Institute of Neurological and Communicative Disorders and Stroke. Compounds were injected intraperitoneally into mice as suspensions in either 30% polyethylene glycol 400 or 0.5% methylcellulose. After the time indicated in Table I, the animal was subjected to either a subcutaneous Metrazol (scMet) challenge (85 mg/kg), a maximal electroshock (MES) challenge (produced with 60 cycle AC at 50 mA for 0.2 s via corneal electrodes), or a rotorod toxicity test. The details of these procedures have been published.[10]

Acknowledgment. We thank Gill Gladding and James Stables of the Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, for the whole animal anticonvulsant assays. We are also indebted to Dr. Michael Henry at J. T. Baker Chemical Co. for identifying the Chiralcel OD column as appropriate for the resolution of 1. Finally, we thank the NIH for generous support of this work (Grant No. NS23866).

Registry No. (±)-1, 120666-79-7; (+)-1, 120710-26-1; (−)-1, 120710-27-2; (±)-2, 101053-00-3; (+)-2, 77207-58-0; (−)-2, 120710-28-3; (±)-3, 120666-80-0; (+)-3, 120710-29-4; (−)-3, 120710-30-7; 4, 24256-91-5.

Exploration of Phenyl-Spaced 2-Amino-(5–9)-phosphonoalkanoic Acids as Competitive *N*-Methyl-D-aspartic Acid Antagonists

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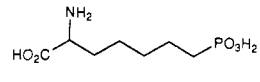
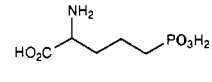
To investigate the preferred spatial relationship of the distal phosphonic acid to the α-amino acid group of the established competitive *N*-methyl-D-aspartic acid (NMDA) antagonists APH (1) and APV (2), we have prepared a series of ortho-, meta-, and para-substituted (phosphonoalkyl)phenylglycine and -phenylalanine derivatives. With use of a [^{3}H]CPP receptor binding assay, significant binding activity was observed to be critically dependent on both the position of substitution and length of alkyl spacing groups. Two compounds, 4-(phosphonomethyl)-phenylglycine (**6**, PD 129635) and 3-(phosphonomethyl)phenylalanine (**15**, PD 130527), displayed receptor-binding affinity comparable to that of APH. Like APH, these compounds were also effective in antagonizing both the proconvulsant and lethal action of NMDA-administered retrobulbar in the mouse. Data are also provided which compare directly the binding efficacy of these compounds against that disclosed recently for the related NMDA antagonist 18 (NPC 451). A preliminary comparison of the structures showing good receptor-binding affinity and in vivo antagonist activity suggests that the NMDA receptor prefers a "folded" rather than "extended" conformation.

The growing awareness of a critical role for excitatory amino acids in the pathology of increasing numbers of neurodegenerative disorders,[1-4] combined with the availability of several selective receptor ligands, has stimulated significant 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, for example, from hypoglycemia[5] and ischemia[6]) are antagonists specific for the *N*-methyl-D-aspartate (NMDA) preferring receptor. Recent advances in the understanding of this area have been made possible by the availability of selective NMDA antagonists such as 2-amino-7-phosphonoheptanoic acid (APH)[7] (**1**), 2-amino-5-phosphonovaleric acid[8] (APV) (**2**),



1



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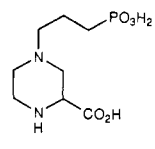

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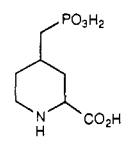

4

4-(3-phosphonopropyl)-2-piperazinecarboxylic acid (CPP)[9,10] (**3**), and most recently *cis*-4-(phosphonomethyl)-2-piperidinecarboxylic acid (CGS 19755)[11] (**4**).

Several reports have described the synthesis and biochemical and electrophysiological action of conformationally restricted NMDA-receptor agonists.[12-16b] Combined, these studies have afforded limited insight into the

† Department of Chemistry.
‡ Department of Pharmacology.

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nature of the agonist pharmacophore. However, the generation of an equivalent antagonist structure–activity relationship is still in its infancy. In large part, this situation arises from the limited structural variation but very high conformational flexibility inherent in the majority of the antagonists such as APH, APV, and CPP.

It seemed to us that substitution of the aliphatic backbone of these compounds by an aromatic ring offered the opportunity to both reduce the conformational flexibility of these compounds and permit the more ready investigation of the preferred spatial relationship of the amino acid residue to the distal phosphonic acid. In addition, such compounds would also act to probe the NMDA receptor for additional areas of steric tolerance. In the following paper, we wish to describe our results on the preparation, preliminary biological characterization, and SAR observations of the complementary series of meta- and para-substituted phenyl-spaced NMDA antagonists.

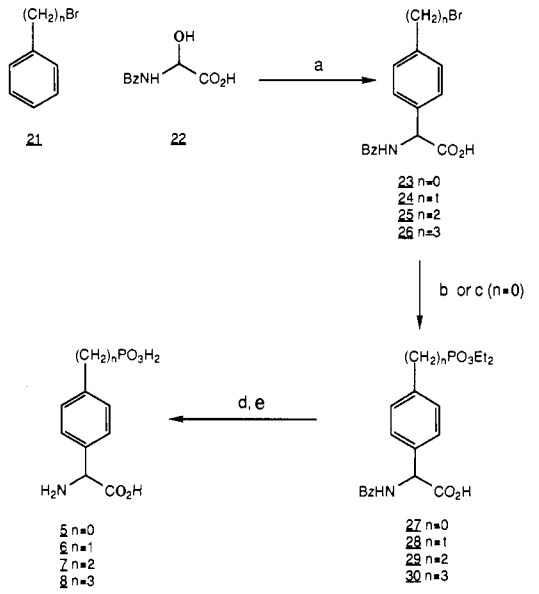
Prior to our studies, an early report[17] had described the synthesis of two related ortho-substituted phenyl-spaced APH analogues. More recently, the synthesis and biological activity of this class of compounds has also been disclosed in a United States patent.[18] One compound from this latter series, 18 (recently referred to as NPC 451), is reported to have been selected for further pharmacological evaluation. In addition to reviewing our own results in this area, we will also evaluate the reported receptor binding and in vivo activity of 18 and several of its "active" analogues.

Chemistry

Each of the compounds prepared in this paper contains an aromatic ring to establish a rigid spacing between an α-amino acid and a distally positioned phosphonic acid. The phosphonic acids were incorporated into the aryl framework as phosphonate esters by one of three methods. Directly bonded phosphonates were prepared by palladium (0)-catalyzed replacement of aryl bromides following recent literature precedent.[19,20] Most of the alkylphosphonates were obtained by direct displacement of an alkyl halide or mesylate. In some cases, where a two-carbon spacer was required, a sequence involving palladium(II)-catalyzed replacement of an aryl bromide with diethyl vinylphosphonate followed by reduction of the double bond was utilized to introduce the phosphonate. This direct aromatic addition of vinylphosphonate is an extension of the reported palladium-catalyzed addition of acrylates to aromatic halides.[21]

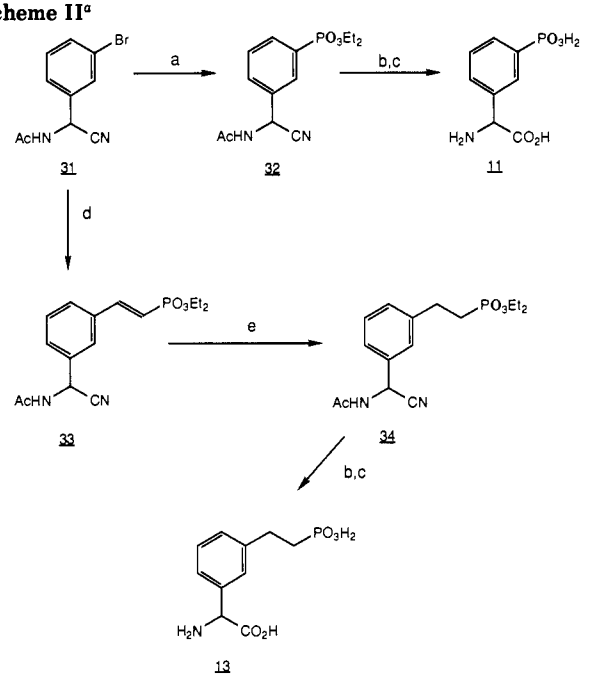
The masked amino acid functionality for most of the compounds was introduced via traditional methodology (e.g., modified Strecker synthesis to give α-amino nitriles or by displacement of aliphatic halides with diethyl acetamidomalonate). In the series of para-substituted phenylglycine analogues described in Scheme I, however, amidoalkylation of aromatic rings as described by Ben-Ishai et al.[22,23] with α-hydroxyhippuric acid (22) provided an

Scheme I[a]



[a] (a) Methanesulfonic acid, α-hydroxyhippuric acid; (b) sodium diethyl phosphite, tetrahydrofuran; (c) tetrakis(triphenylphosphine)palladium(0), diethyl phosphite, triethylamine, toluene, 105 °C; (d) 6 N HCl, reflux; (e) propylene oxide, ethanol.

Scheme II[a]



[a] (a) Tetrakis(triphenylphosphine)palladium(0), diethyl phosphite, triethylamine, toluene, 90–110 °C; (b) 6 N HCl, reflux; (c) propylene oxide, ethanol; (d) palladium(II) acetate, tri-*o*-tolylphosphine, tri-*n*-butylamine, 120 °C; (e) hydrogen gas, 10% palladium on carbon.

expedient route to the desired aryl amino acids **23–26** in a protected form. After introduction of the phosphonate ester, the compounds were hydrolyzed in refluxing 6 N HCl for 16–24 h; this procedure was general for the hydrolyses of all the penultimate intermediates described. In some cases, the hydrochloride salts obtained by this deprotection method were converted to the free bases by treatment with propylene oxide in alcohol to scavenge the hydrochloric

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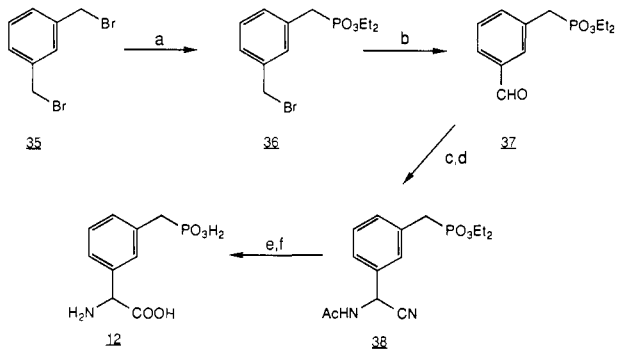
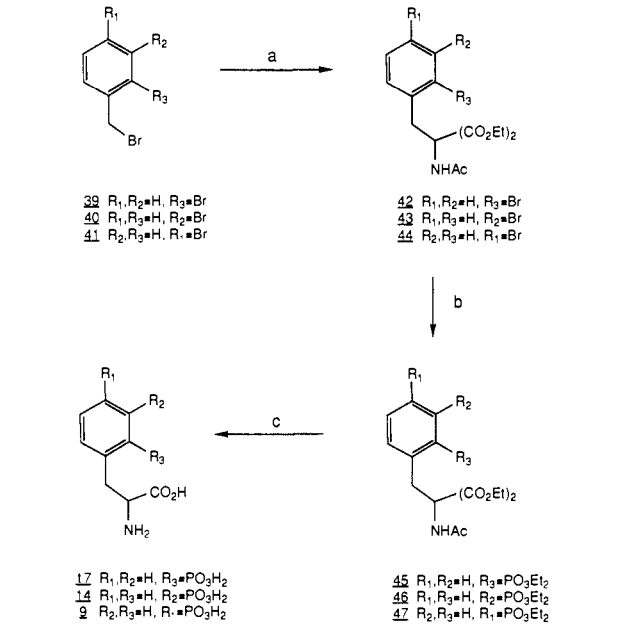
Scheme III[a]



[a] (a) Sodium diethyl phosphite, diethyl ether, −40 °C; (b) 2-nitropropane, sodium ethoxide, ethanol; (c) ammonium hydroxide, sodium metabisulfite, potassium cyanide, water, methanol; (d) acetic anhydride, pyridine; (e) 6 N HCl, reflux; (f) propylene oxide, methanol.

Scheme IV[a]



[a] (a) Diethyl acetamidomalonate, sodium ethoxide, ethanol; (b) tetrakis(triphenylphosphine)palladium(0), sodium diethyl phosphite, triethylamine, toluene, 100 °C; (c) 6 N HCl, reflux, propylene oxide, methanol.

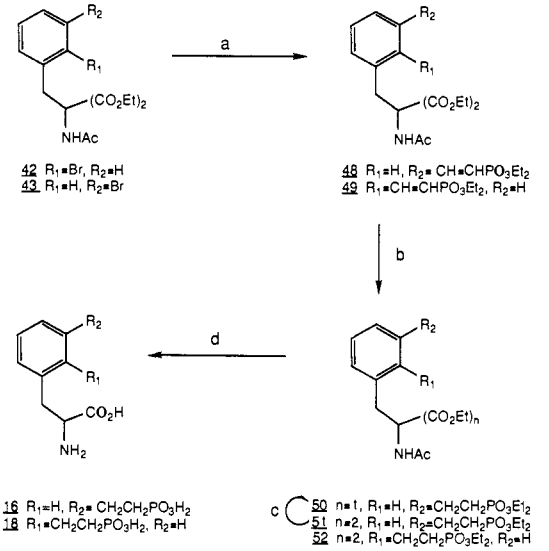
acid. This provided the products as nonhygroscopic precipitates, but occasionally produced small amounts of propylene oxide derived esters contaminating the phosphonic acid targets.

Scheme II illustrates the preparation of the meta-substituted phenylglycine derivatives via the palladium-catalyzed introduction of phosphonate ester groups. The *m*-bromo precursor **31** was either directly phosphonylated in a reaction catalyzed by palladium(0) then hydrolyzed to the $m = 0$ analogue **11** or converted to the *trans*-vinylphosphonate **33** via palladium(II) catalysis. Reduction of the olefinic bond followed by hydrolysis afforded the target **13**.[24]

The synthesis of 3-(phosphonomethyl)phenylglycine (**12**) is given in Scheme III. Preparation of **36**, where only one phosphonate ester is introduced, proceeded in low overall yield (25–31%) which was independent of variations in reaction solvent, temperature, and order of addition of

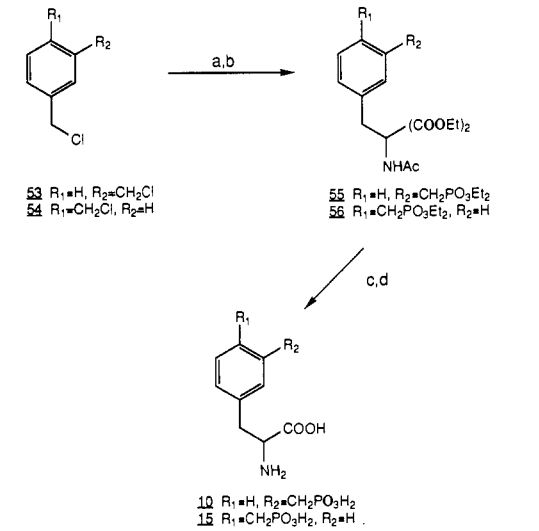
(24) Attempts to hydrolyze the vinyl phosphonate ester directly with 6 N HCl produced a decomposition product which was not characterized further.

Scheme V[a]



[a] (a) Palladium(II) acetate, tri-*o*-tolylphosphine, diethyl vinylphosphonate, tri-*n*-butylamine, toluene, 90–135 °C; or bis(triphenylphosphine)palladium(II) chloride, diethyl vinylphosphonate, triethylamine, dimethylformamide, 90 °C; (b) hydrogen gas, 10% palladium on carbon, ethanol; (c) **50** decarboxylated upon heating; (d) 6 N HCl, reflux, propylene oxide, ethanol.

Scheme VI[a]



[a] (a) Diethyl acetamidomalonate, sodium ethoxide, ethanol; (b) sodium diethyl phosphite, tetrahydrofuran; (c) 6 N HCl, reflux; (d) propylene oxide, ethanol.

reagents. The benzylic bromide **36** served as a masked-aldehyde equivalent[25] which was elaborated into an amino acid using a modified Strecker reaction.[26] Compound **12** was isolated after hydrolysis as previously described and conversion to the free base.

All the (phosphonoaryl)- and (phosphonoalkyl)phenylalanine targets were prepared with combinations of the methodology already described.[27,28] In each case the amino

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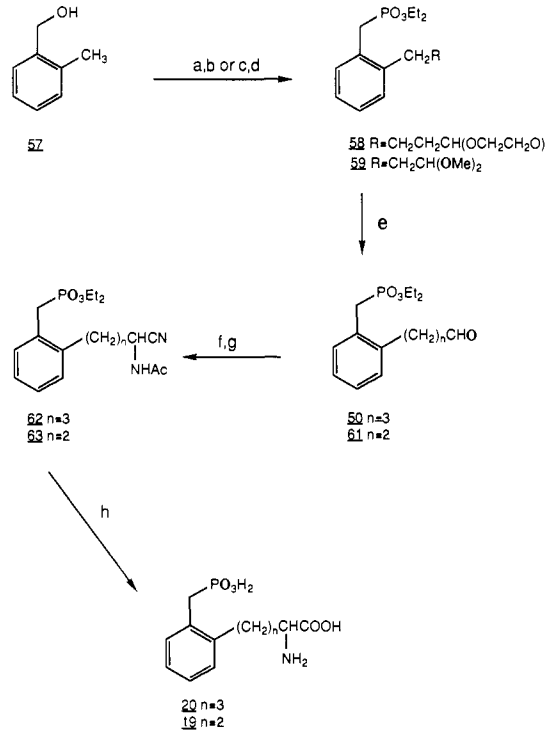
(27) In the reaction to vinylate the aryl bromide **42**, significant amounts of the monodecarboxylated derivative of **48** was formed as the reaction temperature was raised over ca 125 °C (Scheme V).
(28) A preparation of 4-(phosphonomethyl)phenylalanine has been reported recently. Marseigne, I.; Roques, B. P. *J. Org. Chem.* **1988**, *53*, 3621.

Scheme VII[a]



[a] (a) *n*-Butyllithium (2.2 equiv), ether, reflux; (b) 2-(2-bromoethyl)-1,3-dioxolane, ether, 0 °C to room temperature; (c) bromoacetaldehyde dimethylacetal, 0 °C to room temperature; (d) methanesulfonyl chloride, triethylamine, methylene chloride, and then sodium diethyl phosphite, tetrahydrofuran; (e) 1:1 3 N HCl in tetrahydrofuran; (f) ammonium hydroxide, sodium cyanide, ammonium chloride, ethanol; (g) acetic anhydride, pyridine; (h) 6 N HCl, reflux, propylene oxide, methanol.

acid portion of the molecule was introduced by the displacement of a benzylic halide with diethyl acetamidomalonate (see Schemes IV–VI); in the sequences in which a dibenzylic halide was employed (Scheme VI), dialkylation was observed and the disubstituted products could be separated by utilizing the decreased solubility of the diadducts in ether or by column chromatography. As shown in Scheme V, this methodology offers an alternative to that reported for the synthesis[18] of **18**.

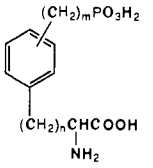
The ortho-substituted analogues **19** and **20**[18] were prepared by new routes as described in Scheme VII. The necessary aryl spacers were assembled from 2-methylbenzyl alcohol (**57**) by α-metalation (2 equiv *n*-BuLi/ether) followed by alkylation with a fragment containing a masked-aldehyde group. The benzylic alcohol was mesylated and displaced with sodium diethyl phosphite to introduce the phosphonic acid and the aldehyde was unmasked to serve as the amino acid precursor.

Pharmacology

Receptor Binding. Compounds **5–20** were evaluated as NMDA ligands by their ability to displace [^{3}H]CPP[29,30] from rat cortical membranes. The results are summarized in Table I. Of the group comprised of 4-phosphono- and 4-(phosphonoalkyl)-substituted phenylglycines (**5–8**), only the 4-(phosphonomethyl)phenylglycine (**6**, PD 129635), showed significant receptor binding with an IC_{50} essentially identical with that of APH itself. Changing the point of ring substitution to the 3-position was also tolerated, with both the directly bonded phosphonate and the phosphonomethyl (**11**, **12**) inhibiting [^{3}H]CPP binding with a similar potency. In addition to the activity of substituted phenylglycines, significant NMDA-receptor binding was also observed for **15** (PD 130527), which is derived formally from phenylalanine. Noteworthy is the relatively poor binding affinity of **18**. Relative to APH, this compound was significantly less potent (IC_{50} = 61 μM) in our assay system than had been reported for a [^{3}H]APH-mediated NMDA receptor binding assay.[18] Compounds **19** and **20**, also reported to be significantly more potent,[18] showed a similar lack of receptor affinity in our [^{3}H]CPP assay.

In Vitro Evaluation in Cultured Rat Cortical Neurons. For those compounds binding most potently to the NMDA receptor, identification of an antagonist action in vitro was made by demonstrating their ability to inhibit glutamate-induced accumulation of $^{45}Ca^{2+}$ in cultured rat cortical neurons[31] (Table II). In this assay, **6** was essen-

Table I. Inhibition of [^{3}H]CPP Binding by Phenyl-Spaced APH Derivatives



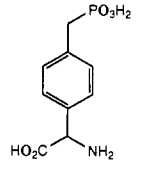
compd	subst	*n*	*m*	[^{3}H]CPP inhibn %I[a]	[^{3}H]CPP inhibn IC_{50}, μM
1 (APH)					0.80
3 (CPP)					0.08
5	p	0	0	10	
6	p	0	1		1.01
7	p	0	2	4	
8	p	0	3	20	
9	p	1	0	10	
10	m	1	1	1	
11	m	0	0		17.45
12	m	0	1		9.3
13	m	0	2	35	
14	m	1	0	15	
15	p	1	1		3.3
16	m	1	2	19	
17	o	1	0	3	
18 (NPC 451)	o	1	2		60.9
19	o	2	1	54	
20	o	3	1	46	

[a] %I = percent inhibition of binding at 100 μM. [b] IC_{50}'s were established where %I > 55.

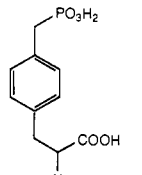
Table II. NMDA-Antagonism Assays

compd	in vitro inhibn of glutamate-induced Ca^{2+} accumulation: IC_{50}, μM	in vivo inhibn of NMDA-induced lethality and ataxia: ED_{50}, mg/kg	
		lethality	ataxia
APH	46	56	17
6	58	8	19
15	378	37	74
18	*a*	*b*	*b*

[a] Inactive up to 250 μM. [b] Inactive at 30 mg/kg, when examined either 5 min or 30 min after iv dosing.



6



15

(29) Olverman, H. J.; Monaghan, D. T.; Cotman, C. W.; Watkins, J. C. *Eur. J. Pharmacol.* **1986**, *131*, 161.
(30) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778.

tially equipotent with APH. **15** was about 7.5 times less potent than APH and **18** was inactive up to 250 μM.

In Vivo Evaluation in Mice. Additional confirmation of an NMDA antagonist action in vivo was demonstrated by the inhibition of NMDA-induced lethality in the mouse. In this assay, intravenously administered NMDA in sufficient doses rapidly induces seizures and results in the death of 100% of the animals. APH antagonizes NMDA-induced lethality with an ED_{50} of 56 mg/kg (Table II). In the same assay a measure of gross behavioral disruption termed ataxia was also determined.[32] Immediately prior to NMDA injection, but 30 min after administration of the putative NMDA antagonist, the mice were examined on an inverted screen. Ataxia was measured as the inability of the mice to cling to the screen platform upon its inversion. APH produces ataxia in this paradigm with an ED_{50} of 17 mg/kg (Table II).

Although similar in receptor binding potency to APH, **6** was about 7 times more potent in vivo as an antagonist of the lethal actions of NMDA. Although less potent than APH at the receptor and as an antagonist in vitro, **15** showed similar potency as an NMDA antagonist in vivo (Table II). In agreement with their weak receptor-binding affinity, compounds **11**, **12**, and **18** were, at the maximum doses examined (30, 60, and 30 mg/kg, respectively), essentially inactive in protecting against NMDA-induced death (data not shown).

Discussion

It is clear from the foregoing that despite the critical dependence of receptor binding on the spatial relationship of the α-amino acid and distal phosphonic acid about the phenyl ring, the NMDA receptor can tolerate the insertion of an aromatic ring into existing antagonists with no loss in binding potency. However, the clear periodicity of binding activity observed for the series AP5, AP6, and AP7[33,34] is not mirrored in these phenyl-spaced analogues. Here, extending **11**, a formal AP5 analogue, by one methylene unit gives the AP6-equivalent **12**. This modification results in a slight improvement in binding affinity. Further extending the phosphonic acid spacing by a second methylene unit affords the AP7-equivalent **13**. This compound is essentially devoid of binding activity. However, the alternate insertion of the second methylene between the amino acid residue and the phenyl ring affords **15**. In this compound, receptor-binding potency is now restored to a level approaching that of the most potent compound of these series, 4-(phosphonomethyl)phenylglycine (**6**). Clearly, from this short series, it can be concluded that the position of the phenyl ring relative to the two pendant acid groups is critically important for biological activity.

Given the restricted conformational mobility of these phenyl-spaced derivatives, these compounds might therefore be considered to generally define the minimum and maximum tolerated spacing of the α-amino acid from the distal phosphonic acid necessary for antagonism of the NMDA receptor. Taken together, these observations further suggest that APH uses its additional aliphatic chain flexibility to bind to the NMDA receptor in a partially folded rather than an extended conformation.[34]

Clearly, the insertion of a phenyl ring between the phosphonic and α-amino acid moieties severely limits the range of conformations and distances that can be achieved by these substituents.[35] For the ortho-substituted derivatives **18–20**, however, conformational flexibility is in large measure restored. It is noteworthy, therefore, that, despite this additional flexibility, these compounds display similar but weaker activity in our [^{3}H]CPP-mediated NMDA receptor binding assay. The diminished binding affinity of these compounds might reflect a reduced steric tolerance for the now essentially pendant aromatic ring.

The novel competitive NMDA antagonists described in this report are extremely polar compounds and as a result are not expected to penetrate the blood–brain barrier well. However, it is reassuring to note that the in vivo activity of **6** and **15** in antagonizing NMDA-induced lethality is predicted both by their receptor-binding affinity and their ability to inhibit glutamate-stimulated $^{45}Ca^{2+}$ accumulation in vitro. Indeed, both compounds are somewhat more potent than expected, perhaps reflecting a slightly increased CNS bioavailability over that of APH.

Conclusion

The conformationally restricted compounds discussed above have provided useful insight into the spatial requirements of the NMDA receptor binding site. A preliminary examination of the antagonists with good affinity for the NMDA receptor suggests that they fit into a model for a folded conformation. We are currently using these results to develop a more refined model of the NMDA receptor.

Experimental Section

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 spectrometer, 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 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.

α-(Benzoylamino)-4-bromobenzeneacetic Acid (23). A solution of bromobenzene (78.5 g, 0.5 mol) in methanesulfonic acid (125 mL) was treated with α-hydroxyhippuric acid (25 g, 0.13 mol) and stirred at room temperature for 5 days. The reaction mixture was poured over ice/water (1 L) and extracted twice with ethyl acetate (500 mL, 250 mL). The combined organic layer was washed successively with water (3 × 250 mL) and saturated sodium bicarbonate (250 mL) The bicarbonate solution was layered with ethyl acetate (500 mL) and acidified with 4 N HCl. The organic layer was separated and washed with water (250 mL),

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(35) The aryl rings of the two compounds with the most potent binding activity (**6** and **15**) were reduced to evaluate the effect on potency. Reduction of the aromatic ring in both compounds was accomplished with 10% Ru/C at 1500 psi at 125 °C. Evaluation of the relative stereochemistry was obstructed by the large number of aliphatic absorbances in the ^{1}H NMR spectrum, therefore the cis/trans ratio of the final products, although assumed to be mainly cis, was, however, not determined. Both reduced compounds displayed some loss in binding affinity over that of their aromatic precursors.

dried over magnesium sulfate, filtered, evaporated, and chromatographed over silica gel to yield an orange-yellow oil (4 g). Standing in ether afforded the free acid as a fluffy, white solid (0.95 g): mp 182–184 °C (changed to compact form at 173–176 °C).

α-Amino-4-phosphonobenzeneacetic Acid Hydrochloride (5). A solution of triethylamine (5 mL) and diethyl phosphite (0.47 g, 3.38 mmol) under a nitrogen atmosphere was charged with tetrakis(triphenylphosphine)palladium(0) (0.2 g, 0.17 mmol) and stirred. A degassed solution of **23** (0.35 g, 0.97 mmol) in toluene (10 mL) was added to the reaction mixture and heated in an oil bath at 105 °C for 2.5 h. After cooling to room temperature, diethyl ether (75 mL) was added and the insoluble precipitate was removed by filtration. The filtrate was evaporated and the viscous, yellow residue was chromatographed on silica gel (50 g) be eluting with a 1:1 mixture of ethyl acetate and pentane, followed by 2% methanol. The fractions were combined and evaporated to yield α-(benzoylamino)-4-(diethoxyphosphinyl)benzeneacetic acid (**27**, 0.47 g) in quantitative yield (one spot on TLC).

Without further characterization, **27** was lightly refluxed in 6 N HCl for 18 h. After cooling, the mixture was refrigerated and crystalline benzoic acid was removed by filtration. The filtrate was evaporated and the residue was triturated with acetone and collected by filtration to give **5** (0.34 g, 1.27 mmol): mp >295 °C; ^{1}H NMR (NaOD) 7.72–7.62 (m, 2 H), 7.38–7.34 (m, 2 H), 4.37 (s, 1 H); MS (FAB) 232 (MH^+). Anal. ($C_8H_{10}NO_5P$·HCl) C, H, N.

α-(Benzoylamino)-4-(bromomethyl)benzeneacetic Acid (24). **24** was prepared as described in the literature.[22]

α-(Benzoylamino)-4-[(diethoxyphosphiny)methyl]benzeneacetic Acid (28). Sodium diethyl phosphite was generated from a solution of diethyl phosphite (9.3 g, 67.2 mmol) in dry tetrahydrofuran (120 mL) treated with sodium pieces (1.4 g, 60.5 mmol). A solution of **24** (6.8 g, 22.4 mmol, impure) in dry tetrahydrofuran (50 mL) was added dropwise and the yellow mixture was stirred overnight, concentrated, and then taken up in a mixture of ethyl acetate (250 mL) and water (250 mL). The aqueous solution was washed with ethyl acetate (1 × 200 mL) and the organic extracts were combined, dried over magnesium sulfate, and concentrated to a white semisolid (12.6 g). Chloroform trituration gave a pure sample (2.3 g, 26%). An additional lot (0.61 g, 7%) was recovered from the mother liquors by concentration and recrystallized from methanol/ethyl acetate (1:10): mp 196–197 °C. Anal. ($C_{20}H_{24}NO_6P$) C (calcd 59.26, found 58.36), H, N.

α-Amino-4-(phosphonomethyl)benzeneacetic Acid (6). **28** (2.7 g, 6.7 mmol) was refluxed in 6 N HCl for 20 h, concentrated, and then reconcentrated successively from water and ethanol. The residue was dissolved in ethanol (25 mL) and treated with propylene oxide (1.2 g, 20 mmol) at room temperature. The free base was collected by filtration and dried at 110 °C in vacuo to give a white solid (1.1 g, 69%). An analytical sample was obtained by trituration with hot methanol: mp >275 °C dec; ^{1}H NMR (200 MHz, D_2O) 7.36 (s, 4 H), 3.99 (s, 1 H), 3.10 (d, 2 H, J = 20 Hz); MS (FAB) 246 (MH^+). Anal. ($C_9H_{12}NO_5P$) C, H, N.

α-(Benzoylamino)-4-(2-bromoethyl)benzeneacetic Acid (25). A solution of (2-bromoethyl)benzene (74 g, 0.4 mol) in methanesulfonic acid (100 mL) was treated with α-hydroxyhippuric acid (20 g, 0.102 mol). After stirring for 60 h at room temperature, the mixture was poured over ice/water (1 L) and extracted twice with ethyl acetate (500 mL, 250 mL) and the combined organic layer was washed successively with water (6 × 250 mL) and saturated sodium bicarbonate (500 mL). The sodium bicarbonate solution was layered with ethyl acetate (500 mL) and acidified with concentrated HCl to pH 2. The ethyl acetate solution was washed with water, dried over magnesium sulfate, filtered, and evaporated to yield the crude product as an oil (30 g), which solidified upon standing. The solid was triturated with diethyl ether and a white powder (10.08 g, first crop) collected by filtration. **25** was recrystallized from toluene: mp 133–136 °C. Anal. ($C_{17}H_{16}BrNO_3$) C, H, N.

α-(Benzoylamino)-4-[2-(diethoxyphosphinyl)ethyl]benzeneacetic Acid (29). Sodium diethyl phosphite was generated from sodium hydride (60% in oil, 0.80 g, 20 mmol) and a solution of diethyl phosphite (3.45 g, 25 mmol) in dry tetrahydrofuran (5 mL). After 30 min a solution of **25** (1.8 g, 5.0 mmol) in dry tetrahydrofuran (5 mL) was added and the reaction was stirred overnight. The solvent was concentrated and the residue was taken up in ethyl acetate (50 mL) and washed with 1 N HCl (10 mL, then 5 mL). The ethyl acetate solution was then washed with saturated sodium bicarbonate. The bicarbonate solution was layered with ethyl acetate, stirred, and carefully acidified with concentrated sulfuric acid. The organic phase was washed with saturated sodium chloride, dried over magnesium sulfate, and chromatographed on silica (0–20% methanol in ethyl acetate as eluant) to give **29** as a hygroscopic foam (1.4 g, 67%).

α-Amino-4-(2-phosphonoethyl)benzeneacetic Acid (7). Hydrolysis of **29** in 6 N HCl for 20 h, followed by reconcentration from water and trituration of the solid residue with methanol gave the pure free base of **7** in 34% yield: mp >280 °C dec; ^{1}H NMR (200 MHz, NaOD) 7.32 (s, 4 H), 4.34 (s, 1 H), 2.82–2.77 (m, 2 H), 1.78–1.62 (m, 2 H); MS (FAB) 260 (MH^+). Anal. ($C_{10}H_{14}NO_5P$) C, H, N, Cl.

α-(Benzoylamino)-4-(3-bromopropyl)benzeneacetic Acid (26). (3-Bromopropyl)benzene (78 g, 0.4 mol) was combined with methanesulfonic acid (100 mL), and the two phases were vigorously stirred as α-hydroxyhippuric acid (19.5 g, 0.1 mol) was added in portions. After 48 h the reaction was poured onto water (1 L) and was extracted with ethyl acetate (2 × 500 mL). The organic layer was washed with 1 N sodium hydroxide (3 × 250 mL) and the aqueous phase was washed with toluene (1 × 250 mL), acidified to pH 2 with 3 N HCl, and extracted with ethyl acetate (2 × 250 mL). The ethyl acetate solutions were combined, dried over magnesium sulfate, and concentrated to a solid (33.2 g). Recrystallization from toluene/ethyl acetate (250 mL of a 4:1 mixture) gave **26** as a white solid (16.1 g, 43%): mp 135–6 °C. Anal. ($C_{18}H_{18}BrNO_3$) C, H, N.

α-(Benzoylamino)-4-[3-(diethoxyphosphinyl)propyl]benzeneacetic Acid (30). **26** (8.0 g, 21.3 mmol) was converted to **30** as described for the n = 2 analogue (**29**). Recrystallization of the crude reaction mixture was accomplished by dissolving it in hot methanol (200 mL) and diluting it with ether (400 mL) to give **30** (7.1 g, 77%): mp 167–8 °C. Anal. ($C_{22}H_{28}NO_6P$) C, H, N.

α-Amino-4-(3-phosphonopropyl)benzeneacetic Acid (8). **30** (6.0 g, 13.8 mmol) was refluxed 24 h in 6 N HCl (300 mL) and then cooled in the refrigerator. Benzoic acid was removed by filtration and the aqueous solution was concentrated to a syrup. The residue was dissolved in water and evaporated to give upon standing a dry, green solid, which was suspended in acetone, filtered, washed with ether, and dried at 78 °C to give the hydrochloride salt as a white solid (4.0 g, 94%): mp foams at 210 °C; ^{1}H NMR (200 MHz, D_2O) 7.46–7.36 (m, 4 H), 5.14 (s, 1 H), 2.76 (t, 2 H, J = 6.8 Hz), 1.96–1.70 (m, 4 H); MS (FAB) 274 (MH^+). Anal. ($C_{11}H_{16}NO_5P$·HCl) C, H, N.

α-(Acetylamino)-3-bromobenzeneacetonitrile (31). A solution of ammonium chloride (5.35 g, 0.1 mol) in water (12.5 mL) was added to a stirred solution of 3-bromobenzaldehyde (18.5 g, 0.1 mol) in methanol (15 mL) and a precipitate formed. After 30 min a solution of potassium cyanide (6.5 g, 0.1 mol) in water (12.5 mL) was added and the reaction was stirred overnight at room temperature and then refluxed for 1 h. The reaction mixture was cooled and an oil separated, which was extracted into diethyl ether and dried over magnesium sulfate, filtered, and finally treated with dry HCl in ether to give a brown oil. The ether was decanted and the oil was dissolved in methanol (100 mL), diluted with ether (350 mL), and refrigerated overnight.

α-Amino-3-bromobenzeneacetonitrile was collected by filtration as a tan, crystalline solid (3.5 g, 15%): mp 182–183 °C dec.

A solution of the amine (2.7 g, 10.9 mmol) in pyridine (25 mL) was cooled in an ice bath and the solution was stirred for 20 min and then was treated with acetic anhydride (2.2 g, 21.8 mmol). After 30 min the reaction mixture was allowed to warm to room temperature and was stirred for 2 h. Concentration of the reaction mixture followed by the addition of water (25 mL) gave a solid, which was recrystallized by dissolving in a minimum amount of ethyl acetate on a steam bath and then by diluting with heptane until cloudy. The N-acetylaryl bromide was obtained as an off-white solid upon cooling and was collected by filtration (2.3 g, 83%): mp 128–129 °C. Anal. ($C_{10}H_9BrN_2O$) C, H, N.

Diethyl [3-[(Acetylamino)cyanomethyl]phenyl]phosphonate (32). Toluene was degassed and under a nitrogen

atmosphere was charged with the protected aryl bromide **31** (3.0 g, 11.2 mmol), diethyl phosphite (1.7 g, 12.3 mmol), triethylamine (1.4 g, 13.4 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.65 g, 0.56 mmol). The reaction was heated to 90 °C for 4 h and then concentrated. The residue was suspended in ethyl acetate to give a white solid, which was collected by filtration, washed with a small volume of ethyl acetate, and resuspended in water. The remaining solid was collected, washed successively with water and diethyl ether, and dried to give a white solid (1.3 g, 37%): mp 117–118 °C. Anal. ($C_{14}H_{19}N_2O_4P$) C, H, N.

α-Amino-3-phosphonobenzeneacetic Acid (11). **32** (1.05 g, 3.4 mmol) was refluxed in 6 N HCl (40 mL) for 20 h, concentrated, and then reconcentrated twice from water (20 mL) and dried. The resulting foam was dissolved in methanol (10 mL) and treated with propylene oxide (0.24 g, 4.1 mmol) and the precipitate which formed was collected by filtration and washed consecutively with methanol and ether and dried (0.56 g, 71%). An analytical sample was obtained by dissolving the solid in water (ca. 1 mL), precipitating it with 2-propanol (1 mL), and then drying it (0.42 g, 54%): mp >305 °C; ^{1}H NMR (100 MHz, NaOD) 7.85–7.40 (m, 4 H), 4.35 (s, 1 H); MS (FAB) 239 (100), 232 (MH^+, 39). Anal. ($C_8H_{10}NO_5P \cdot 0.25H_2O$) C, H, N.

(*E*)-Diethyl [2-[3-[(Acetylamino)cyanomethyl]phenyl]ethenyl]phosphonate (33). Xylene (6 mL) was degassed and under a nitrogen atmosphere was charged with palladium(II) acetate (0.04 g, 0.17 mmol), tri-*o*-tolylphosphine (0.21 g, 0.69 mmol), freshly distilled tri-*n*-butylamine (2.0 g, 10.9 mmol), freshly distilled diethyl vinylphosphonate (1.8 g, 10.9 mmol), and acetylated **31** (2.2 g, 8.7 mmol). The reaction mixture was heated to 115 °C for 1.5 h, allowed to cool, and diluted with xylene to 25 mL. The reaction was adsorbed onto a pad of silica (20 g) and washed with ether and then eluted with a gradient of 50–100% ethyl acetate in heptane to give an oil (4.6 g) contaminated with butylamine. The oil was dissolved in ethyl acetate (50 mL) and extracted with 1 N HCl (2 × 10 mL) and saturated sodium bicarbonate (1 × 10 mL) and then dried over magnesium sulfate and concentrated to a yellow glass (2.4 g, 83%).

Diethyl [2-[3-[(Acetylamino)cyanomethyl]phenyl]ethyl]phosphonate (34). A solution of **33** (2.3 g, 6.84 mmol) in ethanol (100 mL) was reduced over 5% Pd/C (0.2 g) with hydrogen at 50 psi. After completion, the reaction was filtered through Celite and concentrated and the product was isolated by chromatography on silica (ethyl acetate as eluant) to give a viscous oil (1.30 g, 56%).

α-Amino-3-(2-phosphonoethyl)benzeneacetic Acid (13). **34** (1.4 g, 4.1 mmol) was refluxed for 20 h in 6 N HCl and concentrated. After redissolving and evaporating successively with water and acetone, the residue was dissolved in methanol (25 mL). A fine insoluble material was removed by filtration and the filtrate was treated with propylene oxide (0.28 g, 4.8 mmol) in methanol (1 mL) to give a white solid (0.59 g, 56%): mp >295 °C; ^{1}H NMR (200 MHz, D_2O) 7.44–7.27 (m, 4 H), 4.73 (s, 1 H), 2.95–2.82 (m, 2 H), 1.90–1.71 (m, 2 H); MS (FAB) 288 (100). Anal. ($C_{10}H_{14}NO_5P \cdot 0.25H_2O$) C, H, N.

Diethyl [[3-(Bromomethyl)phenyl]methyl]phosphonate (36). A 60% sodium hydride dispersion in oil (3.2 g, 0.08 mol) was washed with heptane, suspended in dry tetrahydrofuran (50 mL), and treated dropwise over 15 min with a solution of diethyl phosphite (11.0 g, 0.08 mol) in dry tetrahydrofuran (20 mL). The reagent was transferred to an addition funnel and added dropwise to a solution of 1,3-bis(bromomethyl)benzene (40 g, 0.15 mol) in dry tetrahydrofuran (200 mL) at reflux over a period of 30 min. The reaction was refluxed 30 min, concentrated, and taken up in a mixture of ethyl acetate (250 mL) and water (50 mL). The ethyl acetate solution was washed with water (25 mL) and concentrated and the oil was dissolved in toluene (250 mL) and chromatographed on silica (20–80% ethyl acetate in heptane as eluant) to give a colorless oil (8.0 g, 31%). Anal. ($C_{12}H_{18}BrO_3P$) C, H, N.

Diethyl [(3-Formylphenyl)methyl]phosphonate (37). Sodium ethoxide, generated from sodium (0.61 g, 26.5 mmol) in absolute ethanol (27 mL), was treated with freshly distilled 2-nitropropane (3.0 g, 33.7 mmol) to give a precipitate. A solution of bromide **36** (7.7 g, 24.1 mmol) in absolute ethanol (20 mL) was added dropwise and the mixture was stirred for 3 days at ambient temperature. The reaction solids were removed by filtration and the filtrate was evaporated. The residue was dissolved in ethyl acetate (150 mL) and washed consecutively with water (50 mL), 1 N sodium hydroxide (2 × 30 mL), 1 N HCl (1 × 30 mL), and saturated sodium bicarbonate (1 × 30 mL), dried over magnesium sulfate, and concentrated to an oil (6.0 g, 97%). Anal. ($C_{12}H_{17}O_4P$) C (calcd 56.25, found 55.52), H, N.

Diethyl [[3-[(Acetylamino)cyanomethyl]phenyl]methyl]phosphonate (38). **37** (5.5 g, 21.5 mmol) was treated successively with a solution of sodium metabisulfite (2.05 g, 10.7 mmol) in water (20 mL) and concentrated ammonium hydroxide (4.2 mL). The reaction mixture was stirred for 15 min and treated with solid sodium cyanide (1.05 g, 21.5 mmol) added in one portion. After 4.5 h, the reaction was extracted with ethyl acetate (3 × 25 mL) and the combined organic extracts were washed with 1 N HCl (2 × 30 mL). The aqueous phase was washed with ethyl acetate (1 × 25 mL), diluted with ethyl acetate (50 mL), and neutralized with solid sodium carbonate. The aqueous phase was extracted with ethyl acetate (3 × 25 mL), and the organic extracts were combined, dried over magnesium sulfate, and concentrated to afford the intermediate amino nitrile as an oil (2.7 g).

Without further purification, the amino nitrile was dissolved in pyridine (18 mL), cooled in an ice bath, and treated with acetic anhydride (1.8 g, 17.6 mmol). After 3.5 h at room temperature, the reaction was concentrated and the residue was taken up in ethyl acetate (25 mL), washed successively with 1 N HCl (2 × 5 mL) and saturated sodium bicarbonate (5 mL), dried over magnesium sulfate, and filtered. Concentration afforded a viscous oil, which was purified by chromatography on silica (50–100% ethyl acetate in heptane as eluant) to give **38** as a viscous oil (1.6 g, 23%).

α-Amino-3-(phosphonomethyl)benzeneacetic Acid (12). **38** (1.3 g, 4.4 mmol) was refluxed 20 h in 6 N HCl (25 mL) and the reaction was concentrated twice from water. A solution of the residue in methanol (20 mL) was treated with propylene oxide (0.77 g, 13.3 mmol) while stirring. A precipitate was collected by filtration, washed with ether, and dried to give a white solid (0.54 g, 49%). An analytical sample was obtained by precipitation from methanol with diethyl ether: mp foams >180 °C. Anal. ($C_9H_{12}NO_5P \cdot 0.5H_2O$) C, H, N.

Diethyl (Acetylamino)[(2-bromophenyl)methyl]propanedioate (42). Sodium ethoxide generated from sodium (0.46 g, 20 mmol) in absolute ethanol (40 mL) was treated with diethyl acetamidomalonate (4.8 g, 22 mmol) in one portion and the reaction was stirred. After 15 min, a solution of *o*-bromobenzyl bromide (5.0 g, 20 mmol) in dry tetrahydrofuran (15 mL) was added dropwise and the reaction was stirred overnight at room temperature, diluted with ether, and filtered. The filtrate was concentrated to a solid, which was recrystallized from heptane to give **42** as a white solid (7.0 g, 91%): mp 97–97.5 °C. Anal. ($C_{16}H_{20}BrNO_5$) C, H, N.

Diethyl (Acetylamino)[(3-bromophenyl)methyl]propanedioate (43). The meta isomer was prepared from *m*-bromobenzyl bromide as described for **42**, except that the amount of each reagent was halved. **43** was obtained as a crystalline solid from heptane (3.25 g, 84%): mp 99–100 °C. Anal. ($C_{16}H_{20}BrNO_5$) C, H, N.

Diethyl (Acetylamino)[(4-bromophenyl)methyl]propanedioate (44). The para isomer was prepared exactly as described for **42**. The product was recrystallized by heating in ethyl acetate (ca. 5 mL) and diluting with heptane (70 mL) to give needles on cooling (4.84 g, 63%): mp 132–132.5 °C. Anal. ($C_{16}H_{20}BrNO_5$) C, H, N.

Diethyl (Acetylamino)[[2-(diethoxyphosphinyl)phenyl]methyl]propanedioate (45). Toluene (41 mL) was degassed and charged with diethyl phosphite (2.4 g, 17.2 mmol), triethylamine (1.74 g, 17.2 mmol), tetrakis(triphenylphosphine)palladium(0) (0.9 g, 0.8 mmol), and the aryl bromide **42** (6.0 g, 15.6 mmol). The reaction was stirred under nitrogen in an oil bath at 90 °C for 24 h, cooled, and then diluted with ether. The solvent was decanted, evaporated, and chromatographed on silica (a gradient of ethyl acetate in heptane as eluant) to give the product as an oil (2.1 g, 30.4%). (Additional product was later isolated from the insoluble material.)

Diethyl (Acetylamino)[[3-(diethoxyphosphinyl)phenyl]methyl]propanedioate (46). The meta phosphonate was prepared from **43** (3.0 g, 7.8 mmol) as described for **45**, except that

the amount of each reagent was halved. After 5 h at 90 °C, the reaction mixture was allowed to cool, diluted with ether (175 mL), and filtered. The filtrate was concentrated and chromatographed as above to give **46** as an oil (2.9 g, 83%).

Diethyl (Acetylamino)[[4-(diethoxyphosphinyl)phenyl]methyl]propanedioate (47). The para phosphonate was prepared from **44** (4.1 g, 10.6 mmol), as described for **45**, to give **47** as a heavy oil (4.6 g, 98%). The dark material was decolorized with activated carbon in ethyl acetate prior to hydrolysis.

2-Phosphono-DL-phenylalanine (17). **45** (1.8 g, 4.1 mmol) was refluxed overnight in 6 N HCl (30 mL) and the water removed by rotoevaporation in vacuo. The residue was reconcentrated successively from water and tetrahydrofuran to give a foam. A solution of the residue in absolute ethanol (25 mL) was treated with propylene oxide (3 mL) with stirring and the resulting precipitate (0.26 g, impure) was collected by filtration and washed successively with ethanol and diethyl ether. A second, analytical crop was isolated from the filtrate and was dried at 110 °C in vacuo (0.45 g, 45%): mp >220 °C dec; ^{1}H-NMR (100 MHz, D_2O) 8.00–7.73 (m, 1 H), 7.70–7.33 (m, 3 H), 4.36 (t, 1 H, J = 6.7 Hz), 3.92–3.50 (m, 2 H). Anal. ($C_9H_{12}NO_5P$) C, H, N.

3-Phosphono-DL-phenylalanine (14). **46** (2.5 g, 5.64 mmol) was refluxed for 18 h in 6 N HCl (50 mL) and the water was removed by rotoevaporation in vacuo. The residue was concentrated twice from tetrahydrofuran to give a gum, which was dissolved in 2-propanol (50 mL), stirred, and treated with propylene oxide (5 mL). A fine precipitate formed, which was collected by filtration after 10 min, washed with 2-propanol and diethyl ether, and dried at 78 °C in vacuo to give an off-white solid (0.55 g, 40%): mp foams >95 °C dec; ^{1}H NMR (100 MHz, D_2O) 7.89–7.46 (m, 4 H), 4.30 (dd, 1 H, J = 7.34, 5.32 Hz), 3.57–3.07 (m, 2 H); MS (FAB) 245 (M). Anal. ($C_9H_{12}NO_5P$) C, H, N.

4-Phosphono-DL-phenylalanine (9). **47** (4.4 g, 9.9 mmol) was refluxed for 20 h in 6 N HCl (200 mL) and the aqueous solution was rotoevaporated in vacuo. The residue was reconcentrated from water to give a solid, which was dissolved in absolute ethanol (75 mL) and treated with propylene oxide (5 mL). The solution was refrigerated for 3 h and the solid (2.1 g, 86%) obtained was washed successively with ethanol and diethyl ether and dried at 110 °C in vacuo. An analytical sample was obtained by recrystallization from water/ethanol (0.25 g, 10%): mp >235 °C dec; ^{1}H NMR (100 MHz, D_2O) 7.77 (dd, 2 H, J = 11.7, 6.8 Hz), 7.74–7.26 (m, 2 H), 4.25 (dd, 1 H, J = 6.8, 5.0 Hz), 3.55–3.03 (m, 2 H); MS (FAB) 246 (MH^+). Anal. ($C_9H_{12}NO_5P \cdot 0.75H_2O$) C, H, N, H_2O.

Diethyl (Acetylamino)[[3-[2-(diethoxyphosphinyl)ethenyl]phenyl]methyl]propanedioate (48). Xylene (9 mL) was degassed, stirred under nitrogen, and charged with palladium(II) acetate (0.064 g, 0.26 mmol), tri-*o*-tolylphosphine (0.32 g, 1.0 mmol), **43** (5.0 g, 13.0 mmol), freshly distilled diethyl vinylphosphonate (2.7 g, 16.3 mmol), and freshly distilled tri-*n*-butylamine (3.0 g, 16.3 mmol). The reaction was heated to 110 °C and maintained there for 2 h, heated to 120 °C for 1 h, and then finally heated to 135 °C for 2 h. A secondary product was observed to be forming, so the reaction mixture was cooled and then diluted with ethyl acetate (10 mL) and 1 N HCl (10 mL), and the aqueous portion was extracted repeatedly with ethyl acetate. The combined organic extracts were washed with 1 N HCl (5 mL), dried over magnesium sulfate, filtered, and concentrated to a mixture of a solid and an oil. Trituration with toluene gave **48** (0.53 g, 8%), and the filtrate was chromatographed on silica (50–100% ethyl acetate in heptane as eluant) to give additional product (1.4 g, 23%). Elution with 5% methanol in ethyl acetate afforded the decarboxylated product as a viscous oil (1.9 g, 37%). For **48** (1.93 g, 31%): mp 144–146 °C. Anal. ($C_{22}H_{32}NO_8P$) C, H, N.

Diethyl (Acetylamino)[[3-[2-(diethoxyphosphinyl)ethyl]phenyl]methyl]propanedioate (51). Reduction of **48** (1.41 g, 3.0 mmol) was accomplished as described for **34**. Concentration of the ethanol afforded an oil (1.5 g, quantitative), which crystallized on standing. Trituration with ether gave a pure sample (0.61 g, 43%): mp 86–87 °C. Anal. ($C_{22}H_{34}NO_8P$) C, H, N.

3-(2-Phosphonoethyl)-DL-phenylalanine (16). **51** (0.54 g, 1.1 mmol) was refluxed in 6 N HCl (20 mL) for 24 h and evaporated to dryness. The residue was successively concentrated from water and twice from acetone to give a foam which was dried at 78 °C in vacuo (0.30 g, 88%): mp foams at 90–100 °C; ^{1}H NMR (200 MHz, D_2O) 7.41–7.16 (m, 4 H), 4.22–4.15 (m, 1 H), 3.38–3.10 (m, 2 H), 2.94–2.82 (m, 2 H), 2.01–1.88 (m, 2 H); MS (FAB) 274 (MH^+). Anal. ($C_{11}H_{16}NO_5P \cdot HCl \cdot 0.5$ acetone) C, H, N.

Diethyl (Acetylamino)[[2-[(diethoxyphosphinyl)ethenyl]phenyl]methyl]propanedioate (49). A solution of **42** (5.00 g, 13.0 mmol), diethyl vinylphosphonate (2.71 g, 16.5 mmol), and bis(triphenylphosphine)palladium(II) chloride (460 mg, 0.66 mmol) in 60 mL 5:1 dimethylformamide/triethylamine was heated at 90 °C under an nitrogen atmosphere for 18 h. The reaction mixture was concentrated and the residue was dissolved in ethyl acetate and washed with water. The organic phase was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by flash chromatography (silica gel, ethyl acetate) to give the crude product as an amber oil (3.61 g), which was recrystallized by dissolution in 10 mL of tetrahydrofuran and trituration with diisopropyl ether (90 mL) to give **49** (1.86 g, 3.97 mmol, 30%) as a white crystalline solid: mp 104 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) 7.71–7.51 (m, 2 H), 7.32–7.24 (m, 2 H), 7.08–7.03 (m, 2 H), 6.48 (s, 1 H), 6.23 (dd, 1 H, J = 18.05, 17.29 Hz), 4.35–4.08 (m, 8 H), 3.80 (s, 2 H), 2.02 (s, 3 H), 1.37 (t, 3 H, J = 7.1 Hz), 1.28 (t, 3 H, J = 7.2 Hz); MS (EI) 470 (M^+, 24).

Diethyl (Acetylamino)[[2-[2-(diethoxyphosphinyl)ethyl]phenyl]methyl]propanedioate (52). A mixture of **49** (2.25 g, 4.79 mmol) and 5% palladium on carbon (500 mg) in 100 mL ethanol was reduced under hydrogen at 52 psi for 28 h. The reaction mixture was concentrated and purified by chromatography (silica gel, ethyl acetate) to give **52** (1.10 g, 2.33 mmol, 48.7%) as a viscous oil: ^{1}H NMR ($CDCl_3$, 200 MHz) 7.21–7.10 (m, 3 H), 6.99 (d, 1 H, J = 7.3 Hz), 6.57 (s, 1 H), 4.35–4.20 (m, 4 H), 4.19–4.04 (m, 4 H), 3.70 (s, 2 H), 2.91–2.78 (m, 2 H), 2.02 (s, 3 H), 2.03–1.86 (m, 2 H), 1.34 (t, 3 H, J = 7.0 Hz), 1.28 (t, 3 H, J = 7.2 Hz); MS (EI) 472 (M^+, 80).

2-(2-Phosphonoethyl)-DL-phenylalanine (18). A solution of **52** (1.05 g, 2.23 mmol) in 20 mL of 6 N HCl was heated at reflux for 36 h. The reaction mixture was concentrated and the foamy product was dissolved in 20 mL of water and freeze-dried. The residual solid was dried under vacuum at 100 °C to give **18** (548 mg, 1.84 mmol, 82.3%): ^{1}H NMR (200 MHz, D_2O) 7.40–7.28 (m, 4 H), 4.26 (t, 1 H, J = 6.9 Hz), 3.49–3.16 (m, 2 H), 3.00–2.87 (m, 2 H), 2.11–1.94 (m, 2 H); MS (FAB) 274 (MH^+). Anal. C, H, N, Cl.

Diethyl (Acetylamino)[[3-[(diethoxyphosphinyl)methyl]phenyl]methyl]propanedioate (55). Sodium ethoxide generated from sodium (1.15 g, 50 mmol) in absolute ethanol (100 mL) was treated with diethyl acetamidomalonate (10.9 g, 50 mmol) in one portion and the reaction was stirred for 30 min. A solution of 1,3-bis(chloromethyl)benzene (8.75 g, 50 mmol) in dry tetrahydrofuran (40 mL) was added dropwise and the reaction was stirred overnight. The solution was concentrated and the unreacted dichloride was removed by precipitation of the reaction solids from diethyl ether with heptane. The less soluble diadduct was removed by heating the solids in a small volume of ether and removed by filtration. The product was obtained from the filtrate by diluting with heptane until cloudy, cooling and collecting the precipitate that formed. The sample was dried to give diethyl 2-(acetylamino)-2-[[3-(chloromethyl)phenyl]methyl]propanedioic acid as a solid (7.1 g, 40%), which was used without further purification.

A solution of sodium diethyl phosphite, generated in situ from a solution of diethyl phosphite (3.9 g, 28.2 mmol) in dry tetrahydrofuran (15 mL) and sodium hydride (60% dispersion in oil, 0.98 g, 24.4 mmol), was treated dropwise over 15 min with a solution of the chloromethyl derivative above (6.7 g, 18.8 mmol) in dry tetrahydrofuran (15 mL), and the reaction was stirred overnight. The solvent was concentrated and the residue was taken up in ethyl acetate (200 mL), washed twice with water (50 mL), dried over sodium sulfate, filtered, and concentrated to an oil. The oil was heated in heptane on a steam bath and a minimum amount of ethyl acetate was added to dissolve the oil. The refrigerated solution gave **55** as a solid, which was collected by filtration, washed with heptane, and dried (5.2 g, 61%): mp 92–93 °C. Anal. ($C_{21}H_{32}NO_8P$) C, H, N.

3-(Phosphonomethyl)-DL-phenylalanine (10). **55** (5.1 g, 11.2 mmol) was refluxed for 20 h in 6 N HCl (200 mL) and the reaction was concentrated at 50 °C in vacuo. The residue was concentrated

twice from water to a foam, which was dissolved in ethanol (125 mL) and treated with propylene oxide (1.0 g, 17.2 mmol). A fine precipitate was collected by filtration, washed successively with ethanol (2 × 100 mL) and diethyl ether (1 × 100 mL) and dried at 78 °C in vacuo (1.90 g, 66%): mp >215 °C dec; ^{1}H NMR (100 MHz, D_2O) 7.50–7.09 (m, 4 H), 4.29 (dd, 1 H, J = 7.5, 4.3 Hz), 3.49–3.09 (m, 2 H), 3.21 (d, 2 H, J = 20 Hz); MS (FAB) 260 (MH^+). Anal. ($C_{10}H_{14}NO_5P \cdot 0.25H_2O$) C, H, N.

Diethyl (Acetylamino)[[4-[(diethoxyphosphinyl)methyl]phenyl]methyl]propanedioate (56). Diethyl 2-(acetylamino)-2-[[4-(chloromethyl)phenyl]methyl]propanedioic acid was prepared exactly as described for the meta isomer (see preparation of **55** above). After filtration of the insoluble material, the filtrate was concentrated, taken up in diethyl ether, refiltered, and chromatographed on silica (diethyl ether as eluant) to give a white solid (3.0 g, 19%), which was used directly.

The resulting benzyl chloride (2.9 g, 8.2 mmol) was alkylated with sodium diethyl phosphite as described for the meta isomer **55** to give a white solid (2.5 g, 68%): mp 95–96 °C. Anal. ($C_{21}H_{32}NO_8P$) C, H, N.

4-(Phosphonomethyl)phenylalanine (15). **56** (2.45 g, 5.34 mmol) was refluxed for 20 h in 6 N HCl (100 mL) and concentrated. The solid residue was reconcentrated from water (50 mL) and triturated with acetone (50 mL) to give a fine white solid as the hydrochloride salt; it was dried at 78 °C in vacuo (1.50 g, 95%): mp >205 °C dec; ^{1}H NMR (100 MHz, D_2O) 7.30 (s, 4 H), 4.46–4.26 (m, 1 H), 3.43–3.07 (m, 4 H); MS (FAB) 260 (MH^+). Anal. ($C_{10}H_{14}NO_5P \cdot HCl$) C, H, N.

Diethyl [[2-[3-(1,3-Dioxolan-2-yl)propyl]phenyl]methyl]phosphonate (58). According to the method of Braun,[36] a solution of 2-methylbenzyl alcohol (20.0 g, 0.164 mol) in 500 mL of tetrahydrofuran was treated with n-butyllithium (200 mL of a 1.6 M solution in hexane) at 0 °C. The resulting orange anion was then warmed to 45 °C for 4 h. After cooling to 0 °C, a solution of 2-(2-bromoethyl)-1,3-dioxolane (29.7 g, 0.164 mol) in 50 mL tetrahydrofuran was added dropwise. The reaction mixture was warmed to room temperature and stirred for 48 h. The reaction mixture was quenched with 100 mL of saturated aqueous ammonium chloride solution. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (2 × 100 mL). The combined organic extracts were dried over magnesium sulfate, filtered, and concentrated. The residue was purified by chromatography (silica gel, 1:1 heptane/ethyl acetate) to give 2-[3-(1,3-dioxolan-2-yl)propyl]benzenemethanol (6.75 g, 29.4 mmol, 18%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) 7.40–7.35 (m, 1 H), 7.25–7.16 (m, 3 H), 4.90–4.86 (m, 1 H), 4.71 (s, 2 H), 3.99–3.80 (m, 4 H), 2.77–2.04 (m, 2 H), 1.90 (brs, 1 H), 1.80–1.70 (m, 4 H); MS (CI) 205 ($MH^+ - H_2O$, 100).

A solution of the hydroxymethyl derivative prepared above (5.75 g, 25.1 mmol) of triethylamine (2.76 g, 27.3 mmol) in 50 mL or methylene chloride was cooled to 0 °C and methanesulfonyl chloride (2.89 g, 25.2 mmol) in 20 mL of methylene chloride was added dropwise and allowed to stir for 30 min. The reaction mixture was warmed to room temperature and washed with saturated aqueous sodium bicarbonate (20 mL). The organic phase was separated and dried over sodium sulfate and concentrated. The crude mesylate residue was dissolved in tetrahydrofuran (20 mL) and added dropwise to a solution of sodium diethyl phosphite (3.26 M, 100 mL) at reflux. After 2 h, the reaction was cooled to room temperature and quenched with water (25 mL). The resulting mixture was extracted with ethyl acetate and the organic phase was dried over magnesium sulfate, filtered, and concentrated. Purification by chromatography (silica gel, ethyl acetate) afforded **58** (7.10 g, 20.7 mmol, 82.6%) as an oil.

Diethyl [[2-(4-Oxobutyl)phenyl]methyl]phosphonate (60). A solution of **58** (6.0 g, 17.5 mmol) in 200 mL of a 1:1 tetrahydrofuran/3 N HCl mixture was stirred at 25 °C for 5 h. The reaction mixture was extracted into ethyl acetate (2 × 100 mL), and the combined extracts were washed with saturated aqueous sodium bicarbonate (25 mL), dried over magnesium sulfate, filtered, and concentrated to give **60** (4.92 g, 16.5 mmol, 92.4%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) 9.72 (t, 1 H, J = 1.4 Hz), 7.26–7.05 (m, 4 H), 4.92–4.65 (m, 4 H), 3.14 (d, 2 H, J = 22.1 Hz), 2.68 (t, 2 H, J = 7.8 Hz), 2.45 (dt, 2 H, J = 7.2, 1.4 Hz), 1.91–1.79 (m, 2 H), 1.16 (t, 6 H, J = 7.0 Hz); MS (EI) 299 (M^+, 10), 255 (100).

Diethyl [[2-[4-(Acetylamino)-4-cyanobutyl]phenyl]methyl]phosphonate (62). A solution of sodium cyanide (0.85 g, 18.9 mmol) and ammonium chloride (1.0 g, 18.7 mmol) in 5 mL of concentrated ammonium hydroxide was treated dropwise with a solution of **60** (2.52 g, 8.45 mmol) in 4 mL of ethanol. The reaction mixture was stirred at room temperature for 24 h, diluted with water (30 mL), and extracted into ethyl acetate (4 × 50 mL). The combined organic extracts were washed with water (3 × 30 mL) and dried over sodium sulfate, filtered, and concentrated.

The residue (2.65 g) was dissolved in pyridine (15 mL), cooled to 0 °C and acetic anhydride (1.52 mL, 16.2 mmol) was added. The resulting solution was stirred at 0 °C for 1 h. The reaction mixture was concentrated; the residue was dissolved in 100 mL of ethyl acetate and washed sequentially with 1 N HCL (20 mL), water (20 mL), and saturated aqueous sodium bicarbonate (20 mL). The organic phase was dried over magnesium sulfate, filtered, and concentrated. Purification by flash chromatography (silica gel, ethyl acetate) furnished **62** (1.48 g, 4.04 mmol, 47.8%) as an oil: ^{1}H NMR ($CDCl_3$, 200 MHz) 7.85 (d, 1 H, J = 6.5 Hz), 7.21–7.06 (m, 4 H), 4.76–4.70 (m, 1 H), 4.07–3.87 (m, 4 H), 3.24–3.04 (m, 2 H), 2.86–2.77 (m, 1 H), 2.69–2.59 (m, 1 H), 1.91 (s, 3 H), 1.90–1.67 (m, 4 H), 1.24 (dt, 6 H, J = 10.5, 7.0 Hz); MS (EI) 366 (M^+).

α-Amino-2-(phosphonomethyl)benzenepentanoic Acid (20). A solution of **62** (1.40 g, 3.82 mmol) in 30 mL of 6 N HCl was heated at reflux for 24 h. The reaction mixture was cooled, decolorized with activated charcoal, filtered, and concentrated. The residue was dissolved in water (20 mL) and freeze-dried. The resulting solid was purified by ion-exchange chromatography (Dowex 50X4 400 ion-exchange resin) eluting with 2 M ammonium hydroxide solution, the freeze-dried eluant was dried under vacuum at 100 °C over phosphorus pentoxide to give **20** (708 mg, 2.22 mmol, 58.2%) as a white solid: ^{1}H NMR (D_2O, 200 MHz) 7.38 (d, 1 H, J = 5.6 Hz), 7.29–7.17 (m, 3 H), 3.75 (t, 1 H, J = 5.5 Hz), 2.99 (d, 2 H, J = 20.5 Hz), 2.84–2.74 (m, 2 H), 2.05–1.83 (m, 2 H), 1.82–1.60 (m, 2 H); MS (FAB) 288 (MH^+, 100). Anal. ($C_{12}H_{18}NO_5P$) C, H, N.

Diethyl [[2-(3,3-Dimethoxypropyl)phenyl]methyl]phosphonate (59). Reaction of 2-methylbenzyl alcohol (20.0 g, 0.164 mol) by the method of Braun[36] described in the preparation of **58** gave the protected hydroxymethylbenzene intermediate (2.91 g, 8.41%) as a pale yellow oil: ^{1}H NMR ($CDCl_3$, 200 MHz) 7.41–7.19 (m, 4 H), 4.72 (s, 2 H), 4.38 (t, 1 H, J = 5.7 Hz), 3.31 (s, 6 H), 2.77 (apparent t, 2 H), 2.20 (brs, 1 H), 1.99–1.94 (m, 2 H); MS (CI) 193 ($MH^+ - H_2O$).

The mesylate was prepared from the above hydroxymethyl derivative (2.91 g, 13.8 mmol) as described in the preparation of **58** and was treated with sodium diethyl phosphite as previously described to give the phosphonomethyl derivative **59** (2.28 g, 7.03 mmol, 50.9%) as an oil.

Diethyl [[2-(3-Oxopropyl)phenyl]methyl]phosphonate (61). Deprotection of **59** (2.28 g, 7.03 mmol) with 1:1 3 N HCl in tetrahydrofuran, as previously described in the preparation of **60**, gave **61** (1.92 g, 100%).

Diethyl [[2-[3-(Acetylamino)-3-cyanopropyl]phenyl]methyl]phosphonate (63). **61** (1.88 g, 6.61 mmol) was treated with sodium cyanide, ammonium chloride, and concentrated ammonium hydroxide as described in the preparation of **62** to give **63** (0.29 g, 0.82 mmol, 12.4%) as an oil: ^{1}H NMR ($CDCl_3$, 200 MHz) 8.24 (d, 1 H, J = 6.7 Hz), 7.22–7.11 (m, 4 H), 4.70–4.64 (m, 1 H), 4.03–3.81 (m, 4 H), 3.25–3.06 (m, 2 H), 2.84–2.74 (m, 2 H), 2.28–2.14 (m, 2 H), 1.87 (s, 3 H), 1.20 (dt, 6 H, J = 17.9, 7.1 Hz); MS (EI) 255 ($M - C_4H_5NO_2$, 100).

α-Amino-2-(phosphonomethyl)benzenebutanoic Acid (19). **63** (279 mg, 0.79 mmol) was hydrolyzed in 6 N HCl and purified on Dowex 50X4 400 as previously described in the preparation of **20** to give **19** (72 mg, 0.23 mmol, 30%) as a tan solid: ^{1}H NMR (D_2O, 200 MHz) 7.37–7.21 (m, 4 H), 3.82–3.78 (m, 1 H), 3.07–2.76 (m, 4 H), 2.36–2.24 (m, 1 H), 2.19–2.05 (m, 1 H); MS (FAB) 274 (MH^+, 100). Anal. ($C_{11}H_{16}NO_5P$) C, H, N.

Biology. Binding of [^{3}H]-3-(2-Carboxypiperazin-4-yl)-propylphosphonic Acid (CPP) to *N*-Methyl-D-aspartate (NMDA) Receptors in Rat Brain Crude Synaptic Mem-

(36) Braun, M.; Ringer, E. *Tetrahedron Lett.* **1983**, *24*, 1233–1234.

branes. Method. Binding assays with [^{3}H]CPP were carried out essentially by methods previously described.[37]

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), DL-2-amino-4-phosphonobutyric acid (AP4), and quisqualate were purchased from Sigma Chemical Co.; CPP was synthesized by Sheryl Hays (Parke-Davis).

Membrane Preparation. Long–Evans male rats (180–200 g) were sacrificed by decapitation, and the whole brain (minus cerebellum and brainstem) was removed and placed in 15 volumes of ice-cold 0.32 M sucrose. The brain was disrupted for 30 s with a glass–Teflon homogenizer (Potter-Elvejhem) at setting 5. The suspension was centrifuged at 1000*g* for 20 min. The pellet (P2) was disrupted in ice-cold water for 30 seconds with a polytron (Brinkman PT-10) homogenizer and centrifuged at 8000*g* for 20 min. The soft, buffy coat was collected with the supernatant, and this suspension was then centrifuged at 48000*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 48 000*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 1, where Y is the percent inhibition, T is the specific binding

$$Y = [(T - S)/T] \times 100 \quad (1)$$

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.[38]

Cell Culture Technique. Sprague–Dawley rats in their 18th day of gestation were placed under halothane anesthesia during uterus removal. The uterus was placed 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 50%/50% 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 (30–70K) 1 day prior 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 4 days following isolation by the addition of 15 μg/mL 5-fluoro-2′-deoxyuridine and 35 μg/mL uridine for a period of 2 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. Base-line 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 base line from control measurements. Previous studies have demonstrated that glutamate-induced toxicity to neurons in related paradigms is blocked specifically by NMDA antagonists.[31,39] 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.

NMDA-Induced Lethality and Ataxia in Mice. Lethal seizures were produced in CF-1 mice by intravenous injection of *N*-methyl-D-aspartic acid (NMDA). Mice weighing 22–28 g were injected behind the lateral aspect of the right eye (retrobulbar) with an appropriate doses of test agent or vehicle control (0.9% saline and a volume of 5 mL/kg), five minutes prior to NMDA injection. Inverted screen ataxia assessments were made immediately prior to NMDA injection using an inverted-screen technique. NMDA (25 mg/kg) dissolved in saline was administered as a bolus left eye retrobulbar injection. Untreated controls develop seizures immediately following NMDA administration. Seizures are between 10–25 s in duration and are terminated by expiration of the animals. Test agents were assessed for their ability to prevent lethality following NMDA injections. ED_{50} doses were determined from dose–response curves constructed from at least three doses of half-log intervals with 10 mice per dose.

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Registry No. **5** (free base), 120667-15-4; **5**·HCl, 120667-18-7; **6**, 120667-19-8; **7**, 120667-20-1; **8** (free base), 120667-16-5; **8**·HCl, 120667-21-2; **9**, 120667-22-3; **10**, 118076-86-1; **11**, 120667-23-4; **12**, 120667-24-5; **13**, 120667-25-6; **14**, 120667-26-7; **15** (free base), 120667-17-6; **15**·HCl, 114791-27-4; **16**, 120667-27-8; **17**, 120710-37-4;

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18, 120710-38-5; 19, 120667-28-9; 20, 120667-29-0; 21 (n = 0), 108-86-1; 21 (n = 2), 103-63-9; 21 (n = 3), 637-59-2; 22, 109125-34-0; 23, 120667-30-3; 24, 120667-31-4; 25, 120667-32-5; 26, 120667-33-6; 27, 120667-34-7; 28, 120667-35-8; 29, 120667-36-9; 30, 120667-37-0; 31, 120667-38-1; 32, 120667-39-2; 33, 120667-40-5; 34, 120667-41-6; 35, 626-15-3; 36, 120667-42-7; 37, 120667-43-8; 38, 120667-44-9; 38 (de-N-acetyl derivative), 120667-59-6; 39, 3433-80-5; 40, 823-78-9; 41, 589-15-1; 42, 120667-45-0; 43, 15017-44-4; 44, 71078-92-7; 45, 117571-56-9; 46, 120667-46-1; 47, 120667-47-2; 48, 120667-48-3; 49, 120667-49-4; 51, 120667-50-7; 52, 110762-21-5; 53, 626-16-4; 54, 623-25-6; 55, 120667-51-8; 56, 114791-26-3; 57, 89-95-2; 58, 120667-52-9; 59, 120667-53-0; 60, 120667-54-1; 61, 120667-55-2; 62, 120667-56-3; 63, 120667-57-4; NMDA, 6384-92-5; 3-BrC_6H_4CHO, 3132-99-8; $H_2C{=}CHP(O)(OEt)_2$, 682-30-4; (±)-$H_2NCH(3\text{-}BrC_6H_4)CN$, 120667-58-5; $EtOCOCH(NHAc)COOEt$, 1068-90-2; $EtOCOC(NHAc)(3\text{-}ClCH_2C_6H_4CH_2)COOEt$, 120667-60-9; $EtOCOC(NHAc)(4\text{-}ClCH_2C_6H_4CH_2)COOEt$, 114791-24-1; $BrCH_2CH(OMe)_2$, 7252-83-7; $2\text{-}(HOCH_2)C_6H_4CH_2CH_2CH(OMe)_2$, 120667-62-1; 2-(2-bromoethyl)-1,3-dioxolane, 18742-02-4; 2-[3-(1,3-dioxolan-2-yl)propyl]benzenemethanol, 120667-61-0.

Dimethylsulfonium Analogues of the Muscarinic Agent McN-A-343: [4-[[*N*-(3- or 4-Halophenyl)carbamoyl]oxy]-2-butynyl]dimethylsulfonium Perchlorates

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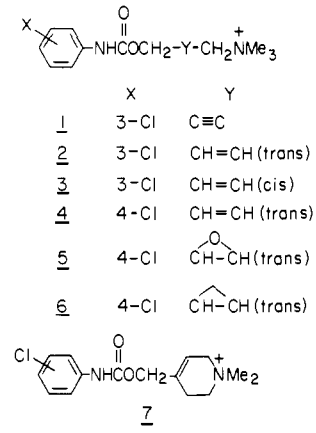
Some 3- and 4-bromophenyl and dimethylsulfonium analogues of the muscarinic agent [4-[[*N*-(3-chlorophenyl)-carbamoyl]oxy]-2-butynyl]trimethylammonium chloride (McN-A-343) (1) were synthesized. The new compounds were assayed for effects on arterial blood pressure in the pithed rat (ganglionic muscarinic activity). The dimethylsulfonium salts (**13a–d**) appeared to be partial agonists in relation to 1. The 4-bromophenyl-substituted trimethylammonium iodide **10d** exceeded 1 in potency by 3-fold. The compounds retained the selectivity for ganglionic muscarinic receptors shown by 1 since they had only weak effects on the guinea pig ileum in vitro.

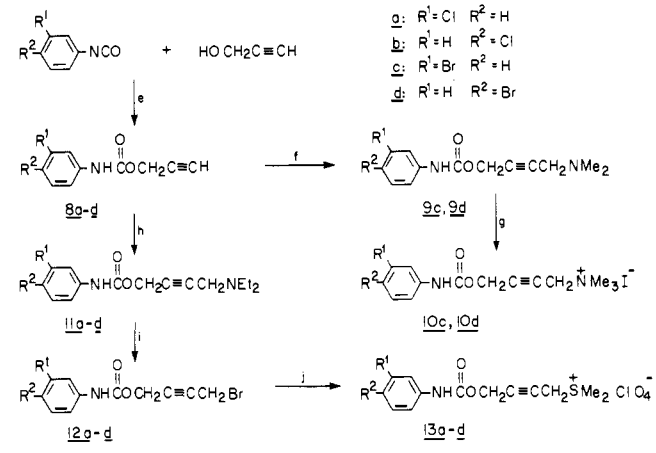
McN-A-343 (1; see Chart I) is potent in stimulating muscarinic receptors in sympathetic ganglia but has only weak direct actions on the heart and on smooth muscles.[1] The selective actions of 1 have been claimed[2,3] to be mediated by a subtype of muscarinic receptors (M1 receptors). The selectivity, however, has also been explained by a combination of low intrinsic efficacy and tissue differences in receptor reserve.[4]

The structural requirements for McN-A-343-like activity appear to be quite specific. Thus N-demethylation of 1 abolished activity whereas replacement of the trimethylammonium group by a triethylammonium group yielded an antagonist. A shift of the chlorine atom of the phenyl ring from the 3- to the 4-position produced a 3-fold enhancement of ganglionic stimulant activity. The corresponding 2-chloro derivative had only one-tenth of the potency of 1.[5] The trans olefinic derivative **2** had about half the potency of 1 as a ganglionic stimulant in the anesthetized cat whereas the cis olefin **3** was much less active. Both **2** and **3** were weak partial muscarinic agonists on the rabbit ileum. The 4-chlorophenyl analogue **4** was more potent than **2** but was less selective.[6] The trans epoxide **5** approached 1 in potency at the ganglion and was very selective since it showed little or no muscarinic activity on smooth muscle. Its cis isomer as well as the cyclopropane derivative **6** was inactive.[7] Some quaternary isoarecolinol derivatives (**7**) of **2** and **4** in which the conformational flexibility of the cationic head is reduced were about one-fifth as potent muscarinic ganglionic stimulants as 1. The corresponding tertiary amines were virtually inactive.[8] Collectively, the structure–activity studies on analogues of 1 showed that a quaternary nitrogen, unsaturation at C-2 of the amino alcohol moiety, and a distance of about 57 nm between the ether oxygen and the ammonium group are necessary for high muscarinic ganglion-stimulating activity.[7,8]

Chart I



Scheme I[a]



[a] Reagents: (e) neat; (f) bis(dimethylamino)methane, HOAc, $CuCl_2$, dioxane; (g) MeI, acetone; (h) $HNEt_2$, CH_2O, $CuCl_2$, dioxane; (i) BrCN, CH_2Cl_2; (j) SMe_2, $AgClO_4$, acetonitrile.

We have shown that substitution of a sulfonium group for the ammonium group in analogues of oxotremorine,

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