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Articles

Progestin 16α,17α-Dioxolane Ketals as Molecular Probes for the Progesterone Receptor: Synthesis, Binding Affinity, and Photochemical Evaluation

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Chemical probes for steroid receptors have proven useful in providing molecular details about important hormone-receptor interactions. A series of progestin 16α,17α-dioxolane ketals of acetophenone or substituted acetophenones that bind to the progesterone receptor (PgR) with comparable or higher affinities than the natural ligand, progesterone, have been prepared and evaluated as potential in vitro and in vivo probes for the progesterone receptor. *p*-Azidoacetophenone ketal **6**, the tetrafluoro analog **8**, and the *p*-(benzoyl)acetophenone ketal **9** demonstrate the required combination of high relative binding affinity (RBA) (**6** = 15%, **8** = 14%, **9** = 6.6%, progesterone = 13%, R5020 = 100%) and photoinactivation efficiency (**6** = 80%, **8** = 77%, **9** = 29% at 30 min) required for potential photoaffinity labeling reagents for the PgR. The synthesis of azide **6** has been modified to accommodate a palladium-catalyzed tritium gas hydrogenolysis of an iodoaryl precursor in the final stage of the synthetic sequence; this procedure has been verified by hydrogenation. In addition, the progestin *p*-fluoroacetophenone ketal **10** was selected for preparation in fluorine-18-labeled form, on the basis of its high affinity for the PgR (RBA = 53%). Fluorine-18-labeled progestins may be evaluated as potential diagnostic imaging agents for PgR-positive breast tumors. The radiochemical syntheses and further biochemical results with the fluorine-18-labeled ketal **10** and the tritium-labeled aryl azide **6** will be presented in an accompanying paper and elsewhere.

Introduction

The interaction of the progesterone receptor (PgR) with its natural hormone, progesterone, is a critical event that initiates a cascade of biological actions. In addition to producing normal physiological effects, progestins have been implicated in a number of pathophysiological processes including breast cancer,[1] endometriosis,[2] and most recently osteoporosis.[3] The mechanism of action of progestins at the molecular level, however, is currently only poorly understood, and efforts to delineate important aspects have been hampered by insufficient information on critical hormone-receptor binding interactions. In the absence of crystallographic data for the progesterone receptor, we are left with direct chemical probe methods such as affinity labeling and indirect approaches such as mutagenesis to define critical binding interactions. Identification of these important binding interactions through in vitro experiments may ultimately facilitate the rational design of progestin-based in vivo probes for medicinal applications.

The search for effective and selective photoaffinity labeling (PAL) reagents for the progesterone receptor has, up to now, relied primarily on progestins containing A,B-ring dienone systems as the photoreactive functionality.[4] While some of these compounds bind with high affinity to the progesterone receptor, none of them demonstrate the required combination of high receptor binding affinity, good photocovalent attachment efficiency, and low nonspecific binding necessary for efficient and selective

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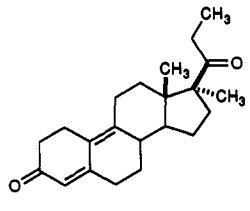
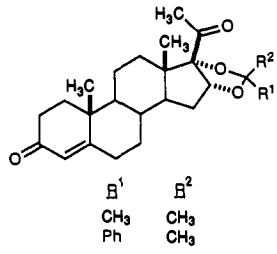


R5020

Figure 1. Roussel-Uclaf's R5020, the most commonly used photoaffinity labeling reagent for the progesterone receptor.



R¹	R²
CH_3	CH_3
Ph	CH_3

Figure 2. 16α,17α-(Methylenedioxy)progesterone derivatives that demonstrate in vivo progestational activity.[8]

labeling of the PgR. The Roussel-Uclaf compound, promegestone (R5020, Figure 1), is currently the most widely used PAL reagent for the PgR, in spite of displaying photocovalent attachment efficiencies of only 2–5%.[4a] While low photoattachment efficiencies may be sufficient for identifying some sites of ligand-receptor contact, more efficient photocovalent attachment may be necessary to identify other residues in the PgR hormone binding domain that are critical for ligand binding.

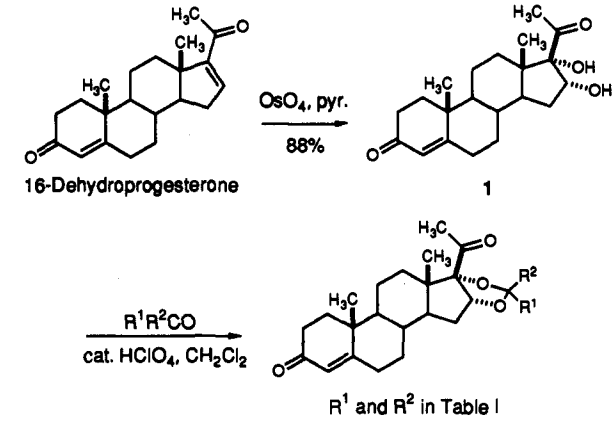
Protio and tetrafluoro aryl azides incorporated into a 3-aroyl-2-arylbenzo[*b*]thiophene molecular scaffold have recently been shown to have relatively high photocovalent attachment efficiencies (20–30%) in photoaffinity-labeling studies with the estrogen receptor.[5] The photoreactive benzophenone functionality has also recently been used to efficiently label the protein synthesis initiation factor eIF-4E.[6] While these types of photoreactive functionalities may offer efficient pathways to photoattachment, linking these groups to the progestin backbone results in a significant increase in the size of the progestin ligand. The structure–activity profile of high-affinity progestins indicates that there are only limited regions in which bulky substituents can be tolerated without loss in binding affinity to the PgR.[7] Fried has demonstrated that the 16α,17α-region of the progestin backbone is amenable to sterically demanding substituents by preparing 16α,17α-(methylenedioxy)progesterone derivatives that exhibit strong in vivo activity (Figure 2).[8]

In this paper, we describe the modification of 16α,17α-progestin ketals to include photoreactive functionalities and a preliminary biochemical evaluation of their potential use as photoaffinity labeling agents for the PgR. In addition, we present high-affinity fluorine- and iodine-substituted 16α,17α-(methylenedioxy)progesterone derivatives as potential lead compounds to positron-emitting and γ-emitting radiopharmaceuticals that may be used as diagnostic imaging agents for PgR-positive breast tumors. The synthesis and biological uptake in rats of a fluorine-18-labeled progestin analog is presented in an accompanying paper.[9]

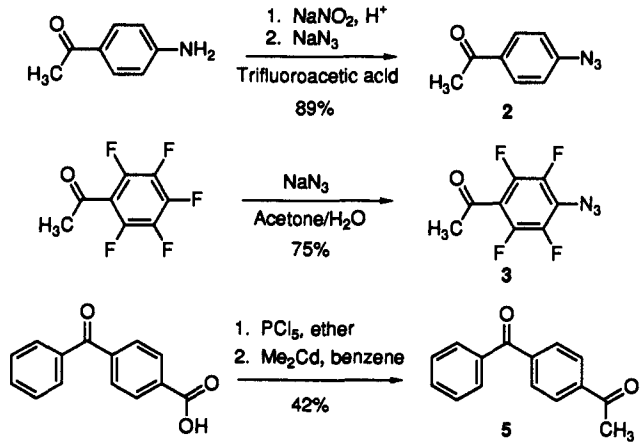
Results and Discussion

Synthesis of Progestin 16α,17α-Ketals. The target 16α,17α-(methylenedioxy)progesterone derivatives are

Scheme I



Scheme II

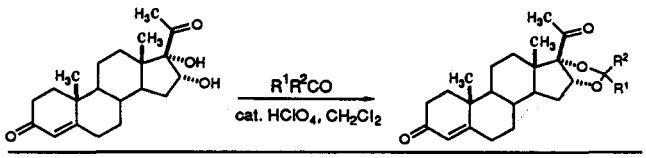


available in high yield in two steps from commercially available 16-dehydroprogesterone (Scheme I). Oxidation with stoichiometric osmium tetroxide in pyridine provides 16α,17α-dihydroxyprogesterone, 1, with high regio- and stereoselectivity in high yield (88%).[10] Attempts at diol formation using other oxidative conditions (catalytic osmium tetroxide with stoichiometric *N*-methylmorpholine *N*-oxide, potassium permanganate, or cetyltrimethylammonium permanganate) were less selective and efficient.

The photoreactive portions of the potential progestin photoaffinity-labeling reagents were assembled as acetophenone derivatives prior to attachment to the progestin backbone (Scheme II). Diazotization of 4′-aminoacetophenone with sodium nitrite, followed by displacement with sodium azide, afforded the aromatic azide **2** in high yield. The tetrafluoroaryl azide **3** was prepared by displacement of the para-situated fluorine in pentafluoroacetophenone with sodium azide in refluxing aqueous acetone.[11] Treatment of 4′-benzoylbenzoic acid with phosphorus pentachloride provided the stable acid chloride **4** in high yield. Methyl ketone synthesis was accomplished using dimethyl cadmium, prepared from cadmium chloride and methyl magnesium bromide, to provide the benzophenone **5** in moderate yield.[12]

The progestindiol **1** was most efficiently converted to the desired 16α,17α-dioxolanes (Table I) by stirring with a large excess of the appropriate ketone (10–40 equiv) and a catalytic amount of perchloric acid in methylene chloride.[8a] Ketones that were liquids were stirred neat with diol **1**. Ketalization of the 16α,17α-diol proceeds in high yield when the aromatic ring of the acetophenone

Table I



compd	R¹ (endo)	R² (exo)	% yield	21-CH₃ δ (ppm from TMS)
6	p-$C_6H_4N_3$	CH_3	82	2.32
7	CH_3	p-$C_6H_4N_3$	46	1.83
8	p-$C_6F_4N_3$	CH_3	39	2.32
9	p-$C_6H_4(CO)C_6H_5$	CH_3	40	2.35
10	p-C_6H_4F	CH_3	78	2.32
11	CH_3	p-C_6H_4F	56	1.82
12	C_6F_5	CH_3	7.4	2.32
13	p-C_6H_4I	CH_3	81	2.33
22	$C_6H_3IN_3$	CH_3	50	2.31

derivative is either unsubstituted or monosubstituted with an electron-withdrawing group. Electron-donating groups (p-NH_2) deactivate the ketone to attack by the 16α,17α-diol and prevent formation of the 16α,17α-progestin dioxolanes. Multiple electron-withdrawing substituents (fluorine) also decrease the efficiency of ketalization, as these reactions require longer reaction times and proceed in lower yields. Ketalization must be performed at 23 °C or below, as higher temperatures result in acid-catalyzed ring opening of the progestin D-ring.

Formation of unsymmetrically substituted 16α,17α-(methylenedioxy)progesterone derivatives introduces an additional stereogenic center at the ketal bridgehead methylene. Determination of stereochemistry at this position is critical, as only one of the diastereomers has been found to be biologically active. The absolute stereochemistry of the ketal epimeric center was assigned by comparison of the 21-CH_3 resonances in the ^1H NMR spectra to the 21-CH_3 resonances in 16α,17α-progestin ketals prepared by Fried and Sabo.[8a] Positioning of a phenyl group in the exo position (Figure 3) has a shielding effect on the 21-CH_3 group; in this diastereomer the 21-CH_3 group lies above the center of the phenyl ring, resulting in an 0.3–0.5 ppm upfield shift in these proton resonances. The three-dimensional structure of the *endo*-phenyl diastereomer was proven in the crystal structure of 21-bromo-9α-fluoro-11β-hydroxy-16α,17α-[β-methyl-α-phenyl(methylenedioxy)]-4-pregnene-3,20-dione.[15]

The stereochemistry of ketal formation is dependent on the reaction conditions and is a classic case of kinetic vs thermodynamic control: under conditions of kinetic control (0.02–0.05 M $HClO_4$), the biologically active diastereomer, in which the methyl group is exo and the phenyl group is endo, can be obtained almost exclusively, whereas the biologically inactive diastereomer, in which the methyl group is endo and the phenyl group is exo, is favored under equilibrium conditions (1.0 M $HClO_4$). In the ketalization of 4′-fluoroacetophenone with progestin-diol 1, the epimeric ketals 10 and 11 can be observed by fluorine-19 NMR: At 0.05 M $HClO_4$, only the resonance due to the *endo*-phenyl progestin 10 is observed (ϕ = −113.3 ppm), whereas at 1.0 M $HClO_4$, this resonance is the major peak at 3 min, but a second peak at −112.8 ppm, due to the *exo*-phenyl diastereomer 11 grows in, so that by 3 h equilibrium is attained (the ratio of 11:10 is at 3:1).

The isothiocyanate **15**, which is isosteric to the azide **6**, was envisaged as a potential electrophilic affinity labeling reagent for the progesterone receptor. Reduction of azide **6** with stannous chloride in methanol afforded amine **14** in high yield (Scheme III).[16] This compound was not available through direct ketalization with 4′-aminoacetophenone, as the strong electron-donating ability of the amine renders the ketone unreactive to ketalization. Treatment with thiophosgene and triethylamine provided the isothiocyanate **15** in high yield.[17]

Binding Affinity for the Progesterone Receptor. The relative binding affinity (RBA)[18] of the 16α,17α-(methylenedioxy)progesterones was determined at 0 °C by a competitive radiometric binding assay using [^3H]-R5020 as tracer. The RBA values (Table II) are reported relative to R5020, which is assigned a value of 100%. The natural hormone, progesterone, has an RBA of 13%. Progestins **16–19**, which had been previously prepared and evaluated by the in vivo Clauberg assay by Fried,[8] were prepared and evaluated for their relative binding affinities in our in vitro competitive radiometric binding assay. Progestin **16**, which has the phenyl ring endo and the methyl group exo, has an RBA of 16%, while the opposite diasteromer **17** demonstrates low affinity for the progesterone receptor, RBA = 1.2%. The furan derivative **19**, which demonstrated high in vivo activity in the Clauberg assay,[8a] demonstrated a surprisingly low RBA of 5.4%. This represents an unusual example in which potent in vivo activity is not well correlated with high in vitro binding affinity.

Progestin derivatives that were substituted with fluorine or iodine and exhibited comparable or higher binding affinity than the natural hormone for the progesterone receptor were considered as candidates for radiolabeling. Other high-affinity fluorine-18-labeled progestins have previously been investigated as possible progestin-based breast tumor imaging agents for positron emission tomography (PET).[19] The fluorine-substituted progestin **10** with an RBA of 53% was identified as a target compound for fluorine-18 radiolabeling. The opposite diastereomer **11**, isolated using conditions of thermodynamic control, did not bind well to the PgR, RBA = 0.56%. Our strategy for radiolabeling progestin **10** along with the in vivo tissue uptake data in rats is presented in an accompanying paper.[9]

The relative binding affinities of the potential PAL reagents are 15% for the protioaryl azide **6**, 14% for the tetrafluoroaryl azide **8**, and 6.6% for the benzophenone progestin derivative **9**. Each of these potential PAL reagents exhibited affinity for the PgR comparable to that of progesterone and was therefore further evaluated for its photochemical properties. The thermodynamic diastereomer of the protioaryl azide **7** exhibited low binding affinity for the receptor, RBA = 0.18%. The isothiocyanate **15**, a potential electrophilic affinity labeling reagent, was also found to bind to the PgR with only low affinity, RBA = 1.3%.

Binding Affinities to Mineralocorticoid and Glucocorticoid Receptors. The progestin 16α,17α-ketal derivatives that exhibited high binding affinity for the PgR were also evaluated for binding affinities to the rat kidney mineralocorticoid receptor (MR) and the rat liver glucocorticoid receptor (GR) (Table III), as progestins may show substantial heterologous binding to these receptors. While all of the ketals assayed (except **10**) bound with low affinity to the MR, affinity for the GR was dependent on the specific ketal formed. The iodinated progestin **13** and benzophenone-substituted progestin **9** bound to the GR with highest affinity, 20% and 15% respectively. The

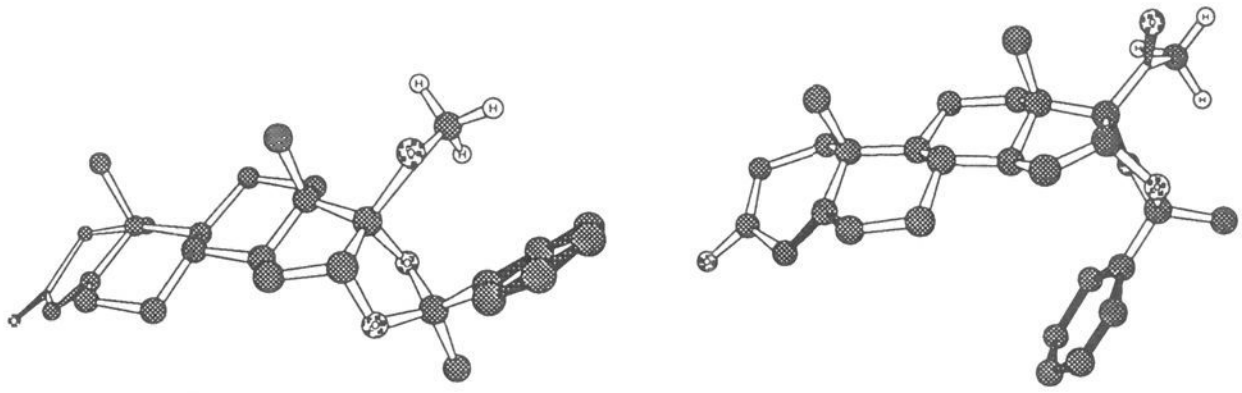


Figure 3. Two diastereomers of 16α,17α-[1′-phenyl(ethylidenedioxy)]pregn-4-ene-3,20-dione. The phenyl exo diastereomer exhibits shielding of the 21-CH_3 resonance in ^1H NMR. The structure of the phenyl endo diastereomer was obtained by retrieval of the crystal structure of 21-bromo-9α-fluoro-11β-hydroxy-16α,17α-[β-methyl-α-phenyl(methylenedioxy)]-4-pregnene-3,20-dione from the Cambridge Structural Database, conversion of the proper heteroatoms to protons and energy minimization in Macromodel version 3.1[13] (MM2[14] force field). The phenyl exo isomer was obtained by isomerization at the 2′ center, followed by minimization (MM2[14] force field).

Scheme III

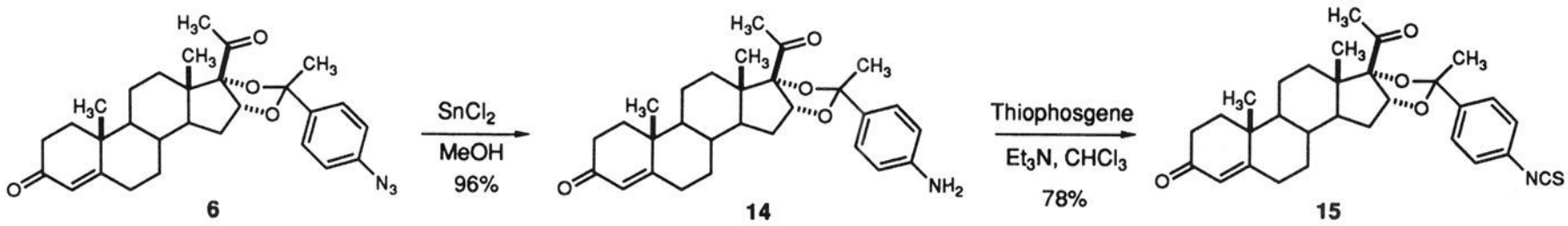


Table II. Relative Binding Affinities (RBAs) of 16α,17α-(Methylenedioxy)progesterones for the Progesterone Receptor (Progesterone = 13%, R5020 = 100%)

compd	R^1 (endo)	R^2 (exo)	RBA
6	p-$C_6H_4N_3$	CH_3	15
7	CH_3	p-$C_6H_4N_3$	0.18
8	p-$C_6F_4N_3$	CH_3	14
9	p-$C_6H_4(CO)C_6H_5$	CH_3	6.6
10	p-C_6H_4F	CH_3	53
11	CH_3	p-C_6H_4F	0.56
12	C_6F_5	CH_3	20
13	p-C_6H_4I	CH_3	25
14	p-$C_6H_4NH_2$	CH_3	0.01
15	p-C_6H_4NCS	CH_3	1.3
16	C_6H_5	CH_3	16
17	CH_3	C_6H_5	1.2
18	CH_3	CH_3	2.0
19	C_4H_3O	CH_3	5.5
22	$C_6H_3IN_3$	CH_3	2.9

[a] Relative binding affinity determined in a competitive radiometric binding assay; details are given in the Experimental Section. Values are expressed as percentages relative to the affinity of R5020 as tracer. The RBAs of compounds which exhibit affinities > 1.0% are the average of two or more determinations, which are generally reproducible to within 30% (relative error).

fluorinated progestins **10** and **12** demonstrate high receptor selectivity for binding to the PgR relative to the MR and GR, while the aryl azides **6** and **8** are moderately selective in binding to PgR over GR.

Ultraviolet Spectra and Preliminary Photoinactivation Studies of Progestin Ketals 6, 8, and 9. Prior to photolysis studies with the progesterone receptor, UV spectra were taken to the potential PAL reagents. The protioaryl azide **6** (PAA) and the tetrafluoroaryl azide **8** (TFAA) demonstrate strong absorbances for the π–π^* transitions of the aryl azide (PAA λ_{max} = 246 nm, ϵ = 10 300; TFAA λ_{max} = 246, ϵ = 9400) and broad, weak absorbances for the forbidden n–π^* transitions. The n–π^* transition is evident as a tail to the absorbance at 246 nm that extends out to 340 nm (Figure 4). The benzophenone analog **9** demonstrates a strong absorbance for the π–π^* transition of its ketone (λ_{max} = 248 nm, ϵ = 38 400) and a weak absorbance for the n–π^* transition that is observable out to 360 nm (data not shown). Although the λ_{max} values indicated that photolysis would be most efficient using 254-nm light, the high energy of this wavelength has been shown to induce protein damage.[20] Therefore, photolysis of PAL reagent–PgR preparations were evaluated at both >315 and 254 nm.[21]

A preliminary study designed to investigate the photochemical behavior of **6**, **8**, and **9** in the active site of the PgR was performed. The photoinactivation efficiency (PIE) of the PAL reagents for PgR was determined by photolysis of PgR complexes formed by incubation of the photoreactive compounds **6**, **8**, and **9** with a rat uterine cytosol preparation of PgR for 4 h at 0 °C.[21] Irradiation of the receptor–ligand complexes was conducted at 254 or >315 nm, and the loss of reversible binding capacity of the PgR was measured by an exchange assay using [^3H]-R5020. Control experiments in which the receptor sites were blocked with the nonphotoactive progestin ORG2058 demonstrated high levels of PgR decomposition when irradiation was carried out at 254 nm. Irradiation at >315 nm did not cause this protein damage and resulted in highly specific photoinactivation of the PgR (Figure 5).

The aryl azides **6** and **8** demonstrate the highest photoinactivation efficiencies (**6** = 80%, **8** = 77%) of any potential photoaffinity labeling reagent for the PgR tested to date by this method.[4i] Furthermore, the ability of **6** and **8** to inactivate the receptor upon photolysis was shown to be completely specific for the PgR binding site. Blocking of the PgR binding site with ORG2058 prior to incubation with PAL ligand **6** or **8** eliminated any photoinactivation of PgR reversible binding capacity. The benzophenone analog **9** also demonstrated specific photoinactivation of the PgR with lower efficiency (PIE = 29%, data not shown).

While these preliminary results are encouraging, the actual efficiency of photoattachment cannot be determined until a target molecule is prepared in radiolabeled form. The PIE assay cannot discriminate between covalent ligand attachment or other protein damaging events (photooxidation–photoreduction) that could render the

Scheme IV

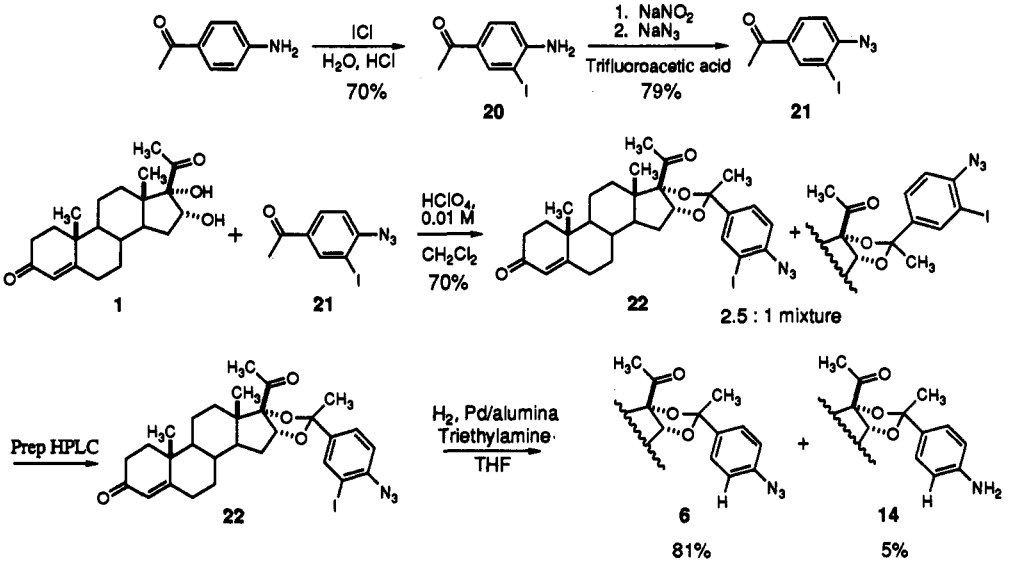


Table III. Receptor Selectivity: Relative Binding Affinities (RBAs) of 16α,17α-(Methylenedioxy)progesterones for the PgR, MR, and GR

compd	PgR (R5020 = 100)	MR (aldosterone = 100)	GR (RU28362 = 100)
progesterone	13	not assayed	0.56
6	15	0.13	7.2
8	14	0.95	5.7
9	6.6	<0.003	15
10	53	6.0	9.4
12	20	0.65	4.4
13	25	0.13	20
15	1.3	0.09	1.7
16	16	1.6	9.7

[a] Relative binding affinity determined in a competitive radiometric binding assay; details are given in the Experimental Section. Values are expressed as percentages relative to the affinity of the indicated tracer and are the average of two or more determinations, which are generally reproducible to within 30% (relative error). R5020 is 17α-,21-dimethyl-19-norpregna-4,9-diene-3,20-dione. RU28362 is 11β,17β-dihydroxy-6-methyl-17α-propynylandrosta-1,4,6-triene-3-one.

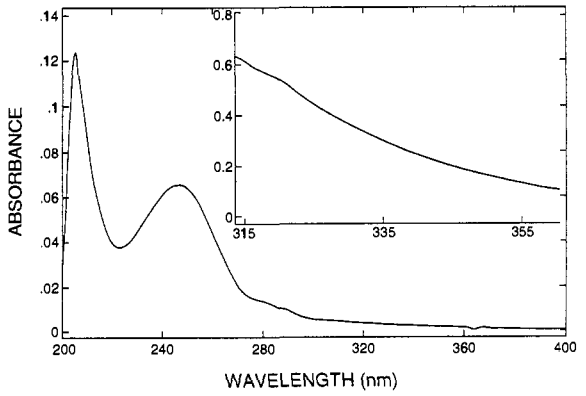


Figure 4. Ultraviolet absorbance spectrum of 6. Photolysis was carried out at >315 nm (inset).

receptor unable to bind ligands.[21] The protioaryl azide 6 has been selected for tritium labeling, on the basis of its high RBA, high PIE, and its synthetic accessibility.

Synthetic Adaptations To Accommodate Radioisotope Incorporation. In order to prepare azide 6 in tritium-labeled form, we have modified our synthetic route to accommodate a palladium-catalyzed tritium gas hydrogenolysis of an iodine-substituted precursor in the last step of the synthetic pathway. In this work we present only the reactions leading up to the tritium-exchange

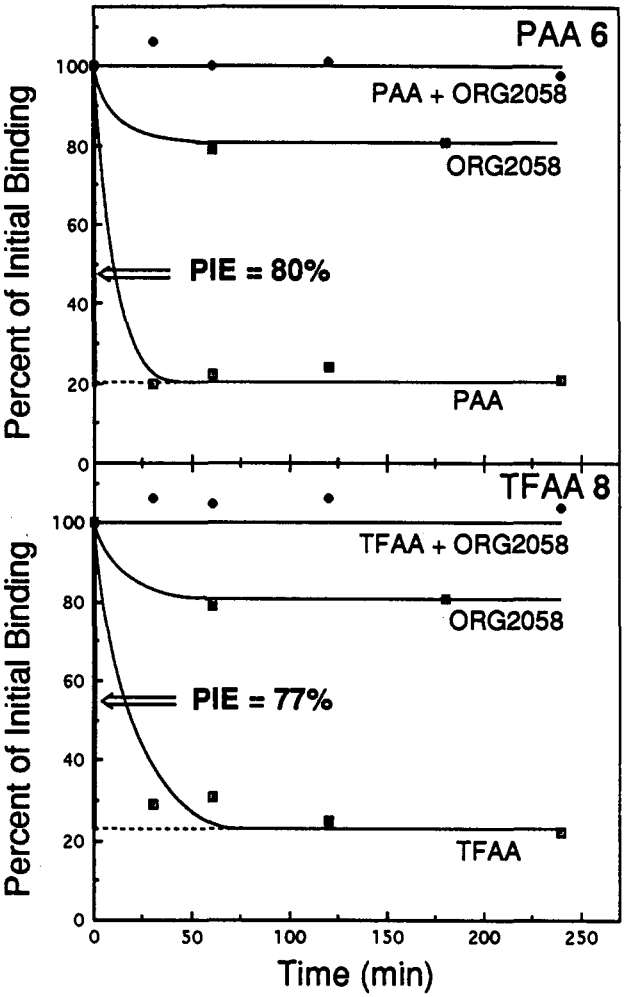


Figure 5. Photoinactivation of PgR in rat uterine cytosol by PAA, 6 (top), and TFAA, 8 (bottom). Cytosol was incubated with PAA or TFAA in the absence or presence of a 100-fold excess of ORG2058 for 1 h at 0 °C and then photolyzed at >315 nm for various times. Following charcoal–dextran treatment to remove free ligand, the cytosol was exchanged at room temperature for 2 h against [^{3}H]R5020. Photoinactivation (PIE) is seen as a loss in exchange sites and plotted as a percentage of the initial binding before photolysis.

reaction, as that reaction will be presented elsewhere, along with further biological results. Iodine was introduced onto

the aromatic ring by electrophilic iodination of 4'-aminoacetophenone with iodine monochloride to provide **20** in good yield (Scheme IV).[22] Diazotization with sodium nitrite, followed by displacement with sodium azide, afforded the iodinated azide **21**. Perchloric acid (0.05 M) catalyzed ketalization with the progestin diol **1** resulted in a 2.5:1 mixture of diastereomers of which the desired product was the major component. The desired diastereomer was isolated in pure form by preparative normal-phase HPLC. Model palladium-catalyzed hydrogen-exchange reactions were performed with hydrogen gas to provide azide **6** in 81% yield. Triethylamine was added to sufficiently poison the palladium catalyst, so the amine **18**, the overreduction product, was isolated in only 5% yield.

Conclusion

In this report, we have described the preparation and receptor binding affinities of a series of 16α,17α-(methylenedioxy)progesterone derivatives developed as potential in vitro and in vivo probes for the PgR. Compounds containing photoreactive functionalities (protioaryl azide, tetrafluoroaryl azide, or benzophenone) were evaluated as potential photoaffinity labeling reagents for the PgR by an indirect photoinactivation assay. The protioaryl azide **6** was identified as a promising potential photoaffinity-labeling reagent, on the basis of its high relative binding affinity (RBA = 15%), high photoinactivation efficiency (PIE = 80%), and synthetic accessibility. An efficient synthesis that should allow for tritium incorporation in the ultimate step of the synthetic pathway has been developed. The results of the radiolabeling and photoaffinity labeling studies will be presented elsewhere.

Several fluorinated and iodinated 16α,17α-(methylenedioxy)progesterone derivatives, potential in vivo tumor imaging agents, were also prepared and evaluated for their binding affinities to the PgR. The fluorinated progestin **10** was found to bind to the PgR with 4 times the affinity of the natural hormone. The synthesis of **10** in fluorine-18-labeled form and its in vivo biodistribution in rats is presented in an accompanying paper.[9]

Experimental Section

General. Reaction progress was monitored by analytical thin-layer chromatograhy using 0.25-mm silica gel glass-backed plates with F-254 indicator (Merck), or gas chromatography on a Hewlett-Packard 5790A GC equipped with a Hewlett-Packard Ultra 1 fused silica capillary column. Flash chromatography was performed with Woelm 32–63 μm silica gel packing. Visualization was accomplished by phosphomolybdic acid or ninhydrin spray reagents, iodine, or UV illumination.

Proton magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz, and chemical shifts are reported as ppm downfield from an internal tetramethylsilane standard (δ scale). The data are reported in the following form: chemical shift (multiplicity, coupling constant in hertz (if applicable), number of protons, assignment). Fluorine magnetic resonance (^{19}F NMR) spectra were recorded on at 376.3 MHz and chemical shifts are reported as ppm upfield from an external $CFCl_3$ standard at 0 ppm (φ scale). Only characteristic infrared (IR) bands were reported. Electron ionization (EI) mass spectra data were obtained at 70 eV and are reported in the following form: *m/z* (intensity relative to base peak = 100). High pressure liquid chromatography (HPLC) was performed with ultraviolet detection at 254 nm using a 5-μm analytical SiO_2 column (4.6 mm × 30 cm, Varian Si-5 Micro-Pak) or 10-μm semipreparative SiO_2 column (0.9 cm × 50 cm, Whatman Partisil M-9). Reverse-phase HPLC was performed on a Varian 5000 using a Partisil M9 0.9 cm × 50 cm ODS-2 column.

Solvents and reagents used were purchased as analytical reagent grade, and the following were further purified by distillation from the indicated drying agent: CH_2Cl_2(P_2O_5), Et_3N (CaH_2), DMF ($MgSO_4$), THF (sodium benzophenone ketyl), ether (sodium benzophenone ketyl), and pyridine (CaH_2). A standard method for product isolation was used; it involved an aqueous quench and organic extraction, drying of the extract, and removal of solvent in vacuo. The components used are given in parentheses following the phrase "product isolation".

4'-Azido-2',3',5',6'-tetrafluoroacetophenone (**3**) was prepared according to the method of Keana.[11] 4'-Benzoylacetophenone (**5**) was prepared according to the method of Zelinski.[12b]

16α,17α-Dihydroxypregn-4-ene-3,20-dione (1) was prepared by modification of Bernstein's procedure.[10] To a stirred solution of 16-dehydroprogesterone (0.612 g, 1.96 mmol) in pyridine (25 mL) was added osmium tetroxide (0.50 g, 1.97 mmol). The black solution was stirred for 2 h at which point the osmate ester was quenched with a solution of sodium bisulfate (3.5 g in 30 mL of H_2O and 10 mL of pyridine). Product isolation (H_2O, EtOAc, aqueous 5% HCl, brine, $MgSO_4$) and removal of the remaining pyridine on the high-vacuum pump (0.1 Torr) gave 0.63 g (93%) of crude diol. Recrystallization from ethyl acetate afforded 0.59 g (88%) of diol **1** as a white solid: mp 215–217 °C (lit.[10] mp 219–223 °C); ^{1}H NMR ($CDCl_3$) δ 5.73 (s, 1 H, 4-H), 5.06 (ddd, *J* = 9.1, 5.9, 2.2 Hz, 1 H, 16β-H), 3.82 (s, 1 H, 16-OH), 2.25 (s, 3 H, 21-CH_3), 1.17 (s, 3 H, 19-CH_3), 0.715 (s, 3 H, 18-CH_3); MS (70 eV) *m/z* (relative intensity) 346 (M^+, 26), 303 (100), 285 (56), 229 (62); HRMS (EI) calcd for $C_{21}H_{30}O_4$ 346.2141, found 346.2141. Anal. ($C_{21}H_{30}O_4$) C, H.

4'-Azidoacetophenone (2). Sodium nitrite (2.04 g, 29.6 mmol) was added as a solid to a 60-mL solution of 4'-aminoacetophenone (2.0 g, 14.8 mmol) in trifluoroacetic acid at 0 °C. After 45 min of stirring, NaN_3 (9.62 g, 148 mmol) was added slowly, so the temperature would not exceed 5 °C. An aliquot of ether was added (60 mL), and the reaction was allowed to stir in the dark for 2 h. Product isolation (H_2O, ether, brine, $MgSO_4$) followed by removal of CF_3CO_2H as an azeotrope with benzene afforded a tan solid. Purification by flash chromatography (5:1 hexanes/EtOAc) gave **2** as a pale yellow solid (2.12 g, 88.6%): mp 43–45 °C (lit.[23] mp 44 °C); ^{1}H NMR δ 7.95 (d, *J* = 8.6 Hz, 2 H, ArH meta to N_3), 7.07 (d, *J* = 8.6 Hz, 2 H, ArH ortho to N_3), 2.57 (s, 3 H, CH_3); IR ($CHCl_3$) ν 2132 (d), 1680 cm^{-1}; MS (70 eV) *m/z* 161 (M^+, 40) 133 (100), 118 (7), 106 (32), 90 (60); HRMS calcd for $C_8H_7ON_3$ 161.0589, found 161.0586. Anal. ($C_8H_7ON_3$) C, H, N.

4'-Benzoylbenzoyl Chloride (4). 4'-Benzoylbenzoic acid (2.71 g, 11.98 mmol) was combined with PCl_5 (2.62 g, 12.58 mmol) in 10 mL of dry ether. The reaction mixture was vigorously shaken by hand to effect dissolution of the PCl_5. Removal of ether in vacuo afforded **4** as a white solid (2.85 g, 97.1%): mp 98–99 °C (lit.[24] mp 95–96 °C); ^{1}H NMR ($CDCl_3$) δ 8.23 (d, *J* = 8.2 Hz, 2 H, ArH ortho to COCl), 7.88 (d, *J* = 8.2 Hz, 2 H, ArH meta to COCl), 7.80 (d, *J* = 7.4 Hz, 2 H, ArH ortho to ClCOArCO), 7.63 (d, *J* = 7.4 Hz, 1 H, ArH para to ClCOArCO), 7.53 (d, *J* = 7.4 Hz, 2 H, ArH meta to ClCOArCO); IR ($CHCl_3$) ν 3019, 1755 (d), 765.671 cm^{-1}; MS (70 eV) *m/z* 244 (M^+, 27), 209 (100), 105 (62), 77 (51), 51 (21). Anal. ($C_{14}H_9ClO_2$) C, H, Cl.

General Procedure for the Synthesis of Progestin 16α,17α-Ketals from 16α,17α-Dihydroxypregn-4-ene-3,20-dione and the Appropriately Substituted Acetophenone Derivative. Ketalization of 16α,17α-dihydroxypregn-4-ene-3,20-dione was effected by adding a catalytic amount of 70% perchloric acid to a suspension of diol **1** and the appropriate acetophenone derivative. Reactions utilizing acetophenone derivatives that were liquids were performed neat, while reactions using acetophenone derivatives that were solids were performed with at least a 40-fold excess of ketone derivative dissolved in 1 mL of CH_2Cl_2. Ketalization under reaction conditions of kinetic control was performed with the perchloric acid concentration kept below 0.05 M. Ketalization under reaction conditions of thermodynamic control was performed with the perchloric acid concentration at 1.0 M. The reactions were monitored by TLC (silica gel, 2:1 hexanes/EtOAc) and quenched by pouring onto iced $NaHCO_3$ (saturated). Product isolation (CH_2Cl_2, H_2O, brine, $MgSO_4$) followed by flash chromatography (2:1 hexanes/EtOAc) afforded the progestin 16α,17α-ketals. Further purification by HPLC [Whatman M-9, 85% hexane/15% (5% *i*-PrOH–CH_2Cl_2), 4 mL/

min] gave a sample for binding affinity measurement. Purity was verified by reinjection on an HPLC analytical column.

16α,17α-[(*R*)-1′-(4-Azidophenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (6). Ketalization of diol 1 (0.095 g, 0.22 mmol) with 4′-azidoacetophenone (0.99 g, 6.2 mmol) using reaction conditions of kinetic control afforded progestin ketal 6 as a white powder (0.083 g, 82%): mp 133–136 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (d, J = 8.5 Hz, 2 H, ArH ortho to N_3), 7.02 (d, J = 8.5 Hz, 2 H, ArH meta to N_3), 5.69 (s, 1 H, 4-H), 5.22 (d, J = 5.0 Hz, 1 H, 16β-H), 2.32 (s, 3 H, 21-CH_3), 1.44 (s, 3 H, ketal CH_3), 1.14 (s, 3 H, 19-CH_3), 0.66 (s, 3 H, 18-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.36, 199.26, 170.37, 139.96, 139.74, 126.35, 124.04, 118.64, 111.06, 99.72, 82.11, 53.43, 52.98, 48.77, 45.99, 38.44, 35.50, 34.61, 33.87, 33.29, 32.53, 31.92, 31.78, 29.70, 27.85, 27.66, 20.32, 17.29, 14.86; IR ($CHCl_3$) ν 2947, 2130, 1730, 1662 cm^{-1}; MS (FAB) m/z 490 (M + 1), 462, 309, 275; HRMS calcd for $C_{29}H_{36}O_4N_3$ 490.2706, found 490.2705.

16α,17α-[(*S*)-1′-(4-Azidophenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (7). Ketalization of diol 1 (0.025 g, 0.072 mmol) with 4′-azidoacetophenone (0.430 g, 2.67 mmol) using reaction conditions of thermodynamic control afforded a 3:1 mixture of progestin ketals 7 and 6 (0.025 g, 71%). Purification by preparative HPLC [Whatman M-9, 85% hexane/15% (5% i-PrOH–CH_2Cl_2), 4 mL/min] afforded pure 7 (0.016 g, 46%): mp 160–192 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.35 (d, J = 8.5 Hz, 2 H, ArH ortho to N_3), 6.93 (d, J = 8.5 Hz, 2 H, ArH meta to N_3), 5.76 (s, 1 H, 4-H), 5.08 (d, J = 5.4 Hz, 1 H, 16β-H), 1.83 (s, 3 H, 21-CH_3), 1.68 (s, 3 H, ketal CH_3) 1.19 (s, 3 H, 19-CH_3) 0.58 (s, 3 H, 18-CH_3); MS (FAB) m/z 490 (M + 1), 462, 279, 261, 222; HRMS (FAB) calcd for $C_{29}H_{36}N_3O_4$ 490.2706, found 490.2695.

16α,17α-[(*R*)-1′-(4′-Azido-2′,3′,5′,6′-tetrafluorophenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (8). Ketalization of diol 1 (0.035 g, 0.101 mmol) with 4′-azido-2′,3′,5′,6′-tetrafluoroacetophenone[11] under kinetic reaction conditions afforded the progestin ketal 8 as an opaque glass (0.022 g, 39%) after 100 h of stirring at 23 °C: ^{1}H NMR ($CDCl_3$) δ 5.74 (s, 1 H, 4-H), 5.01 (d, J = 5.3 Hz, 1 H, 16β-H), 2.23 (s, 3 H, 21-CH_3), 1.47 (s, 3 H, ketal CH_3), 1.17 (s, 3 H, 10-CH_3), 0.64 (s, 3 H, 18-CH_3); MS (FAB) m/z 562 (M + 1), 536, 433, 419, 309. HRMS calcd for $C_{29}H_{32}F_4N_3O_4$ 562.2330, found 562.2341.

16α,17α-[(*R*)-1′-(4′-Benzoylphenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (9). Ketalization of diol 1 (0.055, 0.16 mmol) with 4′-benzoylacetophenone[12b] under kinetic reaction conditions afforded a mixture of 9 and the dimeric compound resulting from ketalization of two molecules of diol 1 with the bis-ketone 5 (0.048 g, 55%). Purification by preparative HPLC [Whatman M-9, 85% hexane/15% (5% i-PrOH–CH_2Cl_2), 4 mL/min] afforded pure 9 as a white solid (0.035 g, 40%): mp 91–93 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (d, J = 8.3 Hz, 2 H, ArH ortho to COPh), 7.81–7.78 (m, 2 H, ArH ortho to $(RO)_2CPhCO$), 7.66 (d, J = 8.3 Hz, 2 H, ArH meta to COPh), 7.61–7.58 (m, 1 H, ArH para to $(RO)_2CPhCO$), 7.48 (dd, J = 7.8, 7.4 Hz, 2 H, ArH meta to $(RO)_2CPhCO$), 5.67 (s, 1 H, 4-H), 5.27 (d, J = 5.18, 1 H, 16β-H), 2.35 (s, 3 H, 21-CH_3), 1.50 (s, 3 H, ketal CH_3), 1.14 (s, 3 H, 10-CH_3), 0.67 (s, 3 H, 18-CH_3); MS (FAB) m/z 553 (M + 1), 309; HRMS calcd for $C_{36}H_{41}O_5$ 553.2954, found 553.2963.

16α,17α-[(*R*)-1′-(4-Fluorophenyl(ethylidenedioxy)]pregn-4-ene-3,20-dione (10). Ketalization of diol 1 (0.037 g, 0.085 mmol) with 4′-fluoroacetophenone (1 mL) under kinetic conditions afforded progestin ketal 10 as a white powder (0.031 g, 78%): mp 109–111 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, J = 8.6 Hz, 1 H, ArH ortho to F), 7.50 (d, J = 8.7 Hz, 1 H, ArH ortho to F), 7.04 (d, J = 8.7 Hz, 1 H, ArH meta to F), 7.02 (d, J = 8.6 Hz, 1 H, ArH meta to F), 5.68, (s, 1 H, 4 CH), 5.22 (d, J = 5.2 Hz, 1 H, 16β-H), 2.32 (s, 3 H, 21-CH_3), 1.45 (s, 3 H, ketal CH_3), 1.14 (s, 3 H, 19-CH_3), 0.65 (s, 3 H, 18-CH_3); ^{19}F NMR (400 MHz, $CDCl_3$) φ −113.3 (tt, J = 8.7, 5.5 Hz, 1 F, ArF). IR ($CDCl_3$) ν 2949, 1709, 1663, 1510, 1435, 1360, 1225, 1157, 1082, 925, 914, 837, 762, 746, 719, 646 cm^{-1}; EIMS (70 eV) m/z 466 (2), 451 (9), 423 (100), 328 (20), 285 (21), 267 (8), 230 (17), 139 (21), 123 (25), 44 (76); HRMS (EI) calcd for $C_{29}H_{35}O_4F$ 466.2519, found 466.2502. Anal. ($C_{29}H_{35}O_4F$) C, H, F.

16α,17α-[(*S*)-1′-(4-Fluorophenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (11). Ketalization of diol 1 (0.020 g, 0.058 mmol) with 4′-fluoroacetophenone (1 mL) under thermodynamic conditions afforded a 3:1 mixture of progestin ketals 11 and 10 (0.020 g, 74%). Purification of preparative HPLC [Whatman M-9, 85% hexane/15% (5% i-PrOH–CH_2Cl_2), 4 mL/min] afforded pure 11 (0.015 g, 56%): mp 209–211 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 5.4 Hz, 2 H, ArH ortho to F) 7.32 (d, J = 5.4 Hz, 2 H, ArH meta to F) 5.76 (s, 1 H, 4-H) 5.07 (d, J = 5.4 Hz, 1 H 16β-H) 1.82 (s, 3 H, 21-CH_3) 1.68 (s, 3 H, ketal CH_3) 1.19 (s, 3 H, 19-CH_3) 0.57 (s, 3 H, 18-CH_3); ^{19}F NMR (400 MHz, $CDCl_3$) φ −112.8 (tt, J = 8.7 Hz, 5.5 Hz, 1 F, ArF); MS (FAB) 467 (M + 1), 329; HRMS calcd for $C_{29}H_{36}O_4F$ 467.2598, found 467.2599. Anal. ($C_{29}H_{35}O_4F$) C, H.

16α,17α-[(*R*)-1′-(2′,3′,4′+,5′,6′-Pentafluorophenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (12). Ketalization of diol 1 (0.030 g, 0.087 mmol) with 2′,3′,4′,5′,6′-pentafluoroacetophenone (1 mL) under kinetic conditions afforded progestin ketal 12 as a glass (0.003 g, 7.4%): ^{1}H NMR ($CDCl_3$) δ 5.70 (s, 1 H, 4-H), 5.23 (d, J = 5.7, 1 H, 16β-H), 2.32 (s, 3 H, 21-CH_3), 1.54 (s, 3 H, ketal CH_3), 1.15 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3); MS (FAB) m/z 539 (M + 1), 387, 313; HRMS (FAB) calcd for $C_{29}H_{32}F_5O$ 539.2221, found 539.2232.

16α,17α-[(*R*)-1′-(4-Iodophenyl)(ethylenedioxy)]pregn-4-ene-3,20-dione (13). Ketalization of diol 1 (0.025 g, 0.072 mmol) with 4′-iodoacetophenone (0.400 g, 1.63 mmol) provided the progestin ketal 13 as a white solid (0.058 g, 81%): mp 109–112 °C; ^{1}H NMR ($CDCl_3$) δ 7.69 (dd, J = 8.3, 1.4 Hz, 2 H, ArH ortho to I) 7.27 (dd, J = 8.3, 1.4 Hz, 2 H, ArH meta to I) 5.69 (s, 1 H, 4-H), 5.21 (d, J = 6.2 Hz, 1 H, 16β-H) 2.31 (s, 3 H, 21-CH_3) 1.42 (s, 3 H, ketal CH_3) 1.14 (s, 3 H, 19-CH_3) 0.65 (s, 3 H, 18-CH_3); MS (FAB) 575 (M + 1), 449; HRMS calcd for $C_{29}H_{36}IO_4$ 575.1658, found 575.1644. Anal. ($C_{29}H_{35}IO_4$) C, H, I.

16α,17α-[(*R*)-1′-(4-Aminophenyl)(ethylidenedioxy)]pregn-4-ene-3,20-dione (14). To a solution of 6 (0.022 g, 0.045 mmol) in MeOH (10 mL) at 0 °C was added $SnCl_2$ (0.013 g, 0.067 mmol) in 2 mL of MeOH. The solution was allowed to warm to room temperature and stirred for 1 h. Product isolation (H_2O, EtOAc, brine, $MgSO_4$) followed by flash chromatography (1.5:1 hexanes/ethyl acetate) afforded 14 as a white foam (0.020 g, 96%): ^{1}H NMR ($CDCl_3$) δ 7.38–7.72 (m, 4 H, ArH), 5.77 (s, 1 H, 4-H), 5.22 (d, J = 5.1 Hz, 1 H, 16β-H), 2.21 (s, 3 H, 21-CH_3), 1.47 (s, 3 H, ketal CH_3), 1.29 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3); MS (FAB) m/z 464 (M + 1), 448, 383, 269; HRMS calcd for $C_{29}H_{38}O_4N$ 464.2781, found 464.2791.

16α,17α-[(*R*)-1′-(4-Isothiocyanatophenyl)(ethylenedioxy)]-pregn-4-ene-3,20-dione (15). Thiophosgene (0.007 g, 0.060 mmol), dissolved in 2 mL of $CHCl_3$, was added dropwise to the progestin 14 (0.020 g, 0.043 mmol) in 5 mL of $CHCl_3$ and 0.030 mL of triethylamine. After 1 h of stirring at 23 °C, product isolation (H_2O, $CHCl_3$, $MgSO_4$) followed by flash chromatography (2:1 hexanes/EtOAc) afforded 15 as a white solid (0.017 g, 78%): mp 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (d, J = 8.4 Hz, 2 H, ArH ortho to NCS), 7.22 (d, J = 8.4 Hz, 2 H, ArH meta to NCS), 5.69 (s, 1 H, 4 CH), 5.23 (d, J = 5.5 Hz, 1 H, 16β-H), 2.32 (s, 3 H, 21-CH_3), 1.43 (s, 3 H, 2′ CH_3), 1.14 (s, 3 H, 19-CH_3), 0.65 (s, 3 H, 18 CH_3); IR ($CDCl_3$) ν 2980, 2251, 1709, 1415, 1361, 972, 891, 833 cm^{-1}; MS (FAB) m/z 506 (M + 1); HRMS (FAB) calcd for $C_{30}H_{36}NO_4S$ 506.2365, found 506.2377. Anal. ($C_{30}H_{35}NOS$) C, H, N.

Progestin 16α,17α-ketals 16–19 were prepared according to Fried's procedure.[8] Spectroscopic characterization not provided in the literature are reported here.

16α,17α-[(*R*)-1′-phenyl(ethylenedioxy)]pregn-4-ene-3,20-dione (16): yield 73%; mp 149–152 °C (lit.[8a] mp 150–152 °C); ^{1}H NMR ($CDCl_3$) δ 7.35 (d, J = 7.3 Hz, 2 H, ArH ortho to ketal), 7.38–7.34 (m, 3 H, ArH meta and para to ketal), 5.67 (s, 1 H, 4-H), 5.23 (d, 1 H, J = 5.0 Hz, 16β-H), 2.33 (s, 3 H, 21-CH_3), 1.48 (s, 3 H, ketal CH_3), 1.13 (s, 3 H, 19-CH_3), 0.65 (s, 3 H, 18-CH_3); MS (EI) m/z 448 (M^+, 2), 433 (10), 405 (100), 328 (20), 285 (22), 230 (16); HRMS calcd for $C_{29}H_{36}O_4$ 448.2614, found 448.2637.

16α,17α-[(*S*)-1′-Phenyl(ethylenedioxy)]pregn-4-ene-3,20-dione (17): yield 40% after HPLC separation [Whatman M-9, 85% hexane/15% (5% i-PrOH–CH_2Cl_2), 4 mL/min] from the *endo*-phenyl diastereomer; mp 229–232 °C (lit.[8a] mp 231–232 °C); ^{1}H NMR ($CDCl_3$) δ 7.38–7.26 (m, 5 H, ArH), 5.76 (s, 1 H, 4-H), 5.30 (d, 1 H, J = 5.0 Hz, 16β-H), 1.80 (s, 3 H, 21-CH_3), 1.71 (s, 3 H, ketal CH_3), 1.19 (s, 3 H, 19-CH_3), 0.57 (s, 3 H, 18-CH_3); MS (EI) m/z 448 (M^+, 2), 419 (3), 405 (100), 391 (7), 285 (22), 267 (18), 257 (15), 243 (13), 230 (16), 121 (90); HRMS calcd for $C_{29}H_{36}O_4$ 448.2614, found: 448.2615.

16α,17α-[1′-Isopropylidenedioxy]pregn-4-ene-3,20-dione (18): yield 81%, mp 205–208 °C (lit.[8a] mp 208–209 °C); ^{1}H NMR ($CDCl_3$) δ 5.72 (s, 1 H, 4-H), 4.99 (d, J = 5.3 Hz, 1 H, 16β-H), 2.21 (s, 3 H, 21-CH_3), 1.45 (s, 3-H, ketal CH_3 endo), 1.17 (s, 3 H, ketal CH_3 exo), 1.15 (s, 3 H, 19-CH_3), 0.620 (s, 3 H, 18-CH_3); MS (FAB) m/z 387 (M + 1); HRMS calcd for $C_{24}H_{35}O_4$ 387.2535, found 387.2521.

16α,17α-[(*R*)-1′-α-Furyl(ethylidenedioxy)]pregn-4-ene-3,-20-dione (19): yield 41% after reverse-phase HPLC separation (3:1 CH_3CN/H_2O) from *exo*-furyl diastereomer: mp 220–222 °C (lit.[8a] mp 223–224 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.42 (m, 1 H, furyl C4-H), 6.42 (dd, J = 3.3, 1.8 Hz, 1 H, furyl C2-H), 6.36 (dd, J = 3.3, 1.8 Hz, furyl C3-H), 5.74 (s, 1 H, 4-H), 5.19 (d, J = 5.7 Hz, 1 H, 16β-H), 2.28 (s, 3 H, 21-CH_3), 1.54 (s, 3 H, ketal CH_3), 1.18 (s, 3 H, 19-CH_3), 0.67 (s, 3 H, 18-CH_3); EIMS (70 eV) 438 (M^+, 1), 423 (10), 395 (100), 328 (31), 311 (8), 285 (72), 267 (22), 257 (17), 230 (32), 149 (36), 111 (100). HRMS (EI) calcd for $C_{27}H_{34}O_5$ 438.2406, found 438.2408.

4′-Amino-3′-iodoacetophenone (20). Iodine monochloride (2.52 g, 15.5 mmol) was added to a solution of 4′-aminoacetophenone (2.00 g, 14.8 mmol) in 30 mL of 6 M HCl. After stirring for 1 h, the solution was neutralized with iced 6.0 N NaOH. Product isolation (EtOAc, brine, $MgSO_4$) followed by flash chromatography (2:1 hexanes/EtOAc) provided **20** as a yellow solid (2.70 g, 70%): mp 50–52 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (d, J = 1.9 Hz, 1 H, ArH ortho to I), 7.76 (dd, J = 8.2, 1.9 Hz, 1 H, ArH para to I), 6.71 (d, J = 8.5, 1 H, ArH meta to I), 2.49 (s, 3 H, CH_3); MS (70 eV) m/z 261 (M^+, 68), 246 (100), 218 (12), 119 (18), 105 (6), 91 (57), 77 (12), 63 (35), 52 (25), 43 (33); HRMS calcd for C_8H_8INO 260.9651, found 260.9651. Anal. (C_8H_8INDO) C, H, I, N.

4′-Azido-3′-iodoacetophenone (21). Sodium nitrite (0.57 g, 8.23 mmol) was added as a solid to a 30-mL solution of 4′-amino-3′-iodoacetophenone (1.07 g, 4.11 mmol) in trifluoroacetic acid at 0 °C. After 45 min of stirring, NaN_3 (2.67, 41.1 mmol) was added slowly, not allowing the temperature to exceed 5 °C. An aliquot of ether was added (60 mL), and the reaction was allowed to stir in the dark for 2 h. The reaction mixture was filuted with 120 mL of H_2O and product isolation (Et_2O, H_2O, brine, $MgSO_4$) followed by the removal of CF_3CO_2H as an azeotrope with benzene gave the crude product as a tan solid. Purification by flash chromatography (5:1 hexanes/EtOAc) afforded a white solid (0.93 g, 79%): mp 61–62 °C; ^{1}H NMR ($CDCl_3$) δ 8.36 (d, J = 2.0 Hz, 1 H, ArH ortho to I), 7.97 (dd, J = 8.4, 2.0 Hz, 1 H, ArH para to I), 7.18 (d, J = 8.4 Hz, 1 H, ArH meta to I), 2.56 (s, 3 H, CH_3); MS (70 eV) m/z 287 (M^+, 5), 259 (24), 217 (5), 132 (4), 117 (4), 104 (3), 89 (8), 77 (8), 62 (10), 43 (100); HRMS calcd for $C_8H_6IN_3O$ 286.9556, found 286.9556. Anal. ($C_8H_6IN_3O$) C, H, I, N.

16α,17α-[(*R*)-1′-(4-Azido-3-iodophenyl)(ethylenedioxy)]-pregn-4-ene-3,20-dione (22). Ketalization of diol **1** (0.020 g, 0.058 mmol) with **21** (0.200 g, 0.697 mmol) under kinetic reaction conditions afforded a 2.5:1 mixture of the kinetic:thermodynamic diastereomers progestin ketal **22** (0.027 g, 75%). Purification by preparative HPLC [Whatman M-9, 85% hexane/15% (5% *i*-PrOH-CH_2Cl_2), 4 mL/min] afforded pure **22** as a white solid (0.018 g, 50%): ^{1}H NMR ($CDCl_3$) δ 7.96 (d, J = 1.8 Hz, 1 H, ArH ortho to I), 7.55 (dd, J = 8.2, 1.8 Hz, 1 H, ArH para to I), 7.11 (d, J = 8.2 Hz, 1 H, ArH meta to I), 5.70 (s, 1 H, 4-H), 5.21 (d, J = 5.2 Hz, 1 H, 16β-H), 2.31 (s, 3 H, 21-CH_3), 1.43 (s, 3 H, ketal CH_3), 1.14 (s, 3 H, 19-CH_3), 0.66 (s, 3 H, 18-CH_3); MS (FAB) m/z 616 (M + 1), 588; HRMS calcd for $C_{29}H_{35}IN_3O_4$ 616.1672, found 616.1657.

Biological Procedures. Relative Binding Affinity (RBA) Determinations. Relative binding affinities of the progestin ketals were determined for several receptor systems using competitive radiometric binding assays. Measurements were carried out according to procedures reported in previous publications: progesterone receptor (PgR),[25] mineralocorticoid receptor (MR),[4i] and glucocorticoid receptor (GR).[4i] Tritium-labeled standards for the RBA measurements were R5020 (K_d = 0.4 nM), aldosterone (K_d = 3.9 nM), and RU 28362 (K_d = 11 nM) for PgR, MR, and GR, respectively. By definition, the standards have RBA values of 100.

Photolysis and Inactivation Assay. Photolysis was routinely carried out at >315 nm [450-W mercury vapor lamp, Hanovia L679A, surrounded by a solution filter of saturated aqueous copper(II) sulfate at 2–4 °C employing Pyrex reaction vessels] as previously described.[21] Photolysis was also carried out at 254 nm in a Rayonet reactor utilizing four 8-W germicidal bulbs at 0–4 °C as previously described.[21] Covalent binding of photolyzed ligands was estimated by a modification of the photolysis-exchange assay previously described for the estrogen receptor.[21]

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