Preparation and Biodistribution of 1-[2-(3-[^{125}I]Iodo-4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine and 1-[2-(3-[^{125}I]Iodo-4-azidophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine

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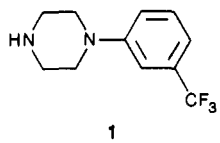
The iodinated analogue of 1-[2-(4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (PAPP), IPAPP (4), and the corresponding azido compound azido-IPAPP (5) were synthesized. The corresponding no-carrier-added ^{125}I ($T_{1/2}$ = 60 days, 35–60 keV) labeled compounds were also prepared. High specific binding was observed from in vitro binding studies using rat brain tissue preparation; K_i = 20 and 17.5 nM against [^{3}H]-5-HT. In vivo biodistribution studies in rats showed that azido-[^{125}I]IPAPP passed through intact blood–brain barrier and localized in the brain. Ex vivo autoradiography of rat brain sections exhibited a diffuse uptake pattern, which may be due to specific and nonspecific binding. The results indicate that IPAPP and azido-IPAPP may not be suitable to image the serotonin receptor in the brain.

Recently, 1-[3-(trifluoromethyl)phenyl]piperazine, TFMPP (1),[1,2] and its related compounds, 1-[2-(4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine, PAPP (2), and 1-[2-(4-azidophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine, azido-PAPP (3), have been



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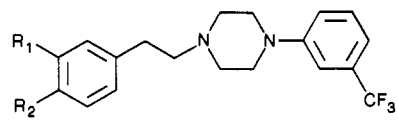

	R_1	R_2
2	NH_2	H
3	N_3	H
4	NH_2	I
5	N_3	I

employed as specific binding agents for the serotonin 1A receptor.[3-6] The azido compound labeled with ^{3}H displayed good affinity with the serotonin receptor; however, the limited specific activity of tritium (~85 Ci/mmol) prevents the further characterization of the receptor with this tracer. In order to improve the specific activity, we have chosen to use ^{125}I as the tracer isotope ($T_{1/2}$ = 60 days, max sp ac. 2200 Ci/mmol). In addition, the corresponding ^{123}I ($T_{1/2}$ = 13 h, 159 keV) labeled compounds may also be potentially useful for external imaging of the same serotonin receptor in vivo. The imaging procedure may provide a clinical tool for evaluation of the sertonin receptor in normal and disease stages. The iodinated analogue of PAPP, 1-[2-(3-iodo-4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine, IPAPP (4), and the corresponding azido compound azido-IPAPP (5) were synthesized. The no-carrier-added ^{125}I ($T_{1/2}$ = 60 days, 35–60 keV) labeled compounds 7 and 8 were also prepared. The in vivo biodistribution in rats and in vitro binding were evaluated.

Chemistry

The uniodinated compound 2 was prepared by reactions shown in Scheme I. The amide 6 was prepared by condensation of *p*-nitrophenylacetic acid with 1-[*m*-(trifluoromethyl)phenyl]piperazine via an activated ester (method A) or acyl chloride intermediate (method B). Method A employed DCC as the coupling agent for the synthesis of succinimido ester, while method B[3] used oxalyl chloride for the formation of activated acyl chloride. The latter gives a higher yield (80%). The amide 6 was then treated with borane/THF to reduce the keto functional group, followed by another reduction step with stannous chloride in hydrochloric acid/95% ethanol to convert the aromatic nitro compound to amine 2. The final product, IPAPP (4), was prepared by reacting 2 with iodomonochloride in acetic acid in good yield (>62%) (Scheme II). After diazotization of amine 4, the diazo group was displaced with an azide to give 5 (yield 23.4%).

Table I. Partition Coefficient of [^{125}I]IPAPP and Azido-[^{125}I]IPAPP (Octanol/Buffer)

pH	[^{125}I]IPAPP	azido-[^{125}I]IPAPP
7	4101 ± 740	1909 ± 710
7.4	4872 ± 1571	2125 ± 218

Radiolabeling

Radioactive labeling of IPAPP (7) was accomplished by mixing sodium [^{125}I]iodide (no-carrier-added, ~2000 Ci/mmol) and Chloramine-T at pH 3 (Scheme II). The product 7 was compared with chemically pure nonradioactive 4 by HPLC using simultaneous UV and radioactivity detection. It was determined to be the desired product on the basis of the same elution profile coeluted with the authentic cold compound (Figure 1). The radiolabeling yield was 60–70%. The product was purified by HPLC using a reverse-phase column and an acetonitrile/buffer solvent mixture to remove the uniodinated starting material. The radiochemical purity of 7 was greater than 95% and the specific activity ~2000 Ci/mmol.

The carrier-free azido-[^{125}I]IPAPP (8) was synthesized in the same manner as that for the synthesis of nonradioactive 5, the only difference being that the carrier-free [^{125}I]IPAPP (7) was used as the starting material. The radiolabeled 7 was diazotized and the diazo group was displaced with an azide to produce 8 (yield 50–70%). The

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Table II. Biodistribution of [^{125}I]IPAPP and Azido-[^{125}I]IPAPP in Rats after an Intravenous Injection

	% dose/organ, av of three rats[a]					
	[^{125}I]IPAPP			azido-[^{125}I]IPAPP		
organ	2 min	15 min	30 min	2 min	15 min	30 min
blood	6.99 (5.70–9.10)	2.23 (2.05–2.33)	1.96 (1.80–2.25)	9.74 (8.96–11.2)	3.21 (2.77–3.50)	2.76 (2.23–3.05)
muscle	6.51 (4.05–11.1)	13.6 (11.6–16.5)	11.8 (10.1–12.9)	11.8 (9.89–13.6)	16.6 (14.0–20.5)	14.4 (11.4–16.4)
heart	2.34 (1.70–3.54)	0.309 (0.277–0.329)	0.262 (0.214–0.358)	1.40 (1.26–1.57)	0.548 (0.542–0.557)	0.372 (0.274–0.493)
lungs (2)	14.2 (11.3–19.8)	3.15 (2.67–3.54)	4.16 (2.51–5.16)	6.50 (4.68–8.01)	2.15 (1.65–2.40)	1.73 (1.38–2.21)
liver	13.1 (8.70–17.0)	19.4 (19.0–19.9)	18.9 (17.6–20.2)	32.5 (30.1–36.1)	34.3 (28.9–37.8)	28.5 (26.8–29.5)
brain	1.68 (1.27–2.45)	0.910 (0.811–1.06)	0.935 (0.742–1.19)	0.503 (0.148–0.578)	0.667 (0.599–0.765)	0.750 (0.599–0.881)
brain/blood ratio	2.63	4.92	4.76	0.499	1.99	2.53

[a] % dose/gram.

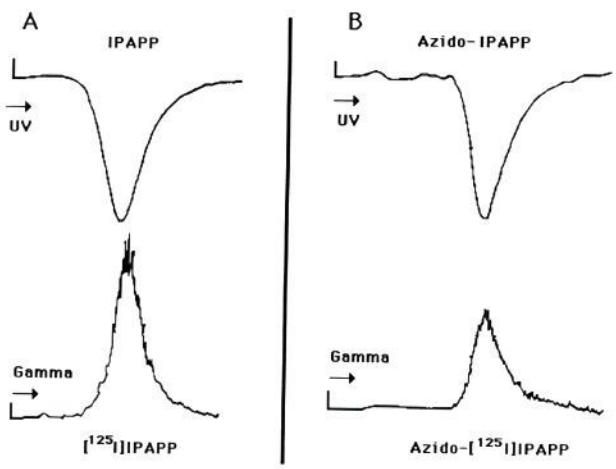

Figure 1. HPLC profiles of **A** (compounds **5** and **8**) and **B** (compounds **4** and **7**). Both of the UV and γ tracings show very similar profiles, indicating that the coeluted "cold" and radioactive compounds are identical.

radiochemical purity was again greater than 95% as indicated in Figure 1.

Biodistribution and Autoradiography

As expected, IPAPP (**7**) and azido-IPAPP (**8**) displayed high lipid solubility (Table I). After an iv injection, both agents showed good initial brain uptake, with a total brain uptake of 1.68 and 0.5% dose/organ at 2 min for [^{125}I]-IPAPP and azido-[^{125}I]IPAPP, respectively (Table II). At later time points (15 and 30 min) [^{125}I]IPAPP displayed ~30% washout from the brain, while the corresponding azido compound showed a dramatic increase of brain uptake. It is likely that azido-[^{125}I]IPAPP may form an irreversible covalent bond with components of brain tissue, while [^{125}I]IPAPP was simply washed out of the brain. The brain to blood ratio for both compounds increased with time, but azido-[^{125}I]IPAPP showed more prominent increases, indicating that the irreversible binding may be contributing to the retention in the brain tissue.

In a similar series of compounds for dopamine receptor binding, azido-[^{125}I]iodoclebopride showed higher brain uptake and retention in vivo than those for iodoclebopride.[7] The same type of irreversible binding to the brain tissue was attributed to the brain retention, most likely due to the presence of an azido group.

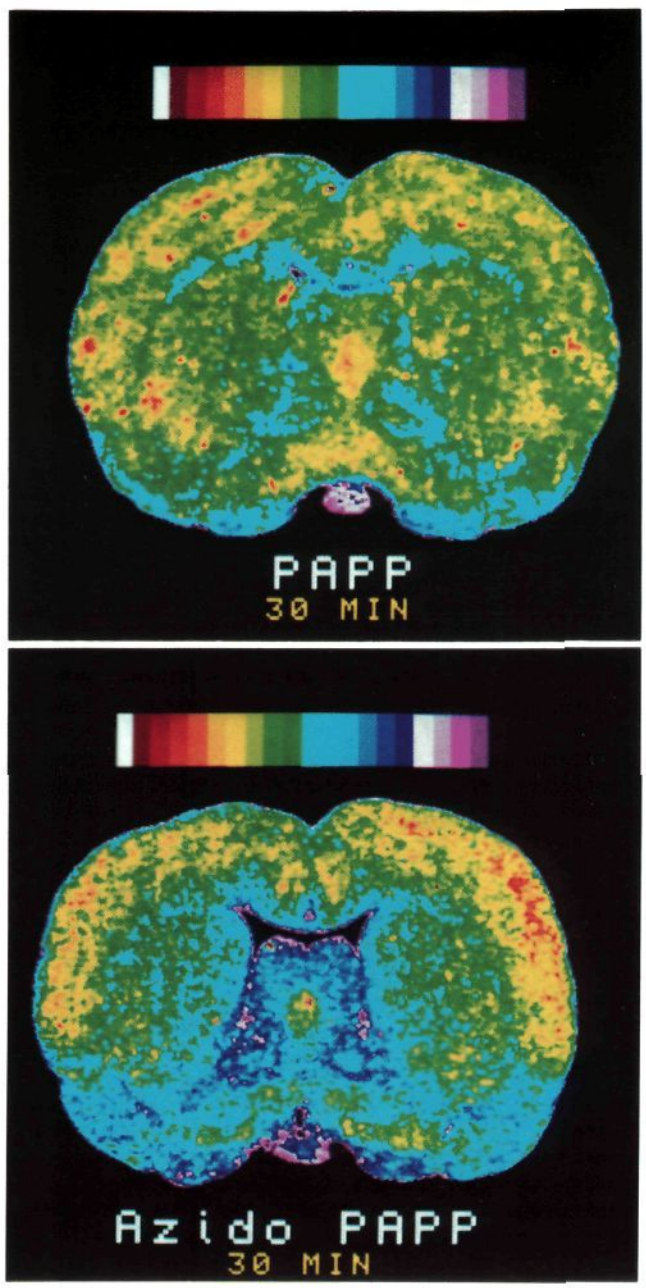

Figure 2. Autoradiographs of ex vivo brain sections of rats injected with [^{125}I]IPAPP and azido-[^{125}I]IPAPP.

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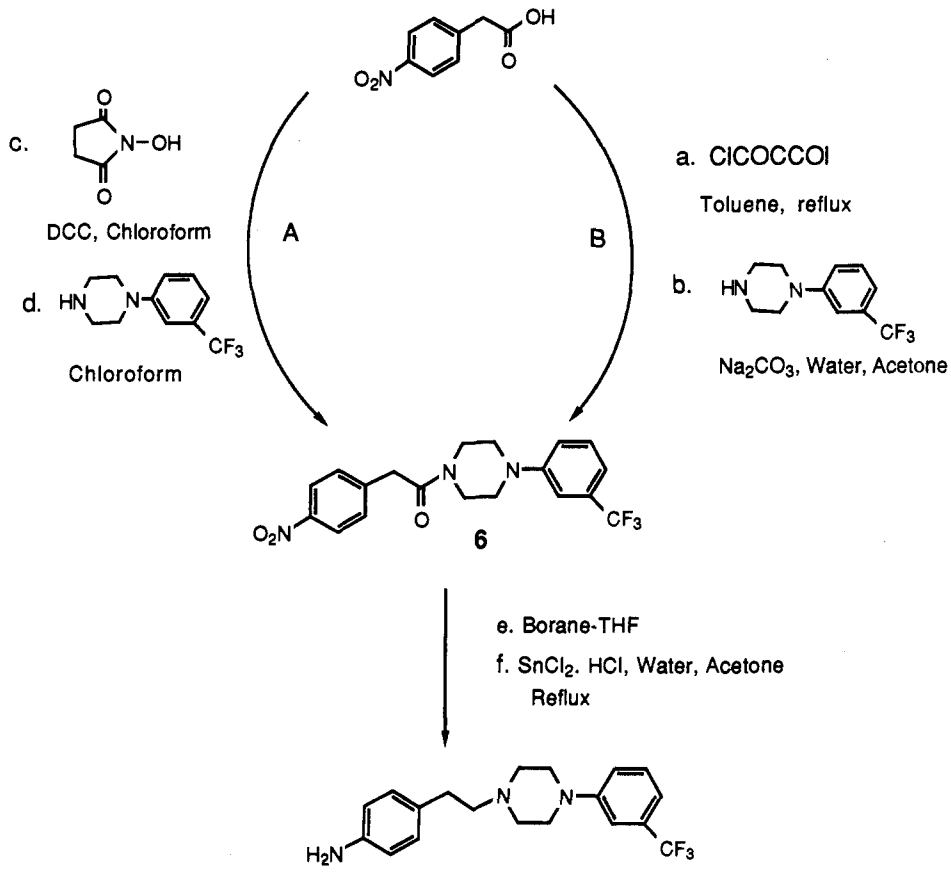
Scheme I



No specific binding and little regional differential localization was observed by autoradiography of rat brain sections after iv injection of azido-[125I]IPAPP. These data suggest that the nonspecific binding (probably due to the high lipid solubility of the compound) is the key factor in determining the regional distribution in the brain. The results strongly indicate that the binding may not be specific to the serotonin receptor. Additional biological studies are needed to further characterize the in vivo binding properties of this agent in the brain.

In Vitro Binding

In vitro binding studies (Table III) show that azido-IPAPP and IPAPP had similar potencies to displace specific [3H]DPAT binding. However, the potency was decreased about 4-fold when compared with PAPP. Nevertheless, they still remain as selective ligands to the 5-HT-1A receptor (because [3H]DPAT is a specific ligand for the 5-HT-1A receptor).

The 5-HT-1 binding characteristics in bovine hippocampus are very similar to that of the rat brain. Therefore, similar displacement of [3H]-5-HT and [3H]DPAT binding by azido-IPAPP and amino-IPAPP would be expected. It may be possible that there is significant nonspecific binding both in vitro and in vivo; however, in vitro binding experiments better characterize the receptor–ligand interaction.

In conclusion, a potential irreversible binding agent for CNS serotonin A receptor, azido-[125I]IPAPP (8), was prepared and characterized. Both IPAPP (7) and azido-IPAPP (8) showed high lipid solubility. However, high nonspecific binding was also seen. High specific binding was observed from in vitro binding studies; K_i = 20 and 17.5 nM against [3H]-5-HT. In vivo biodistribution studies in rats showed that the brain uptake of azido-[125I]IPAPP increased with time, suggesting irreversible trapping in brain tissue. Ex vivo autoradiography of rat brain sections exhibited a diffuse uptake pattern, which may be due to specific and nonspecific binding. More experiments are needed to characterize these compounds further.

Table III. In Vitro Binding Study (Bovine Hippocampus)

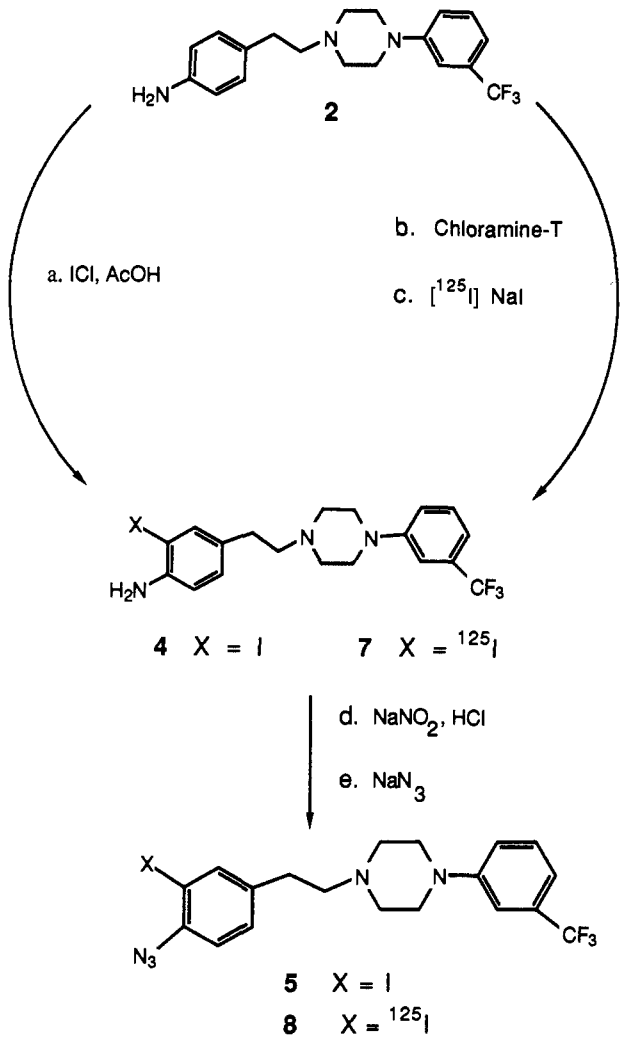
	IC_{50}, nM	K_i, nM
Displacement of [3H]-5-HT (2 nM)		
PAPP	8.1	4.0
azido-PAPP	5.0	2.5
5-HT	5.6	2.8
azido-IPAPP	35.0	17.5
IPAPP	40.0	20.0
Displacement of [3H]DPAT (1 nM)		
PAPP	10.0	5.0
azido-PAPP	3.1	1.5
5-HT	6.0	3.0
azido-IPAPP	40.5	20.3
IPAPP	38.0	19.0
Displacement of Azido-[125I]IPAPP (0.25 nM)		
DPAT	2.4	
5-HT	2.0	

Experimental Section

Proton NMR spectra were recorded on a Varian EM360A spectrometer. The chemical shifts were reported in ppm downfield from an internal tetramethylsilane standard. Infrared spectra were obtained with either a Perkin-Elmer Model 727B spectrometer or a Mattson Polaris FT-IR spectrometer. Melting points were determined on a Meltemp apparatus and are reported uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., of Atlanta, GA, and were within 0.4% of the theoretical values.

1-[2-(4-Nitrophenyl)acetyl]-4-[3-(trifluoromethyl)-phenyl]piperazine (6). Method A. The mixture of 4-nitro-

Scheme II



phenyl acetic acid (9.05 g, 0.05 mol) and *N*-hydroxysuccinimide (5.75 g, 0.05 mol) in chloroform (150 mL) was cooled to ca. 4 °C in an ice bath, and 1,3-dicyclohexylcarbodiimide (10.32 g 0.05 mol) was added to the mixture. The reaction mixture was stirred overnight at room temperature. The mixture was then washed with saturated sodium bicarbonate solution, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to give an orange solid. The crude solid was dissolved in chloroform (100 mL) and was added to TFMPP (1). The mixture was stirred overnight at room temperature. The reaction mixture was washed with 10% sodium bicarbonate solution and water, dried, and concentrated under reduced pressure. The red solid was chromatographed on a column (silica gel, ethyl acetate) to give a pure product of **6** (21%). The NMR and IR spectra of the compound matched those of **6** in the literature.[3]

Method B. Compound **6** was prepared according to the literature procedure.[3]

1-[2-(4-Nitrophenyl)ethyl]-4-[3-(trifluoromethyl)-phenyl]piperazine. The amide **6** (5.1 g, 13.1 mmol) in dry THF was added dropwise to a 1 M solution of borane/THF (38.7 mL, 38.7 mmol). The mixture was stirred for 24 h at room temperature and was cooled in an ice bath. The 2 M hydrochloric acid (23.5 mL) was slowly added to the cooled reaction mixture. The mixture was concentrated and the residue dissolved in 6 M hydrochloric acid (70.0 mL). The mixture was refluxed for 1 h and was basicified with 5 M sodium hydroxide with cooling. The alkaline mixture was extracted with ether. The combined extracts were washed with water and saturated sodium chloride solution and dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure to give 4.4 g (89%) of the desired product, which was used in the next step without purification.

1-[2-(4-Aminophenyl)ethyl]-4-[3-(trifluoromethyl)-phenyl]piperazine (2). A solution of stannous chloride (9.6 g, 50.0 mmol) in concentrated hydrochloric acid (18.2 mL) was added to a solution of nitropiperazine (4.7 g, 12.4 mmol) in 95% ethanol with stirring. The mixture was heated under reflux for 3 h at which time no starting material was detected (TLC, 95:5 chloroform/ethyl acetate). After cooling to room temperature, the solution was made basic with 5 M sodium hydroxide and extracted three times with ether. The combined extracts were washed with water, dried over anhydrous sodium sulfate, and concentrated to give an oil, 3.85 g (88.9%). The oil of **8** was made into the hydrochloride salt and recrystallized with ethanol. The NMR and IR spectra of the compound matched those of **8** in the literature.[3]

1-[2-(3-Iodo-4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (4). PAPP (**2**; 2.10 g, 6.00 mmol) was added to a solution of iodomonochloride (0.97 g, 6.00 mmol) in acetic acid (75 mL). The dark red mixture was stirred at room temperature for 3.5 h (at longer reaction times the diiodinated product was also isolated). The mixture was poured into water and the aqueous layer was extracted three times with dichloromethane. The combined extracts were washed twice with a saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate and concentrated on a rotary evaporator to produce a dark red oil. Purification by column chromatography (silica gel, ethyl acetate) produced 1.79 g (62.60%) of a slightly brown solid: mp 126–128 °C; IR (KBr) 3400–3250 (br, NH_2), 2750–2700 (s, CH_2), 1540, 1410, and 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.18–6.20 (m, 7 H, ArH), 5.72 (br, 2 H, NH_2), 3.21–1.90 (m, 12 H, CH_2). Anal. Calcd for $C_{19}H_{21}F_3IN_3$: C, H, N.

1-[2-(3-Iodo-4-azidophenyl)ethyl]-4-[3-(trifluoromethyl)-phenyl]piperazine (5). The mixture of **4** (1.79 g, 3.80 mmol), water (10 mL) and hydrochloric acid (1 mL) was cooled to 0–5 °C with stirring. A solution of sodium nitrite (0.28 g) in water (2 mL) was added dropwise to the cooled mixture and the diazotization reaction was stirred for 1 h at 0–5 °C. A solution of sodium azide (0.25 g) in water (2 mL) was then added to a reaction mixture. After 30 min the product was filtered and recrystallized from ethanol/water to give 0.45 g (23.40%) of product: mp 155–158 °C decomposed; FT-IR (KBr) 2122 cm^{-1} (s, N_3); ^1H NMR ($CDCl_3$) δ 7.25–6.12 (m, 7 H, ArH), 3.80–2.48 (m, 12 H, CH_2); UV (CH_3OH) 275. Anal. Calcd for $C_{19}H_{19}F_3IN_5 \cdot HCl$: C, H, N.

Radiolabeling. 1-[2-(3-[^{125}I]Iodo-4-aminophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (7). Chloramine-T solution (50 mL, 1 mg/mL) was added to a mixture of **2** (20 mL, 1 mg/mL), sodium [^{125}I]iodide (20 mL, 1–5 mCi, no-carrier-added, sp act. 2200 Ci/mmol), and pH 3 phosphate buffer (0.3 mL) in a sealed vial. The reaction mixture was allowed to stand at room temperature for 1–5 min. Sodium bisulfite solution (50 mL, 1 mg/mL) was added to terminate the reaction and to reduce unreacted radioiodine. To neutralize the mixture, 0.1 M sodium bicarbonate solution (0.4 mL) was added. The mixture was extracted three times with chloroform. The combined organic layers were dried by being passed through an anhydrous sodium sulfate column (0.2 cm × 5.0 cm). The organic solution was concentrated under a nitrogen stream. The desired product **7** was purified by HPLC on a reverse-phase column (PRP-1, Hamilton Inc.), using an isocratic solvent system: 85:15 acetonitrile/pH 7 buffer (10 mmol, 3,3-dimethylglutaric acid). The product fractions were partially concentrated and extracted three times with chloroform. The combined chloroform layers were washed once with water, dried, and concentrated to give **7**, which was redissolved in absolute ethanol. The product yield was 56.3% and radiochemical purity was more than 95%. This agent was used in the biological studies and in the preparation of **8**.

1-[2-(3-[^{125}I]Iodo-4-azidophenyl)ethyl]-4-[3-(trifluoromethyl)phenyl]piperazine (8). The mixture of carrier-free **7** (0.2 mL, 1 mCi/ethanol), hydrochloric acid solution (0.4 mL, 36%), and sodium nitrite solution (0.1 mL, 10 mg/mL) was stirred in a sealed vial at 0 °C for 30 min. A solution of sodium azide (0.1 mL, 10 mg/mL) was added. After 5 min of stirring, the reaction mixture was extracted once with chloroform to eliminate organic impurities. The aqueous layer was then made basic (pH >10) with 0.2 M sodium hydroxide solution and extracted twice with chloroform. The combined organic layers were dried by being passed through an anhydrous sodium sulfate column. The solution was concentrated under a nitrogen stream. The radiochemical purity was 98% (total yield ~50%). This agent was used in the

biological studies.

Biodistribution in Rats. The biodistribution patterns of 7 and 8 were studied in male Sprague–Dawley rats (225–275 g), which were allowed access to food and water ad libitum. A saline solution containing radioligand in a volume of 0.2 mL was injected into the femoral vein while the rats were under ether anesthesia, and the animals were sacrificed by cardiac excision at various time points postinjection. The organs of interest were removed and counted with a Beckman Gamma 4000 automatic counter (at an efficiency ~50%). The percentage dose per organ was calculated by dividing the tissue counts to suitably diluted aliquots of injected material. A dissection procedure for removing the cortex, striatum, hippocampus, and cerebellum was used to determine the regional brain uptake.[8]

Autoradiography. Two Sprague–Dawley rats were each injected intravenously under light ether anesthesia with 0.2 mL of a saline solution containing 2.9 mCi of radioligand. At 30 and 120 min after the injection, the rats were sacrificed, and brain tissue was removed and frozen in embedding medium (Miles Laboratory) with dry ice. After equilibration to −20 °C, consecutive 20-μm coronal sections were cut on a cryostat microtome (American Optical) and thaw mounted on acid-washed microscope slides. The slides were air-dried for 1 h at room temperature. These slides together with 20 m thick ^{125}I standards (Amersham, Arlington Hts, IL) were opposed simultaneously to DuPont X-ray film in an autoradiographic cassette. The appropriately exposed film was developed with a Kodak automatic processor. The optical densities were determined with an image analysis system (Imagitronics S-100) developed by Lear[9] and the relationship between optical density and concentration of tissue radioactivity for various exposure times was determined.

In Vitro Binding. The radioligand binding assay was similar to that described previously by Shih and Young[10] and Muakkassah-Kelly.[11] Bovine brain was purchased from a local slaughterhouse, and the brains were rapidly removed and dissected on ice. The hippocampi were homogenized with a motor-driven pestle in 10–20 volumes of ice-cold 0.32 M sucrose with 10 strokes of a Teflon–glass homogenizer. The homogenate was centrifuged at 750*g* for 10 min at 4 °C, and the resulting supernatant was subsequently centrifuged at 50000*g* for 10 min. The 50000*g* pellet was then resuspended in 40 volumes of 50 mM Tris·HCl, pH 7.4. The tissue suspension was next incubated at 37 °C for 15 min to remove endogenous 5-HT[12] and then centrifuged at 50000*g* for 10 min. Membrane pellets were stored at −70 °C. Protein concentrations were measured by the method of Lowry.[13] The buffer for [^3H]-5-HT and [^3H]DPAT binding assays consists of 50 mM Tris·HCl, pH 7.4, containing 5 mM $MnCl_2$, 1 mM EGTA, 0.1% ascorbic acid, 10 mM pargyline. These assays were carried out in a mixture of 0.1-mL aliquot of tritiated ligand and a 0.8-mL aliquot of membrane suspension, in the presence and absence of 10 μM of nonradioactive ligand in a final volume of 1 mL. The final membrane concentration was approximately 15 mg original wet weight/mL. The ligand concentrations are indicated in the text. Assay tubes were mixed and incubated for 10 min with shaking at 37 °C followed by filtration using a Brandel Cell Harvester M-24R and glass fiber filter sheets (Schleicher and Schuell no. 32). The contents of each assay tube were diluted to 6 mL with cold wash buffer (1:5 dilution of incubation buffer), filtered, and then washed two times with 6 mL of wash buffer. Filter sheets were dried and the individual filters were placed in 6 mL of Liquiscint (National Diagnostics) for determination of radioactivity by liquid scintillation spectrometry. Azido-[^{125}I]-IPAPP binding experiments were performed as described for [^3H]-5-HT except that 0.1% bovine serum albumin (BSA) was included in the incubation buffer to reduce nonspecific filter binding of the radioligand. It was found that inclusion of BSA in the incubation mixture was more effective and more economical for reducing this nonspecific binding than using only wash buffer. At this concentration, BSA had no effect on either specific [^3H]-5-HT or azido-[^{125}I]IPAPP binding or on the displacement of either radioligand by any compound studied. In addition, BSA did not alter the time required to reach equilibrium at 37 °C. Specific azido-[^{125}I]IPAPP binding was defined by the difference between the binding in the absence and presence of 10 μM 5-HT.

Registry No. 2, 1814-64-8; 4, 118249-07-3; 5, 118249-08-4; 7, 118249-09-5; 8, 118249-10-8; *p*-nitrophenylacetic acid, 104-03-0; 1-[*m*-(trifluoromethyl)phenyl]piperazine, 15532-75-9.

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