-5}$	$>10^{-5}$	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.

Structural superimposition analysis was also implemented in our study with Accelrys Discovery Studio program package. The minimum energy conformations of compounds **1**, **2** and OSW-1 were calculated and aligned as shown in Figure 2. The result showed no obvious difference between compounds **1** and **2** in topological structure of molecules. In consideration of the great



Scheme 4. Reagents and conditions: (a) NIS, CH_2Cl_2/H_2O, rt, 3 h, (b) DBU, CCl_3CN, CH_2Cl_2, −15 °C, 10 h, 86% for **23**, 70% for **24** for two steps; (c) TMSOTf, CH_2Cl_2, 4 Å MS, −25 °C, 60% for **25**, 42% for **27**, 25% for **28**; (d) PDC, CH_2Cl_2, rt, 84% for **26**, 80% for **29**, (e) DDQ, CH_2Cl_2/H_2O, then $Pd(CH_3CN)_2Cl_2$, acetone/H_2O, 96% for **1**, 75% for **2**, 59% for **3**.

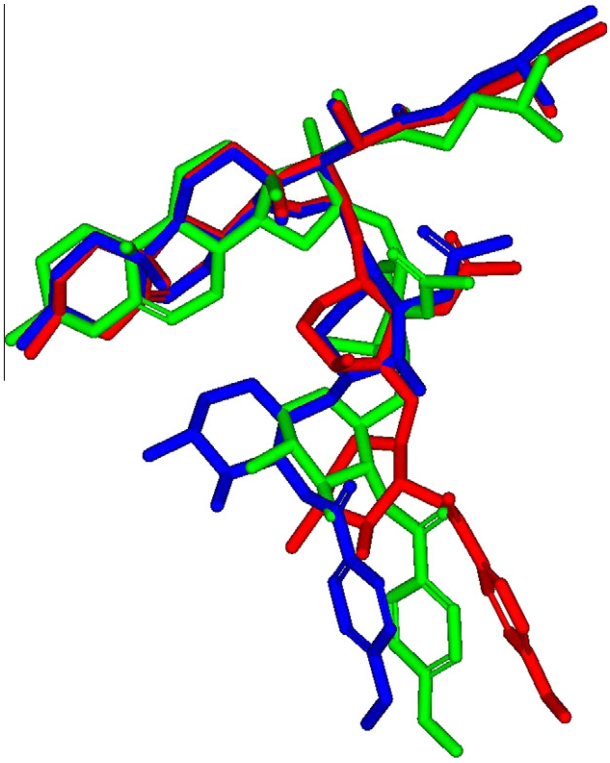


Figure 2. Superimposition of compounds **1** (red), **2** (blue) and OSW-1 (green).

activity difference of these two isomers, it is indicated that the ara-4-OH may play an important role for the biological activity, which is in accord with Tschamber's[19] research findings.

In summary, based on the previous SAR data on the cytotoxicity of OSW saponins, three glycosides bearing the OSW-1 disaccharide or its 1→4-linked analogue were synthesized in a facile way and their cytotoxicities were determined. These results suggested that the 17-OH might not be of importance for antitumor activity, and the 1→3 glycosidic linkage in disaccharide is critical to the cytotoxicities.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.085.

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