

Synthesis of A-Ring Fluorinated Derivatives of (17 α ,20*E/Z*)-[¹²⁵I]Iodovinylestradiols: Effect on Receptor Binding and Receptor-Mediated Target Tissue Uptake¹

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 April 28, 1993*

We have prepared a series of 2- and 4-fluoro derivatives of the isomeric (17 α ,20*E*)- and (17 α ,20*Z*)iodovinylestradiols (IVE₂) and also the analogs substituted with either a 7 α -methyl (7 α -Me-IVE₂) or 11 β -methoxy group (11 β -OMe-IVE₂) and evaluated their *in vitro* and *in vivo* properties. Electrophilic substitution of the estrone derivatives with *N*-fluoropyridinium salt gave the 2- and 4-fluoro analogs which were subsequently converted to the 17 α -ethynyl derivatives. The tributylstannyl intermediates were obtained from the corresponding 17 α -ethynyl analogs using azobisisobutyronitrile or triethylborane as catalyst. All 12 products were also prepared as their no-carrier-added [¹²⁵I]iodovinyl analogs via destannylation of the tributylstannyl precursors. Binding affinity for the estrogen receptor (ER) was in general higher for the 4-F derivatives as compared to the 2-F derivatives, while the 20*Z* isomers of the same compounds showed somewhat higher ER binding affinity as compared to the 20*E* isomers. The combination of an A-ring fluoro and 7 α - or 11 β -substituent decreased ER binding affinity. Substitution of a fluoro atom at C-4 on either the 17 α -ethynylestradiol or isomeric 17 α -IVE₂ enhanced the affinity of the parent molecule for the ER. A-ring fluorination of all other analogues tested had no effect or depressed ER binding affinity. Varying incubation conditions showed substantial differences in ER binding kinetics between the 20*E* and 20*Z* isomers. Tissue distribution in immature female rats showed that the highest uterus uptake and uterus to blood/nontarget ratios in the IVE₂ series were obtained with the 4-F-(17 α ,20*Z*)IVE₂ isomer. The combination of A-ring fluoro and 7 α - or 11 β -substitution decreased uterus uptake but had little or no effect on uterus to blood/nontarget ratios. The highest uterus to blood ratios were observed for the 4-F-(17 α ,20*E*)11 β -OMe-IVE₂ (75 at 6 h and 125 at 12 h pi) reflecting rapid blood clearance and *in vivo* stability, as confirmed by the low levels of thyroid radioactivity. The lack of correlation between ER binding affinities and uterus uptake, and/or uptake ratios, suggests that other factors, including nonspecific binding and metabolic processes, also are involved in the tissue localization process. Our data suggest that 4-F substitution onto (17 α ,20*Z*)IVE₂ and (17 α ,20*E*)11 β -OMe-IVE₂ enhances the potential of these compounds to function as SPECT imaging agents of ER-rich tissues.

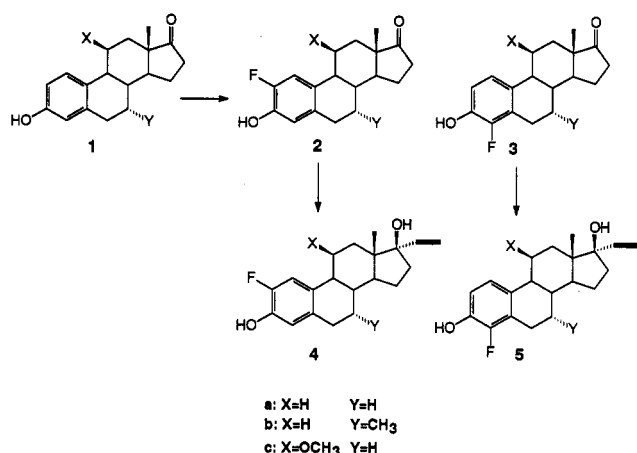
Introduction

The potential use of a radiolabeled estrogen for estrogen receptor (ER) imaging in human breast cancer evaluation and treatment has received a great deal of attention.² Synthesis of such radiopharmaceuticals requires the attachment of the functional element onto the parent molecule, estradiol, at positions providing minimum interference with the receptor binding process. Among the different radioisotopes which have been used for this purpose, radioiodine has the advantage of its availability as ¹²⁵I and ¹³¹I (syntheses and animal studies) as well as ¹²³I (clinical SPECT imaging). Sites of attachment of radioiodine onto estradiol are limited and only 16 α -iodo-³ and 17 α -iodovinyl⁴ derivatives were found to retain target tissue selectivity, combined with *in vivo* stability. These parent molecules were further substituted for optimal *in vivo* imaging properties. The synthesis of the 20*E* and 20*Z* iodovinyl derivatives of estradiol (20*E/Z*-IEV₂) has previously been reported, and receptor binding and tissue distribution studies suggested that the 20*Z* isomer has the preferred configuration.^{4a} This work was subsequently extended to include the 11 β -methoxy/ethoxy and 7 α -methyl derivatives in order to diminish nonspecific binding and to increase the stability of the steroid receptor complex.^{4b}

The main metabolic pathways of estradiol derivatives involves modification of the A- and D-rings.⁵ In the design of biologically active steroids, substitution of fluorine atoms for hydrogen is an important strategy. The resulting small steric alteration often facilitates interactions with receptor molecules while the electrostatic effects can change the biological properties of the analog dramatically. Introduction of fluorine at positions 2 or 4 of estradiol does not affect hormonal activity or binding affinities for the ER but influences A-ring catabolic rates.⁶ The 4-fluoroestradiol, with its high uterotrophic potency of 140% relative to estradiol,⁷ has been suggested as a prostate scanning agent upon labeling with the short-lived ¹⁸F-isotope.⁸ However, the poor synthetic yield and low specific activity of the ¹⁸F-analog rendered the radiolabeled compound unsuitable for this purpose. These studies revealed however that F-substituted steroids are stable under *in vivo* conditions. In view of these observations and to develop additional structure-function relationships for optimal imaging properties, we introduced a fluoro atom at the 2- and 4-positions of the A-ring of the isomeric (20*E/Z*)IVE₂ as well as their 7 α -methyl and 11 β -methoxy derivatives and evaluated ER binding affinities, receptor mediated target tissue uptake, and biodistribution pattern.

* Abstract published in *Advance ACS Abstracts*, September 1, 1993.

Scheme I

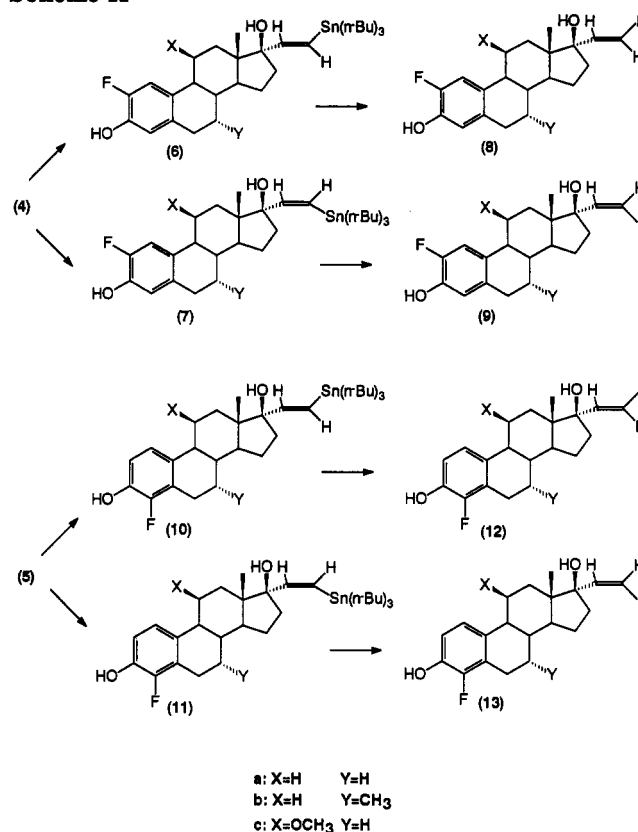


Results

Synthesis of A-Ring Fluorinated (17 α ,20*E/Z*)-Iodovinylestradiol Derivatives. Commercially available 2- and 4-fluoroestrone was used to prepare the corresponding fluoroestrone derivatives 2a and 3a via oxidation with Jones reagent. Attempted fluorination of the A-ring of 11 β -methoxyestrone (1c) with cesium fluorooxysulfate was unsuccessful, although unsubstituted estrone reacts smoothly with this reagent.⁹ The usefulness of the *N*-fluoropyridinium triflate as a fluorinating agent¹⁰ for the preparation of 2- and 4-fluoroestrone (2a and 3a) has recently been reported.¹¹ We extended this method to the synthesis of the 7 α -methyl- and 11 β -methoxyfluoroestrone derivatives 2b, 3b and 2c, 3c (Scheme I). The 11 β -methoxyestrone (1c) reacted much faster with this reagent than the parent molecule, estrone (1a), whereas the 11 β -methoxyestradiol gave a complex mixture from which it was difficult to purify the desired product. Treatment of the 11 β -ethoxyestrone with this reagent likewise did not give the fluoro derivative in appreciable amounts. The fluorinated estrone derivatives 2a–c and 3a–c were subsequently converted to the 17 α -ethynyl analogs 4a–c and 5a–c (Scheme I) via treatment with lithium acetylide–ethylene diamine complex.¹²

Hydrostannylation is a useful reaction for the preparation of carbon-functional organostannanes and is the simplest and most direct route to transform alkynes into vinylstannanes.¹³ For the introduction of the iodine onto the vinyl substituent of the 17 α -iodovinylestradiol we used the destannylation method. To obtain either the 20*E* or 20*Z* stannyl intermediates we previously^{4a,b} established a method using tri-*n*-butyltin hydride, with or without catalyst and with different reaction conditions (reaction time, temperature, and solvent polarity) to favor formation of either isomer. For some of the fluorine substitutions this method was not suitable, leading to a search of an alternative catalyst. Napotitano *et al.*¹⁴ reported the selective synthesis of the 20*E* and 20*Z* isomers involving UV light or thermal reaction conditions, on the phenol-protected derivative. However, Nozaki *et al.*¹⁵ reported a more convenient method using a triethylborane as a catalyst for the formation of vinylstannanes from acetylenic compounds and tri-*n*-butyltin hydride. Likewise, we found that 4 and 5 readily reacted with tri-*n*-butyltin hydride in the presence of triethylborane catalyst to yield a mixture of the 20*E/Z* isomeric vinylstannanes 6, 7 and 10, 11 in moderate to high yield (Scheme II). The reaction was complete at room temperature over a period of 15 min to

Scheme II



1 h, depending on the substrate used and the isomeric ratio required. Prolonged reaction times or increased amounts of tributylstannyl hydride in the reaction mixture resulted in a decrease in the 20*Z/E* isomer ratio. The temperature also exerted a dramatic effect on the stereochemical composition of the reaction mixture; at elevated temperature the 20*E* isomer became dominant. This suggests the possibility that formation of the 20*Z* isomer is under kinetic control and that this isomer may be stereochemically less stable, resulting in isomerization to the 20*E* isomer under our reaction condition. Separation of the 20*E/Z* isomers was readily achieved by chromatographic methods. The 20*Z* isomers are less polar, *R_f* values (silica gel TLC, hexane/ethyl acetate) are slightly higher, and retention times on reverse-phase HPLC columns (water/methanol) are longer than those observed for the corresponding 20*E* isomers. The tin intermediates were characterized via mass spectroscopy by their weak molecular ion, or base peak corresponding to loss of C₄H₉, with characteristic tin-isotope clusters. Addition of a 0.1 M solution of iodine in chloroform to the isomeric tin intermediates 6, 7 and 10, 11 resulted in an immediate destannylation to give the iodovinylestradiol derivatives 8, 9 and 12, 13 (Scheme II), with retention of configuration, in 60–80% yield. In the case of 7a and 11a we also isolated the 2-F-17 α -vinylestradiol (2-F-VE₂) and 4-F-17 α -vinylestradiol (4-F-VE₂) as minor products. The iodovinyl derivatives gave the expected molecular ion peak in the mass spectrum, and their assigned stereochemistry was established from the ¹H NMR coupling constant of the vinyl hydrogens (20*E* isomer, *J* = 14 Hz; 20*Z* isomer, *J* = 8 Hz). The 20*Z* isomers were in general less polar than the 20*E* isomers and possessed different solubility properties. The physical and spectroscopic properties of the various products are summarized in Table I.

To prepare the noncarrier-added ^{125}I -labeled isomeric (20E/Z) analogs, different methods were used. The 20E isomers [^{125}I]-8a-c and [^{125}I]-12a-c were obtained by the treatment of the stannyl precursors with [^{125}I]NaI/ H_2O_2 . We showed previously^{4a} that this procedure gives low labeling yields in the case of the corresponding 20Z isomers and that the preferred method for the latter isomers involves treatment of the stannyl precursors with [^{125}I]NaI/chloramine-T in EtOH.

Binding Affinity for the Estrogen Receptor. The relative binding affinities (RBA) were determined by a competitive binding assay with [^3H]estradiol.¹⁶ The RBA values are expressed relative to unsubstituted estradiol; the 17 α -ethynyl derivative has a similar binding affinity to the ER (RBA = 100).¹⁷ The 2-F-VE₂ exhibited lower ER binding affinity than the 4-F-VE₂ (RBA = 18 vs 74). The receptor binding properties of the other A-ring fluorinated estradiol derivatives and their corresponding non-fluorinated compounds are given in Table II. For most derivatives 2F-substitution diminished ER binding, whereas 4F-substitution had a variable effect, in some cases augmenting the RBA. Synergism between the F-substitution and the configuration about the iodovinyl substituent was also observed. In general 20Z isomers showed higher RBA values than the corresponding 20E isomers. Adding two additional substituents, e.g., a fluoro and a 7 α -methyl or 11 β -methoxy group, resulted in lower RBA values. In order to evaluate whether equilibrium conditions were reached under the experimental conditions, RBA values of the 17 α -ethynyl series lacking 7 α - or 11 β -substituent were also established at elevated temperatures (25 °C, 2 h) and reduced incubation times (4 °C, 2 h) (Table III). Higher temperatures or longer incubation times resulted in higher RBA values in the case of the 20Z isomers 9a and 13a, while the corresponding 20E isomers 8a and 12a gave substantially lower RBA values under these conditions.

Tissue Distribution in Immature Rats. The tissue distribution data of the fluorinated isomeric [^{125}I]iodovinylestradiol derivatives in immature female Long Evans rats at various time intervals after injection is shown in Tables IV–VI. For the 1-h time point, a second group of animals was coinjected with nonlabeled estradiol (60 μg) to block ER, thus providing the level of nonspecific uptake. The radioactivity concentrations in the various organs are presented as the percent of the injected dose per gram of tissue (% ID/g) together with the standard error. The uterus to blood and nontarget (lung, spleen, and muscle) ratios are also included in the tables. High ER-mediated uterus uptake (9–12% ID/g at 3–5 h pi) was observed with the 4-F-(17 α ,20Z)IVE₂ (13a) as well as the 2-F-(17 α ,20E)-, 4-F-(17 α ,20Z)- and 4-F-(17 α ,20E)11 β -OMe-IVE₂ (9c, 13c, and 12c) Figure 1. These same derivatives show advantageous uterus to nontarget ratios of >25 Figure 2. The rapid blood clearance of the 4-F-(17 α ,20E)11 β -OMe-IVE₂ (12c) Figure 4 leads to high uterus to blood ratios of >50 (Figure 3). Thyroid uptake of the [^{125}I]iodovinyl steroids is a measure for the *in vivo* stability of the C–I bond and the % ID/g of the thyroid can be used to estimate the percent deiodination. It can be seen Figure 5 that a synergism between the configuration about the 20-position and the various substituents does exist, leading to varying *in vivo* instabilities of analogs in the series.

In general, blood clearance of the 4-fluorinated compounds is slower than that of the 2-fluorinated analogs,

with the exception of the fluorinated (17 α ,20E)11 β -OMe-IVE₂, whereas blood clearance of the IVE₂ derivatives with the 20Z configuration, in general, is slower than that of the analogous compounds with the 20E configuration.

Discussion

We have described a simple synthetic method to obtain 2- and 4-fluoro substituted iodovinylestradiols and adapted the use of a triethylborane catalyst for the synthesis of the isomeric 20E/Z stannylvinyl intermediates (Schemes I and II). The latter are readily transformed to the corresponding iodo derivatives in a procedure suitable for use with short-lived radioiodine isotopes of medical importance. The potential of these derivatives as scanning agents for ER in nuclear medicine was evaluated in an *in vivo* assay, whereas possible structure–function relationships were studied by an *in vitro* receptor binding assay. Selection of substituents and their site of attachment onto the estradiol molecule was based on our current knowledge of steroid interactions with receptors, nonspecific proteins, and metabolic enzymes. Thus, 11 β -substitution is known to increase the stability of the ER-steroid complex¹⁸ while reducing nonspecific binding, resulting in enhanced uptake by target tissue. Likewise, introduction of a 7 α -methyl group results in increased estrogenic activity and higher affinity for the ER receptor.^{20,21} Finally, 17 α -substitution blocks D-ring metabolism, whereas the 2-fluoro substituent strongly inhibits formation of the catechol estrogens.⁵

The receptor binding data in Table II reveal a systemic pattern of changes in RBA values following structural modifications to the estradiol skeleton. Addition of a 4F-substituent onto 17 α -ethynylestradiol (EE₂) increased ER binding (RBA = 119 vs 100), whereas 2F-substitution significantly lowered ER binding (RBA = 64 vs 100), which is consistent with reported data for 2- and 4-fluoroestradiols.^{6,7} The F-VE₂ exhibited substantially lower ER binding affinities than the corresponding F-EE₂. Addition of the 7 α -methyl onto EE₂ lowers ER binding (RBA = 73 vs 100); subsequent addition of a 4-F has little effect (RBA = 62 vs 73), whereas addition of a 2-F lowers ER binding even further (RBA = 33 vs 73). A similar ER binding affinity pattern was observed when 7 α -methyl derivatives were substituted at the 2- or 4-position with a chlorine atom.¹⁹ As previously reported,²⁰ addition of the 11 β -methoxy onto EE₂ strongly reduces ER binding; further substitution with either 2-F or 4-F does not significantly alter the RBA value (RBA = 15–17).

Substitution(s) of (17 α ,20E/Z)IVE₂ at the 2-, 4-, 7 α -, or 11 β -position(s) results in similar changes in RBA values as those observed for the equivalent substitutions of EE₂. However, in some cases striking synergistic effects can be noted. Thus, 2-F substitution onto the (17 α ,20E)IVE₂, including the 7 α -methyl or 11 β -methoxy derivatives, results in an unexpected large drop in RBA values of 20–50% (Table II). This suggests either a high instability of the 20E isomers in general or unfavorable conformational changes in regard to interactions with the ER.

In order to further evaluate the relative stability of the fluorinated 20E/Z isomers and their ER complexes we compared their RBA values at different incubation times as well as increased temperature (Table III). No differences in the stability of the ER after incubation for 2 h at 4 and 25 °C was observed. In the case of the 4-F-EE₂ (5a) and both the 2- and 4-F-(17 α ,20Z)IVE₂ (9a and 13a)

Table I. Physical and Spectroscopic Data of Products

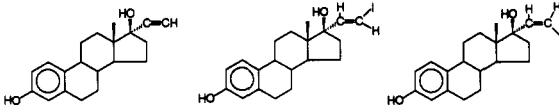
product	mp (0 °C)	HPLC system ^a (t _R)	MS (70 eV) m/z (%)	empirical formula	analysis	¹ H NMR (δ) ^b
4a	101–103	A 17 min	314 (M ⁺ , 21), 246 (15), 231 (100)	C ₂₀ H ₂₃ FO ₂	C,H,F	0.88 (s, 3 H, 18-CH ₃), 2.6 (s, 1 H, C(21)-H), 6.76 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.85 (d, <i>J</i> = 12.6 Hz, 1 H, C(1)-H); ¹⁹ F NMR -62.177 (s, 1 F, C(2)-H)
5a	150–152	B 16	314 (M ⁺ , 23), 246 (12), 268 (5), 231 (100)	C ₂₀ H ₂₃ FO ₂	C,H,F	0.88 (s, 3 H, 18-CH ₃), 2.6 (s, 1 H, C(21)-H), 6.79 (t, <i>J</i> = 9 Hz, 1 H, C(2)-H), 6.96 (d, <i>J</i> = 9 Hz, 1 H, C(1)-H); ¹⁹ F NMR -63.525 (s, 1 F, C(2)-H)
2-F-VE ₂		B 23	316 (M ⁺ , 13), 298 (13), 244 (39), 231 (71), 178 (100)	C ₂₀ H ₂₃ FO ₂	C,H	0.836 (s, 3 H, 18-CH ₃), 4.99–5.12 (m, 2 H, =CH ₂), 5.94–6.05 (m, 1 H, -CH=), 6.56 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.80 (d, <i>J</i> = 13 Hz, 1 H, C(1)-H)
8a	88	A 35	442 (M ⁺ , 12), 315 (12), 297 (14), 178 (62), 151 (100)	C ₂₀ H ₂₄ FIO ₂	C,H,F,I	0.60 (s, 3 H, 18-CH ₃), 5.94 (d, <i>J</i> = 14 Hz, 1 H, =CHI), 6.35 (d, <i>J</i> = 9 Hz, 1 H, C(4)-H), 6.56 (d, <i>J</i> = 14 Hz, 1 H, -CH=), 6.60 (d, <i>J</i> = 13 Hz, 1 H, C(1)-H)
9a	160–162	A 34	442 (M ⁺ , 15), 315 (11), 297 (14), 178 (70), 151 (100)	C ₂₀ H ₂₄ FIO ₂ ·H ₂ O	C,H,I	0.29 (s, 3 H, 18-CH ₃), 5.75 (d, <i>J</i> = 8.6 Hz, 1 H, =CHI), 5.99 (d, <i>J</i> = 9 Hz, 1 H, C(4)-H), 6.21 (d, <i>J</i> = 8.6 Hz, 1 H, -CH=), 6.27 (d, <i>J</i> = 13 Hz, 1 H, C(1)-H)
12a	95–97	A 38	442 (M ⁺ , 15), 315 (19), 297 (16), 231 (40), 178 (15), 151 (100)	C ₂₀ H ₂₄ FIO ₂	C,H,F,I	0.378 (s, 3 H, 18-CH ₃), 5.61 (d, <i>J</i> = 14 Hz, 1 H, =CHI), 6.12 (t, <i>J</i> = 13 Hz, 1 H, C(2)-H), 6.18 (d, <i>J</i> = 14 Hz, 1 H, -CH=), 6.23 (d, <i>J</i> = 9 Hz, 1 H, C(1)-H), 8.3 (s, 1 H, -OH)
13a	98–100	A 37	442 (M ⁺ , 15), 315 (12), 297 (14), 231 (45), 178 (60), 151 (18)	C ₂₀ H ₂₄ FIO ₂	C,H,F,I	0.74 (s, 3 H, 18-CH ₃), 6.17 (d, <i>J</i> = 8.5 Hz, 1 H, =CHI), 6.55 (t, <i>J</i> = 9 Hz, 1 H, C(2)-H), 6.61 (d, <i>J</i> = 8.5 Hz, 1 H, -CH=), 6.67 (d, <i>J</i> = 9 Hz, 1 H, C(1)-H), 8.44 (s, 1 H, -OH)
4-F-VE ₂		A 25	316 (M ⁺ , 12), 298 (14), 244 (48), 231 (65), 178 (100)	C ₂₀ H ₂₄ FIO ₂	C,H	0.94 (s, 3 H, 18-CH ₃), 4.9–5.23 (m, 2 H, =CH ₂), 6.04–6.5 (m, 1 H, -CH=), 6.80 (t, <i>J</i> = 13 Hz, 1 H, C(2)-H), 6.90 (d, <i>J</i> = 8.6 Hz, 1 H, C(1)-H)
4b	101–103	A 19	328 (M ⁺ , 43), 271 (8), 245 (100)	C ₂₁ H ₂₆ FO ₂	C,H	0.84 (d, <i>J</i> = 7 Hz, 7α-CH ₃), 0.89 (s, 3 H, 18-CH ₃), 2.31 (s, 1 H, C(21)-H), 6.68 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.98 (d, <i>J</i> = 12.5 Hz, 1 H, C(1)-H)
5b	102–105	D 35 A 19	328 (M ⁺ , 38), 271 (7), 245 (100)	C ₂₁ H ₂₆ FO ₂	C,H,F	0.89 (d, <i>J</i> = 7 Hz, 7α-CH ₃), 0.92 (s, 3 H, 18-CH ₃), 2.39 (s, 1 H, C(21)-H), 6.84 (t, <i>J</i> = 13 Hz, 1 H, C(2)-H), 7.0 (d, <i>J</i> = 8.6 Hz, 1 H, C(1)-H)
8b	110–115	D 38 B 22	456 (M ⁺ , 43), 328 (40), 310 (27), 271 (21), 245 (100)	C ₂₁ H ₂₆ FIO ₂ ·H ₂ O	C,H,I	0.83 (d, <i>J</i> = 7 Hz, 7α-CH ₃), 0.927 (s, 3 H, 18-CH ₃), 6.3 (d, <i>J</i> = 14.4 Hz, 1 H, =CHI), 6.67 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.78 (d, <i>J</i> = 14.4 Hz, 1 H, -CH=), 6.96 (d, <i>J</i> = 12.6 Hz, 1 H, C(1)-H)
9b	112–114	B 21	456 (M ⁺ , 30), 328 (18), 310 (22), 271 (21), 245 (100)	C ₂₁ H ₂₆ FIO ₂ ·H ₂ O	C,H,I	0.70 (d, <i>J</i> = 7 Hz, 7α-CH ₃), 0.84 (s, 3 H, 18-CH ₃), 6.27 (d, <i>J</i> = 8.5 Hz, 1 H, =CHI), 6.53 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.69 (d, <i>J</i> = 8.6 Hz, 1 H, -CH=), 6.80 (d, <i>J</i> = 12.6 Hz, 1 H, C(1)-H)
12b	100–105	B 20	456 (M ⁺ , 34), 328 (50), 310 (35), 271 (24), 258 (27), 245 (100)	C ₂₁ H ₂₆ FIO ₂ ·H ₂ O	C,H,I	0.844 (d, <i>J</i> = 7 Hz, 7α-CH ₃), 0.926 (s, 3 H, 18-CH ₃), 6.32 (d, <i>J</i> = 14.4 Hz, 1 H, =CH), 6.69 (t, <i>J</i> = 9 Hz, 1 H, C(2)-H), 6.78 (d, <i>J</i> = 14.4 Hz, 1 H, -CH=), 6.94 (d, <i>J</i> = 8.6 Hz, 1 H, C(1)-H)
13b	102–107	B 21	456 (M ⁺ , 43), 328 (35), 310 (15), 271 (26), 245 (100)	C ₂₁ H ₂₆ FIO ₂	C,H,F,I	0.83 (d, <i>J</i> = 7 Hz, 7α-CH ₃), 0.97 (s, 3 H, 18-CH ₃), 6.39 (d, <i>J</i> = 8.5 Hz, 1 H, =CHI), 6.79 (t, <i>J</i> = 9 Hz, 1 H, C(2)-H), 6.83 (d, <i>J</i> = 8.5 Hz, 1 H, -CH=), 6.95 (d, <i>J</i> = 8.6 Hz, 1 H, C(1)-H)
4c	126–132	A 11	344 (M ⁺ , 66), 313 (12), 295 (11), 280 (100)	C ₂₁ H ₂₆ FO ₃	C,H,F	0.90 (s, 3 H, 18-CH ₃), 2.58 (s, 1 H, C(21)-H), 3.15 (s, 3 H, 11β-OCH ₃), 3.92–3.95 (m, 1 H, 11α-H), 6.49 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.65 (d, <i>J</i> = 13.1 Hz, 1 H, C(1)-H), 7.86 (brs, 1 H, -OH), 8.37 (br, 1 H, -OH); ¹⁹ F NMR -61.073 (s, 1 F, C(2)-H)
5c	210–300	C 23 A 11	344 (M ⁺ , 98), 312 (22), 295 (42), 267 (100)	C ₂₁ H ₂₆ FO ₃	C,H,F	1.0 (s, 3 H, 18-CH ₃), 2.53 (s, 1 H, C(21)-H), 3.21 (s, 3 H, 11β-OCH ₃), 4.09–4.116 (m, 1 H, 11α-H), 6.68–6.73 (m, 1 H, C(2)-H), 6.75 (d, <i>J</i> = 8.6 Hz, 1 H, C(1)-H), 7.85 (brs, 1 H, -OH); ¹⁹ F NMR -59.92 (s, 1 F, C(4)-H)
8c	decomp 215–217	C 27 A 21	472 (M ⁺ , 20), 360 (17), 344 (20), 312 (39), 294 (35), 243 (89), 174 (100)	C ₂₁ H ₂₆ FIO ₃ ·H ₂ O	C,H,F,I	1.127 (s, 3 H, 18-CH ₃), 3.38 (s, 3 H, 11β-OCH ₃), 4.06–4.07 (m, 1 H, 11α-H), 6.33 (d, <i>J</i> = 14.4 Hz, 1 H, =CH), 6.56 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.74 (d, <i>J</i> = 11.3 Hz, 1 H, C(1)-H), 6.77 (d, <i>J</i> = 14.4 Hz, 1 H, -CH=)
9c	245–250	A 20	472 (M ⁺ , 16), 344 (22), 312 (28), 294 (28), 243 (100)	C ₂₁ H ₂₆ FIO ₃ ·H ₂ O	C,H,I	1.0 (s, 3 H, 18-CH ₃), 3.16 (s, 3 H, 11β-OCH ₃), 3.9 (m, 1 H, 11α-H), 6.24 (d, <i>J</i> = 8.8 Hz, 1 H, =CHI), 6.50 (d, <i>J</i> = 9.2 Hz, 1 H, C(4)-H), 6.63 (d, <i>J</i> = 11.5 Hz, 1 H, C(1)-H), 6.64 (d, <i>J</i> = 8.8 Hz, 1 H, -CH=)

Table I (Continued)

product	mp (°C)	HPLC system ^a (<i>t_R</i>)	MS (70 eV) <i>m/z</i> (%)	empirical formula	analysis	¹ H NMR (δ) ^b
12c	215–217	A 22	472 (M ⁺ , 13), 360 (20), 344 (18), 312 (42), 294 (31), 243 (83), 174 (100)	C ₂₁ H ₂₆ FIO ₃ ·H ₂ O	C, H, F, I	1.06 (s, 3 H, 18-CH ₃), 3.20 (s, 3 H, 11β-OCH ₃), 4.05–4.06 (m, 1 H, 11α-H), 6.24 (d, <i>J</i> = 14 Hz, 1 H, =CH), 6.72 (d, <i>J</i> = 14 Hz, 1 H, -CH=), 6.74 (d, <i>J</i> = 8 Hz, 1 H, C(1)-H)
13c	205–208	A 20	472 (M ⁺ , 13), 360 (11), 344 (27), 312 (46), 294 (26), 243 (100)	C ₂₁ H ₂₆ FIO ₃	C, H, F, I	1.0 (s, 3 H, 18-CH ₃), 3.16 (s, 3 H, 11β-OCH ₃), 4.01–4.02 (m, 1 H, 11α-H), 6.26 (d, <i>J</i> = 8.6 Hz, 1 H, =CH), 6.60–6.65 (m, 1 H, C(2)-H), 6.67 (d, <i>J</i> = 8.6 Hz, 1 H, -CH=), 6.68 (d, <i>J</i> = 8.6 Hz, 1 H), 8.21 (brs, 1 H, -OH)

^a Retention time (*t_R*) in min on reverse phase HPLC (2 mL/min). Solvent systems: (A) methanol/water (75:25); (B) methanol/water (70:30); (C) *n*-heptane/ethyl acetate (70:30); (D) *n*-heptane/ethyl acetate (90:10). ^b Solvent was 10% DMSO in deuteriochloroform. Spectra were taken at 250 MHz.

Table II. Relative Binding Affinities^a of the Various Steroids for Murine Cytoplasmic Estrogen Receptors

compound			
			
		(17α,20E)	(17α,20Z)
Unsubstituted			
C-2H	100 ^b	39.7 (0.7) ^b	46.7 ^b
C-2F	64.3 (8.7)	6.2 (0.6)	53.7 (11.9)
C-4F	119 (11.7)	55.9 (16.7)	66.9 (12.3)
7α-Methyl			
C-2H	73.1 (12.2) ^b	43.2 (7.5) ^b	44.9 (0.9) ^b
C-2F	33.3 (4.4)	12.1 (1.0)	28.6 (2.0)
C-4F	62.4 (3.9)	36.4 (3.3)	36.8 (3.8)
11β-Methoxy			
C-2H	15.3 (0.5) ^b	27.7 (2.9) ^b	32 (6.6) ^b
C-2F	15.9 (1.9)	13.5 (2.2)	27.9 (1.1)
C-4F	16.7 (1.7)	27.7 (1.0)	37.9 (1.1)

^a The relative binding affinities (RBA) were determined by competitive binding assays. RBA is defined as 100 times the ratio between competitor and unlabeled estradiol concentration required for 50% competition to specific [³H]estradiol binding. Murine cytoplasmic extracts were incubated at 0–4 °C for 18 h with 20 nM of [³H]estradiol in the absence and presence of 2 nM to 20 μM unlabeled steroids. The concentration required for 50% competition was used to calculate the RBA values (mean of three experiments and standard deviation). ^b Value taken from ref 4b.

Table III. Relative Binding Affinities of Estradiol Derivatives for Murine Cytoplasmic Estrogen Receptor at Different Temperatures and Incubation Times

compd no.	substituents	relative binding affinity		
		4 °C (2 h)	4 °C (18 h)	25 °C (2 h)
5a	4-F-17α-ethynyl E ₂	80 (4.5)	119 (11.7)	166.5 (78)
8a	2-F-(17α,20E)iodovinyl E ₂	30.4 (8.5)	6.2 (0.6)	5.3 (0.4)
9a	2-F-(17α,20Z)iodovinyl E ₂	40.1 (6.2)	53.7 (11.9)	99 (8.5)
12a	4-F-(17α,20E)iodovinyl E ₂	58.7 (5.4)	55.9 (12.3)	29.1 (2.4)
13a	4-F-(17α,20Z)iodovinyl E ₂	47.4 (7.4)	66.9 (16.7)	203 (4.2)

isomers, RBA values increased with prolonged incubation times (35–50%) as well as augmented temperature (100–300%), suggesting that their dissociation rates from the ER complex is slow or that possible metabolites exhibit strong ER binding affinity. In contrast, RBA values of the 2-F-(17α,20E)IVE₂ isomer (8a) decreased sharply (80%) following change in either experimental conditions. In the case of the 4-F-(17α,20E)IVE₂ isomer (12a), increasing the incubation time from 2 to 18 h at 4 °C had little effect on the RBA value, whereas the RBA decreased

by 50% upon raising the incubation temperature to 25 °C. The differences between the 20E and 20Z binding properties at various temperatures showed pattern similar to those reported for the IVE₂ lacking fluoro substituents.¹⁴ The lower RBA values observed with the 20E isomers after increasing the incubation temperature have been suggested to result from the rapid dissociation of the steroid, ensuring that the equilibrium is already reached at the lower incubation temperature.¹⁴ However, the large drop in binding affinity after either increasing the temperature or the incubation time in the case of the 20E isomer 8a cannot be explained by dissociation phenomena only but rather suggests an instability of the ER–ligand complex or metabolism of the steroid to a derivative with low binding affinity for the ER. These observations thus do not only provide an indication of ER affinity but also reveal the relative rate of interaction of the ligand with the receptor. Incubation conditions influence the kinetics of formation and dissociation of the hormone–receptor complex; varying these conditions makes it possible to derive different RBA values which may predict the relative *in vivo* stability of the ER–ligand complex, the dynamics of the biological response, and possible metabolism of the ligand. The RBA values measured at 4 °C reflect association rates, while those at 25 °C more closely reflect equilibrium conditions. Our results contrast the prediction that 2-F substitution, which blocks estradiol catabolism, would be the preferred modification for optimal interaction with ER.

In this study we also evaluated the biodistribution in immature female Long Evans rats of the 2- and 4-fluoro derivatives of the (17α,20E/Z)[¹²⁵I]IVE₂ and their 7α-methyl and 11β-methoxy analogs (Tables IV–VI). Distribution pattern in the uterus, thyroid, and blood as well as uterus to blood/nontarget ratios are compared with previously published data on the corresponding nonfluorinated analogs (Figures 1–5). These earlier studies were done in female Fischer rats, and some variation in distribution pattern can be expected due to differences in receptor/transport protein levels between the species. Direct comparison of the distribution pattern between the two species was done with the radioiodinated 4-F-(17α,20E)IVE₂ revealing about two times higher radioactivity retention in the uterus of Fischer rats at 1 h pi. However, radioactivity levels of blood and nontarget organs were also elevated, resulting in comparable uterus to blood/nontarget ratios between the two species (data not shown).

Fluorination of the (17α,20E/Z)IVE₂ at the 4-position (12a and 13a) substantially enhanced uterus uptake over

Table IV. Tissue Distribution of the Isomeric 2- and 4-Fluoro-(17 α ,20E/Z)-[¹²⁵I]iodovinylestradiols in Immature Female Rats

tissue	%ID/g (SE) ^a				
	1 h	1 h (+E ₂)	3 h	5 h	12 h
[¹²⁵ I]-8a (20E Isomer, 2-F)					
uterus	1.00 (0.13)	0.52 (0.01)	0.53 (0.07)	0.34 (0.05)	0.08 (0.00)
blood	0.53 (0.19)	0.49 (0.10)	0.42 (0.20)	0.29 (0.04)	0.08 (0.01)
thyroid	27 (4.71)	22 (5.37)	39.10 (9.27)	43.46 (15.90)	163 (31.50)
muscle	0.73 (0.25)	0.54 (0.29)	0.37 (0.19)	0.28 (0.19)	0.03 (0.01)
fat	3.45 (0.34)	2.93 (0.70)	3.17 (0.92)	2.39 (0.44)	0.41 (0.18)
kidneys	0.87 (0.31)	0.66 (0.56)	0.47 (0.22)	0.25 (0.06)	0.06 (0.01)
spleen	0.57 (0.19)	0.56 (0.15)	0.32 (0.06)	0.27 (0.11)	0.05 (0.00)
lungs	1.00 (0.46)	0.93 (0.19)	0.48 (0.22)	0.54 (0.33)	0.10 (0.04)
liver	3.95 (1.11)	3.86 (0.79)	2.87 (0.81)	2.12 (0.43)	1.58 (0.48)
uterus/blood	2.21 (0.19)	1.09 (0.14)	1.40 (0.26)	1.15 (0.12)	0.92 (0.07)
uterus/nontarget ^b	1.42 (0.09)	0.81 (0.17)	1.49 (0.29)	1.14 (0.27)	1.25 (0.11)
[¹²⁵ I]-9a (20Z Isomer, 2-F)					
uterus	4.41 (0.25)	0.68 (0.06)	5.44 (0.15)	3.67 (0.39)	2.51 (0.29)
blood	0.61 (0.12)	0.58 (0.08)	0.42 (0.03)	0.30 (0.03)	0.17 (0.04)
thyroid	39 (4.09)	36.11 (0.51)	75.30 (7.48)	149.9 (19.81)	365 (30.37)
muscle	0.57 (0.18)	2.16 (1.87)	0.28 (0.06)	0.14 (0.02)	0.05 (0.01)
fat	2.19 (0.20)	2.88 (1.72)	2.21 (0.18)	1.13 (0.18)	0.19 (0.01)
kidneys	0.88 (0.27)	0.65 (0.10)	0.53 (0.06)	0.30 (0.05)	0.14 (0.03)
spleen	0.55 (0.19)	1.00 (0.84)	0.29 (0.04)	0.20 (0.00)	0.17 (0.02)
lungs	0.81 (0.27)	0.75 (0.08)	0.39 (0.03)	0.24 (0.02)	0.12 (0.03)
liver	2.25 (0.76)	2.58 (0.29)	1.76 (0.19)	1.16 (0.13)	0.81 (0.16)
uterus/blood	7.34 (0.43)	1.20 (0.12)	12.98 (0.48)	12.09 (0.59)	15.69 (3.65)
uterus/nontarget ^b	7.32 (0.17)	0.51 (0.05)	17.64 (0.76)	18.05 (1.08)	20.54 (4.34)
[¹²⁵ I]-12a (20E Isomer, 4-F)					
uterus	3.75 (0.22)	0.77 (0.15)	4.24 (0.50)	1.82 (0.45)	0.43 (0.05)
blood	0.78 (0.13)	0.81 (0.42)	0.96 (0.33)	0.26 (0.08)	0.16 (0.07)
thyroid	71.61 (11.50)	65.69 (5.16)	128 (24.82)	208 (27.51)	385 (44.64)
muscle	0.46 (0.06)	0.58 (0.15)	0.31 (0.03)	0.14 (0.01)	0.06 (0.00)
fat	2.97 (0.41)	1.14 (0.25)	1.59 (0.46)	1.67 (0.20)	0.30 (0.05)
kidneys	0.93 (0.06)	0.87 (0.20)	0.59 (0.05)	0.26 (0.01)	0.08 (0.00)
spleen	0.59 (0.11)	0.51 (0.13)	0.36 (0.03)	0.17 (0.02)	0.10 (0.03)
lungs	0.89 (0.06)	0.75 (0.02)	0.57 (0.05)	0.28 (0.03)	0.10 (0.02)
liver	2.91 (0.37)	3.06 (0.37)	2.20 (0.13)	1.21 (0.15)	0.59 (0.04)
uterus/blood	5.42 (0.17)	1.02 (0.18)	4.56 (0.31)	9.01 (4.38)	2.76 (0.16)
uterus/nontarget ^b	6.39 (0.23)	1.17 (0.15)	11.38 (0.56)	10.41 (2.60)	5.40 (0.48)
[¹²⁵ I]-13a (20Z Isomer, 4-F)					
uterus	10.44 (0.43)	0.86 (0.05)	7.96 (0.73)	10.22 (1.55)	6.02 (0.40)
blood	0.71 (0.42)	0.55 (0.15)	0.48 (0.09)	0.47 (0.13)	0.24 (0.12)
thyroid	42.20 (3.78)	50.60 (1.61)	87.39 (35.39)	117 (15.04)	488 (111.37)
muscle	1.26 (0.36)	0.51 (0.07)	0.82 (0.55)	0.38 (0.14)	0.37 (0.04)
fat	4.30 (3.27)	3.41 (0.58)	1.96 (0.17)	1.71 (0.54)	0.75 (0.23)
kidneys	1.87 (1.14)	0.85 (0.16)	0.82 (0.13)	0.58 (0.16)	0.74 (0.79)
spleen	1.02 (0.30)	0.56 (0.20)	0.48 (0.16)	0.40 (0.19)	0.43 (0.41)
lungs	1.89 (0.73)	1.14 (0.15)	0.85 (0.12)	0.56 (0.10)	0.80 (0.95)
liver	3.49 (0.66)	3.04 (0.46)	1.95 (0.53)	1.64 (0.81)	1.78 (1.36)
uterus/blood	15.19 (1.45)	1.65 (0.27)	17.08 (2.52)	22.60 (4.13)	27.32 (7.87)
uterus/nontarget ^b	8.31 (0.86)	1.21 (0.18)	12.16 (2.62)	28.59 (8.02)	31.56 (4.74)

^a Mean organ uptake (percent injected dose per gram of tissue) and standard error (SE) for immature female Long Evans rats (three to five animals), after injection of 3 μ Ci (111 kBq) of [¹²⁵I]-labeled steroids in the presence (+E₂) or absence of 60 μ g co-injected estradiol. ^b Nontarget organs include the muscle, spleen, and lungs.

the total period studied, whereas addition of a 2-F had no effect on the uterus uptake profile of the 20E isomer (8a) and a small positive effect in the case of the 20Z isomer (9a). Furthermore, these uptake values show that the 4-F-(20Z) isomer (13a) reaches 2–3 times higher uterus levels as compared to either the 4-F-(20E) isomer (12a) or the 2-F-(20Z) isomer (9a). The highest uterus to blood/nontarget ratios of this series were also observed with 13a. These uterus uptake levels correlate to some extent with the ER binding affinities of these IVE₂ in that the highest RBA value was obtained for 13a (Table II).

Addition of a 7 α -methyl substituent to the IVE₂ increased uterus uptake substantially for both the 20E and the 20Z isomer; however, further substitution of the A-ring (2-F or 4-F) resulted in suppression of uterus uptake. These data parallel the decrease in RBA values observed

upon simultaneous addition of 7 α -methyl and fluoro substituents onto estradiol (Table II). A similar uterus uptake pattern was observed with the 11 β -methoxy series. In both cases, 4-F addition onto the 20E isomer resulted in higher uterus uptake as compared to 2-F addition, while in the case of the 20Z isomer no significant differences were observed between 2-F or 4-F addition (Figure 1). Uterus to nontarget ratios were not affected, or only slightly depressed, by A-ring fluorination. Rapid blood clearance (Figure 4) of the 4-F-(17 α ,20E)11 β -OMe-IVE₂ (12c) results in particularly favorable uterus to blood ratios of 75 and 125 at 5 h and 12 h pi, respectively (Figure 3).

The *in vivo* stability of the radioiodinated steroids is reflected in the thyroid radioactivity profiles (Figure 5). It can be seen from the low thyroid radioactivity levels that the 20E isomers are in general more stable than the

Table V. Tissue Distribution of the Isomeric 2- and 4-Fluoro-7 α -methyl-(17 α ,20E/Z)-[¹²⁵I]iodovinylestradiols in Immature Female Rats

tissue	% ID/g (SE) ^a				
	1 h	1 h (+E ₂)	3 h	5 h	12 h
[¹²⁵ I]-8b (20E Isomer, 2-F, 7 α -Methyl)					
uterus	1.03 (0.30)	0.39 (0.11)	0.85 (0.16)	0.63 (0.09)	0.35 (0.04)
blood	0.29 (0.01)	0.29 (0.02)	0.20 (0.02)	0.17 (0.02)	0.12 (0.01)
thyroid	78 (1.65)	114 (7.38)	197 (20.02)	203 (12.18)	446 (71.82)
muscle	0.61 (0.25)	0.31 (0.04)	0.15 (0.00)	0.21 (0.02)	0.17 (0.04)
fat	1.50 (0.20)	1.48 (0.13)	1.36 (0.13)	1.28 (0.13)	0.92 (0.01)
kidneys	0.63 (0.06)	0.49 (0.04)	0.29 (0.03)	0.26 (0.03)	0.15 (0.03)
spleen	0.31 (0.01)	0.45 (0.12)	0.43 (0.14)	0.18 (0.02)	0.12 (0.01)
lungs	0.54 (0.03)	0.53 (0.03)	0.28 (0.03)	0.22 (0.04)	0.12 (0.02)
liver	2.29 (0.23)	2.28 (0.41)	1.53 (0.21)	1.38 (0.04)	1.00 (0.13)
uterus/blood	4.49 (0.16)	1.34 (0.31)	4.11 (0.37)	3.69 (0.09)	2.99 (0.23)
uterus/nontarget ^b	3.30 (0.32)	1.09 (0.17)	3.00 (0.06)	3.61 (0.58)	2.55 (0.14)
[¹²⁵ I]-9b (20Z Isomer, 2-F, 7 α -Methyl)					
uterus	3.76 (0.02)	0.39 (0.02)	4.03 (0.66)	3.40 (0.02)	3.84 (0.72)
blood	0.45 (0.02)	0.32 (0.01)	0.32 (0.00)	0.33 (0.06)	0.32 (0.01)
thyroid	205 (45.10)	204 (20.61)	202 (23.24)	424 (54.88)	745 (46.03)
muscle	0.53 (0.05)	0.29 (0.00)	0.32 (0.11)	0.29 (0.05)	0.19 (0.06)
fat	2.02 (0.26)	1.58 (0.27)	1.54 (0.16)	2.20 (0.26)	0.84 (0.29)
kidneys	1.11 (0.14)	0.62 (0.03)	0.69 (0.12)	0.54 (0.10)	0.36 (0.08)
spleen	1.12 (0.16)	0.56 (0.01)	0.74 (0.07)	0.51 (0.02)	0.47 (0.15)
lungs	0.56 (0.06)	0.42 (0.02)	0.36 (0.07)	0.37 (0.07)	0.25 (0.05)
liver	2.19 (0.42)	1.83 (0.12)	1.81 (0.34)	2.51 (0.59)	1.69 (0.41)
uterus/blood	8.30 (0.04)	1.20 (0.12)	14.81 (0.68)	11.08 (2.13)	18.13 (4.20)
uterus/nontarget ^b	5.13 (0.35)	0.76 (0.23)	7.35 (0.41)	8.94 (1.07)	15.99 (4.24)
[¹²⁵ I]-12b (20E Isomer, 4-F, 7 α -Methyl)					
uterus	4.71 (0.45)	1.14 (0.13)	5.29 (0.67)	6.44 (0.71)	3.02 (0.74)
blood	0.38 (0.04)	0.44 (0.05)	0.32 (0.06)	0.39 (0.04)	0.17 (0.03)
thyroid	61 (8.70)	56 (2.10)	70 (6.37)	114 (5.88)	217 (27.71)
muscle	0.75 (0.10)	0.97 (0.06)	0.43 (0.03)	0.56 (0.08)	0.16 (0.04)
fat	0.03 (0.44)	5.65 (1.61)	4.00 (0.19)	4.59 (0.45)	1.67 (0.19)
kidneys	1.41 (0.19)	1.82 (0.23)	0.95 (0.07)	0.89 (0.12)	0.32 (0.10)
spleen	0.68 (0.11)	1.29 (0.17)	0.91 (0.22)	0.80 (0.21)	0.19 (0.05)
lungs	1.47 (0.24)	1.86 (0.23)	0.93 (0.09)	0.77 (0.08)	0.23 (0.04)
liver	4.36 (0.68)	5.84 (0.66)	3.44 (0.32)	3.60 (0.52)	1.57 (0.31)
uterus/blood	12.53 (0.60)	2.68 (0.46)	17.28 (1.84)	16.40 (0.37)	17.87 (2.44)
uterus/nontarget ^b	5.03 (0.37)	0.85 (0.15)	7.15 (1.06)	9.38 (0.74)	15.57 (1.82)
[¹²⁵ I]-13b (20Z Isomer, 4-F, 7 α -Methyl)					
uterus	3.55 (0.48)	0.50 (0.05)	4.11 (0.76)	4.39 (0.73)	3.45 (0.05)
blood	0.44 (0.06)	0.45 (0.08)	0.39 (0.08)	0.36 (0.05)	0.21 (0.05)
thyroid	145 (20.75)	98 (6.27)	304 (47.46)	380 (38.64)	898
muscle	0.45 (0.05)	0.44 (0.09)	0.28 (0.06)	0.34 (0.08)	0.16 (0.04)
fat	1.30 (0.28)	1.39 (0.21)	1.42 (0.40)	1.70 (0.37)	0.89 (0.21)
kidneys	1.07 (0.11)	0.79 (0.13)	0.85 (0.13)	0.67 (0.10)	0.29 (0.08)
spleen	1.16 (0.50)	0.69 (0.22)	0.72 (0.13)	0.57 (0.06)	0.25 (0.07)
lungs	0.71 (0.09)	0.67 (0.11)	0.46 (0.09)	0.48 (0.09)	0.24 (0.06)
liver	1.35 (0.18)	1.54 (0.39)	1.18 (0.30)	1.36 (0.34)	0.80 (0.17)
uterus/blood	8.35 (0.91)	1.16 (0.09)	10.60 (0.45)	10.01 (1.11)	16.64 (1.52)
uterus/nontarget ^b	5.41 (1.23)	0.88 (0.12)	8.32 (0.98)	9.44 (0.52)	17.02 (2.92)

^a Mean organ uptake (percent injected dose per gram of tissue) and standard error (SE) for immature female Long Evans rats (three to five animals), after injection of 3 μ Ci (111 kBq) of ¹²⁵I-labeled steroids in the presence (+E₂) or absence of 60 μ g to co-injected estradiol.

^b Nontarget organs include the muscle, spleen, and lungs.

corresponding 20Z isomers and that 11 β -methoxy substitution enhances *in vivo* deiodination. Fluorination appears to have little effect on the *in vivo* stability of the C-I bond. ER involvement in the uterus uptake process was evaluated by coinjecting some animals for the 1-h time point with unlabeled estradiol. Uterus uptake of all compounds tested decreased substantially to reach similar radioactivity levels as those observed for nontarget organs.

In conclusion, our data show that 4-F substitution of radiolabeled iodovinylestradiols favors uterus uptake whereas 2-F substitution results generally in lower target tissue specificity and diminished ER binding affinity. Both our *in vitro* and *in vivo* results confirm the importance of steroid catabolism in the localization process. Fluorination at C-2 inhibits catechol formation,⁶ and this may be reflected in the less favorable ER binding data as well as

the limited uterus uptake *in vivo*. In contrast, a C-4 fluoro substituent enhances catechol formation⁶ which coincides with favorable ER binding properties *in vitro* and good ER-mediated tissue localization *in vivo*. Our results contradict the suggestion that blocking steroid catabolism via 2-F addition could provide an improved ER-based imaging agent.²² In general, F-substitution along with 7 α -methyl or 11 β -methoxy substitution reduces uterus uptake. Similarly, simultaneous substitution with a 7 α -methyl and a 11 β -methoxy group is known to result in loss of ER binding affinity.²⁰ This suggests that more than two substituents on the estradiol molecule inhibit ligand-receptor interactions, possibly due to small conformational changes of the steroid skeleton or purely physicochemical parameters (van der Waals' volume and isopotential surfaces surrounding the molecule).

Table VI. Tissue Distribution of the Isomeric 2- and 4-Fluoro-11 β -methoxy-(17 α ,20 E/Z)-[¹²⁵I]iodovinylestradiols in Immature Female Rats

tissue	% ID/g (SE) ^a				
	1 h	1 h (+E ₂)	3 h	5 h	12 h
[¹²⁵ I]-8c (20 E Isomer, 2-F, 11 β -Methoxy)					
uterus	4.41 (1.30)	0.81 (0.16)	3.23 (0.85)	3.90 (0.45)	1.20 (0.40)
blood	0.45 (0.03)	0.51 (0.13)	0.38 (0.08)	0.32 (0.07)	0.11 (0.02)
thyroid	224 (15.53)	243 (14.94)	355 (70.27)	412 (44.81)	571 (9.23)
muscle	0.87 (0.06)	1.02 (0.09)	0.56 (0.13)	0.45 (0.08)	0.07 (0.00)
fat	3.34 (0.37)	4.31 (0.37)	1.82 (0.15)	1.57 (0.14)	0.45 (0.01)
kidneys	1.44 (0.23)	1.58 (0.12)	0.96 (0.21)	1.52 (0.62)	0.15 (0.01)
spleen	0.83 (0.07)	1.04 (0.05)	0.80 (0.23)	0.82 (0.25)	0.18 (0.01)
lungs	0.95 (0.09)	1.22 (0.06)	0.62 (0.13)	0.51 (0.06)	0.13 (0.00)
liver	3.50 (0.31)	4.40 (0.17)	3.56 (0.30)	3.11 (0.51)	1.48 (0.11)
uterus/blood	9.82 (2.91)	1.60 (0.10)	8.28 (2.83)	12.41 (3.06)	10.45 (1.43)
uterus/nontarget ^b	4.78 (0.56)	0.86 (0.07)	8.20 (0.71)	8.56 (2.28)	6.75 (0.69)
[¹²⁵ I]-9c (20 Z Isomer, 2-F, 11 β -Methoxy)					
uterus	8.36 (1.03)	0.94 (0.21)	9.59 (2.59)	8.81 (1.32)	6.45 (0.72)
blood	0.54 (0.08)	0.87 (0.37)	0.45 (0.09)	0.34 (0.08)	0.17 (0.08)
thyroid	217 (4.52)	170 (5.52)	882 (48.96)	1210 (58.87)	1899 (344.9)
muscle	0.58 (0.06)	2.30 (0.09)	0.38 (0.04)	0.29 (0.02)	0.10 (0.01)
fat	1.71 (0.20)	4.78 ()	1.15 (0.12)	0.66 (0.11)	0.13 (0.01)
kidneys	1.56 (0.13)	2.83 (0.70)	1.02 (0.07)	0.71 (0.05)	0.29 (0.01)
spleen	0.59 (0.09)	5.30 (1.14)	0.39 (0.54)	0.38 (0.03)	0.22 (0.04)
lungs	0.76 (0.04)	1.52 (0.40)	0.54 (0.05)	0.35 (0.03)	0.13 (0.01)
liver	2.04 (0.48)	10.39 (4.47)	1.51 (0.10)	1.12 (0.05)	0.65 (0.09)
uterus/blood	15.71 (0.97)	1.12 (0.16)	21.19 (2.61)	27.80 (2.46)	50.35 (6.35)
uterus/nontarget ^b	13.04 (0.16)	0.64 (0.16)	21.65 (2.11)	25.79 (1.44)	33.82 (5.57)
[¹²⁵ I]-12c (20 E Isomer, 4-F, 11 β -Methoxy)					
uterus	8.02 (0.45)	1.02 (0.02)	10.45 (1.53)	12.04 (2.17)	12.64 (0.37)
blood	0.26 (0.01)	0.28 (0.02)	0.26 (0.05)	0.17 (0.01)	0.10 (0.00)
thyroid	61 (2.84)	71 (6.60)	126 (14.01)	274 (36.73)	475 (35.19)
muscle	0.98 (0.04)	0.91 (0.05)	0.56 (0.03)	0.45 (0.10)	0.25 (0.01)
fat	2.68 (0.32)	2.30 (0.50)	2.04 (0.10)	1.69 (0.16)	0.83 (0.13)
kidneys	2.03 (0.13)	1.28 (0.09)	1.30 (0.05)	0.94 (0.11)	0.49 (0.03)
spleen	0.79 (0.04)	0.78 (0.07)	0.48 (0.03)	0.39 (0.04)	0.22 (0.01)
lungs	1.07 (0.06)	1.95 (0.74)	0.69 (0.03)	0.52 (0.03)	0.30 (0.02)
liver	3.32 (0.14)	3.24 (0.34)	2.65 (0.28)	2.72 (0.21)	2.28 (0.15)
uterus/blood	30.35 (0.68)	3.57 (0.28)	44.23 (11.02)	68.66 (8.33)	122.53 (4.27)
uterus/nontarget ^b	9.17 (0.71)	1.02 (0.09)	16.64 (1.15)	26.50 (2.70)	50.90 (2.50)
[¹²⁵ I]-13c (20 Z Isomer, 4-F, 11 β -Methoxy)					
uterus	6.87 (0.70)	1.06 (0.13)	7.48 (0.40)	10.96 (0.46)	8.31 (0.50)
blood	0.66 (0.03)	0.81 (0.04)	0.56 (0.02)	0.51 (0.05)	0.25 (0.04)
thyroid	551 (96.45)	441 (26.49)	1446 (178.9)	2369 (229.7)	4651 (662.1)
muscle	0.66 (0.06)	0.77 (0.08)	0.48 (0.02)	0.40 (0.01)	0.27 (0.04)
fat	1.43 (0.11)	1.82 (0.35)	1.13 (0.06)	0.75 (0.08)	0.47 (0.10)
kidneys	1.64 (0.04)	1.36 (0.18)	1.08 (0.07)	1.08 (0.15)	1.23 (0.36)
spleen	2.04 (1.24)	1.28 (0.45)	0.72 (0.15)	0.66 (0.06)	0.70 (0.14)
lungs	0.84 (0.05)	0.95 (0.04)	0.66 (0.05)	0.51 (0.03)	0.37 (0.02)
liver	2.00 (0.12)	2.77 (0.33)	1.21 (0.43)	1.42 (0.17)	0.83 (0.08)
uterus/blood	10.55 (1.21)	1.30 (0.11)	13.35 (0.12)	20.08 (1.34)	34.58 (6.05)
uterus/nontarget ^b	7.30 (1.45)	1.11 (0.04)	11.77 (0.57)	21.03 (1.45)	19.35 (3.49)

^a Mean organ uptake (percent injected dose per gram of tissue) and standard error (SE) for immature female Long Evans rats (three to five animals), after injection of 3 μ Ci (111 kBq) of [¹²⁵I]-labeled steroids in the presence (+E₂) or absence of 60 μ g co-injected estradiol. ^b Nontarget organs include the muscle, spleen, and lungs.

Finally, rapid blood clearance of some of the multi-substituted fluorinated estradiols results in high uterus to blood ratios, which could render selected derivatives, particularly the 4-F-(17 α ,20 E)11 β -OMe-IVE₂ (12c), suitable for ¹²⁵I labeling and SPECT imaging of ER-rich tissues.

Experimental Section

All chemicals used are commercially available and were of the highest chemical grade available; carrier-free [¹²⁵I]NaI was purchased from Amersham Canada Ltd. Steroids were purchased from Steraloids Inc. or Sigma. 17-Ethynedioxyestra-1,3,5(10)-triene-3,11 β -diol was synthesized according to Baran²³ and converted to 11 β -methoxyestrone with methyl iodide. Melting points (mp) were determined on a Fisher-Johns apparatus and are uncorrected.

¹H NMR spectra were obtained with Bruker WM 25 spectrometer, in CDCl₃ + DMSO-*d*₆. Chemical shifts are reported in ppm downfield from tetramethylsilane as an integral standard (δ scale). Low-resolution electron impact mass spectra (EIMS) were acquired at 70 eV with a Hewlett-Packard Model 5988A quadrupole instrument. Microanalysis data were obtained by Guelph Laboratories Ltd., Canada.

Analytical thin-layer chromatography (TLC) was performed on polygram silica gel plates coated with fluorescent indicator (UV 254). Visualization was achieved with shortwave ultraviolet light absorbance and/or color response upon spraying with H₂SO₄/EtOH and heating at 120 °C. Column chromatography was performed on silica gel (60–200 mesh). High performance liquid chromatography (HPLC) was performed on a reverse-phase column (C-18, ODS-2 spherisorb, 5 μ m, 25 \times 0.94 cm, CSC, Montreal, Canada), and the compounds were detected at 280 nm

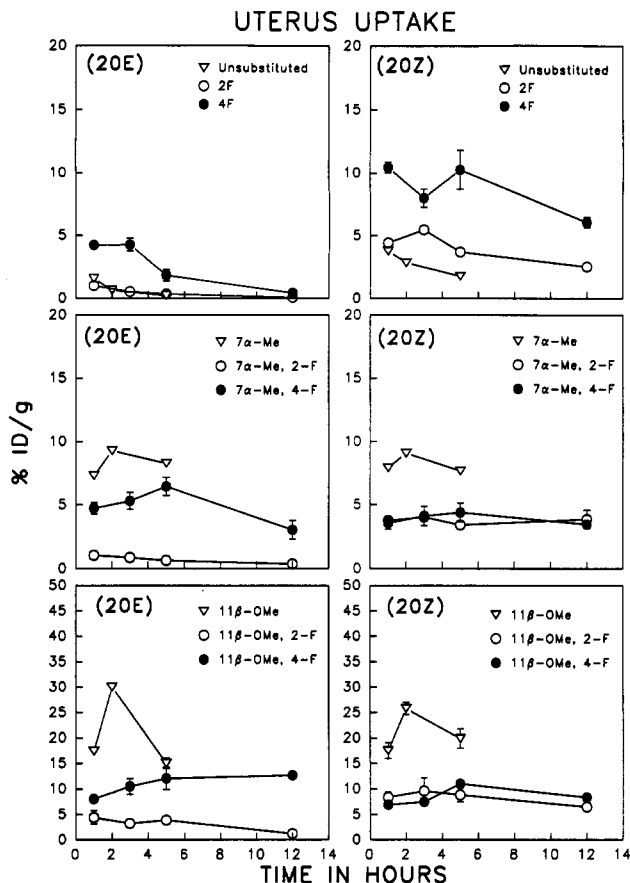


Figure 1. Uterus uptake of ^{125}I -labeled 2-F and 4-F-(17 α ,20E/Z)iodovinylestradiols and the corresponding 7 α -methyl and 11 β -methoxy derivatives in immature female Long Evans rats. Values for the nonfluorinated derivatives were taken from the literature.^{4a,b} The error bars represent the standard error: uterus radioactivity in % ID/g.

and where appropriate, by their γ -radiation which was registered via a sodium iodide detector.

Reaction of *N*-Fluoropyridinium triflate with Estrone Derivatives 1a–c. 7 α -Methylestrone (1b) (1 g) or 11 β -methoxyestrone (1c) (1 g) was refluxed in 1,1,2-trichloroethane (10–20 mL) under nitrogen for 4–6 h, in the presence of *N*-fluoropyridinium triflate (1.5 g). Solvent was removed under reduced pressure, and the residue was extracted with dichloromethane, washed with water, and dried over sodium sulfate. After filtration, solvent was removed under reduced pressure, and the dark residue was purified by column chromatography over silica gel. Elution with 10–30% ethyl acetate in hexane gave the following compounds.

2-Fluoro-7 α -methylestrone (2b) and 4-Fluoro-7 α -methylestrone (3b) (mixture). ^1H NMR (δ) 0.80 (d, J = 8 Hz, 3H, 7 α -CH₃), 0.83 (d, J = 8 Hz, 3H, 7 α -CH₃), 1.19 (s, 6H, 18-CH₃), 6.68 (d, J = 9 Hz, 1H, C₄-H), 6.9 (d, J = 13 Hz, 1H, C₁-H), 6.8–6.85 (m, 1H, C₂ and C₁-H); MS m/z (rel intensity) 302 (M^+ , 100), 269 (10), 245 (19).

2-Fluoro-11 β -methoxyestrone (2c). HPLC in 50:50 acetonitrile/ H_2O , t_R = 20 min; ^1H NMR (δ) 0.96 (s, 3H, 18-CH₃), 3.18 (s, 3H, 11 β -OCH₃), 3.96 (m, 1H, 11 α -H), 6.54 (d, J = 9 Hz, 1H, C₄-H), 6.66 (d, J = 13 Hz, C₁-H); MS m/z (rel intensity) 318 (100), 286 (20), 259 (54).

4-Fluoro-11 β -methoxyestrone (3c). HPLC in 50:50 acetonitrile/ H_2O , t_R = 24 min; ^1H NMR 0.636 (s, 3H, 18-CH₃), 2.84 (s, 3H, 11 β -OCH₃), 3.70 (m, 1H, 11 α -H), 6.29–6.34 (m, C₂ and C₁-H); MS m/z (rel intensity) 318 (60), 277 (24), 270 (100), 258 (28).

Reaction of Lithium Acetylide–Ethylenediamine Complex with 2a–c and 3a–c. A solution of 2 or 3 (1 mmol) in dry dimethyl sulfoxide (10 mL) under nitrogen was treated with lithium acetylide–ethylenediamine complex (300 mg), and the mixture was stirred at room temperature for 16–20 h. The mixture

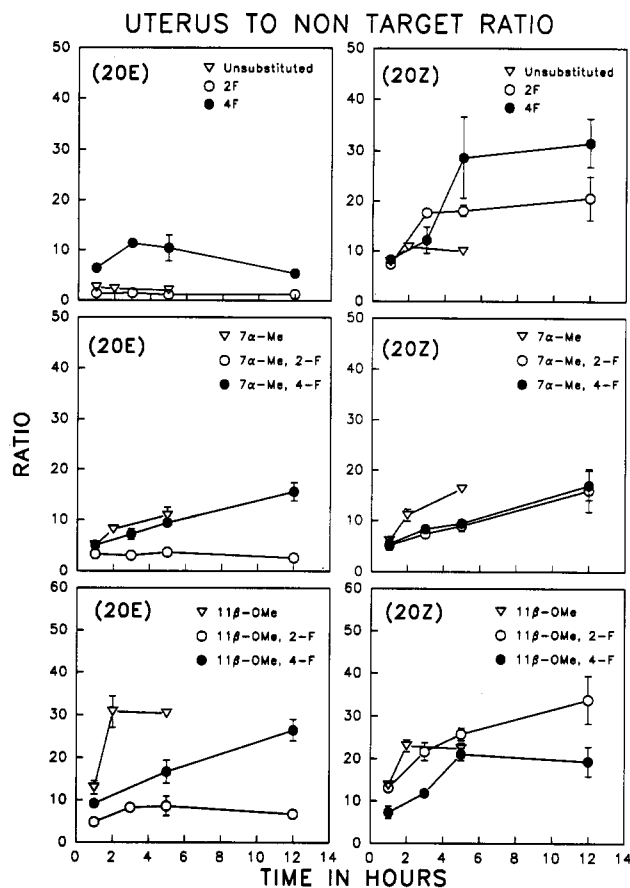


Figure 2. See Figure 1: uterus to nontarget (lungs, spleen, and muscle) ratios.

was poured into cold water, acidified with dilute acetic acid, extracted with ethyl acetate, washed with water and brine, and dried over sodium sulfate. After evaporation of the solvent under reduced pressure, the residue was chromatographed on silica gel with 10–20% ethyl acetate in hexane to yield the desired compound. All melting points and spectral properties of the products are presented in Table I.

Synthesis of Fluorinated (17 α ,20E)- and (17 α ,20Z)-21-(Tri-*n*-butylstannyl)vinylestradiols. Method A: With Triethylborane. A hexane solution of triethylborane (1 M, 0.1 mL, 0.1 mmol) was added to a solution of 4b, 5b, 4c, or 5c (0.1 mmol) and tri-*n*-butyltin hydride (0.2 mmol) in THF (5 mL) at room temperature under nitrogen. After stirring 30–60 min at room temperature, the THF was removed under reduced pressure.

Method B: With Azobisisobutyronitrile. A mixture of 4a or 5a (0.1 mmol) in 5 mL of toluene and 0.1 mL of tri-*n*-butyltin hydride (0.33 mmol) was heated at 95–100 °C for 1–2 h in the presence of azobisisobutyronitrile (8 mg, 0.5 mmol) under nitrogen.

Purification. The residue obtained with either method A or B was chromatographed on silica gel (10 g). Elution with 3–5% EtOAc in hexane gave mixtures which were further purified by HPLC on a reverse phase semipreparative column with a gradient of 5% H_2O in MeOH to 100% MeOH (20 min).

2-Fluoro-(17 α ,20E)-21-(tri-*n*-butylstannyl)vinylestradiol (6a). HPLC, t_R = 20 min; MS m/z (rel intensity) 604 (M^+ , 0.2), 602 (M^+ , 0.2), 586 (M^+ – H_2O , 16), 548 (M^+ – C_4H_9 , 100), 547 (49), 546 (77), 544 (43), 530 (41), 493 (22), 491 (18), 475 (43), 315 (54).

2-Fluoro-(17 α ,20Z)-21-(tri-*n*-butylstannyl)vinylestradiol (7a). HPLC, t_R = 24 min; MS m/z (rel intensity) 604 (M^+ , 0.2), 602 (M^+ , 0.2), 565 (M^+ – H_2O , 0.3), 544 (7), 531 (32), 529 (78), 525 (10), 489 (5), 487 (3), 435 (12), 433 (10), 360 (22), 315 (100).

4-Fluoro-(17 α ,20E)-21-(tri-*n*-butylstannyl)vinylestradiol (10a). HPLC, t_R = 17 min; MS m/z (rel intensity) 604 (M^+ , 0.2), 548 (M^+ – C_4H_9 , 100), 545 (21), 546 (77), 544 (43), 531 (29), 497 (4), 495 (4), 493 (21), 491 (18), 491 (187), 489 (10), 417 (8), 415 (8), 352 (4), 315 (23).

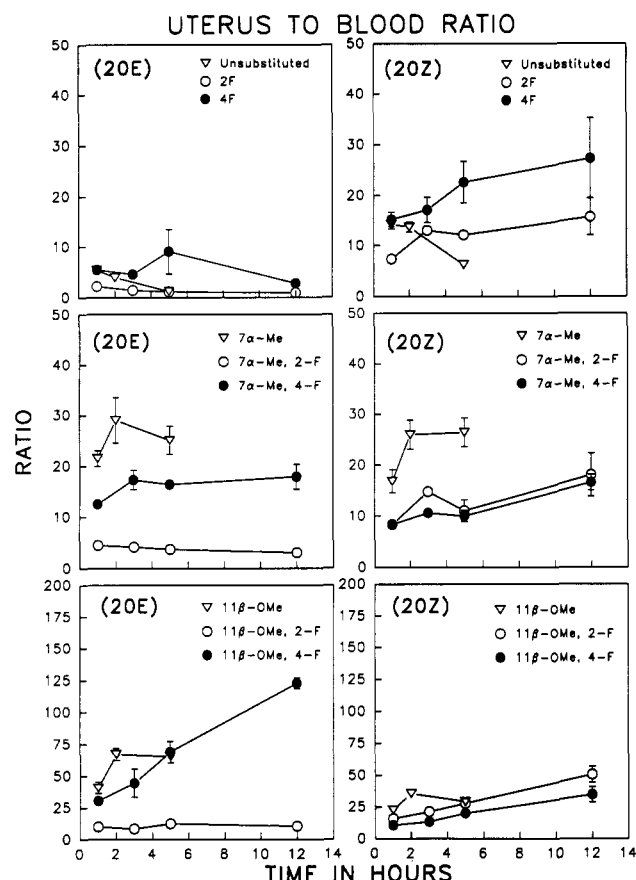


Figure 3. See Figure 1: uterus to blood ratios.

4-Fluoro-(17 α ,20Z)-21-(tri-*n*-butylstannyl)vinylestradiol (11a). HPLC, t_R = 23 min; MS m/z (rel intensity) 604 (M^+ , 2), 548 (M^+ - C_4H_9 , 54), 547 (M^+ - C_4H_9 , 78), 545 (M^+ - C_4H_9 , 21), 531 (56), 530 (43), 493 (10), 491 (21), 489 (19), 435 (48), 434 (22), 417 (27), 415 (23), 360 (23), 358 (18), 315 (77), 314 (100).

2-Fluoro-7 α -methyl-(17 α ,20E)-21-(tri-*n*-butylstannyl)vinylestradiol (6b). HPLC, t_R = 18 min; MS m/z (rel intensity) 618 (M^+ , 0.3), 567 (16), 563 (M^+ - C_4H_9 , 100), 562 (50), 561 (78), 507 (23), 505 (18), 489 (5), 461 (4), 431 (11), 366 (3), 329 (35).

2-Fluoro-7 α -methyl-(17 α ,20Z)-21-(tri-*n*-butylstannyl)vinylestradiol (7b). HPLC, t_R = 25 min; MS m/z (rel intensity) 618 (M^+ , 1), 562 (M^+ - C_4H_9 , 18), 560 (16), 545 (19), 544 (13), 543 (16), 485 (6), 449 (19), 447 (20), 431 (14), 429 (13), 360 (31), 358 (25), 329 (18), 316 (20), 314 (100).

4-Fluoro-7 α -methyl-(17 α ,20E)-21-(tri-*n*-butylstannyl)vinylestradiol (10b). HPLC, t_R = 16 min; MS m/z (relative intensity) 563 (M^+ - C_4H_9 , 100), 562 (48), 561 (54), 548 (38), 507 (19), 505 (13), 429 (10), 329 (5), 310 (26), 290 (23), 245 (43).

4-Fluoro-7 α -methyl-(17 α ,20Z)-21-(tri-*n*-butylstannyl)vinylestradiol (11b). HPLC, t_R = 23 min; MS m/z (rel intensity) 619 (3), 564 (M^+ - C_4H_9 , 14), 546 (17), 544 (19), 449 (11), 447 (12), 445 (10), 329 (10), 314 (55), 290 (38), 235 (100).

2-Fluoro-11 β -methoxy-(17 α ,20E)-21-(tri-*n*-butylstannyl)vinylestradiol (6c). HPLC, t_R = 12 min; MS m/z (rel intensity) 583 (19), 580 (31), 579 (M^+ - C_4H_9 , 100), 578 (52), 577 (72), 576 (35), 575 (42), 523 (10), 321 (8), 294 (19), 290 (19), 242 (18), 232 (26).

2-Fluoro-11 β -methoxy-(17 α ,20Z)-21-(tri-*n*-butylstannyl)vinylestradiol (7c). HPLC, t_R = 16 min; MS m/z (rel intensity) 638 (M^+ , 5), 634 (M^+ , 3), 579 (M^+ - C_4H_9 , 49), 576 (43), 574 (22), 560 (100), 559 (51), 558 (72), 556 (45), 526 (26), 465 (17), 413 (18), 294 (24), 268 (29), 244 (41).

4-Fluoro-11 β -methoxy-(17 α ,20E)-21-(tri-*n*-butylstannyl)vinylestradiol (10c). HPLC, t_R = 15 min; MS m/z (rel intensity) 638 (6), 637 (M^+ , 6), 635 (M^+ , 6), 633 (3), 584 (5), 579 (M^+ - C_4H_9 , 21), 577 (18), 561 (25), 560 (26), 559 (30), 466 (9), 432 (11), 393 (11), 315 (29), 310 (16), 295 (23), 291 (27), 248 (22), 235 (100).

4-Fluoro-11 β -methoxy-(17 α ,20Z)-21-(tri-*n*-butylstannyl)vinylestradiol (11c). HPLC, t_R = 21 min; MS m/z (rel intensity)

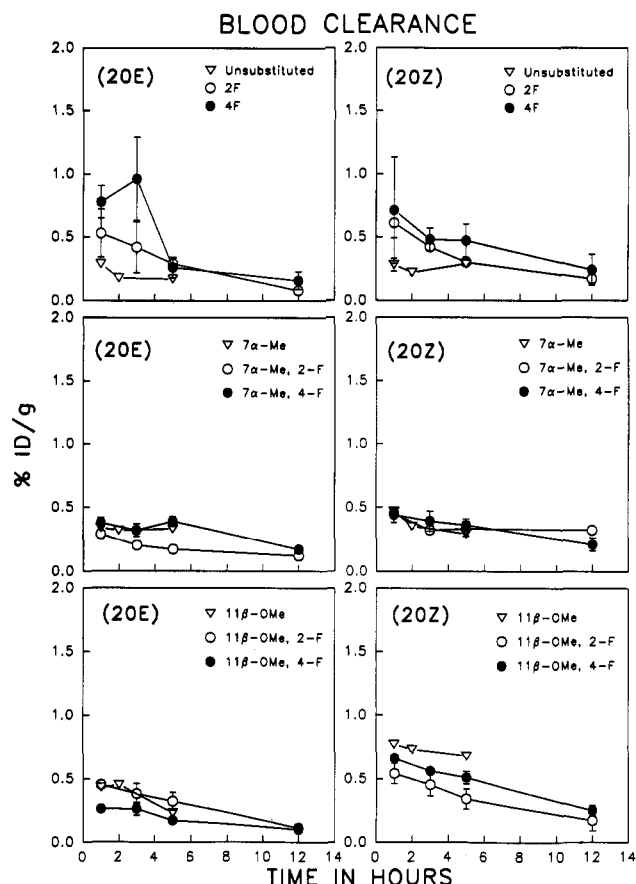


Figure 4. Blood clearance in % ID/g of ^{125}I -labeled 2-F and 4-F-(17 α ,20E/Z)iodovinylestradiols and the corresponding 7 α -methyl and 11 β -methoxy derivatives in immature female Long Evans rats. Values for the nonfluorinated derivatives were taken from the literature.^{4a,b} The error bars represent the standard error.

638 (M^+ , 2), 636 (2), 635 (M^+ , 2), 583 (15), 580 (18), 579 (M^+ - C_4H_9 , 95), 578 (41), 577 (41), 576 (61), 575 (49), 473 (3), 414 (7), 291 (41), 231 (100).

Conditions for the Preparation of (17 α ,20E/Z)Iodovinyl Derivatives from the Corresponding (17 α ,20E/Z)-21-(Tri-*n*-butylstannyl)vinylestradiol Intermediates. To compounds 6a-c, 7a-c, 10a-c, and 11a-c (0.1 mmol) in chloroform (5 mL) was gradually added at room temperature a 0.1 M solution of iodine in chloroform until the color of iodine persisted. This was followed sequentially by the addition of 0.2 mL of 1 M KF in methanol and 0.2 mL of 5% aqueous sodium bisulfite. The mixture was then extracted with chloroform (2 \times 15 mL). The organic phase was dried over magnesium sulfate (anhydrous), filtered, and evaporated to dryness. The residue was purified on a C-18 reverse-phase semipreparative HPLC column in methanol/water. All physical and spectroscopic data of the products are given in Table I.

2- and 4-Fluoro-(17 α ,20E)-21-[^{125}I]iodovinylestradiols ([^{125}I]-8a-c and [^{125}I]-12a-c). To a mixture of 6a-c or 10a-c (100 μg) and 50 μL of a 5% (w/v) solution of NaOAc in glacial AcOH was added [^{125}I]NaI (500 μCi), followed by 50 μL of an oxidant solution consisting of a 2:1 mixture (v/v) of H_2O_2 (30%)/AcOH. After stirring at room temperature for 10 min, the reaction was terminated by the addition of 25 μL of an aqueous 5% NaHSO_3 solution (w/v). The mixture was extracted with CH_2Cl_2 and dried under a stream of nitrogen. The residue (450 μCi , 90%) was dissolved in MeOH and purified on an analytical C-18 reverse phase HPLC column operated at a slow rate of 1 mL/min. Elution with 70:30 MeOH/ H_2O gave [^{125}I]-8a (350 μCi , t_R = 23 min), [^{125}I]-12a (350 μCi , t_R = 21 min), [^{125}I]-8b (350–400 μCi , 70–80%, t_R = 15 min), [^{125}I]-12b, t_R = 21 min; [^{125}I]-8c, t_R = 28 min; and [^{125}I]-12c, t_R = 23 min. The retention times of free iodine was 4 min.

2- and 4-Fluoro-(17 α ,20Z)-21-[^{125}I]iodovinylestradiols ([^{125}I]-9a-c and [^{125}I]-13a-c). To a solution of [^{125}I]NaI (500

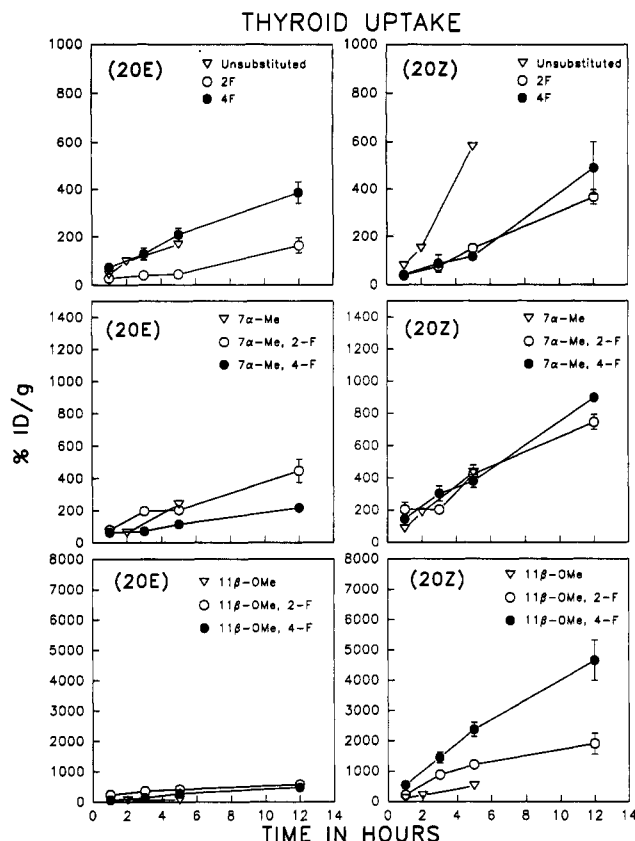


Figure 5. Thyroid uptake in % ID/g of ^{125}I -labeled 2-F and 4-F ($17\alpha,20E/Z$)iodovinylestradiols and the corresponding 7α -methyl and 11β -methoxy derivatives in immature female Long Evans rats. Values for the nonfluorinated derivatives were taken from the literature.^{4a,b} The error bars represent the standard error.

μCi) in 0.5 mL of H_2O was added 0.2 mg of chloramine-T in 0.2 mL of H_2O and of 2 mL of CHCl_3 . The reaction mixture was stirred for 5 min, the CHCl_3 layer was allowed to separate, 100 μg of 7α -c or 11α -c in CHCl_3 (0.2 mL) was added to the iodine solution, and the mixture was stirred at room temperature for 5–8 min. The reaction was terminated by the addition of 25 μL of a 5% NaHSO_3 solution in water. Products were purified in a similar manner as described above. After HPLC purification we obtained in 60–70% overall yield: $[^{125}\text{I}]\text{-9a}$, $t_R = 22$ min; $[^{125}\text{I}]\text{-13a}$, $t_R = 21$ min; $[^{125}\text{I}]\text{-9b}$, $t_R = 29$ min; $[^{125}\text{I}]\text{-13b}$, $t_R = 26$ min; $[^{125}\text{I}]\text{-9c}$, $t_R = 21$ min; $[^{125}\text{I}]\text{-13c}$, $t_R = 18$ min.

Estrogen Receptor Binding Assay. Affinity of the estradiol derivatives for estrogen receptors was determined by a competitive binding assay and is expressed as the relative binding affinity.¹⁶ The RBA is defined as 100 times the ratio between competitor and the unlabeled estradiol concentrations required for 50% competition to specific $[^3\text{H}]\text{estradiol}$ binding. Murine uterine cytoplasmic extracts were incubated at 0–4 $^\circ\text{C}$ for 18 h with 20 nM of $[^3\text{H}]\text{estradiol}$ in the absence and presence of competitive steroids ranging from 2 nM to 20 μM . Selected compounds were also incubated for 2 h at 4 and at 25 $^\circ\text{C}$. 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 in accordance with the recommendations of the Canadian Council on Animal Care and of the in-house Ethic Committee for Animal Experiments as previously described.^{4b} Briefly, immature female Long Evans rats (21–24 days, 45–70 g) from our in-house breeding facilities were injected with 200 μL of the ^{125}I -labeled preparation (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. Prior to injection all solutions were filtered over a 0.22 μm filter. Animals were sacrificed under deep ether anesthesia by severing the axillary artery, followed by chest opening.²⁴ 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 gamma counter (LKB Wallac, Finland), concentrations were expressed as percent of the injected dose per gram of tissue (% ID/g), and the standard error was used as a measure of the dispersion around the mean.²⁵

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 Quebec. We thank Dr. G. Shyamala from the Lady Davis Institute for Medical Research, Montreal, Canada, for conducting the receptor binding assays and Ms. E. Horvath for skillful technical assistance.

References

- (1) Presented in part at the IXth International Symposium on Radiopharmaceutical Chemistry, Paris, France, 6–10 April 1992; Ali, H.; Rousseau, J.; van Lier, J. E. Synthesis, target-tissue uptake of the 7α -methyl and 11β -methoxy derivatives of the 20Z and 20E isomers of 17α - $[^{125}\text{I}]\text{iodovinyl-2- and 4-fluoroestradiol}$. *J. Label. Compd. Radiopharm.* 1993, 32, 225–227.
- (2) (a) Counsell, R. E.; Klausmeier, W. H. Radiotracer interactions with sex steroid hormone receptor proteins (receptor mapping). In *Principles of Radiopharmacology*; Colombetti, L. G., Ed.; CRC Press: Boca Raton, FL, 1979; Vol. II, pp 59–91. (b) Eckelman, W. C.; Reba, R. C. Labeled estrogens and analogues. In *Radiopharmaceuticals: Structure-Activity Relationships*; Spencer, R. P., Ed.; Grune and Stratton: New York, 1981; pp 449–458. (c) Katzenellenbogen, J. A. The pharmacology of steroid radiopharmaceuticals: specific and non-specific binding and uptake selectivity. In *Radiopharmaceuticals Chemistry and Pharmacology*; Nunn, A. D., Ed.; Marcel Dekker: New York, NY, 1992; pp 297–331.
- (3) Hochberg, R. B. Iodine-125-labeled estradiol: a gamma emitting analog of estradiol that binds to the estrogen receptor. *Science* 1979, 205, 1138–1140. (b) Zielinski, J. E.; Yabuki, H.; Pahuja, S. L.; Larner, J. M.; Hochberg, R. B. 16α - $[^{125}\text{I}]\text{iodo-11}\beta$ -methoxy-17 β -estradiol: a radiochemical probe for estrogen-sensitive tissues. *Endocrinology* 1986, 119, 130–139. (c) Zielinski, J. E.; Larner, J. M.; Hoffer, P. B.; Hochberg, R. B. The synthesis of 11β -methoxy- $[16\alpha\text{-}^{125}\text{I}]\text{iodoestradiol}$ and its interaction with the estrogen receptor in vivo and in vitro. *J. Nucl. Med.* 1989, 30, 209–215. (d) Ali, H.; Rousseau, J.; van Lier, J. E. 7α -Methyl- and 11β -ethoxy-substitution of $[^{125}\text{I}]\text{-16}\alpha$ -iodoestradiol: effect on estrogen receptor-mediated target tissue uptake. *J. Med. Chem.* 1993, 36, 264–271.
- (4) (a) Ali, H.; Rousseau, J.; Ghaffari, M. A.; van Lier, J. E. Synthesis, receptor binding and tissue distribution of ($17\alpha,20E$)- and ($17\alpha,20Z$)-21- $[^{125}\text{I}]\text{iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol}$. *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 ($17\alpha,20E$)- and ($17\alpha,20Z$)-21- $[^{125}\text{I}]\text{iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diols}$. *J. Med. Chem.* 1991, 34, 854–860. (c) Jagoda, E. M.; Gibson, R. E.; Goodgold, H.; Ferreira, N.; Francis, B. E.; Reba, R. C.; Rzeszutarski, W. J.; Eckelman, W. C. [^{125}I]17 α -iodovinyl 11β -methoxyestradiol: in vivo and in vitro properties of a high-affinity estrogen-receptor radiopharmaceutical. *J. Nucl. Med.* 1984, 25, 472–477. (d) Nakatsuka, I.; Ferreira, N. L.; Eckelman, W. C.; Francis, B. E.; Rzeszutarski, W. J.; Gibson, R. E.; Jagoda, E. M.; Reba, R. C. Synthesis and evaluation of ($17\alpha,20E$)-21- $[^{125}\text{I}]\text{iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol}$ and ($17\alpha,20E$)-21- $[^{125}\text{I}]\text{iodo-11}\beta$ -methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (17α -iodovinyl)estradiol derivatives) as high specific activity potential radiopharmaceuticals. *J. Med. Chem.* 1984, 27, 1287–1291. (e) McManaway, M. E.; Jagoda, E. M.; Kasid, A.; Eckelman, W. C.; Francis, B. E.; Larson, S. M.; Gibson, R. E.; Reba, R. C.; Lippman, M. E. [^{125}I]17- α -iodovinyl 11β -Methoxyestradiol interaction in vivo with estrogen receptors in hormone-independent MCF-7 human breast cancer transfected with the V-ras^H oncogene. *Cancer Res.* 1987, 47, 2945–2949. (f) Hanson, R. N.; Franke, L. A.; Kaplan, M. M. Radioiodinated ligands for the estrogen receptor: Tissue distribution of 17α - $[^{125}\text{I}]\text{iodovinyl estradiol}$ derivatives in normal and tumor-bearing adult female rats. *Nucl. Med. Biol.* 1990, 17, 239–245. (g) Merrick, M. V.; Corrie, J. E. T.; Millar, A. M.; Hawkins, R. A. A reevaluation of an agent

- proposed for imaging oestrogen receptors: 17 α -[¹²⁵I]iodovinyl-11 β -methoxyoestradiol-3-methyl ether ([¹²⁵I]VMEME). *Nucl. Med. Biol.* 1988, 15, 327-332. (h) Hanson, R. N.; Franke, L. A.; Kaplan, M. L. Synthesis and evaluation of (17 α ,20E)-21-[¹²⁵I]iodo-11-substituted-19-norpregna-1,3,5(10),20-tetraene-3,17 β -diols: the influence of 11-stereochemistry on tissue distribution of radioiodinated estrogens. *Nucl. Med. Biol.* 1989, 16, 3-9. (i) Foulon, C.; Guilloteau, D.; Baulieu, J. L.; Ribeiro-Barras, M. J.; Desplanches, G.; Frangin, Y.; Besnard, J. C. Estrogen receptor imaging with 17 α -[¹²⁵I]iodovinyl-11 β -methoxyestradiol (MIVE₂)-Part I. Radiotracer preparation and characterization. *Nucl. Med. Biol.* 1992, 19, 257-261. (j) Ribeiro-Barras, M. J.; Foulon, C.; Baulieu, J. L.; Guilloteau, D.; Bougnoux, P.; Lansac, J.; Besnard, J. C. Estrogen receptor imaging with 17 α -[¹²⁵I]iodovinyl-11 β -methoxy estradiol (MIVE₂)-Part II. Preliminary results in patients with breast carcinoma. *Nucl. Med. Biol.* 1992, 19, 263-267.
- (5) Guengerich, F. P. Metabolism of 17 α -ethynylestradiol in humans. *Life Science* 1990, 47, 1981-1988.
- (6) (a) Li, J. J.; Purdy, R. H.; Appelman, E. H.; Klicka, J. K.; Li, S. A. Catechol formation of fluoro- and bromo-substituted estradiols by hamster liver microsomes: evidence for dehalogenation. *Mol. Pharmacol.* 1985, 27, 559-565. (b) Utne, T.; Jobson, R. B.; Babson, R. D. The synthesis of 2- and 4-fluoroestradiol. *J. Org. Chem.* 1968, 33, 2469-2473. (c) Liehr, J. G. 2-Fluoroestradiol: separation of estrogenicity from carcinogenicity. *Mol. Pharmacol.* 1983, 23, 278-281.
- (7) Longcope, C.; Rafkind, I.; Arunachalam, T.; Caspi, E. Biological activities of 4-fluoroestrogen analogues. *J. Steroid Biochem.* 1983, 19, 1325-1328.
- (8) Eakins, M. N.; Palmer, A. J.; Waters, S. L. Studies in the rat with ¹⁸F-4-fluoroestradiol and ¹⁸F-4-fluoroestrone as potential prostate scanning agents: comparison with ¹²⁵I-2-Iodoestradiol and ¹²⁵I-2,4-Diiodoestradiol. *Int. J. Applied Rad. and Isotopes* 1979, 30, 695-700.
- (9) Patrick, T. B.; Darling, D. L. Fluorination of activated aromatic system with cesium fluoroxysulfate. *J. Org. Chem.* 1986, 51, 3242-3244.
- (10) Umemoto, T.; Fukami, S.; Tomizawa, G.; Harasawa, K.; Kawada, K.; Tomita, K. Power and structure-variable fluorinating agents. The *N*-fluoropyridinium salt system. *J. Am. Chem. Soc.* 1990, 112, 8563-8575.
- (11) Bulman Page, P. C.; Hussain, F.; Maggs, J. L.; Morgan, P.; Kevin Park, B. Efficient regioselective A-ring functionalization of oestrogens. *Tetrahedron* 1990, 46, 2059-2068.
- (12) Huffman, J. W.; Arapakos, P. G. Synthesis of some tricyclic steroid analogs. *J. Org. Chem.* 1965, 30, 1604-1607.
- (13) Hanson, R. N.; El-Wakil, H. Electrophilic destannylation: Stereospecific introduction of electrophiles at the 21 position of (17 α)-19-Norpregna-1,3,5(10),20-tetraene-3-17 β -diols. *J. Org. Chem.* 1987, 52, 3687-3688.
- (14) (a) Napolitano, E.; Fiaschi, R.; Hanson, R. N. Structure-activity relationship of estrogenic ligands: synthesis and evaluation of (17 α ,20E)- and (17 α ,20Z)-21-halo-19-norpregna-1,3,5(10),20-tetraene-3,17 β -diols. *J. Med. Chem.* 1991, 34, 2754-2759. (b) Hanson, R. N.; Napolitano, E.; Fiaschi, R. Structure-activity relationships for 17 α -E/Z vinyl and 11-substituted-17 α -E/Z vinyl estradiols. *J. Label. Compds. Radiopharm.* 1991, 30, 437-438.
- (15) Nozaki, K.; Oshima, K.; Utimoto, K. Et₃B-induced radical addition of R₃SnH to acetylenes and its application to cyclization reaction. *J. Am. Chem. Soc.* 1987, 109, 2547-2549.
- (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) DaSilva, J. N.; van Lier, J. E. Synthesis and structure-affinity of a series of 7 α -undecylestradiol derivatives: a potential vector for therapy and imaging of estrogen receptor-positive cancers. *J. Med. Chem.* 1990, 33, 430-434.
- (18) Raynaud, J. P.; Ojasoo, T. Receptor binding as a tool in the development of selective new bioactive steroids and non steroids. In *Innovative Approaches in Drug Research*; Harms, A. F., Ed.; Elsevier Science: Amsterdam, 1986; pp 47-72.
- (19) Ali, H.; van Lier, J. E. Synthesis and receptor binding affinity of 7 α - and 17 α -substituted 2- and 4-chloro estradiol derivatives. *J. Chem. Soc., PT 1*, 1991, 2485-2491.
- (20) Raynaud, J. P.; Ojasoo, T.; Bouton, M. M.; Brignon, E.; Pons, M.; Crastes de Paulet, A. In *Estrogens in the Environment*; McLachlan, J. A., Ed.; Elsevier Science: New York, 1985; pp 24-42.
- (21) Kalvoda, von J.; Krähenbühl, Ch.; Desaulles, P. A.; Anner, G. A. 7 α -Methylöstrogene. *Helv. Chim. Acta* 1967, 50, 281-288.
- (22) Hanson, R. N. The influence of structure modification on the metabolic transformation of radiolabeled estrogen derivatives. In *The chemistry and pharmacology of radiopharmaceuticals*; Nunn, A. D., Ed.; Marcel Dekker: New York, NY 1992; pp 333-364.
- (23) Baran, J. S. A synthesis of 11 β -hydroxyestrone and related 16- and 17-hydroxyestratrienes. *J. Med. Chem.* 1967, 10, 1188-1190.
- (24) Waynforth, H. B. In *Experimental and Surgical Technique in the Rat*; Academic: London, 1980.
- (25) Sherrer, B. *Biostatistique*; Morin, G., Ed.; Chicoutimi, Quebec, Canada, 1984.