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Synthesis of 5(6)-dihydro-OSW-1 analogs bearing three kinds of disaccharides linking at 15-hydroxy and their antitumor activities

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ABSTRACT

In order to study the SAR of 5(6)-dihydro-OSW-1, eight 15(α)β-*O*-glycosyl analogs (**26**–**33**) carrying three kinds of disaccharides including [β-D-Xyl*p*-(1–3)-α-L-Ara*p*], [β-D-Xyl*p*-(1–4)-α-L-Ara*p*] and [α-L-Rha*p*-(1–2)-(α)β-D-Glc*p*] were designed and synthesized. Their in vitro antitumor activities were tested by the standard MTT assay which disclosed that compound **33** (IC_{50} = 0.28–0.52 μM) showed potential antitumor activities.

OSW-1 with considerable antitumor activities was isolated from *Ornithogalum saundersiae* bulbs in 1992 by the group of Sashida and co-workers,[1] which belongs to a family of cholestane glycosides characterized by the attachment of a disaccharide to the C-16 position of the steroid aglycone. It was tested against the NCI (the US National Cancer Institute) 60 cell lines. The bioassay results showed that OSW-1 was 10–100 times more potency compared to those of the clinically applied anticancer agents, for example, mitomycin C, cisplatin, adriamycin.[2]

Yu and co-workers reported that 5(6)-dihydro-OSW-1 (Fig. 1) was slightly more potent than OSW-1against the tested three cancer cell lines [including AGS (stomach cancer cells) IC_{50} 0.71 μM, 7404 (liver carcinoma cells) IC_{50} 0.025 μM, and MCF-7 (breast cancer cells) IC_{50} 0.029 μM].[3] This structurally simplified and easily prepared compound in contrast with OSW-1 was synthesized through the modification of 5-androsten-3β-ol-17-one.[3] Tian and co-workers also explored another routine to synthesize 5(6)-dihydro-OSW-1 fully, rationally utilizing the intact skeleton of tigogenin which was an abundant and cheap steroidal sapogenin isolated from industrial waste.[4] (Fig. 1)

The biological study showed that OSW-1 can damage the membrane and cristae of cancer cell's mitochondria which plays an important role in mediating the anticancer activities, leading to the loss of transmembrane potential, a significant increase of cytosolic calcium, and activation of calcium-dependant apoptosis. The evident disclosed that OSW-1 may cause cancer cell's death by apoptosis.[5] However, the detailed mechanism in vivo is still unknown. By using 1D and 2D ^{1}H NMR technique, Cao and co-workers found that 23-oxa-OSW-1, an analog of OSW-1, has an interaction with the selected DNA fragment, because NMR experiment showed that the chemical shifts of the 14-H and 15-H changed obviously compared with that of the free saponin.[6] This finding revealed that 14 and 15 positions in the structure of 23-oxa-OSW-1 could have an important impact on the interaction between the saponin and the DNA fragment.

According to the research work of the Cao and co-workers, the 15-OH of 5(6)-dihydro-OSW-1 analogs may be related to the antitumor activities.[6] However, the impact of glycosylation of 15-OH to the antitumor activities has not been studied yet. Moreover, 15-OH cholestane exists in some natural products, such as, pavoninin 5, which showed potent antiproliferative activity toward KB cells with an IC_{50} of 6.48 μg/mL.[7] Therefore, we infer that 15-OH of 5(6)-dihydro-OSW-1 analogs might play a role in antitumor activities.

In order to study the structural influence to bioactivities, which were caused by the alteration of the disaccharide types and glycosylated position, we designed and synthesized the 15(α)β-*O*-glycosyl analogs bearing three kinds of disaccharide side chains including [β-D-Xyl*p*-(1–3)-α-L-Ara*p*],[8] [β-D-Xyl*p*-(1–4)-α-L-Ara*p*][8] and [α-L-Rha*p*-(1–2)-(α)β-D-Glc*p*].[9] [β-D-Xyl*p*-(1–4)-α-L-Ara*p*], which was produced simultaneously when [β-D-Xyl*p*-(1–3)-α-L-Ara*p*] was synthesized, was chosen to survey the tolerance of the OSW-1 disaccharide for structural changes. The disaccharide [α-L-Rha*p*-(1–2)-

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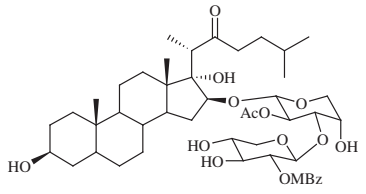


Figure 1. Structure of 5(6)-dihydro-OSW-1.

(α)β-D-Glc*p*] residue, which commonly exits in the skeleton of many steroidal glycosides with potential antitumor activities, such as icogenin,[10] methyl protodioscin,[11] polyphyllin D,[12] and dioscin,[13] according to the reports previously, contributes to the cytotoxic activities of the molecule. It is meaningful to examine the bioactive contributions of [α-L-Rha*p*-(1–2)-(α)β-D-Glc*p*] residue to bioactivities of the whole molecule when [α-L-Rha*p*-(1–2)-(α)β-D-Glc*p*] is the structural part of 5(6)-dihydro-OSW-1 analogs.

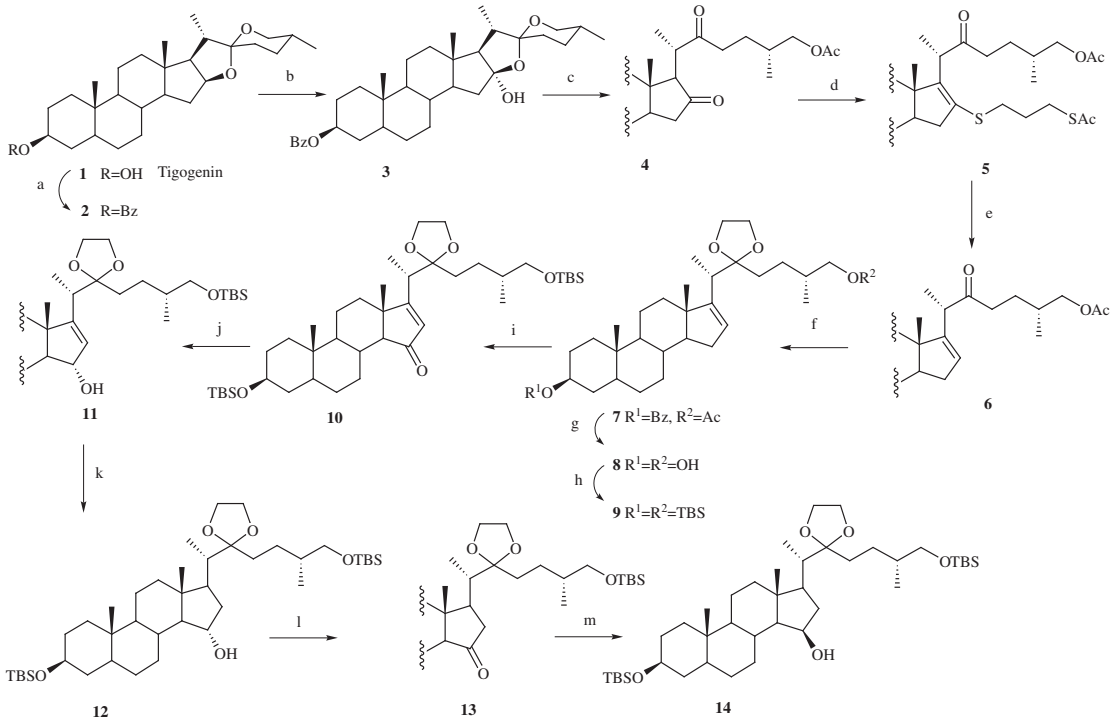
Adopting modification of Cheng and Tian's procedures, we synthesized the desired 15-OH aglycones.[4,14,15] Preparation of compounds **12** and **14** are depicted in Scheme 1. Benzoylation of tigogenin afforded **2** which underwent regioselective oxone oxidation of C-16 to produce **3**.[14] The E/F ring-opened 26-*O*-acetylated product **4** in a good yield of 80% for two steps was achieved by the promotion of Zn/KI in HOAc/Ac_2O.[9] The thioketalization and thioketal-opening-acetylization reaction of **4** was catalyzed with $BF_3{\cdot}Et_2O$ in one pot to give **5**.[15]

Desulfurization of **5** with freshly prepared W-2 Raney nickel at room temperature produced compound **6** in a yield of 74%.[15] Surprisingly, if W-2 Raney nickel was set and stored for a few days, only the terminal SAc was removed. The 22-ketone in **6** was protected by ethylene glycol ketal group to produce **7** which was deprotected by 1 M MeONa/MeOH to give **8** in a satisfactory 89% yield, and then 3, 26-diol **8** was protected by TBS groups to give the target molecule **9** quantitatively. (Scheme 1)

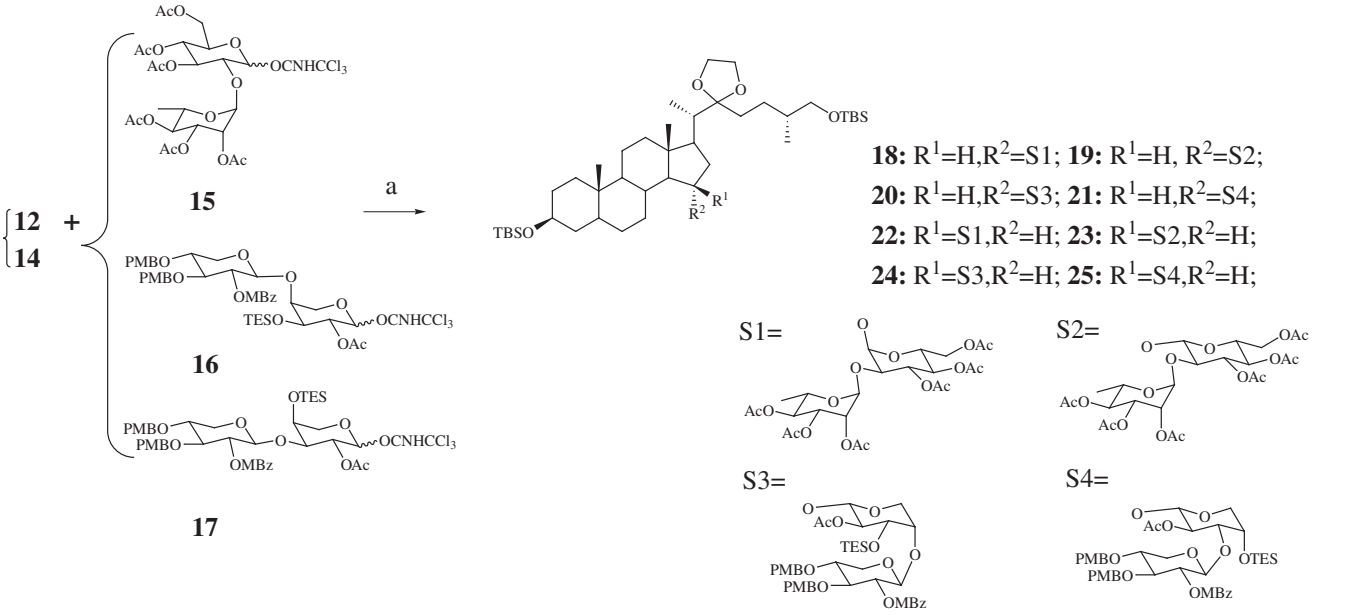
The oxidation of olefin **9** employing $Na_2Cr_2O_7{\cdot}H_2O$/*N*-hydroxysuccinimide system gave the Δ^{16}-15-oxo compound **10** in a moderate yield of 50%.[16] Reduction of the enone **10** under Luche conditions afforded only the allylic C-15α alcohol **11** in a yield of 84%.[16] Catalytic hydrogenation of the 16-olefin in **11** using $Pd(OH)_2$/C and minor amount of 5% NaOAc aqueous solution afforded the saturated alcohol **12** in an excellent yield (93%).[16] As for the catalytic hydrogenation, only adding $Pd(OH)_2$/C in reaction solution resulted in extensive hydrogenolysis of C–O bond at C-3, C-26 and C-22.[17] Fortunately, addition of minor amount of 5% NaOAc aqueous solution to the reaction mixture can perfectly avoid the hydrogenolysis.[18] The configuration of 15-hydroxy in **11** (15β-H, δ 4.52, J = 7.8, 7.8 Hz) was confirmed by One-Dimensional NOE which demonstrated that the 15-hydrogen and the 18-CH_3 have a relative peak. The 15α hydroxyl in **12** was converted stereoselectively to 15β hydroxyl in **14** through the oxidation of 15α hydroxyl to ketone by PDC in CH_2Cl_2 and then reduction by $LiAlH_4$ in THF at −78 °C to afford only 15β hydroxyl **14** in excellent yield of 91%.[6] In the procedure of the reduction of 15-one $NaBH_4/CeCl_3{\cdot}7H_2O$ was also attempted, however, two isomer of 15α-ol **12** (16%) and 15β-ol **14** (62%) which were readily distinguished by their ^1H NMR spectra were produced at the same time.[19] In isomer **12**, the signal of 15-proton appeared at 3.93 ppm, while it was shifted downfield to 4.16 ppm in **14**. Thus, we synthesized the protected target aglycones **12** and **14**.

The disaccharide donors **15**, **16**, **17** (see Scheme 2) were prepared according to the procedures developed by our research group.[20]

Coupling of the aglycone **12** with the disaccharide trichloroacetimidate **15** in the presence of TMSOTf (0.10 equiv) provided two isomers: **18** and **19** (**18**:**19** = 4/1). The ^1H NMR spectra showed that the signal of 1′-β-anomeric proton of epimer **18** appeared at



Scheme 1. Reagents and conditions: (a) BzCl, pyridine, CH_2Cl_2, 0 °C, 90%; (b) oxone, $NaHCO_3$, CH_2Cl_2/H_2O/acetone(4/1/5), rt; (c) Zn, KI, HOAc/Ac_2O, rt, 80% for b, c two steps; (d) $HSCH_2CH_2SH$, $BF_3{\cdot}Et_2O$, HOAc, 3 h, then Ac_2O, 20 min, 54%; (e) W-2 Raney Ni, anhydrous EtOH, rt, 74%; (f) $HOCH_2CH_2OH$, $HC(OEt)_3$, PTSA, rt,80%; (g) 1 M MeONa/MeOH, CH_2Cl_2/MeOH, reflux, 89%; (h) TBDMSCl, imidazole, DMAP, DMF, rt, 99%; (i) $Na_2Cr_2O_7{\cdot}H_2O$, NOS, acetone, 40 °C, 50%; (j) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF/MeOH(2/1), rt, 84%; (k) $Pd(OH)_2$/C, 5% NaOAc aqueous, 1 atm H_2, rt, 93%; (l) PDC, CH_2Cl_2, rt, 86%; (m) $LiAlH_4$, THF, −78 °C, 91%.

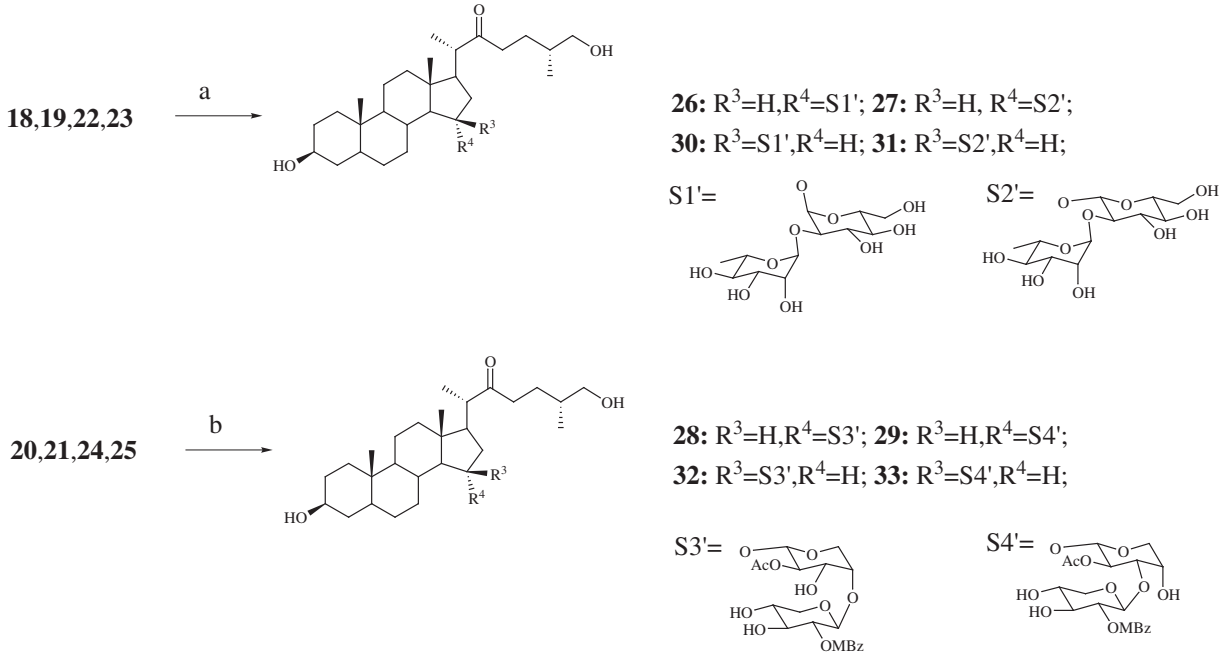


Scheme 2. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 4 Å MS, Ar_2, −20 °C,45% (for **18**); 11.0% (for **19**); 51% (for **20**); 51% (for **21**); 10% (for **22**); 40% (for **23**); 54% (for **24**); 63% (for **25**).

δ ~5.12 ppm (d, 1H, $J_{1,2}$ = 3 Hz), and the 1′-α-anomeric proton of epimer **19** appeared at δ ~4.46 ppm (d, 1H, $J_{1,2}$ = 7.8 Hz). Following the same procedure, **14**was glycosylated with **15** to afford another pair of isomers: **22** and **23** (**22**:**23** = 1/4). The ^{1}H NMR spectra showed that the signal of 1′-β-anomeric proton of epimer **22** appeared at δ ~5.08 ppm (br s, 1H), and the 1′-α-anomeric proton of epimer **23** appeared at δ ~4.49 ppm (d, 1H, $J_{1,2}$ = 8 Hz). The glycosylation of the aglycones (**12**, **14**) with the disaccharide trichloroacetimidates (**16**, **17**) in the presence of TMSOTf (0.10 equiv) provided the desired glycosides (**20**, **21**, **24**, **25**). The resulting saponins bearing Ac, ethylene glycol acetal and silyl protections (**18**, **19**, **22**, **23**) were deprotected using 1 M MeONa/MeOH and followed by $Pd(CH_3CN)_2Cl_2$, providing the desired products (**26**, **27**, **30**, **31**). The another group of resulting saponins bearing PMB, ethylene glycol acetal and silyl protections (**20**, **21**, **24**, **25**) were deprotected using DDQ and followed by $Pd(CH_3CN)_2Cl_2$ to give the expected products (**28**, **29**, **32**, **33**) (Schemes 2 and 3).

The in vitro antitumor activities of compounds **26**–**33** against HCT-8, BEL-7402, Ketr3, A2780, MCF-7, A549 and BGC-823 were evaluated by the standard MTT assay using dioscin as a positive control. The results were listed in Table 1. The IC_{50} values of dioscin against these seven cell lines used in our assays are consistent with those determined by others.[9,21]

Unexpectedly, compounds **26**, **27**, **30**, **31** bearing the disaccharide [α-L-Rha*p*-(1–2)-(α) β-D-Glc*p*] linked at 15-OH showed no inhibition against the seven tumor cells. The consequences proved



Scheme 3. Reagents and conditions: (a) 1 M MeONa/MeOH, CH_2Cl_2/MeOH, reflux, then $Pd(CH_3CN)_2Cl_2$, acetone/H_2O (10/1), rt, 86% (for **26**); 96% (for **27**); 78% (for **30**); 75% (for **31**); (b) DDQ, CH_2Cl_2/H_2O(10/1), rt, then $Pd(CH_3CN)_2Cl_2$, acetone/H_2O (10/1), rt, 85% (for **28**); 82% (for **29**); 86% (for **32**); 76% (for **33**).

Table 1
The in vitro cytotoxicities (IC_{50}, μM) of synthetic 5(6)-dihydro-OSW-1 analogs and dioscin[a]

Cell lines	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	Dioscin
HCT-8	>10	>10	>10	>10	>10	>10	>10	$1 > IC_{50} > 0.1$	0.351
BEL-7402	>10	>10	>10	>10	>10	>10	>10	0.522	0.930
Ketr3	>10	>10	>10	>10	>10	>10	>10	$1 > IC_{50} > 0.1$	0.447
A2780	>10	>10	>10	>10	>10	>10	>10	0.545	0.581
MCF-7	>10	>10	>10	>10	>10	>10	>10	0.499	0.879
A549	>10	>10	>10	>10	>10	>10	>10	0.843	0.454
BGC-823	>10	>10	>10	>10	>10	>10	>10	0.283	0.268

[a] The in vitro cytotoxic activities against HCT-8 (colon cancer), BEL-7402 (liver cancer), Ketr3 (renal cancer), A2780 (ovarian cancer), MCF-7 (breast cancer), A549 (lung cancer) and BGC-823 (stomach cancer) cell lines were evaluated by the standard MTT assay using dioscin as a positive control.

that the introduction of [α-L-Rha*p*-(1–2)-(α) β-D-Glc*p*] did not improve the cytotoxicities of the whole molecule. That the IC_{50} of compounds **28**, **32** carrying the disaccharide [β-D-Xyl*p*-(1–4)-α-L-Ara*p*] were beyond 10 exposed the alteration of glycosylation in arabinose can diminish the activities. Whereas, compound **33** (IC_{50} = 0.28–0.52 μM) bearing the disaccharide [β-D-Xyl*p*-(1–3)-α-L-Ara*p*] displayed potential activities against the seven cancer lines. Nevertheless, its 15-epimer (**29**) was not biologically active. This result revealed that the β configuration of 15-OH is essential to keep the antitumor activities of this kind of saponins.

Thus far, it has been clearly demonstrated that the disaccharide [β-D-Xyl*p*-(1–3)-α-L-Ara*p*] and the β configuration of 15-OH are essential to the antitumor activities of 15-*O*-glycosyl 5(6)-dihydro-OSW-1 analogs. The disaccharides [α-L-Rha*p*-(1–2)-(α) β-D-Glc*p*] and [β-D-Xyl*p*-(1–4)-α-L-Ara*p*] were meaningless to the antitumor activities of 15-*O*-glycosyl 5(6)-dihydro-OSW-1 analogs.

Anyway, compound **33** still retains potential activities which are as potent as 5(6)-dihydro-OSW-1 against liver cancer cells or 10 times lower against breast cancer cells etc. and could be a promising candidate for antitumor agents.

Acknowledgments

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Supplementary data

Supplementary data (^{1}H NMR, ^{13}C NMR, HRMS data of compounds **2**–**14**, **18**–**33** and experimental procedures of these compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.065.

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