Synthesis of A-Ring Fluorinated Derivatives of (17α,20E/Z)-[125I]Iodovinylestradiols: Effect on Receptor Binding and Receptor-Mediated Target Tissue Uptake[1]

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We have prepared a series of 2- and 4-fluoro derivatives of the isomeric (17α,20E)- and (17α,20Z)iodovinylestradiols (IVE_2) and also the analogs substituted with either a 7α-methyl (7α-Me-IVE_2) or 11β-methoxy group (11β-OMe-IVE_2) 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 17α-ethynyl derivatives. The tributylstannyl intermediates were obtained from the corresponding 17α-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 20Z isomers of the same compounds showed somewhat higher ER binding affinity as compared to the 20E isomers. The combination of an A-ring fluoro and 7α- or 11β-substituent decreased ER binding affinity. Substitution of a fluoro atom at C-4 on either the 17α-ethynylestradiol or isomeric 17α-IVE_2 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 20E and 20Z isomers. Tissue distribution in immature female rats showed that the highest uterus uptake and uterus to blood/nontarget ratios in the IVE_2 series were obtained with the 4-F-(17α,20Z)IVE_2 isomer. The combination of A-ring fluoro and 7α- or 11β-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-(17α,20E)11β-OMe-IVE_2 (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 (17α,20Z)IVE_2 and (17α,20E)11β-OMe-IVE_2 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 16α-iodo-[3] and 17α-iodovinyl[4] 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 20E and 20Z iodovinyl derivatives of estradiol (20E/Z-IEV_2) has previously been reported, and receptor binding and tissue distribution studies suggested that the 20Z isomer has the preferred configuration.[4a] This work was subsequently extended to include the 11β-methoxy/ethoxy and 7α-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 uterotropic 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 (20E/Z)IVE_2 as well as their 7α-methyl and 11β-methoxy derivatives and evaluated ER binding affinities, receptor mediated target tissue uptake, and biodistribution pattern.

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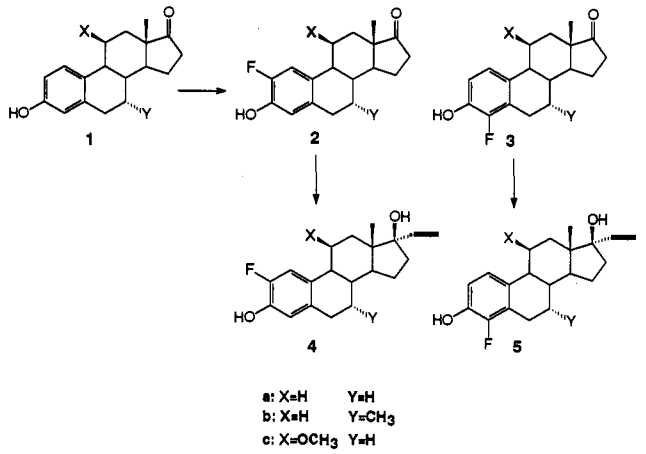
Scheme I

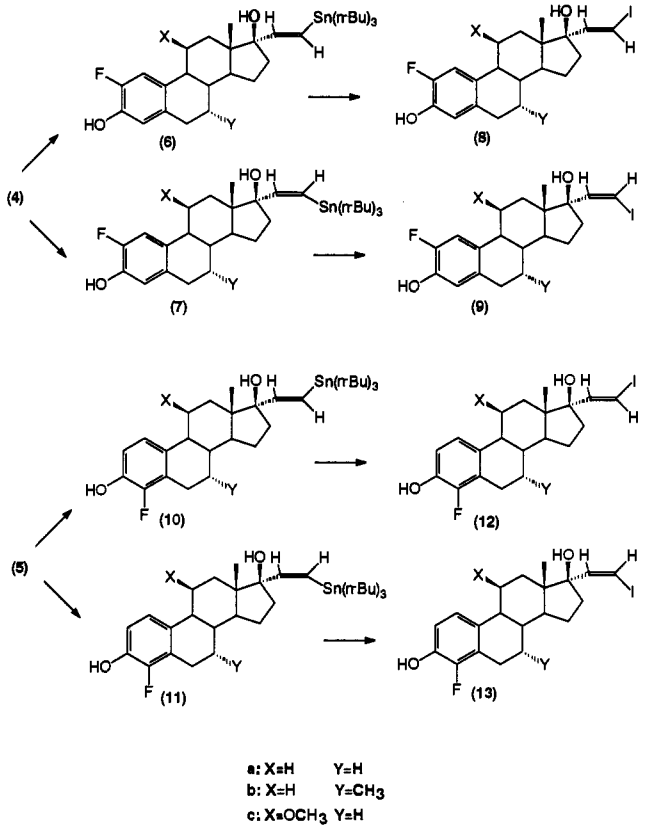


Results

Synthesis of A-Ring Fluorinated (17α,20*E*/*Z*)-Iodovinylestradiol Derivatives. Commercially available 2- and 4-fluoroestradiol was used to prepare the corresponding fluoroestrones **2a** and **3a** via oxidation with Jones reagent. Attempted fluorination of the A-ring of 11β-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 agent[10] 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 7α-methyl- and 11β-methoxyfluoroestrone derivatives **2b, 3b** and **2c, 3c** (Scheme I). The 11β-methoxyestrone (**1c**) reacted much faster with this reagent than the parent molecule, estrone (**1a**), whereas the 11β-methoxyestradiol gave a complex mixture from which it was difficult to purify the desired product. Treatment of the 11β-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 17α-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 organostannes and is the simplest and most direct route to transform alkynes into vinylstannes.[13] For the introduction of the iodine onto the vinyl substituent of the 17α-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 vinylstannes 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 vinylstannes **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 tributyl stannyl 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 C_4H_9, 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-17α-vinylestradiol (2-F-VE_2) and 4-F-17α-vinylestradiol (4-F-VE_2) 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 20*E* 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 20*Z* 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 [^{3}H]estradiol.[16] The RBA values are expressed relative to unsubstituted estradiol; the 17α-ethynyl derivative has a similar binding affinity to the ER (RBA = 100).[17] The 2-F-VE_2 exhibited lower ER binding affinity than the 4-F-VE_2 (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 20*Z* isomers showed higher RBA values than the corresponding 20*E* isomers. Adding two additional substituents, e.g., a fluoro and a 7α-methyl or 11β-methoxy group, resulted in lower RBA values. In order to evaluate whether equilibrium conditions were reached under the experimental conditions, RBA values of the 17α-ethynyl series lacking 7α- or 11β-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 20*Z* isomers **9a** and **13a**, while the corresponding 20*E* 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-(17α,20*Z*)IVE_2 (**13a**) as well as the 2-F-(17α,20*E*)-, 4-F-(17α,20*Z*)- and 4-F-(17α,20*E*)11β-OMe-IVE_2 (**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-(17α,20*E*)11β-OMe-IVE_2 (**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 (17α,20*E*)11β-OMe-IVE_2, whereas blood clearance of the IVE_2 derivatives with the 20*Z* configuration, in general, is slower than that of the analogous compounds with the 20*E* 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 20*E/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, 11β-substitution is known to increase the stability of the ER-steroid complex[18] while reducing nonspecific binding, resulting in enhanced uptake by target tissue. Likewise, introduction of a 7α-methyl group results in increased estrogenic activity and higher affinity for the ER receptor.[20,21] Finally, 17α-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 17α-ethynylestradiol (EE_2) 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-VE_2 exhibited substantially lower ER binding affinities than the corresponding F-EE_2. Addition of the 7α-methyl onto EE_2 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 7α-methyl derivatives were substituted at the 2- or 4-position with a chlorine atom.[19] As previously reported,[20] addition of the 11β-methoxy onto EE_2 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 (17α,20*E/Z*)IVE_2 at the 2-, 4-, 7α-, or 11β-position(s) results in similar changes in RBA values as those observed for the equivalent substitutions of EE_2. However, in some cases striking synergistic effects can be noted. Thus, 2-F substitution onto the (17α,20*E*)IVE_2, including the 7α-methyl or 11β-methoxy derivatives, results in an unexpected large drop in RBA values of 20–50% (Table II). This suggests either a high instability of the 20*E* 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 20*E/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-EE_2 (**5a**) and both the 2- and 4-F-(17α,20*Z*)IVE_2 (**9a** and **13a**)

Table I. Physical and Spectroscopic Data of Products

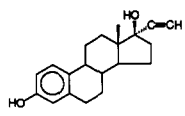
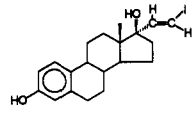
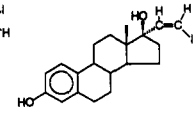
product	mp (0 °C)	HPLC system[a] (t_R)	MS (70 eV) *m/z* (%)	empirical formula	analysis	^{1}H NMR (δ)[b]
4a	101–103	A 17 min	314 (M^+, 21), 246 (15), 231 (100)	$C_{20}H_{23}FO_2$	C,H,F	0.88 (s, 3 H, 18-CH_3), 2.6 (s, 1 H, C(21)–H), 6.76 (d, J = 9.2 Hz, 1 H, C(4)–H), 6.85 (d, J = 12.6 Hz, 1 H, C(1)–H); ^{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, J = 9 Hz, 1 H, C(2)–H), 6.96 (d, J = 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, J = 9.2 Hz, 1 H, C(4)–H), 6.80 (d, J = 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, J = 14 Hz, 1 H, =CHI), 6.35 (d, J = 9 Hz, 1 H, C(4)–H), 6.56 (d, J = 14 Hz, 1 H, –CH=), 6.60 (d, J = 13 Hz, 1 H, C(1)–H)
9a	160–162	A 34	442 (M^+, 15), 315 (11), 297 (14), 178 (70), 151 (100)	$C_{20}H_{24}FIO_2 \cdot H_2O$	C,H,I	0.29 (s, 3 H, 18-CH_3), 5.75 (d, J = 8.6 Hz, 1 H, =CHI), 5.99 (d, J = 9 Hz, 1 H, C(4)–H), 6.21 (d, J = 8.6 Hz, 1 H, –CH=), 6.27 (d, J = 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, J = 14 Hz, 1 H, =CHI), 6.12 (t, J = 13 Hz, 1 H, C(2)–H), 6.18 (d, J = 14 Hz, 1 H, –CH=), 6.23 (d, J = 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, J = 8.5 Hz, 1 H, =CHI), 6.55 (t, J = 9 Hz, 1 H, C(2)–H), 6.61 (d, J = 8.5 Hz, 1 H, –CH=), 6.67 (d, J = 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, J = 13 Hz, 1 H, C(2)–H), 6.90 (d, J = 8.6 Hz, 1 H, C(1)–H)
4b	101–103	A 19	328 (M^+, 43), 271(8), 245 (100)	$C_{21}H_{25}FO_2$	C,H	0.84 (d, J = 7 Hz, 7α-CH_3), 0.89 (s, 3 H, 18-CH_3), 2.31 (s, 1 H, C(21)–H), 6.68 (d, J = 9.2 Hz, 1 H, C(4)–H), 6.98 (d, J = 12.5 Hz, 1 H, C(1)–H)
		D 35				
5b	102–105	A 19	328 (M^+, 38), 271 (7), 245 (100)	$C_{21}H_{25}FO_2$	C,H,F	0.89 (d, J = 7 Hz, 7α-CH_3), 0.92 (s, 3 H, 18-CH_3), 2.39 (s, 1 H, C(21)–H), 6.84 (t, J = 13 Hz, 1 H, C(2)–H), 7.0 (d, J = 8.6 Hz, 1 H, C(1)–H)
		D 38				
8b	110–115	B 22	456 (M^+, 43), 328 (40), 310 (27), 271 (21), 245 (100)	$C_{21}H_{26}FIO_2 \cdot H_2O$	C,H,I	0.83 (d, J = 7 Hz, 7α-CH_3), 0.927 (s, 3 H, 18-CH_3), 6.3 (d, J = 14.4 Hz, 1 H, =CHI), 6.67 (d, J = 9.2 Hz, 1 H, C(4)–H), 6.78 (d, J = 14.4 Hz, 1 H, –CH=), 6.96 (d, J = 12.6 Hz, 1 H, C(1)–H)
9b	112–114	B 21	456 (M^+, 30), 328 (18), 310 (22), 271 (21), 245 (100)	$C_{21}H_{26}FIO_2 \cdot H_2O$	C,H,I	0.70 (d, J = 7 Hz, 7α-CH_3), 0.84 (s, 3 H, 18-CH_3), 6.27 (d, J = 8.5 Hz, 1 H, =CHI), 6.53 (d, J = 9.2 Hz, 1 H, C(4)–H), 6.69 (d, J = 8.6 Hz, 1 H, –CH=), 6.80 (d, J = 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)	$C_{21}H_{26}FIO_2 \cdot H_2O$	C,H,I	0.844 (d, J = 7 Hz, 7α-CH_3), 0.926 (s, 3 H, 18-CH_3), 6.32 (d, J = 14.4 Hz, 1 H, =CH), 6.69 (t, J = 9 Hz, 1 H, C(2)–H), 6.78 (d, J = 14.4 Hz, 1 H, –CH=), 6.94 (d, J = 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, J = 7 Hz, 7α-CH_3), 0.97 (s, 3 H, 18-CH_3), 6.39 (d, J = 8.5 Hz, 1 H, =CHI), 6.79 (t, J = 9 Hz, 1 H, C(2)–H), 6.83 (d, J = 8.5 Hz, 1 H, –CH=), 6.95 (d, J = 8.6 Hz, 1 H, C(1)–H)
4c	126–132	A 11	344 (M^+, 66), 313 (12), 295 (11), 280 (100)	$C_{21}H_{25}FO_3$	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, J = 9.2 Hz, 1 H, C(4)–H), 6.65 (d, J = 13.1 Hz, 1 H, C(1H), 7.86 (brs, 1 H, –OH), 8.37 (br, 1 H, –OH); ^{19}F NMR –61.073 (s, 1 F, C(2)–H)
		C 23				
5c	210–300	A 11	344 (M^+, 98), 312 (22), 295 (42), 267 (100)	$C_{21}H_{25}FO_3$	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, J = 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)
	decomp	C 27				
8c	215–217	A 21	472 (M^+, 20), 360 (17), 344 (20), 312 (39), 294 (35), 243 (89), 174 (100)	$C_{21}H_{26}FIO_3 \cdot H_2O$	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, J = 14.4 Hz, 1 H, =CH), 6.56 (d, J = 9.2 Hz, 1 H, C(4)–H), 6.74 (d, J = 11.3 Hz, 1 H, C(1)–H), 6.77 (d, J = 14.4 Hz, 1 H, –CH=)
9c	245–250	A 20	472 (M^+, 16), 344 (22), 312 (28), 294 (28), 243 (100)	$C_{21}H_{26}FIO_3 \cdot H_2O$	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, J = 8.8 Hz, 1 H, =CHI), 6.50 (d, J = 9.2 Hz, 1 H, C(4)–H), 6.63 (d, J = 11.5 Hz, 1 H, C(1)–H), 6.64 (d, J = 8.8 Hz, 1 H, –CH=)

Table I (Continued)

product	mp (0 °C)	HPLC system[a] (t_R)	MS (70 eV) m/z (%)	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)	$C_{21}H_{26}FIO_3 \cdot H_2O$	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, J = 14 Hz, 1 H, =CHI), 6.72 (d, J = 14 Hz, 1 H, –CH=), 6.74 (d, J = 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, J = 8.6 Hz, 1 H, =CH), 6.60–6.65 (m, 1 H, C(2)–H), 6.67 (d, J = 8.6 Hz, 1 H, –CH=), 6.68 (d, J = 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 [^{3}H]estradiol binding. Murine cytoplasmic extracts were incubated at 0–4 °C for 18 h with 20 nM of [^{3}H]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)IVE$_2$ isomer (**8a**) decreased sharply (80%) following change in either experimental conditions. In the case of the 4-F-(17α,20E)IVE$_2$ 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 IVE$_2$ 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)[^{125}I]IVE$_2$ 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)IVE$_2$ 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)IVE$_2$ at the 4-position (**12a** and **13a**) substantially enhanced uterus uptake over

Table IV. Tissue Distribution of the Isomeric 2- and 4-Fluoro-(17α,20*E*/*Z*)-[^{125}I]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 (20*E* 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 (20*Z* 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 (20*E* 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 (20*Z* 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 μCi (111 kBq) of ^{125}I-labeled steroids in the presence (+E_2) or absence of 60 μg 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 20*E* isomer (**8a**) and a small positive effect in the case of the 20*Z* isomer (**9a**). Furthermore, these uptake values show that the 4-F-(20*Z*) isomer (**13a**) reaches 2–3 times higher uterus levels as compared to either the 4-F-(20*E*) isomer (**12a**) or the 2-F-(20*Z*) 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 IVE_2 in that the highest RBA value was obtained for **13a** (Table II).

Addition of a 7α-methyl substituent to the IVE_2 increased uterus uptake substantially for both the 20*E* and the 20*Z* 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 7α-methyl and fluoro substituents onto estradiol (Table II). A similar uterus uptake pattern was observed with the 11β-methoxy series. In both cases, 4-F addition onto the 20*E* isomer resulted in higher uterus uptake as compared to 2-F addition, while in the case of the 20*Z* 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-(17α,20*E*)11β-OMe-IVE_2 (**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 20*E* isomers are in general more stable than the

Table V. Tissue Distribution of the Isomeric 2- and 4-Fluoro-7α-methyl-(17α,20E/Z)-[^{125}I]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 (20*E* Isomer, 2-F, 7α-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 (20*Z* Isomer, 2-F, 7α-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 (20*E* Isomer, 4-F, 7α-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 (20*Z* Isomer, 4-F, 7α-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 μCi (111 kBq) of ^{125}I-labeled steroids in the presence (+E_2) or absence of 60 μg to co-injected estradiol. [b] Nontarget organs include the muscle, spleen, and lungs.

corresponding 20*Z* isomers and that 11β-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 formation[6] 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 7α-methyl or 11β-methoxy substitution reduces uterus uptake. Similarly, simultaneous substitution with a 7α-methyl and a 11β-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-11β-methoxy-(17α,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
	[125I]-8c (20E Isomer, 2-F, 11β-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)
	[125I]-9c (20Z Isomer, 2-F, 11β-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)
	[125I]-12c (20E Isomer, 4-F, 11β-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)
	[125I]-13c (20Z Isomer, 4-F, 11β-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 μCi (111 kBq) of 125I-labeled steroids in the presence (+E_2) or absence of 60 μg 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-(17α,20E)11β-OMe-IVE$_2$ (12c), suitable for 123I 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-Ethylenedioxyestra-1,3,5(10)-triene-3,11β-diol was synthesized according to Baran[23] and converted to 11β-methoxyestrone with methyl iodide. Melting points (mp) were determined on a Fisher-Johns apparatus and are uncorrected.

^{1}H NMR spectra were obtained with Bruker WM 25 spectrometer, in $CDCl_3$ + 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 H_2SO_4/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 μm, 25 × 0.94 cm, CSC, Montreal, Canada), and the compounds were detected at 280 nm

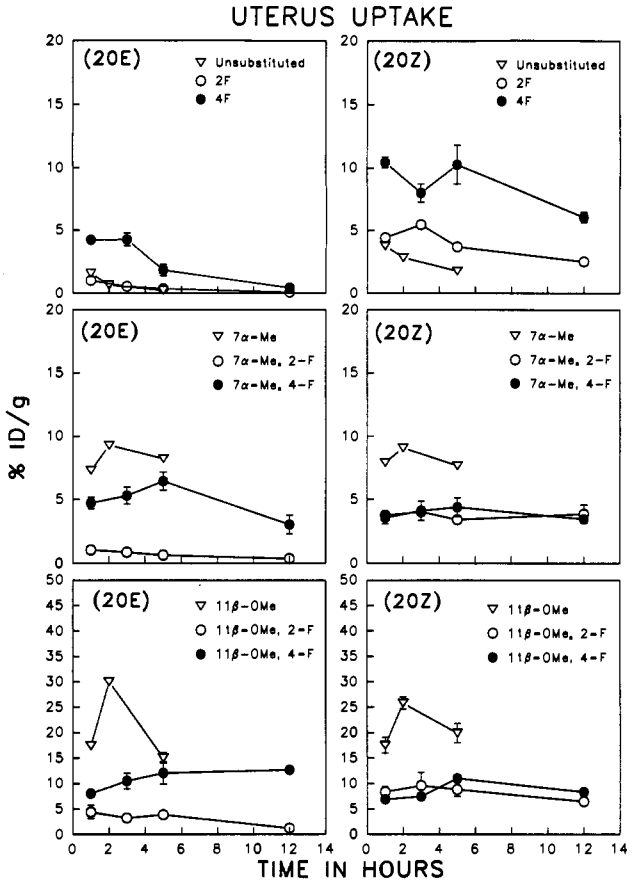


Figure 1. Uterus uptake of ^{125}I-labeled 2-F and 4-F-(17α,20*E*/*Z*)iodovinylestradiols and the corresponding 7α-methyl and 11β-methoxy derivatives in immature female Long Evans rats. Values for the nonfluorinated derivatives were taken from the literature.[4a,b] The error bars represent the standard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

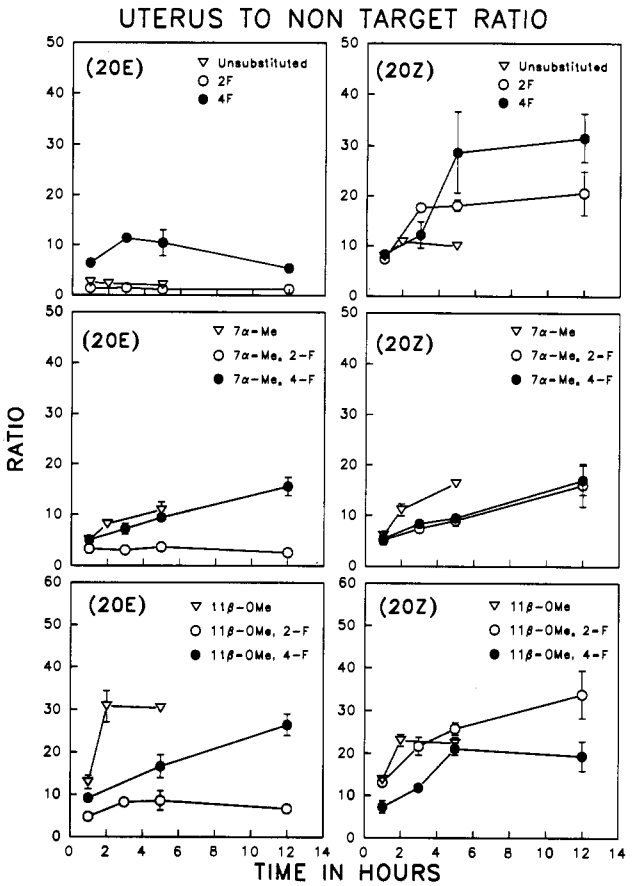


Figure 2. See Figure 1: uterus to nontarget (lungs, spleen, and muscle) ratios.

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. 7α-Methylestrone (1b) (1 g) or 11β-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-7α-methylestrone (2b) and 4-Fluoro-7α-methylestrone (3b) (mixture). ^{1}H NMR (δ) 0.80 (d, J = 8 Hz, 3H, 7α-CH_3), 0.83 (d, J = 8 Hz, 3H, 7α-CH_3), 1.19 (s, 6H, 18-CH_3), 6.68 (d, J = 9 z, 1H, C_4-H), 6.9 (d, J = 13 Hz, 1H, C_1-H), 6.8–6.85 (m, 1H, C_2 and C_1-H); MS *m/z* (rel intensity) 302 (M^+, 100), 269 (10), 245 (19).

2-Fluoro-11β-methoxyestrone (2c). HPLC in 50:50 acetonitrile/H_2O, t_R = 20 min; ^{1}H NMR (δ) 0.96 (s, 3H, 18-CH_3), 3.18 (s, 3H, 11β-OCH_3), 3.96 (m, 1H, 11α-H), 6.54 (d, J = 9 Hz, 1H, C_4-H), 6.66 (d, J = 13 Hz, C_1-H); MS *m/z* (rel intensity) 318 (100), 286 (20), 259 (54).

4-Fluoro-11β-methoxyestrone (3c). HPLC in 50:50 acetonitrile/H_2O, t_R = 24 min; ^{1}H NMR 0.636 (s, 3H, 18-CH_3), 2.84 (s, 3H, 11β-OCH_3), 3.70 (m, 1H, 11α-H), 6.29–6.34 (m, C_2 and C_1-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 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 (17α,20*E*)- and (17α,20*Z*)-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-(17α,20*E*)-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-(17α,20*Z*)-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-(17α,20*E*)-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).

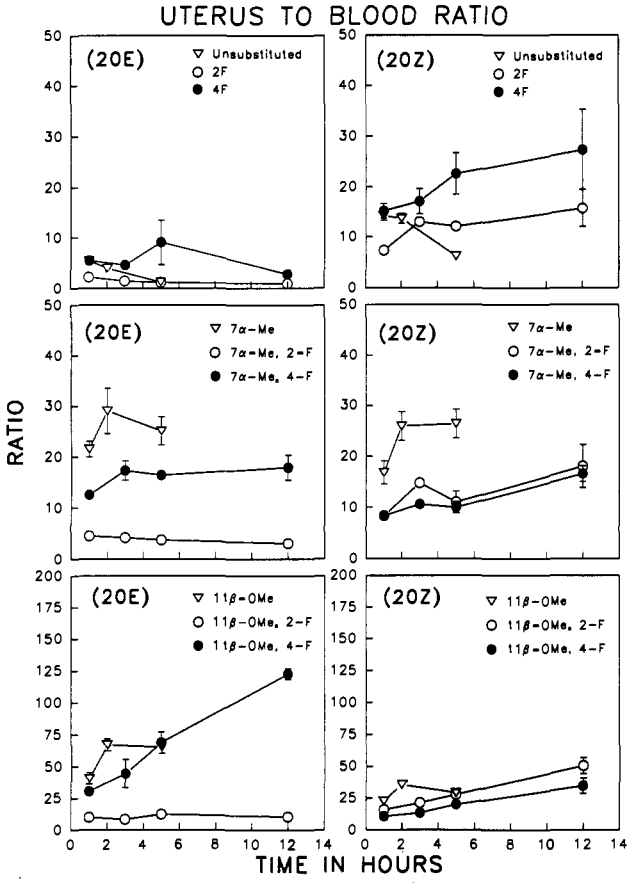


Figure 3. See Figure 1: uterus to blood ratios.

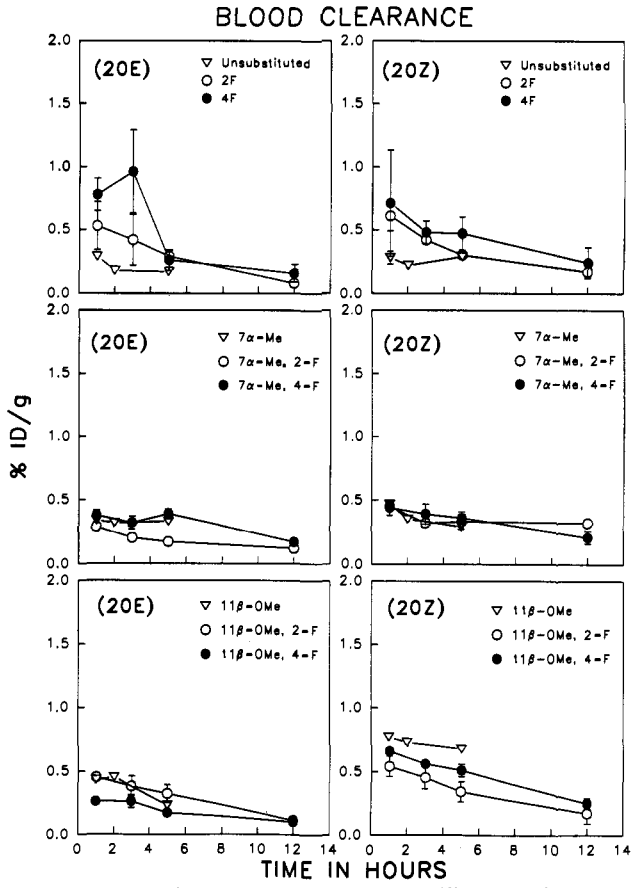


Figure 4. Blood clearance in % ID/g of ^{125}I-labeled 2-F and 4-F-(17α,20*E*/*Z*)iodovinylestradiols and the corresponding 7α-methyl and 11β-methoxy derivatives in immature female Long Evans rats. Values for the nonfluorinated derivatives were taken from the literature.[4a,b] The error bars represent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

4-Fluoro-(17α,20*Z*)-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-7α-methyl-(17α,20*E*)-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-7α-methyl-(17α,20*Z*)-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-7α-methyl-(17α,20*E*)-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), 558 (38), 507 (19), 505 (13), 429 (10), 329 (5), 310 (26), 290 (23), 245 (43).

4-Fluoro-7α-methyl-(17α,20*Z*)-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-11β-methoxy-(17α,20*E*)-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-11β-methoxy-(17α,20*Z*)-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-11β-methoxy-(17α,20*E*)-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-11β-methoxy-(17α,20*Z*)-21-(tri-*n*-butylstannyl)vinylestradiol (11c). HPLC, t_R = 21 min; MS *m*/*z* (rel intensity) 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 (17α,20*E*/*Z*)Iodovinyl Derivatives from the Corresponding (17α,20*E*/*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 × 15 mL). The organic phase was dried over magnesium sulfate (anhydrous), filtered, and evaporated to dryness. The residue were 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-(17α,20*E*)-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-(17α,20*Z*)-21-[^{125}I]iodovinylestradiols ([^{125}I]-9a-c and [^{125}I]-13a-c). To a solution of [^{125}I]NaI (500

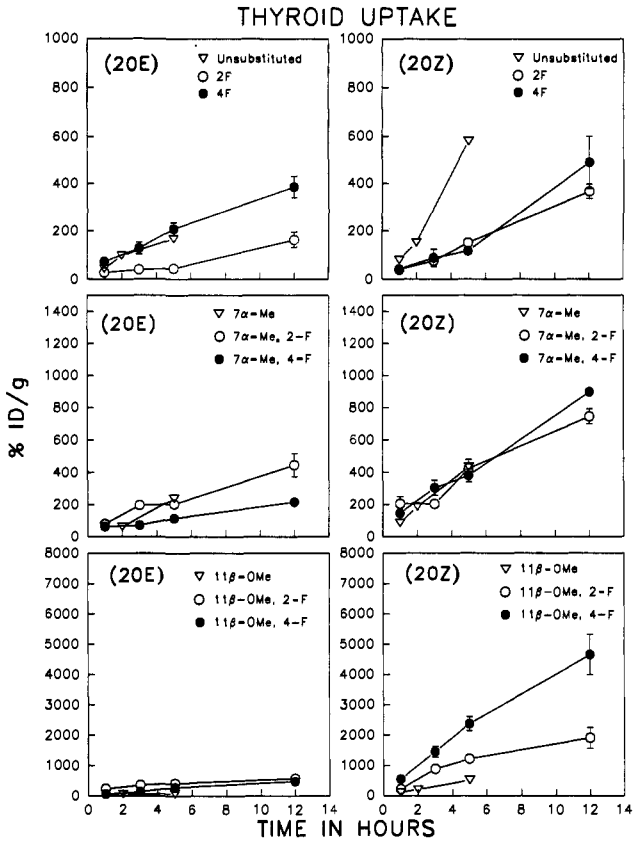


Figure 5. Thyroid uptake in %ID/g of ^{125}I-labeled 2-F and 4-F-(17α,20*E*/*Z*)iodovinylestradiols and the corresponding 7α-methyl and 11β-methoxy derivatives in immature female Long Evans rats. Values for the nonfluorinated derivatives were taken from the literature.[4a,b] The error bars represent 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 **7a-c** or **11a-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}I]-**9a**, t_R = 22 min; [^{125}I]-**13a**, t_R = 21 min; [^{125}I]-**9b**, t_R = 29 min; [^{125}I]-**13b**, t_R = 26 min; [^{125}I]-**9c**, t_R = 21 min; [^{125}I]-**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}H]estradiol binding. Murine uterine cytoplasmic extracts were incubated at 0–4 °C for 18 h with 20 nM of [^{3}H]estradiol in the absence and presence of competitive steroids ranging from 2 nM to 20 μM. Selected compounds were also incubated for 2 h at 4 and at 25 °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]

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