Synthesis of N,N'-Substituted Piperazine and Homopiperazine Derivatives with Polyamine-like Actions at N-Methyl-D-aspartate Receptors

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A series of N,N'-substituted piperazine and homopiperazine derivatives have been synthesized with the objective of producing compounds that interact with polyamine modulatory sites on N-methyl-D-aspartate (NMDA) receptors. These novel compounds exhibited polyamine-like actions, enhancing [^{3}H]MK-801 binding to NMDA receptors in rat forebrain membranes. The potencies of N,N'-bis(2-aminoacetyl)homopiperazine (**15**), N,N'-bis(N-methyl-4-aminobutyl)-piperazine (**7**), and N,N'-bis(3-aminopropyl)homopiperazine (**11**) (EC_{50} 18.0, 21.3, and 24.4 μM, respectively) to enhance [^{3}H]MK-801 binding were comparable to that of spermine (EC_{50} 5.2 μM). However, the efficacies of **15**, **7**, and **11** in this measure were lower (by ~40%, 32%, and 24%, respectively) than spermine, which may be indicative of partial agonist actions. Like spermine, the ability of these piperazine and homopiperazine derivatives to enhance [^{3}H]MK-801 binding could be inhibited by both a competitive polyamine antagonist (arcaine) and a specific, noncompetitive polyamine antagonist (conantokin-G). However, unlike endogenous polyamines, high concentrations (up to 1 mM) of these novel polyamine-like compounds did not inhibit [^{3}H]MK-801 binding. N,N'-Aminoalkylated and aminoacylated piperazine and homopiperazine derivatives may prove useful for studying polyamine recognition sites associated with NMDA receptors.

Introduction

N-Methyl-D-aspartate (NMDA) receptors are a family of ligand-gated channels that play a pivotal role in both physiological and pathophysiological processes in the mammalian central nervous system. For example, activation of NMDA receptors appears required for the induction of long-term potentiation (LTP) in hippocampus, a process that may form the basis for certain types of learning and memory.[1,2] Moreover, converging lines of evidence indicate that excessive activation of NMDA receptors substantially contributes to the neuronal cell death resulting from cerebral ischemia and other neurodegenerative disorders.[3–6]

Both neurochemical and electrophysiological studies indicate that endogenous polyamines such as spermine (N,N'-bis(3-aminopropyl)-1,4-butanediamine) and spermidine (N-(3-aminopropyl)-1,4-butanediamine) have multiple effects at NMDA receptors. For example, these polyamines act as positive modulators at NMDA receptors, increasing the binding of use-dependent channel blockers like [^{3}H]MK-801 to brain membranes in the nominal absence of glutamate and glycine[7–10] and augmenting NMDA currents in both wild type and recombinant receptors.[11–13] Polyamines also increase the apparent affinity of glycine at strychnine-insensitive glycine receptors[14,15] and enhance the potency of glycine to augment NMDA-mediated currents.[11,13] The positive modulatory actions of polyamines appear to be mediated via extracellular recognition sites[16–18] and are dependent upon NMDA receptor subunit composition.[13,19] At higher concentrations, polyamines produce a voltage-dependent block of NMDA receptors[11,16] and inhibit the binding of voltage-dependent channel blockers such as [^{3}H]MK-801.[8–10,20]

These multiple actions have made it difficult to assess the contribution of endogenous polyamines to the operation of NMDA receptors under physiological conditions. Nonetheless, there is evidence to suggest that polyamines contribute to the neuropathology associated with excessive activation of NMDA receptors. For example, an increased efflux of polyamines has been observed following mechanical brain trauma or middle cerebral artery occlusion.[17] Moreover, inhibitors of polyamine biosynthesis reduce NMDA-mediated neurotoxicity both *in vitro*[21] and *in vivo*.[22] Finally, NMDA antagonists that act at polyamine-associated sites reduce ischemic brain injury[17] and block NMDA-mediated neurotoxicity *in vitro*.[23] While several studies have examined the structure–activity relationship of linear polyamines,[9,24] far less is known about the potential of heterocyclic compounds to act as polyamine site ligands.[25]

The objective of the present study was to synthesize heterocyclic polyamine derivatives that modulate NMDA receptors through polyamine sites. To accomplish this objective, we introduced ω-aminoalkyl and ω-aminoacyl moieties to the nitrogen atoms of piperazine and homopiperazine. Several of these derivatives enhanced [^{3}H]MK-801 binding to NMDA receptors through the polyamine site with potencies comparable to, but efficacies lower than, spermine. Unlike many linear polyamines, these compounds did not inhibit [^{3}H]MK-801 binding at concentrations of up to 1 mM. This latter observation indicates that these heterocyclic polyamines may not produce a voltage-dependent block of NMDA receptors characteristic of endogenous polyamines such as spermine.

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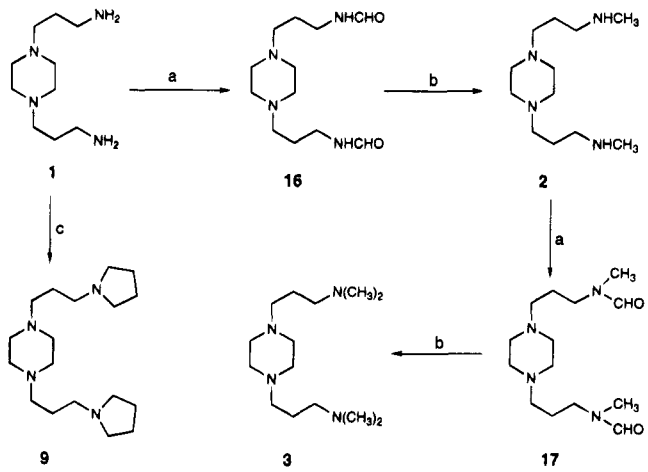
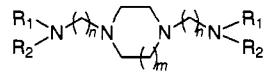
Scheme 1[a]

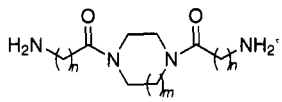


[a] (a) HCOOEt, HCOOH, reflux, N_2; (b) $LiAlH_4$/THF, reflux, N_2; (c) 1,4-dibromobutane, K_2CO_3, DMF, 60 °C, N_2.

Table 1. Structures of *N,N'*-Substituted Piperazines and Homopiperazines



no.	m	n	R_1	R_2
1	1	3	H	H
2	1	3	H	CH_3
3	1	3	CH_3	CH_3
4	1	2	H	CH_3
4	1	2	H	CH_3
5	1	2	CH_3	CH_3
6	1	4	H	H
7	1	4	H	CH_3
8	1	4	CH_3	CH_3
9	1	3	$-(CH_2)_4-$	
10	2	2	H	H
11	2	3	H	H
12	2	4	H	H



no.	m	n
13	1	1
14	1	3
15	2	1

Chemistry

N,N'-Bis(3-(methylamino)propyl)piperazine (**2**) was synthesized starting from the commercially available *N,N'*-bis(3-aminopropyl)piperazine (**1**) (Scheme 1, Tables 1 and 2): *N*-Formylation was accomplished by refluxing **1** in ethyl formate with a trace of formic acid to afford *N,N'*-bis(3-(formylamino)propyl)piperazine (**16**). This was followed by lithium aluminum hydride ($LiAlH_4$) reduction[27] to yield **2**. *N,N'*-Bis(3-(dimethylamino)-propyl)piperazine (**3**) was prepared from **2** by formylation and reduction as described for the preparation of **2** (Scheme 1, Tables 1 and 2). *N,N'*-Bis(3-pyrrolidinyl-propyl)piperazine (**9**) was obtained by treating **1** with 1,4-dibromobutane. The isolated yield of **9** was low due to the high polarity and water solubility of this compound (Scheme 1, Table 2).

Piperazine and homopiperazine were used as starting materials for preparation of target compounds **4–15** (Scheme 2, Tables 1 and 2). Coupling of piperazine with 4-(*t*-Boc-amino)butyric acid in the presence of 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide afforded *N,N'*-bis(4-(*t*-Boc-amino)butyryl)piperazine (**20**). Removal of the *N-t*-butoxycarbonyl protecting group of **20** with CF_3COOH in $CHCl_3$ afforded *N,N'*-bis(4-aminobutyryl)piperazine **14**, which was readily reduced by alane (AlH_3) (1.0 M alane in THF prepared as described[26]) at room temperature to yield *N,N'*-bis(4-aminobutyl)piperazine (**6**). Using the same synthetic route illustrated in Scheme 2, the homopiperazine derivatives **10–12** were obtained.

Amides **18** and **20** served as the precursors in preparation of compounds **4, 5, 7,** and **8**. **18** and **20** were reduced by $LiAlH_4$ to afford **4** and **7** (Scheme 2), respectively. Dimethylamines **5** and **8** were synthesized by the same methods as described for the preparation of **3** (Schemes 1 and 2).

Results and Discussion

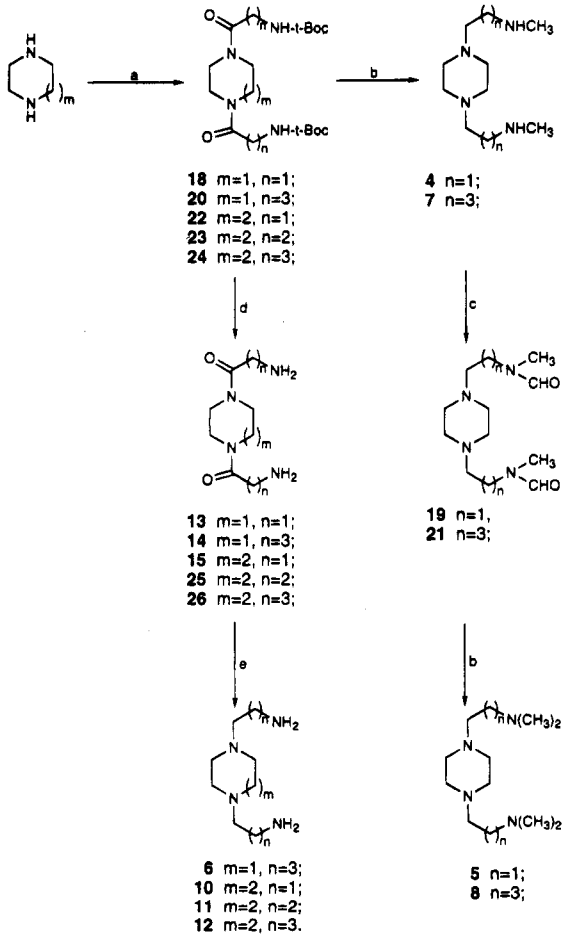
On the basis of the ability of linear di-, tri-, and tetraamines to modulate NMDA receptors,[9,24] a series of *N,N'*-substituted piperazine and homopiperazine derivatives were synthesized with the objective of developing high-affinity polyamine site ligands. These piperazine and homopiperazine analogs (Table 1) were evaluated using [^{3}H]MK-801 binding assays under non-equilibrium conditions, since this strategy is a sensitive and specific means of detecting substances that either facilitate or reduce activity at NMDA receptors.[7, 28–30] Consistent with previous reports,[7–10] spermine enhanced [^{3}H]MK-801 binding in a concentration dependent manner [Figure 1, EC_{50} 5.2 ± 0.3 μM (n = 6)]. At concentrations ≥50 μM, a second effect of spermine was apparent, characterized by a progressive reduction in [^{3}H]MK-801 binding (Figure 1). The novel piperazine and homopiperazine derivatives enhanced [^{3}H]MK-801 binding with EC_{50} values ranging from ~18 to >200 μM (Figure 1; Table 3). Among these compounds, **7, 11** and **15** exhibited potencies closest to spermine, with EC_{50} values of 18.0, 21.3, and 24.4 μM, respectively (Table 3 and Figure 1). However, the maximum increases in [^{3}H]-MK-801 binding produced by these compounds (as well as several of the less potent homopiperazine and piperazine derivatives) were lower than that produced by spermine (Figure 1 and Table 3), indicative of partial agonist actions. Partial agonist actions of synthetic, linear polyamines have also been observed.[31]

While the synthesized piperazine and homopiperazine derivatives were designed as polyamine site ligands, additional experiments were performed to confirm that the enhancement of [^{3}H]MK-801 binding by the most potent derivatives was produced by a polyamine-like action. Arcaine, which can competitively inhibit the positive modulatory effects of polyamines at NMDA receptors,[32,33] produced a parallel rightward shift of the spermine concentration effect curve (Figure 2A). Arcaine produced a similar rightward shift in the concentration effect curve of **11** (Figure 2B). Conantokin-G is an NMDA antagonist[18,23,34] which exhibits a potent, non-competitive inhibiton of polyamine-enhanced [^{3}H]MK-801 binding. In contrast, this peptide has little or no effect on glutamate- or glycine-enhanced [^{3}H]MK-801 binding.[18] Conantokin-G inhibited spermine, **7**, and **15**-enhanced [^{3}H]MK-801 binding in a concentration dependent fashion with similar IC_{50} values (Table 4). This observation, when taken together with the noncompeti-

Table 2. Physical and Chemical Data

no.	salt	solvent	mp (°C)	met	CIMS, m/z (MH^+)	formula	yield (%)
2	4HBr	2-PrOH	250–252	C	$C_{12}H_{29}N_4$	$C_{12}H_{32}N_4Br_4$	77
3	4HBr	2-PrOH	272–275 dec	C	$C_{14}H_{33}N_4$	$C_{14}H_{36}N_4Br_4$	78
4	4HBr	2-PrOH	280–281 dec	C	$C_{10}H_{25}N_4$	$C_{10}H_{28}N_4Br_4$	73
5	4HBr	2-PrOH	260–262 dec	C	$C_{12}H_{29}N_4$	$C_{12}H_{32}N_4Br_4 \cdot 0.5H_2O$	63
6	4fum	EtOH	198–201 dec	E	$C_{12}H_{29}N_4$	$C_{28}H_{44}N_4O_6$	40
7	4HBr	2-PrOH	273–275 dec	C	$C_{14}H_{33}N_4$	$C_{14}H_{36}N_4Br_4$	43
8	4HBr	2-PrOH	272–274 dec	C	$C_{16}H_{37}N_4$	$C_{16}H_{40}N_4Br_4$	75
9	4HBr	MeOH–EtOH	270–273 dec	F	$C_{18}H_{37}N_4$	$C_{18}H_{40}N_4Br_4$	9
10	4oxo	EtOH	158–161 dec	E	$C_9H_{22}N_4$	$C_{17}H_{30}N_4O_4$	35
11	4fum	EtOH–MeOH	165–166 dec	E	$C_{11}H_{27}N_4$	$C_{27}H_{42}N_4O_{16}$	21
12	4fum	EtOH	145–147	E	$C_{14}H_{31}N_4$	$C_{30}H_{46}N_4O_{16}$	46
13	4HBr	MeOH–EtOH	>310	D	$C_8H_{17}N_4O_2$	$C_8H_{18}N_4Br_2O_2$	91
14	4HBr	EtOH	218–220	D	$C_{12}H_{25}N_4O_2$	$C_{12}H_{26}N_4Br_2O_2$	84
15	4HBr	2-PrOH–MeOH	156–161	D	$C_9H_{19}N_4O_2$	$C_9H_{20}N_4Br_2O_2 \cdot H_2O$	89
16	2fum	2-PrOH	198–200 dec	A	$C_{12}H_{25}N_4O_2$	$C_{20}H_{32}N_4O_{10}$	81
	base	2-PrOH–hexanes	80–83		$C_{12}H_{25}N_4O_2$	$C_{12}H_{24}N_4O_2$	
17	2fum	2-PrOH	170–173	A	$C_{14}H_{29}N_4O_2$	$C_{22}H_{36}N_4O_{10}$	94
18		EtOAc–hexanes	177–179	B	$C_{18}H_{33}N_4O_6$	$C_{18}H_{32}N_4O_6$	84
19	2fum	2-PrOH	174–176	A	$C_{12}H_{25}N_4O_2$	$C_{20}H_{32}N_4O_{10}$	91
20		EtOAc–hexanes	130–131	B	$C_{22}H_{41}N_4O_6$	$C_{22}H_{40}N_4O_6$	22
21	2fum	2-PrOH	178–179	A	$C_{16}H_{33}N_4O_2$	$C_{24}H_{40}N_4O_{10}$	96
22		EtOAc–hexanes	145–146	B	$C_{19}H_{35}N_4O_6$	$C_{19}H_{34}N_4O_6$	49
23	oil			B	$C_{21}H_{39}N_4O_6$	$C_{21}H_{38}N_4O_6 \cdot 0.75CH_2Cl_2$	87
24	oil			B	$C_{23}H_{43}N_4O_6$	$C_{23}H_{42}N_4O_6 \cdot 0.33CH_2Cl_2$	59
25	oil			D	$C_{11}H_{23}N_4O_2$	$C_{11}H_{22}N_4O_2$	73
26	oil			D	$C_{13}H_{27}N_4O_2$	$C_{13}H_{26}N_4O_2$	67

Scheme 2[a]



[a] (a) ω-*N*-*t*-Boc-amino acids, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, Et_3N, CH_2Cl_2, room temperature; (b) $LiAlH_4$/THF, reflux, N_2; (c) HCOOEt, HCOOH, reflux, N_2; (d) TFA, $CHCl_3$; (e) AlH_3, THF.

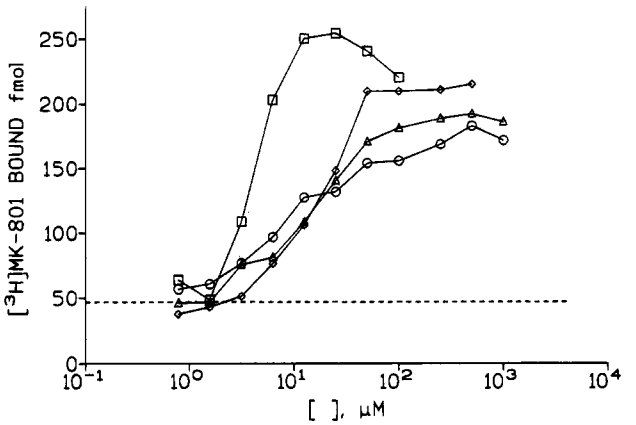


Figure 1. Effects of spermine, **7**, **11**, and **15** on [^{3}H]MK-801 binding. Symbols: squares, spermine; diamonds, **11**; triangles, **7**; and circles, **15**. The data shown are representative of results obtained in Table 3. In these representative experiments, the EC_{50} values for spermine, **7**, **11**, and **15** were 4.0, 13.7, 15.8, and 8.5 μM, respectively. In this experiment, basal [^{3}H]MK-801 binding (dotted line) was 45 fmol/assay.

tive action of conantokin-G (Figure 3), provides additional evidence that these *N,N'*-substituted piperazines and homopiperazines are acting at polyamine sites.

These *N,N'*-substituted piperazines and homopiperazines mimick the positive modulatory action of spermine at NMDA receptors, but appear to lack the low-affinity, inhibitory effect on [^{3}H]MK-801 binding produced by spermine and related polyamines (Figure 1). Thus, the inhibitory effects of spermine on [^{3}H]MK-801 binding can readily be observed at concentrations ≥20-fold its EC_{50} value, while no inhibition was observed at concentrations of **7**, **11**, and **15** corresponding to values ~50-fold higher than their EC_{50}'s (Figure 1). A similar profile has been reported for another heterocyclic compound acting through polyamine sites, 1,5-(diethylamino)piperidine.[25] If the low-affinity inhibition of [^{3}H]MK-801 binding produced by spermine corresponds to the voltage-dependent inhibition of NMDA responses,[11] then compounds such as **7**, **11**, and **15** may lack this action.

Table 3. Potencies and Efficacies of *N,N'*-Substituted Piperazines and Homopiperazines to Enhance [^{3}H]MK-801 Binding: Comparison with Spermine[a]

compound	EC_{50} (μM)	efficacy (% $spermine_{max}$)
spermine	5.2 ± 0.3	100 ± 7
1	50.1 ± 10	80 ± 2
2	35.3 ± 3.0	81 ± 10
3	36.8 ± 4.2	66 ± 9
4	64.5 ± 7.9	50 ± 3
5	112.2 ± 4.2	51 ± 4
6	55.9 ± 4.0	71 ± 3
7	21.4 ± 2.3	68 ± 9
8	77.5 ± 4.8	84 ± 16
9	45.9 ± 2.8	64 ± 10
10	50.7 ± 4.7	43 ± 4
11	24.4 ± 2.6	76 ± 3
12	37.5 ± 8.5	76 ± 2
13	96.5 ± 7.7	90 ± 5
14	238.5 ± 12.2	45 ± 4
15	18.0 ± 3.4	60 ± 2

[a] Values represent the X ± SEM of ≥3 experiments. The efficacy of spermine in these series of experiments was 164 ± 11 fmol/assay. Basal binding was 26 ± 3 fmol/assay. Efficacies are presented as a percentage of the increase in [^{3}H]MK-801 binding produced by spermine. The concentration range of **1**–**15** was 1–1000 μM. EC_{50} and E_{max} values were estimated using GraphPad Inplot.

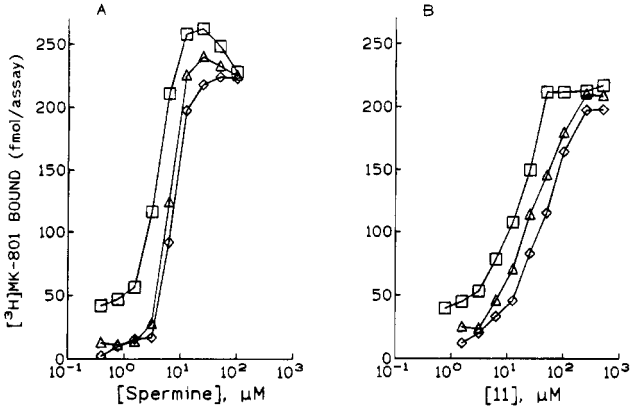


Figure 2. Inhibition of spermine and **11**-enhanced [^{3}H]MK-801 binding by arcaine. The effects of spermine (0.78–100 μM) and **11** (0.78–500 μM) were determined in the presence and absence of arcaine. Left panel: squares, spermine; triangles, spermine + arcaine (10 μM); diamonds, spermine+ arcaine (15 μM). Right panel: squares, **11**; triangles, **11** + arcaine (3 μM); diamonds, **11** + arcaine (6 μM). The EC_{50} values for spermine were 4.0, 6.1, and 6.9 μM in the absence and presence of 10 and 15 μM arcaine, respectively. The EC_{50} values for **11** in the absence and presence of 3 and 6 μM arcaine were 15.8, 30.1, and 43.1 μM, respectively. This representative experiment was replicated three times.

Table 4. Conantokin-G Inhibits [^{3}H]MK-801 Binding Stimulated by **7**, **15**, and Spermine[a]

compound	conantokin IC_{50} (nM)
spermine	232 ± 36
7	196 ± 51
15	307 ± 67

[a] [^{3}H]MK-801 binding was assayed in the presence of maximally effective concentrations of **7** (100 μM), **15** (100 μM), and spermine (12.5 μM). The concentration range of conantokin-G was 0.024–12.5 μM. Values represent the X ± SEM of ≥3 experiments. The IC_{50} values of conantokin-G to inhibit spermine, **7**, and **15**-enhanced [^{3}H]MK-801 binding were not statistically significantly different (F = 1.17; NS).

Electrophysiological experiments will be required to test this hypothesis.

Several linear tetraamines with $(CH_2)_{2-3}$ spacers are significantly more potent than spermine as positive

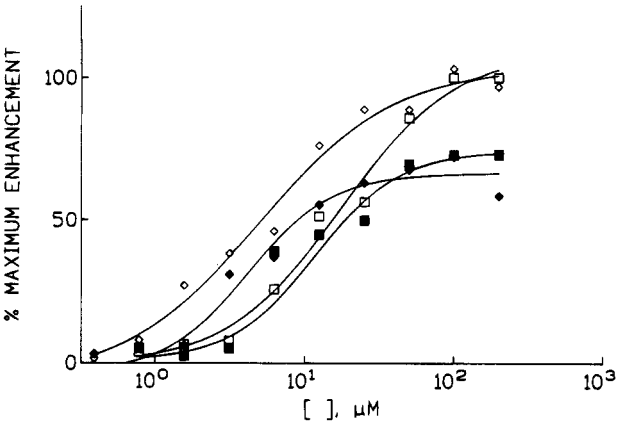


Figure 3. Conantokin-G noncompetitvely inhibits **7** and **15**-enhanced [^{3}H]MK-801 binding. In these representative experiments, **7** (squares) and **15** (diamonds) were incubated in the absence (open symbols) or presence (closed symbols) of conantokin-G (24 nM). The EC_{50} values for **7** and **15** in the absence of conantokin-G were 17.8 and 5.6 μM, respectively and 11.7 and 5.5 μM, respectively, in the presence of conantokin-G. In these representative experiments, the maximum effect of **7** and **15** was reduced 29 and 27% by conantokin-G. This experiment was repeated twice with similar results.

modulators of NMDA receptors.[9,35] For example, *N,N'*-bis(3-aminopropyl)ethylenediamine and *N,N'*-bis(3-aminopropyl)-1,3-propanediamine are ~2.5- and 7.5-fold more potent, respectively, than spermine to enhance [^{3}H]-*N*-(1-thienylcyclohexyl)piperidine (TCP) binding to NMDA receptors.[9] The *N,N'*-substituted piperazines and homopiperazines described here were synthesized in an attempt to develop heterocyclic tetraamine derivatives with affinities comparable to or greater than these linear tetraamines. The preparation of derivatives with terminally alkylated rather than primary amines was intended to increase the accessibility of these compounds to the central nervous system and reduce the potential for metabolism through oxidative deamination.

Among these *N,N'*-substituted piperazine and homopiperazine derivatives, **7**, **11**, and **15** exhibited potencies (~18–24 μM) closest to spermine and spermidine. Since the homopiperazine derivative **11** may be considered as a structural analog of *N,N'*-bis(3-aminopropyl)-1,3-propanediamine, it is concluded that cyclization of the two central diamine segments results in a significant reduction in potency as well as effficacy. It may be hypothesized that alterations in either the electron density or rigidity of the two central nitrogen atoms may contribute to these changes. Since primary amines at termini and secondary amines at other positions appear required for a high-affinity activation of NMDA receptors by linear polyamines,[9,35] the potency of **7** (EC_{50} ~21.4 μM) is remarkable since it contains a secondary amine at each amino terminus and two tertiary amines in the ring system. However, based on this series of compounds it would appear that at least one primary amino terminus is required for high affinity, since the potency of an analog of **7** containing a tertiary amine at both amino termini (**8**) was reduced by ~4-fold (EC_{50} ~77.5 μM). Nonetheless, while the manuscript was in preparation, it was reported[36] that *N,N'*-terminal dialkylated polyamines (including substitutions as large as adamantyl) are potent modulators of [^{3}H]MK-801 binding, producing a biphasic effect in this measure characteristic of polyamines. While compounds **13**–**15** were intermediates in the preparation of *N*-alkylated

piperazine and homopiperazine targets, **15** proved to be the most potent compound in the entire series (EC_{50} ~18 μM). Thus, it appears that addition of ω-aminoacyl moieties at the *N,N'*-positions of piperazine and homopiperazine may also provide an approach to the synthesis of compounds with polyamine-like actions at NMDA receptors. Such heterocycles, particularly those with secondary amine termini, may provide novel tools for studying the role of polyamines and polyamine-associated sites at NMDA receptors.

Experimental Section

Membrane Preparation. Membranes were prepared from adult, male Sprague–Dawley rats (175–300 g, Taconic farms, Germantown, NY) essentially as described.[18] Rats were killed by decapitation, and the forebrains were removed. Tissues were disrupted with a Polytron (30 s, setting 6) in 10 volumes (w/v) of 5 mM HEPES/4.5 mM Tris buffer (pH 7.7) containing 0.32 M sucrose. Unless otherwise stated, all procedures were carried out at 4 °C. The homogenate was diluted to 50 volumes with this buffer and centrifuged at 1000*g* for 10 min. The supernatant was decanted and recentrifuged at 20000*g* for 20 min. The resulting pellet was resuspended in 50 volumes of 5 mM HEPES/4.5 mM Tris buffer (assay buffer) and centrifuged at 8000*g* for 20 min. The supernatant and outer "buffy" pellet coat was centrifuged at 20000*g* for 20 min and the remaining pellet core discarded. The resulting supernatant was discarded, the pellet was resuspended in 50 volumes of assay buffer containing 1 mM EDTA, and the suspension was recentrifuged. This resuspension/centrifugation procedure was repeated four times, with the last cycle performed using assay buffer without EDTA. The resulting pellet was resuspended in 5 volumes of assay buffer, frozen over solid CO_2, and stored at −70 °C until used. On the day of assay, the tissue was thawed, diluted 10-fold with assay buffer, and centrifuged at 20000*g* for 20 min. The pellet was resuspended with 50 volumes of assay buffer, and this suspension used in radioligand binding assays. Tissues were extensively washed in order to remove endogenous materials such as glutamate, glycine, and polyamines that modulate [^{3}H]MK-801 binding.

[^{3}H]MK-801 Binding. Assays were performed in the total volume of 1 mL containing 0.5 mL (~100 μg of protein) of membrane suspension, 0.1 mL of [^{3}H]MK-801 (final concentration 4–5 nM), and test compounds (in buffer) or buffer to final volume. Assays were incubated at room temperature for 2 h and terminated by rapid filtration under partial vacuum (Brandel cell harvester, model M-24R) over glass fiber filters (GF/B) that were presoaked in 0.01% polyethylenimine. This filtration was followed by two 5 mL washes with ice-cold assay buffer. Nonspecific binding was determined using phencyclidine hydrochloride (100 μM) and represented 15–50% of the total binding in the absence of modulatory agents. Protein content was determined using the BCA Protein Assay Reagent (Pierce, Rockford, IL).

Chemistry Materials and Methods. [^{3}H]MK-801 (sp act. 22.4 Ci/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA). Spermine and arcaine were purchased from Sigma. Conantokin-G was obtained from Dr. L. Otvos (Wistar Institute) through Symphony Pharmaceuticals (Malvern, PA). Phencyclidine hydrochloride was donated by NIDA (Rockville, MD). All other materials were obtained from standard commercial sources. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta, GA. Values for C, H, and N are within ±0.4% of the theoretical values unless indicated otherwise, and molecular formulas are indicated in Table 2. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron-impact mass spectra (EIMS) and high-resolution mass measurements (HRMS) were obtained on a VG-Micro Mass 7070F mass spectrometer. ^{1}H NMR spectra were obtained using a Varian XL-300 spectrometer. Results were recorded in ppm relative to trimethylsilane as the internal reference. Thin-layer chromatography (TLC) was performed on 250 μm Analtech GHLF silica gel plates. The eluting solvents were (A) $CHCl_3$–MeOH–concentrated aqueous NH_3 (90:9:1) and (B) $CHCl_3$–MeOH–concentrated aqueous NH_3 (80:18:2). The yields shown in Table 2 are unoptimized.

General Method A. A mixture of 0.5 mL of formic acid (88%) and the primary or secondary amine (20 mmol) in ethyl formate (50 mL) was refluxed overnight. The solvent was evaporated to give the product as a colorless oil or solid. Further purification was achieved by crystallization or salt formation (Table 2).

General Method B. 1-(3-(Dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (33 mmol) was added to a stirred solution of ω-*N*-*t*-Boc-protected amino acids (32 mmol) in alcohol-free CH_2Cl_2 (150 mL). After 5 min of stirring at room temperature, a solution of piperazine or homopiperazine (13 mmol) in CH_2Cl_2 (50 mL) was added followed by triethylamine (75 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred overnight at room temperature, washed with 10% aqueous Na_2CO_3 (50 mL), 15% aqueous citric acid (2 × 50 mL), and water (50 mL), and dried (Na_2SO_4). Evaporation of the solvent in vacuo afforded the products as a colorless crystal or oil.

General Method C. Compounds containing amides and/or *N*-*t*-Boc structure features (as a free base or neutral compound) in THF were added to a stirred $LiAlH_4$ in THF (1.0 M, 5 equiv for amide and 10 equiv for N-*t*-Boc-amide) at room temperature. The mixture was refluxed until TLC indicated complete consumption of the starting material (solvent A for *t*-Boc amino amides and solvent B for amides). The reaction mixture was cooled to room temperature, and water was added. If necessary, additional fresh THF was added prior to quenching. The solution was stirred for 1 h, and the granular inorganic precipitate was filtered and washed with THF. Evaporation of the solvent afforded the target amine which was purified by crystallization of the corresponding hydrobromide salts from suitable solvents (see Table 2).

General Method D. CF_3COOH (12 mL) was added to a stirred solution of *N*-*t*-Boc-amino amides (5 mmol) in $CHCl_3$ (40 mL). The reaction mixture was stirred at room temperature overnight. The mixture was evaporated to dryness and the residue dissolved in the appropriate solvents. HBr (48%) was added to pH 3 (ice–water bath) to give crystals (Table 2).

General Method E. *N,N'*-Acylpiperazine or homopiperazine was added to a stirred solution of freshly prepared alane (5 equiv for amides), and the progress of the reaction was monitored by TLC (solvent system B). The reaction was completed in 5–10 min. The mixture was carefully poured into ice-cold 15% aqueous NaOH, and CH_2Cl_2 was added. The organic extract was separated, dried over Na_2SO_4, and evaporated to give the target compound. Purification was performed by crystallization of corresponding salts from suitable solvents (see Table 2).

Method F. 1,4-Dibromobutane (22 mmol, 1:1 equiv in 50 mL of DMF) was added to a stirred solution of *N,N'*-bis(3-aminopropyl)piperazine (10 mmol) in dry DMF (50 mL), and the mixture was heated at 60 °C for 48 h. Anhydrous K_2CO_3 (22 mmol, 1:1 equiv) was added, and the mixture was heated for an additional 24 h. The reaction mixture was cooled and poured into 400 mL of 1:1 (v/v) Et_2O/H_2O. The aqueous layer was basified with NaOH (60 g, in ice–water bath) and extracted with $CHCl_3$. The Et_2O layer was combined with the $CHCl_3$ extract, dried (Na_2SO_4), and evaporated in vacuo. The resulting oil (1.6 g) was crystallized as the HBr salt in boiling MeOH–EtOH to give 0.55 g of a pale-yellow solid. The crude product was further purified by columnn chromatography using solvent system B/MeOH (1:1) to give the final product **9** (Scheme 1 and Table 2).

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Supporting Information Available: ^{1}H NMR data for compounds **2**–**26** (4 pages). Ordering information is given on any current masthead page.

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