Synthesis and Structure–Activity Studies on Acidic Amino Acids and Related Diacids as NMDA Receptor Ligands

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The 3-isoxazolol amino acids (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid [(*S*)-AMPA, **2**] and (*R,S*)-2-amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA, **5a**) (Figure 1) are potent and specific agonists at the AMPA and *N*-methyl-D-aspartic acid (NMDA) subtypes, respectively, of (*S*)-glutamic acid (**1**) receptors. A number of amino acids and diacids structurally related to AMAA were synthesized and tested electrophysiologically and in receptor-binding assays. The hydroxymethyl analogue **7c** of AMAA was an NMDA agonist approximately equipotent with AMAA in the [^{3}H]CPP-binding assay (IC_{50} = 7 ± 3 μM) and electropharmacologically in the rat cortical wedge model (EC_{50} = 8 ± 2 μM). In contrast to this, the *tert*-butyl analogue **7a** of AMAA turned out to be an antagonist at NMDA and AMPA receptors. The conformational characteristics of AMAA and **7a,c** were studied by molecular mechanics calculations. Compound **7a** possesses extra steric bulk and shows significant restriction of conformational flexibility compared to AMAA and **7c**, which may be determining factors for the observed differences in biological activity. Although the nitrogen atom of quinolinic acid (**6**) has very weak basic character, **6** is a, perhaps subtype-selective, NMDA receptor agonist and a potent neurotoxic agent. These aspects prompted us to synthesize and test the diacids **8a,b**, in which the amino group of AMAA has been replaced by a methylthio and methoxy group, respectively. Neither compound showed significant affinity for nor depolarizing effects at NMDA receptors. The hydroxymethyl AMPA analogue **3c** showed no interaction with NMDA receptors and only weak AMPA agonist effects.

Introduction

(*S*)-Glutamic acid (Glu, 1) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system (CNS).[1-4] Glu hyperactivity of as yet unknown nature has been implicated in a number of neurodegenerative disorders.[2-6] Hypoactivity at Glu-operated synapses may, on the other hand, contribute to the clinical manifestations of Alzheimer's disease[7-9] (impaired memory and learning) and schizophrenic disorders.[10,11] Whereas Glu receptor antagonists may be useful neuroprotective agents in the former class of progressive diseases,[12] nontoxic compounds capable of stimulating Glu receptors or enhancing the function of such receptors may have therapeutic interest in Alzheimer's disease[8] or schizophrenia.[10] These aspects raise a number of complex and in some cases apparently paradoxical pharmacological and therapeutic problems. In any case, a prerequisite for design on a rational basis of therapeutically useful drugs in this field is systematic and detailed mapping of the structural parameters determining agonism, partial agonism, and antagonism at each subtype of Glu receptor.[3,6,13]

The central EAA receptors are at present classified into four, perhaps five, main classes, all of which are heterogeneous.[1-6,14] Among these multiple EAA receptors, three classes are most extensively characterized pharmacologically and studied in relation to CNS disorders: (1) AMPA receptors, at which (*S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid [(*S*)-AMPA, **2**],[15] (*R,S*)-2-amino-3-(3-hydroxy-5-*tert*-butyl-4-isoxazolyl)propionic acid (ATPA, **3a**),[16] and (*R,S*)-2-amino-3-[3-hydroxy-5-(bromomethyl)-4-isoxazolyl]propionic acid (ABPA, **3b**)[17] (Figure 1) are potent and highly selective agonists. 2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo[*f*]quinoxazoline (NBQX) is a potent and selective AMPA receptor antagonist.[18] (2) NMDA receptors, at which *N*-methyl-D-aspartic acid (NMDA, **4**),[19] (*R,S*)-2-amino-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA, **5a**), (*R,S*)-2-(*N*-methylamino)-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (*N*-Me-AMAA, **5b**),[20] and quinolinic acid (**6**)[5,19] are selective agonists, whereas glycine is a coagonist at the NMDA receptor complex.[21] (*R,S*)-[3-(2-Carboxy-4-piperazinyl)prop-1-yl]phosphonic acid (CPP)[22] and (*R*)-2-amino-5-phosphonopentanoic acid (D-AP5)[23] are competitive NMDA antagonists, whereas (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,-10-imine (MK-801) is a noncompetitive NMDA antagonist.[24] Finally, (3) kainic acid receptors, at which the naturally occurring neurotoxin, kainic acid, is a potent agonist.[3,6]

As part of our current project focusing on the development of compounds capable of enhancing AMPA receptor mechanisms[25-28] or activating, as full or partial agonists, NMDA[20,29] or AMPA[13,15-17,30] receptors, we now describe the synthesis and pharmacology of a series of compounds (Figure 1) using AMPA and the NMDA agonist AMAA as leads. In light of the very weak basic character of the nitrogen atom of the NMDA agonist quinolinic acid (**6**) ($pK_a(N) \leq 2.0$), we also synthesized and tested the two diacids (*R,S*)-2-(methylthio)-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (**8a**) and (*R,S*)-2-methoxy-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid

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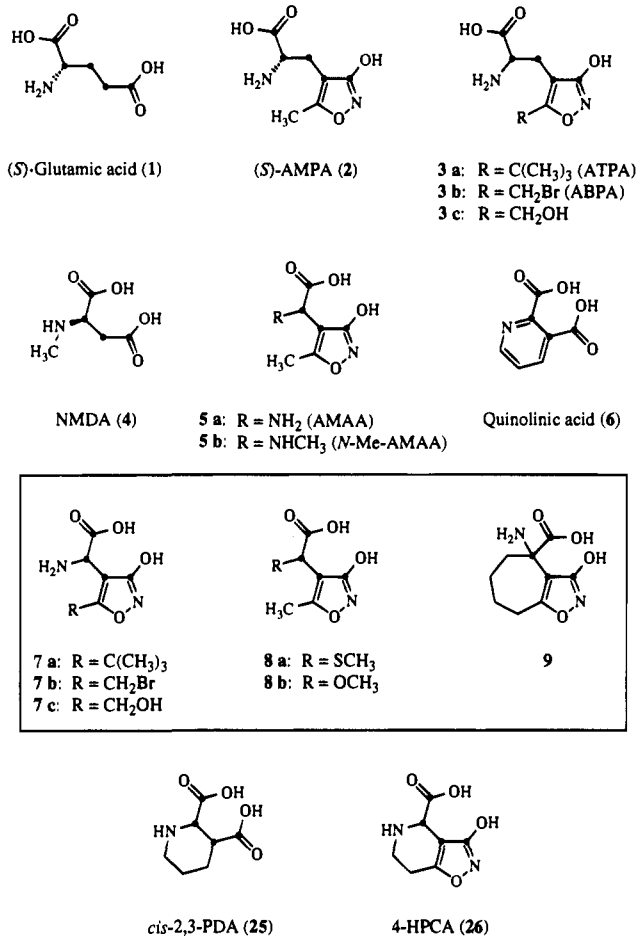
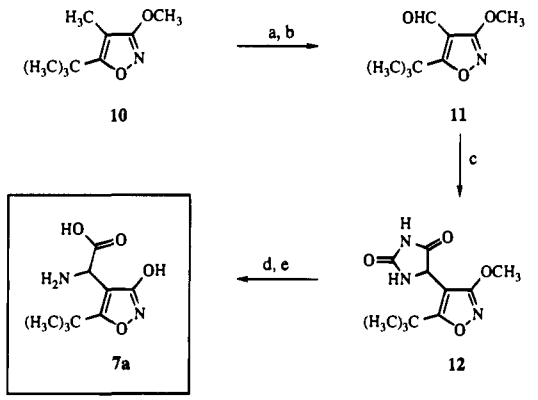


Figure 1. Structures of (*S*)-glutamic acid (1), (*S*)-AMPA (**2**), NMDA (**4**), AMAA (**5a**), quinolinic acid (**6**), and related analogues.

Scheme 1[a]



[a] Reagents: (a) NBS; (b) H_2O, reflux; (c) KCN, $(NH_4)_2CO_3$; (d) 6 M HCl; (e) IRA-400.

(**8b**). In continuation of studies on the relationship between relative efficacy at NMDA receptors and conformational flexibility of cyclic Glu and aspartic acid analogues,[29] we synthesized and tested the conformationally restricted analogue of AMAA, (*R,S*)-4-amino-3-hydroxy-5,6,7,8-tetrahydro-4*H*-cycloheptatrieno[1,2-*d*]isoxazole-4-carboxylic acid (**9**).

Results

Chemistry. Compound **7a** was synthesized as shown in Scheme 1. Bromination of 5-*tert*-butyl-3-methoxy-4-methylisoxazole (**10**),[16] using a large excess of *N*-bromosuccinimide (NBS), primarily resulted in dibromination, and after hydrolysis, the aldehyde **11** was obtained in 30% yield. Using **11** in a Bucherer–Berg synthesis afforded the hydantoin 12, which was hydrolyzed and deprotected by reflux in 6 M hydrochloric acid to give the amino acid **7a**, isolated in the zwitterionic form.

The compounds **7c** and **8a,b** were synthesized according to Scheme 2. Treatment of ethyl (*R,S*)-2-bromo-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate (**13**)[20] with liquid ammonia afforded compound **14**. Stepwise protection of the amino group of **14** using methyl chloroformate followed by BOC anhydride resulted in the *N,N*-diprotected α-amino ester **16**, which was subsequently NBS brominated to give compound **17**. Deprotection of **17** and simultaneous hydrolysis of the bromomethyl substituent afforded the 5-hydroxymethyl analogue of AMAA, **7c**.

Diprotection of the amino group as seen in compound **16** was chosen after a number of unsuccessful attempts to brominate other intermediates. Bromination of derivatives of compound **14** with only one protecting group (methoxycarbonyl or BOC) on the amino group were unsuccessful, resulting in conversion of the *N*-monoprotected α-amino esters into α-keto esters (not illustrated). However, using an analogue of compound **16**, diprotected with two BOC groups, resulted in selective bromination of the 5-methyl substituent accompanied by minor degradation of the *N,N*-diprotected α-amino ester. Unfortunately, this bromination was very slow and did not run to completion, even when using a large excess of NBS, probably due to sterical hindrance by the two BOC groups.

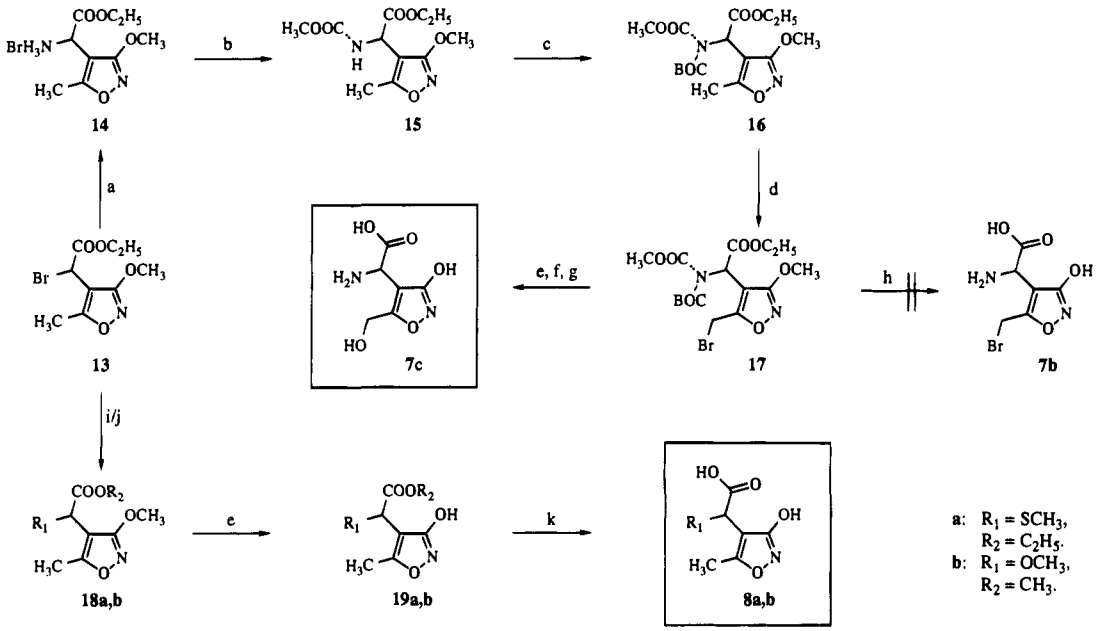
Attempts to prepare the 5-bromomethyl analogue of AMAA, **7b**, were unsuccessful. Deprotection of compound **17** using concentrated hydrobromic acid resulted in a complex reaction mixture containing a compound, which, according to TLC and NMR spectroscopic analysis, was identical with compound **7c**.

Using compound **13** as starting material, the two diacids **8a,b** were synthesized (Scheme 2). Substitution of the bromo atom of **13** using either sodium methylthiolate or sodium methanolate led to the compounds **18a,b**, respectively. Attempts to deprotect the 3-methoxyisoxazole group of **18b** with hydrogen bromide in glacial acetic acid (33%) was accompanied by partial cleavage of the 2-methoxy group. To diminish the extent of this unwanted side reaction, the deprotection was stopped before completion, and the starting material **18b** and compound **19b** were then isolated in 29% yield each. Final deprotection of the esters **19a,b**, to give the diacids **8a,b** was performed by treatment in 1 M sodium hydroxide at room temperature.

As shown in Scheme 3, the bicyclic acidic amino acid **9** was prepared *via* the hydantoin **21** obtained by a Bucherer–Berg synthesis using 3-methoxy-5,6,7,8-tetrahydro-4*H*-cycloheptatrieno[1,2-*d*]isoxazol-4-one (**20**)[31] as starting material. Deprotection of compound **21** under strongly acidic conditions resulted in hydrolytic cleavage of both the hydantoin and the methoxy group, affording compound **9** after ion exchange chromatography.

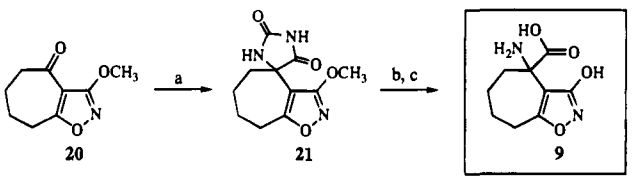
The synthesis of the hydroxymethyl analogue of AMPA, **3c**, is shown in Scheme 4. Selective *O*-benzoylation of methyl 2-(acetylamino)-2-(methoxycarbonyl)-

Scheme 2[a]



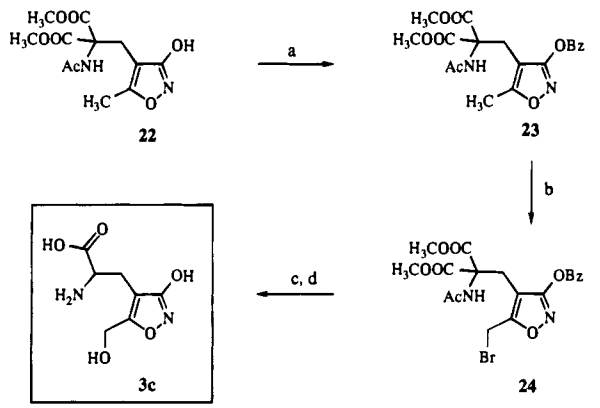
[a] Reagents: (a) NH_3; (b) $ClCOOCH_3$, K_2CO_3; (c) BOC_2O, DMAP; (d) NBS; (e) HBr, AcOH; (f) H_2O, reflux; (g) IRA-400; (h) HBr, H_2O; (i) $NaSCH_3$; (j) $NaOCH_3$; (k) 1 M NaOH.

Scheme 3[a]



[a] Reagents: (a) KCN, $(NH_4)_2CO_3$; (b) 6 M HCl; (c) IRA-400.

Scheme 4[a]



[a] Reagents: (a) BzCl, Et_3N; (b) NBS; (c) 1 M HBr; (d) IRA-400.

3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate[32] (**22**) was carried out using benzoyl chloride and triethylamine. Bromination of **23** using NBS gave compound **24**, and subsequent reflux in 1 M hydrobromic acid resulted in deprotection, hydrolysis, and decarboxylation to give compound **3c** after ion exchange chromatography.

Molecular Mechanics Calculations. Molecular mechanics calculations were performed using the MM3* force field as included in the MacroModel program (version 4.0).[33] Parameters for the isoxazole substructure were obtained from the MM3(92) force field.[34] The conformational flexibility of the glycine moieties for the *S*-forms of compounds AMAA and **7a,c** were systematically analyzed by the torsional drive technique using MULTIC for generation of potential energy curves and maps. The compounds analyzed were in the nonionized forms, as no parameters have been evaluated and made available in the MM3(92) force field for the ionized 3-isoxazolol moiety. Calculations have been performed on the molecules in vacuo and in a simulated aqueous environment.[35]

The calculated potential energy curves for different orientations of the glycine moiety are illustrated in Figure 2. For all three compounds, minimum energy conformations are observed for approximately the same values of the torsion angle, $\tau_1 = -90°$ and 120°. These conformations place the carboxyl group nearly perpendicular to the isoxazole ring and the C_α hydrogen atom and the amino group close to the plane of the ring. The conformation with the torsion angle −90° represents the lowest energy minimum. In this conformation, the amino group is close to the hydroxy group of the isoxazole moiety and an intramolecular hydrogen bond can be formed (N-H···O). The results of the calculations in vacuo and in a simulated water environment are nearly identical for AMAA and compound **7a**, indicating that the aqueous environment has no significant effect on the preferred conformations of the two compounds. However, the curves for compound **7c** in Figure 2, illustrating the two types of calculations, are quite different. Energy minima similar to those observed for the other compounds are found, but the discontinuity of the in vacuo curve for **7c** is very pronounced. The flexibility of all three compounds can not be completely described by analyzing one torsion angle, τ_1. The amino and the carboxyl groups of the glycine moiety can change orientation as well, and this explains the small "jumps". The discontinuity of the potential energy curve for **7c** is due to the presence of the hydroxymethyl group. To describe the flexibility of this compound, two torsion angles have been systematically analyzed by the torsional drive technique using MULTIC. The calculated potential energy maps (Figure 3, top) reveal that the minimum energy areas are located approximately at the expected values of the torsion angle for the glycine

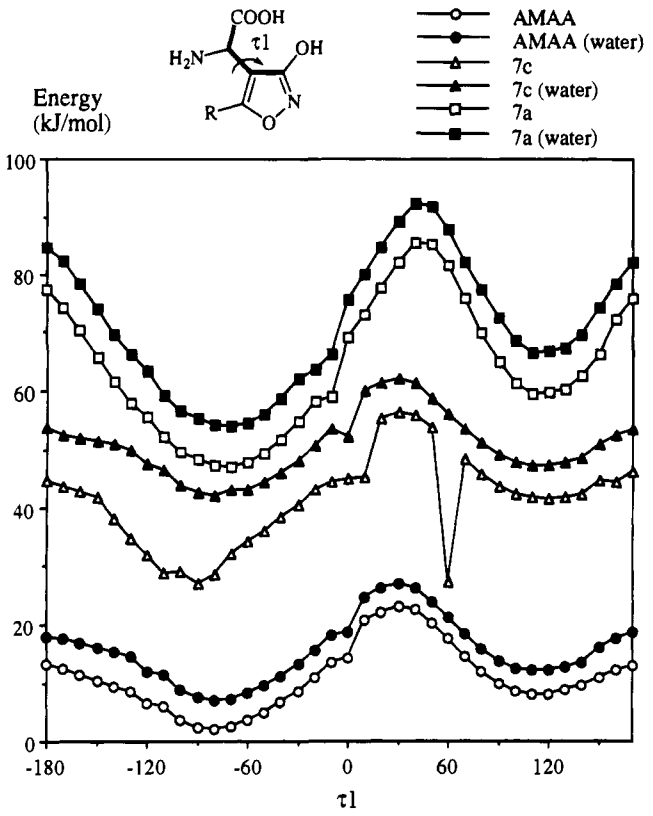


Figure 2. Potential energy curves (relative energies) calculated for *S*-enantiomers of AMAA and **7a,c** in vacuo (open symbols) and in a simulated aqueous environment (filled symbols). The curves are obtained by rotation of the glycine moiety as indicated using the MacroModel program.[33]

moiety ($\tau_1 = -90°$ and 100°), but the level of the energies is highly dependent on the orientation of the hydroxymethyl moiety. A comparison of the potential energy maps from the calculations in vacuo (Figure 3, top left) and in the simulated aqueous environment (Figure 3, top right) further illustrates the difference observed for the curves in Figure 2 for compound **7c**. The minimum energy areas are located at approximately the same positions, but the energy contours are highly different. The lowest energy minimum is the conformation with τ_1 and τ_2 approximately 90° and 0°, respectively. For this conformation, an intramolecular hydrogen bond is observed between the amine and the hydroxymethyl group (N-H···O). This intramolecular hydrogen bond is favorable for the calculations in vacuo. In the simulated water environment, the molecules can make similar contacts to the water environment, making the energy differences between the minima smaller and the energy barriers smaller as well. For comparison, the two torsion angles of compound **7a** have been analyzed (Figure 3, bottom). As expected, the symmetry of the *tert*-butyl group is reflected in the potential energy map.

The calculations show the rotation barriers for the glycine moities of AMAA and **7c** to be nearly identical in the simulated aqueous environment (Figure 2), where the effects of intramolecular hydrogen bonding for **7c** is small. Significantly more energy is required for rotation of the glycine moiety in **7a**.

In Vitro Pharmacology. The affinities for EAA receptor sites in rat cortical membranes of the compounds **3c**, **7a,c**, **8a,b**, and **9** were evaluated in binding assays using [^{3}H]CPP,[22] [^{3}H]MK-801 (stimulation experiments),[24] [^{3}H]glycine,[36] [^{3}H]AMPA,[37] and [^{3}H]kainic acid[38] as radioligands, and the in vitro electropharmacological activities were determined in the rat cortical wedge preparation.[39,40] None of the compounds synthesized showed significant affinity in the [^{3}H]glycine- or [^{3}H]kainic acid-binding assays (IC_{50} > 100 μM). The receptor-binding data are summarized in Table 1.

Table 1. Receptor-Binding and in Vitro Electropharmacological Data (Mean ± SEM; n = 3–5)

compd	[^{3}H]-CPP IC_{50} (μM)	[^{3}H]-MK-801[a] EC_{50} (μM)	[^{3}H]-AMPA IC_{50} (μM)	cortical wedge EC_{50} (μM)
AMAA	4.5[b]	0.45[b]	>100[b]	4.3 ± 1
7a	>100[c]	>100	>100[d]	antagonist
7c	7 ± 2.7	5 ± 2	>100	8 ± 2
N-Me-AMAA	18.3[b]	50[b]	>100[b]	70[b]
quinolinic acid	>100[b]	nt	>100[b]	1500[b]
8a	>100	>100	>100	>5000
8b	>100	>100	>100	>5000
9	>100	>100	>100	2500[e]
AMPA	>100[b]	>100	0.04[b]	3.5 ± 0.2
ABPA	nt	>100	0.03[f]	13[e]
ATPA	>100	>100	11[g]	48[g]
3c	>100	>100	0.4 ± 0.04	110[e]

[a] [^{3}H]MK-801 binding in well-washed membranes with a fixed concentration of glycine and variable concentrations of test compound (stimulation experiments). [b] Reference 20. [c] Inhibition at 100 μM: 25%. [d] Inhibition at 100 μM: 8%. [e] n = 2 (only small amounts of compounds were available). [f] Reference 41. [g] Reference 42. nt, not tested.

The 5-hydroxymethyl analogue of AMAA, compound **7c**, was shown to be an inhibitor of [^{3}H]CPP binding, approximately equipotent with AMAA, but **7c** was weaker than AMAA in stimulating [^{3}H]MK-801 binding (Table 1). In the rat cortical wedge preparation, compound **7c** was a slightly weaker agonist than AMAA (Table 1 and Figure 4, top). The pharmacological profile of compound **7c** is similar to that of AMAA, as the responses to both of these agonists were fully antagonized by D-AP5 (5 μM) and were unaffected by NBQX (2 μM) (data not shown).

The 5-*tert*-butyl analogue of AMAA, compound **7a**, showed very weak affinity for [^{3}H]CPP- and [^{3}H]AMPA-binding sites, 25% and 8% inhibition at 100 μM, respectively, and the compounds **8a,b** and **9** did not show significant affinity (IC_{50} > 100 μM) in any of the performed binding assays. In the cortical wedge preparation, compound **7a** (100 μM) was, however, shown to be an equipotent blocker of responses induced by NMDA (10 μM) and AMPA (5 μM), whereas no significant reduction of responses to kainic acid (10 μM) were observed even at concentrations up to 1 mM (Figure 5). The antagonist profile of compound **7a** is shown in Figure 6, and the dose-dependent inhibition of the NMDA response is illustrated in Figure 7. A Hill coefficient of 1.06 ± 0.19 is obtained from this inhibition curve.

The diacids **8a,b** showed no agonist activity in the cortical wedge preparation at concentrations up to 1 mM. In the same preparation, the bicyclic compound **9** elicited a weak response, which was sensitive to D-AP5 (5 μM) but unaffected by the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (5 μM) (data not shown). NMDA (10 μM)-, AMPA (5 μM)-, and kainic acid (10 μM)-induced depolarizations were unaffected by coadministration of **8a** (100 μM), **8b** (500 μM), or **9** (500 μM) (data not shown).

The 5-hydroxymethyl analogue of AMPA, compound **3c**, was a selective displacer of [^{3}H]AMPA binding,

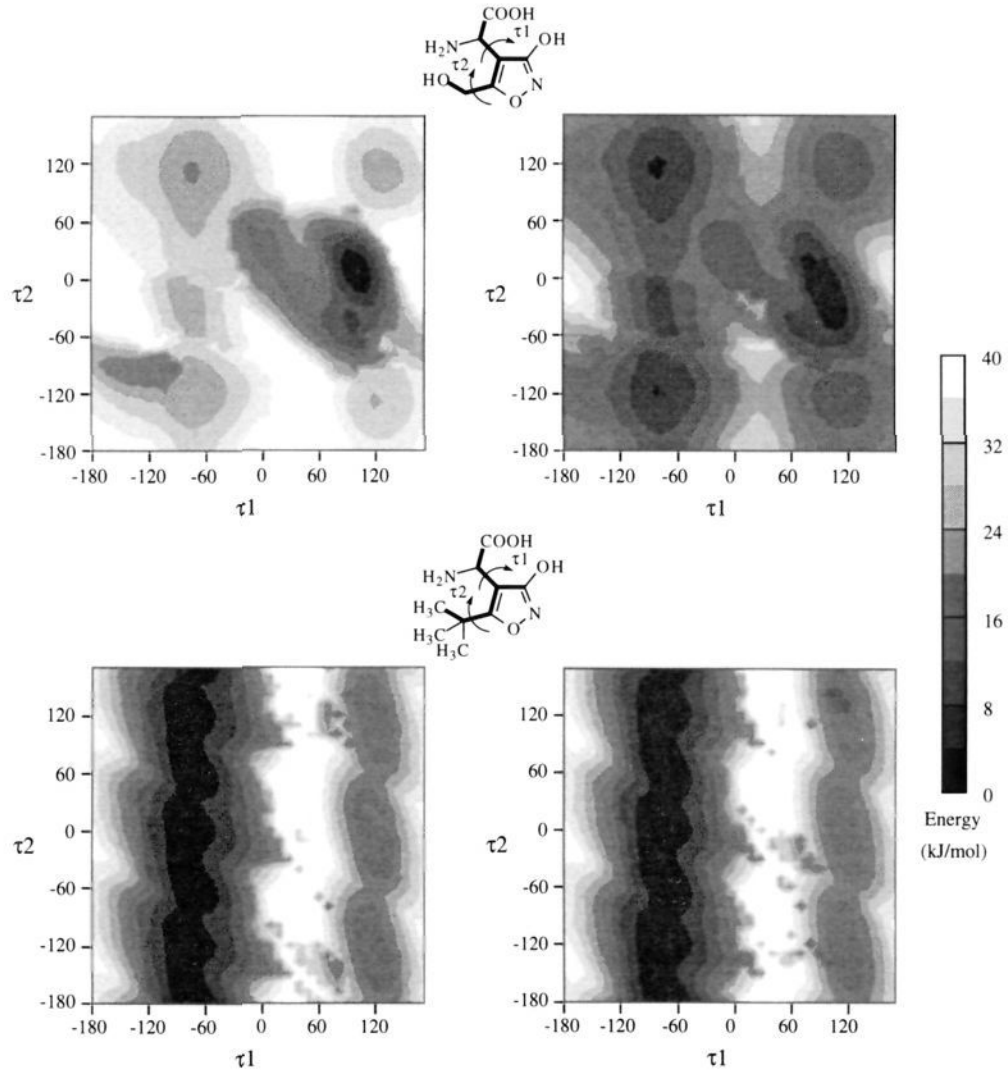


Figure 3. Potential energy maps (relative energies) calculated for *S*-enantiomers of **7c** (top) and **7a** (bottom) in vacuo (left) and in a simulated aqueous environment (right) using the MacroModel program.[33]

showing an affinity approximately 10 times lower than that of AMPA and without significant affinity (IC_{50} > 100 μM) in other binding assays (Table 1). In the cortical wedge preparation, compound **3c** induced a response sensitive to NBQX (2 μM) but insensitive to D-AP5 (5 μM) (data not shown). Compound **3c** was approximately 30 times weaker than AMPA in this electropharmacological test system as illustrated by the dose-response curves (Figure 4, bottom).

Discussion

The structural requirements for interaction with NMDA receptors have been investigated by the synthesis, molecular mechanics calculations, and testing of a number of analogues of the NMDA receptor agonist AMAA. The pharmacological profile of compound **7c** was similar to that of AMAA. Both compounds are selective NMDA receptor agonists with similar affinity in the [^{3}H]CPP-binding assay (Table 1), and they show approximately equipotent D-AP5 sensitive depolarizing activity in the cortical wedge preparation (Figure 4). This indicates that the hydroxymethyl substituent of **7c**, like the 5-methyl substituent of AMAA, is tolerated at the activated state of the NMDA receptor(s). In contrast to this, substitution of a bulky *tert*-butyl group for the 5-methyl group of AMAA dramatically changes the pharmacological profile. Thus, compound **7a** turned out to be an EAA receptor antagonist, capable of inhibiting NMDA and AMPA but not kainic acid-induced depolarizations in the rat cortical wedge preparation (Figures 5 and 6). Thus, the nature of the 5-substituent in analogues of AMAA has a pronounced influence on the pharmacological profile of such compounds. It is noteworthy that although **7a** is equally effective as an antagonist at NMDA and AMPA receptors, the latter effect is not accompanied by any significant effect on kainic acid receptors.

The molecular mechanics calculations performed on AMAA and **7a**,**c** showed the energy barrier for rotation of the glycine moiety of compound **7a** to be significantly higher than for AMAA and **7c**. This restriction of conformational flexibility for compound **7a** is related to the bulky *tert*-butyl substituent. In Figure 8, low-energy conformations of the three compounds obtained from calculations in the simulated water environment are shown. For AMAA and **7a**, the lowest energy conformations are chosen, and for **7c**, the low-energy conformation (ΔE < 4 kJ/mol) with the same spatial orientation

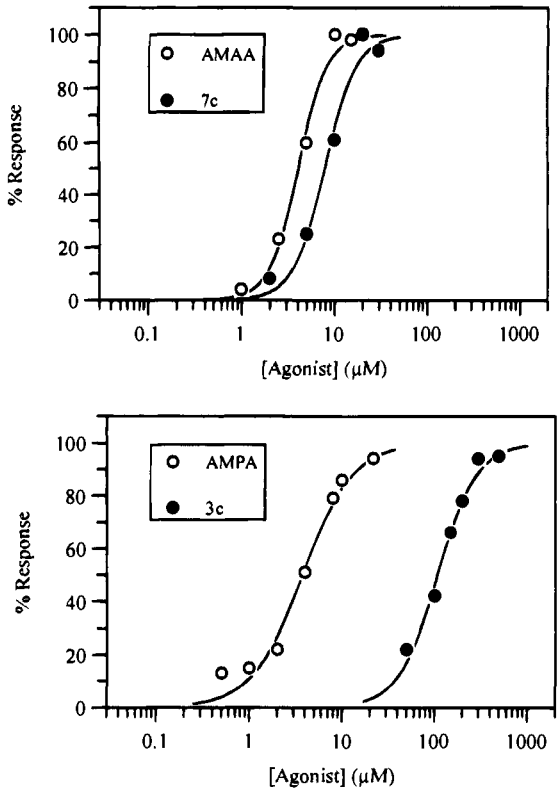


Figure 4. Dose–response curves as determined in the rat cortical wedge preparation for compound **7c** compared to AMAA (top) and for compound **3c** compared to AMPA (bottom). The data are from single experiments, which were repeated at least twice (see Table 1).

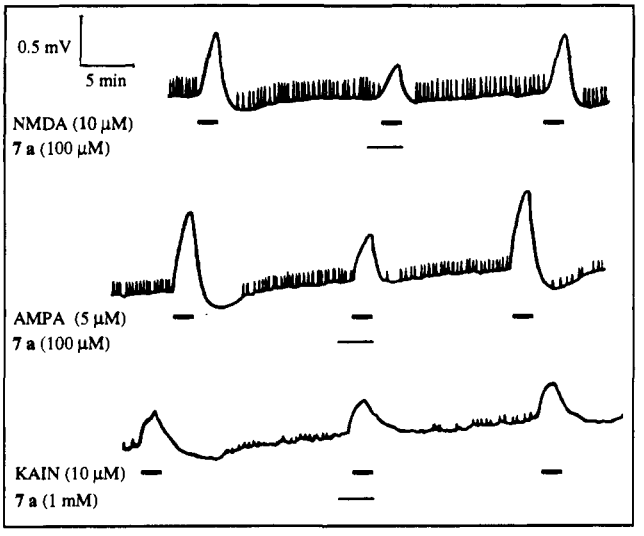


Figure 5. Recordings from rat cortical neurones depolarized by administration of NMDA (top), AMPA (middle), and kainic acid (KAIN, bottom), antagonism of these agonist effects by compound **7a**, and recovery.

of the glycine moiety has been selected. The compounds AMAA and **7c** occupy almost the same volume in space (Figure 8, bottom left), although the extra volume occupied by the hydroxy group of **7c** can be orientated in different directions, due to the rotational flexibility. Compound **7a** prefers conformations of the glycine moiety similar to that of AMAA and **7c**, but the bulky *tert*-butyl group produces a significant extra volume (Figure 8, bottom right). This difference in steric bulk may be a determining factor for the distinctly different pharmacological profiles. Alternatively, the marked

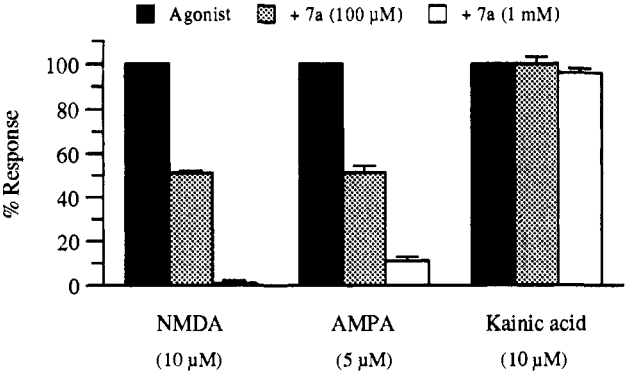


Figure 6. Effects of compound **7a** on rat cortical depolarizations induced by NMDA, AMPA, or kainic acid. Normalized responses ± SEM, $n = 3–5$.

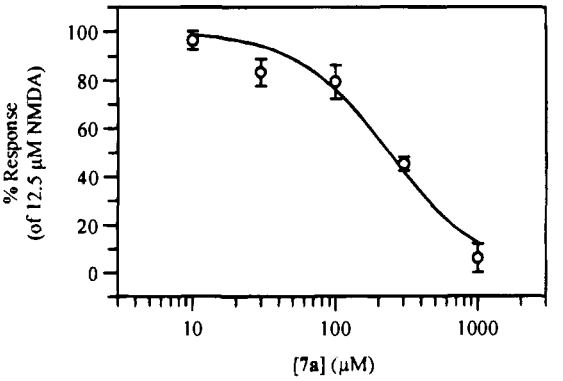


Figure 7. Inhibition by compound **7a** of responses induced by NMDA (12.5 μM) in the rat cortical wedge preparation, $n = 4$.

difference in the pharmacology of **7a** and the agonists AMAA and **7c** may be explained by different degrees of rotational flexibility of the glycine moieties of these compounds as indicated in Figure 2. The rotational flexibility of this common structure element may be of importance not only for receptor selectivity but also for the nature of the receptor interaction (agonist/antagonist profile) of these compounds. The lack of agonist activity of compound **7a** seems to agree with earlier observations indicating that a certain conformational flexibility is a factor of importance for NMDA agonist activity of certain types of cyclic amino acids.[29]

The activity of compound **7a** as an NMDA and AMPA receptor antagonist is not reflected in the [^{3}H]CPP- and [^{3}H]AMPA-binding assays. However, experiments performed with the competitive non-NMDA antagonists (*R*)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionic acid [(*R*)-APPA] and (*R*,*S*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]propionic acid (AMOA) have previously shown that these α-amino acid-based antagonists exhibit no or very small affinity in the [^{3}H]-AMPA-binding assay (IC_{50} values for AMOA and (*R*)-APPA are 90 and >100 μM, respectively).[32,43] A similar result has been obtained with *cis*-2,3-piperidinedicarboxylic acid (*cis*-2,3-PDA, **25**) (Figure 1), a partial NMDA agonist with dominating antagonist activity in the rat cortical wedge preparation.[29] *cis*-2,3-PDA shows very weak affinity in [^{3}H]CPP binding ($IC_{50} = 56 \pm 11$ μM), whereas **7a** has an IC_{50} value above 100 μM (25% inhibition at 100 μM). Thus, these amino acid antagonists at AMPA and NMDA receptors seem to be poor inhibitors of [^{3}H]AMPA and [^{3}H]CPP binding, respectively. NMDA responses are reduced by **7a** in a dose-

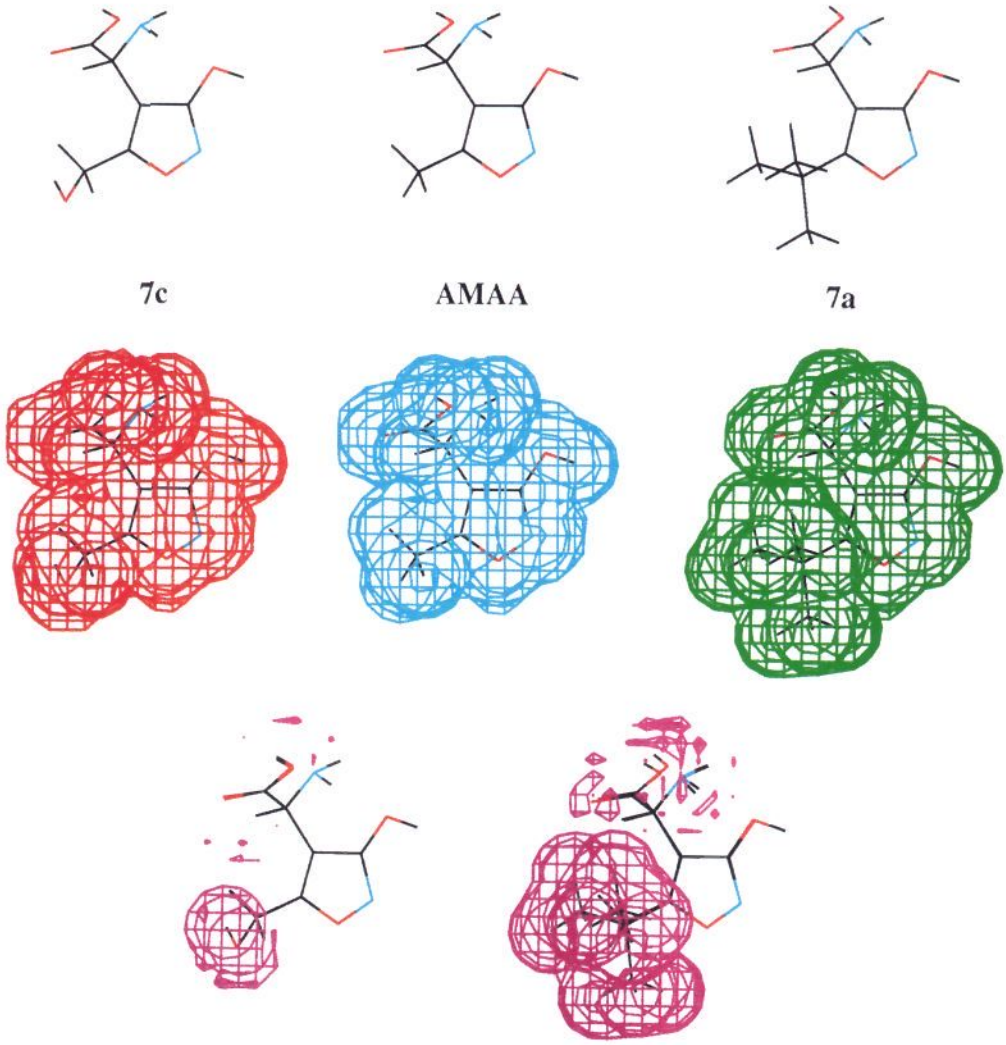


Figure 8. Structures (top) and van der Waals surfaces (middle) of low-energy conformations in the simulated aqueous environment of the *S*-enantiomers of AMAA (cyan), **7a** (green), and **7c** (red). Superposition of low-energy conformations (bottom) showing differences (magenta) in van der Waals volumes of compound **7c** and AMAA (left) and of compound **7a** and AMAA (right). The molecules are displayed using the SYBYL program version 6.03 (Tripos Associates Inc., St. Louis, MO 63144).

dependent manner (Figure 7), and analysis of the dose–response curve shows a Hill coefficient of 1.06. These data further indicate that **7a** is a competitive antagonist of NMDA receptor-mediated responses.

The NMDA and AMPA antagonist profile of compound **7a** is interesting since compound **7a**, unlike other competitive NMDA receptor antagonists such as D-AP5 and CPP as well as the competitive non-NMDA receptor antagonist AMOA,[32] has a short aspartic acid-like backbone separating the two acidic groups. Whether one or both enantiomers of **7a** possess pharmacological activity has not yet been determined, as compound **7a** was synthesized as the racemate.

The two diacids **8a**,**b**, having structural similarities to NMDA and in particular to the NMDA agonist **5b** (*N*-Me-AMAA), were biologically inactive. This indicates that the basic methylamino substituent in **5b** plays an important role for the NMDA agonist activity of this compound and that this substituent cannot be replaced in a classical isosteric manner without loss of biological activity. The nitrogen atom of quinolinic acid (**6**) is practically devoid of basic character ($pK_a(N) \leq 2.0$), consistent with the observation that the activity of quinolinic acid at the NMDA receptor is mimicked by the diacid phthalic acid.[44] In light of this, the total inactivity of **8a**,**b** is quite surprising and may reflect that the agonist activities of **5b** and quinolinic acid are mediated by different subtypes of NMDA receptors.

Conformational restriction of the molecule of AMAA by incorporation into the bicyclic structure of compound **9** is accompanied by total loss of NMDA receptor affinity, which may be due to unfavorable steric interactions between the cycloheptено moiety of **9** and the NMDA receptor site. Alternatively, the conformations represented by the conformationally restricted compound **9** may not reflect the receptor-active conformation(s) of AMAA. Another conformationally restricted analogue of AMAA, (*R*,*S*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-4-carboxylic acid (4-HPCA, **26**) (Figure 1), in which the amino group is incorporated into a bicyclic tetrahydropyridine ring system, has previously been shown to be a very weak NMDA agonist. The conformations of the acidic amino acid backbones as well as the volume of the ring systems of 4-HPCA and compound **9** are different.[20,45]

AMPA analogues having different substituents in the 5-position of the isoxazole ring, including ATPA (**3a**) and ABPA (**3b**) (Figure 1), have been described prev-

iously.[15-17,30] Structure–activity studies on these compounds indicate that the AMPA receptor tolerates analogues of AMPA carrying bulky hydrophobic substituents in the 5-position without marked loss of agonist activity. Furthermore, in light of the fully reversible interaction of ABPA with the AMPA receptor in vivo and in vitro, the chemically reactive bromomethyl group of ABPA presumably does not get into close contact with the AMPA receptor or, if it does, a suitable nucleophile may not be present.[17,41] On the basis of these findings, the presence of a cavity at the AMPA receptor capable of accommodating hydrophobic substituents of agonist molecules has been proposed.[46] Compound **3c**, the analogue of AMPA with a polar 5-hydroxymethyl substituent, is considerably weaker than both AMPA, ABPA, and ATPA as an agonist in the cortical wedge preparation (Table 1 and Figure 4), indicating that the AMPA receptor is not easily activated by analogues of AMPA with more hydrophilic substituents in the 5-position.

In conclusion, variations of the 5-substituents of the NMDA agonist, AMAA, and the AMPA agonist, AMPA, have markedly different consequences for the biological activity. Whereas, the hydroxymethyl analogue **7c** of AMAA is equipotent with AMAA as an NMDA agonist, the hydroxymethyl analogue of AMPA, **3c**, is a weak AMPA agonist. Replacement of the methyl groups of AMAA and AMPA by *tert*-butyl groups provides a mixed NMDA/AMPA antagonist, **7a**, and a potent AMPA agonist, **3a**, respectively. These observations probably reflect different amino acid residue constructions of the binding sites of the NMDA and AMPA receptors, in agreement with the dissimilar primary structures of the subunit proteins of these ionotropic receptors.[47]

Experimental Section

Chemistry. General Procedures. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, LEO Pharmaceutical Products, Denmark, or by Mr. P. Hansen, Department of General and Organic Chemistry, University of Copenhagen, and are within ± 0.4% of the calculated values, unless otherwise stated. IR spectra were recorded from KBr disks or as liquid sandwiches (NaCl) on a Perkin-Elmer 781 grating infrared spectrophotometer. Evaporations were performed under vacuum on a rotary evaporator at 15 mmHg. The 60 MHz ^{1}H NMR spectrum (compound **21**) and 200 MHz ^{1}H NMR spectra (all other compounds under study) were obtained on a Varian EM 360L spectrometer or on a Bruker AC-200F NMR spectrometer, respectively, using 1,4-dioxane as an internal standard for spectra recorded in D_2O and TMS for spectra recorded in $CDCl_3$ or DMSO-d_6. Column chromatography (CC) and TLC were performed on silica gel 60 (70–230 mesh, ASTM, Merck) and silica gel F_{254} plates (Merck), respectively. Compounds containing the 3-hydroxyisoxazole unit were visualized on TLC plates using UV light and a $FeCl_3$ spraying reagent (yellow color). Compounds containing amino groups were visualized using a ninhydrin spraying reagent, and all compounds under study were also detected on TLC plates using a $KMnO_4$ spraying reagent. The three pK_a values of quinolinic acid (**6**) (4.6, 2.0, <1.5) were estimated by titration using a Radiometer pH meter 26.

5-*tert*-Butyl-3-methoxyisoxazole-4-carbaldehyde (11). To 5-*tert*-butyl-3-methoxy-4-methylisoxazole (**10**)[16] (3.0 g, 18 mmol) dissolved in CCl_4 (100 mL) were added NBS (15.7 g, 88 mmol) and benzoyl peroxide (4.3 g, 18 mmol), and the mixture was refluxed for 15 h. Upon cooling, the reaction mixture was added to light petroleum (50 mL), filtered, and evaporated. The residue was dissolved in THF (20 mL), H_2O (20 mL) was added, and the mixture was refluxed for 5 h. After the volume of the reaction mixture was reduced to approximately 20 mL, the mixture was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated. CC [tol–EtOAc (19:1)] afforded **11** (1.0 g, 30%) as a crystalline residue. An analytical sample was recrystallized (light petroleum) to give **11**: mp 102.0–103.0 °C. ^{1}H NMR ($CDCl_3$): δ 9.90 (s, 1 H), 4.06 (s, 3 H), 1.45 (s, 9 H). IR (KBr): 2970 (m), 2950 (m–w), 2870 (w), 1690 (s), 1585 (s), 1515 (m), 1485 (s) cm^{-1}. Anal. ($C_9H_{13}NO_3$) H, N; C: calcd, 58.99; found, 58.55.

(*R,S*)-5-(5-*tert*-Butyl-3-methoxy-4-isoxazolyl)hydantoin (12). A solution of compound **11** (0.95 g, 5.2 mmol), potassium cyanide (1.69 g, 26 mmol), and ammonium carbonate (4.98 g, 51.9 mmol) in 50% aqueous MeOH (30 mL) was refluxed for 20 h. After evaporation of the reaction mixture to approximately 20 mL, the resulting mixture was extracted with EtOAc (5 × 30 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated. CC [tol–EtOAc (1:1)] followed by recrystallization (2-propanol–EtOAc) gave **12** (0.58 g, 44%): mp 235.5–236.0 °C. ^{1}H NMR ($CDCl_3$): δ 7.89 (br s, 1 H), 5.58 (br s, 1 H), 5.34 (s, 1 H), 3.96 (s, 3 H), 1.42 (s, 9 H). IR (KBr): 3300–3200 (br, m), 3160 (m), 3080 (m), 2960 (m), 1760 (s), 1740 (s), 1710 (s), 1630 (m), 1530 (m) cm^{-1}. Anal. ($C_{11}H_{15}N_3O_4$) C, H, N.

(*R,S*)-2-Amino-2-(5-*tert*-butyl-3-hydroxy-4-isoxazolyl)-acetic Acid (7a). A solution of **12** (0.58 g, 2.3 mmol) in 6 M hydrochloric acid (20 mL) was refluxed for 30 h and then evaporated. Treatment of the residue with activated charcoal and filtration through Celite was followed by evaporation and ion exchange chromatography (IRA-400) using 1 M AcOH as eluent. Evaporation of ninhydrin-active fractions afforded, after recrystallization (H_2O), compound **7a** (0.28 g, 57%): mp 202–203 °C. ^{1}H NMR (D_2O): δ 5.03 (s, 1 H), 1.35 (s, 9 H). IR (KBr): 3450 (br, m), 3200–2900 (br, m–s), 1680–1645 (several bands, m–s), 1580 (m), 1550–1525 (m), 1490 (s) cm^{-1}. Anal. ($C_9H_{14}N_2O_4$) C, H, N.

Ethyl (*R,S*)-2-Amino-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate Hydrobromide (14). A solution of ethyl (*R,S*)-2-bromo-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate (**13**)[20] (1.0 g, 3.6 mmol) in Et_2O (10 mL) was added to liquid ammonia (60 mL) during a period of 10 min. Stirring for 2 h at room temperature in an unstoppered flask resulted in evaporation of the ammonia and the Et_2O. To the residue was added an ice-cooled aqueous solution of sodium carbonate (20 mL, 2 M), and the mixture was extracted with EtOAc (5 × 30 mL). Upon drying ($MgSO_4$) and filtration, the organic phase was added to a solution of HBr in EtOAc (1.5 mL, 4 M) and evaporated. Two recrystallizations (EtOH–EtOAc) of the residue gave compound **14** (460 mg, 43%): mp 188–189 °C. ^{1}H NMR (D_2O): δ 5.22 (s, 1 H), 4.28 (m, 2 H), 3.91 (s, 3 H), 2.37 (s, 3 H), 1.20 (t, J = 7.2 Hz, 3 H). IR (KBr): 2950 (m), 2890 (s), 2840 (s), 2720 (m), 2660 (m), 2620 (m), 1750 (s), 1650 (m), 1535 (s), 1515 (m) cm^{-1}. Anal. ($C_9H_{15}N_2O_4Br$) C, H, N, Br.

Ethyl (*R,S*)-2-[*N*-(Methoxycarbonyl)amino]-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate (15). To an ice-cooled solution of **14** (170 mg, 0.58 mmol) and potassium carbonate (200 mg, 1.44 mmol) in H_2O (1 mL) was added methyl chloroformate (65 mg, 0.69 mmol). Stirring for 30 min and addition of H_2O (5 mL) was followed by extraction with EtOAc (3 × 5 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated. CC [tol–EtOAc (10:1)] gave compound **15** (140 mg, 89%) as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 5.8 (br d, J = 8 Hz, 1 H), 5.21 (d, J = 8.0 Hz, 1 H), 4.21 (q, J = 7.1 Hz, 2 H), 3.96 (s, 3 H), 3.69 (s, 3 H), 2.42 (s, 3 H), 1.24 (t, J = 7.1 Hz, 3 H). IR (liquid sandwich): 3450–3250 (br, m), 2980 (m), 2960 (m), 1760–1700 (several bands, s), 1650 (m), 1530 (s) cm^{-1}. Anal. ($C_{11}H_{16}N_2O_6$) H, N; C: calcd, 48.51; found, 49.18.

Ethyl (*R,S*)-2-[*N*-(*tert*-Butoxycarbonyl)-*N*-(methoxycarbonyl)amino]-2-(3-methoxy-5-methyl-4-isoxazolyl)-acetate (16). A solution of **15** (1.5 g, 5.5 mmol) and 4-(dimethylamino)pyridine (75 mg, 0.6 mmol) in dry acetonitrile (15 mL) was added to BOC anhydride (1.31 g, 6.0 mmol) and stirred at room temperature for 3 h. After evaporation, the oily residue was dissolved in Et_2O (50 mL) and washed with aqueous sodium hydrogen sulfate (5 × 25 mL, 2 M), saturated

aqueous sodium hydrogen carbonate (25 mL), and finally saturated aqueous sodium chloride (25 mL). The dried ($MgSO_4$) organic phase was evaporated, and after CC [tol–EtOAc (9:1)], compound **16** (1.6 g, 78%) could be isolated as a viscous oil. ^1H NMR ($CDCl_3$): δ 5.95 (s, 1 H), 4.26 (m, 2 H), 3.95 (s, 3 H), 3.78 (s, 3 H), 2.33 (s, 3 H), 1.50 (s, 9 H), 1.28 (t, J = 7.2 Hz, 3 H). IR (liquid sandwich): 2980 (m), 2960 (m), 2940 (w–m), 1770–1740 (several bands, s), 1710 (s), 1645 (m), 1530 (s) cm^{-1}. Anal. ($C_{16}H_{24}N_2O_8$) C, H, N.

Ethyl (*R,S*)-2-[*N*-(*tert*-Butoxycarbonyl)-*N*-(methoxycarbonyl)amino]-2-[5-(bromomethyl)-3-methoxy-4-isoxazolyl]acetate (17). A mixture of **16** (0.52 g, 1.4 mmol), NBS (1.0 g, 5.6 mmol), and benzoyl peroxide (85 mg, 0.35 mmol) was refluxed under dry conditions in freshly distilled CCl_4 (15 mL) for 115 h. The reaction mixture was cooled, filtered, and evaporated. CC [tol–EtOAc (19:1)] gave compound **17** (278 mg, 44%) as a viscous pale yellow oil. ^1H NMR ($CDCl_3$): δ 6.01 (s, 1 H), 4.49 (s, 2 H), 4.28 (m, 2 H), 3.99 (s, 3 H), 3.82 (s, 3 H), 1.51 (s, 9 H), 1.29 (t, J = 7.2 Hz, 3 H). IR (liquid sandwich): 2980 (m), 2950 (m), 2930 (w–m), 1770–1730 (several bands, s), 1705 (s), 1640 (w–m), 1525 (s). Anal. ($C_{16}H_{23}N_2O_8Br$) C, H, N; Br: calcd, 17.70; found, 19.05.

(*R,S*)-2-Amino-2-[3-hydroxy-5-(hydroxymethyl)-4-isoxazolyl]acetic Acid (7c). Compound **17** (0.21 g, 0.47 mmol) was dissolved in a solution of HBr in HOAc (10 mL, 33%) and stirred at room temperature for 18 h. The reaction mixture was evaporated, dissolved in H_2O (20 mL), and refluxed for 7 h. After evaporation, the residue was subjected to ion exchange chromatography (IRA-400) using 1 M HOAc as an eluent. Evaporation of ninhydrin-active fractions gave, after recrystallization (H_2O–EtOH), compound **7c** (30 mg, 34%): mp 182–184 dec. ^1H NMR (D_2O): δ 4.82 (s, 1 H), 4.64 (s, 2 H). IR (KBr): 3500–3400 (br, m), 3300–2500 (several bands, m–s), 1660 (s), 1630 (m), 1505 (s) cm^{-1}. Anal. ($C_6H_8N_2O_5$) C, H; N: calcd, 14.94; found, 14.48.

Ethyl (*R,S*)-2-(Methylthio)-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate (18a). To a solution of **13** (0.80 g, 2.9 mmol) in freshly distilled THF (10 mL) was added sodium methylthiolate (0.21 g, 3.0 mmol), and the suspension was refluxed for 6 h. After evaporation, the residue was added to H_2O (15 mL) and extracted with Et_2O (3 × 15 mL). Drying of the combined organic phases ($MgSO_4$), filtration, and evaporation resulted in a residue, which, after CC [tol–EtOAc (19:1)] and recrystallization (light petroleum), gave compound **18a** (0.52 g, 74%): mp 53.0–53.5 °C. ^1H NMR ($CDCl_3$): δ 4.34 (s, 1 H), 4.22 (q, J = 7.1 Hz, 2 H), 3.99 (s, 3 H), 2.43 (s, 3 H), 2.18 (s, 3 H), 1.30 (t, J = 7.1 Hz, 3 H). IR (KBr): 2995 (m), 2900 (m), 1725 (s), 1650 (m), 1520 (s) cm^{-1}. Anal. ($C_{10}H_{15}NO_4S$) C, H, N, S.

Methyl (*R,S*)-2-Methoxy-2-(3-methoxy-5-methyl-4-isoxazolyl)acetate (18b). **13** (1.0 g, 3.6 mmol), dissolved in MeOH (5 mL), was added to a solution of NaOMe in MeOH (10 mL, 0.4 M). The resulting solution was stirred for 1 h at room temperature and evaporated to dryness. Addition of H_2O (10 mL), neutralization with HOAc (pH = 6), and extraction with CH_2Cl_2 (3 × 10 mL) were followed by drying ($MgSO_4$), filtration, and evaporation of the combined organic phases. Subsequent CC [tol–EtOAc (20:1)] afforded **18b** (0.42 g, 54%) as an oil. An analytical sample was ball–tube distilled (155–160 °C; 0.4 mmHg). ^1H NMR ($CDCl_3$): δ 4.66 (s, 1 H), 3.99 (s, 3 H), 3.77 (s, 3 H), 3.40 (s, 3 H), 2.37 (s, 3 H). IR (liquid sandwich): 2950 (m), 1755 (s), 1645 (m), 1525 (s) cm^{-1}. Anal. ($C_9H_{13}NO_5$) C, H, N.

Ethyl (*R,S*)-2-(Methylthio)-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetate (19a). Compound **18a** (0.30 g, 1.2 mmol) was dissolved in a solution of HBr in HOAc (5 mL, 33%) and stirred at room temperature for 72 h. Upon evaporation and recrystallization (cyclohexane) of the residue, compound **19a** (0.18 g, 64%) was obtained, mp 71.5–72.0 °C. ^1H NMR ($CDCl_3$): δ 4.41 (s, 1 H), 4.28 (m, 2 H), 2.40 (s, 3 H), 2.19 (s, 3 H), 1.34 (t, J = 7.1 Hz). IR (KBr): 2980 (m), 2920 (m), 2700 (m), 2600 (m), 1735 (s), 1730 (s), 1650 (m), 1540 (s), 1530 (s) cm^{-1}. Anal. ($C_9H_{13}NO_4S$) H, N; C: calcd, 46.73; found, 46.00. S: calcd, 13.86; found, 13.38.

Methyl (*R,S*)-2-Methoxy-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetate (19b). Compound **18b** (0.55 g, 2.6 mmol) was dissolved in a solution of HBr in HOAc (5 mL, 33%), stirred at room temperature for 7 h, and evaporated. The residue was subjected to CC [tol containing EtOAc (10–25%) and HOAc (1%)], affording starting material **18b** (0.16 g, 29%) and a residue which, after recrystallization (tol), gave **19b** (0.15 g, 29%): mp 92.0–92.5 °C. ^1H NMR ($CDCl_3$): δ 4.72 (s, 1 H), 3.79 (s, 3 H), 3.42 (s, 3 H), 2.37 (s, 3 H). IR (KBr): 3010 (m), 2950 (m), 2815 (m), 2680 (m), 2605 (m), 1755 (s), 1650 (m–s), 1520 (s) cm^{-1}. Anal. ($C_8H_{11}NO_5$) C, H, N.

(*R,S*)-2-(Methylthio)-2-(3-hydroxy-5-methyl-4-isoxazolyl)acetic Acid (8a). Compound **19a** (0.13 g, 0.56 mmol) was dissolved in a 1 M solution of sodium hydroxide (5 mL) and stirred at room temperature for 1 h. After acidification with 4 M hydrochloric acid to pH *ca.* 1, the reaction mixture was extracted with EtOAc (3 × 10 mL). Drying of the combined organic phases ($MgSO_4$) followed by filtration, evaporation, and treatment with light petroleum gave a crystalline residue, which, after recrystallization twice (EtOAc–tol), gave compound **8a** (68 mg, 60%): mp 131.5–132.0 °C. ^1H NMR ($CDCl_3$/DMSO-d_6): δ 5.88 (s, 2 H), 4.39 (s, 1 H), 2.37 (s, 3 H), 2.18 (s, 3 H). IR (KBr): 3100–2500 (several bands, m), 1715 (s), 1705 (s), 1650 (m), 1540 (s), 1520 (s) cm^{-1}. Anal. ($C_7H_9NO_4S$) H, N; C: calcd, 41.36; found, 41.78. S: calcd, 15.77; found, 15.21.

(*R,S*)-2-Methoxy-2-(3-hydroxy-5-methyl-4-isoxazolyl)-acetic Acid (8b). To a solution of compound **19b** (80 mg, 0.40 mmol) in THF (1 mL) was added a 1 M solution of sodium hydroxide (3 mL). After stirring for 1.5 h, the solution was acidified to pH *ca.* 1 using 4 M hydrochloric acid and extracted with EtOAc (4 × 10 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated. Recrystallization of the residue (EtOH–tol) afforded **8b** (38 mg, 51%): mp 97.0–98.0 °C. ^1H NMR (D_2O): δ 4.80 (s, 1 H), 3.33 (s, 3 H), 2.30 (s, 3 H). IR (KBr): 3520–3400 (br, s), 2980–2930 (several bands, m), 2840 (m), 2700 (m), 2590 (m), 1730 (s), 1710 (s), 1655 (s), 1530 (s) cm^{-1}. Anal. ($C_7H_9NO_5 \cdot H_2O$) C, H, N.

(*R,S*)-Spiro[3-methoxy-5,6,7,8-tetrahydro-4*H*-cyclohepta[1,2-*d*]isoxazole-4,5′-hydantoin] (21). 3-Methoxy-5,6,7,8-tetrahydro-4*H*-cycloheptatrieno[1,2-*d*]isoxazol-4-one (**20**)[31] (0.25 g, 1.4 mmol), potasium cyanide (0.46 g, 7.0 mmol), and ammonium carbonate (1.34 g, 14 mmol) dissolved in a 50% aqueous MeOH (5 mL) were refluxed for 20 h. After evaporation of MeOH and addition of H_2O (10 mL), the aqueous solution was extracted with EtOAc (3 × 10 mL). Drying of the combined organic phases ($MgSO_4$), filtration, and evaporation were followed by recrystallization (EtOH–EtOAc) to give compound **21** (210 mg, 61%): mp 210–211 °C. ^1H NMR (DMSO-d_6): δ 10.80 (br s, 1 H), 8.40 (br s, 1 H), 3.80 (s, 3 H), 2.95–2.65 (m, 2 H), 2.00–1.70 (m, 6 H). IR (KBr): 3350 (m), 3140 (m), 3060 (m), 2960 (m), 2930 (m), 2860 (m), 2760 (m), 1780 (s), 1745 (s), 1720 (s), 1640 (m), 1525 (s) cm^{-1}. Anal. ($C_{11}H_{13}N_3O_4$) C, H, N.

(*R,S*)-4-Amino-3-hydroxy-5,6,7,8-tetrahydro-4*H*-cyclohepta[1,2-*d*]isoxazole-4-carboxylic Acid (9). A suspension of compound **21** (45 mg, 0.18 mmol) in 6 M hydrochloric acid (5 mL) was refluxed for 85 h. After evaporation and re-evaporation from H_2O, the residue was subjected to ion exchange chromatography (Dowex 50) using a 1 M aqueous solution of ammonia as an eluent. Ninhydrin-active fractions were evaporated. The residue was dissolved in H_2O, and the solution was made acidic (pH = 4) using 0.1 M hydrochloric acid. Evaporation and recrystallization (H_2O–EtOH) gave **9** (21 mg, 53%): mp 207–208 °C. ^1H NMR (D_2O): δ 2.93–2.72 (m, 2 H), 2.36–2.22 (m, 1 H), 2.18–2.04 (m, 1 H), 1.95–1.72 (m, 4 H). IR (KBr): 3400 (br, s), 3230 (m), 3100–2700 (br, m–s), 1660 (s), 1650 (s), 1635 (s), 1625 (s), 1580 (s), 1510 (s) cm^{-1}. Anal. ($C_9H_{12}N_2O_4 \cdot {}^1/_2H_2O$) C, H, N.

Methyl 2-(Acetylamino)-2-(methoxycarbonyl)-3-[3-(benzoyloxy)-5-methyl-4-isoxazolyl]propionate (23). To a solution of methyl 2-(acetylamino)-2-(methoxycarbonyl)-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate[32] (**22**) (1.51 g, 5.0 mmol) in CH_2Cl_2 (50 mL) were added Et_3N (0.72 mL, 5.2 mmol) and benzoyl chloride (0.58 mL, 5.0 mmol), and the resulting solution was stirred for 30 min. Evaporation followed by CC [tol–EtOAc (2:1)] afforded **23** (1.73 g, 85%). Recrystallization of an analytical sample (EtOAc–Et_2O) gave **23** as cubic

crystals: mp 76.5–78.0 °C. ^{1}H NMR ($CDCl_3$): δ 8.16 (m, 2 H), 7.67 (m, 1 H), 7.52 (m, 2 H), 6.65 (br s, 1 H), 3.68 (s, 6 H), 3.49 (s, 2 H), 2.32 (s, 3 H), 1.85 (s, 3 H). IR (KBr): 3600–3300 (br, m), 3020 (w), 2970 (w), 1770 (br, s), 1695 (m), 1660 (m), 1520 (m), 1500 (m) cm^{-1}. Anal. ($C_{19}H_{20}N_2O_8$) C, H, N.

Methyl 2-(Acetylamino)-2-(methoxycarbonyl)-3-[3-(benzoyloxy)-5-(bromomethyl)-4-isoxazolyl]propionate (24). A mixture of **23** (1.51 g, 3.74 mmol), freshly recrystallized NBS (a total of 0.66 g, 3.71 mmol), and benzoyl peroxide (a total of 30 mg, 0.12 mmol) in CCl_4 (100 mL) was refluxed for 20 h. NBS and benzoyl peroxide were added to the reaction mixture in quarter portions each hour in the beginning of the reaction. Upon cooling, filtration, and evaporation, the residue was subjected to CC [tol–EtOAc (4:1)] which gave crude **24** (0.56 g, 31%) as an oily residue. ^{1}H NMR ($CDCl_3$): δ 8.12 (m, 2 H), 7.66 (m, 1 H), 7.50 (m, 2 H), 6.81 (br s, 1 H), 4.34 (s, 2 H), 3.66 (s, 6 H), 3.54 (s, 2 H), 1.91 (s, 3 H).

***(R,S)*-2-Amino-3-[3-hydroxy-5-(hydroxymethyl)-4-isoxazolyl]propionic Acid (3c).** Crude **24** (0.10 g, 0.21 mmol) was dissolved in 1 M hydrobromic acid (5 mL) and refluxed for 16 h. After evaporation, the residue was dissolved in H_2O (2 mL), treated with activated charcoal, filtered through Celite, and subsequently subjected to ion exchange chromatography (IRA-400) using 1 M HOAc as an eluent. Evaporation of ninhydrin-active fractions and recrystallization (H_2O–EtOH) gave **3c** (19 mg, 45%): mp 204–205 °C dec. ^{1}H NMR (D_2O): δ 4.54 (s, 2 H), 3.91 (t, J = 5.8 Hz, 1 H), 2.94 (d, J = 5.8 Hz, 2 H). IR (KBr): 3500–3400 (br, s), 3300–2900 (several bands, m–s), 1680–1640 (several bands, s), 1590 (m), 1550–1510 (several bands, s) cm^{-1}. Anal. ($C_7H_{10}N_2O_5 \cdot {}^1/_4H_2O$) C, H, N.

Receptor-Binding Assays. The membrane preparations used in all the receptor-binding experiments were prepared according to the method of Ransom and Stec.[36] [^{3}H]CPP-, [^{3}H]-glycine-, [^{3}H]AMPA-, and [^{3}H]kainic acid-binding studies were performed as described in refs 22 and 36–38, respectively. Stimulation of [^{3}H]MK-801 binding was studied using a published procedure[24] with the following modifications: The membrane preparations were washed additionally four times with subsequent freezing. A fixed concentration of 100 nM glycine was used in the assay.

In Vitro Electropharmacology. A rat cortical wedge preparation for testing the depolarizing activity of EAAs described by Harrison and Simmonds[39] was used in a modified version.[40] 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 form 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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Supplementary Material Available: Coordinates for AMAA, **7a**, and **7c** shown in Figure 8 (6 pages). Ordering information is given on any current masthead page.

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