Effects of Altering the Electronics of 2-Methoxyestradiol on Cell Proliferation, on Cytotoxicity in Human Cancer Cell Cultures, and on Tubulin Polymerization

Allison B. Edsall,[†] Arasambattu K. Mohanakrishnan,[†] Donglai Yang,[†] Philip E. Fanwick,[‡] Ernest Hamel,[§] Arthur D. Hanson,[||] Gregory E. Agoston,[||] and Mark Cushman*,[†]

Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy and Pharmacal Sciences, and Department of Chemistry, Purdue University, West Lafayette, Indiana 47907, Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute at Frederick, National Institutes of Health, Frederick, Maryland 21702, and EntreMed, Inc., Rockville, Maryland 20850

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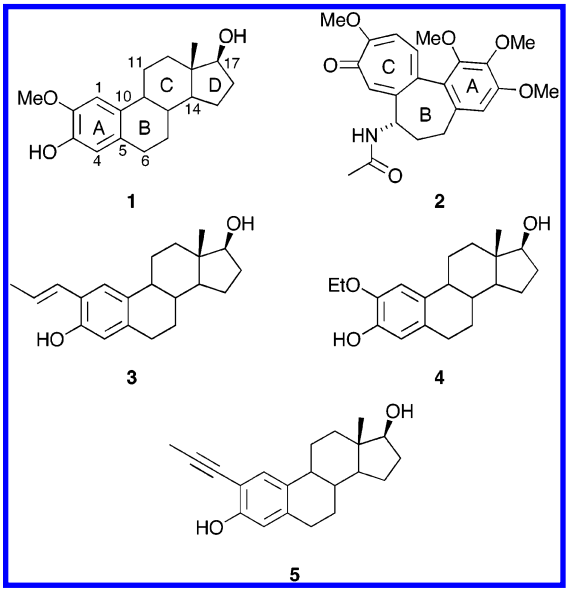
A series of new analogues of 2-methoxyestradiol (**1**) were synthesized to further elucidate the relationships between structure and activity. The compounds were designed to diminish the potential for metabolic deactivation at positions 2 and 17 and were analyzed as inhibitors of tubulin polymerization and for cytotoxicity. 17α-Methyl-*β*-estradiol (**30**), 2-propynyl-17α-methylestradiol (**39**), 2-ethoxy-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**50**) and 2-ethoxy-17α-methylestradiol (**51**) showed similar or greater tubulin polymerization inhibition than 2-methoxyestradiol (**1**) and contained moieties that are expected to inhibit deactivating metabolic processes. All of the compounds tested were cytotoxic in the panel of 55 human cancer cell cultures, and generally, the derivatives that displayed the most activity against tubulin were also the most cytotoxic.

Introduction

The endogenous estrogen metabolite 2-methoxyestradiol (**1**, 2ME2), formed by hepatic cytochrome P450 2-hydroxylation of *β*-estradiol and 2-O-methylation via catechol *O*-methyltranseferase, has generated significant interest as a potential anticancer agent resulting from its potent inhibition of tumor vasculature and tumor cell growth.[1−4] Many preclinical studies have demonstrated that solid tumor growth is dependent upon angiogenesis, the growth of new blood vessels; therefore, the significant antiangiogenic activity (IC_{50} 100 nM) and tubulin polymerization inhibition of 2ME2 in vivo are of considerable therapeutic value and have warranted further investigation in clinical trials.[4−9]

Numerous studies have elucidated possible mechanisms responsible for the cytotoxic effects of 2ME2. Cytotoxicity has been associated with uneven chromosome distribution, inhibition of mitosis, and a quantitative increase in abnormal metaphases.[5,6] Competitive binding studies with [^{3}H]colchicine indicated that the inhibitory effect of 2ME2 on tubulin polymerization was mediated through the colchicine binding site on tubulin.[10,11] Although the antiangiogenic mechanism of action of 2ME2 has not been thoroughly elucidated, disruption of microtubule polymerization resulted in the inhibition of hypoxia-inducible factor-1 (HIF) at the posttranscriptional level.[12] This dysregulation of HIF downstream of the 2ME2/tubulin interaction inhibited the transcriptional activation of HIF response genes including vascular endothelial growth factor (VEGF), a major component of angiogenesis.[12]



Various metabolic processes, similar to those that metabolize *β*-estradiol and other steroid hormones in vivo, are believed to generate inactive metabolites of 2ME2.[13,14] Oxidation of the position 17 alcohol of estradiol to estrone via 17-*β*-hydroxysteroid dehydrogenase and conjugation of the position 3 and 17 hydroxyl moieties to form a sulfate or glucuronide are two main routes for metabolic deactivation and steroid clearance.[13] Oxidation of the 17-hydroxyl moiety is believed to decrease the potency of 2ME2 in vivo since 2-methoxyestrone displayed 1% of the growth inhibitory potency of 2ME2 in endothelial cells.[4,15] Demethylation of the 2-methyl ether could also be detrimental to the activity of 2ME2 because the resulting 2,3-catechol may oxidize to a quinone, a species that has been shown to form DNA adducts.[16,17] More useful analogues of 2ME2

* To whom correspondence should be addressed. Tel. (765) 494-1465. Fax (765) 494-6790. E-mail: cushman@pharmacy.purdue.edu.
† Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University.
‡ Department of Chemistry, Purdue University.
§ Screening Technologies Branch, NIH.
|| EntreMed, Inc.

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should retain its inhibitory effects on tubulin polymerization and cell growth while addressing these metabolic issues.

Previous investigations have elucidated structural similarities between colchicine and 2ME2 that contribute to their ability to inhibit tubulin polymerization.[10,18,19] Most importantly, the A-ring of 2ME2 probably corresponds to the C-ring of colchicine (**2**), and the C- and D-rings of 2ME2 are functionally equivalent to the A-ring of colchicine. Researchers have focused on changing substituents at the 2- and 6-positions of 2-methoxyestradiol to increase cytotoxicity and tubulin polymerization inhibition, and their efforts have produced 2-((*E*)-1′-propenyl)estradiol (**3**), 2-ethoxyestradiol (**4**), and 2-(1′-propynyl)estradiol (**5**).[20−22] Additionally, the 17-position contributes significantly to the anticancer activity and pharmacokinetic profile of 2ME2.[4,15,23] The goal of the present research was to further elucidate the structural and electronic requirements necessary to inhibit tubulin polymerization, increase the cytotoxicity, and enhance the metabolic stability of analogues of 2ME2. Specifically, we have designed and synthesized unique analogues with modifications at positions 2 and 17 in an attempt to maintain pharmacological activity and decrease metabolic deactivation.

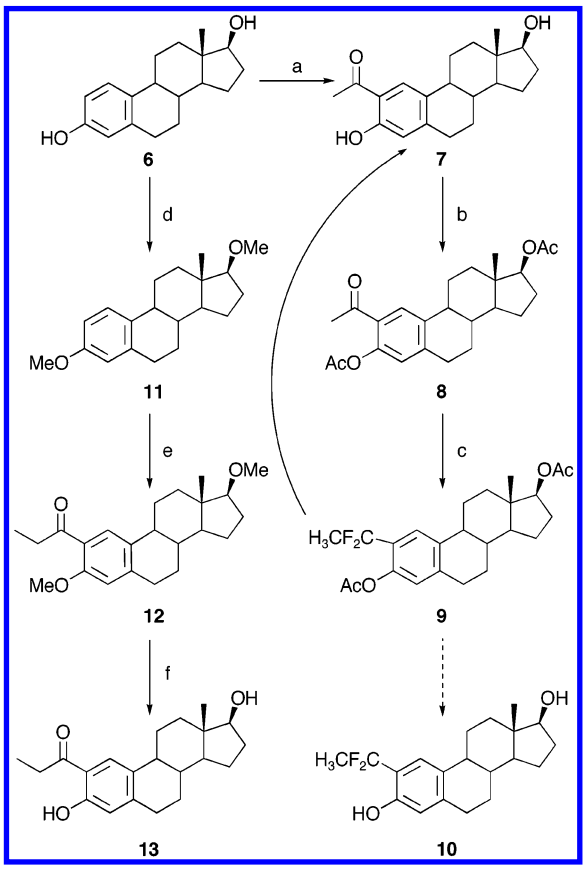
Chemistry. Friedel−Crafts acylation of *β*-estradiol (**6**) using aluminum chloride and acetyl chloride gave a mixture of three products, which, when followed by workup with potassium hydroxide and methanol, afforded the acetylated product **7** (Scheme 1).[23] Reaction of **7** with pyridine and acetic anhydride yielded **8**. Treatment of **8** with [bis(2-methoxyethyl)amino]sulfur trifluoride (BEAST) and ethanol in a bomb reactor provided the difluoroethyl analogue **9**. All attempts to generate **10** following deprotection of **9** were unsuccessful and, instead, yielded the acetyl derivative **7**. However, the diacetate intermediate **9** was still of interest as a possible prodrug of compound **10**. *β*-Estradiol was also methylated using sodium hydride and iodomethane to form **11**. Formation of the mixed anhydride derived from acetylation of propionic acid with trifluoroacetic anhydride, followed by reaction with alumina and **11**, afforded the propionyl derivative **12**. Deprotection of **12** with in situ-generated iodotrimethylsilane provided compound **13**.

Ullmann coupling of 2-iodoestradiol (**14**),[24] prepared according to published methods, with copper and perfluoroethyl iodide in a bomb reactor gave the perfluoroethyl congener **15**, a relatively stable deprotected alternative to the unstable compound **10** (Scheme 2). Protection of **14** with imidazole and *tert*-butyldimethylsilyl chloride, followed by Negishi coupling using allylmagnesium bromide, zinc bromide, and tetrakis(triphenylphosphine)palladium gave **17**.[24] Deprotection of **17** with dilute hydrochloric acid using sonication gave the desired diol **18**.

Compounds **19**−**24**[25−27] and **27**[28] were prepared according to literature methods with the exception that **22** was synthesized using sodium hydride as the base in tetrahydrofuran rather than potassium hydroxide in dimethyl sulfoxide (Schemes 3 and 4). 2-Formylestradiol (**24**) was reacted with cyclopropylamine in the presence of magnesium sulfate and with 3-*N*,*N*-dimethylaminopropylamine to provide compounds **25** and **26**, respectively.

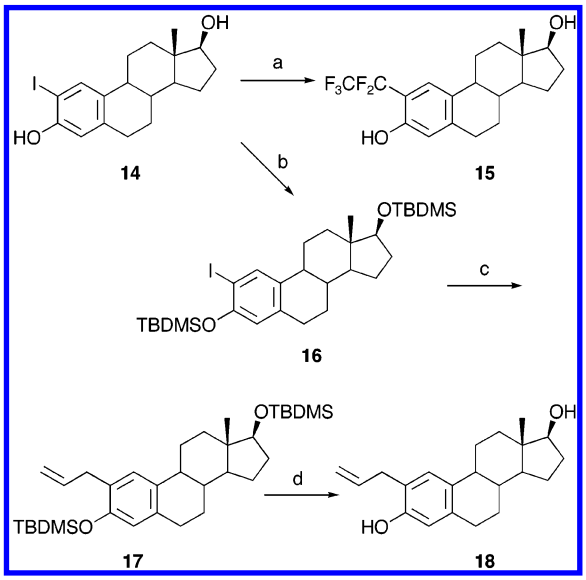
Estrone (**28**) was reacted with BEAST to provide the 17,17-difluoro analogue **29** and with methylmagnesium

Scheme 1[a]



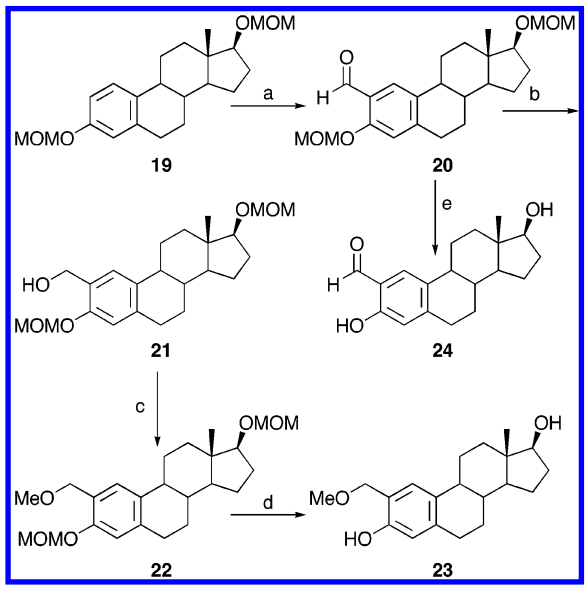
[a] Reagents and conditions: (a) (1) AcCl, $AlCl_3$, 0 °C to room temperature (25 h); (2) KOH, MeOH (10 h). (b) Ac_2O, Pyr (20 h). (c) BEAST, EtOH, 70 °C (11 h). (d) NaH, MeI, THF, 0 °C−rt (24 h). (e) Alumina, CH_3CH_2COOH, $(CF_3CO)_2O$ (15 h). (f) NaI, TMSCl, CH_3CN, 0 °C (48 h).

Scheme 2[a]



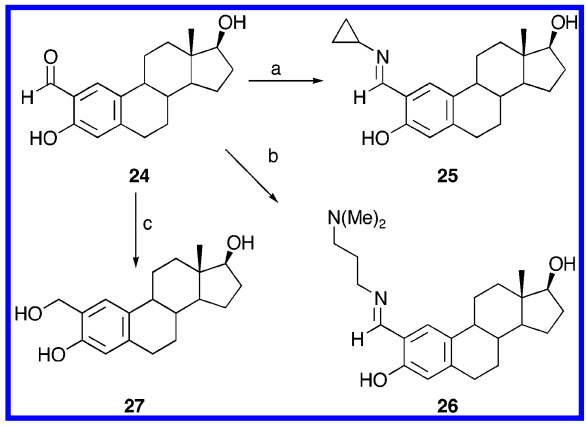
[a] Reagents and conditions: (a) Cu, CF_3CF_2I, DMSO, 0−80 °C (16 h). (b) TBDMSCl, DMF, imidazole, rt (16 h). (c) (1) CH_2=CH-CH_2MgBr, $ZnBr_2$, THF, rt (5 min); (2) $Pd(PPh_3)_4$, **16**, rt (12 h). (d) HCl, EtOH, 2-PrOH (3 h).

Scheme 3[a]



[a] Reagents and conditions: (a) (1) *sec*-BuLi, THF, −78 °C (2 h);[28] (2) DMF, −78 °C−rt (12 h). (b) $NaBH_4$, MeOH, 23 °C, (2.5 h).[25] (c) NaH, MeI, THF, 0 °C-rt (7 h). (d) PPTS, MeOH, reflux (18 h). (e) HCl, MeOH, rt.

Scheme 4[a]

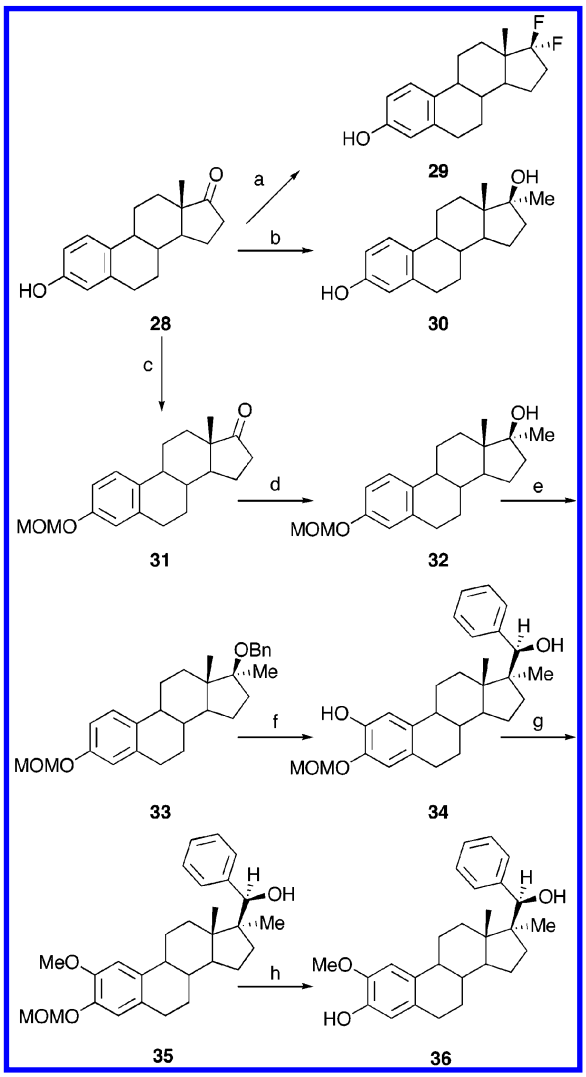


[a] Reagents and conditions: (a) Cyclopropylamine, $MgSO_4$, rt (8 h). (b) 3-*N*,*N*-Dimethylaminopropylamine, reflux (1 h). (c) $LiAlH_4$, THF, 0 °C−rt (12 h).[25]

bromide to create 17α-methylestradiol (**30**, Scheme 5). The methoxymethyl-protected derivative **31**, formed by reacting estrone with diisopropylethylamine and chloromethylmethyl ether, was alkylated with methylmagnesium bromide to yield **32**. Benzyl protection of **32** yielded **33**. When **33** was reacted with *sec*-butyllithium followed by addition of trimethyl borate and hydrogen peroxide, an unexpected Wittig rearrangement occurred to form compound **34**. The phenol of compound **34** was methylated using potassium carbonate and iodomethane. The stereochemistry of **35**, determined via X-ray crystallography (Figure 1), showed that the product had retained the original C-17 stereochemistry of **33** during the radical anion rearrangment to form the new C-17−C-17(1′) linkage. Removal of the methoxymethyl protecting group of **35** with concentrated hydrochloric acid in methanol provided the phenol **36**.

Compound **32** was selectively deprotonated with *sec*-butyllithium and iodinated with iodine to provide intermediate **37** (Scheme 6). Deprotection of **37** with concentrated hydrochloric acid and subsequent Stille

Scheme 5[a]



[a] Reagents and conditions: (a) BEAST, CH_2Cl_2, EtOH (62 h). (b) MeMgBr, THF, $PhCH_3$, 0 °C−rt (8 h). (c) DIEA, MOMCl, THF, 0 °C−reflux (24 h). (d) MeMgBr, THF, 0 °C−rt (20 h). (e) (1) NaH, DMF, 0 °C (15 min); (2) BnBr, 0 °C−rt (6 h). (f) (1) *sec*-BuLi, −78 to −30 °C (5 h); (2) $(MeO)_3B$, −78 °C-rt (8 h); (3) 30% H_2O_2, NaOH (aq), 0 °C (8 h). (g) K_2CO_3, MeI, $(CH_3)_2CO$, rt (24 h). (h) HCl, MeOH, 2-PrOH, rt (6 h).

coupling of **38** with tributylpropynylstannane and tetrakis(triphenylphosphine)palladium yielded the alkyne **39**.

Compound **40** was isolated by reaction of **19** with *sec*-butyllithium, followed by introduction of trimethylborate, and then hydrogen peroxide (Scheme 7). Conversion of **40** to 2-methoxyestrone (**41**) was completed by methylation and deprotection using known methods followed by an Oppenauer oxidation with aluminum isopropoxide and cyclohexanone.[29] Potassium carbonate and chloroacetone were reacted with **40** to generate, upon stirring in hydrochloric acid, the hemiacetal **42**. Reaction of intermediate **42** with hydroxylamine hydrochloride at reflux yielded the oxime **43**. 2-Methoxyestrone (**41**) was benzyl protected using potassium carbonate and methanol to produce **44**, which was subsequently reacted with trifluoromethyltrimethylsilane and a catalytic amount of tetrabutylammonium fluoride generating intermediate **45**. Deprotection of **45** by catalytic hydrogenation, followed by treatment with acid, resulted in compound **46**. Alkylation of 2-methoxy-

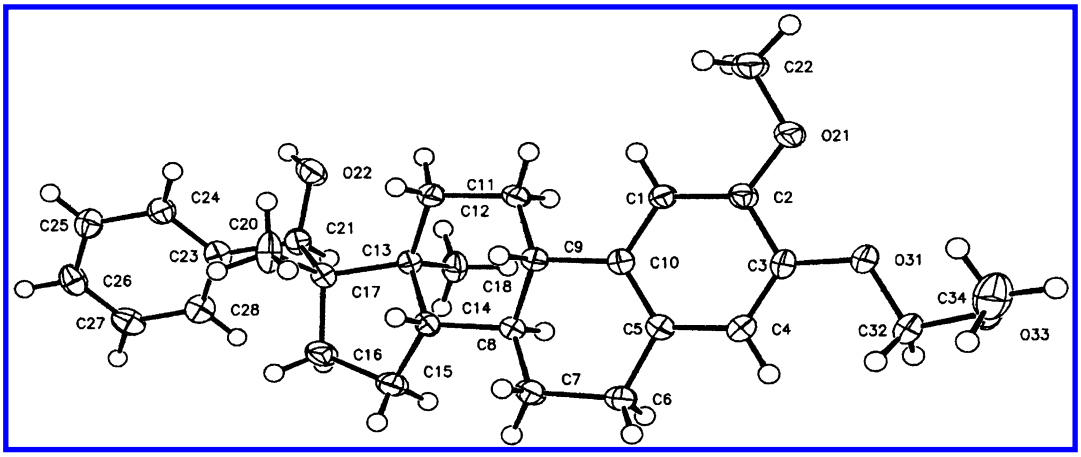
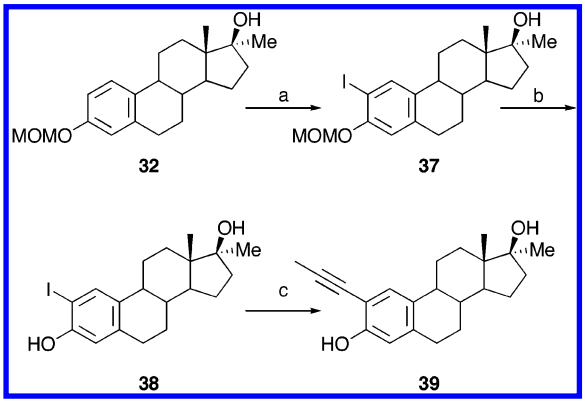


Figure 1. Crystal structure of 17-*β*-(*S*)-(hydroxyphenylmethyl)-2-methoxy-3-*O*-methoxymethyl-17α-methyl-1,3,5(10)-estratriene (**35**).

Scheme 6[a]



[a] Reagents and conditions: (a) (1) *sec*-BuLi, THF, cyclohexane, −78 °C (2 h); (2) I_2, −78 °C-rt (6 h). (b) HCl, THF, rt (6 h). (c) Tributylpropynylstannane, $Pd(PPh_3)_4$, THF, reflux (10 h).

estrone (**41**) using methylmagnesium bromide yielded analogue **47**, while fluorination of **41** with BEAST provided the 17,17-difluoro derivative **48**.

Oppenauer oxidation of 2-ethoxyestradiol (**4**) produced **49** (Scheme 8). Utilizing methyltriphenylphosphonium iodide and the dimsyl anion, the Wittig reaction successfully converted **49** to the alkene **50**. A Grignard reaction of methylmagnesium bromide with 2-ethoxyestrone (**49**) provided the target compound **51**.

Biological Results and Discussion

The antiproliferative activities of the new 2ME2 analogues were determined in the NCI screen, in which the activity of each compound was examined using approximately 55 different human cancer cell lines of diverse tumor origins. The antiproliferative assays provided individual cell line GI_{50} values and mean graph midpoints (MGMs). The MGM values were calculated by averaging the GI_{50} values for all the cell lines tested, where the GI_{50} values below and above the tested concentration range (10^{-4} to 10^{-8} M) were taken as the minimum (10^{-8} M) and maximum (10^{-4} M) analogue concentrations used in the screen.[30]

The analogues were also evaluated for their inhibitory effects on purified bovine brain tubulin using assay conditions as described previously.[10] The tubulin polymerization inhibition values, representative cell line antiproliferative GI_{50} values and MGM values are compiled in Table 1.

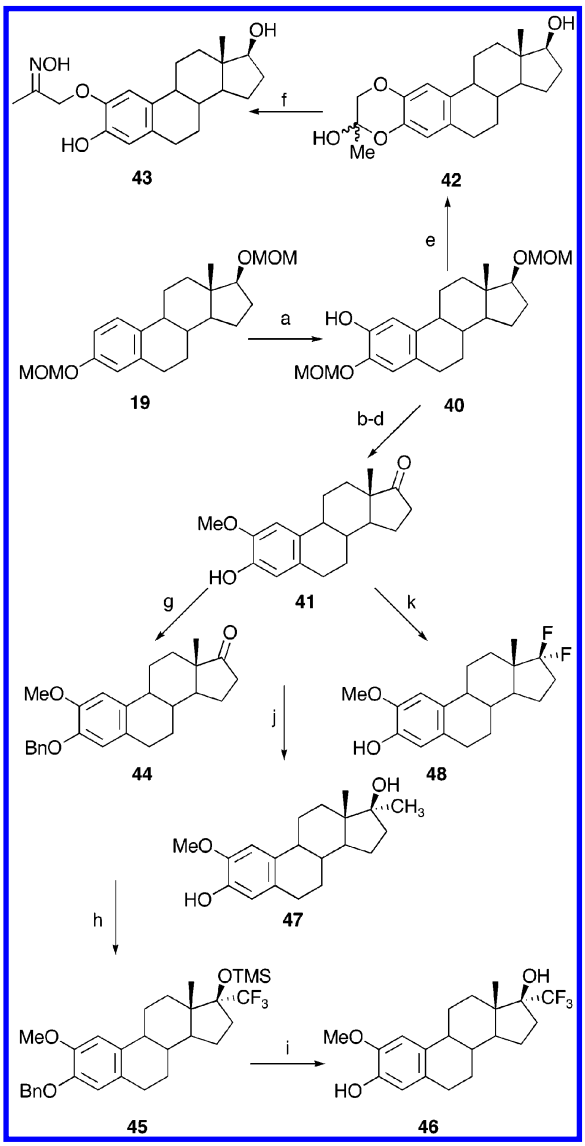
The antiproliferative activities of the synthesized compounds were assessed also in the MDA-MB-231 breast cancer cell line and the human umbilical vein endothelial cell (HUVEC) line (Table 2). The breast carcinoma cell line MDA-MB-231 was chosen as an initial screen for antiproliferative activity in tumor cells because it has been shown that breast carcinomas are among the most sensitive cell lines to 2ME2 (NCI cell screen data), and it is estrogen receptor negative. Additionally, this cell line is used as an orthotopic tumor model for lead compounds that are screened in vivo.

With the exception of compounds **30**, **39**, **50**, and **51**, the position 2 and 17 derivatives had little or no activity as tubulin polymerization inhibitors. The inactivity of **7**, **9**, **13**, **25**, **26**, **29**, **36**, and **43** is reasonably consistent with previously established data indicating that branching of the position 2 side chain and steric bulk at position 2 and position 17 are not well tolerated. However, the inactivity of some of the other compounds containing favorable steric features, specifically **15**, **18**, and **23**, was surprising since previously published results have indicated that straight chain substituents at position 2 containing two or three non-hydrogen atoms conferred maximum tubulin polymerization inhibition.[20−23,26]

Because difluoromethylene moieties have been used previously as isosteric replacements for the oxygen atoms of ethers, compounds **9** and **15** were synthesized to inhibit the potential for oxidative *O*-demethylation of 2-methoxyestradiol.[31] The acetate protecting groups of **9** could not be deprotected without reversion to compound **7**, so **15** was designed as a stable, sterically similar alternative to **9**.[32] 2-Perfluoroethylestradiol (**15**) was also expected to be similar in size and shape to 2-methoxyestradiol.[21] However, compound **15** displayed no activity as an inhibitor of tubulin polymerization. The surprising inactivity of this compound and the previously reported 2-cyanoestradiol[22] indicate that, while steric effects are important characteristics for inhibiting tubulin polymerization, electronic effects are also a component that contributes to the activity of 2-methoxyestradiol analogues.

To illustrate the electronic differences between select molecules, electron density contours (Figures 2 and 3) were examined using the molecular modeling program Sybyl (Tripos, Inc., St. Louis, MO). The contours are

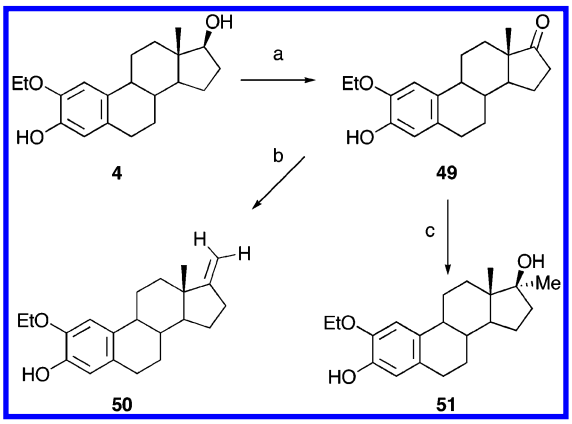
Scheme 7[a]



[a] Reagents and conditions: (a) (1) *sec*-BuLi, THF, −78 °C (2 h); (2) $(MeO)_3B$, −78 °C−rt (5 h); (3) 30% H_2O_2, NaOH (aq), 0 °C (8 h). (b) K_2CO_3, MeI, DMF, TBAI, rt (25 h). (c) 6 N HCl, THF, rt. (6 h). (d) Al(*i*PrO)$_3$, cyclohexanone, toluene, reflux (20 h). (e) (1) K_2CO_3, CH_3COCH_2Cl, CH_3CN, reflux (6 h); (2) 6 M HCl, THF, rt (8 h). (f) NH_2OH·HCl/Pyr, 90 °C (3 h). (g) K_2CO_3, BnBr, DMF, rt (19 h). (h) CF_3TMS, TBAF, THF, rt (10 h). (i) (1) Pd/C, H_2, MeOH, rt (8 h); (2) dilute HCl, MeOH, rt, (20 min). (j) MeMgBr, THF, 0 °C−rt (48 h). (k) BEAST, EtOH, CH_2Cl_2, 0 °C−rt (48 h).

colored according to electrostatic potential (EP, kcal/mol) ranging from regions of high electron density in blue to regions of low electron density in red. When comparing the electronic characteristics (Figure 2) of 2-methoxyestradiol (**1**, upper left) to the sterically similar, yet inactive, 2-pentafluoroethyl derivative (**15**, upper right), the differences between the electron densities of the aromatic rings and position 2 side chain become obvious. The pentafluoroethyl moiety of **15** has a dramatic effect, lowering the overall electron density of the aromatic ring, resulting in a green region of less electron density than the blue aromatic portion of 2-methoxyestradiol. Also, the 2-position ethyl moiety has lowered electron density (represented by yellow), while the fluorines increase the position 2 surface polarity relative to the 2-methoxy-side chain. However, the inhibitory activity

Scheme 8[a]



[a] Reagents and conditions: (a) Al(*i*-PrO)$_3$, cyclohexanone, toluene, reflux (20 h). (b) (1) NaH, DMSO, rt − 62 °C (1.5 h); (2) $CH_3P(Ph)_3I$, rt (30 min); (3) **49** (18 h). (c) MeMgBr, THF, rt (3 h).

of 2-(2′,2′,2′-trifluoroethoxy)estra-1,3,5(10)-triene-3,17β-diol (MGM 2.6 μM; IC_{50} 1.5 μM)[20] was more potent than 2ME2, indicating that the electronic withdrawing effects imposed upon the A-ring by the perfluoroethyl substituent, rather than the surface polarity, caused the inactivity of **15**. The visible differences in electron density between 2-methoxyestradiol and **15**, rather than in steric bulk, shown by the models in Figure 2 give credence to the hypothesis that electronic effects account for the observed disparity in biological activity.

The inactivity of compounds **18** and **23** also seems to indicate that the activity of 2-((*E*)-1′-propenyl)estradiol (**3**, MGM 0.14 μM; IC_{50} 1.1 μM) and 2-ethoxyestradiol (**4**, MGM 0.076 μM; IC_{50} 0.91 μM) result from the electronic effects afforded by the overall electronic characteristics of the 2-positon moieties including the π-conjugation of the 1′-alkene and electron density of the oxygen, respectively.[21] The 2-position substituent of compound **18** possesses similar steric characteristics but differs from the 2-(1′-propenyl) analogue (**3**) in that the alkene is shifted from the 1′-2′ to the 2′-3′ position. Additionally, the inactive methoxymethyl moiety of compound **23** is an alternative atom arrangement of the 2-ethoxy group of 2-ethoxyestradiol (**4**). Analysis of the electronic effects (Figure 2) of analogues **3** (lower left) and **18** (lower right) show a disruption in the electron density of **18**. While the steric bulk of both molecules is comparable and similar to other position 2 alkyl analogues that have displayed at least some activity, the electron density afforded by the π-conjugation of the 1′,2′-alkene of **3** probably accounts for its observed inhibition of tubulin polymerization. Together, the inactivity of 2-cyanoestradiol, **15**, **18**, and **23** and analysis of the electron density maps seem to indicate that the correct size and shape of the substituents at position 2 are not sufficient to inhibit tubulin polymerization by themselves. To maintain or improve activity, the 2-position substituents must contribute to the electron density or at least not withdraw electron density from the aromatic ring in addition to maintaining the critical size restrictions.

The compounds with position 17 alterations were designed to inhibit oxidation of the 17-hydroxyl to the less potent ketone. Oxidation of the tertiary alcohols of compounds **30**, **39**, **46**, **47**, and **51** to a ketone would be inhibited by the 17α-methyl or 17α-trifluoromethyl

Table 1. Cytotoxicities and Tubulin Polymerization Inhibition of 2-Methoxyestradiol and Analogues

	cytotoxicity (GI_{50} in μM)[a]									inhibition of tubulin polymerization (IC_{50} μM ± SD)[c]
no.	lung HOP-62	colon HCT-116	CNS SF-539	melanoma UACC-62	ovarian OVCAR-3	renal SN12C	prostate DU-145	breast MDA-MB-435	MGM[b]	
1	0.70	0.47	0.32	0.36	0.21	0.95	1.8	0.080	1.3	5.3 ± 0.2
7	5.4	ND[d]	18.6	24.5	25.7	53.7	55.0	4.3	17.4	>20
9	31.6	10.7	20.0	12.3	1.9	27.5	13.8	20.9	11.7	>40
13	15.1	9.3	11.7	11.5	4.0	13.8	15.5	2.4	12.0	>40
15	ND[d]	17.8	18.2	13.8	13.2	16.6	14.5	12.0	18.6	>40
18	13.8	14.1	21.9	13.2	21.9	18.2	24.0	15.1	18.2	>40
23	83.2	33.1	33.9	24.5	40.7	56.2	64.6	7.8	38.0	>40
25	24.0	12.3	17.0	16.2	14.5	70.8	16.6	16.2	16.6	>40
26	21.4	12.6	18.2	13.8	19.5	25.7	20.9	22.9	20.0	>40
27	72.4	74.1	38.0	23.4	31.6	63.1	70.8	24.0	34.7	>40
29	19.5	14.5	17.4	14.1	11.0	18.6	16.6	15.8	16.6	>40
30	18.2	0.70	0.35	1.48	0.66	10.2	2.0	0.19	2.5	5.6 ± 0.5
36	18.2	17.0	17.8	13.5	14.1	14.1	5.1	16.2	14.5	>40
39	3.1 ± 0.1	4.3 ± 0.0	2.6 ± 0.2	4.0 ± 0.0	1.5 ± 0.1	7.4 ± 0.1	4.1 ± 0.1	0.3 ± 0.0	3.2 ± 0.0	11 ± 0.5
43	23.4	18.2	20.9	15.5	20.4	27.5	29.5	24.0	24.0	>40
46	ND[d]	22.4	19.5	18.2	15.8	24.5	18.2	16.2	19.5	>40
47	15.2 ± 1.2	2.8 ± 0.6	6.9 ± 1.5	6.3 ± 1.1	7.1 ± 0.4	7.8 ± 0.9	12.3 ± 1.6	1.4 ± 0.6	10.2 ± 1.0	>40
48	81.3	66.1	31.6	58.9	43.7	24.5	56.2	35.5	40.7	>40
50	0.56	0.60	0.12	0.54	0.28	0.19	0.47	0.08	0.79	2.9 ± 0.5
51	5.0 ± 0.0	3.5 ± 0.1	2.8 ± 0.2	3.74 ± 0.1	2.8 ± 0.1	4.5 ± 0.3	5.2 ± 0.0	1.3 ± 0.3	4.7 ± 0.1	4.8 ± 0.4

[a] The GI_{50} values, denoting cytotoxicity, are the concentrations affording 50% growth inhibition of the individual human cancer cell lines. [b] MGM corresponds to the average concentration causing growth inhibition in all of the human cancer cell lines tested. [c] Reaction conditions conducted using tubulin concentration of 1.2 mg/mL (12 μM). [d] ND indicates that the value was not determined.

Table 2. Antitumor and Antiangiogenic Activities of 2-Methoxyestradiol Analogues

no.	MDA-MB-231 IC_{50} (μM)[a]	HUVEC IC_{50} (μM)[b]
1	1.00 ± 0.05	0.84 ± 0.02
3	0.65 ± 0.11	0.59 ± 0.01
4	0.55 ± 0.33	0.12 ± 0.05
5	3.99 ± 1.12	0.52 ± 0.14
7	13.61 ± 0.86	15.64 ± 2.11
9	95.91 ± 4.82	32.27 ± 13.10
13	15.83 ± 2.52	22.5 ± 2.41
15	47.49 ± 3.13	25.04 ± 3.55
18	33.40 ± 8.77	8.51 ± 0.87
23	53.96 ± 2.02	28.75 ± 4.47
27	99.25 ± 2.66	7.73 ± 0.49
29	54.43 ± 6.15	6.60 ± 1.96
30	7.14 ± 0.88	3.09 ± 0.16
36	47.17 ± 0.92	21.04 ± 0.1
39	5.99 ± 0.32	1.94 ± 0.01
46	49.27 ± 1.32	40.04 ± 3.56
47	3.33 ± 0.23	2.40 ± 0.45
48	70.81 ± 11.79	58.27 ± 11.62
50	0.7 ± 0.8	0.26 ± 0.04
51	18.35 ± 3.36	5.70 ± 0.61

[a] The IC_{50} values are the concentrations affording 50% growth inhibition of the MDA-MB-231 cancer cell lines. [b] The IC_{50} values are the concentrations affording 50% growth inhibition of the HUVEC lines.

substituents, while the 17-position moieties of **29**, **48**, and **50** were synthesized as substitutes to inhibit the potential for oxidation and conjugation of the position 17 hydroxyl group. Of the position 17 analogues, compounds **30**, **39**, **50**, and **51** were inhibitors of tubulin polymerization with activity comparable to 2-methoxyestradiol (IC_{50} 5.3 μM). Because estradiol (**6**) is not an inhibitor of tubulin polymerization, the surprising activity of 17α-methylestradiol (**30**, IC_{50} 5.6 μM) indicates that the 17α-methyl moiety is tolerated. The active position 2 substituents of 2-(1′-propynyl)estradiol[20] (**5**, IC_{50} 4.9 μM), 2-ethoxyestradiol[21] (**4**, IC_{50} 0.91 μM), and 2-methoxyestradiol (**1**, IC_{50} 5.3 μM) were combined with the 17α-methyl moiety to provide the active compounds **39** (IC_{50} 11 μM), and **51** (IC_{50} 4.8 μM) and the inactive compound **47** (IC_{50} > 40 μM), respectively.

Although the 17α-methyl moiety of **51** was tolerated, the 17α-methyl of **47** and the 17α-trifluoromethyl moiety of **46** (IC_{50} > 40 μM) effectively abolished tubulin polymerization inhibition at the concentrations tested. Additionally, while fluorine is generally regarded as an adequate isosteric replacement for both a hydroxyl group and hydrogen, 17,17-difluoromethylene-2-methoxyestradiol (**48**, IC_{50} > 40 μM) was completely inactive.

The electron density contours in Figure 3 of 2-ethoxyestradiol (**4**, upper left), 2-ethoxy-17α-methyl estradiol (**51**, upper right), 17,17-difluoro-2-methoxy-1,3,5(10)-estratriene-3-ol (**48**, lower left), and 2-ethoxy-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**50**, lower right) show that both steric and electronic effects of position 17 may contribute to analogue activity. Analogue **51** (upper right) has similar electronic distribution in the C- and D-rings to the highly active 2-ethoxyestradiol (**4**, upper left), but also contains additional steric bulk afforded by the 17α-methyl substituent. This additional bulk results in a loss of activity against tubulin. Significantly, the 17α-methyl moiety of 2-methoxy-17α-methylestradiol (**47**) rendered this congener completely inactive. The trifluoromethyl moiety of compound **46** (not shown), while similar in steric bulk to **47**, differs in electron density at this position, but the electronic differences were not sufficient to restore activity.

The 17,17-difluoromethylene of derivative **48** (lower left) was sterically similar to 2-ethoxyestradiol (**4**, upper left) and noticeably smaller in size than the 17α-methyl moiety of **51** (upper right). However, the electronegativity of the fluorines causes an increase in the electron density of the α-face of the steroid. Because fluorine is commonly used as an isosteric replacement for both oxygen and hydrogen, the lack of activity associated with compound **48** is likely a result of the electronic influence exerted by the electronegative fluorines rather than the slight increase in steric bulk.

2-Ethoxy-17-(1′-methylene)estra-1,3,5(10)-triene-3-ol (**50**, IC_{50} 2.9 μM, lower right) was overall the most active compound of the series and, interestingly, also

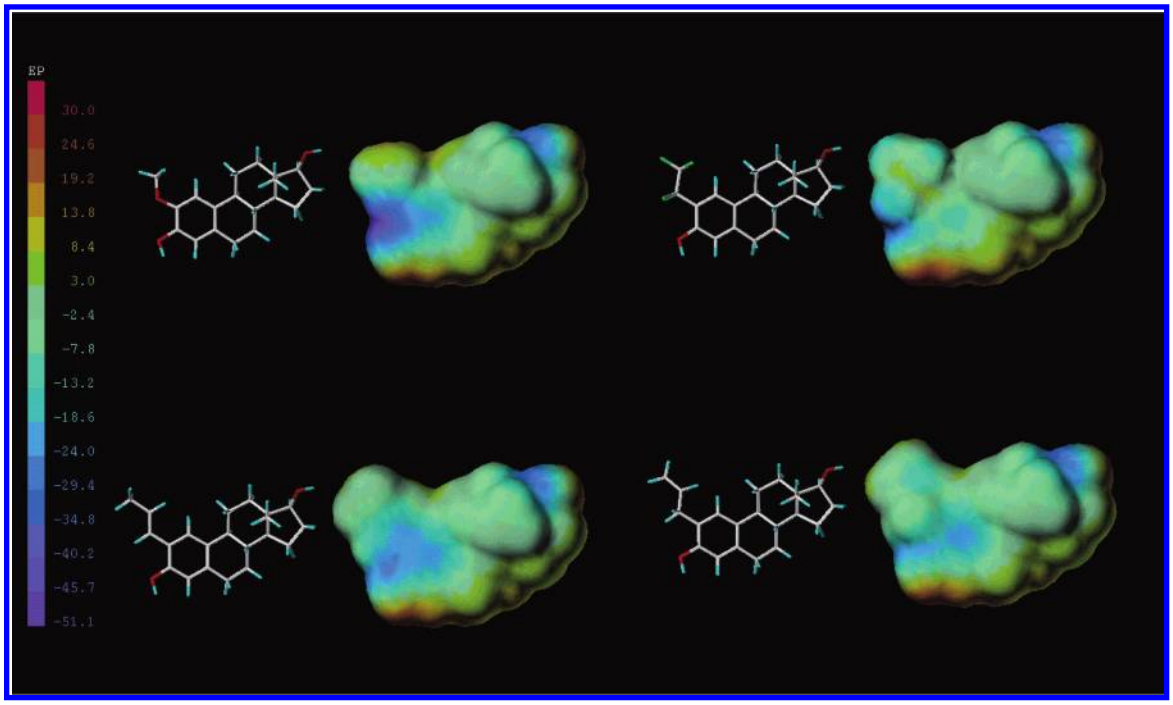


Figure 2. Electron density contour diagram comparing the electrostatic potential (EP) of the position 2 moieties of 2-methoxyestradiol (**1**, upper left), 2-((*E*)-1′-propenyl)estradiol (**3**, lower left), 2-pentafluoroethyl-*β*-estradiol (**15**, upper right), and 2-allylestradiol (**18**, lower right). The EP range (maximum 30.0 kcal/mol and minimum −51.1 kcal/mol) was applied globally to generate comparable regions of high (blue) and low (red) electron density. The orientation of the molecules displayed by the electron density contours is from the *β*-face as shown by the capped-stick models to their immediate left.

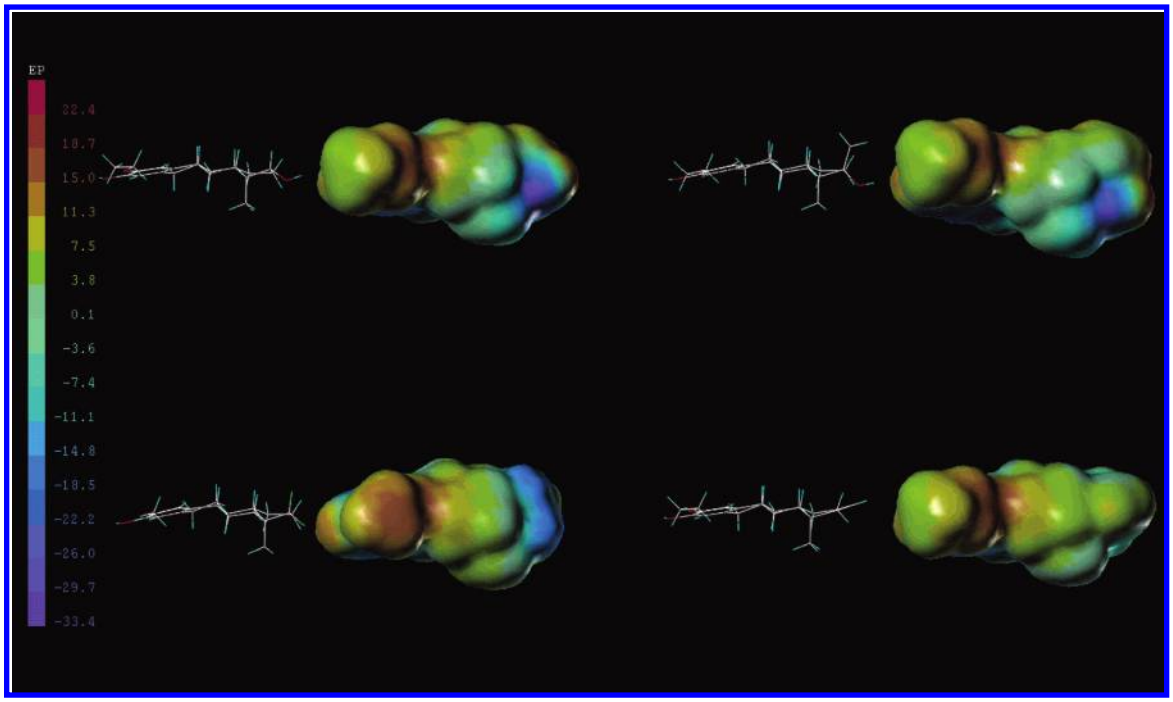


Figure 3. Electron density contour diagram comparing the electrostatic potential (EP) of the position 17 moieties from the "top view". The diagram compares 2-ethoxyestradiol (**4**, upper left), 17,17-difluoro-2-methoxy-1,3,5(10)-estratrien-3-ol (**48**, lower left), 2-ethoxy-17α-methylestradiol (**51**, upper right), and 2-ethoxy-17-(1′-methylene)estra-1,3,5(10)-trien-3-ol (**50**, lower right). The EP range (maximum 22.4 kcal/mol and minimum −33.4 kcal/mol) was applied globally to generate comparable regions of high (blue) and low (red) electron density. The orientation of electron density contours is from "top view" of the steroids as shown by the line models directly to the left of each. The plane containing the aromatic ring is perpendicular to the plane of the page with the α- and *β*-faces extending above and below the plane containing the aromatic ring, respectively. The position 17 moieties are shown nearest to the viewer at the right side of each molecule.

contained the least polar position 17 substituent (shown in yellow). Although the steric and electronic characteristics of the 17-position of **50** may not be solely responsible for the observed activity because it also contained a position 2 ethoxy group, the results indicate that sterically small alternatives to the 17-hydroxyl substituent with low levels of electron density can be used to develop new analogues that inhibit tubulin

polymerization. Collectively, these analogues indicate that small increases in steric bulk are tolerated at position 17, but significantly more polar substituents of comparable size and shape are not. This trend may also account for the notable disparity between the activities of the sterically similar 2-methoxyestrone and 2-methoxyestradiol. Additionally, the activity of compounds **30**, **39**, **50**, and **51** indicates that substitution of 2-methoxyestradiol analogues at position 17 for the purpose of inhibiting metabolic deactivation can result in the retention of some tubulin polymerization inhibitory activity provided appropriate substituents are also located at position 2.

Regarding the cytotoxicities of the tested compounds, each of them displayed some activity in human cancer cell lines. As in earlier studies, the most cytotoxic compounds also inhibited tubulin polymerization at the lowest concentrations, and the least cytotoxic compounds had no significant activity against tubulin. Of the cytotoxic analogues, compound **50** (MGM 0.79 μM) and compound **30** (MGM 2.5 μM) were the most cytotoxic followed by **39** (MGM 3.2 μM), **51** (MGM 4.8 μM), and **47** (MGM 10.2 μM). Compound **50** was more cytotoxic and a more potent inhibitor of tubulin polymerization than 2-methoxyestradiol (MGM 1.3 μM). Moreover, the cytotoxicity and antitubulin activity of 17α-methylestradiol (**30**) is unprecedented and unexpected, because previously examined estrogen derivatives that inhibited tubulin polymerization were all substituted at position 2.

The antiproliferitive activities of the analogues were contrasted in epithelial and endothelial cell lines to determine their preliminary antiangiogenic potential (Table 2). Compounds **30**, **39**, **47**, and **50**, as well as **1**, **3**, **4**, and **5** showed the most activity against the MDA-MB-231 breast cancer cell line. These analogues, with the addition of **18**, **27**, and **29** were also active against the HUVEC line. Of the new analogues, as with the NCI cell screen, only analogue **50** was more active than 2ME2. Congeners with highly polar substituents at both position 2 and position 17, again, displayed less activity relative to the less polar analogues. Compounds **27** and **29** showed the greatest selectivity for the endothelial cell lines over the tumor cell lines. However, both were much less active against the HUVEC line than 2ME2 and **3**, **4**, **5**, and **50**.

In conclusion, the electronic effects of 2- and 17-position substituents significantly affect tubulin polymerization inhibition of analogues of 2-methoxyestradiol. Of the position 2 analogues, compounds **15**, **18**, **23**, and **27** with similar steric characteristics to 2-methoxyestradiol (**1**), 2-ethoxyestradiol (**4**), and 2-((*E*)-1′-propenyl)-estradiol (**3**) were completely inactive as tubulin polymerization inhibitors and displayed low levels of cytotoxicity. The overall inactivity of these derivatives is likely a result of the altered electronic properties of the 2-position substituents upon the A-ring of estradiol. The 17α-methyl moiety of **30**, **39**, and **51** is expected to reduce the deactivating oxidative metabolism of the 17-hydroxyl group. Some of the analogues containing this substituent were comparable to 2ME2 in their antitubulin and antiproliferative activities. The D-ring substituents in **46**−**48** resulted in inactive compounds, indicating that both steric and electronic effects at position 17 are important considerations for designing active analogues. Compound **50** was the most cytotoxic of the newly synthesized analogues and inhibited tubulin polymerization at lower concentrations than 2-methoxyestradiol. The most promising of these analogues will be further evaluated in other in vitro and in vivo screens.

Experimental Section

General. Melting points were determined using capillary tubes with a Mel-Temp apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded using either an ARX300 300 MHz Bruker NMR spectrometer or a DMX500 500 MHz Bruker spectrometer. IR spectra were recorded using a Perkin-Elmer 1600 series FTIR spectrometer. Flash and gravity chromatographic purification were performed using 230−400 mesh silica gel unless otherwise noted. Combustion microanalyses were performed at the Purdue University Microanalysis Laboratory, and the reported values were within 0.4% of the calculated composition.

Tubulin Polymerization Inhibition Assays. Tubulin, purified from bovine brain as described elsewhere,[33] was preincubated with various compound concentrations. The method to determine the IC_{50} values for the inhibition of purified tubulin polymerization was described previously,[10] but Beckman DU7400/7500 spectrophotometers containing "high performance" temperature controllers were used. To enhance cooling of the electronic elements of the temperature control units, these were modified to include a water circuit. Software for operating the spectrophotometers was provided by MDB Analytical Associates, South Plainfield, NJ. After chilling the preincubated mixtures of compound and tubulin on ice, GTP was added and polymerization was monitored for 20 min at 26 °C by tubidimetry at 350 nm. The IC_{50} value was defined as the compound concentration required to inhibit tubulin assembly at 20 min by 50%. All compounds were examined in at least two independent assays.

Cell Culture. Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics (San Diego, CA), and MDA-MB-231 cells were obtained from ATCC. HUVEC cultures were maintained for up to five passages in EGM containing bovine brain extract (Clonetics) and 1X antibiotic-antimycotic (BioWhittaker, Walkersville, MD). MDA-MB-231 cells were maintained in DMEM/F12 (1:1) containing 10% (v/v) fetal bovine serum (Hyclone Laboratories, Logan, UT) and 1X antibiotic-antimycotic.

Proliferation Assays. Proliferation was measured by cell counting using a Coulter Z1 cell counter (Coulter Corporation, Hialeah, FL) or by evaluation of DNA synthesis. Each condition was done in triplicate, and experiments were repeated at least twice. To determine antitumor and antiangiogenic activities of the analogues, proliferation assays were performed by evaluating DNA synthesis using 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, antitumor activity) or 3000 cells/well (HUVEC, antiangiogenic activity) in a 96 well plate, allowed to attach overnight and then exposed to the compound for 48 h.

2-Acetyl-β-estradiol (7). Anhydrous chlorobenzene (75 mL), acetyl chloride (3.2 mL, 45.0 mmol), and aluminum chloride (8.00 g, 60.0 mmol) were combined in a flame-dried round-bottomed flask and cooled to 0 °C under argon. β-Estradiol (**6**, 4.00 g, 14.68 mmol) was added slowly in four portions over 1 h. After stirring for 25 h, 3 N HCl (60 mL) was added dropwise to decompose the orange/red colored material. The mixture was extracted with ethyl acetate (3 × 200 mL), washed with water (200 mL), and dried over anhydrous sodium sulfate. Condensation of the organic layer gave the mixture of three products as a yellow oil. The products were dissolved in methanol (100 mL). Methanol (10 mL) saturated with potassium hydroxide was added to the solution and stirred for 10 h. Following the removal of solvent, the basic

solution was diluted with ice−water (250 mL), extracted with ethyl acetate (3 × 150 mL), washed with water (100 mL) and brine (100 mL), and dried over anhydrous sodium sulfate. After condensation, the orange oil was triturated with methanol, resulting in precipitation of the white solid, **7** (3.73 g, 81%): mp 195−196 °C (lit.[23] 192−195 °C). ^{1}H NMR ($CDCl_3$) δ 12.05 (s, 1 H, exchangeable with D_2O), 7.61 (s, 1 H), 6.70 (s, 1 H), 3.77 (t, J = 17.07 Hz, 1 H), 2.87 (m, 2 H), 2.74−1.32 (m, 17 H), 0.80 (s, 3 H); IR (film) 3472, 2923, 1715, 1640, 1490, 1368, 1264, 1224, 1056, and 1020 cm^{-1}; low resolution ESIMS: *m*/*z* (rel intensity) 313 (MH^+, 100). Anal. ($C_{20}H_{26}O_3$) C, H.

2-Acetyl-3,17β-dihydroxyestra-1,3,5(10)triene 3,17-Diacetate (8). Compound **7** (1.5 g, 4.77 mmol) was dissolved in pyridine (60 mL), and acetic anhydride (30 mL) was added. After stirring the reaction mixture for 20 h, it was diluted with ice−water (800 mL), acidified with 6 M HCl (100 mL), extracted with ethyl acetate (3 × 300 mL), washed with sodium bicarbonate (300 mL) and dried over anhydrous sodium sulfate. After condensation, the desired product **8** (1.30 g, 68%) was precipitated as a white solid with methanol: mp 148−150 °C. ^{1}H NMR ($CDCl_3$) δ 7.74 (s, 1 H), 6.81 (s, 1 H), 4.70 (t, J = 16.92 Hz, 1 H), 2.90 (m, 2 H), 2.53 (s, 3 H), 2.26 (m, 4 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 1.92 (d, J = 12.75 Hz, 3 H), 1.72 (m, 2 H) 1.47−1.25 (m, 6 H), 0.84 (s, 1 H); IR (film) 2929, 1767, 1732, 1683, 1612, 1369, 1250, 1208, 1174, 1048, 1020, and 917 cm^{-1}; low resolution (ESI): *m*/*z* (rel intensity) 399 (MH^+, 75); 357 (100, $MH^+ - C_2H_3O$). Anal. ($C_{24}H_{30}O_5$) C, H.

2-(1′,1′-Difluoroethyl)-β-estradiol 3,17-Diacetate (9). [Bis(2-methoxyethyl)amino]sulfur trifluoride (BEAST, 2.0 mL, 10.85 mmol) and **8** (1.0 g, 2.64 mmol) were dissolved in anhydrous dichloromethane (25 mL) in a dry bomb reactor. A drop of anhydrous ethanol (20 μL) was added to the solution, and the sealed bomb reactor was placed in an oven and heated at 70 °C for 11 h. After cooling, the reaction mixture was poured into ice−water (50 mL), extracted with dichloromethane (3 × 50 mL), washed with brine (50 mL), and concentrated in vacuo. Compound **9** was isolated via flash chromatography (silica gel, hexanes−ethyl acetate 12:1 by volume, 0.33 g, 30%) and recrystallized from a mixture of ethyl acetate and methanol: mp 154−156 °C. ^{1}H NMR ($CDCl_3$) δ 7.22 (s, 1 H), 6.60 (s, 1 H), 4.47 (t, J = 8.52 Hz, 1 H), 2.65 (m, 2 H), 2.07 (s, 3 H), 2.04 (m, 4 H), 1.76 (t, J = 18.42 Hz, 3 H and m, 1 H overlapped), 1.33 (m, 5 H), 1.27−1.04 (m, 6 H), 0.61 (s, 3 H); IR (film) 2935, 2867, 1773, 1733, 1617, 1575, 1498, 1430, 1409, 1374, 1252, 1201, 1173, 1047, 1018, 925, 871, and 801 cm^{-1}; low resolution CIMS: *m*/*z* (rel intensity) 401.7 ($M - HF^+$, 100). Anal. ($C_{24}H_{30}F_2O_4$) C, H, F.

3,17-Dimethoxy-β-estra-1,3,5(10)-triene (11). β-Estradiol (**6**, 2.00 g, 7.34 mmol) was dissolved in anhydrous THF (25 mL) in a flame-dried round-bottomed flask under argon and cooled to 0 °C. Sodium hydride (0.40 g, 16.52 mmol) was added, and the reaction mixture was allowed to stir for 15 min. Iodomethane (1.03 mL, 16.52 mmol) was added, and the reaction mixture was allowed to warm to room temperature over 24 h. After pouring the reaction mixture into ice−water (25 mL), the product was extracted into ethyl acetate (3 × 25 mL), washed with saturated aqueous sodium bicarbonate (25 mL), and dried over anhydrous magnesium sulfate. Purification via flash chromatography (silica gel, hexanes−ethyl acetate 6:1 by volume) gave **11** (0.87 g, 40%): mp 156−159 °C. ^{1}H NMR ($CDCl_3$) δ 7.18 (d, J = 8.63 Hz, 1 H), 6.68 (d, J = 8.54 Hz, 1 H), 6.60 (s, 1 H), 3.75 (s, 3 H), 3.36 (s, 3 H), 3.29 (t, J = 16.62 Hz, 1 H), 2.84 (m, 2 H), 2.25 (m, 1 H), 2.15 (t, J = 21.87 Hz, 1 H), 2.02 (m, 2 H), 1.84 (m, 1 H), 1.66 (m, 1 H), 1.52−1.17 (m, 7 H), 0.77 (s, 3 H); IR (film) 2945, 2914, 1608, 1504, 1449, 1234, 1104, and 1034 cm^{-1}; low resolution CIMS: *m*/*z* (rel intensity) 301 (MH^+, 100). Anal. Calcd for $C_{20}H_{28}O_2$: C, H.

2-(1′-Ketopropyl)-3,17-β-dimethoxyestra-1,3,5(10)-triene (12). Activated alumina was prepared by heating it to 200 °C in a round-bottom flask under vacuum for 8 h and allowing it to cool under argon. In a sealed tube, **11** (0.500 g, 1.66 mmol), alumina (1 g), propionic acid (0.12 mL, 1.66 mmol), and trifluoroacetic anhydride (0.23 mL, 1.66 mmol) were combined and shaken for 15 h. The red reaction mixture was extracted with ethyl ether (3 × 50 mL), and alumina was removed via suction filtration. The organic solution was washed with saturated aqueous sodium bicarbonate (25 mL) and brine (25 mL) and dried over anhydrous sodium sulfate. Condensation of the organic layer and subsequent flash chromatography (silica gel, hexane:ethyl acetate 15:1 by volume) gave **12** (0.21 g, 36%) as a yellow solid: mp 114 °C. ^{1}H NMR ($CDCl_3$) δ 7.62 (s, 1 H), 6.63 (s, 1 H), 3.84 (s, 3 H), 3.36 (s, 3 H), 3.29 (t, J = 16.54 Hz, 1 H), 2.95 (m, J = 11.29 Hz, 2 H), 2.84 (m, 2 H), 2.34 (m, 1 H), 2.14 (t, J = 17.64 Hz, 1 H), 2.05 (m, 1 H), 1.66 (m, 1 H), 1.52−1.17 (m, 9 H), 1.14 (t, J = 18.32 Hz, 3 H), 0.76 (s, 3 H); IR (film) 2930, 2362, 1664, 1605, 1406, 1260, 1129, and 1032 cm^{-1}; low resolution ESIMS: *m*/*z* (rel intensity) 357 (MH^+, 100). Anal. ($C_{23}H_{32}O_3$) C, H.

2-(1′-Ketopropyl)-17-β-estradiol (13). Chlorotrimethylsilane (0.22 mL, 1.75 mmol) was added to a solution of sodium iodide (0.263 g, 1.75 mmol) and **12** (0.25 g, 0.701 mmol) in anhydrous acetonitrile (10 mL) at 0 °C in a flame-dried round-bottomed flask under argon. The reaction mixture was allowed to stir at room temperature for 48 h, and it developed a deep orange color. Methanol (15 mL) was added. The solution was condensed, and the remaining oil was dissolved in ethyl acetate (25 mL). The organic layer was washed with saturated aqueous $Na_2S_2O_3$ (25 mL), $NaHCO_3$ (25 mL), and brine (25 mL) and dried over anhydrous $MgSO_4$. Concentration of the organic solution yielded a yellow oil, which, upon purification via flash chromatography (column 1: silica gel, hexanes−ethyl acetate 8:1 by volume, column 2: silica gel, chloroform), gave **13** (80 mg, 35%): mp 140−146 °C. ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 1 H), 6.69 (s, 1 H), 3.76 (m, 1 H), 3.02 (dq, J = 1.24 Hz, J = 6.41 Hz, 2 H), 2.86 (m, 2 H), 2.31−1.26 (m, 15 H), 1.21 (t, J = 7.25 Hz, 3 H), 0.80 (s, 3 H); IR (film) 3423, 2933, 1735, 1701, 1642, 1490, 1375, 1270, 1055, and 816 cm^{-1}; low resolution CIMS: *m*/*z* (rel intensity) 329 (MH^+, 100). Anal. ($C_{21}H_{28}O_3 \cdot 0.3\ H_2O$) C, H.

2-Pentafluoroethyl-β-estradiol (15). Anhydrous dimethyl sulfoxide (5 mL), copper powder (40 mg, 0.63 mmol), and 2-iodoestradiol (**14**,[19] 100 mg, 0.25 mmol) were combined in a Teflon bomb reactor, and the Teflon sleeve was cooled to 0 °C in an ice bath. Perfluoroethyl iodide was cooled to −78 °C in a round-bottomed flask, and the liquid (0.055 mL, 0.30 mmol) was added via syringe to the bomb. After sealing the bomb reactor, it was placed in the oven at 80 °C for 16 h. The bomb was cooled to 0 °C before water (20 mL) and ethyl acetate (10 mL) were added. The organic mixture was washed with water (3 × 5 mL) and dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to give a crude oil, which was purified via flash chromatography (silica gel, 5:1 hexanes−ethyl acetate) to give a light yellow oil. The oil was triturated with hexane to give **15** as a white solid (40 mg, 41%): mp 120 °C (dec). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (s, 1 H), 6.69 (s, 1 H), 3.74 (t, J = 8.6 Hz, 1 H), 2.85 (m, 2 H), 2.14−1.20 (m, 15 H), 0.79 (s, 3 H); ^{19}F NMR (282 MHz, MeOH, TFA standard) δ −7.25 (s, 3 F), −34.5 (s, 2 F); IR (film) 3271, 2925, 1595, 1404, 1261, 1132, 1053, 1010, and 751 cm^{-1}; low resolution CIMS: *m*/*z* (rel intensity) 391 (MH^+, 9), 373 ($MH^+ - H_2O$). Anal. ($C_{20}H_{23}F_5O_2$) C, H.

2-Allyl-3,17-bis(*tert*-butyldimethylsilyl)-β-estradiol (17). Anhydrous zinc bromide (0.29 g, 1.28 mmol) was dissolved in anhydrous THF (30 mL) under argon in a flame-dried round-bottomed flask. A solution of allylmagnesium bromide (1.28 mL, 1.0 M in diethyl ether) was added via syringe, and the resulting mixture was allowed to stir at room temperature for approximately 10 min until it became turbid. Tetrakis(triphenylphosphine)palladium(0) (0.074 g, 0.064 mmol) and compound **16**[19] (400 mg, 0.64 mmol) were added, and the mixture was allowed to stir at room temperature for 12 h. The reaction mixture was quenched with water (100 mL), extracted with ethyl acetate (2 × 30 mL), washed with brine (40 mL), and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo and purification via gravity chromatography (silica gel, hexane) yielded **17** (0.26 g, 75%) as a white solid: mp 66 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (s, 1 H), 6.48 (s,

1 H), 5.96 (m, 1 H), 5.00 (m, 2 H), 4.13 (t, $J = 8.03$ Hz, 1 H), 3.31 (d, $J = 5.74$ Hz, 2 H), 2.78 (m, 2 H), 2.29−1.04 (m, 13 H), 0.99 (s, 9 H), 0.88 (s, 9 H), 0.73 (s, 3 H), 0.21 (s, 6 H), 0.03 (s, 3 H), 0.01 (s, 3 H); IR (neat) 2929, 2858, 1501, 1256, 1118, 1100, 895, 806, and 777 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 541 (MH^+, 100). Anal. ($C_{33}H_{56}O_2Si_2$) C, H.

2-Allyl-β-estradiol (18). Compound **17** (100 mg, 0.185 mmol) was dissolved in a solution of 2% hydrochloric acid in 95% ethanol (5 mL) and 2-propanol (5 mL). The reaction mixture was sonicated for 3 h. The volume of the reaction mixture was reduced in volume by ∼80%, and water (5 mL) was added. The aqueous mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic extracts were dried over anhydrous sodium sulfate. The organic solvent was removed in vacuo, and the crude yellow oil was purified by flash chromatography (silica gel: 1% MeOH in $CHCl_3$) yielding **18**. Compound **18** was recrystallized from dichloromethane and hexane to afford a solid (20 mg, 35%): mp 84−85 °C (lit.[34] 82−84 °C). ^{1}H NMR (300 MHz, DMSO) δ 9.02 (s, 1 H), 6.88 (s, 1 H), 6.48 (s, 1 H), 5.89 (m, 1 H), 4.96 (m, 2 H), 3.50 (t, $J = 8.28$ Hz, 1 H), 3.19 (d, $J = 6.37$ Hz, 2 H), 2.66 (m, 2 H), 2.20−0.69 (m, 14 H), 0.64 (s, 3 H).

2-(1′-Hydroxymethyl)-3,17-β-*O*-bis(methoxymethyl)-estradiol (21).[25] Compound **20**[28] (500 mg, 1.28 mmol) was dissolved in a solution of $NaBH_4$ (0.010 g, 2.60 mmol) in methanol (65 mL), and the mixture was stirred at room temperature for 2.5 h. The reaction mixture was quenched with water (50 mL) and acidified to pH 1 with 3 N HCl. The reaction mixture was extracted into dichloromethane (3 × 100 mL), washed with water (50 mL), dried over anhydrous sodium sulfate, and concentrated to yield **21** as a clear oil (0.500 g, 100%). ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 1 H), 6.61 (s, 1 H), 5.17 (s, 2 H), 4.49 (s, 2 H), 4.48 (s, 2 H), 3.44 (t, $J = 8.36$ Hz, 1 H), 3.31 (s, 3 H), 3.19 (s, 3 H), 2.66 (m, 2 H), 2.34 (m, 1 H), 2.14 (t, $J = 17.64$ Hz, 1 H), 2.05 (m, 1 H), 2.16−0.87 (m, 11 H), 0.63 (s, 3 H); IR (film) 3422, 2930, 2362, 1616, 1500, 1467, 1397 1341, 1257, 1206, 1150, 1117, 1056, 1022, 1001, 918 and 868 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 373 (MH^+, 100). Anal. ($C_{23}H_{34}O_5 \cdot 0.4\ H_2O$) C, H.

2-(1′-Methoxymethyl)-3,17-β-*O*-bis(methoxymethyl)-estradiol (22).[26] Compound **21** (500 mg, 1.28 mmol) was dissolved in anhydrous THF (10 mL) in a flame-dried round-bottomed flask under argon and cooled to 0 °C. Sodium hydride (0.048 g, 2.0 mmol) was added, followed by addition of iodomethane (0.125 mL, 2.0 mmol) after hydrogen gas had ceased to evolve. The reaction mixture was allowed to warm to room temperature and stir for 7 h. The solution was poured into ice−water (15 mL), extracted into ethyl acetate (3 × 20 mL), washed with saturated aqueous sodium bicarbonate (20 mL), and dried over anhydrous magnesium sulfate. Condensation of the organic layer gave the desired product **22** (450 mg, 87%) as an oil. ^{1}H NMR ($CDCl_3$) δ 7.24 (s, 1 H), 6.78 (s, 1 H), 5.15 (s, 2 H), 4.63 (s, 2 H), 4.45 (s, 2 H), 3.59 (t, $J = 12.39$ Hz, 1 H), 3.45 (s, 3 H), 3.38 (s, 3 H), 3.35 (s, 3 H), 2.80 (m, 2 H), 2.30 (m, 1 H), 2.15 (s, 2 H), 2.01−1.13 (m, 13 H), 0.78 (s, 3 H); IR (film) 3365, 2928, 1684, 1611, 1501, 1447, 1382, 1260, 1150, 1058, and 919 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 405 (1, M^+), 373 ($MH^+ - CH_3OH$, 100).

2-Methoxymethyl-β-estradiol (23).[26] Compound **22** (150 mg, 0.37 mmol) and pyridinium *p*-toluenesulfonate (0.93 g, 3.7 mmol) were dissolved in methanol (30 mL), and the reaction mixture was heated at reflux for 18 h. The organic material was extracted into ethyl acetate (3 × 50 mL), washed with brine (2 × 50 mL), and dried over anhydrous sodium sulfate to yield impure **23** as a brown oil. Concentration and purification via flash chromatography (silica gel, hexane:ethyl acetate 2:1 by volume) gave a yellow solid which was recrystallized from ethyl acetate to provide pure **23** (60 mg, 51%) as a white solid: mp 171−173 °C (lit.[26] 169 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (s, 1 H), 6.92 (s, 1 H), 6.69 (s, 1 H), 4.65 (d, $J = 12.28$ Hz, 1 H), 4.59 (d, $J = 12.31$ Hz, 1 H), 3.73 (m, 1 H), 3.43 (s, 3 H), 2.80 (m, 2 H), 2.31−1.15 (m, 15 H), 0.78 (s, 3 H).

2-Cyclopropyliminomethylestradiol (25). Cyclopropylamine (1 mL, 14.4 mmol) and anhydrous magnesium sulfate (1 g) were added to substrate **24**[18] (0.3 g, 1 mmol), and the reaction mixture was stirred at room temperature for 8 h. The mixture was diluted with ethyl acetate (40 mL) and filtered. The filtrate was evaporated to give the pure imine **25** as a pale yellow solid (0.32 g, 94%): mp 188−190 °C. ^{1}H NMR ($CDCl_3$) δ 8.43 (s, 1 H), 7.11 (s, 1 H), 6.64 (s, 1 H), 3.74 (m, 1 H), 2.95−2.82 (m, 3 H), 2.35−1.12 (m, 15 H), 0.96−0.91 (m, 4 H), 0.78 (s, 3 H); IR (KBr) 3475, 2920, 2862, and 1624 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 340 (MH^+, 100). Anal. ($C_{22}H_{29}NO_2$) C, H, N.

2-(3-*N*,*N*-Dimethylaminopropyl)iminomethylestradiol (26). 3-*N*,*N*-Dimethylaminopropylamine (1.675 mL, 13.3 mmol) was added to substrate **24**[18] (0.4 g, 1.33 mmol), and the suspension was heated at reflux for 1 h. The excess amine was removed in vacuo, and the crude product was purified by passing it through a column of silica gel (230−400 mesh; methanol) to give **26** as a pale yellow solid (0.42 g, 82%): mp 104 °C. ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1 H), 7.13 (s, 1 H), 6.68 (s, 1 H), 3.75−3.69 (t, $J = 9.00$ Hz, 1 H), 2.87−2.83 (m, 2 H), 2.34−1.10 (m, 27 H), 0.78 (s, 3 H); IR (KBr) 3397, 2926, 2865, 1634 cm^{-1}; CIMS *m/z* (rel intensity) 385 (MH^+, 100). Anal. ($C_{24}H_{36}N_2O_2 \cdot 0.5\ H_2O$) C, H, N.

17,17-Difluoro-β-estra-1,3,5(10)-trien-3-ol (29). Compound **28** (500 mg, 1.85 mmol) and [bis(2-methoxyethyl)amino]-sulfur trifluoride (1.70 mL, 9.25 mmol) were dissolved in dichloromethane (15 mL) under argon in a plastic bottle. The reaction mixture was stirred for 48 h. The argon inlet was removed, one drop of ethanol was added to the reaction mixture, and the reaction mixture was allowed to stir under nonanhydrous conditions for an additional 24 h. The reaction mixture was poured into water (10 mL). The aqueous layer was extracted with dichloromethane (2 × 15 mL), washed with brine (15 mL), and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave an impure yellow oil, which upon flash chromatography (silica gel, hexanes−ethyl acetate 8:1 by volume) yielded the yellow solid **29** (160 mg, 30%): mp 156−158 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (d, $J = 8.43$ Hz, 1 H), 6.62 (dd, $J = 8.32$ and 2.60 Hz, 1 H), 6.55 (bs, 1 H), 4.43 (s, 1 H), 2.80 (m, 2 H), 2.37−1.24 (m, 13 H), 0.89 (d, $J = 1.71$, 3 H); IR (film) 3331, 2942, 2357, 1614, 1588, 1501, 1454, 1384, 1342, 1316, 1289, 1233, 1166, 1103, 1067, 372, 929 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 293 (MH^+, 100) Anal. ($C_{18}H_{22}F_2O$) C, H,

17α-Methyl-β-estradiol (30). A solution of estrone (**28**, 500 mg, 1.85 mmol) in anhydrous THF (50 mL) was cooled to 0 °C in flame-dried round-bottomed flask under argon. A 1.4 M solution of methylmagnesium bromide (36.99 mmol) in toluene (19.8 mL), and THF (6.6 mL) was added slowly to the cold solution via syringe. The reaction mixture was allowed to stir at room temperature for 8 h before it was quenched with saturated aqueous ammonium chloride (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 50 mL), and the combined organic layers were dried over anhydrous sodium sulfate. Condensation and purification via flash chromatography (silica gel, hexanes−ethyl acetate 10:1 by volume) of the crude material gave the white solid **30** (428 mg, 81%): mp 215−216 °C (lit.[35] 191−193 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (d, $J = 8.53$ Hz, 1 H), 6.60 (d, $J = 8.42$ Hz, 1 H), 6.54 (s, 1 H), 4.64 (s, 1 H), 2.81 (m, 2 H), 2.29−1.30 (m, 12 H), 1.25 (s, 3 H), 0.87 (s, 3 H).

3-*O*-Methoxymethylestrone (31). Diisopropylethylamine (4.83 mL, 27.7 mmol) and estrone (**28**, 5 g, 18.5 mmol) were dissolved in anhydrous THF (50 mL), and the mixture was stirred at 0 °C under argon for 0.5 h. Chloromethylmethyl ether (2.11 mL, 27.7 mmol) was added dropwise at 0 °C. The solution was allowed to warm to room temperature for 1 h and was stirred at reflux for 24 h. Following addition of saturated ammonium chloride (100 mL), the solution was extracted with anhydrous diethyl ether (3 × 100 mL), washed with brine (100 mL), and dried over anhydrous magnesium sulfate. Concentration of the product gave a crude orange oil which upon purification via flash chromatography (silica gel, 6:1 hexanes−

ethyl acetate) gave **31** (5.2 g, 89%) as a white solid: mp 93−95 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (d, J = 8.54 Hz, 1 H), 6.82 (dd, J = 8.53 and 2.47 Hz, 1 H), 6.78 (bs, 1 H), 5.13 (s, 2 H), 3.46 (s, 3 H), 2.87 (m, 2 H), 2.53−1.42 (m, 13 H), 0.89 (s, 3 H); IR (film) 2928, 1739, 1607, 1498, 1152, and 1005 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 315 (MH^+, 100). Anal. ($C_{20}H_{26}O_3 \cdot 0.3H_2O$) C, H.

3-*O*-Methoxymethyl-17α-methyl-*β*-estradiol (32). A solution of **31** (3 g, 9.54 mmol) in anhydrous THF (50 mL) was cooled to 0 °C in a flame-dried round-bottomed flask under an argon atmosphere. A 3.0 M solution of methylmagnesium bromide (16 mL, 47.7 mmol) in diethyl ether was added slowly to the cold solution via syringe. The reaction mixture was allowed to stir at room temperature for 20 h and was quenched with saturated aqueous ammonium chloride (50 mL). The aqueous layer was acidified to pH 7 and extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried over anhydrous sodium sulfate. Evaporation of the solvent and purification of the crude material via flash chromatography (silica gel, hexanes−ethyl acetate 6:1 by volume) gave **32** as a white solid (2.3 g, 73%): mp 86−89 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (d, J = 8.46 Hz, 1 H), 6.81 (dd, J = 8.46 and 2.58 Hz, 1 H), 6.75 (d, J = 2.44 Hz, 1 H), 5.13 (s, 2 H), 3.46 (s, 3 H), 2.83 (m, 2 H), 2.31−1.29 (m, 14 H), 1.26 (s, 3 H), 0.89 (s, 3 H); IR (film) 3435, 2932, 1609, 1497, 1370, 1241, 1151, and 1016 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 313 ($MH^+ - H_2O$, 100). Anal. ($C_{21}H_{30}O_3 \cdot 0.23H_2O$) C, H.

17*β*-*O*-Benzyl-3-*O*-methoxymethyl-17α-methylestradiol (33). Compound **32** (3.25 g, 9.84 mmol) was dissolved in anhydrous DMF (30 mL) in a flame-dried round-bottomed flask under argon. The reaction mixture was cooled to 0 °C, and sodium hydride (0.71 g, 29.5 mmol) was added. After allowing the reaction mixture to stir at this temperature for 15 min, benzyl bromide (5.9 mL, 49.2 mmol) was added via syringe, and the mixture was allowed to stir for 6 h. The reaction mixture was cooled to 0 °C, quenched with 50% aqueous ethanol (20 mL), acidified with 3 N HCl to pH 5, and extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with water (50 mL) and brine (40 mL) and dried over anhydrous magnesium sulfate. The organic solution was concentrated in vacuo to give a yellow oil, which, upon purification via flash chromatography (silica gel, 20:1 hexanes−ethyl acetate by volume), gave **33** as a clear oil (2.9 g, 70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.14 (m, 6 H), 6.77 (dd, J = 8.49 and 2.60 Hz, 1 H), 6.71 (d, J = 2.47 Hz, 1 H), 5.09 (s, 2 H), 4.70 (d, J = 11.76 Hz, 1 H), 4.43 (d, J = 11.77 Hz, 1 H), 3.41 (s, 3 H), 2.80 (m, 2 H), 2.26−1.27 (m, 13 H), 1.26 (s, 3 H), 0.94 (s, 3 H); IR (film) 2932, 1608, 1242, 1152, 1017, 922, and 734 cm^{-1}; low resolution ESIMS: *m/z* (rel intensity) 313 ($MH^+ - C_7H_8O$, 100). Anal. ($C_{28}H_{36}O_3$) C, H.

2-Hydroxy-17-*β*-(*S*)-(hydroxyphenylmethyl)-3-*O*-methoxymethyl-17α-methyl-1,3,5(10)-estratrien-2-ol (34). Compound **33** (2.1 g, 4.99 mmol) was dissolved in anhydrous THF (50 mL) in a flame-dried round-bottomed flask under argon. After cooling to −78 °C, *sec*-butyllithium (1.3 M in cyclohexanes, 19.2 mL, 25 mmol) was added. The reaction mixture was allowed to warm to −30 °C over 5 h before trimethyl borate (5.59 mL, 49.9 mmol) was added via syringe. The reaction mixture was allowed to warm to room temperature and stir for 8 h. After the reaction mixture was cooled to 0 °C, 30% H_2O_2 (5.65 mL, 49.9 mmol) and a solution of sodium hydroxide (200 mg, 4.99 mmol) in water (5.4 mL) was slowly added. The reaction mixture was allowed to warm to room temperature and stir for 8 h before it was once again cooled to 0 °C. The peroxide was quenched with saturated aqueous sodium thiosulfate. The aqueous mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo to give a colorless oil. Purification via flash chromatography (silica gel, hexanes−ethyl acetate 10:1 by volume) gave **34** (1.5 g, 70%) as a white solid: mp 150−153 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.26 (m, 5 H), 6.92 (s, 1 H), 6.80 (s, 1 H), 5.73 (s, 1 H), 5.16 (s, 2 H), 4.97 (s, 1 H), 3.51 (s, 3 H), 2.78 (m, 2 H), 2.21−1.26 (m, 14 H), 1.07 (s, 3 H), 0.90 (s, 3 H); IR (film) 3498, 2934, 1591, 1504, 1278, 1152, 1064, 1002, 984, and 710 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 437 (55, MH^+), 419 ($MH^+ - H_2O$, 100). Anal. ($C_{28}H_{36}O_4$) C, H.

17-*β*-(*S*)-(Hydroxyphenylmethyl)-2-methoxy-3-*O*-methoxymethyl-17α-methyl-1,3,5(10)-estratriene (35). Compound **34** (1.0 g, 2.29 mmol) was dissolved in acetone (10 mL) and potassium carbonate (1.58 g, 11.45 mmol) was added. Iodomethane (0.71 mL, 11.45 mmol) was added via syringe, and the reaction mixture was allowed to stir at room temperature for 24 h. Water (20 mL) was added, and the aqueous mixture was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous magnesium sulfate, and concentrated in vacuo to give a brown oil. Purification via flash chromatography (silica gel, 8:1 hexanes−ethyl acetate) and recrystallization from ethyl acetate and hexane gave colorless crystals of **35** (0.90 g, 87%), whose molecular connectivity was confirmed by X-ray analysis: mp 181−182 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.26 (m, 5 H), 6.87 (s, 1 H), 6.86 (s, 1 H), 5.20 (s, 2 H), 4.97 (s, 1 H), 3.87 (s, 3 H), 3.52 (s, 3 H), 2.78 (m, 2 H), 2.05−1.26 (m, 14 H), 1.08 (s, 3 H), 0.95 (s, 3 H); IR (film) 3526, 2934, 1607, 1507, 1262, 1152, 1069, 1022, 980, and 710 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 451 (MH^+, 55). Anal. ($C_{29}H_{38}O_4$) C, H.

17*β*-(*S*)-(Hydroxyphenylmethyl)-2-methoxy-17α-methyl-1,3,5(10)-estratrien-3-ol (36). Compound **35** (80 mg, 0.18 mmol) was dissolved in methanol (10 mL), and 2-propanol (10 mL) and 6 N HCl (10 mL) were added. The reaction mixture was allowed to stir at room temperature for 6 h, and water (20 mL) was added. The aqueous mixture was reduced in volume by evaporation, yielding a turbid white aqueous mixture, which was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo. Purification via flash chromatography (silica gel, hexane:ethyl acetate 5:1 by volume) yielded **36** (50 mg, 69%) as a white solid: mp 183−185 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.24 (m, 5 H), 6.81 (s, 1 H), 6.65 (s, 1 H), 5.41 (s, 1 H), 4.98 (s, 1 H), 3.87 (s, 3 H), 2.76 (m, 2 H), 2.23−1.27 (m, 14 H), 1.08 (s, 3 H), 0.95 (s, 3 H); IR (film) 3436, 2933, 1506, 1450, 1276, 1242, 1122, 1023, 873, and 710 cm^{-1}; low resolution (CI): *m/z* (rel intensity) 407 (MH^+, 100). Anal. ($C_{27}H_{34}O_3$) C, H.

2-Iodo-3-*O*-(methoxymethyl)-17α-methylestradiol (37). *sec*-Butyllithium (1.3 M in cyclohexane, 9.9 mL, 12.9 mmol) was added to a solution of **32** (0.85 g, 2.57 mmol) in anhydrous THF (30 mL) at −78 °C under argon, and the reaction mixture was stirred at the same temperature for 2 h. A solution of iodine (3.27 g, 12.9 mmol) in dry THF (30 mL) was added to the resultant pale yellow-colored solution of the anion, and the reaction mixture was slowly raised to room temperature over 6 h. It was poured into ice−water (100 mL). The crude product was extracted into ethyl acetate (2 × 40 mL), washed with a solution of saturated sodium thiosulfate (2 × 20 mL), and dried (Na_2SO_4). The removal of the solvent gave the crude compound **37** as a brown oil. Purification by column chromatography (silica gel, 15:1 hexanes−ethyl acetate) yielded **37** (1.0 g, 85%) as an off-white solid; mp 54−57 °C. ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 1 H), 6.79 (s, 1 H), 5.19 (s, 2 H), 3.51 (s, 3 H), 2.83−2.79 (m, 2 H), 2.42−1.33 (m, 14 H), 1.28 (s, 3 H), 0.88 (s, 3 H); IR (film) 3436, 2934, 1482, 1380, 1239, 1152, 1086, 1016, 1000, 925, and 736 cm^{-1}; low resolution (CI): *m/z* (rel intensity) 457 (MH^+, 5), 330 ($MH^+ - I$, 100). Anal. ($C_{21}H_{29}IO_3 \cdot 0.45\ C_6H_{14}$) C, H.

2-Iodo-17α-methylestradiol (38). Hydrochloric acid (6 M, 30 mL) was added to a solution of **37** (1.2 g, 2.6 mmol) in THF (30 mL), and the reaction mixture was stirred at room temperature for 6 h. The reaction mixture was poured into ice−water (100 mL) and extracted into ethyl acetate (3 × 20 mL). The combined organic phases were dried (Na_2SO_4). Removal of solvent followed by column chromatographic purification (silica gel, 1:5 ethyl acetate−hexane) gave **38** (0.55 g, 52%) as a colorless solid: mp 140−142 °C. ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 1 H), 6.71 (s, 1 H), 2.81−2.76 (dd, J = 6.0 Hz, 2 H), 2.26−2.23 (m, 1 H), 2.18−2.10 (m, 1 H), 1.89−1.31 (m, 13 H),

1.27 (s, 3 H), 0.89 (s, 3 H); IR (film) 3235, 2932, 1657, 1594, 1449, 1150, 1088, and 955 cm^{-1}; low resolution EIMS: *m/z* (rel intensity) 412 (M^+, 100). Anal. ($C_{19}H_{25}IO_2 \cdot 0.7C_6H_{14}$) C, H.

2-Propynyl-17α-methylestradiol (39). Tributylpropynylstannane (0.48 g, 1.45 mmol) was added to a solution of **38** (0.3 g, 0.73 mmol) in dry THF (40 mL). Tetrakis(triphenylphosphine)palladium (0.084 g, 0.073 mmol) was added, and the reaction mixture was heated at reflux under argon for 10 h. Removal of the solvent followed by chromatographic purification (silica gel, 6:1 hexanes−ethyl acetate) of the crude product gave pure **39** (0.18 g, 76%) as a colorless solid: mp 188−190 °C. ^{1}H NMR ($CDCl_3$) δ 7.21 (s, 1 H), 6.64 (s, 1 H), 5.59 (s, 1 H, exchangeable with D_2O), 2.83−2.79 (dd, J = 6.0 Hz, 2 H), 2.32−2.24 (m, 1 H), 2.12 (s, 3 H), 1.86−1.30 (m, 13 H), 1.27 (s, 3 H), 0.88 (s, 3 H); IR (film) 3401, 2933, 1495, 1372, 1344, 1241, 1086, and 931 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 307 ($MH^+ - H_2O$, 100), 325 (MH^+, 88). Anal. ($C_{22}H_{28}O_2$) C, H.

2-Hydroxy-3,17-*O*-bis(methoxymethyl)estradiol (40). Compound **19** (4.0 g, 11.1 mmol) was dissolved in anhydrous THF (150 mL) in a flame-dried round-bottomed flask under argon. After cooling to −78 °C, *sec*-BuLi (1.3 M in cyclohexane, 26 mL, 33.3 mmol) was added. The reaction mixture was allowed to stir at this temperature for 2 h before trimethyl borate (12.4 mL, 110.9 mmol) was added via syringe. The reaction mixture was allowed to warm to room temperature and stir for 5 h. After the reaction mixture was cooled to 0 °C, 30% H_2O_2 (6.4 mL, 110.9 mmol) and a solution of 1 M aqueous sodium hydroxide (1 mL) were slowly added. The reaction mixture was allowed to warm to room temperature over 8 h. It was cooled to 0 °C, and the peroxides were quenched with saturated aqueous sodium thiosulfate. The aqueous mixture was extracted with ethyl acetate (4 × 50 mL), and the combined organic layers were washed with water (1 × 100 mL). The organic material was dried over anhydrous magnesium sulfate and concentrated in vacuo to give a colorless oil. Purification via flash chromatography (silica gel, hexanes−ethyl acetate 8:1 by volume) gave **40** (3.96 mg, 95%) as a clear oil: ^{1}H NMR ($CDCl_3$) δ 6.89 (s, 1 H), 6.79 (s, 1 H), 5.75 (s, 1 H), 5.16 (s, 2 H), 4.66 (s, 2 H), 3.61 (t, J = 8.24 Hz, 1 H), 3.51 (s, 3 H), 3.37 (s, 3 H), 2.77 (m, 2 H), 2.26−1.18 (m, 13 H), 0.81 (s, 3 H); low resolution CIMS: *m/z* (rel intensity) 377 (MH^+, 40), 345 ($MH^+ - OCH_3$, 60), 315 ($MH^+ - OCH_2OCH_3$, 100). Anal. ($C_{22}H_{32}O_5$) C, H.

2-Methoxyestrone (41). 2-Methoxyestradiol (**1**,[29] 1.50 g, 4.96 mmol) was placed in a 250 mL round-bottomed flask that was equipped with a 25 mL Dean−Stark trap and a reflux condenser. The entire apparatus was flame-dried under an argon atmosphere. Toluene (60 mL) was added to dissolve the starting material. Aluminum isopropoxide (5.1 g, 24.8 mmol) and cyclohexanone (20.5 mL, 198.4 mmol) were added, and the entire reaction mixture was heated at reflux (145−150 °C) for 20 h. The reaction mixture was allowed to cool to room temperature and saturated aqueous sodium bicarbonate solution (100 mL) was added. The organic material was extracted with dichloromethane (3 × 150 mL). The aqueous emulsion was acidified with 3 N HCl (∼20 mL) until the emulsion separated. The aqueous layer was again extracted with dichloromethane (100 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, concentrated in vacuo, and purified via flash chromatography (silica gel, hexanes−ethyl acetate 10:1 by volume) to yield the desired product **41**. Fractions containing **41**, contaminated with cyclohexanone, were combined and concentrated in vacuo. The resulting oil was triturated with hexane to give additional pure product (1.32 g, 89%): mp 187−190 °C (lit.[36] 190−192 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1 H), 6.66 (s, 1 H), 3.86 (s, 3 H), 2.82 (m, 2 H), 2.53−1.37 (m, 14 H), 0.92 (s, 3 H); IR (neat) 3333, 2927, 2854, 1720, 1587, 1515, 1504, 1447, 1431, 1357, 1326, 1291, 1268, 1201, 1026, 873, 827, 581 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 300 (M^+, 100). Anal. ($C_{19}H_{24}O_3$) C, H.

5′,6′-Dihydroestra-1,3,5(10)-triene-[2,3-β]-1′,4′-dioxin-3′-methyl-3′,17β-diol (42). Anhydrous potassium carbonate (4.4 g, 31.8 mmol) and chloroacetone (1.27 mL, 15. 9 mmol) were added to a solution of **40** (1.2 g, 3.18 mmol) in acetonitrile (40 mL), and the reaction mixture was heated at reflux under argon for 6 h. After in vacuo removal of the solvent, the crude product was extracted into ethyl acetate (3 × 30 mL), washed with water (50 mL), and dried (Na_2SO_4). The organic solvent was removed, and the sticky residue was dissolved in THF (50 mL). Following addition of 6 M hydrochloric acid (50 mL), the reaction mixture was stirred at room temperature for 8 h. The reaction mixture was poured into ice−water (50 mL), extracted into ethyl acetate (4 × 20 mL), and dried (Na_2SO_4). The removal of the solvent followed by trituration with dichloromethane gave the pure product **42** (0.62 g, 56%) as a colorless solid: mp 190−192 °C. ^{1}H NMR (DMSO-d_6) δ 6.80 (s, 1 H), 6.71 (s, 1 H), 6.45 (s, 1 H), 4.45 (s, 1 H), 3.90−3.87 (d, J = 9.0 Hz, 1 H), 3.79−3.76 (d, J = 9.0 Hz, 1 H), 3.53−3.48 (t, J = 9.0 Hz, 1 H), 2.68−2.66 (m, 2 H), 2.19−1.53 (m, 5 H), 1.39 (s, 3 H), 1.36−1.07 (m, 8 H), 0.78 (s, 3 H); IR (KBr) 3396, 3224, 2932, 2872, 1586, and 1501 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 345 (MH^+, 100). Anal. ($C_{21}H_{28}O_4 \cdot 0.2CH_3CO_2Et$) C, H.

2-(2′-Hydroxyiminopropyloxy)estradiol (43). Hydroxylamine hydrochloride (0.374 g, 5.0 mmol) was added to a solution of compound **42** (0.344 g, 1.0 mmol) in pyridine (20 mL). The resulting mixture was stirred at 90 °C for 3 h. The pyridine was removed, and the residue was dissolved in ethyl acetate (100 mL) and water (50 mL). The organic layer was washed with brine (2 × 50 mL) and dried (Na_2SO_4). Removal of solvent followed by column chromatography (silica gel, 10:1 ethyl acetate−methanol) gave pure oxime **43** as a fluffy solid (0.3 g, 84%): mp 160−162 °C. ^{1}H NMR ($CDCl_3$) δ 6.89 (s, 1 H), 6.66 (s, 1 H), 4.64 (s, 2 H), 3.76−3.70 (t, J = 9.0 Hz, 1 H), 2.79−2.74 (m, 2 H), 2.27−1.15 (m, 19 H), 0.78 (s, 3 H); IR (KBr) 3327, 2925, 1590, and 1504 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 360 (MH^+, 100). Anal. ($C_{21}H_{29}NO_4 \cdot 0.6H_2O$) C, H, N.

2-Methoxyestrone 3-Benzyl Ether (44). 2-Methoxyestrone (**41**, 100 mg, 0.33 mmol) and potassium carbonate (68 mg, 0.495 mmol) were dissolved in anhydrous DMF (5 mL) in a flame-dried round-bottomed flask under argon. Benzyl bromide (0.39 mL, 3.3 mmol) was added, and the reaction mixture was allowed to warm to room temperature and stir for 19 h. The reaction was quenched with water (5 mL), acidified with 3 N HCl and extracted with diethyl ether (3 × 15 mL). The combined organic layers were washed with water (5 mL) and brine (5 mL) and dried over anhydrous magnesium sulfate. After condensation of the solvent in vacuo, purification by flash chromatography (silica gel: 10:1 hexanes−ethyl acetate by volume) yielded the desired product **44** (113 mg, 88%): mp 89−92 °C (lit.[23] 154−156 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50−7.23 (m, 5 H), 6.87 (s, 1 H), 6.67 (s, 1 H), 5.15 (s, 2 H), 3.91 (s, 3 H), 3.21−1.27 (m, 16 H), 1.01 (s, 1 H); IR (film) 2929, 2364, 2735, 1513, 1453, 1262, 1215, 1015, and 697 cm^{-1}.

3-Benzyloxy-2-methoxy-17α-trifluoromethyl-1,3,5(10)-estratriene-17β-trimethylsilane (45). Compound **44** (130 mg, 0.33 mmol) was dissolved in anhydrous THF (3 mL) in a flame-dried round-bottomed flask under argon and trifluoromethyltrimethylsilane (0.074 mL, 0.50 mmol) was added, followed by one drop of tetrabutylammonium fluoride (1.0 M in THF). The reaction mixture was allowed to stir at room temperature for 10 h, and water (5 mL) was added. The aqueous mixture was extracted with ether (3 × 10 mL), and the combined organic layers were washed with brine (10 mL) and dried over anhydrous magnesium sulfate. After condensing the mixture in vacuo, the crude solid was recrystallized from ethyl acetate to give **45** (110 mg, 70%) as yellow crystals: mp 44−46 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.15 (m, 5 H), 6.69 (s, 1 H), 6.48 (s, 1 H), 4.95 (s, 2 H), 3.71 (s, 3 H), 2.59 (m, 2 H), 2.08−1.09 (m, 13 H), 0.74 (s, 3 H), 0.01 (s, 9 H); IR (film) 2953, 1514, 1255, 1168, 1144, 1028, 841, and 756 cm^{-1}; low resolution CIMS *m/z* (rel intensity) 533 (MH^+, 100). Anal. ($C_{30}H_{39}F_3O_3Si$) C, H.

2-Methoxy-17α-trifluoromethyl-β-estradiol (46). Compound **45** (100 mg, 0.188 mmol) and palladium on carbon (10

wt % on carbon, 0.01 mmol) were combined in a round-bottomed flask under argon and covered with methanol (2 mL). The reaction flask atmosphere was filled with argon and evacuated three times before it was evacuated and filled with hydrogen gas. The reaction mixture was allowed to stir for 8 h. After filtering through a pad of Celite and washing the pad with methanol, dilute hydrochloric acid (10%, 1 mL) was added, and the reaction mixture was allowed to stir for 20 min. The reaction mixture was concentrated in vacuo and purified via flash chromatography (silica gel, hexanes−ethyl acetate 5:1) to give pure **46** as a white solid (70 mg, 100%): mp 58−63 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1 H), 6.66 (s, 1 H), 5.49 (s, 1 H), 3.86 (s, 3 H), 2.80 (m, 2 H), 2.49−1.26 (m, 14 H), 0.92 (s, 3 H); IR (film) 3436, 2930, 1624, 1508, 1447, 1276, 1167, 1131, 1107, 1034, and 875 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 371 (MH^+, 7), 301 ($MH^+ - HCF_3$, 100). Anal. ($C_{20}H_{25}F_3O_3 \cdot 0.16C_6H_{14}$) C, H.

2-Methoxy-17α-methyl-*β*-estradiol (47). Methylmagnesium bromide (1.4 M in toluene, 6.0 mL, 8.4 mmol) was added dropwise to a solution of 2-methoxyestrone (**41**, 0.30 g, 1.0 mmol) in dry THF (40 mL) at 0 °C. The reaction mixture was stirred at room temperature for 48 h. The reaction mixture was quenched with ammonium chloride solution (10 mL). The mixture was extracted into ethyl acetate (3 × 20 mL), dried (Na_2SO_4), and purified by column chromatography on silica gel (15 g, ethyl acetate−hexane 1:2) to afford the product **47** (0.22 g, 70%) as a white solid: mp 158−160 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (s, 1 H), 6.64 (s, 1 H), 5.44 (s, 1 H), 3.86 (s, 3 H), 2.77 (m, 2 H), 1.84−1.21 (m, 14 H), 1.28 (s, overlapped, 3 H), 0.92 (s, 3 H); IR (film) 3542, 2931, 1507, 1273, 1121 and 1085 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 316 (M^+, 100). Anal. ($C_{20}H_{28}O_3$) C, H.

17,17-Difluoro-2-methoxy-1,3,5(10)-estratrien-3-ol (48). BEAST (0.46 mL, 2.50 mmol) and 2-methoxyestrone (**41**, 150 mg, 0.50 mmol) were dissolved in anhydrous dichloromethane (10 mL) in a plastic bottle under argon. One drop of anhydrous ethanol (∼20 μL) was added to the solution, and the reaction mixture was allowed to stir at room temperature for 2 days. The reaction mixture was cooled to 0 °C, and water (20 mL) was added. The reaction mixture was extracted with dichloromethane (3 × 30 mL), and the combined organic phases were dried over anhydrous magnesium sulfate and concentrated in vacuo to give a crude brown oil. Compound **48** was isolated as a yellow oil via flash chromatography (silica gel, hexanes−ethyl acetate 15:1 by volume, 30 mg, 19%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.79 (s, 1 H), 6.65 (s, 1 H), 5.45 (s, 1 H), 3.87 (s, 3 H), 2.78 (m, 2 H), 2.35−1.36 (m, 13 H), 0.93 (s, 3 H); ^{19}F NMR [282 MHz, $CDCl_3$ (TFA standard)] δ −24.14 (dt, J_{FF} = 217.0 Hz, J_{HF} = 25.2 Hz, 1 F), −36.58 (dd, J_{FF} = 218.0 Hz, J_{HF} = 11 Hz, 1 F); IR (film) 3544, 2936, 1508, 1463, 1317, 1277, 1236, 1165, 1127, 1020, and 875 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 322 (M^+, 100). Anal. ($C_{19}H_{24}F_2O_2 \cdot 0.6H_2O$) C, H.

2-Ethoxyestrone (49). 2-Ethoxyestradiol (**4**, 1.88 g, 5.67 mmol) was placed in a 250 mL round-bottomed flask that was equipped with a 25 mL Dean−Stark trap and a reflux condenser. The entire apparatus had been flame dried under an argon atmosphere. Toluene (50 mL) was added to dissolve the starting material. Aluminum isopropoxide (5.7 g, 28.4 mmol) and cyclohexanone (23.5 mL, 226.8 mmol) were added, and the entire reaction mixture was heated at reflux (145−150 °C) for 20 h. The reaction mixture was cooled to room temperature and saturated aqueous sodium bicarbonate solution (100 mL) was added. The organic material was extracted with dichloromethane (3 × 150 mL). The aqueous emulsion was acidified with 3 N HCl (∼20 mL) until it separated, and again the aqueous layer was again extracted with ethyl acetate (2 × 75 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated in vacuo. The crude product was purified via flash chromatography (silica gel, hexanes−ethyl acetate 8:1 by volume) to yield the **49** as a white solid (1.7 g, 94%): mp 140−142 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1 H), 6.66 (s, 1 H), 5.49 (s, 1 H), 4.07 (dq, *J* = 6.9 and 1.6 Hz, 2 H), 2.83 (m, 2 H), 2.52 (m, 2 H), 2.36−1.37 (m, 11 H), 1.42 (t, *J* = 7.0 Hz, 3 H), 0.92 (s, 3 H); IR (film) 3436, 2930, 1738, 1506, 1276, 1199, 1051, and 875 cm-^{1}; low resolution (CI): *m/z* (rel intensity) 315 (MH^+, 100). Anal. ($C_{20}H_{26}O_3 \cdot 0.27H_2O$) C, H.

2-Ethoxy-17-(1′-methylene)estra-1,3,5(10)-trien-3-ol (50). Anhydrous DMSO (10 mL) was added via syringe to a flame-dried round-bottomed flask containing sodium hydride (130 mg, 5.12 mmol) under argon. The reaction mixture was heated to 62 °C behind a blast shield for 1.5 h until a dark green color was observed. (Warning: the dimsyl anion is potentially explosive at temperatures above 60 °C.) The reaction mixture was allowed to cool to room temperature, and methyltriphenylphosphonium iodide (2.07 g, 5.12 mmol) was added. The reaction mixture was allowed to stir for 30 min while a red/brown color emerged. A solution of **49** (200 mg, 0.64 mmol) in anhydrous DMSO (5 mL) was added via syringe, and the reaction mixture was allowed to stir at room temperature for 18 h. After cooling to 0 °C, ice−water (20 mL) was added, and the aqueous mixture was extracted with diethyl ether (3 × 50 mL). The combined ether phases were washed with water (2 × 25 mL) and concentrated in vacuo to give an orange oil. Purification via flash chromatography (silica gel, hexanes−ethyl acetate 10:1 by volume) gave **50** as an orange oil (35 mg, 18%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (s, 1 H), 6.67 (s, 1 H), 5.55 (s, 1 H), 4.71 [d (overlapped), *J* = 2.16 Hz, 1 H], 4.70 [d (overlapped), *J* = 2.14 Hz, 1 H], 4.11 (m, 2 H), 2.84−1.26 (m, 15 H), 1.45 (t, *J* = 7.0 Hz, 3 H), 0.85 (s, 3 H); IR (film) 3548, 2928, 1654, 1592, 1505, 1274, 1197, 1097, 1041, and 875 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 313 (MH^+, 100). Anal. ($C_{21}H_{28}O_2 \cdot 0.6H_2O$) C, H.

2-Ethoxy-17α-methylestradiol (51). Methylmagnesium bromide (1.4 M in toluene, 6.83 mL, 9.6 mmol) was added to a solution of **49** (0.3 g, 0.95 mmol) in anhydrous THF (30 mL) under argon. The reaction mixture was stirred at room temperature for 3 h, and the excess Grignard reagent was carefully quenched with a saturated aqueous solution of ammonium chloride (10 mL). The crude product was extracted into ethyl acetate (3 × 20 mL). The combined organic phases were dried (Na_2SO_4) and purified by column chromatography on silica gel (230−400 mesh, 1:2 ethyl acetate−hexane) to give **51** (0.24 g, 76%) as a white solid: mp 139−143 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1 H), 6.65 (s, 1 H), 4.11−4.05 (q, *J* = 6 Hz, 2 H), 2.80−2.74 (m, 2 H), 2.27−1.30 (m, 18 H), 1.25 (s, 3 H), 0.90 (s, 3 H); IR (film) 3420, 2930, and 1507 cm^{-1}; low resolution CIMS: *m/z* (rel intensity) 313 (MH^+, 100). Anal. ($C_{21}H_{30}O_3 \cdot 0.7$EtOAc) C, H.

X-ray Crystallographic Analysis of 35. A colorless chunk of **35**, $C_{29}H_{38}O_4$ (0.48 mm × 0.44 mm × 0.35 mm), was mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed using Mo Kα radiation (λ = 0.71073 Å) on a Nonius KappaCCD diffractometer. The cell constants and an orientation matrix for data collection were obtained from least-squares refinement, using the setting angles of 9815 reflections in the range of 1° < θ < 27°. The data were collected at a temperature of 150 ± 1 K. A total of 9815 reflections were collected, of which 4581 were unique. The structure was solved by direct methods using SIR2002.[37] The remaining atoms were located in succeeding difference Fourier syntheses. Hydrogen atoms were included in the refinement but restrained to ride on the atom to which they were bonded. The structure was refined in full-matrix least squares where the function minimized was $\Sigma w(|F_o|^2 - |F_c|^2)^2$. Refinement was performed on an AlphaServer 2100 using SHELX-97. Crystallographic drawings were done using programs ORTEP and PLUTON.

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Supporting Information Available: Table of elemental analyses.This material is available free of charge via the Internet at http://pubs.acs.org.

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