RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Preparation of Four Fluorine-18-Labeled Estrogens and Their Selective Uptakes in Target Tissues of Immature Rats

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Four fluorine-18-labeled estrogens—16α-[^{18}F]fluoro-estradiol-17β (1), 16β-[^{18}F]fluoro-estradiol-17β (2), (*2R*,3S)-1-[^{18}F]fluoro-2,3-bis(4-hydroxyphenyl)-pentane (1-[^{18}F]fluoropentestrol) (3), and (*3R*,4S**)-1-[^{18}F]fluoro-3,4-bis(4-hydroxyphenyl)hexane (1-[^{18}F]fluorohexestrol) (4)—have been prepared by simple displacement reactions utilizing reactive trifluoromethane sulfonate (triflate) precursors and F-18 fluoride ion. All four fluoroestrogens have high affinity for the estrogen receptor. In immature female rats, they are taken up by target tissues, such as the uterus, with very high selectivity: uterus-to-blood ratios at 1 hr are: Compound 1, 39; Compound 2, 12; Compound 3, 13; and Compound 4, 19. Average uterus-to-blood ratios exceed 80 for Compound 1 at 2 hr. That the uptake process involves an estrogen-specific binder of limited capacity is demonstrated by the suppressive effect of coadministered unlabeled estradiol on target tissue uptake.**

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The measurement of the estrogen-receptor content of human breast tumors has proved useful in selecting the most suitable form of treatment for metastatic disease: in general, tumors devoid of estrogen receptor are not responsive to hormonal therapy, whereas those that contain receptor have a greater response rate than unselected cases (*1–4*). Since the estrogen receptor is a protein that binds estrogens with high affinity, it also provides a potential mechanism for the selective accumulation of estrogens within receptor-positive tumors (*2,4*). Thus, in principle, a gamma-emitting estrogen derivative with suitable biochemical, binding, and pharmacokinetic properties could act as an imaging agent for such receptor-positive tumors. We have outlined in as quantitative a form as possible the characteristics of the estrogen-receptor uptake system, in terms of its binding capacity and its potential for selective uptake, and we have considered the implications of these characteristics in terms of the design of such imaging agents (*5,6*).

In earlier publications, we (*7–12*) and others (*13–16*) have described the preparation and tissue-uptake characteristics of estrogens labeled with the single-photon emitters, iodine-125 and bromine-77. Although some of these agents had very good receptor binding and pharmacokinetic characteristics, it was apparent from our preliminary studies in humans (*11*) that the successful application of these agents to image breast tumors would require radionuclides capable of providing very high-quality images. Thus, the possibility of utilizing positron-emitting estrogens together with positron tomography appeared particularly appealing.

There have been several reports of the preparation of estrogens labeled with the positron emitters C-11 (*17–19*) or F-18 (*20*), but usually these preparations have either been inefficient or have produced material

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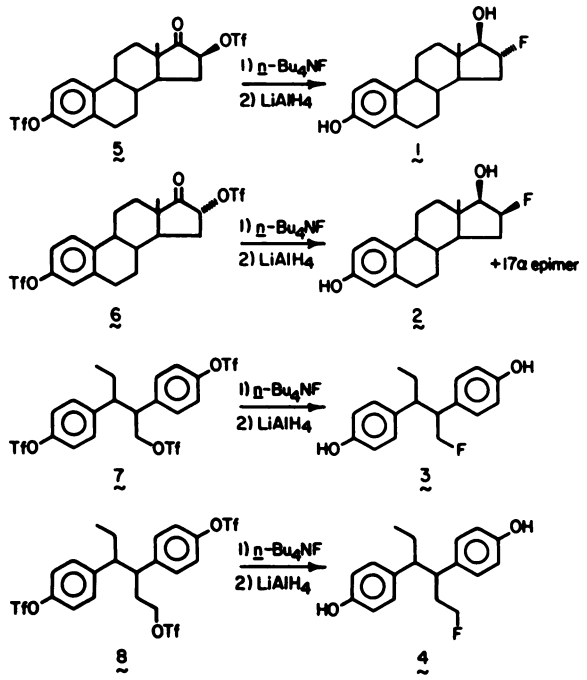


Scheme 1. Routes for [^{18}F]-Fluoride ion incorporation and preparation of fluoroestrogens.

of very low specific activity. We have recently described the details of the synthetic preparation of estrogens labeled with F-18 (*21*, unpublished data, Kiesewetter DO et al.), and presented preliminary tissue-uptake studies on one of these compounds (*21*). In this report we describe the preparation and receptor-binding characteristics of four fluoroestrogens (Scheme 1), 16α-[^{18}F]-fluoroestradiol-17β (**1**), 16β-[^{18}F]fluoroestradiol-17β (**2**), (*2R*,3S**)-1-[^{18}F]fluoro-2,3-bis(4-hydroxyphenyl)pentane, (1-[^{18}F]fluoropentestrol) (**3**), and (*3R*,4S**)-1-[^{18}F]fluoro-3,4-bis(4-hydroxyphenyl)-hexane, (1-[^{18}F]fluorohexestrol) (**4**), and we present studies on their tissue uptake in rats. Our findings indicate that these compounds are taken up with very high selectivity by target tissues, by a receptor-mediated process. An extension of the preparative methods to produce these compounds in somewhat higher specific activity should permit their evaluation as breast-tumor imaging agents in humans.

MATERIALS AND METHODS

Tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenoneketyl. Solutions of lithium aluminum hydride in ether or THF were prepared and standardized as previously described (*7*) to give solutions of 1.0–1.5 *M*.

All reactions were conducted without precautions to exclude atmospheric moisture in glass culture tubes. Extraction solvents were removed by nitrogen purge with gentle heating. Radioactivity was determined using either a sodium iodide well counter or a dose calibrator.

High-performance liquid chromatography (HPLC) was performed using a 5-μm analytical silica gel column (0.46 cm × 30 cm)* or a 10-μm preparative silica gel column (1.25 × 50 cm).† The eluant was monitored with a variable-wavelength detector‡ set at either 275 nm or 254 nm (at 0.1 absorbance units full scale) and a sodium iodide scintillation detector.

Authentic samples of products were prepared as previously described (*23,24*, unpublished data, Kiesewetter DO, et al.) The identity of radiolabeled compounds was supported by HPLC coinjection studies.

Preparation of F-18 fluoride. Fluorine-18 was prepared from [^{18}O]H_2O by the ^{18}O (p,n)^{18}F reaction in a 2-ml nickel-plated copper cyclotron target (*22*). The activity thus obtained was added to 2 μmol of nBu$_4$NOH and taken to dryness under a stream of nitrogen at 110°C in a platinum crucible. The residue was further dried by two azeotropic distillations using 100 μl of CH_3CN. Approximately 40–70% of the dried activity can then be solubilized in 400 μl THF and transferred to a glass test tube. The entire procedure takes less than 10 min.

16α[F-18]-Fluoro-estra-1,3,5(10)-triene-3,17β-diol (Compound 1). In a typical experiment, triflate **5** (1 mg, 1.8 μmol) was added to a THF solution containing 39.8 mCi of F-18 fluoride. After 5 min, the THF was removed under a stream of nitrogen, 300 μl ether added, and the solution cooled to −78°C. $LiAlH_4$ (100 μl, 1 *M* in ether) was added at −78°C, and after 2 min the reaction was allowed to warm to room temperature. The reaction was then quenched with 6 *N* HCl, extracted with ether, and the ether layers dried with Na_2SO_4. The ether was removed and the residue was dissolved in CH_2ClCH_2Cl; 20.4 mCi of 25 mCi was injected onto the preparative HPLC column (70% hexane, 28% CH_2Cl_2, 2% isopropyl alcohol, 5 ml/min) to yield 10.3 mCi (45%, decay-corrected) of Compound **1** (t_R = 20 min) and 2.9 mCi (12.6% decay-corrected) of the 17α-epimer (t_R = 12 min). 17β-Estradiol elutes at t_R = 22 min (Fig. 1).

16β-[F-18]-Fluoro-estra-1,3,5(10)-triene-3,17β-diol (Compound 2). In a typical experiment, triflate **6** (20 μl of a 14 μg/μl solution of THF, 280 μg, 0.51 μmol) was added directly to a THF solution containing 45.9 mCi of [^{18}F]fluoride. After 5 min, TLC analysis indicated 54% incorporation of [^{18}F]fluoride ion into the product. After 17 min, 100 μl of 1 *M* $LiAlH_4$ was added to the reaction vessel, and after an additional 3 min the reaction was quenched with 6 *N* HCl. A single pentane extract and two ether extracts were each dried by passage through a column of Na_2SO_4; the combined organic phases contained (43%, decay-corrected) F-18 activity. The solvent was removed, and 13.9 mCi (out of a total residue of 16.7 mCi) was injected onto an analytical HPLC column (70% hexane/28% CH_2Cl_2/2% iPrOH, 1 ml/min), to yield 9.5 mCi (29%, decay-corrected) of

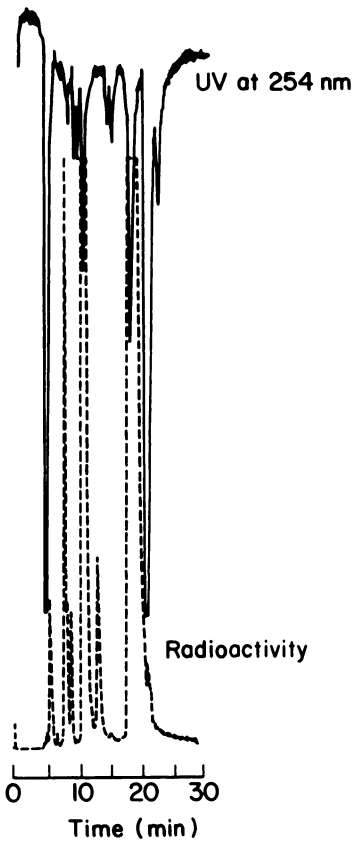


FIG. 1. HPLC trace of purification of 16α-[F-18]fluoroestradiol (Compound 1). Analysis was performed on preparative 10-μm silica gel HPLC column (1.25 × 50 cm) eluted with hexane/CH_2Cl_2/iPrOH (70%:28%:2%), at 5 ml/min. UV detection was at 275 nm [(0.1 (AUFS)]; radioactivity was detected with flow-crystal scintillation counter. Large radioactivity peak at ~20 min is Compound 1, smaller peak at ~12 min is the 17α-epimer. Other radioactive peaks are unidentified. The major uv peak at 22 min is 17β-estradiol. Note that uv peaks exactly coelute with both 16α-fluoroestradiol epimers.

Compound **2**. No byproducts elute in the vicinity of this material (t_R = 17 min).

1-[^{18}F]Fluoropentestrol (Compound 3). The preparation of this compound has been described (*21*). The procedure followed was essentially that used to prepare Compound **2** using precursor **7** (1 mg). Purification was conducted on a preparative HPLC column (90% hexane, 8% CH_2Cl_2, 2% iPrOH, 5 ml/min, t_R = 16 min). The major byproduct is the reduction product pentestrol, which elutes at t_R = 14.8 min.

***Erythro*-3,4-Bis(4-trifluoromethanesulfonyloxyphenyl)-1-hexyl trifluoromethanesulfonate (Compound 8).** *erythro*-3,4-Bis(4-hydroxyphenyl)-1-hexanol (*24*) (143 mg, 0.5 mmol) was dissolved in 0.7 ml of 2,6-dimethylpyridine (dried by distillation from BaO) under nitrogen and diluted with 3 ml of dichloromethane (dried over 0.4Å molecular sieves). The mixture was cooled in an ice/salt bath, and 0.30 ml of trifluoromethanesulfonic anhydride (1.75 mmol) was added. After being stirred for 30 min, the reaction was quenched by addition of 2 ml of 1 *M* trifluoromethanesulfonic acid. The layers were separated, and the organic layer washed successively with a further 2 ml of trifluoromethanesulfonic acid and 2 ml of water, dried ($MgSO_4$), and concentrated under a stream of nitrogen while cooling in an ice bath. The product was purified immediately by chromatography (MPLC, SiO_2 column 1 × 50 cm, 1:1 pentane/dichloromethane) to give 222 mg (65%) of the tris-trifluoromethanesulfonate, Compound **8**, a nearly colorless oil, as the first compound to elute: ^{1}H-NMR (C_6D_6) δ 0.36 (t, 3, J = 6 Hz, CH_3), 0.85–1.65 (m, 4, CH_2 of C-2 and C-5), 2.20 (t,d, 1, J = 5, 10 Hz, C(4)H), 2.57 (t,d, 1, J = 4, 10 Hz, C(3)H), 3.33–3.85 (m, 2, C(1)H_2), 6.70 (d, 4, J = 8 Hz, Ar H), 6.93 (d, 4, J = 8 Hz, Ar H); ^{19}F-NMR ($CDCl_3$) φ −73.29 (s, 3, $ArOSO_2CF_3$), −73.31 (s, 3, $ArOSO_2CF_3$), −75.29 (s, 3, alkyl-OSO_2CF_3); mass spectrum (70 eV) *m/z* (rel. intensity) 682 (0.023, M^+), 267 (100), 239 (47), 69 (16). This compound is thermally unstable and decomposes fairly rapidly at room temperature or above, but it may be stored for several months at −20°C without degradation.

1-[^{18}F]Fluorohexestrol (Compound 4). The preparation of Compound **4** was conducted using precursor **8** (1 mg), following the method used for the preparation of Compound **2**. The product was purified using an analytical HPLC column (91.4% hexane, 7% CH_2Cl_2, 1.6% iPrOH, 1.5 ml/min, t_R = 18 min). As in the preparation of Compound **3** (*21*), the major side product is the reduction product of the precursor **8** (meso-hexestrol) (t_R = 16 min).

In vitro binding assays. In earlier work (*5,25*) we have described in detail the competitive radioreceptor binding assays used to determine the effective specific activity of the fluoroestrogens and their relative binding affinities for the estrogen receptor.

In vivo uptake studies. After HPLC purification and solvent removal, each compound (**1, 2, 3,** and **4**) was dissolved in a small amount of ethanol and diluted to the proper dosage (50 μCi or 5 μCi) with 0.9% NaCl. The ethanol concentration of the injectate was ≤5%. Animals (Sprague-Dawley female rats, 21 days old, 50–60 g) under ether anesthesia were injected by femoral vein with ~0.1 ml of the radiopharmaceutical. At the indicated times, the animals were killed by decapitation, and samples of blood and tissues were weighed and immediately assayed for radioactivity in a gamma counter with 60% efficiency; total counts of several hundred to several hundred thousand were obtained in a 1-min counting period; background was 20 cpm. In experiments to show the blocking of uterine uptake, 15 μg of unlabeled estradiol were coinjected with the radiopharmaceutical (*7,9*).

RESULTS

Synthesis of F-18-labeled estrogens. The synthesis of the fluoroestrogens by fluoride-ion displacements on

reactive trifluoromethane sulfonate (triflate) precursors has been described (*23,24*, unpublished data, Kiesewetter DO, et al.); the final steps in the synthetic sequences are shown in Scheme 1. Since displacement reactions with unlabeled fluoride ion operate in high yield—even with substoichiometric quantities of fluoride ion (*21*, unpublished data, Kiesewetter DO et al.), the key to achieving good yields with the cyclotron-produced F-18 fluoride was to obtain F-18 fluoride ion in a form that is soluble in organic solvents and free from contaminants that reduce its nucleophilic reactivity or compete for consumption of the triflate precursor.

The F-18 activity from the water target (20–100 mCi) could be dried and solubilized most conveniently and effectively by adding base (2 μmol Bu_4NOH) to the target water and evaporating the water in a platinum vessel, with additional azeotropic drying with acetonitrile. After this treatment, 40–70% of the activity could be solubilized readily in tetrahydrofuran. At room temperature, the F-18 fluoride reacted rapidly with the triflates, reaching maximum uptake within 5 min. Since the excess hydroxide ion does compete with fluoride for reaction with the triflate substrate, approximately 1 mg (2 μmol) of triflate was required for most of the displacement reactions. Subsequent treatment with $LiAlH_4$ effected cleavage of the phenolic triflate protective groups and reduced the fluoroestrones to the estradiols. The 16β-[^{18}F]fluoroestradiol and the [^{18}F]fluoropentestrol and hexestrol precursors were reduced in tetrahydrofuran; 16α-[^{18}F]fluoroestrone 3-triflate, however, was reduced in ethyl ether, since this produced a greater proportion of the desired 17β-fluoroestradiol epimer (unpublished data, Kiesewetter DO et al.).

Careful purification of the fluoroestrogens by HPLC is important for the attainment of high chemical purity and effective specific activity. While purification of 1-fluorohexestrol (**4**) and 16β-fluoroestradiol-17β (**2**) was relatively straightforward because the desired products were well separated from both labeled and unlabeled byproducts, separation of 1-[^{18}F]fluoropentestrol (**3**) is complicated by the fact that the reduction product of unreacted triflate, pentestrol, which has a high binding affinity for the estrogen receptor (*23*), elutes on normal-phase HPLC immediately ahead of the desired F-18-labeled product. Fortunately we could achieve adequate separation by careful selection of HPLC conditions (*21*). The reduction product from the precursor for 16α-fluoroestradiol (**1**) is estradiol, which elutes directly behind the fluoroestrogen. Again, careful selection of elution conditions gave adequate separation of these species (cf. Fig. 1).

The yields (decay-corrected) obtained after purification are shown in Table 1, Column 1. We have been able to produce multimillicurie quantities of the epimeric 16-fluoroestradiols, starting from 20–100 mCi quantities of [^{18}F]fluoride in the target water. The overall time for fluoride ion drying and resolubilization, displacement, reduction-deprotection, product isolation, and purification, is 70–110 min, that is, a half-life or slightly less. Thus, the overall yields based on fluoride ion produced by the target are 8–18% (13–26% when decay-corrected) or, based on resolubilized fluoride, they are 14–36% (29–57% when decay-corrected). A small part of the remaining F-18 activity is in minor organic byproducts (17α-OH epimer, etc), but the bulk is present as unreacted inorganic fluoride.

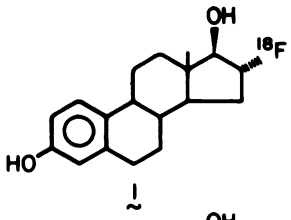
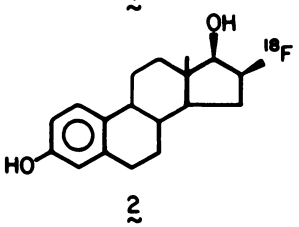
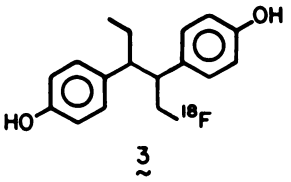
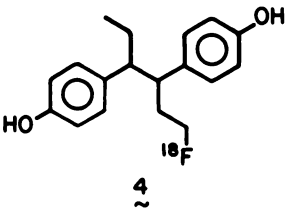
Radiochemical purity, specific activity, and effective specific activity. After isolation by HPLC, the [^{18}F]-fluoroestradiols have radiochemical purity in excess of 99%. The only detectable chemical impurities coelute exactly with the [^{18}F]fluoroestrogens in all solvent systems, and thus appear to be unlabeled material that is being coproduced during these fluorine-18 labeling procedures (see, for example, Fig. 1). The uv peak that coelutes with the intermediate 16β-[^{18}F]fluoroestrone-3-triflate was converted upon reduction to a uv peak that coelutes with 16β-[^{18}F]fluoroestradiol (**2**). Also, the uv peak eluting with 16β-[^{18}F]fluoro-17β-estradiol (**2**) was collected; it produced a mass spectrum with the molecular-ion and characteristic fragment peaks of the unlabeled fluoroestradiol.

Because these estrogens have reasonably strong uv absorbance at 275 nm (ε ca. 2000–3000), as little as 0.1–0.2 μg of product can easily be quantified by HPLC with uv detection. Thus, using external calibration, we were able to estimate the specific activity of the labeled compounds as 200–275 Ci/mmol. Effective specific activity (6)—that is, the activity per equivalent of receptor-binding substance—was estimated by a competitive radioreceptor-binding assay of a decayed product, using tritiated estradiol as a tracer and lamb uterine cytosol as a source of estrogen receptor (*25*). Using this assay, and the previously determined measurement of the relative binding affinities of these compounds for receptor (Table 1), we estimated the effective specific activities of these compounds to be 100–170 Ci/mmol.

These specific activities are several orders of magnitude lower than theoretical maximum for fluorine-18 (1,700,000 Ci/mmol). Further studies, to be detailed elsewhere, have indicated that carrier fluoride is introduced during the manipulations or the bombardment of the water sample. Steps have been taken to reduce this introduction of carrier so that material with at least tenfold higher specific activity can now be produced.

Receptor binding properties of the four fluorine-substituted estrogens. Table 1 presents the binding affinities of the four fluorine-substituted estrogens for rat uterine estrogen receptor, determined in a competitive radioreceptor assay using [^{3}H]estradiol as tracer and charcoal-dextran as an adsorbant of free ligand. It is evident

TABLE 1. YIELD, SPECIFIC ACTIVITY, SYNTHESIS TIME, AND RECEPTOR-BINDING AFFINITY FOR FLUOROESTROGENS

Compound	Radiochemical yield (%)*	Specific activity (Ci/mmol)†	Total time (min)‡	RBA§
 1	43%	166	75–90 min	80
 2	29%	103	110 min	30
 3	57%	160	100 min	129
 4	31%	107	70 min	127

* Radiochemical yield (decay corrected) based on resolubilized F-18 fluoride activity. (Resolubilization efficiency is 40–70%).

† Determined by a competitive radioreceptor assay (*25*). Specific activities determined by HPLC are 200–275 Ci/mmol.

‡ Elapsed time from end of bombardment to elution of final product from HPLC.

§ RBA, relative binding affinity, is a measure of the affinity for the estrogen receptor, expressed as a percentage of that of estradiol. This value is determined by a competitive binding radioreceptor assay, as described in Ref. *25*.

that all four compounds have high to very high receptor-binding affinities.

Tissue uptake selectivity in immature rats. Immature Sprague-Dawley female rats, injected intravenously with a 50 μCi (0.3–0.5 nmol) dose of Compounds **1, 2, 3,** and **4**, were killed at 30, 60, 120 min, and tissue samples were removed and assayed for radioactivity. These data are presented as percent injected dose per gram (Tables 4–7) or as uterus-to-blood and uterus-to-nontarget ratios (Tables 2 and 3 and Fig. 2).

These compounds exhibit highly selective uptake by the uterus, with uterus-to-blood ratios ranging from 11 to 38 at 1 hr. Accumulation and retention of these compounds in the uterus is indicated by the fact that the uterus-to-blood ratios increase and remain high throughout the 2-hr period of observation. This accumulation and retention is consistent with the pharmacodynamics expected for a compound having high binding selectivity. That this uptake is mediated by a high-affinity, limited-capacity system is supported by the selective depression of uterine uptake upon coadministration of a blocking dose of unlabeled estradiol (*7,9*); uterus-to-blood ratios fall from 12–39 at 1 hr to 1.5–1.9 upon coadministration of 15 μg of unlabeled estradiol (Table 2, compare lines 2 and 4). It appears that the 50-μCi dose of these compounds is not recep-

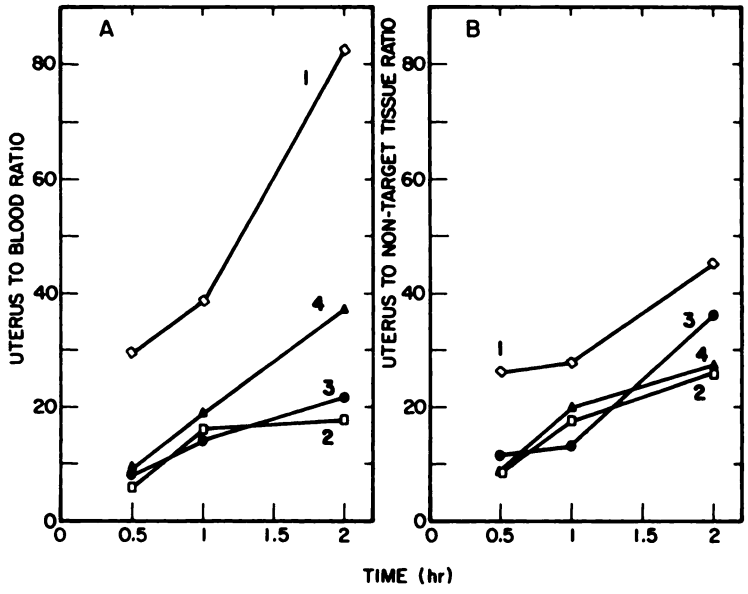


FIG. 2. Uptake selectivity of four F-18-labeled estrogens in immature rats, expressed as uterus-to-blood ratios (Panel A) or uterus-to-nontarget tissue ratios (Panel B). These data are taken from Tables 2 and 3, respectively.

tor-saturating, since when a dose one-tenth as great is administered, uterine uptakes at 1 hr do not change significantly (Table 3, compare lines 2 and 3). The ovaries provide the only other tissue that shows evidence of receptor-mediated uptake (Tables 4–7).

The kidney and liver, the primary organs of metabolism and excretion, take up a great deal of radioactivity, as has been previously observed, particularly at early times, but this uptake is not receptor-mediated. There is evidence of some defluorination in the case of 1-[^{18}F]fluorohexestrol 4, since an elevated uptake and retention of activity is noted in bone. The other three compounds apparently do not undergo substantial defluorination in vivo.

From the graphical representation of uterus-to-blood or uterus-to-nontarget tissue ratios of the four com-

TABLE 2. UTERUS-TO-BLOOD RATIOS OF THE FLUOROESTROGENS*

Time	Compound 16αF-E_2 (1)	16βF-E_2 (2)	1F-pent (3)	1F-hex (4)
1. ½ hr	30 ± 3.5†	5.3 ± 0.47	8.4 ± 4.2	9.2 ± 2.5
2. 1 hr	39 ± 16	12 ± 2.4	13 ± 4.4	19 ± 7.1
3. 1 hr (low dose)	61 ± 34	16 ± 4.4	16 ± 6.1	34 ± 12
4. 1 hr (+E_2)	1.5 ± 1.0	1.2 ± 0.54	1.9 ± 0.8	1.9 ± 0.83
5. 2 hr	83 ± 35	17 ± 11	22 ± 7.7	37 ± 9.4

* Data taken from Tables 4–7.
† Mean ± s.d.

TABLE 3. UTERUS TO NONTARGET TISSUE RATIOS OF THE FLUOROESTROGENS*

Time	16αF-E_2 (1)†	16βF-E_2 (2)	1F-pent (3)	1F-hex (4)
1. 1/2 hr	26 ± 8.1	8.0 ± 1.5	11 ± 3.9	8.0 ± 1.2
2. 1 hr	28 ± 4.8	16 ± 8.9	12 ± 3.7	19 ± 6.8
3. 1 hr (low dose)	23 ± 12	20 ± 4.8	19 ± 5.5	15 ± 5.0
4. 1 hr (+E_2)	2.2 ± 0.44	2.0 ± 0.49	3.0 ± 1.0	2.3 ± 0.73
5. 2 hr	45 ± 10	26 ± 10	37 ± 18	27 ± 10

* Data taken from Tables 4–7. Nontarget tissues = average of uptake by esophagus, spleen, lung, and muscle.
† Mean ± s.d.

TABLE 4. TISSUE DISTRIBUTION OF 16α-[^{18}F]FLUORO-17β-ESTRADIOL (1) IN IMMATURE FEMALE RATS (mean ± s.d.)*

		% I.D. per gram			
Tissue	½ hr†	1 hr†	1 hr‡ (low dose)	1 hr§ (+E_2)	2 hr†
Blood	0.16 ± 0.03	0.14 ± 0.07	0.14 ± 0.07	0.50 ± 0.24	0.10 ± 0.05
Uterus	4.87 ± 1.17	4.67 ± 1.50	7.09 ± 1.04	0.61 ± 0.12	8.58 ± 7.01
Ovaries	1.62 ± 0.41	1.59 ± 0.60	2.48 ± 0.52	0.45 ± 0.13	2.25 ± 1.52
Muscle	0.21 ± 0.09	0.17 ± 0.08	0.42 ± 0.26	0.12 ± 0.02	0.18 ± 0.15
Liver	2.10 ± 0.67	1.29 ± 0.25	1.24 ± 0.36	2.02 ± 0.56	1.26 ± 0.88
Spleen	0.14 ± 0.04	0.11 ± 0.04	0.15 ± 0.05	2.18 ± 0.03	0.17 ± 0.15
Kidney	1.22 ± 0.33	0.81 ± 0.14	1.07 ± 0.13	0.74 ± 0.12	0.57 ± 0.22
Esophagus	0.18 ± 0.04	0.23 ± 0.09	0.81 ± 0.79	0.40 ± 0.07	0.22 ± 0.17
Lung	0.24 ± 0.06	0.17 ± 0.04	0.29 ± 0.07	0.41 ± 0.16	0.17 ± 0.07
Bone	0.18 ± 0.06	0.17 ± 0.07	0.27 ± 0.09	0.23 ± 0.07	0.26 ± 0.15

* Five animals were used at each time point.
† Injected with 50 μCi Compound 1.
‡ Injected with 5 μCi Compound 1.
§ Injected with 50 μCi Compound 1 + 15 μg estradiol.

pounds (Fig. 1), it is apparent that 16α[^{18}F]fluoro-17β-estradiol **1** exhibits the highest selectivity. The uterus-to-blood ratio of 83 at 2 hr (or uterus-to-nontarget tissue ratio of 45) is the highest among the potential tumor-imaging agents we have prepared. Although the other three compounds display somewhat lower selectivity ratios, they may still be sufficiently selective to be useful as tumor-imaging agents.

The reason for the higher uptake selectivity of Compound **1** is interesting: Comparison between the uterus-to-blood ratios (Fig. 2A, Table 2) and the uterus-to-nontarget tissue ratios (Fig. 2B, Table 3) shows that Compounds **2**, **3**, and **4** have higher uterus-to-nontarget tissue ratios than uterus-to-blood ratios, whereas Compound **1** has a higher uterus-to-blood ratio at all times. Comparisons of the uptake data in Table 4 with those in Tables 5–7 indicate that this difference is due largely to the low blood levels for Compound **1**. Thus,

TABLE 5. TISSUE DISTRIBUTION OF 16β-[^{18}F]FLUORO-17β-ESTRADIOL (2) IN IMMATURE FEMALE RATS

		% I.D. per gram			
Tissue	½ hr†	1 hr†	1 hr‡ (low dose)	1 hr§ (+E_2)	2 hr†
Blood	0.76 ± 0.24	0.26 ± 0.07	0.26 ± 0.06	0.23 ± 0.03	0.15 ± 0.13
Uterus	3.95 ± 0.95	2.99 ± 2.83	4.26 ± 0.67	0.28 ± 0.14	1.75 ± 0.51
Ovaries	2.09 ±1.05	1.17 ± 0.50	1.26 ± 0.24	0.32 ± 0.22	0.49 ± 0.21
Muscle	0.38 ± 0.12	0.14 ± 0.06	0.19 ± 0.11	0.11 ± 0.09	0.05 ± 0.03
Liver	4.46 ± 1.26	1.56 ± 0.38	1.79 ± 0.48	1.61 ± 0.36	0.66 ± 0.27
Spleen	0.36 ± 0.16	0.10 ± 0.03	0.14 ± 0.02	0.08 ± 0.02	0.04 ± 0.02
Kidney	2.27 ± 0.53	0.86 ± 0.31	1.00 ± 0.20	0.68 ± 0.18	0.29 ± 0.14
Esophagus	0.45 ± 0.07	0.14 ± 0.07	0.20 ± 0.02	0.12 ± 0.02	0.10 ± 0.07
Lung	0.84 ± 0.16	0.24 ± 0.10	0.33 ± 0.07	0.23 ± 0.05	0.10 ± 0.02
Bone	0.61 ± 0.16	0.45 ± 0.22	0.53 ± 0.24	0.44 ± 0.31	0.31 ± 0.16

* Five animals were used at each time point.
† Injected with 50 μCi Compound 2.
‡ Injected with 5 μCi Compound 2.
§ Injected with 50 μCi Compound 2 + 15 μg estradiol.

TABLE 6. TISSUE DISTRIBUTION OF [^{18}F]FLUOROPENTESTROL (3) IN IMMATURE FEMALE RATS (mean ± s.d.)*

Tissue	% I.D. per gram ½ hr†	1 hr†	1 hr‡ (low dose)	1 hr§ (+E_2)	2 hr†
Blood	0.64 ± 0.29	0.38 ± 0.22	0.27 ± 0.10	0.37 ± 0.34	0.18 ± 0.08
Uterus	4.49 ± 0.99	4.77 ± 2.37	3.91 ± 0.65	0.67 ± 0.26	3.84 ± 1.64
Ovaries	2.07 ± 0.31	2.35 ± 1.98	1.49 ±0.39	0.55 ± 0.34	2.03 ± 1.90
Muscle	0.43 ± 0.12	0.38 ± 0.19	0.20 ± 0.04	0.18 ± 0.07	0.13 ± 0.11
Liver	2.85 ± 1.21	1.56 ± 0.48	1.30 ± 0.31	1.40 ± 0.66	1.12 ± 0.27
Spleen	0.30 ± 0.08	0.17 ± 0.08	0.13 ± 0.04	0.11 ± 0.07	0.09 ± 0.05
Kidney	2.01 ± 0.61	1.19 ± 0.44	0.99 ± 0.18	0.76 ± 0.39	0.38 ± 0.25
Esophagus	0.50 ± 0.18	0.62 ± 0.30	0.28 ± 0.09	0.25 ± 0.17	0.16 ± 0.11
Lung	0.61 ± 0.20	0.35 ± 0.15	0.27 ± 0.06	0.36 ± 0.22	0.15 ± 0.06
Bone	0.36 ± 0.11	0.28 ± 0.14	0.23 ± 0.06	0.23 ± 0.10	0.16 ± 0.09

* Five animals were used at each time point. Data in this table have been taken from Ref. *21*, and are included for comparison purposes.
† Injected with 50 μCi Compound 3.
‡ Injected with 5 μCi Compound 3.
§ Injected with 50 μCi Compound 3 + 15 μg estradiol.

this compound appears to undergo a more rapid and complete clearance than the other three.

DISCUSSION

In this study we have described the preparation of four F-18-labeled estrogens by a fluoride-displacement reaction on trifluoromethane sulfonate (triflate) precursors. This method is rapid, efficient, and convenient (at least in comparison with the preparation of other complex F-18-labeled radiopharmaceuticals), and produces materials with high chemical and radiochemical purity and adequate specific activity. The entire preparation and purification time requires one half-life or less.

All four compounds exhibit selective uptake by the uterus in immature rats, and somewhat lower uptake by the ovaries. This uptake can be blocked by coadministration of unlabeled estradiol. Of the four compounds, 16α-[^{18}F]fluoro-17β-estradiol (**1**) exhibits the highest uptake selectivity, with uterus-to-blood and uterus-to-

TABLE 7. TISSUE DISTRIBUTION OF 1-[^{18}F]FLUOROHEXESTROL (4) IN IMMATURE FEMALE RATS (mean ± s.d.)*

Tissue	% I.D. per gram ½ hr†	1 hr†	1 hr‡ (low dose)	1 hr§ (+E_2)	2 hr†
Blood	0.48 ± 0.27	0.40 ± 0.28	0.24 ± 0.09	0.41 ± 0.19	0.11 ± 0.02
Uterus	3.98 ± 1.41	6.17 ± 2.39	7.46 ± 2.68	0.66 ± 0.16	3.91 ± 0.59
Ovaries	2.50 ± 1.41	1.89 ± 0.50	2.33 ± 0.44	0.47 ± 0.04	1.21 ± 0.36
Muscle	0.52 ± 0.17	0.37 ± 0.15	0.56 ± 0.22	0.22 ± 0.03	0.13 ± 0.03
Liver	2.40 ± 1.70	1.25 ± 0.52	1.56 ± 0.61	2.17 ± 0.63	0.79 ± 0.15
Spleen	0.39 ± 0.19	0.19 ± 0.08	0.26 ± 0.09	0.17 ± 0.04	0.17 ± 0.22
Kidney	1.76 ± 1.45	0.93 ± 0.36	1.72 ± 0.69	1.20 ± 0.44	0.35 ± 0.18
Esophagus	0.49 ± 0.25	0.36 ± 0.15	0.41 ± 0.13	0.31 ± 0.09	0.11 ± 0.02
Lung	0.64 ± 0.23	0.45 ± 0.16	0.57 ± 0.20	0.48 ± 0.12	0.31 ± 0.28
Bone	1.04 ± 0.44	1.10 ± 0.68	1.88 ± 1.13	2.13 ± 0.31	1.70 ± 0.38

* Five animals were used at each time point.
† Injected with 50 μCi Compound 4.
‡ Injected with 5 μCi Compound 4.
§ Injected with 50 μCi Compound 4 + 15 μg estradiol.

nontarget tissues ratios of 83 and 45, respectively, at 2 hr. This uterus-to-nontarget ratio is considerably higher than that previously reported for 11β-methoxy-16α-[Br-77]bromo-17β-estradiol (*8*, *9*), which was, up to now, the best gamma-emitting estrogen we had prepared. The other three agents also show high uterine uptake selectivities, though their selectivity is somewhat less than that of Compound **1**.

It is evident from the time course of the target tissue-to-blood or the target tissue-to-nontarget tissue ratios (Fig. 2) that the "contrast," (i.e., the target-to-background ratio) with these fluoroestrogens is higher at 2 hr than at 1 hr after injection—and might, in fact, continue to increase beyond the 2-hr point. Thus, it may be best to image with a considerable delay after injection in order to obtain optimal images of estrogen-receptor-positive tumors. If this is the case, it may be preferable to use F-18-labeled estrogens, rather than those labeled with the shorter-lived-carbon-11, since the latter would have decayed through several half-lives before reaching the optimal imaging time.

We are continuing to improve the preparation of these F-18-labeled estrogens in order to obtain material in suitable quantity and with sufficiently high specific activity for use in breast-cancer patients.

FOOTNOTES

* Varian SI-5.
† Whatman Partisil Magnum M-9.
‡ Waters Lamda Max Model 480.

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REFERENCES

1. MCGUIRE WL: Steroid receptor sites in cancer therapy. *Adv Intern Med* 24:127-140, 1979
2. THOMPSON EB, LIPPMAN ME, eds: *Steroid Receptors and the Management of Cancer.* Vol 1, Boca Raton, Florida, CRC Press, 1979, pp 1-248
3. HORWITZ KB, MCGUIRE WL: Antiestrogen: Mechanism of action and effect in breast cancer. In *Breast Cancer: Advances in Research and Treatment.* Vol. 2, McGuire WL, ed. New York, Plenum, 1978, p 155
4. ALLEGRA JC, LIPPMAN ME, THOMPSON EG, et al: Distribution, frequency, and quantitative analysis of estrogen, progesterone, androgen, and glucocorticoid receptors in human breast cancer. *Cancer Res* 29:1447-1454, 1979
5. KATZENELLENBOGEN JA, HEIMAN DF, CARLSON KE, et al: In vitro and in vivo steroid receptor assays in the design of estrogen radiopharmaceuticals. In *Receptor Binding Radiopharmaceuticals.* Eckelman WC, ed. Boca Raton, Florida, CRC Press, 1982, Vol. 1, pp 93-126
6. KATZENELLENBOGEN JA: Trends in the design of diagnostic agents. In *Radiopharmaceutical Chemistry.* Jones AG, Welch MJ, eds. The Hague, Netherlands, Martinus Nijhoff: in press
7. KATZENELLENBOGEN JA, SENDEROFF SG, MCELVANY KD, et al: 16α-[^{77}Br]Bromoestradiol-17β: A high specific activity gamma-emitting tracer with uptake in rat uterus and induced mammary tumors. *J Nucl Med* 22:42-47, 1981
8. SENDEROFF SG, MCELVANY KD, CARLSON KE, et al: Synthetic methodology for the preparation of 16α-[^{77}Br]-bromoestradiol-17β and 16α-[^{77}Br]-bromo-11β-methoxy-estradiol-17β. Two high specific activity estrogen receptor-binding radiopharmaceuticals. *Int J Appl Radiat Isotop* 33:545-551, 1982
9. KATZENELLENBOGEN JA, MCELVANY KD, SENDEROFF SG, et al: 16α-[^{77}Br]-Bromo-11β-methoxyestradiol-17β. A gamma-emitting estrogen imaging agent with high uptake and retention by target organs. *J Nucl Med* 23:411-419, 1982
10. MCELVANY KD, CARLSON KE, WELCH MJ, et al: In vivo comparison of 16α-[^{77}Br]Bromoestradiol-17β and 16α-[^{125}I]-iodoestradiol-17β, *J Nucl Med* 23:420-424, 1982
11. MCELVANY KD, KATZENELLENBOGEN JA, SHAFER KE, et al: 16α-[^{77}Br]-bromoestradiol: Dosimetry and preliminary clinical studies. *J Nucl Med 23:425-430, 1982*
12. LANDVATTER SW, KATZENELLENBOGEN JA, MCELVANY KD, et al: (2R*,3S*)-1-[^{125}I]iodo-2,3-bis(4-hydroxyphenyl)pentane ([^{125}I] iodonorhexestrol) and (2R*,3S*)-1-[^{77}Br]bromo-2,3-bis(4-hydroxyphenyl)pentane ([^{77}Br]-bromonorhexestrol), two γ-emitting estrogens that show receptor-mediated uptake by target tissues in vivo. *J Med Chem* 25:1307-1312, 1982
13. GATLEY SJ, SHAUGHNESSY WJ, INHORN L, et al: Studies with 17β(16-α-[^{125}I]-iodo)estradiol, an estrogen receptor binding radiopharmaceutical, in rats bearing mammary tumors. *J Nucl Med* 22:459-464, 1981
14. HANSON RN, SEITZ DE, BOTTARO JC: 17α-[^{125}I]iodovinylestradiol: An estrogen receptor seeking radiopharmaceutical. *J Nucl Med* 23:431-436, 1982
15. HOCHBERG RB, ROSNER W: Interaction of 16α-[^{125}I]-iodo-estradiol with estrogen receptor and other steroid binding proteins. *Proc Nat Acad Sci USA* 77:320-332, 1980
16. JAGODA EM, GIBSON RE, GOODGOLD H, et al: [I-125] 17α-Iodovinyl-11β-Methoxyestradiol: in vivo and in vitro properties of a high-affinity estrogen-receptor radiopharmaceutical. *J Nucl Med* 25:472-477, 1984
17. REIFFERS S, VAALBURG W, WIEGMAN T, et al: Carbon-11 labelled methyllithium as methyl donating agent: The addition to 17-keto steroids. *Int J Appl Radiat Isotop* 31:535-539, 1980
18. VAALBURG W, REIFFERS S, BEERLING E, et al: The preparation of carbon-11 labeled 17α-ethynylestradiol. *J Lab Comp Radiopharm* 13:200-201, 1977
19. VAALBURG W, FEENSTRA A, WIEGMAN T, et al: Carbon-11 labeled moxestrol and 17α-methyl-estradiol as receptor binding radiopharmaceuticals. *J Lab Comp Radiopharm* 18:100-101, 1981
20. PALMER AJ, WIDDOWSON DA: The preparation of ^{18}F-labelled 4-fluoroestrone and 4-fluoroestradiol. *J Lab Comp Radiopharm* 16:14-16, 1979
21. LANDVATTER SW, KIESEWETTER DO, KILBOURN MR, et al: (2*R**,3*S**)-1-[^{18}F]fluoro-2,3-bis(4-hydroxyphenyl)-pentane ([^{18}F]fluoro norhexestrol), a positron-emitting estrogen that shows highly-selective, receptor-mediated uptake by target tissues in vivo. *Life Sciences* 33;1933-1938, 1983
22. KILBOURN MR, HOOD JT, WELCH MJ: A simple 0-18 water target for F-18 production. *Int J Appl Radiat Isotop* 35:599-602, 1984
23. LANDVATTER SW, KATZENELLENBOGEN JA: Nonster-

oidal estrogens: Synthesis and estrogen receptor binding affinity of derivatives of (3R*,4S*)-3,4-bis(4-hydroxyphenyl)-hexane (hexestrol) and (2R*,3S*)-2,3-bis(4-hydroxy-phenyl)pentane (norhexestrol) functionalized on the side chain. *J Med Chem* 25:1300–1307, 1982

24. GOSWAMI R, HARSY SG, HEIMAN DF, et al: Estrogen receptor-based imaging agents. 2. The synthesis and receptor binding affinity of side-chain halogenated hexestrol derivatives. *J Med Chem* 23:1002–1008, 1980

25. KATZENELLENBOGEN JA, JOHNSON HJ, MYERS HN: Photoaffinity labels for estrogen binding proteins of rat uterus. *Biochemistry* 12:4085–4092, 1973

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On January 1, 1985, Thomas P. Haynie, M.D. will assume the Editorship of *The Journal of Nuclear Medicine.* To ensure a smooth transition of duties, it is necessary that Dr. Haynie initiate manuscript processing in September, 1984. Beginning September 15, 1984, all manuscripts, original submissions, and revisions, should be forwarded to:

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