Articles

Synthesis and Evaluation of 11β-Substituted 21-Chloro/Iodo-(17α,20*E*/*Z*)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols: High-Affinity Ligands for the Estrogen Receptor

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We have synthesized six new estrogens substituted at the 11β-position with a methoxy or vinyl group and at the 17α-position with an (*E*)- or (*Z*)-chloro/iodovinyl moiety. The products were obtained in good overall yields from the corresponding tri-*n*-butylstannylvinyl intermediates using the electrophilic halodestannylation methodology. The six new ligands were compared to the 11β-unsubstituted chloro/iodovinyl derivatives and the 11β-methoxy (*E*)- and (*Z*)-iodovinyl estrogens to evaluate the effects of 11β-substitution and 20*E*/*Z*-stereochemistry. While all the compounds exhibited high affinity for the estrogen receptor, the 20*Z*-isomers demonstrated higher affinity than the corresponding 20*E*-isomers. In addition, the presence of the lipophilic 11β-substituent was favored over either no substituent or a polar (methoxy) group. Within each isomeric series, the presence of the 21-halo substituent had different effects. For the 20*E*-series, the 21-chloro products had a higher affinity than the 21-iodo analogue, whereas for the 20*Z*-series the effect was reversed. These results provide additional insights into the interaction of substituted estradiols with the hormone binding domain of the estrogen receptor.

As part of our program to prepare novel high-affinity ligands for the estrogen receptor, we have undertaken the systematic development of novel probes for the hormone binding domain (HBD). In our previous studies we have reported the preparation of derivatives of estradiol bearing substituents at either the 11β-position (compounds **1a**−**f**)[1−3] or the 17α-position (compounds **2a**−**d, 3a**−**d**)[4−7] (Figure 1). In each case, structure−activity relationships were developed which identified the effect of the substituent's properties on the ligand's affinity. For the 11β-group, small lipophilic substituents such as vinyl, ethyl, or fluoroethyl were more favored than larger and/or more hydrophilic groups such as methoxy or hydroxyethyl. At the 17α-position, *Z*-substituted vinyl groups were generally better tolerated than the corresponding *E*-isomers. This effect appeared to hold whether the substituent was small, e.g., chloro, or large, e.g., phenyl or thiophenyl. These results gave rise to a model of the HBD in which steric tolerance existed in the 17α-binding region and steric/hydrophobic interactions were present in the 11β-binding region. This is consistent with the recent pharmacophoric model proposed by Anstead et al.[8] Except for the few cases which we and others[9−12] have reported, little had been published specifically describing the effects of simultaneous 11β/17α-X-vinyl substitution.

The present study represents our initial effort to address this situation. The results of this study support our earlier observations that the (17α*E*)- and (17α*Z*)-halovinyl substituents interact with different components of the HBD, generating individual structure−activity relationships. The effects of the 11β-substituent, however, remain consistent for both series. The inter-

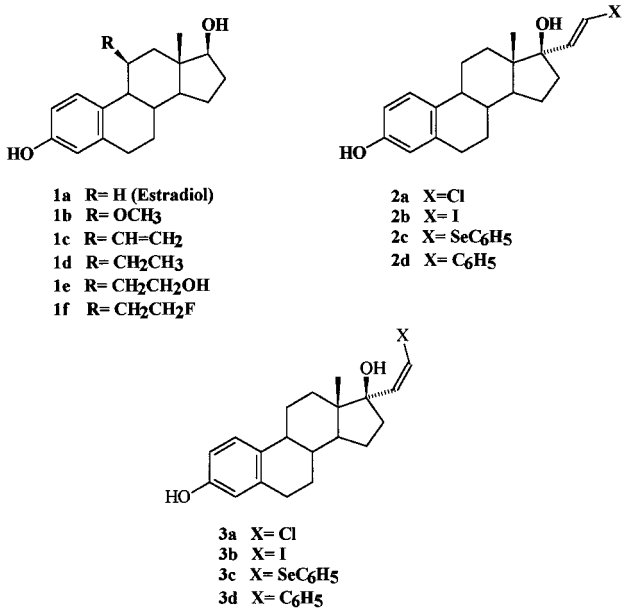

Figure 1. Previously synthesized 11β-substituted estradiols **1a**−**f**, 17α*E*-substituted vinylestradiols **2a**−**d**, and 17α*Z*-substituted vinylestradiols **3a**−**d**.

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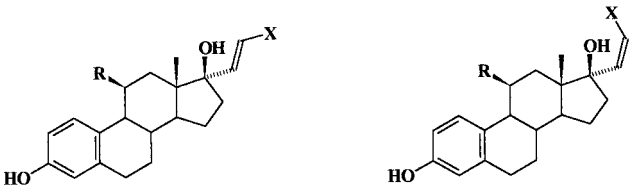


2a R= H, X= Cl
2b R= H, X= I
4a R= OCH_3, X= Cl
4b R= OCH_3, X= I
5a R= $CH{=}CH_2$, X= Cl
5b R= $CH{=}CH_2$, X= I

3a R= H, X= Cl
3b R= H, X= I
6a R= OCH_3, X= Cl
6b R= OCH_3, X= I
7a R= $CH{=}CH_2$, X= Cl
7b R= $CH{=}CH_2$, X= I

Figure 2. Target estrogen receptor ligands bearing 11β- and (17α*E*/*Z*)-halovinyl substituents.

pretation of these results contributes to a growing inferential model of the interaction between the steroidal ligand and the receptor. The recently reported structure for the liganded estrogen receptor HBD suggests that the estradiol interacts strongly with the glutamate (Glu-353) and histidine (His-524) resides.[13] In this orientation, the 11β-substituents would be directed toward the lipophilic amino acids (Leu-525 and -540) of helix-12. The 17α-groups, however, would be directed toward the interface of helices-7 and -11, a region that is largely hydrophobic in character (Phe-425, Ileu-424, Leu-428). The results of this study suggest that the 17α-directed groups are important and may provide the basis for designing newer probes that can identify specific residues in this region of the receptor (Figure 2).

Chemistry

Synthesis of (17α,20*E*/*Z*)-11β-Substituted 21-Halo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols (Substituted Vinylestradiols). The synthesis of the target 21-substituted estrogens (chloro- and iodovinylestradiols) is illustrated in Schemes 1 and 2. On the basis of the susceptibility of the phenolic tributylstannylvinylestradiol to undergo protiodestannylation, as noted in our previous studies,[5] we elected to employ the substituted 17α-ethynylestradiol 3-acetates as the starting materials. Whereas 17α-ethynylestradiol and moxestrol, the precursors for **8a,b**, were available from commercial and industrial sources, **8c** was prepared from 11β-ethenylestrone 3-acetate (**12**), which itself required a 9-step synthesis[2] from estrone 3-methyl ether (**11**) (Scheme 2). The addition of lithium trimethylsilylacetylide to the ketone **12** followed by deprotection with potassium fluoride in DMF gave the 11β-ethenyl-17α-ethynylestradiol in a 52% overall yield for two steps (80% based upon recovered starting material). Conversion to the acetate **8c** using acetic anhydride and triethylamine in dichloromethane was essentially quantitative.

The preparation of the (17α*E*/*Z*)-tri-*n*-butylstannylvinyl intermediates **9a**−**c** and **10a**−**c** was achieved by three methods, each with a particular advantage. In method A, irradiation of a gently warmed THF solution of the 17α-ethynylestradiol 3-acetate and tri-*n*-butylstannane gave a high yield (80−90%) of the (17α*E*)-tri-*n*-butylstannylvinylestradiol with only small quantities of the *Z*-isomer and recovered starting material. In method B, heating of the THF solution of the 17α-ethynylestradiol 3-acetate and tri-*n*-butylstannane at 60 °C in the absence of irradiation or a radical initiator gave a conversion to 40−45% of the (17α*Z*)-tri-*n*-butylstannylvinylestradiol, 5−10% of the *E*-isomer, and 40−50% of the recovered starting material. In the third method, which was applicable to multimillimole scale reactions, conditions could be modulated to provide either isomer as the predominating species. The addition of triethylborane to a THF solution of the 17α-ethynylestradiol 3-acetate and tri-*n*-butylstannane gave primarily the *Z*-isomer at lower stannane:estrogen ratios and short reaction times and predominately the *E*-isomer at high stannane:estrogen ratios. This last method was our primary route in this study when significant quantities of the intermediates were required. The overall conversion rates were good, and the separation of the (*Z*)- and (*E*)-tri-*n*-butylstannylvinyl isomers could be readily achieved by column chromatography.

The target (*E*/*Z*)-chloro- or -iodovinylestradiols were obtained by the methods previously reported.[5] Chlorodestannylation of the intermediate (*E*)- or (*Z*)-tri-*n*-butylstannylvinylestradiol with chlorine gas in cyclohexane at −78 °C gave the crude intermediate which was hydrolyzed with sodium hydroxide in methanol. Workup and purification by column chromatography gave the products in 85−90% overall yields. The (*E*)- and (*Z*)-iodovinylestradiols were prepared by dropwise addition at −18 °C of iodine in CCl_4 to a solution of the (17α*E*/*Z*)-tri-*n*-butylstannylvinylestradiol in CCl_4. The reaction solution was evaporated, and treatment of the crude intermediate with sodium hydroxide in methanol gave, following workup and purification, the (17α*E*/*Z*)-iodovinylestradiols in 85−90% isolated yields. Within limits of detection, the reactions proceeded stereospecifically to yield the ipso-substituted product. The final products, not previously reported, were characterized by thin-layer chromatography, mass spectrometry, optical rotation, and NMR. The stereochemistry of the 20-double bond could readily be established by the coupling constant, e.g., for the *E*-isomers $J = 13-15$ Hz and for the *Z*-isomers $J = 8-9$ Hz.[5]

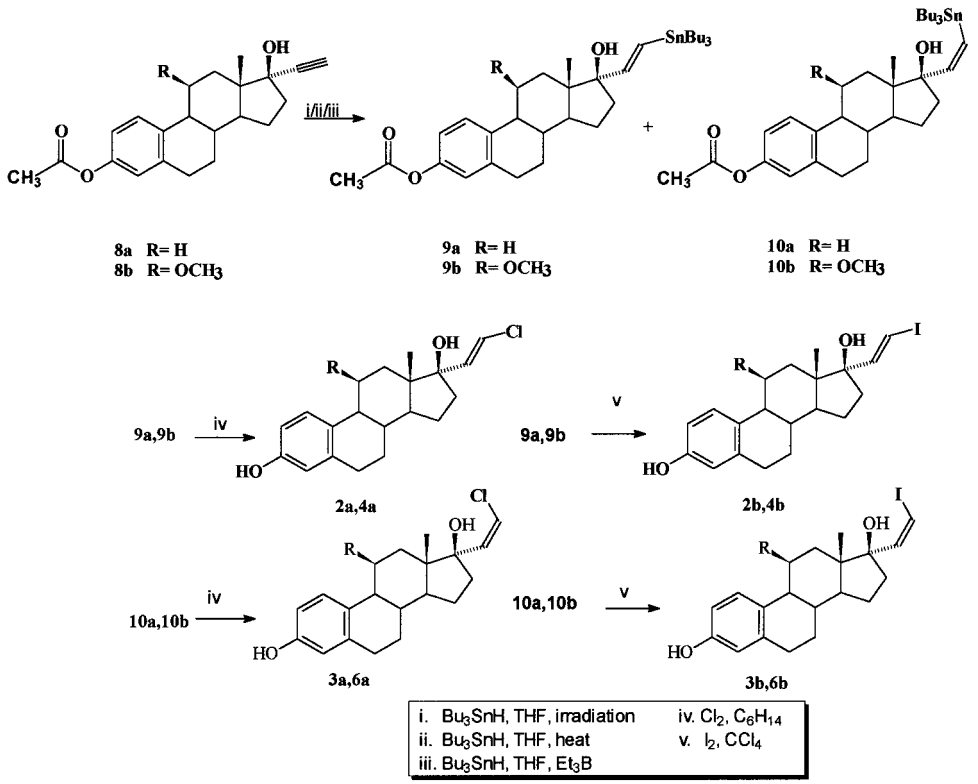
Receptor Binding Assays

Table 1 summarizes the binding data for the compounds prepared in this study as well as the values previously reported. The receptor binding affinity (RBA) values were measured at 4 and 25 °C to represent both the kinetic and equilibrium effects.[14−16]

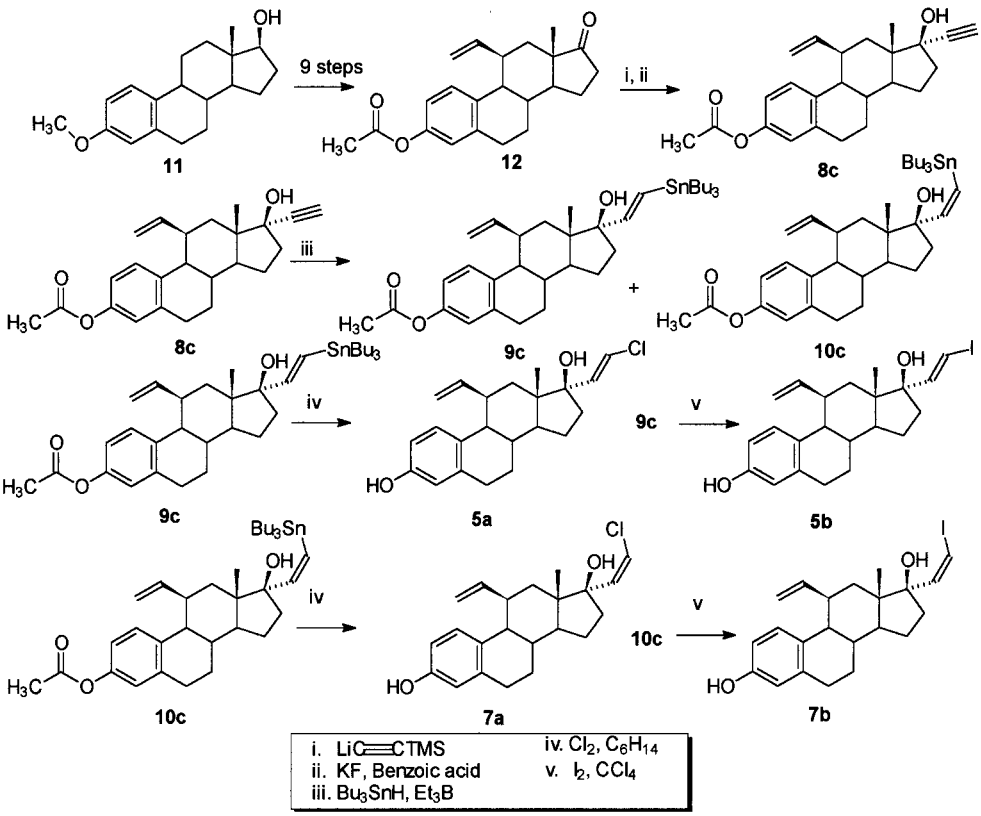
The data indicated that all of the compounds possessed a high affinity for the estrogen receptor, i.e., >25. If one examines the kinetic (4 °C) data, the presence of the small, lipophilic 11β-vinyl substituent has a negligible effect upon receptor association. The more polar 11β-methoxy group, on the other hand, significantly reduces the receptor affinity by a factor of 2−4-fold. This effect is observed in both the (17α*E*)- and (17α*Z*)-halovinyl series, suggesting that both sets of ligands interact initially in a similar fashion with the receptor. In addition, because the 11β-methoxy and 11β-ethyl substituents are relatively similar in size and orientation, the differences in RBA values may represent more of a hydrophobic−hydrophilic difference than a steric factor.

The results from the equilibrium binding assays (25 °C) provide a different picture for the (17α*E*)- and

Scheme 1

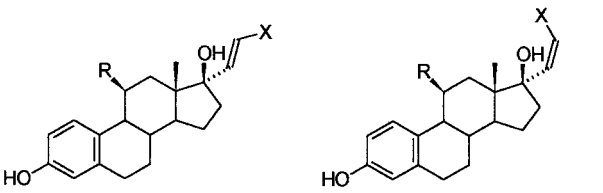


Scheme 2



(17α*Z*)-halovinyl estrogens. The presence of the (17α*Z*)-halovinyl substituent significantly enhances the RBA value compared to estradiol. As previously noted, the parent steroids **3a,b** exhibit affinities 2−8 times greater than estradiol.[5] This level of enhancement is observed for the 11*β*-methoxy pair **6a,b** even though their kinetic (4 °C) affinities are much less than that of estradiol. The highest RBA values are demonstrated by the 11*β*-vinyl-substituted estrogens **7a,b** where the RBA values are 7−12 times greater than that of estradiol. Within the (17α*E*)-halovinyl series, only the 11*β*-vinyl pair **5a,b** demonstrated affinities greater than estradiol. The

Table 1. Relative Binding Affinities (RBA) of *E*- and *Z*-Isomers of Estrogenic Ligands for Estrogen Receptors from Lamb Uterine Cytosol



		RBA[a]			
		E-isomer		*Z*-isomer	
R	X	4 °C	25 °C	4 °C	25 °C
H	Cl	102[b]	89[b]	126[b]	199[b]
H	I	77[b]	62[b]	202[b]	776[b]
OCH_3	Cl	29	65	22	195
OCH_3	I	34[b]	65[b]	48[b]	513[b]
$CH{=}CH_2$	Cl	107	724	101	704
$CH{=}CH_2$	I	78	447	98	1202

[a] The RBA values were determined by competitive radiometric binding assays according to the procedures previously described in refs 15 and 16. [b] RBA values published in ref 5.

unsubstituted compounds **2a,b** and the 11β-methoxy pair **4a,b** possess significant ER affinity but still less than that of estradiol (RBA = 62−89%).

An examination of the influence of the halogen upon the receptor binding under both kinetic and equilibrium conditions results in a further divergence in their structure−activity relationships. At 4 °C, increasing the size of the halogen in the *E*-series has a neutral or a slightly negative effect on binding, i.e., Cl ≥ I. Within the *Z*-series, increasing the size of the halogen has a neutral or positive effect on binding, i.e., I ≥ Cl. This same order of effects is maintained when the binding is performed under equilibrium conditions at 25 °C. The magnitude of these effects is interesting. Within the *E*-series, the maximal reduction in RBA, whether at 4 or 25 °C, is about 35% (RBA 724 vs 447). For the *Z*-series, however, the enhancement of the RBA is more substantial, i.e., up to 250−350% (RBA 195 vs 503 or 199 vs 776).

Similar binding results have been observed with other estrogenic substances. Our earlier studies had indicated that the (21*Z*)-phenyl- and -phenylthiovinylestradiols demonstrated enhanced kinetic and equilibrium properties when compared to the corresponding 21*E*-isomers.[6,7] In a series of 11β-substituted estradiols the (11β*Z*)-iodovinylestradiol demonstrated higher RBA at 0 °C than the *E*-isomer and an enhancement of affinity under equilibrium conditions.[17] It is of note that both isomers had significantly lower RBA values than the unsubstituted 11β-vinylestradiol under both conditions.

Conclusions

The syntheses of these compounds completed the initial series of 11β-substituted (17α*E*/*Z*)-halovinylestradiols whose binding properties would illustrate the effects of the 11β- and 17α-groups on estrogen receptor binding. Previous studies had shown that small, lipophilic substituents substantially improved estrogen receptor binding, whereas small polar groups had the effect of reducing receptor affinity. The influence of the 17α-halovinyl group depended greatly on the stereochemistry of the double bond. The presence of the iodo group in the *Z*-configuration at the 17α-vinyl position and an 11β-substituent produced ligands with RBA values comparable to those of the 17α-unsubstituted compounds. The chloro derivatives were less effective in retaining this affinity. The (*E*)-halovinyl analogues possessed significantly lower affinities than the corresponding 17α-unsubstituted estrogens. In this subset, iodine was less effective in retaining the RBA property than chlorine. For the 11β,17α-disubstituted estrogens, the overall binding behavior is dependent upon the nature of the 11β-substituent, its stereochemistry of the halovinyl group, and the identity of the halide.

In combination with the recent report on the structure of hormone−ligand binding domain, these data provide additional information on the nature of estrogen−ER binding. The studies on the RXR and TR, as well as the ER, suggested a "mouse trap" model of ligand−receptor interaction in which there is considerable peptide movement following the initial association of the hormone ligand with its binding site.[1−3,18,19] The steps in this process are illustrated in Figure 3. The initial associative process may be relatively independent of the 17α-substituents but affected by the size and character of the 11β-substituent to yield intermediate **1**. This is consistent with interactions with the side chains of Leu-525 and -540 which may form a lipophilic pocket. As the receptor protein "molds" itself around the 11β-substituents, interactions at the 17α-position assume more importance. In assuming its ultimate binding conformation, the size, character, and orientation of the R_1, R_2 groups contribute to the interactions with the lipophilic interface of helices-7 and -11. Subtle differences affect the intensity of the intermolecular forces involved in the ligand binding process. Ultimately, the receptor accommodates the ligand in the observed structure−activity relationships, 11β-small lipophilic > small polar and 17α*Z*-substituents exert some influence on the others. This process also works for the 11β- or 17α-unsubstituted series as well. In the series examined here, all of the compounds are capable of associating easily with the unliganded binding site; however, only those with the lipophilic 11β-substituent and/or the (17α*Z*)-halovinyl group form more stable complexes than estradiol. These results suggest that the use of an appropriate 11β-substituent would permit the incorporation of new groups at the 17α-position that can access hither unexplored regions of the estrogen receptor ligand binding domain. Studies in this regard are currently underway.

Experimental Section

Melting points were determined with a Meltemp apparatus using open capillaries and are uncorrected. Flash chromatography was performed with silica gel according to the procedure of Still.[20] ^{1}H NMR spectra were obtained with a Varian XL 300 in the noted deuterated solvents, and chemical shifts are reported in ppm downfield from tetramethylsilane as an internal standard. Optical rotations were obtained with a Perkin-Elmer model 241 polarimeter. Infrared spectra were obtained with a Perkin-Elmer model 1600 FTIR. Thin-layer chromatography was performed on Bakerflex plastic-backed sheets (200-μm thickness). Mass spectra were run at 70 eV with a VG70SE double focusing mass spectrometer and are expressed as *m*/*z* (% base peak). All solvents and reagents were obtained as reagent grade and used without further purification. Elemental analyses (C_1H) were performed by

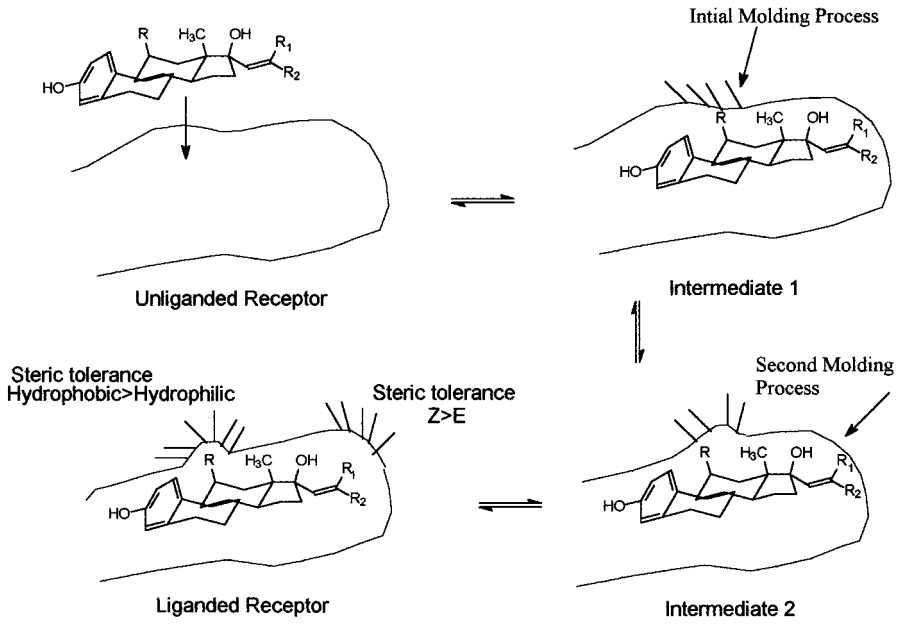


Figure 3. Hypothetical steps in the interaction of the substituted ligands with the steroid binding site of the estrogen receptor.

Atlantic Microlab, Inc. (Norcross, GA) and were within ±0.4% unless otherwise noted.

3-Acetoxy-11*β*-(1-ethenyl)-(17α)-19-norpregna-1,3,5(10)-trien-20-yn-17*β*-ol, 1c. To a solution of 11*β*-vinylestrone 3-acetate[11] (2.0 g, 5.9 mmol) in 20 mL of THF at −78 °C were added dropwise with stirring 15 mL of a 0.5 M solution of lithium trimethylsilylacetylide in THF. The resultant solution was warmed to −20 °C and stirred for 2 h. The reaction was terminated by the addition of ammonium acetate, the solvent was removed, and the resultant mixture was separated by column chromatography (silica gel, hexane:EtOAc, 2:1) to give the desired product (0.7 g), deacetylated product (0.7 g), and recovered starting material (0.65 g). The deacetylated product was converted to the desired product with acetyl chloride and triethylamine in dichloromethane. Removal of the trimethylsilyl group was accomplished by stirring a solution of the intermediate 17α-trimethylsilylacetylide (1 equiv), potassium fluoride (1.2 equiv), and benzoic acid (1.0 equiv in DMF:H_2O, 20:1) for 16 h at ambient temperature. The reaction mixture was partitioned between water and dichloromethane. The organic phase was washed with 10% sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. Chromatography on silica gel using hexane:ethyl acetate (2:1) as the eluent gave the product in a 85% isolated yield: mp 142−144 °C; NMR ($CDCl_3$) 0.92 (s, 3H, C_{18}−H), 1.22−2.08 (m, 8H), 2.27(s, 3H, CH_3CO), 2.22−2.39 (m, 2H), 2.56 (m, 1H), 2.63 (s, 1H), 2.75−2.95 (m, 2H), 3.39 (m, 1H, C_{11}−H), 4.95 (d, 1H, J = 10.5 Hz), 5.02 (d, 1H, J = 18.0 Hz), 5.70 (ddd, 1H, J = 7.5, 10.5, 18.0 Hz), 6.76−6.83 (m, 2H, C_2−H, C_4−H), 7.10 (d, 1H, J = 8.5 Hz, C−H).

General Procedures for Hydrostannation of 17α-Ethynylestradiol 3-Acetates 8a−c. Method A. A solution of the 17α-ethynylestradiol 3-acetate and tri-*n*-butylstannane (1:3 molar ratio) in THF was prepared under nitrogen in a sealed quartz tube. The portion of the tube containing the reaction solution was immersed in a water bath (50−56 °C). The reaction solution was magnetically stirred and simultaneously irradiated for 2.0 h with a UV sun lamp (GE, 275W). The reaction solution was removed from the radiation and cooled to ambient temperature. The solvent was removed, and the reaction mixture was purified by column chromatography using hexane:ethyl acetate (6:1) as the eluent to give the (17α-*E*)-tri-*n*-butylstannyl isomers **9a−c** as the major product (80−90% isolated yield).

Method B. A solution of the 17α-ethynylestradiol 3-acetate and tri-*n*-butylstannane (1:1.5 molar ratio) in THF was sealed in an ampule and heated at 60 °C for 2 h. The solvent was removed, and unreacted starting material was removed by trituration with hexane. The filtrate was applied to a chromatographic column (silica gel) and eluted with hexane:ethyl acetate (6:1). This procedure gave a 40−45% yield of the (*Z*)-tri-*n*-butylstannylvinyl products **10a−c**, 5−10% of the *E*-isomers **9a−c**, and 50% recovery of starting materials **8a−c**.

Method C. To a solution of the 17α-ethynylestradiol 3-acetate and tri-*n*-butylstannane (1:1.5−3.0 molar ratio) in THF at ambient temperature was added 0.1 equiv of triethylborane in THF. The reaction mixture was stirred, and the course of the reaction was followed by TLC on silica gel using hexane:ethyl acetate (4:1) as the eluent. When the product ratio reached the targeted range, the reaction solution was evaporated to dryness, applied to a chromatographic column (silica gel), and eluted with hexane:ethyl acetate (6:1). Depending upon reactant ratios and reaction times, this procedure would produce *Z*:*E* ratios ranging from 10:1 to 1:10, thereby providing the requisite quantities of targeted isomers. The isolated yields of hydrostannated products ranged from 45% for *Z*:*E* = 10:1 to 95% for *Z*:*E* = 1:10 with the remainder being unreacted alkyne.

General Conditions for Chlorodestannylation. Method D. To a solution of the (17α*E*/*Z*)-tri-*n*-butylstannylvinylestradiol 3-acetate (0.2 mmol) in hexane (10 mL) stirred at −78 °C was added chlorine gas (4.5 mL, 0.2 mmol). To the reaction solution was added a few drops of cyclohexane, and the solution was slowly brought to ambient temperature. The solvent was removed by rotary evaporation, and the residue was dissolved in methanol (10 mL) containing sodium hydroxide (0.2 mL of a 10 N aqueous solution). After stirring for 5 min, the solution was acidified by the dropwise addition of glacial acetic acid and partitioned between ethyl acetate and water. The organic phase was washed with sodium bicarbonate (10%), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The resulting residue was then purified by column chromatography using hexanes:ethyl acetate (3:1) as the eluent to give the desired (17α*E*/*Z*)-chlorovinylestradiol in an isolated yield of 85−95%.

General Conditions for Iododestannylation. Method E. To a solution of the (17α*E*/*Z*)-tri-*n*-butylstannylvinylestradiol 3-acetate (0.2 mmol) in carbon tetrachloride (10 mL) stirred at −18 °C was added a solution of iodine (0.2 mmol) in carbon tetrachloride (5 mL). The reaction solution was warmed to ambient temperature and then evaporated to dryness. The residue was dissolved in methanol (10 mL) containing sodium hydroxide (0.2 mL of a 10 N aqueous solution). After stirring for 5 min, the solution was acidified by the addition of glacial acetic acid and partitioned between ethyl acetate and water. The organic phase was washed with

sodium bicarbonate (10%), dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The resulting residue was purified by column chromatography on silica gel using hexanes:ethyl acetate (3:1) as the eluent to give the desired (17α*E*/*Z*)-iodovinylestradiol in an isolated yield of 85−95%.

3-**Acetoxy-11*β*-(1-ethenyl)-21-tri-*n*-butylstannyl-(17α, 20*E*)-19-norpregna-1,3,5(10),20-tetraen-17*β*-ol, 9a.** This product was prepared from **8c** using method A and was isolated as an oil: NMR ($CDCl_3$) 0.80−2.10 (m, 41H), 2.26 (s, 3H, CH_3−CO−), 2.42 (dd, 1H, J = 4.0, 10.0 Hz), 2.74−2.96 (m, 2H), 3.30 (m, 1H, C_{11}−H), 4.92 (d, 1H, J = 10.5 Hz), 4.98 (d, 1H, J = 18.0 Hz), 5.69 (d, dd,1H, J = 7.5, 10.5, 18.0 Hz), 6.06 (d, 1H, J = 21 Hz), 6.20 (d, 1H, J = 21 Hz), 6.72−6.88 (m, 2H, C_2−H, C_4−H), 7.06 (d, 1H, J = 8.5 Hz, C_1−H).

3-Acetoxy-11*β*-(1-ethenyl)-21-tri-*n*-butystannyl-(17α, 20*Z*)-19-norpregna-1,3,5(10),20-tetraen-17*β*-ol, 10a. The product was prepared from **8c** using method B and was isolated as an oil: NMR ($CDCl_3$) 0.70−2.05 (m, 40H), 2.26 (s, 3H, CH_3CO−), 2.50 (dd, 1H, J = 3.5, Hz, 11.0 Hz), 2.74−2.94 (m, 2H), 3.36 (m, 1H, C_{11}−H), 4.76 (d, 1H, J = 18.0 Hz), 4.93 (d, 1H, J = 10.5 Hz), 5.69 (ddd, 1H, J = 7.5, 10.5, 18.0 Hz), 5.87 (d, 1H, J = 13.0 Hz), 6.72−6.83 (m, 3H, C_2−H, C_4−H, CH=CHSn), 7.08 (d, 1H, J = 8.5 Hz, C_1−H).

11*β*-Methoxy-21-chloro-(17α,20*E*)-19-norpregna-1,3,5-(10),20-tetraene-3,17*β*-diol, 4b: mp 196−198 °C dec; $[\alpha]^{25}_D$ +39.2 ($c = 0.77 \times 10^{-3}$ ethyl acetate); R_f = 0.42 (Hex:EtOAc, 1:1); NMR ($CDCl_3$, DMSO-d_6) 1.17 (s, 3H, C_{18}−H), 1.20−2.05 (m, 8H), 2.10 (d, 1H, J = 1.5 Hz), 2.17 (dd, 1H, J = 2.5, 15.0 Hz), 2.35 (bd, 2H, J = 11 Hz), 2.44 (d, 1H, J = 2.5 Hz), 2.68−2.90 (m, 2H), 3.27 (s, 3H), 4.12 (m, 1H, C_{11}−H), 6.16 (d, 1H, J = 16.5 Hz), 6.23 (d, 1H, J = 16.5 Hz), 6.55 (d, 1H, J = 2.5 Hz, C_4−1H), 6.65 (dd, 1H, J = 2.5, 8.0 Hz, C_2-H), 6.95 (d, 1H, J = 8.0 Hz, C_1−H). Anal. $C_{21}H_{27}ClO_3$: C, H. MS: (EI) 362 (50), 330 (35), 226 (100); (CI) 363 (65), 331 (95), 313 (100).

11*β*-(1-Ethenyl)-21-chloro-(17α,20*E*)-19-norpregna-1,3,5-(10),20-tetraene-3,1*β*-diol,4c: mp 150−152 °C dec; $[\alpha]^{25}_D$ +9.3 ($c = 0.65 \times 10^{-3}$, ethyl acetate); R_f = 0.27 (Hex:EtOAc, 3:1); NMR ($CDCl_3$, DMSO-d_6) 0.95 (s, 3H, C_{18}−H), 1.20−2.25 (m, 13H), 2.44 (dd, 1H, J = 5.0, 10.0 Hz), 2.55 (bs, 1H), 2.66−2.90 (m, 2H), 3.78 (m, 1H, C_{12}H), 4.91 (d, 1H, J = 10 Hz), 4.98 (d, 1H, J = 18 Hz), 5.70 (dd, 1H, J = 10.5, 1.8 Hz), 6.20 (s, 2H), 6.55 (d, 1H, J = 2.5 Hz C_4−H), 6.60 (dd, 1H, J = 2.5, 8.5 Hz, C_2−H), 6.89 (d, 1H, J = 8.5 Hz, C_1−H), 7.75 (bs, 1H). Anal. $C_{22}H_{27}ClO_2$: C, H. MS: (EI) 358 (45), 340 (100): (CI) 359 (80), 341 (100).

11*β*-(1-Ethenyl)-21-iodo-(17α,20*E*)-19-norpregna-1,3,5-(10),20-tetraene-3,17*β*-diol, 5c: mp 136−130 °C dec; $[\alpha]^{25}_D$ −32.8 ($c = 0.94 \times 10^{-3}$, ethyl acetate); R_f = 0.21 (Hex:EtOAc, 3:1); NMR ($CDCl_3$, DMSO-d_6) 0.95 (s, 3H, C_{18}−H), 1.20−2.05 (m, 14H), 2.45 (m, 1H), 2.70−2.90 (m, 2H), 3.32 (m, 1H, C_{11}−H), 4.92−5.03 (m, 2H), 5.68 (ddd, 1H, J = 7.5, 10.5, 18.0 Hz), 6.30 (d, 1H, J = 14.5 Hz), 6.53 (d, 1H, J = 2.5 Hz, C_4−H), 6.58 (dd, 1H, J = 2.5, 8.5 Hz, C_2−H), 6.78 (d, 1H, J = 14.5 Hz), 6.99 (d, 1H, J = 8.5 Hz, C_1−H). Anal. $C_{22}H_{27}IO_2$: C, H. MS: (EI) 450 (90), 432 (100), 323 (85); (CI) 451 (50), 433 (100), 323 (45).

11*β*-Methoxy-21-chloro-(17α, 20*Z*)-19-norpregna-1,3,5-(10),20-tetraene-3,17*β*-diol, 6b: mp 190−192 °C dec; $[\alpha]^{25}_D$ +81.20 ($c = 0.25 \times 10^{-3}$, ethyl acetate); R_f = 0.25 (Hex:EtOAc, 3:1); NMR ($CDCl_3$, DMSO-d_6) 1.14 (s, 3H, C_{18}−H), 1.20−2.37 (m, 14H), 2.66−2.70 (m, 2H), 3.27 (s, 3H, OCH_3), 4.15 (m, 1H, C_{11}−H), 5.99 (d, 1H, J = 9 Hz), 6.17 (d, 1H, J = 9 Hz), 6.55 (d, 1H, J = 2.5 Hz, C_4−H), 6.66 (dd, 1H, J = 2.5, 8.5 Hz, C_2−H), 6.96 (d, 1H, J = 8.5 Hz, C_2−H). Anal. $C_{21}H_{27}ClO_3$: C, H. MS: (EI) 362 (25), 330 (20), 226 (100); (CI) 363 (40), 331 (50), 313 (100).

11*β*-(1-Ethenyl)-21-chloro-(17α,20*Z*)-19-norpregna-1,3,5-(10),20-tetraene-3,17*β*-diol, 6c: mp 94−96 °C; $[\alpha]^{25}_D$ +52.0 ($c = 0.71 \times 10^{-3}$, ethyl acetate); R_f = 0.25 (Hex:EtOAc, 3:1); NMR ($CDCl_3$, DMSO-d_6) 0.98 (s, 3H, C_{18}H), 1.22−2.36 (m, 13H), 2.44 (dd, 1H, J = 4.5, 12 Hz), 2.70−2.90 (m, 3H), 3.33 (m, 1H, C_{11}H), 4.60 (s, 1H), 4.95 (dd, 1H, J = 3.0, 10.5 Hz), 5.00 (dd, 1H, J = 3.0, 18.9 Hz), 5.71 (ddd, 1H, J = 7.5, 10.5, 18.0 Hz), 6.02 (d, 1H, J = 8.0 Hz), 6.17 (d, 1H, J = 8. Hz), 6.53 (d, 1H, J = 2.5 Hz, C_4H), 6.59 (dd, 1H, J = 2.5, 8.5 Hz, C_2H), 6.95 (d, 1H, J = 8.5 Hz, C_1H). Anal. $C_{22}H_{27}ClO_2$: C, H. MS: (EI) 359 (60), 341 (100), 325 (70), 397 (90); (CI) 358 (80), 239 (80), 226 (70), 200 (80), 146 (100).

11*β*-(1-Ethenyl)-21-iodo-(17α,20*Z*)-19-norpregna-1,3,5-(10),20-tetraene-3,17*β*-diol, 7c: glass; $[\alpha]^{25}_D$ +61.92 ($c = 0.65 \times 10^{-3}$, ethyl acetate) R_f = 0.29 (Hex:EtOAC, 3:1); NMR ($CDCl_3$, DMSO-d_6) 0.99 (s, 3H, C_{18}H), 1.20−2.22 (m, 13H), 2.38−2.50 (m, 2H), 2.64−2.91 (m, 2H), 3.33 (m, 1H, C_{11}H), 4.75 (s, 1H), 4.95 (dd, 1H, J = 1.5, 10.5 Hz), 5.02 (dd, 1H, J = 1.5, 17.5 Hz), 5.71 (ddd, 1H, J = 2.0, 10.5, 17.5 Hz), 6.40 (d, 1H, J = 8.5 Hz), 6.54 (d, 1H, J = 2.5 Hz, C_4H), 6.57 (dd, 1H, J = 2.5, 8.5 Hz, C_2H), 6.85 (d, 1H, J = 8.5 Hz), 6.95 (d, 1H, J = 8.5 Hz, C_1H). Anal. $C_{22}H_{27}IO_2$: C,H. MS: (EI) 450 (50), 432 (100); (CI) 451 (30), 433 (100).

Competitive Receptor Binding Assay. All cytosol for the estrogen receptor were prepared and stored in TEA buffer (0.01 M Tris-HCl, 0.0015 M EDTA, 0.02% sodium azide, pH 7.4 at 25 °C). Lamb uterine cytosol was prepared and stored as described by Katzenellenbogen et al.[15] The competitive receptor binding assays were performed as previously described by Katzenellenbogen et al.,[15,16] and the results were tabulated as relative binding affinities (RBA) relative to estradiol (RBA = 100).

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