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The First Synthesis of the Aglycone of the Potent Anti-tumor Steroidal Saponin OSW-1[1]

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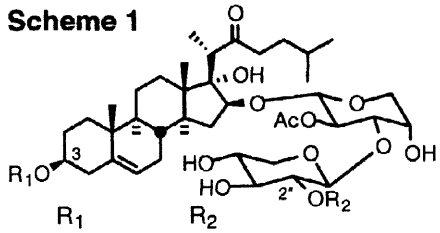
Abstract: The protected aglycone (**21e**-β) of the potent anti-tumor agent OSW-1 (**1**) was synthesized in 9 steps from 5-androsten-3β-ol-17-one (**10**) in 55% overall yield. Key reactions involve ene installation of the side chain, regio and stereoselective dihydroxylation and diastereoselective reduction of the C16 ketone.

The steroidal saponin OSW-1 (**1**) belongs to a family of cholestane glycosides isolated from *Ornithogalum saundersiae* by Sashida and his coworkers at Tokyo University of Pharmacy and Life Science.[2] They feature the same steroidal unit, namely 3β,16β,17α-trihydroxycholest-5-en-22-one. Structural variations arise from different substituents at the 2" positions of the disaccharide moiety and at the C3 oxygen moiety.

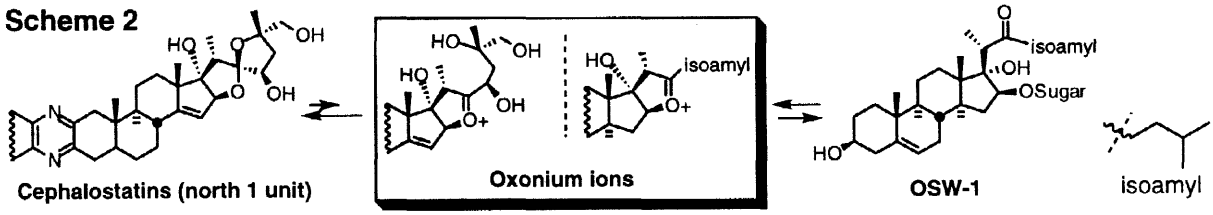
Scheme 1



	R_1	R_2
1	H	*P*-methoxybenzoyl (OSW-1)
2	H	3,4-dimethoxybenzoyl
3	H	(*E*)-cinnamoyl
4	Glc	*P*-methoxybenzoyl
5	Glc	(*E*)-cinnamoyl

Since all five of these saponins show potent cytotoxicity against leukemia HL-60 cancer cells with IC_{50} values between 0.1 to 0.3 nM, and OSW-1 (**1**) is the major component from the extraction, its anti-tumor activity was studied in more detail.[2b] An *in vivo* study showed that this saponin prolonged the life span of P388 leukemia infected mice by 59% via a single administration of **1** at 10 μg/kg. While OSW-1 is exceptionally cytotoxic against various malignant tumor cells, it showed little toxicity (IC_{50} 1500 nM) to normal human pulmonary cells. The compound was also tested in the NCI 60 cell line *in vitro* screen, with an average GI_{50} of 0.78 nM. More interestingly, ***the cytotoxicity profile of OSW-1 against different cancer cell lines is very similar to that of the cephalostatins[3] with correlation coefficients between 0.60 to 0.83***.[2b] The cephalostatins belong to a family of more than forty bis-steroidal pyrazines[3] with broad spectrum of anti-tumor activities. During the last five years, several research groups including our own have actively investigated the evolving SAR of the growing family of these compounds by synthesizing the natural products and their analogs.[4] The cytotoxicity correlation between the cephalostatins and OSW-1 indicates that they might have the same mechanism of action.

Scheme 2



After comparison of the structure of these two families, we hypothesize that the active intermediate might be an oxonium ion, which could be generated from both types of compounds (Scheme 2). The OSW-1 disaccharide unit might serve as a recognition element or a polarity modifier. Since OSW-1 contains relatively simple steroid skeleton, its aglycone should be an excellent tool to probe the mechanism of action of both drug families. To test

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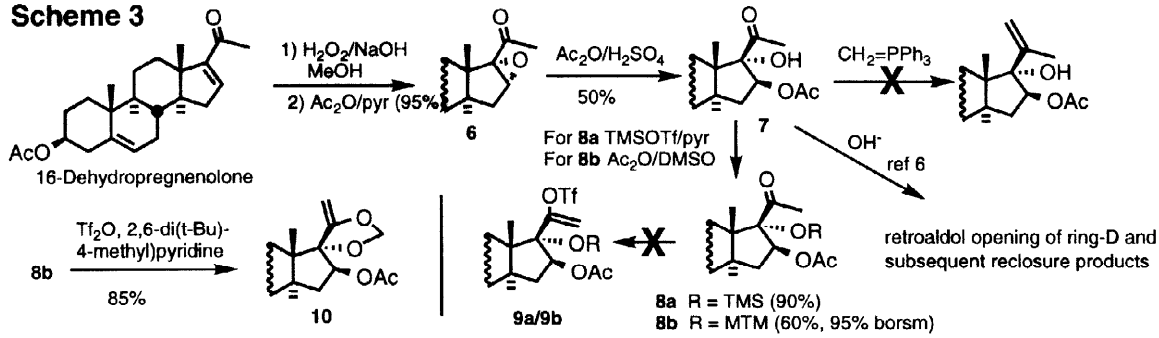
our hypothesis, we needed to first synthesize the aglycone of OSW-1. Because it was reported that the aglycone decomposed during acid hydrolysis of **1**, a protected aglycone should be a valuable intermediate for synthesis of OSW-1 analogs with different substituents at the 16β-alcohol.

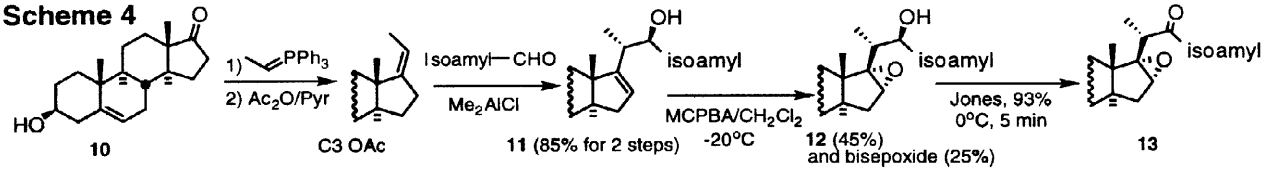
Our initial route involved the 16,17-dioxygenated ketone **7**. 16-Dehydropregnenolone acetate was regio and stereoselectively oxidized to 16α,17α-epoxide (**6**).[5] Subsequent acetolysis[6] gave 16β-acetoxy, 17α-alcohol (**7**). Unfortunately, all attempts to introduce the side chain by Wittig reaction failed presumably due to the known alkali-catalyzed retroaldol opening of the D-ring.[7] The 17α-alcohol was therefore protected as either the TMS or MTM[8] ether (**8a**, **8b**) under forcing conditions. A revised plan to install the side chain by Nozaki-Hiyama coupling[9] required transformation of the C20 ketone to a vinyl triflate (**9a** or **9b**). In the presence of the C17 TMS ether (**8a**), the C20 ketone does not undergo reaction. By comparison, treatment of **8b** with Tf_2O and 2,6-(t-butyl)-4-methylpyridine[10a] resulted in conversion of MTM ether **8b** to the undesired cyclic enol ether **10**. Conditions involving LDA/Tf_2NPh[10b] returned starting material in all cases.

Scheme 3



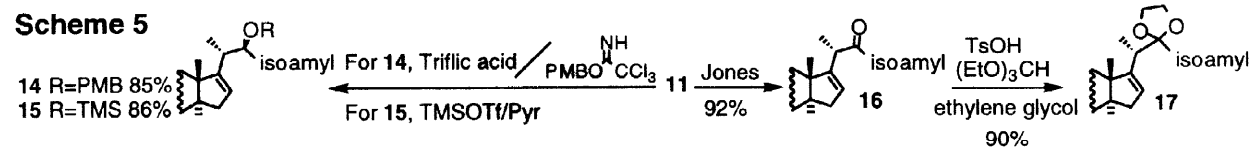
Since it proved difficult to install the side chain with a functionalized D-ring, we elected to invert these two operations. After Wittig olefination of ketone **10**, the C22 oxygenated side chain was introduced by diastereoselective ene reaction.[11] Upon treatment with MCPBA at -20°C, the dienol **11** was non-selectively converted to 16α,17α-epoxide (**12**, 45%) and diepoxide (25%). The C22 alcohol of **12** was then oxidized to ketone (**13**) by Jones oxidation (93%). Alkali-catalyzed alcoholysis of the epoxide **13** only led to cleavage of the C3 acetate, while acid-catalyzed epoxide cleavage resulted in total decomposition of the starting material.

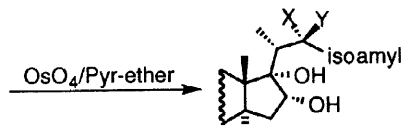
Scheme 4



To avoid problems associated with the epoxide **13**, we turned to dihydroxylation. Dienol **11** was converted to 16α,17α,22(S)-triol **18a** (OsO_4 (1eq) in pyridine-ether at -50°C) in excellent yield (>90%) with nearly exclusive regio and stereoselectivity. The C22 oxygenated center was next protected because the C22

Scheme 5



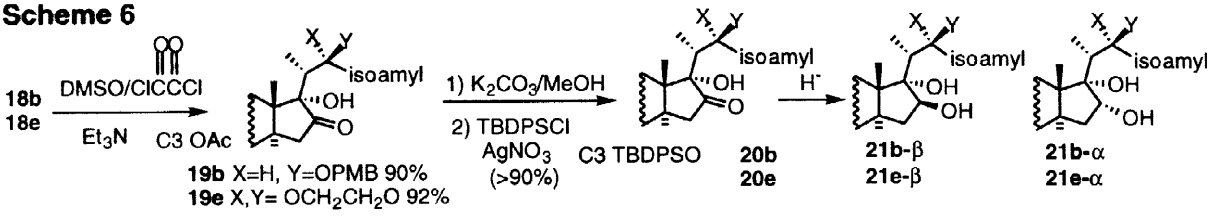


	Temperature (°C)/time
18a X=H Y=OH (>90%)	-60 to -35 (4h)
18b X=H Y=OPMB (80%)	-50 to -30 (5h)
18c X=H Y=OTMS (50%)	-40 to -20 (20h)
18d X=Y=O (40%)	-40 to -20 (20h)
18e X,Y= OCH_2CH_2O (81%)	-30 to -20 (36h)

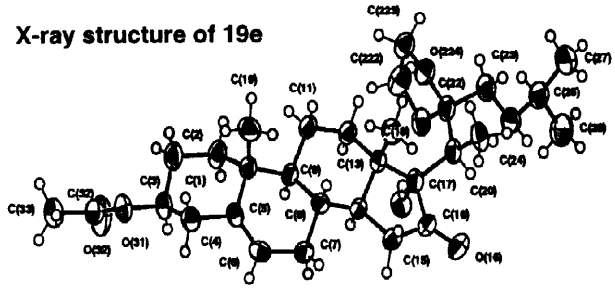
alcohol would interfere during later transformations. Substrates (**14-17**) with different functionalities at C22were subjected to the osmylation reaction. Both C22 PMB ether (**14**) and C22 ketal (**17**) were found to give 16,17-diols **18b** and **18e** in good yield (~80%), along with 5,6-diols (10%) in addition to the starting 16-ene (**14/17**, 10%). Catalytic osmylation on **17** only afforded the diol **18e** in low yield.

Diols **18b** and **18e** were separately carried forward. Attempts to invert the C16 alcohol of **18b** and **18e** under Mitsunobu conditions resulted in no reaction. Oxidation of the C16 alcohol of **18b** and **18e** to the corresponding ketone was found to be non-trivial. Jones oxidation caused decomposition. Starting material was recovered from pyridinium-CrO_3. Dess-Martin periodate oxidatively cleaved the diol to give the D ring-opened 17-keto-16-aldehyde (not shown) instead of affording the α-hydroxyketone. Fortunately, Swern oxidation (oxalyl chloride/DMSO)[12] of *cis*-diols **18b** and **18e** afforded the desired α-hydroxyketones **19b** and **19e** in excellent yield (90%). At this stage, replacement of the C3 acetate with a TBDPS ether was necessary due to anticipated selectivity problems ultimately arising from the presence of 2' and 2" esters in the disaccharide moiety. The C3 acetate (**19b** and **19e**) was hydrolyzed upon treatment with basic methanol. The resulting alcohols (not shown) were protected as TBDPS ethers **20b** and **20e** in excellent yield.[13] Several conditions were tested for stereoselective reduction of the C16 ketone to the *trans*-diol. In all cases, the PMB ether **20b** gave mainly the unwanted *cis*-diol **21b**-α. By contrast, the ketal **20e** undergoes reduction with $CeCl_3/NaBH_4$ at low temperature to exclusively afford the *trans*-diol **21e**-β in excellent yield (95%). The stereochemistry of the **21e**-β was confirmed by single crystal X-ray.[14a]

Scheme 6



X-ray structure of 19e



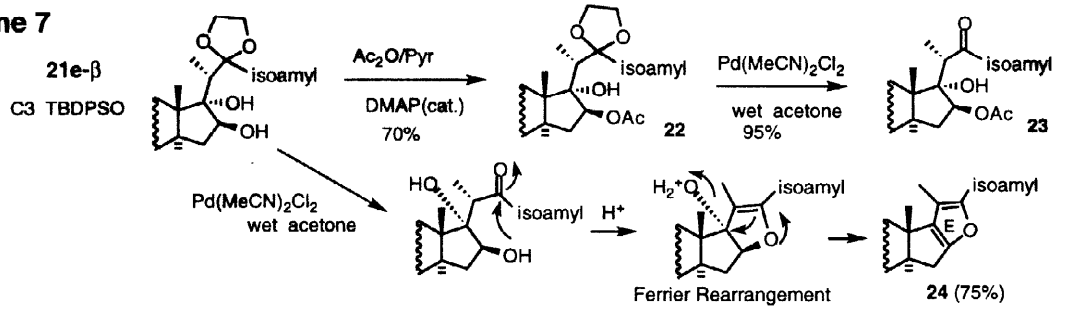
Conditions	**21b** (β:α)	**21e** (β:α)
$LiBH_4$ (-20°C,THF)	50% (1:3)	55% (5:1)
DIBAL(-0°C, THF)	20% (1:4)	15% (4:1)
$NaBH_4/CeCl_3$ (-40 to -30°C, THF)	90% (1: 2.5)	95% (α not obs)

The structure of 16-keto, 22-ketal **19e** was indicated by an NOE experiment[14b] and unambiguously established by single crystal X-ray (Scheme 6).[15] The most stable conformation of ketone **19e** (CAChe v3.7) is basically the same as the conformation shown in Scheme 6. The C22 ketal is pointing inside (the paper) and the 21 methyl is extended toward the C16 ketone (outside the paper). The β-face of C16 ketone is thus blocked by the C21 methyl and the hydride can only be delivered from the α-face. This distinct difference in stereochemical crowding between the two faces of the C16 ketone is likely responsible to the observed highly stereoselective formation of *trans*-diol **21e**-β upon hydride reduction. Overall, the protected aglycone **21e**-β was synthesized in 9 steps (~55%) from 5-androsten-3β-ol-17-one (**10**).

With the 3,22-protected OSW-1 aglycone **21e**-β in hand, mild conditions for unmasking the C22 ketone (**21e**-β) were required (Scheme 7). Deketalization of C16β acetate **22** was achieved in excellent yield (95%) by treatment with $Pd(MeCN)_2Cl_2$ (cat.) in wet acetone.[16] It should be noted that these conditions will not cleave THP ethers,[16] which are less stable than the glycoside bonds required in OSW-1 analogs. On the other hand, the aromatized furan (E ring of **24**) was produced from **21e**-β with free C16 hydroxyl via Ferrier type

rearrangement[17] in 75% yield under the same conditions. Since it is not easy to prepare the aglycone directly from OSW-1,[2] the 3,22-protected aglycone **21e-β** should serve as a key precursor for syntheses of OSW-1 analogs with different substituents at the 16β-alcohol. Further studies in this area are underway.

Scheme 7



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References and Notes

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14 (a) The *trans*-diol **21e-β** with a C3 acetate was crystallized from its pyridine-ether solution. Details of thecrystal structure investigations may be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, GB-Cambridge CB2 1EZ (UK); (b) For compound **19e**, 3% NOE effect was observed between H20 (δ 3.13 ppm, q, J=7.3 Hz) and Me-18 protons (δ 1.05 ppm) in pyridine-d_5

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