Notes

2-(Hydroxyalkyl)estradiols: Synthesis and Biological Evaluation[1,2]

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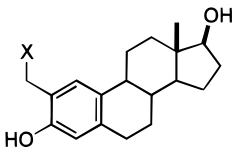
Synthetic estrogens possessing hydroxyalkyl side chains at the C-2 position of the A-ring were designed in order to further elucidate the structural and electronic requirements of the estrogen receptor to A-ring modifications. Furthermore, these compounds were envisaged as being stable analogs of the estradiol metabolite 2-hydroxyestradiol. The homologous series of 2-(hydroxyalkyl)estradiols **1**−**3** has been prepared by chain extension of 2-formylestradiol **6**, which, in turn, was prepared *via* ortholithiation of estradiol. The substituted estradiols **1**−**3** were assayed for their abilities to bind to the estrogen receptor in MCF-7 cells and induce estrogen-responsive gene expression. The estradiol homologs exhibited significantly weaker affinity than estradiol for the MCF-7 cell estrogen receptor, with relative binding affinities (estradiol = 100) ranging from 1.11 for 2-(hydroxymethyl)estradiol (**1**) to 0.073 for 2-(hydroxypropyl)estradiol (**3**). The relative activities for mRNA induction of the *pS2* gene by the estradiol homologs closely parallel the relative binding affinities for the estrogen receptor in MCF-7 cells. 2-(Hydroxymethyl)-estradiol (**1**) exhibited similar estrogen receptor affinity and *pS2* gene induction to the catechol estrogen 2-hydroxyestradiol and may prove useful in examination of the further biological effects of 2-hydroxyestrogen homologs.

Introduction

The structure−activity relationships of estrogens have received extensive investigations over the past 3 decades, with studies focusing on the evaluation of uterotrophic activity, estrogen receptor affinities, and stimulation of cell growth.[3] Estradiol is the most potent endogenous estrogen, and the estrogen metabolites estrone and estriol exhibit limited estrogenic activity compared to estradiol. Two other metabolites, 2-hydroxyestradiol and 4-hydroxyestradiol, also have significantly lower estrogenic activity than estradiol.[4] More recently, the ability of estrogenic substances to induce gene transcription at the mRNA level of estrogen-responsive sequences has been investigated.[5] The results of these studies have shed light on some of the structural and electronic requirements of the estrogen receptor and the mechanisms through which estrogens elicit a biological response.[3]

It has long been believed that only minor modifications in the A-ring can be made without losing significant binding affinity.[6] However, a recent report has shown that larger substituents can be tolerated and that electronic considerations may be important.[7] In addition, estrogen analogs containing the hydroxyl group at different positions of the A-ring (at C-2, C-3, or C-4) have demonstrated subtle differences in estrogen receptor affinity but significant differences in abilities to induce the expression of various estrogen-responsive genes.[8] In order to further the understanding of the structural and electronic requirements of the estrogen receptor to A-ring modifications, estradiols **1**−**3** possessing a hydroxyalkyl side chain were designed. Furthermore, these previously unprepared compounds were envisaged as being stable analogs of the estradiol metabolite 2-hydroxyestradiol.[1] Estradiol is metabolized *in vivo* by oxidation by a cytochrome P450 enzyme complex termed 2/4-hydroxylase. The catechol estradiols are then rapidly methylated by catechol *O*-methyltransferase, which renders the products much less estrogenic.



1: X=OH, **2**: X=CH_2OH, **3**: X=CH_2CH_2OH

Figure 1. Structure of 2-(hydroxyalkyl)estradiols.

The catechol estradiols have been implicated as a possible causitive agent in estrogen-induced tumorigenesis.[9] It has been postulated that the mechanism of carcinogenesis stems from the formation of further oxidized species, such as semiquinones or orthoquinones which form protein or possibly DNA adducts leading to changes in function.[10,11] A further postulate is that these quinone species undergo redox cycling producing hydroxyl radicals, leading to DNA damage.[12] We were interested in examining the potential of oxidative damage of the catechol estradiols in tumorigenesis; however, as these compounds are both chemically and biochemically short-lived, the use of them *in vitro* and *in vivo* is difficult. The synthetic compounds **1**−**3** (Figure 1) should not suffer from chemical instability and would not be susceptible to quinone/semiquinone formation.

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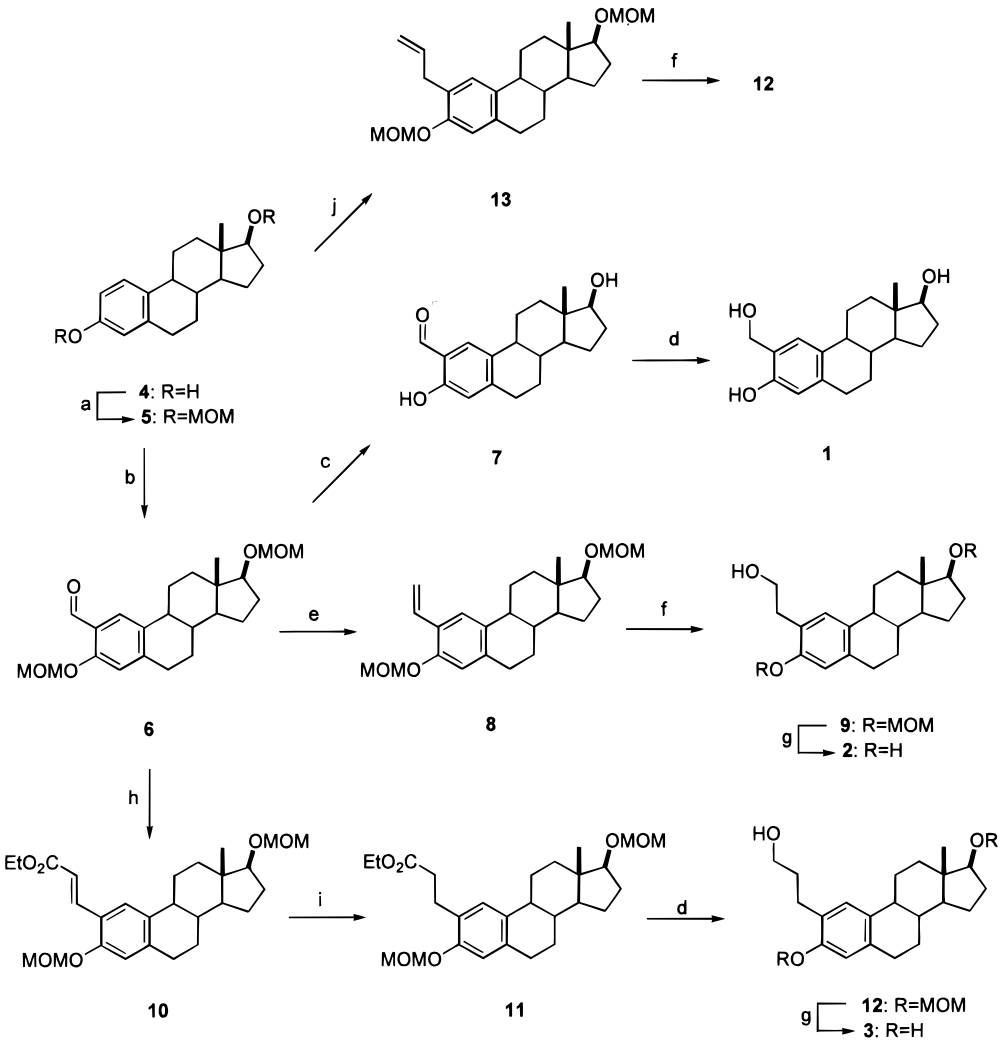
Scheme 1[a]



[a] Reagents and conditions: (a) MOMCl, *i*-Pr_2NEt, THF, Δ, 96%; (b) (i) *s*-BuLi, THF, −78 °C, (ii) DMF, −78 °C → room temperature, 86%; (c) HCl, THF, H_2O, room temperature, quantitative; (d) $LiAlH_4$, THF, 0 °C → room temperature, **7** → **1**, 71%, **11** → **12**, quantitative; (e) $Ph_3P{=}CH_2$, THF, room temperature, 90%; (f) (i) BH_3·THF, THF, 0 °C, (ii) NaOH, H_2O_2, Δ, **8** → **9**, 80%, **13** → **12**, 77%; (g) PPTS, MeOH, Δ, **9** → **2**, quantitative, **12** → **3**, 72%; (h) $EtO_2CCH_2P(O)(OEt)_2$, KHMDS, THF, RT, 81%; (i) $PhI(OAc)_2$, NH_2NH_2, CH_2Cl_2, room temperature, 93%; (j) (i) *s*-BuLi, THF, −78 °C, (ii) CuI, (iii) allyl bromide, 66%.

If redox cycling of catechol estrogens is important for tumor formation, then these homologs would be expected to have greatly diminished activity. On the other hand, compounds **1**−**3** do contain hydroxyl groups at positions 2 and 3 available for hydrogen bonding in protein interactions (receptors or enzymes) and may exhibit classical estrogenic activity. Therefore, these homologs may prove useful as chemical probes for differentiating receptor-mediated vs redox-mediated events which operate in estrogen-induced tumorigenesis. This report describes the synthesis of an initial series of 2-(hydroxyalkyl)estradiols **1**−**3**, their relative estrogen receptor affinities, and their ability to induce estrogen-responsive gene expression.

Results and Discussion

Chemistry. The synthesis of the catechol analogs began by converting estradiol **4** to the known bisMOM ether **5** in 96% yield by treatment with methoxymethyl chloride and diisopropylethylamine in THF at reflux.[13] The protected estradiol **5** was formylated in the 2-position according to the procedure of Pert and Ridley.[14] Thus, the 2-position was lithiated by treatment of **5** with *s*-BuLi at low temperature, and the resulting anion was quenched with dimethylformamide to give the 2-formylated estradiol **6** in 86% yield as a single regioisomer. For this reaction to be successful, it was essential to distill the dimethylformamide from calcium hydride just prior to use, otherwise the reaction would fail. It is noteworthy that the formylated product was obtained as a single regioisomer, as evidenced by one aldehyde proton signal observed in the ^1H NMR spectrum of the crude product. It had previously demonstrated that **6** could be deprotected with aqueous acid to give **7**.[14] After removal of the MOM ethers, **7** was reduced with lithium aluminum hydride to give the first estratriol **1** in 71% yield (Scheme 1).

It had originally been our intention to prepare the hydroxyethyl and hydroxypropyl analogs by lithiation of **5** and subsequent alkylation with ethylene oxide or allyl bromide, respectively. Several experiments were performed to probe the workability of this approach; however, they were uniformally unsuccessful. Although we were later able to prepare the propyl derivative in this way (*vide infra*), it was decided to synthesize the higher analogs *via* homologation of **6**.

Table 1. Estrogen Receptor Affinities of Estradiol Homologs

compd	EC_{50} (M)	RBA[a]
Estrogen Receptor Preparations		
estradiol	9.96×10^{-10}	100.00
1	1.46×10^{-7}	0.68
Whole Cell Estrogen Receptor Assays		
estradiol	3.80×10^{-9}	100.00
1	3.42×10^{-7}	1.11
2	4.89×10^{-6}	0.078
3	5.22×10^{-6}	0.073
7	7.82×10^{-7}	0.486
2-hydroxyestradiol	1.66×10^{-6}	0.229

[a] The relative binding affinity (RBA) for each synthetic homolog was determined using the following equation: RBA = (EC_{50} for estradiol)/(EC_{50} for homolog) × 100.

Although several homologation strategies were explored, simple Wittig methylenation of **6** with $Ph_3P{=}CH_2$ was the most successful, giving the vinylestradiol **8** in 90% yield (Scheme 1). Hydroboration of the double bond followed by oxidative workup gave the (hydroxyethyl)estradiol **9** in 80% yield. The triol **2** could then be readily obtained in good yield by exposure of **9** to PPTS in refluxing methanol.[15]

The hydroxypropyl compound **3** was obtained in a somewhat similar fashion. Thus, treatment of **6** with the anion generated from triethyl phosphonacetate and potassium hexamethyldisilazide gave the cinnamate **10** in 81% yield as a 20:1 mixture of *E*/*Z* isomers. Attempted reduction of the double bond with $NaBH_4$ or by catalytic hydrogenation was unsuccessful; however, use of diimide, generated *in situ* from hydrazine hydrate and iodobenzene diacetate, gave the dihydrocinnamate **11** in 93% yield.[16] The ester was reduced in quantitative yield with lithium aluminum hydride to the propyl alcohol **12**; removal of the MOM groups was then achieved as before by refluxing a methanol solution of **12** with PPTS.

After preparing the triol **3** by homologation of **6**, it was subsequently found that the anion generated by ortholithiation of **5** could be alkylated efficiently with allyl bromide provided certain modifications of the reaction conditions were carried out. Thus, if the anion generated from **5** and *s*-BuLi was transmetalated with copper iodide to form the cuprate, followed by treatment with allyl bromide, then the 2-allylated estradiol **13** could be obtained in 65% (89% based on recovered starting material) yield.[17] Hydroboration of **13** followed by oxidative workup gave **12** in 77% yield (Scheme 1).

Biological Evaluations. The affinities of the synthetic estradiol homologs for the estrogen receptor were assessed in isolated estrogen receptor preparations[6] and whole cell estrogen receptor binding assays using MCF-7 human mammary cancer cells.[18] The EC_{50} value for estradiol binding to the estrogen receptor preparations using 2.0 nM [^{3}H]estradiol was found to be 0.996 nM, while the affinity for compound **1** was 146 nM (Table 1). The EC_{50} value for estradiol binding to the estrogen receptor in these whole cell assays using 3.0 nM [^{3}H]-estradiol was found to be 3.80 nM. The estradiol homologs exhibited significantly weaker affinity for the estrogen receptor than estradiol, with relative binding affinities (RBA; estradiol = 100) ranging from 1.11 for compound **1** to 0.073 for compound **3** (Table 1, Figure 2).

Estradiol acts through the estrogen receptor to induce the transcription of a variety of hormone-responsive

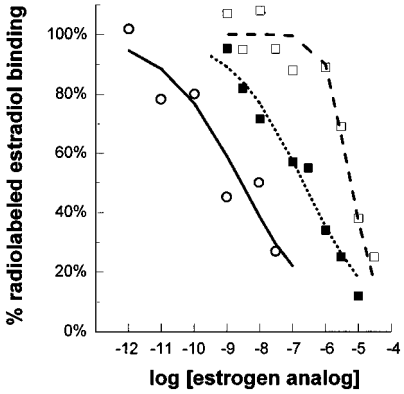


Figure 2. Estrogen receptor binding for estradiol (○), 2-(hydroxymethyl)estradiol (■), and 2-(hydroxyethyl)estradiol (□).

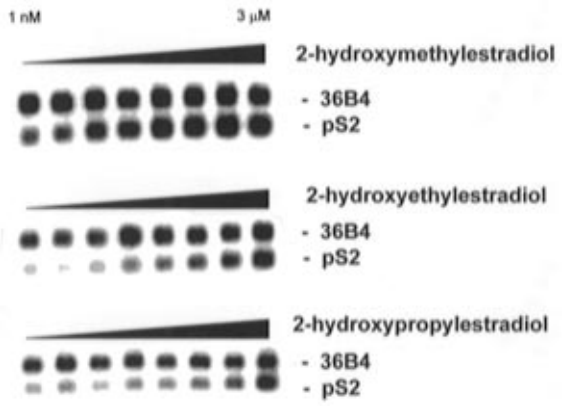


Figure 3. Northern analysis of mRNA levels for estrogen-responsive *pS2* gene and control *36B4* gene expressed by 2-(hydroxymethyl)estradiol, 2-(hydroxyethyl)estradiol, and 2-(hydroxypropyl)estradiol.

Table 2. Induction of *pS2* Gene Expression by Estradiol Homologs

compd	EC_{50} (M)	relative activity[a]
estradiol	0.996×10^{-10}	100.00
1	1.03×10^{-8}	0.967
2	2.72×10^{-7}	0.0367
3	4.95×10^{-7}	0.0201
2-hydroxyestradiol	5.04×10^{-7}	0.0198

[a] The relative activity for each synthetic homolog was determined using the following equation: relative activity = (EC_{50} for estradiol)/(EC_{50} for homolog) × 100.

genes. The induction of transcription of the *pS2* gene in human MCF-7 mammary carcinoma cells is a primary response to estrogen.[19] The induction of pS2 mRNA expression by estradiol and estrogen homologs was determined by Northern analysis (Figure 3). The EC_{50} value for estradiol induction of pS2 mRNA was found to be 0.292 nM. The estradiol homologs exhibited significantly weaker activity than estradiol for pS2 mRNA induction, with relative activities (estradiol = 100) ranging from 2.79 for compound **1** to 0.004 for compound **3** (Table 2, Figure 4).

Thus, the 2-(hydroxyalkyl)estradiols **1**−**3** exhibited significantly weaker affinity for the estrogen receptor than estradiol. These results are congruous with the established structure−activity relationships of estrogens and the limitations for A-ring substitutions. Furthermore, the relative activities for pS2 mRNA induction of the estradiol homologs closely parallel the relative

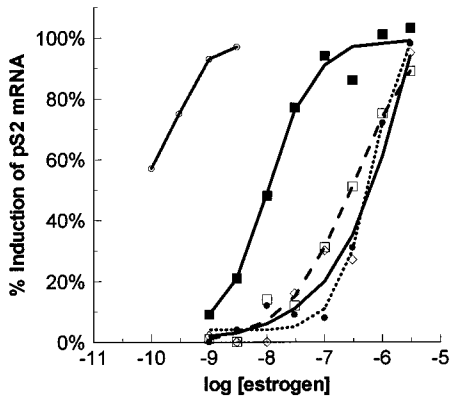


Figure 4. Induction of *pS2* gene expression by estradiol (○), 2-(hydroxymethyl)estradiol (■), 2-(hydroxyethyl)estradiol (□), 2-(hydroxypropyl)estradiol (◇), and 2-hydroxyestradiol (●).

binding affinities for the estrogen receptor in MCF-7 cells. Interestingly, 2-(hydroxymethyl)estradiol (**1**) exhibited similar estrogen receptor affinity and *pS2* gene induction to the catechol estrogen 2-hydroxyestradiol. This catechol estradiol has been implicated as a possible causitive agent in estrogen-induced tumorigenesis; however, *in vitro* and *in vivo* investigations with 2-hydroxyestradiol are difficult due to its chemical and biochemical instability. Thus, 2-(hydroxymethyl)estradiol (**1**) may be viewed as a chemically stable catechol estrogen homolog and may therefore prove useful in examination of the role of catechol estrogens in normal physiology and pathological states, such as estrogen-induced tumorigenesis.

Materials and Methods

Synthesis. General Information. Steroids were purchased from Steraloids (Wilton, NH); all other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received unless otherwise indicated. Anhydrous solvents were dried by standard procedures, and amines were stirred over CaH_2, distilled, and stored over KOH pellets. Silica gel TLC plates (60 F_{254}) were purchased from Analtech Inc. (Newark, NE) and visualized with a UV lamp and/or 5% ethanolic phosphomolybdic acid followed by charring. All intermediates were purified by flash column chromatography on silica gel (Merck Kieselgel 60) using the indicated mixtures of hexanes and ethyl acetate. Melting points were determined in open capillaries on a Thomas Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Laser Precision Analytical RFX-40 FTIR spectrometer in the phase indicated. ^1H and ^{13}C NMR were recorded on an IBM AF/250 spectrometer at 250 and 67.5 MHz, respectively, in $CDCl_3$ solutions unless otherwise indicated using the residual protiosolvent signal as internal reference. NMR data are reported in δ units. Mass spectra were obtained at The Ohio State University Chemical Instrumentation Center on either a VG 70-2505, a Nicolet FTMS-200, or a Finnigan MAT-900 mass spectrometer. Elemental Analyses were performed by Oneida Research Services, Inc. (Whitesboro, NY).

Estra-1,3,5(10)-triene-3,17β-diol 3,17β-Bis(methoxymethoxy) Ether (5). MOMCl (9.1 mL, 120 mmol) was added dropwise to a cold (0 °C) solution of estradiol (6.50 g, 23.9 mmol) and diisopropylethylamine (34 mL, 143 mmol) in THF (200 mL). On completion of the addition, the reaction mixture was allowed to warm up to room temperature, stirred for 1 h at the same temperature, and then heated at reflux overnight. The mixture was allowed to cool; then saturated NH_4Cl solution (100 mL) was added. After extraction with Et_2O (4 × 100 mL), the combined organics were washed with saturated brine (100 mL), dried ($MgSO_4$), and concentrated. The crude product was purified by chromatography (4:1) to afford 8.30 g (97%) of the title compound as a colorless oil: IR (neat, cm^{-1}) 2925−2787, 1610, 1576, 1498, 1471, 1446, 1403, 1248, 1205, 1151, 1134, 1116, 1105, 1076, 962, 922, 873; ^1H NMR 7.19 (1H, d, J = 8.5 Hz), 6.82 (1H, dd, J = 8.5, 2.7 Hz), 6.76 (1H, d, J = 3.0 Hz), 5.14 (2H, s), 4.65 (2H, ABq, J = 6.6 Hz, $\Delta\nu$ = 3.4 Hz), 3.61 (1H, t, J = 8.4 Hz), 3.46 (3H, s), 3.37 (3H, s), 2.87−2.81 (2H, m), 0.80 (3H, s); ^{13}C NMR 155.2, 138.1, 134.1, 126.3, 116.4, 113.8, 96.1, 94.6, 86.7, 55.8, 55.1, 50.2, 44.1, 43.1, 38.7, 37.4, 29.1, 28.1, 27.2, 26.3, 23.1, 11.7; MS m/z (M^+) calcd 360.2301, obsd 360.2306.

2-Formylestra-1,3,5(10)-triene-3,17β-diol 3,17β-Bis-(methoxymethoxy) Ether (6). *s*-BuLi (50 mL, 65.0 mmol) was added dropwise to a cold (−78 °C) solution of **5** (6.00 g, 16.7 mmol) in THF (100 mL). The resulting brown solution was stirred at −78 °C for 2 h, at which time freshly distilled DMF was added and the reaction mixture was allowed to warm up to room temperature overnight. The reaction mixture was cooled to 0 °C; then 10% HCl (40 mL) was added cautiously. After separation of the organic layer, the aqueous layer was extracted with Et_2O (4 × 100 mL). The combined organic extracts were washed with water (2 × 100 mL) and saturated brine (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (3:1) to afford 5.56 g (86%) of the desired product as a colorless oil: IR (neat, cm^{-1}) 2929−2871, 1684, 1608, 1491, 1471, 1446, 1423, 1392, 1261, 1209, 1151, 1105, 1054, 1025, 997, 919; ^1H NMR 10.41 (1H, s), 7.75 (1H, s), 6.90 (1H, s), 5.25 (2H, s), 4.65 (2H, ABq, J = 6.7 Hz, $\Delta\nu$ = 3.1 Hz), 3.60 (1H, t, J = 8.4 Hz), 3.50 (3H, s), 3.36 (3H, s), 2.91−2.87 (2H, m), 0.79 (3H, s); ^{13}C NMR 189.4, 157.5, 146.2, 134.6, 125.3, 123.5, 115.1, 96.0, 94.7, 86.5, 56.5, 55.1, 50.1, 43.7, 43.0, 38.4, 37.1, 30.3, 28.1, 26.8, 26.1, 23.0, 11.6; MS m/z (M^+) calcd 388.2250, obsd 388.2273.

2-Formylestra-1,3,5(10)-triene-3,17β-diol (7). A solution of **6** (2.00 g, 5.15 mmol), 6 M aqueous HCl (25 mL), and THF (25 mL) was stirred at room temperature for 3 h. The reaction mixture was poured into water and then extracted with EtOAc (4 × 50 mL). The combined organics were dried ($MgSO_4$), concentrated, and then chromatographed (1:3 increasing to 1:2) to afford the title compound (1.52 g, 98%) as a pale yellow, crystalline solid: mp 225−227 °C (lit.[14] mp 231−233 °C); IR (KBr, cm^{-1}) 3361, 3116, 3023, 2964, 2927, 2865, 1658, 1612, 1442, 1384, 1352, 1342, 1290, 1267, 1242, 1117, 1051, 1020, 870; ^1H NMR (DMSO) 10.41 (1H, brs), 10.12 (1H, s), 7.55 (1H, s), 6.66 (1H, s), 4.50 (1H, d, J = 4.7 Hz), 3.52 (1H, q, J = 8.2 Hz), 2.49 (2H, brm), 0.65 (3H, s); ^{13}C NMR (DMSO) 192.2, 158.2, 146.6, 131.9, 126.5, 120.0, 116.4, 79.9, 49.4, 42.9, 42.6, 38.1, 36.3, 29.8, 29.3, 26.3, 25.8, 22.6, 11.0; MS m/z (M^+) calcd 300.1725, obsd 300.1732.

2-(Hydroxymethyl)estra-1,3,5(10)-triene-3,17β-diol (1). $LiAlH_4$ (0.20 g, 5.20 mmol) was added portionwise to a solution of the aldehyde **7** (0.21 g, 0.70 mmol) in cold (0 °C) THF (10 mL). The resulting mixture was stirred for 2 h while warming to room temperature. The reaction was quenched by the addition of water (0.2 mL), 15% NaOH (0.2 mL), and water (0.6 mL). The granular precipitate was removed by filtration through a pad of Celite and $MgSO_4$ (1:1). After concentration, the residue was recrystallized from water and methanol to give the desired triol (150 mg, 71%) as a colorless solid: mp > 270 °C; IR (KBr, cm^{-1}) 3514, 3319, 3147, 2954, 2922, 2866, 1614, 1508, 1431, 1381, 1354, 1340, 1319, 1286, 1252, 1213, 1186, 1130, 1115, 1095, 1070, 1052, 1034, 1016, 972, 866; ^1H NMR (DMSO) 8.92 (1H, s), 7.15 (1H, s), 6.41 (1H, s), 4.80 (1H, t, J = 5.6 Hz), 4.49 (1H, d, J = 4.8 Hz), 4.40 (2H, d, J = 5.5 Hz), 2.48−2.66 (2H, m), 0.65 (3H, s); ^{13}C NMR (DMSO) 151.8, 135.0, 130.0, 125.4, 124.4, 114.4, 80.0, 58.4, 49.5, 43.5, 42.7, 38.5, 36.5, 29.8, 28.7, 26.9, 26.1, 22.7, 11.1; MS m/z (M^+) calcd 302.1875, obsd 302.1882. Anal. Calcd for $C_{19}H_{26}O_3 \cdot 0.25H_2O$: C, 74.36; H, 8.70. Found: C, 74.25; H, 8.46.

2-Ethenylestra-1,3,5(10)-triene-3,17β-diol 3,17β-Bis-(methoxymethoxy) Ether (8). A solution of KHMDS (0.64 g, 3.22 mmol) in THF (10 mL) was added by cannula to a suspension of methyltriphenylphosphonium iodide (1.30 g, 3.22 mmol) in THF (10 mL) at room temperature followed by stirring for 1 h at the same temperature. **6** (0.50 g, 1.29 mmol) was added to the ylide solution and then stirred for an additional 2 h at room temperature. Saturated NH_4Cl (50 mL) was added; then the aqueous solution was extracted with

EtOAc (3 × 100 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (4:1) to afford 0.48 g (96%) of **8** as a colorless oil: IR (neat, cm^{-1}) 2927, 1496, 1149, 1105, 1065, 1043, 1022, 999, 918; ^1H NMR 7.40 (1H, s), 7.01 (1H, dd, J = 11.2, 17.8 Hz), 6.80 (1H, s), 5.68 (1H, dd, J = 1.6, 17.7 Hz), 5.20 (1H, dd, J = 1.6, 11.2 Hz), 5.17 (2H, s), 4.66 (2H, ABq, J = 6.6 Hz, $\Delta\nu$ = 3.5 Hz), 3.61 (1H, t, J = 8.4 Hz), 3.45 (3H, s), 3.37 (3H, s), 2.85−2.81 (2H, m), 0.80 (3H, s); ^{13}C NMR 152.4, 137.8, 134.0, 132.0, 125.1, 123.5, 115.2, 113.5, 96.1, 95.0, 86.7, 56.1, 55.1, 50.2, 44.1, 43.1, 38.7, 29.7, 28.2, 27.3, 26.3, 23.1, 11.7; MS m/z (M^+) calcd 386.2457, obsd 386.2462. Anal. Calcd for $C_{24}H_{34}O_4$: C, 74.58; H, 8.87. Found: C, 74.64; H, 8.89.

2-(2′-Hydroxyethyl)estra-1,3,5(10)-triene-3,17β-diol 3,-17β-Bis(methoxymethoxy) Ether (9). BH_3·THF complex in THF (5.0 mL, 5.00 mmol) was added dropwise to a solution of **8** (0.48 g, 1.24 mol) in THF (10 mL) at 0 °C. The cooling bath was removed, and stirring was continued for 1 h; 1 N NaOH (10 mL) was cautiously added followed by 30% H_2O_2 (10 mL). The resulting mixture was heated to a gentle reflux for 1 h. After cooling, the reaction mixture was extracted with EtOAc (3 × 50 mL), washed with brine (2 × 50 mL), dried ($MgSO_4$), and concentrated. The residue was chromatographed (1:1) to give 0.40 g (80%) of **9** as a colorless oil: IR (neat, cm^{-1}) 3344, 2931, 1502, 1471, 1444, 1417, 1400, 1382, 1382, 1369, 1356, 1340, 1317, 1284, 1259, 1207, 1182, 1149, 1117, 1105, 1061, 920; ^1H NMR 7.08 (1H, s), 6.80 (1H, s), 5.16 (2H, s), 4.65 (2H, s), 3.87−3.79 (3H, m), 3.60 (1H, t, J = 8.4 Hz), 3.46 (3H, s), 3.37 (3H, s), 2.89−2.79 (4H, m), 0.79 (3H, s); ^{13}C NMR 153.3, 136.4, 133.9, 128.0, 124.8, 114.4, 96.1, 94.7, 86.7, 63.1, 56.1, 55.1, 50.1, 44.0, 43.1, 38.7, 37.4, 34.1, 29.7, 28.2, 27.3, 26.4, 23.1, 11.8; MS m/z (M^+) calcd 404.2563, obsd 404.2563. Anal. Calcd for $C_{24}H_{36}O_5$: C, 71.26; H, 8.97. Found: C, 70.93; H, 8.87.

2-(2′-Hydroxyethyl)estra-1,3,5(10)-triene-3,17β-diol (2). A solution containing **9** (120 mg, 0.30 mmol) and pyridinium *p*-toluenesulfonate (0.34 g, 1.35 mmol) in methanol (5 mL) was heated at reflux overnight. After cooling to room temperature, the reaction mixture was poured into water (50 mL) which was then extracted with EtOAc (3 × 50 mL). The combined organics were washed with brine (50 mL), dried ($MgSO_4$), and concentrated. The residue was recrystallized from methanol and water to give 94 mg (quantitative) of a colorless crystalline solid: mp 201−202 °C; IR (KBr, cm^{-1}) 3400, 2919, 2870, 2856, 2840, 1512, 1469, 1437, 1421, 1383, 1354, 1340, 1282, 1267, 1253, 1213, 1209, 1186, 1117, 1087, 1053, 1009, 989; ^1H NMR ($CDCl_3$/CD_3OD) 7.00 (1H, s), 6.51 (1H, s), 3.77 (2H, t, J = 6.8 Hz), 3.67 (1H, t, J = 8.5 Hz), 2.84−2.67 (2H, m), 2.82 (2H, t, J = 7.1 Hz), 0.78 (3H, s); ^{13}C NMR 153.7, 136.7, 132.5, 128.5, 123.9, 116.3, 82.2, 63.6, 51.0, 44.9, 44.0, 40.0, 37.7, 35.0, 30.0, 28.2, 27.3, 23.8, 11.5; MS m/z (M^+) calcd 330.2195, obsd 330.2194. Anal. Calcd for $C_{20}H_{28}O_3$·$0.25H_2O$: C, 74.85; H, 8.94. Found: C, 74.79; H, 8.94.

Ethyl 3,17β-Bis(methoxymethoxy)estra-1,3,5(10)-triene-2-propenoate (10). Triethyl phosphonacetate (0.52 mL, 3.34 mmol) was added dropwise to a solution of KHMDS (0.67 g, 3.34 mmol) in THF (10 mL) at room temperature and stirred for 1 h. The resulting solution was added by cannula to a solution of **6** (0.65 g, 1.68 mmol) in THF (10 mL) followed by 4 h of stirring at room temperature. The reaction was quenched with water (50 mL), and then the aqueous mixture was extracted with EtOAc (4 × 50 mL). The combined organic solutions were dried ($MgSO_4$), concentrated, and chromatographed (4:1) giving 0.62 g (81%) of **10** as a colorless oil (20:1, E/Z): IR (neat, cm^{-1}) 2873, 2931, 1739, 1712, 1629, 1608, 1496, 1467, 1446, 1421, 1398, 1367, 1315, 1278, 1252, 1213, 1151, 1117, 1105, 1047, 989, 920, 867; ^1H NMR 7.98 (1H, d, J = 16.2 Hz), 7.44 (1H, s), 6.85 (1H, s), 6.46 (1H, d, J = 16.2 Hz), 5.21 (2H, s), 4.65 (2H, ABq, J = 6.6 Hz, $\Delta\nu$ = 3.4 Hz), 4.24 (2H, q, J = 7.1 Hz), 3.61 (1H, t, J = 8.3 Hz), 3.48 (3H, s), 3.38 (3H, s), 2.87−2.82 (2H, m), 1.32 (3H, t, J = 7.1 Hz), 0.80 (3H, s); ^{13}C NMR 167.5, 154.0, 141.1, 140.3, 134.2, 125.6, 121.8, 117.8, 115.1, 96.1, 94.7, 86.6, 60.2, 56.2, 55.1, 50.1, 43.8, 43.0, 38.5, 37.2, 29.9, 28.1, 27.1, 26.3, 23.1, 14.4, 11.7; MS m/z (M^+) calcd 458.2668, obsd 458.2671. Anal. Calcd for $C_{27}H_{38}O_6$: C, 70.72; H, 8.35. Found: C, 70.81; H, 8.34.

Ethyl 3,17β-Bis(methoxymethoxy)estra-1,3,5(10)triene-2-propionoate (11). Iodobenzene diacetate (0.57 g, 1.77 mmol) in CH_2Cl_2 (15 mL) was added dropwise to a stirred solution of **10** (0.54 g, 1.18 mmol) and hydrazine hydrate (0.24 g, 4.72 mmol) in CH_2Cl_2 (5 mL) at room temperature. The reaction mixture was stirred overnight; then the same amount of reagents was added neat and followed by overnight stirring. This procedure was repeated until the reaction was complete (TLC), requiring a total of 4 days. Aqueous $NaHCO_3$ solution (50 mL) was added to the reaction mixture, which was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried ($MgSO_4$), concentrated, and chromatographed (6:1) to yield 0.50 g (93%) of **13** as a colorless oil: IR (neat, cm^{-1}) 2929−2825, 1736, 1502, 1468, 1446, 1371, 1286, 1252, 1178, 1105; ^1H NMR 7.07 (1H, s), 6.78 (1H, s), 5.16 (2H, s), 4.65 (2H, s), 4.12 (2H, q, J = 7.1 Hz), 3.60 (1H, t, J = 8.4 Hz), 3.47 (3H, s), 3.36 (3H, s), 2.91 (2H, t, J = 8.0 Hz), 2.81−2.78 (2H, m), 2.58 (2H, t, J = 8.0 Hz), 1.24 (3H, t, J = 7.1 Hz). 0.79 (3H, s); ^{13}C NMR 173.3, 153.1, 136.0, 133.6, 127.0, 126.8, 114.2, 96.1, 94.5, 86.7, 60.2, 56.0, 55.1, 50.2, 44.1, 43.1, 38.7, 37.4, 34.8, 29.6, 28.2, 27.3, 26.4, 26.1, 23.1, 14.2, 11.2; MS m/z (M^+) calcd 460.2825, obsd 460.2822. Anal. Calcd for $C_{27}H_{40}O_6$: C, 70.41; H, 8.50. Found: C, 70.41; H, 8.75.

2-(3′-Hydroxypropyl)estra-1,3,5(10)-triene-3,17β-diol 3,-17β-Bis(methoxymethoxy) Ether (12). 11 (0.48 g, 1.04 mmol) in THF (25 mL) was added dropwise to a cold (0 °C) suspension of $LiAlH_4$ (198 mg, 5.20 mmol). On completion of the addition, the mixture was allowed to warm to room temperature and then stirred for 9 h. The reaction was quenched by the addition of water (0.2 mL), 15% NaOH (0.2 mL), and water (0.6 mL), and the resulting granular precipitate was removed by filtration through a 1:1 mixture of Celite and $MgSO_4$. The filtrate was concentrated and purified by chromatography (1:1) to give 0.43 g (quantitative) of the alcohol as a colorless oil: IR (neat, cm^{-1}) 3855−3435, 3028−2927, 1614, 1576, 1502, 1473, 1448, 1417, 1400, 1382, 1355, 1149, 1117, 1105, 920; ^1H NMR 7.25 (1H, s), 7.07 (1H, s), 5.18 (2H, s), 4.65 (2H, s), 3.85−3.50 (3H, m), 3.48 (3H, s), 3.37 (3H, s), 2.83−2.81 (2H, m), 2.69 (2H, t, J = 7.5 Hz), 1.84 (2H, m), 0.80 (3H, s); ^{13}C NMR 153.1, 135.7, 133.8, 127.9, 127.2, 114.4, 96.1, 94.9, 86.8, 62.2, 56.1, 55.1, 50.2, 44.1, 43.1, 38.7, 37.4, 33.4, 29.6, 28.2, 27.3, 26.4, 26.2, 23.1, 11.8; MS m/z (M^+) calcd 418.2719, obsd 418.2717. Anal. Calcd for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.46; H, 8.88.

2-(3′-Hydroxypropyl)estra-1,3,5(10)-triene-3,17β-diol (3). A solution containing **12** (0.56 g, 1.33 mmol) and pyridium *p*-toluenesulfonate (2.96 g, 13.30 mmol) in methanol (25 mL) was heated at reflux overnight. After cooling, water (50 mL) and EtOAc (50 mL) were added. The separated aqueous layer was extracted with EtOAc (2 × 50 mL); the combined organics were washed with brine (50 mL), dried ($MgSO_4$), and concentrated. The crude product was chromatographed (1:1) to give the desired product, which was recrystallized from methanol and water to afford 315 mg (72%) of **3** as a colorless crystalline solid: mp 203−204 °C; IR (KBr, cm^{-1}) 3411, 2924, 2866, 1509, 1471, 1423, 1371, 1284, 1263, 1205, 1055, 1010; ^1H NMR ($CDCl_3$/CD_3OD) 7.00 (1H, s), 6.49 (1H, s), 3.67 (1H, t, J = 8.5 Hz), 3.59 (2H, t, J = 6.5 Hz), 2.80−2.75 (2H, m), 2.63 (2H, t, J = 7.5 Hz), 1.82 (2H, t, J = 7.3 Hz), 0.78 (3H, s); ^{13}C NMR ($CDCl_3$/CD_3OD) 153.3, 136.0, 132.5, 127.7, 126.3, 115.9, 82.2, 62.2, 51.0, 44.9, 44.0, 40.0, 37.7, 33.7, 30.5, 30.0, 28.2, 27.3, 26.8, 23.7, 11.5; MS m/z (M^+) calcd 330.2195, obsd 330.2193. Anal. Calcd for $C_{21}H_{30}O_3$·$0.25H_2O$: C, 75.30; H, 9.17. Found: C, 75.71; H, 9.12.

2-(2′-Propenyl)estra-1,3,5(10)-triene-3,17β-diol 3,17β-Bis(methoxymethoxy) Ether (13). *s*-BuLi (1.3 M, 9.7 mL, 12.6 mmol) was added dropwise to a cold (−78 °C) solution of **5** (0.97 g, 2.69 mmol) in THF (30 mL). After stirring for 2 h at −78 °C, the reaction mixture was transferred by cannula to a suspension of flame-dried CuI (2.81 g, 14.7 mmol) in THF (5 mL) and maintained at −78 °C for 1 h. Allyl bromide (2.4 mL, 15.4 mmol) was added, and then the reaction mixture was allowed to warm to room temperature overnight. Any precipitated salts were removed by filtration through Celite, and

NH_4Cl (50 mL) was added to the filtrate. The organic layer was separated, and then the aqueous solutions were extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with brine (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (4:1) to afford 696 mg (65%) of the desired alkene as a colorless oil and 260 mg (27%) of unreacted starting material: IR (neat, cm^{-1}) 3050−2775, 1620, 1600, 1500, 1175, 1150, 1100, 1050, 975; ^1H NMR 7.07 (1H, s), 6.79 (1H, s), 6.04−5.91 (1H, m), 5.16 (2H, s), 5.10−4.99 (2H, m), 4.66 (2H, s), 3.62 (1H, t, J = 8.3 Hz), 3.48 (3H, s), 3.38 (3H, s), 3.46−3.36 (2H, m), 2.86−2.87 (2H, m), 0.81 (3H, s); ^{13}C NMR 152.9, 137.4, 135.7, 133.7, 127.0, 126.6, 115.0, 114.5, 96.1, 94.7, 86.8, 55.9, 55.1, 50.2, 44.2, 43.1, 38.8, 37.4, 34.4, 29.6, 28.2, 27.3, 26.4, 23.1, 11.8; MS m/z (M^+) calcd 400.2618, obsd 400.2616.

2-(3′-Hydroxypropyl)estra-1,3,5(10)-triene-3,17β-diol 3,-17β-Bis(methoxymethoxy) Ether (12). BH_3·THF (5.2 mL, 5.2 mmol) was added dropwise to a solution of the alkene **13** (560 mg, 1.40 mmol) in THF (10 mL) at 0 °C. On completion of the addition, the mixture was allowed to warm to room temperature and stirred for 1 h. NaOH (1 M, 10 mL) was added cautiously followed by 30% H_2O_2 (10 mL), and the resulting mixture was heated to a gentle reflux for 1 h. After cooling, the reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic solutions were washed with brine (100 mL), dried ($MgSO_4$), and concentrated. The residue was purified by chromatography (4:1) and then MPLC (3:1) to give the desired alcohol (445 mg, 76%) as a colorless oil.

Biological Evaluations. General Information. [2,4,6,7-^{3}H]Estradiol (98.4 Ci/mmol; ^3H-E_2) was purchased from DuPont/NEN (Boston, MA) and used as received. MCF-7 human breast adenocarcinoma cells were obtained from ATCC, and cells were incubated in a humidified CO_2 incubator (Forma model 3052) with 5% CO_2 atmosphere. A modified Eagle's minimum essential medium (MEM) supplemented with essential amino acids (1.5×), vitamins (1.5×), nonessential amino acids (2×), and *l*-glutamine (1×) was obtained from Gibco BRL (Long Island, NY) and used for maintaining the cells. The sterilized liquid medium was prepared by The OSU Comprehensive Cancer Center by dissolving the powder in water containing sodium chloride (0.487 g/L), pyruvic acid (0.11 g/L), and sodium bicarbonate (1.5 g/L), and the pH was adjusted to 6.8. Fetal calf serum was obtained from Gibco BRL. Steroids were removed from the fetal calf serum by two treatments with dextran-coated charcoal. Tissue culture flasks and supplies were obtained from Corning Glass Works (Corning, NY). Biochemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Radioactive samples were detected with a Beckman LS 6800 scintillation counter using Formula 963 (DuPont/NEN) as the counting solution. Probes for Northern analysis (pS2:ATCC 57137, 36B4:ATCC 65917) were obtained as purified plasmids from the American Type Culture Collection and amplified by PCR for use in hybridization. Primers used were synthesized by OLIGOS, ETC (Wilsonville, OR) and were for pS2:sense, 5′-ATC CCT GAC TCG GGG TCG CCT TTG-3′; antisense, 5′-CAA TCT GTG TTG TGA GCC GAG GCA CAG-3′; for 36B4:sense, 5′-AAA CTG CTG CCT CAT ATC CG-3′; antisense, 5′-TTT CAG CAA GTG GGA AGG TG-3′.

Probes were labeled by random priming with Klenow fragment. Analysis of the Northern blots was carried out with an Ambis 100 instrument to obtain values for the cpm/mm^2. Permanent visual records were made by exposing the membranes to Kodak XOMAT film at −80 °C for 1−4 days.

Estrogen Receptor Preparations.[6] MCF-7 mammary cells were grown in 75 cm^2 plastic flasks at 37 °C in a modified Eagle's MEM culture medium containing 10% fetal calf serum and gentamycin (20 mg/L). Estrogen receptors were isolated from homogenized cells by differential centrifugation, and binding affinity was determined by competitive ligand binding assays using 2 nM [^{3}H]estradiol, as previously described by Brueggemeier *et al.*[6]

Whole Cell Estrogen Receptor Studies.[18] MCF-7 cells were maintained in a similar fashion as described above. Cells from 90−100% confluent cultures were harvested by treatment with 0.01% trypsin solution, and the washed cell pellet was divided into 9.4 cm^2 wells on a six-well plate at 1.5−2 × 10^5 cells/well in modified MEM (2−3 mL) containing 10% steroid free fetal calf serum and gentamycin (20 mg/mL). After 12−24 h at 37 °C, the culture media were replaced, and the culture was continued for a further 48 h. The media were removed, and then serum free MEM media (888 μL) containing insulin (5.0 mg/L), transferrin (5.0 mg/L), glutamine (2 mM), albumin (2.0 mg/mL), and the synthetic estrogens **1**−**3** at various concentrations ((3−1) × 10^{-5} M, 100 μL) were added and incubated for 10 min at 37 °C. To determine total binding, [^{3}H]estradiol (3.0 nM, 1.0 μCi) was added and the plates were then incubated for 1 h at 37 °C. The cells were washed twice with PBS at 4 °C; then 95% ethanol (1 mL) was added followed by standing for 30 min at room temperature. An aliquot (500 μL) of the ethanol solution was added to Formula 963 and counted on a liquid scintillation counter. The blank samples with no cells and the nonspecific binding samples, containing 6 μM unlabeled estradiol, were performed in a comparable manner. Specific binding of [^{3}H]estradiol was calculated by subtracting the nonspecific binding data from total binding data. The EC_{50} value for each synthetic estrogen homolog represents the concentration of homolog to produce a half-maximal displacement of specific [^{3}H]estradiol binding and was calculated by a nonlinear regression analysis using the Marquardt method (SAS Institute, Cary, NC).

pS2 Induction. MCF-7 cells were maintained in a similar fashion as described above. Cells were placed on defined media for 3 days. Twelve hours prior to RNA isolation, the cells were treated with **1**−**3** (10^{-3}−10^{-6}M), estradiol, or carrier (95% ethanol). The final concentration of ethanol was 0.1% in all flasks. Total RNA was isolated by an adaptation of the method of Chomczynski and Sacchi.[20] The cells were lysed with a 4 M guanidine isothiocyanate solution and transferred to Phase-Lock Gel tubes (5Prime-3Prime, Inc.). After acidification with 3 M NaOAc, pH 5.2 (1:10 vol), RNA was extracted with phenol:chloroform:isoamyl alcohol (60:24:1), phenol:chloroform:isoamyl alcohol (25:24:1), and then chloroform:isoamyl alcohol (24:1). RNA was collected by ethanol precipitation. The pellets were washed with 70% ethanol (3×) and then resuspended in RNase−DNase free water for spectral analysis. Aliquots of RNA equal to 10 μg were loaded onto a 1.5% agarose: 0.66 M formaldehyde gel and electrophoresed for 2 h. Northern transfer was accomplished by downward capillary action under neutral conditions using a turboblotter (Schleicher and Scheull, Keene, NH). Nylon membranes were prehybridized at 42 °C for 1 h in a buffer of 50% formamide, 2.5× SSPE, 2.5× Denhardt's solution, and 2% SDS. Membranes were hybridized simultaneously with pS2 and 36B4 probes (10^6 cpm/mL each; specific activity of *ca.* 1 × 10^9 cpm/μg each) for 12−20 h in a similar solution lacking SDS. The membranes were washed in 5× SSPE, 15′, 55 °C; 2× SSPE, 15′, 60 °C; and 0.1× SSPE, 20′, 65 °C; all wash solutions contained 1% SDS. The extent of pS2 induction was normalized to the 36B4 signal in each lane and corrected for baseline pS2 expression on an Ambis scanning proportional counter. The EC_{50} value for each synthetic estrogen homolog represents the concentration of homolog to produce a half-maximal induction of pS2 and was calculated by a nonlinear regression analysis using the Marquardt method (SAS Institute, Cary, NC).

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(1) A preliminary communication has appeared on the synthetic aspects of this work; see: Lovely, C. J.; Brueggemeier, R. W. Synthesis of 2-substituted hydroxyalkyl and aminoalkylestradiols. *Tetrahedron Lett.* **1994**, *35*, 8735−8738.

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Gilbert, N. E.; Liberto, M. M.; Burak, W. E. Synthesis of Catechol Estrogen Analogs and Evaluation in Estrogen-Responsive Human Breast Cells. Presented at the IXth International Congress on Hormonal Steroids, Sept. 24−29, 1994, Dallas, TX.

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