New and Versatile Approaches to the Synthesis of CPP-Related Competitive NMDA Antagonists. Preliminary Structure–Activity Relationships and Pharmacological Evaluation[1]

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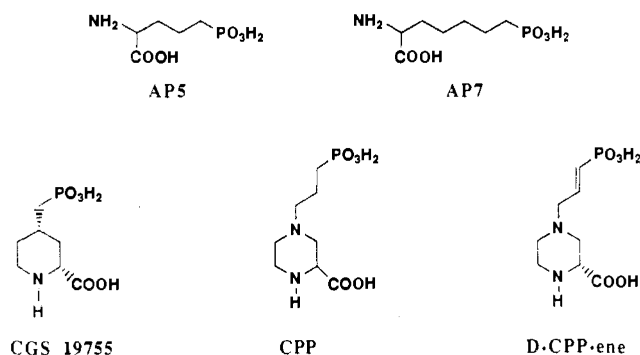
Fourteen new CPP analogues have been prepared with methyl 1-(phenylmethyl) (±)-1,2-piperazinedicarboxylate **3** as a versatile synthetic intermediate. Derivatives were evaluated as NMDA ligands by their ability to displace [^{3}H]CPP from rat cortical membranes. The binding affinity of various chain lengths at the N^4-position of the CPP analogues, **5a**, **5b**, and **9a** mimics the binding affinity observed for the acyclic derivatives AP6, AP8, and AP5. Analogue **9a**, with a single methylene group in its phosphonate side chain, exhibited diminished affinity for the NMDA receptor when compared to the structurally similar piperidine compound CGS 19755. Replacement of the phosphonic acid moiety with monoionizable acidic groups such as a carboxylate or a phosphinate resulted in a reduction of binding affinity. An aryl spacer between the N^4-nitrogen and the distal acidic group was detrimental to binding as was alkylation at the N^1-position. Steric bulk, however, was better tolerated when a phenyl group was positioned α to the phosphonate, as seen with analogues **21** and **22**.

Introduction

Excitatory amino acids aspartate and glutamate are major neurotransmitters within the mammalian central nervous system (CNS).[2-5] There are at least three subtypes of glutamate receptors which are most often characterized by the prototypical agonists *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainic acid (KA).[6] Of these subtypes, the NMDA receptor has received the most attention because of its possible involvement in a variety of neuropathologies. NMDA antagonists have been shown to reduce epileptiform activity in brain slices[7] and in intact animals[8] and have prevented neuronal degeneration produced by hypoxia[9] and hypoglycemia.[10] Antagonists of the NMDA receptor, therefore, could have therapeutic utility in several CNS disorders, particularly for the treatment of epilepsy, and for the amelioration of neuronal damage from cerebral ischemia.[11]

2-Amino-5-phosphonopentanoic acid (AP5)[12] and 2-amino-7-phosphonoheptanoic acid (AP7)[13] were among the first excitatory amino acid antagonists to display high potency and specificity for the NMDA receptor subtype. More recently, 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP)[14] and *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS 19755)[15,16] have been reported to have 10-fold greater affinity for the NMDA receptor than AP7. Furthermore, for both CPP and an unsaturated side chain analogue (CPP-ene), potent NMDA antagonism is shown only by the *R* enantiomer.[17]



With the objective of developing the structure–activity relationship (SAR) for CPP-type compounds at the NMDA receptor and of increasing the lipophilicity of the parent CPP molecule, we have prepared a series of CPP-related compounds. The known method by which CPP had been prepared was, in our hands, poorly reproducible and inflexible. In this paper we describe the synthetic utility of methyl 1-(phenylmethyl) (±)-1,2-piperazinedicarboxylate **3**,[18] which has enabled us to selectively functionalize either the N^1- or N^4-nitrogen of the piperazine ring of CPP. In addition, the preliminary structure–activity relationships of CPP analogues have been explored.

(1) Presented in part at the 198th American Chemical Society Meeting, Miami Beach, FL, 1989, Abstr. 198 (0) MEDI 10.

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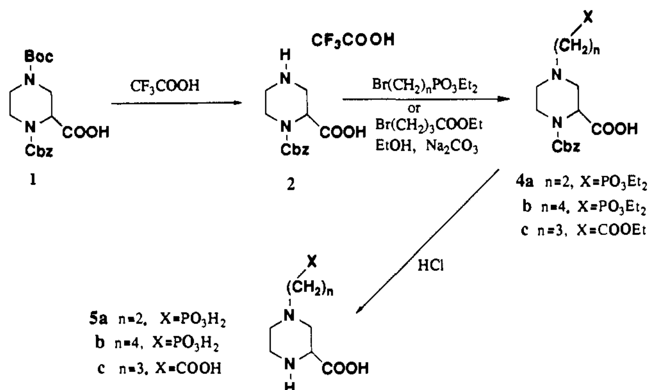
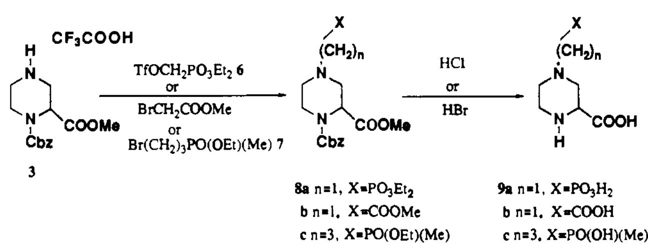
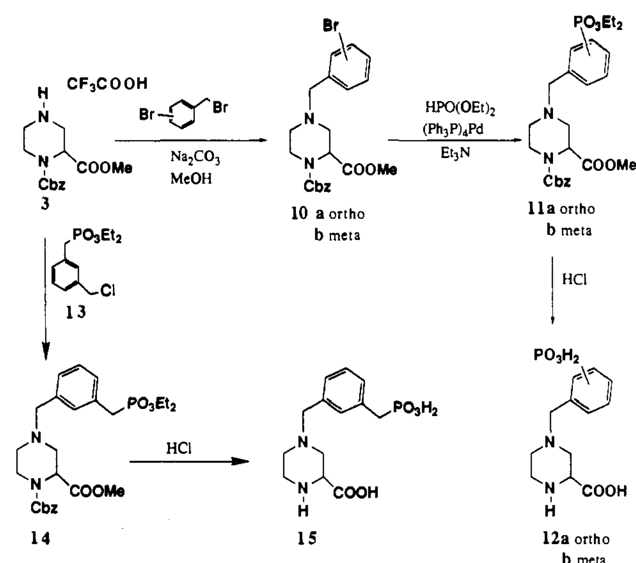
Scheme I



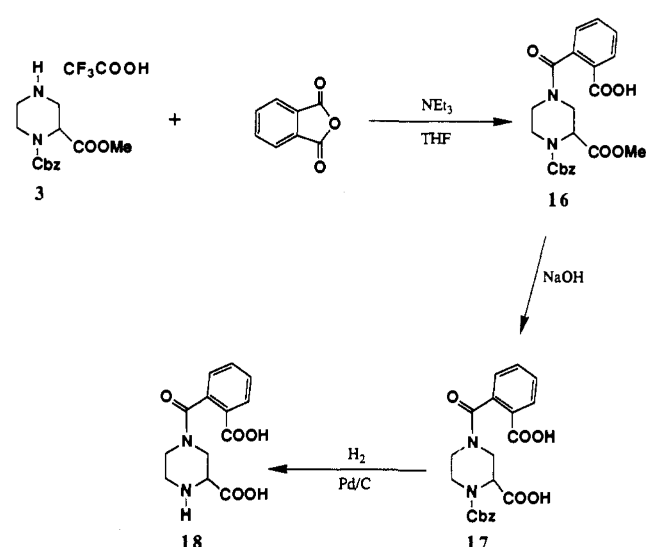
Scheme II



Scheme III



Scheme IV



The length and nature of the methylene spacer between the N[4]-nitrogen and the phosphonic acid moiety of CPP has been varied and the distal acidic group altered. Additionally the effect of adding steric bulk to various parts of the CPP molecule has been investigated.

Chemistry

The N^4-Boc group of intermediate **1** was selectively removed with trifluoroacetic acid to give amino acid **2** in 90% yield as shown in Scheme I. Alternatively, esterification of **1** with diazomethane followed by the removal of the N^4-Boc group yielded N[1]-protected ester **3** as described previously.[18] Both compounds **2** and **3** were readily alkylated at the N[4]-position. However, purification of the alkylated product was made easier and yields were substantially improved when **3** was used as the starting intermediate.

The synthesis of analogues **5a–c** is shown in Scheme I. The Cbz-protected piperazinecarboxylate **2** was alkylated with the bromoalkyl esters in ethanol with sodium carbonate to yield th N[4]-substituted products **4a–c** in 58–63% yields. The N^1-Cbz and the ester-protecting groups of **4a–c** were removed with refluxing hydrochloric acid to produce the desired phosphonoalkyl amino acids **5a–c**.[19] The (bromoalkyl)phosphonates used in these alkylations were synthesized by the Arbuzov reaction.[20] Attempted distillation of the resulting phosphonate esters led to substantial product decomposition. Alternative purification by silica gel chromatography produced acceptable yields of these alkylating agents on a multigram scale.

The versatility of piperazine methyl ester **3** as a precursor for CPP analogues is shown in Scheme II. Use of **3** as an intermediate for the synthesis of N^4-(phosphonomethyl) CPP derivative **9a** was imperative since N[4]-alkylation of amino acid **2** with dimethyl (chloromethyl)phosphonate was unsuccessful. [(Diethoxyphosphinyl)methyl]trifluoromethyl sulfonate (**6**), although a much more reactive electrophile,[21] also produced none of the alkylated product when **2** was used as the amine nucleophile. However, when **3** was treated with triflate **6** in the presence of potassium carbonate the desired alkylated product **8a** was obtained in a 41% yield. Deprotection of **8a** to amino acid **9a** was achieved in 86% yield with refluxing 6 M hydrochloric acid. Treatment of **3** with methyl bromoacetate produced the alkylated analogue **8b** in a 90% yield. Diester **8b** was then hydrolyzed to unprotected amino acid **9b**.[19] Ethyl (3-bromopropyl)methylphosphinate **7** was prepared via a modified Arbuzov reaction between diethylmethyl phosphite and 1,3-dibromopropane. Phosphinate ester **8c** was produced as a mixture of diastereomers after alkylation of **4** with the (bromopropyl)phosphinate ester **7**. The mixture was deprotected with 48% hydrobromic acid to yield **9c**.

Aryl phosphonates **12a** and **12b** were synthesized as shown in Scheme III. The free amine of **3** was alkylated with the appropriate bromobenzyl bromide to produce the aryl bromides **10a** and **10b** in excellent yields. Treatment of **10a** or **10b** with diethyl phosphite in the presence of catalytic tetrakis(triphenylphosphine)palladium and triethylamine, following recent literature precedent[22] pro-

(19) Compounds **5a**, **5b**, and **9b** have been synthesized by an alternative procedure. Watkins, A. J.; Jones, A. W. Eur. Pat. Appl. EP 0159889A2, 1985.

(20) Kamiya, T.; Hashimoto, M.; Hemmi, K.; Takeno, H. U. S. Patent 4,206,156, 1980.

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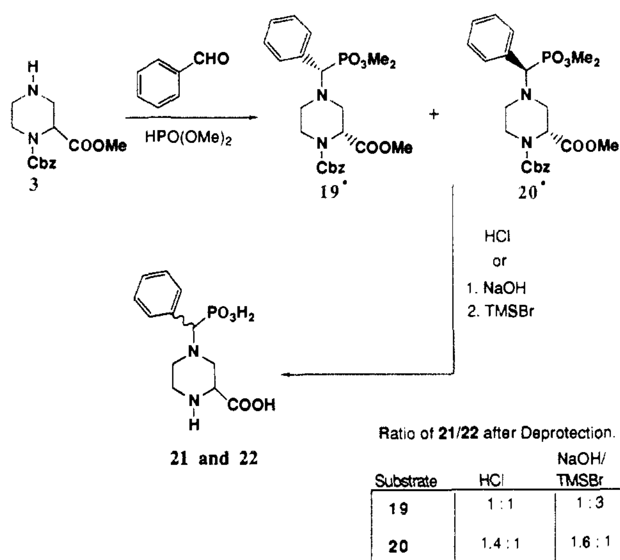
Scheme V



Ratio of 21/22 after Deprotection.

Substrate	HCl	NaOH/ TMSBr
19	1 : 1	1 : 3
20	1.4 : 1	1.6 : 1

*Stereochemistry has been arbitrarily assigned.

duced aryl phosphonates **11a** and **11b** in 44–53% yield. Hydrolysis of the protecting groups with hydrochloric acid completed the synthesis to produce amino acids **12a** and **12b**. Benzyl phosphonate **15** was obtained by alkylation of **3** with diethyl [[3-(chloromethyl)phenyl]methyl]-phosphonate (**13**) to give **14**, which was subsequently deprotected to the desired derivative **15**.

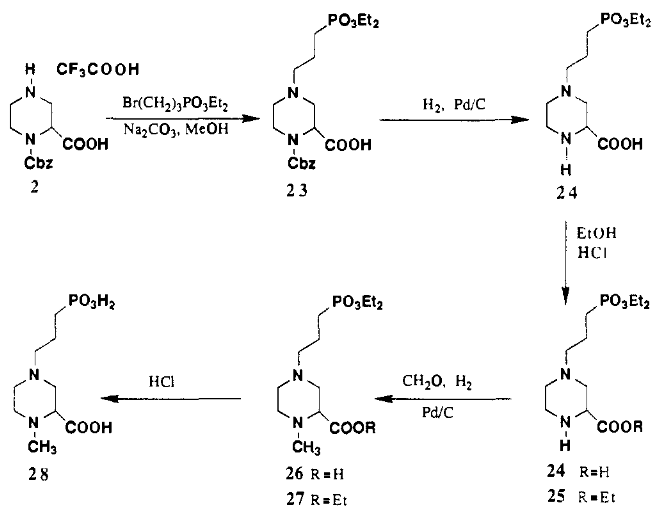
Benzoic acid analogue **18** was synthesized as shown in Scheme IV. Precursor **3** was treated with phthalic anhydride to give amide **16** in quantitative yield. Saponification of **16** provided diacid **17** in 65% yield and, subsequently, the carbobenzyloxy group was removed by catalytic hydrogenation to give **18** in low yield.[23]

Scheme V shows the syntheses of phenylmethyl analogues **21** and **22**. Amine **3** was treated with benzaldehyde and dimethyl phosphite in refluxing methanol to yield diastereomers **19** and **20**, which were separated by silica gel chromatography. By NMR experiments, we were unable to determine the relative stereochemical relationship for each isomer. Deprotection of each diastereomer by either hydrochloric acid or a two-step deprotection sequence employing sodium hydroxide and trimethylsilyl bromide produced a mixture of diastereomers **21** and **22**. Scheme V shows that the two-step deprotection resulted in an improved ratio of one diastereomer over the other compared to the acid deprotection method. Product ratios were determined by derivatization of the product with *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide followed by capillary GC analysis.

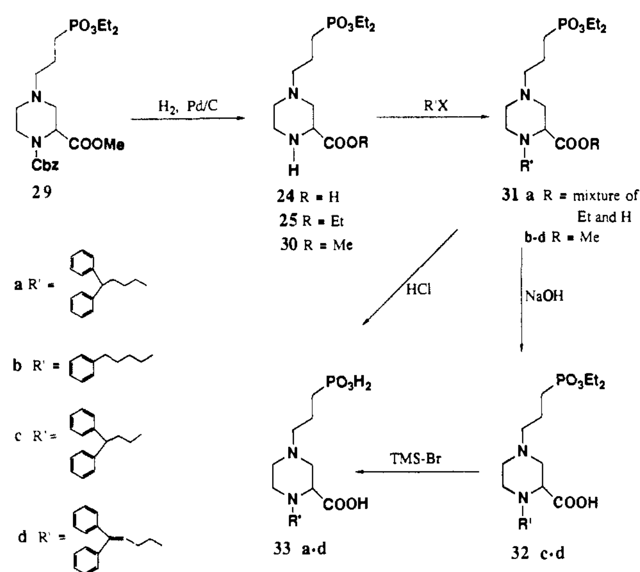
The N^1-substituted analogues were initially synthesized with amino acid precursor **2**. Alkylation of **2** with diethyl (bromopropyl)phosphonate, as shown in Scheme VI, was followed by removal of the N^1-Cbz group by catalytic hydrogenation to give amine **24**. Esterification of **24** was incomplete and produced a mixture of the desired ester **25** and the starting acid **24**. The resulting mixture was treated with formaldehyde under reducing conditions to produce a mixture of N^1-methylated acid **26** and ester **27**. The acid/ester mixture was then hydrolyzed to the desired N^1-methylated analogue **28**. The overall yield from the

(22) Hirao, T.; Masunanaga, T.; Ohshiro, Y.; Agawa, T. *Synthesis* 1981, 56.
(23) This low yield was a result of poor elution of the compound from the ion-exchange resin during chromatography.

Scheme VI



Scheme VII



alkylated product **23** was 73%.[24] In NMR experiments on **28**, strong nuclear Overhauser enhancements were observed between the N^1-methyl group and the protons in the 2- and 6-positions of the piperazine ring, thus confirming our structural assignment for **28**. N^1-Alkylated derivative **33a** was prepared by alkylation of the mixture of **24** and **25** as shown in Scheme VII. Reverse-phase chromatography provided acid/ester mixture **31a**, which was hydrolyzed to yield diphenylbutyl analogue **33a**. A more convenient route to the N^1-alkylated derivatives utilized phosphonopropyl derivative **29**[18] as a starting material, as shown in Scheme VII. Hydrogenolysis of the Cbz group of **29** produced free amine **30** in 96% yield. Treatment of **30** with selected arylalkyl bromides resulted in the formation of the N^1-alkylated products **31b–d** in 37–71% yield. The phenylbutyl analogue **31b** was deprotected with hydrochloric acid to give **33b** in 55% yield. Derivatives **31c,d** were saponified to the corresponding acids **32c,d** with sodium hydroxide, and then the phosphonate esters were removed with trimethylsilyl bromide[25] to obtain the desired products **33c** and **33d** in 34 and 61%

(24) It is likely that this sequence could be simplified by omitting the esterification step and purification procedures that followed.
(25) McKenna, C. E.; Schmidhauser, J. *J. Chem. Soc., Chem. Commun.* 1979, 739.

yields, respectively. This two-step deprotection was required because of the N^1-dealkylation which occurred to a small extent with hydrochloric acid deprotection.[26] Analysis for CPP contamination was performed by derivatization of the final product with *N*-methyl-*N*-(butyl dimethylsilyl)trifluoroacetamide, followed by capillary GC analysis. Additional N^1-substituted CPP analogues such as benzyl and propargyl were prepared by methodology similar to that described above but could not be deprotected without some N-dealkylation occurring even with the milder two-step procedure of saponification followed by treatment with trimethylsilyl bromide.

Results and Discussion

Compounds targeted for synthesis were evaluated as NMDA ligands by their ability to displace [^{3}H]CPP[27,28] from rat cortical membranes. The results for these and selected reference compounds are summarized in Table I. Additionally, a simple biochemical assay for distinguishing NMDA receptor agonists from antagonists was performed.[29] This assay is based on the observation that agonists, such as glutamate and NMDA, facilitate access of noncompetitive NMDA antagonists, such as 1-[1-(2-thienyl)-cyclohexyl]piperidine (TCP), to the 1-(1-phenylcyclohexyl)piperidine (PCP) binding site. Conversely, antagonists block this stimulatory effect of glutamate at the agonist recognition site. The results of this glutamate-stimulated TCP binding assay (GSTCP) are also recorded in Table I.

Varying the length of the methylene spacer of CPP analogues **5a**, **5b**, and **9a** alters the binding affinity in the following order: $n = 3 > 1 \gg 2 > 4$ (CPP, **9a**, **5a**, and **5b**, respectively).[30] This order grossly mimics the periodicity of binding affinity observed for the acyclic derivatives. For comparison, DL-AP5 was found to possess just over 2 times greater affinity for the NMDA receptor compared to DL-AP7 and 26 times greater affinity than DL-2-amino-6-phosphonohexanoic acid (AP6).[27,31] The longer chain compound DL-2-amino-8-phosphonooctanoic acid (AP8) had over 500-fold reduced affinity for the receptor when compared to AP5.[31]

Despite similarities to AP5 and AP7, analogue **9a**, with a single methylene group, exhibited diminished affinity for the NMDA receptor when compared to CPP (IC_{50} = 0.32 versus 0.079 μM). This was unexpected since the structurally similar piperidine compound CGS 19755 exhibits improved affinity compared to CPP. There are several possible reasons for this discrepancy. We initially speculated that the decreased affinity of **9a** may be due to the formation of an internal five-membered hydrogen-bonded ring between the phosphonic acid and the N^4-nitrogen of the piperazine ring, stabilizing an unfavorable conformation of the phosphonic acid side chain. However, modeling studies suggest that the required phosphonate OH to N^4-nitrogen hydrogen bond would be weak because the relatively long carbon to phosphorous and phosphorous to oxygen bond lengths preclude an optimum approach of the two heteroatoms. Alternatively, an electrostatic repulsion between the lone pair of the N^4-nitrogen and the negatively charged PO_3 moiety may be preventing the distal acidic group from assuming a conformation required for binding. An additional possibility is that the decreased lipophilicity of a piperazine ring versus a piperidine ring may have a detrimental effect. It is assumed that the rapid interconversion of the methylene phosphonate side chain at the N^4-nitrogen of **9a** would allow the compound to adopt a cis stereochemistry similar to that of CGS 19755. Replacing the phosphonate of **9a** with a carboxylate reduces the binding affinity by an additional 50-fold (see **9b**, Table I). Once again, either an internal hydrogen bond between the COOH and the piperazine N^4-atom or electrostatic repulsion between negatively charged centers may be stabilizing unfavorable conformations of the side chain. In contrast, other CPP analogues where the phosphonate has been replaced with an acidic group that has only a single ionizable proton (carboxylate **5c** and phosphinate **9c**) demonstrate only a 10-fold reduction in affinity for the NMDA receptor when compared to CPP.

CPP has a log *P* of -3.4[32] and is poorly absorbed into the central nervous system due to its polar character.[33] To increase the lipophilicity, the addition of steric bulk at various positions of the CPP molecule was explored. The incorporation of an aryl spacer between the N^4-nitrogen and the distal acidic group (**12a**, **12b**, **15**, and **18**) resulted in greatly reduced or complete loss of binding affinity. The phenylmethylene analogues **21** and **22** were examined as enriched mixtures of the two diastereomers. The discrepancy in binding affinities for the two mixtures suggests that a phenyl group may be tolerated by the receptor if properly positioned. The mixture enriched with **22**, with an IC_{50} of 5.8 μM, has approximately a 10-fold decrease in receptor affinity when compared to analogue **9a**, which does not contain the phenyl group.

N^1-alkylation clearly results in a dramatic reduction in binding affinity. N^1-methyl CPP **28** exhibits 3 orders of magnitude less affinity for the receptor when compared to the parent CPP. In an attempt to explore the potential for an auxiliary receptor binding site, aromatic functionality was attached through an alkyl spacer to the N^1-position of CPP. This approach was unsuccessful as all binding affinity was abolished for analogues **33a–d**.

All analogues tested in the GSTCP assay demonstrated antagonist properties. Although several dicarboxylic acids in addition to aspartate and glutamate have been reported to have agonist properties,[34] it was particularly interesting to note that carboxylic acids **5c** and **9b** exhibited full antagonist properties.

(26) Although this acid-mediated dealkylation occurs only to the extent of a few percent, the parent compound, CPP, is the byproduct and even small quantities of CPP in the product had profound effects on the results of the NMDA receptor binding assays.

(27) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. *J. Pharm. Exp. Ther.* **1987**, *240*, 778.
(28) Bigge, C. F.; Drummond, J. T.; Johnson, G.; Malone, T.; Probert, A. J.; Marcoux, F. W.; Coughenour, L. L.; Brahce, L. J. *J. Med. Chem.* **1989**, *32*, 1580.
(29) Thomas, J. W.; Hood, W. F.; Monahan, J. B.; Contreras, P. C.; O'Donohue, T. L. *Brain Res.* **1988**, *442*, 396.

(30) Binding results for compounds **5a**, **5b**, and **9b** have been summarized previously by Watkins, J. C.; Olverman, H. J. In *Excitatory Amino Acids in Health and Disease*; Lodge, D., Ed.; John Wiley and Sons, Inc.: New York, 1988.

(31) Olverman, H. J.; Monaghan, D. T.; Cotman, C. W.; Watkins, J. C. *Eur. J. Pharmacol.* **1986**, *131*, 161.

(32) The log *P* of CPP was determined experimentally with tritiated CPP partitioned between 1-octanol and phosphate buffer (pH 7.55) according to methods described by Purcell et al.: Purcell, W. P.; Bass, G. E.; Clayton, J. M. *Strategy of Drug Design. A Molecular Guide to Biological Activity*; John Wiley and Sons, Inc.: New York, 1973; Appendix I.

(33) The level of radioactivity in the brain of structurally similar [^{3}H]AP7 corresponded to approximately 0.1% of the total amount of tritium injected. Compton, R. P.; Hood, W. F.; Monahan, J. B. *Neurosci. Lett.* **1988**, *84*, 339. Chapman, A. G.; Collins, J. F.; Meldrum, B. S.; Westerberg, E. *Neurosci. Lett.* **1983**, *37*, 75.

(34) Madson, U.; Brehm, L.; Schaumburg, K.; Jorgensen, F. S.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1990**, *33*, 374.

Table I. Inhibition of [^{3}H]CPP Binding

compound	R_1	R_2	[^{3}H]CPP binding: IC_{50}, μM	SEM[b]	GSTCP act.[d]
L-glutamate			0.171	±0.012	ag
NMDA			4.55	±1.42	ag
CPP	$(CH_2)_3PO_3H_2$	H	0.079	±0.007	ant.
CGS 19755			0.065	±0.010	ant.
AP5			0.290	±0.048	ant.
AP7			0.76	±0.24	ant.
9a	$CH_2PO_3H_2$	H	0.32	±0.09	ant.
5a	$(CH_2)_2PO_3H_2$	H	9.8	±0.84	ant.
5b	$(CH_2)_4PO_3H_2$	H	48.0	±7.4	ant.
5c	$(CH_2)_3COOH$	H	0.85[c]	±0.14	ant.
9b	CH_2COOH	H	16.0[c]		ant.
9c	$(CH_2)_3P(=O)(OH)CH_3$	H	0.78[c]		ant.
12a	PO_3H_2	H	>100		*a*
12b	PO_3H_2	H	83.0[c]		*a*
15	PO_3H_2	H	>100		*a*
18	COOH	H	>100		*a*
21/22 (1:3)	PO_3H_2	H	5.8	1.1	ant.
21/22 (1.6:1)	PO_3H_2	H	13.3	0.33	ant.
28	$(CH_2)_3PO_3H_2$	CH_3	47.7	11.4	*a*
33a	$(CH_2)_3PO_3H_2$		>100		*a*
33b	$(CH_2)_3PO_3H_2$		>100		*a*
33c	$(CH_2)_3PO_3H_2$		>100		*a*
33d	$(CH_2)_3PO_3H_2$		>100		*a*

[a] Not tested in GSTCP because of weak affinity for the NMDA receptor. [b] SEM = standard error of the mean. [c] This determination was performed with $N = 1$. [d] Ag = agonist; ant. = antagonist.

In conclusion, 14 new CPP analogues have been synthesized and their affinity for the NMDA receptor has been examined. The binding affinity of the various chain lengths at the N[4]-position of the CPP derivatives is in agreement with the binding affinity of the acyclic derivatives AP5, AP6, and AP7. Replacement of the phosphonic acid moiety with monoionizable acidic groups results in a reduction of binding affinity. An aryl spacer between

the N[4]-nitrogen and the distal acidic group was detrimental to binding affinity, as was alkylation at the N[1]-position. Steric bulk, however, was better tolerated when a phenyl group was positioned α to the phosphonate as seen with analogue 22. These analogues have been helpful in the development and evaluation of a predictive pharmacophore model of the competitive NMDA receptor. The details of this model will be presented in the near future.[35]

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Melting points of foams are not reported since they decomposed over a wide temperature range. IR spectra were obtained on a Nicolet MX-1 FT spectrometer, but are not reported. The ^1H NMR spectra were recorded on an IBM W-P100SY NMR spectrometer (100 MHz), a Varian XL200 NMR spectrometer (200 MHz), or a Varian XL 300 equipped with a 5-mm broad-band switchable probe. All spectra were consistent with the proposed structures. The peaks are described in ppm downfield from TMS (internal standard). The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG Analytical 7070E/HF mass spectrometer. Although all intermediates were characterized by NMR, IR, and MS, ^1H NMR and MS data are given only on final products and selected intermediates. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values; values outside the limits are indicated. TLC was carried out with 0.25-mm silica gel F254 (E. Merck) glass plates. Some intermediate products were used directly without further purification or characterization.

Diethyl (Bromoalkyl)phosphonates. The diethyl alkylphosphonates were prepared by an Arbuzov reaction as described previously[20] with 5 equiv of the dibromoalkanes and 1 equiv of triethyl phosphite heated to 140 °C. The workup was modified to include chromatography of the crude oil on a large silica gel plug. The silica gel was eluted initially with hexane to recover the excess dibromoalkane. The eluant was then changed to ethyl acetate to elute off the desired diethyl (bromoalkyl)phosphonate in 60–80% yields. GC analysis on a OV-17 column indicated that the products were 85–95% pure. The bromides were used without additional purification.

1-(**Phenylmethyl) (±)-1,2-Piperazinedicarboxylate Trifluoroacetate (1:1) Salt (2).** 4-(1,1-Dimethylethyl) 1-(phenylmethyl) (+/−)-1,2,4-piperazinetricarboxylate (1) was synthesized as described previously.[18] Crude, BOC-protected compound 1 (38.5 g, 0.106 mol) was dissolved in methylene chloride (200 mL) and trifluoroacetic acid (100 mL). The reaction was stirred at 25 °C for 18 h and the solvent was removed under reduced pressure. Diethyl ether was added to the clear syrup and the product precipitated as a white solid (36.3 g, 90% yield): mp 196–198 °C, ^1H NMR (100 MHz, DMSO) δ 9.3 (b, 2 H), 7.4 (s, 5 H), 5.5 (b, 1 H), 5.2 (s, 2 H), 4.9 (m, 1 H), 4.2–2.8 (m, 6 H); MS (EI) 264 (M^+). Anal. ($C_{13}H_{16}N_2O_4 \cdot CF_3COOH$) C, H, N.

(±)-4-(2-Phosphonoethyl)-2-piperazinecarboxylic Acid (5a). Amino acid 2 (5.0 g, 13.2 mmol) was slurried in absolute ethanol, and sodium carbonate (5.6 g, 52.8 mmol) and diethyl (bromoethyl)phosphonate (3.3 g, 13.5 mmol) were added. The reaction was refluxed for 24 h and cooled and the ethanol solution was concentrated under reduced pressure. The oily brown residue was placed on a silica gel column eluted initially with methylene chloride followed by methylene chloride/methanol (19:1). Product **4a** was isolated as a pale yellow oil (3.48 g, 62%) which was suspended in 6 M HCl (150 mL) and heated to reflux for 24 h. The water was removed under reduced pressure and the crude material was dissolved in a minimum of water and washed with methylene chloride. The two layers were separated and the aqueous layer was chromatographed on a cation-exchange column (Dowex 50 × 8-400, hydrogen form). The column was eluted first with water (250 mL) followed by 2 M pyridine. The first 100 mL of the pyridine eluant was discarded. The next 200 mL was concentrated under reduced pressure to produce a white foam. The foam was triturated with acetone and filtered to obtain amino acid **5a** (1.52 g, 79% from **4a**): ^1H NMR (100 MHz, D_2O) δ 4.3–3.1 (m, 9 H), 2.3–1.78 (m, 2 H); MS (FAB) 239 (MH^+). Anal. ($C_7H_{15}N_2O_5P \cdot 0.5H_2O$) C, H, N.

(±)-4-(2-Phosphonobutyl)-2-piperazinecarboxylic Acid (5b). Amino acid 2 (5.0 g, 13.2 mmol) and sodium carbonate (5.6 g, 52.8 mmol) were slurried in ethanol (100 mL) and diethyl (bromobutyl)phosphonate (3.8 g, 13.8 mmol) was added in one batch and the reaction was refluxed for 20 h. The reaction was worked up and chromatographed as described for **5a** to produce alkylated product **4b** as a white foam (3.5 g, 63% yield). Phosphonate ester **4b** was hydrolyzed, worked up, and chromatographed on an ion-exchange resin as described for **5a**. Product **5b** was isolated as a tan foam (2.14 g, 61% yield from **4b**): ^1H NMR (100 MHz, D_2O) δ 4.2–3.0 (m, 9 H), 2.1–1.3 (m, 6 H); MS (FAB) 267 (MH^+). Anal. ($C_9H_{19}N_2O_5P \cdot 0.1C_5H_5N$) C, H, N.

(±)-3-Carboxy-1-piperazinebutanoic Acid (5c). Amino acid 2 (5.0 g, 13.2 mmol) and sodium carbonate (5.6 g, 52.8 mmol) were slurried in ethanol (100 mL), ethyl 4-bromobutyrate (2.7 g, 13.8 mmol) was added in one batch, and the reaction was refluxed for 20 h. The reaction was worked up and chromatographed as described for **5a** to produce **4c** as a yellow oil (2.9 g, 58% yield). Ester **4c** (2.7 g, 7.1 mmol) was dissolved in 2 M HCl (100 mL) and the solution was refluxed for 20 h. The reaction was worked up and chromatographed as described for **5a**. Product **5c** was isolated as a tan foam (1.23 g, 80% yield from **4c**): ^1H NMR (200 MHz, D_2O) δ 4.1–2.7 (m, 9 H), 2.4 (t, 2 H), 2.0 (m, 2 H); MS (EI) 216 (M^+). Anal. ($C_9H_{16}N_2O_4$) C, H, N.

(±)-4-(Phosphonomethyl)-2-piperazinecarboxylic Acid (9a). Protected amino acid 3[18] (1.34 g, 3.4 mmol) was dissolved in acetonitrile (80 mL), and potassium carbonate (1.88 g, 13.6 mmol) and (diethoxyphosphinyl)methyl trifluoromethanesulfonate[21] (6; 1.53 g, 5.1 mmol) were added. The reaction was refluxed for 4 h and then stirred at 25 °C for 2.5 days. The reaction was filtered and the solid was washed with acetonitrile. The filtrate was concentrated and the residue was partitioned between methylene chloride and water. The methylene chloride was dried with sodium sulfate, filtered, and concentrated. The crude residue was chromatographed on a silica gel column eluted with hexane/ethyl acetate (1:4). Product **8a** was isolated as a clear oil (0.6 g, 41% yield). Phosphonate ester **8a** was mixed with 6 M hydrochloric acid (100 mL) and refluxed for 24 h. The reaction was cooled to 25 °C and stirred for an additional 3.5 days. The reaction was concentrated and the residue was partitioned between water and methylene chloride. The layers were separated, and the methylene chloride layer was discarded. The water layer was applied to the top of a cation-exchange column (Dowex 50X 8-400, hydrogen form) and eluted with water (300 mL) which was discarded, followed by 2 M pyridine (300 mL). The aqueous pyridine was concentrated under reduced pressure to yield amino acid **9a** as a tan foam (0.30 g, 86% yield from **8a**): ^1H NMR (200 MHz, D_2O) δ 4.1 (dd, 1 H), 4.0–3.6 (m, 3 H), 3.5–3.1 (m, 5 H). Anal. ($C_6H_{13}N_2O_5P \cdot 0.075C_5H_5N$) C, H, N.

(±)-3-Carboxy-1-piperazineacetic Acid Dihydrochloride (9b). The trifluoroacetic acid salt of 3 (1.5 g, 3.8 mmol) was dissolved in methanol (10 mL), and sodium carbonate (2.0 g, 18.9 mmol) and methyl bromoacetate (0.93 g, 6.11 mmol) were added. The reaction was stirred at 25 °C for 24 h. The reaction was worked up as described for **8a** and chromatographed on a silica gel column eluted with methanol/methylene chloride (1:19) to produce **8b** as a clear oil (1.17 g, 88% yield). Diester **8b** was dissolved in 2 M hydrochloric acid and refluxed for 24 h. The reaction was cooled to 25 °C and the reaction was washed with methylene chloride. The aqueous layer was separated and concentrated under reduced pressure to yield amino acid **9b** as a foam (0.73 g, 90% yield from **8b**) which was triturated with ethyl ether and filtered: ^1H NMR (200 MHz, D_2O) δ 4.5 (dd, 1 H), 4.2 (s, 2 H), 4.1 (m, 1 H), 3.8 (m, 2 H), 3.7–3.4 (m, 3 H); MS (CI) 189 (MH^+). Anal. ($C_7H_{12}N_2O_4 \cdot 2.0HCl$) (C, H, N, Cl).

Ethyl (3-Bromopropyl)methylphosphinate (7). A mixture of 1,3-dibromopropane (50 g, 0.247 mol) and diethyl methylphosphonate (9.0 g, 0.066 mol) was heated at reflux for 4 h. The excess dibromopropane was removed by distillation in vacuo. The pot residue was fractionated by column chromatography on silica gel eluted initially with chloroform followed by chloroform/

(35) Humblet, C.; Johnson, G.; Malone, T.; Ortwine, D. F. Proceedings from the 11th International Symposium on Medicinal Chemistry, Jerusalem, Israel, Sept. 2, 1990; to be published in *Pure Appl. Chem.*

methanol (40:1) to give a clean separation of the starting phosphonate, the disphosphine adduct, and the desired product 7 (5.6 g, 37%) as a clear oil: ^{1}H NMR (90 MHz, $CDCl_3$) δ 4.2–3.8 (dt, 2 H, J = 5.7 Hz), 3.5–3.3 (t, 2 H), 2.3–1.6 (4 H, m), 1.48 (d, 3 H, J = 12.9 Hz), 1.3 (t, 3 H); MS (EI) M^+ (229). No elemental analysis was obtained.

(±)-4-[(3-Methylphosphinyl)propyl]-2-piperazinecarboxylic Acid (9c). Protected amino acid **3** (1.5 g, 3.9 mmol) was dissolved in methanol (10 mL), and sodium carbonate (2.0 g, 18.9 mmol) and ethyl (3-bromopropyl)methylphosphinate (**7**; 1.4 g, 6.11 mmol) were added. The reaction was stirred for 18 h at 25 °C and additional bromo phosphinate **7** (0.6 g, 2.6 mmol) was added. The reaction was refluxed for 18 h, cooled, and filtered. The filtrate was concentrated under reduced pressure to yield an oily–solid residue which was chromatographed on a silica gel column eluted with methanol/chloroform/heptane (10:95:95). Alkylated product **8c** was isolated as a pale yellow oil which was dissolved directly in 48% HBr (20 mL) and was refluxed for 18 h. The water was removed as much as possible by rotary evaporation at 50 °C. The reaction was then lyophilized to remove the additional water. The solid residue was dissolved in water and chromatographed on a Whatman OD8-3 P40 ion-exchange resin (10 g). The column was eluted initially with water (6 × 12.5 mL) followed by methanol/water (1:19). The solution was evaporated and the residue was redissolved in water and lyophilized to give a hygroscopic foam (**9c**): ^{1}H NMR (200 MHz, DMSO) δ 4.62–4.56 (dd, 1 H), 3.98–3.65 (m, 3 H), 3.48–3.16 (m, 5 H), 2.02–1.81 (m, 2 H), 1.78–1.64 (m, 2 H), 1.38 (d, 3 H, J = 15 Hz); MS (FAB) 251 (MH^+). Anal. ($C_9H_{19}N_2O_4P{\cdot}0.2H_2O$) C, H, N.

(±)-4-[(2-Phosphonophenyl)methyl]-2-piperazinecarboxylic Acid (12a). Protected amino acid **3** (2.0 g, 5.1 mmol) was dissolved in methanol (10 mL), and sodium carbonate (2.2 g, 20.4 mmol) was added. After stirring for 10 min, 2-bromobenzyl bromide (1.4 g, 5.6 mmol) was added and the reaction was stirred for 24 h at 25 °C. The reaction was diluted with diethyl ether and filtered. The filtrate was concentrated and the residue was partitioned between water and diethyl ether. The diethyl ether layer was dried over magnesium sulfate, filtered, and concentrated. The residue was chromatographed on silica gel eluted initially with heptane to remove the excess alkylating agent followed by elution with diethyl ether. Product **10a** was isolated as a viscous gum (2.34 g, 100% yield) which solidified upon standing. Aryl bromide **10a** (2.2 g, 4.9 mmol) was dissolved in toluene and concentrated to 15 mL. Diethyl phosphite (0.75 g, 5.4 mmol), triethylamine (0.55 g, 5.4 mmol), and tetrakis(triphenylphosphine)palladium (0.35 g, 0.29 mmol) were added under a nitrogen atmosphere. The reaction was heated to 90 °C for 6 h and cooled to 25 °C. The reaction was washed with water and the toluene layer was dried over magnesium sulfate, filtered, and concentrated. The residue was chromatographed on a silica gel column eluted with ethyl acetate to yield **11a** as a colorless oil (1.1 g, 44% yield from **10a**). The aryl phosphonate (1.0 g, 2.0 mmol) was refluxed in 6 M hydrochloric acid (20 mL) for 20 h, cooled to 25 °C, and stirred an additional 18 h. The reaction was extracted between water and ethyl acetate. The water layer was concentrated and the residue was taken up in water and chromatographed on a Dowex 50W X 4 ion-exchange column. The column was eluted with water (200 mL) followed by 1 M ammonium hydroxide (300 mL). The product was lyophilized twice to yield monoammonium salt **12a** (0.15 g, 22% yield from **11a**): ^{1}H NMR (200 MHz, D_2O) δ 7.94–7.84 (m, 1 H), 7.56–7.42 (m, 3 H), 4.29 (s, 2 H), 3.84 (dd, 1 H), 3.58–3.47 (m, 2 H), 3.26–3.20 (m, 2 H), 2.97–2.84 (m 2 H); MS (FAB) 301 (MH^+). Anal. ($C_{12}H_{17}N_2O_5P{\cdot}NH_3{\cdot}1.5H_2O$) C, H, N, H_2O.

(±)-4-[(3-Phosphonophenyl)methyl]-2-piperazinecarboxylic Acid (12b). Protected amino acid **3** (2.0 g, 5.1 mmol) was dissolved in methanol, and sodium carbonate (2.2 g, 20.4 mmol) was added. After stirring for 10 min, 3-bromobenzyl bromide (1.4 g, 5.6 mmol) was added. The reaction was run and worked up as described for **12a** to yield product **10b** as a clear oil (2.1 g, 92% yield). Aryl bromide **10b** (2.0 g, 4.5 mmol) was dissolved in toluene and concentrated to 15 mL. Diethyl phosphite (0.68 g, 5.0 mmol), triethylamine (0.50 g, 5.0 mmol), and tetrakis(triphenylphosphine)palladium (0.35 g, 0.29 mmol) were added under a nitrogen atmosphere. The reaction was run and worked up as described for **12a** to yield **11b** as a colorless oil (1.2 g, 53% yield from **21**). The aryl phosphonate (1.0 g, 2.0 mmol) was refluxed in 6 M hydrochloric acid (15 mL) for 20 h and cooled to 25 °C. The reaction was extracted between water and diethyl ether. The water layer was concentrated. The residue was concentrated again from water (10 mL) and then from ethanol (25 mL). The residue was taken up in water and chromatographed on a Dowex 50W X-4 ion-exchange column. The column was eluted with water (200 mL) followed by 1 M ammonium hydroxide. The product was lyophilized twice to yield diammonium salt **12b** (0.40 g, 57% yield from **11b**): ^{1}H NMR (100 MHz, D_2O) 7.8–7.5 (m, 2 H), 7.5–7.3 (m, 2 H), 3.96–3.57 (m, 3 H), 3.53–3.2 (m, 2 H), 3.2–2.87 (m, 2 H), 2.7–2.27 (m, 2 H); MS (FAB) 301 (MH^+). Anal. ($C_{12}H_{17}N_2O_5P{\cdot}2.0NH_3{\cdot}H_2O$).

(±)-4-[[3-(Phosphonomethyl)phenyl]methyl]-2-piperazinecarboxylic Acid (15). Protected amino acid **3** (1.6 g, 4.0 mmol) was dissolved in methanol (8 mL), sodium carbonate (1.7 g, 16 mmol) was added, and the reaction was stirred at 25 °C for 30 min. Diethyl [[(3-chloromethyl)phenyl]methyl]phosphonate (**13**;[28] 1.1 g, 4.0 mmol) was dissolved in methanol (4 mL) and added to the reaction. The reaction was refluxed 2 h, cooled to 25 °C, and filtered. The filtrate was partitioned between ethyl acetate (100 mL) and water (15 mL). The ethyl acetate was washed with additional water (2 × 10 mL), dried over magnesium sulfate, filtered, and concentrated to produce crude product **14** as a viscous gum. The residue was chromatographed on a silica gel column eluted initially with ethyl acetate/heptane (4:6) with increasing proportions of ethyl acetate up to 7:3. Alkylated product **14** was isolated as a clear thick oil (0.9 g, 43% yield). Intermediate **14** (0.8 g, 1.5 mmol) was refluxed in 6 M hydrochloric acid (20 mL) for 22 h. The reaction was concentrated under reduced pressure, water (10 mL) was added, and the reaction, was concentrated again. The foam was triturated with acetone and the tan solid **15** (0.43 g, 74% yield from **14**) was filtered: ^{1}H NMR (100 MHz, D_2O) δ 7.43 (bs, 4 H), 4.5 (s, 2 H), 4.33 (dd, 1 H), 4.16–3.25 (m, 6 H), 3.27 (d, 2 H, J = 21.7 Hz); MS (FAB) 315 (MH^+). Anal. ($C_{13}H_{19}N_2O_5P{\cdot}2.0HCl$) C, H, N.

(±)-4-(2-Carboxybenzoyl)-2-piperazinecarboxylic Acid (18). Methyl ester **3** (1.5 g, 3.8 mmol) and phthalic anhydride (0.57 g, 3.8 mmol) were dissolved in anhydrous tetrahydrofuran (15 mL). Triethylamine (1.0 g, 9.5 mmol) was slowly added to the reaction flask and the reaction was stirred at 25 °C for 20 h. The reaction was concentrated under reduced pressure and slowly acidified with 1 M hydrochloric acid. A gum formed which was extracted into ethyl acetate. The two layers were separated, and the ethyl acetate layer was dried over magnesium sulfate, filtered, and concentrated to yield amide **16** (1.72 g, 100%) as a crude oil which was used without additional purification. Amide **16** (1.6 g, 3.7 mmol) was added to a solution of 50% sodium hydroxide (0.66 g) in water (20 mL) and stirred at 25 °C for 20 h. The reaction was slowly acidified with 1 M hydrochloric acid to give a gummy precipitate which was extracted into ethyl acetate. The layers were separated, and the ethyl acetate layer was dried over magnesium sulfate, filtered, and concentrated. The residue was crystallized from chloroform/diethyl ether to yield diacid **17** (1.0 g, 65% yield from **16**). Analysis of **17** indicated that it was slightly low in nitrogen, but it was carried on without additional purification. Intermediate **17** (0.9 g, 2.2 mmol) was dissolved in tetrahydrofuran (3 mL), 1 M ammonium hydroxide (2.5 mL), and water (0.6 mL). 10% Pd/C (0.10 g) was added and the reaction was hydrogenated for 18 h at 25 °C. The catalyst was removed by filtration through a Celite pad and the filtrate was concentrated. The residue was dissolved in water and chromatographed on a Dowex 50W X-8 column eluted first with water followed by 1 M ammonium hydroxide. The ammonium hydroxide fractions were concentrated to produce amino acid **18** (0.027 g, 4.1% from **17**): ^{1}H NMR (200 MHz, D_2O) δ 8.04–7.96 (m, 1 H), 7.70–7.57 (m, 2 H), 7.38–7.35 (d, 1 H), 4.1 (dd, 1 H), 4.0–3.05 (m, 6 H); MS (FAB) 279 (MH^+). Anal. ($C_{13}H_{14}N_2O_5{\cdot}H_2O$) C, H, N.

2-Methyl 1-Phenylmethyl 4-[(Dimethoxyphosphinyl)phenylmethyl]-1,2-piperazinedicarboxylate (19 and 20). The free base of methyl ester **3** (3.88 g, 13.9 mmol) was dissolved in acetonitrile (75 mL), and benzaldehyde (1.49 g, 14.0 mmol) and dimethyl phosphite (2.03 g, 18.0 mmol) were added. The reaction was heated to 80 °C for 48 h, cooled, and concentrated under reduced pressure. The crude oil was partitioned between water

and methylene chloride. The methylene chloride layer was washed with water (30 mL), 5% sodium bisulfite (30 mL), saturated sodium bicarbonate (30 mL), and water (30 mL). The methylene chloride layer was dried over sodium sulfate, filtered, and concentrated. The residue was chromatographed on a silica gel column eluted initially with methylene chloride followed by ethyl acetate. Three products were eluted from the column. The diastereomer of the desired product **19** (2.0 g, 30% yield) was eluted from the column first followed by the other diastereomer **20** (1.62, 25% yield). No assignment of the relative stereochemistry for these two compounds could be made and they are arbitrarily assigned in Scheme V. The third product was the N^4-methylated compound 2-methyl 1-(phenylmethyl) 4-methyl-1,2-piperazinedicarboxylate. **19**: ^1H NMR (200 MHz, $CDCl_3$) δ 7.4–7.3 (m, 10 H), 5.1 (s, 2 H), 4.8 (d, 1 H), 4.0–3.7 (m, 8 H), 3.5–3.4 (m, 6 H), 2.6 (m, 1 H), 2.0 (m, 1 H). Anal. ($C_{23}H_{29}N_2O_7P$) C, H, N. **20**: ^1H NMR (200 MHz, $CDCl_3$) δ 7.4–7.3 (m, 10 H), 5.1 (d, 2 H), 4.8 (d, 1 H), 3.9–3.7 (m, 9 H), 3.4 (d, 4 H), 3.0 (d, 1 H), 2.5–2.1 (m, 2 H). Anal. ($C_{23}H_{29}N_2O_7P$) C, H, N.

4-(Phosphonophenylmethyl)-2-piperazinecarboxylic Acid (21 and 22). A. Deprotection of 19 with Refluxing Hydrochloric Acid. Protected amino acid 19 (0.82 g, 1.7 mmol) was dissolved in 6 M hydrochloric acid and was heated to reflux for 1.5 days. The reaction was concentrated under reduced pressure and the crude foam was dissolved in a minimum of absolute ethanol and treated with propylene oxide (120 μL). The white solid (0.38 g, 66% yield) was filtered and washed with isopropyl alcohol followed by ethyl ether. Analysis of the product by capillary GC, as described in a later section, showed the product to be a mixture of the two diastereomers **21** and **22** (1:1): t_R for **21**, 15.2 min; for **22**, 15.5 min; ^1H NMR (200 MHz, D_2O) δ 7.6–7.5 (m, 5 H), 4.7–3.3 (m, 8 H); MS (FAB) 301 (H^+). Anal. ($C_{12}H_{17}N_2O_5P{\cdot}2.0H_2O$) C, H, N.

B. Deprotection of 33 with Refluxing Hydrochloric Acid. Protected amino acid **20** (0.65 g, 1.4 mmol) was dissolved in 6 M hydrochloric acid and was heated to reflux for 1.5 days. The reaction was worked up as described for **19** to yield a white, amorphous powder (0.40 g, 88% yield). Analysis of the product by capillary GC, as described in a later section, showed the product to be a mixture of the two diastereomers **21** and **22** (1.4:1): ^1H NMR (200 MHz, D_2O) as above for part A; MS (FAB) 301 (MH^+). Anal. ($C_{12}H_{17}N_2O_5P{\cdot}2.0H_2O$) C, H, N.

C. Deprotection of 19 with Sodium Hydroxide Followed by Trimethylsilyl Bromide. Protected amino acid **19** (0.5 g, 1.0 mmol) was dissolved in dioxane, and 1 M sodium hydroxide (1.1 mL) was added. The reaction was stirred at 25 °C for 18 h. Additional 1 M sodium hydroxide (0.3 mL) was added and the reaction was stirred another 7 h. The reaction was concentrated under reduced pressure and the residue was partitioned between saturated sodium bicarbonate and ethyl acetate. The sodium bicarbonate layer was acidified with 6 M hydrochloric acid and the aqueous layer was extracted with ethyl acetate. This second ethyl acetate layer was dried over sodium sulfate, filtered, and concentrated to yield the carboxylic acid as a clear oil (0.39 g, 84% yield) which was used without additional purification. The acid (0.39 g, 0.84 mmol) was dissolved in acetonitrile (5 mL), and trimethylsilyl bromide (0.77 g, 5.0 mmol) was added. The reaction was stirred at 25 °C for 24 h. Water (1 mL) was added and the reaction was stirred for 1 h. The reaction was concentrated under reduced pressure and the yellow foam (0.33 g, 75% yield) was washed with ethyl ether and filtered. Analysis of the product by capillary GC, as described in a later section, showed the product to be a mixture of the two diastereomers **21** and **22** (1:3): ^1H NMR (200 MHz, D_2O) as above for part A; MS (FAB) 301 (MH^+). Anal. ($C_{12}H_{17}H_2O_5P{\cdot}1.6HBr{\cdot}0.85H_2O$) C, H, N.

D. Deprotection of 20 with Sodium Hydroxide Followed by Trimethylsilyl Bromide. Protected amino acid **20** (0.3 g, 0.63 mmol) was dissolved in dioxane (10 mL) and 1 M sodium hydroxide (0.7 mL) was added. The reaction was stirred at 25 °C for 24 h. Additional 1 M sodium hydroxide (0.7 mL) was added and the reaction was stirred another 3 days. The reaction was worked up as described for part C to yield the carboxylic acid as a clear oil (0.24 g, 83% yield). The acid (0.24 g, 0.52 mmol) was dissolved in acetonitrile (5 mL), and trimethylsilyl bromide (0.35 g, 2.3 mmol) was added. The reaction was stirred at 25 °C for 24 h. Water (1 mL) was added and the reaction was stirred for 1 h. The reaction was concentrated under reduced pressure and the yellow oil was dissolved in water and chromatographed on a cation-exchange column (Dowex 50 X2-200) eluted first with water followed by 2 M pyridine. The pyridine fractions were concentrated to produce a beige foam (0.081 g, 48% yield). Analysis of the product by capillary GC, as described in a later section, showed the product to be a mixture of the two diastereomers **21** and **22** (1.6:1): ^1H NMR (200 MHz, D_2O) δ 7.6–7.5 (m, 5 H), 4.0–3.2 (m, 6 H), 2.9–2.5 (m, 2 H); MS (FAB) 301 (MH^+). Anal. ($C_{12}H_{17}N_2O_5P{\cdot}0.25C_5H_5N{\cdot}0.5H_2O$) C, H, N.

(±)-4-[3-(Diethoxyphosphinyl)propyl]-1-methyl-2-piperazinecarboxylic Acid (28). Amino Acid **2** (18.9 g, 0.05 mol) and sodium carbonate (21.2 g, 0.2 mol) were slurried in ethanol (400 mL), diethyl (bromopropyl)phosphonate (13.1 g, 0.051 mol) was added in one batch, and the reaction was refluxed for 20 h. The reaction was worked up and chromatographed as described for **5a** to produce alkylated product **23** as a white foam (12.1 g, 55% yield). 1-(Phenylmethyl) (±)-4-[3-(diethoxyphosphinyl)propyl]-1,2-piperazinedicarboxylate **23** (10.7 g, 24.2 mmol) was dissolved in ethanol (100 mL) and 20% Pd/C (1.0 g) was added. The reaction was hydrogenated for 4 h, filtered through a Celite pad, and concentrated to yield a tan foam (**24**; 7.4 g, 100%) which was used without additional purification. Acid **24** (7.4 g, 24 mmol) was dissolved in ethanolic hydrochloric acid (200 mL) and refluxed for 18 h. The ethanol was removed in vacuo and an NMR of the crude material indicated that the product was a mixture of ethyl ester **25** and starting acid **24**. The tan foam (8.3 g) was used without additional purification. The mixture of **24** and **25** (3.0 g) was dissolved in methanol (85 mL) with 37% aqueous formaldehyde (1.5 mL) and 10% Pd/C (1.0 g). The reaction was hydrogenated for 1 h, filtered through a Celite pad, and concentrated to yield a yellow oil. The crude oil was partitioned between 5% sodium carbonate and methylene chloride. The methylene chloride layer was dried over sodium sulfate, filtered, and concentrated to yield ester **27** as a pale yellow oil (0.56 g). The water layer was evaporated in vacuo to yield a white solid which was mixed with absolute ethanol and sonicated for 15 min. The mixture was filtered and the process was repeated again. The combined ethanol filtrate was evaporated to yield a white hygroscopic foam which NMR suggested was mainly acid **26**. Crude acid **26** was chromatographed on an ion-exchange (Dowex 50 X2-200) column eluted initially with water (200 mL) followed by 2 M pyridine (200 mL). The pyridine fractions were concentrated to produce acid **26** as a tan foam (1.49 g). Both acid **26** and ester **27** were combined in 6 M hydrochloric acid at 90 °C for 2.5 days. The H_2O was removed and the residue was chromatographed on an ion-exchange column (Dowex 50 X2-200). The column was eluted initially with water (200 mL) followed by 0.5 M pyridine. The pyridine fractions were concentrated to yield the desired amino acid as a foam (**28**; 1.73 g, 73% yield from **23**): ^1H NMR (300 MHz, D_2O) δ 4.0 (t, 2 H), 3.8 (d, 2 H), 3.5–3.3 (m, 5 H), 3.3 (s, 3 H), 2.0 (m, 2 H), 1.9–1.6 (m, 2 H). Nuclear Overhauser effects were measured by subtracting a spectrum recorded with selective irradiation of the CH_3 resonance during a 3-s relaxation delay from a spectrum recorded with off-resonance irradiation. MS (FAB) 267 (MH^+). Anal. ($C_9H_{19}N_2O_5P{\cdot}0.33H_2O$) C, H, N.

(±)-1-(4,4-Diphenylbutyl)-4-(3-phosphonopropyl)-2-piperazinecarboxylic Acid (33a). A mixture of ethyl ester **25** and acid **24** (3.1 g) as described in the synthesis of **28** was dissolved in absolute ethanol (150 mL), and sodium carbonate (3.22 g, 30.4 mmol) was added. 1,1′-(4-Bromobutylidene)bis[benzene][36] (2.3 g, 8 mmol) was added and the reaction was refluxed for 2.5 days. The reaction was filtered and the solid was washed with ethanol. The ethanol was concentrated to produce a brown oily residue. The residue was taken up in water containing a few drops of 2 M hydrochloric acid and chromatographed on a reverse-phase C-18 column eluted with water and increasing amount of ethanol. The acid followed by the ethyl ester were eluted from the column with water/ethanol (1:1). The acid and ester **31a** were combined to produce 1.2 g of alkylated product (4:1 ratio, acid/ester), which was dissolved in 6 M hydrochloric acid (100 mL) and refluxed

(36) Maercker, A.; Passlack, M. *Chem. Ber.* 1983, *116*, 710.

for 24 h. The water was concentrated under reduced pressure to produce a tan foam. The solid was triturated with acetone and filtered to produce the desired amino acid **33a** (0.79 g, 16% yield from **23**): ^{1}H NMR (100 MHz, D_2O) δ 7.3 (s, 10 H), 4.2–2.9 (m, 12 H), 2.5–1.5 (m, 8 H); MS (FAB) 461 (MH^+). Anal. ($C_{24}H_{33}N_2O_5P\cdot2HCl\cdot H_2O$) C, H, N.

Methyl (±)-4-[3-(Diethoxyphosphinyl)propyl]-2-piperazinecarboxylate (30). 2-Methyl 1-(phenylmethyl) (±)-4-[3-(diethoxyphosphinyl)propyl]-1,2-piperazinedicarboxylate[18] (**29**; 41.0 g, 0.090 mol) was dissolved in methanol (600 mL) and 20% Pd/C (2 g) was added. The reaction was hydrogenated for 18 h. The reaction solution was filtered through Celite and concentrated under reduced pressure. The crude product was purified by silica gel chromatography eluting with methanol/methylene chloride (1:4). Amine **30** was obtained as a colorless oil (27.8 g, 96%): ^{1}H NMR (200 MHz, $CDCl_3$) 4.0 (m, 4 H), 3.6 (s, 3 H), 3.5 (m, 1 H), 3.0 (m, 1 H), 2.75 (m, 2 H), 2.5 (m, 1 H), 2.3 (m, 3 H), 2.1 (m, 2 H), 1.7 (m, 4 H), 1.2 (t, 6 H, 7.1 Hz); MS (EI) 323 (MH^+). Anal. ($C_{13}H_{27}N_2O_5P$) C, H, N.

(±)-1-(4-Phenylbutyl)-4-(3-phosphonopropyl)-2-piperazinecarboxylic Acid (33b). Amine **30** (0.5 g, 1.55 mmol) and (4-bromobutyl)benzene[36] (0.4 g, 1.9 mmol) were dissolved in absolute ethanol (15 mL). Sodium carbonate (0.8 g, 7.6 mmol) was added and the reaction was refluxed for 48 h. The mixture was filtered and the filtrate was concentrated to yield the crude product which was chromatographed on a silica gel column eluted initially with methylene chloride followed by methanol/methylene chloride (3:97). Alkylated product **31b** (0.31 g, 44% yield) was dissolved in 6 M hydrochloric acid (10 mL) and heated to 93 °C for 48 h. The reaction was concentrated under reduced pressure and the product was recrystallized from ethanol to yield amino acid **33b** (0.16 g, 55% yield from **31b**): mp 206 °C dec; ^{1}H NMR (200 MHz, DMSO) δ 7.3 (m, 5 H), 4.3 (m, 1 H), 3.9–3.10 (m, 10 H), 2.7 (m, 2 H), 1.9 (m, 2 H), 1.6 (m, 6 H); MS (FAB) 385 (MH^+). Anal. ($C_{18}H_{29}N_2O_5P\cdot0.82HCl\cdot0.72H_2O$) C, H, N, Cl.

(±)-1-(3,3-Diphenylpropyl)-4-(3-phosphonopropyl)-2-piperazinecarboxylic Acid (33c). Amine **30** (1.2 g, 3.63 mmol) and 1,1′-(3-bromo-1,1-propylidene)bis[benzene][37] (1.2 g, 4.36 mmol) were dissolved in absolute ethanol (20 mL). Sodium carbonate (1.92 g, 18.15 mmol) was added and the reaction was refluxed for 72 h. The mixture was filtered and the filtrate was concentrated to yield the crude product which was chromatographed on a silica gel column eluted initially with methylene chloride followed by methanol/methylene chloride (3:97). Alkylated product **31c** (1.34 g, 71% yield) was isolated as an oil which was dissolved in methanol (10 mL) and 4 M sodium hydroxide (10 mL) and stirred for 18 h at 25 °C. The reaction was concentrated and the residue was partitioned between water (20 mL) and diethyl ether (100 mL). The aqueous layer was acidified to pH 2 and saturated with solid sodium chloride. The aqueous layer was extracted with methylene chloride (250 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to produce a light yellow solid (**32c**). Acid **32c** was dissolved in acetonitrile (15 mL), and trimethylsilyl bromide (2.38 g, 15.14 mmol) was added. The reaction was stirred at 25 °C for 24 h. Water (5 mL) was then added and the reaction was stirred an additional 30 min. The reaction was concentrated and the crude product was recrystallized from ethanol to yield **33c** (0.39 g, 34% yield from **31c**): mp 195–196 °C; ^{1}H NMR (200 MHz, DMSO) δ 7.3 (m, 10 H), 4.0 (m, 1 H), 3.6 (m, 1 H), 3.3–3.0 (m, 8 H), 2.7 (m, 2 H), 2.3 (m, 2 H), 1.8 (m, 2 H), 1.6 (m, 2 H); MS (FAB) 447 (MH^+). Anal. ($C_{23}H_{31}N_2O_5P\cdot HBr\cdot0.5H_2O$) C, H, N.

(±)-1-(4,4-Diphenyl-3-butenyl)-4-(3-phosphonopropyl)-2-piperazinecarboxylic Acid (33d). Amine **30** (1.66 g, 5.15 mmol) and 1,1′-(4-chloro-1-buten-1,1-ylidene)bis[benzene][38] (1.50 g, 6.18 mmol) were dissolved in absolute ethanol (20 mL). Sodium carbonate (2.73 g, 25.75 mmol) was added and the reaction was run and worked up as described for compound **33c**. Alkylated product **31d** (1.00 g, 37% yield) was converted to a white solid (**32d**) by using the procedure described for **33c**. Acid **32d** was dissolved in acetonitrile (15 mL), and trimethylsilyl bromide (1.74 g, 11.4 mmol) was added. The reaction was stirred at 25 °C for 24 h, water (10 mL) was then added, and the reaction was stirred for an additional 3 h. The reaction was concentrated, the crude product was dissolved in ethanol, and propylene oxide was added (0.2 mL). The solid was filtered and washed with ethanol to yield **33d** (0.53 g, 61% yield from **31d**): mp 207–208 °C; ^{1}H NMR (200 MHz, DMSO) δ 7.6–7.1 (m, 10 H(, 6.1 (t, 1 H, J = 7.27 Hz), 4.0 (m, 1 H), 3.6–2.9 (m, 10 H), 2.4–2.3 (m, 2 H), 2.0–1.8 (m, 2 H), 1.7–1.5 (m, 2 H); MS (FAB) 459 (MH^+). Anal. ($C_{24}H_{31}N_2O_5P\cdot0.82HBr\cdot0.75H_2O$) C, H, N.

Gas Chromatographic Analysis of CPP and Analogues. The sample (2 mg) was dissolved in 200 μL of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide/pyridine/triethylamine (2:2:1) and heated to 140 °C for 90 min. The derivatized sample was analyzed on a DB-5 fused silica capillary column (o.d. 0.40 mm, i.d. 0.25 mm, film thickness 0.25 μm, length 30 m) with an fid detector (injector temperature, 300 °C; detector temperature, 325 °C, oven temperature program 230 °C for 2 min, 230 to 320 °C increasing 4 °C/min, hold 320 °C for 11 min; t_R for CPP, 13.2 min with hydrogen carrier gas having a linear velocity of 55.4 cm/s).

Biology. Binding of [^{3}H]-4-(3-Phosphonopropyl)-2-piperazinecarboxylic Acid (CPP) to *N*-Methyl-D-aspartate (NMDA) Receptors in Rat Brain Crude Synaptic Membranes. Method. Binding assays with [^{3}H]CPP were carried out essentially by methods previously described.[27,28]

Glutamate-Stimulated [^{3}H]-1-[1-(2-Thienyl)cyclohexyl]-piperidine (GSTCP) Assay. Method. Binding of [^{3}H]TCP to rat brain membranes was performed as described previously[27] with several modifications. Crude synaptic membranes were prepared from rat whole brains minus cerebellum and brain stem and stored at −70 °C until use. On the day of the assay, the membranes were thawed, placed in 20 volumes of 0.01% Triton X-100 in 50 mM Tris-HCl (pH 7.6) and incubated at 37 °C for 30 min. The membranes were pelleted by centrifugation at 48000*g* for 15 min and then washed at least three times by resuspension in 50 mM Tris-HCl (pH 7.6). Test agents were incubated in 5 mM Tris-HCl (pH 8.0) with 15 mg of tissue (wet weight) and [^{3}H]TCP (1 nM) for 1 h at 25 °C. The samples were filtered through glass-fiber filters which had been pretreated with 0.5% polyethyleneimine. The filters were washed three times with 3 mL of ice-cold 5 m*M* Tris-HCl (pH 7.7). Following extraction, radioactivity on the filters was determined with liquid-scintillation spectrophotometry. Nonspecific binding was defined as the binding remaining in the presence of 100 μM (+)-SKF 10047.

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