Fluorine-18-Labeled Androgens: Radiochemical Synthesis and Tissue Distribution Studies on Six Fluorine-Substituted Androgens, Potential Imaging Agents for Prostatic Cancer

Aijun Liu, Carmen S. Dence, Michael J. Welch, and John A. Katzenellenbogen

Department of Chemistry, University of Illinois, Urbana, Illinois and Division of Radiation Sciences, Mallinckrodt Institute, Washington University Medical School, St. Louis, Missouri

We have synthesized six androgens labeled with ^{18}F as potential imaging agents for prostatic cancer. These include 16β-fluorine-substituted testosterone, dihydrotestosterone and mibolerone, 16α- and 16β-fluorine substituted 7α-methyl-19-nortestosterone, and 20-fluoro-R1881 (metribolone). All of the radiochemical preparations proceeded in satisfactory yield, giving material with adequately high effective specific activity for the in vivo studies. In the tissue distribution studies in diethylstilbestrol-treated male rats, high selective uptake by the prostate was observed that ranged from 0.39% to 1.21% injected dose (ID)/g at 1 hr and 0.20 to 0.47 at 4 hr, with prostate-to-blood and prostate-to-muscle ratios ranging from 3.28 to 9.45, respectively, at 1 hr and 4.06 to 35.0, respectively, at 4 hr. Those compounds that are likely to be metabolized rapidly showed lower prostate uptake but higher uptake selectivity at 4 hr; at earlier times, uptake selectivities were more comparable. Compounds with a 16β-fluorine substituent showed extensive metabolic defluorination, resulting in ca. 50% of the dose being deposited in bone at 4 hr. This is consistent with a 16α-hydroxylation process that may proceed rapidly with these compounds, but would be retarded by a 17α-methylation, blocked by inversion of stereochemistry at C-16, and would not affect fluorine at the C-20 position. These fluoroandrogens, together with 20-fluoromibolerone described previously, are the first positron-emitting androgens to show high affinity and selective uptake by androgen target tissues in vivo, and they may be useful as in vivo prostate imaging agents in man.

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The assay of androgen receptor $(AR)^+$ level in prostatic cancer has been used to predict tumor responsiveness to hormonal therapy (*1–11*). The correlations between AR positivity and response, however, has been less good (*1–3*), for example, than that between estrogen or progesterone receptor positivity and response to hormonal therapy in breast cancer (*12*). The poor correlation in prostatic cancer may be due, in part, to the fact that partial tissue sampling techniques (needle biopsy or transurethral resection) may be selecting a nonrepresentative fraction of the tumor (*1*). Thus, a method for assessing AR levels in the prostate and tumor as a whole, such as an AR-based imaging agent, might provide a more meaningful basis for predicting the success of hormonal therapy. Agents to image the prostate and prostatic tumors based on their AR content might also be useful in staging the disease and monitoring the course of therapy. In this regard, AR is a propitious target, since typical hormonal treatments that involve orchidectomy or the administration of estrogens or high doses of gonadotropin releasing hormones (*13*) reduce circulating androgen levels and increase the fraction of unoccupied AR in the prostate and in tumors (*14–17*).

There have been a few reports on the development of AR-based imaging agents (*18–23*). However, in most cases, the compounds prepared have had either relatively low affinity for AR or their tissue distribution properties were not adequately studied. Recently, Salman and Chamness reported on the synthesis of an iodinated androgen with high binding affinity, but not on its distribution (*24*). Still, it is clear from two early studies (*25,26*), plus a more recent one of ours (*27*), that tritium-labeled androgens are taken up selectively by the prostate in rats, that good contrast between target (prostate) and nontarget tissues (muscle and blood) ranging from 3.7 to 23 can be achieved within reasonable times (1–4 hr) and that the target tissue uptake is mediated by a limited capacity binding system that can be blocked by co-administration of an excess of unlabeled androgen.

We have outlined the structural characteristics of androgens that are important for high affinity binding to AR and moderate affinity binding to competing binding sites (other steroid receptors such as progesterone receptor (PgR) and serum and tissue binding proteins, sex steroid binding protein (SBP) and androgen binding protein

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For reprints contact: John A. Katzenellenbogen, Department of Chemistry, University of Illinois, 461 Roger Adams Laboratory, Box 37, 1209 West California St., Urbana, IL 61801.

(ABP), respectively) (*28–30*). We have also described the synthesis and determined the AR binding affinity of a number of fluorine-substituted androgens that might eventually be prepared in ^{18}F-labeled form for prostate imaging with positron emission tomography (PET) (*31*). Recently, we described a series of fluorine-substituted androgens with very desirable binding properties for imaging (*30*) and reported on the favorable tissue distribution in rats for one of these compounds, 20-fluoromibolerone, prepared in ^{18}F-labeled form (*32*).

In this study, we describe the synthesis of six additional ^{18}F-labeled androgens and their tissue distribution in diethylstilbestrol-treated rats. The target tissue uptake efficiency and selectivity of these compounds is high and at later times seems to be related in a direct and reciprocal fashion, respectively, to their presumed clearance rates; at earlier times, their uptake selectivities are more comparable. In addition, the pattern of metabolic defluorination of these compounds suggests that hydroxylation at the 16α-position can occur and that this process can be suppressed by certain structural features. A comparison of the uptake and metabolism data suggests which compounds should be studied further as AR-based imaging agents for the prostate.

EXPERIMENTAL PROTOCOL

Radiochemical Synthesis

Fluorine-18 was produced from [^{18}O]H_2O by the ^{18}O (p,n) ^{18}F reaction as previously described (*33*). Resolubilization was carried out as follows. A suitable amount of radioactivity in H_2O was added to 0.65–0.85 eq. (relative to triflate or cyclic sulfate substrate) of nBu_4 NOH (1 *M* solution in H_2O) in a Vacutainer® (*34*). The vessel was placed in an oil bath at 110°C, and water was removed under a gentle stream of N_2, assisted by three azeotropic distillations, each employing 0.5–0.8 ml CH_3CN; the last segment of the final distillation was carried out without heating. The dried activity was redissolved in 2 × 100 μl of freshly distilled tetrahydrofuran (THF) and transferred to a 4-ml glass ample vial containing 1–2 mg of substrate and a stirring bar. The entire process requires 10–15 min, and ~80% of the initial activity is transferred. All reactions were done without carrier addition.

High-performance liquid chromatography (HPLC) was performed isocratically on a Spectra-Physics Model 8700 or a Varian 5060 liquid chromatograph with an analytical 5-μm SiO_2 column (4.6 mm × 30 cm, Varian Si-5 Micro Pak, operated at 1 ml/min), a preparative 10 μm SiO_2 column (Whatman Partisil M-9, 0.9 cm × 50 cm, operated at 5 ml/min) or an analytical reverse-phase C-18 column (Whatman Partisil 10 ODS, 4.6 mm × 25 cm, operated at 1 ml/min). The eluent was monitored by UV absorbance at 254 nm for compounds with the conjugated enone function (0.1 absorbance units full scale), and a refractive index detector for compounds with only ketone and hydroxy functions. Radioactivity of the eluent was monitored with a flow-through sodium iodide scintillation detector, where appropriate, and the identity of the radiolabeled compounds was confirmed by coelution with authentic unlabeled standard on an analytical HPLC. Radioactivity levels >100 μCi were quantitated with a dose calibrator. Effective activities (*35*) were determined by a competitive radiometric binding assay to AR using a modification of the procedure previously reported (*36*).

16β-[^{18}F]Fluorodihydrotestosterone (16β-F-DHT; ^{18}F-2). A sample vial containing 1.7 mg (3.54 μmol) of the trifluoromethane sulfonate (triflate) precursor **1** and the resolubilized radioactivity ($nBu_4N^{18}F$ + nBuNOH) in 200 μl of THF was capped with a Teflon-lined cap and placed in an oil bath at 55°C. It was stirred for 5 min, cooled to room temperature and then cooled to −78°C. To this reaction mixture was added via syringe 0.15 ml of $LiAlH_4$ solution (1 *M* in Et_2O, Aldrich). The mixture was stirred at −78°C for 10 min and without the cooling bath for 1 min before being quenched with 0.5 ml of 3 N aq. HCl (Excess HCl is needed for the ketal deprotection step). At this point, 0.2 ml of acetone was added, and the solution was stirred at 55°C for 10 min. It was then extracted with Et_2O three times, dried with anhydrous $MgSO_4$ and passed through a parfait column (short pipette filled sequentially with glass wool, 0.3 cm of SiO_2 and 0.5 cm of Na_2SO_4) with Et_2O. The solvents were evaporated in vacuo and the residue was redissolved in 1 ml of an HPLC solvent mixture (83% hexane/17% CH_2Cl_2 (containing 5% iPrOH)). It was injected onto a preparative normal-phase HPLC column through a syringe filter and eluted with the HPLC solvent mixture mentioned above; the desired compound eluted at ~28 min. The total manipulation time was ca. 90 min, and ^{18}F-2 was produced in decay-corrected radiochemical yields of 30.7%–48.1% with an effective specific activity of 1151 Ci/mmol.

16β-[^{18}F]Fluorotestosterone (16β-F-T; ^{18}F-4). A solution of the triflate precursor **3** (2 mg, 3.54 μmol) and resolubilized activity in 200 μl of THF was capped with a Teflon-lined cap and placed in an oil bath at 55°C. It was stirred for 5 min and then cooled to room temperature and −78°C. To this mixture was added via syringe 0.12 ml of $LiAlH_4$ solution (1 *M* in Et_2O). The mixture was stirred at −78°C for 12 min before being quenched with 0.1 ml of EtOAc while kept cold. It was warmed up to room temperature and further quenched with 0.1 ml of MeOH. KOH/MeOH solution (0.1 ml × 5 N) was then added, and the mixture was stirred at 55°C for 12 min to remove the triflate protection function at the 3-position. Finally, the mixture was acidified with 0.7 ml of aq. HCl (2 N) solution and worked up and dried as described above. After the solvents were evaporated in vacuo, the residue was redissolved with an HPLC solvent mixture (78% hexane/22% CH_2Cl_2 (containing 5% iPrOH) and injected onto a preparative normal-phase HPLC column. It was eluted with the same HPLC solvent mixture and the desired product eluted at ~43 min. The total reaction and purification time was about 140 min, and decay-corrected radiochemical yields were 19.2%–30.2%. Collected fractions of ^{18}F-4 for animal studies had a specific activity of 295 Ci/mmol.

16β-^{18}F]Fluoromibolerone (16β-F-Mib; ^{18}F-6) and 16β-[^{18}F]fluoro-7α-methyl-19-nortestosterone (16β-F-MNT; ^{18}F-7). A sample vial containing 2 mg (3.54 μmol) of the 16α-triflate precursor **5** and the resolubilized radioactivity in 200 μl of THF was placed in an oil bath at 58–59°C. It was stirred for 5 min, then cooled to room temperature and −78°C. To the mixture was added via syringe 0.17 ml of $LiAlH_4$ solution (1 *M* in Et_2O) and 0.1 ml of dry THF (for ^{18}F-6, 0.22 ml of 3 *M* MeMgBr solution in Et_2O was added), and the mixture was stirred at −78°C for 14 min (for ^{18}F-6, it was at −15 to −10°C for 10 min and without the cooling bath for 2 min). The mixture was then quenched with 0.1 ml of EtOAc, then stirred at −78°C for 1 min as well as at room temperature for 2 min. The mixture was further quenched with

0.5 ml of aq. HCl (2N) (for ^{18}F-6, it was directly quenched with 0.5 ml × 2 N of aq. HCl) and worked up as before. At this point, 0.2 ml of MeOH was added and the solvents, except MeOH, were evaporated in vacuo (without heating). To the concentrate were added 0.2 ml more of MeOH as well as 75 μl of KOH/MeOH (5 N) solution, and the mixture was stirred at 58°C for 12 min. It was then diluted with Et_2O and passed through a parfait column with EtOAc. The solvents were evaporated in vacuo, and the residue was redissolved in 1 ml of an HPLC solvent mixture (for ^{18}F-6, 80% hexane/20% CH_2Cl_2 (containing 5% iPrOH) and for ^{18}F-7, 76% hexane/24% CH_2Cl_2 (containing 5% iPrOH)). The samples were injected onto a preparative normal phase HPLC column and eluted with the HPLC solvent mixtures mentioned above. The R_t of the compound ^{18}F-6 was ~34 min, and ^{18}F-7 was ~31 min. The total manipulation time required was about 130 min for ^{18}F-6 and 140 min for ^{18}F-7, and decay-corrected radiochemical yields were 5.0%–8.4% and 20.5%–25.8%, respectively. Collected fractions of ^{18}F-6 for in vivo animal studies had an effective specific activity of 1693 Ci/mmol, and ^{18}F-7 804 Ci/mmol.

16α-[^{18}]Fluoro-7α-methyl-19-nortestosterone (16α-F-MNT; ^{18}F-9). A solution of the 16β triflate precursor **8** (2 mg, 3.54 μmol) and resolubilized activity in 200 μl of freshly distilled THF was capped and placed in an oil bath at 55°C. It was stirred at 55°C for 5 min and cooled to room temperature for 1 min. The THF solvent was then evaporated under a stream of N_2, followed by addition of 0.2 ml of dry Et_2O and evaporation to make sure that THF was completely removed. The residue was taken up in 0.2 ml of dry Et_2O and cooled to −78°C. To the solution was added 0.5 ml of $LiAlH_4$ (1 *M* in Et_2O) solution slowly via syringe. The mixture was stirred at −78°C for 15 min before being quenched with 0.1 ml of EtOAc while being kept cold. The rest of the procedure was the same as that in synthesis of ^{18}F-6 and ^{18}F-7 described above. The HPLC solvent for this compound was 75.5% hexane 24.5% CH_2Cl_2 (containing 5% iPrOH), and the desired product eluted at ~49 min. Since there is a large mass peak that coeluted with the desired compound, the effective specific activity of collected fractions was only 15.6 Ci/mmol. The total manipulation time was ca. 160 min, and ^{18}F-9 was produced in decay-corrected radiochemical yields of 14.5%–19.5%.

20-[^{18}F]Fluoro-R1881 (20-F-R1881; ^{18}F-11). The resolubilized activity in 200 μl of dry THF was added to a solution of the cyclic sulfate precursor **10** (1.3 mg, 3.59 μmol) in 100 μl of dry THF, during which time the solution changed from colorless to greenish blue. The reaction vial was capped, placed in an oil bath at 72–73°C and stirred for 15 min before being cooled to room temperature. To the solution was added 0.1 ml of HCl/MeOH solution (2 N), and the mixture, now a wine red color, was stirred at room temperature for 7 min and at 72°C for 3 min. It was then quenched with 0.8 ml of saturated aq. $NaHCO_3$ solution, extracted with Et_2O three times and passed through a parfait column (see above). The solvents were evaporated in vacuo, and the residue was redissolved in an HPLC solvent mixture (77% hexane 23% CH_2Cl_2 (containing 5% iPrOH)). It was then injected onto a preparative normal-phase HPLC column and was purified with the solvent mixture mentioned above. Retention time of the desired product ^{18}F-11 was about 36 min, and the collected fractions for animal studies had an effective specific activity of 128 Ci/mmol. The reaction and purification took about 2 hr, and decay-corrected radiochemical yields were 25.5%–37.7%.

Tissue Distribution Studies

Mature male Sprague-Dawley rats (175 g average) (Sasco Laboratory Animals, Omaha, NE) were either untreated or pretreated with diethylstilbestrol (DES) to suppress endogenous androgen synthesis. The treated animals were injected subcutaneously with 1 mg DES in 0.2 ml of sunflower seed oil at 24 hr and 3 hr prior to the experiment, following the method of Symes (*26*).

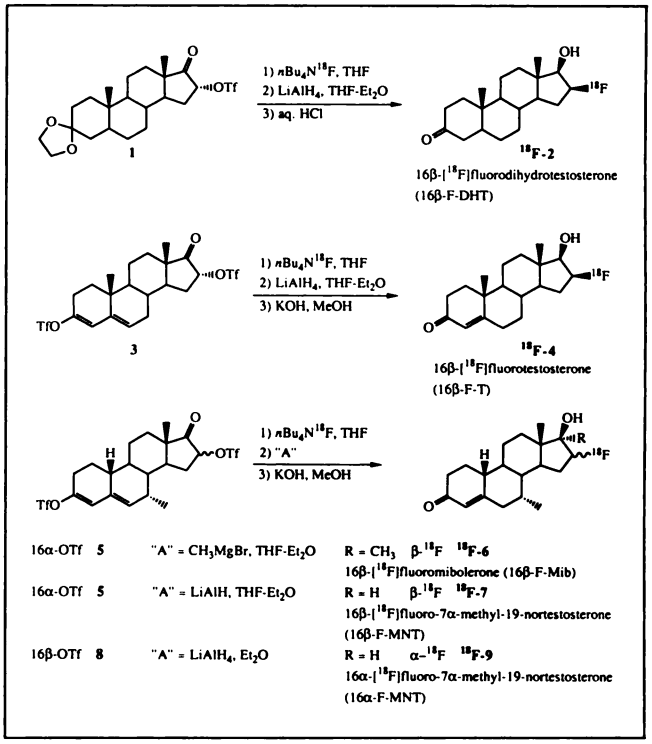
Fluorine-18-labeled androgens were dissolved in ethanol and diluted with physiologic saline to give a final solution of 10% ethanol-saline. The animals were injected intravenously under ether anesthesia with 100 μCi (~200 μl) of the test compound. To block receptor-mediated uptake, 36 μg of testosterone were added to the injected dose for one set of animals. All were provided with rat chow and water ad libitum.

At the indicated time points, 1, 2 and 4 hr, the animals were killed, samples of blood and tissue were excised, weighed and the radioactivity determined as previously reported (*36*). The injected dose was calculated from standards prepared from the injection solution, and the data were expressed as percent injected dose per gram of tissue (%ID/g).

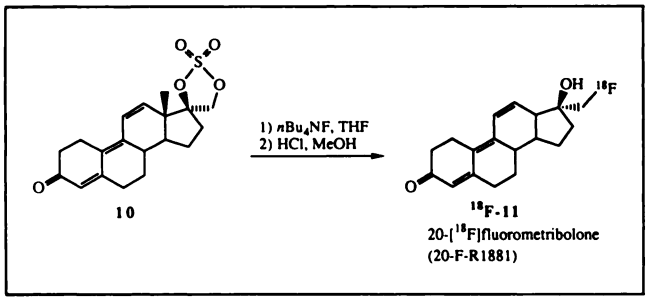
RESULTS

Radiochemical Synthesis

The preparative routes to the six fluorine-substituted androgens previously described were designed so that fluorine could be introduced into the steroid late in the synthesis by a fluoride ion displacement of a reactive triflate or cyclic sulfate derivative (*30*). Thus, these sequences could be readily adapted to the synthesis with no-carrier-added ^{18}F, using tetrabutylammonium [^{18}F]fluoride prepared as described previously (*33,34*) (Schemes 1



SCHEME 1. Synthesis of ^{18}F-labeled androgens: ^{18}F-2, ^{18}F-4, ^{18}F-6, ^{18}F-7 and ^{18}F-9.



SCHEME 2. Synthesis of ^{18}F-labeled androgen: ^{18}F-11.

and 2). The effective specific activities (*35*) of the ^{18}F-labeled androgens were determined by competitive AR radioreceptor binding assay on a decayed sample of known activity using a modification of the procedure reported (*36*).

Triflates were utilized as precursors in the synthesis of all five 16-[^{18}F]fluoro androgens, as shown in Scheme 1, the 16α-triflates for the corresponding 16β-[^{18}F]fluoro compounds and the 16β-triflate for the 16α-[^{18}F]fluoro compound. The 3-ketone function in the saturated A ring was protected as a ketal, while that in the 4(5)-unsaturated A ring was protected in the form of the dienol triflate.

In the preparation of 16β-F-DHT (^{18}F-2), [^{18}F]fluoride ion displacement of the 16α-triflate proceeded in excellent yield; the $LiAlH_4$ reduction in a mixed Et_2O-THF solution proceeded with high stereoselectivity in this series, and ketal deprotection was rapid. The synthesis and subsequent HPLC purification took only about 90 min and produced 16β-F-DHT in a decay-corrected radiochemical yield of 31%-48%. The effective specific activity of 16β-F-DHT was 1151 Ci/mmol.

The purification of 16β-F-DHT by normal-phase HPLC was complicated by the fact that this compound has no chromophore that absorbs beyond the wavelength cut off of CH_2Cl_2 (245 nm), the major solvent for normal phase HPLC. Pure fractions were collected by following the radioactivity trace, and these were subsequently reanalyzed by reversed-phase analytical HPLC in a CH_3CN-H_2O system, where 16β-F-DHT can be detected either by UV absorbance at 215 nm or by refractive index. The latter detector has a stable base line when the solvents are premixed. By this method, the material collected from the preparative column was shown to be radiochemically homogeneous and to co-elute with an authentic standard.

Synthesis of the other four 16-[^{18}F]fluoro androgens also included three steps: [^{18}F]fluoride substitution of the 16-triflates, $LiAlH_4$ reduction of or CH_3MgBr addition to the 16-fluoro-17-ketones, and removal of the 3-triflates under basic conditions. The [^{18}F]fluorine incorporation gave good yield at 60°C in all the cases, and the other two steps went smoothly in the synthesis of the 16β-F-T but proved to be troublesome in the synthesis of the three 7α-methyl compounds. As was observed in the original preparation of the 7α-methyl-substituted compounds (*30*), a much larger excess of reagent was needed, and control of the reaction conditions proved to be critical. The best conditions known so far are described in the experimental. As a general practice, the excess reagent present in the $LiAlH_4$ reduction was first quenched with EtOAc and then with either KOH/MeOH solution or aq. HCl, depending on the next operation; the initial aprotic quench was done to prevent reduction of the double bonds by the H_2 gas that forms during an aqueous quenching process. The $LiAlH_4$ reduction as well as the Grignard reaction of the three 16β-[^{18}F]fluoro compounds were carried out in a mixture of THF and Et_2O, as nucleophilic addition at C-17 proceeds with high α-stereoselectivity with a 16β-disposed fluorine substituent. However, for 16α-F-MNT (^{18}F-9), $LiAlH_4$ reduction was done in Et_2O, as this solvent is known to give higher stereoselectivity (*30*). For 16β-F-DHT, removal of the 3-triflate protecting group with base proceeded satisfactorily without product isolation after the $LiAlH_4$ reduction. In the three 7α-methyl androgen cases, however, it proved important to verify the completeness of nucleophilic addition to the C-17 ketone before deprotection and isolation of the 16-fluoro-17-hydroxy intermediate after reduction but prior to deprotection of the A-ring dienol triflate was required, otherwise the deprotection yield was very low. All four [^{18}F]fluorinated androgens were purified by normal-phase HPLC, and the collected fractions were analyzed by analytical reversed-phase HPLC, with UV detection at 254 nm to monitor the eluted mass. For compounds 16β-F-T (^{18}F-4), 16β-F-Mib (^{18}F-6) and 16β-F-MNT (^{18}F-7), the total time for synthesis and purification was 140 min. Decay-corrected radiochemical yields were 19.2%–30.2%, 5.0%–8.4% and 20.5%–25.8%, respectively, and the effective specific activities were 295 Ci/mmol, 804 Ci/mmol and 1693 Ci/mmol, respectively.

The preparation of 16α-F-MNT (^{18}F-9) proved to be the most difficult. In the $LiAlH_4$ reduction, slow addition of a large excess of the reagent with careful control of the temperature was essential. Isolation of the intermediate after the reduction was time-consuming, and an impurity that is slightly less polar than the product (possibly the protio analog, 7α-methyl-19-nortestosterone, formed by $LiAlH_4$ reduction of the 16β-triflate function in the excess starting material (*37*) that remains after the fluoride ion displacement step) was difficult to separate from the product by normal-phase HPLC, even with long retention times. The synthesis and purification took 160 min and gave a decay-corrected radiochemical yield of 14.5%–19.5% and an effective specific activity of only 15.6 Ci/mmol.

The C-20 position of R1881 (metribolone) was activated as the spiro cyclic sulfate, as shown in Scheme 2. Fluorine-18-fluoride ion displacement of the spiro cyclic sulfate was followed by hydrolysis of the [^{18}F]fluorobisulfate intermediate with HCl/MeOH to give the 20-[^{18}F]fluoroandrogen. Even though this sequence requires harsher reaction conditions and gives a lower yield of [^{18}F]fluorine incorpora-

tion than triflate displacement, the overall process has fewer steps and is thus less time-consuming. As discussed before in the synthesis of 20-F-R1881 (^{18}F-11) (*30*), the solution color changed from colorless to greenish blue, wine red and colorless again in order, upon addition of $nBu_4N^{18}F$ (including nBu_4NOH), HCl/MeOH and aq. $NaHCO_3$. The total time for the synthesis and purification was about 120 min, and ^{18}F-11 was furnished in a decay-corrected radiochemical yield of 25.5%–37.7%, with an effective specific activity of 1201 Ci/mmol.

In Vivo Tissue Distribution Studies

For in vivo uptake studies, the purified ^{18}F-labeled androgens were reconstituted in 10% ethanol-saline, and a 100-μCi portion was injected into each Sprague-Dawley male rat (i.v. femoral vein). In order to suppress in vivo androgen biosynthesis so as to increase concentration of unoccupied androgen receptors (AR), all rats were treated with 1 mg of DES in 0.2 ml sunflower oil per rat, at 24 hr and 3 hr prior to injection of the radiolabeled androgens (*26*). Tissue distribution of radioactivity was determined at 1 hr, 2 hr and 4 hr. To ascertain that the uptake was mediated by a high-affinity, limited-capacity system, one set of rats was also given 36 μg of unlabeled testosterone to occupy the androgen receptors and thus block receptor-mediated uptake of the ^{18}F-labeled compound (2 hr "blocked").

The tissue distribution results of 16β-F-DHT (^{18}F-2) and 16β-F-T (^{18}F-4), shown in Tables 1 and 2, indicate that they are associating with AR in the prostate: (a) uptake ratios of prostate to blood and prostate to muscle were ca. 5 and 7, respectively, for 16β-F-DHT, and 4.5 and 8.5 for 16β-F-T at 1 hr, and went up to 11 and 22 for the former, and 12 and 19 for the latter, at 4 hr; (b) the prostate uptake for the 2-hr unblocked animals were 3.4–4-fold higher than that of the 2-hr blocked animals for both compounds. There is much radioactivity in bone for both compounds, indicating that extensive metabolic defluorination of these two compounds is occurring in vivo (see Discussion).

The in vivo tissue distribution results of all three ^{18}F-labeled 7α-methyl androgens, shown in Tables 3–5, demonstrate that they were selectively taken up by the prostate and that the target tissue uptake was receptor-mediated: (a) the highest ratios of prostate-to-muscle or prostate-to-blood (at 4 hr) for the three compounds were 12.30 and 7.17, respectively; (b) the target uptake for the 2-hr unblocked animals was 2–5-fold higher than that of the 2-hr blocked animals. High bone activity was seen for the 16β-F-MNT (^{18}F-7). However, bone activity was much lower for 16β-F-Mib (^{18}F-6) and was not significant at all for the 16α-F-MNT (^{18}F-9). This suggests that the 17α-methyl group retards, and the 16α-orientation of the fluorine blocks, the in vivo defluorination process.

20-F-R1881 (^{18}F-11) was selectively taken up by the target tissue prostate, but was cleared rather quickly (Table 6). Even though the uptake of this compound by the prostate was higher than that of the other molecules, the prostate-to-blood and the prostate-to-muscle ratios were not high (ca. 5) and remained unchanged between the 1- and 4-hr time points. The activity in blood and muscle cleared quite slowly and at about the same rate as that in the prostate. The ^{18}F label on 20-F-R1881 was not rapidly cleaved, so that low bone radioactivity was observed.

TABLE 1
Tissue Distribution of 16β-[^{18}F]Fluorodihydrotestosterone (16β-F-DHT; ^{18}F-2)

Organ	1 hr	2 hr	2 hr (blocked)†	4 hr
%ID/g ± s.e.m.*				
Blood	0.083 ± 0.010	0.055 ± 0.020	0.056 ± 0.037	0.022 ± 0.008
Bone	1.862 ± 0.617	4.147 ± 2.045	2.745 ± 0.223	3.235 ± 0.553
Muscle	0.055 ± 0.007	0.034 ± 0.009	0.024 ± 0.006	0.011 ± 0.005
Spleen	0.094 ± 0.035	0.136 ± 0.028	0.086 ± 0.029	0.060 ± 0.031
Lung	0.119 ± 0.014	0.075 ± 0.022	0.062 ± 0.021	0.030 ± 0.004
Liver	0.514 ± 0.095	0.457 ± 0.143	0.378 ± 0.153	0.252 ± 0.072
Fat	0.040 ± 0.020	0.027 ± 0.004	0.019 ± 0.006	0.009 ± 0.001
Kidney	0.288 ± 0.039	0.206 ± 0.061	0.153 ± 0.058	0.085 ± 0.011
Prostate (v)‡	0.432 ± 0.183	0.416 ± 0.094	0.134 ± 0.044	0.249 ± 0.061
Prostate (d)	0.386 ± 0.196	0.479 ± 0.200	0.134 ± 0.050	0.198 ± 0.046
Ratios ± s.e.m.				
Prostate/Muscle (v)	7.604 ± 2.183	12.641 ± 2.612	5.721 ± 1.608	24.870 ± 8.393
(d)	6.751 ± 2.446	14.196 ± 3.579	5.819 ± 2.127	20.093 ± 8.048
Prostate/Blood (v)	5.152 ± 1.795	7.903 ± 1.118	3.163 ± 1.391	12.783 ± 4.721
(d)	4.620 ± 2.058	8.723 ± 1.124	3.187 ± 1.561	10.083 ± 3.641

* %ID/g and s.e.m.: percent injected dose per gram tissue and standard error of the mean (n = 4). All rats were treated with DES 24 hr and 3 hr prior to injection with the ^{18}F-labeled androgen.

† Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.

‡ v = ventral and d = dorsal.

TABLE 2
Tissue Distribution of 16β-[^{18}F]Fluorotestosterone (16β-F-T; ^{18}F-4)

Organ	1 hr	2 hr	2 hr (blocked)†	4 hr
%ID/g ± s.e.m.*				
Blood	0.137 ± 0.033	0.055 ± 0.009	0.054 ± 0.021	0.020 ± 0.001
Bone	2.120 ± 1.037	3.239 ± 0.475	2.684 ± 0.367	2.808 ± 1.042
Muscle	0.071 ± 0.009	0.033 ± 0.010	0.029 ± 0.006	0.012 ± 0.002
Spleen	0.075 ± 0.003	0.031 ± 0.007	0.030 ± 0.005	0.014 ± 0.002
Lung	0.134 ± 0.021	0.059 ± 0.006	0.051 ± 0.010	0.022 ± 0.002
Liver	0.848 ± 0.064	0.451 ± 0.080	0.347 ± 0.071	0.213 ± 0.040
Fat	0.071 ± 0.007	0.028 ± 0.003	0.027 ± 0.005	0.012 ± 0.002
Kidney	0.369 ± 0.053	0.169 ± 0.022	0.154 ± 0.034	0.078 ± 0.014
Prostate (v)‡	0.566 ± 0.078	0.395 ± 0.028	0.108 ± 0.021	0.248 ± 0.039
Prostate (d)	0.665 ± 0.097	0.404 ± 0.063	0.092 ± 0.018	0.224 ± 0.062
Ratios ± s.e.m.				
Prostate/Muscle (v)	8.037 ± 0.882	12.681 ± 3.098	3.830 ± 0.605	20.116 ± 1.922
(d)	9.448 ± 1.129	13.051 ± 4.053	3.325 ± 0.855	17.841 ± 1.712
Prostate/Blood (v)	4.282 ± 0.810	7.265 ± 1.040	2.288 ± 0.953	12.335 ± 1.581
(d)	5.067 ± 1.174	7.510 ± 1.700	1.894 ± 0.686	11.105 ± 2.485

* %ID/g and s.e.m.: percent injected dose per gram tissue and standard error of the mean (n = 4). All rats were treated with DES 24 hr and 3 hr prior to injection with the ^{18}F-labeled androgen.
† Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.
‡ v = ventral and d = dorsal.

DISCUSSION

We have synthesized six ^{18}F-substituted androgens and measured their tissue distribution in diethylstilbestrol-treated male rats. Although in some cases, careful attention needs to be given to the details of the reaction conditions, all of the radiochemical syntheses proceeded in satisfactory yield and produced material with high effective specific activity (ranging from 295–1693 Ci/mmol) in all cases but one. Even though the effective specific activity of the 16α-F-MNT was not high (15.6 Ci/mmol), because of contamination by some co-eluting material, the injected mass per adult rat was no more than 2 μg (calculated based on 100 μCi injected dose per rat and assuming 306 as the molecular weight of the impurity) and could be as low as 0.5 μg if the impurity was the protio analog 7α-methyl-19-nortes-

TABLE 3
Tissue Disbribution of 16β-[^{18}F]Fluoromibolerone (16β-F-Mib; ^{18}F-6)

Organ	1 hr	2 hr	2 hr (blocked)†	4 hr
%ID/g ± s.e.m.*				
Blood	0.158 ± 0.031	0.082 ± 0.004	0.080 ± 0.017	0.061 ± 0.008
Bone	0.353 ± 0.113	0.474 ± 0.080	0.321 ± 0.038	0.567 ± 0.243
Muscle	0.180 ± 0.030	0.066 ± 0.002	0.069 ± 0.018	0.038 ± 0.005
Spleen	0.147 ± 0.021	0.071 ± 0.002	0.071 ± 0.018	0.050 ± 0.005
Lung	0.240 ± 0.020	0.104 ± 0.008	0.088 ± 0.020	0.058 ± 0.006
Liver	1.323 ± 0.260	0.740 ± 0.080	0.654 ± 0.050	0.439 ± 0.073
Fat	0.413 ± 0.134	0.185 ± 0.021	0.198 ± 0.030	0.123 ± 0.041
Kidney	0.554 ± 0.094	0.282 ± 0.020	0.223 ± 0.029	0.148 ± 0.018
Prostate (v)‡	0.667 ± 0.165	0.620 ± 0.092	0.255 ± 0.012	0.402 ± 0.156
Prostate (d)	0.756 ± 0.167	0.600 ± 0.053	0.253 ± 0.027	0.468 ± 0.090
Ratios ± s.e.m.				
Prostate/Muscle (v)	3.769 ± 0.874	9.386 ± 1.170	3.868 ± 0.694	10.908 ± 3.982
(d)	4.304 ± 1.024	9.082 ± 0.651	3.924 ± 1.096	12.632 ± 2.798
Prostate/Blood (v)	4.418 ± 1.399	7.547 ± 1.081	3.302 ± 0.478	6.762 ± 2.402
(d)	4.954 ± 1.346	7.299 ± 0.692	3.331 ± 0.780	7.844 ± 1.663

* %ID/g and s.e.m.: percent injected dose per gram tissue and standard error of the mean (n = 4). All rats were treated with DES 24 hr and 3 hr prior to injection with the ^{18}F-labeled androgen.
† Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.
‡ v = ventral and d = dorsal.

TABLE 4
Tissue Distribution of 16β-[^{18}F]Fluoro-7α-methyl-19-nortestosterone (16β-F-MNT; ^{18}F-7)

Organ	1 hr	2 hr	2 hr (blocked)†	4 hr
%ID/g ± s.e.m.*				
Blood	0.124 ± 0.033	0.058 ± 0.013	0.049 ± 0.002	0.034 ± 0.008
Bone	1.853 ± 0.524	1.968 ± 0.437	2.425 ± 0.214	2.888 ± 0.462
Muscle	0.111 ± 0.021	0.051 ± 0.006	0.030 ± 0.002	0.020 ± 0.004
Spleen	0.100 ± 0.016	0.046 ± 0.007	0.036 ± 0.002	0.025 ± 0.005
Lung	0.164 ± 0.023	0.082 ± 0.009	0.047 ± 0.004	0.036 ± 0.004
Liver	0.519 ± 0.073	0.270 ± 0.024	0.265 ± 0.076	0.146 ± 0.029
Fat	0.104 ± 0.005	0.052 ± 0.006	0.044 ± 0.006	0.020 ± 0.003
Kidney	0.431 ± 0.090	0.220 ± 0.027	0.130 ± 0.017	0.088 ± 0.018
Prostate (v)‡	0.572 ± 0.057	0.571 ± 0.092	0.149 ± 0.016	0.299 ± 0.106
Prostate (d)	0.622 ± 0.051	0.473 ± 0.089	0.232 ± 0.170	0.343 ± 0.112
Ratios ± s.e.m.				
Prostate/Muscle (v)	5.366 ± 1.164	11.341 ± 1.847	4.947 ± 0.651	14.698 ± 3.244
(d)	5.792 ± 1.053	9.262 ± 0.730	7.827 ± 5.077	17.090 ± 4.286
Prostate/Blood (v)	4.950 ± 1.385	10.156 ± 2.416	3.067 ± 0.346	8.936 ± 3.018
(d)	5.319 ± 1.258	8.200 ± 0.753	4.784 ± 2.957	10.386 ± 3.713

* %ID/g and s.e.m.: percent injected dose per gram tissue and standard error of the mean (n = 4). All rats were treated with DES 24 hr and 3 hr prior to injection with the ^{18}F-labeled androgen.
† Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.
‡ v = ventral and d = dorsal.

tosterone. Regardless, this level of mass should not cause saturation of the receptor, as was attested to by the selective prostate uptake of 16α-F-MNT and its effective blockage by excess unlabeled androgens.

The uptake of the six ^{18}F-labeled androgens in the prostate target organ was receptor-mediated, blockable and persistent. The major metabolic and clearance pathways of the compounds appear to be through the kidney and liver, and the compounds are not so lipophilic as to accumulate in fat tissue. To evaluate the tissue distribution properties of the ^{18}F-labeled androgens, we have compared several aspects of their in vivo behavior. We have included in this comparison the uptake data of 20-F-mibolerone, a compound we have described recently (*32*). The receptor binding data on these compounds, reported earlier (*30, 32*), are summarized briefly in Table 7.

TABLE 5
Tissue Distribution of 16α-[^{18}F]Fluoro-7α-methyl-19-nortestosterone (16α-F-MNT; ^{18}F-9)

Organ	1 hr	2 hr	2 hr (blocked)†	4 hr
%ID/g ± s.e.m.*				
Blood	0.149 ± 0.054	0.115 ± 0.024	0.115 ± 0.042	0.024 ± 0.012
Bone	0.268 ± 0.272	0.282 ± 0.238	0.428 ± 0.213	0.188 ± 0.173
Muscle	0.082 ± 0.016	0.050 ± 0.010	0.040 ± 0.009	0.012 ± 0.004
Spleen	0.059 ± 0.011	0.039 ± 0.011	0.041 ± 0.011	0.010 ± 0.004
Lung	0.141 ± 0.033	0.094 ± 0.009	0.082 ± 0.016	0.022 ± 0.007
Liver	0.991 ± 0.402	0.485 ± 0.150	0.480 ± 0.230	0.103 ± 0.043
Fat	0.148 ± 0.041	0.081 ± 0.013	0.071 ± 0.030	0.103 ± 0.141
Kidney	0.962 ± 0.501	0.438 ± 0.092	0.443 ± 0.196	0.097 ± 0.030
Prostate (v)‡	0.527 ± 0.219	0.554 ± 0.244	0.097 ± 0.007	0.376 ± 0.086
Prostate (d)	0.519 ± 0.142	0.585 ± 0.244	0.123 ± 0.063	0.288 ± 0.134
Ratios ± s.e.m.				
Prostate/Muscle (v)	6.593 ± 2.966	11.026 ± 2.825	2.472 ± 0.408	34.995 ± 9.841
(d)	6.110 ± 1.486	11.525 ± 2.682	3.420 ± 2.257	25.855 ± 10.35
Prostate/Blood (v)	3.859 ± 2.163	5.373 ± 3.270	0.927 ± 0.279	19.564 ± 8.416
(d)	3.275 ± 0.569	5.598 ± 3.092	1.296 ± 0.860	14.267 ± 6.716

* %ID/g and s.e.m.: percent injected dose per gram tissue and standard error of the mean (n = 4). All rats were treated with DES 24 hr and 3 hr prior to injection with the ^{18}F-labeled androgen.
† Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.
‡ v = ventral and d = dorsal.

TABLE 6
Tissue Distribution of 20-[^{18}F]Fluoro-R1881 (20-F-R1881; ^{18}F-11)

Organ	1 hr	2 hr	2 hr (blocked)†	4 hr
%ID/g ± s.e.m.*				
Blood	0.234 ± 0.050	0.166 ± 0.010	0.179 ± 0.009	0.091 ± 0.015
Bone	0.182 ± 0.043	0.219 ± 0.028	0.248 ± 0.028	0.318 ± 0.037
Muscle	0.218 ± 0.038	0.129 ± 0.009	0.127 ± 0.007	0.066 ± 0.014
Spleen	0.192 ± 0.039	0.120 ± 0.008	0.120 ± 0.008	0.060 ± 0.009
Lung	0.286 ± 0.046	0.177 ± 0.018	0.178 ± 0.013	0.082 ± 0.011
Liver	1.475 ± 0.369	0.906 ± 0.143	1.085 ± 0.135	0.481 ± 0.156
Fat	0.334 ± 0.080	0.125 ± 0.024	0.113 ± 0.022	0.049 ± 0.016
Kidney	0.691 ± 0.132	0.522 ± 0.048	0.551 ± 0.042	0.247 ± 0.056
Prostate (v)‡	1.128 ± 0.217	0.746 ± 0.125	0.202 ± 0.021	0.383 ± 0.107
Prostate (d)	1.206 ± 0.190	0.812 ± 0.120	0.252 ± 0.083	0.366 ± 0.113
Ratios ± s.e.m.				
Prostate/Muscle (v)	5.350 ± 1.495	5.772 ± 0.848	1.600 ± 0.172	5.950 ± 1.584
(d)	5.627 ± 0.961	6.276 ± 0.787	2.002 ± 0.649	5.693 ± 1.639
Prostate/Blood (v)	5.112 ± 1.678	4.479 ± 0.500	1.136 ± 0.126	4.250 ± 0.981
(d)	5.347 ± 1.164	4.870 ± 0.408	1.429 ± 0.502	4.059 ± 1.026

* %ID/g and s.e.m.: percent injected dose per gram tissue and standard error of the mean (n = 4 for 2 hr and 4 hr data and n = 5 for others). All rats were treated with DES 24 hr and 3 hr prior to injection with the ^{18}F-labeled androgen.

† Blocked: in order to block receptor-mediated uptake, 36 μg of testosterone was added to each injected dose.

‡ v = ventral and d = dorsal.

A comparison of the prostate uptake (%ID/g) and the prostate-to-muscle and the prostate-to-blood uptake ratios at 1, 2 and 4 hr (Fig. 1) demonstrates the importance of binding and metabolic characteristics in establishing an efficient target site uptake and a good contrast (high ratio) between the target organ and the background, blood as well as muscle. (Although muscle contains AR, levels are very low (*38*); so, in terms of in vivo uptake it may be considered to be a "nontarget" organ.) In nearly every case, the highest ratios were observed at 4 hr for all seven compounds (Fig. 1C), but the values at this time vary significantly. The prostate uptake (%ID/g) of the three 16β-fluorine androgens that defluorinated rapidly in vivo was low (16β-F-MNT, 16β-F-DHT, 16β-F-T), even though their RBA values are relatively high (cf. Table 1; note 16β-F-T has a low RBA-2.1, but may be converted to 16β-F-DHT in vivo; see below). However, their target-to-background uptake ratios were quite high. The defluorination process, therefore, might account for this phenomenon, as rapid metabolism of these steroids could curtail the blood activity curve. This would have the effect of reducing the target tissue uptake, while at the same time clearing radioactivity from nontarget areas, thus enhancing uptake selectivity. For the other four fluorinated androgens that did not show rapid defluorination in vivo, the prostate uptake at 4 hr was higher for the ones with a higher RBA. However, the uptake selectivity ratios at 4 hr did not follow the RBA order: even though 20-F-R1881 and 16α-F-MNT have similar RBA values (~20), the latter has the highest ratios of all seven compounds, while the former

TABLE 7
Relative Binding Affinity (RBA) of Fluorine-Substituted Androgens for AR, PgR and SBP*

Compounds	RBA for Androgen Receptor (AR)	RBA for Progesterone Receptor (PR)	RBA for Sex Steroid Binding Protein (SBP)
16β-F-DHT (**2**)	42.7	0.12	385
16β-F-T (**4**)	2.1	—	—
16β-F-Mib (**6**)	30.8	3.0	1.3
16β-F-MNT (**7**)	36.5	1.6	3.8
16α-F-MNT (**9**)	21.9	5.7	4.0
20-F-R1881 (**11**)	18.8	10.5	2.2
20-F-Mib†	53.2	10.3	3.0
R1881	100	43.7	4.0

* Binding data for all compounds except 20-F-Mib are from ref. 30. The radiotracer and reference ligands (RBA = 100) are: AR = R1881 (K_d = 0.6 n*M*); PgR = R5020 (K_d = 0.4 n*M*); SBP = estradiol (K_d = 1.6 n*M*).

† Binding data are from ref. 32.

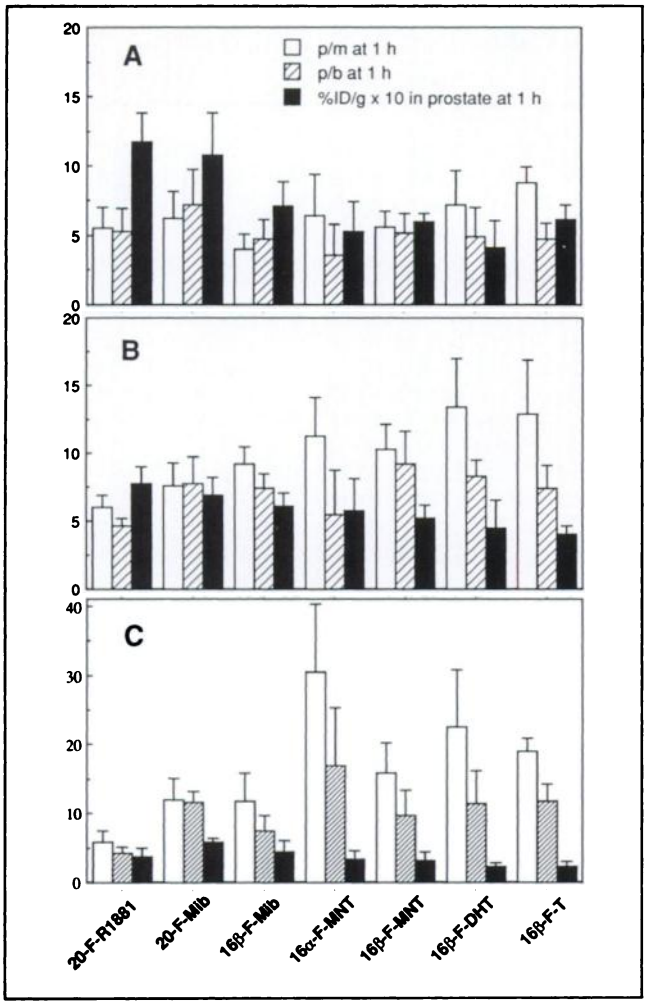


FIGURE 1. Prostate uptake of seven fluorine-labeled androgens in diethylstilbestrol-treated rats. Uptake is shown as %ID/g × 10 (solid black bars), prostate-to-blood ratio (slashed bars) and prostate-to-muscle ratio (open bars), and data are from 1 hr (A), 2 hr (B) and 4 hr (C; note change of scale). In each case, the prostate values are the average of the ventral and dorsal lobes. (Data are taken from Tables 1–6, and ref. 32).

has the lowest. The latter compound, without the 17α-methyl group that is known to retard steroidal metabolism (*39,40*), may be metabolized quickly and thus, cleared from the nontarget tissues, while the former has a longer half-life in the body and is thus more persistent in nontarget as well as in target tissues.

While we see the highest prostate-to-muscle or prostate-to-blood ratios in rats at 4 hr with these seven compounds, a 4-hr imaging time in rats would correspond to a very long imaging time in humans because of the difference in metabolic rate between rats and humans. At earlier times (1 and 2 hr, Fig. 1B), there is a less pronounced difference in uptake selectivity among the seven compounds; in fact, rarely is there more than a 2-fold difference in the prostate-to-muscle or prostate-to-blood ratios. Thus, at the 1-hr time point (Fig. 1A), the two compounds with the highest uptake, 20-F-R1881 and 20-F-Mib, also have uptake ratios nearly equal to those of all the other compounds. (The higher affinity of these two synthetic androgen derivatives for the progesterone receptor (Table 7) should be noted, however.) Aside from 16β-F-DHT, none of these compounds have substantial affinity for SBP (Table 7), the sex steroid binding globulin found in the serum of humans but not rats (*32*); although not tested, 16β-F-T would be expected to bind well to SBP.

Testosterone, the major circulating form of androgens in vivo, has relatively low affinity for AR (RBA = 5.9) (*30*), but is converted to the higher binding DHT (RBA = 60.2) (*30*) in target tissue by the action of 5α-reductase. It appears that the same process is occurring with 16β-F-T (RBA = 2.1), since its tissue distribution properties (prostate uptake and prostate-to-blood and muscle ratios) are similar to that of 16β-F-DHT (RBA = 42.7). Thus, the 16β-fluorine substituent on testosterone does not appear to interfere with this A-ring metabolism step.

When we make comparisons between the tissue distribution properties of these fluorine-substituted androgens with that of the tritium-labeled analogs that we studied earlier (*27*), we find that in most cases, the prostate uptake of the ^{18}F-labeled and tritium-labeled compound are comparable, but the uptake selectivities (prostate-to-muscle or blood ratios) are higher for the ^{18}F-labeled androgens. The generation from the tritium-labeled steroids of a higher level of metabolites (possibly including tritiated water) that continue to circulate and can enter nontarget tissues, could account for their lower uptake selectivity. Both the uptake efficiency and selectivity of 20-F-R1881 at 4 hr was lower than its protio analog R1881.

As depicted in Figure 2, all 16β-fluorine-18 labeled androgens except the 16β-[^{18}F]F-Mib showed rapid defluorination in vivo, whereas the 16α- and 20-fluorine labeled ones showed little defluorination; the defluorination of the 16β-[^{18}F]F-Mib was dramatically less than that of the other 16β-fluorine substituted androgens. In the three cases where defluorination was rapid, total bone

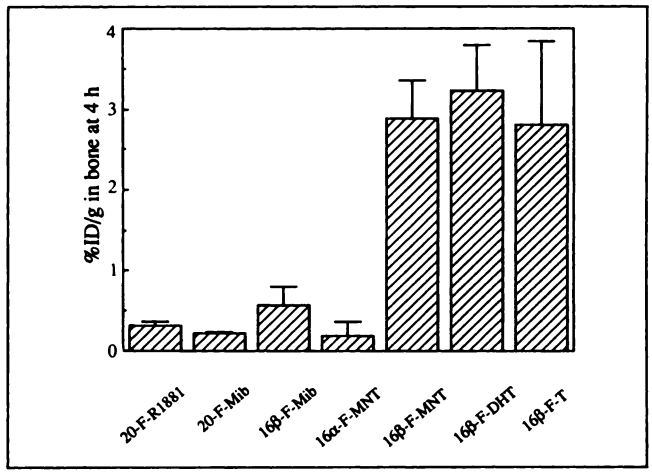


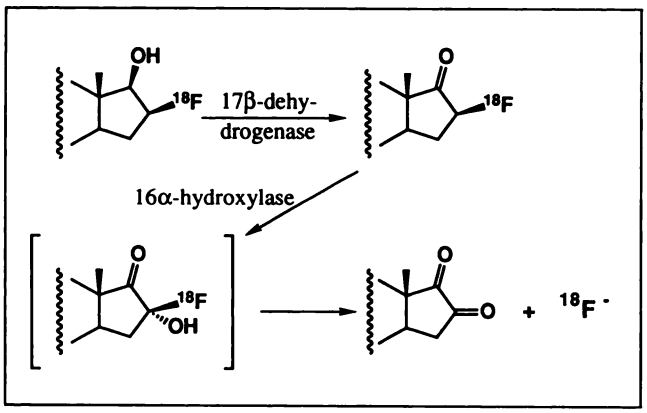
FIGURE 2. Bone radioactivity comparison of fluorinated androgens. (Data are taken from Tables 1–6, and ref. 32).

radioactivity at 4 hr was estimated to be about 50% of the injected dose. This could be a disadvantage for in vivo imaging, because it would result in interference by the high radioactivity in the bone that might obscure the detection of skeletal metastasis. Although bone has very low levels of AR (*41*), the extensive bone uptake we observed with three of these ^{18}F-labeled androgens is most likely due to the well known avidity of bone for free fluoride ion (*42,43*).

The rapid defluorination in vivo might be the result of the metabolic reaction outlined in Scheme 3, which is patterned after the biosynthetic conversion of estradiol to estriol via 16α-hydroxyestrone (*44*). Oxidation of the 17β-hydroxy function to a ketone by a 17β-dehydrogenase would activate the C-16 position that would then undergo 16α-hydroxylation (*44*). This 16α-hydroxy group in the 16β-fluoro steroids would be part of a highly reactive α-keto fluorohydrin that would collapse to an α-dione, releasing the fluoride ion that then accumulates in bone. Based on this speculation, it is easy to explain why the 17α-methyl function retards defluorination of the 16β-F-Mib and the 16α-orientation of the fluorine blocks cleavage of fluorine in 16α-F-MNT nearly completely. It is also evident why the 20-fluoro androgens were not readily defluorinated.

CONCLUSION

These seven fluorinated compounds are the first ^{18}F-labeled androgens that show relatively high binding affinity for AR as well as selective uptake by target tissue in vivo. For imaging at early times, 20-F-R1881 and 20-F-Mib might be the best candidates, while 16α-F-MNT, which shows the best uptake and highest contrast at 4 hr, might be preferred for imaging at later times. The uptake of these compounds and their possible radioactive metabolites by other tissues was not high, and activity cleared rapidly from nontarget areas. Also, their in vivo defluorination is low. Therefore, good contrast and a lower radiation dose could be obtained from imaging with these three probes.



SCHEME 3. Possible mechanism for 16β-defluorination.

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