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# Synthesis of OSW-1 Analogues and a Dimer and Their Antitumor Activities

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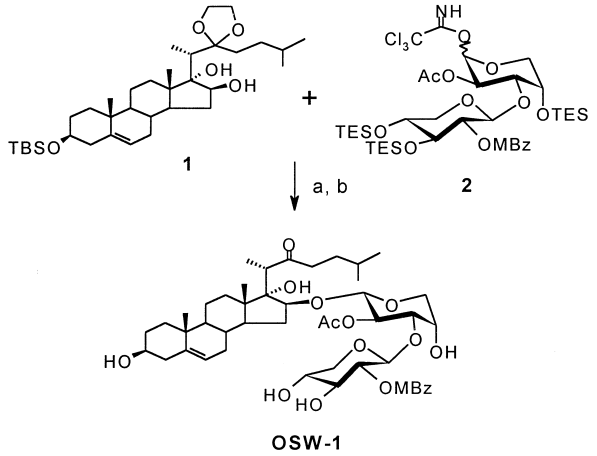
**Abstract**—Five analogues, including a 16-epi-isomer (**6**), and a 3-terephthalic acid linked dimer (**8**) of OSW-1 were synthesized. Their inhibitory activities on P388 and A-549 cells were detected. 

OSW-1 is the major component of a group of cholestane saponins isolated by Sashida et al. from the bulbs of *Ornithogalum saudersiae*, a species of the lily family.[1] In vitro assays showed that OSW-1 was extremely toxic to a broad spectrum of malignant tumor cells, with $IC_{50}$ being between 0.1 and 0.7 nM, which is about 10–100 times more potent than those of the clinically applied anticancer agents, such as mitomycin C, adriamycin, cisplatin, camptothecin, and taxol.[1b] Removal of the acetyl (Ac) and the 4-methoxybenzoyl (MBz) groups on the disaccharide moiety diminished the cytotoxicity by 1000 times.[1b,c] In addition, the synthetic aglycone analogues showed only marginal cytotoxicity ($ED_{50}$ around 10 μM) toward tumor cells,[2] and so were the synthetic glycosides bearing the disaccharide but a totally disparate steroid aglycone of OSW-1, which did not show any cytotoxicity at concentrations lower than 10 μM.[3] These results demonstrate that both the disaccharide and the aglycone are important to the antitumor activities of OSW-1. Therefore, we are attracted to synthesize compounds with structures more close to OSW-1 and test their antitumor activities.

The first total synthesis of OSW-1 was accomplished by our group in 1998.[4] Shortly before that, Fuchs et al. reported the synthesis of the OSW-1 aglycone.[5] Recently, Morzycki et al. reported their synthetic experiments toward the OSW-1 aglycone,[6] and Jin et al. reported the second synthetic route to OSW-1.[7] Both our group and Jin et al. completed the target molecule with the coupling of aglycone **1** with a disaccharide imidate (**2** and its 3′,4′-di-*O*-*p*-methoxybenzyl analogue) and a similar protection strategy; the *tert*-butyldimethylsilyl (TBS), triethylsilyl (TES), and ethylene glycol acetal protections were finally removed using $Pd(CN)_2Cl_2$ (Scheme 1). OSW-1 analogues **3**–**7** and dimer **8** were synthesized in a similar pattern (Fig. 1).

The aglycones **11**[6b] and **12**[4] were intermediates, while **9**, **10**, **13**, and **14** were readily prepared in a few steps from the corresponding intermediates (**15**, **17**, **1**, and **19**, respectively) along the synthetic route toward OSW-1




**Scheme 1.** Reagents and conditions: (a) TMSOTf (0.05 equiv), 4 Å MS, $CH_2Cl_2$, −20 °C, 45 min, 69%; (b) $Pd(CN)_2Cl_2$, acetone/water (v/v, 20:1), rt, overnight, 79%.

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aglycone[4] (Scheme 2). Most of the transformation conditions involved herewith have already been applied in the OSW-1 synthesis. It is worth noting that treatment of the bis-5,16-diene **20** with $OsO_4$ under similar conditions gave the corresponding mono-16α,17α-diol mainly (73%), which was charged with $OsO_4$ again to give the expected bis-16α,17α-diol **21** in 83% yield (totally 69% yield for two steps). Reverse of the configuration of the 16-OH using a Swern oxidation–$NaBH_4/CeCl_3$ reduction sequence worked well on the dimeric substrates (Fig. 2).

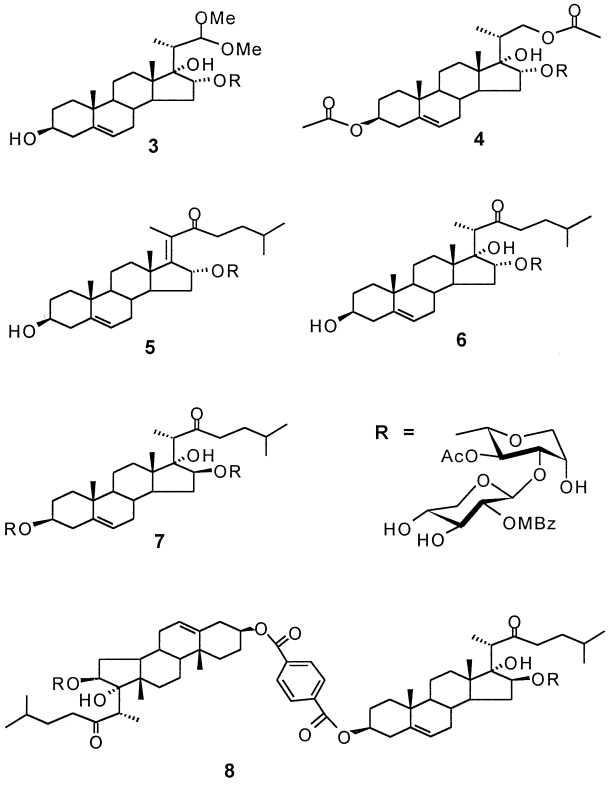



**Figure 1.** OSW-1 analogues **3**–**7** and the dimer **8**.

Coupling of the aglycones (**9**–**14**) with disaccharide trichloroacetimidate **2** in the presence of TMSOTf (0.05 equiv) provided the desired glycosides **22**–**27** smoothly (Table 1). For the 16α,17α-diols (**9**, **10**, and **12**), only the 16-*O*-glycosyalted products (**22**, **23**, and **25**) were isolated, in yields of 80, 62, and 44%, respectively. For 3,16,17-triol **13**, the 3,16-di-*O*-glycosylated product (**26**) was isolated as the major product in a yield of 35% (equal to a 60% yield per glycosylation) when 3.0 equivalents of the donor **2** were used. To drive the reaction to completion, 15 equivalents of the donor **2** were used in the coupling with dimeric aglycone **14**, giving the dimer **27** in 81% yield. In comparison, glycosylation of the 16α-OH is a little more difficult than of the 16β-OH (cf. glycosylation with epimer **1** and **12**, especially).

The resulting saponins bearing ethylene glycol acetal and silyl protections (**22**, **25**–**27**) were deprotected using $Pd(CN)_2Cl_2$, providing the desired products (**3**, **6**–**8**) in 70–82% yields. It should be noted that removal of the ethylene glycol acetal protection of the 22-aldehyde of **22** afforded dimethyl acetal **3** upon chromatography on a silica gel column eluted with $CH_2Cl_2$/MeOH (v/v, 8:1). Cleavage of the TES protection on **23** was realized in 70% HOAc at 65 °C, affording **4** in 91% yield. The 3α,5α-cyclo-6β-methoxy protection and TES groups on

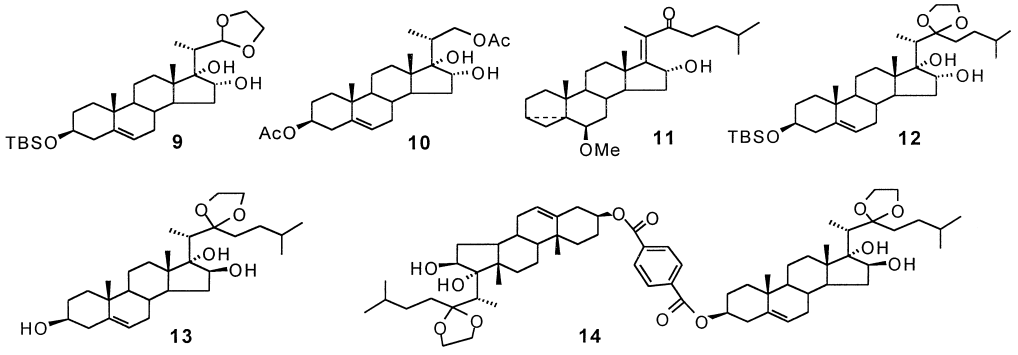
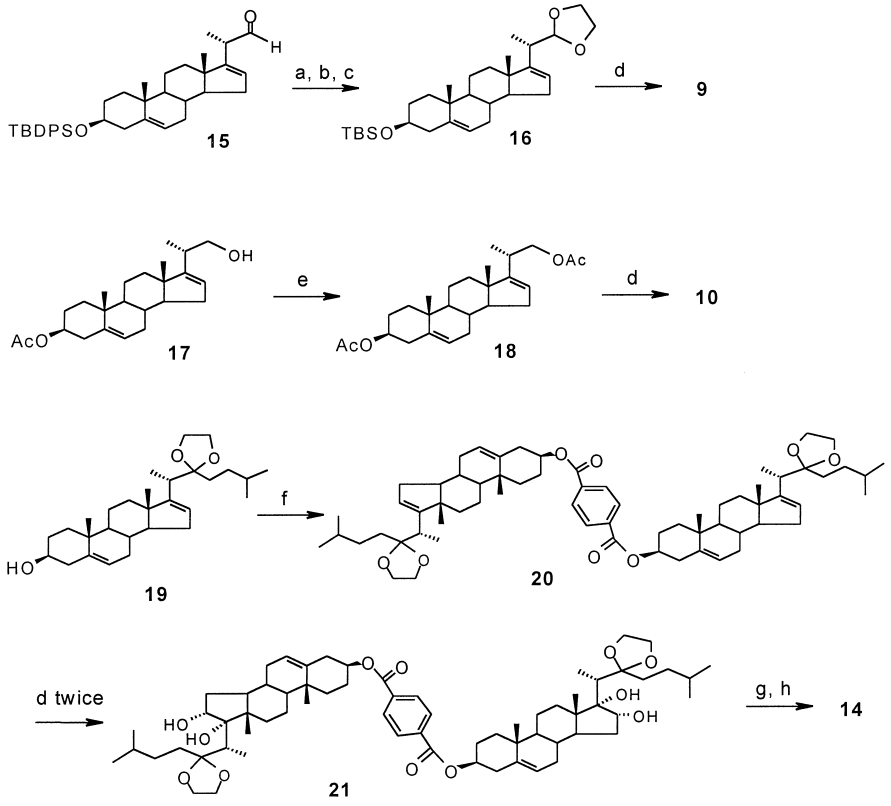



**Figure 2.** The corresponding aglycones **9**–**14**.

**Table 1.** Glycosylation of aglycones **9**–**14** with disaccharide imidate **2** and deprotection of the coupling products to prepare the OSW-1 analogues **3**–**8**[a,b,9]

| Entry | Aglycone | Coupling product (Yield%) | Deprotection conditions | Deprotected product (Yield %) |
|---|---|---|---|---|
| 1 | **9** | **22** (80) | A | **3** (82) |
| 2 | **10** | **23** (62) | B | **4** (91) |
| 3 | **11** | **24** (61) | C | **5** (99) |
| 4 | **12** | **25** (44) | A | **6** (80) |
| 5 | **13** | **26** (35) | A | **7** (71) |
| 6 | **14** | **27** (81) | A | **8** (70) |

[a]Coupling conditions: donor **2** (1.0 equiv), acceptors **9**–**11** or **13** (0.8 equiv), or **12** (0.37 equiv), or **14** (0.06 equiv), TMSOTf (0.05 equiv), 4 Å MS, $CH_2Cl_2$, −20 °C to rt, 30 min.
[b]A, $Pd(CN)_2Cl_2$, acetone/water (v/v, 20:1), rt, 4–8 h; B, 70% HOAc, 65%, 3 h; C, *p*-TsOH·$H_2O$, dioxane/water (v/v, 3:1), 80 °C, 1 h.




**Scheme 2.** Preparation of the aglycones **9**, **10**, and **14.** Reagents and conditions: (a) $HOCH_2CH_2OH$, $CH(OEt)_3$, $CH_2Cl_2$, $TsOH{\cdot}H_2O$ (cat.), rt, overnight, 100%; (b) TBAF, THF, rt, overnight, 97%; (c) TBSCl, imidazole, DMF, rt, overnight, 94%; (d) $OsO_4$, pyridine, $Et_2O$, −20 °C to rt, 83% (for **9** and **10**), 69% (for **14**); (e) $Ac_2O$, pyridine, rt, overnight, 100%; (f) teraphthaloyl chloride, benzene, pyridine, 55 °C, 2 days, 84%; (g) ClCO-COCl, DMSO, $CH_2Cl_2$, −78 °C, 30 min, 70%; (h) $NaBH_4$, $CeCl_3{\cdot}7H_2O$, THF, 0 °C, 4 h, 93%.

**24** were released in dioxane/water (v/v, 3:1) in the presence of a catalytic amount of *p*-TsOH at 80 °C, giving **5** in 99% yield.

The in vitro antitumor activities of OSW-1 and its analogues **3**–**7** and the dimer **8** against P388 (mouse leukemia) and A-549 (human lung adenocarcinoma) were evaluated by the standard MTT assay.[8] The results were listed in Table 2. OSW-1, which has a reported $IC_{50}$ of 0.13 nM against P388 and of 0.68 nM against A-549,[1b] showed 100 and 98.2% growth inhibition rate, respectively, against P388 and A-549 at 0.01 μM in the present testing. However, its 16-epimer (**6**) showed only marginal activities at 0.1 μM. This result demonstrates that the $C_{16}$ configuration is essential to the antitumor activities of OSW-1. It is therefore reasonable that compounds **3**–**5**, which have both an opposite configuration at $C_{16}$ and a modified $C_{17}$ side chain, were much less potent, which showed little or no inhibition against P388 and A-549 at 1.0 μM concentration. It was reported that a 3-*O*-glucopyranosyl derivative of OSW-1 was as active as OSW-1 against HL-60 cells.[1b] However, the 3-*O*-disaccharide derivative of OSW-1 (**7**) showed high growth inhibition rate only at 1.0 μM, which is about 1000 times less active than that of **1**. Interestingly, the 3-*O*-teraphthaloyl linked dimer **8** showed 65.6 and 52.8% growth inhibition rate against P388 and A-549 cells, respectively, at 0.01 μM.

Thus far, it has been clearly demonstrated that the acyl groups on the disaccharide moiety and the $C_{16}$ configuration are essential to the antitumor activities of OSW-1. The importance of the $C_{17}$ side chain to the antitumor activities of OSW-1 has also been implied. The 3-OH of OSW-1 is most likely the holder for further derivatization without significantly affecting the antitumor activities. Synthesizing conjugates with biotin and fluorescent groups linked at the 3-OH of OSW-1 to facilitate the mechanistic studies of its antitumor activities is our current interest, and the results will be published in due course.

**Table 2.** Growth inhibition rate (%) of OSW-1 and its analogues **3**–**8** on tumor cells (P388 and A-549)

| | P388 | | | A-549 | | |
|---|---|---|---|---|---|---|
| Compound | $10^{-6}$ M | $10^{-7}$ M | $10^{-8}$ M | $10^{-6}$ M | $10^{-7}$ M | $10^{-8}$ M |
| **OSW-1** | 100 | 100 | 100 | 99.3 | 99.1 | 98.2 |
| **3** | — | — | — | — | — | — |
| **4** | 20.4 | 15.5 | 17.9 | 6.2 | 0.8 | — |
| **5** | — | — | — | — | — | — |
| **6** | 66.7 | 35.4 | 15.5 | 53.8 | 29.5 | — |
| **7** | 90.7 | 52.0 | — | 80.9 | 6.7 | 0.7 |
| **8** | 99.7 | 98.7 | 65.6 | 89.7 | 74.1 | 52.8 |

## Acknowledgements

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9. Selected analytical data for compounds **3**–**8**. **3**: $[\alpha]_D^{17} = -22.3°$ (*c* 0.44, $CH_3OH$); ESI-MS: 871 (M + Na); $^1H$ NMR (pyridine-$d_5$, 300 MHz): 8.00 (d, 2H, $J = 9.0$ Hz), 6.86 (d, 2H, $J = 9.0$ Hz), 5.32 (brs., 1H), 5.30–5.20 (m, 3H), 4.88–4.78 (t-like, 2H, $J = 7.7$, 5.3 Hz), 3.76 (s, 3H), 3.37 (s, 3H), 1.56 (s, 3H), 1.03 (d, 3H, $J = 6.9$ Hz), 1.02 (s, 3H), 0.73 (s, 3H). **4**: $[\alpha]_D^{17} = -19.8°$ (*c* 0.59, $CH_3OH$); ESI-MS: 911 (M + Na); $^1H$ NMR ($CDCl_3$, 300 MHz): 7.96 (d, 2H, $J = 8.7$ Hz), 6.91 (d, 2H, $J = 8.7$ Hz), 5.35 (br, 1H), 4.99 (t-like, 2H, $J = 7.8$, 6.3 Hz), 4.79 (d, 1H, $J = 5.5$ Hz), 4.60 (m, 1H), 4.38 (d, 1H, $J = 6.6$ Hz), 4.32 (brs., 1H), 3.86 (s, 3H), 3.47 (d, 1H, $J = 11.0$ Hz), 3.44 (dd, 1H, $J = 12.4$, 6.5 Hz), 2.06 (s, 3H), 2.04 (s, 3H), 1.80 (s, 3H), 1.05 (d, 3H, $J = 6.6$ Hz), 1.00 (s, 3H), 0.78 (s, 3H). **5**: $[\alpha]_D^{17} = -42.3°$ (*c* 0.45, $CH_3OH$); ESI-MS: 878 (M + Na); $^1H$ NMR ($CDCl_3$, 300 MHz): 7.97 (d, 2H, $J = 8.8$ Hz), 6.91 (d, 2H, $J = 8.8$ Hz), 5.30 (d, 1H, $J = 3.9$ Hz), 5.00 (m, 2H), 4.75 (d, 1H, $J = 6.3$ Hz), 4.72 (d, 1H, $J = 5.0$ Hz), 4.38 (d, 1H, $J = 6.6$ Hz), 4.31 (t-like, 1H, $J = 6.9$, 6.6 Hz), 3.86 (s, 3H), 1.90 (s, 3H), 1.73 (s, 3H), 0.97 (s, 3H), 0.90 (d, 6H, $J = 6.3$ Hz), 0.89 (s, 3H). **6**: $[\alpha]_D^{17} = -16.3°$ (*c* 0.45, $CH_3OH$); ESI-MS: 896 (M + Na); $^1H$ NMR (pyridine-$d_5$, 300 MHz): 8.00 (d, 2H, $J = 9.0$ Hz), 6.77 (d, 2H, $J = 9.0$ Hz), 5.42 (q-like, 2H, $J = 9.3$, 8.0 Hz), 5.14 (brd., 1H, $J = 3.8$ Hz), 4.93 (d, 1H, $J = 8.0$ Hz), 4.35 (d, 1H, $J = 8.0$ Hz), 4.20 (brs., 1H), 3.93 (dd, 2H, $J = 10.2$, 3.3 Hz), 3.73 (s, 3H), 3.45 (s, 3H), 1.57 (s, 3H), 1.01 (d, 3H, $J = 6.8$ Hz), 0.82 (s, 3H), 0.71 (d, 3H, $J = 6.3$ Hz), 0.67 (d, 3H, $J = 6.3$ Hz), 0.60 (s, 3H). **7**: $[\alpha]_D^{17} = -22.7°$ (*c* 0.34, $CH_3OH$); ESI-MS: 1335 (M + Na); $^1H$ NMR (pyridine-$d_5$, 300 MHz): 8.06, 6.84 (AB, 8H), 5.64 (dd, 1H, $J = 9.3$, 7.4 Hz), 5.46 (m, 3H), 5.33 (dd, 1H, $J = 8.2$, 6.6 Hz), 5.10 (brs., 1H), 4.95 (d, 1H, $J = 8.0$ Hz), 4.90 (d, 1H, $J = 7.7$ Hz), 4.53 (d, 1H, $J = 7.4$ Hz), 4.36 (d, 1H, $J = 5.8$ Hz), 4.27 (brs., 1H), 4.18 (brs., 1H), 3.52 (s, 3H), 3.48 (s, 3H), 2.96 (q, 1H, $J = 7.7$ Hz), 1.75 (s, 3H), 1.65 (s, 3H), 1.05 (d, 3H, $J = 7.4$ Hz), 0.74 (s, 3H), 0.69 (s, 3H), 0.66 (d, 3H, $J = 6.6$ Hz), 0.62 (d, 3H, $J = 6.6$ Hz). Anal. calcd for $C_{67}H_{92}O_{26}$: C, 61.27; H, 7.06. Found: C, 60.94; H, 7.60. **8**: $[\alpha]_D^{19} = -24.5°$ (*c* 0.38, $CH_3OH$); $^1H$ NMR (pyridine-$d_5$, 600 MHz): 8.34, 7.02 (AB, 8H), 5.93 (t, 2H, $J = 9.6$ Hz), 5.77 (dd, 1H, $J = 10.0$ Hz, 7.8), 5.64 (d, 2H, $J = 8.0$ Hz), 5.57 (dd, 1H, $J = 9.6$ Hz, 7.8), 5.24 (brs., 2H), 5.12 (d, 2H, $J = 7.8$ Hz), 5.00 (m, 2H), 4.58 (d, 2H, $J = 7.8$ Hz), 4.31–4.19 (m, 6H), 4.18–4.06 (m, 6H), 4.00 (brs., 2H), 3.70 (s, 6H), 1.83 (s, 6H), 1.20 (d, 6H, $J = 7.2$ Hz), 1.19 (s, 6H), 1.01 (s, 6H), 0.88 (d, 12H, $J = 6.6$ Hz). $^{13}C$ NMR (pyridine-$d_5$, 150 MHz): 223.5, 168.4, 165.3, 164.2, 163.8, 139.3, 132.4, 132.3, 130.0, 114.2, 114.1, 110.3, 103.7, 95.3, 88.9, 85.3, 81.8, 78.9, 76.4, 75.3, 71.5, 71.0, 70.9, 69.3, 69.0, 67.1, 67.0, 58.9, 55.5, 54.5, 46.8, 43.4, 38.2, 36.4, 35.1, 33.2, 32.0, 30.0, 27.8, 24.2, 23.0, 22.6, 22.5, 20.9, 18.3, 15.9, 14.2, 14.0, 8.6.