Synthesis and Evaluation of B-, C-, and D-Ring-Substituted Estradiol Carboxylic Acid Esters as Locally Active Estrogens

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We have synthesized derivatives of estradiol that are structurally modified to serve as "soft" estrogens and act within a geographically limited area of the body; estrogens without systemic action. We have previously shown with 16α-substituted analogues of estradiol that carboxylates proximal to the steroid ring neither bind to the estrogen receptor nor activate estrogen-responsive genes. However, when the carboxylic acid is masked as an ester, they bind to the receptor and stimulate estrogenic responses. Enzymatic hydrolysis through nonspecific esterases can inactivate these estrogens and thereby limit their area of action. Here, we describe our continued studies to design "soft" estrogens by synthesizing carboxylic acid esters of estradiol at the 7α-, 11β-, and 15α-positions in the steroid nucleus at which bulky substituents are accommodated by the estrogen receptor. These compounds were tested for estrogen receptor binding (estrogen receptors α and β), stimulation of an estrogen sensitive gene in Ishikawa cells in culture, and as substrates for enzymatic hydrolysis. Likely candidates were tested in in vivo assays for systemic and local estrogenic action. The biological studies showed that regardless of the point of attachment, all of the short-chain carboxylic acids, C-1 to C-3, were devoid of hormonal action, while many of the esters were estrogenic. The site on the steroid nucleus had great influence on hormonal activity and esterase hydrolysis. Formate esters at 7α and 15α were good estrogens, but lengthening the chain to acetate dramatically decreased hormonal activity. However, the 7α-formate esters were not enzymatically hydrolyzed. At 11β, the acetate (methyl ester) was an effective estrogen, but increasing the chain length to propionate dramatically reduced hormonal activity. In general, the length of the alcohol from methyl to butyl had only a small effect on receptor binding, and as the size of the alcohol increased, so did esterase hydrolysis. One exception was the 11β-acetate esters where increasing the alcohol moiety from methyl to ethyl eliminated estrogenic activity (Ishikawa cells) without affecting estrogen receptor binding. Several of the esters were tested in vivo, and two, the methyl and ethyl esters of estradiol-15α-formate, appeared to have the requisite properties (high local and low systemic activity) of superior "soft" estrogens.

Introduction

Estrogen replacement therapy for menopausal women is one of the most common therapeutic regimens. It is prescribed for the relief of a number of menopausal symptoms, including hot flashes, vaginal dyspareunia, and the prevention of osteoporosis as well as heart disease. However, it has been shown that estrogen therapy and/or estrogen–progestin therapy is not without its risks[1–4](see editorial[5]). It has been our goal to synthesize a locally active estrogen that could be used to treat vaginal dyspareunia without the risks involved in systemic treatment. Therapeutic agents whose biological actions are limited to the regions to which they are applied have been termed "soft drugs".[6] These drugs have geographically limited actions through rapid metabolism into inactive products in tissues and blood. The most common structural feature of these "soft" drugs are ester groups that can be hydrolyzed to inactive carboxylic acids through the action of nonspecific and ubiquitous esterases.[7]

Recently, we described a series of compounds in which analogues of estradiol (E_2) were modified at 16α with a series of carboxylic esters to produce a locally active estrogen that could be applied directly into and act solely within the vagina, without producing systemic effects.[8] While various preparations of estrogens are currently available for direct vaginal application, they are adsorbed from the vagina into the blood stream, and thus, they act at sites throughout the body.[9–12] Our design for a locally active estrogen is based upon the synthesis of analogues of estradiol containing carboxylates in proximity to the steroid nucleus. These organic acids have poor affinity for the estrogen receptor while esters of these same carboxylic acids bind very well to this receptor.[8] To be restricted to local action, these potent estrogens would be rapidly inactivated by esterases. The 16α-carboxylates of E_2 that we described were designed and tested for the following character-

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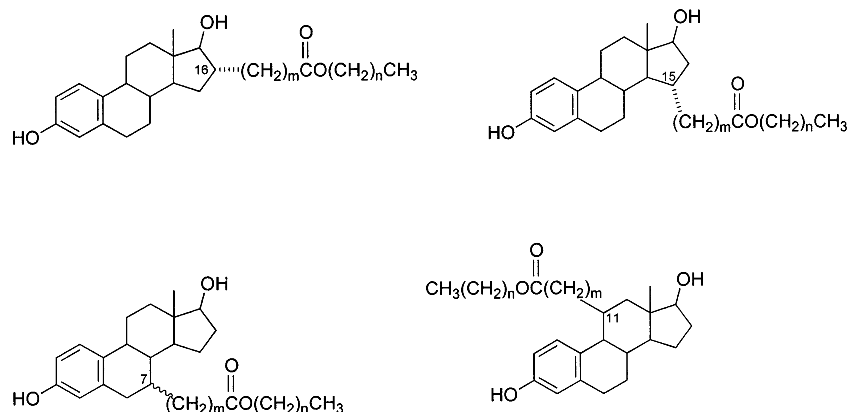


Figure 1. Abbreviation key for the E_2-carboxy esters. E*x*-(m + 1),(n + 1): where *x* is the position in the steroid nucleus from which the ester chain originates and the quantity (m + 1) is the number of carbon atoms in the acid and (n + 1), the alcohol portion of the chain containing the ester. For example, E11-2,2, lower right in the figure, steroid position (*x*) is 11, m and n = 1. In the case of C-7, both epimers were prepared and their stereochemistry is provided within the abbreviation.

istics: affinity for the estrogen receptor; biological activity in an in vitro estrogen sensitive model (endometrial cells in culture); as substrates for esterase hydrolysis; local action through in vivo estrogenic stimulation of the vagina; systemic activity (uterotrophic action). Two of these E_2 analogues, the ethyl and 2′-monofluorethyl esters of E_2-16α-formate, showed the requisite properties with significant differences in their systemic and local actions.

Our aim in the present study was to design a second generation of local estrogens with increased estrogenic potency leading to greater local action and yet decreased systemic action. To this end, we synthesized carboxylic esters of estradiol at positions in the steroid nucleus, 7α, 11β, and 15α (Figure 1), at which substituents are known to be tolerated by the estrogen receptor.[13] In addition, several 7β analogues were isolated during the synthesis of the 7α analogues. These esters and carboxylic acids were tested for estrogenic potential (binding to the estrogen receptor; in vitro bioassay; in vivo, vaginal and uterotrophic assays) and as substrates for esterase enzyme(s).

Chemistry

The synthesis of the 7α-formyl ester anologues of estradiol **8**−**12** is shown in Scheme 1. A cyano group is introduced stereoselectively at the 7α-position of dienone **1** with diethylaluminum cyanide in THF.[14−16] The signal for H-7 in the ^{1}H NMR of **2** appears as a ddd at δ 3.01 with J = 2, 4, and 5.6 Hz consistent with that of an equatorial hydrogen. Aromatization of the A-ring of **2** with $CuBr_2$−LiBr in acetonitrile at reflux[15,17] followed by reduction of the cyano group of **3** with DIBAL-H in toluene gave the α-aldehyde **4**[14] along with removal of the protecting groups. Reprotection with Ac_2O in pyridine followed by Jones oxidation[16] of the aldehyde **5** gave the carboxylic acid **6**. Deprotection with MeOH/HCl (1:1) at room temperature produced the acid E7α-1,0 **7**. The esters **8**−**12** were prepared by reacting **7** with the appropriate alcohols in the presence of $SOCl_2$ or H_2SO_4. The signal for H-7β in the ^{1}H NMR spectrum of these esters appears as a poorly resolved dd at 2.88 ppm with J = 3.6, 5.6 Hz consistent with that of an equatorial hydrogen.

For the synthesis of the 7β-formyl analogues, hydrolysis of 7α-cyanoestradiol **3** in KOH/ethylene glycol produces a 1:5 mixture of the acid E7α-1,0 **7** and E7β-1,0 **13** and can be separated by reversed-phase HPLC (Scheme 1). Esterification of **13** as above gave the esters **14** and **15** whose ^{1}H NMR spectra have a ddd signal at δ 2.62 ppm with J = 6, 8.5, and 9 Hz for H-7α consistent with its axial orientation.

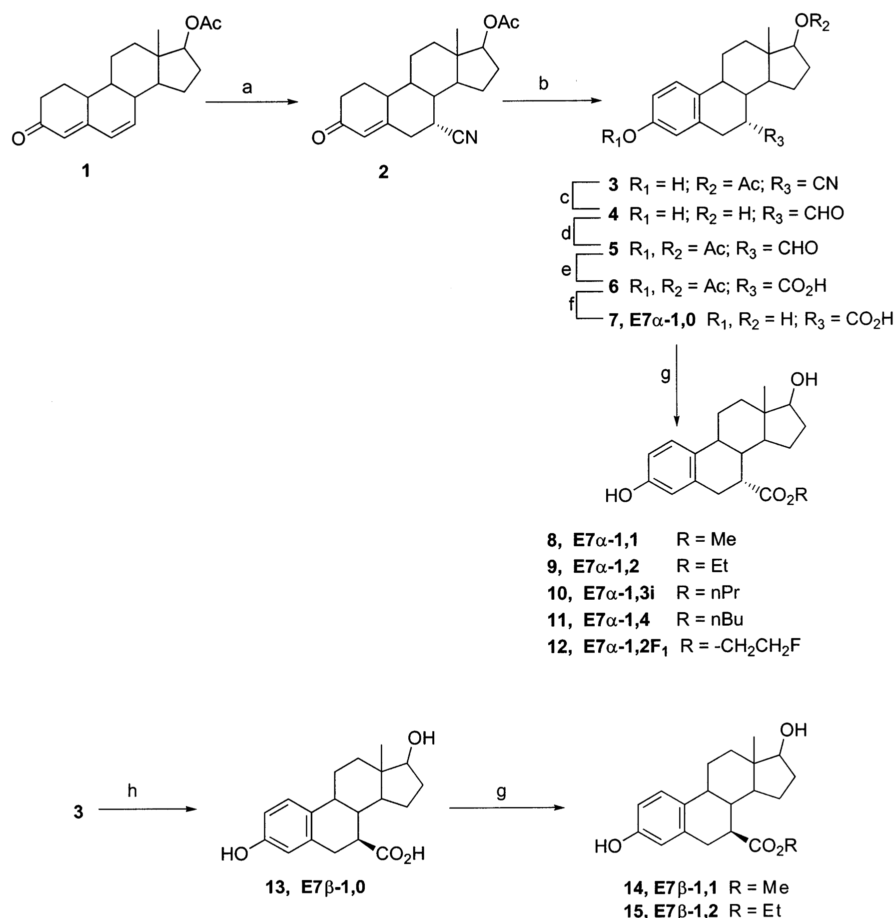
The synthesis of the 7α-carboxymethyl analogues of estradiol **19**−**21** is shown in Scheme 2. Aldehyde **5** was cleanly reduced to the alcohol **16** using $NaBH_4$ in ethanol at 0 °C. That the stereochemistry of C-7 was unchanged during the $NaBH_4$ reduction of **5** to **16** is indicated by the fact that reduction of **5** and **16** with $LiAlH_4$ in ether, conditions known not to affect epimerizable asymmetric centers,[18,19] gives the same product by inspection of the ^{1}H NMR (data not shown). Tosylation of **16** followed by cyanation gave **18**, which was hydrolyzed to the acid E7α-2,0 **19**. Esterification as above gave E7α-2,1 **20** and E7α-2,2 **21**.

The synthesis of the 11β-carboxymethyl analogues of estradiol **32**−**34** is shown in Scheme 3. Estradiol was protected with 3- and 17-benzyl ether groups and oxidized with DDQ in methanol and dioxane to give **24**. Hydroboration and oxidation followed by further oxidation with PCC with methodology used previously[18,20] gave the 11-ketone **26**, which was converted to the 11-methylene compound **27** by Peterson olefination.[21,22] Hydroboration/oxidation of 11-methylene-substituted steroids occurs with delivery of the reagent to the less stearically hindered α-face giving the 11β-hydroxymethyl substituent.[23−25] Olefin **27** was converted to **28** in this manner. Tosylation of the hydroxyl group followed by displacement with cyanide gave the nitrile **30**, which was hydrolyzed to the acid **31**. Removal of the benzyl groups produced E11-2,0 **32**. Esterification as above gave E11-2,1 **33** and E11-2,2 **34**.

Synthesis of the E11-1 formyl ester series was attempted by oxidation of hydroxymethyl steroid **28** with Jones reagent. However, oxidation went no further than the aldehyde, which was stable to a variety of oxidants (TPAP/NMO,[26] H_2O_2, $NaClO_2$,[27] *m*-CPBA, Ag_2O), and only nonpolar degradation products were obtained with $KMnO_4$, Cr_2O_3, and NaOCl.[28]

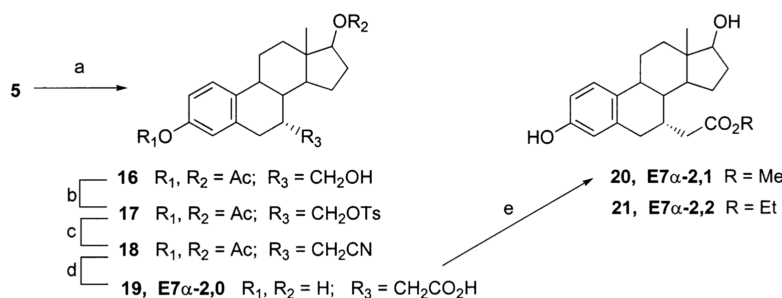
The synthesis of the 11β-carboxyethyl analogues of estradiol **39**−**41** is shown in Scheme 4 and uses an intermediate **36** prepared with methodology used to prepare similar 11β-alkyl-substituted steroids.[20] Addi-

Scheme 1[a]



[a] Key: (a) Et_2AlCN, THF (**1** → **2**); (b) $CuBr_2$, LiBr (**2** → **3**); (c) DIBAL, toluene (**3** → **4**); (d) Ac_2O, pyridine (**4** → **5**); (e) CrO_3, H_2SO_4, acetone (**5** → **6**); (f) MeOH, aq HCl (**6** → **7**); (g) ROH, $SOCl_2$ or H_2SO_4; (h) KOH, ethylene glycol, 120 °C (**3** → **13**).

Scheme 2[a]



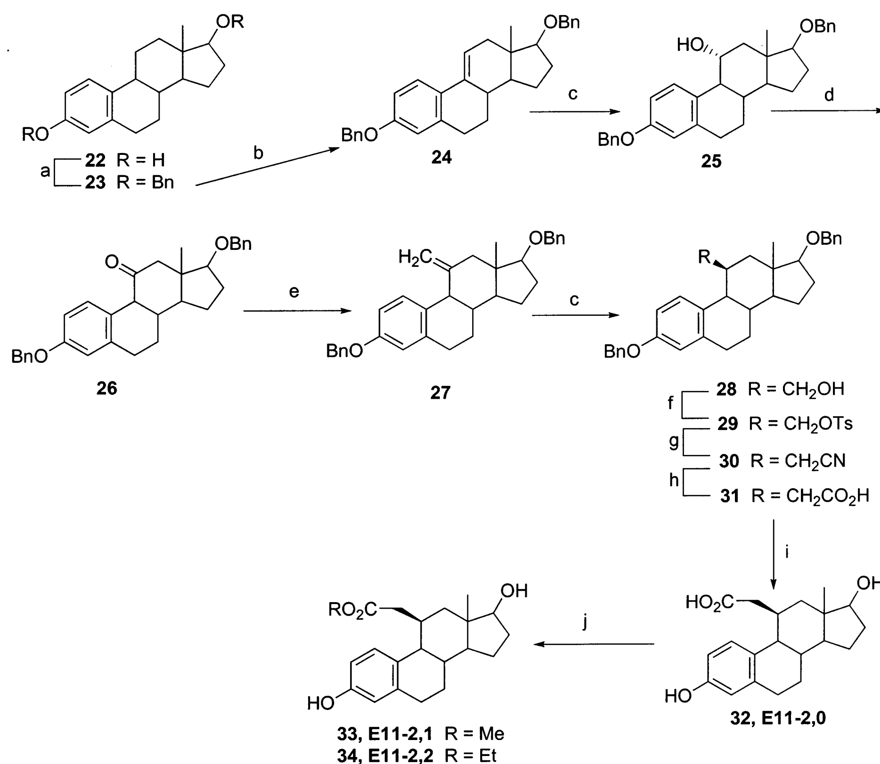
[a] Key: (a) $NaBH_4$, EtOH 0 °C (**5** → **16**); (b) pTsCl, pyridine (**16** → **17**); (c) NaCN, DMSO, 90 °C (**17** → **18**); (d) KOH, ethylene glycol, 150 °C (**18** → **19**); (e) ROH, $SOCl_2$ (**19** → **20**, **21**).

tion of allylmagnesium bromide to ketone **26** from the less stearically hindered α-face yields the 11α-allylhydroxy steroid **35**. Reduction with triethylsilane and $BF_3\cdot OEt_2$ occurs with inversion of C-11 giving the 11β-allyl steroid **36**. Hydroboration/oxidation of the terminal olefin followed by Jones' oxidation produced the acid **38** which was deprotected to yield E11-3,0 **39**. Esterification as above gave E11-3,1 **40** and E11-3,2 **41**.

The synthesis of the 15α-formyl ester analogues of estradiol **49**–**54** is shown in Scheme 5 and employs methodology used previously by Bernstein to prepare 15α-carboxyl substituted estradiol derivatives.[29,30] Ketal **42**[31] was protected as the 3-benzyl ether **43** and carefully deketalized with pTsOH in aqueous acetone at room temperature to give enone **44**. Conjugate addition of NaCN in aqueous THF at reflux gave the 15β-cyano steroid **45**. Ketone reduction followed by nitrile hydrolysis gave the 15α-carboxylic acid **47** via epimerization of the intermediate carboxamide. Hydrogenolysis of **47** with 5% Pd–C/H_2 gave the acid **48**, E151,0. The methyl, ethyl, trifluoroethyl, *n*-propyl, isopropyl, and *n*-butyl esters [E15-1,1 (**49**), E15-1,2 (**50**), E15-1,2 F_1 (**51**), E15-1,3 (**52**), E15-1,3i (**53**), and E15-1,4 (**54**)] were prepared by reacting **48** with the appropriate alcohol in the presence of a catalytic amount of H_2SO_4.

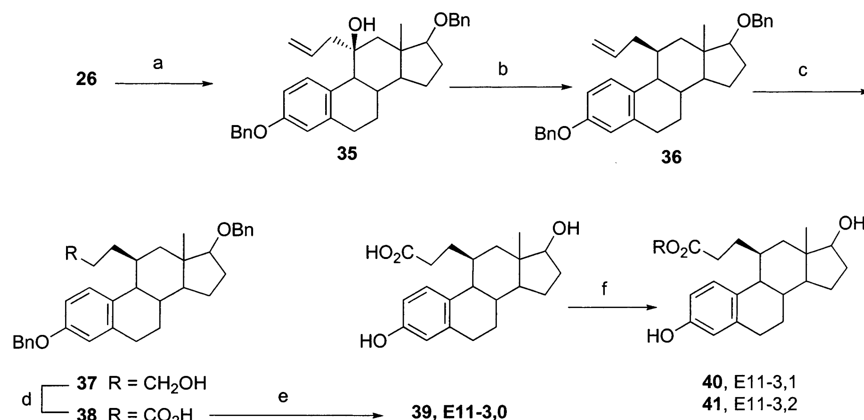
To support the assignment of stereochemistry at C-15, the ester function of E15-1,2 (**50**) was converted to a methyl group giving the known 3-methoxy-15α-methyl-1,3,5(10)-estratriene-17β-ol **60**[32] as follows (Scheme 5). Methylation of the phenolic hydroxyl group followed by

Scheme 3[a]



[a] Key: (a) (i) NaH, **22**, DMF, THF, (ii) BnBr (**22** → **23**); (b) DDQ, MeOH, dioxane (**23** → **24**); (c) (i) catecholborane, $LiBH_4$, THF, (ii) NaOH, H_2O_2; (d) PCC, CH_2Cl_2 (**25** → **26**); (e) (i) Me_3SiCH_2MgCl, Et_2O, (ii) HCl, acetone (**26** → **27**); (f) pTsCl, pyridine (**28** → **29**); (g) NaCN, DMSO, 90 °C (**29** → **30**); (h) KOH, ethylene glycol, 140 °C (**30** → **31**); (i) BCl_3, CH_2Cl_2, 0 °C (**31** → **32**); (j) BCl_3, ROH (**32** → **33**, **34**).

Scheme 4[a]

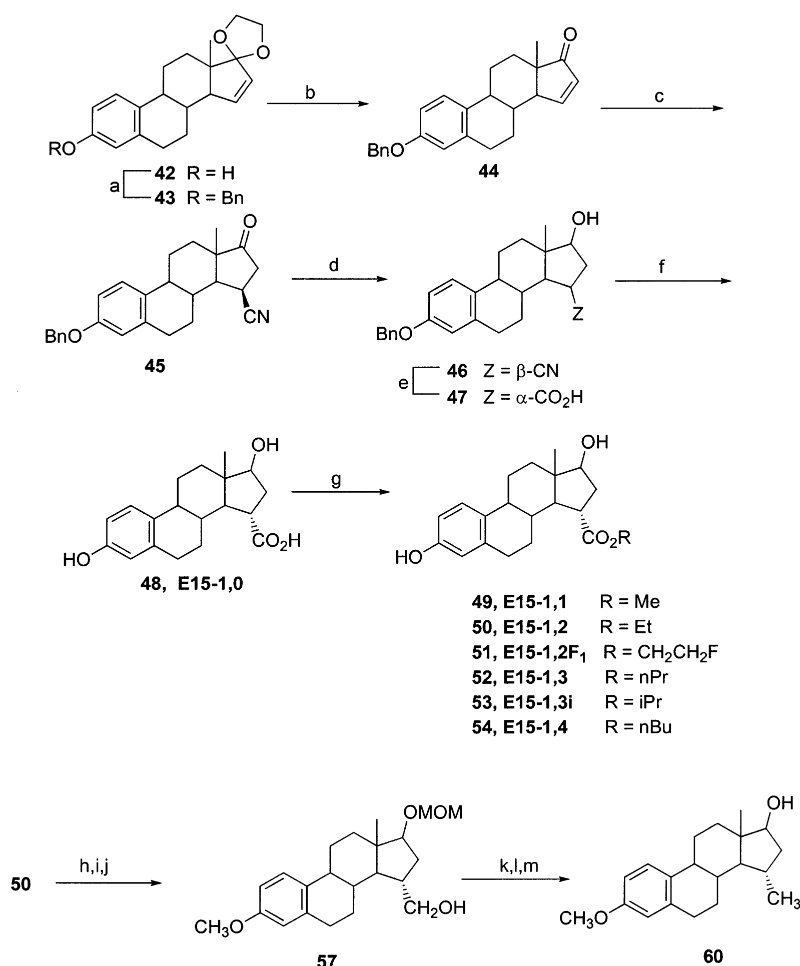


[a] Key: (a) allylmagnesium bromide, THF (**26** → **35**); (b) $HSiEt_3$, $BF_3{\cdot}Et_2O$, 0 °C (**35** → **36**); (c) (i) catecholborane, $LiBH_4$, THF, (ii) NaOH, H_2O_2; (**36** → **37**); (d) CrO_3, H_2SO_4, acetone (**37** → **38**); (e) BCl_3, CH_2Cl_2, 0 °C (**38** → **39**); (f) BCl_3, ROH (**39** → **40**, **41**).

protection of the 17β-hydroxyl group as the MOM-ether gave **56**. Reduction of the 15-ester with $LiAlH_4$, a reagent known not to affect epimerizable asymmetric centers[18,19] gave the hydroxymethyl steroid **57**, which was tosylated giving **58**. Reductive removal of the tosyl group followed by deprotection of the 17-hydroxyl group yielded **60** whose ^{1}H NMR was identical with that reported in the literature.[32] In addition, there is a triplet signal (J = 10.2 Hz) for H-14α at δ 0.90 ppm in the ^{1}H NMR spectrum of **60**, indicating a trans-diaxial relationship with both H-8 and H-15, and is in accord with that seen with other 15α-substituted estradiol compounds.[33]

The synthesis of the 15α-carboxymethyl analogues of estradiol **65**−**67** is shown in Scheme 6 and uses the procedure employed by Kojima to prepare 15α-carboxymethyltestosterone derivatives.[34] Sodium diethylmalonate was added to enone **44** in a Michael reaction to give mainly the α-epimer of **61**. Ester hydrolysis followed by decarboxylation produced only the 15α-carboxymethyl steroid **63**. Ketone reduction with $NaBH_4$ followed by deprotection with 5% Pd−C/H_2 gave the acid E15-2,0, **65**. The esters E15-2,1 **66** and E15-2,2 **67** were prepared by reacting **65** with the appropriate alcohol in the presence of $SOCl_2$. Confirmation of the stereochemistry at C-15 was provided by conversion of **67** to the known 15α-allylestradiol **69**[35] (Scheme 6) by DIBAL reduction to **68** followed by methyleneation with Nystead reagent to **69**. The ^{1}H NMR spectrum of **69** is identical to that reported for 15α-allylestradiol. In

Scheme 5[a]



[a] Key: (a) BnBr, K_2CO_3, $^i Pr_2NEt$, acetone (**42** → **43**); (b) pTsOH, acetone (**43** → **44**); (c) NaCN, THF, 75 °C (**44** → **45**); (d) $NaBH_4$, MeOH, THF (**45** → **46**); (e) KOH, ethylene glycol 160 °C (**46** → **47**); (f) 5% Pd–C/H_2, EtOH (**47** → **48**); (g) ROH, H_2SO_4 (**48** → **49**–**54**); (h) CH_3I, K_2CO_3, acetone 60 °C (**50** → **55**); (i) MOMCl, $^i PrEt_2N$, toluene (**55** → **56**); (j) $LiAlH_4$, Et_2O (**56** → **57**); (k) pTsCl, pyridine, 4 °C (**57** → **58**); (l) $LiEt_3BH$, THF, 65 °C (**58** → **59**); (m) HCl–MeOH (**59** → **60**).

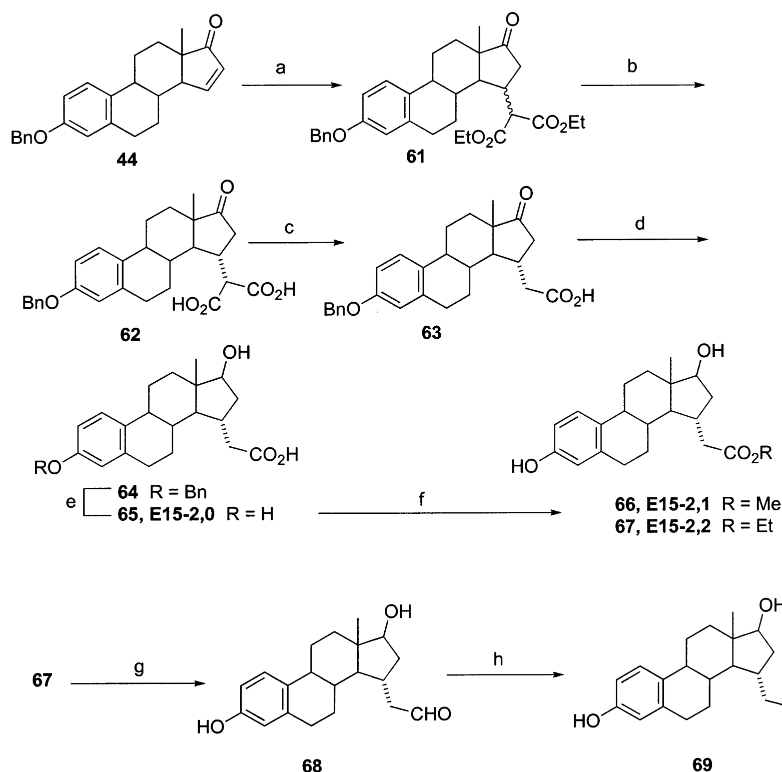
particular the signal for H-14α of 15α-allylestradiol **69** appears as a distinct triplet at δ 1.00 ppm with $J = 10.0$ Hz, whereas the signal for H-14α of the β-allyl epimer would be contained in a region of overlapping signals δ 1.1–1.5 ppm.[35] In addition, this triplet signal at δ 1.00 ppm is present in the ^1H NMR spectrum of each of the 15α-carboxymethyl steroids **65**-**67**.

The synthesis of the 15α-carboxyethyl analogues of estradiol **77**–**79** is shown in Scheme 7 and is based on methodology used previously by Bojack et al.[33] and Dionne et al.[35] to prepare 15α-allylestradiol. The 1, 2-addition of allylmagnesium chloride to enone **44** in THF at 0 °C gave **70** as the only isomer. Anionic oxy-Cope rearrangement with KH and 18-crown-6 in THF produced exclusively the 15α-allylestrone **71**. Reduction of the 17-ketone followed by acetylation of the resulting 17β-alcohol gave **73**. Hydroboration of **73** followed by oxidation with trimethylamine *N*-oxide in diglyme produced the alcohol **74**, which was oxidized with CrO_3–H_2SO_4 to give the acid **75**. Saponification of the acetate group followed by hydrogenolysis of the benzyl group gave E15-3,0 **77**. Esterification of **77** with the appropriate alcohol and $SOCl_2$ gave E15-3,1 **78** and E15-3,2 **79**.

Results and Discussion

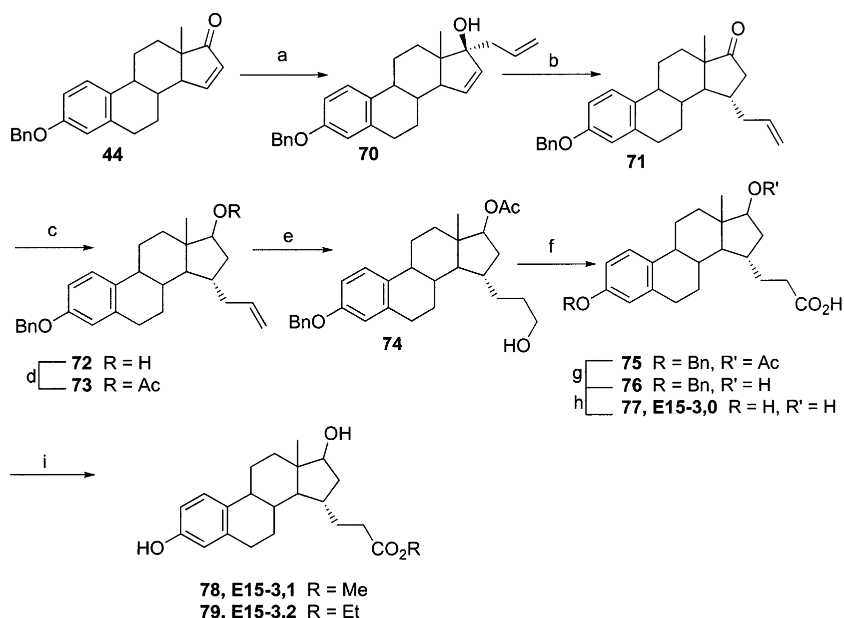
In our previous study of 16α-carboxylate esters of E_2, several general observations could be made about their structure/activity relationships.[8] Increasing the length of the carboxyl chain from C-1 to C-3 (formate, acetate, and propionate) progressively decreased binding to the estrogen receptor. Accordingly, as the carboxylate chain length increased the biological activity (Ishikawa cells) decreased, whereas the length of the alcohol group in the esters had a negligible affect on estrogen receptor binding. Thus, within the limits of the small-chain esters that were synthesized, the size of the alcohol was not as important as the length of the carboxyl moiety. Consequently, esters of exactly the same size but with different carboxyl and alcohol groups had very different affinities for the estrogen receptor, for example, E16-1,3 and E16-2,2 or E16-3,1. Increasing the length of either the alcohol or the carboxylic substituents increased the rate of enzymatic hydrolysis. In studying the potent 16α-formate esters, we noted that while the effect of the length of the alcohol substituent on estrogen receptor binding was minor, the biological effectiveness of the various esters decreased markedly as the alcohol was lengthened greater than ethyl. Since longer alcohol moieties do not appreciably affect estrogen receptor binding but are hydrolyzed faster, it is likely that the decrease in biological estrogenic activity is due to the increase in esterase hydrolysis. Therefore, esterase susceptibility of these compounds is an important

Scheme 6[a]



[a] Key: (a) (i) NaH, diethyl malonate, THF, (ii) **44** (**44** → **61**α,*β*); (b) NaOH, EtOH, H_2O (**61** → **62**); (c) diglyme, 162 °C (**62** → **63**); (d) $NaBH_4$, EtOH (**63** → **64**); (e) 5% Pd−C/H_2, EtOH (**64** → **65**); (f) $SOCl_2$, ROH, 40 °C (**65** → **66,67**); (g) DIBAL, toluene, −60 °C (**67** → **68**); (h) Nystead reagent, $BF_3 \cdot OEt_2$, THF (**68** → **69**).

Scheme 7[a]



[a] Key: (a) allylmagnesium chloride, THF, 0 °C (**44** → **70**); (b) KH, THF, 18-crown-6 (**70** → **71**); (c) $NaBH_4$ THF (**71** → **72**); (d) Ac_2O, pyridine (**72** → **73**); (e) (i) $BH_3 \cdot THF$, THF, (ii) Me_3NO, 150 °C (**73** → **74**); (f) CrO_3−H_2SO_4, acetone, 0 °C (**74** → **75**); (g) KOH, MeOH, 55 °C (**75** → **76**); (h) 5% Pd−C/H_2, EtOH (**76** → **77**); (i) $SOCl_2$, ROH (**77** → **78**, **79**).

determinant in their estrogenic action, and therefore, in their ability to act as "soft" estrogens.

In this study, we synthesized 4 different families of carboxylic acid analogues of E_2 at 7α, 7*β*, 11*β*, 15α and their esters and evaluated them as "soft" estrogens in several different types of assays designed to measure their inherent estrogenic potency as well as to differentiate their systemic and local actions.

7α- and 7*β*- Substitution. As can be seen in Table 1, the 7α-formate derivatives appear to have the requisite estrogenic characteristics that are desired for a "soft" estrogen. The carboxylate E7α-1,0 has very poor affinity for the estrogen receptor and shows almost no activity in the estrogen bioassay with cultured Ishikawa cells. As desired, the methyl and ethyl esters bind to the estrogen receptor with affinities equal to or greater

Table 1. Estrogenic Properties of E_2-alkyl Esters

compd[a]	estrogen receptor (RBA[b])	Ishikawa cell AlkP(RSA[c])	esterase (RHA[d])
E_2	100	100	
E_1	9 ± 6	4 ± 2	
E16-1,2	14 ± 4	9 ± 3	100
E7α-1,0 (**7**)	<0.1	0.3 ± 0.3	
E7α-1,1 (**8**)	12 ± 4	40 ± 25	0
E7α-1,2 (**9**)	10 ± 4	15 ± 12	0
E7α-1,2F_1 (**12**)	3 ± 0.3	2 ± 1	0
E7α-1,3 (**10**)	4 ± 1	1 ± 0.7	0
E7α-1,4 (**11**)	9 ± 2	0.6 ± 0.3	0
E7α-2,0 (**19**)	0.2 ± 0.2	0	
E7α-2,1 (**20**)	3 ± 0.8	0.8 ±0.3	30 ± 1
E7α-2,2 (**21**)	1 ± 0.2	0.2 ± 0.03	70 ± 5
E7β-1,1 (**14**)	0.1 ± 0.1	0.4 ± 0.1	0
E7β-1,2 (**15**)	0.4 ± 0.4	0.4 ± 0.3	0
E11-2,0 (**32**)	0.3 ± 0.2	0.3 ± 0.3	
E11-2,1 (**33**)	24 ± 8	16 ± 10	106 ± 17
E11-2,2 (**34**)	45 ± 19	*e*	152 ± 15
E11-3,0 (**39**)	0.2 ± 0.2	*e*	
E11-3,1 (**40**)	18 ± 3	*e*	64 ± 5
E11-3,2 (**41**)	25 ± 6	*e*	542 ± 84
E15-1,0 (**48**)	0	0	
E15-1,1 (**49**)	20 ± 10	11 ± 5	1.7 ± 0.1
E15-1,2 (**50**)	25 ± 12	18 ± 10	2.3 ± 0.1
E15-1,2F_1 (**51**)	8 ± 3	3 ± 0.2	4.5 ± 0.1
E15-1,3 (**52**)	17 ± 5	2 ± 1	7.8 ± 0.4
E15-1,3i (**53**)	5 ± 4	0.3 ± 0.06	0.4 ± 0.1
E15-1,4 (**54**)	8 ± 2	0.7 ± 0.4	7 ± 0.5
E15-2,0 (**65**)	<0.1	0.2 ± 0.2	
E15-2,1 (**66**)	5 ± 3	1 ± 1	31 ± 6
E15-2,2 (**67**)	2 ± 0	0.4 ± 0.5	71 ± 7
E15-3,0 (**77**)	0	0	
E15-3,1 (**78**)	0.8 ± 0.1	0	248 ± 26
E15-3,2 (**79**)	<0.1	0	674 ± 14

[a] Abbreviations are in Figure 1, with examples as follows. E15-2,0 is the 15α-propionic acid analogue of E_2. E15-2,1 is the methyl ester, 3i, the propyl ester, etc. The stereochemistry is not assigned in the abbreviations C-11, C-15, and C16; they are 11β, 15α, and 16α. [b] RBA is the relative binding affinity in the ER assay, where $E_2 = 100$. [c] RSA is the relative stimulatory activity in the induction of alkaline phosphatase (AlkP) activity in the Ishikawa estrogen bioassay, where $E_2 = 100$. [d] RHA is the relative hydrolytic activity in the esterase assay with hepatic microsomes in comparison to E16-1,2. [e] These compounds produced a small estrogenic response at high concentrations, reaching a plateau at a value of 10−20% of that produced by E_2. The dash (−) indicates not done. All values are ± SD.

than estrone (E_1), and for these types of E_2-esters, they have very high activity in the Ishikawa cell assay. However, they are not hydrolyzed by the nonspecific esterase from rat hepatic microsomes. They are completely stable to enzymatic hydrolysis even after incubation with esterase preparations for 4 h, conditions under which the standard E16-1,2 was hydrolyzed completely in 2 min (not shown). In an attempt to increase the rate of esterase hydrolysis, we synthesized 3 other esters of E7α-1; lengthening the alcohol moiety, E7α-1,3 and E7α-1,4; or employing fluorine in the alcohol E7α-1,-2F_1. In our previous study, both strategies increased the rate of E16-1 ester hydrolysis without having deleterious effects on their estrogenic potency.[8] However, as can be seen in Table 1, neither the insertion of fluorine nor the extension of the alcohol substituent to C-3 or C-4 produced compounds that were hydrolyzed appreciably, and in contrast to the 16α-formate esters their estrogenic potency decreased dramatically. In the case of the 16α-esters, lengthening the carboxylate group from formate to acetate increased esterase hydrolysis. This tactic did increase the hydrolysis of the 7α-2 esters dramatically: E7α-2,1 and E7α-2,2 were hydrolyzed at rates almost comparable to E16-1,2 (30% and 70% respectively). However, like the 16α- analogues, their affinity for the estrogen receptor and their biological activity is exceedingly low. The 7β-formate esters E7β-1,1 and E7β-1,2 are also not hydrolyzed, and more importantly, they are very poor estrogens.

11β-Substitution. We synthesized and tested the 11β-acetate (E11-2) and propionate (E11-3) analogues of E_2. As can be seen in Table 1, the acetate-substituted esters (E11-2,1 and E11-2,2) are hydrolyzed by the esterase preparation at about the same rate as E16-1,2. The propionate analogue E11-3,1 is also cleaved at about the same rate (RHA = 64%), while E11-3,2 is hydrolyzed very rapidly (RHA = 542%). As we anticipated, both E11-2,0 and E11-3,0 are poor ligands for the estrogen receptor, and consequently, they both have low activity in the Ishikawa cell assay. While this activity is low it is nevertheless significant. This residual activity in both assays is higher than any of the other parent carboxylates, formic acid derivatives at 7α, 15α, and 16α. E11-2,1 is a good ligand for the ER (RBA of 24%) with high biological activity in the Ishikawa assay (RSA 16%). However, the results of the Ishikawa assay with E11-2,2 were unexpected. This ester has a very high binding affinity in the estrogen receptor assay, RBA = 45%, which is much higher than all of the other compounds that we tested in this study and yet it demonstrates only very little estrogenic activity in the Ishikawa assay. The esters of the propionate analogue, E11-3,1 and E11-3,2, also have relatively good affinity for the estrogen receptor, RBA = 18% and 25%, respectively, and they too show low activity in the Ishikawa cell assay. This discrepancy between receptor binding and estrogenic activity appears not to be related to rapid hydrolysis to the parent carboxylates since similar esters at 16α with lower affinities for the estrogen receptor and similar or much more rapid rates of enzymatic hydrolysis, have relatively high activity in the Ishikawa cell assay.[8] Preliminary studies indicate that these compounds are strong antiestrogens, and experiments are currently underway in this laboratory to uncover the underlying mechanism.

15α-Substitution. In addition to the above B- and C-ring-substituted esters, we also synthesized a series of D-ring carboxylates and esters at C-15α. Like the E7 and E11 series, the E15-3 (propionate) esters are very poor ligands for the estrogen receptor, and consequently, they are devoid of estrogenic activity in the Ishikawa cell assay. While the C15-2 (acetate) analogues were somewhat better ligands for the estrogen receptor than the E15-3 esters, with RBAs for E15-2,1 and E15-2,2 of 5% and 2%, respectively, their biological activity in the Ishikawa bioassay is low. The E15-1 compounds (formate) have very good estrogenic potential. The formate analogue E15-1,0 does not bind to the estrogen receptor, nor does it have estrogenic activity in the Ishikawa cell assay. However, as would be desired for "soft" hormones, the esters E15-1,1 and E15-1,2 are very potent ligands for estrogen receptor (RBA = 20% and 25%) with very good activity in the Ishikawa cell assay (RSA = 11% and 18%). However, the rate of enzymatic hydrolysis is relatively slow for both (RHA: E15-1,1 = 1.7% and E15-1,2 = 2.3%). As with the 7α-analogues, we attempted

Table 2. Binding of E_2-Alkyl Esters to the LBD of Human ERα and ERβ

compd	ERα[a]	ERβ[a]	ERα/ERβ
E_2	100	100	1
E7α-1,2 (**9**)	11 ± 2	9 ± 2	1 ± 0.1
E11-2,2 (**34**)	66 ± 11	70 ± 14	1 ± 0.04
E11-3,2 (**41**)	66 ± 3	64 ± 7	1 ± 0.4
E15-1,2 (**50**)	22 ± 5	7 ± 2	3 ± 0.6
E16-1,2	27 ± 6	0.3 ± 0.1	95 ± 47

[a] RBA of the indicated ester compared to E_2. Values are ± SD. The inhibition of the binding of $[^3H]E_2$ in lysates of *E. coli* in which the LBD of human ERα and ERβ were separately expressed. Abbreviations are in Table 1. LBD is the ligand binding domain.

Table 3. In Vivo Estrogenic Action: Systemic (Uterotrophic) and Local (Vaginal) Activity[a]

compd	uterotrophic effect 100 μg dose (ng E_2 equiv)	uterotrophic R.A. × 10^3 [b]	vaginal effect 50 ng dose (pg E_2 equiv)	vaginal R.A. × 10^3 [b]
E16-1,2F_1	2[c]	0.02	21[c]	0.42
E7α-1,1 (**8**)	10.5 (10−11)	0.11	50[c]	1.0
E7α-1,2 (**9**)	4 (3.5−4.4)	0.04	22[c]	0.44
E11-2,1 (**33**)	6.5[c]	0.07	21[c]	0.42
E15-1,1 (**49**)	n.s. (0−1.3)	n.s.	19 (15−23)	0.38
E15-1,2 (**50**)	n.s. (0.7−3)	n.s.	21 (18−25)	0.42

[a] In the uterotrophic assay, the results are compared to the effect of 5 ng (total dose administered over 3 days) of E_2 injected subcutaneously in immature rats; in the vaginal assay the results are compared to 50 pg of E_2 administered vaginally to ovariectomized adult mice. In the uterotrophic assays $n = 5$, and in the vaginal assays $n = 5-6$. Values in parentheses show the range. [b] R.A. activity relative to E_2. n.s. = not significantly different from the control. Except where noted, each compound was assayed in three different experiments. [c] Data are from a single experiment.

to increase the hydrolytic rate by introducing fluorine (E15-1,2F_1) or by lengthening the alcohol moiety of the ester (E15-1,3 and E15-1,4). While the hydrolytic rates do increase, the estrogenic activity of these three compounds decreases precipitously when compared to the methyl or ethyl esters. Steric factors are important determinants in the rate of enzymatic hydrolysis: the isopropyl ester E15-1,3i has an RHA = 0.3%, which is much slower than the propyl ester E15−1,3, RHA = 7. Again, lengthening the carboxylic acid chain increases the rate of hydrolysis; compare the RHA of E15-3 > E15-2 > E15-1 esters containing the same alcohols. However, as discussed above, the esters in the E15-2 and E15-3 series have low affinity for the estrogen receptor and poor estrogenic action.

Binding to ERα and ERβ. In addition to the classical estrogen receptor, now called estrogen receptor α (ERα), there is another subtype of estrogen receptor, termed ERβ. These estrogen-activated transcription factors are expressed differently in various tissues, and although they both bind E_2 avidly, they have a somewhat different affinity for other estrogens.[36,37] Various substitutions at 16α of E_2 have a profound differential effect on the binding of the two estrogen receptor subtypes, with preferential binding to ERα.[8,38] In this study, we investigated the relative binding of representative ethyl esters of the analogues at 7α, 11β, and 15α (E7α-1,2, E11-2,2, and E15-1,2) to the ligand binding domain (LBD) of ERα and ERβ. For comparison, E16-1,2, which is ERα specific,[8] was also included in these experiments. As can be seen in Table 2, of the five estrogens, E7α-1,2, E11-2,2, and E11-3,2 bound about equally to ERα and ERβ, while E15-1,2 showed a small selectivity, about 3-fold for ERα. Again, E16-1,2 was a highly selective ligand with about a 95-fold preference for ERα.

In Vivo Studies. Several of the analogues were tested for estrogenic potency in in vivo assays for systemic (uterotrophic assay) and local (vaginal assay) action. In both of these assays, the esters were compared to E_2; 50 pg of E_2 in the vaginal assay (within the dose range that produces a linear response) and 5 ng of E_2 in the uterotrophic assay (the minimum dose that we found reproducibly produces a statistically significant uterine stimulation). The objective of this study was to determine which compounds show the greatest differential between local (high) and systemic (low) action. The results are in Table 3. E7α-1,1 and E7α-1,2, have relatively high activity in the vaginal assay. However, both are also very active in the uterotrophic assay. Likewise, E11-2,1, which is estrogenically active in the Ishikawa assay, is also estrogenic in the vaginal assay, and it too is uterotrophic with an activity even greater than E7α-1,2. The E15-1,1 and E15-1,2 analogues are estrogenic in vitro and in the vaginal assay (50 ng, equivalent to approximately 20 pg of E_2—about the same potency as E7α-1,2 and E11-2,1). However, unlike the other three analogues tested in vivo, neither of these 15α-substituted estrogens produced a statistically significant uterotrophic response at the 100 μg dose. Both were slightly estrogenic at a dose of 300 μg, producing a statistically significant stimulation ($P < 0.05$), E15-1,1 and E15-1,2, equivalent to 4 and 5 ng of E_2, respectively. For comparative purposes, in one of the uterotrophic assays with E15-1,1 and E15-1,2 we included a 100 μg dose of the E16-1,2F_1 group. This compound showed the best differential in comparing local vs systemic estrogenic effects in our previous study.[8] In this assay, 100 μg of E16-1,2F_1 produces a statistically significant ($P < 0.01$) uterotrophic stimulation equivalent to 2 ng of E_2. This is approximately what was observed previously, although in those experiments the standard deviations were higher and the stimulation was not statistically significant.

The relatively high estrogenic potency of the 7α-carboxy esters in both types of in vivo assays is easily explained because these esters are very active in the estrogen receptor binding assay and the Ishikawa cell bioassay and because they cannot be enzymatically hydrolyzed. The esterase enzyme apparently cannot attack the formate esters at 7α (or 7β) due to steric constraints of the proximal steroid ring system. Lengthening the carboxylate by one methylene group to E7α-2 allows esterase access to the ester function, but the E7α-2,1 and E7α-2,2 have very low activity in estrogen receptor and Ishikawa assays. It is evident that while the methyl and ethyl formate esters (E7α-1,1 and E7α-1,2) have the requisite estrogenic action, their resistance to enzymatic hydrolysis made it predictable that they would be estrogenic in the systemic assay. Consequently, they cannot act as local estrogens since they are not inactivated by hydrolysis to E7α-1,0.

Our previous studies showed that esters of carboxylic analogues of E_2 at C-16α that have high estrogenic potential (estrogen receptor binding and Ishikawa cell stimulation) and rapid esterase hydrolysis (E16-1,2 and E16-1,2F_1) generate a large differential between local

and systemic estrogenic activity in vivo.[8] In this view, analogues such as E7α-1 that have high estrogenic potential but are not enzymatically hydrolyzed do not provide this divergence since they have both high local activity as well as high systemic action. Thus, the results of the studies with the E7α and E16 analogues support the rationale behind the design of a "soft" estrogen.

However, the analogues E11-2,1, E15-1,1, and E15-1,2 do not appear to behave according to this model. E11-2,1 is a good ligand for the ER with an RBA considerably higher than estrone; it is also more than four times as active as estrone in the Ishikawa assay, and therefore, as expected, it is a relatively potent estrogen in the vaginal assay. As this ester is cleaved fairly rapidly (RHA = 106%) in the esterase assay, it might be surprising that it is active in the systemic uterotrophic assay. It is even more active than E7α-1,2, which is not hydrolyzed by esterase. Additionally, the results with E15-1,1 and E15-1,2 are unexpected. Both are highly potent estrogens in receptor binding and in the Ishikawa assay, more potent than either E_1 or E16-1,2. Consequently, they are very active in the vaginal assay. Since they are cleaved by esterase(s) at a relatively low rate, RHAs of about 2%, our model would predict them to have a relatively high systemic (uterotrophic) action. However, neither of these 15α-alkyl esters produce a statistically significant estrogenic response in the uterus at the 100 μg dose. As described above, the systemic effect of the 15α-analogues is lower than that produced by E16-1,2F_1, which we previously found shows the greatest differential action. Thus, the uterotrophic activity of E15-1,1 and E15-1,2 was unusually low for all of these E_2-alkyl esters.

The fact that esters such as E11-2,1, E15-1,1, and E15-1,2 show such divergence between the Ishikawa assay (where they are almost as potent as E_2) and the uterotrophic assay (where they are either inactive or almost inactive) demonstrates their unusual susceptibility to catabolism, likely through esterase hydrolysis. This discrepancy between the uterotropic and Ishikawa assays is strong evidence that the esters of the E_2-carboxylates are acting as labile estrogens, since generally the potency of estrogens in the Ishikawa assay closely mirrors in vivo activity.[39] The high systemic activity of E11-2,1 even with its high esterase hydrolysis and (conversely) the low systemic activity of E15-1,1 and E15-1,2, despite their low enzymatic hydrolysis, appear to contradict the "soft" estrogen model. How can estrogens that are enzymatically hydrolyzed at low rates (E15-1,1 and E15-1,2) seem to have the qualities of local estrogens, while an estrogen that is rapidly cleaved (E11-2,1) has such high systemic action? The answer for the high residual activity of E11-2,1 results from the relatively high estrogen receptor binding and Ishikawa bioactivity of the parent carboxylate, E11-2,0. As discussed above both E11-2,0 and E11-3,0 showed measurable estrogen receptor binding and Ishikawa bioactivity. Although the potency of E11-2,0 in both systems is less than 1% of estradiol, it is considerably higher than all of the other carboxylates at C-7, -15, and -16. Apparently, E11-2,0 is of sufficient potency to produce a systemic estrogenic response at high dose. In contrast, the RBA and RSA of the carboxylate, E15-1,0, is very low, not measurable, but as discussed above the hydrolysis of the 15-esters by esterase is relatively slow. However, this rate of hydrolysis is evidently sufficient to hydrolytically deactivate these E15-1 esters. Additionally, the rate of esterase hydrolysis of the E_2-analogues measures only one of the potential catabolic routes of metabolism, albeit the one designed to play the major role. It is well-known that the major secretory estrogen, E_2, is metabolized by a large number of catabolic enzymes. While in the case of the E_2 analogues the esterase enzyme probably plays the most important role, there are other enzymatic routes that also inactivate these steroids. Substituents on E_2 analogues are known to affect these enzymes and play an important role in their metabolic clearance. For example, substituents at C-11 protect steroidal estrogens from metabolism, specifically from the metabolically important 2-hydroxylase[40] and thus, such substituents at C-11 have a major impact, decreasing metabolic clearance and increasing potency.[41] In this view, substitution at 11β as in E11-2,1 protects the estrogen from other catabolic enzymes, thereby extending the estrogenic effect of the parent E11-2,0. This would result in a more systemically potent estrogen. Conversely, substitution at 15α could increase metabolism and therefore decrease the biological $t_{1/2}$ of the E15-1 analogues. Thus, increased metabolism in concert with enzymatic hydrolysis would eliminate systemic activity.

Nevertheless, regardless of the reason, the 15α-alkyl esters E15-1,1 and E15-1,2 have the characteristics of a "soft" estrogen. Interestingly, E15-1,1 and E15-1,2 are as potent in stimulating a vaginal response as E16-1,-2F_1, the best compound in our previous study.[8] However, as discussed above, E15-1,1 and E15-1,2 were inactive in the uterotrophic assay, in contrast to E16-1,2F_1, which produced a small but statistically significant response. Thus, the E15-esters have an improved local to systemic estrogenic profile. E15-1,1 and E15-1,2 have excellent potential for being useful "soft" therapeutic agents for the local treatment of estrogen deprivation.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded with a Bruker AM500 and chemical shifts are reported relative to residual $CHCl_3$ (7.27 ppm) or DMSO (2.5 ppm). Purification by flash chromatography was performed according to the procedure of Still[42] using 230−400 mesh silica gel (EM Science, Darmstadt Germany). High-resolution mass spectra were obtained by electrospray ionization on a Micromass Q-Tof spectrometer by Dr. Walter J. McMurray at the Yale University Comprehensive Cancer Center using either PEG as an internal standard with NH_4OAc or NaI as an internal standard. Elemental analyses were performed by Schwarzkopf Micro Analytical Laboratory, Woodside, NY. The computer program Prism was purchased from GraphPad Software, Inc. (San Diego, CA). The cell culture reagents were obtained from Gibco-BRL (Grand Island, NH). Unless otherwise indicated, solvents (analytical or HPLC grade) and reagents were used as supplied, and all reactions were carried out under nitrogen.

Chromatographic Systems. Thin-layer chromatography (TLC) was performed using Merck silica gel plates (F_{254}) (EM Science) and visualized using phosphomolybdic acid or UV illumination. TLC systems: **T-1**, hexanes/EtOAc (2:1); **T-2**, hexanes/EtOAc (1:1); **T-3**, $CHCl_3$/MeOH (5:1); **T-4**, hexanes/EtOAc (4:1). Analytical high-performance liquid chromatography (HPLC) was performed on a Waters 600E system (Waters Co. Milford MA) equipped with a 484 variable wavelength detector set at 280 nm using the following columns

and systems. Ultrasphere ODS column (5 μm, 10 mm × 25 cm, Altex Scientific Operations Co.) with the following solvent systems at 3 mL/min: **H-1**, HOAc/CH_3CN/H_2O (0.15:25:74.85); **H-2**, CH_3OH/H_2O (60:40); **H-3**, CH_3CN/H_2O (40:60); **H-4**, HOAc/CH_3CN/H_2O (0.13:35:64.87); **H-5**, CH_3CN/H_2O (50:50); **H-6**, HOAc/CH_3CN/H_2O (0.14:30:69.86); **H-7**, HOAc/CH_3CN/H_2O (0.12:40:59.88); **H-8**, CH_3CN/H_2O (45:55). LiChrospher 100 Diol column (5 μm, 4.6 mm × 25 cm, EM Science) with the following solvent systems at 1 mL/min: **H-9**, $CH_2Cl_2/{}^i$PrOH (90:10); **H-10**, $CH_2Cl_2/{}^i$PrOH (99:1); **H-11**, $CH_2Cl_2/{}^i$PrOH (98:2); **H-12**, $CH_2Cl_2/{}^i$PrOH (95:5); **H-13**, HOAc/$CH_2Cl_2/{}^i$PrOH (0.094:94.25:5.65); **H-14**, HOAc/$CH_2Cl_2/{}^i$PrOH (0.1:6:93.9); **H-15**, CH_2Cl_2; **H-16**, CH_2Cl_2/isooctane (80:20); **H-17**, HOAc/$CH_2Cl_2/{}^i$PrOH (0.1:3:96.9); **H-18**, CH_2Cl_2/isooctane (90:10). Protein I-60 column (7.8 mm × 30 cm, Waters Co.) with the following solvent systems at 3 mL/min: **H-19**, HOAc/^{i}PrOH/CH_2Cl_2 (0.1:6:93.9); **H-20**, HOAc/^{i}PrOH/CH_2Cl_2 (0.1:5.99:93.91); **H-21**, CH_2Cl_2; **H-22**, HOAc/^{i}PrOH/CH_2Cl_2 (0.1:3:96.9). Beckman System Gold HPLC system (Beckman Coulter, Inc. Fullerton, CA) consisting of a model 126 solvent module and a model 168 diode array detector set at 280 nm using a Microsorb-MV C18 column (5 μm, 4.6 mm × 25 cm, Varian Analytical Instruments) in the following solvent systems at 1 mL/min: **H-23**, HOAc/CH_3CN/H_2O (0.15:25:74.85); **H-24**, CH_3CN/H_2O (35:65); **H-25**, CH_3CN/H_2O (40:60); **H-26**, CH_3CN/H_2O (50:50); **H-27**, HOAc/CH_3CN/H_2O (0.13:35:64.87); **H-28**, CH_3CN/H_2O (45:55); **H-29**, HOAc/CH_3CN/H_2O (0.14:30:69.86); **H-30**, HOAc/CH_3CN/H_2O (0.13:33:66.87).

17β-Acetoxy-7α-cyanoestr-4-ene-3-one (2). To a solution of 1.96 g of 6-dehydro-19-nortestosterone acetate **1** (6.2 mmol) in THF (100 mL) was added 23 mL of diethylaluminum cyanide (1.0 M solution in toluene, Aldrich). The reaction mixture was stirred at room temperature for 1 h, poured into cold aqueous NaOH solution (0.5 N, 300 mL), and extracted with CH_2Cl_2 (3×, 70 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 1.56 g (74%) of **2**.[14] Data for **2**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, H-18), 2.06 (s, 3H, OAc), 3.01 (ddd, 1H, J = 2.4, 3.6, 5.6 Hz, H-7β), 4.70 (dd, 1H, J = 8.8, 7 Hz, H-17α), 5.98 (s, 1H, H-4).

7α-Cyano-3-hydroxyestra-1,3,5(10)-trien-17β-yl Acetate (3). Compound **3** was prepared by aromatization of **2** (1.56 g, 4.6 mmol) with $CuBr_2$ (2 g, 9 mmol) and LiBr (0.38 g, 4.4 mmol) as described in the literature.[14] Purification by flash chromatography on a 2 × 17 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 0.94 g (60%) of **3**. Data for **3**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H, H-18), 2.07 (s, 3H, OAc), 3.10 (m, 2H, H-6), 4.76 (t, 1H, J = 8.1 Hz, H-17α), 4.88 (s, 1H, 3-OH), 6.57 (d, 1H, J = 2.7 Hz, H-4), 6.70 (dd, 1H, J = 2.5 Hz, 8.3 Hz, H-2), 7.20 (d, 1H, J = 8.4 Hz, H-1).

3,17β-Dihydroxyestra-1,3,5(10)-triene-7α-carboxaldehyde (4). To a solution of 0.25 mL of DIBAL-H (25% in toluene) in toluene (1 mL) was added 50 mg of **3** (0.15 mmol). The reaction mixture was shaken vigorously at room temperature for 3 h. A solution of MeOH (0.2 mL) and 2 N HCl (0.35 mL) was added carefully. The mixture was stirred for 15 min and extracted with EtOAc (3×, 1 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 27 mg (61%) of **4**. Data for **4**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.67 (s, 3H, H-18), 3.54 (t, 1H, J = 8.4 Hz, H-17α), 6.50 (m, 2H, H-2 & 4), 7.05 (d, 1H, J = 8.5 Hz, H-1), 9.76 (s, 1H, CHO).

7α-Formylestra-1,3,5(10)-triene-3,17β-diyl Diacetate (5). A solution of 25 mg of **4** (0.08 mmol) in Ac_2O (0.25 mL) and pyridine (0.5 mL) was stirred at room temperature for 17 h. The mixture was poured into H_2O (5 mL), acidified with concentrated HCl (0.45 mL), and extracted with EtOAc (1 × 25 mL). The organic extract was washed with 1 N HCl (2 × 5 mL) and H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 18 mg (56%) of **5**. Data for **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H, H-18), 2.07 (s, 3H, OAc), 2.29 (s, 3H, OAc), 2.76 (m, 1H, H-7β), 3.07 (m, 2H, H-6), 4.73 (t, 1H, J = 8.4 Hz, H-17α), 6.89 (m, 2H, H-2 & 4), 7.29 (d, 1H, J = 9.2 Hz, H-1), 9.85 (d, 1H, J = 1.9 Hz, CHO).

3,17β-Diacetoxyestra-1,3,5(10)-triene-7α-carboxylic Acid (6). Jones' reagent[43] solution (8 N CrO_3 in aqueous H_2SO_4, 0.1 mL) was added to a solution of 240 mg of **5** (0.6 mmol) in acetone (13 mL) that was cooled to 0 °C. After the mixture was stirred for 30 min, MeOH (50% in H_2O, 5 mL) was added, and the mixture was extracted with EtOAc (3×, 50 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a column of silica gel using hexanes/EtOAc (1.5:1) as eluent gave 0.12 g (48%) of **6**.

3,17β-Dihydroxyestra-1,3,5(10)-triene-7α-carboxylic Acid (7, E7α-1,0). To a mixture of MeOH (5 mL) and concentrated HCl (5 mL) was added 120 mg of **6** (0.3 mmol). The solution was stirred at room temperature for 4 h and then evaporated under a N_2 stream. Purification by preparative reversed-phase HPLC using system H-1 (t_R = 15 min) as eluent gave 52 mg (54%) of **7**. Data for **7**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.65 (s, 3H, H-18); 2.80 (m, 2H, H-6), 3.48 (m, 1H, H-17α), 6.48 (d, 1H, J = 2.6 Hz, H-4), 6.54 (dd, 1H, J = 2.4, 8 Hz, H-2), 7.00 (d, 1H, J = 8.2 Hz, H-1); HRMS (ES$^+$) calcd for $C_{19}H_{24}O_4Na$ (M + Na$^+$) m/e 339.1572, found m/e 339.1580. HPLC system H-9, t_R = 7.1 min, and system H-23, t_R = 14.8 min, >99% pure.

Methyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)formate (8, E7α-1,1). To a solution of 2 mg of **7** (0.006 mmol) in MeOH (1 mL) was added $SOCl_2$ (10 μL). The mixture was stirred at 50 °C for 17 h, evaporated under a N_2 stream diluted with EtOAc (5 mL), washed with saturated aqueous $NaHCO_3$ (2 mL) and H_2O, dried over Na_2SO_4, and evaporated. Purification by preparative reversed-phase HPLC using system H-2 as eluent gave 1.5 mg (75%) of **8**. Data for **8**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (s, 3H, H-18), 2.89 (dd, 1H, J = 3.6, 5.6 Hz, H-7β), 3.00 (m, 2H, H-6), 3.60 (s, 3H, OCH_3), 3.76 (t, 1H, J = 8.2 Hz, H-17α), 4.70 (s, 1H, OH), 6.57 (d, 1H, J = 2.6 Hz, H-4), 6.63 (dd, 1H, J = 2.6, 8.4 Hz, H-2), 7.15 (d, 1H, J = 8.4 Hz, H-1); HRMS (ES$^+$) calcd for $C_{20}H_{26}O_4Na$ (M + Na$^+$) m/e 353.1729, found m/e 353.1718. HPLC system H-10, t_R = 9.7 min, and system H-24, t_R = 10.7 min, >99% pure.

Ethyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)formate (9, E7α-1,2). Compound **9** was prepared by esterification of the acid **7** (8 mg, 0.025 mmol) with EtOH as described for the preparation of **8**. Purification of this material by preparative reversed-phase HPLC using system H-3 (t_R = 12 min) gave 5 mg (57%) of **9**. Data for **9**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 (s, 3H, H-18), 1.12 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 2.79 (m, 1H, H-7β), 2.94 (m, 2H, H-6), 3.67 (m, 1H, H-17α), 4.00 (m, 2H, OCH_2CH_3), 4.94 (s, 1H, OH), 6.53 (m, 2H, H-2 & 4), 7.07 (d, 1H, J = 8.4 Hz, H-1); HRMS (ES$^+$) calcd for $C_{21}H_{28}O_4Na$ (M + Na$^+$) m/e 367.1885, found m/e 367.1895. HPLC system H-11, t_R = 7.3 min, and system H-25, t_R = 10.4 min, >99% pure.

***n*-Propyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)formate (10, E7α-1,3).** Compound **10** was prepared by esterification of the acid **7** (6 mg, 0.019 mmol) with *n*-PrOH as described for the preparation of **8**. Purification of this material by preparative reversed-phase HPLC using system H-3 (t_R = 17 min) gave 2 mg (29%) of **10**. Data for **10**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.80 (s, 3H, H-18), 0.84 (t, 3H, J = 7.2 Hz, $OCH_2CH_2CH_3$), 2.88 (m, 1H, H-7β), 3.05 (m, 2H, H-6), 3.78 (m, 1H, H-17α), 4.00 (m, 2H, $OCH_2CH_2CH_3$), 4.49 (s, 1H, OH), 6.59 (d, 1H, J = 2.7 Hz, H-4), 6.64 (dd, 1H, J = 2.4, 8.3 Hz, H-2), 7.16 (d, 1H, J = 8.6 Hz, H-1), HRMS (ES$^+$) calcd for $C_{22}H_{30}O_4Na$ (M + Na$^+$) m/e 381.2042, found m/e 381.2050. HPLC system H-12, t_R = 9 min, and system H-25, t_R = 15.4 min, >99% pure.

***n*-Butyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)formate (11, E7α-1,4).** Compound **11** was prepared by esterification of the acid **7** (6 mg, 0.019 mmol) with *n*BuOH as described for the preparation of **8**. Purification of this material by preparative reversed-phase HPLC using system H-3 (t_R = 23 min) gave 1.2 mg (17%) of **11**. Data for **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, H-18), 2.88 (m, 1H, H-7β), 3.04

(m, 2H, H-6), 3.78 (m, 1H, H-17α), 4.03 (m, 2H, OCH_2CH_3), 4.48 (s, 1H, OH), 6.59 (d, 1H, J = 2.6 Hz, H-4), 6.64 (dd, 1H, J = 2.5, 8.5 Hz, H-2), 7.16 (d, 1H, J = 8.5 Hz, H-1); HRMS (ES^+) calcd for $C_{23}H_{32}O_4Na$ (M + Na^+) *m/e* 395.2198, found *m/e* 395.2201. HPLC system H-11, t_R = 6.4 min, and system H-26, t_R = 9.8 min, >99% pure.

2′-Fluoroethyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)formate (12, E7α-1,2F1). Compound **12** was prepared by esterification of the acid **7** (5 mg, 0.014 mmol) with fluoroethanol as described for the preparation of **8**. Purification of this material by preparative reversed-phase HPLC using system H-3 (t_R = 10.9 min) gave 0.7 mg (14%) of **12**. Data for **12**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.71 (s, 3H, H-18), 2.87 (m, 1H, H-7β), 2.96 (m, 2H, H-6), 3.67 (m, 1H, H-17α), 4.30 (m, 4H, OCH_2CH_2F), 6.50 (d, 1H, J = 2.5 Hz, H-4), 6.57 (dd, 1H, J = 2.7, 8.4 Hz, H-2), 7.07 (d, 1H, J = 8.4 Hz, H-1); HRMS (ES^+) calcd for $C_{21}H_{27}FO_4Na$ (M + Na^+) *m/e* 385.1791, found *m/e* 385.1788. HPLC system H-10, t_R = 9.8 min, and system H-25, t_R = 8.3 min, >99% pure.

3,17β-Dihydroxyestra-1,3,5(10)-trien-7β-carboxylic Acid (13, E7β-1,0). A solution of 40 mg of **3** (0.12 mmol), 200 mg KOH (3.6 mmol), and ethylene glycol (2 mL) was heated at 120 °C overnight. The reaction mixture was diluted with H_2O (50 mL) and extracted with ether (3×, 50 mL). The combined organic extracts were coevaporated with toluene and purified by preparative reversed-phase HPLC using system H-1 to give 3 mg (8%) of **7** (t_R = 15 min) and 15 mg (41%) of **13** (t_R= 17 min). Data for **13**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.65 (s, 3H, H-18), 2.77 (m, 2H, H-6), 3.47 (m, 1H, H-17α), 6.50 (m, 2H, H-2 & 4), 6.98 (d, 1H, J = 8.3 Hz, H-1); HRMS (ES^+) calcd for $C_{19}H_{24}O_4Na$ (M + Na^+) *m/e* 339.1572, found *m/e* 339.1587. HPLC system H-9, t_R = 7.2 min, and system H-23, t_R = 15.1 min, >96% pure.

Methyl 3,17β-Dihydroxyestra-1,3,5(10)-trien-7β-yl)formate (14, E7β-1,1). Compound **14** was prepared by esterification of the acid **13** (2 mg, 0.006 mmole) with MeOH as described for the preparation of **8** gave 1 mg (50%). Data for **14**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, H-18), 2.23 (s, 3H, OCH_3), 2.62 (ddd, 1H, J = 8.5, 5.8, 9.2 Hz, H-7α), 2.97 (m, 1H, H-6), 3.70 (t, 1H, J = 8.2 Hz, H-17α), 4.71 (s, 1H, OH), 6.57 (d, 1H, J = 2.7 Hz, H-4), 6.67 (dd, 1H, J = 2.6, 8.3 Hz, H-2), 7.15 (d, 1H, J = 8.4 Hz, H-1); HRMS (ES^+) calcd for $C_{20}H_{26}O_4Na$ (M + Na^+) *m/e* 353.1729, found *m/e* 353.1734. HPLC system H-10, t_R = 11 min, and system H-24, t_R = 11.7 min. >99% pure.

Ethyl 3,17β-Dihydroxyestra-1,3,5(10)-trien-7β-yl)formate (15, E7β-1,2). Compound **15** was prepared by esterification of the acid **13** (7 mg, 0.022 mmol) with EtOH as described for the preparation of **8**. Purification of this material by preparative reversed-phase HPLC using system H-3 (t_R = 10 min) gave 3 mg (39%) of **15**. Data for **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 1.29 (t, 3H, J = 7.3 Hz, OCH_2CH_3), 2.62 (m, 1H, H-7α), 2.90(m, 1H, H-6), 3.68 (m, 1H, H-17α), 4.15 (m, 2H, OCH_2CH_3), 4.50 (s, 1H, OH), 6.58 (d, 1H, J = 2.7 Hz, H-4), 6.68 (dd, 1H, J = 2.8, 8.3 Hz, H-2), 7.16 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES^+) calcd for $C_{21}H_{28}O_4Na$ (M + Na^+) *m/e* 367.1885, found *m/e* 367.1888. HPLC system H-10, t_R = 10.2 min, and system H-25, t_R = 11.1 min, >99% pure.

7α-Hydroxymethylestra-1,3,5(10)-trien-3,17β-diyl Diacetate (16). To a solution of **5** (5 mg, 0.013 mmol) in ethanol (0.5 mL) at 0 °C was added 2 mg of $NaBH_4$. After being stirred at 0 °C for 50 min, the mixture was extracted with EtOAc (10 mL), washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 2.5:1 hexanes/EtOAc as eluent gave 2 mg (40%) of **16**. Data for **16**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 2.06 (s, 3H, 17-AcO), 2.28 (s, 3H, 3-AcO), 3.47&3.76 (m, 2H, OCH_2), 4.70 (dd, 1H, J = 7.6, 8.4 Hz, H-17α), 6.84 (m, 2H, H-2 & 4), 7.28 (d, 1H, J = 8.4 Hz, H-1).

3,17β-Diacetoxy-(7α-toluenesulfonyloxymethyl)estra-1,3,5(10)-trien (17). To a solution of 24 mg of **16** (0.06 mmol) in pyridine (2 mL) was added TsCl (40 mg). After the mixture was stirred for 17 h at room temperature, another portion of TsCl (40 mg) was added and the stirring was continued for 3 h. The solution was poured into saturated aqueous $NaHCO_3$ (200 mL) and extracted with EtOAc (3×, 60 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a column of silica gel using 3:1 hexanes/EtOAc as eluent gave 15 mg (46%) of **17**. Data for **17**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (s, 3H, H-18), 2.05 (s, 3H, OAc), 2.28 (s, 3H, OAc), 2.43 (s, 3H, $ArCH_3$), 3.80 (t, 1H, J = 9.4 Hz, H-17α), 4.12 & 4.62 (m, 2H, CH_2OTs), 6.65 (d, 1H, J = 2.3 Hz, H-4), 6.84 (dd, 1H, J = 2.3, 8.5 Hz, H-2), 7.21 (d, 1H, J = 8.9 Hz, H-1), 7.30 (d, 2H, J = 8.5 Hz, ArH), 7.70 (d, 2H, J = 8.3 Hz, ArH).

7α-Cyanomethylestra-1,3,5(10)-triene-3,17β-diyl Diacetate (18). A mixture of 15 mg of **17** (0.028 mmol), NaCN (20 mg), and DMSO (1 mL) was stirred at 90 °C for 2 h. The solution was cooled to room temperature and poured into saturated aqueous NH_4Cl (50 mL). The mixture was extracted with CH_2Cl_2 (3×, 100 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 6:1 hexanes/EtOAc as eluent gave 10 mg (89%) of **18**.

(3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)acetic Acid (19, E7α-2,0). A solution of **18** (10 mg, 0.025 mmol), KOH (0.1 g), and ethylene glycol (1 mL) was heated at 150 °C for 5 days, poured into H_2O, and extracted with EtOAc. Purification by preparative reversed-phase HPLC with system H-6 as eluent gave 6 mg (71%) of **19**. Data for **19**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.66 (s, 3H, H-18), 3.52 (t, 1H, J = 8 Hz, H-17α), 6.40 (d, 1H, J = 2.4 Hz, H-4), 6.52 (dd, 1H, J = 2.6, 8.4 Hz, H-2), 7.07 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES^+) calcd for $C_{20}H_{26}O_4Na$ (M + Na^+) *m/e* 353.1729, found *m/e* 353.1731. HPLC system H-13, t_R = 12.2 min, and system H-29, t_R = 8.6 min >99% pure.

Methyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)acetate (20, E7α-2,1). A solution of 4 mg of **19** (0.012 mmol), MeOH (1 mL), and $SOCl_2$ (20 μL) was stirred at 55 °C for 15 h. The solvent was evaporated under a N_2 stream, and the residue dissolved in EtOAc (50 mL). The solution was washed with saturated aqueous NaHCO, dried over $MgSO_4$, and evaporated. Purification by preparative reversed-phase HPLC using system H-3 gave 2 mg (48%) of **20**. Data for **20**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (s, 3H, H-18), 3.59 (s, 3H, OCH_3), 3.67 (m, 1H, H-17α), 4.46 (s, 1H, OH), 6.48 (d, 1H, J = 2.5 Hz, H-4), 6.58 (dd, 1H, J = 2.6, 8.3 Hz, H-2), 7.12 (d, 1H, J = 8.4 Hz, H-1); HRMS (ES^+) calcd for $C_{21}H_{28}O_4Na$ (M + Na^+) *m/e* 367.1885, found *m/e* 367.1892. HPLC system H-11, t_R = 7.2 min, and system H-24, t_R = 14.2 min >99% pure.

Ethyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-7α-yl)acetate (21, E7α-2,2). Compound **21** was prepared by esterification of **19** (4 mg, 0.012 mmol) with EtOH as described for the preparation of **20**. Purification with preparative reversed-phase HPLC using system H-5 as eluent gave 1.4 mg (32%) of **21**. Data for **21**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.72 (s, 3H, H-18), 1.15 (t, 3H, J = 7.2 Hz, OCH_2CH_3), 3.66 (m, 1H, H-17α), 4.04 (q, 2H, J = 7.2 Hz, OCH_2CH_3), 4.51 (s, 1H, OH), 6.47 (d, 1H, J = 2.6 Hz, H-4), 6.57 (dd, 1H, J = 2.6, 8.4 Hz, H-2), 7.05 (d, 1H, J = 8.5 Hz, H-1); HRMS (ES^+) calcd for $C_{22}H_{30}O_4Na$ (M + Na^+) *m/e* 381.2042, found *m/e* 381.2035. HPLC system H-10, t_R = 8.7 min, and system H-28, t_R = 8.7 min >99% pure.

3,17β-Dibenzyloxyestra-1,3,5(10)-triene (23). A suspension of 20 g of NaH (0.8 mmol) in DMF (100 mL) was cooled to 0 °C with ice bath. Then 25 g of estradiol **22** (92 mmol) in THF (100 mL) was added dropwise over 40 min followed by the addition of BnBr (27 mL) dropwise over 30 min. The reaction was stirred at room temperature for 20 h, diluted with H_2O, and extracted with EtOAc (2×, 250 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. The residue was diluted with methanol (150 mL), and the resulting solid was filtered and washed with MeOH giving 40 g (96%) of **23**: mp 68−70 °C.

3,17β-Dibenzyloxyestra-1,3,5(10),9(11)-tetraene (24). To a solution of 1 g of **23** (2.2 mmol) in MeOH (50 mL) and dioxane (10 mL) was added 0.7 g of DDQ (3 mmol). The reaction was stirred at room temperature for 22 h. The solvent was evaporated at 50 °C, and the residue was dissolved in CH_2Cl_2 and filtered. The filtrate was evaporated and diluted with

MeOH. The resulting solid was collected and washed with MeOH giving 0.44 g (44%) of **24**:[20,44] mp 105−110 °C.

3,17*β*-Dibenzyloxyestra-1,3,5(10)-trien-11α-ol (25). The compound **25** was prepared by hydroxylation of **24** (3 g, 6.6 mmol) with $LiBH_4$ (0.2 g, 10 mmol) and catecholborane (20 mL, 1.0 M in THF, Aldrich) as described in the literature.[20] The solution was stirred at room temperature for 18 h and added dropwise over 30 min to an ice-cold mixture of NaOH (3 g), water (10 mL), EtOH (30 mL), and H_2O_2 (35%, 20 mL). The solution was stirred at room temperature for 5 h and extracted with EtOAc (3×, 100 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 20:1 CH_2Cl_2/EtOAc as eluent gave 2.4 g (78%) of **25**.

3,17*β*-Dibenzyloxyestra-1,3,5(10)-trien-11-one (26) To the solution of 1.0 g of **25** (2.1 mmol) in CH_2Cl_2 (20 mL) was added 1.0 g of PCC (4.8 mmol). The reaction was stirred at room temperature for 3 h, diluted with Et_2O (20 mL), filtered through Florisil, and washed through with CH_2Cl_2/Et_2O (1:1). The filtrate was washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a column of silica gel using hexanes−EtOAc (5:1) as eluent gave 680 mg (68%) of **26**.

3,17*β*-Dibenzyloxy-11-methylenestra-1,3,5(10)-triene (27). To a solution of **26** (0.52 g, 1.1 mmol) in Et_2O (15 mL) was added trimethylsilylmethylmagnesium chloride (15 mL, 1.0 M in ether). The solution was stirred at room temperature for 17 h, quenched with saturated aqueous NH_4Cl (250 mL), and extracted with $CHCl_3$ (500 mL). The organic extract was washed with H_2O, dried over $MgSO_4$, and evaporated. The resulting white solid (0.7 g) was dissolved in a mixture of acetone (10 mL) and concentrated HCl (30*μ*L) and stirred at room temperature for 17 h. The mixture was evaporated to dryness with a N_2 stream, dissolved in EtOAc (50 mL), washed with saturated aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 15:1 hexanes/EtOAc as eluent gave 0.37 g (72%) of **27**. Data for **27**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.84 (s, 3H, H-18), 2.90 (m, 1H, H-6), 3.58 (t, 1H, J = 8.2 Hz, H-17α), 4.57 (s, 2H, benzylic-H), 4.85 (d, 2H, J = 11.2 Hz, $=CH_2$), 5.03 (s, 2H, benzylic-H), 6.71 (d, 1H, J = 2.4 Hz, H-4), 6.78 (dd, 1H, J = 2.5, 8.6 Hz, H-2), 7.35 (m, 11 H, ArH).

3,17*β*-Dibenzyloxy-11*β*-hydroxymethylestra-1,3,5(10)-triene (28). Compound **28** was prepared by hydroxylation of the olefin **27** (0.15 g, 0.3 mmol) with catecholborane as described for the preparation of **25**. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 0.11 g (76%) of **28**. Data for **28**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.01 (s, 3H, H-18), 3.52 (t, 1H, J = 8.5 Hz, H-17α), 3.59&3.72 (m, 2H, CH_2OH), 4.61 (s, 2H, benzylic-H), 5.04 (s, 2H, benzylic-H), 6.71 (d, 1H, J = 2.8 Hz, H-4), 6.80 (dd, 1H, J = 2.7, 8.4 Hz, H-2), 7.24 (d, 1H, J = 8.4 Hz, H-1), 7.38 (m, 10 H, ArH).

3,17*β*-Dibenzyloxy(11*β*-toluenesulfonyloxymethyl)estra-1,3,5(10)-triene (29). To a solution of 200 mg of **28** (0.4 mmol) in pyridine (5 mL) was added 400 mg of pTsCl (2 mmol). The reaction was stirred for 28 h, poured into saturated aqueous $NaHCO_3$ (200 mL), and extracted with EtOAc (3×, 100 mL). Combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 4:1 hexanes/EtOAc as eluent gave 0.2 g (76%) of **29**. Data for **29**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 2.43 (s, 3H, $ArCH_3$), 3.48 (t, 1H, J = 8 Hz, H-17α), 4.00&4.06 (m, 2H, $TsOCH_2$), 4.57 (d, 2H, J = 3 Hz, benzylic-H), 5.06 (s, 2H, benzylic-H), 6.68 (d, 1H, J = 2.6 Hz, H-4), 6.72 (dd, 1H, J = 2.5, 8.4 Hz, H-2), 6.97 (d, 1H, J = 8.7 Hz, H-1), 7.43 (m, 12H, ArH), 7.71 (d, 2H, J = 8.4 Hz, ArH).

3,17*β*-Dibenzyloxy-11*β*-cyanomethylestra-1,3,5(10)-triene (30). The compound **30** was prepared by cyanation of **29** (200 mg, 0.3 mmol) as described for the preparation of **18**. Purification by flash chromatography on a column of silica gel using 6:1 hexanes−EtOAc as eluent gave 140 mg (93%) of **30**. Data for **30**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 3H, H-18), 3.57 (t, 1H, J = 7.4 Hz, H-17α), 4.64 (s, 2H, benzylic-H), 5.09 (s, 2H, benzylic-H), 6.77 (m, 2H, H-2 & 4), 7.09 (d, 1H, J = 8.6 Hz, H-1), 7.48 (m, 10H, ArH).

(3,17*β*-Dibenzyloxyestra-1,3,5(10)-trien-11*β*-yl)acetic Acid (31). A mixture of 140 mg of **30** (0.29 mmol), 700 mg of KOH (13 mmol), and ethylene glycol (5 mL) was heated at 140 °C for 5 days. The mixture was extracted with EtOAc (3×, 10 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 1:2 hexanes/EtOAc as eluent gave 34 mg (23%) of **31**. Data for **31**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (s, 3H, H-18), 3.49 (t, 1H, J = 8.6 Hz, H-17α), 4.56 (s, 2H, benzylic-H), 5.03 (s, 2H, benzylic-H), 6.70 (d, 1H, J = 2.6 Hz, H-4), 6.80 (dd, 1H, J = 2.7, 8.3 Hz, H-2), 7.12 (d, 1H, J = 8.3 Hz, H-1), 7.40 (m, 10H, ArH).

(3,17*β*-Dihydroxyestra-1,3,5(10)-trien-11*β*-yl)acetic Acid (32, E11−2,0). To a solution of 15 mg of **31** (0.03 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C was added BCl_3 (0.6 mL, 1 M in CH_2Cl_2, Aldrich). The reaction was stirred at room temperature for 40 min, quenched with 10 mL of H_2O, and extracted with EtOAc (3×, 20 mL). The combined organic extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by preparative reversed-phase HPLC using system H-4 (t_R = 14 min) as eluent gave 5 mg (51.5%) of **32**. Data for **32**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.78 (s, 3H, H-18), 3.49 (t, 1H, J = 8 Hz, H-17α), 6.43 (d, 1H, J = 2.8 Hz, H-4), 6.54 (dd, 1H, J = 2.8, 8.4 Hz, H-2), 6.90 (d, 1H, J =8.4 Hz, H-1); HRMS (ES$^+$) calcd for $C_{20}H_{26}O_4Na$ (M + Na$^+$) *m*/*e* 353.1729, found *m*/*e* 353.1738. HPLC system H-13, t_R = 18.5 min, and system H-27, t_R = 9.6 min, >99% pure.

Methyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-11*β*-yl)acetate (33, E11−2,1). At 0 °C, BCl_3 (0.4 mL, 1.0 M in CH_2Cl_2) was added to a solution of 7 mg of **31** (0.014 mmol) in CH_2Cl_2 (0.6 mL). The reaction was stirred at 0 °C for 40 min, and then MeOH (0.5 mL) was added. The mixture was stirred for 2 h, diluted with 0.5 mL of saturated aqueous $NaHCO_3$, and extracted with CH_2Cl_2 (3×, 20 mL). Combined extracts were evaporated under N_2 stream, and the residue was purified by preparative reversed-phase HPLC with system H-3 (t_R = 15 min) gave 3 mg (64%) of **33**. Data for **33**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 3H, H-18), 3.79 (s, 3H, OCH_3), 3.87 (t, 1H, J = 7.8 Hz, H-17α), 6.73 (d, 1H, J = 2 Hz, H-4), 6.80 (dd, 1H, J = 2, 8.6 Hz, H-2), 7.20 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES$^+$) calcd for $C_{21}H_{28}O_4Na$ (M + Na$^+$) *m*/*e* 367.1885, found *m*/*e* 367.1885. System H-11, t_R = 7.4 min, and system H-25, t_R = 11.2 min, >99% pure.

Ethyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-11*β*-yl)acetate (34, E11−2,2). The compound **34** was prepared by deprotection of **31** (9 mg, 0.018 mmol) and esterification with EtOH as described for the preparation of **33**. Purification with preparative reversed-phase HPLC with system H-5 (t_R = 8 min) gave 2 mg (31%) of **34**. Data for **34**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (s, 3H, H-18), 1.23 (t, 3H, J = 7 Hz, OCH_2CH_3), 3.72 (t, 1H, J = 8.1 Hz, H-17α), 4.10 (m, 2H, OCH_2CH_3), 4.56 (s, 1H, OH), 6.54 (d, 1H, J = 2.8 Hz, H-4), 6.64 (dd, 1H, J = 2.9, 8.8 Hz, H-2), 7.09 (d, 1H, J = 8.7 Hz, H-1); HRMS (ES$^+$) calcd for $C_{22}H_{30}O_4Na$ (M + Na$^+$) *m*/*e* 381.2042, found *m*/*e* 381.2038. HPLC system H-11, t_R = 6.5 min, and system H-28, t_R = 10.5 min >99% pure.

11α-Allyl-3,17*β*-dibenzyloxyestra-1,3,5(10)-trien-11*β*-ol (35). Allylmagnesium bromide (2.5 mL, 1.0 M in ether, Aldrich) was added to a solution of **26** (0.12 g, 0.26 mmol) in THF (2.5 mL) under N_2. The reaction was stirred at room temperature for 1.5 h, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3×, 20 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 4:1 hexanes/EtOAc as eluent gave 98 mg (74%) of **35**.

11*β*-Allyl-3,17*β*-dibenzyloxyestra-1,3,5(10)-triene (36). To a solution of 98 mg of **35** (0.19 mmol) in CH_2Cl_2 (10 mL) was added $HSiEt_3$ (1 mL). The mixture was cooled to 0 °C,

and $BF_3 \cdot Et_2O$ (2 mL) was added. The reaction was stirred at 0 °C for 40 min, washed with saturated aqueous $NaHCO_3$ followed by H_2O, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 20:1 hexanes/EtOAc as eluent gave 90 mg (95%) of **36**. Data for **36**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3H, H-18), 3.48 (t, 1H, J = 8.5 Hz, H-17α), 4.57 (s, 2H, benzylic-H), 5.00 (m, 2H, C=CH_2), 5.02 (s, 2H, benzylic-H), 5.80 (m, 1H, C−CH=C), 6.69 (d, 1H, J = 2.8 Hz, H-4), 6.78 (dd, 1H, J = 2.9, 8.6 Hz, H-2), 7.09 (d, 1H, J = 8.6 Hz, H-1), 7.36 (m, 10H, ArH).

3,17β-Dibenzyloxy-11β-(3′-hydroxypropyl)estra-1,3,5-(10)-triene (37). Compound **37** was prepared by hydroxylation of the olefin **36** (20 mg, 0.04 mmol) with $LiBH_4$ (1.5 mg) and catecholborane (0.2 mL) as described for the preparation of **25**. Purification by flash chromatography on a 1 × 15 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 16 mg (78%) of **37**. Data for **37**: ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (s, 3H, H-18), 2.75 (m, 2H, H-6), 3.50 (t, 1H, J = 7.2 Hz, H-17α), 3.59 (m, 2H, CH_2O), 4.60 (s, 2H, benzylic-H), 5.04 (s, 2H, benzylic-H), 6.71 (d, 1H, J = 2.4 Hz, H-4), 6.80 (dd, 1H, J = 2.7, 8.7 Hz, H-2), 7.08 (d, 1H, J = 8.6 Hz, H-1), 7.38 (m, 10 H, ArH).

3-(3,17β-Dibenzyloxyestra-1,3,5(10)-trien-11β-yl)propanoic acid (38). Compound **38** was prepared by CrO_3 oxidation of **37** (40 mg, 0.08 mmol) as described for the preparation of **6**. Purification by flash chromatography on a column of silica gel using 4:1 hexanes/EtOAc as eluent gave 15 mg (36%) of **38**. Data for **38**: ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3H, H-18), 3.49 (t, 1H, J = 8.5 Hz, H-17α), 4.59 (d, 2H, J = 4.6 Hz, benzylic-H), 5.04 (s, 2H, benzylic-H), 6.70 (d, 1H, J = 2.5 Hz, H-4), 6.81 (dd, 1H, J = 2.6, 8.5 Hz, H-2), 7.09 (d, 1H, J = 8.6 Hz, H-1), 7.38 (m, 10 H, ArH), 8.4 (broad, 1H, COOH).

3-(3,17β-Dihydroxyestra-1,3,5(10)-trien-11β-yl)propanoic Acid (39), Methyl Ester (40), and Ethyl Ester (41). BCl_3 (0.6 mL) was added to a solution of **38** (15 mg, 0.029 mmol) in CH_2Cl_2 (1.5 mL) at 0 °C and the mixture stirred for 40 min. The solution was divided into three aliquots, H_2O (0.5 mL), MeOH (0.5 mL), or EtOH (0.5 mL), respectively, was added, and the reactions were stirred at room temperature for 3 h. The three mixtures were separately extracted with EtOAc (3×, 10 mL). The organic extracts from each were washed with H_2O, dried over Na_2SO_4 and evaporated. The residues were purified by preparative reversed-phase HPLC.

3-(3,17β-Dihydroxyestra-1,3,5(10)-trien-11β-yl)propanoic acid (39, E11-3,0) using system H-7, t_R = 11 min, gave 1.2 mg (36%). Data for **39**: ^1H NMR (400 MHz, DMSO-d_6) δ 0.77 (s, 3H, H-18), 3.47 (t, 1H, J = 8 Hz, H-17α), 6.40 (d, 1H, J = 2.1 Hz, H-4), 6.52 (dd, 1H, J = 2.1, 8.1 Hz, H-2), 6.95 (d, 1H, J = 8.7 Hz, H-1); HRMS (ES^+) calcd. for $C_{21}H_{28}O_4Na$ (M + Na^+) m/e 367.1885, found m/e 367.1878. HPLC system H-9, t_R = 7 min, and system H-27, t_R = 11.8 min >99% pure.

Methyl 3-(3,17β-dihydroxyestra-1,3,5(10)-trien-11β-yl)-propanoate (40, E11-3,1) using system H-8, t_R = 11 min, gave 2 mg (59%). Data for **40**: ^1H NMR (400 MHz, $CDCl_3$): δ 0.94 (s, 3H, H-18), 3.62 (s, 3H, OCH_3), 3.73 (t, 1H, J = 8 Hz, H-17α), 4.52 (s, 1H, OH), 6.55 (d, 1H, J = 2.6 Hz, H-4), 6.66 (dd, 1H, J = 2.7, 8.5 Hz, H-2), 7.05 (d, 1H, J = 8.3 Hz, H-1); HRMS (ES^+) calcd. for $C_{22}H_{30}O_4Na$ (M + Na^+) m/e 381.2042, found m/e 381.2045. HPLC system H-10, t_R = 8.6 min, and system H-28, t_R = 9.6 min >99% pure.

Ethyl 3-(3,17β-dihydroxyestra-1,3,5(10)-trien-11β-yl)-propanoate (41, E11-3,2) using system H-5, t_R = 11 min, gave 2 mg, (56%). Data for **41**: ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (s, 3H, H-18), 3.73 (m, 1H, H-17α), 4.12 (q, 2H, J = 7.2 Hz, OCH_2), 4.53 (s, 1H, OH), 6.55 (d, 1H, J = 2.5 Hz, H-4), 6.67 (dd, 1H, J = 2.6, 8.6 Hz, H-2), 7.06 (d, 1H, J = 8.7 Hz, H-1); HRMS (ES^+) calcd for $C_{23}H_{32}O_4Na$ (M + Na^+) m/e 395.2198, found m/e 395.2208. HPLC system H-10, t_R = 8 min, and system H-28, t_R = 12.9 min >99% pure.

3-Benzyloxy-17,17-ethylenedioxyestra-1,3,5(10),15-tetraene (43). A solution of 1.90 g (6.07 mmol) of **42**,[31] 940 μL (7.89 mmol) of benzyl bromide, 1.09 (7.89 mmol) of K_2CO_3, and 1.06 mL (6.07 mmol) of diisopropylethylamine in 50 mL acetone was stirred at room temperature for 3 days. The reaction mixture was poured into H_2O (150 mL) and extracted with EtOAc (3×, 100 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 2.37 g (97%) of **43**. Data for **43**: TLC, T-1, R_f 0.54.

3-Benzyloxyestra-1,3,5(10),15-tetraen-17-one (44). A solution of 1.04 g (2.57 mmol) of **43**, 117 mg (0.617 mmol) of pTsOH in acetone (70.3 mL), and H_2O (11.4 mL) was stirred at room temperature for 1.5 h. The reaction mixture was adjusted to pH 7 with 5% $NaHCO_3$ and concentrated by rotovap to about 30 mL without heating. The solution was poured into H_2O (50 mL) and extracted with EtOAc (3×, 70 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a white solid. Crystallization of the residue from CH_2Cl_2/hexanes gave 477 g (52%) of **44** as fine white needles. Data for **44**: TLC, T-1, R_f 0.5; ^1H NMR (400 MHz, $CDCl_3$) δ 1.12 (s, 3H, H-18), 5.05 (s, 2H, benzylic-H), 6.10 (dd, 1H, J = 5.9, 3.2 Hz, H-15), 6.76 (d, 1H, J = 2.7 Hz, H-4), 6.81 (dd, 1H, J = 8.6, 2.7 Hz, H-2), 7.22 (d, 1H, J = 8.6 Hz, H-1), 7.31−7.45 (m, 5H, Ar−H), 7.64 (dd, 1H, J = 5.9, 1.1 Hz, H-16).

3-Benzyloxy-15β-cyanoestra-1,3,5(10)-trien-17-one (45). This procedure is based on the literature method.[29,30] A solution of 1.03 g (2.88 mmol) of **44** and 2.03 g (41.5 mmol) of NaCN in THF (30 mL) with 21 drops of H_2O was stirred and heated at 75 °C under reflux for 2 h. The reaction mixture was poured into ice−water (300 mL) and extracted with CH_2Cl_2 (3×, 100 mL). Combined organic extracts were washed with H_2O (100 mL), dried over Na_2SO_4, and concentrated in vacuo giving a brown oil. Purification by flash chromatography on a 3 × 17 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 557 mg (50%) of **45** as a white solid. Data for **45**: TLC, T-1, R_f 0.3; ^1H NMR (400 MHz, $CDCl_3$) δ 1.24 (s, 3H, H-18), 5.06 (s, 2H, benzylic-H), 6.76 (d, 1H, J = 2.7 Hz, H-4), 6.81 (dd, 1H, J = 8.6, 2.7 Hz, H-2), 7.20 (d, 1H, J = 8.6 Hz, H-1), 7.33−7.45 (m, 5H, Ar-H).

3-Benzyloxy-15β-cyanoestra-1,3,5(10)-trien-17β-ol (46). A solution of 558 mg (1.45 mmol) of **45** in THF (6.6 mL) and MeOH (34 mL) was stirred at room temperature as 400 mg (10.6 mmol) of $NaBH_4$ was added in small portions over 10 min. The reaction was stirred at room temperature under N_2 for 3.5 h, the solvent was evaporated, and the residue was dissolved in EtOAc (100 mL) and H_2O (50 mL). The phases were separated, and the aqueous phase was extracted with EtOAc (2×, 70 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow foam which was used without purification in the next step. Data for **46**: TLC, T-2, R_f 0.22; ^1H NMR (400 MHz, $CDCl_3$) δ 1.07 (s, 3H, H-18), 3.75 (t, 1H, J = 8.7 Hz, H-17α), 5.05 (s, 2H, benzylic-H), 6.75 (d, 1H, J = 2.7 Hz, H-4), 6.80 (dd, 1H, J = 8.5, 2.7 Hz, H-2), 7.21 (d, 1H, J = 8.5 Hz, H-1), 7.33−7.45 (m, 5H, Ar-H).

3-Benzyloxy-17β-hydroxyestra-1,3,5(10)-triene-15α-carboxylic Acid (47). A solution of 571 mg (1.47 mmol) of **46** crude, 2.54 g (45.3 mmol) of KOH in H_2O (6 mL), and ethylene glycol (34 mL) was stirred and heated at 160 °C for 112 h without a reflux condenser to allow H_2O to evaporate. The reaction mixture was poured into H_2O (700 mL) and washed with Et_2O (2×, 100 mL). The aqueous phase was adjusted to pH 2 with concentrated HCl and extracted with Et_2O (3×, 100 mL). Combined organic extracts were washed with 10% sodium metabisulfite (50 mL) and H_2O (50 mL), dried over Na_2SO_4, and concentrated in vacuo giving 535 mg (89%, two steps) of **47** as a tan solid. Data for **47**: TLC, T-3, R_f 0.575; ^1H NMR (400 MHz, DMSO-d_6) δ 0.69 (s, 3H, H-18), 3.61 (ddd, 1H, J = 8.7, 8.7, 5.0 Hz, H-17α), 4.73 (d, 1H, J = 5.0 Hz, 17-OH), 5.04 (s, 2H, benzylic-H), 6.67 (d, 1H, J = 2.6 Hz, H-4), 6.74 (dd, 1H, J = 8.7, 2.6 Hz, H-2), 7.17 (d, 1H, J = 8.7 Hz, H-1), 7.31−7.42 (m, 5H, Ar-H), 12.07 (s, 1H, OH).

3,17β-Dihydroxyestra-1,3,5(10)-triene-15α-carboxylic Acid (48, E15-1,0). A solution of 479 mg (1.18 mmol) of **47** in

EtOH (10 mL) was warmed to dissolve solid, cooled to room temperature, and added to a suspension of 50 mg 5% Pd on carbon in EtOH (5 mL) and stirred at room temperature under an atmosphere of H_2 for 20 h. The reaction mixture was filtered through a 1 in. plug of Celite and washed through with EtOH (50 mL). The solvent was evaporated giving 368 mg (99%) of **48** as a white solid. Purification of 25.5 mg of this material by HPLC in system H-20 followed by acid/base extraction gave 11.9 mg **48** for bioassay. Data for **48**: TLC, T-3, R_f 0.475; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.69 (s, 3H, H-18), 3.60 (ddd, 1H, J = 8.7, 8.7, 5.0 Hz, H-17α), 4.72 (d, 1H, J = 5.0 Hz, OH), 6.40 (d, 1H, J = 2.3 Hz, H-4), 6.49 (dd, 1H, J = 8.5, 2.3 Hz, H-2), 7.04 (d, 1H, J = 8.5 Hz, H-1), 9.00 (s, 1H, OH), 12.05 (br s, 1H, OH); HRMS (ES$^-$) calcd for $C_{19}H_{23}O_4$ (M−H) *m*/*e* 315.1597, found *m*/*e* 315.1603. HPLC system H-14, t_R = 12.85 min, and system H-23, t_R = 12.08 min, >99% pure.

Methyl (3,17*β*-Dihydroxyestra-1,3,5(10)-estratrien-15α-yl)formate (49, E15-1,1). A solution of 41.8 mg (0.132 mmol) of **48** and 1 drop of concentrated H_2SO_4 in MeOH (2 mL) was heated at 60 °C in a sealed vial for 75 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (50 mL) and extracted with EtOAc (3×, 70 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow oil. Purification of the residue by flash chromatography on a 2 × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 21.2 mg of **49** as a white solid. Further purification of this material by HPLC with system H-21 (t_R = 12−14 min) gave 19.1 mg (44%) of **49**. Crystallization from acetone−petroleum ether gave 16.9 mg (39%) of **49** as white needles for bioassay. Data for **49**: TLC, T-2, R_f 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 3.71 (s, 3H, OCH_3), 3.93 (t, 1H, J = 8.8 Hz, H-17α), 4.56 (br s, 1H, OH), 6.55 (d, 1H, J = 2.8 Hz, H-4), 6.63 (dd, 1H, J = 8.6, 2.8 Hz, H-2), 7.16 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES$^+$) calcd for $C_{20}H_{26}O_4Na$ (M + Na$^+$) *m*/*e* 353.1729, found *m*/*e* 353.1737. HPLC system H-15, t_R = 13.21 min, and system H-25, t_R = 8.15 min, >99% pure.

Ethyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-15α-yl)formate (50, E15-1,2). Compound **50** was prepared by esterification of **48** (43.1 mg, 0.136 mmol) with EtOH as described for **49**. Purification of the residue by flash chromatography on a 2 × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent followed by HPLC with system H-21 gave 23.3 mg (50%) of **50**. Crystallization from acetone−petroleum ether gave 18.6 mg (40%) of **50** as white needles for bioassay. Data for **50**: TLC, T-2, R_f 0.375; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 1.29 (t, 3H, J = 7.1 Hz, $-OCH_2CH_3$), 3.93 (t, 1H, J = 8.9 Hz, H-17α), 4.17 (q, 2H, J = 7.2 Hz, $-OCH_2CH_3$), 4.59 (br s, 1H, OH), 6.55 (d, 1H, J = 2.7 Hz, H-4), 6.63 (dd, 1H, J = 8.6, 2.7 Hz, H-2), 7.16 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES$^+$) calcd for $C_{21}H_{28}O_4Na$ (M + Na$^+$) *m*/*e* 367.1885, found *m*/*e* 367.1882. HPLC system H-15, t_R = 12.55 min, and system H-25, t_R = 11.77 min, >99% pure.

2′-Fluoroethyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-15α-yl)formate (51, E15-1,2F$_1$). Compound **51** was prepared by esterification of **48** (83.9 mg, 0.265 mmol) with 2′-fluoroethanol as described for **49**. Purification of the residue by flash chromatography on a 3 × 21 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 33.6 mg of **51**. Further purification by HPLC with system H-21 in 3 portions gave 29.1 mg (29%) of **51** as a white solid. Crystallization from acetone-petroleum ether gave 24.9 mg (25%) of **51** as white needles for bioassay. Data for **51**: TLC, T-3, R_f 0.65; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H, H-18), 3.93 (t, 1H, J = 8.8 Hz, H-17α), 4.36 (m, 2H, $-OCH_2CH_2F$), 4.63 (dt, 2H, J = 47.5, 4.1, 4.1 Hz, $-OCH_2CH_2F$), 6.55 (d, 1H, J = 2.7 Hz, H-4), 6.63 (dd, 1H, J = 8.3, 2.7 Hz, H-2), 7.16 (d, 1H, J = 8.3 Hz, H-1); HRMS (ES$^+$) calcd for $C_{21}H_{27}FO_4Na$ (M + Na$^+$) *m*/*e* 385.1791, found *m*/*e* 385.1791. HPLC system H-15, t_R = 12.69 min, and system H-25, t_R = 9.37 min, >99% pure.

Propyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-15α-yl)formate (52, E15-1,3). Compound **52** was prepared by esterification of **48** (33.9 mg, 0.107 mmol) with ^{n}PrOH as described for **49**. Purification of the residue by flash chromatography on a 2 × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 21.9 mg of **52**. Further purification by HPLC with system H-21 in 3 portions gave 22.3 mg (58%) of **52** as a white solid. Crystallization from acetone-petroleum ether gave 12 mg (31%) of **52** as white needles. Data for **52**: TLC, T-3, R_f 0.64; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 0.98 (t, 3H, J = 7.5 Hz, $OCH_2CH_2CH_3$), 3.93 (t, 1H, J = 8.6 Hz, H-17α), 4.05−4.08 (m, 2H, $OCH_2CH_2CH_3$), 6.54 (d, 1H, J = 2.7 Hz, H-4), 6.63 (dd, 1H, J = 8.2, 2.7 Hz, H-2), 7.20 (d, 1H, J = 8.2 Hz, H-1); HRMS (ES$^+$) calcd for $C_{22}H_{30}O_4Na$ (M + Na$^+$) *m*/*e* 381.2042, found *m*/*e* 381.2034. HPLC system H-15, t_R = 11.97 min, and system H-25, t_R = 19.35 min, >99% pure.

Isopropyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-15α-yl)formate (53, E15-1,3i). Compound **53** was prepared by esterification of **48** (34.6 mg, 0.109 mmol) with ^{i}PrOH as described for **49**. Purification of the residue by flash chromatography on a 2 × 17 cm column of silical gel using 1:1 hexanes/EtOAc gave 21.3 mg of **53**. Further purification by HPLC with system H-21 in 4 portions gave 16 mg (41%) of **53** as a white solid. Crystallization from Et_2O-petroleum ether gave 13.6 mg (35%) of **53** as white needles. Data for **53**: TLC, T-3, R_f 0.74; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 1.26 (d, 6H, J = 6.3 Hz, $-OCH(CH_3)_2$), 3.91 (t, 1H, J = 8.5 Hz, H-17α), 5.04 (septet, 1H, J = 6.3 Hz, $-OCH(CH_3)_2$), 6.55 (d, 1H, J = 2.6 Hz, H-4), 6.63 (dd, 1H, J = 8.5, 2.6 Hz, H-2), 7.16 (d, 1H, J = 8.5 Hz, H-1); HRMS (ES$^+$) calcd for $C_{22}H_{30}O_4Na$ (M + Na$^+$) *m*/*e* 381.2042, found *m*/*e* 381.2038. HPLC system H-16, t_R = 11.3 min, and system H-28, t_R = 11.4 min, >99% pure.

Butyl (3,17*β*-Dihydroxyestra-1,3,5(10)-trien-15α-yl)formate (54, E15-1,4). Compound **54** was prepared by esterification of **48** (29.1 mg, 0.0920 mmol) with ^{n}BuOH as described for **49**. Purification of the residue by flash chromatography on a 3 × 21 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 16.2 mg of **54**. Further purification by HPLC with system H-21 in 4 portions gave 10.5 mg (31%) of **54** as a white solid. Crystallization from Et_2O-petroleum ether gave 9.6 mg (28%) of **54** as white needles. Data for **54**: TLC, T-3, R_f 0.85; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 0.96 (t, 3H, J = 7.3 Hz, $-OCH_2CH_2CH_2CH_3$), 3.92 (t, 1H, J = 8.8 Hz, H-17α), 4.11 (t, 2H, J = 6.6 Hz, $-OCH_2$-), 6.55 (d, 1H, J = 2.5 Hz, H-4), 6.63 (dd, 1H, J = 8.6, 2.5 Hz, H-2), 7.16 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES$^+$) calcd for $C_{23}H_{32}ONa$ (M + Na$^+$) *m*/*e* 395.2198, found *m*/*e* 395.2193. HPLC system H-15, t_R = 14.78 min, and system H-25, t_R =32.53 min, >99% pure.

Ethyl (17*β*-Hydroxy-3-methoxyestra-1,3,5(10)-trien-15α-yl)formate (55). A solution of 53.7 mg (0.144 mmol) of **50**, 400 μL (6.40 mmol) of CH_3I, and 96 mg (0.695 mmol) of K_2CO_3 in acetone (5 mL) was stirred at room temperature for 19 h and then heated at 60 °C for 6 h. The reaction mixture was allowed to cool to room temperature, poured into H_2O (70 mL), and extracted with EtOAc (3×, 50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography on a 2 × 15 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 55.7 mg (100%) of **55** TLC, T-2, R_f 0.51.

Ethyl (3-Methoxy-17*β*-(methoxymethoxy)estra-1,3,5-(10)-trien-15α-yl)formate (56). A solution of 59.9 mg (0.155 mmol) of **55**, 269 μL (1.55 mmol) of ^{i}PrEt$_2$N, 118 μL (1.55 mmol) of MOMCl in anhydrous toluene (2 mL) was stirred at room temperature for 22 h. The reaction mixture was poured into H_2O (70 mL) and extracted with CH_2Cl_2 (3x, 50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 53.7 mg (80%) of **56**. Data for **56**: TLC, T-2, R_f 0.8; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (s, 3H, H-18), 1.29 (t, 3H, J = 7.2 Hz, CH_2CH_3), 3.37 (s, 3H, $-CH_2OCH_3$), 3.77 (t, 1H, J = 8.7 Hz, H-17α), 3.78 (s, 3H, $ArOCH_3$), 4.20−4.14 (m, 2H, $-CH_2CH_3$), 4.66 (s, 2H, OCH_2O), 6.61 (d, 1H, J = 2.8 Hz, H-4), 6.71 (dd, 1H, J = 8.8, 2.8 Hz, H-2), 7.21 (d, 1H, J = 8.8 Hz, H-1).

15α-Hydroxymethyl-3-methoxy-17*β*-(methoxymethoxy)-estra-1,3,5(10)-triene (57). A solution of 53.7 mg (0.125

mmol) of **56** in anhydrous Et_2O (2 mL) was stirred at room temperature as 47.3 mg (1.25 mmol) of $LiAlH_4$ was added and reaction was stirred at room temperature for 2 h. The reaction mixture was poured into saturated Na-K tartrate (100 mL) and extracted with EtOAc (3×, 70 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving 47.1 mg (87%) of **57** as a clear colorless oil which was a single isomer as judged by ^{1}H NMR and used without further purification in the next step. Data for **57**: TLC, T-2, R_f 0.31; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, H-18), 1.05 (t, 1H, J = 10.6 Hz, H-14), 3.38 (s, 3H, $-CH_2OCH_3$), 3.42 (dd, 1H, J = 10.3, 8.2 Hz, CH_2OH), 3.60 (t, 1H, J = 8.9 Hz, H-17α), 3.78 (s, 3H, $ArOCH_3$), 3.89 (dd, 1H, J = 10.3, 3.2 Hz, $-CH_2OH$), 4.66 & 4.69 (AB quartet, 2H, J_{AB} = 6.5 Hz, OCH_2O), 6.62 (d, 1H, J = 2.6 Hz, H-4), 6.72 (dd, 1H, J = 8.7, 2.6 Hz, H-2), 7.22 (d, 1H, J = 8.7 Hz, H-1).

3-Methoxy-17*β*-methoxymethoxy-15α-toluenesulfonyloxymethylestra-1,3,5(10)-triene (58). A solution of 47.1 mg (0.109 mmol) of **57** and 457 mg (2.39 mmol) of pTsCl in pyridine (7 mL) was allowed to stand in a sealed vial at 4 °C for 24 h. The reaction mixture was poured into H_2O (100 mL) and extracted with CH_2Cl_2 (3×, 70 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 60 mg (94%) of **58**. Data for **58**: TLC, T-2, R_f 0.73; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 0.99 (t, 1H, J = 10.9 Hz, H-14), 2.45 (s, 3H, $ArCH_3$), 3.35 (s, 3H, CH_2OCH_3), 3.52 (t, 1H, J = 8.8 Hz, H-17α), 3.78 (s, 3H, $ArOCH_3$), 3.80 (dd, 1H, J = 9.5, 8.0 Hz, CH_2OH), 4.26 (dd, 1H, J = 9.5, 3.1 Hz, CH_2OH), 4.61 (s, 2H, OCH_2O), 6.60 (d, 1H, J = 2.7 Hz, H-4), 6.71 (dd, 1H, 8.6, 2.7 Hz, H-2), 7.19 (d, 1H, J = 8.6 Hz, H-1), 7.37 (d, 2H, J = 8.1 Hz, Ar−H), 7.82 (d, 2H, J = 8.1 Hz, Ar-H).

3-Methoxy-17*β*-methoxymethoxy-15α-methylestra-1,3,5-(10)-triene (59). A solution of 15.8 mg (0.0269 mmol) of **58** in anhydrous THF (500 μL) was stirred at room temperature as 108 μL of a 1 M solution of $LiEt_3BH$ in THF (0.108 mmol) was added, and the reaction was stirred and heated at 65 °C for 5 h and then quenched at room temperature with EtOH (1 mL). To the reaction mixture were added diglyme (2 mL) and 48 mg (0.431 mmol) Et_3NO, and the reaction was stirred and heated at 150 °C for 1 h allowing the THF to evaporate. The reaction mixture was allowed to cool to room temperature, poured into H_2O (50 mL), and extracted with EtOAc (3×, 50 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ (30 mL), H_2O (30 mL), dried over Na_2SO_4, and concentrated in vacuo giving a colorless oil. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 4:1 hexanes/EtOAc as eluent gave 9 mg (97%) of **59**. Data for **59**: TLC, T-1, R_f 0.73; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.86 (s, 3H, H-18), 0.90 (t, 1H, J = 10.2 Hz, H-14), 1.12 (d, 3H, J = 6.2 Hz, 15α-CH_3), 3.38 (s, 3H, CH_2OCH_3), 3.64 (t, 1H, J = 8.6 Hz, H-17α), 3.79 (s, 3H, $ArOCH_3$), 4.65−4.67 (AB quartet, 2H, J_{AB} = 6.6 Hz, OCH_2O), 6.62 (d, 1H, J = 2.8 Hz, H-4), 6.72 (dd, 1H, J = 8.6, 2.8 Hz, H-2), 7.22 (d, 1H, J = 8.6 Hz, H-1).

3-Methoxy-15α-methylestra-1,3,5(10)-trien-17*β*-ol (60). A solution of 9 mg (0.026 mmol) of **59** and 5 μL of concentrated HCl in MeOH (2 mL) was stirred at room temperature for 15 min then heated at 60 °C for 30 min. Another 25 mL of concentrated HCl was added, and the reaction was stirred at 60 °C for 1 h. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (5 mL) and extracted with EtOAc (3×, 5 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a clear yellow oil. Purification by flash chromatography on 2 × 17 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 7.3 mg (93%) of **60**.[32] Data for **60**: TLC, T-1, R_f 0.31; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 0.90 (t, 1H, J = 10.2 Hz, H-14) 1.13 (d, 3H, J = 6.3 Hz, 15α-CH_3), 3.76 (t, 1H, J = 8.7 Hz, H-17α), 3.79 (s, 3H, $ArOCH_3$), 6.63 (d, 1H, J = 2.7 Hz, H-4), 6.72 (dd, 1H, J = 8.6, 2.7 Hz, H-2), 7.22 (d, 1H, J = 8.6 Hz, H-1).

Diethyl (3-Benzyloxy-17-oxoestra-1,3,5(10)-trien-15α-yl)malonate (61α) and Diethyl (3-Benzyloxy-17-oxoestra-1,3,5(10)-trien-15*β*-yl)malonate (61*β*). A suspension of 46.1 mg of NaH (76.8 mg of 60% dispersion in oil, washed with hexanes, 1.92 mmol) in anhydrous THF (1 mL) was stirred at room temperature as 264 μL (1.74 mmol) of diethyl malonate was added, and the reaction mixture was stirred at room temperature for 30 min. To this was added a solution of 312.5 mg (0.872 mmol) of **44** in THF (2 mL), and the reaction was stirred at room temperature for 1.5 h, poured into saturated aqueous NH_4Cl, and extracted with CH_2Cl_2 (3×, 30 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a slightly yellow oil. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 392 mg (87%) of **61** as an inseparable 5:1 mixture of 15α and 15*β* epimers. Data for **61**α: TLC, T-1, R_f 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 3H, H-18), 1.27 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 1.29 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 4.01 (d, 1H, J = 4.0 Hz, $HC(CO_2Et)_2$), 4.22 (m, 4H, OCH_2CH_3), 5.04 (s, 2H, benzylic-H), 6.72 (d, 1H, J = 2.6 Hz, H-4), 6.80 (dd, 1H, J = 8.7, 2.6 Hz, H-2), 7.21 (d, 1H, J = 8.7 Hz, H-1), 7.33−7.44 (m, 5H, Ar-H). Data attributed to **61***β*: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (s, 3H, H-18), 3.72 (d, 1H, J = 3.5 Hz, $HC(CO_2Et)_2$).

(3-Benzyloxy-17-oxoestra-1,3,5(10)-trien-15α-yl)malonic Acid (62). A solution of 392 mg (0.756 mmol) of **61** and 2 g NaOH in 30 mL of EtOH and 10 mL of H_2O was stirred at room temperature for 17 h. The reaction mixture was concentrated to about 15 mL and poured into 100 mL of H_2O and washed with Et_2O (1×, 20 mL). The aqueous phase was adjusted to pH 2 with concentrated HCl and extracted with EtOAc (3×, 70 mL). Combined organic extracts were washed with saturated aqueous NaCl (1×, 50 mL), dried over Na_2SO_4, and concentrated in vacuo giving 297 mg (85%) of **62** as a white solid. It was one isomer by inspection of the ^{1}H NMR and it was used without purification in the next step. Data for **62**: TLC, T-3, R_f 0.08; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3H, H-18), 4.14 (d, 1H, J = 3.7 Hz, $HC(CO_2H)$), 5.04 (s, 2H, benzylic-H), 6.72 (s, 1H, J = 2.7 Hz, H-4), 6.80 (dd, 1H, J = 8.7, 2.7 Hz, H-2), 7.20 (d, 1H, J = 8.7 Hz, H-1), 7.33−7.45 (m, 5H, Ar-H).

(3-Benzyloxy-17-oxoestra-1,3,5(10)-trien-15α-yl)acetic Acid (63). A solution of 297 mg (0.642 mmol) of **62** crude in 10 mL of 2-methoxyethyl ether was stirred and heated at 162 °C for 15 min. The reaction mixture was allowed to cool to room temperature, poured into H_2O (100 mL), and extracted with EtOAc (3x, 70 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ and concentrated in vacuo giving a yellow oil which was used without purification in the next step. Data for **63**: TLC, T-3, R_f 0.58; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (s, 3H, H-18), 5.04 (s, 2H, benzylic-H), 6.72 (d, 1H, J = 2.6 Hz, H-4), 6.81 (dd, 1H, J = 8.5, 2.6 Hz, H-2), 7.22 (d, 1H, J = 8.5 Hz, H-1), 7.30−7.45 (m, 5H, Ar-H).

(3-Benzyloxy-17*β*-hydroxyestra-1,3,5(10)-trien-15α-yl)-acetic Acid (64). A solution of crude **63** and 97 mg (2.57 mmol) of $NaBH_4$ in EtOH (20 mL) was stirred at room temperature for 22 h. The reaction mixture was poured into saturated Na_2CO_3 (70 mL) and washed with Et_2O (1×, 50 mL). The aqueous phase was adjusted to pH 1 and extracted with EtOAc (2×, 70 mL). Combined organic extracts were washed with H_2O (2×, 50 mL), dried over Na_2SO_4, and concentrated in vacuo giving 220 mg (82%, 2 steps) of **64** as a white solid. Data for **64**: TLC, T-3, R_f 0.46; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H, H-18), 1.03 (t, 1H, J = 10.5 Hz, H-14), 3.77 (t, 1H, J = 8.9 Hz, H-17α), 5.04 (s, 2H, benzylic-H), 6.71 (d, 1H, J = 2.9 Hz, H-4), 6.80 (dd, 1H, J = 8.3, 2.9 Hz, H-2), 7.22 (d, 1H, J = 8.3 Hz, H-1), 7.33−7.45 (m, 5H, Ar−H).

(3,17*β*-Dihydroxyestra-1,3,5(10)-trien-15α-yl)acetic Acid (65, E15-2,0). Compound **65** was prepared from **64** (220 mg, 0.523 mmol) as described for **48** giving a yellow oil which crystallized on standing. Further purification of 33.9 mg of this material by HPLC with system H-20 gave 15.8 mg of **65** for bioassay. Data for **65**: TLC, T-3, R_f 0.33; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.70 (s, 3H, H-18), 0.91 (t, 1H, J = 9.9 Hz, H-14), 3.51 (t, 1H, J = 8.9 Hz, H-17α), 6.41 (d, 1H, J = 2.7 Hz, H-4), 6.50 (dd, 1H, J =8.6, 2.7 Hz, H-2), 7.04 (d, 1H, J = 8.6 Hz,

H-1); HRMS (ES$^+$) calcd for $C_{20}H_{26}O_4Na$ (M + Na$^+$) *m/e* 353.1729, found *m/e* 353.1731. HPLC system H-20, t_R = 13.57 min, and system H-23, t_R = 21.28 min, >99% pure.

Methyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-15α-yl)-acetate (66, E15-2,1). Compound **66** was prepared from **65** (50.6 mg, 0.153 mmol) as described for **8**. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 2:1 EtOAc/hexanes as eluent gave 38.5 mg of **66**. Further purification by HPLC with system H-21 gave 27.9 mg of **66**. Crystallization from Et_2O-petroleum ether gave 22.4 mg (43%) of **66** as white needles. Data for **66**: TLC, T-3, R_f 0.61; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H, H-18), 1.00 (t, 1H, *J* = 10.5 Hz, H-14), 3.69 (s, 3H, OCH_3), 3.74 (t, 1H, *J* = 8.4 Hz, H-17α), 6.55 (d, 1H, *J* = 2.6 Hz, H-4), 6.64 (dd, 1H, *J* = 8.5, 2.6 Hz, H-2), 7.17 (d, 1H, *J* = 8.5 Hz, H-1); HRMS (ES$^+$) calcd for $C_{21}H_{28}O_4Na$ (M + Na$^+$) *m/e* 367.1885, found *m/e* 367.1872. HPLC system H-21, t_R = 16.5 min, and system H-25, t_R = 10.78 min, >99% pure.

Ethyl (3,17β-Dihydroxyestra-1,3,5(10)-trien-15α-yl)acetate (67, E15-2,2). Compound **67** was prepared from **65** (41.9 mg, 0.127 mmol) with EtOH as described for **8**. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 2:1 EtOAc/hexanes as eluent gave 36.7 mg of **67**. Further purification by HPLC with system H-21 gave 36 mg of **67**. Crystallization from Et_2O-petroleum ether gave 26.6 mg (58%) of **67** as fine needles. Data for **67**: TLC, T-3, R_f 0.67; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (s, 3H, H-18), 1.01 (t, 1H, *J* = 10.5 Hz, H-14), 1.28 (t, 3H, *J* = 7.0 Hz, CH_2CH_3), 3.74 (br t, 1H, *J* = 8.5 Hz, H-17α), 4.15 (q, 1H, *J* = 7.0 Hz, CH_2CH_3), 6.56 (d, 1H, *J* = 2.7 Hz, H-4), 6.64 (dd, 1H, *J* = 8.5, 2.7 Hz, H-2), 7.17 (d, 1H, *J* = 8.5 Hz, H-1); HRMS (ES$^+$) calcd for $C_{22}H_{30}O_4Na$ (M + Na$^+$) *m/e* 381.2042, found *m/e* 381.2028. HPLC system H-21, t_R = 15.70 min, and system H-25, t_R = 16.38 min, >99% pure.

3-Benzyloxy-17β-hydroxyestsra-1,3,5(10)-trien-15α-yl)-acetaldehyde (68). A solution of 10 mg (0.0279 mmol) of **67** in anhydrous toluene (500 μL)was stirred at −60 °C as 200 μL (0.3 mmol) of a 1.5 M solution of DIBAL in toluene was added. The reaction mixture was stirred at −60 °C for 1.5 h, poured into H_2O (3 mL), and extracted with EtOAc (2×, 3 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a clear colorless oil. Purification by flash chromatography on a 1 × 17 cm column of silica gel using 2:1 EtOAc/hexanes gave 1.8 mg (20%) of **68**. Data for **68**: TLC, T-2, R_f 0.2; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.85 (s, 3H, H-18), 1.05 (t, 1H, *J* = 10.5 Hz, H-14), 3.75 (br t, 1H, *J* = 9.7 Hz, H-17α), 4.53 (s, 1H, OH), 6.56 (d, 1H, *J* = 2.8 Hz, H-4), 6.64 (dd, 1H, *J* = 8.5, 2.8 Hz, H-2), 7.17 (d, 1H, *J* = 8.5 Hz, H-1), 9.79 (d, 1H, *J* = 1.6 Hz, CHO).

15α-Allylestra-1,3,5(10)-triene-3,17β-diol (69). A solution of 1.8 mg (0.0057 mmol) of **68** in anhydrous THF (500 μL) was stirred at 0 °C as 110 μL (0.048 mmol) of Nystead reagent and 1 μL of $BF_3·OEt_2$ was added. The reaction mixture was allowed to warm to room temperature, stirred for 2 h, transferred to 1 N HCl (3 mL), and extracted with EtOAc (3×, 2 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a clear colorless oil. Purification by flash chromatography on a 1 × 12 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 1 mg (55%) of **69**.[35] Data for **69**: TLC, T-2, R_f 0.54; ^{1}H NMR (500 MHz, acetone-d_6) δ 0.83 (s, 3H, H-18), 1.02 (t, 1H, *J* = 10.3 Hz, H-14), 3.63 (t, 1H, *J* = 8.9 Hz, H-17α), 4.97 (d, 1H, *J* = 10.5 Hz, =CH_2), 5.03 (d, 1H, *J* = 17.1 Hz, =CH_2), 5.81−5.90 (m, 1H, −*CH*=CH_2), 6.51 (d, 1H, *J* = 2.6 Hz, H-4), 6.59 (dd, 1H, *J* = 8.5, 2.6 Hz, H-2), 7.10 (d, 1H, *J* = 8.5 Hz, H-1).

17α-Allyl-3-benzyloxyestra-1,3,5(10),15-tetraen-17β-ol (70). A solution of 477 mg (1.33 mmol) of **44** in anhydrous THF (5.15 mL) was stirred at 0 °C as 1.99 mL (3.99 mmol) of a 2 M solution of allylmagnesium chloride in THF was added dropwise slowly over 10 min. The reaction mixture was stirred at 0 °C for 3 h, poured into saturated aqueous NH_4Cl (70 mL) and extracted with EtOAc (3×, 70 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ (30 mL), H_2O (50 mL), dried over Na_2SO_4, and concentrated in vacuo giving a white solid. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 3:1 hexanes/EtOAc as eluent gave 389 mg (73%) of **70** as a white solid. Data for **70**: TLC, T-1, R_f 0.61; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (s, 3H, H-18), 5.05 (s, 2H, benzylic-H), 5.16−5.20 (m, 2H, =CH_2), 5.67 (dd, 1H, *J* = 6.0, 3.2 Hz, H-15), 5.90−5.98 (m, 1H, −HC=), 5.99 (dd, 1H, *J* = 6.0, 1.6 Hz, H-16), 6.74 (d, 1H, *J* = 2.7 Hz, H-4), 6.80 (dd, 1H, *J* = 8.6, 2.7 Hz, H-2), 7.21 (d, 1H, *J* = 8.5 Hz, H-1), 7.31−7.45 (m, 5H, ArH).

15α-Allyl-3-benzyloxyestra-1,3,5(10)-trien-17-one (71). A 556 mg portion (4.85 mmol) of a 35% oil dispersion of KH was washed with hexanes and suspended in anhydrous THF (2 mL). To this was added a solution of 389 mg (0.971 mmol) of **70** and 1.28 g (4.85 mmol) of 18-crown-6 in THF (6 mL). The reaction mixture was stirred at room temperature for 3 h, transferred dropwise to EtOH (10 mL), diluted with saturated aqueous NH_4Cl (70 mL), and extracted with CH_2Cl_2 (3×, 70 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ (30 mL) and H_2O (30 mL), dried over Na_2SO_4, and concentrated in vacuo giving an orange oil. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 4:1 hexanes/EtOAc as eluent gave 311 mg (80%) of **71** as a white solid. Data for **71**: TLC, T-1, R_f 0.37; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (s, 3H, H-18), 1.36 (t, 1H, *J* = 10.7 Hz, H-14), 5.01−5.05 (m, 2H, =CH_2), 5.05 (s, 2H, benzylic-H), 5.74−5.85 (m, 1H, −HC=), 6.73 (d, 1H, *J* = 2.6 Hz, H-4), 6.80 (dd, 1H, *J* = 8.7, 2.8 Hz, H-2), 7.22 (d, 1H, *J* = 8.7 Hz, H-1), 7.33−7.45 (m, 5H, ArH).

15α-Allyl-3-benzyloxyestra-1,3,5(10)-trien-17β-ol (72). A solution of 341 mg (0.850 mmol) of **71** and 129 mg (3.40 mmol) of $NaBH_4$ in THF (9 mL) with 37 drops of H_2O was stirred at room temperature for 3 h, poured into saturated aqueous NH_4Cl (50 mL), and extracted with EtOAc (3x, 50 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ (30 mL) and H_2O (30 mL), dried over Na_2SO_4, and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 2:1 hexanes/EtOAc as eluent gave 311 mg (91%) of **72**. Data for **72**: TLC, T-1, R_f 0.37; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 1.02 (t, 1H, *J* = 10.3 Hz, H-14), 3.70 (t, 1H, *J* = 9.2 Hz, H-17α), 5.01−5.06 (m, 2H, =CH_2), 5.04 (s, 2H, benzylic-H), 5.78−5.86 (m, 1H, −CH=), 6.72 (d, 1H, *J* = 2.7 Hz, H-4), 6.79 (dd, 1H, *J* = 8.6, 2.7 Hz, H-2), 7.22 (d, 1H, *J* = 8.6 Hz, H-1), 7.31−7.44 (m, 5H, ArH).

(15α-Allyl-3-benzyloxyestra-1,3,5(10)-trien-17β-yl) Acetate (73). A solution of 311 mg (0.770 mmol) of **72** and 2 mL of Ac_2O in pyridine (4 mL) was stirred at room temperature for 17 h. The reaction mixture was poured into H_2O (70 mL) and extracted with CH_2Cl_2 (3×, 50 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 5:1 hexanes/EtOAc as eluent gave 323 mg (94%) of **73**. Data for **73**: TLC, T-4, R_f 0.45; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (s, 3H, H-18), 1.11 (t, 1H, *J* = 10.2 Hz, H-14), 2.07 (s, 3H, OAc), 4.69 (t, 1H, *J* = 8.8 Hz, H-17α), 5.01−5.07 (m, 2H, =CH_2), 5.04 (s, 2H, benzylic-H), 5.76−5.87 (m, 1H, −HC=), 6.71 (d, 1H, *J* = 2.7 Hz, H-4), 6.79 (dd, 1H, *J* = 8.6, 2.7 Hz, H-2), 7.21 (d, 1H, 8.6 Hz, H-1), 7.31−7.45 (m, 5H, ArH).

(3-Benzyloxy-15α-(3′-hydroxypropyl)estra-1,3,5(10)-trien-17β-yl) Acetate (74). A solution of 304 mg (0.685 mmol) of **73** in anhydrous THF (4.9 mL) was stirred at 0 °C as 890 μL (0.890 mmol) of a 1 M solution of BH_3−THF in THF was added dropwise. The reaction mixture was stirred at room temperature for 2 h and diluted with EtOH (2 mL) and diglyme (10 mL). To this was slowly added 396 mg (3.56 mmol) trimethylamine *N*-oxide dihydrate and the reaction was heated at 150 °C for 1 h allowing the THF to evaporate. The reaction was cooled to room temperature, poured into H_2O (100 mL) and extracted with EtOAc (3×, 70 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ (60 mL), H_2O (60 mL), dried over Na_2SO_4, and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 3 × 21 cm column of silica gel using 1.5:1 hexanes/EtOAc as eluent gave

261 mg (82%) of **74**. Data for **74**: TLC, T-3, R_f 0.59; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, H-18), 1.06 (t, 1H, J = 10.2 Hz, H-19), 2.07 (s, 3H, OAc), 3.66 (t, 2H, J = 6.6 Hz, CH_2OH), 4.69 (t, 1H, J = 8.6 Hz, H-17α), 5.04 (s, 2H, benzylic-H), 6.71 (d, 1H, J = 2.9 Hz, H-4), 6.79 (dd, 1H, J = 8.6, 2.9 Hz, H-2), 7.21 (d, 1H, J = 8.6 Hz, H-1), 7.33−7.45 (m, 5H, Ar-H).

3-(17β-Acetoxy-3-benzyloxyestra-1,3,5(10)-trien-15α-yl)-propanioc Acid (75). A solution of 261 mg (0.564 mmol) **74**in acetone (35 mL) was stirred at 0 °C as 211 μL (0.564 mmol) of 2.64 M CrO_3−H_2SO_4[43] in H_2O was added. The reaction mixture was stirred at 0 °C for 20 min, poured into 1:1 MeOH/H_2O (120 mL), concentrated to about 60 mL, and extracted with EtOAc (3×, 80 mL). Combined organic extracts were washed with H_2O (20 mL), dried over Na_2SO_4, and concentrated in vacuo giving 213 mg of **75** as a yellow foam which was used in the next step without purification. Data for **75**: TLC, T-3, R_f 0.54; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (s, 3H, H-18), 1.07 (t, 1H, J = 10.2 Hz, H-14), 2.06 (s, 3H, OAc), 4.68 (t, 1H, J = 8.5 Hz, H-17α), 5.04 (s, 2H, benzylic-H), 6.71 (d, 1H, J = 2.7 Hz, H-4), 6.79 (dd, 1H, J = 8.6, 2.7 Hz, H-2), 7.21 (d, 1H, J = 8.6 Hz, H-1), 7.31−7.45 (m, 5H, Ar-H).

3-(3-Benzyloxy-17β-hydroxyestra-1,3,5(10)-trien-15α-yl)propanoic Acid (76). A solution of 213 mg (0.446 mmol) of **75** in 5% aqueous KOH (20 mL) and MeOH (20 mL) was stirred and heated at 55 °C for 3 h. The reaction mixture was allowed to cool to room temperature, poured into H_2O (70 mL), adjusted to pH 2 with 10% HCl, and extracted with Et_2O (3x, 40 mL). Combined organic extracts were washed with 10% $Na_2S_2O_5$ (30 mL), H_2O (30 mL), dried over Na_2SO_4, and concentrated in vacuo giving a white foam. Purification by flash chromatography on a 2 × 15 cm column of silica gel using 0.5:5 EtOH/$CHCl_3$ gave 134 mg (55%, two steps) of **76**. Data for **76**: TLC, T-3, R_f 0.46; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.82 (s, 3H, H-18), 1.00 (t, 1H, J = 10.0 Hz, H-14), 3.73 (t, 1H, J = 8.6 Hz, H-17α), 5.04 (s, 2H, benzylic-H), 6.72 (d, 1H, J = 2.8 Hz, H-4), 6.79 (dd, 1H, J = 8.5, 2.8 Hz, H-2), 7.22 (d, 1H, J = 8.5 Hz, H-1), 7.21−7.44 (m, 5H, Ar-H).

3-(3,17β-Dihydroxyestra-1,3,5(10)-trien-15α-yl)propionic Acid (77, E15-3,0). Compound **77** was prepared from **76** (104 mg, 0.239 mmol) as described for **48**. The filtrate was concentrated in vacuo giving 82 mg (100%) of **77** as a yellow oil. HPLC purification of a 43.3 mg portion of this material using system H-22 gave 36.2 mg of **77** for bioassay. Data for **77**: TLC, T-3, R_f 0.44; ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.68 (s, 3H, H-18), 3.48 (t, 1H, J = 8.7 Hz, H-17α), 6.41 (d, 1H, J = 2.6 Hz, H-4), 6.50 (dd, 1H, J = 8.6, 2.6 Hz, H-2), 7.04 (d, 1H, J = 8.6 Hz, H-1); HRMS (ES$^+$) calcd for $C_{21}H_{28}O_4Na$ (M + Na$^+$) m/e 367.1885, found m/e 367.1882. HPLC system H-17, t_R = 11.2 min, and system H-23, t_R = 32.9 min, >99% pure.

Methyl 3-(3,17β-Dihydroxyestra-1,3,5(10)-trien-15α-yl)-propionate (78, E15-3,1). Compound **78** was prepared from **77** (51.6 mg, 0.150 mmol) as described for **8**. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 48.5 mg of **78**. Further purification by HPLC with system H-21 gave 42.3 mg. Crystallization from Et_2O−petroleum ether gave 31.2 mg (58%) of **78** as white needles. Data for **78**: TLC, T-3, R_f 0.62; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (s, 2H, H-18), 0.98 (t, 1H, J = 10.2 Hz, H-14), 3.71 (s, 3H, OCH_3), 3.73 (t, 1H, J = 8.5 Hz, H-17α), 6.57 (d, 1H, J = 2.7 Hz, H-4), 6.65 (dd, 1H, J = 8.3, 2.7 Hz, H-2), 7.18 (d, 1H, J = 8.3 Hz, H-1); HRMS (ES$^+$) calcd for $C_{22}H_{30}O_4Na$ (M + Na$^+$) m/e 381.2042, found m/e 381.2032. HPLC system H-18, t_R = 8.5 min, and system H-25, t_R = 14.45 min, >99% pure.

Ethyl 3-(3,17β-Dihydroxyestra-1,3,5(10)-trien-15α-yl)-propionate (79, E15-3,2). Compound **79** was prepared from **77** (51.65 mg,0.150 mmol) with EtOH as described for **8**. Purification by flash chromatography on a 2 × 17 cm column of silica gel using 1:1 hexanes/EtOAc as eluent gave 48.7 mg. Further purification by HPLC with system H-21 gave 41.3 mg. Crystallization from Et_2O−petroleum ether gave 30.9 mg (56%) of **79** as white needles. Data for **79**: TLC, T-3, R_f 0.71; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (s, 3H, H-18), 0.98 (t, 1H, J = 10.1 Hz, H-14), 1.28 (t, 3H, J = 7.1 Hz, OCH_2CH_3), 3.72 (t, 1H, J = 8.4 Hz, H-17α), 4.12−4.18 (m, 2H, OCH_2CH_3), 6.56 (d, 1H, J = 2.7 Hz, H-4), 6.63 (dd, 1H, J = 8.3, 2.7 Hz, H-2), 7.17 (d, 1H, J = 8.3 Hz, H-1); HRMS (ES$^+$) calcd for $C_{23}H_{32}O_4$-Na (M + Na$^+$) m/e 395.2198, found m/e 395.2198. HPLC system H-18, t_R = 8.2 min, and system H-25, t_R = 21.98 min, >99% pure.

Competitive Binding to the Estrogen Receptor ERα and ERβ. Binding affinities relative to E_2 were performed in incubations with the ER (ERα[36]) in uterine cytosol prepared from Sprague Dawley rats that had been castrated and 24 h prior to sacrifice. For assay, the cytosol was incubated with 1 nM [^{3}H]E_2 in the presence and absence of nonradioactive E_2, estrone (E_1), E16-1,2 and the E_2-carboxy analogues over a range of concentrations from 10^{-12} to 10^{-6} M. Incubations were carried out on ice overnight and bound radioactivity was separated from free by adsorption with dextran coated charcoal and quantified by counting.[45] The details of the assay are as we previously described.[8] Binding affinity (RBA) was determined by analysis of the displacement curves by the curve-fitting program Prism. The results shown in Table 1 are from at least three separate experiments performed in duplicate.[52] A subset of the E_2-alkyl esters was also compared for binding to the ligand binding domain (LBD) of human ERα (M_{250}−V_{595})[46] and human ERβ (M_{214}−Q_{530}).[47] The assay was performed in competition with [^{3}H]E_2 in lysates of *Escherichia coli* in which the LBDs are expressed as described, with the exception that the incubation was performed overnight at 0−2 °C.[48] The results, the average of 3 experiments, each performed in duplicate, as RBAs compared to E_2 and the ratio, RBA of ERα/ERβ, are shown in Table 2.

Estrogenic Potency in Ishikawa Cells. The estrogenic potency of the E_2-analogues was determined in an estrogen bioassay, the induction of alkaline phosphatase (AlkP) in human endometrial adenocarcinoma cells (Ishikawa) grown in 96-well microtiter plates as we have previously described.[39] In short, the cells are grown in phenol red free medium with estrogen depleted (charcoal stripped) bovine serum in the presence or absence of varying amounts of the steroids, across a dose range of at least 6 orders of magnitude. E_2, E_1, and E16-1,2 were included for comparison. After 3 days, the cells are washed, frozen, thawed, and then incubated with 5 mM *p*-nitrophenyl phosphate, a chromogenic substrate for the AlkP enzyme, at pH 9.8. To ensure linear enzymatic analysis, the plates are monitored kinetically for the production of *p*-nitrophenol at 405 nm. The relative stimulatory activity (RSA) represents the ratio of EC_{50} of E_2 to that of the steroid analogue × 100, using the curve fitting program Prism to determine the EC_{50}. Each compound was analyzed in at least three separate experiments performed in duplicate.

In Vivo Estrogen Bioassays: Uterine Weight. Systemic estrogenic potency was determined by an uterotrophic assay in immature rats as described.[49] Female Sprague−Dawley rats, 22 days old, were injected subcutaneously daily for 3 days with a solution of 0.1 mL of the various steroids in sesame oil. Control animals received sesame oil. On the fourth day, the animals were killed, the uteri were removed, dissected, blotted, and weighed. Except where noted, each compound was assayed in 2 separate experiments with n = 5. The results comparing 100 μg (total dose) of the E_2-analogues to 5 ng of E_2 run concurrently are presented in Table 3.

In Vivo Estrogen Bioassays: Vaginal Reductases. The estrogenic action of locally applied estrogens on the vagina was determined by measuring the induction of vaginal reductases.[50] Female CD-1 mice were ovariectomized, and 1 week later the E_2-alkyl esters or E_2 were instilled into the vagina in 10 μL of sesame oil. (The details of the assay and the use of sesame oil to increase the $t_{1/2}$ of the steroid has been previously discussed.[8]) Briefly, the next morning 2,3,5-triphenyltetrazolium chloride is injected into the vagina and 30 min later the animals are euthanized and the vaginas removed, washed, and extracted with ethanol/tetrachloroethylene (3:1). The formazan product in the organic extract is quantified at 500 nm. Except where noted, each compound was assayed on at least two

separate occasions with at least five to six replicates each time. The results, compared to a 50 pg dose of E_2, are presented in Table 3.

Esterase. Esterase activity was measured in rat hepatic microsomes essentially using the conditions described[51] with some minor modifications. Briefly, rat hepatic microsomes were incubated with 50 μM E2-alkyl ester. Since the rates of reaction are widely different for the various esters, the incubation times and enzyme concentration were varied accordingly to obtain linear kinetics. In every experiment, E16-1,2 was run concurrently to normalize the rate of hydrolysis of each compound. At several appropriate time points during the incubation (37 °C), 100 μL aliquots were quenched with a solution of 2 μg of the internal standard, 6-ketoestradiol in 33 μL of CH_3CN, 33 μL of THF, and 5 μL of glacial HOAc. The mixture was centrifuged and analyzed for the esterase-hydrolysis product (the corresponding E_2-carboxylic acid) by reversed-phase HPLC: E16-1,0; E15-1,0; E15-2,0; E15-3,0 and E7α-2,0 by HPLC with system H-29 and E11-2,0 and E11-3,0 by HPLC with system H-30. Peaks for the hydrolysis product (E16-1,0 $t_R = 10.7$ min; E15-1,0 $t_R = 6.9$ min; E15-2,0 $t_R = 9.9$ min; E15-3,0 $t_R = 14$ min; E11-2,0 $t_R = 11.9$ min; E11-3,0 $t_R = 14.8$ min; E7α-2,0 $t_R = 8.6$ min) and the internal standard ($t_R = 11.8$ min for system H-29 and $t_R = 8.8$ min for system H-30 were quantified at 280 nm on the HPLC UV detector. The UV absorbance was converted to nmoles of product by comparison to standard curves and corrected for recovery of the internal standard, 6-ketoestradiol. The velocity of the reaction for each ester, in nmol product/min/mg protein, was then normalized to the ester, E16-1,2 and is shown in Table 1 as relative hydrolytic activity (RHA) The enzymatic velocity for the hydrolysis of E16-1,2 was 2.34 $\pm$ 0.8 (S.D.) nmol product/min/mg protein over the various experiments. All compounds were tested in triplicate in at least two separate experiments.

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(52) The results of the estrogen receptor assay are somewhat different from those we previously reported.[8] In those experiments the K_d for E_2 averaged 1.75 nM and in these experiments 0.77 nM, whereas the K_d for the other weaker estrogens such as estrone and the 16α-alkoxy analogues are as were reported. Thus, the RBA, which is relative to E_2, of the various analogues is decreased, while their ratio compared to estrone is unchanged. The reason for this shift in the measured E_2 affinity is unknown. Consequently, for comparative purposes, the receptor binding activity of estrone and E16-1,2 which were also run in parallel, are included in Table 1. The behavior of the estrogens in the Ishikawa bioassay did not change.

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