internal standard with $CDCl_3$ and D_2O as solvents. Infrared spectra were obtained with a Beckmann Acculab 4 spectrometer with carbon tetrachloride and KBr disks.

3-Alkyl(or Aryl)-4-amino-D_3-trishomocubanes 8a-h General Procedure. Concentrated sulfuric acid (12 mL was added slowly to a well-stirred nitrile (40 mL) (Table I) at 0 °C. The stirred mixture was cooled to -10 °C and the tertiary alcohol **5** (0.5 g) was added slowly to keep the reaction temperature at -10 °C. The reaction mixture was allowed to reach room temperature, stirred for another 3 h, and then poured onto ice, whereupon the mixture was mde alkaline with aqueous sodium hydroxide (10%). Precipitated amide **7** was filtered, dried, and either hydrolyzed or reduced without further purification. The amide was reduced with LiAlH in anhydrous ether under reflux for 2 h to the desired 4-amino-D_3-trishomocubane **8c-h**. The excess $LiAlH_4$ was decomposed with ice-cold water, and the amine was extracted with ether (3 × 50 mL). The organic layer was dried (sodium sulfate) and evaporated (20 mbar), yielding 4-amino-D_3-trishomocubanes **8c-H**. The amine was dissolved in anhydrous ether, and hydrogen chloride was bubbled through the solution, whereupon the hydrochloride salts of the 4-amino-D_3-trishomocubanes (Table I) crystallized. Amide **7** was hydrolyzed under reflux conditions in concentrated hydrochloric acid (72 h) to yield the corresponding primary 4-amino-D_3-trishomocubane **8a,b**. The reaction mixture was made alkaline, whereupon the amine was extracted with ether (3 × 50 mL). The organic layer was then treated as described above, yielding the hydrochloride salt of the primary 4-amino-D_3-trishomocubane **8a,b** (Table I).

Pharmacology. Irwin Dose-Range Study/Acute Toxicity in Mice. Male CD-1 mice were deprived of food for 18 h prior to the experiment, but water was available ad libitum except during the observation period. The test compounds were prepared in 1% tragacanth and administered orally to groups of four mice. The test compound were tested at 1000, 464, 214, and 100 mg/kg; the dose volume remained constant at 10 mL/kg. The animals were observed daily for 7 days postdose and any mortalities noted. The LD_{50} values were estimated by using the method of Horn.[28]

Anti-Oxotremorine Test in Mice. Male CD-1 mice were deprived of food for 18 h prior to the experiment, but water was available ad libitum except during the observation period. The test compounds were prepared in 1% tragacanth and administered orally to groups of 10 mice. The test compounds were tested at doses of 100, 30, and 10 mg/kg at a constant dose volume of 10 mL/kg. Thirty minutes after administration of the test compound, vehicle, or reference standard, the mouse received an intraperitoneal injection of oxotremorine (0.4 mg/kg). The intensity of salivation and tremor was scored for all mice on a 0-3 scale, at 10, 20, and 30 min post-oxotremorine. Only the concentrations of the test compounds exhibiting the most promising anti-oxotremorine activity appear in Table III.

Antagonism of Reserpine-Induced Catalepsy in Mice. Male CD-1 mice were deprived of food for 18 h prior to the experiment, but water was available ad libitum except during the observation period. The test compounds were prepared in 1% tragacanth and administered orally to groups of five mice. Four hours prior to the administration of the test compounds or vehicle, each mouse received an intraperitoneal dose of reserpine (5 mg/kg). Forty-five minutes after the administration of the test compounds or vehicle, each mouse was placed with its forepaws on a 5-cm high cork, in order to assess the presence or absence of catalepsy. Mice which remain in this position for 5 min were considered cataleptic. The ED_{50} (i.e. the dose of the test compound causing a reduction of the catalepsy score to 50% of that of the control group) values were determined. Each test compound was tested at doses 100, 30, and 10 mg/kg. A constant dose volume of 10 mL/kg was employed.

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Synthesis, Receptor Binding, and Tissue Distribution of 7α- and 11β-Substituted (17α,20*E*)- and (17α,20*Z*)-21-[^{125}I]Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diols†

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The 11β-methoxy, 11β-ethoxy, and 7α-methyl derivatives of the isomeric (17α,20*E*)- and (17α,20*Z*)-(iodovinyl)estradiols **3** and **6**, and their no-carrier-added [^{125}I]iodovinyl analogues, were evaluated for their relative target-tissue retention and binding affinity for the estrogen receptor. The isomeric iodovinyl and [^{125}I]iodovinyl derivatives were prepared via destannylation of the corresponding tributylstannyl precursors in the presence of H_2O_2 or chloroamine-T, with retention of configuration. The 20*Z* isomers **6** exhibited slightly higher receptor binding affinities than the 20*E* isomers **3**, with all eight isomeric products giving relative binding affinity values in the 20-50 range. The 11β- and 7α-substituted (iodovinyl)estradiols gave substantially higher estrogen receptor-mediated uterus uptake as compared to the nonsubstituted parent molecule. Synergism between the effect of 11β- or 7α-substituents and the configuration of the iodovinyl group was evident from the in vivo distribution pattern of [^{125}I]-**3** and -**6**. The best uterus uptake was observed, at 2 h postinjection, with the 20*E* isomer of 11β-methoxy derivative **3b**. However, at 5 h postinjection the 20*Z* isomer **6b** reached higher uterus concentrations than the 20*E* isomer **3b**, and furthermore, these values are now comparable to those observed with the 20*Z* isomer of the 11β-ethoxy derivative **6c**. In the case of the 7α-methyl derivatives the differences in in vivo stability between the 20*E* and 20*Z* isomers was less pronounced, whereas the 20*Z* isomer **6d** reached somewhat higher uterus to blood as well as nontarget ratios.

A radiolabeled estrogen analogue with high binding affinity for estrogen receptors could play an important role in the characterization, delineation, as well as management of breast cancer. Among the many radiolabeled estrogens advanced to this end,[1] the 17α-iodovinyl derivatives of substituted estradiols are particularly promising.[2] Comparative studies with such derivatives in transplanted tumors containing various concentration of receptor proteins suggested that their level of tissue uptake qualitatively relates to the estrogen receptor concentration.[3,4] The 3-methyl ether derivatives (e.g. **3b**) have also been suggested as a potential imaging agents;[2d] however, their insignificant receptor-binding affinity and high lipid solubility render these derivatives unsuitable for imaging of small receptor-containing tumors.[5] Most studies to data

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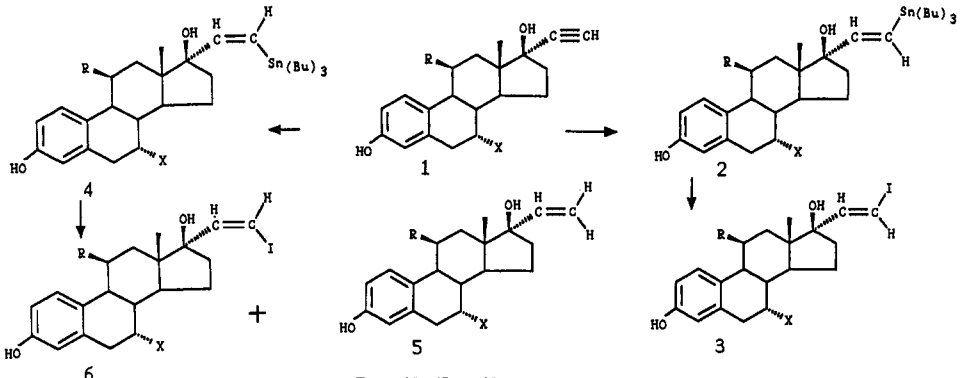
0022-2623/91/1834-0854$02.50/0

Scheme I



a: R = H; X = H
b: R = OCH_3; X = H
c: R = OC_2H_5; X = H
d: R = H; X = CH_3

with 17α-(iodovinyl)estradiol derivatives were concerned with the 20*E* isomers. We recently reported that the 20*Z* isomer of 17α-(iodovinyl)estradiol localizes better in estrogen receptor rich rat uterus as compared to the 20*E* isomer.[6] Introduction of the 11β-substituent in estrogens increases the stability of the estrogen receptor complex[7] and in the case of the (17α,20*E*)-(iodovinyl)estradiol addition of the 11β-methoxy dramatically enhances receptor-mediated tissue localization of the radioiodinated analogue.[2] Likewise, introduction of a 7α-methyl group results in increased estrogenic activity and higher affinity for the estrogen receptor.[8,9] Attachment of long, linear chains at the 7α-position of estradiol, although useful for the preparation of affinity columns for receptor purification,[10] did not give a product with appreciable affinity for the estrogen receptor.[11] Introduction of a combination of a 7α- and 11β-substituent onto the same estradiol molecule leads to a compound with little affinity for the estrogen receptor.[8] In this paper we have studied a possible synergism between the iodovinyl configuration of (17α,20*E*/*Z*)-(iodovinyl)estradiol and substituents which are known to enhance target specificity, i.e. the 11β-methoxy, 11β-ethoxy, and 7α-methyl groups.

Chemistry. 17α-Ethynylestradiol derivatives (**1b–d**) were treated for 4 h with tri-n-butyltin hydride in toluene at 90–100 °C, in the presence of azobisisobutyronitrile as a catalyst, to yield (17α,20*E*)-[2-(tributylstannyl)vinyl]-estradiols **2** as major products in up to 75% yield. Substitution of toluene for a polar solvent (e.g. hexamethylphosphoric triamide), omission of catalyst, and lowering of the reaction temperature to 65–70 °C favored formation of the 20*Z* isomers **4** (Scheme I). The products required chromatographic purification to separate the starting material and unwanted products. The tin intermediates gave weak molecular ions in the mass spectrum. Addition of a solution of iodine in chloroform to these intermediates (**2** and **4**) gave the (iodovinyl)estradiol derivatives (**3** and **6**), with retention of configuration, in 50–80% yield. Compounds **3** and **6** gave the appropriate molecular ions in the mass spectrum and their configurations were established by ^1H and ^{13}C NMR. The assigned stereochemistry of the iodine on C-21 was based on the coupling constants of the two vinylic protons at C-20 and C-21 in the ^1H NMR spectrum and the chemical shifts of the carbon signals in the ^{13}C NMR. Thus in the ^1H NMR spectrum two doublets centered at δ = 6.12–6.22 for the protons at C-20 and at δ = 6.63–6.68 for the proton at C-21 with J = 14 Hz are indicative of trans (*E*) incorporated iodine (**3**) while the two doublets centered at δ = 6.22–6.39 and δ = 6.62–6.84 with J = 8 Hz are indicative of the cis (*Z*) configuration. Signals centered at δ = 72.96–74.02 for C-21 and at δ = 150.8–151.16 for C-20 in the ^{13}C NMR spectrum correspond to the *E* configuration while in the case of *Z* isomers the C-21 signal was shifted downfield and appeared at δ 75.7–77.51. The C-20 signals were shifted upfield and appeared centered at δ = 143.5–143.73. The remaining carbon signals were unaffected by the configuration about C-20 and appeared at almost the same position in both isomeric products. The 20*Z* isomers were less stable than the 20*E* isomers and they also exhibited different solubility. The *E* and *Z* tin intermediates were well separated on silica gel TLC in mixtures of EtOAc and hexane as well as on C-18 reverse-phase HPLC columns using a mixture of water and methanol, while the iodo derivatives **3** and **6** were difficult to separate.

Radiochemistry. The stannyl intermediates **2** and **4** were converted to the radioiodinated products [^{125}I]-**3** and -**6** by using different reaction conditions for each isomer. The 20*E* isomers [^{125}I]-**3** were readily obtained by treatment of the corresponding **2** with [^{125}I]NaI in the presence of H_2O_2,[12] while for the preparation of the 20*Z* isomers

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Table I. Relative Binding Affinities of the Various Steroids for Murine Cytoplasmic Estrogen Receptors

compd	RBA (SD)	compd	RBA (SD)
1b (mexestrol)	15.3 (0.5)	**5b**	13.5 (4.1)
1c	21.0 (1.9)		
1d	73.1 (12.2)	**5d**	58.1 (10.6)
	Iodinated Products		
3a (*E*)	32.8[b]	**6a** (*Z*)	46.7[b]
3b (*E*)	27.7 (2.9)	**6b** (*Z*)	32.0 (6.6)
3c (*E*)	21.6 (0.3)	**6c** (*Z*)	30.8 (1.1)
3d (*E*)	43.2 (7.5)	**6d** (*Z*)	44.9 (0.9)

[a] Competitive binding between 10^{-9} M [^{3}H]estradiol and 2×10^{-9}–2×10^{-12} M unlabeled estradiol and the derivatives **1**, **5**, **3**, and **6** was plotted and the concentration required for 50% competition was used to calculate the RBA values (mean of three experiments). [b] Datum taken from the literature.[6]

[^{125}I]-**6**, we used [^{125}I]NaI and chloroamine-T in EtOH. After solvent extraction and chromatographic purification on a reverse-phase HPLC system the ^{125}I-labeled products were obtained in 75–80% overall yield. The identity of the labeled material was confirmed by coinjection with the authentic iodinated products on HPLC. During the HPLC purification, eluting ^{125}I-labeled **3** or **6** could not be detected by the UV monitor and it is consequently assumed that their specific activities were in the same range as that of the starting [^{125}I]NaI, e.g. up to 2200 Ci/mmol.

Biological Properties. The relative binding affinities (RBA) of **1**, **3**, **5**, and **6** for estrogen receptors were determined by competitive binding assays with [^{3}H]estradiol.[13] The concentration required for 50% competition was used to calculate RBA values.[6] For all substituted and nonsubstituted isomeric (iodovinyl)estradiols, the 20*Z* isomers (**6**) exhibited somewhat higher RBA values than the corresponding 20*E* isomers (**3**); however, only in the case of the 11β-ethoxy derivatives **3c** (*E*) and **6c** (*Z*) were such differences found to be significant (Table I).

The biodistribution and uterus uptake of the ^{125}I-labeled estradiols was studied in immature Fischer female rats. The animals were injected through the tail vein with 3 μCi (111 kBq) of the HPLC-purified ^{125}I-labeled steroid in 200 μL of a 9% ethanol–saline solution containing 1% Tween-80. The animals were sacrificed at 1, 2, and 5 h postinjection. In order to establish that the uterus uptake of the labeled steroid was receptor mediated, some rats of the 1 h group were coinjected with 60 μg of unlabeled estradiol. The radioactivity concentration in the various organs are presented in Table II. With the exception of the blood, uterus, and thyroid, the distribution patterns were similar for all isomeric (iodovinyl)estrogens: ^{125}I uptake reaches a maximum within 1 h postinjection followed by a continuous decrease in activity. Uterus concentrations (Table II) and uterus to blood and nontarget ratios of the differently substituted [^{125}I]-**3** and [^{125}I]-**6** reached levels well in excess of what was previously observed for the analogues ^{125}I-labeled estrogens lacking 11β- or 7α-substitutents (Figure 1).[6] Peak uterus radioactivity concentrations occurred at 2 h postinjection. With the exception of the uterus to nontarget tissue ratios of the *E* and *Z* isomers of the 7α-methyl derivatives (**3d** and **6d**), which like the nonsubstituted analogues (**3a** and **6a**) gave the highest uptake ratios for the *Z* isomer, the highest uterus uptake ratios were observed with the *E* isomers (Figure 1). In vivo deiodination, as measured via the ^{125}I uptake by the thyroid, was consistently higher for the *Z* isomers and, furthermore, strongly influenced by the nature of the substituents (Figure 2). Particularly, addition of the 11β-ethoxy group, i.e. compound **6c**, drastically

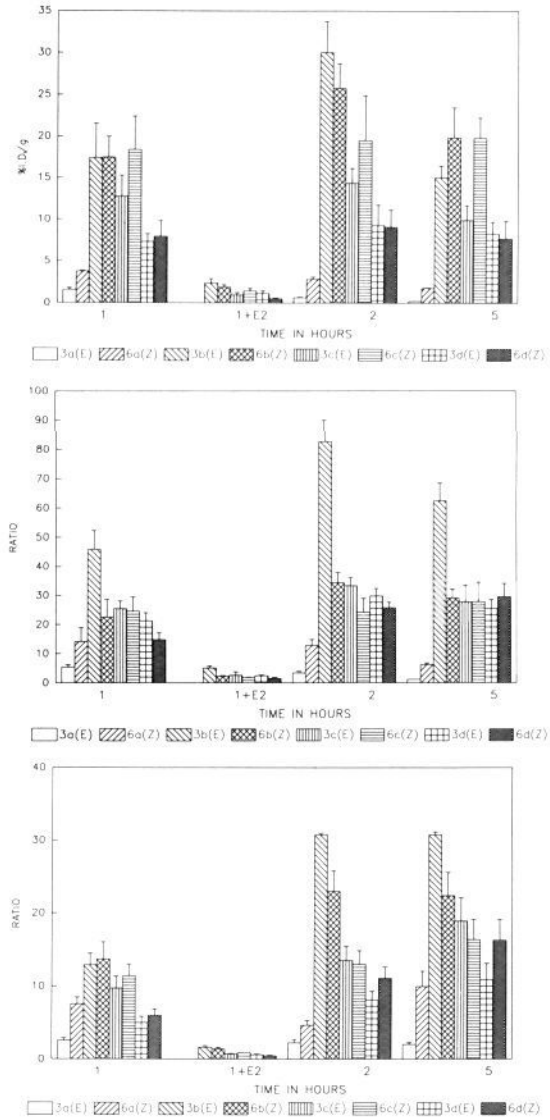

Figure 1. Uterus uptake in % ID/g (top), uterus to blood ratios (middle), and target (uterus) to nontarget (lung, spleen, and muscle) ratios (bottom) of 11β- and 7α-substituted ^{125}I-labeled (17β,20*E*/*Z*)-(iodovinyl)estradiols in immature female Fischer rats. The error bars represent the standard deviations.

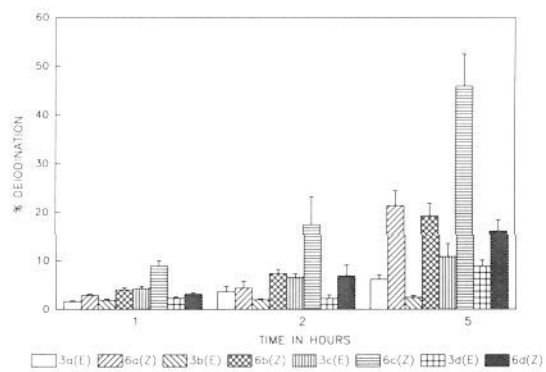

Figure 2. Percent deiodination of 11β- and 7α-substituted ^{125}I-labeled (17β,20*E*/*Z*)-(iodovinyl)estradiols at 1, 2, and 5 h postinjection was calculated from the thyroid uptake. The ^{125}I-uptake at the same time interval after [^{125}]NaI injection was taken as 100%. The error bars represent the standard deviation.

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Table II. Tissue Distribution of 11β- and 7α-Substituted ^{125}I-Labeled (17β,20*E*/*Z*)-(Iodovinyl)estradiols in Immature Female Fischer Rats

tissue	% ID/g (% CV)[a] 1 h	1 h (+E_2)	2 h	5 h	tissue	% ID/g (% CV)[a] 1 h	1 h (+E_2)	2 h	5 h
	[^{125}I]-**3b** (20*E* Isomer, 11β-Methoxy)					[^{125}I]-**6b** (20*Z* Isomer, 11β-Methoxy)			
blood	0.43 (23)	0.47 (14)	0.45 (25)	0.23 (15)	blood	0.77 (15)	0.83 (11)	0.73 (3)	0.68 (11)
uterus	17.41 (25)	2.38 (22)	30.02 (13)	15.02 (10)	uterus	17.49 (15)	1.87 (17)	25.77 (12)	19.86 (19)
plasma	0.50 (24)	0.54 (13)	0.55 (27)	0.28 (16)	plasma	0.87 (15)	1.00 (11)	0.87 (5)	0.85 (9)
thyroid	51.19 (12)	38.91 (18)	52.97 (16)	66.68 (29)	thyroid	108.06 (12)	177.28 (17)	199.33 (12)	517.77 (9)
muscle	1.46 (22)	1.63 (13)	1.11 (11)	0.47 (47)	muscle	1.15 (16)	1.32 (47)	1.04 (13)	0.73 (15)
fat	5.02 (26)	4.75 (15)	2.95 (16)	0.96 (14)	fat	2.68 (9)	3.19 (14)	1.47 (7)	1.07 (16)
brain	1.32 (29)	1.40 (14)	0.73 (11)	0.25 (16)	brain	1.11 (43)	1.04 (12)	0.52 (19)	0.37 (13)
kidneys	2.95 (27)	2.16 (19)	2.10 (6)	0.97 (17)	kidneys	3.40 (21)	1.77 (9)	3.01 (4)	2.24 (6)
spleen	0.76 (20)	1.13 (11)	0.52 (33)		spleen	0.83 (17)	1.32 (26)	0.70 (26)	0.53 (17)
lungs	1.83 (25)	1.66 (17)	1.30 (24)	0.52 (19)	lungs	1.85 (11)	1.35 (13)	1.62 (12)	1.40 (11)
liver	5.63 (23)	5.38 (8)	5.03 (16)	2.97 (12)	liver	3.73 (8)	4.30 (11)	2.64 (18)	2.49 (19)
	[^{125}I]-**3c** (20*E* Isomer, 11β-Ethoxy)					[^{125}I]-**6c** (20*Z* Isomer, 11β-Ethoxy)			
blood	0.50 (12)	0.52 (17)	0.43 (16)	0.46 (45)	blood	0.76 (14)	0.77 (27)	0.80 (8)	0.75 (33)
uterus	12.74 (21)	0.95 (32)	14.38 (13)	9.89 (19)	uterus	18.38 (23)	1.45 (22)	19.52 (29)	19.80 (13)
ovaries	4.76 (25)	2.00 (29)	5.14 (12)	3.23 (24)	ovaries	6.72 (24)	2.88 (55)	6.57 (16)	5.65 (15)
adrenals	4.12 (18)	4.17 (25)	2.67 (31)	0.95 (44)	adrenals	4.83 (9)	3.18 (29)	3.07 (24)	1.43 (10)
thyroid	115.81 (21)	98.12 (5)	177.22 (41)	561.61 (26)	thyroid	241.28 (9)	207.21 (46)	472.28 (13)	1240.11 (16)
muscle	1.28 (39)	1.35 (15)	0.96 (25)	0.37 (36)	muscle	1.24 (12)	1.78 (6)	1.06 (30)	0.78 (20)
fat	4.73 (15)	5.46 (29)	3.23 (23)	1.27 (20)	fat	4.85 (20)	5.17 (44)	2.81 (24)	1.23 (18)
brain	0.82 (19)	0.90 (22)	0.45 (19)	0.16 (23)	brain	0.97 (15)	0.98 (34)	0.60 (13)	0.37 (20)
kidneys	2.12 (14)	2.04 (25)	1.65 (20)	0.75 (30)	kidneys	3.61 (14)	1.91 (32)	2.98 (11)	2.02 (17)
spleen	1.23 (10)	1.21 (13)	1.21 (20)	0.72 (45)	spleen	1.72 (7)	1.67 (45)	1.96 (17)	1.67 (35)
lungs	1.43 (16)	1.49 (26)	1.02 (22)	0.48 (20)	lungs	1.88 (18)	1.59 (28)	1.48 (19)	1.18 (16)
liver	5.81 (10)	5.44 (15)	4.31 (8)	3.78 (7)	liver	4.73 (11)	4.63 (17)	3.65 (13)	2.93 (16)
	[^{125}I]-**3d** (20*E* Isomer, 7α-Methyl)					[^{125}I]-**6d** (20*Z* Isomer, 7α-Methyl)			
blood	0.34 (14)	0.46 (27)	0.32 (35)	0.33 (25)	blood	0.47 (29)	0.33 (7)	0.35 (21)	0.29 (14)
uterus	7.30 (14)	1.14 (33)	9.27 (28)	8.26 (18)	uterus	7.92 (26)	0.51 (23)	9.08 (24)	7.66 (29)
ovaries	4.35 (18)	3.78 (18)	4.15 (29)	2.91 (21)	ovaries	3.54 (22)	1.37 (22)	2.57 (24)	2.24 (37)
adrenals	3.33 (11)	4.69 (24)	2.49 (16)	1.57 (26)	adrenals	2.84 (27)	1.56 (8)	1.49 (22)	0.88 (10)
thyroid	61.58 (14)	54.71 (35)	60.78 (30)	237.90 (14)	thyroid	84.78 (22)	83.77 (1)	185.08 (9)	434.36 (6)
muscle	0.98 (5)	1.26 (26)	0.65 (13)	0.65 (31)	muscle	1.13 (32)	0.53 (32)	0.59 (36)	0.31 (18)
fat	5.03 (16)	6.57 (14)	3.44 (10)	2.59 (23)	fat	4.51 (14)	2.40 (4)	1.69 (22)	0.84 (20)
brain	0.90 (9)	1.21 (21)	0.61 (29)	0.31 (31)	brain	0.77 (17)	0.53 (8)	0.24 (22)	0.10 (11)
kidneys	2.83 (8)	3.18 (19)	2.10 (29)	1.19 (23)	kidneys	1.90 (18)	1.02 (9)	1.17 (23)	0.75 (12)
spleen	1.23 (17)	1.47 (21)	1.09 (47)	0.64 (19)	spleen	1.29 (32)	2.04 (33)	1.13 (50)	0.59 (15)
lungs	2.17 (13)	2.88 (24)	1.68 (21)	0.99 (29)	lungs	1.53 (22)	0.85 (21)	0.74 (24)	0.51 (11)
liver	12.44 (5)	14.87 (19)	8.15 (25)	4.99 (20)	liver	3.60 (17)	2.13 (3)	1.63 (18)	1.10 (16)

[a] Average % ID/g for 4 or 5 rats. Each rat was injected iv with 3 μCi (111 kBq) of nca ^{125}I-labeled steroid in the presence (+E_2) or absence of 60 μg coinjected estradiol. The incertitude is given in brackets as the % CV, e.g. the percent coefficient of variation corrected for small sample size effect.[20]

lowers the in vivo stability of the (17α,20*Z*)-iodovinyl substituents.

Discussion

The reaction sequences, workup, and radiochemical yield of the 20*E* and 20*Z* isomers of the 11β- and 7α-substituted ^{125}I-labeled (17α,20*E*/*Z*)-(iodovinyl)estradiols **3b–d** and **6b–d**, is very similar to that reported for the analogous isomeric products [^{125}I]-**3a** and [^{125}I]-**6a** lacking these substituents.[6]

Our earlier biological studies[6] with the 20*E* and 20*Z* isomers of (17α,20*E*/*Z*)-(iodovinyl)estradiol **3a** and **6a** suggested that the 20*Z* configuration, although somewhat less stable, provided for the best in vivo localization of the isomeric 17α-(iodovinyl) steroids. All 7α-methyl derivatives exhibited higher binding affinities for the estrogen receptor than the 11β-methoxy or -ethoxy derivatives (Table I). All eight isomeric products **3a–d** and **6a–d** exhibited RBA values, measured at 0–4 °C, in the 30–50 range (Table I). Among all four pairs of isomers, the *Z* isomers exhibited slightly higher RBA values (Table I) which is consistent with the higher uterus uptake of the *Z* isomers at 1 h postinjection (Figure 1). In the case of the isomeric 11β-substituted derivatives **3b,c** and **6b,c** the higher RBA's of the *Z* isomers parallel their prolonged receptor-mediated uterus uptake (Figure 1). Uterus uptake was significantly higher for all 11β- and 7α-substituted estradiol derivatives tested, as compared to the 17α-(iodovinyl)estradiols lacking such substituents.[6] This is in agreement with earlier observations[2] on the 11β-methoxy 20*E* isomer **3a** and reflects the increased stability of the estrogen-receptor complex as a result of the added substituents.[7] Uterus retention of the 20*Z* isomers [^{125}I]-**6** is somewhat more persistent as compared to that of the 20*E* isomers [^{125}I]-**3** (Table II). However, uterus to blood or nontarget ratios are more favorable for the 20*E* isomers **3b,c** (Figure 1). This is most likely due to the higher instability of the corresponding 20*Z* isomers, which is evident from the high thyroid ^{125}I uptake (percent deiodination, Figure 2) and which may also be reflected in the higher ^{125}I-blood levels of the 20*Z* isomers (Table I). The in vivo stability of the isomeric [^{125}I]-**3** and -**6** are strongly influenced by the nature of the substituents, and in particular, addition of the 11β-ethoxy group renders the (17α,20*Z*)-(iodovinyl)estradiol prone to deiodination (Figure 2). Thus, contrary to our conclusion concerning the isomeric (17α,20*E*/*Z*)-(iodovinyl)estradiols **3a** and **6a**, where the higher instability of the 20*Z* isomer was com-

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pensated for by its better tissue-localizing properties, the 20*E* isomers of 11β- and 7α-substituted (17α,20*E*/*Z*)-(iodovinyl)estradiols (**3**) possess the advantageous configuration for radiopharmaceutical applications due to their higher in vivo stability. The influence of the 11-stereochemistry in [^{125}I]-**3** has recently been addressed by Hanson et al.,[14] confirming the essential 11β-configuration for optimal uterine localization. Among the isomeric products tested in this study, the 20*E* isomer of the 11β-methoxy derivatives (**3b**) reached the highest target to nontarget and target to blood ratios. Our uterus to blood ratios and absolute uterus uptake values for **3b–d** and **6b–d** are comparable to those reported for the analogous (17α,20*E*)-(iodovinyl)-11β-ethylestradiol.[15] These data point at a possible synergism between 11β/7α-substituents and the configuration at C-20 in terms of in vivo (target uptake, stability) and in vitro (RBA) properties. However more derivatives will be required to fully exploit such a relationship for the design of an improved radiopharmaceutical and work to this end is in progress in our laboratory.

In summary, we have presented methods for the synthesis of 20*E*,*Z* isomeric 17α-(iodovinyl)estradiols substituted at the 11β- and 7α-positions and provided evidence for the improved target localization of the radioiodinated analogues. The labeling procedures can readily be used with ^{123}I and only clinical trials with the ^{123}I-analogues will be able to determine the usefulness of these isomeric radiopharmaceuticals in the management of breast cancer.

Experimental Section

Melting points (mp) were determined on a Fisher-Johns apparatus and are uncorrected. Spectral data (UV, Varian UV/vis 2200 spectrophotometer; ^{1}H and ^{13}C NMR, Bruker WM 25 Spectrometer, in $CDCl_3$ + DMSO-d_6 with Me_4Si as an integral standard; EIMS, Hewlett-Packard Model 5988A quadrupole instrument) were recorded. Microanalysis data were obtained by Guelph Laboratories Ltd., Guelph, Ont., Canada. Silica gel (60–200 mesh) was used for column chromatography. Silica gel plates coated with fluorescent indicator (UV 254) were used for analytical thin-layer chromatography (TLC) and the compounds were located by their UV absorbance/or color response upon spraying with H_2SO_4/EtOH and heating at 120 °C. The following two solvent systems were used for TLC: system I, 3:7 EtOAc/hexane; system II, 10:1 $CHCl_3/CH_3CN$. 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 and, where appropriate, by their γ-radiation, which was registered via a sodium iodide detector. All chemicals used are commercially available and were of the highest chemical grade available; carrier-free (nca) [^{125}I]NaI was purchased from Amersham Canada Ltd. Steroids were purchased from Steraloids Inc. 17-(Ethylenedioxy)estra-1,3,5(10)-triene-3,11β-diol was synthesized according to Baran[16] which was converted to 11β-methoxy or 11β-ethoxy 3-hydroxyestra-1,3,5(10)trien-17-one with methyl iodide or ethyl iodide.[17] The ketone derivatives were subsequently converted to **1b,c** by treatment with lithium acetylide–ethylenediamine complex.[18]

(17α,20*E*)-21-(Tributylstannyl)-11β-methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (2b). A mixture of 11β-methoxy-17α-ethynylestradiol (**1b**; 50 mg, 0.153 mmol) in 5 mL of toluene and 0.1 mL of tri-*n*-butyltin hydride (0.37 mmol) was heated at 95–100 °C for 4 h in the presence of azobisisobutyronitrile (5.3 mg, 0.34 mmol) under nitrogen. The solvent was evaporated under reduced pressure and the residue was chromatographed on a silica gel column. Elution with 10% EtOAc in hexane gave **2b** as a single major product (55 mg, 50%): mp 131 °C; TLC R_f (system I) 0.45, (system II) 0.44; HPLC, gradient of 5% H_2O in MeOH to 100% MeOH (20 min) gave a single peak at t_R = 17 min; MS *m*/*z* (relative intensity) 618 ($M^{\cdot+}$, 0.5), 617 ($M^{\cdot+}$, 1.5), 616 ($M^{\cdot+}$, 0.7), 561 (100), 553 (3), 543 (7), 453 (5), 397 (11), 277 (23).

(17α,20*E*)-21-Iodo-11β-methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (3b). To compound **2b** (31 mg, 0.05 mmol) in $CHCl_3$ (3 mL) was gradually added at room temperature a 0.1 M solution of iodine in $CHCl_3$ until the color of iodine persisted. This was followed sequentially by the addition of 0.2 mL of 1 M KF in MeOH and 0.2 mL of 5% aqueous $NaHSO_3$. The mixture was then extracted with $CHCl_3$ (2 × 10 mL). The organic phase was dried over $MgSO_4$ (anhydrous), filtered, and evaporated to dryness. The residue was purified on a C-18 reverse-phase semipreparative HPLC column in 70:30 MeOH/H_2O. The desired compound **3b** eluted after 32 min and was crystallized from MeOH (18 mg, 79.2%): mp 125–135 °C (lit.[2c] mp 130–132 °C); TLC R_f (system I) 0.23, (system II) 0.27; UV (EtOH) λ_{max} 280 nm; ^{1}H NMR δ 0.84 (s, 3 H, 18-CH_3), 1.0–3.0 (br m, steroid nucleus), 3.14 (s, 3 H, 11β-OCH_3), 4.01 (m, 1 H, 11α-H), 6.22 (d, *J* = 14 Hz, 1 H, =CHI); 6.68 (d, *J* = 14 Hz, 1 H, —CH=), 6.38–6.82 (m, 2 H, 1-CH and 4-CH), 7.0–7.12 (m, 1 H, 2-CH), 8.52 (3, 1 H, PhOH); ^{13}C NMR δ 14.2 (C-18), 22 (C-15), 26.4 (C-7), 28.6 (C-6), 31.4 (C-16), 33.8 (C-12), 34.9 (C-8), 45.9 (C-13), 47.9 (C-9), 49.1 (C-14), 54.9 (C-11), 73.05 (C-21), 75.5 (11β-OCH_3), 85.5 (C-17), 112.2 (C-2), 114.3 (C-4), 125.6 (C-1), 126.3 (C-10), 137.0 (C-5), 150.8 (C-20), 153.8 (C-3); MS *m*/*z* (relative intensity) 454 ($M^{\cdot+}$, 20), 404 (4), 389 (8), 327 (27), 277 (45), 251 (14), 235 (14), 226 (58), 200 (49), 180 (20), 181 (81), 157 (100), 145 (90), 131 (80). Anal. ($C_{21}H_{27}IO_3$) C, H, I.

(17α,20*Z*)-21-(Tributylstannyl)-11β-methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (4b). A mixture of **1b** (70 mg, 0.214 mmol) and 0.1 mL (0.37 mmol) of tributyltinhydride was stirred in 2 mL of hexamethylphosphoric triamide at 60–65 °C for 5 h. The reaction mixture was diluted with EtOAc (20 mL), washed with water, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed over silica gel. Elution with 10% EtOAc in hexane gave compound **4b** (60 mg, 45.3%), TLC R_f (system I) 0.64, (system II) 0.72; HPLC, gradient of 5% H_2O and MeOH to 100% MeOH (20 min) t_R = 26 min; mp 162–154 °C; MS *m*/*z* (relative intensity) 618 ($M^{\cdot+}$, 0.3), 617 ($M^{\cdot+}$, 2), 616 ($M^{\cdot+}$, 3), 561 (23), 543 (69), 509 (20), 328 (30), 251 (50), 226 (97), 211 (100).

Further elution of the silica gel column with the same solvent mixture gave **2** as a minor product. A final elution with 10% EtOAc in hexane gave unreacted starting material.

(17α,20*Z*)-21-Iodo-11β-methoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (6b). To **4b** (40 mg, 0.064 mmol) in $CHCl_3$ (3 mL) was gradually added at room temperature a 0.1 M solution of I_2 in $CHCl_3$ until the color of iodine persisted. This was followed sequentially by the addition of 0.2 mL of 1 M KF in MeOH and 0.2 mL of 5% aqueous $NaHSO_3$. The mixture was then extracted with $CHCl_3$ (2 × 10 mL). The organic phase was worked up as usual and the residue was purified by HPLC over a C-18 reverse-phase column with 70:30 MeOH/H_2O. Compound **5b** eluted after 21 min as a minor product (6 mg, 28.5%): TLC R_f (system I) 0.21, (system II) 0.24; mp 125–135 °C; ^{1}H NMR δ 0.93 (s, 3 H, 18-CH_3), 1.0–3.0 (br m, steroid nucleus), 3.07 (s, 3 H, 11β-OCH_3), 3.96 (m, 1 H, 11α-H), 4.9–5.1 (m, 2 H, =CH_2), 5.9 (m, 1 H, —CH=), 6.26–6.8 (m, 2 H, 1-H and 4-H), 6.78 (m, 1 H, 2-H); ^{13}C NMR δ 14.2 (C-18), 21.9 (C-5), 26.3 (C-7), 28.5 (C-6), 31.10 (C-16), 33.6 (C-12), 34.5 (C-8), 45.4 (C-13), 48.5 (C-9,C-14), 54.7 (C-11), 75.30 (C-21), 75.50 (OCH_3), 82.5 (C-17), 112 (C-2), 114.2 (C-4), 125.5 (C-1), 126.4 (C-10), 137 (C-5), 142.9 (C-20), 153.5 (C-3); MS *m*/*z* (relative intensity) 328 ($M^{\cdot+}$, 28), 310 (18), 298 (18), 278 (16), 252 (14), 251 (17), 241 (18), 227 (19), 226 (100). Anal. ($C_{21}H_{28}O_3$) C, H.

Further elution with the same solvent mixture gave after 32 min compound **6b**, which was crystallized from MeOH (15 mg, 51.5%); mp 221–223 °C; TLC R_f (system I) 0.27, (system II) 0.36; UV (EtOH) λ_{max} 280 nm; ^{1}H NMR δ 0.98 (s, 3 H, 18-CH_3), 1.0–3.0 (br m, steroid nucleus), 3.04 (s, 3 H, 11β-OCH_3), 3.96 (m, 1 H, 11α-H), 6.22 (d, *J* = 8 Hz, 1 H, =CHI), 6.63 (d, *J* = 8 Hz, 1 H,

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—CH=), 6.26–6.8 (m, 2 H, 1-H and 4-H), 6.82 (m, 1 H, 2-H); ^{13}C NMR δ 14.5 (C-18), 22.3 (C-15), 26.6 (C-7), 28.8 (C-6), 31.1 (C-16), 33.9 (C-12), 35.8 (C-8), 47.4 (C-13), 48 (C-19), 49.4 (C-14), 55.2 (C-11), 75.7 (C-21), 83.5 (C-17), 114.6 (C-2), 114.6 (C-4), 125.7 (C-1), 126.6 (C-10), 137.4 (C-5), 143.5 (C-20), 153.9 (C-3); MS *m/z* (relative intensity) 454 ($M^{\cdot+}$, 13), 404 (16), 389 (40), 327 (15), 296 (4), 295 (25), 277 (55), 251 (46), 211 (54), 160 (63), 157 (86), 145 (73), 127 (100). Anal. ($C_{21}H_{27}IO_3$) C, H, I.

(17α,20*E*)-21-(Tributylstannyl)-11β-ethoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (2c). A mixture of 11β-ethoxy-17α-ethynylestradiol (**1c**; 50 mg, 0.152 mmol) in 5 mL of toluene and 0.1 mL of tri-*n*-butyltin hydride (0.37 mmol) was heated at 60–65 °C for 5 h in the presence of azobisisobutyronitrile (5.3 mg, 0.34 mmol) under nitrogen and worked up in a similar manner as described above and chromatographed on a silica gel column. Elution with 5% EtOAc in hexane gave **2c** as a single major product (55 mg, 58%): mp 112–113 °C; HPLC, gradient of 5% H_2O in MeOH to 100% (20 min) t_R = 14 min; ^{1}H NMR ($CDCl_3$) δ 0.80–2.8 (m, steroid nucleus plus *n*-Bu_3), 3.31–3.62 (q, *J* = 7 Hz, 2 H, 11β-OCH_2CH_3), 4.18 (m, 1 H, 11α-H), 6.15 (sextet, 2 H, *J* = 2 Hz, CH=CH), 6.52 (d, *J* = 2 H, 1 H, 4-H), 6.62 (dd, *J* = 2 and 8 Hz, 1 H, 2-H), 6.98 (d, *J* = 8 Hz, 1 H, 1-H); MS *m/z* (relative intensity), 632 ($M^{\cdot+}$, 1), 631 (M·1+, 1), 575 (100), 574 (49), 571 (41), 557 (10), 519 (51), 489 (10), 397 (12), 342 (12), 315 (22).

(17α,20*E*)-21-Iodo-11β-ethoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (3c). To compound **2c** (80 mg, 0.126 mmol) in $CHCl_3$ (4 mL) at room temperature was added a 0.1 mL solution of iodine in $CHCl_3$ as described above, extracted with $CHCl_3$, and worked up in the usual manner. The residue was purified on a C-18 reverse-phase semipreparative HPLC colmn in 70:30 MeOH/H_2O. The desired compound **3c** eluted after 31 min and was crystallized from methanol (50 mg, 84.8%): mp 126–129 °C; ^{1}H NMR δ 0.89 (t, *J* = 7 Hz, 3 H, 11β-OCH_2CH_3), 0.97 (s, 3 H, 18-CH_3), 3.14, 3.48 (q, *J* = 7 Hz, 2H, 11β-OCH_2CH_3), 4.07 (m, 1 H, 11α-H), 6.12 (d, *J* = 14 Hz, 1 H, =CHI), 6.40 (d, *J* = 2 H, 1 H, 4-H), 6.48 (dd, *J* = 2 and 8 Hz, 1 H, 2-H), 6.63 (d, *J* = 14 Hz, 1 H, —CH=), 6.80 (d, *J* = 8 Hz, 1 H, 1-H); ^{13}C NMR δ 14.89 (C-18), 15.16 (11β-OCH_2CH_3), 22.86 (C-15), 27.30 (C-7), 29.34 (C-6), 33.22 (C-16), 33.45 (C-12), 36.01 (C-8), 48.66 (C-13), 50.02 (C-14), 63.46 (11β-OCH_2), 74.02 (C-21), 77.04 (C-11), 84.54 (C-17), 112.75 (C-2), 115.10 (C-4), 126.62 (C-1), 127.54 (C-10), 151.16 (C-20); MS *m/z* (relative intensity) 468 ($M^{\cdot+}$, 7), 422 (3), 389 (11), 341 (21), 295 (45), 277 (42), 251 (21), 226 (59), 211 (62), 181 (54), 172 (32), 157 (100). Anal. ($C_{22}H_{29}IO_3$) C, H, I.

(17α,20*Z*)-21-(Tributylstannyl)-11β-ethoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (4c). A mixture of 11β-ethoxy-17α-ethynylestradiol (**1c**; 50 mg, 0.152 mmol) in 2 mL of hexamethylphosphoric triamide and 0.1 mL of tri-*n*-butyltin hydride (0.37 mmol) was heated at 60–65 °C for 5 h under nitrogen and worked up in a similar manner as described above and chromatographed on a silica gel column. Elution with 5% EtOAc in hexane gave **4c** as a major product (45 mg, 47%): 148–149 °C; HPLC, gradient of 5% H_2O in MeOH to 100% MeOH (20 min) t_R = 20 min; ^{1}H NMR ($CDCl_3$) δ 0.80–2.6 (m, steroid nucleus plus *n*-Bu_3), 3.31–3.62 (q, *J* = 7 Hz, 2 H, 11β-OCH_2CH_3), 4.35 (m, 1 H, 11α-H), 5.86 (d, 1 H, *J* = 17 Hz, CH=CH), 6.52 (d, *J* = 2 H, 1 H, 4-H), 6.70 (dd, *J* = 2 and 8 Hz, 1 H, 2-H), 6.99 (d, *J* = 8 Hz, 1 H, 1-H); MS *m/z* (relative intensity) 633 ($M^{\cdot+}$, 4) 632 ($M^{\cdot+}$, 3), 631 ($M^{\cdot+}$, 3), 575 (64), 573 (46), 571 (26), 557 (100), 553 (51), 509 (51), 461 (21), 415 (27), 413 (20), 395 (30) 313 (36).

Further elution with the same solvent gave compound **2c** (10–15%). Elution with 10% EtOAc in hexane gave unreacted starting material (10%).

(17α,20*Z*)-21-Iodo-11β-ethoxy-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (6c). Compound **4c** (63 mg, 0.1 mmol) in $CHCl_3$ (4 mL) was treated at room temperature with 0.1 mL of a solution of iodine in $CHCl_3$ until the color of iodine persisted as described above, extracted with $CHCl_3$, and worked up in the usual manner. The residue was purified on a C-18 reverse-phase semipreparative HPLC column in 70:30 MeOH/H_2O. The compound eluted at 17 min was identified as minor component **5c** (4 mg, 11.7%): mp 220–222 °C; ^{1}H NMR δ 0.69 (t, *J* = 7 Hz, 3 H, 11β-OCH_2CH_3), 0.79 (s, 3 H, 18-CH_3), 3.29 (q, *J* = 7 Hz, 2 H, 11β-OCH_2CH_3), 3.85 (m, 1 H, 11α-H), 4.72–4.88 (m, 2 H, =CH_2), 5.69–5.80 (m, 1 H, —CH=), 6.18 (d, *J* = 2 Hz, 1 H, 4-H), 6.25 (dd, *J* = 2 and 8 Hz, 1 H, 2-H), 6.6 (d, *J* = 8 Hz, 1 H, 1-H); MS *m/z* (relative intensity) 342 ($M^{\cdot+}$, 18) 324 (18), 296 (16), 251 (34), 226 (100), 211 (82), 200 (65). Anal. ($C_{22}H_{30}O_3$) C, H, I.

Further elution with the same solvent mixture gave after 28 min compound **6c**, which was crystallized from MeOH (30 mg, 64%): mp 214–215 °C; ^{1}H NMR δ 0.89 (t, *J* = 1 Hz, 3 H, 11β-OCH_2CH_3), 1.01 (s, 3 H, 18-CH_3), 3.14, 3.50 (q, *J* = 7 Hz, 2 H, 11β-OCH_2CH_3), 4.10 (m, 1 H, 11α-H), 6.22 (d, *J* = 8 Hz, 1 H, =CHI), 6.39 (d, *J* = 2 H, 1 H, 4-H), 6.52 (dd, *J* = 2 and 8 Hz, 1 H, 2-H), 6.65 (d, *J* = 8 Hz, 1 H, —CH=), 6.80 (d, *J* = 8 Hz, 1 H, 1-H); ^{13}C NMR δ 14.91 (C-18), 15.23 (11β-OCH_2CH_3), 22.84 (C-15), 27.28 (C-7), 29.30 (C-6), 32.66 (C-16), 33.35 (C-12), 36.95 (C-8), 48.62 (C-13), 50.22 (C-14), 63.48 (11β-OCH_2), 74.04 (C-11), 77.51 (C-21), 84.54 (C-17), 112.71 (C-2), 115.06 (C-4), 126.53 (C-1), 127.53 (C-10), 143.73 (C-20); MS *m/z* (relative intensity) 469 ($M^{\cdot+}$, 10), 404 (9), 389 (11), 341 (25), 295 (41), 277 (40), 226 (78), 223 (21), 211 (64), 200 (49), 1181 (54), 157 (100). Anal. ($C_{22}H_{29}IO_3$) C, H, I.

(17α,20*E*)-21-(Tributylstannyl)-7α-methyl-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (2d). A mixture of 7α-methyl-17α-ethynylestradiol (**1d**, 100 mg, 0.323 mmol) in 5 mL of toluene and 0.3 mL of tri-*n*-butyltin hydride (0.1 mmol) was heated at 90 °C for 5 h in the presence of azobisisobutyronitrile (16.6 mg, 1 mmol) under nitrogen. The solvent was evaporated to dryness under reduced pressure and the residue was chromatographed on a silica gel column. Elution with 5–10% EtOAc in hexane gave **2d** as a single major product, which crystallized out as a white solid from hexane (130 mg, 0.216 mmol, 67%): mp 105 °C; HPLC, gradient 5% H_2O in MeOH to 100% MeOH (20 min), t_R = 21 min; ^{1}H NMR ($CDCl_3$) δ 0.8–2.4 (m, steroid nucleus + *n*-Bu_3), 2.8–3.1 (3 H), 5.6 (1 H), 6.08–6.7 (3 H), 6.9–7.2 (2 H); MS *m/z* (relative intensity) 602 ($M^{\cdot+}$, 7.1), 598 ($M^{\cdot+}$, 17.8), 545 (28), 543 (35), 541 (17), 228 (100).

(17α,20*E*)-21-Iodo-7α-methyl-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (3d). To compound **2d** (120 mg, 0.2 mmol) in 4 mL of $CHCl_3$ 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 0.2 mL of 1 M KF in MeOH and 0.2 mL of 5% $NaHSO_3$. The mixture was then extracted with EtOAc (2 × 10 mL). The organic phase was dried over $MgSO_4$ (anhydrous), filtered, and evaporated to dryness. The residue was purified on a reverse-phase HPLC column in 80:20 MeOH/H_2O. The desired compound **3d** eluted for 18 min and was crystallized from MeOH (60 mg, 68.5%): mp 146–148 °C; UV (EtOH) λ_{max} 280 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 0.82 (d, *J* = 8 Hz, 3 H, 7α-CH_3), 0.88 (s, 3 H, 18-CH_3), 1.0–3.0 (steroid nucleus), 6.2 (d, *J* = 14 Hz, 1 H, =CHI), 6.74 (d *J* = 14 Hz, 1 H, HC=), 6.6 (d, 1 H, *J* = 2 Hz, 4-H), 6.80 (dd, *J* = 2 and 8 Hz, 1 H, 2-H), 7.08 (d, *J* = 8 Hz, 1 H, 1-H); ^{13}C NMR δ 11.82 (7α-CH_3), 13.19 (C-18), 21.61 (C-15), 26.05 (C-11), 26.57 (C-7), 31.75 (C-16), 35.08 (C-12), 36.05 (C-6), 37.12 (C-8), 40.92 (C-9), 45.11 (C-13), 46.24 (C-14), 72.96 (C-21), 85.53 (C-17), 112.11 (C-2), 115.08 (C-4), 125.64 (C-1), 128.92 (C-10), 135.37 (C-5), 150.98 (C-20), 154.03 (C-3); MS *m/z* (relative intensity) 438 ($M^{\cdot+}$, 65), 311 (34), 310 (52), 293 (10), 286 (17), 227 (100). Anal. ($C_{21}H_{27}IO_3$) C, H, I.

(17α,20*Z*)-21-(Tributylstannyl)-7α-methyl-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (4d). Compound **1d** (50 mg, 0.16 mmol) in 3 mL of hexamethylphosphoric triamide and 0.2 mL of tri-*n*-butyltin hydride was heated at 65 °C under nitrogen for 50 h and worked up in the usual manner. The residue was purified on a silica gel column. Elution with 5% EtOAc in hexane gave a single major product (**4d**; 45 mg, 47%) which failed to solidify: HPLC, gradient of 5% H_2O in MeOH to 100% MeOH (20 min) t_R = 29 min; MS *m/z* (relative intensity) 602 ($M^{\cdot+}$, 1), 598 (M^+, 1), 548 (8), 527 (23), 413 (6), 312 (23), 291 (6), 225 (8), 177 (10), 55 (100).

Continuous elution with 5% EtOAc in hexane gave 10–15% of **2d**, further elution with 10% EtOAc in hexane gave 15–20% unreacted starting material.

(17α,20*Z*)-21-Iodo-7α-methyl-19-norpregna-1-3,5(10),20-tetraene-3,17-diol (6d). Compound **4d** (60 mg, 0.1 mmol) in $CHCl_3$ (4 mL) was treated at room temperature with 0.1 mL of a soltion of iodine in $CHCl_3$, analogous to the method described above for the preparation of **3d**. After extraction with $CHCl_3$ the residue was purified on a C-18 reverse-phase semipreparative HPLC olumn in 70:30 MeOH/H_2O. The compound eluting at

22 min as a minor component was identified as **5d** (9 mg, 11%): mp 75–85 °C; ^{1}H NMR δ 0.69 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.80 (s, 3 H, 18-CH_3), 4.96–5.08 (m, 2 H, =CH_2), 5.93–6.04 (m, 1 H, —CH=), 6.40 (d, J = 2 Hz, 1 H, 4-H), 6.50 (dd, J = 2 and 8 Hz, 1 H, 2-H), 6.95 (d, J = 8 Hz, 1 H, 1-H); MS m/z (relative intensity) 312 ($M^{\cdot+}$, 31), 294 (6), 279 (5), 240 (230), 227 (94), 235 (25) 213 (15), 200, (17), 185 (27), 174 (100), 157 (58). Anal. ($C_{21}H_{28}O_2$) C, H.

Further elution with the same solvent mixture gave after 32 min a major fraction identified as **6d**, crystallized from MeOH (35 mg, 80%): mp 108–112 °C; ^{1}H NMR δ 0.83 (d, J = 7 Hz, 3 H, 7α-CH_3), 0.96 (s, 3 H, 18-CH_3), 6.39 (d, J = 8 Hz, 1 H, =CHI), 6.53 (d, J = 2 H, 1 H, 3-H), 6.63 (dd, J = 2 and 8 Hz, 1 H, 2-H), 6.84 (d, J = 8 Hz, 1 H, —CH=), 7.15 (d, J = 8 Hz, 1 H, 1-H); ^{13}C NMR δ 12.16 (7α-CH_3), 13.53 (C-18), 21.94 (C-15), 26.34 (C-11), 28.84 (C-7), 31.45 (C-16), 35.46 (C-12), 36.33 (C-6), 37.4 (C-8), 41.4 (C-9), 41.54 (C-13), 47.74 (C-14), 75.90 (C-21), 83.6 (C-17), 112.39 (C-2), 115.4 (C-4), 125.4 (C-1), 129.27 (C-10), 135.76 (C-5), 143.72 (C-20), 154.30 (C-3); MS m/z (relative intensity) 438 ($M^{\cdot+}$, 37), 420 (3), 311 (14), 254 (19), 227 (91), 181 (30), 174 (74), 171 (67), 174 (83), 55 (100). Anal. ($C_{21}H_{27}IO_2$) C, H, I.

(17α,20*E*)-21-([^{125}I]Iodovinyl)estradiol Derivatives ([^{125}I]-3b–d). To a mixture of **2b**, **2c**, or **2d** (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 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 Altech column operated at a flow rate of 1 mL/min. Elution with 70:30 MeOH/H_2O gave [^{125}I]-**3b** (350 μCi, 70%, t_R = 18 min), [^{125}I]-**3c** (350 μCi, 70%, t_R = 21 min), and [^{125}I]-**3d** (350–400 μCi, 70–80%, t_R = 15 min). The retention times of free iodine was 4 min.

(17α,10*Z*)-21-([^{125}I]Iodovinyl)estradiol Derivatives ([^{125}I]-6b–d). To a solution of [^{125}I]NaI (500 μCi) in 0.5 mL in H_2O was added 0.2 mg of chloroamine-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 **4b**, **4c**, and **4d** 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 for [^{125}I]-**3** and after HPLC were obtained in 60–70% overall yield ([^{125}I]-**6b**, t_R= 21 min; [^{125}I]-**6c**, t_R = 25 min; [^{125}I]-**6d**, t_R = 15 min).

Estrogen Receptor Binding Assay. Affinity of the estradiol derivatives for estrogen receptors was determined by a competitive binding assay[13] and is expressed as the relative binding affinity. The RBA is defined as 100 times the ratio between competitor and the unlabeled estradiol concentrations required for 50% competition to specific [^{3}H]estradiol binding. Murine uterine cytoplasmic extracts were incubated at 0–4 °C for 18 h with 20 nM of [^{3}H]estradiol in the absence and presence of competitive steroids ranging from 2 nM to 20 μM. The bound steroid was separated from free steroid by Sephadex LH-20 chromatography. The nonspecific binding (equivalent to that observed in the presence of a 100-fold excess of unlabeled estradiol) was 3–4% of the total binding which was subtracted from the total binding to estimate the specific binding. The specific binding (average of three experiments) in the receptor preparation was equivalent to 6.3 nM.

In Vivo Studies. The animal experiments were conducted in accordance with the recommendations of the Canadian Council of Animal Care and of the in-house Ethic Committee for Animal Experiments as previously described.[6] Briefly, immature female Fischer rats (24 days old, 55–60 g, Charles River) 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.[19] 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 γ-counter (LKB Wallac, Finland) and concentrations were expressed as percent of the injected dose per gram of tissue (% ID/g). Statistical variations are presented as the percent coefficient of variation corrected for small sample size effect (% CV).[20]

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