Estrogen Receptor Subtype-Selective Ligands: Asymmetric Synthesis and Biological Evaluation of *cis*- and *trans*-5,11-Dialkyl-5,6,11,12-tetrahydrochrysenes

Marvin J. Meyers,[†] Jun Sun,[‡] Kathryn E. Carlson,[†] Benita S. Katzenellenbogen,[‡,§] and John A. Katzenellenbogen*[,†]

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

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We have recently reported that racemic 5,11-*cis*-diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (THC, *rac*-**2b**) acts as an agonist on estrogen receptor alpha (ERα) and as a complete antagonist on estrogen receptor beta (ER*β*) (Sun et al. *Endocrinology* **1999**, *140*, 800−804). To further investigate this novel ER subtype-selective estrogenic activity, we have synthesized a series of *cis*- and *trans*-dialkyl THCs. *cis*-Dimethyl, -diethyl, and -dipropyl THCs **2a**−**c** were prepared in a highly enantio- and diastereoselective manner by the acyloin condensation of enantiomerically pure α-alkyl-*β*-arylpropionic esters, followed by a Lewis acid-mediated double cyclization under conditions of minimal epimerization. ERα and ER*β* binding affinity of both cis and trans isomers of dimethyl, diethyl, and dipropyl THCs was determined in competitive binding assays, and their transcriptional activity was determined in reporter gene assays in mammalian cells. Nearly all THCs examined were found to be affinity-selective for ER*β*. All these THCs are agonists on ERα, and THCs with small substituents are agonists on both ERα and ER*β*. As substituent size was increased, ER*β*-selective antagonism developed first in the (*R,R*)-cis enantiomer series and finally in the trans diastereomer and (*S,S*)-cis enantiomer series. The most potent and selective ligand was identified as (*R,R*)-*cis*-diethyl THC **2b**, which mimicked the ER*β*-selective antagonist character of racemic *cis*-diethyl THC **2b**. This study illustrates that the antagonist character in THC ligands for ER*β* depends in a progressive way on the size and geometric disposition of substituent groups and suggests that the induction of an antagonist conformation in ER*β* can be achieved with these ligands with less steric perturbation than in ERα. Furthermore, antagonists that are selectively effective on ER*β* can have structures that are very different from the typical antiestrogens tamoxifen and raloxifene, which are antagonists on both ERα and ER*β*.

Introduction

The estrogen receptor (ER), a member of the nuclear hormone receptor superfamily, mediates the activity of estrogens in the regulation of a number of important physiological processes, including the development and function of the female reproductive system and the maintenance of bone mineral density and cardiovascular health. While the stimulation of processes in these tissues has important health benefits, the stimulation of other tissues, such as the breast and uterus, can increase the risk of cancer at these sites. Intriguingly, some pharmaceutical agents, such as tamoxifen and raloxifene, act as antagonists in some tissues, such as the breast and uterus, while acting as agonists in other tissues, such as the liver and vasculature.[1] This mixed response has raised the interesting issue of tissue-, cell-, and gene-specific activity of estrogens based on the ligand, the receptor, and the effector site, which has been termed "tripartite receptor pharmacology".[2] Today, extensive efforts are being made to develop ligands which selectively antagonize undesirable estrogenic effects, such as the stimulation of breast cancer, while promoting positive estrogen effects in maintaining bone and cardiovascular health.

Until recently, it had been assumed that estrogen-related events were mediated by only one estrogen receptor. However, the recent discovery of a second estrogen receptor (ER*β*)[3,4] has suggested that tissue and cell selectivity of certain estrogens may be due, in part, to their action through ER*β*, separate from or in conjunction with the classical estrogen receptor (ERα). This possibility has been supported by the difference in tissue distribution between ERα and ER*β*.[3,5−7] Furthermore, it has been shown that the pharmacology of traditional ER agonists and antagonists is reversed for ER*β* in the context of certain ER effector sites.[8,9]

Although the two ER subtypes are both activated by binding estradiol (**1**, E_2), the ligand-binding domain (LBD) and activation function-2 (AF-2) region of the proteins are only 56% conserved and the A/B domain/activation function-1 (AF-1) is only 20% conserved.[3,4] This suggests that ligands may be developed which have different affinities, potencies, and agonist versus antagonist behavior for the two ER subtypes. Indeed, some

* Address correspondence to: John A. Katzenellenbogen, Department of Chemistry, University of Illinois, 461 Roger Adams Laboratory, Box 37-5, 600 S. Mathews Ave., Urbana, IL 61801. Tel: (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.

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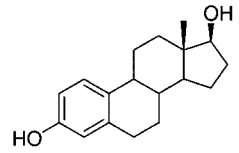
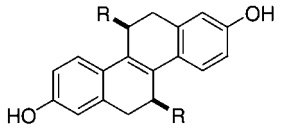
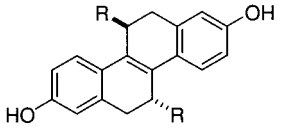


1 Estradiol (E_2)



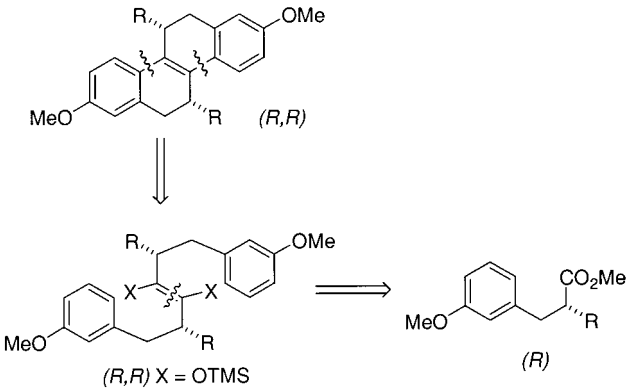
cis-THC (racemic)
2a R = Me
2b R = Et
2c R = Pr



trans-THC (meso)
3a R = Me
3b R = Et
3c R = Pr

known ligands have been shown to have subtype-selective affinities and a degree of subtype-selective agonist/antagonist character.[5,10]

Scheme 1



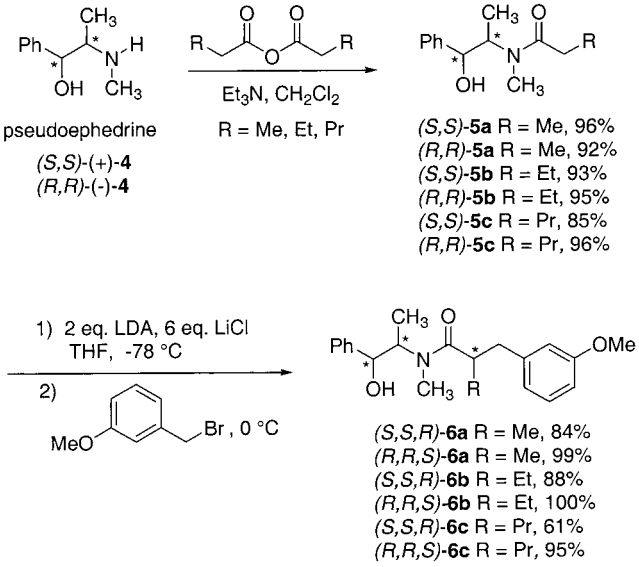
(R,R) X = OTMS

We have recently surveyed a number of compounds previously synthesized in our laboratories and found that the racemic *cis*-diethyltetrahydrochrysene (THC) (*rac*-**2b**) is an agonist on ERα and a complete antagonist on ERβ and has a 10-fold higher affinity for ERβ relative to ERα.[11] Furthermore, the (*R*,*R*)-enantiomer of *cis*-diethyl THC was found to be a more potent antagonist than *rac*-**2b**, while the (*S*,*S*)-enantiomer was an agonist on both receptor subtypes.[11] The work described herein details the asymmetric synthesis of a series of dialkyl-substituted *cis*-THCs and the evaluation of both the relative binding affinity (RBA) and agonist/antagonist selectivity of both *cis*-THCs (**2a**−**c**) and *trans*-THCs (**3a**−**c**). From an analysis of the activity of the whole series of compounds, it is clear that the (*R*,*R*)-enantiomers develop ERβ-selective antagonism with smaller substituents than do the (*S*,*S*)-enantiomers and trans isomers. This suggests that the induction of an antagonist conformation in ERβ can be achieved with these ligands with less steric perturbation than in ERα.

Results and Discussion

Retrosynthesis of *cis*-Dialkyl THCs. Following a strategy previously described,[12,13] we reasoned that the acyloin condensation of an enantiomerically pure ester, followed by a double Friedel−Crafts cyclization under conditions of minimal epimerization, would selectively furnish the *cis*-THC isomer as a single enantiomer (Scheme 1).

Scheme 2



pseudoephedrine
(S,S)-(+)-**4**
(R,R)-(-)-**4**

Et_3N, CH_2Cl_2
R = Me, Et, Pr

(S,S)-**5a** R = Me, 96%
(R,R)-**5a** R = Me, 92%
(S,S)-**5b** R = Et, 93%
(R,R)-**5b** R = Et, 95%
(S,S)-**5c** R = Pr, 85%
(R,R)-**5c** R = Pr, 96%

1) 2 eq. LDA, 6 eq. LiCl
THF, -78 °C
2) MeO, Br, 0 °C

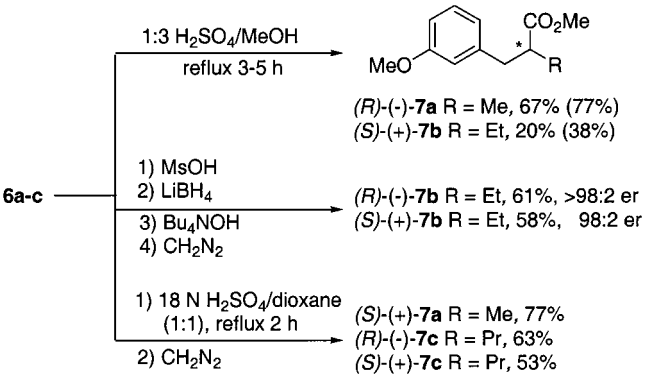
(S,S,R)-**6a** R = Me, 84%
(R,R,S)-**6a** R = Me, 99%
(S,S,R)-**6b** R = Et, 88%
(R,R,S)-**6b** R = Et, 100%
(S,S,R)-**6c** R = Pr, 61%
(R,R,S)-**6c** R = Pr, 95%

In previous work, we have described the synthesis of *trans*-THC isomers **3b,c** and racemic cis isomers **2a,b** by the same approach. However, we used racemic esters, and we did not evaluate the extent of epimerization that occurred when PPA was used as the cyclization reagent.[13] By this earlier approach, the dimethyl THC was obtained primarily as the cis isomer, but the diethyl and dipropyl isomers were obtained as mixtures of cis and trans isomers. Even though the *cis*- and *trans*-diethyl THCs were separable by recrystallization (and their stereochemistry confirmed by X-ray crystallographic analysis), we desired a *cis*-selective route to each of the dialkyl-substituted THCs. We surmised that if epimerization could be avoided throughout the sequence, then the asymmetric strategy presented in Scheme 1 should be a feasible means for preparing the cis isomers diastereoselectively.

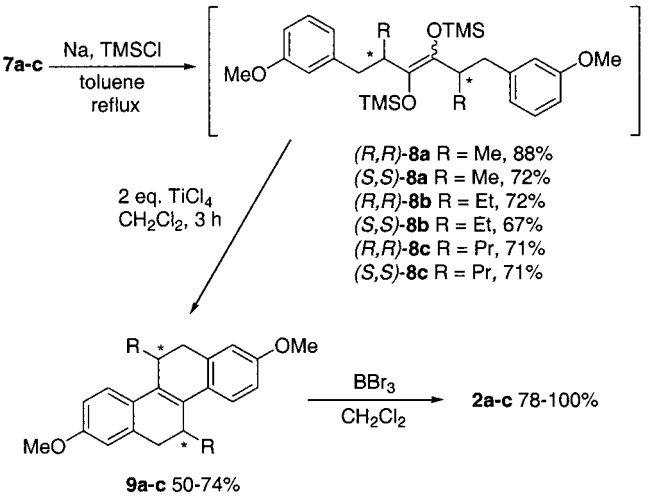
Asymmetric Synthesis of α-Alkyl Esters 7a−c. Although several methods are available for the asymmetric α-alkylation of carboxylic acid derivatives, we decided to use Myers' pseudoephedrine chiral auxiliary method because of its ease of use, high yields, and high diastereoselectivities.[14] The acylation of pseudoephedrine amines **4** with the appropriate anhydrides proceeded in excellent yield, to afford amides **5a**−**c** (Scheme 2). Enolate formation from **5a**−**c** with LDA and asymmetric alkylation with *m*-methoxybenzyl bromide gave amides **6a**−**c** in excellent yields. In our hands, however, many of the amides **5a**−**c** and **6a**−**c** were thick viscous oils, which prevented the enrichment of diastereomeric ratios by crystallization.

The hydrolysis of amides **6a**−**c** proved to be challenging (Scheme 3). Initial attempts to effect methanolysis with sulfuric acid and methanol furnished ester (*R*)-**7a** in good yields after 3 h at reflux; however, as the alkyl substituent increased in steric bulk, the hydrolysis became quite sluggish (e.g., ester (*S*)-**7b** was furnished in only 20% yield after 5 h at reflux). Thus, two methods described by Myers and co-workers[14] were utilized to prepare the crude carboxylic acids. To prepare esters **7b**, a one-pot MsOH/$LiBH_4$/*n*-Bu_4NOH hydrolysis[14] of amides **6b** provided the crude carboxylic acids, which, after a simple workup, were then treated with excess

Scheme 3



Scheme 4



diazomethane to furnish esters **7b** in moderate yields and excellent enantiomeric ratios (er ≥ 98:2). Alternatively, hydrolysis of amides (*R,R,S*)-**6a** and **6c** with aqueous sulfuric acid in dioxane followed by methylation with diazomethane also furnished esters (*S*)-**7a** and **7c** in moderate to good yields (53−77%). Presumably, the yields in these hydrolyses could be improved by extending the hydrolysis times; however, Myers has shown that this is likely to compromise the enantiopurity.[14] The absolute stereochemistry of esters **7a**−**c** was assigned by analogy to (*R*)-α-methylbenzenepropionic acid, prepared by Myers by an analogous route.[14]

Acyloin Condensation. The acyloin coupling of esters **7a**−**c** proceeded in good yields to afford silyl ethers **8a**−**c** with minimal, if any, epimerization, as assessed by ^{1}H NMR (Scheme 4). TMSCl was used to trap the enediolate as the bis-silyl ether; it evidently also functioned as a methoxide scavenger,[15] thus minimizing epimerization of the product. An attempt to couple the aldehyde corresponding to ester (*S*)-**7a** using Stetter chemistry to provide the acyloin[16] was unsuccessful, presumably because of steric hindrance by the α-alkyl substituent.[17] After minimal purification, the silyl ethers were cyclized with the appropriate acid to furnish THCs **9a**−**c** (see below).

Double Cyclization. Initial cyclization attempts of silyl ether (*R,R*)-**8a** with PPA and with MsOH yielded **9a** as a 2:1 mixture of cis:trans isomers (entries 1−3, Table 1). When the cyclization was quenched at about 50% completion, the same cis:trans ratio was observed, suggesting that, under these conditions, epimerization occurs very quickly (entry 2). To find a reagent which would avoid this epimerization, we surveyed a number of nonprotic Lewis acids ($AlCl_3$, BF_3−OEt_2, $TiCl_4$, $POCl_3$, $ZnCl_2$, $ZnCl_2/POCl_3$, $LiClO_4$, and $SnCl_4$) with silyl ether *rac*-**8b**, prepared as previously described.[13] $TiCl_4$ and BF_3−etherate were found to be optimal reagents, in terms of their reactivity, cleanliness of reaction, and stereoselectivity, with the former being considerably more efficient and consistent than the latter. Both reagents effected the cyclization of (*R,R*)-**8a** to dimethyl THC (*R,R*)-**9a** with high stereoselectivity (entries 4 and 5). It is notable that the ratio of 13:1 cis:trans for THC (*R,R*)-**9a** amounts to only 3−4% overall epimerization in the acyloin condensation and cyclization steps.

Table 1. Optimization of the Cyclization of Silyl Ether (*R,R*)-**8a**

entry	reagents	conditions	cis:trans[a]
1	PPA, neat	rt, 1 h	2:1
2	PPA, neat	rt, 4 min	2:1
3	10 equiv MsOH, CH_2Cl_2	0 °C, 2 h	2:1
4	10 equiv BF_3·OEt_2, CH_2Cl_2	rt, 23 h	9:1
5	2 equiv $TiCl_4$, CH_2Cl_2	rt, 3 h	13:1

[a] Determined by integration of ^{1}H NMR peaks.

Table 2. Cyclization of Silyl Ethers **8a**−**c** with $TiCl_4$

product	R	yield, %	crude cis:trans[a]	recrystallized cis:trans[a]	er[b]
(*R,R*)-**9a**	Me	50	13:1	64:1	98:2
(*S,S*)-**9a**	Me	42	ND[c]	66:1	>99:1
(*R,R*)-**9b**	Et	71	15:1	>100:1	>99:1
(*S,S*)-**9b**	Et	65	18:1	>100:1	>99:1
(*R,R*)-**9c**	Pr	69	∼20:1	NA[d]	>99:1
(*S,S*)-**9c**	Pr	74	∼20:1	NA	>99:1

[a] Determined by integration of ^{1}H NMR peaks. [b] Determined by CSP HPLC (*R,R* Whelk-O 1). [c] ND, not determined. [d] NA, not applicable.

Cyclization of silyl ethers **8a**−**c** with $TiCl_4$ (2 equiv) furnished optically active *cis*-THCs **9a**−**c** in moderate to excellent yields with minimal epimerization, as evident from their high cis:trans ratios and enantiopurities (Table 2). The stereochemical assignments and ratios were determined by comparison of ^{1}H NMR spectra with known *cis*- and *trans*-THC isomers (see below). The effect of epimerization, which produces the trans isomer, could be reduced by careful recrystallization. In the case of diethyl THCs **9b**, all of the trans isomer was removed by recrystallization, as ascertained by ^{1}H NMR. Unfortunately, attempts to crystallize the propyl analogues (**9c**) were not successful. Handling of these compounds required care, as these THCs are sensitive to light and air (see below).[13]

Deprotection of methyl ethers **9b** (100% cis) with $AlBr_3$ and EtSH furnished the desired phenols, but with considerable epimerization (approximately 10−15%), presumably because of the strong protic acid conditions. The bis-phenol products obtained by this method were also quite sensitive to oxygen and light. During workup and purification by chromatography, the products rapidly turned yellow-orange, presumably due to oxidation. However, the extent of this decomposition proved to be minimal (less than 5%), and the decomposition products could be removed by chromatography and recrystallization in the presence of trace levels of ascorbic acid added as an antioxidant.

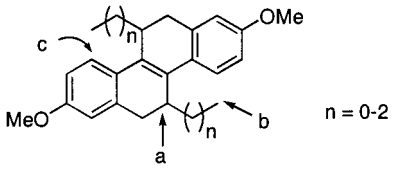
Fortunately, deprotection with boron tribromide proved to be much more suitable, as no epimerization was

Table 3. Deprotection of THCs **9a**−**c** with BBr_3

product	R	yield, %	crude cis:trans[a]	recrystallized cis:trans[a]	er[b]
(*R,R*)-**2a**	Me	100	ND[c]	100:0	98:2
(*S,S*)-**2a**	Me	88	ND	83:1	>99:1
(*R,R*)-**2b**	Et	78	100:0	100:0	>99:1
(*S,S*)-**2b**	Et	100	100:0	100:0	>99:1
(*R,R*)-**2c**	Pr	93	33:1	73:1	>99:1
(*S,S*)-**2c**	Pr	79	23:1	67:1	>99:1

[a] Determined by integration of ^{1}H NMR peaks. [b] Determined by CSP HPLC (ChiralPak AS). [c] ND, not determined.

Table 4. Selected ^{1}H NMR Chemical Shifts for *cis*- and *trans*-THC Isomers



entry	THC	isomer	δ a, ppm	δ b, ppm	δ c, ppm
1	(*R,R*)-**9a**	cis	2.92	1.07	7.31
2	dimethyl	cis[a]	2.90	1.05	7.30
3	*trans*-**9a**	trans[b]	3.19	0.90	7.35
4	(*R,R*)-**9b**	cis	2.58	0.96	7.23
5	*rac*-**9b**	cis[c]	2.59	0.97	7.23
6	diethyl	trans[c,d]	2.94	0.79	7.33
7	(*R,R*)-**9c**	cis	2.72	0.93	7.27
8	dipropyl	trans[d]	2.98	0.78	7.34

[a] Reported as trans,[13] actually cis. [b] Minor isomer indicated in Table 1. [c] Stereochemistry confirmed by X-ray crystallography.[13] [d] Data from compounds prepared previously.[13]

detected in any of the bis-phenolic products **2a**−**c** (Scheme 4 and Table 3). Also, at the bis-phenol stage, the cis:trans ratio of the propyl analogues could be improved to approximately 70:1 by recrystallization. Curiously, decomposition problems of the bis-phenols were much less significant when they were generated by the BBr_3 deprotection than by the $AlBr_3$/EtSH deprotection. Thus, both the (*R,R*)- and (*S,S*)-enantiomers of the *cis*-dimethyl, -diethyl, and -dipropyl THC diols were prepared in high enantio- and diastereoselectivity (er ≥ 98:2, dr > 98:2 cis:trans).

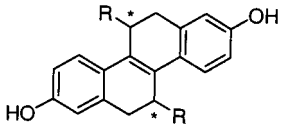
THC Stereochemistry Confirmation and Synthesis of *trans*-Dimethyl THC 3a. The cyclization of racemic dimethylsilyl ether *rac*-**8a** with PPA was originally thought to give predominantly the *trans*-dimethyl THC **3a**;[13] this original report was incorrect. The ^{1}H NMR spectra of the major isomer produced in all of the cyclizations presented in Table 1 match what had previously been reported as the *trans*-dimethyl isomer (Table 4, entries 1−3). However, it is now clear that the major dimethyl THC isomer is cis, because the starting ester was of high enantiopurity and the THC is formed with minimal epimerization. In the major isomer, the chemical shifts of the allylic protons are shifted upfield (0.26 ppm), the methyl protons downfield (0.17 ppm), and the meta ArH protons upfield (0.04 ppm) relative to the minor isomer (entries 1 and 3). This matches the pattern seen in the diethyl THC series for the cis isomer relative to the trans isomer (entries 4−6, stereochemistry confirmed by X-ray crystallography).[13] In addition, the product (**9a**) of the cyclization with $TiCl_4$ was recrystallized to enhance the isomer ratio to 64:1. This purified compound was found to be optically active ($[\alpha]_D^{24}$ −109° (*c* 0.98, $CHCl_3$)), which also distinguishes it from the trans isomer, a meso compound, which is optically inactive. The *cis*-dipropyl THC **9c** gave spectra which were shifted in the same fashion from the previously described *trans*-dipropyl THC,[13] as described above for **9a,b** (entries 7 and 8).

The synthesis of *trans*-**3a** was accomplished by the cyclization of (*R,R*)-**8a** with PPA to furnish a 1:2 ratio of trans:cis isomers of **9a** in 60% yield. Since this isomeric mixture was inseparable by recrystallization, the mixture was deprotected with BBr_3 and recrystallized twice from MeOH to provide exclusively *trans*-**3a**.

Estrogen Receptor Binding Affinities. THC diols **2a**−**c, 3a**−**c**, and unsubstituted THC **10** (prepared as previously described)[13] were evaluated in competitive radiometric binding assays to determine their affinities for the ER (Table 5).[18,19] Relative binding affinity (RBA) values were determined with lamb uterine cytosol ER preparations and with purified full-length human ERα and ER*β*, and they are reported relative to estradiol (E_2), which is set at 100%.

All of the *cis*-THCs examined show a binding preference for ER*β* relative to ERα (*β*/α ratio 3−16), with some

Table 5. Relative Binding Affinities[a] of THCs for the Estrogen Receptors α and *β*



entry	ligand	R	uterine ER	hERα	hER*β*	*β*/α ratio
1	estradiol	H	100	100	100	
2	**10**[b]	H	8.1 ± 0.5	3.0 ± 0.7	6.5 ± 0.3	2
3	*trans*-**3a**	Me	252 ± 25	222 ± 18	254 ± 57	1
4	(*R,R*)- **2a**	Me	13 ± 3	24 ± 6	76 ± 18	3
5	(*S,S*)-**2a**	Me	3.2 ± 0.2	9.3 ± 2.7	75 ± 12	8
6	*trans*-**3b**[b]	Et	137 ± 16	221 ± 42	432 ± 21	2
7	*rac*-**2b**[b]	Et	14 ± 5	14 ± 3	67 ± 5	5
8	(*R,R*)-**2b**	Et	17 ± 7	23 ± 11	144 ± 5	6
9	(*S,S*)-**2b**	Et	1.5 ± 0.4	0.9 ± 0.2	14 ± 4	16
10	*trans*-**3c**[b]	Pr	59 ± 16	33.6 ± 2.8	92.3 ± 4.5	3
11	(*R,R*)-**2c**	Pr	4.5 ± 0.7	5.2 ± 1.0	26 ± 10	5
12	(*S,S*)-**2c**	Pr	1.4 ± 0.3	1.6 ± 0.4	5.1 ± 4.0	3

[a] Determined by a competitive radiometric binding assay with [^{3}H]estradiol; cytosol preparations of lamb uterus or full-length human ERα and ER*β* (PanVera) were used; see Experimental Section.[18,19] Values are reported as the mean ± SD (n > 2) or range (n = 2) under these conditions; the K_d for estradiol is 0.3 nM. [b] Prepared as previously described.[13]

of the (*S,S*)-enantiomers showing high β/α selectivity. The (*R,R*)-enantiomers, with lower β/α subtype selectivities (β/α ratio 3−6), have up to 26-fold greater absolute affinities for either ER subtype relative to their (*S,S*)-enantiomers. The difference in relative affinities between the (*R,R*)- and (*S,S*)-enantiomers is especially apparent in the diethyl series, where (*R,R*)-**2b** has a 10-fold greater affinity for ERβ and a 26-fold greater affinity for ERα relative to (*S,S*)-**2b**. All of the *trans*-THC isomers **3a**−**c** have significantly greater affinities for both receptor subtypes than the corresponding cis isomers (∼4−16-fold). However, the trans isomers show only minimal ERβ subtype selectivity (β/α ratio 1−3). The unsubstituted THC **10** (Table 5, entry 2) also shows minimal ERβ selectivity (∼2-fold). The RBAs from uterine cytosol ER preparations match the RBA values for full-length ERα, as expected, because uterine ER is predominantly ERα.[3,5]

Transcriptional Activation Assays. The transcriptional activities of THCs **10**, **2a**−**c**, and **3a**−**c** were assayed in human endometrial cancer (HEC-1) cells with ERα and ERβ and an estrogen-responsive reporter gene construct, $(ERE)_3$-pS2-CAT, containing these estrogen response elements, the estrogen-responsive pS2 promoter, and the chloramphenicol acetyltransferase reporter gene. Activities are normalized to that of 10^{-8} M E_2 which is set at 100, as previously described.[11] Dose−response curves are presented in Figure 1. Antagonist activity of all of the THCs was determined with 1 nM E_2 and 1 μM THC diol concentrations; those that showed significant antagonist activity were subsequently assayed over a larger concentration range.

Unsubstituted THC diol **10** does not show pronounced agonist/antagonist selectivity, exhibiting mixed agonist/antagonist character for both ERα and ERβ (Figure 1A,E). The *trans*-dimethyl THC **3a** is a full or nearly full agonist on both ERα and ERβ (Figure 1B,F). The *cis*-dimethyl THC enantiomers (*R,R*)-**2a** and (*S,S*)-**2a** exhibit somewhat different agonist/antagonist character (Figure 1B,F). (*R,R*)-**2a** is a full agonist on ERα, but it is a very weak agonist on ERβ, with considerable antagonistic character. (*S,S*)-**2a** shows a curious and reproducible biphasic dose−response curve on both ER subtypes: It is a full agonist at concentrations up to 10^{-7} M but shows antagonistic character at 10^{-6} M on both ER subtypes. All of the *cis*-diethyl and *cis*-dipropyl THC diols **2b,c** are agonists on ERα in a manner similar to racemic diethyl THC (*rac*)-**2b** (Figure 1C,D).[11] However, on ERβ, the diethyl (*R,R*)-**2b** and both *cis*-dipropyl THC **2c** enantiomers exhibit no, or very low, transcriptional activity, and they appear to very effectively antagonize the effect of estradiol (Figure 1G,H). In contrast, diethyl (*S,S*)-**2b** is an agonist on ERβ and begins to antagonize the effect of estradiol weakly only at very high concentrations (Figure 1G). *trans*-Diethyl THC diol **3b** is an agonist with some weak antagonist character on ERβ (Figure 1G). In contrast, *trans*-dipropyl THC diol **3c**, like the corresponding *cis*-dipropyl enantiomers, exhibits only minimal transcriptional activity on ERβ and very effectively antagonizes the effect of estradiol on ERβ (Figure 1H). Although *trans*-dipropyl THC **3c** is more potent as an ERβ antagonist than *cis*-diethyl *(R,R)*-**2b**, it has some minimal activity in the absence of E_2. The ERβ antagonist character of the dipropyl (*S,S*)-**2c** and *trans*-dipropyl **3c** is surprising, given the agonist character of the (*S,S*)-**2b** and *trans*-**3b**. To the best of our knowledge, *cis*-diethyl (*R,R*)-**2b**, both *cis*-dipropyl THC enantiomers **2c,** and *trans*-dipropyl THC **3c** are the first compounds described as pure ERβ-selective antagonists.

Discussion

Enantioselective Synthesis of *cis*-5,11-Dialkyl-5,6,11,12-tetrahydrochrysene-2,8-diols. We have described a method for the asymmetric synthesis of *cis*-5,11-dialkyl-5,6,11,12-tetrahydrochrysene-2,8-diols in a six-step sequence utilizing Myers' pseudoephedrine chiral auxiliary methodology.[14] The crucial acyloin and cyclization steps were achieved with minimal epimerization by careful choice of reaction conditions and reagents, allowing the enantio- and diastereoselective formation of *cis*-dialkyl THCs with high enantiopurity. These compounds, together with the corresponding trans stereoisomers, provide an interesting set of ligands and transcriptional modulators for the estrogen receptor subtypes ERα and ERβ that includes compounds—agonists on ERα but complete antagonists on ERβ—that could prove to be useful as pharmacological probes for determining the biological effects mediated by the individual ER subtypes.

Structure−Binding Affinity Relationships. The size of the substituent at C-5 and C-11 in the THCs has a major effect on ER binding affinity, but the optimum size also depends on substituent stereochemistry and ER subtype. On ERα, the highest affinities are obtained with trans-disposed Me or Et groups; although the binding affinities of the corresponding *cis*-THC diastereomers were uniformly lower, Me and Et also were preferred in the (*R,R*)-series and Me in the (*S,S*)-series. Substituent−affinity trends on ERβ are similar: Again, the trans isomers bind better than the cis isomers in all cases, with an Et substituent preferred in the trans and (*R,R*)-series and a Me in the (*S,S*)-series. The lower affinity of the Pr-substituted THCs relative to the smaller congeners in all cases suggests that substituent tolerance may be exceeded by groups of this size.

Although the *trans*-THC isomers have higher affinity for both ER subtypes than do their corresponding cis isomers, they show no or only modest ERβ subtype affinity selectivity (1−3-fold). By contrast, the cis stereoisomers have a more distinct binding preference for ERβ (3−16-fold), with the (*S,S*)-enantiomers generally having greater preference. Interestingly, the (*R,R*)-enantiomers usually have higher affinities for both ERα and ERβ.

Others have reported on compounds that show differences in their binding affinity for ERα and ERβ.[5] ERα affinity selectivities up to 5-fold have been found for certain substituted steroidal estrogens, especially those with 17α-substituents. The highest ERβ affinity selectivities of about 7-fold have been reported for certain nonsteroidal phytoestrogens, such as genistein. A number of the *cis*-dialkyl THCs that we have prepared show equally high or greater ERβ affinity selectivities.

In our initial report on the biological activity of the *cis*-diethyl THCs,[11] we reported relative binding affinities for the purified LBDs of ERα and ERβ. Subsequently, we have found that these ER LBD RBA values, while exhibiting similar trends, are quantitatively dif-

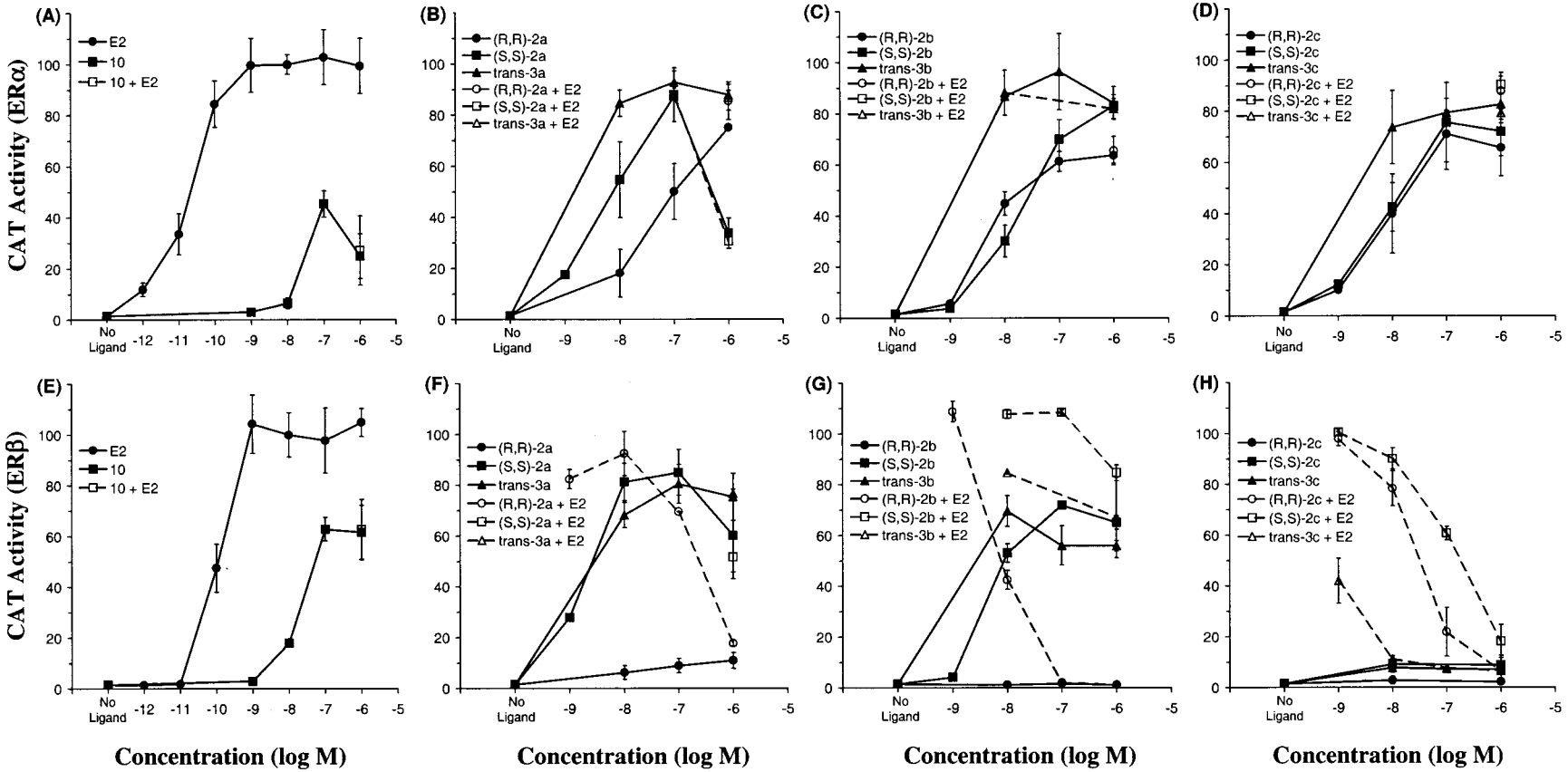


Figure 1. Transcription activation by ERα (upper panels, A−D) and ERβ (lower panels, E−H) in response to THCs **2a**−**c**, **3a**−**c**, and **10**. Human endometrial cancer (HEC-1) cells were transfected with expression vectors for ERα or ERβ and the estrogen-responsive $(ERE)_3$-pS2-CAT reporter gene and were treated with the indicated concentrations of estradiol (E_2) or ligand (**2a**−**c**, **3a**−**c**, or **10**) for 24 h (solid lines and symbols). The antagonist activity of **2a**−**c**, **3a**−**c**, and **10** on ERα and ERβ was assayed in the presence of 1 nM E_2 (dashed lines and open symbols). CAT activity was normalized for β-galactosidase activity from an internal control plasmid. Values are the mean ± SD for three or more separate experiments and are expressed as a percent of the ERα or ERβ response with 10^{-8} M E_2. For some values, error bars are too small to be visible.

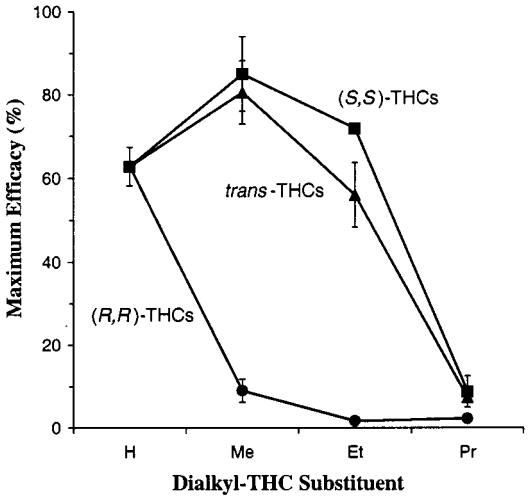


Figure 2. Maximum efficacy of THCs as a function of substituent size and stereochemistry. Maximum efficacy is the level of transcription activation by ERβ in response to THCs **2a**−**c**, **3a**−**c**, and **10** at a ligand concentration of 10^{-7} M and is expressed as a percent of the transcriptional stimulation with 10^{-8} M E_2. Transfection assays were conducted in HEC-1 cells using the $(ERE)_3$-pS2-CAT reporter gene construct as described in Figure 1.

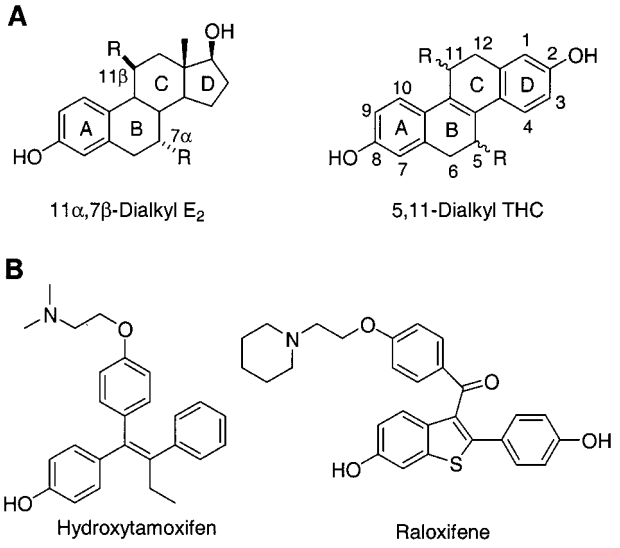


Figure 3. Ligands for the estrogen receptor: (A) numbering scheme for 7α,11β-disubstituted E_2 and 5,11-disubstituted THCs and (B) antiestrogens hydroxytamoxifen and raloxifene.

ferent from the corresponding values for *full-length* ERα/β RBAs, which we are reporting here. The difference is particularly marked in the (*S*,*S*)-**2b** affinity for full-length ERβ, which is about 10-fold higher than its affinity for the corresponding purified ERβ LBD.[11] This difference is reproducible, but at this point an explanation is not apparent.

Structure−Transcriptional Efficacy and Potency Relationships. In cell-based reporter gene transcription assays, all of the THCs are agonists through ERα; those with small substituents are also agonists through ERβ, but as substituent size grows, they become antagonists. This relationship between the efficacy (i.e., agonist and antagonist character of these THC ligands) and the size and geometry of their alkyl substituents is illustrated in a direct fashion in Figure 2. THCs with smaller substituents (H, Me) in all cases but one are agonists on both receptors. As substituent size is increased, ERβ-selective antagonism develops first in the (*R*,*R*)-THC series, being clearly evident at the Me substituent size and complete at the Et and Pr sizes. Eventually, the ERβ-selective antagonism develops as well in the *trans*-THC and (*S*,*S*)-THC series, being complete only at the Pr substituent size (Figure 2).

As has been noted by others, the binding affinity of a ligand is not always perfectly reflected in its potency in a receptor transcriptional activation assay.[10,11] As far as we have examined within the THC series, however, there appears to be reasonable concordance between a compound's relative binding affinity and its relative transcriptional potency. Thus, in general, those THCs with the highest affinities (the trans isomers and the (*R*,*R*)-enantiomers) were also the most potent as agonists or antagonists, although this is not uniformly the case (see, for example, (*S*,*S*)-dimethyl THC, which is more potent as an agonist on ERα than the (*R*,*R*)-isomer but has lower affinity, and *trans*-dipropyl THC, which is a more potent ERβ antagonist than (*R*,*R*)-diethyl THC but has lower affinity). However, overall, the major difference between affinity and potency in this THC series is that relative to estradiol; all of the THCs are somewhat less potent as transcriptional modulators than would be predicted from their affinities. The fact that there is not always a direct correlation between ligand binding affinity and transcriptional potency suggests that factors beyond ligand−receptor interaction, such as receptor−coactivator interactions or differential utilization of AF-1 and AF-2, are likely to be important determinants of transcriptional potency.[2]

Ligand Binding Affinity and Estrogen Receptor Structure. The THCs that we have examined bear substituents at C-5 and C-11, positions that correspond to the C-7 and C-11 positions in estradiol (Figure 3A). From an earlier analysis of the tolerance of ERα for substituents on the steroidal ligand estradiol,[20] we had ascertained that there was considerable "preformed pocket volume" in ERα that would accommodate sizable substituents at the 7α- and 11β-positions; these positions are, respectively, below and above the plane of the steroid skeleton. Recently reported X-ray crystal structures of ERα with the agonists estradiol[21,22] and diethylstilbestrol (DES)[23] have delineated these pockets in greater detail and have shown that they have a combined volume of nearly 200 Å^3. In fact, in the ERα−DES structure, the two ethyl groups of the ligand fill the 7α- and 11β-pockets in preference to the peripheral regions of the receptor that are normally occupied by the B- and C-rings of steroidal ligands.[23]

The THC ligands that we have studied have a tetracyclic structure that is closely related to steroidal ligands such as estradiol, so one may reasonably presume that they fit into the binding site in ER in a fashion similar to that of estradiol. In this orientation, the two substituents in the *trans*-THCs, which project below and above the plane of the ligand core, can both be nicely accommodated in the 7α and 11β preformed pockets in ERα (Figure 4), consistent with the high binding affinity of the trans isomers. The disposition of the substituents in the cis isomers, however, is less clear, because both substituents project out from the

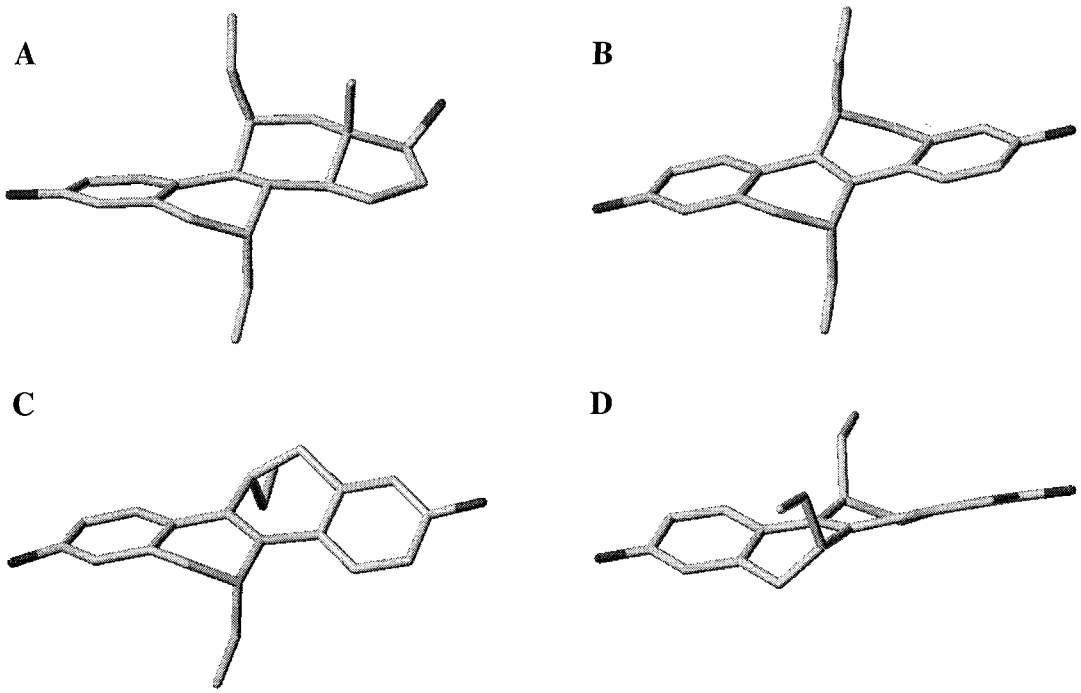


Figure 4. Stereorenditions of (A) 7α,11*β*-diethyl E_2,[16] minimized with SYBYL 6.5 using the Tripos force field; (B) *trans*-5,11-diethyl THC **3b**; (C) (*R,R*)-5,11-diethyl THC **2b**; (D) (*S,S*)-5,11-diethyl THC **2b**. Structures in B−D were taken from X-ray crystal structures of the *trans*- and racemic *cis*-THCs reported previously.[13]

same side of the ligand core structure. If one assumes that the tetracyclic core of the *cis*-THC ligands is also bound in the same manner as that of estradiol and the *trans*-THCs, then the two substituents in the cis isomers would both project above the plane in the cis (*S,S*)-series or below the plane in the cis (*R,R*)-series (Figure 4).[24] In the cis isomers, steric interactions between the two substituents and the ring system also result in a flexing of the central two rings, giving the tetracyclic core an overall twist (Figure 4C,D).

Clearly, the binding affinity measurements indicate that in this geometry, the *cis*-THC ligands are less well-accommodated by both ERα and ER*β* than are the trans isomers. Interestingly, however, with all *cis*-THCs on both ER subtypes (except the *cis*-dimethyl THCs on ER*β*), the (*R,R*)-enantiomer, with the two substituents projecting downward, binds better than the (*S,S*)-enantiomer. Again, all of the THCs we have studied, regardless of substituent size and stereochemistry, bind better to ER*β* than to ERα. Thus, the two ER subtypes have a significantly different tolerance to the size and stereochemistry of substituents on the THC ring system and to conformational distortions of the THC core ring structure. What is most intriguing, however, is the very different levels of transcriptional efficacy that some of the THC ligands have on the two ER subtypes.

Structural Basis for the ER*β*-Selective Antagonist Activity of the Tetrahydrochrysenes. The THC compounds that we have studied differ in activity from well-known antiestrogenic compounds hydroxytamoxifen and raloxifene, which are full antagonists on ER*β* but usually have only minimal agonist activity on ERα and substantially antagonize E_2 agonism when assayed in systems similar to those used here.[5,10,25−27] For example, in HEC-1 cells, hydroxytamoxifen has 25% the agonist activity of estradiol through ERα but is a pure antagonist through ER*β*.[26,27] In contrast, *cis*-diethyl (*R,R*)-**2b**, both enantiomers of *cis*-dipropyl **2c**, and *trans*-dipropyl **3c** are nearly full agonists on ERα.

These THC ligands are also quite different structurally from hydroxytamoxifen and raloxifene, which have bulky substituents that project outward from the ligand core and contain basic tertiary amine side chains (Figure 3B). From the recent ERα X-ray crystal structures, [21,23] these substituents were seen to project *upward* from the ligand core toward helix 12 and result in a change in the tertiary structure of the ERα LBD: By steric interactions, the bulky substituent displaces helix 12 from its normal docking site in the ERα−agonist structures and repositions it into a groove in which the amphipathic helix motif LXXLL, found in co-activator proteins, is thought to interact. In this conformation, the basic amine function on the large substituent is stabilized by salt bridge formation with a specific aspartic acid residue (D351).

The antagonist activity of raloxifene and hydroxytamoxifen, then, is presumed to arise from the blocking of co-activator LXXLL helix interaction by the repositioned helix 12. Because they are also antagonists on ER*β*, one would presume that hydroxytamoxifen and raloxifene would induce an "antagonist conformation" in ER*β* by a similar repositioning of helix 12 into the co-activator helix binding groove of this ER subtype.

But how is it that some of the THCs, (*R,R*)-diethyl THC and all three dipropyl THCs, can be full or nearly full agonists on ERα yet full antagonists on ER*β*? None of these ER*β*-selective antagonists have substituents of the size and character found in tamoxifen and raloxifene. Also, with increase in substituent size, ER*β*-selective antagonism developed first in the (*R,R*)-*cis*-THC series, in which the substituents project *downward* from the ligand core, yet with two propyl substituents, antagonism was induced in ER*β* with all three of the THCs. Thus, it would appear that an antagonist con-

formation can be induced in ERβ by a change in tertiary structure that may be different from the displacement of helix 12 by a large, basic substituent on a ligand, as has been observed in ERα with hydroxytamoxifen and raloxifene.

The groove into which the LXXLL helix from co-activators binds is made up of portions of helices 3, 4, and 5, as well as helix 12.[23] It is possible that ligand-induced motions of one or more helices—other than helix 12—could distort this groove so that the co-activator helix would no longer be able to bind. Such an alternative ligand-induced conformational change blocking co-activator helix binding would, of course, need to be induced by the appropriate THCs more easily in ERβ than in ERα. Clearly, additional X-ray crystallographic structural work, comparing the structure of the ERα and ERβ LBDs complexed with ERβ-selective antagonists, is needed to settle this issue definitively.

In summary, we have developed an enantioselective asymmetric synthesis of *cis*-dimethyl-, -diethyl-, and -dipropyltetrahydrochrysenes and their trans congeners which has provided us with a set of novel ligands for the estrogen receptor that are interesting for studying the tolerance of both ERα and ERβ to ligand substitution and stereochemistry and as probes for the conformational basis of agonist/antagonist character. Both the *cis*- and *trans*-THC series were found to have higher binding affinities for ERβ relative to ERα. THCs with small substituents (at the 5- and 11-ring positions) are agonists on both ER subtypes; however, as substituent size is increased, ERβ-selective antagonism develops first in the (*R,R*)-enantiomer of the *cis*-THC series and finally in the trans and (*S,S*)-enantiomer THC series. Thus, *cis-(R,R)*-diethyl THC, *trans*-dipropyl THC **3c**, and both *cis*-dipropyl enantiomers **2c** were full or nearly full antagonists on ERβ yet are full or nearly full agonists on ERα. These compounds are the first to be described that are full agonists on ERα and full antagonists on ERβ.

These studies illustrate that the antagonist character in THC ligands for ERβ depends in a progressive way on the size and geometric disposition of substituent groups. Furthermore, antagonists that are selectively effective on ERβ can have structures that are very different from the typical antiestrogens tamoxifen and raloxifene, all of which have very bulky substituents and are complete or nearly complete antagonists on both ER subtypes. This suggests, at least for ERβ, that an antagonist state of the receptor can be reached by conformational change that is different from that which has been demonstrated so far in antagonist complexes with ERα. The subtype-selective efficacy of some of these THCs should be useful in evaluating the biological role of ERβ through studies in various in vitro and in vivo test systems and in examining the conformation of ERα and ERβ agonist/antagonist complexes by X-ray crystallography.

Experimental Section

General. The synthesis of compounds **3b,c** and **10** has been described previously.[13] Reagents and solvents were purchased from Aldrich, Fisher, and Mallinckrodt. THF was distilled immediately prior to use from sodium/benzophenone. CH_2Cl_2 and toluene were distilled from CaH_2. *n*-Butyllithium was titrated against *N*-pivaloyl-*o*-toluidine. Et_3N and TMSCl were distilled over CaH_2. LiCl was dried in vacuo at 130 °C overnight and stored in a desiccator. Diazomethane was prepared from *N*-methyl-*N*-nitrosourea as previously described.[28] All reactions were carried out under nitrogen or argon, using oven- or flame-dried glassware, unless stated otherwise. Reaction progress was monitored by analytical thin-layer chromatography using 0.25-mm HLF silica plates with UV254 indicator (Analtech), and visualization was achieved by UV light (254 nm) or phosphomolybdic acid indicator. Hexane was distilled prior to use in chromatography. Flash chromatography was performed using Woelm 32−63-μm silica gel packing. Radial preparative-layer chromatography was performed on a Chromatotron instrument (Harrison Research, Inc., Palo Alto, CA) using EM Science silica gel Kieselgel 60 PF_{254} as adsorbent.

Melting points were determined on a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were obtained with Varian Unity 400- and 500-MHz spectrometers. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane and referenced from solvent references. NMR coupling constants are reported in hertz (Hz). ^{13}C NMR spectra were determined using attached proton test (APT) experiment. Low- and high-resolution electron impact (EI) mass spectra were obtained on a Micromass 70-VSE spectrometer. Low- and high-resolution fast atom bombardment (FAB) spectra were obtained on Micromass ZAB-SE and 70-SE-4F spectrometers, respectively. Elemental analyses were performed by the Microanalytic Service Laboratory of the University of Illinois.

Cis:trans isomer ratios were determined by integration of ^1H NMR peaks with comparison to known compounds.[13] Enantiomeric ratios were determined with one of three HPLC columns from Regis Technologies, Inc. and Chiral Technologies: (A) *S,S* Whelk-O 1 (4.6 mm × 25 cm), (B) *R,R* Whelk-O 1 (4.6 mm × 25 cm), or (C) ChiralPak AS (4.6 mm × 25 cm).

General Method for Pseudoephedrine Acylation. According to the method of Myers,[14] the appropriate anhydride (1.2 equiv) was added slowly to a stirred 0.5 M solution of (+)- or (−)-pseudoephedrine (1 equiv) and triethylamine (1.2 equiv) in CH_2Cl_2 at room temperature. After having been stirred for 1 h, the reaction mixture was quenched with water. The organic layer was washed with half-saturated $NaHCO_3$ (aqueous), 1 M HCl, and brine. The extract was dried over Na_2SO_4 and concentrated to provide amides **5a**−**c**.

(1*S*,2*S*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methylpropionamide ((*S,S*)-5a). Amide (*S,S*)-**5a** was obtained as a white solid (5.38 g, 96%): mp 112−113.5 °C (lit.[14] mp 114−115 °C); spectroscopic data matched those previously reported.[14] Anal. ($C_{13}H_{19}NO_2$) C, H, N.

(1*R*,2*R*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methylpropionamide ((*R,R*)-5a). Recrystallization from hot toluene provided amide (*R,R*)-**5a** as a white solid (3.67 g, 92%): mp 113−114 °C; spectroscopic data identical to those for (*S,S*)-**5a**.[14] Anal. ($C_{13}H_{19}NO_2$) C, H, N.

(1*S*,2*S*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methylbutyramide ((*S,S*)-5b). Recrystallization from hot toluene furnished amide (*S,S*)-**5b** as a white crystalline solid (7.70 g, 93%): mp 82.5−84 °C; ^1H NMR (3:1 rotamer ratio, asterisk denotes minor rotamer peaks, 500 MHz, C_6D_6) δ 7.32 (d, 2H, J = 7.2), 7.05−7.20 (m, 3H), 4.94 (br s, 1H), 4.53 (t, 1H, J = 7.2), 4.22 (br, 1H), 4.19* (dd, 1H, J = 8.8, 2.7), 3.78* (quint, 1H, J = 6.7), 2.83* (s, 3H), 2.44* (sextet, 1H, J = 7.8), 2.18* (m, 1H), 2.12 (s, 3H), 1.72−1.85 (m, 2H), 1.58 (sextet, 2H, J = 7.4), 0.97 (d, 3H, J = 7.1), 0.94* (t, 3H, J = 7.4), 0.81 (t, 3H, J = 7.4), 0.60* (d, 3H, J = 6.8); ^{13}C NMR (asterisk denotes minor rotamer peaks, 125 MHz, C_6D_6) δ 174.8, 174.0*, 144.2, 143.3*, 128.9, 128.7, 127.8, 127.8, 127.3, 76.8, 75.8*, 58.9, 36.5, 36.0*, 27.1, 19.4*, 19.0, 15.7*, 14.7, 14.7*, 14.4; MS (FAB) m/z 236 (MH^+). Anal. ($C_{14}H_{21}NO_2$) C, H, N.

(1*R*,2*R*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methylbutyramide ((*R,R*)-5b). Recrystallization from hot toluene furnished amide (*R,R*)-**5b** as a white crystalline solid (7.83 g, 95%): mp 85−86.5 °C; spectroscopic data identical to those of (*S,S*)-**5b**. Anal. ($C_{14}H_{21}NO_2$) C, H, N.

(1*S*,2*S*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methylpentanamide ((*S*,*S*)-5c). Flash chromatography (20% acetone/benzene) provided (*S*,*S*)-**5c** as a clear viscous oil (3.85 g, 85%): ^{1}H NMR (3:1 rotamer ratio, asterisk denotes minor rotamer peaks, 500 MHz, C_6D_6) δ 7.33 (d, 2H, J = 7.4), 7.05−7.21 (m, 3H), 4.99 (br s, 1H), 4.54 (d, 1H, J = 7.2), 4.21 (br, 1H), 4.18* (d, 1H, J = 8.7), 3.80* (quint, 1H, J = 7.0), 2.83* (s, 3H), 2.49* (m, 1H), 2.26* (m, 1H), 2.14 (s, 3H), 1.85 (ABt, 2H, ν_A = 1.87 ppm, ν_B = 1.83 ppm, J_{AB} = 15.5, J = 7.5), 1.74−1.81* (m, 2H), 1.55 (quint, 2H, J = 7.4), 1.29−1.39* (m, 2H), 1.20 (sextet, 2H, J = 7.5), 0.98 (d, 3H, J = 7.0), 0.90* (t, 3H, J = 7.4), 0.83 (t, 3H, J = 7.3), 0.60* (d, 3H, J = 6.8); ^{13}C NMR (asterisk denotes minor rotamer peaks, 100 MHz, C_6D_6) δ 175.1, 174.2*, 144.2, 143.0*, 129.0, 128.8, 127.8, 127.7, 127.2, 76.9, 75.9*, 58.9, 34.4, 33.9*, 28.2*, 27.7, 23.4*, 23.1, 14.8, 14.5; MS (FAB) m/z 250 (MH^+). Anal. ($C_{15}H_{23}NO_2$) C, H, N.

(1*R*,2*R*)-*N*-(2-Hydroxy-1-methyl-2-phenylethyl)-*N*-methylpentanamide ((*R*,*R*)-5c). Flash chromatography (20% acetone/benzene) provided amide (*R*,*R*)-**5c** as a clear viscous oil (4.32 g, 96%): spectroscopic data identical to those of (*S*,*S*)-**5c**. Anal. ($C_{15}H_{23}NO_2$) C, H, N.

General Method for Asymmetric Alkylation. According to the method of Myers,[14] *n*-butyllithium (2.08 equiv) was added to a mixture of LiCl (≥6 equiv) and diisopropylamine (2.25 equiv) in THF (~1.5 M) at −78 °C. The flask was warmed to 0 °C for 15 min before recooling to −78 °C. An ice-cooled solution of amides **5a**−**c** (1 equiv) in THF (~0.33 M) was added via cannula. The resulting suspension was stirred at −78 °C for 1 h, 0 °C for 15 min, and room temperature for 5 min. After the mixture was recooled to 0 °C, 3-methoxybenzyl bromide (1.5 equiv) was added and stirring was continued at 0 °C until the reaction was complete as indicated by TLC (1−2 h). The reaction was quenched with saturated NH_4Cl (aqueous) and EtOAc. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to provide amides **6a**−**c**.

(1′*S*,2′*S*,2*R*)-*N*-(2′-Hydroxy-1′-methyl-2′-phenylethyl)-*N*-methyl-2-(3-methoxybenzyl)propionamide ((*S*,*S*,*R*)-6a). Flash chromatography (20% acetone/benzene) provided amide (*S*,*S*,*R*)-**6a** as a light-yellow highly viscous oil (645 mg, 1.89 mmol, 84%): ^{1}H NMR (3:1 rotamer ratio, asterisk denotes minor rotamer peaks, 500 MHz, C_6D_6) δ 7.24 (d, 2H, J = 7.6), 7.01−7.18 (m, 4H), 6.82 (br, 1H), 6.72 (d, 1H, J = 7.1), 6.66 (dd, 1H J = 8.2, 2.5), 4.47 (t, 1H, J = 6.4), 4.41 (br s, 1H), 4.34 (br, 1H), 4.07* (dd, 1H, J = 8.3, 2.9), 3.85* (quintet, 1H, J = 7.3), 3.40* (s, 3H), 3.33 (s, 3H), 3.09* (m, 1H), 3.04 (dd, 1H, J = 13.1, 8.2), 2.77* (m, 1H), 2.74* (s, 3H), 2.62 (sextet, 1H, J = 6.8), 2.51 (dd, 1H, J = 13.1, 6.1), 2.13 (s, 3H), 1.09* (d, 3H, J = 6.8), 1.04 (d, 3H, J = 6.8), 0.85 (d, 3H, J = 6.3), 0.62* (d, 3H, J = 6.6); ^{13}C NMR (asterisk denotes minor rotamer peaks, 125 MHz, C_6D_6) δ 177.5, 176.9*, 160.3*, 160.2, 143.5, 142.9*, 142.8*, 142.3, 129.5*, 129.5, 128.6, 128.5, 128.3, 127.4, 127.3*, 127.0, 122.1*, 121.7, 115.4*, 115.2, 112.0*, 111.8, 76.3, 75.4*, 58.2, 54.7, 40.7, 40.5*, 38.9, 38.2*, 17.7, 17.6*, 15.4*, 14.2; MS (FAB) m/z 342 (MH^+). Anal. ($C_{21}H_{27}NO_3$) C, H, N.

(1′*R*,2′*R*,2*S*)-*N*-(2′-Hydroxy-1′-methyl-2′-phenylethyl)-*N*-methyl-2-(3-methoxybenzyl)propionamide ((*R*,*R*,*S*)-6a). Flash chromatography (35−50% EtOAc/hexane) furnished amide (*R*,*R*,*S*)-**6a** as a clear viscous oil (5.37 g, 99%): spectroscopic data identical to those for (*S*,*S*,*R*)-**6a**. Anal. ($C_{21}H_{27}NO_3$) C, H, N.

(1′*S*,2′*S*,2*R*)-*N*-(2′-Hydroxy-1′-methyl-2′-phenylethyl)-*N*-methyl-2-(3-methoxybenzyl)butyramide ((*S*,*S*,*R*)-6b). Flash chromatography (20% acetone/benzene) furnished amide (*S*,*S*,*R*)-**6b** as a clear viscous oil (9.48 g, 88%): ^{1}H NMR (5:1 rotamer ratio, asterisk denotes minor rotamer peaks, 500 MHz, C_6D_6) δ 7.24 (d, 2H, J = 7.1), 7.01−7.13 (m, 4H), 6.82 (t, 1H, J = 1.8), 6.72 (d, 1H, J = 7.5), 6.65 (dd, 1H J = 8.1, 2.0), 4.47 (br s, 1H), 4.43 (t, 1H, J = 7.0), 4.21 (br, 1H), 4.05* (dd, 1H, J = 8.7, 3.7), 3.93* (quintet, 1H, J = 7.0), 3.40* (s, 3H), 3.33 (s, 3H), 2.99 (dd, 1H, J = 12.3, 8.5), 2.78* (dd, 1H, J = 13.1, 6.7), 2.75* (s, 3H), 2.55−2.64 (m, 2H), 2.14 (s, 3H), 1.83−1.87 (m, 1H), 1.36−1.40 (m, 1H), 0.83 (t, 3H, J = 7.4), 0.76−0.81 (m, 3H + 3H*), 0.63* (d, 3H, J = 6.8); ^{13}C NMR (asterisk denotes minor rotamer peaks, 125 MHz, C_6D_6) δ 177.2, 160.2, 143.5, 142.2, 129.5, 129.5*, 128.6*, 128.4, 128.3*, 127.5, 127.3*, 126.9, 122.0*, 121.6, 115.5*, 115.2, 112.0*, 111.8, 76.6, 75.3*, 65.7, 54.6, 46.4, 39.8, 26.6, 14.4, 11.9; MS (FAB) m/z 356 (MH^+); HRMS (FAB) calcd for $C_{22}H_{30}NO_3$ 356.2226, found 356.2227.

(1′*R*,2′*R*,2*S*)-*N*-(2′-Hydroxy-1′-methyl-2′-phenylethyl)-*N*-methyl-2-(3-methoxybenzyl)butyramide ((*R*,*R*,*S*)-6b). Flash chromatography (20% acetone/benzene) furnished amide (*R*,*R*,*S*)-**6b** as a clear viscous oil (11.8 g, quantitative): spectroscopic data identical to those for (*S*,*S*,*R*)-**6b**; HRMS (FAB) calcd for $C_{22}H_{30}NO_3$ 356.2226, found 356.2224.

(1′*S*,2′*S*,2*R*)-*N*-(2′-Hydroxy-1′-methyl-2′-phenylethyl)-*N*-methyl-2-(3-methoxybenzyl)pentanamide ((*S*,*S*,*R*)-6c). Flash chromatography (35−50% EtOAc/hexane) furnished amide (*S*,*S*,*R*)-**6c** as a yellow viscous oil (3.79 g, 61%): ^{1}H NMR (4:1 rotamer ratio, asterisk denotes minor rotamer peaks, 400 MHz, C_6D_6) δ 7.27 (d, 2H, J = 7.1), 6.98−7.19 (m, 4H), 6.84 (dd, 1H, J = 2.3, 1.7), 6.74 (d, 1H, J = 7.4), 6.70* (ddd, 1H J = 8.1, 2.5, 0.8), 6.64 (ddd, 1H J = 8.1, 2.5, 0.8), 4.58 (br s, 1H), 4.44 (t, 1H, J = 6.2), 4.31 (br, 1H), 4.15* (dd, 1H, J = 8.7, 2.6), 4.01* (quintet, 1H, J = 6.8), 3.41* (s, 3H), 3.32 (s, 3H), 3.09−3.16* (m, 1H), 2.99 (dd, 1H, J = 12.9, 9.5), 2.81* (dd, 1H, J = 13.2, 7.5), 2.77* (s, 3H), 2.66−2.75 (m, 1H), 2.57 (dd, 1H, J = 12.9, 5.1), 2.17 (s, 3H), 1.79−1.92 (m, 1H), 1.38−1.47* (m, 1H), 1.23−1.37 (m, 2H), 1.05−1.17 (m, 1H), 0.98−1.17* (m, 1H), 0.82 (t, 3H, J = 7.1), 0.78 (d, 3H, J = 6.7), 0.76* (t, 3H, J = 7.2), 0.66* (d, 3H, J = 6.7); ^{13}C NMR (asterisk denotes minor rotamer peaks, 100 MHz, C_6D_6) δ 177.6, 160.5, 143.8, 143.3*, 143.0*, 142.6, 130.1*, 129.9, 129.0*, 128.8, 127.9, 127.7*, 127.4, 122.4*, 122.0, 115.9*, 115.6, 112.4*, 112.2, 76.8, 75.6*, 58.9, 55.1, 45.1, 40.4, 36.2, 21.2, 14.9, 14.7; MS (FAB) m/z 370 (MH^+); HRMS (FAB) calcd for $C_{23}H_{32}NO_3$ 370.2382, found 370.2383. Anal. ($C_{23}H_{31}NO_3$) C, H, N.

(1′*R*,2′*R*,2*S*)-*N*-(2′-Hydroxy-1′-methyl-2′-phenylethyl)-*N*-methyl-2-(3-methoxybenzyl)pentanamide ((*R*,*R*,*S*)-6c). Flash chromatography (35−60% EtOAc/hexane) furnished amide (*R*,*R*,*S*)-**6c** as a clear viscous oil (5.81 g, 95%): spectroscopic data identical to those for (*S*,*S*,*R*)-**6c**; HRMS (FAB) calcd for $C_{23}H_{32}NO_3$ 370.2382, found 370.2383. Anal. ($C_{23}H_{31}NO_3$) C, H, N.

Methyl (2*R*)-3-(3-Methoxyphenyl)-2-methylpropionate ((*R*)-7a). A solution of amide (*S*,*S*,*R*)-**6a** (1.16 g, 3.40 mmol) in 1:3 concentrated H_2SO_4/MeOH (10 mL) was refluxed for 4 h. The reaction was neutralized with 1 M NaOH and extracted with EtOAc. The organic layers were dried over Na_2SO_4 and concentrated. Flash chromatography (20% acetone/benzene) yielded (*R*)-**7a** as a clear oil (475 mg, 67% yield, 77% corrected for starting material consumed): $[\alpha]_{589}^{28}$ −19.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (t, 1H, J = 7.8), 6.75 (dd, 2H, J = 7.9, 2.1), 6.71 (t, 1H, J = 2.0), 3.79 (s, 3H), 3.65 (s, 3H), 3.01 (dd, 1H, J = 13.3, 6.7), 2.74 (sextet, 1H, J = 7.0), 2.63 (dd, 1H, J = 13.4, 7.8), 1.15 (d, 3H, J = 6.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.5, 159.5, 140.9, 129.3, 121.3, 114.6, 111.6, 55.0, 51.6, 41.3, 39.6, 16.7; MS (EI, 70 eV) m/z 208 (M^+, 73), 148 (M − CO_2Me, 58). Anal. ($C_{12}H_{16}O_3$) C, H.

Methyl (2*S*)-3-(3-Methoxyphenyl)-2-methylpropionate ((*S*)-7a). A solution of amide (*R*,*R*,*S*)-**6a** (5.21 g, 15.2 mmol) in 1:1 18 N aqueous H_2SO_4/dioxane (52 mL) was refluxed for 2 h. After cooling, the solution was concentrated and partitioned between water (200 mL) and EtOAc (200 mL). The organic extract was washed with 3 N HCl and brine and was dried over Na_2SO_4. Concentration gave the crude carboxylic acid as a golden brown oil. The crude acid was dissolved in diethyl ether (80 mL) and treated with excess diazomethane at 0 °C. The reaction was quenched with a few drops of glacial acetic acid. Concentration and bulb-to-bulb distillation (~85−100 °C at 0.2 Torr) gave (*S*)-**7a** as a clear liquid (2.44 g, 77%): $[\alpha]_{589}^{28}$ +28.8° (*c* 0.82, $CHCl_3$); spectroscopic data identical to those of ester (*R*)-**7a**. Anal. ($C_{12}H_{16}O_3$) C, H.

Methyl (2*R*)-2-(3-Methoxybenzyl)butyrate ((*R*)-7b). Methanesulfonic acid (2.5 mL, 38.4 mmol) was added to a solution of amide (*S*,*S*,*R*)-**6b** (9.10 g, 25.6 mmol) in THF (100 mL) and refluxed for 3 h. The solution was cooled to 0 °C, and $LiBH_4$ (2.0 M in THF, 19.2 mL, 38.4 mmol) was added slowly.

Water (55 mL) was added cautiously, followed by tetrabutylammonium hydroxide (40% w/w in H_2O, 85 mL, 128 mmol). The heterogeneous mixture was stirred for 1 h. The solution was acidified to pH < 2 with 3 N HCl, extracted with ether, and concentrated to give 4.5 g of the crude acid. A solution of the crude acid in ether was treated with excess diazomethane at 0 °C. After the mixture was quenched with glacial acetic acid and concentrated, bulb-to-bulb distillation (105−110 °C at 0.45 Torr) gave ester (*R*)-**7b** as a clear liquid (3.45 g, 15.5 mmol, 61%): er ≥ 98:2 (HPLC column A, 0.25% IPA/hexanes, t_R = 20.5 min); $[\alpha]_{589}^{24}$ −30.3° (*c* 1.02, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (t, 1H, *J* = 7.9), 6.74 (dd, 2H, *J* = 7.9, 2.1), 6.70 (s, 1H), 3.79 (s, 3H), 3.62 (s, 3H), 2.92 (dd, 1H, *J* = 13.7, 8.2), 2.71 (dd, 1H, *J* = 13.5, 6.8), 2.60 (tdd, 1H, *J* = 8.4, 6.8, 5.1), 1.63 (ddq, 1H, *J* = 13.6, 8.7, 7.5), 1.58 (dqd, 1H, *J* = 13.7, 7.5, 5.2), 0.91 (t, 3H, *J* = 7.5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 176.0, 159.6, 141.1, 129.3, 121.2, 114.5, 111.6, 55.1, 51.4, 49.0, 38.1, 25.1, 11.7; MS (EI, 70 eV) *m*/*z* 222 (M^+, 62), 162 (M − CO_2Me, 41). Anal. ($C_{13}H_{18}O_3$) C, H.

Methyl (2*S*)-2-(3-Methoxybenzyl)butyrate ((*S*)-7b). Ester (*S*)-**7b** was prepared by the same method described for (*R*)-**7b** from amide (*R*,*R*,*S*)-**6b** (9.32 g, 26.2 mmol). Isolation of the second distillate from bulb-to-bulb distillation (∼90−100 °C at 0.3 Torr) furnished ester (*S*)-**7b** as a clear liquid (3.36 g, 58%): er = 98:2 (HPLC column A, 0.25% IPA/hexanes, t_R = 18.9 min); $[\alpha]_{589}^{24}$ +32.6° (*c* 1.0, $CHCl_3$); spectroscopic data identical to those of ester (*R*)-**7b**. Anal. ($C_{13}H_{18}O_3$) C, H.

Methyl (2*R*)-2-(3-Methoxybenzyl)pentanoate ((*R*)-7c). Ester (*R*)-**7c** was prepared by the same method as ester (*S*)-**7a** from amide (*S*,*S*,*R*)-**6c** (3.66 g, 9.91 mmol). Bulb-to-bulb distillation provided (*R*)-**7c** as a clear liquid (1.48 g, 63%): $[\alpha]_{589}^{26}$ −24.4° (*c* 1.19, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (t, 1H, *J* = 7.8), 6.74 (dd, 2H, *J* = 7.9, 2.1), 6.70 (t, 1H, *J* = 1.9), 3.79 (s, 3H), 3.61 (s, 3H), 2.87−2.96 (m, 1H), 2.62−2.74 (m, 2H), 1.57−1.69 (m, 1H), 1.42−1.52 (m, 1H), 1.21−1.40 (m, 2H), 0.89 (t, 3H, *J* = 7.3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.2, 159.6, 141.1, 129.3, 121.1, 114.5, 111.5, 55.1, 51.5, 47.4, 38.5, 34.2, 20.6, 13.9; MS (EI, 70 eV) *m*/*z* 236 (M^+, 67), 176 (M − CO_2Me, 41). Anal. ($C_{14}H_{20}O_3$) C, H.

Methyl (2*S*)-2-(3-Methoxybenzyl)pentanoate ((*S*)-7c). Ester (*S*)-**7c** was prepared by the same method as ester (*S*)-**7a** from amide (*R*,*R*,*S*)-**6c** (4.85 g, 13.1 mmol). Bulb-to-bulb distillation gave (*S*)-**7c** as a clear liquid (1.65 g, 53%): $[\alpha]_{589}^{26}$ +19.9° (*c* 1.08, $CHCl_3$); spectroscopic data identical to that of ester (*R*)-**7c**. Anal. ($C_{14}H_{20}O_3$) C, H.

General Method for Acyloin Condensation and Lewis Acid Cyclization. In a three-necked round-bottom flask, sodium metal (3 equiv) was melted in refluxing toluene (∼0.5 M) under an Ar atmosphere. A solution of the appropriate esters **7a**−**c** (1 equiv) and TMSCl (6 equiv) in toluene (∼1.5 M) was added dropwise via an addition funnel, and reflux was continued for 18−24 h. After the heterogeneous purple mixture was cooled to room temperature, the mixture was filtered to remove sodium metal and salts. The precipitate was washed with anhydrous ether. The combined organic solutions were washed with 1 M HCl, washed with brine (2 times), and dried over $MgSO_4$. Concentration and flash chromatography (10% ether/hexanes) provided silyl ethers **8a**−**c** as clear oils (67−88% yield). Solutions of silyl ethers **8a**−**c** (1 equiv) in CH_2Cl_2 (0.15 M) were cooled to −78 °C and treated with $TiCl_4$ (1.0 M in CH_2Cl_2, 2 equiv). The reaction was protected from light with aluminum foil, allowed to warm to room temperature, and stirred for 3−4 h. The reaction was quenched with half-saturated $NaHCO_3$ (aqueous) and partitioned with ether. The organic layer was washed with water and brine and dried over $MgSO_4$. Concentration provided crude tetrahydrochrysenes **9a**−**c**.

(5*R*,11*R*)-2,8-Dimethoxy-5,11-dimethyl-5,6,11,12-tetrahydrochrysene ((*R*,*R*)-9a). Flash chromatography (10% ether/hexanes) provided silyl ether (*R*,*R*)-**8a** as a clear oil (88%) which cyclized upon treatment with $TiCl_4$ to provide the crude THC (*R*,*R*)-**9a** as a white solid in a ratio of 12.5:1 of the cis:trans isomers. Recrystallization from ether furnished (*R*,*R*)-**9a** as a white solid (175 mg, 50%, 33:1 cis:trans). Repeated recrystallizations from diethyl ether provided (*R*,*R*)-**9a** in a cis:trans ratio of 64:1: mp 196.5−197.5 °C; er = 98:2 (HPLC column B, 0.5% IPA/hexanes, t_R = 13.1 min); $[\alpha]_{589}^{24}$ −109° (*c* 0.98, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (d, 2H, *J* = 8.3), 6.76−6.79 (m, 4H), 3.84 (s, 6H), 3.14 (dd, 2H, *J* = 15.1, 6.1), 2.92 (quint.d, 2H, *J* = 6.2, 1.5), 2.59 (dd, 2H, *J* = 15.2, 1.5), 1.07 (d, 6H, *J* = 6.8); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2, 136.0, 131.6, 127.9, 122.8, 114.7, 111.0, 55.2, 36.4, 28.9, 17.4; MS (EI, 70 eV) *m*/*z* 320 (M^+, 100), 305 (M − Me, 39). Anal. ($C_{22}H_{24}O_2$) C, H.

(5*S*,11*S*)-2,8-Dimethoxy-5,11-dimethyl-5,6,11,12-tetrahydrochrysene ((*S*,*S*)-9a). Flash chromatography (10% ether/hexanes) provided silyl ether (*S*,*S*)-**8a** as a clear oil (72%) which cyclized upon treatment with $TiCl_4$. Recrystallization from ether provided THC (*S*,*S*)-**9a** as a white solid (487 mg, 42% yield, 33:1 cis:trans). Further recrystallization from EtOAc furnished (*S*,*S*)-**9a** in a 66:1 cis:trans ratio: mp 195−196.5 °C; er ≥ 99:1 (HPLC column B, 0.5% IPA/hexanes, t_R = 10.9 min); $[\alpha]_{589}^{28}$ +106° (*c* 1.0, $CHCl_3$); spectroscopic data identical to those for (*R*,*R*)-**9a**. Anal. ($C_{22}H_{24}O_2$) C, H.

(5*R*,11*R*)-2,8-Dimethoxy-5,11-diethyl-5,6,11,12-tetrahydrochrysene ((*R*,*R*)-9b). Flash chromatography (10% ether/hexanes) provided silyl ether (*R*,*R*)-**8b** as a clear oil (72%) which cyclized upon treatment with $TiCl_4$. Flash chromatography (10% ether/hexanes) provided THC (*R*,*R*)-**9b** as a white solid (1.16 g, 71% yield, 15:1 cis:trans). Repeated recrystallizations from ether/hexanes furnished (*R*,*R*)-**9b** (100% cis isomer): mp 132.5−134 °C; er ≥ 99:1 (HPLC column B, 0.5% IPA/hexanes, t_R = 11.8 min); $[\alpha]_{589}^{24}$ −209° (*c* 0.97, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (d, 2H, *J* = 8.0), 6.74−6.77 (m, 4H), 3.84 (s, 6H), 2.94−3.06 (br m, 2H), 2.82 (dd, 2H, *J* = 15.6, 1.4), 2.59 (br m, 2H), 1.49 (dqd, 2H, *J* = 15.0, 7.4, 3.4), 1.33 (ddq, 2H, *J* = 14.7, 10.6, 7.3), 0.97 (t, 6H, *J* = 7.4); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.2, 136.2, 131.7, 128.3, 122.9, 114.7, 110.9, 55.2, 35.8, 32.1, 23.2, 12.0; MS (EI, 70 eV) *m*/*z* 348 (M^+, 100), 319 (M − Et, 64); HRMS (EI, 70 eV) calcd for $C_{24}H_{28}O_2$ 348.2089, found 348.2084. Anal. ($C_{24}H_{28}O_2$) C, H.

(5*S*,11*S*)-2,8-Dimethoxy-5,11-diethyl-5,6,11,12-tetrahydrochrysene ((*S*,*S*)-9b). Flash chromatography (10% ether/hexanes) provided silyl ether (*S*,*S*)-**8b** as a clear oil (67%) which cyclized upon treatment with $TiCl_4$. Flash chromatography (10% ether/hexanes) provided THC (*S*,*S*)-**9b** as a white solid (966 mg, 65% yield, 17.6:1 cis:trans). Repeated recrystallizations from ether/hexanes furnished (*S*,*S*)-**9b** (100% cis isomer): mp 131.5−133 °C; er ≥ 99:1 (HPLC column B, 0.5% IPA/hexanes, t_R = 9.5 min); $[\alpha]_{589}^{24}$ +192° (*c* 0.99, $CHCl_3$); spectroscopic data was identical to those for (*R*,*R*)-**9b**; HRMS (EI, 70 eV) calcd for $C_{24}H_{28}O_2$ 348.2089, found 348.2086. Anal. ($C_{24}H_{28}O_2$) C, H.

(5*R*,11*R*)-2,8-Dimethoxy-5,11-dipropyl-5,6,11,12-tetrahydrochrysene ((*R*,*R*)-9c). Flash chromatography (5% ether/hexanes) provided silyl ether (*R*,*R*)-**8c** as a clear oil (71%) which cyclized upon treatment with $TiCl_4$. Flash chromatography (5% ether/hexanes) provided THC (*R*,*R*)-**9c** as an oil (495 mg, 69% yield, ∼20:1 cis:trans). Flash chromatography (20% CH_2Cl_2/hexanes) and crystallization attempts were unsuccessful in improving the cis:trans ratio: er ≥ 99:1 (HPLC column B, 0.5% IPA/hexanes, t_R = 11.9 min); $[\alpha]_{589}^{28}$ −118° (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (d, 2H, *J* = 8.4), 6.78 (dd, 2H, *J* = 8.3, 2.6), 6.76 (d, 2H, *J* = 2.4), 3.85 (s, 6H), 3.01 (dd, 2H, *J* = 15.6, 6.0), 2.81 (dd, 2H, *J* = 15.6, 1.4), 2.70−2.75 (br m, 2H), 1.47−1.58 (m, 2H), 1.33−1.42 (m, 6H), 0.93 (t, 6H, *J* = 6.8); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.2, 136.2, 131.7, 128.3, 122.8, 114.8, 110.8, 55.2, 33.8, 32.4, 32.3, 20.6, 14.2; MS (EI, 70 eV) *m*/*z* 376 (M^+, 100), 333 (M − Pr, 91); HRMS (EI, 70 eV) calcd for $C_{26}H_{32}O_2$ 376.2402, found 376.2397.

(5*S*,11*S*)-2,8-Dimethoxy-5,11-dipropyl-5,6,11,12-tetrahydrochrysene ((*S*,*S*)-9c). Flash chromatography (5% ether/hexanes) provided silyl ether (*S*,*S*)-**8c** as a clear oil (71%) which cyclized upon treatment with $TiCl_4$. Flash chromatography (5% ether/hexanes) provided THC (*S*,*S*)-**9c** as an oil (585 mg, 74% yield, ∼20:1 cis:trans). Flash chromatography (20% CH_2Cl_2/hexanes) and crystallization attempts were unsuccessful in improving the cis:trans ratio: er ≥ 99:1 (HPLC column B, 0.5%

IPA/hexanes, t_R = 10.1 min); $[\alpha]_{589}^{28}$ +100° (*c* 0.5, $CHCl_3$); spectroscopic data identical to those for (*R,R*)-**9c**; HRMS (EI, 70 eV) calcd for $C_{26}H_{32}O_2$ 376.2402, found 376.2393.

2,8-Dimethoxy-5,11-*trans*-dimethyl-5,6,11,12-tetrahydrochrysene (*trans*-9a). A solution of (*R,R*)-**8a** (200 mg, 0.4 mmol) in PPA (1.8 g) was stirred with a mechanical stirrer for 75 min. Water and EtOAc were added, and the mixture was stirred until all of the material had dissolved. The organic layer was washed with saturated $NaHCO_3$ and brine and dried over Na_2SO_4. Concentration provided crude *trans*-**9a**:(*R,R*)-**9a** as a 1:2 mixture. Recrystallization from EtOAc furnished the same ratio of isomers as white crystals (77 mg, 60%). Further recrystallizations did not remove (*R,R*)-**9a** from the product mixture. *trans*-**9a**: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, 2H, J = 9.4), 6.75−6.79 (m, 4H), 3.84 (s, 6H), 3.20 (quint d, 2H, J = 6.9, 1.2), 3.10 (dd, 2H, J = 15.3, 6.8), 2.68 (dd, 2H, J = 15.4, 1.2), 0.91 (d, 6H, J = 6.9); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.3, 136.6, 131.7, 127.0, 123.7, 114.9, 110.9, 55.2, 36.3, 26.1, 17.9; MS (EI, 70 eV) *m/z* 320 (M^+, 100), 305 (M − Me, 46); HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O_2$ 320.1776, found 320.1777.

General Method for Methyl Ether Deprotection. BBr_3 (1.0 M in CH_2Cl_2, 3 equiv) was added to a solution of methyl ethers **9a**−**c** (1 equiv) in CH_2Cl_2 (∼0.1 M) at −78 °C. The flask was wrapped in foil and allowed to warm to room temperature for 6−12 h. The reaction was quenched with water and partitioned between water and EtOAc, which was acidified with 3 N HCl. The organic layer was washed with brine and dried over Na_2SO_4. Concentration provided diols **2a**−**c**.

(5*R*,11*R*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol ((*R,R*)-2a). Recrystallization from EtOAc/hexanes provided diol (*R,R*)-**2a** as yellow-tinted crystals (42 mg, quantitative yield, 54:1 cis:trans). Further recrystallization from $CHCl_3$/acetone/hexanes furnished (*R,R*)-**2a** as a white solid (100% cis isomer): mp 200.5−202.5 °C; er = 98:2 (HPLC column C, 15% IPA/hexanes, t_R = 13.2 min); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 2H), 7.21 (d, 2H, J = 9.1), 6.66−6.71 (m, 4H), 3.01 (dd, 2H, J = 15.2, 6.2), 2.86 (quint.d, 2H, J = 6.4, 1.6), 2.55 (dd, 2H, J = 15.2, 1.6), 1.00 (d, 6H, J = 6.9); ^{13}C NMR (100 MHz, acetone-d_6) δ 156.9, 136.5, 131.8, 127.5, 123.7, 116.6, 113.6, 36.8, 29.6, 17.7; MS (EI, 70 eV) *m/z* 292 (M^+, 48), 277 (M − Me, 24); HRMS (EI, 70 eV) calcd for $C_{20}H_{20}O_2$ 292.1463, found 292.1464. Anal. ($C_{20}H_{20}O_2 \cdot 0.8H_2O$) C, H.

(5*S*,11*S*)-5,11-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol ((*S,S*)-2a). Recrystallization from EtOAc/hexanes furnished diol (*S,S*)-**2a** as yellow crystals (81 mg, 88% yield, 82.6:1 cis:trans): mp 199.5−202 °C; er ≥ 99:1 (HPLC column C, 15% IPA/hexanes, t_R = 10.7 min); spectroscopic data identical to those for diol (*R,R*)-**2a**; MS (EI, 70 eV) *m/z* 292 (M^+, 100), 277 (M − Me, 50); HRMS (EI, 70 eV) calcd for $C_{20}H_{20}O_2$ 292.1463, found 292.1465. Anal. ($C_{20}H_{20}O_2 \cdot 0.7H_2O$) C, H.

(5*R*,11*R*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol ((*R,R*)-2b). Flash chromatography (25% EtOAc/hexanes) provided diol (*R,R*)-**2b** as an off-white powder (145 mg, 78%). Recrystallization from 25% EtOAc/hexanes provided (*R,R*)-**2b** as off-white crystals (100% cis isomer): mp 241−243 °C; er ≥ 99:1 (HPLC column C, 15% IPA/hexanes, t_R = 10.7 min); ^{1}H NMR (400 MHz, acetone-d_6) δ 8.23 (s, 2H), 7.16 (d, 2H, J = 8.2), 6.65−6.72 (m, 4H), 2.89 (dd, 2H, J = 15.6, 5.5), 2.80 (dd, 2H, J = 15.6, 1.7), 2.52−2.60 (br m, 2H), 1.43 (dqd, 2H, J = 14.9, 7.6, 3.2), 1.27 (ddq, 2H, J = 14.4, 10.4, 7.4), 0.95 (t, 6H, J = 7.4); ^{13}C NMR (100 MHz, acetone-d_6) δ 157.0, 136.7, 132.0, 128.0, 123.9, 116.7, 113.7, 36.7, 32.6, 24.0, 12.3; MS (EI, 70 eV) *m/z* 320 (M^+, 75), 291 (M − Et, 96); HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O_2$ 320.1776, found 320.1771. Anal. ($C_{22}H_{24}O_2$) C, H.

(5*S*,11*S*)-5,11-Diethyl-5,6,11,12-tetrahydrochrysene-2,8-diol ((*S,S*)-2b). Flash chromatography (25% EtOAc/hexanes) provided diol (*S,S*)-**2b** as a yellow powder (96 mg, quantitative). Recrystallization from 25% EtOAc/hexanes provided (*S,S*)-**2b** as off-white crystals (100% cis isomer): mp 241−243 °C dec; er ≥ 99:1 (HPLC column C, 15% IPA/hexanes, t_R = 7.4 min); spectroscopic data identical to those for (*R,R*)-**2b**; HRMS (EI, 70 eV) calcd for $C_{22}H_{24}O_2$ 320.1776, found 320.1772. Anal. ($C_{22}H_{24}O_2 \cdot 0.2H_2O$) C, H.

(5*R*,11*R*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol ((*R,R*)-2c). Flash chromatography (25% EtOAc/hexanes) provided diol (*R,R*)-**2c** as a pink foam (202 mg, 93% yield, 33:1 cis:trans). Recrystallization from $CHCl_3$ provided (*R,R*)-**2c** as off-white crystals (73:1 cis:trans): mp 182−184.5 °C; er ≥ 99:1 (HPLC column C, 15% IPA/hexanes, t_R = 9.3 min); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.21 (s, 2H), 7.17 (d, 2H, J = 7.9), 6.66−6.69 (m, 4H), 2.88 (dd, 2H, J = 15.5, 5.7), 2.78 (dd, 2H, J = 15.5, 1.7), 2.65−2.70 (br m, 2H), 1.45−1.56 (m, 2H), 1.26−1.42 (m, 6H), 0.88 (t, 6H, J = 7.1); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0, 136.5, 131.6, 128.3, 123.0, 115.8, 112.7, 33.8, 32.3, 32.3, 20.6, 14.2; MS (EI, 70 eV) *m/z* 348 (M^+, 100), 305 (M − Pr, 100); HRMS (EI, 70 eV) calcd for $C_{24}H_{28}O_2$ 348.2089, found 348.2099. Anal. ($C_{24}H_{28}O_2 \cdot 0.3H_2O$) C, H.

(5*S*,11*S*)-5,11-Dipropyl-5,6,11,12-tetrahydrochrysene-2,8-diol ((*S,S*)-2c). Flash chromatography (25% EtOAc/hexanes) provided diol (*S,S*)-**2c** as a yellow foam (192 mg, 79% yield, 23:1 cis:trans). Recrystallization from $CHCl_3$ provided (*S,S*)-**2c** as off-white crystals (67:1 cis:trans): mp 182−184.5 °C; er ≥ 99:1 (HPLC column C, 15% IPA/hexanes, t_R = 6.5 min); spectroscopic data identical to those for (*R,R*)-**2c**; HRMS (EI, 70 eV) calcd for $C_{24}H_{28}O_2$ 348.2089, found 348.2099. Anal. ($C_{24}H_{28}O_2 \cdot 0.7H_2O$) C, H.

5,11-*trans*-Dimethyl-5,6,11,12-tetrahydrochrysene-2,8-diol (3a). A 1:2 mixture of *trans*-**9a** and (*R,R*)-**9a** (25 mg, 0.078 mmol) was deprotected with BBr_3 as described above to yield **3a** as a 1:2 trans:cis mixture (25 mg, quantitative). Recrystallization twice from MeOH furnished exclusively the trans isomer **3a** as a white powder (2.7 mg): ^{1}H NMR (500 MHz, acetone-d_6) δ 8.23 (s, 2H), 7.30 (d, 2H, J = 8.2), 6.68−6.71 (m, 4H), 3.20 (quint d, 2H, J = 7.0, 1.4), 2.97 (dd, 2H, J = 15.4, 6.8), 2.64 (dd, 2H, J = 15.4, 1.4), 0.84 (d, 6H, J = 7.1); ^{13}C NMR (125 MHz, acetone-d_6) δ 157.1, 137.1, 132.1, 126.7, 124.7, 116.9, 113.6, 36.7, 26.8, 18.2; MS (EI, 70 eV) *m/z* 292 (M^+, 44), 277 (M − Me, 23); HRMS (EI, 70 eV) calcd for $C_{20}H_{20}O_2$ 292.1463, found 292.1470. Anal. ($C_{20}H_{20}O_2 \cdot 0.85H_2O$) C; H: calcd, 7.11; found, 6.63.

Biological Procedures. 1. Relative Binding Affinity Assay. Relative binding affinities were determined by competitive radiometric binding assays using 10 nM [^{3}H]E_2 as tracer as previously described,[18,19] using either lamb uterine cytosol diluted to approximately 1.5 nM of receptor or purified full-length human ERα and ERβ purchased from Pan Vera. Free ligand was removed by adsorption to dextran-coated charcoal for cytosol RBAs.[18] Hydroxyapatite was used to absorb the purified receptor−ligand complexes.[19] Incubations were done at 0 °C for 18−24 h.

2. Transcriptional Activation Assay. Human endometrial cancer (HEC-1) cells were maintained in culture and transfected as described previously.[25,26] Transfection of HEC-1 cells in 60-mm dishes used 0.4 mL of a calcium phosphate precipitate containing 0.5 μg of pCMVβGal as internal control, 2 μg of the reporter gene plasmid, 100 ng of ER expression vector, and carrier DNA to a total of 5 μg of DNA. CAT activity, normalized for the internal control β-galactosidase activity, was assayed as previously described.[25,26]

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Supporting Information Available: Proton NMR spectra for **2a**−**c** and **3a** and chiral stationary-phase HPLC traces for **2a**−**c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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