## RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

# 16α-[$^{77}$Br]Bromo-11β-methoxyestradiol-17β: A Gamma-Emitting Estrogen Imaging Agent with High Uptake and Retention by Target Organs

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**16α-[$^{77}$Br]Bromo-11β-methoxyestradiol-17β [MBE(Br-77)], a compound with high affinity for the estrogen receptor and with low nonspecific binding, has been prepared with an effective specific activity of 770–1450 Ci per mmole at the time of synthesis. In immature female rats, this compound is taken up selectively by the uterus and is retained for prolonged periods. This is presumably due to the binding of this compound to the estrogen receptor, as uterine uptake is blocked selectively by coadministration of an excess of unlabeled estradiol, and administration of a chase dose of unlabeled estradiol results in a rapid decrease in activity in the uterus. In double-label experiments with 16α-[$^{125}$I]estradiol and MBE(Br-77), the two compounds showed equally selective uterine uptake at 1 hr, but the bromine-labeled compound became increasingly more selective at 3 and 6 hr. MBE(Br-77) may prove to be a more favorable agent for imaging human breast tumors than our previously described compound, 16α-[$^{77}$Br]bromoestradiol-17β.**

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Estrogens are taken up selectively and retained by estrogen target tissues and certain tumors derived from these tissues, by virtue of their binding with high affinity to the estrogen receptor present in these tissues (*1–7*). Because of this selective, receptor-mediated uptake, an estrogen labeled with a suitable gamma-emitting radionuclide might provide an imaging agent for target tissues, and in particular might be useful as an imaging agent for estrogen-receptor-positive tumors of the human breast (*8,9*). Such breast-tumor imaging agents would provide a new diagnostic tool, since they might permit the identification of both primary and mestastatic tumors and the determination of the extent of axillary lymph-node involvement noninvasively. They might also be useful for characterizing in vivo the dynamics of estrogen-receptor interaction in the tumor.

In an earlier publication (*10*) we outlined the chemical and biochemical characteristics required of a compound for it to be useful as an imaging agent for breast tumors, and we have reviewed earlier work in this area. We have recently described the synthesis of 16α-[$^{77}$Br]bromoestradiol-17β [BE(Br-77)] and its selective, receptor-mediated uptake by estrogen target tissues and by dimethylbenz(a)anthracene-induced mammary tumors in rats. We have also presented preliminary breast-tumor imaging studies in humans (*11–14*). In this report, we describe the preparation of a new analog, 16α-[$^{77}$Br]-bromo-11β-methoxyestradiol-17β MBE(Br-77)], that shows a more selective uptake and, in particular, a longer retention by estrogen target tissues in rats than the previously prepared compound. We also discuss the characteristics with which this gamma-emitting estrogen binds to the estrogen receptor and to nonreceptor proteins in relation to its behavior in vivo.

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## MATERIALS AND METHODS

The preparation of the enol acetate precursor (Compound 1) from 17,17-ethylenedioxyestra-1,3,5(10)-

triene-3,11β-diol has been described elsewhere (*12*), as have important details in the radiobromination (preparation of Compound 2) and hydride reduction (preparation of Compounds 3 and 4) (*12*). An abbreviated procedure is given below. All unlabeled halogenated estrogens were prepared as described previously (*12*). Bromine-77 was obtained as a spallation product (*15*).

High-pressure liquid chromatography was performed using a 10-μm silica-gel column (9 × 500 mm)* eluted isocratically with hexane/methylene chloride/2-propanol (85:12:3); the eluate was monitored with an ultraviolet detector at 254 nm and a sodium iodide scintillation detector. The identity of all peaks observed with the UV and radioactivity detectors was confirmed by coinjection with authentic samples.

All reactions were conducted under a nitrogen atmosphere in glass culture tubes with magnetic stirring, and solutions were transferred using a nitrogen-purged syringe. Organic extracts were dried by passage through a Pasteur pipette filled with 2–3 cm of anhydrous sodium sulfate, and solvents were removed under a gentle stream of nitrogen with a barrier to prevent the entrainment of air. As has been described previously (*12*), ascorbic acid (50 μl of a saturated solution in ethanol to each HPLC fraction) was added to the final purified sample of Compound 3(Br-77) to retard oxidative degradation. Radioactivity was determined using either a sodium iodide well counter or a dose calibrator.

**16α-[$^{77}$Br]Bromo-11β-methoxyestrone 3-Acetate (compound 2).** 11β-Methoxyestrone 3-acetate 17-enol acetate [Compound 1 (*12*), 50–200 μg, 12.5–50 μl of a 4 μg/μl stock solution in 3:2 THF-ether] was added to Na$^{77}$Br (3.5–30 mCi), previously evaporated to dryness under a stream of nitrogen and reconstituted in 100 μl acetate buffer (2.81 g KAc to 50 ml 85° HAc, freshly prepared), with magnetic stirring. To the solution was added premixed oxidant solution (150 μl of 2:1 v/v 30% hydrogen peroxide glacial acetic acid, mixed 1–2 hr before use and stored at room temperature). The course of the reaction was followed by periodic removal of a 1-μl aliquot, partitioning the aliquot between 1 ml each of ether and water, and counting each separated layer. After reaching *ca.* 80–90% organic incorporation (30–45 min), the reaction was quenched by the addition of 1.25 ml saturated $Na_2HPO_4$ solution. Ether (1 ml) was added to the mixture, and the organic phase was dried and concentrated to give, in 82–90% radiochemical yield, activity that comigrates with authentic Compound 2 by radio-TLC analysis (silica gel, 20% ethyl acetate in benzene).

**16α-[$^{77}$Br]Bromo-11β-methoxyestradiol-17β and -17α (compounds 3 and 4).** Radiobromoketone 2 (6–25 mCi) was taken up in THF (0.3 ml) under dry nitrogen and chilled to −78°C. A solution of lithium aluminum hydride in THF (0.5 *M*, 0.3 ml) was added very slowly, and after 10 min the reaction was quenched by the slow addition of 0.5 ml of 1:1 v/v THF-ethyl acetate (prechilled to −78°). After 5 min the reaction mixture was allowed to warm to 0°C, and 1.25 ml of a 10% aqueous hydrochloric acid solution was added. Extraction was done with ether-pentane to give a combined radiochemical yield of 82–91% for Compounds 3 and 4. HPLC separation gave Compound 3 as the second of the two radioactive peaks (Fig. 2). Compounds 3 and 4 were produced in a ratio of 1.4–3.1 to 1, and purified Compound 3 was obtained in an overall radiochemical yield of 50% from Compound 1. The HPLC elution solvents were immediately evaporated from the peak fractions containing Compound 3, and the fractions were redissolved in ethanol, combined, and stored at 0°. The identity of Compound 3 was confirmed by radio-TLC comparison with authentic, nonradioactive material. Its radiochemical purity was 96–98%, and it was free from chemical impurities detectable by UV analysis during the HPLC purification. The specific activity of Compound 3 ranged from 770–1450 Ci/mmole at time of synthesis, as determined by Scatchard analysis (cf. Fig. 4).

**In vitro studies.** Competitive-binding assays (cf. Fig. 3) were performed by a method described previously (*16*), using lamb-uterus cytosol as a source of receptor, 10 n*M* estradiol(H-3) as a tracer, and dextran-coated charcoal as an adsorbant for free tracer. Incubations contained 7% dimethylformamide to reduce nonspecific binding (*10,17*). Direct binding assays (cf. Fig. 4) were also done according to previously described methods (*12*).

The measurement of low-affinity, nonreceptor binding was done by equilibrium dialysis. Various concentrations of lamb-uterus cytosol (0.4 ml of solution of 0.1–5 mg protein per ml) were placed in dialysis bags made from 10-mm Spectrophor-4 tubing,† and bags were dialyzed together for ≥20 hr at 4°C against 3 μ*M* [$^3$H]estradiol, BE(Br-77), or MBE(Br-77) in a volume of buffer (10 m*M* Tris, 1.5 m*M* EDTA, 0.02% $Na_3N$, pH 7.4) ten times that of the total internal volume of the bags. The external solution and the bag contents were assayed in duplicate to determine the concentration of free and bound ligand (cf. Fig. 5).

**In vivo studies.** Immature female Sprague-Dawley rats (21–25 day, *ca.* 50 g) were used. Uterotropic assays were done according to previously described methods (*18*). For tissue uptake studies, animals under ether anesthesia were injected by jugular vein with *ca.* 0.1 ml of injection solutions that were prepared by dilution of an ethanol solution of the radiopharmaceutical MBE(Br-77) or 16α-[$^{125}$I]iodoestradiol-17β‡ [IE I-125] with 0.9% NaCl (final ethanol concentration ≤5%). At the indicated times, the animals were killed by decapitation, and samples of blood and tissues were weighed and assayed for radioactivity. In experiments to show the blocking

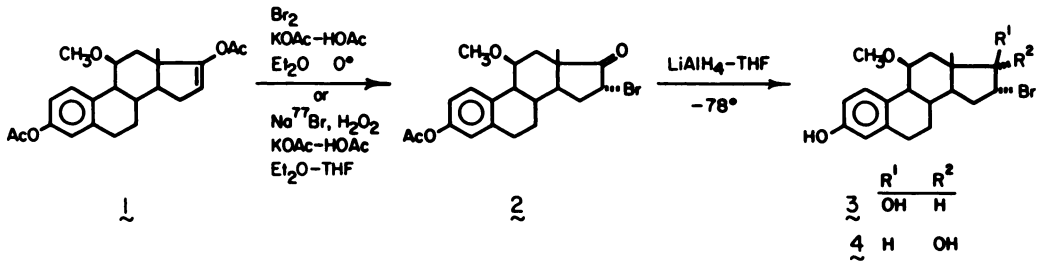


FIG. 1. Reaction scheme for the synthesis of 16α-[$^{77}$Br]Bromo-11β-methoxyestradiol-17β (Compound 3, MBE).

of uterine uptake, 18 μg of unlabeled estradiol was coinjected with the radiotracer; in the washout studies, 18 μg unlabeled estradiol was injected intraperitoneally at 1 hr after the radiotracer. Doses in this range are used to ensure that >95% of the estrogen receptor sites will become occupied by the unlabeled ligand.

Clearance of Compound 3 MBE(Br-77) was measured in a female pigtail monkey. The animal was injected intravenously with 1.0 mCi of the radiolabeled estrogen, and feces and urine were collected daily and assayed for radioactivity over a period of 144 hr.

## RESULTS

**Synthesis and radiosynthesis of 16α-bromo-11β-methoxyestradiol-17β (compound 3, MBE).** Compound 3 was prepared according to the scheme outlined in Fig. 1. In the preparation of nonradiolabeled material, 3,17-diacetoxy-11β-methoxyestra-1,3,5(10),16-tetraene (1) was treated with bromine in buffered acetic acid, furnishing the 16α-bromoketone acetate, 2, which, after reduction with lithium aluminum hydride at low temperature, gave a mixture of 16α-bromo-11β-methoxyestradiols epimeric at C-17 (3 and 4). These isomers were separated by column chromatography on silica gel (*12*).

The radiochemical synthesis followed the same course of reaction, except that the reaction was scaled down; bromination was accomplished by in situ oxidation of sodium [$^{77}$Br]bromide with hydrogen peroxide in acetic acid, and the final separation was achieved by HPLC. A radiochromatogram illustrating the separation of the epimeric bromoestradiols is presented in Fig. 2. The synthesis of the enol acetate prescursor, 1, and details concerning the synthesis and radiosynthesis of MBE(Br-77) have been discussed in a recent publication (*12*).

**Studies of estrogen-receptor binding.** The interaction of MBE with the estrogen receptor can be demonstrated by binding assays. Competitive binding curves for MBE and several analogs for the estrogen receptor from lamb uterus are shown in Fig. 3. The introduction of bromine at the 16α position increases the affinity of both compounds for receptor (*19*); the 11β-methoxy group reduces receptor affinity several fold (*20*).

High-affinity binding of MBE(Br-77) to the estrogen receptor can be demonstrated directly, and a Scatchard plot is shown in Fig. 4A. By this assay, the affinity of MBE for the receptor appears to be comparable to that of estradiol. It is also possible to determine the effective specific activity of MBE(Br-77) by comparing the maximum high-affinity binding observed in this assay

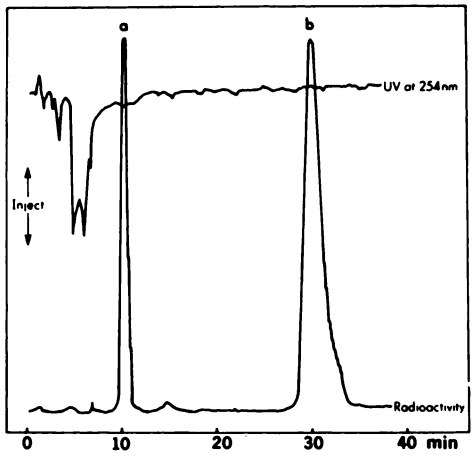


FIG. 2. HPLC purification of MBE. Epimeric products from the radiochemical synthesis (Compounds 3 and 4) were separated by HPLC on a silica-gel column. Compound 4 elutes at 10 (peak a) min and Compound 3 at 30 min (peak b).

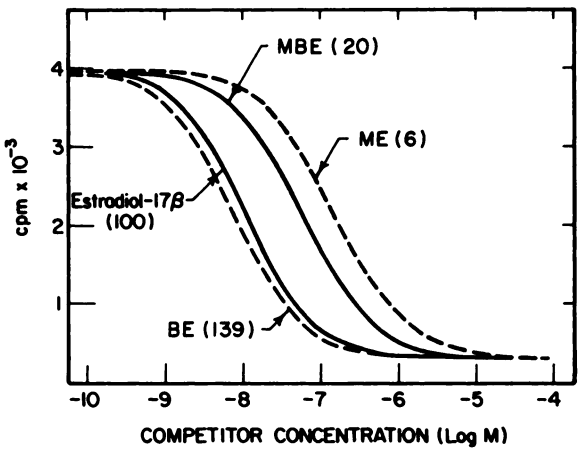


FIG. 3. Competitive-binding assay of estrogen analogs. Binding affinities of 16α-bromoestradiol (BE), 11β-methoxyestradiol (ME), and 16α-bromo-11β-methoxyestradiol (MBE) were measured relative to that of estradiol by a competitive binding assay using [$^{3}$H]estradiol. Affinities, expressed as percentages of binding of estradiol (considered to be 100%), are shown in parentheses.

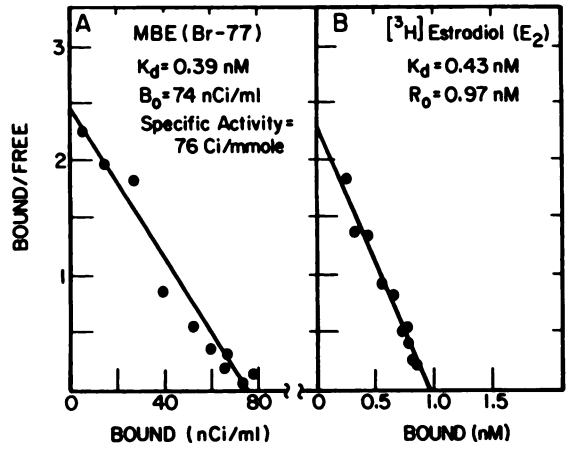


FIG. 4. High-affinity binding of MBE(Br-77) (Panel A) and [$^3$H]estradiol (Panel B). Binding was determined in lamb-uterus cytosol using dextran-coated charcoal. Correction has been made for nonspecific binding.

($B_0$, Fig. 4A) with the receptor-site concentration measured in a parallel assay ($R_0$, Fig. 4B), utilizing tritium-labeled estradiol of known specific activity (*12*). In this case, the effective specific activity of MBE(Br-77) was determined to be 76 Ci per mmole. As this measurement was made 3.5 half-lives after the preparation of the compound, the specific activity extrapolates back to 840 Ci per mmole at the time of synthesis of MBE(Br-77). Effective specific activities at the time of synthesis have typically ranged from 770 to 1450 Ci per mmole. The term "effective" specific activity is used to indicate that the receptor-binding assay is measuring all receptor-binding substances, which may include certain by-products from the preparation of MBC(Br-77). In biochemical and medical uses of this receptor-binding

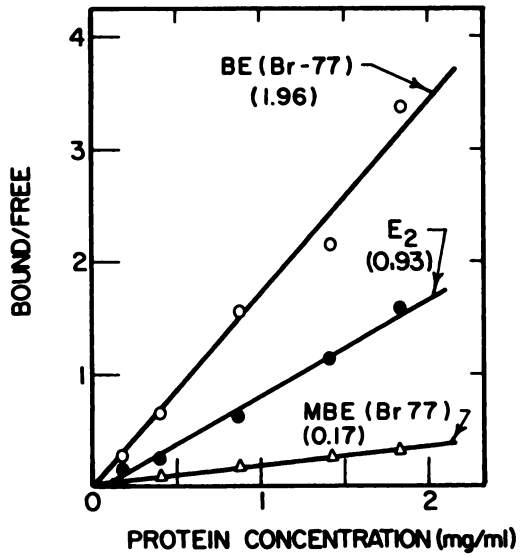


FIG. 5. Low affinity, nonreceptor binding (nonspecific binding) of BE(Br-77), [$^3$H]estradiol ($E_2$), and MBE(Br-77). Binding was measured by equilibrium dialysis in lamb-uterus cytosol with compounds at low specific activity. Numbers in parentheses are binding indices (expressed in ml per mg protein).

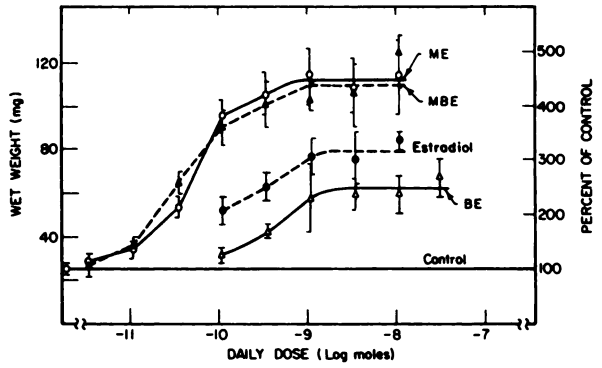


FIG. 6. Uterotrophic activity of estradiol, 11$\beta$-methoxyestradiol (ME), 16$\alpha$-bromoestradiol (BE), and Compound 3 (MBE). Immature rats (four animals per group) received three daily injections of indicated doses, and uterine weights were determined 24 hr after last dose.

radiopharmaceutical, however, it is effective specific activity that is important rather than true specific activity.

It is also possible to measure by equilibrium dialysis (*10*) the extent to which MBE(Br-77) binds to low-affinity, nonreceptor (i.e., nonspecific) binding sites in uterine cytosol preparations. It is evident from the results presented in Fig. 5 that the nonspecific binding of this compound is very low (*20*): only 18% that of estradiol itself and 9% that of 16$\alpha$-bromoestradiol.

**Physiological and pharmacological activity of MBE.** The activities of MBE and several related estrogens in a standard 3-day uterine weight-gain assay are shown in Fig. 6. It is evident that MBE and 11$\beta$-methoxyestradiol are more potent and effective uterotropic agents than estradiol (*20*); 16$\alpha$-bromoestradiol is somewhat less potent than estradiol. The enhanced potency of the 11$\beta$-methoxy-substituted compounds is thought to arise from their prolonged retention in the uterus (*vide infra* and Refs. *20* and *21*).

The clearance of MBE(Br-77) from a female monkey is shown in Fig. 7. This compound is eliminated almost

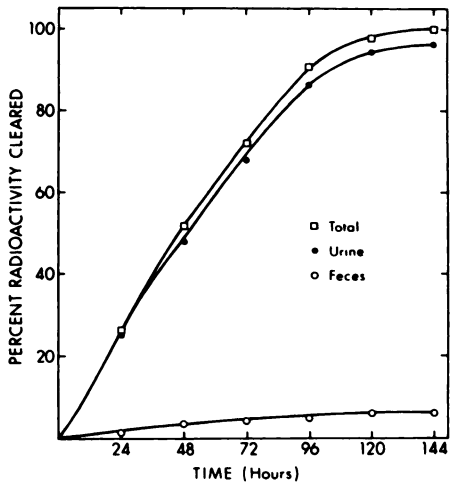


FIG. 7. Clearance of MBE(Br-77) in a female pigtail monkey.

**TABLE 1. TISSUE-TO-BLOOD RATIO OF 16α-[$^{77}$Br]BROMO-11β-METHOXYESTRADIOL-17β IN IMMATURE FEMALE RATS (MEAN ± s.d. FOR ≥5 RATS)**

| | Tissue-to-blood ratio | | | | | |
|---|---|---|---|---|---|---|
| | 1 hr | 1 hr* | 3 hr | 6 hr | 24 hr | 48 hr |
| Blood | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ovaries | 5.87± 1.20 | 1.20 ± 0.22 | 4.79 ± 1.30 | 4.34 ± 0.79 | 3.20 ± 0.79 | 1.92 ± 0.76 |
| Uterus | 19.7 ± 3.5 | 2.57 ± 1.13 | 16.5 ± 5.7 | 16.2 ± 4.0 | 16.0 ± 2.4 | 10.9 ± 8.0 |
| Muscle | 0.77 ± 0.17 | 0.67 ± 0.38 | 0.40 ± 0.08 | 0.39 ± 0.07 | 0.37 ± 0.10 | 0.20 ± 0.16 |
| Spleen | 0.84 ± 0.15 | 0.59 ± 0.16 | 0.58 ± 0.07 | 0.59 ± 0.04 | 0.62 ± 0.10 | 0.53 ± 0.09 |
| Kidney | 3.21 ± 0.59 | 1.09 ± 0.12 | 1.99 ± 0.50 | 1.47 ± 0.19 | 1.07 ± 0.25 | 0.70 ± 0.08 |
| Liver | 4.21 ± 1.29 | 2.46 ± 0.24 | 2.60 ± 1.16 | 1.70 ± 0.79 | 0.67 ± 0.12 | 0.53 ± 0.06 |
| Esophagus | 0.88 ± 0.36 | 0.87 ± 0.15 | 0.70 ± 0.15 | 0.74 ± 0.20 | 0.77 ± 0.19 | 0.42 ± 0.14 |
| Lung | 1.22 ± 0.10 | 0.97 ± 0.30 | 0.96 ± 0.15 | 0.92 ± 0.06 | 0.97 ± 0.15 | 0.78 ± 0.01 |

* +18 μg unlabeled estradiol.

exclusively by a urinary route, in contrast to 16α-bromoestradiol, which was excreted equally by urinary and fecal routes (*14*).

**Tissue uptake selectivity in immature rats.** Immature female rats were injected intravenously with 2–20 μCi of MBE(Br-77) (40–400 pmole/kg), and at various times they were killed and tissue samples were removed and assayed for radioactivity. These data are expressed as organ-to-blood ratios (Table 1) or percent injected dose per gram (Table 2). The uptake of this compound shows a striking selectivity for the uterus, as is evident by the high percentage of uptake by this tissue and by the high tissue-to-blood ratios. The high effective specific activity of this bromo-estrogen was also evident from the fact that equally selective uterine uptake was observed after administration of a 2- or a 20-μCi dose to immature rats (data not shown).

As expected for tissue uptake mediated by a high-affinity saturable binder (presumed to be the estrogen receptor), the uptake by the uterus could be blocked very effectively by coadministration of 18 μg of unlabeled estradiol—enough to occupy fully the receptor sites in the uterus. By such treatment, uptake into the uterus was depressed nearly to the level of that in blood and nontarget tissues. Aside from the uterus, only the ovaries showed any indication of receptor-mediated uptake; all the other tissues showed equivalent uptake in the absence and presence of the displacing dose of unlabeled estradiol.

The selective uterine uptake of MBE(Br-77) and its pronounced retention in the uterus are particularly striking from the data presented in Fig. 8, in which the activity in the uterus is presented as a function of time in Panel A as a percent of the injected dose, or in Panel B as a ratio to the average activity in four nontarget tissues. It is remarkable that the uterus-to-nontarget tissue ratio (Panel B) remains at or above 20 for the period from 1 to 48 hr. For comparison, the uterine uptake of BE(Br-77) is shown on the same figure. It is clear that the latter compound is taken up somewhat less efficiently by the uterus, and that its retention is less pronounced.

**TABLE 2. TISSUE DISTRIBUTION OF 16α-[$^{77}$Br]BROMO-11β-METHOXYESTRADIOL-17β IN IMMATURE FEMALE RATS (MEAN ± s.d. FOR ≥5 RATS)**

| | % injected dose per gram | | | | | |
|---|---|---|---|---|---|---|
| | 1 hr | 1 hr* | 3 hr | 6 hr | 24 hr | 48 hr |
| Blood | 0.59 ± 0.07 | 0.65 ± 0.21 | 0.69 ± 0.08 | 0.54 ± 0.13 | 0.46 ± 0.24 | 0.28 ± 0.02 |
| Ovaries | 3.69 ± 0.71 | 1.78 ± 1.24 | 3.36 ± 0.57 | 2.26 ± 0.38 | 1.23 ± 0.51 | 0.53 ± 0.17 |
| Uterus | 12.3 ± 1.84 | 2.97 ± 1.32 | 12.4 ± 3.5 | 8.42 ± 1.80 | 6.89 ± 4.02 | 2.97 ± 1.96 |
| Muscle | 0.48 ± 0.11 | 0.48 ± 0.10 | 0.29 ± 0.06 | 0.21 ± 0.06 | 0.14 ± 0.02 | 0.06 ± 0.05 |
| Spleen | 0.52 ± 0.10 | 0.57 ± 0.15 | 0.37 ± 0.06 | 0.31 ± 0.09 | 0.25 ± 0.10 | 0.15 ± 0.03 |
| Kidney | 2.02 ± 0.31 | 1.05 ± 0.11 | 1.43 ± 0.32 | 0.81 ± 0.21 | 0.46 ± 0.10 | 0.20 ± 0.01 |
| Liver | 2.52 ± 0.54 | 2.38 ± 0.23 | 1.95 ± 0.65 | 0.86 ± 0.27 | 0.27 ± 0.10 | 0.15 ± 0.01 |
| Esophagus | 0.56 ± 0.17 | 0.84 ± 0.14 | 0.54 ± 0.10 | 0.38 ± 0.11 | 0.32 ± 0.06 | 0.12 ± 0.03 |
| Lung | 0.78 ± 0.17 | 0.82 ± 0.16 | 0.68 ± 0.05 | 0.50 ± 0.14 | 0.41 ± 0.15 | 0.21 ± 0.04 |

* +18 μg unlabeled estradiol.

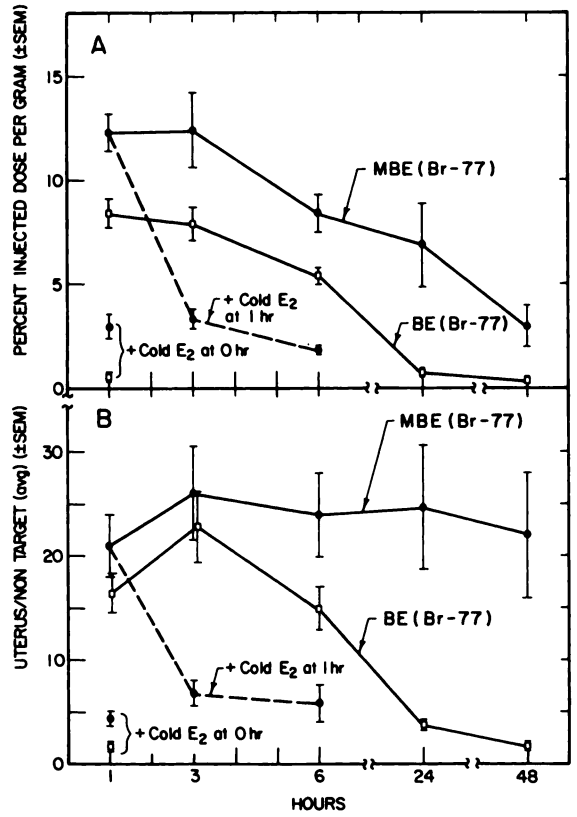


**FIG. 8.** Time course of uterine activity after administration of MBE(Br-77) or BE(Br-77) to immature rats. Data are expressed as percent of injected dose per gram uterus (Panel A) or ratio of uterine activity to average activity in four nontarget tissues (muscle, lung, spleen, esophagus). Low points at 1 hr are from animals that received a blocking dose of unlabeled estradiol ($E_2$) at 0 hr; dashed lines show data from animals that received a chase dose of unlabeled estradiol ($E_2$) at 1 hr.

A process that may be responsible for the retention of a receptor-binding radiopharmaceutical by a target tissue is ligand "recapture"—the phenomenon by which a dissociated ligand fails to escape the target tissue because it becomes bound again by available, unoccupied receptor sites before escape. Thus, one would expect that the loss of a receptor-binding radiopharmaceutical from a target tissue would be accelerated by an excess of an unlabeled ligand administered after the phase of initial uptake. Such a washout experiment is also presented in Fig. 8. When an excess of unlabeled estradiol is injected into rats 1 hr after the initial dose of MBE(Br-77), activity in the uterus decreases rapidly; when washout is complete (at 6 hr), the uterine content of MBE(Br-77) reaches the level observed in animals in which initial uptake was blocked by pretreatment with unlabeled estradiol.

We have recently discussed (*13*) how complications can arise in attempts to make quantitative comparisons between different receptor-binding radiopharmaceuticals because of physiological differences between individual animals. We also suggested that such comparison could be made more reliably by measuring uptake relative to an internal standard. In this study, as before, we have used commercial 16$\alpha$-[$^{125}$I]-iodoestradiol-17$\beta$ [IE(I-125)] (*22,23*) as an internal standard, and in Tables 3 and 4 we have made a comparison of the relative uptake of the two radiopharmaceuticals in terms of the uterus-to-blood or uterus-to-nontarget tissue ratio or the percent injected dose per gram. Paired-sample t-tests were used to compare the uterine uptake of MBE(Br-77) with that of the IE(I-125) in individual animals. The results of this analysis to determine the significance of any difference in uptake of the two radiolabeled compounds are shown in Table 4. Whereas the uterine uptake ratios of the two radiopharmaceuticals are not significantly different at 1 hr, at 3 and 6 hr MBE(Br-77) demonstrates a significant and increasingly greater selectivity for the uterus in terms of uterus-to-blood ratio and percent injected dose per gram.

In these studies we also determined the uptake of radioactivity in the thyroid after injection of MBE(Br-77) and IE(I-125) (Table 3). From these data it is evident that some iodide is being released from IE(I-125); it is accumulated by the thyroid progressively with time and can reach high levels. On the other hand, there is no accumulation of radiobromide by the thyroid, which is in accord with the known absence of a bromide-concentrating mechanism in this organ (*24*).

**Dosimetry.** The radiation absorbed doses to a human resulting from intravenous administration of MBE(Br-77) were determined from tissue-distribution data, obtained in immature female rats, and the MIRD tables (*26*). Table 5 shows the results of these calculations. The primary critical organ is the upper large intestine, which receives 0.87 rad/mCi injected, followed by the small intestine, with 0.52 rad/mCi. These doses are slightly higher than those reported for BE(Br-77) (*14*).

Because the intestine receives the greatest radiation dose, we examined the chemical nature of the radioactivity in this organ at 1 and 6 hr after injection (three animals at each time), by ether-water partitioning and charcoal adsorption of extracts of intestinal wall and intestinal contents. At 1 hr >50% of the intestinal activity is in the contents, and at 6 hr >90%. At both times, less than 10% of the activity in the intestinal contents is unmetabolized compound, but only 6.8 ± 2.9% at 1 hr and 7.1 ± 2.2% at 6 hr appears to be free bromide. There is even less free bromide in the intestinal walls. Therefore, most of the activity in the intestine is in the contents and is due to polar metabolites and conjugates rather than to free bromide.

## DISCUSSION

In this study we have reported the synthesis of MBE(Br-77), a gamma-emitting estrogen that can be prepared conveniently and in high specific activity by a

**TABLE 3. UTERINE AND THYROID UPTAKE OF 16α[$^{77}$Br]BROMO-11β-METHOXYESTRADIOL-17β (COMPOUND 3) AND 16α-[$^{125}$I]IODOESTRADIOL-17β IN IMMATURE FEMALE RATS (MEAN ± s.d. FOR 5 RATS)**

| | Uterus-to-blood ratio | | Uterus-to-nontarget ratio* | | % I.D. per gram uterus | |
|---|---|---|---|---|---|---|
| | Br-77 | I-125 | Br-77 | I-125 | Br-77 | I-125 |
| 1 hr | 18.2 ± 3.6 | 12.1 ± 4.4 | 19.8 ± 1.8 | 15.8 ± 6.8 | 12.5 ± 2.2 | 10.9 ± 2.0 |
| 1 hr† | 2.57 ± 1.13 | 1.44 ± 0.43 | 4.00 ± 2.26 | 2.06 ± 0.51 | 2.97 ± 1.31 | 1.25 ± 0.37 |
| 3 hr | 18.6 ± 4.1 | 10.4 ± 3.2 | 27.2 ± 6.7 | 19.5 ± 7.6 | 12.5 ± 2.9 | 6.87 ± 1.51 |
| 6 hr | 17.6 ± 4.7 | 8.30 ± 3.56 | 26.0 ± 5.7 | 18.1 ± 3.4 | 8.28 ± 1.74 | 1.74 ± 0.51 |

| | Thyroid‡-to-blood ratio | | % I.D. per gram thyroid | |
|---|---|---|---|---|
| | Br-77 | I-125 | Br-77 | I-125 |
| 1 hr | 1.37 ± 0.38 | 73.7 ± 23.9 | 0.31 ± 0.08 | 9.92 ± 2.80 |
| 1 hr† | 1.51 ± 0.67 | 62.9 ± 38.6 | 0.21 ± 0.05 | 4.51 ± 1.94 |
| 3 hr | 0.92 ± 0.12 | 114.3 ± 26.5 | 0.21 ± 0.04 | 16.2 ± 6.1 |
| 6 hr | 0.49 ± 0.05 | 444.4 ± 218 | 0.08 ± 0.02 | 29.3 ± 10.1 |

* Nontarget uptake is average of uptake in lung, muscle, spleen, and esophagus.

† +18 μg unlabeled estradiol coinjected.

‡ Thyroid weight is taken to be 5 mg per 100 g body weight (*25*).

route that parallels our earlier preparation of 16α-[$^{77}$Br]bromoestradiol-17β [BE(Br-77)]. This compound has an affinity for the estrogen receptor comparable with, or somewhat less than, that of estradiol, but has very low nonspecific binding. It is a more potent uterotropic agent than estradiol.

As was the case with our earlier-described agent, BE(Br-77), MBE(Br-77) shows selective uptake by the uterus (and to a lesser extent ovaries) in immature rats, uptake that can be blocked selectively by coadministration of a saturating dose of unlabeled estradiol. The activity in the uterus appears to be retained by virtue of a recapture process involving a high-affinity binder, since subsequent treatment with a displacing dose of estradiol leads to rapid washout of activity.

One of the most striking features of this compound is its very long retention by the uterus. In fact, even after 48 hr, uterus-to-blood ratios are greater than 20:1, and at 24 hr the percent of injected dose in the uterus is still more than half of that at 1 hr. This long-term retention by the uterus also can account for the greater uterotropic potency of this compound compared with estradiol, since it is known that effective stimulation of late uterine responses to estrogen, such as tissue growth, requires relatively prolonged occupancy of nuclear estrogen-receptor sites (*21*). The molecular basis of the prolonged uterine retention of MBE is probably the result of its very low affinity for nonreceptor binding proteins. Because its interaction with the nonreceptor binding proteins in

**TABLE 4. IN VIVO COMPARISON OF THE UTERINE UPTAKE OF 16α-[$^{77}$Br]BROMO-11β-METHOXYESTRADIOL-17β AND 16α-[$^{125}$I]-IODOESTRADIOL-17β IN IMMATURE FEMALE RATS (MEAN ± s.d. FOR 5 RATS)**

| | Ratio of Br-77 to I-125 | | |
|---|---|---|---|
| | Uterus-to-blood ratio | Uterus-to-non-target ratio | % I.D. per gram uterus |
| 1 hr | 1.71 ± 0.72 | 1.81 ± 0.90 | 1.16 ± 0.20 |
| | (p = 0.010)† | (p = 0.141) | (p = 0.135) |
| 1 hr* | 2.31 ± 0.95 | 2.48 ± 0.97 | 2.57 ± 1.05 |
| | (p = 0.021) | (p = 0.044) | (p = 0.017) |
| 3 hr | 2.26 ± 0.88 | 1.97 ± 1.30 | 2.03 ± 0.45 |
| | (p = 0.029) | (p = 0.133) | (p = 0.006) |
| 6 hr | 2.38 ± 0.95 | 1.84 ± 0.69 | 5.54 ± 2.27 |
| | (p = 0.008) | (p = 0.007) | (p = 0.001) |

* +18 μg unlabeled estradiol.

† p values determined using paired t-test.

**TABLE 5. RADIATION ABSORBED DOSES FOR 16α-[$^{77}$Br]BROMO-11β-METHOXYESTRADIOL-17β**

| Target organ | rads/mCi |
|---|---|
| Stomach | 0.13 |
| Small intestine | 0.52 |
| Upper large intestine | 0.87 |
| Lower large intestine | 0.25 |
| Kidneys | 0.11 |
| Liver | 0.08 |
| Lungs | 0.02 |
| Ovaries | 0.31 |
| Spleen | 0.06 |
| Uterus | 0.22 |
| Whole body | 0.05 |

tissues and blood is so low, there is no binding system (e.g., blood binders) that competes effectively with the binding of MBE to the estrogen receptor, once it has been taken up by the uterus. In this regard, MBE differs from 16$\alpha$-bromoestradiol (BE), which has greater nonreceptor binding than estradiol and is thus lost from the uterus at a greater rate than MBE. The high ratio of receptor-to-nonreceptor binding affinity of MBE may also account for its more efficient uptake by the uterus, compared with BE since one would expect that the fraction extracted by the uterus would increase for a compound with an increased ratio of receptor-binding affinity (i.e., binding by target tissue) to nonreceptor binding affinity (i.e., binding in blood).

In comparing uptake selectivities, we have used ratios based on total radioactivity in the uterus over that in blood or in nontarget tissues; these ratios, however, may not represent the actual ratios of concentration of the administered compound in the different tissues because of differential metabolism. We have done preliminary studies on the metabolism of these bromo-estrogens by thin-layer chromatography, and have found that the ratio of compound concentration is, in fact, much greater than the ratio of radioactivity. For example, at 2 hr the percentages of MBE(Br-77) and BE(Br-77) that are unmetabolized are ~90% in the uterus, 40–70% in the lung and spleen, and ~10% in the blood. Thus, relative to the ratio of radioactivity, the ratio of compound concentration would be 1.3–2.3 times higher for uterus to nontarget tissues, and 9 times higher for uterus to blood.

Because of the accumulation of metabolites in nontarget tissues—and especially in blood—the most reliable comparisons between compounds can be made on the basis of total radioactivity in the uterus, since even at later times this represents largely unmetabolized radiopharmaceutical. Thus, in comparing MBE with IE, it is best to consider for each compound the percent injected dose per gram of uterus. On the basis of such a comparison, MBE appears to have a uterine uptake selectivity equivalent to that of IE at 1 hr, whereas at 3 and 6 hr, the selectivities are 2 and 5.5 times as high, owing to the prolonged target-tissue retention.

In conclusion, we have described the preparation of MBE(Br-77) with high effective specific activity, and we have shown that this compound is accumulated selectively by estrogen target tissues in the rat and is retained by these tissues for long periods of time. Both with respect to the selectivity of target-tissue accumulation and the duration of this retention, this compound appears to be superior to BE(Br-77), a compound we have described earlier (*11*,*13*), which suggests that the former may prove to be still more useful as an agent for imaging human breast tumors or metastases that contain estrogen receptors, and for providing a dynamic assay of estrogen uptake by these tumors in vivo.

## FOOTNOTES

* Alltech Corp, Chicago, IL.
† Spectrum Medical Industries, Los Angeles, CA.
‡ New England Nuclear Corp., Boston, MA.

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