Synthesis and Evaluation of (17α,20*Z*)-21-(4-Substituted-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diols as Ligands for the Estrogen Receptor-α Hormone Binding Domain: Comparison with 20*E*-Isomers

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As part of our ongoing program to develop probes for the hormone binding domain of the estrogen receptor-α (ERα), we prepared and evaluated a series of 17α,*Z*-(4-substituted-phenyl)-vinyl estradiol derivatives. The results indicated that the relative binding affinities (RBAs) at 25 °C for the new compounds were significant (RBA = 9−57) although less than that of estradiol (RBA = 100) or of the parent unsubstituted phenylvinyl estradiol (RBA = 66). All of the *Z*-compounds were full agonists in the uterotrophic assay, indicating that the ligands formed estrogen-like complexes with the estrogen receptor-α hormone binding domain (ERα-HBD). Comparison of corresponding *Z*- and *E*-4-substituted phenylvinyl ligands complexed with the ERα-HBD indicated small but significant differences in binding modes that may account for the differing trends seen in the structure−activity relationships for the two series.

Introduction

The estrogen receptor (ER), a member of the nuclear receptor (NR) superfamily, regulates a wide range of developmental and physiological responses including reproductive functions, cardiovascular modulation, and bone density.[1−4] This receptor, similar to the other NRs, is characterized by a distinctive structural homology consisting of six domains (A−F) that include the N-terminal transcription activation function-1 (AF-1) domain, a DNA-binding domain (DBD), a hinge region, a hormone-binding domain (HBD), and a transcription activation function-2 (AF-2) domain. Sequencing and cloning studies revealed that the DBD utilized two zinc fingers to recognize the cognate portion of the ER-response element (ERE), while selective components of the HBD provided the binding site for the hormone. Subsequent studies have generated an increasingly complex picture of how the interactions of hormone agonists and antagonists with the ER initiate intracellular events that eventually express the pharmacological response.[5−8] Differential expression of ERα and ER*β* subtypes, coupled with variable coactivator or corepressor proteins, may contribute to the explanation of how the same hormonal ligand can elicit a variety of effects in different tissues. These newer models of estrogen action help to understand the roles of the hormone in pathological states, such as carcinoma of the breast, in which cellular growth and differentiation have been altered.[9,10]

Our research program has focused on the development of novel estradiol derivatives, both as probes for the estrogen receptor hormone binding domain (ERα/*β*-HBD) and as potential therapeutic agents for the treatment of hormone-responsive disorders, including breast cancer. Observations that ERα is overexpressed in most breast cancers (>75%) and that activation of that receptor stimulates breast cancer cell proliferation strongly suggested that agents that selectively block hormone-initiated processes would have significant therapeutic potential.[11−13] Other investigators have described efforts to develop selective estrogen receptor modulators (SERMs) as potential chemotherapeutic agents.[14−22] Additional studies[23,24] highlighting the linkage between the estrogen receptor and tumor progression have made identification of new estrogen receptor ligands that clarify the activation process an important research target.

Our previous publications in the field described the identification of the 17α-phenyl-X-vinyl or halovinyl estradiols as potential ER ligands and synthetic methods for their preparation.[25−29] Subsequent studies suggested that modifications on structurally similar hormone receptor ligands may elicit subtle changes in receptor conformations that modulate the biological responses of these compounds.[30−34] Our recent evaluation of the series of 17α,*E*-(2-, 3-, 4-trifluoromethylphenylvinyl) estradiols in the uterotrophic assay indicated a range of estrogenic potencies that was only partially accounted for by the relative binding affinities (RBAs)[35] (Figure 1). On the basis of these observations, we prepared a series of new ER ligands encompassing a number of 17α,*E*-(4-substituted-phenyl)vinyl estradiols as probes for the interactions between the ligand and binding site of the ERα-HBD (Figure 2).[36] Understanding those interactions would allow us to design and prepare compounds having higher affinity for the receptor, possessing selectivity for specific subtype and

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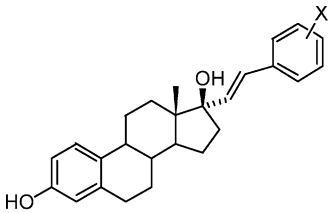

Figure 1. Phenyl ring isomers.

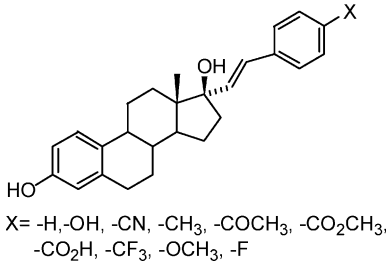

Figure 2. Para substitution on phenyl ring.

expressing a more appropriate transcriptional response. The results from this work described the synthetic feasibility of Pd(0)-catalyzed reactions. The molecular modeling methods used for the docking studies suggested that the steroidal portion of the compounds bound in a manner similar to estradiol in the published crystal structures. However, the 17α-substituent occupied a space bounded by phenylalanine-525 and three methionine residues, a region not normally accessed by other steroidal and nonsteroidal ligands. This docking process generated calculated binding energies that gave a strong correlation with the observed RBA values. In this study we report the extension of the synthetic methods to the preparation of 17α,*Z*-(4-substituted-phenyl)vinyl estradiols, their evaluation as estrogenic agents, and the use of molecular modeling to describe their use as probes for the ERα-HBD. As the results indicate, these compounds are potent estrogenic agents with structure−activity relationships that differ from those of 17α,*E*-analogues. This difference can be explained by an alternative binding mode as determined by docking studies.

Results

Synthesis of Estrogenic Ligands. The target compounds selected in this study were the *Z*-isomers of the 17α,*E*-(4-substituted-phenyl)vinyl estradiols from the previous series.[36] We had observed in earlier studies that synthesis of the *Z*-phenylvinyl estradiols was more complex that of the *E*-isomers. The requisite *Z*-tri-*n*-butylstannylvinyl estradiol was more difficult to obtain in high yields and, being the thermodynamically less favored product, was also much more prone to proteolytic decomposition. Therefore, different synthetic strategies were used to prepare the target compounds (Scheme 1).

The initial approach, method A, was a variation of that used for the *E*-isomers. Hydrostannation of ethynyl estradiol 3-acetate **1** with tri-*n*-butyltin hydride using triethylborane as the radical initiator gave reasonable yields of the *Z*-vinylstannane **2** when tin/alkyne ratios were low (<2), when the reaction was run at ambient temperature, and when the reaction time was relatively short (<4 h). The isolated yields for **2** were 30−40%, with the *E*-isomer **3** being present in 5−15% yields. The remaining material under these conditions was mostly unreacted ethynyl estradiol 3-acetate **1** accompanied by small amounts of the proteodestannylated **4**. Efforts to increase the conversion to the *Z*-isomer by modifying this method gave higher yields of the *E*-isomer. Although there are other literature methods for preparing *Z*-vinylstannanes,[37,38] we did not investigate them because we could obtain sufficient quantities of pure intermediate for our immediate studies.

The *Z*-stannylvinyl estradiol 3-acetate **2** was then coupled to the corresponding aryl iodide or bromide using the Stille coupling conditions.[36] As the results in Table 1 indicate, yields of the *Z*-phenylvinyl estradiol 3-acetates **5a**,**c**−**h** were low using this method (2−35%). Under these conditions some starting material **2** was recovered and the coupled products accounted for only a small percentage of the reaction mixture. The major product was the protiodestannylated vinyl estradiol 3-acetate **4**. The coupled intermediates **5** were hydrolyzed with sodium hydroxide−methanol to give the target 17α,*Z*-(4-substituted-phenyl)vinyl estradiols **6a**,**c**−**h**, which were characterized by ^{1}H and ^{13}C NMR and elemental analysis. The *Z*-stereochemistry was established by ^{1}H NMR, where the coupling constant for the vinylic protons was $J = 12-13$ Hz. No isomerization of products was noted during the reaction or purification steps.

The low yields and difficulties encountered in separation led us to examine alternative synthetic methods. We previously reported the essentially quantitative conversion of the 17α,*E*- and *Z*-stannylvinyl estradiols to the corresponding 17α,*E*- and *Z*-iodovinyl estradiols.[25,29] We anticipated that *Z*-iodovinyl estradiol **7** would then participate in the reverse coupling reaction with either the arylstannane (reverse Stille coupling, method B) or arylboronic acid (Suzuki coupling, method C). Although iododestannylation proceeded quantitatively, the product **7** proved to be photosensitive, requiring protection against ambient exposure to light. The iodovinyl estradiol **7** was purified by flash chromatography and used immediately with the arylstannane or arylboronic acid. One reverse Stille coupling with the 4-tributylstannylacetophenone was performed to demonstrate the feasibility of this method. Although arylboronic acids are more readily available and more stable than the stannanes, only one Suzuki coupling was performed with 4-fluorophenylboronic acid. Yields of the *Z*-phenylvinyl estradiol 3-acetates **5b**,**i** were 34% and 52%, respectively, although the vinyl estradiol **4** was still a significant component of the reaction mixture. The intermediates were hydrolyzed to remove the 3-acetyl group, and the products **6b**,**i** were characterized by NMR spectrometry and elemental analysis.

Relative Binding Affinities. Our evaluative strategy involved an initial screening of the new compounds and standard ligands for their relative binding affinities (RBAs) at 4 and 25 °C using the ERα-HBD isolated from the transfected BL21 cells.[39] Competitive radiometric binding assays using this protein have provided reproducible RBA values for ERα-HBD. Ligands that demonstrate significant affinity (RBA > 10) for the ER-HBD

Scheme 1. Synthesis of *Z*-17α-(4-Substituted-phenyl)vinyl Estradiols

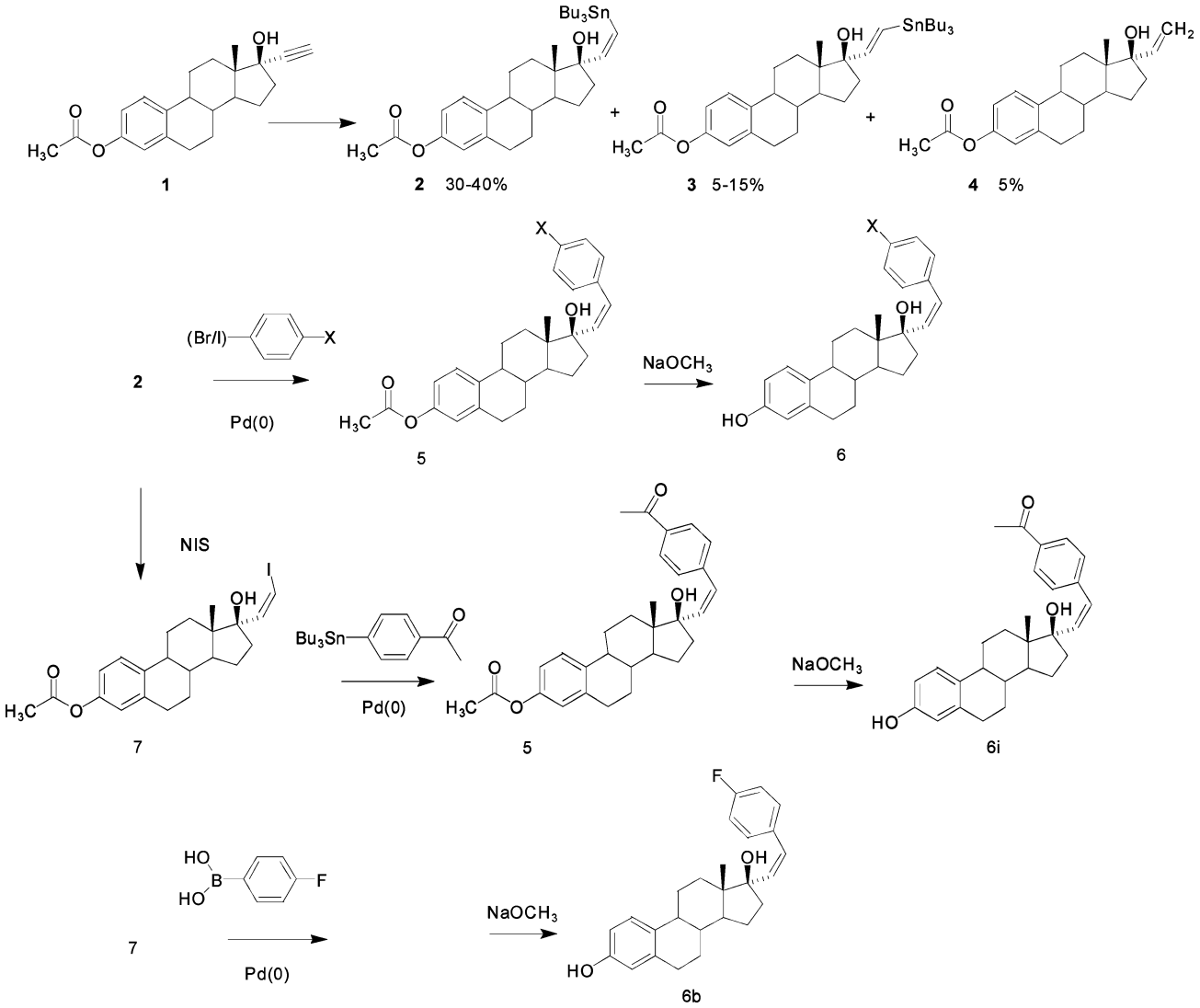

Table 1. Yields of 17α-*Z*-(4-Substituted-phenyl)vinyl Estradiol 3-Acetates and 3-OH Products

X	yield of -3-acetate, %	yield of -3-OH, %
H	10	89
F	44	31
OH	10	55
CN	2	90
CH_3	32	44
OCH_3	25	94
NH_2	35	68
$COCH_3$	5	51

would subsequently be evaluated for efficacy in the in vivo rat uterotrophic growth assay.[40] The products were compared to both estradiol and the unsubstituted phenylvinyl estradiol **6a** using this assay in order to evaluate the effects of substituents on both the basic steroidal interactions and on the phenylvinyl group. The results of the competitive binding assays, shown in Table 2, indicated that all of the compounds retained significant affinity for the estrogen receptor. In most cases, the differences between the 4 °C RBA values (kinetic) and the 25 °C RBA values (equilibrium) were not dramatic.[41] RBA values for the corresponding *E*-isomers[36] are included for comparison purposes.

As previously noted, the introduction of the *Z*-phenylvinyl group at the 17α-position of estradiol

Table 2. Relative Binding Affinity for 17α-*Z*-(4-Substitituted-phenyl)vinyl Estradiols

		RBA, %			
compd	X	at 4 °C for *Z*	at 4 °C for *E*	at 25 °C for *Z*	at 25 °C for *E*
6a	H	57	16	66	9
6b	F	43	24	44	22
6c	OH	12	21	25	25
6d	CN	20	9	12	27
6e	CH_3	10	10	9	18
6f	CF_3	5	5	9	8
6g	OCH_3	20	36	39	32
6h	NH_2	22		26	
6i	$COCH_3$	22	53	29	60

results in a modest reduction in the relative binding affinity at 4 and 25 °C (RBA = 57%, 66%) while the *E*-isomer produces a more significant decrease (RBA = 16%, 9%). Introduction of substituents at the para position of the phenyl ring leads to a further reduction in the RBA values. Compounds **6d**−**f** have only $^1/_8$ to $^1/_6$ the binding affinity (RBA = 9−12%) of the unsubstituted compound, while compounds **6c**,**h**,**i** are approximately 30−40% as effective as **6a** as estrogen receptor ligands. Only two compounds in this series, **6b** and **6g**, had affinities (RBA = 39−57) greater than half of the parent compound, and none were more potent.

Table 3. Uterotrophic Growth Assay for Selected *Z*- and *E*-Isomers

	ED_{50},[a] nmol	
R	for *Z*	for *E*
CH_3	4.2	5.5
CF_3	1.1	10.6
F	2.3	72

[a] ED_{50}: dose equal to 50% response of 10 nmol estradiol. ED_{50}(estradiol) = 0.06 nmol.

This is in contrast to the *E*-(4-substituted-phenyl)vinyl estradiol series in which almost every compound had an RBA value greater than that of the unsubstituted parent compound. In that series the best derivatives were 3−6 times more effective than the parent compound. Therefore, while there is a general similarity between the two series of compounds, i.e., the individual RBA values rarely differed by more than a factor of 2, there exist significant differences in ERα-HBD binding that require reconciliation.

Uterotrophic Growth Assays. The second component of our biological evaluation process involved assaying the compounds possessing significant RBA values for estrogenic efficacy. Initially we had selected RBA > 10 as the criterion for in vivo evaluation; however, because all of the compounds had appropriate binding values, we elected to completely evaluate only three compounds from each series for this study. The immature rat uterotrophic growth assay is well-established for demonstrating estrogenic and antiestrogenic responses mediated though ERα.[40] Although reporter assays have been developed more recently to define estrogenic activity, they were not available at the time to assay this series of compounds. Three compounds in each series were evaluated over a 4 log dose range (0.01−100 nmol) for their uterotrophic potency. All of the compounds in the series were full agonists and at the highest tested doses yielded uterotrophic growth that was comparable to 10 nmol of estradiol. Therefore, the complex that the compounds formed with the intact receptor was competent to elicit a full agonist response. The results for the uterotrophic assay are shown in Table 3. Although the compounds are significantly less potent than estradiol in this assay, the results indicate two trends. First, the *Z*-isomers are generally more potent than the *E*-isomers. Compounds **6b** and **6f** are approximately 30 and 10 times more potent than the corresponding *E*-isomer. The *Z*- and *E*-4-methylphenylvinyl estradiols are essentially equipotent in this assay (4.2 vs 5.5 nmol). The second trend is that there exists essentially no obvious correlation between the RBA values and the ED_{50} values obtained in this study. The *Z*-4-methyl and 4-fluoro compounds had higher RBA values than the *Z*-4-trifluoromethyl ligand but expressed roughly $^1/_2$ to $^1/_4$ the in vivo potency. The same absence of a correlation between the in vitro binding affinity and in vivo uterotrophic growth potency was also observed for the *E*-4-fluoro and *E*-4-trifluoromethyl compounds.

Molecular Modeling. The objectives of the molecular modeling component of the study were threefold. The first aim was to determine the preferred ligand−HBD conformation compared to estradiol. Then, on the basis of that binding mode, the effect of the 4-substituent could be evaluated. Third, we would compare the binding interactions observed with the *Z*-isomers to those predicted for the corresponding *E*-phenylvinyl isomers. We used a molecular modeling approach similar to that described in our earlier study[36] to evaluate the interaction of the new probes with the ERα-HBD and explain the observed biological effects. On the basis of the known pharmacology of the *Z*-isomers, we selected the estradiol−ERα-HBD complex (PDB code 1G50)[42] for which the coordinates were readily available. The estradiol−ERα-HBD monomer was optimized using molecular mechanics methods and then was used for the binding studies. Our ligands were superimposed on the estradiol structure (steroidal components), the estradiol was deleted, and the new complex was subjected to a docking procedure previously described. Best docking poses, selected from the docking study, and additional poses, where the steroidal skeleton was superimposed on estradiol and the phenylvinyl moiety had different starting conformations, were optimized using simulated annealing procedures. Alternative modes in which the molecules were rotated around the 3−17 axis were also examined.

The binding mode in which the steroid maintained the "standard" orientation and the dihedral angle between it and the vinyl group was about −80° had the lowest binding energy (Figure 3). In this ligand−ERα-HBD conformation, the steroidal portion of the ligand occupies essentially the same position as estradiol, only translated by about 1.4 Å toward Glu-353 and Arg-394 at the end of the binding pocket. This shift increases the distance between the 17-OH and His-524, possibly reducing the hydrogen-bonding effect at that site. The phenyl ring is located in a hydrophobic pocket on the α-face of the steroidal C,D-region, bounded by two phenylalanine residues (-404 and -425), two leucines (-391 and -402), isoleucine-424, and methionine-421. This pocket is formed by the conjunction of the β sheet, helices 3, 5, 6, 7 and the 6−7 loop. The leucines in particular provide a cap to that portion of the binding pocket juxtaposed to the para position of the phenyl group. Therefore, we examined the influence of the 4-substituent on the ligand−receptor binding. Although the 4-substituent had little effect on the ultimate conformation of the ligand−HBD complex, interactions between the 4-substituent and the leucine side chains were observed. Even though all of the substituents evaluated were sterically undemanding, still some movement of the receptor side chains was required to generate optimal binding.

Initial efforts to obtain a direct correlation between the calculated binding energy and the observed RBA values were not as successful as for the *E*-isomers ($r^2 = 0.83$); nevertheless, a general trend existed indicating that lower protein flexibility at this site led to lower binding affinity (Figure 4A). However, when the protein energy was factored in, the correlation between the RBA values and the calculated binding energy was more significant ($r^2 = 0.96$) (Figure 4B).

Because the correlations for this series were different from those observed for the *E*-isomers, we compared the two predicted binding modes. For this study we selected the 4-fluorophenylvinyl estradiol isomers in their most probable binding poses. The protein backbones were superimposed, and residues that exhibited essentially

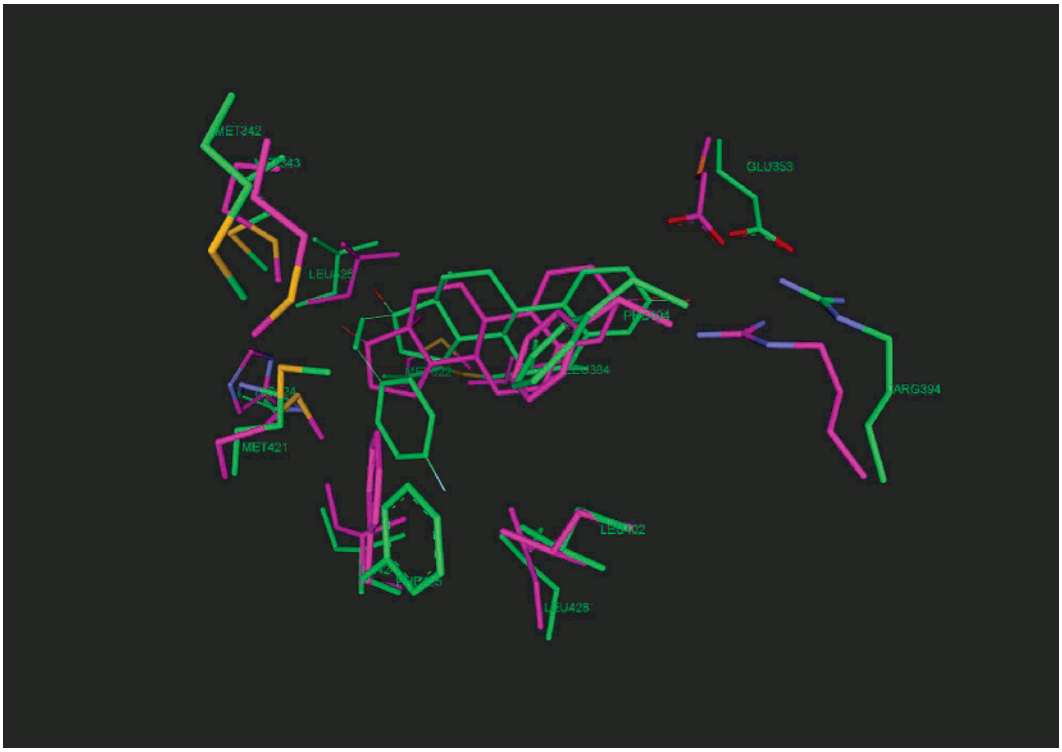

Figure 3. Docking of *Z*-4-fluorophenylvinyl estradiol (green) and estradiol (purple) in ERα-LBD. An approximate 1 Å translation of the steroid ring yields movement of Arg-394 and Glu-353 but permits positioning of substituted phenylvinyl group in a hydrophobic pocket bounded by Phe-, Leu-428, Leu-402 residues.

no deviation were removed (Figure 5). The *Z*-4-fluorophenylvinyl group occupies a hydrophobic pocket on the α-face of the ligand binding pocket, roughly in the same region as that observed for the *E*-4-fluorophenylvinyl estradiol. However, the identity of the amino acids lining the binding pocket for the 4-fluorophenylvinyl group is different for each isomer. The *Z*-stereochemistry, coupled with the torsional rotation around the 17α-vinyl bond, forces the phenyl ring into a binding pocket bounded by Phe-404 and -425, Leu-402 and -391, Ile-424, and Met-421, as opposed to the Phe-425, Met-341, -342, and -421, Leu-346 and -410, and Val-418 grouping previously identified for the *E*-isomers. Although the steroidal portions of the two series occupy similar domains, having a number of residues in common, the environment for the terminal phenyl rings is significantly different. For example, the degree of interaction with Met-421 is different for the two isomers and the pocket termini are different.

Discussion

Chemistry. The choice of *Z*-(4-substituted-phenyl)-vinyl estradiols was based on the biological activity expressed by the corresponding *Z*-halovinyl and *Z*-phenyl(seleno or thio)vinyl estradiols. However, the chemistry of C−C coupling reactions with *Z*-vinylstannanes, especially functionalized vinylstannanes is less well-described than for *E*-vinylstannanes. Our studies were hampered by the fact that hydrostannation under radical conditions gave the *Z*-isomer as the minor product unless the time, temperature, and stoichiometry of the reaction were carefully controlled. Under kinetically dominating conditions we were able to isolate the *Z*-vinylstannane as the major product although in modest yields. The second problem involved the appropriate coupling conditions that were developed for the more stable *E*-vinylstannanes. The use of the standard Pd(0) catalyst [tetrakis(triphenylphosphine)-palladium(0)] to provide the *Z*-coupled materials (2−35% isolated yields) required reaction conditions, usually elevated temperatures, that gave the protiodestannylated material **4** and other unidentified compounds as the major products. Recent studies of Pd(0)-catalyzed coupling reactions suggest that more sterically hindered, electron-rich phosphines may be even better Pd(0) ligands.[43,44] These catalysts are compatible with aryl chlorides, opening this methodology to a wider variety of substrates. We also were able to demonstrate that the target compounds were accessible by alternative means. Stille coupling in the reverse fashion (method B), with vinyl iodide and arylstannane, was also successful as was the Suzuki coupling (method C) with the vinyl iodide and arylboronic acid. Substituted arylstannanes and boronic acids are commercially available and therefore also provide access to many substituted phenylvinyl estradiols. However, should more highly substituted phenylvinyl derivatives become necessary, methods B and C would be limited by the availability or synthesis of the requisite arylstannanes and boronic acids. Efforts to improve coupling yields remain to be done, but the concept is valid. As in the case of the *E*-isomers, saponification of the 3-acetate intermediates was uneventful and gave the final products in yields of 31−94%. The *Z*-stereochemistry of the olefin was established by ^{1}H NMR, which gave a coupling constant of J = 12.5−13.5 Hz.

Biological Evaluation. The results of the biological assays indicated that the *Z*-(4-substituted-phenyl)vinyl estradiol derivatives that we prepared were potent estrogenic ligands both in vitro and in vivo. The range

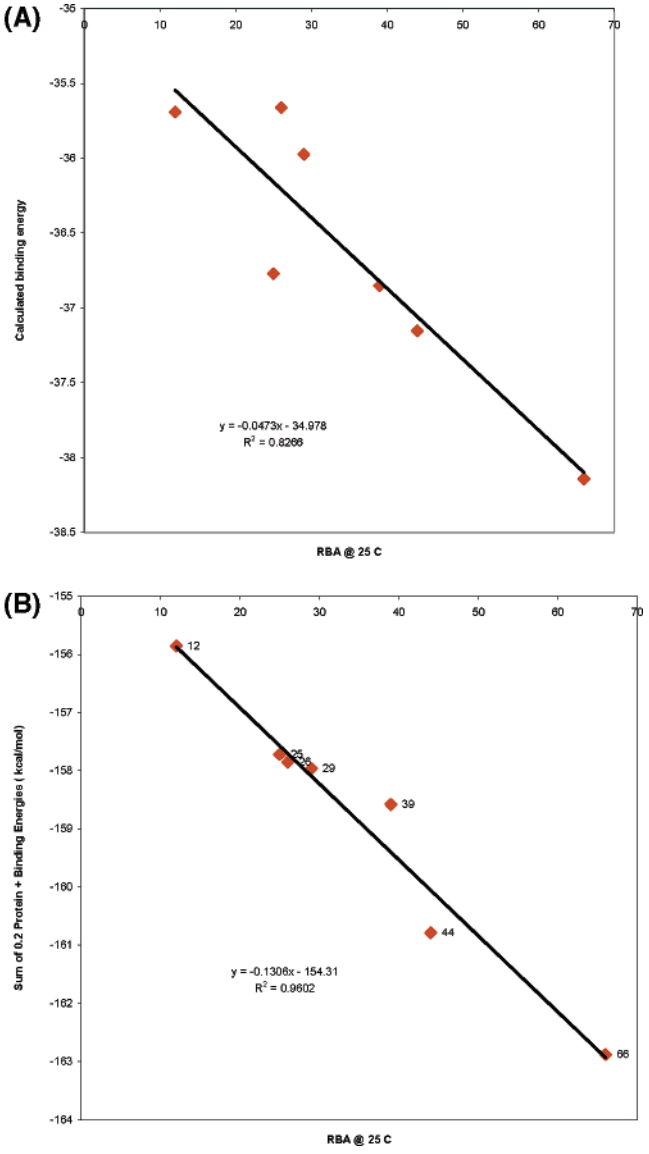

Figure 4. (A) Calculated binding energy vs RBA at 25 °C without *p*-CH_3. (B) Sum of (0.2)(protein) plus binding energies vs RBA at 25 °C without *p*-CH_3.

of RBAs at 25 °C was from 9 to 66, and these values were, except for **6d** (X = CN), equal to or greater than the values obtained at 4 °C. Although there was a variation in the order of potencies, the binding behavior was generally similar to that expressed by the corresponding *E*-isomers (Table 2) Where differences existed, e.g., X = F, CN, CH_3, and $COCH_3$, the magnitude was usually less than a factor of 2. The data suggested that the compounds may interact with the receptor in similar binding modes.

On the basis of the in vitro data, three pairs of compounds from the *Z*- and *E*-series were selected for evaluation in the immature female rat uterotrophic growth assay. In this case, there was no obvious direct relationship between the RBAs and the in vivo response. Comparing the corresponding *E*- and *Z*-phenylvinyl isomers is illustrative. Both 4-fluorophenyl isomers are potent in vitro ER-HBD binding agents (*Z* RBA = 44 vs *E* RBA = 22), but the *Z*-isomer is more potent in vivo by a factor of 30-fold (*Z* ED_{50} = 2.3 nmol vs *E* ED_{50} = 72 nmol). The two trifluoromethyl isomers have essentially the same in vitro RBA values (RBA = 9 vs 8); however, the *Z*-isomer is 10 times more potent in vivo (*Z* ED_{50} = 1.1 nmol vs *E* ED_{50} = 10.6 nmol). On the other hand, of the methylphenyl isomers, the *E*-isomer has a higher RBA value but the compounds are essentially equipotent in vivo. Even within the isomeric series, the order of ED_{50} values does not match the order of RBA values. As has been suggested by others,[45] results from ER-HBD studies do not necessarily reflect the intact ER. The presence of the additional functional group at the para position and the stereochemistry of the vinyl group may influence the orientation of the peptide backbone or the surface features. Such minor alterations in secondary or tertiary structure may affect the ability of the ER-HBD to elicit appropriate conformational adaptations of the other domains of the intact ER responsible for dimerization and DNA recognition. Therefore, evaluation of the data provide important information not only for structure−activity relationships in this series and its relationship to the corresponding *E*-series but also for understanding the effect of ligand binding on the subsequent biological response.

Structure−Activity Relationships in the *Z*-(4-Substituted-phenyl)vinyl Estradiols. We prepared and evaluated the *E*-(4-substituted-phenyl)vinyl estradiols as part of our program to develop chemical probes to explore the binding pocket of the estrogen receptor-α. Because the compounds demonstrated significant ERα-HBD affinity, we believed that interactions between the ligand and the residues within the binding pocket could be evaluated by molecular modeling. Other investigators have applied a variety of computational methods to evaluate ligand binding to the estrogen receptor subtypes.[46−52] We had synthesized several *Z*-phenylvinyl estradiols and examined their preferred solution and gas-phase conformations using a combination of NMR and computational methods.[53] Those results suggested that the *Z*-isomers should exhibit different binding modes compared to the *E*-isomers.

The first relevant finding from this study was that all of the 4-substituted derivatives that were evaluated retained significant affinity for the ER-HBD. Although previous studies indicated that *Z*-phenyl-(X)-vinyl estradiols generally displayed reasonable affinity for the receptor, the effect of para substitution had not been examined. The data clearly indicate that the presence of a 4-substituent did not disrupt receptor binding. However, in contrast to the *E*-isomers, where substitution generally increased receptor affinity compared to the unsubstituted parent compound, in the *Z*-series all of the substituted derivatives had RBA values less than the parent compound. Because individual electronic, hydrophobic, or steric parameters of the substituents did not correlate directly with the observed order of binding affinity, our interpretation of the results required evaluation of the presumed binding modes as determined by molecular modeling and its comparison with our previous modeling study.

Docking studies were performed with the *E*- and *Z*-(4-fluorophenyl)vinyl estradiol isomers as a basis for interpreting the observed biological results. Both isomers adopted low-energy complexes with the ERα-HBD structure, with the steroidal rings essentially overlapping the estradiol skeleton (Figure 3). The most notable effect for the steroidal component was an approximately

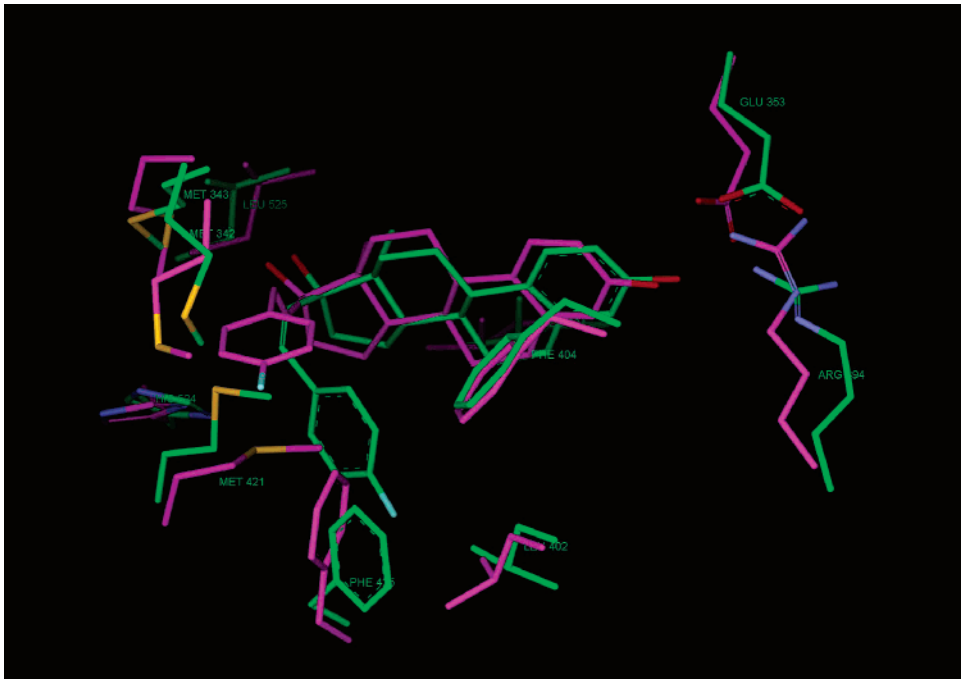

Figure 5. Docking of *E*- (purple) and *Z*--4-fluorophenylvinyl estradiols (green) in ERα-HBD. The position of steroid skeleton within the binding pocket is similar for both isomers; however, the orientation of the 4-substituted phenylvinyl groups is different. The *E*-isomer is bounded by Met-421, -341, -342 and Phe-425, whereas the *Z*-isomer is bounded by Phe-404 and -425 and Leu-402 and -428.

1.4 Å translational movement toward the Arg-394, Glu-353 residues that hydrogen-bond to the A-ring phenolic hydroxyl. Such movements have been observed in the crystal structures of other ER-HBD complexes and apparently are well-tolerated.[48] Other amino acids intimately associated with estradiol binding and the agonist response, His-524, Phe-404, and Met-342, -343, 421, undergo only minor perturbations, i.e., <1 Å. The movement of the ligand along that axis allows the *Z*-phenylvinyl ring to adopt a conformation that inserts the aromatic ring into a previously unidentified hydrophobic pocket similar but not identical to that occupied by the *E*-phenylvinyl moiety (Figure 5). The pocket that surrounds the terminal phenyl group comprises Phe-404 and -425, Leu-391 and -402, Ile-424, and Met-421. Unlike the binding pocket for the *E*-isomer, the presence of an amino acid residue (Leu-402) immediately juxtaposed to the para position imposes significant steric constraints. As the receptor binding values (RBAs) with the ERα-HBD suggest, introduction of the 4-substituent causes a reduction in binding affinity. That binding is not affected more indicates that the phenylvinyl group or the binding pocket or both can adapt to achieve a lower energy complex. The influence of protein energy on ligand binding is reflected in its contribution to the correlation between calculated binding energies and observed RBA values (Figure 4). It is notable that for the *E*-isomers where no receptor residue is juxtaposed to the para substituent and steric factors are not significantly involved, there was essentially no contribution of the protein energy to the correlation equation.

The adaptive response by the ligand and receptor clearly occurs within the agonist conformation because all of the compounds, *E*- and *Z*-isomers, generate full estrogenic responses in vivo. The interaction between the ligand and the intact receptor present within the normal cell is not fully represented by the model developed with the ER-HBD. This is apparent from the lack of a clear correlation between the in vitro RBA values and the in vivo ED_{50} values. The presence of the other receptor domains in the full-length receptor and the coregulatory (activator) proteins provides influences that modify the biological response in ways not readily accessible to computational analysis. Slight perturbations in the receptor peptide backbone may enhance or suppress the interactions between the DBD and the ERE of the DNA. Similarly, perturbations at the surface of the HBD can enhance, suppress, or modify the selectivity of the coactivator protein binding required for the transcriptional response. Therefore, it remains an important consideration of binding studies not to attach excessive significance to binding affinities and computational modeling in predicting the in vivo biological activity of NR ligands.

The docking study does provide the basis for additional considerations of these compounds as potential ER-subtype selective ligands. Although the homology between the ERα-HBD and ERβ-HBD is less than 60%,[54] within the binding site itself there are only two mutations. One of these, Leu-384 → Met- occurs on the β-face of the steroid and would not be significantly involved with interactions with the phenylvinyl group of our compounds. The second, however, is Met-421→ Ile-, and this specific amino acid is one of three methionine residues that we predicted would line the hydrophobic pocket enfolding the *E*-phenylvinyl moiety. It is also associated with the *Z*-phenylvinyl binding mode, although not in the same manner. If our proposed binding model is correct, the *E*-phenylvinyl estradiol derivatives should display greater ER-subtype selectivity than the corresponding *Z*-isomers.

In summary, using three Pd(0)-catalyzed coupling reactions, we have prepared and characterized a series of unique probes for the ERα-HBD. Competitive binding

assays indicated that the new substituted compounds possessed significant receptor binding affinity compared to the unsubstituted parent compound and to estradiol. All of the new compounds expressed full estrogenic activity in vivo, but potency levels did not correlate with the observed in vitro RBA values. Molecular modeling of the ligand−ERα-HBD complexes provided a rationale for the observed binding results but not the in vivo biological activity. The model suggests that the *E*- and *Z*-substituted phenylvinyl estradiols access different hydrophobic pockets within the ERα-HBD and that these pockets are the result of an adaptive response by the receptor to the presence of the ligand. Modeling of the interactions between putative ligands and the individual receptor subtype binding pockets provides the basis for designing ligands with enhanced receptor affinity and receptor subtype selectivity. Studies directed toward these goals are in progress.

Experimental Section

General Methods. All reagents and solvents were purchased from Aldrich or Fisher Scientific. THF and toluene were distilled from sodium/benzophenone. Reactions were monitored by TLC, performed on 0.2 mm silica gel plastic backed sheets containing F-254 indicator. Visualization on TLC was achieved using UV light, iodine vapor, and/or phosphomolybdic acid reagent. Column chromatography was performed with 32−63 μm silica gel packing. Melting points were determined using an Electrotherm capillary melting point apparatus and are uncorrected. NMR spectra chemical shifts are reported in parts per million downfield from TMS and referenced to TMS internal standard for deuterochloroform ($DCCl_3$) or deuteroacetone (CD_3COCD_3) solvent peak. Coupling constants are reported in hertz. All compounds gave satisfactory elemental analyses, ±0.4% (Atlantic Microchemical Laboratories, Inc., Norcross, GA), unless otherwise stated.

Synthetic Methods. 3-Acetoxy-(17α,20*Z*)-21-(tri-*n*-butylstannyl)-19-norpregna-1,3,5(10),20-tetraene-17β-ol (2). To a solution of **1** (6.76 g, 20 mmol) in tetrahydrofuran (20 mL) was added tri-*n*-butyltin hydride (8.5 mL, 31 mmol) and 1 M triethylborane (1 mL, 8.8 mmol). The reaction mixture was stirred under nitrogen at room temperature for 10 h. The THF was removed under reduced pressure, and the resulting oil was separated via silica gel column chromatography using hexane/ethyl acetate (5:1) as both the packing and eluting solvent. The product **2** was isolated as an oil that solidified upon standing (4.6 g, 36%; 63% based on recovered starting material): $R_f = 0.68$ (hexane/ethyl acetate 5:1); mp 80−82 °C; ^{1}H NMR in $CDCl_3$ δ 0.8−2.8 (m, b, 42H, steroid and tributyltin), 2.89 (s, 3H, −OCOC*H*$_3$), 5.87 (d, 1H, J_{20-21} = 13.2 Hz, 20-H), 6.78 (d, 1H, J_{21-20} = 12.0 Hz, 21-H), 6.79 (s, 1H, 4-H), 6.84 (d, 1H, J_{2-1} = 8.4 Hz, 2-H), 7.28 (d, 1H + $CDCl_3$, J_{1-2} = 9 Hz, 1-H); ^{13}C NMR in acetone-d_6 δ 13.00 (−(*C*H$_2$CH$_2$CH$_2$CH$_3$)$_3$), 14.07 (−(CH$_2$CH$_2$CH$_2$*C*H$_3$)$_3$), 14.63 (C-18), 20.93 (−OCO*C*H$_3$), 24.07 (C-15), 27.10 (C-11), 28.02 (C-7), 28.16 (−(CH$_2$*C*H$_2$CH$_2$CH$_3$)$_3$), 29.01 (−(CH$_2$*C*H$_2$CH$_2$CH$_3$)$_3$), 33.77 (C-12), 39.58 (C-16), 40.29 (C-8), 44.83(C-9), 47.69 (C-13), 50.47 (C-14), 85.43 (C-17), 119.59 (C-2), 122.37 (C-4), 124.99 (C-21), 126.98 (C-1), 138.55 (C-10), 138.72 (C-5), 149.72 (C-20), 151.45 (C-3), 169.69 (−*C*OCH$_3$). Further elution gave 3-acetoxy-(17α,20*E*)-21-(tri-*n*-butylstannyl)-19-norpregna-1,3,5(10),20-tetraene-17β-ol (**3**) (1.2 g, 10%; based on recovered starting material, 26%).

Method A. Stille Coupling. General Procedure. A mixture of the 4-substituted aryl bromide or iodide (1.0 mmol), tetraksi(triphenylphosphine)palladium(0) (0.02 mmol), one crystal of 2,6-di-*tert*-butyl-4-methylphenol, and **2** (0.9 mmol) was stirred in anhydrous toluene (10 mL). The reaction mixture was heated at reflux under a nitrogen atmosphere and monitored for the disappearance of **2**. The reaction mixture was cooled to ambient temperature, a 10% KF−CH_3OH (10 mL) solution was added, and the mixture was stirred for 8 h. The mixture was partitioned between ethyl acetate and water (100 mL:100 mL), and the Bu_3SnF was removed by filtration. The organic layer was separated, and the aqueous layer was extracted twice with ethyl acetate. The organic layers were combined, washed with NH_4Cl/H_2O (100 mL), water (2 × 100 mL), and brine (2 × 100 mL), dried over magnesium sulfate, and concentrated. The residue was purified by chromatography on silica gel using hexanes/ethyl acetate (5:1) as the eluent. Evaporation of the solvent gave the purified **5**. In most cases the purified material was used directly for the hydrolysis step.

3-Acetoxy-(17α,20*Z*)-21-(phenyl)-19-norpregna-1,3,5-(10),20-tetraene-17β-ol (5a). A mixture of bromobenzene (0.17 g, 1.1 mmol), tetrakis(triphenylphosphine)palladium(0) (0.024 g, 0.02 mmol), one crystal of 2,6-di-*tert*-butyl-4-methylphenol, and **4** (0.6 g, 0.9 mmol) was reacted using method A. The residue was chromatographed on silica gel (16 g), using hexane/ethyl acetate (5:1) to afford recovered **4** (0.1 g) and **5a** (0.06 g, 18%, based on recovered staring material): $R_f = 0.35$ (hexane/ethyl acetate 5:1).

3-Acetoxy-(17α,20*Z*)-21-[(4-hydroxy)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5c). A mixture of 4-iodophenol (0.29 g, 1.4 mmol), tetrakis(triphenylphosphine)-palladium(0) (0.024 g, 0.02 mmol), and **4** (0.41 g, 0.65 mmol) in anhydrous toluene (25 mL) was reacted using method A. The residue was chromatographed on silica gel (6 g) using hexane/ethyl acetate (5:1) to afford recovered **4** (0.1 g) and **5c** as a mixture (0.04 g, 14%; based on recovered staring material, 19%): $R_f = 0.12$ (hexane/ethyl acetate 5:1).

3-Acetoxy-(17α,20*Z*)-21-[(4-cyano)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5d). A mixture of 4-bromobenzonitrile (0.13 g, 0.7 mmol), tetrakis(triphenylphosphine)-palladium(0) (0.016 g, 0.014 mmol), and **2** (0.31 g, 0.5 mmol) in toluene (5 mL) was reacted under method A conditions for 72 h. The residue was chromatographed on silica gel (50 g) and eluted with hexane/ethyl acetate (5:1) to yield **5d** (0.005 g, 2%).

3-Acetoxy-(17α,20*Z*)-21-[(4-methyl)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5e). A mixture of *p*-iodotoluene (0.32 g, 1.5 mmol), tetrakis(triphenylphosphine)-palladium(0) (0.005 g, 0.004 mmol), **2** (0.69 g, 1.1 mmol), and 1 crystal of 2,6-di-*tert*-butyl-4-methylphenol were heated with refluxing anhydrous toluene (15 mL) for 7 h using method A. The residue was chromatographed on silica gel using hexane/ethyl acetate (5:1) to afford **5e** (0.14 g, 32%): $R_f = 0.25$ (hexane/ethyl acetate 5:1); ^{1}H NMR in $CDCl_3$ δ 0.90 (s, 3H, 18-CH_3), 1.2−2.8 (m, 18H, steroid nucleus), 5.89 (d, 1H, J_{20-21} = 12.80 Hz, 20-H), 6.49 (d, 1H, J_{21-20} = 12.72 Hz, 21-H), 6.56 (d, 1H, J_{4-2} = 2.6 Hz, 4-H), 6.62 (dd, 1H, J_{2-1} = 8.49 Hz, J_{2-4} = 2.73 Hz, 2-H), 7.14 (m, 3H, 1-H, 23-H, 27-H), 7.34 (d, 2H, J_{24-23} and J_{26-27} = 7.89 Hz, 24-H and 26-H).

3-Acetoxy-(17α,20*Z*)-21-[(4-methoxy)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5g). A mixture of *p*-iodoanisole (0.93 g, 4 mmol), tetrakis(triphenylphosphine)-palladium(0) (0.016 g, 0.014 mmol), **4** (1.26 g, 2 mmol), and one crystal of 2,6-di-*tert*-butyl-4-methylphenol were heated in refluxing anhydrous toluene (25 mL) for 12 h. The residue was chromatographed on silica gel using with hexane/ethyl acetate (4:1) to afford **5g** (0.22 g, 25%): $R_f = 0.35$ (hexane/ethyl acetate 4:1); ^{1}H NMR in acetone-d_6 δ 0.90 (s, 3H, 18-CH_3), 1.2−2.8 (m, 15H, steroid nucleus), 3.79 (s, 3H, OCH_3), 5.87 (d, 1H, J_{20-21} = 13.08 Hz, 20-H), 6.39 (d, 1H, J_{21-20} = 13.08 Hz, 21-H), 6.82 (m, 4H, 2-H, 4-H, 24-H, and 26-H), 7.30 (d, 1H, J_{1-2} = 8.79).

3-Acetoxy-(17α,20*Z*)-21-[(4-amino)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5h). A mixture of *p*-iodoaniline (0.22 g, 5.7 mmol), tetrakis(triphenylphosphine)palladium(0) (0.028 g, 0.02 mmol), **4** (1.7 g, 2.8 mmol), and one crystal of 2,6-di-*tert*-butyl-4-methylphenol were heated in refluxing anhydrous toluene (25 mL) for17 h. The residue was chromatographed on silica gel using hexane/ethyl acetate (4:1) to afford **5h** (0.06 g pure and 0.5 g mixture, >35%): $R_f = 0.32$ (hexane/ethyl acetate 5:1); ^{1}H NMR in acetone-d_6 δ 0.97 (s, 3H,

18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 5.98 (d, 1H, J_{20-21} = 13.2 Hz, 20-H), 6.46 (d, 1H, J_{20-21} = 12.9 Hz, 21-H), 6.78 (d, 1H, J_{4-2} = 2.1 Hz, 4-H), 6.84 (dd, 1H, J_{2-4} = 2.4 Hz, J_{2-1} = 8.7 Hz, 2-H), 7.21 (d, 1H, J_{1-2} = 7.2 Hz, 1-H), 7.30 (m, 4H, 24-H, 26-H and $CDCl_3$ peak), 7.60 (d, 4H, J_{23-24} and J_{27-26} = 7.2 Hz, 23-H and 27-H), 7.62 (d, 2H, J_{23-24} and J_{27-26} = 8.91 Hz, 23-H and 27-H); ^{13}C NMR in acetone-d_6 δ 14.43 (C-18), 20.80 (−OCO*C*H_3), 23.65 (C-15), 27.02 (C-11), 27.80 (C-7), 28.89 (C-6), 32.50 (C-12), 38.19 (C-16), 40.20 (C-8), 44.57 (C-9), 48.60 (C-13), 49.82 (C-14), 55.25 (−O*C*H_3), 83.68 (C-17), 113.53 (C-24 and C-26), 119.46 (C-2), 122.25 (C-4), 126.88 (C-1), 129.71 (C-21), 130.42 (C-22), 132.35 (C-23 and C-27), 134.92 (C-10), 138.34 (C-20), 138.61 (C-5), 149.53 (C-3), 159.36 (C-25), 169.58 (−*C*OCH$_3$).

3-Acetoxy-(17α,20*Z*)-21-(iodo)-19-norpregna-1,3,5(10),-20-tetraene-17β-ol (7). To a solution of **2** (0.77 g, 1.22 mmol) in chloroform/methylene chloride (1:1, 10 mL) was added a slurry of *N*-iodosuccinimide (0.36 g, 1.6 mmol) in the same solvent solution. The reaction mixture was stirred, under aluminum foil, at −78 °C for 2 h. The reaction mixture was washed with saturated sodium bicarbonate/water (20 mL). Aqueous and organic layers were separated. The aqueous layer was extracted with chloroform (20 mL × 2). Organic layers were combined washed with water (20 mL × 2) and brine (20 mL × 2), dried over magnesium sulfate, and concentrated. The yellow oil (1.26 g) was purifed on a silica gel column (40 g) and covered with aluminum foil, using hexane/ethyl acetate (5: 1) as the eluting solvent to give **7** as a colorless crystals (0.34 g, 83%): R_f = 0.25 (hexane/ethyl acetate 5:1); ^{1}H NMR in $CDCl_3$-d_6 δ 0.96 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 6.38 (d, 1H, J_{21-20} = 8.73 Hz, 21-H), 6.79 (d, 1H, J_{4-2} = 2.46 Hz, 4-H), 6.85 (dd, 1H, J_{2-4} = 2.55 Hz, 2-H), 6.85 (d, 1H, J_{20-21} = 8.52 Hz, 20-H), 7.27 (d, 1H and $CDCl_3$ peak, J_{1-2} = 8.31 Hz, 1-H); ^{13}C NMR in $CDCl_3$ δ 14.13 (C-18), 23.24 (−OCO*C*H_3), 21.11 (C-15), 26.09 (C-11), 27.20 (C-7), 29.50 (C-6), 32.02 (C-12), 37.72 (C-16), 39.10 (C-8), 43.83 (C-9), 48.41 (C-13), 49.67 (C-14), 72.91 (C-21), 84.88 (C-17), 118.61 (C-2), 121.50 (C-4), 126.39 (C-1), 137.77 (C-10), 138.17 (C-5), 143.40 (C-20), 148.41 (C-3), 169.85 (−*C*OCH$_3$).

4-Tributylstannylacetophenone (8). A solution of 4-bromoacetophenone (1.08 g, 5.4 mmol), bis(tributyltin) (5 mL, 10 mmol), tetrakis(triphenylphosphine)palladium(0) (0.08 g, 0.07 mmol), and toluene (50 mL) was stirred under nitrogen for 24 h at reflux. The black solution was concentrated and chromatographed on a 45 g silica column packed with hexane and eluted with 5:1 hexane/ethyl acetate to yield an oil **12** (1.22 g, 55%): R_f = 0.82 (hexane/ethyl acetate 5:1); ^{1}H NMR in $CDCl_3$ δ 0.8−1.7 (m, 27H, $SnBu_3$), 2.58 (s, 3H, $COCH_3$), 1.59 (d, 2H, J_{2-3} = 8 Hz, 2-H and 6-H), 7.88 (d, 2H, J_{3-2} = 8 Hz, 3-H and 5-H).

Method B. Reversed Stille Coupling. 3-Acetoxy-(17α,-20*Z*)-21-[(4-acetyl)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5i). A mixture of **12** (0.40 g, 1 mmol), tetrakis-(triphenylphosphine)palladium(0) (0.009 g, 0.008 mmol), and **8** (0.24 g, 0.51 mmol) was stirred under nitrogen in anhydrous toluene (20 mL) at reflux for 15 min. The reaction vessel was protected from light by wrapping it in aluminum foil. A 10% KF/H_2O (20 mL) solution was added, and the mixture was stirred for 1 h. The solution was partitioned between ethyl acetate and water (100 mL:100 mL), and the precipitate ($SnBu_3F$) was filtered off. The aqueous layer was extracted with ethyl acetate (2 × 100 mL). Organic layers were combined and washed with water (100 mL) and brine (2 × 100 mL), dried over magnesium sulfate, and concentrated. The residue was chromatographed on a silica gel column (30 g), using hexane/ethyl acetate (5:1) to afford **5i** (0.012 g, 5%): R_f = 0.05 (hexane/ethyl acetate 5:1); ^{1}H NMR in $CDCl_3$ δ 0.91 (s, 3H, 18-CH_3), 1.2−2.8 (m, 21H, steroid nucleus), 6.01 (d, 1H, J_{20-21} = 12.93 Hz, 20-H), 6.52 (d, 1H, J_{21-20} = 13.1 Hz, 21-H), 6.79 (d, 1H, J_{4-2} = 2.4 Hz, 4-H), 6.86 (dd, 1H, J_{2-1} = 8.3 Hz, J_{2-4} = 2.46 Hz, 2-H), 7.29 (d, 1H, J_{1-2} = 8.79 Hz, 1-H), 7.54 (d, 2H, J_{23-24} and J_{27-26} = 8.40 Hz, 23-H, 27-H), 7.90 (d, 2H, J_{24-23} and J_{26-27} = 8.31 Hz, 24-H and 26- Hz,).

Method C. Suzuki Coupling. 3-Acetoxy-(17α,20*Z*)-21-[(4-fluoro)phenyl]-19-norpregna-1,3,5(10),20-tetraene-17β-ol (5b). To a solution of **7** (0.46 g, 1 mmol) in THF (15 mL) was added tris(dibenzylideneacetone)dipalladium (0.15 g, 0.17 mmol), sodium bicarbonate (0.43 g, 4 mmols, 4 equiv, in 10 mL of water), and 4-fluorobenzene boronic acid (0.29 g, 2.1 mmol). The reaction mixture was protected from light and stirred at room temperature for 14 h. The mixture was extracted with ethyl acetate (2 × 50 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over magnesium sulfate, filtered, and concentrated to yield a brown gummy residue. The residue was chromatographed on a silica gel column (30 g) using 98:2 chloroform/methanol as the eluting solvent to give **5b** as a crude product (0.19 g, 44%): R_f = 0.45 (chloroform/methanol 98:2); ^{1}H NMR in $CDCl_3$ δ 0.97 (s, 3H 18-CH_3), 1.2−2.9 (m, b, 15H, steroid nucleus), 5.90 (d, 1H, J_{20-21} = 13.08 Hz, 20-H), 6.45 (d, 1H, J_{21-20} = 12.7 Hz, 21-H), 6.78 (d, 1H, J_{4-2} = 2.0 Hz, 4-H), 6.83 (dd, 1H, 2-H), 6.9 (∼t, 2H, 24-H and 26-H), 7.04 (d, contaminant), 7.26 (d, 1H and $CDCl_3$ peak, 1-H), 7.4 (m, 2H, 25-H and 27-H), 7.6−7.7 (m, contaminant). Further elution yielded the *E*-isomer **5o** (0.8 g, 17.5%).

Hydrolysis of 3-Acetoxy Intermediates. General Procedure. Compound **5** (0.3 mmol) was stirred in methanol (10 mL) with 10 N sodium hydroxide (0.1 mL) for 20 min. The solution was reacidified with a few drops of acetic acid. The methanol was removed under vacuum, and the residue was partitioned between ethyl acetate and water (100 mL:100 mL). The aqueous layer was extracted with ethyl acetate. Organic layers were combined, washed with water (100 mL), dried over magnesium sulfate, and concentrated to yield **6**. Recrystallization from acetone/hexane afforded colorless crystals (50−90%).

(17α,20*Z*)-21-(Phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol (6a). Compound **5a** (0.06 g, 0.15 mmol) was deprotected to yield an off-white powder (0.057 g, 0.15 mmol, 100%). Recrystallization in ethyl acetate afforded a white powder (0.05 g, 0.13 mmol, 89%): R_f = 0.03 (hexane/ethyl acetate 5:1); mp 125−127 °C; ^{1}H NMR in $CDCl_3$ δ 0.90 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 4.58 (s, 1H, 17-OH), 5.93 (d, 1H, J_{20-21} = 12.78 Hz, 20-H), 6.53 (d, 1H, J_{21-20} = 12.81 Hz, 21-H), 6.57 (s, 1H, J_{4-2} = 2.19 Hz), 6.62 (dd, 1H, J_{2-1} = 8.58 Hz and J_{2-4} = 2.7 Hz, 2-H), 7.17 (d, 1H, J_{1-2} = 8.79 Hz, 1-H), 7.25 (1H and $CDCl_3$, m, 24-H), 7.27 (t, 2H, J = 7.71 and 7.05 Hz, 23-H and 25-H), 7.44 (d, J = 7.35 Hz, 22-H and 26-H); ^{13}C NMR in $CDCl_3$ δ 14.07 (C-18) 23.30 (C-15), 27.46 (C-11), 29.67 (C-7), 26.52 (C-6), 32.58 (C-12), 38.62 (C-16), 39.66 (C-8), 43.83 (C-9), 48.04 (C-13), 49.72 (C-14), 85.09 (C-17), 112.71 (C-2), 115.27 (C-4), 126.55 (C-25), 127.13 (C-1), 127.91 (C-24 and C-26), 128.10 (C-23 and C-27), 129.09 (C-21), 136.04 (C-22), 132.78 (C-10), 137.44 (C-20), 138.33 (C-5), 153.65 (C-25), 153.34 (C-3).

(17α,20*Z*)-21-[(4-Fluoro)phenyl]-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (6b). The crude acetylated product **5b** (0.19 g, mmol) was deprotected to yield **6b** (0.053 g, 31%): R_f = 0.15 (hexane/ethyl acetate 5:1); mp 105−108 °C; ^{1}H NMR in $CDCl_3$ δ 0.91 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 4.6 (s, b, 17-OH), 5.90 (d, 1H, J_{20-21} = 12.84 Hz, 20-H), 6.45 (d, 1H, J_{21-20} = 12.72 Hz, 20-H), 6.56 (d, 1H, J_{4-2} = 2.67 Hz, 4-H), 6.63 (dd, 1H, J_{2-1} = 8.46 Hz, J_{2-4} = 2.67 Hz, 2-H), 7.00 (ddd∼t, 2H, 24-H and 26-H), 7.16 (d, 1H, J_{1-2} = 8.31 Hz, 1-H), 7.26 (s, $CDCl_3$), 7.45 (dd∼t, 2H, 23-H and 27-H); ^{13}C NMR in $CDCl_3$ δ 14.04, (C-18), 24.25 (C-15), 27.45 (C-11), 27.41 (C-7), 29.65 (C-6), 32.35 (C-12), 38.45 (C-16), 39.59 (C-8), 43.76 (C-9), 48.02 (C-13), 49.55 (C-14), 84.88 (C-17), 112.68 (C-2), 114.81 (d, J_{CCF} = 21 Hz, C-24 and C-26), 115.25 (C-4), 126.54 (C-22), 127.26 (C-1), 131.03 (C-21), 131.08 (d, J_{CCCF} = 7.7 Hz, C-23 and C-27), 132.61 (C-10), 135.86 (C-20), 138.29 (C-5), 153.34 (C-3), 161.88 (d, J_{C-F} = 246 Hz, C-25); ^{19}F NMR in acetone-d_6 δ −16.42;

(17α,20*Z*)-21-[(4-Hydroxy)phenyl]-19-norpregna-1,3,5-(10),20-tetraene-3,17β-diol (6c). Compound **5c** (0.04 g, 0.09 mmol) was deacetylated using the hydrolytic procedure to yield a yellow powder (0.023 g, 63%). Recrystallization in

acetone/hexane afforded an off-white powder (0.020 g, 55 %): R_f = 0.03 (hexane/ethyl acetate 4:1); mp 133−136 °C; ^{1}H NMR in acetone-d_6 δ 0.96 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 5.83 (d, 1H, J_{20-21} = 12.93 Hz, 20-H), 6.35 (d, 1H, J_{21-20} = 12.93 Hz, 21-H), 6.55 (s, 1H, J_{4-2} = 2.34 Hz), 6.60 (dd, 1H, J_{2-1} =8.4 Hz and J_{2-4} = 2.43 Hz, 2-H), 6.77 (d, 2H, J_{23-24} = 8.64 Hz, 23-H and 27-H), 7.11 (d, 1H, J_{1-2} = 8.31 Hz, 1-H), 7.53 (d, 2H, J_{24-23} = 8.61 Hz, 24-H and 26-H), 8.01 (s, 1H, 3-OH), 8.43 (s, 1H, 25-OH); ^{13}C NMR in acetone-d_6 δ 14.27 (C-18) 23.52 (C-15), 28.11 (C-11), 28.16 (C-7), ∼29 under acetone peak (C-6), 32.39 (C-12), 38.00 (C-16), 40.58 (C-8), 44.57 (C-9), 48.62 (C-13), 50.14 (C-14), 83.36 (C-17), 113.31 (C-2), 114.95 (C-24 and C-26), 115.66 (C-4), 126.80 (C-23 and C-27), 127.82 (C-1), 129.20 (C-21), 131.32 (C-22), 132.32 (C-10), 134.22 (C-20), 138.16 (C-5), 153.65 (C-25), 154.00 (C-3).

(17α,20*Z*)-21-[(4-Cyano)phenyl]-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (5d). Compound **5d** (0.005g) was hydrolyzed to yield **6d** (0.004 g, 90%): R_f = 0.22 (hexane/ethyl acetate 3:1); mp 99−105 °C; ^{1}H NMR in acetone-d_6 δ 0.90 (s, 3H, 18-CH_3), 1.2−2.8 (m, 15H, steroid nucleus), 6.15 (d, 1H, J_{20-21} = 13.20 Hz, 20-H), 6.48 (d, 1H, J_{21-20} = 13.20 Hz, 21-H), 6.54 (d, 1H, J_{4-2} = 2.79, 4-H), 6.60 (dd, 1H, J_{2-4} = 2.8 Hz, J_{2-1} = 8.32 Hz, 2-H), 7.11 (d, 1H, J_{1-2} = 8.31 Hz, 1-H), (d, 2H, J_{23-24} and J_{27-26} = 8.58 Hz, 23-H and 27-H) (d, 2H, J_{24-23} and J_{26-27} = 8.55 Hz, 24-H and 26-H), 7.96 (s, 1H, 3-OH); ^{13}C NMR in acetone-d_6 δ 14.42 (C-18), 23.81 (C-15), 27.23 (C-11), 28.16 (C-7), ∼29 under acetone peak (C-6), 32.93 (C-12), 38.91 (C-16), 40.67 (C-8), 44.46 (C-9), 48.87 (C-13), 50.17 (C-14), 84.51 (C-17), 110.23 (C-25), 113.46 (C-2), 115.80 (C-4), 119.52 (−CN), 126.91 (C-24 and C-26), 127.80 (C-1), 131.58 (C-21), 131.71 (C-23 and 27), 131.85 (C-10), 138.27 (C-5), 140.41 (C-20), 143.61 (C-22), 155.82 (C-3).

(17α,20*Z*)-21-[(4-Methyl)phenyl]-19-norpregna-1,3,5-(10),20-tetraene-3,17β-diol (6e). Compound **5e** (0.14 g, 0.32 mmol) was hydrolyzed to yield **6e** (0.060 g, 48%). Recrystallization in chloroform afforded a white powder (0.055 g, 44%): R_f = 0.38 (hexane/ethyl acetate 4:1); mp 95−96 °C; ^{1}H NMR in $CDCl_3$ δ 0.90 (s, 3H, 18-CH_3), 1.2−2.8 (m, 18H, steroid nucleus), 5.89 (d, 1H, J_{20-21} = 12.81 Hz, 20-H), 6.50 (d, 1H, J_{21-20} = 12.75 Hz, 21-H), 6.56 (d, 1H, J_{4-2} = 2.46 Hz, 4-H), 6.62 (dd, 1H, J_{2-1} = 8.40 Hz, J_{2-4} = 2.82 Hz, 2-H), 7.15 (m, 3H, 1-H, 23-H, 27-H), 7.34 (d, 2H, J_{24-23} and J_{26-27} = 8.19 Hz, 24-H and 26-H); ^{13}C NMR in $CDCl_3$-d_6 δ 14.07 (C-18), 21.18 (C-28), 23.26 (C-15), 26.48 (C-11), 27.44 (C-7), 29.68 (C-6), 32.49 (C-12), 38.49 (C-16), 39.61 (C-8), 43.79 (C-9), 47.99 (C-13), 49.58 (C-14), 85.08 (C-17), 112.70 (C-2), 115.26 (C-4), 126.55 (C-24 and C-26), 127.91 (C-1), 128.87 (C-21), 129.01 (C-23 and C-27),132.66 (C-10), 134.31 (C-22), 135.46 (C-25), 136.95 (C-20), 138.30 (C-5), 153.38 (C-3).

(17α,20*Z*)-21-[(4-Methoxy)phenyl]-19-norpregna-1,3,5-(10),20-tetraene-3,17β-diol (6g). Compound **5g** (0.14 g, 0.314 mmol) was hydrolyzed using the general procedure to yield **6g** (0.125 g, 98%), and recrystallization in acetone/hexane afforded white crystalline needles (0.120 g, 94%): R_f = 0.21 (hexane/ethyl acetate 4:1); mp 164−165 °C; ^{1}H NMR in acetone-d_6 δ 0.97 (s, 3H 18-CH_3), 1.2−2.9 (m, b, 15H, steroid nucleus), 3.79 (s, 3H, OCH_3), 5.87 (d, 1H, J_{20-21} = 12.96 Hz, 20-H), 6.38 (d, 1H, J_{21-20} = 12.96 Hz, 21-H), 6.53 (d, 1H, J_{4-2} = 2.55 Hz, 4-H), 6.62 (dd, 1H, J_{2-4} = 2.43 Hz, J_{2-1} = 8.43 Hz, 2-H), 6.86 (d, 2H, J_{24-23} and J_{26-27} = 8.91 Hz, 24-H and 26-H), 7.11 (d, 1H, J_{1-2} = 8.26 Hz, 1-H), 7.62 (d, 2H, J_{23-24} and J_{27-26} = 8.64 Hz, 23-H and 27-H), 7.95 (s, 1H, 3-OH); ^{13}C NMR in acetone-d_6 δ 14.50 (C-18), 23.71 (C-15), 27.27 (C-11), 26.16 (C-7), ∼29 under acetone peak (C-6), 32.60 (C-12), 38.26 (C-16), 40.73 (C-8), 44.52 (C-9), 48.70 (C-13), 49.87 (C-14), 55.29 (−OCH_3), 83.78 (C-17), 113.46 (C-2), 113.58 (C-24 and C-26), 115.81 (C-4), 126.95 (C-1), 129.67 (C-21), 130.52 (C-22), 131.89 (C-10), 132.37 (C-23 and C-27), 135.06 (C-20), 138.32 (C-5), 155.80 (C-3), 159.41 (C-25).

(17α,20*Z*)-21-[(4-Amino)phenyl]-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (6h). The crude acetylated product **5h** (∼0.5 g) was deprotected to yield a dark-orange solid **6h** (0.34 g, 75.3%) followed by three recrystalizations from acetone/chloroform to yield a light-orange powder (0.31 g, 68%): R_f = 0.47 (1:1 hexane/ethyl acetate); mp 140−142 °C; ^{1}H NMR in $CDCl_3$ δ 0.91 (s, 3H, 18-CH_3), 1.2−2.9 (m, 15H, steroid nucleus), 3.8 (s, b, 2H, −NH_2), 4.6 (s, b, 17-OH), 5.79 (d, 1H, J_{20-21} = 12.90 Hz, 20-H), 6.40 (d, 1H, J_{21-20} = 12.60 Hz, 20-H), 6.56 (d, 1H, J_{4-2} = 2.7 Hz, 4-H), 6.64 (m, 4H, 2-H, 4-H, 24-H and 26-H), 7.16 (d, 1H, J_{1-2} = 8.4 Hz, 1-H), 7.26 (s, $CDCl_3$), 7.32 (d, 2H, J = 8.4 Hz, 23-H and 27-H); ^{13}C NMR in acetone-d_6 δ 15.04 (C-18), 24.17 (C-15), 27.79 (C-11), 28.66 (C-7), under acetone peak (C-6), 32.94 (C-12), 38.51 (C-16), 41.25 (C-8), 45.03 (C-9), 49.12(C-13), 50.12 (C-14), 83.99 (C-17), 113.90 (C-2), 114.58 (C-24 and C-26), 116.25 (C-4), 127.41 (C-1), 131.14 (C-21), 132.33 (C-10), 132.66 (C-23 and C-27), 133.03 (C-20), 138.76 (C-5), 150.02 (C-25), 156.29 (C-3).

(17α,20*Z*)-21-[(4-Acetyl)phenyl]-19-norpregna-1,3,5(10),-20-tetraene-3,17β-diol (6i). Compound **5i** (0.012 g, 0.026 mmol) was hydrolyzed using the general procedure to yield **6i** (0.0056 g, 51%): R_f = 0.12 (hexane/ethyl acetate 5:1); ^{1}H NMR in $CDCl_3$ δ 0.91 (s, 3H, 18-CH_3), 1.2−2.8 (m, 18H, steroid nucleus), 6.01 (d, 1H, J_{20-21} = 12.99 Hz, 20-H), 6.51 (d, 1H, J_{21-20} = 12.81 Hz, 21-H), 6.57 (d, 1H, J_{4-2} = 2.7 Hz, 4-H), 6.64 (dd, 1H, J_{2-1} = 8.3 Hz, 2-H), 7.16 (d, 1H, J_{1-2} = 8.31 Hz, 1-H), 7.55 (d, 2H, J_{23-24} and J_{27-26} = 8.46 Hz, 23-H, 27-H), 7.90 (d, 2H, J_{24-23} and J_{26-27} = 8.37 Hz, 24-H and 26-H); ^{13}C NMR in $CDCl_3$ δ 14.04 (C-18), 23.31 (C-15), 26.43 (−CO*C*H_3), 26.62 (C-11), 27.43 (C-7), 29.63 (C-6), 32.45 (C-12), 38.69 (C-16), 39.59 (C-8), 43.75 (C-9), 48.10 (C-13), 49.68 (C-14), 85.07 (C-17), 112.71 (C-2), 115.26 (C-4), 126.55 (C-21), 127.31 (C-1), 127.91 (C-23 and C-27), 129.52 (C-24 and C-26), 132.53 (C-10), 135.50 (C-25), 137.77 (C-20), 138.27 (C-5), 142.69 (C-22), 153.37 (C-3), 197.71 (−*C*OCH_3).

Molecular Modeling and Dynamics. We initially evaluated the conformations of our ligands **6a**−**i** using the Builder module from Insight II. Potentials for each atom were assigned automatically or manually, when necessary. Low-energy conformations were generated using the molecular mechanics method (Discover program, 100 steps, 0.001 final convergence) and compared to solution conformations determined by NMR.[39] The ERα-HBD used in our study was obtained from the Protein Data Bank (PDB code 1G50, wild-type ERα-HBD cocrystallized with estradiol). From the available monomers (A, B), monomer A from the A/C homodimer was selected for the docking and molecular dynamics studies. All water molecules present in the crystal structure were deleted. The monomer contains all the amino acid residues between ASN 304 and HIS 550. All manipulations were performed using the Builder module in Insight II. The complex of the ERα-HBD monomer and estradiol bound within the binding cavity was minimized using the molecular mechanics method with restraints applied to the backbone atoms of the protein (Discover_3 module, CVFF force field, dielectric constant 2.0, conjugate gradient minimization 10 000 steps or until 0.001 final convergence). All ligands used in this study were constructed using the Builder module from Insight II. Potentials for each atom were assigned automatically or manually when necessary. Each ligand was optimized using the molecular mechanics method as done with the receptor. Partial charges for each atom were calculated using the Mopac program from the Ampac/Mopac module in the Insight II package. In addition, ligands were further optimized using the semiemperical method (calculation method. PM3; calculation type, optimization; optimizer type, native).

The Affinity program within the Docking module in InsightII was used to perform the docking studies of the ligands with the ERα-HBD.[55] This module includes elements from Monte Carlo, simulated annealing, and minimization for automatically docking and finding the best structures of the ligand complexed to the receptor based on the energy of the ligand−receptor complex. The ligand was superimposed on the estradiol molecule (A-ring over A-ring), and the estradiol was then deleted. The complex was subjected to energy minimization to obtain a starting structure in which bad steric contacts are removed and internal energies are relieved. During the

docking procedure both the ligand and the protein residues within the ligand binding cavity (amino acids within 15 Å of the ligand as well as all amino acids in helix-12, loops 11−12, 1−3, 6−7) were allowed to flex while the backbone atoms and the rest of the protein were restrained in their original positions. In addition, the phenylvinyl side chain of the ligand was rotated by a maximum of 180° increments in order to more fully explore the potential binding modes of the conformational choices of the ligand. After each docking procedure, structures within 10 kcal/mol of the lowest energy structure and with an rms distance of greater than 0.125 Å were selected and used in simulated annealing studies. At the beginning of each run, the ligand−receptor complex was minimized over 5000 steps or until 0.001 final convergence. Then each structure was heated from 300 to 500 K over 5000 fs and allowed to equilibrate for and additional 5000 fs. Each structure was allowed to cool to 300 K in 20 stages with 10 K decrements for each stage and 100 fs long equilibration periods for each stage. The structure at the end of the final stage was recorded in an archive file and further minimized with 200 steps. Each of the dynamics and simulated annealing cycles was repeated 10 times. During these calculations additional restraints were applied to amino acids facing the outer surface of the protein. All calculations involving docking and refinement of generated structures were performed with a dielectric constant of 2.0.

Results of the docking studies were analyzed using a combination of modules: Analysis, Discover_3, Docking, and Viewer. Each structure generated during the docking, simulated annealing, and dynamics runs was analyzed in terms of binding energy, ligand energy, and protein energy. Values of the binding energy $\Delta E_{\text{binding}}$ were calculated as the difference between the potential energy of the complex (E_{complex}) and the potential energy of the ligand (E_{ligand}) and receptor (E_{receptor}).[52,56] Binding energy calculations were performed using the Energy Analysis macro in the Discover_3 module.

Receptor Binding Studies. In Vitro Competitive Binding Assay. The compounds were screened for their affinity for the ERα-HBD isolated from BL 21 cells that overexpressed the 33 kDa PER-23d ERG vector. The cells were induced with 0.6 mM isopropyl-β-thiogalactopyranoside for 3 h at room temperature, pelleted by centrifugation, frozen, and stored at −75 °C. The cells were thawed and lysed by sonication (4 × 20 s) in four volumes of lysis buffer (50 mM Tris, 50 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 1 M urea, pH 7.4) several times. Clarified fractions, obtained at 30000*g* for 30 min, were pooled, assayed for receptor binding, and diluted to 50 nM in ER, and 100 μL aliquots were frozen and stored at −75 °C until ready for use. Then 80 μL of the ERα-HBD-containing extract was incubated with 10 μL of 10 nM 6,7-[H-3]estradiol (specific activity of 51 Ci/mmol) and 10 μL of buffer, unlabeled estradiol, or test ligand in 100 μL total volume. The final concentrations were 1 nM 6,7-[H-3]estradiol, 2 nM unlabeled estradiol (using 200 nM estradiol to define specific binding), and 0.5−5000 nM of the test ligand. In all cases, 10 μL of each incubation solution was removed for assay of the actual initial concentration of [H-3]estradiol and the remainder was incubated at 4 or 25 °C for 18 h. After incubation, 100 μL of dextran-coated charcoal suspension (fines removed) was added to adsorb the unbound [H-3]estradiol, incubated for 10 min, and centrifuged, and 100 μL samples were taken from the supernatant fraction for assay of radioactivity. The results were calculated and plotted as % specific binding as a function of log of competitor concentration using the best fit equation for the binding inhibition to define the 50% inhibition level. The relative binding affinity (RBA) was calculated as 100 times [E]/[C], where [E] was the concentration of unlabeled estradiol needed to reduce the specific binding of [H-3]estradiol by 50% and [C] was the concentration of test ligand needed to reduce the specific binding by 50%.

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Supporting Information Available: Results from elemental analysis of the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Evans, R. M. The steroid and thyroid hormone receptor superfamily. *Science* **1988**, *240*, 889−895.
(2) Tsai, M.-J.; O'Malley, B. W. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu. Rev. Biochem.* **1994**, *63*, 451−486.
(3) Nilsson, S.; Gustafsson, J.-A. Biological role of estrogen and estrogen receptors. *Crit. Rev. Biochem. Mol. Biol.* **2002**, *37*, 1−28.
(4) Hart, L. L.; Davie, J. R. The estrogen receptor: more than the average transcription factor. *Biochem. Cell Biol.* **2002**, *80*, 335−341.
(5) Pike, A. C. W.; Brzozowski, A. M.; Hubbard, R. E. A structural biologist's view of the oestrogen receptor. *J. Steroid Biochem. Mol. Biol.* **2000**, *74*, 261−268.
(6) Katzenellenbogen, B. S.; Choi, I.; Delage-Mourroux, R.; Ediger, T. R.; Martini, P. G. V.; Montano, M.; Sun, J.; Weis, K.; Katzenellenbogen, J. A. Molecular mechanisms of estrogen action: selectve ligands and receptor pharmacology. *J. Steroid Biochem. Mol. Biol.* **2000**, *74*, 279−285.
(7) McDonnell, D. P.; Dana, S. L.; Hoener, P. A.; Lieberman, B. A.; Imhof, M. O.; Stein, R. B. Cellular mechanisms which distinguish between hormone- and antihormone-activated estrogen receptor. *Ann. N.Y. Acad. Sci.* **2001**, *949*, 121−137.
(8) Dutertre, M.; Smith, C. L. Molecular mechanisms of selective estrogen receptor modulator (SERM) action. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 431−437.
(9) Krishnan, V.; Heath, H.; Bryant, H. U. Mechanism of action of estrogens and selective estrogen receptor modulators. *Vitam. Horm.* **2001**, *60*, 123−147.
(10) Katzenellenbogen, B. S.; Sun, J.; Harrington, W. R.; Kraichely, D. M.; Ganessunker, D.; Katzenellenbogen, J. A. Structure−function relationships in estrogen receptors and the characterization of novel selective estrogen receptor modulators with unique pharmacological profiles. *Ann. N.Y. Acad. Sci.* **2001**, *949*, 6−15.
(11) Leclercq, G. Molecular forms of the estrogen receptor in breast cancer. *J. Steroid Biochem. Mol. Biol*. **2002**, *80*, 259−272.
(12) Miner, J. N.; Tyree, C. M. Drug discovery and the intracellular receptor family. *Vitam. Horm.* **2001**, *62*, 253−280.
(13) Karamouzis, M. V.; Gorgoulis, V. G.; Papavassiliou, A. G. Transcription factors and neoplasia: vistas in novel drug design. *Clin. Cancer Res*. **2002**, *8*, 949−961.
(14) Johnston, S. R. D.; Riddle, S.; Haynes, B. P.; Hern, R. A.; Smith, I. E.; Jarman, M.; Dowsett, M. The novel anti-estrogen iodoxifene inhibits the growth of human cancer xenografts and reduces the frequency of acquired anti-estrogen resistance. *Br. J. Cancer* **1997**, *75*, 804−809.
(15) Jones, C. D.; Jevnikar, M. G.; Pike, A. J.; Peters, M. K.; Black, L. J.; Falcone, J. F.; Clemens, J. A. Anti-estrogens. 2. Structure−activity studies in a series of 3-aroyl-2-arylbenzo[*b*]thiophene derivatives leading to [6-hydroxy-2-(4-hydroxyphenyl)benzo[*b*]-thien-2-yl][4-[2-(1-piperidinyl)ethoxy]phenyl]methanone hydrochloride LY 156758, a remarkably effective estrogen antagonist with only minimal intrinsic activity. *J. Med. Chem.* **1984**, *27*, 1057−1066.
(16) Palkowitz, A. D.; Glasebrook, A. L.; Thrasher, K. J.; Hauser, K. L.; Short, L. L.; Phillips, D. L.; Muehl, B. S.; Sato, M.; Shetler, P. K.; Cullinan, G. L.; Pell, T. R.; Bryant, H. U. Discovery and synthesis of [6-hydroxy-3-[4-[2-(1-piperidinyl)-ethoxy]phenoxy]-2-(4-hydroxyphenyl)]benzo[*b*]thiophene: a novel, highly potent, selective estrogen receptor modulator. *J. Med. Chem*. **1997**, *40*, 1407−1416.
(17) LaBrie, F.; LaBrie, C.; Belanger, A.; Simard, J.; Gauthier, S.; Luu-The, V.; Merand, Y.; Giguere, V.; Candas, B.; Luo, S.; Martel, C.; Singh, S. M.; Fournier, M.; Coquet, A.; Richard, V.; Charbonneau, R.; Charpanet, G.; Tremblay, A.; Cusan, L.; Villeux, R. EM-652 (SCH 57068), a third generation SERM acting as a pure anti-estrogen in the mammary gland and endometrium. *J. Steroid Biochem. Mol. Biol.* **1999**, *69*, 51−84.
(18) Greenberger, L. M.; Annablr, T.; Collins, K. I.; Komm, B. S.; Lyttle, C. R.; Miller, C. P.; Satyaswaroop, P. G.; Zhang, Y.; Frost, P. A new anti-estrogen, 2-(4-hydroxy-phenyl)-3-methyl-1-[4-(2-

piperidin-2-yl-ethoxy-)benzyl]-1*H*-indol-5-ol hydrochloride (ERA-923), inhibits the growth of tamoxifen-sensitive and -resistant tumors and is devoid of uterotrophic effects in mice and rats. *Clin. Cancer Res.* **2001**, *7*, 3166−3177.

(19) Meegan, M. J.; Hughes, R. B.; Lloyd, D. G.; Williams, D. C.; Zisterer, D. M. Ethyl side-chain modifications in novel flexible anti-estrogens—design, synthesis and biological efficacy in assay against the MCF-7 breast tumor cell line. *Anti-Cancer Drug Des.* **2001**, *16*, 57−69.

(20) Weatherman, R. V.; Carroll, D. C.; Scanlan, T. S. Activity of a tamoxifen−raloxifene hybrid ligand for estrogen receptors at an AP-1 site. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 3129−3131.

(21) Rosati, R. L.; Da Silva, J.; Cameron, K. O.; Thompson, D. D.; Ke, H. Z.; Toler, S. M.; Brown, T. A.; Pan, L. C.; Ebbinghaus, C. F.; Reinhold, A. R.; Elliott, N. C.; Newhouse, B. N.; Tjoa, C. M.; Sweetnam, P. M.; Cole, M. J.; Arriola, M. W.; Gauthier, J. W.; Crawford, D. T.; Nickerson, D. F.; Pirie, C. M.; Qi, H.; Simmons, H. A.; Tkalcevic, G. T. Discovery and preclinical pharmacology of a novel, potent, nonsteroidal estrogen receptor agonist/antagonist, CP 336156, a diaryltetrahydronaphthalene. *J. Med. Chem.* **1998**, *41*, 2928−2931.

(22) Bury, P. S.; Christiansen, L. B.; Jacobsen, P.; Jorgensen, A. S.; Kanstrup, A.; Naerum, L.; Bain, S.; Fledelius, C.; Gissel, B.; Hansen, B. S.; Korsgaard, N.; Thorpe, S. M.; Wasserman, K. Synthesis and pharmacological evaluation of novel *cis*-3,4-diarylhydroxychromanes as high affinity partial agonists for the estrogen receptor. *Bioorg. Med. Chem.* **2002**, *10*, 125−145.

(23) Levenson, A. S.; MacGregor-Schafer, J. I.; Bentrm, D. J.; Pease, K. M.; Jordan, V. C. Control of the estrogen-like action of tamoxifen−estrogen receptor complex by the surface amino acid at position 351. *J. Steroid Biochem. Mol. Biol.* **2001**, *76*, 61−70.

(24) Jordan, V. C.; MacGregor-Schafer, J.; Levenson, A. S.; Liu, H.; Pease, K. M.; Simons, L. A.; Zapf, J. W. Molecular classification of estrogens. *Cancer Res*. **2001**, *61*, 6619−6623.

(25) Napolitano, E.: Fiaschi, R.; Hanson, R. N. Structure−activity relationships of estrogenic ligands. Synthesis and evaluation of (17α,20*E*/*Z*)-21-halogenated-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diols. *J. Med. Chem*. **1991**, *34*, 2754−2759.

(26) Napolitano, E.; Fiaschi, R.; Herman, L. W.; Hanson, R. N. Synthesis and estrogen receptor binding of (17α,20E)- and (17α,-20*Z*)-21-phenylthio- and phenylseleno-19-norpregna-1,3,5(10),-20-tetraene-3,17*β*-diols. *Steroids* **1996**, *61*, 384−387.

(27) Hanson, R. N.; Herman, L. W.; Fiaschi, R.; Napolitano, E. Stereochemical probes for the estrogen receptor: Synthesis and receptor binding of (17α,20*E*/*Z*)-21-phenyl-19-norpregna-1,3,5-(10),20-tetraene-3,17*β*-diols. *Steroids* **1996**, *61*, 718−722.

(28) Hanson, R. N.; Napolitano, E.; Fiaschi, R. Novel high affinity steroidal estrogenic ligands: Synthesis and receptor binding of 11*β*-vinyl-17α-*E*/*Z*-phenylselenovinyl estradiols. *Steroids* **1998**, *63*, 479−483.

(29) Hanson, R. N.; Napolitano, E.; Fiaschi, R. Synthesis and evaluation of 11*β*-substituted-21-chloro/iodo-(17α,20*E*/*Z*)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diols: High affinity ligands for the estrogen receptor. *J. Med. Chem*. **1998**, *41*, 4686−4692.

(30) McKenna, N. J.; O'Malley, B. W. From ligand to response: generating diversity in nuclear coregulator function. *J. Steroid Biochem. Mol. Biol*. **2000**, *74*, 351−356.

(31) Connor, C. E.; Norris, J. D.; Broadwater, G.; Willson, T. M.; Gottardis, M. M.; Dewhirst, M. W.; McDonnell, D. P. Circumventing tamoxifen resistance in breast cancers using antiestrogens that induce unique conformational changes in the estrogen receptor. *Cancer Res*. **2001**, *61*, 2917−2922.

(32) Sathya, G.; Jansen, M. S.; Nagel, S. C.; Cook, C. E.; McDonnell, D. P. Identification and characterization of novel estrogen receptor-*β*-sparing antiprogestins. *Endocrinology* **2002**, *143*, 3071−3082.

(33) Liu, H.; Lee, E.-S.; De Los Reyes, A.; Zapf, J. W.; Jordan, V. C. Silencing and reactivation of the selective estrogen receptor modulator−estrogen receptor α complex. *Cancer Res*. **2001**, *61*, 3632−3639.

(34) Levenson, A. S.; Svoboda, K. M.; Pease, K. M.; Kaiser, S. A.; Chen, B.; Simons, L. A.; Jovanovic, B. D.; Dyck, P. A.; Jordan, V. C. Gene expression profiles with activation of the estrogen receptor α-selective estrogen receptor modulator complex in breast cancer cells expressing wild-type estrogen receptor. *Cancer Res*. **2002**, *62*, 4419−4426.

(35) Hanson, R. N.; Lee, C. Y.; Friel, C. J.; DeSombre, E. R.; Hughes, A. Evaluation of 17α-(trifluoromethylphenyl)vinyl estradiols as novel estrogen receptor ligands. *Steroids* **2003**, *68*, 143−148.

(36) Hanson, R. N.; Lee, C. Y.; Friel, C. J.; Dilis, R.; DeSombre, E. R.; Hughes, A. Synthesis and evaluation of (17α,20*E*)-21-(4-substituted-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diols as probes for the estrogen receptor-α (ERα) hormone binding domain. *J. Med. Chem.* **2003**, *46*, 2865−2876.

(37) Lebl, T.; Holecek, J.; Dymal, M.; Steinborn, D. Synthesis and characterization and reactivity of 2-functionalized vinylstannanes. *J. Organomet. Chem.* **2001**, *625*, 86−94.

(38) Lipshutz, B. H.; Keil, R.; Barton, J. C. Preparation of *Z*-vinylstannanes via hydrozirconation of stannylacetylenes. *Tetrahedron Lett.* **1992**, *33*, 5861−5864.

(39) Sielstad, D. A.; Carlson, K. E.; Katzenellenbogen, J. A.; Kushner, P. J.; Greene, G. L. Molecular characterization by mass spectrometry of the human estrogen receptor ligand-binding domain expressed in *Escherichia coli*. *Mol. Endocrinol.* **1993**, *9*, 647−658.

(40) DeSombre, E. R.; Mease, R. C.; Sanghavi, J.; Singh, T.; Seevers, R. H.; Hughes, A. Estrogen receptor binding affinity and uterotrophic activity of triphenylhaloethylenes. *J. Steroid Biochem.* **1988**, *29*, 583−590.

(41) Raynaud, J. P.; Ojasoo, T.; Bouton, M. M.; Philibert, D. Receptor binding as a tool in the development of new bioactive steroids. In *Drug Design*; Ariens, E. J., Ed.; Academic Press: New York, 1979; Vol. VIII, pp 169−214.

(42) Eiler, S.; Gangloff, M.; Duclaud, S.; Moras, D.; Ruff, M. Overexpression, purification and crystal structure of native ER alpha LBD. *Protein Expression Purif*. **2001**, *20*, 165-

(43) Littke, A. F.; Fu, G. C. The first general method for Stille cross-couplings of aryl chlorides. *Angew. Chem., Int. Ed*. **1999**, *38*, 2411−2416.

(44) Littke, A. F.; Fu, G. C. Palladium-catalyzed coupling reactions of aryl chlorides. *Angew. Chem., Int. Ed.* **2002**, *41*, 4176−4211.

(45) Bapat, A. R.; Frail, D. E. Full-length estrogen receptor α and its ligand-binding domain adopt different conformations upon ligand binding. *J. Steroid Biochem. Mol. Biol*. **2003**, *86*, 143−149.

(46) Wurtz, J.-M.; Egner, U.; Heinrich, N.; Moras, D.; Mueller-Fahrnow, A. Three-dimensional models of estrogen receptor ligand binding domain complexes, based on related crystal structures and mutational and structure−activity relationships. *J. Med. Chem.* **1998**, *41*, 1803−1814.

(47) Sippl, W. Receptor-based 3-D QSAR analysis of estrogen receptor ligands—merging the accuracy of receptor-based alignments with the computational efficiency of ligand-based methods. *J. Comput.-Aided Mol. Des.* **2000**, *14*, 559−572.

(48) Egner, U.; Heinrich, N.; Ruff, M.; Gangloff, M.; Mueller-Fahrnow, A.; Wurtz, J.-M. Different ligands−different receptor conformations: modeling of the hERα-LBD in complexes with agonists and antagonists. *Med. Res. Rev.* **2001**, *21*, 523−539.

(49) Wolohan, P.; Reichert, D. E. CoMFA and docking study of novel estrogen receptor subtype selective ligands. *J. Comput.-Aided Mol. Des.* **2003**, *17*, 313−328.

(50) Schipira, M.; Abagyan, R.; Totrov, M. Nuclear hormone receptor targeted virtual screening. *J. Med. Chem.* **2003**, *46*, 3045−3059.

(51) van Lipzig, M. M. H.; ter Laak, A. M.; Jongejan, A.; Vermeulen, N. P. E.; Wamelink, M.; Geerke, D.; Meerman, J. H. N. Prediction of ligand binding affinity and orientation of xenoestrogens to the estrogen receptor by molecular dynamics simulations and the linear interaction energy method. *J. Med. Chem*., in press.

(52) DeLisle, R. K.; Yu, S.-J.; Nair A. C.; Welsh, W. J. Homology modeling of the estrogen receptor subtype *β* (ER*β*) and calculation of ligand binding affinities. *J. Mol. Graphics Modell.* **2001**, *20*, 155−167.

(53) Sebag, A. B.; Friel, C. J.; Hanson, R. N.; Forsyth, D. A. Conformational studies on (17α,20*Z*)-21-(X-phenyl)-19-norpregna-1,3,5(10),20-tetraene-3,17*β*-diols using 1D and 2D NMR spectroscopy and GIAO calculations of ^{13}C shieldings. *J. Org. Chem*. **2000**, *65*, 7902−7912.

(54) Katzenellenbogen, B. S.; Sun, J.; Harrington, W. R.; Kraichely, D. M.; Ganessunker, D.; Katzenellenbogen, J. A. Structure−function relationships in the estrogen receptors and the characterization of novel selective estrogen receptor modulators with unique pharmacological profiles. *Ann. N. Y. Acad. Sci*., submitted.

(55) I*nsight II, 97.2* and *Discover 2.9.7*; MSI Inc.: San Diego, CA.

(56) Nair, A. C.; Miertus, S.; Tossi, A.; Romeo, D. A computational study of the resistance of HIV-1 aspartic protease to inhibitors ABT-538 and VX-478 and design of new analogues. *Biochem. Biophys. Res. Commun.* **1998**, *242*, 545−551.

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