Design and Synthesis of [2-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic Acid (EAA-090), a Potent *N*-Methyl-D-aspartate Antagonist, via the Use of 3-Cyclobutene-1,2-dione as an Achiral α-Amino Acid Bioisostere

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The diazabicyclic amino acid phosphonate **15**, [2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl]phosphonic acid, was identified as a potent NMDA antagonist. It contains the α-amino acid bioisostere 3,4-diamino-3-cyclobutene-1,2-dione and an additional ring for conformational rigidity. Compound **15** was as potent as CGS-19755 (**5**) in the [^{3}H]CPP binding assay, the stimulated [^{3}H]TCP binding assay, and the NMDA-induced lethality model in mice. A single bolus dose of compound **15**, administered intravenously following permanent occlusion of middle cerebral artery (MCA) in the rat, reduced the size of infarcted tissue by 57%. Structure−activity relationship (SAR) studies have indicated that the six- and eight-membered ring derivatives had diminished activity and that the two-carbon side chain length was optimum for NMDA receptor affinity. Substitution on the ring was found to be counterproductive in the case of sterically demanding dimethyl groups and of no consequence in the case of an H-bonding hydroxyl group. Replacement of the phosphonic acid group by either a carboxylic acid or a tetrazole group was unproductive. The potent bicyclic NMDA antagonists were synthesized efficiently by virtue of their achiral nature and the ease of vinylgous amide formation from squaric acid esters. Compound **15**, being a unique NMDA antagonist structural type with a favorable preclinical profile, may offer advantages over existing NMDA antagonists for the treatment of neurological disorders such as stroke and head trauma. Compound **15** is currently under clinical evaluation as a neuroprotective agent for stroke.

A growing body of evidence supports the pivotal role of excitotoxic mechanisms in the pathogenesis of neuronal death due to acute brain ischemia[1] and central nervous system (CNS) trauma.[2] The excitotoxin theory of neurodegeneration indicates that the aberrant release of excitatory amino acid (EAA) neurotransmitters, in response to injury, results in the overstimulation of glutamate receptors, in particular the *N*-methyl-D-aspartate (NMDA) class of glutamate receptors.[3] The ensuing acceleration in calcium ion influx through this receptor channel leads to a cascade of events resulting in cell death.[4] Numerous reports in the literature indicate that the EAA-mediated toxicity contributes to neuronal cell loss in clinical conditions that are both rapid in onset (stroke, head trauma, spinal injury[5−7]) and chronically slow in progression (Huntington's disease, amyotropic lateral sclerosis, epilepsy, AIDS dementia, Alzheimer disease[8−10]). Therefore, antagonizing the effect of glutamic acid at the NMDA receptor as a therapeutic strategy for treating these neurodegenerative conditions has been the subject of intense research in recent years.[10−13] In hope of identifying an agent which has a different preclinical or clinical profile than existing competitive NMDA antagonists, novel functionalities were sought which might substitute for the α-amino acid functionality contained in typical antagonists, such as 2-amino-5-phophonopentanoic acid (**1**) and 2-amino-7-phosphonoheptanoic acid (**2**) (Chart 1). In a recent report,[14] the 3,4-diamino-3-cyclobutene-1,2-dione group was described as a useful α-amino acid bioisostere, and [2-[(2-amino-3,4-dioxo-1-cyclobuten-1-yl)amino]ethyl]phosphonic acid (**3**) was found to be equipotent to **2** both in the [^{3}H]CPP binding assay and in the NMDA-induced lethality model in mice. This novel bioisostere has sufficient electronic similarities to facilitate NMDA receptor binding but lacks other properties of an amino acid such as basicity, acidity, or nucleophilicity. The lack of these other features may be a disadvantage for the broader application of this bioisostere to other amino acid-containing ligands which require such features for molecular recognition with their target; however, such a functionality may be advantageous on selectivity grounds. By having only one of the four mentioned attributes of an amino acid, the 3,4-diamino-3-cyclobutene-1,2-dione group has potential to be more selective in its effects at NMDA versus other receptors, limiting the potential for deleterious side effects. For example, amino acid substrates bind to decarboxylase enzymes by the nucleophilic nitrogen reacting with pyridoxal phosphate to form an imine;[15] a 3,4-diamino-3-cyclobutene-1,2-dione-containing substrate probably would not bind to such a receptor because of its lack of a nucleophilic nitrogen. The discovery of **3** was an exciting finding in the NMDA antagonist area; however, **3** lacks the potency of earlier NMDA antagonists such as 4-(3-phosphonopropyl)-2-

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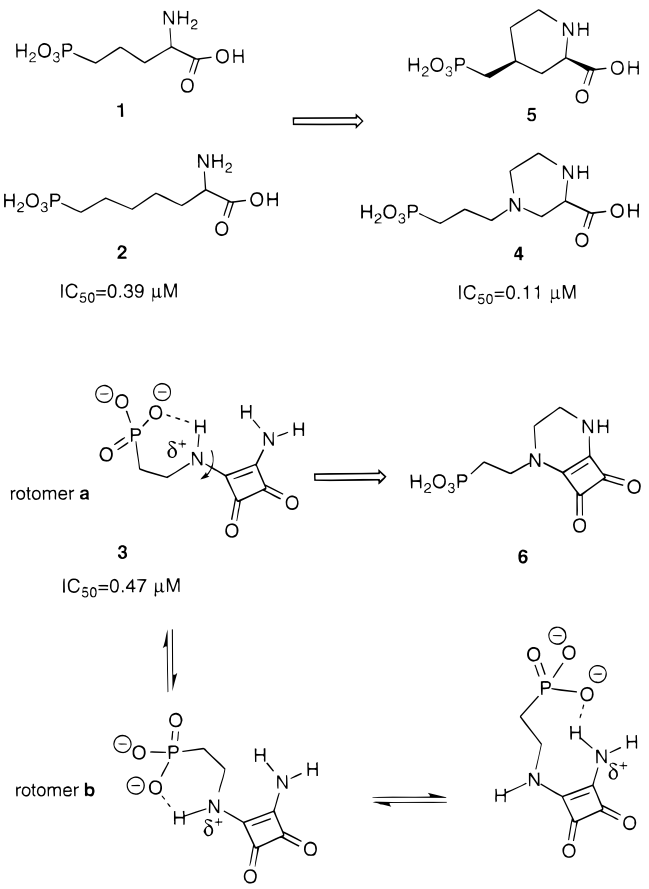
Chart 1



piperazinecarboxylic acid (**4**, CPP)[16] and *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (**5**).[17]

Since **4** is 4-fold more potent in vitro than the corresponding acyclic molecule **2**, it was anticipated that a similar increase in potency could be achieved in the case of cyclized cyclobutenedione derivative **6** (Chart 1). The acyclic molecule **3** has two rotomeric forms by virtue of the sp^2-hybridized amide nitrogens. Rotomer a has one opportunity for intramolecular hydrogen bonding, whereas rotomer b has an additional hydrogen-bonding interaction with the remote amide nitrogen. Based on the assumption that a molecule which is not hydrogen-bonded intramolecularly would be more available to hydrogen bond to a receptor, compound **6** would be an appealing target in that it lacks any such intramolecular interactions. Alternatively, the traditional argument could be made for **6**, that it is restricted to a conformation resembling the active conformation predicted within **5**.

Chemistry

The synthesis of the 7,8-dioxo-2,5-diazabicyclo[4.2.0]-oct-1(6)-ene ring system contained in target **6** was achieved from the (phosphonoethyl)ethylenediamine **9** (Scheme 1). The requisite diamine component was prepared by combining the CBZ-protected diamine **7**[18] with diethyl (2-oxoethyl)phosphonate[19] under reductive amination conditions[20] to yield **8**, which was then converted to **9** by catalytic transfer hydrogenation.[21] Solutions of **9** and 3,4-diethoxy-3-cyclobutene-1,2-dione in ethanol were coinjected in separate syringes over a 3-h period into a refluxing ethanol solution.[22] This procedure was meant to minimize the many possible side reactions of multiple additions of either reagent to the other. A somewhat variable yield of **10** was obtained, though in adequate quantities to proceed to the target **6**. The low yield is mostly due to the great degree of strain in **10** by virtue of its inability to compensate for the strain in the cyclobutenedione ring by opening up the C=C−N bond angles. Deprotection of the phosphonate ester was achieved with bromotrimethylsilane in refluxing 1,2-dichloroethane for 20 min to give a good yield of pure phosphonic acid.

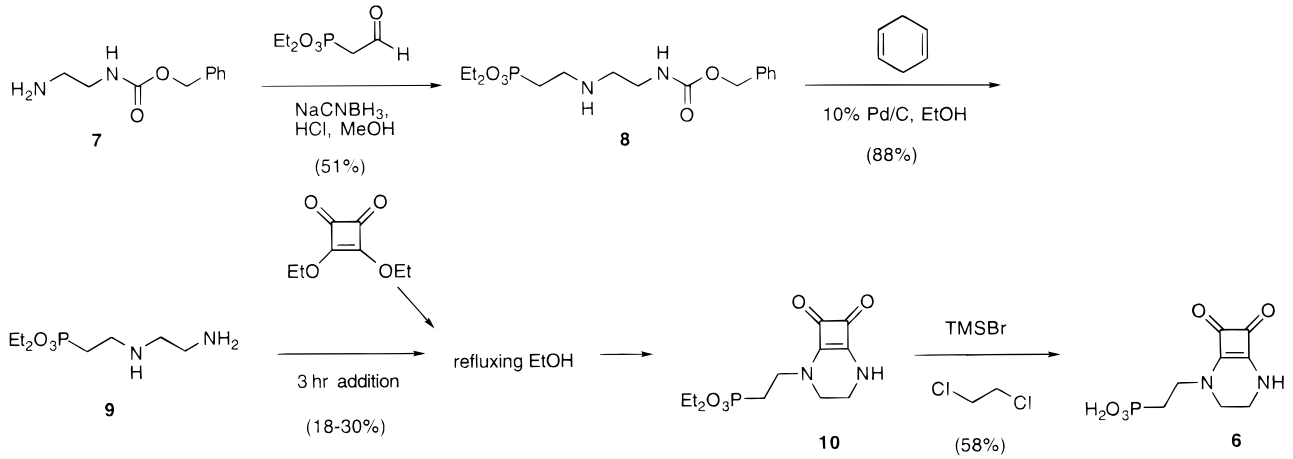
An improved stepwise cyclization scheme was demonstrated in the original discovery syntheses of the seven-membered ring containing derivatives **15** and **16** (Scheme 2). The CBZ-protected propylenediamine **11** was combined with (2-oxoethyl)phosphonate as before to yield the phosphonoethyl-substituted propylenediamine **12a** in poor yield. Alternatively, for the other analogue (**16**) alkylation of **11** with (3-bromopropyl)-phosphonate afforded **12b**. Taking advantage of the protecting group, the free amine was acylated with 3,4-diethoxy-3-cyclobutene-1,2-dione to give the monoamide **13** in excellent yield. The *N*-benzyloxycarbonyl group was cleaved under hydrogenation conditions to free the other amine, which cyclized spontaneously at room temperature to the 8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-ene ring system in good yield; the phosphonic acid **15** was obtained as before. The improved yield (62% vs 30%) of **14** is due to the stepwise cyclization strategy and the lower strain energy of **14** relative to **10**; in the case of **14** the seven-membered ring confers on the molecule the ability to increase the C=C−N bond angle.

In the syntheses of derivatives **22** and **42**, the *tert*-butyloxycarbonyl protecting group was utilized (Scheme 3). For example, reaction of 1,3-diamino-2-hydroxypropane (**17**) with di-*tert*-butyl dicarbonate[23] in acetonitrile afforded a modest yield of **18**. The phosphonoethyl group was introduced by alkylation with diethyl (2-bromoethyl)phosphonate in the presence of sodium carbonate in refluxing ethanol, which was an improvement over the reductive amination procedure (Schemes 1 and 2). The amine **19** was combined with 3,4-diethoxy-3-cyclobutene-1,2-dione to afford **20**. The *N*-BOC group was cleaved with formic acid, and the resulting crude formate salt was treated with diisopropylethylamine in refluxing ethanol overnight to yield **21**, which was hydrolyzed to the phosphonic acid **22**.

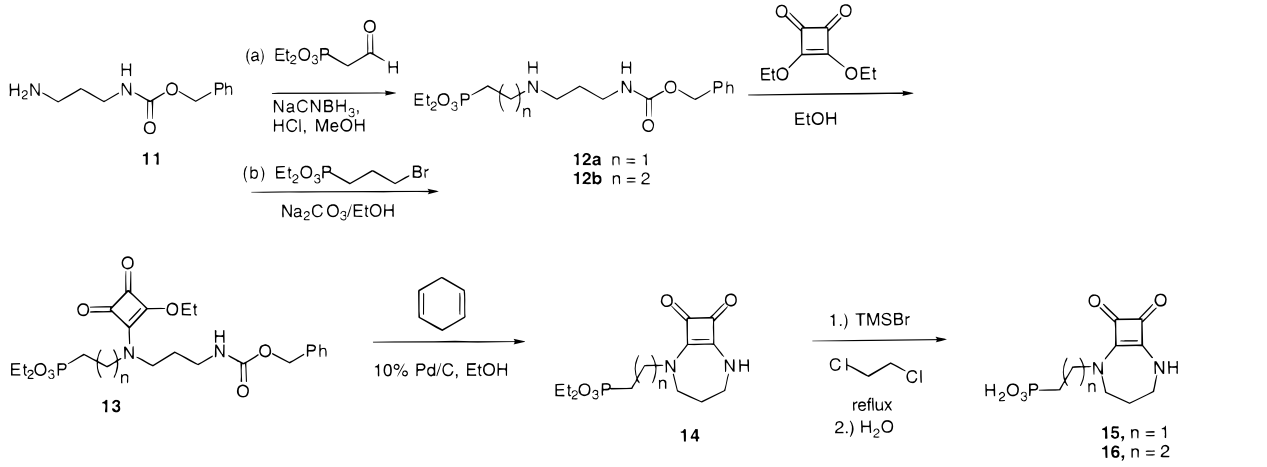
The carboxylic acids **28** and **29** (Scheme 4) were prepared using the CBZ-protected diamine **11** as the starting material. For example, alkylation with the corresponding *tert*-butyl bromo ester in a solution of diisopropylethylamine in dimethylformamide furnished **25** in good yield. The stepwise addition of **25** to 3,4-diethoxy-3-cyclobutene-1,2-dione was accomplished as before (Scheme 2) to yield **27**. Conversion to the free acid **28** was best achieved under basic conditions, and the sodium salt was isolated. The tetrazoles **34** and **35** (Scheme 5) were prepared from the corresponding nitriles **33** by reacting with sodium azide and ammonium chloride in DMF at 125 °C.[24] Nitriles **33** were prepared from the BOC-protected propylenediamine **30** as before, but no base was necessary for the cyclization step.

Improvements needed in the synthesis of **14** for scale-

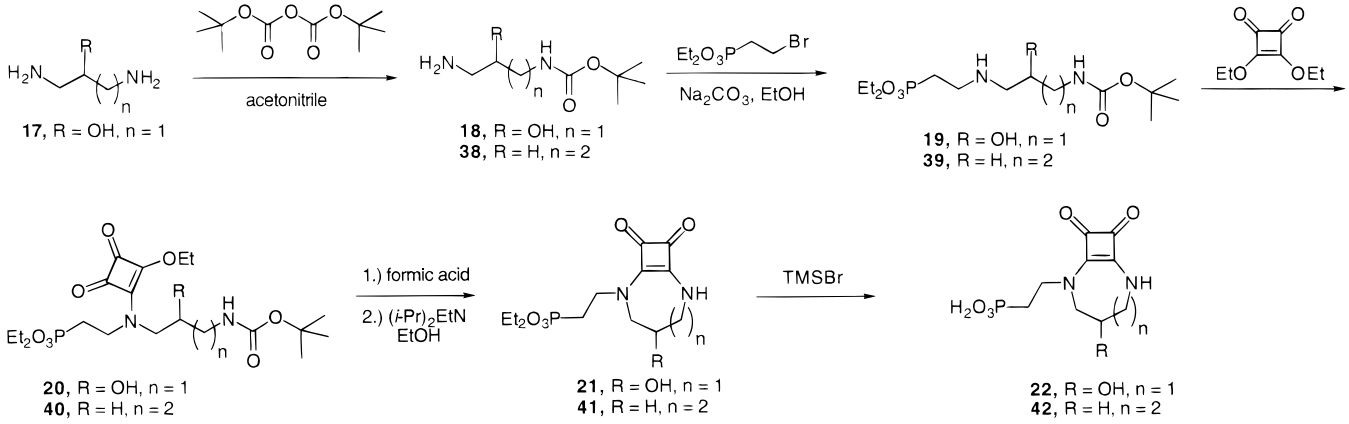
Scheme 1



Scheme 2



Scheme 3

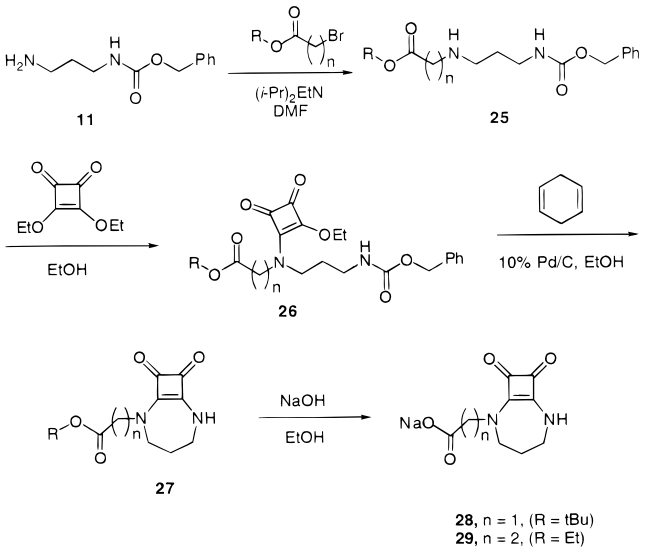


up and pilot plant implementation are illustrated in Scheme 6. The alkylation of **30** was accomplished with diethyl vinylphosphonate in methanol at room temperature over 48 h in good yield to afford the aminoethyl phosphonate intermediate **36**. Reaction of the intermediate **36** with 3,4-diethoxy-3-cyclobutene-1,2-dione afforded **37** in excellent yield, which was deprotected with trifluoroacetic acid and cyclized with triethylamine at room temperature to yield **14**. Other derivatives (**23** and **24**) were prepared by the procedures described in Scheme 3 and are detailed in the Experimental Section, following the method by which they were prepared from appropriate starting materials.

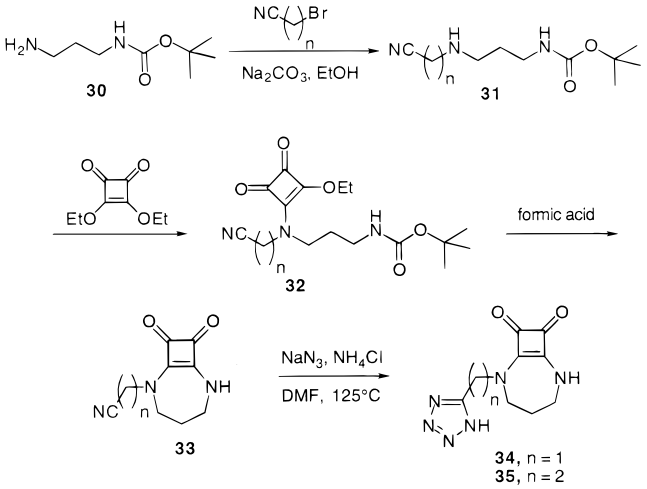
Biological Results and Discussion

The cyclic 3,4-diamino-3-cyclobutene-1,2-diones (Table 1) were evaluated in vitro for NMDA affinity in the [^{3}H]-CPP binding assay and for NMDA antagonist activity in the stimulated [^{3}H]TCP binding assay. The compounds were also tested for their efficacy in blocking NMDA-induced lethality in mice, as a functional model of selective NMDA receptor antagonism in vivo.[25] (±)-

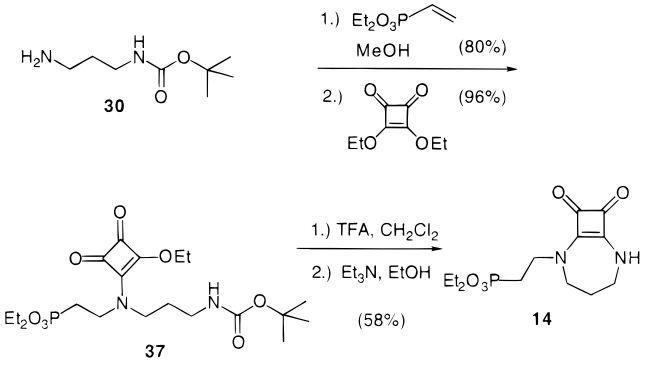
Scheme 4



Scheme 5



Scheme 6



CGS-19,755 (**5**) was used as the positive control, and all drugs were administered intraperitoneally (Table 1). The six-membered ring derivative **6** demonstrated very good affinity for the NMDA receptor (IC_{50} = 92 nM) and was 5 times more potent than the corresponding acyclic molecule **3**. This is a particularly striking result in that previously it was shown in this series that substitution with methyl groups on either nitrogen led to a decrease in NMDA affinity.[14] Presumably, the gain from blocking intramolecular H-bonding interactions (Chart 1) and constraining the molecule to a conformation capable of binding overwhelms the steric effect; also, the methylenes tied back in a ring would be expected to be less sterically demanding than a methyl group. Compound **6** was also 5-fold more potent than **3** in vivo (ED_{50} of 6.1 mg/kg) and only 4-fold less potent than **5**.

Because the side chain length requirement had already been addressed in the acyclic series,[14] variation of the ring size was the next obvious challenge. The seven-membered ring derivative **15**, which contains greater conformational stability than **6** by examining molecular models, was also a more readily accessible target. When the ^{1}H NMR spectrum of **15** was compared to that of **6**, the exocyclic methylene resonances adjacent to nitrogen were found to be shifted downfield by 0.28 ppm in **15**, suggesting that this cyclobutenedione was more deshielding and contained a greater dipole; the greater dipole was probably due to better donation of the nitrogen lone pair because of less conformational strain. It was hoped that the greater separation of charge would lead to tighter affinity for **15**, and indeed a 3-fold increase in affinity and in vivo efficacy was observed (Table 1). Compound **15** was equipotent to **5** in the [^{3}H]CPP binding assay and in the NMDA-induced lethality model, but **15** was slightly more potent in the stimulated [^{3}H]TCP binding model, which measures NMDA antagonism in vitro. The eight-membered ring derivative **42** displayed similar binding affinity but was clearly a weaker antagonist in the in vitro functional assay; this result is likely due to the eight-membered ring being more sterically demanding than the seven-membered ring.

Although the two-carbon side chain length requirement had been optimum for NMDA affinity in the acyclic series, it was deemed necessary to confirm this expectation in the cyclic series. The propenyl side chain (**24**) was chosen because it would be intermediate in length between the ethyl (**15**) and propyl (**16**) side chains. Compound **24** would also possess additional structural constraints, which proved productive in the propenyl derivative of **4** (CPP-ene).[26] Compounds **24** and **16** were found to be inactive (<50% displacement) at 10 and 1 μM, respectively, in the [^{3}H]CPP binding assay.

Substitution on the ring methylenes was pursued next to investigate potential van der Waal's or H-bonding interactions with the NMDA receptor. The dimethyl-substituted derivative **23** was designed to investigate the former but was found to be 10-fold less potent than **15** in vitro and in vivo; the additional lipophilic groups do nothing to enhance the molecule's bioavailability. Compound **22** provides a hydroxyl probe to investigate hydrophilicity and H-bond interactions in a particular region of space. Initial binding results suggested a slight improvement over **15** in the [^{3}H]CPP binding assay, but **22** was indistinguishable from **15** upon further evaluation. There was no apparent change in bioavailability upon increasing hydrophilicity, indicating that the polar phosphonate group dominates these more subtle changes in lipophilic character.

To improve bioavailability, the carboxylic acid and tetrazole analogues of **15** were prepared. What might be lost in binding affinity was expected to be gained in bioavailability for these less-polar substrates. The carboxylic acids **28** and **29** were found predictably to be less potent in the [^{3}H]CPP binding assay by 1 order

Table 1. Phosphonoalkyl 3,4-Diamino-3-cyclobutene-1,2-dione Derivatives

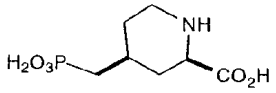
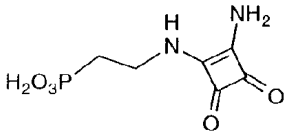
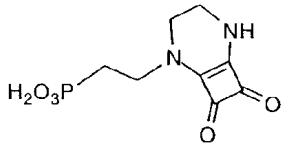
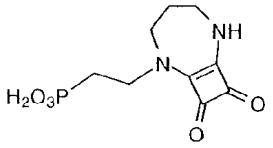
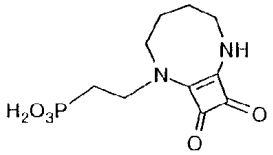
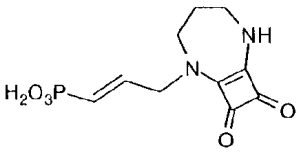
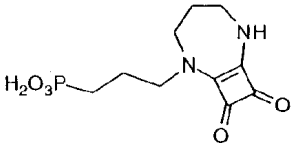
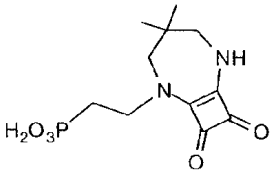
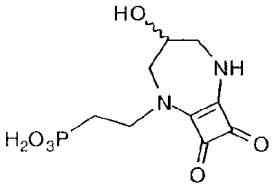
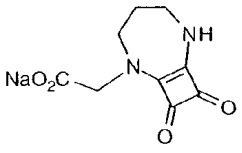
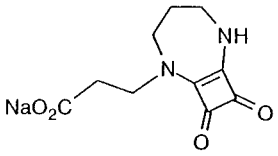
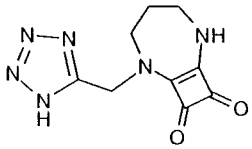
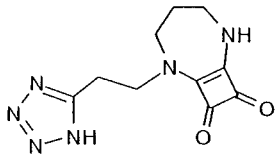
Entry	Structure	NMDA Affinity IC_{50} (nM)[a]	Stimulated TCP Binding IC_{50} (μM)[a]	NMDA-Induced Lethality ED_{50} (mg/kg, ip)[a]
5		28 (22-34)	22 (18-28)	1.5 (0.95-2.4)
3		470 (360-610)	Nt[b]	29 (16-51)
6		92 (74-115)	29 (24-35)	6.1 (4.3-8.7)
15		30 (20-43)	7.6 (6.5-8.8)	2.1 (1.4-3.1)
42		49 (36-68)	36 (33-38)	>3
24		> 10,000	>100	>5
16		>1,000	>100	>10
23		280 (220-350)	59% (100)[c]	22 (15-34)
22		19 (13-28)	10 (8-12)	1.8 (1.5-2.3))
28		550 (440-690)	64% (100)[c]	>20

Table 1 (Continued)

Entry	Structure	NMDA Affinity IC_{50} (nM)[a]	Stimulated TCP Binding IC_{50} (μM)[a]	NMDA-Induced Lethality ED_{50} (mg/kg, ip)[a]
29		340 (270-450)	53% (100)[c]	>20
34		38 (30-49)	18 (16-20)	>10
35		41% (10,000)[c]	>100	>5

[a] 95% confidence limits in parentheses. [b] Not tested in this assay. [c] Percent inhibition at concentration in parentheses.

Table 2. Effect of Compound **15** on Infarct Volume in the Rat Permanent Focal Ischemia Model[a]

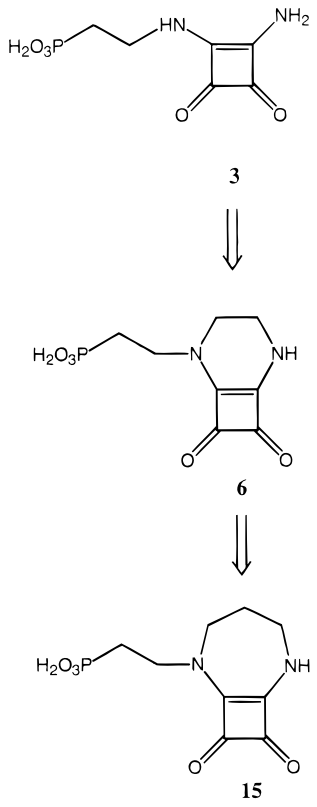
treatment	no. of animals	infarct vol (mm^3)	% decrease in infarct vol
vehicle	19	100.4 ± 10.9	
compd **15**	20	43.2 ± 6.1	57

[a] Values are shown as mean ± SE. Compound **15** was dissolved in 0.9% NaCl (vehicle) and was administered 5 min following the MCA occlusion as a single intravenous bolus injection at a dose of 10 mg/kg. Brain infarct size was quantified at 24 h postocclusion. $p < 0.05$ as compared to the vehicle group.

of magnitude over **15**. Disappointingly, they were inactive in vivo at 20 mg/kg, which is 10 times the ED_{50} of **15**, suggesting lesser bioavailability for these agents. Ornstein[27] has demonstrated that tetrazoles are useful phosphonic acid substitutes at the NMDA receptor and at least in some cases confer greater bioavailability. The two-carbon derivative **35** was expected to possess activity based on the literature precedent, but it was found to be weakly active at 10 μM. Suspecting that the tetrazole was extending the negative charge too far relative to the cyclobutenedione functionality, the one-carbon tetrazole **34** was synthesized. Compound **34** was found to have robust ability to displace [^{3}H]CPP (IC_{50} = 38 nM), rivaling **15**, but surprisingly was less bioavailable; it was unable to prevent NMDA-induced lethality at up to 10 mg/kg, which is 5 times the ED_{50} for **15**.

The in vivo neuroprotective efficacy of compound **15** was tested in the rat focal ischemia model involving occlusion of the middle cerebral artery (MCA). This model is considered by many investigators to be a relevant animal model of severe human stroke.[28] As shown in Table 2, compound **15**, administered as a single iv bolus 5 min following the MCA occlusion, markedly ameliorated the ischemic damage to the CNS. The volume of infarcted brain tissue in animals treated with compound **15** was reduced by 57% as compared to the vehicle-treated control group ($p > 0.05$). These data support a promising neuroprotective potential for compound **15**.

Chart 2



Conclusion

The use of 3,4-diamino-3-cyclobutene-1,2-dione as an achiral α-amino acid bioisostere has led to the synthesis of novel NMDA competitive antagonists (Chart 2). Optimization of **3** via the incorporation of a six-membered ring has led to a 5-fold increase in in vitro

and in vivo potency (compound **6**). Further optimization of **6** led to the synthesis of compounds with greater conformational stability, one of which ([2-(8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl]phosphonic acid, **15**) has enhanced in vitro and in vivo potency. Compound **15** has been identified as a potent in vitro and in vivo NMDA antagonist with marked neuroprotectant activity in a focal ischemia model. The six- and eight-membered derivatives had diminished activity, and the two-carbon side chain length was determined to be optimum. Substitution on the ring was found to be counterproductive in the case of a lipophilic group and of no consequence in the case of a hydroxyl group. Replacement of the phosphonic acid group by either a carboxylic acid or a tetrazole group was unproductive.

The usefulness of the 3,4-diamino-3-cyclobutene-1,2-dione group as an α-amino acid bioisostere in these potent cyclic achiral derivatives is noteworthy, as well as their straightforward synthesis. In this regard, further evaluation of compound **15** may confirm initial behavioral observations of an improved therapeutic ratio[29] for this structurally novel compound relative to existing potent NMDA antagonists, which almost universally contain the α-amino acid and phosphonic acid functionalities, the tetrazoles of Ornstein being an exception. Therefore, compound **15** shows a particularly promising profile as a neuroprotective agent for the treatment of neurological disorders such as stroke and head trauma in which overstimulation of NMDA receptors plays an important role. Compound **15** is undergoing clinical evaluation as a neuroprotective agent for stroke.

Experimental Section

Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 781 spectrophotometer. ^{1}H NMR spectra were obtained at either 200 or 400 MHz on a Varian XL-200 or Bruker AM-400 spectrometer, respectively. Mass spectra were measured on either a Finnigan 8230 or Hewlett-Packard 5995A mass spectrometer. Elemental analyses were obtained on a Control Equipment 240-XA elemental analyzer. Flash chromatography refers to the technique described by Still.[30] The diameter of the column used is noted, but the height of silica gel 60 (400−230 mesh) was 20 cm in all cases.

[2-[[2-(Diethoxyphosphinyl)ethyl]amino]ethyl]-carbamic Acid Benzyl ester (8). A solution of **7**[18] (3.06 g, 16 mmol), (2-oxoethyl)phosphonic acid diethyl ester[19] (2.88 g, 16 mmol), and sodium cyanoborohydride (1.00 g, 16 mmol) in dry methanol (90 mL) was prepared under nitrogen. Methanolic hydrogen chloride was added until the solution remained slightly acidic (pH 6.5). After 3 h, additional sodium cyanoborohydride (0.25 g, 4.0 mmol) was introduced, and the reaction was left overnight. After the mixture was acidified to pH 1.5 with concentrated hydrochloric acid, the methanol was removed in vacuo and the residue was diluted with water (25 mL). After washing with diethyl ether (3 × 25 mL), the aqueous layer was basified to pH 10 with a 1 N sodium hydroxide solution, saturated with solid sodium chloride, and then extracted with chloroform (3 × 50 mL). The dried (Na_2SO_4) organic layer was preadsorbed onto silica gel and purified by flash chromatography (3-cm diameter, gradient elution with 5−10% methanol in dichloromethane) to yield **8** as a pale yellow oil (2.90 g, 51%): IR (neat, cm^{-1}) 3300, 1715; MS (+FAB) 359 (MH^+, 100), 91 (70); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36 (m, 5H), 5.46 (br s, NH), 5.10 (s, 2H), 4.16−4.02 (m, 4H), 3.29 (q, J = 5.5 Hz, 2H), 2.89 (d of t, J = 17, 7 Hz, 2H), 2.75 (t, J = 5.5 Hz, 2H), 1.95 (d of t, J = 18, 7 Hz, 2H), 1.82 (br s, NH), 1.31 (t, J = 7 Hz, 6H). Anal. ($C_{16}H_{27}N_2O_5P\cdot{}^4/_5H_2O$).

[2-[(2-Aminoethyl)amino]ethyl]phosphonic Acid Diethyl Ester (9). To a flask containing 10% palladium on carbon (3.79 g) under nitrogen was added **8** (3.79 g, 10 mmol) in ethanol (50 mL) followed by 1,4-cyclohexadiene (10.4 mL, 110 mmol). After the suspension stirred overnight, it was filtered through Celite, preadsorbed onto silica gel, and purified by flash chromatography (7-cm diameter, elution with dried ($MgSO_4$) 5/10/85 ammonium hydroxide/methanol/dichloromethane) to afford **9** as a yellow oil (1.98 g, 88%): MS (+FAB) 225 (MH^+, 9), 194 (100), 166 (34), 138 (71), 120 (38), 57 (39), 44 (32); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.18−4.04 (m, 4H), 2.91 (d of t, J = 15, 7 Hz, 2H), 2.80 (t, J = 5.5 Hz, 2H), 2.68 (t, J = 5.5 Hz, 2H), 1.98 (d of t, J = 18, 7 Hz, 2H), 1.68 (br s, 3 NH), 1.33 (t, J = 7 Hz, 6H).

[2-(7,8-Dioxo-2,5-diazabicyclo[4.2.0]oct-1(6)-en-2-yl)-ethyl]phosphonic Acid Diethyl Ester (10). Solutions (10 mL each) of 3,4-diethoxy-3-cyclobutene-1,2-dione (1.27 mL, 8.6 mmol) and **9** (1.92 g, 8.6 mmol) in ethanol were injected separately via syringe pump into refluxing ethanol (22 mL) over 3 h. After refluxing overnight, the red-brown solution was preadsorbed onto silica gel and purified by flash chromatography (7-cm diameter, gradient elution with 2.5−10% methanol in ethyl acetate) and recrystallization (methanol in ethyl acetate) to yield **10** as a beige solid (0.78 g, 30%, mp 115−116 °C): IR (KBr, cm^{-1}) 3170, 1780, 1660, 1580; MS (+FAB) 303 (MH^+, 100), 109 (38); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.64 (br s, NH), 4.16−4.06 (m, 4H), 3.80−3.73 (m, 2H), 3.63−3.60 (m, 2H), 3.48 (t, J = 5 Hz, 2H), 2.31 (d of t, J = 18, 7.5 Hz, 2H), 1.33 (t, J = 7 Hz, 6H). Anal. ($C_{12}H_{19}N_2O_5P$).

[2-(7,8-Dioxo-2,5-diazabicyclo[4.2.0]oct-1(6)-en-2-yl)-ethyl]phosphonic Acid (6). A solution of **10** (0.78 g, 2.6 mmol) and bromotrimethylsilane (2.1 mL, 16 mmol) in dry 1,2-dichloroethane (30 mL) was refluxed under nitrogen for 20 min. The cooled reaction mixture was concentrated in vacuo, and the residue was dissolved in water (100 mL) and washed with diethyl ether (3 × 50 mL). After the aqueous layer was concentrated, the residue was recrystallized from water (25 mL) and methanol (300 mL) to yield a solid beige impurity which was removed by filtration. The filtrate was concentrated and recrystallized from water in 2-propanol to yield **6** as a yellow solid (0.37 g, 58%, mp 220−270 °C dec): IR (KBr, cm^{-1}) 1800; MS (−FAB) 245 (M − H); ^{1}H NMR (DMSO/drop of DCl, 400 MHz) δ 3.58−3.51 (m, 2H), 3.40−3.33 (m, 4H), 2.07−1.98 (m, 2H). Anal. ($C_8H_{11}N_2O_5P$).

[3-[[2-(Diethoxyphosphinyl)ethyl]amino]propyl]-carbamic Acid Benzyl Ester (12a). This was prepared following the procedure of **8** with the exception that compound **11** was used as starting material to yield **12a** as a waxy solid (3.86 g, 36%): IR (neat, cm^{-1}) 3300, 1720, 1250, 1030; MS (+FAB) 373 (MH^+, 100), 91 (90); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.36−7.29 (m, 5H), 5.58 (br s, NH), 5.09 (s, 2H), 4.15−4.04 (m, 4H), 3.29 (q, J = 6 Hz, 2H), 2.91 (d of t, J = 16, 7 Hz, 2H), 2.71 (t, J = 6 Hz, 2H), 1.98 (d of t, J = 18, 7 Hz, 2H), 1.70 (p, J = 6 Hz, 2H), 1.31 (t, J = 7 Hz, 6H).

[3-[[2-(Diethoxyphosphinyl)ethyl](2-ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]propyl]carbamic Acid Benzyl Ester (13). A solution of **12** (3.17 g, 8.5 mmol) in absolute ethanol (40 mL) was added over 45 min to 3,4-diethoxy-3-cyclobutene-1,2-dione (2.3 mL, 16 mmol) dissolved ethanol (55 mL). After standing overnight, the reaction mixture was preadsorbed onto silica gel and purified by flash chromatography (7-cm diameter, gradient elution with 2.5−10% methanol in dichloromethane) to yield **13** as a viscous oil (3.75 g, 89%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (m, 5H), 5.45 (br m, NH), 5.09 (s, 2H), 4.80−4.71 (m, 2H), 4.16−4.09 (m, 4H), 3.90−3.48 (m, 4H), 3.23−3.20 (m, 2H), 2.16−2.05 (m, 2H), 1.85−1.79 (m, 2H), 1.47, 1.41 (t, J = 7 Hz, 3H), 1.34 (t, J = 7 Hz, 6H).

[2-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic Acid Diethyl Ester (14). To a flask containing 10% palladium on carbon (2.11 g) under nitrogen was added **13** (2.11 g, 4.2 mmol) in absolute ethanol (180 mL) followed by 1,4-cyclohexadiene (4.3 mL, 45 mmol). After stirring for 5 h, the reaction mixture was filtered through

Celite and concentrated in vacuo to yield a crude solid, which was recrystallized from methanol and ethyl acetate (total volume = 20 mL), filtered, and washed with hexane to afford **14** hemihydrate as a cream-colored solid (0.82 g, 62%, mp 148−149 °C): IR (KBr, cm^{-1}) 3220, 1810, 1665, 1605, 1550; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50 (br s, NH), 4.18−4.10 (m, 4H), 4.09−4.00 (m, 2H), 3.51−3.46 (m, 4H), 2.19 (d of t, J = 18, 7.5 Hz, 2H), 2.10−2.04 (m, 2H), 1.34 (t, J = 7 Hz, 6H). Anal. ($C_{13}H_{21}N_2O_5P\cdot{}^1/_2H_2O$).

[2-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic Acid (15). A solution of **14** (1.41 g, 4.5 mmol) and bromotrimethylsilane (4.2 mL, 32 mmol) in dry 1,2-dichloroethane (50 mL) was refluxed for 20 min under nitrogen. The cooled reaction mixture was concentrated in vacuo, and the residue was dissolved in water (100 mL) and washed with diethyl ether (3 × 75 mL). The water was removed in vacuo, and the resulting solid was recrystallized from water and 2-propanol to afford **15** $^1/_5$hydrate as a yellow solid (0.91 g, 78%, mp 260−278 °C dec): IR (KBr, cm^{-1}) 3440, 3250, 1800, 1650, 1590, 1550; ^1H NMR (DMSO, 1 drop of DCl, 400 MHz) δ 3.85−3.79 (m, 2H), 3.35−3.32 (m, 2H), 3.25−3.23 (m, 2H), 1.97−1.87 (m, 4H). Anal. ($C_9H_{13}N_2O_5P\cdot{}^1/_5H_2O$).

[3-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-propyl]phosphonic Acid (16). This was prepared following the procedures of **15**. However, the phosphonopropyl group was introduced by alkylation with diethyl (3-bromopropyl)-phosphonate,[31] as in example **22**, to give **16** (23% overall from **11**, mp 244−248 °C): ^1H NMR (DMSO, 400 MHz) δ 8.50 (br s, NH), 3.69 (t, J = 6.7 Hz, 2H), 3.34−3.28 (m, 4H), 1.94−1.87 (m, 2H), 1.80−1.70 (m, 2H), 1.52−1.43 (m, 2H). Anal. ($C_{10}H_{15}N_2O_5P$).

(3-Amino-2-hydroxypropyl)carbamic Acid 1,1-Dimethylethyl Ester (18). A solution of 1,3-diamino-2-hydroxypropane (**17**; 27.0 g, 0.300 mol) in dry acetonitrile (270 mL) was maintained at ambient temperature with a water bath, was treated with di-*tert*-butyl dicarbonate (23.0 mL, 0.100 mol) in acetonitrile (90 mL) over 2 h with vigorous mechanical stirring, and then was left overnight. The reaction mixture was concentrated in vacuo, and the residue was dissolved in brine (250 mL) to which bromocresol green was added. The mixture was treated with a 1 N hydrochloric acid solution until it just turned yellow, washed with dichloromethane (3 × 250 mL), and then made basic (pH 12) by the addition of a 2.5 N sodium hydroxide solution. The product was extracted into chloroform (15 × 250 mL), dried with sodium sulfate, and concentrated to yield **18** as a white solid (7.76 g, 41%, mp 77−79 °C): IR (KBr, cm^{-1}) 3360, 1680; MS (+FAB) 191 (MH^+, 42), 135 (100), 58 (78); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.04 (br s, NH), 3.65−3.60 (m, 1H), 3.34−3.22 (m, 1H), 3.12−3.05 (m, 1H), 2.83 (d of d, J = 13, 4 Hz, 1H), 2.63 (d of d, J = 13, 7.5 Hz, 1H), 1.44 (s, 9H).

[3-[[2-(Diethoxyphosphinyl)ethyl]amino]-2-hydroxypropyl]carbamic Acid 1,1-Dimethylethyl Ester (19). A solution of **18** (7.73 g, 41 mmol), sodium carbonate (6.52 g, 62 mmol), and diethyl (2-bromoethyl)phosphonate (11.8 mL, 65 mmol) in absolute ethanol (150 mL) was prepared under nitrogen. This mixture was refluxed overnight and then concentrated in vacuo. The residue was dissolved in chloroform (150 mL), then preadsorbed onto silica gel, and purified by flash chromatography (7-cm diameter, gradient elution with 2.5−20% methanol in dichloromethane) to yield **19** as a colorless oil (11.16 g, 77%): IR (neat, cm^{-1}) 3320, 1710, 1250, 1170, 1040; MS (+FAB) 355 (MH^+, 68), 255 (90), 58 (100); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.23 (br s, NH), 4.16−4.05 (m, 4H), 3.77 (m, 1H), 3.32−2.90 (m, 4H), 2.73 (d of d, J = 12, 3.5 Hz, 1H), 2.58 (d of d, J = 12, 8.5 Hz, 1H), 2.00 (d of t, J = 18, 7 Hz, 2H), 1.43 (s, 9H), 1.32 (t, J = 7 Hz, 6H).

[3-[[2-(Diethoxyphosphinyl)ethyl](2-ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]-2-hydroxypropyl]carbamic Acid 1,1-Dimethylethyl Ester (20). To a solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (5.0 mL, 34 mmol) in absolute ethanol (180 mL) prepared under nitrogen was added a solution of **19** (12.11 g, 34 mmol) in ethanol (60 mL) over 1 h. The reaction mixture was stirred at room temperature overnight and then concentrated in vacuo. The residue was dissolved in chloroform (150 mL), preadsorbed onto silica gel, and purified by flash chromatography (7-cm diameter, gradient elution with 2.5−15% methanol in dichloromethane) to yield **20** as a clear viscous oil (11.34 g, 70%): IR (neat, cm^{-1}) 3350, 1800, 1700, 1600; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.37, 5.19 (br s, NH), 4.82−4.72 (m, 3H), 4.16−3.11 (m, 11H), 2.24−2.14 (m, 2H), 1.47−1.43 (m, 12H), 1.32 (t, J = 7 Hz, 6H).

[2-(4-Hydroxy-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl]phosphonic Acid Diethyl Ester (21). A solution of **20** (11.34 g, 24 mmol) in 96% formic acid (100 mL) was prepared under nitrogen and stirred at room temperature overnight. The reaction mixture was then concentrated in vacuo to yield a thick yellow oil, which was dissolved in absolute ethanol (120 mL) and added dropwise over 1.5 h to a solution of diisopropylethylamine (16.7 mL, 96 mmol) in absolute ethanol (360 mL). After refluxing overnight, the reaction mixture was concentrated in vacuo, and the residue was dissolved in chloroform (150 mL), preadsorbed onto silica gel, and purified by flash chromatography (9-cm diameter, gradient elution with 5−20% methanol in dichloromethane) to yield **21** as a white solid (5.94 g, 74%, mp 169−171 °C): IR (KBr, cm^{-1}) 3330, 3200, 1800, 1660, 1620, 1560, 1250, 1030; MS (+FAB) 333 (MH^+, 100), 185 (50), 179 (78); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.18 (br s, NH), 4.52−4.44 (m, 1H), 4.20 (m, 1H), 4.14−4.04 (m, 4H), 3.79−3.70 (m, 2H), 3.57−3.54 (m, 2H), 3.36 (d, J = 14 Hz, 1H), 2.33−2.13 (m, 2H), 1.32 (d of t, J = 13, 7 Hz, 6H). Anal. ($C_{13}H_{21}N_2O_6P$).

[2-(4-Hydroxy-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl]phosphonic Acid (22). To a solution of **21** (2.5 g, 7.5 mmol) in dry 1,2-dichloroethane (100 mL) under nitrogen was added bromotrimethylsilane (9.2 mL, 60 mmol). The reaction mixture was refluxed for 20 min and then concentrated in vacuo to yield a yellow-orange foam, which was dissolved in water (100 mL). The water was washed with ether (3 × 100 mL) and then concentrated in vacuo to yield a solid which was recrystallized from water in 2-propanol to yield **22** $^1/_6$hydrate as a pale yellow solid (1.79 g, 86%, mp 268−270 °C dec): IR (KBr, cm^{-1}) 3480, 3220, 1820, 1650, 1610, 1550; MS (−FAB) 275 (M − H, 22), 148 (100); ^1H NMR (DMSO, 1 drop of DCl, 400 MHz) δ 3.90 (m, 1H), 3.85−3.74 (m, 2H), 3.46 (d, J = 13 Hz, 1H), 3.34 (d of d, J = 13, 7 Hz, 1H), 3.28 (m, 2H), 1.98−1.89 (m, 2H). Anal. ($C_9H_{13}N_2O_6P\cdot{}^1/_6H_2O$).

[2-(4,4-Dimethyl-8,9-dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)ethyl]phosphonic Acid (23). Following the procedure of **22**, with the exception that 2,2-dimethyl-1,3-propanediamine was employed as the reactant, gave **23** as a monohydrate (4% overall from the diamine, mp 265−282 °C dec): ^1H NMR (DMSO, 400 MHz) δ 8.43 (br s, NH), 3.84−3.78 (m, 2H), 3.11 (s, 2H), 3.00 (d, J = 2.5 Hz, 2H), 1.94−1.85 (m, 2H), 0.90 (s, 6H). Anal. ($C_{11}H_{17}N_2O_5P\cdot H_2O$).

[(*E*)-3-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-1-propenyl]phosphonic Acid (24). Following the procedure of **22**, with the exception that **30** and diethyl (*E*)-(3-chloro-1-propenyl)phosphonate were the reactants, gave **24** as a $^1/_6$hydrate (18% from **30**, mp 255−275 °C dec): ^1H NMR (D_2O, 400 MHz) δ 6.56−6.44 (m, 1H), 6.02−5.93 (m, 1H), 4.44−4.41 (m, 2H), 3.50−3.47 (m, 4H), 2.14−2.08 (m, 2H). Anal. ($C_{10}H_{13}N_2O_5P\cdot{}^1/_6H_2O$).

[3-[[[[(1,1-Dimethylethyl)oxy]carbonyl]methyl]amino]-propyl]carbamic Acid Benzyl Ester (25). A solution of **11**[18] (7.08 g, 34 mmol) and diisopropylethylamine (4.5 mL, 26 mmol) in anhydrous dimethylformamide (100 mL) under nitrogen was cooled to 10 °C and treated with *tert*-butyl bromoacetate (2.80 mL, 17 mmol) over 5 min. After 1 h, the bath was removed, and the reaction mixture was stirred overnight, poured into water (500 mL), and made basic (pH 12) with a 2.5 N sodium hydroxide solution. The aqueous layer was extracted with dichloromethane (2 × 250 mL), which was dried over sodium sulfate and concentrated in vacuo with heat to remove dimethylformamide. The residue was purified by flash chromatography (9-cm diameter, gradient elution with 2.5−3.5% methanol in dichloromethane) to yield **25** as a pale yellow oil (4.50 g, 82%): ^1H NMR ($CDCl_3$, 200 MHz) δ 7.35 (m, 5H), 5.43

(br s, NH), 5.09 (s, 2H), 3.35−3.24 (m, 4H), 2.68 (t, J = 6.5 Hz, 2H), 1.68 (p, J = 6.5 Hz, 2H), 1.46 (s, 9H).

[3-[[[(1,1-Dimethylethyl)oxy]carbonyl]methyl](2-ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]propyl]carbamic Acid Benzyl Ester (26). An ethanolic solution (60 mL) of **25** (4.49 g, 13.9 mmol) under nitrogen was added to 3,4-diethoxy-3-cyclobutene-1,2-dione (2.0 mL, 13 mmol) in the same solvent (60 mL) over 1 h. After 3.5 more hours, the reaction mixture was preadsorbed onto silica gel and purified by flash chromatography (7.5-cm diameter, gradient elution with 30−60% ethyl acetate in petroleum ether) to afford **26** as a viscous colorless oil (5.26 g, 91%): MS (+CI) 447 (MH^+, 100), 391 (38); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (m, 5H), 5.55, 4.97 (br m, NH), 5.10 (s, 2H), 4.75, 4.73 (q, J = 7 Hz, 2H), 4.29, 3.99 (s, 2H), 3.74, 3.49 (t, J = 6.5 Hz, 2H), 3.29−3.22 (m, 2H), 1.84−1.75 (m, 2H), 1.48, 1.47 (s, 9H), 1.45−1.40 (m, 3H).

(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetic Acid 1,1-Dimethylethyl Ester (27). To a cooled (20 °C) flask containing 10% palladium on carbon (5.25 g) under nitrogen was added **26** (5.25 g, 11.8 mmol) in ethanol (500 mL) followed by 1,4-cyclohexadiene (11 mL, 0.12 mol) over 5 min. After 5 h, the suspension was filtered through Celite with generous ethanol washing (1 L). The ethanol was evaporated, and the residue was dissolved in dichloromethane and purified by flash chromatography (7.5-cm diameter, gradient elution with 2.5−3% methanol in dichloromethane) to yield **27** as a white solid (2.59 g, 82%, mp 167−168 °C): IR (KBr, cm^{-1}) 3200, 1800, 1720, 1660, 1610; MS (EI) 266 (M^+, 42), 210 (33), 166 (37), 165 (100), 154 (58), 138 (37), 70 (58); ^{1}H NMR (DMSO, 400 MHz) δ 8.62 (br s, NH), 4.38 (s, 2H), 3.36−3.27 (m, 4H), 1.91 (m, 2H), 1.40 (s, 9H). Anal. ($C_{13}H_{18}N_2O_4$).

(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetic Acid Sodium Salt (28). An ethanolic (86 mL) solution of **27** (2.29 g, 8.60 mmol) was treated at room temperature with a 2.5 N sodium hydroxide solution (3.5 mL, 8.7 mmol) and left stirring overnight. The suspension was filtered and washed with ethyl acetate to give a solid which was recrystallized from methanol/water/2-propanol (final volume 50 mL) to afford **28** sodium salt hydrate (1.43 g, 66%, mp 280−300 °C dec): MS (−FAB) 231 (M − H, 37), 209 (M − Na, 100); ^{1}H NMR (DMSO, 1 drop of DCl, 400 MHz) δ 4.40 (s, 2H), 3.36−3.25 (m, 4H), 1.89 (m, 2H). Anal. ($C_9H_9N_2NaO_4 \cdot H_2O$).

3-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-propanoic Acid (29). Following the procedure of **28**, with the exception that ethyl 3-bromopropionate was employed as a reactant, afforded **29** as the sodium salt sesquihydrate (33% overall from **11**, mp 310 °C dec): ^{1}H NMR (D_2O, 400 MHz) δ 3.96 (t, J = 7 Hz, 2H), 3.57−3.47 (m, 4H), 2.54 (t, J = 7 Hz, 2H), 2.12−2.07 (m, 2H). Anal. ($C_{10}H_{11}N_2NaO_4 \cdot 1.5H_2O$).

[3-[(Cyanomethyl)amino]propyl]carbamic Acid 1,1-Dimethylethyl Ester (31). A solution of (3-aminopropyl)-carbamic acid 1,1-dimethylethyl ester (**30**; 6.00 g, 34 mmol) in absolute ethanol (90 mL) was treated with sodium carbonate (3.96 g, 37 mmol) followed by a solution of bromoacetonitrile (2.6 mL, 37 mmol) in ethanol (30 mL) over 45 min. After stirring at room temperature overnight, the contents of the flask were filtered, preadsorbed onto silica gel, and purified by flash chromatography (7-cm diameter, elution with 2.5% methanol in dichloromethane) to afford **31** as a yellow oil (3.99 g, 55%): IR (neat, cm^{-1}) 3330, 2240, 1700; MS (+CI) 214 (MH^+, 20), 187 (76), 158 (100), 131 (44); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.80 (br s, NH), 3.61 (s, 2H), 3.22 (q, J = 6.5 Hz, 2H), 2.79 (t, J = 6.5 Hz, 2H), 1.69 (p, J = 6.5 Hz, 2H), 1.45 (s, 9H).

[3-[(Cyanomethyl)(2-ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]propyl]carbamic Acid 1,1-Dimethylethyl Ester (32). A solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (2.6 mL, 18 mmol) in absolute ethanol (90 mL) was treated with **31** (3.90 g, 18 mmol) in ethanol (30 mL) over 90 min. After 40 h, the reaction mixture was evaporated, dissolved in dichloromethane, preadsorbed onto silica gel, and purified by flash chromatography (7-cm diameter, gradient elution with 0−5% methanol in dichloromethane) to yield **32** as a yellow oil (5.48 g, 90%): IR (neat, cm^{-1}) 3360, 1800, 1600; MS (+FAB) 282 (76), 238 (98), 158 (100); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.81 (q, J = 7 Hz, 2H), 4.98, 4.75 (br s, NH), 4.80, 4.45 (br s, 2H), 3.84, 3.60 (br m, 2H), 3.26−3.15 (m, 2H), 1.91 (p, J = 6.5 Hz, 2H), 1.50 (t, J = 7 Hz, 3H), 1.45 (s, 9H).

(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)acetonitrile (33). A solution of **32** (5.48 g, 16 mmol) in 96% formic acid (40 mL) under nitrogen was prepared. After 24 h, the solvent was removed and the residue was dissolved in 2-propanol and concentrated several times. Ethanol (40 mL) was added, and the suspension was stirred overnight and filtered to afford **33** $^1/_8$hydrate as a white solid (1.50 g, 48%, mp 215−218 °C): IR (KBr, cm^{-1}) 3220, 1810, 1670, 1620, 1550; MS (DEI) 191 (M^+, 57), 135 (41), 70 (41), 43 (70), 41 (100); ^{1}H NMR (DMSO, 400 MHz) δ 8.83 (br s, NH), 4.84 (s, 2H), 3.40−3.30 (m, 4H), 1.95 (m, 2H). Anal. ($C_9H_9N_3O_2 \cdot {}^1/_8H_2O$).

2-[(1*H*-Tetrazol-5-yl)methyl]-2,6-diazabicyclo[5.2.0]non-1(7)-ene-8,9-dione (34). To a solution of **33** $^1/_8$hydrate (1.54 g, 8.0 mmol) in dry dimethylformamide (35 mL) under nitrogen were added sodium azide (0.79 g, 12 mmol) and ammonium chloride (0.65 g, 12 mmol). The reaction mixture was slowly heated to 125 °C, maintained at this temperature for 2 h, cooled to room temperature, and then filtered. The concentrated filtrate was dissolved in water and evaporated. The residue was recrystallized from methanol to yield **34** $^1/_3$hydrate as off-white crystals (1.18 g, 61%, mp 264−267 °C): IR (KBr, cm^{-1}) 3200, 1810, 1660, 1540; ^{1}H NMR (DMSO, 400 MHz) δ 8.70 (s, NH), 5.24 (s, 2H), 3.34−3.28 (m, 4H), 1.92 (m, 2H). Anal. ($C_9H_{10}N_6O_2 \cdot {}^1/_3H_2O$).

2-[2-(1*H*-Tetrazol-5-yl)ethyl]-2,6-diazabicyclo[5.2.0]non-1(7)-ene-8,9-dione (35). Following the procedure of **34**, with the exception that 3-bromopropionitrile was employed as a reactant, delivered **35** (20% from **30**, mp 255−262 °C dec): ^{1}H NMR (DMSO, 400 MHz) δ 8.52 (br s, NH), 4.01 (t, J = 6.8 Hz, 2H), 3.34−3.23 (m, 4H), 3.23 (t, J = 6.8 Hz, 2H), 1.89−1.84 (m, 2H). Anal. ($C_{10}H_{12}N_6O_2$).

[2-[*N*-[3-[(*tert*-Butyloxycarbonyl)amino]propyl]-*N*-(4-ethoxy-2,3-dioxocyclobut-3-en-3-yl)amino]ethyl]phosphonic Acid Diethyl Ester (37). To a solution of **30** (77 g, 0.44 mol) in methanol (500 mL) was added diethyl vinylphosphonate (97%, 75 g, 0.44 mol) under nitrogen, and the mixture was kept in a water bath at 20 °C for 48 h. The reaction mixture was concentrated in vacuo, and the residue (160 g) was added to a pad of Florisil (3 in. × 6 in.) and eluted with dichloromethane/hexane (1/1), dichloromethane, and finally 10% methanol/dichloromethane to furnish [2-[*N*-[3-[(*tert*-butyloxycarbonyl)amino]propyl]amino]ethyl]phosphonic acid diethyl ester (**36**) as a colorless oil (121 g, 80%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.12 (br s, NH), 4.09 (m, 4H), 3.19 (br q, J = 6 Hz, 2H), 2.90 (m, 2H), 2.67 (t, J = 6.5 Hz, 2H), 2.51 (br s, NH), 1.98 (dt, J = 18, 7 Hz, 2H), 1.66 (p, J = 6.5 Hz, 2H), 1.42 (s, 9H), 1.31 (t, 6H).

To a solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (45 g, 0.265 mol) in absolute ethanol (1.2 L) under nitrogen was added dropwise a solution of **36** (80 g, 0.24 mol) in absolute ethanol (600 mL), and the reaction mixture was stirred at ambient temperature 15 h. The reaction mixture was concentrated in vacuo, and the resulting residue was applied to a pad of silica gel (6 in. × 4 in.) and eluted with a mixture of dichloromethane/hexane (1/1) to remove excessive 3,4-diethoxy-3-cyclobutene-1,2-dione and 10% methanol/dichloromethane to yield **37** after evaporation as a viscous oil (107 g, 96%): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.30 (br s, NH), 4.77 (q, J = 7 Hz, 2H), 4.10 (m, 4H), 3.90 (m, 1H), 3.72 (t, J = 7 Hz, 1H), 3.66 (m, 1H), 3.49 (t, J = 7 Hz, 1H), 3.13 (m, 2H), 2.12 (m, 2H), 1.80 (m, 2H), 1.46 (t, J = 7 Hz, 3H), 1.43 (s, 9H), 1.33 (t, J = 7 Hz, 6H).

[2-(8,9-Dioxo-2,6-diazabicyclo[5.2.0]non-1(7)-en-2-yl)-ethyl]phosphonic Acid Diethyl Ester (14). A solution of **37** (100 g, 0.22 mol) in dichloromethane (600 mL) was cooled in ice and treated with trifluoroacetic acid (300 mL). The reaction mixture was left to warm to ambient temperature overnight. The solution was concentrated in vacuo at 40 °C and coevaporated with toluene (2 × 500 mL) to yield a viscous oil (159.5 g), which was dissolved in absolute ethanol (1.5 L), added dropwise over 8 h to a solution of triethylamine (350

mL) in ethanol (1.5 L), and stirred for 8 h at room temperature The reaction mixture was concentrated in vacuo to an oil which was taken up in ethyl acetate (1 L). After the compound crystallized, the mixture was cooled in ice, filtered, and washed with ethyl acetate and finally hexane to give **14** (40 g, 58%). LC analysis: column, Microsorb-MV C18, 150 × 4.6 mm; eluent, 30/70 MeOH/0.01 M $NH_4H_2PO_4$, pH 4.7; flow rate, 1 mL/min; UV detector, 210 nm.

(4-Aminobutyl)carbamic Acid 1,1-Dimethylethyl Ester (38). A solution of 1,4-diaminobutane (30 mL, 0.30 mol) in dry tetrahydrofuran (90 mL) was maintained at 0 °C, treated with di-*tert*-butyl dicarbonate (23 mL, 0.10 mol) in tetrahydrofuran (90 mL) over 1.5 h with vigorous mechanical stirring, and then left overnight. The reaction mixture was concentrated in vacuo, and the residue was dissolved in water (100 mL) to which bromocresol green was added. The mixture was treated with a 1 N hydrochloric acid solution until it just turned yellow, washed with dichloromethane (2 × 250 mL), and then made basic (pH 12) by the addition of a 2.5 N sodium hydroxide solution. The product was extracted into chloroform (6 × 250 mL), dried with sodium sulfate, and concentrated to yield **38** as a yellow oil (13.4 g, 71%): IR (neat, cm^{-1}) 3350, 1700; MS (EI) 188 (M^+, 4), 132 (56), 115 (33), 70 (100); ^1H NMR ($CDCl_3$, 400 MHz) δ 4.76 (br s, NH), 3.11 (q, J = 6 Hz, 2H), 2.70 (t, J = 6 Hz, 2H), 1.58−1.45 (m, 4H), 1.43 (s, 9H).

[4-[[2-(Diethoxyphosphinyl)ethyl]amino]butyl]-carbamic Acid 1,1-Dimethylethyl Ester (39). A solution of **38** (13.3 g, 71 mmol), sodium carbonate (11.25 g, 106 mmol), and diethyl (2-bromoethyl)phosphonate (20 mL, 104 mmol) in absolute ethanol (150 mL) was prepared under nitrogen. This mixture was refluxed overnight and then concentrated in vacuo. The residue was dissolved in chloroform (150 mL), then preadsorbed onto silica gel, and purified by flash chromatography (7-cm diameter, gradient elution with 5−30% methanol in dichloromethane) to yield **39** as a colorless oil (13.7 g, 55%): MS (+FAB) 353 (MH^+, 100), 150 (68), 56 (83); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.01 (br s, NH), 4.17−4.03 (m, 4H), 3.15−3.08 (m, 2H), 2.92 (d of t, J = 15, 7.5 Hz, 2H), 2.84 (br s, NH), 2.66 (br t, J = 7 Hz, 2H), 2.02 (d of t, J = 18, 7.5 Hz, 2H), 1.56−1.52 (m, 4H), 1.43 (s, 9H), 1.33 (t, J = 7 Hz, 6H).

[2-(9,10-Dioxo-2,7-diazabicyclo[6.2.0]dec-1(8)-en-2-yl)-ethyl]phosphonic Acid Diethyl Ester (41). To a solution of 3,4-diethoxy-3-cyclobutene-1,2-dione (5.8 mL, 39 mmol) in absolute ethanol (200 mL) prepared under nitrogen was added a solution of **39** (13.7 g, 39 mmol) in ethanol (75 mL) over 1.75 h. The reaction mixture was stirred at room temperature overnight and then concentrated in vacuo. The residue was dissolved in chloroform (150 mL), preadsorbed onto silica gel, and purified by flash chromatography (9-cm diameter, elution with 2.5% methanol in dichloromethane) to yield [4-[[2-(diethoxyphosphinyl)ethyl](2-ethoxy-3,4-dioxo-1-cyclobuten-1-yl)amino]butyl]carbamic acid 1,1-dimethylethyl ester (**40**) as a yellow viscous oil (16.8 g, 90%).

A solution of **40** (16.8 g, 35 mmol) in 96% formic acid (100 mL) was prepared under nitrogen and stirred at room temperature overnight. The reaction mixture was then concentrated in vacuo to yield a thick yellow oil, which was dissolved in absolute ethanol (125 mL) and added dropwise over 1.5 h to a solution of diisopropylethylamine (17 mL, 98 mmol) in absolute ethanol (375 mL). After refluxing overnight the reaction mixture was concentrated in vacuo, and the residue was dissolved in chloroform (150 mL), preadsorbed onto silica gel, and purified by flash chromatography (9-cm diameter, gradient elution with 5−10% methanol in dichloromethane) to yield **41** as a pale yellow solid (9.65 g, 83%, mp 103−105 °C): IR (KBr, cm^{-1}) 3440, 3230, 1795, 1660, 1580, 1540; MS (+FAB) 331 (MH^+, 100), 109 (31); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.14 (br s, NH), 4.16−4.05 (m, 6H), 3.39−3.36 (m, 4H), 2.17 (d of t, J = 19, 7.5 Hz, 2H), 1.74−1.68 (m, 4H), 1.33 (t, J = 7 Hz, 6H). Anal. ($C_{14}H_{23}N_2O_5P$).

[2-(9,10-Dioxo-2,7-diazabicyclo[6.2.0]dec-1(8)-en-2-yl)-ethyl]phosphonic Acid (42). To a solution of **41** (7.60 g, 23 mmol) in dry 1,2-dichloroethane (300 mL) under nitrogen was added bromotrimethylsilane (25 g, 160 mmol). The reaction mixture was refluxed for 25 min and then concentrated in vacuo to yield a dark rust oil, which was dissolved in water (250 mL). The water was washed with ether (3 × 250 mL) and then concentrated in vacuo to yield a yellow-orange solid which was recrystallized from water (100 mL) to yield **42** as a tan solid (3.80 g, 60%, mp 266−271 °C dec): IR (KBr, cm^{-1}) 3350, 3260, 1800, 1650, 1580, 1560; MS (−FAB) 273 (M − H); ^1H NMR (DMSO, 400 MHz) δ 8.32 (br s, NH), 3.94−3.87 (m, 2H), 3.31−3.28 (m, 2H), 3.22−3.18 (m, 2H), 1.96−1.87 (m, 2H), 1.61−1.49 (m, 4H). Anal. ($C_{10}H_{15}N_2O_5P\cdot H_2O$).

Binding Assays. Crude synaptic membrane preparations (CSMs) were prepared from rat brains as described by Murphy[32] and subsequently treated with 0.04% Triton X-100 (Eastman Kodak, Rochester, NY) as described by Enna and Snyder.[33] The CSM pellets were then frozen at −70 °C for storage. Prior to use in the NMDA receptor binding assay and the stimulated [^3H]TCP binding assay, the CSMs were thawed, washed once in Tris-HCl buffer, and resuspended in buffer to a final concentration of 0.3−0.5 mg of protein/mL as determined by the method of Lowry.[34] Displacement of [^3H]CPP binding, as described by Murphy,[35] to CSMs was utilized to determine NMDA receptor affinity.

The stimulated [^3H]TCP binding assay was a modification of the [^3H]MK-801 binding assay of Ransom[36] using the CSMs described above. In triplicate, 100-μL samples of the CSMs were incubated at 25 °C for 60 min in the presence of 2.5 nM [^3H]TCP (specific activity 45−50 Ci/mmol; DuPont NEN, Boston, MA), 3 μM L-glutamic acid, 1 μM glycine, one of various test drugs or concentrations thereof, and an appropriate volume of buffer for a final incubation volume of 2 mL. Tris buffer and a 100 μM solution of MK-801 were substituted for the test solution in separate triplicates to define total and nonspecific binding, respectively. The tissue homogenates were then filtered under vacuum, using 0.1% poly(ethylenimine)-pretreated filters, and rinsed with three 2-mL rinses of ice-cold buffer. The filters were placed into individual 20-mL glass scintillation vials and prepared for counting using conventional liquid spectroscopy. The concentration of test compound which displaced 50% of [^3H]TCP binding and its 95% confidence limits were determined from concentration−response (5−10 concentrations) curves derived using a nonlinear logistic regression of counts versus the log of the test drug concentration.[37]

NMDA-Induced Lethality.[25] The compounds were evaluated for NMDA antagonist properties in male Swiss albino mice (CD-1 strain, 18−22 g; Charles River, Wilmington, DE). The mice were acclimated for 30 min prior to treatment (10 mL/kg ip) with the representative test compounds or vehicle (saline or saline with Tris buffer) followed 30 min later with NMDA (195 mg/kg, ip, the LD_{90} dose). The mice were then observed for 30 min, noting the latency of onset of generalized myoclonus and death. From the latter, percentage survival was determined. Animals were tested in groups of 10 mice/dose level, and dose−response data were analyzed using the probit analysis program PS NONLIN (natural response rate version) to determine the dose (ED_{50}) which provides 50% protection and the 95% confidence interval.

Focal Brain Ischemia in the Rat. Male Fisher-344 rats (290−310 g) were subjected to permanent occlusion of the distal MCA and ipsilateral common carotid arteries according to the method of Brint et al.[38] Briefly, animals were anesthetized with 2−4% isofluorane followed by the occlusion of the right common carotid artery. The right MCA was exposed transcranially without removing the zygomatic arch. The artery was then electrocoagulated using bipolar electrocautery forceps. Body temperature was maintained throughout the surgical procedure at 37 ± 1 °C. Test compound or vehicle was administered as an intravenous bolus 5 min following the occlusion of MCA in a single bolus iv injection of 1 mL/kg via the tail vein; 24 h later animals were sacrificed. Frozen brain sections (20 μm) were cut at 400-μm intervals and stained with cresyl violet to demarcate the infarcted tissue. The infarct area of each section was measured by image analysis and the total infarct volume (mm^3) calculated from the sum of all

sectional areas multiplied by the interval thickness. The results are presented as means ± SE. A one-way ANOVA followed by least significant difference analysis was used to determine statistical significance of differences in infarct volume between groups.

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