2- and 4-Fluorinated 16α-[^{125}I]Iodoestradiol Derivatives: Synthesis and Effect on Estrogen Receptor Binding and Receptor-Mediated Target Tissue Uptake[1]

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

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

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The effect of 2- and 4-fluoro substitution on the estrogen receptor-mediated tissue localization of radioiodinated 16α-iodoestradiol (16α-IE$_2$) and its 11β-methoxy analogue (11β-OMe-16α-IE$_2$) was evaluated. Electrophilic substitution of estrone or 11β-methoxyestrone with *N*-fluoropyridinium salt gave the 2- and 4-fluoro derivatives which were subsequently converted to the 3,17β-enol diacetate and brominated to yield exclusively the 16α-bromo analogues. Epimerization gave the corresponding 16β-bromoestrones which were reduced to the 17β-hydroxy derivatives. Halogen exchange with NaI or Na[^{125}I]I provided the A-ring fluorinated 16α-iodoestradiols. The 4-F analogue exhibited higher affinity for estrogen receptors than the corresponding 2-F analogue, and these differences were more pronounced at higher incubation temperatures. Biodistribution studies in immature female rats showed that 4-fluoro substitution had only a moderate effect on receptor-mediated tissue uptake of the parent molecules whereas 2-fluoro substitution resulted in strongly diminished tissue specificity. The lower target selectivity of the 2-F, compared to the 4-F, analogue correlates to some extent with their different receptor binding properties; however, the rate of catabolism may also be involved. Differences in blood clearance further accentuated the localization properties to yield particularly high uterus to blood ratios in the case of the 4-F-11β-OMe-16α-IE$_2$, suggesting the potential of the analog labeled with ^{123}I as a radiopharmaceutical for receptor imaging in nuclear medicine. The isopotential maps of the fluorinated steroids, obtained via semiempirical computer modeling on the molecular structures, show striking differences between the 4-F and 2-F derivatives reflecting their varying biological properties.

Introduction

Estrogen analogues labeled with γ-emitting radionuclides have the potential to delineate tissues rich in estrogen receptors (ER). *In vivo* quantification of these receptors could provide significant diagnostic information for the selection of therapeutic approaches in the management of human breast tumors (hormonal *vs* chemotherapeutic or radiation).[2] Several radiolabeled estrogens substituted with various functional groups have been synthesized[3] over the past years and evaluated as radiopharmaceuticals in both preclinical and clinical assays.[4] 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 ER.[5-7] 16α-[^{125}I]-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.[8-10] The usefulness of a γ-emitting 16α-IE$_2$ analogue for *in vivo* ER imaging is, however, limited due to rapid metabolism of this steroid.[11,12] Earlier studies indicate that the receptor binding site tolerates selected substituents at the 7α-, 11β-, 16α-, and 17α-positions of estradiol. Substitution at the 11β-position increases the stability of the ER–steroid complex and reduces nonspecific binding while a 7α-methyl enhances interaction with ER.[13]

The major metabolic routes of estradiol involve A- and D-ring oxidation and/or reduction.[14] Addition of a 11β-methoxy group decreases metabolism of 16α-IE$_2$ while the iodine at C-16 prevents interaction with 17β-hydroxysteroid dehydrogenase,[15] the enzyme which converts estradiol to estrone. Among the estradiols tested, the 11β-OMe-16α-IE$_2$ possess the highest selectivity for ER imaging.[16] The major metabolic A-ring transformation of estradiol occurs at C-2 to give catechol estrogens.[14] Fluorine has been introduced into biologically active molecules to block metabolism while exhibiting very little steric effects on the molecule. Metabolism of estradiol can be slowed down via the introduction of fluorine at C-2. In contrast, the addition of fluorine at C-4 enhances catechol formation.[17] Whereas 4-F addition onto estradiol has little effect on ER binding affinities, 2-F addition results on a marked decrease in receptor affinity.[18]

Radioiodinated 17α-iodovinylestradiol derivatives constitute another class of promising ligands where uptake by ER-rich target tissues has been shown to improve substantially upon 11β- or 7α-substitution.[19] We have recently shown that addition of a 2- or 4-fluoro atom to 17α-iodovinylestradiol (IVE$_2$) derivatives significantly altered the *in vitro* and *in vivo* behavior of the steroid.[20] On account of this, we extended this study to include the effect of 2- and 4-fluorine substitution onto 16α-[^{125}I]IE$_2$ and the 11β-methoxy analogue (11β-OMe-16α-IE$_2$). The ^{125}I-labeled products were prepared via a rapid halogen exchange reaction, and tissue distribution and ER-mediated uterus uptake was established in immature female rats.

Results

Chemistry. A-ring fluorinated 16α-iodinated estradiol derivatives were prepared (Scheme I) in a way similar to that described earlier for the synthesis of 7α-methyl- and 11β-ethoxy-substituted 16α-iodoestradiols.[21] The 2- and 4-fluoroestrone derivatives **2a–d** were prepared using *N*-fluoropyridinium triflate as a fluorinating agent.[22] Compounds **2a–d** were converted to the enol diacetates **3a–d** with isopropenyl acetate in the presence of acid

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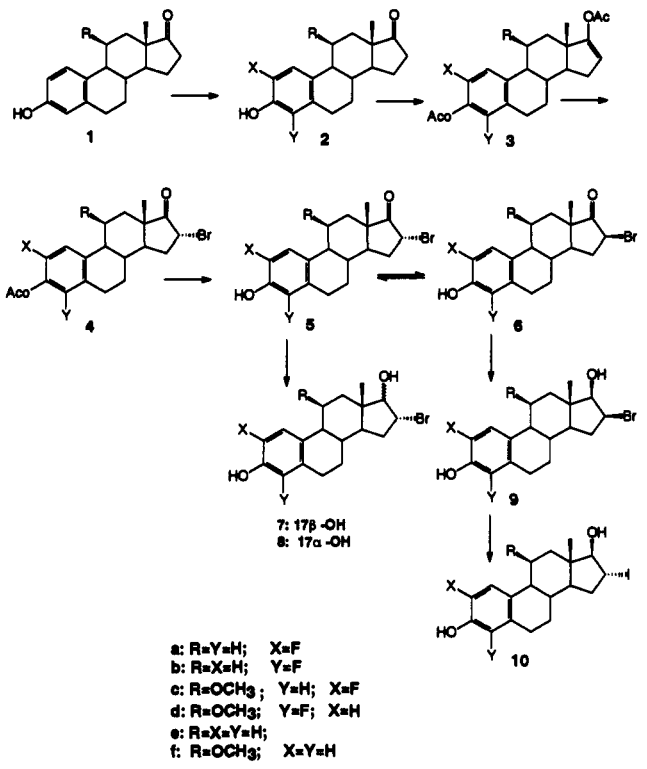
Scheme I



catalyst. Treatment of **3a–d** with Br_2 in acetic acid, according to the procedure of Johnson and Johns,[23] gave exclusively the 16α-bromoestrone 3-acetate derivatives **4a–d**. Acid hydrolysis of the 3-acetate group of **4a–d** with concentrated sulfuric acid in absolute ethanol at room temperature gave the 16α-bromoestrones **5a–d**, which were subsequently converted to the 16β-bromoestrones **6a–d** 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 ^1H NMR.[21] The 16α-bromoestrones **5a–d** were reduced with lithium aluminum hydride to give a mixture of 16α-bromo diols, epimeric at the C-17 position (**7a–d**, **8a–d**). The mass spectra of **7a–d** and **8a–d** showed pairs of molecular ions in about 1:1 ratios indicating the presence of bromine in all compounds. The C17-H was shifted downfield toward the C16-H signal, which is consistent with the *cis* stereochemistry of the C17α-H and the 16-bromo substituent. Reduction of the 16β-bromo derivatives **6a–d** with sodium borohydride in ethanol yielded the 16β-bromo-17β-estradiols **9a–d** along with the debrominated products. The 16α-iodoestradiol derivatives **10a–d** were obtained by halogen exchange of the 16β-bromo in **9a–d** using NaI. Radioiodinated **10a–d** were obtained by the halogen-exchange reaction (2 h) using Na[^{125}I]I (60–75% yield). The compounds were purified by HPLC on a reversed-phase column in MeOH/H_2O.

Biological Properties

Immature female Long Evans rats were injected via the tail vein with HPLC-purified 16α-[^{125}I]IE_2 substituted either with 2-F (**10a**) or 4-F (**10b**) and the corresponding 11β-OMe-16α-[^{125}I]IE_2 likewise substituted with 2-F (**10c**) or 4-F (**10d**). Each animal received 3 μCi (111 KBq) of the radioiodinated steroids in 200 μL of 9% ethanol–saline containing 1% Tween-80. Animals were sacrificed at 1, 3, 5, 12, and 24 h postinjection and dissected, and tissue samples were counted for ^{125}I. The biodistribution and uterus uptake of the ^{125}I-labeled compounds is presented in Table I. Blood clearance (Figure 1), uterus uptake, and uptake ratios (Figure 2) are compared to earlier reported data[16] on the corresponding nonfluorinated 16α-[^{125}I]IE_2 (**10e**) and 11β-OMe-16α-[^{125}I]IE_2 (**10f**). In all cases, the highest uterus radioactivity levels were observed at 1 h postinjection, whereafter uterus radioactivity decreased steadily (Figure 2a). Addition of the 11β-OMe substituent to the 2- and 4-F-16α-IE_2 improved uterus uptake, with the 4-fluorinated steroids giving higher values as compared to the 2-fluorinated analogues (Figure 2a). The uterus to blood (Figure 2b) and nontarget (Figure 2c) ratios follow a similar pattern, with the 4-F-11β-OMe-16α-IE_2 (**10d**) giving the highest ratios. Receptor-mediated uterine uptake was evaluated in the 1 h time group by blocking ER via coinjection with 60 μg of unlabeled estradiol (Table I, 1 + E_2). Uterus uptake of the 11β-OMe compounds **10c,d** decreased by 85–94% in the presence of nonradioactive estrogen confirming high specificity for the ER. In the case of the 2-F-16α-IE_2 (**10a**) the effect of added E_2 was less pronounced (58% decrease), reflecting substantial nonspecific binding. Radioactivity uptake in the nontarget tissues was not affected by the coinjection of unlabeled estradiol. Estrogen receptor binding properties were determined for the 11β-OMe derivatives only, e.g., the analogues which showed good uterus retention. The relative binding affinities (RBA) were performed at two different incubation temperatures (0 °C and room temperature) using lamb cytosol (Table II).

Discussion

In this paper we report the synthesis of 2- and 4-fluoro-16α-iodoestradiols and their 11β-OMe derivatives. The analogous ^{125}I-labeled derivatives were obtained via the halogen-exchange method (16β-Br → 16α-I). The entire radiochemical synthesis, including purification, can be completed within 4–6 h to give the radioiodinated estrogens in 65–85% radiochemical yield. After HPLC purification, the radiochemical purity of [^{125}I]**10a–d** was in excess of 95%. The identities of the products were confirmed by their chromatographic mobilities (HPLC) which were identical to those of the corresponding unlabeled analogues. On the basis of the specific activity of the nca Na[^{125}I]I employed (2125 mCi/mmol) the specific activity of these compounds should exceed 500 Ci/mmol. The reversed-phase HPLC system readily separates the 16α-iodo products from the 16β-bromo reactants, as well as possible products from side reactions, such as the 17-ketosteroids formed by dehydrohalogenation and the 16α-bromosteroids resulting from epimerization. Sodium thiosulfate was added to the ^{125}I-labeled solution to reduce free iodine to I^-, and benzoic acid was added to buffer the reaction mixture to protect the alkali-sensitive bromohydrins. This rapid synthesis could readily be adapted for use with the medicinally important ^{123}I isotope ($t_{1/2}$ = 13.3 h) to yield radiopharmaceuticals for SPECT imaging.

The receptor binding affinities of the 2- and 4-F-11β-OMe-16α-IE_2 (**10c** and **10d**) (Table II) show that the 4-F analogue has higher affinity for ER as compared to the 2-F analogue (RBA = 23 vs 17, at 0 °C) and that these differences are further accentuated when the incubation temperature is raised to room temperature (RBA = 43 vs 14, at 25 °C). The 90% increase in the RBA in the case of the 4-F analogue suggests that dissociation of the 4-F analogue **10d** from the ER–steroid complex is slow or,

Table I. Tissue Distribution of 2- and 4-Fluorinated 16α-[^{125}I]Iodoestradiol Derivatives in Immature Female Long Evans Rats

tissue	% ID/g (SE)[a] 1 h	(1 + E_2)	3 h	5 h	12 h	24 h
			2-F-16α-[^{125}I]IE_2 (**10a**)			
uterus	0.46 (0.06)	0.19 (0.01)	0.13 (0.02)	0.08 (0.01)	0.04 (0.01)	0.02 (0.01)
blood	0.31 (0.04)	0.26 (0.08)	0.20 (0.04)	0.18 (0.04)	0.08 (0.02)	0.04 (0.01)
thyroid	57.96 (9.47)	56.72 (5.86)	149.73 (10.26)	240.49 (20.54)	423.43 (48.15)	440.21 (44.87)
muscle	0.14 (0.01)	0.15 (0.02)	0.04 (0.00)	0.03 (0.01)	0.02 (0.00)	0.01 (0.00)
fat	1.23 (0.08)	1.07 (0.24)	0.47 (0.08)	0.14 (0.03)	0.04 (0.01)	0.02 (0.00)
kidneys	0.54 (0.11)	0.46 (0.11)	0.26 (0.06)	0.20 (0.03)	0.08 (0.02)	0.04 (0.00)
spleen	0.10 (0.03)	0.14 (0.02)	0.07 (0.01)	0.06 (0.01)	0.03 (0.01)	0.02 (0.00)
lungs	0.28 (0.05)	1.39 (0.14)	0.10 (0.02)	0.10 (0.02)	0.04 (0.01)	0.03 (0.00)
liver	1.84 (0.16)	1.48 (0.31)	0.24 (0.23)	1.22 (0.24)	0.89 (0.09)	0.60 (0.09)
uterus/blood	1.53 (0.14)	0.86 (0.22)	0.71 (0.15)	0.43 (0.03)	0.48 (0.05)	0.62 (0.07)
uterus/nontarget[b]	2.92 (0.63)	0.96 (0.21)	1.94 (0.26)	1.25 (0.06)	1.20 (0.19)	1.25 (0.25)
			4-F-16α-[^{125}I]IE_2 (**10b**)			
uterus	2.70 (0.10)	0.40 (0.05)	1.03 (0.12)	0.56 (0.06)	0.15 (0.03)	0.02 (0.00)
blood	0.37 (0.04)	0.47 (0.13)	0.29 (0.01)	0.21 (0.02)	0.11 (0.02)	0.03 (0.00)
thyroid	43.73 (5.76)	28.88 (4.52)	101.59 (20.13)	129.36 (9.57)	23.75 (6.52)	352.36 (44.40)
muscle	0.14 (0.02)	0.14 (0.01)	0.06 (0.00)	0.04 (0.00)	0.03 (0.00)	0.01 (0.00)
fat	1.51 (0.28)	1.00 (0.09)	0.32 (0.07)	0.15 (0.02)	0.04 (0.00)	0.01 (0.00)
kidneys	0.41 (0.06)	0.51 (0.10)	0.37 (0.04)	0.23 (0.03)	0.11 (0.02)	0.04 (0.00)
spleen	0.17 (0.00)	0.17 (0.01)	0.10 (0.01)	0.08 (0.00)	0.10 (0.02)	0.03 (0.00)
lungs	0.24 (0.02)	0.30 (0.03)	0.15 (0.01)	0.11 (0.01)	0.06 (0.01)	0.02 (0.00)
liver	0.53 (0.03)	0.65 (0.12)	0.53 (0.01)	0.40 (0.06)	0.26 (0.05)	0.11 (0.01)
uterus/blood	7.40 (0.70)	0.92 (0.12)	3.50 (0.40)	2.80 (0.27)	1.33 (0.09)	0.73 (0.07)
uterus/nontarget[b]	14.55 (0.93)	1.93 (0.12)	9.95 (0.70)	7.39 (0.89)	2.36 (0.25)	0.82 (0.12)
			2-F-11β-OMe-16α-[^{125}I]IE_2 (**10c**)			
uterus	4.72 (0.33)	0.30 (0.03)	2.81 (0.40)	2.34 (0.33)	0.73 (0.06)	0.13 (0.05)
blood	0.24 (0.02)	0.27 (0.03)	0.24 (0.01)	0.29 (0.02)	0.16 (0.03)	0.04 (0.01)
thyroid	107.92 (24.31)	69.54 (17.20)	309.91 (53.02)	344.85 (42.08)	1281.42 (76.85)	535.76 (125.61)
muscle	0.23 (0.05)	0.30 (0.03)	0.23 (0.09)	0.19 (0.01)	0.16 (0.03)	0.03 (0.01)
fat	1.91 (1.09)	0.87 (0.10)	0.81 (0.31)	0.28 (0.05)	0.19 (0.04)	0.12 (0.03)
kidneys	0.45 (0.04)	0.47 (0.08)	0.44 (0.00)	0.52 (0.12)	0.21 (0.04)	0.05 (0.01)
spleen	0.56 (0.19)	0.38 (0.07)	0.38 (0.05)	0.65 (0.22)	0.24 (0.08)	0.03 (0.00)
lungs	0.25 (0.02)	0.28 (0.03)	0.19 (0.02)	0.23 (0.02)	0.12 (0.02)	0.31 (0.02)
liver	1.52 (0.03)	1.27 (0.07)	1.56 (0.35)	1.30 (0.29)	0.78 (0.11)	4.29 (1.43)
uterus/blood	19.79 (2.69)	1.11 (0.05)	11.50 (1.46)	7.91 (0.83)	4.84 (0.67)	3.07 (0.76)
uterus/nontarget[b]	14.47 (1.61)	0.96 (0.13)	10.40 (0.89)	6.93 (1.31)	4.48 (0.89)	4.29 (1.43)
			4-F-11β-OMe-16α-[^{125}I]IE_2 (**10d**)			
uterus	5.46 (0.64)	0.32 (0.03)	3.67 (0.55)	4.05 (0.60)	3.19 (0.54)	2.26 (0.28)
blood	0.27 (0.02)	0.30 (0.02)	0.21 (0.02)	0.20 (0.03)	0.10 (0.01)	0.05 (0.01)
thyroid	63.83 (11.87)	64.03 (5.75)	202.81 (39.73)	384.70 (47.05)	653.00 (86.58)	708.62 (35.97)
muscle	1.08 (0.72)	2.06 (1.84)	0.18 (0.00)	0.25 (0.07)	0.12 (0.06)	0.05 (0.01)
fat	3.54 (1.63)	1.62 (1.11)	0.93 (0.48)	0.35 (0.18)	0.24 (0.13)	0.07 (0.01)
kidneys	0.80 (0.09)	0.46 (0.07)	0.46 (0.06)	0.33 (0.00)	0.35 (0.21)	0.09 (0.02)
spleen	0.46 (0.04)	0.43 (0.07)	0.07 (0.23)	0.26 (0.06)	0.16 (0.03)	0.10 (0.02)
lungs	0.32 (0.03)	0.32 (0.03)	0.23 (0.01)	0.19 (0.02)	0.09 (0.01)	0.04 (0.01)
liver	0.85 (0.02)	0.93 (0.24)	0.81 (0.23)	0.56 (0.04)	0.35 (0.02)	0.21 (0.40)
uterus/blood	20.76 (3.39)	1.08 (0.04)	18.46 (4.19)	21.81 (6.07)	33.78 (9.97)	48.83 (17.02)
uterus/nontarget[b]	14.71 (2.54)	0.73 (0.31)	10.83 (2.30)	19.02 (5.07)	24.99 (2.03)	37.03 (8.82)

[a] Mean organ uptake (% injected dose per gram of tissue) and standard error (SE) for immature female Long Evans rats three to four animals) after injection of 3 μCi (111 kBq) of [^{125}I]**10a–d** in the presence (+E_2) or absence of 60 μg of coinjected estradiol. [b] Nontarget organs include the muscle, spleen, and lungs.

alternatively, that a high affinity metabolite is formed. In contrast, the RBA of the 2-F analogue **10c** decreased by 15% at higher incubation temperature suggesting instability of the ER–steroid complex or the formation of low-affinity metabolites. Similar patterns were observed with the 2- and 4-fluoro derivatives of (17α,20*E*/*Z*)iodovinylestradiols.[20]

Comparisons of the biodistribution pattern in female rats of our 2- and 4-fluorinated 16α-IE_2 with earlier reported data on the nonfluorinated analogues[16] were made (Figures 1 and 2). Striking differences between the effect of 2-F and 4-F substitution on the uterus uptake pattern are observed, particularly in the case of the 11β-OMe-16α-IE_2 (Figure 2). The highest uterus uptake values were observed with the nonfluorinated 11β-OMe-16α-IE_2 (**10f**) although target to nontarget ratios were similar to those observed with the 4-F analogue **10d**. However, the earlier reported uterus uptake studies on the nonfluorinated compounds were done in Sprague–Dawley rats whereas our present studies were done in Long Evans rats. Differences in the organ uptake levels between the species, due to different receptor and transport protein levels as well as metabolic enzyme activities, can be expected and uptake ratios appear to be a better parameter for comparative studies. Addition of a 2-F or 4-F substituent reduces uterus uptake substantially (Figure 2a), although the 4-F-11β-OMe-16α-IE_2 (**10d**) reaches similar uterus concentrations as the nonfluorinated **10f** at the 24-h time point. At 1 h postinjection the 2-F and 4-F derivatives **10c** and **10d** exhibit similar uterus uptake values; however, **10c** was rapidly released from the target tissue to reach background levels at 24 h postinjection.

In most cases uterus to blood and nontarget tissue ratios were lower for the fluorinated as compared to the nonfluorinated 16α-IE_2, with the exception of the 4-F-11β-OMe-16α-IE_2 (**10d**) which gave the highest uterus to

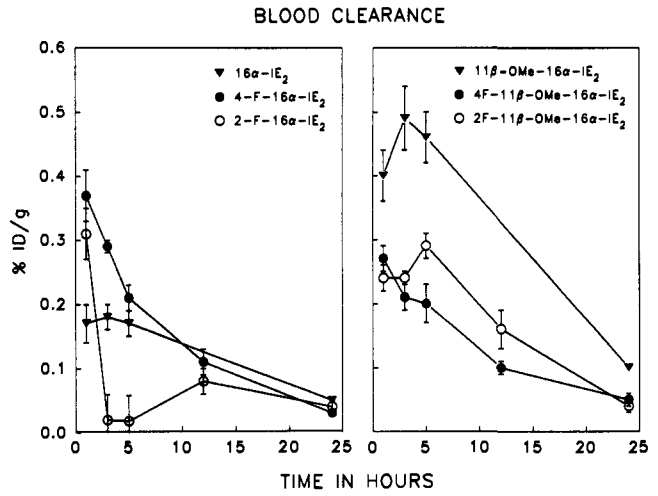


Figure 1. Blood clearance in %ID/g of ^{125}I-labeled 2- and 4-F-16α-IE$_2$ (**10a,b**) and the corresponding 11β-methoxy derivatives **10c,d** in immature female rats. The error bars represent the standard error. Values for the nonfluorinated derivatives were taken from the literature.[16a]

blood ratios among all compounds tested (Figure 2b). The same analogue also gave good uterus to nontarget (>25) and uterus to liver (>10) ratios (Figure 2c,d). These observations can be explained by examining the blood clearance profiles (Figure 1). 4-F addition to 11β-OMe-16α-IE$_2$ results in augmented blood clearance accounting for the favorable uterus to blood ratios of **10d**. Addition of 2-F to either the 11β-OMe-16α-IE$_2$ or 16α-IE$_2$ to yield **10c** or **10a** resulted in diminished target specificity (Figure 2). This lack of specificity in the case of **10c** correlates with its low ER binding affinity observed at room temperature (Table II). A similar effect on the biodistribution pattern was observed upon 2- and 4-F addition to the (17α,20*E*/*Z*)-IVE$_2$ derivatives.[20] The C-2 position is involved in the principal metabolic pathway of the A-ring of estrogens to yield the catechol derivatives, and its availability thus appears of importance for optimal pharmacokinetics. The *in vivo* stability of the iodosteroids also plays a role in the localization process, and this can be assessed from the uptake of radioiodine by the thyroid (Table I). Similar thyroid retention profiles are observed for the 2-F and 4-F analogues suggesting that this parameter is not involved in the observed differences in biodistribution pattern.

In order to explain the dramatic differences in the biological effects between the 4-F and 2-F additions onto the 11β-OMe-16α-IE$_2$ at the molecular level, we evaluated a number of physicochemical parameters of the molecular structures, using semiempirical computer modeling. In general, the optimized molecular geometries of the A, B, C ring-aligned structures of 16α-IE$_2$ (**10e**), 11β-OMe-16α-IE$_2$ (**10f**), 2- and 4-F-11β-OMe-16α-IE$_2$ (**10c** and **10d**) do not show any significant conformational changes, apart from a 0.1–0.2-Å disalignment of the D-ring in the case of the 11-OMe-substituted steroids **10c,d,f**. Comparison of the steric effects on a VDW space-filling model shows that the addition of the 16α-I onto E$_2$ to yield 16α-IE$_2$ increases the overall molecular volume by 10.9% (282.2 Å^3 vs 254.4 Å^3). Further substitution of the 16α-IE$_2$ with either the 2-F or 4-F, to yield **10a** (284.9 Å^3) or **10b** (284.2 Å^3), has little effect. Addition of the 11β-OMe to 16α-IE$_2$ to yield **10f** increases the overall volume by an additional 9.4% (308.7 Å^3 vs 282.2 Å^3). As for the 16α-IE$_2$ lacking the 11β-OMe, further substitution of **10f** with either 2-F or 4-F has little effect (**10c**, 310.7 Å^3; **10d**, 310.4 Å^3).

In order to study variations in the electrostatic molecular potential (EMP) we selected the same series of substituted 16α-IE$_2$. Figure 3 shows the EMP maps of **10e**, **10f**, **10c**, and **10d**. Comparison of the 3D-isopotential surfaces surrounding the various substituted estradiols reveals some striking differences which can be correlated with the different biological properties. Thus, the shape of the positive isopotential maps (Figure 3, yellow lines) of 16α-IE$_2$ (**10e**), 11β-OMe-16α-IE$_2$ (**10f**), and 4-F-11β-OMe-16α-IE$_2$ (**10d**) are very similar whereas the map of the 2-F-11β-OMe-16α-IE$_2$ (**10c**) shows a distinct shift in the positive electrostatic potential distribution surrounding the molecule. 2-F substitution induces the formation of two separate positive cavities about the A- and C-rings, whereas the 4-F substituent provides a marked increase of the negative isopotential surface in synergy with the existing isopotential surfaces surrounding the C-3 OH. There are two negative minima in **10e** (Figure 3, red lines) due to the oxygen atoms at C-3 and C-17. The minimum at C-3 is located largely over the aromatic A-ring. In the case of the compounds featuring the 11β-OMe substituent (**10f**) this negative surface is extended up to the C-ring. Further substitution with 4-F to yield **10d** does not significantly change this pattern but shows an increase of the minima outward from C-3 and C-4. In contrast, 2-F substitution to yield **10c** spreads the negative minima, extending from C-1 to C-4, creating a totally different pattern (Figure 3). The same effect of 2-F or 4-F substitution on the EMP is observed in the case of the 16α-IE$_2$, i.e., the analog lacking the 11β-OMe (data not shown). These changes correlate well with the loss in uptake of the modified 2-F-estradiols by ER-rich target tissues, suggesting the importance of charge homogeneity surrounding the hormone for optimal interaction with receptors and catabolic enzymes.

In conclusion, our data show that 4-F substituted 16α-IE$_2$ and 11β-OMe-16α-IE$_2$ exhibit higher affinity for ER and better ER-mediated target tissue uptake as compared to the 2-F substituted analogues. Since it is well known[17] that catechol formation is retarded by 2-F substitution, whereas 4-F substitution enhances this matabolic pathway, our findings support an intimate role of catechol formation in the tissue localization process. Furthermore, these varying biological activities between 4-F and 2-F derivatives appears to be reflected in characteristic differences between the isopotential maps of these closely related molecular structures.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. ^{1}H NMR spectra were taken in chloroform, with or without deuterated dimethyl sulfoxide, on a Bruker WM 25 spectrometer using Me$_4$Si as an integral standard, with chemical shift values expressed in ppm (δ) relative to the standard and coupling constants (*J*) in Hz. Mass spectra were obtained on a Hewlett-Packard Model 5988A quadrupled mass spectrometer. The relative intensity of the salient fragment ions to the base peak (100) is given in parentheses. All chemicals used are commercially available and were of the highest chemical grade available; nca Na[^{125}I]I was purchased from Amersham Canada Ltd. Steroids were purchased from Steraloids Inc. Column chromatography was performed using silica gel (60–200 mesh). Microanalysis data were obtained by Guelph Laboratories Ltd., Canada.

Analytical thin-layer chromatography (TLC) was routinely used to check homogeneity of all the compounds by silica gel

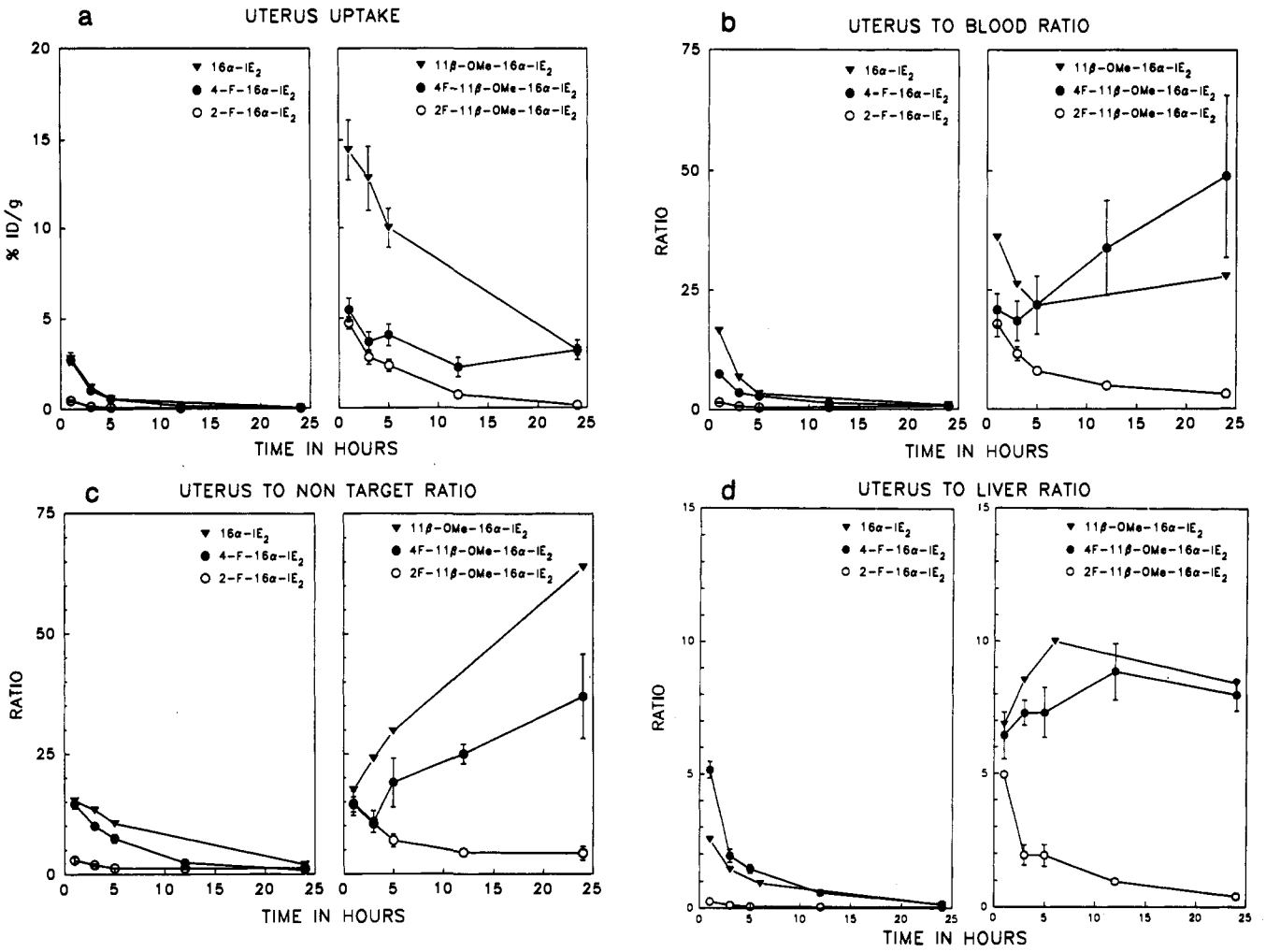


Figure 2. Uterus uptake ^{125}I-labeled 2- and 4-F-16α-IE$_2$ (**10a,b**) and the corresponding 11β-methoxy derivatives **10c,d** in immature female Long Evans rats. The vertical bars represent the standard error. Values for the nonfluorinated derivatives were taken from the literature.[16a] (a) Uterus radioactivity in % ID/g. (b) Uterus to nontarget (lungs, spleen and muscle) ratios. (c) Uterus to blood ratios. (d) Uterus to liver ratios.

Table II. Relative Binding Affinities[a] of Estradiol Derivatives for Lamb Cytosol Estrogen Receptors

compd		relative binding affinity	
no.	substituents	0 °C	room temp
10c	2-F-11β-OMe-16αIE$_2$	17.2 (± 3.3)	14.2 (± 0.9)
10d	4-F-11β-OMe-16αIE$_2$	23.2 (± 4.5)	43.4 (± 11.2)

[a] The relative binding affinity (RBA) were determined relative to estradiol (RBA = 100). Various concentrations of unlabeled **10c** and **10d** were incubated with [^{3}H]estradiol in lamb uterine cytosol at 0 °C and room temperature.[32]

plates coated with fluorescent indicator (UV 254) and also by high-performance liquid chromatography (HPLC). The compounds were located by their UV absorbance and/or color response upon spraying with H_2SO_4/EtOH and heating at 120 °C. HPLC was performed on a reversed-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. 17-Ethylenedioxyestra-1,3,5(10)-triene-3-11β-diol was synthesized according to Baran[24] and converted to 11β-methoxy-3-hydroxyestra-1,3,5(10)trien-17-one with methyl iodide.

Estra-1,3,5(10),16-tetraene-3,17-diol Diacetate Derivatives 3. A solution of **2** (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 anhydrous Na_2SO_4, 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 **3**.

2-Fluoroestra-1,3,5(10),16-tetraene-3,17-diol diacetate (3a) and **4-fluoroestra-1,3,5(10),16-tetraene-3,17-diol diacetate (3b)** as mixture: MS *m/z* (relative intensity) 372 (M^+, 5), 330 ($M^+ - CH_2CO$, 26), 287 ($M^+ - 2\ CH_2CO$, 100), 272 (40).

2-Fluoro-11β-methoxyestra-1,3,5(10),16-tetraene-3,17-diol diacetate (3c): ^{1}H NMR 1.12 (s, 3H, 18-CH$_3$), 2.0 (s, 3H, 3-OCOCH$_3$), 2.13 (s, 3H, 17-OCOCH$_3$, 3.27 (s, 3H, 11β-OCH$_3$), 5.52 (dd, 1H, J = 1.5 and 3.6 Hz, 16-H), 6.73 (d, 1H, J = 8.5 Hz, C4-H), 6.83 (d, 1H, J = 12 Hz, C1-H); MS *m/z* (relative intensity) 402 (M^+, 38), 360 ($M^+ - CH_2CO$, 88), 317 ($M^+ - CH_2CO$, 100), 299 (42), 259 (34).

4-Fluoro-11β-methoxyestra-1,3,5(10),16-tetraene-3,17-diol diacetate (3d): ^{1}H NMR 1.14 (s, 3H, 18-CH$_3$), 2.19 (s, 3H, 3-OCOCH$_3$), 2.30 (s, 3H, 17-OCOCH$_3$), 3.28 (s, 3H, 11β-OCH$_3$), 5.48 (dd, 1H, J = 1.5 and 3.6 Hz, 16-H), 6.90 (m, 2H, C1 and C2-H); MS *m/z* (relative intensity) 402 (M^+, 29), 360 ($M^+ - CH_2CO$, 68), 31 ($M^+ - 2CH_2CO$, 100), 299 (70), 286 (81).

2- and 4-Fluoro-16α-bromo-3-acetoxyestra-1,3,5(10)-trien-17-one Derivatives 4. A solution of **3** (0.2 mmol) in ether (4 mL) and acetate buffer (4 mL, 85% acetic acid–200 mg 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 Br_2 and 6.75 mL glacial acetic acid) with continuous stirring, and then the solution was stirred for an additional 10–15 min. The reaction was terminated by the addition of water, and then the

organic phase was washed with water, 5% sodium thiosulfate, and 5% sodium bicarbonate, dried over sodium sulfate (anhydrous), filtered, and evaporated to dryness to afford 4.

2-Fluoro-16α-bromo-3-acetoxyestra-1,3,5(10)-trien-17-one (4a): mp 140–142 °C; HPLC 85:15 (hexane/EtOAc) 20 min; ^{1}H NMR 0.946 (s, 3H, 18-CH_3), 2.32 (s, 3H, 3-$OCOCH_3$), 4.58 (dd, 1H, J = 1.5 and 5 Hz, 16β-H), 6.83 (d, 1H, J = 8.5 Hz, C4-H), 7.09 (d, 1H, J = 12 Hz, C1-H); MS m/z (relative intensity) 410 (M^+, 3), 408 (M^+, 3), 368 ($M^+ - CH_2CO$, 92), 36 ($M^+ - CH_2CO$, 100), 337 (4), 287 (15), 232 (87).

4-Fluoro-16α-bromo-3-acetoxyestra-1,3,5(10)-trien-17-one (4b): HPLC 85:15 (hexane/EtOAc) 18 min; ^{1}H NMR 0.945 (s, 3H, 18-CH_3), 2.33 (s, 3H, 3-$OCOCH_3$), 4.58 (dd, 1H, J = 1.5 and 5 Hz, 16β-H), 6.92 (t, 1H, J = 8 Hz, C2-H), 7.05 (d, 1H, J = 8 Hz, C1-H); MS m/z (relative intensity) 410 (M^+, 4), 408 (M^+, 3), 368 ($M^+ - CH_2CO$, 84), 366 ($M^+ - CH_2CO$, 82), 337 (4), 287 (20), 232 (100).

2-Fluoro-16α-bromo-3-acetoxy-11β-methoxyestra-1,3,5(10)-trien-17-one (4c): ^{1}H NMR 1.14 (s, 3H, 18-CH_3), 2.31 (s, 3H, 3-$OCOCH_3$), 3.3 (s, 3H, 11β-OCH_3), 4.14 (m, 1H, 11α-H), 4.62 (dd, 1H, J = 1.5 and 5 Hz, 16β-H), 6.84 (d, 1H, J = 7.9 Hz, C4-H), 6.92 (d, 1H, J = 12 Hz, C1-H); MS m/z (relative intensity) 442 (M^+, 12), 440 (M^+, 12), 361 (67), 339 (67), 358 (12), 316 (100).

4-Fluoro-16α-bromo-3-acetoxy-11β-methoxyestra-1,3,5(10)-trien-17-one (4d): ^{1}H NMR 0.98 (s, 3H, 18-CH_3), 2.15 (s, 3H, 3-$OCOCH_3$), 3.13 (s, 3H, 11β-OCH_3), 4.08 (m, 1H, 11α-H), 4.54 (dd, 1H, J = 1.5 and 5 Hz, 16β-H), 6.75 (brs, 2H, C1 and C2-H); MS m/z (relative intensity) 442 (M^+, 8), 440 (M^+, 9), 397 (29), 395 (30), 358 (11), 316 (100), 285 (69).

Acid Hydrolysis of 4. 2- and 4-fluoro-16α-bromoestrone acetate derivatives 4 (0.74 mmol) were 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 5.

2-Fluoro-16α-bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (5a): mp 180–182 °C; HPLC 85:15 (hexane/EtOAc) 22 min; ^{1}H NMR 0.84 (s, 3H, 18-CH_3), 4.50 (dd, 1H, J = 1.8 and 5.4 Hz, 16β-H), 6.57 (d, 1H, J = 9.2 Hz, C4-H), 6.82 (d, J = 13.4 Hz, 1H, C1-H); MS m/z (relative intensity) 368 (M^+, 68), 366 (M^+, 69), 338 (15), 287 (M^+ - HBr, 26), 232 (100). Anal. ($C_{18}H_{20}BrFO_2$) C, H, Br.

4-Fluoro-16α-bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (5b): mp 185–190 °C; HPLC 50:50 (THF/H_2O) 23 min; ^{1}H NMR 0.95 (s, 3H, 18-CH_3), 4.57 (dd, 1H, J = 1.9 and 5.4 Hz, 16β-H), 6.81 (t, 1H, J = 8.7 Hz, C2-H), 6.94 (d, J = 8 Hz, 1H, C1-H); MS m/z (relative intensity) 368 (M^+, 97), 366 (M^+, 100), 305 (10), 287 (M^+ - HBr, 42). Anal. ($C_{18}H_{20}BrFO_2$) C, H, Br.

2-Fluoro-16α-bromo-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (5c): mp 233–235 °C; HPLC 80:20 (hexane/EtOAc) 22 min; ^{1}H NMR 1.02 (s, 3H, 18-CH_3), 3.22 (s, 3H, 11β-OCH_3), 3.98 (m, 1H, 11α-H), 4.55 (dd, 1H, J = 1.4 and 5 Hz, 16α-H), 6.58 (d, 1H, J = 8 Hz, C4-H), 6.72 (d, 1H, J = 12 Hz, C1-H); MS m/z (relative intensity) 398 (M^+, 43), 396 (M^+, 42), 316 (M^+ - HBr, 100), 285 (95). Anal. ($C_{19}H_{22}BrFO_3$) C, H, Br.

4-Fluoro-16α-bromo-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (5d): mp 265–270 °C; HPLC 80:20 (hexane/EtOAc) 25 min; ^{1}H NMR 1.08 (s, 3H, 18-CH_3), 3.37 (s, 3H, 11β-OCH_3), 4.16 (m, 1H, 11α-H), 4.61 (dd, 1H, J = 1.4 and 5 Hz, 16α-H), 6.2–6.35 (m, C1 and C2-H); MS m/z (relative intensity) 398 (M^+, 31), 396 (M^+, 33), 316 (M^+ - HBr, 100), 285 (92). Anal. ($C_{18}H_{22}BrFO_3$) C, H, Br.

Reduction of 2- and 4-Fluoro-16α-bromo-3-hydroxyestra-1,3,5(10)-trien-17-one Derivatives (5). A solution of 5 (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 anhydrous Na_2SO_4, 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 7 and 8.

2-Fluoro-16α-bromoestra-1,3,5(10)-triene-3,17α-diol (8a): HPLC 15:85 (EtOAc/hexane) 13.7 min; ^{1}H NMR 0.79 (s, 3H, 18-CH_3), 3.66 (d, 1H, J = 4.5 Hz, 17β-H), 4.68 (m, 1H, 16β-H), 6.70 (d, J = 9.2 Hz, 1H, C4-H), 6.98 (d, J = 9.2 Hz, 1H, C1-H); MS m/z (relative intensity) 370 (M^+, 98), 368 (M^+, 100), 290 (M^+ - HBr, 30), 269 (50).

2-Fluoro-16α-bromoestra-1,3,5(10)-triene-3,17β-diol (7a): mp 200–203 °C; HPLC 15:85 (EtOAc/hexane) 26 min; ^{1}H NMR 0.68 (s, 3H, 18-CH_3), 3.86 (d, 1H, J = 7 Hz, 17α-H), 4.08 (m, 1H, 16β-H), 6.58 (d, J = 9.2 Hz, C4-H), 6.84 (d, J = 13 Hz, C1-H); MS m/z (relative intensity) 370 (M^+, 97), 368 (M^+, 100), 291 (M^+ - HBr, 6) 271 (17).

4-Fluoro-16α-bromoestra-1,3,5(10)-triene-3,17α-diol (8b): mp 210–212 °C (lit.[18c] mp 242–244 °C); HPLC 15:85 (EtOAc/hexane) 30 min; ^{1}H NMR 0.42 (s, 3H, 18-CH_3), 3.28 (t, J = 2.0 Hz, 1H), 3.57 (t, 1H, J = 6.6 Hz, 17β-H), 4.60 (d, 1H, 16β-H), 6.40 (t, J = 8.6 Hz, C2-H), 6.51 (d, J = 8.6 Hz, C1-H); MS m/z (relative intensity) 370 (M^+, 98), 368 (M^+, 100), 291 (M^+ - HBr, 26).

4-Fluoro-16α-bromoestra-1,3,5(10)-triene-3,17β-diol (7b): mp 175–178 °C (lit.[18c] mp 214–216 °C); HPLC 85:15 (EtOAc/hexane) 30 min; ^{1}H NMR 0.92 (s, 3H, 18-CH_3), 3.46 (t, 1H, J = 9.3 Hz, 17α-H), 4.66 (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, 1H, 16β-H), 6.80 (t, J = 8.7 Hz, C1-H), 6.94 (d, J = 8.7 Hz, C2-H); MS m/z (relative intensity) 370 (M^+, 98), 368 (M^+, 100), 288 (M^+ - HBr, 37), 271 (62), 232 (89).

4-Fluoro-16α-bromo-11β-methoxyestra-1,3,5(10)-triene-3,17α-diol (8d): mp 295 °C; HPLC 70:30 (MeOH/H_2O) 20 min; ^{1}H NMR 1.04 (s, 3H, 18-CH_3), 3.18 (s, 3H, 11β-OCH_3), 3.35 (d, 1H, J = 9 Hz, 17β-H), 3.95–4.05 (m, 1H, 11α-H), 4.5 (m, 1H, 16β-H), 6.65–6.70 (m, 2H, C1 and C2-H); MS m/z (relative intensity) 400 (M^+, 24), 398 (M^+, 25), 318 (M^+ - HBr, 23), 286 (59), 215 (100).

4-Fluoro-16α-bromo-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (7d): mp 250–255 °C; HPLC 70:30 (MeOH/H_2O) 22 min; ^{1}H NMR 0.84 (s, 3H, 18-CH_3), 3.15 (s, 3H, 11β-OCH_3), 3.78 (m, 1H, 11α-H, 1H), 3.94 (d, 1H, J = 6.5 Hz, 17α-H), 4.05 (d, 1H, J = 7.8 Hz, 16β-H), 6.58–6.75 (m, 2H, C1-H and C2-H); MS m/z (relative intensity) 400 (M^+, 61), 398 (M^+, 68), 318 (M^+ - HBr, 20), 313 (38), 286 (77), 268 (67), 242 (53), 231 (74), 174 (100).

Epimerization of 16α-Bromo 5 into 16β-Bromo Derivatives 6. A mixture of 16α-bromoestrone (5, 0.383 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 6.

2-Fluoro-16β-bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (6a): mp 191–195 °C; HPLC 85:15 (hexane/EtOAc) 18 min; ^{1}H NMR 0.95 (s, 3H, 18-CH_3), 4.15 (t, 1H, J = 8.5 Hz, 16α-H), 5.01 (brs, 1H, –OH), 6.72 (d, 1H, J = 9.1 Hz, C4-H), 6.96 (d, J = 12.4 Hz, 1H, C1-H); MS m/z (relative intensity) 368 (M^+, 97), 366 (M^+, 100), 339 (11), 267 (16). Anal. ($C_{18}H_{20}BrFO_2$) C, H, Br.

4-Fluoro-16β-bromo-3-hydroxyestra-1,3,5(10)-trien-17-one (6b): mp 84–86 °C; HPLC 50:50 (THF/H_2O) 26 min; ^{1}H NMR 0.94 (s, 3H, 18-CH_3), 4.58 (t, 1H, J = 6.5 Hz, 16α-H), 6.8 (t, 1H, J = 8.8 Hz, C2-H), 6.95 (d, 1H, J = 8 Hz, C1-H); MS m/z (relative intensity) 368 (M^+, 97), 366 (M^+, 100), 286 (M^+ - HBr, 22), 233 (100). Anal. ($C_{18}H_{20}BrFO_2$) C, H, Br.

2-Fluoro-16β-bromo-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (6c): mp 190–230 °C dec; HPLC 80:20 (hexane/EtOAc) 18 min; ^{1}H NMR 1.05 (s, 3H, 18-CH_3), 3.09 (s, 3H, 11β-OCH_3), 3.86 (m, 1H, 11α-H), 3.94 (t, 1H, J = 16α-H), 6.44 (d, 1H, J = 7.9 Hz, C4-H), 6.58 (d, 1H, J = 12 Hz, C1-H); MS m/z (relative intensity) 398 (M^+, 26), 396 (M^+, 26), 316 (M^+ - HBr, 47), 285 (74), 163 (100). Anal. ($C_{19}H_{22}BrFO_3$) C, H, Br.

4-Fluoro-16β-bromo-3-hydroxy-11β-methoxyestra-1,3,5(10)-trien-17-one (6d): mp 260–262 °C; HPLC 80:20 (hexane/EtOAc) 23 min; ^{1}H NMR 1.08 (s, 3H, 18-CH_3), 3.08 (s, 3H, 11β-OCH_3), 3.90–4.0 (m, 2H, 11α-H and 16α-H), 6.48–6.62 (m, 2H, C1 and C4-H); MS m/z (relative intensity) 398 (M^+, 24), 396 (M^+, 21), 316 (M^+ - Br, 76), 284 (100). Anal. ($C_{19}H_{22}BrFO_3$) C, H, Br.

Reduction of 2- and 4-Fluoro-16β-bromo-3-hydroxyestra-1,3,5(10)-trien-17-one Derivatives 6. A solution of $NaBH_4$ (80 mg) in EtOH (5 mL) was added to 16β-bromoestrone derivatives 6 (0.22 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

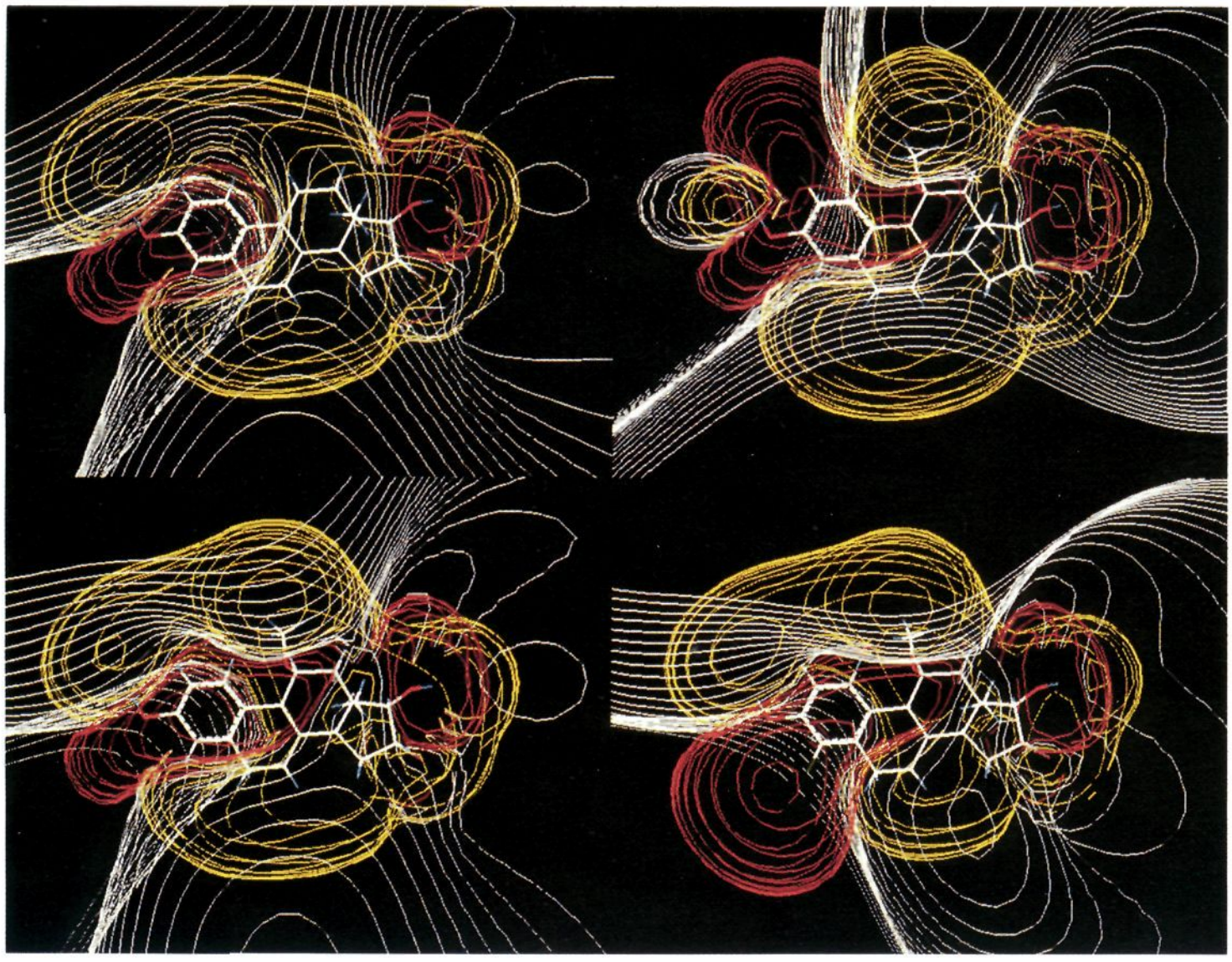


Figure 3. Electrostatic isopotential maps of 16α-IE_2 (**10e**: top left), the 11β-OMe-16α-IE_2 (**10f**: bottom left), the 2-F-11β-OMe-16α-IE_2 (**10c**: top right) and the 4-F-11β-OMe-16α-IE_2 (**10d**: bottom right). Negative fields are presented in red, neutral fields in white, and positive fields in yellow.

EtOAc, washed with water, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography on silica gel with 10% EtOAc in hexane to yield **9**.

2-Fluoro-16β-bromoestra-1,3,5(10)-triene-3,17β-diol (9a): mp 187–192 °C; HPLC 75:25 (MeOH/H_2O) 19 min; ^{1}H NMR 0.68 (s, 3H, 18-CH_3), 3.24 (t, 1H, J = 7.8 Hz, 17α-H), 3.43 (t, 1H, J = 8.4 Hz), 4.34 (t, J = 1.8 and 8 Hz, 16α-H), 6.40 (d, 1H, J = 9.2 Hz, C4-H), 6.66 (d, 1H, J = 13 Hz, C1-H); MS m/z (relative intensity) 370 (M^+, 97), 368 (M^+, 100), 288 (M^+ – HBr, 41). Anal. ($C_{18}H_{22}BrFO_2$) C, H, Br.

4-Fluoro-16β-bromoestra-1,3,5(10)-triene-3,17β-diol (9b): mp 173–175 °C; HPLC 80:20 (MeOH/H_2O) 17 min, HPLC 80:20 (EtOAc/hexane) 20 min; ^{1}H NMR 0.92 (s, 3H, 18-CH_3), 4.0 (d, 1H, J = 6.9 Hz, 17α-H), 4.62 (q, 1H, J = 6.3 Hz, 16α-H), 6.79 (t, 1H, J = 8.7 Hz, C2-H), 6.95 (d, J = 8.6 Hz, C1-H); MS m/z (relative intensity) 370 (M^+, 30), 368 (M^+, 32), 272 (10), 266 (100). Anal. ($C_{18}H_{22}BrFO_2$) C, H, Br.

2-Fluoro-16β-bromo-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (9c): mp 212–215 °C; HPLC 70:30 (MeOH/H_2O) 15 min; ^{1}H NMR 0.94 (s, 3H, 18-CH_3), 3.15 (s, 3H, 11β-OCH_3), 3.29 (d, 1H, J = 6 Hz, 17α-H) 3.85 (m, 1H, 11α-H), 4.42 (q, 1H, J = 8 Hz, 16α-H), 6.45 (d, 1H, J = 9 Hz, C4-H), 6.62 (d, 1H, J = 13 Hz, C1-H), 6.65–6.70 (m, 2H, C1 and C4-H); MS m/z (relative intensity) 400 (M^+, 49), 398 (M^+, 50), 318 (M^+ – HBr, 21), 286 (69), 268 (58), 230 (48), 174 (100). Anal. ($C_{19}H_{24}BrFO_3$) C, H, Br.

4-Fluoro-16β-bromo-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (9d): mp 222–226 °C; HPLC 75:25 (MeOH/H_2O) 11 min; ^{1}H NMR 1.04 (s, 3H, 18-CH_3), 3.19 (s, 3H, 11β-OCH_3), 3.35 (d, 1H, J = 6 Hz, 17α-H), 3.95 (m, 1H, 11α-H), 4.48 (q, J = 8 Hz, 16α-H), 6.65–6.70 (m, 2H, C1 and C2-H); MS m/z (relative intensity) 400 (M^+, 49), 398 (M^+, 51), 318 (M^+ – HBr, 21), 286 (69), 268 (59), 230 (50), 164 (81). Anal. ($C_{19}H_{24}BrFO_3$) C, H, Br.

Further elution with 15% EtOAc in hexane gave the corresponding estradiol derivative (20%).

2- and 4-Fluoro-16α-iodoestra-1,3,5(10)-triene-3,17β-diol Derivatives 10. A mixture of 16β-bromo-3,17β-estradiol derivatives (9, 0.1 mmol) and 7 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 to yield **10**.

2-Fluoro-16α-iodoestra-1,3,5(10)-triene-3,17β-diol (10a): mp 214–216 °C; HPLC 75:25 (MeOH/H_2O) 25 min; ^{1}H NMR 0.68 (s, 3H, 18-CH_3), 4.04 (m, 1H, 16β-H), 4.06 (m, 1H, 17α-H), 6.56 (d, 1H, J = 9.2 Hz, C4-H), 6.86 (d, J = 9.2 Hz, 1H, C1-H); MS m/z (relative intensity) 416 (M^+, 100), 287 (M^+ – HI, 15), 271 (30), 233 (77). Anal. ($C_{18}H_{22}FIO_2$) C, H, I.

4-Fluoro-16α-iodoestra-1,3,5(10)-triene-3,17β-diol (10b): mp 200–203 °C (lit.[18c] mp 195–197 °C); HPLC 80:20 (EtOAc/hexane) 18 min; ^{1}H NMR 0.76 (s, 3H, 18-CH_3), 4.1 (m, 2H, 16β-H and 17α-H), 6.75 (t, J = 8.7 Hz, 1H, C2-H), 6.94 (d, 1H, J = 8.6 Hz, C1-H); MS m/z (relative intensity) 416 (M^+, 48), 287 (M^+ – HI, 12), 272 (34), 232 (100).

2-Fluoro-16α-iodo-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (10c): mp 222–226 °C; HPLC 70:30 (MeOH/H_2O) 22 min; ^{1}H NMR 0.96 (s, 3H, 18-CH_3), 3.31 (s, 3H, 11β-OCH_3), 4.01–4.12 (m, 3H, 11α-H, 17α-H and 16β-H), 6.67 (d, J = 9.2 Hz, C4-H), 6.80 (d, J = 12.4 Hz, 1H, C1-H); MS m/z (relative intensity) 446 (M^+, 54), 368 (M^+ – HI, 8), 318 (17), 286 (41), 269 (53), 231 (100). Anal. ($C_{19}H_{24}FIO_3$) C, H, I.

4-Fluoro-16α-iodo-11β-methoxyestra-1,3,5(10)-triene-3,17β-diol (10d): mp 238–244 °C; HPLC 70:30 (MeOH/H_2O) 18 min; ^{1}H NMR 1.04 (s, 3H, 18-CH_3), 3.19 (s, 3H, 11β-OCH_3), 3.35 (d, 1H, J = 6 Hz, 16β-H), 3.95 (m, 1H, 11α-H and 17α-H), 6.65–6.70 (m, 2H, C1 and C2-H); MS m/z (relative intensity) 446 (M^+, 100), 318 (M^+ – HI, 15), 286 (42), 268 (42), 242 (38), 230 (71), 174 (75). Anal. ($C_{19}H_{24}FIO_3$) C, H, I.

Synthesis of 2-Fluoro-16α-[125I]iodoestradiols 10a, 4-Fluoro-16α-[125I]iodoestradiols 10b, 2-Fluoro-11β-methoxy-16α-[125I]iodoestradiols 10c, and 4-Fluoro-11β-methoxy-16α-[125I]-iodoestradiols 10d by Na125I Halogen Exchange. 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 **9a**, **9b**, **9c**, and **9d** (10 μg) or **1** in 20 μL 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 reversed-phase column with EtOH and water. The compound [^{125}I]**10a** eluted at 24 min, [^{125}I]**10b** at 21 min, [^{125}I]-**10c** at 15 min, and [^{125}I]**10d** at 13 min in 70:30 MeOH/water, respectively.

In Vivo Studies. The animal experiments were conducted as previously described[19a] 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 Long Evans rats, 24 days old, 55–60 g (Charles River) were injected with 200 μL of ^{125}I-labeled **10a**, **10b**, **10c**, or **10d** (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.[25] Blood was collected, tissues of interest were removed, washed with 0.154 M KCl, and blotted dry, and samples were weighted. The radioactivity was counted in a Model 1282 Compugamma counter (LKB Wallac, Finland), and concentrations were expressed as percent of the injected dose per gram of tissue (% ID/g). Statistical variations are presented as the standard error on the mean.[26]

Molecular Modeling. The computer models of estradiol derivatives were obtained using MNDO (modified neglect of diatomic overlap) semiempirical computations with the MOPAC[28] software (versions 5.1–6.0) on UNIX DISC 6000 work stations. Because of the relative rigidity of the steroid nucleus, the basic model used was unsubstituted estradiol with atomic coordinates obtained from the Cambridge Crystallographic Data Base.[29] Molecular orbital computations were performed introducing (imposing) complete geometrical optimizations (bond lengths angles and dihedral angles). Atomic charges and banal orders were derived from Mulliken population analysis.[30] The graphics presentations were obtained using SYBYL software.[31] The molecules were aligned using root mean square computations, superimposing all atoms from the A, B, and C steroidal nucleus (average weighted root mean square distance ≈ 0.06 Å). Electrostatic isopotential maps (based on Mulliken charge distribution were performed using the standard Coulombic potential as introduced in the SYBYL software.

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