11β-Substituted Estradiol Derivatives. 2. Potential Carbon-11- and Iodine-Labeled Probes for the Estrogen Receptor

Elio Napolitano,[†,‡] Rita Fiaschi,[‡] Kathryn E. Carlson,[§] and John A. Katzenellenbogen*,[§]

Scuola Normale Superiore, Piazza dei Cavalieri, 56100 Pisa, Italy, Dipartimento di Chimica Bioorganica, Università di Pisa, Via Bonanno 33, 56126 Pisa, Italy, and Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801

Received February 13, 1995[⊗]

Four new classes of 11β-substituted estradiol and estriol derivatives (cyanoalkyl, ethynyl, propynyl, and iodovinyl) have been synthesized, and their binding affinity for the estrogen receptor has been evaluated. The binding affinity values indicate that the estrogen receptor has tolerance for estradiol derivatives bearing 11β-groups whose size, rigidity, and polarity are limited. The estradiol derivatives have higher affinity than the estriol derivatives. The potential of these agents as imaging agent for estrogen receptor-positive breast tumors is discussed. On the basis of the results of this and a previously reported study (Napolitano, E.; Fiaschi, R.; Carlson, K. E.; Katzenellenbogen, J. A. 11β-Substituted Estradiol Derivatives, Potential High-Affinity Carbon-11-Labeled Probes for the Estrogen Receptor: A Structure-Affinity Relationship Study. *J. Med. Chem.* **1995**, *38*, 429–434), a general strategy for designing high-affinity probes for the estrogen receptor is proposed.

Introduction

The presence of estrogen receptors (ER) in certain breast tumors provides a potential mechanism for the selective uptake of suitably radiolabeled estrogens. A number of different classes of these ER-based breast tumor-imaging radiopharmaceuticals have been described, including many labeled with the positron-emitting radionuclide fluorine-18 ($t_{1/2}$ = 110 min)[1] and several labeled with radioactive isotopes of bromine[1d,2] or iodine.[1d,3] Imaging studies have been done in human breast cancer patients by PET with the fluorine-18-labeled estrogen 16α-[^{18}F]fluoroestradiol (FES)[4] and by conventional planar imaging with the iodine-123 labeled analog and certain of its derivatives.[5]

The prospect of using the shorter-lived, positron-emitting radionuclide carbon-11 ($t_{1/2}$ = 20 min) has been considered. The shorter half-life of this isotope might enable repeat images on a patient to be done in a single session, and radiation doses could be reduced. Despite these potential advantages, however, the logistical problems involved in the preparation of estrogens labeled with such a short-lived isotope are considerable, so that this challenge that has only recently been seriously taken up.[6]

The preparation of 17α-[^{11}C]methylestradiol was reported Vaalburg in 1983, but only limited tissue distribution studies were performed on this compound.[6a] Recently, we described an improved synthesis of this species, and in tissue distribution studies in rats, we found that it showed receptor-mediated, selective uptake in target tissues.[6b] The behavior of this compound, however, was not ideal; its affinity for ER is only about one-half that of estradiol, and its in vivo target site uptake efficiency and selectivity were considerably poorer than that of FES.

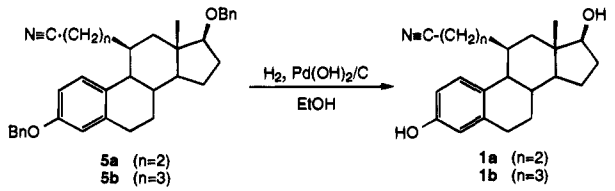
There are a number of functional alterations that could be introduced into carbon-11-labeled estrogen radiopharmaceuticals that might improve their behavior, changes that could increase their ER binding affinity and improve the rate of target tissue contrast development by accelerating their rate of clearance.[7] Recently, we presented a study of estrogens that might be labeled with carbon-11 via [^{11}C]methyl iodide or [^{11}C]methyllithium and some related derivatives that incorporate some of these features.[6c] Several of these compounds have high affinity for ER and appear to be favorable candidates for further study. In a continuation of this investigation, aimed at studying alternative possibilities for introducing carbon-11 labels and improving the pharmacodynamic behavior of labeled probes, we report here on the synthesis and estrogen receptor binding affinity of several members of four new classes of estradiol derivatives **1**–**4**.

(Cyanoalkyl)estradiols **1a,b** and 1-propynyl derivatives **2a,b** are the actual candidates for labeling with carbon-11 and were designed for the incorporation of the nucleophilic carbon-11 cyanide ion and an electrophilic carbon-11 methyl group, respectively. The rationale for choosing estradiols with this substitution pattern was that short 11β-carbon substituents generally improve the binding affinity of estradiol derivatives.[8] Thus, it was expected that the 11β-substituents of **1** and **2** might secure a high affinity for the estrogen receptor and provide, at the same time, a site for the convenient incorporation of the radiolabel. This strategy has already proven successful in designing 11β-(2-fluoroethyl)estradiol, the steroidal fluoroestrogen endowed with the highest affinity for the estrogen receptor.[9,10] Furthermore, estradiols **1a,b** and **2a,b** differ from the previously reported series[6c] by the absence of substituents in the 17α-position. Carbon substituents at this position are easily introduced, amenable for incorporation of the carbon-11 label, and even capable of improving the binding affinity of the derivative. However, they can also retard the phase I and phase II metabolism that is a prelude to elimination of these compounds. Since rapid clearance from non-target areas is crucial when dealing with imaging agents

* Address Correspondence to this author at University of Illinois, Department of Chemistry, 461 Roger Adams Laboratory, Box 37, 600 S. Mathews Ave., Urbana, IL 61801. Telephone: (217) 333-6310. Fax: (217) 333-7325. e-mail: katzenel@aries.scs.uiuc.edu.
† Scuola Normale Superiore.
‡ Università di Pisa.
§ University of Illinois.
⊗ Abstract published in *Advance ACS Abstracts*, June 15, 1995.

0022-2623/95/1838-2774$09.00/0

Scheme 1



labeled with short-lived isotopes such as carbon-11, compounds **1a,b** and **2a,b** were designed with a particular attention to this problem. Ethynyl derivatives **3** are not amenable for labeling with carbon-11 in an obvious way, nor are the halogenated derivatives **4**, which are rather candidates for labeling with iodine radionuclides. Nevertheless, they are included in this study of structure–affinity relationships because they are readily available from intermediates synthesized in the course of the preparation of **2**, and we expected that their behavior might contribute to a better understanding of the factors controlling the affinity of steroidal estrogens for the estrogen receptor.

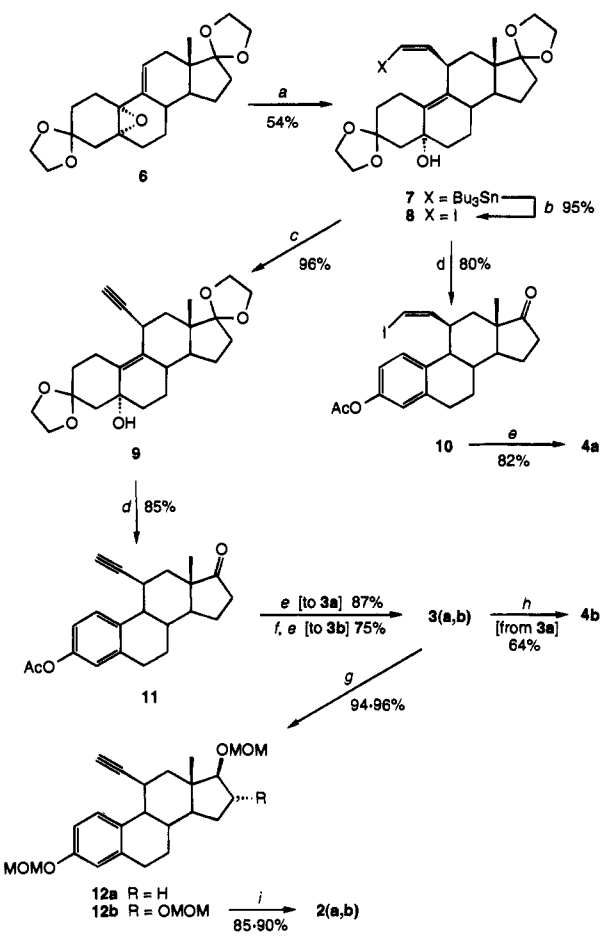
Results and Discussion

Chemistry. (Cyanoalkyl)estradiol derivatives were prepared by hydrogenolysis of the corresponding benzyl ethers **5** (Scheme 1) whose preparation was described previously.[8]

The compounds in the 11β-alkynyl and -alkenyl series **2–4** were obtained through the sequence presented in Scheme 2, which is an adaptation of the general approach to 11β-substituted steroid derivatives devised by Roussel-Uclaf.[8] Thus, the stannylvinyl cuprate reagent, prepared according to Marino,[11] was allowed to react with unsaturated epoxide **6**[12] in a stereo- and regioselective way to give the stannylvinyl alcohol **7** in fair yields; lower-order stannylvinyl cuprates gave less satisfactory results. Reaction with iodine cleanly converted the (stannylvinyl)estrane derivative **7** to the corresponding iodovinyl derivative **8**, which was subsequently converted to the alkynyl derivative **9** by dehydroiodination (lithium diisopropylamide in tetrahydrofuran). Hydrolysis of the acetal group (70% acetic acid) and aromatization (acetyl bromide and acetic anhydride in methylene chloride) gave the estrone **11** in good overall yield from **9**. The estrone **11** was reduced and deacetylated in one step (sodium borohydride and sodium hydroxide in methanol) to afford the estradiol **3a**.[13]

In order to obtain the estriol derivative **3b**, the estrone **11** was converted to the corresponding silyl enol ether (trimethylsilyl triflate and triethylamine in methylene chloride), which was then oxidized using dimethyldioxirane generated in situ in a two-phase system (aqueous oxone, acetone/methylene chloride, in the presence of tetrabutylammonium hydrogen sulfate as a phase transfer catalyst).[14] No attempt was made to analyze the mixture from the above step, which contained products from partial desilylation and deacetylation of the actual oxidation product. However, when the crude material from the oxidation step was treated with a methanol solution containing sodium borohydride and sodium hydroxide, which completed the deacetylation and the desilylation and also reduced the 17-ketone, the estriol **3b** was obtained in fair yield. The stereochemistry of estriol **3b** at C-16 and C-17 was based on ^{1}H-NMR precedent.[15] The compounds **3a,b** were then condensed

Scheme 2[a]



[a] (*a*) Bu_3SnLi, CuCN, LiCl, HC≡CH; (*b*) I_2, CCl_4; (*c*) LDA, THF; (*d*) (1) H_2O, MeCOOH; (2) MeCOBr, $(MeCO)_2O$, CH_2Cl_2; (*e*) $NaBH_4$, NaOH, MeOH; (*f*) (1) Me_3SiOTf, *i*-Pr_2EtN, CH_2Cl_2; (2) dimethyldioxirane; (*g*) methoxymethyl (MOM) chloride, *i*-Pr_2EtN, MeCN; (*h*) (1) $Pd(OH)_2$, Bu_3SnH, (2) I_2; (*i*) (1) BuLi, THF; (2) MeI; (3) HCl, MeOH.

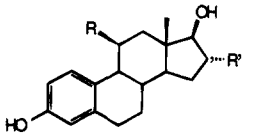
with chloromethyl methyl ether to give the respective *O*-methoxymethyl (MOM)-protected compounds **12a,b**, respectively, which were finally alkylated (butyllithium followed by quenching with methyl iodide) to give the target compounds **2a,b** after removal of the protecting groups (hydrochloric acid in methanol). The sequences leading to **1a,b** and **2a,b** are amenable to radiosynthesis using [^{11}C]cyanide or [^{11}C]methyl iodide, respectively.

For the synthesis of (*Z*)-iodovinyl **4a**, the ketalized intermediate **8** was hydrolyzed and aromatized to the estrone acetate **10**, which gave **4a** after reduction and saponification. For the synthesis of **4b**, tributyltin hydride was added to ethynylestradiol **3a** in the presence of Pd,[16] and the intermediate vinylstannane was iodinated with molecular iodine.

Receptor Binding Affinity. Table 1 summarizes the receptor binding affinity[17] (RBA) values of the compounds prepared in this study, together with certain other data taken from the literature for comparison. RBA values were measured at both 0 and 25 °C after an 18 h incubation, the latter being considered to represent those of more fully equilibrated ligand–receptor complexes.[18]

The cyanoalkyl derivatives **1a,b** bind with modest stability to the estrogen receptor. As in the case of fluoroalkyl derivatives containing the same number of

Table 1. Binding Affinities Relative to Estradiol (RBA) of Estradiol Derivatives **1**–**4** for the Estrogen Receptor of Lamb Uterine Cytosol at 0 and 25 °C, after 18 h Incubation



entry	compd no. (or ref)[a]	R	R'	RBA 0 °C	RBA 25 °C
	estradiol	H-	-H	100	100
	estriol	H-	-OH	21	5
1	**1a**	NC-$(CH_2)_2$-	-H	18	10
2	**1b**	NC-$(CH_2)_3$-	-H	31	15
3	**2a**	Me-C≡C-	-H	25	20
4	**2b**	Me-C≡C-	-OH	6	1
5	**3a**	H-C≡C-	-H	51	447
6	**3b**	H-C≡C-	-OH	7	21
7	**4a**	(*Z*)-I-CH=CH-	-H	63	139
8	**4b**	(*E*)-I-CH=CH-	-H	54	31
10	(21)	CH_3CH_2-	-H	120	1479
11	(10)	CH_2=CH-	-H	123	1230
12	(9)	F$(CH_2)_2$-	-H	77	1820
13	(9)	F$(CH_2)_3$-	-H	57	76
14	(9)	F$(CH_2)_4$-	-H	112	132
15	(10)	HO$(CH_2)_2$-	-H	13	74

[a] Entries 10–15 are included for comparison purposes. Data come from references given in parentheses.

methylene groups (entries 12–14),[10] the length of the chain does not substantially affect the affinity. Compared to the (fluoroalkyl)estradiols, however, the presence of a more polar and potentially more rigid CH_2-C≡N atom array (in **1a,b**), in place of the more flexible $CH_2CH_2CH_2$-F group (in entries 13 and 14), causes the binding affinity to be uniformly lower by almost 1 order of magnitude. The polar hydroxyalkyl group (entry 15) has a similar deleterious effect on binding. These data confirm the limited tolerance that the 11β-position has for hydrophilic groups. However, in a different series, it is known that a polar substituent at 11β (such as a methoxy group) can improve the estrogenic potency, in vitro binding selectivity, and target tissue uptake selectivity of estrogens because it depresses nonreceptor (high- and low-affinity) binding to plasma proteins more than it depresses binding to the estrogen receptor.[19] Thus, a study of the tissue distribution of **1a,b**, whose affinity is borderline between poor and acceptable ligands, might be interesting.

Because of the favorable effect of small nonpolar substituents at the 11β-position, we expected that an ethynyl group at this position (compound **3a**) would enhance the affinity for the estrogen receptor. This enhancement, however, is only about one-third as great as that produced by the either the ethenyl, ethyl, or 2-fluoroethyl groups (entries 10–12). The modest positive effect of the ethynyl group is probably due to its unique ability to invade the β-hemispace above the plane of the molecule in a directionally restricted manner. This region of the receptor is evidently an area where a repulsive interaction with extended substituents may develop rapidly, as is evident from the further sharp decrease in affinity observed on moving from **3a** to its higher homolog **2a**. In contrast to these findings are the relatively high affinities shown by a new class of steroidal anti-estrogens that bear *p*-substituted phenyl substituents at the 11β-position.[20] So, size, polarity, and orientation are all important facets in determining the effect of an 11β-group in increasing or decreasing the binding affinity of estrogens relative to estradiol. It is of note, as well, that the propynyl derivatives **2** show an inverted temperature dependence with respect to the corresponding ethynyl derivatives **3**, suggesting that they may have quite a different binding mode for the estrogen receptors. Because of their low affinity, however, neither of the propynyl derivatives **2a,b** are suitable for labeling with [^{11}C]methyl iodide. Where comparison can be made, the estriol derivatives **2b** and **3b** have lower affinity than the corresponding estradiol derivatives **2a** and **3b**. These differences reflect the relative affinities of the parent ligands.

The isomeric iodovinyl derivatives **4a,b** exhibit quite different binding behavior: whereas the Z isomer **4a** exhibits a higher affinity than estradiol and a positive dependence of RBA value from the temperature, the *E* isomer **4b** has a lower affinity and a negative dependence on temperature. In agreement with what was observed with the alkynyl derivatives, the rigid, more extended *E* isomer **4b** has lower affinity, consistent with its greater invasion of the limited hemispace above C-11β. Estrogens labeled with iodine at the other positions (16α-iodo and 17α-iodovinyl) are known and have higher affinity than compounds **4a,b**.[1c] It is of note that in the 17α-iodovinyl series, the (*Z*)-iodovinyl group also gives higher affinity derivatives than the (*E*)-iodovinyl isomer.[1c]

Conclusions

In conclusion, four novel series of 11β-substituted estradiol derivatives, differing in the size, polarity, shape, and orientation of the 11β-substituent, were synthesized, and their affinity to the estrogen receptor was determined. Cyanoethyl and cyanopropyl groups at position 11β have a detrimental effect on the binding properties, which is insensitive to the length of the chain. The effect of a 1-alkynyl group at the 11β-position on binding depends on the length of the chain: with the ethynyl group, the binding increases, whereas with the higher homolog 1-propynyl group, it undergoes a marked drop. With iodovinyl estradiols, the binding behavior is strongly dependent on the stereochemistry; the *Z* isomer binds well, but the *E* isomer is a poor ligand for the estrogen receptor.

All these data, together with those reported previously, allow us to outline a general strategy for optimizing the binding affinity of estradiol derivatives as candidate receptor-based imaging agents. The possibility for markedly improving the binding affinity of moderately sized estradiol derivatives by placing a substituent at the 11β-position has definite constraints, since the enhancement of binding appears limited to nonpolar groups whose volume is comparable to or lower than that of an ethyl group. Therefore, as far as the binding affinity is concerned, in designing a receptor-based imaging agent it will probably not be a useful strategy to use an 11β-group to improve binding *and* to provide a site for the incorporation of the label, if this will make the 11β-group too polar or too large. Rather, it appears to be more fruitful to secure high-binding affinity by using a simple 11β-group (such as a chloromethyl or an ethyl) and then locate the label in a different part of the estradiol molecule.

Experimental Section

Chemical Synthesis. General. Melting points were taken with either a Thomas-Hoover capillary melting point or a Kofler hot plate apparatus and are uncorrected. For analytical thin-layer chromatography, Merck silica gel F-254 glass-backed plates were used; for the visualization of the spots, the plates were soaked with an ethanol solution containing phosphomolybdic acid (5%) and sulfuric acid (5%) and heated with a heat gun. For column chomatography, the technique described by Still was adopted using, unless otherwise stated, mixtures of hexane (H) and ethyl acetate (EA) as eluants.[22] ^{1}H-NMR (200 MHz) spectra were obtained using either a Varian XL200 or a Bruker AC200 spectrometer; chemical shifts (δ) are relative to tetramethylsilane as internal standard. High-resolution electron impact mass spectra (HREIMS) were obtained with a Varian MAT 731 mass spectrometer. All the reactions involving organometallic reagents were performed under nitrogen in solvents distilled from sodium benzophenone ketyl. Reagents were obtained from Aldrich or Fisher.

11β-(2-Cyanoethyl)estradiol (1a). A solution of **5a**[10] (500 mg, 1 mmol) in ethyl acetate (20 mL) was stirred in a hydrogen atmosphere in the presence of 10% $Pd(OH)_2$/C (20 mg) until **5a** was converted to a single more polar compound. The catalyst was removed by filtration and the solution evaporated to afford a residue from which **1a** (330 mg, 93%) was obtained after chromatography (H/EA, 3:7) as a solid: mp 213–216 °C; ^{1}H-NMR ($CDCl_3$) δ 0.90 (s, 3 H, 18-H_3), 3.74 (m, 1 H, 17-H), 6.56 (d, J = 2.5 Hz, 1 H, 4-H), 6.67 (dd, J = 2.5, 8.5 Hz, 1 H, 2-H), 7.05 (d, J = 8.5 Hz, 1 H, 1-H). Anal. C, H. HREIMS: calcd for $C_{21}H_{27}O_2N$, 325.2041; found, 325.2047.

11β-(3-Cyanopropyl)estra-1,3,5(10)-triene-3,17β-diol (1b). This compound was obtained from **5b**[10] in the same way as **1a** was from **5a**: solid (89% yield); mp 133–136 °C; ^{1}H-NMR ($CDCl_3$) δ 0.93 (s, 3 H, 18-H_3), 3.71 (m, 1 H, 17-H), 6.55 (d, J = 2.5 Hz, 1 H, 4-H), 6.64 (dd, J = 2.5, 8.5 Hz, 1 H, 2-H), 6.99 (d, J = 8.5 Hz, 1 H, 1-H). Anal. C, H. HREIMS: calcd for $C_{22}H_{29}O_2N$, 339.2199; found, 339.2190.

5α-Hydroxy-11β-[(*Z*)-2-(tributylstannyl)vinyl]estr-9-ene-3,17-dione Bis(ethylene acetal) (7). To a cooled solution (−30 °C) of the stannylvinyl cuprate reagent prepared according to Marino[11] (20 mmol), was added **6** (5 g, 13 mmol, containing small amounts of the epimeric β-epoxide[12]) as a solution in THF (20 mL). The reaction mixture was allowed to equilibrate with room temperature over 5 h and stirred for an additional 12 h. It was then partitioned between ether and ice-cold saturated ammonium chloride containing some concentrated ammonia. The organic phase was washed with brine, dried over sodium carbonate, and evaporated to leave a residue from which **7** (5 g, 54%) was obtained by chromatography (H/EA, 3:1) as a viscous oil: ^{1}H-NMR ($CDCl_3$) δ 0.8–1.00 (m, 12 H, CH_3), 2.30 (m, 1 H), 2.55 (m, 1 H), 3.45 (m, 1 H), 3.75–4.05 (m, 8 H, OCH_2), 5.65 (d, J = 12.5 Hz, 1 H, SnC*H*=CH), 6.57 (dd, J = 8.7, 12.5 Hz, 1 H, SnCH=C*H*).[23]

5α-Hydroxy-11β-((*Z*)-2-iodovinyl)estr-9-ene-3,17-dione Bis(ethylene acetal) (8). To a solution of **7** (5 g, 7 mmol) in carbon tetrachloride was added a saturated solution of iodine in carbon tetrachloride in small portions until a stable pink color was reached and **7** was completely consumed (TLC monitoring with H/EA, 7:3). The solution was washed with 10% aqueous sodium thiosulfate, dried over sodium carbonate, and evaporated to give a residue from which **8** (3.5 g, 95%) was obtained after chromatography (H/EA, 7:3) as an oil which solidified on standing: mp 95–98 °C; ^{1}H-NMR ($CDCl_3$) δ 0.90 (s, 3 H, 18-H_3), 2.23–2.43 (m, 1 H), 2.48–2.61 (m, 1 H), 3.75–4.05 (m, 8 H, OCH_2), 6.05 (d, J = 7.3 Hz, 1 H, IC*H*=CH), 6.24 (dd, J = 7.3, 7.8 Hz, 1 H, ICH=C*H*).[23]

11β-Ethynyl-5α-hydroxyestr-9-ene-3,17-dione Bis(ethylene acetal) (9). To a solution of **8** (3 g, 5.7 mmol) in THF (30 mL) cooled in a dry ice–acetone bath was added lithium diisopropylamide (20 mmol, 10 mL of a 2 M solution in hexane) dropwise, and the reaction mixture was allowed to equilibrate with room temperature. After 1 h, the mixture was partitioned between ether and water. The organic phase was washed with brine, dried over sodium sulfate, and evaporated to afford a residue from which **9** (2.2 g, 96%) was obtained by recrystallization from hexane: mp 169–170 °C; ^{1}H-NMR ($CDCl_3$) δ 1.19 (s, 3 H, 18-H_3), 2.10–2.23 (m, 1 H), 2.23–2.44 (m, 1 H), 2.68–2.80 (m, 1 H), 3.76–4.08 (m, 8 H, CH_2O). HREIMS: calcd for $C_{24}H_{32}O_5$, 400.2249; found, 400.2249.

3-Acetoxy-11β-ethynylestra-1,3,5(10)-trien-17-one (11). A solution of **9** (2 g, 5 mmol) in 70% aqueous acetic acid (30 mL) was heated at 80 °C for 3 h. The solution was partitioned between water and ether. The organic phase was washed with 10% sodium carbonate, dried over magnesium sulfate, and evaporated. The residue was dissolved in methylene chloride (50 mL), and to the cooled solution (0 °C) were added acetic anhydride (5 mL) and acetyl bromide (5 mL), in that order. After 5 h stirring at room temperature, water and solution sodium bicarbonate were added to consume the excess of acid. The organic phase was dried over magnesium sulfate, filtered through a short layer of silica gel to remove colored impurities, and evaporated to afford a residue from which 11 (1.43 g, 85%) was obtained by trituration with ether. A sample was chromatographed (H/EA, 2:1) and recrystallized from methylene chloride/ether; mp 154–156 °C; ^{1}H-NMR ($CDCl_3$) δ 1.25 (s, 3 H, 18-H_3), 1.91 (d, J = 2.5 Hz, 1 H, C≡CH), 2.27 (s, 3 H, CH_3-CO), 2.45–2.60 (m, 2 H), 2.70–3.05 (m, 2 H), 3.61 (m, 1 H, 11-H), 6.83 (d, J = 2.3 Hz, 1 H, 4-H), 6.89 (dd, J = 2.3, 8.8 Hz, 1 H, 2-H), 7.24 (d, J = 8.8 Hz, 1 H, 1-H). HREIMS: calcd for $C_{22}H_{24}O_3$, 336.1725; found, 336.1725.

11β-Ethynylestra-1,3,5(10)-triene-3,17β-diol (3a). Compound 11 (330 mg, 1 mmol) was allowed to dissolve in a stirred solution (5 mL) containing sodium borohydride (38 mg, 1 mmol) and sodium hydroxide (80 mg, 2 mmol). After stirring for 1 h to effect complete dissolution, the reaction mixture was diluted with water, a few drops of acetic acid were added to remove the excess of hydride, and the mixture was extracted with ethyl acetate. The organic extracts were dried over magnesium sulfate and evaporated to yield a residue. **3a** (260 mg, 87%) was obtained after chromatography (H/EA, 1:1) as a solid: mp 153–158 °C; ^{1}H-NMR ($CDCl_3$) δ 1.12 (s, 3 H, 18-H_3), 1.89 (d, J = 2.5 Hz, 1 H, C≡CH), 2.65–2.95 (m, 2H), 3.50 (m, 1 H, 11-H), 3.68 (m, 1 H, 17-H), 6.54 (d, J = 2.3 Hz, 1 H, 4-H), 6.64 (dd, J = 2.3, 8.5 Hz, 1 H, 2-H), 7.01 (d, J = 8.5 Hz, 1 H, 1-H). HREIMS: calcd for $C_{20}H_{24}O_2$, 296.1776; found, 296.1777.

11β-Ethynylestra-1,3,5(10)-triene-3,16α,17β-triol (3b). To a solution of 11 (330 mg, 1 mmol) and triethylamine (0.5 mL, 0.36 g, 3.6 mmol) in methylene chloride (10 mL) was added trimethylsilyl triflate (0.5 mL, 5.7 g, 2.6 mmol). After 1 h, the reaction mixture was diluted with methylene chloride. The solution was washed with 10% aqueous sodium bicarbonate and added to a slurry of oxone (2 g, 3.25 mmol), acetone (0.5 mL), tetrabutylammonium hydrogen sulfate (100 mg), and water (10 mL). To the stirred mixture was added solid sodium carbonate in small portions (carbon dioxide evolution) until the silyl ether was consumed (TLC monitoring with H/EA, 1:1). The mixture was partitioned between water and methylene chloride. The organic phase was evaporated to afford a residue which was dissolved in a methanol solution (10 mL) containing sodium borohydride (50 mg, 1.3 mmol) and sodium hydroxide (100 mg, 2.5 mmol). After 1 h, acetic acid was added dropwise to decompose excess of hydride and base. The mixture was diluted with water and extracted with ethyl acetate. Organic extracts, dried over magnesium sulfate and evaporated, gave a residue from which **3b** (230 mg, 75%) was obtained by chromatography (ethyl acetate) as a solid: mp 175–177 °C; ^{1}H-NMR (methanol-d_6) δ 1.11 (s, 3 H, 18-H_3), 2.04 (d, J = 2.5 Hz, 1 H, C≡CH), 3.40 (d, J = 5.4 Hz, 1 H, 17-H), 3.50 (m, 1 H, 11-H), 4.05 (m, 1 H, 16-H), 6.46 (d, J = 2.5 Hz, 1 H, 4-H), 6.53 (dd, J = 2.5, 8.5 Hz, 1 H, 2-H), 7.02 (d, J = 8.5 Hz, 1 H, 1-H). HREIMS: calcd for $C_{20}H_{24}O_3$, 312.1725; found, 312.1725.

11β-Ethynyl-3,17β-bis(methoxymethoxy)estra-1,3,5(10)-triene (12a). A solution of **3a** (200 mg, 0.67 mmol), diisopropylethylamine (0.6 mL, 440 mg, 3.44 mmol), and chloromethyl methyl ether (0.3 mL, 0.312 g, 3.87 mmol) in acetonitrile (5 mL) was heated in a capped vial at 80 °C for 1 h. The mixture was then partitioned between ether and aqueous sodium bicarbonate. The organic phase was dried over sodium carbonate and evaporated to afford a residue from which **12a**

(0.24 g, 96%) was obtained as an oil after chromatography (H/EA, 4:1): ^{1}H-NMR ($CDCl_3$) δ 1.14 (s, 3 H, 18-H_3), 1.88 (d, J = 2.3 Hz, 1H, C≡CH), 2.28–2.45 (m, 2 H), 2.70–2.98 (m, 2 H), 3.36 and 3.45 (2 s, 2 × 3 H, OCH_3), 3.50 (m, 2 H, 11-H, 17-H), 4.62 and 5.12 (2 s, 2 × 2 H, OCH_2), 6.73 (d, J = 2.6 Hz, 1 H, 4-H), 6.82 (dd, J = 2.6, 8.5 Hz, 1 H, 2-H), 7.10 (d, J = 8.5 Hz, 1 H, 1-H).[23]

11β-Ethynyl-3,16α,17β-tris(methoxymethoxy)estra-1,3,5(10)-triene (12b). This compound was obtained as an oil from **3b** in the same way as **12a** was obtained from **3a** (94% yield): ^{1}H-NMR ($CDCl_3$) δ 1.27 (s, 3 H, 18-H_3), 3.49, 3.52, and 3.57 (3 s, 3 × 3 H, OMe), 3.55–3.20 (m, 2 H, 11-H, 17-H), 4.25 (m, 1 H, 16-H), 4.75–4.95 (m, 4 H, 17-OCH_2, 16-OCH_2), 5.24 (s, 2 H, 3-OCH_2), 6.86 (d, J = 2.6 Hz, 1 H, 4-H), 6.95 (dd, J = 2.6, 8.5 Hz, 1 H, 2-H), 7.25 (d, J = 8.5 Hz, 1 H, 1-H).[23]

11β-(1-Propynyl)estra-1,3,5(10)-triene-3,17β-diol (2a). To a solution of **12a** (50 mg, 0.13 mmol) and triphenylmethane (10 mg) in tetrahydrofuran was added butyllithium (0.2 mL of a solution 1.6 M in hexane) until a stable brown color was developed. Excess of methyl iodide was then added, causing an immediate color discharge. The reaction mixture was partitioned between ether and water, the organic phase was evaporated, and the residue was dissolved in 1 M methanolic hydrochloric acid (5 mL). After heating for 5 min at 80 °C, the reaction mixture was partitioned between water and ethyl acetate, and the organic phase was dried over magnesium sulfate and evaporated to afford a residue from which **2a** (36 mg, 90%) was obtained after chromatography (H/EA, 1:1) as a solid: mp 139–144 °C: ^{1}H-NMR ($CDCl_3$) δ 1.11 (s, 3 H, 18-H_3), 1.59 (d, J = 2.5 Hz, 3 H, C≡CCH_3), 2.60–2.95 (m, 2 H), 3.44 (m, 1 H, 11-H), 3.68 (m, 1 H, 17-H), 6.53 (d, J = 2.6 Hz, 1 H, 4-H), 6.63 (dd, J = 2.6, 8.5 Hz, 1 H, 2-H), 7.07 (d, J = 8.5 Hz, 1 H, 1-H). Anal. C, H. HREIMS: calcd for $C_{21}H_{26}O_2$, 310.1933; found, 310.1934.

11β-(1-Propynyl)estra-1,3,5(10)-triene-3,16α,17β-triol (2b). This compound was obtained from **12b** in the same way as **2a** was obtained from **12a** (final chromatography with ethyl acetate): solid (85% yield); mp 173–175 °C: ^{1}H-NMR (methanol-d_6) δ 1.11 (s, 3 H, 18-H_3), 1.55 (d, J = 2.5 Hz, 3 H, C≡CCH_3), 2.16 (dd, J = 1.6, 19.9 Hz, 1 H), 2.37 (dd, J = 4.7, 10.5 Hz, 1 H), 2.65–2.90 (m, 2 H), 3.38 (d, J = 5.5 Hz, 1 H, 17-H), 3.44 (m, 1 H, 11-H), 4.05 (m, 1H, 16-H), 6.45 (d, J = 2.6 Hz, 1 H, 4-H), 6.54 (dd, J = 2.6, 8.5 Hz, 1 H, 2-H), 7.01 (d, J = 8.5 Hz, 1 H, 1-H). Anal. C, H. HREIMS: calcd for $C_{21}H_{26}O_2$, 326.1882; found, 326.1882.

(Z)-3-Acetoxy-11β-(2-iodoethenyl)estra-1,3,5(10)-trien-17-one (10). This compound was obtained from acetal **8** as described for the conversion of **9** to 11: solid (80% yield); mp 185–187 °C: ^{1}H-NMR ($CDCl_3$) δ 0.88 (s, 3 H, 18-H_3), 2.27 (s, 3 H, MeCO), 2.89 (m, 2 H), 3.50 (m, 1 H, 11-H), 6.04 (t, J = 7.5 Hz, 1 H, IHC=CH), 6.21 (d, J = 7.5 Hz, 1 H, I*H*C=CH), 6.78–6.83 (m, 2 H, 2-H, 4-H), 6.95 (d, J = 8.5 Hz, 1 H, 1-H). HREIMS: calcd for $C_{22}H_{25}IO_3$, 464.0850; found, 464.0852.

(Z)-11β-(2-Iodoethenyl)estra-1,3,5(10)-triene-3,17β-diol (4a). This compound was obtained from **10** by reduction with sodium borohydride followed by saponification, as detailed for the conversion of 11 to **3a**: solid (82% yield); mp 118–120 °C; ^{1}H-NMR (acetone-d_6) δ 0.74 (s, 3 H, 18-H_3), 2.25 (dd, J = 2 and 13 Hz, 1H), 2.48 (dd, J = 4.5, 10.5 Hz, 1 H), 2.60–2.80 (m, 2 H), 3.35 (m, 1 H, 11-H), 3.70 (m, 1 H, 17-H), 6.09–6.2 (m, 1 H, IHC=CH), 6.49 (d, J = 2.5 Hz, 1 H, 4-H), 6.54 (dd, J = 2.5, 8 Hz, 1 H, 2-H), 6.74 (d, J = 8 Hz, 1 H, 1-H). Anal. C, H. HREIMS: calcd for $C_{20}H_{25}IO_2$, 424.0899; found, 424.0899.

(E)-11β-(2-Iodoethenyl)estra-1,3,5(10)-triene-3,17β-diol (4b). To a suspension of 20% $Pd(OH)_2$ on charcoal (5 mg) in an ethyl acetate (10 mL) solution of **3a** (50 mg, 0.17 mmol), stirred in an ice–water bath, was added tributyltin hydride (0.5 mL) over 10 min (H_2 evolution). The mixture was evaporated and the residue chromatographed (H/EA, 4:1), yielding (*E*)-11β-[2-(tributylstannyl)ethenyl]estra-1,3,5(10)-triene-3,17β-diol (80 mg, 80% yield) as a waxy solid: ^{1}H-NMR ($CHCl_3$) δ 0.80 (s, 3 H, 18-H_3), 0.82 (t, J = 7 Hz, 9 H, CH_2CH_3), 2.21 (dd, J = 2, 13 Hz, 1 H), 2.44 (dd, J = 4.5, 10.5 Hz, 1 H), 2.60–2.80 (m, 2 H), 3.28 (m, 1 H, 11-H), 3.71 (m, 1 H, 17-H), 5.77 (dd, J = 6, 19 Hz, 1 H, SnHC=C*H*), 5.95 (d, J = 19 Hz, 1 H, Sn*H*C=CH), 6.48–6.59 (m, 2 H, 2-H, 4-H), 6.92 (d, J = 8 Hz, 1 H, 1-H).

The above stannane was allowed to react with iodine in carbon tetrachloride as described in detail for the conversion of **7** to **8** to obtain **4b** (80% yield) as a solid: mp 134 °C; ^{1}H-NMR ($CHCl_3$) δ 0.79 (s, 3 H, 18-H_3), 2.16 (dd, J = 2, 13 Hz, 1H), 2.44 (dd, J = 4.5, 10.5 Hz, 1H), **3.31** (m, 1 H, 11-H), 3.65 (m, 1 H, 17-H), 5.87 (dd, J = 1, 15 Hz, 1 H, I*H*C=CH), 6.37 (dd, J = 7, 15 Hz, 1 H, IHC=C*H*), 6.51 (d, J = 2.5 Hz, 1 H, 4-H), 6.58 (dd, J = 2.5, 8 Hz, 1 H, 2-H), 6.83 (d, J = 8 Hz, 1 H, 1-H). Anal. C, H. HREIMS: calcd for $C_{20}H_{25}IO_2$, 424.0899; found, 424.0899.

Determination of Receptor Binding Affinity. The binding affinities reported in Table 1 (which are relative to estradiol, given the value of 100%) were determined by previously reported methods.[17] The radiotracer was [^{3}H]-estradiol (Amersham; 51 Ci/mmol). Lamb uterine cytosol was the source of estrogen receptor. Separate assays were performed by incubation for 18 h at both 0 and 25 °C. The free steroids were absorbed onto dextran-coated charcoal (15 min, 0 °C). The reproducibility of these values is generally ±30%, relative.

Acknowledgment. We are grateful for support of this work through grants from MURST (Roma) and CNR (Roma) (to E.N. and R.F.) and the National Institutes of Health (PHS 5R01 CA25836).

References

(1) (a) Pomper, M. G.; VanBrocklin, H.; Thieme, A. M.; Thomas, R. D.; Kiesewetter, D. O.; Carlson, K. E.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A. 11β-Methoxy-, 11β-ethyl-, and 17α-Ethynyl-substituted 16α-Fluoroestradiols: Receptor-Based Imaging Agents with Enhanced Uptake Efficiency and Selectivity. *J. Med. Chem.* **1990**, *33*, 3143–3155. (b) VanBrocklin, H. F.; Pomper, M. G.; Carlson, K. E.; Welch, M. J.; Katzenellenbogen, J. A. Preparation of Evaluation of 17-Ethynyl-Substituted 16α-[^{18}F]Fluoroestradiols: Selective Receptor-Based PET Imaging Agents. *Nucl. Med. Biol.* **1992**, *19*, 363–374. (c) VanBrocklin, H. F.; Carlson, K. E.; Katzenellenbogen, J. A.; Welch, M. J. 16β-([^{18}F]Fluoro)estrogens: Systematic Investigation of a New Series of Fluorine-18 Labeled Estrogens as Potential Imaging Agents for Estrogen-Receptor-Positive Breast Tumors. *J. Med. Chem.* **1993**, *36*, 1619–1629. (d) Cummins, C. H. Radiolabeled Steroidal Estrogens in Cancer Research. *Steroids* **1993**, *58*, 245–259.

(2) (a) Katzenellenbogen, J. A.; Senderoff, S. G.; McElvany, K. D.; O'Brien, H. A., Jr.; Welch, M. J. [^{77}Br]-16α-Bromoestradiol-17β: A High Specific-Activity Gamma-Emitting Tracer with Uptake in Rat Uterus and Induced Mammary Tumors. *J. Nucl. Med.* **1981**, *22*, 42–47. (b) McElvany, K. D.; Carlson, K. E.; Welch, M. J.; Senderoff, S. G.; Katzenellenbogen, J. A. *In Vivo* Comparison of 16α-[^{77}Br]-Bromoestradiol-17β and 16α-[^{125}I]-Iodoestradiol-17β. *J. Nucl. Med.* **1982**, *23*, 420–424.

(3) Kenady, D.; Pavlik, E.; Nelson, K.; van Nagell, F.; Gallion, H.; DePriest, P.; Ryo, U.; Baranczuk, R. Images of Estrogen-Receptor-Positive Breast Tumors Produced by Estradiol Labeled with Iodine I-123 at 16 Alphà. *Arch. Surg.* **1993**, *128*, 1373–1381.

(4) (a) Mintun, M. A.; Welch, M. J.; Siegel, B. A.; Mathias, C. J.; Brodack, J. W.; McGuire, A. H.; Katzenellenbogen, J. A. Breast Cancer: PET Imaging of Estrogen Receptors. *Radiology* **1988**, *169*, 45–48. (b) McGuire, A. H.; Dehdashti, F.; Siegel, B. A.; Lyss, A. P.; Brodack, J. W.; Mathias, C. J.; Mintun, M. A.; Katzenellenbogen, J. A.; Welch, M. J. Positron Tomographic Assessment of 16α-[^{18}F]Fluoro-17β-Estradiol Uptake in Metastatic Breast Carcinoma. *J. Nucl. Med.* **1991**, *32*, 1526–1531.

(5) Ribeiro-Barras, M.; Coulon, C.; Baulieu, J.; Guilloteau, D.; Bougnoux, P.; Lansac, J.; Besnard, J. Estrogen Receptor Imaging with 17α-[123I]iodovinyl-11β-methoxyestradiol (MIVE2)—Part II. Preliminary Results in Patients with Breast Carcinoma. *Nuc. Med. Biol.* **1992**, *19*, 263–267.

(6) (a) Feenstra, A.; Vaalburg, G. M. J.; Reiffers, S.; Talma, A. G.; Wiegman, T.; van der Molen, H. D.; Woldring, M. G. Estrogen Receptor Binding Radiopharmaceuticals: II. Tissue Distribution of 17α-Methylestradiol in Normal and Tumor-Bearing Rats. *J. Nucl. Med.* **1983**, *24*, 522–528. (b) Napolitano, E. N.; Dence, C. S.; Welch, M. J.; Katzenellenbogen, J. A. Carbon-11 Labeled Estrogens as Imaging Agents for Breast Tumors. The Society of Nuclear Medicine 41st Annual Meeting, Orlando, FL, June 1994; *J. Nucl. Med.* **1994**, *35S*, 250P. (c) Napolitano, E.; Fiaschi, R.;

Carlson, K. E.; Katzenellenbogen, J. A. 11β-Substituted Estradiol Derivatives, Potential High-Affinity Carbon-11-Labeled Probes for the Estrogen Receptor: A Structure-Affinity Relationship Study. *J. Med. Chem.* **1995**, *38*, 429–434.
(7) Flood, C.; Pratt, J. H.; Longcope, C. The Metabolic Clearance and Blood Production Rates of Estriol in Normal, Non-Pregnant Women. *J. Clin. Endocrinol. Metab.* **1976**, *42*, 1–7.
(8) (a) Teutsch, G.; Belanger, A.; Philibert, D.; Tournemine, C. Synthesis of 11β-Vinyl-19-norsteroids as Potent Progestins. *Steroids* **1982**, *39*, 607–615. (b) Belanger, A.; Philibert, D.; Teutsch, G. Regio and Stereospecific Synthesis of 11β-Substituted-19-norsteroids. Influence of 11β -Substitution on Progesterone Receptor Afinity. *Steroids* **1981**, *37*, 361–382.
(9) French, A. N.; Napolitano, E.; VanBrocklin, H. F.; Hanson, R. N.; Welch, M. J.; Katzenellenbogen, J. A. Synthesis, Radiolabeling and Tissue Distribution of 11β-Fluoroalkyl and 11β-Fluoroalkoxy-Substituted Estrogens. Target Tissue Uptake Selectivity and Defluorination of a Homologous Series of Fluorine-18-Labeled Estrogens. *Nucl. Med. Biol.* **1993**, *20*, 31–47.
(10) Hanson, R. N.; Napolitano, E.; Fiaschi, R. Synthesis and Estrogen Receptor Binding Affinity of Novel 11β-Substituted Estra-1,3,5,(10)-triene-3,17β-diols. *J. Med. Chem.* **1990**, *33*, 3155–3160.
(11) Marino, J. P.; Emonds, M. V. M.; Stengel, P. J.; Oliveira, A. R. M.; Simonelli, J. T. B. Cis-2-tributylstannylvinyl Cuprates: A New Synthon for the Cis-1,2-ethene Dianion. *Tetrahedron Lett.* **1992**, *33*, 49–52.
(12) Napolitano, E.; Fiaschi, R.; Hanson, R. N. Epoxidation of Estra-5(10),9(11)-diene Derivatives: A Convenient Synthesis of 11β-Vinylestrone Acetate. *Gazz. Chim. Ital.* **1990**, *120*, 323–326.
(13) For a more recent and improved preparation of coumpound **3a**, see: Tedesco, R.; Fiaschi, R.; Napolitano, E. Novel Stereoselective Synthesis of 11β-carbon substituted Estradiol Derivatives. *J. Org. Chem.* **1995**, *60*, in press.
(14) For a recent use of *in situ* generated dimethyldioxiranes, see: Denmark, S. E.; Forbes, D. C.; Hays, D. S.; DePoe, D. S.; Wilde, R. C. Catalytic Epoxidation of Alkenes with Oxone. *J. Org. Chem.* **1995**, *60*, 1391–1407.
(15) Schoenecker, B.; Tresselt, D.; Draffehn, J.; Ponsold, K.; Engelhardt, G.; Zeigan, D.; Schneider, G.; Weisz-Vincze, I.; Dombi, G. Steroids LIII. Proton NMR Studies. Configurational Assignment of 16-substituted Steroids. *J. Pract. Chem.* **1977**, *319*, 419–431.
(16) Zhang, H. X.; Guibe, F.; Balavoine, G. Palladium- and Molybdenum-catalyzed Hydrostannation of Alkynes. A Novel Access to Regio- and Stereodefined Vinylstannanes. *J. Org. Chem.* **1990**, *55*, 1957–1967.
(17) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. Photoaffinity Labels for Estrogen Binding Proteins of Rat Uterus. *Biochemistry* **1973**, *12*, 4085–4092.
(18) Aranyi, P. Kinetics of the Hormone-Receptor Interaction. Competition Experiments with Slowly Equilibrating Ligands. *Biochim. Biophys. Acta* **1980**, *628*, 220–227.
(19) Raynaud, J. P.; Bouton, M.; Gallet-Bourquin, D.; Philibert, D.; Tournemine, C.; Azadian-Boulanger, G. Comparative Study of Estrogen Action. *Mol. Pharmacol.* **1973**, *9*, 520–533.
(20) (a) Van de Velde, P.; Nique, F.; Bouchoux, F.; Brémaud, J.; Hameau, M.-C.; Lucas, D.; Moratille, C.; Viet, S.; Philibert, D.; Teutsch, G. RU 58 668, a New Pure Antiestrogen Inducing a Regression of Human Mammary Carcinoma Implanted in Nude Mice. *J. Steroid Biochem. Mol. Biol.* **1994**, *48*, 187–196. (b) Nique, F.; Van de Velde, P.; Brémaud, J.; Hardy, M.; Philibert, D.; Teutsch, G. 11β-Amidoalkoxyphenyl Estradiols, a New Series of Pure Antiestrogens. *J. Steroid Biochem. Mol. Biol.* **1994**, *50*, 21–29.
(21) Pomper, M. G.; VanBrocklin, H.; Thieme, A. M.; Thomas, R. D.; Kiesewetter, D. O.; Carlson, K. E.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A. 11β-Methoxy-, 11β-Ethyl-, and 17α-Ethynyl-Substituted 16α-Fluoroestradiols: Receptor-Based Imaging Agents with Enhanced Uptake Efficiency and Selectivity. *J. Med. Chem.* **1990**, *33*, 3143–3155.
(22) Still, W. C.; Kahn, M.; Mitra, A. P. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(23) This compound failed to give satisfactory microanalysis (retained solvent) or high-resolution mass spectrometry (molecular ion absent).

JM950119W