# 20-[$^{18}$F]Fluoromibolerone, a Positron-Emitting Radiotracer for Androgen Receptors: Synthesis and Tissue Distribution Studies

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To develop an androgen receptor-based, positron-emitting imaging agent for prostate tumors, we have prepared 20-fluoromibolerone (F-Mib) and evaluated its tissue distribution. This compound was synthesized in eight steps from 7$\alpha$-methyl-19-nortestosterone, with fluorine introduced in the penultimate step by fluoride ion displacement on a spirocyclic sulfate. Fluoromibolerone was obtained in 9%–19% radiochemical yield (decay corrected), at 1.5 hr after bombardment, with an effective specific activity of 217–283 Ci/mmol. The relative binding affinity of F-Mib is 53 (versus R1881 = 100 or mibolerone = 118). In tissue distribution studies in diethylstilbestrol-treated male rats, $^{18}$F-Mib demonstrates high target/tissue uptake efficiency and selectivity: the prostate uptake at 0.5 hr and 4 hr is 1.0%–1.3% injected dose/gram tissue (ID/g) and 0.5%–0.6% ID/g, respectively; the prostate-to-blood and the prostate-to-muscle (non-target) ratios are both ca. 4 at 0.5 hr, and increase to ca. 12 by 4 hr after injection. The observed distribution of $^{18}$F-Mib suggests that it may be useful for in vivo imaging of prostatic tumors in man by positron emission tomography.

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Steroid hormone receptors, present in target tissue and receptor-positive tumors, form the basis for the development of fluorine-18- ($^{18}$F) labeled steroid radiotracers as in vivo positron emission tomographic (PET) imaging agents. We have demonstrated the utility of $^{18}$F-labeled estrogens in imaging estrogen receptor-positive breast tumors (*1*). It should likewise be possible to develop analogous $^{18}$F-labeled androgens to image prostatic tumors, since many prostatic tumors contain androgen receptors.

In vivo imaging of prostatic carcinoma has been an attractive goal for many researchers because of the inadequacies of current diagnostic methods. First, two kinds of tumor cells are present in prostatic tumor tissue, receptor-rich cells requiring androgens for growth and receptor-poor cells capable of growing without androgens; thus, even though there is a good correlation between tumor androgen receptor (AR) concentration and tumor responsiveness to hormonal treatment (*2–8*), partial tissue sampling techniques such as transurethral resection or needle biopsy may not provide fully representative tissue samples, which makes in vitro measurement of AR levels less accurate and less reproducible (*9*). Second, since the effectiveness of surgical treatment of prostatic tumors depends largely on whether the cancer has disseminated to surrounding tissue or not, an in vivo image of the extent of metastasis, especially by a noninvasive method, would be helpful in confirming the choice of surgical treatment.

Various derivatives of the androgens have been labeled with the gamma emitters bromine-77, bromine-82, iodine-125, and selenium-175, as well as the positron emitter $^{18}$F, in the search for AR-based prostatic imaging agents (*10–16*). However, none of these has proved to be useful for imaging prostate tumors, probably because they suffer from rapid metabolic cleavage of the radiolabel, low receptor binding affinity, or inadequate specific activity.

Mibolerone (Mib) is a synthetic androgen with a relative receptor binding affinity of 118 (versus methyltrienolone (R1881) = 100), which is higher than that of the natural AR ligands DHT (60.2) and testosterone (5.9). Unlike the natural ligands, Mib does not bind to sex binding protein (SBP), a serum-steroid carrier found in humans, and it is relatively resistant to some of the steroid-metabolizing enzymes (*17,18*). In a recent study of the in vivo uptake of five tritium-labeled androgens (including Mib) by tissues in the rat, we found that the uptake of Mib by target tissues (prostate) is highly selective (*19*). Therefore, Mib has been chosen in this work for labeling. To investigate mibolerone derivatives suitable for PET imaging, we prepared 20-fluoromibolerone (F-Mib). The C-20 position is a good site for fluorine attachment: $^{18}$F can be introduced conveniently by [$^{18}$F]fluoride ion displacement upon a cyclic

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sulfate (*20,21*), and fluorine at C-20 is stable in vivo and interferes minimally with receptor binding affinity. 20-[$^{18}$F]fluoromibolerone ($^{18}$F-Mib) thus produced is the first $^{18}$F-labeled androgen that shows highly selective uptake by prostate tissue of male rats. Thus, it appears to be a good candidate for imaging prostatic carcinoma in vivo.

## EXPERIMENTAL PROTOCOL

### Chemical Synthesis

The chemical synthesis section is presented in the Appendix that follows this article.

### Radiochemical Syntheses

*20-[$^{18}$F]Fluoromibolerone ($^{18}$F-9, $^{18}$F-Mib).* Aqueous $^{18}$F-labeled fluoride ion, produced in water by proton bombardment of an oxygen-18 enriched water target, was added to n-$Bu_4NOH$ (tetra-n-butylammoniumhydroxide) (1 $M H_2O$ solution 0.85 equal to cyclic sulfate samples) in a Vacutainer® (*22*) and then resolubilizing by performing three azeotropic distillations each employing 0.5-0.8 ml of acetonitrile. It was then transferred to a 4-ml glass sample vial containing 1.5 mg of cyclic sulfate 8 with 100 μl of freshly distilled THF. The mixture was stirred at 70°C for 10 min, cooled at 25°C for 1 min, and treated with 2 drops (~40 μl) of 2N HCl at 25°C for 3 min. It was diluted with 10 drops (~200 μl) of water and extracted with 1 ml ether. The organic layer was filtered through a column (short pipette filled with glass wool, 0.8 cm $Na_2SO_4$ and then 0.7 cm of $SiO_2$) with 2 × 0.6 ml of ethyl acetate. The solvent was evaporated under a gentle stream of $N_2$ and the residue was redissolved in 1 ml 1,2-dichloroethane and injected onto normal phase high-performance liquid chromatography (HPLC) (Whatman Partisil M-9, 0.9 cm × 50 cm), It was eluted with 67% hexane/33% (iPr-OH (5%)-$CH_2Cl_2$) at 5 ml/min (UV detection, 254 nm; radioactivity detection, flow-through scintillation detector), the desired product, 20-[$^{18}$F]fluoromibolerone, had a retention time of 16 min. Reanalysis by analytical HPLC of the material collected from the preparative column indicated that it was radiochemically homogeneous; there was no evidence of chemical impurities with elution times different from that of F-Mib.

### Biologic Methods

*Binding Assays.* Relative binding affinities were determined in several receptor and binding protein systems as described previously: AR (*21,23*), progesterone receptor (*21,24*), mineralocorticoid receptor (*25*), sex steroid binding globulin (*26*).

The relative specific activity (*27*) of $^{18}$F-Mib was determined from a decayed sample of known activity by competitive AR radioreceptor binding assay by a modification of the procedure previously reported (*28*).

### 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 sunflower seed oil at 24 hr and 3 hr prior to the experiment, following the regime of Symes (*29*).

Fluorine-18-Mib was 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 (Ca. 200 μl) of $^{18}$F-Mib. 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 times, 0.5, 1, 2, and 4 hr, the animals were killed, samples of blood and tissue were excised, weighed, and the radioactivity determined as previously reported (*28*). The injected dose was calculated from standards prepared from the injection solution and the data were expressed as percent injected dose per gram tissue (%ID/g).

## RESULTS

### Synthesis of F-Mib and $^{18}$F-Mib

The synthetic route used to prepare F-Mib is illustrated in Figure 1. The 3-oxo function of the starting material, 7α-methyl-19-nortestosterone 1, was protected as the dithiolane (*30*); the 17β-hydroxy group in 2 was oxidized with pyridinium chlorochromate (PCC) and the C-17 ketone 3 was treated with an excess of dimethylsulfonium methylide (*21*) to furnish epoxide 4. Vigorous base treatment of this epoxide produced diol 5, but only in a moderate yield, due to competing polymerization. Cyclic sulfite 6 as a mixture of diastereomers was prepared from diol 5, using thionyl chloride (*21*). Removal of the 3,3-ethylenedithio group proved to be troublesome, but could be done best by the methods shown to produce cyclic sulfite 7 (*31*). Oxidation of cyclic sulfite 7 to cyclic sulfate 8 also proved difficult. The oxidation reagent, $NaIO_4/RuCl_3 \cdot 3H_2O$, which is typically used (*21,32*), also oxidizes the 4(5)-double bond in compound 7. Fortunately, tetra-n-butylammonium monopersulfate (OXONE®, Aldrich) ($Bu_4NS(O_3)OOH$; TBA-oxone) (*23*) was found to be suitable, and oxidation proceeded readily with TBA-oxone, together with tetrabutylammonium fluoride ($Bu_4NF$) or $Bu_4NOH$. The effect of these bases might be to remove the acidic proton in TBA-oxone and thus improve its nucleophilicity. Finally, reaction of the cyclic sulfate 8 with excess $Bu_4NF$, followed by acid hydrolysis of the fluorobissulfate intermediate (*20,21*), furnished F-Mib in 48% yield. The whole synthesis involved eight steps and gave F-Mib 9 in 9% overall yield from 7α-methyl-19-nortestosterone 1.

Tetra-n-butylammonium [$^{18}$]F-fluoride, prepared as previously described (*22,33*), was allowed to react with cyclic sulfate 8 to give a fluorobisulfate intermediate; hydrolysis with $HCl/H_2O$ produced $^{18}$F-Mib (9), which was purified by normal-phase HPLC. Reinjection of a collected sample on a normal-phase analytical HPLC column showed that there was very little mass that coeluted with $^{18}$F-Mib (data not shown). Fluorine-18-labeled Mib was produced in 9%–19% radiochemical yield (decay corrected at 1.5 hr after bombardment) with an effective specific activity of 217–283 Ci/mmol (*27,28*).

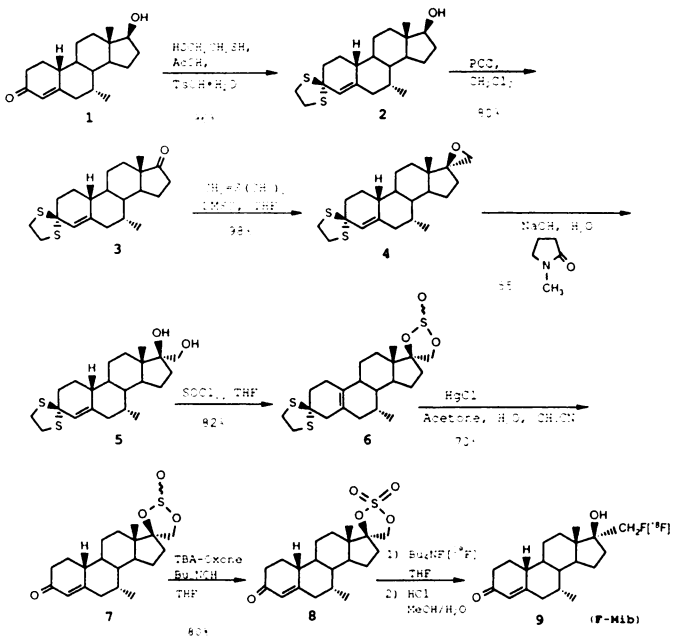


**FIGURE 1**
Synthetic route used for the preparation of F-Mib.

### Binding Affinities of Androgens-to-Androgen Receptor, Progesterone Receptor, Mineralocorticoid Receptor, and Sex Steroid Binding Protein

The binding affinity of F-Mib (*9*), synthetic precursors, intermediates, derivatives and some common androgens toward AR and other important targets were measured; the data are shown in Table 1.

The binding affinity of F-Mib for AR was found to be 53 (relative to R1881 = 100), about half the affinity of its parent compound (mibolerone = 118). The reduction in affinity may be due to the larger size of the fluorine atom (versus the tritium atom) or its high electronegativity. The 17$\alpha$-hydroxymethyl analog has a much lower affinity than Mib; the 17$\alpha$-unsubstituted analog 1 has the highest affinity overall. The well known effects of methylation at 7$\alpha$ (increases affinity – 1 versus norT) and 17$\alpha$ (little change in affinity - Mib versus 1) (*34,35*) are reproduced here. The spiro-epoxide and the cyclic sulfites (7, evaluated as partially separated mixtures of diastereomers), have low affinity for AR.

Mibolerone has substantial binding affinity for progesterone receptor (*18*). While rat prostate does not contain progesterone receptor (*18,36*), human prostate does, so this heterologous binding can interfere with AR measurements in humans (*18*). 20-Fluoromibolerone binds to the progesterone receptor with half the affinity of Mib (and one-quarter the affinity of R1881). All the other androgens have yet lower affinity for progesterone receptor. Some androgens have significant affinity for corticosteroid receptors (*37*). Mib is known to have only very low affinity for glucocorticoid receptors (*18,19*), yet its affinity for mineralocorticoid receptors is substantial (Table 1 and Ref. *19*). The affinity of F-Mib for mineralocorticoid receptors, however, is very low, plus this receptor is known not to be found in prostatic tissues (*26*).

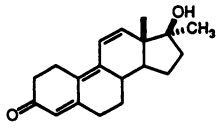
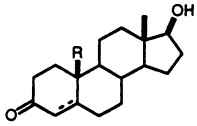
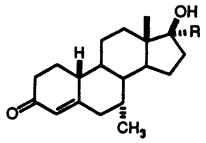
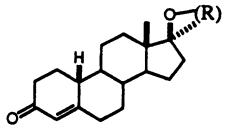
Certain androgens (most notably DHT) bind with high affinity to SBP, a serum globulin found in humans but not in rats (*18,38*). The synthetic androgens R1881 and Mib were designed to have much lower affinity for SBP than the natural androgens (*18,39*), and the affinity of F-Mib is yet lower.

### Tissue Distribution Studies of $^{18}$F-Mib

Purified $^{18}$F-Mib was injected (i.v. femoral vein) into male rats. The tissue distribution of radioactivity is shown in Table 2. All but one set of rats were treated with high levels of DES to suppress in vivo androgen biosynthesis, thus increasing the concentration of unoccupied androgen receptors (*29*). Since many prostatic cancer patients are given estrogen therapy, it was important to measure the tissue distribution of $^{18}$F-Mib under conditions which resemble those likely to be encountered in the clinical setting. For comparison, one set of rats was untreated (2 hr "untreated"). To ascertain whether the uptake was mediated by a high-affinity, limited-capacity system, one set of rats was given 36 $\mu$g of unlabeled testosterone together with $^{18}$F-Mib. This dose of testosterone is sufficient to fully occupy the androgen receptors and thus block receptor-mediated uptake of the $^{18}$F compound (2 hr "blocked").

The uptake of $^{18}$F-Mib is highly selective for target tissues. Prostate-to-blood and prostate-to-muscle (nontarget tissue) ratios increased quickly from ca. 4 for dorsal and ventral prostate at 0.5 hr to ca. 12 for dorsal and ventral prostate at 4 hr after injection. As expected, the prostate uptake in "untreated" and especially in "blocked" animals was lower than that of DES-treated

**TABLE 1**
Binding Affinities of Androgen Derivatives to Androgen, Progestin and Mineralocorticoid Receptors, and Sex Steroid Binding Protein

| Compound | Relative Binding Affinity* AR† | PgR‡ | MR§ | SBP¶ |
|---|---|---|---|---|
|  | | | | |
| R1881 (Methyltrienolone) | 100** | 43.7 ± 9.1 | 25.4 ± 1.3 | 4.04 ± 0.9 |
|  | | | | |
| R = $CH_3$, $\Delta^4$, Testosterone (T) | 5.99 ± 1.18 | 0.09 ± 0.02 | 4.19 ± 3.44 | 417 ± 88 |
| R = $CH_3$, 5α-Dihydrotestosterone (DHT) | 60.9 ± 17.2 | 0.23 ± 0.10 | 1.17 ± 0.14 | 2125 ± 999 |
| R = H, $\Delta^4$ 19-nor-testosterone (nor-T) | 30.6 ± 1.5 | 3.10 ± 0.99 | 2.00 ± 1.20 | 29.9 ± 6.8 |
|  | | | | |
| R = H (1) | 155 ± 21 | 3.72 ± 0.12 | 0.74 ± 0.32 | 28.7 ± 9.4 |
| R = $CH_3$ Mibolerone (Mib) | 118 ± 4 | 20.4 ± 4.2 | 5.70 ± 0.47 | 19.0 ± 8.3 |
| R = $CH_2F$ 20-Fluoromibolerone (9, F-Mib) | 53.2 ± 4.3 | 10.3 ± 2.0 | 0.86 ± 0.07 | 3.00 ± 1.90 |
| R = $CH_2OH$ | 1.37 ± 0.06 | — | — | — |
|  | | | | |
| R = $CH_2$ | 2.16 ± 1.32 | 0.81†† | 0.85†† | — |
| R = S(O)-O-$CH_2$ A*** (7) | 2.47 ± 0.98 | 2.82†† | 7.54†† | — |
| R = S(O)-O-$CH_2$ B*** (7) | 1.81 ± 1.07 | — | — | — |

* Relative binding affinity was determined by a competitive radiometric binding assay. Values are the average of two or more determinations ± range (n = 2) or s.d. (n ≥ 3) and are expressed on a percent scale relative to the affinity of the tritium-labeled tracer (23).

† AR = androgen receptor. The tracer was [$^3$H]R1881 and the assay was performed according to the method described in Refs. 21 and 24. The $K_d$ for R1881 is 0.6 n*M*.

‡ PgR = progesterone receptor. The tracer was [$^3$H]R5020 and the assay was performed according to the method described in Ref. 21. The $K_d$ for R5020 is 0.4 n*M*.

§ MR = mineralocorticoid receptor. The tracer was [$^3$H]aldosterone and the assay was performed according to the method described in Ref. 25. The $K_d$ for aldosterone is 3.9 n*M*; in the presence of RU28362 the $K_d$ of aldosterone to block glucocorticoid receptors is 0.42 n*M*.

¶ SBP = sex steroid binding protein. The tracer was [$^3$H]estradiol and the assay was performed according to the method described in Ref. 26. The $K_d$ for estradiol is 1.6 n*M*.

** Relative affinity of tracer compound is 100 by definition.

†† Single determination.

*** Mixture A: the ratio of diastereomer a and b is ca. 70:30; mixture B: the ratio is ca. 30:70.

rats. This, together with the persistence of high uptake even after 4 hr, is consistent with the receptor-mediated nature of the prostate uptake. There was considerable activity in liver and kidney, known organs for metabolism and excretion of steroids in rodents (*40*). The low radioactivity in bone suggests that the metabolic cleavage of the $^{18}$F label in $^{18}$F-Mib is not facile (*41,42*). The low uptake of this compound by fat tissue and its rapid clearance are desirable for in vivo imaging of the prostate, since this organ is surrounded by a considerable amount of adipose tissue.

## DISCUSSION

20-[$^{18}$F]fluoromibolerone is the first $^{18}$F-labeled androgen having a high receptor binding affinity and

**TABLE 2**
Tissue Radioactivity Distribution of 20-[$^{18}$F]Fluoromibolerone*

| Organ | 0.5 hr | 1 hr | 2 hr | 2 hr (untreated) | 2 hr (blocked)† | 4 hr |
|---|---|---|---|---|---|---|
| **%ID/g ± s.e.m.** | | | | | | |
| Blood | 0.234 ± 0.003 | 0.159 ± 0.014 | 0.091 ± 0.006 | 0.064 ± 0.002 | 0.081 ± 0.011 | 0.050 ± 0.001 |
| Bone | 0.189 ± 0.011 | 0.197 ± 0.010 | 0.251 ± 0.014 | 0.184 ± 0.017 (a) | 0.220 ± 0.007 | 0.226 ± 0.006 |
| Muscle | 0.232 ± 0.006 | 0.177 ± 0.008 | 0.092 ± 0.005 | 0.069 ± 0.003 | 0.067 ± 0.011 | 0.050 ± 0.009 |
| Spleen | 0.237 ± 0.011 | 0.170 ± 0.013 | 0.092 ± 0.006 | 0.064 ± 0.002 | 0.070 ± 0.011 | 0.045 ± 0.001 |
| Lung | 0.417 ± 0.011 | 0.300 ± 0.012 | 0.165 ± 0.011 | 0.107 ± 0.004 | 0.078 ± 0.011 | 0.080 ± 0.006 |
| Liver | 2.362 ± 0.075 | 1.839 ± 0.095 | 0.805 ± 0.032 | 0.514 ± 0.036 | 0.739 ± 0.069 | 0.402 ± 0.029 |
| Fat | 0.573 ± 0.049 | 0.264 ± 0.012 | 0.132 ± 0.020 | 0.112 ± 0.014 | 0.137 ± 0.020 | 0.067 ± 0.003 |
| Kidney | 0.880 ± 0.038 | 0.753 ± 0.035 | 0.423 ± 0.018 | 0.266 ± 0.012 | 0.361 ± 0.078 | 0.174 ± 0.008 |
| Prostate (v)‡ | 0.886 ± 0.037 | 0.969 ± 0.140 | 0.601 ± 0.056 | 0.384 ± 0.073 | 0.150 ± 0.034 (a) | 0.611 ± 0.034 |
| Prostate (d)‡ | 0.923 ± 0.028 | 1.188 ± 0.304 | 0.775 ± 0.135 | 0.393 ± 0.068 | 0.111 ± 0.009 | 0.545 ± 0.057 (a) |
| **Ratio ± s.e.m.** | | | | | | |
| Prostate/(v) | 3.83 ± 0.19 | 5.53 ± 0.94 | 6.54 ± 0.54 | 5.62 ± 1.11 | 2.77 ± 0.39 (a) | 13.25 ± 2.22 |
| Muscle (d) | 3.99 ± 0.14 | 6.91 ± 1.92 | 8.56 ± 1.78 | 5.71 ± 0.92 | 1.76 ± 0.31 | 10.82 ± 3.14 |
| Prostate/(v) | 3.79 ± 0.11 | 6.385 ± 1.30 | 6.71 ± 0.67 | 6.13 ± 1.39 | 2.16 ± 0.30 (a) | 12.41 ± 0.92 |
| Blood (d) | 3.95 ± 0.10 | 8.021 ± 2.50 | 8.82 ± 1.95 | 6.20 ± 1.20 | 1.43 ± 0.19 | 10.95 ± 1.46 (a) |

* In the study, mature male Sprague-Dawley rats were injected with a 100-$\mu$Ci dose of 20-[$^{18}$F]fluoromibolerone in 10% ethanol-saline. Average animal weight at time of experiment was 173 ± 16 g. s.e.m. is standard error of mean. All rats were treated with 1 mg of DES in 0.2 ml sunflower oil per rat, 24 hr and 3 hr prior to injection of radioactivity, except the 2 hr (untreated) animals which did not receive DES. n = 4, except where noted otherwise (a).

† Blocked. In order to block receptor-mediated uptake, 36 $\mu$g of testosterone was added to each injected dose.

‡ v = ventral and d = dorsal.

showing selective uptake by prostate tissue. Studies in mature male rats treated with DES (an endocrine manipulation that corresponds to certain hormonal therapies in human prostatic cancer patients that increase available AR by suppressing endogenous androgen production) show high uptake of $^{18}$F-Mib into prostate. This uptake is suppressed by coinjection of excess testosterone (blocked) or by endogenous androgens in intact animals (untreated), confirming that the uptake is mediated by a high-affinity, low-capacity receptor system, that is presumed to be AR. There is high uptake into liver and kidney, both organs involved in metabolism and elimination of steroids. This uptake drops rapidly and shows little or no blocking by excess androgens (blocked, untreated), indicating that uptake is not receptor mediated.

In a recent study, we compared the uptake of five tritium-labeled androgens in rats (*19*). These animals were larger (250 g versus 175 g here) and, in most cases, the animals were castrated 24 hr prior to the experiment; only in a few cases, were they treated with DES. Nevertheless, if the prostate uptake is expressed on the basis of %ID kg rat/g tissue to correct for the differences in animal weight, the prostate uptake we observed at 1 hr with $^{18}$F-Mib (0.18% ID kg/g) is comparable to that observed with Mib (0.14% ID kg/g in castrated animals, 0.18% ID kg/g in DES-treated animals) and R1881 (0.17% ID kg/g in castrated animals, 0.14% ID kg/g in DES-treated animals), and greater than that for testosterone, dihydrotestosterone, and 19-nortestosterone in castrated animals (0.11%, 0.10%, and 0.10% ID kg/g, respectively).

The uptake characteristics of $^{18}$F-Mib in the rat are very favorable and suggest that this compound may be suitable for imaging AR-positive prostatic tumors in humans. However, it is important to consider that in two respects, the rat is not an ideal model for the human to evaluate the uptake of labeled androgens by target tissues. First, the human prostate has significant levels of progesterone receptor (*18*), so that in the human, progesterone receptor rather than AR might mediate the uptake of an androgen that has significant heterologous binding affinity for the progesterone receptor. Second, human serum also contains SBP binding protein, which has high affinity for some androgens (*18,38*), and might result in elevated blood levels of androgens in humans, diminishing target tissue-to-blood ratios. Such a phenomenon has been observed with certain estrogens in rodents due to their binding to serum alphafetoprotein (*26*).

We believe, however, that these two factors will not prove to be substantive problems with F-Mib in the human. 20-[$^{18}$F]fluoromibolerone binding to the progesterone receptor is relatively modest and is, in fact, less than that of Mib. Also, unlike the natural androgens testosterone and DHT, the binding of Mib and F-Mib to SPB is very modest.

In conclusion, F-Mib, a $^{18}$F-labeled androgen, has been prepared at high-specific activity and shows selective uptake into the prostate. These characteristics sug-

gest that it may be useful as an agent to image AR-positive prostate tumors and metastases in humans, thus providing the clinician with a noninvasive method to help select a program of therapy and to monitor the progress and success of the treatment.

## APPENDIX

### Chemical Synthesis

*General.* Part of the Mib used in this study was a generous gift from Upjohn and part was synthesized in our laboratory from 19-nortestosterone (*43*). Tetra-n-butylammonium monopersulfate (TBA-oxone) was prepared according to Trost's procedure (*44*). Tetrahydrofuran (THF) and ethyl ether were distilled from sodium/benzophenone ketyl immediately prior to use. DMSO, methylene chloride, and hexane were distilled from calcium hydride. Flash chromatography was performed according to Still (*45*). Melting points are uncorrected. $^{19}F$ NMR was obtained on a Nicolet NT-360 (Fremont, CA) (at 338 MHz) spectrometer, using fluorotrichloromethane as an internal standard and deuterochloroform as a solvent. Both low- and high- resolution fast atom bombardment (FAB) mass spectra were obtained on a VG Instruments ZAB HF (Manchester, UK), employing a dithiothreitol matrix.

*3,3-Ethylenedithio-7α-methyl-4-estren-17β-ol (Compound 2).* A solution of 7α-methyl-19-nortestosterone 1 (3.50 g, 12.10 mmol) in 10 ml acetic acid was treated with 1.02 ml (1.72 g, 12.10 mmol) ethylenedithiol and a solution of toluenesulfonic acid (1.72 g, 4.52 mmol) in another 10 ml of acetic acid. The mixture was allowed to stir at room temperature for 5 min. A copious white precipitate was collected, washed (2N NaOH solution and water), and recrystallized from ethanol. White crystals of 2 (3.19 g) were collected. The mother liquor, containing some 17-acetate, was concentrated to dryness and treated with NaOMe in methanol, affording an additional 1.01 g of purified product 2 (95.5% total yield): mp 205°C decomposed; IR (KBr), 3485 (br.s, OH) $cm^{-1}$, no C = 0 band; $^1H$ NMR ($CDCl_3$) δ5.57 (br.s, 1H, 4-H), 3.65 (t, 1H, J = 8.26 Hz, 17α-H), 3.42-3.17 (m, 4H, 3,3-ethylenedithio), 0.76 (s, 3H, 18-H), 0.72 (d, 3H, J = 7.39 Hz, 7α-$CH_3$); mass spectrum (70 eV), m/e (rel. intensity), 364 (95, M+), 336 (100), 304 (97), 131 (26), 123 (39), 91 (86), 81 (51), 55 (63); HRMS calculated for $C_{21}H_{32}OS_2$: 364.1895; found: 364.1885; analysis calculated for $C_{21}H_{32}OS_2$: C, 69.18; H, 8.85; S, 17.59; found: C, 69.11; H, 8.93; S, 17.43.

*3,3-Ethylenedithio-7α-methyl-4-estren-17-one (Compound 3).* The 17β-hydroxy compound 2 (3.02 g, 8.27 mmol) was dissolved in 192 ml of methylene chloride and added with stirring to a solution of pyridinium chlorochromate (PCC) (3.00 g, 13.92 mmol) in 50 ml of methylene chloride at 10°C. The mixture was allowed to stir for 4 hr at room temperature and then was diluted with ether. The dark brown solution was filtered through Celite with ether, washed with 2N NaOH solution once and water twice, and dried ($Na_2SO_4$). After concentration in vacuo, the residue was triturated with ethanol to give 2.41 g of purified product 3 (80.3% yield). An analytical sample was further purified by flash chromatography (hexane: EtOAc = 4:1) (mp 261-263°C partially decomposed). IR (KBr), 1767 (s, C = O) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ5.60 (br.s, 1H, 4-H), 3.52-3.20 (m, 4H, 3,3-ethylenedithio), 0.89 (s, 3H, 18-H), 0.77 (d, 3H, J = 6.32 Hz, 7α-$CH_3$); mass spectrum (70 eV), m/e (rel. intensity), 362 (79, M+), 337 (100), 302 (93), 131 (26), 123 (39), 91 (81), 81 (38), 54 (48); analysis calculated for $C_{21}H_{30}OS_2$: C, 69.60; H, 8.34; S, 17.69. found: C, 69.60; H, 8.43; S, 17.61.

*17S-Spiro-2'-(1'-oxacyclopropane)-3,3-ethylenedithio-7α-methyl-4-estrene (Compound 4).* Sodium hydride (2.20 g, 60%, 55.20 mmol) was rinsed four times with distilled hexane under $N_2$ protection to remove oil prior to addition of 40 ml of dry DMSO. The suspension of NaH in DMSO was then heated at 70–75°C for 45 min. At this point, 50 ml of THF was added and the mixture was cooled to 0°C. A solution of trimethylsulfonium iodide (10.45 g, 51.05 mmol) in 35 ml DMSO was added to the cold reaction mixture dropwise with stirring and after completion of addition, stirring was continued 10 min longer. Ketone 3 (1.15 g, 3.17 mmol) was dissolved in 150 ml of THF and added dropwise to the newly formed $CH_2 = S(CH_3)_2$ solution at 0°C. The resulting milky mixture was kept at 0°C for 1 hr and at room temperature for 2 hr. Product isolation involved quenching with water, extraction with ether, and washing twice with water. After the ether extract was dried ($Na_2SO_4$) and concentrated, the product was purified by flash chromatography (hexane: EtOAc = 2:1) to afford 1.16 g of a white solid 4 (97.5% yield, mp 167–169°C); IR (KBr), 1275 (s, epoxide) $cm^{-1}$, no C = 0 band; $^1H$ NMR ($CDCl_3$) δ 5.57 (br.s, 1H, 4-H), 3.18–3.42 (m, 4H, 3,3-ethylenedithio), 2.76 (AB quartet, 2H, Δν = 56 Hz, J = 5.00 Hz, 17 spiro epoxide $CH_2$), 0.90 (s, 3H, 18-H), 0.75 (d, 3H, J = 8.12 Hz, 7α-$CH_3$); mass spectrum (70 eV), m/e (rel. intensity), 376 (97, M+), 348 (100), 316 (92), 159 (23), 131 (13), 122 (30), 105 (29), 91 (57); analysis calculated for $C_{22}H_{32}OS_2$: C, 70.16; H, 8.57; S, 17.03; found: C, 70.14; H, 8.60; S, 17.05.

*3,3-Ethylenedithio-17α-hydroxymethyl-7α-methyl-4-estren-17β-ol (Compound 5).* 1-Methyl-2-pyrrolidinone (5 ml) was added to a flask containing epoxide 4 (100 mg, 0.267 mmol); after the solid dissolved, 5 ml of 2N NaOH solution was added. The cloudy mixture was allowed to stir at 90–92°C for 23 hr and then cooled to room temperature. It was diluted with 20 ml of water, extracted with ether twice, acidified with 3N HCl solution to pH 3 and extracted with ether again. The combined organic layer was washed with 15 ml of water and dried over $Na_2SO_4$. Flash chromatography (hexane: EtOAc = 1:1) gave 57.2 mg of purified product 5 (54.9% yield, mp 182–183°C); IR (KBr), 3600–3010 (very br. s, two OH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 5.57 (br.s, 1H, 4-H), 3.58 (AB quartet, 2H, Δν = 66 Hz, J = 10.4 Hz, 17α $CH_2OH$), 3.40-3.17 (m, 4H, 3.3-ethylenedithio), 0.89 (s, 3H, 18-H), 0.70 (d, 3H, J = 7.83 Hz, 7α-$CH_3$); mass spectrum (70 eV), m/e (rel. intensity), 394 (100, M+), 366 (53), 348 (33), 334 (73), 131 (11), 123 (17), 105 (19), 91 (26), 81 (22), 55 (17); analysis calculated for $C_{22}H_{34}O_2S_2$: C, 66.96; H, 8.68; S, 16.25; found: C, 66.44; H, 8.76; S, 16.18.

*17S-Spiro-3'-(1'-oxo-2',5'-dioxa-1'-thiacyclopentane)-3,3-ethylenedithio-7α-methyl-5(10)-estrene (Compound 6).* A flame-dried flask was charged with diol 5 (28.7 mg, 0.073 mmol) and 1.7 ml of THF and then cooled to −10°C in a ice-salt bath. Thionyl chloride (22.2 μl, 0.298 mmol) was added dropwise and the resulting mixture was stirred at −10–0°C for about 1 hr. It was quenched in the cold with saturated aqueous $NaHCO_3$ and extracted three times with ether. The organic layer was washed with water and dried over $Na_2SO_4$. Flash chromatography (hexane:EtOAc = 1:1), performed rapidly,

gave 26.2 mg of a gelatinous mixture of two epimers of the product 6 (81.6% yield); $^1$H NMR ($CDCl_3$) $\delta$ 4.73, 3.97 (two doublets, J = 8.70 Hz, Ha, and Hb of the methylene group of one epimer of the cyclic sulfite), 4.41 (AB quartet, $\Delta\nu$ = 50 Hz, J = 8.70 Hz, Ha, and Hb of the other cyclic sulfite epimer), 4 peaks totaled 2H, 3.38–3.24 (m, 4H, 3.3-ethylenedithio), 0.99, 0.93 (two singlets, 3H, 18-H of two epimers), 0.86, 0.75 (two doublets, 3H, J = 6.96 Hz, 6.52 Hz, 7$\alpha$-$CH_3$ of two epimers); mass spectrum (70 eV), m/e (rel. intensity), 440 (68, M+), 412 (94), 380 (100), 348 (35), 316 (36), 225 (22), 123 (42), 105 (53), 91 (96), 81 (56), 55 (81); HRMS calculated for $C_{22}H_{32}O_3S_3$: 440.1514; found: 440.1523.

*17S-Spiro-3'-(1'-oxo-2',5'-dioxa-1'-thiacyclopentane)-7α-methyl-4-estren-3-one (Compound 7).* Dithiolane 6 (95.7 mg, 0.217 mmol) was dissolved in 11.5 ml of acetonitrile and 7.7 ml of acetone before addition of 2.11 ml water. Mercuric chloride (259.5 mg, 0.950 mmol) as a fine powder was added to the resulting solution and the mixture was stirred at 25°C until (thin-layer chromatography) analysis results showed complete consumption of the starting material (~20 min). It was filtered through Celite, and the cake was washed with $CH_2Cl_2$ four times. The combined filtrate was then washed with saturated $NaHCO_3/H_2O$ solution and water and dried over anhydrous $Na_2SO_4$. After being concentrated to dryness, the crude product was purified by flash chromatography (hexane: EtOAc = 1:1), affording 55.9 mg of pure product 7 (70.2% yield, gelatinous mixture of two epimers); $^1$H NMR ($CDCl_3$) $\delta$ 5.84 (s, 1H, 4-H), 4.70, 3.93 (two doublets, J = 8.12 Hz, Ha, and Hb of cyclic sulfite methylene of one epimer), 4.40 (AB quartet, $\Delta\nu$ = 50 Hz, J = 8.12 Hz, Ha, and Hb of the other cyclic sulfite epimer), 4 peaks totaled 2H, 1.03, 0.98 (two singlets, 3H, 18-H of two epimers), 0.86, 0.77 (two doublets, 3H, J = 6.25 Hz, 7$\alpha$-$CH_3$ of two epimers); mass spectrum (70 eV), m/e (rel. intensity), 364 (30, M+), 300 (100), 242 (69), 229 (55), 159 (28), 136 (41), 107 (34), 91 (47), 79 (45), 55 (41); HRMS calculated for $C_{20}H_{28}O_4S$: 364.1708; found: 364.1715.

*17S-Spiro-3'-(1',1'-dioxo-2',5'-dioxa-1'-thiacyclopentane)-7α-methyl-4-estren-3-one (Compound 8).* Cyclic sulfite 7 (10 mg, 0.027 mmol) and tetrabutylammonium monoperoxysulfate (OXONE®, 40 mg, 0.11 mmol), in that order, were dissolved in 1.4 ml of $CH_2Cl_2$ and the solution was cooled to 0°C. A solution (0.5 ml, 1 *M* in THF) of $Bu_4NF$, was then added dropwise and the mixture was stirred at 0°C for 20 min. At this point, an additional 0.4 ml of $Bu_4NF$ was added, and stirring was continued for another 20 min. Finally, the mixture was quenched with a cold saturated $NaHCO_3$ solution, extracted three times with ether, washed with water, and dried over $Na_2SO_4$. The solvent was removed under a gentle stream of nitrogen while the system was kept below 0°C, since the product is not stable at 25°C. Flash chromatography (hex:EtOAc = 1:1, pre-chilled solvent) was performed quickly, and the peak fractions were collected in a flask cooled to 0°C and were concentrated first under a stream of nitrogen and then under vacuum to yield 8.3 mg pure product 8 (79.8% yield, white crystals). This material was not stable on silica gel and gradually decomposed at 25°C. However, it is stable when stored below −20°C; mp 113°C (with decomposition). $^1$H NMR ($CDCl_3$) $\delta$ 5.83 (s, 1H, 4-H), 4.57 (AB quartet, 2H, $\Delta\nu$ = 70.0 Hz, J = 9.1 Hz, Ha, and Hb of cyclic sulfate methylene), 1.07 (s, 3H, 18-H), 0.75 (d, 3H, J = 7.0 Hz, 7$\alpha$-$CH_3$); mass spectrum (FAB) m/e (rel. intensity), 381 (49, M + 1), 329 (12), 252 (10), 153 (34), 121 (23): HRMS (FAB) calculated for $C_{20}H_{28}O_5S$: M + 1, 381.1736; found: M + 1, 381.1743.

*20-Fluoromibolerone (Compound 9, F-Mib).* Cyclic sulfate 8 (4 mg, 0.011 mmol) was dissolved in 1.2 ml of THF, and to it was added dropwise 0.15 ml of $Bu_4NF$ (1 *M* in THF, 0.15 mmol) at 25°C. The mixture was stirred at 25°C until TLC showed complete consumption of starting material (~15 min). Then 0.5 ml of 2N HCl/MeOH solution was added, and the mixture was stirred at 25°C for 10 min. It was diluted with water, extracted three times with ether, and dried over $Na_2SO_4$. Flash chromatography (hex: EtOAc = 1:1) gave white product 1.6 mg (47.5% yield) mp 174–176°C; $^1$H NMR $\delta$ 5.83 (s, 1H, 4-H), 4.38 (d, AB quartet, 2H, $J_{HF}$ = 47.0 Hz, $\Delta\nu$ = 57.4 Hz, $J_{HH}$ = 9.1 Hz, 17$\alpha$-$CH_2F$), 0.98 (s, 3H, 18-H), 0.76 (d, 3H, J = 7.8 Hz, 7$\alpha$-$CH_3$); $^{19}$F NMR $\delta$-233.64 (br t, J = 47.6 Hz); mass spectrum (70 eV) m/e (rel. intensity), 320 (61, M+), 300 (75), 282 (20), 269 (46), 245 (64), 229 (75), 187 (45), 119 (73), 105 (89), 91 (100), 79 (95), 55 (96): HRMS calculated for $C_{20}H_{29}O_2F$: 320.2151; found: 320.2157.

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## REFERENCES

1. Mintun MA, Welch MJ, Siegel BA, et al. Positron tomographic imaging of estrogen receptors in human breast tumors. *Radiology* 1988;169:45–48.
2. Pertschuk LP, Rosenthal HE, Macchia RJ, et al. Correlation of histochemical and biochemical analysis of androgen binding in prostatic cancer: relation to therapeutic response. *Cancer* 1982;49:984–993.
3. Mobbs BG, Johnson IE. Basal and estrogen-stimulated hormone receptor profiles in four R3327 rat prostatic carcinoma sublines in relation to histopathology and androgen sensitivity. *Cancer Res* 1988;48:3077–3083.
4. Diamond DA, Barrack ER. The relationship of androgen receptor level to androgen responsiveness in the Dunning R3327 rat prostate tumor sublines. *J Urology* 1984;32:821–827.
5. Müntzing J, Kirdani RY, Murphy GP, Sandberg AA. A rat prostatic adenocarcinoma as a model for the human disease. *Invest Urology* 1979;17:37–41.
6. Barrack ER, Tindall DJ. A critical evaluation of the use of androgen receptor assays to predict the androgen responsiveness of prostatic cancer. In: *Current concepts and approaches to the study of prostate cancer.* New York: Alan R. Liss, Inc.; 1987:155–187.
7. Ekman P, Dahlberg E, Gustafsson JA, Högberg B, Pousette A, Snochowski M. Present and future clinical value of steroid receptor assays in human prostatic carcinoma. In: Iacobelli S, ed. *Hormone and cancer.* New York: Raven Press; 1980:361–370.
8. Ekman P, Snochowski M, Dahlberg E, Gustafsson JA. Steroid receptors in metastatic carcinoma of the human prostate. *Eur J Cancer* 1979;15:257–262.

9. Ekman P. The application of steroid receptor assay in human prostate cancer research and clinical management. *Anticancer Res* 1982;2:163–172.
10. Counsell RE, Klausmeier EH, Weinhold PA, Skinner RWS. Radiolabeled androgens and their analogs. In: Spencer RP, ed. *Radiopharmaceuticals: structure-activity relationships.* New York: Grune and Stratton; 1981:425–448.
11. Ghanadian R, Waters SL, Chisholm GD. Investigations into the use of $^{77}$Br-labeled 5α-dihydrotestosterone for scanning of the prostate. *Eur J Nucl Med* 1977;2:155–157.
12. Eakins MN, Waters SL. The synthesis of $^{77}$Br-labeled 5α-dihydrotestosterone and a comparison of its distribution in rats with $^{77}$Br-bromide. *Int J Appl Radiat Isot* 1979;30:701–703.
13. Hoyte RM, Rosner W, Hochberg RB. Synthesis of 16-[$^{125}$I] Iodo-5α-dihydrotestosterone and evaluation of its affinity for the androgen receptor. *J Steroid Biochem* 1982;16:621–628.
14. Tarle M, Padovan R, Spaventi S. The uptake of radioiodinated 5-alpha-dihydrotestosterone by the prostate of intact and castrated rats. *Eur J Nucl Med* 1981;6:79–83.
15. Skinner RWS, Pozerac RV, Counsell RE, Hsu CF, Weinhold PA. Androgen receptor protein binding properties and tissue distribution of 2-selena-A-nor-5α-androstan-17β-ol in the rat. *Steroids* 1977;30:15–23.
16. Spitzgale LA, Eng RR, Marino CA, Kasina S. In: Structure—distribution studies with fluorine-18 labeled steroids. Spencer RP, ed. *Radiopharmaceutical: structure-activity relationships* New York: Grune and Stratton; 1981:459–475.
17. Schilling K, Liao S. The use of radioactive 7α,17α-dimethyl-19-nortestosterone (mibolerone) in the assay of androgen receptors. *The Prostate* 1984;5:581–588.
18. Murthy LR, Johnson MP, Rowley DR, Young CYF, Scardino PT, Tindall DJ. Characterization of steroid receptors in human prostate using mibolerone. *The Prostate* 1986;8:241–253.
19. Carlson KE, Katzenellenbogen JA. A comparative study of the selectivity and efficiency of target tissue uptake of five tritium-labeled androgens in the rat. *J Ster Biochem* 1990; 36:549–561.
20. Berridge MS, Franceschini MP, Rosenfeld E, Tewson TJ. Cyclic sulfate: useful substrates for selective nucleophilic substitution. *J Org Chem* 1990;55:1211–1217.
21. Brandes SJ, Katzenellenbogen JA. Fluorinated androgens and progestins: molecular probes for androgen and progesterone receptors with potential use in positron emission tomography. *Mol Pharmacol* 1987;32:391–403.
22. Brodack JW, Kilbourn MR, Welch MJ, Katzenellenbogen JA. 16α-[$^{18}$F]Fluoroestradiol-17β: the effect of reaction vessel on fluorine-18 resolubilization, product yield, and effective specific activity. *Int J Appl Radiat Isot* 1986;37:217–221.
23. Katzenellenbogen JA, Johnson HJ, Jr., Myers HN. Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* 1973;12:4085–4092.
24. Zava DT, Landrum B, Horwitz KB, McGuire WL. Androgen receptor assay with [$^{3}$H]methyltrienolone (R1881) in the presence of progesterone receptors. *Endocrinology* 1979;104: 1007–1012.
25. Pinney KG, Carlson KE, Katzenellenbogen JA. [$^{3}$H] DU41165: a high affinity ligand and novel photoaffinity labeling reagent for the progesterone receptor. *J Steroid Biochem* 1990; 35:179–189.
26. McElvany KD, Carlson KE, Katzenellenbogen JA, Welch MJ. Factors affecting the target site uptake selectivity of estrogen radiopharmaceuticals: serum binding and endogenous estrogens. *J Steroid Biochem* 1983;18:635–641.
27. Senderoff SG, McElvany KD, Carlson KE, Heiman DF, Katzenellenbogen JA, Welch MJ. Methodology for the synthesis and specific activity determination of 16α-[$^{77}$Br]-bromoestradiol-17β-and 16α-[$^{77}$Br]-11β-methoxyestradiol-17β,two estrogen receptor-binding radiopharmaceuticals. *Int J Appl Radiat Isot* 1982;33:545–551.
28. Kiesewetter DO, Kilbourn MR, Landvatter SW, Heiman DF, Katzenellenbogen JA, Welch MJ. Preparation of four fluorine-18-labeled estrogens and their selective uptakes in target tissues of immature rats. *J Nucl Med* 1984;25:1212–1221.
29. Symes EK. Uptake and retention of androgens by the rat ventral prostate and consideration of their use as site directing agents. *Biochem Pharmacol* 1982;31:3231–3236.
30. Ralls JW, Riegel B. Selective mercaptole formation of steroid ketones. *J Am Chem Soc* 1954;76:4479–4480.
31. Corey EJ, Erickson BW. Oxidative hydrolysis of 1,3-dithiane derivatives to carbonyl compounds using N-halosuccinimide reagents. *J Org Chem* 1971;36:3553–3558.
32. Gao Y, Sharpless KB. Vicinal diol cyclic sulfates: like epoxides only more reactive. *J Am Chem Soc* 1988;110:7538–7539.
33. Kilbourn MR, Hood JT, Welch MJ. A simple $^{18}$O-water target for $^{18}$F production. *Int J Appl Radiat Isot* 1984;35:599–602.
34. Schmit JP, Guivy JI, Rousseau GG. The androgen receptor binding sites: a conformational study of steroids in relation with affinity. I. Interaction with the D-ring. *J Steroid Biochem* 1980;13:1387–1394.
35. Tindall DJ, Cunningham GR, Means AR. Structural requirements for steroid binding to androgen binding proteins. In: *Endocrine approach to male contraception. International Journal of Andrology* (suppl 2), 5th Annual Workshop on the Testis, Geilo, Norway, April 1978.
36. Mainwaring WIP, Milroy EJG. Characterization of the specific androgen receptors in the human prostate gland. *J Endocrinol* 1973;57:371–384.
37. Ojasoo T, Raynaud J-P. Unique steroid congeners for receptor studies. *Cancer Res* 1978;38:4186–4198.
38. Katzenellenbogen JA, Heiman DF, Carlson KE, Lloyd JE. In vivo and in vitro steroid receptor assays in the design of estrogen pharmaceuticals In: Eckelman WC, ed. *Receptor-binding radiotracers, volume 1.* Orlando: CRC Press; 1982:96–126.
39. Bonne C, Raynaud J-P. Methyltrienolone, a specific ligand for cellular androgen receptors. *Steroids* 1975;26:227–232.
40. Hanson RN. In: The influence of structure modification on the metabolic transformations of radiolabled steroid derivatives. Nunn A, ed. *Chemistry and pharmacology of radiopharmaceuticals.* New York: Dekker; 1991: in press.
41. Fiserova-Bergerova V. Changes of fluoride content in bone: an index of drug defluorination in vivo. *Anesthesiology* 1973;38:345–351.
42. Wallace-Durbin P. The metabolism of fluorine in rats using F-18 as a tracer. *J Dental Res* 1957;33:789–900.
43. Bucort B, Vignau M, Torelli V. New biospecific adsorbents for the purification of estradiol receptor. *J Biol Chem* 1978;253:8221–8228.
44. Trost BM, Braslau R. Tetra-n-butylammonium oxone. Oxidation under anhydrous conditions. *J Org Chem* 1988;53: 532–537.
45. Still WC, Kahn M, Mitra A. Rapid chromatographic technique for preparative separations with moderate resolution. *J Org Chem* 1978;43:2923–2925.