A 3-Amino-4-hydroxy-3-cyclobutene-1,2-dione-Containing Glutamate Analogue Exhibiting High Affinity to Excitatory Amino Acid Receptors

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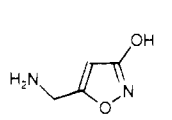
The syntheses of several novel *N*-(hydroxydioxocyclobutenyl)-containing analogues of γ-aminobutyric acid and L-glutamate were undertaken to test the hypothesis that derivatives of 3,4-dihydroxy-3-cyclobutene-1,2-dione (squaric acid), such as 3-amino-4-hydroxy-3-cyclobutene-1,2-dione, could serve as a replacement for the carboxylate moiety in neurochemically interesting molecules. The syntheses were successfully accomplished by preparation of a suitably protected diamine or diamino acid followed by reaction with diethyl squarate. Subsequent deprotection resulted in the isolation of the corresponding *N*-(hydroxydioxocyclobutenyl)-containing analogues **13**, **14**, and **18**. These analogues were screened as displacers in various neurochemical binding site assays. The L-glutamate analogue **18**, which showed high affinity as a displacer for kainate and AMPA binding, was also examined for agonist potency for CA1 pyramidal neurons of the rat hippocampal slice preparation. It rivaled AMPA as one of the most potent agonists for depolarizing pyramidal neurons in medium containing 2.4 mM Mg^{-2} ions in which kainate/AMPA receptors are active but NMDA receptors are inhibited (IC_{50} = 1.1 μM). It was 1 order of magnitude less potent for depolarizing pyramidal neurons under conditions in which kainate/AMPA receptors were inhibited by 10 μM CNQX but NMDA receptors were active in 0.1 mM Mg^{-2}-containing medium (IC_{50} = 10 μM). Compound **18** did not induce sensitization of CA1 pyramidal cells to depolarization by phosphonate analogues of glutamate (the QUIS-effect).

Introduction

The availability of bioisosteric replacements[1] for the carboxylic acid group has been critical to the development of novel medicinal agents, especially in the area of neurochemistry. Substitution of a carboxylate group in γ-aminobutyric acid (GABA) and L-glutamate and their analogues by chemical moieties such as tetrazole, isoxazole, 3,5-dioxo-1,2,4-oxadiazolidine, phosphonate, and sulfonate among others have led to the development of several derivatives exhibiting potent biological properties with high affinity for various receptors.[2,3] For example, compounds such as muscimol (**1**), kojic amine (**2**), and 3-aminopropanesulfonic acid (3APS) (**3**) and other analogues containing carboxylate bioisosteres have been useful in increasing our understanding of the receptors involved in GABA physiology. Similarly,



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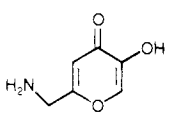

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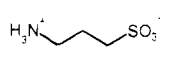

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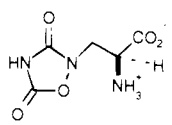

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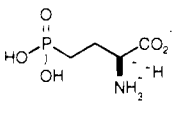

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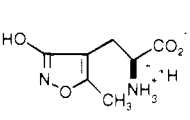

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L-quisqualic acid (**4**), L-AP4 (**5**), and L-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (**6**) have been useful in the elucidation of excitatory amino acid biochemistry with these compounds acting as prototypic ligands for these receptors.[4-6] With recent reports on the overexpression and characterization of various recombinant GABA and excitatory amino acid receptors, intense interest is currently focused on the development of novel GABA and excitatory amino acid receptor ligands both for fundamental studies as well as for medicinal purposes.[7,8] Excitatory amino acid analogues have been suggested to be potential therapeutics for the treatment of various neurodegenerative disorders[9] and for the treatment of traumatic brain injury.[10] In addition, these analogues may have therapeutic utility as anticonvulsants[11] and neuroprotectants, and several clinical trials are currently underway to evaluate these possibilities.[12]

Despite the high level of investigation into carboxylic acid bioisosteres, derivatives of 3,4-dihydroxy-3-cyclobutene-1,2-dione, surprisingly, have not been studied to as great an extent as other carboxyl surrogates. It has been incorporated into an analogue of the antibiotic lactivicin,[13] which resulted in an analogue exhibiting moderate antibacterial activity. In addition, the moiety has been incorporated into phosphonoformic acid[14] and into an analogue of the angiotensin-II antagonist losartan;[15] in both cases the biological activities of the analogues were maintained. The functionality has also found use as an "enolate metaphor".[16] Very recently, Kinney and co-workers have reported the use of 3,4-diamino-3-cyclobutene-1,2-dione as a replacement for the entire α-amino carboxylic acid functionality in various NMDA antagonists[17] and in the synthesis[18] of C-linked squarate analogues of glycine, β-alanine, and γ-aminobutyric acid. These findings have prompted us to report our own results on the substitution of the carboxylate group (**A**) with 3-amino-4-hydroxy-3-cyclobutene-1,2-dione (**B**), which has led to the production

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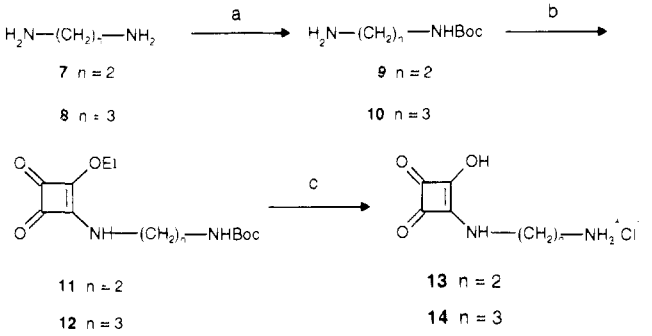
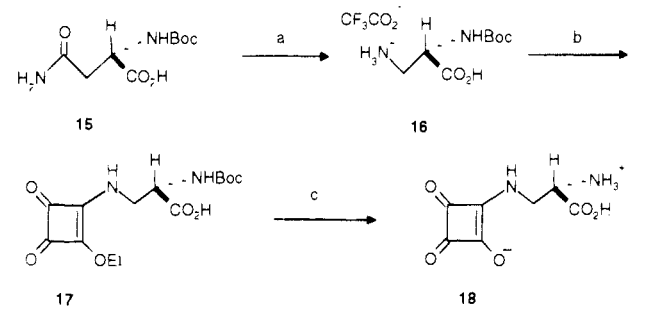
Scheme 1[a]



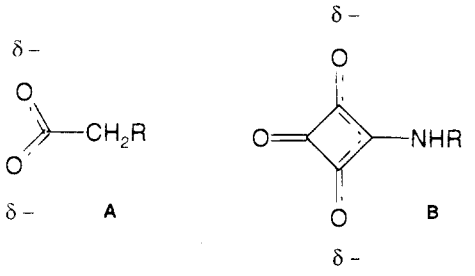
[a] Reaction conditions: (a) MeOH, di-*tert*-butyl dicarbonate, 1.0 equiv, 0 °C; (b) THF, diethyl squarate 1.0 equiv, 23 °C; (c) HCl-saturated aqueous THF, 23 °C.

Scheme 2[a]



[a] Reaction conditions: (a) $PhI(OCOCF_3)_2$, ref 20; (b) EtOH, diethyl squarate 1 equiv, Et_3N, 23 °C; (c) HCl-saturated aqueous dioxane, 23 °C, 16 h.

of an N-linked squarate analogue of L-glutamic acid exhibiting high affinity to kainic acid and AMPA receptors.



Chemistry

Initial attempts to evaluate the usefulness of squaric acid derivatives as carboxylic acid replacements commenced with the synthesis of GABA analogues **13** and **14**. Diamines **7** and **8** were reacted with 1 equiv of di-*tert*-butyl dicarbonate in methanol to afford *N*-(*tert*-butyloxycarbonyl)-1,2-diaminoethane (**9**) and *N*-(*tert*-butyloxycarbonyl)-1,3-diaminopropane (**10**) in approximately 30% yields. Reaction of the monoprotected amines with diethyl squarate[19] in tetrahydrofuran for 5 h resulted in the isolation of the fully protected amines **11** and **12** in greater than 75% yield (Scheme 1). Deprotection in 10% aqueous tetrahydrofuran saturated with HCl at room temperature for 24 h yielded the desired analogues **13** and **14** in 87% and 82% yields, respectively. As shown in Scheme 2, the synthetic approach to **18** involved the Hoffmann rearrangement of *N*-(*tert*-butyloxycarbonyl)-L-asparagine (**15**) catalyzed by bis(trifluoroacetoxy)iodobenzene to produce N^{α}-(*tert*-butyloxycarbonyl)-2,3-diamino-L-propionic acid trifluoroacetate (**16**).[20] Reaction with diethyl squarate in anhydrous ethanol in the presence of triethylamine for 48 h resulted in the production of the protected amino acid analogue **17**. Acidic deprotection (HCl saturated 10% aqueous dioxane) for 16 h yielded desired glutamate analogue **18** in 88% yield.

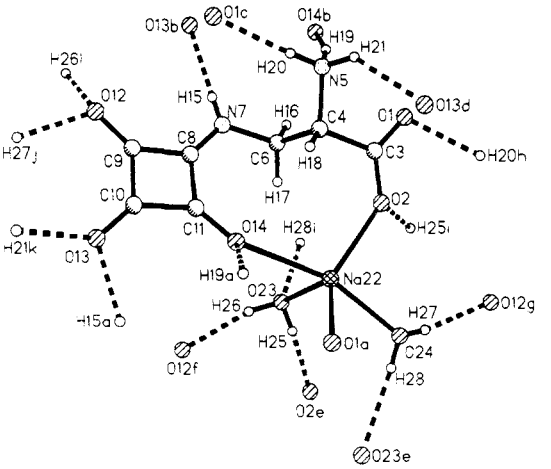


Figure 1. Molecular structure of compound **18** including all H-bond contacts.

X-ray Crystallography and Molecular Modeling

Single crystal X-ray analysis of the monosodium salt of **18** was performed and revealed C3–C4–C6–N7, C4–C6–N7–C8, and C6–N7–C8–C11 dihedral angles of 180°, −80.5°, and 3.6° respectively, and O14–N5, O14–O1, and O14–O2 intramolecular distances of 4.62, 5.47, and 3.90 Å, respectively (Figure 1). The structure corrresponds to one of several low energy conformers (within 5 kcal/mol of the global minimum) obtained by a systematic conformational energy search (GridSearch) of the molecule by variation of the C3–C4–C6–N7 and C4–C6–N7–C8 dihedral angles (10° increments) followed by energy minimization (Tripos Maximin2 force field in SYBYL[21]). The results were qualitatively similar for uncharged **18** and for **18** having formal charges in a high dielectric constant medium.

Biological Results and Discussion

Preliminary broad screening of the analogues as displacers in several receptor binding assays was performed to determine their affinity profiles.[22] At 10 μM the diaminopropane analogue **14** exhibited poor affinity for various binding sites including those for $GABA_A$, $GABA_B$, kainic acid, NMDA, and AMPA. Although the diaminoethane analogue **13** also showed little affinity to $GABA_A$ and $GABA_B$ binding sites, it did exhibit a greater than 40% inhibition of [^{3}H]AMPA (20 nM) binding at 10 μM using rat forebrain membranes.[23] A conformational comparison of **13** and **14** with 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol (THIP, **19**), a potent agonist of $GABA_A$ receptors,[7] indicated that both **13** and **14** have several minimum energy conformations (within 5 kcal/mol of the global minimum[21]) with distances between the OH and NH_2 groups similar to that found in THIP (5.18 Å[24]). The lack of binding affinity of **13** and **14** may therefore indicate the inability to effectively populate these conformations and/or that structural features other than the OH–NH_2 distance exert important roles in binding affinity. In contrast to the GABA analogues, the corresponding glutamate

Table 1. Potency of Squarate Compounds for Inhibition of Stimulus-Evoked Kainate/AMPA Receptor Responses in CA1 Pyramidal Cells of the Rat Hippocampus

compound	before QUIS exposure IC_{50} (mM) ± SD (n_H)[a]	after QUIS exposure IC_{50} (mM) ± SD (n_H)[a]	after reversal with αAA IC_{50} (mM) ± SD (n_H)[a]
18	0.0011 ± 0.0006 (3.4)	0.00055 ± 0.000 17 (4.4)	0.0010 ± 0.0003 (3.1)
13	>10	>10	ND
14	>10	>10	ND

[a] The IC_{50} value for inhibition of the stimulus-evoked Schaffer collateral–CA1 pyramidal cell extracellular synaptic field potential was measured. The slice was exposed to 16 μM L-quisqualic acid for 5 min. After washout of the quisqualic acid, the IC_{50} value measurement was repeated. The slice was exposed to 2 mM L-α-aminoadipic acid (αAA) for 5 min, and after washout, the IC_{50} value measurement was again repeated. (SD is the sample standard deviation (n = 4–5); n_H is the Hill coefficient, estimated from the maximum slope of the log concentration - response curve).

Table 2. Potency of Squarate Compounds for Inhibition of Stimulus-Evoked Lateral and Medial Perforant Path–Dentate Granule Cell Responses and Inhibiton of Stimulus-Evoked CA1 Pyramidal Cell NMDA Receptor Responses of the Rat Hippocampus

compound	lateral perforant path IC_{50} (mM) ± SD (n_H)[a]	medial perforant path IC_{50} (mM) ± SD (n_H)[a]	NMDA of CA1 IC_{50} (mM) ± SD (n_H)[a]
18	0.0034 ± 0.0003 (2.7)	0.0059 ± 0.0016 (2.2)	0.010 ± 0.003 (3.8)
13	>10	ND	3.5 ± 1.2
14	>10	ND	5.3 ± 2.2

[a] SD is the sample standard deviation (n = 4–5); n_H is the Hill coefficient, estimated from the maximum slope of the log concentration–response curve). ND = not determined.

analogue **18** exhibited quite potent binding site affinities. Preliminary screening of glutamate analogue **18** in a range of binding assays indicated high affinity for kainic acid and AMPA binding sites as exemplified by a greater than 50% inhibition of specific binding of [^{3}H]kainic acid (20 nM) and [^{3}H]AMPA (20 nM) to rat forebrain membranes at 10 μM. Preliminary analysis of analogue **18** indicated IC_{50} values of 190 and 72 nM for [^{3}H]AMPA (20 nM) and [^{3}H]kainic acid[25] (20 nM) binding, respectively.

To provide pharmacological information, compounds **13**, **14**, and **18** were evaluated for pharmacological actions on stimulus-evoked extracellular synaptic field potentials in rat hippocampal slices. Inhibition of stimulus-evoked extracellular synaptic field potentials by bath application of the squarate analogues were examined for the following systems: (1) Schaffer collateral–CA1 pyramidal cell synapses, (2) NMDA receptor-evoked responses of CA1 pyramidal cells evoked by Schaffer collateral axon stimulation, and (3) medial and lateral perforant path–dentate granule cell synapses. Compounds **13** and **14** showed little activity at millimolar concentrations in this assay (Tables 1 and 2). In contrast, **18** rivaled AMPA as one of the most potent agonists for depolarizing pyramidal neurons in medium containing 2.4 mM Mg^{2+} ions in which kainate/AMPA receptors are active but NMDA receptors are inhibited (IC_{50} = 1.1 μM for **18** (Table 1); IC_{50} values for prototype agonists: L-α-kainic acid, 1.7 μM; L-quisqualic acid, 24 μM; AMPA, 1.1 μM). The concentration–response curves for inhibition by **18** showed Hill coefficients ≈ 2–4, and inhibition was accompanied by appearance of population spikes, extracellular criteria that **18** acts as an agonist to depolarize the CA1 pyramidal neurons, not as an antagonist for their kainate/AMPA receptors.[26] It should be noted that the receptors activated by **18** may be located anywhere on the surface of the CA1 pyramidal neurons, not necessarily at Schaffer collateral synapses, to show this pattern of inhibition.[26] Likewise, the 10-fold less potent activation of NMDA responses by **18**, evoked under conditions where kainate/AMPA receptors were inhibited by CNQX but in 0.1 mM Mg^{2+}-containing medium where NMDA receptors are active, may involve the postsynaptic NMDA receptors of the Schaffer collateral–CA1 pyramidal synapses and/or NMDA receptors located elsewhere on the pyramidal neurons.

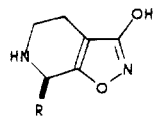
Quisqualate sensitization of neurons (QUIS-effect) is a dramatic change of neuronal sensitivity induced by an active transport-mediated uptake of L-quisqualic acid.[27,28] At least two sites which interact with excitatory amino acids are thought to be involved in this phenomenon: the cellular uptake site and one or more receptors that mediate depolarization of the neurons. The latter site or sites is activated by two known classes of EAA analogues, the excitatory amino acid phosphonate analogues AP4, AP5, and AP6[29] and certain structural analogues of L-quisqualic acid including L-quisqualic acid itself.[30] Compounds **13**, **14**, and **18** did not induce sensitization of CA1 pyramidal neurons, and they did not inhibit the sensitization induced by exposure to L-quisqualic acid (data not shown). However, **18** is among the small number of known compounds which show higher potency for depolarization of CA1 pyramidal neurons after the slice is exposed to L-quisqualic acid.[30] Although sensitivity to **18** was only enhanced 2-fold, it was reversed by exposure of the slice to L-α-aminoadipic acid, a hallmark of the QUIS-effect (Table 1).

Since the related compounds 3,4-dihydroxy-3-cyclobutene-1,2-dione (pK_1 = 1.2 and pK_2 = 3.5) and 3-hydroxy-4-methyl-3-cyclobutene-1,2-dione (pK = 0.69) are acidic,[31,32] compound **18** is expected to be fully ionized at physiological pH. On the basis of the predicted resonance structures for substituted squaric acid analogues[33] and on the presence of only three ^{13}C signals for the squaramide moiety in **18**, it is likely that the negative charge on the squaramide moiety is partially delocalized onto oxygens O14 and O12.

It has been shown[34] that the free energy of activation for the rotation about the C–N bond in various squaric acid amide esters (approximately 75 kJ/mol) is comparable to that found for vinylogous amide esters, and therefore, the N-linked squaric acid moiety more closely resembles the carbamate than the carboxylate moiety. The close to 0° dihedral angle of C6–N7–C8–C11 in the X-ray structure of **18** as well as the shortened N7–C8 bond length (1.32 Å) are consistent with these

observations. A qualitatively similar preference for binding to AMPA/kainic acid over NMDA receptors has been reported for two other compounds containing N-linked bioisosteres of the carboxyl functionality, β-(*N*-oxalylamino)-L-alanine (BOAA)[35] and L-quisqualic acid.[6] In contrast, AMPA, which contains the 3-hydroxyisoxazole bioisostere, is selective for AMPA receptors.[6] However, the molecular context in which these bioisosteres are placed is a major factor in determining their receptor affinities.[6]

It has been suggested[36] that the receptor-bound conformation of AMPA may not, in fact, be satisfactorily represented by its reported X-ray crystal structure but rather by a structure more related to the active but conformationally-restricted agonist, (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA, **20**). Although the X-ray structure determined for **18** does not conform to one similar to **20**, a systematic conformational search of **18** produced several low energy conformations (less than 5 kcal/mol in energy from the global minimum) that indeed satisfy the required distances between the OH and amino (5.32 Å) and the OH and carboxyl oxygen functionalities (6.49, 6.19 Å) found in the X-ray structure of **20**.



19 R = H

20 R = CO_2H

Conclusions

The facile introduction of the 3-amino-4-hydroxy-3-cyclobutene-1,2-dione group (**B**) into various molecules and the potency produced by its introduction as exemplified by novel glutamate analogue **18** encourages further exploitation of this moiety in medicinal chemistry and especially in the design of novel neurochemical agents. The importance of other structural features of these analogues to receptor selectivity and potency is being actively investigated.

Experimental Section

Chemistry. Melting points were determined in sealed capillary tubes with a Mel-Temp melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and are within ±0.4% of the theoretical values. NMR (^1H, ^{13}C) spectra were recorded at ambient temperature on a Bruker AM250 spectrometer, and chemical shifts are reported as δ values (ppm) relative to TMS. Mass spectra were recorded at the McMaster Regional Centre For Mass Spectrometry at the Department of Chemistry, McMaster University, Hamilton, Ontario, Canada. Electrospray mass spectra were performed on a Sciex API3 at Sciex Inc., Thornhill, Ontario. Tetrahydrofuran was distilled from Na/benzophenone. All other solvents used were reagent grade and were used without further purification. Optical rotations were measured with a JASCO DIP-360 digital polarimeter.

***N*-(*tert*-Butyloxycarbonyl)diaminoalkanes 9 and 10.** These compounds were made by modifications of the procedures reported by Essien et al.[37] and Huang et al.[38] To a solution of diaminoalkane **7** or **8** (10 mmol) in MeOH (150 mL) at 0 °C was added slowly a solution of di-*tert*-butyl dicarbonate (10 mmol) in MeOH (50 mL). The mixture was stirred at 0 °C for 1 h. The solvent was evaporated under reduced pressure. The desired product was then isolated as a colorless oil by silica gel column chromatography (MeOH:EtOAc:CH_2Cl_2 = 1:2:2). Yields of approximately 35% were routinely obtained. *N*-*tert*-Butyloxycarbonyl-1,2-diaminoethane (**9**): ^1H NMR (250 MHz, $CDCl_3$ + D_2O exchange) δ 1.44 (s, 9H), 2.78 (t, J = 6 Hz, 2H), 3.16 (t, J = 6 Hz, 2H); ^{13}C NMR (50.3 MHz, $CDCl_3$) δ 27.9, 41.1, 42.6, 78.5, 156.0. *N*-*tert*-Butyloxycarbonyl-1,3-diaminopropane (**10**): ^1H NMR (250 MHz, D_2O) δ 1.27 (s, 9H), 1.57 (quintet, J = 7 Hz, 2H), 2.68 (t, J = 7 Hz, 2H), 2.98 (t, J = 7 Hz, 2H); ^{13}C NMR (62.9 MHz, D_2O) δ 30.3, 31.9, 40.1 (2C), 83.6, 160.3.

***N*-(2-Hydroxy-3,4-dioxo-1-cyclobutenyl)-1,2-ethanediamine Hydrochloride (13).** To a solution of diethyl squarate[39] (1.07 g, 6.29 mmol) in THF (35 mL) was added slowly a solution of **9** (1.01 g, 6.30 mmol) in THF (15 mL). The mixture was stirred at room temperature for 5 h. The solvent was then removed under reduced pressure. The residue was recrystallized from ethyl acetate/hexanes to give 1.35 g (75%) of the desired product, *N*-(2-ethoxy-3,4-dioxo-1-cyclobutenyl)-*N*′-*tert*-butyloxycarbonyl-1,2-diaminoethane (**11**): mp 114.5–115.5 °C; ^1H NMR (250 MHz, acetone-d_6) δ 1.38 (bs, 12 H), 3.31 (m, 2 H), 3.53 (bs, 1 H), 3.69 (bs, 1 H), 4.69 (m, 2 H), 6.20 (bs, 1 H), 7.60 (bs, 1 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 15.8, 28.5, 41.0, 45.3, 70.0, 80.0, 156.5, 173.0, 177.6, 183.0, 189.5. The product was used directly in the next step of the reaction scheme. Compound **11** (250 mg, 0.88 mmol) was added to a solution (10 mL) of 10% aqueous THF saturated with HCl. The mixture was stirred at room temperature for 24 h. The white precipitate formed was collected, washed with ether, and dried under vacuum to give 142 mg (87%) of the desired product **13** as a crystalline solid: mp 230–232 °C dec. ^1H NMR (250 MHz, D_2O) δ 2.50 (t, J = 6 Hz, 2 H), 3.30 (t, J = 6 Hz, 2 H); ^{13}C NMR (62.9 MHz, DMSO) δ 39.3, 41.0, 174.1, 184.4(b), 185.0; MS (EI) m/z 156 (M^+), 138, 110, 82, 54. Anal. ($C_6H_9ClN_2O_3$) C, H, N.

***N*-(2-Hydroxy-3,4-dioxo-1-cyclobutenyl)-1,3-propanediamine Hydrochloride (14).** To a solution of diethyl squarate (820 mg, 4.80 mmol) in THF (35 mL) was added compound **10** (816 mg, 4.70 mmol) in THF (15 mL). The mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure. The residue was then purified by silica gel column chromatography (EtOAc:hexanes = 2:1) to give 1.07 g (78%) of the desired product, *N*-(2-ethoxy-3,4-dioxo-1-cyclobutenyl)-*N*′-*tert*-butyloxycarbonyl-1,3-diaminopropane (**12**), as a white solid: mp 68–70 °C; ^1H NMR (250 MHz, $CDCl_3$) δ 1.43 (bs, 12 H), 1.73–1.90 (m, 2 H), 3.10–3.30 (m, 2 H), 3.43–3.60 (m, 1 H), 3.63–3.80 (m, 1 H), 4.68–4.83 (m, 2 H), 5.26 (bs, 1 H), 7.72 (bs, 1 H); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 15.5, 28.5, 31.0, 37.0, 41.5, 69.6, 79.4, 156.5, 172.6, 176.9, 182.7, 189.2. The product was used directly in the next step of the reaction. Compound **12** (250 mg, 0.85 mmol) was added to a solution (10 mL) of 10% aqueous THF saturated with HCl. The mixture was stirred at room temperature for 24 h. The white precipitate formed was collected, washed with ether, and dried under vacuum to give 137 mg (82%) of the desired product as a crystalline solid: mp 219–220 °C dec; ^1H NMR (250 MHz, D_2O) δ 1.45 (quintet, J = 7 Hz, 2 H), 2.36 (t, J = 7 Hz, 2 H), 3.31 (t, J = 7 Hz, 2H); ^{13}C NMR (62.9 MHz, D_2O) δ 28.2, 36.9, 41.3, 176.6, 184.7, 187.3; MS (EI) m/z 170 (M^+), 152 (100), 124, 96, 68. Anal. ($C_7H_{11}ClN_2O_3$) C, H, N.

***N*$^{\beta}$-(2-Hydroxy-3,4-dioxo-1-cyclobutenyl)-2,3-diamino-L-propionic Acid (18).** To a solution of the trifluoracetate salt of *N*$^{\alpha}$-*tert*-butyloxycarbonyl-2,3-diamino-L-propionic acid[40] (1.21 g, 5 mmol) prepared from *N*$^{\alpha}$-*tert*-butyloxycarbonyl-asparagine by Hoffmann rearrangement using bis(trifluoroacetoxy)iodobenzene in anhydrous EtOH (35 mL) was added Et_3N (0.75 mL; 5.4 mmol) followed by diethyl squarate (0.85 g, 5 mmol) in EtOH (15 mL). The mixture was stirred at room temperature for 2 days. Solvent was then removed under reduced pressure. The residue was redissolved in 1 N NaOH (10 mL) and washed twice with CH_2Cl_2 (10 mL). The aqueous layer was neutralized with 1 N HCl (10 mL) and was extracted with CH_2Cl_2. After evaporation under reduced pressure, the initial adduct **17** was redissolved in HCl saturated aqueous dioxane (20 mL; 10% H_2O). The mixture was stirred at room temperature for 16 h. The precipitate formed was collected, washed with ether, and dried under reduced pressure to give 1.04 g (88%) of the desired product as a crystalline solid: mp

286–287 °C dec; $[\alpha]^{23}_D$ −50.2° (c = 1.357g/dL, H_2O); ^{1}H NMR (250 MHz, D_2O/NaOD) δ 3.93 (AB of ABC, J = 6.7, 7.3, 17.7 Hz, 2H), 3.88 (C of ABC, J = 6.7, 7.3 Hz, 1H); ^{13}C NMR (62.9 MHz, D_2O/NaOD) δ 46.7, 57.8, 174.4, 184.3, 191.3, 198.8; electrospray mass spectrum (of disodium salt prepared by reaction with sodium carbonate) 245 (M + 1, 100), 223, 201, 155. Anal. ($C_7H_8N_2O_5 \cdot 0.25H_2O$) C, H, N.

Single-Crystal X-ray Analysis of 18 (Monosodium Salt). Colorless polyhedral-shaped crystals were grown from water. The compound crystallized in the orthorhombic space group $P2_12_12_1$ with unit the following cell dimensions: a = 6.501(1) Å; b = 10.839(2) Å; c = 14.506(3) Å; V = 1022.2(4) Å^3; Z = 4; ρ(calcd) = 1.678 g/mL; F(000) = 536; T = 180 K; λ(Mo Kα) = 0.710 73 Å; crystal size (mm) 0.36{101} × 0.24{011}; $C_7H_{11}N_2NaO_7$; M = 258.2.

A Nicolet LT2 equipped R3m/V diffractometer was used for crystallographic data collection. Two standard reflections used to monitor crystallographic data collection did not show significant deviations within the data collection period. Unit cell constants were determined by a least-squares fit of 25 reflections having $22° < 2\theta < 32°$. Data were collected by the ω scan method ($4.0° \leq 2\theta \leq 60°$): the index ranges were $0 \leq h \leq 9$, $-15 \leq k \leq 15$, and $0 \leq l \leq 20$. A total of 3321 reflections were collected and corrected for Lorentz, polarization, face-indexed analytical absorption, and extinction effects. Of these 1734 were unique (R_{merg} 2.49%), and 1545 reflections with $F \geq 6.0\sigma(F)$ were considered observed. The structure was solved by direct methods and refined by full-matrix least-squares methods[39] using Siemens SHELXTL PLUS (VMS) software. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were located by difference Fourier synthesis and included in the refinement with isotropic thermal parameters. In the final cycles, a weighting scheme based on counting statistics was employed ($\omega^{-1} = \sigma^2(F)$). The final residuals were R = 2.65% ($R = \sum||F_o| - |F_c||/\sum|F_o|$) and wR = 2.66% ($wR = [\sum w(|F_o| - |F_c|)^2/\sum w F_o^2]^{1/2}$) with the GoF = 1.90 ($GoF = [\sum w(|F_o| - |F_c|)^2/(N_{obs} - N_{var})]^{1/2}$). The enantiomorph was not determined but fixed by prior knowledge.

Biological Methods. Tissue Preparation. Transverse hippocampal slices were obtained from 30–50 day old male Sprague–Dawley rats. Rats were anesthetized with urethane (1.5 g/kg i.p.) 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).[40] 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 to 95% O_2/5% CO_2 and incubated for 15 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, 4–30 V, 0.1 Hz) was delivered to the Schaffer collateral axons in stratum radiatum of region CA1 or to the perforant path of the dentate gyrus via a twisted pair of Teflon-coated stainless steel wires (0.003 in.). A glass recording microelectrode (2–14 MΩ impedance filled with 2 M NaCl) was placed in the stratum radiatum of CA1 or in the synaptic field of the dentate granule cells innervated by the lateral or medial perforant path. To measure evoked NMDA receptor responses in CA1, the concentration of magnesium ions in the incubation medium was reduced to 0.1 mM to disinhibit NMDA receptor responses, and 10 μM CNQX was included in the medium to inhibit kainate/AMPA receptor responses.[41,42] The evoked extracellular synaptic field potentials were observed using a storage oscilloscope, and their peak amplitudes were electronically sampled and recorded with a strip 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.[43]

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 than 70% or the bath concentration of the drug exceeded 8 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 at least four experiments.

QUIS-Effect Data. Compounds **13**, **14**, and **18** were examined for their ability to induce sensitization of CA1 pyramidal neurons to depolarization by L-2-amino-6-phosphonohexanoic acid (L-AP6) (QUIS-effect).[27–29] They were also tested for capacity to "preblock" slices, by prior exposure of slices to the test compound, to subsequent sensitization by L-quisqualic acid. Finally, they were tested for increase of their potency as agonists for depolarizing CA1 pyramidal cells after the slices were exposed to quisqualic acid. For compound **18**, to which the neurons were sensitized by exposure of the slice to L-quisqualic acid, reversal of sensitatization by L-α-aminoadipic acid was also investigated.

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Supporting Information Available: Receptor assay screens and X-ray data (23 pages). Ordering information is given on any current masthead page.

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