in THF (10 mL) was added dropwise, and then the mixture was stirred at −65 °C for a further 0.75 h. The reaction mixture was allowed to attain room temperature, stirred for 1.75 h, and cooled (10 °C). Water (10 mL) and dichloromethane (20 mL) were then added. The organic layer was separated, the aqueous phase was further extracted with methylene chloride (2 × 20 mL), and the combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica gel (50 g) to give 1-amino-6,7-dimethoxy-3-[4-(*tert*-butoxycarbonyl)piperazin-1-yl]isoquinoline (**10**) (0.59 g, 21%), NMR.

(b) A solution of the product (0.58 g, 0.9 mmol) from (a) in ethyl acetate (20 mL) was stirred with HCl (3 mL, 3 N) at room temperature for 2.75 h. Ether (50 mL) was then added, the supernatant liquid was decanted, and the solid product was further triturated with ether. The crude residue was cooled (10 °C) and treated with chloroform (40 mL) and sodium hydroxide (20 mL, 5 N), and the organic layer was separated. The aqueous phase was further extracted with chloroform (3 × 20 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), and evaporated to leave 1-amino-6,7-dimethoxy-3-piperazin-1-ylisoquinoline (0.38 g, 88%), NMR.

(c) A solution of furan-2-carbonyl chloride (0.10 g, 0.8 mmol) in chloroform (5 mL) was added dropwise to a stirred solution of the crude product (0.34 g, 0.8 mmol) from (b) in chloroform (15 mL) and triethylamine (0.11 g, 1.1 mmol) at 7 °C. After 0.75 h, sodium carbonate solution (5 mL, 10%) was added, the organic layer was separated, and the aqueous phase was further extracted with chloroform (2 × 20 mL). The combined organic extracts were washed with water, dried (Na_2SO_4), and evaporated, and then the residue was purified by chromatography on silica gel (25 g) with dichloromethane/methanol (100:0 → 96:4) as eluant. The product was dissolved in chloroform, treated with ethereal HCl, and then evaporated, and the residue was recrystallized from ethanol/methanol to give 1-amino-6,7-dimethoxy-3-[4-(furan-2-ylcarbonyl)piperazin-1-yl]isoquinoline hydrochloride $^1/_3$-ethanolate (0.19 g, 56%): mp 258–261 °C; MS, (M^+) 382; NMR (DMSO-d_6) δ 3.35 (4 H, br, s), 3.87 (10 H, br m), 6.38 (1 H, s, exchanges with TFA-*d*), 6.67 (1 H, m), 7.08 (1 H, m), 7.17 (1 H, s), 7.75 (1 H, s), 7.88 (1 H, s), 8.54 (2 H, br s, exchanges with TFA-*d*). Anal. ($C_{20}H_{22}N_4O_4 \cdot HCl \cdot 0.33EtOH$) H, N; C: calcd, 57.2; found, 56.6.

1-Cyano-4-(*tert*-butoxycarbonyl)piperazine. A solution of di-*tert*-butyl dicarbonate (3.24 g, 15 mmol) in THF (25 mL) was added dropwise to a stirred solution of 1-cyanopiperazine[21] (1.5 g, 13.5 mmol) in THF (25 mL) at 5–10 °C under an atmosphere of nitrogen. The reaction mixture was then allowed to attain room temperature and was stirred for a further 2 h. The mixture was evaporated, and the residue was dissolved in ethyl acetate, washed with citric acid solution and brine, dried (Na_2SO_4), and evaporated. The residue was purified by chromatography on silica gel (25 g), and the product was recrystalllized from ethyl acetate/hexane to give 1-cyano-4-(*tert*-butoxycarbonyl)piperazine (1.48 g, 52%): mp 101 °C; MS, (M^+) 211. Anal. ($C_{10}H_{17}N_3O_2$) C, H, N.

Biology. Experimental details for evaluation of α-adrenoceptor binding and antihypertensive activities have been provided previously.[1]

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Registry No. **3**, 58814-69-0; **4**, 113533-94-1; **4** (free base), 113533-95-2; **5**, 113533-96-3; **5** (free base), 113533-97-4; **6**, 113533-98-5; **6** (free base), 23023-37-2; **8**, 113533-99-6; **8** (free base), 113534-00-2; **9**, 40172-93-8; **10**, 113534-01-3; Me_2NCN, 1467-79-4; 2-furoyl chloride, 527-69-5; *N*-cyanopiperidine, 1530-87-6; 1-cyano-4-(*tert*-butoxycarbonyl)piperazine, 113534-02-4; 1-amino-6,7-dimethoxy-3-piperazin-1-ylisoquinoline, 113534-03-5; 1-cyanopiperazine, 34065-01-5.

Supplementary Material Available: X-ray data are available for 1-amino-6,7-dimethoxy-3-[4-(furan-2-ylcarbonyl)piperazin-1-yl]isoquinoline hydrochloride (**8**) (9 pages). Ordering information is given on any current masthead page.

Dopamine D-2 Receptor Imaging Radiopharmaceuticals: Synthesis, Radiolabeling, and in Vitro Binding of (*R*)-(+)- and (*S*)-(−)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide

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In developing central nervous system (CNS) dopamine D-2 receptor imaging agents, enantiomers, *R*-(+) and *S*-(−) isomers, of 3-[^{125}I]iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide, [^{125}I]IBZM, were synthesized, and their in vitro binding characteristics were evaluated in rat striatum tissue preparation. The (*S*)-(−)-[^{125}I]IBZM showed high specific dopamine D-2 receptor binding (K_d = 0.43 nM, B_{max} = 0.48 pmol/mg of protein). Competition data of various ligands for IBZM binding displayed the following rank order of potency: spiperone > (*S*)-(−)-IBZM > (+)-butaclamol ≫ (*R*)-(+)-IBZM > (*S*)-(−)-BZM > dopamine > ketanserin > SCH23390 ≫ propanolol. The results indicate that [^{125}I]IBZM binds specifically to the dopamine D-2 receptor with stereospecificity. The [^{123}I]IBZM is potentially useful as an imaging agent for the investigation of dopamine D-2 receptors in humans.

A variety of substituted benzamide derivatives possessing antidopaminergic properties has been reported.[1-6] Of these sulpiride,[7,8] raclopride,[9] eticlopride,[10,11] iodoclebopride, and iodoazidoclebopride[12] show specific antagonistic activity, high affinity constants (K_d) in rat striatum tissue preparations (Table I), and relatively low nonspecific binding.

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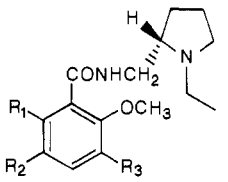
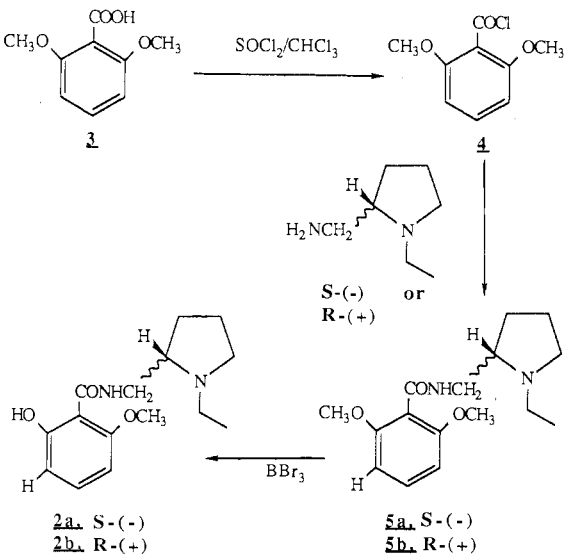
Table I. Chemical Structures and in Vitro Binding Constants of Benzamides



	R_1	R_2	R_3	K_d, nM
sulpiride	H	SO_2NH_2	H	1.5
raclopride	OH	Cl	Cl	1.1
eticlopride	OH	C_2H_5	Cl	0.17
IBZM	OH	I	H	0.43
BZM	OH	H	H	31.1

Scheme I. Synthesis of (*S*)-(−)- and (*R*)-(+)-BZM



Some of these benzamide derivatives, if congugated with a suitable radioisotope of short half-life and appropriate γ-ray energies, may find clinical use as diagnostic agents for imaging dopamine receptors. In fact, the preparation and central nervous system (CNS) dopamine D-2 receptor imaging studies of [^{11}C]raclopride have been recently reported in humans.[13-17] A high ratio of specific striatal to nonspecific cerebellar binding in living human brain was observed. By use of an equilibrium model and Scatchard plots, the affinity constant (K_d = 7.1 nM, B_{max} = 24.6 pmol/mL) in a living human brain was measured by positron emmision tomography (PET).[13,14] The values for the dopamine D-2 receptor were comparable to those determined earlier by using a different imaging agent: *N*-methylspiperone (K_d = 0.097 nM, B_{max} = 16.6 pmol/g).[18,19]

Because of the cost effectiveness of SPECT (single photon emmission computed tomography) methodology

Scheme II. Synthesis of (*S*)-(−)- and (*R*)-(+)-IBZM

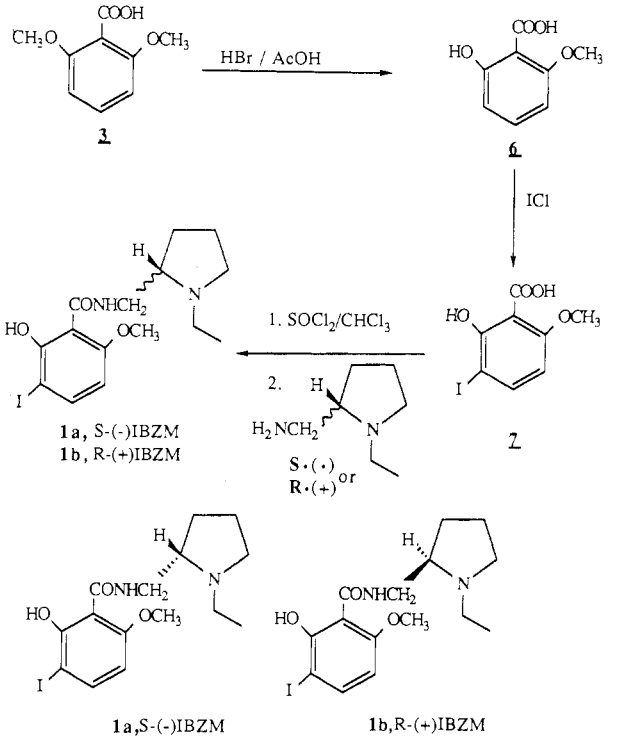


Table II. Comparison of Caculated and Observed Chemical Shifts of ^{13}C NMR Spectra for **7**

carbon	calcd ppm	obsd ppm	carbon	calcd ppm	obsd ppm
COOH	172.6	170.9	C_3	75.3	75.7
OCH_3	54.0	56.5	C_4	145.5	142.7
C_1	106.1	105.6	C_5	109.0	106.6
C_2	167.8	160.2	C_6	161.3	159.5

and as well as the ever increasing resolution images offered by SPECT cameras, an agent that could be used in conjunction with this technology would be highly desirable. This paper describes the syntheses of (*R*)-(+)- and (*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (IBZM). The corresponding no-carrier-added ^{125}I ($T_{1/2}$ = 60 days, 35–60 keV) labeled compounds were also prepared. In vitro binding properties of these agents were determined with rat brain tissue preparations. The radiolabeling method is readily adaptable for the preparation of [^{123}I]IBZM ($T_{1/2}$ = 13 h, 159 keV), which may provide a useful tool for in vivo imaging of the dopamine D-2 receptors in humans in conjunction with SPECT.

Chemistry. Enantiomers of cold IBZM and the corresponding uniodinated compound, BZM, were synthesized by improved methods similar to the ones used for the preparation of various benzamides.[2-5] The synthesis of (*S*)-(−)- and (*R*)-(+)-BZM, **2a** and **2b**, respectively, is outlined in Scheme I.

Treatment of 2,6-dimethoxybenzoic acid, **3**, with thionyl chloride in dry chloroform containing a catalytic amount of dimethylforamide gave the acid chloride, **4**, which was treated in crude form with an excess of either (*S*)-(−)- or (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine[20] to give the corresponding benzamide **5a** or **5b** in moderate to high yields (41–81%). Treatment of **5a** or **5b** with 1.1 equiv of boron tribromide in dry dichloromethane gave the mono-

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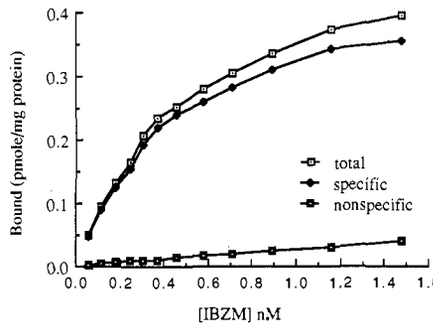

Figure 1. Saturation binding of [^{125}I]IBZM in rat striatum. The radiolabeled [^{125}I]IBZM binds to rat striatal homogenate with high affinity. The saturation curve indicates that this ligand demonstrates a very low nonspecific binding (approximately 5% at K_d).

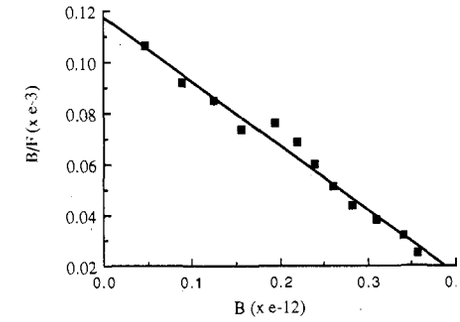

Figure 2. Scatchard plot of [^{125}I]IBZM binding to rat striatal tissue preparation. On the basis of the scatchard plot, the specific binding of [^{125}I]IBZM was calculated; K_d = 0.426 ± 0.082 nM and B_{max} = 0.48 ± 0.0215 pmol/mg of protein.

dealkylated compound **2a** or **2b** as light yellow oils. Reaction of (*S*)-(−)-BZM, **2a**, with L-(+)-tartaric acid in anhydrous methanol yielded the tartarate salt **2c** as a white foam in quantitative yield.

The optically pure cold IBZM, **1a** or **1b**, was prepared by the sequence of reactions outlined in Scheme II. Treatment of **3** with 1.1 equiv of 30% hydrobromic acid–acetic acid in dry chloroform gave the monodealkylated benzoic acid **6**; iodination of **6** was achieved by using iodine monochloride in glacial acetic acid to give the monoiodinated product **7** in good yield (70%). The ^{13}C NMR spectra of **7** is consistent with the iodine being ortho to the phenol (Table II). This observation is similar to the preparation of the bromo derivatives where the bromine atom is found to be at the position ortho to the hydroxy group on the phenyl ring.[4,5] Conversion of **7** to the corresponding acid chloride was achieved by using the reaction conditions described above. Reaction of the crude acid chloride with an excess of (*S*)-(−)- or (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine[20] gave (*S*)-(−)- and (*R*)-(+)-IBZM, **1a** and **1b**, respectively, as light yellow oils. Treatment of **1a** or **1b** with L-(+)-tartaric acid in methanol gave the tartarate salt of (*S*)-(−)- and (*R*)-(+)-IBZM, **1c** or **1d**, as off-white foams. Stereoisomers, (*S*)-(−)- and (*R*)-(+)-BZM, **2a** and **2b**, were also prepared by condensing the acid chloride of 6-methoxysalicyclic acid (**6**) with the optically pure (*S*)-(−)- or (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine, in 60% yield. The compounds prepared by this method were identical with the ones obtained by Scheme I in chromatographic and spectrometric characterization.

Radiolabeling. Radioactive labeling of IBZM was accomplished by the chloramine-T method. The product obtained was compared with chemically pure nonradioactive IBZM by HPLC with simultaneous UV and radioactivity detection and was determined to be the desired product on the basis of its elution profile.[21] At pH 3, the labeling produced an excellent yield of >95%. As previously reported, there was a significant decrease in labeling yield at higher pH values.[21] The desired product was purified by HPLC with use of a reverse-phase column and an acetonitrile–buffer solvent mixture (radiochemical purity >95%). The specific activity was determined by direct UV measurement: >600 Ci/mmol (the detection limit of this method).

In Vitro Binding. Similarly to earlier reports concerning the in vivo autoradiographic binding of [^{125}I]IBZM in rat brain,[21,22] this ligand bound with high affinity to rat striatal homogenate in vitro. The saturation curve shown in Figure 1 indicates that this ligand has a very low nonspecific binding (approximately 5% at K_d) in contrast to the other two substituted benzamides, [^{3}H]sulpiride and [^{3}H]sultopride (60% and 40% nonspecific binding, respectively).[23,24] The specific binding of [^{125}I]IBZM was found to be saturable and displayed a K_d of 0.426 ± 0.082 nM and a B_{max} of 0.48 ± 0.0215 pmol/mg of protein (Figure 2). These values were comparable to the numbers for eticlopride, another substituted benzamide selective for D-2 receptors, measured under similar conditions.[10,11] Competition data of various compounds for IBZM binding are listed in Table III with the following rank order of potency: spiperone > (*S*)-(−)-IBZM > (+)-butaclamol > (*R*)-(+)-IBZM ≥ (*S*)-(−)-BZM > dopamine > ketanserin > SCH23390 ≫ propanolol. The results indicate that [^{125}I]IBZM binds specifically to the dopamine D-2 receptor with high selectivity and stereospecificity.

Table III. Inhibition Constants of Compounds on [^{125}I]IBZM Binding to Rat Striatal Membranes[a]

compound	K_i, nM ± SD	compound	K_i, nM ± SD
(*S*)-(−)-IBZM	0.633 ± 0.049	dopamine	296 ± 59
(*R*)-(+)-IBZM	30.3 ± 0.84	SCH23390	600 ± 15
(*S*)-(−)-BZM	31.1 ± 5.78	ketanserin	359 ± 79
spiperone	0.149 ± 0.010	propranolol	12902 ± 387
(+)-butaclamol	0.851 ± 0.174		

[a] 0.15–0.4 nM [^{125}I]BZM was incubated in the presence of the indicated compounds in 7–11 concentrations and of membrane preparation from rat striatum. Each value represents the mean ± SEM of four to six determinations.

In conclusion, the in vitro binding data presented in this paper together with the in vivo autoradiography reported earlier suggests that [^{123}I]IBZM may be a useful ligand for SPECT imaging of D-2 dopamine receptors in living human brain.

Experimental Section

Proton NMR and carbon-13 NMR spectra were recorded on Varian EM360A and JEOL FX-90Q spectrometers, respectively. The chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with either a Perkin-Elmer Model 727B or a Mattson Polaris FT-IR spectrometer. Low-resolution mass spectra were determined on a Krados MS 80 mass spectrometer. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. Melting points were determined on a Meltemp apparatus and are reported un-

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corrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Atlanta, GA, and were within 0.4% of the theoretical values.

Resolution of (*RS*)-(+)-*N*-Ethyl-2-(aminomethyl)-pyrrolidine. Racemic *N*-ethyl-2-(aminomethyl)pyrrolidine was resolved into optically active *R* and *S* enantiomers through the formation of its ditartarate salt, according to the published procedure.[20] (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine ditartarate: mp 157–159 °C (lit.[20] mp 158–159 °C); $[\alpha]_D$ −38.9° (c 20, H_2O) (lit.[20] $[\alpha]_D$ −39 ± 0.5°). (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine ditartarate: mp 158–160 °C (lit.[20] mp 161–162 °C); $[\alpha]_D$ +38.7° (c 20, H_2O) (lit.[20] $[\alpha]_D$ +39 ± 0.5°).

(*S*)-(−)-*N*-[(Ethyl-2-pyrrolidinyl)methyl]-2,6-dimethoxybenzamide (5a). Thionyl chloride (4.86 mL, 66.6 mmol) was added to a solution of 2,6-dimethoxybenzoic acid (**3**) (3.64 g, 20 mmol) and DMF (0.1 mL) in dry chloroform (20 mL), and the reaction mixture was stirred at reflux for 2 h. Volatile components were removed in vacuo to give a yellow foam that was dissolved in dry chloroform (20 mL) and added slowly under gentle reflux to a solution of (*S*)-(−)-*N*-ethyl-2-(aminomethyl)-1-ethylpyrrolidine (6.0 g, 46.8 mmol) in dry chloroform (10 mL), and the reaction mixture was stirred at ambient temperature for 24 h. Voltile components were removed in vacuo to give an orange oil that was dissolved in water (50 mL) and adjusted to a pH of 8.0 by dropwise addition of aqueous 15% sodium hydroxide. The aqueous solution was washed with chloroform (2 × 75 mL), and the combined organic layers were dried (sodium sulfate) and concentrated in vacuo to give an orange oil that was purified by silica gel column chromatography (acetone) to give the product, **5a**, as a colorless oil that solidified on storage at 5 °C for 24 h (4.70 g, 81%): mp 102–103 °C (lit.[5] mp 100–102 °C); NMR ($CDCl_3$/TMS) δ 1.05 (t, *J* = 7 Hz, 3 H, CH_3), 1.4–3.35 (complex m, 11 H), 3.75 (s, 6 H, OCH_3), 5.79–6.26 (br s, 1 H), 6.41 (d, *J* = 8 Hz, 2 H), 6.98–7.35 (m, 1 H); IR (film) 3400, 3320, 2980, 2860, 2800, 1670, 1600, 1520, 1475, 1380, 1300, 1270, 1120, 1030, 800, and 730 cm^{-1}.

(*S*)-(−)-2-Hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (2a). Dry hydrogen chloride gas was bubbled through a solution of **5a** (3.0 g, 10.3 mmol) in dry chloroform (70 mL) for 15 min. Volatile components were removed in vacuo to give a yellow oil that was dissolved in dry dichloromethane (20 mL) and treated with a solution of 1 M boron tribromide in dichloromethane (11.3 mL, 11.33 mmol), and the reaction mixture was stirred at ambient temperature for 1.5 h. A solution of 2 N ammonium hydroxide (25 mL) was added, and the aqueous layer was washed with dichloromethane (2 × 25 mL); the combined organic layer was dried (sodium sulfate) and concentrated in vacuo to give a yellow oil that was purified by silica gel column chromatography (acetone) to give **2a** as a light yellow oil (2.0 g, 70%): low-resolution MS, *m/e* 278.8 (M^+); NMR ($CDCl_3$/TMS) δ 1.10 (t, *J* = 7 Hz, 3 H), 1.30–3.80 (complex m, 12 H), 3.85 (s, 3 H), 6.20–6.65 (m, 2 H), 7.15 (d, d, *J* = 8 Hz, 1 H), 8.55–9.05 (br s, 1 H); IR (neat) 3350, 2975, 2875, 2800, 1640, 1590, 1530, 1460, 1360, 1310, 1240, 1185, 1145, 1090, 875, and 800 cm^{-1}; UV (310 nm, ε 4.03 × 10^6).

(*S*)-(−)-2-Hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (+)-Tartarate (2c). To a 1 M solution of (*S*)-(−)-BZM (**2a**) in anhydrous methanol was added an equivalent amount of L-(+)-tartaric acid, and the mixture was stirred at 25 °C for 30 min. Solvent was evaporated in vacuo and residue (foam) triturated with anhydrous ether. The desired product, **2c** (quantitative yield), was filtered under argon as a white foam: mp 43–50 °C. Anal. ($C_{19}H_{28}O_9N_2 \cdot H_2O$) C, H, N.

(*R*)-(+)-2-Hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (2b). The *R*-(+) isomer was prepared by employing the identical reaction conditions described for the *S*-(−) isomer (yield 1.2 g, 42%): NMR ($CDCl_3$/TMS) δ 1.15 (t, *J* = 7 Hz, 3 H), 1.45–3.75 (complex m, 12 H), 3.90 (s, 3 H), 6.15–6.60 (m, 2 H), 7.20 (dd, *J* = 8 Hz, 1 H), 8.70–9.15 (br s, 1 H); IR (neat) 3320, 3000, 2950, 2700, 2500, 1630, 1600, 1540, 1460, 1370, 1310, 1250, 1100, 880, 820, and 760 cm^{-1}.

6-Methoxysalicylic Acid (6). A solution of 30% hydrobromic acid in acetic acid (18 g, 67 mmol) in dry chloroform (60 mL) was added slowly to a stirred suspension of 2,6-dimethoxybenzoic acid (**3**, 10.92 g, 60 mmol) in dry chloroform (90 mL), and the reaction mixture was stirred at ambient temperature for 20 h. Volatile components were removed in vacuo to give a pink solid that was suspended in ether–hexanes (1:4) and filtered. Recrystallization from ethanol afforded 6-methoxysalicylic acid (**6**) as fine white needles (6.08 g, 60%): mp 134.5–136 °C (lit.[25] mp 135–136.3 °C); NMR ($CDCl_3$/DMSO-d_6/TMS) δ 3.76, (s, 3 H, OCH_3), 6.33 (m, 2 H, C-3 and C-5 aromatic H), 7.12 (dd, *J* = 8 Hz, 1 H, C-4 H), and 10.43–11.25 (br s, 2 H); IR (KBr) 3125, 2950, 1690, 1620, 1580, 1450, 1390, 1330, 1305, 1240, 1185, 1085, 1060, 940, 810, 730 cm^{-1}.

3-Iodo-6-methoxysalicylic Acid (7). A solution of iodine monochloride (4 g, 25 mmol) in glacial acetic acid (30 mL) was added dropwise over 20 min to a stirred suspension of 6-methoxysalicyclic acid (**6**, 3.4 g, 20 mmol) in glacial acetic acid (30 mL). The reaction mixture was stirred at reflux for 3 h and then stirred at ambient temperature for an additional 18 h. The dark brown solution was decolorized by addition of 10% aqueous sodium thiosulfate (10 mL), the volatile components were removed in vacuo, and the resultant precipitate was suspended in cold water (50 mL) and filtered. Recrystallization from ethanol gave 3-iodo-6-methoxysalicyclic acid (**7**) as fluffy tan needles (4.14 g, 70%): mp 178.5–180.5 °C; NMR ($CDCl_3$/DMSO-d_6/TMS) δ 3.84 (s, 3 H, OCH_3), 6.38 (d, *J* = 7 Hz, 1 H), and 7.75 (d, *J* = 7 Hz, 1 H); ^{13}C NMR ($CDCl_3$/TMS) δ 56.5 (OCH_3), 75.7 (C_3), 105.6 (C_1), 142.7 (C_4), 159.5 (C_6), 160.2 (C_2), and 170.9 (COOH); IR (film) 3150, 1675, 1600, 1560, 1420, 1380, 1300, 1275, 1220, 1180, 1150, 1100, 1075, 950, and 800 cm^{-1}. Anal. ($C_8H_7IO_4$) C, H, I.

(*S*)-(−)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (1a). A solution of 3-iodo-6-methoxysalicylic acid (**7**, 5.0 g, 17 mmol) in chloroform (20 mL) and thionyl chloride (3.72 mL, 51 mmol) containing a catalytic amount of dimethylformamide (0.5 mL) was heated to reflux. After 2 h the solvent was removed in vacuo, and chloroform (5 mL) was added to the resulting residue. A solution of (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine [prepared by chlorofrom extraction (2 × 20 mL) of a solution of 25 g (0.058 mol) of 2-(aminomethyl)-1-ethylpyrrolidine ditartarate in 5 N NaOH (35 mL)] was added at 25 °C. After the mixture was stirred under argon for 2 h, the solvent was removed, and residue was dissolved in 1 N HCl (30 mL). The aqueous layer was washed with ether and basified to pH 8 with 5 N NaOH. The resulting oil was extracted with chloroform (3 × 100 mL). Column chromatography on silica gel with acetone as eluent gave the desired product, **1a** (4.3 g, 62.6%), as a pale yellow oil: NMR ($CDCl_3$/TMS) δ 1.11 (t, 3 H, CH_3), 1.32–3.86 (complex m, 12 H), 3.91 (s, 3 H, OCH_3), 6.23 (d, 1 H, H-5), 7.68 (d, 1 H, H-4), 8.98 (br s, 1 H, CONH); ^{13}C NMR ($CDCl_3$) δ 14.01, 22.78, 28.24, 40.63, 47.55, 53.35, 56.08, 61.54, 103.04, 104.16, 141.51, 158.91, 162.47, 169.30; IR (neat) 3350, 2970, 2865, 2930, 2790, 1635, 1575, 1525, 1430, 1380, 1355, 1290, 1230, 1210, 1185, 1145, 1100, 1050, 1015, 955, 920, 800, 780, 730, 675, and 620 cm^{-1}; low-resolution MS, *m/e* 405.2 (M + 1); UV (322 nm, ε 4.9 × 10^6).

(*S*)-(−)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (+)-Tartarate (1c). To a 1 M solution of (*S*)-(−)-IBZM (**1a**) in anhydrous methanol was added an equivalent amount of L-(+)-tartaric acid, and the mixture was stirred at ambient for 30 min. Solvent was evaporated in vacuo, and the foam residue was triturated with anhydrous ether. The desired product (**1c**, quantitative yield) was filtered under argon as an off-white solid: mp 50–58 °C. Anal. ($C_{19}H_{27}IN_2O_9$) C, H, I, N.

(*R*)-(+)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (1b). The (*R*)-(+)-benzamide (**1b**) was produced in a similar fashion from 3-iodo-6-methoxysalicylic acid (**7**) and (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine: ^1H NMR ($CDCl_3$/TMS) δ 1.10 (t, 3 H, CH_3), 1.32–3.85 (complex m, 12 H), 3.93 (s, 3 H, OCH_3), 6.25 (d, 1 H, H-5), 7.69 (d, 1 H, H-4), 8.98 (br s, 1 H, CONH); IR (neat) 3350, 2970, 2930, 2870, 2795, 1635, 1575, 1525, 1425, 1380, 1360, 1290, 1230, 1185, 1145, 1100, 1020, 925, 800, 780, 750, 680, 630, and 570 cm^{-1}.

(*R*)-(+)-3-Iodo-2-hydroxy-6-methoxy-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide (+)-Tartarate (1d). To a 1 M solution of (*R*)-(+)-IBZM (**1b**) in anhydrous methanol was added an equivalent amount of L-(+)-tartaric acid, and the mixture was stirred at ambient for 30 min. Solvent was evaporated in vacuo, and the foam residue was triturated with anhydrous ether.

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The desired product (quantitative yield) was filtered under argon as an off-white solid: mp 50–60 °C. Anal. ($C_{19}H_{27}IN_2O_9$) C, H, I, N.

Radiolabeling. Chloramine-T solution of (50 μL, 1 mg/mL) was added to a mixture of BZM (50 μL, 1 mg/mL), sodium [^{125}I]iodide (10 μL, 1–5 mCi, no-carrier added, sp act. 2200 Ci/mmol), and pH 3 phosphate solution (0.3 mL) in a sealed vial. The reaction was allowed to proceed at room temperature for 1.5 min. The reaction was terminated by addition of sodium bisulfite (0.1 mL, 10 mg/mL) and neutralized with of 0.4 N sodium bicarbonate (0.5 mL). The product was extracted with ethyl acetate (3 × 1 mL). The combined organic layers were dried by passing through an anhydrous sodium sulfate column (0.2 cm × 5 cm). The organic solution was evaporated under a stream of nitrogen, and the residue was dissolved in absolute ethanol (50–200 μL). The desired product, [^{125}I]IBZM, was isolated from the unreacted BZM and a small amount of unknown radioactive impurities by HPLC on a reverse phase column (PRP-1, Hamilton Inc.), with an isocratic solvent system: 82:18 acetonitrile–pH 7.0 buffer (10 mM, 3,3-dimethylglutaric acid). After the appropriate fractions were collected, the solvent was evaporated under a stream of nitrogen, and the product was reextracted with ethyl acetate (1 × 3 mL). The solution containing the no-carrier-added product was condensed to dryness and redissolved in absolute ethanol (radiochemical purity >95%, overall yield 60%). This agent was used in the binding studies after dilution with saline.

Tissue Preparation. Male Sprague–Dawley rats (200–250 g) were decapitated, and the brains were removed and placed in ice. Striatal tissues were excised, pooled, and homogenized in 100 volumes (w/v) of ice-cold Tris·HCl buffer (50 mM), pH 7.4. The homogenates were centrifuged at 20000*g* for 20 min. The resultant pellets were rehomogenized in the same buffer and centrifuged again. The final pellets were resuspended in assay buffer containing 50 mM Tris buffer, pH 7.4, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 1 mM $MgCl_2$.

Binding Assays. The binding assays were performed by incubating 50 μL of tissue preparations containing 40–60 μg of protein with appropriate amounts of [^{125}I]IBZM ligand and competitors in a total volume of 0.2 mL of the assay buffer. After an incubation period of 15 min at 37 °C (with stirring), the samples were rapidly filtered in the cell harvester (Brandel M-24R) under vacuum through Whatman GF/B glass fiber filters pretreated with 0.2% poly-L-lysine and washed with cold (4 °C) 50 mM Tris·HCl buffer, pH 7.4 (3 × 5 mL). The nonspecific binding was obtained in the presence of 10 μM spiperone. The filters were counted in a γ counter (Beckman 5500) at an efficiency of 70%.

Data Analysis. Both Scatchard and competition experiments, were analyzed by using the iterative nonlinear least-squares curve-fitting program LIGAND.[26]

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Registry No. **1a**, 84226-06-2; [^{125}I]-**1a**, 113452-80-5; **1b**, 113452-78-1; [^{125}I]-**1b**, 113452-81-6; **1c**, 113452-77-0; **1d**, 113452-79-2; **2a**, 84226-04-0; **2b**, 113452-75-8; **2c**, 113474-70-7; **3**, 1466-76-8; **5a**, 96947-76-1; **5b**, 113531-33-2; **6**, 3147-64-6; **7**, 113452-76-9; (*S*)-(−)-*N*-ethyl-2-(aminomethyl)pyrrolidine, 22795-99-9; (*R*)-(+)-*N*-ethyl-2-(aminomethyl)pyrrolidine, 22795-97-7.

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Synthesis and Biodistribution of ^{99m}Tc-Labeled Piperidinyl Bis(aminoethanethiol) Complexes: Potential Brain Perfusion Imaging Agents for Single Photon Emission Computed Tomography

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In developing clinically useful ^{99m}Tc-labeled radiopharmaceuticals for the evaluation of regional cerebral perfusion with single photon emission computed tomography (SPECT), a number of substituted alkyl(aryl)piperidinyl bis(aminoethanethiol) ligands for chelating [^{99m}Tc]TcO(III) were synthesized. Each ligand forms two diastereomers, syn and anti, after reacting with a racemic mixture of the ligand. The diastereomers were separated by high-pressure liquid chromatography. In biodistribution studies conducted in rats, the diastereomers exhibit widely disparate brain uptake values; however, this disparity seems to diminish as the steric bulk of the substituent at the C-4 position of the piperidinyl moiety increases. Furthermore, all the complexes evaluated failed to show a prolonged retention in the rat brain, suggesting that further structural modification may be necessary to obtain clinically useful complexes from this class of compounds.

The basic thrust of the second generation of brain-imaging agents is the evaluation of changes of brain perfusion and metabolism in the living brain (with intact blood/brain barrier). The potential diagnostic utility of brain perfusion imaging lies in the recognition that functional imbalance, which may be manifested as a perfusion abnormality, generally precedes anatomic manifestations of disease. The ideal brain perfusion imaging agents are expected to exhibit characteristics that should include the following: (a) extraction, which is linearly proportional to a flow over a wide range of blood flow; (b) blood brain barrier penetrability (which in many cases is the result of lipid solubility); (c) high initial brain uptake; (d) prolonged brain retention; (e) a fixed regional distribution–no change from its original perfusion pattern; and (f) a high brain/blood ratio. In conjunction with tomographic modalities such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), a neutral, lipid-soluble, radiolabeled molecule could be utilized to evaluate regional cerebral perfusion. Such molecules have, in fact, been synthesized and evaluated. For SPECT, these are notably *N*,*N*,*N*^{1}-trimethyl-*N*^{1}-(2-hydroxyl-3-methyl-5-[^{123}I]iodobenzyl)-1,3-propanediamine ([^{123}I]HIPDM) and *N*-isopropyl-*p*-[^{123}I]iodoamphetamine ([^{123}I]IMP).[1-4] Both compounds have been used with SPECT in the evaluation

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