algorithm, using a dynamic weighting of the randomly chosen rotation axes to be modified. The starting data set is minimized using a function that randomly selects the rotation axes. During the first part of the calculation, the "heaviest" rotation axes are favored, next the "lightest" ones (second part), and finally the "heaviest" ones again (third part). One hundred iteractions with maximums or dE less than 0.1 kcal/mol were performed for optimization, according to Newton-Raphson.

Crystal Analysis of 10. A colorless crystal suitable for X-ray diffraction studies for both compound **10** and (*R*)-lorglumide, prepared by slow cooling of an ethyl acetate solution, was mounted on an ENRAF-NONIUS CAD-4 diffractometer and irradiated with monochromatized Mo Kα radiation using the $\theta/2\theta$ scan technique. The intensities were correlated for Lorentz and polarization factors. The structures were solved by direct methods with MULTAN-80[29] and refined with SHELX-76[30] using blocked full-matrix least-square refinement. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms, located by different Fourier maps or by theoretical calculations, were refined isotropically. The main crystal data obtained for both compound **10** and (*R*)-lorglumide are registered in Table VII.

Supplementary Material Available: Tables containing the atomic coordinates, bond distances, bond angles, torsion angles, and weighted least-square planes through the selected atoms from X-ray diffraction studies for compound **10** (coded CR 2093) and (*R*)-lorglumide, tables containing the atomic coordinates, bond distances, bond angles, and torsion angles from computer-aided design for compound **10**, (*R*)-lorglumide, and tetragastrin (39 pages). Ordering information is given on any current masthead page.

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Synthesis, Characterization, and Biological Evaluation of a Novel Class of *N*-(Arylethyl)-*N*-alkyl-2-(1-pyrrolidinyl)ethylamines: Structural Requirements and Binding Affinity at the σ Receptor

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By synthesizing and testing a part-structure, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**3**), derived from our previously reported high affinity σ receptor ligands (1*S*,2*R*)-(−)-*N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(−)-**2**] and (+)-**2**, we have identified a novel class of superpotent (subnanomolar affinity) σ ligands specific for the σ receptor labeled by [^{3}H]-(+)-3-PPP. When **3** was tested for its capacity to displace [^{3}H]-(+)-3-PPP from guinea pig brain membranes, it exhibited a K_i of 0.34 nM, which is better than either of its parent compounds (−)-**2** (K_i = 1.3 nM) and (+)-**2** (K_i = 6.0 nM). Other compounds related to **3** such as *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-homopiperidinyl)ethylamine (**19**) exhibited K_i = 0.17 nM ([^{3}H]-(+)-3-PPP). The determinants for high σ receptor affinity of **3** were examined by manipulation of this structure in a number of different ways. The high efficacy of these compounds for the σ receptor, their relative chemical simplicity and ease of synthesis, and their high degree of selectivity identifies *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**3**) and related compounds as a highly promising base for determination of the functional role of σ receptors as well as the development of novel therapeutic agents.

Introduction

σ receptors have attracted much attention due to their ability to bind with significant affinity a number of psychoactive compounds or compounds with other activities (see ref 1 for a review). Among these are haloperidol and other typical neuroleptics,[2,3] the dissociative anesthetic phencyclidine,[4] the antitussive dextromethorphan,[5] and the steroid hormone progesterone.[6] Though many σ ligands bind to other receptors, σ sites are distinct from any known neurotransmitter or hormone receptor. Attempts to define a functional role(s) for σ sites have resulted in its implication in several physiological and biochemical processes. Among these are (1) regulation of motor behavior and postural tone,[7-9] (2) negative modulation of the phosphoinositide response to muscarinic cholinergic agonists,[10-12] (3) regulation of smooth muscle contraction,[13-15] and (4) neuroprotective activity.[16] The ability of σ ligands to affect motor systems and protect from neuronal damage suggests that selective σ compounds may be useful therapeutic agents in the treatment of motor disorders such as dystonia[17] and protection from the

† Brown University.

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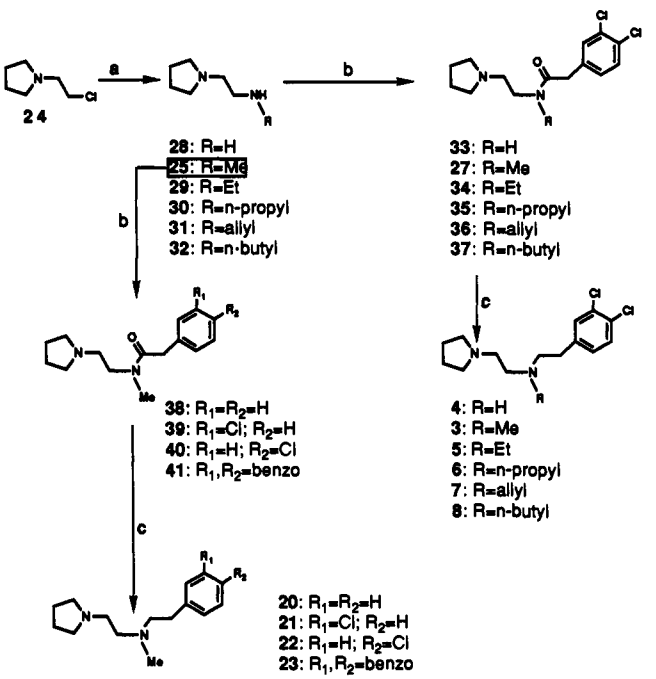
damaging effects of ischemia and stroke.[16]

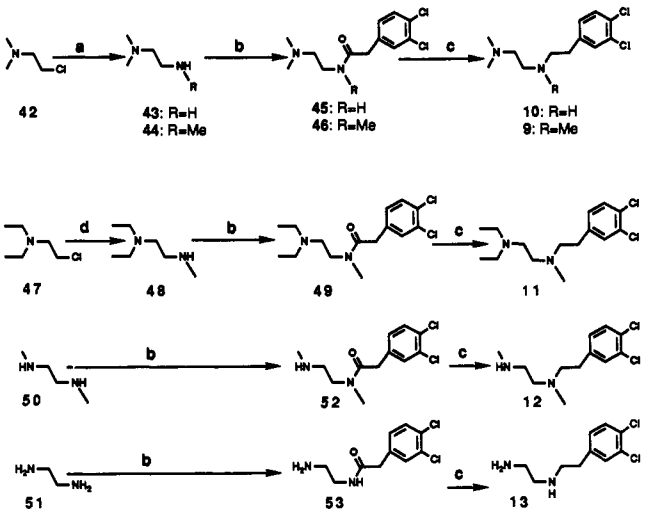
However, our ability to further define the function and properties of σ sites has been hampered by lack of a large variety of ligands which exhibit both high affinity and selectivity. We have therefore attempted to identify novel classes of compounds which have these properties. Thus, we recently reported that the cis diastereoisomers (-)- and (+)-1 of the κ selective agonist, U50,488, are potent ligands for the σ receptor, exhibiting K_i values of 81 and 221 nM, respectively, against displacement of [^{3}H]-(+)-3-PPP.[18] Compounds (-)- and (+)-1 displayed negligible affinity for dopamine-D_2, κ, or phencyclidine receptors.[18] This finding led us to pursue a systematic structure–activity relationship (SAR) study involving manipulation of the arylacetamide moiety of (-)- and (+)-1 to further improve the efficacy of these compounds for the σ receptor.[19] This study subsequently resulted in the identification of cyclohexanediamines (-)- and (+)-2, both of which exhibited extremely high potency and efficacy for σ receptors (K_i values of 1.3 and 6.0 nM, respectively, against [^{3}H]-(+)-3-PPP). The more potent and selective (-)-2 failed to bind to κ, dopamine-D_2, or PCP receptors, which are binding sites that commonly cross-react with σ ligands.[19] Further manipulation of the N-substituent of (+)- and (-)-2 resulted in still further efficaceous σ receptor ligands.[20]

Scheme I[a]



[a] (a) RNH_2; (b) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (c) AlH_3/THF.

Scheme II. Synthesis of Truncated Versions of *N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine[a]



[a] (a) RNH_2; (b) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (c) AlH_3/THF; (d) $MeNH_2$

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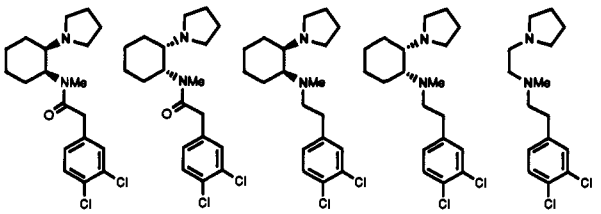


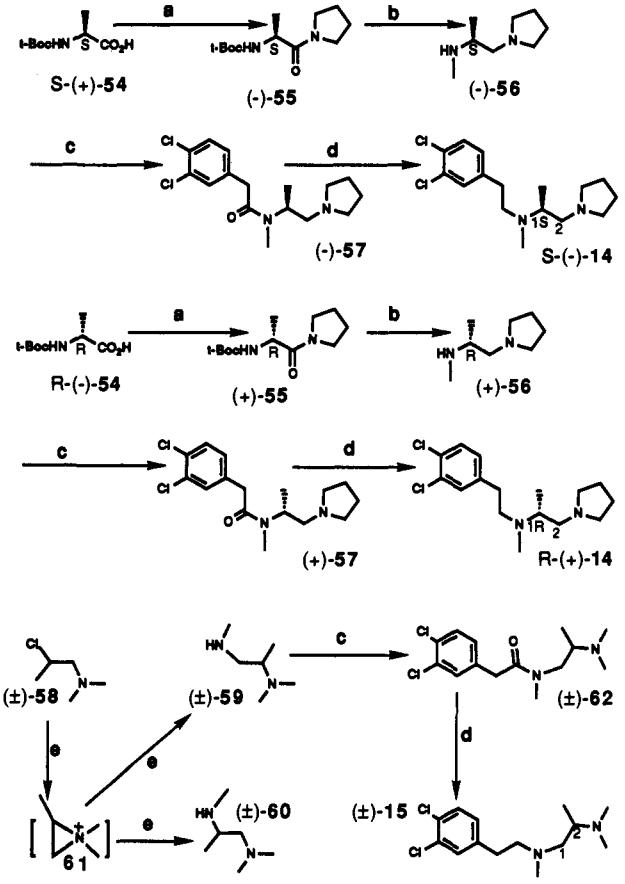
(-)-1 (+)-1 (-)-2 (+)-2 3

In the present study, we wished to find a simple substructure of (-)- and (+)-2 that retains σ receptor activity and then to manipulate this substructure further to define the determinants for activity. The most logical change to obtain such a substructure was removal of the cyclohexane ring of (-)- and (+)-2, a change that removes stereochemical constraints and gives rise to ethylenediamine 3. We found in doing this that 3 is more potent at the σ receptor than either of the parent compounds (-)- or (+)-2. In this paper, we investigated the σ receptor binding effects of modifying the phenethylamino *N*-alkyl substituent, the pyrrolidino ring, the substituent pattern on the aromatic ring, and the length of the alkyl chain between the two nitrogen atoms of the diamine moiety of compound 3. Selected compounds (those with subnanomolar affinity) (Table VIII) were examined for selectivity across receptor types that commonly cross-react with σ receptor ligands; these include σ, κ, PCP, and dopamine-D_2 receptors. We report here the synthesis, characterization, and σ receptor binding data of compounds 3–23 to define the optimal structural features of this novel σ receptor pharmacophore.

Chemistry

Compound 3 was synthesized in three steps starting from the readily available *N*-(2-chloroethyl)pyrrolidine hydrochloride (24·HCl) (Scheme I and Table I): methylaminolysis of 24 at ambient temperature in water in the presence of a 10-fold excess of methylamine afforded *N*-methyl-2-(1-pyrrolidinyl)ethylamine (25) in 65% redistilled yield. Coupling of this compound with 3,4-dichlorophenylacetic acid (26) in the presence of DCC afforded amide 27 in 98% yield. Alane (AlH_3) reduction[21] of 27 afforded 3 in 74% yield. Compounds 4–8, respectively (Scheme I and Table I), were similarly obtained in high yields. Aryl analogues 20–23 were similarly obtained from 25 starting with the respective arylacetic acids (Scheme I). Truncated compounds 9, 10, and 11 were synthesized via aminolysis of *N*-(2-chloroethyl)dimethylamine hydrochloride (42), *N*-(2-chloroethyl)diethylamine hydrochloride (47), and commercially available *N,N*-dimethylethylenediamine (43) (Scheme II and Table I). Compounds 12 and 13 were synthesized starting with symmetrical diamines 50 and 51, respectively (Scheme II and Table I). (1*R*)-(+)- and (1*S*)-(-)-methyl analogues of parent compound 3 were synthesized starting with (*R*)-(-)- and (*S*)-(+)-*N*-*t*-Boc-protected alanines [(-)- and (+)-54] (Scheme III and Table I). Coupling of these precursors with pyrrolidine in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride and hydroxybenzotriazole (HOBT) gave amides (-)- and (+)-55.[22,23] $LiAlH_4$ reduction of (-)- and (+)-55 afforded

Scheme III. Synthesis of (*R*)- and (*S*)-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-1-methyl-2-(1-pyrrolidinyl)ethylamines and (±)-*N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-methyl-2-(dimethylamino)ethylamine[a]



[a] (a) 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, pyrrolidine, hydroxybenzotriazole, CH_2Cl_2; (b) $LiAlH_4$, THF; (c) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (d) AlH_3/THF; (e) $MeNH_2$.

diamines (*S*)-(-)- and (*R*)-(+)-56 (85% yield).[23] The optical purity (>98%) of these diamines was determined by ^1H NMR analysis of the ureas 75 and 76 (Scheme VI) formed in quantitative yield by reaction of these amines with optically pure (*S*)-(+)-α-methylbenzyl isocyanate in $CHCl_3$.[24] Coupling of (1*R*)-(+)- and (1*S*)-(-)-56 with 3,4-dichlorophenylacetic acid gave amides (1*R*)-(+)- and (1*S*)-(-)-57,[23] respectively. The target compounds (1*R*)-(+)- and (1*S*)-(-)-14 (Scheme III and Table I) were furnished by AlH_3 reduction[21] of these amides. The corresponding (±)-2-methyl derivative of 9 (Scheme II), compound (±)-15 (Scheme III), was synthesized via methylaminolysis of *N,N*-dimethyl-2-chloropropylamine hydrochloride [(±)-58]; this reaction afforded an 8.2:1.8 mixture of isomeric *N*-methyl-2-(dimethylamino)propylamine [(±)-59] and *N,N*-dimethyl-2-(methylamino)propylamine [(±)-60]. Com-

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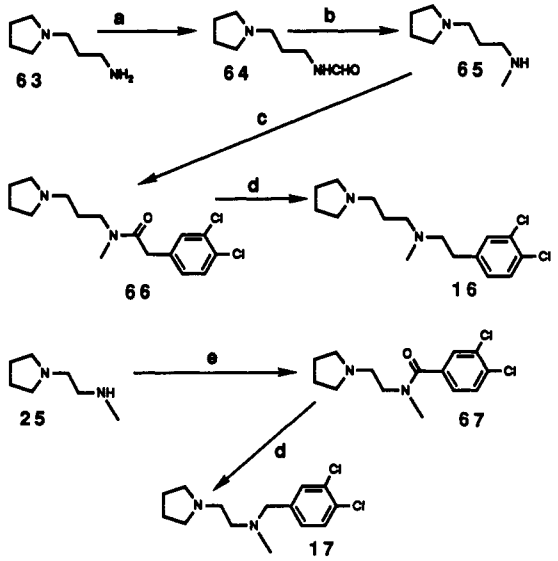
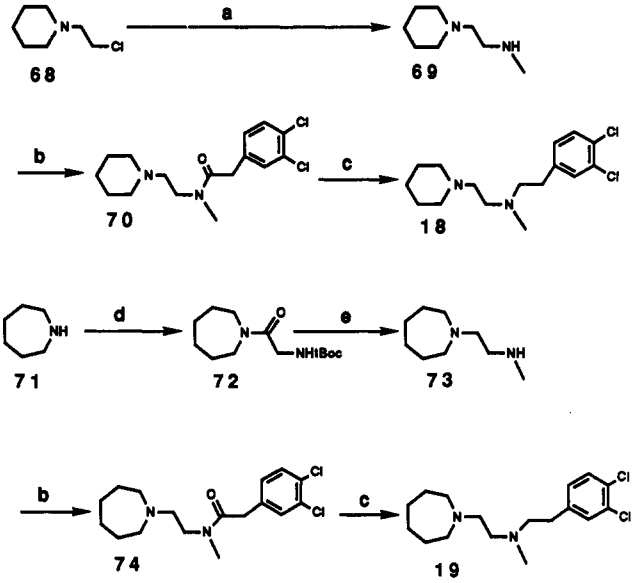
Scheme IV. Synthesis of Miscellaneous (3,4-Dichlorophenyl)-alkyldiamines[a]



[a] (a) Ethyl formate; (b) $LiAlH_4$, THF; (c) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (d) AlH_3/THF; (e) 3,4-dichlorobenzoic acid, DCC, CH_2Cl_2.

Scheme V. Synthesis of 6- and 7-Membered-Ring Homologues of *N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)-ethylamine[a]



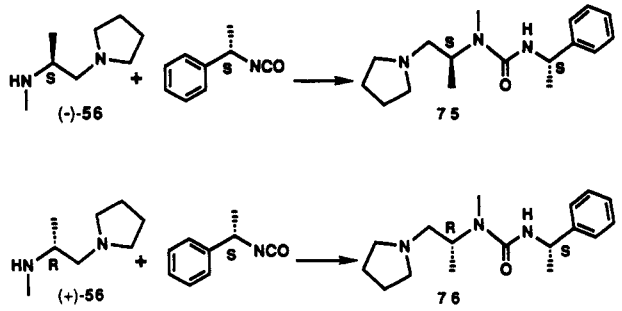
[a] (a) $MeNH_2$; (b) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (c) AlH_3/THF; (d) 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride, *N*-(*t*-Boc)glycine, HOBT, hydroxybenzotriazole, CH_2Cl_2; (e) $LiAlH_4$, THF.

pound **16** (Scheme IV and Table I) was obtained starting with commercially available 1-(3-aminopropyl)pyrrolidine (**63**). To observe the effect of ring size, compounds **18** and **19** (Scheme V and Table I) were synthesized. Compound **19** was obtained starting from homopiperidine (**71**) and the aminoethyl side chain introduced via *N*-(*t*-Boc)glycine (Scheme V).

Results and Discussion

The results of this study indicate that part-structure **3** of our originally reported σ ligands (−)- and (+)-*cis*-*N*-methyl-*N*-[2-(3,4-dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)cyclohexylamines [(−)- and (+)-**2**] (K_i = 1.3 and 6.0 nM against [^{3}H]-(+)-3-PPP, respectively)[19] is an extremely potent (K_i = 0.34 nM) (Table II) and selective

Scheme VI. Determination of Optical Purity of Chiral Diamine Intermediates (+)- and (−)-**56**



(Table VIII) ligand for σ receptors. This result indicates that by increasing the conformational freedom of (−)- and (+)-**2**, we have identified a novel and highly potent (subnanomolar potency) class of σ receptor ligands related to **3**. Since we demonstrated earlier (by comparison of the binding of the corresponding trans diastereomers) that the cis relationship (dihedral angle = 60°) between the C–N bonds of (−)- and (+)-**2** contributes significantly to the high σ receptor affinity of this class of compounds,[20] we were initially surprised to observe the increase in σ receptor affinity in going from (−)-**2** and (+)-**2** to **3**. However, preliminary molecular modeling studies[25] of monoprotonated **3** indicated that the added proton bridges the two nitrogen atoms by a hydrogen bonding type interaction, thereby constraining the C–N bonds of **3** in a cis relationship (dihedral angle of ca. 40°), which could account for the very high σ receptor affinity of **3** and related compounds.

In order to examine the effect of *N*-alkyl substitution on **3**, the *N*-H, *N*-Et, *N*-Pr, *N*-allyl, and *N*-butyl homologues were tested for their ability to displace [^{3}H]-(+)-3-PPP (Table II). The results indicate that *N*-Me is the optimal size for maximum affinity at the σ receptor labeled by [^{3}H]-(+)-3-PPP. The H substituent (compound **4**) exhibited a 3-fold decreased affinity relative to **3**. In general, increase in the size of the N-substituent from H to Pr resulted in only a slight overall decrease in receptor affinity. However, a dramatic (10-fold) decrease in affinity was observed in going from propyl to butyl; this result indicates that propyl may represent a border of the σ receptor domain since only a small decrease in binding was observed in going from hydrogen to propyl while a large (10-fold) decrease occurred in changing from propyl to butyl; however, all compounds (including the *N*-butyl compound) in this series are, relatively speaking, potent σ ligands. As expected because of their similarity in size, the propyl and allyl derivatives (compounds **6** and **7**) showed almost identical affinity for the σ receptor.

In the second stage of the study, the effect of truncation of the parent structure **3** was investigated (Table III). It was found that opening up of the pyrrolidine ring to give *N*,*N*-dimethyl derivative **9** resulted in only a 4-fold decrease in affinity (K_i = 1.26 nM). Breakage of only one bond in **3**, as represented by compound **11**, resulted in a 2-fold decrease in affinity compared with the parent molecule **3**. Further simplification of the structure as in **10** and **12** reduced the affinity by 16- and 5-fold, respectively, compared with **9**. The completely unsubstituted compound **13** which represents the basic backbone of **3** exhibited a more than 800-fold decrease in σ receptor affinity compared with **3**; the affinity of **13** (K_i = 277 nM) is still significant when compared with prototypic σ re-

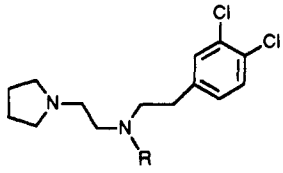
(25) Dr. Joannes Linders, personal communication.

Table I. Physical and Chemical Properties of Target Compounds and Their Intermediates

compound	molecular formula	solvent[a]	method	% yield[b]	mp (°C)
3·HBr[c]	$C_{15}H_{24}Br_2Cl_2N_2$	EtOH	C	74	264–265 dec
4·HBr	$C_{14}H_{22}Br_2Cl_2N_2$	EtOH	C	70	244–245 dec
5·HBr	$C_{16}H_{26}Br_2Cl_2N_2$	EtOH	C	77	188–189
6·HBr	$C_{17}H_{28}Br_2Cl_2N_2$	EtOH	C	65	190–191
7·HBr	$C_{17}H_{26}Br_2Cl_2N_2$	2-PrOH	C	78	178–179
8·oxalate	$C_{22}H_{32}Cl_2N_2O_8$	MeOH	C	81	203–204 dec
9·HBr	$C_{13}H_{22}Br_2Cl_2N_2$	EtOH	C	38	253–255 dec
10·HBr	$C_{12}H_{20}Br_2Cl_2N_2$	EtOH	C	73	222–223 dec
11·HBr	$C_{15}H_{26}Br_2Cl_2N_2$	EtOH	C	68	179–180
12·HBr	$C_{12}H_{20}Br_2Cl_2N_2$	EtOH	C	59	215–217
13·HBr	$C_{10}H_{16}Br_2Cl_2N_2$	EtOH	C	44	264–267 dec
(*R*)-**14**·oxalate[d]	$C_{20}H_{28}Cl_2N_2O_8$	MeOH/DMF (2:1)	C	52	198–200
(*S*)-**14**·oxalate[e]	$C_{20}H_{28}Cl_2N_2O_8$	MeOH/DMF (2:1)	C	50	198–200
(±)-**15**·oxalate	$C_{18}H_{26}Cl_2N_2O_8$	EtOH	C	84	152–153
16·HBr	$C_{16}H_{26}Br_2Cl_2N_2$	MeOH	C	85	269–270 dec
17·HCl[c]	$C_{14}H_{22}Cl_4N_2$	EtOH	C	69	258–259 dec
18·HBr	$C_{16}H_{26}Br_2Cl_2N_2$	MeOH	C	71	264–265 dec
19·HCl	$C_{17}H_{28}Cl_4N_2$	MeOH	C	73	260.5–261.5 dec
20·HCl	$C_{15}H_{26}Cl_2N_2$	EtOH	H	73	266–267 dec
21·HCl	$C_{15}H_{25}Cl_3N_2$	EtOH	C	62	258–259 dec
22·HCl	$C_{15}H_{25}Cl_3N_2$	EtOH	C	67	257–258 dec
23·HBr	$C_{19}H_{28}Br_2N_2$	DMF	H	71	259–260 dec
25·oxalate	$C_{11}H_{20}N_2O_8$	MeOH	A	69	206–207[f]
27·HCl	$C_{15}H_{21}Cl_3N_2O$	EtOAc	B	98	169.5–170.5[g]
28·fumarate	$C_{14}H_{22}N_2O_8$	MeOH	A	47	165–166
29·oxalate	$C_{12}H_{22}N_2O_8$	MeOH	A	54	230–231 dec[h]
30·HCl	$C_9H_{22}Cl_2N_2$	EtOH	A	83	211–212[i]
31·fumarate	$C_{17}H_{26}N_2O_8$	EtOH	D[j]	64	133–135[k]
32·fumarate	$C_{18}H_{30}N_2O_8$	EtOH	A[j]	80	163–164[l]
33 (base)	$C_{14}H_{18}Cl_2N_2O$	isooctane/EtOAc	B	73	74–75
34·oxalate	$C_{18}H_{24}Cl_2N_2O_5$	2-PrOH	B	91	143.5–144
35·oxalate	$C_{19}H_{26}Cl_2N_2O_5$	2-PrOH	B	96	170.5–171.5
36·fumarate	$C_{21}H_{26}Cl_2N_2O_5$	EtOH	B	97	153–154
37·oxalate	$C_{20}H_{28}Cl_2N_2O_5$	2-PrOH	B	100	150.5–151
38·oxalate	$C_{17}H_{24}N_2O_5$	2-PrOH	B	100	159–160
39·oxalate	$C_{17}H_{23}ClN_2O_5$	2-PrOH	B	100	142.5–143
40·oxalate	$C_{17}H_{23}ClN_2O_5$	2-PrOH	B	88	151–153
41·fumarate	$C_{23}H_{28}N_2O_5$	2-PrOH	B	100	128–130
44·fumarate[m]	$C_{13}H_{22}N_2O_8$	MeOH	A	97	176–176.5
45 (base)	$C_{12}H_{16}Cl_2N_2O$	isooctane	B	82	63–64
46·HCl	$C_{13}H_{19}Cl_3N_2O$	2-PrOH	B	62	168–169
48·fumarate[n]	$C_{15}H_{26}N_2O_8$	2-PrOH	A	77	116–117
49·oxalate	$C_{17}H_{24}Cl_2N_2O_5$	EtOAc	B	76	100–101
52·oxalate	$C_{14}H_{18}Cl_2N_2O_5$	EtOH	G	78	193–195
53·fumarate	$C_{14}H_{16}Cl_2N_2O_5$	2-PrOH	G	56	133–135
(*S*)-(−)-**55**[o,p]	$C_{12}H_{22}N_2O_3$	isooctane	I	100	69–70
(*R*)-(+)-**55**[q,r]	$C_{12}H_{22}N_2O_3$	isooctane	I	100	65.5–67
(*S*)-(−)-**56**·oxalate[s]	$C_{12}H_{22}N_2O_8$	EtOH	J	83	141–144
(*R*)-(+)-**56**·oxalate[t]	$C_{12}H_{22}N_2O_8$	EtOH	J	85	141–144
(*S*)-(−)-**57**·oxalate[u]	$C_{18}H_{24}Cl_2N_2O_5$	2-PrOH	B	70	114–115[v]
(*R*)-(+)-**57**·oxalate[w]	$C_{18}H_{24}Cl_2N_2O_5 \cdot H_2O$	2-PrOH	B	72	114–115[x]
(±)-**59**·oxalate	$C_{10}H_{20}N_2O_8$	MeOH	A	90[y]	170–172
(±)-**62**·oxalate	$C_{16}H_{22}Cl_2N_2O_5$	2-PrOH	B	73	151.5–152.5
64·oxalate	$C_{10}H_{18}N_2O_5$	2-PrOH	E	97	114–116
65·fumarate	$C_{16}H_{26}N_2O_8$	2-PrOH	F	81	140–141[z]
66·oxalate	$C_{18}H_{24}Cl_2N_2O_5$	2-PrOH	B	94	147–147.5
67·fumarate	$C_{18}H_{22}Cl_2N_2O_5$	2-PrOH	B	88	149–150
69·fumarate[aa]	$C_{16}H_{26}N_2O_8$	EtOH	A	79	161–162
70·oxalate	$C_{18}H_{24}Cl_2N_2O_5$	2-PrOH	B	100	148–149
72[bb]	$C_{13}H_{24}N_2O_3$	–	I	71	oil
73·oxalate[cc]	$C_{13}H_{24}N_2O_8$	MeOH	J	80	170–180
74·oxalate	$C_{19}H_{26}Cl_2N_2O_5$	2-PrOH	B	75	142–143
75 (base)	$C_{17}H_{27}N_3O$	–	K	100	oil[dd]
76 (base)	$C_{17}H_{27}N_3O$	–	K	100	oil[ee]

[a] All crystallizations were performed in ca. 1:10 weight/volume ratio of salt to solvent. [b] All yields are nonoptimized. [c] Concentrated aqueous HBr (47–48%) and concentrated aqueous HCl were utilized for formation of all the HBr and HCl salts in this Table. [d] $[\alpha]_D = +10°$ (*c* 0.64, H_2O). [e] $[\alpha]_D = -11.5°$ (*c* 0.77, H_2O). [f] Compound reported in literature[23] but no physical data is given. [g] Lit.[23] mp for oxalate salt, 152–155 °C. [h] Base bp 49 °C (1.3 mmHg). [i] Base bp 56 °C (0.7 mmHg). [j] Reaction required greater than 24 h at room temperature for completion. [k] Base bp 73 °C (0.8 mmHg). [l] Base bp 88 °C (0.8 mmHg). [m] This compound (CAS registry no. 142-25-6) is described in the literature,[27] but no synthesis is given. [n] Compound **48** (CAS registry no. 104-79-0) is described in the literature,[28] but no physical data is given. [o] M^+ (calc for $C_{12}H_{22}N_2O_3$) 242.1630, M^+ (found) 242.1658. [p] $[\alpha]_D = -27.6°$ (*c* 2.29, MeOH). [q] M^+ (calc for $C_{12}H_{22}N_2O_3$) 242.1630, M^+ (found) 242.1618. [r] $[\alpha]_D = +29.3°$ (*c* 1.34, MeOH). [s] $[\alpha]_D = -12°$ (*c* 0.61, H_2O), compound is reported in the literature,[23] but no physical data is given. [t] $[\alpha]_D = +15°$ (*c* 0.58, H_2O), compound is reported in the literature,[23] but no physical data is given. [u] $[\alpha]_D = -33°$ (*c* 0.56, H_2O), lit.[23] on HCl salt $[\alpha]_D = -55.7°$ (*c* 1.0, $CHCl_3$). [v] Lit.[23] mp on HCl salt 173–174 °C. [w] $[\alpha]_D = +37°$ (*c* 0.59, H_2O), lit.[23] on HCl salt $[\alpha]_D = +56.5°$ (*c* 1.0, $CHCl_3$). [x] Lit.[23] mp on HCl salt 174–176 °C. [y] This corresponds to the product distribution of 8.2:1.8 of (±)-**59** to (±)-**60**. [z] Base bp 64 °C (1.3 mmHg). [aa] This compound (CAS registry no. 41239-39-8) was first described in ref 29, but no physical data was reported for the fumarate salt. [bb] M^+ (calc for $C_{13}H_{24}N_2O_3$) 256.1787, M^+ (found) 256.1790. [cc] Intermediate **73** (CAS registry no. 118808-13-2) structure and use reported in ref 30, but no synthetic or physical data was given. [dd] M^+ (calc for $C_{17}H_{27}N_3O$) 289.2154, M^+ (found) 289.2145. [ee] M^+ (calc for $C_{17}H_{27}N_3O$) 289.2154, M^+ (found) 289.2144.

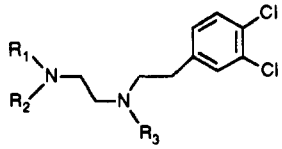
Table II. σ Receptor Binding Affinities of N-Alkyl-Substituted N-[2-(3,4-Dichlorophenyl)ethyl]-2-(1-pyrrolidinyl)ethylamines[a]



compd	R	K_i (nM) ([^{3}H]-(+)-3-PPP)
4	H	1.11 ± 0.11
3	Me	0.34 ± 0.07
5	Et	0.51 ± 0.08
6	n-Pr	1.35 ± 0.15
7	allyl	1.39 ± 0.03
8	n-Bu	11.7 ± 2.5

[a] Each compound was initially screened at concentrations of 10, 100, and 1000 nM in order to obtain an estimate of σ binding affinity and to determine the appropriate concentration range to use in 12-point competition curves. For most compounds in the study, a concentration range of 0.0005–100 nM was appropriate. A range of 0.005–1000 nM or 0.05–10 000 nM was used for the less potent compounds. Twelve concentrations of unlabeled ligand were incubated with 3 nM [^{3}H]-(+)-3-PPP as described in Methods. The CDATA iterative curve-fitting program (EMF Software, Inc., Baltimore, MD) was used to determine IC_{50} values. Values are the average of 2–4 experiments ± SEM. Each experiment was carried out in duplicate. The Cheng–Prussoff equation[31] was then used to convert IC_{50} values to apparent K_i values. The K_d for [^{3}H]-(+)-3-PPP (27.4 nM) was determined in independent experiments using guinea pig brain membranes.

Table III. σ Receptor Affinities of Truncated Forms of N-[2-(3,4-Dichlorophenyl)ethyl]-N-methyl-2-(1-pyrrolidinyl)ethylamine (3)[a]



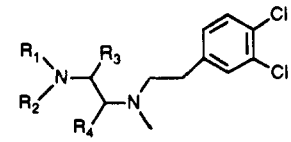
compd	R_1	R_2	R_3	K_i (nM) ([^{3}H]-(+)-3-PPP)
3	$-(CH_2)_4-$		Me	0.34 ± 0.07
9	Me	Me	Me	1.26 ± 0.26
10	Me	Me	H	20.18 ± 0.22
11	Et	Et	Me	0.73 ± 0.11
12	Me	H	Me	5.78 ± 0.45
13	H	H	H	277.0 ± 75.6

[a] See footnote a of Table II.

ceptor ligands such as SKF10,047. It can be concluded from this brief study of the effect of N-substitution that at least two tertiary N atoms are necessary for high affinity at the σ receptor and that 3 may represent an optimal structure for high σ receptor affinity among this class of ligands.

Methyl-substituted derivatives of 3, compounds (+)- and (−)-14, were synthesized (Scheme III and Table IV) in order to observe the effect of sterically restricting rotation about the C–N/C–N′ bond of 3 and perhaps favoring a conformation that would further improve the affinity at the σ receptor compared with 3. However, a modest (2.5-fold) decrease in affinity was observed compared with 3. As has been noted previously for a number of other σ receptor ligands from unrelated structural classes,[1] very little enantioselectivity was observed between (+)- and (−)-14. Similarly, with the alternative C-methyl-substituted derivative of 9, compound (±)-15, an insignificant change in σ receptor affinity was observed compared with 9. This study with C-methyl substitution of compounds 3 and 9

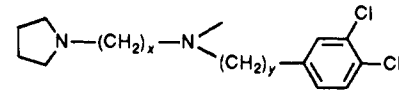
Table IV. σ Receptor Affinities of 1- and 2-Methyl Analogues of 3 and 9[a]



compd code	R_1, R_2	R_3	R_4	K_i (nM) ([^{3}H]-(+)-3-PPP)
3	$-(CH_2)_4-$	H	H	0.34 ± 0.07
(R)-(+)-14	$-(CH_2)_4-$	H	α-Me	0.62 ± 0.07
(S)-(−)-14	$-(CH_2)_4-$	H	β-Me	0.83 ± 0.17
(±)-15	Me, Me	Me	H	1.46 ± 0.17

[a] See footnote a of Table II.

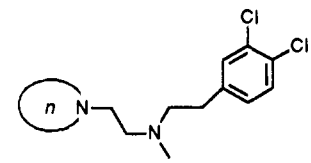
Table V. Effect of Varying the Distance between the 3,4-Dichlorophenyl and N-Methyl Moieties as Well as the Pyrrolidinyl and N-Methyl Groups on σ Receptor Binding Affinity[a]



compd	x	y	K_i (nM) ([^{3}H]-(+)-3-PPP)
3	2	2	0.34 ± 0.07
16	3	2	1.76 ± 0.02
17	2	1	0.38 ± 0.03

[a] See footnote a of Table II.

Table VI. Effect of Ring Size on the σ Receptor Binding Affinity of N-[2-(3,4-Dichlorophenyl)ethyl]-N-methyl-2-(1-cycloalkylamino)ethylamines[a]



compd	n	K_i (nM) ([^{3}H]-(+)-3-PPP)
3	5	0.34 ± 0.07
18	6	0.26 ± 0.07
19	7	0.17 ± 0.03

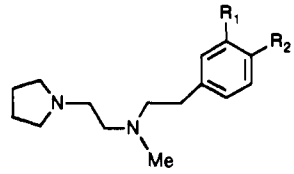
[a] See footnote a of Table II.

indicates that addition of a 1- or 2-methyl group into 3 or 9 does not favor efficacy at σ receptors. However, the methyl group appears to improve the σ receptor selectivity of (R)-(+)-14 (see Table VIII).

In order to determine the optimal number of carbon atoms between the two N atoms and between the N-methyl N atom of 3 and the aromatic ring of 3, a limited study was executed involving three compounds (3, 16, and 17) (Table V). This limited study showed that all of the compounds were still very high affinity σ ligands with K_i values below 2 nM. Increasing the number of carbon atoms between the pyrrolidine N atom and the N-methyl N atom of 3 resulted in a 5-fold decrease in affinity at σ receptors while decreasing the number of carbon atoms between the N-Me nitrogen atom and aromatic ring resulted in no significant change in σ receptor affinity. This limited study thus indicates that the optimal structure for potent affinity at σ receptors is represented by 3 or perhaps 17.

The effect of ring size was examined to determine if the pyrrolidine ring of 3 is optimal for high affinity at the σ receptor. The 5-membered ring of 3 was compared with

Table VII. Effect of Aromatic Substitution on the σ Receptor Binding Affinity of 3[a]



compd	R_1	R_2	K_i (nM) ([3H]-(+)-3-PPP)
20	H	H	7.40 ± 0.14
21	Cl	H	0.84 ± 0.03
22	H	Cl	1.47 ± 0.08
3	Cl	Cl	0.34 ± 0.07
23	R_1,R_2 = benzo		2.09 ± 0.24

[a] See footnote a of Table II.

the 6- and 7-membered rings of 18 and 19 (Table VI). The results indicated that 5-, 6-, and 7-membered-ring homologues were very potent (subnanomolar affinity) ligands at the σ receptor. The 6-membered-ring homologue (18) showed a slight increase in affinity while the 7-membered homologue (19) exhibited an even greater increase in affinity compared with 3; compound 19 proved to be the most potent compound in the entire series (K_i = 0.17 nM, Table VI), and indeed ever tested by us. Thus it appears that the 5-membered heterocycle of 3 is not optimal within this series for highest affinity at the σ receptor and can be improved further by increasing ring size. Furthermore, the requirement of larger ring systems for optimal σ affinity is consistent with the decrease in affinity upon opening of the pyrrolidine ring as discussed above.

To test which of the chlorine atoms of 3 are important for high σ receptor affinity or whether they are important at all, we synthesized the deschloro compound 20 and the *m*- and *o*-monochloro analogues of 3 (Scheme I and Table VII). Also synthesized was the benzo analogue of 3 to examine whether the 3,4-dichloro unit of 3 could be effectively replaced by a benzo unit as we have observed previously in a different but related class of σ receptor ligands.[19] The results (Table VII) indicated that the deschloro compound 20 was still a high-affinity σ receptor ligand but that it was 22-fold lowered in affinity compared with 3. The *m*-monochloro analogue 21 exhibited a 2.5-fold lowered affinity compared with 3 while the para isomer 22 was 4-fold lowered in affinity. The naphtho compound 23 was less potent compared with 3 and roughly 3.5 times as potent as unsubstituted compound 20, indicating that the benzo group of 23 can substitute for the 3,4-chlorine atoms of 3, but not as effectively. These results indicate that the monochloro compounds are more potent than the deschloro compound and that the chlorine atoms of 3 exert a synergistic effect resulting in the very high σ receptor affinity of 3 compared with 20. These results with the 3,4-chlorine atoms of 3 and the benzo group of 23 suggest that the lipophilic groups in the 3,4-positions of 3 may occupy a hydrophobic pocket at the receptor site which serves to augment binding affinity of the 3,4-dichloro-substituted σ ligands at this receptor.

Finally, all of the compounds in this paper showing subnanomolar affinity at the σ receptor (8 out of the 22 compounds examined in this paper) were tested for selectivity at σ receptors labeled by [3H]-(+)-3-PPP by measuring their ability to displace [3H]bremazocine (κ receptor), [3H]TCP (PCP receptor), and [3H]sulpiride (dopamine-D_2 receptor). κ opioid receptors were investigated since these compounds are distantly related to U50,488, a selective κ receptor agonist,[18,19] while PCP and dopamine-D_2 receptors were investigated since several σ ligands are known to cross-react with these receptors.[1] The results of this selectivity study indicated that all of the compounds tested were highly selective for the σ receptor. All eight compounds showed K_i > 10000 nM for κ receptor labeled by [3H]bremazocine and K_i > 10000 nM for PCP receptor labeled by [3H]TCP except (*R*)-(+)-14 which exhibited a K_i = 3042 for PCP receptor (5000-fold selective for σ over PCP receptors) and 19 with K_i = 6943 nM (41000-fold selective for σ over PCP receptors). In terms of their dopamine-D_2 receptor binding, the eight compounds tested showed affinities ranging from 1112 nM for 3 to 11235 nM for (*R*)-(+)-14. However, all of the compounds showing subnanomolar affinity for the σ receptor exhibited selectivity ratios ranging from 3265 for 5 to 39720 for 19. Interestingly, the C-1-methyl-substituted σ ligand (*R*)-(+)-14, although exhibiting increased affinity for PCP receptors compared with other compounds in Table VIII, showed decreased affinity for the dopamine-D_2 receptors compared with the rest of the compounds; this result suggests that in some cases, receptor selectivity may be a trade-off between one receptor (for example dopamine-D_2) and another (for example PCP).

Table VIII. σ ([3H]-(+)-3-PPP), κ ([3H]Bremazocine), PCP ([3H]TCP), and Dopamine-D_2 ([3H]Sulpiride) Receptor Binding of Selected Compounds from Tables II–VII[a]

	K_i (nM)			
compd	σ [3H](+)PPP	κ [3H]brem	PCP [3H]TCP	dopamine-D_2 [3H]sulpiride
3	0.34 ± 0.07	no inhibn[b]	no inhibn[b]	1112 ± 74
5	0.51 ± 0.08	no inhibn[b]	no inhibn[b]	1665 ± 69
11	0.73 ± 0.11	no inhibn[b]	no inhibn[b]	3006 ± 322
(*R*)-(+)-14	0.62 ± 0.07	no inhibn[b]	3042 ± 716	11235 ± 161
17	0.38 ± 0.03	no inhibn[b]	no inhibn[b]	1301 ± 221
18	0.26 ± 0.07	no inhibn[b]	no inhibn[b]	1209 ± 15
19	0.17 ± 0.03	no inhibn[b]	6943 ± 1046	6752 ± 836
21	0.84 ± 0.03	no inhibn[b]	no inhibn[b]	6497 ± 322

[a] In order to obtain an initial estimate of binding affinity, three concentrations of each compound (100, 1000, and 10,000 nM) were incubated with the indicated radioligand for dopamine-D_2, κ opiate, or PCP receptors. Assay conditions were as described in Methods. Compounds eliciting >30% inhibition were investigated further using 12 concentrations of unlabeled ligand ranging from 0.5 to 100000 nM. Data were analyzed as described in footnote a of Tables II–VII. Values are the average of 2–3 experiments ± SEM, each carried out in duplicate. The following K_d values (as determined in independent experiments) were employed to calculate K_i: [3H](−)-sulpiride (rat brain), K_d = 10.3 nM; [3H]bremazocine (guinea pig brain), K_d = 0.64 nM; [3H]TCP (guinea pig brain), K_d = 25 nM. [b] No IC_{50} or K_i value was determined since the compound produced less than 30% inhibition of control binding at a concentration of 10000 nM. σ receptor binding data are taken from Tables II–VII.

Conclusion

We have identified a novel class of exceptionally potent and selective σ receptor ligands as a result of synthesizing and testing 3, a part-structure of our previously reported *cis*-cyclohexanediamine σ ligands (+)- and (−)-2. Sixteen out of the 22 compounds tested exhibited a σ affinity less than 2 nM while 8 of these compounds displayed subnanomolar affinity. Structural variations of the *N*-alkyl substituent of 3 revealed that the Me group is optimal for high σ receptor affinity. Examination of truncated forms of 3 indicated that an intact pyrrolidine ring results in higher affinity compounds than a simple *N*,*N*-dialkyl group. *C*-Alkyl substitution of 3 resulted in lowered affinity as did changing the distances between the two N atoms or the NMe group and aromatic ring; the optimal spacing appears to be as it is in 3, but it is possible that reducing the distance between the NMe group and aro-

matic ring to one carbon is not deleterious toward affinity. The study of different-sized heterocyclic rings in **3** revealed that the 5-membered ring of **3** is not optimal and that the affinity of **3** could be further increased to 0.17 nM by substitution of the 5-membered ring for a 7-membered ring. The 3- and 4-chlorine atoms of **3** contribute significantly to its σ receptor affinity, perhaps by occupation of a lipophilic pocket at the receptor site, as we have proposed previously for the arylacetamide-related σ ligands.[19]

Since many σ ligands commonly cross-react with κ, PCP, and dopamine-D_2 receptors, this new class of compounds should prove to be very valuable in the study of σ receptor biochemistry and pharmacology and may provide a base for new therapeutic agents. Compounds such as **19** (K_i = 0.17 nM) which are 160-fold higher in binding affinity than prototypic σ ligands such as (+)-3-PPP (K_d = 27.4 nM) and showing insignificant affinity for PCP, dopamine-D_2, or κ receptors, sites which commonly cross-react with σ receptor ligands, should prove useful in functional studies of σ receptors.

Experimental Section

Materials and Methods. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Specific rotation determinations at the sodium D line were obtained in a 1-dm cell using a Perkin-Elmer 241-MC polarimeter. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA; where molecular formulae are indicated in Table I, elemental analyses for C, H, and N are within ±0.4% of the theoretical values unless indicated otherwise. 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. TLC system A corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (90:9:1); TLC system B corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (80:18:2). No attempt was made to optimize the yields reported in Table I. All intermediates and final products synthesized afforded ^{1}H NMR and mass spectral data consistent with their assigned structures. Spectral (^{1}H NMR and MS) data for the compounds described in the methods below are provided as supplementary material.

***N*-Methyl-2-(1-pyrrolidinyl)ethylamine (25). Method A.** To a stirred solution of 40% aqueous methylamine (913 mL, 11.8 mol, 10 equiv) was added during 20 min a solution of *N*-(2-chloroethyl)pyrrolidine hydrochloride (**24**·HCl) (200 g, 1.18 mol) in distilled water (300 mL). The solution became warm during the addition. TLC (solvent system B) 30 min after completion of the addition indicated that the reaction was complete. The reaction mixture was basified by the addition of 370 g of NaOH pellets. No external cooling was necessary during the addition of NaOH since the vigorous evolution of gaseous methylamine removed the heat generated as heat of vaporization. The desired product separated as a yellow oil after complete addition of the NaOH. The oily reaction mixture was set aside and allowed to cool to room temperature. The cooled solution was extracted with ether (4 × 500 mL), and the combined organic layer was evaporated in vacuo to afford the product as a pale yellow oil. The oil was distilled under aspirator vacuum, collecting the central fraction, bp 95–105 °C, yield = 98 g (65%). Crystallization of the oxalate salt from MeOH afforded an analytically pure sample of **25**·bisoxalate (Table I): mp 206–207 °C. **25**·bisoxalate: Anal. ($C_{11}H_{20}N_2O_8$) C, H, N.

***N*-[2-(3,4-Dichlorophenyl)acetyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (27). Method B.** To a stirred solution of *N*,*N*′-dicyclohexylcarbodiimide (DCC) (123 g, 0.596 mol) in CH_2Cl_2 (500 mL) was added a solution of 3,4-dichlorophenylacetic acid (91.7 g, 0.447 mol) in CH_2Cl_2 (500 mL), and the solution was stirred for 10 min at room temperature. To this solution was added **25** (38.0 g, 0.297 mol), and the solution was stirred for 20 min at room temperature when TLC (solvent system A) indicated that the reaction was complete. The precipitated *N*,*N*′-dicyclohexylurea was filtered off, and the filter cake was washed with ether (500 mL). The filtrate was diluted to a total volume of 1500 mL with ether. The diluted filtrate was extracted with 10% aqueous citric acid (1000 mL), and the organic layer was discarded. The aqueous citric acid extract was washed with a further 2 × 500 mL of ether, basified by addition of excess concentrated aqueous ammonia solution, and then extracted with 2 × 500 mL of CH_2Cl_2. The CH_2Cl_2 layer was back-washed with water (200 mL), dried over Na_2SO_4, and evaporated to give 91.3 g (98%) of **27** as a colorless oil, which was of high enough purity (99% by GC analysis) for use in the next step. For purposes of further characterization, a small amount of this material was converted to **27**·HCl in ethyl acetate (Table I): mp 169.5–170.5 °C. **27**·HCl: Anal. ($C_{15}H_{21}Cl_3N_2O$) C, H, N.

***N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (3). Method C.** To a stirred solution of freshly prepared alane (1450 mL of a 1.0 M solution in THF prepared as described below and in ref 21) (1.45 mol) was added dropwise over 15 min a solution of **27** base (91.2 g, 0.289 mol). In order to prevent the reaction mixture from heating to the point of reflux of the THF, it was periodically immersed in an ice bath to keep the temperature around room temperature. Five minutes after addition of **27** was complete, progress of the reaction was monitored by TLC (solvent system A); TLC indicated that the reduction was complete. The reaction mixture was carefully poured into a large beaker (2–4-L) containing 600 mL of 15% aqueous NaOH, and the mixture was vigorously stirred during the addition. After the quenching was complete, the aqueous mixture was cooled to room temperature and enough chloroform (ca. 1500 mL) was added to result in a lower organic layer. The organic extract was separated and dried through a pad of Na_2SO_4, and the solvent was evaporated to give the crude product as a pale yellow oil. The oil was dissolved in MeOH to a volume of 1000 mL, and the stirred solution was heated to boiling point and then the heat was removed. To the stirred solution was added 100 mL of 47% HBr. The solution continued to boil as a result of heat of neutralization. After the addition of the HBr was complete and the boiling had subsided, crystallization began to occur spontaneously. During the crystallization process, the solution continued to boil as a result of heat of crystallization. The flask was set aside to cool to room temperature, and the crystals were filtered, washed twice with cold (0 °C) MeOH and once with ether, and dried in vacuo at 80 °C to give analytically pure **3**·HBr (99 g, 74%) (Table I): mp 264–265 °C dec. **3**·2HBr: Anal. ($C_{15}H_{24}Br_2Cl_2N_2$) C, H, N.

***N*-Allyl-2-(1-pyrrolidinyl)ethylamine (31). Method D.** To a stirred solution of allylamine (38.7 g, 0.679 mole) in MeOH (200 mL) was added dropwise at room temperature a solution of 1-(2-chloroethyl)pyrrolidine hydrochloride (**24**·HCl) (20.0 g, 0.177 mol) in MeOH (50 mL). Analysis of the reaction by TLC (solvent system B) indicated that the reaction was complete after 4 days. The solvent was evaporated in vacuo, and the oily residue was dissolved in distilled water (200 mL), cooled to 0 °C, and treated with KOH pellets (60 g) with continued cooling from an ice bath. A separate organic layer of crude **31** formed after complete addition of the KOH. The cooled mixture was extracted with $CHCl_3$ (2 × 100 mL), and the combined extract was evaporated to give a yellow oil; distillation under high vacuum afforded **31** (11.6 g, 64%) as a colorless oil: bp 73 °C (0.8 mmHg) (Table I); **31**·bisfumarate mp 133–135 °C. **31**·bisfumarate: Anal. ($C_{17}H_{26}N_2O_8$) C, H, N.

***N*,*N*,*N*′-Trimethylethylenediamine (44).** The same procedure was used as for the synthesis of **25** starting with *N*-(2-chloroethyl)dimethylamine hydrochloride (**42**·HCl, Scheme II) (50 g, 0.347 mol) in 100 mL of water and aqueous methylamine (40%) (404 mL, 15 equiv). Reaction was complete within 1 h (TLC; solvent system A). The product was isolated by addition of NaOH (150 g) and extraction with $CHCl_3$ (3 × 200 mL): yield 34.3 g (97%) as a colorless oil (Table I); **44**·bisfumarate mp 176–176.5 °C. **44**·bisfumarate: Anal. ($C_{13}H_{22}N_2O_8$) C, H, N.

***N*-(3-Formamidopropyl)pyrrolidine (64). Method E.** A mixture of *N*-(3-aminopropyl)pyrrolidine (Fluka) (**63**) (20.3 g, 158.6 mmol) and ethyl formate (150 mL) was refluxed overnight under an argon atmosphere when TLC (solvent system A) indicated that the reaction was complete. The solvent was evaporated in vacuo, and the oily residue was distilled in vacuo to give **64** (24.0 g, 97%)

as a colorless oil which formed a crystalline oxalate salt (Table I): mp 114–116 °C. **64**·oxalate: Anal. ($C_{10}H_{18}N_2O_5$) C, H, N.

(±)-2-(Dimethylamino)-*N*-methylpropylamine [(±)-59]. To a stirred solution of aqueous methylamine (40%) (246 mL, 10 equiv) at room temperature was added dropwise a solution of 2-chloro-*N*,*N*-dimethylpropylamine hydrochloride [(±)-**58**·HCl] (50 g, 316.5 mmol) in water (100 mL). Reaction was found to be complete within 30 min (TLC; solvent system A). KOH pellets (103.8 g) were added, and the product was isolated by extraction with $CHCl_3$ (3 × 200 mL) as described earlier for **25** to give a mixture of (±)-**59** together with (±)-**60** as the minor isomer (8.2:1.8 ratio as determined by ^1H NMR comparison of the C(Me) group of (±)-**59** with that of (±)-**60**: yield 32.9 g as a colorless liquid which was subjected to separation as described below.

Separation of (±)-59 and (±)-60. Mixed amines [(±)-**59** and (±)-**60**] from above (25.4 g, 0.219 mol) was dissolved in MeOH, the solution was heated to boiling point, and the heat source was removed. To this stirred solution was added a boiling solution of oxalic acid (39.4 g, 0.44 mol) in MeOH (200 mL). The solution began to boil again during the addition as a result of heat of neutralization. As soon as all of the oxalic acid had been added, exothermic crystallization occurred. The crystallization mixture was cooled to room temperature and filtered to give (±)-**59**·bisoxalate (Table I): mp 170–172 °C. (±)-**59**·bisoxalate: Anal. ($C_{10}H_{20}N_2O_8$) C, H, N.

***N*-[3-(Methylamino)propyl]pyrrolidine (65). Method F.** To a stirred suspension of $LiAlH_4$ (7.55 g, 199 mmol) in dry THF (200 mL) was added dropwise with cooling from an ice bath a solution of **64** (7.55 g, 48.4 mmol) in THF (200 mL). The solution was stirred at room temperature under an N_2 atmosphere overnight, or until complete by TLC (solvent system B). The reaction was quenched by dropwise (with cooling) addition of water (7.55 mL), 15% NaOH (7.55 mL), and finally water (22.6 mL). The precipitated aluminum salts were filtered, and the filter cake was washed with THF (100 mL). The combined filtrate and washings were evaporated in vacuo to give **65** in quantitative yield as a colorless oil: bp 64 °C (1.3 mmHg) (Table I). **65**·bisfumarate: mp 140–141 °C. **65**·bisfumarate: Anal. ($C_{16}H_{26}N_2O_8$) C, H, N.

***N*-[2-(*N*'-Methylamino)ethyl]-*N*-methyl-3,4-dichlorophenylacetamide (52). Method G.** The complex formed by allowing 3,4-dichlorophenylacetic acid (4.1 g, 20.0 mmol) and *N*,*N*'-dicyclohexylcarbodiimide (5.5 g, 26.7 mmol) to react together in CH_2Cl_2 (50 mL) was added in one portion to a rapidly stirred solution of *N*,*N*'-dimethylethylenediamine (Aldrich) (17.62 g, 10 equiv) in CH_2Cl_2 (50 mL). After being stirred overnight, the solution was filtered, and the filter cake was washed with ether. The combined filtrate and washings were diluted to 200 mL with ether and extracted with 10% aqueous citric acid (200 mL). The aqueous layer was washed with ether (2 × 100 mL), basified by addition of excess concentrated aqueous NH_3, and extracted with CH_2Cl_2 (5 × 50 mL). The combined CH_2Cl_2 extract was dried (Na_2SO_4), and the solvent was evaporated in vacuo to give **52** (4.3 g, 78%) as a colorless oil. **52**·oxalate crystallized from EtOH (see Table I): mp 193–195 °C. **52**·oxalate: Anal. ($C_{14}H_{18}Cl_2N_2O_5$) C, H, N.

Preparation of 1.0 M Alane in THF.[21] To a vigorously stirred solution of $LiAlH_4$ in THF (1600 mL of a 1.0 M solution) was added, dropwise from a dropping funnel, sulfuric acid (78.08 g, 0.5 equiv) at such a rate that the solution temperature was maintained at 20 °C; this was accomplished by periodic dipping of the reaction vessel in an ice bath. When the addition was complete, the solution was stirred for a further 1 h at 20 °C and then allowed to stand overnight at this temperature until the precipitated Li_2SO_4 had settled to the bottom of the flask. The 1.0 M alane was decanted from these salts prior to use. The shelf-life (2 weeks) could be increased to 1 year by decanting the 1.0 M alane solution into a clean dry bottle and storing it under a nitrogen atmosphere at −30 °C.

***N*-(2-Phenylethyl)-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (20). Method H.** To a stirred solution of $LiAlH_4$ in THF (72.4 mL of a 1.0 M solution, 72.4 mmol) was added dropwise from a dropping funnel a solution of amide **38** (8.9 g, 36.2 mmol) in dry THF (50 mL). TLC (solvent system A) indicated the reaction to be almost complete after 10 min at room temperature. The reaction was driven to completion by brief refluxing and then cooled to room temperature. Additional THF (200 mL) was added, and then excess $LiAlH_4$ was destroyed by dropwise addition of water (2.75 mL), 15% aqueous NaOH (2.75 mL), and finally water (8.25 mL). The reaction mixture was stirred for 1 h and filtered, and the filtrate was evaporated to give the crude **20** as an oil which formed crystalline **20**·HCl (8.06 g) from EtOH (50 mL) on addition of concentrated HCl (6 mL) (Table I): mp 266–267 °C dec. **20**·HCl: Anal. ($C_{15}H_{26}Cl_2N_2$) C, H, N.

(*R*)-(+)-*N*-(*tert*-Butyloxycarbonyl)-*N*',*N*'-tetramethylenealaninamide [(*R*)-(+)-55]. Method I. A solution of *t*-Boc-D-alanine (13.2 g, 0.0696 mol), HOBT (7.5 g, 0.0556 mol), and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (20 g, 0.1043 mol) in 30 mL of CH_2Cl_2 was stirred at room temperature for 1 h. Pyrrolidine (5.9 g, 0.0835 mol) was then added, and the mixture was stirred for an additional 3 h. The solvent was removed in vacuo, and the mixture was diluted in 50 mL of EtOAc. This organic layer was washed with water (2 × 50 mL), 5% citric acid (2 × 50 mL), and saturated $NaHCO_3$ (2 × 50 mL) and dried with Na_2SO_4. (*R*)-(+)-**55** was obtained as a colorless oil (10.68 g, 100%) which was used in the next step without further purification. For the purposes of characterization, however, a portion of this compound was obtained in analytically pure form by recrystallization from isooctane (Table I): R_f 0.24 (hexane–EtOAc, 1:1); mp 65.5–67 °C. **55**: Anal. ($C_{12}H_{22}N_2O_3$) C, H, N.

(*R*)-(+)-2-(1-Pyrrolidinyl)-1-methyl-*N*-methylethylamine [(*R*)-(+)-56]. Method J. (*R*)-*N*-(*tert*-Butyloxycarbonyl)-*N*,*N*'-tetramethylenealaninamide (8.4 g, 0.0348 mol) was dissolved in 100 mL of THF. A 1 M solution of $LiAlH_4$ in THF was then added dropwise (100 mL, 0.1044 mol), and the mixture was refluxed for 3 h. Water (4 mL), then 10% NaOH (4 mL), and water again (8 mL) were added. Filtration led to a clean solution which was diluted with Et_2O (100 mL). This organic layer was extracted with 5% citric acid (3 × 60 mL). The aqueous solution was basified with KOH pellets and extracted with $CHCl_3$ (4 × 40 mL). The resulting organic layer was dried (Na_2SO_4), and the solvent was removed in vacuo to give 4.94 g of crude diamine. This could be purified by crystallization of its oxalate salt from EtOH to give (+)-**56**·bisoxalate (85% based on amount of t-Boc-D-alanine) (see Table I): mp 141–144 °C. (+)-**56**·bisoxalate: Anal. ($C_{12}H_{22}N_2O_8$) C, H, N.

***N*-[1-(1-Pyrrolidinyl)-2(*S*)-propyl]-*N*-methyl-*N*'-[(*S*)-α-methylbenzyl]urea (75). Method K.** To a stirred solution of (*S*)-(−)-**56** (12.1 mg, 0.0854 mmol) in dry $CHCl_3$ (0.5 mL) was added (*S*)-(−)-1-phenylethyl isocyanate (12.6 mg, 0.0854 mmol), and the solution was stirred at room temperature under an N_2 atmosphere until complete by TLC (solvent system A). The solvent was evaporated in vacuo to give **75** in quantitative yields as a colorless oil (Table I): HRMS M^+ (found) 289.2145, M^+ ($C_{17}H_{27}N_3O$) requires 289.2154.

***N*-[1-(1-Pyrrolidinyl)-2(*R*)-propyl]-*N*-methyl-*N*'-[(*S*)-α-methylbenzyl]urea (76).** The same method was followed as described above for **75** starting with (*R*)-(+)-**56** (16.5 mg, 0.116 mmol), dry $CHCl_3$ (0.5 mL), and (*S*)-(−)-1-phenylethyl isocyanate (12.6 mg, 0.0854 mmol) to give **76** in quantitative yield as a colorless oil (Table I): HRMS M^+ (found) 289.2144, M^+ ($C_{17}H_{27}N_3O$) requires 289.2154.

Biological Materials and Methods. Membrane Preparation. Receptor binding assays were performed using the crude synaptosomal (P_2) membrane fraction of guinea pig brain (σ, κ, and PCP receptors) or rat brain (dopamine-D_2 receptors).

Crude P_2 membrane fractions were prepared from frozen (−80 °C) guinea pig brains (Pel-Freeze, Rogers, AK), minus cerebella. After removal of cerebella, brains were allowed to thaw slowly on ice and placed in ice-cold 10 mM Tris-HCl, pH 7.4, containing 320 mM sucrose (Tris-sucrose buffer). Brains were then homogenized in a Potter–Elvehjem homogenizer by 10 strokes of a motor driven Teflon pestle in a volume of 10 mL/g tissue wet weight. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C, and the supernatants were saved. The pellets were resuspended by vortexing in 2 mL/g ice-cold Tris-sucrose and centrifuged again at 1000*g* for 10 min. The combined 1000*g* supernatant was centrifuged at 31000*g* for 15 min at 4 °C. The pellets were resuspended by vortexing in 3 mL/gm of 10 mM Tris-HCl, pH 7.4, and the suspension was allowed to incubate at 25 °C for 15 min. Following centrifugation at 31000*g* for 15 min, the pellets were resuspended by gentle Potter–Elvehjem

homogenization to a final volume of 1.53 mL/g in 10 mM Tris-HCl, pH 7.4. Aliquots were stored at −80 °C until use. Protein concentration was determined by the method of Lowry et al.[26] using bovine serum albumin (BSA) as standard.

To prepare rat brain crude P_2 membranes, male Sprague–Dawley rats (150–200 g, Charles River, Boston, MA) were killed by decapitation. Brains (minus cerebella) were then treated as described above.

Receptor Binding Assays. σ Receptors. σ receptors were labeled with [^{3}H]-(+)-3-PPP [1-*n*-propyl-3-(3-hydroxyphenyl)-piperidine] (109 Ci/mmol). Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 500 μg of membrane protein and 3 nM [^{3}H]-(+)-3-PPP. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by the addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass-fiber filters (Schleicher and Schuell). Filters were then washed twice with 5 mL of ice-cold Tris-HCl buffer. Filters were soaked in 0.5% polyethylenimine for at least 30 min at 25 °C prior to use.

κ Opiate Receptors. κ receptors were labeled with [^{3}H]bremazocine (17.3 Ci/mmol) in the presence of [D-Ala^2,*N*-methyl-Phe^4,Gly-ol^5]enkephalin (DAGO) and [D-Ser^2,Leu^5,Thr^6]enkephalin (DSTLE) as μ and δ opiate receptor blockers, respectively. Incubations were carried out in 0.5 mL of 10 mM Tris-HCl, pH 7.4, for 90 min at 25 °C with 500 μg of membrane protein, 100 nM DAGO, 100 nM DSTLE, and 2 nM [^{3}H]bremazocine. Assays were terminated by the addition of 5 mL of ice-cold buffer and filtration through glass-fiber filters (Schleicher and Schuell) under reduced pressure. Filters were then washed twice with 5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 10 μM levallorphan.

Phencyclidine (PCP) Receptors. PCP [1-(1-phenylcyclohexyl)piperidine] receptors were labeled using [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP) (48.9 Ci/mmol). Incubations were carried out in 5 mM Tris-HCl, pH 7.4, for 60 min at 4 °C in a volume of 0.5 mL with 500 μg of membrane protein and 5 nM [^{3}H]TCP. Assays were terminated by addition of 5 mL of ice-cold buffer and filtration through glass-fiber filters under reduced pressure. Filters were then washed twice with 5 mL of ice-cold buffer. Filters were soaked in 0.3% polyethylenimine for at least 30 min at 25 °C prior to use. Nonspecific binding was determined in the presence of 10 μM cyclazocine.

Dopamine-D_2 Receptors. Dopamine-D_2 receptors were labeled with 5 nM [^{3}H](−)-sulpiride (73.1 Ci/mmol) using rat brain membranes. Incubations were carried out for 60 min at 25 °C in 0.5 mL of 50 mM Tris-HCl, pH 7.7, containing 120 mM NaCl and 500 μg of membrane protein. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by the addition of 5 mL of ice-cold incubation buffer and vacuum filtration through glass-fiber filters (Schleicher and Schuell). Filters were then washed twice with ice-cold incubation buffer.

Chemicals. All scintillation counting was performed with a Packard Model 4450 scintillation spectrometer using Ecoscint cocktail (National Diagnostics, Manville, NJ) after overnight extraction of the counts from the filters. Radioligands were purchased from DuPont/New England Nuclear (Boston, MA). Haloperidol, polyethyleneimine, and Tris were purchased from Sigma Chemicals (St. Louis, MO). Cyclazocine and levallorphan were obtained from the National Institute on Drug Abuse (Rockville, MD). [^{3}H]-(+)-3-PPP, [^{3}H]bremazocine, [^{3}H]TCP, and [^{3}H](−)-sulpiride were purchased from Dupont/New England Nuclear, Boston, MA.

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Supplementary Material Available: NMR and MS data for **3, 20, 25, 27, 31, 44, 52, 55, 56, 59, 64, 65, 75,** and **76** (4 pages). Ordering information is given on any current masthead page.

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