# 7α-Iodo and 7α-Fluoro Steroids as Androgen Receptor-Mediated Imaging Agents

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We have synthesized several 7α-fluoro (F) and 7α-iodo (I) analogues of 5α-dihydrotestosterone (5α-DHT) and 19-nor-5α-dihydrotestosterone (5α-NDHT) and tested them for binding to the androgen receptor and for their biological activity in an in vitro assay with cells that have been engineered to respond to androgens. The relative binding affinity to the androgen receptor determined in competition assays showed that in the androstane series the fluoro steroids have the highest affinity and that F-17α-$CH_3$-DHT (**4**) has a higher affinity than 5α-DHT. All other steroids were somewhat less potent than 5α-DHT with F-DHT (**2**) = I-17α-$CH_3$-DHT (**3**) ≥ F-NDHT (**6**) > F-17α-$CH_3$-NDHT (**8**) = I-DHT (**1**) ≥ I-NDHT (**5**) > I-17α-$CH_3$-NDHT (**7**). The relative biological activity in cells transfected with the androgen receptor and an androgen responsive reporter gene is **4** ≫ 5α-DHT > **2** > **6** > **3** ≥ **1** ≥ **8** ≥ **5** > **7**. The iodinated compound, I-17α-$CH_3$-DHT (**3**)**,** with the highest binding activity was synthesized labeled with $^{125}$I and was shown to bind with high affinity, $K_a = 1.9 \times 10^{10}$ L/mol, and low nonspecific binding to the androgen receptor in rat prostatic cytosol. However, when radiolabeled [$^{125}$I]-17α-$CH_3$-DHT ([$^{125}$I]**3**) was injected into castrated male rats, it showed very poor androgen receptor-mediated uptake into the rat prostate. This was unexpected in light of its superior receptor binding properties and its protection by the 17α-methyl group from metabolic oxidation at C-17. However, the biological potency of I-17α-$CH_3$-DHT (**3**) was not as high as would have been expected. When I-DHT (**1**) and I-17α-$CH_3$-DHT (**3**) were incubated in aqueous media at 37 °C they rapidly decomposed, but they were stable at 0 °C. The fluorinated analogue **4** treated similarly at 37 °C was completely stable. The products of the decomposition reaction of I-DHT (**1**) at 37 °C were identified as iodide and principally 17β-hydroxy-5α-androst-7-en-3-one. The temperature dependence of this elimination reaction explains the inconsistency between the high binding to the androgen receptor (measured at 0 °C) and the low biological activity, as well as the poor androgen receptor mediated concentration in vivo. The fluorinated analogue F-17α-$CH_3$-DHT (**4**) has both high affinity for the androgen receptor and high stability in aqueous media. Of the compounds tested, **4** has the highest affinity for the androgen receptor as well as the highest androgenic activity. Thus it is likely that F-17α-$CH_3$-DHT **4** labeled with $^{18}$F will be an excellent receptor-mediated diagnostic imaging agent.

## Introduction

The design and synthesis of ligands for steroid receptors that are labeled with various radioactive halogens has led to important imaging agents as well as probes for the study of hormone action. Different strategies have produced estrogens and progestins substituted with radioactive halogens at various positions in the steroid nucleus or at short side chains. Some halogenated estrogens[1−4] and progestins[5−8] have been shown to bind with high affinity to their receptors and concentrate in steroid target tissues in a receptor-dependent manner, and thus they have the potential to act as imaging agents for the detection of hormone responsive cancer. For this reason, isotopically labeled androgens have been sought to detect hormone responsive prostate tumors and metastases. Androgens labeled with $^{18}$F at C-11β, C-16α, C-16β, or C-20 (at a 17α-methyl substituent) for positron emission tomography (PET) have been shown to bind with high affinity and specificity to the androgen receptor.[9−11] Several have very good androgen target organ (prostate) uptake in vivo. To the contrary, synthesis of androgens with radioiodine for single-photon emission computed tomography (SPECT) has met with only limited success. It has been noted that this has been a difficult problem because the androgen receptor has a more specific binding region which discriminates to a much greater extent than the estrogen receptor.[12]

Although androgens and estrogens with obvious exceptions share many of the same characteristics necessary for high-affinity binding to their receptors, the strategies that produced excellent iodinated ligands for the estrogen receptor did not succeed with androgens. For example, while C-16α radioiodine-labeled estrogens have excellent receptor binding characteristics, 5α-dihydrotestosterone[13] as well as testosterone[14] labeled with iodine at 16α bind very poorly to the androgen receptor. However, as noted above, these same compounds when substituted at C-16 with fluorine are excellent ligands. Likewise, the 17α-iodovinyl group leads to excellent ligands for the estrogen receptor[4] as

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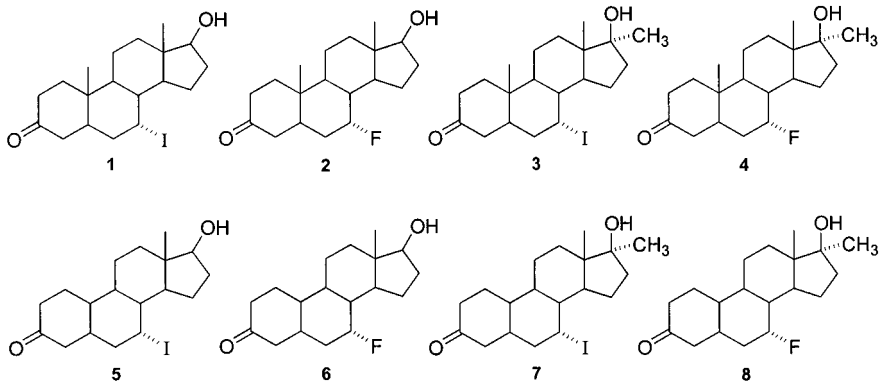



**Figure 1.** Structure of 7α-halosteroids **1**−**8**.

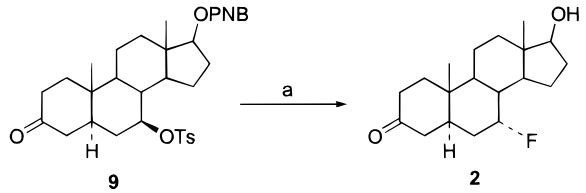
well as the progesterone receptor,[5,6] but this same group, (*E*- or *Z*- 2′-[$^{125}$I]iodovinyl) linked to 5α-dihydrotestosterone leads to poor ligands for the androgen receptor that do not concentrate in androgen target organs in vivo.[15a] Similarly, 7α-methyl-17α-(*E*-2′-[$^{125}$I]-iodovinyl)-19-nortestosterone, an analogue of mibolerone, binds with high affinity to the androgen receptor,[15b] but it and the *Z*-isomer concentrated only slightly in androgen target organs in vivo.[8] While, in general, the major problem in obtaining an androgen receptor-mediated SPECT ligand has been low binding affinity, some have shown good binding to the androgen receptor but none have been useful in vivo.

More recently we have utilized the 7α-postion as a site for radioiodine substitution in order to synthesize a SPECT agent that could be labeled with $^{123}$I.[16,17] We chose substitution at C-7 because androgenic action is potentiated in steroids substituted with a methyl group at the 7α-position.[18] In addition, C-7 is sufficiently distant from C-3 and C-17 that halogen substitution has minimal steric or stereoelectronic effects which might interfere with the important interactions between the receptor and the carbonyl and hydroxyl groups of the steroid. We had synthesized 7α-iodo-5α-dihydrotestosterone (I-DHT) and found that it bound with high affinity and specificity to the androgen receptor. Nevertheless, this iodinated analogue concentrated only poorly in target organs. These results suggested that I-DHT was rapidly catabolized producing inactive metabolites in vivo that were not capable of androgen receptor-mediated localization and that other analogues of 7α-halogenated steroids might be superior imaging agents. Herein, we describe experiments in which we synthesized several 7α-iodo and 7α-fluoro analogues of 5α-DHT and 19-nor-5α-DHT (estranes), some of which contain a 17α-methyl group designed to protect them from oxidation of the 17$\beta$-hydroxyl (Figure 1). We tested these steroids as ligands for the androgen receptor and for their androgenic activity. The most active iodinated analogue, 7α-iodo-17α-methyl-5α-dihydrotestosterone (**3**), was synthesized labeled with radioiodine, [$^{125}$I]**3**, and we determined its binding to the androgen receptor in vitro and its organ distribution in vivo.

## Chemistry

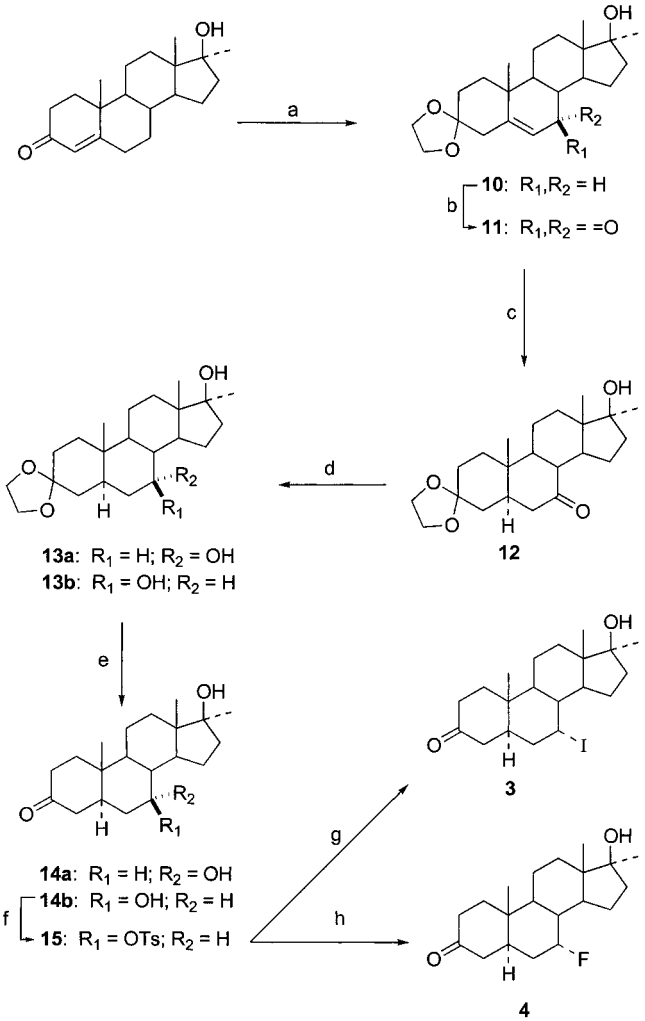
F-DHT (**2**) was prepared (Scheme 1) by fluorination of tosylate **9** (obtained as previously described[17]) with $nBu_4NF$ in MEK. The 17α-$CH_3$ halosteroids **3** and **4** were synthesized from 17α-methyltestosterone as shown in Scheme 2. Ketalization of 17α-methyltestosterone

**Scheme 1**[a]



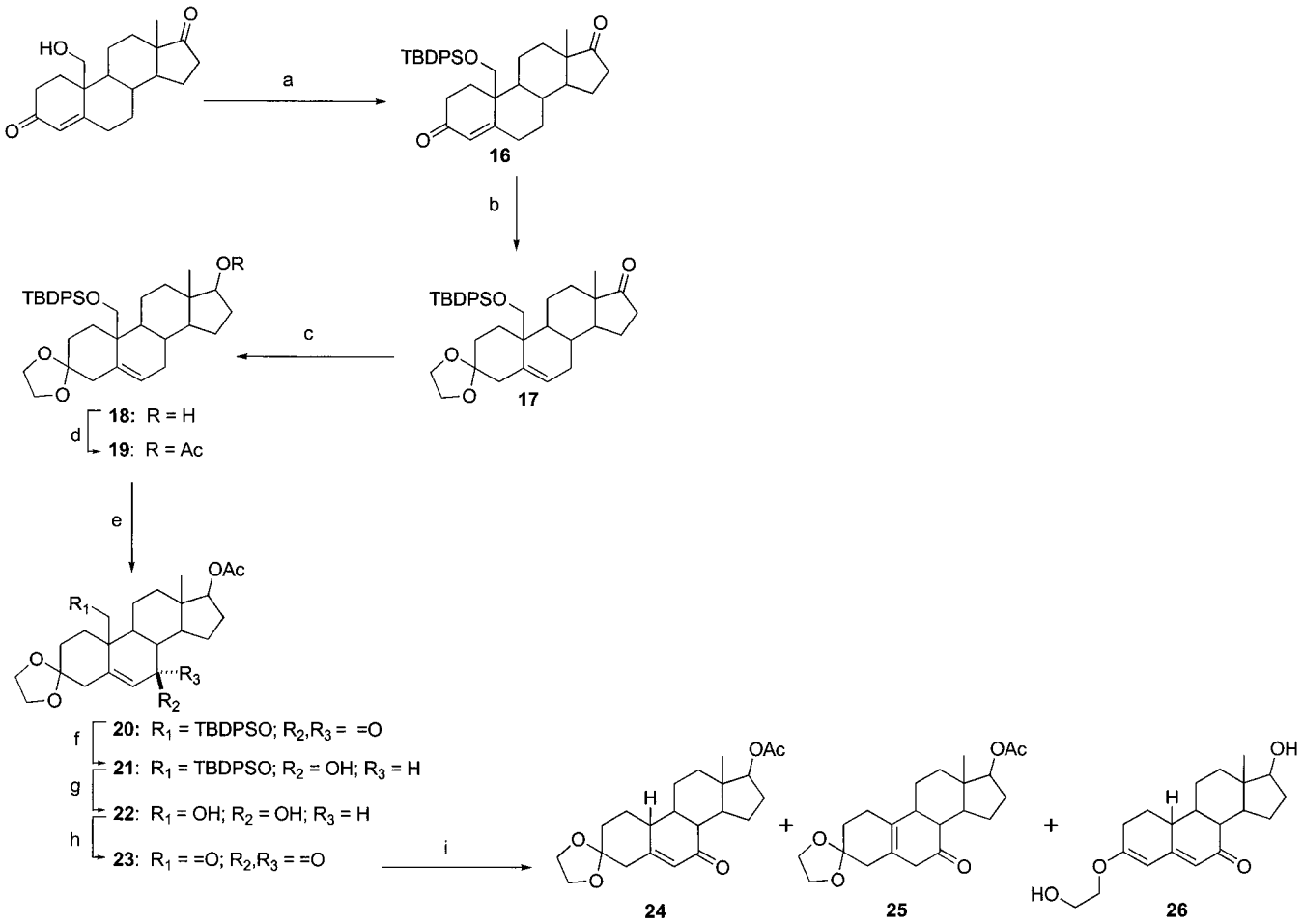

[a] (a) $nBu_4NF$, MEK, 90 °C.

**Scheme 2**[a]




[a] (a) Ethylene glycol, pTsOH, benzene, reflux (17α-methyltestosterone → **10**); (b) $CrO_3$−3,5-dimethylpyrazole, $CH_2Cl_2$, −23 °C (**10** → **11**); (c) 10% Pd/C, $H_2$, MeOH (**11** → **12**); (d) $NaBH_4$−$CeCl_3$, THF−$H_2O$−MeOH (**12** → **13a,b**); (e) pTsOH, acetone−$H_2O$, (**13a,b** → **14a,b**); (f) pTsCl, pyridine, 4 °C (**14b** → **15**); (g) NaI, $CH_3CN$, 83 °C (**15** → **3**); (h) $nBu_4NF$, MEK, 85 °C (**15** → **4**).

**Scheme 3**[a]



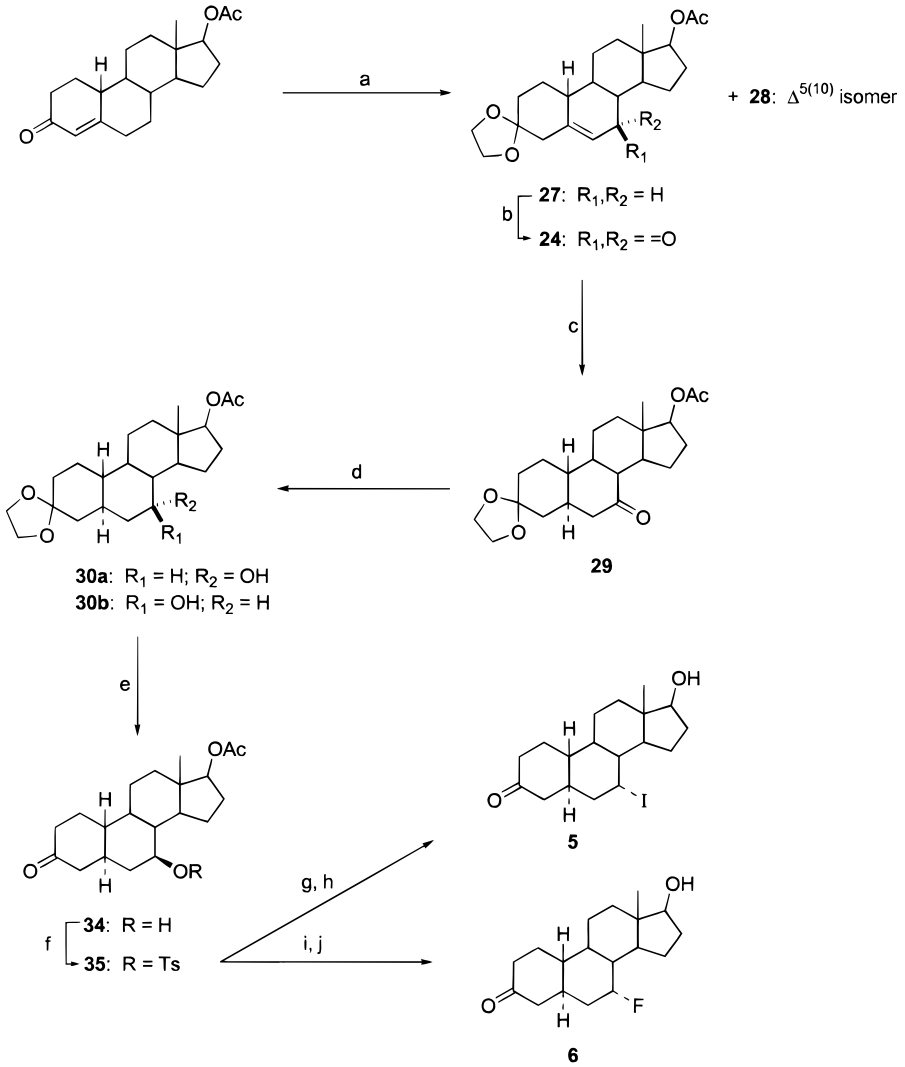

[a] (a) TBDPSCl, imidazole, DMF (19-hydroxyandrostenedione → **16**); (b) pTsOH, 2-ethyl-2-methyl-1,3-dioxolane, 83 °C (**16** → **17**); (c) $NaBH_4$, THF−$H_2O$ (**17** → **18**); (d) $Ac_2O$, pyridine (**18** → **19**); (e) $CrO_3$−3,5-dimethylpyrazole, $CH_2Cl_2$, −23 °C (**19** → **20**); (f) $NaBH_4$−$CeCl_3$, THF−MeOH (**20** → **21**); (g) $nBu_4NF$, THF (**21** → **22**); (h) $CrO_3$−pyridine, $CH_2Cl_2$ (**22** → **23**); (i) KOH, MeOH, 4 °C (**23** → **24**−**26**).

followed by allylic oxidation of the resulting $\Delta^{5(6)}$-ketal with $CrO_3$-3,5-dimethylpyrazole complex[19] gave enone **11**. Hydrogenation followed by reduction of the 7-ketone gave a 1:6 mixture of 7α- and 7β-epimeric C-7 alcohols. After removal of the C-3 ketal with pTsOH/acetone, the desired 7β-alcohol **14b** was isolated by flash chromatography. The 7β-tosylate **15** was prepared from **14b** by reaction with pTsCl in pyridine.[20] The halosteroids **3** and **4** were synthesized by exchange with NaI or $nBu_4NF$, respectively.

Two different routes were attempted for the synthesis of the 19-norsteroids **5**−**8**. In the first route (Scheme 3) the commercially available 19-hydroxyandrost-4-ene-3,17-dione was used as starting material in order to direct isomerization of the $\Delta^{4,5}$-double bond to $\Delta^{5,6}$ during the ketalization reaction and avoid formation of the $\Delta^{5,10}$-steroid. Ordinarily, when a C-10 methyl group is present, as it is in methyltestosterone (Scheme 2), only the $\Delta^{5,6}$-double bond is obtained. The advantage of the C-10 hydroxymethyl group is that it can be facilely removed after conversion to an aldehyde.[21] First the 19-hydroxyl group was protected as the *tert*-butyldiphenylsilyl ether **16**, the 3-ketone was protected as the ketal **17**, and the 17-ketone was reduced with $NaBH_4$ to form the 17β-alcohol **18**. Acetylation of **18** led to **19**, followed by allylic oxidation gave **20**. To remove the silyl group without ketal opening to the dienol ether, the C-7 ketone had to be reduced to the allylic alcohol **21** with $NaBH_4$−$CeCl_3$.[22] The silyl group was removed with $nBu_4NF$ forming **22**. Oxidation of both the 7- and 19-alcohols with $CrO_3$−pyridine gave **23**. Deformylation with methanolic KOH produced the desired enone **24** and the $\Delta^{5(10)}$ isomer **25** as an inseparable mixture as well as the dienol ether **26**. Attempts to isomerize **25** to **24** with continued base treatment led to the formation of **26**. Hydrogenation of the mixture of **24** and **25** led to a further mixture of saturated ketones (uncharacterized). However, this route produced a standard of **24** which was essential in the identification of this intermediate in the complex reaction mixture produced in the allylic oxidation reaction of the next scheme.

The second route (Scheme 4) to **5**−**8** involves the allylic oxidation of 19-nortestosterone 3-ketal. Although a complex mixture of oxidation products was obtained, hence a low yield, the desired enone **24** was obtained cleanly and from an inexpensive starting material. Ketalization of 19-nortestosterone acetate gave a mixture of the $\Delta^{5(6)}$- and $\Delta^{5(10)}$-isomers in 1/1.6 ratio. Allylic oxidation of this mixture using $CrO_3$−3,5-dimethylpyrazole complex gave a mixture of several different oxidation products. Purification by flash chromatography in two different systems followed by crystallization gave **24** in an 8% yield. Hydrogenation of **24** with 10% Pd/C gave **29**, and reduction with $NaBH_4$−$CeCl_3$ gave a 1/3.5

**Scheme 4**[a]




[a] (a) pTsOH, 2-ethyl-2-methyl-1,3-dioxolane, 83 °C (19-nortestosterone acetate → **27**, **28**); (b) $CrO_3$−3,5-dimethylpyrazole, $CH_2Cl_2$, −23 °C (**27** → **24**); (c) 10% Pd/C, $H_2$, MeOH (**24** → **29**); (d) $NaBH_4$−$CeCl_3$, THF−$H_2O$−MeOH (**29** → **30a,b**); (e) pTsOH, acetone−$H_2O$ (**30a,b** → **34**); (f) pTsCl, pyridine, 4 °C (**34** → **35**); (g) NaI, $CH_3CN$, 83 °C (**35** → **36**); (h) $NaCO_3$, MeOH−$H_2O$ (**36** → **5**); (i) $nBu_4NF$, MEK, 85 °C (**35** → **37**); (j) $NaCO_3$, MeOH−$H_2O$ (**37** → **6**).

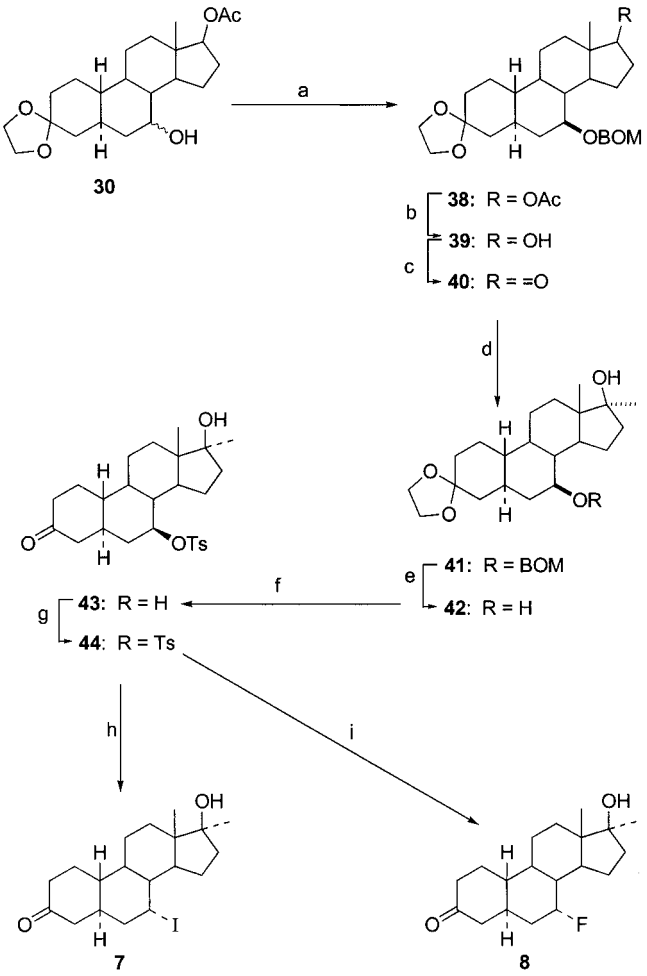
mixture of 7α- and 7*β*-epimeric C-7 alcohols **30a**, **30b**. (Proof that the hydrogenation yielded the 5α-steroid was obtained by converting **30b** into the known 17*β*-hydroxy-5α-estran-3-one (**33**) by tosylation, reductive cleavage with $LiEt_3BH$, and deketalization.) The mixture of **30a**, **30b** was deketalized, and the resulting 3-ketones were purified by chromatography yielding the pure 7*β*-alcohol **34**. Tosylation followed by exchange with NaI or $nBu_4NF$ and deprotection produced **5** and **6**, respectively.

The 17α-methyl 19-norsteroids **7** and **8** were synthesized from the mixture of **30a, 30b** as follows (Scheme 5): protection of the hydroxyl group as the benzyloxymethyl (BOM) ether **38**, deprotection of the 17*β*-hydroxyl group **39**, and oxidation with $CrO_3$−pyridine yielded the 17-ketone **40**. Reaction with $CH_3MgI$ gave the 17α-methylsteroid **41**. Deprotection of the BOM ether by hydrogenolysis followed by deketalization yielded **43**. Tosylation followed by exchange with NaI or $nBu_4NF$ produced **7** and **8**, respectively.

## Results and Discussion

We have synthesized several different steroids (Figure 1) with either iodine or fluorine at C-7α. Previously, we showed that one of these compounds, **1**, is an excellent ligand, binding with high affinity and specificity to the androgen receptor.[16,17] The 5α-reduced structure was chosen because it was analogous to the natural androgen, 5α-DHT. We also attempted to synthesize the corresponding 7α-iodinated ring A unsaturated $\Delta^4$-3-ketones, analogous to testosterone, using a scheme similar to that described for the 5α-reduced steroids. Thus, we developed a method for the stereospecific synthesis of the required precursor, 7*β*-hydroxytestosterone.[23] While we could convert this alcohol to the tosylate, attempted halogen displacement of the latter invariably led to elimination (not described). In all, eight iodinated or fluorinated analogues of 5α-DHT and 19-nor-5α-DHT (Figure 1) were synthesized and tested for binding to the androgen receptor in rat prostate cytosol by competition for the binding of [$^3$H]R1881. As can be seen in Table 1, all of the 7α-halogenated steroids showed fairly good binding to the androgen receptor. Of the iodinated series, both of the 19-nor compounds **5** (47%) and **7** (25%) had the lowest RBAs. The fluoro 19-norsteroids **6** (63%) and **8** (55%) had a somewhat higher affinity for the receptor. Of the 5α-DHT analogues,

**Scheme 5**[a]




[a] (a) BOMCl, $iPr_2EtN$, $CH_2Cl_2$ (**30b** → **38**); (b) 1% KOH−MeOH, 50 °C (**38** → **39**); (c) $CrO_3$−pyridine, $CH_2Cl_2$ (**39** → **40**); (d) $CH_3MgI$, THF (**40** → **41**); (e) 10% Pd/C, $H_2$, THF−$H_2O$ (**41** → **42**); (f) pTsOH, acetone−$H_2O$ (**42** → **43**); (g) pTsCl, pyridine, 4 °C (**43** → **44**); (h) NaI, $CH_3CN$, 83 °C (**44** → **7**); (i) $nBu_4NF$, MEK, 85 °C (**44** → **8**).

**Table 1.** Relative Binding Affinity (RBA) to the Androgen Receptor and Relative Androgenic Activity (RSA)[a]

| compounds | RBA (mean ± SD) | RSA (mean ± SD) |
|---|---|---|
| 5α-DHT | 100 | 100 |
| I-DHT (**1**) | 54 ± 8 | 20 ± 8[b] |
| F-DHT (**2**) | 78 ± 15 | 60 ± 13 |
| I-17α-$CH_3$-DHT (**3**) | 71 ± 6 | 28 ± 11[b] |
| F-17α-$CH_3$-DHT (**4**) | 123 ± 29 | 418 ± 272 |
| I-NDHT (**5**) | 47 ± 5 | 13 ± 4[b] |
| F-NDHT (**6**) | 63 ± 7 | 44 ± 3 |
| I-17α-$CH_3$-NDHT (**7**) | 25 ± 4 | 8 ± 4[b] |
| F-17α-$CH_3$-NDHT (**8**) | 55 ± 7 | 16 ± 7 |

[a] Competition binding analysis: relative binding affinity (RBA) ± SD, compared to 5α-DHT were determined in incubations of rat prostate cytosol at 0−2 °C for 20 h with [$^3$H]R1881. In vitro androgen bioassay: relative stimulatory activity (RSA) ± SEM compared to 5α-DHT was determined with monkey kidney CV1 cells transiently transfected with genes for the androgen receptor and an androgen responsive reporter. Cells were treated with various concentrations of steroids and incubated for 24 h at 37 °C. [b] The RSA values for the iodinated steroids **1**, **3**, **5**, and **7**, do not represent the biological potency of the specified steroids; rather it is probable that the measured potencies are predominantly those of elimination products formed by dehydrohalogenation (see text).

I-17α-$CH_3$-DHT (**3**) (71%) had a higher RBA than I-DHT (**1**) (54%), the original 7α-iodo steroid that we synthesized as a ligand for the androgen receptor.[16,17] In a similar manner, the 17α-methyl fluoro analogue, F-17α-$CH_3$-DHT (**4**) (123%), was a stronger competitor than F-DHT (**2**) (78%). Fluoro analogue **4** proved to be the highest affinity ligand of all the compounds tested.

To better understand the biological activity of these halogenated androgens, their androgenic potency was measured in monkey kidney CVI cells that were transiently transfected with genes for the androgen receptor and an androgen responsive reporter.[24] In contrast to the receptor experiments, the cells are metabolically active. Consequently, the steroids can be metabolized, and we expected that the protection afforded by the 17α-methyl group would be markedly apparent. Thus, we anticipated a large disparity between the androgen receptor competition experiments and the androgen response assay when comparing the same compounds with and without the 17α-methyl group, but this was only found for the fluoro androstane pair **4** (418%) and **2** (60%) (Table 1). The opposite was found for the fluoro estranes, where **8** (16%) was weaker than **6** (44%). In the iodo series the 17α-methyl group appeared not to confer an advantage in androgenic activity: in the androstane series, **3** (28%) versus **1** (20%), and in the estrane series, **7** (8%) versus **5** (13%).

An analysis of the androgen receptor binding data and the androgenic potency data allowed for the formulation of some structure−activity correlations. Considering that the order of decreasing receptor binding affinity (Table 1) of the steroids was F-17α-$CH_3$-DHT (**4**) > 5α-DHT > F-DHT (**2**) = I-17α-$CH_3$-DHT (**3**) ≥ F-NDHT (**6**) > F-17α-$CH_3$-NDHT (**8**) = I-DHT (**1**) ≥ I-NDHT (**5**) > I-17α-$CH_3$-DHT (**7**), the following correlations were noted. (1) All fluoro steroids have higher affinity than their corresponding iodo analogue. (2) For both fluoro and iodo steroids in the androstane series, the presence of a 17α-methyl group increased affinity, while in the estrane series this group decreased receptor affinity. (3) For both fluoro and iodo steroids with a 17α-methyl group, androstanes were better ligands than estranes, whereas for compounds without a 17α-methyl group, androstanes and estranes had similar binding affinity. In the biological assay with the CV1 cells only the fluoro steroids can be compared (discussed below). The order of decreasing stimulatory activity was F-17α-$CH_3$-DHT (**4**) ≫ 5α-DHT > F-DHT (**2**) > F-NDHT (**6**) > F-17α-$CH_3$-NDHT (**8**). Overall, the same structure−activity trends were observed in the bioassay as in the receptor binding assay. Of all the steroids tested only F-$CH_3$-DHT (**4**) was significantly more potent than 5α-DHT.

Since **3** was the most potent of the iodinated steroids, we synthesized it labeled with $^{125}$I, to be able to measure directly the binding of [$^{125}$I]**3** to the androgen receptor. Varying concentrations of [$^{125}$I]**3** were incubated overnight at ∼0 °C with cytosol from rat prostate gland. The binding of the radiolabeled steroid in the presence or absence of saturating amounts of 5α-DHT to determine nonspecific binding is shown in Figure 2. As can be seen, saturation of binding occurred at very low concentrations of the steroid, and nonspecific binding was low. Scatchard analysis (Figure 2, inset) indicated that **3** binds to the androgen receptor with high affinity, $K_d$ = 0.7 nM. Comparison of the saturation analysis with that for [$^3$H]5α-DHT indicated that the [$^{125}$I]**3** was carrier-free, approximately 2200 Ci/mmol. The characteristics

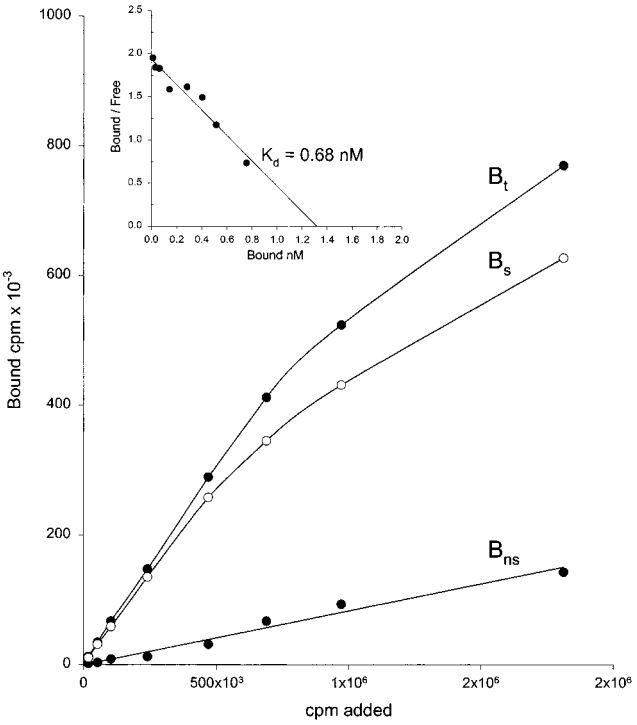



**Figure 2.** Saturation analysis of the binding of [$^{125}$I]**3** to the androgen receptor in rat prostate cytosol. $B_t$, total binding; $B_{ns}$, nonspecific binding determined with a saturating amount, 1 mM, of 5α-DHT; $B_s$, specific binding calculated as $B_s = B_t - B_{ns}$. The graph in the insert is the Scatchard representation of the $B_s$ data.

of [$^{125}$I]**3**—high affinity for the androgen receptor, high specific activity, and low nonspecific binding—indicated that the $^{125}$I steroid was an excellent analytical ligand for the androgen receptor. However, more importantly, because **3** was a better ligand for the androgen receptor than **1** and because the 17α-methyl group affords protection of the 17$\beta$-hydroxyl, it seemed likely that **3** would be a good androgen receptor-mediated imaging agent and that it would be concentrated in vivo in androgen target receptor organs. Consequently, we tested the tissue distribution of [$^{125}$I]**3** injected into castrated male rats. Animals that were injected with sufficient 5α-DHT to saturate the androgen receptor (blocked animals) were included as controls. At different times after the injection of the [$^{125}$I]**3** steroids, the animals were killed, various tissues were removed and weighed, and the radioactivity was counted. The amount of radioactivity per weight of each tissue was calculated and compared to the prostate. As can be seen in Table 2, there was a somewhat greater amount of $^{125}$I in the prostate of the animals injected with [$^{125}$I]**3** at 1 h, 0.39, compared to the androgen-blocked animals 0.23 (% injected dose/g of tissue). There were also higher ratios of prostate/blood and prostate/control tissues in the castrated animals compared to the animals that received saturating amounts of 5α-DHT. Similar results were seen at 2 and 4 h. Nevertheless, the amount of radioactivity in the prostate was very low as was the prostate-to-control tissues ratio. High levels of radioactivity were found in the thyroid, indicative of free iodide from degradation of the iodo steroid. In a similar experiment we compared the distribution of [$^{125}$I]**3** to that of [$^{125}$I]**1**. There was little difference between the two steroids. There was evidence of receptor-mediated uptake in the prostate for both steroids, but the concentration in the target organ was low. In this experiment performed at a single time point, 1 h, the prostate-to-blood ratio of [$^{125}$I]**1** was 1.34 ± 0.06 (5α-DHT blocked = 0.94 ± 0.05) and that of [$^{125}$I]**3** was 0.99 ± 0.08 (α-DHT blocked = 0.75 ± 0.04). The prostate-to-control tissues ratio (spleen, muscle, lung) of [$^{125}$I]**1** was 2.13 ± 0.06 (5α-DHT blocked 1.58 ± 0.09) and that of [$^{125}$I]**3** was 1.82 ± 0.15 (5α-DHT blocked 1.33 ± 0.05). Thus, as seen in the bioassay where **3** was about equipotent with **1** (Table 1), the 17α-methyl group in **3** did not result in a higher concentration in the prostate in the in vivo experiments.

The low uptake of [$^{125}$I]**1** into the prostate seemed likely to be caused by rapid catabolism of the steroid. This was evident by the much lower biological activity of **3** compared to **4**. Although, **3** has a lower RBA for the receptor than **4**, it seemed unlikely that this difference, 71% versus 123%, respectively, could be sufficient by itself to account for the extremely large difference in their androgenic potency. We suspected that oxidation of the 17$\beta$-hydroxyl group might not be the limiting factor in the metabolism of the C-7-iodinated compounds. Thus, we designed an experiment to compare the metabolism of **1** and **3**, measuring their disappearance when incubated with rat liver microsomes. We found that the $^{125}$I steroids were "metabolized" even in heat-denatured microsomal preparations.

**Table 2.** Distribution of Tissue Radioactivity following Intravenous Injection of 7α-[$^{125}$I]-17α-$CH_3$-5α-DHT ([$^{125}$I]**3**) into Castrated Male Rats[a]

| | % injected dose/g | | | | |
|---|---|---|---|---|---|
| tissue | 0.5 h | 1 h | 1 h (blocked)[b] | 2 h | 4 h |
| prostate | 0.21 ± 0.006 | 0.39 ± 0.05 | 0.23 ± 0.01 | 0.44 ± 0.03 | 0.44 ± 0.07 |
| blood | 0.30 ± 0.01 | 0.33 ± 0.03 | 0.35 ± 0.02 | 0.31 ± 0.03 | 0.37 ± 0.07 |
| muscle | 0.06 ± 0.006 | 0.07 ± 0.007 | 0.08 ± 0.01 | 0.07 ± 0.005 | 0.07 ± 0.008 |
| spleen | 0.13 ± 0.03 | 0.16 ± 0.01 | 0.18 ± 0.01 | 0.15 ± 0.008 | 0.17 ± 0.04 |
| fat | 0.20 ± 0.02 | 0.25 ± 0.03 | 0.29 ± 0.04 | 0.20 ± 0.02 | 0.17 ± 0.02 |
| lung | 0.22 ± 0.006 | 0.24 ± 0.02 | 0.28 ± 0.02 | 0.24 ± 0.02 | 0.28 ± 0.04 |
| kidney | 0.26 ± 0.004 | 0.27 ± 0.02 | 0.30 ± 0.03 | 0.25 ± 0.01 | 0.23 ± 0.03 |
| liver | 0.31 ± 0.01 | 0.29 ± 0.007 | 0.36 ± 0.06 | 0.23 ± 0.006 | 0.24 ± 0.03 |
| thyroid[c] | 1.58 ± 0.16 | 4.07 ± 0.95 | 3.55 ± 0.70 | 3.55 ± 0.70 | 9.70 ± 1.14 |
| prostate/blood | 0.70 ± 0.01 | 1.17 ± 0.09 | 0.66 ± 0.03 | 1.43 ± 0.06 | 1.19 ± 0.04 |
| prostate/ Sp.Mu.Lu.[d] | 1.54 ± 0.03 | 2.45 ± 0.14 | 1.30 ± 0.08 | 2.93 ± 0.02 | 2.54 ± 0.08 |

[a] Castrated male rats were injected subcutaneously with [$^{125}$I]**3**, and at the indicated times the animals were killed and the tissues were dissected, weighed, and counted. [b] Blocked animals were injected subcutaneously with 500 mg of 5α-DHT 15 min before the administration of the $^{125}$I tracer. [c] Thyroid also contained adhering esophagus. [d] Sp.Mu.Lu., average of the tissues spleen, muscle and lung; $n = 5$. Values are ± SEM.

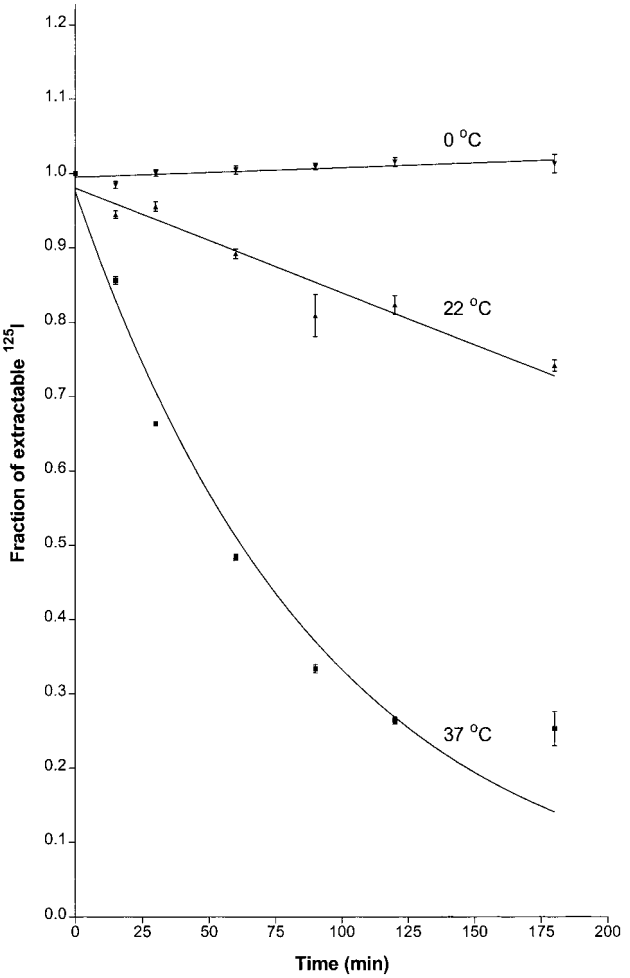



**Figure 3.** Elimination of [$^{125}$I]**3** in aqueous solution. The $^{125}$I-labeled analogue **3** was incubated in buffer at the temperatures and times indicated. The graph shows the plot of the radioactivity that is extracted with EtOAc from the reaction mixture.

Subsequently, we incubated the steroids in buffer at 37, 22, and 0 °C(Figure 3). Both iodinated steroids decomposed in a temperature-dependent manner. Thus, at 37 °C the $t_{1/2}$ for **1** is 134 min and for **3**, 65 min. At 22 °C the decompositions were slower with half-lives of 421 and 951 min, respectively. In contrast, at 0 °C both steroids were stable indefinitely. These studies explained the contradiction between the high affinity and low nonspecific binding of these compounds to the androgen receptor and the poor receptor-mediated uptake in vivo. The former experiments were all performed at 0 °C at which temperature the steroids were stable, in contrast to the in vivo physiological temperature of 37 °C, at which the steroids rapidly decomposed.

To determine the chemistry of the apparent decomposition of the 7-iodo steroids, experiments were carried out to identify the products. The nature of the radioactive [$^{125}$I]product formed at 37 °C from [$^{125}$I]**1** in these experiments was analyzed by TLC, and it was found to be iodide ion, $^{125}I^-$, indicating that dehydrohalogenation had occurred. In separate experiments, the steroid product formed during the incubation of **1** in aqueous buffer at 37 °C was investigated. A single spot was detected by TLC. It was isolated by column chromatography and characterized by HRMS, GC−MS, and $^1$H NMR spectroscopy. GC−MS analysis showed one major component, **45c**, of 84% and two minor components, **45a** (5%) and **45b** (11%), all having the same *m*/*z* (288). $^1$H NMR analysis indicated that the major component **45c** was the $\Delta^7$-steroid, 17$\beta$-hydroxy-5α-androst-7-en-3-one. The two minor components were not isolated separately, but presumably, they are isomeric elimination products. Thus, it was concluded that the 7α-iodo-5α-androstanes are labile under physiological conditions. The dramatic temperature dependence of the elimination reaction explained why the receptor binding studies which were performed at 0 °C showed no hint of the elimination of iodide. [$^{125}$I]Iodide would have been detected as nonspecifically bound radioactivity. Of note, the RBA values in Table 1, which are determined at 0 °C, very likely reflect the affinity of the iodinated compounds for the androgen receptor. On the other hand, since the gene activation studies in transfected cells were performed for 24 h at 37 °C, the temperature at which the iodinated steroids rapidly eliminate, it is likely that the measured potencies were predominantly those of the elimination products. The rapid elimination of the 7α-iodo group in aqueous solution at 37 °C explained why [$^{125}$I]**3**, although it bound with high affinity to the androgen receptor and was protected from facile enzymatic oxidation at C-17, was concentrated only poorly in the prostate of castrated male rats. It also explained the high uptake of radioactivity by the thyroid (Table 2) in the in vivo study. Although we recognized that the axial cycloalkyl C−I bond is susceptible to substitution and elimination, we did not presume that the 7α-iodo group would eliminate so rapidly at 37 °C. It is obvious that the 7α-iodo steroids are too labile for use as receptor-based imaging agents. However, when labeled with $^{125}$I, they can be excellent ligands for sensitive receptor binding studies performed at 0 °C.

The fluoro steroids strongly resist elimination. This was apparent in both the chemical and biological studies (Table 1) of **4**. If **4**, like **3**, had been eliminated, then the same product, **45**, would have been produced and the relative stimulatory activity of the two would have been similar. That the potency of **4** was more than 10 times greater than that of **3** indicated that rapid elimination did not occur. Indeed, **2** and **4** were shown to be stable. When incubated in aqueous media under the same conditions in which **1** was completely eliminated, **2** and **4** were totally unchanged. Thus, it appears likely that the 7α-fluoro steroid **4** will be an extremely useful imaging agent. Of all the 7α-halogens described in this study, this compound has the highest receptor affinity as well as androgenic potency (Table 1). The C−F bond is considerably stronger than the C−I bond, and many $^{18}$F-labeled steroids (or ligands for their receptors) have been shown to have the requisite properties of high affinity and stability for in vivo studies.[9−11,25,26] Thus, **4** labeled with $^{18}$F should be an excellent PET probe for androgen receptor-mediated imaging of prostate tumors and metastases.

## Experimental Section

**General.** Melting points were obtained in a Mel-temp apparatus or a Thomas-Hoover hot stage and are uncorrected. IR spectra were recorded on a Perkin-Elmer model 1600 FT-IR or Beckman AccuLab 4 instruments. $^1$H NMR spectra were recorded with the following instruments: Bruker WP100SY

(100 MHz), GE Omega-300 (300 MHz), or Bruker AM500 (500 MHz); chemical shifts are reported relative to residual $CHCl_3$. Purification by flash chromatography was performed according to the procedure of Still[27] using 230−400 mesh silica gel (EM Science). Solvents for chromatography and extraction were reagent or HPLC grade. GC−MS experiments were performed with a Hewlett-Packard system consisting of a model 5890 GC interfaced with a model 5972 mass selective detector at 70 eV. The injector temperature was 250 °C, and the detector temperature was 280 °C. The GC column was operating at a flow rate of 1 mL/min (He) at an initial temperature of 200 °C for 5 min, then a temperature gradient of 10 °C/min for 5 min to 250 °C, and held. Low- and high-resolution fast atom bombardment (FAB) mass spectra were obtained on a VG instrument (FAB SE) using a matrix of *m*-nitrobenzyl alcohol or Magic Bullet + PEG by DR. Walter J. McMurray at the Yale University Comprehensive Cancer Center. Elemental analyses were performed by Schwarzkopf Micro Analytical Laboratory, Woodside, NY. The computer program Prism was purchased from GraphPad Software Inc. (San Diego, CA).

The cell culture reagents were obtained from Gibco-BRL (Grand Island, NH). Monkey kidney CV1 cells were from American Type Culture Collection (Rockville, MD). pZ523 columns were from 5 Prime→ 3 Prime, Inc. (Boulder, CO). Poly-L-lysine was purchased from Sigma (St Louis, MO). Carrier-free $Na^{125}I$, [17α-methyl-$^3$H]methyltrienolnone (R1881), 83.3 Ci/mmol, [$^3$H]5α-DHT, 81 Ci mmol, and [$^3$H]choramphenicol were from DuPont New England Nuclear Co., North Billerica, MA. 19-Hydroxyandrostene-3,17-dione was from Fluka, Buchs, Switzerland; all other nonradioactive steroids were from Steraloids Inc., Newport, RI. Tetrahydrofuran (THF) was distilled from benzophenone ketyl under $N_2$ immediately before use. Other reagents were from Aldrich (Milwaukee, WI) and were used without further purification.

**Chromatographic Systems.** Thin-layer chromatography (TLC) was performed using Merck silica gel plates ($F_{254}$) (EM Science, Darmstadt, Germany) and visualized using phosphomolybdic acid or UV illumination. TLC systems: T-1, hexanes/EtOAc (1:1); T-2, hexanes/EtOAc (2:1); T-3, hexanes/EtOAc (1:3); T-4, hexanes/EtOAc (1:2), T-5, hexanes/EtOAc (3:1); T-6, toluene/EtOAc (8:1); T-7, isooctane/EtOAc (1.5:1); T-8, acetone/butanol/30% aqueous $NH_4OH/H_2O$ (65:20:10:5). Analytical high-performance liquid chromatography (HPLC) was performed isocratically on a Waters 600E system equipped with an Omniscribe recorder and a Waters 484 variable wavelength detector. Radiolabeled compounds were purified by HPLC using a Waters modular system consisting of a U6K injector, a M-45 pump, a model 440 detector set at 254 nm, and a reverse-phase column (Altex Ultrasphere 5 μm ODS, 4.6 mm i.d. × 25 cm). Analytical HPLC systems were all at 1 mL/min: H-1, Diol column (LiChrosorb Diol, 10 mm, 4.6 mm × 25 cm, EM Science, Darmstardt, Germany) isooctane/iPrOH (9:1); H-2, RP-18 column (LiChrosorb RP-18, 5 μm, 4.6 mm × 25 cm, EM Science) $CH_3CN/H_2O$ (1:1); H-3, Altex Ultrasphere ODS (5 μm 4.6 mm × 25 cm) MeOH/$H_2O$ (65:35); H-4, Altex Ultrasphere ODS (5 μm, 4.6 mm × 25 cm) MeOH/$H_2O$ (70:30). Semipreparative HPLC (system H-p) was performed on a Waters Protein I-60 column (7.8 mm × 30 cm), isooctane/iPrOH (9:1), at 3 mL/min.

**17β-Hydroxy-7α-fluoro-5α-androstan-3-one (2).** A solution of tosylate **9**[17] (54.3 mg, 0.089 mmol), $nBu_4NF$ (1.32 mL of a 1.0 M solution in THF, 1.32 mmol) in anhydrous methyl ethyl ketone (7 mL) was stirred and heated at 85 °C for 4 h under Ar. The reaction mixture was poured into $H_2O$ (30 mL) and extracted with $CH_2Cl_2$ (3×, 50 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving an orange oil. Purification by flash chromatography on a 2- × 15-cm column of silica gel using hexanes−EtOAc (2:1) followed by flash chromatography on a 2- × 15-cm column of silica gel using $CHCl_3$−EtOAc (6.7:1) gave 9.8 mg of **2** as a white solid containing 10% of a close migrating impurity. Semipreparative HPLC in system H-p gave 6.2 mg (23%) of **2** as a white solid. Data for **2**: mp 230−233 °C (acetone); T-1, $R_f$ 0.43; $^1$H NMR (300 MHz, $CDCl_3$) δ 0.77 (s, 3H, H-18), 1.03 (s, 3H, H-19), 3.71 (dd, 1H, $J$ = 8.5, 8.5 Hz, H-17α), 4.65 (br d, 1H, $J_{HF}$ = 49.3 Hz, H-7β); HRMS (FAB+, glycerol + TFA) calcd for $C_{19}H_{30}O_2F$ 309.222984, found 309.221640. HPLC analysis in both system H-1 (285 nm), $t_R$ = 10 min, and system H-2 (285 nm), $t_R$ = 7.25 min, indicated a purity of >99%.

**3,3-(Ethylenedioxy)-17α-methyl-5-androsten-17β-ol (10).** A solution of 17α-methyltestosterone (5 g, 16.5 mmol), ethylene glycol (25 mL, 448 mmol), pTsOH (100 mg, 0.5 mmol) in anhydrous benzene (280 mL) was heated at reflux in a 500-mL round-bottom flask equipped with a Dean−Stark trap and a mechanical stirrer for 4.5 h. The reaction mixture was allowed to cool to room temperature, poured into saturated aqueous $NaHCO_3$, and extracted with $CH_2Cl_2$ (2×, 300 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a white solid. Purification by flash chromatography on a 5- × 14-cm column of silica gel using hexanes−EtOAc (3:1) as eluent gave 4.7 g (82%) of **10** as a white solid. Data for **10**: mp 110−115 °C (acetone/trace pyridine); TLC system T-2, $R_f$ 0.35; IR (KBr) 3450 cm$^{-1}$, 1110; $^1$H NMR (100 MHz, $CDCl_3$) δ 0.95 (s, 3H, H-18), 1.10 (s, 3H, H-19), 1.20 (s, 3H, 17-$CH_3$), 4.01 (m, 4H, 3-ketal).

**3,3-(Ethylenedioxy)-17β-hydroxy-17α-methyl-5-androsten-7-one (11).** This procedure is based on the literature method.[19] A mixture of $CH_2Cl_2$ (171 mL) and anhydrous $CrO_3$ (33.7 g, 337 mmol) was stirred at −23 °C (dry ice/$CCl_4$ bath) in a 500-mL round-bottom flask under $N_2$ for 2 min. A portion of 3,5-dimethylpyrazole (32.6 g, 339 mmol) was added, and the mixture was stirred for 15 min at −23 °C. To this was added compound **10** (3.6 g, 10.5 mmol), and stirring was continued at −23 °C for 2.5 h. The mixture was allowed to warm to 0 °C, and 5 N NaOH (171 mL) was added. After stirring at 0 °C for 1 h, the mixture was poured into $Et_2O$ (300 mL) and washed with 250 mL each of $H_2O$, cold 0.2 N HCl, 5% NaOH, $H_2O$, and brine. The organic phase was dried ($Na_2SO_4$) and concentrated in vacuo giving a brown solid. Purification by flash chromatography using toluene−EtOAc (3:1) as eluent gave 1.32 g (34%) of **11** as a white solid. Data for **11**: mp 207−209 °C (methanol/trace pyridine); TLC system T-1, $R_f$ 0.23; IR (KBr) 1650 cm$^{-1}$; $^1$H NMR (100 MHz, $CDCl_3$) δ 0.89 (s, 3H, H-18), 1.23 (s, 3H, H-19), 1.60 (s, 3H, 17-$CH_3$), 3.94 (s, 4H, 3-ketal), 5.70 (s, 1H, H-6).

**3,3-(Ethylenedioxy)-17β-hydroxy-17α-methyl-5α-androstan-7-one (12).** Ketone **11** (245 mg, 686 mmol) in MeOH (22 mL) was hydrogenated using 10% Pd on charcoal (150 mg) for 3 h under 50 psi of $H_2$ in a Parr low-pressure apparatus. The mixture was filtered to remove catalyst and the catalyst washed with MeOH. Concentration of the filtrate in vacuo gave 220 mg (90%) of **12** as a white solid. Data for **12**: mp 224−225 °C ($CH_2Cl_2$/petroleum ether); TLC system T-1, $R_f$ 0.33; IR (KBr) 1700 cm$^{-1}$; $^1$H NMR (100 MHz, $CDCl_3$) δ 0.90 (s, 3H, H-18), 1.22 (s, 3H, H-19), 1.60 (s, 3H, 17-$CH_3$), 3.92 (s, 4H, 3-ketal).

**3,3-(Ethylenedioxy)-17α-methyl-5α-androstane-7α,17β-diol (13a) and 3,3-(Ethylenedioxy)-17α-methyl-5α-androstane-7β,17β-diol (13b).** A solution of ketone **12** (263 mg, 0.725 mmol), $CeCl_3$ (3.78 g, 10.1 mmol), $H_2O$ (7.5 mL) in THF (82 mL) and MeOH (83 mL) was stirred at room temperature as $NaBH_4$ (383 mg, 10.1 mmol) was added slowly over 5 min. The mixture was stirred at room temperature for 0.5 h, poured into saturated aqueous $NH_4Cl$ (150 mL), and extracted with $CH_2Cl_2$ (3×, 150 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving 270 mg (100%) of **13** as a white foam. This material was an inseparable mixture of 7α- and 7β-isomers **13a** and **13b** in a 1/6 ratio as determined by inspection of the $^1$H NMR spectrum and was used without further purification. Data for **13a** and **13b**: TLC system T-1, $R_f$ 0.13; IR (KBr) 3455 cm$^{-1}$; $^1$H NMR (100 MHz, $CDCl_3$) δ 3.43 (m, 1H, H-7α), 3.86 (m, 1H, H-7β).

**7α,17β-Dihydroxy-17α-methyl-5α-androstan-3-one (14a) and 7β,17β-dihydroxy-17α-methyl-5α-androstan-3-one (14b).** A solution of the ketal mixture **13a** and **13b** (143 mg, 0.392 mmol), pTsOH (82 mg, 0.43 mmol) in acetone (21 mL) with 40 drops of $H_2O$ was stirred and heated at 65 °C in a flask equipped with a reflux condenser for 1 h 45 min under

$N_2$. The reaction mixture was allowed to cool to room temperature, poured into saturated aqueous $NaHCO_3$, and extracted with $CH_2Cl_2$ (3x, 50 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 2- × 17-cm column of silica gel using EtOAc−isooctane (3:1) as eluent gave 5.9 mg (4.7%) of **14a**, 5.9 mg (4.7%) of a 1:1 mixture of **14a** and **14b**, and 88 mg (70%) of **14b** as a white solid. Data for **14a**: mp 114−116 °C ($CH_2Cl_2$−petroleum ether); TLC system T-3, $R_f$ 0.38; IR (KBr) 3460 $cm^{-1}$, 1700; $^1H$ NMR (100 MHz, $CDCl_3$) $\delta$ 0.88 (s, 3H, H-18), 1.03 (s, 3H, H-19), 1.25 (s, 3H, 17-$CH_3$), 3.93 (m, 1H, H-7$\beta$). Data for **14b**: mp 198−200 °C ($CH_2Cl_2$−petroleum ether); TLC system T-3, $R_f$ 0.31; IR (KBr) 3460 $cm^{-1}$, 1700; $^1H$ NMR (100 MHz, $CDCl_3$) $\delta$ 0.90 (s, 3H, H-18), 1.06 (s, 3H, H-19), 1.22 (s, 3H, 17-$CH_3$), 3.42 (br m, 1H, half-width 25 Hz, H-7α). Anal. ($C_{20}H_{32}O_3$) C, H.

**17β-Hydroxy-17α-methyl-7β-(*p*-toluenesulfonyloxy)-5α-androstan-3-one (15).** A solution of 7β-hydroxysteroid **14b** (88 mg, 0.27 mmol), *p*-toluenesulfonyl chloride (1.04 g, 5.49 mmol) in anhydrous pyridine (36 mL) was allowed to stand at 4 °C for 5 days in a sealed flask.[20] The reaction mixture was poured into $CH_2Cl_2$ (100 mL) and washed with $H_2O$ (50 mL). The aqueous layer was back-extracted with $CH_2Cl_2$ (2x, 50 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a yellow solid. Purification of the residue by flash chromatography on a 2- × 15-cm column of silica gel using hexanes−EtOAc (1:1) as eluent gave 85.3 mg (65%) of **15**. Crystallization from acetone/petroleum ether gave the analytical sample as white needles. Data for **15**: mp 150−151 °C (acetone/petroleum ether); TLC system T-1, $R_f$ 0.25; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 0.86 (s, 3H, H-18), 1.03 (s, 3H, H-19), 1.21 (s, 3H, 17-$CH_3$), 2.46 (s, 3H, Ar$CH_3$), 4.48 (ddd, 1H, $J$ = 10.4, 10.4, 5.3 Hz, H-7α), 7.33 (d, 2H, $J$ = 7.9 Hz, ArH), 7.78 (d, 2H, $J$ = 8.1 Hz, ArH). Anal. ($C_{27}H_{38}SO_5$) C, H, S.

**17β-Hydroxy-7α-iodo-17α-methyl-5α-androstan-3-one (3).** A solution of tosylate **15** (57.8 mg, 0.122 mmol), NaI (182 mg, 1.22 mmol) in anhydrous acetonitrile was stirred and heated at 83 °C for 1.75 h. The reaction mixture was allowed to cool to room temperature, diluted with $CH_2Cl_2$ (75 mL), and washed with 10% aqueous sodium thiosulfate solution (20 mL) and $H_2O$ (20 mL). The organic phase was dried over $Na_2SO_4$ and concentrated in vacuo giving a yellow foam. Purification of the residue by flash chromatography on a 2- × 17-cm column of silica gel using hexanes−EtOAc (3:1) as eluent gave 47 mg (89%) of **3** as a white solid. Data for **3**: mp 121−122 °C dec (acetone/petroleum ether); TLC system T-1, $R_f$ 0.48; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.93 (s, 3H, H-18), 1.09 (s, 3H, H-19), 1.26 (s, 3H, 17-$CH_3$), 4.66 (apparent quartet, 1H, $J$ = 2.75 Hz, H-7$\beta$); HRMS (FAB+) calcd for $C_{20}H_{32}O_2I$ 431.144708, found 431.144030. Anal. ($C_{20}H_{31}O_2I$) C, H, I.

**17β-Hydroxy-7α-fluoro-17α-methyl-5α-androstan-3-one (4).** A solution of tosylate **15** (178.8 mg, 0.377 mmol) in anhydrous 2-butanone (47 mL) was stirred at room temperature as a 1 M solution of $nBu_4NF$ in THF (5.27 mL, 5.27 mmol) was added. The reaction mixture was stirred at 85 °C for 4 h, allowed to cool to room temperature, poured into $H_2O$ (100 mL), and extracted with $CH_2Cl_2$ (3x, 100 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a yellow oil. Purification by repeated flash chromatography, first on a 3- × 20-cm column of silica gel using hexanes−EtOAc (1:1) as eluent and then a 3- × 20-cm column of silica gel using isooctane−EtOAc (1:1), followed by a 3- × 20-cm column of silica gel using $CHCl_3$−EtOAc (6.7:1), gave 43.7 mg (36%) of **4** as a white solid. Crystallization from acetone/petroleum ether gave the analytical sample as white plates. Data for **4**: mp 206−210 °C (acetone/petroleum ether); TLC system T-4, $R_f$ 0.56; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.87 (s, 3H, H-18), 1.03 (s, 3H, H-19), 1.25 (s, 3H, 17-$CH_3$), 4.64 (br d, 1H $J_{HF}$ = 48.7 Hz, H-7$\beta$); HRMS (FAB+) calcd for $C_{20}H_{32}O_2F$ 323.238634, found 323.237470. HPLC analysis in both system H-1 (285 nm), $t_R$ = 8.5 min, and H-2 (285 nm), $t_R$ = 9 min, indicated a purity of >99%. Anal. ($C_{20}H_{31}O_2F$) C, H, F.

**19-(*tert*-Butyldiphenylsiloxy)-4-androstene-3,17-dione (16).** A solution of 19-hydroxyandrost-4-ene-3,17-dione (266 mg, 0.878 mmol), imidazole (120 mg, 1.75 mmol), *tert*-butyldiphenylsilyl chloride (297 μL, 1.14 mmol) in anhydrous dimethylformamide (1.8 mL) was stirred at room temperature under $N_2$ for 17 h. The reaction mixture was poured into $H_2O$ (100 mL) and extracted with $CH_2Cl_2$ (3x, 70 mL). Combined organic extracts were washed with brine (30 mL), dried over $Na_2SO_4$, and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 2- × 17 cm column of silica gel using hexanes−EtOAc (2:1) as eluent gave 432 mg (91%) of **16** as a white foam. Data for **16**: TLC system T-1, $R_f$ 0.55; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 0.76 (s, 3H, H-18), 1.03 (s, 9H, tBu), 3.84 and 3.93 (AB quartet, 2H, $J$ = 10.3 Hz, H-19), 5.90 (s, 1H, H-4), 7.39 (m, 6H, ArH), 7.61 (m, 4H, ArH); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{35}H_{45}O_3Si$ 541.313799, found 541.313633.

**19-(*tert*-Butyldiphenylsiloxy)-3,3-(ethylenedioxy)-5-androsten-17-one (17).** A solution of enone **16** (3.65 g, 6.75 mmol), *p*-toluenesulfonic acid (15 mg, 0.079 mmol) in 2-ethyl-2-methyl-1,3-dioxolane (115.8 mL) was stirred and heated at 83 °C under Ar for 7.5 h. The reaction mixture was allowed to cool to room temperature, poured into saturated aqueous $NaHCO_3$ (70 mL), and extracted with $CH_2Cl_2$ (3x, 90 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving an oil. Purification of the residue by flash chromatography on a 3- × 21-cm column of silica gel using hexanes−EtOAc (3:1) as eluent gave 1.79 g (45%) of **17** and 1.28 g of recovered starting material. Data for **17**: mp 189−191 °C (EtOAc/petroleum ether); TLC system T-5, $R_f$ 0.67; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 0.77 (s, 3H, H-18), 1.07 (s, 9H, tBu), 3.70 and 3.71 (AB quartet, 2H, $J$ = 11.5 Hz, H-19), 3.90 (m, 4H, 3-ketal), 5.57 (s, 1H, H-6), 7.40 (m, 6H, ArH), 7.65 (m, 4H, ArH); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{37}H_{49}O_4Si$ 585.340014, found 585.339600.

**19-(*tert*-Butyldiphenylsiloxy)-3,3-(ethylenedioxy)-5-androsten-17β-ol (18).** A solution of ketone **17** (1.35 g, 2.31 mmol) in tetrahydrofuran (29.4 mL) and $H_2O$ (1 mL) was stirred at room temperature as $NaBH_4$ was added. The reaction mixture was stirred for 6 h under Ar, poured into saturated aqueous $NH_4Cl$ (70 mL), and extracted with $CH_2Cl_2$ (3x, 70 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography on a 3- × 21-cm column of silica gel using hexanes−EtOAc (2.5:1) as eluent gave 1.13 g (84%) of **18**. Data for **18**: TLC system T-5, $R_f$ 0.19; HRMS (FAB+) calcd for $C_{37}H_{51}O_4Si$ 587.355664, found 587.354990.

**19-(*tert*-Butyldiphenylsiloxy)-3,3-(ethylenedioxy)-5-androsten-17β-yl Acetate (19).** A solution of alcohol **18** (808 mg, 1.38 mmol) and acetic anhydride (5.0 mL, 5.3 mmol) in pyridine (10 mL) was stirred at room temperature for 24 h under Ar. Reaction mixture was poured into saturated aqueous $CuSO_4$ (200 mL) and extracted with $CH_2Cl_2$ (4x, 100 mL). Combined organic extracts were washed with brine (100 mL), dried over $Na_2SO_4$, and concentrated in vacuo giving a yellow solid. Purification of the residue by flash chromatography on a 3- × 20-cm column of silica gel using hexanes−EtOAc (5:1) as eluent gave 797 mg (92%) of **19**. Data for **19**: mp 165−166 °C (acetone/petroleum ether); TLC system T-5, $R_f$ 0.42; $^1H$ NMR (300 MHz, $CDCl_3$) $\delta$ 0.75 (s, 3H, H-18), 1.07 (s, 9H, tBu), 2.05 (s, 3H, OAc), 3.65 and 3.69 (AB quartet, 2H, $J$ = 11.1 Hz, H-19), 3.88 (m, 4H, 3-ketal), 4.58 (dd, 1H, $J$ = 8.9, 7.7 Hz, H-17α), 5.52 (br s, 1H, H-6), 7.44−7.34 (m, 6H, ArH), 7.65 (m, 4H, ArH); MS (M + H)$^+$ 629; HRMS (FAB+) calcd for $C_{39}H_{53}O_5Si$ 629.366229, found 629.365480.

**19-(*tert*-Butyldiphenylsiloxy)-3,3-(ethylenedioxy)-7-oxoandrost-5-en-17β-yl Acetate (20).** Compound **19** (545 mg, 0.867 mmol) was oxidized using the procedure described in the preparation of compound **11**. Purification by flash chromatography using hexanes−EtOAc (2.5:1) as eluent gave 470 mg (84%) of **20** as a clear colorless oil. Data for **20**: TLC system T-5, $R_f$ 0.135; $^1H$ NMR (500 MHz, $CDCl_3$) $\delta$ 0.84 (s, 3H, H-18), 1.03 (s, 9H, tBu), 2.05 (s, 3H, OAc), 3.00 (dd, 1H, $J$ = 11.8, 11.8 Hz, H-8), 3.78 (d, 1 part of AB quartet, 1H, $J$ = 10.5 Hz, H-19), 3.89 (m, 5H, H-19 & 3-ketal), 4.63 (dd, 1H, $J$ = 8.4, 8.4 Hz, H-17α), 5.85 (br s, 1H, H-6), 7.48−7.38 (m, 6H,

ArH), 7.65−7.61 (m, 4H, ArH); HRMS (FAB+) calcd for $C_{39}H_{51}O_6Si$ 643.345493, found 643.344360.

**19-(*tert*-Butyldiphenylsiloxy)-3,3-(ethylenedioxy)-7*β*-hydroxyandrost-5-en-17-yl Acetate (21).** Compound **21** was prepared by reduction of of enone **20** (2.25 g, 3.50 mmol) as described for the preparation of **13a** and **13b**. Flash chromatography on a 5- × 14-cm column of silica gel using hexanes−EtOAc (1.5:1) as eluent gave 1.84 g (82%) of **21** as a white foam. Data for **21**: TLC system T-1, $R_f$ 0.43; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.79 (s, 3H, H-18), 1.09 (s, 9H, tBu), 2.05 (s, 3H, OAc), 3.66 and 3.72 (AB quartet, 2H, $J$ = 10.9 Hz, H-19), 3.89 (m, 4H, 3-ketal), 3.90 (m, 1H, H-7α), 4.59 (dd, 1H, $J$ = 8.2 Hz, H-17α), 5.46 (br s, 1H, H-6), 7.36−7.46 (m, 6H, Ar−H), 7.62−7.68 (m, 4H, Ar−H); HRMS (FAB+) calcd for $C_{39}H_{51}O_5Si$ 627.350579, found 627.349770.

**7*β*,19-Dihydroxy-3,3-(ethylenedioxy)-5-androsten-17*β*-yl Acetate (22).** A solution of allylic alcohol **21** (1.80 g, 2.79 mmol) and $nBu_4NF$ (8.37 mL of a 1 M solution in THF, 8.37 mmol) in THF (50 mL) was stirred at room temperature under Ar for 24 h. The reaction mixture was poured into saturated aqueous $NH_4Cl$ (150 mL) and extracted with $CH_2Cl_2$ (3x, 150 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a white solid. Purification by flash chromatography on a 3- × 13-cm column of silica gel using EtOAc as eluent gave 916 mg (81%) of **22** as a white solid. Data for **22**: TLC system T-3, $R_f$ 0.155; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.88 (s, 3H, H-18), 2.05 (s, 3H, OAc), 3.67 and 3.85 (AB quartet, 2H, $J$ = 11.4 Hz, H-19), 3.95 (m, 4H, 3-ketal), 4.00 (m, 1H, H-7α), 4.61 (dd, 1H, $J$ = 7.2 Hz, H-17α), 5.64 (s, 1H, H-6); HRMS (FAB+) calcd for $C_{23}H_{33}O_5$ 389.232800, found 389.233560.

**7,19-Dioxo-3,3-(ethylenedioxy)-5-androsten-17*β*-yl Acetate (23).** Chromium trioxide (2.7 g, 27 mmol) was added to a magnetically stirred solution of pyridine (4.37 mL, 54 mmol) in $CH_2Cl_2$ (50 mL). The solution was stirred at room temperature for 15 min, then diol **22** (916 mg, 2.25 mmol) in $CH_2Cl_2$ (15 mL) was added by syringe, and the reaction was stirred at room temperature for 1 h under Ar. The reaction mixture was diluted with 2-propanol (10 mL) and $H_2O$ (100 mL) and extracted with $CH_2Cl_2$ (3x, 150 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo. Purification of the residue by flash chromatography on a 3- × 14-cm column of silica gel using hexanes−EtOAc (1:1) gave 788 mg (87%) of **23** as a white solid. Data for **23**: mp 202−211 °C dec ($CH_2Cl_2$/hexanes); TLC system T-3, $R_f$ 0.67; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.76 (s, 3H, H-18), 2.05 (s, 3H, OAc), 3.98 (m, 4H, 3-ketal), 4.63 (dd, 1H, $J$ = 7.8, 7.8 Hz, H-17α), 6.02 (s, 1H, H-6), 9.88 (s, 1H, H-19); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{23}H_{31}O_6$ 403.212064, found 403.212767.

**3,3-(Ethylenedioxy)-5-estren-17*β*-yl Acetate (27) and 3,3-(Ethylenedioxy)-5(10)-estren-17*β*-yl Acetate (28).** Testosterone acetate (19 g, 60 mmol) was ketalized as described in the preparation of **17**. Purification on a 5- × 16-cm column of flash silica gel using hexanes−EtOAc (4:1) as eluent gave 14.2 g (66%) of **27** as a mixture of $\Delta^{5,6}$- and $\Delta^{5(10)}$-isomers in a 1/1.6 ratio as determined by inspection of the $^1$H NMR spectrum. Crystallization of a 3.12-g portion of this mixture with absolute EtOH gave 0.58 g of the $\Delta^{5,6}$-isomer **27** as fine needles. A 569-mg portion of the mixture was chromatographed on a 2- × 20-cm column of flash silica gel using toluene−EtOAc (8:1) as eluent and gave 55.8 mg of pure $\Delta^{5(10)}$-isomer **28**, 6.7 mg of pure $\Delta^{5,6}$-isomer **27**, and 407 mg of a mixture of $\Delta^{5,6}$- and $\Delta^{5(10)}$-isomers. Data for **27**: mp 164−168 °C (EtOH); TLC system T-6, $R_f$ 0.38; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.81 (s, 3H, H-18), 2.03 (s, 3H, OAc), 3.95 (m, 4H, 3-ketal), 4.61 (dd, 1H, $J$ = 8.7, 8.7 Hz, H-17α), 5.46 (br d, 1H, $J$ = 5.4 Hz, H-6); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{22}H_{33}O_4$ 361.2379, found 361.2373. Data for **28**: TLC system T-6, $R_f$ 0.44; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.79 (s, 3H, H-18), 2.03 (s, 3H, OAc), 3.98 (m, 4H, 3-ketal), 4.63 (dd, 1H, $J$ = 8.6, 8.6 Hz, H-17α).

**3,3-(Ethylenedioxy)-7-oxoestra-5-en-17*β*-yl Acetate (24). Method A. Oxidation of 27:** A mixture of a solution of **27** and **28** (4.12 g, 11.4 mmol) was oxidized as described for the preparation of **11**. Purification by flash chromatography using hexanes−EtOAc (2:1) as eluent and then rechromatography using toluene−EtOAc (4:1) as eluent followed by crystallization of the product-containing fractions from MeOH gave 357 mg (8%) of **24** as a white solid. Data for **24**: mp 224−227 °C (MeOH); TLC system T-1, $R_f$ 0.49; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.81 (s, 3H, H-18), 2.03 (s, 3H, OAc), 3.96 (m, 4H, 3-ketal), 4.64 (dd, 1H, $J$ = 8.3, 8.3 Hz, H-17α), 5.75 (s, 1H, H-6); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{22}H_{31}O_5$ 375.21715, found 375.21665.

**Method B. Deformylation of 23:** A solution of aldehyde **23** (78 mg, 0.19 mmol) in MeOH (1.58 mL) and THF (3.32 mL) was stirred at 4 °C as a solution of methanolic KOH (22.8 mg KOH in 1.74 mL of MeOH) was added. Mixture was allowed to stand at 4 °C for 1 h, poured into saturated aqueous $NH_4Cl$ (30 mL), and extracted with $CH_2Cl_2$ (3x, 30 mL) and EtOAc (1x, 30 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo. Purification by flash chromatography on a 2- × 16-cm column of silica gel using hexanes−EtOAc (2:1) as eluent gave 32.5 mg (45%) of **24** and **25** as an inseparable mixture of $\Delta^{5,6}$- and $\Delta^{5(10)}$-isomers in a 4/6 ratio as a white solid and 36.2 mg (50%) of **26** as a yellow oil. Data for **25**: TLC system T-4, $R_f$ 0.68; $^1$H NMR (attributed to **25**, 300 MHz, $CDCl_3$) $\delta$ 0.79 (s, 3H, H-18), 2.03 (s, 3H, OAc), 2.95 (br d, 1H, $J$ = 21.0 Hz, H-6eq), 3.98 (m, 4H, 3-ketal), 4.62 (dd, 1H, $J$ = 7.4, 7.4 Hz, H-17α); HRMS (FAB+) calcd for $C_{22}H_{31}O_5$ 375.21715, found 375.21581. Data for **26**: TLC system T-4, $R_f$ 0.15; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.79 (s, 3H, H-18), 3.67 (dd, 1H, $J$ = 8.5, 8.5 Hz, H-17α), 3.92 (m, 4H, $OCH_2CH_2O$), 5.39 (s, 1H, H-6), 5.64 (s, 1H, H-4).

**3,3-(Ethylenedioxy)-7-oxo-5α-estran-17*β*-yl Acetate (29).** A solution of enone **24** (23.9 mg, 0.0638 mmol) and 10% Pd on carbon (16.9 mg) in MeOH (3 mL) was stirred under 1 atm of $H_2$ for 1.5 h. The reaction mixture was filtered through Celite, and the solid was washed with MeOH (30 mL). The filtrate was concentrated in vacuo and purified by flash chromatography on a 2- × 18-cm column of silica gel using hexanes−EtOAc (2:1) as eluent to give 19.6 mg (82%) of **29** as a white solid. Data for **29**: mp 132−135 °C (MeOH); TLC system T-1, $R_f$ 0.55; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.79 (s, 3H, H-18), 2.03 (s, 3H, OAc), 3.92 (m, 4H, 3-ketal), 4.64 (dd, 1H, $J$ = 8.4, 8.4 Hz, H-17α); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{22}H_{33}O_5$ 377.232800, found 377.232100.

**3,3-(Ethylenedioxy)-7α-hydroxy-5α-estran-17*β*-yl Acetate (30a) and 3,3-(Ethylenedioxy)-7*β*-hydroxy-5α-estran-17*β*-yl Acetate (30b).** Ketone **29** (308 mg, 0.812 mmol) was reduced using the method described in the preparation of **13a** and **13b** giving a 1:3.5 mixture of 7α- and 7*β*-isomers as a white foam. Purification by flash column chromatography on a 3- × 20-cm column of silica gel using isooctane−EtOAc (1.5:1) as eluent gave 8 mg of pure **30a** and 236 mg (79%) of **30a** and **30b** as a 1:4 mixture of 7α- and 7*β*-epimers as seen by inspection of the $^1$H NMR spectrum. Data for **30a**: TLC system T-7, $R_f$ 0.28; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.82 (s, 3H, H-18), 2.05 (s, 3H, OAc), 3.89 (br s, 1H, H-7*β*), 3.94 (m, 4H, 3-ketal), 4.66 (dd, 1H, $J$ = 8.5, 8.5 Hz, H-17α); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{22}H_{35}O_5$ 379.248450, found 379.247700. Data for **30b**: TLC system T-7, $R_f$ 0.24; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.82 (s, 3H, H-18), 2.04 (s, 3H, OAc), 3.41 (ddd, 1H, $J$ = 9.9, 9.9, 4.4 Hz, H-7α), 3.93 (m, 4H, 3-ketal), 4.59 (dd, 1H, $J$ = 8.8, 8.8 Hz, H-17α); HRMS (FAB, mNBA + PEG + NaOAc) calcd for $C_{22}H_{35}O_5$ 379.248450, found 379.248200.

**3,3-(Ethylenedioxy)-7*β*-(*p*-toluenesulfonyloxy)-5α-estran-17*β*-yl Acetate (31).** Tosylation of **30b** (46.8 mg, 0.124 mmol) was carried out as described for **15**. Purification by flash column chromatography on a 2- × 16-cm column of silica gel using hexanes−EtOAc (2:1) as eluent gave 53 mg (80%) of **31** as a white foam. Data for **31**: TLC system T-2, $R_f$ 0.28; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.78 (s, 3H, H-18), 2.03 (s, 3H, OAc), 2.45 (s, 3H, $ArCH_3$), 3.91 (m, 4H, 3-ketal), 4.48 (ddd, 1H, $J$ = 10.6, 10.6, 4.4 Hz, H-7α), 4.57 (dd, 1H, $J$ = 8.4, 8.4

Hz, H-17α), 7.32 (d, 2H, $J$ = 8.0, Ar−H), 7.77 (d, 2H, $J$ = 8.0, Ar−H); HRMS (FAB+) calcd for $C_{29}H_{41}O_7S$ 533.257301, found 533.256050.

**3,3-(Ethylenedioxy)-5α-estran-17β-ol (32).** A solution of tosylate **31** (30.1 mg, 0.0565 mmol) in anhydrous THF (650 mL) was stirred at room temperature as $LiEt_3BH$ (226 μL of a 1 M solution in THF, 0.226 mmol) was added by syringe. The reaction mixture was stirred and heated at 65 °C for 5 h, cooled to room temperature, and quenched with water (60 drops). To this were added 3 N NaOH (125 μL) and 30% $H_2O_2$ (125 μL). The reaction mixture was allowed to stand at room temperature for 5 min and extracted with $CH_2Cl_2$ (3x, 10 mL). Combined organic extracts were washed with aqueous 10% sodium thiosulfate (5 mL) and concentrated in vacuo. Purification by flash column chromatography on a 2- × 16-cm column of silica gel eluting with hexanes−EtOAc (2:1) gave 16.9 mg (94%) of **32** as a white solid. Data for **32**: TLC system T-2, $R_f$ 0.31; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.75 (s, 3H, H-18), 3.65 (dd, 1H, $J$ = 8.6 Hz, H-17α), 3.94 (m, 4H, 3-ketal); HRMS (EI+) calcd for $C_{20}H_{32}O_3$ 320.235145, found 320.235100.

**17β-Hydroxy-5α-estran-3-one (33).** Deketalization of **32** (8.6 mg, 0.027 mmol) was carried out as described for **14a** and **14b**. Purification by flash column chromatography on a 1- × 12-cm column of silica gel using hexanes−EtOAc (1.5:1) as eluent gave 6.9 mg (93%) of **33** as a white solid. The physical data as follows was identical to an authentic standard purchased from Steraloids. Data for **33**: TLC system T-2, $R_f$ 0.19; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.78 (s, 3H, H-18), 3.66 (dd, 1H, $J$ = 8.5 Hz, H-17α); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 11.1, 23.2, 25.8, 30.2, 30.5, 30.6, 33.9, 36.6, 41.1, 41.3, 43.1, 43.7, 45.8, 47.8, 48.7, 50.0, 81.9, 211.8; HRMS (EI+) calcd for $C_{18}H_{28}O_2$ 276.208930, found 276.208667.

**7β-Hydroxy-3-oxo-5α-estran-17β-yl Acetate (34).** Deketalization of **30a** and **30b** (126 mg, 0.333 mmol) was carried out as described for **14a** and **14b**. Purification by flash chromatography on a 3- × 18-cm column of silica gel using EtOAc−hexanes (2:1) as eluent gave 16.8 mg (15%) of the 7α-hydroxy isomer, 76.2 mg (69%) of the 7β-hydroxy isomer **34**, and 4.6 mg of a 1:1 mixture of these isomers. Data for **34**: mp 200.5−202 °C (acetone/petroleum ether); TLC system T-3, $R_f$ 0.43; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.86 (s, 3H, H-18), 2.05 (s, 3H, OAc), 3.44 (m, 1H, halfwidth = 21.9 Hz, H-7α), 4.61 (dd, 1H, $J$ = 8.4, 8.4 Hz, H-17α); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 12.1, 21.2, 25.6, 26.3, 27.7, 30.2, 36.7, 41.0, 41.2, 43.4, 43.5, 44.8, 45.5, 47.8, 48.0, 49.2, 73.8, 82.3, 171.2, 211.0; HRMS (FAB, mNBA + PEG +NaOAc) calcd for $C_{20}H_{31}O_4$ 335.222235, found 335.222733. Anal. ($C_{20}H_{30}O_4$) C,H.

**3-Oxo-7β-(*p*-toluenesulfonyloxy)-5α-estran-17β-yl Acetate (35).** Compound **34** (61.6 mg, 0.184 mmol) was tosylated as described for the preparation of **15**. Purification by flash chromatography on a 2- × 18-cm column of silica gel using hexanes−EtOAc (2:1) as eluent gave 88.6 mg of **35** as a yellow foam. Crystallization from acetone−petroleum ether gave 81 mg (90%) of **35** as white needles. Data for **35**: mp 175−177 °C (acetone/petroleum ether); TLC system T-1, $R_f$ 0.51; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.81 (s, 3H, H-18), 2.04 (s, 3H, OAc), 2.46 (s, 3H, Ar−$CH_3$), 4.47 (ddd, 1H, $J$ = 10.6, 10.6, 4.5 Hz, H-7α), 4.58 (dd, 1H, $J$ = 9.1, 8.1 Hz, H-17α), 7.34 (d, 2H, $J$ = 8.1 Hz, Ar−H), 7.78 (d, 2H, $J$ = 8.7 Hz, Ar−H); HRMS (FAB+) calcd for $C_{27}H_{37}O_6S$ 489.231086, found 489.230080.

**7α-Iodo-3-oxo-5α-estran-17β-yl Acetate (36).** Tosylate **35** (24.8 mg, 0.051 mmol), was iodinated as described for the preparation of **3**. Purification by flash chromatography on a 2- × 17-cm column of silica gel using hexanes−EtOAc (3:1) as eluent gave 19.4 mg (84%) of **36**. Data for **36**: TLC system T-1, $R_f$ 0.76; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.88 (s, 3H, H-18), 2.05 (s, 3H, OAc), 4.55 (ddd, 1H, $J$ = 3.1, 2.4, 2.4 Hz, H-7β), 4.66 (dd, 1H, $J$ = 8.7, 8.7 Hz, H-17α).

**7α-Iodo-17β-hydroxy-5α-estran-3-one (5).** A solution of acetate **36** (9.2 mg, 0.021 mmol) in saturated aqueous $Na_2CO_3$ (0.3 mL) and MeOH (2.3 mL) was stirred at room temperature for 42 h. The reaction mixture was poured into $H_2O$ (20 mL) and extracted with $CH_2Cl_2$ (2x, 30 mL) and then EtOAc (2x, 30 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 1- × 13-cm column of silica gel using hexanes−EtOAc (1:1) as eluent gave 7 mg (84%) of **5** as a white solid. Data for **5**: mp 154−156 °C (acetone/petroleum ether); TLC system T-1, $R_f$ 0.29; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 3.72 (dd, 1H, $J$ = 8.6, 8.6 Hz, H-17α), 4.57 (br d, 1H, $J$ = 2.4 Hz, half width = 9.0 Hz, H-7β); HRMS (EI) calcd for $C_{18}H_{28}O_2I$ 403.113407, found 403.112350. HPLC system H-1 (280 nm), $t_R$ = 11 min, and system H-2 (280 nm), $t_R$ = 15 min, >99% pure.

**7α-Fluoro-3-oxo-5α-estran-17β-yl Acetate (37).** Tosylate **35** (5.1 mg, 0.0104 mmol) was fluorinated as described for the preparation of **4**. Purification by flash chromatography on a 1- × 17-cm column of silica gel using hexanes−EtOAc (3:1) as eluent gave 1.7 mg (48%) of **37** as an oil. Data for **37**: TLC system T-1, $R_f$ 0.64; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 2.05 (s, 3H, OAc), 4.67 (dd, 1H, $J$ = 8.6, 7.9 Hz, H-17α), 4.70 (br d, 1H, $J$ = 49 Hz, H-7α).

**7α-Fluoro-17β-hydroxy-5α-estran-3-one (6).** Compound **37** (10.8 mg, 0.0321 mmol) was saponified as described for the preparation of **5**. Purification by flash chromatography on a 1- × 13-cm column of silica gel using 1:1 hexanes−EtOAc gave 5.9 mg (63%) of **6** as a white solid. Data for **6**: mp 105−106.5 °C (acetone/petroleum ether); TLC system T-1, $R_f$ 0.26; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.78 (s, 3H, H-18), 3.72 (dd, 1H, $J$ = 8.8, 8.8 Hz, H-17α), 4.70 (br d, 1H, $J_{HF}$ = 48.6 Hz, H-7β); HRMS (EI+) calcd for $C_{18}H_{27}O_2F$ 294.199509, found 294.199500. HPLC system H-1 (285 nm), $t_R$ = 10 min, and system H-2 (285 nm), $t_R$ = 6.5 min, >99% pure.

**3,3-(Ethylenedioxy)-7β-[(phenylmethoxy)methoxy]-5α-estran-17β-yl Acetate (38).** A solution of the 1:4 mixure of epimers **30a, 30b** (75.6 mg, 0.200 mmol), benzyl chloromethyl ether (555 μL, 3.99 mmol), diisopropylethylamine (696 μL, 3.99 mmol) in anhydrous $CH_2Cl_2$ (4 mL) was stirred at room temperature for 2 h under Ar. The reaction mixture was poured into $H_2O$ (10 mL) and extracted with $CH_2Cl_2$ (3x, 15 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving an orange oil. Purification by flash chromatography on a 1- × 17-cm column of silica gel using hexanes−EtOAc (5:1) as eluent gave 100 mg (100%) of **38** as a clear colorless oil; $^1$H NMR analysis showed no evidence of the 7α-epimer. Data for **38**: TLC system T-1, $R_f$ 0.74; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.83 (s, 3H, H-18), 2.04 (s, 3H, OAc), 3.27 (ddd, 1H, $J$ = 10.3, 10.3, 4.2 Hz, H-7α), 3.93 (m, 4H, 3-ketal), 4.51 and 4.69 (AB quartet, 2H, $J$ = 11.8 Hz, Ph$CH_2$O), 4.58 (dd, 1H, $J$ = 8.7, 8.7 Hz, H-17α), 4.74 and 4.84 (AB quartet, 2H, $J$ = 7.0 Hz, $OCH_2O$), 7.35 (m, 5H, Ar−H); HRMS (FAB, mNBA + PEG +NaOAc) calcd for $C_{30}H_{43}O_6$ 499.305965, found 499.304967.

**3,3-(Ethylenedioxy)-7β-[(phenylmethoxy)methoxy]-5α-estran-17β-ol (39).** A solution of acetate **38** (277 mg, 0.556 mmol) in 1% KOH−MeOH (50 mL) was stirred and heated at 50 °C for 3 h under $N_2$. The reaction mixture was allowed to cool to room temperature, poured into $H_2O$ (50 mL), and extracted with $CH_2Cl_2$ (3x, 50 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a white wax. Purification by flash chromatography on a 2- × 20-cm column of silica gel using hexanes−EtOAc (1.5:1) as eluent gave 173 mg (75%) of **39** as a white solid. Data for **39**: mp 107−108 °C (acetone/petroleum ether); TLC system T-1, $R_f$ 0.54; $^1$H NMR (500 MHz, $CDCl_3$) δ 0.78 (s, 3H, H-18), 3.25 (ddd, 1H, $J$ = 10.2, 10.2, 3.8 Hz, H-7α), 3.60 (dd, 1H, $J$ = 8.7, 8.7 H-17α), 3.93 (m, 4H, 3-ketal), 4.68 and 4.50 (AB quartet, 2H, $J$ = 11.7 Hz, Ph$CH_2$O), 4.83 and 4.73 (AB quartet, 2H, $J$ = 6.9 Hz, $OCH_2O$), 7.33 (m, 5H, Ar−H); HRMS (FAB, mNBA + PEG +NaOAc) calcd for $C_{28}H_{41}O_5$ 457.2954, found 457.2962.

**3,3-(Ethylenedioxy)-7β-[(phenylmethoxy)methoxy]-5α-estran-17-one (40).** A solution of pyridine (315 μL, 3.9 mmol) in anhydrous $CH_2Cl_2$ (12 mL) was stirred at room temperature as $CrO_3$ (195 mg, 1.95 mmol) was added. The reaction mixture was stirred for 15 min, and a solution of alcohol **39** (148 mg, 325 mmol) in $CH_2Cl_2$ (28 mL) was added. The reaction mixture was stirred at room temperature for 45 min under $N_2$, quenched with iPrOH (10 mL), poured into $H_2O$ (50 mL), and

extracted with $CH_2Cl_2$ (3x, 75 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo. Purification of the residue by flash column chromatography on a 2- × 18-cm column of silica gel using hexanes−EtOAc (3:1) as eluent gave 134 mg (88%) of **40** as a white solid. Data for **40**: mp 159−161 °C (acetone/petroleum ether); TLC system T-1, $R_f$ 0.47; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.92 (s, 3H, H-18), 2.42 (dd, 1H, $J$ = 18.7, 9.0 Hz, H-16$\beta$), 3.39 (m, 1H, halfwidth 21.5 Hz, H-7α), 3.94 (m, 4H, 3-ketal), 4.71 and 4.54 (AB quartet, 2H, $J$ = 11.8 Hz, Ph$CH_2$O), 4.89 and 4.80 (AB quartet 2H, $J$ = 6.6 Hz, $OCH_2O$), 7.35 (m, 5H, Ar−H).

**3,3-(Ethylenedioxy)-7$\beta$-[(phenylmethoxy)methoxy]-17α-methyl-5α-estran-17$\beta$-ol (41).** A solution of ketone **40** (55.6 mg, 0.122 mmol) and methylmagnesium iodide (5.2 mL of a 3.0 M solution in $Et_2O$, 15.6 mmol) in anhydrous THF (1.7 mL) was stirred at room temperature for 48 h under $N_2$. The reaction mixture was poured into saturated aqueous $NH_4Cl$ (20 mL) and extracted with $CH_2Cl_2$ (2x, 20 mL) and EtOAc (2x, 20 mL). Combined organic extracts were dried over $Na_2SO_4$ and concentrated in vacuo giving a yellow oil. Purification by flash chromatography on a 2- × 15-cm column of silica gel using hexanes−EtOAc (2:1) as eluent gave 36 mg (62%) of **41** as a white solid. Data for **41**: TLC system T-1, $R_f$ 0.54; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.89 (s, 3H, H-18), 1.20 (s, 3H, 17-$CH_3$), 3.25 (m, 1H, halfwidth = 23.4 Hz, H-7α), 3.92 (m, 4H, 3-ketal), 4.68 and 4.50 (AB quartet, 2H, $J$ = 11.8 Hz, Ph$CH_2$O), 4.82 and 4.73 (AB quartet, 2H, $J$ = 6.9 Hz, $OCH_2O$), 7.33 (m, 5H, Ar−H).

**3,3-(Ethylenedioxy)-17α-methyl-5α-estrane-7$\beta$,17$\beta$-diol (42).** To a suspension of 10% palladium on carbon (293 mg) in $H_2O$ (4.5 mL) and THF (10 mL) was added a solution of alcohol **41** (38 mg, 0.081 mmol) in THF (8 mL). The reaction mixture was stirred under an atm of $H_2$ for 3 h and poured through Celite. The solid was washed with MeOH, and the filtrate was concentrated in vacuo giving a white solid. Purification by flash chromatography on a 1- × 15-cm column of silica gel using EtOAc−hexanes (2:1) as eluent gave 27 mg (96%) of **42** as a white solid. Data for **42**: TLC system T-1, $R_f$ 0.14; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.89 (s, 3H, H-18), 1.22 (s, 3H, 17α-$CH_3$), 3.40 (ddd, 1H, $J$ = 10.1, 10.1, 4.0 Hz, H-7α), 3.93 (m, 4H, 3-ketal); HRMS (EI+) calcd for $C_{21}H_{34}O_4$ 350.245710, found 350.246000.

**7$\beta$,17$\beta$-Hydroxy-17α-methyl-5α-estran-3-one (43).** Deketalization of **42** (17.5 mg, 0.0499 mmol) was carried out as described for the preparation of **14a**, **14b**. Purification by flash chromatography on a 1- × 17-cm column of silica gel using EtOAc−hexanes (3:1) as eluent gave 15 mg (98%) of **43** as a white solid. Data for **43**: mp 179−181 °C (acetone/petroleum ether); TLC system T-3, $R_f$ 0.23; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.92 (s, 3H, H-18), 1.23 (s, 3H, 17α-$CH_3$), 3.43 (ddd, 1H, $J$ = 10.8, 9.3, 4.5 Hz, H-7α); $^{13}$C NMR (125.8 MHz, $CDCl_3$) $\delta$ 14.1, 25.7, 25.9, 26.0, 30.3, 31.4, 39.2, 41.1, 41.4, 43.6, 45.0, 45.6, 46.4, 48.0, 49.0, 49.1, 74.3, 80.6, 210.9; HRMS (EI+) calcd for $C_{19}H_{30}O_3$ 306.219495, found 306.219400. Anal. ($C_{19}H_{30}O_3$) C, H.

**17$\beta$-Hydroxy-17α-methyl-7$\beta$-(*p*-toluenesulfonyloxy)-5α-estran-3-one (44).** Tosylation of **43** (13.6 mg, 0.0444 mmol) was achieved as described in the preparation of **15**. Purification by flash chromatography on a 1- × 20-cm column of silica gel using hexanes−EtOAc (1:1) as eluent gave 18 mg (88%) of **44** as a white solid. Data for **44**: mp 149−152 °C (acetone/petroleum ether); TLC system T-3, $R_f$ 0.57; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.88 (s, 3H, H-18), 1.21 (s, 3H, 17-$CH_3$), 2.46 (s, 3H, Ar−$CH_3$), 4.49 (ddd, 1H, $J$ = 10.5, 10.5, 4.3 Hz, H-7α), 7.34 (d, 2H, $J$ = 8.0, Ar−H), 7.78 (d, 2H, $J$ = 8.0, Ar−H).

**7α-Iodo-17$\beta$-hydroxy-17α-methyl-5α-estran-3-one (7).** Tosylate **44** (6.7 mg, 0.014 mmol) was iodinated as described for the preparation of **3**. Purification by flash chromatography on a 1- × 18-cm column of silica gel using hexanes−EtOAc (1:1) gave 5.2 mg (87%) of **7** as a white solid. Data for **7**: mp 74−76 °C (acetone/petroleum ether); TLC system T-4, $R_f$ 0.5; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.95 (s, 3H, H-18), 1.26 (s, 3H, 17-$CH_3$), 4.58 (ddd, 1H, $J$ = 3.2, 3.2, 2.8 Hz, H-7$\beta$); HRMS (FAB+, glycerol + TFA) calcd for $C_{19}H_{30}O_2I$ 417.129057, found 417.129650. HPLC system H-1 (280 nm), $t_R$ = 9.5 min, and system H-2 (280 nm), $t_R$ = 17 min, >99% pure.

**7α-Fluoro-17$\beta$-hydroxy-17α-methyl-5α-estran-3-one (8).** Tosylate **44** (10 mg, 0.022 mmol) was fluorinated as described in the preparation of **4**. Purification by flash chromatography on a 1- × 19-cm column of silica gel eluting with hexanes−EtOAc (1:1) gave 3.9 mg (58%) of **8**. Data for **8**: mp 205−209 °C (acetone/hexanes); TLC system T-1, $R_f$ 0.26; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 0.89 (s, 3H, H-18), 1.26 (s, 3H, 17α-$CH_3$), 4.71 (br d, 1H, $J_{HF}$ = 49.1 Hz, H-7$\beta$); HRMS (EI) calcd for $C_{19}H_{30}O_2F$ 309.222984, found 309.221920. HPLC system H-1 (285 nm), $t_R$ = 10 min, and system H-2 (285 nm), $t_R$ = 8 min >99% pure.

**Radiosynthesis of 17$\beta$-Hydroxy-7α-[$^{125}$I]iodo-5α-androstan-3-one ([$^{125}$I]1) and 17$\beta$-Hydroxy-7α-[$^{125}$I]iodo-17α-methyl-5α-androstan-3-one ([$^{125}$I]3).** The synthesis of [$^{125}$I]**1** by substitution reaction with Na$^{125}$I and tosylate **9** followed by saponification with 1% KOH/MeOH was performed as we have previously described[17] except that the purification of the intermediate *p*-nitrobenzoyl ester was omitted; e.g., the crude iodination product was directly hydrolyzed and then purified by HPLC in system H-3. Radiolabeled [$^{125}$I]**3** was synthesized in a similar manner by the exchange of tosylate **15** with Na$^{125}$I. A solution of Na$^{125}$I (5 mCi, 2.47 nmol, low pH) in $H_2O$ was mixed with 20 $\mu$L of a solution containing 1 mg/mL sodium thiosulfate in acetonitrile/$H_2O$ (9:1) and evaporated to dryness under vacuum. To ensure removal of $H_2O$, 20 $\mu$L of acetonitrile was added to the vial and evaporated again; 20 $\mu$L of a solution containing 23 $\mu$g of tosylate **15** in acetonitrile was added; the vial was sealed and heated at 83 °C for 110 min. The reaction was allowed to cool to room temperature, diluted with 1 mg/mL aqueous $Na_2S_2O_3$ solution (20 $\mu$L), and vented through a charcoal filter. The mixture was diluted with 100 $\mu$L of MeOH/$H_2O$ (70/30) and purified by HPLC in system H-4. Material eluting as a peak containing the bulk of the radioactivity at 13−15 min was collected and concentrated in vacuo to give [$^{125}$I]**3** (65% radiochemical yield). The dried residue was dissolved in benzene:ethanol (4/1) and stored at 4 °C under argon where it was stable for months. This material comigrated with authentic **3** by TLC (system T-1), $R_f$ 0.48.

**Androgen Receptor Analysis.** Cytosol prepared from rat prostate were used to compare the binding of compounds **1**−**8** to 5α-DHT in competition experiments and for saturation binding studies of [$^{125}$I]**3** using the methods previously described.[16,17] Prostate glands were obtained from Sprague−Dawley rats that had been castrated 48 h before sacrifice. The glands were suspended and homogenized in ice-cold TEGDMo buffer (10 mM Tris, 1.5 mM $Na_2$-EDTA, 10% (v/v) glycerol, 1.0 mM dithiothreitol, 25 mM sodium molybdate, pH 7.4 at 4 °C) at 1.5 mL/prostate and centrifuged at 105 000*g* for 45 min at 4 °C. The supernatant (cytosol) was frozen on dry ice and stored at −80 °C until assay. For assay, the cytosol was defrosted on ice and diluted with TEGDMo buffer to a concentration of 6−10 mg of protein/mL.

**Competitive Binding.** Binding affinities relative to 5α-DHT were determined by incubating aliquots of rat prostate cytosol at 4 °C for 20 h with [$^3$H]R1881 in the presence or absence of compounds **1**−**8** or 5α-DHT.[16] The final concentration of [$^3$H]R1881 was 2−4 nM, and the competitor concentrations ranged from 0.005 nM to 3 $\mu$M. All incubates contained 1 $\mu$M triamcinolone acetonide to prevent potential binding to the progesterone receptor. Bound radioligand was separated from free by LH-20 Sephadex gel filtration and quantified by counting. Displacement curves were analyzed by a curve-fitting method with the use of the computer program Prism (GraphPad Software Inc., San Diego, CA). The results of three experiments performed in duplicate are presented in Table 1.

**Saturation Binding Analysis.** Androgen receptor binding of [$^{125}$I]**3** was performed with rat prostate cytosols as we previously described.[17] Aliquots of the cytosol were incubated for 20 h at 4 °C with a range of concentrations of [$^{125}$I]**3**. Nonspecific binding was assessed in parallel incubations containing 1 $\mu$M radioinert 5α-DHT. As above all incubates also contained 1 $\mu$M triamcinolone acetonide. Bound [$^{125}$I]**3** was separated from free by gel filtration on LH-20 Sephadex

columns. The column eluents containing the receptor-bound steroid were counted and specific binding was calculated as the difference between total binding and nonspecific binding (measured in the presence of excess radioinert 5α-DHT). The resulting data were analyzed by the method of Scatchard using a computer-assisted nonlinear curve-fitting method (Prism). Comparison of the saturation analysis with that for [$^3$H]5α-DHT performed simultaneously as we have previously described[17] indicated that the $^{125}$I product was carrier-free; e.g. the specific activity was within error of theoretical, approximately 2200 Ci/mmol (not shown).

**In Vitro Androgen Bioassay.** Steroids were assayed for androgenic activity in a bioassay using cells transfected with the androgen receptor and an androgen responsive reporter gene. The human androgen receptor construct, an expression vector driven by the cytomegalovirus promoter, was provided by Dr. Michael McPhaul.[28] The GRE$_2$E1bCAT reporter construct was provided by Dr. John Cidlowski.[29] It has two glucocorticoid/androgen response elements from the tyrosine aminotransferase promoter, followed by the TATA box of the adenovirus E1b gene and the chloramphenicol acetyl transferase (CAT) gene. Plasmid DNA was purified using pZ523 columns. Monkey kidney CV1 cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 100 μg/mL penicillin, and 100 μg/mL streptomycin in incubators with humidified air and 5% carbon dioxide at 37 °C. The day before transfection, cells were washed with Hanks buffered saline solution, trypsinized, and plated at a density of $2 \times 10^5$ cells/well in six-well plates in DMEM with 10% charcoal-stripped serum. At the time of transfection, the medium was replaced with DMEM devoid of serum. The CV1 cells were transfected by the nonrecombinant adenoviral-mediated DNA transfer procedure as described earlier.[24,30] For each well, 1 ng of androgen receptor DNA and 0.5 μg of GRE$_2$-E1bCAT reporter plasmid were incubated with $5 \times 10^7$ virus particles. The virus−DNA complex was allowed to infect cells for 2 h in DMEM devoid of serum, following which the medium was supplemented with charcoal-stripped serum to a final concentration of 5% and the cells and virus−DNA mix were incubated for 24 h.

Twenty-four hours following infection, the cells were treated separately with compounds **1**−**8**, 5α-DHT, in ethanol (from $10^{-12}$ to $10^{-8}$ M) or ethanol vehicle alone (final ethanol concentration = 0.05%) and incubated for 24 h. (After the incubation had begun, the ethanolic stock solutions of the steroids were evaporated. Several days later they were analyzed by TLC. There was no evidence of decomposition of the steroids.) Following incubation the cells were harvested by scraping in TEN buffer (40 mM Tris, 1 mM EDTA, 150 mM NaCl, pH 8.0), collected by centrifugation, resuspended in 0.25 M Tris, pH 7.5, and lysed by three rounds of freeze−thawing. The CAT activity was determined by incubating 2.5−10 μg of total protein[31] with [$^3$H]chloramphenicol (20 μCi/μmol) and butyryl CoA substrate as described earlier.[32] A 2:1 mixture of tetramethylpentadecane:xylenes was used to extract acylated chloramphenicol, which was then counted. The data were analyzed using a computer-assisted nonlinear curve-fitting method (Prism) to determine the ED$_{50}$ for each compound. The results of three experiments performed in triplicate are presented in Table 1. The relative stimulatory activity (RSA) of each steroid is shown as compared to the potency of 5α-DHT.

**Distribution of [$^{125}$I]3 in Vivo.** Male Sprague−Dawley rats, approximately 250 g, were castrated and on the following day injected in the tail vein with 5.4 μCi (2.4 pmol) of [$^{125}$I]**3** in 100 μL of ethanol/saline (1:4). Blocked animals were injected subcutaneously with 500 μg of 5α-DHT in 250 μL of propylene glycol/saline (3:2) 15 min before the administration of the $^{125}$I tracer. Each group contained 5−6 animals. At 0.5, 1, 2, and 4 h after the injection of the tracer, the animals were anesthetized with ether and killed by exsanguination. Immediately afterward, tissues were extirpated, weighed, and counted. The entire thyroid was taken, which contained some adhering esophagus. The results are presented in Table 2. In another experiment performed in a similar manner, the tissue distribution of [$^{125}$I]**3** was compared to that of [$^{125}$I]**1**. Animals were killed, and tissues were removed, weighed, and counted at a single time point, 1 h. The results are presented in the Results section.

**Stability of 7α-Iodo and 7α-Fluoro Steroids in Aqueous Solution.** A 10-μL aliquot of a $1 \times 10^5$ cpm/μL solution of [$^{125}$I]**1** or [$^{125}$I]**3** in EtOH was added to 1 mL of 0.1 M Tris-HCl buffer at pH 7.4 and incubated for 0, 15, 30, 60, 120, or 180 min at 0, 22, and 37 °C. Aliquots of the incubation mixtures were extracted with EtOAc (1x, 3 mL) and (1x, 2 mL). The combined organic extracts were evaporated under a $N_2$ stream and counted. Incubations were performed in triplicate, and the results for [$^{125}$I]**3** are presented in Figure 3. The plot of the fraction of initial radioactivity extracted in the organic phase after incubation versus time was analyzed using a one-phase exponential fit with Prism software (GraphPad Software Inc., San Diego, CA) to determine the half-life.

An aliquot of the aqueous phase from a 1 h incubation of [$^{125}$I]**3** at 37 °C was spotted on a TLC plate with a standard of NaI and developed with system T-8. The radioactivity was contained in the band migrating with carrier iodide ($R_f$ 0.7) which was visualized using bromocresol green indicator. Thus, the radioactivity left in the aqueous solution after incubation at 37 °C is in the form of free iodide. The identity of the dehalogenated steroid resulting from the elimination reaction in the aqueous incubation was determined as follows. A solution of 3 mg (0.007 mmol) of **1** in 30 mL of 0.017 M sodium phosphate buffer, pH 7.4, containing 42% (v/v) EtOH was stirred and heated at 37 °C for 48 h. The reaction mixture was poured into $H_2O$ (50 mL), extracted with $CH_2Cl_2$ (3x, 50 mL), dried over $Na_2SO_4$, and concentrated in vacuo. TLC analysis in system T-2 showed a single product with $R_f = 0.27$. Purification of the residue by flash column chromatography on a 1- × 20-cm column of silica gel using 1.5/1 hexanes/EtOAc as eluent gave 0.5 mg of compound **45** as a white solid. Data for **45**: $^1$H NMR (500 MHz, $CDCl_3$) δ 0.64 (s, 3H, H-18), 1.04 (s, 3H, H-19), 3.77 (dd, 1H, $J$ = 9.0, 7.9 Hz, H-17α), 5.20 (m, 1H, H-7); HRMS (FAB, $NH_4$ + PEG) calcd for $C_{19}H_{29}O_2$ 289.2168, found 289.2161. GC−MS analysis (baseline resolution) showed three products: **45a**,**b**,**c** (5:11:84). The GC−MS analysis established the presumptive molecular ion at *m*/*z* 288 for all three components, consistent with the expected $C_{19}H_{28}O_2$: **45a**, 10.89 min, *m*/*z* 288 (100); **45b**, 11.16 min, *m*/*z* 288 (98); **45c**, 11.72 min, *m*/*z* 288 (98). The fragmentation patterns of all three were very similar and did not allow for definitive assignments of structure.

Fluorinated steroids **2** and **4** were subjected to the same aqueous conditions as described above for **1**. Compounds **2** (132 μg, 0.43 μmol) in 540 μL of EtOH, 1.25 mL of 0.017 M sodium phosphate buffer, pH 7.4; **4** (161 μg, 0.5 μmol) in 620 μL of EtOH, 1.46 mL of buffer were stirred at 37 °C for 48 h. The reaction mixtures were extracted with $CH_2Cl_2$ (3x, 2 mL). The combined organic extracts were evaporated under $N_2$ and analyzed by TLC in system T-2. In both cases only unchanged starting material was detected; i.e., no elimination product **45** was found.

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## References

(1) Hochberg, R. B. 16α-iodo-17β-estradiol: A Highly specific radioiodinated probe for the estrogen receptor. In *Cytotoxic Estrogens in Hormone Receptive Tumors*; Raus, J., Martens, H., Leclercq, G., Eds.; Academic Press: New York, 1980; pp 123−140.

(2) Hochberg, R. B.; Rosner, W. The Interaction of 16α-[$^{125}$I]-Iodoestradiol With Estrogen Receptor and Other Binding Proteins. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 328−332.

(3) Zielinski, J. E.; Yabuki, H.; Pahuja, S. L.; Larner, J. M.; Hochberg, R. B. 16α-[$^{125}$I]Iodo-11$\beta$-Methoxy-17$\beta$-Estradiol: A Radiochemical Probe for Estrogen Sensitive Tissues. *Endocrinology* **1986**, *119*, 130−139.
(4) Hanson, R. N.; Franke, L. A. Preparation and Evaluation of 17α-[$^{125}$I]Iodovinyl-11$\beta$-Methoxyestradiol As a Highly Selective Radioligand for Tissues Containing Estrogen Receptors. *J. Nucl. Med.* **1984**, *25*, 998−1002.
(5) Hochberg, R. B.; Hoyte, R. M.; Rosner, W. E-17α-(2-[$^{125}$I]-Iodovinyl)-19-Nortestosterone: The Synthesis of a Gamma-Emitting Ligand for the Progesterone Receptor. *Endocrinology* **1985**, *117*, 2550−2552.
(6) Grill, H.-J.; Pollow, K.; Heubner, A.; Laurent, H.; Hoffmeister, H.; Schulze, P. E.; Elger, W. Iodine 125-Labeleled Vinyl Nortestosterone: a New High Affinity Ligand for Progesterone Receptor Determination. *Acta Endocrinol.* **1985**, *108*, 125−126.
(7) Pomper, M. G.; Katzenellenbogen, J. A.; Welch, M.; Brodack, J. W.; Mathias, C. J. 21-[18F]Fluoro-16 Alpha-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.
(8) Ali, H.; Rousseau, J.; Van Lier, J. E. Synthesis of (17α,20E/Z) Iodovinyl Testosterone and 19-Nortestosterone Derivatives As Potential Radioligands for Androgen and Progesterone Receptors. *J. Steroid Biochem. Mol. Biol.* **1994**, *49*, 15−29.
(9) Choe, Y. S.; Lidstrom, P. J.; Chi, D. Y.; Bonasera, T. A.; Welch, M. J.; Katzenellenbogen, J. A. Synthesis of 11$\beta$-[$^{18}$F]Fluoro-5α-Dihydrotestosterone and 11$\beta$-[$^{18}$F]Fluoro-19-nor-5α-Dihydrotestosterone: Preparation Via Halofluorination-Reduction, Receptor Binding, and Tissue Distribution. *J. Med. Chem.* **1995**, *38*, 816−825.
(10) Liu, A.; Katzenellenbogen, J. A.; VanBrocklin, H. F.; Mathias, C. J.; Welch, M. J. 20-[$^{18}$F]Fluoromibolerone, a Positron-Emitting Radiotracer for Androgen Receptors: Synthesis and Tissue Distribution Studies. *J. Nucl. Med.* **1991**, *32*, 81−88.
(11) Liu, A.; Dence, C. S.; Welch, M. J.; Katzenellenbogen, J. A. Fluorine-18-Labeled Androgens: Radiochemical Synthesis and Tissue Distribution Studies on Six Fluorine-Substituted Androgens, Potential Imaging Agents for Prostatic Cancer. *J. Nucl. Med.* **1992**, *33*, 724−734.
(12) Welch, M. J.; Downer, J. B.; Katzenellenbogen, J. A. Steroid hormone receptors as targets for diagnostic imaging. In *Current Directions in Radiopharmaceutical Research and Development*; Matias, J. R., Ed.; Kluwer Academic Publishers: Dordrecht, Netherlands, 1996; pp 137−156.
(13) Hoyte, R. M.; Rosner, W.; Hochberg, R. B. Synthesis of [16α-$^{125}$I]-Iodo-5α-Dihydrotestosterone and Evaluation of Its Affinity for the Androgen Receptor. *J. Steroid Biochem.* **1982**, *16*, 621−628.
(14) Symes, E. K.; Coulson, W. F.; Farrant, R. D.; Milroy, E. J. G. 16α-Iodo-Testosterone: Chemical Synthesis and Evaluation As a Potential Radiopharmaceutical. *Biochem. Pharmacol.* **1985**, *34*, 3173−3178.
(15) (a) Hoyte, R. M.; MacLusky, N. J.; Hochberg, R. B. The Synthesis and Testing of E-17α-(2-Iodovinyl)-5α-Dihydrotestosterone and Z-17α-(2-Iodovinyl)-5α-Dihydrotestosterone As $\gamma$-Emitting Ligands for the Androgen Receptor. *J. Steroid Biochem.* **1990**, *36*, 125−132. (b) Hoyte, R. M.; Brown, T. J.; MacLusky, N. J.; Hochberg, R. B. 7α-Methyl-17α-(E-2′-[$^{125}$I]Iodovinyl)-19-Nortestosterone: a New Radioligand for the Detection of Androgen Receptor. *Steroids* **1993**, *58*, 13−23.
(16) Hoyte, R. M.; Borderon, K.; Bryson, K.; Allen, R.; Hochberg, R. B.; Brown, T. J. Synthesis and Evaluation of 7α-Iodo-5α-Dihydrotestosterone As a Potential Radioligand for Androgen Receptor. *J. Med. Chem.* **1994**, *37*, 1224−1230.
(17) Labaree, D. C.; Brown, T. J.; Hoyte, R. M.; Hochberg, R. B. 7α-[$^{125}$I]Iodo-5α-Dihydrotestosterone: a Radiolabeled Ligand for the Androgen Receptor. *J. Nucl. Med.* **1997**, *38*, 402−409.
(18) Segaloff, A. The Enhanced Local Androgenic Activity of 19-nor Steroids and Stabilization of Their Structure by 7α and 17α-Methyl Substituents to Highly Potent Androgens by Any Route of Administration. *Steroids* **1963**, *1*, 299−315.
(19) Salmond, W. G.; Barta, M. A.; Havens, J. L. Allylic Oxidation With 3,5-Dimethylpyrazole. Chromium Trioxide Complex. Steroidal $\alpha^5$-7-Ketones. *J. Org. Chem.* **1978**, *43*, 2057−2059.
(20) Cragg, G. M. L.; Davey, C. W.; Hall, D. N.; Meakins, G. D.; Richards, E. E.; Whateley, T. L. Hydroxy-Steroids. Part IV. The Preparation and Spectra of Steroid Olefins. *J. Chem. Soc.* **1966**, 1266−1276.
(21) Hagiwara, H.; Noguchi, S.; Nishikawa, M. Syntheses of 19-Nor-3-Ketosteroids. *Chem. Pharm. Bull. (Jpn)* **1960**, *8*, 84−85.
(22) Kumar, V.; Amann, A.; Ourisson, G.; Luu, B. Stereospecific Syntheses of 7$\beta$- and 7α-Hydroxycholesterols. *Synth. Commun.* **1987**, *17*, 1279−1286.
(23) Labaree, D. C.; Hoyte, R. M.; Hochberg, R. B. A Direct Stereoselective Synthesis of 7-$\beta$-Hydroxytestosterone. *Steroids* **1997**, *62*, 482−486.
(24) Nazareth, L. V.; Weigel, N. L. Activation of the Human Androgen Receptor Through a Protein Kinase A Signaling Pathway. *J. Biol. Chem.* **1996**, *271*, 19900−19907.
(25) Kiesewetter, D. O.; Kilbourn, M. R.; Landvatter, S. W.; Heiman, D. F.; Katzenellenbogen, J. A.; Welch, M. J. Preparation of Four Fluorine- 18-Labeled Estrogens and Their Selective Uptakes in Target Tissues of Immature Rats. *J. Nucl. Med.* **1984**, *25*, 1212−1221.
(26) French, A. N.; Wilson, S. R.; Welch, M. J.; Katzenellenbogen, J. A. A Synthesis of 7 Alpha-Substituted Estradiols: Synthesis and Biological Evaluation of a 7 Alpha-Pentyl-Substituted BODIPY Fluorescent Conjugate and a Fluorine-18-Labeled 7 Alpha-Pentylestradiol Analogue. *Steroids* **1993**, *58*, 157−169.
(27) Still, C. W.; Kahn, M.; Mitra, A. Rapid Chomatographic Technique for Preparative Separations With Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923−2925.
(28) Tilley, W. D.; Wilson, C. M.; Marcelli, M.; McPhaul, M. J. Androgen Receptor Gene Expression in Human Prostate Carcinoma Cell Lines. *Cancer Res.* **1990**, *50*, 5382−5386.
(29) Allgood, V. E.; Oakley, R. H.; Cidlowski, J. A. Modulation by Vitamin B6 of Glucocorticoid Receptor-Mediated Gene Expression Requires Transcription Factors in Addition to the Glucocorticoid Receptor. *J. Biol. Chem.* **1993**, *268*, 20870−20876.
(30) Allgood, V. E.; Zhang, Y.; O'Malley, B. W.; Weigel, N. L. Analysis of Chicken Progesterone Receptor Function and Phosphorylation Using an Adenovirus-Mediated Procedure for High-Efficiency DNA Transfer. *Biochemistry* **1997**, *36*, 224−232.
(31) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, *248−54*, 248−254.
(32) Zhang, Y.; Bai, W.; Allgood, V. E.; Weigel, N. L. Multiple Signaling Pathways Activate the Chicken Progesterone Receptor. *Mol. Endocrinol.* **1994**, *8*, 577−584.

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