over a 2-h period. The reaction was quenched by cautious addition of EtOAc. Pentane and 5% HCl were added, the layers were separated, and the organic layer was dried ($MgSO_4$). Solvent removal and recrystallization from benzene gave 44 mg (83%) of norhexestrol (37) as white needles: mp 183.5 °C; IR 3460 (OH) cm^{-1}; ^1H NMR (acetone-d_6) δ 0.98 (d, 3 H, $CHCH_3$); mass spectrum (70 eV), m/z (relative intensity) 257 (2, M^+), 135 (100). Anal. ($C_{17}H_{20}O_2$) C, H.

Binding Affinity to the Uterine Estrogen Receptor. The determination of the binding affinity of these derivatives to the estrogen receptor in cytosol preparations from lamb uterus was measured in a competitive binding assay with [^{3}H]estradiol as a tracer and charcoal–dextran as an adsorbant of free ligand. In earlier competitive binding studies, receptor preparations from both rat[6] and lamb[7] uterus have been used with essentially equivalent results. The full details of this method have been described.[6]

Acknowledgment. This work was supported by a grant from the National Institutes of Health (PHS 5R01 CA 25836). We are grateful to Kathryn Carlson for her assistance and to Joontaik Park for the preparation of compounds **12**, **14**, **16**, and **18**. The high-resolution mass spectrometry equipment used in this study was provided by a grant from the National Cancer Institute (CA 11388).

(2*R**,3*S**)-1-[^{125}I]Iodo-2,3-bis(4-hydroxyphenyl)pentane ([^{125}I]Iodonorhexestrol) and (2*R**,3*S**)-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

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Two γ-emitting estrogen analogues, (2*R**,3*S**)-1-[^{125}I]iodo-2,3-bis(4-hydroxyphenyl)pentane ([^{125}I]iodonorhexestrol) and (2*R**,3*S**)-1-[^{77}Br]bromo-2,3-bis(4-hydroxyphenyl)pentane ([^{77}Br]bromonorhexestrol), have been prepared by halide ion displacement on a labile trifluoromethanesulfonate derivative of a suitably protected precursor, followed by mild acid deprotection. Although halide displacement on a more stable tristrifluoromethanesulfonate derivative was successful, the basic conditions required for deprotection of this precursor resulted in destruction of the products by a base-induced spiroelimination reaction. In immature female rats, both of these halonorhexestrols demonstrated preferential uptake by the uterus that could be blocked selectively by coadministration of a large dose of unlabeled estradiol. In a double label comparison with 16α-[^{125}I]iodo-17β-estradiol the uterine uptake of [^{77}Br]bromonorhexestrol was notably less selective. Stability studies in vitro and in vivo have indicated that both iodo- and bromonorhexestrol are quite labile, and this lability compromises the selectivity of their uptake by estrogen target tissues in vivo. *p*-Hydroxyphenethyl halides are known to be unusually prone to a base-catalyzed solvolysis, via cyclization of the phenolate to a spirocyclohexadienone intermediate. This unusual solvolytic mechanism may contribute to the lability of these halonorhexestrols in vivo.

The estrogen receptor, a specific, high-affinity binding protein present in estrogen-sensitive tissues, is thought to be the principal mediator of estrogen action. A large portion of human breast tumors are also found to have significant levels of estrogen receptor.[1] The measurement of estrogen receptor levels in these tumors is of vital importance, since tumor receptor content has provided a resonable basis for selecting the most appropriate therapeutic approach for managing the progress of breast cancer.[2,3]

Through selective uptake mediated by the estrogen receptor, estrogens at physiological concentrations are known to be concentrated in target tissues several-fold over nontarget tissues.[4–7] Thus, selective localization of a γ-emitting estrogen in a receptor-positive breast tumor should allow its receptor content to be assayed noninvasively. Similarly, this selective uptake would provide a means of detecting primary and metastatic tumors.

The achievement of a high uptake selectivity with such a radiopharmaceutical reagent is predicated upon an estrogen that has high affinity for estrogen receptor, low binding to other estrogen-specific binding proteins, reasonable metabolic stability, and high specific activity.[8] We have recently synthesized two γ-emitting steroidal estrogens, 16α-[^{77}Br]bromo-17β-estradiol[9] and 16α-[^{77}Br]-bromo-11β-methoxy-17β-estradiol,[10] which satisfy these requirements. We are also exploring the use of non-

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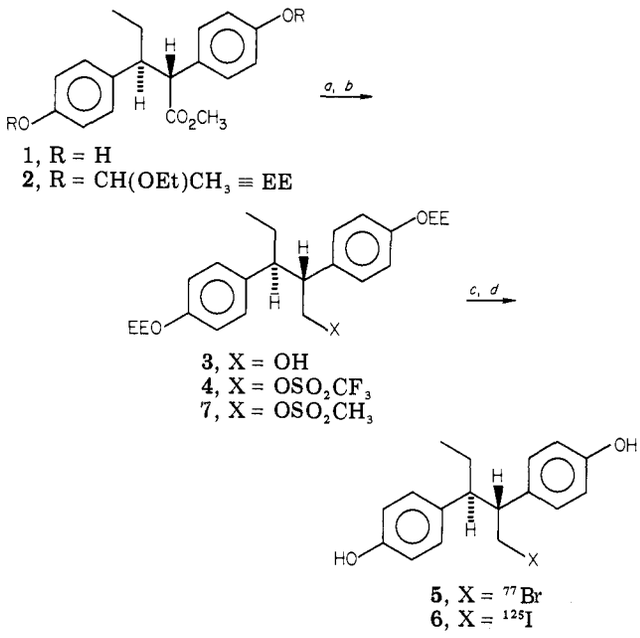
Scheme I



[a] $LiAlH_4$, NaOMe, −78 °C (95%). [b] $(CF_3SO_2)_2O$, 2,6-lutidine, −78 °C (84%). [c] NaX. [d] Dowex 50W-X8.

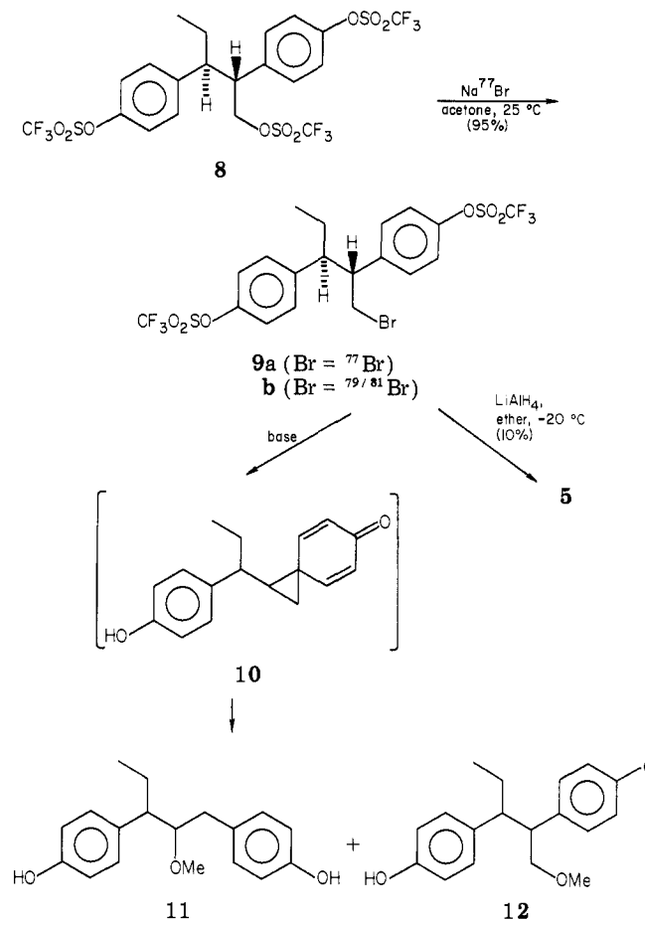
steroidal estrogens based on hexestrol as potential breast tumor imaging agents.[11,12] In this paper we describe the synthesis of two such derivatives, (2*R**,3*S**)-1-[^{125}I]iodo-2,3-bis(4-hydroxyphenyl)pentane (1-[^{125}I]iodonorhexestrol, **6**) and (2*R**,3*S**)-1-[^{77}Br]bromo-2,3-bis(4-hydroxyphenyl)pentane (1-[^{77}Br]bromonorhexestrol, **5**), and we present studies on their in vivo distribution and their stability.

Results

Chemical Synthesis. The precursor for both the 1-bromo- and 1-iodonorhexestrols (**5** and **6**) is the bis(ethoxyethyl ether) protected norhexestrol trifluoromethanesulfonate (triflate) **4**. The alcohol (**3**) used in the preparation of this triflate is obtained from the norhexestrol acid methyl ester (**1**)[13] by reaction with ethyl vinyl ether, followed by reduction with lithium aluminum hydride (Scheme I). The inclusion of sodium methoxide in the latter reaction is essential to avoid loss of the acetal protecting group that is catalyzed by traces of trivalent aluminum impurities.[14]

Treatment of **3** with trifluoromethanesulfonic anhydride and 2,6-lutidine gives the desired triflate (**4**). This material is extremely unstable and decomposes within minutes at room temperature; however, it may be stored for several days at −78 °C. The triflate reacts with no-carrier-added sodium [^{77}Br]bromide in acetone at 0 °C, giving a 60% radiochemical incorporation within 2 h. Cleavage of the ethoxyethyl ether groups with Dowex 50W-X8 in methanol, followed by HPLC purification, furnishes the desired 1-[^{77}Br]bromonorhexestrol (**5**) in 41% overall radiochemical yield. The synthesis of the corresponding iodo compound (**6**), starting from no-carrier-added sodium [^{125}I]-iodide, is achieved by the same method in 25% overall radiochemical yield. The γ-emitting halonorhexestrols appear to have high effective specific activities: ≥1600 Ci/mmol for **5** and ≥130 Ci/mmol for **6**.[15]

Scheme II



In earlier investigations, we prepared the ethoxyethyl ether protected norhexestrol methanesulfonate (**7**). Although this substance is much more stable than the corresponding triflate and can be used to prepare the bromo- and iodonorhexestrols with the unlabeled halides on a milligram scale, it fails to give significant incorporation on the no-carrier-added scale.

Another precursor, the tris(triflate) **8**, appeared to be attractive because it embodies the highly reactive aliphatic triflate, while the phenolic rings are deactivated as the triflate esters. In fact, the tris(triflate) is quite a stable species, and displacement with no-carrier-added sodium [^{77}Br]bromide is extremely facile, the [^{77}Br]bromobis(triflate) **9a** being obtained in 95% radiochemical yield after 30 min at room temperature (Scheme II). Unfortunately, the usual mode of triflate hydrolysis is incompatible with our system, since treatment of unlabeled bromobis(triflate) **9b** with potassium carbonate in methanol results in formation of methyl ethers **11** and **12**, with **11** being the major product. These methyl ethers presumably arise from phenoxide ion formation, followed by intramolecular elimination of bromide, to give the spirocyclohexadienone intermediate **10**. Attack by methanol on the cyclopropane ring of this intermediate gives the

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(15) Determined by Scatchard analysis. See Experimental Section and ref 10 for details.

Table I. Tissue Distribution of [^{125}I]Iodonorhexestrol (6) in Immature Female Rats (Mean ± SD)[a]

tissue	tissue to blood ratio		% inject. dose/g[b]	
	1 h	1 h[c]	1 h	1 h[c]
blood	1.00	1.00	1.3 ± 0.2	1.5 ± 0.1
uterus	11.3 ± 5.8	3.3 ± 0.8	15.7 ± 10.2	4.9 ± 0.6
ovaries	3.7 ± 0.7	3.2 ± 0.3	4.8 ± 0.6	4.9 ± 0.9
muscle	1.2 ± 0.3	1.9 ± 1.3	1.6 ± 0.3	2.7 ± 1.7
liver	1.0 ± 0.6	1.6 ± 0.0	2.0 ± 0.4	2.5 ± 0.3
spleen	0.9 ± 0.2	0.9 ± 0.1	1.2 ± 0.3	1.3 ± 0.0
kidney	1.4 ± 0.3	1.2 ± 0.1	1.8 ± 0.3	1.9 ± 0.1
esophagus	6.3 ± 4.9	3.6 ± 0.8	8.3 ± 6.6	5.4 ± 0.6
lung	1.1 ± 0.3	1.2 ± 0.1	1.4 ± 0.4	1.8 ± 0.1
thyroid[c]	494.8 ± 191.2	278.4 ± 28.4	619.0 ± 223.6	474.5 ± 97.7

[a] Eight animals were used in this study; five with compound 6, three with 6 plus estradiol. [b] Percent injected dose per gram tissue. [c] Unlabeled estradiol (9 μg) was coinjected in this experiment. [d] A thyroid weight of 0.005 g per 100 g of body weight was assumed for each animal (see ref 17).

Table II. Tissue Distribution of [^{77}Br]Bromonorhexestrol (5) and 16α-[^{125}I]Iodo-17β-estradiol in Immature Female Rats

tissue	tissue to blood ratio (mean ± SD)[a]							
	1 h		1 h[b]		3 h		6 h	
	^{77}Br	^{125}I	^{77}Br	^{125}I	^{77}Br	^{125}I	^{77}Br	^{125}I
blood	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
uterus	4.49 ± 0.98	9.54 ± 4.99	0.98 ± 0.09	1.37 ± 0.49	2.86 ± 0.60	7.36 ± 2.61	2.51 ± 0.55	9.14 ± 3.35
ovaries	1.98 ± 0.44	3.02 ± 1.66	0.74 ± 0.18	1.36 ± 0.67	1.23 ± 0.30	2.03 ± 0.51	1.41 ± 0.79	2.49 ± 1.25
muscle	0.48 ± 0.17	0.42 ± 0.03	0.31 ± 0.03	0.50 ± 0.22	0.29 ± 0.04	0.25 ± 0.06	0.26 ± 0.04	0.27 ± 0.04
liver	1.69 ± 0.06	3.55 ± 0.31	1.51 ± 0.25	3.95 ± 1.19	0.84 ± 0.13	4.76 ± 1.32	0.69 ± 0.12	6.28 ± 1.71
spleen	0.52 ± 0.05	0.46 ± 0.12	0.51 ± 0.04	0.67 ± 0.17	0.48 ± 0.01	0.27 ± 0.05	0.55 ± 0.08	0.35 ± 0.14
kidney	1.29 ± 0.13	2.00 ± 0.40	0.84 ± 0.31	1.40 ± 0.55	0.76 ± 0.05	1.83 ± 0.32	0.89 ± 0.43	3.49 ± 3.11
esophagus	0.54 ± 0.10	0.62 ± 0.27	0.59 ± 0.14	0.95 ± 0.60	0.49 ± 0.07	0.47 ± 0.16	0.50 ± 0.07	0.76 ± 0.29
lung	0.60 ± 0.24	0.86 ± 0.10	0.82 ± 0.04	1.08 ± 1.04	0.68 ± 0.21	0.67 ± 0.11	0.76 ± 0.02	0.93 ± 0.33
thyroid[c]	0.32 ± 0.12	30.8 ± 18.2	0.25 ± 0.05	44.5 ± 28.9	0.08 ± 0.05	192.8 ± 72.6	0.13 ± 0.04	472.6 ± 399.8

[a] Five animals were used at each time point. [b] Unlabeled estradiol (18 μg) was coinjected. [c] A thyroid weight of 0.005 g per 100 g of body weight was assumed for each animal (see ref 17).

observed products. Winstein[16] observed a similar reaction sequence as a base-catalyzed component in the solvolysis of *p*-hydroxyphenylethyl bromide. We were able to obtain low yields (10%) of the desired bromine-77 labeled bisphenolic norhexestrol bromide **5** by cleaving the phenolic triflates in **9a** with lithium aluminum hydride at −20 °C. However, in spite of our efforts to optimize this reductive cleavage, the major portion of material still undergoes debromination.

Tissue Uptake Selectivity in Immature Rats. The tissue distribution of radioactivity observed in immature female rats injected with [^{125}I]iodonorhexestrol (**6**) is summarized in Table I. The uterus to blood ratio in immature rats at 1 h is high (11.3 ± 5.8), while most of the nontarget tissues (muscle, liver, spleen, kidney, and lung) show levels not significantly different from blood. That the uterine uptake is mediated by a high-affinity, limited-capacity uptake system is demonstrated by the fact that coadministration of a quantity of unlabeled estradiol sufficient to occupy >95% of the estrogen receptors causes a selective depression of uptake by uterus, with the uterus to blood ratios decreasing from 11.3 ± 5.8 to 3.3 ± 0.8. Uptake into other organs is not significantly affected by the coadministration of this blocking dose of unlabeled estradiol. The very high thyroid uptake observed at 1 h is consistent with the lability of this compound and the formation of free iodide (see below). The moderate uptake by the esophagus is curious; this uptake is not significantly suppressed by unlabeled estradiol, which is consistent with the fact that the esophagus does not have detectable levels of estrogen receptor.

We have recently discussed[18] complictions that can arise in attempts to make quantitative comparisons between different receptor-binding radiopharmaceuticals due to physiological differences between individual animals, and we have developed a solution to these problems by using double label–internal standardization methods. Thus, in studying [^{77}Br]bromonorhexestrol **5**, we have used commercially available 16α-[^{125}I]iodo-17β-estradiol[19,20] (1000 Ci/mmol) as an internal standard.

A comparison of the relative uptake of the two radiopharmaceuticals in terms of tissue to blood ratios of radioactivity, percent injected dose per gram, and isotope ratios is shown in Tables II–IV. Again, as was the case with compound **6** at 1 h, [^{77}Br]bromonorhexestrol (**5**) demonstrates a preferential uptake by the uterus that is selectively suppressed at 1 h by coadministration of a blocking dose of unlabeled estradiol; the elevated uterine levels persist up to 6 h after injection (Tables II and III).

Some notable comparisons can be made between the uptake of the bromo compound **5** and 16α-[^{125}I]iodoestradiol: (a) Both compounds appear to show modest, estrogen-specific uptake by the ovary. (b) In addition to its preferential accumulation in the uterus and ovaries, iodoestradiol also shows considerable uptake by the liver and kidney (Table II); the uptake in these tissues increases with time in terms of tissue to blood ratios and does not appear to be suppressible at 1 h by the blocking dose of unlabeled estradiol. (c) It can be noted from Table IV that [^{77}Br]bromonorhexestrol, both in terms of uterus to blood

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Table III. Tissue Distribution of [^{77}Br]Bromonorhexestrol (5) and 16α-[^{125}I]Iodo-17β-estradiol in Immature Rats

	% inject. dose/g[b] (mean ± SD)[a]							
	1 h		1 h[c]		3 h		6 h	
tissue	^{77}Br	^{125}I	^{77}Br	^{125}I	^{77}Br	^{125}I	^{77}Br	^{125}I
blood	0.75 ± 0.34	0.47 ± 0.17	0.98 ± 0.24	1.22 ± 0.20	1.24 ± 0.37	0.46 ± 0.13	1.36 ± 0.14	0.22 ± 0.07
uterus	3.16 ± 0.95	9.59 ± 1.85	0.95 ± 0.18	1.81 ± 0.24	3.59 ± 0.78	6.83 ± 1.76	3.19 ± 0.81	1.57 ± 0.47
ovaries	1.38 ± 0.39	4.17 ± 1.05	0.72 ± 0.22	2.47 ± 0.68	1.28 ± 0.41	1.75 ± 0.28	1.93 ± 1.12	0.52 ± 0.15
muscle	0.29 ± 0.09	0.53 ± 0.20	0.30 ± 0.09	0.92 ± 0.47	0.37 ± 0.15	0.15 ± 0.05	0.35 ± 0.08	0.05 ± 0.02
liver	1.28 ± 0.61	2.37 ± 0.24	1.46 ± 0.29	4.91 ± 1.10	1.05 ± 0.37	1.96 ± 0.69	0.93 ± 0.22	0.90 ± 0.23
spleen	0.38 ± 0.16	0.43 ± 0.12	0.34 ± 0.09	0.79 ± 0.24	0.60 ± 0.19	0.18 ± 0.07	0.69 ± 0.10	0.08 ± 0.02
kidney	0.94 ± 0.38	1.48 ± 0.42	0.70 ± 0.21	2.77 ± 0.78	0.84 ± 0.13	0.94 ± 0.30	0.90 ± 0.11	0.40 ± 0.17
esophagus	0.38 ± 0.15	0.49 ± 0.15	0.56 ± 0.13	1.07 ± 0.30	0.61 ± 0.22	0.32 ± 0.12	0.68 ± 0.16	0.14 ± 0.05
lung	0.42 ± 0.19	0.75 ± 0.23	0.80 ± 0.16	1.76 ± 0.49	0.95 ± 0.32	0.36 ± 0.12	1.02 ± 0.11	0.14 ± 0.04
thyroid[d]	0.26 ± 0.01	7.46 ± 3.47	0.25 ± 0.05	11.9 ± 7.1	0.09 ± 0.04	190.9 ± 110.7	0.18 ± 0.07	276.6 ± 91.9

[a] Five animals were used at each time point. [b] Percent injected dose per gram tissue. [c] Unlabeled estradiol (18 μg) was coinjected. [d] A thyroid weight of 0.005 g per 100 g of body weight was assumed for each animal (see ref 17).

Table IV. Uptake Ratio of [^{77}Br]Bromonorhexestrol (5) and 16α-[^{125}I]Iodo-17β-estradiol in Immature Rats

time, h	^{77}Br/^{125}I Ratio[a]		% inject. dose/g of uterus
	uterus to blood	uterus to nontarget[b]	
1	0.47	0.58	0.33
3	0.39	0.30	0.53
6	0.27	0.31	2.03

[a] Ratios are the mean values of the ^{77}Br/^{125}I ratios calculated for each rat. [b] Uterus uptake compared to average uptake in four nontarget tissues: esophagus, spleen, lung, and muscle.

and uterus to nontarget tissue ratios, has a uterine uptake selectivity that is only about one-half that of 16α-[^{125}I]-iodo-17β-estradiol at 1 h and somewhat less than that at 3 and 6 h. In terms of percent injected dose per gram, however, the uterine uptake selectivity of the bromonorhexestrol increases with time with respect to iodoestradiol. (d) Finally, there is no selective uptake of bromine radioactivity in the thyroid after treatment with [^{77}Br]-bromonorhexestrol; this is consistent with the fact that the thyroid is known not to concentrate bromide.[21] However, with [^{125}I]iodoestradiol, there is a progressive increase in iodine radioactivity in the thyroid that increases to very high levels. This is indicative of deiodination of iodoestradiol, with trapping of radioiodide by the thyroid. The extent of deiodination of iodoestradiol (thyroid to blood ratio of 30.8 ± 18.2 at 1 h) is, however, much less than the deiodination of iodonorhexestrol (thyroid to blood ratio of 494.8 ± 191.2 at 1 h).

Stability of Bromo- and Iodonorhexestrol in Vitro and in Vivo. Thin-layer chromatographic analysis was used to determine the stability of the norhexestrol halides. [^{77}Br]Bromonorhexestrol underwent a slow decomposition at room temperature in physiological saline, with radiochemical purity falling from an initial value of >99% to 85% at 1 h an 70% at 2 h. All of the extraneous activity was at the origin of the chromatogram and was presumed to be bromide ion.

Thin-layer chromatographic analysis of extracts of tissues after injection of [^{77}Br]bromonorhexestrol indicates that the compound is very unstable in vivo (Figure 1). Even at 1 h, very little of the activity in the blood corresponds to authentic material. There is considerably more unaltered compound in two nontarget tissues, lung and spleen, at 1 h, but compound purity in these tissues declines rapidly with time. Activity in the uterus shows the highest fraction of unaltered compound and the slowest

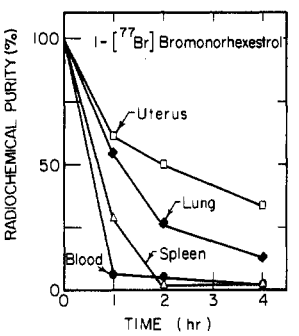

Figure 1. Radiochemical purity of 1-[^{77}Br]bromonorhexestrol (5) in different tissues of the rat. Extracts of tissues and blood were subjected to thin-layer chromatographic analysis to determine the radiochemical purity, as detailed under Experimental Section.

Table V. Solvolytic Stability of Several Halogenated Norhexestrol, Hexestrol, and Estradiol Derivatives Relative to *p*-Hydroxyphenylethyl Bromide[a]

compd	$1/k_{rel}$[b]
p-hydroxyphenylethyl bromide	1.00
1-iodonorhexestrol	0.71 ± 0.11
1-bromonorhexestrol	1.17 ± 0.17
1-bromohexestrol[c]	233 ± 45
16α-(bromomethyl)-17β-estradiol[d]	451 ± 104
16α-bromo-17β-estradiol[e]	1276 ± 433

[a] Rates were measured in 40% ethanol-water at 50 °C buffered to pH 7. [b] $1/k_{rel} = (k_{compd}/k_{p\text{-hydroxyphenylethyl bromide}})^{-1}$. [c] See ref 12. [d] Compound prepared by M. K. Mao and J. A. Katzenellenbogen, unpublished results. [e] See ref 9.

decline in purity. This is presumably due to the fact that most of the activity in the uterus is associated with the estrogen receptor and, hence, protected from solvolytic and metabolic degradation. In all cases, the radiochemical impurities are found at the origin. While this chromatographic behavior does not preclude formation of a polar metabolite, it seems more likely to be free bromide ion, especially in view of the solvolytic instability of this compound (vide infra).

Less extensive stability studies performed with iodonorhexestrol also indicate that this compound is quite unstable in vitro and in vivo. In fact, even in organic solvents at room temperature, it is stable for only a few hours. After 1 h in vivo, none of the original iodo compound was detectable in the blood, while 39% of the radioactivity in the uterus was found to be the authentic iodo compound.

Solvolytic Stability of Bromo- and Iodonorhexestrol. Further evidence for the extreme instability

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of bromo- and iodonorhexestrol was obtained with solvolysis in 40% ethanol–water as a model for in vivo stability. The solvolysis of several steroidal and nonsteroidal estrogens was examined relative to that of *p*-hydroxyphenylethyl bromide, a compound which is known to solvolyze with great facility (see below).[16] The data shown in Table V clearly indicate extreme differences in solvolytic behavior between the norhexestrols, on the one hand, and the hexestrols and steroidal estrogens, on the other. Both bromo- and iodonorhexestrol solvolyze at a rate roughly the same as the phenethyl bromide. Bromohexestrol[12] solvolyzes over 200 times more slowly than the phenethyl bromide, while 16α-(bromomethyl)estradiol (M. K. Mao and J. A. Katzenellenbogen, unpublished) and 16α-bromoestradiol[9] solvolyze 451 and 1276 times more slowly, respectively.

Discussion

A sequence for the synthesis of 1-[^{77}Br]bromonorhexestrol (**5**) and 1-[^{125}I]iodonorhexestrol (**6**) has been described. While producing material with sufficiently high specific activity for studying receptor mediated uptake, these syntheses suffer from relatively low radiochemical yields (41 and 25%, respectively) and instability of starting material (triflate **4**). Studies in immature female rats show a pattern of tissue uptake commensurate with a receptor-mediated process: Uterine uptake is moderately high and is selectively depressed by coadministration of an excess of unlabeled estradiol.

The 11.3 uterus to blood ratio obtained at 1 h with 1-[^{125}I]iodonorhexestrol (**6**), while comparable to both 16α-[^{125}I]iodo-17β-estradiol and our previously prepared compound 16α-[^{77}Br]bromo-17β-estradiol, is substantially less than that which we obtained recently with 16α-[^{77}Br]bromo-11β-methoxy-17β-estradiol[10] (uterus to blood ratio of 19.7). Bromine-77 labeled bromonorhexestrol **5**, on the other hand, shows a poorer selectivity (uterus to blood ratio of 4.5 at 1 h).

In the preceeding study, we found that the receptor-binding affinity of the iodo- and bromonorhexestrols to be 150–200% that of estradiol by competitive radioreceptor binding assay,[22] and based on the predicted nonspecific binding of these compounds, we expected that their interaction with the estrogen receptor would be of comparable selectivity to that of estradiol.[8] The selectivity of uterine uptake that we observed was, thus, somewhat less than we expected. The "deficiency" with these particular nonsteroidal estrogens appears to result from their solvolytic and metabolic lability. This can account for their relatively modest uptake selectively by the uterus, since destruction of the compound would liberate free halide ion and other radioactive byproducts that would elevate background radiation in the blood and depress uterus to blood ratios.

The unusual facility with which *p*-hydroxyphenylalkyl halides undergo solvolysis was studied in detail by Winstein[16] and has been ascribed to the intermediacy of spirocyclohexadienones species resulting from anchimeric assistance in the ionization of the halide by the phenoxide form of the aryl substituent (cf. Scheme II). While we were aware that the norhexestrol halides are, in fact, both *p*-hydroxyphenethyl and *p*-hydroxyphenylpropyl systems (with respect to the proximal and distal hydroxyphenyl groups, respectively), the neighboring group participation described by Winstein occurred only under basic conditions, when the hydroxyphenyl group was in the form of the phenoxide ion. Thus, we did not expect the observed lability of the norhexestrol halides at neutral pH. Nonetheless, the data clearly indicate the extreme facility with which the norhexestrol halides solvolyze.

The contrasting solvolytic labilities of the norhexestrol vs. hexestrol halides are striking. For example, bromohexestrol, being a *p*-hydroxyphenylpropyl and *p*-hydroxyphenylbutyl system, which does not undergo this type of internal elimination to form a cyclohexadienone intermediate, solvolyzes over 200 times more slowly than bromonorhexestrol. The two steroidal estrogens, as might be expected, appear to be quite stable to solvolysis. The lower reactivity of the 16α-bromo derivative compared to the 16α-bromomethyl derivative probably results from a stabilizing effect exerted by the 17β-hydroxy function or greater steric hindrance.

In conclusion, we have prepared two γ-emitting estrogens, one labeled with bromine-77 and the other with iodine-125, which have sufficiently high specific activity to demonstrate receptor-mediated uptake into estrogen-sensitive target tissues. However, the chemical and metabolic instability of these compounds in vitro and in vivo severely limits their usefulness as human breast tumor imaging agents and serves to reemphasize the need for chemical and metabolic stability in such radiopharmaceutical agents.

Experimental Section

General. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl. Ethyl vinyl ether was distilled prior to use. A lithium aluminum hydride solution (2.0 M) in ether was prepared and standardized as described earlier.[9] Trifluoromethanesulfonic anhydride was prepared by distillation of trifluoromethanesulfonic acid from phosphorus pentoxide.[23] Sodium [^{125}I]iodide in 0.1 N sodium hydroxide and 16α-[^{125}I]iodo-17β-estradiol (1000 Ci/mmol) were obtained from New England Nuclear. Sodium [^{77}Br]bromide was obtained as a spallation product from Los Alamos Scientific Laboratory.[24] All unlabeled halogenated norhexestrols were obtained as previously described.[22]

High-pressure liquid chromatography was performed with 12 μm of silica gel in a 25 cm × 4.6 mm column eluted with 90:8:2 hexane/CH_2Cl_2/*i*-PrOH. Radioactivity was determined with either a sodium iodide well counter or a Nuclear-Chicago liquid scintillation counter.

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were measured on a Beckman IR-12 spectrophotometer (KBr pellet). Proton magnetic resonance spectra (^{1}H NMR) were obtained at 90 MHz on a Varian EM-390 spectrometer; proton chemical shifts are reported in parts per million downfield from tetramethylsilane as an internal standard (δ scale). Electron-impact mass spectra (70 eV) were obtained on a Varian Model CH-5 spectrometer. Microanalytical data were provided by the Microanalytical Service Laboratory of the University of Illinois.

Radiochemical purities were determined in the following manner: 1 μL of radiolabeled compound was spotted on top of 1 μg of cold compound on a plastic-backed TLC plate without UV indicator. After development and visualization with iodine, the plate was cut into 0.5-cm strips, and radioactivity was counted. Radiochemical purity (RCP) is a percentage of total counts eluting with authentic spot. Count recovery from the plates was quantitative.

Chemical and Metabolic Stability Determination. The radiochemical purity of samples of the bromo- or iodonorhexestrols, after the treatments described in the text, was assayed by thin-layer chromatography as described above. After stability studies in vitro, samples were applied directly to the chroma-

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tography plates; samples from the in vivo studies were obtained as follows: Tissues and blood were homogenized in a small volume of buffer (0.01 M Tris, pH 7.4 at 25 °C); ethanol corresponding to twice the homogenate volume was added, and the sample was centrifuged (800 g for 10 min) to remove precipitated protein. The ethanol–water supernatant was evaporated under a stream of nitrogen and redissolved in a small volume of ethanol for application to the chromatograms. By this procedure, ca. 90% of the tissue or blood activity can be applied to the chromatogram.

Solvolysis Studies. Solvolyses were carried out in 40% ethanol–water with 0.001 M halogenated substrate, 0.015 M phosphate buffer (KH_2PO_4/Na_2HPO_4), and a small amount of 2-acetylnaphthalene as an internal standard, at 50 °C in a constant-temperature bath. Rates were determined by following the disappearance of the starting halides; these rates were monitored by reverse-phase HPLC (Supelco 25 cm × 4.6 mm, 5 μm silica gel column, LC-18, eluted with 40:60 ethanol/water, or a Varian MCH-10 Micro-Pak 30 cm × 4.6 mm column, eluted with 45:55 ethanol/water). Multiple kinetic runs were performed on *p*-hydroxyphenylethyl bromide with various concentrations of buffer (0.05–0.20 M); the rate was found to be independent of buffer concentration.

Specific Activity Determination. A binding curve for the γ-emitting, halogenated norhexestrol for the extrogen receptor in lamb uterine cytosol was measured in parallel with a binding determination with 17β-[^{3}H]estradiol of known specific activity. Specific activity of the γ emitter was determined by comparison of the maximum estrogen-specific binding observed with the γ-emitter, with the receptor site concentration determined with the estradiol. The method has been described in detail elsewhere.[10]

In Vivo Tissue Uptake Studies. After purification of HPLC, compounds **5** and **6** were evaporated to dryness and dissolved in 50 μL of ethanol. Prior to injection, a mixture of 0.9% NaCl and rat serum (1:1) was added to the solution to prevent adsorption of the radiolabeled estrogen to the syringe. Tissue uptake studies were carried out in immature (25 day) Sprague–Dawley female rats (ca. 50 g). The rats were injected intravenously (jugular or tail vein) with 2 μCi (≤15 pmol/dose or ≤300 pmol/kg) of compound **6** or 2 μCi each of **5** (≤1.25 pmol/dose or ≤25 pmol/kg) and 16α-[^{125}I]iodoestradiol (2 pmol/dose or 40 pmol/kg) and killed 1, 3, or 6 h after injection. Samples of blood and 11 tissues were measured, weighed, and assayed for radioactivity. Blocking of receptor-mediated uptake was achieved by simultaneous injection of 9 or 18 μg of unlabeled estradiol.

Methyl (2*R,3*S**)-2,3-Bis[4-[(ethoxyethyl)oxy]phenyl]-pentanoate (2).** Methyl (2*R**,3*S**)-2,3-bis(4-hydroxyphenyl)-pentanoate (1; 200 mg, 0.67 mmol)[15] was dissolved in 5 mL of THF and cooled to 0 °C. Ethyl vinyl ether (0.129 mL, 2.0 mmol) was added dropwise. To this was added a few crystals of *p*-toluenesulfonic acid, and the mixture was stirred for 3 h. The THF was removed in vacuo, and the residue was taken up in 5% EtOAc–CH_2Cl and filtered through neutral alumina to give 277 mg (93%) of **2** as a clear, colorless oil: IR (KBr) 1745 (C=O) cm^{-1}; ^{1}H NMR (CCl_4) δ 0.57 (t, 3, J = 7 Hz, CH_2CH_3), 1.16 (t, 3, J = 7 Hz, OCH_2CH_3), 1.18 (t, 3, J = 7 Hz, OCH_2CH_3),~1.3 (m, 2, CH_2CH_3), 1.41 (d, 3, J = 6 Hz, $CHCH_3$), 1.43 (d, 3, J = 6 Hz, $CHCH_3$), 2.98 (dt, 1, J = 5 and 9 Hz, $CHCH_2$), 3.30 (s, 3, CO_2CH_3), 3.33–3.87 (m, 5, OCH_2CH_3 + $CHCOO$), 5.27 (quartet, 2, J = 6 Hz, $CHCH_3$), 6.82 (d, 2, J = 9 Hz, Ar H ortho to Ar OR), 6.87 (d, 2, J = 9 Hz, Ar H ortho to Ar OR), 7.03 (d, 2, J = 9 Hz, Ar H ortho to alkyl), 7.21 (d, 2, J = 9 Hz, Ar H ortho to alkyl); mass spectrum (35 eV), m/z (relative intensity) 332 (1), 103 (38), 73 (100), 45 (97). Anal. ($C_{26}H_{36}O_6$) C, H.

(2*R,3*S**)-2,3-Bis[4-[(ethoxyethyl)oxy]phenyl]-1-pentanol (3).** Ethoxyethyl ether methyl ester **2** (444 mg, 1 mmol) was dissolved in 15 mL of ether and cooled to −78 °C. To this was added 1 mL of a 2 M solution of $LiAlH_4$ in ether, along with a small amount (ca. 10 mg) of sodium methoxide. After stirring for 1 h at −78 °C, the reaction was quenched by successive addition of 76 μL of H_2O, 76 μL of 15% NaOH, and 228 μL of H_2O. The solution was filtered, and the aluminum salts were washed with ether. Removal of solvent gave 396 mg (95%) of pentanol **3** as a clear, colorless oil: IR (KBr) 3460 (OH) cm^{-1}; ^{1}H NMR (CCl_4) δ 0.57 (t, 3, J = 7 Hz, CH_2CH_3), 1.20 (t, 6, J = 7 Hz, OCH_2CH_3), ~1.3 (m, 2, CH_2CH_3), 1.44 (d, 6, J = 5 Hz, $CHCH_3$), 2.48–2.80 (m, 2, benzylic *H*), 3.47–3.90 (m, 6, OCH_2 + $CHCH_2OH$), 5.27 (quartet, 2, J = 5 Hz, $CHCH_3$), 6.89 (d, 4, J = 9 Hz, Ar H ortho to Ar OR), 7.03 (d, 2, J = 9 Hz, Ar H ortho to alkyl), 7.08 (d, 2, J = 9 Hz, Ar H ortho to alkyl); mass spectrum (70 eV), m/z (relative intensity) 416 (1, M$^+$), 135 (100), 73 (57), 45 (53). Anal. ($C_{25}H_{36}O_5$) C, H.

(2*R,3*S**)-2,3-Bis[4-[(ethoxyethyl)oxy]phenyl]-1-pentyl Trifluoromethanesulfonate (4).** Ethoxyethyl ether alcohol **3** (145 mg, 0.35 mmol) was dissolved in 0.4 mL of 2,6-lutidine, diluted with 3 mL of CH_2Cl_2, and cooled to −78 °C. Trifluoromethanesulfonic anhydride (60 μL, 0.35 mmol) was added dropwise, and the mixture was stirred for 0.5 h at −78 °C. The reaction mixture was washed (0.5 M $CuSO_4$) and the organic layer was dried over Na_2SO_4 while the temperature was maintained at −78 °C. Filtration through neutral alumina and solvent removal in vacuo at −78 °C gave 162 mg (84%) of triflate **4** as a pale yellow oil. Thin-layer chromatography in 5% EtOAc–CH_2Cl_2 on Al_2O_3 gives an R_f of 0.80. This compound is extremely unstable and decomposes at room temperature in about 3 min. The product may be stored at −78 °C for a few days. The compound was used directly without further purification or characterization.

(2*R,3*S**)-1-[^{125}I]Iodo-2,3-bis(4-hydroxyphenyl)pentane (6).** Na^{125}I in 0.1 N NaOH (0.2 mL, ~1 mCi) was taken to dryness under a stream of dry N_2. To this was added 0.2 mL of 0.1 N HOAc in acetone, and the mixture was cooled to 0 °C. Triflate **4** (0.2 mg in 0.2 mL of acetone) was added, and the mixture was stirred for 2 h. Ether–water partitioning of a 1-μL aliquot indicated 81% uptake of radioiodide into the organic (ether) layer. The reaction mixture was taken to dryness under a stream of dry N_2, and the residue was taken up in MeOH and suspended in a column of Dowex 50W-X8. After 30 min, the column was rinsed with 20% EtOAc in CH_2Cl_2, and the solvents were removed under a stream of dry N_2. The residue was partitioned between ether and H_2O, the layers were separated, and the organic layer was dried by passage through a column of Na_2SO_4. Purification by HPLC gave 250 μCi (25% radiochemical yield) of **6** (elution time ~7.5 min at a flow rate of 1.5 mL/min). Analysis by TLC shows compound **12** to comigrate with authentic unlabeled iodonorhexestrol (20% EtOAc in CH_2Cl_2) and to be >99% radiochemically pure.

(2*R,3*S**)-1-[^{77}Br]Bromo-2,3-bis(4-hydroxyphenyl)pentane (5).** This compound was prepared in 41% yield in similar fashion as the iodine-125 labeled compound (**6**), except that bromide incorporation took place over 0.5 H. TLC analysis after HPLC purification (elution time ~11.1 min) again shows **5** to migrate with authentic nonradiolabeled bromonorhexestrol and have an RCP >99%.

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