16β-([18F]Fluoro)estrogens: Systematic Investigation of a New Series of Fluorine-18-Labeled Estrogens as Potential Imaging Agents for Estrogen-Receptor-Positive Breast Tumors

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Received January 14, 1993

In order to understand the structural features that might lead to an estrogen receptor (ER) based breast tumor imaging agent with improved uptake characteristics, we have synthesized several new analogs of 16β-fluoroestradiol (βFES) and studied their tissue distribution in immature rats. The compounds we prepared were 11β-methoxy-βFES (**7a**), 11β-ethyl-βFES (**7b**), 17α-ethynyl-βFES (**8c**), 17α-ethynyl-11β-methoxy-βFES (**8a**), and 11β-ethyl-17α-ethynyl-βFES (**8b**). All of the analogs exhibit good affinity for ER, ranging at 25 °C from 10 to 460, with estradiol equal to 100. Measurement of their octanol/water partition coefficients by an HPLC method allowed us to estimate their level of nonspecific binding and thereby to predict their binding selectivity indices (BSI, i.e., the ratio of their ER-specific to nonspecific binding); the BSI values of three fluorine-substituted analogs exceed that of estradiol. These ligands have been labeled in the 16β position with fluorine-18 by the nucleophilic displacement of an α-disposed trifluoromethanesulfonate by [18F]fluoride ion. Reduction with lithium aluminum hydride produced the estradiol series ([18F]-**7a–c**), while treatment with lithium trimethylsilylacetylide afforded the ethynylated series ([18F]-**8a–c**). The synthesis time was 85 min for [18F]-**7a–c** and 120 min for [18F]-**8a–c**, with radiochemical yields ranging from 16 to 43%, and effective specific activities being 90–2900 Ci/mmol (3.3–107 TBq/mmol). In tissue distribution studies in immature female rats, all of the labeled analogs demonstrated ER-selective uptake in the principal target tissues, the uterus and the ovaries, and also in organs with lower titers of ER, the secondary target sites kidney, thymus, fat, and muscle. Although factors other than specific and nonspecific binding obviously affect the tissue distribution of these 16β-fluoroestrogens, we find that their ER-specific uptake by both the principal and the secondary target tissues correlates with their BSI values at a high level of statistical significance in most cases. The ethynylated-11β-methoxy analog [18F]-**8a** had high selectivity (uterus to blood ratio) after 3 h and exhibited the highest uterine uptake (percent injected dose/gram) of any fluorine-substituted estradiol ligand we have studied to date. This compound has been chosen for more detailed studies (to be described elsewhere), including clinical trials in human patients diagnosed with primary breast cancer.

Introduction

The assessment of estrogen receptor (ER) concentration in human breast carcinoma has significant clinical applications for the selection of effective therapeutic regimens.[1] Currently, an invasive biopsy coupled with an *in vitro* assay are the principal means by which receptor content is ascertained. Shortcomings in this assay and the search for noninvasive methods have prompted the investigation of *in vivo* receptor quantitation using radiolabeled estrogens.[2] The most promising *in vivo* agent to date is 16α-[18F]fluoroestradiol (FES).[3] In human studies, FES demonstrated favorable uptake in both primary and metastatic breast lesions.[4] A strong correlation was shown in primary tumors between the estrogen receptor concentration measured by *in vitro* assay and the tumor uptake of labeled FES measured by positron emission tomography (PET).[4a]

Effective ER imaging agents must possess (a) high specific activity, (b) high receptor affinity and binding selectivity, and (c) appropriate distribution and clearance characteristics of labeled metabolites.[5,6] In our attempts to improve upon the *in vivo* distribution behavior of FES, we have prepared a number of estradiol analogs bearing additional substituents in the 11β position (methoxy and ethyl) and at the 17α position (ethynyl).[7,8] All members of this series were initially labeled with fluorine at the 16α position, because the 16α epimer binds to the estrogen receptor with higher affinity than the 16β epimer (relative binding affinities (RBA) are estradiol = 100; 16α-fluoroestradiol = 54; 16β-fluoroestradiol = 12).[3b] As we have described,[7,8] these 11β- and 17α-substituted 16α-fluoroestrogens cover a range of estrogen receptor and nonspecific binding affinities, and in tissue distribution studies in immature rats, most show efficient and selective receptor-mediated uptake into the major target site, the uterus.

However, despite these favorable results, two factors have led us to make a further investigation of fluoroestrogens, which has extended now into the corresponding 16β-fluoro epimeric series. First, although we found in the 16α-fluoroestrogen series that there was a reasonably good correlation of target tissue uptake selectivity directly with

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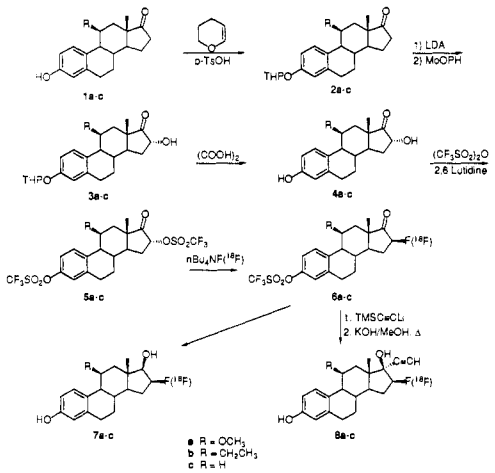
0022-2623/93/1836-1619$04.00/0

Scheme I



estrogen receptor binding affinity and inversely with lipophilicity,[5] this correlation did not hold in all cases.[7,8] Second, in an *in vivo* titration study of 16α-fluoroestradiol itself,[9] we found that the uptake by a receptor-rich target tissue such as the uterus was limited by blood flow and tissue permeability characteristics, and thus may not directly reflect a compound's affinity for the estrogen receptor, nor its potential for efficient, selective uptake by tissues and tumors that are less receptor rich.

For these reasons, we have extended our investigation of fluorine-substituted estrogens into the 16β-fluoro series. In certain cases, we have investigated the extent of receptor-mediated uptake not only by the principal target tissues, the uterus and the ovaries, but also by secondary target tissues, e.g. kidney, thymus, fat, and muscle, that is, those tissues with lower estrogen receptor content in which uptake should not be flow limited; the uptake efficiency of the radiolabeled estrogens by these secondary target tissues may better reflect the estrogen receptor binding characteristics of the estrogens and their potential for uptake by human breast tumors. In this study, we have prepared six estradiol analogs in the 16β-fluoro epimeric series with substituents in the 11β and 17α positions. All of the ligands have good affinity for the estrogen receptor and show receptor-mediated uptake in estrogen receptor-rich target tissues in immature rats, and in some cases also in secondary target tissues. One compound in particular, 17α-ethynyl-16β-fluoro-11β-methoxyestradiol (16β-fluoromoxestrol), appears to have unusually favorable target tissue uptake efficiencies and selectivity, and has been selected for further study.[10]

Results

Synthesis of Fluorine-Substituted Estrogens 7a–c. The 11β-methoxy- and 11β-ethyl-substituted 16β-fluoroestradiol analogs **7a** and **7b** were prepared from their respective 11β-substituted estrones **1a** and **1b**. The 11β-substituted estrones were synthesized from 1-dehydroandrenosterone as reported by Pomper.[7] The 11-unsubstituted 16β-fluoroestradiol **7c** was prepared from estrone.[3]

The estrone analogs **1a–c** were converted to their respective 3,16α-bis(trifluoromethanesulfonates) (triflates) in four steps, as shown in Scheme I. The phenolic functions were protected as tetrahydropyranyl (THP) ethers. The α-disposed hydroxy group was introduced at C-16 by direct hydroxylation of the enolate with the molybdenum peroxide species $MoOPH$[11] (MoO_5·Py·HMPA). This reaction proceeds stereoselectivity, yielding exclusively the 16α-hydroxy diastereomer. The stereochemical assignments at C-16 were inferred from their ^{1}H-NMR spectra: The 16α-protons of the corresponding previously reported 16β-hydroxy derivatives appear as triplets at 4.0 ppm, while the 16β-protons in the 16α-hydroxy derivatives **3a–c** resonate at 4.4 ppm and appear as doublets.[3a,7] Acid hydrolysis of the THP ethers provides the corresponding 3,16α-dihydroxyestrones **4a–c**. The 3- and 16-hydroxyl groups are simultaneously triflated (**5a–c**) with triflic anhydride and 2,6-lutidine to activate the 16β-alcohol toward displacement and protect the 3-phenol.

Treatment of the bistriflates **5a–c** with 1 equiv of nBu_4NF in THF produces the 16β-fluoroestrone 3-triflates **6a–c**. Reduction of the C-17 ketone with $LiAlH_4$ proceeds stereoselectivity to yield exclusively the 3,17β-diols **7a–c**. The 16β-fluoro group and the 13-methyl group hinder the attack of LAH on the β face of the molecule, thereby directing attack on the α face, providing the desired 17β-hydroxy derivatives with very high stereoselectivity. Reduction with LAH also cleaves the phenolic triflates, leaving the free phenol upon workup.

Synthesis of Fluorine-Substituted Estrogens 8a–c. The synthesis of the 17α-ethynyl-16β FES (Scheme I) analogs **8a–c** parallels the 16β-FES synthesis with the exception that lithium trimethylsilylacetylide is added in place of LAH to the 16β-fluoroestrone 3-triflates. The nucleophilic addition of the acetylide to the C-17 carbonyl is highly stereoselective and proceeds from the α face, due to steric hinderance of the β face, producing only the desired 17β-hydroxy derivatives. Removal of the trimethylsilyl group and the 3-*O*-triflate under aqueous base conditions yields the 17α-ethynyl-16β-fluoro-11-protio and 11-substituted estradiols **8a–c**.

Synthesis of Fluorine-18-Labeled Estrogens. The fluorine-18-labeled 16β-fluoroestrogens were synthesized along the same pathway as the unlabeled analogs. [^{18}F]-Fluoride ion was produced by the proton bombardment of an enriched $H_2{}^{18}O$ target.[12] The water was azeotropically removed in the presence of nBu_4NOH. The residue was resolubilized in dry THF and added to the bistriflate precursors **5a–c**. The 16α-triflate moiety was displaced by [^{18}F]fluoride with gentle heating. Subsequent reduction of the C-17 keto group with LAH or C-17 ethynylation and base deprotection afforded the 16β-fluoroestradiols [^{18}F]-**7a–c** and [^{18}F]-**8a–c**. All six labeled estradiols were purified by semipreparative normal-phase HPLC. Radio-HPLC and radio-TLC chromatograms indicate the formation of the desired 17β-hydroxy derivatives to the exclusion of the 17α epimer in all six compounds. This parallels and confirms the selectivity seen with the unlabeled compounds.

Total synthesis and purification time for the 16β-fluoroestradiols [^{18}F]-**7a–c** and the ethynylated 16β-fluoroestradiols [^{18}F]-**8a–c** was 85 and 120 min, respectively, from the end of bombardment. The decay-corrected

Table I. Decay-Corrected Radiochemical Yield Ranges for 16α- and 16β-Fluoroestra-3,17β-diols

ligand	range of yield (%) 16α-^{18}F[a]	16β-^{18}F
fluoroestradiol (FES) (7c)	11–47	17–41
11β-methoxy-FES (7a)	12–35	7–42
11β-ethyl-FES (7b)	7–20	18–35
17α-ethynylfluoroestradiol (FEES) (8c)	2–21	16–35
11β-methoxy-FEES (8a)	3–13	24–44
11β-ethyl-FEES (8b)	1–6	19–30

[a] Data are taken from refs 3, 7, and 8.

Table II. Relative Binding Affinities, Nonspecific Binding Coefficients, and Binding Selectivity Indices for the Estrogen Receptor Ligands

ligand	ER 0 °C[a]	ER 25 °C	log P[b]	NSB[c]	BSI[d]	SBP[a]	AFP[a]
estradiol (ES)	100	100	3.26	1.00	100	100	100
11β-methoxyestradiol	9.7	86	2.72	0.57	151	1.72	0.26
11β-ethylestradiol	133	1360	3.9	2.10	648	40.8	1.21
17α-ethynylestradiol	112	272	3.42	1.18	231	1.81	3.51
17α-ethynyl-11β-methoxyestradiol	13.9	185	3.01	0.76	243	0.071	0.023
11β-ethyl-17α-ethynylestradiol	88.1	946	4.28	2.86	331	41.2	0.347
16β-FES (7c)	38	12	2.81	0.63	19	8.9	20
11β-methoxy-16β-FES (7a)	5.2	13	2.35	0.39	34	0.087	0.062
11β-ethyl-16β-FES (7b)	32	253	3.72	1.60	158	0.55	0.079
17α-ethynyl-16β-FES (8c)	54	28	3.30	1.04	27	0.589	22
17α-ethynyl-11β-methoxy-16β-FES (8a)	10	78	2.87	0.67	116	0.037	<0.01
11β-ethyl-17α-ethynyl-16β-FES (8b)	59	461	4.12	2.43	189	11	0.147

[a] The relative binding affinity values were determined by competitive radiometric binding assays by previously described methods for estrogen receptor (ER),[14a] alphafetoprotein (AFP)[16a] and sex steroid binding protein (SBP).[15] Values represent the mean of two to seven separate determinations (uncertainty lies within ±30%). [b] The log $P_{o/w}$ values were extrapolated from a standard curve based on HPLC derived k'_w values.[17,7] (uncertainty lies within ±10%). [c] The nonspecific binding coefficients were calculated from HPLC-derived estimated octanol/water partition coefficients as described in the text. (uncertainty ± 10%). [d] The BSI values are a ratio of the RBA at 25 °C and the NSB.

radiochemical yield ranges for the 16β-fluoroestradiols [^{18}F]-7a–c and [^{18}F]-8a–c and for the corresponding 16α-fluoro epimers are given in Table I. The yields of 16βFES (7c) and 11β-methoxy-16βFES (7a) are comparable to the 16α-fluoro analogs, while the yields of the rest of the 16β-fluoro series (7b, 8a–c) are significantly greater than analogous 16α-fluoro ligands. The effective specific activities measured by competitive binding on a decayed sample versus [^{3}H]estradiol for the estrogen receptor[3b,13] ranged from 90 to 2900 Ci/mmol (33–107 TBq/mmol), similar to those obtained for previously reported synthetic [^{18}F]fluorinated estrogens.[3b,7,8]

Estrogen Receptor (ER), Alphafetoprotein (AFP), and Sex Steroid Binding Protein (SBP) Binding Affinity of the 16β-Fluoroestradiols. The estrogen receptor binding affinities for the six 16β-fluoro-ligands are given in Table II, along with the values for the nonfluorinated parent compounds. Competitive radiometric binding assays were utilized to determine the binding affinities relative to estradiol. The values are generally reproducible with a coefficient of variation of 0.3. Estrogen receptor binding measurements were conducted to both 0 and 25 °C, the latter most likely reflecting the true relative binding affinities, as an equilibrium between the receptor and the ligand has been established more completely.[14]

In general, the effect of single substituents (16β-fluoro, 11β-methoxy, 11β-ethyl, or 17α-ethynyl) on the binding affinity to ER appears to be consistent at each temperature. Variations in the trends between the two temperatures may reflect differing degrees of equilibration.[14] The 16β-fluoro substituent lowers the binding affinity 1.4–8-fold relative to the corresponding nonfluorinated ligands. In all cases, addition of an 11β-methoxy substituent lowers the affinity at 0 °C; however, in the 16β fluoro series it increases the affinity slightly at 25 °C. Addition of an 11β-ethyl group has little effect at 0 °C, while it enhances the binding at 25 °C. The 17α-ethynyl moiety increases the affinity 1.4–6-fold at both temperatures. This binding pattern is similar to that reported previously for the 16α-fluoroestrogens.[7,8]

Alphafetoprotein (AFP), found in the serum of immature rats, and sex steroid binding protein (SBP), found in humans, can have a profound affect on the *in vivo* biodistribution of the labeled steroids in their respective species.[15,16] Structural modification, however, can alter the binding characteristics of these steroids to the serum proteins, as shown in Table II. Compared to the nonfluorinated estrogens, the 16β-fluoro derivatives, in general, demonstrate an overall reduction in binding to SBP and AFP. However, appreciable binding to both serum proteins was still seen with 16β-fluoroestradiol itself. The 11β- and 17α-substituents tend to drastically reduce the serum protein binding, with the exception being the doubly substituted 11β-ethyl-17α-ethynylestradiol, where the binding to SBP was elevated or unchanged. This trend, however, did not hold for binding to AFP. The compound with the lowest affinity for both proteins was the 17α-ethynyl-11β-methoxy-16β FES (8a).

Octanol/Water Partition Coefficient Determination, Estimation of Nonspecific Binding and Binding Selectivity. The lipophilicity of a steroidal ligand has been found to be predictive of its binding to low-affinity, nonspecific sites.[5] Additionally, the lipophilicity can affect the tissue permeability properties of a ligand, thus affecting its ability to enter target tissues. These two factors affect the *in vivo* distribution of the fluorinated estrogens. To estimate the lipophilicity of these steroidal ligands, we have measured their octanol/water partition coefficients using a reversed-phase HPLC method.[17] We have previously utilized this method to measure the lipophilicities of other substituted estrogens.[7,8] The values of log $P_{o/w}$ for the 17β-fluoroestradiols are shown in Table II. The lipophilicities follow the expected trends, with the 11β-ethyl and 17α-ethynyl groups increasing the log P, while the 11β-methoxy moiety decreases log P. These values are reproducible with a coefficient of variation of 0.1.

We have shown that the nonspecific binding affinity (NSB) of a substituted estrogen can be estimated from the difference between the log P of the new compound and the log P of estradiol, as shown in eq 1.[5a] The

$$\log \mathrm{NSB} = 0.447(\log P_{\mathrm{compound}} - \log P_{\mathrm{estradiol}}) \quad (1)$$

calculated values for the NSB are given in Table II and are relative to estradiol, which is given the value 1. The NSB values for the 16β-fluoro series are consistent with the trends noted earlier for the 16α-fluoro series.[7,8]

The value of binding selectivity index (BSI), a ratio of the RBA to the NSB, has been correlated with the selectivity and the efficiency of uterine uptake.[5,7,8] The BSI has been shown to be a better indicator of uterine selectivity than simply the RBA of the ligand[18] and may also be a better predictor of uptake selectivity in tissues

Table III. Biodistribution of 11β-Methoxy-16β-([^{18}F]fluoro)estra-3,17β-diol (7a) in 25-Day-Old Sprague–Dawley Female Rats[a]

	% injected dose/g		
tissue	1-h	1 h blocked[b]	3 h
blood	0.148 ± 0.032	0.184 ± 0.039	0.117 ± 0.057
liver	1.178 ± 0.211	1.397 ± 0.186	0.893 ± 0.408
kidney	0.946 ± 0.175	0.579 ± 0.096	0.429 ± 0.181
muscle	0.251 ± 0.082	0.149 ± 0.019	0.107 ± 0.054
fat	0.516 ± 0.102	0.338 ± 0.103	0.402 ± 0.247
bone	0.515 ± 0.066	0.604 ± 0.185	0.646 ± 0.301
uterus	6.122 ± 1.489	0.671 ± 0.140	4.813 ± 1.653
ovaries	2.466 ± 0.967	0.417 ± 0.031	1.409 ± 0.744
thymus	0.391 ± 0.089	0.135 ± 0.013	0.175 ± 0.074
uterus/blood	41.70 ± 5.49	3.73 ± 0.94	43.47 ± 10.17
uterus/muscle	25.57 ± 6.40	4.49 ± 0.60	47.73 ± 11.42

[a] Female Sprague–Dawley rats (~50 g) were injected iv with 50 μCi of 7a (effective specific activity, 770 Ci/mmol) in 10% ethanol saline. Tissue distribution values are % injected dose/g and presented as mean ± standard deviation (n = 5). [b] Coinjection of 50 μCi of 7a and 15 μg of estradiol.

Table IV. Biodistribution of 11β-Ethyl-16β-([^{18}F]fluoro)estra-3,17β-diol ([^{18}F]-7b) in 25-Day-Old Sprague–Dawley Female Rats[a]

	% injected dose/g		
tissue	1 h	1 h blocked[b]	3 h
blood	0.108 ± 0.019	0.202 ± 0.048	0.084 ± 0.007
liver	2.331 ± 0.418	3.615 ± 1.133	1.822 ± 0.274
kidney	2.534 ± 0.331	1.716 ± 0.606	1.413 ± 0.377
muscle	0.513 ± 0.038	0.189 ± 0.061	0.307 ± 0.038
fat	1.692 ± 0.569	1.054 ± 0.347	1.294 ± 0.391
bone	0.889 ± 0.093	1.057 ± 0.184	1.077 ± 0.138
uterus	5.570 ± 1.443	1.214 ± 0.212	6.894 ± 1.541
ovaries	2.991 ± 0.663	1.063 ± 0.201	2.742 ± 0.267
thymus	0.779 ± 0.115	0.186 ± 0.070	0.453 ± 0.054
uterus/blood	52.37 ± 12.58	6.30 ± 1.71	82.19 ± 15.72
uterus/muscle	10.81 ± 2.22	7.11 ± 2.66	22.88 ± 6.40

[a] Female Sprague–Dawley rats (~50 g) were injected iv with 15 μCi of 7b (effective specific activity, 214 Ci/mmol) in 10% ethanol saline. Tissue distribution values are % injected dose/g and presented as mean ± standard deviation (n = 5). [b] Coinjection of 15 μCi of 7b and 15 μg of estradiol.

Table V. Biodistribution of 16β-([^{18}F]fluoro)estra-3,17β-diol (16β-[^{18}F]FES, [^{18}F]-7c) in 25-Day-Old Sprague–Dawley Female Rats[a]

	% injected dose/g		
tissue	1 h (6)[b]	1 h blocked (4)[c]	3 h (5)
blood	0.949 ± 0.360	1.486 ± 0.178	0.534 ± 0.159
liver	2.957 ± 1.186	3.771 ± 0.264	1.483 ± 0.533
kidney	2.051 ± 0.899	3.388 ± 0.944	0.821 ± 0.279
muscle	0.402 ± 0.237	0.466 ± 0.074	0.151 ± 0.085
fat	0.310 ± 0.122	0.391 ± 0.047	0.131 ± 0.045
bone	1.008 ± 0.243	1.216 ± 0.212	1.201 ± 0.463
uterus	4.994 ± 1.524	1.257 ± 0.425	1.176 ± 0.362
ovaries	1.908 ± 0.815	1.189 ± 0.210	0.615 ± 0.220
uterus/blood	5.631 ± 1.463	0.857 ± 0.324	2.273 ± 0.537
uterus/muscle	15.22 ± 6.53	2.685 ± 0.744	9.046 ± 3.326

[a] Female Sprague–Dawley rats (40 g) were injected iv with 120 μCi of 16β-FES (7c) (effective specific activity, 1306 Ci/mmol) in 10% ethanol saline. Tissue distribution values are % injected dose/g and presented as mean ± standard deviation. [b] The number in parentheses represents the number of animals in each group. [c] Coinjection of 120 μCi of 7c and 15 μg of estradiol.

with lower ER titer, i.e., the muscle, thymus, and kidney.[9] The BSI values using the RBA at 25 °C for the 16β-fluoro ligands are given in Table II.

***In Vivo* Biodistribution of the 16β-Fluoroestradiols in Immature Female Rats.** The biodistribution of the 16β-fluoro analogs **7a–c** and **8a–c** in 25-day-old female Sprague–Dawley rats is presented in Tables III–VIII. The rats, five per time point, were injected iv with the labeled

Table VI. Biodistribution of 17α-Ethynyl-11β-methoxy-16β-([^{18}F]fluoro)estra-3,17β-diol ([^{18}F]-8a) in 25-Day-Old Sprague–Dawley Female Rats[a]

	% injected dose/g		
tissue	1 h	1 h blocked[b]	3 h
blood	0.284 ± 0.134	0.380 ± 0.127	0.101 ± 0.026
liver	3.244 ± 1.155	3.708 ± 1.652	2.129 ± 0.512
kidney	1.961 ± 0.561	1.038 ± 0.363	0.973 ± 0.184
muscle	0.739 ± 0.184	0.525 ± 0.155	0.389 ± 0.076
fat	1.538 ± 0.742	0.679 ± 0.253	0.858 ± 0.200
bone	1.384 ± 0.630	1.477 ± 0.725	2.007 ± 0.541
uterus	18.26 ± 7.850	2.548 ± 0.663	12.99 ± 3.760
ovaries	5.197 ± 2.457	1.661 ± 0.239	3.510 ± 1.260
thymus	0.852 ± 0.205	0.440 ± 0.133	0.454 ± 0.080
uterus/blood	66.26 ± 9.38	7.52 ± 3.44	129.8 ± 30.4
uterus/muscle	24.31 ± 6.14	5.25 ± 1.79	32.76 ± 5.83

[a] Female Sprague–Dawley rats (~50 grams) were injected iv with 50 μCi of 8a (effective specific activity, 2860 Ci/mmol) in 10% ethanol saline. Tissue distribution values are % injected dose/g and presented as mean ± standard deviation (n = 5). [b] Coinjection of 50 μCi of 8a and 15 μg of estradiol.

Table VII. Biodistribution of 17α-Ethynyl-11β-ethyl-16β-([^{18}F]fluoro)estra-3,17β-diol ([^{18}F]-8b) in 25-Day-Old Sprague–Dawley Female Rats[a]

	% injected dose/g		
tissue	1 h	1 h blocked[b]	3 h
blood	0.264 ± 0.110	0.227 ± 0.051	0.144 ± 0.038
liver	4.023 ± 1.303	3.888 ± 0.769	4.003 ± 0.505
kidney	1.886 ± 0.512	0.709 ± 0.114	1.030 ± 0.149
muscle	0.725 ± 0.173	0.262 ± 0.058	0.382 ± 0.035
fat	3.375 ± 0.903	2.174 ± 0.219	1.805 ± 0.410
bone	1.365 ± 0.320	1.037 ± 0.180	1.606 ± 0.276
uterus	8.197 ± 1.644	0.880 ± 0.068	9.091 ± 2.507
ovaries	3.934 ± 0.607	1.712 ± 0.170	3.115 ± 0.985
thymus	0.815 ± 0.133	0.277 ± 0.054	0.493 ± 0.066
uterus/blood	33.61 ± 8.55	4.03 ± 0.780	64.53 ± 13.21
uterus/muscle	11.44 ± 1.61	3.50 ± 0.770	23.55 ± 4.09

[a] Female Sprague–Dawley rats (~50 g) were injected iv with 50 μCi of 8b (effective specific activity 92 Ci/mmol) in 10% ethanol saline. Tissue distribution values are % injected dose/g and presented as mean ± standard deviation (n = 5). [b] Coinjection of 50 μCi of 8b and 15 μg of estradiol.

estrogen and sacrificed 1 and 3 h postinjection. The blocked studies involved the coadministration of 15 μg of unlabeled estradiol together with the radiolabeled dose (to block uptake by an ER-mediated process), with sacrifice after 1 h.

All of the 16β-fluoro derivatives exhibited selective uptake in the ER-rich target tissues, uterus and ovaries. Uterine levels for the 17α-ethynylated (**8a–c**) analogs were significantly higher ($p < 0.035$) than for the 17α-protio (**7a–c**) analogs, with the 17α-ethynyl-11β-methoxy derivative **8a** possessing the highest uptake ever achieved among positron-emitting estrogen radiopharmaceuticals. In the blocking experiment, uterine- and ovarian-uptake levels decrease by 75–93% and 40–65%, respectively, demonstrating that the uptake of these ligands in these receptor-rich tissues is ER-mediated. Receptor-mediated uptake can also be seen in the kidney, thymus, fat, and muscle, tissues known to contain low concentrations of estrogen receptors.[19–22] A further analysis of the uptake characteristics of these 16β-fluoro estrogens and correlations with their *in vitro* binding characteristics is given in the Discussion section.

Discussion

We have prepared six fluorine-18-labeled analogs of 16β-fluoroestradiol and have studied their chemical and *in*

Table VIII. Biodistribution of 17α-Ethynyl-16β-([^{18}F]fluoro)estra-3,17β-diol ([^{18}F]-8c) in 25-Day-Old Sprague–Dawley Female Rats[a]

	% injected dose/g		
tissue	1 h	1 h blocked[b]	3 h
blood	0.449 ± 0.065	0.576 ± 0.048	0.358 ± 0.058
liver	2.249 ± 0.206	2.412 ± 0.139	2.204 ± 0.153
kidney	0.906 ± 0.122	0.752 ± 0.089	0.376 ± 0.055
muscle	0.307 ± 0.032	0.220 ± 0.027	0.133 ± 0.021
fat	0.337 ± 0.038	0.206 ± 0.029	0.122 ± 0.022
bone	0.608 ± 0.072	0.635 ± 0.091	0.557 ± 0.122
uterus	7.466 ± 1.308	0.533 ± 0.116	4.312 ± 0.707
ovaries	2.159 ± 0.127	0.863 ± 0.167	1.274 ± 0.257
uterus/blood	16.87 ± 3.56	0.920 ± 0.145	12.21 ± 2.13
uterus/muscle	24.42 ± 4.08	2.424 ± 0.429	32.37 ± 2.12

[a] Female Sprague–Dawley rats (53 g) were injected iv with 100 μCi of 8c (effective specific activity, 312 Ci/mmol) in 10% ethanol saline. Tissue distribution values are % injected dose/g and presented as mean ± standard deviation (n = 5). [b] Coinjection of 100 μCi of 8c and 15 μg of estradiol.

vitro binding properties, as well as their *in vivo* distribution characteristics in immature female rats. This study was a logical extension of our work with 16α-fluoroestradiol, an ER imaging agent currently under investigation in human clinical trials,[4] with the overall purpose of finding an imaging agent with improved *in vivo* characteristics for the noninvasive quantitation of ER in human breast tumors.

On the basis of previous studies, it is known that substituents at the 11β and 17α positions of estrogens modulate the chemical and metabolic properties of these molecules and affect their binding by the estrogen receptor.[23] We have studied these effects in the 16α-[^{18}F]fluoroestradiol series, where we found a broad spectrum of receptor binding affinities, lipophilicities, and rates of *in vitro* hepatocyte metabolism.[7,8,24] In contrast to the *in vitro* data, however, we found that the *in vivo* receptor-mediated uterine uptake of these 16α-fluoro ligands spanned a more limited range. Thus, in order to complete our investigation of substituent effects on distribution and metabolism of fluorine-18-labeled estrogens, we investigated the behavior of 11β- and 17α-substituted compounds in the 16β-fluoroestradiol series.

Synthetic Facility of Radiolabeling in the 16β-Fluoroestrogen Series. The 16β-fluoroestradiols offer an advantage over the corresponding 16α-fluoro analogs in terms of the ease of their synthesis. The reduction or ethynylation of the 17-keto group occurs with very high stereoselectively, yielding the desired 17β-hydroxy derivative to the complete exclusion of the 17α-hydroxy compound. The β-disposed fluorine combined with the positioning of the 18-methyl group hinders the β face of the molecule, forcing attack from the α face. This is particularly advantageous in the radiolabeling reaction, where the label resides only in the desired 17β-hydroxy product; by contrast with the 16α-fluoroestradiols, 25–90% of the label (depending on the 11β substituent) was associated with the undesired 17α-hydroxy product.[3,7,8] The stereoselectivity of reaction at C-17 is also reflected in the decay-corrected yield ranges shown in Table I. The yields are markedly greater in the 16β-fluoro series, except for fluoroestradiol (FES) and 11β-methoxy-FES.[3,7,8]

Effect of 11β- and 17α-Substituents on the Binding Characteristics of Estrogens. The 17α-ethynyl group is known to improve the oral potency of the estrogens, presumably by blocking the enzymatic oxidation of the 17β-hydroxy group by 17β-dehydrogenase.[25] The ethynyl group, in all the systems we have studied, increases the affinity of an estrogen for ER to a greater degree than it increases its lipophilicity; the same is true, to an even greater extent, for the 11β-ethyl moiety. The 11β-methoxy exhibits the opposite effect, decreasing affinity but reducing lipophilicity to a greater degree. The result of these changes is that the substituents at 11β and 17α all increase the BSI values of the parent ligands.

It was expected that these substituents would reduce the affinity of the estrogens for the plasma proteins, alphafetoprotein, and sex binding globulin.[7,8] This holds true, in general, with the exception of 17α-ethynyl-16β-FES (8c), which binds to AFP better than the nonfluorinated 17α-ethynyl-E_2, and 11β-ethyl-17α-ethynyl-16β-FES (8b), which binds to SBP with an affinity of 11%. All are lower, however, than estradiol itself. Substituents at both the 11β and 17α sites are known to suppress A and D ring metabolism.[23,24] Although we have not studied the metabolism of the 16β-substituted fluoroestradiols, we found, in the 16α-fluoro series, that the compounds doubly substituted at 11β and 17α exhibited a synergistic reduction in metabolism.[8b,24]

Correlations between *in Vitro* Binding Characteristics and *in Vivo* Uptake. In our efforts to develop estrogen radiopharmaceuticals for imaging ER-positive tumors, we have sought to find correlations between their *in vivo* uptake properties and their *in vitro* binding characteristics to ER, to specific serum binding proteins, and to nonreceptor (nonspecific) binders (the last being directly related to their lipophilicity).[5] Initially, in a comparison of a limited number of radiolabeled estrogens spanning a wide range of receptor binding affinities and lipophilicities, we found a satisfying relationship between *in vivo* uptake selectivity (defined as the ratio of target tissue (uterus) activity to nontarget tissue activity) and *in vitro* binding selectivity (defined as the ratio of affinities for the ER vs nonspecific binders, i.e., the BSI).[18] As we began to study compounds with increasingly favorable binding characteristics, it became apparent that such a simple correlation might not hold in general.[7-9]

Several factors that complicate such a simple analysis are evident: First, the uptake selectivity expressed simply as a ratio of target to nontarget activity overlooks the fact that the activity in nontarget tissues is often mainly due to metabolites,[7,8a] so this ratio does not represent the distribution of the compound itself. Second, from *in vivo* titration studies, it became evident that the uptake of some compounds by receptor-rich target tissues (such as the uterus and ovaries) was flow limited.[9] Third, other tissues not normally considered to be principal targets for estrogen action have significant levels of estrogen receptors and do show receptor-mediated uptake.[9] Finally, the metabolic clearance rate of these compounds also varies considerably, so that their blood activity curves, which represent the quantity of agent being presented to the target tissue, would be different.[10]

Without performing an exhaustive study of blood activity curves, metabolism, and flow limitation, which is beyond the scope of this work, we have examined the uptake data of these six new 16β-fluoro-substituted estrogens for possible correlations with their binding properties. While being neither exhaustive nor conclusive, the results are at least instructive. Rather than using, as before, the uterus to nontarget tissue activity ratio as the *in vivo* uptake parameter, we have used tissue *specific* uptake, defined as the difference in uptake between experiments conducted in the absence (total uptake) and

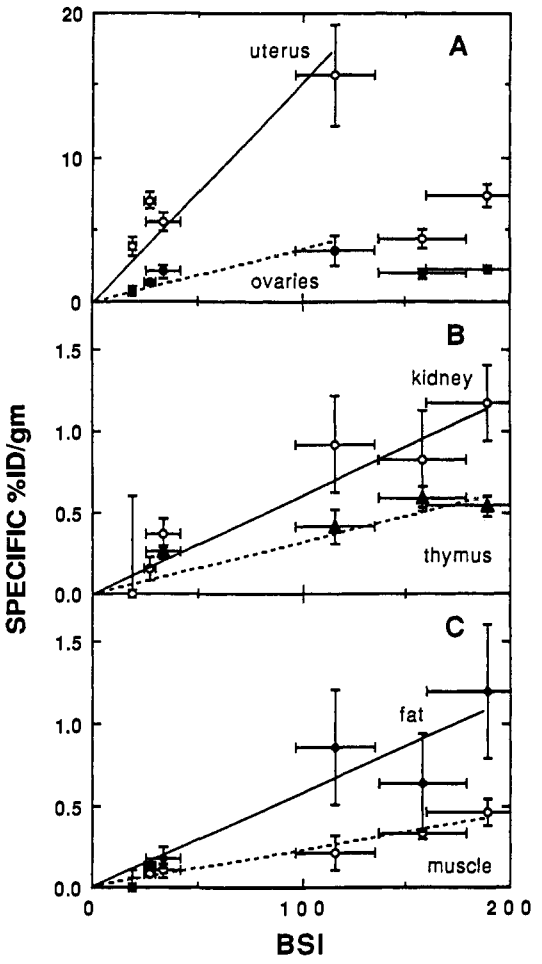


Figure 1. Correlation between ER-specific tissue uptake of the 16β-fluoroestrogens and their binding selectivity index (BSI) values. (BSI is the ratio of receptor binding affinity (RBA) to nonspecific binding (NSB).) In each case, the ER-specific uptake was calculated as the difference between the uptake at 1 h in the absence and presence of a blocking dose of unlabeled estradiol. The errors in each determination were propagated by standard methods (root mean square values) and are expressed as the standard errors of the mean. The errors in the BSI values are estimated from historical coefficients of variation, being 0.3 and 0.1, respectively, for the RBA and the NSB values, giving a propagated coefficient of variation of 0.32.

presence (nonspecific uptake) of a blocking dose of unlabeled estradiol;[26] these data are available for the 1-h uptake only. This parameter of *in vivo* uptake should represent unmetabolized activity bound to the ER. The results of these correlations are shown in Figure 1.

With four of the six β fluoroestrogens, there is a good correlation between the BSI value and the 1-h *specific* uptake in the principal target organs, uterus and ovaries (r^2 = 0.969 in uterus and 0.937 in ovaries) Figure 1A). The two compounds that do not fit the correlation, the two 11β-ethyl derivatives **7b** and **8b**, have very high BSI values; they show lower than expected uptake at 1 h, perhaps due to flow limitations.[9] Also, in contrast with the other four compounds, the 11β-ethyl derivatives **7b** and **8b** show increased uptake at 3 h compared to 1 h, suggesting that these steroids are still being taken up by the uterus over the 1–3-h period. Thus, rapid metabolism and clearance cannot account for their low uptake.

Kidney, thymus, and muscle are not usually considered primary target tissues for estrogens, but they do have low titers of estrogen receptor (kidney, 0.4–0.7 pmol/g;[19] thymus, 0.2–0.4 pmol/g;[20] and muscle, 0.06–0.12 pmol/g of tissue[21]). While some uptake into kidney is receptor mediated, the majority is probably associated with the urinary excretion function of the kidneys.[27] Likewise with muscle, a portion of the uptake is receptor associated, but nearly 50% is nonspecific (Tables III–VIII). The *specific* component of uptake by these tissues correlates well with the BSI values of all six of the 16β-fluoroestrogens; r^2 values are 0.94 and greater (Figure 1B,C). In these tissues, the 11β ethyl derivatives show uptake consistent with their BSI. As we have discussed previously, in these lower ER titer tissues, uptake is less likely to be flow limited.[9]

We have evaluated the correlations between the specific uptake by other tissues and the BSI value, as well as uptake correlations simply with receptor binding affinity (RBA at 0 or 25 °C) or simply with nonspecific binding (NSB). While some positive correlations are seen (muscle vs NSB, r^2 = 0.771; thymus vs NSB, r^2 = 0.675), in most cases, these correlations are very poor compared to those with the BSI. An exception of interest is the correlation between the specific uptake in fat and the BSI value (r^2 = 0.884, Figure 1C). While one might imagine fat tissue to be simply a site for nonspecific uptake, due to drug lipid interactions, the metabolism of fat is affected by estrogens,[28] and estrogen receptors have been detected in adipocytes ~0.3 pmol/g.[22] Thus, in terms of the rapid distribution of these steroids, fat tissue may be, like kidney, thymus, and muscle, simply a secondary target tissue.

There are a few other aspects of the *in vivo* uptake characteristics of these compounds that are worthy of note. An overall comparison of selected 1-h tissue distribution of the six β-fluoro analogs is given in Table IX. The 1-h blood levels for the 11β-substituted analogs **7a,b** and **8a,b** were low, while the levels for the 11β-protio compounds **7c** and **8c** were significantly elevated. This has a noticeable effect on the uterus to blood ratios, which range from 2 to 17 for the 11β-unsubstituted ligands and from 40 to 130 for the 11β-substituted ligands. Although, in immature rats, AFP titers have generally decreased to undetectable levels by day 25,[16b] small remaining amounts could greatly affect the blood-uptake levels, since the 11β-protio compounds have up to a 300-fold greater affinity for AFP than do the 11β-substituted compounds (Table II). The level of uptake into bone, indicative of metabolic release of fluorine ion, is moderate. The greatest bone uptake (with 11β-methoxy-17α-ethynyl-16β-fluoro-E_2, **8a**), corresponds to about 9% of the injected dose (the total skeleton accounts for ~16% of the body weight of a 50-g, 3-week-old rat[29]).

Factors Considered in Selecting Imaging Agents. The criteria for the selection of an optimal imaging agent are multifaceted. Not only must one consider the affinity for the receptor, target tissues levels, and selectivity, one must also consider the lipophilicity and levels of nonspecific uptake, binding by steroid carrier proteins in serum, and metabolism characteristics, including the potential for redistribution of labeled metabolites, all factors that may alter the overall distribution and reduce target to nontarget contrast. For example, based purely on their selectivity and uptake efficiency in the secondary target organs, and their *in vitro* receptor binding characteristics, one might have considered the 11β-ethyl analogs to be the most promising imaging agents among those we have studied here. However, when the lipophilicity data, enhanced levels of nonspecific uptake, and blood-flow limitations are considered, one can see that these molecules are not the most suitable ER imaging agents. Thus, ligand selection based largely on *in vitro* binding and physical

Table IX. Comparison of the 1-h Distribution of the Six 16β-Fluoroestradiols in Immature Rats[a]

	% injected dose/g					
tissue	7a	7b	7c	8a	8b	8c
blood	0.148 ± 0.032	0.108 ± 0.019	0.949 ± 0.360	0.284 ± 0.134	0.264 ± 0.110	0.449 ± 0.065
liver	1.178 ± 0.211	2.331 ± 0.418	2.957 ± 1.186	3.244 ± 1.155	4.023 ± 1.303	2.249 ± 0.206
kidney	0.946 ± 0.175	2.534 ± 0.331	2.051 ± 0.899	1.961 ± 0.561	1.886 ± 0.512	0.906 ± 0.122
muscle	0.251 ± 0.082	0.513 ± 0.038	0.402 ± 0.237	0.739 ± 0.184	0.725 ± 0.173	0.307 ± 0.032
fat	0.516 ± 0.102	1.692 ± 0.569	0.310 ± 0.122	1.538 ± 0.742	3.375 ± 0.903	0.337 ± 0.038
bone	0.515 ± 0.066	0.889 ± 0.093	1.008 ± 0.243	1.384 ± 0.630	1.365 ± 0.320	0.608 ± 0.072
uterus	6.122 ± 1.489	5.570 ± 1.443	4.994 ± 1.524	18.26 ± 7.850	8.197 ± 1.644	7.466 ± 1.308
ovaries	2.466 ± 0.967	2.991 ± 0.663	1.908 ± 0.815	5.197 ± 2.457	3.934 ± 0.607	2.159 ± 0.127
thymus	0.391 ± 0.089	0.779 ± 0.115		0.852 ± 0.205	0.815 ± 0.133	
uterus/blood	41.70 ± 5.49	52.37 ± 12.58	5.63 ± 1.46	66.26 ± 9.38	33.61 ± 8.55	16.87 ± 3.56
uterus/muscle	25.57 ± 6.40	10.81 ± 2.22	15.22 ± 6.53	24.31 ± 6.14	11.44 ± 1.61	24.42 ± 4.08

[a] Data are selected from Tables III–VIII.

characteristics may prove insufficient, as it may not adequately predict all aspects of a ligand's potential imaging ability.

16β-Fluoromoxestrol. The most promising compound of all the fluoroestradiols studied to date appears to be 17α-ethynyl-11β-methoxy-16β-fluoroestradiol (16β-fluoromoxestrol). The uptake level of this compound is nearly twice that of the clinically useful 16α-fluoroestradiol. The governing factor that gives rise to this elevated uptake appears to be the reduced metabolic consumption rate of this analog.[10] This affords an extended blood-activity curve and thus an extended bioavailability of this compound, such that it can accumulate in the ER-rich uterus. 16β-Fluoromoxestrol also demonstrates very significant selective uptake by tissues—kidney, thymus, and muscle—that have relatively low titers of estrogen receptor. As this was not the case with 16α-fluoroestradiol, it suggests that 16β-fluoromoxestrol may be more effective in imaging human breast tumors with low estrogen receptor titer. While its nonspecific uptake is not the lowest, it is considerably less than that of the 11β-ethyl analog. We are presenting elsewhere a more detailed study of the uptake and metabolism characteristics of 16β-fluoromoxestrol,[10] and an investigation of its effectiveness as a tumor imaging agent in human breast cancer patients is underway.[30]

Experimental Section

Chemical Synthesis. General. Melting points are uncorrected. Analytical thin-layer chromatography was performed on Kodak Chromatogram plastic-backed or Merck silica gel F-254 glass-backed plates, with visualization by UV (253.7 and 375 nm) and/or phosphomolybdic acid indicator. Flash column chromatography was performed as described by Still.[30] Solvents and column packing dimensions are given parenthetically. Proton (^{1}H) NMR spectra were obtained at 200 and 300 MHz and are reported in ppm (δ) relative to internal tetramethylsilane (0.00 ppm). Fluorine (^{19}F) NMR spectra, obtained at 283.2 MHz, are reported in ppm (φ) relative to external hexafluorobenzene (−163 ppm). Electron impact mass spectra (EIMS) for an electron energy of 70 eV are presented as *m/e* (intensity relative to base peak = 100). High-resolution exact mass determinations were obtained on a Varian MAT 731 spectrometer. Elemental analyses were performed by the Microanalytical Services Laboratory of the University of Illinois. High-performance liquid chromatography (HPLC) was performed on a Whatman Partisil M-9 (9 mm × 50 cm) semipreparative silica gel column and monitored at 254 nm. The solvents and flow parameters are given parenthetically. HPLC effluent for the radiochemical preparations was also monitored by a NaI (Tl) radioactivity detector. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl. Chemicals were purchased from the following sources: Aldrich, Fisher, Kodak, or Sigma.

11β-Methoxy-3-(tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (2a). 11β-Methoxyestrone 1a[7] (552 mg, 1.84 mmol) was dissolved in 1.5 mL of dry THF and 1.5 mL of dry ether. Dihydropyran (0.75 mL, excess) was added to the solution followed by *p*-toluenesulfonic acid monohydrate (15 mg, catalyst). TLC analyses confirmed the consumption of the starting material after 1 h. EtOAc was added and the solution was washed with aqueous $NaHCO_3$. The organic layer was dried over Na_2SO_4 and evaporated to dryness *in vacuo*. The residual yellow oil was passed through a 20-g column of 70 mesh silica gel (50% EtOAc/hexane). Concentration yielded a white crystalline solid (643 mg, 91%): mp 140–141 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.08 (s, 3H, 13-CH_3), 3.28 (s, 3H, 11β-OCH_3), 3.40–4.00 (m, 4H), 4.19 (m, 1H, 11α-H), 5.37 (m, 1H, PhOC*H*(CH_2)O), 6.77 (s, 1H, 4-H), 6.84 (dd, 1H, *J* = 8.7, 2.5 Hz, 2-H), 7.03 (d, 1H, *J* = 8.7 Hz, 1-H); EIMS (35 eV) 384 (M^+, 0.3) (6.0), 170 (12), 146 (21), 85 (100). Anal. ($C_{24}H_{32}O_4$) C, H.

11β-Ethyl-3-(tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (2b). 11β-Ethylestrone 1b[7] (200 mg, 0.671 mmol) was converted into its THP ether 2b according to the preceding method. Flash column chromatography (15-g silica column, 20% EtOAc/hexane) gave **2b** (0.191 g, 74.4%) as a glassy solid: mp 50–52 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 3H, *J* = 7.1 Hz, CH_2CH_3), 1.02 (s, 3H, 13-CH_3), 5.38 (m, 1H, PhOC*H*(CH_2)O), 6.77 (s, 1H, 4-H), 6.84 (dd, 1H, *J* = 9.0, 2.5 Hz, 2-H), 7.05 (d, 1H, *J* = 8.6 Hz, 1-H); EIMS 382 (M^+, 1.5), 298 (100), 185 (53), 172 (35), 146 (59). Anal. (exact mass, HREIMS) calcd for $C_{25}H_{34}O_3$ *m/e* 382.2508, found 382.2525.

3-(Tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (2c). Estrone 1c (1 g, 3.7 mmol) was converted into its THP ether 2c according to the method outlined above. The crude product was subjected to recrystallization from warm MeOH to yield white crystals (1.1 g, 92%): mp 135–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 3H, 13-CH_3), 5.37 (m, 1H, PhOC*H*(CH_2)O), 6.78 (s, 1H, 4-H), 6.83 (d, 1H, *J* = 8.5 Hz, 2-H), 7.17 (d, 1H, *J* = 8.5 Hz, 1-H); EIMS (35 eV) 354 (M^+, 0.3), 270 (17), 185 (13), 146 (16), 85 (100). Anal. ($C_{23}H_{30}O_3$) C, H.

16α-Hydroxy-11β-methoxy-3-(tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (3a). 11β-Methoxyestrone-OTHP **2a** (300 mg, 780 μmol) was dissolved in 5 mL of freshly distilled THF and added dropwise to a freshly prepared cold lithium diisopropylamide (LDA) solution (2.0 mmol of LDA in 5 mL THF formed at −78 °C and warmed to −23 °C). The reaction was stirred at −23 °C for 30 min followed by the addition of MoOPH[11] (1.36 g, 3.12 mmol) from a solid addition tube. The mixture was stirred for 1 h at −23 °C and the color changed from orange to green. The reaction was quenched with 5 mL of saturated aqueous Na_2SO_3 and warmed to room temperature, and stirring was continued until the color remained unchanged. The aqueous solution was extracted three times with EtOAc. The organic portion was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to flash column chromatograph (silica, 10 mm × 5 in., 30% EtOAc/hexane) and concentrated to yield an off-white solid (180 mg, 58%): mp 204–206 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (s, 3H, 13-CH_3), 3.26 (s, 3H, 11β-OCH_3), 4.18 (m, 1H, 11α-H), 4.41 (d, 1H, *J* = 7.8 Hz, 16β-H), 5.37 (m, 1H, PhOC*H*(CH_2)O), 6.77 (s, 1H, 4-H), 6.83 (dd, 1H, *J* = 6.1, 2.4 Hz, 2-H), 7.02 (d, 1H, *J* = 7.5 Hz, 1-H); EIMS (35 eV) 400 (M^+, 1.5), 375 (16), 316 (11), 259 (14), 197 (45), 146 (65), 85 (100). Anal. (exact mass, HREIMS) calcd for $C_{24}H_{32}O_5$ *m/e* 400.2250, found 400.2244.

11β-Ethyl-16α-hydroxy-3-(tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (3b). 11β-Ethylestrone–OTHP **2b** (102 mg, 267 μmol) was converted at −23 °C to **3b** according to the preceding procedure. The orange oil was subjected to flash column chromatography (silica, 10 mm × 5 in., 50% EtOAc/hexane) to yield an orange solid (38.5 mg, 36%): mp 154–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.2 (s, 3H, 13-CH_3), 4.46 (d, 1H, J = 7.1 Hz, 16β-H), 5.39 (m, 1H, PhOCH(CH_2)O, 6.77 (1s, 1H, 4-H), 6.85 (dd, 1H, J = 8.7, 2.6 Hz, 2-H), 7.05 (d, 1H, J = 8.6 Hz, 1-H); EIMS (70 eV, offscale) 398 (M^+, 1.7), 314 (100), 268 (100), 242 (93), 213 (42), 186 (65), 172 (100), 146 (100), 85 (100). Anal. (exact mass, HREIMS) calcd for $C_{25}H_{34}O_4$ m/e 398.2457, found 398.2459.

16α-Hydroxy-3-(tetrahydropyran-2-yloxy)estra-1,3,5(10)-trien-17-one (3c). Estrone-OTHP **2c** (200 mg, 565 μmol) was converted at −23 °C to **3c** according to the procedure outlined for **3a**. The yellow oil was subjected to flash column chromatography (silica, 10 mm × 5 in., 30% EtOAc/hexane) and concentrated to yield **3c** as an off-white foam (83 mg, 40%): mp 154–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (s, 3H, 13-CH_3), 4.39 (d, 1H, J = 7.7 Hz, 16β-H), 5.37 (m, 1H, PhOCH(CH_2)O), 6.78 (d, 1H, J = 2.5 Hz, 4-H), 6.83 (dd, 1H, J = 8.6, 2.6 Hz, 2-H), 7.16 (d, 1H, J = 8.8 Hz, 1-H); EIMS (70 eV) 370 (M^+, 1.3), 286 (100), 214(38), 85 (43). Anal. (exact mass, HREMS) calcd for $C_{23}H_{30}O_4$ m/e 370.2144, found 370.2145.

3,16α-Dihydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (4a). 16α-OH-estrone–OTHP **3a** (150 mg, 375 μmol) was dissolved in 10 mL of THF. Oxalic acid (1 M in water, 1.5 mL, 1.5 mmol) was added to the THF solution. The mixture was warmed to 60 °C for 3–4 h. Saturated $NaHCO_3$ was added to neutralize the reaction. The aqueous solution was extracted three times with ether. The ethereal solution was dried over $MgSO_4$ and evaporated *in vacuo*, leaving a colorless residue. The residue was subjected to silica gel chromatography (30 g silica, 50% EtOAc/hexane) and concentrated to yield **4a** (96 mg, 81%) as a white powder: mp 227–229 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (s, 3H, 13-CH_3), 1.20–3.00 (m, 11H), 3.29 (s, 3H, 11β-OCH_3), 4.18 (m, 1H, 11α-H), 4.43 (d, 1H, J = 8 Hz, 16β-H), 6.55 (d, 1H, J = 2.5 Hz, 4-H), 6.63 (dd, 1H, J = 7, 2.5 Hz, 2-H), 6.98 (d, 1H, J = 7.5 Hz, 1-H); EIMS (35 eV) 316 (M^+, 4), 157 (10), 146 (52), 71 (100). Anal. (exact mass, HREMS) calcd for $C_{19}H_{24}O_4$ m/e 316.1675, found 316.1680.

3,16α-Dihydroxy-11β-ethylestra-1,3,5(10)-trien-17-one (4b). 11β-Ethylhydroxyestrone–OTHP **3b** (35 mg, 88 μmol) was dissolved in 2 mL of THF. Oxalic acid (1 M in H_2O, 1 mL, 1 mmol) was added and the solution was heated to 60 °C for 3 h. Upon cooling, the THF was removed *in vacuo*. Saturated $NaHCO_3$ was added to neutralize the aqueous solution. The mixture was extracted three times with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to flash column chromatography (silica, 4, mL, 30% EtOAc/hexane) to yield **4b** (21 mg, 76%) as a white solid: mp 200–202 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, 3H, J = 7.3 Hz, CH_2CH_3), 1.08 (s, 3H, 13-CH_3), 4.45 (d, 1H, J = 6.7 Hz, 16β-H), 6.54 (s, 1H, 4-H), 6.62 (dd, 1H, J = 8.6, 2.5 Hz, 2-H), 6.96 (d, 1H, J = 8.6 Hz, 1-H); EIMS (70 eV) 314 (M^+, 52), 268 (37), 242 (25), 213 (16), 186 (30), 172 (53), 146 (100). Anal. (exact mass, HREIMS) calcd for $C_{20}H_{26}O_3$ m/e 314.1882, found 314.1884.

3,16α-Dihydroxyestra-1,3,5(10)-trien-17-one (4c). Hydroxyestrone–OTHP **3c** (4.1 mg, 11.1 μmol) was dissolved in 0.5 mL of THF. Oxalic acid (1 M in 50% MeOH/H_2O, 1 mL, 1 mmol) was added to the solution and the mixture was stirred for 2 h at 60–70 °C. The solution was neutralized with saturated $NaHCO_3$ and extracted with ether. The etheral solution was dried over Na_2SO_4 and evaporated to dryness *in vacuo*, affording a clear residue (2 mg, 63%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (s, 3H, 13-CH_3), 4.25 (t, 1H, J = 5 Hz, 16β-H), 5.42 (d, 1H, J = 8 Hz, 16α-OH), 6.47 (s, 1H, 4-H), 6.54 (d, 1H, J = 8.5 Hz, 2-H), 7.07 (d, 1H, J = 8 Hz, 1-H); EIMS (70 eV) 286 (M^+, 100), 214 (80), 172 (37), 159 (30), 146 (23). Anal. (exact mass, HREMS) calcd for $C_{18}H_{22}O_3$ m/e 286.1569, found 286.1566.

3,16α-Bis[[(trifluoromethyl)sulfonyl]oxy]-11β-methoxyestra-1,3,5(10)-trien-17-one (5a). Dihydroxyestrone **4a** (100 mg, 316 μmol) was dissolved in 2,6-lutidine (300 mL, 2.5 mmol) and 2 mL of dry CH_2Cl_2. The solution was cooled to 0 °C followed by the addition of excess triflic anhydride (1 mL). The mixture was stirred for 30 min at 0 °C and then quenched with 3 mL of water. The bistriflate **5a** was extracted into EtOAc. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to flash column chromatography (silica, 15 mm × 5 in., 20% EtOAc/hexane) and was concentrated to give **5a** as a white foam (139 mg, 76%). An analytical sample was recrystallized from EtOAc/hexane: mp 126–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 3H, 13-CH_3), 3.29 (s, 3H, 11β-OCH_3), 4.23 (m, 1H, 11α-H), 5.43 (d, 1H, J = 7.7 Hz, 16β-H), 6.98 (d, 1H, J = 2.5 Hz, 4-H), 7.05 (dd, 1H, J = 8.7, 2.7 Hz, 2-H), 7.17 (d, 1H, J = 8.8 Hz, 1-H); ^{19}F NMR (60 MHz, $CDCl_3$) φ −73.0 (s), −74.4 (s); EIMS (70 eV) 432 (M^+, 25), 419 (100), 398 (16), 302 (41), 246 (25). Anal. (exact mass, HREMS) calcd for $C_{21}H_{22}O_8S_2F_6$ m/e 580.0661, found 580.0662.

3,16α-Bis[[(trifluoromethyl)sulfonyl]oxy]-11β-ethylestra-1,3,5(10)-trien-17-one (5b). Bishydroxyestrone **4b** (20 mg, 63.5 μmol) was dissolved in 0.5 mL of CH_2Cl_2 (freshly distilled from CaH_2, followed by the addition of 2,6-lutidine (60 mL, 515 mmol). The solution was cooled to 0 °C. Triflic anhydride (200 mL, 1.18 mmol) was added and the cooled mixture was stirred for 30 min. The reaction mixture was diluted with 1 mL of hexane and passed through a 3-mL silica column with 30% EtOAc/hexane, leaving a yellow oil upon concentration. Further purification by flash column chromatography (silica, 4 mL, 10% EtOAc/hexane) yielded **5b** (23.8 mg, 62.7%): mp 62–65 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7.2 Hz, CH_2CH_3), 1.09 (s, 3H, 13-CH_3), 5.42 (dd, 1H, J = 6.5, 1.6 Hz, 16β-H), 6.95 (s, 1H, 4-H), 7.01 (dd, 1H, J = 9.0, 2.9 Hz, 2-H), 7.18 (d, 1H, J = 10.3 Hz, 1-H); EIMS (70 eV) 578 (M^+, 20), 522 (18), 373 (100), 241 (46), 185 (40), 145 (43). Anal. (exact mass, HREMS) calcd for $C_{22}H_{24}S_2O_7F_6$ m/e 578.0876, found 578.0863.

3,16α-Bis[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (5c). Dihydroxyestrone **4c** (8.7 mg, 30 μmol) was converted to its bistriflate **5c** by the method described for **5a**. Flash chromatography (silica, 20% EtOAc/hexane) and concentration gave **5c** (8 mg, 48%). mp 139–141 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 3H, 13-CH_3), 5.42 (dd, 1H, J = 6.5, 1.6 Hz, 16β-H), 7.01 (d, 1H, J = 2.5 Hz, 4-H), 7.05 (dd, 1H, J = 8.5, 2.8 Hz, 2-H), 7.33 (d, 1H, J = 8.5 Hz, 1-H); EIMS (70 eV) 550 (M^+, 23), 346 (37), 345 (100), 213 (50). Anal. ($C_{23}H_{30}F_6O_7S_2$) C, H, F, S.

16β-Fluoro-11β-methoxy-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (6a). Bistriflate **5a** (52 mg, 90 μmol) was dissolved in 200 mL of freshly distilled THF. Tetrabutylammonium fluoride (1 M in THF, 90 μL, 90 μmol) was added and the solution was stirred for 1 h at room temperature. The solvent was removed *in vacuo* and the residue was subjected to column chromatography (3 g, 70–230 mesh silica, 30% EtOAc/hexane) affording 35.5 mg (88%) of **6a** as a white foam: mp 126–127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (s, 3H, 13-CH_3), 3.29 (s, 3H, 11β-OCH_3), 4.23 (m, 1H, 11α-H), 4.72 (dt, 1H, J = 50.3, 8 Hz, 16α-H), 6.98 (s, 1H, 4-H), 7.04 (dd, 1H, J = 8.5, 1.8 Hz, 2-H), 7.17 (d, 1H, J = 8.8 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −73.3 (s, Ph-F), −184.5 (dd, J = 49.7, 22.3 Hz, 16β-F); EIMS 450 (M^+, 45), 412 (25), 376 (26), 278 (100). Anal. (exact mass, HREMS) calcd for $C_{20}H_{22}O_5S_1F_4$ m/e 450.1124, found 450.1127.

11β-Ethyl-16β-fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (6b). Bistriflate **5b** (15.5 mg, 27 μmol) was dissolved in 1 mL of freshly distilled THF. Tetrabutylammonium fluoride (1 M in THF, 34 μL, 34 μmol) was added and the mixture was stirred at room temperature for 30 min. The solvent was evaporated *in vacuo* and the residue was passed through a 250-mg silica plug with 30% EtOAc/hexane, yielding a pale yellow oil, **6b** (11.9 mg, 99%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 3H, J = 7.3 Hz), 1.09 (s, 3H, 13-CH_3), 4.66 (dt, 1H, J = 49.9, 8.1 Hz, 16β-H), 6.92 (s, 1H, 4-H), 6.98 (dd, 1H, J = 8.6, 2.5 Hz, 2-H), 7.15 (d, 1H, J = 8.7 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −74.3 (s, Ph-F), −184.5 (dd, J = 48.1, 22.6 Hz, 16β-F); EIMS (10 eV) 448 (M^+, 100), 392 (41), 374 (68), 318 (21), 278 (30), 241 (23), 142 (60). Anal. (exact mass, HREMS) calcd for $C_{21}H_{24}O_4S_1F_4$ m/e 448.1331, found 448.1333.

16β-Fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (6c). Bistriflate **5c** (37 mg, 68 μmol) was dissolved in 200 μL of freshly distilled THF. Tetrabutylammonium fluoride (1 M in THF, 68 μL, 68 μmol) was added and the solution was stirred for 1 h at room temperature. The solvent

was removed *in vacuo* and the residue was passed through a silica plug (50% EtOAc/hexane), affording **6c** (18.7 mg, 95%) as a white foam: mp 162–164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (s, 3H, 13-CH_3), 4.77 (dt, 1H, J = 50, 8.5 Hz, 16α-H), 7.01 (s, 1H, 4-H), 7.05 (dd, 1H, J = 9, 3 Hz, 2-H), 7.33 (d, 1H, J = 8.8 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −73.4 (s, Ph-F) −185.4 (dd, J = 50, 22 Hz, 16β-F); EIMS (70 eV): 420 (M^+, 31), 346 (45), 213 (100). Anal. (exact mass, HREMS) calcd for $C_{19}H_{20}O_4SF_4$ m/e 420.1018, found 420.1013.

16β-Fluoro-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (7a). 16β-Fluoroestrone 3-triflate **6a** (15.4 mg, 37.2 μmol) was dissolved in 0.5 mL of freshly distilled ether and cooled to −78 °C. $LiAlH_4$ (1 M in ether, 1 mL, 1 mmol) was added to the cold solution. After 5 min the mixture was allowed to warm to room temperature over the next 8 min. The reaction was quenched with EtOAc followed by the addition of 6 N HCl and water to dissolve the precipitates. The aqueous layer was extracted three times with EtOAc. The organic extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue was subjected to normal-phase preparative HPLC (Whatman M9/50, silica, 65% hexane, 33.25% CH_2Cl_2, 1.75% 2-propanol, 5 mL/min, t_R = 22 min), yielding **7a** as a white powder (9.4 mg, 86.4%) upon concentration: mp 240–241 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H, 13-CH_3), 3.07 (s, 3H, 11β-OCH_3), 3.22 (m, 1H, 17α-H), 3.92 (m, 1H, 11α-H), 4.74 (dtd, 1H, J = 55.9, 7.4, 3.6 Hz, 16α-H), 6.29 (s, 1H), 6.38 (dd, 1H, J = 8.4, 2.9 Hz, 2-H), 6.77 (d, 1H, J = 9.6 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −185.38 (dddd, J = 47.9, 34.3, 12.2, 19.8 Hz, 16β-F); EIMS (70 eV): 320 (M^+, 100), 288 (35), 172 (73), 146 (89). Anal. (exact mass, HREMS) calcd for $C_{19}H_{25}O_3F$ for m/e 320.1788, found 320.1789.

11β-Ethyl-16β-fluoroestra-1,3,5(10)-triene-3,17β-diol (7b). Fluoroketone **6b** (2.6 mg, 5.8 μmol) was dissolved in 1 mL of dry ether and cooled to −78 °C. Following the addition of $LiAlH_4$ (5 mg, 112 mmol), the reaction was stirred at −78 °C for 15 min and then allowed to warm to room temperature over 15 min. The reaction was quenched with EtOAc, 6 N HCl, and water. The aqueous layer was extracted three times with EtOAc. The organic extracts were dried over Na_2SO_4 and evaporated to dryness *in vacuo*. Purification on a normal-phase semipreparative HPLC column (Whatman M9/50 silica, 75% hexane, 23.75% CH_2Cl_2, 1.25% 2-propanol, 5 mL/min) gave **7b** (0.6 mg, 33%) as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.0 Hz, CH_2CH_3), 1.00 (s, 3H, 13CH_3), 4.97 (dm, 1H, J = 54 Hz, 16α-H), 6.55 (s, 1H, 4-H), 6.64 (dd, 1H, J = 8.4, 2.9 Hz, 2-H), 7.02 (d, 1H, J = 8.9 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −188.1 (m, 16βF); EIMS (70 eV) 318 (M^+, 27), 243 (100), 165 (27), 146 (14). Anal. (exact mass, HREMS) calcd for $C_{20}H_{27}O_2F$ m/e 318.1984, found 318.1989.

16β-Fluoroestra-1,3,5(10)-triene-3,17β-diol (7c). 16β-Fluoroestrone triflate **6c** (20 mg, 37.2 μmol) was converted to **7c** according to the procedure outlined for **7a**. The residue was subjected to normal-phase semipreparative HPLC (Whatman M9/50, silica, 80% hexane, 19% CH_2Cl_2, 1% 2-propanol, 5 mL/min t_R = 17.6 min) yielding **7c** as a white powder (9.4 mg, 86%): mp 225–227 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 3H, 13-CH_3), 3.47 (ddd, 1H, J = 20, 10, 6 Hz, 17α-H), 4.59 (br s, 1H, Ph-OH), 5.00 (dm, 1H, J = 54 Hz, 16α-H), 6.56 (d, 1H, J = 2.5 Hz, 4-H), 6.63 (dd, 1H, J = 8.5, 2.6 Hz, 2-H), 7.15 (d, 1H, J = 8.8 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −180.8 (dddd, J = 54, 37, 23, 13 Hz, 16α-F); EIMS 290 (M^+, 8), 270 (100), 185 (45), 146 (50). Anal. (exact mass, calcd for HREMS) $C_{18}H_{23}O_2F$ m/e 290.1682, found 290.1683.

17α-Ethynyl-16β-fluoro-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (8a). (Trimethylsilyl)acetylene (50 μL, 355 μmol) was dissolved in 0.5 mL of pentane cooled to 0 °C. Butyllithium (1.6 M in hexane, 200 μL, 320 μmol) was added to the acetylene solution forming a white precipitate (TMSC≡CLi). The precipitate was redissolved by adding 50 μL of freshly distilled THF. The 16β-fluoroestrone **6a** (18.2 mg, 40.2 μmol) was dissolved in 0.5 mL of freshly distilled THF and cooled to 0 °C. The lithium–acetylide solution was added to the estrone solution and stirred while the mixture warmed to room temperature. The mixture turned yellow-orange with time. The reaction was quenched with aqueous NH_4Cl (5 M, 65 μL, 325 μmol) after 45 min. The reaction mixture was extracted three times with EtOAc. The solvent was removed *in vacuo* leaving a dark orange-brown residue.

The intermediate residue was dissolved in 250 μL of MeOH followed by the addition of aqueous KOH (5 M, 100 μL, 500 μmol). The solution was heated at 60 °C for 30 min. The reaction was quenched with aqueous NH_4Cl (1 M, 500 μL, 500 μmol) and extracted three times with EtOAc. The extracts were dried with Na_2SO_4 and evaporated to dryness *in vacuo*. Purification by normal phase semipreparative HPLC (Whatman M9/50 Silica, 65% hexane, 33.25% CH_2Cl_2, 1.75% 2-propanol, 5 mL/min) afforded **8a** (8.0 mg, 58% t_R = 16 min): mp 251–254 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (s, 3H, 13-CH_3), 2.60 (s, 1H, ≡CH), 3.28 (s, 3H, 11β-OCH_3), 4.18 (m, 1H, 11α-H), 4.62 (s, 1H, 17-OH), 4.96 (ddd, 1H, J = 55, 7.7, 3.7 Hz, 16α-H), 6.50 (s, 1H, 4-H), 6.61 (dd, 1H, J = 8.5, 2.7 Hz, 2-H), 6.98 (d, 1H, J = 8.4 Hz, 1-H); ^{19}F-NMR (283 MHz, $CDCl_3$) φ −168.98 (dddd, J = 56.6, 34, 8.5, 14.2 Hz, 16β-F); EIMS (70 eV) 344 (M^+, 100), 312 (14), 267 (40), 211 (48), 146 (74). Anal. (exact mass, HREMS) calcd for $C_{21}H_{25}O_3F$ m/e 344.1788, found 344.1790.

11β-Ethyl-17α-ethynyl-16β-fluoroestra-1,3,5(10)-triene-3,17β-diol (8b). Fluoroketone **6b** (2.6 mg, 5.8 μmol) was dissolved in 1.5 mL of dry THF and cooled to 0 °C. Conversion to **8b** was achieved according to the preceding procedure. The residue was subjected to normal-phase semipreparative HPLC (Whatman M9/50 silica, 75% hexane, 23.75% CH_2Cl_2, 1.25% 2-propanol, 5 mL/min), yielding **8b** (1.3 mg, 65%) as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, 3H, J = 6.04 Hz, CH_2CH_3), 1.05 (s, 3H, 13-CH_3), 2.53 (s, 1H, ≡CH), 4.97 (ddd, 1H, J = 55.3, 7.8, 5.1 Hz, 16α-H), 6.54 (s, 1H, 4-H), 6.64 (dd, 1H, J = 7.2, 3.0 Hz, 2-H), 7.03 (d, 1H, J = 7.2 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −170.76 (ddd, J = 52.4, 34.4, 5.8 Hz, 16β-F); EIMS (70 eV) 342 (M^+, 13), 318 (7), 243 (100), 165 (43), 146 (9). Anal. (exact mass, HREMS) calcd for $C_{22}H_{27}O_2F$ m/e 342.1995, found 342.2004.

17α-Ethynyl-16β-fluoroestra-1,3,5(10)-triene-3,17β-diol (8c). 16β-Fluoroestrone triflate **6c** (15 mg, 36 μmol) was converted to **8c** by the procedure outlined for **8a**. Purification by normal-phase preparative HPLC (Whatman M9/50 Silica, 80% hexane, 19% CH_2Cl_2, 1% 2-propanol, 5 mL/min) afforded **8c** (5.25 mg, 24%, t_R = 29.3 min) mp 78–81 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (s, 3H, 13-CH_3), 2.61 (s, 1H, ≡CH), 2.65 (d, 1H, J = 17 Hz, 17β-OH), 4.56 (s, 1H, Ph-OH), 4.90 (ddd, 1H, J = 53, 8, 2 Hz, 16α-H), 6.57 (s, 1H, 4-H), 6.64 (dd, 1H, J = 9, 2.6 Hz, 2-H), 7.16 (d, 1H, J = 8 Hz, 1-H); ^{19}F NMR (283 MHz, $CDCl_3$) φ −168.8 (dddd, J = 55, 41, 13, 7 Hz, 16α-F); EIMS (70 eV): 314 (M^+, 47), 213 (100). Anal. HREIMS (exact mass HREMS) calcd for $C_{20}H_{23}O_3F$ m/e 314.1682, found 314.1680.

Radiochemical Synthesis. General. Fluorine-18 was produced by the ^{18}O(p,n)^{18}F reaction on an enriched water target.[12] Oxygen-18 water containing the ^{18}F ion was transferred to a Vacutainer containing tetrabutylammonium hydroxide, nBu_4NOH, (1 M in water, 2 μL, 2 μmol). The water was removed azeotropically with the continuous addition of 0.5–1.5 mL of acetonitrile at 105–110 °C (oil bath) under a gentle stream of nitrogen. Before being completely dried, the Vacutainer was removed from the oil bath and the final drying process was completed by the gentle stream of nitrogen at room temperature. Once dry, the $nBu_4N^{18}F$ residue was taken up in 200–300 μL of freshly distilled THF and transferred to a borosilicate glass vial (15mm × 45mm, Teflon-lined cap) containing 1.5 mg of the desired substrate. The resolubilization procedure takes 10–15 min with 85–95% of the initial activity being recovered. Radioactive thin-layer chromatography was performed on a 20-cm glass-backed silica gel plates without fluorescent indicator. Visualization was achieved on a Berthold Tracemaster 20 Automatic TLC linear analyzer coupled to a PC workstation. All reactions were performed under no-carrier-added conditions. End-of-synthesis yields are based on resolubilized activity and presented as decay-corrected ranges. Effective specific activities (SA) were measured by *in vitro* competitive binding assays performed on fully decayed samples.[3b,13] Radioactivity was measured in a Capintec well counter.

16β-([^{18}F]Fluoro)-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-7a). Bistriflate **5a** (1.5 mg, 2.6 μmol) was vacuum dried 12–15 h prior to labeling. [^{18}F]Fluoride was added to the bistriflate and the solution was mixed vigorously and warmed to 50 °C. The THF was removed under a gentle stream of nitrogen. The labeled substrate was solubilized in freshly distilled diethyl ether (0.1 mL) and placed in a −78 °C bath (CO_2/2-propanol). $LiAlH_4$ (1 M in diethyl ether, 0.1 mL, 0.1 mmol) was added to

the cold solution. After 3 min the mixture was removed from the cold bath and allowed to warm to room temperature over 5 min. The reaction was quenched by the addition of HCl (6 N, 0.1 mL, 0.6 mmol) and extracted (3× ether, 2×, 50/50 CH_2Cl_2/hexane). The organic extracts were pooled and passed through a drying column (0.5 cm i.d. × 1 cm) consisting of 50/50 Na_2SO_4 over $MgSO_4$. The organic eluent was applied to normal-phase HPLC column (Whatman M9/50 silica, 65% hexane, 33.25% CH_2Cl_2, 1.75% 2-propanol, 5 mL/min. [^{18}F]-**7a**: (typical range 7–20%); t_R = 22 min; SA = 770 Ci/mmol.

11β-Ethyl-16β-([^{18}F]fluoro)estra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-7b). Bistriflate **5b** (1.5 mg, 2.6 μmol) was vacuum dried 12–15 h prior to labeling. Conversion of **6b** to [^{18}F]-**7b** was carried out according to the preceding procedure. The organic eluent was applied to semipreparative normal-phase HPLC column (Whatman M9/50 silica, 75% hexane, 23.75% CH_2Cl_2, 1.25% 2-propanol, 5 mL/min). [^{18}F]-**7b**: (typical range 18–35%) t_R = 22 min; SA = 214 Ci/mmol.

16β-([^{18}F]Fluoro)estra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-7c). Bistriflate **5c** (1.5 mg, 2.7 μmol) was vacuum dried 12–15 h prior to labeling. Conversion of **6c** to [^{18}F]-**7c** was carried out following the procedure outlined for [^{18}F]-**7a**. The organic eluent was applied to semipreparative normal-phase HPLC column (Whatman M9/50 silica, 80% hexane, 19% CH_2Cl_2, 1% 2-propanol, 5 mL/min). [^{18}F]-**7c**: (typical range 17–41%) t_R = 17.6 min; SA = 1306 Ci/mmol.

17α-Ethynyl-16β-([^{18}F]fluoro)-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-8a). Bistriflate **5a** (1.5 mg, 2.6 μmol) was vacuum dried for 12–15 h prior to labeling. ^{18}F-fluoride was added to the dry bistriflate and the reaction mixture was warmed to 60 °C while the lithium (trimethylsilyl)acetylide was prepared. ([Trimethylsilyl)acetylene (28 μL, 200 μmol) was dissolved in 0.5 mL of pentane cooled to 0 °C. Butyllithium (1.6 M in hexane, 120 μL, 192 μmol) was added to the acetylide solution forming a white precipitate (TMSC≡CLi). The precipitate was redissolved by adding 50 μL of freshly distilled THF. The TMSC≡CLi (150 μL, 56 μmol) was added to the cooled (0 °C) reaction mixture, and the resulting solution was allowed to warm to room temperature over 10 min. The reaction was quenched with NH_4Cl (1 M, 50 μL, 50 μmol) and evaporated to dryness *in vacuo*. The residue was resolubilized in 250 μL of MeOH and KOH (5 N, 100 μL, 500 μmol). The mixture was capped and heated with stirring at 60 °C for 30 min. The reaction was cooled and quenched with NH_4Cl (5 N, 100 μL, 500 μmol). The solution was diluted with 20 mL of H_2O and passed, in two portions, through a prepared C-18 Sep Pak (5 mL of MeOH, 10 mL of H_2O). The loaded Sep Pak was washed with pentane and the labeled product was eluted with CH_2Cl_2. The CH_2Cl_2 was diluted with an equal volume of hexane and injected onto a normal-phase semipreparative HPLC column (Whatman M9/50 silica, 65% hexane, 33.25% CH_2Cl_2, 1.75% 2-propanol, 5 mL/min). [^{18}F]-**8a**: (typical range 24–44%) t_R = 16 min; SA = 2860 Ci/mmol.

11β-Ethyl-17α-ethynyl-16β-([^{18}F]fluoroestra)-1,3,5(10)-triene-3,17β-diol ([^{18}F]-8b). Bistriflate **5b** (1.5 mg, 2.6 μmol) was vacuum dried 12–15 h prior to labeling. Conversion to [^{18}F]-8b was achieved by the preceding procedure. The compound was purified on a semipreparative normal phase HPLC column (Whatman M9/50 silica, 75% Hexane, 23.75% CH_2Cl_2, 1.25% 2-propanol, 5 mL/min). [^{18}F]-**8b**: (typical range 19–30%) t_R = 9.2 min; SA = 724 Ci/mmol.

17α-Ethynyl-16β-[^{18}F]fluoroestra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-8c). Bistriflate **5c** (1.5 mg, 2.6 μmol) was vacuum dried 12–15 h prior to labeling. Conversion according to the procedure outlined for [^{18}F]-**8a** yielded [^{18}F]-**8c**. Purification was achieved on a semipreparative normal phase HPLC column (Whatman M9/50 silica, 70% hexane, 28.5% CH_2Cl_2, 1.5% 2-propanol, 5 mL/min) [^{18}F]-**8c**: (typical range 16–35%) t_R = 12.3 min; SA 312 Ci/mmol.

Biological Methods. Determination of Receptor Binding Affinity. The binding affinity of the six 16β-fluoro ligands for the estrogen receptor (ER), alphafetoprotein (AFP), and sex binding protein (SBP) was determined by previously reported methods.[14-16a] The radiotracer for all three assays was [^{3}H]estradiol (Amersham, 51 Ci/mmol). Immature female rat uterine cytosol was the source of ER for the ER assay and the free steroid was absorbed on dextran-coated charcoal.[14a] Separate assays were performed at both 0 and 25 °C.[31] Rat amniotic fluid was the source of AFP and the unbound steroid was removed by hydroxylapatite.[16a] Third trimester human pregnancy serum served as the receptor source of SBP with removal of the free steroid by hydroxylapatite.[15] The affinities are reported relative to estradiol, given the value of 100%.

Measurement of the Octanol/Water Partition Coefficient. The log *P* values were estimated from log k'_w values determined by reversed-phase HPLC following the method outlined by Minick.[17] Full experimental details have been presented previously.[7] A Chromegabond C8 silica (5 mm, 60 Å, ES Industries) 15 cm × 4.6 mm column served as the stationary phase. The organic mobile phase was methanol containing 0.25% (v/v) 1-octanol, and the aqueous phase consisted of octanol-saturated water containing 0.02 M MOPS (3-morpholinopropanesulfonic acid, Sigma) buffer and 0.15% (v/v) *n*-decylamine, adjusted to pH 7.4. The flow rate was 1 mL/min.

***In Vivo* Biodistribution Studies.** The ^{18}F-labeled estrogens, purified by HPLC, were concentrated *in vacuo*, and redissolved in 250 mL of 100% ethanol and 250 mL of isotonic saline. The solution was passed through an ethanol-wetted filter. The filtered solution was diluted to 2.5 mL (final solution 10% ethanol/saline) with isotonic saline. Ether-anesthetized Sprague–Dawley female rats (25-days-old, >50 g)[32] were injected in the femoral vein with the desired doses (5–50 mCi) of the labeled compound. At specified time points postinjection the rats were sacrificed by decapitation and blood and organs were removed, weighed, and counted in a Beckman Gamma 6000 counter. Uterine uptake blocking studies were accomplished by a coinjection of 15 mg of estradiol and the labeled steroid.

Acknowledgment. We are grateful for support of this work through grants from the Department of Energy (DOE FG02 ER84 60218 to M.J.W.) and from the National Institutes of Health (CA 25836 to J.A.K. and HL13851 to M.J.W.). Additional support for this work was granted to H.F.V. through an Alexander Hollaender Distinguished Postdoctoral Fellowship, administered by the Oak Ridge Institutes for Science and Education. We thank Dr. Elio Napolitano for supplying the precursor to compound **1b**. We also greatly appreciate the assistance of Pamela Rocque, Henry Lee, and Zoe Ann McKinnon with the animal experiments. High-resolution mass spectra were obtained on instruments supported by the National Institutes of Health (GM 27029).

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