A New Approach to the Design of σ-2-Selective Ligands: Synthesis and Evaluation of *N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine-Related Polyamines at σ-1 and σ-2 Receptor Subtypes

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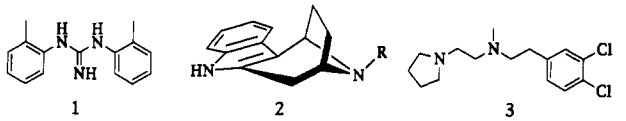
Received August 12, 1993®

A series of polyamines based on the high affinity σ receptor ligand *N*-[2-(3,4-dichlorophenyl)-ethyl]-*N*-methyl-2-(1-pyrrolidinyl)ethylamine (**3**) were developed and evaluated for their binding characteristics at σ-1 and σ-2 receptor subtypes. The data indicated that a considerable degree of structural variation is possible while still retaining nanomolar affinity at σ receptors. As the structure of the polyamines was varied, their binding at σ-1 and σ-2 subtypes showed quite different and in some cases opposite trends, supporting the belief that these are pharmacologically distinct entities. Polyamines containing two nitrogen atoms showed optimal binding at both σ-1 and σ-2 receptor subtypes. Although additional nitrogen atoms resulted in decreased affinity at σ-1 and σ-2 subtypes, an increase in selectivity for σ-2 subtypes was evident; the parent **3** showed greater selectivity for σ-1 subtypes. Internitrogen spacings had a large effect on binding affinity and subtype selectivity. For example, the difference between *N*-[3-(1-pyrrolidinyl)propyl]-*N'*-(3,4-dichlorobenzyl)-*N,N'*-dimethylethylenediamine (**8**) [K_i = 29.9 nM at σ-1 receptor and 18.3 nM at σ-2 receptor] to *N*-[3-(1-pyrrolidinyl)propyl]-*N'*-(3,4-dichlorobenzyl)-*N,N'*-dimethylethylenediamine (**10**) [K_i = 1.49 nM at σ-1 receptor and 12.1 nM at σ-2 receptor] illustrates the importance of internitrogen spacing. Triamines **11** and **13** [$K_i(\sigma\text{-}2)/K_i(\sigma\text{-}1)$ = 0.19 and 0.10, respectively] containing the N–N–N–Ar spacings 3–3–2 and 4–4–2, proved to be the most σ-2 subtype selective of the 15 polyamines examined in this study. The N–N–N spacings appear to be an important factor in their σ-2 subtype selectivity. These compounds will serve as templates in the design of still further σ-2 subtype selective ligands. The pyrrolidine ring (present in most of the polyamines tested in this series) proved to be an important recognition site for σ receptor binding activity. Furthermore, alkyl substitution also appears to be important since the stripped down polyamines *N*-[2-(3,4-dichlorophenyl)ethyl]ethylenediamine (**15**) and *N*¹-[2-(3,4-dichlorophenyl)ethyl]diethylenetriamine (**16**) exhibited relatively low binding affinity.

Introduction

Investigation of the physiological and pharmacological role of σ receptors is a relatively new area which has been facilitated by the development of high affinity and selective σ receptor ligands.[1] The recent introduction of selective σ receptor antagonists is further facilitating functional studies of this receptor.[2] Many different classes of drugs[1b] ranging from steroids such as progesterone[3] to neuroleptics such as haloperidol[4] exhibit high affinity for σ receptors. The σ binding activity of most neuroleptic drugs suggested that σ receptors may be involved in at least some of the motor side effects of these drugs. This was subsequently confirmed by the identification of altered σ receptors in genetically dystonic (dt) rats[5] as well as motor disturbances in rats following administration of σ ligands.[6] The involvement of σ receptors in several physiological and biological effects ranging from psychotic behavior[7] to inhibition of cholinergic agonist stimulated phosphoinositide (PI) turnover[8] have been reviewed in detail elsewhere.[1a] More recent studies have suggested the utility of σ receptor ligands in the development of neuroprotective[9] and atypical antipsychotic drugs.[10] Certain σ ligands have also been shown to inhibit the behavioral effects of cocaine.[11]

Chart 1



1 2 3

In view of the diversity of compounds that bind the σ receptor, coupled with their spectrum of activities in functional assays, it was apparent to us that σ receptor subtypes may account for the differences between these compounds. The existence of at least two σ receptor subtypes (designated σ-1 and σ-2)[12] has been confirmed in several independent studies.[13] The vast majority of σ ligands exhibit high selectivity for the σ-1 subtype.[14] Compounds which bind selectively to σ-1 receptors include (+)-benzomorphans such as (+)-pentazocine, (+)-3-phenyl-1-propylpiperidine ([³H]-(+)-3-PPP), haloperidol, (+)-morphinans such as dextrallorphan, *cis*-cyclohexane-1,2-diamines, and ethylenediamines.[14] The disubstituted guanidine DTG (**1**) (Chart 1) is an exception since it displays roughly equal affinity for both σ subtypes,[15] while haloperidol and (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP] show only slight preference for σ-1 sites.[12,13a,15] The 5,6,7,8,9,10-hexahydro-7,10-iminocyclohept[*b*]indoles (**2**) have been recently identified as selective σ-2 ligands.[16] As part of our program to develop σ-2 selective ligands, we focused our attention on the high-

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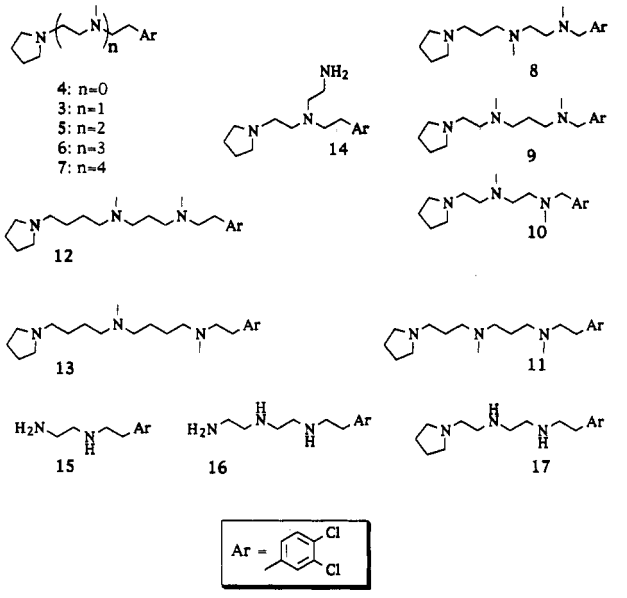
® Abstract published in *Advance ACS Abstracts*, December 1, 1993.

0022-2623/94/1837-0314$04.50/0

Chart 2. Polyamino σ Ligands



affinity ethylenediamine σ ligand **3**.[17] Binding studies of aromatic ring substituted derivatives of **3** indicated it to be a potential template for the design of σ-2-selective ligands.[15]

Our interest in the development of σ-2 subtype selective ligands has been spurred by recent studies which indicate that this subtype mediates the motor effects of σ ligands.[18,19] This is supported by our observation that DTG which binds with lower affinity to σ-1 receptors than (+)-pentazocine is nonetheless much more potent at causing dystonia in rats.[19] It is notable that DTG is roughly equipotent at both σ-1 and σ-2 subtypes whereas pentazocine is ca. 500-fold more selective for σ-1 over σ-2 subtypes.[13a,15]

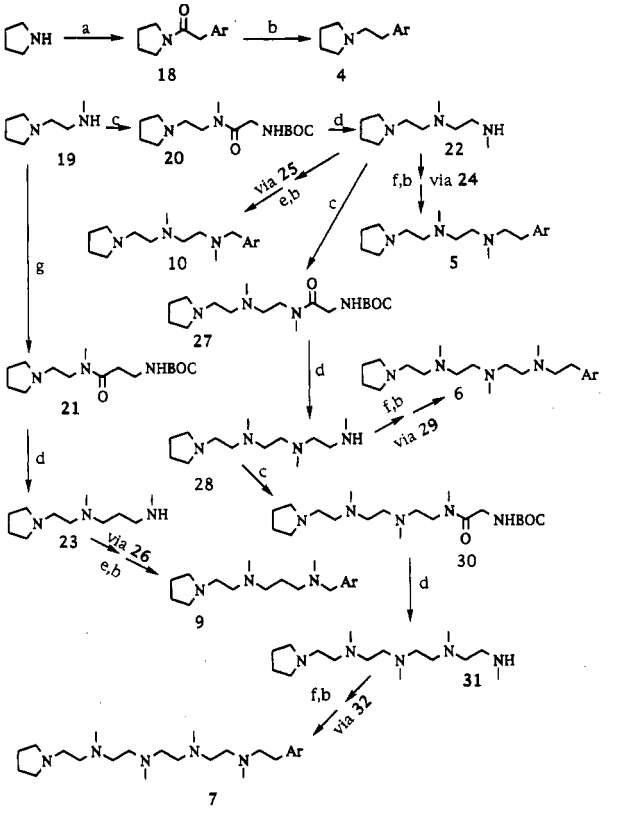
We report here the synthesis and evaluation of a series of novel polyamines **4–17** (Chart 2) based on **3**. Polyamines are important cellular regulators[20] with pharmacologically distinct binding sites on the *N*-methyl-D-aspartate (NMDA) receptor channel complex.[21] The biological properties of synthetic and naturally occurring polyamines have been reviewed in detail elsewhere (see ref 22). The endogenous polyamine "agonists" spermine and spermidine typically increase both the binding of open channel blockers such as MK801 as well as the sensitivity of neurons to excitatory amino acids (EAA's). Synthetic polyamine antagonists such as arcaine or diethylenetriamine (DET) competitively inhibit the effects of polyamine agonists on neuronal excitability.[22] Polyamines have a diverse array of uses such as the targeting of tumorous cells with anticancer drugs,[23] novel antimalarial agents,[24] and development of anticholesterolemic agents[25] to name a few examples.

Using **4–17**, we probe the effect of number and spacing of the N atoms on σ receptor subtype selectivity with the aim of discovering subtype selective agents. It has previously been shown that simple polyamines are weak noncompetitive inhibitors of σ-1 receptors labeled by [^{3}H]-(+)-3-PPP.[26]

Chemistry

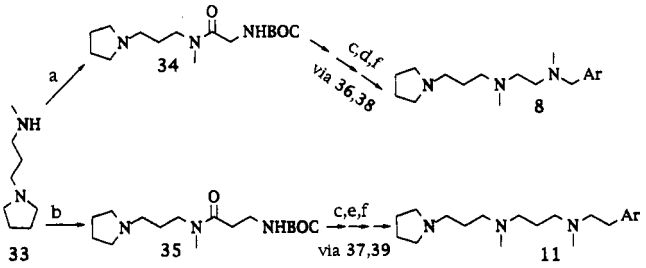
Amines **3** and **15** (Chart 2) were obtained as described previously.[17] Monoamine **4** was obtained via alane reduction[27] of amide **18** (Scheme 1). Triamines **5** and **10**

Scheme 1[a]



[a] (a) 3,4-Dichlorophenylacetic acid, DEC, CH_2Cl_2; (b) AlH_3, THF, room temperature; (c) BOC-Gly, DCC, CH_2Cl_2; (d) $LiAlH_4$, THF, reflux; (e) 3,4-dichlorobenzoic acid, DCC, CH_2Cl_2; (f) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (g) BOC-β-Ala, DCC, CH_2Cl_2; "Ar" = 3,4-dichlorophenyl.

Scheme 2[a]



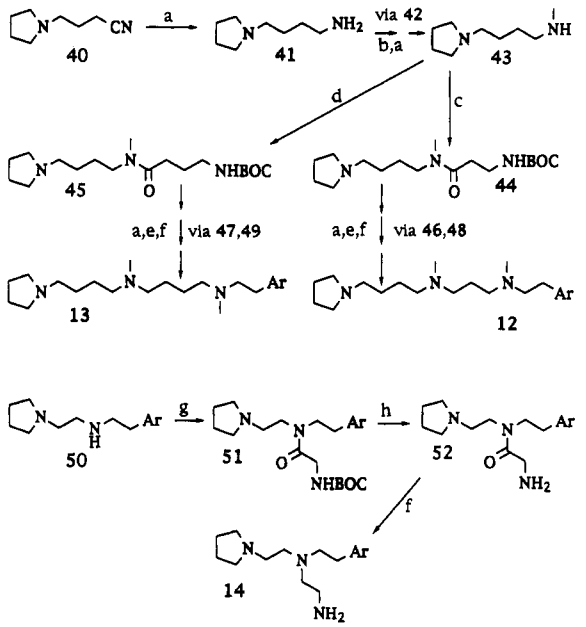
[a] (a) BOC-Gly, DCC, CH_2Cl_2; (b) BOC-β-Ala, DCC, CH_2Cl_2; (c) $LiAlH_4$, THF, reflux; (d) 3,4-dichlorobenzoic acid, DCC, CH_2Cl_2; (e) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (f) AlH_3, THF, room temperature; "Ar" = 3,4-dichlorophenyl.

(Scheme 1) were synthesized from **19**[17] through the sequence of DCC coupling with BOC-Gly (to **20**) followed by $LiAlH_4$ reduction (to **22**), coupling with 3,4-dichlorophenylacetyl and 3,4-dichlorobenzoyl chlorides, respectively, and alane reduction. Triamine **9**, tetraamine **6**, and pentaamine **7** were similarly obtained (Scheme 1).

Starting with diamine **33**[17] (Scheme 2), a similar methodology (via coupling with BOC-Gly and BOC-β-Ala) afforded triamines **8** and **11**. The intermediate triamines **36** and **37** were obtained via $LiAlH_4$ reduction of amido carbamates **34** and **35**. Alane reduction of the intermediate amides **38** and **39** furnished the target compounds in high yield.

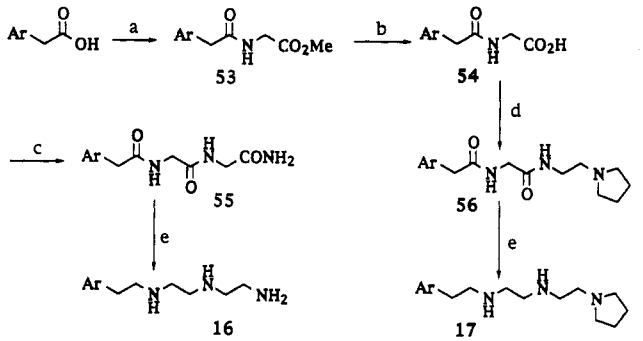
4-(1-Pyrrolidinyl)butyronitrile **40** (Aldrich Chemical Co., Milwaukee, WI) served as starting material for triamines **12** and **13** (Scheme 3). Thus, the sequence of reduction of **40** with $LiAlH_4$, N-formylation (HCO_2Et), and finally

Scheme 3[a]



[a] (a) $LiAlH_4$, THF, reflux; (b) HCO_2Et, reflux; (c) BOC-β-Ala, DCC, CH_2Cl_2; (d) BOC-GABA, DCC, CH_2Cl_2; (e) 3,4-dichlorophenylacetic acid, DCC, CH_2Cl_2; (f) AlH_3, THF, room temperature; (g) BOC-Gly, DCC, CH_2Cl_2; (h) CF_3CO_2H, $CHCl_3$, room temperature; "Ar" = 3,4-dichlorophenyl.

Scheme 4[a]



[a] (a) GlyOMe·HCl, DEC, Et_3N, CH_2Cl_2; (b) 10% KOH/MeOH; (c) glycinamide, DEC, CH_2Cl_2; (d) 1-(aminoethyl)pyrrolidine, DEC, CH_2Cl_2; (e) AlH_3, THF, room temperature; "Ar" = 3,4-dichlorophenyl.

$LiAlH_4$ reduction afforded **43**. Using the same methodology as in Scheme 2, DCC coupling with BOC-β-Ala and BOC-GABA gave **44** and **45** which on $LiAlH_4$ reduction followed by further transformation gave **12** and **13**.

Diamine **50**[17] (Scheme 3) served as a precursor for triamine **14**. DCC coupling of **50** with BOC-Gly gave **51** which was N-deprotected (66%) by treatment with CF_3CO_2H at room temperature to give **52**. This was readily reduced to **14**.

Unsubstituted triamine **16** (Scheme 4) was synthesized starting with 3,4-dichlorophenylacetic acid. DEC coupling of this acid with GlyOMe gave **53** (83%) which was hydrolyzed to carboxylic acid **54** (95%) with 10% KOH/MeOH. DEC coupling of **54** with glycinamide and 1-(aminoethyl)pyrrolidine afforded **55** and **56**, respectively. Alane reduction of **55** and **56** furnished the target compounds **16** and **17**.

Results and Discussion

The binding data (Table 2) indicates that a considerable degree of structural variation is possible while still retaining high (nanomolar) affinity for the σ receptor. The results show a clear difference between σ-1 and σ-2 receptor subtypes in their response to structural variations of these polyamines. This is also reflected in the wide variations of the ratio $K_i(\sigma\text{-}1)/K_i(\sigma\text{-}2)$. This supports the hypothesis that σ-1 and σ-2 receptor subtypes are pharmacologically and structurally distinct entities.[13] Comparison of the series **3**, **4**, **5**, **6**, and **7** (Chart 2) (all containing a 3,4-dichlorophenylethyl and internitrogen spacings of CH_2-CH_2) indicates that in this series, two amine nitrogens are optimal for binding both σ-1 and σ-2 receptor subtypes. Although the affinity at both σ-1 and σ-2 subtypes drops in the series **4** → **3** → **5** → **6** → **7**, an increase in σ-2 selectivity (decrease in $K_i(\sigma\text{-}2)/K_i(\sigma\text{-}1)$ ratio) is evident, suggesting that additional basic amine nitrogen atoms encourage σ-2 selectivity. It is possible that the lower σ binding affinity of compounds such as pentaamine **7** may be due to its very high hydrophilicity which may prevent it from interacting with the receptor. In this same series, compound **5** (i.e., three amine nitrogens) represents a cutoff or boundary point since σ-1 binding affinity drops 8-fold and σ-2 binding affinity drops 4-fold. Compounds **5** and **8** possess the same length from pyrrolidine ring to aromatic ring and contain the same number of amine nitrogens. The fact that they differ in internitrogen spacings and N-aromatic ring spacings appears to make no difference to their binding at σ-2 receptor subtypes, but does affect their binding affinity at σ-1 subtypes. This also holds roughly true for all of the compounds in the table.

Removal of one CH_2 group from in between the pyrrolidine ring and nitrogen atom as in **8** → **10** results in a 20-fold improvement in binding affinity to the σ-1 subtype with little or no change in binding at the σ-2 subtype.

That compound **3** binds with higher affinity than compound **4** corroborates our earlier observation that at least two basic nitrogen atoms may constitute the σ receptor pharmacophore. Relative to diamine **3**, the improved σ-2 subtype selectivity of triamines **11**, **12**, and **13** indicates that triamines or polyamines containing the correct internitrogen and N-aromatic ring spacings may represent the σ receptor pharmacophore more closely. Compounds **11** and **13** containing the N–N–N–Ar spacings 3–3–2 and 4–4–2 proved to be among the most selective σ-2 subtype selective ligands [$K_i(\sigma\text{-}2)/K_i(\sigma\text{-}1)$ = 0.19 and 0.10, respectively] in this series. The N–N spacings of at least three or four methylenes may be a factor in their high σ-2 subtype selectivity.

The previously reported **15** which is an N-dealkylated version of **3** showed 190-fold reduced affinity at σ-1 and 300-fold reduced affinity at σ-2 receptor subtypes relative to **3**. A slight increase in the σ-1 subtype selectivity was evident. Compound **16** which is a dealkylated form of triamine **5** also showed considerable loss of binding affinity. The 4.5-fold (at σ-1) and 4.8-fold improved (at σ-2) binding affinity of **17** compared with **16** indicates that the pyrrolidine ring may be an important recognition site in the σ binding of these and related compounds.

The prototypic σ ligands shown at the bottom of Table 2 all displayed high affinity for σ-1 receptor subtypes and low (DTG) to high [(+)-pentazocine] σ-1 subtype selectivities. The very high σ-1 subtype selectivity is typical of the (+)-benzomorphans and (+)-morphinans as well as most other classes of σ ligands.[1a,13a,29]

The potent binding affinity of these polyamines is surprising in view of their high polarity. Prototypic σ

Table 1. Physical and Chemical Data

no.[e]	salt[a]	method[b]	yield[c] (%)	cryst solvent	mp, °C	CIMS, m/z (MH+)	anal. found[d]
4	HBr	B	85	2-PrOH	222–223 dec	244	$C_{12}H_{16}BrCl_2N$
5	3HBr	B	89	EtOH	255–256 dec	358	$C_{18}H_{32}Br_3Cl_2N_3$
6	4HBr	B	63	MeOH	256–257 dec	415	$C_{21}H_{40}Br_4Cl_2N_4$
7	5HBr	B	52	MeOH	248–250 dec	472	$C_{24}H_{48}Br_5Cl_2N_5$
8	3HBr	B	90	EtOH	262–263 dec	358	$C_{18}H_{32}Br_3Cl_2N_3$
9	3HBr	B	66	EtOH	270–271 dec	358	$C_{18}H_{32}Br_3Cl_2N_3$
10	3HBr	B	88	MeOH	266–267 dec	344	$C_{17}H_{30}Br_3Cl_2N_3$
11	3HBr	B	77	EtOH	267–270 dec	386	$C_{20}H_{36}Br_3Cl_2N_3 \cdot H_2O$
12	3HBr	B	90	2-PrOH	255–256	400	$C_{21}H_{38}Br_3Cl_2N_3$
13	3HBr	B	60	EtOH	250–252	414	$C_{22}H_{40}Br_3Cl_2N_3$
14	3oxalate	B	63	MeOH	170–171	330	$C_{22}H_{31}Cl_2N_3O_{12}$
16	3HBr	B	53	EtOH	232–233	276	$C_{12}H_{22}Br_3Cl_2N_3$
17	3HBr	B	66	EtOH	242–243 dec	330	$C_{16}H_{28}Br_3Cl_2N_3$
18		A	85	isooctane	99–100	258	$C_{12}H_{12}Cl_2NO$
20	fumarate	D	44	EtOAc	136–137	286	$C_{18}H_{31}N_3O_7$
21	oxalate	D	46	EtOAc	86–88	300	$C_{17}H_{31}N_3O_7 \cdot 0.5H_2O$
22	3HBr	C	82	EtOH	238–240 dec	186	$C_{10}H_{28}Br_3N_3$
23	3HBr	C	76	EtOH	229–231	200	$C_{11}H_{28}Br_3N_3$
24	2fumarate	D	93	EtOH	169–171	371 (M^+)	$C_{26}H_{35}Cl_2N_3O_9$
25	2fumarate	D	81	MeOH–EtOH	168–169	358	$C_{25}H_{33}Cl_2N_3O_9$
26	2fumarate	D	100	EtOH	175–176	372	$C_{26}H_{35}Cl_2N_3O_9$
27	2oxalate	D	92	MeOH	186–187 dec	343	$C_{21}H_{38}N_4O_{11}$
28	4HBr	C	56	MeOH	240–241 dec	243	$C_{13}H_{34}Br_4N_4$
29	3oxalate	D	100	MeOH	223–224 dec	429	$C_{27}H_{40}Cl_2N_4O_{13}$
30[f]	oil	D	100				
31	5HBr	C	46	MeOH–EtOH	248–250 dec	300	$C_{16}H_{42}Br_5N_5$
32	4oxalate	D	75	MeOH	222–223 dec	486	$C_{32}H_{49}Cl_2N_5O_{17} \cdot 0.5H_2O$
34	oxalate	D	77	MeOH	123–124	300	$C_{17}H_{31}N_3O_7$
35[f]	oil	D	100			314	$C_{16}H_{31}N_3O_3$
36	3HBr	C	77	MeOH	225–230	200	$C_{11}H_{28}Br_3N_3$
37	3HBr	C	70	2-PrOH	231–233	214	$C_{12}H_{30}Br_3N_3$
38	2oxalate	D	64	MeOH–EtOH	187–188	372	$C_{22}H_{31}Cl_2N_3O_9$
39	2oxalate	D	54	MeOH	194–195	400	$C_{24}H_{35}Cl_2N_3O_9 \cdot 1.25H_2O$
41	2fumarate	C	37	EtOH	131–132	143	$C_{16}H_{26}N_2O_8$
42	oxalate		96	2-PrOH	84–85	171	$C_{11}H_{20}N_2O_5 \cdot 0.25H_2O$
43	2fumarate	C	85	2-PrOH	116–118	157	$C_{17}H_{28}N_2O_8$
44[f]	oil	D	100			328	$C_{17}H_{33}N_3O_3$
45[f]	oil	D	100			342	$C_{18}H_{35}N_3O_3$
46	3HBr	C	70	2-PrOH	207–209	228	$C_{13}H_{32}Br_3N_3$
47	3HBr	C	66	2-PrOH	234–236	242	$C_{14}H_{34}Br_3N_3$
48	2oxalate	D	63	EtOH	101–103	414	$C_{25}H_{37}Cl_2N_3O_9 \cdot 0.5H_2O$
49	2oxalate	D	85	EtOH	89–91	428	$C_{28}H_{39}Cl_2N_3O_9 \cdot H_2O$
51	oxalate	D	75	EtOAc	121–122	444	$C_{23}H_{33}Cl_2N_3O_7$
52	1.5oxalate		66	EtOH	178–179	344	$C_{19}H_{26}Cl_2N_3O_7$
53[g]		A	83	EtOAc–hexanes (1:3)	87–90	275 (M^+)	$C_{11}H_{11}Cl_2NO_3$
54			95	hot H_2O	144–146	279 (MNH_4^+)	$C_{10}H_9Cl_2NO_3$
55		A	72	hot H_2O	222–223	318	$C_{12}H_{13}Cl_2N_3O_3$
56		D	81	EtOAc–hexanes (1:3)	123–125	358	$C_{16}H_{21}Cl_2N_3O_2$

[a] Salts were formed in approximately 1:10 weight/volume salt/solvent. [b] General methods as described in the Experimental Section. [c] All yields are non-optimized. [d] Elemental compositions (%) were found to be within ±0.4% of the theoretical values for C, H, and N unless stated otherwise. [e] See ref 35 for ^{1}H-NMR data of all of the compounds reported in this table. [f] No attempt was made to purify or further characterize this intermediate; it was used directly for the next step. 35: HRMS $M^+(C_{16}H_{31}N_3O_3)$ requires 313.2360, M^+ (found) 313.2365. 44: HRMS $M^+(C_{17}H_{33}N_3O_3)$ requires 327.2529, M^+ (found) 327.2522. 45: HRMS $M^+(C_{18}H_{35}N_3O_3)$ requires 341.2965, M^+ (found) 341.2678. [g] This compound failed to yield a satisfactory elemental analysis (anal. calcd for $C_{11}H_{11}Cl_2NO_3$: C, 47.85; H, 4.02; N, 5.07. Found: C, 47.58; H, 4.37; N, 6.04) due to solvation; HRMS M^+ ($C_{11}H_{11}Cl_2NO_3$) requires 275.0116, M^+ (found) 275.0112.

ligands such as the butyrophenones, (+)-benzomorphans, and octahydrobenz[f]quinolines[1] are lipophilic compounds which show further improvements in binding with increasing hydrophobicity of the N-substituents. Certain of the polyamine part-structures corresponding to removal of the 3,4-dichlorophenylethyl group of 3–17 completely abolished binding affinity at σ-1 receptor subtypes, indicating that the aromatic ring is an essential component for the binding of these compounds.

The involvement of σ-2 receptor subtypes in the motor effects of σ ligands and neuroleptic drugs[18,19] has prompted studies toward the design of σ-2 subtype selective ligands. Study of the physiological role of this subtype has been hindered by the paucity of σ-2-selective ligands. The polyamines shown in Table 2 will provide a base for the development of such compounds, as well as the identification of new σ receptor subtypes. It is likely that manipulation of the aromatic ring substitution of σ-2 selective polyamines such as 11 and 13 will result in further improvements in their σ-2 profile. Studies in this direction are presently in progress. Furthermore, 3–17 are undergoing evaluation for their σ agonist/antagonist properties in several functional assays for σ receptors.

Experimental Section

Biological Materials and Methods. σ Binding Assays. σ-1 Binding sites were labeled using the σ-1-selective ligand, [^{3}H]-(+)-pentazocine[28] and guinea pig brain membranes, as described previously.[29] Rat liver membranes have been shown previously to be a rich source of σ-2 sites[30,31] and are labeled using [^{3}H]DTG in the presence of dextrallorphan to mask σ-1 sites.[15,29,32]

Membrane Preparation. Crude P_2 membrane fraction was prepared from frozen guinea pig brains (Pel-Freeze, Rogers, AK),

Table 2. σ Binding of Polyamino σ Ligands 3–17 (Chart 2) and Comparison with the Prototypic Ligands

compd[a]	K_i ([^{3}H]-(+)-pent) (nM, gp) (σ_1)	K_i ([^{3}H]DTG) (nM, rat liver) (σ_2)	σ_2/σ_1
3	2.1 ± 0.8	8.1 ± 2.2	3.9
4	28.7 ± 4.9	77.5 ± 8.0	2.7
5	13.8 ± 2.2	14.4 ± 4.7	1.0
6	110 ● 16	53.6 ± 4.6	0.49
7	227 ● 31	162 ± 9	0.71
8	29.9 ● 4.2	18.3 ± 0.3	0.61
9	8.0 ± 1.2	52.6 ● 4.1	6.6
10	1.49 ± 0.07	12.1 ± 0.4	8.1
11	77.5 ± 5.9	14.9 ± 3.0	0.19
12	128 ● 20	152 ± 18	1.2
13	1122 ± 93	115 ± 28	0.10
14	40.7 ± 0.9	135 ± 6	3.3
15	390 ± 36	2364 ± 613	6.1
16	243 ± 16	537 ± 60	2.2
17	54 ± 7	111 ± 6	2.1
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 17. Values are averages ± SEM of two to four experiments, each carried out in triplicate.

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/g 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/g in 10 mM Tris-HCl, pH 7.4, and aliquots were stored at −80 °C until use. Protein concentration of the suspension was determined by the method of Lowry[33] and was 20–25 mg of protein/mL.

Various concentrations of the test ligand ranging from 0.005 to 1000 nM or from 0.05 to 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[34] and K_d values that were predetermined in independent experiments.

σ-1 Binding Assay. Guinea pig brain membranes (325–500 μg of protein) were incubated with 3 nM [^{3}H]-(+)-pentazocine (51.7 Ci/mmol) 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 10 μM (+)-pentazocine. Test compounds were added in concentrations ranging from 0.005 to 1000 or from 0.05 to 10000 nM. Assays were terminated by the addition of 5 mL 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% polyethylenimine 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 to 1000 or from 0.05 to 10000 nM. Assays were terminated by the addition of 5 mL 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% polyethylenimine 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.

Chemicals. [^{3}H]DTG was purchased from Dupont/New England Nuclear (Boston, MA). [^{3}H]-(+)-Pentazocine was synthesized as described previously.[28] Dextrallorphan and (+)-pentazocine were synthesized in the Laboratory of Medicinal Chemistry, NIDDK, NIH. DTG was purchased from Aldrich Chemical Co. (Milwaukee, WI). Polyethylenimine, and Tris-HCl were purchased from Sigma Chemical Co. (St. Louis, MO).

Chemistry 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 recorded from $CDCl_3$ solutions using a Varian XL-300 spectrometer; results are recorded as ppm downfield of the TMS signal. ^{1}H-NMR spectral data for all amines is reported for the free base form of these compounds. Thin-layer chromatography (TLC) was performed on 250 μM Analtech GHLF silica gel plates. TLC solvent system A refers to concentrated aqueous ammonia–MeOH–$CHCl_3$ (1:9:90). TLC solvent system B refers to concentrated aqueous ammonia–MeOH–$CHCl_3$ (2:18:80). No attempt was made to optimize the yields shown in Table 1.

General Method A. To a stirred solution of acid (37 mmol) in dry CH_2Cl_2 (150 mL) was added a solution of 1-((dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (8.53 g, 1.2 equiv). The solution was stirred for 10 min at rt, and then amine (1.2 equiv) was added in one portion. The reaction was stirred at room temperature overnight or until complete by TLC (solvent system A). The solvent was evaporated in vacuo, and the residue was partitioned between water (200 mL) and EtOAc (200 mL). The aqueous layer was discarded. The organic layer was washed with 2 M HCl (2 × 200 mL), aqueous 10% K_2CO_3 (200 mL), and saturated NaCl (200 mL) and dried (Na_2SO_4). Evaporation of the solvent in vacuo gave the products which were purified further by recrystallization from the appropriate solvent (Table 1).

In cases where amino acid ester hydrochlorides were coupled with carboxylic acids, all proportions were the same except that triethylamine (11.25g, 3 equiv) was also added to the reaction mixture.

General Method B. To a stirred solution of freshly prepared 1.0 M AlH_3 in THF[27] (50 mL 50 mmol, 5 equiv) was added, dropwise at room temperature, a solution of amide (10 mmol in dry THF, 20 mL). The solution was stirred for 10–20 min at room temperature or until complete by TLC (solvent system A for monoamines and diamines and solvent system B for triamines and higher). The reaction mixture was carefully quenched by pouring it into aqueous 15% NaOH (100 mL). The aqueous mixture was extracted with $CHCl_3$ (2 × 200 mL). The combined organic extract was dried (anhydrous Na_2CO_3) and evaporated in vacuo to give the crude products as oils which were purified by crystallization of the appropriate salts from suitable solvents (Table 1).

General Method C. To a stirred solution of $LiAlH_4$ in THF (1.0 M) (3 equiv for nitriles or monoamides, 5 equiv for amido carbamates and triamides) was added the appropriate substrate (dissolved in a volume of THF equal to half that of the $LiAlH_4$ solution used) at room temperature. The reaction mixture was boiled under reflux until complete by TLC (solvent system A for monoamines and diamines and solvent system B for triamines and higher), cooled to room temperature, and treated dropwise with water (1 mL/g of $LiAlH_4$), 15% aqueous NaOH (1 mL/g of $LiAlH_4$), and finally water (3 mL/g of $LiAlH_4$). If neccessary, more fresh THF was added prior to quenching. The solution was stirred for 45 min, and then the granular inorganic precipitate was filtered and washed with a little THF. Evaporation of the solvent afforded the target amines which were purified either by

distillation or by crystallization of the appropriate salts from suitable solvents (Table 1).

General Method D. To a stirred solution of acid (50 mmol, 1.5 equiv) in dry CH_2Cl_2 (100 mL) was added a solution of DCC (13.8 g, 66.7 mmol, 2 equiv) in CH_2Cl_2 (100 mL). The solution was stirred for 10 min at room temperature and then amine (33.3 mmol) was added in one portion. The reaction was stirred overnight at room temperature or until complete by TLC (solvent system A). The precipitated 1,3-dicyclohexylurea was removed by filtration, and the filter cake was washed with a little ether. The filtrate was evaporated, and the residue was taken up in ether (200 mL). This solution was then extracted with 30% aqueous citric acid (50 mL), and the organic layer was discarded. The aqueous layer was washed with ether (2 × 50 mL), and the combined ethereal washings were discarded. The solution was basified by addition of concentrated aqueous ammonia solution (to pH ca. 9.5) and extracted with CH_2Cl_2 (3 × 100 mL). The combined CH_2Cl_2 extract was dried (Na_2SO_4) and evaporated in vacuo to give pure products. Crystalline samples were obtained by salt formation from a suitable solvent in most cases (Table 1).

[4-(1-Pyrrolidinyl)butyl]formamide (42). Primary amine **41** (18.78 g, 130 mmol) was dissolved in HCO_2Et (150 mL) and boiled under reflux under a nitrogen atmosphere until homogeneous by TLC (solvent system A). The reaction mixture was cooled, and the solvent was evaporated in vacuo. The residue was purified by high vacuum distillation (154–156 °C (0.6 mmHg)) to give **42** (21.29 g, 96%) as a colorless, viscous oil. **42**·oxalate crystallized from 2-propanol (Table 1).

***N*-[2-(3,4-Dichlorophenyl)ethyl]-*N*-glycyl-2-(1-pyrrolidinyl)ethylamine (52).** To a stirred solution of **51** (2.2 g, 4.95 mmol) in $CHCl_3$ (30 mL) was added CF_3CO_2H (15 mL). The reaction was stirred for 2 h at room temperature or until complete by TLC (solvent system B). The solvent was evaporated, and the residue was dissolved in water (10 mL) and treated with K_2CO_3 to give a 30% w/v solution. The solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic extract was dried (Na_2SO_4), and the solvent was evaporated in vacuo to give **52** (1.5 g, 88%). **52**·oxalate salt crystallized from hot EtOH (Table 1).

***N*-(3,4-Dichlorophenylacetyl)glycine (54).** Methyl ester **53** (3.0 g, 10.9 mmol) was dissolved in 10% KOH/MeOH (50 mL), and the reaction mixture was stirred overnight at rt. The solvent was evaporated in vacuo, and water (100 mL) was added. The aqueous solution was acidified (to pH 2) by addition of concentrated aqueous HCl. The precipitated **54** was filtered, washed with a little cold water, and dried in vacuo. A pure sample was obtained by recrystallization from hot water (Table 1).

Acknowledgment. X.-S.H. acknowledges financial support from the Fogarty Foundation. The authors offer their appreciation to Noel Whittaker and Wesley White for performing mass spectral analysis of all compounds in this paper.

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(35) ^{1}H-NMR (taken in $CDCl_3$ unless stated otherwise) data of the compounds reported in Table 1 is as follows. **4**: δ 7.34 (d, J = 8.2 Hz, 1H, ArH^5), 7.30 (d, J = 2.0 Hz, 1H, ArH^2), 7.05 (dd, J = 2.0, 8.2 Hz, 1H, ArH^6), 2.78 (m, 2H), 2.67 (m, 2H), 2.55 (m, 4H, pyrrolidinyl CH_2), 1.80 (m, 4H, pyrrolidinyl CH_2). **5**: δ 7.34 (d, J = 8.3 Hz, 1H, ArH^5), 7.29 (d, J = 2.1 Hz, 1H, ArH^2), 7.03 (dd, J = 2.1, 8.3 Hz, 1H, ArH^6), 2.73 (m, 2H), 2.61 (m, 2H), 2.46–2.58 (complex m, 12H), 2.30 (s, 3H, NCH_3), 2.27 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **6**: δ 7.34 (d, J = 8.1 Hz, 1H, ArH^5), 7.29 (d, J = 2.1 Hz, 1H, ArH^2), 7.03 (dd, J = 2.1, 8.1 Hz, 1H, ArH^6), 2.73 (dist t, J = 5.5 Hz, 2H, CH_2CH_2Ar), 2.46–2.64 (complex m, 18H), 2.30 (s, 3H, NCH_3), 2.27 (s, 3H, NCH_3), 2.26 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **7**: δ 7.34 (d, J = 8.1 Hz, 1H, ArH^5), 7.29 (d, J = 2.1 Hz, 1H, ArH^2), 7.03 (dd, J = 2.1, 8.1 Hz, 1H, ArH^6), 2.73 (m, 2H), 2.61 (m, 2H), 2.44–2.58 (complex m, 20H), 2.30 (s, 3H, NCH_3), 2.27 (s, 3H, NCH_3), 2.25 (s, 6H, 2xNCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **8**: δ 7.44 (m, 1H, ArH^2), 7.37 (d, J = 8.1 Hz, 1H, ArH^5), 7.16 (dd, J = 2.0, 8.1 Hz, 1H, ArH^6), 3.46 (s, 2H, ArCH_2), 2.34–2.53 (complex m, 12H), 2.22 (s, 3H, NCH_3), 2.21 (s, 3H, NCH_3), 1.78 (m, 4H, pyrrolidinyl CH_2), 1.70 (m, 2H). **9**: δ 7.43 (br s, 1H, ArH^2), 7.37 (d, J = 7.7 Hz, 1H, ArH^5), 7.15 (dm, J = 7.7 Hz, 1H, ArH^6), 3.42 (s, 2H, ArCH_2), 2.47–2.63 (complex m, 8H), 2.39 (m, 4H), 2.25 (s, 3H, NCH_3), 2.17 (s, 3H, NCH_3), 1.78 (m, 4H, pyrrolidinyl CH_2), 1.67 (quintet, J = 7.4 Hz, 2H, NCH$_2$CH_2CH$_2$N). **10**: δ 7.44 (m, 1H, ArH^2), 7.37 (d, J = 8.1 Hz, ArH^5), 7.16 (dm, J = 8.1 Hz, ArH^6), 3.46 (s, 2H, ArCH_2), 2.45–2.62 (complex m, 12H), 2.25 (s, 3H, NCH_3), 2.21 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **11**: δ 7.34 (d, J = 8.3 Hz, 1H, ArH^5), 7.30 (d, J = 2.0 Hz, 1H, ArH^2), 7.03 (dd, J = 2.0, 8.3 Hz, 1H, ArH^6), 2.72 (dist t, J = 7.4 Hz, 2H), 2.56 (dist t, J = 7.4 Hz, 2H), 2.28–2.53 (complex m, 12H), 2.28 (s, 3H, NCH_3), 2.21 (s, 3H, NCH_3), 1.78 (m, 4H, pyrrolidinyl CH_2), 1.68 (complex m, 4H, 2 × NCH$_2$CH_2CH$_2$CH$_2$N). **12**: δ 7.33 (d, J = 8.2 Hz, ArH^5), 7.30 (d, J = 2.1 Hz, 1H, ArH^2), 7.03 (dd, J = 2.1, 8.2 Hz, 1H, ArH^6), 2.72 (dist t, J = 7.8 Hz, 2H), 2.57 (dist t, J = 7.8 Hz, 2H), 2.29–2.51 (complex m, 10H), 2.27 (s, 3H, NCH_3), 2.19 (s, 3H, NCH_3), 1.74–1.86 (m, 6H), 1.63 (quintet, J = 7.5 Hz, 2H, NCH$_2$CH_2CH$_2$N), 1.50 (m, 4H). **13**: δ 7.33 (d, J = 8.1 Hz, 1H, ArH^5), 7.30 (d, J = 2.0 Hz, 1H, ArH^2), 7.03 (dd, J = 2.0, 8.1 Hz, ArH^6), 2.72 (dist t, J = 7.7 Hz, 2H, CH_2CH_2Ar), 2.56 (dist t, J = 7.7 Hz, 2H, CH_2CH_2Ar), 2.28–2.52 (complex m, 12H), 2.27 (s, 3H, NCH_3), 2.19 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.40–1.56 (complex m, 8H). **14**: δ 7.34 (d, J = 8.2 Hz, 1H, ArH^2), 7.30 (d, J = 2.1 Hz, 1H, ArH^5), 7.03 (dd, J = 2.1, 8.2 Hz, 1H, ArH^6), 2.63–2.74 (complex m, 8H), 2.47–2.60 (complex m, 8H), 1.78 (m, 4H, pyrrolidinyl CH_2). **16**: δ 7.35 (d, J = 8.1 Hz, 1H, ArH^5), 7.31 (d, J = 1.9 Hz, 1H, ArH^2), 7.04 (dd, J = 1.9, 8.1 Hz, 1H, ArH^6), 2.86 (dist t, J = 6.7 Hz, 2H), 2.69–2.81 (complex m, 8H), 2.65 (dist t, J = 5.8 Hz, 2H). **17**: δ 7.35 (d, J = 8.2 Hz, 1H, ArH^5), 7.30 (d, J = 2.0 Hz, 1H, ArH^2), 7.04 (dd, J = 2.0, 8.2 Hz, 1H, ArH^6), 2.86 (dist t, J = 6.2 Hz, 2H), 2.69–2.78 (complex m, 8H), 2.57 (dist t, J = 6.5 Hz, 2H), 2.49 (m, 4H, pyrrolidinyl CH_2), 1.76 (m, 4H, pyrrolidinyl CH_2). **18**: δ 7.38 (d, J = 8.3 Hz, 1H, ArH^5), 7.34 (d, J = 2.0 Hz, 1H, ArH^2), 7.13 (dd, J = 2.0, 8.3 Hz, 1H, ArH^6), 3.59 (s, 2H, ArCH_2), 3.49 (t, J = 6.9 Hz, 2H), 3.44 (t, J = 6.8 Hz, 2H), 1.81–2.02 (m, 4H). **20**: δ 5.53 (br s, 1H, NH), 4.00 (40% rotamer), 3.94 (60% rotamer) (d, J = 4.3 Hz, 2H, COCH_2NH), 3.55 (60% rotamer), 3.35 (40% rotamer) (t, J = 7.3 Hz, 2H, CH_2CH_2NCO), 2.99 (s, 3H, NCH_3), 2.64 (t, J = 7.3 Hz, 2H, NCH_2CH$_2$NCO), 2.50–2.60 (m, 4H, pyrrolidinyl CH_2), 1.79 (m, 4H, pyrrolidinyl CH_2), 1.45 (s, 9H, tBu). **21**: δ 5.54 (55% rotamer), 5.39 (45% rotamer) (br s, 1H, NH), 3.54 (55% rotamer), 3.42 (45% rotamer) (t, J = 7.1 Hz, 2H), 3.41 (m, 2H), 2.99 (55% rotamer), 2.95 (45% rotamer) (s, 3H, NCH_3), 2.46–2.65 (complex m, 8H), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.43 (s, 9H, tBu). **22**: δ 2.63 (m, 2H), 2.46–2.59 (complex m, 10H), 2.43 (br s, 3H, NHCH_3), 2.25 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **23**: δ 2.85 (t, J = 6.2 Hz, 2H), 2.46-2.64 (complex m, 10H), 2.50 (s, 3H, NHCH_3), 2.30 (s, 3H, NCH_3), 1.79–1.90 (m, 6H, pyrrolidinyl CH_2 and NCH$_2$CH_2CH$_2$N). **24**: δ 7.38 (d, J = 8.3 Hz, 1H, ArH^5), 7.37 (m, 1H, ArH^2), 7.11 (dm, J = 8.3 Hz, 1H, ArH^6), 3.70 (45% rotamer), 3.64 (55% rotamer) (s, 2H, COCH_2Ar), 3.50 (55% rotamer), 3.38 (45% rotamer) (t, J = 6.8 Hz, 2H, CH_2CH_2NCO), 3.03 (55% rotamer), 2.96 (45% rotamer) (s, 3H, CONCH_3), 2.46–2.58 (complex m, 10H), 2.29 (s, 3H, NCH_3), 1.76 (m, 4H, pyrrolidinyl CH_2). **25**: δ 7.50–7.60 (m, 1H, ArH^2), 7.48 (d, J = 8.2 Hz, 1H, ArH^5), 7.26 (dd, J = 1.9, 8.2 Hz, 1H, ArH^6), 3.62 (m, 1H), 3.31 (m, 1H), 3.04 (m, 2H), 2.00–2.72 (complex m, 14H), 1.76 (m, 4H, pyrrolidinyl CH_2). **26**: δ 7.50 (d, J = 1.9 Hz, 1H, ArH^2), 7.48 (d, J = 8.3 Hz, 1H, ArH^5), 7.24 (dd, J = 1.9, 8.3 Hz, 1H, ArH^6), 3.54 (50% rotamer), 3.28 (50% rotamer) (m, 2H, CH_2NCO), 3.06 (50% rotamer), 2.96 (50% rotamer) (s, 3H, CONCH_3), 2.36–2.66 (m, 8H), 1.96–2.34 (complex m, 5H), 1.77 (m, 6H, NCH$_2$CH_2CH$_2$N and pyrrolidinyl CH_2). **27**: δ 5.54 (br s, 1H, NH), 4.00 (38% rotamer), 3.95 (62% rotamer) (d, J = 4.2 Hz, 2H, COCH_2NH), 3.49 (62% rotamer), 3.30 (38% rotamer) (t, J = 6.9 Hz, 2H, CH_2CH_2NCO), 2.97 (s, 3H, CONCH_3), 2.46–2.57 (complex m, 10H), 2.29 (s, 3H, CH_2N(CH_3)CH$_2$), 1.76 (m, 4H, pyrrolidinyl CH_2), 1.44 (s, 9H, tBu). **28**: δ 2.43–2.66 (complex m, 16H), 2.42 (br s, 3H, NHCH_3), 2.27 (s, 3H, NCH_3), 2.23 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **29**: δ 7.38 (d, J = 8.3 Hz, 1H, ArH^5), 7.36 (m, 1H, ArH^2), 7.11 (dm, J = 8.3 Hz, 1H, ArH^6), 3.70 (38% rotamer), 3.65 (62% rotamer) (s, 2H, CH_2Ar), 3.50 (62% rotamer), 3.39 (38% rotamer) (t, J = 6.9 Hz, 2H, CH_2CH_2NCO), 3.02 (62% rotamer), 2.96 (38% rotamer) (s, 3H, CONCH_3), 2.43–2.63 (complex m, 14H), 2.27 (62% rotamer, s, 3H, NCH_3), 2.25 (62% rotamer, s, 3H, N"CH_3), 1.74–1.84 (m, 4H, pyrrolidinyl CH_2). **31**: δ 2.63 (dist t, J = 6.1 Hz, 2H), 2.46–2.60 (complex m, 18H), 2.42 (s, 3H, NHCH_3), 2.27 (s, 3H, NCH_3), 2.25 (s, 3H, NCH_3), 2.23 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2). **32**: δ 7.38 (d, J = 8.3 Hz, 1H, ArH^5), 7.36 (m, 1H, ArH^2), 7.11 (dm, J = 8.3 Hz, 1H, ArH^6), 3.70 (42% rotamer), 3.64 (58% rotamer) (s, 2H, COCH_2Ar), 3.50 (58% rotamer), 3.38 (42% rotamer) (t, J = 6.9 Hz, 2H, CH_2CH_2NCO), 3.02 (58% rotamer), 2.96 (42% rotamer) (s, 3H, CONCH_3), 2.40–2.63 (complex m, 14H), 2.27 (58% rotamer), 2.24 (42% rotamer) (s, 3H, NCH_3), 2.26 (s, 3H, NCH_3), 1.77 (m, 4H). **34**: δ 5.54 (br s, 1H, NH), 4.02 (45% rotamer), 3.93 (55% rotamer) (d, J = 3.9 Hz, 2H, COCH_2NH), 3.45 (55% rotamer), 3.30 (45% rotamer) (t, J = 7.3 Hz, 2H, CH_2NCO), 2.95 (s, 3H, NCH_3), 2.38–2.52 (complex m, 6H), 1.72–1.82 (complex m, 6H), 1.45 (s, 9H, tBu). **35**: δ 5.37 (br s, 1H, NH), 3.30–3.47 (complex m, 6H, CH_2-NCOCH_2CH$_2$NHBOC), 2.97 (50% rotamer), 2.92 (50% rotamer) (s, 3H, NCH_3), 2.40–2.69 (complex m, 6H), 1.72–1.89 (complex m, 6H), 1.43 (50% rotamer), 1.42 (50% rotamer) (s, 9H, tBu). **36**: δ 2.32–2.68 (complex m, 12H), 2.24 (s, 3H, NHCH_3), 2.21 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.68 (quintet, J = 7.5 Hz, 2H, $CH_2CH_2CH_2$). **37**: δ 2.60 (m, 2H), 2.49 (m, 4H, pyrrolidinyl CH_2), 2.32–2.46 (complex m, 6H), 2.41 (br s, 3H, NHCH_3), 2.21 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.60–1.74 (complex m, 4H, NCH$_2$CH_2CH$_2$N × 2). **38**: δ 7.50–7.58 (m, 1H, ArH), 7.48 (d, J = 8.2 Hz, 1H, ArH^5), 7.23–7.29 (m, 1H, ArH), 3.62 (m, 1H), 3.31 (m, 1H), 3.04 (m, 2H), 1.96–2.70 (complex m, 14H), 1.78 (m, 4H, pyrrolidinyl CH_2), 1.56–1.86 (complex m, 2H). **39**: δ 7.39 (50% rotamer), 7.38 (50% rotamer) (d, J = 8.3 Hz, 1H, ArH^5), 7.36 (br s, 1H, ArH^2), 7.10 (dm, J = 8.3 Hz, 1H, ArH^6), 3.72 (50% rotamer), 3.64 (50% rotamer) (s, 2H, COCH_2Ar), 3.40 (50% rotamer), 3.34 (50 % rotamer) (t, J = 7.3 Hz, 2H, CH_2NCO), 2.99 (50% rotamer), 2.93 (50% rotamer) (s, 3H, CONCH_3), 2.20-2.53 (complex m, 10 H), 2.18 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.69 (m, 4H,

$NCH_2CH_2CH_2NCH_2CH_2CH_2N$). **41**: δ 2.71 (t, J = 6.8 Hz, 2H), 2.49 (m, 4H, pyrrolidinyl CH_2), 2.44 (t, J = 6.8 Hz, 2H), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.41–1.62 (complex m, 4H). **42**: δ 8.13 (s, 1H, CHO), 6.99 (br s, 1H, NH), 3.30 (m, 2H, CH_2NHCHO), 2.50 (m, 6H), 1.78 (m, 4H, pyrrolidinyl CH_2), 1.59 (m, 4H, $NCH_2CH_2CH_2CH_2N$). **43**: δ 2.58 (t, J = 6.8 Hz, 2H, $NHCH_2CH_2$), 2.40–2.52 (m, 6H), 2.42 (s, 3H, $NHCH_3$), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.53 (m, 4H, $NCH_2CH_2CH_2CH_2N$). **44**: δ 5.41 (br s, 1H, NH), 3.40 (m, 4H), 3.26 (45% rotamer), 2.97 (55% rotamer) (t, J = 7.3 Hz, 2H, $CH_2CH_2NCH_3CO$), 2.95 (55% rotamer), 2.92 (45% rotamer) (s, 3H, NCH_3), 2.64 (m, 2H), 2.51 (m, 4H, pyrrolidinyl CH_2), 1.83 (m, 4H, pyrrolidinyl CH_2), 1.57 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 1.43 (s, 9H, *t*Bu). **45**: δ 4.90 (br s, 1H, NH), 3.38 (65% rotamer), 3.28 (35% rotamer) (t, J = 6.8 Hz, 2H, $NCOCH_2CH_2$), 3.22–3.48 (m, 2H), 3.16 (m, 2H, CH_2CH_2NHBOC), 2.97 (65% rotamer), 2.91 (35% rotamer) (s, 3H, $CONCH_3$), 2.66–2.82 (m, 2H), 2.50–2.62 (m, 2H), 2.35 (m, 2H), 1.91 (m, 4H, pyrrolidinyl CH_2), 1.83 (m, 2H, $COCH_2CH_2CH_2N$), 1.60 (m, 4H, $NCH_2CH_2CH_2CH_2N$), 1.43 (s, 9H, *t*Bu). **46**: δ 2.60 (t, J = 7.0 Hz, 2H), 2.48 (m, 4H, pyrrolidinyl CH_2), 2.42 (s, 3H, $NHCH_3$), 2.30–2.42 (m, 6H), 2.20 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.66 (quintet, J = 7.1 Hz, 2H, $NCH_2CH_2CH_2N$), 1.50 (m, 4H, $NCH_2CH_2CH_2CH_2N$). **47**: δ 2.40–2.60 (complex m, 8H), 2.42 (s, 3H, $NHCH_3$), 2.33 (m, 4H), 2.20 (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.50 (m, 8H). **48**: δ 7.38 (50% rotamer), 7.37 (50% rotamer) (d, J = 8.3 Hz, 1H, ArH^5), 7.36 (d, J = 1.9 Hz, 1H, ArH^2), 7.10 (dm, J = 7.3 Hz, 1H, ArH^6), 3.73 (50% rotamer), 3.64 (50% rotamer) (s, 2H, CH_2Ar), 3.40 (50% rotamer), 3.34 (50% rotamer) (t, J = 7.4 Hz, 2H, CH_2CH_2NCO), 2.99 (50% rotamer), 2.93 (50% rotamer) (s, 3H, $CONCH_3$), 2.38–2.52 (complex m, 6H), 2.24–2.35 (m, 4H), 2.17 (br s, 3H, NCH_3), 1.73–1.80 (m, 4H, pyrrolidinyl CH_2), 1.68 (quintet, J = 6.6 Hz, 2H, $NCH_2CH_2CH_2N$), 1.42–1.56 (m, 4H, $NCH_2CH_2CH_2CH_2N$). **49**: δ 7.38 (d, J = 8.2 Hz, 1H, ArH^5), 7.36 (m, 1H, ArH^2), 7.10 (dm, J = 8.2 Hz, 1H, ArH^6), 3.66 (45% rotamer), 3.63 (55% rotamer) (s, 2H, $COCH_2Ar$), 3.39 (55% rotamer), 3.29 (45% rotamer) (t, J = 7.3 Hz, 2H, CH_2CH_2NCO), 2.98 (55% rotamer), 2.94 (45% rotamer) (s, 3H, $CONCH_3$), 2.39–2.52 (m, 6H), 2.31 (m, 4H), 2.18 (55% rotamer), 2.17 (45% rotamer) (s, 3H, NCH_3), 1.77 (m, 4H, pyrrolidinyl CH_2), 1.36–1.60 (m, 8H, $NCH_2CH_2CH_2CH_2N \times 2$). **51**: δ 7.39 (33% rotamer), 7.36 (67% rotamer) (d, J = 8.0 Hz, 1H, ArH^5), 7.31 (67% rotamer), 7.27 (33% rotamer) (d, J = 2.0 Hz, 1H, ArH^2), 7.06 (67% rotamer), 7.00 (33% rotamer) (dd, J = 2.0, 8.0 Hz, 1H, ArH^6), 3.53 (t, J = 7.6 Hz, 2H), 3.49 (67% rotamer), 3.32 (33% rotamer) (s, 2H, $COCH_2NH$), 3.44 (67% rotamer), 3.24 (33% rotamer) (t, J = 7.6 Hz, 2H), 2.83 (m, 2H), 2.47–2.69 (complex m, 6H), 1.78 (m, 4H), 1.62 (br s, 9H, *t*Bu). **52**: δ 7.39 (33% rotamer), 7.36 (67% rotamer) (d, J = 8.1 Hz, 1H, ArH^5), 7.31 (d, J = 2.0 Hz, 1H, ArH^2), 7.06 (67% rotamer), 7.00 (33% rotamer) (dd, J = 2.0, 8.1 Hz, 1H, ArH^6), 3.53 (dist t, J = 7.9 Hz, 2H), 3.49 (67% rotamer), 3.32 (33% rotamer) (s, 2H, $COCH_2NH_2$), 3.44 (33% rotamer), 3.24 (67% rotamer) (t, J = 7.6 Hz, 2H), 2.83 (dist t, J = 7.9 Hz, 2H), 2.65 (33% rotamer), 2.56 (67% rotamer) (t, J = 7.1 Hz, 2H), 2.51 (m, 4H), 1.78 (m, 4H, pyrrolidinyl CH_2). **53**: δ 7.43 (d, J = 8.8 Hz, 1H, ArH^5), 7.41 (m, 1H, ArH^2), 7.15 (dd, J = 2.2, 8.8 Hz, 1H, ArH^6), 5.97 (br s, 1H, NH), 4.04 (d, J = 5.4 Hz, 2H), 3.76 (s, 3H, CO_2CH_3), 3.56 (s, 2H). **54** (CD_3OD): δ 7.49 (d, J = 2.1 Hz, 1H, ArH^2), 7.44 (d, J = 8.3 Hz, 1H, ArH^5), 7.23 (dd, J = 2.1, 8.3 Hz, 1H, ArH^6), 3.90 (s, 2H), 3.56 (s, 2H). **55** (CD_3OD): δ 7.49 (d, J = 2.0 Hz, 1H, ArH^2), 7.45 (d, J = 8.2 Hz, 1H, ArH^5), 7.24 (dd, J = 2.0, 8.7 Hz, 1H, ArH^6), 3.88 (s, 2H), 3.84 (s, 2H), 3.59 (s, 2H). **56**: δ 7.42 (d, J = 8.3 Hz, 1H, ArH^5), 7.40 (d, J = 2.2 Hz, 1H, ArH^5), 7.14 (dd, J = 2.2, 8.3 Hz, 1H, ArH^6), 6.40 (br s, 2H, 2 x NH), 3.91 (d, J = 4.9 Hz, 2H), 3.54 (s, 2H), 3.36 (q, J = 5.6 Hz, 2H), 2.59 (t, J = 6.0 Hz, 2H), 2.51 (m, 4H, pyrrolidinyl CH_2), 1.70–1.84 (m, 4H, pyrrolidinyl CH_2) ppm.