Heterocyclic Excitatory Amino Acids. Synthesis and Biological Activity of Novel Analogues of AMPA

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The novel acidic amino acids **6a–c**, **7**, and **8** have been synthesized via 1,3-dipolar cycloadditions, using nitrile oxides and alkynes. The prepared compounds are heterocyclic analogues of glutamic acid with differing chain lengths. One of these compounds, (*RS*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA, **8**), was shown in [^{3}H]AMPA binding studies to be more active than AMPA itself (IC_{50} = 20 nM compared to IC_{50} = 79 nM for AMPA). No affinity for NMDA receptors (NMDA-sensitive [^{3}H]glutamic acid binding) was found, and only weak affinity in [^{3}H]kainic acid binding (IC_{50} = 6.3 μM) was detected. The excitatory activity in rat cortical wedge also showed that ACPA was more potent than AMPA (EC_{50} = 1.0 μM compared to EC_{50} = 3.5 μM for AMPA). The depolarizing effect of ACPA could be fully antagonized by the selective non-NMDA antagonist 6-cyano-7-nitroquinoxazoline-2,3-dione (CNQX), but was unaffected by the selective NMDA antagonist D-2-amino-5-phosphonovaleric acid (AP5).

Receptors for excitatory amino acids are at present subdivided into five subtypes,[1-4] four of which are coupled to the opening of an ion channel: (1) NMDA receptors at which *N*-methyl-D-aspartic acid (NMDA) acts as a selective agonist, (2) AMPA receptors selectively activated by (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), (3) kainic acid receptors activated by kainic acid, and (4) AP4 receptors at which the electrophysiological responses are antagonized by L-2-amino-4-phosphonobutyric acid (AP4). The fifth glutamate receptor subtype, the metabotropic receptor,[4-6] is associated with hydrolysis of phosphoinositides and activated by agonists such as glutamic acid (GLU), quisqualic acid, and *trans*-1-amino-3-carboxycyclopentanecarboxylic acid.

The NMDA receptors (i.e. the NMDA receptor complex) have been investigated extensively.[1,3,7] The availability of a number of both competitive and noncompetitive antagonists has made it possible to obtain detailed information about the physiological and pharmacological relevance of this receptor type. Nevertheless, it remains important to design and synthesize new ligands (agonists and antagonists) for the NMDA receptors in order to address the therapeutic potential associated with manipulations of this receptor. As for the non-NMDA receptors, the lack of selective and potent ligands has limited our understanding of the structure and function of these receptors. A number of 3-hydroxyisoxazole amino acids have been used in structure–activity studies (e.g. refs 8–10). These bioisosteres of GLU (**1**) have been designed using ibotenic acid (IBO, **2**) (Figure 1) as a lead structure. (*RS*)-2-Amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA, **3**) has been shown to be a potent and selective NMDA agonist in cortical tissue,[10] whereas AMPA (**5**), due to its potency and selectivity, is the agonist of choice at the AMPA receptors.[1,4,11] Compared to GLU these 3-hydroxyisoxazoles have afforded compounds which act selectively at different receptor subtypes, but there is no simple relationship determining receptor selectivity in relation to, for example, carbon backbone length in the molecules. Thus IBO and AMAA, with five and four carbon atom backbones, respectively, both interact with the NMDA receptors, whereas AMPA and (*RS*)-2-amino-3-(3-hydroxy-4-methyl-5-isoxazolyl)propionic acid[8] (4-methylhomoibotenic acid, 4-Me-HIBO, **4**) (Figure 1), with five and six carbon atom backbones, respectively, activate AMPA receptors.

In order to extend these structure–activity studies, a number of analogues have been synthesized (**6a–c**, **7**, and **8**) (Figure 1), in which the 3-hydroxy group at the isoxazole ring has been substituted for a carboxylic acid side chain of different chain length. The activities of the prepared compounds have been investigated in receptor binding assays as well as electrophysiologically using a rat cortical slice preparation.

Chemistry

The acidic amino acids synthesized (**6a–c**, **7**, and **8**) have all been prepared through 1,3-dipolar cycloadditions, using a nitrile oxide reacting with an alkyne, substituted with an acetamidomalonate ester moiety. Reaction of compound **9**,[12] prepared from propargyl chloride through a

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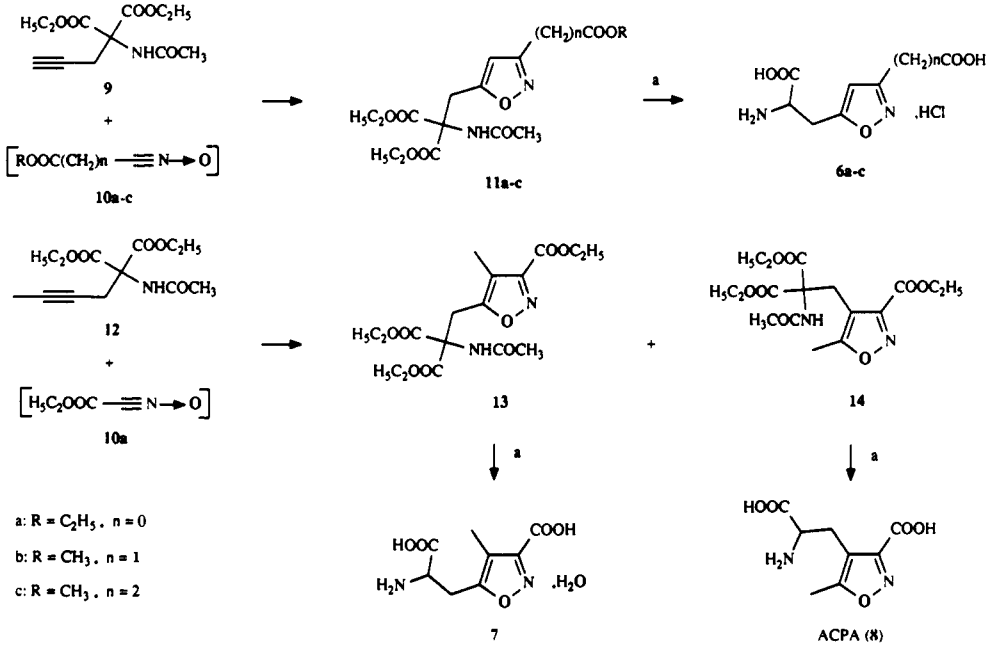
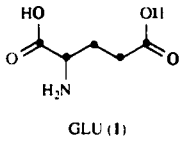
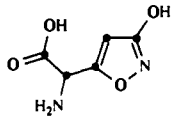
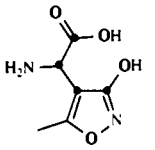
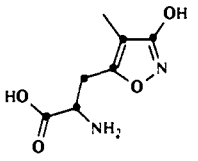
Scheme I

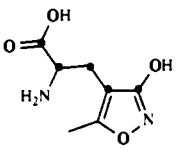


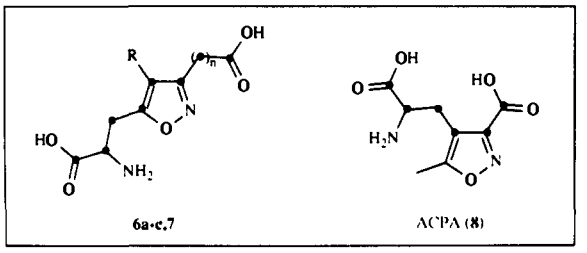

GLU (1)


IBO (2)


AMAA (3)

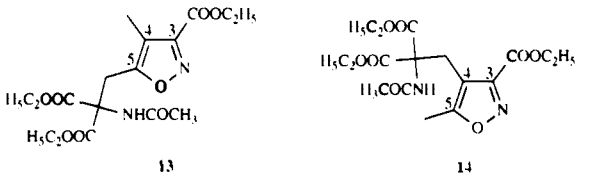

4-Me-HIBO (4)


AMPA (5)



6: R = H, a: n = 0 b: n = 1 c: n = 2 7: R = CH3, n = 0

Figure 1. Glutamic acid (GLU, 1) and heterocyclic analogues.



C3	δ 154.8 q ^3J = 3.5 Hz	δ 154.8 t ^3J = 4.4 Hz
C4	δ 114.7 qt ^2J = 6.8 Hz, ^3J = 2.6 Hz	δ 109.4 tq ^2J = 9.3 Hz, ^3J = 3.1 Hz
C5	δ 165.5 tq ^2J = 10.6 Hz, ^3J = 5.3 Hz	δ 170.5 qt ^2J = 6.9 Hz, ^3J = 5.7 Hz

Figure 2. ^{13}C NMR data for compound **13** and **14**: chemical shift values (δ) in ppm, multiplicity of ^{13}C signals, and ^{13}C-^1H coupling constants.

Sorensen synthesis, with nitrile oxides **10a–c** gave intermediates **11a–c** (Scheme I). These intermediates were obtained in 21–55% yields. Nitrile oxides **10a–c** were generated with phenyl isocyanate and triethylamine (TEA) from ethyl nitroacetate, methyl 3-nitropropionate, or methyl 4-nitrobutyrate, respectively. Compound **11a** was also prepared using ethyl chloro(hydroxyimino)acetate for generation of nitrile oxide **10a**,[13] but a lower yield was obtained. In each cycloaddition reaction only one isomer was formed, i.e. **11a–c**, and the predicted structure with the acetamidomalonate moiety in the 5-position of the isoxazole was established by ^{13}C NMR. C4 in the isoxazole ring (δ ca. 105 ppm) was shown by DEPT NMR to be tertiary, whereas C3 and C5 (δ ca. 160 and 170 ppm, respectively) were quaternary.

Compound **12**[14] was prepared from 3-butyn-1-yl chloride (obtained from 3-butyn-1-ol) and the sodium salt of diethyl acetamidomalonate. This substrate (**12**) proved to be very resistant toward cycloaddition reactions with nitrile oxide **10a**. When **10a** was prepared from ethyl chloro(hydroxyimino)acetate, using different reaction conditions, no products could be detected, except for the cyclic dimer of ethyl chloro(hydroxyimino)acetate. Generation of nitrile oxide **10a** from ethyl nitroacetate (using phenyl isocyanate and TEA) and reaction with **12** by reflux for 3 h in toluene gave products **13** and **14** in very low yields (ca. 2% of each). Compounds **13** and **14** were isolated from a very compli-

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Table I. Receptor Binding and in Vitro Electrophysiological Data for Glutamic Acid (GLU), Kainic Acid (KAIN), AMPA, and ACPA[a]

	IC_{50}, μM			EC_{50}, μM, electrophys
	[^{3}H]AMPA	[^{3}H]KAIN	[^{3}H]GLU[b]	
GLU	0.50 ± 0.25	0.40 ± 0.1	0.20 ± 0.12	nt
KAIN	4.0 ± 0.9	0.016 ± 0.004	>100	nt
AMPA	0.079 ± 0.012	50.1 ± 15.8	>100	3.5 ± 0.2
ACPA	0.020 ± 0.012	6.3 ± 1.6	>100	1.0 ± 0.1

[a] Mean ± SEM; n = 4. nt = not tested. [b] NMDA sensitive.

cated reaction mixture, in which **12** was the major component and difficult to separate from the products. The structure of the two isomers **13** and **14** was established by ^{13}C NMR. The long-range C–H coupling constants were determined as seen in Figure 2. Reaction of alkyne **12** with nitrile oxides generated from methyl 3-nitropropionate and methyl 4-nitrobutyrate, respectively, was also investigated, but gave very complicated reaction mixtures (TLC) as seen for the synthesis of **13** and **14**. Starting material **12** was the major component and the yield for the supposed cyclization products was poor (very weak spots on TLC with lower R_f value compared to that of **12**). Therefore these reactions were not pursued on a large scale.

Intermediates **11a–c**, **13**, and **14** were all deprotected by reflux overnight in 1 N HCl. Compounds **6a–c** were isolated as hydrochlorides, whereas **7** and **8** were isolated as zwitterions.

Biological Activity

The receptor affinity of the products **6a–c**, **7**, and **8** (ACPA) were evaluated in three receptor binding assays: [^{3}H]AMPA,[15] [^{3}H]kainic acid,[16] and NMDA-sensitive [^{3}H]glutamic acid binding.[17] As shown in Table I, ACPA proved to be a very potent displacer of [^{3}H]AMPA binding, with affinity greater than that of AMPA (means ± SEM, n = 4). It has weak affinity for [^{3}H]kainic acid binding, and no significant affinity for NMDA-sensitive [^{3}H]glutamic acid binding (IC_{50} > 100 μM). Compounds **6a–c** and **7** showed no significant affinity in any of the three binding assays (IC_{50} > 100 μM).

Similar pharmacological profiles were found in the rat cortical slice model.[18] The excitatory activity of ACPA in this functional model indicated it to be an agonist with potency greater than that of AMPA (Table I). In contrast, only very weak excitatory activity for **6a–c** and **7** was detected (EC_{50} > 1 mM). The excitatory activity of ACPA (and AMPA) was fully antagonized by the antagonist 6-cyano-7-nitroquinoxazoline-2,3-dione (CNQX),[19] whereas no antagonism was observed with the selective NMDA receptor antagonist, D-2-amino-5-phosphonovaleric acid[20]

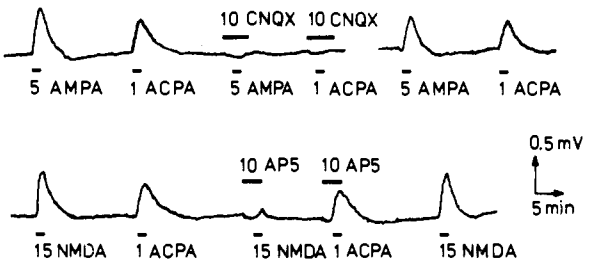

Figure 3. Recordings from cortical slice neurones depolarized by administration of AMPA, ACPA, and NMDA and antagonism of these by CNQX (top) or AP5 (bottom) and recovery. The numbers are concentrations (in μM).

(AP5) (Figure 3). Since no potency (EC_{50}) values could be determined for compounds **6a–c** and **7**, because of the low activity, it follows that unambiguous antagonist experiments of these weak excitatory activities could not be performed.

Compounds **6a–c** and **7** were also tested for possible antagonistic activities. No significant effects of 250 μM **6a–c** or **7** (at which none of the compounds showed any excitatory activity) were shown on the excitatory effects elicited by NMDA (15 μM), AMPA (5 μM), quisqualic acid (10 μM), or kainic acid (10 μM).

Discussion

Activity at excitatory amino acid receptors is closely related to the existence of an α-amino acid moiety and an ω-acidic moiety (most often a carboxylic acid for agonists or a phosphonic acid for NMDA antagonists) in the ligands. This requirement is indeed fulfilled for the putative transmitter GLU, which activates all excitatory amino acid receptors. Several structurally restricted analogues, in which the carbon backbone of GLU is incorporated, have shown selective agonistic action at different receptor subtypes or have been shown to be antagonists, e.g. the naturally occurring heterocycles kainic acid, quinolinic acid, and quisqualic acid, synthetic analogues such as piperidinedicarboxylic acids,[21,22] and cyclopropane,[23] -butane,[24] and -pentane[6,21] GLU analogues.

A number of isoxazole analogues of GLU, in which a 3-hydroxyisoxazole moiety acts as a bioisostere to the ω-carboxyl group, have previously shown potent excitatory activity at different receptor subtypes (e.g. refs 8–10). Ligands with a four or five carbon atom backbone seem to be preferred for NMDA agonists, e.g. AMAA and IBO

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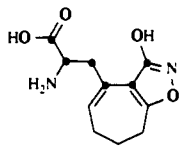
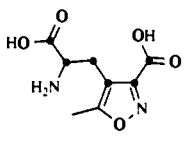
(Figure 1), whereas the most potent AMPA agonists are obtained with five or six carbon atom backbones, e.g. AMPA and 4-Me-HIBO (Figure 1).

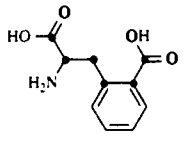
In this paper the 3-hydroxy group in these isoxazole analogues has been replaced by carboxylic acid side chains of different chain length. ACPA, a homologue of AMPA, has proven to be the most potent agonist identified so far at AMPA receptors in cortical tissue. This has been shown in [^{3}H]AMPA binding studies and by in vitro electrophysiological experiments (Table I). It is noteworthy that 4-Me-HIBO, another homologue of AMPA, in contrast to ACPA, is considerably less potent than AMPA. Another potent AMPA agonist with a C_6 backbone is (*RS*)-2-amino-3-(3-hydroxy-7,8-dihydro-6*H*-cyclohepta[1,2-*d*]-isoxazol-4-yl)propionic acid[25] (4-AHCP, **15**). Neither ACPA, 4-AHCP, nor 4-Me-HIBO have shown any antagonist activity in spite of having a chain length analogous to NMDA antagonists such as D-2-aminoadipic acid[26] and AP5.



4-AHCP (15)



ACPA (8)



16

A previously reported aromatic analogue of ACPA, (*RS*)-2-(2-carboxyphenyl)alanine (**16**), was found to be a very weak agonist at cat spinal neurones compared to AMPA and 4-AHCP.[25] Thus, the benzene ring affords almost complete loss of activity in spite of structural similarity among **16**, ACPA, and 4-AHCP. The substitution pattern on the isoxazoles is also a determining factor as an amino acid side chain in position 4 of the ring is preferred for AMPA agonists, AMPA, 4-AHCP, and ACPA being potent and highly selective agonists. The selectivity of ACPA as an AMPA agonist is likely due to the AMPA structure incorporated in the ACPA molecule, whereas the enhanced potency could possibly be ascribed to the greater acidity of the 3-carboxylic acid group compared to the 3-hydroxyisoxazole moiety in AMPA, 4-AHCP, and 4-Me-HIBO. It is striking that both substitution with a carboxylate group in the 3-position and the use of a 3-hydroxy group on the isoxazole ring afford compounds with high receptor selectivity and potency. This effect of the isoxazole ring compared to aliphatic analogues such as aspartic acid and GLU and the benzene analogue (**16**) is not understood. The other compounds prepared, **6a–c** and **7**, with chain lengths of C_7, C_8, or C_9, were found to have very weak agonistic activities. This indicates that there is an upper limit for chain length in order to retain potency.

Utilization of an isoxazole ring in the design of a number of GLU analogues has facilitated ligands with high potency and receptor selectivity. The differences in activities, compared to the activities of structurally nonrestricted ligands such as GLU itself, may be due to steric, conformational, and/or electronic properties of the molecules. These structure–activity aspects are at present being further pursued in order to extend the knowledge about structure in relation to receptor selectivity, potency, and efficacy at these excitatory amino acid receptors.

Experimental Section

Chemistry. General Procedures. Melting points were determined on a Hoover-Thomas apparatus and are uncorrected. All compounds were detected as single spots on TLC plates and visualized using UV light and $KMnO_4$ spraying reagent. Compounds containing amino groups were also visualized using ninhydrin as spraying reagent. Infrared spectra were recorded on a Nicolet SPC FT-IR spectrophotometer as KBr pellets for solids and between NaCl disks for liquids. NMR spectra (300 MHz) were obtained on a Bruker WM 300 spectrometer in $CDCl_3$ solution using TMS as internal standard, unless otherwise indicated. Microanalyses were within ±0.4% of calculated values, unless otherwise indicated, and were performed by the Analytical Department, Syntex Research.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[3-(ethoxycarbonyl)-5-isoxazolyl]propionate (11a). A solution of ethyl 2-acetamido-2-(ethoxycarbonyl)-4-pentynoate[12] (**9**) (1.4 g, 5.5 mmol), phenyl isocyanate (1.2 mL, 11 mmol), ethyl nitroacetate (0.6 mL, 5.5 mmol), and triethylamine (3 drops) in toluene (5 mL) was stirred for 1 h and then heated to reflux for 3 h. The cooled reaction mixture was filtered and evaporated, methylene chloride was added, and the solution was extracted with 1 N NaOH and then water. The organic phase was dried, evaporated, and subjected to column chromatography [hexane–methylene chloride–ethyl acetate (3:2:1 with 1% AcOH)], which, after recrystallization (ether–light petroleum), gave **11a** (854 mg, 42%). Mp: 88.5-89.8 °C. IR: 3245 (m), 2985 (m), 1760 (s), 1745 (s), 1735 (s), 1645 (s), 1515 (m) cm^{-1}. ^{1}H NMR: δ 6.74 (1 H, s), 6.44 (1 H, s), 4.43 (2 H, q, J = 7 Hz), 4.30 (2 × 2 H, q, J = 7 Hz), 3.96 (2 H, s), 2.04 (3 H, s), 1.41 (3 H, t, J = 7 Hz), 1.30 (2 × 3 H, t, J = 7 Hz). Anal. ($C_{16}H_{22}N_2O_8$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[3-(methoxyacetyl)-5-isoxazolyl]propionate (11b). A solution of ethyl 2-acetamido-2-(ethoxycarbonyl)-4-pentynoate[12] (**9**) (3.8 g, 15 mmol), phenyl isocyanate (3.2 mL, 30 mmol), methyl 3-nitropropionate (2.0 g, 15 mmol), and triethylamine (4 drops) in toluene (15 mL) was stirred at room temperature for 1 h and then refluxed for 2 h. After cooling, filtration, and evaporation, column chromatography (hexane–methylene chloride–acetone 5:4:1) gave **11b** (1.2 g, 21%) as a yellow oil. IR: 3370 (m), 2985 (m), 1745 (s), 1680 (s), 1605 (m), 1505 (s) cm^{-1}. ^{1}H NMR: δ 6.73 (1 H, s), 6.08 (1 H, s), 4.28 (2 × 2 H, q, J = 7 Hz), 3.87 (2 H, s), 3.73 (3 H, s), 3.69 (2 H, s), 2.02 (3 H, s), 1.28 (2 × 3 H, t, J = 7 Hz). Anal. ($C_{16}H_{22}N_2O_8$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[3-(3-methoxypropionyl)-5-isoxazolyl]propionate (11c). A solution of ethyl 2-acetamido-2-(ethoxycarbonyl)-4-pentynoate[12] (**9**) (4.0 g, 15.6 mmol), phenyl isocyanate (3.4 mL, 31.2 mmol), methyl 4-nitrobutyrate (2.0 mL, 15.6 mmol), and triethylamine (3 drops) in toluene (15 mL) was stirred for 1 h at room temperature and then refluxed for 3 h. The reaction mixture was cooled, filtered, and evaporated, after which column chromatography [hexane–ethyl acetate (1:1 → 1:2)] gave **11c** (3.2 g, 55%) as a liquid which slowly crystallized at room temperature. Mp: 37–40 °C. IR: 3415 (m), 2985 (m), 1745 (s), 1680 (s), 1605 (m), 1500 (s) cm^{-1}. ^{1}H NMR: δ 6.72 (1 H, s), 5.87 (1 H, s), 4.27 (2 × 2 H, q, J = 7 Hz), 3.83 (2 H, s), 3.68 (3 H, s), 2.93 (2 H, t, J = 7.4 Hz), 2.68 (2 H, t, J = 7.4 Hz), 2.01 (3 H, s), 1.27 (2 × 3 H, t, J = 7 Hz). Anal. ($C_{17}H_{24}N_2O_8$) C, H, N.

(*RS*)-2-Amino-3-(3-carboxy-5-isoxazolyl)propionic Acid Hydrochloride (6a). Compound **11a** (100 mg, 0.27 mmol) was heated to reflux in 1 N HCl (10 mL) for 12 h. The solution was cooled and extracted with ethyl acetate and the aqueous phase evaporated and recrystallized (glacial acetic acid) to give **6a** (46 mg, 72%). Mp: 187–190 °C dec. IR: 3400 (br, m), 3300–2500 (multiple, m–s), 1745 (s), 1705 (s), 1600 (m), 1495 (s) cm^{-1}. ^{1}H NMR (D_2O, DMSO-d_6): δ 6.74 (1 H, s), 4.34 (1 H, t, J = 6 Hz), 3.42 (2 × 1 H, m). Anal. ($C_7H_9N_2O_5Cl$) C, H, N, Cl.

(*RS*)-2-Amino-3-[3-(carboxymethyl)-5-isoxazolyl]-propionic Acid Hydrochloride (6b). Compound **11b** (210 mg, 0.55 mmol) was heated to reflux in 1 N HCl for 16 h. The reaction mixture was cooled and extracted with ethyl acetate. The aqueous phase was evaporated and recrystallized (glacial acetic acid) to

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give **6b** (66 mg, 48%). Mp: 168–172 °C dec. IR: 3600–2500 (multiple, m–s), 1750 (br, s), 1615 (s), 1590 (s), 1495 (s) cm^{-1}. ^1H NMR (D_2O): δ 6.46 (1 H, s), 4.31 (1 H, dd, J = 6.8 and 5.5 Hz), 3.51 (2 × 1 H, m). Anal. ($C_8H_{11}N_2O_5Cl$) H, N. C: calcd, 38.34; found, 38.96.

(*RS*)-2-Amino-3-[3-(carboxyethyl)-5-isoxazolyl]propionic Acid Hydrochloride (6c). Compound 11c (500 mg, 1.3 mmol) was heated to reflux in 1 N HCl for 16 h. The solution was cooled and extracted with ethyl acetate. The aqueous phase was evaporated and gave after recrystallization (glacial acetic acid) **6c** (310 mg, 90%). Mp: 207–210 °C dec. IR: 3400 (br, m), 3300–2400 (multiple, m-s), 1735 (s), 1715 (s), 1610 (s), 1490 (s) cm^{-1}. ^1H NMR (D_2O): δ 6.26 (1 H, s), 4.39 (1 H, t, J = 6 Hz), 3.41 (2 × 1 H, m), 2.86 (2 H, t, J = 7 Hz), 2.68 (2 H, t, J = 7 Hz). Anal. ($C_9H_{13}N_2O_5Cl$) H, N. C: calcd, 40.84; found, 41.45.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[3-(ethoxycarbonyl)-4-methyl-5-isoxazolyl]propionate (13) and Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[3-(ethoxycarbonyl)-5-methyl-4-isoxazolyl]propionate (14). A solution of ethyl 2-acetamido-2-(ethoxycarbonyl)-4-hexynoate[14] (**12**) (13.45 g, 50 mmol), phenyl isocyanate (10.8 mL, 100 mmol), ethyl nitroacetate (5.54 mL, 50 mmol), and triethylamine (20 drops) in toluene (40 mL) was stirred at room temperature for 1 h and heated to reflux for 3 h. The mixture was cooled, filtered, and evaporated, water was added, and the mixture was extracted with methylene chloride. The dried and evaporated organic phases were subjected to column chromatography [hexane–methylene chloride–acetone (5:4:1 with 1% AcOH)]. The fractions enriched in **13** and **14** were treated with decoloring carbon and rechromatographed twice [hexane–methylene chloride–acetone (5:4:1)], which afforded **13** and **14**. The following are data for **13** (424 mg, 2.2%). Mp: 130.5–132 °C (ether–light petroleum). IR: 3410 (br, w), 3240 (m), 2985 (m), 1755 (s), 1725 (s), 1640 (s) cm^{-1}. ^1H NMR: δ 6.65 (1 H, s), 4.40 (2 H, q, J = 7 Hz), 4.28 (2 × 2 H, q, J = 7 Hz), 3.86 (2 H, s), 2.05 (3 H, s), 1.99 (3 H, s), 1.39 (3 H, t, J = 7 Hz), 1.29 (2 × 3 H, t, J = 7 Hz). Anal. ($C_{17}H_{24}N_2O_8$) C, H, N. The following are data for **14** (310 mg, 1.6%). Mp: 96–97.2 °C (ether–light petroleum). IR: 3420 (br, m), 3250 (m), 2985 (m), 1740 (s), 1640 (s) cm^{-1}. ^1H NMR: δ 6.51 (1 H, s), 4.35 (2 H, q, J = 7 Hz), 4.26 (2 H, q, J = 7 Hz), 4.17 (2 H, q, J = 7 Hz), 3.63 (2 H, s), 2.33 (3 H, s), 1.93 (3 H, s), 1.38 (3 H, t, J = 7 Hz), 1.25 (3 H, t, J = 7 Hz). Anal. ($C_{17}H_{24}N_2O_8$) C, H, N.

(*RS*)-2-Amino-3-(3-carboxy-4-methyl-5-isoxazolyl)-propionic Acid Zwitterion Monohydrate (7). Compound **13** (384 mg, 1 mmol) was heated to reflux for 15 h in 1 N HCl (20 mL). The solution was cooled, extracted with ethyl acetate, evaporated, and re-evaporated twice from water and then recrystallized (water) to give **7** (201 mg, 86.6%). Mp: 202–205 °C dec. IR: 3500–2500 (multiple, m–s), 1700 (s), 1600 (s), 1510 (s) cm^{-1}. ^1H NMR (D_2O): δ 4.08 (1 H, t, J = 5.3 Hz), 2.85 (2 H, m), 1.79 (3 H, t, J = 2.5 Hz). Anal. ($C_8H_{10}N_2O_5,H_2O$) H, N. C: calcd, 41.38; found, 41.83.

(*RS*)-2-Amino-3-(3-carboxy-5-methyl-4-isoxazolyl)-propionic Acid Zwitterion (ACPA, 8). Compound **14** (180 mg, 0.47 mmol) was heated to reflux for 17 h in 1 N HCl (12 mL). The solution was cooled, extracted with ethyl acetate, evaporated, and reevaporated from water. The residue was dissolved in water (ca. 0.25 mL), ethanol (1 mL) and ethyl acetate (1 mL) were added, and the pH was adjusted to ca. 4 with triethylamine. The precipitate was filtered off and recrystallized from water to give **8** (57 mg, 57%). Mp: 204–214 °C dec. IR: 3600–2500 (multiple, m–s), 1685 (br, s), 1620 (s), 1585 (br, s), cm^{-1}. ^1H NMR (D_2O): δ 4.14 (1 H, t, J = 6.2 Hz), 3.29 (1 H, dd, J = 15 and 6.2 Hz), 3.17 (1 H, dd, J = 15 and 6.2 Hz), 2.43 (3 H, s). Anal. ($C_8H_{10}N_2O_5$) H, N. C: calcd, 44.86; found, 45,37.

Receptor Binding Assays. [^3H]AMPA, [^3H]kainic acid, and NMDA-sensitive [^3H]glutamic acid binding studies were performed as described in refs 15, 16, and 17, respectively.

In Vitro Electrophysiology. A rat cortical slice preparation for testing the depolarizing activity of excitatory amino acids described by Harrison and Simmonds[18] was used in a modified version. Wedges (500 μM thick) of rat brain containing cerebral cortex and corpus callosum were placed with the cortex part between two layers of absorbent fiber ("nappy liner") and the corpus callosum part between two other layers of absorbent fiber. The two halves were electrically insulated from each other by a grease gap. The cortical part was constantly perfused with a Mg^{2+}-free, oxygenated Krebs buffer to which the compounds tested were added, whereas the corpus callosum part was perfused with a Mg^{2+}- and Ca^{2+}-free Krebs buffer. The two parts were each in contact with an Ag/AgCl electrode through which DC potentials were measured and via a DC amplifier plotted on a chart recorder.

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Registry No. **6a**·HCl, 137091-92-0; **6a** (free base), 137091-93-1; **6b**·HCl, 137091-94-2; **6b** (free base), 137091-95-3; **6c**·HCl, 137091-96-4; **6c** (free base), 137091-97-5; **7**, 137091-98-6; **8**, 137091-99-7; **9**, 61172-60-9; **11a**, 137092-02-5; **11b**, 137092-03-6; **11c**, 137092-04-7; **12**, 19013-58-2; **13**, 137092-00-3; **14**, 137092-01-4; O_2NCH_2COOEt, 626-35-7; $O_2NCH_2CH_2COOMe$, 20497-95-4; $O_2N(CH_2)_3COOMe$, 13013-02-0.