Synthesis and Biological Activity of Novel Nonsteroidal Progesterone Receptor Antagonists Based on Cyclocymopol Monomethyl Ether

Lawrence G. Hamann,*[,†] Luc J. Farmer,[†] Michael G. Johnson,[†] Steven L. Bender,[†,‡] Dale E. Mais,[§] Ming-Wei Wang,[§] Diane Crombie,[§] Mark E. Goldman,[⊥,∥] and Todd K. Jones[†]

Departments of Endocrine Chemistry Research, Endocrine Research, and New Leads Discovery, Ligand Pharmaceuticals, Inc., 9393 Towne Centre Drive, San Diego, California 92121

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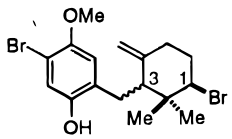
A novel class of nonsteroidal progesterone receptor antagonists has been synthesized and was shown to exhibit moderate binding affinity for hPR-A, the ability to inhibit the transcriptional activity of human progesterone receptor (hPR) in cell-based assays, and anti-progestational activity in a murine model. Cyclocymopol monomethyl ether, a component of the marine alga *Cymopolia barbata* was weakly active in random screening against PR. Investigations into the SAR surrounding the core of this natural product lead structure resulted in improved *in vitro* activity. In contrast to the cross-reactivity profiles observed with known steroidal antiprogestins, compounds of the general structural class described display a high degree of selectivity for the progesterone receptor and no functional activity on the glucocorticoid receptor.

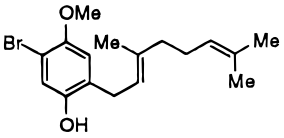
Introduction

Small molecule antagonists of the progesterone receptor (PR) have important roles in health care, including potential use as therapeutic agents for treatment of uterine leiomyomas,[1a] endometriosis,[1b] breast cancer,[1c] and meningiomas,[1d] as well as potential use in fertility control.[1e] PR is a member of the superfamily of intracellular receptors (IRs), including sex steroid receptors, which act as prototypic ligand-dependent transcription factors (TFs) to control gene expression, thereby influencing cell growth, differentiation, and other physiologic processes.[2] All of the known PR antagonists currently available or in clinical development are derived from steroids, and consequently, many have significant cross-reactivities with other steroid hormone receptors.[3] As part of a program designed to identify and develop compounds of novel structural classes which more selectively interact with the PR to regulate its various functions, we have synthesized and observed PR antagonist activity in analogues of cyclocymopol monomethyl ether, a marine natural product from the alga *Cymopolia barbata*, found in shallow coastal waters near the Florida Keys.[4] Beginning with this weakly active natural product as a lead structure, we have prepared numerous analogues through total synthesis and semisynthetic efforts which have been shown to exhibit moderate affinity for human PR-A (hPR-A), inhibit the transcriptional activity of hPR in cell-based assays, and display pharmacological efficacy in a standard murine model.[5] This series of analogues is highly selective for PR over human glucocorticoid receptor (hGR), with virtually no functional activity on hGR, and PR-A/GR affinity ratios ranging from 8- to 237-fold, in contrast to that observed with the known steroidal antiprogestins, such as RU486 and ZK98,299.[6] However, some analogues have moderate affinities for the human androgen receptor (hAR). The greater selectivity exhibited by these nonsteroidal PR antagonists should provide therapeutic benefit without steroid-related liabilities in any potential chronic clinical use.

As part of a high-throughput screening effort using the cotransfection assay,[7] we discovered that a crude organic extract of the marine alga *C. barbata* exhibited activity against hPR-B1. The active components were isolated from the extract and fully characterized as two diastereomers of cyclocymopol monomethyl ether (**1** and **2**).[8] These natural products are of mixed biogenetic origin, containing both a terpenoid aliphatic portion and a polyketide-derived aromatic portion.



1 (3*R*)
2 (3*S*)



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The 3(*R*) isomer (**1**) inhibited progesterone-stimulated reporter gene expression in the cotransfection assay with an IC_{50} value of 549 ± 55 nM. The 3(*S*) isomer (**2**) exhibited agonist activity 80% of that of progesterone, with an EC_{50} value of 35 ± 2 nM. None of the other related compounds isolated from the complex mixture of secondary metabolites of the alga, notably cymopol (**3**),[9] were active in the hPR cotransfection assay. An effort was initiated to further examine this novel pharmacophore and to elucidate structure−activity relationships using several biological assays. These include the cotransfection assay with hPR-B1, a high-throughput T-47D (human breast carcinoma cell) alkaline phosphatase assay, hPR-A receptor binding,[7] and a murine uterine decidualization assay.[10]

Chemistry

Previous synthetic efforts toward the cyclocymopols have successfully utilized a biomimetic approach,[11] though a more convergent approach was desired for our purposes. A modular chemical synthesis of the general

† Department of Endocrine Chemistry Research.
‡ Present address: Agouron Pharmaceuticals Inc., 10350 N. Torrey Pines Rd., Suite 100, La Jolla, CA 92037.
§ Department of Endocrine Research.
⊥ Department of New Leads Discovery.
∥ Present address: Signal Pharmaceuticals Inc., 5555 Oberlin Dr., San Diego, CA 92121.
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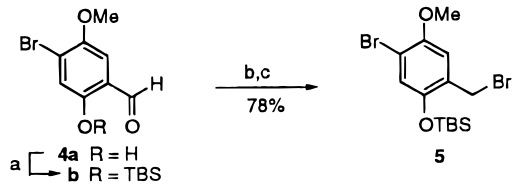
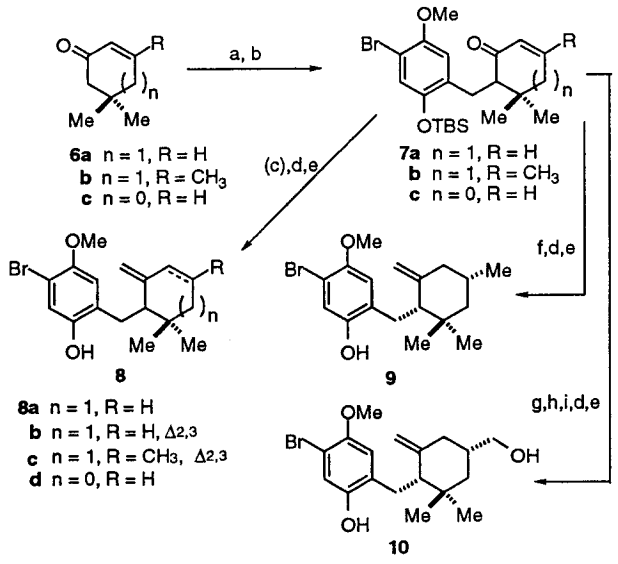
Scheme 1[a]



[a] (a) TBSCl, imidazole, DMAP, CH_2Cl_2, rt; (b) $NaBH_4$, MeOH, 0 °C; (c) Ph_3P, Br_2, DMF, 0 °C.

Scheme 2[a]

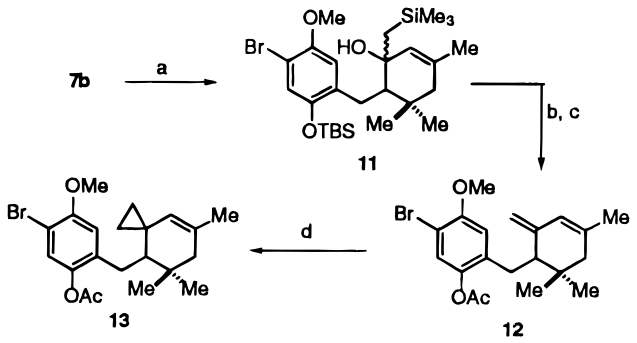


[a] (a) LDA, THF −78 °C; (b) **5**, → rt; (c) H_2, 5% Pd on C, EtOAc; (d) $TMSCH_2Li$, THF, −78 °C; (e) HF/py, THF; (f) MeMgBr, CuI, THF, −55 °C; (g) $(CH_2{=}CH)_2MgBr$, $CuBr{\cdot}Me_2S$, THF, −50 °C; (h) O_3, MeOH, −78 °C, then Me_2S; (i) LiTEPA, THF, 0 °C.

structural framework of the cyclocymopols was designed to allow for flexibility in the construction of diverse analogues. This strategy was retrosynthetically based on dissection of the molecule into electrophilic aromatic and nucleophilic aliphatic fragments, each readily amenable to variation prior to coupling through enolate alkylation chemistry. Electrophilic benzyl bromide **5** was prepared in multigram quantities in three steps from the known 4-bromo-2-hydroxy-5-methoxybenzaldehyde (**4a**)[12] (Scheme 1). This was accomplished by silylation of the phenol, followed by sodium borohydride reduction of the aldehyde and conversion of the resultant benzylic alcohol to the corresponding bromide using triphenylphosphine dibromide.[13]

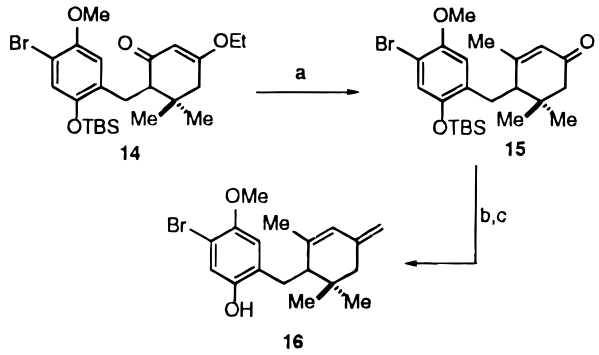
The general synthetic route for cyclocymopol analogue synthesis is outlined in Scheme 2. The lithium enolate of 5,5-dimethyl-2-cyclohexenone (**6a**) was trapped with benzyl bromide **5** to provide the key intermediate enone **7a**. An alternate route to this intermediate through TMSCl-assisted conjugate addition of lithiomethyl cyanocuprate to 3-methyl-2-cyclohexenone, followed by Lewis acid-mediated ($TiCl_4$ or $BF_3{\cdot}OEt_2$) alkylation[14] of the intermediate silyl−enol ether with benzyl bromide **5**, failed to give the desired product in a synthetically useful yield. While the lithium enolates reacted slowly at room temperature, these metalated enolate species most likely lacked sufficient reactivity to overcome steric congestion around the neopentyl carbon. Catalytic hydrogenation of enone **7a**, followed by Peterson methylenation[15] of the resultant ketone, gave **8a** (Wittig methylenation failed to give the desired product). Methylenation of **7a** gave diene **8b**. Compounds **8c** and **8d** were prepared from isophorone and 4,4-dimethylcyclopent-2-en-1-one respectively in an analogous fashion, though preparation of **8d** required use of the corresponding organocerium methylenation reagent.[16] Conjugate addition of methyl cuprate to enone **7a**, followed by Peterson methylenation/desilylation, gave **9**. Similarly, conjugate addition of vinyl cuprate[17] to enone **7a** allowed installation of varied functionality at this position. Ozonolysis of the vinyl group gave an aldehyde which was selectively reduced using lithium tris[(3-ethyl-3-pentyl)oxy]aluminohydride (LiTEPA)[18] to afford the corresponding primary alcohol. Silylation, followed by Peterson methylenation/desilylation gave **10**.

Scheme 3[a]



[a] (a) $TMSCH_2Li$, THF, −78 °C; (b) TBAF, THF, Ac_2O, 0 °C; (c) HF/py, THF; (d) Et_2Zn, $ClCH_2I$, 1,2-DCE.

Scheme 4[a]



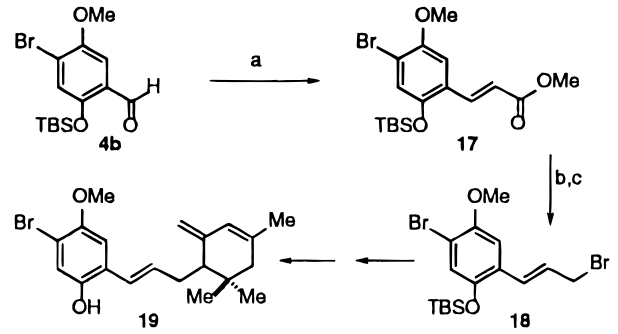
[a] (a) MeLi, Et_2O, −78 °C, then 1 N HCl; (b) $TMSCH_2Li$, THF, −78 °C; (c) HF/py, THF, rt.

Deprotection−reprotection of the phenol group on intermediate tertiary alcohol **11** prior to introduction of the *exo*-methylene group was accomplished in a one-step procedure by treatment with tetra-*n*-butylammonium fluoride (TBAF) and acetic anhydride (Scheme 3). Subsequent elimination of TMSOH provided diene **12**. Regioselective cyclopropanation of **12** using diethylzinc and chloroiodomethane[19] provided spirocyclopropyl derivative **13**.

Compound **16** was prepared by treatment of the lithium enolate of 3-ethoxy-5,5-dimethyl-2-cyclohexenone with bromide **5** affording **14** (Scheme 4). Enone transpositioning was accomplished using methyllithium, followed by Peterson methylenation to give the desired diene **16**, isomeric to **8c**.

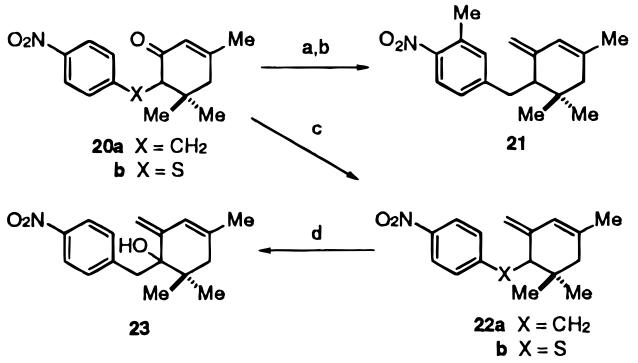
Compound **19** was prepared from isophorone and allylic bromide **18**, followed by methylenation and deprotection (Scheme 5). Bromide **18** was prepared in a straightforward fashion by Horner−Emmons reaction of aldehyde **4b** with methyl (dimethylphosphono)ac-

Scheme 5[a]



[a] (a) Methyl (dimethylphosphono)acetate, NaH, PhH, rt; (b) DIBAL-H, THF, −78 °C; (c) Ph_3P, Br_2, DMF, 0 °C.

Scheme 6[a]

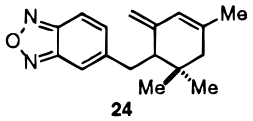


[a] (a) $TMSCH_2Li$, THF, −78 °C; (b) HF/py, THF, rt; (c) Tebbe's reagent, THF/tol, 0 °C; (d) SeO_2, *t*-BuOOH, CH_2Cl_2, rt.

etate, affording cinnamate **17**. Reduction of **17** with DIBAL-H and subsequent bromination of the resultant primary allylic alcohol gave **18**.

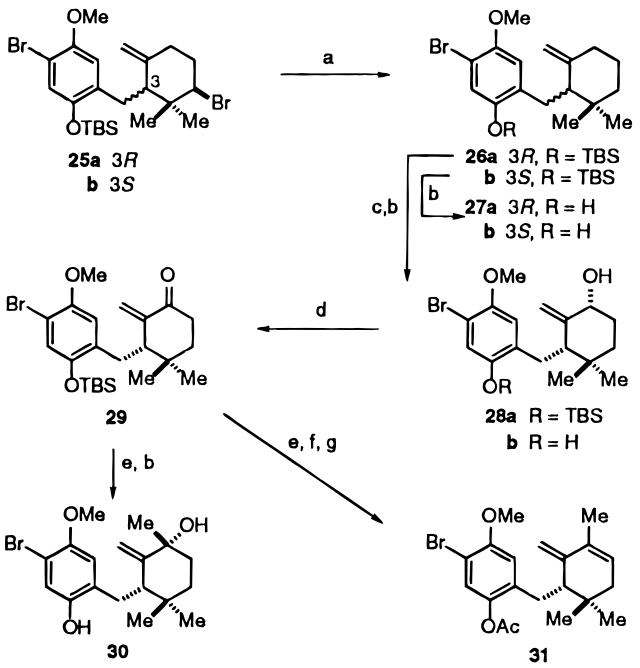
The use of Tebbe's reagent[20] was required to prepare compound **22a** from **20a** (Scheme 6), as attempted Peterson methylenation gave a low yield (16%) of the product of bis-addition, **21**. Allylic oxidation[21] of **22a** afforded a low yield (14%) of the corresponding tertiary alcohol **23**. Compound **22b** was prepared in two steps. The reaction of the lithium enolate of isophorone with *p*-nitrobenzene disulfide gave intermediate **20b**, which required the use of Tebbe's reagent for conversion to **22b**, giving the desired product in low yield.

Analogue **24** was prepared using the route described for **8c**, starting with isophorone and the corresponding benzylic bromide, prepared by bromination of 5-methylbenzofurazan with NBS.



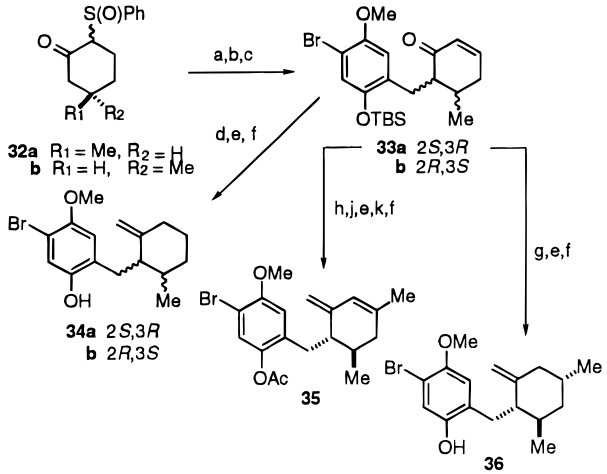
All semisynthetic compounds were prepared from silyl-protected natural cyclocymopols **25a** and **25b**. Completely selective aliphatic debromination of each independent diastereomer followed by desilylation afforded the respective enantiomers **26b** and **27b** (Scheme 7). Compound **28b** was prepared by allylic oxidation of **26a**, affording a readily separable diastereomeric mixture of allylic alcohols, followed by desilylation. Oxidation of the diastereomeric mixture was achieved using Dess−Martin periodinane[22] which afforded enone **29**. Methyllithium addition to the enone gave a separable

Scheme 7[a]



[a] (a) *n*-Bu_3SnH, AIBN, PhH, rt; (b) TBAF, THF, rt; (c) SeO_2, CH_2Cl_2, *t*-BuOOH; (d) Dess−Martin periodinane, CH_2Cl_2; (e) MeLi, THF, −78 °C; (f) Burgess reagent, PhH, rt; (g) TBAF, THF, Ac_2O, 0 °C.

Scheme 8[a]



[a] (a) 2.2 equiv of LDA, THF/HMPA, −35 °C; (b) **5**, THF, −35 °C; (c) $CaCO_3$, CCl_4, 65 °C; (d) H_2, 10% Pd on C, EtOAc; (e) $TMSCH_2Li$, THF, −78 °C; (f) HF/py, THF, rt; (g) MeMgBr, CuI, Et_2O, 0 °C; (h) g, then PhSeBr, 0 °C; (j) H_2O_2(aq), CH_2Cl_2, 35 °C; (k) TBAF, Ac_2O, THF, 0 °C.

mixture of diastereomeric tertiary alcohols, which upon elimination using Burgess' reagent[23] gave, after desilylation/acetylation, **31**.

In addition to those enantiomerically-pure analogues arrived at through semisynthesis, several optically-pure analogues (**34**−**36**) were synthesized through total synthetic approaches (Scheme 8). The pivotal intermediate in the synthesis of **34a**, enone **33a**, was prepared in four steps from commercial (*R*)-3-methylcyclohexanone. The first two steps used the same procedure as for the preparation of **8**. The dianion[24] of **32a** was treated with benzylic bromide **5**, affording a benzylated *β*-keto sulfoxide which was subjected to dehydrosulfenylation to produce enone **33a**. Enantiomer **34b** was synthesized using the same route, starting with (*S*)-3-

Table 1. Activities of Nonsteroidal Compounds on hPR-B1 in Cotransfected CV-1 Cells[a]

	agonist		antagonist	
compound	efficacy[b] (%)	potency[c] EC_{50} (nM)	efficacy[b] (%)	potency IC_{50} (nM)
RU486	na[d]		96 ± 1	0.18 ± 0.02
ZK98,299	na		99 ± 0	1.60 ± 0.35
1	na		83 ± 3	549 ± 55
2	84 ± 7	35 ± 2	na	
8a	39 ± 2	4020 ± 230	55 ± 9	405 ± 93
8b	na		71 ± 5	783 ± 83
8c	25 ± 9	1295 ± 283	37 ± 8	3567 ± 2247
8d	na		98[e]	1200
9	115[e]	458	na	
10	ND[f]		87[e]	3300
12	23[e]	1457	42[e]	710
13	72[e]	2117	26[e]	160
16	na		66[e]	580
19	ND		61[e]	3300
21	na		85 ± 4	1800 ± 0
22a	na		90 ± 3	1050 ± 235
22b	na		44 ± 23	1350 ± 71
23	na		87 ± 1	260 ± 62
24	na		72 ± 1	1950 ± 1200
27a	na		94 ± 1	741 ± 98
27b	50 ± 8	3264 ± 358	45 ± 5	597 ± 206
28b	na		73[e]	3400
30	na		78[e]	2000
31	na		83[e]	660
34a	na		93 ± 4	1050 ± 495
34b	42[e]	3011	62 ± 4	320 ± 93
35	63 ± 11	3005 ± 849	52 ± 1	350 ± 14
36	ND		63 ± 19	985 ± 615
37	na		93[e]	2443

[a] Values represent triplicate determinations. [b] Agonist efficacies relative to progesterone (100%), antagonist efficacies determined as percent of maximal inhibition. [c] EC_{50}/IC_{50} values calculated as the concentration of ligand required to give half-maximal activation/inhibition, respectively. [d] Not active; defined as efficacy <20%, potency >10 000 nM. [e] Assayed once. [f] Not determined.

methylcyclohexanone, which was accessible from (*S*)-pulegone by a retro aldol reaction.[25] Compounds **35** and **36** were prepared from **33a**. Diene **35** was prepared by methyl cuprate addition, followed by trapping of the enolate with phenylselenenyl bromide to afford the phenylselenenoketone intermediate, which was eliminated using hydrogen peroxide in pyridine. Conversion of this intermediate as previously described yielded **35**. Compound **36** was prepared from **33a** as previously described for **9**.

Biological Results and Discussion

Cotransfection Assays. Using the cotransfection assay, specific ligand-dependent modulation of gene transcription by individual IRs can be studied experimentally. This assay is thus an effective tool for functional characterization of the interactions of small molecule agonists or antagonists with hPR. In the cotransfection assay, progesterone causes a concentration-dependent increase of reporter gene (luciferase) activity that can be reversed by PR antagonists. The cotransfection assays used to measure antagonist activity utilized a modification of hPR-B, hPR-B1,[6a,b] the results of which are summarized in Table 1. The nonsteroidal PR antagonist analogues do not possess intrinsic hPR agonist activity. Antagonist effects on hPR-B1 were determined by evaluating compounds in the presence of progesterone at 1 nM, its EC_{50}. The hPR-B1 cotransfection assay data demonstrate that these nonsteroidal PR antagonists inhibit a progesterone-mediated response in a concentration-dependent manner.

Cross-reactivities with other IRs were assessed by determining the activities as agonists or antagonists of hGR,[6d] hAR,[6c] human mineralocorticoid receptor (hMR),[6e] and human estrogen receptor (hER)[6f] in cotransfection assays (Table 2). Unlike the steroidal PR antagonists, none of the nonsteroidal PR antagonist analogues were agonists in cotransfection assays using hGR, hAR, hMR, or hER. A few analogues in this series were weak antagonists only of hAR in cotransfection assays, but demonstrated no appreciable hER, hGR, or MR antagonist activities.

Receptor Binding Assays. Binding K_i values were determined using baculovirus expressed[26] hPR-A to provide information about the interaction of a molecule with the receptor independent of issues such as distribution, metabolism, and protein binding and are summarized in Table 3. Binding affinities to baculovirus expressed hGR, hAR, and hER were also determined to evaluate the potential for cross-reactivities involving these receptors.

T-47D Alkaline Phosphatase Assays. The T-47D cell line is derived from a human breast cancer.[27] It expresses both endogenous wild-type human PR isoforms (hPR-A and hPR-B). This cellular context may therefore allow observation of biological properties of these analogues distinct from those observed using the cotransfection assay with hPR-B1.[28] Incubation of T-47D cells with PR agonists stimulates expression of alkaline phosphatase. Table 4 summarizes the IC_{50} values for a selection of PR antagonist analogues in the T-47D alkaline phosphatase assay. The nonsteroidal PR antagonist analogues were devoid of agonist properties and, consistent with the cotransfection assay data, inhibited progesterone-induced alkaline phosphatase expression in a concentration-dependent manner.

Decidualization Assay in Mice. To further establish this pharmacophore as a structurally novel PR antagonist lead, some of the more potent analogues underwent limited *in vivo* testing using a murine uterine decidualization assay. A successful pregnancy requires not only fusion of egg and spermatozoon, but also the provision of a receptive and supportive uterus. During early pregnancy (days 3−6) in mice, there is a steep increase in progesterone concentrations in the circulation, which peaks at about 30 ng/mL on day 6, whereas 17β-estradiol concentrations remain relatively constant during the same period.[29] This endocrine profile coincides with the window during which the uterus undergoes an important transformation, *i.e.*, decidualization, in order to provide a receptive and supportive environment for blastocyst implantation. Progesterone acts upon the estrogen-primed uterus to facilitate this process.[30]

Pseudopregnant mice (fertile females mated with vasectomized males) were used to establish the assay system. These *in vivo* experiments were designed to utilize the high concentrations of endogenous progesterone present during early days of pseudopregnancy and then induce uterine decidualization with an artificial stimulus (oil).[31] Subsequent manipulation of the endocrine status was achieved by perturbing progesterone action with various PR antagonists in an attempt to

Table 2. Antagonist[a] Cross-Reactivities on hAR, hER, hGR, and hMR[a]

compound	IC_{50} (nM) [efficacy[b] (%)]			
	hAR	hER	hGR	hMR
progesterone	37 ± 2 [46 ± 7]	>1000 [<20]	>1000 [39 ± 8]	14 ± 4 [83 ± 6]
RU486	5 ± 2 [75 ± 2]	>1000 [40 ± 7]	0.8 ± 0.1 [98 ± 1]	>1000 [77 ± 5]
ZK98,299	269 ± 57 [93 ± 4]	>1000 [27 ± 4]	27 ± 4 [100 ± 0]	>1000 [34 ± 9]
1	449 ± 69 [79 ± 8]	na[c]	na	ND[d]
2	238 ± 123 [89 ± 3]	na	na	ND
22a	250 [21][e]	na	na	na
27a	377 ± 49 [90 ± 1]	na	na	1500 [40][e]

[a] Values represent triplicate determinations. [b] Antagonist efficacies were determined as a function (%) of maximal inhibition in the presence of an EC_{50} concentration of DHT, estradiol, dexamethasone, or aldosterone for hAR, hER, hGR, and hMR, respectively. [c] Not active; defined as efficacy <20%, potency >10 000 nM. [d] Not determined. [e] Assayed once.

Table 3. Binding Affinities for hPR-A, hGR, hAR, and hER

compound	K_i: mean (nM) ± SEM			
	hPR-A	hGR[a]	hAR	hER
progesterone	3.5 ± 0.2	30.5 ± 1.9	8.5 ± 3.1	na[c]
dexamethasone	ND[b]	5.2 ± 1.2	ND	ND
DHT	ND	ND	1.1 ± 0.3	ND
estradiol	ND	ND	ND	0.38 ± 0.11
RU486	0.58 ± 0.07	0.68 ± 0.06	10.6 ± 2.9	na
ZK98,299	18 ± 3	41.8 ± 4.6	>100	na
1	490 ± 30	3210 ± 486	ND	ND
2	343 ± 23	2465 ± 412	ND	ND
8a	77 ± 5	2206 ± 333	1542 ± 239	ND
8b	156 ± 4	2781 ± 499	1518 ± 8.9	ND
8c	31 ± 2.5	1261 ± 114	1449 ± 167	ND
8d	441 ± 58	4532 ± 369	1923 ± 273	ND
9	22 ± 3	1036 ± 14	696 ± 136	ND
10	3247 ± 480	6386 ± 562	6810 ± 3199	ND
12	59.6 ± 2.7	2199 ± 235	1942 ± 1492	ND
13	98 ± 11	2570 ± 578	3008 ± 484	ND
16	109 ± 14	2670 ± 678	1591 ± 346	ND
19	84 ± 12	4417 ± 785	2318 ± 354	ND
21	28.8 ± 0.8	6812 ± 437	2698 ± 1748	ND
22a	53.5 ± 1.2	5424 ± 679	6303 ± 3708	na
22b	119 ± 21	8215 ± 1785	na	ND
23	243 ± 39	8511 ± 1489	6454 ± 3556	ND
24	88 ± 7	8356 ± 1644	na	ND
27a	218 ± 23	1766 ± 142	737 ± 195	na
27b	34 ± 5.8	1777 ± 166	1099 ± 736	ND
28b	na	na	na	ND
30	275 ± 21	na	6662 ± 3348	ND
31	455 ± 38	4109 ± 757	6387 ± 3623	ND
34a	71 ± 9	3697 ± 571	1644 ± 135	ND
34b	69 ± 8	2286 ± 242	570 ± 177	ND
35	21 ± 3	2379 ± 734	1497 ± 905	ND
36	84 ± 7	1657 ± 1043	1139 ± 32	ND
37	na	na	na	ND

[a] GR prepared from CV-1 extract. [b] Not determined. [c] K_i > 10 000 nM.

block oil-induced decidual cell reaction. Percent inhibition was estimated using the following formula:

$$\% \text{ inhibition} = 100 - \frac{\text{weight gain in treated group}}{\text{weight gain in control group}} \times 100$$

As shown in Figure 1, control mice showed a dramatic increase in net uterine wet weight gain (323.8 ± 27.6 mg) in response to the decidual stimulus. RU486 completely blocked this response at a dose of 0.5 mg/day orally or 0.1 mg/day intraluminally (Figure 2), resulting in net increases of only 1.0% (3.2 ± 1.0 mg) of the control value (estimated ED_{50} for RU486 = 0.05 mg).

Block of Decidualization by 22a and 27a. Figures 2 and 3 show that both **22a** and **27a** block oil-induced decidualization. Compound **27a** showed no efficacy at 1.0 mg/day (data not shown), but blocked decidualization by 85% when given at a dose of 5.0 mg/day. Compound **22a** showed comparable potency, inhibiting deciduomata formation by 80% at a dose of 5.0 mg/day.

Table 4. Antagonist Efficacies and Potencies in the T-47D Alkaline Phosphatase Assay[a]

compound	efficacy[b] (%)	potency[c] IC_{50} (nM)
RU486	83 ± 9	0.35 ± 0.15
ZK98,299	91 ± 4	3.2 ± 0.3
1	84 ± 5	354 ± 70
2	66 ± 12	3500 ± 350
8a	72 ± 1	163 ± 24
8b	72[d]	280
8c	26[d]	1200
8d	75 ± 8	250 ± 25
9	<20	>10000
10	73[d]	2000
12	59[d]	770
13	68[d]	440
16	75[d]	1300
19	65[d]	1600
21	81[d]	510
22a	80 ± 3	186 ± 42
22b	89 ± 2	420 ± 15
23	81 ± 4	94 ± 6
24	71 ± 3	775 ± 325
27a	85 ± 5	210 ± 58
27b	51 ± 0	290 ± 20
28b	83[d]	1700
30	81[d]	430
31	81[d]	210
34a	85[d]	300
34b	63[d]	200
35	50 ± 10	147 ± 48
36	65 ± 1	350 ± 50

[a] Values represent triplicate determinations. [b] Efficacies were determined as a function (%) of maximal inhibition. [c] IC_{50} values were calculated as the concentration of ligand required to give half-maximal inhibition in the presence of progesterone at its EC_{50}. [d] Assayed once.

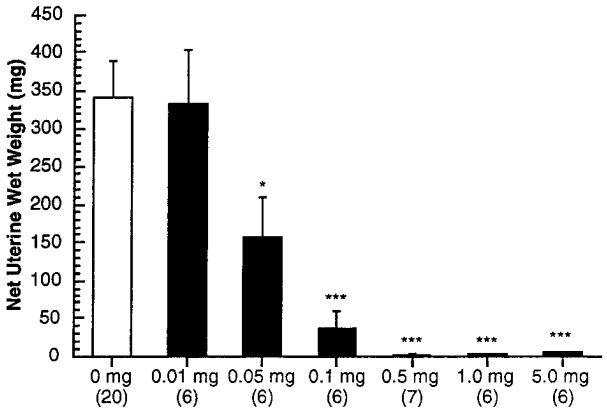


Figure 1. Effect of RU486 on oil-induced uterine decidualization when given orally once daily for three days (day 3 to day 5 of pseudopregnancy); *$p < 0.05$; ***$p < 0.001$ *vs* control. Number of mice is given in parentheses.

The PR antagonist RU486 was highly effective in blocking deciduomata formation, indicating that the endometrial sensitivity was greatly reduced by this

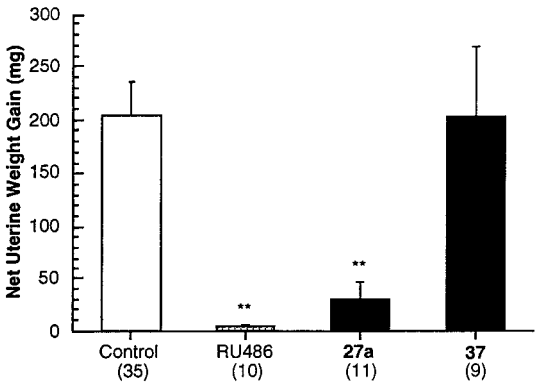


Figure 2. Effect of RU486 (0.10 mg, intrauterine), **27a** (5 mg intrauterine), and **37** (5.0 mg intrauterine) on oil-induced uterine decidualization (single dose, day 4 of pseudopregnancy) in BALB/c mice; $^{**}p < 0.01$ *vs* control. Numbers of mice in each group are given in parentheses.

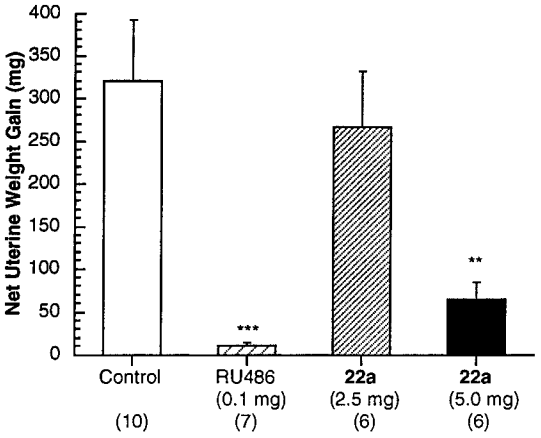
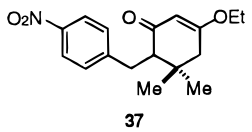


Figure 3. Effect of RU486 (0.10 mg, intrauterine) and **22a** (2.5 and 5.0 mg intrauterine) on oil-induced uterine decidualization (single dose, day 4 of pseudopregnancy) in ICR mice; $^{**}p < 0.01$ *vs* control. Numbers of mice in each group are given in parentheses.

compound. The ability of RU486 to prevent uterine responses to various stimuli has also been shown in rats,[32] monkeys,[33] and ewes.[34] Vinijsanun and Martin[35] have demonstrated further that the inhibitory effects of RU486 and another anti-progestin, ZK98,734, on mouse uterine decidualization were restricted to the actions mediated by progesterone. Like RU486, the two nonsteroidal PR modulators tested in this assay displayed pharmacologically effective PR antagonist activities *in vivo*. Both compounds blocked mouse uterine decidualization in a dose-dependent manner, and the effect was nearly maximal at 5.0 mg/day. A third compound, **37**, which is structurally similar yet inactive in cotransfection and binding assays, was also examined in this model; this was to rule out the possibility that *in vivo* activity of these compounds was due to toxicity at the doses given. The control compound (**37**) did not block decidualization, consistent with the inactivity observed using *in vitro* assays.

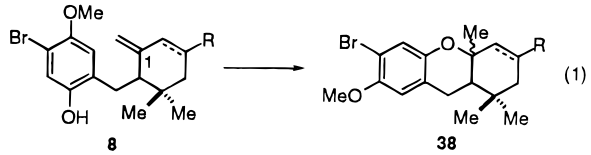


37

Discussion of Structure–Activity Relationships

Initial synthetic studies focused both on total synthetic and semisynthetic approaches to analogues of the natural product lead compounds. By removal of the aliphatic bromine in each of the respective natural cyclocymopols (**1** and **2**), the resultant optically-pure forms of previously-synthesized racemate **8a** (**27a** and **27b**) were prepared and were observed to exhibit enhanced PR antagonist activity relative to that of the parent compounds. Interestingly, analysis of the cotransfection assay data for these three analogues indicates that while the parent natural product with the 3(*S*) configuration (**2**) displayed full agonist properties, the debromo analogue (**27b**) had only weak yet measurable partial agonist properties, and instead exhibited antagonist properties with slightly greater potency than its enantiomer (**27a**). On the basis of biological data for these and related analogues, it appears that the absolute stereochemistry at C_3 is of lesser significance relative to the biological activity than the conformational orientation of the benzylic moiety with respect to the aliphatic ring. Use of molecular modeling helped to elucidate the preferred conformation of the benzylic group about the cyclohexyl ring. Structures were minimized using the Quanta interface to the CHARMM force field with the conjugate gradient minimization method. The axial conformation was calculated to be favored by approximately 4 kcal/mol, due to unfavorable van der Waals interactions (allylic strain) with the methylidene group.[36] Substituents at the other neopentyl position on the cyclohexyl ring (C_1 in parent compounds **1** and **2**) can exert a counterinfluence to this conformational preference to avoid unfavorable 1,3-diaxial interactions. Since antagonist activity was generally superior in analogues without the aliphatic bromine, and this allows the molecule to adopt a more energetically favorable conformation, analogues without substitution at this position or with substituents trans to the benzylic substituent were generally pursued.

The diene **8c**, with an aliphatic portion derived from isophorone, showed a substantial improvement in binding affinity to hPR-A ($K_i = 31$ nM) relative to the parent compounds **1** and **2**, yet was weakly active in the cotransfection assay ($IC_{50} = 3567$ nM). Relative to binding assays which are performed using a cell-free receptor preparation, the cotransfection assay more closely mimics an *in vivo* setting, where components of the intact cell have the potential to metabolize test compounds. Additionally, the cotransfection assays are typically of 40 h duration at physiologic temperature (37 °C), as compared with binding assays performed at 4 °C for 16 h. Compound instability under the cotransfection assay conditions was suspected to be a potential cause of the disparity in data obtained in these two assays, and studies were therefore undertaken to examine this possibility. The two most likely sources of chemical instability of these analogues were addressed: unwanted cyclization of the phenol group onto the *exo*-methylene under mildly acidic conditions to yield inactive compounds with cymobarbatol[37] skeleton **38** (eq 1) and oxidation of the *p*-methoxyphenol to an inactive quinone.[38]



This cyclization, observed in certain analogues to occur to a measurable extent under the conditions of the cotransfection assay, also occurs in nature, as compounds of the general cymobarbatol structure are also present in some algal extracts containing the cyclocymopols. In particular, the cymobarbatol compound corresponding to **8c** was isolated in approximately 30% from the cotransfection assay medium upon incubation of **8c** for 40 h at 37 °C (results not shown). Structural features present in **8c** and other diene analogues can stabilize a positive charge at C_1 in the transition state leading to cymobarbatol formation to a greater degree than the parent compounds, due to allylic delocalization.

To circumvent issues of chemical instability, several analogues were synthesized with the intent of finding biologically suitable replacements for both the phenol-substituted aryl group and the *exo*-methylene group. A systematic examination of varied aryl groups resulted in the observation that a *p*-nitrophenyl group could replace the aryl group of the natural cyclocymopols without loss of activity, giving active structures typified by **22a**. This substitution greatly simplified the synthetic route to analogues of this type and also eliminated the possibility for phenolic oxidation. In general, electron-deficient aryl groups (Ar = *p*-nitrophenyl, *p*-bromophenyl, *p*-acetylphenyl) exerted a beneficial influence on activity as compared with electron-rich aryl groups (Ar = *p*-methoxyphenyl, *p*-dimethylaminophenyl, *m*-methoxyphenyl). Parallel to these efforts, it was also discovered that a spirocyclopropyl group could function as a bioisosteric surrogate[39] for the *exo*-methylene unit (**13**) without loss of activity, yet other substitutions for this *exo*-olefin (*i.e.* ketone, hydroxyl, methyl) destroyed activity.

Conclusion

We have synthesized PR antagonists of a novel structural type which contribute to our understanding of key molecular features critical for development of more potent, highly selective PR antagonists. Relative to the parent compounds, the synthetic nonsteroidal PR antagonist analogues have been observed to exhibit improved binding affinities for hPR-A and an enhancement in the ability to repress PR-mediated gene expression in the hPR-B1 cotransfection assay in CV-1 cells. The compounds displayed moderate cross-reactivity as antagonists of hAR, but were not antagonists or agonists in cotransfection assays using hER, hGR, or hMR. The binding affinities of these analogues were selective for hPR relative to other IRs. In addition, compounds in this series of analogues were shown to possess PR antagonist activity *in vivo*. The structure−activity relationships around this pharmacophore elucidated to date have demonstrated that these compounds can serve as a useful template to prepare more potent and selective PR antagonists.

Experimental Section

General Chemical Procedures. Proton nuclear magnetic resonance (^{1}H NMR) and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded with $CDCl_3$ as the solvent at 400 and 100 MHz, respectively (Brüker AC 400). Chemical shifts are given in parts per million (ppm) downfield from internal reference tetramethylsilane in δ units, and coupling constants (J values) are given in hertz (Hz). Selected data are reported in the following manner: chemical shift, multiplicity, coupling constants, and assignment. Infrared (IR) spectra were recorded on a Mattson Galaxy Series 3000 FT infrared spectrometer. Liquid samples were measured as neat films on NaCl plates; solid samples were measured as KBr pellets. The reported frequencies are given in reciprocal centimeters (cm^{-1}) with the following relative intensities: s (strong, 70−100%), m (medium, 40−70%), w (weak, 20−40%), br (broad). Optical rotations were obtained on a Perkin-Elmer 241 polarimeter using a cell 1 dm in length and are reported as follows: $[\alpha]^{temp}_{wavelength}$ (concentration in g/100 mL, solvent). Elemental analyses were performed by Oneida Research Services, Inc., Whitesboro, NY, or Galbraith Laboratories, Inc., Knoxville, TN. Flash column chromatography refers to the method of Still[40] using Merck 230−400 mesh silica gel. Gradient elution refers to applying the compound as a solution in hexanes to the hexanes-equilibrated column and then eluting with progressively more polar hexanes/EtOAc solutions. Analytical thin layer chromatography (TLC) was performed using Merck 60-F-254 0.25 mm precoated silica gel plates. Compounds were visualized using ultraviolet light, iodine vapor, or cerium molybdate/sulfuric acid/methanol. Preparative thin layer chromatography (PTLC) was performed using Merck 60-F-254 0.50 or 1.00 mm precoated silica gel plates. High-performance liquid chromatography (HPLC) was performed on a Beckman System Gold 126 chromatograph using a 4.6 × 250 mm Beckman Ultrasphere ODS column. Preparative HPLC was performed on a Waters Delta Prep 4000. The detector wavelength was set to 254 nm. Ethyl ether and tetrahydrofuran were distilled directly prior to use from sodium/benzophenone ketyl. Dichloromethane (CH_2Cl_2), benzene, and toluene were dried and stored under nitrogen over 4 Å molecular sieves. Organic amines were distilled from CaH_2 and stored over solid KOH pellets under nitrogen. Unless otherwise specified, solutions of common inorganic salts used in workups are aqueous solutions. All moisture sensitive reactions were carried out using oven-dried or flame-dried round-bottomed (rb) flasks and glassware under an atmosphere of dry nitrogen.

General Procedure for Cycloalkenone Alkylation with Benzylic or Allylic Halides. 6-[[4-Bromo-2-[(*tert*-butyldimethylsilyl)oxy]-5-methoxyphenyl]methyl]-5,5-dimethylcyclohex-2-en-1-one (7a). To a flame-dried 50-mL rb flask containing diisopropylamine (0.19 mL, 1.3 mmol, 1.1 equiv) in 10 mL of THF at −78 °C was added *n*-BuLi (0.58 mL of a 2.2 M solution in hexanes, 1.3 mmol, 1.1 equiv). After 20 min at −78 °C, enone **6a** (0.15 g, 1.2 mmol) was added as a solution in 1 mL of THF, and the reaction mixture was allowed to stir at −78 °C for 15 min before removal of the cooling bath. When the temperature of the reaction mixture reached −5 °C, benzylic bromide **5** (1.00 g, 2.44 mmol, 2.00 equiv) was added as a solution in 2 mL of THF. The reaction mixture was then allowed to warm to room temperature (rt), and after 3 h, TLC analysis indicated complete consumption of the enone starting material and the formation of a less polar product (R_f 0.59, 2:1 hexanes/EtOAc), and the reaction was quenched by the addition of 5 mL of saturated NH_4Cl. The contents of the flask were transferred to a separatory funnel and extracted with 60 mL of EtOAc, and the resultant organic phase was washed with 30 mL of brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, gradient elution) afforded 0.459 g (83%) of the benzylated enone as a colorless low-melting solid: ^{1}H NMR 0.21 and 0.23 [2s, 2 × 3H, Si(CH_3)$_2$], 0.99 [s, 9H, SiC(CH_3)$_3$], 1.02 and 1.03 (2s, 2 × 3H, geminal CH_3's), 2.25 and 2.32 (dd of AB q, 2H, J_{AB} = 20.0, J_A = 3.9, 2.0, J_B = 4.1, 2.2, 4-H), 2.51 (dd, 1H, J = 8.7, 5.0, 6-H), 2.78 and 2.88 (d of AB q, 2H, J_{AB} = 14.2, J_A = 8.7, J_B = 5.1, benzylic CH_2), 3.80 (s, 3H, OCH_3), 5.96 (ddd, 1H, J = 10.0, 2.1, 1.9, 2-H), 6.68 (s, 1H, 6′-H), 6.77 (ddd, 1H, J = 9.9, 4.1, 3.8, 3-H), 6.94 (s, 1H, 3′-H); ^{13}C NMR −4.0, 18.4, 24.4, 25.9, 26.1, 28.6, 37.1, 39.5, 56.8, 58.3, 108.4, 114.4, 123.1, 128.5, 131.2, 146.3, 147.6, 150.1, 201.7.

General Procedure for Enone C=C Bond Reduction. 2-[[4-Bromo-2-[(*tert*-butyldimethylsilyl)oxy]-5-methoxyphenyl]-methyl]-3,3-dimethylcyclohexan-1-one. To a flame-dried 100-

mL rb flask containing enone **7a** (0.154 g, 0.454 mmol) in 22 mL of EtOAc (predried over K_2CO_3) at rt was added 20 mg of 5% palladium on carbon (ca. 2 mol %), and after flushing/evacuating the vessel three times with nitrogen, a hydrogen atmosphere was introduced and maintained by use of a balloon. After 24 h, the flask was again flushed several times with nitrogen, and the contents of the flask were filtered, rinsing with an additional 100 mL of EtOAc. Rotary evaporation of the solvent afforded 0.156 g (99%) of the saturated ketone as a colorless syrup (R_f 0.67, 2:1 hexanes/EtOAc): ^{1}H NMR 0.22 and 0.25 [2s, 2 × 3H, Si(CH_3)$_2$], 0.87 and 1.12 (2s, 2 × 3H, geminal CH_3's), 1.01 [s, 9H, SiC(CH_3)$_3$], 1.61−1.95 (m, 4H, 4,5-H), 2.19−2.38 (m, 2H, 6-H), 2.67 and 2.88 (dd of AB q, 2H, J_{AB} = 14.0, J_A = 6.1, 3.1, J_B = 9.8, 0, benzylic CH_2), 3.71 (s, 3H, OCH_3), 6.79 (s, 1H, 6′-H), 6.91 (s, 1H, 3′-H).

General Procedure for Peterson Methylenation of Ketones. 2-[(4-Bromo-2-hydroxy-5-methoxyphenyl)methyl]-3,3-dimethyl-1-methylidenecyclohexane (8a). To a flame-dried 50-mL rb flask containing 2-[[4-bromo-2-[(*tert*-butyldimethylsilyl)oxy]-5-methoxyphenyl]methyl]-3,3-dimethylcyclohexanone (0.13 g, 0.28 mmol) in 5 mL of THF at −78 °C was added [(trimethylsilyl)methyl]lithium (0.42 mL of a 1.0 M solution in pentane, 0.42 mmol, 1.5 equiv). An immediate change from colorless to a yellow reaction solution was observed, and TLC analysis indicated complete consumption of starting material and the formation of a less polar product (R_f 0.79, 2:1 hexanes/EtOAc). The reaction was then quenched with 4 mL of saturated NH_4Cl. EtOAc (30 mL) extraction of the reaction mixture, drying (Na_2SO_4), and concentration under reduced pressure gave 0.152 g (99%) of a crude product, which was a single diastereomer of the ketone addition product by ^{1}H NMR analysis. A portion of this crude intermediate (0.015 g, 0.028 mmol) was placed in a 10-mL Nalgene vial containing 2 mL of THF, 0.2 mL of HF/pyridine complex was added, and the mixture was allowed to stir at rt for 32 h, at which time TLC analysis indicated complete consumption of starting material and formation of a more polar product. The contents of the reaction vessel were transferred to a separatory funnel containing 20 mL EtOAc and 10 mL 1.0 M $NaHSO_4$. The layers were separated, and the resultant organic phase was washed with 10 mL brine, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, gradient elution) afforded 8.7 mg (92%) of the phenolic olefin as a colorless oil: ^{1}H NMR 0.97 and 1.00 (2s, 2 × 3H, geminal CH_3's), 1.25−1.35 (m, 1H, 5-H), 1.53−1.64 (m, 3H, 4,5-H), 2.05 (ddd, 1H, J = 12.9, 8.7, 4.3, 6-H$_{ax}$), 2.12 (dd, 1H, J = 10.9, 3.9, 2-H), 2.21−2.28 (m, 1H, 6-H$_{eq}$), 2.65 and 2.80 (d of AB q, 2H, J_{AB} = 14.1, J_A = 10.9, J_B = 3.5, benzylic CH_2), 3.80 (s, 3H, OCH_3), 4.36 (d, 1H, J = 1.0) and 4.63 (s, 1H) [methylidene CH_2], 6.59 (s, 1H, 6′-H), 6.94 (s, 1H, 3′-H); ^{13}C NMR 23.5, 26.7, 28.1, 28.4, 32.4, 35.2, 36.1, 54.3, 57.0, 108.3, 110.3, 115.0, 120.1, 128.8, 148.0, 148.5, 150.0; IR (neat) 3437 (br, m); HRMS calcd for $C_{17}H_{23}BrO_2$ *m/z* 338.0882, found *m/z* 338.0867.

General Procedure for Peterson Methylenation of Enones. 6-[(4-Bromo-2-hydroxy-5-methoxyphenyl)methyl]-5,5-dimethyl-1-methylidenecyclohex-2-ene (8b). This compound was prepared from enone **7a** (0.075 g, 0.17 mmol) in the manner previously described for olefin **8a**, with the following procedural changes. After formation of the initial [(trimethylsilyl)methyl]lithium addition adduct to enone **7a**, the phenolic protecting group was exchanged prior to effecting elimination of TMSOH. The crude addition product (0.090 g, 0.17 mmol) was dissolved in 5 mL of THF containing 0.20 mL of acetic anhydride (2.1 mmol) and cooled to 0 °C. Tetra-*n*-butylammonium fluoride (0.20 mL of a 1.0 M solution in THF, 0.20 mmol, 1.2 equiv) was added, and the mixture was allowed to warm to rt. The contents of the flask were then poured into a separatory funnel containing 30 mL of EtOAc and 10 mL of 1.0 M $NaHSO_4$, the layers were separated, and the organic phase was washed with 10 mL brine, dried (Na_2SO_4), and concentrated under reduced pressure. The crude material thus obtained was transferred to a 10 mL nalgene vial containing 3 mL of THF, and 0.3 mL of HF/pyridine complex was added. After stirring overnight at rt, the reaction mixture was worked up as previously described, and purification by flash column chromatography (silica gel, hexanes/EtOAc, gradient elution) afforded 38 mg (64%) of the desired acetoxy diene as a colorless, low-melting solid: ^{1}H NMR 0.89 and 1.11 (2s, 2 × 3H, geminal CH_3's), 1.84 and 2.13 (d of AB q, 2H, J_{AB} = 18.6, J_A = 5.4, J_B = 0, 4-H), 2.27 (s, 3H, acetate CH_3), 1.94 and 2.80 (d of AB q, 2H, J_{AB} = 12.9, J_A = 2.6, J_B = 2.9, benzylic CH_2), 3.83 (s, 3H, OCH_3), 4.14 and 4.67 (2s, 2 × 1H, C=CH_2), 5.70 (dd, 1H, J = 7.6, 7.3, 2-H), 6.04 (dd, 1H, J = 9.8, 0.4, 3-H), 6.52 (s, 1H, 6′-H), 7.19 (s, 1H, 3′-H). The phenolic acetate was then hydrolyzed under basic conditions to give the desired phenol. In a 10 mL test tube was combined the above acetate (10.0 mg, 0.030 mmol) and 2.0 mL of 5% methanolic K_2CO_3. After 10 min at rt, the methanol was removed by rotary evaporation, and the residue was dissolved in 20 mL of EtOAc. The organic solution was then washed with 20 mL of saturated NH_4Cl, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash column chromatography (silica gel deactivated with approx. 0.5% triethylamine in hexanes/EtOAc, gradient elution) afforded 7.1 mg (81%) of the phenolic diene as a colorless, low-melting solid: ^{1}H NMR 0.90 and 1.15 (2s, 2 × 3H, geminal CH_3's), 1.86 and 2.22 (d of AB q, 2H, J_{AB} = 18.8, J_A = 5.5, J_B = 0, 4-H), 2.02 and 2.84 (d of AB q, 2H, J_{AB} = 11.5, J_A = 3.2, J_B = 3.3, benzylic CH_2), 3.81 (s, 3H, OCH_3), 4.23 and 4.71 (2s, 2 × 1H, methylidene CH_2), 4.46 (br s, 1H, O*H*), 5.78 (dd, 1H, J = 9.8, 5.2, 2-H), 6.09 (dd, 1H, J = 9.8, 2.3, 3-H), 6.51 (s, 1H, 6′-H), 6.98 (s, 1H, 3′-H); ^{13}C NMR 20.8, 25.5, 28.4, 30.8, 36.0, 47.2, 56.2, 108.4, 112.5, 115.0, 122.2, 127.2, 133.0, 134.0, 148.1, 148.5, 152.1.

***cis*-2-[(4-Bromo-2-hydroxy-5-methoxyphenyl)methyl]-3,3,5-trimethyl-1-methylidenecyclohexane (9).** To a suspension of copper(I) iodide (2 mg) in 1 mL of ether at 0 °C was added methylmagnesium bromide (0.11 mL of a 3.0 M solution in ether, 0.32 mmol, 1.0 equiv). Enone **7a** (149 mg, 0.320 mmol) in 2 mL of ether was then added slowly while maintaining the temperature below 5 °C, and upon completion of the addition, the reaction mixture was stirred at 0 °C for 3 h before quenching with a 4:1 mixture of saturated NH_4Cl/NH_4OH (5 mL). The resultant biphasic mixture was extracted with 50 mL of ether, dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, 9:1) afforded 36 mg (30%) of the desired *cis*-2-[[4-bromo-2-[(*tert*-butyldimethylsilyl)oxy]-5-methoxyphenyl]methyl]-3,3,5-trimethylcyclohexan-1-one as a colorless, low-melting solid: ^{1}H NMR 0.22 (s, 6H, Si(CH_3)$_2$), 1.00 [m, 18H, 3,3,5-CH_3 and SiC(CH_3)$_3$], 1.50 (dd, 2H, J = 15.1, 6.5, 4-H), 2.02 (m, 1H, 5-H), 2.14 and 2.23 (AB q, 2H, J_{AB} = 12.0, 6-H), 2.42 (dd, 1H, J = 10.4, 6.4, 2-H), 2.88 and 2.89 (d of AB q, 2H, J_{AB} = 12.0, J_A = 10.4, J_B = 6.3, benzylic CH_2), 3.82 (s, 3H, OCH_3), 6.65 (s, 1H, 6′-H), 6.92 (s, 1H, 3′-H). This conjugate addition product (7.50 mg, 0.0200 mmol) was then methylenated in the manner previously described for **8a**, affording 2.7 mg (70%) of the desired phenol as a colorless syrup: ^{1}H NMR 0.92 and 1.05 (2s, 2 × 3H, geminal CH_3's), 0.93 (d, 3H, J = 7.0, 5-CH_3), 1.25 (m, 2H, 4-H), 1.70 (m, 1H, 5-H), 1.85 (dd, 1H, J = 12.5, 12.5, 6-H$_{ax}$), 2.04 (dd, 1H, J = 11.0, 3.4, 2-H), 2.10 (dd, 1H, J = 13.3, 4.2, 6-H$_{eq}$), 2.58 and 2.81 (d of AB q, 2H, J_{AB} = 13.6, J_A = 11.2, J_B = 3.7, benzylic CH_2), 3.81 (s, 3H, OCH_3), 4.27 and 4.58 (2s, 2 × 1H, methylidene CH_2), 4.46 (s, 1H, O*H*), 6.53 (s, 1H, 6′-H), 6.94 (s, 1H, 3′-H); ^{13}C NMR 22.6, 28.4, 28.9, 29.1, 29.3, 34.8, 39.2, 43.1, 54.4, 57.0, 108.3, 111.1, 115.2, 120.2, 128.8, 147.9, 148.4, 149.9; IR (neat) 3406 (br, s) 2951 (s), 2870 (m), 1496 (s), 1400 (m), 1202 (s). Anal. ($C_{18}H_{27}BrO_2$) C, H.

***cis*-2-[(4-Bromo-2-hydroxy-5-methoxyphenyl)methyl]-4-(hydroxymethyl)-3,3-dimethyl-1-methylidenecyclohexane (10).** To a suspension of copper(I) bromide/methyl sulfide complex (57.4 mg, 0.280 mmol, 0.500 equiv) in 3.5 mL of anhydrous DMS/THF (1:6) at −55 °C was added enone **7a** (260 mg, 0.56 mmol) in 2 mL of THF. Vinylmagnesium bromide (2.79 mL of a 1.0 M solution in THF, 2.79 mmol, 5.00 equiv) was then added slowly over 1 h while maintaining the temperature between −50 and −55 °C, and upon completion of the addition, the reaction mixture was stirred for 1 h before quenching at 0 °C with 2 N HCl (15 mL). The resultant biphasic mixture was extracted with 100 mL of EtOAc, dried (Na_2SO_4), and concentrated under reduced pressure. Purifica-

tion by flash column chromatography (silica gel, hexanes/EtOAc, 9:1) afforded 150 mg (55%) of the desired cis conjugate addition product as a colorless, low-melting solid, along with 73 mg (27%) of the trans diastereomer: ^{1}H NMR 0.22 and 0.23 [2s, 2 × 3H, Si(CH_3)$_2$], 0.99 and 1.07 (2s, 2 × 3H, geminal CH_3's), 1.02 [s, 9H, SiC(CH_3)$_3$], 1.48 (dd, 1H, J = 13.3, 4.4, 4-H$_{eq}$), 1.74 (dd, 1H, J = 13.4, 12.8, 4-H$_{ax}$), 2.16 (dd, 1H, J = 13.3, 4.4, 6-H$_{eq}$), 2.35 (dd, 1H, J = 13.2, 12.8, 6-H$_{ax}$), 2.47 (dd, 1H, J = 11.4, 5.6, 2-H), 2.58 (m, 1H, 5-H), 2.84 and 2.91 (d of AB q, 2H, J_{AB} = 14.4, J_A = 5.6, J_B = 11.7, benzylic CH_2), 3.82 (s, 3H, OCH_3), 5.00 (d, 1H, J = 9.7) and 5.03 (d, 1H, J = 15.4) [CH=CH_2], 5.77 (ddd, 1H, J = 16.9, 10.3, 6.3, CH=CH$_2$), 6.66 (s, 1H, 6′-H), 6.93 (s, 1H, 3′-H). To a solution of this ketone (20 mg, 0.04 mmol) in 0.6 mL of 2:1 acetone/water was added 4-methylmorpholine *N*-oxide hydrate (8 mg, 0.07 mmol, 2 equiv) and osmium tetraoxide (20 μL of a 0.1 M solution in 2-methyl-2-propanol, 0.02 mmol, 0.05 equiv), and the mixture was stirred at rt for 24 h. The reaction mixture was then cooled to 0 °C, and $NaIO_4$ (17 mg, 0.081 mmol, 2.0 equiv) in 0.5 mL of water was added. After 3 h of stirring at 0 °C, 0.5 mL of 10% $Na_2S_2O_3$ was added, and the mixture was extracted with EtOAc (3 × 5 mL). The combined organics were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, 9:1) afforded 16 mg (81%) of the desired keto aldehyde as a colorless oil which was used directly in the next step. To an oven-dried 10 mL rb flask containing aldehyde (55 mg, 0.11 mmol) in 2 mL THF at −78 °C was added LiTEPA (0.26 mL of a 0.5 M solution in THF, 0.14 mmol, 1.2 equiv). After 4 h at −78 °C, 0.1 mL of methanol was added, and the reaction mixture was allowed to warm to rt. Water (1 mL) was added, and the mixture was extracted with EtOAc (3 × 5 mL). The combined organics were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, 85:15) afforded 52 mg (94%) of the desired hydroxy ketone as a colorless oil: ^{1}H NMR 0.22 and 0.23 [2s, 2 × 3H, Si(CH_3)$_2$], 0.98 and 1.08 (2s, 2 × 3H, geminal CH_3's), 1.01 [s, 9H, SiC(CH_3)$_3$], 1.47 (m, 1H, 4-H$_{eq}$), 1.73 (dd, 1H, J = 11.5, 11.5, 4-H$_{ax}$), 2.16 (m, 2H, 5,6-H$_{eq}$), 2.28 (dd, 1H, J = 11.7, 11.7, 6-H$_{ax}$), 2.46 (dd, 1H, J = 8.6, 8.6, 2-H), 2.87 (m, 2H, benzylic CH_2), 3.58 (s, 2H, CH_2OH), 3.82 (s, 3H, OCH_3), 6.66 (s, 1H, 6′-H), 6.93 (s, 1H, 3′-H). This hydroxy ketone (45 mg, 0.090 mmol) was then methylenated in the manner previously described for **8d**, affording 7.0 mg (21%) of the desired olefin as a colorless oil: ^{1}H NMR 1.09 and 0.95 (2s, 2 × 3H, geminal CH_3's), 1.33 (m, 2H, 4-H), 1.85 (m, 1H, 5-H$_{ax}$), 1.97 (dd, 1H, J = 12.9, 12.9, 6-H$_{ax}$), 2.09 (dd, 1H, J = 11.4, 3.7, 2-H$_{eq}$), 2.21 (dd, 1H, J = 13.0, 4.1, 6-H$_{eq}$), 2.82 and 2.57 (d of AB q, 2H, J_{AB} = 13.5, J_A = 3.7, J_B = 11.4, benzylic CH_2), 3.54 (s, 2H, CH_2OH), 3.80 (s, 3H, OCH_3), 4.29 (s, 1H, methylidene H), 4.54 (s, 1H, Ar-OH), 4.62 (s, 1H, methylidene H), 6.53 (s, 1H, 6′-H), 6.94 (s, 1H, 3′-H); ^{13}C NMR 28.9, 33.4, 34.4, 37.0, 54.7, 57.2, 68.1, 108.2, 112.5, 115.1, 120.5, 129.3, 147.5, 148.1; IR (neat) 3367 (br s), 2930 (s), 2872 (m), 1499 (s), 1404 (s), 1206 (s); HRMS calcd for $C_{18}H_{25}BrO_3$ m/z 368.0987, found m/z 368.0971.

8-[(2-Acetoxy-4-bromo-5-methoxyphenyl)methyl]-5,7,7-trimethylspiro[2.5]oct-4-ene (13). To a flame-dried 10 mL rb flask containing 20 mg (0.05 mmol) of diene **12** in 1 mL of 1,2-dichloroethane at 0 °C was added diethylzinc (250 μL of a 1.0 M solution in hexanes, 0.26 mmol, 5.0 equiv). Chloroiodomethane (37 μL, 0.50 mmol, 10.0 equiv) was added dropwise, and the mixture was allowed to warm to rt. After 9 h, the reaction mixture was quenched at 0 °C with 10 mL of saturated NH_4Cl, and the reaction mixture was extracted with EtOAc (50 mL). The organic phase was washed with brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, gradient) afforded 20 mg (96%) of the spirocyclopropane as a colorless syrup: ^{1}H NMR −0.07 (ddd, 1H, J = 11.0, 9.3, 5.9, cyclopropyl H), 0.16 (ddd, 1H, J = 11.5, 9.4, 6.1, cyclopropyl H), 0.32 (ddd, 1H, J = 10.1, 5.8, 5.8, cyclopropyl H), 0.43 (ddd, 1H, J = 9.8, 6.1, 6.1, cyclopropyl H), 1.03 and 1.06 (2s, 2 × 3H, geminal CH_3's), 1.60 and 1.98 (AB q, 2H, J_{AB} = 17.8, 6-H), 1.69 (s, 3H, 5-CH_3), 2.25 (s, 3H, acetate CH_3), 2.39 and 2.65 (d of AB q, 2H, J_{AB} = 13.6, J_A = 10.0, J_B = 3.4, benzylic CH_2), 3.86 (s, 3H, OCH_3), 4.68 (s, 4-H), 6.69 (s, 1H, 6′-H), 7.18 (s, 1H, 3′-H); ^{13}C NMR 13.9, 12.4, 15.9, 20.1, 20.9, 21.4, 22.8, 27.5, 30.9, 34.4, 47.0, 56.8, 108.6, 112.0, 114.3, 120.1, 128.2, 148.1, 149.0, 152.2, 169.7; IR (neat) 1732 (s).

3,5,5-Trimethyl-6-[(4-nitrophenyl)methyl]cyclohex-2-en-1-one (20a). This compound was prepared from isophorone (2.065 g, 14.94 mmol) and *p*-nitrobenzyl bromide (4.06 g, 18.8 mmol, 1.25 equiv) in the manner previously described for enone **7a**, affording 1.891 g (46%), of the nitro enone as a pale yellow oil: ^{1}H NMR 0.98 and 1.13 (2s, 2 × 3H, geminal CH_3's), 1.92 (s, 3H, 3-CH_3), 2.17 and 2.31 (AB q, 2H, J_{AB} = 18.5, 4-H), 2.38 (dd, 1H, J = 9.0, 3.3, 6-H), 2.75 and 3.05 (d of AB q, 2H, J_{AB} = 14.0, J_A = 3.2, J_B = 8.9, benzylic CH_2), 5.84 (s, 1H, 2-H), 7.33 and 7.53 (AB q, 4H, J_{AB} = 8.2, Ar-H); ^{13}C NMR 22.2, 23.6, 28.5, 30.6, 36.6, 45.3, 58.5, 122.9, 123.2, 124.4, 128.9, 129.5, 145.6, 149.8, 158.3, 199.6; IR (neat) 2963 (br, m), 1667 (s). Anal. ($C_{16}H_{19}NO_2$) C, H, N.

3,5,5-Trimethyl-6-[(3-methyl-4-nitrophenyl)methyl]-1-methylidenecyclohex-2-ene (21). This compound was prepared from enone **20a** (21 mg, 0.080 mmol) in the manner previously described for olefin **8a**, using 3 equiv of [(trimethylsilyl)methyl]lithium, and allowing 18 h for the subsequent elimination step, affording 3.5 mg (16%) of the nitro diene as a pale yellow oil: ^{1}H NMR 0.89 and 1.15 (2s, 2 × 3H, geminal CH_3's), 1.71 and 2.11 (AB q, 2H, J_{AB} = 18.1, 4-H), 1.78 (s, 3H, 3-CH_3), 1.97 (dd, 1H, J = 11.3, 2.7) and 2.91 (dd, 1H, J = 12.8, 2.9) [benzylic CH_2], 2.58 (s, 3H, Ar-CH_3), 3.96 and 4.55 (2s, 2 × 1H, methylidene CH_2), 5.82 (s, 1H, 2-H), 6.98 (s, 1H, 2′-H), 6.99 (d, 1H, J = 7.6, 6′-H), 7.88 (d, 1H, J = 8.5, 5′-H); ^{13}C NMR 21.0, 23.8, 27.9, 28.4, 33.4, 35.5, 41.6, 53.8, 112.0, 122.8, 124.6, 128.2, 133.5, 134.0, 136.1, 144.1, 147.1, 148.4; IR (neat) 2928 (w, br), 1518 (s), 1342 (s). Anal. ($C_{18}H_{23}NO_2$) C, H, N.

3,5,5-Trimethyl-1-methylidene-6-[(4-nitrophenyl)-methyl]cyclohex-2-ene (22a). To a flame-dried 10 mL rb flask containing enone **20a** (114 mg, 0.417 mmol) in 2.0 mL of THF at 0 °C was added Tebbe's reagent [(μ-chloro-μ-methylene)[bis(cyclopentadienyl)titanium]dimethylaluminum] (1.7 mL of a 0.50 M solution in toluene, 0.84 mmol, 2.0 equiv) dropwise over a period of 5 min. The reaction mixture was then allowed to warm to rt and stirred 45 min before cooling to 0 °C and the addition of 0.5 mL of 1 N NaOH. The reaction mixture was then diluted with hexanes (50 mL) and filtered through a pad of Celite and silica gel. The organic solution was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, 95:5) afforded 16.1 mg (14%) of the desired diene as a colorless oil: ^{1}H NMR 0.89 and 1.13 (2s, 2 × 3H, geminal CH_3's), 1.71 and 2.11 (AB q, 2H, J_{AB} = 18.1, 4-H), 1.78 (s, 3H, 3-CH_3), 1.96 (dd, 1H, J = 11.4, 3.1) and 2.98 (dd, 1H, J = 12.7, 3.3) [benzylic CH_2], 2.30 (dd, 1H, J = 12.5, 11.6, 6-H), 3.91 and 4.53 (2s, 2 × 1H, methylidene CH_2), 5.81 (s, 1H, 2-H), 7.17 (d, 2H, J = 8.6, 2′,6′-H), 8.08 (d, 2H, J = 8.6, 3′,5′-H); ^{13}C NMR 23.6, 27.7, 28.2, 33.2, 35.5, 41.3, 53.7, 111.8, 122.4, 123.0 (2C), 130.3 (2C), 135.9, 143.7, 146.1, 149.9; IR (neat) 2930 (br, w), 1518 (s), 1344 (s). Anal. ($C_{17}H_{21}NO_2$) C, H, N.

1-Hydroxy-4,6,6-trimethyl-2-methylidene-1-[(4-nitrophenyl)methyl]cyclohex-3-ene (23). To a flame-dried 10 mL rb flask containing 31 mg (0.12 mmol) of diene **22a** in 1.6 mL of CH_2Cl_2 at rt was added selenium(IV) oxide (SeO_2) (6.4 mg, 0.060 mmol, 0.50 equiv), followed by (*tert*-butyl)hydroperoxide (76 μL of a 3.0 M solution in 2,2,4-trimethylpentane, 0.23 mmol, 2.0 equiv), and the reaction mixture was allowed to stir at rt for 42 h. The solvent was then removed under reduced pressure, and the residue was purified by flash column chromatography (silica gel, hexanes/EtOAc, 15:1), affording 4.2 mg (13%) of the desired tertiary alcohol as a pale yellow oil: ^{1}H NMR 0.89 and 1.17 (2s, 2 × 3H, geminal CH_3's), 1.81 (s, 3H, 3-CH_3), 1.95 and 2.35 (AB q, 2H, J_{AB} = 18.3, 4-H), 2.69 and 3.02 (AB q, 2H, J_{AB} = 13.0, benzylic CH_2), 4.23 and 4.61 (2s, 2 × 1H, methylidene CH_2), 5.88 (s, 1H, 2-H), 7.22 (d, 2H, J = 9.6, 2′,6′-H), 8.05 (d, 2H, J = 9.6, 3′,5′-H); ^{13}C NMR 23.2 (2C), 29.7, 38.0, 39.5, 45.6, 78.4, 110.3, 122.3, 124.1, 131.9, 135.8, 146.2, 146.5, 146.6; IR (neat) 3577 (br, m), 2966 (m), 1518 (s), 1344 (s). Anal. ($C_{17}H_{21}NO_3$) C, H, N.

3,5,5-Trimethyl-1-methylidene-6-[(4-nitrophenyl)thio]-cyclohex-2-ene (22b). To a flame-dried 100 mL rb flask containing diisopropylamine (338 μL, 2.41 mmol, 1.10 equiv) in 10 mL of THF at −72 °C was added *n*-BuLi (1.00 mL of a 2.41 M solution in hexanes, 2.41 mmol, 1.10 equiv). After 20 min at −78 °C, isophorone (329 μL, 2.19 mmol) was added dropwise as a solution in 2 mL of THF, and the reaction mixture was allowed to gradually warm to 0 °C over 90 min before the addition of 4-nitrophenyl disulfide (1.19 g, 3.29 mmol, 1.50 equiv) as a solution in 5 mL of THF. The resultant dark-orange reaction solution was allowed to warm to rt and stirred for 15 h before cooling to 0 °C, and the reaction was quenched with 10 mL saturated NH_4Cl. The reaction mixture was then extracted with 60 mL of EtOAc, and the organic phase was washed with brine (40 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, 6:1) afforded 462 mg (72%) of the thiophenyl-substituted enone as an orange oil (R_f 0.52, 2:1 hexanes/EtOAc). This intermediate enone was then carried on to the next step without further purification. To a flame-dried 10 mL rb flask containing the thiophenyl-substituted isophorone (25 mg, 0.090 mmol) in 0.5 mL of THF at 0 °C was added Tebbe's reagent (350 μL of a 0.50 M solution in toluene, 0.17 mmol, 2.00 equiv) dropwise over a period of 5 min. The reaction mixture was then allowed to warm to rt and stirred 4 h before cooling to 0 °C and the addition of 0.5 mL of methanol. The reaction mixture was then diluted with 4:1 hexanes/EtOAc (50 mL) and filtered through a pad of Celite and silica gel. The organic solution was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (basic alumina, Brockman, hexanes/EtOAc, 10:1) afforded 2.8 mg (11%) of the desired diene as a colorless oil (R_f 0.67, 2:1 hexanes/EtOAc): ^{1}H NMR 1.04 and 1.15 (2s, 2 × 3H, geminal CH_3's), 1.79 (s, 3H, 3-CH_3), 1.84 and 2.23 (AB q, 2H, J_{AB} = 17.4, 4-H), 3.63 (s, 1H, 6-H), 4.62 and 4.74 (2s, 2 × 1H, methylidene CH_2), 5.87 (s, 1H, 2-H), 7.46 (d, 2H, J = 9.6, 2′,6′-H), 8.11 (d, 2H, J = 9.6, 3′,5′-H); HRMS calcd for $C_{16}H_{19}NO_2S$ *m/z* 289.1137, found *m/z* 289.1152.

(3*R*)-2′-[(*tert*-Butyldimethylsilyl)oxy]cyclocymopol Monomethyl Ether (25a). To a flame-dried 100 mL rb flask containing **1** (1.38 g, 3.30 mmol) in 10 mL of anhydrous CH_2Cl_2 at rt were added *tert*-butylchlorodimethylsilane (0.62 g, 4.1 mmol, 1.3 equiv), imidazole (0.57 g, 8.3 mmol, 2.5 equiv), and DMAP (50 mg, 0.4 mmol, 0.1 equiv), and the mixture was stirred at rt for 6 h. The reaction mixture was then diluted with 50 mL of CH_2Cl_2 and successively washed with 1.0 M $NaHSO_4$ (30 mL) and brine (30 mL). The organic solution was then dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, hexanes/EtOAc, gradient elution) afforded 1.72 g (98%) of the silylated phenol as a white solid: ^{1}H NMR 0.22 and 0.23 [2s, 2 × 3H, Si(CH_3)$_2$], 1.01 [s, 9H, SiC(CH_3)$_3$], 1.08 and 1.18 (2s, 2 × 3H, geminal CH_3's), 2.02−2.33 (m, 4H, 5,6-H), 2.58 (s, 1H, 3-H), 2.60 (dd, 1H, J = 20.1, 11.9) and 2.88 (d, 1H, J = 20.1, 11.4) [benzylic CH_2], 3.80 (s, 3H, OCH_3), 4.30 and 4.59 (2s, 2 × 1H, methylidene CH_2), 4.44 (dd, 1H, J = 11.1, 4.2, 1-H), 6.55 (s, 1H, 6′-H), 6.92 (s, 1H, 3′-H); ^{13}C NMR −4.1, −4.0, 18.3, 24.2, 25.9, 27.2, 28.0, 32.0, 34.7, 39.9, 53.8, 56.8, 63.5, 108.1, 112.4, 114.4, 123.0, 131.1, 144.9, 147.7, 149.9.

General Procedure for Selective Aliphatic Debromination of Natural Cyclocymopols 1 and 2. (2*R*)-2-[[4-Bromo-2-[(*tert*-butyldimethylsilyl)oxy]-5-methoxyphenyl]-methyl]-3,3-dimethyl-1-methylidenecyclohexane (26a). To a flame-dried 100 mL rb flask containing **25a** (1.00 g, 1.88 mmol) in 50 mL of anhydrous benzene with 10 mg of AIBN at rt was added *n*-Bu_3SnH (2.02 mL, 7.51 mmol, 4.00 equiv). After 18 h, TLC analysis indicated complete consumption of starting material and formation of a slightly less polar product. Carbon tetrachloride (10 mL) was added, and after 3 h at rt the mixture was diluted to 200 mL with CH_2Cl_2 and washed with brine (100 mL). The resultant organic solution was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, 10% EtOAc in hexanes) afforded 0.748 g (88%) of the debrominated compound as a colorless, low-melting solid: ^{1}H NMR 0.21 and 0.22 [2s, 2 × 3H, Si(CH_3)$_2$], 0.96 and 0.98 (2s, 2 × 3H, geminal CH_3's), 1.03 [s, 9H, SiC(CH_3)$_3$], 1.23−1.32 (m, 2H, 5-H), 1.53−1.60 (m, 2H, 4-H), 1.98 (ddd, 1H, J = 12.9, 8.7, 4.3, 6-H_{ax}), 2.14−2.20 (m, 1H, 6-H_{eq}), 2.24 (dd, 1H, J = 10.9, 3.8, 2-H), 2.65 and 2.78 (d of AB q, 2H, J_{AB} = 13.9, J_A = 3.4, J_B = 11.5, benzylic CH_2), 3.80 (s, 3H, OCH_3), 4.30 and 4.58 (2s, 2 × 1H, methylidene CH_2), 6.61 (s, 1H, 6′-H), 6.92 (s, 1H, 3′-H).

(3*R*)-1-Debromocyclocymopol Monomethyl Ether (27a). To a 10-mL rb flask containing **26a** (16 mg, 0.036 mmol) at 0 °C in 2 mL of THF was added TBAF (200 μL of a 1.00 M solution in THF, 0.2 mmol, 6 equiv). After 10 min at 0 °C, pH 7 potassium phosphate buffer was added, and the reaction mixture was extracted with hexanes. The resultant organic solution was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, 10% EtOAc in hexanes) afforded 7.5 mg (61%) of the debromophenol as a colorless oil. The ^{1}H NMR spectrum and TLC elution properties of this compound were identical to those reported for the racemic compound (**8a**): $[\alpha]^{24}_D$ +16.4° (1.00, $CHCl_3$). Anal. ($C_{17}H_{23}BrO_2$) C, H.

(3*S*)-2′-[(*tert*-Butyldimethylsilyl)oxy]cyclocymopol Monomethyl Ether (25b). This silylated natural product was prepared from **2** (0.41 g, 0.99 mmol) in the manner previously described for **25a**, affording 0.52 g (98%) of the desired silyl ether as a colorless syrup: ^{1}H NMR 0.20 and 0.24 [2s, 2 × 3H, Si(CH_3)$_2$], 0.98 and 1.24 (2s, 2 × 3H, geminal CH_3's), 1.02 [s, 9H, SiC(CH_3)$_3$], 2.03−2.10 (m, 2H, 6-H), 2.27−2.37 (m, 3H, 3,5-H), 2.78 and 2.95 (d of AB q, 2H, J_{AB} = 15.7, J_A = 3.1, J_B = 11.0, benzylic CH_2), 3.79 (s, 3H, OCH_3), 4.23 (dd, 1H, J = 11.0, 4.2, 1-H), 4.61 and 4.81 (2s, 2 × 1H, methylidene CH_2), 6.62 (s, 1H, 6′-H), 6.93 (s, 1H, 3′-H).

(3*S*)-1-Debromocyclocymopol Monomethyl Ether (27b). This compound was prepared from **25b** (0.40 g, 0.75 mmol) in two steps in the manner previously described for the **27a**, affording 140 mg (55%) of the debromophenol as a colorless oil. The ^{1}H NMR spectrum and TLC elution properties of this compound were identical to those reported for the racemic compound (**8a**).

Cotransfection Assays. Cotransfection assays using hPR-B1,[7a,b] hAR,[7c] hGR,[7d] hER,[7f] and hMR[7e] were performed in CV-1 cells as described in the literature.[8] CV-1 cells (African green monkey kidney fibroblasts) were cultured in the presence of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% charcoal resin-stripped fetal bovine serum and then transferred to 96-well microtiter plates one day prior to transfection. pRShPR-B1 is a pBR322 plasmid containing hPR-B (from D. McDonnell and B. O'Malley, Baylor College of Medicine) with the Tau-1 region of hGR inserted into the N-terminal region as a *Bgl*II fragment at the unique *Hin*cII site of hPR using BglII linkers. This results in 175 amino acids of hGR being inserted after amino acid residue 456 of hPR-B. In addition, two amino acid residues specified by the linker were included at either side of the Tau-1 domain. The GR Tau-1 domain was added to hPR in order to enhance the trancriptional gain of the receptor, giving better signal-to-noise in the cotransfection assay. The modified receptor is termed hPR-B1. The insertion is in the region of hPR N-terminal to the DNA binding domain and leaves the ligand binding domain unchanged. Cells were transiently transfected by the calcium phosphate coprecipitation procedure with the following plasmids: pRShPR-B1, MMTV-LUC reporter, pRS-β-Gal and filler DNA (pGEM). The receptor plasmid, pRShPR-B1, contained the hPR-B1 under constitutive control of the SV-40 promoter. The reporter plasmid, MMTV-LUC, contained the cDNA for firefly luciferase (LUC) under control of the mouse mammary tumor virus (MMTV) long terminal repeat, a conditional promoter containing a progesterone response element. pRS-β-Gal, coding for constitutive expression of *E. coli* β-galactosidase (β-Gal), was included as an internal control for evaluation of transfection efficiency and compound toxicity. After transfection, media were removed and the cells were washed with phosphate-buffered saline (PBS). Media-containing reference compounds (*i.e.*, progesterone and RU486) or test compounds in concentrations ranging from 10^{-12} to 10^{-5} M were added to the cells. Three to four replicates were used for each sample. After incubation, the cells were washed with PBS, lysed with

a Triton X-100 buffer, and assayed for LUC and β-Gal activities using a luminometer or spectrophotometer, respectively. Data evaluation was performed using the Oracle relational database management system with analysis reports and programs designed at Ligand. For each replicate, the normalized response (NR) was calculated as LUC response/β-Gal rate where β-Gal rate = β-Gal $\times$ 1 $\times$ 10^{-5}/β-Gal incubation time. The mean and standard error of the mean (SEM) of the NR were calculated. Data were plotted as the response of the compound compared to the reference compounds over the range of the concentration−response curve. For agonist experiments, the effective concentration that produced 50% of the maximum response (EC_{50}) was quantified. Agonist efficacy (%) was a function of LUC expression relative to the maximum LUC production by the reference agonist, progesterone. Antagonist activity was determined by testing the amount of LUC expression in the presence of progesterone at its EC_{50} concentration. The concentration of test compound that inhibited 50% of LUC expression induced by progesterone was quantified (IC_{50}). In addition, efficacy of antagonists was determined as a function (%) of maximal inhibition. Cotransfection studies with hAR, hGR, and hMR with the MMTV-LUC reporter and hER with the MMTV-ERE5-LUC reporter were carried out as described above to determine cross-reactivity of test compounds.

Receptor Binding Assays. Binding assays using baculovirus-expressed hPR-A, hAR, hER, hMR, and hGR obtained from CV-1 cell extract were performed as described in the literature.[8,26] Binding assays with hER were performed using overexpressed receptor extracted from yeast. The receptor protein is intact structurally as indicated by Western blot analysis and is present as a single species. Binding of receptor-containing extracts (total protein of 10 μg per well) to tritiated ligand (5 nM) equilibrated at 4 °C for 16 h in the presence of increasing amounts of unlabeled test compounds (1 pM to 10 μM). Binding buffer contained 0.3 M KCl, 10 mM Tris, pH 7.5, and 5 mM DTT. Nonspecific binding was determined by incubating receptor extract and tritiated ligand in the absence or presence of a 200-fold molar excess of unlabeled estradiol. At the end of the incubation period, bound and free ligand were separated by hydroxylapatite, and the resultant ligand−receptor complex bound to hydroxylapatite was washed with ice-cold buffer containing 0.3 M KCl, 10 mM Tris, pH 7.5, three times; bound radioactivity was determined using a scintillation counter. After correcting for nonspecific binding, IC_{50} values were determined. The IC_{50} value is defined as the concentration of competing ligand required to decrease specific binding by 50%. The IC_{50} value was determined graphically from a log-logit plot of the data. K_i values were determined by application of the Cheng−Prusoff equation [$K_i = IC_{50}/(1 + [L]/K_d)$] to the IC_{50} values, where [L] is the concentration of labeled ligand and K_d is the dissociation constant of the labeled ligand.

T-47D Alkaline Phosphatase Assay. T-47D alkaline phosphatase assays were performed as described in the literature.[8,27] The T-47D human breast-carcinoma cell line (obtained from the American Type Culture Collection) was grown in the presence of RPMI 1640 media (BioWhittaker) with 10% (v/v) fetal bovine serum (Hyclone), 2.5 mM L-glutamine, 60 μg/mL gentamycin, and 0.2 μg/mL insulin. Three days before experiments, cells were transferred from 10 cm dishes to 96 well plates (1×10^4 cells/well). At the beginning of each experiment, the media were removed and replaced with fresh media [2% (v/v) charcoal resin-stripped fetal bovine serum] containing either test compound or test compound plus progesterone (1×10^{-9} M) and returned to a 37 °C incubator (5% CO_2). After 18−20 h, media were aspirated and the cells were fixed for 30 min with 100 μL of 5% formalin (in PBS). The fixed cells were then washed and 75 μL of assay buffer (1 mg/mL *p*-nitrophenol phosphate in 1 M diethanolamine pH 9, 2 mM $MgCl_2$ was added. Following incubation at 19 °C for 70 min, the reaction was terminated with the addition of 100 μL of 1 M NaOH. The absorbance at 405 nm was measured (Biomek). T-47D cells were prepared as described above. Cells were incubated in the presence of 1 nM progesterone and test compound. The ordinate (% of control) represents alkaline phosphatase expression as a function of 1 nM progesterone alone.

Murine Uterine Decidualization Assay. Mature virgin BALB/c (22 g mean weight) or ICR (30 g mean weight) mice were housed in a light- (14 h light, 10 h darkness; lights off at 20.00 h) and temperature- (22 °C) controlled room and fed and watered *ad libitum*. Females were caged with vasectomized males of the same strain between 17.00 and 10.00 h, and pseudopregnancy was dated from the morning when a vaginal plug was detected (day 1). Mating was presumed to have taken place at 02.00 (time 0).[41] Pseudopregnant mice were treated intraluminally with either standard compounds (RU486) or test compounds (**27a**, **37**, and **22a**) at the specified times *post coitum*. Control animals received an equivalent volume of sesame oil. RU486 was first dissolved in 100% ethanol and then diluted with sesame oil. Test compounds were dissolved in sesame oil and kept at rt. On day 4 (16.00 h), 10 μL of sesame oil was injected intraluminally into the right uterine horn (stimulated), and the left horn was left undisturbed to serve as an internal control (nonstimulated). At necropsy 72 h after application of the decidual stimulus, the uterine horns were removed, trimmed, blotted and weighed. Decidual response to trauma at the injection site was easily distinguished from the response to oil which stimulated deciduomata along the length of the uterus. Uterine wet weight gain was calculated by subtracting the weight of the nonstimulated horn from that of the stimulated horn. Animals were treated following the protocols: doses of RU486 ranging from 0.01 to 5.0 mg; doses of **27a**, **37**, and **22a** ranging from 1.0 to 5.0 mg; and sesame oil (0.1−0.2 mL) control, all given intraluminally between day 3 and day 5 of pseudopregnancy.

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Supporting Information Available: Synthetic procedures and chemical characterization data for compounds **5**, **8c**,**d**, **12**, **16**, **18**, **19**, **24**, **28b, 30, 31, 34a**,**b**, and **35**−**37** (16 pages). Ordering information is given on any current masthead page.

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