Synthesis of Oxadiazolidinedione Derivatives as Quisqualic Acid Analogues and Their Evaluation at a Quisqualate-Sensitized Site in the Rat Hippocampus

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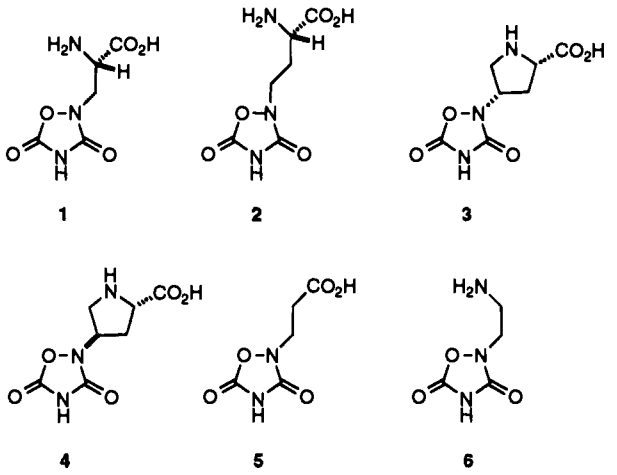
The ability of quisqualic acid (1) to sensitize neurons to depolarization by ω-phosphono α-amino acid analogues of excitatory amino acids is a highly specific phenomenon and is termed the QUIS effect. In an attempt to elucidate the structure–activity relationships for this sensitization, analogues **2-6** of quisqualic acid have been synthesized. Compounds **4, 5**, and **6** showed no quisqualate sensitization with respect to L-2-amino-6-phosphonohexanoic acid (L-AP6), while compounds **2** and **3** were 1/10 and 1/1000, respectively, as active as quisqualic acid in sensitizing neurons toward L-AP6.

Introduction

Quisqualic acid (**1**) is an unique analogue of glutamic acid in that it affects a number of systems within the central nervous system (CNS) that are associated with excitatory amino acid neurotransmission.[1,2] Quisqualic acid is a potent agonist at several excitatory amino acid receptor subtypes including the kainate,[3,4] AMPA,[5] and metabotropic receptors.[6,7] It also inhibits the enzyme *N*-acetyl α-linked acidic dipeptidase which hydrolyzes the brain dipeptide *N*-acetyl-L-aspartyl-L-glutamic acid[8] and the Ca^{2+}/Cl^--dependent glutamic acid uptake system in brain synaptic plasma membrane preparations.[9] Exposure of hippocampal slices to quisqualic acid produces a sensitization of neurons to depolarization by other excitatory amino acid analogues, in particular amino acid phosphonates.[10,12] This effect has been termed the QUIS effect and 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] An uptake model for the QUIS effect has been proposed.[13] Furthermore, the site which is sensitized by quisqualic acid and the site to which quisqualic acid binds in order to bring about this sensitization are novel sites of action different from the classical AMPA and AP4 receptors.[14,15] 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 the changes of excitability that occur in disease states such as epilepsy, we have been interested in delineating the structure–activity relationships of this phenomenon.

Previously, we synthesized a series of quisqualic acid analogues in which the oxadiazolidinedione ring system was replaced with other heterocyclic ring systems.[16] In the present study, we have undertaken the synthesis of a number of analogues of quisqualic acid, compounds **2–6**, in which the oxadiazolidinedione ring has been retained while modifications have been made in the moiety attached to this heterocyclic ring system. Analogue **2** is a homologue of quisqualic acid in that it has an additional methylene unit in the side chain, while the pyrrolidine derivatives **3** and **4** have been synthesized as conformationally restricted analogues of quisqualic acid. Analogues **5** and **6** have been made to determine the importance of the amino and carboxyl moieties of quisqualic acid, respectively, for quisqualate sensitization.



Chemistry

The synthetic strategies for **2–6** centered on the construction of the oxadiazolidinedione ring on a suitable precursor. In the case of analogues **2**, **3**, and **5**, the synthetic methodology was similar to that developed for the synthesis of L-quisqualic acid.[17,18] The synthesis of **2** is outlined in Scheme 1. The previous synthesis of this compound by alternative methods has been reported in the literature.[19] However, this previous report did not provide any experimental details or physical properties. In our approach, aldehyde **7** was condensed with *O*-benzylhydroxylamine in the presence of sodium cyanoborohydride to give the *N*-benzyloxyamino derivative **8**, which was then acylated with ethoxycarbonyl isocyanate to yield **9**. Removal of the benzyl protecting groups from **9** by hydrogenolysis gave the *N*-hydroxy carboxylic acid **10**. Treatment of **10** under basic conditions led to formation of the oxadiazolidinedione ring, and subsequent removal of the *tert*-butoxycarbonyl protecting group with acid provided **2** as its HCl salt.

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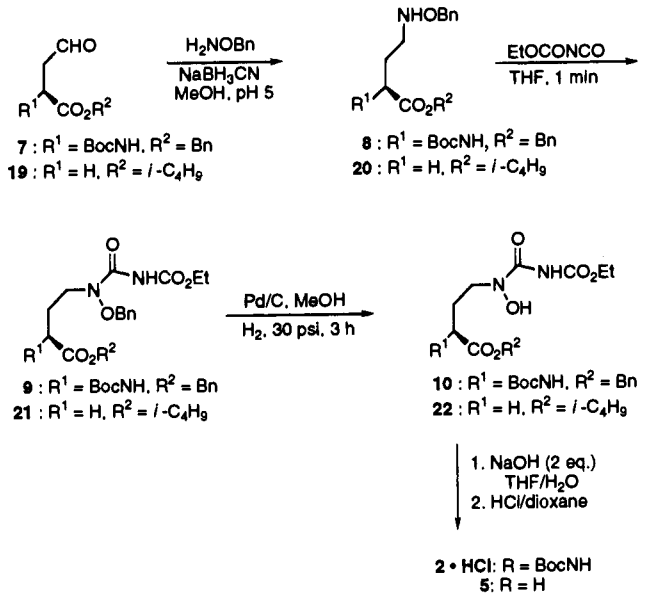
Scheme 1



An analogous sequence of reactions was used to synthesize the *cis*-pyrrolidine analogue, compound **3**, starting from the 4-oxoproline derivative **11**[20] (Scheme 2). Reductive amination of **11** provided a mixture of *cis* (**12**) and *trans* *N*-benzyloxyamino derivatives in a ratio of 45:1, as determined by HPLC. The small traces of the undesired *trans* isomer were carried through the next two reaction steps before being ultimately removed during the recrystallization of **14**.

Synthesis of the *trans*-pyrrolidine analogue, compound **4**, was accomplished by an entirely different set of reaction sequences (Scheme 3). A Mitsunobu reaction between *N*-Boc-*cis*-4-hydroxyproline methyl ester (**16**) and *N,O*-bis(phenoxycarbonyl)hydroxylamine[21] efficiently provided the corresponding *N,O*-bis(phenoxycarbonyl)hydroxylamine intermediate with complete inversion of the configuration at the 4-position. This intermediate was unstable and thus was immediately treated with an excess of methanolic ammonia to provide the *N*-hydroxyurea **17**. This compound was cyclized to the oxadiazolidinedione derivative **18** by treatment with ethyl chloroformate and sodium hydroxide. The *tert*-butoxycarbonyl group was removed with acid and the desired product **4** isolated after treatment with propylene oxide.

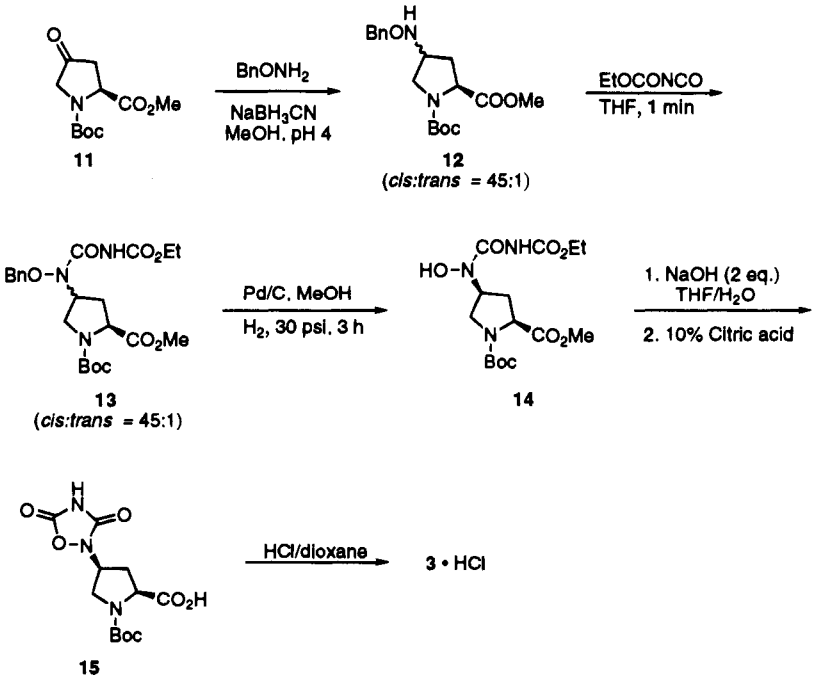
The desaminoquisqualic acid analogue **5** was obtained via the intermediates **19**–**22** (Scheme 1) using the same general reaction sequence that was employed to make **2**. Although the synthesis of the descarboxy analogue **6** had been previously reported in literature,[22] the reported route did not work well in our hands. Instead, **6** was synthesized from the known compound *N*-hydroxy[2-[(*tert*-butyloxycarbonyl)amino]ethyl]urea[21] by treating this compound with ethyl chloroformate and sodium hydroxide followed by HCl and dioxane to remove the *tert*-butoxycarbonyl protecting group.

Biological Results and Discussion

The oxadiazolidinedione derivatives were first tested for their ability to directly depolarize CA1 pyramidal neurons in preparations of rat hippocampal slices. In this study, minimal levels of **2**–**6** were used to estimate the IC_{50} value of each compound prior to the tissue slices being treated with quisqualic acid. After this determination, the hippocampal slices were exposed to 20 μM quisqualic acid for 5 min to induce quisqualate sensitization. The IC_{50} values of **2**–**6** were then again determined. Finally, quisqualate sensitization was reversed by a 10 min exposure to 2 mM L-α-aminoadipic acid (L-α-AA), and the IC_{50} value of each compound after reversal was measured. The data for this set of experiments are shown in Table 1.

The rationale for conducting this study first is that it allowed us to identify the maximum level of a test compound that could be utilized during tests of its ability to induce quisqualate-like sensitization. In

Scheme 2



Scheme 3

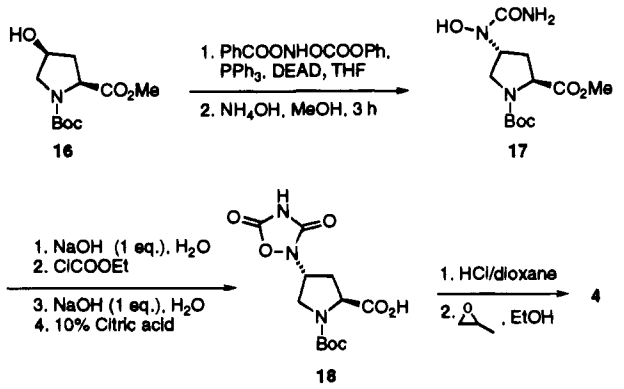


Table 1. Potency of Test Compounds in Schaffer Collateral-CA1 Pyramidal Neurons

	IC_{50} values (μM) ± SD[a]		
compound	before exposure to quisqualic acid	after exposure to quisqualic acid[b]	after reversal with L-α-AA[c]
L-quisqualic acid	7.0 ± 0.8	4.0 ± 0.8	26 ± 4
2	9.8 ± 0.8	5.5 ± 0.5	20 ± 3
3	1000 ± 130	530 ± 150	1100 ± 130
4	15 ± 0.4	14 ± 2.1	nd[d]
5	>10000	>10000	nd
6	5400 ± 2100	3800 ± 1000	nd

[a] SD: sample standard deviation. [b] Exposure for 5 min with 20 μM quisqualic acid. [c] Treatment for 10 min with 2 mM L-α-aminoadipic acid (L-α-AA). [d] nd: not determined.

previous studies we found that a 20 min exposure to a test compound at twice its IC_{50} level caused reversible inhibition of the electrophysiological response in the hippocampal CA1 pathway, but increasing either the concentration or time of exposure to the test compound resulted in some irreversible loss of electrophysiological activity. In this study, two of the test compounds, **2** and **4**, exhibited a potency (IC_{50} value) in the CA1 pathway of approximately 10 μM which is equivalent to quisqualic acid itself. Two other compounds, **3** and **6**, exhibited weak activity in the millimolar range. Compound **5** was inactive at a 10 mM concentration. In subsequent investigations of quisqualate sensitization, we employed the test compounds at approximately twice their IC_{50} values prior to quisqualic acid pretreatment or 4 mM, whichever was lower.

One interesting phenomenon we observed involves the ability of quisqualic acid to induce "self-sensitization". Minimal levels of quisqualic acid were used to estimate an IC_{50} value of 7 μM before pretreatment with larger doses. Following exposure of hippocampal slices to higher levels (20 μM) of quisqualic acid, it was observed that the IC_{50} value decreased to 4 μM. After reversal with L-α-AA, the IC_{50} value was 26 μM. A plausible interpretation of these data is that the initial exposure of hippocampal slices to quisqualic acid during measurement of the IC_{50} value results in partial self-sensitization, so that the initial value does not reflect the actual receptor response in the absence of quisqualate sensitization. The IC_{50} value measured after reversal probably corresponds to the actual response of non-NMDA receptors to quisqualic acid where there is no quisqualate sensitization. Consistent with this interpretation is the observation that other excitatory amino acids we have tested previously (whose response in CA1 is increased by quisqualate sensitization, but which do not themselves initiate quisqualate sensitization) exhibit essentially unchanged IC_{50} values before exposure to quisqualic acid and after reversal with L-α-AA. The interesting observation in the present study is that compound **2** shows the same upward shift in IC_{50} values as quisqualic acid. This presumably is related to the ability of **2** to induce quisqualate sensitization as shown below.

Table 2. Sensitization of CA1 Pyramidal Neurons to Depolarization by L-AP6[a] after Exposure to Quisqualic Acid Analogues

		IC_{50} values for L-AP6 (mM) ± SD[b]		
compound	exposure time (min) [concn, mM]	before exposure	after exposure	after reversal with L-α-AA[c]
quisqualic acid[d]	4 [0.016]	>10	0.04 ± 0.008	1.9 ± 0.1
2	20 [0.02]	11 ± 1.4	0.17 ± 0.10	12 ± 1.1
3	20 [2]	>10	0.78 ± 0.37	>10
4	20 [0.02]	>10	>10	nd[e]
5	20 [4]	>10	>10	nd
6	20 [4]	>10	>10	nd

[a] L-AP6: L-2-amino-6-phosphonohexanoic acid. [b] SD: sample standard deviation. [c] Treatment with 2 mM L-α-aminoadipic acid (L-α-AA) for 10 min. [d] Data from ref 23. [e] nd: not determined.

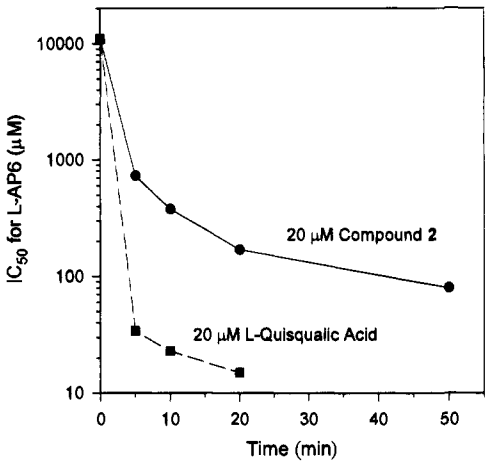


Figure 1. Time course for sensitization of CA1 pyramidal neurons to L-2-amino-6-phosphonohexanoic acid (L-AP6). Hippocampal slices were exposed to 20 μM **2** or L-quisqualic acid for various time intervals. After each exposure, the IC_{50} value for depolarization of CA1 pyramidal neurons by L-AP6 was measured.

The data in Table 2 show the effectiveness of the analogues for inducing sensitization to depolarization by L-2-amino-6-phosphonohexanoic acid (L-AP6). When hippocampal slices were exposed to the test compounds for 20 min, a significant increase in neuronal sensitivity to L-AP6 was observed with compounds **2** (>50-fold) and **3** (>10-fold). This compares to a 250-fold sensitization elicited by a 4 min exposure to quisqualic acid.[23] Because compound **2** appeared to be the most potent analogue of quisqualic acid, it was subjected to further analysis. As with quisqualic acid, the sensitization produced by **2** was long lasting, persisting with little change for more than 1 h (data not shown). Similarly, the sensitization produced by **2** could be reversed by a 10 min exposure to 2 mM L-α-AA. The data indicate that **2** induces sensitization in a manner analogous to quisqualic acid, but with a 10-fold lower potency. This conclusion is supported by the data in Figure 1, which show the time dependency for sensitization of pyramidal

Table 3. Sensitization of CA1 Pyramidal Neurons by **2** to Depolarization by L-AP4, L-AP5, and L-AP6[a]

compound	IC_{50} values (mM) ± SD[b] before exposure to **2**	after exposure to **2**	after reversal with L-α-AA
L-AP4	1.5 ± 0.1	0.26 ± 0.06	1.7 ± 0.3
L-AP5	2.3 ± 0.4	0.31 ± 0.1	2.2 ± 0.9
L-AP6	11 ± 1.4	0.17 ± 0.1	12 ± 1.1

[a] L-AP4, L-2-amino-4-phosphonobutanoic acid; L-AP5, L-2-amino-5-phosphonopentanoic acid; L-AP6, L-2-amino-6-phosphonohexanoic acid. [b] Stimulating and recording electrodes were placed in stratum radiatum of CA1, and the extracellular synaptic field potentials were recorded. The IC_{50} values for the phosphonate agonists were measured before and after a 20 min exposure to 20 mM **2** and again after a 20 min exposure to 2 mM L-α-aminoadipic acid (L-α-AA). SD: sample standard deviation.

neurons to L-AP6. At each time point the sensitization promoted by **2** is approximately 10-fold lower than that achieved with quisqualic acid. The ability of **2** to produce sensitization to other phosphonates is shown in Table 3. Analogous to quisqualic acid,[23] **2** produces increased neuronal sensitization with L-2-amino-4-phosphonobutanoic acid (L-AP4), L-2-amino-5-phosphonopentanoic acid (L-AP5), and L-AP6.

A detectable level of sensitization was also observed with compound **3**, one of the conformationally restricted analogues of quisqualic acid. Taking into account the time of exposure (20 min) and the concentration used (2 mM), it appears that compound **3** is approximately $^1/_{1000}$ as effective as quisqualic acid in promoting sensitization. The other conformationally restricted analogue, **4**, elicited no detectable sensitization in our study. However, this compound could not be tested above 20 μM concentration because of its low IC_{50} value for depolarization of CA1 pyramidal neurons. It is possible that barring toxic effects, **4** would also produce some sensitization at higher levels.

The inactivity of compounds **5** and **6** indicates that the amino and carboxylic acid functional groups of quisqualic acid are necessary for quisqualate sensitization. The activity profile of homologue **2**, however, shows that minor modification of the carbon chain is tolerated at the quisqualic acid site which brings about the sensitization. Furthermore, the partial activity of conformationally constrained analogue **3** coupled with the inactivity of its diastereoisomer, **4**, provides a clue to the relative spatial requirements of the carboxylic acid and oxadiazolidinedione ring of quisqualic acid and suggests a starting point for the synthesis of other conformationally constrained analogues to explore the relative spatial requirements of these pharmacophoric groups.

We have proposed that quisqualic acid must be transported into an intracellular compartment in order to induce sensitization.[13] We demonstrated concentrative cellular uptake of quisqualic acid with HPLC methodology[13] and immunocytochemical methods.[24] In the present study, we compared the rate of uptake of quisqualic acid and **2** into hippocampal slices and found that **2** is transported at about 50% the rate observed with quisqualic acid. The rate of uptake of **2** at 20 μM concentration in the medium was 21 pmol/(mg of protein·min) and remained constant for the 30 min duration of the experiments, whereas the rate of uptake of 20 μM quisqualic acid was 43 pmol/(mg of protein·min) under comparable conditions. Since **2** induces sensitization at approximately 10% the rate of quisqualic acid, some, but not all, of the difference may be explained by the slower uptake of **2**.

Quisqualic acid also sensitizes the neurons to depolarization by itself and by **2**. This suggests that these compounds also interact directly with the "quisqualate-sensitized site". This result also contradicts the hypothesis that enhanced depolarization after exposure to quisqualic acid involves heteroexchange of sequestered quisqualic acid with an exogenously applied agonist, since the self-sensitization following exposure to quisqualic acid does not introduce another compound.[25]

In summary, the data indicate that **2** mimics the actions of quisqualic acid, with respect to its ability to sensitize neurons to depolarization by L-AP4, L-AP5, and L-AP6, and by the reversal of this sensitization by L-α-AA. Although the sensitization by **2** follows a slower time course than sensitization by quisqualic acid, the sensitivity to depolarization by L-AP6 is eventually enhanced at least 100-fold. Thus, **2** represents the first reported analogue of quisqualic acid capable of inducing sensitization in a potent and sustained manner.

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 an IBM 200-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 MeOH-d_4 or acetone-d_6 and to TSP in D_2O. ^{13}C-NMR was performed on either an IBM 200-MHz or a GE 300-MHz spectrometer at 50 and 75-MHz, respectively. For those compounds where rotamers about the carbamate bond were observed, both ^{1}H and ^{13}C resonances are listed. FAB mass spectra were obtained on a Finnigan 4000 spectrometer. Column chromatography was performed on silica gel (Merck, grade 60, 240−400 mesh, 60 Å) from Aldrich Chemical Co. Thin-layer chromatography (TLC) was carried out on Analtech 250 μm silica gel GHLF Uniplates. Visualization was achieved with either UV, I_2, or ninhydrin spray. THF was freshly distilled from sodium and benzophenone. CH_2Cl_2 was distilled from sodium hydride.

(2*S*)-Benzyl 4-[(*O*-Benzylhydroxy)amino]-2-[[(1,1-dimethylethoxy)carbonyl]amino]butanoate (8). *O*-Benzylhydroxylamine (0.273 g, 2.2 mmol) and sodium cyanoborohydride (0.278 g, 4.4 mmol) were dissolved in 25 mL of anhydrous MeOH. The pH of the solution was brought to 5 by addition of HCl in dioxane. (2*S*)-Benzyl 4-oxo-2-[[(1,1-dimethylethoxy)-carbonyl]amino]butanoate[26] was added to the above solution and stirred for 48 h at room temperature. The pH of the solution was brought to 8 by addition of 1 M $NaHCO_3$. The solvents were removed under aspirator pressure. The crude product was partitioned between EtOAc (50 mL) and H_2O (25 mL). The organic layer was washed with H_2O, brine, and dried. The product was purified by flash column chromatography with hexanes/EtOAc (3:1) as the eluent. The product was obtained as an oil in a yield of 0.56 g (60%): TLC (hexanes/EtOAc, 3:1) R_f = 0.28; $[\alpha]^{20}$ −111.3° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.43 (br s, 10 H, Ar-H), 5.68 (br s, 1 H, HNOBn), 5.16 (d, *J* = 6 Hz, 2 H, $NOCH_2Ph$) 4.77 (s, 2 H, $PhCH_2O$), 4.31−4.45 (m, 1 H, α-H), 2.83−3.06 (m, 2 H, γ-H), 2.04−2.16 (m, 1 H, β-H), 1.74−1.87 (m, 1 H, β-H), 1.55 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 28.51 ($C(CH_3)_3$), 29.50, (β-C), 48.36 (NC), 52.36 (α-C), 67.11 ($NOCH_2Ph$), 76.38 (OCH_2Ph), 79.87 ($OC(CH_3)_3$), 127.02−128.99, 135.66, 137.94 (Ar-C), 155.71 (NCO_2), 172.68 (CO_2); FAB-MS (MNBA matrix) *m*/*z* 415 (100) $[M + H]^+$. Anal. ($C_{23}H_{30}N_2O_5$) C, H, N.

(2S)-Benzyl 4-[N-(Benzyloxy)-N'-(ethoxycarbonyl)ureido]-2-[[(1,1-dimethylethoxy)carbonyl]amino]butanoate (9). Compound **8** (0.81 g, 1.95 mmol) was dissolved in 5 mL of dry THF under N_2, ethoxycarbonyl isocyanate (0.22 g, 2.05 mmol) was added, and the reaction mixture was stirred for 1 min. The solvent was removed on a rotary evaporator under aspirator pressure. The crude product was purified by flash chromatography with hexanes/EtOAc (1:1) as an eluent. The product was obtained as an oil in a yield of 0.90 g (90%): TLC (hexanes/EtOAc, 1:1) R_f = 0.61; $[\alpha]^{20}$ −68.3° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.77 (br s, 1 H, CONHCO), 7.21 (br s, 10 H, Ar-H), 5.45 (br s, 1 H, OCONH), 4.97 (s, 2 H, $NOCH_2Ph$) 4.62 (s, 2 H, $PhCH_2O$), 4.10−4.21 (m, 1 H, α-H), 4.00 (q, J = 6 Hz, 2 H, OCH_2CH_3), 3.47−3.53 (m, 2 H, γ-H), 1.86−2.04 (m, 2 H, β-H), 1.28 (s, 9 H, $C(CH_3)_3$), 1.05 (t, J = 7.2 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 14.19 (OCH_2CH_3), 28.24 ($C(CH_3)_3$), 29.07 (β-C), 44.29 (γ-C), 51.68 (α-C), 61.77 (OCH_2CH_3), 62.38 ($NOCH_2Ph$), 77.18 (OCH_2Ph), 79.68 ($OC(CH_3)_3$), 128.20, 129.48, 134.21, 135.43 (Ar-C), 150.76 (NCONH), 152.38 ($NCONHCO_2$), 155.51 ($NCO_2C(CH_3)_3$), 171.90 (CO_2Me); FAB-MS (thioglycerol) m/z 430 (70) [M − $CO_2C(CH_3)_3$], 315 (100) [M − ($CO_2C(CH_3)_3$ and $CONHCO_2Et$)]. Anal. ($C_{27}H_{35}N_3O_8$) C, H, N.

2(S)-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-[N'-(ethoxycarbonyl)-N-hydroxyureido]butanoic Acid (10). Compound **9** (1.4 g, 2.8 mmol) was dissolved in 20 mL of MeOH, and 10% Pd/C (0.2 g) was added. The solution was hydrogenated under 30 psi of pressure for 3 h. The solution was then filtered through Celite, and the solvent was removed from the filtrate on a rotary evaporator under aspirator pressure. The crude compound was purified by flash chromatography with CH_2Cl_2/MeOH (30:1) as the eluent. The product was obtained as a crystalline solid from CH_2Cl_2/MeOH in a yield of 0.82g (85%): mp 93−95 °C; TLC (CH_2Cl_2/MeOH, 10:1) R_f = 0.41; $[\alpha]^{20}$ −18.6° (*c* 1.0, MeOH); ^{1}H NMR (acetone-d_6) δ 9.02 (br s, 1 H, NOH), 8.45 (br s, 1 H, CONHCO), 4.19−4.23 (m, 1 H, α-H), 4.09−4.15 (q, J = 6 Hz, 2 H, OCH_2CH_3), 3.33−3.67 (m, 2 H, NCH_2), 1.97−2.20 (m, 2 H, β-H), 1.38 (s, 9 H, $C(CH_3)_3$), 1.24 (t, J = 6 Hz, 3 H, OCH_2CH_3). ^{13}C NMR (acetone-d_6) δ 14.22 (OCH_2CH_3), 28.28 ($C(CH_3)_3$), 45.95 (NCH_2), 51.22 (α-C), 62.63 (OCH_2), 80.544 ($C(CH_3)_3$), 152.02 (NCONH), 154.15 ($NCONCO_2$); 156.35 ($NCO_2C(CH_3)_3$), 174.83 (CO_2CH_2); FAB-MS (MNBA matrix) m/z 372.2 (100) [M + Na]$^+$. Anal. ($C_{13}H_{23}N_3O_8$) C, H, N.

2(S)-Amino-4-[2'-(3',5'-dioxo-1',2',4'-oxadiazolidinyl)]-butanoic Acid Hydrochloride (2•HCl). Compound **10** (0.5 g, 1.65 mmol) was dissolved in 6 mL of 4 N HCl in dioxane under N_2. The reaction mixture was stirred at room temperature for 30 min, at which time TLC indicated the disappearance of starting material. The solvent was removed under aspirator pressure, and the crude material was crystallized from MeOH/EtOAc to give the product in a yield of 0.29 g (74%): TLC (EtOAc/MeOH, 9:1) R_f = 0.11; $[\alpha]^{20}$ −14.3° (*c* 1.0, H_2O); ^{1}H NMR (D_2O) δ 4.08−4.15 (m, 1 H, α-H), 3.82−3.94 (m, 2 H, γ-H), 2.02−2.37 (m, 2 H, β-H); ^{13}C NMR (D_2O) δ 27.98 (β-C), 45.89 (γ-C), 51.03 (α-C), 154.02, (NCON), 158.43-(OCONH), 171.98 (CO_2H); FAB-MS (MNBA matrix) m/z 204.1 (90) [M + H]$^+$. Anal ($C_6H_{10}N_3O_5Cl$) C, H, N.

(2S)-Methyl 4(R,S)-[(O-Benzylhydroxy)amino]-1-[(1,1-dimethylethoxy)carbonyl]pyrrolidine-2-carboxylate (12). (2*S*)-Methyl 1-[(1,1-dimethylethoxy)carbonyl]-4-oxopyrrolidine-2-carboxylate[20] (**11**, 3 g, 12.9 mmol) and *O*-benzylhydroxylamine (2.0 g, 1.7 mmol) were dissolved in 25 mL of MeOH at room temperature followed by 1 mL of HOAc and 5 g of dry 4 Å molecular sieves. After 3 h, the MeOH was removed on a rotary evaporator under aspirator pressure. The crude material was partitioned between EtOAc (25 mL) and 1% HCl (25 mL). The organic layer was washed with brine solution and then dried over $MgSO_4$. The solvent was removed on a rotary evaporator, and the crude imine product was purified by flash column chromatography with hexanes/EtOAc (2:1) to give a thick viscous oil in a yield of 4.4 g (100%). This material (3 g, 8.6 mmol) was dissolved in 25 mL of dry MeOH. Bromocresol green was added as an indicator followed by sodium cyanoborohydride (0.54 g, 8.6 mmol). The pH of the solution was brought to 4 and maintained at 4 by addition of HCl in MeOH at regular intervals of time. After 5 h, TLC indicated the presence of some starting material along with the product. Aqueous 10% $NaHCO_3$ solution was added to bring the pH to 7.7−8.0. The MeOH was removed on a rotary evaporator under aspirator pressure. The crude material was partitioned between EtOAc (25 mL) and brine solution (25 mL). The organic layer was dried with $MgSO_4$, and solvent was removed on a rotary evaporator under aspirator pressure. Flash column chromatography of the crude product using hexanes/EtOAc (1:1) as an eluent gave two fractions. The first fraction was identified as the imine intermediate (0.3 g, 10% recovery), and the second fraction was the desired product (2.65 g, 100% yield based on the recovery of starting material). HPLC analysis (Waters Novapak 8 × 100 mm, silica cartridge column, 2 mL/min, hexanes/EtOAc, 2.5:1) indicated the product was a mixture of diastereoisomers in the ratio of 45:1. HPLC retention times for the major and minor diastereoisomers are 14 and 16 min, respectively: ^{1}H NMR ($CDCl_3$) δ 7.31 (br s, 5 H, Ar-H), 5.77 (br s, 1 H, HNOBn), 4.76 (s, 2 H, $PhCH_2O$), 4.34 (dd, J = 6 and 4 Hz, 1H, α-H), 3.70 (s, 4 H, OCH_3, γ-H), 3.56−3.42 (m, 2 H, δ-H), 2.30−2.11 (m, 2 H, β-H), 1.45, 1.39 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 28.14 ($C(CH_3)_3$), 32.45, 33.46 (β-C), 49.48, 49.87 (δ-C), 51.79, 51.90 (OCH_3); 57.85 (γ-C), 58.36, 59.36 (α-C), 76.13 (OCH_2Ph), 79.63 ($OC(CH_3)_3$), 127.62, 128.18, 137.87 (Ar-C), 154.10 (NCO), 173.30 (CO_2Me); FAB-MS (MBNA matrix) m/z 351 (40) [M + H]$^+$, 295 (100) [M − $C(CH_3)_3$]. Anal. ($C_{18}H_{26}N_2O_5$) C, H, N.

(2S)-Methyl 4(R,S)-[N-(Benzyloxy)-N'-(ethoxycarbonyl)ureido]-1-[(1,1-dimethylethoxy)carbonyl]pyrrolidine-2-carboxylate (13). Compound **12** (0.75 g, 2.1 mmol) was dissolved in 5 mL of dry THF under N_2, and ethoxycarbonyl isocyanate (0.246 g, 2.1 mmol) was added, after which the reaction mixture was stirred for 1 min. The solvent was removed on a rotary evaporator under aspirator pressure. The crude product was purified using flash chromatography with hexanes/EtOAc (1:1) as the eluent. The product was obtained as an oil in a yield of 0.98 g (100%). TLC (hexanes/EtOAc, 1:1) R_f = 0.25; both the ^{1}H and ^{13}C spectra indicated the presence of rotamers about the carbamate bond; ^{1}H NMR ($CDCl_3$) δ 7.72 (br s, 1 H, CONHCO), 7.19 (br s, 5 H, Ar-H), 4.73−4.45 (m, 3 H, $PhCH_2O$ and γ-H), 4.09−3.86 (m, 3 H, OCH_2 and α-H), 3.66−3.60 (m, 1 H, δ-H), 3.50 (s, 3 H, OCH_3), 3.39−3.27 (m, 1 H, δ-H), 2.34−2.25 (m, 1 H, β-H), 2.05−1.99 (m, 1 H, β-H), 1.24, 1.20 (s, 9 H, $C(CH_3)_3$), 1.03 (t, J = 4.6 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 13.91 (OCH_2CH_3), 28.03 ($C(CH_3)_3$), 30.74, 31.52 (β-C), 46.65, 46.18 (δ-C), 51.79, 51.86 (OCH_3), 56.34, 56.79 (α-C), 57.03, 57.20 (γ-C), 61.72, 62.14 (OCH_2), 79.61 (OCH_2Ph), 80.01, 80.17 ($OC(CH_3)_3$), 127.49, 129.25, 133.65 (Ar-C), 150.23 (NCONH), 153.74 ($NCONHCO_2$), 153.86 ($NCO_2C(CH_3)_3$), 172.21, 172.48 (CO_2Me); FAB-MS (thioglycerol) m/z 466 (10) [M + H]$^+$, 410 (100) [M − $C(CH_3)_3$]. Anal. ($C_{22}H_{31}N_3O_8$) C, H, N.

(2S,4S)-Methyl 1-[(1,1-Dimethylethoxy)carbonyl]-4-[N'-(ethoxycarbonyl)-N-hydroxyureido]pyrrolidine-2-carboxylate (14). Compound **13** (0.7 g, 1.5 mmol) was dissolved in 20 mL of MeOH, and 0.2 g of 10% Pd/C was added. Hydrogenation was carried out under 30 psi of pressure for 3 h. The solution was then filtered through Celite, and the solvent was removed on a rotary evaporator under aspirator pressure. The crude compound was purified by flash chromatography with CH_2Cl_2/MeOH (30:1) as the eluent. The product was obtained as a solid in a yield of 0.42 g (100%). Recrystallization was accomplished from EtOAc and hexanes: mp 85−87 °C. HPLC analysis of the recrystallized product (Waters Novapak 8 × 100 mm silica cartridge column, 2 mL/min, CH_2Cl_2/MeOH, 50:1) indicated the product was a single diastereoisomer with a retention time of 20 min. $[\alpha]^{20}$ −23.3° (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 9.1 (br s, 1 H, NOH), 8.37 (d, J = 7.5 H, CONHCO), 4.86 (m, 1 H, γ-H), 4.27−4.13 (m, 3 H, OCH_2 and α-H,), 3.79−3.62 (m, 2 H, δ-H), 3.72 (s, 3 H, OCH_3), 2.44 (m, 1 H, β-H), 2.25 (m, 1 H, β-H), 1.41, 1.36 (s, 9 H, $C(CH_3)_3$), 1.16 (t, J = 3.5 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 14.28 (OCH_2CH_3), 28.24 ($C(CH_3)_3$), 33.88 (β-C), 46.85

(δ-C), 52.58 (OCH_3), 54.76 (α-C), 57.36 (γ-C), 62.11 (OCH_2), 80.75 ($OC(CH_3)_3$), 151.65 (NCONH), 153.44 (NCO_2Et), 174.79 (CO_2Me); FAB (MNBA matrix) m/z 374 (25) $[M - H]^-$, 328 (100) $[M - OCH_2CH_3]$. Anal. ($C_{15}H_{25}N_3O_8$) C, H, N.

(2S,4S)-1-[(1,1-Dimethylethoxy)carbonyl]-4-[2′-(3′,5′-dioxo-1′,2′,4′-oxadiazolidinyl)]pyrrolidine-2-carboxylic Acid (15). Compound **14** (0.7 g, 1.87 mmol) was dissolved in a mixture of 5 mL of THF and 5 mL of water. Sodium hydroxide (0.15 g, 3.7 mmol) was added, and the reaction mixture was stirred at room temperature for 30 min. The reaction was quenched by addition of 3 mL of 10% citric acid solution, and the product was extracted with 3 × 10 mL of EtOAc. The organic layer was dried over $MgSO_4$, and the solvent was removed under aspirator pressure to give 0.5 g (80%) of the product as a solid. Recrystallization from EtOAc and hexanes gave 0.47 g of product: mp 112–114 °C. A 1-D NOE experiment indicated the product was the *cis* diastereoisomer: TLC (CH_2Cl_2/MeOH, 10:1) R_f = 0.13; $[\alpha]^{20}$ −20.3° (*c* 1.0, MeOH); both the ^1H and ^{13}C spectra indicated the presence of rotamers about the carbamate bond; ^1H NMR (acetone-d_6) δ 10.07 (br s, 1 H, COOH), 4.73–4.65 (m, 1 H, γ-H), 4.36–4.29 (m, 1 H, α-H), 3.93–3.84 (m, 1 H, δ-H), 3.60–3.49 (m, 1 H, δ-H), 2.79–2.66 (m, 1 H, β-H), 2.38–2.28 (m, 1 H, β-H), 1.41, 1.36 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (acetone-d_6) δ 27.52, 27.67 ($C(CH_3)_3$), 31.39, 32.23 (β-C), 47.40, 47.80 (δ-C), 56.80, 57.09 (α-C), 57.33, 57.53 (γ-C), 79.91 ($OC(CH_3)_3$), 151.25, (NCONH), 153.32 (NOCON), 156.79, 156.82 ($NCO_2C(CH_3)_3$), 172.42, 172.86 (CO_2H); FAB-MS (thioglycerol) m/z 260 (100) $[M - C(CH_3)_3]$. Anal. ($C_{12}H_{17}N_3O_7$) C, H, N.

(2S,4S)-4-[2′-(3′,5′-Dioxo-1′,2′,4′-oxadiazolidinyl)]pyrrolidine-2-carboxylic Acid Hydrochloride (3·HCl). Compound **15** (0.5 g, 1.5 mmol) was dissolved in 5 mL of 4 N HCl/dioxane under a N_2 atmosphere, and the reaction mixture was stirred at room temperature for 30 min. After TLC analysis indicated the disappearance of starting material, the solvent was removed under aspirator pressure and the solid was triturated with EtOAc. The solid product was collected and then recrystallized from H_2O/MeOH to yield 0.26 g (64.4%) of a white solid: TLC (CH_2Cl_2/MeOH/AcOH, 10:1:1) R_f = 0.10; $[\alpha]^{20}$ −14.3° (*c* 1.0, MeOH); ^1H NMR (D_2O) δ 4.76 (m, 1 H, γ-H), 4.22 (dd, J = 3 and 16.5 Hz, 1 H, α-H), 3.89 (dd, J = 4.2 and 17.1 Hz, 1 H, δ-H), 3.60 (dd, J = 7.2 and 17.1 Hz, 1 H, δ-H), 2.64–2.74 (m, 1 H, β-H), 2.46–2.54 (m, 1 H, β-H); ^{13}C NMR (D_2O) δ 33.52 (β-C), 50.38 (δ-C), 60.23 (α-C), 61.48 (γ-C), 155.21 (NCONH), 159.76 (NOCON), 173.26 (CO_2H); FAB-MS (thioglycerol) m/z 216.1 (5) $[M + H]^+$. Anal ($C_7H_{10}N_3O_5Cl$) C, H, N.

(2S,4R)-Methyl 1-[(1,1-Dimethylethoxy)carbonyl]-4-(*N*-hydroxyureido]pyrrolidine-2-carboxylate (17). To a dry flask under N_2 was added freshly distilled dry THF (50 mL), followed by *N*-Boc-*cis*-4-hydroxyproline methyl ester[20] (**16**, 1.4 g, 5.71 mmol), PPh_3 (1.5 g, 5.71 mmol), and bis(phenoxycarbonyl)hydroxylamine[27] (1.14 g, 5.71 mmol). The solution was cooled to 0 °C, and diethyl azodicarboxylate (1.0 g, 5.71 mmol) was slowly added, while keeping the temperature at 0 °C. The mixture was stirred overnight at room temperature. The solvent was removed on a rotary evaporator under aspirator pressure. The intermediate product was isolated by flash chromatography with hexane/EtOAc (4:1) as the eluent. The fraction containing the component with a R_f value of 0.51 was isolated. As the intermediate is unstable, it was treated with 18 M NH_4OH (50 mL) solution in MeOH (20 mL) without any further purification. The solvents were removed on a rotary evaporator under aspirator pressure. Trituration of the crude product with EtOAc (10 mL) yielded a white solid (0.5 g, 50%): mp 108–111 °C; $[\alpha]^{20}$ −86.4° (*c* 1.0, MeOH); both the ^1H and ^{13}C spectra indicated the presence of rotamers about the carbamate bond; ^1H NMR (MeOH-d_4) δ 4.91 (m, 1 H, γ-H), 4.34 (dd, J = 6 and 3.6 Hz, 1H, α-H), 3.72 (s, 3 H, OCH_3), 3.66–3.42 (m, 2 H, δ-H), 2.52–2.42 (m, 1 H, β-H), 2.10–2.02 (m, 1 H, β-H), 1.45, 1.39 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (MeOH-d_4) δ 27.93, 27.79 ($C(CH_3)_3$), 31.49, 32.34 (β-C), 52.04 (δ-C), 55.54, 56.23 (γ-C), 58.76, 59.33 (α-C), 80.98 ($OC(CH_3)_3$), 154.10, 155.39 (NCO), 163.78 (NCON), 173.30 (CO_2Me); FAB-MS (MBNA matrix) m/z 304.2 (30) $[M + H]^+$. Anal. ($C_{12}H_{21}N_3O_6$) C, H, N.

(2S,4R)-1-[(1,1-Dimethylethoxy)carbonyl]-4-[2′-(3′,5′-dioxo-1′,2′,4′-oxadiazolidinyl)]pyrrolidine-2-carboxylic Acid. (18). In a 50 mL flask was dissolved compound **17** (0.50 g, 1.67 mmol) in H_2O (40 mL). Sodium hydroxide (0.095 g, 1.67 mmol) was added, and the mixture was stirred for 15 min. Ethyl chloroformate (0.16 g, 1.67 mmol) was added, and the mixture was stirred for 1 h. The second equivalent of NaOH (0.20 g, 3.4 mmol) was added, and the mixture was stirred for 3 h. Thirty milliliters of 10% citric acid was added, and the product was extracted with 25 mL of EtOAc. The organic layer was washed with a brine solution and dried with anhydrous $MgSO_4$. The solvent was removed on a rotary evaporator under aspirator pressure. The product was obtained as a white solid (0.2 g, 40%): mp 89–93° C; TLC (CH_2Cl_2/MeOH, 10:1) R_f = 0.13; $[\alpha]^{20}$ −18.3° (*c* 1.0, MeOH); both the ^1H and ^{13}C spectra indicated the presence of rotamers about the carbamate bond; ^1H NMR (MeOH-d_4) δ 4.33–4.21 (m, 2 H, γ-H, α-H), 3.61–3.55 (dd, J = 5.4 Hz, 5.4 Hz, 1 H, δ-H), 3.34–3.49 (dd, J = 7.5 Hz, 2.4 Hz, 1 H, δ-H), 2.43–2.38 (m, 1 H, β-H), 2.11–1.97 (m, 1 H, β-H), 1.44, 1.41 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (MeOH-d_4) δ 27.79, 27.97 ($C(CH_3)_3$), 36.68, 38.83 (β-C), 54.17, 55.28 (δ-C), 58.31, 58.51 (α-C), 69.23, 69.41 (γ-C), 80.80 ($OC(CH_3)_3$), 151.25, (NCONH), 153.32 (NOCON), 156.79, 156.82 ($NCO_2C(CH_3)_3$), 175.66 (CO_2H); FAB-MS (MNBA matrix) m/z 314.1 (100) $[M - H]^-$. Anal. ($C_{12}H_{17}N_3O_7$) C, H, N.

(2S,4R)-[2′-(3′,5′-Dioxo-1′,2′,4′-oxadiazolidinyl)]pyrrolidine-2-carboxylic Acid (4). Compound **18** (0.5 g, 1.5 mmol) was dissolved in 5 mL of 4 N HCl/dioxane under a N_2 atmosphere, and the reaction mixture was stirred at room temperature for 30 min. The solvent was removed under aspirator pressure, and the solid was triturated with EtOAc. The triturated product was dissolved in EtOH (15 mL), propylene oxide (5 mL) was added, and the solution was refluxed for 15 min. The solvents were removed, and the solid was dissolved in water and lyophilized to yield 0.20 g (64.4%) of a white solid product: TLC (CH_2Cl_2/MeOH/AcOH, 10:1:1) R_f = 0.10; $[\alpha]^{20}$ −14.3° (*c* 1.0, MeOH); ^1H NMR (D_2O) δ 4.63 (m, 1 H, γ-H), 4.29 (t, J = 5.8 Hz, 1 H, α-H), 3.42 (dd, J = 3.9 and 3.5 Hz, 1 H, δ-H), 3.30 (m, 1 H, δ-H), 2.42–2.34 (m, 1 H, β-H), 2.16–2.06 (m, 1 H, β-H); ^{13}C NMR (D_2O) δ 33.52 (β-C), 49.33 (δ-C), 60.29 (α-C), 60.54 (γ-C), 153.21 (NCONH), 159.76 (NOCON), 174.41 (CO_2H); FAB-MS (MNBA) m/z 215.1 (60) $[M + H]^+$. Anal. ($C_7H_{10}N_3O_5Cl$) C, H, N.

2′-Methylpropyl 3-[(*O*-Benzylhydroxy)amino]propionate (20). Isobutyl vinylacetate (5.0 g, 35.2 mmol) was dissolved in 50 mL of dry CH_2Cl_2 under N_2, and the solution was cooled to −78 ° C. Ozone was passed through the solution for 1 h at −78 °C. After the ozone gas was stopped, the solution was purged with N_2 until the solution turned from blue to colorless. The solution was transferred to a round bottom flask fitted with a reflux condenser, and then 2 mL of dimethyl sulfide was added at −78 °C. The solution was then allowed to warm to room temperature, where the CH_2Cl_2 was distilled off at atmospheric pressure. The crude product 2′-methylpropyl 3-oxopropionate (**19**) was used without any further purification. *O*-Benzylhydroxylamine hydrochloride (7.5 g, 46.9 mmol) in 100 mL of dry MeOH was added to the above solution followed by sodium cyanoborohydride (2.96 g, 47 mmol). The pH of the solution was brought to 5 by the addition of HCl in dioxane. The solution was stirred for 48 h, whereupon the pH of the solution is brought to 7.8 and the solvent was removed in vacuo. The crude mixture was partitioned between EtOAc (100 mL) and H_2O (50 mL). The aqueous layer was extracted with 2 × 25 mL of EtOAc. The organic layers were combined, washed with brine solution, and dried. The solvent was removed in vacuo. The crude product (5.0 g, 56%) was purified by flash chromatography with hexanes/EtOAc (1:1) as an eluent. The product was obtained as an oil in a yield of 4.0 g (44%): TLC (hexanes/EtOAc, 1:1) R_f = 0.71; ^1H NMR ($CDCl_3$) δ 7.36 (br s, 5 H, Ar-H), 4.69 (s, 2 H, $PhCH_2O$), 3.84 (d, J = 6.6 Hz, 2 H, OCH_2), 3.24 (t, J = 6.4 Hz, 2 H, NCH_2), 2.62 (t, J = 6.3 Hz, 2 H, CH_2CO), 1.90 (m, 1 H, $CH(CH_3)_2$), 0.93 (d, J = 6.8 Hz, 6 H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 19.76 ($C(CH_3)_2$), 28.33 ($C(CH_3)_2$), 32.89 (CH_2CO), 48.18 (NCH_2), 71.27 (OCH_2), 76.82 (OCH_2Ph), 128.49, 129.05,

138.48 (Ar-C) 173.27 (C=O); FAB-MS (MNBA matrix) m/z 252.2 (100) $[M + H]^+$. Anal. ($C_{14}H_{21}NO_3$) C, H, N.

2'-Methylpropyl 3-[*N*-(Benzyloxy)-*N'*-(ethoxycarbonyl)ureido]propionate (21). Compound **20** (1.6 g, 6.4 mmol) was dissolved in 5 mL of dry THF under nitrogen, and ethoxycarbonyl isocyanate (0.73 g, 6.4 mmol) was added. The reaction mixture was stirred for 1 min, and then the solvent was removed under reduced pressure. The crude product was purified by flash chromatography with hexanes/EtOAc (1:1) as the eluent. The product was obtained as an oil in a yield of 2.6 g (100%): TLC (hexanes/EtOAc, 1:1) $R_f = 0.51$; ^{1}H NMR ($CDCl_3$) δ 7.77 (br s, 1 H, CONHCO), 7.39 (br s, 5 H, Ar-H), 4.82 (s, 2 H, $PhCH_2O$), 4.19 (q, $J = 4.2$ Hz, 2 H, OCH_2CH_3), 3.91 (t, $J = 4.2$ Hz, 2 H, NCH_2), 3.84 (d, $J = 6.6$ Hz, 2 H, OCH_2-CH), 2.61 (t, $J = 4.2$ Hz, 2 H, CH_2CO), 1.86 (m, 1 H, *H* C-$(CH_3)_2$), 1.26 (t, $J = 4.2$ Hz, 3 H, CH_3), 0.88 (d, $J = 6.6$ Hz, 6 H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 14.86 (OCH_2CH_3), 19.66 ($C(CH_3)_2$), 28.19 ($C(CH_3)_2$), 32.36 (CH_2CO), 44.15 (NCH_2), 62.63 (OCH_2), 71.48 ($OCH_2CH(CH_3)_2$), 77.83 (OCH_2Ph), 129.62, 130.04, 130.25, 130.38, 134.58 (Ar-C), 151.36 (NCONH), 153.66 (CO_2Et); 172.34 (CO_2Bu^i); FAB-MS (MBNA matrix) m/z 367.1 (100) $[M + H]^+$. Anal. ($C_{18}H_{26}N_2O_6$) C, H, N.

2'-Methylpropyl 3-[*N*-Hydroxy-*N'*-(ethoxycarbonyl)ureido]propionate (22). The compound **21** (1.5 g, 4 mmol) was dissolved in 20 mL of MeOH, and 10% Pd/C (0.2 g) was added. The solution was hydrogenated under 30 psi of pressure for 3 h. The solution was then filtered through Celite, and the solvent was removed under reduced pressure. The crude compound was purified by flash chromatography with CH_2Cl_2/MeOH (30:1) as the eluent. Product was obtained as a crystalline solid in a yield of 1.10 g (100%): mp 93 °C; TLC (CH_2Cl_2/MeOH, 10:1) $R_f = 0.61$; ^{1}H NMR ($CDCl_3$) δ 9.02 (br s, 1 H, NOH), 8.45 (br s, 1 H, CONHCO), 4.22 (d, $J = 7.2$ Hz, 2 H, OCH_2CH); 3.87 (m, 4 H, OCH_2CH_3 and NCH_2), 2.73 (t, $J = 6.6$ Hz, 2 H, CH_2CO), 1.90 (m, 1 H, *H* $C(CH_3)_2$), 1.28 (t, $J = 3$ Hz, 3 H, CH_3), 0.91 (d, $J = 6.6$ Hz, 6 H, $C(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 14.86 (OCH_2CH_3), 19.67 ($C(CH_3)_2$), 28.24 ($C(CH_3)_2$), 32.73 (CH_2CO), 45.38 (NCH_2), 62.63 (OCH_2), 71.84 (OCH_2Ph), 152.39 (NCONH), 153.76 (CO_2Et); 173.82 (CO_2CH_2); FAB-MS (MBNA matrix) m/z 277.1 (100) $[M + H]^+$. Anal. ($C_{11}H_{20}N_2O_6$) C, H, N.

3-[2'-(3',5'-Dioxo-1',2',4'-oxadiazolidinyl)]propionic Acid (5). Compound **21** (100 mg, 0.36 mmol) was dissolved in 5 mL of THF and 5 mL of H_2O followed by the addition of KOH (40 mg, 0.72 mmol). The solution was stirred at room temperature overnight. TLC indicated a complete disappearance of starting material, and the product showed a positive test with 2,6-dichlorophenol–indophenol spray. The solvents were removed under vacuum, followed by the addition of 1 mL of 4 N HCl in dioxane. Acetone 20 mL was added, and the reaction mixture was stirred for 30 min. The solution was then filtered to remove inorganic salts. The solvent was removed from the filtrate under aspirator pressure to give a solid product which crystallized from EtOAc in a yield of 25 mg (40%) of **5**: mp 40–41 °C; TLC (EtOAc/MeOH, 9:1) $R_f = 0.13$; ^{1}H NMR (acetone-d_6) δ 10.89 (br s, 1 H, COOH), 3.96 (t, $J = 4.2$ Hz, 2 H, NCH_2), 2.73 (t, $J = 4.2$ Hz, 2 H, CH_2CO); ^{13}C NMR (acetone-d_6) δ 32.73 (CH_2CO), 45.38 (NCH_2), 152.71 (NCONH), 158.72 ($NHCO_2$), 173.61 (CO_2H); FAB-MS (MNBA matrix) m/z 173.1 (100) $[M - H]^-$. Anal. ($C_5H_6N_2O_5$) C, H, N.

2-[2'-(3',5'-Dioxo-1',2',4'-oxadiazolidinyl)]ethylamine Hydrochloride (6·HCl). In a 50 mL flask was added *N*-hydroxy-[2-[(*tert*-butoxycarbonyl)amino]ethyl]urea (0.10 g, 0.67 mmol) as a solution in H_2O (10 mL). Sodium hydroxide (0.025 g, 0.67 mmol) was added, and the reaction mixture was stirred for 15 min. Ethyl chloroformate (0.072 g, 0.67 mmol) then was added, and the reaction mixture was stirred for an additional hour. The second equivalent of NaOH (0.03 g, 0.7 mmol) was added, and the mixture was stirred for 3 h. Then 10 mL of 10% citric acid was added, and the product was extracted with 25 mL of EtOAc. The organic layer was washed with brine solution and dried with anhydrous $MgSO_4$. The solvent was removed on a rotary evaporator under aspirator pressure. The above compound was placed in a culture tube under argon, 4 N HCl/dioxane (0.1 mL, 0.4 mmol) was added, and the reaction mixture was stirred for 60 min. The solvent was removed on a rotary evaporator under aspirator pressure. Water (15 mL) was added, and the solution was lyophilized to yield 15 mg (39%) of the product: TLC (CH_2Cl_2/MeOH, 5:1) $R_f = 0.10$; ^{1}H NMR (D_2O) δ 3.81 (t, $J = 4.8$, 2 H, NCH_2), 3.16 (t, $J = 3.3$, 2 H, $ONCH_2$); FAB-MS (MNBA matrix) m/z 146.1 (100) $[M + H]^+$.

Tissue Slice Preparation. Detailed procedures for tissue slice preparation have been published.[13] Transverse slices of hippocampus (500 μM) were obtained from 25–60 day old male Sprague–Dawley rats, preincubated for 20–30 min in high Mg^{2+}–low Ca^{2+} preparatory medium, and maintained at 28 °C as previously described. For pharmacological experiments, individual slices were transferred to a microperfusion chamber where submerged slices were maintained at 34 °C in rapidly stirred medium equilibrated with an atmosphere of 95% O_2/5% CO_2. The peak amplitudes of the evoked field potentials were sampled and recorded using a strip chart recorder. Test compounds were added and removed using a push/pull device allowing a complete change of medium within 30 s.

Electrophysiology. Detailed procedures for the electrophysiological experiments have been published.[13] When evaluating test compounds, stimulation was delivered to the Schaffer collaterals with the recording electrode placed in the stratum radiatum of regio superior. Slices were maintained in incubation medium throughout the experiment. For the potency test outlined in Table 1, concentration–response data were collected for the test compound both before and after a 5 min exposure of the slices to quisqualic acid and also after reversal of quisqualate sensitization by a 10 min exposure to 2 mM L-α-AA. The sequence in which these were measured was as follows: (1) test compound (to measure its IC_{50} before exposure to quisqualic acid); (2) quisqualic acid; (3) test compound (to measure its IC_{50} value after exposure to quisqualic acid); (4) L-α-AA (2 mM); (5) test compound (to measure its IC_{50} value after reversal). Slices were washed between each addition of new drug until the peak amplitude of the evoked extracellular field potential returned to its pretest level. 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 compound. The concentration which produced a 50% inhibition of the peak amplitude of the field potential (IC_{50}) was interpolated from the graph. All reported IC_{50} values represent the mean value for four experiments. A similar protocol was used to evaluate sensitization of neurons to L-AP4, L-AP5, and L-AP6 (Tables 2 and 3) except that IC_{50} values were measured for L-AP6 and the test compounds were substituted for quisqualic acid.

Cellular Uptake. Cellular uptake of **2** by hippocampal slices was measured by precolumn derivatization of tissue samples with *o*-phthalaldehyde and HPLC separation on a reverse-phase hydrophobic C18 column. Details of these procedures have been published previously by us for quisqualic acid .[13]

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