Synthesis and Binding Characteristics of Potential SPECT Imaging Agents for σ-1 and σ-2 Binding Sites

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2-, 3-, and 4-iodophenyl derivatives of the high-affinity σ ligand *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (1) were synthesized in two to four steps starting from *N*-methyl-2-(1-pyrrolidinyl)ethylamine. These compounds were evaluated for their capacity to label both σ_1 and σ_2 subtypes in vitro. σ-1 binding affinity was determined by measuring competition with [^{3}H]-(+)-pentazocine binding to guinea pig brain membranes while σ_2 binding was evaluated through competition with [^{3}H]DTG binding to rat liver membranes in the presence of excess dextrallorphan. The binding data revealed that *N*-[2-(3-iodophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (2) and *N*-[2-(4-iodophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (3) displayed almost identical binding affinity at σ_1 sites to the parent compound 1. This suggests that the 3- or 4-iodo group can effectively substitute for the 3,4-dichloro substituents of 1. In this series of compounds, K_i's at the σ_1 site varied from 2.0 nM for *N*-(4-iodobenzyl)-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (6) to 26.6 nM for *N*-(2-iodobenzyl)-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (4). K_i's for σ_2 site ranged from 8.1 nM for 1 to 220 nM for *N*-(3-bromobenzyl)-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (11) while the σ_2/σ_1 ratio varied from 1.8 for 4 to 25 for 11. Comparing halogen substitution, the trend Cl = I > Br > F was observed for binding affinity at σ_1 sites; no such trend was observed at σ_2 sites. On the basis of the binding data, compounds 2 and 3 were selected for labeling with ^{123}I. Thus, treatment of the corresponding 3- and 4-(tributylstannyl) intermediates (7 and 8) with Na^{123}I in the presence of excess CH_3CO_3H furnished [^{123}I]-2 and [^{123}I]-3 in up to 70% radiochemical yield. Preliminary in vitro binding with [^{123}I]-3 indicated up to 97% specific binding with guinea pig brain membranes.

Introduction

Positron emission computerized tomography (PET) and single photon emission computed tomography (SPECT) ligands have been successfully employed for the study of CNS receptors such as benzodiazepine,[1] dopamine,[2] muscarinic,[3] and opiate.[4] Although of comparable resolution,[5] SPECT offers several advantages over PET which include commercial availability of ^{123}I and adaptability to the small hospital or clinic. Until very recently,[6] no ligands were available for PET/SPECT imaging of σ sites. We therefore wished to develop novel σ ligands amenable to high specific activity labeling with ^{123}I as potential SPECT imaging agents for these sites.

Sigma sites are high affinity, saturable, stereospecific binding sites found both in the CNS and the periphery; they have been the focus of intense study in recent years because of their potential to offer new insights into the mechanisms underlying psychoses,[7] movement disorders,[8] and neurodegeneration[9] with which they have been implicated (for an exhaustive review, see ref 8). At the time of their discovery, σ sites were confused with opiate and later with phencyclidine receptors but have since been shown to be distinct from either of these sites.[8] Evidence has been presented for at least two σ binding subtypes, termed σ_1 and σ_2, which may mediate the physiological and pharmacological effects of structurally diverse σ ligands.[10]

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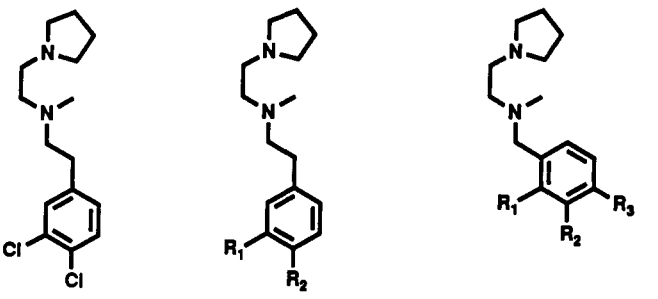
Chart I



1

2: R_1=I, R_2=H
3: R_1=H, R_2=I
7: R_1=$SnBu_3$, R_2=H
8: R_1=H, R_2=$SnBu_3$

4: R_1=I, R_2,R_3=H
5: R_1,R_3=H, R_2=I
6: R_1,R_2=H, R_3=I

The availability of σ specific SPECT ligands that are amenable to labeling to high specific activity (with ^{123}I) would provide tools for investigating the functional characteristics of this binding site in vivo. At the same time, such agents would allow clinical correlates to be formulated through comparison of differences in σ binding site distribution and density in normal versus disease states.

We recently reported the design, synthesis, and biological evaluation of a series of *N*-(arylethyl)ethylenediamines many of which showed exceptionally high affinity (subnanomolar K_i's) for σ binding sites labeled by [^{3}H]-

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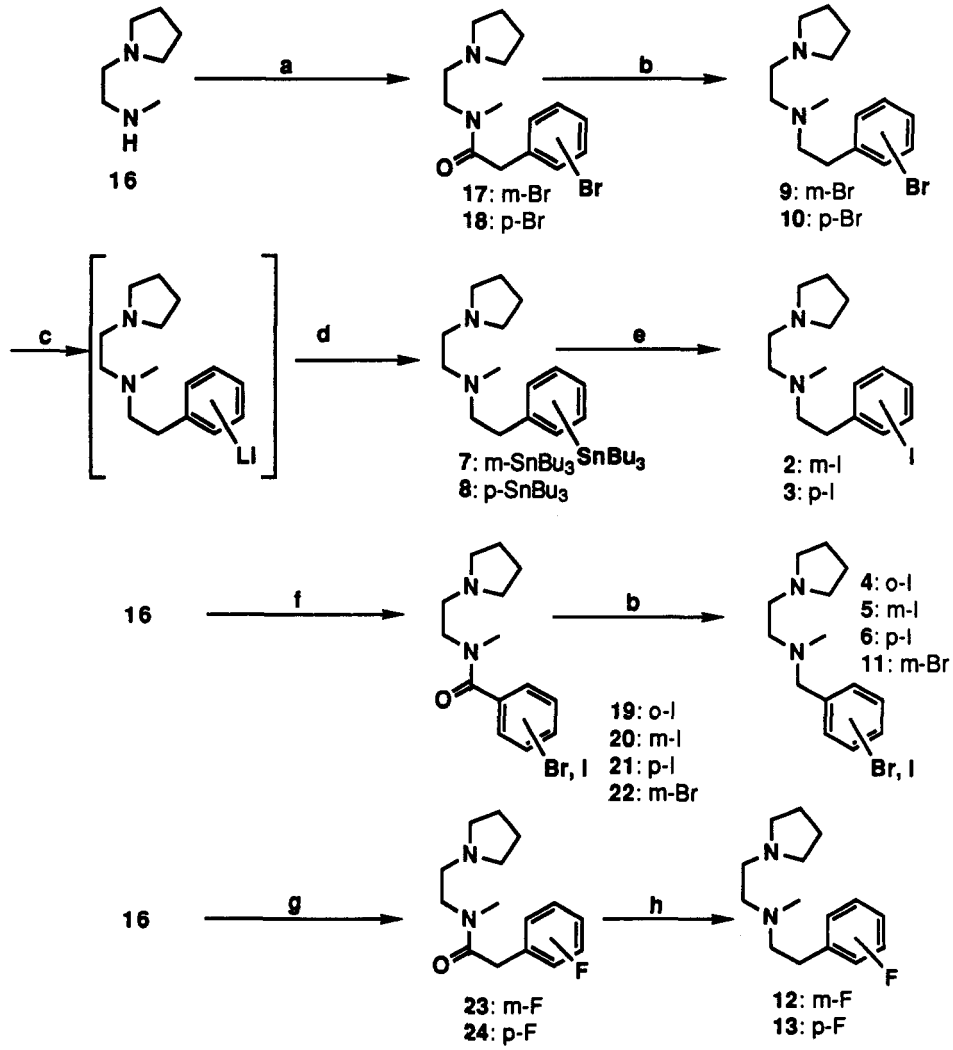
Table I. Physical and Chemical Data

no.	salt	method	yield (%)[e]	cryst solvent	mp, °C	CIMS *m/z* (MH^+)	anal.[c]
2[f]	2HBr	D	46.7	EtOH	233–235 dec	359	$C_{15}H_{25}Br_2IN_2$
3[f]	2HBr	D	47.1	EtOH	247–247.5 dec	359	$C_{15}H_{25}Br_2IN_2$
4[f]	2HBr[a]	B	54.3	iPrOH	216–217	345	$C_{14}H_{23}Br_2IN_2$
5[f]	2HBr	B	77.1	EtOH	232–233	345	$C_{14}H_{23}Br_2IN_2$
6[f]	2HBr	B	76.1	EtOH	245–245.5	345	$C_{14}H_{23}Br_2IN_2$
7[f]	base	C	76.8	oil[b]	oil	*d*	$C_{27}H_{50}N_2Sn$
8[f]	base	C	46	oil[b]	oil	*d*	$C_{27}H_{50}N_2Sn$
9	2HBr	B	38.9	EtOH	245–246	311	$C_{15}H_{25}Br_3N_2$
10	2HBr	B	84.7	EtOH	244–244.5	311	$C_{15}H_{25}Br_3N_2$
11	2HBr	B	92.6	EtOH	237–239	297	$C_{14}H_{23}Br_3N_2$
12[f]	2HBr	E	65	iPrOH	248.5–249.5 dec	251	$C_{15}H_{25}Br_2FN_2$
13[f]	2HBr	E	53	iPrOH	248–248.5 dec	251	$C_{15}H_{25}Br_2FN_2$
17[f]	fumarate	A	86.4	iPrOH	119–120	325	$C_{19}H_{25}BrN_2O_5$
18	base	A	67.8	i-octane	70–71.5	325	$C_{15}H_{21}BrN_2O$
19	oxalate	A	62.7	EtOH	154–155	359	$C_{16}H_{21}IN_2O_5$
20[f]	fumarate	A	90.4	iPrOH	141–143	359	$C_{18}H_{23}IN_2O_5$
21	base	A	70.5	i-octane	109–110.5	359	$C_{14}H_{19}IN_2O$
22	oxalate	A	95.7	iPrOH	155–156	311	$C_{16}H_{21}BrN_2O_5$
23	oxalate	A	79	EtOAc	144–145	265	$C_{17}H_{23}FN_2O_5$
24	fumarate	A	90	EtOAc	152.5–153	265	$C_{19}H_{25}FN_2O_5$

[a] This compound was purified by silica gel chromatography prior to salt formation. [b] Purified by silica gel chromatography prior to the next reaction step. [c] Elemental compositions (%) were found to be within ±0.4% of the theoretical values for C, H, and N. [d] These compounds failed to yield molecular ions by chemical ionization mass spectrometry. [e] All reported yields are nonoptimized. [f] See ref 28 for ^{1}H-NMR data.

Scheme I.[a] Synthesis of Halogenated/Iodo-Substituted Ethylenediamines as SPECT Probes for σ Binding Sites



[a] (a) *m*- or *p*-bromophenylacetic acid, DCC, CH_2Cl_2, 20 °C; (b) AlH_3, THF, 20 °C, 5–20 min; (c) *n*-BuLi, THF, −78 °C; (d) Bu_3SnCl, THF, −78 °C; (e) I_2, $CHCl_3$; (f) *o*-, *m*-, *p*-iodobenzoic or *m*-bromobenzoic acids, DCC, CH_2Cl_2, 20 °C; (g) *m*- or *p*-fluorophenylacetic acid, DCC, CH_2Cl_2, 20 °C; (h) $LiAlH_4$, THF, reflux.

(+)-3-PPP.[11] All of the ligands in this series exhibited no significant cross-reactivity with those receptors, most notably dopamine-D_2, phencyclidine, and kappa opiate, that bind with and limit the utility of most σ ligands.[11,22]

The high affinity and selectivity of these ethylenediamines for σ binding sites has identified them as a base suitable for development of σ SPECT imaging agents. The relatively high polarity of these ligands may lower nonspecific binding which is an advantage in central nervous system (CNS) receptor imaging.

Compound 1 (Chart I) (K_i = 0.34 nM against [^{3}H]-(+)-3-PPP and 2.1 nM against [^{3}H]-(+)-pentazocine) proved to be among the most potent and selective σ ligands in the ethylenediamine class.[11,22] We therefore selected this compound as a template for the development of novel agents for σ SPECT (Scheme I and Table I). Another consideration in the selection of 1 as a template is the very high selectivity of this series of compounds and negligible cross-reactivity with opiate, phencyclidine, and cholinergic receptors.[11] We report here the synthesis, radiosynthesis, and in vitro σ binding characteristics (Table II) of monoiodinated ethylenediamines 2–6 (Chart I) to assess their suitability as SPECT agents. We also report the effect of the halogen of 2 and 3 on σ subtype selectivity and potency. [^{3}H]-(+)-Pentazocine was utilized for labeling of the σ_1 subtype in guinea pig brain homogenates[23,24] and [^{3}H]DTG in the presence of excess dextrallorphan for the σ_2 subtype in rat liver membranes.[16,17,25]

Finally, we investigate the synthesis and radiolabeling of 2 and 3 (Scheme II) to high specific activity with ^{123}I (via iodine/tin exchange[12] of tributyltin precursors 7 and 8) (Chart I). Our main assumption in this study was that an iodine atom would substitute effectively for chlorine in these compounds.

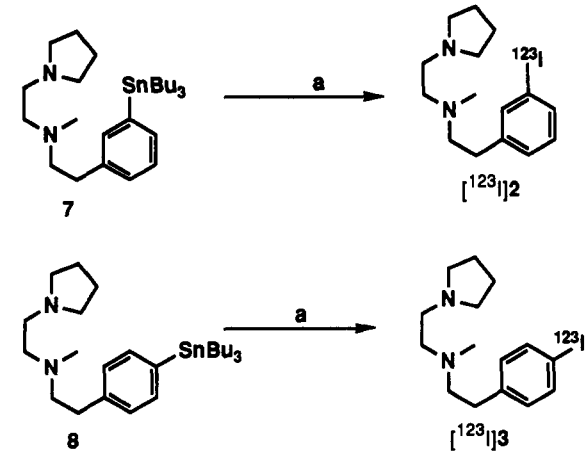
Chemistry

N-Methyl-2-(1-pyrrolidinyl)ethylamine (16) was synthesized as described previously[11] and used as precursor for all of the compounds reported herein (Schemes I and II). 1,3-Dicyclohexylcarbodiimide (DCC) coupling of this compound with *m*- and *p*-bromophenylacetic acids afforded the corresponding bromo acetamides 17 (86%) and 18 (68%) (see Table I). Iodobenzamides 19–21 were similarly obtained. Careful (5–10 min at 20 °C) AlH_3 (alane) reduction[13] of iodobenzamides 19–21 afforded *N*-(iodobenzyl)ethylenediamines 4–6 (54–76%). Prolonged reaction times resulted in halogenolysis. The (bromophenyl)acetamides 17 and 18 and bromobenzamide 22 were similarly reduced (20 min or less at 20 °C) to diamines 9, 10, and 11 (39–93%), but proved to be more stable to halogenolysis than the corresponding iodides.

Treatment of bromo intermediates 9 and 10 (Scheme I) with *n*-butyllithium at −78 °C resulted in facile bromine/lithium exchange. It is likely that this exchange reaction is assisted by complexation of *n*-BuLi with the ethylenediamine moiety of these compounds. Quenching of the corresponding lithio derivatives with tributyltin chloride at −78 °C gave the desired tin derivatives 7 and 8 (Scheme I and Table I). Generation of the lithio intermediates at room temperature instead of −78 °C resulted in lowered product yields. Treatment of 7 and 8 with elemental iodine in $CHCl_3$ afforded the desired iodides 2 and 3 (47%) as the major products. Similar treatment of 7 and 8 with Na^{123}I in the presence of CF_3CO_3H (Scheme II) afforded high specific activity [^{123}I]-2 and [^{123}I]-3 in up to 70% radiochemical yield.

The fluoro derivatives 12 and 13 were obtained via $LiAlH_4$ reduction of the intermediate fluoro acetamides 23 and 24. AlH_3 reduction was unnecessary in these cases because of the greater stability of C–F bond to $LiAlH_4$ compared with the other halogens.

Scheme II. [a] Radiochemical Synthesis of [^{123}I]Iodo-Substituted Ethylenediamines as σ SPECT Probes



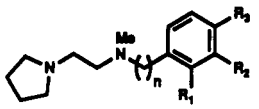
[a] (a) [^{123}I]NaI, aqueous CH_3CO_3H, EtOH, HCl, 5 min, room temperature.

Results and Discussion

A series of (haloaryl)alkyl-substituted ethylenediamines were synthesized based upon the high affinity σ ligand *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (1).[11] The series allowed investigation of the effect of halogen on σ subtype selectivity and potency as well as the selection of targets suitable for labeling with ^{123}I as σ SPECT imaging agents. The data (Table II) reveal that both σ_1 and σ_2 binding are markedly affected by the nature of the halogen substituent. In general, affinity for σ_2 binding sites proved to be lower than those at the σ_1 site. Replacement of the 3,4-dichloro substituents of 1 with a single halogen in the meta or para position as in 2, 3, 10, 14, and 15 resulted in no major change in the affinity at σ_1 sites whereas 9, 12, and 13 showed only slightly lowered affinities. Affinity at σ_2 sites showed considerably greater variation with nature and substitution of the halogen. Inspection of Table II reveals that, overall, affinity at σ_1 binding sites follows the trend Cl = I > Br > F. The effect of halogen substitution on σ_2 binding is less clear. m- or *p*-Halo (e.g. iodo) substituents in the corresponding benzyl analogs as in 5 and 6 resulted in almost the same binding affinities at σ_1 subtypes as their phenylethyl homologs 2 and 3 suggesting that the aryl–nitrogen distance is not under rigid control for high affinity in this series.

We had previously observed that *o*-chloro-substituted σ ligands in the related arylacetamide series[14] possessed lower σ binding affinities (higher K_i values) than the corresponding *m*- and *p*-substituted isomers. This is the case also with compound 4 which shows roughly 7–14-fold reduced affinity (increased K_i) at σ_1 sites compared with its meta and para isomers (5 and 6). Interestingly, the affinity at σ_2 subtypes was not significantly affected. Thus, although lower in affinity (at σ_1), 4 proved to be the most σ_2 selective (σ_2/σ_1 = 1.8) of all the compounds examined in this series. Compound 1 and the *m*-iodo derivative 2 displayed the highest σ_2 affinity in this series. Interestingly, the majority of σ ligands so-far reported in the literature show either similar affinity at σ_1 and σ_2 sites or greater affinity for the σ_1 site compared with the σ_2

Table II. σ Binding of Halogenated σ Ligands 1–15 and Comparison with the Prototypic Ligands



compd	R_1	R_2	R_3	n	K'_i([^{3}H]-(+)-pent) in guinea pig (σ_1), nM	K_i([^{3}H]DTG + dextrallorphan) in rat liver (σ_2), nM	σ_2/σ_1
1[a]	H	Cl	Cl	2	2.1 ± 0.8	8.1 ● 2.2	3.6
2	H	I	H	2	2.5 ± 0.9	9.1 ● 3.6	3.7
3	H	H	I	2	2.5 ± 0.3	43.1 ± 3.2	17
4	I	H	H	1	26.6 ± 3.0	48.2 ± 9.2	1.8
5	H	I	H	1	3.6 ● 0.07	33.7 ± 0.6	9.4
6	H	H	I	1	2.0 ± 0.2	24.5 ± 0.6	13
9	H	Br	H	2	4.3 ± 0.2	59.8 ● 8.4	14
10	H	H	Br	2	2.9 ± 0.03	32.5 ± 2.2	11
11	H	Br	H	1	8.9 ± 0.9	220 ± 26	25
12	H	F	H	2	5.4 ● 0.1	103.6 ● 9.8	19
13	H	H	F	2	6.0 ± 0.9	124 ± 5.9	21
14[a]	H	Cl	H	2	2.2 ± 0.5	28.3 ± 1.8	13
15[a]	H	H	Cl	2	2.4 ± 0.4	50.5 ± 12.7	21
haloperidol					3.7 ± 0.6	12.0 ± 1.7	3.2
DTG					27.7 ● 4.3	12.8 ± 2.1	0.46
(+)-pentazocine					3.1 ± 0.3	1540 ± 313	497

[a] Previously reported compounds. See ref 11.

site.[10b,15,16,17] For example, (+)-pentazocine is 500–700-fold selective for the σ_1 site compared with σ_2 whereas haloperidol and DTG have much less ability to discriminate the two sites (see ref 10b and standard compounds in Table II).

The high affinity for σ binding sites and ease of radioiodination (see below) of iodo compounds **2**, **3**, **5**, and **6** identifies them as agents suitable for SPECT imaging of σ binding sites in living animals. Iodinated ligands **2** and **4** may prove to be useful agents for imaging the σ_2 subtype if used in the presence of a selective σ_1 blocker. The possible involvement of the σ_2 subtype in the motor effects of σ ligands[18,19] suggests that those compounds showing enhanced σ_2-subtype binding may be important tools for examining possible alterations in σ binding site distribution in subjects with movement disorders such as dystonia.

The aryl iodides reported herein offer the advantage over alkyl iodide-based SPECT ligands in that they are likely to display enhanced metabolic stability. Organotin derivatives **7** and **8** proved to be efficient precursors for [^{123}I]-**2** and [^{123}I]-**3** since they afforded these compounds in up to 70% radiochemical yield and quantitative isotopic incorporation (an advantage of Sn/I exchange methodology) after treatment with carrier-free Na^{123}I in the presence of CH_3CO_3H.[20] The products were readily separated from unreacted **7** and **8** either by TLC or HPLC. Preliminary binding with [^{123}I]-**3** indicated that it could be displaced by several prototypic σ ligands and exhibited up to 97% specific binding in guinea pig brain homogenates (as determined in the presence and absence of 10 μM unlabeled **3**).[21]

The relatively high affinity and selectivity of the aryl fluoride derivatives **12** and **13** for σ_1 subtypes suggest that they may be useful agents for PET studies of these sites; they should be complementary to our previously reported alkyl fluoride σ PET agents.[22]

Experimental Section

Chemical Materials and Methods. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high resolution mass measurements (HRMS) were obtained using a VG-Micro Mass 7070F mass spectrometer. ^{1}H-NMR spectra were obtained from $CDCl_3$ solutions using a Varian XL-300 spectrometer. Thin-layer chromatography (TLC) was performed on 250-μm Analtech GHLF silica gel plates.

General Method A. To a stirred solution of carboxylic acid in CH_2Cl_2 (1 g of acid to 20 mL of CH_2Cl_2) was added a solution of DCC in CH_2Cl_2 (20 mL of CH_2Cl_2 to 1 g of DCC). After 10 min at room temperature, a solution of *N*-methyl-2-(1-pyrrolidinyl)ethylamine[11] (**16**) in CH_2Cl_2 (20 mL of CH_2Cl_2 to 1 g of **16**) was added. The molar ratio of diamine/carboxylic acid/DCC was always 1:1.5:2. The reaction mixture was stirred overnight at room temperature and then filtered. The filter cake was washed thoroughly with Et_2O. The combined organic layer was extracted with 15% aqueous citric acid solution (2 × 30 mL), the combined aqueous extract was washed with ether (×2), the ether washings were discarded, and the aqueous layer was basified to pH 9.5 with concentrated aqueous ammonia solution. The resulting solution was extracted with CH_2Cl_2 (2 × 100 mL) and the combined extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give the corresponding free bases which were purified by salt formation followed by recrystallization (see Table I).

Method B. To a stirred, freshly prepared 1.0 M solution of AlH_3 in THF[11,13] was added dropwise a solution of amide in THF (equivolume to the 1.0 M AlH_3) at room temperature under N_2. The molar ratio of amide to AlH_3 was 1:5. The reaction was found to be complete (TLC, concentrated aqueous NH_4OH/MeOH/$CHCl_3$, 1:9:90) within 5 min. at room temperature. The reaction mixture was poured into 15% NaOH and extracted twice with $CHCl_3$. The combined $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give the corresponding diamines as colorless oils. These were readily purified by recrystallization of the appropriate crystalline salts (Table I).

Method C. The bromo compound (free base, 1 mmol) in dry THF (5 ml) was cooled to −78 °C in a dry ice/acetone bath. To this solution, *n*-butyllithium (0.8 mL, 1.28 mmol, 1.6 M in hexane) was added dropwise with stirring. After 20 min at −78 °C, tri-*n*-butyltin chloride (0.3 ml, 1.1 mmol) was added dropwise. The reaction mixture was stirred at −78 °C under N_2 for 30 min and then quenched with saturated NH_4Cl solution (0.6 mL). The mixture was allowed to warm to room temperature and extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The residue was chromatographed on silica gel (eluting with CH_2Cl_2/MeOH/concentrated aqueous NH_4OH, 100:5:1) to give the desired organotin derivative (Table I).

Method D. To a solution of tri-*n*-butyltin derivative (0.5 mmol) in hydrocarbon-stabilized $CHCl_3$ (5 mL) at room temperature was added a 0.1 M solution of iodine in hydrocarbon-stabilized $CHCl_3$ until the color of iodine persisted. The mixture was stirred overnight at room temperature and then treated with a solution of KF (1.0 M, 1 mL, 1 mmol) in MeOH followed by a 5% aqueous sodium bisulfite solution (1 mL). After 5 min stirring, water (2 mL) was added. The mixture was extracted with $CHCl_3$ (2 × 20 mL) and the combined $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated in vacuo to give the crude products which were purified by chromatography on silica gel (CH_2Cl_2/MeOH/concentrated aqueous NH_4OH, 100:5:1) followed by formation of suitable crystalline salts (Table I).

Method E. To a stirred solution of $LiAlH_4$ in THF (3.0 equiv of a 1.0 M solution) at room temperature was added dropwise a solution of (fluorophenyl)acetamide (1.0 equiv) in THF (10 mL of THF to 1.0 g of substrate). The solution was boiled under reflux for 1 h or until complete by TLC (concentrated aqueous NH_4OH/MeOH/$CHCl_3$, 1:9:90). The reaction mixture was doubled in volume by addition of fresh THF, cooled to 0 °C, and treated dropwise with stirring with 1.0 mL/g $LiAlH_4$ of water, 1.0 mL/g $LiAlH_4$ of 15% aqueous NaOH, and finally 3.0 mL/g $LiAlH_4$ of water. The mixture was stirred for 40 min at room temperature and the granular precipitate of aluminum salts was removed by filtration. The filter cake was washed with THF and the

combined filtrate and washings were evaporated in vacuo to give the crude diamines. These were purified by appropriate salt formation (Table I).

Radiochemistry. Radiochemical Materials and Methods. All steps involving the use of ^{123}I were first performed with unlabeled materials, and the structures of the products were confirmed spectroscopically. All manipulations with ^{123}I were performed in a radioiodination hood surrounded with 0.25-in. thick lead shielding encased in a stainless steel jacket and fitted with a 0.5-in. thick lead glass window. Chromatographic separations of radioisotopes were accomplished using 250-μm analytical silica gel plates (GTLC, Macherey-Nagel, Germany). Radioactivity determinations were made using a Radcal Model 4050 Radionuclide calibrator (Radcal Corporation). Radioactivity was detected on TLC plates using a Bioscan system 200 imaging scanner. $Na^{123}I$ [specific activity 240000 Ci/mmol produced by Xe (p, 2n) reaction] was purchased from Nordion International Inc.,[20] Vancouver, Canada and used immediately upon receipt. Reported radiochemical yields are optimized.

[^{123}I]-*N*-[2-(4-Iodophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine [[^{123}I]-3]. Aqueous peracetic acid (100 μL of a 0.32% w/v solution) was added to a mixture of the tri-*n*-butylstannyl derivative 8 (100 μL, 0.192 μmol of a solution made by dissolving 1.0 mg of 8 in 1 mL of EtOH), EtOH (200 μL), 1 M HCl (40 μL), and no carrier added $Na^{123}I$ (5 mCi)[20] in a sealed vial. The reaction mixture was allowed to stand for 5 min at room temperature and then the reaction was quenched by the addition of $NaHSO_3$ (20 mg) followed by an aqueous solution of $NaHCO_3$ (25 mg in 1 mL of water) to render the mixture basic. The aqueous solution was subsequently extracted with ethyl acetate (3 × 1 mL). The combined organic extract was backwashed with 1 mL of water, and the organic layer was evaporated to dryness under a stream of N_2. EtOH (100–200 μL) was added to the residue, and the product mixture was then purified by HPLC using a reverse-phase column (PRP-1, C-18, 250 × 4.1 mm, 5-μm particle size, Hamilton Co.). Elution was isocratic (flow rate 1 mL/min using a solvent consisting of acetonitrile/water/trifluoroacetic acid (35:65:0.1). Fractions containing the product appeared after ca. 6 min. Unreacted 8 eluted after 20 min. The appropriate fractions were collected and evaporated to dryness under a stream of N_2. The product was redissolved in ethanol for final storage and was used within 36 h of synthesis. The radiochemical yield was determined to be 50–70% after purification (>99% radiochemically pure).

[^{123}I]-*N*-[2-(3-Iodophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine [[^{123}I]-2]. This was synthesized as described above for [^{123}I]-3 starting with tributyltin precursor 7 (0.1 mg, 0.192 μmol). The elution profile of this compound was found to be very similar to [^{123}I]-3. As for [^{123}I]-3, the radiochemical yield was determined to be between 50–70% (>99% radiochemically pure) after purification.

Biological Materials and Methods. σ Binding Assays. σ_1 Binding sites were labeled using the σ_1 selective ligand, [^{3}H]-(+)-pentazocine and guinea pig brain membranes, as described previously.[23,24] Rat liver membranes have been shown previously to be a rich source of σ_2 sites[16,17] and are labeled using [^{3}H]DTG in the presence of dextrallorphan to mask σ_1 sites.[25]

Membrane Preparation. Crude P_2 membrane fraction was prepared from frozen guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebellum. Brains were allowed to thaw slowly on ice before homogenization. Crude P_2 membrane fraction was also prepared from the livers of male Sprague-Dawley rats (150–200 g, Taconic Farms). Animals were killed by decapitation and the livers removed and minced before homogenization.

Tissue homogenization was carried out at 4 °C in 10 mL/g tissue weight of 10 mM Tris-HCl/0.32 M sucrose, pH 7.4 using 10 motor-driven strokes in a Potter-Elvehjem Teflon-glass homogenizer. The crude homogenate was centrifuged for 10 min at 1000*g* and the pellet discarded. The resultant supernatant was centrifuged at 31000*g* for 15 min. The pellet was resuspended in 3 mL/gram 10 mM Tris-HCl, pH 7.4 by vortexing, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellet was resuspended to 1.53 mL/gram in 10 mM Tris-HCl, pH 7.4 and aliquots were stored at −80 °C until used. Protein concentration of the suspension was determined by the method of Lowry[26] and was 20–25 mg protein/mL.

Various concentrations of the test ligand ranging from 0.005–1000 nM or 0.05–10000 nM were incubated with guinea pig brain membranes (σ_1) or rat liver membranes (σ_2) and radioligand. Assays were carried out using the conditions described below: σ_1, 3 nM [^{3}H]-(+)-pentazocine; σ_2, 3 nM [^{3}H]DTG + 1 μM dextrallorphan. IC_{50} values were derived using the computerized iterative curve-fitting program GraphPAD. K_i values were calculated from IC_{50} values using the Cheng-Prusoff equation[27] and K_d values that were predetermined in independent experiments ([^{3}H]-(+)-pentazocine, 4.8 nM; [^{3}H]DTG, 17.9 nM). Values are averages ± SEM of two to four experiments, each carried out in triplicate.

σ_1 Binding Assay. Guinea pig brain membranes (325–500 μg protein) were incubated with 3 nM [^{3}H]-(+)-pentazocine (51.7 Ci/mmol) in 0.5 mL of 50 mM Tris-HCl for 120 min at 25 °C. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine. Test compounds were added in concentrations ranging from 0.005–1000 or 0.05–10000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris–HCl, pH 8.0 followed by rapid filtration through glass fiber filters using a Brandel cell harvester (Gaithersburg, MD). Filters were then washed twice with 5 mL of ice-cold buffer. Prior to use, filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25 °C.

σ_2 Binding Assay. Rat liver membranes (160–200 μg of protein) were incubated with 3 nM [^{3}H]DTG (39.4 Ci/mmol) in the presence of 1 μM unlabeled dextrallorphan. Incubations were carried out in 0.5 mL of 50 mM Tris-HCl, pH 8.0 for 120 min at 25 °C. Nonspecific binding was determined in the presence of 5 μM haloperidol. Test compounds were added in concentrations ranging from 0.005–1000 or 0.05–10000 nM. Assays were terminated by the addition of ice-cold 10 mM Tris-HCl, pH 8.0 followed by rapid filtration through glass fiber filters using a Brandel cell harvester (Gaithersburg, MD). Filters were then washed twice with 5 mL of ice-cold buffer. Prior to use, filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25 °C.

All scintillation counting was carried out in Ecoscint (National Diagnostics, Manville, NJ) after an overnight extraction of counts. Protein was determined by the method of Lowry et al.[26]

Chemicals. [^{3}H]DTG was purchased from Dupont/New England Nuclear (Boston, MA). [^{3}H]-(+)-Pentazocine was synthesized as described previously.[23] Dextrallorphan and (+)-pentazocine was synthesized in the Laboratory of Medicinal Chemistry, NIDDK, NIH. DTG was purchased from Aldrich Chemical Co. (Milwaukee, WI). Haloperidol, polyethyleneimine, and Tris-HCl were purchased from Sigma Chemicals (St. Louis, MO).

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