Medicinal Chemistry

23-Oxa-Analogues of OSW-1: Efficient Synthesis and Extremely Potent Antitumor Activity**

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Saponin OSW-1, which contains a novel 16β,17α-dihydroxycholest-22-one aglycone unit with an acylated disaccharide at the 16-OH group, was discovered by Sashida, Mimaki, and coworkers in 1992 in the bulbs of *Ornithogalum saudersiae*, a perennial garden plant of the lily family widely cultivated in southern Africa.[1] Tremendous attention has been given to this compound since its antitumor activity was tested in 1995: OSW-1 has a low toxicity for normal cells but inhibits the growth of a variety of malignant tumor cells and is 10–100 times more potent than clinically applied anticancer agents such as mitomycin C, adriamycin, cisplatin, camptothecin, and taxol.[2] Further research on *O. saudersiae* and taxonomically related plants has revealed a family of 16β,17α-dihydroxycholest-22-one saponins.[3] Considerable effort has been directed toward the synthesis of OSW-1, the first and most abundant member of the family having been isolated.[4–7] The aglycone was first synthesized by Fuchs and Guo in 1998.[4] Shortly afterwards, we coupled the aglycone with the disaccharide moiety to complete the total synthesis of OSW-1.[5] Jin and Yu recently greatly improved this synthesis.[6] However, studies on the structure–activity relationships (SAR) of this novel type of anticancer agent have so far been limited;[3,8] the ascertained structural requirements for the exceptionally strong antitumor activity of OSW-1 are the presence of Ac and MBz groups on the disaccharide moiety and β orientation of the 16-*O*-sugar unit.[3,8b] Detailed biological, toxicological, and pharmacokinetic studies await the outcome of attempts to overcome a new synthetic challenge: the preparation of a variety of analogues, derivatives, and hopefully a lead compound in multigram quantities. Herein we report a novel and efficient approach to the construction of the 16β,17α-dihydroxycholest-22-one structure. One of the

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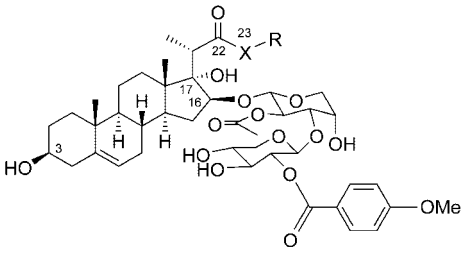
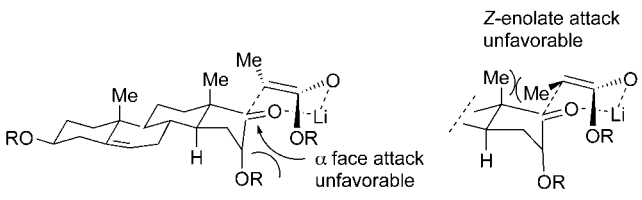
23-oxa-analogues of OSW-1 (**1**–**3**) prepared by this method is a more potent inhibitor of the growth of tumor cells than OSW-1.



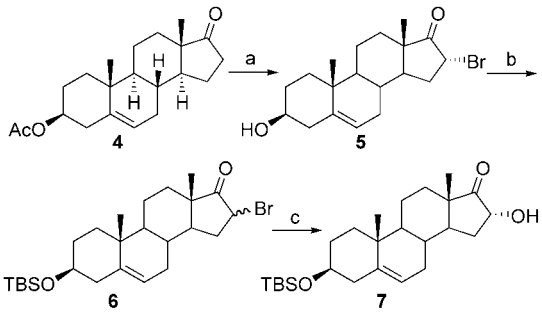
OSW-1: X = CH_2, R = $CH_2CH(CH_3)_2$
1: X = O, R = $CH_2CH(CH_3)_2$
2: X = O, R = CH_2CH_3
3: X = O, R = $(CH_2)_{11}CH_3$

A major challenge in the synthesis of OSW-1 is the elaboration of the 16β,17α-dihydroxycholest-22-one structure. Fuchs and Guo assembled the side chain on 5-androsten-3β-ol-17-one by a Wittig olefination and ene reaction sequence. The 16β,17α-diol was then introduced by dihydroxylation of the 16,17-ene group (1 equiv OsO_4) followed by inversion of the resulting 16α-OH group.[4] Jin and Yu developed a novel protocol for the synthesis of OSW-1 that involves 1,4-addition of an α-alkoxy vinyl cuprate to a 17(20)-en-16-one. The 17α-OH group was introduced to the resulting 16,17-enolate by treatment with Davis reagent.[6] Morzycki et al. used intramolecular ring opening of the 16α,17α-epoxide by the 22-carbonyl function as a starting point for further elaboration.[7a,b] We reanalyzed the characteristic 16β,17α-dihydroxycholest-22-one structure of OSW-1 saponins and envisioned the synthetic target as an α-methyl-β,γ-diol-one. This evaluation suggested that aldol condensation (between an α-hydroxy ketone and a propionate) would be the most direct approach to synthesis of the desired product.[9] We hoped to achieve stereocontrol by using a rigid 16α-hydroxy-17-oxo steroid as the ketone substrate, which would lead to the desired natural stereochemistry at C17 and C20 if the bulky 16α-OR group could force the enolate to approach from the β face (by nonchelation control,[10] Scheme 1, left). The chair transition state (Zimmerman–Traxler model)[10a] favors the *E* enolate over the *Z* enolate since the *E* conformation avoids steric interaction of the 18-methyl group with the methyl group of the propionate enolate (right).



Scheme 1. Proposed construction of the 21α-methyl-16α,17α-diol steroid structure by stereocontrolled aldol condensation.

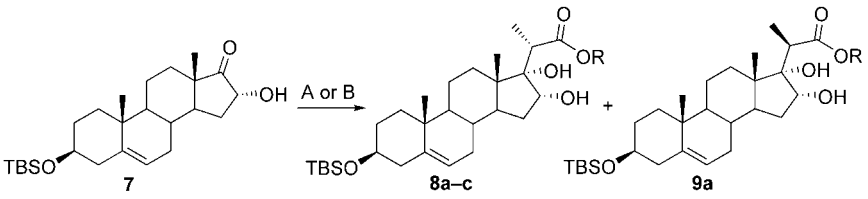
Encouragingly, Gros and Doller found that β-face attack of *tert*-butyl propionate by a lithium enolate leads to a 16α-acetoxy-17-oxo-androstane.[11]



Scheme 2. Introduction of the 16α-OH group. a) $CuBr_2$ (3.0 equiv), CH_3OH, reflux, 91%; b) TBSCl, imidazole, DMF, RT, overnight, 96%; c) NaOH (1.3 equiv), DMF/CH_2Cl_2/H_2O (3:2:1, v/v/v), RT, overnight, 95%. TBS = *tert*-butyldimethylsilyl, DMF = dimethylformamide.

The required 16α-hydroxy-5-androsten-17-one **7**, with the 3-OH group protected by a TBS ether, was readily prepared from the industrially produced acetate **4** by using a modified version of a literature procedure (Scheme 2).[12] Bromination of ketone **4** by treatment with $CuBr_2$ (3 equiv) in methanol under reflux provided 16α-bromide **5** in 91% yield (full cleavage of the 3-OAc group). Protection of the 3-OH group by treatment with TBSCl in the presence of imidazole in DMF gave **6**. Epimerization of the 16α-bromide and its 16β isomer took place readily. S_N2 displacement of the 16β-bromide by a hydroxide ion in DMF afforded the 16α-alcohol **7** in 95% yield.

The crucial aldol condensation of 16α-hydroxy-17-one **7** with propionate enolates was examined carefully (Scheme 3). The lithium *E* enolate of ethyl propionate was generated by

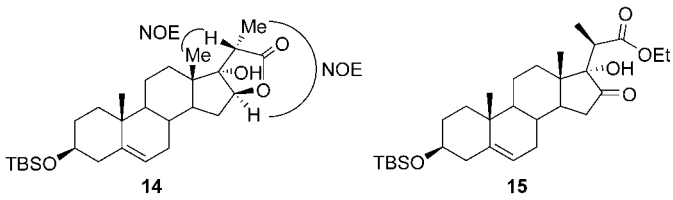


Scheme 3. Aldol condensation of 16α-hydroxy-17-one **7** with propionate enolates. Conditions: A) 1. iPr_2NH, *n*BuLi, −78°C, 15 min; 2. HMPA, THF, −78°C, then ethyl propionate, 0.5 h; 3. **7**, −78°C, 63% (for **8a**). B) 1. iPr_2NH, *n*BuLi, −78°C, 15 min; 2. propionates, THF, −78°C, 0.5 h; 3. **7**, −78°C, 75% (**8a**), 12% (**9a**), 78% (**8b**), 81% (**8c**). HMPA = hexamethyl phosphoramide, THF = tetrahydrofuran.

the method described by Ireland et al.[13] When **7** was treated with this enolate (A, Scheme 3), only the expected 17α-hydroxy-21α-methyl product **8a** was produced (in 63% yield). Without the use of stereocontrol to generate exclusively the *E* enolate of ethyl propionate (B, Scheme 3), the condensation reaction predominantly led to the desired 21α-methyl product **8a** (75% yield), but its 21β-methyl isomer **9a** was also isolated in 12% yield. Condensation with isobutyl

and dodecyl propionate under conditions B gave predominantly the *E* enolates as a result of the greater size of the isobutyl and dodecyl groups compared to the ethyl group;[14] the desired 17α-hydroxy-21α-methyl compounds (**8b** and **8c**) were the only isolated products (78% and 81% yield, respectively).

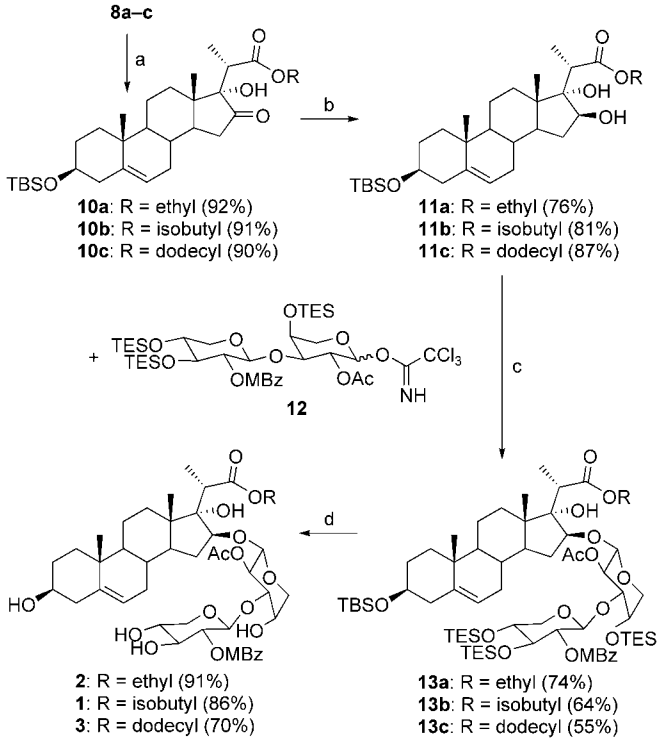
The stereochemistry of the aldol products was confirmed by analysis of their reaction products **14** and **15**. Lactone **14**



was produced in the course of inversion of the 16α-OH group of **8** (**10**→**11**, Scheme 4) and was analyzed by NMR spectroscopy. NOE interactions were observed between the α-oriented protons at position 16 and on the 21-methyl group, and between the β-oriented protons at position 20 and on the 18-methyl group. Single-crystal X-ray diffraction analysis was performed on **15**,[15] which was prepared by oxidation of the 16-OH group of the minor aldol product **9a** (conditions similar to those for **8**→**10**, Scheme 4).

Once the desired 17α-hydroxy-21α-methyl-22-one architecture had been constructed, synthesis of OSW-1 and its congeners was straightforward. However, we first turned our attention to the synthesis of the 23-oxa analogues of OSW-1, **1**–**3**, which we obtained from **8a–c** in four steps by employing transformations similar to those used in our previously described OSW-1 synthesis.[5] The results are shown in Scheme 4. Oxidation of the 16α-OH group of **8b** by treatment with TPAP/NMO[16] gave the 16-keto compound **10b** in 91% yield. Reduction of the resulting 16-ketone with $NaBH_4$ in the presence of $CeCl_3$ afforded 16β-ol **11b** stereoselectively in 81% yield. This reaction had to be quenched at −40°C, as higher temperatures resulted in the production of lactone **14**. The lactonization also took place in $CDCl_3$ during NMR measurement. Coupling of 16β,17α-diol **11b** with disaccharide imidate **12**[5] in the presence of TMSOTf (0.1 equiv) and 4-Å MS gave glycoside **13b** in a satisfactory yield (64%), without production of a significant amount of lactone **14**. Finally, deprotection of the silyl groups by treatment with $[PdCl_2(MeCN)_2]$[17] afforded the desired product **1** in 86% yield. The congeners **2** and **3**, which have a shorter or a longer side chain than **1**, were similarly prepared from **8a** and **8c**, respectively.

23-Oxa-OSW-1 (**1**) strongly suppressed the growth of the three types of malignant tumor cells tested. The concentration of **1** required for 50% inhibition (IC_{50}) was 0.031–3.1 μM, which indicates that this compound is as potent as OSW-1 and is 8–60 times more potent than the anticancer drug cisplatin in the assay we used (Table 1). The short congener **2** was slightly



Scheme 4. Completion of the synthesis of 23-oxa-OSW-1 (**1**) and its congeners **2** and **3**. a) TPAP, NMO, 4-Å MS, CH_2Cl_2, RT, overnight. b) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, THF, −10°C, then −40°C, MeOH, 15 min. c) TMSOTf (0.1 equiv), 4-Å MS, CH_2Cl_2, −20°C, 2 h. d) $[PdCl_2(MeCN)_2]$, acetone/water (20:1, v/v), RT, overnight. MBz = 4-methylbenzoyl, MS = molecular sieves, NMO = *N*-methylmorpholine *N*-oxide, TES = triethylsilyl, TMSOTf = trimethylsilyl trifluoromethanesulfonate, TPAP = tetrapropylammonium perruthenate.

Table 1: Cytotoxic activities of **1**–**3** and the controls OSW-1 and cisplatin against tumor cells.[a]

Tumor cells[b]	IC_{50} [μM]				
	1	**2**	**3**	OSW-1	cisplatin
AGS	3.10	11.82	0.40	1.42	24.1
7404	0.031	0.30	0.004	0.10	8.37
MCF-7	0.31	1.48	0.052	0.27	18.7

[a] The standard MTT assay was used (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The IC_{50} values determined for cisplatin against the three cell lines used in our assay are consistent with those determined by others.[15] [b] AGS = human stomach cancer cell line, 7404 = human liver carcinoma cell line, MCF-7 = human breast cancer cell line.

less potent than **1**. Interestingly, the longer congener **3** was considerably more potent than OSW-1 (as much as 25 times more potent against the human liver carcinoma cell line 7404).

In summary, a novel and efficient approach to the construction of the 16β,17α-dihydroxycholest-22-one steroidal architecture characteristic of the saponin OSW-1 family was developed that uses an aldol condensation as the key reaction. Members of this family of compounds have exceptionally potent antitumor activities. The 23-oxa analogues of OSW-1 **1**–**3** were synthesized from the industrially produced steroid **4** in 26, 30, and 20% overall yield, respectively, by a

linear eight-step procedure. The 23-oxa-OSW-1 (**1**) showed similar antitumor potency to that of OSW-1, while the longer congener **3** was even more potent. Progress toward an understanding of the SARs and antitumor mechanisms of OSW-1 saponins should be greatly accelerated by the development described herein. The results of our ongoing research will be reported in due course.

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