7α-Methyl- and 11β-Ethoxy-Substitution of [^{125}I]-16α-Iodoestradiol: Effect on Estrogen Receptor-Mediated Target Tissue Uptake†

Hasrat Ali, Jacques Rousseau, and Johan E. van Lier*

MRC Group in the Radiation Sciences, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4

Received August 24, 1992

The 7α-methyl and 11β-ethoxy derivatives of 16α-[^{125}I]iodoestradiol were prepared via halogen exchange with ^{125}I of the corresponding 16β-bromoestradiol precursors. The 16α-bromo derivatives were obtained via halogenation of the analogous 17-enol acetate, epimerization to the 16β-isomer, and hydride reduction. Stereochemical assignments were based on high resolution ^{1}H NMR. To evaluate the effect of the nature and stereochemistry of the 16-halo substituent on the relative binding affinity for the estrogen receptor, the analogous 16-chloro derivatives were also prepared. The highest binding affinities were observed with the 7α-methyl-16α-haloestradiols, particularly the bromo and chloro derivatives while the 16α-iodo derivatives gave somewhat lower values. Both the 11β-ethoxy and 7α-methyl-16α-[^{125}I]iodoestradiols localize in the uteri of immature female rats via a receptor-mediated process. Rapid blood clearance of the ^{125}I-labeled 7α-methyl derivative results in lower ^{125}I uptake by the uterus as well as nontarget organs as compared to the 11β-substituted estradiol analogs. However, uterus to blood and nontarget ratios are more favorable for the 7α-methyl-16α-[^{125}I]iodoestradiol as compared to the analogous 11β-ethoxy derivatives suggesting that this compound substituted with ^{123}I may be useful for the in vivo imaging of estrogen receptor-rich breast tumors by single photon emission computerized tomography.

Introduction

Early detection of mammary tumors is perceived as the best way to reduce the mortality rate from breast cancer in women and also provides the basis for selective therapeutic approaches in the management of this disease. Since a large percentage of breast cancers are rich in estrogen receptor (ER), many attempts to use γ-emitting estrogens for ER detection have been made.[1] Radioisotopes which have been used to label estrogens for receptor imaging include ^{131}I, ^{123}I, ^{18}F, ^{77}Br, and ^{11}C. Although in vivo images of ER-rich tissues have been reported with some derivatives, none of these products have found routine clinical applications and studies to develop a suitable radiopharmaceutical for ER imaging are in progress in several laboratories. A radioiodinated estrogen analog with high binding affinity for ER could have important clinical applications through the use of the ^{123}I-analog and single photon emission computerized tomography (SPECT) imaging. Many estrogen analogs have been studied for this purpose[1] and among them 16α-iodoestradiol (16α-IE$_2$) stands out as a simple derivative which closely resembles the parent molecule, estradiol, and which exhibits high affinity for the estrogen receptor.[2-4] 16α-^{125}IE$_2$ has proven to be an excellent ligand for the ER and it is now routinely used for the in vitro quantification of ER in biopsy material. The usefulness of a γ-emitting 16α-IE$_2$ analog for in vivo ER imaging is however limited due to rapid metabolism of this steroid.[5,6] Attempts to slow down its metabolism via substitution at the 11β-position have shown promise.[7] Addition of a 11β-methoxy group decreases metabolism of 16α-IE$_2$ while the iodine at C-16 prevents interaction with 17β-hydroxy steroid dehydrogenase,[8] the enzyme which converts estradiol to estrone. Furthermore, substitution at the 11β-position increases the stability of the ER–steroid complex and reduces nonspecific binding. Likewise, addition of a 7α-methyl substituent has shown to enhance interaction of estradiol with ER.[9] Radioiodinated 17α-(iodovinyl)-estradiol derivatives[10] constitute another class of promising ligands where uptake by ER-rich target tissues has been shown to improve substantially upon 11β- or 7α-substitution.[10b]

In this study we describe the synthesis, characterization, and structure–activity relationships of the 7α-methyl and 11β-ethoxy derivatives of the 16α-halo- (I, Br, Cl) and [^{125}I]16α-iodoestradiols in relation to the earlier reported 11β-methoxy analogs.[11] The ^{125}I-labeled products were prepared via a rapid halogen exchange reaction. Relative binding affinities (RBA) for the estrogen receptor were measured via an in vitro assay, and tissue distribution and ER-mediated uterus uptake was established in immature female rats.

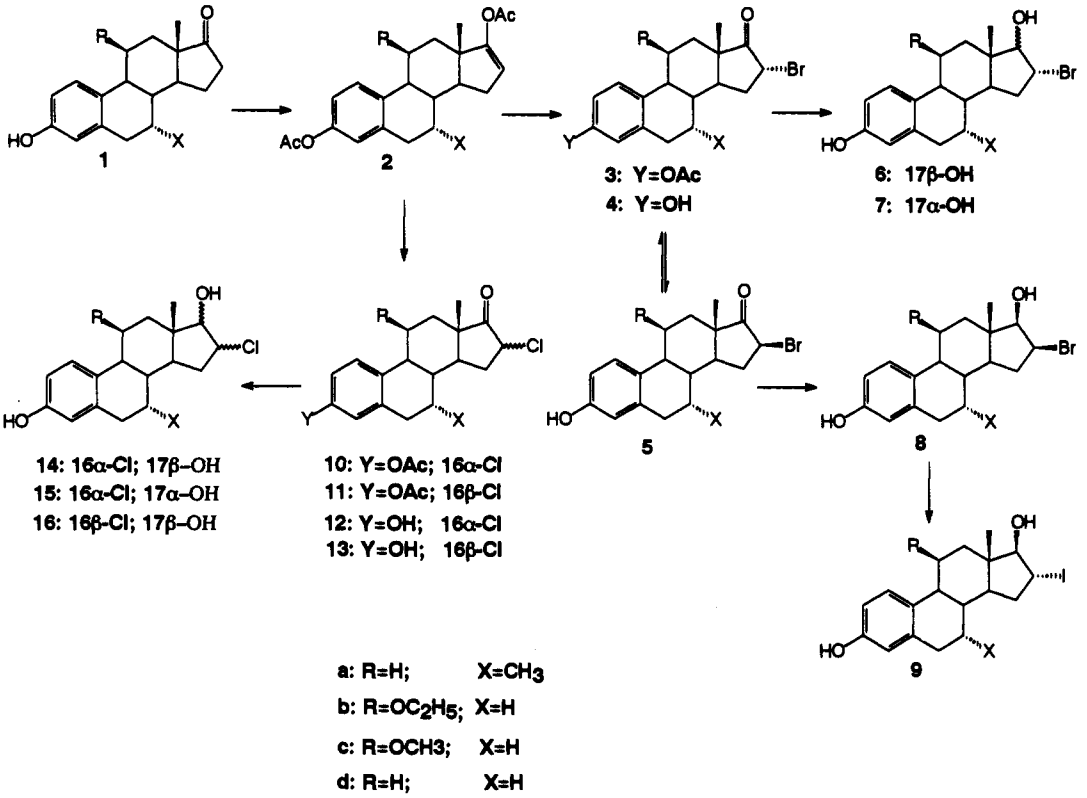
Chemistry

The 16-substituted halo steroids were prepared by an adaptation of the Johnson and Johns procedure[12] (Scheme I). The estrone derivatives (**1a,b**) were converted to the estrone 3-acetate 17-enol acetate (**2a,b**) and brominated with Br$_2$ in acetic acid to give exclusively the 16α-bromoestrone 3-acetate derivatives (**3a,b**). The acetate group was then hydrolyzed with concentrated sulfuric acid in absolute ethanol at room temperature to give the 16α-bromoestrones (**4a,b**), which were converted to the 16β-bromoestrones (**5a,b**) via epimerization with LiBr in 2-butanone. The 16β-configuration in the final products was confirmed by the characteristic downfield shift of the C-18 protons in the ^{1}H NMR.[13] Whereas the epimeric 7α-methyl derivatives **4a** and **5a** separated well on a silica gel column, the analogous 11β-ethoxy epimers **4b** and **5b** did not separate under these conditions. The 16α-bromo estrones (**4a,b**) were reduced with lithium aluminum hydride to give a mixture of 16α-bromo diols, epimeric at the C-17 position (**6a,7a** and **6b,7b**). The mass spectra of **6a,7a** and **6b,7b** showed pairs of molecular ions in about

† Presented in part at the Eighth International Symposium on Radiopharmaceutical Chemistry, Princeton University, Princeton, NJ, June 24-29, 1990.

0022-2623/93/1836-0264$04.00/0

Scheme I



1:1 ratios indicating the presence of bromine in both compounds. The C17-H was shifted downfield towards the C16-H, which is consistent with the cis stereochemistry of the C17α-H and the 16-bromo substituent.[13] Reduction of the 16β-bromo derivatives **5a,b** with sodium borohydride in ethanol yielded the 16β-bromo-17β-estradiols **8a,b** together with the debrominated products. The 16α-iodoestradiol derivatives **9a,b** were obtained by halogen exchange of the 16β-bromo in **8a,b** using NaI or [125I]-NaI.[11a,14] The halogen exchange reaction (2 h) gave the radioiodinated products in 60–75% yield. The compounds were purified by HPLC on a reverse-phase column in MeOH/H_2O. The analogous 16-chloroestradiols were obtained by treatment of the diacetates **2a,b** with *tert*-butyl hypochlorite,[15] which afforded a 3:1 isomeric mixture of the 16α/β-chloroestrone-3-acetates **10a,11a** and **10b,11b** which were converted to the free phenols **12** and **13**, respectively, via acid hydrolysis. The 16-chloro epimers **12a,13a** and **12b,13b** were separated on a silica gel column. The 16α-chloroestrone derivatives **12a,b** were reduced with sodium borohydride to the C17-epimeric alcohols **14a, 15a** and **14b, 15b**, whereas reduction of the 16β-chloro analog **13a** gave exclusively the 17β-alcohol analog **16a**.

Biological Properties

The relative binding affinities (RBA) of **4–9**, **12**, and **14–16** for the ER were measured via a competitive binding assay with [3H]estradiol[16] and are summarized in Table I. The highest affinities are observed with the 16α-halogenated steroids containing the natural 17β-hydroxy configuration in the order of Br > Cl > I which is in agreement with literature data on related compounds.[15] Between the 7α-methyl and 11β-ethoxy derivatives the former gave substantially higher RBA values. Halogenated estrone derivatives showed only a weak binding affinity for the ER.

Table I. Relative Binding Affinities[a] of Substituted 16-Haloestrogens for Murine Cytoplasmic Estrogen Receptors

	substituents				
compd	7α	11β	17	16	RBA ± SD
4a	Me	-	keto	α-Br	28.4 ± 3.7
4b	-	OEt	keto	α-Br	0.8 ± 0.1
7a	Me	-	α-OH	α-Br	13.6 ± 0.3
7b	-	OEt	α-OH	α-Br	1.1 ± 0.1
6a	Me	-	β-OH	α-Br	79.1 ± 11.8
6b	-	OEt	β-OH	α-Br	16.8 ± 1.0
6c	-	OMe	β-OH	α-Br	20.0[b]
5a	Me	-	keto	β-Br	5.3 ± 0.4
8a	Me	-	β-OH	β-Br	7.1 ± 0.7
8b	-	OEt	β-OH	β-Br	0.6 ± 0.3
9a	Me	-	β-OH	α-I	55.2 ± 3.6
9b	-	OEt	β-OH	α-I	16.5 ± 1.1
12a	Me	-	keto	α-Cl	33.8 ± 3.7
12b	-	OEt	keto	α-Cl	0.8 ± 0.7
14a	Me	-	β-OH	α-Cl	71.5 ± 7.6
14b	-	OEt	β-OH	α-Cl	14.9 ± 0.4
15a	Me	-	α-OH	α-Cl	16.6 ± 0.6
15b	-	OEt	α-OH	α-Cl	0.8 ± 0.0
16a	Me	-	β-OH	β-Cl	4.3 ± 0.2

[a] The realtive binding affinity (RBA) is defined as 100 times the ratio between competitor and unlabeled estradiol concentration required for 50% competition to specific [3H]estradiol binding. Competitive binding between 10^{-9} M [3H]estradiol and 2×10^{-9} to 2×10^{-12} M unlabeled ligands was plotted, and the concentration required for 50% competition was used to calculate the RBA values (mean of three experiments and SD, standard deviation). [b] Value taken from ref 11b.

The biodistribution and uterus uptake of the 125I-labeled estradiol derivatives **9a** and **9b** was studied in immature female Fischer rats. The animals were injected via the tail vein with HPLC-purified [125I]-**9a** or [125I]-**9b** (3 μCi, 111 KBq) in 200 μL of 9% ethanol–saline containing 1% Tween-80 and sacrificed at 0.5, 1, 2, 5, and 24 h postinjection. Receptor-mediated uterine uptake was evaluated in the 1 h time group by blocking ER via coinjection with

Table II. Tissue Uptake of ^{125}I after Administration of ^{125}I-Labeled 11β-Ethoxy- and 7α-Methyl-16α-Iodoestradiol to Immature Female Fischer Rats

	%ID/g (%CV)[a]					
tissue	0.5 h (n = 3)	1 h (n = 5)	1 h (+E_2) (n = 3)	2 h (n = 4)	5 h (n = 4)	24 h (n = 3)
			[^{125}I]-**9b** (7α-methyl)			
blood	0.45 (2)	0.38 (19)	0.33 (9)	0.32 (21)	0.28 (14)	0.03 (18)
uterus	4.32 (25)	5.25 (28)	0.34 (11)	6.35 (26)	3.86 (35)	1.64 (13)
thyroid	40.33 (31)	45.15 (24)	43.50 (15)	66.19 (40)	320.54 (35)	334.84 (20)
muscle	0.54 (1)	0.32 (11)	0.27 (5)	0.15 (32)	0.06 (33)	0.01 (44)
fat	2.20 (49)	1.79 (28)	1.64 (8)	1.12 (25)	0.38 (41)	0.03 (11)
kidneys	1.30 (6)	0.83 (3)	0.51 (9)	0.50 (24)	0.25 (22)	0.30 (19)
plasma	0.68 (2)	0.58 (19)	0.51 (11)	0.49 (24)	0.41 (12)	0.04 (19)
spleen	0.57 (4)	0.43 (6)	0.43 (20)	0.33 (12)	0.22 (29)	0.08 (27)
lungs	0.82 (13)	0.54 (7)	0.43 (12)	0.32 (18)	0.19 (15)	0.03 (13)
liver	1.94 (10)	1.26 (8)	1.09 (9)	1.09 (10)	0.72 (19)	0.13 (7)
			[^{125}I]-**9c** (11β-ethoxy)			
blood	1.29 (11)	1.01 (17)	1.09 (9)	0.89 (16)	0.70 (13)	0.17 (18)
uterus	7.85 (17)	11.67 (18)	0.72 (9)	13.08 (18)	7.76 (19)	1.64 (35)
thyroid	105.65 (19)	110.96 (32)	156.51 (34)	218.87 (21)	703.10 (10)	773.37 (45)
muscle	1.05 (22)	0.69 (21)	0.72 (20)	0.52 (35)	0.35 (29)	0.13 (52)
fat	2.47 (23)	1.51 (24)	1.63 (20)	0.94 (32)	0.52 (14)	0.15 (24)
brain	0.68 (16)	0.39 (21)	0.42 (25)	0.25 (33)	0.14 (22)	0.04 (17)
kidneys	2.26 (10)	1.83 (25)	1.61 (6)	1.78 (28)	0.91 (17)	0.24 (21)
spleen	1.44 (21)	1.49 (19)	1.60 (23)	1.76 (19)	1.28 (21)	0.70 (23)
lungs	1.72 (13)	1.30 (22)	1.18 (14)	1.01 (19)	0.76 (8)	0.23 (11)
liver	5.91 (30)	3.48 (26)	4.50 (19)	3.24 (18)	2.31 (9)	0.96 (11)

[a] Average %ID/g for 3–5 rats. Each rat was injected iv with 3 μCi (111 kBq) of n.c.a. ^{125}I-labeled steroid in the presence (+E_2) or absence of 60 μg coinjected estradiol. The incertitude is given in brackets as the %CV, e.g. the % coefficient of variation corrected for small sample size effect.[21]

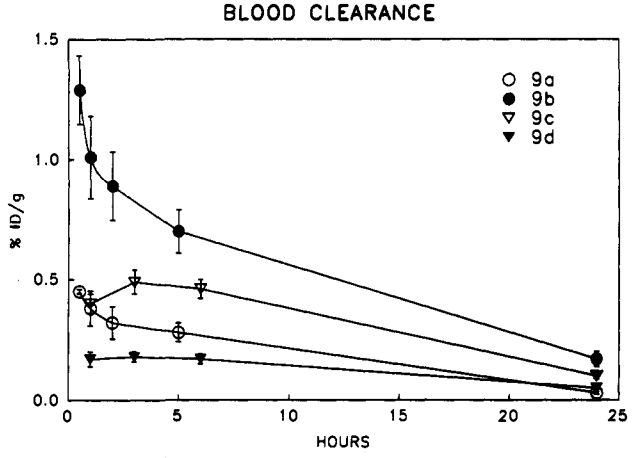


Figure 1. Blood clearance in immature female Fischer rats after iv injection with 3 μCi of ^{125}I-labeled 16α-iodo-7α-methylestradiol-1,3,5(10)-triene-3,17β-diol (**9a**) or 16α-iodo-11β-ethoxy-1,3,5(10)-triene-3,17β-diol (**9b**). The values for the 11β-methoxy analog **9c** and the nonsubstituted 16α-iodoestradiol (**9d**) were taken from the literature.[11]

60 μg of unlabeled estradiol. Tissue uptake of radioiodine after administration of [^{125}I]-**9a** or -**9b** is presented in Table II as the % of injected dose per gram tissue (%ID/g). Blood clearance, uterus uptake, and uptake ratios are compared to earlier reported data on the [^{125}I]-16α-iodoestradiol (**9d**) and the [^{125}I]-11β-methoxy-16α-iodoestradiol (**9c**)[11] in Figures 1 and 2. Overall tissue distribution pattern of **9a** and **9b** resemble those reported for **9c**[11] although some distinct differences are observed in uterine uptake pattern (Figure 2). The uterus uptake of **9a** and **9b** reaches a maximum between 1–2 h postinjection, at which time the ^{125}I concentration is the highest of all organs counted, with the exception of the thyroid (Table II). Apart from the uterus and thyroid, ^{125}I-radioactivity level decrease steadily from the first time point measured at 0.5 h, for both [^{125}I]-**9a** and -**9b**. Compared to the 11β-methoxy derivative **9c** and the nonsubstituted 16α-iodoestradiol **9d**, the 7α-methyl and 11β-ethoxy analogs **9a** and **9b** exhibit strong ER-mediated uterus uptake and similar uterus to blood/nontarget ratios (Figure 2).[11] The 7α-methyl derivative **9a** shows a 2–3 fold lower uterus uptake as compared to the 11β-substituted derivatives however, its rapid blood and systemic clearance results in higher uterus to blood/nontarget ratios at 2 h postinjection (Figure 2).

Discussion

Among the steroidal estrogens tested bearing different halogens on the D-ring at the 16α-position, the 16α-Br derivatives have the highest affinities for the ER, confirming earlier reports by Heimann et al.,[15] Levesque et al.,[17] and Longcope et al.[18] (Table I). The RBA values of all 11β-ethoxy derivatives are much lower than those of the corresponding 7α-methyl analogs. 16α-Bromo-7α-methylestradiol (**6a**) has the highest binding affinity for the ER (RBA = 79). The RBA values decrease by substitution of Br for Cl (**14a**) or I (**9a**) (RBA = 71 and 55, respectively). A similar structure–activity relationship has been observed in the unsubstituted estradiol series.[15] The corresponding 16β-epimers exhibited substantially diminished binding affinities for the ER. In the 11β-ethoxy series, the highest binding affinity was likewise observed for the 17β-hydroxy, 16α-bromo derivative **6b** (RBA = 16.8) (Table I).

The radiochemical purity of [^{125}I]-**9a** and [^{125}I]-**9b**, after HPLC purification, was in excess of 95%; no other radioactive material could be detected on the chromatogram. The identity of the products were confirmed by their chromatographic mobilities (HPLC) which were identical to those of the corresponding unlabeled analogs. Specific activities could not be calculated for these compounds since no UV absorption at the most sensitive detector setting was observed in the region of the radioactive HPLC peak. Based on the specific activity of the n.c.a. [^{125}I]NaI employed (2125 mCi/mmol) the specific activity of these compounds should exceed 500 Ci/mmol. The reverse-phase HPLC system readily separates the

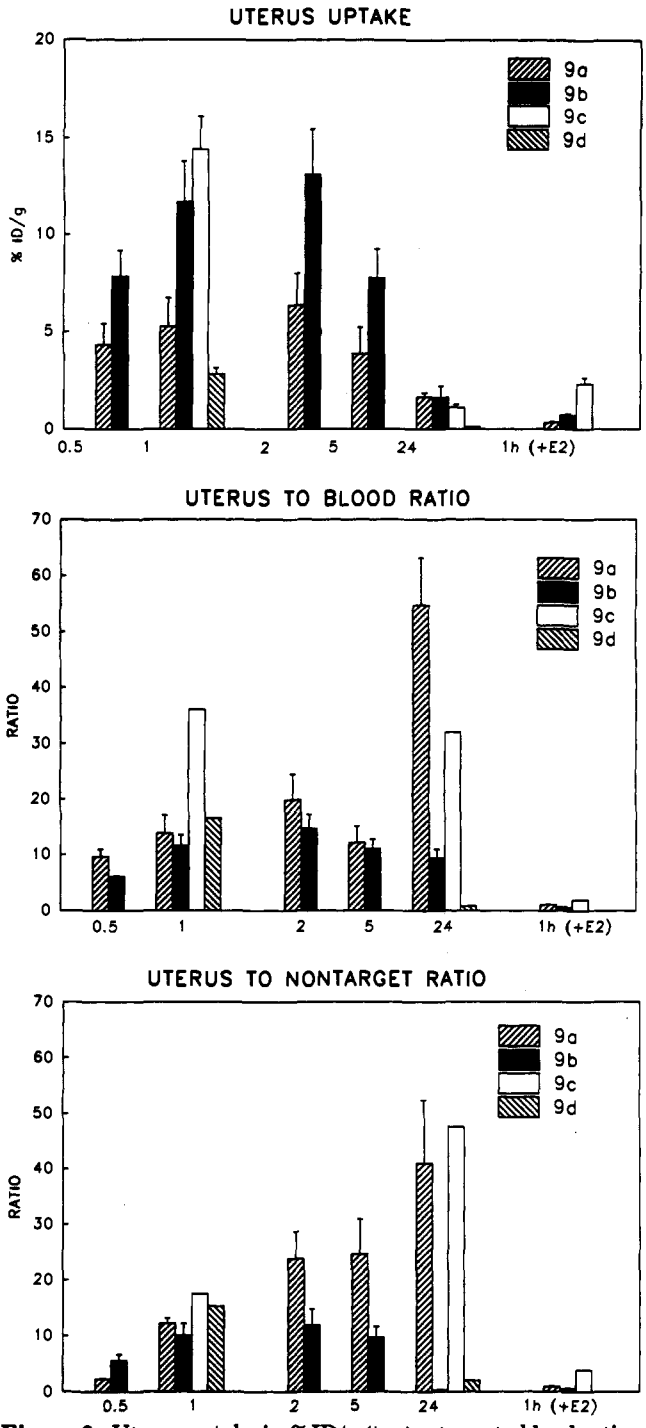


Figure 2. Uterus uptake in % ID/g (top), uterus to blood ratios (middle), and uterus to nontarget (lung, spleen, and muscle) ratios (bottom) of ^{125}I-labeled 7α-methyl (**9a**), 11β-ethoxy (**9b**), 11β-methoxy (**9c**), and nonsubstituted 16α-iodoestradiol (**9d**). Values for **9c** and **9d** were taken from the literature.[11]

16α-iodo products from the 16β-bromo reactants, as well as possible products from side reactions, such as the 17-keto steroids formed by dehydrohalogenation and the 16α-bromo steroids resulting from epimerization. Sodium thiosulfate was added to the ^{125}I-labeled solution to reduce free iodine to I^- since commercial [^{125}I]NaI upon standing was found to contain a significant amount of I_2. In addition, benzoic acid was added to buffer the reaction mixture and to protect the alkali-sensitive bromohydrins. The entire radiochemical synthesis, including purification, can be completed within 4–6 h to give the radioiodinated estrogens in 65–85% radiochemical yield. This rapid synthesis could readily be adapted for use with the medicinally important ^{123}I ($t_{1/2}$ = 13.3 h) isotope.

The biodistribution studies with these ^{125}I-labeled steroids revealed persistent retention by the uterus as the most striking property (Table II). In the case of the 7α-methyl derivative ^{125}I-**9a**, uterus to blood ratios were particularly accentuated due to rapid blood clearance (Figure 1). After 24 h, uterus to blood ratios for ^{125}I-**9a** were greater than 40:1 while at 5 h postinjection the % ID/g values still are over 50% of those measured at the maximum concentrations at 1–2 h postinjection. Thus, although the absolute uterus uptake values in % ID/g are higher for the 11β-methoxy/ethoxy derivatives as compared to the 7α-methyl derivative, the latter gave the best target to nontarget ratios (Figure 2). Accordingly, the ^{123}I-labeled 7α-methyl-16α-iodoestradiol could be of particular interest for SPECT imaging of estrogen receptors in breast cancer.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Spectral data (^{1}H NMR, Bruker WM 25 spectrometer with Me_4Si as an integral standard; mass spectra, Hewlett-Packard Model 5988A quadrupled) were recorded. Microanalyses data were obtained by Guelph Laboratories Ltd., Canada. Silica gel (60–200 mesh) was used for column chromatography. Silica gel plates coated with fluorescent indicator (UV 254) were used for analytical thin-layer chromatography (TLC), and the compounds were located by their UV absorbance and/or color response upon spraying with H_2SO_4/EtOH and heating at 120 °C. High performance liquid chromatography (HPLC) was performed on a reverse-phase column (C-18, ODS-2 spherisorb, 5 μm, 25 × 0.94 cm, CSC, Montreal), and the compounds were detected at 280 nm and where appropriate, by their γ-radiation which was registered via a sodium iodide detector. All chemicals used are commercially available and were of the highest chemical grade available; n.c.a. (non-carrier added) [^{125}I]NaI was purchased from Amersham Canada Ltd. Steroids were purchased from Steraloids Inc. 17-Ethylenedioxyestra-1,3,5-(10)-triene-3,11β-diol was synthesized according to Baran[19] and converted to 11β-ethoxy-3-hydroxyestra-1,3,5(10)trien-17-one with ethyl iodide.

7α-Methylestra-1,3,5(10),16-tetraene-3,17-diol Diacetate (2a). A solution of 7α-methyl estrone (**1a**, 375 mg, 1.32 mmol) in 3.5 mL of isopropenyl acetate and 0.15 mL of catalyst solution (prepared by mixing 4 mL of isopropenyl acetate + 0.1 mL of H_2SO_4) was refluxed for 2 h. Approximately 1 mL of the solvent was slowly distilled over a period of 1 h. An additional 2 mL of isopropenyl acetate and 0.1 mL of catalyst were added, and the solution was concentrated to one-half of the volume by slow distillation for 1 h. The solution was chilled and EtOAc was added. The EtOAc solution was washed with ice-chilled sodium bicarbonate (5%) in water and dried over sodium sulfate (anhydrous) and the solvent was removed under reduced pressure. The residue was purified on a column packed with alumina oxide. Elution with 10% EtOAc in hexane furnished **2a** (200 mg, 0.54 mmol, 54%), which failed to crystallize: ^{1}H NMR ($CDCl_3$) δ 0.86 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.92 (s, 3 H, 18-CH_3), 2.13 (s, 3 H, 17-$OCOCH_3$), 2.23 (s, 3 H, 3-$OCOCH_3$), 5.48 (brs, 1 H, 16-H), 6.86, 6.96, 7.18 (3 H); MS m/z (rel inten), 368 (M^+, 3), 326 (10), 284 (29), 270 (36), 255 (15), 159 (11).

Further elution gave 7α-methylestrone acetate (100 mg, 0.3 mmol) (23%): ^{1}H NMR ($CDCl_3$) 0.82 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.92 (s, 3 H, 18-CH_3), 2.28 (s, 3 H, 3-$OCOCH_3$), 6.64, 6.92, 7.18 (3 H), MS m/z (rel inten) 326 (M^+, 4), 312 (3), 284 (35), 270 (34), 185 (11), 145 (13).

11β-Ethoxyestra-1,3,5(10),16-tetraene-3,17-diol Diacetate (2b). A solution of 11β-ethoxyestrone (**1b**, 970 mg, 3.08 mmol) was treated as described for **2a** to furnish **2b**, which failed to crystallize (620 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 1.15 (s, 3 H, 18-CH_3), 2.18 (s, 3 H, 17-$OCOCH_3$) 2.26 (s, 3 H, 3-$OCOCH_3$), 3.28, 3.67 (q, J = 7 Hz,

2 H, 11β-OCH_2CH_3), 4.26 (m, 1 H, 11α-H), 5.47 (brs, 1 H, 16-H), 6.78 (d, J = 2 Hz, 1 H, 2-CH), 6.82 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 7.08 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 389 (M^+, 3), 356 (5), 338 (5), 268 (5), 250 (5), 188 (14), 146 (100).

Further elution of the alumina oxide column with 10% EtOAc gave 11β-ethoxyestrone acetate (360 mg, 23%): mp 120–122 °C; ^1H NMR ($CDCl_3$) 0.93 (t, 3 H, J = 7 Hz, 11β-OCH_2CH_3), 1.01 (s, 3 H, 18-CH_3), 2.15 (s, 3 H, 3-$OCOCH_3$), 3.22, 3.56 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 4.21 (m, 1 H, 11α-H), 6.71 (d, J = 2 Hz, 1 H, 4-CH), 6.76 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 7.04 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 356 (M^+, 28), 314 (7), 283 (16), 268 (22), 241 (14), 212 (36), 188 (17), 170 (31), 146 (100).

Bromination of 2a and 2b. A solution of **2a** (73.6 mg, 0.2 mmol) or **2b** (550 mg, 1.41 mmol) in ether (4 mL) and acetate buffer (4 mL, 85% acetic acid, 200 mg of potassium acetate) was cooled to 0 °C. To this solution was dropwise added 0.68 mL of a molecular bromine solution (prepared from 0.08 mL of Br_2 and 6.75 mL of glacial acetic acid) with continuous stirring, whereafter the solution was stirred for an additional 10–15 min. The reaction was terminated by the addition of water, whereafter the organic phase was washed with water, 5% sodium thiosulfate, and 5% aqueous sodium bicarbonate, dried over sodium sulfate (anhydrous), filtered, and evaporated to dryness to afford **3a** and **3b**.

16α-Bromo-3-acetoxy-7α-methylestra-1,3,5(10)-trien-17-one (3a). White crystalline solid (55 mg, 65%): recrystallized from hexane, mp 158–160 °C; ^1H NMR ($CDCl_3$), δ 0.92 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.98 (s, 3 H, 18-CH_3), 2.22 (s, 3 H, 3-$OCOCH_3$), 4.50 (dd, J = 1.5 and 4.5 Hz, 1 H, 16β-H), 6.66, 6.92, 7.18 (3 H); MS m/z (rel inten) 406 (M^+, 3), 364 (38), 362 (40), 350 (27), 348 (29), 284 (8), 214 (12), 172 (11).

16α-Bromo-3-acetoxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (3b). White crystalline solid (370 mg, 60.5%): recrystallized from hexane, mp 214–217 °C; ^1H NMR ($CDCl_3$) δ 1.02 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 1.14 (s, 3 H, 18-CH_3) 2.27 (s, 3 H, 3-$OCOCH_3$), 3.32, 3.65 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3) 4.31 (m, 1 H, 11α-H), 4.63 (dd, J = 1.5 and 4.5 Hz, 1 H, 16β-H), 6.80 (d, J = 2 Hz, 1 H, 4-CH), 6.86 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 7.12 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 436 (6), 434 (M^+, 6), 355 (28), 313 (15), 309 (33), 267 (48), 239 (19), 167 (50), 157 (28), 147 (100).

Acid Hydrolysis of 3a and 3b. 16α-Bromo-7α-methylestrone acetate (**3a**) (300 mg, 0.74 mmol) or 16α-bromo-11β-ethoxyestrone acetate (**3b**) (320 mg, 0.74 mmol) was hydrolyzed by treatment with concentrated sulfuric acid (0.1 mL) in anhydrous ethanol (5 mL) at room temperature. The compound was extracted with $CHCl_3$ and worked up in the usual manner. Evaporation of the solvent afforded **4a** and **4b**.

16α-Bromo-3-hydroxy-7α-methylestra-1,3,5(10)-trien-17-one (4a). White crystalline solid (200 mg, 74.5%): mp 228–233 °C; HPLC (70:30 MeOH/H_2O) t_R = 26 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.917 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.946 (s, 3 H, 18-CH_3) 4.57 (dd, 1 H, J = 1.5 and 5 Hz, 16β-H), 6.57 (d, J = 2 Hz, 1 H, 4-CH), 6.67 (dd, J = 2 Hz, 1 H, 2-CH), 7.16 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 364 (55), 362 (M^+, 60), 322 (5), 284 (29), 255 (6), 228 (31), 213 (92), 171 (58), 157 (100). Anal. ($C_{19}H_{23}BrO_2$) C, H, Br.

16α-Bromo-3-hydroxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (4b). White crystalline solid (270 mg, 93%): mp 250–252 °C; HPLC (75:25 MeOH/H_2O) t_R = 17 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.85 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 0.96 (s, 3 H, 18-CH_3), 3.16, 3.47 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 4.10 (m, 1 H, 11α-H), 4.48 (dd, J = 1.5 and 5 Hz, 1 H, 16β-H), 6.38 (d, J = 2 Hz, 1 H, 4-CH), 6.46 (dd, J = 2 Hz, and 8 Hz, 1 H, 2-CH), 6.78 (d, 1 H, J = 8 Hz, 1-CH); MS m/z (rel inten) 392 (7), 394 (M^+, 6), 313 (46), 267 (52), 239 (16), 212 (16), 197 (18), 167 (34), 159 (26), 157 (41), 146 (100). Anal. ($C_{20}H_{25}BrO_3$) C, H, Br.

16α-Bromo-7α-methylestra-1,3,5(10)-triene-3,17α-diol (7a) and 16α-Bromo-7α-methylestra-1,3,5(10)-triene-3,17β-diol (6a). A solution of 16α-bromo-7α-methyl-3-hydroxyestra-1,3,5-(10)-trien-17-one (**4a**, 30 mg, 0.082 mmol) in THF (5 mL) was added dropwise to a stirred solution of 1 M $LiAlH_4$ in THF (0.4 mL) at 0 °C. The reaction mixture was stirred for 2 h and then quenched by the careful addition of water, followed by 10% hydrochloric acid until the solution was strongly acidic. The product was extracted with EtOAc, washed with water, and dried over Na_2SO_4 (anhydrous) and the solvent was evaporated under reduced pressure. TLC showed the presence of several compounds, and the mixture was purified by column chromatography over silica gel. Elution with 10% EtOAc in hexane furnished a white crystalline compound **7a** (8 mg; 27%): mp 178–184 °C; HPLC (70:30 MeOH/H_2O) t_R = 34 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.80 (s, 3 H, 18-CH_3), 0.87 (d, J = 7 Hz, 3 H, 7α-CH_3), 3.65 (d, J = 5 Hz, 1 H, 17β-H), 4.65 (m, 1 H, 16β-H), 6.54 (d, J = 1 Hz, 1 H, 4-CH), 6.64 (dd, J = 2 and 9 Hz, 1 H, 2-CH) 7.17 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 366 (38), 364 (M^+, 36), 267 (33), 229 (45), 211 (20), 199 (29), 171 (82), 157 (67), 147 (100). Anal. ($C_{19}H_{25}BrO_2$) C, H, Br.

Further elution with 15% EtOAc in hexane furnished a crystalline compound **6a** (15 mg, 50%): mp 110–115 °C; HPLC (70:30 MeOH/H_2O) t_R = 31 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.80 (s, 3 H, 18-CH_3), 0.87 (d, J = 7 Hz, 3 H, 7α-CH_3), 4.0 (d, J = 5.8 Hz, 1 H, 17α-H), 4.13 (ddd, $J_{16\beta,17\alpha}$ = 4.3 Hz; $J_{16\beta,15\alpha}$ = 2.4 Hz, $J_{16\beta,15\beta}$ = 7.8 Hz, 1 H, 16β-H), 6.55 (d, J = 2 Hz, 1 H, 4-CH), 6.64 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 7.14 (d, 1 H, J = 8 Hz, 1-CH); MS m/z (rel inten) 366 (38), 364 (M^+, 38), 285 (10), 267 (18), 243 (13), 229 (56), 199 (34), 171 (59), 147 (100). Anal. ($C_{19}H_{25}BrO_2$) C, H, Br. Further elution with 20% EtOAc in hexane afforded 7α-methyl-3,17β-estradiol.

16α-Bromo-11β-ethoxyestra-1,3,5(10)-triene-3,17α-diol (7b) and 16α-bromo-11β-ethoxyestra-1,3,5(10)-triene-3,17β-diol (6b). A solution of 16α-bromo-11β-ethoxy-3-hydroxy-estra-1,3,5-(10)-trien-17-one (**4b**, 50 mg; 0.127 mmol) was treated as described for **6a**. Elution from the silica gel column with 20% EtOAc in hexane furnished a white crystalline compound **7b** (16 mg, 32%): mp 260–264 °C; HPLC (75:25 MeOH/H_2O) t_R = 16 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.83 (s, 3 H, 18-CH_3) 0.84 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 3.15, 3.45 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.45 (d, J = 5 Hz, 1 H, 17β-H), 4.11 (m, 1 H, 11α-H), 4.52–4.58 (m, 1 H, 16β-H), 6.37 (d, J = 2 Hz, 1 H, 4-CH), 6.47 (dd, J = 2 and 9 Hz, 1 H, 2-CH), 6.80 (d, J = 8 Hz, 1 H, 1-CH), MS m/z (rel inten) 394 (22), 396 (M^+, 19), 350 (13), 348 (13), 269 (58), 251 (53), 241 (22), 213 (57), 185 (43), 157 (69), 149 (100). Anal. ($C_{20}H_{27}BrO_3$) C, H, Br.

Continuous elution with 20% EtOAc in hexane furnished **6b** as a crystalline compound (25 mg, 50%): mp 235–237 °C; HPLC (75:25 MeOH/H_2O) t_R = 16 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.69 (s, 3 H, 18-CH_3), 0.75 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 3.02, 3.35 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.62 (d, J = 6.5 Hz, 1 H, 17α-H), 3.86–3.93 (m, 2 H, 11α-H and 16β-H), 6.26 (d, J = 2 Hz, 1 H, 4-CH), 6.34 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 6.67 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 394 (70), 396 (M^+, 63), 315 (29), 269 (100), 213 (83), 157 (86). Anal. ($C_{20}H_{27}BrO_3$) C, H, Br.

Epimerization of 16α-Bromo into 16β-Bromo Ketone. A mixture of 16α-bromo-7α-methylestrone (**4a**, 140 mg, 0.383 mmol) or 16α-bromo-3-hydroxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (**4b**, 50 mg, 0.127 mmol) and LiBr (140 mg) in 1 mL of 2-butanone was heated for 1 h at 80 °C in a closed 5-mL vial. The reaction mixture was poured into 15 mL of water and extracted with EtOAc, washed with water, dried over Na_2SO_4 (anhydrous) and evaporated to dryness. Column chromatography on silica gel with 8% EtOAc in hexane gave **5a** and **5b**.

16β-Bromo-3-hydroxy-7α-methylestra-1,3,5(10)-trien-17-one (5a): 80 mg, 57%; HPLC (50:50 THF/H_2O) t_R = 22 min; mp 101–105 °C; ^1H NMR ($CDCl_3$) δ 0.88 (d, J = 7 Hz, 3 H, 7α-CH_3), 1.12 (s, 3 H, 18-CH_3), 4.17 (t, J = 8.5 Hz, 1 H, 16α-H), 6.57 (d, J = 1 Hz, 1 H, 4-CH), 6.65 (dd, J = 2 and 7 Hz, 1 H, 2-CH), 7.14 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 364 (40), 362 (M^+, 44), 284 (19), 283 (28), 228 (30), 213 (79), 205 (79), 171 (54), 145 (100). Anal. ($C_{19}H_{23}BrO_2$) C, H, Br.

16β-Bromo-3-hydroxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (5b). ^1H NMR analysis indicated that **5b** consisted of a 50:50 mixture of the 16α- and β-bromo isomers, which we were unable to separate. The ^1H NMR values obtained for the 16β-isomer, after subtracting those of the 16α-isomer, include ^1H NMR ($CDCl_3$) 0.73 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 0.98 (s, 3 H, 18-CH_3), 3.03, 3.34 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.88 (t, J = 8.7 Hz, 1 H, 16α-H), 3.98 (m, 1 H, 11α-H), 6.23 (d, J = 1 Hz, 1 H, 4-CH), 6.31 (dd, J = 2 and 7 Hz, 1 H, 2-CH), 6.64 (d, J = 8 Hz, 1 H, 1-CH).

Reduction of 16β-Bromo Ketones 5a and 5b with $NaBH_4$. A solution of $NaBH_4$ (80 mg) in EtOH (5 mL) was added to

16β-bromo-7α-methylestrone (5a, 80 mg, 0.22 mmol) or 16β-bromo-11β-ethoxyestrone (5b, 115 mg, 0.292 mmol) in EtOH (5 mL). The mixture was stirred overnight at room temperature, excess $NaBH_4$ was destroyed with acetone, and the reaction mixture was poured into water, extracted with EtOAc, washed with water, dried over Na_2SO_4 (anhydrous), and evaporated to dryness. The residue was purified by column chromatography on silica gel with 10% EtOAc in hexane to yield 8a or 8b.

16β-Bromo-7α-methylestra-1,3,5(10)-triene-3,17β-diol (8a): 32 mg, 40%; mp 98–104 °C; HPLC (70:30 MeOH/H_2O) t_R = 24 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.82 (d, J = 6 Hz, 3 H, 7α-CH_3), 0.93 (s, 3 H, 18-CH_3), 3.48 (t, J = 7 Hz, 1 H, 17α-H), 4.68 (td, J = 7 Hz, 1 H, 16α-H), 6.56 (d, J = 2 Hz, 1 H, 4-CH), 6.65 (dd, J = 2 and 9 Hz, 1 H, 2-CH), 7.14 (d, J = 8 Hz, 1 H, 1-CH), 8.01 (s, 1 H, 17α-OH); MS m/z (rel inten) 366 (33), 364 (M^+, 35), 284 (54), 267 (23), 229 (33), 213 (25), 199 (44), 185 (22), 171 (59), 147 (83). Anal. ($C_{19}H_{25}BrO_2$) C, H, Br. Further elution with 15% EtOAc in hexane gave 7α-methylestradiol (20%).

16β-Bromo-11β-ethoxyestra-1,3,5(10)-triene-3,17β-diol (8b): 50 mg, 43.6%; mp 217–220 °C; HPLC (65:35 MeOH/H_2O) t_R = 21 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.78 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 0.87 (s, 3 H, 18-CH_3), 3.04, 3.36 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.19 (t, J = 8 Hz, 1 H, 17α-H), 3.95 (m, 1 H, 11α-H), 4.32 (q, J = 8 Hz, 1 H, 16α-H), 6.26 (d, J = 2 Hz, 1 H, 4-CH), 6.36 (dd, 1 H, J = 2 and 8 Hz, 2-CH), 6.69 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 394 (42), 396 (M^+, 41), 350 (41), 315 (25), 269 (71), 251 (52), 241 (24), 225 (17), 213 (62), 197 (29), 185 (32), 147 (100). Anal. ($C_{20}H_{27}BrO_3$) C, H, Br. Continuous elution with the same solvent gave a mixture of 6b and 7b (42 mg, 36%), t_R = 26 min.

Iodination of 8a and 8b. A mixture of 16β-bromo-7α-methyl-3,17β-estradiol (8a, 40 mg, 0.109 mmol) or 16β-bromo-11β-ethoxyestra-3,17-diol (8b, 19 mg, 0.048 mmol) and 7–14 mg of NaI in 1 mL of 2-butanone (methyl ethyl ketone) was heated for 1 h at 105 °C in a closed vial. After cooling, the mixture was poured into 15 mL of water and extracted with EtOAc (15 mL, twice). The extracts were combined, washed with water, dried over Na_2SO_4 (anhydrous) and evaporated. The reaction mixture was purified by HPLC.

16α-Iodo-7α-methylestra-1,3,5(10)-triene-3,17β-diol (9a): 35 mg, 80%; mp 184–186 °C; HPLC (70:30 MeOH/H_2O) t_R = 37 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) 0.617 (s, 3 H, 18-CH_3), 0.734 (d, J = 7 Hz, 3 H, 7α-CH_3), 3.93 (td, J = 7 Hz, 1 H, 16β-H), 4.01 (dd, J = 2 and 5 Hz, 1 H, 17α-H), 6.50 (dd, J = 2 and 5 Hz, 1 H, 2-CH), 6.96 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 412 (M^+, 100), 284 (12), 267 (31), 229 (79), 173 (64), 171 (60), 157 (50). Anal. ($C_{19}H_{25}IO_2$) C, H, I.

16α-Iodo-11β-ethoxyestra-1,3,5(10)-triene-3,17β-diol (9b): 18 mg, 84%; mp 245–246 °C; HPLC (70:30 MeOH/H_2O) t_R = 21 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.68 (s, 3 H, 18-CH_3), 0.77 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 3.04, 3.36 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.74 (td, J = 6 Hz, 1 H, 16β-H), 3.85–3.94 (m, 2 H, 11α-H and 17α-H), 4.5 (d, J = 6 Hz, 1 H, 17β-OH), 6.28 (d, J = 2 Hz, 1 H, 4-CH), 6.36 (dd, J = 5 and 2 Hz, 2-CH), 6.70 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 442 (M^+, 48), 315 (8), 269 (49), 251 (41), 225 (31), 213 (67), 157 (100). Anal. ($C_{20}H_{27}IO_3$) C, H, I.

16α-Chloro-3-acetoxy-7α-methylestra-1,3,5(10)-trien-17-one (10a) and 16β-Chloro-3-acetoxy-7α-methylestra-1,3,5-(10)-trien-17-one (11a). A solution of the enol diacetate 2a (400 mg, mmol) in acetone (20 mL), buffered with sodium acetate (400 mg) and glacial acetic acid (0.5 mL) and water (4 mL), was treated with *tert*-butyl hypochlorite (0.2 mL). The clear solution was warmed to 50–55 °C, stirred for 90 min, poured into water, extracted with EtOAc, washed with water and aqueous sodium bicarbonate (5%), and dried over Na_2SO_4 (anhydrous). Evaporation of the sovlent under reduced pressure furnished a white crystalline solid, which according to the ^1H NMR consisted of a 3:1 mixture of 16α- and 16β-chloro isomers 10a and 11a: mp 158–160 °C; ^1H NMR ($CDCl_3$) δ 0.90 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.98 (s, 3 H, 18-CH_3), 2.29 (s, 3 H, 3-$OCOCH_3$), 4.18 (dd, J = 2 and 8 Hz, 1 H, 16β-H), 4.43 (t, J = 8 Hz, 1 H, 16α-H), 6.60, 6.80, 7.2 (3 H); MS m/z (rel inten) 362 (3), 360 (M^+, 8), 320 (33), 318 (100) 284 (8), 254 (16), 228 (24), 213 (38).

16α-Chloro-3-acetoxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (10b) and 16β-Chloro-3-acetoxy-11β-ethoxyestra-1,3,5-(10)-trien-17-one (11b). A solution of the enol acetate 2b (300 mg, 0.77 mmol) was treated as described for 12a to afford a white crystalline solid which according to the ^1H NMR consisted of a 3:1 mixture of 16α- and 16β-chloro isomers 10b and 11b (260 mg, 86%): ^1H NMR ($CDCl_3$) δ 1.01 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 1.15 (s, 3 H, 18-CH_3), 2.26 (s, 3 H, 3-$OCOCH_3$), 3.3, 3.6 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 4.07 (t, J = 4 Hz, 1 H, 16α-H), 4.30 (m, 1 H, 11α-H), 4.50 (d, J = 7 Hz, 1 H, 16β-H), 6.79 (d, J = 2 Hz, 1 H, 4-CH), 6.82 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 6.86 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 390 (29), 392 (M^+, 11), 348 (15), 336 (8), 274 (10), 267 (13), 258 (32), 212 (100).

Acid Hydrolysis of 10a,b and 11a,b. A mixture of 16α- and 16β-chloro-7α-methylestrone acetate 10a and 10b (250 mg, 0.64 mmol) and a mixture of 16α- and 16β-chloro-11β-ethoxyestrone acetate 10b and 11b (200 mg, 0.51 mmol) was hydrolyzed with H_2SO_4 (1 mL) in absolute EtOH (20 mL) at room temperature for 12 h. The usual workup with EtOAc gave 200 mg of crude product which was purified by column chromatography on silica gel. Elution with 12–15% EtOAc in hexane furnished 13a, 12a, 13b, and 12b.

16β-Chloro-3-hydroxy-7α-methylestra-1,3,5(10)-trien-17-one (13a): 35 mg, 17%; mp 105–112 °C; HPLC (50:50 THF/H_2O) t_R = 22 min; ^1H NMR ($CDCl_3$) δ 0.88 (d, J = 7 Hz, 3 H, 7α-CH_3), 1.07 (s, 3 H, 18-CH_3), 4.05 (t, J = 8 Hz, 1 H, 16α-H), 6.57 (d, J = 1 Hz, 1 H, 4-CH), 6.64 (dd, J = 2 Hz and 6 Hz, 1 H, 2-CH), 7.14 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 320 (22), 318 (M^+, 61), 254 (30), 228 (47), 213 (100). Anal. ($C_{19}H_{23}ClO_2$) C, H, Cl.

16α-Chloro-3-hydroxy-7α-methylestra-1,3,5(10)-trien-17-one (12a): 125 mg, 61%; mp 222–225 °C; HPLC (50:50 THF/H_2O) t_R = 25 min; ^1H NMR ($CDCl_3$) δ 0.92 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.96 (s, 3 H, 18-CH_3), 4.5 (dd, J = 1 and 8 Hz, 1 H, 16β-H), 6.57 (d, J = 2 Hz, 1 H, 4-CH), 6.65 (dd, J = 2 and 6 Hz, 1 H, 2-CH), 7.20 (d, J = 8 Hz, 1 H, 2-CH); MS m/z (rel inten) 320 (27), 318 (M^+, 76), 283 (5), 254 (20), 228 (45), 213 (100). Anal. ($C_{19}H_{23}ClO_2$) C, H, Cl.

16β-Chloro-3-hydroxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (13b): 4 mg, 2.2%; mp 215–230 °C; HPLC (50:50 THF/H_2O) t_R = 16 min; ^1H NMR ($CDCl_3$) δ 0.92 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 1.13 (s, 3 H, 18-CH_3), 3.20, 3.53 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.90 (t, J = 8.5 Hz, 1 H, 16α-H), 4.15 (m, J = 2 Hz, 1 H, 11α-H), 6.45 (d, J = 2 Hz, 1 H, 4-CH), 6.53 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 6.83 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 348 (86), 350 (M^+, 30), 312 (11), 302 (18), 284 (12), 275 (29), 267 (37), 258 (27), 240 (24), 212 (100). Anal. ($C_{20}H_{25}ClO_3$) C, H, Cl.

16α-Chloro-3-hydroxy-11β-ethoxyestra-1,3,5(10)-trien-17-one (12b): 90 mg, 50%; mp 225–245 °C; HPLC (50:50 THF/H_2O) t_R = 17 min; ^1H NMR ($CDCl_3$) δ 0.67 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 0.80 (s, 3 H, 18-CH_3), 2.98, 3.29 (q, J = 8 Hz, 2 H, 11β-OCH_2CH_3), 3.92 (m, 1 H, 11α-H), 4.22 (d, J = 8 Hz, 1 H, 16β-H), 6.17 (d, J = 2 Hz, 1 H, 2-CH), 6.25 (dd, J = 2 and 6 Hz, 1 H, 2-CH), 6.60 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 348 (99), 350 (M^+, 31), 312 (14), 302 (16), 275 (34), 267 (30), 258 (29), 240 (18), 212 (100). Anal. ($C_{20}H_{25}ClO_3$) C, H; Cl: calcd, 10.16; found, 9.71.

Reduction of 12a, 12b, and 13a with $LiAlH_4$. 16α-Chloro-7α-methylestrone (12a, 50 mg, 0.156 mmol), 16α-chloro-11β-ethoxyestrone (12b, 67 mg, 0.19 mmol), or 16β-chloro-7α-methylestrone (13a, 31 mg, 0.01 mmol) in THF (10 mL) was cooled to 0 °C, and a solution of $LiAlH_4$ in THF (1 M, 0.4 mL) was added dropwise with vigorous stirring. After continuous stirring for 60 min, the reaction mixture was stopped by the addition of EtOAc, followed by water and 10% HCl until strongly acidic. The mixture was extracted with EtOAc, washed with water, dried over Na_2SO_4 (anhydrous) and brought to dryness under reduced pressure. The products were purified by column chromatography on silica gel with 10–20% EtOAc in hexane.

16a-Chloro-7α-methylestra-1,3,5(10)-triene-3,17β-diol (14a): 25 mg, 50%; mp 129 °C; HPLC (70:30 MeOH/H_2O) t_R = 25 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.79 (s, 3 H, 18-CH_3), 0.83 (d, J = 7 Hz, 3 H, 7α-CH_3), 3.71 (d, J = 4 Hz, 1 H, 17α-H), 4.62 (m, 1 H, 16β-H), 6.5 (d, J = 2 Hz, 1 H, 4-CH), 6.64 (dd, J = 2 Hz, 1 H, 2-CH), 7.16 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten), 322 (36), 320 (M^+, 100), 278 (17), 234 (19), 225 (30), 213

(27), 199 (29), 186 (21), 174 (50), 159 (80), 145 (71). Anal. ($C_{19}H_{25}ClO_2$), C, H, Cl.

16α-Chloro-7α-methylestra-1,3,5(10)-triene-3,17α-diol (15a): 10 mg, 20; mp 107–113 °C; HPLC (70:30 MeOH/H_2O) t_R = 25 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.80 (s, 3 H, 18-CH_3), 0.865 (d, J = 7 Hz, 3 H, 7α-CH_3), 3.85 (d, J = 6 Hz, 1 H, 17α-H), 4.11 (m, ddd $J_{16\beta,17\alpha}$ = 4.2 Hz, $J_{16\beta,15\alpha}$ = 2.5 Hz, $J_{16\beta,15\beta}$ = 8.5 Hz, 1 H, 16β-H), 6.55 (d, J = 2 Hz, 1 H, 4-CH), 6.64 (dd, J = 2 and 7 Hz, 1 H, 2-H), 7.14 (d, J = 8 Hz, 1 H, 1-H); MS m/z (rel inten) 322 (36), 320 (M^+, 100), 242 (19), 234 (24), 227 (25), 213 (24), 199 (35), 174 (47), 147 (53). Anal. ($C_{19}H_{25}ClO_2$) C, H, Cl.

16α-Chloro-11β-ethoxyestra-1,3,5(10)-triene-3,17α-diol (15b): 12.2 mg, 18.4%; mp 225–235 °C; HPLC (50:50 THF/H_2O) t_R = 17 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.65 (s, 3 H, 18-CH_3), 0.68 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3) 2.95, 3.30 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.68 (d, J = 2.5 Hz, 1 H, 17β-H), 3.92 (m, 1 H, 11α-H), 4.28 (m, 1 H, 16β-H), 6.16 (d, J = 2 Hz, 1 H, 4-CH), 6.25 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 6.60 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten), 350 (7), 352 (M^+, 15), 304 (11), 269 (5), 251 (9), 212 (21), 197 (10), 172 (16), 157 (36), 146 (100). Anal. ($C_{20}H_{27}ClO_3$) C, H; Cl: calcd, 10.10; found, 9.47.

16α-Chloro-11β-ethoxyestra-1,3,5(10)-triene-3,17β-diol (14b): 22 mg, 33%; mp 228–240 °C; HPLC (50:50 THF/H_2O) t_R = 14 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.71 (s, 3 H, 18-CH_3), 0.76 (t, J = 7 Hz, 3 H, 11β-OCH_2CH_3), 3.03, 3.35 (q, J = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.43 (t, J = 6 Hz, 1 H, 16β-H), 3.86 (dt, J = 1 and 6 Hz, 1 H, 17α-H), 3.94 (m, 1 H, 11α-H), 4.54 (d, J = 5.5 Hz, 1 H, 17β-OH), 6.25 (d, J = 2 Hz, 1 H, 4-CH), 6.34 (dd, J = 2 and 8 Hz, 1 H, 2-CH), 6.68 (d, J = 8 Hz, 1 H, 1-CH); MS m/z (rel inten) 350 (10), 352 (M^+, 26), 304 (7), 251 (7), 225 (8), 212 (20), 185 (13), 172 (27), 157 (42), 146 (100). Anal. ($C_{20}H_{27}ClO_3$) C, H, Cl: calcd, 10.10; found, 9.46.

16β-Chloro-7α-methylestra-1,3,5(10)-triene-3,17β-diol (16a): 20 mg, 68%; mp 104–105 °C; HPLC (70:30 MeOH/H_2O) t_R = 24 min; ^1H NMR ($CDCl_3$ + DMSO-d_6) δ 0.83 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.89 (s, 3 H, 18-CH_3), 3.65 (t, J = 8 Hz, 1 H, 17α-H), 4.53 (dt, J = 6 Hz, 1 H, 16α-H), 6.57 (d, J = 2 Hz, 1 H, 4-CH), 6.65 (dd, J = 2 and 6 Hz, 1 H, 2-CH), 7.12 (d, 1 H, J = 8 Hz, 1-CH); MS m/z (rel inten) 322 (35), 320 (M^+, 100), 248 (13), 227 (35), 213 (30), 199 (33), 186 (23), 171 (58), 159 (83), 145 (92). Further elution with 15% EtOAc in hexane gave 7α-methyl-3,17β-estradiol (20%).

Synthesis of 7α-methyl- and 11β-Ethoxy-16α-[^{125}I]iodoestradiols, [^{125}I]-9a and [^{125}I]-9b, by Halogen Exchange. The radiosynthesis of [^{125}I]-**9a** and [^{125}I]-**9b** was performed by a published method.[18] Two mCi of Na^{125}I in 20 μL of water containing 20 mg of $Na_2S_2O_3$ was added to a reaction vial and evaporated (N_2) close to dryness at 60–65 °C; 200 mL of acetonitrile was added and evaporation was repeated. A solution of either 7α-methyl-16β-bromoestradiol (**8a**, 10 μg) or 11β-ethoxy-16β-estradiol (**8b**, 10 μg) in 20 μL of methanol was added to the reaction vial. The reaction vial was tightly sealed and heated at 105 °C for 90 min; 100 μL of water was added and the reaction mixture was purified by HPLC on a reverse-phase column with EtOH and water. The compound [^{125}I]-**9a** eluted at 22 min and [^{125}I]-**9b** at 24 min in 65:35 and 70:30 MeOH/water, respectively.

Estrogen Receptor Binding Assay. Affinity of the estradiol derivatives for estrogen receptors (ER) was determined by a competitive binding assay[14] and is expressed as the relative binding affinity (RBA). The RBA is defined as 100 times the ratio between competitor and the unlabeled estradiol concentrations required for 50% competition to specific [^{3}H]estradiol binding. Murine uterine cytoplasmic extracts were incubated at 0–4 °C for 18 h with 20 nM of [^{3}H]estradiol in the absence and presence of competitive steroids ranging from 2 nM to 20 μM. The bound steroid was separated from free steroid by Sephadex LH-20 chromatography. The nonspecific binding (equivalent to that observed in the presence of a 100-fold excess of unlabeled estradiol) was 3–4% of the total binding which was subtracted from the total binding to estimate the specific binding. The specific binding (average of three experiments) in the receptor preparation was equivalent to 6.3 nM.

In Vivo Studies. The animal experiments were conducted as previously described[10a,b] and in accordance with the recommendations of the Canadian Council on Animal Care and an in-house Ethics Committee for Animal Experiments. Briefly, immature female Fischer rats, 24 days old, 55–60 g (Charles River), were injected with 200 μL of ^{125}I-labeled **9a** or **9b** (3 μCi, 111 KBq) via the lateral tail vein. The animals were placed in retention cages and therefore not anesthetized during the injection procedure. The radiopharmaceutical was dissolved in ethanol and diluted with sterile physiological saline (0.9% NaCl in H_2O) containing 1% Tween-80 to give a final ethanol concentration of 9%. For the receptor saturation studies, 60 μg of unlabeled estradiol was coinjected with the radiopharmaceutical. Animals were sacrificed under deep ether anesthesia by severing the axillary artery, followed by chest opening.[20] Blood was collected, tissues of interest were removed, washed with 0.154 M KCl, and blotted dry, and samples were weighed. The radioactivity was counted in a Model 1282 Compugamma counter (LKB Wallac, Finland) and concentrations were expressed as % of the injected dose per gram of tissue (%ID/g). Statistical variations are presented as the % coefficient of variation corrected for small sample size effect (%CV).[21]

Acknowledgment. Generous financial support for this work was provided by the Medical Research Council of Canada and the "Programme d'Actions structurantes" of the MESS of Québec. We thank Dr. G. Shyamala from the Lady Davis Institute for Medical Research, Montreal, for conducting the receptor binding assays.

References

(1) (a) Counsell, R. E.; Klausmeier, W. H. In *Principles of Radiopharmacology*; Colombetti, L. G., Ed.; CRC Press: Boca Raton, 1979; Vol. II, pp 59–91. (b) Eckelman, W. C.; Reba, R. C. In *Radiopharmaceuticals: Structure-Activity Relationships*; Spencer, R. P., Ed.; Grune and Stratton: New York, 1981; pp 449–458. (c) Katzenellenbogen, J. A.; Heiman, D. F.; Carlson, K. E. *et al.* In *Receptor-Binding Radiotracers*; Eckelman, W. C., Ed.; CRC Press: Boca Raton, *1982*; Vol. I, pp 93–126.

(2) Hochberg, R. B. Iodine-125-labeled estradiol: a gamma emitting analog of estradiol that binds to the estrogen receptor. *Science* 1979, *205*, 1138–1140.

(3) Hochberg, R. B.; Rosner, W. Interaction of 16α-[^{125}I]iodo-estradiol with estrogen receptor and other steroid-binding protein. *Proc. Natl. Acad. Sci. U.S.A.* 1980, *77*, 328–332.

(4) Lippman, M. E.; Do, H. M. T.; Hochberg, R. B. Specific estrogen receptor binding and biological effects of 16α-iodoestradiol on human breast cancer cells. *Cancer Res.* 1981, *41*, 3150–3154.

(5) Symes, E. K.; Coulson, W. F.; Ralph, D. N. L. The synthesis of 16α-[^{131}I]iodo-estradiol and evaluation of its use as a radiotracer for estrogen receptor positive breast tumors. *J. Steroid Biochem.* 1985, *22*, 155–160.

(6) Hochberg, R. B.; MacLusky, N. J.; Chambers, J.; Eisenfield, A. J.; Naftolin, F.; Schwartz, P. E. Concentration of [16α-^{125}I]iodoestradiol in human ovarian tumors in vivo and correlation with estrogen receptor content. *Steroids* 1985, *46*, 775–788.

(7) Salmon, J.; Coussediere, D.; Cousty, C.; Raynaud, J. P. Pharmacokinetics and metabolism of moxestrol in animals - rat, dog and monkey. *J. Steroid Biochem.* 1983, *19*, 1223–1234.

(8) Grill, H. J.; Moebius, U.; Manz, B.; Pollow, K. 16α-Iodo-3,17β-estradiol: a stable ligand for estrogen receptor determinations in tissue with high 17β-hydroxysteroid dehydrogenase activity. *J. Steroid Biochem.* 1983, *19*, 1887–1688.

(9) (a) Kalvoda, J.; Krahenbuhl, Ch.; Desaulles, P. A.; Anner, G. A. 7α-Methyl oestrogene. *Helv. Chim. Acta* 1967, *50*, 281–288. (b) Bucourt, R.; Vignau, M.; Torelli, V.; Richard-Foy, H.; Geynet, C.; Secco-Millet, C.; Redavilh, G.; Baulieu, E. E. New biospecific adsorbents for the purification of estradiol receptor. *J. Biol. Chem.* 1978, *253*, 8221–8228.

(10) (a) Ali, H.; Rousseau, J.; Ghaffari, M. A.; van Lier, J. E. Synthesis, receptor binding and tissue distribution of (17α,20E)- and (17α,20Z)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol. *J. Med. Chem.* 1988, *31*, 1946–1950. (b) Ali, H.; Rousseau, J.; Ghaffari, M. A.; van Lier, J. E. Synthesis, receptor binding, and tissue distribution of 7α- and 11β-substituted (17α,20E)- and (17α,20Z)-21-[^{125}I]iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diols. *J. Med. Chem.* 1991, *34*, 854–860. (c) Napolitano, E.; Fiaschi, R.; Hanson, R. N. Structure–activity relationship of estrogenic ligands: synthesis and evaluation of (17α,20E)- and (17α,20Z)-21-halo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols. *J. Med. Chem.* 1991, *34*, 2754–2759. (d) Jagoda, E. M.; Gibson, R. E.; Goodgold, H.; Ferreira, N.; Francis, B. E.; Reba, R. C.; Rzeszotarski, W. J.; Eckelman, W. C. [I-125]17α-iodovinyl 11β-methoxyestradiol: in vivo and in vitro properties of a high-affinity estrogen-receptor radiopharmaceutical. *J. Nucl. Med.* 1984, *25*, 472–477. (e) Nakatsuka, I.; Ferreira, N. L.; Eckelman, W. C.; Francis, B. E.; Rzeszotarski, W. J.; Gibson, R. E.; Jagoda, E. M.; Reba, R. C. Synthesis and evaluation of (17α,20E)-21-[I-125]iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol and

(17α,20E)-21-[I-125]iodo-11β-methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (17α-[iodovinyl]estradiol derivatives) as high specific activity potential radiopharmaceuticals. *J. Med. Chem.* **1984**, *27*, 1287–1291.

(11) (a) Zielinski, J. E.; Yabuki, H.; Pahuja, S.; Larner, J. M.; Hochberg, R. B. 16α-[^{125}I]Iodo-11β-methoxy-17β-estradiol: a radiochemical probe for estrogen-sensitive tissues. *Endocrinology* **1986**, *119*, 130–139. (b) Katzenellenbogen, J. A.; McElvany, K. D.; Senderoff, S. G.; Carlson, K. E.; Landvatter, S. W.; Welch, M. J. 16α-[^{77}Br]Bromo-11β-methoxyestradiol-17β: a gamma-emitting estrogen imaging agent with high uptake and retention by target-organs. *J. Nucl. Med.* **1982**, *23*, 411–419.

(12) Johnson, W. S.; Johns, W. F. 14-Isoestrone methyl ether and its identity with totally synthetic material. *J. Am. Chem. Soc.* **1957**, *79*, 2005–2009.

(13) Arunachalan, T.; Caspi, E. Synthesis of seleno estrogens. *J. Org. Chem.* **1981**, *46*, 3415–3420.

(14) Zielinski, J. E.; Larner, J. M.; Hoffter, P. B.; Hochberg, R. B. The synthesis of 11β-methoxy-[16α-^{123}I]iodoestradiol and its interaction with the estrogen receptor in vivo and in vitro. *J. Nucl. Med.* **1989**, *30*, 209–215.

(15) Heiman, D. F.; Stephen, G.; Senderoff, J.; Katzenellenbogen, J. A.; Neeley, R. J. Estrogen receptor based imaging agents. 1. Synthesis and receptor binding affinity of some aromatic and D-ring halogenated estrogens. *J. Med. Chem.* **1980**, *23*, 994–1002.

(16) Schneider, W.; Shyamala, G. Glucocorticoid receptors in primary culture of mouse mammary epithelial cells: characterization and modulation by prolactin and cortisol. *Endocrinology* **1982**, *116*, 2656–2662.

(17) Lévesque, C.; Mérand, Y.; Dufour, J. M.; Labrie, C.; Labrie, F. Synthesis and biological activity of new halo-steroidal antiestrogens. *J. Med. Chem.* **1991**, *34*, 1624–1630.

(18) Longcope, C.; Rafkind, I.; Arunachalam, T.; Caspi, E. Biological activities of 4-fluoroestrogen analogues. *J. Steroid Biochem.* **1983**, *19*, 1325–1328.

(19) Baran, J. S. A synthesis of 11β-hydroxyestrone and related 16- and 17-hydroxyestratrienes. *J. Med. Chem.* **1967**, *10*, 1188–1190.

(20) Waynforth, H. B. In *Experimental and Surgical Technique in the Rat*; Academic: London, 1980.

(21) Sherrer, B. *Biostatistique*; Morin, G., Ed.; Chicoutimi, Quebec, Canada, 1984.