Exploration of N-Phosphonoalkyl-, N-Phosphonoalkenyl-, and N-(Phosphonoalkyl)phenyl-Spaced α-Amino Acids as Competitive N-Methyl-D-aspartic Acid Antagonists†

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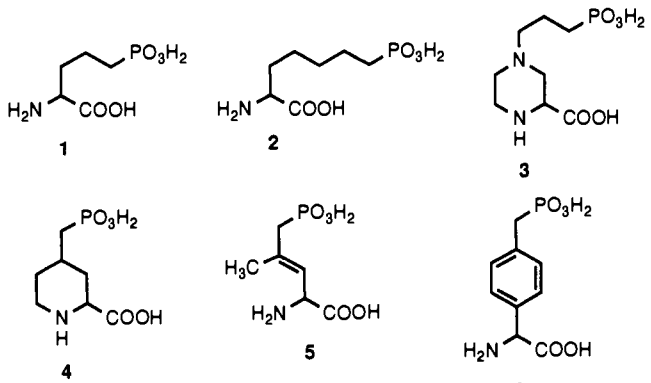
A series of N-substituted α-amino acids containing terminal phosphonic acid groups has been synthesized as potential N-methyl-D-aspartate (NMDA) receptor antagonists. NMDA receptor affinity was determined by displacement of a known ligand ([^{3}H]CPP) from crude rat brain synaptic membranes; an antagonist action was demonstrated by the inhibition of glutamate-induced accumulation of [$^{45}Ca^{2+}$] in cultured rat cortical neurons. Receptor affinity was significantly correlated with antagonist activity (Figure 1). Moderate affinity (IC_{50} = 1–2 μM) was retained for analogues (**31** and **32**, Table I; and **59** and **66**, Table II) with reduced flexibility in their phosphonate side chains and is consistent with entropy playing a role in determining receptor affinity. Modeling studies suggest a folded conformation that brings the distal phosphonic acid group into close proximity with the α-carboxylate is required for binding. Each of the active analogues possess entropy-limiting features (double bonds, phenyl rings) in their side chains that allows the superposition of their key NH_2, α-COOH, and distal PO_3H_2 groups with those of known competitive antagonists. Affinity decreased for analogues with α-carbon substitution, presumably because the α-substituent inhibits the folding of these structures into a bioactive conformation and occupies receptor-excluded volume. A complete description of the NMDA antagonist pharmacophore model is provided in a companion paper.[1]

Introduction

The growing awareness of a critical role for excitatory amino acids in the pathology of a number of neurodegenerative disorders[2-5] and epilepsy has stimulated interest in the search for new and more potent excitatory amino acid antagonists. Particularly interesting from their potential pharmaceutical use in the treatment of cerebral infarction, resulting from hypoglycemia[6] and ischemia,[7] are antagonists specific for the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors. Advances in the understanding of this area have been made possible by the availability of selective NMDA antagonists such as 2-amino-5-phosphonovaleric acid (APV, **1**),[8] 2-amino-7-phosphonoheptanoic acid (APH, **2**),[9] 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP, **3**),[10,11] and 4-(phosphonomethyl)-2-piperidinecarboxylic acid (**4**).[12,13] More recently, the list of NMDA-selective ligands has been extended to include (*E*)-2-amino-4-methyl-5-phosphono-



3-pentenoic acid (**5**),[14] the (*Z*)- and (*E*)-4-(phosphonoalkenyl)-2-carboxypiperidines,[15] and a series of phenyl-spaced APH analogues exemplified by 4-(phosphonomethyl)phenylglycine (**6**).[16] In addition, the chiral syntheses of D-APV,[17] and D-CPP[18] have been observed.

The NMDA receptor appears to be sensitive to both entropic and steric factors. This is illustrated by agonists

† This paper is dedicated to the memory of Mathias P. Mertes, Professor of Medicinal Chemistry at the University of Kansas, who died suddenly of a stroke on April 6, 1989. Professor Mertes was a mentor and friend and is deeply missed.

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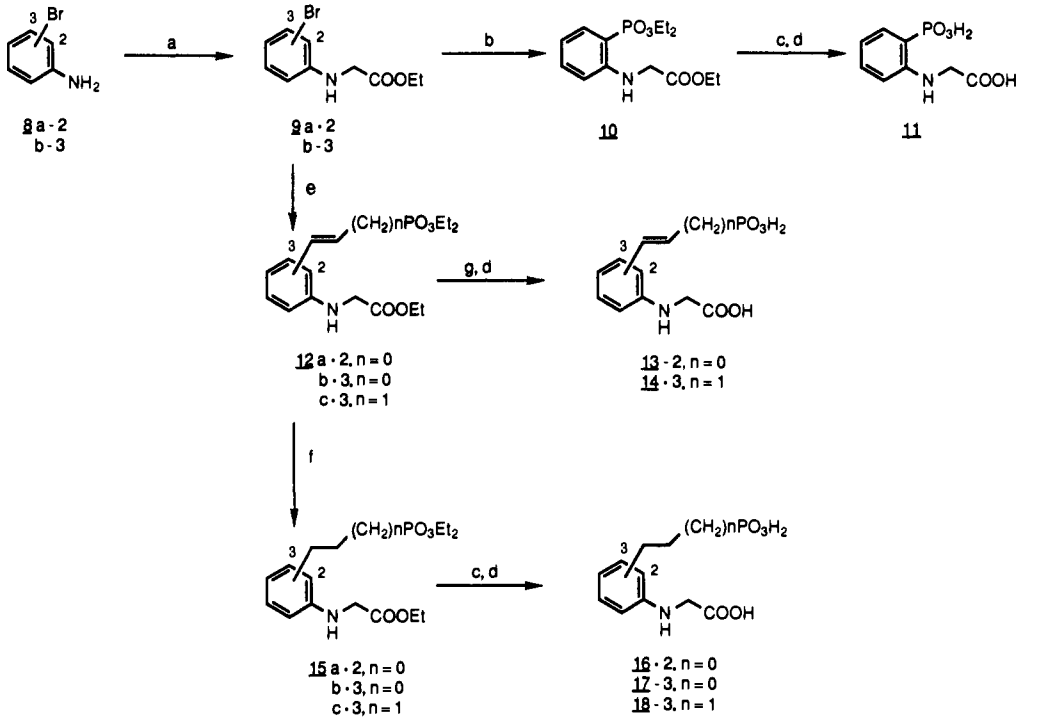
Scheme I[a]



[a] (a) $BrCH_2COOEt$, NEt_3, DMAP (cat.), EtOH, reflux; (b) HPO_3Et_2, NEt_3, $(Ph_3P)_4Pd^0$, toluene, N_2, 115–125 °C; (c) 6 N HCl, reflux; (d) Dowex 50; (e) $CH_2{=}CH(CH_2)_nPO_3Et_2$, NEt_3, $(Ph_3P)_2PdCl_2$, DMF, 90–100 °C; (f) H_2 (50 psi), 10% Pd/C, EtOH; (g) $(Me_3)_3SiBr$, CH_3CN; aq KOH (or $(CH_3)_3SiOK$, THF).

such as glutamate and aspartate that stimulate the receptor and, more strikingly, by potent antagonists such as the ring-constrained derivatives **3** and **4** and the alkenyl APV analogue **5**. Each of these structures has entropy-limiting features that affect the spatial positioning of the terminal phosphonic acid moiety. Additionally, the order of affinity, **5** > **4** > **3**, follows the decrease in steric demands placed upon the receptor. However, areas of steric tolerance within the NMDA receptor exist, as demonstrated by the moderate affinity of selected aryl- and cycloalkyl-spaced APH derivatives.[16,19]

In order to further explore the steric and conformational requirements of the NMDA receptor, a series of *N*-(phosphonoalkenyl)- and *N*-(phosphonoaryl)glycine and -alanine derivatives has been prepared. These compounds differ from known acyclic NMDA antagonists such as **1** and **2** in that their distal alkylphosphonic acid group is connected through nitrogen rather than the α-carbon of the amino acid. As such, they provide a different direction of attack to a presumed common phosphonate binding site among antagonists, and they have been used in the refinement of a pharmacophore model of the NMDA receptor.[1] This communication details the synthesis, pharmacology, and SAR of these novel antagonists.

Chemistry

Bromoaniline derivatives were readily transformed into the desired *N*-[(phosphonoalkyl)phenyl]glycines (**11**, **13**, **14**, and **16–18**) by first N-alkylation of bromoaniline with ethyl bromoacetate and by elaboration of the aryl bromide into the phosphonoalkyl side chain (Scheme I). Tetrakis(triphenylphosphine)palladium(0) was used to catalyze the direct attachment of diethyl phosphite to the aromatic

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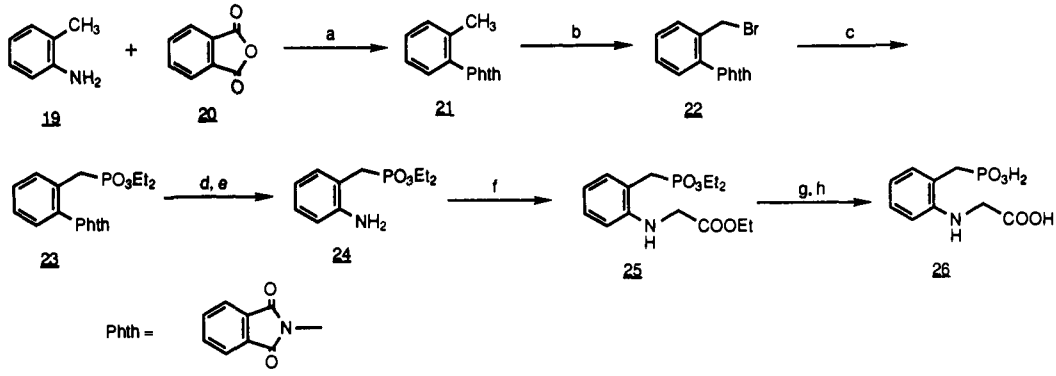
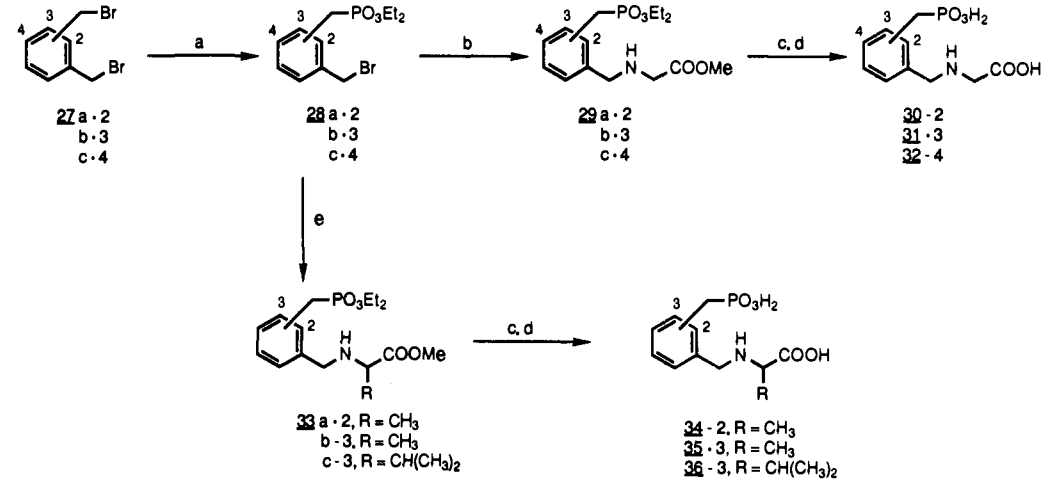
Scheme II[a]



[a] (a) NEt_3, toluene, reflux ($-H_2O$); (b) NBS, AIBN, CCl_4, *hν*; (c) $P(OEt)_3$, 100 °C; (d) $NaBH_4$, *i*-PrOH/H_2O (5:1); (e) 1 N HCl/EtOH (1:1); 75 °C; neutralize; (f) $BrCH_2COOEt$, NEt_3, DMAP (cat.), EtOH, reflux; (g) 6 N HCl, reflux; (h) Dowex 50.

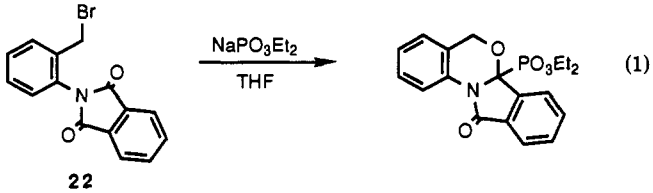
Scheme III[a]



[a] (a) $NaPO_3Et_2$, THF; (b) $HCl{\cdot}H_2NCH_2COOMe$, NEt_3, MeOH, reflux; (c) 6 N HCl, reflux; (d) Dowex 50; (e) $HCl{\cdot}H_2NCHRCOOMe$, NEt_3, MeOH, reflux.

ring.[20,21] Alternatively, in a Heck addition reaction, palladium(II) effected the coupling of the aryl bromide and either diethyl vinylphosphonate[22] or diethyl allylphosphonate to establish a two- or three-carbon alkenyl spacer. After the phosphonate and amino acid functionalities were integrated into the molecules, hydrolysis of the triesters was accomplished generally by refluxing for 20 h in 6 N HCl. To accomodate acid-sensitive substrates, some derivatives were hydrolyzed by sequential treatment with bromotrimethylsilane to remove the phosphonate esters and aqueous potassium hydroxide solution to saponify the carboxylic acid ester. Following each hydrolysis procedure, the deprotected material was neutralized and further purified on a Dowex 50 ion exchange resin if necessary.

The *o*-phosphonomethyl analogue **26** was prepared by the alternate sequence illustrated in Scheme II. The α-tolylphthalimide (**21**) was photolytically brominated with *N*-bromosuccinimide to give the benzyl bromide **22**. An



(1)

initial attempt to subtitute **22** with sodium diethyl phosphite gave predominantly (>95%) the fused tetracycle shown in eq 1, with only a trace of the desired product seen on TLC. However, an Arbuzov reaction of **22** with triethyl phosphite gave **23** quantitatively. After removal of the phthalimide protecting group,[23] the (phosphonomethyl)aniline **24** was alkylated, and subsequently hydrolyzed to give **26**.

The *N*-(phosphonomethyl)benzyl amino acids **30–32** and **34–36** were obtained (Scheme III) in a two step alkylation of the dibromoxylenes **27a–c**, followed by hydrolysis. Initial displacement of one of the bromides with sodium diethyl phosphonate gave after chromatography the pure monophosphonates **28a–c**, which were then treated with α-amino acid esters to provide the N-alkylated intermediates **29a–c** and **33a–c**.

The amidine derivative **41** was prepared as shown in Scheme IV. The key step in this synthesis was conversion of the amidate hydrochloride **39** into the glycine amidine **40**. Mild hydrolysis of the phosphonate ester with bromotrimethylsilane gave the free phosphonic acid without affecting the amidine linkage.

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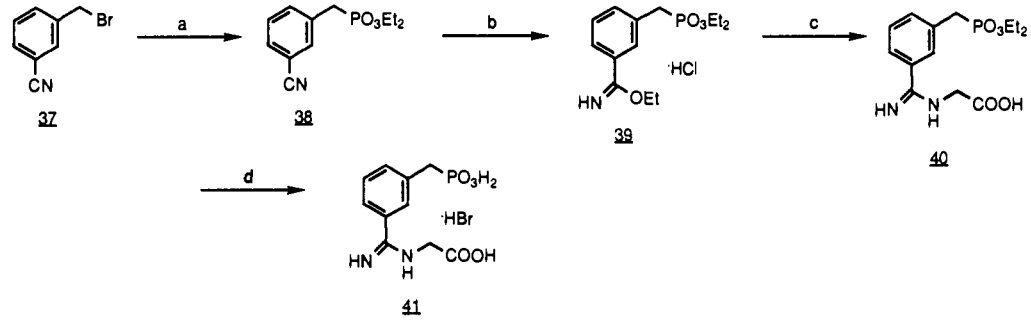
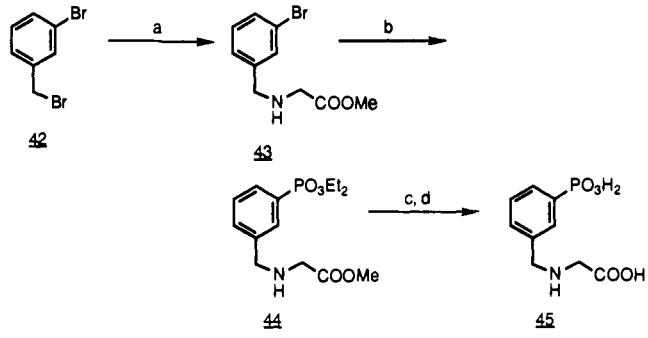
Scheme IV[a]



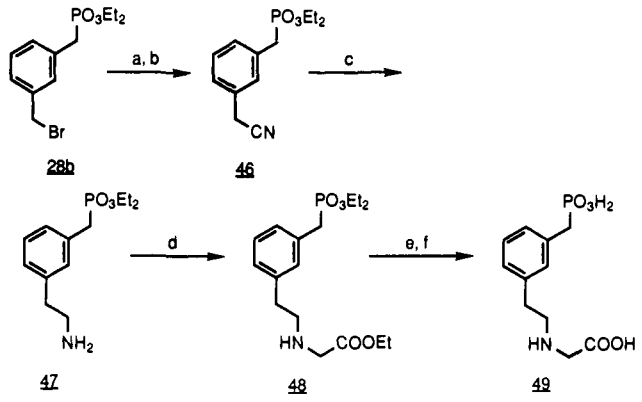
[a] (a) $NaPO_3Et_2$, THF; (b) EtOH, Et_2O, HCl (g); (c) $NaHCO_3$, glycine, EtOAc; (d) $(Me_3)_3SiBr$, CH_3CN.

Scheme V[a]



[a] (a) $HCl \cdot H_2NCH_2COOMe$, NEt_3, MeOH, reflux; (b) HPO_3Et_2, $(Ph_3P)_4Pd^0$, toluene, 105 °C; (c) 6 N HCl, reflux; (d) Dowex 50.

Scheme VI[a]



[a] (a) NaI, acetone; (b) KCN, H_2O, reflux; (c) H_2 (50 psi), Raney Ni, EtOH; (d) $BrCH_2COOEt$, NEt_3, toluene; (e) 6 N HCl, reflux; (f) Dowex 50.

The amino acid **45**, which has the phosphonate group directly bound to the aromatic ring, was prepared by coupling 3-bromobenzyl bromide with glycine methyl ester, followed by conversion into the arylphosphonate via palladium(0) chemistry (Scheme V). Derivative **49**, with the phosphonate and the amino acid groups extended from the aromatic ring by one and two methylene units, respectively, was prepared by the elaboration of **28b** into the phenethylamine **47**, followed by alkylation with ethyl bromoacetate and hydrolysis (Scheme VI).

The *N*-(phosphonoalkyl)glycine compounds **56–60** and **66** were obtained by alkylation of the phosphonoalkyl or alkenyl halide with glycine methyl ester (Schemes VII and VIII). Although 1-bromo-2-(diethoxyphosphinyl)ethane and 1-bromo-3-(diethoxyphosphinyl)propane coupled readily with glycine methyl ester in ethanol and triethylamine at reflux to give the N-alkylated products **55a** and **55b** in good yields, other unactivated phosphonoalkyl halides (or mesylates) gave low yields of N-alkylation with α-amino acids. Indeed, 1-bromo-4-(diethoxyphosphinyl)butane coupled so poorly with glycine methyl ester, that **58** was more readily prepared (Scheme VII) via reduction of the butenyl intermediate **54a**, obtained by substitution of the more reactive allylic bromide **51**. The key intermediate in the synthesis of **66** was the mesylate **64**, which was prepared from the tetrahydropyranyl protected butynol **61** by conversion into the butynylphosphonate **62**, deprotection, and mesylation of the corresponding alcohol. Partial reduction of the triple bond gave **64** in 50% yield from **62**. The unactivated mesylate was displaced with glycine methyl ester in poor yield, and the triester **65** was hydrolyzed with bromotrimethylsilane followed by potassium hydroxide to afford amino acid **66**.

The sulfide analogue **70** was prepared as shown in Scheme IX. The [(acetylthio)methyl]phosphonate **67**[24] was treated with sodium methoxide, and the resulting sulfide anion was reacted with 1,2-dibromomethane to give the monobromo intermediate **68**. Displacement of the alkyl halide with glycine methyl ester, followed by hydrolysis of the ester functionalities gave **70**. The sulfoxide derivative **71** was readily obtained by oxidation of **70** in neutral hydrogen peroxide solution.[25]

Pharmacology

Receptor Binding. Compounds were evaluated as NMDA ligands by their ability to displace [^{3}H]CPP[26,27] from rat cortical membranes. The results for the *N*-phosphonoaryl and -alkyl series of compounds are summarized in Tables I and II, respectively. In Table I, significant binding affinity for the NMDA receptor was restricted to compounds **7**, **31**, and **32**. Modest receptor affinity was observed for compounds **41** and **49**. In Table II, compounds **59** and **66** showed affinity for the NMDA receptor comparable to that of the reference receptor antagonist APH (IC_{50} = 0.8 μM). The alanine derivative **60** displayed significantly reduced affinity.

In Vitro Evaluation in Cultured Rat Cortical Neurons. For those compounds with a calculated IC_{50} value

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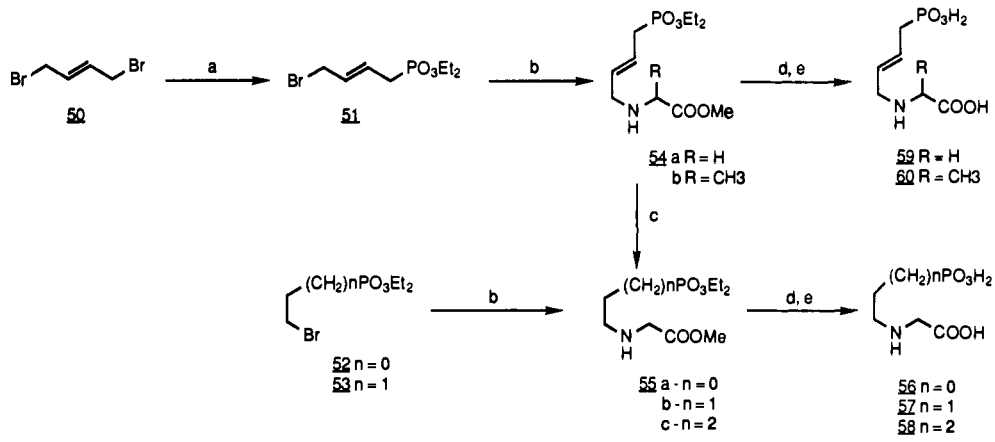
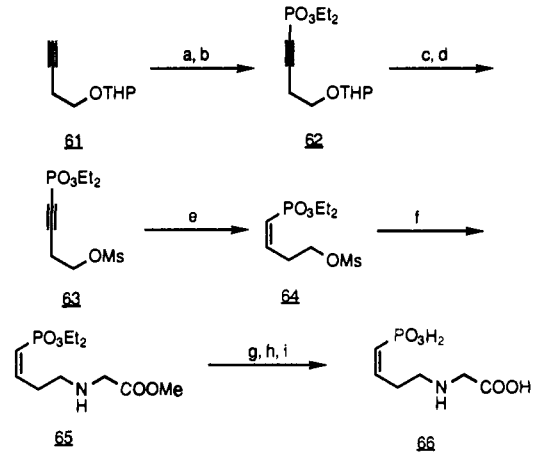
Scheme VII[a]



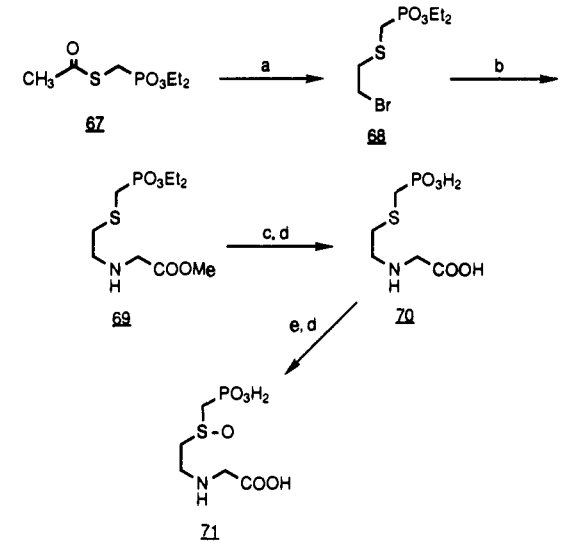
[a] (a) $P(OEt)_3$, 100 °C; (b) $HCl \cdot H_2NCHRCOOMe$, NEt_3, DMAP (cat.), EtOH, reflux; (c) H_2 (50 psi), 10% Pd/C, EtOH; (d) 6 N HCl, reflux; (e) Dowex 50.

Scheme VIII[a]



[a] (a) *n*-BuLi, THF, −78 °C; (b) $ClPO_3Et_2$; (c) TsOH, MeOH; (d) CH_3SO_2Cl, NEt_3, CH_2Cl_2; (e) H_2 (50 psi), 10% Pd/$BaSO_4$, THF/pyridine (9:1); (f) $HCl \cdot H_2NCH_2COOMe$, NEt_3, DMAP (cat.), DMSO, 60 °C; (g) TMSBr, CH_3CN; (h) KOH (aq); (i) Dowex 50.

Scheme IX[a]



[a] (a) MeONa, MeOH, $BrCH_2CH_2Br$; (b) $HCl \cdot H_2NCH_2COOMe$, K_2CO_3, DMF, 60 °C; (c) 6 N HCl, reflux; (d) Dowex 50; (e) 30% H_2O_2, H_2O.

in NMDA receptor binding, an antagonist action was demonstrated by examining their ability to inhibit glutamate-induced accumulation of [$^{45}Ca^{2+}$] in cultured rat cortical neurons.[28] NMDA receptor binding and NMDA antagonist effect were significantly correlated (Figure 1).

Discussion

The compounds described in this paper were synthesized to further explore the structural requirements of the NMDA receptor binding site for competitive antagonists. The N-substituted analogues were viewed as excellent vehicles that would allow the introduction of conformational constraints and added steric bulk in unique regions.

Previous studies[8-16] have demonstrated that high receptor affinity was dictated by a proper juxtaposition of the key NH_2, COOH, and PO_3H_2 functional groups. A pharmacophore model for competitive NMDA antagonists, described in a companion paper,[1] suggests that strong binding to the NMDA receptor requires the carboxylate and phosphonic acid moieties to be directed to the same side of the molecule. This implies that antagonists such as APV (1) and APH (2) bind to the NMDA receptor in a folded conformation that allows the α-carboxylic acid and phosphonic acid moieties to be in close proximity to one another; the active conformation of 4 also places the car-

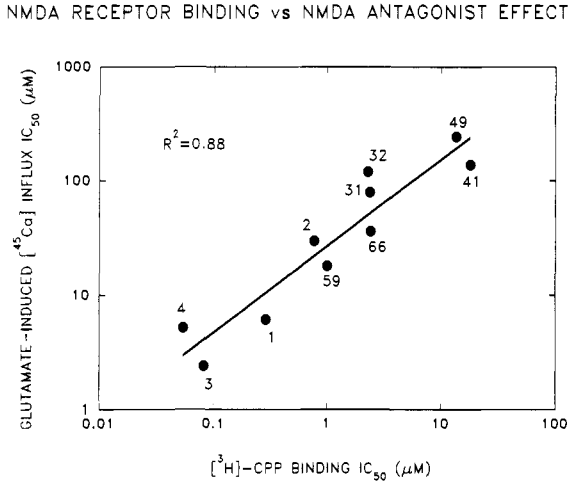
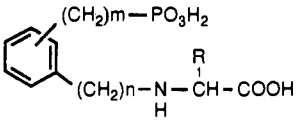


Figure 1. Correlation between calculated IC_{50} values in the receptor binding and cell culture assays. The relevant equation is log (IC_{50}, cell culture assay) = 0.71 (0.10) log (IC_{50}, receptor binding) + 1.4 [n = 10, r^2 = 0.87, F = 55, s = 0.24].

(28) Marcoux, F. W.; Goodrich, J. E.; Probert, A. W., Jr.; Dominick, M. A. In *Sigma and Phencyclidine-like Compounds as Molecular Probes in Biology*; Domino, E. F., Kamenka, J.-M., Eds.; NPP Books: Ann Arbor, MI, 1988; pp 735–746.

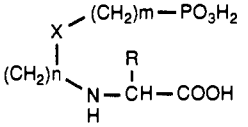
Table I. Inhibition of [^{3}H]CPP NMDA Receptor Binding and Glutamate-Induced Calcium Influx in Cultured Neuronal Cells by *N*-(Phosphonoalkyl)phenyl-Spaced α-Amino Acids



no.	subst	*n*	*m*	R	[^{3}H]CPP binding %I[a]	[^{3}H]CPP binding IC_{50},[b] μM	glutamate-induced calcium influx: IC_{50}, μM
APV (1						0.29 ± 0.048	6.1
APH (2)						0.77 ± 0.24	30
7	o	0	0 (COOH)[c]	H		4.0 ± 1.22	>250
11	o	0	0	H	27		
26	o	0	1	H	48		
16	o	0	2	H	0		
13	o	0	2 (*E*)[d]	H	9		
30	o	1	1	H	21		
34	o	1	1	CH_3	4		
17	*m*	0	2	H	0		
14	*m*	0	3 (*E*)[d]	H	13		
18	*m*	0	3	H	5		
45	*m*	1	0	H	21		
31	*m*	1	1	H		2.4 ± 0.19	40
41	*m* (=NH)[e]	1	1	H		18.0 ± 1.38	137
35	*m*	1	1	CH_3	31		
36	*m*	1	1	$CH(CH_3)_2$	0		
49	*m*	2	1	H		13.7 ± 1.35	144
32	*p*	1	1	H		2.3 ± 0.11	59

[a] % Inhibition values were determined at 10^{-4} and 10^{-5} M and are reported here for 10^{-4} M. [b] IC_{50} values were obtained for those compounds that displayed >50% inhibition at 10^{-5} M. [c] The phosphonate group is replaced by COOH. [d] *E* (entgegen) refers to the geometry of the double bond. [e] For the structure of 41, see Scheme IV.

Table II. Inhibition of [^{3}H]CPP NMDA Receptor Binding and Glutamate-Induced Calcium Influx in Cultured Neuronal Cells by *N*-(Phosphonoalkyl) α-Amino Acids



no.	*n*	*m*	X	R	[^{3}H]CPP binding %I[a]	[^{3}H]CPP binding IC_{50},[b] μM	glutamate-induced calcium influx: IC_{50}, μM
APV (1)						0.29 ± 0.048	6.1
APH (2)						0.77 ± 0.24	30
CPP (3)						0.079 ± 0.007	2.4
CGS 19755 (4)						0.065 ± 0.010	5.2
56	2	0	-	H	14		
57	3	0	-	H	3		
58	4	0	-	H	17		
59	1	1	CH=CH (*E*)[c]	H		1.0 ± 0.05	16.7
60	1	1	CH=CH (*E*)[c]	CH_3		42.4 ± 2.61	
66	2	0	CH=CH (*Z*,*E*)[c,d]	H		2.25 ± 0.12	36
70	2	1	S	H	60		
71	2	1	S(O)	H	4		

[a] % Inhibition values were determined at 10^{-4} and 10^{-5} M and are reported here for 10^{-4} M. [b] In general, IC_{50} values were obtained for those compounds that displayed >50% inhibition at 10^{-5} M. [c] *E* (entgegen) and *Z* (zuzammen) refer to the geometry of the double bond. [d] (*Z*,*E*) refers to a mixture of the *Z* and *E* geometric isomers.

boxylic and phosphonic acids in a syn orientation (Figure 2). A similar conclusion has been reached previously.[15]

When the NH and the α-COOH groups of the present series of molecules are fit to the pharmacophore model, the *N*-phosphonoalkyl moiety must have sufficient length and flexibility to allow the phosphonic acid group to be stretched across the molecule and fit the proposed distal acidic binding site that is in close proximity to the α-carboxylic acid. This process generally introduces additional volume relative to the α-carbon substituted analogues **1** and **2**, and additional flexible bonds relative to the cyclic analogue **4**, which effectively increases the entropy of these systems. For example, low energy conformers (Table III) of the *m*- and *p*-benzyl-substituted glycine derivatives **31** and **32** can be fit to the pharmacophore model, but both occupy additional areas of volume relative to the 10–80-fold more potent compounds **1**, **2**, and **4** (Figure 3).

A further reduction in potency, seen with the higher homologue **49** (Table I, *m* = 2, IC_{50} = 13.6 μM, versus 2.4 μM for **31**), can be rationalized either by increased entropy (an additional freely rotatable bond has been introduced), or additional volume consumption when it is fit to the pharmacophore model. Placing α-methyl and α-isopropyl groups on **31** resulted in compounds with markedly reduced and no affinity, respectively, for the NMDA receptor

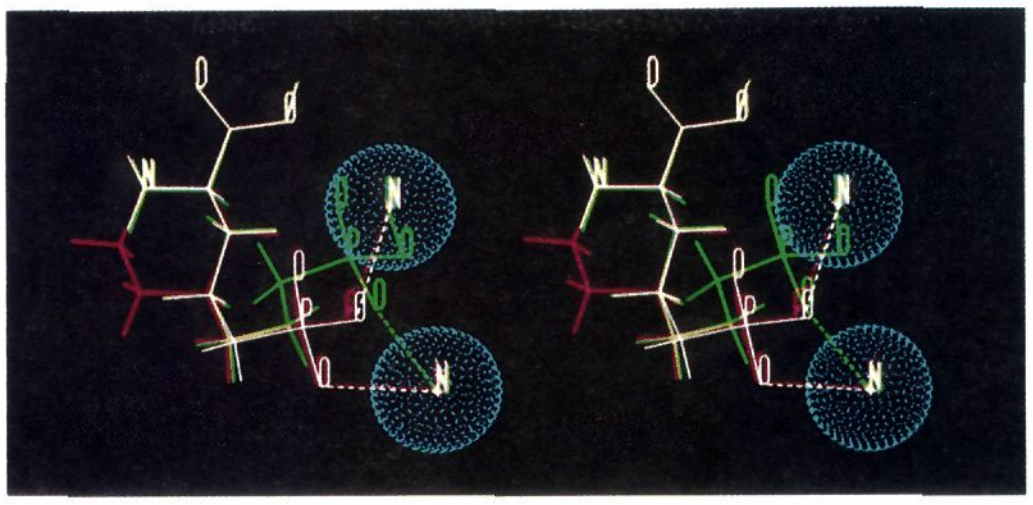


Figure 2. Stereoview of the proposed bioactive conformations of the reference antagonists D-APV (**1**, white), D-APH (**2**, green), and CGS 19755 (**4**, red). Receptor interaction points from the pharmacophore model[1] (nitrogen atoms) are included, and are surrounded by blue dots.

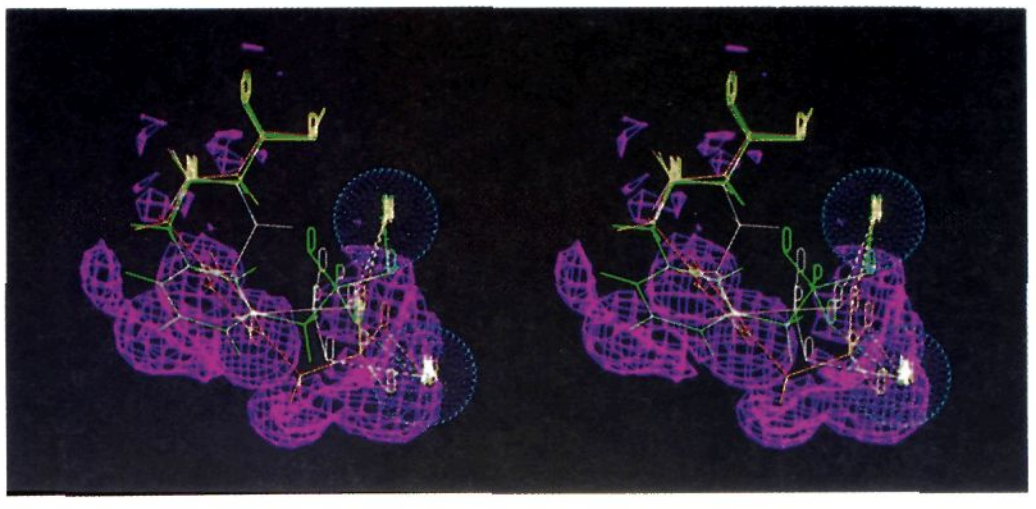


Figure 3. Stereoview of the superposition of **31** (green) and **32** (yellow) onto **1** (white), with the receptor interaction points (blue dots) and the composite additional volume occupation (in purple) of **31** and **32** over that occupied by the potent antagonists depicted in Table III of ref 1.

Table III. Fitting Energies[a] for Selected *N*-Phosphonoalkyl-, *N*-Phosphonoalkenyl-, and *N*-(Phosphonoalkyl)phenyl-Spaced α-Amino Acids

		energies, kcal/mol			
no.	RMS[b]	spring[c]	total[d]	relaxed[e]	cost[f]
1 (APV)[g]	0.04	0.2	−0.8	−1.4	0.3
2 (APH)[g]	0.03	0.1	−0.8	−1.7	0.7
3 (CPP)[g]	0.04	0.1	−0.5	−1.1	0.5
4 (CGS 19755)[g]	0.05	0.3	−2.7	−3.3	0.3
7[h]	0.1	2.1	18.5	8.3	8.0
30	0.1	1.4	2.7	−0.1	1.4
31	0.1	1.0	3.3	1.4	1.0
32[g]	0.1	1.2	2.0	0.1	0.6
35	0.1	2.1	10.6	4.2	4.3
36	0.1	2.2	10.4	2.7	5.6
41	0.09	1.0	9.4	7.6	0.8
45	0.06	0.4	8.2	5.6	2.2
49	0.1	1.4	1.0	−2.0	1.6
57	0.2	2.2	12.4	3.6	6.6
58	0.06	0.5	5.7	3.0	2.2
59[g]	0.05	0.3	1.9	0.8	0.7
60	0.1	1.4	7.0	1.4	4.3

[a] Compounds were either used in the formation of, or fit to, a reference compound (**4**) from the competitive antagonist pharmacophore model described in ref 1. [b] Deviation, in Å, between fit atoms. Higher values reflect a less precise fit. [c] Spring energy between fit atoms. *High values reflect a less precise fit. See ref 35, p* 142. [d] Includes the spring energy contribution. [e] No fitting constraints applied. [f] Total−spring−relaxed energy; the difference in energy between fit and relaxed versions. [g] Used in the formation of the competitive antagonist pharmacophore model; ref 1. [h] Fit to only one (the primary[1]) receptor interaction point.

(Table I, **35** and **36**). The α-alkyl groups act as steric buffers, causing an increase in energy cost to fit the pharmacophore (Table II: compare the energy costs in fitting **31**, **35**, and **36**), as well as consuming excluded volume in a region critical for receptor interactions (Figure 4).

In the series that we have examined, compounds with phosphonates directly bonded to aryl moieties generally display low NMDA receptor affinities.[16] Consistent with our earlier findings, **11** and **45** show low affinity for the NMDA receptor when compared to their higher homologues, **26** and **31**, respectively. This may be due to alterations in the pK_a of the phosphonate, direct steric intolerance of the phenyl group, or in the case of **11**, the phenyl ring may be preventing the phosphonate from assuming the appropriate geometry for strong receptor interactions. In general, analogues containing *o*-phenyl spacers (**11**, **13**, **16**, **26**, **30**, and **34**) displayed low receptor affinities. When examined in the pharmacophore model, steric and/or conformational constraints prevented the PO_3H_2 of these analogues from optimally interacting with the distal acidic binding sites. Derivatives with a phenyl directly attached to the amine (**11**, **13**, **14**, and **16**–**18**) also were less potent. Possible reasons include reduced basicity of the resulting anilines, or steric and conformational effects of the phenyl ring.

Although not a phosphonic acid derivative, the commercially available carboxylic acid **7** was tested because of its similarity to our synthetic targets. We were surprised by its moderate NMDA receptor affinity since it has a rather high energy cost in fitting to the pharmacophore model (and can fit only one of the two receptor interaction sites), and the phosphonic acid analogue **11** had no affinity. Because carboxylic acid derivatives generally display weaker NMDA antagonist activity than the corresponding phosphonate, **7** was not expected to display antagonist properties, and indeed, results showed that **7** does not

Figure 4. Stereoview of the superposition of **31** (green) onto **1** (white) in their proposed bioactive conformations, with the receptor interaction points added (blue dots). Addition of an α-*i*-Pr to give **36** (orange) generates a steric contact that results in an increase in energy cost to fit the pharmacophore model (Table III), additional volume occupation (red), and an altered location and conformation of the PO_3H_2 moiety.

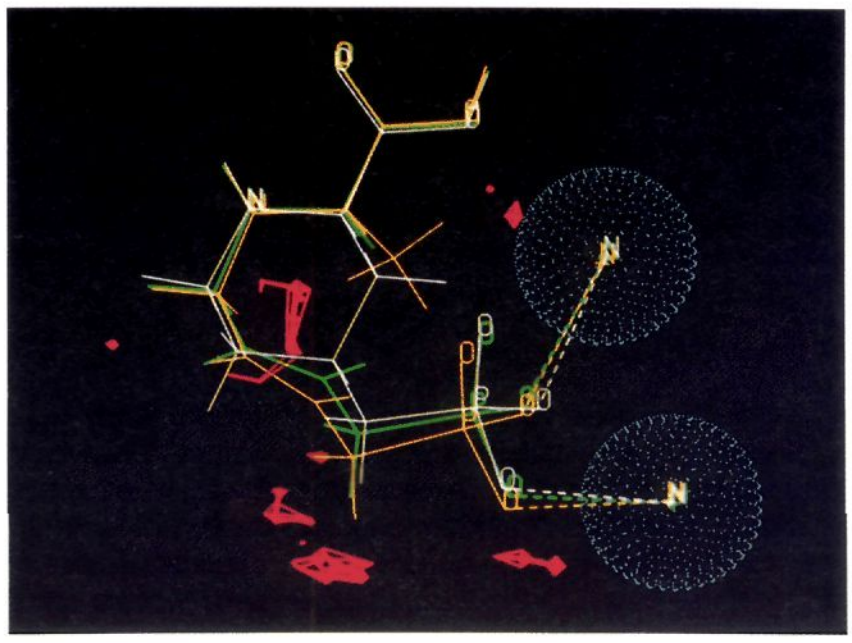


Figure 5. Superposition of the proposed bioactive conformations of **4** (white) and **59** (green) with the receptor interaction points added. Similar to the phenyl-spaced analogues, addition of an α-Me to give **60** (orange) generates a steric contact that results in an increase in energy cost to fit the pharmacophore and a minor amount of exclude volume (red).

reduce [$^{45}Ca^{2+}$] accumulation or glutamate toxicity in the cell culture assay (Table I). Because of its structural resemblance to aspartate and glutamate (compact structure, distal COOH instead of PO_3H_2), **7** was also tested for agonist properties. However, when examined in the absence of glutamate in the cell culture assay at concentrations ranging from 0.24–63 μM, no agonist-like activity could be detected.

The amidine derivative **41** was prepared to determine whether an increase in electron density at the amino moiety would lead to an enhancement in the NMDA receptor interaction. The observed reduction in receptor affinity is probably due to an adverse electronic and/or steric effect of the amidine NH.

Among aliphatic *N*-alkylphosphonate derivatives, NMDA antagonist activity was observed only for the butenyl derivatives **59** and **66** (Table II). The corresponding reduced derivative **58** displayed no affinity, as did the shorter chain derivatives **56** and **57**. These observations are consistent in part with entropy considerations. Although **59** can exist in a low energy conformation that superimposes closely onto the reference compound **4** (Figure 5), the entropy of this analogue would be expected to be higher due to an increase in freely rotating bonds (four between the NH and PO_3H_2 moieties versus two for **4**). Saturating the double bond to give **58** further increased the entropy and led to a greater reduction in potency. The shorter chain derivatives may be less potent because they cannot project their phosphonate moiety far enough into the acidic binding site or in the proper direction for optimal binding. Similar to **35** and **36** in the phenyl-spaced series above, the loss in affinity of **60** may be due to excluded volume consumption by the α-methyl group and/or an increase in energy cost to fit the pharmacophore due to a steric interaction between the α-methyl and a vinyl hydrogen atom (Figure 5).

Conclusion

Similar to other literature reports,[8–16] the SAR surrounding the *N*-phosphonoalkyl-, -alkenyl-, and -arylglycines reported herein illustrates that the requirements for high affinity at the NMDA receptor are very strict. Small structural changes frequently resulted in a transition from high to low or no affinity. The potent compounds **31**, **32**, **59**, and **66** are consistent with a hypothesis that the two acidic functionalities in common to competitive NMDA antagonists must exist in close proximity to one another. This implies that antagonists such as APV and APH exist in a "folded" conformation and that the active conformation of the rigid analog **4** is one in which the carboxylic and the phosphonic acids are situated on the same side of the molecule (syn).[15] These antagonists have also proven useful in the formation and validation of an NMDA competitive antagonist pharmacophore model.[1]

Experimental Section

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] IC_{50} values were obtaned in triplicate.

Materials. [^{3}H]CPP (specific activity 27.0 Ci/mmol) was purchased from New England Nuclear Corp. Tris(hydroxymethyl)aminomethane was purchased from Calbiochem-Behring; Triton X-100, L-glutamic acid, DL-2-amino-5-phosphonovaleric acid (APV), (+/−)-2-amino-4-phosphonobutanoic acid (AP4), and quisqualate were purchased from Sigma Chemical Co.; CPP was synthesized by the Stroke Chemistry Group (Parke-Davis).[29,30]

(29) Bigge, C. F.; Hays, S. J.; Novak, P. M.; Drummond, J. T.; Johnson, G.; Bobovski, T. P. New Preparations of the N-Methyl-D-Aspartate Receptor Antagonist, 4-(3-Phosphonopropyl)-2-piperazinecarboxylic Acid (CPP). *Tetrahedron Lett.* **1989**, *30*, 5193–5196.

(30) Hays, S. J.; Bigge, C. F.; Novak, P. M.; Drummond, J. T.; Bobovski, T. P.; Rice, M. J.; Johnson, G.; Brahce, L. J.; Coughenour, L. L. New and Versatile Approaches to the Synthesis of CPP-related Competitive NMDA Antagonists. Preliminary Structure-Activity Relationships and Pharmacological Evaluation. *J. Med. Chem.* **1990**, *33*, 2916–2924.

Membrane Preparation. Long-Evans male rats (180–200 g) were sacrificed by decapitation, and the whole brain (minus cerebellum and brainstem) was removed, placed in 15 volumes of ice-cold 0.32 M sucrose and homogenized in a Potter–Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 1000*g* for 10 min at 4 °C; the supernatant was saved and recentrifuged at 20000*g* for 20 min. The pellet (P2) was disrupted in ice-cold water for 30 s with a polytron (Brinkman PT-10) homogenizer and centrifuged at 8000*g* for 20 min. The final pellet was resuspended in ice-cold water, recentrifuged at 48000*g* for 20 min, and finally frozen at −70 °C until used in the assay.

Assay Conditions. On the day of the assay, the crude synaptic membranes (CSMs) were routinely treated with 0.01% Triton X-100 at 37 °C for 30 min to remove endogenous glutamate. The suspension was then washed three times (centrifugation at 48000*g* for 10 min, followed by homogenization in ice-cold 50 mM Tris-HCl, pH 7.6). The membranes (200–400 μg of protein/mL) were incubated with 10 nM [^{3}H]CPP and various concentrations of test agents in a total volume of 1.0 mL of 50 mM Tris-HCl, pH 7.6. The order of additions was test compound (100 μL), [^{3}H]CPP (100 μL), and then membranes (800 μL). All incubations were done in triplicate for 15 min at 23 °C in plastic scintillation minivials (Beckman Instruments). The incubations were terminated by centrifugation at 48000*g* for 10 min at 4 °C, after which the vials were immediately placed on ice. The pellets were rapidly washed twice with 3-mL aliquots of ice-cold 50 mM Tris-HCl, pH 7.6. Any remaining liquid on the inside of the vials was removed with cotton-tipped applicators. Beckman HP Readi-Solv scintillation cocktail (5 mL) was added to the pellets; the samples were extracted overnight, which dissolves the pellet, and then shaken on a mechanical shaker for 1 h. The sample radioactivity was determined by liquid-scintillation spectrophotometry. Nonspecific binding was defined as the binding in the presence of 1 mM L-glutamate. A typical assay yielded 75–80% specific binding.

Data Analysis. Specific binding was defined as total binding minus nonspecific binding. Results were reported as percent inhibition of control (specific binding without test agent), shown in eq 2, where Y is the percent inhibition, T is the specific binding

$$Y = [(T - S)/T] \times 100 \quad (2)$$

without test agent, and S is the specific binding in the presence of test agent. The concentration of test agents which inhibited 50% of the specific binding (IC_{50}) was determined from four or more concentrations of test agents by a nonlinear least-squares curve-fitting program.[31]

Cell Culture Technique. Sprague-Dawley rats in their 18th day of gestation were placed under halothane anesthesia during uterus removal. The uterus was place in a bath of chilled calcium and magnesium-free Hanks' balanced salt solution buffered with 15 mM HEPES (HBSS). Cerebral hemispheres were isolated from each fetus while under constant bathing in chilled HBSS. Hemispheres were minced and exposed to 15 min of porcine trypsin digestion (0.25%) followed by rapid trituration in 5 mL of HBSS. Cells were adjusted to a final concentration of 640 000 cells/mL using a 1:1 Dulbecco's modified Eagle's/Ham's nutrient mixture F-12 medium supplemented with 5 mg/L bovine insulin, 50 mg/L human transferrin, 16 mg/L putrescine hydrochloride, and 3 g/L D-glucose (DME/F12) and containing 10% horse and 6% fetal calf serum. The medium was buffered with 30 mM sodium bicarbonate yielding a pH of 7.4 (all culturing reagents were purchased from Sigma).

Ninety-six-well tissue-culture plates (0.32 cm^2/well) were coated with poly-L-lysine (30–70 K) one day to cell plating. Following triple washing with HBSS to remove excess poly-L-lysine, the wells were incubated overnight with 100 μL of DME/F12 medium containing 10% horse and 6% fetal calf serum.

A 100-μL aliquot of the freshly dissociated neurons was added to each well yielding a final concentration of 200 000 cells/cm^2. Cells were then placed in a humidified, 37 °C, 2.5% CO_2 atmosphere. One day following cell isolation, 100 μL of medium was removed from each well and replaced with fresh DME/F12 containing 10% horse serum. All further medium manipulation prior to experimentation used this recipe. Cell division was halted four days following isolation by the addition of 15 μg/mL 5-fluoro-2′-deoxyuridine and 35 μg/mL uridine for a period of two days. Additional feedings were performed when deemed necessary.

Cell Culture Experimental. Experiments were performed on cells on their 14th to 16th day after plating. Basic medium for all experiments was a serum and magnesium-free HBSS (Sigma) containing 1.4 mM $CaCl_2$. A final pH of 7.4 was maintained with 35 mM sodium bicarbonate as the buffering agent. All experiments were performed in a humidified, 37 °C, 5% CO_2 atmosphere.

Thirty minutes prior to glutamate exposure, maintainance medium was replaced with 50 μL of calcium-containing HBSS. Cultures were exposed to glutamate by the addition of 50 μL of 200 μM L-glutamic acid (100 μM final concentration) in the presence or absence of test agents. A trace amount of [$^{45}Ca^{2+}$] (2 μCi/mL) was added to the exposure medium to estimate calcium accumulation intracellularly [Ca^{2+}_i]. In all cases, cells were subjected to a 30-min exposure followed by a triple washing with 0.9% saline solution. Cells were lysed with distilled water and individual well lysates were counted for β-emissions. Baseline [Ca^{2+}_i] accumulation was estimated in cultures incubated for 30 min with [$^{45}Ca^{2+}$]-containing HBSS without glutamate. Control [Ca^{2+}_i] accumulation was estimated in cultures incubated for 30 min with [$^{45}Ca^{2+}$]-containing HBSS and glutamate. Glutamate-induced [Ca^{2+}_i] accumulation was calculated by subtracting baseline from control measurements. Previous studies have demonstrated that glutamate-induced toxicity to neurons in related paradigms is blocked specifically by NMDA antagonists.[32] In this study, putative NMDA antagonists were assessed as inhibitors of glutamate-induced [Ca^{2+}_i] accumulation by adding them to the control exposure medium in increasing concentrations. IC_{50} concentrations were determined from concentration–inhibition curves constructed from at least four concentrations at half-log intervals with at least six replicate experiments per concentration.

Molecular Modeling. Molecules were built using the SYBYL software package,[33] starting from a compendium of average X-ray structures available within the program,[34] and minimized using the MAXIMIN[35] procedure (phenyl ring carbon atoms aggregated; electrostatics considered, using CNDO/2 charges calculated within the CHEMLAB-II program;[36] all other defaults taken). All structures were oriented with the basic amine at the origin, the α-methylene along the positive x-axis, and the COOH carbon in the positive x–y quadrant. The structures were either used in the formation of a competitive antagonist pharmacophore model[1] (**32** and **59**) or were fit to a reference structure (**4**) from the model. Fitting was accomplished using the MULTIFIT procedure[35] in SYBYL. Each molecule was first prepared by adding a lone pair (LP) to the basic amine, defining a 2-Å tensor normal to the plane of the α-COOH, passing through the OH oxygen, and defining two hypothetical receptor interaction points (N.3 atoms were used) that were attached to each OH oxygen of the distal PO_3H_2 (replacing the hydrogen atom), 2.8 Å from the oxygen atoms, with a P–O–N valence angle of 125°. These distance and angle values were determined by searching crystal databases for PO_3H_2 hydrogen bonding interactions; see the companion article[1] for details. To maintain the receptor interaction points at an appropriate hydrogen-bonding distance from the PO_3H_2 oxygens but provide

(31) Parker, R. B.; Waud, D. R. Pharmacological Estimation of Drug-Receptor Dissociation Constants. Statistical Evaluatiofn. I. Agonists *J. Pharmacol. Exp. Ther.* **1973**, *183*, 1–12.

(32) Choi, D. W.; Koh, J.-Y.; Peters, S. Pharmacology of Glutamate Neurotoxicity in Cortical Cell Culture: Attenuation by NMDA Antagonists. *J. Neurosci.* **1988**, *8*, 185–96.

(33) Commercially available from Tripos Associates, 1699 South Hanley Road, St. Louis, MO 63144. Version 3.5, operating on a VAX 11/785, was used for the calculations. Version 5.3.2 was used to generate the figures.

(34) SYBYL Standard Fragment Library; Tripos Associates, Inc.

(35) Labanowski, J.; Motoc, I.; Naylor, C. B.; Mayer, D.; Dammkoehler, R. A. Three-Dimensional Quantitative Structure-Activity Relationships. Conformational Mimicry and Topographical Similarity of Flexible Molecules. *Quant. Struct.-Act. Relat.* **1986**, *5*, 138–52.

for a certain amount of flexibility in fitting, an equilibrium bond length of 2.8 Å and a stretching constant of 600 kcal/mol was specified for the O–N bonds for the fitting and subsequent minimizations. For the fitting analyses and subsequent minimizations, the N-LP, CO_2 + tensor atoms, and the phenyl ring carbons were separately aggregated. The atoms used to fit the structures included the basic nitrogen, its LP, the endpoints of the tensor associated with the α-COOH, and the two receptor interaction points. Spring constants at 20 kcal/mol were used on all fitting atoms (*SYBYL* default), except for the secondary receptor interaction point. The spring constant for this atom was reduced to 10 kcal/mol to reflect the somewhat reduced importance of interaction with this site for maintenance of high affinity to the NMDA receptor. The energies of the fit and relaxed versions are given in Table III; the fit structures of two of the active analogues (**32** and **59**) are included in the supplementary material in SYBYL.MOL2 file format.

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet MX-1 FT spectrophotometer, but are not reported. The ^{1}H-NMR spectra were recorded on an IBM WP100SY NMR spectrometer (100 MHz) or a Varian XL200 NMR spectrometer (200 MHz) and were consistent with the proposed structures. The peaks are described in parts per million (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. ^{1}H-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.

A. General Procedure for the Acid Hydrolysis of Triesters to Triacids. *N*-[[3-(Phosphonomethyl)phenyl]methyl]glycine Hydrochloride (31). A solution of **29b** (330 mg, 1 mmol) in 6 N HCl (50 mL) was refluxed for 20 h. The solution was concentrated, and the residue was dried in vacuo to give a nonhygroscopic white solid (0.29 g, 95%): ^{1}H NMR (D_2O) 7.47–7.41 (m, 4 H), 4.31 (s, 2 H), 3.95 (s, 2 H), 3.21 (d, 2 H); MS (FAB) *m/e* 260 (MH^+). Anal. ($C_{10}H_{14}NO_5P\cdot HCl\cdot 0.5H_2O$) C, H, N, Cl.

B. General Procedure for the Deprotection of Acid-Sensitive Triesters to Triacids. (*E*)- and (*Z*)-*N*-(4-Phosphono-3-butenyl)glycine Diammonium Salt (66). A solution of **65** (0.3 g, 1 mmol) in anhydrous acetonitrile (25 mL) under a nitrogen atmosphere was treated with bromotrimethylsilane (4 mL) and stirred for 15 h. The solvent was removed in vacuo, the residue was dissolved in an aqueous solution of potassium hydroxide (4 mmol) in water (2 mL), and the mixture was stirred for 22 h. (Potassium trimethylsilanolate in refluxing tetrahydrofuran could be used instead of potassium hydroxide to hydrolyze the carboxylate esters.) The solution was neutralized to pH 5 and was purified on Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants). The ninhydrin-positive fractions were lyophilized and the tan solid (0.13 g, 53%) dried in vacuo: ^{1}H NMR (D_2O) 6.04–5.80 (m, 2 H), 3.56 (s, 2 H), 3.20 (t, 2 H), 2.90–2.75 (m, 2 H); MS (FAB) *m/e* 208 (MH^+). Anal. ($C_6H_{12}NO_5P\cdot 2NH_3$) C, H, N.

C. General Procedure for N-Alkylation of Glycine Methyl Ester. Methyl *N*-[[2-[(Diethylphosphinyl)methyl]phenyl]methyl]glycinate (29a). A solution of **28a** (0.9 g, 2.8 mmol) in methanol (25 mL) and triethylamine (5 mL) was treated with glycine methyl ester hydrochloride (0.71 g, 5.6 mmol) and was heated at reflux in an oil bath for 19 h. The solvent was removed in vacuo, and the residue was dissolved in water and extracted with chloroform. The chloroform layer was backwashed with water and then dried over magnesium sulfate, filtered, and evaporated. The oily residue was purified via silica gel chromatography (2–4% methanol in ethyl acetate as eluant) to give an oil (0.76 g, 82%): ^{1}H NMR ($CDCl_3$) 7.35–7.2 (m, 4 H), 4.08–3.85 (m, 4 H), 3.88 (s, 2 H), 3.74 (s, 3 H), 3.46 (s, 2 H), 3.40 (d, 2 H), 1.97 (br s, 1 H), 1.27–1.14 (m, 6 H); MS (EI) *m/e* 330 (M + 1, 100).

D. General Procedure for N-Alkylation of Ethyl Bromoacetate. Ethyl *N*-(3-Bromophenyl)glycinate (9b). A solution of *m*-bromoaniline (10 g, 58 mmol), ethyl bromoacetate (10 g, 60 mmol), triethylamine (5 mL), and (dimethylamino)pyridine (0.2 g) in ethanol (50 mL) was refluxed overnight. The solution was cooled in an ice bath, and the crystals which formed were collected by filtration and washed with methanol to give **9b** (8 g, 53%). Anal. ($C_{10}H_{12}BrNO_2$) C, H, N, Br.

E. General Procedure for the Preparation of (Phosphonomethyl)benzyl Bromides 28a–c. A suspension of sodium hydride (60% in oil, 1.4 g, 35 mmol) in anhydrous tetrahydrofuran (75 mL) was stirred under a nitrogen atmosphere and a solution of diethyl phosphite (4.82 g, 34.9 mmol) in tetrahydrofuran (20 mL) was added dropwise. The reaction mixture stirred an additional 20 min. A solution of *o*-dibromoxylene (or meta or para derivatives, 10.56 g, 40 mmol) in anhydrous tetrahydrofuran (150 mL) was treated dropwise with the solution of sodium diethyl phosphite generated above. The reaction mixture was stirred 6 h at room temperature and then quenched with water and diluted with diethyl ether. The ether layer was separated, washed with water (2 × 100 mL), and dried over magnesium sulfate. After filtration and evaporation, the residue was chromatographed on silica gel (ethyl acetate as eluant) to give a clear oil (1.8 g, 18%). Yields were generally between 20 and 25%.

Ethyl *N*-(2-Bromophenyl)glycinate (9a). *o*-Bromoaniline (10 g, 58.1 mmol) was used to prepare **9a** (6.3 g, 22%) according to general procedure D.

Ethyl *N*-[2-(Diethoxyphosphinyl)phenyl]glycinate (10). A solution of diethyl phosphite (10 mL), triethylamine (5.5 mL), and tetrakis(triphenylphosphine)palladium(0) (0.74 g) was stirred under nitrogen. A solution of **9a** (4.3 g, 16.7 mmol) in toluene (40 mL) was degassed in vacuo and added to the reaction mixture. After heating at 115–125 °C for 16 h, the reaction mixture was cooled and diluted with diethyl ether. An insoluble precipitate was removed by filtration and the filtrate evaporated. The residue was purified by silica gel column chromatography (0–1% methanol in chloroform as eluant) to give recovered starting material (3.5 g, 81%) and the product as a white solid (60 mg, 1%), mp 77–78 °C.

***N*-(2-Phosphonophenyl)glycine Diammonium Salt (11).** Compound **10** (40 mg, 0.13 mmol) was hydrolyzed according to general procedure A. The residue was purified on Dowex 50 × 4 400 ion exchange resin (water, then 2 N ammonium hydroxide as eluants). Lyophilization of the ninhydrin-positive fraction gave a creamy white powder (20 mg, 57%): ^{1}H NMR (D_2O) 7.36 (dd, 2 H), 6.64 (dd, 2 H), 3.70 (s, 2 H); MS (FAB) *m/e* 232 (MH^+).

Ethyl (*E*)-*N*-[2-[2-(Diethoxyphosphinyl)ethenyl]phenyl]glycinate (12a). A solution of diethyl vinylphosphonate (5.4 mL, 35 mmol), **9a** (4.83 g, 18.7 mmol), and bis(triphenylphosphine)palladium(II) acetate (1 g) in dimethylformamide (15 mL) and triethylamine (5 mL) was heated in an oil bath at 90–100 °C for 20 h. The solvent was removed in vacuo, and the residue was suspended in water and extracted with chloroform (2 × 100 mL). The chloroform layer was washed with saturated sodium chloride solution, dried over magnesium sulfate, filtered, and evaporated. The residue was purified over silica gel (step gradient from 1:1 to 4:1 ethyl acetate and pentane) to give a yellow oil (4.2 g, 66%): MS (CI) *m/e* 342 (MH^+).

Ethyl (*E*)-*N*-[3-[2-(Diethoxyphosphinyl)ethenyl]phenyl]glycinate (12b). A solution of **9b** (4 g, 15.5 mmol), diethyl vinylphosphonate (4.92 g, 30 mmol), and bis(triphenylphosphine)palladium(II) chloride (1 g) in dimethylformamide (15 mL) and triethylamine (5 mL) was heated in an oil bath at 95 °C and stirred for 20 h. After cooling the dimethylformamide was removed in vacuo and the oily brown residue was dissolved

(36) (a) Pearlstein, R. A. *CHEMLAB-II Reference Manual*; Chemlab Inc. (software available from Molecular Design Limited, San Leandro, CA). (b) Childress, T., Ed. Revised MDL Edition, March, 1985; Molecular Design Limited, San Leandro, CA. (c) Potenzone, R., Jr.; Cavicchi, E.; Weintraub, H. J. R.; Hopfinger, A. J. Molecular Mechanics and the CAMSEQ Processor. *J. Comput. Chem.* **1977**, *1*, 187–94. (d) Weintraub, H. J. R.; Hopfinger, A. J. CAMSEQ (Conformational Analysis of Molecules in Solution by Empirical and Quantum Mechanical Techniques) Software System in Drug Design Calculations. *Int. J. Quantum Chem. Quantum Biol. Symp.* **1975**, 203–8.

in saturated sodium chloride solution and extracted with chloroform. The chloroform layer was dried over magnesium sulfate, filtered, and evaporated. The residue was purified by silica gel chromatography (ethyl acetate as eluant) to give the product as an oil (3.2 g, 60%) with a single fluorescent spot on TLC (R_f = 0.5, ethyl acetate): ^{1}H NMR ($CDCl_3$) 7.71–6.92 (m, 4 H), 6.89–6.13 (m, 2 H), 4.13 (q, 2 H), 4.10–4.03 (m, 4 H), 3.92 (s, 2 H), 1.38–1.23 (m, 9 H); MS (EI) m/e 341 (M^+, 19).

Ethyl (*E*)-*N*-[3-[3-(Diethoxyphosphinyl)-1-propenyl]phenyl]glycinate (12c). A solution of diethyl allylphosphonate (2 mL), **9b** (2.08 g, 8.06 mmol), and bis(triphenylphosphine)-palladium(II) chloride (0.5 g) in dimethylformamide (15 mL) and triethylamine (5 mL) was heated in an oil bath at 100 °C and stirred for 5 h. The solvent was removed in vacuo, and the residue was suspended in water and extracted with chloroform. The chloroform layer was further washed with water and then was dried over magnesium sulfate, filtered, and evaporated. The residue was purified by silica gel chromatography (0–2% methanol in chloroform as eluant) gave only partial purification; ethyl acetate as eluant gave reasonably pure product (2.05 g, 72%): ^{1}H NMR ($CDCl_3$) 7.71–6.41 (m, 4 H), 6.20–5.71 (m, 1 H), 5.27–5.18 (m, 1 H), 4.25 (q, 2 H), 4.17–3.97 (m, 4 H), 3.91 (s, 2 H), 2.81–2.56 (m, 2 H), 1.35–1.25 (m, 9 H); MS (FAB) m/e 356 (MH^+).

(*E*)-*N*-[2-(2-Phosphonoethenyl)phenyl]glycine (13). Compound **12a** (1.3 g, 3.8 mmol) was hydrolyzed according to general procedure B to give a hygroscopic white solid (0.89 g, 91%): ^{1}H NMR (D_2O) 7.58–7.33 (m, 3 H), 7.05 (t, 1 H), 6.88 (d, 1 H), 6.44 (dd, 1 H), 4.09 (s, 2 H). Anal. ($C_{10}H_{12}NO_5P$) C, H, N.

(*E*)-*N*-[3-(3-Phosphono-1-propenyl)phenyl]glycine Diammonium Salt (14). Compound **12c** (0.35 g, 0.98 mmol) was hydrolyzed according to general procedure B to give a white solid (0.22 g, 69%): ^{1}H NMR (D_2O) 7.23 (t, 1 H), 6.92 (d, 1 H), 6.80 (s, 1 H), 6.62 (d, 1 H), 6.49–6.29 (m, 2 H), 3.73 (s, 2 H), 2.54 (dd, 2 H). Anal. ($C_{11}H_{14}NO_5P\cdot 2NH_3\cdot H_2O$) H, N; C: calcd, 40.86; found, 40.24.

Ethyl *N*-[2-[2-(Diethoxyphosphinyl)ethyl]phenyl]glycinate (15a). A solution of **12a** (2 g, 5.9 mmol) was reduced under a hydrogen atmosphere (50 psi) with 10% palladium on carbon (0.5 g) as catalyst. After filtration and evaporation, the residue was purified by silica gel chromatography (5–20% ethyl acetate in chloroform as eluant) to give a clear oil (0.42 g, 27%): ^{1}H NMR ($CDCl_3$) 7.17–7.06 (m, 2 H), 6.72 (dd, 1 H), 6.50 (d, 1 H), 4.32 (q, 2 H), 4.19–4.0 (m, 4 H), 3.93 (d, 2 H), 2.89–2.78 (m, 2 H), 1.79–1.66 (m, 2 H), 1.36–1.10 (m, 9 H); MS (EI) m/e 343 (M^+).

Ethyl *N*-[3-[2-(Diethoxyphosphinyl)ethyl]phenyl]glycinate (15b). A solution of **12b** (1.5 g, 4.4 mmol) in methanol was reduced under a hydrogen atmosphere (50 psi) using 10% palladium on carbon as catalyst. After filtration and evaporation, the residue was purified by silica gel chromatography (10–50% ethyl acetate in 1:1 chloroform/pentane as eluant) to give a clear oil (0.84 g, 73%). Anal. ($C_{16}H_{26}NO_5P$) C, H, N.

Ethyl *N*-[3-[3-(Diethoxyphosphinyl)propyl]phenyl]glycinate (15c). A solution of **12c** (1.7 g, 4.8 mmol) in ethanol (75 mL) was reduced under a hydrogen atmosphere (50 psi) using 10% palladium on carbon as catalyst. After filtration and evaporation of the solvent, the residue was purified by silica gel chromatography (ethyl acetate as eluant) to give a clear oil (1.24 g, 72%): MS (FAB) m/e 356 (MH^+).

***N*-[2-(2-Phosphonoethyl)phenyl]glycine Diammonium Salt (16).** **15a** (0.48 g, 1.4 mmol) was hydrolyzed according to the general procedure A. The product was purified over Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants) to give a colored solid (0.14 g, 34%): ^{1}H NMR (D_2O) 7.21–6.76 (m, 4 H), 3.75 (s, 2 H), 2.80–2.66 (m, 2 H), 1.76–1.63 (m, 2 H); MS (FAB) m/e 260 (MH^+). Anal. ($C_{10}H_{14}NO_5P\cdot 2NH_3$) C, H, N.

***N*-[3-(2-Phosphonoethyl)phenyl]glycine Monoammonium Salt (17).** Compound **15b** (0.78 g, 2.27 mmol) was hydrolyzed according to the general procedure A. The residue was purified on Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants) to give an off-white solid (0.26 g, 41%): ^{1}H NMR (D_2O) 7.21 (t, 1 H), 6.90–6.69 (m, 2 H), 6.57 (dd, 1 H), 3.72 (s, 2 H), 2.80–2.70 (m, 2 H), 1.79–1.66 (m, 2 H); MS (FAB) m/e 260 (MH^+). Anal. ($C_{10}H_{14}NO_5P\cdot NH_3$) C, H, N.

***N*-[3-(3-Phosphonopropyl)phenyl]glycine Diammonium Salt (18).** Compound **15c** (1.2 g, 3.4 mmol) was hydrolyzed according to the general procedure A. The residue was purified on Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants) to give a gray-brown solid (0.44 g, 42%): ^{1}H NMR (D_2O) 7.27 (s, 1 H), 7.14 (t, 1 H), 6.67–6.57 (m, 2 H), 3.93 (s, 2 H), 2.63 (t, 2 H), 1.90–1.60 (m, 4 H); MS (FAB) m/e 274 (MH^+). Anal. ($C_{11}H_{16}NO_3P\cdot 2NH_3\cdot H_2O$) C, H, N.

2-(2-Methylphenyl)-1*H*-isoindole-1,3(2*H*)-dione (21). A solution of 2-methylbenzenamine (10.7 g, 0.1 mol), phthalic anhydride (0.1 mol), triethylamine (5 mL), and toluene (150 mL) was refluxed for 2 h with a Dean–Stark trap to remove water. After removal of solvent, the remaining solid was dissolved in boiling ethanol and crystallized (first crop 4.62 g, 20%), mp 180–181 °C. Anal. ($C_{15}H_{11}NO_2$) C, H, N.

2-[2-(Bromomethyl)phenyl]-1*H*-isoindole-1,3(2*H*)-dione (22). A suspension of **21** (3.6 g, 15.2 mmol) in carbon tetrachloride (50 mL) and chloroform (15 mL) was treated with *N*-bromosuccinimide (2.7 g, 15.2 mmol) and AIBN (50 mg) and then irradiated for 90 min with a flood lamp (condenser required to maintain solvent volume). After evaporation of the solvent, the residue was chromatographed on silica gel (3:2 chloroform and hexane as eluant) to give white crystals (3.6 g, 75%), mp 157–162 °C. Anal. ($C_{15}H_{10}BrNO_2$) C, H, N.

Diethyl [[2-(1,3-Dihydro-1,3-dioxo-2*H*-isoindol-2-yl)phenyl]methyl]phosphonate (23). A suspension of **22** (0.98 g, 3.1 mmol) and triethyl phosphite (10 mL) was heated at 100 °C for 3 h. The excess triethyl phosphite was removed in vacuo, and the residue was purified on silica gel (30:1 chloroform and methanol as eluant). The resulting white solid was triturated in diethyl ether and collected by filtration (1.1 g, 95%): mp 127–130 °C; MS (EI) m/e 374 (M + 1).

Diethyl [(2-Aminophenyl)methyl]phosphonate (24). A suspension of **23** (1.1 g, 2.92 mmol) in 5:1 2-propanol/water (18 mL) was treated with sodium borohydride (0.6 g, 15.9 mmol) in one portion and stirred at room temperature. After 2 h an additional portion of sodium borohydride (0.2 g) was added. After 1 h, the reaction mixture was quenched with water and extracted with ethyl acetate. The organic layer was washed with saturated sodium chloride solution, dried over magnesium sulfate, filtered, and evaporated to give a white solid (1.07 g, 97%, mp 117–121 °C). A solution of the solid in 1:1 ethanol/1 N HCl (20 mL) was heated at 75 °C for 1 h. The ethanol was removed by evaporation and the aqueous solution extracted with chloroform. The aqueous phase was basified with saturated sodium bicarbonate and extracted with chloroform. The combined chloroform extracts were dried over sodium sulfate, filtered, and evaporated. The crude product was chromatographed on silica gel (ethyl acetate, then 60:1 ethyl acetate and triethylamine as eluant) to give a ninhydrin-positive, clear oil (0.36 g, 51% overall).

Ethyl *N*-[2-[(Diethoxyphosphinyl)methyl]phenyl]glycinate (25). 24 (0.36 g, 1.48 mmol) was treated according to the general procedure D to give **25**. Silica gel chromatography (applied to the column in chloroform, and eluted with ethyl acetate) gave a clear film (0.3 g, 61%): ^{1}H NMR ($CDCl_3$) 7.3–7.0 (m, 2 H), 6.9–6.5 (m, 2 H), 5.5 (br t, 1 H), 4.25 (q, 2 H), 4.2–3.9 (m, 6 H), 3.2 (d, 2 H), 1.4–1.15 (m, 9 H).

***N*-[2-(Phosphonomethyl)phenyl]glycine Diammonium Salt (26). 25** (0.3 g, 0.9 mmol) was hydrolyzed according to the general procedure A. The product was purified on Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants) to give a white foam (0.15 g, 60%): ^{1}H NMR (D_2O) 7.23–7.14 (m, 2 H), 6.82 (t, 1 H), 6.59 (d, 1 H), 3.73 (s, 2 H), 2.89 (d, 2 H); MS (FAB) m/e 246 (MH^+). Anal. ($C_9H_{12}NO_5P\cdot 2.7NH_3$) C, H, N.

Methyl *N*-[[3-[(Diethoxyphosphinyl)methyl]phenyl]methyl]glycinate (29b). Compound **28b** (2.2 g, 6.85 mmol) was N-alkylated with glycine methyl ester hydrochloride according to general procedure C. The residue was chromatographed over silica gel (10% methanol in 1:1 ethyl acetate and pentane as eluant) to give an oil (0.35 g, 15%): ^{1}H NMR ($CDCl_3$) 7.26–7.21 (m, 4 H), 4.06–3.92 (m, 4 H), 3.77 (s, 2 H), 3.71 (s, 3 H), 3.40 (s, 2 H), 3.12 (d, 2 H), 2.11 (s, 1 H), 1.22 (t, 6 H); MS (EI) m/e 329 (M^+).

Methyl *N*-[[4-[(Diethoxyphosphinyl)methyl]phenyl]methyl]glycinate (29c). Compound **28c** (0.43 g, 1.34 mmol) was N-alkylated with glycine methyl ester hydrochloride according

to general procedure C. The residue was purified by silica gel chromatography (2% methanol in chloroform as eluant) to give an oil (0.12 g, 28%).

***N*-[[2-(Phosphonomethyl)phenyl]methyl]glycine Hydrochloride (30).** Compound **29a** (0.5 g, 1.5 mmol) was hydrolyzed according to general procedure A to give a white solid (0.4 g, 90%): ^{1}H NMR (D_2O) 7.57–7.33 (m, 4 H), 4.33 (s, 2 H), 3.97 (s, 2 H), 3.28 (d, 2 H). Anal. ($C_{10}H_{14}NO_5P$·0.75HCl) C, H; N: calcd, 4.89; found, 4.45.

***N*-[[4-(Phosphonomethyl)phenyl]methyl]glycine Hydrochloride (32).** Compound **29c** (0.12 g, 0.36 mmol) was hydrolyzed according to general procedure A to give a white solid (0.1 g, 92%): ^{1}H NMR (D_2O) 7.4 (s, 4 H), 4.2 (s, 2 H), 3.95 (s, 2 H), 3.15 (d, 2 H). Anal. ($C_{10}H_{14}NO_5P$·HCl·2H_2O) C, H, N.

Methyl *N*-[[2-[(Diethoxyphosphinyl)methyl]phenyl]methyl]-DL-alaninate (33a). Compound **28a** (0.24 g, 0.75 mmol) was treated according to general procedure C, except that DL-alanine methyl ester hydrochloride (0.21 g, 1.5 mmol) was substituted for glycine. The oily residue was chromatographed on silica gel (2% methanol in chloroform as eluant) to give a film (90 mg, 35%): ^{1}H NMR ($CDCl_3$) 7.35–7.1 (br s, 4 H), 4.1–3.7 (m, 4 H), 3.67 (s, 5 H), 3.33 (q, 1 H), 3.33 (d, 2 H), 1.97 (br s, 1 H), 1.4–1.05 (m, 9 H).

Methyl *N*-[[3-[(Diethoxyphosphinyl)methyl]phenyl]methyl]-DL-alaninate (33b). Compound **28b** (0.9 g, 2.8 mmol) was treated according to general procedure C, except that DL-alanine methyl ester hydrochloride (0.84 g, 6 mmol) was substituted for glycine. The residue was chromatographed on silica gel (0–4% methanol in chloroform as eluant) to give a clear oil (0.15 g, 16%): ^{1}H NMR ($CDCl_3$) 7.17 (br s, 4 H), 4.2–3.75 (m, 4 H), 3.7 (s, 5 H), 3.5–3.2 (m, 1 H), 3.06 (d, 2 H), 2.83 (br s, 1 H), 1.35–1.05 (m, 9 H).

Methyl *N*-[[3-[(Diethoxyphosphinyl)methyl]phenyl]methyl]-DL-valinate (33c). Compound **28b** (0.9 g, 2.8 mmol) was treated according to general procedure C, except that DL-valine methyl ester hydrochloride (1.0 g, 6 mmol) was substituted for glycine. The residue was chromatographed on silica gel (0–2% methanol as eluant) to give a film (0.2 g, 19%): ^{1}H NMR ($CDCl_3$) 7.17 (m, 4 H), 4.1–3.77 (m, 4 H), 3.67 (s, 3 H), 3.7–3.4 (m, 1 H), 3.4–2.9 (m, 2 H), 3.07 (d, 2 H), 2.0–1.65 (m, 2 H), 1.2 (t, 6 H), 0.9 (d, 6 H).

***N*-[[2-(Phosphonomethyl)phenyl]methyl]-DL-alanine Hydrochloride (34).** Compound **33a** (0.1 g, 0.29 mmol) was hydrolyzed according to general procedure A to give a nonhygroscopic white solid (0.06 g, 76%): ^{1}H NMR (D_2O) 7.51–7.33 (m, 4 H), 4.40–4.22 (m, 2 H), 4.11 (q, 1 H), 3.27 (d, 2 H), 1.61 (d, 3 H). Anal. ($C_{11}H_{16}NO_5P$·1.2HCl·H_2O) C, H, N, Cl.

***N*-[[3-(Phosphonomethyl)phenyl]methyl]-DL-alanine Hydrochloride (35).** Compound **33b** (0.15 g, 0.44 mmol) was hydrolyzed according to general procedure A to give a hygroscopic white solid (0.12 g, 84%): ^{1}H NMR (D_2O) 7.46–7.37 (m, 4 H), 4.28 (s, 2 H), 4.07 (q, 1 H), 3.18 (d, 2 H), 1.60 (d, 3 H). Anal. ($C_{11}H_{16}NO_5P$·1.25HCl·0.4H_2O) H, N, Cl; C: calcd, 45.03; found, 45.47.

***N*-[[3-(Phosphonomethyl)phenyl]methyl]-DL-valine Hydrochloride (36).** Compound **33c** (0.2 g, 0.53 mmol) was hydrolyzed according to the general procedure A to give a white solid (0.13 g, 72%): ^{1}H NMR (D_2O) 7.4 (br s, 4 H), 4.3 (s, 2 H), 3.85 (d, 1 H), 3.2 (d, 2 H), 2.5–2.1 (m, 1 H), 1.0 (t, 6 H). Anal. ($C_{13}H_{20}NO_5P$·HCl·0.5H_2O) C, H, N; Cl: calcd, 10.22; found, 10.83.

Diethyl [(3-Cyanophenyl)methyl]phosphonate (38). A solution of 3-(bromomethyl)benzonitrile (10 g, 51.3 mmol) in anhydrous tetrahydrofuran (60 mL) was added dropwise over 30 min to a solution of sodium diethyl phosphite (51.3 mmol) in tetrahydrofuran (100 mL) cooled in an ice bath. The reaction mixture was removed from the ice bath and warmed to room temperature. The tetrahydrofuran was evaporated, and the residue was dissolved in ethyl acetate and washed with water. The organic solution was dried over magnesium sulfate, filtered, and evaporated to give a light colored oil (11.2 g, 97%).

Ethyl 3-[(Diethoxyphosphinyl)methyl]benzenecarboximidate Monohydrochloride (39). The nitrile **38** (11 g, 43.4 mmol, purified on silica gel) was dissolved in a mixture of diethyl ether (7 mL) and ethanol (2.2 g, 47.8 mmol) and cooled in an ice bath. The solution was saturated with hydrogen chloride gas and stirred under nitrogen at room temperature for 16 h. The product was obtained by collection of a first crop of solids (1.05 g) by filtration and washing with ether. The mother liquor was resaturated with hydrogen chloride gas and a second crop (1.1 g) was obtained (16.1%).

***N*-[[3-[(Diethoxyphosphinyl)methyl]phenyl]iminomethyl]glycinate (40).** The hydrochloride salt of **39** (1.65 g, 4.9 mmol) was stirred in a solution of saturated sodium bicarbonate (50 mL) layer with 50 mL of ethyl acetate. After separation, the organic layer was dried over magnesium sulfate, filtered, and evaporated. The resulting oil was dissolved in methanol (10 mL), treated with glycine (0.39 g, 5.2 mmol), and stirred for 72 h at room temperature. A trace of unreacted glycine was removed by filtration, and the methanol was removed by evaporation. The residue was suspended in ether and collected by filtration to give a white solid (1.2 g, 82%). Anal. ($C_{14}H_{21}N_2O_5P$) C, H, N.

***N*-[Imino[3-(phosphonomethyl)phenyl]methyl]glycine Hydrobromide (41).** A solution of the amidine **39** (1.1 g, 3.35 mmol) in acetonitrile (7 mL) was treated under a nitrogen atmosphere with trimethylsilyl bromide (3.5 mL, 27 mmol) to give a gummy solid. After the solid was allowed to sit overnight, methanol (5 mL) was added carefully, followed by the addition of 25 mL of water. The reaction mixture was stirred for 10 min and then extracted with methylene chloride. The aqueous solution was lyophilyzed to give a foam (1.4 g). The foam was triturated with ether to give a hygroscopic powder (1.2 g, 96%): MS (FAB) *m/e* 273 (MH^+). Anal. ($C_{10}H_{13}N_2O_5P$·HBr·H_2O) H, N; C: calcd, 33.16; found, 31.27; Br: calcd, 22.06; found, 17.53; H_2O.

Methyl *N*-[(3-Bromophenyl)methyl]glycinate (43). 3-Bromobenzyl bromide (4.3 g, 17.2 mmol) was N-alkylated with glycine methyl ester hydrochloride according to general procedure C. The crude product was chromatographed on silica gel (0–2% methanol in ethyl acetate as eluant) to give a yellow oil (1.62 g, 36.5%). Anal. ($C_{10}H_{12}BrNO_2$) C, H, N.

Methyl *N*-[[3-(Diethoxyphosphinyl)phenyl]methyl]glycinate (44). A solution of diethyl phosphite (1 mL, 6.75 mmol) and triethylamine (5 mL) were stirred under a nitrogen atmosphere, and then treated with tetrakis(triphenylphosphine)palladium(0) (0.4 g). A solution of **43** (1.62 g, 6.28 mmol) in toluene (10 mL) was degassed in vacuo and added to the palladium catalyst mixture, and the reaction vessel was heated at 105 °C for 17 h. The reaction mixture was cooled to room temperature and diluted with diethyl ether. An insoluble precipitate was removed by filtration, and the filtrate was evaporated. The yellow residue was chromatographed on silica gel (0–5% methanol in chloroform as eluant) to give a yellow film (0.31 g, 15.6%): ^{1}H NMR ($CDCl_3$) 7.9–7.3 (m, 4 H), 4.25–3.95 (m, 4 H), 3.8 (s, 2 H), 3.7 (s, 3 H), 3.4 (s, 2 H), 1.25 (t, 6 H).

***N*-[(3-Phosphonophenyl)methyl]glycinate Hydrochloride (45).** **44** (0.18 g, 0.57 mmol) was hydrolyzed according to the general procedure A to give a white solid (0.12 g, 70%): ^{1}H NMR (D_2O) 7.85–7.80 (m, 2 H), 7.64–7.59 (m, 2 H), 4.38 (s, 2 H), 3.98 (s, 2 H). Anal. ($C_9H_{12}NO_5P$·HCl·2H_2O) C, H, N.

Diethyl [[3-(Cyanomethyl)phenyl]methyl]phosphonate (46). A solution of **28b** (7.0 g, 21.8 mmol) in acetone (25 mL) was treated with sodium iodide (0.33 g). A precipitate formed, and the reaction mixture was further treated with a solution of potassium cyanide (2.1 g, 32.7 mmol) in water (7.5 mL), and the reaction mixture was heated for 30 min at reflux. After evaporation of the acetone, the remaining aqueous phase was extracted with ethyl acetate, and the organic layer was dried over magnesium sulfate, filtered, and evaporated. The residue was purified over silica gel to give an oil (3.7 g, 71%).

Diethyl [[3-(2-Aminoethyl)phenyl]methyl]phosphonate (47). A solution of **46** (3.7 g, 15.54 mmol) in methanolic ammonia was reduced under a hydrogen atmosphere (50 psi) with Raney nickel as catalyst. The solvent was evaporated to give an oil (3.5 g, 93%).

Ethyl *N*-[2-[3-[(Diethoxyphosphinyl)methyl]phenyl]ethyl]glycinate (48). Compound **47** (3.6 g, 13.2 mmol) was converted to **48** using the general procedure D, except that toluene was used instead of ethanol and no (dimethylamino)pyridine was used. The crude product was chromatographed on silica gel to give a colorless oil (2.9 g, 45%). Anal. ($C_{17}H_{28}NO_5P$) C, H, N.

***N*-[2-[3-(Phosphonomethyl)phenyl]ethyl]glycine Hydrochloride (49).** Compound **48** (2.75 g, 7.7 mmol) was hydrolyzed according to general procedure A to give a tan solid (1.95 g, 82%):

mp 175–180 °C. Anal. ($C_{11}H_{16}NO_5P$·HCl) C, H, N.

N-(2-Phosphonoethyl)glycine Hydrochloride (56). A solution of glycine methyl ester hydrochloride (1.38 g, 11 mmol) and diethyl (2-bromoethyl)phosphonate (2.45 g, 10 mmol) in ethanol (15 mL) and triethylamine (3.5 mL) was heated at reflux for 17 h. The solvent was removed, and the residue was dissolved in water and extracted with chloroform. The organic layer was dried over magnesium sulfate, filtered, and evaporated. The product was obtained as a 1:3 mixture of methyl and ethyl esters after purification on silica gel (0–3% methanol in chloroform as eluant). Hydrolysis via the general procedure A gave a white solid (1.15 g, 52%): mp 177–179 °C. Anal. ($C_4H_{10}NO_5P$·HCl) C, H, N, Cl.

N-(3-Phosphonopropyl)glycine Hydrochloride (57). (3-Bromopropyl)phosphonate (2.6 g, 10 mmol) was treated as in the preparation of **24** with glycine methyl ester hydrochloride to give the N-alkylated intermediate. Purification on silica gel (0–2.5% methanol in chloroform as eluant) gave a clear oil (1.05 g). Hydrolysis via the general procedure A gave a white solid (0.6 g, 26%): mp 98–103 °C. Anal. ($C_5H_{12}NO_5P$·HCl) C, H, N.

Diethyl (E)-(4-Bromo-2-butenyl)phosphonate (51). A solution of 1,4-dibromo-2-(*E*)-butene (20 g, 93.5 mmol) and triethyl phosphite (23.7 g, 142.6 mmol) was heated at 85 °C under reduced pressure for 4 h. The reaction mixture was purified on silica gel (ethyl acetate as eluant) to give a clear oil (14 g, 62%).

Methyl (E)-N-[4-(Diethoxyphosphinyl)-2-butenyl]glycinate (54a). A solution of glycine methyl ester hydrochloride (1.6 g, 12.7 mmol) and **51** (3.14 g, 11.6 mmol) in methanol and triethylamine (5 mL) was heated at reflux for 16 h. The solvent was removed in vacuo, and the residue was dissolved in water and extracted with chloroform (2 × 70 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. The residue was chromatographed on silica gel (0–2.5% methanol in chloroform as eluant) to give a clear oil (1.1 g, 38%): ^{1}H NMR ($CDCl_3$) 5.69–5.59 (m, 2 H), 4.18–4.02 (m, 4 H), 3.73 (s, 3 H), 3.40 (s, 2 H), 3.30–3.19 (m, 2 H), 2.59 (dd, 2 H), 1.66 (br s, 1 H), 1.31 (t, 6 H).

Methyl (E)-N-[4-(Diethoxyphosphinyl)-2-butenyl]-DL-alaninate (54b). A solution of **51** (2.56 g, 9.44 mmol) and DL-alanine methyl ester hydrochloride (2 g, 29.2 mmol) in methanol (20 mL) and triethylamine (5 mL) was heated at reflux for 16 h. The solvent was evaporated and the residue dissolved in water and extracted with chloroform (2 × 70 mL). The organic layer was dried over magnesium sulfate, filtered, and evaporated. The resulting oil was purified over silica gel (0–2.5% methanol in chloroform as eluant) to give an oil (2.1 g, 84%): ^{1}H NMR ($CDCl_3$) 5.59–5.50 (m, 2 H), 4.08–3.97 (m, 4 H), 3.65 (s, 3 H), 3.29 (q, 1 H), 3.22–3.04 (m, 2 H), 2.57–2.46 (m, 2 H), 1.60 (br s, 1 H), 1.29–1.20 (m, 9 H).

Methyl N-[4-(Diethoxyphosphinyl)butyl]glycinate (55c). A solution of **53a** (1.1 g, 4.4 mmol) in methanol was treated with 10% palladium on carbon and reduced under a hydrogen atmosphere (50 psi). After filtration and evaporation of the filtrate, a colorless oil was obtained (1.05 g, 95%): ^{1}H NMR ($CDCl_3$) 4.17–4.02 (m, 4 H), 3.81 (s, 3 H), 3.76 (s, 2 H), 3.01 (t, 2 H), 2.04–1.64 (m, 6 H), 1.33 (t, 6 H).

N-(4-Phosphonobutyl)glycine Hydrochloride (58). Compound **55c** (1.05 g, 3.7 mmol) was hydrolyzed according to the general procedure A to give a white solid (0.82 g, 83%): ^{1}H NMR (D_2O) 3.94 (s, 2 H), 3.14 (t, 2 H), 1.88–1.61 (m, 6 H). Anal. ($C_6H_{14}NO_5P$·HCl·H_2O) C, H, N.

(E)-N-(4-Phosphono-2-butenyl)glycine Hydrochloride (59). **54a** (0.7 g, 2.5 mmol) was hydrolyzed according to the general procedure A. The crude product was dissolved in water and extracted with ether, and the water layer was lyophilized to give a white solid (0.3 g, 46%): ^{1}H NMR (D_2O) 6.08–5.97 (m, 1 H), 5.77–5.61 (m, 1 H), 3.97 (s, 2 H), 3.73 (dd, 2 H), 2.71–2.57 (m, 2 H). Anal. ($C_6H_{12}NO_5P$·HCl·H_2O) C, H, N, Cl.

(E)-N-(4-Phosphono-2-butenyl)-DL-alanine Hydrochloride (60). **54b** (2 g, 6.8 mmol) was hydrolyzed according to the general procedure A and extracted with ether as in the preparation of **27** to give a white solid (1.4 g, 79%): ^{1}H NMR (D_2O) 6.07–5.95 (m, 1 H), 5.75–5.60 (m, 1 H), 3.95 (q, 1 H), 3.8–3.67 (m, 2 H), 2.65–2.50 (m, 2 H), 1.54 (d, 3 H). Anal. ($C_7H_{14}NO_5P$·HCl·H_2O) C, H, N.

4-(Diethoxyphosphinyl)-3-butynyl Methanesulfonate (63). A solution of 4-(diethylphosphono)-3-butyn-1-ol (6.3 g, 30.6 mmol) in methylene chloride (30 mL) was cooled in an ice bath and treated with triethylamine (5 mL). A solution of methanesulfonyl chloride (3.9 g, 34 mmol) in methylene chloride (10 mL) was added dropwise. The reaction mixture was warmed to room temperature and stirred for 16 h. After the mixture was quenched with water, the aqueous layer was extracted with methylene chloride (2 × 100 mL), and the combined organic layers were dried over magnesium sulfate, filtered, and evaporated. The crude oil was chromatographed over silica gel (0–1% methanol in chloroform as eluant) to give a light colored oil (7 g, 80%).

4-(Diethoxyphosphinyl)-3-butenyl Methanesulfonate (64). A solution of **63** (5 g, 17.6 mmol) in 9:1 tetrahydrofuran/pyridine was reduced under a hydrogen atmosphere (50 psi) with 10% palladium on barium sulfate as catalyst. The filtrate was evaporated to a yellow oil, which was chromatographed on silica gel (0–1% methanol in chloroform as eluant) to give a clear oil (3.15 g, 63%).

Methyl N-[4-(Diethoxyphosphinyl)-3-butenyl]glycinate (65). A solution of **64** (2.9 g, 10.1 mmol) and glycine methyl ester hydrochloride (1.4 g, 11.1 mmol) and (dimethylamino)pyridine (50 mg) in ethanol (20 mL) and triethylamine (2 mL) was heated at reflux for 20 h. The solvent was removed in vacuo, and the residue was dissolved in water and extracted with chloroform (3 × 30 mL). The chloroform layer was dried over magnesium sulfate, filtered, and evaporated to give a yellow oil. Chromatography on silica gel (0–5% methanol in chloroform as eluant) gave a clear oil (0.3 g, 11%): ^{1}H NMR ($CDCl_3$) 6.71–6.42 (m, 1 H), 5.82–5.56 (m, 1 H), 4.23–3.94 (m, 6 H), 3.73 (s, 3 H), 3.45–3.39 (m, 2 H), 2.80–2.77 (m, 2 H), 2.21 (br s, 1 H), 1.36–1.24 (m, 6 H); MS (EI) *m/e* 280 (M + 1).

Diethyl [[(2-Bromoethyl)thio]methyl]phosphonate (68). A solution of tetra-*n*-butylammonium thioacetate (12.8 g, 41 mmol) and diethyl (chloromethyl)phosphonate (5 g, 27 mmol) in tetrahydrofuran (30 mL) was stirred overnight. The solvent was evaporated, and the residue was purified on silica gel (2:1 heptane/acetone as eluant) to give **67**. A solution of **67** (4 g, 17.7 mmol) in anhydrous methanol was treated with sodium methoxide (11.8 mL of 1.5 M) and the solution was stirred at 0 °C for 2 h. The reaction mixture was added slowly to a cold solution of 1,2-dibromoethane (10 g, 53 mmol) in methanol (50 mL). The solvent was evaporated, and the residue dissolved in methylene chloride and washed with water. The organic layer was dried over sodium sulfate, filtered, and evaporated. The residue was purified on silica gel (2:1 heptane/acetone as eluant) to give an oil (4.4 g, 59%): ^{1}H NMR ($CDCl_3$) 4.21–4.09 (m, 4 H), 3.53 (t, 2 H), 3.12 (t, 2 H), 2.86 (d, 2 H), 1.36–1.29 (m, 6 H).

Methyl N-[2-[[(Diethoxyphosphinyl)methyl]thio]ethyl]glycinate (69). A suspension of potassium carbonate (0.42 g, 3 mmol) in anhydrous dimethylformamide (3 mL) was treated with glycine methyl ester hydrochloride (0.25 g, 2 mmol) and stirred under a nitrogen atmosphere. After 5 min, **68** (0.28 g, 1.08 mmol) was added as a solution in dimethylformamide (0.5 mL). After the mixture was stirred for 48 h, the dimethylformamide was removed in vacuo, and the residue was dissolved in water and extracted with chloroform. The organic layer was dried over sodium sulfate, filtered, and evaporated. The residue was chromatographed on silica gel (60:1 chloroform/methanol as eluant) to give a clear film (0.18 g, 56%): ^{1}H NMR ($CDCl_3$) 4.27–3.93 (m, 4 H), 3.67 (s, 3 H), 3.38 (s, 2 H), 2.82 (br s, 4 H), 2.77 (d, 2 H), 2.15–2.0 (m, 1 H), 1.30 (t, 6 H).

N-[2-[(Phosphonomethyl)thio]ethyl]glycine Diammonium Salt (70). Compound **69** (0.16 g, 0.54 mmol) was hydrolyzed according to the general procedure A. The compound was further purified on Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants) to give a white solid (0.95 g, 67%): ^{1}H NMR (D_2O) 3.62 (s, 2 H), 3.29 (t, 2 H), 2.94 (t, 2 H), 2.56 (d, 2 H). Anal. ($C_5H_{12}NO_5PS$·2NH_3·H_2O) C, H, N, S.

N-[2-[(Phosphonomethyl)sulfinyl]ethyl]glycine Diammonium Salt (71). A solution of **70** (0.45 g, 1.7 mmol) in water was treated with 30% hydrogen peroxide (1 equiv) and stirred overnight. The product was purified on Dowex 50 × 4 400 ion exchange resin (water and then 2 N ammonium hydroxide as eluants), and the ninhydrin-positive fraction was lyophilyzed to give a white solid (0.17 g, 35%): ^{1}H NMR (D_2O) 3.71 (s, 2 H), 3.78–3.43 (m, 2 H), 3.30 (d, 2 H), 3.3–3.1 (m, 2 H).

Acknowledgment. The authors extend their thanks to Donald Johnson and Norman Colbry for their work on catalytic reductions and the analytical chemistry group for their spectroscopy support. We also thank Thomas Malone and Sheryl Hays for useful discussions on competitive NMDA antagonists. Special thanks are reserved for Christine Humblet for her commitment to excellence in regard to the molecular modeling.

Supplementary Material Available: The fit structures of active analogues **32** and **59**, in SYBYL-MOL2 file format (4 pages). Ordering information is given on any current masthead page.