11β-Methoxy-, 11β-Ethyl- and 17α-Ethynyl-Substituted 16α-Fluoroestradiols: Receptor-Based Imaging Agents with Enhanced Uptake Efficiency and Selectivity†,[1]

Martin G. Pomper,‡ Henry VanBrocklin,§ Andrea M. Thieme,‡ Ralph D. Thomas,‡ Dale O. Kiesewetter,‡ Kathryn E. Carlson,‡ Carla J. Mathias,§ Michael J. Welch,§ and John A. Katzenellenbogen*,‡

Department of Chemistry, University of Illinois, 1209 West California Street, Urbana, Illinois 61801, and Division of Radiation Sciences, Mallinckrodt Institute of Radiology, Washington University Medical School, 510 South Kingshighway, St. Louis, Missouri 63110. *Received October 23, 1989*

We have prepared three analogues of 16α-fluoroestradiol (FES) substituted either with an 11β-methoxy group (1, 11β-MeO-FES), an 11β-ethyl group (2, 11β-Et-FES), or a 17α-ethynyl group (3, 17α-ethynyl-FES). These substituents all lower the binding of FES to the serum proteins alphafetoprotein and sex steroid binding protein, but their effect on estrogen receptor binding varies: Receptor binding is increased by the 11β-ethyl and 17α-ethynyl groups, but decreased by the 11β-methoxy group. These substituents also have a parallel effect on the lipophilicity, and hence the nonspecific binding estimated for these compounds. All three compounds were prepared in fluorine-18 labeled form, at effective specific activities of 90–1600 Ci/mmol, by fluoride ion displacement reactions as done previously with FES. Tissue distribution studies in immature rats show high uptake selectivity by target tissue (uterus) and effective competition by an excess of unlabeled estradiol. Percent injected dose per gram values (% ID/g) at 1 h are 6% for 11β-MeO-FES and 11–13% for 11β-Et-FES and 17α-ethynyl-FES (FES itself has a % ID/g of 9%). Uptake selectivity in terms of uterus to blood or muscle ratios at 1 h is highest for 11β-MeO-FES and 17α-ethynyl-FES (43–149). Metabolic consumption studies show that most activity in uterus is unmetabolized and in blood is rapidly and nearly completely metabolized. In muscle, FES and the substituted estrogens show intermediate levels of metabolic consumption; in some cases activity in muscle extracts is nearly unmetabolized. Thus, the substituents on FES cause major alterations in receptor and nonreceptor binding affinity, uptake efficiency and selectivity, and extent of metabolism. It is not readily clear, however, whether the alterations in uptake efficiency and selectivity are the result of differences in receptor or nonreceptor binding or lipophilicity, or altered patterns of metabolism. Nevertheless, these compounds should be useful in providing a spectrum of uptake properties that could be used for imaging different estrogen-receptor-containing structures.

Introduction

There has been great interest in the development of receptor-binding radiopharmaceuticals as agents for the in vivo imaging of receptor-containing tissues and tumors.[2] The receptors for steroid hormones provide a system with binding characteristics favorable for the uptake and selective retention of such radiopharmaceuticals for imaging purposes,[3] and radiolabeled estrogens and progestins with suitable binding properties (high affinity for the receptor and moderate to low nonspecific binding) have been shown to be taken up by target sites with high efficiency and great selectivity.[4]

We have been most interested in developing estrogens labeled with the positron-emitting radionuclide fluorine-18, as the half-life of this isotope (110 min) is suitable for radiochemical synthesis and permits imaging periods consistent with the development of adequate target to nontarget tissue contrast. As a result of an earlier investigation,[4c,5] we focused our efforts on 16α-fluoroestradiol (FES), a compound whose binding properties were very similar to those of estradiol, and we found that this compound shows high uptake in target tissues of the rat[4c] and that it can be used to image primary and metastatic estrogen-receptor-positive breast tumors[6] and receptor-rich regions of the brain in humans.[7]

The utility of such an imaging agent would be maximized if its uptake properties by estrogen-receptor regions were optimized. Therefore, in an attempt to improve upon the properties of FES, we have prepared three analogues of 16α-fluoroestradiol, two substituted at the 11β position with a methoxy and ethyl group, respectively (11β-MeO-

* Address correspondence to: Dr. John A. Katzenellenbogen, 461 Roger Adams Laboratory, Box 37, 1209 West California Street, University of Illinois at Urbana-Champaign, Urbana, IL 61801. Telephone: (217) 333 6310. Fax: (217) 244 8068.

† This paper is dedicated to the late Roger Adams on the occasion of the 100th anniversary of his birth; January, 1889.

‡ University of Illinois.

§ Washington University Medical School.

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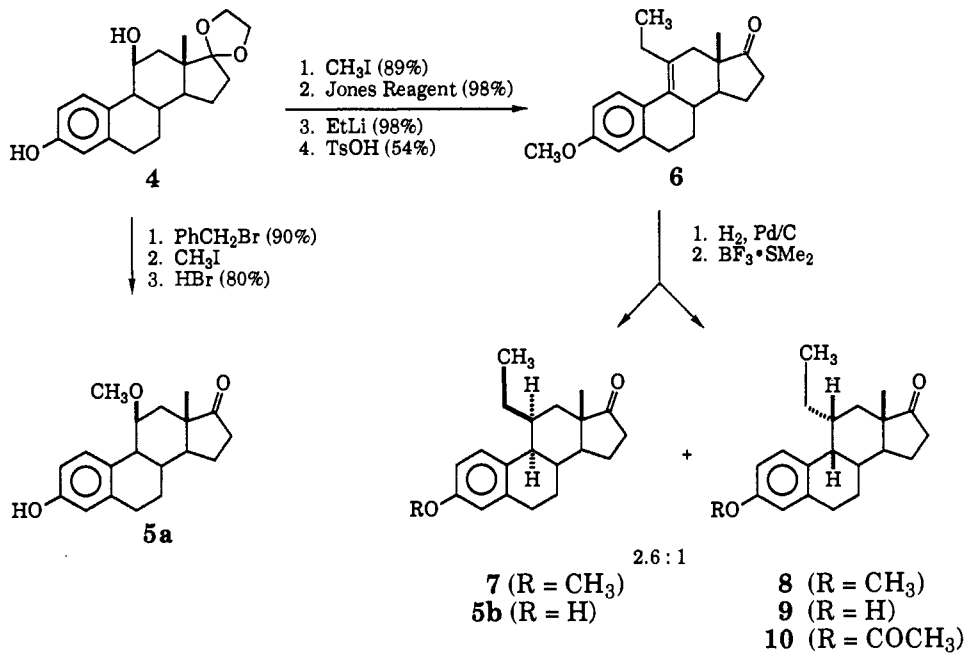
Scheme I. Synthesis of 11β-Substituted Estrone Derivatives **5a** and **5b**



4 **6** **5a**

7 (R = CH_3) **8** (R = CH_3)
5b (R = H) **9** (R = H)
10 (R = $COCH_3$)

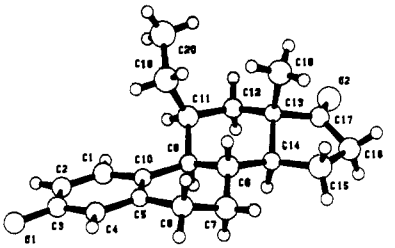
FES and 11β-Et-FES), and a third with a 17α-ethynyl substituent (17α-ethynyl-FES). These substituents are all known to affect the binding properties and to alter the course of metabolism of estrogens in ways that either increase their potency in vivo or improve their in vivo uptake properties.[8] We have labeled these three derivatives with fluorine-18 and studied the time course of their uptake in vivo and certain aspects of their metabolism in the immature rat. These substituents have favorable effects on the uptake efficiency and selectivity of these compounds in target sites so that they provide a spectrum of uptake behavior that may be useful in providing optimum images of tumor and target sites with varying levels of receptor and differing permeability characteristics.

Results

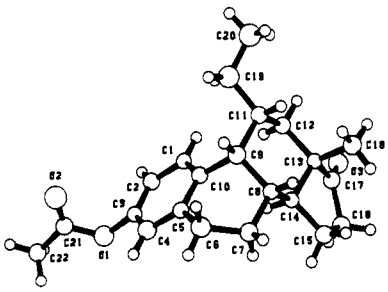
Synthesis of 11β-Methoxy-16α-fluoroestradiol (11β-MeO-FES, 1), 11β-Ethyl-16α-fluoroestradiol (11β-Et-FES, 2), and 17α-Ethynyl-16α-fluoroestradiol (17α-Ethynyl-FES, 3).

The 11β-methoxy- and 11β-ethyl-substituted analogues of 16α-fluoroestradiol (**1** and **2**) were synthesized (Scheme II) from the corresponding 11β-substituted estrone derivatives (**5a** and **5b**) (Scheme I). These derivatives, in turn, were prepared from a common intermediate **4**, which itself is produced by a route reported by Baran,[8a] starting from 1-dehydroadrenosterone.

We have described the preparation of 11β-methoxyestrone (**5a**) from the precursor **4** (Scheme I).[9] The route to 11β-ethylestrone (**5b**), shown in Scheme I, parallels that



11β-Ethylestrone (5b)



11α-Ethyl-9β-estrone 3-O-Acetate (10)

Figure 1. Plots of the X-ray structures of 11β-ethylestrone (**5b**) and 11α-ethyl-9α-estrone 3-*O*-acetate (**10**).

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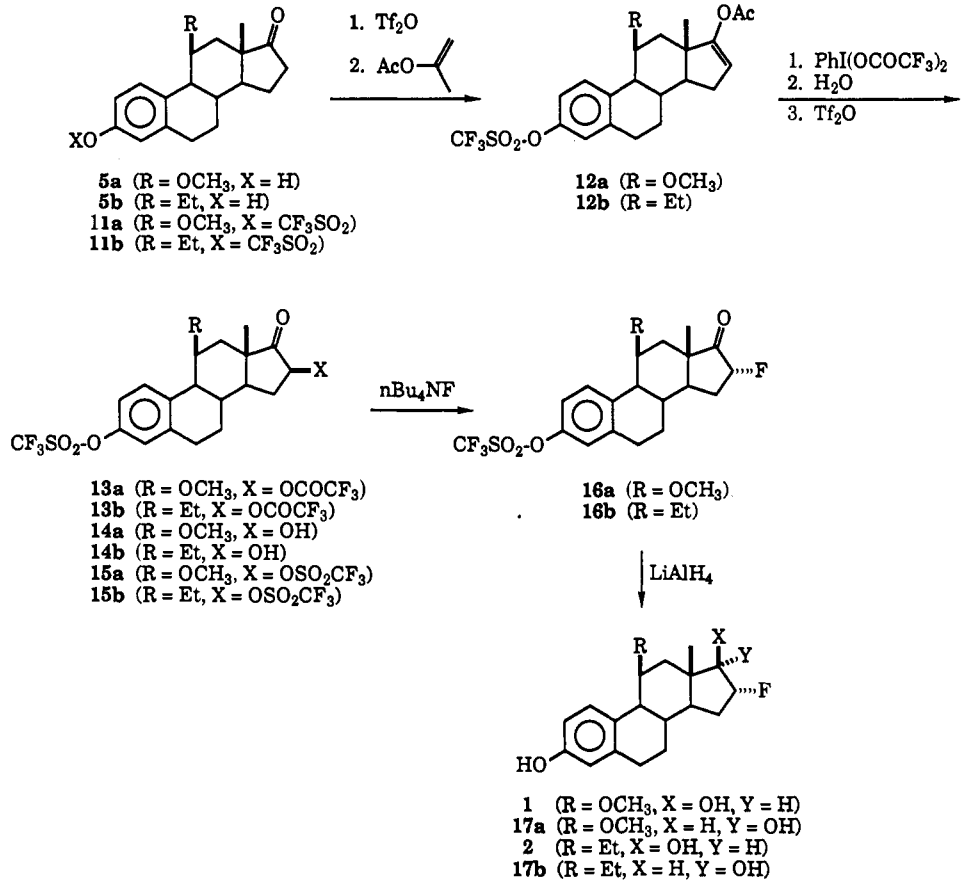
Scheme II. Conversion of 11β-Substituted Estrone Derivatives (**5a** and **5b**) into the 16α-Fluoroestradiol Derivatives 1 and 2



5a (R = OCH_3, X = H)
5b (R = Et, X = H)
11a (R = OCH_3, X = CF_3SO_2)
11b (R = Et, X = CF_3SO_2)

12a (R = OCH_3)
12b (R = Et)

13a (R = OCH_3, X = $OCOCF_3$)
13b (R = Et, X = $OCOCF_3$)
14a (R = OCH_3, X = OH)
14b (R = Et, X = OH)
15a (R = OCH_3, X = OSO_2CF_3)
15b (R = Et, X = OSO_2CF_3)

16a (R = OCH_3)
16b (R = Et)

1 (R = OCH_3, X = OH, Y = H)
17a (R = OCH_3, X = H, Y = OH)
2 (R = Et, X = OH, Y = H)
17b (R = Et, X = H, Y = OH)

described by Baran.[8a] The phenol was protected as the methyl ether; oxidation with Jones reagent gave the C-11 ketone, and ethyllithium addition and acid-catalyzed dehydration gave the 9(11)-dehydro compound **6**. Catalytic hydrogenation of the 9(11)-double bond in **6** proceeds to give a 2.6:1 mixture of predominantly two stereoisomers, the desired 11β-ethyl-B/C-trans fused system **7** (resulting from α attack of hydrogen) and the 11α-ethyl-B/C-cis fused system **8** (resulting from β attack of hydrogen), respectively. Although these stereoisomers can be separated by GC on an analytical scale, their separation by chromatographic methods on a preparative scale, either at the methyl ether stage (**7** and **8**) or as the free phenol (**5b** and **9**), has proved difficult. We found, however, that careful trituration of the mixture of **5b** and **9** in ether furnished nearly pure 11β-ethylestrogen **5b**. Further recrystallization gave the 11β-isomer **5b** in pure form; the 11α-isomer **9** furnished a crystalline acetate **10** that was further purified by crystallization.

The stereochemical assignment of the epimeric 11-ethyl estrogens **5b** and **9** is suggested by their ^1H NMR spectra: Situated nearly in the plane of the aromatic ring, the methyl protons of the 11α-ethyl group (δ 0.98) are deshielded relative to those of the 11β-ethyl group in **5b** (δ 0.91); interaction of the C-1 proton with the 11α-ethyl substituent in **9** causes a substantial shift (δ 7.33) relative to that of the less crowded 11β epimer **5b** (δ 7.08). The stereochemistry of the isomeric 11-ethyl estrogens was established definitively by X-ray crystallography. The crystal structures (Figure 1) show clearly the 9α-hydrogen and 11β-ethyl stereochemistry in **5b**, and the 9β-hydrogen and 11α-ethyl stereochemistry in **9**. (X-ray coordinate data are furnished in the supplementary material.)

The two 11β-substituted estrogen derivatives (**5a** and **5b**) were converted into the corresponding 16α-fluoroestradiols (**1** and **2**) by the sequence that we have used previously for the synthesis of FES,[5] i.e., preparation of the 3-trifluoromethanesulfonates (triflate), followed by conversion to the 17-enol acetates. It proved significantly more difficult to prepare the enol acetates from the 11β-substituted estrones than from estrone itself. The reaction failed to go to completion, and the yields shown are based on reacted starting material. The unreacted material was recycled. Perhaps a steric buttressing effect, due to the 11β-substituent, transmitted through the 18-methyl group, retards the rate of acetylation or destabilizes the enol acetate product. The 16β-trifluoroacetoxylation, hydrolysis, and triflation proceeded in the substituted systems with ease comparable to that of the unsubstituted one, to give compounds **15a** and **15b**, respectively. Displacement with fluoride ion and hydride reduction to deprotect at 3-0-triflate and reduce the 17-ketone, proceeded in yields comparable to those of the unsubstituted system, furnishing the 11-substituted fluoroestrogens **1** and **2**.

Hydride reduction of unsubstituted D-ring keto steroids is known to proceed via α-face attack,[10] but the 11β-substituent in these systems was found to have a significant influence on the stereoselectivity of this reduction. For FES, the 17β- and 17α-hydroxy isomers are produced in a ratio of 3:1,[5] whereas, this ratio is 11:1 and 1.6:1 in the 11β-methoxy- and 11β-ethyl series, respectively. That the 11β-ethyl compound showed lower 17β-ol:17α-ol selectivity than the unsubstituted case is surprising in view of the expected shielding of the β-face of the molecule to hydride attack at the C-17 carbonyl, due to the buttressing effect alluded to earlier. Additionally, comparison of the dihedral angles C_{14}–C_{13}–C_{17}–carbonyl oxygen between estrone[11] and

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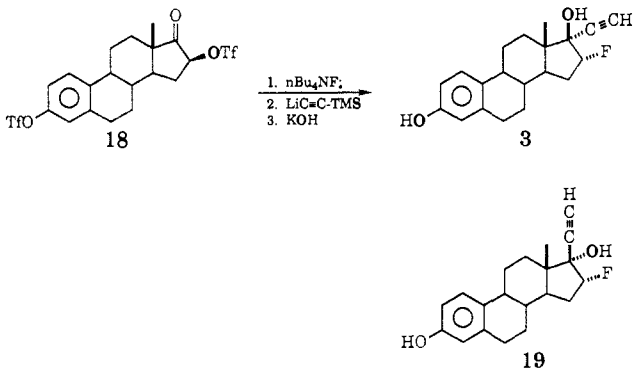
Table I. Binding Affinity of 16α-Fluoroestrogen Derivatives for the Estrogen Receptor (ER), Alphafetoprotein (AFP), Sex Steroid Binding Protein (SBP) and Their Nonspecific Binding (NSB) and Binding-Selectivity Index (BSI)

	relative binding affinity (RBA) [estradiol = 100][a]						
	ER				log P		
compd	25 °C	0 °C	AFP	SBP	(from k'_w)	NSB[b]	BSI[c]
estradiol	100	100	100	100	3.261	1.00	100
11β-methoxyestradiol	86 ± 9.6	9.7 ± 2.6	0.26 ± 0.04	1.72 ± 0.20	2.550	0.48	179
11β-ethylestradiol	1360 ± 494	133 ± 30	1.21 ± 0.33	40.8 ± 8.6	3.799	1.74	782
17α-ethynylestradiol	272 ± 22	122 ± 5	3.51 ± 1.12	1.81 ± 0.47	3.421	1.18	231
16α-fluoroestradiol (FES)	54 ± 9.4	77 ± 4	101 ± 7	9.5 ± 5.4	2.982	0.75	72
11β-OMe-FES (1)	26 ± 3	4.6 ± 0.3	0.51 ± 0.10	0.53 ± 0.36	2.302	0.37	70
11β-Et-FES (2)	1630 ± 1050	122 ± 41	1.47 ± 0.12	2.24 ± 0.14	3.732	1.62	1003
17α-ethynyl-FES (3)	108 ± 21	66 ± 4	0.94 ± 0.22	0.43 ± 0.09	3.075	0.83	131

[a] The relative binding affinity (RBA) values are determined by competitive radiometric binding assays, according to previously described methods: estrogen receptor (ER),[13] alphafetoprotein (AFP),[14] and sex steroid binding protein (SBP).[15] In each case, estradiol was given a relative value of 100; the actual equilibrium dissociation constant for estradiol is 0.3 nM (ER), 25 nM (AFP), and 2 nM (SBP).[3a] Values shown are the average ± standard deviation or range for 2–7 determinations. [b] The nonspecific binding (NSB) was estimated from the calculated partition coefficient by using an empirically derived expression previously described by this laboratory;[3a] estradiol is given a relative value of 1.00. [c] The binding selectivity index (BSI) is calculated by dividing the RBA (25 °C) by NSB.

Scheme III. Synthesis of 17α-Ethynyl-16α-fluoroestradiol (3)



11β-ethyl estrone, derived from crystallographic data, shows them to be nearly identical (~148°), implying minimal differences in steric hindrance of hydride attack to C-17 from the α-face.

The synthesis of 17α-ethynyl-FES (3) (Scheme III) has been described by us previously.[5] The route parallels that for the preparation of FES, except that lithium trimethylsilylacetylide is added to the 16α-fluoroestrone, followed by treatment with base to remove the ethynyl trimethylsilyl group and the triflate at 3-O. The 17α-hydroxy- and 17β-hydroxy stereoisomers of 17-ethynyl-FES are produced in a 1:1 ratio.

Synthesis of 11β-Methoxy-16α-[^{18}F]fluoroestradiol ([^{18}F]-1), 11β-Ethyl-16α-[^{18}F]fluoroestradiol ([^{18}F]-2), and 17α-Ethynyl-16α-[^{18}F]fluoroestradiol ([^{18}F]-3). The title compounds were prepared in fluorine-18 labeled form by the same route used to prepare the unlabeled compounds. [^{18}F]Fluoride ion in water, prepared by proton bombardment of an oxygen-18 water target,[12] was treated with nBu_4NOH, dried, and resolubilized in tetrahydrofuran (THF), as previously described.[4c] [^{18}F]Fluoride ion displacement of the 16β-trifloxy groups in **6**, **9**, and **10**, followed by hydride reduction (for **1** and **2**) or ethynylation and base treatment (for **3**), produced the substituted 16α-fluoroestradiols (**1–3**).[5] All of the final products were purified by normal-phase HPLC.

In each case, radio-HPLC revealed two products, corresponding to the 17-hydroxy epimers. The ratio of 17β-ol:17α-ol products was 1.8:1 and 0.5:1 for the 11β-methoxy and 11β-ethyl cases, respectively. The selectivity was decreased in each case relative to the unlabeled synthesis (11:1 for methoxy, 1.6:1 for ethyl). The reason for this decrease is not apparent.

Typical synthesis times are 90–120 min from the end of bombardment; radiochemical yields are 2–32% (decay corrected); and effective specific activities[9] range from 90–1600 Ci/mmol. (The effective specific activities of the preparations used in the tissue uptake experiments are given in the footnotes to Tables II–IV.) These are all comparable to those we have obtained previously for FES.

Binding Affinity of the Substituted 16α-Fluoroestradiols (1, 2, and 3) for the Estrogen Receptor and the Serum Proteins Alphafetoprotein (AFP) and Sex Steroid Binding Protein (SBP). The receptor[13] and serum protein[14,15] binding properties of the three substituted 16α-fluoroestradiols and some related compounds are given in Table I. This table also shows the estimated nonspecific binding affinity of these compounds.

As was known previously,[8c] the 11β-methoxy substituent lowers binding affinity to the estrogen receptor and 11β-ethyl substitution increases binding; this is apparent both in the estradiol and in the 16α-fluoroestradiol series. The 17α-ethynyl substituent also raises binding affinity, but less markedly than the 11β-ethyl substituent. It is of note that the receptor binding affinities are all significantly greater when the binding assay is incubated at 25 °C, than at 4 °C for 18 h. This increase in relative binding affinity is ascribed either to a slow approach to equilibrium[16] or to the induction of enhanced binding affinity due to receptor transformation.[17] The relative error in the binding affinity determinations of the two highest affinity compounds, 11β-ethyl estradiol and 11β-ethyl-FES is the greatest. This is a problem inherent in saturation binding analyses, where the displacement curves for very high affinity compounds tend to converge to a titration curve, increasing the relative error.[8i]

Two serum proteins with high affinity for estrogens are potential factors that may perturb in vivo uptake selectivity. In rodents (but not in humans), a fetal albumin, alphafetoprotein (AFP), binds estradiol with a K_d of 15

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nM (about 100-fold lower affinity than the estrogen receptor; K_d = 0.3 nM).[3a] AFP titers in newborn rats are very high (300 μM), but decline to undetectable levels by about day 25.[3a] Nevertheless, we have found that with the age range of rats that we use (day 22–25), AFP can have a profound effect on uptake selectivity.[15] While the 16α-fluoro substituent does not alter the binding affinity of estradiol for AFP, substituents at 11β and 17α cause a precipitous drop (70–200-fold) in binding affinity, thus eliminating potential problems from this serum binding protein.

Sex steroid binding protein, a serum β-globulin found in human serum,[3a] could affect the binding distribution of these compounds in humans. Although the affinity of SBP for estradiol is 2 nM, its titer in humans is relatively low (nonpregnant females 50–100 nM; pregnant females 200–400 nM).[3a] The 16α-fluoro substituent causes a 10-fold decrease in estradiol binding to SBP. The 11β and 17α substituents cause an additional 4–20-fold decrease, again, minimizing the potential effect of this serum binding protein.

Measurement of Octanol/Water Partition Coefficients of the Estrogens and Estimation of Their Nonspecific Binding. In the past, in correlating the in vitro binding properties of radiolabeled estrogens with their in vivo target tissue uptake selectivity, we found it useful to consider their nonspecific, as well as their receptor binding affinity.[3ab,18] Nonspecific binding is generally not structure specific, but, like solvent partitioning, depends on the lipophilicity of the molecule.[19] As have others, we found that the nonspecific binding of steroids correlates well with their octanol/water partition coefficients,[3a] and we derived an empirical relationship that correlates the relative nonspecific binding of various estrogens (toward nonspecific binding proteins in target tissues) with both experimentally measured (shake-flask) and calculated octanol/water partition coefficients (log $P_{o/w}$).[3a] Since it is tedious and experimentally demanding to measure octanol–water partition coefficients of highly nonpolar substances by the shake-flask method and since the calculation methods give only an approximation of log $P_{o/w}$ (the rules cannot take into account all of the functional, stereochemical, and proximity relationships within a large range of compounds[20], we utilized the reversed-phase HPLC method of Minick[21] to estimate octanol/water partition coefficients of the estrogens.

Minick's method involves the measurement of the chromatographic capacity factors (k') for the unknown compounds on a C-8 reversed-phase column at various concentrations of methanol (containing 0.25% octanol) and an aqueous phase consisting of 0.15% *n*-decylamine in 0.02 M MOPS (3-morpholinopropanesulfonic acid) buffer pH 7.4 (prepared in 1-octanol-saturated water). These capacity factors (k') are extrapolated to 100% of the aqueous component (k'_w). As documented by Minick,[21] this chromatographic system reliably gives a linear relationship between log k' and the solvent composition. The octanol/water partition coefficients are then estimated from a standard curve generated from compounds with experimentally verified partition coefficients. The standard curve is shown in Figure 2. Examples of such plots for the fluorine-substituted estrogens are shown in Figure 3, and the estimated octanol/water partition coefficients are summarized in Table I.

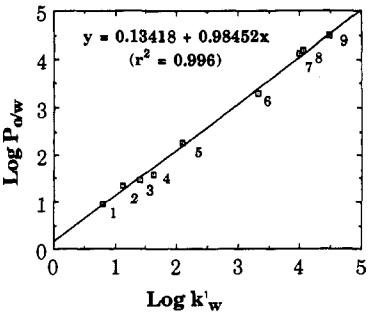

Figure 2. The correlation for standard compounds between the octanol/water partition coefficients (log $P_{o/w}$) and the log k'_w values derived by linear extrapolation of the measured chromatographic capacity values (log k') at several volume fractions of methanol (ϕ). The numbers refer to the standard compounds listed below. The substituted estrogens in this study had log k'_w values between 2.39 and 3.80. The regression line is shown in the figure. Standards used were (compound no., name, log $P_{o/w}$[ref], measured log k'_w): 1. 4-methoxyaniline, 0.95[21], 0.802; 2. 4-methoxyphenol, 1.34[21], 1.112; 3. phenol, 1.46[21], 1.392; 4. acetophenone, 1.58[21], 1.619; 5. 4-bromoaniline, 2.26[21], 2.096; 6. naphthalene, 3.30[21], 3.314; 7. *tert*-butylbenzene, 4.11[22], 3.990; 8. anthracene, 4.20[23], 4.052; 9. pyrene, 4.50[23], 4.469.

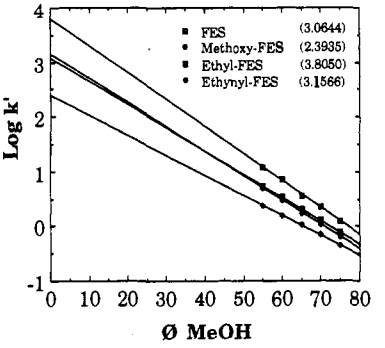

Figure 3. log k' values at five volume fractions of methanol (ϕ) for the four fluoroestrogens, extrapolated to zero ϕ methanol (100% aqueous) = k'_w. The log k'_w values are given in parentheses. For all the lines, the r^2 values from the linear regression were greater than 0.998.

The regression line relating the log k'_w values of the standards to their log P values (Figure 2) shows very high statistical reliability, but of equal importance it has a slope very nearly equal to one. A correlation such as this is generally considered to indicate that the chromatographic method is accurately reproducing octanol/water partition values that would be measured by the shake-flask method.[22] Since the reliable measurement range for log k' is ca. −0.5 to 1.5, the plots of log k' vs volume mole fraction of methanol (Figure 3) indicate that the regression line, particularly with the more lipophilic compounds, must be extrapolated quite a way to reach ϕ = 0. Nevertheless, as documented by Minick,[21] this chromatographic system gives good linearity and reliable extrapolation. (This lin-

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Table II. Tissue Distribution of 11β-Methoxy-16α[^{18}F]fluoroestradiol (11β-MeO-FES, 1) in Immature Rats (% ID/g ± SD; n = 5)[a]

tissue	1 h	1 h (low)	1 h[b] (blocked)	3 h
blood	0.127 ± 0.051	0.147 ± 0.047	0.152 ± 0.025	0.086 ± 0.022
lung	0.135 ± 0.032	0.274 ± 0.048	0.123 ± 0.017	0.075 ± 0.008
liver	2.30 ± 1.60	2.00 ± 0.88	2.04 ± 0.60	0.467 ± 0.168
kidney	1.22 ± 0.80	1.88 ± 0.68	1.05 ± 0.23	0.336 ± 0.110
muscle	0.135 ± 0.023	0.330 ± 0.095	0.075 ± 0.010	0.056 ± 0.012
fat	0.440 ± 0.084	0.500 ± 0.220	0.133 ± 0.047	0.164 ± 0.017
bone	0.083 ± 0.024	0.208 ± 0.174	0.131 ± 0.112	0.167 ± 0.137
uterus	5.80 ± 1.02	6.76 ± 0.74	0.147 ± 0.760	2.77 ± 0.26
ovaries	1.89 ± 0.59	1.90 ± 0.82	0.105 ± 0.020	1.07 ± 0.25
uterus/blood	48.1 ± 1.0	49.2 ± 13	0.93 ± 0.47	35.6 ± 7.0
uterus/muscle	43.2 ± 5.6	19.8 ± 12	1.93 ± 0.80	50.5 ± 9.2

[a] Rats were injected iv with 50 μCi of 11β-MeO-FES (effective specific activity was 90 Ci/mmol) in 10% ethanol/saline. Animals in the 1-h (low) experiment received a dose of 5 μCi, and those in the 1-h (blocked) experiment received 15 μg of unlabeled estradiol by coinjection. [b] n = 4.

Table III. Tissue Distribution of 11β-Ethyl-16α[^{18}F]fluoroestradiol (11β-Et-FES, 2) in Immature Rats (% ID/g ± SD; n = 5)[a]

tissue	1 h	1 h (low)	1 h[b] (blocked)	3 h
blood	1.09 ± 0.46	0.82 ± 0.11	1.10 ± 0.23	0.844 ± 0.550
lung	0.99 ± 0.23	1.35 ± 0.37	0.918 ± 0.182	0.710 ± 0.074
liver	3.31 ± 2.12	1.94 ± 0.57	3.35 ± 1.50	2.47 ± 0.90
kidney	4.05 ± 2.40	3.29 ± 0.65	3.06 ± 1.16	2.52 ± 0.87
muscle	0.75 ± 0.15	0.82 ± 0.15	0.236 ± 0.04	0.390 ± 0.027
fat	2.27 ± 0.88	1.42 ± 0.04	1.86 ± 0.43	0.978 ± 0.156
bone	0.444 ± 0.096	0.51 ± 0.18	0.38 ± 0.13	0.35 ± 0.17
uterus	12.59 ± 3.40	13.50 ± 3.26	0.48 ± 0.15	11.49 ± 2.11
ovaries	5.62 ± 1.41	5.08 ± 0.66	0.76 ± 0.27	4.61 ± 0.70
uterus/blood	13.0 ± 2.1	16.6 ± 3.4	0.52 ± 0.19	13.7 ± 2.8
uterus/muscle	17.0 ± 4.5	17.2 ± 6.3	2.12 ± 0.59	32.5 ± 5.7

[a] Rats were injected iv with 50 μCi of 11β-Et-FES (effective specific activity was 439 Ci/mmol) in 10% ethanol/saline. Animals in the 1-h (low) experiment received a dose of 5 μCi, and those in the 1-h (blocked) experiment received 15 μg of unlabeled estradiol by coinjection. [b] n = 4.

Table IV. Tissue Distribution of 17α-Ethynyl-16α[^{18}F]fluoroestradiol (3) (17α-Ethynyl-FES), in Immature Rats (% ID/g ± SD; n = 5)[a]

tissue	1 h	1 h (low)	1 h[b] (blocked)	3 h
blood	0.087 ± 0.037	0.089 ± 0.032	0.129 ± 0.116	0.128 ± 0.058
lung	0.438 ± 0.139	0.446 ± 0.203	0.147 ± 0.109	0.261 ± 0.069
liver	1.548 ± 0.530	1.472 ± 0.458	1.975 ± 1.052	1.901 ± 1.103
kidney	0.950 ± 0.075	1.056 ± 0.186	0.296 ± 0.208	0.430 ± 0.089
muscle	0.307 ± 0.033	0.339 ± 0.046	0.085 ± 0.083	0.245 ± 0.179
fat	0.571 ± 0.172	0.750 ± 0.240	0.068 ± 0.030	0.374 ± 0.083
bone	1.268 ± 0.176	1.071 ± 0.205	1.259 ± 0.341	1.510 ± 0.425
uterus	11.152 ± 3.747	12.145 ± 5.113	0.510 ± 0.206	6.578 ± 1.351
ovaries	2.444 ± 0.648	3.053 ± 1.592	0.348 ± 0.219	2.150 ± 0.538
uterus/blood	149 ± 81	154 ± 89	5.27 ± 2.88	58.3 ± 24.8
uterus/muscle	35.9 ± 10.0	35.5 ± 14.6	8.24 ± 4.23	36.7 ± 19.1

[a] Rats were injected iv with 50 μCi of 17α-ethynyl-FES (3) (effective specific activity was 1590 Ci/mmol) in 10% ethanol/saline. ID is injected dose. SD is standard deviation. Animals in the 1-h (low) experiment received a dose of 5 μCi, and those in the 1-h (blocked) experiment received 15 μg of unlabeled estradiol by coinjection. [b] n = 4.

earity was found, as well, for the standards presented in Figure 2.)

The equation below describes an experimentally determined relationship between octanol/water partition coefficients and the nonspecific binding (NSB) of various steroidal and non-steroidal estrogens.[3ab,18] Using this equation, we have calculated the relative nonspecific binding of the estrogens studied here. These values, which are all scaled to estradiol = 1, are also given in Table I.

$$\log \text{NSB} = 0.447(\log P_{\text{cpd}} - \log P_{\text{E}_2})$$

From these data, it is apparent that the 11β-methoxy group decreases nonspecific binding consistently by about a factor of 2, while the 11β-ethyl substituent increases it by about the same factor. Somewhat less consistently, the 17α-ethynyl group increases nonspecific binding by a factor of 1.4, and the 16α-fluoro group decreases it by 1.25.

We have termed the ratio of a compound's receptor binding affinity (RBA) to its nonspecific binding (NSB) to be its binding-selectivity index (BSI).[3ab,18] Because it accounts for both receptor and nonspecific binding, the binding-selectivity index in some cases correlated with uptake selectivity better than did the binding affinity measurement alone. The values for the BSI of these estrogens are also given in Table I.

Tissue Distribution of the Substituted 16α-Fluoroestradiols (1, 2, and 3) in Immature Rats. The tissue distribution of the substituted fluoroestrogens 1–3 in immature female rats at various times after injection is shown in Tables II–IV. In each case, distribution data are given at 1 and 3 h at a normal dose (ca. 50 μCi), at 1 h at a low dose (5 μCi), and at 1 h together with a blocking dose of unlabeled estradiol. The last experiment establishes the level of nonspecific uptake, since the large dose of unlabeled ligand blocks receptor-mediated uptake.

It is evident that uptake by the uterus is highly selective for all three compounds. In all cases and at all times (except the 1-h blocked experiments), uterus to blood, or uterus to muscle ratios are at least 10, and in some cases as high as 150. Evidence for receptor mediation of uterine uptake comes from the 1-h blocked experiment, where coinjection of an excess of unlabeled estradiol lowers

Table V. Comparison of the Tissue Distribution of the Three Substituted FES Compounds (1-3) with FES itself in the Immature Rat at 1 h (% ID/g ± SD (n = 5))

tissue	FES[a]	11β-MeO-FES (1)[b]	11β-Et-FES (2)[c]	17α-Ethynyl-FES (3)[d]
uterus	8.96 ± 1.20	5.80 ± 1.02	12.59 ± 3.40	11.15 ± 3.75
ovaries	2.53 ± 0.31	1.89 ± 0.59	5.62 ± 1.41	2.44 ± 0.65
blood	0.72 ± 0.21	0.13 ± 0.05	1.09 ± 0.46	0.09 ± 0.04
muscle	0.37 ± 0.14	0.14 ± 0.02	0.75 ± 0.15	0.31 ± 0.03
lung	0.17 ± 0.07	0.14 ± 0.03	0.99 ± 0.23	0.44 ± 0.14
kidney	1.48 ± 0.29	1.22 ± 0.80	4.05 ± 2.40	0.95 ± 0.08
liver	2.10 ± 0.50	2.30 ± 1.60	3.31 ± 2.12	1.55 ± 0.53
fat	-	0.44 ± 0.08	2.27 ± 0.88	0.57 ± 0.17
bone	0.40 ± 0.18	0.08 ± 0.02	0.44 ± 0.10	1.27 ± 0.18
uterus/blood	13.5 ± 4.3	48.1 ± 8.5	13.0 ± 2.1	149 ± 81
uterus/muscle	25.9 ± 5.2	43.2 ± 5.6	17.0 ± 4.5	35.9 ± 10.0

[a] Data are from H. VanBrocklin, unpublished. [b] Data are from Table II. [c] Data are from Table III. [d] Data are from Table IV.

uterine uptake to the level of a nontarget tissue. Uterus to blood or muscle ratios generally fall to 1-2; only with 17α-ethynyl-FES are the blocked ratios somewhat greater. In no case does the uptake in the 1-h (low) experiment differ significantly from the 1-h experiment, confirming that the uptake we have observed is not limited by effective specific activity.

The comparative aspects of the tissue distribution of these three substituted fluoroestradiols can be appreciated more readily by reference to Table V, where the uptake at 1 h is presented, together with the uptake for FES itself. The uptake for FES shown here is taken from a recent experiment (H. VanBrocklin, unpublished) in rats of age (22-25 days), comparable to those used for compounds 1-3 in this report; our original report on the uptake of FES used younger animals (estimated 19-21 days old), and the uptake recorded there (% ID/g = 4.67 ± 1.50)[4c] was somewhat lower than is presented here (% ID/g = 8.96 ± 1.20).

The % ID/g uterus varies considerably among these four compounds. Compared to FES, the uptake of the 11β-MeO-FES (1) is somewhat lower, and that of the 11β-ethyl and 17α-ethynyl derivatives considerably greater. These trends in uptake efficiency parallel the receptor binding affinity of these compounds (cf. Table I); however, it is possible that the differences in uptake do not arise from their different affinities, since uptake of high affinity estrogens by a receptor-rich target tissue such as the uterus is probably limited by tissue permeability (flow limited).[24] Therefore, the tissue accumulation will probably also reflect the relative permeabilities of these compounds, which is likely to be related to their relative lipophilicities (cf. Table I).

The activity ratios of uterus to blood and uterus to nontarget tissues (such as muscle) have traditionally been used as indicators of uptake selectivity. This may be valid in an empirical sense, since it is this difference that is responsible for the contrast between target and nontarget areas needed for an image to develop. However, one may question the degree to which these ratios should be related to the receptor binding and physical properties of the radiopharmaceuticals. Although most of the activity in the target tissue is not metabolized, much of the activity in nontarget areas arises from metabolites, not the parent compounds (see next section for a more complete discussion). Nevertheless, with these limitations understood, it is interesting that 11β-MeO-FES (1), the compound with the lowest uterine uptake, has one of the highest uptake selectivities, matched only by 17α-ethynyl-FES (3). By contrast, 11β-Et-FES (2), which has the highest level of uterine uptake, has the lowest uptake selectivity, since uptake of this compound by nontarget tissues is also relatively high.

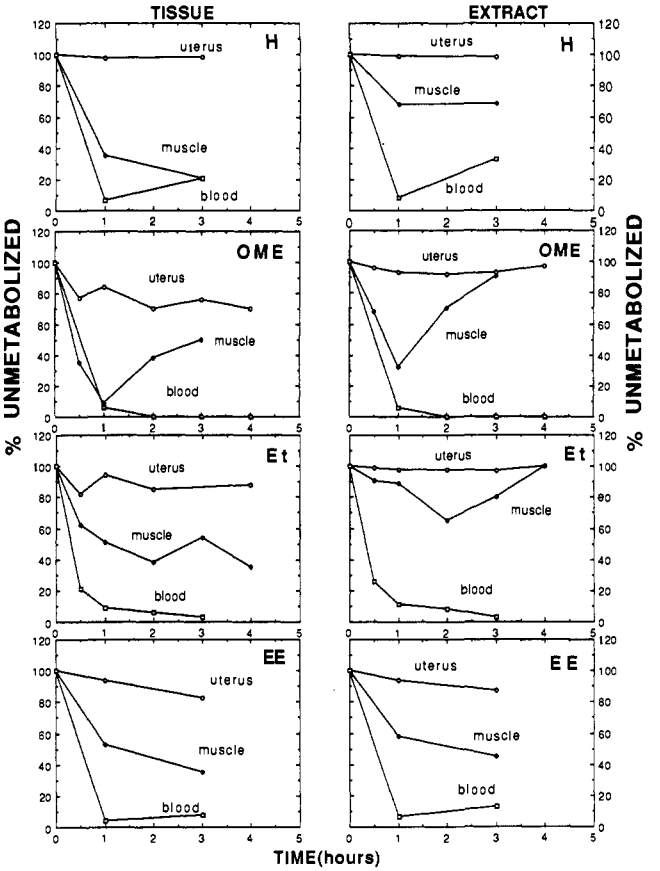

Figure 4. Metabolism of 11β-MeO-FES (1), 11β-Et-FES (2), and 17α-ethynyl-FES (3). In each case, the radiochemical purity (% unmetabolized) is determined by thin-layer chromatographic analysis of an ethanol extract of a homogenate of the indicated tissue (EXTRACT column, right). These data are corrected by the fraction of the activity that is extractable, relative to the extraction of a radiochemically pure standard, to give the data in the TISSUE column (left). For a complete explanation, see Experimental Section and ref 25.

All three compounds show relatively low activity levels in bone. As the bone activity is due, most likely, to free fluoride ion,[25] liberated by metabolic defluorination processes, this indicates that there is relatively little defluorination in these compounds.

Time Course of Metabolic Consumption of FES and the Substituted FES Derivatives (1-3) in Uterus,

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Blood, and Muscle. The radioactivity in uterus, blood, and muscle at various times after injection was extracted and analyzed by thin-layer chromatography to establish the fraction of activity that is extractable and the fraction of the extract that is unmetabolized. The level of metabolic consumption of the fluorosteroids in these tissues, plotted as a function of time, is presented in Figure 4. The panels on the right show the percent of activity in the tissue extracts that is unmetabolized; those on the left are estimates of the percent of activity in the tissue itself that is unmetabolized. These latter values are obtained by multiplying the percent unmetabolized in the extract by the percent extraction, corrected for the extraction efficiency of the unmetabolized compound itself in the same tissue preparation.[26] In all cases, the unmetabolized compounds were extracted from tissues with >90% efficiency. The metabolism data for FES shown here, differs somewhat from that in an earlier publication.[26] The metabolism study presented here was done in immature rats (day 22–25), whereas the earlier study was done in mature rats.

In all cases, the percent unmetabolized in the target tissue remains high, whereas that in the blood rapidly drops to low values. The situation with muscle is intermediate: With FES and 17α-ethynyl-FES, activity in muscle extract drops; with the other two compounds, the percent unmetabolized in muscle extract drops initially, but then later rises until nearly all the activity in the extracts is unmetabolized.

While this time course for muscle metabolites may at first appear curious, it is consistent with a situation in which the extractable metabolites are cleared from muscle more rapidly than the unmetabolized material. Thus, whereas metabolites might accumulate in muscle at early times (as they are generated elsewhere, e.g., in liver, and circulate freely), their more rapid clearance will leave an increasing percentage of unmetabolized estrogen in the muscle. This model requires that muscle retain unmetabolized compound in preference to metabolites. Muscle is generally considered to be a nontarget tissue, and it is possible that nonspecifically bound, unmetabolized radiotracer may be retained in preference to its metabolites. These are, however, low levels of estrogen receptor in muscle;[27] so, the selective retention that is observed may be assisted by binding to this high affinity site. In fact, in other studies (C. J. Mathias et al., unpublished), we have shown that low levels of a saturable, high affinity binding component can be detected by FES uptake in muscle.

Discussion

We have described the synthesis, and studied the receptor and serum protein binding, in vivo uptake, and metabolic consumption of three new fluorine-18 labeled estrogens that are analogues of 16α-fluoroestradiol (FES), a compound that we have studied extensively in the past.[4c,5,6,18] Selection of the substituents and their site of attachment was based on substitutions that are known from previous work to increase the in vivo potency[8] or to improve the uptake selectivity of other types of estrogens,[4b,f,h-j] either by enhancing binding to the estrogen receptor, reducing nonspecific or serum binding, or altering the route or reducing the rate of metabolism.

The 17α-ethynyl group is a frequently utilized substituent on estrogens, known to increase the oral potency of estrogen pharmaceuticals.[28] This effect is presumed to be due to its suppression of D-ring metabolism (to estrone and ultimately estriol). The ethynyl group also increases receptor binding affinity and reduces binding to serum proteins. The 11β-position on estrogens is a site where small to moderately sized substituents are well tolerated by the receptor.[8d] In fact, lipophilic substituents at this site (ethyl or chloromethyl) give compounds with affinities as much as 30-fold greater than estradiol;[8i] hydrophilic substituents, such as methoxy, reduce receptor binding modestly,[8c] but, as do the lipophilic substituents, they still enhance potency.[8a,c,e] The hydrophilic groups decrease binding by nonspecific sites and all the 11β substituents reduce binding to certain serum proteins and are thought to retard both A- and D-ring metabolism.[8c] So, these substituents seemed to be favorable ones to investigate in the FES series. The effect of 11β-methoxy substitution in other γ-emitting estrogens has been investigated and has resulted in compounds with improved uptake selectivity (11β-methoxy-16α-bromo or iodoestradiol, and 11β-methoxy-17α-(iodovinyl)estradiol);[4b,f,h,i] carbon-11 labeled 17α-ethynylestradiol has been prepared, but at low specific activity.[4j] There is only a preliminary report of the use of 11β-ethyl substituents in γ-labeled estrogens.[29]

The 11β- and 17α-substituents in FES were found to have the expected effect on binding to the estrogen receptor and to serum proteins, and to have consistent effect on lipophilicity (measured by log $P_{o/w}$) and hence on predicted nonspecific binding affinity. Their effect on tissue distribution in vivo, however, was complex. Whereas the uptake efficiency (% ID/g of FES) by the uterus was increased by 11β-ethyl or 17α-ethynyl substitution, it was decreased by 11β-methoxy substitution. While this trend parallels the receptor binding affinity of these compounds, it also parallels their lipophilicity. Thus, since the uptake of these agents by a receptor-rich target tissue like the uterus is probably limited by tissue permeability,[24] the differences in their uptake may simply be due to their differences in lipophilicity. The tissue uptake may also be affected by the blood activity curve: The blood activity curve of those compounds that have substituents that retard their metabolism may be more extended than in the unsubstituted analogue, and thus their uptake greater. This effect would account for the increased uptake of 11β-Et-FES and 17α-ethynyl-FES, relative to FES, but not the decreased uptake of 11β-MeO-FES.

Overall, uptake selectivity (uterus to blood or uterus to muscle ratios) was best for the 11β-methoxy and 17α-ethynyl estradiols. These two compounds have selectivities that exceed that of any positron-emitting estrogen radiopharmaceutical previously described. The 11β-ethyl compound, while having the highest receptor binding affinity, is also the most lipophilic, and may thereby suffer from higher nonspecific uptake.

Our study of the metabolic consumption of FES and these three substituted analogues allows for some interesting comparisons: Since most of the activity in the target

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tissue is sequestered by receptor and hence protected from metabolism, uterine radioactivity is nearly all due to unmetabolized compound, while blood contains mostly metabolites after 1 h. Differences, however, are noted in muscle, where intermediate levels of metabolites are found; at later times with the 11β-substituted FES derivatives, nearly all radioactivity (at least in the extractable fraction) is accounted for by unmetabolized radiotracer. The situation encountered with a compound like 11β-ethyl-FES, where about half of the activity in the muscle is due to the authentic radiotracer over the period 0.5–3 h, may enable pharmacokinetic models that utilize target and nontarget tissue activity levels to be developed more readily.[30]

In the past, we found that the in vivo uptake selectivity (target to nontarget tissue ratio) of a series of radiolabeled estrogens correlated very significantly with their binding-selectivity indices (BSI);[3ab,18] however, this was not the case with the four fluoroestrogens studied here. There are a number of explanations: In the earlier study, compounds with a relatively narrow range of receptor binding affinities but widely differing nonspecific binding were used, so the range of binding selectivity indices was lower and quite wide (BSI 12–100); these correlated with a wider range of both target and nontarget tissue uptake levels. In the present study, we have compounds with both higher BSI values and much higher uptake efficiency and selectivity. Thus, it is perhaps not surprising that there is not a clear correlation between the narrower range of in vitro binding characteristics and in vivo uptake properties. In fact, one may question the expectation that the in vivo distribution of activity between target and nontarget sites should correlate directly with simple in vitro measures of receptor specific and nonspecific binding: The uptake of high affinity estrogen radiopharmaceuticals by receptor-rich target tissues is probably flow limited,[24] which means that the observed uptake will not directly reflect binding affinity, but will be affected by tissue permeability (which may correlate directly with log $P_{o/w}$, rather than inversely); the total activity taken up and retained by the target tissue will also be related to the blood-activity curve, which may vary with structure, as additional functionality affects various routes and rates of metabolic transformation and clearance. Finally, the activity in nontarget tissues is, in many cases, largely due to metabolites, and the proportion of unmetabolized compound to metabolites can vary to a great extent. Thus, nontarget activity level is a poor reference value for establishing uptake selectivity.

Nevertheless, among the new substituted FES derivatives that we have prepared are those that have the highest uptake efficiency and uptake selectivity that have been observed among positron-emitting estrogen radiopharmaceuticals. These agents provide a variety of uptake features that may prove useful in optimizing the imaging of different estrogen-receptor-containing tissue and organs.

Experimental Section

Chemical Synthesis. General. Melting points (uncorrected) were determined on a Thomas-Hoover, Electrothermal, or Fisher-Johns apparatus. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 glass-backed plates. Visualization was achieved with short-wave ultraviolet light and/or phosphomolybdic acid spray. Flash chromatography was performed according to Still[31] with use of Woelm 32–63-μm silica gel.

^{1}H NMR spectra were obtained on a Varian XL-200, a General Electric QE-300, a Nicolet NT-360, or a General Electric GN-500 spectrometer. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). ^{19}F NMR spectra were obtained on a Nicolet NT-360 spectrometer at 338.76 MHz and are reported downfield from internal $CFCl_3$. Low-resolution electron impact (LREI) mass spectra were acquired from a Finnigan MAT-CH5 spectrometer. High-resolution electron impact (HREI) mass spectra were obtained on a MAT-731 instrument, while a VG Instruments ZAB-HF mass spectrometer provided spectra via fast-atom bombardment (FAB) employing a dithiothreitol matrix. For EI spectra, the reported data is for an electron energy of 70 eV and is in the form: m/e (intensity relative to base peak = 100). Infrared (IR) spectra were obtained on a Nicolet 7199 FT-IR or an IBM IR/32 FT instrument. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois.

Analytical gas–liquid chromatography (GLC) was performed on a Hewlett-Packard 5793A instrument equipped with flame-ionization detector. Analyses were performed on an Alltech RSL-150 capillary column (0.25 mm × 30 m) or a Hewlett-Packard Ultra 1 capillary column (0.20 mm × 12.5 m). High-performance liquid chromatography (HPLC) was performed isocratically with a Varian 5060 or a Spectra-Physics 8700 liquid chromatograph, using a 5-μm analytical silica gel column (4.6 mm × 30 cm, Varian Si-5 Micro Pak), a 10-μm preparative silica gel column (9 mm × 50 cm, Whatman Partisil M-9), or a C_{18} column (10 mm × 50 cm, Whatman Partisil M-9, ODS-2). HPLC eluent was monitored via UV absorbance at 280 nm; for radiochemical purification, HPLC eluent was also monitored with a NaI(Tl) radioactivity detector. Radioactivity was determined in a dose calibrator.

X-ray crystallography involved diffraction experiments performed at room temperature with Mo radiation ($\lambda(K_\alpha)$ = 0.71073 A) by the X-ray Crystallographic Service Laboratory at the University of Illinois.

Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl; all other solvents were distilled from CaH_2. The $BF_3{\cdot}S(CH_3)_2$ complex was prepared by saturating $(CH_3)_2S$ with BF_3 gas.

Chemicals were obtained from the following sources and were used as received, unless otherwise noted: Baker, Fisher, Mallinckrodt, Aldrich, Sigma, Eastman, or Alfa. 1-Dehydroadrenosterone was obtained from Searle Laboratories, Skokie, IL.

11β-Ethyl-3-methoxyestra-1,3,5(10)-trien-17-one (7) and 11α-Ethyl-3-methoxy-9β-estra-1,3,5(10)-trien-17-one (8). Tetraene 6[32] (938 mg, 3.03 mmol) was dissolved in 64 mL of 20% EtOAc/methanol in a 250-mL round-bottomed flask. Pd/C (5%, 200 mg) was added and the mixture vigorously stirred under hydrogen at 1 atm for 31 h at which time GLC indicated consumption of starting material and production of 7 and 8 in a ratio of 2.6:1, respectively. The reaction mixture was filtered through neutral alumina (7 cm × 25 mm) and then through filter paper. Concentration in vacuo gave a yellow foam (902 mg, 96%) which was used without further purification. Partial separation of the crude mixture via preparative TLC (5:3:2 $CHCl_3$, hexane, CH_2Cl_2, 7 developments) revealed two major products: 7 and 8.

7: GLC (220 °C) t_R, 6.52 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, 3 H, J = 7.6 Hz, CH_2CH_3), 1.04 (s, 3 H, 18-CH_3), 3.78 (s, 3 H, OCH_3), 6.62 (d, 1 H, J = 2.1 Hz, 4-H), 6.73 (dd, 1 H, J = 9.5, 2.1 Hz, 2-H), 7.08 (d, 1 H, J = 9.5 Hz, 1-H); EIMS 312 (M^+, 100), 285 (6), 257 (7), 199 (84), 186 (41), 184 (25), 160 (46), 91 (16).

8: GLC (220 °C) t_R, 5.54 min; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3 H, J = 8.5 Hz, CH_2CH_3), 1.00 (s, 3 H, 18-CH_3), 3.78 (s, 3-H, OCH_3), 6.67 (dd, 1 H, J = 8.4, 2.1 Hz, 2-H), 6.69 (d, 1 H, J = 2.1 Hz, 4-H), 7.73 (d, 1 H, J = 8.4 Hz, 1-H); EIMS 312 (M^+, 100), 199 (72), 186 (31), 160 (43).

11β-Ethyl-3-hydroxyestra-1,3,5(10)-trien-17-one (5b) and 11α-Ethyl-3-hydroxy-9β-estra-1,3,5(10)-trien-17-one (9). A mixture containing 7 and 8 (902 mg, 2.84 mmol) was dissolved in 42 mL of freshly distilled CH_2Cl_2 in a 100-mL round-bottomed flask. The solution was cooled to 0 °C and 5.3 mL of $BF_3{\cdot}S(CH_3)_2$ (vide supra) was added. The reaction mixture was allowed to warm to room temperature as it reacted and was quenched at 24 h with ice water (40 mL). The heterogeneous mixture was washed

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with saturated aqueous $NaHCO_3$ (50 mL), the layers were separated, and the aqueous layer was extracted (3 × 50 mL, CH_2Cl_2). The combined organic layers were washed with brine and dried (Na_2SO_4). Concentration in vacuo followed by ether trituration provided **5b** as a white powder (490 mg, 57%), an analytical sample of which was recrystallized from acetone/hexane to afford white needles; **9** remained as the soluble component and produced a yellow foam (209 mg, 24%).

5b: mp 250–254 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 0.89 (t, 3 H, J = 7.4 Hz, CH_2CH_3) 1.05 (s, 3 H, 18-CH_3), 4.60 (s, 1 H, OH), 6.53 (d, 1 H, J = 2.1 Hz, 4-H), 6.66 (dd, 1 H, J = 10.6, 2.1 Hz, 2-H), 7.04 (d, 1 H, J = 10.4 Hz, 1-H); IR (KBr) 3340 (OH), 1719 cm^{-1} (ketone); EIMS 298 (M^+, 100), 296 (18), 185 (47), 172 (28), 170 (12), 146 (36). Anal. ($C_{20}H_{26}O_2$) C, H.

9: ^1H NMR (300 MHz, $CDCl_3$) δ 0.96 (t, 3 H, J = 7.36 Hz, CH_2CH_3), 1.00 (s, 3 H, 18-CH_3), 4.60 (br s, 1 H, OH), 6.61 (dd, 1 H, J = 8.2, 5.1 Hz, 2-H), 6.62 (br s, 1 H, 4-H), 7.27 (d, 1 H, J = 8.2 Hz, 1-H); IR ($CHCl_3$) 3390 (OH), 1730 cm^{-1} (ketone); EIMS 298 (M^+, 60), 185 (44), 172 (36), 146 (100), 97 (12). Anal. (exact mass, HREIMS) calcd for $C_{20}H_{26}O_2$ m/e 298.1932, found 298.1936.

3-Acetoxy-11α-ethyl-9β-estra-1,3,5(10)-trien-17-one (10). Ketone **9** (7.3 mg, 24.5 μmol) was dissolved in Ac_2O (1 mL, 10.6 mmol) in a 15 mm × 45 mm sample vial equipped with magnetic stirring and a Teflon-lined cap. A small amount (<5 mg) of p-toluenesulfonic acid monohydrate was added to the reaction mixture which was stirred at 70 °C. After 1 h, the mixture was poured onto water, extracted (EtOAc, 2 × 2 mL), washed with brine, dried (Na_2SO_4), and concentrated in vacuo to produce a brown oil. Flash chromatography (25% EtOAc/hexane, 16 cm × 1 cm SiO_2) was followed by recrystallization (ether) to afford white needles (3.4 mg, 41%): mp 167–168 °C; ^1H NMR (360 MHz, $CDCl_3$) δ 0.96 (t, 3 H, J = 7.4 Hz, CH_2CH_3), 1.00 (s, 3 H, 18-CH_3), 2.28 (s, 3 H, $OCOCH_3$), 6.83 (br d, 1 H, J = 8.4 Hz, 2-H), 6.85 (br s, 1 H, 4-H), 7.40 (d, 1 H, 8.4 Hz); IR (KBr) 1754 (C=O, ester), 1730 cm^{-1} (ketone), EIMS 340 (M^+, 18), 298 (100), 185 (44), 146 (90), 43 (21). Anal. ($C_{22}H_{28}O_3$) C, H.

3-[[(Trifluoromethyl)sulfonyl]oxy]-11β-methoxyestra-1,3,5(10)-trien-17-one (11a). Ketone **5a**[9] (30 mg, 0.10 mmol) was added to a 15 mm × 45 mm sample vial, equipped with a Teflon-lined cap and magnetic stirring and was dissolved in freshly distilled CH_2Cl_2 (1.2 mL). The solution was cooled to 0 °C and triflic anhydride (24 μL, 0.150 mmol) was added followed by 2,6-lutidine (18 μL, 0.152 mmol). The reaction mixture was allowed to stir at 0 °C for 20 min at which time it was passed through a 2-cm plug of neutral alumina. Concentration in vacuo was immediately followed by flash chromatography (30% EtOAc/hexane, 15 cm × 15 mm SiO_2) to provide a brown oil (37.8 mg, 88%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 3 H, 18-CH_3), 3.31 (s, 3 H, OCH_3), 4.23 (m, 1 H, 11α-H), 7.00 (d, 1 H, J = 3.3 Hz, 4-H), 7.09 (dd, 1 H, J = 9.8, 3.3 Hz, 2-H), 7.22 (d, 1 H, J = 9.8 Hz, 1-H); IR (KBr) 1730 cm^{-1} (ketone); FABMS 433 (M^+ + H, 21), 415 (25), 401 (100), 152 (45), 135 (44), 119 (60). Anal. (exact mass, HRFABMS) calcd for $C_{20}H_{23}SO_5F_3$ m/e 433.1327, found 433.1301.

3-[[(Trifluoromethyl)sulfonyl]oxy]-11β-ethylestra-1,3,5(10)-trien-17-one (11b). Ketone **5b** (170 mg, 0.57 mmol) was converted according to the preceding method into the triflate **11b**. The crude product was subjected to flash chromatography (15% EtOAc/hexane, 21 cm × 35 mm SiO_2), and a yellow oil (233 mg, 95%) was isolated. An analytical sample was recrystallized from methanol to give white prisms: mp 98–99 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.04 (s, 3 H, 18-CH_3), 6.98 (br s, 1 H, 4-H), 7.05 (dd, 1 H, J = 8.6, 2.6 Hz, 2-H), 7.22 (d, 1 H, J = 8.6 Hz, 1-H); IR (KBr) 1730 cm^{-1} (ketone); EIMS 430 (M^+, 24), 170 (23), 114 (82), 70 (100), 57 (50). Anal. ($C_{21}H_{25}SO_4F_3$) C, H, F, S.

17-Acetoxy-3-[[(trifluoromethyl)sulfonyl]oxy]-11β-methoxyestra-1,3,5(10),16-tetraene (12a). Ketone **11a** (663 mg, 1.53 mmol) was placed in a 100-mL pear-shaped flask equipped with magnetic stirring and a reflux condenser. Isopropenyl acetate (18 mL) was added followed by p-toluenesulfonic acid monohydrate (91 mg, 0.48 mmol). The mixture was allowed to reflux for 4 h, stir at room temperature for 11 h, and was then concentrated to 9 mL over 1 h. Fresh isopropenyl acetate was added, and the reaction mixture was refluxed for an additional 4 h. This cycle of concentration, addition of fresh isopropenyl acetate and heating was repeated once more. Workup consisted of pouring onto 40 mL of water, extraction (3 × 30 mL, EtOAc), drying (Na_2SO_4), passage through a 2-cm plug of neutral alumina, and concentration in vacuo. The resultant brown oil was subjected to flash chromatography (15% EtOAc/hexane, 23 cm × 45 mm SiO_2) to give a white solid (292 mg, 41% or 83%, based on consumed starting material): mp 114–116 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 1.15 (s, 3 H, 18-CH_3), 2.20 (s, 3 H, $OCOCH_3$), 3.30 (s, 3 H, OCH_3), 4.19 (m, 1 H, 11α-H), 5.50 (m, 1 H, 16-H), 6.97 (dd, 1 H, J = 2.6 Hz, 4-H), 7.05 (dd, 1 H, J = 7.8, 2.6 Hz, 2-H), 7.17 (d, 1 H, J = 7.8 Hz, 1-H); EIMS 474 (M^+); Anal. (exact mass, HREIMS) calcd for $C_{22}H_{25}SO_6F_3$ m/e 474.1315, found 474.1329.

17-Acetoxy-11β-ethyl-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10),16-tetraene (12b). Triflate **11b** (226 mg, 0.53 mmol) was converted according to the preceding method above into the enol acetate **12b**. The crude product was subjected to flash chromatography (20% EtOAc/hexane, 14 cm × 30 mm SiO_2), and an off-white solid was produced (98 mg, 40% or 80%, based on consumed starting material): mp 90–91 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.91 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.10 (s, 3 H, 18-CH_3), 2.20 (s, 3 H, $OCOCH_3$), 5.48 (m, 1 H, 16-H), 6.96 (br s, 1 H, 4-H), 7.02 (dd, 1 H, J = 8.6, 2.6 Hz, 2-H), 7.19 (d, 1 H, J = 8.6, 1-H); IR ($CHCl_3$) 1720 cm^{-1} (C=O, ester); EIMS 472 (M^+, 10), 430 (100), 415 (44), 318 (27), 94 (52). Anal. (exact mass, HREIMS) calcd for $C_{23}H_{27}SO_5F_3$ m/e 472.1531, found 472.1540.

16β-(Trifluoroacetoxy)-3-[[(trifluoromethyl)sulfonyl]oxy]-11β-methoxyestra-3,5(10)-trien-17-one (13a). A 10-mL round-bottomed flask equipped with magnetic stirring and nitrogen inlet was charged with [bis-(trifluoroacetoxy)iodo]benzene[33] (103.5 mg, 240.6 mmol) which was subsequently dissolved in CH_3CN (2 mL). Enol acetate **12a** (108.7 mg, 229.1 mmol) was added as a solution in CH_2Cl_2 (620 mL). After stirring 12 h at room temperature, the reaction mixture was concentrated in vacuo to a yellowish oil. This oil underwent flash chromatography (20% EtOAc/hexane, 16 cm × 35 mm oven-dried SiO_2) to give a white solid (117 mg, 94%): mp 128–132 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 1.26 (s, 3 H, 18-CH_3), 3.32 (s, 3 H, 11β-OCH_3), 4.25 (m, 1 H, 11α-H), 5.23 (t, 1 H, J = 9.3 Hz, 16α-H), 7.00 (d, 1 H, J = 1.6 Hz, 4-H), 7.09 (dd, 1 H, J = 8.8, 1.6 Hz, 2 H), 7.24 (d, 1 H, J = 8.8 Hz, 1-H); IR (KBr) 1790 ($OCOCF_3$), 1762 (ketone), 1500, 1425 cm^{-1}; EIMS 544 (M^+, 44), 448 (21), 412 (17), 376 (98), 344 (26), 278 (96), 98 (100); Anal. ($C_{22}H_{22}SO_7F_6$) C, H, F.

11β-Ethyl-16β-(trifluoroacetoxy)-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (13b). Enol acetate **12b** (209 mg, 0.44 mmol) was converted into the trifluoroacetate **13b** by following the preceding method. The crude product, a yellowish oil, was subjected to flash chromatography (20% EtOAc/hexane, 16 cm × 35 mm oven-dried SiO_2), to give a white solid (131 mg, 60% or 76%, based on consumed starting material): mp 138–140 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.15 (s, 3 H, 18-CH_3), 5.20 (t, 1 H, J = 8.4 Hz, 16α-H), 6.99 (d, 1 H, J = 2.3 Hz, 4-H), 7.06 (dd, 1 H, J = 8.7, 2.3 Hz, 2-H), 7.22 (d, 1 H, 8.7 Hz, 1-H); IR (KBr) 1790 ($OCOCF_3$), 1762 (ketone), 1488, 1420 cm^{-1}; EIMS 542 (M^+, 77), 374 (92), 241 (79), 185 (87), 145 (64); Anal. ($C_{23}H_{24}SO_6F_6$) C, H, F.

3-[[(Trifluoromethyl)sulfonyl]oxy]-16β-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (14a). Trifluoroacetate **13a** (43.2 mg, 79.4 μmol), was added to a 5-mL round-bottomed flask and suspended in a mixture of 10% aqueous EtOH (1.4 mL) and THF (1.1 mL). The heterogeneous mixture was magnetically stirred under nitrogen at room temperature for several hours before homogeneity was achieved. After a total of 12 h, solvent was removed in vacuo to give a white foam (34.2 mg, 72.3 μmol, 91%) which was used without further purification: ^1H NMR (200 MHz, $CDCl_3$) δ 1.20 (s, 3 H, 18-CH_3), 3.31 (s, 3 H, OCH_3), 4.00 (t, 1 H, J = 7.8 Hz, 16α-H), 4.24 (m, 1 H, 11α-H), 7.00 (d, 1 H, J = 2.5, 4-H) 7.06 (dd, 1 H, J = 9.3, 2.5 Hz, 2-H), 7.13 (d, 1 H, J = 9.3 Hz, 1-H); IR (KBr) 3440 (OH), 1743 cm^{-1} (ketone); EIMS 448 (M^+, 68), 416 (20), 376 (74), 344 (27), 304 (61), 211 (35), 171 (27), 98 (100). Anal. (exact mass, HREIMS) calcd for $C_{20}H_{23}SO_6F_3$ m/e 448.1162, found 448.1162.

11β-Ethyl-3-[[(trifluoromethyl)sulfonyl]oxy]-16β-hydroxyestra-1,3,5(10)-trien-17-one (14b). Trifluoroacetate

(33) Spyroudis, S.; Varvoglis, A. *Synthesis* **1975**, 445.

13b (140 mg, 0.26 mmol) was dissolved in a mixture of 10% aqueous ethanol (4.6 mL) and THF (3.6 mL) and stirred at room temperature for 12 h. Solvent was removed in vacuo to provide a clear oil which underwent flash chromatography (40% EtOAc/hexane). A white foam was isolated (85 mg, 74%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (t, 3 H, J = 9.1 Hz, CH_2CH_3), 1.12 (s, 3 H, 18-CH_3), 3.98 (br t, 1 H, 16α-H), 6.99 (br s, 1 H, 4-H), 7.05 (dd, 1 H, J = 8.2, 2.9 Hz, 2-H), 7.21 (d, 1 H, J = 8.2 Hz, 1-H); IR (KBr) 3430 (OH), 1750 (ketone), 1420, 1210, 1140 cm^{-1}; EIMS 446 (M^+, 37), 373 (100), 318 (39), 241 (44), 185 (41), 55 (55); Anal. (exact mass, HREIMS) calcd for $C_{21}H_{25}SO_5F_3$ m/e 446.1394, found 446.1384.

3,16β-Bis[[(trifluoromethyl)sulfonyl]oxy]-11β-methoxyestra-1,3,5(10)-trien-17-one (15a). Ketol **14a** (61.2 mg, 0.137 mmol) was dissolved in freshly distilled CH_2Cl_2 (1.7 mL) and cooled to 0 °C. Triflic anhydride (35 μL, 0.206 mmol) was added followed by 2,6-lutidine (24 μL, 0.209 mmol). After 30 min the reaction mixture was diluted with cold EtOAc and then passed through a parfait column (neutral alumina on top, SiO_2 on bottom). After concentration in vacuo, recrystallization was effected in EtOAc/hexane to provide a white powder (54.4 mg, 68%): mp 148–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (s, 3 H, 18-CH_3), 3.32 (s, 3 H, 11β-OCH_3), 4.25 (m, 1 H, 11α-H), 4.97 (t, 1 H, J = 8.6 Hz, 16α-H), 7.00 (d, 1 H, J = 2.1 Hz, 4-H), 7.08 (dd, J = 8.6, 2.1 Hz, 2-H), 7.19 (d, J = 8.6 Hz, 1-H); IR (KBr) 1765 (ketone), 1415, 1210, 1140 cm^{-1}; EIMS 580 (M^+, 6), 419 (42), 376 (37), 211 (40), 98 (100). Anal. (exact mass, HREIMS) calcd for $C_{21}H_{22}S_2O_8F_6$ m/e 580.0661, found 580.0668.

11β-Ethyl-3,16β-bis[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (15b). Ketol **14b** (26 mg, 58 μmol) was converted according to the preceding method to the bistriflate **15b**. Recrystallization was effected in EtOAc/hexane, to provide white flakes (24 mg, 72%): mp 142–143 °C%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.95 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.16 (s, 3 H, 18-CH_3), 4.95 (t, 1 H, J = 9.3 Hz, 16α-H), 7.02 (d, 1 H, J = 2.2 Hz, 4-H), 7.06 (dd, 1 H, J = 8.2, 2.2 Hz, 2-H), 7.21 (d, 1 H, J = 8.2 Hz, 1-H); IR (KBr) 1750 (ketone), 1420, 1210, 1140 cm^{-1}; EIMS 578 (M^+, 24), 373 (100), 241 (36), 185 (32), 145 (34), 69 (63), 55 (58); Anal. (exact mass, HREIMS) calcd for $C_{22}H_{24}S_2O_7F_6$ m/e 578.0868, found 578.0875.

16α-Fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]-11β-methoxyestra-1,3,5(10)-trien-17-one (16a). Bistriflate **15a** (39.5 mg, 68 μmol) was dissolved in dry THF (100 μL); nBu_4NF (1 M in THF, 62 μL, 61 μmol) was added, and the solution was allowed to stir at room temperature for 30 min. The solvent was removed in vacuo and the residue subjected to flash chromatography (15% EtOAc/hexane, 25 cm × 20 mm SiO_2). A clear oil was isolated (13 mg, 42%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.16 (s, 3 H, 18-CH_3), 3.30 (s, 3 H, 11β-OCH_3), 4.24 (m, 1 H, 11α-H), 5.20 (dd, 1 H, J = 5.1, 6.5 Hz, 16β-H), 7.00 (d, 1 H, J = 2.0 Hz, 4-H), 7.07 (dd, 1 H, J = 8.8, 2.0 Hz, 2-H), 7.20 (d, 1 H, J = 8.8 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) φ −73.48, −193.90 (ddd, J = 50, 29, 28 Hz, 16α-F); IR ($CHCl_3$) 1755 (ketone); EIMS 450 (M^+, 28), 376 (24), 278 (65), 211 (25), 116 (100), 98 (62). Anal. (exact mass, HREIMS) calcd for $C_{20}H_{22}SO_5F_4$ m/e 450.1124, found 450.1118.

11β-Ethyl-16α-fluoro-3-[[(trifluoromethyl)sulfonyl]oxy]estra-1,3,5(10)-trien-17-one (16b). Bistriflate **15b** (26 mg, 45 μmol) was converted according to the preceding method into fluoroketone **16b**. A white foam was isolated (11.5 mg, 57%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.92 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 1.09 s, 3 H, 18-CH_3), 5.21 (dd, 1 H, J = 51, 6.6 Hz, 16β-H), 6.99 (d, 1 H, J = 2.5 Hz, 4-H), 7.05 (dd, 1 H, J = 8.7, 2.5 Hz, 2-H), 7.22 (d, 1 H, J = 8.7 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) φ −73.47 (s, OSO_2CF_3), −194 (ddd, J = 50, 29, 27 Hz, 16α-F); IR ($CHCl_3$) 1755 (ketone), 1420 cm^{-1}; EIMS 448 (M^+, 13), 241 (37), 185 (51), 115 (55), 55 (100). Anal. (exact mass, HREIMS) calcd for $C_{21}H_{23}SO_4F_4$ m/e 448.1332, found 448.1335.

16α-Fluoro-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (1) and 16α-Fluoro-11β-methoxyestra-1,3,5(10)-triene-3,17α-diol (17a). The 16α-fluoroketone **16a** (14.4 mg, 32 μmol) was dissolved in dry ether (27 mL) and cooled under argon in a dry ice/2-propanol bath. $LiAlH_4$ (1 M in ether, 420 μL, 0.42 mmol) was added, and the mixture was stirred for 10 min and then allowed to warm to room temperature for 10 min. The reaction was then quenched with EtOAc, acidified with 6 N HCl, and extracted (EtOAc, 2×), and the organic layer was dried by passage through a column of Na_2SO_4. The solvent was removed in vacuo, and the products were separated by preparative HPLC [Whatman M-9, 50% hexane/50% (5% iPrOH/CH_2Cl_2); 6 mL/min] to yield **1** (5.6 mg, 55%, t_R = 29 min) and **17a** (0.5 mg, 5%, t_R = 16 min).

1: mp 246–247 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 0.91 (s, 3 H, 18-CH_3), 3.20 (s, 3 H, 11β-OCH_3), 3.66 (dt, 1 H, J = 37, 5.6 Hz, 17α-H), 4.13 (m, 1 H, 11α-H), 4.87 (dm, 1 H, J = 55 Hz, 16β-H), 6.47 (d, 1 H, J = 2.3 Hz, 4-H), 6.56 (dd, 1 H, J = 8.5, 2.3 Hz, 2-H), 6.99 (d, 1 H, J = 8.5 Hz, 1-H); ^{19}F NMR (338 MHz, CD_3CN) φ −179.16 (dtd, 56, 29, 27 Hz, 16α-F); EIMS 320 (M^+, 18), 172 (14), 146 (18), 105 (88), 85 (100), 43 (62). Anal. (exact mass, HREIMS) calcd for $C_{19}H_{25}O_3F$ m/e 320.1788, found 320.1788.

17a: mp 236–240 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 0.87 (s, 3 H, 18-CH_3), 3.21 (s, 3 H, 11β-OCH_3), 3.71 (m, 1 H, 17β-H), 4.19 (m, 1 H, 11α-H), 5.26 (dm, 1 H, J = 52 Hz, 16α-H), 6.47 (d, 1 H, J = 2.5 Hz, 4-H), 6.56 (dd, 1 H, J = 8.4, 2.5 Hz, 2-H), 6.99 (d, 1 H, J = 8.4 Hz, 1-H); EIMS 320 (M^+, 12), 288 (72), 175 (57), 146 (51), 85 (56). Anal. (exact mass, HREIMS) calcd for $C_{19}H_{25}O_3F$ m/e 320.1788, found 320.1786.

11β-Ethyl-16α-fluoroestra-1,3,5(10)-triene-3,17β-diol (2) and 11β-Ethyl-16α-fluoroestra-1,3,5(10)-triene-3,17α-diol (17b). The 16α-fluoroketone **16b** (10 mg, 22.3 μmol) was converted according to the preceding method to a mixture of the fluoroestrogen **2** and its 17α-epimer. The products were separated by preparative HPLC [Whatman M-9, 75% hexane/25% (5% iPrOH/CH_2Cl_2); 6 mL/min] to yield **2** (1.6 mg, 23%, t_R = 22 min) and the 17α-epimer (1.0 mg, 14%, t_R = 13 min).

2: mp 211–212 °C; ^{1}H NMR (300 MHz, CD_3CN) δ 0.87 (s, 3 H, 18-CH_3), 0.88 (t, 3 H, J = 7.4 Hz, CH_2CH_3), 3.64 (dt, 1 H, J = 30, 4.4 Hz, 17α-H), 4.86 (dm, 1 H, J = 54 Hz, 16β-H), 6.47 (d, 1 H, J = 2.4 Hz, 4-H), 6.57 (dd, 1 H, J = 8.3 Hz, 2.4 Hz, 2-H), 6.98 (d, 1 H, J = 8.3 Hz, 1-H); ^{19}F NMR (338 MHz, CD_3CN) φ −179.26 (dq, J = 55, 28 Hz); EIMS 318 (M^+, 100), 172 (85), 146 (48), 43 (31). Anal. (exact mass HREIMS) calcd for $C_{20}H_{27}O_2F$ m/e 318.1995, found 318.1998.

17b: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (s, 3 H, 18-CH_3), 0.90 (t, 3 H, J = 7.3 Hz, CH_2CH_3), 3.82 (s, 1 H, 17β-H), 4.63 (br s, 1 H, OH), 5.31 (dm, 1 H, J = 52 Hz, 16β-H), 6.54 (d, 1 H, J = 2.2 Hz, 4-H), 6.64 (dd, 1 H, J = 8.4, 2.2 Hz, 2-H), 7.03 (d, 1 H, J = 8.4 Hz, 1-H); ^{19}F NMR (338 MHz, $CDCl_3$) φ −196.97 (dtd, J = 46, 28, 8 Hz); EIMS 318 (M^+, 100), 172 (99), 146 (76), 43 (43), 28 (58). Anal. (exact mass, HREIMS) calcd for $C_{20}H_{27}O_2F$ m/e 318.1995, found 318.1998.

Radiochemical Synthesis. General. Fluorine-18 was prepared from [^{18}O]H_2O by the ^{18}F reaction.[12] Tetrabutylammonium hydroxide (1 M in water, ~3 μmol) was added via Hamilton syringe to the bottom of a Vacutainer. Water containing ^{18}F-fluoride was added to the base followed by acetonitrile (200 μL). The solution was then heated in an oil bath at 110 °C while a gentle stream of nitrogen assisted in the azeotropic removal of water. When almost dry, heat was removed and an additional 200 μL of acetonitrile was added for a second azeotropic distillation. Once nearly dry, the Vacutainer was removed from the oil bath and the final segment of the evaporation was performed without heat. The reaction solvent was then added to the Vacutainer and the resolubilized flouride was transferred to a borosilicate glass vial (15 mm × 45 mm, Teflon-lined cap) containing 1–2 mg of substrate. The entire process requires ~10 min and ~80% of the initial activity is transferred. Yields given are only for reaction in which isolated activity was used for in vivo experiments; yield ranges for smaller activity scale reactions are shown parenthetically. Activity collected is uncorrected for decay while yield (and yield ranges) are corrected. All reactions are with no carrier added. HPLC injection volume was 1 mL. Identity of the radiolabeled compounds was confirmed by coelution with authentic unlabeled standards on HPLC. Specific activities were determined by a competitive radiometric binding assay.[9]

16α-[^{18}F]Fluoro-11β-methoxyestra-1,3,5(10)triene-3,17β-diol ([^{18}F]-1) and 16α-[^{18}F]Fluoro-11β-methoxyestra-1,3,5(10)-triene-3,17α-diol ([^{18}F]-17a). Resolubilization employed 2.0 μL nBu_4NOH. Activity (88.2 mCi) was transferred in THF (300 μL) to a vial containing bistriflate **15a** (2.35 mg, 4.05 μmol). Reaction was allowed to occur at room temperature for 5 min whereupon the THF was removed under a gentle stream of nitrogen. The residue was dissolved in 300 μL of ether and placed in a dry

ice/acetone bath and was then allowed to stir for an additional 10 min. The chalky white reaction mixture was then acidified with 6 N HCl (1 mL), and the resulting two-phase mixture was extracted with ether (2 × 1.5 mL). The organic layer was passed down a 2-cm column of Na_2SO_4, and the solvent was evaporated under a stream of nitrogen. The residue was dissolved in 1,2-dichloroethane and injected onto the Whatman M-9V preparative column [50% hexane/50% (5% iPrOH/CH_2Cl_2), 6 mL/min].

[^{18}F]-1: 12.0 mCi, 29% this run (typical range 12–35%); t_R = 28 min; SA = 89.6 Ci/mmol.

[^{18}F]-17a: 6.5 mCi, 16% this run (typical range 4–16%); t_R = 10 min.

11β-Ethyl-16α-[^{18}F]fluoroestra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-2) and 11β-Ethyl-16α-[^{18}F]fluoroestra-1,3,5(10)-triene-3,17α-diol ([^{18}F]-17b). Resolubilization employed 2.4 μL nBu_4NOH. Activity (126 mCi) was transferred in two separate portions in THF (300 μL each) to a vial containing bistriflate **15b** (2.74 mg, 48 μmol). Conversion according to the preceding method gave [^{18}F]-**2** and [^{18}F]-**17b**. These products were redissolved in 1,2-dichloroethane and injected onto the Whatman M-9V preparative column [75% hexane/25% (5% iPrOH/CH_2Cl_2), 6 mL/min].

[^{18}F]-2: 10.3 mCi, 17% this run (typical range 7–20%); t_R = 30 min; SA = 439 Ci/mmol.

[^{18}F]-17b: 19 mCi, 32% this run (typical range 22–32%); t_R = 16 min.

17α-Ethynyl-16α-[^{18}F]fluoroestra-1,3,5(10)-triene-3,17β-diol ([^{18}F]-3) and 17β-Ethynyl-16α-[^{18}F]fluoroestra-1,3,5(10)-triene-3,17α-diol ([^{18}F]-19). Resolubilization employed 2.0 μmol of nBu_4NOH. Activity (250 mCi) was dissolved in THF (300 μL), transferred to a vial containing bistriflate **18** (1.5 mg, 26 μmol) and allowed to stand at room temperature for 10 min. Lithium trimethylsilylacetylide was prepared by the addition of trimethylsilylacetylene (25 μL, 177 μmol) and *n*-butyllithium (100 μL of a 1.6 M solution in hexane) to 250 μL of pentane at 0 °C. THF (50 μL) was added to dissolve the white precipitate. The reaction vessel was cooled to 0 °C, and a 150-μL portion of the lithium trimethylsilylacetylide solution was added. After 7–10 minutes at room temperature, the reaction was quenched by the addition of 50 μL of 1 M aqueous ammonium chloride. The mixture was evaporated to dryness, and 0.25 mL of methanol, 0.1 mL of 5 N aqueous sodium hydroxide and a stirring bar were added. The mixture was heated to 60 °C with stirring for 30 min. A C-18 Sep Pak (Waters) was washed with 5–6 mL of MeOH and twice with 5–6 mL of distilled water. The reaction vial was cooled and quenched with 0.1 mL of 5M NH_4Cl. A portion of the mixture (200 μL) was transferred to the Sep Pak and washed with 10 mL of water; the remaining portion of the mixture was then transferred to the Sep Pak, followed by 10 mL of water. The Sep Pak was washed with 1 mL of pentane (discarded), followed by 1.5 mL of CH_2Cl_2. This eluate was diluted with 1.5 mL of hexane and injected onto the Whatman M-9 preparative column [75% hexane/25% (5% iPrOH/CH_2Cl_2); 5 mL/min)].

[^{18}F]-3: 7.5 mCi, 3% this run (typical range 2–4%); t_R = 11 min; SA = 1590 Ci/mmol.

[^{18}F]-19: 7.0 mCi, 2.8% this run (typical range 2–4%); t_R = 19 min.

X-ray Crystallography. Crystals of compound **5b** were grown from acetone/hexane. The transparent, colorless, columnar crystal used for data collection was cut from a larger crystal. The resultant crystal had well developed faces and sharply extinguished plane-polarized light. There were no crystallites or other contaminating substances attached to the surface of the sample. The crystal was bound by the inversion related forms: {1 1 0}, {1 −1 0}, and {0 0 1}. Distances from the crystal center to these facial boundaries were 0.05, 0.06, and 0.30 mm, respectively. The crystal was mounted with use of epoxy to a thin glass fiber with the (1 1 3) scattering planes roughly normal to the spindle axis.

Crystals of compound **10** were grown from ether. The transparent, colorless, columnar crystal used for data collection was cut from a larger crystal. The resultant crystal had well developed faces and sharply extinguished plane-polarized light. There were a few crystallites but no other contaminating substances attached to the surface of the sample. The crystal was bound by the inversion related forms: {1 0 0}, {0 0 1}, and {0 1 0}. Distances from the crystal center to these facial boundaries were 0.08, 0.09 and 0.39 mm, respectively. The crystal was mounted with use of epoxy to a thin glass fiber with the (−1 −1 −3) scattering planes roughly normal to the spindle axis.

Table VI. Crystal Data for 11β-Ethylestrone (**5b**) and 11α-Ethyl-9β-estrone 3-O-Acetate (**10**)

	11β-ethylestrone (**5b**)	11α-ethyl-9β-estrone 3-O-acetate (**10**)
formula	$C_{20}H_{26}O_2$	$C_{22}H_{28}O_3$
crystal system	orthorhombic	orthorhombic
space group	$P2_12_12_1$	$P2_12_12_1$
a, Å	7.829 (2)	10.471 (3)
b, Å	9.241 (4)	16.180 (5)
c, Å	22.943 (5)	10.834 (4)
V, Å^3	1661 (1)	1835 (1)
Z	4	4
density calcd, g/cm^3	1.194	1.232
crystallizing solvent	acetone/hexane	ether
crystal habit	columnar (colorless)	columnar (colorless)
crystal dimensions, mm	0.2 × 0.2 × 0.8	0.1 × 0.1 × 0.6
diffractometer	Enrof-Nonius CAD$_4$	Syntex P2$_1$
μ, cm^{-1}	0.70	0.75
transmission factor range	0.989–0.972	0.993–0.989
extinction	not applied	not applied
2θ limit, deg (octants)	46.0 (−*h*, −*k*, −*l*) 12.0 (±*h*, ±*k*, ±*l*)	46.0 (+*h*, +*k*, +*l*) 12.0 (±*h*, ±*k*, ±*l*)
intensities (unique, R_i)	1615 (1376, 0.013)	1771 (1503, 0.015)
intensities >2.58σ(*I*)	733	628
R^a	0.105	0.072
$R_w{}^a$ (for $w = 1/\sigma 2(F_o) + pF_o{}^2$)	0.117 (p = 0.020)	0.068 (p = 0.020)
max density in ΔF map, e/Å^3	0.42	0.38

[a] Conventional *R* factors were defined as

$$R = \sum||F_o| - |F_c||/\sum|F_o|;\ R_w = (\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2)^{1/2}$$

Diffraction experiments were performed at room temperature with Mo radiation (λ(K_α) = 0.71073 Å). Final cell dimensions were obtained by a least-squares fit to the automatically centered settings for 5–15 reflections. Three reference reflections monitored during each experiment showed no significant variation. Intensity data were corrected for Lorentz-polarization effects. Crystal data are listed in Table VI. In both cases, diffraction intensity was weak, but no other problems were encountered in collecting the data and there was no change in the appearance of the sample during the experiment. Shell 2 was collected to confirm the crystal system.

The structure was solved by direct methods (SHELX-86).[34] Correct positions for all non-hydrogen atoms were deduced from an E-map. Owing to the paucity of data, hydrogen atoms were included as fixed contributors in "idealized" positions. In the final cycle of least squares, isotropic thermal coefficients were refined for the non-hydrogen atoms and a group isotropic thermal parameter was varied for the hydrogen atoms. In compound **10**, H01 was never located in the difference map. Successful convergence was indicated by the maximum shift/error for the last cycle. The absolute configuration had been previously determined. The final difference Fourier map had no significant features. A final analysis of variance between observed and calculated structure factors showed no significant errors.

Biological Methods. Receptor Binding Affinity. The binding affinities of the substituted estrogens for the estrogen receptor (ER), alphafetoprotein (AFP), and sex binding protein (SBP) were determined as previously reported.[13-15] All three assays utilized [^{3}H]estradiol (Amersham, 43 Ci/mmol) as the radiotracer, and the affinities are reported relative to estradiol set at 100%. Uterine cytosol from immature female rats was the source of estrogen receptor for the ER assay, and free steroid was removed by the charcoal/dextran method.[13] The affinity to ER was measured at both 0 °C and 25 °C.[8] The assay for AFP used rat amniotic fluid as the receptor source and separated bound steroid from free by the hydroxylapatite assay.[14] The SBP assay used third trimester human pregnancy serum as the receptor

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source and the hydroxylapatite assay to separate bound steroid from free.[15]

log P Determinations. log P values were estimated from the log k'_w values determined by HPLC chromatography following the recommendations of Minick.[21] The solvents were analytical grade methanol (Fisher), 1-octanol (Aldrich), and n-decylamine and 3-morpholinopropanesulfonic acid (MOPS) (Sigma). The standards were obtained from Aldrich and used without further purification. A Spectra Physics SP8700 chromatographic system equipped with a 7125 Rheodyne injector, a Beckman 254-nm fixed wavelength UV detector and a Hewlett Packard 3390A integrator was used. The stationary phase was a 15 cm × 4.6 mm column packed with 5 μm, 60 Å, Chromegabond C8 silica (ES Industries). The organic portion of the mobile phase was composed of methanol containing 0.25% (v/v) 1-octanol. The aqueous portion was prepared from octanol-saturated water containing 0.02 M MOPS buffer, 0.15% (v/v) n-decylamine and adjusted to pH 7.4. A silica saturator column was included in the line, as well as a guard column packed with C8 silica. The flow was 1 mL/min.

The steroids were dissolved at 1 mg/mL in methanol and ~4 μg was injected onto the column. The standards were also dissolved in methanol, but only ~0.04–0.4 μg were used per injection. Column void volume was estimated from the retention time of uracil, which was included as a nonretained internal reference with each injection.[23] The log k'_w was determined by linear extrapolation of log$'\phi$ vs ϕ methanol data acquired in the region $0.55 \leq \phi$ methanol ≤ 0.85.

In Vivo Uptake Studies. In vivo uptake studies were performed as previously reported.[4c] Immature, female Sprague-Dawley rats (22–25 day, 50–60 g) were injected under ether anesthesia, via the femoral vein, with ~0.1 mL of the radiopharmaceutical. At the indicated times, the animals were killed by decapitation and samples of blood and tissue were excised, weighed, and immediately assayed for radioactivity in a gamma counter with 60% efficiency. The ^{18}F radiopharmaceutical was prepared for injection by dissolving in ethanol and diluting to the proper dosage with physiological saline, so that the injection dose contained <20% ethanol. In the blocked experiments, 15 μg of estradiol was coinjected with the isotope.

Metabolism Studies. The metabolism of the ^{18}F-estradiols was evaluated by analysis of target and nontarget tissues and blood samples at various times after injection.[25] The ^{18}F compounds were extracted from the homogenized tissues with ethanol and assayed by silica gel thin-layer chromatography, by comparing them to an authentic unlabeled sample of the substituted estrogen. No attempt was made to identify the metabolites.

Acknowledgment. We are grateful for support of this work by grants from the National Institutes of Health (Grant No. CA25836 to J.A.K.) and (Grant No. HL 13851 to M.J.W.) and from the Department of Energy (Grant No. DOE FG02ER84 60218 to M.J.W.). We greatly appreciate the assistance of Karen Kandl for log P determinations, and Dr. Scott R. Wilson for X-ray structure work. High-resolution mass spectra were obtained on instruments supported by a grant from the National Institutes of Health (Grant No. GM 27029).

Registry No. 1, 129000-37-9; [^{18}F]-1, 129000-38-0; **2**, 129000-39-1; [^{18}F]-**2**, 129000-40-4; [^{18}F]-**3**, 92817-14-6; **5a**, 21375-11-1; **5b**, 64109-55-3; **6**, 67020-41-1; **7**, 17253-50-8; **8**, 129098-85-7; **9**, 129098-86-8; **10**, 129000-41-5; **11a**, 129000-42-6; **11b**, 129000-43-7; **12a**, 129000-44-8; **12b**, 129000-45-9; **13a**, 129000-46-0; **13b**, 129000-47-1; **14a**, 129000-48-2; **14b**, 129000-49-3; **15a**, 129000-50-6; **15b**, 129000-51-7; **16a**, 129000-52-8; **16b**, 129000-53-9; **17a**, 129000-54-0; [^{18}F]-**17a**, 129000-55-1; **17b**, 129000-56-2; [^{18}F]-**17b**, 129000-57-3; **18**, 92817-08-8; [^{18}F]-**19**, 129000-58-4.

Supplementary Material Available: Atomic numbering schemes and tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for compounds 5b and 10 (8 pages). Ordering information is given on any current masthead page.

Synthesis and Estrogen Receptor Binding of Novel 11β-Substituted Estra-1,3,5(10)-triene-3,17β-diols

Robert N. Hanson,* Elio Napolitano,† and Rita Fiaschi†

Section of Medicinal Chemistry, College of Pharmacy & Allied Health Professions, Northeastern University, Boston, Massachusetts 02114

Kay D. Onan

Department of Chemistry, Northeastern University, Boston, Massachusetts 02114. *Received February 6, 1990*

As part of our program to develop estrogenic radioligands for use in nuclear medicine, a study was undertaken to investigate the effect of substituents on the receptor affinity of putative radiochemicals. In the study a synthetic strategy directed toward the introduction of an 11β-(fluoroethyl) substituent was devised. The target compound **9** was prepared via a five-step procedure starting from 11β-vinylestrone 3-acetate (**4**) in an overall 43% yield. The stereochemistry of the 11β-vinyl moiety was established by X-ray crystallography. The final product and several analogues, 11β-ethyl-, -vinyl-, and (hydroxyethyl)estradiols (**11**, **5**, and **12**), were evaluated for their estrogen receptor binding affinity. The results indicate that the target compound and several 11β-substituted analogues possess relative binding affinities greater than of estradiol and its 16α-fluorinated derivatives. The manner in which the target compound **9** was prepared is amenable to use with ^{18}F incorporation.

As part of our program for the development of radiopharmaceuticals for the external visualization of estrogen receptor containing human breast cancer, we have previously focused on radioiodinated and brominated derivatives.[1-6] Because of the desirable properties associated with the positron-emitting radionuclide fluorine-18[7,8] we have sought to include in our program ligands capable of incorporating this radionuclide. The clinical potential for agents labeled with this radionuclide was recently demonstrated by the successful noninvasive imaging of human breast cancer with 16α-[^{18}F]-fluoroestradiol.[9,10] Although the images obtained provide a measure of diagnostic in-

* To whom correspondence should be addressed.
† On leave from the University of Pisa.

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