Design, Synthesis, and Biological Activity of a Potent Inhibitor of the Neuropeptidase N-Acetylated α-Linked Acidic Dipeptidase

Paul F. Jackson,*[,†,§] Derek C. Cole,[†] Barbara S. Slusher,[‡,§] Susan L. Stetz,[‡] Laurie E. Ross,[‡] Bruce A. Donzanti,[‡] and Diane Amy Trainor[†]

Departments of Medicinal Chemistry and Pharmacology, ZENECA Pharmaceuticals, Wilmington, Delaware 19897

Received October 17, 1995[⊗]

A series of substituted phosphonate derivatives were designed and synthesized in order to study the ability of these compounds to inhibit the neuropeptidase N-acetylated α-linked acidic dipeptidase (NAALADase). The molecules were shown to act as inhibitors of the enzyme, with the most potent (compound **3**) having a K_i of 0.275 nM. The potency of this compound is more than 1000 times greater than that of previously reported inhibitors of the enzyme. NAALADase is responsible for the catabolism of the abundant neuropeptide *N*-acetyl-L-aspartylglutamate (NAAG) into *N*-acetylaspartate and glutamate. NAAG has been proposed to be a neurotransmitter at a subpopulation of glutamate receptors; alternatively, NAAG has been suggested to act as a storage form of synaptic glutamate. As a result, inhibition of NAALADase may show utility as a therapeutic intervention in diseases in which altered levels of glutamate are thought to be involved.

Introduction

The dipeptide *N*-acetyl-L-aspartate-L-glutamate (NAAG) is a major peptidic component of the brain, with levels comparable to that of the major inhibitory neurotransmitter γ-aminobutyric acid (GABA). Although NAAG was first isolated in 1964, there was little activity toward elucidating its role in the central nervous system (CNS) until the deleterious nature of excess glutamate in a variety of disease states became apparent. Due to its structural similarity to glutamate, NAAG has been suggested to have a variety of roles similar to those of glutamate itself, including functioning as a neurotransmitter or a cotransmitter in the CNS.[1] In 1985, NAAG was reported to cause excitation of neurons of the lateral olfactory tract; however, it was subsequently demonstrated that these findings were due to an artifact of the preparation.[2] More recently, NAAG was shown to elicit excitatory responses both *in vitro* and *in vivo*.[3] However, under both scenarios, NAAG was less potent than glutamate. In 1988, a brain enzyme was identified which hydrolyzes NAAG to *N*-acetylaspartate (NAA) and glutamate (Figure 1).

This enzyme was found to be specific for the cleavage of N-acetylated α-linked acidic dipeptides and as a result was named NAALADase.[4] The enzyme is a membrane-bound peptidase, with an apparent weight of 94 kDa. NAALADase exhibits a requirement for divalent cations and is inhibited by known metal-chelating agents, suggesting that it belongs to the class of metallopeptidases. The identification and subsequent purification of NAALADase led to the proposal of another role for NAAG: specifically that the dipeptide may serve as a storage form of synaptic glutamate.

In order to further ascertain the exact role of NAALADase and NAAG in the CNS, a number of *in vitro* and *in vivo* studies have been conducted. For example, altered levels of NAAG and NAALADase have been reported in animal models of epilepsy and amyotrophic lateral sclerosis (ALS)[5,7] and in postmortem brain tissue of patients with ALS, schizophrenia, and Alzheimer's disease.[6,8,9] The further investigation of NAALADase activity, however, has been limited by the availability of potent and selective inhibitors of this enzyme. Although there have been several inhibitors of NAALADase reported, they have only weak affinity and specificity for the enzyme.[10,11] As a result, there is a need for the development of potent inhibitors of NAALADase in order to ascertain its role in the CNS. In this paper, we describe the synthesis and activity of such an inhibitor.

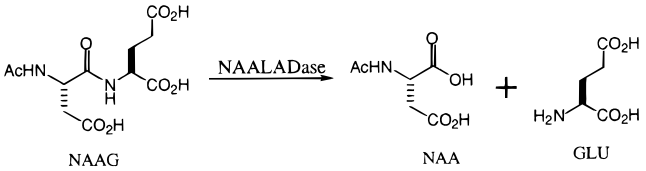


Figure 1. Catabolism of NAAG by the peptidase NAALADase.

Chemistry

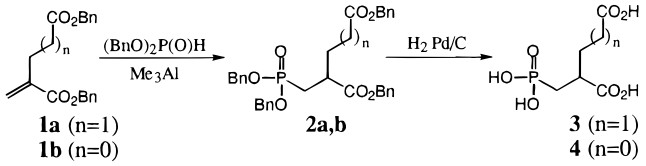
Since there was substantial evidence that NAALADase was a metallopeptidase, we initially focused our attention on small molecules with functional groups that are known to inhibit metallopeptidases, such as hydroxyphosphinyl derivatives, thio derivatives, and hydroxamic acids.[12] On the basis of an initial screening of compounds from our corporate compound collection which contained these functional groups, we began to examine a variety of hydroxyphosphinyl derivatives. In addition, on the basis of literature precedent, we made the further assumption that the glutamate moiety of NAAG was important for recognition by the enzyme and that the aspartate region played a less critical role.[13] As a result, we focused our attention on a series of glutamate-derived hydroxyphosphinyl derivatives whose synthesis is outlined in Schemes 1 and 2.

Treatment of commercially available benzyl acrylate with hexamethylphosphoramide (HMPA) afforded the

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmacology.
[§] Current address: Guilford Pharmaceuticals, 6611 Tributary St., Baltimore, MD 21224.
[⊗] Abstract published in *Advance ACS Abstracts,* December 15, 1995.

0022-2623/96/1839-0619$12.00/0

Scheme 1



Scheme 2

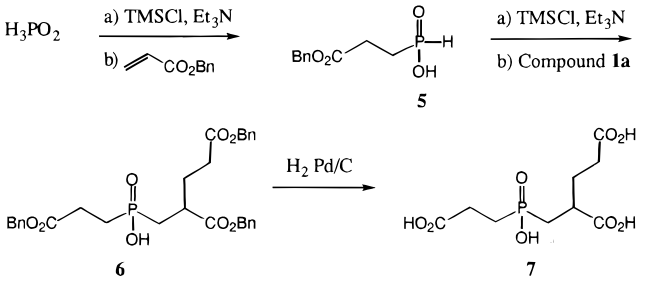


Table 1. *In Vitro* Activity of NAALADAse Inhibitors

compd	K_i (nM)
3	0.275 ± 0.08
4	700 ± 67.3
7	1.89 ± 0.19

α-methylene dibenzyl glutamate **1a**[14] in 60% yield (Scheme 1). A trimethylaluminum-promoted conjugate addition of dibenzyl phosphite then gave the fully protected compound **2a**[15] (50% yield) which was deprotected to afford the (phosphonomethyl)pentanedioic acid **3** in 90% yield. Compound **4** was also prepared by this route starting with the dibenzyl itaconate **1b**[16] (40% overall yield).

The disubstituted phosphinic acid **7** was prepared as shown in Scheme 2. Conjugate addition of the *in situ* generated bis(trimethylsilyl)phosphonite to benzyl acrylate afforded the protected phosphinate **5**. Repetition of the *in situ* phosphonite generation followed by conjugate addition to α-methylene dibenzyl glutarate **1a** provided the protected triester **6**, which was then deprotected to afford compound **7** (Scheme 2).[17,18]

Results and Discussion

Compounds **3**, **4**, and **7** were tested for inhibition of NAALADase activity. Of the compounds tested, the phosphonate **3** showed the best activity, with a K_i of 0.275 nM (Table 1). *The activity of this compound is >1000 times more potent than that of previously described inhibitors.*

The aspartate analog of compound **3** was also prepared. This molecule (**4**) showed a large decrease in efficacy in inhibiting the activity of NAALADase (Table 1), suggesting that a glutamate analog attached to the phosphonic acid is required for potent inhibition of the enzyme. In addition, compound **7**, which contains an additional carboxylic acid side chain similar to the aspartate residue found in NAAG, did not lead to an increase in potency.

The effect of NAALADase inhibition by compound **3** was subsequently examined *in vivo* using intracranial microdialysis, a technique that allows the assessment of endogenous glutamate levels and release in the extracellular space of brain tissue.[19] Male Sprague–Dawley rats (300–350 g) were anesthetized with urethane (1.5 g/kg, ip) and placed in a stereotaxic frame.

Table 2. Effects of Compound **3** on Basal Glutamate Efflux and KCl-Evoked Glutamate Release *in Vitro*[a]

concentration[b] (nM)	basal efflux (%)	K^+-evoked release (% of base line)
control	100	285 ± 16
0.1	151 ± 33	316 ± 27
1.0	137 ± 8	368 ± 46
10.0	167 ± 24	437 ± 99[c]

[a] For all studies, Sprague–Dawley rats were used; $n = 3$–6. See ref 20 for protocol. [b] Compound dissolved in artificial cerebrospinal fluid. [c] $p < 0.05$ vs control.

Microdialysis probes were then lowered into the striatum, a brain region which contains NAAG, NAALADase, and glutamatergic nerve terminals. Ten minute dialysate samples were collected and analyzed for glutamate content using HPLC with electrochemical detection as previously described.[20] Our findings demonstrate that compound **3**, when perfused directly through the dialysis probe, did not decrease the basal or potassium-evoked levels of synaptic glutamate (Table 2). Thus, these results tentatively argue against NAAG serving as a storage form of synaptically available glutamate. It is possible, however, that in our experiments NAAG could have multiple activities including acting as an agonist at presynaptic glutamate receptors or interacting with the glutamate transporters.[21] Further studies will be necessary to explore these possibilities and will be reported in due course.

In conclusion, we have synthesized a potent inhibitor of the neuropeptidase NAALADase. This compound is greater than 1000 times more potent than previously described inhibitors and should serve as a useful pharmacological tool in order to ascertain the function of NAAG in the CNS.

Experimental Section

Chemistry. Proton NMR spectra were obtained using a Bruker AM-300 spectrometer. Chemical shifts are reported in parts per million relative to tetramethylsilane as internal standard. Mass spectra (MS) were recorded on a Kratos MS-80 instrument operating in the chemical ionization (DCI) mode. Elemental analyses for carbon and hydrogen were determined by the ZENECA Pharmaceuticals Analytical Department on a Perkin-Elmer 241 elemental analyzer and are within ±0.4% of theory for the formula given. 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 were 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. HPLC purification was carried out on a Dynamax 300 Å C8 21.4 × 250 mm reversed phase column with a Rainin HPXL solvent delivery system. Fractions were analyzed on a Zorbax Rx-C8 4.6 × 250 mm reversed phase column with a Hewlett Packard HP 1090 liquid chromatograph and a Hewlett Packard HP 1047A RI detector.

Dibenzyl 2-Methylenepentanedioate (1a). HMPA (2.06 g, 11.5 mmol) was added to benzyl acrylate (19.33 g, 119 mmol), and the mixture stirred at room temperature for 16 h. The crude material was then applied directly to a silica gel flash chromatography column (4 × 18 cm) and subsequently eluted with a hexanes–EtOAc (19:1 → 9:1) gradient. This afforded compound **1a** as a clear oil (11.5 g, 59.5%): TLC R_f 0.23 (4:1 hexanes–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.55–2.60 (m, 2 H), 2.65–2.71 (m, 2 H), 5.10 (s, 2 H), 5.19 (s, 2 H),

5.59 (s, 1 H), 6.22 (s, 1 H), 7.29−7.36 (m, 10 H); MS (methane, DCI) *m/z* 325 ($M^+ + 1$).

Dibenzyl 2-[[Bis(benzyloxy)phosphoryl]methyl]pentanedioate (2a). To a solution of dibenzyl phosphite (0.53 g, 2.02 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added trimethylaluminum (1.01 mL, 2.0 M solution in hexanes, 2.02 mmol). After 20 min a solution of **1a** (0.66 g, 2.02 mmol) in CH_2Cl_2 (5 mL) was added. The cooling bath was removed, and stirring at room-temperature continued for 16 h. The reaction was quenched by the slow addition of 1 N hydrochloric acid (20 mL). After stirring for 10 min the phases were separated, and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The organic phases were combined, dried ($MgSO_4$), and concentrated. Flash chromatography over silica gel (3 × 18 cm) with a hexanes−EtOAc (4:1 → 1:1) gradient gave **2a** as a clear oil (0.58 g, 48%): TLC R_f 0.50 (1:1 hexanes−EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.80−1.99 (m, 3 H), 2.25−2.35 (m, 3 H), 2.78−2.90 (m, 1 H), 4.88−5.01 (m, 6 H), 5.05 (s, 2 H), 7.23−7.35 (m, 20 H); MS (methane, DCI) *m/z* 587 ($M^+ + 1$). Anal. Calcd for $C_{34}H_{35}O_7P$: C, 69.61; H, 6.01. Found: C, 69.21; H, 6.00.

2-(Phosphonomethyl)pentanedioic Acid (3). To a solution of **2a** (2.89 g, 4.93 mmol) in methanol (10 mL) was added 10% palladium on carbon (0.26 g, 5 mol %). After shaking at 40 psi for 24 h, the mixture was filtered through Celite and the filter cake washed with methanol. The combined filtrate was concentrated *in vacuo* to afford **3** in quantitative yield. Purification of **3** was carried out by HPLC: semipreparative C8 column, linear gradient of water and methanol (0% → 20%) over 20 min, 10 mL/min, 20 × 10 mL fractions. HPLC analysis, Zorbax C8 column, isocratic water−methanol (4:1), showed the product (t_R = 1.6 min) was contained in fractions 7−10. These fractions were combined and lyophilized to afford **3** (0.62 g, 55%): ^{1}H NMR (300 MHz, D_2O) δ 1.75−2.21 (m, 4 H), 2.39−2.49 (m, 2 H), 2.66−2.83 (m, 1 H); MS (methane, DCI−) *m/z* 225 ($M^+ - 1$). Anal. Calcd for $C_6H_{11}O_7P \cdot 1.25H_2O$: C, 28.98; H, 5.47. Found: C, 28.98; H, 5.45.

Dibenzyl Itaconate (1b). A solution of itaconic acid (1.23 g, 9.45 mmol), triethylamine (18.9 mmol, 1.92 g), and benzyl bromide (23.62 mmol, 4.04 g) in toluene (30 mL) was heated at 80 °C for 16 h. After cooling to room temperature, the solution was diluted with ether (100 mL), washed with 1 N hydrochloric acid (20 mL) and water (20 mL), dried ($MgSO_4$), and concentrated. Flash chromatography over silica gel (3 × 18 cm) with a hexane−EtOAc (20:1 → 10:1) gradient gave **2b** as a clear oil (1.84 g, 63%): TLC R_f 0.28 (9:1 hexanes−EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.41 (s, 2 H), 5.09 (s, 2 H), 5.17 (s, 2 H), 5.73 (s, 1 H), 6.39 (s, 1 H), 7.23−7.36 (m, 10 H); MS (methane, DCI) *m/z* 311 ($M^+ + 1$).

Dibenzyl 2-[[Bis(benzyloxy)phosphoryl]methyl]succinate (2b). To a solution of dibenzyl phosphite (3.04 g, 11.6 mmol) in THF (20 mL) at 0 °C was added *n*-butyllithium (11.6 mmol, 4.6 mL, 2.5 M solution in hexanes) followed after 10 min by trimethylaluminum (11.6 mmol, 5.8 mL, 2.0 M solution in hexanes). After 20 min a solution of **1b** (3.0 g, 9.7 mmol) in THF (5 mL) was added. The cooling bath was removed, and the resulting solution stirred at room temperature for 16 h. The reaction was quenched by the slow addition of 1 N hydrochloric acid (20 mL). After stirring for 10 min the phases were separated, and the aqueous phase was extracted with ethyl acetate (2 × 10 mL). The organic phases were combined, dried ($MgSO_4$), and concentrated. Flash chromatography over silica gel (3 × 18 cm) with a hexane−EtOAc (4:1 → 1:1) gradient gave **2b** as a clear oil (2.6 g, 55%): TLC R_f 0.39 (1:1 hexanes−EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.08 (ddd, *J* = 17.6, 15.5, 8.2 Hz, 1 H), 2.35 (ddd, *J* = 19.2, 15.5, 5.5 Hz, 1 H), 2.83 (d, *J* = 6.5 Hz, 2 H), 3.10−3.27 (m, 1 H), 4.82−5.06 (m, 8 H), 7.22−7.37 (m, 20 H); MS (methane, DCI) *m/z* 573 ($M^+ + 1$). Anal. Calcd for $C_{33}H_{33}O_7P$: C, 69.22; H, 5.81. Found: C, 69.28; H, 5.86.

2-(Phosphonomethyl)succinic Acid (4). The procedure used for the preparation of **3** was followed using **2b** (1.4 g, 2.44 mmol) in methanol (10 mL) with 10% palladium on carbon (0.25 g, 5 mol %) to afford **4** (0.55 g). Purification of **4** was carried out by HPLC: semipreparative C8 column, linear gradient of water and methanol (0% → 20%) over 20 min, 10 mL/min, 20 × 10 mL fractions. HPLC analysis, Zorbax C8 column, isocratic water−methanol (4:1), showed the product (t_R = 1.6 min) was contained in fractions 7−10. These fractions were combined and lyophilized to afford **3** (0.44 g, 38%): ^{1}H NMR (300 MHz, D_2O) δ 1.91 (ddd, *J* = 17.4, 15.5, 7.7 Hz, 1 H), 2.13 (dd, *J* = 18.0, 15.5, 6.1 Hz, 1 H), 2.71−2.92 (m, 2 H) 3.01−3.15 (m, 1 H); MS (methane, DCI−) *m/z* 211 ($M^+ - 1$). Anal. Calcd for $C_5H_9O_7P \cdot 2H_2O$: C, 24.2; H. 5.28. Found: C, 24.2; H, 5.34.

Benzyl 3-(Hydroxyphosphinyl)propionate (5). Hypophosphorous acid (1.82 g, 13.8 mmol, 50% aqueous solution) and triethylamine (0.97 g, 13.8 mmol) were mixed and dried by azeotropic removal of toluene (3 × 20 mL) *in vacuo* at 50 °C. The residue was dissolved in dry CH_2Cl_2 (50 mL) and cooled to 0 °C. Triethylamine (2.4 g, 24.2 mmol) and chlorotrimethylsilane (2.56 g, 23.6 mmol) were added followed after 5 min by benzyl acrylate (0.32 g, 1.98 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 24 h at room temperature and then filtered. The filtrate was washed with 1 N hydrochloric acid (10 mL) and water (10 mL), dried ($MgSO_4$), and concentrated to afford **5** in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (dquintet, *J* = 1.8, 7.7 Hz, 2 H), 2.66 (quintet, *J* = 7.7 Hz, 2 H), 5.12 (s, 2 H), 7.25−7.38 (m, 5 H), 10.89 (brs, 1 H).

Dibenzyl 2-[[[2-(Benzylcarboxy)ethyl]hydroxyphosphinoyl]methyl]pentanedioate (6). Compound **5** (0.82 g, 3.63 mmol) was dried by azeotropic removal of toluene (3 × 20 mL) *in vacuo* at 50 °C. The residue was dissolved in dry CH_2Cl_2 (50 mL) and cooled to 0 °C. Triethylamine (1.5 g, 14.6 mmol) and chlorotrimethylsilane (1.34 mL, 12.4 mmol) were added followed after 5 min by compound **1a** (1.28 g, 3.94 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 24 h at room temperature and then washed with 1 N hydrochloric acid (10 mL) and water (10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography over silica gel (3 × 18 cm) with a hexane−EtOAc (4:1 → 1:1) gradient gave **6** as a clear oil (0.74 g, 37%): TLC R_f 0.1 (1:1 hexanes−EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.70−1.84 (m, 1 H), 1.84−2.05 (m, 4 H), 2.12−2.35 (m, 3 H), 2.51−2.66 (m, 2 H), 2.76−2.94 (m, 1 H), 5.00−5.17 (m, 6 H), 7.20−7.40 (m, 15 H); MS (methane, DCI) *m/z* 553 ($M^+ + 1$).

2-[[(2-Carboxyethyl)hydroxyphosphinoyl]methyl]pentanedioic Acid (7). The procedure used for the preparation of **3** was followed using **6** (0.58 g, 1.06 mmol) in methanol (10 mL) with 10% palladium on carbon (0.11 g, 10 mol %) to afford 0.31 g of compound **7**. Purification of **7** was carried out by HPLC: semipreparative C8 column, linear gradient of water and methanol (0% → 20%) over 20 min, 10 mL/min, 20 × 10 mL fractions. HPLC analysis, Zorbax C8 column, isocratic water−methanol (4:1), showed the product (t_R = 1.9 min) was contained in fractions 12−15. These fractions were combined and lyophilized to afford **7** (0.17 g, 56%): ^{1}H NMR (300 MHz, MeOD) δ 1.82−2.08 (m, 5 H), 2.14−2.27 (m, 1 H), 2.31−2.43 (m, 2 H), 2.51−2.63 (m, 2 H), 2.74−2.91 (m, 1 H); MS (FAB) *m/z* 283 (M^+).

NAALADase Assay. NAALADase activity was assayed as was previously described.[22] In brief, the assay measured the amount of [^{3}H]Glu liberated from [^{3}H]NAAG in 50 mM Tris-Cl buffer in 15 min at 37 °C using 30−50 μg of synaptosomal protein; substrate and product were resolved by anion-exchange liquid chromatography. Duplicate assays were always performed so that no more than 20% of the NAAG was digested, representing the linear range of peptidase activity. Quis (100 μM) was included in parallel assay tubes to confirm the specificity of measurements.

Acknowledgment. The authors would like to thank Dr. Andrew Shaw for helpful discussions during the course of this work.

References

(1) Coyle, J. T.; Stauch-Slusher, B.; Tsai, G.; Rothstein, J.; Meyerhoff, J. L.; Simmons, M.; Blakely, R. D. N-Acetyl-Aspartyl Glutamate: Recent Developments. In *Excitatory Amino Acids*; Meldrum, B. S., Moroni, F., Simon, R. P., Woods, J. H., Eds.; Raven Press: New York, 1991; pp 69−77.

(2) Whittemore, E. R.; Koerner, J. F. An explanation for the purported excitation of pirform cortical neurons by N-Acetyl-L-aspartyl-L-glutamic acid (NAAG). *Proc. Natl. Acad. Sci. U.S.A* **1989**, *86*, 9602−9605.
(3) Koenig, M. L.; Rothbard, P. M.; DeCoster, M. A.; Meyerhoff, J. L. N-Acetyl-aspartyl-glutamate (NAAG) elicits rapid increases in intraneuronal Ca^{2+} *in vitro*. *NeuroReport* **1994**, *5*, 1063−1068.
(4) Robinson, M. B.; Blakely, R. D.; Couto, R.; Coyle, J. T. Hydrolysis of the Brain Dipeptide N-Acetyl-L-aspartyl-L-glutamate. *J. Biol. Chem*. **1987**, *262*, 14498−14506.
(5) Meyerhoff, J. L.; Carter, R. E.; Yourick, D. L.; Slusher, B. S.; Coyle, J. T. Genetically epilepsy-prone rats have increased brain regional activity of an enzyme which liberates glutamate from N-Acetyl-Aspartyl-Glutamate. *Brain Res*. **1992**, *593*, 140−143.
(6) Rothstein, J. D.; Tsai, G.; Kuncl, R.; Clawson, L.; Cornblath, D. R.; Drachman, D. B.; Pestronk, A.; Stauch-Slusher, B.; Coyle, J. T. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann. Neurol*. **1990**, *28*, 18−25.
(7) Tsai, G.; Stauch-Slusher, B.; Sim, L.; Hedreen, J. C.; Rothstein, J. D.; Kuncl, R.; Coyle, J. T. Reductions in acidic amino acids and N-Acetyl-Aspartyl-Glutamate in amyotrophic lateral sclerosis CNS. *Brain Res*. **1991**, *556*, 151−156.
(8) Tsai, G.; Slusher, B. S.; Carter, R. E.; Passani, L.; Casanova, F.; Kleinman, J.; Coyle, J. T. Abnormalities in NAAG and NAALADase levels in schizophrenic brain. *Soc. Neurosci. Abstr*. **1991**, *17*, 1455.
(9) Jaarsma, D.; Veenma-van der Duin, L.; Korf, J. *N*-Acetylaspartate and *N*-acetylaspartylglutamate levels in Alzheimer's disease post-mortem brain tissue. *J. Neurol. Sci*. **1994**, *127*, 230−233.
(10) The NAALADase inhibitors reported to date have IC_{50} values >0.50 μM; see: Subasinghe, N.; Schulte, M.; Chan, M.; Roon, R.; Koerner, J. Synthesis of acrylic and dehydroaspartic acid analogues of Ac-Asp-Glu-OH and their inhibition of rat brain N-acetylated α-linked acidic dipeptidase (NAALA Dipeptidase). *J. Med. Chem*. **1990**, *33*, 2734−2744.
(11) Serval, V.; Galli, T.; Cheramy, A.; Glowinski, J.; Lavielle, S. *In Vitro and In Vivo* inhibition of N-Acetyl-L-aspartyl-L-glutamate-catabolism by N-acylated L-glutamate analogs. *J. Pharmacol. Exp. Ther*. **1992**, *260*, 1093−1100.
(12) For a review, see: Rich, D. Peptidase Inhibitors. In *Comprehensive Medicinal Chemistry*, 1st ed.; Hansch, C., Sammes, P., Taylor, J., Eds.; Pergamon Press: New York, 1990; Vol. 3, pp 391−441.
(13) This observation is consistent with work which was carried out using dipeptides to characterize the enzyme; see ref 4.
(14) Amri, H.; Rambaud, M.; Villiers, J. A short large scale synthesis of (±) sarkomycin esters. *Tetrahedron Lett*. **1989**, *30*, 7381−7382.
(15) Green, K. Trimethylaluminum promoted conjugate additions of dimethylphosphite to α,β-unsaturated esters and ketones. *Tetrahedron Lett*. **1989**, *30*, 4807−4810.
(16) Jpn. Kokai Tokkyo Koho JP 01,135743 [89,135743]; *Chem. Abstr*. **1990**, *112*, 7174z.
(17) Boyd, E. A.; Regan, A. C. Synthesis of γ-keto-substituted phosphinic acids from bis(trimethylsilyl)phosphonite and α,β-unsaturated ketones. *Tetrahedron Lett*. **1992**, *33*, 813−816.
(18) Boyd, E. A.; Corlass, M.; James, K.; Regan, A. C. A versatile route to substituted phosphinic acids. *Tetrahedron Lett*. **1990**, *31*, 2933−2936.
(19) Benveniste, H.; Huttemeier, P. C. Microdialysis-theory and application. *Prog. Neurobiol*. **1990**, *35*, 195−215.
(20) Donzanti, B. A.; Yamamoto, B. K. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. *Life Sci*. **1988**, *43*, 913−922.
(21) Koenig, M. L.; Rothbard, P. M.; DeCoster, M. A.; Meyerhoff, J. L. N-Acetyl-aspartyl-glutamate (NAAG) elicits rapid increase in interneuronal calcium in vitro. *NeuroReport* **1994**, *5*, 1063−1068.
(22) Slusher, B. S.; Robinson, M. B.; Tsai, G.; Simmons, M. L.; Richards, S. S.; Coyle, J. T. Rat brain N-Acetylated-Alpha-Linked Acidic Dipeptidase activity: Purification and immunological characterization. *J. Biol. Chem*. **1990**, *265*, 21297−21301.

JM950801Q