Bridged Bicyclic Cores Containing a 1,1-Diarylethylene Motif Are High-Affinity Subtype-Selective Ligands for the Estrogen Receptor

Rajeev S. Muthyala,[†] Shubin Sheng,[‡] Kathryn E. Carlson,[†] Benita S. Katzenellenbogen,[‡,§] and John. A. Katzenellenbogen*[,†]

Department of Chemistry, Department of Molecular and Integrative Physiology, and Department of Cell and Structural Biology, University of Illinois, Urbana, Illinois 61801

Received October 25, 2002

The actions of estrogens are mediated through the two estrogen receptors, ERα and ERβ. Compounds that interact selectively with ERα or ERβ are of interest because they could be used to explore the biological roles of these ER subtypes and they might be interesting estrogen pharmaceuticals. In a new approach to develop ER subtype-selective ligands, we have embellished the 1,1-diarylethylene motif, common to many nonsteroidal estrogens, with various bridged bicyclic or tricyclic cores, including ones based on bicyclo[3.3.1]nonane, bicyclo[2.2.1]-heptane, and selected bi- and tricyclic terpenoids. This design leads to three-dimensional ER ligands of unusual structure that we have used to probe the size and shape of the ligand binding pocket of ERα and ERβ. Many of these compounds have high binding affinities, with the best having a bicyclo[3.3.1]nonane core and binding 3−5 times better than estradiol to both ER subtypes. Some of the compounds show significant affinity selectivity in favor of ERβ (4- to 5-fold), and in cell-based assays for transcriptional activity most are partial agonists on ERα and full antagonists on ERβ.

Introduction

The estrogen receptor (ER)[1,2] is a ligand-regulated transcription factor that mediates many of the actions of estrogens. The principal endogenous ligand for ER in most species is 17β-OH estradiol (E2). Over the years, many synthetic and natural ligands have been investigated as potential analogues of E2,[3−6] in the search for agents that that would be useful in regulating fertility, in preventing and treating breast cancer, and for menopausal hormone replacement.

The pharmacology of estrogens is complex, and because certain estrogens show tissue selectivity, it is difficult to classify a particular estrogen as being an agonist or an antagonist, without specific reference to a particular response. Thus, the term selective estrogen receptor modulators or SERMs[7] has been coined to emphasize the fact that what an ER ligand does is bind to the ER and stabilize a specific protein conformation. It is the subsequent interactions that this SERM−ER complex has with cell- and promoter-specific factors that then determine whether the ligand has an agonist or an antagonist effect.[8−10]

There are two ER subtypes: ERα and ERβ. They have significant homology in their ligand-binding domain, but their tissue distribution is different.[11] For example, ERα is expressed primarily in breast and uterine tissues whereas ERβ is found mainly in the brain, bone, and vascular epithelium. The gene regulatory activities of the two subtypes are also different. ERα knockout mice have reduced fertility and show greatly muted uterine development, whereas ERβ knockout mice show impairment of cognitive function yet retain normal function in the breast and uterine tissue.[12] Thus, it is possible that some of the tissue-selective pharmacology of estrogens could be achieved by selective binding to and/or activation of one or the other of these two ER subtypes. This possibility has spawned a major effort in academic and industrial labs to identify ER subtype-specific ligands.[13−20]

Structural studies on the ERs have suggested that there is ample unoccupied space within the ligand binding pocket,[21] leading one to hypothesize that an ER ligand with an overall three-dimensional topology would be better able to utilize this available structural space. In fact, a number of recent reports have described both steroidal[22] and nonsteroidal estrogens, such as those derived from ferrocene,[23] carboranes,[24,25] and polycyclics,[26] made up of structural elements having a pronounced overall three-dimensional topology.

In light of these recent reports and in continuation of our interest in nonsteroidal estrogens, we have taken a novel approach to probe the ligand binding pockets in the two ERs. We have started with the 1,1-diarylethylene unit, a motif common to many nonsteroidal estrogens and exemplified in the well-known nonsteroidal estrogen cyclofenil (Figure 1),[27] and embellished it with various bridged bicyclic or tricyclic cores (see the bicyclo[3.3.1]nonane example, Figure 1). These bicyclic and tricyclic cores present unusual sizes and geometries that can be used to probe the size, shape, and flexibility of the ERα and ERβ ligand binding pockets. In particular, this design results in ER ligands having an inherent three-dimensional topology that is much more pronounced than that of the endogenous estrogen, estradiol, and of cyclofenil (compare the three stereo structures

* To whom correspondence should be addressed. Address: Department of Chemistry, 461 Roger Adams Laboratory, Box 37-5, University of Illinois, 600 S. Mathews Avenue, Urbana, Il 61801. Telephone: (217) 333-6310. Fax: (217) 333-7325. E-mail: jkatzene@uiuc.edu.
[†] Department of Chemistry.
[‡] Department of Molecular and Integrative Physiology.
[§] Department of Cell and Structural Biology.

10.1021/jm0204800 CCC: $25.00
Published on Web 04/02/2003

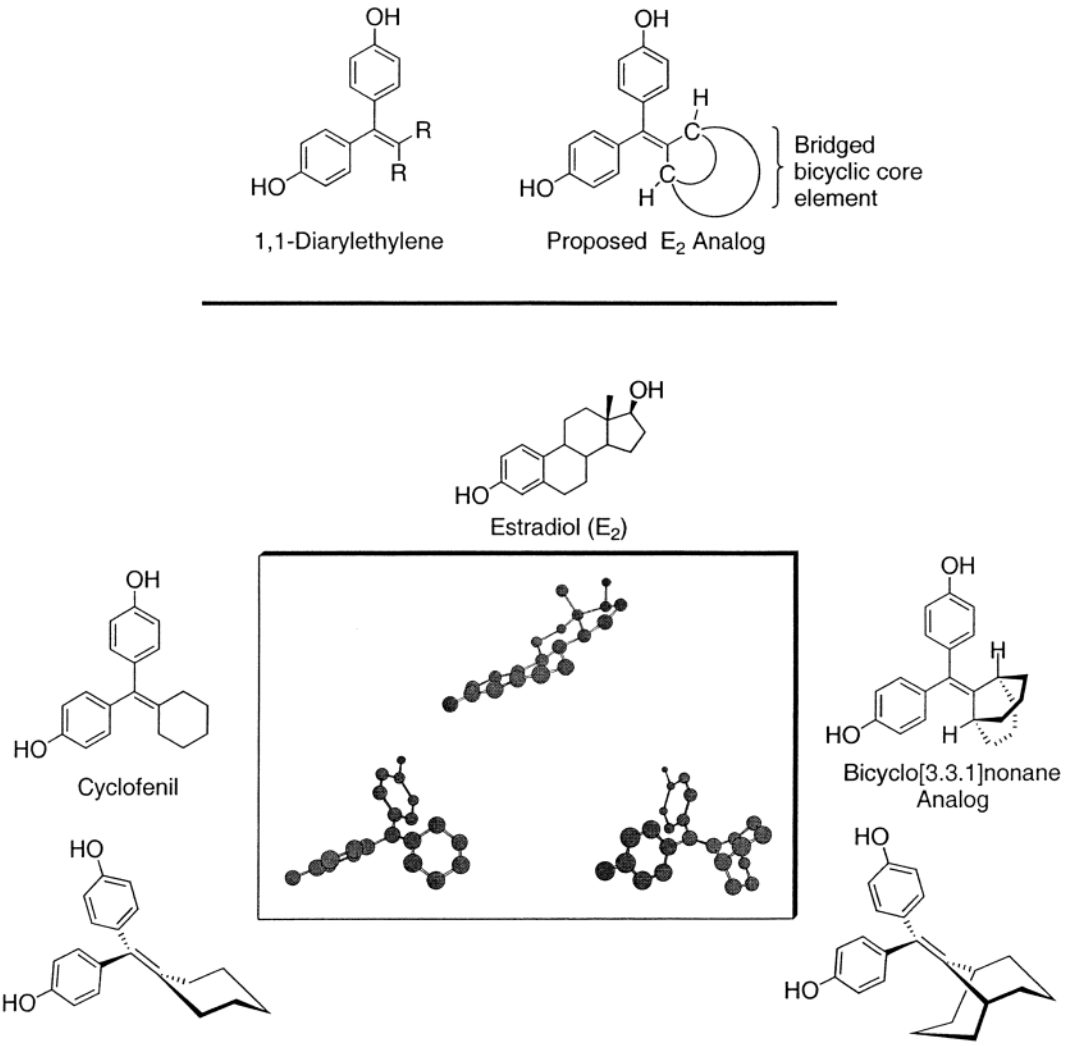


Figure 1. Comparison of the structural motifs for ER ligands. Two- vs three-dimensional ligand cores and their stereo representations.

in Figure 1), as well as that of most other known nonsteroidal estrogens. Thus, our approach here differs from our prior investigations in ER ligand design that have been based on cores that are inherently two-dimensional.[3,17−20] Many of the analogues we have prepared have high ER binding affinities, and some show distinct size and shape discrimination between the two ER subtypes, in some cases favoring ERβ in affinity by up to 5-fold. In cell-based assays for transcriptional activity, certain analogues are also more efficacious on ERα than on ERβ, but they are more potent as antagonists on ERβ.

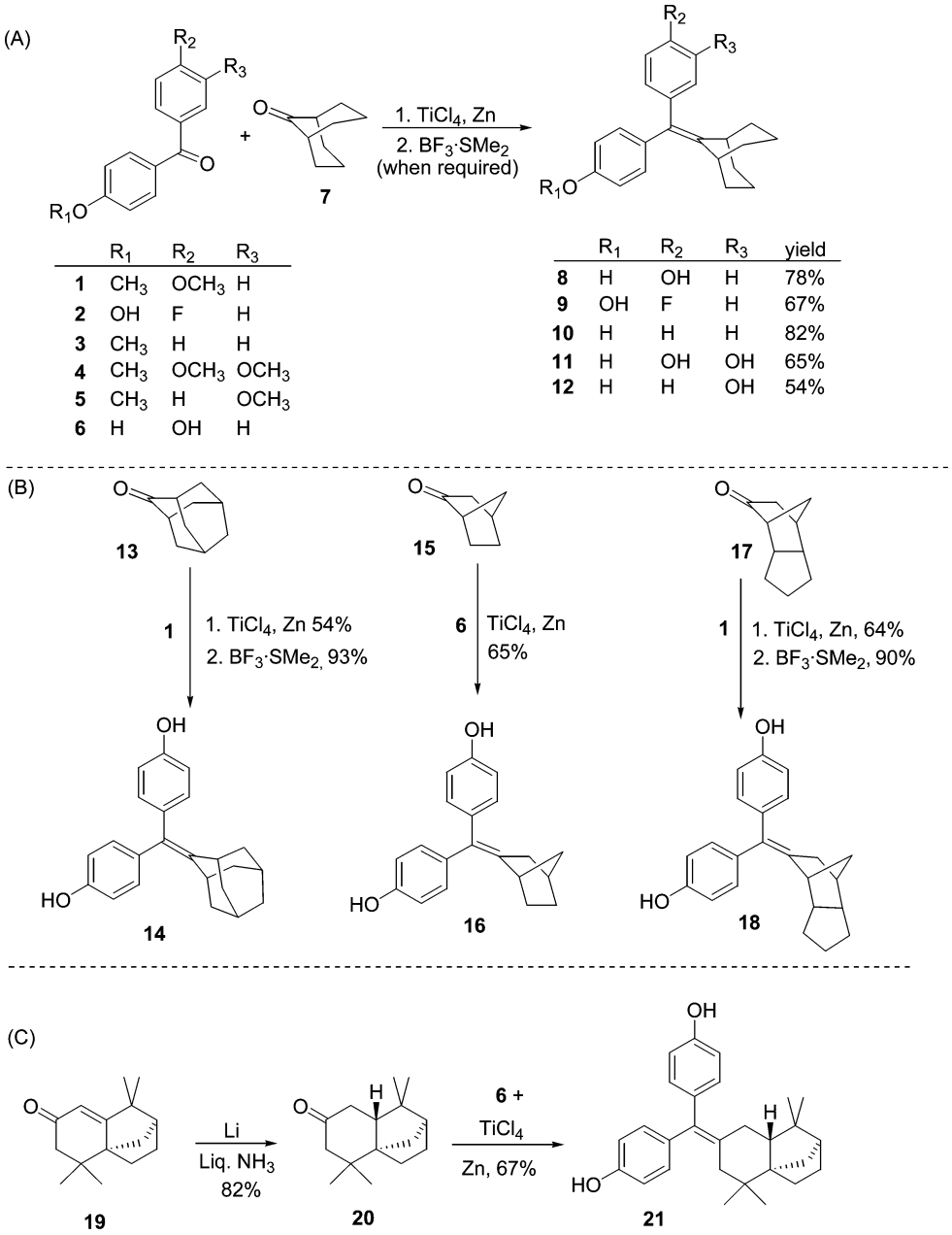
Results and Discussion

Synthesis. The synthetic methods used to prepare the bicyclic core estrogens were tailored to the steric demands of the compounds. For sterically unencumbered ketones, the McMurry coupling[28] was the shortest and most successful route (Scheme 1). As is well precedented, this reductive cross-coupling is most efficient when one of the reactants is a diaryl ketone, in which case the cross-coupled alkene is the predominant or sometimes exclusive product formed.[29] Several compounds with a 1,1-diphenylethylene motif were synthesized starting from benzophenones containing either protected or unprotected phenolic groups and the appropriate bicyclic ketone (Scheme 1).

The McMurry coupling is rather sensitive to steric factors, and it failed with very hindered ketones, camphor (**24**) and fenchone (**25**). To overcome this problem, we investigated an alternative synthetic route (Scheme 2) involving conversion of the cyclic ketone into a gem-dibromo olefin, followed by a double Suzuki arylation reaction. Indeed, in a model experiment with 9-ketobicyclo[3.3.1]nonane, this reaction sequence did give the desired product, albeit in low yield. Unfortunately, we were unable to convert either camphor or fenchone into their respective gem-dibromo olefins by this method, presumably again for steric reasons.

Thus, to prepare the most sterically hindered alkenes, we used the 2-fold extrusion strategy developed by Barton and Kellog,[30] as outlined in Scheme 3. In the first step, the hindered ketones were converted to their thioketo derivatives using tin-assisted sulfuration methodology developed by Steliou and Mrani.[31] The thioketones derived from the hindered bicyclic ketones fenchone and camphor are relatively stable and readily underwent reaction with a diphenyldiazomethane (produced by the MnO_2 oxidation of a hydrazone)[32,33] to give the thiadiazoline precursor. The subsequent loss of

Scheme 1



	R_1	R_2	R_3
1	CH_3	OCH_3	H
2	OH	F	H
3	CH_3	H	H
4	CH_3	OCH_3	OCH_3
5	CH_3	H	OCH_3
6	H	OH	H

	R_1	R_2	R_3	yield
8	H	OH	H	78%
9	OH	F	H	67%
10	H	H	H	82%
11	H	OH	OH	65%
12	H	H	OH	54%

nitrogen to produce the episulfide was facile and even occurred at room temperature, though the desulfurization step proved to be somewhat problematic. In our hands, the latter step was best accomplished by using the more nucleophilic tributylphosphine rather than triphenylphosphine.

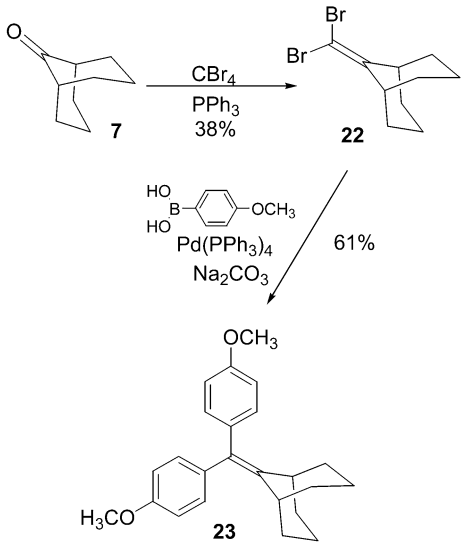
Purification of alkene **31**, a solid, was easily accomplished by recrystallization from methanol, but alkene **30**, an oil, required repeated chromatography to produce an analytically pure sample. Finally, removal of the methyl protecting groups with boron trifluoride/methyl sulfide afforded the phenolic bicyclic compound.

In attempts to reduce the double bond in tetrasubstituted alkene **23**, we found that hydrogenation at atmospheric pressure as well as ionic hydrogenation with triethylsilane/TFA gave only recovered starting material. Hydrogenation at elevated pressures (45 psi) and transfer hydrogenation with ammonium formate in refluxing acetic acid gave a mixture of the product and starting material. Fortunately, however, reduction with lithium in liquid ammonia,[34,35] using aniline as the quenching agent (Scheme 4A), gave very good yields of the reduced product, which after deprotection furnished the saturated analogue **34**.

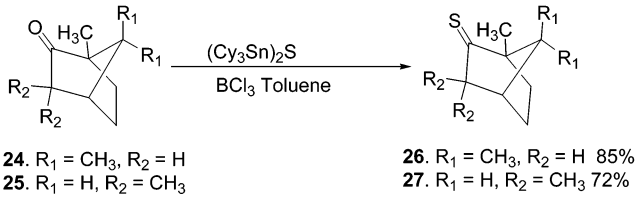
Compound **35**, which bears a structural resemblance to bisphenol A, was prepared (Scheme 4B) by the condensation of the commercially available bicyclic ketone **7** with phenol in the presence of either HCl gas or $BF_3{\cdot}OEt_2$ and a catalytic amount of butane thiol.[36] Presumably, the thiol serves to convert the ketone into a thioketal, which then undergoes displacement by phenol to give the product.

It is of note that of the chiral ligands, compounds **16** and **19** are racemates and compounds **21**, **32**, and **33** are derived from enantiomerically pure natural products, (+)-camphor (**24**), (−)-fenchone (**25**), and (−)-isolongifolenone (**19**). The correct enantiomers are shown for the enantiopure compounds.

Scheme 2

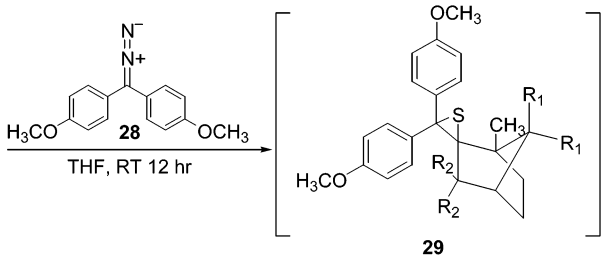


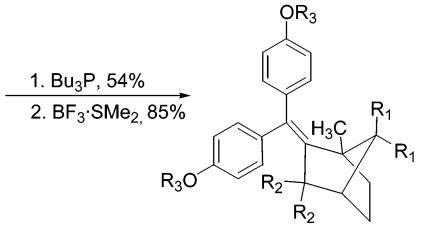
Scheme 3



24. $R_1 = CH_3$, $R_2 = H$
25. $R_1 = H$, $R_2 = CH_3$

26. $R_1 = CH_3$, $R_2 = H$ 85%
27. $R_1 = H$, $R_2 = CH_3$ 72%

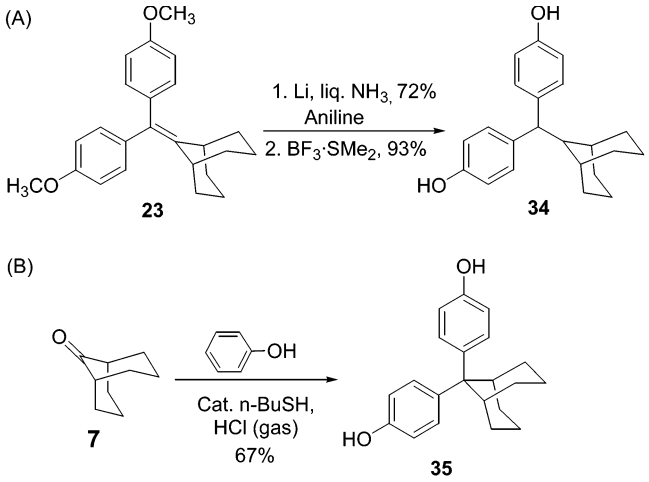




	R_1	R_2	R_3
30	CH_3	H	CH_3
31	H	CH_3	CH_3
32	CH_3	H	H
33	H	CH_3	H

Estrogen Receptor Binding Affinity. The binding affinity of ER ligands prepared in this study for purified human ERα and ERβ receptors was determined using a competitive radiometric binding assay, according to our published procedure.[37] Binding is expressed as relative binding affinity (RBA), where estradiol has an affinity of 100%. The RBA values are listed in Table 1.

Scheme 4

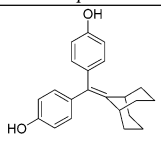
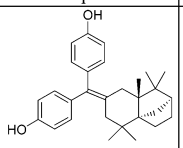
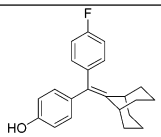
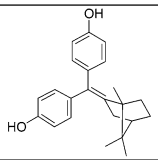
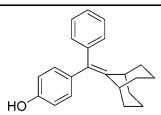
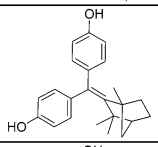
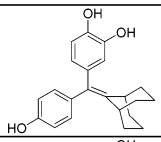
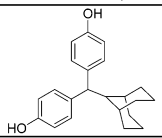
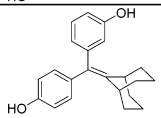
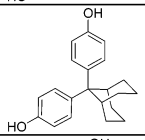
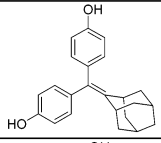
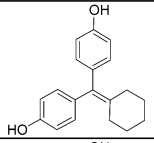
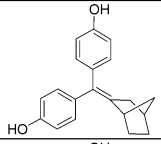
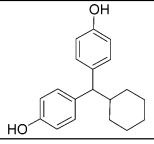
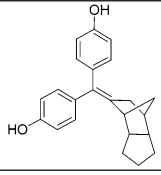


In general, the majority of the compounds show very high binding affinity to both ERα and ERβ, having RBA values that are in the 10−500% range for both ER subtypes. The highest affinity is shown by the ligands having the bicyclo[3.3.1]nonane core linked to the 1,1-diarylethylene unit. Bisphenol **8**, the best of this series with an ERα RBA of 500 and ERβ of 300, serves as a convenient benchmark for all of the others in terms of presenting structure−binding affinity relationships.

The effect of the phenolic functions on binding affinity is evident from a comparison of the bisphenol **8** with compounds **9**−**11**. Removal of one phenolic hydroxyl (**8** vs **10**) or its conversion to a fluoro substituent (**8** vs **9**) results in a 10- to 20-fold reduction in binding affinity to both ERα and ERβ. These large changes in affinity suggest that both phenols are playing important roles in binding with both ER subtypes, an issue that is discussed in the next section. By contrast, introduction of an additional phenolic hydroxyl (**8** vs **11**) or movement of one phenol from a para to a meta position (**8** vs **12**) has a more moderate effect: ERα binding is lowered 5- to 10-fold, but ERβ binding is less affected so that the catechol **11** and the isomeric phenol **12** have a moderate affinity preference for ERβ.

Changes to the size and shape of the bicyclic unit in these ligands (compounds **14**, **16**, **18**, **21**, **32**, **33**, and **36**) have interesting but unpredictable effects on ER binding affinity and subtype selectivity. Simply adding a methylene bridge, which constrains the bicyclo[3.3.1]-nonane system as an adamantane (**8** vs **14**), has a minimal effect on ERα binding (2-fold reduction) and no effect on ERβ binding, giving a ligand that has now lost ERα binding preference. By contrast, the ligand with the smaller bicyclo[2.2.1]heptane core (compound **16**) shows a far greater drop in ERα than in ERβ binding, giving the compound that has the highest ERβ binding preference of the ligands in this series. When the bicyclo[2.2.1]heptane core of compound **16** is altered, binding characteristics continue to change in various ways. Methyl substitution as in the camphor analogue (**16** vs **32**) has minimal effect, and ERβ selectivity is maintained, but the isomeric methylation as in the fenchone analogue (**33**) markedly lowers affinity and eliminates the ERβ selectivity. This is also the case with the isolongifolene-derived analogue (**21**).

Table 1. Relative Binding Affinity (RBA) of 1,1-Diarylethylene Analogues for Estrogen Receptors α and *β* Determined by a Competitive Radiometric Binding Assay with [^{3}H]Estradiol and Full Length Human ERα and ER*β*, Using Methods Described in the Experimental Section[a]

#	Compound	ERα	ERβ	β/α	#	Compound	ERα	ERβ	β/α
8		513±17	301 ±54	0.59	**21**		9±2	13±3	1.4
9		40±0	52±11	1.3	**32**		57±8	189±15	3.3
10		31±3	35±7	1.1	**33**		10±0.5	8±1.5	0.80
11		108±26	187±44	1.7	**34**		15±4	13±1	0.87
12		46±2	89±0	1.9	**35**		0.31±0.02	0.25±0.02	0.81
14		243±28	317±31	1.3	**36**		68±18	334±54	4.9
16		47±13	211±57	4.5	**37**		3.2±0.5	9±1.5	2.8
18		97±5	125±22	1.3					

[a] The RBA of estradiol is 100. Values represent the average ± range or SD of two to three independent determinations.

The other analogues that we have prepared are ones in which different aspects of the diphenylethylene system itself are altered, either by elimination of the double bond or by the way in which the two phenols are attached to the bicyclic system. In both cases we have studied, elimination of the double bond (i.e., **34** vs **8**, and **37** vs **36**) has a remarkable effect on binding affinity, with RBA values dropping 20- to 30-fold for both ER subtypes. While at first glance it might seem curious to see such a large decrease in affinity for what is apparently only a small structural change, elimination of the double bond has a significant effect on the preferred conformation of the ligand. Whereas the alkene fixes the relative orientation of the bisphenol system to the bicyclic (**8**) or monocyclic cores (**36**), in the more conformationally mobile alkane analogues (i.e., **34** and **37**, respectively), the preferred conformations are quite different, and considerable steric repulsion is encountered if the aryl rings in the alkanes are constrained to their alkene-like conformations.

Attachment of the phenols directly to the bicyclo[3.3.1]nonane system (**35** vs **8**) has an even more drastic effect on binding affinity (>1000-fold reduction). This suggests that both ERs have a "constriction" (or at least a limited deformability) somewhere in the middle of the ligand binding pocket.[21] In compound **35**, the bisphenylmethylene unit and the bicyclic unit are simply too close together, and they crowd this constriction site. We believe that a similar effect is noted, though to a lesser degree, in comparing the ligand with the simple bicyclo[2.2.1]heptane (**16**) with that derived from the fenchone analogue (**33**). In the latter analogue, two of the methyl groups are crowding this middle region (constriction site), whereas these methyl groups are more remote from this site in the camphor analogue (**32**). The

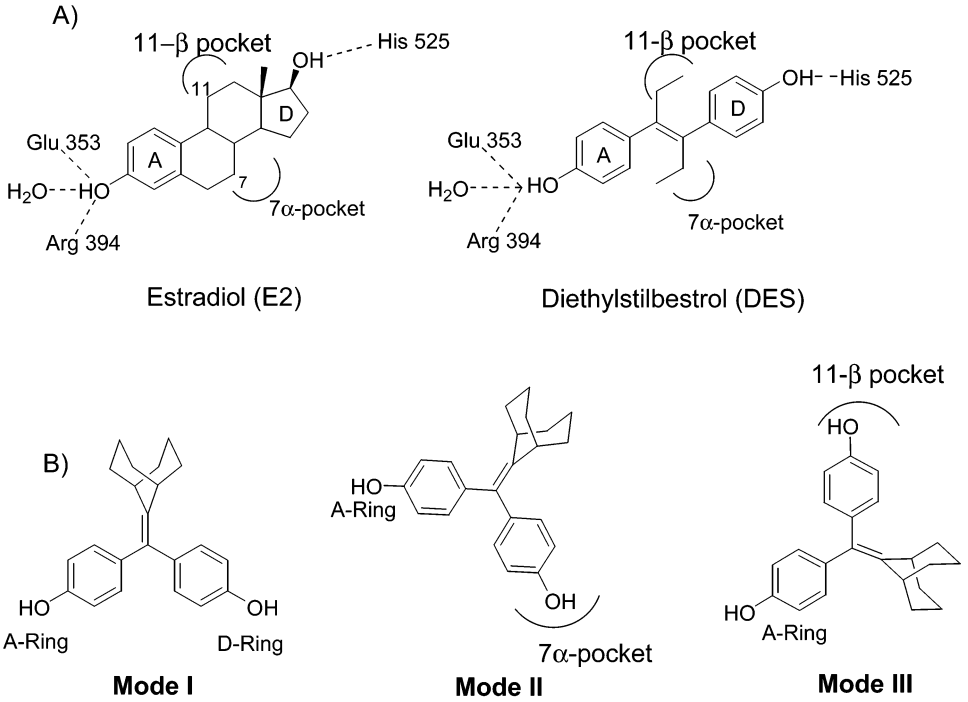


Figure 2. (A) Estradiol (E2) and diethylstilbestrol (DES) and their hydrogen bonding partners in ERα. (B) Schematic illustration of the three different binding modes for compounds containing a 1,1-diphenylethylene motif in the ligand binding pocket of the ER.

crowded isolongifolene-derived analogue (**21**) also encounters this constriction.

An important factor that needs to be taken into account with ligands containing the 1,1-diarylethylene moiety is the torsion angle between the two phenyl groups and the double bond. Owing to steric interactions between the ortho protons and the allylic bridgehead atoms (A1,3-strain), the two aromatic rings are not coplanar. In fact, an early study by Miquel observed a correlation between the estrogenic effects of certain 1,1-diarylethylenes and the torsion angle for the aryl groups.[38] This direct dependence of the RBA values on the torsion angle was attributed to an increase in the overall "thickness" (surface area or volume),[38] which leads to enhanced hydrophobic interactions with nearby protein residues and thus contributes favorably to the RBA values.

A notable exception to the above statement is found in the series of ligands we have examined based on the bicyclo[2.2.1]heptane core (compounds **16**, **32**, and **33**). In this series, the A1,3-strain increases in a progressive manner. The calculated dihedrals (determined in MACROMODEL) are 53°, 71°, and 80° for the unsubstituted (**16**), camphor-derived (**32**), and fenchone-derived (**33**) systems, respectively. From X-ray analysis, the experimentally determined value for compound **31**, the dimethyl ether of **33**, is 87° (for details, see Supporting Information). Thus, if there were a simple linear correlation between torsion angle and the binding affinity, then compound **33** should have a higher RBA value than either **32** or **16**. In our series, however, the relationship between torsion angle and binding affinity is reversed from that in the earlier Miquel study.[38] This indicates that other factors, such as a nonoptimal orientation of the phenyl rings and lack of steric complementarity in the binding pocket, are playing dominant roles rather than simply exposing the hydrophobic surface area.

Overall then, the size and shape of the bicyclic core unit in these ER ligands, as well as the disposition of the appended phenols, all prove to be factors in determining their affinity for the ER subtypes. High affinity is generally found when the diarylmethylene unit is attached to the bicyclic core at a site that is not excessively crowded. Crowding (as in **21** and **33**), reduction of the double bond (as in **34** and **37**), and direct aryl attachment to the bicyclic core (as in **35**) all reduce affinity markedly. ERα selectivity at a modest level is found in only one case (e.g., **8**), but ERβ selectivity is substantial when the cyclic portion is relatively small and unencumbered (e.g., **16** and **36**), as well as in one case that is more hindered (e.g., **32**). The variations in the binding affinities presumably reflect not only the differences in the overall volume of the ligand binding pockets but also its adaptability to accommodate various ligand shapes.

Molecular Modeling. As an aid to understanding the structure−affinity relationships shown in Table 1, we used molecular modeling. The first step in modeling was an evaluation of the orientation that these ligands might prefer to adopt in the binding pocket of the two ER subtypes. At least three different binding modes (Figure 2) can be postulated for compounds containing a 1,1-diarylethylene motif. Each of these was examined, with consideration of possible hydrogen bond formation, using the binding affinity results (Table 1) as a guide. Thus, ligand **8** was docked into the binding pocket of the receptor, and the entire protein−ligand complex was minimized.

In the first binding mode, an attempt was made to have the two phenol rings act as mimics of the 3-OH and the 17β-OH of estradiol (or the two hydroxyls in DES), with the bicyclic core contributing to the overall hydrophobicity of the ligand. In the two remaining binding modes, one phenol is used as the A-ring mimic,

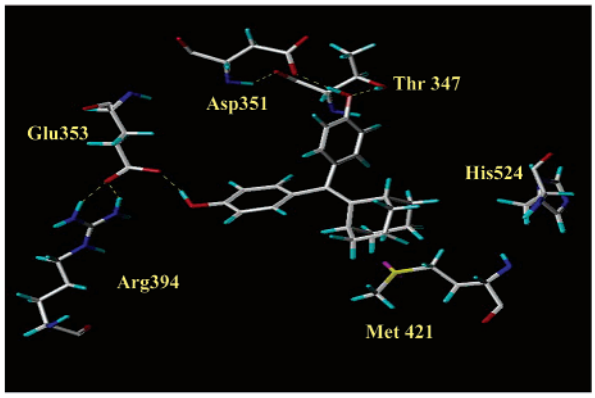


Figure 3. Ligand binding pocket of ERα with compound **8** in mode III.

and the second phenol, depending on its orientation, was extended into either the 7α or the 11β regions of the binding pocket of the receptor. Because there are sizable preformed or empty pockets in these regions of the receptor,[6,39,40] large ligand substituents that project into these regions are usually well tolerated and in most cases engender high binding affinities.[41,42]

A detailed evaluation of the three ligand-binding modes suggested that mode III for ligand binding is the most reasonable. Mode I does not appear to be preferred because the second phenol cannot reach the residue (histidine) that interacts with the 17β-hydroxyl of estradiol. In the remaining two modes, modeling suggested that one of the phenols can function nicely as an A-ring mimic, engaging in the two hydrogen bonds with the glutamate and arginine residues (Figure 3). Mode II, however, appears unlikely because the second phenol, which projects into the 7α binding pocket, is not apparently involved in any favorable interaction with nearby protein residues. By contrast, in mode III the second phenol on ligand **8** is oriented into the 11β pocket, which (unlike mode II) does contain at its edge two polar residues near the N terminus of helix-3. In ERα, this second −OH group is well positioned to engage in hydrogen bonding with Thr 347 and Asp 351 (Figure 3), but in ERβ, only Thr 299 is within hydrogen bonding distance. Furthermore, comparison of the energies of the final minimized protein−ligand complexes revealed that mode III is favored in both receptor ER subtypes by a considerable margin. Therefore, we propose that ligands containing a 1,1-diarylethylene motif bind in mode III and that contributions to their high binding affinities are made largely by several hydrogen bond interactions and the cumulative effects of a number of hydrophobic contacts made by the bicyclic core.

Our preference for mode III is also supported by the following. (1) Deletion of one of the phenolic −OH groups (as in compounds **9** and **10**), which would lead to loss of these hydrogen-bonding interactions, results in a very significant loss in binding affinity in both ER subtypes. (2) In this mode, the second phenol is placed in a position known to be occupied by one of the phenyl groups in the triarylethylene ER ligand hydroxytamoxifen.[39]

Using the ligand fit of mode III as a guide, we can see that the dramatic (25- to 30-fold) reduction in binding affinity when the double bond in ligands **8** and **36** is reduced (producing ligands **34** and **37**, respectively) results from an alteration in ligand shape. When the low-affinity, saturated compound **34** (in its minimum energy conformation with the two hydrogens antiperiplanar) is superimposed with the high-affinity, unsaturated parent **8** in ERα and both complexes are subjected to energy minimization, the reduced ligand **34** moves to a new position where it can no longer interact optimally with key residues on the receptor (Glu 353, Arg 394, Thr 347, and Asp 351).

Activity of 1,1-Diarylethylene Estrogens on Gene Transcription. To evaluate the activity of these bicyclic estrogens as transcriptional agonists and antagonists, cotransfection assays were conducted in human endometrial (HEC-1) cells, using expression plasmids for either ERα or ERβ and an estrogen-responsive reporter gene construct. In an initial screen, the agonist activity of the new compounds was determined at two concentrations, 10^{-8} and 10^{-6} M, and antagonist activity was assayed at 10^{-6} M but in the presence of 10^{-9} M E_2. In all cases, the transcriptional activity is normalized relative to that obtained with 10^{-9} M estradiol, which is set at 100%. These data are summarized in Table 2, where the percent efficacy of the compounds tested as agonists (at 10^{-8} and 10^{-6} M) and as antagonists (at 10^{-6} M) is given.

On the basis of the level of efficacy achieved in the agonist and antagonist modes, the compounds were classified as "agonists" (nearly full efficacy, >80%), "antagonists" (very little efficacy, <15%), "partial agonists" (50−80% efficacy), or "partial antagonists" (15−50% efficacy). In addition, compounds that failed to reach a similar level of efficacy at 10^{-6} M in both agonist and antagonist modes were classifed as "weak", whereas those showing equivalent efficacy at 10^{-8} and 10^{-6} M in agonist and 10^{-6} M in antagonist modes are classified as "potent". Complete dose−response curves were also obtained for seven compounds (**8**, **9**, **11**, **16**, **18**, **32**, and **36**) whose affinity and efficacy seemed most interesting (Figure 4). Again, for convenience, comparisons have been made with compound **8**, the high-affinity bicyclo[3.3.1]nonane ligand, as well as with cyclofenil (**36**).

The general feature of the pharmacology of these cyclic ER ligands is that they are all, with few exceptions, partial agonists on ERα and full antagonists on ERβ. Their potencies as agonists and antagonists are also generally quite good but are not as high as might be expected from their binding affinities. As is well-known, estradiol is a full agonist on ERα and ERβ (Figure 4A), having EC_{50} values of 10^{-11} and 10^{-10} M, respectively. By contrast, where they are ERα agonists, the bicyclo ER ligands typically have EC_{50} values of 10^{-9} to 3×10^{-8} M. Thus, it is curious that even though some of the bicyclic ligands bind to ERα with affinities 5 times greater than that of estradiol, their EC_{50} values are nevertheless considerably higher than that of estradiol. In their full antagonism on ERβ, these compounds have IC_{50} values in the range 3×10^{-9} to 3×10^{-8} M, whereas in their partial antagonism of ERα, they are less potent, with IC_{50} values that are typically 10- to 30-fold higher. This is consistent with the generally higher affinity of these ligands for ERβ than for ERα.

Within these general pharmacological features, there are a number of other interesting trends in the tran-

Table 2. Transcriptional Efficacy of 1,1-Diarylethylene Bicyclic Estrogens and Analogues on ERα and ERβ[a]

	% efficacy on ERα[b]				% efficacy on ERβ[b]			
	agonist[c]		agonist[c]	pharmacological	agonist[c]		agonist[c] (M)	pharmacological
compd	10^{-8} M	10^{-6} M	10^{-6} M	character[d]	10^{-8} M	10^{-6} M	10^{-6}	character[d]
8	25	22	23	potent partial antagonist	2	2	2	potent antagonist
9	58	63	65	partial agonist	7	10	16	partial antagonist
10	71	80	83	agonist	8	17	22	partial antagonist
11	15	20	38	weak partial antagonist	2	2	2	potent antagonist
14	20	20	19	potent partial agonist	2	2	2	potent antagonist
16	25	22	32	partial antagonist	3	3	3	potent antagonist
18	27	26	35	partial antagonist	2	2	2	potent antagonist
21	1	4	30	weak antagonist	1	1	15	weak antagonist
32	24	28	21	potent partial antagonist	3	4	4	potent antagonist
33	5	11	18	weak antagonist	2	3	13	weak antagonist
34	16	41	40	partial antagonist	21	50	55	partial agonist
35	1	6	43	very weak antagonist	2	4	78	very weak
36	9	12	22	weak partial antagonist	2	3	4	potent antagonist

[a] Transcriptional efficacy determined in cotransfection assay in HEC-1 cells using ERα or ERβ expression plasmids and an estrogen-regulated reporter gene plasmid (see Experimental Section for details). Values are percent of the transcriptional response of estradiol at 10^{-9} M. [b] Values are percent of the transcriptional response of estradiol at 10^{-9} M, and they represent the average of triplicate determinations (CV < 0.15). [c] Agonist assays are done with compound alone; antagonist assays are done with compound together with 10^{-9} M estradiol. [d] For a definition of these terms, see text.

scription data. (1) Most of the bicyclic compounds (**8**, **11**, **14**, **16**, **18**, and **32**) have an ERα/ERβ agonist/antagonist balance that is very similar to that of the parent compound cyclofenil (**36**); they have ca. 20−30% efficacy on ERα and are complete antagonists on ERβ (Table 2 and Figure 4). (2) The potencies of these compounds vary considerably, in some cases nicely reflecting differences in their ERα vs ERβ relative binding affinities. For example, compounds **16**, **32**, and **36**, which have the highest (3.3- to 4.9-fold) binding preference for ERβ, also show the greatest selectivity in terms of more potent antagonism of ERβ vs ERα (ca. 50-fold) (see parts E, G, and H of Figure 4). This ERβ selectivity in antagonist potency is considerably greater than that shown by the parent compound **8** (ca. 5-fold) (Figure 4B), consistent with the fact that it does not show ERβ affinity selectivity. (3) The two compounds that lack a second phenolic hydroxyl group (**9** and **10**) are the only compounds that show higher efficacy on both ERα (ca. 60−70%) and ERβ (ca. 20−30% efficacy) compared to those enumerated in point 1 above (Table 2 and Figure 4C). Removal of the second phenol abrogates ligand hydrogen bonding with the helix-3 residues (Asp and Thr), which might also release a constraint on the conformation of this region of the ligand binding domain that now allows helix-12 to pack more comfortably in the agonist conformation, thus engendering the increased level of agonist activity. Loss of this interaction probably also accounts for the reduced binding affinity of ligands **9** and **10**, compared to the parent ligand **8**. (4) Three compounds (**21**, **33**, and **35**) are essentially complete antagonists on both ERα and ERβ (Table 2). These compounds encompass those in which the core structures are very bulky (**21** and **33**) or those that lack the diarylethylene motif (**35**). These all have the lowest affinities and potencies of the compounds we have studied. (5) Compound **34**, which is the saturated analogue of the parent bicycle **8**, is a mixed agonist−antagonist with no particular ER subtype selectivity. It is the only compound that shows marked agonism on ERβ, but it is of low potency (Table 2). (6) For the bisphenolic compounds, the efficacy of transcription as well as the ERα agonist selectivity remains relatively constant despite an increase in the size of the bicyclic core from a C_7 to a C_{10} moiety; however, beyond a certain point, the level of transcriptional efficacy as well as the ERα agonist selectivity decreases. For example, compound **21**, which contains the largest C_{15} core derived from a sesquiterpenoid, is an antagonist on both ER subtypes (Table 2).

An intriguing point that emerges from our study of bicyclic 1,1-diarylethylenes is that their biological profile (partial agonist on ERα and antagonist on ERβ) is very reminiscent of some recently described ER ligands, the tetrahydrochrysenes (THCs)[18] and trisubstituted triazines,[13] yet there is very little structural similarity between the bicyclic 1,1-diarylethylenes and these two classes of ligands. The THCs, of note, even lack a basic side chain, indicating that this functionality is not obligatory for compounds to exhibit antagonism on ERβ. Perhaps the ERβ-selective antagonism of all of these compounds is simply a reflection of the greater facility with which helix 12 in the ERβ ligand-binding domain can be induced to adopt an antagonist conformation, a process that we termed "passive antagonism" in a recent structural study of THC complexes with ERα and ERβ.[43] An X-ray analysis of the ERs bound to ligand **8** would likely resolve this issue as well as confirm the orientation of the ligand in the binding pocket.

Conclusion

In summary, we have prepared novel ligands for the estrogen receptor (ER) that combine a familiar 1,1-diphenylethylene motif, found in the simple ligand cyclofenil (**36**), with various bicyclic core structures to probe the size, shape, and flexibility of the ligand binding pockets of ERα and ERβ. Most of these ligands have very high affinity for the ERs. The best (compound **8**) has a bicyclo[3.3.1]nonane core and binds 3−5 times better than estradiol to both ER subtypes. Two analogues based on the smaller bicyclo[2.2.1]heptane core (compounds **16** and **32**, as well as cyclofenil **36** itself) show affinity selectivity for ERβ as high as 4- to 5-fold.In cell-based transcription assays, most of the compounds were partial agonists on ERα and full antagonists on ERβ, and those that have preferential ERβ binding affinity are considerably more potent as ERβ antagonists than they are as antagonists of ERα.

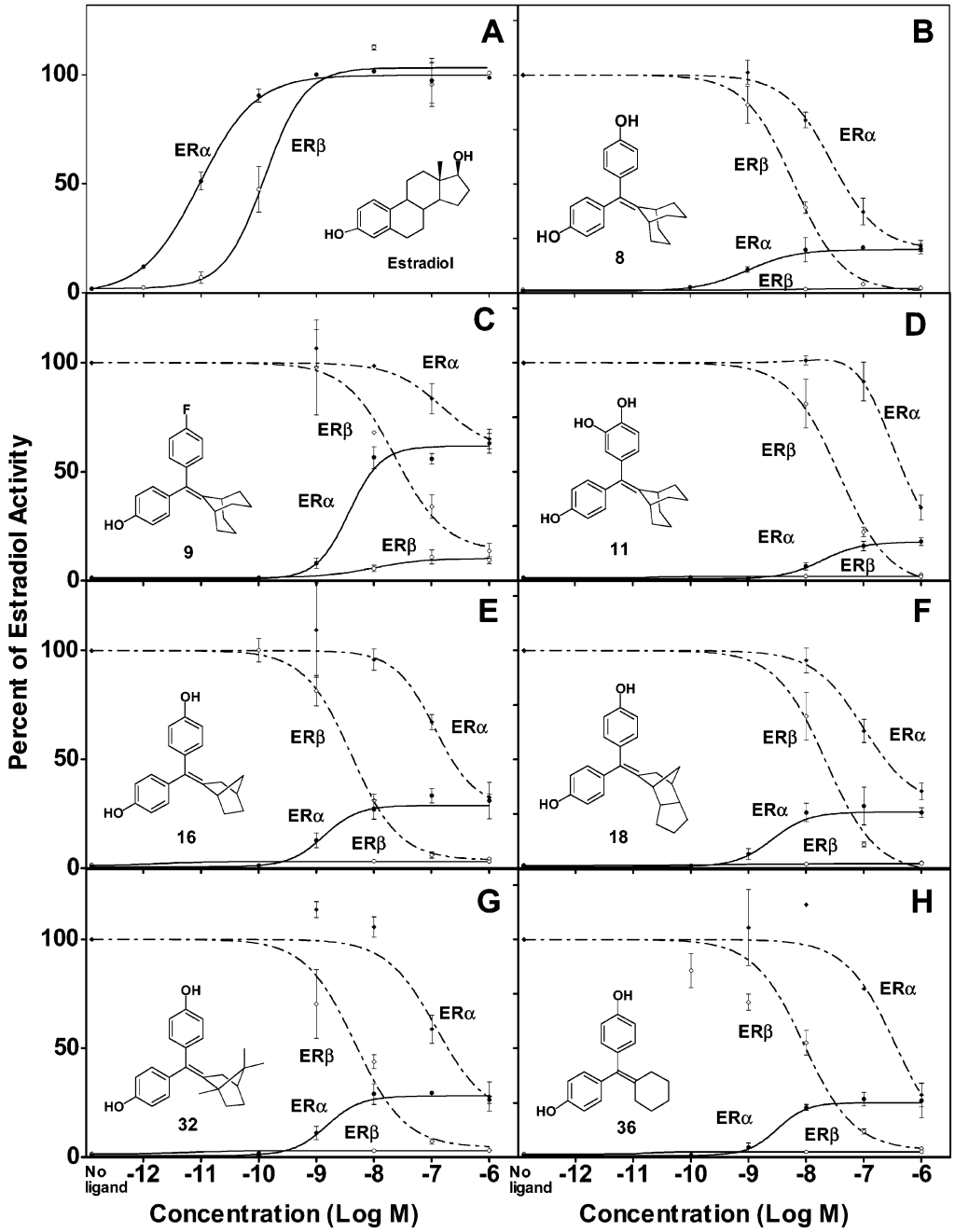


Figure 4. Transcription activation through ERα and ERβ in response to compounds with a 1,1-diphenylethylene motif. Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα, ERβ, and the estrogen responsive gene 2ERE-pS2-Luc and were incubated with the indicated concentrations of estradiol (E2) or ligands **8**, **9**, **11**, **16**, **18**, **32**, **36** (solid lines) for 24 h. Antagonist activity was assayed in the presence of 1 nM E2 (dashed lines). The values given are the mean ± SD of three or more experiments and are expressed as a percent of the ERα or ERβ response with 10^{-9} M E2.

Experimental Section

Materials and Methods. ^{1}H NMR spectra were obtained on a 500 MHz instrument. ^{13}C NMR spectra were acquired at 125 MHz. The chemical shifts are reported in ppm and are referenced to either tetramethylsilane or the solvent. Mass spectra were recorded under electron impact conditions at 70 eV. Melting points were obtained on a Thomas-Hoover Melt-emp apparatus and are uncorrected. Glassware was oven-dried, assembled while hot, and cooled under an inert atmosphere. Unless otherwise stated, all reactions were conducted in an inert atmosphere. Reactions using moisture- or air-sensitive reagents were performed in anhydrous solvents, which were collected when required using a solvent dispensing system built by by J. C. Meyer based on a design developed by Pangborn et al.[44] Benzophenones **4**[45]and **5**[46] were synthesized according to published procedures.

General Procedure A. McMurry Coupling. Titanium tetrachloride (0.420 mL, 3.7 mmol) was added slowly at −10 °C to a stirred suspension of zinc (0.490 g, 7.5 mmol) in 10 mL of anhydrous THF. The cooling was removed, and the reaction mixture was refluxed for 2.5 h. The reaction mixture was then cooled to ∼30 °C. At this point, a mixture of the two ketones (1 mmol each) in 8 mL of THF was added and the reaction mixture was refluxed again. After 1.5 h, the heating was stopped and the reaction mixture was cooled to room temperature and was poured slowly into a 10% K_2CO_3 solution. The mixture was extracted with 50 mL of ether, washed with brine, and dried on $MgSO_4$. The solvent was evaporated to provide the crude cross-coupled products, which were chromatographed using 10% EtOAc/hexane as the eluent.

General Procedure B. Deprotection of Methyl Ethers. Boron trifluoride/dimethyl sulfide (10 equiv) was added slowly

to a solution of methyl ethers (0.5 M solution in CH_2Cl_2) and was stirred at room temperature for 8−12 h. After the mixture was cooled to 0 °C, ethyl acetate (20 mL) was added to dilute the reaction mixture and the reaction was quenched by the addition of 5 mL of 10% HCl. Extraction with an additional 20 mL of EtOAc, followed by evaporation of the solvent in vacuo, furnished the crude phenols, which were subsequently chromatographed using 30% EtOAc/hexane as the eluent.

9-[Bis(4-hydroxyphenyl)methylene]bicyclo[3.3.1]-nonane (8). Following the general procedure A and using ketones **6** and **7** as the reactants, the isolated yield of **8** was 0.240 g (78%). ^{1}H NMR (acetone-d_6): δ 8.18 (s, 2H), 6.97 (d, 8.5 Hz, 4H), 6.75 (d, 8.5 Hz, 4H), 2.69 (br s, 2H), 2.09−1.98 (m, 3H), 1.84−1.72 (m, 10H), 1.59−1.53 (m, 2H). ^{13}C NMR: δ 156.2, 143.9, 135.2, 131.8, 130.7, 115.3, 34.6, 34.0, 22.0. MS (EI, 70 eV) *m*/*z*: 320 (M^+, 100%). HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O_2$, 320.1776; found, 320.1774.

9-[(4-Fluorophenyl)(4-hydroxyphenyl)methylene]-bicyclo[3.3.1]nonane (9). Using ketones **2** and **7** as the reactants and following the general procedure A, the isolated yield of **9** was 0.210 g (67%). ^{1}H NMR (acetone-d_6): δ 8.4 (br s, 1H), 7.26 (dd, 9 Hz, 6.5 Hz, 2H), 7.12 (t, 9 Hz, 2H), 7.09 (d, 9 Hz, 2H), 6.87 (d, 8.5 Hz, 2H), 2.81 (bs. s, 1H), 2.70 (br s, 1H), 2.18−2.09 (m, 2H), 1.93−1.82 (m, 8H), 1.67−1.63 (m, 2H). ^{13}C NMR (acetone-d_6, 125 MHz): δ 162.6, 160.7, 156.3, 145.3, 140.1, 140.08, 134.4, 131.3, 131.2, 130.8, 130.6, 115.3, 115.1, 114.9, 34.6, 34.4, 33.8, 21.9. HRMS (EI, 70 eV) calcd for $C_{22}H_{23}$-FO, 320.1733; found, 320.1730.

9-[(4-Hydroxyphenyl)phenylmethylene]bicyclo[3.3.1]-nonane (10). Following the general procedure A and using ketones **3** and **7** as the reactants, the isolated yield of the cross-coupled product was 0.26 g, (82%). ^{1}H NMR: δ 7.34 (t, 8 Hz, 2H), 7.25 (s, 1H), 7.24 (d, 8 Hz, 2H), 7.16 (d, 8.5 Hz, 2H), 6.89 (d, 8.5 Hz, 2H), 3.84 (s, 3H), 2.82 (br s, 1H), 2.76 (br s, 1H), 2.65−2.06 (m, 2H), 1.96−1.82 (m, 9H), 1.69−1.65 (m, 2H). ^{13}C NMR: δ 157.8, 145.3, 143.3, 135.5, 130.7, 130.3, 129.2, 127.9, 125.8, 113.3, 55.0, 34.1, 34.02, 33.7, 21.6. MS (EI, 70 eV) *m*/*z*: 318 (M^+, 100%). HRMS (EI, 70 eV) calcd for $C_{23}H_{26}O$, 318.1984; found 318.1984.

Deprotection. Starting from 0.1 g (0.31 mmol) of the McMurry coupling product and using the general procedure B, 0.082 g (87%) of compound **10** was obtained. ^{1}H NMR (acetone-d_6): δ 8.2 (br s, 1H), 7.26 (d, 8 Hz, 2H), 7.12 (t, 9 Hz, 2H), 7.09 (d, 9 Hz, 2H), 6.87 (d, 8.5 Hz, 2H), 2.81 (bs. s, 1H), 2.70 (br s, 1H), 2.18−2.09 (m, 2H), 1.93−1.82 (m, 8H), 1.67−1.63 (m, 2H). ^{13}C NMR (acetone-d_6, 125 MHz): δ 161.6, 160.5, 155.3, 143.3, 142.1, 139.08, 131.4, 130.3, 130.2, 129.8, 129.6, 113.3, 112.1, 111.9, 33.6, 32.4, 31.8, 20.9. HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O$, 304.1827; found, 304.1827.

9-[(3,4-Dihydroxyphenyl)(4-hydroxyphenyl)methylene]-bicyclo[3.3.1]nonane (11). Following the general procedure A and using ketones **4** and **7** as the reactants, the isolated yield of the cross-coupled product was 0.210 g (67%). ^{1}H NMR: δ 7.08 (d, 8.5 Hz, 2H), 6.81 (d, 8.5 Hz, 2H), 6.78 (d, 8.5 Hz, 1H), 6.69 (dd, 8.5 Hz, 1.5 Hz, 1H), 6.67 (d, 1.5 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 3.77 (s, 3H), 2.71 (br s, 2H), 2.08−2.00(m, 4H), 1.85−1.78 (m, 8H), 1.61−1.55 (m, 2H). ^{13}C NMR: δ 157.7, 148.2, 147.1, 144.9, 136.2, 135.5, 130.4, 130.2, 121.4, 113.2, 112.6, 110.6, 55.6, 55.0, 34.24, 34.21, 34.02, 33.72, 33.70, 21.6. MS (EI, 70 eV) *m*/*z*: 378 (M^+, 100%), 347 (M − 31, 14%). HRMS (EI, 70 eV) calcd for $C_{25}H_{30}O_3$, 378.2195; found, 378.2193.

Deprotection. Starting from 0.1 g (0.26 mmol) of the McMurry coupling product and using the general procedure B, 0.075 g (85%) of compound **11** was obtained. ^{1}H NMR-(acetone-d_6): δ 6.97 (d, 8.5 Hz, 2H), 6.75 (d, 8 Hz, 1H), 6.73 (d, 8.5 Hz, 2H), 6.62 (d, 2 Hz, 1H), 6.51 (dd, 8 Hz, 2 Hz, 1H), 2.75 (br s, 1H), 2.67 (br s, 1H), 2.07−1.98 (m, 3H), 1.84−1.71 (m, 8H), 1.58−1.52 (m, 2H). ^{13}C NMR (acetone-d_6): δ 155.9, 144.7, 143.7, 143.5, 135.7, 134.9, 131.7, 130.4, 120.9, 116.6, 114.9, 34.3, 33.7, 21.8. HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O_3$, 336.1725; found, 336.1720.

9-[(4-Hydroxyphenyl)(3-hydroxyphenyl)methylene]-bicyclo[3.3.1]nonane (12). Following the general procedure A and using ketones **5** and **7** as the reactants, the isolated yield of the cross-coupled product was 0.21 g (61%). ^{1}H NMR: δ 7.19 (t, 8 Hz, 1H), 7.11(d, 8.5 Hz, 2H), 7.09 (d, 8 Hz, 1H), 6.78 (d, 8.5 Hz, 1H), 6.74 (s, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 2.74 (m,1H), 2.71 (m, 1H), 2.09−2.00 (m, 2H), 1.88−1.77 (m, 8H), 1.63−1.58 (m, 2H). ^{13}C NMR: δ 159.2, 157.8, 145.4, 144.8, 135.3, 130.6, 130.3, 128.8, 121.8, 115.0, 113.3, 111.1, 55.1, 55.0, 34.2, 33.9, 33.7, 21.6. HRMS (EI, 70 eV) calcd for $C_{24}H_{28}O_2$, 348.2089; found, 348.2080.

Deprotection. Starting from 0.1 g (0.26 mmol) of the McMurry coupling product and using the general procedure B, the yield of compound **12** was 0.064 g (69%). ^{1}H NMR: δ 7.17 (t, 8 Hz, 1H), 7.15 (d, 8.2 Hz, 2H), 7.07 (d, 8 Hz, 1H), 6.58 (d, 8.5 Hz, 1H), 6.74 (s, 1H), 2.73 (m,1H), 2.69 (m, 1H), 2.08−2.00 (m, 2H), 1.85−1.74 (m, 8H), 1.61−1.52 (m, 2H). ^{13}C NMR: δ 158.2, 156.8, 145.1, 144.8, 134.6, 129.6, 129.3, 128.5, 120.5, 113.9, 113.4, 111.3, 34.1, 34.0, 33.4, 21.5. HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O_2$, 320.1776; found, 320.1772.

2-[Bis(4-hydroxyphenyl)methylene]adamantane (14). Following the general procedure A and using ketones **1** and **13** as the reactants, the isolated yield of the cross-coupled product was 0.190 g (54%). ^{1}H NMR: δ 7.05 (d, 8.5 Hz, 4H), 6.82 (d, 8.5 Hz, 1H), 3.79 (s, 6H), 2.81 (br s, 2H), 2.1−1.84 (m, 9H). ^{13}C NMR: δ 157.7, 145.7, 135.8, 130.6, 129.5, 113.2, 55.1, 39.5, 39.2, 37.4, 37.1, 28.2. mp 145−146 °C.

Deprotection. Starting from 0.1 g of the McMurry coupling product and using the general procedure B, the yield of compound **14** was 0.085 g (93%). ^{1}H NMR: δ 7.00 (d, 8 Hz, 4H), 6.71 (d, 8 Hz, 1H), 2.86 (br s, 2H), 2.0−1.84 (m, 9H). ^{13}C NMR: δ 153.7, 140.7, 133.8, 130.5, 128.7, 113.0, 39.4, 39.1, 36.4, 38.1, 28.7. HRMS (EI, 70 eV) calcd for $C_{23}H_{24}O_2$, 332.1776; found, 332.1774.

2-[Bis(4-hydroxyphenyl)methylene]bicyclo[2.2.1]-heptane (16). Following the general procedure A and using ketones **6** and **15** as the reactants, the isolated yield of the cross-coupled product **16** was 0.19 g (65%). ^{1}H NMR: δ 8.12 (s, 1H), 8.10 (s, 1H), 7.12 (dd, 8 Hz, 2 Hz, 1H), 7.01 (d, 7 Hz, 2H), 6.86 (dd, 8.5 Hz, 2 Hz, 1H), 6.70 (d, 8.5 Hz, 1H), 6.70 (d, 8.3 Hz, 2H), 6.64 (dd, 8 Hz, 2.5 Hz, 1H), 1.86−1.81 (m, 2H), 1.72 (tt, 4.5 Hz, 11.5 Hz, 1H), 1.62 (br s, 1H), 1.52 (tt, 4.5 Hz, 12.5 Hz, 1H), 1.40 (td, 5 Hz, 12.5 Hz, 1H). ^{13}C NMR: δ 156.05, 156.03, 154.2, 137.7, 136.6, 134.5, 131.5, 130.9, 129.9, 115.3, 114.6, 114.4, 52.2, 50.6, 46.95, 46.93, 46.0, 37.7, 26.41. HRMS (EI, 70 eV) calcd for $C_{20}H_{20}O_2$, 292.1463; found, 292.1460.

5-[Bis(4-hydroxyphenyl)methylene]octahydro-4,7-meth-anoindene (18). Following the general procedure A and using ketones **1** and **17** as the reactants, the isolated yield of the cross-coupled product was 0.23 g, (64%). ^{1}H NMR: δ 7.14 (d, 8.5 Hz, 2H), 7.11 (d, 8.5 HZ, 2H), 6.84 (d, 9 Hz, 2H), 6.83 (d, 9 Hz, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 2.70 (br s, 1H), 2.42 (dd, 16.5 Hz, 4.5 Hz, 1H), 2.22 (dd, 17 Hz, 8 Hz, 1H), 2.13 (d, 4.5 Hz, 1H), 1.98 (dd, 8 Hz, 17 Hz, 1H), 1.95−1.88 (m, 2H), 1.81 (dd, 3 Hz, 16.5 Hz, 1H), 1.70 (dt, 12 Hz, 6 Hz, 1H), 1.49 (d, 10 Hz, 1H), 1.31−1.21 (m, 3H), 1.04−0.95 (m, 2H). ^{13}C NMR: δ 157.63, 157.56, 144.0, 136.1, 135.9, 130.4, 130.3, 129.6, 113.26, 113.19, 113.14, 55.11, 55.09, 47.6, 47.2, 47.1, 40.9, 38.3, 33.2, 32.4, 31.6, 27.6. HRMS (EI, 70 eV) calcd for $C_{25}H_{28}O_2$, 360.2089; found, 360.2086.

Deprotection. Using 0.1 g (0.27 mmol) of the product from the McMurry reaction and using the general procedure B, the yield of compound **18** was 0.082 g (90%). ^{1}H NMR (acetone-d_6): δ 7.14 (d, 8.5 Hz, 2H), 7.11 (d, 8.5 HZ, 2H), 6.84 (d, 9 Hz, 2H), 6.83 (d, 9 Hz, 2H), 2.70 (br s, 1H), 2.42 (dd, 16.5 Hz, 4.5 Hz, 1H), 2.22 (dd, 17 Hz, 8 Hz, 1H), 2.13 (d, 4.5 Hz, 1H), 1.98 (dd, 8 Hz, 17 Hz, 1H), 1.95−1.88 (m, 2H), 1.81 (dd, 3 Hz, 16.5 Hz, 1H), 1.70 (dt, 12 Hz, 6 Hz, 1H), 1.49 (d, 10 Hz, 1H), 1.31−1.21 (m, 3H), 1.04−0.95 (m, 2H). ^{13}C NMR: δ 155.63, 156.5, 141.0, 136.1, 135.9, 130.4, 130.3, 129.6, 113.26, 113.19, 113.14, 55.09, 47.6, 47.2, 47.1, 40.9, 38.3, 33.2, 32.4, 31.6, 27.6. HRMS (EI, 70 eV) calcd for $C_{23}H_{24}O_2$, 332.1776; found, 332.1774.

1,1,5,5-Tetramethyloctahydro-2,4*a*-methanonaphtha-len-7-one (20). Lithium was added in three portions to liquid ammonia at −78 °C. The dark-blue solution was stirred for 15 min. The enone **19** ((−)-isolongifolenone, 0.5 g, 2.3 mmol)

in 5 mL of THF was then added, and the reaction mixture was stirred at −78 °C for 10 min and quenched with solid NH_4Cl (1 g). After complete evaporation of the ammonia, the reaction mixture was diluted with ether, washed with water, dried, and concentrated in vacuo. The residue was chromatographed on silica gel using 5% EtOAc/hexane as the eluent to give a white solid. Yield: 0.41 g (82%). ^{1}H NMR: δ 2.29 (t, 14 Hz, 1H), 2.19 (d, 14 Hz, 1H), 2.09 (dd, 14.% Hz, 1H), 1.91 (dd, 14 Hz, 1.5 Hz, 1H), 1.83−1.79 (m, 2H), 1.69−1.59 (m, 1H), 1.51−1.45 (m, 1H), 1.39−1.32 (m, 2H), 0.96 (s, 3H) 0.92 (s, 3H), 0.9 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (125 MHz): δ 212.9, 54.6, 53.2, 49.4, 48.4, 48.3, 38.6, 36.5, 36.4, 36.3, 31.7, 26.1, 25.9, 24.6, 21.1, 20.3. Mp: 56−58 °C; reported, 60 °C.[47]

7-[Bis(4-hydroxyphenyl)methylene]-1,1,5,5,-tetramethyloctahydro-2,4*a*-methanonaphthalene (21). Following the general procedure A and using ketones **20** and **6** as the reactants, the isolated yield for compound **21** was 0.268 g (67%). ^{1}H NMR: δ 6.94 (d, 8.5 Hz, 4H), 6.70 (d, 8.5 Hz, 4H), 2.19 (t, 14 Hz, 1H), 2.16 (d, 14 Hz, 1H), 2.07 (dd, 14.% Hz, 1H), 1.9 (dd, 14 Hz, 1.5 Hz, 1H), 1.81−1.79 (m, 2H), 1.68−1.59 (m, 1H), 1.51−1.45 (m, 1H), 1.39−1.32 (m, 2H), 0.96 (s, 3H), 0.92 (s, 3H), 0.9 (s, 3H), 0.77 (s, 3H). ^{13}C NMR (125 MHz): δ 155.9, 144.7, 143.7, 143.5, 135.7, 134.9, 131.7, 130.4, 120.9, 116.6, 114.9, 54.6, 53.2, 49.4, 48.4, 48.3, 38.6, 36.5, 36.4, 36.3, 31.7, 26.1, 25.9, 24.6, 21.1, 20.3. HRMS (EI, 70 eV) calcd for $C_{28}H_{32}O_2$, 400.2402; found, 400.2400.

9-Dibromomethylenebicyclo[3.3.1]nonane (22). Bicyclo-[3.3.1]nonanone (0.138 g, 1 mmol), carbon tetrabromide (0.67 g, 2 mmol), and triphenylphosphine (0.78 g, 2.9 mmol) were mixed together in *n*-heptane, and the reaction mixture was refluxed for 48 h. After the mixture was cooled to room temperature, the white solid was filtered and the filtrate was evaporated in vacuo to give a light-yellow oily residue. Chromatography on silica gel using hexane as the eluent gave 0.094 g of the product (38%) as an off-white solid. ^{1}H NMR: δ 3.1 (s, 2H), 2.03−1.93 (m, 2H), 1.82−1.70 (m, 8H), 1.51−1.46 (m, 2H). ^{13}C NMR (125 MHz): δ 164.5, 151.4, 38.5, 32.2, 21.2. MS (EI, 70 eV) *m*/*z*: 294, 211.9 (M − Br). HRMS (EI, 70 eV) calcd for $C_{10}H_{14}Br_2$, 291.9462; found, 291.9462. Mp: 77−79 °C.

9-[Bis(4-methoxyphenyl)methylene]bicyclo[3.3.1]-nonane (23). To a solution of compound **22** (0.09 g, 0.31 mmol) in toluene (5 mL) was added $Pd(PPh_3)_4$ (0.034 g, 0.03 mmol) and an aqueous solution of Na_2CO_3 (0.8 mL of a 1 M solution). This was then followed by the addition of 4-methoxyphenylboronic acid (0.103 g, 0.67 mmol). The mixture was refluxed vigorously for 12 h and cooled to room temperature. After oxidation of the unreacted residual boronic acid with 0.5 mL of 30% H_2O_2, the product was extracted with CH_2Cl_2, washed with brine, and dried on $MgSO_4$. The residue after evaporation of the solvent was chromatographed on silica gel using 5% EtOAC/hexane to give 0.064 g (61%) of compound **23**. ^{1}H NMR: δ 7.07 (d, 8.5 Hz, 4H), 6.82 (d, 8.5 Hz, 4H), 3.78 (s, 6H), 2.71 (br s, 2H), 2.08−1.98 9m, 2H), 1.86−1.75 (m, 8H), 1.61−1.56 (m, 2H). ^{13}C NMR (125 MHz): δ 157.7, 144.9, 135.8, 130.3, 130.2, 113.3, 55.1, 34.1, 33.7, 21.6. HRMS (EI, 70 eV) calcd for $C_{24}H_{28}O_2$, 348.2089; found, 348.2087.

Thiofenchone (27). Boron trichloride (0.84 mL of a 1 M solution in hexane) was injected into a toluene solution containing the tin sulfide (1.7 g mmol) and (−)-fenchone (0.152 g, 1 mmol). The reaction mixture was refluxed for 48 h. After the mixture was cooled to room temperature, the solvent was removed in vacuo and the residue was chromatographed on silica gel using hexane as the eluent to give an orange oil that gradually solidified on cooling (0.14 g, 85%). ^{1}H NMR: δ 2.31 (br s, 1H), 1.82 (dd, 8 Hz, 2 Hz, 1H), 1.77−1.73 (m, 3H), 1.66−1.61 (m, 2H), 1.32 (s, 3H), 1.29−1.22 (m, 2H), 1.17 (s, 3H), 1.13 (s, 3H). ^{13}C NMR: δ 66.3, 57.7, 46.8, 43.6, 35.3, 28.6, 26.3, 24.9, 19.1. Mp: 42−46 °C.

4,4′-Dimethoxydiphenyldiazomethane (28). Hydrazine hydrate (3 g, 60 mmol) was mixed with 4,4-dimethoxybenzophenone (2.43 g, 10 mmol) in 15 mL of absolute ethanol. The reaction mixture was refluxed for 48 h, cooled to room temperature, and filtered. The filtrate was then concentrated in vacuo to yield an off-white solid, which was recrystallized from 95% EtOH. The colorless crystals were collected and dried to give 2.14 g (83%) of 4,4′-dimethoxybenzophenonehydrazone. ^{1}H NMR: δ 7.40 (d, 8.5 Hz, 2H), 7.22 (d, 8.8 Hz, 2H), 7.03 (d, 8.8 Hz, 2H), 6.81 (d, 8.5 Hz, 2H), 3.85 (s, 3H), 3.78 (s, 3H). Mp: 82−83 °C (lit.,[32] 83−85 °C).

Oxidation with MnO_2.[32] 4,4-Dimethoxybenzophenonehydrazone (0.263 g, 0.97 mmol) was dissolved in 10 mL of CH_2Cl_2 and allowed to stir at room temperature. To this was added activated MnO_2 (0.183 g, 2.12 mmol) in one portion. The reaction mixture immediately attained a purple color. Stirring was continued for 4.5 h, and the reaction mixture was filtered through a 1 cm Celite pad. After the Celite pad was washed with 75 mL of CH_2Cl_2, the volatiles were removed in vacuo to yield 0.24 g (100%) of the crude diazoalkane as a purple solid, which was used in subsequent reactions without any further purification.

2-[Bis(4-methoxyphenyl)methylene]-1,3,3-trimethylbicyclo[2.2.1]heptane (30). To the crude diazoalkane **28** (0.306 g, 1.2 mmol) in THF was added thiocamphor (0.110 g, 0.65 mmol). The reaction mixture was stirred at room temperature for 14 h under a nitrogen atmosphere. Tri-*n*-butylphosphine (0.24 g, 1.1 mmol) was added, and the reaction mixture was refluxed for 48 h. After the mixture was cooled to room temperature, the solvent was removed in vacuo. The residue was then dissolved in hexane (2 mL), and 1 mL of iodomethane was carefully added to it at 0 °C. The cooling was removed, and stirring was continued at room temperature for 2 h. The solvent was then removed in vacuo, and the residue was chromatographed twice on silica gel using 2% EtOAc/hexane as the eluent to give 0.16 g (67%) of compound **30** as a colorless oil. ^{1}H NMR: δ 6.79 (d, 8.5 Hz, 2H), 6.78 (d, 8.5 Hz, 2H), 7.06 (d, 8 Hz, 2H), 7.05 (d, 8 Hz, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 2.61 (dt, 16.5 Hz, 4.5 Hz, 1H), 1.85−1.62 (m, 5H), 1.22 (dt, 9 Hz, 4.5 Hz, 1H), 0.917 (s, 3H), 0.82 (s, 3 H), 0.48 (s, 3H). ^{13}C NMR: δ 157.8, 157.5, 147.0, 137.6, 136.0, 132.3, 130.75, 130.72, 129.64, 129.60, 129.2, 113.8, 113.5, 112.8, 55.1, 52.2, 49.4, 44.1, 39.1, 35.9, 28.0, 20.2, 19.1, 14.8. HRMS (EI, 70 eV) calcd for $C_{25}H_{30}O_2$, 362.2246; found, 362.2248.

2-[Bis(4-methoxyphenyl)methylene]-1,3,3-trimethylbicyclo[2.2.1]heptane (31). The experimental procedure was the same as described above for **30**. The amounts of reagents used were as follows: thiofenchone (0.1 g, 0.72 mmol), diphenyldiazomethane (0.28 g, 1.0 mmol), tri-*n*-butylphosphine (0.220 g, 1.0 mmol). The yield of desired product, **33**, was 0.116 g (54%). Mp: 133−135 °C. ^{1}H NMR: δ 7.17 (dd, 8.5 Hz, 2 Hz, 1H), 7.08 (d, 8 Hz, 1H), 6.94 (dd, 8.5 Hz, 2 Hz, 1H), 6.77−6.76 (m, 3H), 6.71 (dd, 8.5 Hz, 3 Hz, 2H), 3.76 (s, 3H), 3.75 (s, 3H), 1.86−1.81 (m, 2H), 1.71−1.66 (m, 1H), 1.65−1.63 (m, 1H), 1.56 (s, 1H), 1.53 (tt, 1H), 1.43 (td, 11.5 Hz, 3.5 Hz, 1H), 1.09 (s, 3H), 0.79 (s, 3H), 0.66 (s, 3H). ^{13}C NMR (125 MHz): δ 157.6, 154.8, 138.1, 137.1, 132.8, 130.9, 130.3, 129.3, 113.5, 112.7, 112.3, 55.1, 51.8, 50.0, 46.7, 45.7, 37.4, 29.8, 26.2, 25.5, 21.7. MS (EI, 70 eV) *m*/*z*: 362 (M^+, 100%), 347 (M − 15, 46%). HRMS (EI, 70 eV) calcd for $C_{25}H_{30}O_2$, 362.2246; found, 362.2250.

2-[Bis(4-hydroxyphenyl)methylene]-1,7,7-trimethylbicyclo[2.2.1]heptane (32). Starting with 0.1 g (0.27 mmol) of **30** and using the general procedure B, the yield of compound **32** was 0.78 g (85%). ^{1}H NMR (acetone-d_6): δ 6.78 (d, 8.5 Hz, 2H), 7.06 (d, 8 Hz, 2H), 7.05 (d, 8 Hz, 2H), 2.61 (dt, 16.5 Hz, 4.5 Hz, 1H), 1.85−1.62 (m, 5H), 1.22 (dt, 9 Hz, 4.5 Hz, 1H), 0.92 (s, 3H), 0.82 (s, 3 H), 0.48 (s, 3H). ^{13}C NMR: δ 157.8, 157.5, 147.0, 137.6, 136.0, 132.3, 130.75, 129.72, 129.64, 129.60, 129.2, 113.8, 113.5, 112.8, 52.2, 49.4, 44.1, 39.1, 35.9, 28.0, 20.2, 19.1, 14.8. HRMS (EI, 70 eV) calcd for $C_{23}H_{26}O_2$, 334.1933; found, 334.1930.

2-[Bis(4-hydroxyphenyl)methylene]-1,3,3-trimethylbicyclo[2.2.1]heptane (33). Starting with 0.1 g (0.27 mmol) of **31** and using the general procedure B, the yield of compound **33** was 0.082 g (89%). ^{1}H NMR: δ 8.12 (s, 1H), 8.10 (s, 1H), 7.12 (dd, 8 Hz, 2 Hz, 1H), 7.01 (d, 7 Hz, 2H), 6.86 (dd, 8.5 Hz, 2 Hz, 1H), 6.70 (d, 8.5 Hz, 1H), 6.70 (d, 8.3 Hz, 2H), 6.64 (dd, 8 Hz, 2.5 Hz, 1H), 1.86−1.81 (m, 2H), 1.72 (tt, 4.5 Hz, 11.5 Hz, 1H), 1.62 (br s, 1H), 1.52 (tt, 4.5 Hz, 12.5 Hz, 1H), 1.40

(td, 5 Hz, 12.5 Hz, 1H), 1.09 (s, 3H), 0.81 (s, 3H), 0.67 (s, 3H). ^{13}C NMR: δ 156.05, 156.03, 154.2, 137.7, 136.6, 134.5, 131.5, 130.9, 129.9, 115.3, 114.6, 114.4, 52.2, 50.6, 46.95, 46.93, 46.0, 37.7, 26.41, 26.42, 25.9, 22.0. HRMS (EI, 70 eV) calcd for $C_{23}H_{26}O_2$, 334.1933; found, 334.1933.

9-[Bis(4-hydroxyxyphenyl)methyl]bicyclo[3.3.1]-nonane (34). Ammonia (25 mL) was distilled at −78 °C into a 50 mL three-necked round-bottomed flask fitted with a coldfinger condenser. The olefin **8** (0.120 g, 0.34 mmol) in 2 mL of THF and aniline, 2 mL, were then added to the liquid ammonia, and the reaction mixture was stirred at −78 °C for 5 min. To this was added lithium in three portions. After stirring for about 7 min at −78 °C, the reaction mixture turned into a dark-blue solution. Stirring was continued for another 5 h, at which point TLC analysis showed complete disappearance of the starting material. Solid NH_4Cl (1.5 g) was then added in four portions, and the cooling was removed. The ammonia was evaporated, and the reaction mixture was diluted with 10 mL of moist THF and 20 mL of ether. The organic layer was extracted with three 25 mL portions of 3 N HCl to remove aniline. After evaporation of the solvent in vacuo, the light-brown oily residue was chromatographed on silica gel using 10% ethyl acetate/hexane as the eluent. The reduced product was collected as a white crystalline solid. Yield: 0.087 g, 72%. ^{1}H NMR: δ 7.13 (d, 8.5 Hz, 4H), 6.69 (d, 8.5 Hz, 4H), 3.96 (d, 12.5 Hz, 1H), 3.63 (s, 6H), 2.08 (d, 12 Hz, 1H), 1.75 (m, 4H), 1.58 (m, 4H), 1.45 (m, 4H), 1.25 (d, 7 Hz, 2H). ^{13}C NMR (125 MHz): δ 157.7, 136.9, 128.6, 113.8, 55.12, 55.11, 50.8, 45.2, 34.0, 28.8, 24.7, 22.5, 21.4. Mp: 158−159 °C. HRMS (EI, 70 eV) calcd for $C_{24}H_{30}O_2$, 350.2246; found, 350.2244.

Deprotection. Following the general procedure B and starting with 0.06 g (0.17 mmol) of the reduced product above, the yield of compound **34** was 0.051 g (93%). ^{1}H NMR (acetone-d_6): 7.10 (d, 8.5 Hz, 4H), 6.78 (d, 8.5 Hz, 4H), 3.7 (d, 12.5 Hz, 1H), 2.08 (d, 12 Hz, 1H), 1.75 (m, 4H), 1.58 (m, 4H), 1.45(m, 4H), 1.25(d, 7 Hz, 2H). ^{13}C NMR (125 MHz): δ 154.7, 135.9, 121.6, 113.8, 54.12, 50.9, 45.2, 31.0, 27.8, 23.7, 20.5, 19.4. HRMS (EI, 70 eV) calcd for $C_{22}H_{26}O_2$, 322.1933; found, 322.1932.

9,9-Bis(4-hydroxyphenyl)bicyclo[3.3.1]nonane (35). Bicyclo[3.3.1]nonanone (0.138 g, 1 mmol) and phenol (0.895 g, 9.5 mmol) were heated to 60 °C. Anhydrous HCl gas was then passed through the reaction mixture for 15 min. Heating was continued for 3 h, at which point the reaction mixture attained a dark-green color. After cooling to room temperature, the reaction mixture was diluted with 100 mL of water, extracted with ethyl acetate, and dried on magnesium sulfate and the solvent was removed in vacuo. The residue was chromatographed on silica gel to yield a colorless white solid, which was recrystallized from hexane/ethyl acetate to give 0.206 g (67%) of the desired compound. ^{1}H NMR (CD_2Cl_2, CD_4-OD, 1:1): δ 7.17 (d, 7 Hz, 4H), 6.62 (d, 7 Hz, 4H), 3.08 (br s, 2H), 2.08−2.04 (m, 4H), 1.97−1.86 (m, 2H), 1.62−1.58 (m, 4H), 1.32 (m, 2H). ^{13}C NMR (125 MHz): δ 154.1, 140.4, 127.5, 115.5, 47.1, 32.3, 32.2, 28.0, 21.6. MS (EI, 70 eV) *m*/*z*: 308 (M^+, 100%). HRMS (EI, 70 eV) calcd for $C_{21}H_{24}O_2$, 308.1776; found, 308.1773.

Estrogen Receptor Binding Affinity Assays. Relative binding affinities were determined by a competitive radiometric binding assay as previously described,[37,48] using 10 nM [^{3}H]-estradiol as tracer ([6,7-^{3}H]estra-1,3,5,(10)-triene-3,17-β-diol, 51−53 Ci/mmol, Amersham BioSciences, Piscataway, NJ), and purified full-length human ERα and ERβ receptors were purchased from Pan Vera (Madison, WI). Incubations were for 18−24 h at 0 °C. Hydroxyapatite (BioRad, Hercules, CA) was used to absorb the receptor−ligand complexes, and free ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values with the RBA of estradiol set to 100%. The values given are the average ± range or SD of two to three independent determinations. Estradiol binds to ERα with a K_d of 0.2 nM and to ERβ with a K_d of 0.5 nM.

Cell Culture and Transient Transfections. Human endometrial cancer (HEC-1) cells were maintained in minimum essential medium (MEM) plus phenol red supplemented with 5% calf serum and 5% fetal calf serum. Cells were plated in phenol-red-free improved MEM and 5% charcoal dextran-treated calf serum (CDCS) and were given fresh medium 24 h before transfection. Transfection assays were performed in 24-well plates using a mixture of 0.35 mL of serum-free improved MEM medium and 0.15 mL of Hank's balanced salt solution containing 5 μL of lipofectin (Life Technologies, Inc., Gaithersburg, MD), 1.6 μg of transferrin (Sigma, St. Louis, MO), 0.5 μg of pCMV β-galactosidase as internal control, 1 μg of 2ERE-pS2-Luc, and 100 ng of ER expression vector per well. The cells were incubated at 37 °C in a 5% CO_2-containing incubator for 5 h. The medium was then replaced with fresh improved MEM supplemented with 5% CDCS plus the desired concentrations of ligands. Cells were harvested 24 h later. Luciferase and β-galactosidase activity were assayed as described.[49]

Molecular Modeling. Small-molecule geometry optimization was carried out in either MACROMODEL or Sybyl (version 6.7, Tripos). MM3 was the force field of choice in the former case, whereas the MMFF94 force field was used in the latter. For protein−ligand complexes, Sybyl (version 6.7) was used exclusively. For ERα, the tamoxifen−ERα ligand binding domain (3ERT) crystal structure was used. The ligand, **8**, was prepostioned by first overlaying it on tamoxifen using a least-squares fit for select atoms. Tamoxifen was then deleted, and in its place, ligand **8** was merged and docked into the receptor using the Flexidock routine of Sybyl. For optimal docking, the hydrogen bond donors/acceptors and select torsional bonds on the ligand were defined. The best docked receptor−ligand complex was then subjected to a three-step minimization routine. In the first step, nonring torsional bonds were minimized using the torsmin command. In the second step, the side chains of amino acid residues within 8 Å of the ligand were minimized while holding the backbone and residues Glu 353 and Arg 394 fixed. In the final step, the protein−ligand complex was minimized with the anneal command, using a hot radius of 8 Å and an interesting radius of 16 Å. All minimizations used the MMFF94 force field with the Powell gradient (final rms < 0.1 kcal mol^{-1} Å^{-1}). An identical routine was followed for ERβ using the genistein−ERβ complex (1QKM).

Acknowledgment. We are grateful for support of this research through grants from the National Institutes of Health (Grants PHS 5R37 DK15556 [J.A.K] and 5R01 CA19118 [B.S.K]) and the U.S. Army Breast Cancer Research Program (Grant DAMD17-97-1-7076). NMR spectra were obtained in the Varian Oxford Instrument Center for Excellence in NMR Laboratory. Funding for the instrumentation was provided in part by the W. M. Keck Foundation, the National Institutes of Health (Grant PHS 1 S10 RR104444-01), and the National Science Foundation (Grant NSF CHE 96-10502). Mass spectra were obtained on instruments supported by grants form the National Institute of General Medical Sciences (Grant GM 27029), the National Instituted of Health (Grant RR 01575), and the National Science Foundation (Grant PCM 8121494). We thank Dr. Jerome Baudry for helpful discussions on molecular modeling and Dr. Scott Wilson for determining the X-ray structure of compound **31**.

Supporting Information Available: X-ray crystallographic data for compound **31** and HPLC purity data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Katzenellenbogen, J. A.; Katzenellenbogen, B. S. Nuclear hormone receptors: ligand-activated regulators of transcription and diverse cell responses. *Chem. Biol.* **1996**, *3*, 529−536.

(2) Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Estrogen receptor transcription and transactivation: Estrogen receptor alpha and estrogen receptor beta: regulation by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res.* **2000**, *2*, 335−344.

(3) Gao, H.; Katzenellenbogen, J. A.; Garg, R.; Hansch, C. Comparative QSAR analysis of estrogen receptor ligands. *Chem. Rev.* **1999**, *99*, 723−744.

(4) Grundy, J. Artificial estrogens. *Chem. Rev.* **1957**, *57*, 281−416.

(5) Solmssen, U. V. Synthetic estrogens and the relation between their structure and their activity. *Chem. Rev.* **1945**, *37*, 481−598.

(6) Anstead, G. M.; Carlson, K. E.; Katzenellenbogen, J. A. The estradiol pharmacophore: ligand structure−estrogen receptor binding affinity relationships and a model for the receptor binding site. *Steroids* **1997**, *62*, 268−303.

(7) Grese, T. A.; Sluka, J. P.; Bryant, H. U.; Cullinan, G. J.; Glasebrook, A. L.; Jones, C. D.; Matsumoto, K.; Palkowitz, A. D.; Sato, M.; Termine, J. D.; Winter, M. A.; Yang, N. N.; Dodge, J. A. Molecular determinants of tissue selectivity in estrogen receptor modulators. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14105−14110.

(8) Katzenellenbogen, J. A.; O'Malley, B. W.; Katzenellenbogen, B. S. Tripartite steroid hormone receptor pharmacology: Interaction with multiple effector sites as a basis for the cell- and promoter-specific action of these hormones. *Mol. Endocrinol.* **1996**, *10*, 119−131.

(9) Shang, Y.; Brown, M. Molecular determinants for the tissue specificity of SERMs. *Science* **2002**, *295*, 2465−2468.

(10) Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Biomedicine. Defining the "S" in SERMs. *Science* **2002**, *295*, 2380−2381.

(11) Kuiper, G. G. J. M.; Carlsson, B.; Grandien, K.; Enmark, E.; Häggblad, J.; Nilsson, S.; Gustafsson, J.-Å. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptor α and *β*. *Endocrinology* **1997**, *138*.

(12) Mueller, S. O.; Korach, K. S. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. *Curr. Opin. Pharmacol.* **2001**, *1*, 613−619.

(13) Henke, B. R.; Consler, T. G.; Go, N.; Hale, R. L.; Hohman, D. R.; Jones, S. A.; Lu, A. T.; Moore, L. B.; Moore, J. T.; Orband-Miller, L. A.; Robinett, R. G.; Shearin, J.; Spearing, P. K.; Stewart, E. L.; Turnbull, P. S.; Weaver, S. L.; Williams, S. P.; Wisely, G. B.; Lambert, M. H. A new series of estrogen receptor modulators that display selectivity for estrogen receptor *β*. *J. Med. Chem.* **2002**, *45*, 5492−5505.

(14) Henke, B. R.; Drewry, D. H.; Jones, S. A.; Stewart, E. L.; Weaver, S. L.; Wiethe, R. W. 2-Amino-4,6-diarylpyridines as novel ligands for the estrogen receptor. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1939−1942.

(15) Larrea, F.; Garcia-Becerra, R.; Lemus, A. E.; Garcia, G. A.; Perez-Palacios, G.; Jackson, K. J.; Coleman, K. M.; Dace, R.; Smith, C. L.; Cooney, A. J. A-ring reduced metabolites of 19-nor synthetic progestins as subtype selective agonists for ER alpha. *Endocrinology* **2001**, *142*, 3791−3799.

(16) Lin, X.; Huebner, V. Non-Steroidal Ligands for Steroid Hormone Receptors. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 383−398.

(17) Mortensen, D. S.; Rodriguez, A. L.; Carlson, K. E.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Synthesis and biological evaluation of a novel series of furans: ligands selective for estrogen receptor alpha. *J. Med. Chem.* **2001**, *44*, 3838−3848.

(18) Meyers, M. J.; Sun, J.; Carlson, K. E.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Estrogen receptor subtype-selective ligands: Asymmetric synthesis and biological evaluation of *cis*- and *trans*-5,11-dialkyl-5,6,11,12-tetrahydrochrysenes. *J. Med. Chem.* **1999**, *42*, 2456−2468.

(19) Meyers, M. J.; Sun, J.; Carlson, K. E.; Marriner, G. A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Estrogen receptor-*β* potency-selective ligands: structure−activity relationship studies of diarylpropionitriles and their acetylene and polar analogues. *J. Med. Chem.* **2001**, *44*, 4230−4251.

(20) Stauffer, S. R.; Coletta, C. J.; Tedesco, R.; Nishiguchi, G.; Carlson, K.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Pyrazole ligands: structure−affinity/activity relationships and estrogen receptor-α-selective agonists. *J. Med. Chem.* **2000**, *43*, 4934−4947.

(21) Pike, A. C.; Brzozowski, A. M.; Hubbard, R. E.; Bonn, T.; Thorsell, A. G.; Engstrom, O.; Ljunggren, J.; Gustafsson, J.; Carlquist, M. Structure of the ligand-binding domain of oestrogen receptor beta in the presence of a partial agonist and a full antagonist. *EMBO J.* **1999**, *18*, 4608−4618.

(22) Bull, J. R.; Hoadley, C.; Mountford, P. G.; Steer, L. M. Synthesis of 14,17-propano analogs of estradiol. *J. Chem. Soc., Perkin Trans. 1* **1997**, 1179−1192.

(23) Top, S.; Vessieres, A.; Cabestaing, C.; Laios, I.; Leclercq, G.; Provot, C.; Jaouen, G. Studies on organometallic selective estrogen receptor modulators. (SERMs) Dual activity in the hydroxy-ferrocifen series. *J. Organomet. Chem.* **2001**, *637−639*, 500−506.

(24) Endo, Y.; Iijima, T.; Yamakoshi, Y.; Fukasawa, H.; Miyaura, C.; Inada, M.; Kubo, A.; Itai, A. Potent estrogen agonists based on carborane as a hydrophobic skeletal structure: a new medicinal application of boron clusters. *Chem. Biol.* **2001**, *8*, 341−355.

(25) Valliant, J. F.; Schaffer, P.; Stephenson, K. A.; Britten, J. F. Synthesis of Boroxifen, A Nido-Carborane Analogue of Tamoxifen. *J. Org. Chem.* **2002**, *67*, 383−387.

(26) Yamakoshi, Y.; Otani, Y.; Fujii, S.; Endo, Y. Dependence of estrogenic activity on the shape of the 4-alkyl substituent in simple phenols. *Biol. Pharm. Bull.* **2000**, *23*, 259−261.

(27) Lunan, C. B.; Klopper, A. Antioestrogens. A review. *Clin. Endocrinol. (Oxford)* **1975**, *4*, 551−572.

(28) Coe, P. L.; Scriven, C. E. Crossed coupling of functionalized ketones by low-valent titanium (the McMurry reaction): a new stereoselective synthesis of Tamoxifen. *J. Chem. Soc., Perkin Trans. 1* **1986**, 475−477.

(29) McMurry, J. E.; Krepski, L. R. Synthesis of Unsymmetrical Olefins by Titanium(0) Induced Mixed Carbonyl Coupling. Some Comments on the Mechanism of the Pinacol Reaction. *J. Org. Chem.* **1976**, *41*, 3929−3930.

(30) Barton, D. H. R.; Guziec, F. S., Jr.; Shahak, I. Olefin synthesis by twofold extrusion processes. II. Synthesis of very hindered olefins. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1794−1799.

(31) Steliou, K.; Mrani, M. Tin Assisted Sulfuration: A Highly Potent Method for the Conversion of Carbonyl Units into Their Corresponding Thiocarbonyl Analogues. *J. Am. Chem. Soc.* **1982**, *104*, 3104−3106.

(32) Nakaya, T.; Wada, H.; Tanaka, M.; Imoto, M. Vinyl polymerization. 269. Polymerization of methyl methacrylate initiated by p′p′-dimethoxydiphenyldiazomethane. *J. Macromol. Sci., Chem.* **1971**, *5*, 1043−1055.

(33) Reimlinger, H. Darstellung und eigenschaften einiger diaryldiazomethane. *Chem. Ber.* **1967**, *97*, 3493−3502.

(34) Douglas, G. H.; Graves, J. M. H.; Hartley, D.; Hughes, G. A.; McLoughlin, B. J.; Siddall, J.; Smith, H. Totally synthetic steroid hormones. I. Estrone, and related estrapolyenes. *J. Chem. Soc.* **1963**, 507294.

(35) Johns, W. F. Estra-4,6,8(14)-trienes. *J. Org. Chem.* **1966**, *31*, 3780−3784.

(36) Liaw, D.-J.; Liaw, B.-Y.; Chung, C.-Y. Synthesis and characterization of new adamantane-type cardo polyamides. *Acta Polym.* **1999**, *50*, 135−140.

(37) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* **1973**, *12*, 4085−4092.

(38) Miquel, J. F. Molecular Structure and Estrogenic Activity−I. Unsymmetrical Diphenylethylenes and Triphenylethylenes. *Tetrahedron* **1960**, *8*, 205−216.

(39) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **1998**, *95*, 927−937.

(40) Brzozowski, A. M.; Pike, A. C.; Dauter, Z.; Hubbard, R. E.; Bonn, T.; Engstrom, O.; Ohman, L.; Greene, G. L.; Gustafsson, J. A.; Carlquist, M. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature* **1997**, *389*, 753−758.

(41) Tedesco, R.; Katzenellenbogen, J. A.; Napolitano, E. 7α,11*β*-Disubstituted estrogens: Probes for the shape of the ligand binding pocket in the estrogen receptor. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2919−2924.

(42) Poupaert, J. H.; Lambert, D. M.; Vamecq, J.; Abul-Hajj, Y. Molecular modeling studies on 11*β*-aminoethoxyphenyl and 7α-aminoethoxyphenyl estradiol. Evidence suggesting a common hydrophobic pocket in estrogen receptor. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 839−842.

(43) Shiau, A. K.; Barstad, D.; Radek, J. T.; Meyers, M. J.; Nettles, K. W.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A.; Agard, D. A.; Greene, G. L. Structural characterization of a subtype-selective ligand reveals a novel mode of estrogen receptor antagonism. *Nat. Struct. Biol.* **2002**, *9*, 359−364.

(44) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. Safe and Convenient Procedure for Solvent Purification. *Organometallics* **1996**, *15*, 1518−1520.

(45) Curtze, J. Preparation of benzophenones as fungicide intermediates. *Ger., Pat.* **1988**, 1−16.

(46) Goossen, L. J.; Ghosh, K. Palladium-catalyzed synthesis of aryl ketones from boronic acids and carboxylic acids or anhydrides. *Angew. Chem., Int. Ed.* **2001**, *40*, 3458−3460.

(47) Banthorpe, D. V.; Curtis, A. J.; Fordham, W. D. Stereochemistry of isolongifolene alcohols. *Tetrahedron Lett.* **1972**, 3865−3868.

(48) Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. Altered ligand binding properties and enhanced stability of a constitutively active estrogen receptor: evidence that an open pocket conformation is required for ligand interaction. *Biochemistry* **1997**, *36*, 14897−14905.

(49) McInerney, E. M.; Tsai, M. J.; O'Malley, B. W.; Katzenellenbogen, B. S. Analysis of estrogen receptor transcriptional enhancement by a nuclear hormone receptor coactivator. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10069−10073.

JM0204800