# Synthesis and Antitumor Activity of the Estrane Analogue of OSW-1

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The estrane analogue of OSW-1, a potent antitumor natural saponin, was efficiently synthesized from estrone in 12 steps. The cytostatic activity of the compound against several human malignant tumor cells was examined and compared to those of the natural OSW-1 and cisplatin, the results suggesting that the modification of the steroidal component could be an effective approach in the search for new candidates of anticancer drugs.


## Introduction

OSW-1 (**1**) is an extraordinarily potent antitumor saponin characterized as a cholestane disaccharide, which has been isolated as a main constituent from the bulbs of *Ornithogalum saundersiae* by Sashida et al.[1] The key characteristics of this series of natural products, including the analogues **2**–**5** isolated from the same natural source (Figure 1), are their antitumor activity, these compounds being over ten times more potent than the clinically used anticancer agents, and the remarkable similarity of the cytotoxicity profile to that of cephalostatins.[2] These attractive bioactivities and the structural novelty have prompted synthetic and bioorganic chemists to perform total and partial synthetic studies of OSW-1.[3] In our ongoing research on the structure-activity relationship of OSW-1 and its analogues, we have recently reported the synthesis of an A-nor B-aromatic steroidal aglycon of OSW-1[4] by *o*-quinodimethane chemistry.[5] To obtain sufficient amounts of compounds for detailed bioassay, however, a synthesis with much higher efficiency is required, and so development of a modified synthetic method to provide final samples effectively has been carried on in our laboratory. On the other hand, Yu and his co-workers reported a highly effective approach for the construction of the OSW-1 aglycon in their first total synthesis of OSW-1, in which dehydroisoandrosterone was used as a starting material.[3a] This synthetic method seemed to be applicable for various keto-steroidal substrates, and was in fact successfully applied for our synthesis of the A-nor B-aromatic steroidal aglycon of OSW-1.[4] As a part of our study on the search for new anticancer drugs based on

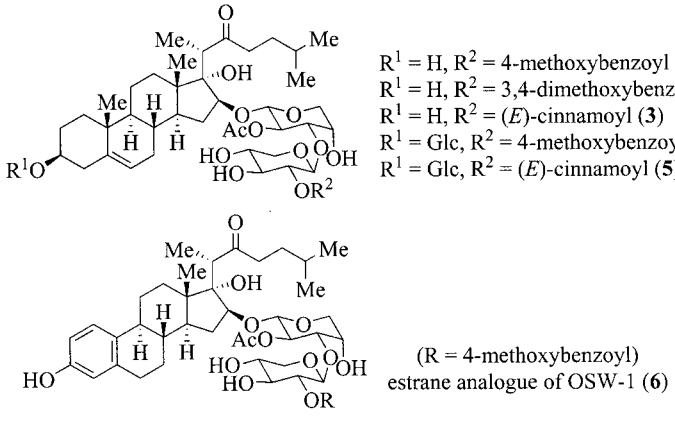


Figure 1. Natural OSW-1 and its analogues

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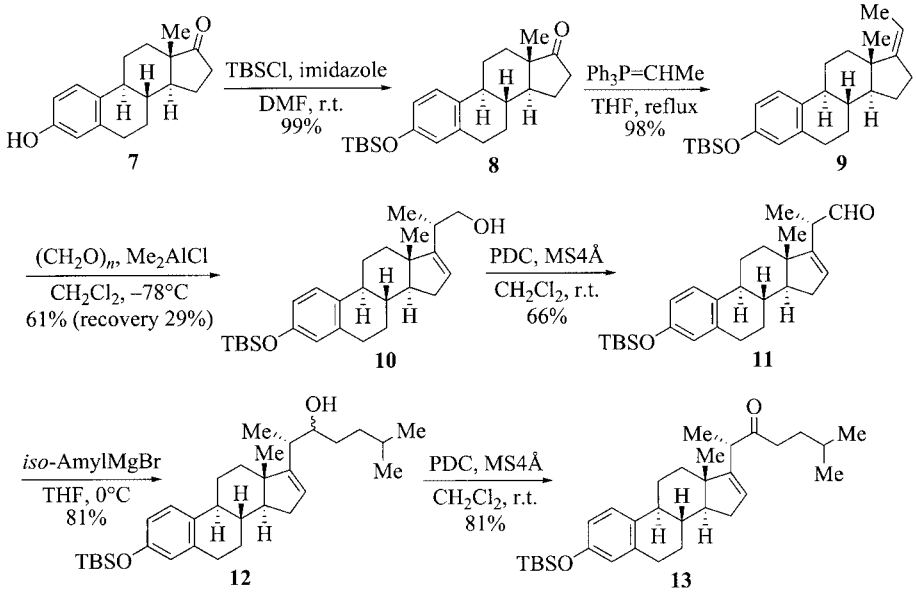
OSW-1, we speculated that the estrane analogue (**6**) of OSW-1 might be readily accessible from commercially available estrone and might provide some insights into the effect of modification of the aglycon moiety for the bioactivity. Here we describe the preparation of the estrane analogue **6** and its cytostatic activity against several human malignant tumor cells.

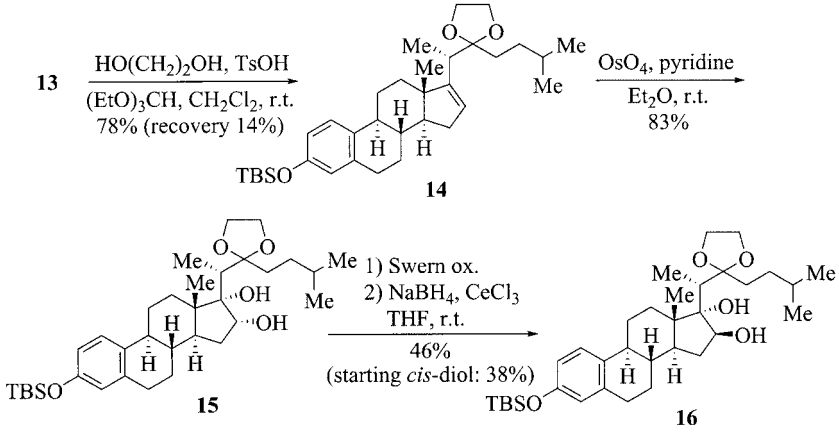
## Results and Discussion

Introduction of the alkyl side chain onto the estrane framework was accomplished as shown in Scheme 1. The phenolic hydroxy group of estrone (**7**) was protected as the TBS ether **8** by treatment with TBSCl and imidazole in DMF. Subsequent treatment of **8** with ethylidenetriphenylphosphorane gave a (*Z*)-olefin **9**, with exclusive stereoselectivity and in high yield. Hydroxymethylation accompanied by the formation of a new chiral center was achieved through a stereoselective ene reaction with paraformaldehyde and dimethylaluminium chloride, which approached from the less congested α-side to afford the alcohol **10**. Reaction time and temperature were important for satisfactory results in this reaction, as aromatic hydroxymethylation on the A-ring competed with the ene reaction when the reaction was carried out for a prolonged time (> 15 min) or at over –60 °C. The resulting alcohol **10** was oxidized with PDC to give the aldehyde **11**, which was treated with isoamylmagnesium bromide in THF to afford the addition product **12** in good yields. The alcohol thus formed was found to be almost the sole stereoisomer by $^{1}H$ and $^{13}C$ NMR spectroscopy. Although the stereostructure of **12** has not been determined, we believe it to the β-alcohol, on the assumption of the simple Felkin–Anh model. PDC oxidation of the alcohol **12** provided the corresponding ketone **13**, possessing the same alkyl side chain as natural OSW-1.

Next, the installation of the *trans*-dihydroxy structure onto the D-ring was performed as shown in Scheme 2. Prior to oxidation of the double bond in the D-ring, the carbonyl

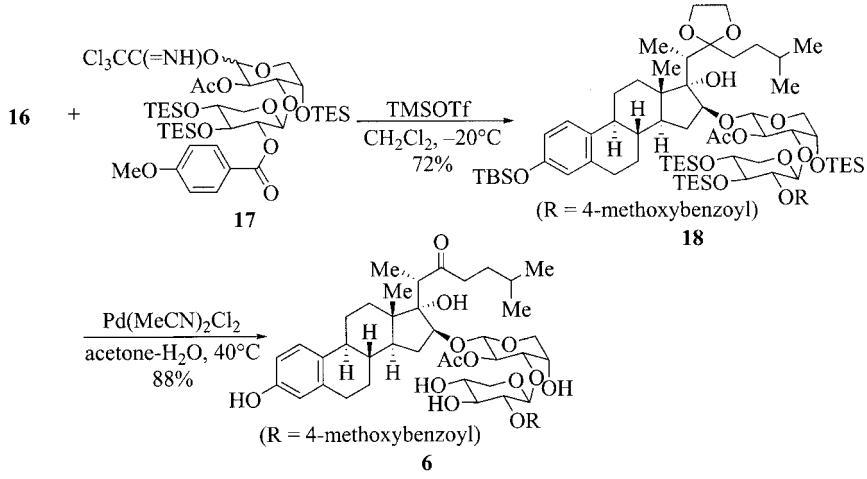



Scheme 1




Scheme 2




Scheme 3

group of **13** was converted into the ethylene ketal by treatment with ethylene glycol in the presence of TsOH and orthoformate for 16 h. Any much longer reaction time should be avoided, to evade cleavage of the phenolic TBS ether. Dihydroxylation of **14** by treatment with osmium tetroxide cleanly proceeded from α-face exclusively to give the *cis*-diol **15** in 83% yield. Inversion of the secondary hydroxy group of **15** was carried out by an oxidation-reduction sequence. Swern oxidation of **15** afforded the corresponding keto alcohol, which was unexpectedly unstable in silica gel and was found to be deketalized during the purification process to form an undesired diketone. The keto alcohol was therefore directly used in crude form for the next reduction step under $NaBH_4/CeCl_3$ condition. Although the selectivity was not as high as expected, the required *trans*-diol **16** was formed in 46% yield, together with the recyclable starting *cis*-diol **15** in 38% yield. The target estrane aglycon **16** was thus satisfactorily synthesized starting from estrone in 10 steps.

The glycosylation of the prepared aglycon **16** with a disaccharide **17** is our next subject. The anomeric mixture of the disaccharide imidate **17**, prepared from D-xylose and L-arabinose by the reported method,[3a] was allowed to react with the aglycon **16** in the presence of TMSOTf in $CH_2Cl_2$ at –20 °C to afford the glycoside **18** in 72% yield. The removal of all the protecting groups, four silyl ethers and one ethylene ketal, was achieved by treatment with (bisacetonitrile)dichloropalladium(II)[6] to complete the synthesis of the final target compound **6**, the estrane analogue of OSW-1 (Scheme 3).

The anticancer activity of the synthesized OSW-1 analogue was evaluated in vitro. While OSW-1 aglycon did not show any tumor cell growth inhibition, the estrane analogue **6** synthesized in this study had anticancer effects on all assayed cancer cell lines (Table 1). In another experiment, some polysaccharides, including OSW-1 disaccharide, had no cytotoxic effects on human cancer cells (data not shown). Therefore, both the saccharide moiety and the steroidal component must be essential for the growth-inhibitory activity of OSW-1. The cytotoxic effects of the estrane analogue in Table 1 are weaker than those of OSW-1 itself as reported in the literature,[2a] but its activity still stands comparison with the activity of cisplatin, which is a clinically used anticancer agent. These results should provide useful information for future design geared towards the discovery of suitable derivatives based on OSW-1 possessing high efficacy without lethal toxicity.

Table 1. Cytotoxic activity of OSW-1 analogues in vitro (NT: not tested)

| Cell | $IC_{50}$ Value (μM) | | |
|---|---|---|---|
| | Cysplatin | Estrane Analogue (**6**) | OSW-1 Aglycon |
| NCI-H460 | 0.660 | 0.439 | > 10 |
| T-47D | 11.5 | 0.709 | NT |
| MDA-MB-231 | 7.85 | 1.01 | NT |
| A498 | 2.11 | 0.430 | NT |
| PC-3 | 3.62 | 1.40 | > 10 |
| DLD-1 | 2.21 | 1.18 | > 10 |

## Experimental Section

**General Remarks:** All nonaqueous reactions were carried out under Ar atmosphere. Reagents were purchased from commercial sources and were used as received. Anhydrous solvents were obtained from commercial sources or were prepared by distillation over $CaH_2$ or $P_2O_5$. $^1H$ and $^{13}C$ NMR spectra were obtained on a Varian UNITY plus 500 (500 MHz for $^1H$ and 125 MHz for $^{13}C$) instrument, with tetramethylsilane or chloroform as an internal reference. Mass spectra were measured on a JEOL AX 505 mass spectrometer. IR spectra were recorded on a Perkin–Elmer 1600 spectrometer. The optical rotations were determined on a JASCO DIP-1000 instrument. Melting points were taken with a Yanagimoto micro melting point apparatus and are uncorrected. Column chromatography was carried out on Cica Silica Gel 60*N* (spherical, neutral, 40–50 μm or 63–210 μm).

**(+)-3-(*tert*-Butyldimethylsilyloxy)-13-methyl-6,7,8,9,11,12,13,-14,15,16-decahydrocyclopenta[*a*]phenanthren-17-one (8):** A solution of TBSCl (900 mg, 6 mmol) in anhydrous DMF (5 mL) was added to an ice-cooled solution of (+)-estrone **7** (1.08 g, 4 mmol) and im-

idazole (680 mg, 10 mmol) in anhydrous DMF (10 mL), and the mixture was stirred at room temperature for 1 h. The mixture was diluted with $H_2O$ and the aqueous solution was extracted with $Et_2O$. The combined organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated to leave a residue, which was chromatographed on silica gel ($CH_2Cl_2$) to afford the TBS ether **8** (1.518 g, 99%) as a colorless solid. m.p. 173–175 °C. $[\alpha]_D^{27}$ = +115.9 (*c* = 0.50, $CHCl_3$). $^1H$ NMR ($CDCl_3$): $\delta$ = 0.20 (s, 6 H), 0.91 (s, 3 H), 0.99 (s, 9 H), 1.39–1.66 (m, 6 H), 1.92–2.09 (m, 3 H), 2.14 (td, *J* = 9.0, 19 Hz, 1 H), 2.21–2.26 (m, 1 H), 2.37–2.41 (m, 1 H), 2.50 (dd, *J* = 9.0, 19 Hz, 1 H), 2.82–2.86 (m, 2 H), 6.57 (d, *J* = 2.6 Hz, 1 H), 6.63 (dd, *J* = 2.6, 8.5 Hz, 1 H), 7.12 ppm (d, 1 H, *J* = 8.5 Hz). $^{13}C$ NMR ($CDCl_3$): $\delta$ = –4.43, 13.83, 18.13, 21.55, 25.66, 25.80, 26.52, 29.44, 31.54, 35.84, 38.25, 43.97, 47.98, 50.39, 117.26, 119.95, 126.09, 132.38, 137.55, 153.42 ppm. IR (KBr) $\tilde{\nu}$ = 1733 $cm^{-1}$. MS (EI) *m*/*z*: 384 ($M^+$). HRMS (EI) calcd. for $C_{24}H_{36}O_2Si$: 384.2485 ($M^+$), found: 384.2463. $C_{24}H_{36}O_2Si$ (384.63): calcd. C 74.94, H 9.43; found C 75.03, H 9.45.

**(+)-3-(*tert*-Butyldimethylsilyloxy)-17(*Z*)-ethylidene-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthrene (9):** *t*BuOK (4.937 g, 44 mmol) was added to a suspension of ethyltriphenylphosphonium bromide (16.34 g, 44 mmol) in anhydrous THF (30 mL), and the mixture was stirred at room temperature for 1 h. After addition of a solution of the ketone **8** (4.254 g, 11 mmol) in anhydrous THF (45 mL), the resulting mixture was heated at reflux for 24 h. The reaction was quenched with sat. $NH_4Cl$, and the aqueous mixture was extracted with $CH_2Cl_2$. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel ($CH_2Cl_2$/hexane 1:9) to afford the olefin **9** (4.27 g, 98%) as a colorless solid. m.p. 96–98 °C. $[\alpha]_D^{27}$ = +49.3 (*c* = 0.50, $CHCl_3$). $^1H$ NMR ($CDCl_3$): $\delta$ = 0.23 (s, 6 H), 0.95 (s, 3 H), 1.02 (s, 9 H), 1.30–1.62 (m, 6 H), 1.73 (td, 3 H, *J* = 1.7, 7.3 Hz), 1.74–1.80 (m, 1 H), 1.92–1.97 (m, 1 H), 2.22–2.39 (m, 3 H), 2.40–2.49 (m, 2 H), 2.81–2.90 (m, 2 H), 5.19 (1 H, tq, *J* = 1.7, 7.3 Hz), 6.59 (d, 1 H, *J* = 2.6 Hz), 6.65 (dd, 1 H, *J* = 2.6, 8.5 Hz), 7.16 ppm (d, 1 H, *J* = 8.5 Hz). $^{13}C$ NMR ($CDCl_3$): $\delta$ = –4.40, 13.14, 16.94, 18.15, 24.13, 25.70, 26.86, 27.55, 29.68, 31.41, 37.21, 38.28, 43.83, 44.54, 55.22, 113.34, 117.07, 119.89, 126.02, 133.22, 137.80, 150.24, 153.22 ppm. IR (KBr) $\tilde{\nu}$ = 2931, 1606, 1495 $cm^{-1}$. MS (EI) *m*/*z*: 396 ($M^+$). HRMS (EI) calcd. for $C_{26}H_{40}OSi$: 396.2848 ($M^+$), found: 396.2817. $C_{26}H_{40}OSi$ (396.68): calcd. C 78.72, H 10.16; found C 78.77, H 10.06.

**(+)-3-(*tert*-Butyldimethylsilyloxy)-17-(2-hydroxy-1-methylethyl)-13-methyl-7,8,9,11,12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthrene (10):** A mixture of the olefin **9** (4.616 g, 11.65 mmol), paraformaldehyde (5.243 g, 175 mmol as HCHO), and anhydrous $CH_2Cl_2$ (120 mL) was cooled to –78 °C, and $Me_2AlCl$ (1 M in hexane, 58.25 mL, 58.25 mmol) was added dropwise to the mixture. After the mixture had been stirred at –78 °C for 15 min, sat. $NaHCO_3$ was added and the resulting heterogeneous mixture was vigorously stirred at 0 °C for 30 min. The insoluble precipitate was filtered off through a Celite pad and the filtrate was extracted with $CH_2Cl_2$. The dried organic layer was evaporated to leave a residue, which was chromatographed on silica gel ($CH_2Cl_2$) to afford the alcohol **10** (3.00 g, 61%) as a colorless oil, as well as the starting olefin **9** (1.324 g, 29%). $[\alpha]_D^{27}$ = +50.0 (*c* = 0.50, $CHCl_3$). $^1H$ NMR ($CDCl_3$): $\delta$ = 0.19 (s, 6 H), 0.83 (s, 3 H), 0.98 (s, 9 H), 1.07 (d, *J* = 6.8 Hz, 3 H), 1.39–1.47 (m, 1 H), 1.55–1.62 (m, 5 H), 1.89–2.00 (m, 3 H), 2.18–2.22 (m, 1 H), 2.22–2.30 (br, 1 H), 2.31–2.38 (m, 1 H), 2.41–2.48 (m, 1 H), 2.79–2.90 (m, 2 H), 3.57 (dd, *J* = 5.9, 11 Hz, 1 H), 3.63 (dd, *J* = 7.3, 11 Hz, 1 H), 5.47 (br. s, 1 H), 6.56 (d, *J* = 2.6 Hz, 1 H), 6.62 (dd, 1 H, *J* = 2.6, 8.5 Hz), 7.11 ppm (d, 1 H, *J* = 8.5 Hz). $^{13}C$ NMR ($CDCl_3$): $\delta$ = –4.42, 16.34, 18.14, 18.17, 25.69, 26.35, 27.76, 29.49, 30.89, 34.85, 35.36, 37.33, 44.37, 47.36, 56.48, 66.51, 117.04, 119.95, 122.78, 125.73, 133.29, 137.83, 153.25, 157.70 ppm. IR (neat) $\tilde{\nu}$ = 3309, 1607 $cm^{-1}$. MS (EI) *m*/*z*: 426 ($M^+$). HRMS (EI) calcd. for $C_{27}H_{42}O_2Si$: 426.2954 ($M^+$), found: 426.2953.

**(+)-3-(*tert*-Butyldimethylsilyloxy)-13-methyl-17-(1-methyl-2-oxoethyl)-7,8,9,11,12,13,14,15-octahydro-6*H*-cyclopenta[*a*]phenanthrene (11):** A mixture of the alcohol **10** (3.00 g, 7 mmol), PDC (13.17 g, 35 mmol), and activated molecular sieves (4 Å, 5.00 g) in anhydrous $CH_2Cl_2$ (200 mL) was stirred at room temperature for 24 h. Insoluble materials were filtered off through a Celite pad, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography ($CH_2Cl_2$) to afford the aldehyde **11** (1.969 g, 66%) as a colorless solid. m.p. 73–75 °C. $[\alpha]_D^{27}$ = +80.5 (*c* = 0.50, $CHCl_3$). $^1H$ NMR ($CDCl_3$): $\delta$ = 0.19 (s, 6 H), 0.81 (s, 3 H), 0.98 (s, 9 H), 1.23 (d, *J* = 6.8 Hz, 3 H), 1.39–1.47 (m, 1 H), 1.50–1.64 (m, 4 H), 1.88–1.95 (m, 2 H), 1.96–2.05 (m, 1 H), 2.21–2.30 (m, 2 H), 2.32–2.38 (m, 1 H), 2.81–2.91 (m, 2 H), 3.08 (q, *J* = 6.8 Hz, 1 H), 5.52 (br. s, 1 H), 6.56 (d, *J* = 2.6 Hz, 1 H), 6.61 (dd, *J* = 2.6, 8.5 Hz, 1 H), 7.10 (d, *J* = 8.5 Hz, 1 H), 9.48 ppm (s, 1 H). $^{13}C$ NMR ($CDCl_3$): $\delta$ = –4.41, 14.38, 16.14, 18.15, 25.69, 26.21, 27.72, 29.47, 31.19, 34.56, 37.30, 44.31, 46.05, 47.44, 56.14, 117.08, 119.96, 125.74, 126.92, 133.10, 137.76, 152.05, 153.30, 201.08 ppm. IR (KBr) $\tilde{\nu}$ = 1717, 1607 $cm^{-1}$. MS (EI) *m*/*z*: 424 ($M^+$). HRMS (EI) calcd. for $C_{27}H_{40}O_2Si$: 424.2798 ($M^+$), found: 424.2780. $C_{27}H_{40}O_2Si$ (424.69): calcd. C 76.36, H 9.49; found C 76.50, H 9.33.

**(+)-2-[3-(*tert*-Butyldimethylsilyloxy)-13-methyl-7,8,9,11,12,13,-14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-17-yl]-6-methylheptan-3-ol (12):** Isoamylmagnesium bromide (0.5 M in THF; prepared from isoamyl bromide and magnesium, 27.54 mL, 13.77 mmol) was added dropwise at 0 °C to a solution of the aldehyde **11** (1.948 g, 4.59 mmol) in anhydrous THF (20 mL), and the reaction mixture was stirred at the same temperature for 30 min. The reaction was quenched with sat. $NH_4Cl$, and the aqueous mixture was extracted with $CH_2Cl_2$. The organic layer was dried over $MgSO_4$ and evaporated to leave a residue, which was chromatographed on silica gel ($CH_2Cl_2$/hexane 1:1) to afford the alcohol **12** (1.852 g, 81%) as a colorless oil. $[\alpha]_D^{27}$ = +31.5 (*c* = 0.50, $CHCl_3$).$^1H$ NMR ($CDCl_3$): $\delta$ = 0.20 (s, 6 H), 0.90–0.95 (m, 9 H), 0.99 (s, 9 H), 1.07 (d, *J* = 6.8 Hz, 3 H), 1.18–1.24 (m, 1 H), 1.38–1.65 (m, 10 H), 1.88–2.02 (m, 3 H), 2.18–2.38 (m, 4 H), 2.80–2.93 (m, 2 H), 3.64–3.70 (m, 1 H), 5.53 (br. s, 1 H), 6.57 (d, *J* = 2.6 Hz, 1 H), 6.63 (dd, *J* = 2.6, 8.5 Hz, 1 H), 7.12 ppm (d, *J* = 8.5 Hz, 1 H). $^{13}C$ NMR ($CDCl_3$): $\delta$ = –4.43, 14.31, 16.79, 18.13, 22.57, 22.65, 25.68, 26.24, 27.72, 28.10, 29.49, 30.89, 32.43, 34.71, 35.50, 37.18, 37.77, 44.30, 47.18, 56.99, 73.08, 117.02, 119.94, 124.04, 125.69, 133.26, 137.82, 153.23, 158.77 ppm. IR (neat) $\tilde{\nu}$ = 3419, 1607 $cm^{-1}$. MS (EI) *m*/*z*: 496 ($M^+$). HRMS (EI) calcd. for $C_{32}H_{52}O_2Si$: 496.3737 ($M^+$), found: 496.3714.

**(+)-2-[3-(*tert*-Butyldimethylsilyloxy)-13-methyl-7,8,9,11,12,13,-14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-17-yl]-6-methylheptan-3-one (13):** The alcohol **12** (1.608 g, 3.24 mmol) was treated with PDC (3.656 g, 9.72 mmol) in anhydrous $CH_2Cl_2$ (100 mL) in the presence of activated molecular sieves (4 Å, 2.5 g) by the procedure used for the synthesis of **11**. Purification by silica gel column chromatography ($CH_2Cl_2$/hexane 1:1) afforded the ketone **13** (1.30 g, 81%) as a colorless solid. m.p. 62–64 °C. $[\alpha]_D^{28}$ = +125.0 (*c* = 0.20, $CHCl_3$). $^1H$ NMR ($CDCl_3$): $\delta$ = 0.20 (s, 6 H), 0.85 (s, 3 H), 0.88 (d, *J* = 6.8 Hz, 6 H), 0.98 (s, 9 H), 1.19 (d, *J* = 6.8 Hz, 3 H), 1.42–1.61 (m, 8 H), 1.89–1.99 (m, 3 H), 2.16–2.29 (m, 2 H), 2.32–2.40 (m, 2 H), 2.48–2.56 (m, 1 H), 2.79–2.90 (m, 2 H), 3.23 (q, *J* =

6.8 Hz, 1 H), 5.39 (br. s, 1 H), 6.57 (d, $J$ = 2.6 Hz, 1 H), 6.62 (dd, $J$ = 2.6, 8.5 Hz, 1 H), 7.11 ppm (d, $J$ = 8.5 Hz, 1 H). $^{13}$C NMR ($CDCl_3$): $\delta$ = –4.43, 16.41, 16.91, 18.13, 22.22, 22.49, 25.67, 26.28, 27.57, 27.70, 29.47, 30.91, 33.01, 34.66, 37.35, 38.26, 44.29, 45.64, 47.71, 56.25, 117.04, 119.94, 124.97, 125.72, 133.15, 137.78, 153.28, 154.45, 211.56 ppm. IR (KBr) $\tilde{\nu}$ = 1709, 1606 cm$^{-1}$. MS (EI) *m*/*z*: 494 ($M^+$). HRMS (EI) calcd. for $C_{32}H_{50}O_2Si$: 494.3580 ($M^+$), found: 494.3580. $C_{32}H_{50}O_2Si$ (494.82): calcd. C 77.67, H 10.18; found C 77.74, H 10.19.

**(+)-2-[3-(*tert*-Butyldimethylsilyloxy)-13-methyl-7,8,9,11,12,13,-14,15-octahydro-6*H*-cyclopenta[*a*]phenanthren-17-yl]-6-methylheptan-3-one 3-Ethylene Ketal (14):** A mixture of the ketone **13** (1.262 g, 2.55 mmol), ethylene glycol (2.13 mL, 38 mmol), triethyl orthoformate (2.12 mL, 12.8 mmol), and *p*-toluenesulfonic acid hydrate (49 mg, 0.255 mmol) in anhydrous $CH_2Cl_2$ (20 mL) was stirred at room temperature for 16 h. After dilution with $CH_2Cl_2$, the organic layer was washed with sat. $NaHCO_3$ and then dried over $MgSO_4$. The solvent was evaporated off to leave a residue, which was chromatographed on silica gel ($CH_2Cl_2$/hexane 1:1) to afford the ethylene ketal **14** (1.075 g, 78%) as a colorless oil, together with the starting ketone **13** (174 mg, 14%). $[\alpha]_D^{28}$ = +44.5 ($c$ = 0.30, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ = 0.19 (s, 6 H), 0.83 (s, 3 H), 0.86 (d, $J$ = 6.8 Hz, 3 H), 0.87 (d, $J$ = 6.8 Hz, 3 H), 0.98 (s, 9 H), 1.07 (d, $J$ = 6.8 Hz, 3 H), 1.21–1.29 (m, 2 H), 1.40–1.61 (m, 6 H), 1.69–1.75 (m, 2 H), 1.83–1.98 (m, 3 H), 2.13–2.19 (m, 1 H), 2.20–2.28 (m, 1 H), 2.30–2.36 (m, 1 H), 2.50 (q, $J$ = 6.8 Hz, 1 H), 2.79–2.90 (m, 2 H), 3.95–4.00 (m, 4 H), 5.70 (br. s, 1 H), 6.56 (d, $J$ = 2.6 Hz, 1 H), 6.61 (dd, $J$ = 2.6, 8.5 Hz, 1 H), 7.11 ppm (d, $J$ = 8.5 Hz, 1 H). $^{13}$C NMR ($CDCl_3$): $\delta$ = –4.40, 15.76, 17.48, 18.15, 22.64, 22.66, 25.69, 26.39, 27.82, 28.37, 29.58, 31.08, 32.39, 33.96, 34.91, 37.47, 39.24, 44.40, 47.88, 56.50, 65.28, 65.85, 113.82, 116.98, 119.93, 123.88, 125.74, 133.51, 137.92, 153.21, 156.73 ppm. IR (neat) $\tilde{\nu}$ = 2928, 1607 cm$^{-1}$. MS (EI) *m*/*z*: 538 ($M^+$). HRMS (EI) calcd. for $C_{34}H_{54}O_3Si$: 538.3842 ($M^+$), found: 538.3815.

**(16α,17α)-(+)-2-[3-(*tert*-Butyldimethylsilyloxy)-16,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-17-yl]-6-methylheptan-3-one 3-Ethylene Ketal (15):** $OsO_4$ (0.1 g ml$^{-1}$ in $Et_2O$, 6.94 mL, 2.73 mmol) was added at room temperature to a solution of the olefin **14** (980 mg, 1.82 mmol) and pyridine (1.5 mL) in $Et_2O$ (20 mL). After the mixture had been stirred at room temperature for 4 h, hydrogen sulfide gas was bubbled through the solution for reductive cleavage of the osmates. After evaporation of the solvent, the residue was purified by silica gel column chromatography ($CH_2Cl_2$) to afford the *cis*-diol **15** (866 mg, 83%) as a colorless solid. m.p. 159–161 °C. $[\alpha]_D^{28}$ = +17.5 ($c$ = 0.50, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ = 0.19 (s, 6 H), 0.80 (s, 3 H), 0.86 (d, $J$ = 6.8 Hz, 3 H), 0.87 (d, $J$ = 6.8 Hz, 3 H), 0.98 (s, 9 H), 1.19 (d, $J$ = 6.8 Hz, 3 H), 1.20–1.52 (m, 6 H), 1.60–2.02 (m, 9 H), 2.16–2.28 (m, 2 H), 2.78–2.82 (m, 2 H), 3.19 (br. s, 1 H), 3.92–4.00 (m, 4 H), 4.08 (br. s, 1 H), 4.31 (d, $J$ = 9.4 Hz, 1 H), 6.54 (d, $J$ = 2.6 Hz, 1 H), 6.60 (dd, $J$ = 2.6, 8.5 Hz, 1 H), 7.10 ppm (d, $J$ = 8.5 Hz, 1 H). $^{13}$C NMR ($CDCl_3$): $\delta$ = –4.42, 13.08, 14.42, 18.14, 22.72, 22.76, 25.68, 26.02, 27.72, 28.10, 29.58, 31.25, 32.75, 32.89, 33.09, 38.55, 43.08, 45.33, 46.80, 50.37, 64.39, 65.81, 75.90, 82.46, 115.65, 117.04, 119.86, 125.90, 133.17, 137.75, 153.18 ppm. IR (KBr) $\tilde{\nu}$ = 3481, 1607 cm$^{-1}$. MS (EI) *m*/*z*: 572 ($M^+$). HRMS (EI) calcd. for $C_{34}H_{56}O_5Si$: 572.3897 ($M^+$), found: 572.3878. $C_{34}H_{56}O_5Si$ (572.89): calcd. C 71.28, H 9.85; found C 71.14, H 9.93.

**(16β,17α)-(+)-2-[3-(*tert*-Butyldimethylsilyloxy)-16,17-dihydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6*H*-cyclopenta[*a*]phenanthren-17-yl]-6-methylheptan-3-one 3-Ethylene Ketal (16):** DMSO (71 μL, 1 mmol) was added at –78 °C to a solution of oxalyl chloride (44 μL, 0.5 mmol) in anhydrous $CH_2Cl_2$ (2 mL). After the mixture had been stirred for 15 min, the *cis*-diol **15** (114 mg, 0.2 mmol) in $CH_2Cl_2$ (3 mL) was added, and the mixture was stirred at –78 °C for 30 min. Triethylamine (279 μL, 2 mmol) was added to the solution, which was then warmed to room temperature over 1 h. The reaction mixture was diluted with $CH_2Cl_2$, washed successively with sat. $NaHCO_3$ and brine, and dried over $MgSO_4$. Evaporation of the solvent afforded the crude ketone, which was used directly for the next reaction. Cerium chloride hydrate (112 mg, 0.3 mmol) and sodium borohydride (46 mg, 1.2 mmol) were added to a solution of the crude ketone in THF (3 mL), and the mixture was stirred at room temperature for 7 h. The mixture was diluted with $NaHCO_3$, and the aqueous solution was extracted with $CH_2Cl_2$, and then dried. Evaporation of the solvent, followed by silica gel column chromatography ($CH_2Cl_2$), afforded the *trans*-diol **16** (53 mg, 46%) as a colorless solid and *cis*-diol **15** (44 mg, 38%). m.p. 225–226 °C. $[\alpha]_D^{28}$ = +23.8 ($c$ = 0.20, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ = 0.19 (s, 6 H), 0.88 (d, $J$ = 6.8 Hz, 3 H), 0.90 (d, $J$ = 6.8 Hz, 3 H), 0.95 (s, 3 H), 0.98 (s, 9 H), 1.14–1.27 (m, 3 H), 1.23 (d, $J$ = 6.8 Hz, 3 H), 1.32–1.55 (m, 5 H), 1.70–1.79 (m, 2 H), 1.80–1.84 (m, 1 H), 1.85–1.91 (m, 1 H), 1.95–2.01 (m, 1 H), 2.15–2.21 (m, 1 H), 2.23–2.28 (m, 1 H), 2.29–2.36 (m, 1 H), 2.62 (q, $J$ = 7.3 Hz, 1 H), 2.74–2.87 (m, 2 H), 3.95–3.99 (m, 1 H), 4.00–4.12 (m, 4 H), 4.13–4.19 (m, 2 H), 6.54 (d, $J$ = 2.6 Hz, 1 H), 6.60 (dd, $J$ = 2.6, 8.5 Hz, 1 H), 7.11 ppm (d, $J$ = 8.5 Hz, 1 H). $^{13}$C NMR ($CDCl_3$): $\delta$ = –4.43, 11.88, 12.72, 18.13, 22.25, 22.71, 25.68, 26.31, 27.62, 28.25, 29.63, 32.73, 33.46, 35.78, 38.58, 43.17, 46.54, 48.29, 62.81, 64.05, 81.58, 86.88, 116.49, 117.01, 119.85, 125.93, 133.31, 137.84, 153.15 ppm. IR (KBr) $\tilde{\nu}$ = 3485, 1607 cm$^{-1}$. MS (EI) *m*/*z*: 572 ($M^+$). HRMS (EI) calcd. for $C_{34}H_{56}O_5Si$: 572.3897 ($M^+$), found: 572.3867. $C_{34}H_{56}O_5Si$ (572.89): calcd. C 71.28, H 9.85; found C 71.33, H 9.73.

**Glycosylation of the Aglycon 16. Synthesis of the Estrane Analogue of OSW-1 (6):** A solution of the disaccharide imidate **17** (52 mg, 55 μmol) and the aglycon **16** (25 mg, 43.6 μmol) in anhydrous $CH_2Cl_2$ (1 mL) containing activated molecular sieves (4 Å) was stirred at room temperature for 15 min. The system was cooled to –20 °C, a solution of TMSOTf (0.5 mL of a solution of 9 μL TMSOTf in 10 mL $CH_2Cl_2$) was added, and the mixture was stirred for 20 min at –20 °C. The end of the reaction was monitored by TLC (AcOEt/cyclohexane 2:8) with the disappearance of the imidate. After the reaction was complete, triethylamine (50 μL) was added and the reaction mixture was evaporated to dryness in vacuo. The residue was subjected to silica gel flash column chromatography (AcOEt/cyclohexane 1:9) to afford the protected glycoside **18** (42 mg, 72%), which was used directly for the next step. A solution of the protected glycoside **18** (32 mg, 23.9 μmol) and $Pd(MeCN)_2Cl_2$ (10 mg) in acetone/$H_2O$ (20:1, 1 mL) was stirred under Ar atmosphere at 35–40 °C overnight. TLC ($CH_2Cl_2$/MeOH 15:1) indicated that all the protecting groups had been removed. (If the TBS group is not entirely removed, add a little portion of the catalyst and continue to stir and heat.) The reaction mixture was evaporated to dryness in vacuo, and the residue was purified by silica gel flash column chromatography ($CH_2Cl_2$/MeOH 25:1 to 15:1) to afford the estrane analogue **6** (18 mg, 88%) as a colorless powder with m.p. 140–142 °C. $[\alpha]_D^{25}$ –8.54 ($c$ = 0.355, $CHCl_3$). $^1$H NMR ($CDCl_3$): $\delta$ = 0.76 (d, $J$ = 6.4 Hz, 3 H), 0.78 (d, $J$ = 6.0 Hz, 3 H), 0.78 (s, 3 H), 1.05 (d, $J$ = 7.3 Hz, 3 H), 1.06–1.15 (m, 1 H), 1.22–1.50 (m, 6 H), 1.60–1.63 (m, 1 H), 1.79–1.92 (m, 4 H), 1.93 (s, 3 H), 2.10–2.32 (m, 4 H), 2.73 (q, $J$ = 7.3 Hz, 1 H), 2.76–2.86 (m, 2 H), 3.38 (t-like, $J$ = 10 Hz, 1 H), 3.48 (dd, $J$ = 3.4, 11 Hz, 1 H), 3.68–3.90 (m, 6 H), 3.87 (s, 3 H), 4.10 (dd, $J$ = 4.7, 12 Hz, 1 H), 4.18 (d, $J$ = 3.4 Hz,

1 H), 4.29 (br, 1 H), 4.68 (d, $J$ = 6.8 Hz, 1 H), 4.74 (dd, $J$ = 3.4, 5.6 Hz, 1 H), 4.92 (dd, $J$ = 6.8, 8.5 Hz, 1 H), 6.55 (d, $J$ = 2.6 Hz, 1 H), 6.62 (dd, $J$ = 2.6, 8.5 Hz, 1 H), 6.96 (d, $J$ = 9.0 Hz, 2 H), 7.11 (d, $J$ = 8.5 Hz, 1 H), 8.06 ppm (d, $J$ = 9.0 Hz, 2 H). $^{13}$C NMR ($CDCl_3$): $\delta$ = 11.68, 13.54, 20.74, 22.19, 22.63, 26.25, 27.24, 27.58, 29.61, 32.06, 32.53, 34.04, 38.57, 38.66, 43.05, 46.02, 46.37, 46.70, 55.51, 64.64, 65.04, 69.70, 71.18, 73.70, 75.04, 79.57, 85.83, 88.36, 99.29, 101.92, 112.67, 114.02, 115.27, 121.34, 126.41, 132.21, 132.86, 138.23, 153.31, 164.10, 166.01, 169.44, 219.00 ppm. IR (KBr) $\tilde{\nu}$ = 3448, 1716, 1606 cm$^{-1}$. MS (FAB) *m/z*: 878 ($M^+ + H + Na$). HRMS (FAB) calcd. for $C_{46}H_{63}NaO_{15}$: 878.4064 ($M^+ + H + Na$), found: 878.4056.

**Procedure for the Cytotoxicity Assay**

**Materials:** Human breast cancer T-47D and MDA-MB-231, human renal cancer A498, human lung cancer NCI-H460, and human prostate cancer PC-3 were purchased from the American Type Culture Collection. Human colon cancer DLD-1 (JCRB9094) was obtained from the Japanese Collection of Research Bioresources. All cells were cultured in RPMI1640 medium containing 10% fetal bovine serum (lot No. 49300604, Moregate Bio Tech, Australia). Some supplements were added to the medium as appropriate. Cisplatin (Nippon Kayaku Co., Ltd. lot Z20024) was used as a control in a cytotoxic assay.

**In vitro Cytotoxicity Assay:** The cytotoxicity against human solid tumor cell lines was assessed by the methylene blue staining method. Briefly, appropriate numbers of cells were inoculated into 96-well microplates. Following overnight culture, serially diluted samples were added into the wells. After a 3-day culture, cells were stained with methylene blue (0.05%) dissolved in Tris buffer (pH 8.5, 10 mM) for 30 min, and then thoroughly washed with distilled water. The stained dye was extracted with HCl (3%), and OD660 was measured with a Benchmark Plus microplate reader (Bio-Rad, USA) to determine cell growth inhibition.

**Supporting Information:** $^1$H and $^{13}$C NMR spectra for all new compounds. See also footnote on the first page of this article.

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