Quisqualic Acid Analogues: Synthesis of β-Heterocyclic 2-Aminopropanoic Acid Derivatives and Their Activity at a Novel Quisqualate-Sensitized Site

Nalin Subasinghe,† Marvin Schulte,‡ Robert J. Roon,‡ James F. Koerner,‡ and Rodney L. Johnson*,†

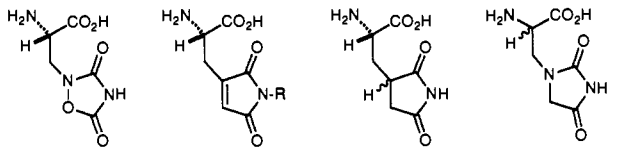
Department of Medicinal Chemistry, College of Pharmacy, and Department of Biochemistry, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

Received July 28, 1992

Hippocampal CA1 pyramidal cell neurons are sensitized over 30-fold to depolarization by L-2-amino-4-phosphonobutanoic acid (L-AP4) following exposure to L-quisqualic acid. This phenomenon has been termed the QUIS effect. In the present study several novel L-quisqualic acid analogues have been synthesized and tested for their interaction with the different components of the QUIS-effect system. Replacement of the oxadiazolidinedione ring of L-quisqualic acid with several other types of heterocyclic rings yielded the following quisqualic acid analogues: maleimide **2**, *N*-methylmaleimide **3**, *N*-(carboxymethyl)maleimide **4**, succinimides **5A** and **5B**, and imidazolidinedione **6**. None of these analogues were able to mimic the effects of L-quisqualic acid and sensitize hippocampal CA1 neurons to depolarization by L-AP4. Also, unlike L-serine *O*-sulfate, L-homocysteinesulfinic acid, or L-α-aminoadipic acid, none of the analogues were able to preblock or reverse the QUIS effect. However, when the IC_{50} values for inhibition of the CA1 synaptic field potential of analogues **2–6** were determined both before and after hippocampal slices were exposed to L-quisqualic acid, the IC_{50} values of analogues **3** and **4** were found to decrease more than 7-fold. Thus, these two compounds behave like L-AP4 rather than L-quisqualic acid in this system in that they exhibit increased potencies in slices that have been pretreated with L-quisqualic acid even though they cannot themselves induce this sensitization. Compounds **3** and **4**, therefore, represent the first non-phosphorus-containing compounds to which hippocampal neurons become sensitized following exposure to L-quisqualic acid. No change in the IC_{50} values was observed for **5A** or **5B**. Analogues **2** and **6**, on the other hand, displayed a high potency for inhibition of the evoked field potential even prior to treatment of the slices with L-quisqualic acid.

Neuronal conduction across synapses in the mammalian central nervous system (CNS) is facilitated by a wide array of neurotransmitters. These neurotransmitters, upon release from the presynaptic neuron, can be either inhibitory or excitatory in action depending upon whether they hyperpolarize or depolarize the postsynaptic neuron. Glutamic acid and aspartic acid have been identified as the major millisecond excitatory neurotransmitters in the mammalian CNS.[1] Five excitatory amino acid (EAA) receptor subtypes have been characterized so far.[2] These are the NMDA, AMPA, kainate, metabotropic, and L-AP4 receptors. The first three EAA receptor types directly gate ion channels, while the last two receptor types operate via second-messenger systems.

L-Quisqualic acid (**1**), an amino acid first isolated from the seeds of *Quisqualis indica* L., is a unique compound in that it is able to function as an agonist at multiple EAA receptor subtypes in the CNS. It has high affinity for the kainate,[3,4] AMPA,[5] and the metabotropic receptors.[6,7] It also inhibits the Ca^{2+}/Cl^--dependent glutamic acid uptake system in brain synaptic plasma membrane preparations[8] and an *N*-acetyl α-linked acidic dipeptidase which hydrolyzes the brain dipeptide Ac-Asp-Glu-OH.[9]



Recently, L-quisqualic acid has been shown to produce a 30–100-fold sensitization of CA1 neurons in rat hippocampal slices to depolarization by D- or L-2-amino-4-phosphonobutanoic acid (AP4) and related phosphonates.[10,11] This phenomenon, which has been termed the

† Department of Medicinal Chemistry.
‡ Department of Biochemistry.

(1) Johnson, R. L.; Koerner, J. F. Excitatory Amino Acid Neurotransmission. *J. Med. Chem.* **1988**, *31*, 2057–2066.
(2) Monaghan, D. J.; Bridges, R. J.; Cotman, C. W. Excitatory Amino Acid Receptors: Their Classes, Pharmacology, and Distinct Properties in the Function of the Central Nervous System. *Annu. Rev. Pharmacol. Toxicol.* **1989**, *29*, 365–402.
(3) Davies, J. D.; Evans, R. H.; Francis, A. A.; Watkins, J. C. Excitatory Amino Acid Receptors and Synaptic Excitation in the Mammalian Central Nervous System. *J. Physiol.* **1979**, *75*, 641–654.
(4) London, E. D.; Coyle, J. T. Specific Binding of ^{3}H-Kainic Acid to Receptor Sites in Rat Brain. *Mol. Pharmacol.* **1979**, *15*, 492–505.
(5) Honore, T.; Lauridsen, J.; Krogsgaard-Larsen, P. The Binding of [^{3}H]AMPA, a Structural Analogue of Glutamic Acid, to Rat Brain Membranes. *J. Neurochem.* **1982**, *38*, 173–178.
(6) Schoepp, D. D.; Johnson, B. G. Excitatory Amino Acid Agonist-Antagonist Interactions at 2-Amino-4-phosphonobutyric Acid-Sensitive Quisqualate Receptors Coupled to Phosphoinositide Hydrolysis in Slices of Rat Hippocampus. *J. Neurochem.* **1988**, *50*, 1605–1613.
(7) Recasens, M.; Guiramand, J.; Nourigat, A.; Sassetti, I.; Devilliers, G. A New Quisqualate Receptor Subtype (sAA_2) Responsible for the Glutamate-induced Inositol Phosphate Formation in Rat Brain Synaptoneurosomes. *Neurochem. Int.* **1988**, *13*, 463–467.
(8) Zaczek, R.; Arlis, S.; Markl, A.; Murphy, T.; Drucker, H.; Coyle, J. T. Characteristics of Chloride-Dependent Incorporation of Glutamate into Brain Membranes Argue Against a Receptor Binding Site. *Neuropharmacology* **1987**, *26*, 281–287.
(9) 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.

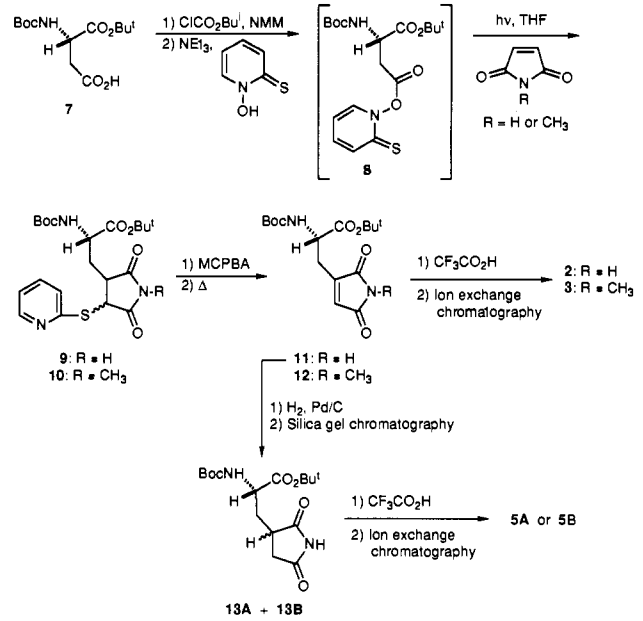
0022-2623/92/1835-4602$03.00/0

QUIS effect, appears to be widely distributed, having been reported for neurons of the CA1 region in the rat and guinea pig brain and the medial perforant path, the lateral olfactory tract, and the cingulate cortex in rat brain.[11,12] The QUIS effect can be blocked by a brief exposure of CA1 neurons to L-homocysteinesulfinic acid, L-α-aminoadipic acid, or L-serine O-sulfate.[13] These compounds have been termed "preblockers" since they are able to block the QUIS effect even when they are removed from the incubation chamber prior to treatment of the slices with L-quisqualic acid.[13] These same compounds are also capable of reversing the QUIS effect after it has been induced. Thus, these compounds are also referred to as "reversers".[13] The AP4 site which is sensitized by L-quisqualic acid and the site to which L-quisqualic acid binds in order to bring about this sensitization are novel sites of action different from the classical AMPA and L-AP4 sites.[10,11,13,14]

Since the dramatic increase of neuronal excitability manifested by the QUIS effect may have significance for understanding mechanisms of neuronal plasticity that occur during learning and memory and for changes of excitability that occur in disease states such as epilepsy, we have undertaken the synthesis of a number of analogues of L-quisqualic acid, compounds **2-6**, in an attempt to gain further insight into structure–activity requirements of the QUIS effect. Since X-ray structural and molecular modeling studies have shown that the ring N^2 nitrogen atom of L-quisqualic acid possesses a trigonal configuration[15] and since the potent activity of L-quisqualic acid at the locust neuromuscular junction has been attributed to this trigonal configuration,[15,16] we designed compounds **2**, **5A**, and **5B** in order to assess the importance of this trigonal configuration in the QUIS-effect system.

Analogue **3** was synthesized in order to determine the importance of the iminodicarbonyl NH function while analogue **4** was made to explore the importance of an acidic functionality in this region of the molecule. Although Bycroft et al.[16] have reported the imidazolidinedione analogue **6** to be inactive at glutamatergic receptors of the locust neuromuscular junction, its activity on mammalian EAA receptors is unknown. In light of its structural similarity to L-quisqualic acid, we prepared **6** and evaluated this analogue in the QUIS effect.

Scheme I



Syntheses

Compounds **2** and **3** were synthesized by the general methodology of Barton et al.[17] (Scheme I). The 2-thiono-*N*-hydroxypyridyl ester of Boc-Asp(OBu^t)-OH (**7**), intermediate **8**, was prepared via the mixed anhydride route with isobutyl chloroformate. This ester was not isolated, but rather was immediately irradiated in the presence of either maleimide or *N*-methylmaleimide to give adducts **9** and **10**, respectively, as diastereoisomeric mixtures. Oxidation of adduct **9** to the sulfoxide followed by pyrolysis gave the maleimido intermediate **11**. A similar sequence of reactions gave the *N*-methylmaleimido intermediate **12** from adduct **10**. Treatment of **11** and **12** with CF_3CO_2H, followed by cation-exchange chromatography, gave the β-maleimido amino acid **2** and the β-(*N*-methylmaleimido) amino acid **3**, respectively.

Catalytic reduction of the olefinic bond of **11** gave the reduced product as a mixture of two diastereoisomers, **13A** and **13B**, in a ratio of 1:1. This mixture was separated by silica gel column chromatography. No attempt was made to determine the absolute configuration of the two isomers. Deprotection of **13A** and **13B** with CF_3CO_2H followed by cation-exchange chromatography afforded the diastereoisomeric β-succinimido analogues **5A** and **5B**, respectively.

The synthesis of analogue **4** was carried out as shown in Scheme II and involved initially the alkylation of **9** with *tert*-butyl bromoacetate. Since it has been shown that silver salts of succinimides undergo alkylation at both the oxygen and nitrogen atoms,[18] while mineral salts give N-alkylated products,[19] the product of this alkylation was believed to be the *N*-carboxymethyl adduct **14**. This was confirmed by IR analysis of the *N*-(carboxymethyl)-maleimido intermediate **15** which was obtained from **14**

(10) Robinson, M. B.; Whittemore, E. R.; Marks, R. L.; Koerner, J. F. Exposure of Hippocampal Slices to Quisqualate Sensitizes Synaptic Responses to Phosphonate-Containing Analogues of Glutamate. *Brain Res.* **1986**, *381*, 187–190.

(11) Whittemore, E. R.; Koerner, J. F. Novel Recognition Site for L-Quisqualate Sensitizes Neurons to Depolarization by L-2-Amino-4-phosphonobutanoate (L-AP4). *Brain Res.* **1989**, *489*, 146–156.

(12) Sheardown, M. J. A New and Specific Non-NMDA Receptor Antagonist, FG 9065, Blocks L-AP4-Evoked Depolarization in Rat Cerebral Cortex. *Eur. J. Pharmacol.* **1988**, *148*, 471–474.

(13) Whittemore, E. R.; Koerner, J. F. Pre-exposure to L-Homocysteinesulfinic Acid Blocks Quisqualate-Induced Sensitization to L-2-Amino-4-phosphonobtanoic Acid. *Eur. J. Pharmacol.* **1991**, *192*, 435–438.

(14) Schulte, M. K.; Whittemore, E.; Johnson, R. L.; Koerner, J. F. Structure-function Relationships for Analogues of L-2-Amino-4-phosphonobutanoic Acid on the Quisqualic Acid-sensitive AP4 Receptor of the Rat Hippocampus. *Brain Res.* **1992**, *582*, 291–298.

(15) Jackson, D. E.; Bycroft, B. W.; King, T. J. Crystallographic Studies and Semi-empirical MNDO Calculations on Quisqualic Acid and its Analogues: Systems Containing Unusual Pyramidal Heterocyclic Ring Nitrogens. *J. Comput-Aided Drug Des.* **1988**, *2*, 321–328.

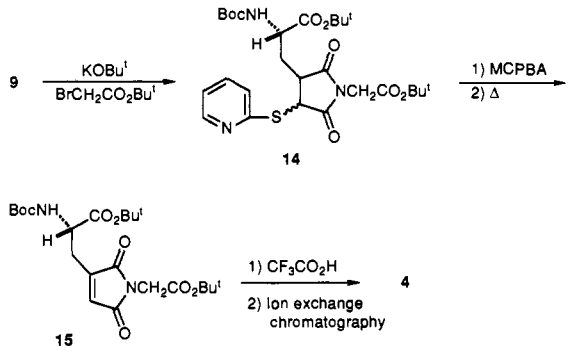
(16) Boden, P.; Bycroft, B. W.; Chhabra, S. R.; Chiplin, J.; Crowley, P. J.; Grout, R. J.; King, T. J.; McDonald, E.; Rafferty, P.; Usherwood, P. N. R. The Action of Natural and Synthetic Isomers of Quisqualic Acid at a Well-Defined Glutamatergic Synapse. *Brain Res.* **1986**, *385*, 205–211.

(17) Barton, D. H. R.; Herve, Y.; Potier, P.; Thierry, J. Synthesis of Novel α-Amino Acids and Derivatives Using Radical Chemistry: Synthesis of L- and D-α-Amino-Adipic Acids, L-α-Aminopimelic Acid and Appropriate Unsaturated Derivatives. *Tetrahedron* **1987**, *43*, 4297–4308.

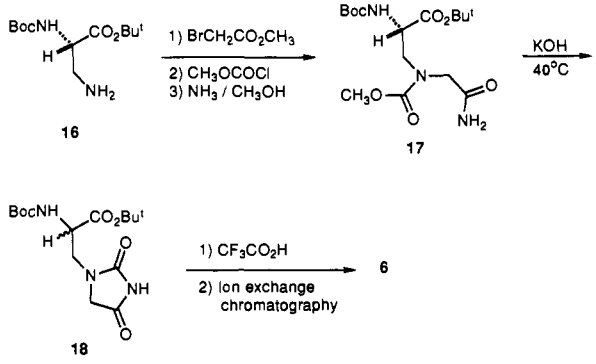
(18) Comstock, W. J.; Wheeler, H. L. On The Preparation of Oxygen-Ethers of Succinimide From Its Silver Salt. *Am. Chem. J.* **1891**, *13*, 520–522.

(19) Hargreaves, M. K.; Pritchard, J. G.; Dave, H. R. Cyclic Carboxylic Monoimides. *Chem. Rev.* **1970**, *70*, 439–469.

Scheme II



Scheme III



by the same set of reactions used to convert **9** to **11**. Structure **15** was differentiated from the possible O-alkylation product by the IR C=C stretching band. This band appeared at 1646 cm^{-1} in **15**. In contrast, the conjugated double bond in the O-alkylated maleimides appears at 1600 cm^{-1}.[20,21] In addition, the strong band that is observed at 1042 cm^{-1} for the ether linkage in O-alkylated maleimides was not observed in **15**. Two bands around 1700 and 1746 cm^{-1} were observed for **15** for the imide and ester carbonyl stretching bands, respectively. Deprotection of **15** with CF_3CO_2H gave the β-[*N*-(carboxymethyl)maleimido] amino acid **4**.

Bycroft et al.[22] had previously reported the synthesis of the imidazolidinedione analogue **6** in racemic form. In an attempt to prepare **6** in an enantiomerically pure form, we carried out the sequence of reactions shown in Scheme III. Even though we were able to obtain **6** by this route, the compound was racemic. The starting point for our synthesis was the chiral diaminopropanoic acid **16**, which was obtained by the method of Otsuka et al.[23] In a sequence of reactions in which the intermediates were not purified, primary amine **16** was first alkylated with methyl 2-bromoacetate under conditions in which **16** was used in excess to minimize multiple alkylation. The secondary amine was then treated with methyl chloroformate to give the *N*-(methoxycarbonyl) derivative. Aminolysis of the methyl ester with methanolic ammonia afforded compound **17**. Cyclization of **17** with KOH at 40 °C afforded the desired protected imidazolidinedione **18**; however, the basic reaction conditions also resulted in the epimerization of the chiral center during this reaction. Under milder basic conditions, the chiral center remained intact, but cyclization failed to occur. Deprotection of **18** with CF_3CO_2H followed by cation-exchange chromatography gave racemic imidazolidinedione **6**.

Table I. IC_{50} Values of Quisqualic Acid Analogues in the QUIS Effect[a]

compound	IC_{50} values ± SEM (μM) before quisqualic acid	IC_{50} values ± SEM (μM) after quisqualic acid	sensitization
2	21 ± 4.4	9.5 ± 2.5	2.2
3	95 ± 19	6.8 ± 2.3	14.0
4	2900 ± 320	375 ± 75	7.7
5A	>10000	>10000	
5B	9200 ± 1100	6800 ± 930	1.3
6	1.4 ± 0.6	0.8 ± 0.26	1.7
D-quisqualic acid	78 ± 8.5	10 ± 1.7	7.5
L-quisqualic acid[b]	3.9	2.9	1.3
L-AP4[b]	1800	55	33
D-AP4[b]	2600	152	17

[a] Electrodes were placed in the CA1 region of the hippocampus. Potencies were determined before and after a 4-min exposure of slices to 16 μM L-quisqualic acid. Sensitization refers to the fractional increase in potency. [b] L-Quisqualic acid and D- and L-2-amino-4-phosphonobutanoic acid data are from ref 14.

Biological Results and Discussion

L-Quisqualic acid analogues **2–6** were tested for their capability of inducing, preblocking and reversing the QUIS effect. Of all the compounds tested only L-quisqualic acid sensitized slices to depolarization by AP4. Thus, L-quisqualic acid remains the only compound known to induce the QUIS effect. In addition, none of the analogues were capable of preblocking or reversing the sensitization induced by L-quisqualic acid. Although a thorough explanation for the unique nature of L-quisqualic acid awaits further studies, it has been suggested that induction of the QUIS effect may involve uptake of L-quisqualic acid.[24] Since, ultimately, L-quisqualic acid must produce its effects by interacting with a receptor molecule, we have proposed that uptake may be followed by action at an intracellular receptor.[13,25] While the precise details are unknown, an induction mechanism involving multiple sites of interaction such as this would explain the apparent strict structural requirements for induction, reversal, and preblocking of the QUIS effect.

The IC_{50} values for inhibition of the evoked synaptic field potential of CA1 neurons of analogues **2–6** were determined both before and after slices were exposed to L-quisqualic acid. Varying degrees of sensitization were observed (Table I). The IC_{50} values of analogues **3** and **4** decreased more than 7-fold after exposure to L-quisqualic acid. Although these compounds are structurally similar to L-quisqualic acid, they do not induce the QUIS effect. Instead, they behave like L-AP4 in this system; i.e., the slices are sensitized to depolarization by these compounds following exposure to L-quisqualic acid. No significant change in IC_{50} values was observed for **5A** or **5B**. While **2** and **6** appeared to show a decrease in their IC_{50} values,

(20) Izawa, Y.; Yokoi, K.; Tomioka, H. Synthesis and Photochemical Reactivity of O-Alkylmaleimide. *Chem. Lett.* **1981**, 1473–1476.
(21) Schwartz, A. L.; Lerner, L. M. Preparation of N-Substituted Maleimides by Direct Coupling of Alkyl or Aralkyl Halides with Heavy Metal Salts of Maleimide. *J. Org. Chem.* **1974**, *39*, 21–23.
(22) Bycroft, B. W.; Chhabra, S. R.; Grout, R. J.; Crowley, P. J. A Convenient Synthesis of the Neuroexcitatory Amino Acid Quisqualic Acid and Its Analogues. *J. Chem. Soc., Chem. Commun.* **1984**, 1156–1157.
(23) Otsuka, M.; Kittaka, A.; Iimori, T.; Yamashita, H.; Kobayashi, S.; Ohno, M. Synthetic Studies on an Antitumor Antibiotic, Bleomycin. XII. Preparation of an L-2,3-Diaminopropionic Acid Derivative as a Synthetic Intermediate. *Chem. Pharm. Bull.* **1985**, *33*, 509–514.
(24) Harris, E. W.; Stevens, D. R.; Cotman, C. W. Hippocampal Cells Primed with Quisqualate are Depolarized by AP4 and AP6, Ligands for a Putative Glutamate Uptake Site. *Brain Res.* **1987**, *418*, 361–365.
(25) Schulte, M. K.; Roon, R. J.; Koerner, J. F. Quisqualic Acid Induced Sensitization and the Active Uptake of L-Quisqualic Acid by Hippocampal Slices. *Brain Res.*, in press.

the decrease was less than the 4-fold change which we have suggested is necessary to clearly establish their potencies at a QUIS-sensitized site.[14] Since **2** and **6** displayed high potency even prior to treatment of hippocampal slices with L-quisqualic acid, much of their activity may be due to action at a site which does not become sensitized by L-quisqualic acid, possibly the classical AMPA receptor.

The only compounds previously shown to exhibit an increase in potency following treatment of hippocampal slices with L-quisqualic acid have been certain phosphorus-containing compounds.[10,14] Thus, analogues **3** and **4** represent the first non-phosphorus compounds to which hippocampal slices are known to become sensitized. These analogues may act either at the previously described QUIS-sensitized AP4 site[14] or at a different QUIS-sensitized site. We have also observed that slices become sensitized to the D-isomer of quisqualic acid (Table I). This observation, in conjunction with the close structural similarity of **3** and **4** to L-quisqualic acid, raises the question as to whether L-quisqualic acid itself is a ligand at a QUIS-sensitized site. Our observation that compounds which preblock the QUIS effect also decrease the sensitivity of hippocampal slices for depolarization by L-quisqualic acid[13] lends further support to the argument that quisqualic acid may be able to sensitize neurons to its own depolarization. Additionally, we have observed that preblocking compounds do not block depolarization by AMPA; suggesting that depolarization of CA1 neurons by L-quisqualic acid may involve action at a QUIS-sensitized site in addition to the classical AMPA receptor.

The activity of the maleimide analogues **3** and **4**, which have a coplanar geometry at the junction of the heterocyclic ring and the 2-aminopropanoic acid side chain, and the poor activity of the succinimide analogues (**5A** and **5B**) with their nonplanar geometry indicate that the trigonal nitrogen configuration invoked to explain the activity of L-quisqualic acid in the locust glutamatergic receptor[16] is not an important factor in this particular mammalian QUIS-sensitized system. Of particular interest is the observation that an ionizable side chain is not required for activity at the QUIS-sensitized site, since the *N*-methylmaleimide analogue **3** showed the best activity in terms of sensitization in this series of compounds.

The results also show that replacement of the oxygen atom of the oxadiazolidinedione ring of L-quisqualic acid with a methylene moiety yields an analogue, imidazolidinedione **6**, which displays an intrinsically high potency for depolarization in the mammalian EAA system. This is in contrast to the locust glutamatergic system where this same compound was inactive.[16]

In summary, modification of the heterocyclic ring of L-quisqualic acid has led to the first known non-phosphorus-containing compounds to which hippocampal CA1 neurons become sensitized following exposure to L-quisqualic acid. This in turn has provided further insight into the unique phenomenon known as the QUIS effect.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt melting point apparatus Model 6406-K and are uncorrected. Specific rotations were measured with a Rudolph Research Autopol III polarimeter at 589 nm (Na D-line) at 24 °C. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Unless otherwise indicated, all analytical results were within ±0.4% of the theoretical values. ^{1}H-NMR spectra were recorded on either a Varian 300-MHz, an IBM 200-MHz, an IBM 300-MHz, or a GE 300-MHz spectrometer. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) in $CDCl_3$ or DMSO-d_6 and to TSP in D_2O. ^{13}C NMR was performed on either a Varian (300 MHz at 75 MHz), an IBM (200 MHz at 50 MHz or 300 MHz at 75 MHz), or a GE (300 MHz at 75 MHz) spectrometer. When DMSO-d_6 was used as solvent, it served as the internal standard at δ 39.5. When D_2O was used, dioxane (δ 64.5) was added as the external standard. FAB mass spectra were obtained on a Kratos MS25 spectrometer. Column chromatography was performed on silica gel (Merck, grade 60, 240–400 mesh, 60 Å) from Aldrich Chemical Co. Cation-exchange chromatography was performed with AG 50W-X8 resin (100–200 mesh) obtained from Bio-Rad Laboratories. Thin-layer chromatography (TLC) was carried out on Analtech 250-μm silica gel GF Uniplates. Visualization was achieved with either UV, I_2, vanillin/sulfuric acid, or ninhydrin spray. D- and L-quisqualic acid were obtained from Tocris Neuramin.

***tert*-Butyl(*S*)-2,5-Dihydro-2,5-dioxo-α-[(*tert*-butoxycarbonyl)amino]-1*H*-pyrrole-3-propanoate (11).** Boc-Asp-OBu^t (0.65 g, 2.2 mmol) was dissolved in THF (6 mL) and the solution cooled to −15 °C under an Ar atmosphere. To this solution was added *N*-methylmorpholine (NMM) (0.22 g, 2.2 mmol) and isobutyl chloroformate (0.30 g, 2.2 mmol). The mixture was stirred for 5 min at −15 °C. To this was added a cooled (−15 °C) solution of 2-mercaptopyridine *N*-oxide (0.3 g, 2.6 mmol) and NEt_3 (0.2 g, 2.7 mmol) in THF (4 mL). The mixture was stirred for 1.5 h at −15 °C in the dark. The solution was then rapidly filtered and the yellow filtrate was irradiated in the presence of maleimide (1.07 g, 11 mmol) with two tungsten lamps (200 W) at ambient temperature under an Ar atmosphere for 30 min. Et_2O was added to the reaction mixture and the solid that precipitated was removed by filtration. The Et_2O layer was washed successively with 0.1 N $NaHCO_3$, H_2O, 0.5 N HCl, H_2O, and saturated NaCl. The Et_2O solution was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow oil. This was purified by silica gel column chromatography with a solvent system consisting of EtOAc and hexane (1:3) to give 0.69 g (70%) of **9** as an oil. ^{1}H NMR resonances at δ 6.96–7.06, 7.12–7.24, 7.42–7.58, and 8.21–8.34 indicated that the 2-thiopyridyl moiety was presented. This mixture of diastereoisomers was used in the next reaction without further purification.

A solution of **9** (0.61 g, 1.35 mmol) in $CHCl_3$ (7 mL) was cooled to 0 °C. To this solution was added a solution of *m*-chloroperoxybenzoic acid (MCPBA) (0.31 g, 1.42 mmol) in $CHCl_3$ (4 mL). The mixture was stirred at room temperature for 1 h after which time CH_2Cl_2 was added. This solution was washed successively with 1 N $NaHCO_3$, H_2O, 0.5 N HCl, H_2O, and saturated NaCl. The solution was dried over anhydrous $MgSO_4$ and stripped of solvent in vacuo and the residue dried under high vacuum for 1 h. This material was then dissolved in anhydrous toluene and heated at reflux for 1 h. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (EtOAc/hexane, 1:5) to give 0.26 g (57% from **9**) of **11** as a colorless oil which solidified when cooled: mp 104–105 °C; $[\alpha]_D$ +9.05° (*c* 1.47, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (s, 9 H, $C(CH_3)_3$), 1.42 (s, 9 H, $C(CH_3)_3$), 2.73 (dd, J = 7.0 and 15.0 Hz, 1 H, β-CH), 2.93 (dd, J = 4.6 and 15.0 Hz, 1 H, β-CH), 4.3–4.5 (m, 1 H, α-CH), 5.33 (br d, J = 7.3 Hz, 1 H, NH), 6.38 (d, J = 0.5 Hz, 1 H, C=CH), 8.27 (br s, 1 H, imide NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.92 ($C(CH_3)_3$), 28.22 ($C(CH_3)_3$), 28.80 (CH_2), 52.40 (α-CH), 80.28 ($C(CH_3)_3$), 83.08 ($C(CH_3)_3$), 129.59 (C=CH), 145.90 (C=CH), 155.21 (urethane C=O), 170.03 (C=O), 170.59 (C=O), 171.51 (C=O); FAB-MS m/z 341 $(MH)^+$. Anal. ($C_{16}H_{24}N_2O_6$) C, H, N.

***(S)*-2,5-Dihydro-2,5-dioxo-α-amino-1*H*-pyrrole-3-propanoic Acid (2).** Compound **11** (0.4 g, 1.17 mmol) was dissolved in CF_3CO_2H and the mixture stirred at room temperature overnight. The excess CF_3CO_2H was evaporated under vacuum and the resulting residue was chromatographed on a cation-exchange column (AG 50W-X8) with water as the eluting solvent. The fractions containing the product were lyophilized to give 0.18 g (83%) of **2** as a white solid: mp 142 °C dec; $[\alpha]_D$ +5° (*c* 0.5, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 3.1–3.2 (m, 2 H, CH_2), 4.42–4.48 (m, 1 H, α-CH), 6.76 (s, 1 H, HC=C); ^{13}C NMR (75 MHz, D_2O) δ 26.02 (CH_2), 52.31 (α-C), 130.87 (C=CH), 144.01

(C=CH), 171.89 (C=O), 173.07 (C=O), 173.53 (C=O). Anal. ($C_7H_8N_2O_4$) C, H, N.

***tert*-Butyl (*S*)-2,5-Dihydro-2,5-dioxo-1-methyl-α-[(*tert*-butoxycarbonyl)amino]pyrrole-3-propanoate (12).** Boc-Asp-OBu^t (5 g, 17.3 mmol) was converted to its 2-thiono-*N*-hydroxypyridyl ester 8 in the same manner as described above in the preparation of 11. A solution of this ester was irradiated in the presence of *N*-methylmaleimide (9.6 g, 86.4 mmol) with two tungsten lamps (200 W) at ambient temperature under an Ar atmosphere for 1 h. Et_2O was added to the reaction mixture and the solid that precipitated was removed by filtration. The Et_2O layer was washed successively with 0.1 N $NaHCO_3$, H_2O, 0.5 N HCl, H_2O, and saturated NaCl. The Et_2O solution was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give a yellow oil. This was purified by silica gel column chromatography with a solvent system consisting of EtOAc and hexane (1:4) to give 6.47 g (80%) of 10 as an oil. This mixture of diastereoisomers was converted to 12 by the same set of reactions described above for the conversion of 9 to 11. The crude product was purified by silica gel column chromatography (EtOAc/hexane, 1:6) to give 2.5 g (50%) of 12 as a colorless oil which solidified when cooled: mp 111–112 °C; $[\alpha]_D$ +8.6° (*c* 1.22, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9 H, $C(CH_3)_3$), 1.44 (s, 9 H, $C(CH_3)_3$), 2.77 (ddd, *J* = 1.2, 7.3, 15.3 Hz, 1 H, β-CH), 2.95–3.00 (m, 1 H, β-CH), 3.00 (s, 3 H, NCH_3), 4.4–4.5 (m, 1 H, α-CH), 5.24–5.26 (m, 1 H, NH), 6.43 (br s, 1 H, C=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 23.73 (β-C), 27.82 ($C(CH_3)_3$), 28.13 ($C(CH_3)_3$), 28.71 (NCH_3), 52.28 (α-C), 80.03 ($C(CH_3)_3$), 82.86 ($C(CH_3)_3$), 128.55 (C=C), 144.94 (C=C), 155.06 (urethane C=O), 169.90 (C=O), 170.47 (C=O), 171.23 (C=O); FAB-MS *m/z* 355 $(MH)^+$. Anal. ($C_{17}H_{26}N_2O_6$) C, H, N.

(*S*)-2,5-Dihydro-2,5-dioxo-α-amino-1-methylpyrrole-3-propanoic Acid (3). Compound 12 (1 g, 2.8 mmol) was dissolved in CF_3CO_2H (20 mL) which had been precooled in an ice bath. This mixture was stirred overnight at room temperature. The acid was removed under vacuum and the residue dried under high vacuum overnight. This residue was dissolved in water (1 mL) and subjected to cation-exchange chromatography (AG 50W-X8, column volume = 3 mL). The column was eluted with water, and the fractions corresponding to product were evaporated to dryness. The residue was dissolved in a small amount of water and lyophilized to give 0.33 g (59%) of 3 as a white solid: mp 190–194 °C dec; $[\alpha]_D$ +2.5° (*c* 0.28, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 2.9 (s, 3 H, CH_3), 2.96–3.0 (m, 2 H, CH_2), 3.97 (dd, *J* = 6.41 and 7.02 Hz, 1 H, α-CH), 6.6 (m, 1 H, C=CH); ^{13}C NMR (75 MHz, D_2O) δ 23.53 (CH_3), 26.57 (CH_2), 52.96 (α-C), 130.07 (C=C), 143.79 (C=C), 172.59 (C=O), 172.72 (C=O), 173.01 (C=O); FAB-MS *m/z* 199 $(MH)^+$. Anal. ($C_8H_{10}N_2O_4 \cdot {}^1/_3H_2O$) C, H, N.

***tert*-Butyl 2,5-Dioxo-α(*S*)-[(*tert*-butoxycarbonyl)amino]-3(*RS*)-pyrrolidinepropanoate (13A and 13B).** A solution of 11 (0.3 g, 0.88 mmol) dissolved in methanol (2 mL) was placed in a Parr bottle along with 10% Pd/C (30 mg). The contents were subjected to a hydrogen pressure of 46 psi and shaken on a Parr apparatus for 5.5 h. The resulting solution was filtered and the MeOH evaporated under vacuum to give a colorless oil, which yielded a gelatinous solid upon trituration with EtOAc and hexane. The diastereoisomers were separated by silica gel column chromatography (ratio of compound to silica gel was 1:200) with EtOAc/hexane (1:3) as the eluting solvent. The elution was carried out over a period of 2 days and yielded 92 mg (30.5%) of 13A and 115.5 mg (38.3%) of 13B as gelatinous solids.

13A: TLC (EtOAc/hexane, 1:1) R_f = 0.42; $[\alpha]_D$ +2.4° (*c* 3.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.4 (s, 9 H, $C(CH_3)_3$), 1.44 (s, 9 H, $C(CH_3)_3$), 1.85 (dt, *J* = 8.3 and 14.2 Hz, 1 H, β-CH), 2.37 (dt, *J* = 5.4 and 14.2 Hz, 1 H, β-CH), 2.63 (dd, *J* = 5.4 and 18.55 Hz, 1 H, CH_2CO), 2.87 (dd, *J* = 9.0 and 18.55 Hz, 1 H, CH_2CO), 3.0–3.04 (m, 1 H, CHCO), 4.2–4.3 (m, 1 H, α-CH), 5.24 (br d, *J* = 8.3 Hz, 1 H, NH), 8.66 (br s, 1 H, imide NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.72 ($C(CH_3)_3$), 28.84 ($C(CH_3)_3$), 33.42 (β-C), 35.18 (CH_2CO), 38.82 (CHCO), 52.35 (α-C), 79.87 ($C(CH_3)_3$), 82.42 ($C(CH_3)_3$), 155.14 (urethane C=O), 170.76 (C=O), 177.18 (C=O), 180.34 (C=O). Anal. ($C_{16}H_{26}N_2O_6$) C, H, N.

13B: TLC (EtOAc/hexane, 1:1) R_f = 0.48; $[\alpha]_D$ +2.8° (*c* 8.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.4 (s, 9 H, $C(CH_3)_3$), 1.43 (s, 9 H, $C(CH_3)_3$), 1.8–2.0 (m, 1 H, β-CH), 2.05–2.2 (m, 1 H, β-CH), 2.5 (dd, *J* = 4.9 and 18.1 Hz, 1 H, CH_2CO), 3.0 (dd, *J* = 9.3 and 18.1 Hz, 1 H, CH_2CO), 2.9–3.0 (m, 1 H, CHCO), 4.1–4.2 (m, 1 H, α-CH), 5.22 (br d, *J* = 8.3 Hz, 1 H, NH), 8.92 (br s, 1 H, imide NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.74 ($C(CH_3)_3$), 28.08 ($C(CH_3)_3$), 34.55 (β-C), 35.77 (CH_2CO), 38.39 (CHCO), 51.90 (α-C), 79.96 ($C(CH_3)_3$), 82.53 ($C(CH_3)_3$), 155.59 (urethane C=O), 170.80 (C=O), 177.19 (C=O), 180.47 (C=O). Anal. ($C_{16}H_{26}N_2O_6$) C, H, N.

2,5-Dioxo-α(*S*)-amino-3(*RS*)-pyrrolidinepropanoic Acid (5A and 5B). Diastereoisomers 13A (0.07 g, 0.2 mmol) and 13B (0.1 g, 0.29 mmol) were each deprotected by the same procedure as that described above for the synthesis of 3 to give 5A and 5B, respectively. Both were isolated as white solids after lyophilization.

5A: Yield = 25 mg (65%); mp 216–219 °C dec; $[\alpha]_D$ −3.04° (*c* 0.69, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 2.0–2.24 (m, 2 H, β-CH_2), 2.44 (dd, *J* = 5.4 and 18.1 Hz, 1 H, CH_2), 2.88 (dd, *J* = 8.79 and 18.1 Hz, 1 H, CH_2), 2.96–3.01 (m, 1 H, CH), 3.77 (t, *J* = 6.0 Hz, 1 H, α-CH); ^{13}C NMR (75 MHz. D_2O) δ 31.07 (CH_2), 35.55 (CH_2), 37.87 (CH), 52.80 (α-C), 173.35 (C=O), 180.91 (C=O), 183.33 (C=O); FAB-MS *m/z* 187 $(MH)^+$. Anal. ($C_7H_{10}N_2O_4 \cdot {}^2/_3H_2O$) C, H, N.

5B: Yield = 36.7 mg (68%); mp 228–230 °C dec; $[\alpha]_D$ +27.9° (*c* 0.62, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 2.04–2.15 (m, 2 H, β-CH_2), 2.47 (dd, *J* = 5.4 and 18 Hz, 1 H, CH_2), 2.89 (dd, *J* = 9.3 and 18 Hz, 1 H, CH_2), 3.0–3.1 (m, 1 H, CH), 3.68 (t, *J* = 5.7 Hz, 1 H, α-CH); ^{13}C NMR (75 MHz, D_2O) δ 31.72 (CH_2), 35.39 (CH_2), 38.87 (CH), 53.44 (α-C), 173.72 (C=O), 180.86 (C=O), 183.24 (C=O); FAB-MS *m/z* 187 $(MH)^+$.

***tert*-Butyl (*S*)-2,5-Dihydro-2,5-dioxo-1-[(*tert*-butoxycarbonyl)methyl]-α-[(*tert*-butoxycarbonyl)amino]pyrrole-3-propanoate (15).** Compound 9 (0.95 g, 2.1 mmol) was dissolved in dry DMF (50 mL). To this solution was added potasium *tert*-butoxide (0.26 g, 2.3 mmol) and the mixture stirred for 45 min at room temperature. *tert*-Butyl bromoacetate (1.9 g, 9.7 mmol) was added and the mixture stirred for a further 3.5 h at room temperature. DMF was removed by rotoevaporation under high vacuum at 40 °C. The resulting crude mixture was purified by column chromatography with EtOAc/hexane (1:2) as the eluting solvent to give 0.8 g (67%) of 14 as an oil. The presence of the 2-thiopyridyl moiety was shown by the ^{1}H NMR resonances at δ 6.93–7.04, 7.15–7.24, 7.44–7.6, and 8.29–8.39. This material was directly converted to 15 by the same set of reaction conditions used above in the conversion of 9 to 11. The crude product was purified by silica gel column chromatography (EtOAc/hexane, 1:7). The oil which was obtained gave a white solid upon trituration with cold hexane. Recrystallization of this solid from hexane gave 0.38 g (59%) of 15: mp 88.5–89.5 °C; $[\alpha]_D$ +11.4° (*c* 0.51, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9 H, $C(CH_3)_3$), 1.45 (s, 9 H, $C(CH_3)_3$), 1.46 (s, 9 H, $C(CH_3)_3$), 2.82 (ddd, *J* = 1.5, 7.8, 15.6 Hz, 1 H, β-CH), 3.03 (dd, *J* = 4.5 and 15.6 Hz, 1 H, β-CH), 4.15 (s, 2 H, NCH_2), 4.46–4.55 (m, 1 H, α-CH), 5.24 (br d, *J* = 3.6 Hz, 1 H, NH), 6.52 (s, 1 H, C=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.87 ($C(CH_3)_3$), 27.91 ($C(CH_3)_3$), 28.18 ($C(CH_3)_3$), 28.68 (β-C), 39.57 (NCH_2), 52.20 (α-C), 80.18 ($C(CH_3)_3$), 82.72 ($C(CH_3)_3$), 83.07 ($C(CH_3)_3$), 128.86 (C=C), 145.27 (C=C), 155.11 (urethane C=O), 166.19 (C=O), 169.63 (C=O), 169.92 (C=O), 170.52 (C=O); FAB-MS *m/z* 455 $(MH)^+$. Anal. ($C_{22}H_{34}N_2O_8$) C, H, N.

(*S*)-2,5-Dihydro-2,5-dioxo-α-amino-1-(carboxymethyl)-pyrrole-3-propanoic Acid (4). Compound 15 (0.3 g, 0.66 mmol) was deprotected using the same method as that used to make 3 and gave 0.11 g (68.8%) of 4 as white solid: mp 125 °C dec; $[\alpha]_D$ −3.0° (*c* 0.61, H_2O); ^{1}H NMR (300 MHz, D_2O) δ 2.9–3.1 (m, 2 H, CH_2), 4.07 (dd, *J* = 6.1 and 7.32 Hz, 1 H, α-CH), 4.18 (s, 2 H, NCH_2), 6.69 (s, 1 H, C=CH); ^{13}C NMR (75 MHz, D_2O) δ 26.29 (CH_2), 39.38 (α-C), 52.33 (NCH_2), 130.51 (C=C), 143.72 (C=C), 171.14 (C=O), 171.72 (C=O), 171.96 (C=O), 172.52 (C=O); FAB-MS *m/z* 243 $(MH)^+$. Anal. ($C_9H_{10}N_2O_6 \cdot {}^1/_3H_2O$) C, H, N.

***tert*-Butyl 2(*S*)-[*N*-(*tert*-Butoxycarbonyl)amino]-3-[*N*-(methoxycarbonyl)-*N*-(carbamoylmethyl)amino]propanoate (17).** *tert*-Butyl 2-[(*tert*-butoxycarbonyl)amino]-3-aminopropanoate (1.73 g, 6.65 mmol) was dissolved in dry DMF (100 mL) and treated with KI (1.2 g, 7.2 mmol) and $NaHCO_3$ (8.5 g, 0.1 mol). To this heterogeneous mixture was added methyl 2-bromoacetate (0.51 g, 3.3 mmol) after which time the mixture

was stirred at room temperature for 24 h. The resulting mixture was concentrated to dryness and the residue mixed with THF. The precipitated salts were removed by rapid filtration and the THF solution was cooled to 0 °C whereupon it was treated with methyl chloroformate (1.08 mL) and Et_3N (0.67 g, 6.6 mmol). This solution was allowed to warm up to room temperature, where it was stirred for 24 h. The resulting solution was concentrated to dryness and the residue was dissolved in EtOAc and washed with 10% citric acid and saturated NaCl. The solution was dried over $MgSO_4$ and concentrated to dryness. This residue was dissolved in methanolic ammonia and stirred overnight at room temperature. The solution was rotoevaporated to dryness and the residue was subjected to silica gel column chromatography (EtOAc/hexane, 5:2) to give 0.83 g (66%) of **17** as an oil: $[\alpha]_D$ −8.7° (*c* 0.58, MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9 H, $C(CH_3)_3$), 1.44 (s, 9 H, $C(CH_3)_3$), 3.6–3.65 (m, 2 H, β-CH_2), 3.69 (s, 3 H, OCH_3), 3.92 (s, 2 H, NCH_2CO), 4.25–4.4 (m, 1 H, α-CH), 5.4–5.5 (m, 1 H, NH), 5.97 (br s, 0.5 H, NH), 6.04 (br s, 0.5 H, NH), 6.50 (br s, 0.5 H, NH), 6.77 (br s, 0.5 H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.82 ($C(CH_3)_3$), 28.18 ($C(CH_3)_3$), 50.10 (β-C), 51.10 (α-C), 51.80 (NCH_2CO), 53.02 (OCH_3), 80.00 ($C(CH_3)_3$), 155.5 (urethane C=O), 157.2 (urethane C=O), 169.66 (amide C=O), 171.73 (ester C=O); FAB-MS *m/z* 376 $(MH)^+$. Anal. ($C_{16}H_{29}N_3O_7$) C, H, N.

***tert*-Butyl 2,4-Dioxo-α(*RS*)-[(*tert*-butoxycarbonyl)amino]-1-imidazolidinepropanoate (18).** Compound **17** (0.06 g, 0.16 mmol) was dissolved in 95% EtOH (3 mL) and treated with 2 N KOH (80 μL, 0.16 mmol). This mixture was heated at 40 °C for 2 h and a further 2 days at room temperature. Another 40 μL of KOH (0.08 mmol) was added to this mixture and heated at 40 °C for a further 2 h, at which time EtOH was removed by rotoevaporation. The residue was partitioned between EtOAc and 10% citric acid. The EtOAc layer was washed with saturated NaCl and concentrated under vacuum. The residue was chromatographed on a silica gel column (EtOAc/hexane, 2:1) to give 19.4 mg (35%) of **18** as a colorless oil which crystallized when cooled: mp 160.5–161.5 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.40 (s, 9 H, $C(CH_3)_3$), 1.45 (s, 9 H, $C(CH_3)_3$), 3.6 (dd, J = 4.89 and 14.34 Hz, 1 H, β-CH_2), 3.74 (dd, J = 7.33 and 14.34 Hz, 1 H, β-CH_2), 3.81 (d, J = 17.4 Hz, 1 H, NCH_2), 4.14 (d, J = 17.4 Hz, 1 H NCH_2), 4.32–4.39 (m, 1 H, α-CH), 5.44 (d, J = 7.63 Hz, 1 H, NH), 9.13 (s, 1 H, imide NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.83 ($C(CH_3)_3$), 28.15 ($C(CH_3)_3$), 44.3 (β-C), 51.46 (NCH_2CO), 52.70 (α-C), 80.31 ($C(CH_3)_3$), 83.25 ($C(CH_3)_3$), 155.35 (urethane C=O), 157.16 (urethane C=O), 169.02 (NCH_2CO), 170.57 (C=O); FAB-MS *m/z* 344 $(MH)^+$. Anal. ($C_{15}H_{25}N_3O_6$) C, H, N.

2,4-Dioxo-α(*RS*)-amino-1-imidazolidinepropanoic Acid (6). Compound **18** (0.15 g, 0.44 mmol) was deprotected by the same procedure used to synthesize **3** to give 50 mg (60%) of **6** as a white solid: ^1H NMR (300 MHz, D_2O) δ 3.71 (dd, J = 7.02 and 16.17 Hz, 1 H, β-CH_2), 3.8–3.94 (m, 2 H, α-CH and β-CH_2), 4.03 (d, J = 1.2 Hz, NCH_2); ^{13}C NMR (75 MHz, D_2O) δ 43.38 (β-C), 52.12 (α-C), 53.58 (NCH_2CO), 159.46 (urea C=O), 171.81 (C=O), 174.40 (C=O). Anal. ($C_6H_9N_3O_4 \cdot {}^1/_2H_2O$) C, H, N.

Tissue Preparation. Transverse hippocampal slices were obtained from 30–100-day-old male Sprague–Dawley rats. Rats were anesthetized with urethane (1.5 g/kg ip) and then decapitated. The brain was removed and placed in ice-cold preparatory medium comprised of 124 mM NaCl, 3.3 mM KCl, 10 mM $MgSO_4$, 0.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 10 mM glucose, and 26 mM $NaHCO_3$ equilibrated with 95% O_2/5% CO_2 (pH 7.4).[26] The hippocampus was then isolated and sliced into 500-μm slices using a Campden Instruments VibroSlice microtome. Slices were submerged in preparatory medium at 28 °C which was aerated with 95% O_2/5% CO_2 and incubated for 45 min. Slices were then transferred to recording medium comprised of 124 mM NaCl, 3.3 mM KCl, 2.4 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 10 mM glucose, and 26 mM $NaHCO_3$ aerated with 95% O_2/5% CO_2 (pH 7.4) and incubated at 28 °C for at least 30 min prior to use.

Electrophysiology. Slices were transferred to a small recording chamber containing the recording medium at 34 °C. Initially the upper surface of the slice was exposed to a humid atmosphere containing 95% O_2/5% CO_2. Bipolar stimulation (0.1 ms, 10–40 V, 0.1 Hz) was delivered to the Schaffer collateral axons via a pair of Teflon-coated stainless steel wires (0.003 in.). Glass microelectrodes (2–14 MΩ inpedance filled with 2 M NaCl) were placed in the stratum radiatum of regio superior to serve as recording electrodes. The evoked extracellular synaptic field potentials were observed using a storage oscilloscope, and the peak amplitudes were sampled and recorded with a chart recorder. When a suitable field potential was obtained, the slice was submerged in oxygenated medium (34 °C) and the response allowed to stabilize. Test compounds were dissolved in oxygenated medium and were added and removed using a push/pull device allowing a complete change of medium within 30 s.[27]

Slices were exposed to L-AP4, L-quisqualic acid, and the test compound in the following sequence, which is a modification of our previous method:[14] (1) 200 μM L-AP4, (2) test compound, (3) 200 μM L-AP4, (4) 16 μM L-quisqualic acid, (5) 200 μM L-AP4, (6) test compound, (7) 200 μM L-AP4. Slices were washed between each addition of new drug until the peak amplitude of the field potential returned to its pretest level. Full concentration–response curves were determined for the test compound both before and after treatment of slices with L-quisqualic acid. In addition to determining the potency of compounds before and after addition of L-quisqualic acid, this protocol was designed to test for the ability of test compounds to induce the QUIS effect (and thus mimic the effects of L-quisqualic acid) and to test for the "preblocking" and "reversal" effects which have been previously observed only for L-homocysteine sulfinic acid, L-serine O-sulfate, and L-α-aminoadipic acid.

Concentration–Response Data. Concentration–response data were obtained by exposing the slice to a concentration of drug which was subthreshold for inhibition of the field potential. Drug concentration was doubled every 4 min until the response had either declined more then 70% or the bath concentration of the drug exceeded 10 mM. A 4-min exposure has been shown to allow sufficient time to equilibrate the slice with the drug. IC_{50} values were obtained by plotting the fractional response remaining at the end of 4 min versus the log of the bath concentration of the drug. The concentration which produced a 50% inhibition of the peak amplitude (IC_{50}) was interpolated from the graph. All reported IC_{50} values are the mean values for four or more experiments.

Acknowledgment. This work was supported by grants from Cortex Pharmaceuticals, Inc. (R.L.J.), the Minnesota Medical Foundation (J.F.K.), and the National Institutes of Health (NS 17944) (J.F.K. and R.L.J.).

(26) Fieg, S.; Lipton, P. N-Methyl-D-aspartate Receptor Activation and Ca^{2+} Account for Poor Pyramidal Cell Structure in Hippocampal Slices. *J. Neurochem.* **1990**, *55*, 473–483.

(27) Koerner, J. F.; Cotman, C. W. A Microperfusion Chamber for Brain Slice Pharmacology. *J. Neurosci. Methods* **1983**, *7*, 243–251.