Synthesis of 11β-[^{18}F]Fluoro-5α-dihydrotestosterone and 11β-[^{18}F]Fluoro-19-nor-5α-dihydrotestosterone: Preparation via Halofluorination-Reduction, Receptor Binding, and Tissue Distribution

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We have prepared 11β-fluoro-5α-dihydrotestosterone (11β-F-DHT, **1**) and 11β-fluoro-19-nor-5α-dihydrotestosterone (11β-F-19-nor-DHT, **2**) in order to investigate the properties of these new androgens labeled with fluorine-18 as potential androgen receptor (AR)-based imaging agents for prostate cancer. These compounds were synthesized in 6 steps from hydrocortisone and in 13 steps from 1,4-androstadiene-3,11,17-trione, respectively. Relative binding affinities (RBA) of 11β-F-DHT and 11β-F-19-nor-DHT to AR are 53.1 and 75.3 (R1881 = 100), respectively, the latter being the highest reported among fluorine-substituted androgens. The fluorination step, which involves addition of halogen fluoride across the 9(11)-double bond, followed by reductive dehalogenation at the 9α-position has been adapted to introduce a fluorine-18-label at the 11β-position of DHT and 19-nor-DHT. The two high-affinity F-18-labeled ligands [^{18}F]-**1** and [^{18}F]-**2** were evaluated *in vivo*, in tissue distribution studies using diethylstilbestrol-pretreated mature male rats. 11β-F-DHT shows high prostate uptake and selective prostate to blood and prostate to muscle uptake ratios, the latter two ratios increasing from 5 and 8 at 1 h to 12 and 19 at 4 h postinjection. Moreover, this compound has low uptake in bone, displaying the lowest *in vivo* defluorination among all androgens labeled with fluorine-18 tested so far. The *in vivo* properties of 11β-F-DHT in rats are thus favorable for imaging of prostate cancer. On the other hand, 11β-F-19-nor-DHT shows low prostate uptake with low selectivity and high uptake in liver, kidney, and bladder. Even though this ligand has the highest RBA and undergoes little metabolic defluorination, it appears to suffer from rapid metabolism *in vivo*. Therefore, it is apparent that the biodistribution properties of androgens are affected by their structure and metabolism as well as by their RBA.

Introduction

Fluorine-18-labeled steroid hormones have potential application as imaging agents for receptor-positive tumors, using positron emission tomography (PET). A number of fluorine-18-labeled estrogens have been prepared and studied extensively in animal model systems, and one compound, 16α-[^{18}F]fluoroestradiol, has been shown to provide clear images of both primary and metastatic estrogen receptor-positive breast tumors.[1] Preliminary work has also been published with fluorine-18-labeled progestins for imaging breast tumors on the basis of their progesterone receptor content.[2,3] Fluorine-18-labeled androgens also have the potential for imaging androgen receptor (AR)-positive tumors of the prostate and metastases derived from these tumors. If such images were to provide a clear delineation of the stage of the cancer, these agents might provide a basis for improved therapy of prostatic carcinoma.

In several earlier publications, we have described the synthesis of a number of fluorine-substituted androgens[4,5] and we have presented tissue distribution studies with seven fluorine-18-labeled androgens in male rats.[6] More recently, we have reported PET imaging of the prostate in a baboon using three of these compounds.[7] All of these agents were labeled with fluorine-18 through substitution reactions involving the displacement of a reactive sulfonate ester or cyclic sulfate group on a suitable precursor. While these reactions generally proceeded in reasonable yield, the products that can be obtained by this approach are limited to those that are substituted at relatively nonhindered sites and can be prepared through simple S_N2 displacement reactions.

In one of our first publications, we reported that androgens substituted with fluorine at the 11β-position had high affinity for AR.[4] In fact, while fluorine substitution at other sites lowers AR binding affinity considerably, fluorine substitution at the 11β-position appeared to maintain or even increase binding affinity. The preparation of 11β-substituted steroids through nucleophilic substitution is complicated by steric hindrance and the tendency of reactive 11-substituted precursors to undergo elimination to produce the 9(11)-dehydro steroid. 11β-Fluoro steroids, however, have been prepared by other methods, such as halogen fluoride addition to the 9(11)-alkene, giving the 11β-fluoro-9α-halo steroid,[8,9] followed by reductive dehalogenation at the 9α-center. Such an approach has so far not been described for radiofluorination.

Some time ago, we described the use of bromofluorination or iodofluorination as a method for introducing

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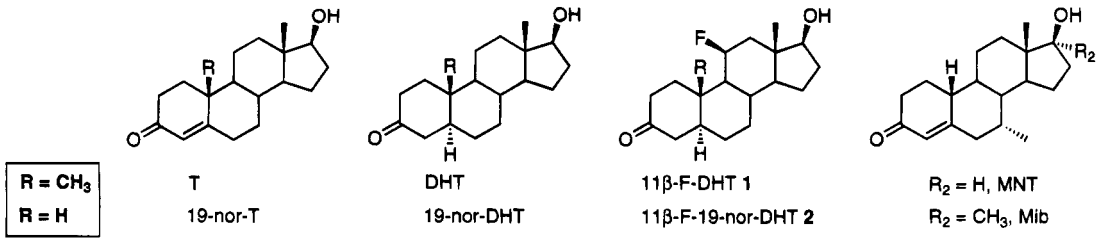
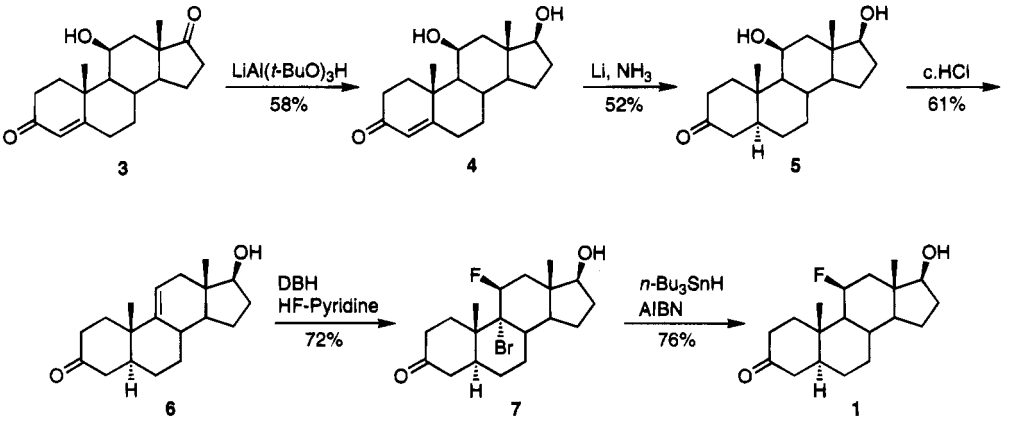


Figure 1. Structures of androgens and fluorinated androgens **1** and **2**.

Scheme 1



fluorine-18 at the substituted position of monosubstituted alkenes,[10] and more recently, we have described an extension of this approach to more highly substituted alkenes.[11] In this report, we describe the preparation of two 11β-fluorine-substituted androgens, 11β-fluoro-5α-dihydrotestosterone (Figure 1; 11β-F-DHT, **1**) and 11β-fluoro-19-nor-5α-dihydrotestosterone (Figure 1; 11β-F-19-nor-DHT, **2**), and their fluorine-18-labeled analogs. Also, we present their *in vitro* receptor binding affinities, their estimated nonspecific binding, and their *in vivo* tissue distributions. 11β-F-DHT (**1**) displays target tissue-selective distribution properties *in vivo* and has the lowest fraction of metabolic defluorination that we have observed for any fluorine-18-labeled steroid.

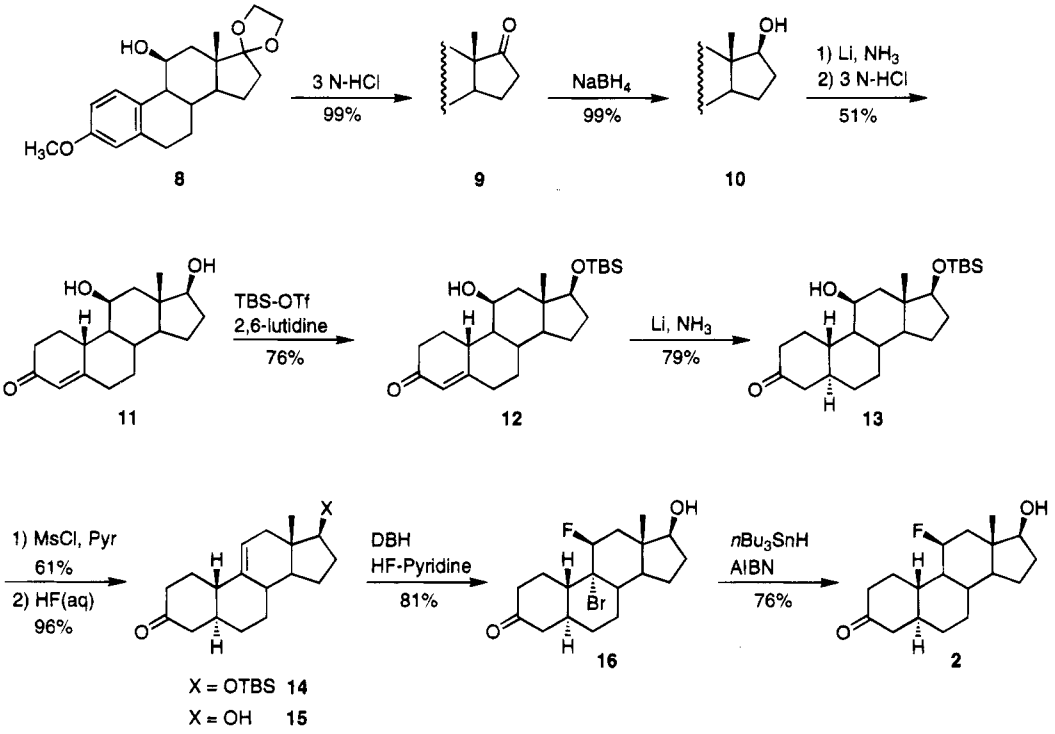
Results and Discussion

Synthesis of 11β-F-DHT (1). Synthesis of 17β-hydroxy-9(11)-androsten-3-one (**6**; Scheme 1), the 9(11)-alkene precursor for halofluorination, begins with 11β-hydroxyandrostenedione **3**, whose preparation from hydrocortisone by bismuthate side chain cleavage has been described (Scheme 1).[12] The androstanediol **5** was prepared from **3** by a sequential reduction of the 17-ketone and the double bond using LiAl(*t*-BuO)$_3$H and Li/NH$_3$,[13] respectively. This two-step reduction provided few byproducts and gave an overall yield of 31%; by contrast, our attempts to effect this double reduction in one step, by reduction with Li/NH$_3$, gave a yield of product **5** that was lower (22%) and produced material that contained inseparable reaction byproducts. Treatment of diol **5** with acid afforded the 9(11)-alkene **6** exclusively. This elimination is facile because the axially positioned 11β-OH group suffers from a number of 1,3-diaxial interactions; formation of the desired regioisomer reflects the relative stability of a trisubstituted vs a disubstituted alkene. Halofluorination of alkene **6** with 1,3-dibromo-5,5-dimethylhydantoin (DBH) and hydrogen fluoride–pyridine[14] resulted in axial attack by fluoride ion on the 9α,11α-bromonium ion.[9] The bromonium ion forms exclusively on the α-side of alkene **6**, thereby avoiding steric hindrance from the 18- and 19-methyl groups on the β-side. Fluoride ion attack on the bromonium ion in this polycyclic system follows the Fürst–Plattner rule (diaxial addition);[15] thus, the overall addition is anti-Markownikoff and gives the 9α-bromo, 11β-fluoro stereoisomer that has the B- and C-rings in the trans-fused configuration. Reductive debromination of the dihalo steroid **7** yielded the desired product **1**.

Synthesis of 11β-F-19-nor-DHT (2). Synthesis of 17β-hydroxy-9(11)-estren-3-one (**15**), the precursor to 11β-F-19-nor-DHT (**2**), involved the longer sequence shown in Scheme 2. Ketal **8** was prepared from 1,4-androstadiene-3,11,17-trione in four steps according to the procedure of Baran.[16] The 17,17-ethylenedioxy group of androgen **8** was removed under mild acidic conditions to give the 17-ketone **9**, which was further reduced selectively to the 17β-alcohol **10**. Reduction of the 17-ketone of androgen **9** at a later step in the synthesis resulted in competing reduction of the 3-ketone. Birch reduction followed by acid hydrolysis of diol **10** provided 11β-hydroxy-19-nortestosterone (**11**). Selective protection of the less hindered 17β-alcohol over the 11β-alcohol to afford steroid **12** was accomplished by adding 1 equiv of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBS triflate)[17] and 1.5 equiv of 2,6-lutidine to diol **11**. The double bond of the 17β-TBS-protected steroid **12** was reduced by Li/NH$_3$ to afford the alcohol **13** with 5α-stereochemistry.[13]

Since in the other series (cf., Scheme 1) we were able to selectively eliminate the 11β-OH group of diol **5** to give the 9(11)-alkene **6** under acidic conditions, we attempted to do the same on the corresponding 19-nor steroid alcohol **13**. However, the reaction yielded mostly decomposition products. Dehydration was accomplished by activation of the 11β-OH group as its mesylate

Scheme 2



followed by base-induced elimination to give the trisubstituted 9(11)-alkene **14** in 61% yield, together with a small amount (7–8%) of the undesired 11(12)-alkene regioisomer. While deprotection of the 17β-TBS ether of alkene **14** was not complete with tetrabutylammonium fluoride, deprotection was accomplished in 96% yield using aqueous HF in acetonitrile.[18] Halofluorination of alkene **15** followed by debromination of the dihalo steroid **16** to afford the final product **2** was carried out as described for synthesis of steroids **7** and **1**. The anti-Markownikoff dihalo adduct, steroid **16**, was the exclusive halofluorination product, indicating that even in the 19-nor steroid, that lacks the bulk of the 19-methyl group on the β-face, the bromonium ion forms selectively on the α-side of the steroid backbone.

The stereochemistry of the C-11 fluorine substituent in androgens **1** and **2** was assigned by both proton and fluorine NMR. In the ^{1}H NMR, both the 18β-CH_3 and 19β-CH_3 groups appear as doublets (J = 2.4, 3.4 Hz, respectively, for **1**; J = 1.8 Hz for **2**) due to long-range coupling between these two methyl groups and the nearby 11β-F atom;[19] the 11α-H (equatorial) experiences a large geminal coupling with 11β-F (J = 48-49 Hz). In the ^{19}F NMR, the 11β-F (axial) experiences a larger antiperiplanar vicinal coupling with the neighboring H atoms at the 9α-H (axial) and 12α-H (axial) positions.

Radiochemical Synthesis of [^{18}F]-1 and [^{18}F]-2. Radiohalofluorination was carried out by adding alkene **6** or **15** to Br[^{18}F]F generated *in situ* from a CH_2Cl_2 solution of F-18 fluoride ion, DBH, and sulfuric acid.[10,11] The reaction conditions were optimized by varying the temperature and the reaction time independently. The reaction required relatively harsh conditions (25–40 °C; 15 min) as compared to those normally used for other alkenes,[10,11] probably because of steric hindrance at the 11β-position.

Reductive debromination was accomplished by refluxing a benzene solution of dihalo steroid [^{18}F]-**7** or [^{18}F]-**16**, tributyltin hydride, and azobis(isobutyronitrile) (AIBN). Excess AIBN was required for the reaction to go to completion in a short period of time. At the end of the reaction, the tributyltin bromide byproduct was converted with NH_4OH to tributyltin amide, which was easily removed by passage through a short plug of silica gel.

Purification of the final tracers [^{18}F]-**1** and [^{18}F]-**2** was accomplished by normal phase HPLC. The desired fractions were collected by monitoring the radioactivity trace, since these ligands do not have a chromophore that absorbs at the wavelength (254 nm) used for normal phase systems. Despite a potential difference in polarity due to the presence of 19-CH_3 group in [^{18}F]-**1**, these two tracers eluted at the same retention time (t_R = 45 min). The collected fractions were further analyzed for both chemical and radiochemical purity by reversed phase analytical HPLC, which was monitored at 210 nm in a CH_3CN–phosphate buffer system. Identification of the tracer was determined by coinjection of the corresponding unlabeled standard. The tracer collected from normal phase HPLC was shown to be radiochemically pure and to coelute with the appropriate standard. The overall radiochemical yields (2.4–3.2%) were relatively low, but this is not atypical for tracer scale radiofluorination reactions, especially those involving more than one step.[1,2,5,6]

Androgen Receptor Binding and Steroid Structure. Relative binding affinities (RBA) of some androgens and the fluorine-substituted androgens **1** and **2** (Figure 1) to AR, progesterone receptor (PgR), and sex steroid binding protein (SBP) are shown in Table 1 (the standards used in each case are shown in Table 1). Because the prostate contains substantial levels of PgR[20] in addition to AR, and the androgen carrier protein SBP is present in the bloodstream of primates, binding affinities to all three proteins should be considered in screening androgens as potential *in vivo* ligands for AR binding in the prostate. The natural androgen testosterone (T) is converted to DHT in the

Table 1. Relative Binding Affinities and Lipophilic Properties of Androgens

compound	RBA[a] AR (R1881 = 100)	RBA[a] PgR (R5020 = 100)	RBA[a] SBP (estradiol = 100)	log $P_{o/w}$[b]	NSB[c]	BSI[d]
T	6.6 ± 1.4	0.09 ± 0.03	417 ± 88	3.45	1.18	5.6
DHT	60.9 ± 17.2	0.23 ± 0.10	1808 ± 699	3.90	1.87	32.6
19-nor-T	30.6 ± 1.5	2.54 ± 1.19	30 ± 6.8	3.17	0.88	34.8
19-nor-DHT	24.3 ± 0.4	1.21 ± 0.12	213 ± 97	3.85[f]	1.78	13.7
11β-F-DHT	53.1 ± 7.6	0.22 ± 0.02	1737 ± 0.7	3.50[f]	1.24	42.8
11β-F-19-nor-DHT	75.3 ± 9.4	0.72[e]	134 ± 20	3.10[f]	0.82	91.8
R1881	100	43.70 ± 9.10	4 ± 0.9	3.29	1.00	100

[a] Relative binding affinity (RBA) values were determined in a competitive radiometric assay; details are described in the Experimental Section. Values are expressed as percentages relative to the affinity of the indicated tritium-labeled tracer and are the average of two or more determinations ± standard deviation. [b] log $P_{o/w}$ (log of octanol–water partition coefficient) was measured according to Minick et al., ref 29. [c] Nonspecific binding (R1881 = 1.00), calculated using an empirically derived relationship, ref 28. [d] Binding selectivity index, ratio of the RBA (to AR) to NSB (R1881 = 100), ref 28. [e] A single determination. [f] HPLC traces of 5α-dihydroandrogens were monitored at 290 nm, and the HPLC void volume marker was monitored at 254 nm.

prostate by 5α-reductase, and the latter binds to AR 10 times better than the former (60.9 vs 6.6). This enhancement in affinity was noted, as well, with the fluorine-substituted T's and DHT's.[4,5,21] On the other hand, 19-nor-DHT, 5α-dihydro-Mibolerone (5α-dihydro-Mib), and 5α-dihydro-7α-methyl-19-nortestosterone (5α-dihydro-MNT; Figure 1) have lower binding affinities to AR than their 19-nor-T counterparts.[22] Thus, 5α-reduction appears to increase AR binding affinity in the T → DHT series, whereas it reduces affinity in the 19-nor T → 19-nor-DHT series. This trend was also observed in 19-nor-DHT and 19-nor-T substituted with fluorine at C-6; these results will be discussed elsewhere.[21]

A fluorine atom has been introduced at positions 2α, (3,3), 6, 11β, 16, or 17β of DHT and at positions 6, 11β, or 17α-CH_3 of 19-nor-DHT.[4,5,23] Also, a fluorine atom has been introduced at positions C-16 or C-20 of the synthetic androgens MNT, Mib, and R1881 (Figure 1).[5] In most cases except the 11β-position, the introduction of a fluorine atom into an androgen decreases its binding affinity to AR. The results suggest that the presence of a fluorine atom near the 3-ketone or the 17β-hydroxyl group disrupts its potential hydrogen bonding to the receptor electronically and/or sterically. Furthermore, replacement of the 3-ketone or the 17β-hydroxyl group in DHT with a fluorine atom causes a nearly complete loss of binding affinity to the receptor,[5] which reiterates the requirement of these groups for high affinity binding to AR. Fluorination at the 11β-position, however, *increases* the binding affinity of 19-nor-DHT by 3-fold and maintains the affinity of DHT.

Natural androgens (particularly DHT) tend to bind tightly to SBP, a protein which functions as a steroid carrier in the bloodstream of primates and serves as a reservoir that protects steroids from metabolic transformations.[24] Therefore, high binding affinity to SBP can affect the availability and therefore the uptake of a steroid by a target tissue in humans. A related serum steroid carrier protein in rodents, alphafetoprotein, has been shown to affect the tissue distribution of estrogens in rats.[25] The effect of SBP on the distribution of androgens, unfortunately, cannot be studied in rats because rats do not have SBP.

The RBA values of natural androgens and fluorine-substituted androgens to SBP are shown in Table 1. Fluorine substitution at the 11β-position has little effect on their binding affinities to SBP. Because the 17β-hydroxyl group is needed for binding to SBP, the RBA of fluorine-substituted androgens for SBP depends on the site of fluorine as well as other substituents. Introduction of a methyl group at the 7α- or 17α-position of T and DHT decreases their binding to SBP by 2- or 3-fold.[26] By the same token, fluorine substitution at C-16 in DHT lowers the RBA to SBP by 5.5–9-fold,[5] probably because the high electronegativity of the adjacent fluorine atom disrupts hydrogen bond formation between the 17β-OH group and the protein. The binding affinity of natural androgens and 19-nor-DHT to PgR is low and slightly diminished by fluorine substitution (Table 1). Thus, there is little concern that prostate uptake of these steroids will be mediated by PgR.[20]

Octanol–Water Partition Coefficients, Nonspecific Binding, and Binding Selectivity Indices. Octanol–water partition coefficients have been used to predict the lipophilicities of fluorine-substituted estrogens and progestins[3,27a,c] and are well correlated with the nonspecific binding (NSB) of these steroids.[28] Therefore, this coefficient was used to estimate the relative lipophilicity of androgens and subsequently their relative nonspecific binding. The log $P_{o/w}$ values of the androgens were measured by the method of Minick[29] and are given in Table 1. Introduction of a fluorine atom at the 11β-position of DHT and 19-nor-DHT lowers their lipophilicities as measured by log $P_{o/w}$ of 0.4 and 0.75, respectively. Using the direct relationship between log $P_{o/w}$ and nonspecific binding that we have determined for estrogens,[28] we can anticipate that this change in log $P_{o/w}$ will result in a reduction in nonspecific binding by a factor of 1.5 and 2.2, respectively. As will be noted below, the low lipophilicity of 11β-F-DHT and 11β-F-19-nor-DHT is consistent with their low fat uptake.

We have termed the ratio of the RBA to the NSB of a steroid as its binding selectivity index (BSI), and we have used these values to correlate the *in vitro* binding of estrogens[27] and progestins[3b] with the efficiency or selectivity of their target tissue uptake *in vivo*. The BSI values of the androgens are given in Table 1; the relationship to their uptake behavior will be discussed in the section on tissue distribution below. 11β-F-DHT (**1**) shows a relatively high BSI value (43), but the BSI value of 11β-F-19-nor-DHT (**2**) is even higher (92); this is comparable to the BSI value of the androgen standard R1881 (100) and reflects the high AR binding affinity and the low NSB of 11β-F-19-nor-DHT.

Table 2. Tissue Distribution of 11β-[^{18}F]Fluoro-DHT ([^{18}F]-**1**)

tissue	% ID/g[a] ± SD[b]				
	1 h	2 h	2 h (low)	2 h (blocked)	4 h
blood	0.092 ± 0.013	0.038 ± 0.016	0.022 ± 0.006	0.048 ± 0.010	0.028 ± 0.009
muscle	0.059 ± 0.007	0.038 ± 0.015	0.026 ± 0.010	0.023 ± 0.008	0.017 ± 0.001
lung	0.111 ± 0.014	0.069 ± 0.021	0.036 ± 0.008	0.039 ± 0.009	0.035 ± 0.007
spleen	0.048 ± 0.008	0.030 ± 0.012	0.017 ± 0.002	0.026 ± 0.007	0.020 ± 0.003
liver	1.160 ± 0.129	0.451 ± 0.136	0.228 ± 0.062	0.468 ± 0.133	0.415 ± 0.201
kidney	0.417 ± 0.068	0.229 ± 0.075	0.145 ± 0.016	0.200 ± 0.027	0.155 ± 0.049
bladder	1.245 ± 1.083	0.165 ± 0.103	0.263 ± 0.259	0.903 ± 0.839	0.268 ± 0.309
fat	0.061 ± 0.008	0.024 ± 0.004	0.020 ± 0.007	0.026 ± 0.010	0.012 ± 0.005
bone	0.064 ± 0.012	0.061 ± 0.004	0.059 ± 0.007	0.061 ± 0.013	0.072 ± 0.011
prostate (v)[c]	0.486 ± 0.210	0.317 ± 0.067	0.212 ± 0.060	0.069 ± 0.046	0.327 ± 0.106
prostate (d)[c]	0.459 ± 0.222	0.352 ± 0.095	0.229 ± 0.041	0.074 ± 0.041	0.291 ± 0.084
prostate (v)/blood[d]	5.409 ± 2.419	10.058 ± 5.898	10.430 ± 4.153	1.357 ± 0.663	12.249 ± 4.080
(d)	5.155 ± 2.639	10.642 ± 4.426	11.358 ± 4.354	1.465 ± 0.618	10.827 ± 2.655
prostate (v)/muscle[d]	8.208 ± 2.981	9.022 ± 3.073	8.864 ± 3.633	2.895 ± 1.055	19.715 ± 6.430
(d)	7.749 ± 3.277	10.141 ± 3.660	9.780 ± 4.134	3.089 ± 0.918	17.573 ± 5.231

[a] %ID/g, percent injected dose per gram of tissue; normal dose, 45 μCi in 0.25 mL of 10% ethanol–saline; low dose, 9.4 μCi in 0.05 mL; blocked, 100 μg of testosterone was added to each dose. Effective specific activity of [^{18}F]-**1** was 231 Ci/mmol. [b] SD is standard deviation (n = 4). [c] v stands for ventral and d for dorsal. All rats were pretreated with 1 mg of DES in 0.2 mL of sunflower seed oil 24 and 3 h prior to the injection of [^{18}F]-**1**. [d] Ratios represent the average ± SD of the ratios of the individual animals.

Table 3. Tissue Distribution of 11β-[^{18}F]Fluoro-19-nor-DHT ([^{18}F]-**2**)

tissue	% ID/g[a] ± SD[b]				
	1 h	2 h	2 h (low)	2 h (blocked)	4 h
blood	0.089 ± 0.037	0.046 ± 0.014	0.047 ± 0.015	0.061 ± 0.014	0.035 ± 0.019
muscle	0.064 ± 0.023	0.035 ± 0.013	0.037 ± 0.011	0.030 ± 0.003	0.027 ± 0.019
lung	0.115 ± 0.041	0.058 ± 0.020	0.061 ± 0.014	0.054 ± 0.008	0.046 ± 0.025
spleen	0.061 ± 0.022	0.036 ± 0.016	0.049 ± 0.032	0.032 ± 0.003	0.029 ± 0.024
liver	1.812 ± 0.274	0.992 ± 0.285	1.045 ± 0.165	1.189 ± 0.201	0.863 ± 0.124
kidney	0.458 ± 0.118	0.276 ± 0.050	0.251 ± 0.055	0.301 ± 0.059	0.201 ± 0.049
bladder	3.331 ± 5.070	4.549 ± 7.623	0.686 ± 0.479	11.404 ± 7.457	0.456 ± 0.242
fat	0.069 ± 0.007	0.041 ± 0.012	0.041 ± 0.021	0.036 ± 0.007	0.022 ± 0.012
bone	0.068 ± 0.019	0.077 ± 0.006	0.096 ± 0.039	0.065 ± 0.011	0.078 ± 0.012
prostate (v)[c]	0.284 ± 0.154	0.120 ± 0.047	0.109 ± 0.024	0.054 ± 0.026	0.066 ± 0.029
prostate (d)[c]	0.210 ± 0.054	0.132 ± 0.035	0.139 ± 0.028	0.048 ± 0.012	0.084 ± 0.026
prostate (v)/blood[d]	3.054 ± 0.703	2.605 ± 0.590	2.427 ± 0.409	0.880 ± 0.380	2.036 ± 0.726
(d)	2.531 ± 0.758	2.950 ± 0.562	3.126 ± 0.770	0.815 ± 0.253	2.682 ± 1.002
prostate (v)/muscle[d]	4.400 ± 1.947	3.404 ± 0.452	2.993 ± 0.333	1.763 ± 0.705	2.919 ± 1.297
(d)	3.473 ± 1.214	3.882 ± 0.395	3.830 ± 0.531	1.610 ± 0.315	3.980 ± 2.208

[a] %ID/g, percent injected dose per gram of tissue; normal dose, 37 μCi in 0.25 mL of 10% ethanol–saline; low dose, 7.4 μCi in 0.05 mL; blocked, 100 μg of testosterone was added to each dose. Effective specific activity of [^{18}F]-**2** was 670 Ci/mmol. [b] SD is standard deviation (n = 4). [c] v stands for ventral and d for dorsal. See Table 2 for animal pretreatment procedure. [d] Ratios represent the average ± SD of the ratios of the individual animals.

Tissue Distribution Studies. Tracer [^{18}F]-**1** and [^{18}F]-**2** were redissolved in 10% ethanol–saline and injected into diethylstilbestrol (DES)-pretreated mature male rats (162–170 g, n = 4). DES pretreatment was to suppress androgen biosynthesis and thus increase the concentration of unoccupied androgen receptors.[6,30] The normal dose per rat was 45 μCi for androgen [^{18}F]-**1** and 37 μCi for [^{18}F]-**2**. *In vivo* distribution data were obtained at 1, 2, and 4 h postinjection and are shown in Tables 2 and 3.

For both androgens [^{18}F]-**2** and [^{18}F]-**1**, prostate uptake at 2 h was reduced by 2.5–4.7-fold, respectively, by coinjection of an excess of unlabeled testosterone (100 μg), indicating that most of the prostate uptake is mediated by a high-affinity, low-capacity receptor system (Tables 2 and 3, 2 h "blocked"). Previously, 36 μg of testosterone was used in blocking experiments,[6] but this lower dose is now believed not to be sufficient, in some cases, to fully saturate AR in the prostate and thus to fully block receptor-mediated uptake by the prostate. This conjecture is supported by Tveter and Attamada's observation that 500 μg of testosterone was needed to block the prostate uptake of [^{3}H]testosterone to background levels in 24 h castrated mature male rats (200–250 g).[31] The amount of testosterone injected to block target site uptake was therefore increased from 36 to 100 μg in this study.

For one set of rats, the injected dose of fluorine-18-labeled androgen was reduced by 5-fold (9.4 and 7.4 μCi for [^{18}F]-**1** and [^{18}F]-**2**, respectively; Tables 2 and 3, 2 h "low dose"). The ratios of prostate to blood or to muscle were comparable in animals injected with both the normal and low doses of androgens. This demonstrates that the receptor was not saturated by unlabeled impurities present in the tracer. It is also consistent with the relatively high specific activity values (231 and 670 Ci/mmol) obtained for androgens [^{18}F]-**1** and [^{18}F]-**2**.

High uptake in liver, kidney, and bladder suggests that tracers [^{18}F]-**1** and [^{18}F]-**2** are metabolized via phase I and II transformations and excreted in the urine. Prostate uptake and ratios of prostate to blood and to muscle for other fluorine-18-labeled androgens[6] are shown in Figure 2 to provide a comparison to those of androgens [^{18}F]-**1** and [^{18}F]-**2**.

Tissue distribution data for the androgen [^{18}F]-**1** demonstrate selective uptake by the prostate (Table 2, Figure 2). Ratios of prostate to blood and to muscle went up from 5–8 at 1 h to 12–19 at 4 h, an indication that the tracer was being cleared more rapidly from

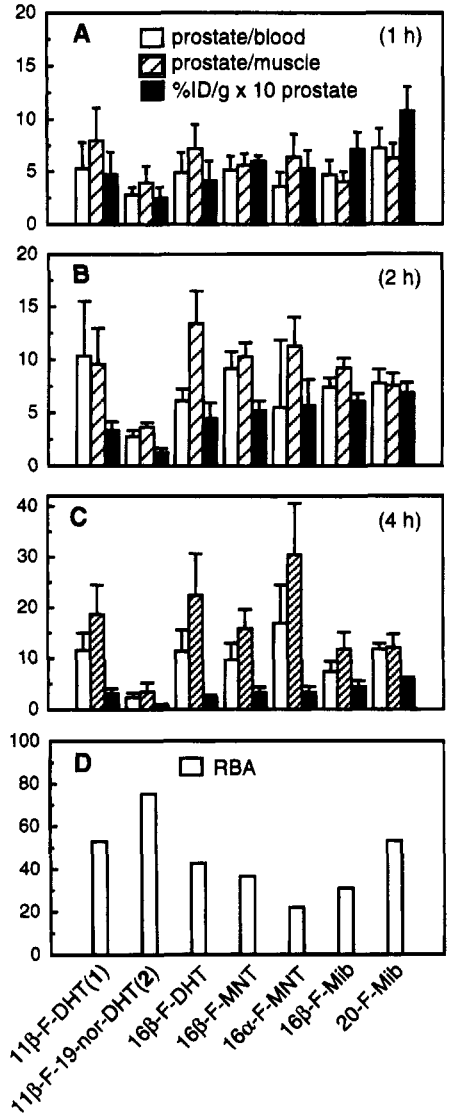


Figure 2. Prostate uptake of fluorine-18-labeled androgens in DES-pretreated rats: 11β-F-DHT—11β-fluoro-5α-dihydrotestosterone (**1**); 11β-F-19-nor-DHT—11β-fluoro-19-nor-5α-dihydrotestosterone (**2**); 16β-F-DHT—16β-fluoro-5α-dihydrotestosterone; 16β-F-MNT—16β-fluoro-7α-methyl-19-nortestosterone; 16α-F-MNT—16α-fluoro-7α-methyl-19-nortestosterone; 16β-F-Mib—16β-fluoro-7α,17α-dimethyl-19-nortestosterone; 20-F-Mib—20-fluoro-7α,17α-dimethyl-19-nortestosterone; A (1 h), B (2 h), and C (4 h), prostate to blood ratio (open bars), prostate to muscle (slashed bars), and prostate uptake shown as % ID/g x 10 (black bars); D, RBA. The prostate values are the average of the uptakes to ventral and dorsal tissues. Data are from Tables 2 and 3 and ref 6.

nontarget tissues than from target tissues; in fact, over this time period, prostate activity levels were retained by 66%. Thus, target to nontarget tissue ratios with [^{18}F]-**1** are better at the 1 and 2 h time points than with other fluorine-18-labeled androgens, including 16β-F-DHT and the synthetic androgens, and only slightly lower than those of 16β-F-DHT and 16α-F-MNT at 4 h (Figure 2). The ligand [^{18}F]-**1** also shows the lowest activity in bone relative to all of the other fluorine-18-labeled androgens (3—45-fold), suffering little metabolic defluorination (Figure 3).

The other high-affinity ligand, [^{18}F]-**2**, shows selective uptake by the prostate but less than other androgens (Table 3, Figure 2). The *in vivo* data indicate that initial uptake is low in prostate and high in liver, kidney, and

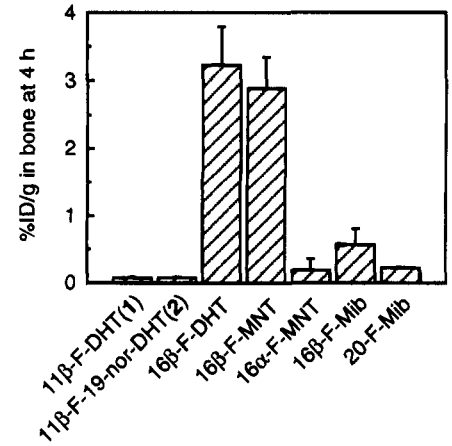


Figure 3. Comparison of bone radioactivity uptake of fluorinated androgens. Data are taken from Tables 2 and 3 and ref 6.

bladder, which may be due to a rapid drop in the blood levels of this androgen (steep blood activity curve), indicative of a high rate of metabolism or clearance. The rapid clearance of the androgen from the target tissue is also of note: only 30% of the initial 1 h prostate uptake was retained at the 4 h time point. As a result, ratios of prostate to blood and to muscle with this 19-nor steroid are the lowest among the fluorine-18-labeled androgens we have studied, and remain at the same level from 1 to 4 h (Figure 2). Bone activity of the tracer [^{18}F]-**2**, however, is comparable to that of the 19-CH_3 counterpart [^{18}F]-**1**, indicating again low *in vivo* defluorination (Figure 3).

The tissue distribution data thus suggest that a fluorine atom at the 11β-position is metabolically stable but that the absence of the 19-CH_3 group makes this androgen metabolically unstable at other sites. This metabolic instability of 19-nor-DHT may also be lessened by the presence of a double bond at the C-4 position (i.e., 19-nor-T), since earlier biodistribution data in castrated male rats demonstrate that [^{3}H]-19-nor-T shows high prostate uptake at 1 h, with 39% of the uptake being retained at 4 h.[32] Moreover, *in vivo* metabolic stability of 19-nor-T can be enhanced by the 7α-CH_3 group with and without the 17α-CH_3 group (MNT and Mib).[6]

Relationship of *in Vitro* Binding Characteristics and *in Vivo* Tissue Distribution. It is convenient to think of the target tissue uptake of a receptor binding radiopharmaceutical in terms of "efficiency" and "selectivity", efficiency being defined as the target tissue uptake (% ID/g) that is specific or receptor mediated (i.e., uptake in untreated animals (total) minus uptake in blocked animals (nonspecific)) and selectivity being defined as the percent of the uptake that is specific (i.e., the specific uptake as a percent of total uptake). In studies of estrogens[27] and progestins,[3b] we have frequently found that the target site uptake efficiency or selectivity (or both) of a series of analogs correlated well with their BSI values. However, no such correlation is found with the two F-18-labeled androgens studied here: while 11β-F-DHT, which has high BSI and RBA values, shows good uptake efficiency and selectivity (0.263% ID/g and 79%, respectively; comparable to the best of the F-18-labeled androgens we have studied so far: 20-F-Mib, 0.558% ID/g and 81%, respectively), 11β-F-19-nor-DHT, which has even *higher* BSI and RBA

values, has *lower* uptake efficiency and selectivity (0.075% ID/g and 60%, respectively).

Ligands with reasonably high binding affinity and low lipophilicity are usually selected for *in vivo* tissue distribution studies. However, in addition to their *in vitro* specific and nonspecific binding affinities, their metabolic fate and pharmacokinetics are also important factors in determining the outcome of *in vivo* tissue distribution studies. From data given in Figure 2, the RBA of most androgens correlates relatively well with their target to nontarget uptake ratios and the extent of their prostate uptake, except for two ligands, 11β-F-19-nor-DHT and 16α-F-MNT (Figure 2). The former shows the poorest prostate uptake and target to nontarget ratio, despite having the highest RBA (75.3) and BSI (92), whereas the latter shows the highest ratios, despite its relatively low binding affinity (21.9). The low initial prostate uptake of 11β-F-19-nor-DHT (Table 3) could be accounted for by rapid metabolism, although this would not explain its poor retention by the target organ. Since the metabolism of fluorine-substituted 19-nor-DHT has not been studied *in vivo*, its metabolic rate and fate are unknown. Even though 19-nor-DHT is expected to be metabolized in a similar fashion to that of its 19-CH_3 counterpart, DHT,[33] 11β-F-19-nor-DHT may be considerably more susceptible to metabolism because it is the most polar and least sterically hindered androgen labeled with fluorine-18. Furthermore, the observation that fluorine-substituted 19-norandrogens Mib and MNT demonstrate good *in vivo* properties leads us to believe that the 4-alkene and the 7α-CH_3 group are required for the metabolic stability of androgens with a 19-nor-DHT structure (Figure 2). This study thus suggests that the structure and metabolism of androgens are major factors in determining their *in vivo* distribution properties.

Potential Applications in Prostate Cancer. Androgens [^{18}F]-**1** and [^{18}F]-**2** were prepared via radiohalofluorination–reduction. Even though the total manipulation time for preparation of [^{18}F]-**1** and [^{18}F]-**2** is relatively long (180 min), and the F-18 incorporation yield (2.4–3%) is rather low, these compounds can be synthesized in yields sufficient for further study. Fluorination at the 11β-position increases or maintains the binding affinity of 19-nor-DHT and DHT to AR (Table 1), and both androgens undergo little *in vivo* defluorination (Figure 3). Ligand [^{18}F]-**2** appears to be rapidly metabolized *in vivo*. On the other hand, ligand [^{18}F]-**1** shows efficient and selective prostate uptake and high metabolic stability and thus has promise as an imaging agent for AR-positive prostate cancer. While we initially thought that the high binding affinity this ligand has to SBP might lower prostate uptake in primates, we no longer have this concern. Another androgen with high SBP binding, 16β-[^{18}F]F-DHT, gave better PET images of the prostate in baboons than either 16β-[^{18}F]F-Mib or 20-[^{18}F]F-Mib, two androgens with low affinity for SBP.[7]

Conclusions

We have synthesized two high-affinity ligands, 11β-F-DHT (**1**) and 11β-F-19-nor-DHT (**2**), and their fluorine-18-labeled analogs [^{18}F]-**1** and [^{18}F]-**2** and studied their *in vitro* binding properties and their *in vivo* biodistributions. These androgens are the first examples of steroids which are labeled with fluorine-18 via radiohalofluorination–reductive debromination, thereby highlighting a way to introduce a fluorine-18 at positions where a fluoride displacement reaction is sometimes not feasible. *In vivo* studies of androgen [^{18}F]-**2** show low prostate uptake and high uptake in liver, kidney, and bladder, indicating that this ligand is rapidly metabolized. On the other hand, *in vivo* studies of [^{18}F]-**1** show that this androgen is selectively taken up by the prostate and is well retained with low *in vivo* defluorination. Therefore, it may hold promise as an imaging agent for receptor-positive prostate cancer.

Experimental Section

Thin-layer chromatography (TLC) was performed using Merck silica gel plates with F-254 indicator (0.25 mm) and visualized by phosphomolybdic acid (PMA) or UV illumination. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was performed isocratically on a Varian 5060 liquid chromatograph with a semipreparative C-18 column (Whatman Partisil 10 ODS, 0.9 × 50 cm). For radiolabeled compounds, HPLC was carried out on a Spectra-Physics Model 8700 instrument with a semipreparative SiO_2 column (Whatman Partisil M-9, 0.9 × 50 cm) and an analytical C-18 column (Whatman Partisil 10 ODS, 0.46 × 25 cm). The eluant was monitored at 210 nm for reversed phase HPLC systems and at 254 nm for normal phase systems. Proton magnetic resonance (^{1}H NMR) spectra were obtained on a General Electric QE-300 (300 MHz) spectrometer, and the data are reported in parts per million relative to deuterated solvent as an internal standard ($CDCl_3$, δ 7.26; CD_3OD, δ 3.3). ^{19}F NMR was obtained on a Varian Unity-400 (376.3 MHz) spectrometer, using $CFCl_3$ as an internal standard and $CDCl_3$ as a solvent. Low-resolution electron-impact (EI) mass spectra were obtained on a Finnigan MAT CH-5 spectrometer. High-resolution EI exact mass spectra were recorded on a Varian MAT 731 spectrometer. Both low- and high-resolution fast atom bombardment (FAB) mass spectra were obtained on a VG instrument (ZAB HF). Both low- and high-resolution chemical ionization (CI) mass spectra were recorded on a 70-VSE spectrometer. Most chemicals were obtained from Aldrich (Milwaukee, WI). Steroids were obtained from Steraloids (Wilton, NH) or Upjohn (Kalamazoo, MI). Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois.

A general procedure for product isolation involved an aqueous quench (or aqueous acids or bases) and organic extraction, drying of the extract over Na_2SO_4, and evaporation of solvent *in vacuo*. Product purification was achieved by flash chromatography.[34]

11β,17β-Dihydroxy-4-androsten-3-one (4). 17-Ketone **3** (0.4 g, 1.32 mmol) was dissolved in freshly distilled THF, and $LiAl(t\text{-}BuO)_3H$ (0.34 g, 1.32 mmol) was added. The reaction mixture was stirred at 0 °C for 1 h. Product isolation (EtOAc) and purification (4:1 EtOAc–hexane) afforded diol **4** (0.23 g, 58%) as a white solid: mp 228–230 °C; ^{1}H NMR (CD_3OD) δ 1.01 (s, 3 H, 18-CH_3), 1.47 (s, 3 H, 19-CH_3), 3.52 (t, 1 H, 17α-H), 4.31 (q, 1 H, 11α-H), 5.64 (s, 1 H, 4-H); MS (CI) *m/z* (rel intensity) 305 (M + H$^+$, 100), 287 (21), 269 (28); HRMS calcd for $C_{19}H_{29}O_3$ 305.2117, found 305.2114. Anal. ($C_{19}H_{28}O_3$) C, H.

11β,17β-Dihydroxyandrostan-3-one (5). Liquid ammonia (30 mL) was dissolved in freshly distilled THF (5 mL) at −40 °C, and lithium metal (5.6 mmol) was added. To the deep blue solution was added a solution of diol **4** (0.1 g, 0.33 mmol) in THF (5 mL). After the mixture was stirred at −40 °C for 15 min, the reaction was quenched by addition of solid NH_4Cl.[13] The ammonia was then evaporated, and the product was isolated in EtOAc and purified (4:1 EtOAc–hexane) affording diol **5** (53 mg, 52%) as a white solid: mp 253–254 °C; ^{1}H NMR (CD_3OD) δ 0.97 (s, 3 H, 18-CH_3), 1.29 (s, 3 H, 19-CH_3), 3.51 (t, 1 H, 17α-H), 4.28 (q, 1 H, 11α-H); MS (CI) *m/z* (rel intensity)

307 ($M + H^+$, 100), 289 (24), 271 (51); HRMS calcd for $C_{19}H_{31}O_3$ 307.2273, found 307.2276. Anal. ($C_{19}H_{30}O_3$) C, H.

Diol **5** was also directly obtained in 22% yield from 17-ketone **3** using the procedure described above, but the product was more difficult to purify due to the presence of coeluting impurities.

17β-Hydroxy-9(11)-androsten-3-one (6). Diol **5** (0.46 g, 1.5 mmol) was refluxed in acidic methanol (1:4 concentrated HCl–methanol) for 8 h. Product isolation (EtOAc) followed by purification (5:1 benzene–ethyl acetate) yielded a white solid (0.262 g, 61%): mp 167–168 °C; ^{1}H NMR ($CDCl_3$) δ 0.72 (s, 3 H, 18-CH_3), 1.16 (s, 3 H, 19-CH_3), 3.74 (t, 1 H, 17α-H), 5.39 (d, 1 H, 11α-H); MS (70 eV) *m/z* (rel intensity) 288 (M^+, 56), 270 (18), 255 (62); HRMS calcd for $C_{19}H_{28}O_2$ 288.2089, found 288.2090. Anal. ($C_{19}H_{28}O_2$) C, H.

9α-Bromo-11β-fluoro-17β-hydroxyandrostan-3-one (7). 1,3-Dibromo-5,5-dimethylhydantoin (DBH) (114 mg, 0.4 mmol) was placed in a poly(ethylene) vial, and CH_2Cl_2 was added. A substantial amount of DBH was not soluble until HF–pyridine (62 μL, 2.17 mmol) was added. Alkene **6** (208 mg, 0.72 mmol) was then added, and the solution was stirred at room temperature for 1.5 h. Product was isolated in CH_2Cl_2 and then purified (4:1 benzene–ethyl acetate), giving dihalo steroid **7** (202 mg, 72%) as a white solid: mp 138–140 °C; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 3 H, J = 2.6 Hz, 18-CH_3), 1.47 (d, 3 H, J = 6.0 Hz, 19-CH_3), 3.76 (dd, 1 H, J = 8.7 Hz, 17α-H), 5.28 (dt, 1 H, J = 48.0, 2.7 Hz, 11α-H); MS (FAB) *m/z* (rel intensity) 389 ($M + H^+$, ^{81}Br, 10), 387 ($M + H^+$, ^{79}Br, 10), 308 (2); HRMS calcd for $C_{19}H_{29}O_2{}^{79}$BrF 387.1335, found 387.1336. Anal. ($C_{19}H_{28}O_2$BrF) C, H, Br, F.

11β-Fluoro-17β-hydroxyandrostan-3-one (1). Dihalo steroid **7** (183 mg, 0.47 mmol) was dissolved in benzene, and tributyltin hydride (317 μL, 1.18 mmol) and AIBN (1 grain) were added. After the solution was refluxed for 1 h, it was cooled down and extracted with diluted NH_4OH (1:1 NH_4OH–H_2O). Product isolation (CH_2Cl_2) and purification (1:1 hexane–EtOAc) furnished fluoro-DHT **1** (110 mg, 76%) as a white solid. The sample for RBA assay was further purified by a semipreparative reversed phase HPLC column (2:1 methanol–water, 3 mL/min). The desired product eluted at 36 min: mp 185–187 °C; IR[4] (KBr) 3406 (OH), 1707 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.91 (d, 3 H, J = 2.4 Hz, 18-CH_3), 1.19 (d, 3 H, J = 3.4 Hz, 19-CH_3), 3.64 (t, 1 H, J = 8.3 Hz, 17α-H), 5.09 (dq, 1 H, J = 49, 2.7 Hz, 11α-H); ^{19}F NMR φ −178.3 (qd, 1 F, J = 46.0, 14.4 Hz, 11β-F); MS (70 eV) *m/z* (rel intensity) 308 (M^+, 7), 288 (55), 273 (19), 255 (12); HRMS calcd for $C_{19}H_{29}O_2F$ 308.2152, found 308.2149. Anal. ($C_{19}H_{29}O_2F$) C, H, F.

11β-Hydroxy-3-methoxyestra-1,3,5(10)-trien-17-one (9). Ketal **8** (5 g, 14.5 mmol) was dissolved in acetone (200 mL) and treated with 3 N HCl (30 mL). The reaction solution was stirred at room temperature for 2 h. Product isolation (EtOAc) afforded ketone **9** (4.32 g, 99%) as a white solid: mp 158–159 °C; ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 3 H, 18-CH_3), 1.56 (s, 3 H, 19-CH_3), 3.79 (s, 3 H, 3-OCH_3), 4.79 (q, 1 H, J = 2.8 Hz, 11α-H), 6.69 (d, 1 H, J = 2.6 Hz, 4-H), 6.77 (dd, 1 H, J = 8.6, 2.7 Hz, 2-H), 7.21 (d, 1 H, J =8.6 Hz, 1-H); MS (70 eV) *m/z* (rel intensity) 300 (M^+, 84), 282 (5), 269 (10); HRMS calcd for $C_{19}H_{24}O_3$ 300.1725, found 300.1725. Anal. ($C_{19}H_{24}O_3$) C, H.

11β-Hydroxy-3-methoxyestra-1,3,5(10)-trien-17β-ol (10). 11β-Hydroxy-17-one **9** (4.3 g, 14.3 mmol) was dissolved in ethanol (200 mL), and $NaBH_4$ (1.09 g, 28.6 mmol) was added. The heterogeneous solution was stirred at room temperature for 1 h. The product isolation (EtOAc) provided diol **10** (4.3 g, 99%) as a white solid: mp 145–147 °C; ^{1}H NMR ($CDCl_3$) δ 1.03 (s, 3 H, 18-CH_3), 1.49 (s, 3 H, 19-CH_3), 3.71 (t, 1 H, 17α-H), 3.78 (s, 3 H, 3-OCH_3), 4.74 (q, 1 H, J = 3 Hz, 11α-H), 6.67 (d, 1 H, J = 2.5 Hz, 4-H), 6.76 (dd, 1 H, J = 8.5, 2.7 Hz, 2-H), 7.21 (d, 1 H, J = 8.5 Hz, 1-H); MS (70 eV) *m/z* (rel intensity) 302 (M^+, 89), 284 (16), 266 (4); HRMS calcd for $C_{19}H_{26}O_3$ 302.1882, found 302.1883. Anal. ($C_{19}H_{26}O_3$) C, H.

11β,17β-Dihydroxy-4-estren-3-one (11). To diol **10** (1.5 g, 4.97 mmol) in THF (80 mL) at −40 °C was added liquid ammonia (150 mL) followed by lithium metal (149 mmol). After the reaction mixture was stirred at −40 °C for 30 min, the reaction was quenched by slow addition of absolute ethanol (20 mL). The ammonia was evaporated, the product was isolated in CH_2Cl_2, and the resulting residue was dissolved in methanol (60 mL) and treated with 3 N HCl (30 mL). The reaction mixture was heated to 65 °C for 1 h, and the product was isolated in CH_2Cl_2. Purification (6:1 EtOAc–hexane) furnished 0.74 g (51%) of the white solid enone **11**: mp 215–216 °C; ^{1}H NMR (CD_3OD) δ 1.00 (s, 3 H, 18-CH_3), 3.55 (t, 1 H, J = 8.2 Hz, 17α-H), 4.14 (d, 1 H, J = 2.9 Hz, 11α-H), 5.82 (s, 1 H, 4-H); MS (CI) *m/z* (rel intensity) 291 ($M + H^+$, 100), 273 (72), 255 (41); HRMS calcd for $C_{18}H_{27}O_3$ 291.1960, found 291.1961. Anal. ($C_{18}H_{26}O_3$) C, H.

17β-[(*tert*-Butyldimethylsilyl)oxy]-11β-hydroxy-4-estren-3-one (12). To a solution of diol **11** (1.16 g, 4 mmol) in CH_2Cl_2 (100 mL) was added 2,6-lutidine (700 μL, 6 mmol) followed by TBS triflate (918 μL, 4 mmol).[17] The reaction mixture was stirred at 0 °C for 30 min. The product isolation (CH_2Cl_2) and purification (2:1 hexane–EtOAc) afforded TBS ether **12** (1.23 g, 76%) as a white solid: mp 192–193 °C; ^{1}H NMR ($CDCl_3$) δ −0.004 (s, 3 H, Si-CH_3), 0.004 (s, 3 H, Si-CH_3), 0.88 (s, 9 H, *t*-Bu), 0.99 (s, 3 H, 18-CH_3), 3.54 (t, 1 H, 17α-H), 4.19 (q, 1 H, J = 2.8 Hz, 11α-H), 5.85 (s, 1 H, 4-H); MS (FAB) *m/z* (rel intensity) 405 ($M + H^+$, 100), 387 (61), 329 (5); HRMS calcd for $C_{24}H_{41}O_3Si$ 405.2825, found 405.2813. Anal. ($C_{24}H_{40}O_3$-Si) C, H, Si.

17β-[(*tert*-Butyldimethylsilyl)oxy]-11β-hydroxyestran-3-one (13). The desired product **13** was obtained using the procedure described for the synthesis of diol **5**. Enone **12** (1.2 g, 2.97 mmol) was added to a THF solution of liquid ammonia (300 mL) and lithium metal (51 mmol) at −40 °C.[13] Product isolation (EtOAc) and purification (3:1 benzene–EtOAc) afforded 0.95 g (79%) of the white solid **13**: mp 181–182 °C; ^{1}H NMR ($CDCl_3$) δ −0.005 (s, 3 H, Si-CH_3), 0.004 (s, 3 H, Si-CH_3), 0.88 (s, 9 H, *t*-Bu), 0.96 (s, 3 H, 18-CH_3), 3.54 (t, 1 H, 17α-H), 4.20 (q, 1 H, J = 2.9 Hz, 11α-H); MS (70 eV) *m/z* (rel intensity) 406 (M^+, 3), 349 (100); HRMS calcd for $C_{24}H_{42}O_3Si$ 406.2903, found 406.2894. Anal. ($C_{24}H_{42}O_3Si$) C, H, Si.

17β-[(*tert*-Butyldimethylsilyl)oxy]-9(11)-estren-3-one (14). TBS ether **13** (0.92 g, 2.37 mmol) was dissolved in DMF (20 mL), and pyridine (20 mL) and methanesulfonyl chloride (1.3 mL, 17.2 mmol) were added.[4] The reaction mixture was heated to 85 °C for 1 h and cooled down to room temperature. The product was isolated in EtOAc after dilution with water and washed with 1 N $CuSO_4$. The off white solid alkene **14** (0.59 g, 68%) was obtained after purification (10:1 hexane–EtOAc) and was contaminated with 7–8% of 17β-[(*tert*-butyldimethylsilyl)oxy]-11-estren-3-one: ^{1}H NMR ($CDCl_3$) δ 0.01 (s, 3 H, Si-CH_3), 0.02 (s, 3 H, Si-CH_3), 0.69 (s, 3 H, 18-CH_3), 0.88 (s, 9 H, *t*-Bu), 3.66 (t, 1 H, J = 8.4 Hz, 17α-H), 5.32 (d, 1 H, J = 4.4 Hz, 11-H); MS (FAB) *m/z* (rel intensity) 389 ($M + H^+$, 68), 371 (65), 331 (14); HRMS calcd for $C_{24}H_{41}O_2Si$ 389.2876, found 389.2882. Anal. ($C_{24}H_{40}O_2Si$) C, H, Si.

17β-Hydroxy-9(11)-estren-3-one (15). A Teflon flask was charged with TBS ether **14** (0.53 g, 1.37 mmol) in CH_3CN (50 mL), and HF (49% in H_2O, 24.5 mmol) was added.[18] The reaction mixture was stirred at room temperature for 10 h and then treated with an excess of 8% $NaHCO_3$ (aqueous). The product isolation (EtOAc) and purification (3:1 benzene–EtOAc) furnished 0.36 g (96%) of the desired product **15** as a white solid: mp 140–142 °C; ^{1}H NMR ($CDCl_3$) δ 0.73 (s, 1 H, 18-CH_3), 3.75 (t, 1 H, J = 8.7 Hz, 17α-H), 5.36 (dd, 1 H, J = 5.8, 1.2 Hz, 11-H); MS (70 eV) *m/z* (rel intensity) 274 (M^+, 47), 256 (22), 241 (13); HRMS calcd for $C_{18}H_{26}O_2$ 274.1933, found 274.1934. Anal. ($C_{18}H_{26}O_2$) C, H.

9α-Bromo-11β-fluoro-17β-hydroxyestran-3-one (16). Dihalo steroid **16** was obtained by the procedure described for the synthesis of dihalo **7**. Alkene **15** (65 mg, 0.24 mmol) was added to a CH_2Cl_2 solution of DBH (37 mg, 0.13 mmol) and HF–pyridine (20 μL, 0.71 mmol). Product isolation in CH_2Cl_2 followed by purification (4:1 benzene–EtOAc) afforded dihalo steroid **16** as a white solid (71 mg, 81%): mp 135–137 °C; ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 2.1 Hz, 18-CH_3), 3.77 (dd, 1 H, J = 8.8 Hz, 17α-H), 5.11 (dt, 1 H, J = 47.1, 2.7 Hz, 11α-H); MS (FAB) *m/z* (rel intensity) 375 ($M + H^+$, ^{81}Br, 20), 373 ($M + H^+$, ^{79}Br, 22); HRMS calcd for $C_{18}H_{27}O_2{}^{79}$BrF 373.1178, found 373.1172. Anal. ($C_{18}H_{26}O_2$BrF) C, H, Br, F.

11β-Fluoro-17β-hydroxyestran-3-one (2). Fluoro-19-nor-DHT **2** was synthesized by the same procedure described for

the synthesis of fluoro-DHT **1**. A benzene solution of dihalo steroid **16** (56 mg, 0.15 mmol), tributyltin hydride (100 μL, 0.38 mmol), and 1 grain of AIBN was refluxed for 1 h. The product isolation (CH_2Cl_2) and purification (5:4 hexane–EtOAc) furnished the white solid fluoro-19-nor-DHT **2** (34 mg) in 76% yield. The sample for RBA assay was further purified by a semipreparative reversed phase HPLC column (1:1 CH_3CN–water, 4.5 mL/min). The desired product eluted at 25 min: mp 163–164.5 °C; IR[4] (neat) 3412 (OH), 1711 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 1.8 Hz, 18-CH_3), 3.65 (t, 1 H, J = 8.2 Hz, 17α-H), 4.98 (dq, 1 H, J = 48, 2.7 Hz, 11α-H); ^{19}F NMR ϕ −187.7 (tdd, 1 F, J = 47.7, 38.3, 13.7 Hz, 11β-F); MS (70 eV) m/z (rel intensity) 294 (M^+, 22), 274 (100); HRMS calcd for $C_{18}H_{27}O_2F$ 294.1995, found 294.1996. Anal. ($C_{18}H_{27}O_2F$) C, H, F.

Radiochemical Synthesis. Fluorine-18 was produced from $[^{18}O]H_2O$ as previously reported.[35] An appropriate amount of radioactivity in $[^{18}O]H_2O$ was placed in a poly(ethylene) vial, and three azeotropic distillations were carried out each time with 0.5 mL CH_3CN in an oil bath at 85 °C under a gentle stream of N_2. The vial was cooled to room temperature, and 400 μL of CH_2Cl_2 was added. To this was added DBH (7.2 mg, 25 μmol) followed by sulfuric acid (0.25 μL, 5 μmol). A substantial amount of DBH was solubilized after addition of the acid. The solution containing a brown droplet of sulfuric acid was vortexed for 1 min, and then the alkene **6** or **15** (7 mg, 25 μmol) was added. The reaction mixture was vortexed for another 1 min and stirred at either room temperature or 40 °C for 15 min (final yields were equivalent). At the end of the reaction, the mixture was passed through a parfait column (a short pipette filled sequentially with a piece of cotton, 0.5 cm of Florisil, and 0.5 cm of Na_2SO_4) with a 1:1 mixture of CH_2Cl_2 and EtOAc. The solvents were removed in a water bath under a gentle stream of N_2. The resulting residue was redissolved in 1 mL of benzene, and tributyltin hydride (10 μL, 37.2 μmol) and AIBN (12–15 mg, 70–90 μmol) were added. The reaction mixture was refluxed in an oil bath at 85 °C for 20 min and cooled down to room temperature before it was treated with NH_4OH (20 μL). The resulting solution containing white precipitates was directly passed through a SiO_2 parfait column as described above. The column was washed with a 1:1 mixture of CH_2Cl_2 and EtOAc. The solvents (3 mL) were evaporated *in vacuo*, and the residue was redissolved in 0.6 mL of CH_2Cl_2 containing 5% *i*-PrOH, filtered through a syringe needle filter, and diluted with 1.4 mL of hexane. The sample was injected onto a semipreparative normal phase HPLC column and eluted with a mixture of HPLC solvents (7:3 hexane–CH_2Cl_2 containing 5% *i*-PrOH, 5 mL/min). The eluant was monitored simultaneously by a UV detector (254 nm) and a NaI radioactivity detector. The desired product, which eluted at 45 min, was collected for tissue distribution studies, radiochemical purity, and effective specific activity determination. The total reaction and purification time was 180 min, and decay-corrected radiochemical yields were 3.1% and 2.4–3.2% for the tracers [^{18}F]-**1** and [^{18}F]-**2**, respectively; their effective specific activities were 231 and 670 Ci/mmol. The tracer was further analyzed by an analytical reversed phase HPLC column (1:1 phosphate buffer (0.01 M)–acetonitrile, 1 mL/min), and the eluant was monitored by a UV detector (210 nm) and a radioactivity detector. Retention times were 14 min for the tracer [^{18}F]-**1** and 12 min for [^{18}F]-**2**.

Relative Binding Affinity, Specific Activity, and Octanol–Water Partition Coefficient Determinations. Relative binding affinities (RBA) of androgens were measured against [^3H]R1881 in AR,[4,36] [^3H]R5020 in PgR,[4,37] and [^3H]-estradiol in SBP[25a] as previously described. RBA values of the standards are 100 by definition.

Samples for determination of effective specific activity of the fluorine-18-labeled androgens were obtained from the same fractions as were injected into rats. The activities[38] were determined from the decayed sample of known radioactivity by competitive binding assays to AR.

Octanol–water partition coefficients were estimated, as previously reported,[27c] from log K'_w values determined by HPLC chromatography, following the recommendations of Minick.[29] NSB and BSI were calculated relative to R1881 as the standard, using an empirically derived relationship.[28]

Tissue Distribution Studies. Mature male Sprague–Dawley rats (162–170 g, four rats per time point) were injected subcutaneously with 1 mg of DES in 0.2 mL of sunflower seed oil at 24 and 3 h prior to the experiment.[6,30] Fluorine-18-labeled androgens collected from HPLC were concentrated *in vacuo*, redissolved in ethanol, and diluted with saline to give a final solution of 10% ethanol in saline. The androgen (37–45 μCi in 0.25 mL of 10% ethanol/saline per rat) was injected into rats intravenously via tail vein. For one set of rats, the injected dose was reduced by 5-fold (7.4–9.4 μCi in 0.05 mL of 10% ethanol/saline; "low" or low dose). The uptake data from this set were used as a control for those administered the normal dose. For another set of rats, 100 μg of testosterone was added to the injected dose to block receptor-mediated uptake.

At the indicated time points (1, 2, and 4 h), the rats were sacrificed, and samples of blood and tissue were removed, weighed, and counted as described previously.[39] The injected dose was calculated from standards prepared from the injection solution. The data were expressed as percent injected dose per gram of tissue (% ID/g).

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