diately on dry ice, and either used fresh or stored at −80 °C until used in binding studies. Striatal tissue was homogenized in 50 mM HEPES buffer (pH 7.4, 25 °C) at a Brinkman Polytron, PCU-2 setting of 3.0 for 5 s. The tissue suspension was then centrifuged at 32000*g* for 15 min, the supernatant discarded, and this wash step repeated. After the second wash the final pellet was resuspended at a wet weight concentration of 1.25 mg of tissue/mL of buffer for use. Radioligand binding was performed in 12 × 75 mm culture tubes at a total assay volume of 1.0 mL. Each tube contained 100 μL of competitor, 100 μL of radioligand, and 800 μL of tissue homogenate prepared as described above. Competing drugs were dissolved in 0.1% tartaric acid at 1.0 mM concentrations and diluted appropriately with buffer. [^{3}H]-SCH23390[9] and [^{3}H]spiperone,[33] at 0.25 and 0.02 nM concentrations, respectively, were diluted from methanol stock solutions with buffer. All tubes in the spiperone assays also contained a final concentration of 50 nM ketanserin in order to mask 5-HT_2 receptor binding. Reactions were initiated by the addition of tissue to tubes already containing radioligand and any competitors. The tubes, which were maintained on ice prior to the addition of tissue, were then vortexed and incubated at 37 °C for 15 min. Binding was terminated by rapid filtration over 1-μm glass fiber filters onto a Skatron cell harvester. IC_{50} values were calculated from a linear regression of a Hill transformation with all n_h values equal to 1 ± 0.1 for these compounds. Therefore, K_i values were calculated on the basis of the Cheng–Prusoff[34] relationship for competitive inhibition.

Adenylate Cyclase Assay. The effects of the test compounds on adenylate cyclase were determined by the method of Schulz and Mailman.[35] The conversion of [^{32}P]ATP to [^{32}P]cyclic AMP (cAMP) in striatal homogenates was quantified by using an automated preparative HPLC procedure, rather than column chromatography,[36] to separate cAMP from other labeled nucleotides. Striatal tissue (obtained as described earlier) was removed and homogenized at 50 mL/g tissue in 5.0 mM HEPES buffer (pH 7.5) containing 2.0 mM EGTA. After nine manual strokes with a Teflon–glass homogenizer, an additional 50 mL/g of 100 mM HEPES–2.0 mM EGTA was added and mixed with one additional stroke. A 20-μL aliquot of this tissue homogenate was added to a prepared reaction mixture, yielding a final volume of 100 μL containing 0.5 mM ATP, [^{32}P]ATP (0.5 μCi), 1.0 mM cAMP, 2.0 mM $MgCl_2$, 0.5 mM IBMX, 0.7 mM HEPES buffer, 2.0 μM GTP, 0–100 μM dopamine, 10 mM phosphocreatine, and 5.0 units of creatine phosphokinase. The reaction was initiated by placing the samples in a water bath at 30 °C and terminated 15 min later by adding 100 μL of 3% SDS. Drugs to be tested (e.g., varying concentrations of a test compound alone or in the presence of 100 μM dopamine) were added in an appropriate vehicle (usually 0.1% tartaric or 0.5 M hydrochloric acid). Subsequent to incubation, proteins and much of the noncyclic nucleotides were precipitated by addition of 300 μL each of 8.0% $ZnSO_4$ and 18% $Ba(OH)_2$ (pH 2.0) to each incubation tube. The samples were centrifuged at 10000*g* for 6 min, and the supernatants were immediately removed and loaded in an autoinjector. The HPLC separations described were carried out with a mobile phase of 100 mM sodium acetate–20% methanol (pH 5.0). A flow rate of 4.0–4.5 mL/min was used for separation. The autoinjector was programmed for a 2-min injection interval, with a rinse between samples. A UV detector equipped for 254 nm detection triggered collection of the cAMP fractions via a fraction collector and a three-way diversion valve. Unlabeled cAMP added to the samples provided the source of UV absorbance.

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Registry No. 1, 104-47-2; 2, 7569-58-6; 3, 7569-87-1; 4, 115514-67-5; 5, 115514-68-6; (±)-6, 115514-69-7; (+)-6, 115589-10-1; (+)-6-(D)-*N*-acetylleucine, 115647-99-9; (−)-6, 115589-11-2; (−)-6-(L)-*N*-acetylleucine, 115648-00-5; (±)-7, 115514-70-0; (+)-7, 115589-13-4; (−)-7, 115589-12-3; (±)-8, 115514-71-1; (+)-8, 115589-15-6; (−)-8, 115589-14-5; (+)-9, 115514-85-7; (−)-9, 115514-86-8; 10, 115514-72-2; 11, 115514-73-3; 12, 115514-74-4; 13, 115514-75-5; 14, 115514-76-6; 15, 2393-23-9; 16, 115514-77-7; 17, 115514-78-8; 18, 115514-79-9; 19, 115514-80-2; 20, 115514-81-3; 21, 115514-82-4; 23, 115514-84-6; 23 (O-methylated), 115514-83-5; styrene oxide, 96-09-3.

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

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The isomeric (17α,20*E*)- and (17α,20*Z*)-(iodovinyl)estradiol derivatives **3** and **6**, and their no-carrier-added (nca) [^{125}I]iodovinyl analogues, were tested for their relative target tissue retention and binding affinity for the estrogen receptor. The (iodovinyl)estradiols **3** and **6** were prepared via destannylation of the (17α,20*E*)- and (17α,20*Z*)-tributylstannyl precursors **2** and **4** with retention of configuration. Selective formation of the *E* or *Z* isomers **2** and **4** during the reaction of 17α-ethynylestradiol **1a** with tri-*n*-butyltin hydride was controlled by the presence or absence of the catalyst, the polarity of the solvent, and the reaction temperature. The nca [^{125}I]iodovinyl analogues [^{125}I]-**3a** and [^{125}I]-**6a** were obtained in good radiochemical yield and high purity by treatment of **2a** and **4a** with [^{125}I]NaI in the presence of H_2O_2 and chloroamine-T, respectively. Of the two isomeric iodovinyl derivatives **3** and **6**, the 20*Z* isomer **6a** exhibited the highest receptor binding affinity and the [^{125}I]-**6a** gave the highest in vivo receptor-mediated target tissue uptake.

Among the various estrogen receptor based radiopharmaceuticals that have been advanced over the past years as possible imaging agents for breast cancer, 17α-iodovinyl derivatives of estradiol and 11β-methoxyestradiol showed the most promising properties. The established synthetic procedures for these 17α-iodovinyl estrogens mainly yield the 20*E* isomers, and, accordingly, only estrogen-receptor binding and in vivo distribution pattern of the latter isomer have been studied.[2-5] However, in the case of the analo-

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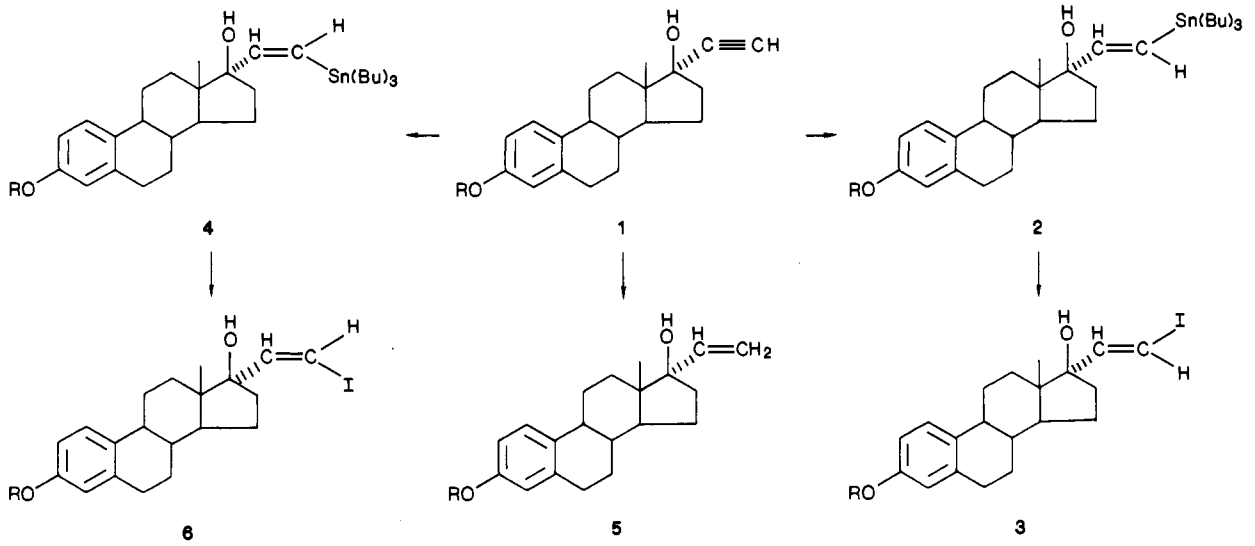
Scheme I



gous 17α-(iodovinyl)nortestosterone, synthesis of both the 20*E* and 20*Z* isomers has been reported,[6,7] and, furthermore, the 20*Z* isomer was found to exhibit the more interesting biological properties.[7] On such accounts we prepared both the 17α,20*E* and 17α,20*Z* isomers of 21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol, together with the ^{125}I-radiolabeled analogues, and compared their estrogen receptor binding affinity and in vivo target tissue uptake. Our data suggest that the 20*Z* configuration provides for the best receptor interaction and the highest receptor-mediated target tissue uptake.

Chemistry. For the introduction of the iodine onto the vinyl substituents of the 17α-(iodovinyl)estradiols **3** and **6** we used the destannylation method. This rapid, electrophilic substitution reaction has previously been used for the preparation of the (17α,20*E*)-(iodovinyl)estradiol **3** and was shown to be stereospecific resulting in good yield and high specific activities of the desired radiopharmaceutical.[1,4] Preparation of the intermediate (17α,20*E*)-21-[(tri-*n*-butylstannyl)vinyl]estradiol **2** via this procedure involved the reaction of estrone with (*E*)-bis(tributylstannyl)ethylene.[1] To obtain both the 20*E* and 20*Z* stannyl intermediates **2** and **4** we adapted an alternative procedure reported for the preparation of the analogous nortestosterone derivatives, e.g. the (17α,20*E*,*Z*)-17β-hydroxy-21-[(tri-*n*-butylstannyl)vinyl]-4-estren-3-ones.[6,7] Both the 17α-ethynylestradiol **1a** and its 3-methyl ether **1b** 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 the (17α,20*E*)-[(tributylstannyl)vinyl]-estradiol **2a**, or its 3-methyl ether **2b**, as major products in up to 75% yield. Substitution of a polar solvent (e.g. hexamethylphosphoric triamide) for toluene, omission of catalyst, and lowering of the reaction temperature to 65–70 °C favored formation of the 20*Z* isomer **4a** or its methyl ether **4b**, respectively. The latter products required chromatographic purification from starting material and unwanted side products, including the 20*E* isomers **2a,b** and the vinyl derivatives **5a,b**. The isomeric tributylstannyl intermediates **2** and **4** were converted with retention of configuration to the corresponding (iodovinyl)estradiols **3a,b** and **6a,b** via the reaction with iodine in chloroform in 70–90% yield. The 20*Z* isomers **6a** and **6b** were less stable than the 20*E* isomers **3a** and **3b** and also exhibited different solubility. The isomeric (iodovinyl)-estradiols **3** and **6** were well separated by silica gel TLC in chloroform/acetonitrile (95:5) but failed to separate on a C-18 reverse-phase HPLC column with mixtures of water and methanol. The intermediate isomeric [(tributylstannyl)vinyl]estradiols **2** and **4** separated well on silica gel TLC in mixtures of ethyl acetate and hexane.

The assigned configurations of the isomeric (17α,20*E*,-*Z*)-(iodovinyl)estradiols were confirmed by ^{1}H and ^{13}C NMR analyses. The ^{1}H NMR spectrum of the 20*E* isomer **3a** gave two doublets at δ 6.18 and 6.80 with coupling constants of J = 14 Hz, characteristic of the 17α-vinyl protons with trans (*E*) incorporated iodine.[6] In contrast, the 20*Z* isomer **6** have doublets at δ 6.22 and 6.72 with coupling constants of J = 8 Hz, characteristic of the 17α-iodovinyl with cis (*Z*) incorporated iodine.[6] ^{13}C NMR spectra of the 20*E* isomer **3** showed characteristic vinyl carbon signals at δ 73.2 (C-21) and 151 (C-20) while the 20*Z* isomer **6** gave corresponding signals at δ 76.3 (C-21) and 144.2 (C-20).

Radiochemistry. The analogous isomeric non-carrier-added [^{125}I]-**3a** and [^{125}I]-**6a** were obtained under different conditions. The 20*E* isomer [^{125}I]-**3a** was readily obtained by the treatment of **2a** with [^{125}I]NaI in the presence of H_2O_2.[4] Under similar conditions the 20*Z* stannyl isomer **4a** gave less than 5% of the desired product [^{125}I]-**6a**. Instead, we used [^{125}I]NaI and chloroamine-T in EtOH followed by organic solvent extraction and chromatographic purification to give [^{125}I]-**6a** in 75–80% overall yield. Eluting ^{125}I-labeled **3a** or **6a** from the reverse-phase HPLC column could not be detected by the UV monitor, and it is assumed that their specific activities is in the same range as that of the starting [^{125}I]NaI, e.g. up to 2200 Ci/mmol.

Biological Properties. Relative binding affinities (RBA) for estrogen receptors of the nonradiolabeled isomer (iodovinyl)estradiols **3a** and **6a** were determined by com-

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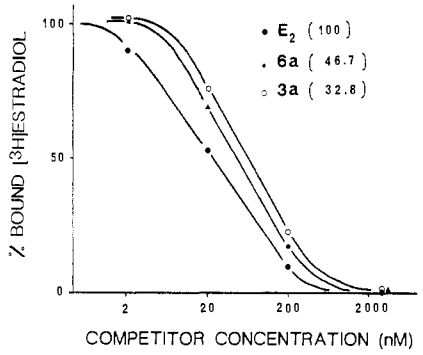

Figure 1. Competitive binding between 10^{-9} M [^{3}H]estradiol and 2×10^{-9} to 2×10^{-12} M unlabeled estradiol (E_2) and the isomeric (17α,20)-(iodovinyl)estradiols **3a** (20*E* isomer) and **6a** (20*Z* isomer). The concentration required for 50% competition was used to calculate RBA values, which are given in brackets with the corresponding displacement curve.

Table I. Organ Uptake of [^{125}I]-3a and [^{125}I]-6a

tissue	PDK × 1000 (SD)[a]			
	1 h	2 h	2 h (+E_2)	5 h
	[^{125}I]-3a (20*E* Isomer)			
blood	17 (0.47)	10 (0.00)	10 (1.48)	9 (0.94)
lungs	49 (3.09)	25 (3.11)	28 (3.77)	8 (0.00)
liver	245 (10.53)	92 (3.20)	106 (20.88)	55 (1.25)
spleen	27 (3.50)	10 (1.12)	11 (1.79)	4 (0.00)
kidneys	71 (4.55)	27 (2.87)	29 (3.83)	10 (0.47)
adrenals	175 (21.20)	72 (8.20)	73 (12.36)	21 (2.45)
uterus	92 (9.63)	35 (4.87)	22 (6.80)	11 (0.94)
ovaries	108 (2.45)	43 (6.18)	39 (10.03)	16 (2.05)
muscle	30 (1.25)	11 (1.50)	13 (2.38)	4 (1.26)
thyroid	1331 (358)	2869 (302)	2303 (331)	4063 (324)
brain	38 (1.25)	12 (1.12)	14 (2.38)	2 (0.00)
	[^{125}I]-6a (20*Z* Isomer)			
blood	16 (1.70)	12 (1.09)	14 (0.83)	17 (2.94)
lungs	44 (4.32)	25 (1.66)	26 (3.00)	18 (0.47)
liver	148 (8.06)	84 (4.90)	83 (5.54)	57 (9.74)
spleen	19 (0.47)	11 (1.22)	10 (1.12)	9 (1.25)
kidneys	58 (3.09)	32 (1.92)	29 (4.03)	20 (1.70)
adrenals	154 (31.72)	67 (9.42)	77 (24.99)	23 (1.89)
uterus	228 (32.07)	166 (22.23)	41 (3.96)	106 (8.73)
ovaries	116 (9.46)	85 (7.36)	36 (4.50)	50 (4.32)
muscle	24 (0.82)	10 (0.83)	10 (9.87)	5 (0.82)
thyroid	2547 (245)	3718 (1021)	4975 (623)	19186 (9727)
brain	34 (2.62)	11 (1.12)	14 (2.59)	2 (0.47)

[a] Mean organ uptake (PDK, percent injected dose × animal weight in kilograms per gram tissue) and standard deviation (SD) for three rats 1, 2, and 5 h after iv injection of 3 μCi (111 kBq) of [^{125}I]-3a (20*E* isomer) or [^{125}I]-6a (20*Z* isomer) in the presence (+E_2) or absence of 60 μg co-injected estradiol.

petitive binding assays with [^{3}H]estradiol.[8] Radioligand binding to murine uterine cytoplasmic extracts, at four competitor concentrations, was measured by Sephadex LH-20 chromatographic separation of bound from free steroid. The RBA is 100 times the ratio between the competitor and unlabeled estradiol concentrations required for 50% competition to specific [^{3}H]estradiol binding. The result of these assays are shown in Figure 1. The RBA values derived from these displacement curves are 32.8 for the 20*E* isomer **3a** and 46.7 for the 20*Z* isomer **6a**. Accordingly, the 20*Z* isomer has the better estrogen-receptor affinity, which is in agreement with the earlier reported more promising biological properties of the 20*Z* isomer of the analogous nortestosterone derivative.[7,8]

The biodistribution and uterus uptake of the ^{125}I-labeled (iodovinyl)estradiols [^{125}I]-**3a** and [^{125}I]-**6a** was studied in immature Fischer female rats. Animals were injected intravenously 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. In order to show receptor-mediated uptake in the uterus, a separate group of animals was co-injected with 60 μg of unlabeled estradiol. Animals were sacrificed 1, 2, and 5 h after injection with the radiotracer, and 2 h in the case of co-injected cold estradiol, and the activities in the various organs, and the uterus to blood ratios were determined (Tables I and II). With the exception of the uterus and the thyroid, biodistribution pattern are similar for both isomeric iodovinyl estrogens [^{125}I]-**3a** and [^{125}I]-**6a**, with maximum uptake for most organs within 2 h postinjection followed by a rapid decrease of the tracer concentration. In accordance with the higher estrogen-receptor affinity of the 20*Z* isomer **6a**, the highest uterine uptake was observed with [^{125}I]-**6a**, e.g. 3.7% dose/g, 1 h postinjection with good activity retention after 2 h (75%) and 5 h (41%). Uterus uptake of the 20*Z* isomer [^{125}I]-**6a** was effectively blocked by co-injecting nonlabeled estradiol, with uterus to blood ratios at 2 h postinjection falling from 13.6 in the absence of cold estradiol to 3.0 in the presence of estradiol (Table II), confirming a receptor mediated uptake mechanism. Uterus to blood ratios for the 20*E* isomer [^{125}I]-**3a** were less elevated, ranging from 3.9 in the absence of estradiol to 2.3 in the presence of cold estradiol. Both isomeric estrogens [^{125}I]-**3a** and [^{125}I]-**6a** exhibited moderate in vivo deiodination, as evidenced by the increased ^{125}I uptake with time by the thyroid (Tables I and II). The extent of deoiodination was estimated by comparing thyroid uptake values of the ^{125}I-labeled steroids with those observed after [^{125}I]NaI injection. These data (Table III) suggest 4.4% deiodination 5 h postinjection for the 20*E* isomer **3a** compared to 17% for the 20*Z* isomer **6a**. The higher in vivo decomposition rate of the 20*Z* isomer [^{125}I]-**6a** is in agreement with the higher chemical instability of this isomer. In spite of the higher instability, the stronger receptor binding affinity and the higher in vivo uptake by target tissue indicate that the 20*Z* isomer of (17α,20)-([^{125}I]iodovinyl)estrogens may be the preferred configuration for radiopharmaceutical applications.

Table II. Effect of Co-Injection with Estradiol on the Uterus to Blood Ratio of [^{125}I]-3a and [^{125}I]-6a after 2 h[a]

compound	uterus to blood ratio (SD)[b]		
	control	co-injected[c]	% change[d]
[^{125}I]-3a (*E* isomer)	3.91 (1.02)	2.32 (0.64)	−41
[^{125}I]-6a (*Z* isomer)	13.64 (2.03)	3.00 (0.25)	−78

[a] Immature Fischer female rats received 3 μCi (111 kBq) of [^{125}I]-3a or [^{125}I]-6a via the tail vein and where sacrificed after 2 h. [b] Mean values calculated from PKD values given in Table I, standard deviation (SD) of the mean for three animals. [c] Co-injected with 60 μg of estradiol (E_2). [d] Percent change in uterus to blood ratios between animals co-injected with estradiol and animals injected with the radiopharmaceutical only.

Table III. In Vivo Deiodination of [^{125}I]-3a and [^{125}I]-6a

time, h	% deiodination[a]	
	[^{125}I]-6a (*Z* isomer)	[^{125}I]-3a (*E* isomer)
1	3.8	2.0
2	2.8	2.1
2 (+E_2)[b]	3.7	1.7
5	17.0	4.4

[a] The percent deiodination at 1, 2, or 5 h postinjection was calculated from the PKD of the thyroid of animals injected with [^{125}I]-3a or [^{125}I]-6a (Table I), taking the thyroid uptake after [^{125}I]NaI injection at the same time interval as 100%. [b] Co-injected with 60 μg of estradiol (E_2).

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Discussion

The reaction sequence, workup, and yield of the 20*E* and 20*Z* isomers of (17α,20)-(iodovinyl)estradiol is appropriate to allow for extention of the procedure to include the short-lived ^{123}I-labeled isotope, of interest for SPECT imaging. Radiochemical yield with ^{125}I was sufficiently high such that no steroid adsorption was detectable upon elution of the ^{125}I-labeled products by its UV absorption, suggesting that the specific activities of [^{125}I]-**3** and [^{125}I]-**6** approaches that of the [^{125}I]NaI used for the labeling reaction, e.g. up to 2200 Ci/mmol. The synthesis of both the 17α,20*E* and 17α,20*Z* isomers of (17α,20)-(iodovinyl)estradiol could readily be applied to other 17α-iodovinyl estrogens containing substituents on the steroid nucleus. Our studies indicate that the 20*Z* configuration, although somewhat less stable, provides for the best in vivo localization of 17α-iodovinyl steroids, and this could further be improved upon by additional substituents that enhance receptor interaction and diminish nonspecific binding. It has previously been shown that addition of the 11β-methoxy group to the 20*E* isomer **3** results in improved target tissue retention and higher target to nontarget ratios,[3] and we recently showed that addition of the 7α-methyl to **3** also results in augmented receptor binding.[9] Accordingly, the 11β-methoxy or 7α-methyl derivative of the 20*Z* isomer **6** should be of particular interest for further studies.

Experimental Section

Melting points were determined on a Fisher–Johns apparatus and are uncorrected. UV spectra were recorded on a Varian UV/vis 2200 spectrophotometer. ^{1}H Nuclear magnetic resonance (NMR) spectra were recorded on a Varian T60 spectrometer with Me_4Si as an internal standard. ^{13}C NMR spectra were determined on a Bruker WM 25 spectrometer with Me_4Si as an internal standard. Mass spectra were obtained on a Hewlett-Packard Model 5988A quadrupole apparatus. Thin-layer chromatography (TLC) was conducted on 0.25 mm thick silica gel plates coated with fluorescent indicator (UV 254) in chloroform containing 5% acetonitrile. Compounds were located on TLC plates by their UV absorbence or/and color response upon spraying with H_2SO_4/EtOH and heating at 120 °C. Column chromatograpy was done on silica gel (60–200 mesh). High-performance liquid chromatography (HPLC) were performed on a reverse-phase column (C-18, ODS-2 spherisorb, 5 μm, 25 cm long × 0.94 cm i.d., from CSC, Montreal) operated at 2 mL min^{-1}. Compounds were detected at 280 nm and where appropriate, by their γ-radiation, which was registered via a sodium iodide detector. Combustion analysis were performed by Guelph Laboratories Ltd, Canada. Steroids were purchased from Steraloid Inc. All chemicals used were commercially available and were of highest chemical grade; carrier-free [^{125}I]NaI was purchased from Amersham Canada Ltd.

(17α,20*E*)-21-(Tributylstannyl)-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (2a). A mixture of 17α-ethynylestradiol **1a** (100 mg, 0.337 mmol) in 5 mL of toluene and 0.3 mL of tri-*n*-butyltin hydride (1.1 mmol) was heated at 90 °C for 3 h in the presence of azobisisobutyronitrile (16.6 mg, 1 mmol) under nitrogen. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column. Elution with 5% EtOAc in hexane gave **2a** as a single major product (135 mg, 76.6%): mp 65–70 °C; R_f 0.59; ^{1}H NMR ($CDCl_3$) δ 0.65–2.4 (41 H), 2.56–3.08 (3 H), 5.92 (1 H), 6.1–6.8 (3 H), 7.0–7.3 (2 H); MS, *m/z* (relative intensity) 588 ($M^{\cdot+}$, 0.5), 586 ($M^{\cdot+}$, 0.4), 584 ($M^{\cdot+}$, 0.4), 531 (80), 529 (60).

(17α,20*E*)-21-Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (3a). To compound **2a** (116.8 mg, 0.2 mmol) in 3 mL of chloroform 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 addition of 0.2 mL of 1 M potassium fluoride in methanol and 0.2 mL of 5% aqueous sodium bisulfite. The mixture was then extracted with EtOAc (2 × 10 mL). The organic phase was dried over magnesium sulfate, filtered, and evaporated to dryness. The residue was purified on a C-18 reverse-phase HPLC column in 80:20 MeOH/H_2O. The desired compound **3a** eluted after 27 min and was crystallized from methanol (70 mg, 82.5%): mp 110–112 °C dec (lit.[3] mp 113–115 °C); R_f 0.47; UV(EtOH) λ_{max} 280 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 0.84 (s, 3 H, 18-CH_3), 1.0–3.0 (br m, steroid nucleus), 6.18 (d, *J* = 14 Hz, 1 H, =CHI); 6.80 (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); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 13.3 (C-18), 22.4 (C-15), 22.5 (C-11), 26.6 (C-7), 28.8 (C-6), 31.7 (C-16), 35.2 (C-12), 39.6 (C-8), 42.8 (C-9), 46.8 (C-13), 48.5 (C-14), 73.2 (C-21), 85.7 (C-17), 112.2 (C-2), 114.5 (C-4) 125.3 (C-1), 130.3 (C-10), 136.9 (C-5), 151 (C-20), 154.2 (C-3); MS, *m/z* (relative intensity) 424 ($M^{\cdot+}$, 77), 297 (32), 296 (1), 279 (20), 213 (100), 160 (63), 159 (55), 145 (30).

(17α,20*Z*)-21-(Tributylstannyl)-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (4a). A mixture of **1a** (1.0 g, 3.37 mmol) and 2.6 mL (9.66 mmol) of tributyltin hydride was stirred in 5 mL of hexamethylphosphoric triamide at 70 °C for 45 h. The reaction mixture was diluted with EtOAc (20 mL), washed with water, dried over magnesium sulfate, and concentrated under reduced pressure. The residue was chromatographed over silica gel. Elution with 5% EtOAc in hexane gave compound **4a** (400 mg, 0.68 mmol, 67%) as an oil: R_f 0.7; ^{1}H NMR ($CDCl_3$) δ 0.67–2.7 (41 H), 2.8–3.2 (3 H), 6.2 (1 H), 6.56–6.98 (3 H), 7.0–7.5 (2 H). MS, *m/z* 588 (M^+, 0.7), 586 (M^+, 0.6), 584 (M^+, 0.4), 531 (3), 539 (3), 527 (2), 399 (3), 397 (3), 395 (2).

Further elution of the silica gel column with the same solvent mixture gave compound **2a** as a minor product (100 mg, 0.17 mmol, 16.9%). A final elution with 10% EtOAc in hexane gave unreacted starting material.

(17α,20*Z*)-21-Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol (6a). To a **4a** (116.8 mg, 0.2 mmol) in 3 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 potassium fluoride in MeOH and 0.2 mL of a 5% aqueous sodium bisulfite solution. The mixture was then extracted with EtOAc (2 × 10 mL). The organic phase was worked up as usual, and the residue was purified by HPLC over a C-18 column with 80:20 MeOH/H_2O. Compound **5a** eluted after 20 min as a minor product (9 mg, 0.03 mmol, 15%): mp 153–154 °C (lit.[10] mp 148–150 °C). Further elution with the same solvent mixture gave after 27 min compound **6a**, which was crystallized from MeOH (65 mg, 0.153 mmol, 76.5%): mp 123–125 °C; R_f 0.59; UV (EtOH) λ_{max} 280 nm; ^{1}H NMR ($CDCl_3$ + DMSO-d_6) δ 0.88 (s, 3 H, 18-CH_3), 1.0–3.0 (br m, steroid nucleus), 6.22 (d, *J* = 8 Hz, 1 H, =CHI), 6.72 (d, *J* = 8 Hz, 1 H, CH=), 6.40–7.0 (m, 2 H, 1-CH and 4-CH), 6.96 (m, 1 H, 2-CH); ^{13}C NMR ($CDCl_3$ + DMSO-d_6) δ 14 (C-18), 23 (C-15), 26.1 (C-11), 27.2 (C-7), 29.4 (C-6), 31.8 (C-16), 36.6 (C-12), 39.5 (C-8), 43.4 (C-9), 48 (C-13), 49.2 (C-14), 76.3 (C-21), 84 (C-17), 112.8 (C-2), 115.2 (C-4), 125 (C-1), 130.8 (C-10), 137.5 (C-5), 144.1 (C-20), 154.8 (C-3); MS, *m/z* (relative intensity) 424 (M^+, 52), 297 (14), 296 (4), 279 (13), 213 (100), 160 (63), 159 (47), 157 (31), 133 (60). Anal. ($C_{20}H_{25}IO_2$) C, H, I.

(17α,20*E*)-21-(Tributylstannyl)-19-norpregna-1,3,5(10),20-tetraene-3,17-diol 3-(Methyl ether) (2b). A mixture of 17α-ethynylestradiol 3-(methyl ether) (**1b**) (310 mg, 1 mmol) and 1.0 mL of tributyltin hydride (3.7 mmol) in 8 mL of toluene were heated at 90 °C in the presence of azobisisobutyronitrile (50 mg, 0.13 mmol), under nitrogen for 3 h. The solvent was evaporated under reduced pressure, and the residue was chromatographed over silica gel. Elution with 3% EtOAc in hexane gave **2b** as a major product (oil, 450 mg, 75%): ^{1}H NMR ($CDCl_3$) δ 0.7–2.6 (41 H), 2.82–3.1 (3 H), 3.72 (s, 3 H, 3-OCH_3), 6.18 (1 H), 6.58–6.84 (3 H), 7.1–7.34 (2 H); MS, *m/z* (relative intensity) 545 (4), 543 (3), 541 (2), 527 (100), 525 (80), 523 (44), 413 (18).

(17α,20*E*)-21-Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol 3-(Methyl ether) (3b). Compound **2b** (240 mg, 0.4 mmol) in 5 mL of $CHCl_3$ was treated with a 0.1 M solution of

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iodine in chloroform as described for compound **3a**. After the usual workup, the compound was purified by C-18 HPLC with a gradient (30 min) of 90–100% MeOH in water. The compound eluting at 30 min was characterized as **3b** and crystallized from methanol (150 mg, 0.34 mmol, 85.6%): mp 136–138 °C; ^{1}H NMR ($CDCl_3$) δ 0.84 (s, 3 H, 18-CH_3), 1.0–2.6 (br m, steroid nucleus), 3.60 (s, 3 H, 3-OCH_3), 6.18 (d, J = 14 Hz, 1 H, =CHI), 6.68 (d, J = 14 Hz, 1 H, CH=), 6.32–6.71 (m, 2 H, 1-CH and 4-CH), 6.98–7.2 (m, 1 H, 2-CH); MS, m/z (relative intensity) 438 (M^+, 87), 420 (7), 311 (25), 293 (16), 242 (28), 227 (100).

(17α,20*Z*)-21-(Tributylstannyl)-19-norpregna-1,3,5(10),20-tetraene-3,17-diol 3-(Methyl ether) (4b). A mixture of **1b** (1.0 g, 3.23 mmol) and 2.5 mL of tributyltin hydride (9.2 mmol) in 5 mL of hexamethylphosphoric triamide was stirred at 65–70 °C for 45 h. The reaction mixture was diluted with EtOAc and worked up in the usual manner as described for **4a**. The residue was chromatographed over silica gel with 3% EtOAc in hexane to give compound **4b** as an oil (500 mg, 0.83 mmol, 64.8%): ^{1}H NMR ($CDCl_3$) δ 0.7–2.6 (41 H), 2.82–3.2 (3 H), 3.74 (s, 3 H, 3-OCH_3), 5.96 (1 H), 6.56–6.86 (3 H), 7.0–7.22 (2 H); MS, m/z (relative intensity) 545 (100), 543 (73), 541 (40), 525 (2), 523 (1), 521 (1), 413 (42).

Continuous elution with the same solvent mixture gave **2b** (90 mg, 0.15 mmol, 11.6%). Further elution with 5–10% EtOAc in hexane gave unreacted starting material (600 mg).

(17α,20*Z*)-21-Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol 3-(Methyl ether) (6b). To **4b** (100 mg, 0.167 mmol) in 3 mL of $CHCl_3$ was added at room temperature a 0.1 M solution of iodine in $CHCl_3$ as described for **4a**. The reaction mixture, after the usual workup, was purified by reverse-phase HPLC with a gradient (30 min) of 90–100% MeOH in water. Elution at 20 min gave **5b** (15 mg, 0.0032 mmol, 19.1%): mp 108 °C (lit.[11] mp 90–95 °C). Elution at 30 min gave **6b**, which was crystallized from MeOH (40 mg, 0.128 mmol, 76.7%): mp 45 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (s, 3 H, 18-CH_3), 1.1–2.8 (br m, steroid nucleus) 3.7 (s, 3 H, 3-OCH_3), 6.36 (d, J = 8 Hz, 1 H, =CHI), 6.74 (d, J = 8 Hz, 1 H, CH=), 6.5–6.78 (m, 2 H, 1-CH and 4-CH), 7.0–7.22 (m, 1 H, 2-CH); MS, m/z (relative intensity) 438 ($M^{\cdot+}$, 67), 420 (5)8, 311 (23), 293 (15), 242 (24), 227 (100). Anal. ($C_{21}H_{27}IO_2$) C, H, I.

Synthesis of (17α,20*E*)-21-[^{125}I]Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol ([^{125}I]-3a). To a mixture of **2a** (100 μg, 166 nmol) and 50 μL of a 5% (w/v) solution of sodium acetate in glacial acetic acid 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%)/acetic acid. After the mixture was stirred at room temperature for 20 min, the reaction was terminated by the addition of 25 μL of a 5% $NaHSO_3$ solution (w/v). The mixture was extracted with dichloromethane and dried under the stream of nitrogen. The residue (450 μCi, 90%) was dissolved in methanol and purified on an analytical C-18 reverse-phase HPLC Altech column operated at a flow rate of 1 mL/min. Elution with MeOH/water (70:30) gave [^{125}I]-**3a** (350 μCi, 70%). The retention times of free iodine and the desired product were 4 and 28 min, respectively. The radiochemical and chemical purity were consistently in excess of 95%. The radioactive product [^{125}I]-**3a** exhibited identical mobilities on TLC and HPLC with those of the nonradioactive product **3a**.

Synthesis of (17α,10*Z*)-21-[^{125}I]Iodo-19-norpregna-1,3,5(10),20-tetraene-3,17-diol ([^{125}I]-6a). To a mixture of **4a** (100 μg, 100 nmol) and [^{125}I]NaI (500 μCi) in 500 μL of ethanol was added 1.75 mg of chloroamine-T in 50 μL of water. The reaction was quenched after 15 min by the addition of 25 μL of a 5% $NaHSO_3$ solution in water. The reaction product was purified in a similar manner as described for [^{125}I]-**3a**, and after HPLC purification the radiolabeled product [^{125}I]-**6a** was obtained in 75–80% overall yield.

Estrogen Receptor Binding Assay. The affinity of the (17α,20*E*)- and (17α,20*Z*)-(iodovinyl)estradiol isomers **3a** and **6a** for estrogen receptors was determined by a competitive binding assay[8] and is expressed as the relative binding assay (RBA). The RBA is defined as 100 times the ratio between the competitor and 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 4% of the total binding, which was substracted from the total binding to estimate the specific binding. The specific binding in the receptor preparation was equivalent to 4.77 nM.

In Vivo Studies. The animal experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care. Tissue distribution studied were conducted in immature female Fischer rats (24 days old, 55–60 g), obtained from Charles River Breeding Farm. The animals were allowed free access to food and water throughout the experiment. All injections were carried out with use of aseptic techniques. Before injection the animals were warmed for a few minutes under an infrared lamp to ensure dilation of the tail vein whereafter 200 μL of the ^{125}I-labeled preparation (3 μCi, 111 kBq) was injected through the lateral tail vein. The injection solutions were prepared by dilution of an ethanolic solution of the radiopharmaceutical with sterile physiological saline (0.9% sodium chloride in water) containing 1% Tween 80, to give a final ethanol concentration of 9%. For the receptor saturation studies 60 μg of unlabeled estradiol was co-injected with the radiopharmaceutical. Prior to injection all solutions were filtered over a 0.22-μm millipore filter (Millex Millipore Co., Bedford, ME) and stored in sterile vaccutainer tubes.

The animals were sacrificed by bleeding, followed by chest opening, while the animal was under deep ether anesthesia. Bleeding was carried by exposing and severing the axillary artery.[12] Tissues of interest were removed, washed with 0.154 M KCl, blotted dry, and placed in sealed preweighed test tubes. The radioactivity in each sample was counted in a Model 1282 Compugamma gamma counter (LKB Wallac, Finland) together with a reference sample of the injected preparation. Radioactivity levels are expressed as percent of the injected dose × animal weight (kg) per gram of tissue (PDK or % dose × kg/g).[13] The extent of in vivo deiodination was estimated from the ^{125}I uptake by the thyroid, by comparing the PDK of the thyroid animals injected with a test compound versus that of animals injected with [^{125}I]NaI.

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Registry No. **1a**, 57-63-6; **1b**, 72-33-3; **2a**, 82123-95-3; **2b**, 94887-68-0; **3a**, 91085-44-8; [^{125}I]-**3a**, 82123-96-4; **3b**, 78479-31-9; **4a**, 115115-60-1; **4b**, 115464-37-4; **5a**, 7678-95-7; **6a**, 104011-19-0; [^{125}I]-**6a**, 104068-30-6; **6b**, 104011-20-3.

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