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Synthesis and antimitotic activity of novel 2-methoxyestradiol analogs—Part II

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ABSTRACT

The syntheses and antimitotic activity of several novel 18a-homo-analogs of 2-methoxyestradiol are described. Structural modifications of the parent 2-methoxy-18a-homoestradiol include introduction of unsaturation in the D-ring and methylation of the 17-OH. Of seven analogs synthesized, one has demonstrated superior biological activities compared to 2-methoxyestradiol. The relationship between biological activity and the conformational preference of the 13-ethyl group as determined by computational analysis is discussed.

1. Introduction

The nonestrogenic endogenous metabolite of estradiol, namely 2-methoxyestradiol (2ME2) has been shown to possess antiangiogenic and antimitotic properties [1,2]. Because of the exciting biological activities of 2ME2, several analogs of this compound have been synthesized and tested for various biological activities. We have earlier reported [3,4] the synthesis of several ring-D modified 2ME2 analogs and observed that by introducing an additional unsaturation in ring-D such as a 14-dehydro or a 15-dehydro derivative, the *in vitro* antiproliferative activity could be considerably enhanced relative to the parent 2ME2 compound. We also observed that the 17-hydroxy analogs are more active *in vitro* than their 17-oxo counterparts. It is not known whether these structural modifications enhance the binding affinity towards tubulin or decrease the rate of metabolic inactivation. In order to explore further the structure-activity relationship in 2ME series of compounds, we have now synthesized additional derivatives of 2ME2 and evaluated their biological activity.

In a recent phase I trial of 2ME2 in patients with metastatic breast cancer, extensive metabolic oxidation of 2ME2 to 2-methoxyestrone (2ME1) was observed [5]. Since it has also been observed that the 17β-hydroxy derivatives of 2ME2 are more active *in vitro* than their corresponding 17-oxo derivatives, it would be desirable to minimize any metabolic oxidation of the 17β-hydroxy group of potential 2ME2 analogs to the corresponding 17-oxo compounds. We reasoned that

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this could possibly be achieved by two basic structural modifications. First by increasing the steric bulk of the 13β-substituent next to the 17β-hydroxy function and second by protecting the 17β-hydroxy group as a methyl ether. 2ME2 has a methyl group at C-13 position adjacent to the 17β-hydroxyl group. If we could replace the C-13 methyl group with a sterically bulkier ethyl group, the 17β-hydroxyl function might be protected from metabolic deactivation. Along similar lines, conversion of the 17β-hydroxy group to the corresponding methyl ether should also decrease metabolic oxidation. Incorporating these structural modifications along with the introduction of additional unsaturation in ring-D, we synthesized seven new 2ME2 derivatives and evaluated their cytotoxic activity in a variety of cell types. In this communication, we now present the details of our investigations.

2. Experimental

2.1. Chemistry

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a General Electric GE-300 (300 MHz) spectrometer as deuterochloroform ($CHCl_3$) solutions using tetramethylsilane (TMS) as an internal standard ($\delta = 0$) unless noted otherwise. Infrared spectra were recorded on Thermo-Nicolet model 370 FT-IR instrument equipped with an attenuated reflectance (ATR) accessory. Combustion analyses were performed by Midwest Microlabs Ltd. (Indianapolis, IN). 'Flash column' chromatography was performed on 32–64 μM silica gel obtained from EM Science, Gibbstown, NJ. 'Dry column' chromatography was performed on 70–230 mesh silica gel, also obtained from EM Science. Thin-layer chromatography (TLC) analyses were carried out on silica gel GF (Analtech) glass plates (2.5 cm × 10 cm with 250 μM layer and prescored). Most chemicals and solvents were analytical grade and used without further purification. Commercial reagents were purchased from Aldrich Chemical Company (Milwaukee, WI). Levonorgestrel was provided as a gift by Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08534-8000.

2.1.1. 3,17β-Dihydroxy-18a-homoestra-1,3,5(10)-triene (2)

3-Hydroxy-18a-homoestra-1,3,5(10)-trien-17-one (**1**) was prepared from levonorgestrel as described earlier [6]. Under nitrogen, the 3-phenol (**1**, 9.4 g, 33 mmol) was dissolved in 50 ml of 1:1 EtOH/H_2O. Sodium borohydride (2.5 g, 66 mmol) was dissolved in 450 ml of 1:1 EtOH/H_2O. The sodium borohydride solution was added to the steroid solution drop wise over 2 h and stirred overnight. Analysis by TLC confirmed complete reaction (5% acetone/CH_2Cl_2). Excess sodium borohydride was decomposed with acetic acid, the solvent evaporated, and the residue extracted with ethyl acetate. The ethyl acetate was washed with water, brine, dried over sodium sulfate and evaporated. The residue was crystallized from methanol/H_2O to give the pure 3,17-diol (**2**, 9.31 g, 98%) as a white powder. mp = 183 °C; FTIR (ATR) ν_{max}: 3377, 3270, 2930, 1614, and 1582 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.92 (t, $J = 7.4$ Hz, 18a), 3.73 (t, $J = 7.1$ Hz, 17-H), 6.65 (t, $J = 3.1$ Hz, 4-H), 6.63 (dd, $J_1 = 8.5$ Hz, $J_2 = 2.6$ Hz, 2-H), 7.15 (d, $J = 8.7$ Hz, 1-H). Analysis calculated for $C_{19}H_{26}O_2 \cdot 2/3MeOH$: C, 76.77; H, 9.38. Found: C, 76.77; H, 9.35.

2.1.2. 18a-Homoestra-1,3,5(10)-trien-3,17β-diyl-diacetate (3)

Under nitrogen, the 3,17-diol (**2**, 9.41 g, 33 mmol) was dissolved in 100 ml of pyridine. Acetic anhydride (11.5 ml, 0.12 mol) was added and the mixture stirred over the weekend. Analysis by TLC confirmed complete reaction (5% acetone/CH_2Cl_2). The reaction was quenched with methanol and the solvent removed in vacuo. This residue was crystallized from methanol to give the pure 3,17-diacetate (**3**, 9.7 g, 80%) as a white powder. mp = 124–126 °C; FTIR (ATR) ν_{max}: 2930, 1758, 1727, 1604, 1576 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.96 (t, $J = 7.6$ Hz, 18a), 2.05 (s, 17-OAc), 2.78 (s, 3-OAc), 4.75 (t, $J = 8.5$ Hz, 17-H), 6.79 (t, $J = 2.6$ Hz, 4-H), 6.83 (dd, $J_1 = 5.7$ Hz, $J_2 = 2.7$ Hz, 2-H), 7.27 (d, $J = 5.7$ Hz, 1-H). Analysis calculated for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.63; H, 8.11.

2.1.3. 2-Acetyl-3-hydroxy-18a-homoestra-1,3,5(10)-trien-17β-yl-acetate (4)

Under nitrogen, the 3,17-diacetate (**3**, 8.91 g, 24 mmol) was dissolved in 650 ml of CH_2Cl_2. Zirconium tetrachloride (26 g, 0.11 mol) was added and the mixture stirred 48 h at room temperature. Analysis by TLC confirmed complete reaction (5% acetone/CH_2Cl_2). The mixture was chilled to 0 °C and quenched with 300 ml of water. The product extracted with CH_2Cl_2 (3×), the organic phase washed with water (3×), and brine. The organic fractions were dried over sodium sulfate, filtered, and concentrated in vacuo. This material was crystallized from methanol to give the pure 2-acetyl compound (**4**, 5.18 g, 63%) as a white powder. mp = 240–242 °C; FTIR (ATR) ν_{max}: 2943, 1714, 1636, 1614, and 1573 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.96 (t, $J = 7.4$ Hz, 18a), 2.05 (s, 17-OAc), 2.62 (s, 3-Ac), 4.76 (t, $J = 8.4$ Hz, 17-H), 6.69 (s, 4-H), 7.59 (s, 1-H), 12.05 (s, 3-OH). Analysis calculated for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.36; H, 8.15.

2.1.4. 2-Acetyl-3-benzyloxy-18a-homoestra-1,3,5(10)-trien-17β-yl-acetate (5)

Under nitrogen, the 2-acetyl compound (**4**, 5.18 g, 14 mmol) was dissolved in 165 ml of DMF. Potassium carbonate (16.2 g, 0.12 mol) and benzyl chloride (7.17 ml, 87 mmol) were added and the mixture stirred at 60 °C for 48 h. Analysis by TLC (5% acetone/CH_2Cl_2) confirmed complete reaction. The reaction was quenched with 500 ml of water whereby a white flocky precipitate formed. The precipitate was filtered, washed with water until neutral, and dried in vacuo. This material was crystallized from methanol/CH_2Cl_2 to give the pure 3-benzyl ether (**5**, 5.59 g, 87%) as a white powder. mp = 213–215 °C; FTIR (ATR) ν_{max}: 2939, 2869, 1734, 1657, and 1603 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.93 (t, $J = 7.4$ Hz, 18a), 2.05 (s, 17-OAc), 2.57 (s, 2-Ac), 4.74 (t, $J = 8.3$ Hz, 17-H), 5.13 (s, benzyl CH_2), 6.73 (s, 4-H), 7.70 (s, 1-H), 7.34-7.45 (m, benzyl aromatic). Analysis calculated for $C_{30}H_{36}O_4 \cdot 1/6CH_3OH$: C, 77.76; H, 7.93. Found: C, 77.89; H, 7.72.

2.1.5. *3-Benzyloxy-18a-homoestra-1,3,5(10)-trien-2,17β-diyl-diacetate (**6**)*

Under nitrogen, the 3-benzyl ether (**5**, 5.59 g, 12 mmol) was dissolved in 160 ml of CH_2Cl_2. Sodium phosphate (4.48 g, 32 mmol) and 3-chloroperoxybenzoic acid (4.17 g, 24 mmol) were added to the steroid solution and the suspension stirred for 24 h at room temperature. Water was added and the mixture extracted with CH_2Cl_2 (3×). The organic phase was washed with water (1×), 10% sodium sulfite (1×), and half saturated sodium bicarbonate (1×). The organic fractions were dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by flash chromatography to give the pure 2,17-diacetate (**6**, 4.57 g, 79%) as a white foam. mp = 98–100 °C; FTIR (ATR) ν_{max}: 2933, 1759, 1729, and 1612 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.92 (t, J = 7.4 Hz, 18a-H), 2.05 (s, 17-OAc), 2.25 (s, 2-OAc), 4.74 (t, J = 8.3 Hz, 17-H), 5.05 (s, benzyl CH_2), 6.72 (s, 4-H), 6.95 (s, 1-H), 7.26–7.38 (m, benzyl aromatic). Analysis calculated for $C_{30}H_{36}O_5$: C, 75.60; H, 7.61. Found: C, 75.85; H, 7.69.

2.1.6. *2,17β-Dihydroxy-3-benzyloxy-18a-homoestra-1,3,5(10)-triene (**7**)*

Under nitrogen, the 2,17-diacetate (**6**, 4.50 g, 9.5 mmol) was dissolved in 250 ml of methanol. To this solution 50 ml of 1M NaOH was added and the mixture stirred at 60 °C for 2 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated an incomplete reaction. An additional 25 ml of 1 M NaOH was added and the mixture stirred an additional hour. Analysis by TLC at that time indicated a complete reaction. The reaction mixture was cooled to room temperature and glacial acetic acid was slowly added with stirring until the mixture was neutral. The mixture was diluted with 300 ml of water whereby a precipitate formed. Methanol was removed in vacuo. The residue was extracted with ethyl acetate (3×), the organic phase washed with water (3×), and brine. The organic fractions were dried over sodium sulfate, filtered and concentrated in vacuo. The solid residue was crystallized from ethyl acetate to give the pure 2,17-diol (**7**, 2.61 g, 71%) as a white powder. mp = 178 °C; FTIR (ATR) ν_{max}: 3531, 3261, 2922, and 1604 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.98 (t, J = 7.55 Hz, 18a), 3.80 (t, J = 8.35 Hz, 17-H), 5.04 (s, benzyl CH_2), 6.66 (s, 4-H), 6.92 (s, 1-H), 7.35–7.45 (m, benzyl aromatic). Analysis calculated for $C_{26}H_{32}O_3$: C, 79.56; H, 8.22. Found: C, 79.38; H, 8.24.

2.1.7. *2-Methoxy-3-benzyloxy-18a-homoestra-1,3,5(10)-trien-17β-ol (**8**)*

Under nitrogen, the 2,17-diol (**7**, 2.057 g, 5.2 mmol) was dissolved in 30 ml of THF. To the solution lithium hydroxide (0.55 g, 1.3 mmol) and dimethyl sulfate (0.55 ml, 7.7 mmol) were added and the mixture refluxed at 60 °C overnight. Analysis by TLC (5% acetone/CH_2Cl_2) confirmed complete reaction and the mixture cooled to room temperature. The solvent was removed in vacuo and the residue purified by flash chromatography (5% acetone/CH_2Cl_2). Crystallization attempts from different solvent systems failed and the compound (**8**) was obtained as a viscous oil which was dried under vacuum to obtain a white foam which melted 240–242 °C; FTIR (ATR) ν_{max}: 3522, 2927, 2861, 1601, and 1506 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.99 (t, J = 7.4 Hz, 18a), 3.87 (s, 2-OCH_3), 3.81 (t, J = 8.4 Hz, 17-H), 5.10 (s, benzyl CH_2), 6.63 (s, 4-H), 6.48 (s, 1-H), 7.30–7.48 (m, benzyl aromatic). Analysis calculated for $C_{27}H_{34}O_3 \cdot 2/3H_2O$ C, 77.48; H, 8.51. Found: C, 77.92; H, 8.26.

2.1.8. *2-Methoxy-3,17β-dihydroxy-18a-homoestra-1,3,5(10)-triene (**9**)*

A mixture of the 2-methoxy compound (**8**, 200 mg, 0.5 mmol) and 5% palladium on carbon (200 mg) in ethanol (50 ml) was hydrogenated in a Parr shaker apparatus at 40 psi hydrogen pressure overnight. Analysis by TLC (3% acetone/CH_2Cl_2) confirmed complete reaction. The mixture was filtered through Celite while rinsing with dichloromethane. The solvent was removed in vacuo. This material was crystallized from dichloromethane/hexanes to give the pure 3,17-diol (**9**, 126 mg, 81%) as a white powder. mp = 114–115 °C; FTIR (ATR) ν_{max}: 3842, 3359, 2921, 2850, 1592, 1505 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 1.00 (t, J = 7.5 Hz, 18a), 3.86 (s, 2-OCH_3), 3.77-3.821 (m, 17-H), 6.65 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{20}H_{28}O_3 \cdot 1/6$hexanes: C, 76.25; H, 9.24. Found: C, 76.20; H, 9.17

2.1.9. *2,17β-Dimethoxy-3-benzyloxy-18a-homoestra-1,3,5(10)-triene (**10**)*

Under nitrogen, the 2-methoxy compound (**8**, 400 mg, 0.95 mmol) was dissolved in 20 ml of THF. This solution was added drop wise to 0.08 g (~2 mmol) of 60% NaH in 10 ml of THF. The mixture was refluxed for 1 h and cooled to room temperature. Methyl iodide (0.27 ml, 4.3 mmol) was added and the mixture stirred for 2 h at room temperature. At this point TLC (5% acetone/CH_2Cl_2) showed no reaction. An additional methyl iodide (6 ml, 9.6 mmol) was added and the mixture stirred overnight at room temperature. Analysis by TLC confirmed complete reaction. The reaction was quenched with water and extracted with ether (3×), the ether fraction was washed with water (3×), and brine. The organic fractions were dried over sodium sulfate, filtered and concentrated in vacuo. This material was crystallized from acetone/hexanes to give the pure 2,17-dimethoxy-3-benzyl ether (**10**, 266 mg, 64%). mp = 113–114 °C; FTIR (ATR) ν_{max}: 2922, 2860, 1604, and 1514 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.98 (t, J = 7.5 Hz, 18a), 3.37 (s, 17-OCH_3), 3.87 (s, 2-OCH_3), 3.35 (t, J = 7.75 Hz, 17-H), 5.11 (s, benzyl CH_2), 6.62 (s, 4-H), 6.85 (s, 1-H), 7.27-7.46 (m, benzyl aromatic). Analysis calculated for $C_{28}H_{36}O_3$: C, 79.96; H, 8.63. Found: C, 80.03; H, 8.68.

2.1.10. *2,17β-Dimethoxy-3-hydroxy-18a-homoestra-1,3,5(10)-triene (**11**)*

A mixture of the 2,17-dimethoxy-3-benzyl ether (**10**, 150 mg, 0.35 mmol) and 5% palladium on carbon (150 mg) in ethanol (60 ml) was hydrogenated in a Parr shaker apparatus at 40 psi hydrogen pressure overnight. Analysis by TLC (3% acetone/CH_2Cl_2) confirmed complete reaction. The mixture was filtered through Celite while rinsing with dichloromethane. The solvent was removed in vacuo and the remaining reside purified by flash chromatography using the TLC solvent system. This material was triturated from cold ether, rinsed with pentane and dried in vacuo to yield the pure 2,17-dimethoxy compound (**11**, 110 mg, 94%). mp = 161–162 °C; FTIR (ATR) ν_{max}: 3392, 2933, 2868, 1591, 1506 cm^{-1}. NMR

(300 MHz, $CDCl_3$), δ_H (ppm): 0.97 (t, $J = 7.4$ Hz, 18a), 3.37 (s, 17-OCH_3), 3.85 (s, 2-OCH_3), 3.49 (t, $J = 7.55$ Hz, 17-H), 6.65 (s, 4-H), 6.80 (s, 1-H). Analysis calculated for $C_{21}H_{30}O_3 \cdot 1/6H_2O$: C, 75.64; H, 9.17. Found: C, 75.54; H, 8.91.

2.1.11. 3,17β-Bis(methoxymethoxy)-18a-homoestra-1,3,5(10)-triene (12)

Under nitrogen, the diol (**2**, 47.2 g, 0.165 mol) was dissolved in 1.2 l of dry THF. Chloromethyl methyl ether (62 ml, 5 equiv.) and diisopropylethylamine (170 ml, 6 equiv.) were added and the reaction heated to 65 °C overnight. The reaction was cooled to room temperature, quenched with 20% NH_4Cl, and extracted with EtOAc (3×). The organic phase washed with water (3×), brine, dried over Na_2SO_4, and concentrated in vacuo to give the pure 3,17β-dimethoxymethyl ether (**12**, 48.2 g, 78 %) as a pale yellow oil. The material was used without further purification. FTIR (ATR) ν_{max}: 2929, 2876, 1608, and 1496 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.98 (t, $J = 7.5$ Hz, 18a), 3.38 (s, 17-OCH_2OCH_3), 3.48 (s, 3-OCH_2OCH_3), 4.64 (dd, $J_1 = 9.0$ Hz, $J_2 = 6.45$ Hz, 17-OCH_2OCH_3), 5.15 (s, 3-OCH_2OCH_3), 6.78 (d, $J = 2.4$ Hz, 4-H), 6.84 (dd, $J_1 = 8..4$ Hz, $J_2 = 2.4$ Hz, 2-H), 7.21 (d, $J = 8.7$ Hz, 1-H). Analysis calculated for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.34; H, 8.86.

2.1.12. 2-Hydroxy-3,17β-bis(methoxymethoxy)-18a-homoestra-1,3,5(10)-triene (13)

Under nitrogen, the dimethoxymethyl ether (**12**, 56.4 g, 0.151 mol) was dissolved in 1 l of dry THF and chilled to −78 °C. To this was added sec-BuLi (215 ml, 1.4 M, 2 equiv.) at such a rate the temperature did not exceed −65 °C and the reaction mixture stirred at −78 °C for 3 h. Trimethyl borate (68 ml, 4 equiv.) was then added maintaining the temperature below −65 °C and the reaction stirred for 15 min at −78 °C and then warmed to 0 °C. The reaction was quenched with 1 l of 20% NH_4Cl, then allowed to come to room temperature and stirred for 1 h. Sodium perborate (93 g, 4 equiv.) was then added at such a rate that the temperature did not exceed 35 °C and the reaction carried out at room temperature overnight. The reaction mixture was concentrated in vacuo and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash choromatography (2% acetone/CH_2Cl_2) to give (**13**, 60.4 g, 84 %) as a yellow oil. FTIR (ATR) ν_{max}: 3407, 2930, 1589, and 1504 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.99 (t, $J = 7.5$ Hz, 18a), 3.36 (s, 17-OCH_2OCH_3), 3.52 (s, 3-OCH_2OCH_3), 4.63 (dd, $J_1 = 9.0$ Hz, $J_2 = 6.6$ Hz, 17-OCH_2OCH_3), 5.16 (s, 3-OCH_2OCH_3), 6.80 (s, 4-H), 6.90 (s, 1-H). The material was used without further purification.

2.1.13. 2-Methoxy-3,17β-bis(methoxymethoxy)-18a-homoestra-1,3,5(10)-triene (14)

Under nitrogen, the 2-hydroxy compound (**13**, 44.7 g, 0.115 mol) was dissolved in 500 ml of dry THF. Lithium hydroxide (6 g, 1.25 equiv.) and dimethyl sulfate (12.2 ml, 1.12 equiv.) were added and the reaction was refluxed for 4 h. Analysis by TLC (CH_2Cl_2) indicated a complete reaction. The reaction was cooled to room temperature, diluted with water, concentrated in vacuo, and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give the 2-methoxy derivative (**14**, 53.7 g, >100%) as a yellow oil which still contained some solvent. FTIR (ATR) ν_{max}: 2930, 1608, and 1507 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.98 (t, $J = 7.5$ Hz, 18a), 3.37 (s, 17-OCH_2OCH_3), 3.51 (s, 3-OCH_2OCH_3), 3.86 (s, 2-OCH_3), 4.63 (dd, $J_1 = 9.0$ Hz, $J_2 = 6.3$ Hz, 17-OCH_2OCH_3), 5.19 (s, 3-OCH_2OCH_3), 6.84 (s, 4-H), 6.86 (s, 1-H). The material was used without further purification.

2.1.14. 2-Methoxy-3,17β-dihydroxy-18a-homoestra-1,3,5(10)-triene (9)

Under nitrogen, 2-methoxy compound (**14**, 53.7 g, 0.132 mol) was hydrolyzed with a mixture of THF (300 ml) and 6M HCl (500 ml) at room temperature overnight. Analysis by TLC (2% Acetone/CH_2Cl_2) indicated a complete reaction. The reaction was diluted with water, concentrated in vacuo and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column (6% Acetone/CH_2Cl_2). This material was crystallized from dichloromethane/hexanes to give the pure 3,17-diol (**9**, 30.3 g, 72%) as yellow solid. mp = 114–115 °C; FTIR (ATR) ν_{max}: 3484, 3362, 2921, 2872, and 1706 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 1.00 (t, $J = 7.5$ Hz, 18a), 3.84 (s, 2-OCH_3), 6.64 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{20}H_{28}O_3 \cdot 1/6$hexanes: C, 76.25; H, 9.24. Found: C, 76.20; H, 9.17

2.1.15. 2-Methoxy-3-acetoxy-18a-homoestra-1,3,5(10)-trien-17β-ol (15)

Under nitrogen, the diol (**9**, 20 g, 62.3 mmol) was selectively acetylated in isopropanol with acetic anhydride (18 ml, 3 equiv.) in the presence of NaOH (95 ml, 2 M, 3 equiv.) at room temperature for 90 min. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was slowly quenched with methanol, diluted with water, concentrated in vacuo and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give the pure 3-acetate (**15**, 20.56 g, 91%) as a yellow foam which resisted crystallization. mp = 84 °C; FTIR (ATR) ν_{max}: 3523, 2931, 2870, 1751, and 1644 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.93 (t, $J = 7.5$ Hz, 18a), 2.29 (s, 3-OAc), 3.80 (s, 2-OCH_3), 6.73 (s, 4-H), 6.89 (s, 1-H). Analysis calculated for $C_{22}H_{30}O_4 \cdot 2/3H_2O$: C, 71.32; H, 8.52. Found: C, 71.15; H, 8.16.

2.1.16. 2-Methoxy-3-acetoxy-18a-homoestra-1,3,5(10)-trien-17-one (16)

Under nitrogen, the 17-hydroxy compound (**15**, 20 g, 55.8 mmol) was dissolved in 400 ml of acetone and chilled to 0 °C. Jones's Reagent was slowly added with stirring until the yellow-orange color persisted (~40 ml). the reaction was stirred an additional 5 min then slowly quenched with isopropanol. The solution was concentrated in vacuo, diluted with water, and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give the 17-ketone (**16**, 19 g, 95.5%). The material was used without further purification. mp = 159 °C; FTIR (ATR) ν_{max}: 2932, 1762, 1731, 1614, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.81 (t, $J = 7.5$ Hz, 18a), 2.31 (s, 3-OAc), 3.81 (s, 2-OCH_3), 6.76 (s, 4-H), 6.89 (s, 1-H). Analysis

calculated for $C_{22}H_{28}O_4 \cdot 1/6H_2O$: C, 73.51; H, 7.94. Found: C, 73.73; H, 7.84.

*2.1.17. 17,17-Ethylenedioxy-2-methoxy-18a-homoestra-1,3,5(10)-trien-3-yl-acetate (**17**)*

Under nitrogen, the 17-ketone (**16**, 19 g, 53 mmol) was dissolved in 300 ml of CH_2Cl_2. To this solution were added triethylorthoformate (28.8 ml, 3.25 equiv.), ethylene glycol (19 ml, 6.4 equiv.) and tosic acid (0.77 g, 0.076 equiv.) and the reaction mixture stirred at room temperature overnight. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a partial reaction. Additional triethylorthoformate (35 ml) and ethylene glycol (10 ml) were added and refluxed for an additional 3 h. The reaction was cooled to room temperature, quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2 (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give a dark yellow oil. Analysis showed an incomplete reaction. The material was re-reacted over the weekend using 60 ml of triethylorthoformate, 40 ml of ethylene glycol and 0.8 g of tosic acid. Subsequent purification and re-acetylation afforded the ketal (**17**, 20 g, 94%) as a yellow foam which resisted crystallization. mp = 97–98 °C; FTIR (ATR) ν_{max}: 2936, 2875, 1763, 1614, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.99 (t, J = 7.35 Hz, 18a), 2.31 (s, 3-OAc), 3.82 (s, 2-OCH_3), 3.90–3.95 (m, 17-ketal CH_2's), 6.74 (s, 4-H), 6.90 (s, 1-H). Analysis calculated for $C_{24}H_{32}O_5 \cdot 3/4H_2O$: C, 69.62; H, 8.16. Found: C, 69.51; H, 8.00.

*2.1.18. 17,17-Ethylenedioxy-16ξ-bromo-2-methoxy-18a-homoestra-1,3,5(10)-trien-3-yl-acetate (**18**)*

Under nitrogen, the 17-ketal (**17**, 20 g, 50 mmol) was dissolved in 400 ml of THF and chilled to −5 °C. Phenyltrimethylammonium tribromide (22.5 g, 1.2 equiv.) was added in 5 g portions over 0.5 h. Once the addition was complete, the reaction mixture was stirred at −5 °C overnight. The reaction was quenched with cold, saturated $NaHCO_3$ and extracted with EtOAc (3×). The organic phase was washed with saturated $NaHCO_3$ (2×), 10% sodium thiosulfate, cold water (2×), and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column and subsequently crystallized from CH_2Cl_2/hexanes to give the pure 16-bromo compound (**12**, 22.94 g, 95%) mp = 194 °C; FTIR (ATR) ν_{max}: 2936, 2878, 1763, 1614, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 1.00 (t, J = 7.050 Hz, 18a), 2.31 (s, 3-OAc), 3.81 (s, 2-OCH_3), 3.96 – 4.21 (m, 17-ketal CH_2's), 4.69 (dd, J_1 = 10.350 Hz, J_2 = 3.450 Hz, 16-H), 6.74 (s, 4-H), 6.90 (s, 1-H). Analysis calculated for $C_{24}H_{31}O_5Br$: C, 60.13; H, 6.52; Br, 16.67. Found: C, 59.96; H, 6.35; Br, 16.85.

*2.1.19. 17,17-Ethylenedioxy-2-methoxy-18a-homoestra-1,3,5(10),14-tetraen-3-ol (**19**) and 17,17-ethylenedioxy-2-methoxy-18a-homoestra-1,3,5(10),15-tetraen-3-ol (**20**)*

Under nitrogen, the 16-bromo compound (**18**, 19 g, 19.8 mmol) was dissolved in 100 ml of dry xylenes. This solution was added to freshly prepared potassium *tert*-butoxide (43 g, 19.3 equiv.) in 500 ml of dry xylenes and refluxed overnight. The reaction was cooled to room temperature, quenched with water and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give a dark brown residue. The material was purified via flash chromatography (1.5% acetone/CH_2Cl_2) to give a mixture of the double bond isomers (**19** and **20**, 6.92 g, 49%) as a dark brown solid. The material was used without further purification.

*2.1.20. 2-Methoxy-3-hydroxy-18a-homoestra-1,3,5(10)14-tetraen-17-one (**21**) and 2-methoxy-3-hydroxy-18a-homoestra-1,3,5(10)15-tetraen-17-one (**22**)*

Under nitrogen, the mixture of the double bond isomers (**19** and **20**, 8 g, 22.4 mmol) was dissolved in 400 ml of 6:1 acetone/water. Tosic acid (0.41 g 0.1 equiv.) was added and the reaction mixture stirred at room temperature for 2.5 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated the reaction was complete. The reaction mixture was evaporated to 1/3 volume and extracted with CH_2Cl_2 (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (2.5% acetone/CH_2Cl_2) to give a mixture of the Δ^{14} and Δ^{15} isomers (**21** and **22**, 2.7 g) as well as the pure Δ^{15} derivative (**22**, 0.46 g, 45%). The Δ^{15} compound (**22**) was crystallized from ether/hexanes. mp = 163 °C; FTIR (ATR) ν_{max}: 3236, 2959, 2929, 1686, 1609, and 1523 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.79 (t, J = 7.5 Hz, 18a), 3.86 (s, 2-OCH_3), 6.06 (dd, J_1 = 6.0 Hz, J_2 = 3.3 Hz, 16-H), 5.72 (s, 3-OH), 6.66 (s, 4-H), 6.77 (s, 1-H), 7.81 (dd, J_1 = 6.0 Hz, J_2 = 2.7 Hz, 15-H). Analysis calculated for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.36; H, 7.71.

*2.1.21. 2-Methoxy-18a-homoestra-1,3,5(10),14,16-pentaen-3-17β-diyl-diacetate (**23**)*

Under nitrogen, a mixture of Δ^{14} and Δ^{15} compounds (**21** and **22**, 2.7 g, 8.64 mmol) was dissolved in 51 ml of acetic anhydride. Isopropenyl acetate (50 ml, 53 equiv.) and Tosic acid (1.0 g, 0.6 equiv.) were added and the reaction mixture was refluxed overnight. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was cooled to room temperature, quenched with water and stirred for 1 h. The mixture was then concentrated in vacuo and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography (2.5% acetone/CH_2Cl_2) and subsequently crystallized from ether/hexanes to give the pure pentaene derivative (**23**, 1.6 g, 46.6%) as a white solid. mp = 133 °C; FTIR (ATR) ν_{max}: 2933, 2865, 1763, and 1615 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.53 (t, J = 7.350 Hz, 18a), 2.23 (s, 17-OAc), 2.31 (s, 3-OAc), 3.81 (s, 2-OCH_3), 5.93 (t, J = 1.95 Hz, 15-H), 6.22 (d, J = 2.7 Hz, 16-H), 6.79 (s, 4-H), 6.89 (s, 1-H). Analysis calculated for $C_{24}H_{28}O_5$: C, 72.71; H, 7.12. Found: C, 72.64; H, 7.14.

*2.1.22. 2-Methoxy-3,17β-dihydroxy-18a-homoestra-1,3,5(10),14-tetraene (**24**)*

Under nitrogen, a solution of the enolacetate (**23**, 1.5 g, 3.8 mmol) in ethanol (20 ml) and THF (20 ml) was chilled to 0 °C in an ice bath. An ice cold solution of sodium borohydride (0.9 g, 6.3 equiv.) in ethanol/water (1:1, 50 ml) was added to the steroid solution. The reaction mixture was stirred for 1 h, allowed to warm to room temperature, and then stirred overnight. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with acetic acid, concentrated to a small volume, and extracted with

EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column to give the pure diol (**24**, 0.93 g, 69%) as an amorphous powder. mp = 65 °C; FTIR (ATR) ν_{max}: 3409, 2920, 2853, 1592, and 1506 cm^{--1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.89 (t, J = 7.5 Hz, 18a), 3.87 (s, 2-OCH_3), 4.21 (t, J = 8.4 Hz, 17-H), 5.21 (m, 15-H), 6.67 (s, 4-H), 6.80 (s, 1-H). Analysis calculated for $C_{20}H_{26}O_3 \cdot 1/2H_2O$: C, 74.27; H, 8.41. Found: C, 74.33; H, 8.21.

2.1.23. 2-Methoxy-3-acetoxy-18a-homoestra-1,3,5(10), 14-tetraen-17β-ol (25)

Under nitrogen, the diol (**24**, 0.7 g, 2.2 mmol) was dissolved in 20 ml of isopropanol. Acetic anhydride (5 ml, 52 mmol) and 2 M NaOH (20 ml) were added and the reaction stirred at room temperature for 2 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with methanol, concentrated to a small volume and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Crystallization from ether/hexanes afforded the pure 3-acetate (**25**, 0.61 g, 77%) as a white solid. mp = 139 °C; FTIR (ATR) ν_{max}: 3510, 2922, 2854, 1755, 1615, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.88 (t, J = 7.35 Hz, 18a), 2.31 (s, 3-OAc), 3.80 (s, 2-OCH_3), 4.19 (t, J = 8.55 Hz, 17-H), 5.34 (m, 15-H), 6.76 (s, 4-H), 6.90 (s, 1-H). Analysis calculated for $C_{22}H_{28}O_4 \cdot 1/2H_2O$: C, 72.30; H, 8.00. Found: C, 72.47; H, 7.70.

2.1.24. 2,17β-Dimethoxy-18a-homoestra-1,3,5(10), 14-tetraen-3-yl-acetate (26)

Under nitrogen, fluoroboric acid (48%, 2.5 ml, 13 equiv.) was added to a solution of the 3-acetate (**25**, 0.5 g, 1.4 mmol) in CH_2Cl_2 (20 ml) chilled to 0 °C, and stirred for 10 min. Trimethylsilyl diazomethane (1 ml, 1.4 equiv.) was slowly added and the reaction mixture stirred for 20 min. An additional amount of trimethylsilyl diazomethane was added every 20 min. After the final addition, the reaction was stirred for an additional 0.5 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with water and extracted with CH_2Cl_2 (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash column (5% acetone/CH_2Cl_2) afforded the pure 17-methoxy derivative (**26**, 0.37 g, 71%) as a yellow foam. The material was used without further purification. FTIR (ATR) ν_{max}: 2934, 2859, 1763, 1613, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.85 (t, J = 7.5 Hz, 18a), 2.30 (s, 2-OAc), 3.41 (s, 17-OMe), 3.74 (t, J = 8.25 Hz, 17-H), 3.80 (s, 2-OCH_3), 5.34 (m, 15-H), 6.75 (s, 4-H), 6.89 (s, 1-H).

2.1.25. 2,17β-Dimethoxy-3-hydroxy-18a-homoestra-1,3,5(10),14-tetraene (27)

Under nitrogen, the 3-acetate (**26**, 0.3 g, 0.81 mmol) was dissolved in 60 ml of 3:1 MeOH/H_2O. Potassium carbonate (0.44 g, 4 equiv.) was added and the reaction mixture stirred at room temperature overnight. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with water, concentrated, and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash chromatography and subsequent crystallization from ether/hexanes afforded the pure 3-hydroxy (**27**, 0.2 g, 75%) as a white solid. mp = 202 °C; FTIR (ATR) ν_{max}: 3383, 2935, 2834, 1618 and 1507 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.85 (t, J = 7.2 Hz, 18a), 3.42 (s, 17-OMe), 3.85 (t, J = 8.25 Hz, 17-H), 3.86 (s, 2-OCH_3), 5.34 (m, 15-H), 6.66 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{21}H_{28}O_3 \cdot 1/6Et_2O$: C, 76.36; H, 8.77. Found: C, 76.14; H, 8.55.

2.1.26. 2-Methoxy-3,17β-dihydroxy-18a-homoestra-1,3,5(10),15-tetraene (28)

To a solution of the 17-ketone (**22**, 0.4 g, 1.28 mmol), in ether (380 ml), chilled to −5 °C, was added solid $LiAlH_4$ (1.0 g, 19.75 equiv.) in small portions, and the reaction mixture stirred for 1 h. Analysis by TLC (5% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with the dropwise addition of 5% H_2SO_4 and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash chromatography (5% acetone/CH_2Cl_2) and subsequent crystallization from methanol afforded the pure diol (**28**, 0.15 g, 37%) as a white solid. mp = 164 °C; FTIR (ATR) ν_{max}: 3519, 3240, 2919, 1589, and 1506 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.91 (t, J = 7.5 Hz, 18a), 3.87 (s, 2-OCH_3), 5.71 (m, 16-H), 5.98 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 15-H), 6.67 (s, 4-H), 6.79 (s, 1-H). Analysis calculated for $C_{20}H_{26}O_3 \cdot 1/2H_2O$: C, 74.27; H, 8.41. Found: C, 74.38; H, 8.15.

2.1.27. 2-Methoxy-3-acetoxy-18a-homoestra-1,3,5(10), 15-tetraen-17β-ol (29)

Under nitrogen, the 3-hydroxy compound (**28**, 0.25 g, 0.79 mmol) was dissolved in 20 ml of isopropanol. Acetic anhydride (5 ml, 52 mmol) and 2 M NaOH (20 ml) were added and the reaction mixture was stirred at room temperature for 3 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with methanol, concentrated to a small volume, and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash column (2% acetone/CH_2Cl_2) to give the pure 3-acetate (**29**, 0.17 g, 61%) as a white foam. The material was used without further purification. FTIR (ATR) ν_{max}: 3427, 2923, 2854, 1751, 1614, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.90 (t, J = 7.5 Hz, 18a), 2.30 (s, 3-OAc), 3.81 (s, 2-OCH_3), 5.71 (m, 16-H), 5.97 (m, 15-H), 6.75 (s, 4-H), 6.89 (s, 1-H).

2.1.28. 2,17β-Dimethoxy-18a-homoestra-1,3,5(10), 15-tetraen-3-yl-acetate (30)

Under nitrogen, 48% fluoroboric acid (1.2 ml, 13 equiv.) was added to a solution of the 3-acetate (**29**, 0.175 g, 0.49 mmol) in CH_2Cl_2 (10 ml) chilled to 0 °C, and stirred for 10 min. Trimethylsilyl diazomethane (8 ml, 32.5 equiv.) was added drop wise over 2.5 h. Once the addition was complete, the reaction was stirred an additional 90 min. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with water and extracted with CH_2Cl_2 (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by flash column (5% acetone/CH_2Cl_2) afforded the pure 17-methoxy (**30**, 0.13 g, 71%) as a white foam which melted at 142 °C; FTIR (ATR)

ν_{max}: 2921, 1764, 1614 and 1511 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.88 (t, J = 7.5 Hz, 18a), 2.30 (s, 3-OAc), 3.47 (s, 17-OCH_3), 3.81 (s, 2-OCH_3), 5.80 (m, 16-H), 5.95 (dd, J_1 = 6.0 Hz, J_2 = 2.1 Hz, 15-H), 6.75 (s, 4-H), 6.89 (s, 1-H). Analysis calculated for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.20; H, 8.30.

*2.1.29. 2,17β-Dimethoxy-3-hydroxy-18a-homoestra-1,3,5(10),15-tetraene (**31**)*

Under nitrogen, the 3-acetate derivative (**30**, 0.1 g, 0.27 mmol) was dissolved in 20 ml of 3:1 MeOH/H_2O. Potassium carbonate (1.0 g, 27 equiv.) was added and the reaction mixture stirred at room temperature for 2 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction was quenched with water, and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash chromatography and subsequent crystallization from CH_2Cl_2/hexanes gave the pure 3-hydroxy compound (**31**, 0.067 g, 76%) as a white solid. mp = 180 °C; FTIR (ATR) ν_{max}: 3378, 2930, 1617, and 1504 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.87 (t, J = 7.5 Hz, 18a), 3.46 (s, 17-OCH_3), 3.87 (s, 2-OCH_3), 5.80 (m, 16-H), 5.96 (m, 15-H), 6.65 (s, 4-H), 6.78 (s, 1-H). Analysis calculated for $C_{21}H_{28}O_3 \cdot 1/10H_2O$: C, 76.38; H, 8.61. Found: C, 76.19; H, 8.52.

*2.1.30. 2-Methoxy-3-hydroxy-18a-homoestra-1,3,5(10)-trien-17-one p-toluenesulfonylhydrazone (**32**)*

To a solution of the ketone (**16**, 1.0 g, 3.1 mmol) in MeOH (20 ml) was added *p*-toluene sulfonhydrazide (0.72 g, 1.25 equiv.) and the reaction mixture refluxed overnight. Analysis by TLC (2% acetone/CH_2Cl_2) indicated an incomplete reaction. Hydrochloric acid (4 drops) was added and the reaction mixture refluxed for an additional 4 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated an incomplete reaction. Additional HCl was added (four drops) and the reflux continued an additional 2 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated approximately 90% completion. The reaction was cooled to room temperature and stored in the refrigerator over the weekend. The reaction mixture was diluted with water and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo to give the *p*-toluenesulfonylhydrazone (**32**, 1.65 g) as a green/brown solid. The material was used without further purification. FTIR (ATR) ν_{max}: 3519, 3225, 2928, 1597, and 1508 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.50 (t, J = 7.2 Hz, 18a), 2.44 (d, J = 3.9 Hz, benzyl CH_3), 3.87 (s, 2-OCH_3), 6.64 (s, 4-H), 6.78 (s, 1-H), 7.316 (d, J = 7.5 Hz, benzyl CH_2's), 7.86 (d, J = 7.8 Hz, benzyl CH_2's).

*2.1.31. 2-Methoxy-3-hydroxy-18a-homoestra-1,3,5(10),16-tetraene (**33**)*

Under nitrogen, the 17-*p*-toluenesulfonylhydrazone (**32**, 1.0 g, 2.1 mmol) was dissolved in dry THF (130 ml) and chilled to 0 °C in an ice/salt bath. To this solution was added chilled butyl lithium solution (3.3 ml, 4 equiv.). Once the addition was complete, the reaction mixture was stirred at room temperature for 72 h. Analysis by TLC (2% acetone/CH_2Cl_2) indicated a complete reaction. The reaction mixture was chilled to 0 °C, quenched with 20% NH_4Cl, concentrated to a small volume, and extracted with EtOAc (3×). The organic phase was washed with water, brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by flash chromatography gave the pure tetraene (**33**, 0.120 g, 19%) as a viscous oil. FTIR (ATR) ν_{max}: 3549, 2923, 2853, 1591 and 1504 cm^{-1}. NMR (300 MHz, $CDCl_3$), δ_H (ppm): 0.79 (t, J = 7.5 Hz, 18a), 3.85 (s, 2-OCH_3), 5.82 (m, 16-H), 6.04 (m, 17-H), 6.64 (s, 4-H), 6.78 (s, 1-H). Analysis calculated for $C_{20}H_{26}O_2 \cdot 1/2H_2O$: C, 79.70; H, 8.81. Found: C, 79.78; H, 8.87.

2.2. Biological assays

Biological assays were carried out by EntreMed Inc., Rockville, MD.

2.3. Cell cultures

Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics (San Diego, CA), MDA-MB-231, and U87-MG cells were obtained from ATCC (American Type Culture Collection, Manassas, VA). HUVEC cultures were maintained for up to five passages in EGM (Endothelial Growth Medium) containing bovine brain extract (Clonetics) and 1× antibiotic–animycotic (BioWhittaker, Walkersville, MD). MDA-MB-231 and U87-MG cells were maintained in DMEM/F12 (1:1) containing 10% (v/v) fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1× antibiotic–antimycotic.

2.4. In vitro inhibition of proliferation

Proliferation assays were performed by evaluating detection of DNA synthesis by the use of the 5-bromo-2′-deoxyuridine (BrdU) cell proliferation colorimetric ELISA kit from Roche (Indianapolis, IN) according to the manufacturer's instructions. The cells were seeded at 1000 cells/well (MDA-MB-231 and U87-MG cells, anti-tumor activity) or 3000 cells/well (HUVEC, anti-angiogenic activity) in a 96 well plate, allowed to attach overnight and then exposed to the compound to be tested for 48 h. IC50 values and standard deviations (S.D.) were calculated for each assay using Sigma Plot (Systat Software Inc., San Jose, CA). Reported IC50 values are the mean and SD of a minimum of three separate assays.

2.5. Computational analysis

All calculations were carried out using the MM3 forcefield [14] as implemented by PCMODEL (Version 9.1, Serena Software, Bloominton, IN). After initial input of basic starting geometries, the conformational space of each compound was explored by means of the GMMX routine (Global Energy Minimization) in PCMODEL using the MM3 forcefield, and an energy window of 20 kcal/mol. All degrees of conformational freedom were considered including ring conformations and rotatable bond orientations. The global minima thus obtained was then used as a starting point for the conformational profile calculations presented in Figs. 7 and 8 whereby the C14–C13–C18–C18a torsional angle was fixed in incremental 5° steps as the remainder of the molecule was minimized. The results were plotted and interpolated (cubic spline) using CurveExpert 1.3 (Hyams Development, Hixson TN).

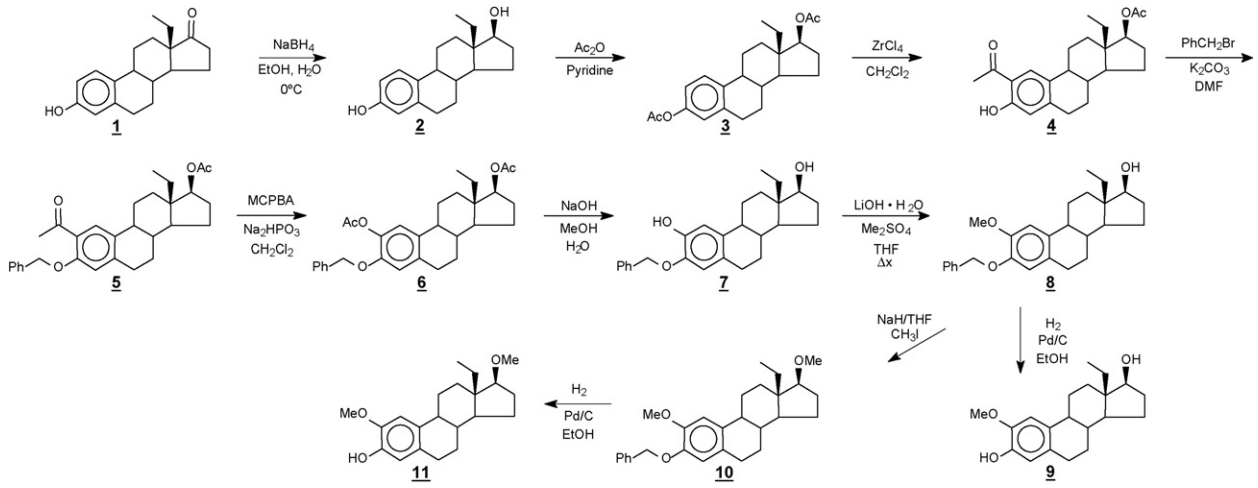


Fig. 1 – Synthesis of compounds (2–11).

3. Results and discussion

3.1. *Chemistry*

The 3-hydroxy-17-ketone derivative (**1**) was prepared from Norgestrel as previously described [5]. The syntheses of compounds (**2–11**) are outlined in Fig. 1 and were based on the procedures we previously developed for the 13-methyl analogs [7]. Reduction of the ketone via sodium borohydride in ethanol/THF at room temperature overnight gave the diol compound (**2**) in 98% yield. Simple acetylation of this material with acetic anhydride in pyridine at room temperature for 48 h gave the 3,17β-diacetate compound (**3**) in 80% yield. Treatment of the diacetate derivative (**3**) with zirconium (IV) chloride in dichloromethane at room temperature for 48 h gave the 2-acetyl-3-hydroxy-17β-acetate compound (**4**) in 63% yield. This compound was then converted to the 3-benzyl ether derivative (**5**) in 87% yield by reaction with benzyl chloride in the presence of potassium carbonate in DMF at 65 °C overnight. Conversion of this material to the diacetate derivative (**6**) was accomplished by reaction with 3-chloroperoxybenzoic acid and sodium phosphate in dichloromethane at room temperature for 48 h in 79% yield. Simple base hydrolysis of the diacetate material (**6**) with sodium hydroxide in methanol/water gave the 2,17β-diol-3-benzyl ether compound (**7**) in 71% yield. Methylation of this material in THF with dimethyl sulfate in the presence of lithium hydroxide monohydrate at 60 °C overnight gave the 2-methoxy derivative (**8**) in 92% yield. Removal of the benzyl ether from compound (**8**) via catalytic hydrogenation with 5% palladium on carbon in ethanol at 40 psi gave compound (**9**) in 81% yield. Compound (**8**) was also converted to the 2,17β-dimethoxy derivative (**10**) in 64% yield by refluxing with solid sodium hydride in THF, followed by the addition of iodomethane. Finally, catalytic hydrogenation of compound (**10**) with 10% palladium on carbon in ethanol at 40 psi gave compound (**11**) in 94% yield.

An alternate synthesis of compound (**9**) is illustrated in Fig. 2 and is based on the procedure described by Paaren and Duff [8]. Treatment of 2,17β-diol derivative (**2**) with chloromethyl methyl ether and *N*,*N*-diisopropylethylamine at 65 °C in THF overnight afforded the dimethoxymethyl ether (**12**) in 78% yield. This material was then reacted with *n*-BuLi in THF at −65 °C followed by the addition of trimethyl borate and sodium perborate to give the 2-hydroxy compound (**13**) in 84% yield. Methylation of this material in THF with dimethyl sulfate in the presence of lithium hydroxide monohydrate at 60 °C overnight gave the 2-methoxy derivative (**13**) in quantitative yield. This material was then hydrolyzed with 6M HCl in THF to give the 3,17β-diol derivative (**9**) in 72% yield.

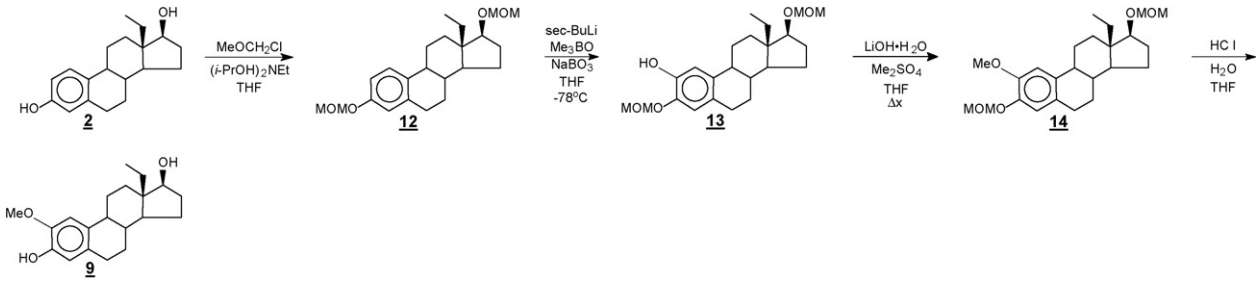


Fig. 2 – Alternative synthesis of 2-methoxy-18a-homoestra-1,3,5(10)-trien-17β-ol (9).

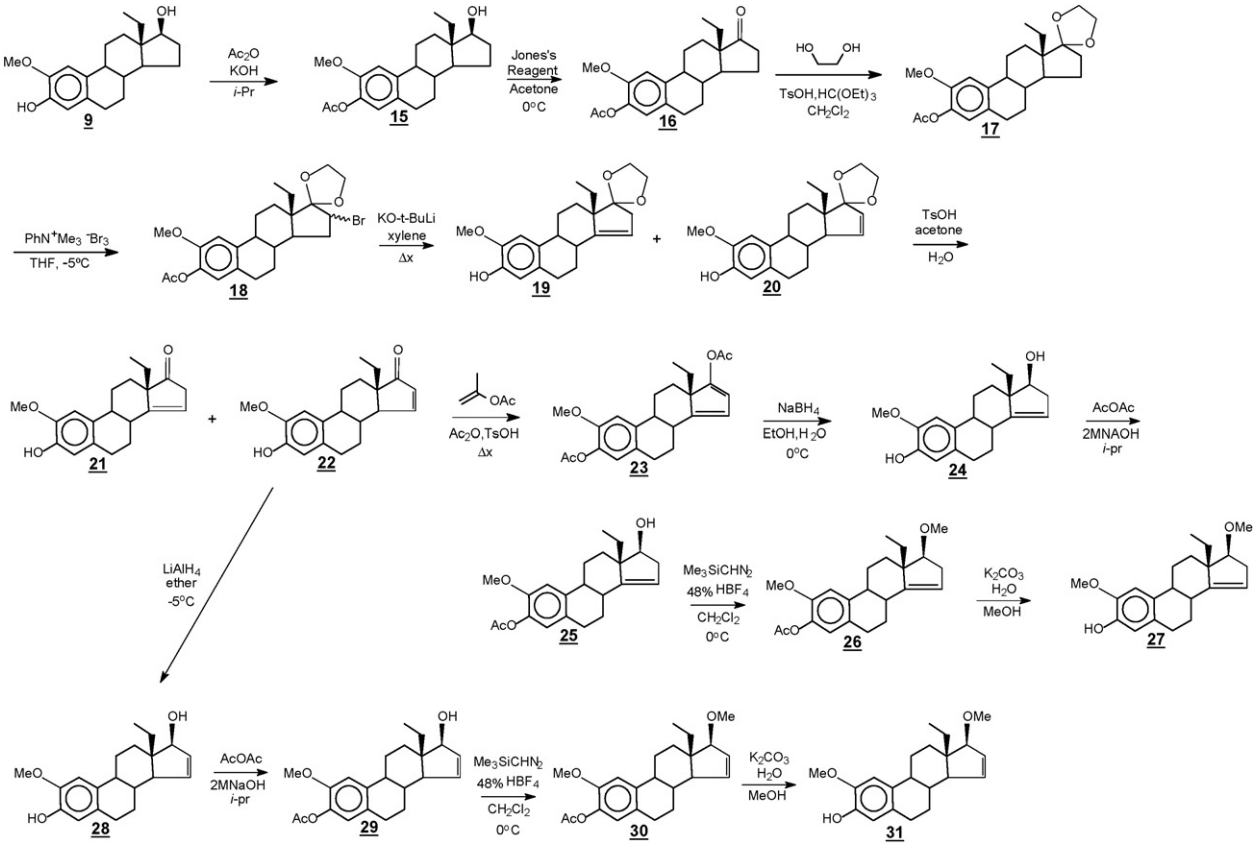


Fig. 3 – Synthesis of ring-D-unsaturated 2-methoxy-18a-homoestra-1,3,5(10)-trien-3-ol derivatives.

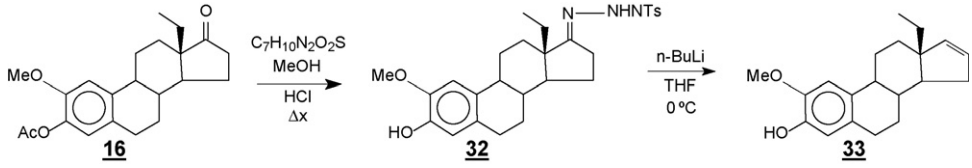


Fig. 4 – Synthesis of 2-methoxy-3-hydroxy-18a-homoestra-1,3,5(10),16-tetraene.

For the synthesis of ring-D-unsaturated compounds (**15–31**), the general procedure we developed earlier [3] was followed and the synthetic scheme is presented in Fig. 3. The 3,17β-diol derivative (**9**) was then selectively acetylated in isopropanol with acetic anhydride in the presence of 2 M KOH to give the 3-acetate derivative (**15**) in 91% yield. Oxidation of this material with Jones's reagent in acetone at 0 °C gave the 17-ketone compound (**16**) in 95% yield. Treating this material with triethylorthoformate, ethylene glycol and tosic acid in methylene chloride at room temperature overnight gave the 17-ketal (**17**) in 94% yield. Reaction of the 17-ketal (**17**) with phenyltrimethylammonium tribromide in THF at −5 °C afforded the 16ξ-bromo-17,17-ethylenedioxy compound (**18**) in 95% yield. Dehydrobromination by refluxing with potassium *t*-butoxide in xylene gave a mixture of the Δ^{14} and Δ^{15} compounds (**19** and **20**) in 49% total yield. The mixture of unsaturated compounds (**19** and **20**) was reacted with tosic acid in 6:1 acetone/water at room temperature to give a mixture of Δ^{14} and Δ^{15} ketone derivatives (**21** and **22**) as well as the pure Δ^{15} compound (**22**), with a total yield of 49%. The mixture

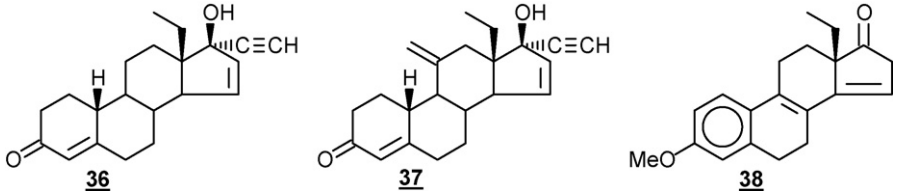


Fig. 5 – Compounds observed with 13-ethyl *gauche* g^+ conformation.

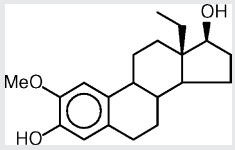
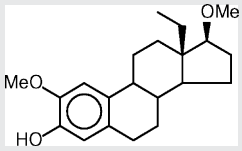
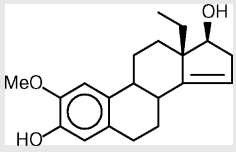
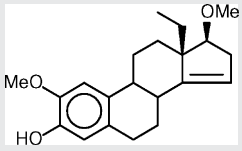
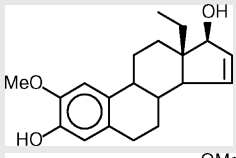
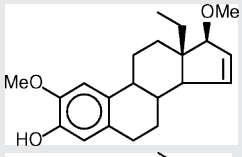
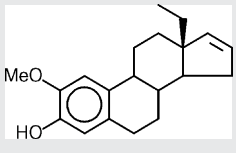
of isomers (**21** and **22**) was refluxed with isopropenyl acetate and tosic acid in acetic anhydride to give the pure $\Delta^{14,16}$-3,17-diacetate compound (**23**) in 46% yield. This material was then reduced with sodium borohydride in 1:1 methanol/water at 0 °C to give the Δ^{14}-3,17β-diol (**24**) in 69% yield.

Selective acetylation of compound (**24**) in isopropanol with acetic anhydride in the presence of 2 M NaOH to gave the 3-acetate material (**25**) in 77% yield. The 3-acetate material (**25**) was then methylated with trimethylsilyl diazomethane in the presence of aqueous fluoroboric acid according to the procedure of Aoyama and Shioiri [9] to give the 17β-methoxy compound (**26**) in 71% yield. Hydrolysis of this material with potassium carbonate in 3:1 methanol/water at room temperature gave the 3-hydroxy compound (**27**) in 75% yield.

Reduction of the Δ^{15} compound (**22**) with lithium aluminum hydride in ether at 0 °C gave the Δ^{15}-3,17β-diol (**28**) in 37% yield. This material was then selectively acetylated in isopropanol with acetic anhydride in the presence of 2 M NaOH to give the 3-acetate derivative (**29**) in 61% yield. Methylation of this material with trimethylsilyl diazomethane in methylene chloride in the presence of aqueous fluoroboric acid (48%) at 0 °C gave the 17β-methoxy compound (**30**) in 71% yield. Hydrolysis of this material with potassium carbonate in 3:1 methanol/water at room temperature gave the 3-hydroxy compound (**31**) in 76% yield.

The synthesis of the tetraene derivative (**33**) is presented in Fig. 4 and is based on the procedure of Berliner et al. [10]. Compound (**16**) was refluxed with *p*-toluenesulfonhydrazide in methanol in the presence of HCl for 16 h to give the 3-hydroxy-17-*p*-toluenesulfonylhydrazone derivative (**32**). This material was then treated with *n*-BuLi in THF at 0 °C for 72 h to give the Δ^{16} material (**33**) in 19% yield.

Table 2 – MM3 relative energies of *trans*- and *gauche* g^+ conformations of 18a-homo steroids

Compound no.	Structure	MM3 minimized conformations			
		Trans		*gauche* g^+	
		MM3 E_{Rel}	Dihedral[a]	MM3 E_{Rel}	Dihedral[a]
9		0.0	191.4	2.50	59.3
11		0.0	191.7	2.50	60.3
24		1.47	189.8	0.0	54.5
27		1.55	190.2	0.0	54.9
28		0.09	191.1	0.0	56.0
31		0.10	191.5	0.0	56.6
33		0.0	172.8	2.24	55.6

[a] Dihedral angle for the C14–C13–C18–C18a bond.

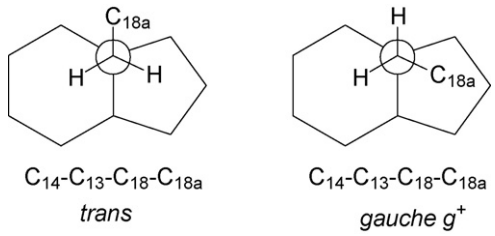


Fig. 6 – Observed conformations of 18a-homosteroids.

3.2. *Biological activity*

The compounds tested for biological activity are shown. 2-Methoxyestradiol (**34**) and the Δ-14 analog (**35**) are included here for comparison.

The IC_{50} values for inhibition of proliferation were generated in three cell types: human umbilical vein endothelial cells (HUVEC), human breast carcinoma cells (MDA-MB-231) and human gliomablastoma cells (U87-MG). The data are presented in Table 1. The IC_{50} value is the concentration at which cell proliferation is inhibited by 50%.

Replacement of the 13-methyl group of 2-methylestradiol with an ethyl group in compound (**9**) gives about equipotent biological activity (0.7- to 1.6-fold). Methylation of the 17-OH to give compound (**11**) results in a substantial increase in biological activity (3- to 5-fold more active). Introduction of the Δ^{14}-bond in compound (**24**) significantly lowers biological activity with the exception of the U87-MG cell line where it is about twice as active. Unlike compound (**9**), 17-*O*-methylation of (**24**) to give (**27**) further decreases biological activity in all cell lines tested. Introduction of the Δ^{15}-bond in compound (**28**) also leads to a considerable decrease in activity, but in this case, *O*-methylation to give compound (**31**) results in a 2- to 4-fold increase in activity compared to compound (**28**). However, compound (**31**) is still about half as active as either (**9**) or the parental 2ME2 (**34**). Finally, introduction of the Δ^{16}-bond with removal of the 17-OH group in (**33**) provides 2- to 6-fold less biological activity compared to 2ME2.

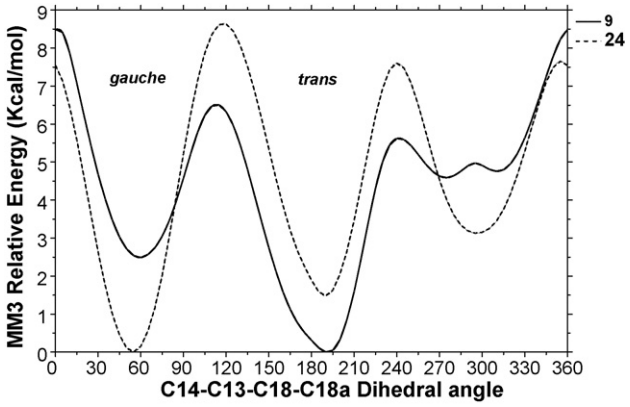


Fig. 7 – MM3 Energy profile for rotation of the 13-ethyl group of compounds 9 and 24.

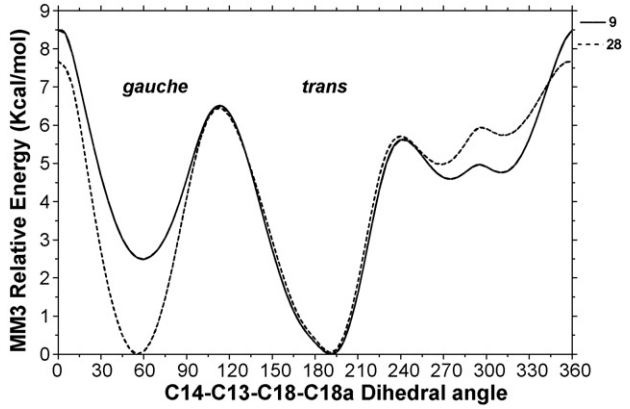


Fig. 8 – MM3 Energy profile for rotation of the 13-ethyl group of compounds 9 and 28.

4. Discussion

The decrease in biological activity for compound (**33**) could be explained by the absence of oxygen functionality at C-17, but the results for compounds (**24**), (**27**), (**28**) and (**31**) are somewhat puzzling in light of our earlier observation that introduction of unsaturation in ring-D leads to increased biological activity [4]. As can be seen from Table 1, introduction of a Δ^{14}-bond in 2ME2 (**34**) to give (**35**) gives a 4- to 37-fold increase in biological activity.

One possible explanation for these results could lie in the observation of crystal structural data reported for certain 18a-homosteroids containing unsaturation in ring-D. van Geerestein et al. [11,12] and Eckle et al. [13] have observed an unusual 18a-methyl orientation in the crystal structure of Gestodene (**36**) and 11-methylenegestodene (**37**). Normally, the observed orientation of the ethyl group in 18a-homosteroids with saturated D-rings is *trans* with respect to the C/D ring junction. However, crystal structures of compounds (**36**) and (**37**) were observed with the C14–C13–C18–C18a bond in the *gauche* g^+ configuration with the 18a-methyl group directly over the D-ring. A similar observation was made by Chekhlov et al. [14] for *rac*-3-methoxy-18a-homoestra-1,3,5(10),8,14-pentaen-17-one (**38**). These compounds along with an illustration of the conformational difference are shown in Figs. 5 and 6.

In order to investigate this possibility for compound (**24**) and (**28**), conformational energy profiles were calculated for these compounds along with the parental compound (**9**) using the MM3 forcefield [15] implemented in PC-Model [16]. The results are shown in Figs. 7 and 8.

While the expected *trans* conformation for compound (**9**) is favored by ~2.5 kcal/mol, these calculations indicate the *gauche* conformation is favored for the Δ^{14} compound (**24**) by about 1.5 kcal/mol and both conformations are about equally populated for the Δ^{15}-compound (**28**). Table 2 summarizes the results of similar calculations carried out for all the 18a-homo derivatives tested in this study.

Experimental evidence in support of these calculations can be seen in the chemical shift data for the C18a methyl group for the 18a-homosteroids in this investigation. Table 3

compares these values along with the data for the corresponding 13-methyl compounds (**34**), (**39**), and (**35**). It can be seen from these data that there is an increase in upfield shifts in the 18a-methyl for the sequences (**9**)–(**28**)–(**24**) and (**11**)–(**31**)–(**27**) which is opposite of the trend observed for the 13-methyl compounds (**34**)–(**39**)–(**35**). This could be explained by increased shielding of the methyl group by the double bond caused by an increased population of the *gauche* g^+ conformation as predicted by the calculations in Figs. 7 and 8 and Table 2.

The net effect of an increased population of the *gauche* g^+ conformations for compounds (**24**), (**27**), (**28**) and (**31**) would be a decrease in accessibility of the *beta*-face of the D-ring for interaction with tubulin. This could explain the decrease in biological activities observed for these compounds compared to (**34**) and (**35**). This hypothesis is in agreement with

Table 1 – IC_{50} values for inhibition of proliferation

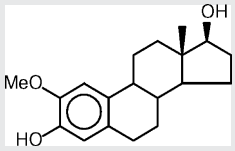
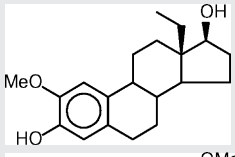
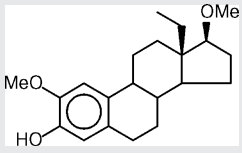
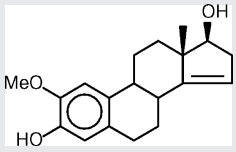
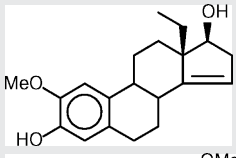
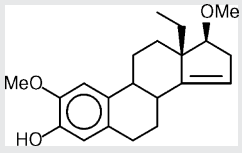
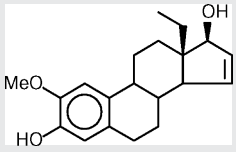
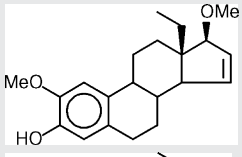
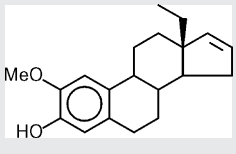
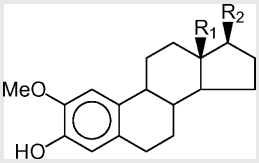
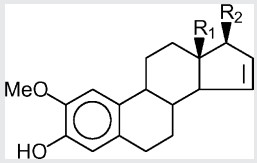
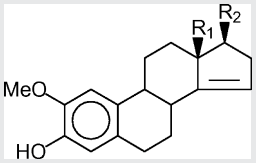
Compound no.	Structure	Inhibition of proliferation IC_{50} (μM) ± S.D.		
		HUVEC	MDA-MB-231	U87-MG
34		0.68 ± 0.15	0.69 ± 0.14	1.48 ± 0.62
9		0.51 ± 0.09	0.75 ± 0.17	0.92 ± 0.03
11		0.17 ± 0.07	0.17 ± 0.05	0.51 ± 0.23
35		0.03 ± 0.04	0.19 ± 0.13	0.04 ± 0.04
24		1.28 ± 0.64	1.11 ± 0.16	0.72 ± 0.01
27		2.76 ± 0.16	1.35 ± 0.62	2.13 ± 0.04
28		2.08 ± 1.86	5.14 ± 3.93	4.98 ± 1.16
31		0.97 ± 0.33	1.26 ± 0.64	2.39 ± 0.01
33		2.32 ± 0.30	3.60 ± 1.58	2.69 ± 0.01

Table 3 – 18-Methyl and 18a-methyl chemical shifts

Compound	18- or 18a-Methyl chemical shifts					
						
	Compound	Shift	Compound	Shift	Compound	Shift
R_1 = Me, R_2 = OH[a]	**34**	0.79	**39**	0.89	**35**	1.02
R_1 = Et, R_2 = OH	**9**	1.00	**28**	0.91	**24**	0.89
R_1 = Et, R_2 = OMe	**11**	0.97	**31**	0.87	**27**	0.85

[a] Data taken from Refs. [3] and [7].

the observation that for 16-substituted or 15,16-disubstituted 2ME2 analogs, the β-isomer is usually less active than the α-isomer [3,17].

Acknowledgements

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