Fluorine-18-Labeled Progestin Ketals: Synthesis and Target Tissue Uptake Selectivity of Potential Imaging Agents for Receptor-Positive Breast Tumors

Monica J. Kochanny,† Henry F. VanBrocklin,†,‡ Philip R. Kym,† Kathryn E. Carlson,† James P. O'Neil,† Thomas A. Bonasera,§ Michael J. Welch,§ and John A. Katzenellenbogen*,†

Department of Chemistry, University of Illinois, 1209 West California Street Urbana, Illinois 61801, and Division of Radiation Sciences, Mallinckrodt Institute of Radiology, Washington University Medical School, 510 South Kingshighway, St. Louis, Missouri 63110

Received November 4, 1992

We have studied two new fluorine-substituted progestins as potential imaging agents for progesterone-receptor-positive human breast tumors. The steroids are 16α,17α-fluoroacetophenone ketals of 16α,17α-dihydroxyprogesterone and 16α,17α,21-trihydroxy-19-norprogesterone. Synthesis of the latter compound in seven steps from 19-norandrost-4-ene-3,17-dione is reported. Both compounds demonstrate high affinity for the progesterone receptor (PgR) (52.5 and 240%, respectively, relative to R5020 = 100). The syntheses were adapted to ^{18}F-labeling with 4′-[^{18}F]-fluoroacetophenone, prepared from 4′-nitroacetophenone by nucleophilic substitution with K^{18}F/Kryptofix. Considerable adjustment of reaction conditions was required to effect ketalization using tracer quantities of the ketone. In tissue distribution studies in estrogen-primed immature female rats, both ketals showed selective uterine uptake, which was blocked by coinjection of a saturating dose of the unlabeled progestin ORG 2058. Additionally, metabolic stability of the radiolabel was indicated by the low radioactivity levels seen in bone. Both compounds showed relatively high uptake in fat, in accord with their relative lipophilicities demonstrated by HPLC-derived octanol–water partition coefficients. The selective uterine uptake and metabolic stability of these compounds suggests that this class of PgR ligands might be promising for the selective imaging of receptor-positive tumors if derivatives of reduced lipophilicity can be prepared.

Introduction

The presence of estrogen and progestin receptors in some breast tumors provides the possibility for in vivo imaging of tumors via the selective uptake of suitably labeled steroid ligands.[1] Development of such imaging agents has focused largely on estrogen receptors. Successful imaging via positron emission tomography (PET) of both primary and metastatic breast tumors in humans has been accomplished using the estrogen receptor ligand 16α-[^{18}F]fluoroestradiol.[2] In principal, a progestin-based imaging agent may be better suited to breast tumor imaging than an estrogen-based imaging agent. Progesterone receptor (PgR) titer in the tumor is usually indicative of a functioning estrogen receptor system, and thus serves as a positive predictive indicator of responsiveness to hormonal therapy.[3] Hormonal therapy involving antiestrogen treatment (e.g., tamoxifen) results in full occupation of estrogen receptors,[4] rendering estrogen-receptor-based imaging impossible. In contrast, progesterone receptors in such patients would remain unoccupied, and receptor levels may actually be elevated in response to hormonal treatment.[5]

Of the positron-emitting progestins prepared to date, the most successful candidate has been 21-[^{18}F]fluoro-16α-ethyl-19-norprogesterone ([^{18}F]FENP, 1),[6,7] a fluorinated analog of the synthetic high-affinity progestin 21-hydroxy-16α-ethyl-19-norprogesterone (ORG 2058, 2) (Figure 1). [^{18}F]FENP showed favorable uptake selectivity and retention in target tissues (uterus, ovaries) in immature female rats, and was evaluated as an imaging agent for progesterone receptor-positive breast tumors in humans.[8] In contrast to the favorable distribution in rats, the human studies were characterized by poor image quality (low target to background ratio) and poor correlation of uptake with tumor progesterone receptor levels. The principal problems associated with [^{18}F]FENP were found to be lipophilicity and susceptibility of the C-21 position to metabolic scission of the radiolabel. The high lipophilicity resulted in elevated uptake in adipose tissue in rat studies and increased liver uptake leading to greater metabolite production in human studies. We sought to address the issues of lipophilicity and metabolic stability in the development of new fluorinated PgR ligands.

In the course of our investigations into photoaffinity labeling reagents for progesterone receptor, our attention was drawn to a series of ketals of 16α,17α-dihydroxyprogesterone first prepared by Fried.[9] In particular, 16α,-17α-dihydroxyprogesterone acetophenide (3) was reported to be twice as potent as progesterone in parenteral activity[10] (Figure 1). The aromatic ring of 3 provides an attractive site for radiolabel introduction, as literature precedent suggested that the aryl carbon–fluorine may be metabolically stable.[11] Accordingly, as is described in an accompanying paper,[12] we prepared the 4′-fluoroacetophenide 4 of dihydroxyprogesterone and found it to bind well to PgR (vide infra). We report herein the preparation of this compound in fluorine-18-labeled form and its biodistribution in estrogen-primed rats.

While progesterone ketal 4 appeared to be a promising candidate in terms of metabolic stability, we sought to improve upon the binding and lipophilicity characteristics of this class of PgR ligand. In this regard, we chose to introduce the ketal moiety onto the 21-hydroxy-19-norprogesterone backbone of ORG 2058. We hypothesized

* Address correspondence to John A. Katzenellenbogen, Department of Chemistry, University of Illinois, Roger Adams Laboratory, Box 37, 1209 W. California St., Urbana, IL 61801.
† University of Illinois.
‡ Current address: Center for Functional Imaging, Lawrence Berkeley Laboratory, University of California, 1 Cyclotron Rd. MS 55/121, Berkeley, CA 94720.
§ Washington University Medical School.

0022-2623/93/1836-1120$04.00/0

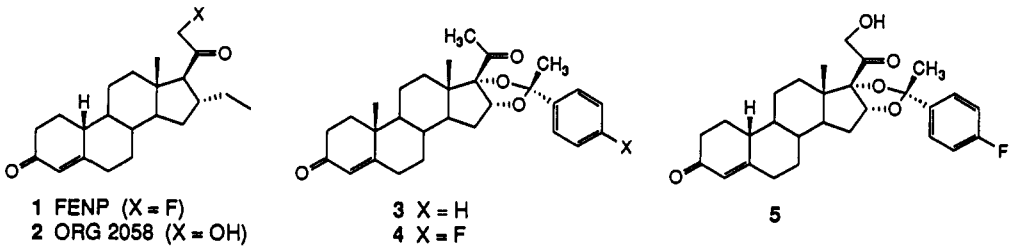
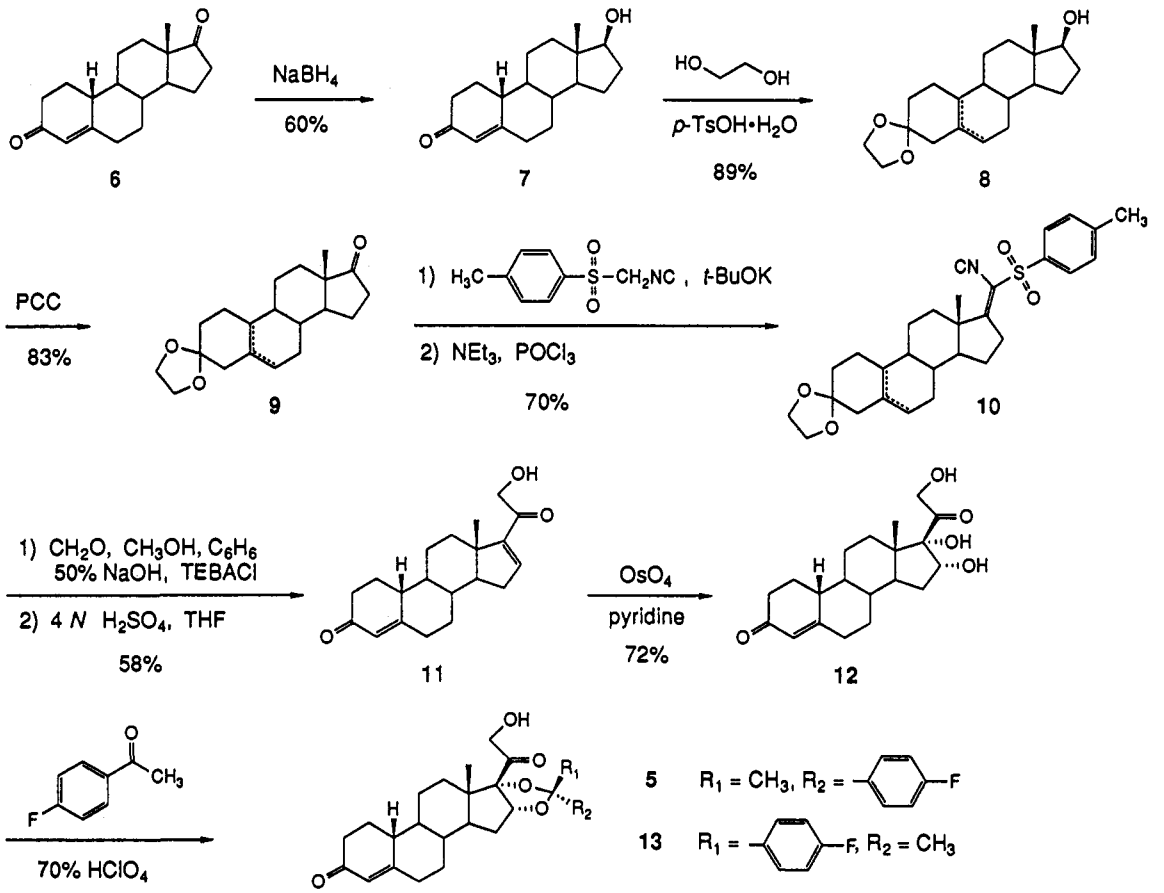


Figure 1. Structures of progestins and fluorinated progestins.

Scheme I



that the 19-norprogesterone backbone would be favorable in terms of binding affinity, while the presence of the 21-hydroxyl group would provide a less lipophilic compound. Thus, herein we also report the synthesis of hydroxynorprogesterone fluoroacetophenide **5**, as well as its preparation in fluorine-18-labeled form and its biological distribution. To our knowledge, the syntheses of [^{18}F]**4** and [^{18}F]**5** represent the first reported examples of ketal formation using a fluorine-18-labeled ketone.

Results and Discussion

Synthesis of Hydroxynorprogesterone Fluoroacetophenides. Synthesis of trihydroxynorprogesterone **12** (Scheme I), the immediate precursor to ketal formation, from readily available starting materials requires the introduction of a hydroxyacetyl side chain as well as a 16,17-double bond. While formation of the corresponding 17-acetyl-Δ^{16}-functionality from 17-oxo steroids is well precedented,[13] few direct methods exist for hydroxyacetyl introduction. Especially suited to this purpose is the method of van Leusen[14] for direct conversion of 17-oxo steroids to 16-dehydro-21-hydroxy-20-keto steroids.

Reduction of 19-norandrostenedione **6** with $NaBH_4$ in methanol afforded 17β-hydroxy-19-nortestosterone (**7**) selectively. Reduction occurs exclusively from the α-face due to hindrance of β-face attack by the angular 18-methyl group. Use of a slight excess of hydride (1.08 equiv) affords reduction only at C-17, with ca. 25% recovery of unreacted ketone **6**. Addition of sufficient $NaBH_4$ to effect complete consumption of starting material resulted in competing reduction at C-3 with no improvement in the isolated yield of nortestosterone (**7**), a result which has also been observed in the reduction of androst-4-ene-3,17-dione to testosterone.[15] Ethylene glycol ketalization provided a mixture of the Δ^5- and $\Delta^{5(10)}$-dioxolanes **8**, which was used without separation in subsequent reactions. Oxidation of the 17-hydroxy group with pyridinium chlorochromate afforded the A-ring-protected ketones **9** required for side-chain introduction.[16]

Treatment of the ketones with the anion of tosylmethyl isocyanide[14] and dehydration of the intermediate formamides with NEt_3 and $POCl_3$ gave the 17-(isocyanosulfonylmethylene) steroids **10**. Reaction of **10** with formaldehyde, methanol, and concentrated base under phase-transfer conditions, followed by acid hydrolysis of the intermediate alkoxyoxazoline with concomitant A-ring deprotection, gave the desired 21-hydroxy-16-dehydro-

Table I. Progestin Receptor Binding Affinities and Lipophilicity Data

compd	relative binding affinity[a] PgR (R5020 = 100)	MR (aldosterone = 100)	GR (RU28362 = 100)	log P_o/w[b]
progesterone	13	not assayed	0.56	3.869 (3.91)[c]
R5020	100	0.93	1.5	4.050 (4.04)[c]
ketal 4	53	6.0	9.4	5.705
ketal 5 (*endo*-phenyl)	240	140	47	4.915
ketal 13 (*exo*-phenyl)	1.3[d]	not assayed	not assayed	4.837
ORG 2058	200	0.92	1.0	4.015
FENP	700	3.2	0.74	4.656

[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). [b] Log of octanol/water partition coefficient, measured by the method of Minick et al., ref 29. [c] log *P* values measured by the shake flask method. [d] RBA value for a single determination. Normal dose 47 μCi of [^{18}F]**5** in 0.1 mL of 10% ethanol/saline; "low dose", 17 μCi in 0.04 mL; for "blocked" animals, 18 μg of ORG 2058 was added to each dose of [^{18}F]**5** in an injection volume of 0.2 mL. Specific activity of [^{18}F]**5** = 409 Ci/mmol.

19-norprogesterone (**11**). Oxidation of the 16,17-double bond of **11** was effected using a modification of published procedures.[17,18] Thus, treatment of **11** in pyridine with 1 equiv of OsO_4 cleanly afforded the osmate ester. The pyridine was distilled off in vacuo and the crude osmate dissolved in ethyl acetate and treated with aqueous sodium bisulfite to afford triol **12** as the sole product in 72% yield. Failure to remove the pyridine before addition of ethyl acetate and bisulfite resulted in slower cleavage of the ester and a lower yield of **12** (ca. 55%), while cleavage of the osmate with bisulfite in aqueous pyridine with no added organic solvent resulted in complete loss of steroid upon organic extraction.[19]

There are several points which should be noted with respect to this synthesis. The reduction and reoxidation steps could be avoided by selective protection of the enone moiety of **6** as either the 3,5-dienol methyl ether or the 3,3-dithiolane, both of which are compatible with the method used for side-chain introduction. However, van Leusen has reported that the dienol ether analog of **10** gave a low yield of the 21-hydroxy steroid **11**.[14c] In our hands, the Δ^4-ethylenedithio analog of **9** gave yields comparable to the dioxolane for side-chain incorporation. However, cleavage of the dithiolane under a wide variety of conditions gave unsatisfactory yields of compound **11** (ca. 45%). As we experienced earlier in the preparation of 16α,17α-dihydroxyprogesterone,[12] attempts to effect catalytic OsO_4 oxidation[20] of the 16,17-double bond were unsuccessful, either returning starting material unchanged or resulting in decomposition. Similarly, potassium permanganate oxidation[21] (performed on the 21-acetate of **11**) afforded the desired diol, however, yields from this reaction also were low (ca. 20%) and proved inconsistent.

Fluoroacetophenone ketals **5** and **13** were prepared from triol **12** according to the ketalization procedure of Fried.[9] Thus, treatment of the triol in 4′-fluoroacetophenone with 70% aqueous $HClO_4$ (0.05 M acid concentration) at ambient temperature for 1 h afforded an 86% yield of the *endo*-phenyl ketal **5** as the sole reaction product. When the reaction was run at higher acid concentration (1 M) for 12 h, a mixture of **5** and the *exo*-phenyl ketal **13** was obtained in a 1:3 *endo*-phenyl:*exo*-phenyl ratio (65% yield of **5** + **13**). Thin-layer chromatographic analysis during the course of reaction showed initial rapid consumption of starting material to form the kinetic ketal **5**, followed by slow isomerization of **5** to the thermodynamic *exo*-phenyl ketal **13**. As we described in the progesterone series,[12] the isomerization can also be observed by ^{1}H NMR or ^{19}F NMR. The two isomers are readily distinguishable by the chemical shifts of the C-21 protons which are shifted upfield by 0.5 ppm for isomer **13** relative to **5** due to shielding by the aromatic ring.[22]

Receptor Binding Affinity. The binding affinities of fluoroacetophenone ketals **4**, **5**, and **13** (along with several progestins selected for comparison) for rat uterine progesterone receptor are given in Table I. The near 5-fold higher affinity for PgR of ketal **5** vs **4** reflects the favorable binding properties of the 19-norprogesterone skeleton; compound **5** has an affinity comparable to that of ORG 2058. In contrast, the *exo*-phenyl isomer **13** shows a drastic reduction in binding affinity. This result is consistent with the observation of Fried that *exo*-phenyl-substituted ketals were invariably of lower biological activity than their *endo*-phenyl counterparts[9] and with our own experience with some epimeric ketals in the progesterone series.[12] While neither ketal **4** nor **5** demonstrates an affinity for PgR as high as that of FENP, both ketals were deemed to be of interest for preparation in ^{18}F-labeled form on the basis of the potential gain in metabolic stability of the *aryl* ^{18}F-label compared to the known metabolic lability of the *aliphatic* ^{18}F-label of [^{18}F]FENP.[6]

Binding affinities of the two *endo*-phenyl ketals, **4** and **5**, were also determined for rat mineralocorticoid receptor (MR) and rat glucocorticoid receptor (GR), as many progestins show substantial heterologous binding to these receptors. Ketal **5** shows rather poor binding selectivity for PgR, demonstrating high affinity for both MR (142% that of aldosterone) and GR (46.8% that of RU 28362). The introduction of a 21-hydroxyl moiety (ketal **5** vs ketal **4**) causes a substantial increase in affinity for MR and GR, in accord with the known selectivity of these receptors for steroids having the Δ^4-3,20-diketo-21-hydroxy substructure.[23] The binding of ketal **5** to GR is consistent with the demonstrated high affinity of this receptor for triamcinolone acetonide and fluocinolone acetonide, both of which bear 16α,17α-ketal substituents.[24]

Synthesis of 4′-[^{18}F]Fluoroacetophenides. 4′-Fluoroacetophenone was prepared in ^{18}F-labeled form from 4′-nitroacetophenone by nucleophilic aromatic substitution using K^{18}F/Kryptofix 222.[25] Excess 4′-nitroacetophenone was separated from the 4′-[^{18}F]fluoroacetophenone on a silica column eluted with 20% ether/pentane, providing the labeled ketone in 65–70% radiochemical yield. Adaptation of the ketalization reaction to use tracer levels of ketone required substantial modification of conditions. In contrast to the synthesis of an unlabeled fluoroacetophenone ketal, performed using a 10–100-fold excess of ketone, the radiochemical synthesis contains only

ca. 10^{-7} equiv of the labeled ketone. Reaction conditions were developed for the radiolabeling of ketals **4** and **5** as described below. Reaction progress was monitored and yields estimated using radioactive thin-layer chromatographic (radio-TLC) analysis.

Investigation of a number of reaction solvents indicated that the ketalization proceeded most readily in a chlorinated solvent. Since a solution of 4′-[^{18}F]fluoroacetophenone in 20% ether/pentane is obtained from the chromatographic purification, solvent mixtures were also investigated. Yields of the 4′-[^{18}F]fluoroacetophenides were found to be comparable in either 100% chlorinated solvent or a 50:50 mixture of a chlorinated solvent and 20% ether/pentane; the latter solvent mixture was selected in order to facilitate the optimization of the radiochemical reactions.

A reaction volume of 100–500 μL gave optimal incorporation yields, requiring concentration of the column eluent (2–5 mL total volume) to obtain sufficient radioactivity for biodistribution studies. However, evaporation of the solvent resulted in the near complete volatilization of the 4′-[^{18}F]fluoroacetophenone. Addition of the steroid substrate (ca. 1 mg) to the ether/pentane solution just prior to solvent evaporation overcame this problem. The pseudocarrier effect of the steroid gave retention of nearly 70% of the ^{18}F-labeled ketone upon solvent evaporation.

The ketalization was promoted by the addition of 1 μL of 70% aqueous $HClO_4$ per 100 μL of solvent. This quantity of $HClO_4$ gives an acid concentration of 0.12 M, which is similar to the acid concentration used to generate the unlabeled kinetic ketals. Typical ketal yields for a 1 h reaction were 17% and 3% for [^{18}F]**4** and [^{18}F]**5**, respectively. Upon addition of triethyl orthoformate (TEOF) to the reactions, 4 μL (24 μmol) per 1 μL of acid to [^{18}F]**4** and 5 μL (30 μmol) per 1 μL of acid to [^{18}F]**5**, the ketalization yields increased to 57% and 35%, respectively. These quantities of TEOF are sufficient to remove most or all of the 26.5 μmol of water present per 1 μL of 70% aqueous $HClO_4$, thus facilitating ketal formation. Upon addition of excess TEOF (i.e., greater than 5 μL), radio-TLC analysis indicated predominantly the formation of uncharacterized radiolabeled byproducts, thought to arise from the aldol condensation of the 4′-[^{18}F]fluoroacetophenone.

Ketals [^{18}F]**4** and [^{18}F]**5** were synthesized for biodistribution studies according to the procedure described above. After stirring for 30–50 min at room temperature, the reactions were quenched by filtration through a SiO_2 column overlaid with $NaHCO_3$ ([^{18}F]**4**), or by triethylamine addition and filtration through SiO_2 ([^{18}F]**5**). Semipreparative normal phase HPLC purification afforded the desired labeled compounds [^{18}F]**4** and [^{18}F]**5** in 15% and 16% decay corrected yield, respectively, with a total synthesis time of ca. 140 min from the end of bombardment. The effective specific activities[26] of [^{18}F]**4** and [^{18}F]-**5** were 30 and 409 Ci/mmol, respectively, considerably lower than the typical specific activity values of 1500 Ci/mmol seen for FENP.

Tissue-Distribution Studies. Purified 4′-[^{18}F]fluoroacetophenides [^{18}F]**4** and [^{18}F]**5** were reconstituted in 10% ethanol–saline and were injected (iv, tail vein) into estrogen-primed immature female rats. Doses employed were 51 μCi/animal for ketal [^{18}F]**4** and 47 μCi/animal for ketal [^{18}F]**5**. Animals were sacrificed at 1 and 3 h postinjection. In order to ascertain that uptake was mediated by a high-affinity, limited capacity system, one set of animals in each study was coinjected with the radiotracer and with 18 μg of unlabeled ORG 2058, a high-affinity progestin, to saturate the progestin receptors (1 h "blocked" values). As the affinity of ORG 2058 for the mineralocorticoid and glucocorticoid receptors is very low (cf. Table I), its blockage of the uptake demonstrates that their uptake is due to their binding to PgR. One set of animals in each study was injected with a reduced dose of radiotracer (30 μCi for compound [^{18}F]**4** and 17 μCi for compound [^{18}F]**5**, 1 h " low dose" values), to insure that target tissue uptake of the tracers was not limited by binding of unlabeled impurities to PgR. Tissue-distribution results are given in Tables II and III. Table IV presents a comparison of the tissue distribution at 1 h postinjection of ketals [^{18}F]**4** and [^{18}F]**5** with that of [^{18}F]-FENP.

The data in Table II indicate that uterine uptake of [^{18}F]**4** is selective, measuring 3–7 times that in muscle (a nontarget tissue) and blood at 1 h. Uterine activity is lost slowly, decreasing by ca. 33% between 1 and 3 h, although

Table II. Tissue Distribution of ^{18}F Activity after Injection of [^{18}F]**4** into Estrogen-Primed Immature Rats[a]

	percent injected dose[b]/g ± SD,[c] $n = 5$			
	1 h			
tissue	normal dose	low dose	blocked	3 h
uterus	3.08 ± 0.43	3.30 ± 0.44	1.27 ± 0.22	2.13 ± 0.33
ovaries	3.93 ± 0.77	3.68 ± 1.49	2.29 ± 0.39	2.19 ± 0.42
blood	0.47 ± 0.04	0.52 ± 0.08	0.43 ± 0.08	0.28 ± 0.06
muscle	0.82 ± 0.15	0.82 ± 0.14	0.76 ± 0.07	0.49 ± 0.20
lung	1.30 ± 0.35	1.46 ± 0.34	1.26 ± 0.26	0.91 ± 0.22
brain	0.56 ± 0.03	0.57 ± 0.10	0.55 ± 0.05	0.20 ± 0.04
liver	3.41 ± 0.26	3.62 ± 0.53	3.16 ± 0.30	1.93 ± 0.22
kidney	2.18 ± 0.12	2.27 ± 0.30	2.18 ± 0.41	1.47 ± 0.42
fat	5.76 ± 1.35	5.39 ± 1.88	3.77 ± 0.72	5.06 ± 0.99
bone	1.76 ± 0.48	1.77 ± 0.32	1.44 ± 0.40	1.69 ± 0.35
uterus/blood	6.59 ± 0.68	6.34 ± 0.98	3.06 ± 0.74	7.56 ± 0.92
uterus/muscle	3.85 ± 0.74	4.08 ± 0.67	1.69 ± 0.92	4.61 ± 0.98

[a] Female Sprague–Dawley rats (23 days old) were estrogen primed by subcutaneous injection with 5 μg of estradiol in 0.1 mL of 20% ethanol/sunflower oil on two successive days and used on the third day (25 days old), 24 h after the last injection. [b] Normal dose, 51 μCi of [^{18}F]**4** in 0.1 mL of 10% ethanol/silane; "low dose", 30 μCi in 0.04 mL; for "blocked" animals, 18 μg of ORG 2058 was added to each dose of [^{18}F]**4** in an injection volume of 0.2 mL. Specific activity of [^{18}F]**4** = 30 Ci/mmol. [c] SD is standard deviation.

Table III. Tissue Distribution of ^{18}F Activity after Injection of [^{18}F]**5** into Estrogen-Primed Immature Rats[a]

	percent injected dose[b]/g ± SD,[c] $n = 5$			
	1 h			
tissue	normal dose	low dose	blocked	3 h
uterus	3.65 ± 1.11	4.06 ± 0.70	0.86 ± 0.26	3.86 ± 0.99
ovaries	2.80 ± 0.70	2.66 ± 0.42	1.37 ± 0.31	1.78 ± 0.36
blood	0.26 ± 0.02	0.28 ± 0.04	0.23 ± 0.03	0.10 ± 0.02
muscle	0.44 ± 0.04	0.46 ± 0.07	0.35 ± 0.03	0.16 ± 0.09
lung	0.60 ± 0.08	0.72 ± 0.15	0.54 ± 0.05	0.22 ± 0.06
brain	0.34 ± 0.05	0.39 ± 0.07	0.28 ± 0.04	0.06 ± 0.01
liver	4.25 ± 0.39	3.93 ± 0.56	3.56 ± 0.40	1.36 ± 0.22
kidney	1.42 ± 0.08	1.57 ± 0.28	1.28 ± 0.17	0.45 ± 0.10
fat	3.53 ± 1.05	3.78 ± 0.99	2.76 ± 0.39	1.64 ± 0.21
bone	0.62 ± 0.08	0.68 ± 0.14	0.60 ± 0.09	0.64 ± 0.11
uterus/blood	14.0 ± 4.2	14.5 ± 2.1	3.69 ± 1.03	38.0 ± 8.3
uterus/muscle	8.2 ± 2.5	8.8 ± 1.3	2.42 ± 0.62	27.9 ± 9.5

[a] See Table II for animal pretreatment protocol. [b] Normal dose 47 μCi of [^{18}F]**5** in 0.1 mL of 10% ethanol/saline", 17 μCi in 0.04 mL; for "blocked" animals, 18 μg of ORG 2058 was added to each dose of [^{18}F]**5**, in an injection volume of 0.2 mL. Specific activity of [^{18}F]**5** = 409 Ci–mmol. [c] SD is standard deviation.

Table IV. Tissue Distribution Summary for Labeled Progestins[a]

tissue	% injected dose/g ± SD[b] at 1 h		
	[18F]4	[18F]5	[18F]FENP
uterus	3.08 ± 0.43	3.65 ± 1.11	6.43 ± 1.53
ovaries	3.93 ± 0.77	2.80 ± 0.70	2.86 ± 0.78
blood	0.47 ± 0.04	0.26 ± 0.02	0.25 ± 0.07
muscle	0.82 ± 0.15	0.44 ± 0.04	0.42 ± 0.10
lung	1.30 ± 0.35	0.60 ± 0.08	0.47 ± 0.20
brain	0.56 ± 0.03	0.34 ± 0.05	0.63 ± 0.16
liver	3.41 ± 0.26	4.25 ± 0.39	3.34 ± 0.90
kidney	2.18 ± 0.12	1.42 ± 0.08	1.05 ± 0.22
fat	5.76 ± 1.35	3.53 ± 1.05	3.56 ± 0.66
bone	1.76 ± 0.48	0.62 ± 0.08	3.18 ± 0.76
uterus/blood	6.59 ± 0.68	14.0 ± 4.2	26.26 ± 6.58
uterus/muscle	3.85 ± 0.74	8.2 ± 2.5	15.98 ± 5.51

[a] Data from Tables II and III and from ref 6. [b] SD is standard deviation.

a more rapid clearance from blood and muscle causes the ratios to rise slightly. Comparable uptake is observed at 1 h for both the normal and low doses, indicating that the low specific activity of this sample is not a limiting factor in the distribution of [18F]4. Uterine uptake is lowered by ca. 60% in the animals receiving the blocking dose, indicating receptor-mediated distribution. High uptake in liver and kidney is consistent with known routes of metabolism and excretion of steroids.[27]

A significant feature of the distribution of ketal [18F]4 is the relatively low level of radioactivity in bone and the notable lack of increase of bone activity between 1 and 3 h. In comparison, bone activity for [18F]FENP increased from 3.2% injected dose/g at 1 h to 5.5% by 3 h.[6] A dramatic improvement in metabolic stability has thus been attained by placement of the 18F label on an aromatic ring. Considerable uptake is observed, however, in fat. Fat uptake is observed with lipophilic compounds, and is significantly higher ($0.01 < p < 0.02$) for compound [18F]4 than for [18F]FENP (cf. Table IV). This is consistent with the higher lipophilicity measured for ketal 4 (vide infra).

Tissue distribution data for [18F]5 (Table III) shows high uterine uptake at 1 h which is well retained at 3 h, with a significant increase in uterus to blood and uterus to muscle ratios between 1 and 3 h. Coinjection of ORG 2058 effectively blocks uterine uptake (76% decrease in uterine activity). Comparison of normal and low doses indicates that the modest specific activity of this sample of [18F]5 does not limit its biodistribution. As with ketal [18F]4, bone activity is low and remains constant through the 3-h study. Uptake in fat, while significantly lower ($0.01 < p < 0.02$) than observed for [18F]4 is still higher than desirable, being comparable to that seen with FENP (cf. Table IV).

Some noteworthy observations can be made by comparing the distribution of compounds [18F]4 and [18F]5 (Table IV). While the uptake in estrogen-primed uterus—a tissue with a high PgR content—is similar for compounds [18F]4 and [118F]5 at 1 h, compound [18F]4 washes out more rapidly, in accord with its lower binding affinity. The second observation concerns the nonspecific (non-receptor-mediated) binding of [18F]4 and [18F]5 in target tissues. The higher activity levels of [18F]4 in fat and muscle suggest a higher degree of nonspecific binding for this compound. Ovarian tissue may have a higher fat content than uterine tissue, possibly causing anomalously high uptake values for highly lipophilic compounds, due to nonspecific binding to fat. In fact, total ovarian uptake of [18F]4 is higher than uterine uptake at 1 h; however, specific uptake (total minus blocked) reflects the lower PgR content of ovarian tissue.[28] Nonspecific binding likely accounts for the fact that blocking of both compounds is less efficient in the ovaries than the uterus.

The relative lipophilicity of a compound, which affects the extent to which binding occurs to low affinity nonspecific sites, can be estimated from its octanol–water partition coefficient.[29] $\log P_{o/w}$ values were measured for ketals 4 and 5, as well as other progestins of interest, according to the method of Minick.[30] The data are shown in Table I. An excellent correlation is found between the $\log P_{o/w}$ values and the degree of fat uptake observed for the radiolabeled compounds. Ketal 4, which showed the highest level of activity in fat, was found to be considerably more lipophilic than the other progestins (higher $\log P_{o/w}$ value). Additionally, ketal 5 and FENP, which show comparable uptake in fat, have similar $\log P_{o/w}$ values.

Development of a successful imaging agent depends on a number of criteria. A compound must demonstrate not only selective receptor-mediated uptake in target tissues but must also have limited uptake in nontarget tissues such as fat and bone, the degree of which is influenced by lipophilicity and metabolic stability of the radiolabel, respectively. The data for compound 5 suggest that any favorable reduction in lipophilicity due to the 21-hydroxyl group and introduction of 16- and 17-oxo substituents is outweighed by the presence of lipophilic alkyl and aryl substituents on the ketal moiety. Compound 5 showed more favorable distribution characteristics than compound 4; however, the lipophilicity of these compounds is still high enough to make them unlikely candidates for PgR-based imaging. Nevertheless, other biodistribution properties (selective, receptor-mediated uterine uptake, dramatic reduction of radiolabel cleavage) indicate that this class of PgR ligands holds potential for the development of a selective imaging agent. The preparation of less lipophilic analogs of ketal 5 is in progress and may result in an improved imaging agent for PET studies of PgR-rich tissues.

Conclusions

16α,17α,21-Trihydroxynorprogesterone 16α,17α-(4-fluoroacetophenide) has been prepared and shows high binding affinity for progesterone receptor. The syntheses of this compound and of 16α,17α-dihydroxyprogesterone 16α,17α-(4-fluoroacetophenide) have been adapted for labeling with fluorine-18. These syntheses represent the first examples of ketal formation using a fluorine-18-labeled ketone. In estrogen-primed immature female rats, the norprogesterone-based ketal shows highly selective target tissue uptake and high metabolic stability of the radiolabel, indicating that analogs of this compound of reduced lipophilicity may hold promise for the selective in vivo imaging of progesterone-receptor-positive tumors of the breast.

Experimental Section

General. Melting points are uncorrected. Flash chromatography was performed according to the method of Still,[30] using Merck silica gel (40–63 μm). Proton magnetic resonance (1H NMR) spectra were obtained at 300 MHz; chemical shifts are reported in ppm downfield from internal tetramethylsilane (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 obtained at 376.3 MHz and chemical shifts are reported in ppm upfield from $CFCl_3$ at 0 ppm (ϕ scale). Only characteristic infrared (IR) bands are reported. High-performance liquid chromatography (HPLC) was performed isocratically using a 5-μm analytical SiO_2 column (4.6 mm × 30 cm, Varian Si-5 Micro-Pak) or a 10-μm preparative SiO_2 column (0.9 cm × 50 cm, Whatman Partisil M-9). Eluant was monitored with a variable-wavelength detector set at 254 nm and a flow-through sodium iodide scintillation detector, where appropriate. Radioactivity was determined with a dose calibrator. Radiochemical yields are corrected for decay.

19-Norandrostenedione was a generous gift from Syntex, or was purchased from Steraloids. THF was distilled from sodium benzophenone ketyl immediately prior to use. CH_2Cl_2, NEt_3, and pyridine were distilled from CaH_2. Other solvents and reagents were used as purchased. Unless otherwise indicated, all reactions were carried out under a nitrogen atmosphere. 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".

The fluoroacetophenone ketal 4 of 16α,17α-dihydroxyprogesterone was prepared as reported in ref 12.

17β-Hydroxy-4-estren-3-one (19-Nortestosterone, 7). A solution of 19-norandrostenedione (6) (4.0 g, 14.7 mmol) in 100 mL of reagent-grade methanol was cooled to 0 °C and treated with $NaBH_4$ (150 mg, 3.96 mmol) for 1 h (air atmosphere). The reaction was quenched by addition of several drops of HOAc and the solution concentrated to dryness in vacuo. Product isolation (H_2O, CH_2Cl_2, $MgSO_4$) followed by flash chromatography (30% EtOAc/hexanes) gave 2.43 g (8.86 mmol, 60%) of alcohol 7 as a white foam and 1.03 g (3.78 mmol, 26%) of unreacted ketone 6. A sample of 7 was recrystallized from ether/petroleum ether: mp 110–112 °C (lit.[32] mp 112 and 124 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (s, 3 H, 18-CH_3), 3.66 (t, J = 8.5 Hz, 1 H, 17α-H), 5.82 (s, 1 H, 4-H); IR (KBr) ν 3409 (OH), 1653 (C=O) cm^{-1}. Anal. ($C_{18}H_{26}O_2$) C, H.

3,3-(Ethylenedioxy)-17β-hydroxy-5-estrene (8). To a solution of alcohol 7 (3.0 g, 10.9 mmol) in benzene (150 mL) were added ethylene glycol (1.8 mL, 32 mmol) and p-toluenesulfonic acid monohydrate (208 mg, 1.1 mmol). The mixture was refluxed for 3 h with azeotropic removal of water (Dean–Stark trap). Product isolation (EtOAc, $NaHCO_3$ (aqueous), Na_2SO_4) followed by flash chromatography (30% EtOAc/hexanes) gave 3.1 g (9.7 mmol, 89%) of ketals 8 as a tacky solid. ^{1}H NMR revealed a 2:1 mixture of $\Delta^{5(10)}$- and Δ^5-3-ethylene ketals based on the ratio of the peaks at δ 5.44 (6-H), 3.95 (ketal), and 3.64 (17α-H). The ketal mixture was used in subsequent reactions. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.72 (s, 18-CH_3, $\Delta^{5(10)}$), 0.74 (s, 18-CH_3, Δ^5), 3.64 (m, 1 H, 17α-H), 3.95 (m, 4 H, ketal), 5.44 (d, J = 5.6 Hz, 6-H, Δ^5), identical with literature values;[33] IR ($CHCl_3$) ν 3447 (OH) cm^{-1}.

3,3-(Ethylenedioxy)-5-estren-17-one (9). A solution of alcohols 8 (2.44 g, 7.66 mmol) in dry CH_2Cl_2 (75 mL) at 0 °C was treated with Celite (2.5 g) and pyridinium chlorochromate (3.3 g, 15.3 mmol). After 30 min, the mixture was warmed to room temperature and an additional 5 g of Celite added. After 2.5 h at room temperature, the mixture was poured into a well-stirred suspension of Celite (17 g) in ether (300 mL) and then filtered and rinsed with ether, and the volume was reduced by half on a rotary evaporator. The organic solution was washed with H_2O until colorless, dried (Na_2SO_4), and evaporated. Flash chromatography (30% EtOAc/hexanes) gave 2.02 g (6.38 mmol, 83%) of ketones 9 as a colorless glass. A sample crystallized from petroleum ether had mp 115–20 °C (lit.[32] mp Δ^5: 157–158 °C; $\Delta^{5(10)}$: 128–130 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (s, 18-CH_3, $\Delta^{5(10)}$), 0.90 (s, 18-CH_3, Δ^5), 3.97 (m, 4 H, ketal), 5.50 (d, J = 5.9, 6-H, Δ^5), identical with literature values;[33] IR (KBr) ν 1740 (C=O) cm^{-1}. Anal. ($C_{20}H_{28}O_3$) C, H.

3,3-(Ethylenedioxy)-17-[isocyano[(*p*-methylphenyl)sulfonyl]methylene]-estr-5-ene (10). Potassium *tert*-butoxide (1.22 g, 11 mmol) was dissolved in 40 mL of dry THF and cooled to −60 °C. A solution of tosylmethyl isocyanide (1.87 g, 9.6 mmol) in 15 mL of THF was added, and the reaction temperature was kept below −50 °C during the addition. Stirring was continued for 20 min. A solution of ketones 9 (2.17 g, 6.9 mmol) in 25 mL of THF was added dropwise. The temperature was then raised to −40 °C and stirring continued for 40 min. HOAc (650 μL, 11.4 mmol) was added and the mixture allowed to slowly start warming. After 5 min, NEt_3 (21 mL, 151 mmol) was added over 5–10 min, followed by addition of $POCl_3$ (2.7 mL, 29 mmol) in one portion. The temperature was brought to 0 °C and stirring continued for 50 min. The reaction mixture was poured into dilute NaCl. Product isolation (CH_2Cl_2, $MgSO_4$) followed by filtration through silica (60% petroleum ether/ether) and evaporation gave a brown oil. Addition of methanol containing a trace of pyridine gave 954 mg (1.93 mmol, 28%) of product as pale yellow needles, mp 178–181 °C. ^{1}H NMR indicated enrichment in the Δ^5-ketal isomer. Flash chromatography (25% EtOAc/hexanes) gave a further 1.43 g (2.9 mmol, 42%) of product as a yellow foam, enriched in the $\Delta^{5(10)}$ isomer. The total yield of 10 was 2.38 g (4.83 mmol, 70%). Δ^5-ketal: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (s, 3 H, 18-CH_3), 2.47 (s, 3 H, Ar-CH_3), 3.96 (m, 4 H, ketal), 5.46 (d, J = 5.5 Hz, 1 H, 6-H), 7.38 (d, J = 8.2 Hz, 2 H, aromatic H), 7.83 (d, J = 8.2 Hz, 2 H, aromatic H). $\Delta^{5(10)}$-ketal: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (s, 3 H, 18-CH_3), 2.47 (s, 3 H, Ar-CH_3), 3.97 (m, 4 H, ketal), 7.38 (d, J = 8.2 Hz, 2 H, aromatic H), 7.83 (d, J = 8.2 Hz, 2 H, aromatic H). Mixture: IR (KBr) 2104 (N=C), 1336 (S=O), 1158 (S=O) cm^{-1}; MS (70 eV) m/z (relative intensity) 155 (34), 131 (11), 99 (69), 91 (100), 86 (34), 85 (32), 83 (50), 65 (31), 47 (14), 39 (14). (70 eV offscale) 494 (M^+ + 1, 1.09), 493 (M^+, 3.07). Anal. ($C_{29}H_{35}NO_4S$) C, H, N, S.

21-Hydroxy-16-dehydro-19-norprogesterone (11). To a solution of the isocyanosulfonylmethylene compounds 10 (2.27 g, 4.6 mmol) in 90 mL of benzene were added formaldehyde (2.06 mL of a 37% w/w solution in H_2O, 27.5 mmol), methanol (1.86 mL, 46 mmol), Triton B (310 μL of a 40% solution in CH_3OH, 0.68 mmol), and 50% w/w aqueous NaOH (32 mL). The two-phase mixture was stirred vigorously for 25 min at room temperature. Product isolation (H_2O, C_6H_6) and filtration over neutral Al_2O_3 (CH_2Cl_2) gave 1.63 g of a pale yellow foam. The foam was dissolved in THF (90 mL) and treated with 4 N H_2SO_4 (27 mL) at room temperature for 24 h. $NaHCO_3$ (8.5 g) and H_2O (90 mL) were added, and the THF was removed in vacuo. Product isolation ($CHCl_3$, $MgSO_4$) gave a pale yellow solid. Crystallization (CH_2Cl_2/Et_2O) gave 834 mg (2.65 mmol, 58%) of diene dione 11 as pale yellow crystals, mp 211–218 °C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.99 (s, 3 H, 18-CH_3), 3.30 (t, J = 4.6 Hz, 1 H, 21-OH), 4.48 (AB q, $\Delta\nu$ = 37 Hz, J = 18 Hz, with additional doublet splitting due to 21-OH, J = 4.6 Hz, 2 H, 21-H), 5.84 (s, 1 H, 4-H), 6.75 (dd, J = 3.3 Hz, 1.9 Hz, 1 H, 16-H); IR (KBr) ν 3401 (OH), 1661 (C=O) cm^{-1}, MS (70 eV) m/z (relative intensity) 314 (M^+, 4.8), 284 (20), 283 (100), 105 (10), 93 (13), 91 (19), 79 (13), 77 (14), 42 (12). Anal. ($C_{20}H_{26}O_3$) C, H.

16α,17α,21-Trihydroxy-19-norprogesterone (12). A solution of diene dione 10 (618 mg, 1.97 mmol) in 30 mL of pyridine was treated with OsO_4 (500 mg, 1.97 mmol) in 5 mL of pyridine, and the dark brown solution stirred at room temperature for 25 min. The pyridine was distilled off in vacuo without heat, leaving the crude osmate ester as a dark brown solid. The solid was dissolved in 120 mL of EtOAc and treated with 40 mL of aqueous $NaHSO_3$ (8 g $NaHSO_3$/100 mL solution). After 20 min, the phases were separated. Product isolation (EtOAc, $MgSO_4$) and flash chromatography (80% EtOAc/hexanes) gave 494 mg (1.42 mmol, 72%) of triol 12 as a white solid. Crystallization (acetone/petroleum ether) gave colorless prisms: mp 192–194 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.73 (s, 3 H, 18-CH_3), 3.79 (br s, 1 H, OH), 4.49 (AB q, $\Delta\nu$ = 117.4 Hz, J = 20.2 Hz, 2 H, 21-H), 5.05 (dd, J = 9.2 Hz, 2.2 Hz, 1 H, 16β-H), 5.83 (s, 1 H, 4-H); IR (KBr) ν 3445 (OH), 1715 (C-20, C=O), 1671 (C-3, C=O) cm^{-1}; MS (70 eV) m/z (relative intensity) 349 (M^+ + 1, 2.3), 348 (M^+, 8.2), 300 (54), 289 (66), 283 (35), 272 (20), 271 (31), 257 (38), 253 (21), 245 (39), 216 (34), 215 (33), 147 (33), 110 (46), 109 (32), 107 (44), 105 (46), 93 (35), 91 (99), 86 (31), 84 (60), 81 (32), 79 (60), 77 (59), 67 (41), 55 (51), 43 (100), 41 (89), 39 (30). Anal. ($C_{20}H_{28}O_5$) C, H.

16α,17α-[(*R*)-1′-(4-Fluorophenyl)(ethylenedioxy)]-21-hydroxy-19-norpregn-4-ene-3,20-dione (5). To a suspension of triol 12 (75 mg, 0.22 mmol) in 4′-fluoroacetophenone (2 mL) was added 70% aqueous $HClO_4$ (9 μL, 0.10 mmol). After 50 min at room temperature, the reaction was quenched by addition of NEt_3 (30 μL, 0.22 mmol). Product isolation (H_2O, $CHCl_3$, Na_2SO_4) followed by flash chromatography (50% EtOAc/hexanes to

elute excess ketone, then 70% EtOAc/hexanes) gave 89 mg (0.19 mmol, 86%) of ketal **5** as a glass. Further purification via HPLC [Whatman M-9, 70% hexane/30% (5% *i*-PrOH–CH_2Cl_2), 5 mL/min] gave a sample for binding affinity measurement: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.68 (s, 3 H, 18-CH_3), 1.46 (s, 3 H, ketal CH_3), 4.57 (AB q, $\Delta\nu$ = 117.4 Hz, J = 20 Hz, 2 H, 21-H), 5.28 (d, J = 5.6 Hz, 1 H, 16β-H), 5.79 (s, 1 H, 4-H), 7.04 (t, $J_{HH} = J_{HF}$ = 8.7 Hz, 2 H, aromatic H ortho to F), 7.50 (dd, J_{HH} = 8.8 Hz, J_{HF} = 5.4 Hz, 2 H, aromatic H meta to F); ^{19}F NMR ($CDCl_3$) ϕ −115.19 (tt, J_{HF} = 9.0 Hz, 5.5 Hz); IR (KBr) 3431 (OH), 1717 (C-20 C=O), 1669 (C-3 C=O); MS (70 eV) m/z (relative intensity) 453 (9), 410 (34), 409 (100), 330 (33), 283 (14), 216 (24), 123 (12), (70 eV offscale) 469 (M^+ + 1, 2.0), 468 (M^+, 6.9); HRMS (CI) calcd for $C_{28}H_{34}FO_5$ (M + H) 469.2390, found 469.2387.

16α,17α-[(*S*)-1′-(4-Fluorophenyl)(ethylenedioxy)]-21-hydroxy-19-norpregn-4-ene-3,20-dione (13). To a suspension of triol **12** (14.8 mg, 0.042 mmol) in 4′-fluoroacetophenone (730 μL) was added 70% aqueous $HClO_4$ (70 μL, 0.78 mmol). After stirring for 23 h at room temperature, NEt_3 (125 μL, 0.90 mmol) was added to quench the reaction. Product isolation (H_2O, CH_2Cl_2, Na_2SO_4) followed by flash chromatography (50% EtOAc/hexanes) gave 9.7 mg (0.021 mmol, 49%) of ketal **13** and 3.1 mg (0.007 mmol, 16%) of ketal **5** as glasses. Further purification of ketal **13** via HPLC [Whatman M-9, 70% hexane/30% (5% *i*-PrOH–CH_2Cl_2), 5 mL/min] gave a sample for binding affinity measurement. Ketal **13**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.58 (s, 3 H, 18-CH_3), 1.70 (s, 3 H, ketal CH_3), 4.06 (AB q, $\Delta\nu$ = 93.1 Hz, J = 19.3 Hz, 2 H, 21-H), 5.17 (d, J = 5.3 Hz, 1 H, 16β-H), 5.86 (s, 1 H, 4-H), 6.97 (t, $J_{HH} = J_{HF}$ = 8.7 Hz, 2 H, aromatic H ortho to F), 7.32 (dd, J_{HH} = 8.8 Hz, J_{HF} = 5.4 Hz, 2 H, aromatic H meta to F); ^{19}F NMR ($CDCl_3$) ϕ −114.48 (tt, J_{HF} = 8.5 Hz, 5.3 Hz); IR (KBr) ν 3432 (OH), 1717 (C-20, C=O), 1663 (C-3, C=O) cm^{-1}; MS (70 eV) m/z (relative intensity) 410 (28), 409 (100), 271 (22), 139 (23), 123 (18), 107 (11), 105 (13), 97 (10), 95 (11), 91 (17), 81 (11), 79 (16), 77 (10), 57 (13), 56 (12), 55 (25), 43 (32), 41 (27), (70 eV offscale) 468 (M^+, 6.9); HRMS calcd for $C_{28}H_{33}FO_5$ 468.2312, found 468.2311.

Radiochemical Synthesis. 4′-[^{18}F]Fluoroacetophenone. 4′-[^{18}F]Fluoroacetophenone was prepared by an adaptation of a previously described procedure.[25] Fluorine-18 was prepared from [^{18}O]H_2O as previously described.[34] A stock solution of K_2CO_3/Kryptofix 222 was prepared: K_2CO_3 (25 mg, 181 μmol) and Kryptofix 222 (125 mg, 332 μmol) were added to 0.5 mL of deionized H_2O, and the mixture was sonicated for 2 min. CH_3CN (12.5 mL) was added and the stock solution stored in a crimp-sealed injection vial. Aqueous [^{18}F]fluoride ion (215 mCi) was added to 500 μL of the stock solution in a 3-mL Reactivial, and the water was removed by azeotropic distillation with CH_3CN at 110 °C under a stream of nitrogen. The resolubilized activity was removed from heat, and 4′-nitroacetophenone (3 mg, 18 μmol) and anhydrous DMSO (500 μL) were added. The capped vial was subjected to microwave radiation (700 W) for 2.5 min, then immediately cooled in ice. The cooled room temperature reaction mixture was applied to a 0.5-cm i.d. column containing 1.5 g of dry SiO_2 (flash chromatography grade) and eluted with 20% ether/pentane, collecting 1 mL fractions. 4′-[^{18}F]Fluoroacetophenone eluted in 7 mL, free of carrier 4′-nitroacetophenone; yield 125 mCi (71%).

General Procedure for 4′-[^{18}F]Fluoroacetophenone Ketal Formation. 4′-[^{18}F]Fluoroacetophenone in 20% ether/pentane was concentrated to dryness *in the presence of* 1 mg of the desired steroid diol under a gentle stream of nitrogen. The residue was redissolved in a 1:1 v/v mixture of CH_2Cl_2/(20% ether–pentane) and transferred to a glass screwcap vial. Triethyl orthoformate (TEOF) was added, followed by 70% aqueous $HClO_4$. After the indicated reaction time, the reaction mixture was passed through a small SiO_2 column overlaid with $NaHCO_3$, rinsed with ca. 3 mL of EtOAc. Alternatively, the reaction was quenched by addition of NEt_3 and the mixture filtered through SiO_2 (EtOAc). The solvent was removed under a stream of nitrogen and the residue dissolved in the indicated HPLC solvent mixture of purification.

16α,17α-[(*R*)-1′-(4-[^{18}F]Fluorophenyl)(ethylenedioxy)]-pregn-4-ene-3,20-dione ([^{18}F]4). The reaction employed 60.2 mCi of 4′-[^{18}F]fluoroacetophenone, 1.0 mg (2.9 μmol) of 16α,17α-dihydroxyprogesterone, 4 μL (24 μmol) of TEOF, and 1 μL (11 μmol) of 70% $HClO_4$ in 100 μL of solvent. Reaction was at room temperature for 45 min, followed by $NaHCO_3/SiO_2$ column purification. Purification via HPLC [Whatman M-9, 85% hexane/15% (5% *i*-PrOH–CH_2Cl_2), 5 mL/min] gave 3.89 mCi (15%), t_R = 25 min, sp act. = 30 Ci/mmol.

16α,17α-[(*R*)-1′-(4-[^{18}F]Fluorophenyl)(ethylenedioxy)]21-hydroxy-19-norpregn-4-ene-3,20-dione ([^{18}F]5). The reaction employed 77.6 mCi of 4′-[^{18}F]fluoroacetophenone, 1.0 mg (2.9 μmol) of triol **12**, 25 μL (150 μmol) of TEOF, and 5 μL (56 μmol) of 70% $HClO_4$ in 500 μL of solvent. Reaction was at room temperature for 30 min, followed by NEt_3 quench (10 μL, 70 μmol) and SiO_2 filtration. Purification via HPLC [Whatman M-9, 70% hexane/30% (5% *i*-PrOH–CH_2Cl_2), 5 mL/min] gave 7.77 mCi (16%), t_R = 28.5 min, sp act. = 409 Ci/mmol.

Biological Procedures Relative Binding Affinity (RBA) and Specific Activity 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 for progesterone receptor (PgR),[35] mineralocorticoid receptor (MR),[35] and glucocorticoid receptor (GR).[36] Tritium-labeled standards for the RBA measurements were R5020 (K_d = 0.4 nM), aldosterone (K_d = 3.9 nM), and RU28362 (K_d = 11 nM) for PgR, MR, and GR, respectively. By definition, the standards have RBA values of 100. Effective specific activities of the ^{18}F-labeled progestins were measured by in vitro competitive binding assays performed on fully decayed samples.[26]

In Vivo Biodistribution Studies. The progesterone receptor levels in the uteri of immature rats were induced by estrogen treatment. Immature female Sprague–Dawley rats (23 days old, >50 g)[37] were given two daily s.c. injections of 5 μg of estradiol in 0.1 mL of 20% ethanol/sunflower seed oil, prepared fresh daily. The experiments were begun 24 h after the last injection. The ^{18}F-labeled progestins, purified by HPLC, were concentrated in vacuo, and redissolved in 250 μL of 100% ethanol and 250 μL of isotonic saline. The solution was passed through an ethanol-wetted filter. The filtered solution was diluted to 2.5 mL with isotonic saline (final solution 10% ethanol/saline). Ether-anesthetized rats were injected in the tail vein with the desired doses (17–51 μCi) of the labeled compound. At specified time points postinjection the rats were sacrificed by decapitation and blood and organs were removed, weighed, and counted in a Beckman Gamma 6000 counter. Uterine uptake blocking studies were accomplished by a coinjection of 18 μg of ORG 2058 and the labeled steroid.

Acknowledgment. We are grateful for the support of this work through grants from the Department of Energy (DE FG02 86ER60401 to J.A.K. and DE FG02 86ER60218 to M.J.W.) and the American Health Assistance Foundation. Additional support for this work was granted to H.F.V. through an Alexander Hollaender Distinguished Postdoctoral Fellowship, administered by the Oak Ridge Institutes for Science and Education. High-field NMR spectra and high-resolution mass spectra were obtained on instruments supported by grants from the National Institutes of Health to the University of Illinois (RR 02299 and GM 27029, respectively). We thank Dr. John Edwards of Syntex for the gift of 19-norandrostenedione, Drs. Filippus J. Zeelen and Willem Bergink of Organon for a gift of ORG 2058, Anita Skariah for measuring the log $P_{o/w}$ values, and Pamela Rocque and Henry Lee for performing the tissue-distribution studies.

References

(1) For a review see: Katzenellenbogen, J. A. The Pharmacology of Steroid Radiopharmaceuticals: Specific and Non-specific Binding and Uptake Selectivity. In *Radiopharmaceuticals: Chemistry and Pharmacology*; Nunn, A., Ed.; Marcel Dekker: New York, 1992; pp 297–331.

(2) (a) Mintun, M. A.; Welch, M. J.; Siegel, B. A.; Mathias, C. J.; Brodack, J. M.; McGuire, A. H.; Katzenellenbogen, J. A. Breast Cancer: PET Imaging of Estrogen Receptors. *Radiology* 1988, *169*, 45–48. (b) McGuire, A. H.; Dedashti, F.; Siegel, B. A.; Lyss,

A. P.; Brodack, J. W.; Mathias, C. J.; Mintun, M. A.; Katzenellenbogen, J. A.; Welch, M. J. Positron Tomographic Assessment of 16α-[^{18}F]Fluoro-17β-Estradiol Uptake in Metastatic Breast Carcinoma. *J. Nucl. Med.* **1991**, *32*, 1526–1531.

(3) (a) Ganz, P. A.; Korenman, S. G. The Clinical Value of Steroid Receptors in Breast Cancer. In *Cancer Diagnosis: New Concepts and Techniques*; Steckle, R. J., Kagan, A. R., Eds.; Grune & Stratton: New York, 1982; pp 32–61. (b) Sledge, G. W., Jr.; McGuire, W. L. Steroid Hormone Receptors in Human Breast Cancer. *Adv. Cancer Res.* **1983**, *38*, 61–75.

(4) Furr, B. J. A.; Jordan, V. C. The Pharmacology and Clinical Uses of Tamoxifen. *Pharmacol. Ther.* **1984**, *25*, 127.

(5) (a) Noguchi, S.; Miyauchi, K.; Nishizawa, Y.; Koyama, H. Induction of Progesterone Receptor with Tamoxifen in Human Breast Cancer with Special Reference to its Behavior over Time. *Cancer* **1988**, *61*, 1345–1349. (b) Howell, A.; Harland, R. N. L.; Barnes, D. M.; Baildam, A. D.; Wilkinson, M. J. S.; Hayward, E.; Swindell, R.; Sellwood, R. A. Endocrine Therapy for Advanced Carcinoma of the Breast: Relationship Between the Effect of Tamoxifen upon Concentrations of Progesterone Receptor and Subsequent Response to Treatment. *Cancer Res.* **1987**, *47*, 300–304.

(6) Pomper, M. G.; Katzenellenbogen, J. A.; Welch, M. J.; Brodack, J. W.; Mathias, C. J. 21-[^{18}F]Fluoro-16α-ethyl-19-norprogesterone: Synthesis and Target Tissue Selective Uptake of a Progestin Receptor Based Radiotracer for Positron Emission Tomography. *J. Med. Chem.* **1988**, *31*, 1360–1363.

(7) The following abbreviations are used: FENP for 21-fluoro-16α-ethyl-19-norpregn-4-ene-3, 20-dione, ORG 2058 for 21-hydroxy-16α-ethyl-19-norpregn-4-ene-3,20-dione, R5020 (promegestone) for 17α,21-dimethyl-19-norpregna-4,9-diene-3,20-dione, RU28362 for 11β,17β-dihydroxy-6-methyl-17α-propynylandrosta-1,4,6-triene-3-one, triamcinolone acetonide for 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16α,17α-acetonide, fluocinolone acetonide for 6α,9α-difluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16α,17α-acetonide, and TEOF for triethyl orthoformate.

(8) Dedashti, F.; McGuire, A. M.; VanBrocklin, H. F.; Siegel, B. A.; Andriole, D. P.; Griffeth, L. K.; Pomper, M. G.; Katzenellenbogen, J. A.; Welch, M. J. Assessment of 21-[^{18}F]Fluoro-16α-Ethyl-19-Norprogesterone as a Positron-Emitting Radiopharmaceutical for the Detection of Progestin Receptors in Human Breast Carcinomas. *J. Nucl. Med.* **1991**, *32*, 1532–1537.

(9) (a) Fried, J.; Sabo, E. F.; Grabowich, P.; Lerner, L. J.; Kessler, W. B.; Brennan, D. M.; Borman, A. Progestationally Active Acetals and Ketals of 16α,17α-Dihydroxyprogesterone. *Chemistry and Industry* **1961**, 465–466. (b) Fried, J.; Sabo, E. F. The Stereochemistry of Unsymmetrically Substituted 16α,17α-methylenedioxyprogesterones. In *Hormonal Steroids, Biochemistry, Pharmacology and Therapeutics: Proceedings of the First International Congress on Hormonal Steroids*; Academic Press: New York, 1965; Vol. 2, pp 15–21.

(10) Lerner, L. J.; Brennan, D. M.; Borman, A. Biological Activities of 16α,17α-Dihydroxyprogesterone Derivatives. *Proc. Soc. Exp. Biol. Med.* **1961**, *106*, 231–234.

(11) (a) Kilbourn, M. J.; Carey, J. E.; Koeppe, R. A.; Haka, M. S.; Hutchins, G. D.; Sherman, P. S.; Kuhl, D. E. Biodistribution, Dosimetry, Metabolism and Monkey PET Studies of [^{18}F]GBR 13119. Imaging the Dopamine Uptake System In Vivo. *Nucl. Med. Biol.* **1989**, *16*, 569–576. (b) Plenevaux, A.; Fowler, J. S.; Dewey, S. L.; Wolf, A. P.; Guillaume, M. The Synthesis of No-carrier-added DL-4-[^{18}F]Fluorodeprenyl via the Nucleophilic Aromatic Substitution Reaction. *Appl. Radiat. Isot.* **1991**, *42*, 121–127.

(12) Kym, P. R.; Carlson, K. E.; Katzenellenbogen, J. A. Progestin 16a,-17a-Dioxolane Ketals as Molecular Probes for the Progesterone Receptor: Synthesis, Binding Affinity, and Photochemical Evaluation. *J. Med. Chem.*, preceding paper in this issue.

(13) Oliveto, E. P. Synthesis and Degradation of the Pregnane Side-Chain. In *Organic Reactions in Steroid Chemistry*; Fried, J., Edwards, J. A., Eds.; Van Nostrand Reinhold: New York, 1972; Vol. 2, pp 127–236, and references therein.

(14) (a) Van Leusen, D.; Van Leusen, A. M. A Novel Method to Build Acetyl and Hydroxyacetyl Side-Chains in 17-Oxosteroids. *Tetrahedron Lett.* **1984**, *25*, 2581–2584. (b) Van Leusen, A. M. 17-(Isocyano-sulfonylmethylene)-steroids, 17-(Formamido-sulfonylmethylene)-steroids and Their Preparation. European Patent #123734 A1, Nov. 7, 1984. (c) Van Leusen, A. M. New Process for the Preparation of 21-Hydroxy-20-keto-delta-16-steroids and New Intermediate Compounds Formed in this Process. European Patent #123736 A1, Nov. 7, 1984.

(15) Norymberski, J. K.; Woods, G. F. Partial Reduction of Steroid Hormones and Related Substances. *J. Chem. Soc.* **1955**, 3426–3430.

(16) On a preparative scale the reduction, ketalization, and reoxidation reactions may be carried out without purification of compounds **5** and **6** without adversely affecting the overall yield. Brad O. Buckman, University of Illinois, unpublished result.

(17) Brown, J. J.; Bernstein, S. 16-Hydroxylated Steroids. XIX. The Synthesis of 16α-Hydroxy-19-norhydrocortisone. *J. Org. Chem.* **1961**, *26*, 5033–5036.

(18) Baran, J. S. Method for the Cleavage of Osmate Esters. *J. Org. Chem.* 1960, *25*, 257.

(19) It is noteworthy that the osmate formed from 16-dehydroprogesterone could be successfully cleaved by addition of aqueous sodium bisulfite to the reaction mixture in pyridine to afford upon extraction with ethyl acetate an 88% yield of 16α,17α-dihydroxyprogesterone.[12]

(20) (a) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. An Improved Catalytic OsO_4 Oxidation of Olefins to *cis*-1,2-glycols using Tertiary Amine Oxides as the Oxidant. *Tetrahedron Lett.* **1976**, *17*, 1973–1976. (b) Minato, M.; Yamamoto, K.; Tsuji, J. Osmium Tetraoxide Catalyzed Vicinal Hydroxylation of Higher Olefins by using Hexacyanoferrate (III) Ion as a Cooxidant. *J. Org. Chem.* **1990**, *55*, 766–768.

(21) Allen, G. R., Jr.; Weiss, M. J. Studies in the Synthesis of Triamcinolone. The Condensation of 16α,17α-Isopropylidenedioxy-4,9(11)-pregnadiene-3,20-dione with Ethyl Oxalate. *J. Am. Chem. Soc.* **1959**, *81*, 4968–4979.

(22) See ref 9b for a discussion of the effect of aryl group orientation on the chemical shift of C-21 protons in 16α,17α-aryl ketals of dihydroxyprogesterone.

(23) Rousseau, G. G.; Schmit, J.-P. Structure-Activity Relationships for Glucocorticoids—I. Determination of Receptor Binding and Biological Activity. *J. Steroid Biochem.* **1977**, *8*, 911–919.

(24) Ojasoo, T.; Doré, J.-C.; Gilbert, J.; Raynaud, J.-P. Binding of Steroids to the Progestin and Glucocorticoid Receptors Analyzed by Correspondence Analysis. *J. Med. Chem.* **1988**, *31*, 1160–1169.

(25) (a) Kilbourn, M. R.; Dence, C. S.; Welch, M. J.; Mathias, C. J. Fluorine-18 Labeling of Proteins. *J. Nucl. Med.* **1987**, *28*, 462–470. (b) Hwang, D.-R.; Dence, C. S.; Gong, J.; Welch, M. J. A New Procedure for Labeling Alkylbenzenes with [^{18}F]Fluoride. *Appl. Radiat. Isot.* **1991**, *42*, 1043–1047.

(26) Senderoff, S. G.; McElvany, K. D.; Carlson, K. E.; Heiman, D. F.; Katzenellenbogen, J. A.; Welch, M. J. Methodology for the Synthesis and Specific Activity Determination of 16α-[^{77}Br]Bromoestradiol-17β and 16α-[^{77}Br]Bromo-11β-methoxyestradiol-17β, Two Estrogen Receptor-Binding Radiopharmaceuticals. *Int. J. Appl. Radiat. Isot.* **1982**, *33*, 545–551.

(27) Hanson, R. N. The Influence of Structure Modification on the Metabolic Transformations of Radiolabeled Estrogen Derivatives. In *Radiopharmaceuticals: Chemistry and Pharmacology*; Nunn, A., Ed.; Marcel Dekker: New York, 1992; pp 333–364, and references therein.

(28) (a) Schreiber, J. R.; Hsueh, A. J. W. Progesterone "Receptor" in Rat Ovary. *Endocrinology* **1979**, *105*, 915–919. (b) Arakawa, S.; Iyo, M.; Ohkawa, R.; Kambegawa, A.; Okinaga, S.; Arai, K. Steroid Hormone Receptors in the Uterus and Ovary of Immature Rats Treated with Gonadotropins. *Endocrinol. Jpn.* **1989**, *36*, 219–228.

(29) Leo, A.; Hansch, C.; Elkins, D. Partition Coefficients and Their Uses. *Chem. Rev.* **1971**, *71*, 525–616.

(30) Minick, D. J.; Fresz, J. H.; Patrick, M. A.; Brent, D. A. A Comprehensive Method for Determining Hydrophobicity Constants by Reversed-phase High-performance Liquid Chromatography. *J. Med. Chem.* **1988**, *31*, 1923–1933.

(31) Still, W. C.; Kahn, M.; Mitra, A. A Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(32) *The Merck Index*, 11th ed.; Merck & Co., Inc.: Rahway, New Jersey, 1989; p 1007.

(33) Saha, N. N. The $\Delta^{5(6)}$- and $\Delta^{5(10)}$-Ethylene Ketals of 19-Nortestosterone. *Steroids* **1968**, *12*, 735–747.

(34) Kilbourn, M. R.; Jerabek, P. A.; Welch, M. J. An Improved [^{18}O]-Water Target for [^{18}F]Fluoride Production. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 327–328.

(35) Brandes, S. J.; Katzenellenbogen, J. A. Fluorinated Androgens and Progestins: Molecular Probes for Androgen and Progesterone Receptors with Potential Use in Positron Emission Tomography. *Mol. Pharmacol.* **1987**, *32*, 391–403.

(36) Pinney, K. G.; Carlson, K. E.; Katzenellenbogen, J. A. [^{3}H]DU41165: A High Affinity Ligand and Novel Photoaffinity Labeling Reagent for the Progesterone Receptor. *J. Steroid Biochem.* **1990**, *35*, 179–189.

(37) VanBrocklin, H. F.; Brodack, J. W.; Mathias, C. J.; Welch, M. J.; Katzenellenbogen, J. A.; Keenan, J. F.; Mizejewski, G. J. Binding of 16α-[^{18}F]Fluoro-17β-estradiol to Alphafetoprotein in Sprague-Dawley Female Rats Affects Blood Levels. *Nucl. Med. Biol.* **1990**, *17*, 769–773.