Design and Pharmacological Activity of Phosphinic Acid Based NAALADase Inhibitors

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A novel series of phosphinic acid based inhibitors of the neuropeptidase NAALADase are described in this work. This series of compounds is the most potent series of inhibitors of the enzyme described to date. In addition, we have shown that these compounds are protective in animal models of neurodegeneration. Compound **34** significantly prevented neurodegeneration in a middle cerebral artery occlusion model of cerebral ischemia. In addition, in the chronic constrictive model of neuropathic pain, compound **34** significantly attenuated the hypersensitivity observed with saline-treated animals. These data suggest that NAALADase inhibition may provide a new approach for the treatment of both neurodegenerative disorders and peripheral neuropathies.

Introduction

The dipeptide N-acetyl-L-aspartyl-L-glutamate (NAAG) was first identified in the brain in 1965[1] as one of the major peptidic components of the central nervous system. NAAG was found in high levels in the brain stem and spinal cord, however, its physiological function was not determined at the time of its discovery. It was not until the early 1980s when NAAG was reisolated that its role as a potential neurotransmitter was explored.[2] To prove the dipeptide was a neurotransmitter, a method of inactivation of NAAG had to be determined. Toward this end, an enzyme responsible for the catabolism of NAAG was identified in 1987.[3] The enzyme was named NAALADase (N-acetylated-α-linked acidic dipeptidase, EC 3.4.17.21) for its specificity as a dipeptidase. Subsequent in vitro and in vivo studies demonstrated that NAALADase cleaves NAAG into N-acetylaspartate and glutamate.[4] The membrane bound enzyme has an apparent molecular weight of 94 kDa and has the characteristics of a metallopeptidase. Human NAALADase has been cloned and was shown to be similar to both prostate specific membrane antigen (PSMA) and folylpoly-γ-glutamate carboxypeptidase (FGCP).[5,6] In addition, work by Barrett has indicated that this enzyme is a type II protein of the cocatalytic class of metallopeptidases and contains two zinc atoms in the active site.[7] Site-directed mutagenesis studies subsequently confirmed the active site residues surrounding the two zinc atoms.[8] In addition, the mutagenesis studies revealed residues potentially responsible for the substrate specificity of the enzyme.

We became interested in the inhibition of NAALADase as a potential way of treating neurological disorders. Because NAAG may serve as a storage form of glutamate, inhibition of NAALADase should decrease synaptic glutamate levels and be effective in preventing damage in diseases in which excess glutamatergic transmission has been implicated.[9] In addition, NAAG has been shown recently to be an agonist at mGluR3 receptors and a mixed agonist/antagonist at the NMDA receptor.[10] Because mGluR3 agonists are neuroprotective, elevation of NAAG levels through NAALADase inhibition could also be beneficial.

The first potent and selective inhibitor of NAALADase, 2-(phosphonomethyl)pentanedioic acid (2-PMPA, **1**), has been previously described.[11] We have reported that this prototype inhibitor provides neuroprotection in an animal model of cerebral ischemia.[12] In this paper, we describe the SAR and synthesis around a series of phosphinic acid derivatives of 2-PMPA. Importantly, we also provide data showing that inhibition of NAALADase with these compounds prevents neurodegeneration in several animal models of CNS and PNS disorders. The results described herein demonstrate that NAALADase inhibition is a new and novel mechanism for therapeutic intervention in neurodegenerative disorders.[13]

Although compound **1** was reported to be a potent and specific inhibitor of the enzyme, the highly polar nature of the molecule (clogD = −13, pH = 7.4) limits penetration into the brain.[12] We began our work by modifying the acidic portions of compound **1** in order to obtain more lipophilic compounds and in turn a more favorable pharmacological profile. In addition, we hoped to gain an understanding of the steric and electronic requirements for effective inhibition of NAALADase.

Synthesis of Phosphonates and Phosphinic Acids

The phosphonates reported in this work were prepared by the general method shown in Scheme 1.[14] By treating diethyl malonate (**2**) with sodium ethoxide and an alkyl halide in ethanol, we obtained the substituted malonate in good yield. Saponification with potasium hydroxide resulted in compound **3**, which was converted to an α,β-unsaturated acid with formaldehyde and

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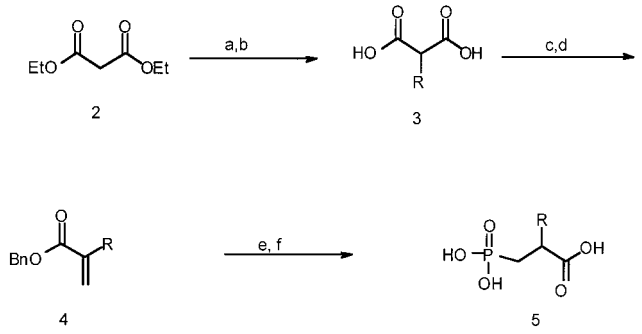
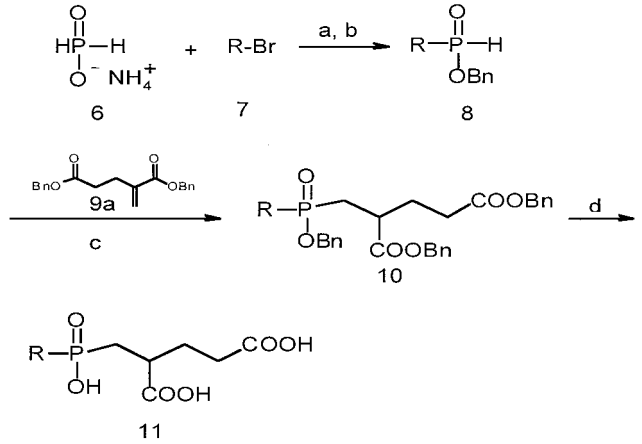
Scheme 1[a]



[a] Reagents: (a) NaOEt, RBr, EtOH, reflux, 8 h; (b) KOH, EtOH, 0 °C to room temperature, 4 h; (c) HCHO, Et_2NH, H_2O, reflux; (d) BnBr, DMF, H_2O, K_2CO_3, rt, 3 h; (e) $(BnO)_2P(O)H$, NaH, THF, 1 h, rt; (f) H_2, Pd/C, H_2O.

Scheme 2[a]



[a] Reagents: (a) HMDS, 100 °C, 2 h; CH_2Cl_2, −78 °C to room temperature, 15 h; (b) HCl, CH_2Cl_2; BnOH, EDC, CH_2Cl_2; (c) NaH, THF, 0 °C to room temperature, 25 min; (d) H_2, Pd/C, H_2O.

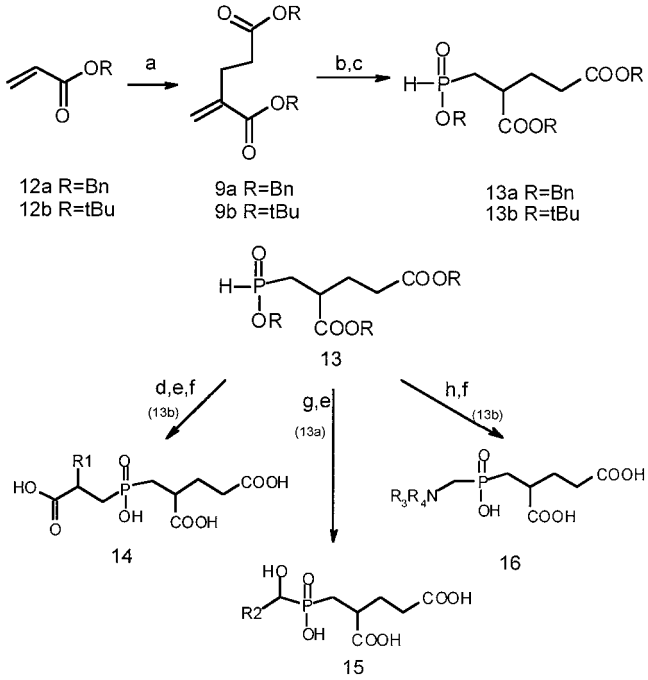
diethylamine. The α,β-unsaturated acid was then converted to benzyl ester **4**. Conjugate addition of dibenzyl phosphite to **4** with NaH (0.4 equiv) as an initiator, followed by hydrogenation of the benzyl protecting groups, provided the monocarboxylic acid phosphonate **5**.

The dicarboxylic phosphinic acids utilized in this work were prepared via several methods, one of which is shown in Scheme 2. A benzyl protected phosphinic acid **8** was prepared by reacting ammonium phosphonate with an alkyl bromide.[15] Phosphinic acid **8** was treated with NaH and the benzyl acrylate dimer **9a** in THF to provide compound **10**. Finally, hydrogenation of the benzyl esters afforded phosphinic acid **11**. Alternatively, an appropriately substituted acrylate (**12**) could be dimerized to give the protected diester **9** (Scheme 3). This was then reacted with ammonium phosphinate and hexamethyldisilazane followed by treatment with tBuOH and EDC to give the protected phosphinate **13**. This could then be utilized as an intermediate in the synthesis of tri and tetra acid derivatives (**14**), α-hydroxy derivatives (**15**), and amino derivatives (**16**) (Table 1).

In Vitro Results

Analysis of the data presented in Table 2 indicates that modification of the propionic acid side chain of compound **1** greatly reduces enzyme inhibition efficacy. The side chain acid is thought to interact with either a

Scheme 3[a]



[a] Reagents: (a) HMPT, 105 °C; (b) ammonium phosphinate, HMDS; (c) *t*BuOH, EDC, CH_2Cl_2; (d) NaH, THF, substituted acrylate; (e) H_2, Pd/C; (f) TFA, DCM; (g) NaH, THF, substituted benzaldehyde; (h) substituted hexahydro-1,3,5-triazine, toluene, 110 °C.

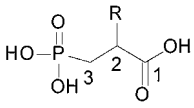
lysine or arginine in the specificity pocket of NAALADase.[6] Shortening the side chain had a greater negative effect than lengthening it (i.e., compounds **17** and **18**), potentially indicating that the propionic acid side chain of compound **1** and the glutamate side chain of NAAG may bind to NAALADase in an extended rather than a folded conformation. The phenyl derivatives **19** and **20** were prepared to see if a favorable cation-π interaction could be achieved with an arginine in the binding pocket. The two aromatic compounds had suprisingly high affinity considering they lacked an acidic functionality. We next prepared compound **21** but did not see an increase in affinity compared to compound **19**. Presumably either the pK_a of the phenol is too dissimilar to that of an acid or there exists an unfavorable orientation of the phenol relative to the basic residues in the binding pocket. Decreasing the pK_a of the phenol via substitution of the ring or changing the position of the hydroxyl group may result in increased affinity for the enzyme. Two additional compounds, nitrile **22** and tetrazole **23**, were prepared but also showed modest activity. Overall, our efforts to alter the propionic acid side chain at C-2 of compound **1** resulted in less potent NAALADase inhibitors.

We then focused on changing the zinc-binding phosphonic acid of compound **1** to a phosphinic acid, leaving the propionic acid side chain unchanged. Table 3 summarizes the compounds prepared via the methods described above and their corresponding IC_{50} values. Within the series of derivatives **24**−**27**, the benzyl derivative **26** demonstrated the best activity. Large groups directly attached to the phosphorus decreased activity (compound **25**). The benzyl series was elaborated to include compounds **28**−**34** but resulted in only a 2-fold decrease in the IC_{50} value. By placing an alkyl

Table 1. Representative Analytical Data

compound	analytical data
dibenzyl 2-methylenepentanedioate (**9a**)	^{1}H NMR ($CDCl_3$) 2.6 (m, 4H), 5.1 (s, 2H), 5.2 (s, 2H), 5.6 (s, 1H), 6.2 (s, 1H), 7.3 (m, 10H)
di-*tert*-butyl 2-methylenepentanedioate (**9b**)	^{1}H NMR ($CDCl_3$) 1.4 (m, 18H), 2.4 (t, 2H), 2.6 (t, 2H), 5.5 (s, 1H), 6.0 (s, 1H)
di-*tert*-butyl 2-[(*tert*-butoxyphosphinyl)methy]-pentanedioate (**13b**)	^{1}H NMR ($CDCl_3$) 1.4 (m, 27 H), 1.8 (m, 1H), 1.9 (m, 2H), 2.1 (m, 1H), 2.3 (m, 2H), 2.7−2.8 (m, 1H), 6.7 & 8.0 (d, 1H, P−H)
2-[[(2-flurobenzylhydoxyphosphinoyl]methyl]-pentanedioic acid (**28**)	^{1}H NMR (D_2O) 1.8−1.9 (m, 3H), 2.0−2.2 (m, 1H), 2.3−2.4 (m, 2H), 2.6−2.7 (m, 1H), 3.28 (d, J = 16.6 Hz, 2H), 7.1−7.5 (m, 4H). Anal. Calcd for $C_{13}H_{16}FO_6P\cdot0.1H_2O$: C, 48.79; H, 5.10. Found: C, 48.84; H, 5.14.
2-[[(4-methylbenzyl)hydroxyphosphinoyl]-methyl]pentanedioic acid (**30**)	^{1}H NMR (D_2O) 1.7−1.9 (m, 3H), 2.13 (ddd, J = 9.3 Hz, 13.3 Hz, 15.5 Hz, 1H), 2.33 (d, J = 2.1 Hz, 3H), 2.40 (dt, J = 2.1 Hz, 7.4 Hz, 2H), 2.5−2.7 (m, 1H), 3.18 (d, J = 16.9 Hz, 2H), 7.23 (s, 4H). Anal. Calcd for $C_{14}H_{19}O_6P\cdot0.30H_2O$: C, 52.60; H, 6.18. Found: C, 52.60; H, 6.28.
2-[[(4-methoxybenzyl)hydroxyphosphinoyl]-methyl]pentanedioic acid (**31**)	^{1}H NMR (D_2O) 1.8−1.9 (m, 3H), 2.0−2.2 (m, 1H), 2.3−2.4 (m, 2H), 2.55−2.70 (m, 1H), 3.16 (d, J = 16.7 Hz, 2H): 3.81 (s, 3H), 6.98 (d, J = 8.7 Hz, 2H), 7.25 (d, J = 8.7 Hz, 2H). Anal. Calcd for $C_{14}H_{19}O_7P\cdot0.30H_2O$: C, 50.09; H, 5.89. Found: C, 49.98; H, 5.80.
2-[[(pentaflurobenzyl)hydroxyphosphinoyl]-methyl]pentanedioic acid (**34**)	^{1}H NMR (D_2O) 1.8−2.0 (m, 3H), 2.1−2.3 (m, 1H), 2.3−2.5 (m, 2H), 2.7−2.9 (m, 1H), 3.29 (d, J = 15.4 Hz, 2H). Anal. Calcd for $C_{13}H_{12}F_5O_6P\cdot0.45H_2O$; C, 39.20; H, 3.26. Found C, 39.17; H, 3.28.
2-[[[2-(benzylcarboxy)propyl]hydroxyphos-phinoyl]methyl]pentanedioic acid (**37**)	^{1}H NMR (D_2O) 1.2 (m, 3 H), 1.6−1.8 (m, 4H), 2.1 (m, 2H), 2.2 (m, 2H), 2.6 (m, 1H), 2.8 (m, 1H), 5.0 (m,2H), 7.3 (m, 5H). Anal. Calcd for $C_{17}H_{23}O_8P\cdot1.0H_2O$: C, 50.50; H, 6.23. Found: C, 50.52; H, 5.92.
2-[[(carboxy)propyl]hydroxyphosphinoyl]-methyl]pentanedioic acid (**38**)	^{1}H NMR (D_2O) 1.2 (d, 3H), 1.9 (m, 4H), 2.2 (m, 2H), 2.4 (m, 2H), 2.8 (m, 2H). Anal. Calcd for $C_{10}H_{17}O_8P\cdot0.2CH_3CN$: C, 41.03; H, 5.83. Found: C, 41.05; H, 5.92.
2-[(methylhydroxyphosphinoyl)methyl]-pentanedioic acid (**39**)	^{1}H NMR ($CDCl_3$) 1.9 (m, 3H), 2.2 (m, 1H), 2.4 (m, 2H), 2.7 (m, 1H) 5.0 (d, 1H), 7.4 (m, 5H). Anal. Calcd for $C_{13}H_{17}O_7P\cdot0.6H_2O$: C, 47.74; H, 5.61. Found: C, 47.73; H, 5.68.
2-[[[benzylamino]methyl](hydoxyphos-phinoyl)methyl]pentanedioic acid (**42**)	^{1}H NMR (D_2O) 1.6−2.0 (m, 4H), 2.2−2.4 (m, 2H), 2.5−2.7 (m, 1H), 3.00 (d, J = 9.0 Hz, 2H), 4.21 (s, 2H), 7.3−7.5 (m, 5H)
2-[[[phenylamino]methyl](hydoxyphos-phinoyl)methyl]pentanedioic acid (**43**)	^{1}H NMR (D_2O) 1.6−2.0 (m, 4H), 2.2−2.4 (m, 2H), 2.5−2.7 (m, 1H), 3.53 (d, J = 8.8 Hz, 2H), 7.3−7.5 (m, 5H)
2-[[[4-methoxyphenylamino]methyl]-(hydoxyphosphinoyl)methyl]-pentanedioic acid (**44**)	^{1}H NMR (D_2O) 1.2−1.3 (m, 1H), 1.6−1.7 (m, 3H), 2.2 (m, 2H), 2.3−2.5 (m, 1H), 3.4 (d, J = 8.9 Hz, 2H), 3.7 (s, 3H), 7.0 (d, J = 12 Hz, 2H), 7.4 (d, J = 12 Hz, 2H)

Table 2[a]



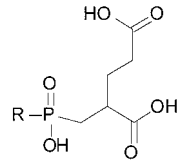
compd	R	IC_{50} (nM)	SD
1	CH_2CH_2COOH	0.3	±0.05
17	CH_2CO_2H	2200	±1960
18	$CH_2CH_2CH_2COOH$	185	±14
19	CH_2Ph	548	±160
20	CH_2CH_2Ph	199	±106
21	CH_2(3-OH)Ph	508	±412
22	CH_2CH_2CN	335	±64
23	$CH_2CH_2(CN_4)$	175	±35

[a] IC_{50} values were determined as previously described in ref 11, with each value representing two to four independent determinations. All of the data reported are for the racemic compounds.

acid group on the phosphorus, we were able to subsequently increase activity (i.e., compounds **35**−**38**).[16]

Because NAALADase contains two zincs in the active site, we next examined a series of compounds containing an additional functional group that could interact with the metal ions. We hoped to recover some of the potency lost by modifying the phosphonic acid in **1** to a phosphinic acid. Toward this end, a series of hydroxy- and methylaminophosphinyl derivatives (**39**−**44**) were prepared. The most potent compounds in the series were **43** and **44**, both aniline derivatives. The low IC_{50} values (4 nM) of the aniline derivatives could be due to an additional interaction between a zinc atom and the amino group.[17] Currently, we are using modeling techniques to examine the interactions of these compounds with both zinc atoms in the active site.

Table 3[a]



compd	R	IC_{50} (nM)	SD
24	*n*-C_3H_7	360	±124
25	Ph	2930	±1250
26	CH_2Ph	53	±33
27	CH_2CH_2Ph	149	±78
28	CH_2(2-F-Ph)	156	±35
29	CH_2(3-NH_2-Ph)	143	±53
30	CH_2(4-CH_3-Ph)	68	±0
31	CH_2(4-OCH_3-Ph)	90	±22
32	CH_2(3,5-CF_3-Ph)	55	±6
33	CH_2(3,5-F-Ph)	49	±16
34	CH_2 (2,3,4,5,6-F-Ph)	82	±14
35	$CH_2CH(CH_2CH_2CO_2H)CO_2H$	0.5	±0.1
36	$CH_2CH(CH_2Ph)CO_2H$	2	±1.8
37	$CH_2CH(CH_3)CO_2Bn$	95	±7.1
38	$CH_2CH(CH_3)CO_2H$	1.5	±0.71
39	CH(OH)Ph	55	±9
40	CH(OH)-4-pyridyl	10	±1.8
41	CH(OH)-(3-F-Ph)	16	±9
42	CH_2 $NHCH_2Ph$	59	±14
43	CH_2NHPh	4	±0
44	CH_2NH(4-OCH_3-Ph)	3	±2.6

[a] IC_{50} values were determined as previously described in ref 11, with each value representing two to four independent determinations. All of the data reported are for the racemic compounds.

NAALADase Inhibition Is Protective in a Rat Middle Cerebral Artery Occlusion Model

Compound **1** was initially used as a prototype compound in the rat transient middle cerebral artery

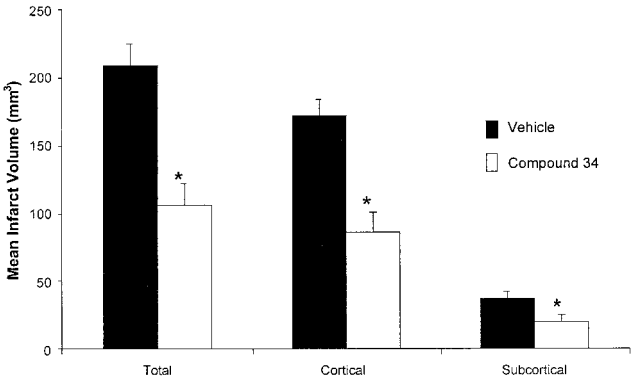


Figure 1. Effect of compound **34** in a transient MCAO model of cerebral ischemia. As described previously, ischemia was produced using a monofilament nylon suture inserted through the internal carotid artery for 2 h. When administered 1 h post ischemia, compound **34** decreased total infarct volume.

occlusion model using the intraluminal filament technique. This compound was shown to be neuroprotective when administered at 60 min post-occlusion (100 mg/kg bolus followed by 20 mg/kg/h iv infusion, 30% protection, $p < 0.05$). A high dose was required due to the low brain levels obtained with this compound.[12] Compound **34** also showed efficacy in this model. For example, when this compound was dosed at 30 mg/kg iv bolus, followed by 6 mg/kg/h iv infusion for 4 h, compound **34** reduced mean total infarct injury by 50% ($p < 0.0001$), as compared to the saline treatment group (Figure 1). In addition, this compound was efficacious at this dose when administered up to 2 h post occlusion, whereas compound **1** was only effective when administered up to 90 min post occlusion. This is remarkable due to the fact that compound **34** is at least 200 times less effective at inhibiting NAALADase in vitro when compared to compound **1**.

NAALADase Inhibition Shows Efficacy in Neuropathic Pain Models

We have also evaluated the utility of NAALADase inhibition for the potential treatment of neuropathic pain. Glutamate is thought to be involved in nociception, and several reports have suggested that glutamate antagonists show efficacy in animal models of pain.[18] More recently, NAALADase has been shown to be present in the peripheral nervous system. Specifically, NAALADase has been shown to be involved in Schwann cell-signaling and appears to be downregulated as myelination begins.[19] Using the chronic constrictive injury model of neuropathic pain, we examined the efficacy of compound **34**.[20] Following sciatic nerve ligation, rats were treated with compound **34** and tested using a Hargreaves apparatus. The difference score (between the latency of the response for the paw on the operated side versus the control side) was determined. Animals received compound **34** (10 mg/kg ip daily) or vehicle starting 10 days post surgery. Treatment with this compound dramatically normalized the difference scores between the two paws compared to the continued hyperalgesic vehicle-treated controls (Figure 2). This effect was significant starting at 12 days of drug treatment and persisted through to the end of the study (for 23 days of daily dosing).

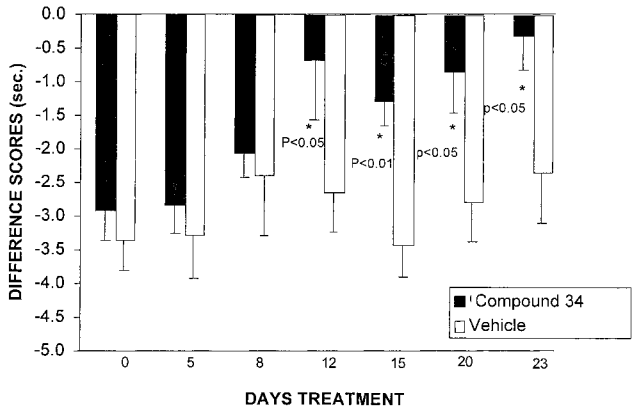


Figure 2. Chronic constrictive injury model of neuropathic pain. Sciatic nerve ligation, consisting of four ligatures being tied loosely around the sciatic nerve at 1 mm intervals proximal to the nerve trifurcation, was performed on rats. As shown, compound **34** significantly attenuated chronic constrictive injury-associated hyperalgesia.

Summary

We have synthesized a series of phosphinic acid based NAALADase inhibitors. The compounds require an acidic moiety in the propionic acid portion of the molecule to obtain good in vitro potency. Alternatively, in the opposing section of the phosphinic acids, a polar group enhances activity. The enhanced activity may be due to an additional interaction with a zinc atom or an amino acid residue near the catalytic site. We have demonstrated for the first time that phosphinic acid containing NAALADase inhibitors show efficacy in animal models of neurodegeneration. Inhibition of this enzyme represents a novel strategy for neuroprotection and may have utility in a variety of disorders such as peripheral neuropathy.

Experimental Section

Chemistry. NMR spectra were recorded on a Bruker 400 instrument. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. Analytical thin-layer chromatography (TLC) was conducted on prelayered silica gel GHLF plates (Analtech, Newark, DE) Visualization of the plates was accomplished by using UV light, phosphomolybdic acid-ethanol, and/or iodoplatinate charring. Flash chromatography was conducted on Kieselgel 60, 230−400 mesh (E. Merck, Darmstadt, West Germany). Solvents are either reagent or HPLC grade. Reactions were run at ambient temperature and under a nitrogen atmosphere unless otherwise noted. Solutions were evaporated under reduced pressure on a Buchi rotary evaporator. Elemental analysis was performed by Atlantic Microlabs, Norcross, GA.

Dibenzyl 2-Methylenepentanedioate (9a). Benzyl acrylate (500 g, 3.0 mol) was heated to 100 °C under an atmosphere of nitrogen. The heating was stopped, and HMPT (10 g, 61 mmol) was added dropwise while maintaining an internal temperature of 135−145 °C. Once addition was complete, the mixture was cooled to room temperature and a slurry of silica with 5:1 hexanes/EtOAc was added. The slurry was then transferred to a column containing a plug of dry silica. The column was then washed with 1:1 Hex/EtOAc and the solvent was collected and evaporated. The clear yellow liquid was distilled under high vacuum (200 μHg) to give an initial fraction of 8 g distilling at 45 °C and then the desired product at 180−185 °C. This afforded 212 g (42%) of a clear and colorless liquid.

Di-*tert*-butyl 2-Methylenepentanedioate (9b). *tert*-Butyl acrylate (465 g, 3.6 mol) was warmed to 100 °C under nitrogen, then HMPT (10 g, 61.2 mmol) was added dropwise, and the

addition rate was adjusted to maintain the reaction temperature at 100 °C. The reaction mixture was allowed to cool, then poured over a plug of silica with 4:1 hexane/ethyl acetate. The solvent was removed under reduced pressure, and the resulting oil was distilled to afford 300 g of the product (65%, bp 67−70 °C at 300 μm) as a clear oil.

Di-*tert*-butyl 2-[(*tert*-Butoxyphosphinyl)methy]pentanedioate (13b). A mixture of ammonium phosphinate (162.6 g, 1.96 mol) and hexamethyldisilazane (316 g, 1.96 mol) was heated to 105 °C for 2 h. The reaction mixture was cooled in an ice bath and di-*tert*-butyl 2-methylenepentane-1,5-dioate (251 g, 0.979 mol) dissolved in dichloromethane (1000 mL) was added dropwise. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was then quenched with distilled water (500 mL), the layers were separated, and the aqueous layer was washed with dichloromethane. The combined organic layers were dried over $MgSO_4$, and the solvent was removed under reduced pressure. This afforded a slightly yellow oil (315 g), which was used without further purification.

To a solution of di-*tert*-butyl 2-[(hydroxyphosphinyl)methyl]-pentane-1,5-dioate (315 g, 0.98 mol) in dichloromethane (1000 mL) cooled in an ice bath and under nitrogen were added *tert*-butyl alcohol (123 g, 1.7 mol), 4-(dimethylamino)pyridine (1 g, 8.2 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (281 g, 1.47 mol). The reaction was allowed to stir overnight. Water was added to the reaction mixture, the dichloromethane layer was retained and dried, and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography, and the desired product was eluted with 1:1 to 2:3 hexane/ethyl acetate. This afforded 260 g of the desired product as a clear oil (70%).

Preparation of 2-[[(4-Methylbenzyl)hydroxyphosphinoyl]methyl]pentanedioic Acid (30). Hexamethyldisilazane (21.1 mL, 100 mmol) was added to vigorously stirred ammonium phosphate (8.30 g, 100 mmol), and the resulting suspension was stirred at 105 °C for 2 h. A solution of 4-methylbenzyl bromide (5.00 g, 27.0 mmol) was then added dropwise to the suspension at 0 °C. The mixture was stirred at room temperature for 19 h. The reaction mixture was then diluted with dichloromethane (50 mL) and washed with 1 N HCl (50 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated to give 4.72 g of a white solid. This was dissolved in dichloromethane (50 mL), and benzyl alcohol (3.24 g, 30 mmol) was added to the solution. 1,3-Dicyclohexylcarbodiimide (DCC) (6.19 g, 30 mmol) was then added to the solution at 0° C, and the suspension was stirred at room temperature for 14 h. The solvent was removed under reduced pressure, and the residue was suspended in EtOAc. The resulting suspension was filtered, and the filtrate was concentrated. The residue was purified by silica gel chromatography (hexanes:EtOAc, 4:1 to 1:1) to give 2.40 g of 4-methylbenzyl-*o*-benzylphosphinic acid as a white solid (34% yield): R_f 0.42 (EtOAc); ^{1}H NMR (DMSO-d_6) 2.30 (s, 3H), 3.29 (d, J = 16.6 Hz, 2H), 5.2 (m, 2H), 7.0 (d, J = 543 Hz, 1H), 7.1−7.2 (m, 4H), 7.3−7.4 (m, 5H).

To a solution of 4-methylbenzyl-*o*-benzylphosphinic acid (2.16 g, 8.3 mmol) in THF (15 mL) was added sodium hydride (0.10 g, 60% dispersion in oil) followed by dibenzyl 2-methylenepentanedioate at 0° C, and the mixture was stirred at room temperature for 4 h. The reaction mixture was then diluted with EtOAc (50 mL) and poured into 1 N HCl (50 mL). The organic layer was separated, dried over Na_2SO_4, and concentrated. This material was purified by silica gel chromatography (hexanes:EtOAc, 4:1 to 1:1) to give 3.41 g of 2,4-di-(benzyloxycarbonyl)butyl(4-methylbenzyl)-*o*-benzylphosphinic acid as a colorless oil (70% yield): R_f 0.56 (EtOAc); ^{1}H NMR ($CDCl_3$) 1.6−1.8 (m, 1H), 1.9−2.0 (m, 2H), 2.1−2.4 (m, 6H), 2.7−2.9 (m, 1H), 3.05 (dd, J = 9.0, 16.8 Hz, 2H), 4.8−5.1 (m, 6 H), 7.0−7.1 (m, 4H), 7.2−7.4 (m, 15 H).

To a solution of 2,4-di(benzyloxycarbonyl)butyl(4-methylbenzyl)-*o*-benzylphosphinic acid (0.70 g, 1.2 mmol) in ethanol (30 mL) was added Pd/C (5%, 0.10 g), and the suspension was shaken under hydrogen (50 psi) for 18 h. The suspension was then filtered through a pad of Celite and concentrated under reduced pressure. The resulting residue was dissolved in distilled water (5 mL), passed through a column of AG 50W-X8 resin (H+ form), and lyophilized to give 0.21 g of 2-[[(4-methylbenzyl)hydroxyphosphinoyl]methyl]pentanedioic acid as a white solid (55% yield).

2-[[[2-(Benzylcarboxy)propyl]hydroxyphosphinoyl]-methyl]pentanedioic Acid (37). To a solution of di-*tert*-butyl 2-[(*tert*-butoxyphosphinyl)methyl]pentanedioate (13.6 g, 36 mmol) and benzyl methacrylate (6.35 g, 36 mmol) in THF (100 mL) under nitrogen was added sodium hydride (0.14 g, 60 5 dispersion in oil, 3.60 mmol). After 3 h, the reaction mixture was poured into water (300 mL), and ether (100 mL) was added. The organic layer was separated, and the combined organic extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The resulting residue was purified by column chromatography, and the product was eluted with 2:3 EtOAc/hexane. This afforded 10.5 g (53%) as a clear oil. ^{1}H NMR ($CDCl_3$) 1.3 (m, 3 H), 1.5 (m, 27H), 1.7 (m, 2H), 1.9 (m, 2H), 7.3 (m, 5H). To a solution of di-*tert*-butyl 2-[[[2-(benzylcarboxy)propyl]*tert*-butoxyphosphinyl]methyl]-pentanedioate (1.6 g, 2.9 mmol) in dichloromethane (10 mL) under nitrogen was added trifluoroacetic acid (10 mL). The reaction mixture was stirred for 2 h and then concentrated under reduced pressure. Additional DCM was added to the reaction residue, and the solvent was removed under reduced pressure. The product was dissolved in EtOAc, washed with water, dried over $MgSO_4$, and the solvent was removed under reduced pressure leaving 800 mg of the desired product as a clear oil (72%).

2-[[(Carboxy)propyl]hydroxyphosphinoyl]methyl]-pentanedioic Acid (38). A solution of 2-[[[2-(benzylcarboxy)-propyl]hydroxyphosphinyl]methyl]pentanedioic acid (8.9 g, 16.1 mmol), palladium on carbon catalyst (10%, 1.0 g), and ethyl acetate (100 mL) was shaken under hydrogen (60 psi) for 16 h. The reaction mixture was filtered over Celite, and the filtrate was concentrated under reduced pressure leaving 7.5 g of a clear oil. To a solution of di-*tert*-butyl 2-[[[2-(carboxy)-propyl]*tert*-butoxyphosphinyl]methyl]pentanedioate (2.1 g, 4.53 mmol) in DCM (10 mL) under nitrogen was added TFA (10 mL). The reaction mixture was stirred for 2 h and then concentrated under reduced pressure. Additional DCM was added and removed under reduced pressure. The resulting residue was triturated with acetonitrile, then dried under reduced pressure leaving the product as a thick clear oil (1.2 g, 89%).

Preparation of 2-[(Methylhydroxyphopshinoyl)methyl]pentanedioic Acid (39). Dry phosphinic acid (100 g, 1.52 mol) was dissolved in 100 mL of $CHCl_3$ and treated with triethylamine (155 g, 1.52 mol). The mixture was evaporated and transferred to a 3 L flask, containing 750 mL of $CHCl_3$. The solution was stirred by means of a mechanical stirrer and the flask cooled to 0 °C. The clear solution was treated with triethylamine (277 g, 2.72 mol) followed by trimethylsilyl chloride (281 g, 2.58 mol). Once addition was complete, dibenzyl 2-methylenepentanedioate in 150 mL of $CHCl_3$ was added, and the mixture warmed to room temperature. After 6 h the thick slurry was filtered and the filtrate cooled to 0 °C. The filtrate was then quenched with 5% HCl and the organic layer removed. The aqueous layer was extracted with $CHCl_3$, the combined organic layers were dried, and the solvent was removed under reduced pressure to afford 55 g of 2,4-di-(benzyloxycarbonyl)butylphosphinic acid as a light yellow liquid. This was purified by flash chromatography and eluted using 3:1 hexanes/EtOAc containing 5% TFA to give 40 g of 2,4-di(benzyloxycarbonyl)butylphosphinic acid. ^{1}H NMR ($CDCl_3$) 2.0 (d, 3H), 2.2 (m, 1H), 2.4 (t, 2H), 2.9 (m, 1H), 5.1 (s, 2H), 7.2 (d, 1H), 7.3 (m, 10H).

To a solution of 2,4-di(benzyloxycarbonyl)butylphosphinic acid (19.3 g, 49,4 mmol) in THF was added benzyl alcohol (5.3 g, 49 mmol) and DMAP (0.5 g). Dicyclohexylcarbodiimide (12 g, 58 mmol) was added, and a white precipitate formed. After 30 min, the white suspension was filtered and the filtrate evaporated under reduced pressure. The clear and colorless

oil was purified by flash chromatography and eluted with 1:1 hexanes/EtOAc to give 2,4-di(benzyloxycarbonyl)butylbenzylphosphinic acid (11.5 g) as a clear and colorless oil. ^{1}H NMR ($CDCl_3$) 1.9 (m, 3H), 2.2 (m, 3H), 2.9 (t, 1H), 5.0 (m, 6H), 7.2 (d, 1H), 7.3 (m, 15H).

2,4-Di-(benzyloxycarbonyl)butylbenzylphosphinic acid in 5 mL of THF was added dropwise to a stirring, cooled mixture of sodium hydride (0.09 g, 2.3 mmol) in 15 mL of THF. After 15 min benzaldehyde (0.23 g, 2.2 mmol) was added via syringe while maintaining a temperature of 0 °C. After 30 min, the reaction was quenched with water and extracted with DCM. The solvent was removed under reduced pressure to give a clear colorless oil which was purified using flash chromatography using 1:1 hexane/EtOAc. This afforded 0.4 g (33%) of 2,4-di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]-benzylphosphinic acid as a clear and colorless oil. ^{1}H NMR ($CDCl_3$) 1.9 (m, 3H), 2.2 (m, 3H), 2.8 (dm, 1H), 4.9 (m, 6H), 5.2 (m, 1H), 7.3 (m, 20H).

2,4-Di(benzyloxycarbonyl)butyl[hydroxy(phenyl)methyl]-benzylphosphinic acid (0.37 g, 0.6 mmol) in 25 mL of water containing 0.10 g of 10% Pd/C was hydrogenated at 40 psi for 6 h. The mixture was filtered through a pad of Celite and lyophilized to give 0.14 g (70%) of 2-[(methylhydroxyphosphinyl)methyl]pentanedioic acid as a white solid.

Synthesis of 2-[[[Benzylamino]methyl](hydoxyphosphinoyl)methyl]pentanedioic Acid (42). A solution of 1,3,5-tribenzylhexahydro-1,3,5-triazine (14.30 g, 40.0 mmol) and di-*tert*-butyl 2-[[[tert-butoxy]phosphinyl]methyl]pentane-1,5-dioate (37.85 g, 100 mmol) in toluene (200 mL) was stirred at 110 °C for 14 h. The solvent was removed under reduced pressure, and the residual yellow oil was purified by silica gel chromatography (hexanes/ethyl acetate 2:1) to give 23.40 g of di-*tert*-butyl 2-[[[*tert*-butoxy](benzyl amino)methyl]phosphinoyl]methyl]-pentane-1,5-dioate as a light yellow oil (43%). ^{1}H NMR ($CDCl_3$) 1.40−1.48 (m, 27H), 1.7−2.1 (m, 4H), 2.2−2.4 (m, 3H), 2.6−3.0 (m, 3H) 3.8−4.0 (m, 2H), 7.2−7.4 (m, 5H).

To a solution of 2-[[[*tert*-butoxy](benzyl amino)methyl]-phosphoryl]methyl]pentane-1,5-dioate (0.498 g, 1.0 mmol) in DCM (10 mL) was added TFA (5 mL) at O °C, and the mixture was stirred at room temperature for 18 h. The solvent was removed under reduced pressure, the resulting taken up in DCM (10 mL), and the solvent was removed under reduced pressure. This process was repeated three times to remove the excess TFA. The resulting oil was crystallized from methanol to give 0.174 g of 2-[[[benzylamino]methyl](hydroxyphosphinoyl)methyl]pentanedioic acid as a white solid.

In Vivo Efficacy Studies. The rat middle cerebral artery occlusion model was performed as previously described.[12] The chronic constrictive injury model of neuropathic pain was performed following the procedure of Bennett.[20] In brief, sciatic nerve ligation, consisting of four ligatures being tied loosely around the sciatic nerve at 1 mm intervals proximal to the nerve trifurcation, was performed on rats. Following this treatment, rats exhibit a thermal hyperalgesia and allodynia. Animals were habituated to the Hargreaves apparatus, and the infrared heat source was directed onto the dorsal surface of the hindpaw and the time taken for the animal to withdraw its paw noted. The difference score (between the latency of the response for the paw on the operated side versus the control side) was determined. Animals received the test compound (10 mg/kg i.p. daily) or vehicle, starting 10 days post surgery. Treatment with compound **34** normalized the difference scores between the two paws compared to the continued hyperalgesic vehicle-treated controls.

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