Structural Determinants of AMPA Agonist Activity in Analogues of 2-Amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic Acid: Synthesis and Pharmacology

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We have previously shown that the 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptor agonist, 2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA, **2**), binds to AMPA receptors in a manner different from that of AMPA (**1**) itself and that **2**, in contrast to **1**, also binds to kainic acid receptor sites. To elucidate the structural requirements for selective activation of the site/conformation of AMPA receptors recognized by **2**, a number of isosteric analogues of **2** have now been synthesized and pharmacologically characterized. The compound 2-amino-3-(5-carboxy-3-methoxy-4-isoxazolyl)propionic acid (**3a**) (IC_{50} = 0.11 μM; EC_{50} = 1.2 μM), which is a regioisostere of **2** with a methoxy group substituted for the methyl group, was approximately equipotent with **2** (IC_{50} = 0.020 μM; EC_{50} = 1.0 μM) as an inhibitor of [^{3}H]AMPA binding and as an AMPA agonist, respectively, whereas the corresponding 3-ethoxy analogue **3b** (IC_{50} = 1.0 μM; EC_{50} = 4.8 μM) was slightly weaker. The analogues **3c**−**e**, containing C3 alkoxy groups, were an order of magnitude weaker than **3b**, whereas the additional steric bulk of the alkoxy groups of **3f**−**i** or the presence of an acidic hydroxyl group at the 3-position of the isoxazole ring of **3j** prevented interaction with AMPA receptor sites. The 2-amino-3-(2-alkyl-5-carboxy-3-oxo-4-isoxazolyl)propionic acids **4a**,**b**,**i**, which are regioisosteric analogues of **3a**,**b**,**i**, showed negligible interaction with AMPA recognition sites. Similarly, replacement of the carboxyl group of **3b** by isosteric tetrazolyl or 1,2,4-triazolyl groups to give **5** and **6**, respectively, or conversion of **3b** into analogue **7**, in which the diaminosquaric acid group has been bioisosterically substituted for the α-aminocarboxylic acid unit, provided compounds completely devoid of effect at AMPA receptors. In contrast to the parent compound ACPA (**2**) (IC_{50} = 6.3 μM), none of the analogues described showed detectable inhibitory effect on [^{3}H]kainic acid receptor binding.

Introduction

The central excitatory neurotransmitter effects of (*S*)-glutamic acid [(*S*)-Glu] are mediated by three heterogeneous classes of ionotropic receptors named *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainic acid receptors[1−3] and a number of subtypes of metabotropic receptors.[3,4] These or perhaps distinct subtypes of these receptors have been associated with certain neurologic and psychiatric diseases and are potential therapeutic targets in such diseases.[3−6]

In recent years, much interest has been directed toward the role of AMPA receptors in the mechanisms associated with cognitive functions,[7−9] and enhancement of AMPA receptor functions has been shown to facilitate learning and memory.[3,10,11] Although AMPA receptor agonists may not be used therapeutically due to potential neurotoxicity, these observations have focused interest on the molecular mechanisms of receptor activation and, thus, on the structural basis of AMPA receptor−agonist interactions.

Recently, the recombinant S1−S2 binding domain of an AMPA receptor subtype, cocrystallized with the atypical AMPA agonist kainic acid, was subjected to an X-ray crystallographic structure analysis,[12] and subsequently, a number of AMPA receptor ligands, including AMPA (**1**), have been cocrystallized with the S1−S2 binding domain.[13] Different structural classes of receptor ligand may interact with the AMPA recognition site(s) in a different manner, and an important step toward design of AMPA receptor ligands on a rational basis is mapping of the structural determinants of receptor−ligand interactions.

Extensive structure−activity studies (SARs) on analogues of AMPA (**1**) (Figure 1), in which a variety of alkyl,[14] aryl,[15] or heteroaryl groups[16−18] have been substituted for the methyl group, have shed some light on the structural requirements for activation of AMPA receptors by this class of ligand. These studies have supported the view that, in addition to binding sites for the three charged groups of AMPA agonists, this receptor contains a pocket capable of accommodating lipophilic groups of limited size.[19]

We have recently shown that AMPA (**1**) and the AMPA receptor agonist 2-amino-3-(3-carboxy-5-methyl-

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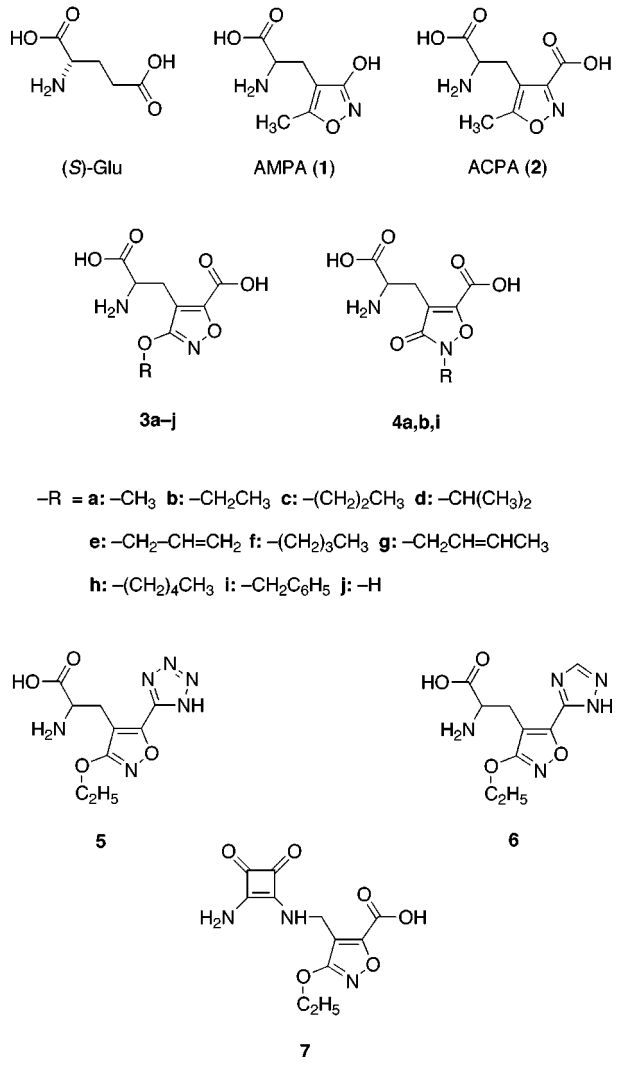


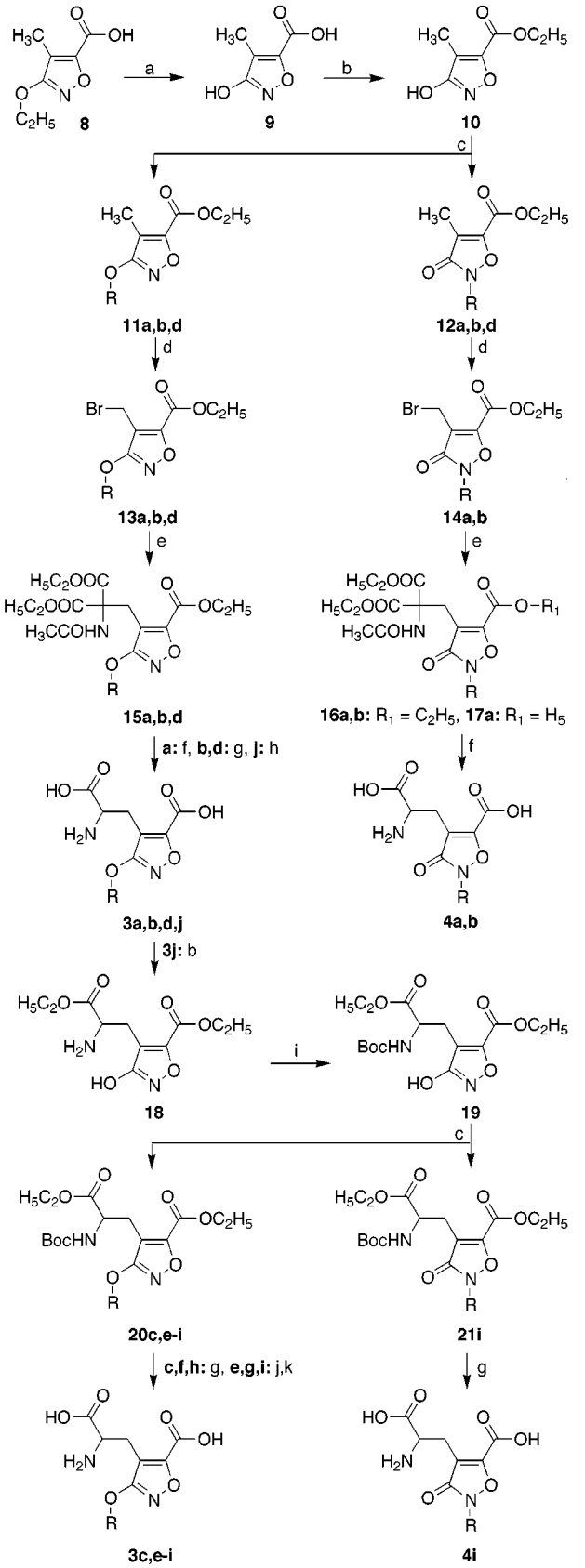
Figure 1. Structures of (*S*)-glutamic acid [(*S*)-Glu], the AMPA receptor agonists 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionic acid (AMPA, **1**) and 2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA, **2**), and the new ACPA analogues and bioisosteres **3a**−**j**, **4a**,**b**,**i**, and **5**−**7**.

4-isoxazolyl)propionic acid (ACPA, **2**)[20] bind to AMPA receptor sites in a dissimilar manner,[21] suggesting that the structural determinants of AMPA receptor activation by **1** and **2** are different. These aspects prompted us to synthesize and pharmacologically characterize a series of compounds (**3a**−**j**, **4a**,**b**,**i**, and **5**−**7**) in which different parts of the molecule of ACPA (**2**) have been isosterically modified (Figure 1).

Results

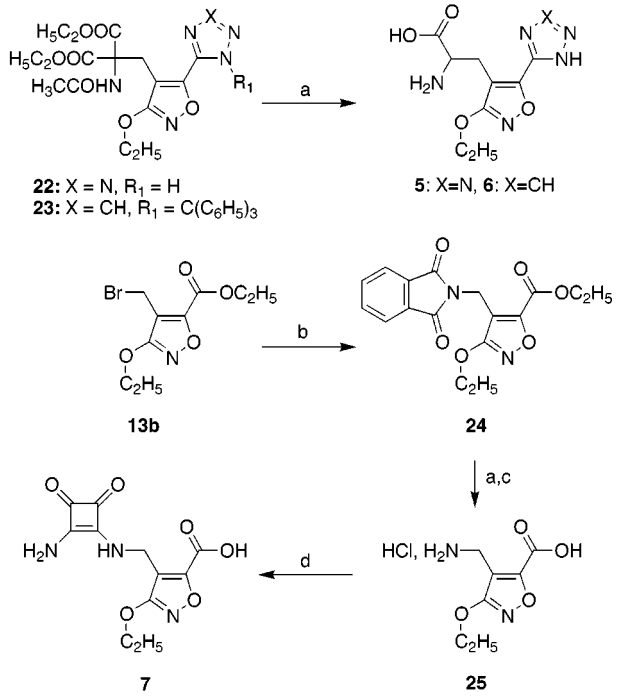
Chemistry. The analogues **3a**−**j**, **4a**,**b**,**i**, and **7** containing a carboxyl group in the 5-position of the isoxazole ring (Figure 1) were synthesized as shown in Schemes 1 and 2, and the analogues **5** and **6** containing heterocyclic carboxyl group bioisosteres were synthesized as outlined in Scheme 2. Two strategies were followed for the synthesis of the former group of analogues, involving introduction of the different *O*- or *N*-alkyl groups at an early stage in the synthetic sequence or as a later synthetic step (Scheme 1). Following the first strategy, the ethoxy group of **8**[16] was hydrolyzed using 47% aqueous HBr to give **9**. The ethyl ester of **9**, compound **10**, was treated with appropriate

Scheme 1[a]



[a] Reagents and conditions: (a) 47% HBr (aq), reflux; (b) C_2H_5OH, HCl, reflux; (c) alkyl halide, K_2CO_3, (DMF, acetone, or EtOH), heat; (d) NBS, dibenzoyl peroxide, CCl_4, reflux; (e) $CH_3CONHCH(COOC_2H_5)_2$, $(CH_3)_3COK$, NMP, rt; (f) 0.5 M HCl (aq), reflux; (g) 1 M HCl (aq), reflux; (h) 47% HBr (aq), reflux; (i) $(Boc)_2O$, TEA, THF, H_2O, rt; (j) 1 M NaOH (aq), EtOH, 90 °C; (k) 1 M HCl (aq), 40 °C.

Scheme 2[a]



[a] Reagents and conditions: (a) 1 M HCl (aq), reflux; (b) potassium phthalimide, DMF, 90 °C; (c) 1 M NaOH (aq), reflux; (d) 3-amino-4-ethoxy-3-cyclobutene-1,2-dione, NaOH, rt.

alkyl halides to give the *O*-alkyl derivatives **11a**,**b**,**d** and the corresponding *N*-alkylated products **12a**,**b**,**d** in different ratios depending on the structure of the alkyl halides. For each alkyl halide, this ratio was strongly dependent on the reaction conditions, in particular the nature of the solvent used. Thus, for example, alkylation of **10** with methyl iodide, using potassium carbonate as base, gave **11a** and **12a** at ratios of 15:1, 2:1, and 1:1 using DMF, acetone, or ethanol, respectively, as solvents (experimental details not given for acetone). The ethoxy derivative **11b** was most conveniently synthesized by esterification of **8**,[16] whereas the *N*-ethyl isomer **12b** was isolated from a 2:1 mixture of **11b** and **12b**, prepared by treatment of **9** with ethyl iodide and potassium carbonate in ethanol.

The intermediates **15a**,**b**,**d** and **16a**,**b** were synthesized by NBS bromination of **11a**,**b**,**d** and **12a**,**b**, respectively, and subsequent treatment with diethyl acetamidomalonate under basic conditions, where **16a** was extensively hydrolyzed to give **17a**. Acid deprotection of these intermediates under different conditions gave the respective target compounds **3a**,**b**,**d**,**j** and **4a**,**b**, which were isolated in the zwitterionic forms.

The second strategy for the synthesis of target compounds containing carboxyl groups in the 5-position of the isoxazole ring was based on **3j** as the starting material (Scheme 1). Compound **3j**, synthesized from **8** via **11b**, **13b**, and **15b**, was converted into the *N*-Boc-protected diester **19**, which was treated with appropriate alkyl halides. Using DMF as solvent, the *O*-alkyl derivatives **20c**,**e**−**h** were obtained in excellent yield (>80%) with formation of the corresponding *N*-alkyl derivatives in trace amounts. Compounds **20i** and **21i** were isolated from a mixture of **20i** and **21i** prepared by treatment of **19** with benzyl bromide using acetone as solvent. Acid deprotection of **20c**,**f**,**h**, and **21i** gave the respective target compounds **3c**,**f**,**h** and **4i**, whereas **20e**,**g**,**i**, which contain unsaturated alkyl substituents or an *O*-benzyl substituent, were deprotected under basic conditions. The deprotections of **20e**,**g** were accompanied by the formation of several byproducts, and purification of **3e**,**g** required ion-exchange chromatographic procedures.

The target compounds **5** and **6** were synthesized from the earlier described intermediates **22** and **23**,[16] respectively (Scheme 2). The squaric acid analogue **7** was synthesized from intermediate **13b**, which via the phthalimide derivative **24** was converted into the amino acid **25**. Under basic conditions, **25** was reacted with 3-amino-4-ethoxy-3-cyclobutene-1,2-dione to give **7**.

In Vitro Pharmacology. The compounds were characterized in receptor binding studies, using rat brain membranes, and electrophysiologically based on the rat cortical wedge preparation for testing depolarizing activity of excitatory amino acid receptor ligands. With the exception of ACPA (**2**) itself, none of the compounds showed significant affinity for [^{3}H]kainic acid binding sites[22] (Table 1). Compounds **3a**,**b** containing a 3-methoxy and a 3-ethoxy group, respectively, were potent inhibitors of [^{3}H]AMPA receptor binding,[23] and these compounds showed agonist potencies comparable with that of **2**. The larger alkoxy groups of **3c**−**e** resulted in reduced affinity for and proportionally reduced agonist activity at AMPA receptors, whereas **3f**−**i** were essentially inactive. Similarly, compound **3j** containing an acidic hydroxy group in the 3-position of the isoxazole ring did not interact with AMPA receptors. The *N*-alkylated isomers of **3a**,**b**, compounds **4a**,**b**, were totally inactive at AMPA receptors, whereas the *N*-benzyl analogue **4i** was a very weak inhibitor of NMDA-induced depolarization (Table 1). Among the compounds tested, only **7** showed weak affinity for NMDA receptor sites labeled by tritiated 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ([^{3}H]CPP),[24] and this affinity turned out to reflect a marginally weak NMDA antagonist effect.

Discussion

Like AMPA (**1**), ACPA (**2**) contains an isoxazole ring, but whereas **1** is a 3-isoxazolol bioisostere of Glu, compound **2** is an aminodicarboxylic acid homologue of Glu (Figure 1). Despite these quite profound dissimilarities, both **1** and **2** are potent AMPA receptor agonists, but in contrast to **1**, compound **2** also displays significant affinity for kainic acid receptor sites (Table 1).

In a number of cases, homologation of heterocyclic Glu analogues showing AMPA agonist activity has been shown to provide metabotropic Glu receptor ligands.[3] Although ACPA (**2**) is a homologue of Glu, neither enantiomer of **2** shows detectable affinity for metabotropic Glu receptor subtypes (H. Bräuner-Osborne and T. N. Johansen, unpublished results). Consequently, none of the ACPA analogues **3**−**7** described here were tested for effect at metabotropic Glu receptors.

On the basis of quite extensive SAR studies on **1** and structurally related compounds, the methyl group of **1** appears to fit into a cavity at the AMPA recognition site capable of accommodating lipophilic groups of limited size.[14−19] The aim of the present project was to elucidate the structural determinants of AMPA agonist activity

Table 1. Receptor Binding Data and AMPA Agonist or NMDA Antagonist Effects (values ± SEM, $n = 3-7$)

	receptor binding IC_{50} (μM)			electropharmacology	
compound	[^{3}H]AMPA	[^{3}H]kainic acid	[^{3}H]CPP	agonism EC_{50} (μM)	antagonism IC_{50} (μM)
AMPA (**1**)	0.040 ± 0.014[a]	> 100[a]	> 100[a]	3.5 ± 0.2[a]	
ACPA (**2**)	0.020 ± 0.012[b]	6.3[b]	Nd	1.0 ± 0.1[b]	
3a	0.11 ± 0.016	> 100	> 100	1.2 ± 0.2	
3b	1.0 ± 0.14	> 100	> 100	4.8 ± 1.8	
3c	14 ± 3	> 100	> 100	79 ± 10	
3d	5.5 ± 0.58	> 100	> 100	37 ± 5	
3e	7.2 ± 0.54	> 100	> 100	40 ± 6	
3f	> 100	> 100	> 100	406 ± 52	
3g	> 100	> 100	> 100	500	
3h	> 100	> 100	> 100	1000	
3i	> 100	> 100	> 100	> 1000	
3j	> 100	> 100	> 100	> 1000	
4a	> 100	> 100	> 100	> 1000	
4b	> 100	> 100	> 100	> 500	
4i	> 100	> 100	> 100		704 ± 140
5	> 100	> 100	> 100	2000	
6	> 100	> 100	> 100	> 1000	
7	> 100	> 100	76 ± 14		603 ± 17

[a] Reference 17. [b] Reference 20.

in analogues of **2** and, furthermore, to develop analogues of **2** devoid of the kainic acid receptor affinity. All of the analogues of **2** synthesized contain an alanine side chain with a 3-oxygenated isoxazole ring structure (Figure 1).

Interestingly, none of the compounds synthesized showed detectable affinity for kainic acid receptor sites (Table 1). In analogy with the observations for analogues of **1** containing different alkyl substituents, the AMPA recognition site was shown to impose constraints on the size of the alkoxy substituents of the analogues of **2**. Whereas **3a**,**b** containing a 3-methoxy and a 3-ethoxy group, respectively, are potent AMPA receptor agonists, **3c**−**e** containing C3 alkoxy groups are markedly weaker, and incorporation of one or more additional C atoms into the alkoxy groups essentially abolished activity. The inactivity of the *N*-alkyl isomers of **3a**,**b**, compounds **4a**,**b**, seems to indicate that only substituents of limited size and proper orientation can be accommodated by the proposed lipophilic pocket, which does not recognize the 3-hydroxy group of **3j**, which is deprotonated at pH 7.

A similar degree of structural specificity was exhibited by the site of the AMPA receptor which binds the heteroaromatic carboxyl groups of **2** and analogues such as **3a**,**b**. Thus, compounds **5** and **6**, in which a tetrazolyl and a 1,2,4-triazolyl group, respectively, have been substituted for the carboxyl group of **3b** (Figure 1), were totally inactive. Since the tetrazolyl group of **5** is markedly more acidic than the 1,2,4-triazolyl group of **6**,[16] steric rather than, or in addition to, pK_a effects of these azole rings seem to prevent interaction with the AMPA recognition site. Compound **7** is not recognized by AMPA or kainic acid receptor sites. The very weak NMDA antagonist effect of **7** is, however, consistent with the reported NMDA antagonist effect of certain amino acids, in which the α-amino acid functionality has been bioisosterically replaced by a 3,4-diamino-3-cyclobutene-1,2-dione unit.[25]

In continuation of the successful X-ray crystallographic analysis of the recombinant S1−S2 binding domain of the AMPA receptor cocrystallized with the atypical AMPA agonist, kainic acid,[12] and the successful crystallization of this binding domain bound to AMPA (**1**) and structurally related potent AMPA agonists,[3,13] attempts will be made to subject ACPA (**2**) and, for example, **3a** to similar biostructural studies. Studies along these lines may pave the way for future design of AMPA receptor ligands on a rational basis.

Experimental Section

Chemistry. All reactions were performed under nitrogen. Reagents were purchased commercially and used without purification. Melting points were determined in capillary tubes (Büchi 535 apparatus) and are uncorrected. All evaporations of solvents were performed in vacuo at 15 mmHg. ^{1}H NMR was recorded on a Bruker AC 250 MHz spectrometer. Unless otherwise stated, chemical shift values (δ) are expressed in ppm relative to TMS. The following abbreviations are used for multiplicity of NMR signals: br = broad, s = singlet, d = doublet, t = triplet, q = quartet, h = heptet, dd = double doublet, ddd = double doublet of doublets, dt = doublet of triplet, dq = doublet of quartet, tdd = triplets of double doublets, m = multiplet. NMR signals corresponding to acidic protons are omitted. Mass spectra were obtained on a Quattro MS−MS system from VG Biotech, Fisons Instruments. The MS−MS system was connected to an HP 1050 modular HPLC system. A volume of 20−50 μL of the sample (10 μg/mL) dissolved in a mixture of acetonitrile/water/acetic acid (250:250:1) or in a mixture of acetonitrile/water/aqueous ammonia (25%) (25:25:1) was introduced via the autosampler at a flow of 30 μL/min into the electrospray source. Spectra were recorded for all target compounds and for selected key intermediates at standard operating conditions to obtain molecular weight information ($(M + H)^+$ or $(M - H)^-$). The background was subtracted. Compounds containing the 3-isoxazolol moiety were visualized on TLC plates (Merck silica gel 60 F_{254}) using UV light and an $FeCl_3$ spraying reagent (yellow color). Compounds containing amino groups were visualized using a ninhydrin spraying reagent. Microanalyses were performed by

the Lundbeck Analytical Department, and results obtained were within ±0.4% of the theoretical values if not otherwise stated.

3-Hydroxy-4-methylisoxazole-5-carboxylic Acid (9). A mixture of **8**[16] (15 g, 88 mmol) and 47% aqueous HBr (150 mL) was heated under reflux for 6 h. The solution was cooled, and crystalline **9** was collected by filtration (8.7 g, 69%): mp 257−259 °C. Water (100 mL) was added to the residual solution, which was left after filtration, and the aqueous phase was extracted with Et_2O (6 × 400 mL). The combined organic phase was washed with brine (100 mL), dried ($MgSO_4$), and concentrated to give crude **9** (3.0 g, 24%): ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3H); MS ($(M - H)^-$) *m*/*z* 142. Anal. ($C_5H_5NO_4$) C, H, N. A mixture of the two crops was used in the next step.

Ethyl 3-Hydroxy-4-methylisoxazole-5-carboxylate (10). A mixture of **9** (6.0 g, 42 mmol) and a saturated solution of HCl in EtOH (110 mL) was heated under reflux for 4 h. The resulting solution was concentrated, and the residue was dissolved in EtOAc. The organic phase was dried ($MgSO_4$) and concentrated to give crude **10** (7.2 g, 100%). A small sample was recrystallized (EtOAc/heptane) to give colorless crystals: mp 133−134 °C; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 2.20 (s, 3H), 4.44 (q, 2H). Anal. ($C_7H_9NO_4$) C, H, N. The crude product was used in the next step without further purification.

Ethyl 3-Methoxy-4-methylisoxazole-5-carboxylate (11a). A mixture of **10** (3.0 g, 17.5 mmol), methyl iodide (1.1 mL, 17.5 mmol), K_2CO_3 (4.8 g, 35.1 mmol), and DMF (40 mL) was heated at 40 °C for 1 h. The mixture was poured onto an ice/water mixture (100 mL), and the aqueous phase was extracted with Et_2O (3 × 100 mL). The combined organic phase was washed with water (2 × 50 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated (2.4 g, 74%; according to ^{1}H NMR, a 15:1 mixture of **11a** and **12a** was obtained). The residue was subjected to flash chromatography [silica gel, eluent: CH_2Cl_2/Et_2O (9:1)] affording crude **11a** as a yellow oil (1.4 g, 43%): ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H), 2.15 (s, 3H), 4.05 (s, 3H), 4.41 (q, 2H). No attempt was made to isolate compound **12a** from this mixture. The crude product was used in the next step without further purification.

Ethyl 3-Methoxy-4-methylisoxazole-5-carboxylate (11a) and Ethyl 2,3-Dihydro-2,4-dimethyl-3-oxoisoxazole-5-carboxylate (12a). A mixture of **10** (2.0 g, 11.6 mmol), K_2CO_3 (4.0 g, 29 mmol), and EtOH (50 mL) was heated at 40 °C for 1 h followed by the addition of methyl iodide (0.8 mL, 13 mmol). The resulting mixture was heated at 40 °C for 25 h, and during this time additional methyl iodide (0.8 mL, 13 mmol) was added three times. The mixture was filtered and concentrated (according to ^{1}H NMR, a 1:1 mixture of **11a** and **12a** was obtained). Flash chromatography [silica gel, eluent: CH_2Cl_2/Et_2O (9:1 then 1:1)] gave compound **11a** as a yellow oil (0.40 g, 18%; for analytical data see above) and compound **12a** (0.45 g, 21%). A small sample of **12a** was recrystallized (EtOAc/heptane) to give colorless crystals: mp 64−65 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (t, 3H), 2.15 (s, 3H), 3.60 (s, 3H), 4.42 (q, 2H). Anal. ($C_8H_{11}NO_4$) C, H, N. Crude **12a** was used in the next step without further purification.

Ethyl 3-Ethoxy-4-methylisoxazole-5-carboxylate (11b). A mixture of **8**[16] (3.6 g, 21 mmol) and a saturated solution of HCl in EtOH (100 mL) was heated under reflux for 2 h. The mixture was concentrated, and the residue was dissolved in CH_2Cl_2 (200 mL), dried ($MgSO_4$), and concentrated. Flash chromatography [silica gel, eluent: heptane/EtOAc (4:1)] gave compound **11b** as a colorless solid (4.0 g, 95%): mp 34−36 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H), 1.45 (t, 3H), 2.15 (s, 3H), 4.36 (q, 2H), 4.40 (q, 2H). Anal. ($C_9H_{13}NO_4$) C, H, N.

Ethyl 3-Ethoxy-4-methylisoxazole-5-carboxylate (11b) and Ethyl 2,3-Dihydro-2-ethyl-4-methyl-3-oxoisoxazole-5-carboxylate (12b). A mixture of **9** (3.7 g, 26 mmol), K_2CO_3 (5.8 g, 42 mmol), ethyl iodide (2.8 mL, 35 mmol), and EtOH (70 mL) was heated under reflux for a total of 68 h. Additional ethyl iodide (2.8 mL, 35 mmol) was added after 16, 24, and 44 h, and additional K_2CO_3 (5.8 g, 42 mmol) was added after 44 h. The mixture was filtered and concentrated (according to ^{1}H NMR, a 2:1 mixture of **11b** and **12b** was obtained). The residue was subjected to flash chromatography [silica gel, eluent: heptane/EtOAc (2:1 then 1:2)] to give compound **11b** as a colorless solid (2.6 g, 47%; for analytical data see above) and crude **12b** which was crystallized (heptane) to give **12b** as yellow crystals (0.64 g, 12%): mp 65−67 °C; ^{1}H NMR ($CDCl_3$) δ 1.36 (t, 3H), 1.41 (t, 3H), 2.15 (s, 3H), 4.15 (q, 2H), 4.42 (q, 2H). Anal. ($C_9H_{13}NO_4$) C, H, N.

Ethyl 3-Isopropoxy-4-methylisoxazole-5-carboxylate (11d) and Ethyl 2,3-Dihydro-2-isopropyl-4-methyl-3-oxoisoxazole-5-carboxylate (12d). A mixture of **10** (3.4 g, 20 mmol), K_2CO_3 (5.5 g, 40 mmol), and acetone (75 mL) was heated under reflux for a total of 40 h. 2-Bromopropane (3.7 mL, 40 mL) was added after 1, 4.5, 20, and 26 h, and additional K_2CO_3 (2.8 g, 20 mmol) was added after 26 h. The mixture was cooled, filtered, and concentrated. Flash chromatography [silica gel, eluent: EtOAc/heptane (1:2)] gave crude **11d** as a yellow oil (3.2 g, 76%): ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 6H), 1.40 (t, 3H), 2.13 (s, 3H), 4.40 (q, 2H), 4.99 (h, 1H). The crude product was used in the next step without further purification. Further elution furnished compound **12d** as a brown oil (0.25 g, 6%). A small sample was crystallized (heptane) to give colorless crystals: mp 67−68 °C; ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 6H), 1.41 (t, 3H), 2.13 (s, 3H), 4.41 (q, 2H), 4.73 (h, 1H). Anal. ($C_{10}H_{15}NO_4$) C, H, N.

Ethyl 4-(Bromomethyl)-3-methoxyisoxazole-5-carboxylate (13a). A mixture of **11a** (1.3 g, 7.0 mmol), NBS (1.4 g, 7.9 mmol), dibenzoyl peroxide (catalytic amount), and CCl_4 (40 mL) was heated under reflux for 10 h. The mixture was cooled to room temperature, filtered, and concentrated to give crude **13a** as a yellow oil (1.8 g, 97%): ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H), 4.10 (s, 3H), 4.46 (q, 2H), 4.48 (s, 2H). The crude product was used in the next step without further purification.

Compounds **13b**,**d** and **14a** were prepared in a similar manner as described for **13a**.

Ethyl 4-(Bromomethyl)-3-ethoxyisoxazole-5-carboxylate (13b). Yield: 5.3 g, 100%; ^{1}H NMR ($CDCl_3$) δ 1.44 (t, 3H), 1.47 (t, 3H), 4.44 (q, 2H), 4.46 (q, 2H), 4.50 (s, 2H). The crude product was used in the next step without further purification.

Ethyl 4-(Bromomethyl)-3-isopropoxyisoxazole-5-carboxylate (13d). Yield: 4.0 g, 100%; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3H), 1.44 (d, 6H), 4.46 (q, 2H), 4.47 (s, 2H), 5.04 (h, 1H). The crude product was used in the next step without further purification.

Ethyl 4-(Bromomethyl)-2,3-dihydro-2-methyl-3-oxoisoxazole-5-carboxylate (14a). Yield: 1.0 g, 100%; ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H), 3.64 (s, 3H), 4.46 (s, 2H), 4.48 (q, 2H). The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[5-(ethoxycarbonyl)-3-methoxy-4-isoxazolyl]propionate (15a). A mixture of diethyl acetamidomalonate (1.6 g, 7.4 mmol) and potassium *tert*-butoxide (0.9 g, 8.0 mmol) in *N*-methylpyrrolidin-2-one (NMP) (30 mL) was stirred at room temperature for 30 min followed by the addition of **13a** (1.8 g, 6.8 mmol) in NMP (10 mL) (temperature 22−28 °C). The resulting mixture was stirred at room temperature for 1.5 h, and the mixture was poured onto an ice/water mixture (100 mL). The aqueous phase was extracted with EtOAc (3 × 150 mL), and the combined organic phase was washed with an aqueous solution of potassium *tert*-butoxide in water, water (100 mL), and brine (100 mL), dried ($MgSO_4$), and concentrated. Flash chromatography [silica gel, eluent: EtOAc/heptane (1:1)] afforded crude **15a** (1.8 g, 66%). A small sample was recrystallized (EtOAc/heptane) to give colorless crystals: mp 78−80 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 6H), 1.39 (t, 3H), 1.95 (s, 3H), 3.68 (s, 2H), 4.00 (s, 3H), 4.10−4.34 (m, 4H), 4.37 (q, 2H), 6.50 (br s, 1H). Anal. ($C_{17}H_{24}N_2O_9$) C, H, N. The crude product was used in the next step without further purification.

Compounds **15b**,**d**, **16a**, and **17** were prepared in a similar manner as described for **15a**.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[3-ethoxy-5-(ethoxycarbonyl)-4-isoxazolyl]propionate (15b). Flash chromatography [silica gel, eluent: EtOAc/heptane (1:2 then 2:1)] afforded crude **15b** as a yellow oil (3.0 g, 67%). A small

sample was crystallized (2-propanol) to give colorless crystals: mp 94−95 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 6H), 1.39 (t, 3H), 1.41 (t, 3H), 1.95 (s, 3H), 3.68 (s, 2H), 4.08−4.33 (m, 4H), 4.33 (q, 2H), 4.36 (q, 2H), 6.49 (br s, 1H). Anal. ($C_{18}H_{26}N_2O_9$) C, H, N. The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[5-(ethoxycarbonyl)-3-isopropoxy-4-isoxazolyl]propionate (15d). Flash chromatography [silica gel, eluent: EtOAc/heptane (1:2 then 1:1)] gave crude **15d** (1.6 g, 73%). A small sample was recrystallized (2-propanol) to give colorless crystals: mp 73−74 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 6H), 1.38 (d, 6H), 1.39 (t, 3H), 1.96 (s, 3H), 3.66 (s, 2H), 4.07−4.37 (m, 4H), 4.36 (q, 2H), 4.95 (h, 1H), 6.50 (br s, 1H). Anal. ($C_{19}H_{28}N_2O_9$) C, H, N. The crude product was used in the next step without further purification.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[5-(ethoxycarbonyl)-2,3-dihydro-2-methyl-3-oxo-4-isoxazolyl]propionate (16a) and Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-(5-carboxy-2,3-dihydro-2-methyl-3-oxo-4-isoxazolyl)propionate (17a). Flash chromatography [silica gel, eluent: EtOAc/heptane (3:1 then 5:1)] followed by crystallization (EtOAc/heptane) gave compound **16a** as colorless crystals (0.10 g, 6%): mp 121−123 °C; ^{1}H NMR ($CDCl_3$) δ 1.28 (t, 6H), 1.39 (t, 3H), 1.95 (s, 3H), 3.57 (s, 3H), 3.65 (s, 2H), 4.18−4.33 (m, 4H), 4.39 (q, 2H), 6.84 (br s, 1H). Anal. ($C_{17}H_{24}N_2O_9$) C, H, N. The potassium *tert*-butoxide aqueous solution, after extracted by EtOAc to give **16a**, was acidified with concentrated aqueous HCl and extracted with EtOAc (3 × 150 mL). The combined organic phase was washed with brine, dried ($MgSO_4$), and concentrated. The residue was crystallized (EtOH/Et_2O) to give compound **17a** as colorless crystals (0.3 g, 20%): mp 203−205 °C; ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 1.27 (t, 3H), 1.97 (s, 3H), 3.56 (s, 3H), 3.60 (s, 2H), 4.15−4.31 (m, 4H), 7.09 (br s, 1H). Anal. ($C_{15}H_{20}N_2O_9$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[5-(ethoxycarbonyl)-2-ethyl-2,3-dihydro-3-oxo-4-isoxazolyl]propionate (16b). A mixture of **12b** (0.56 g, 2.8 mmol), NBS (0.55 g, 3.1 mmol), dibenzoyl peroxide (catalytic amount), and CCl_4 (40 mL) was heated under reflux for 4 h. The mixture was cooled to room temperature, filtered, and concentrated to give crude **14b** as a yellow oil (0.8 g, 100%). The crude product was dissolved in NMP (10 mL) and subsequently added to a mixture of diethyl acetamidomalonate (0.67 g, 3.1 mmol) and potassium *tert*-butoxide (0.38 g, 3.4 mmol) in NMP (20 mL) (temperature 22−28 °C). The reaction mixture was stirred at room temperature for 1 h and poured onto an ice/water mixture (100 mL). The aqueous phase was extracted with EtOAc (4 × 75 mL), and the combined organic phase was washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography [silica gel, eluent: EtOAc/heptane (3:1)] followed by crystallization (EtOAc/heptane) gave compound **16b** as colorless crystals (88 mg, 8%): mp 113−114 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 6H), 1.34 (t, 3H), 1.40 (t, 3H), 1.96 (s, 3H), 3.64 (s, 3H), 3.99 (q, 2H), 4.16−4.36 (m, 4H), 4.39 (q, 2H), 6.86 (br s, 1H). Anal. ($C_{18}H_{26}N_2O_9$) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-3-methoxy-4-isoxazolyl)-propionic Acid Hydrate (3a). A suspension of **15a** (1.2 g, 3.0 mmol) in 0.5 M HCl (100 mL) was heated under reflux for 48 h. The mixture was cooled to room temperature, washed with CH_2Cl_2 (100 mL) and Et_2O (2 × 100 mL), filtered, and concentrated. The residue was dissolved in water (5 mL), and pH of the solution was adjusted to about 3 by addition of NaOH (0.1 and 1 M). The aqueous phase was reduced to about 2 mL, and the precipitate that formed was collected by filtration. The crude product was stirred in water (2 mL) at room temperature for 24 h affording **3a** (70 mg, 10%): mp 222−225 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.88 (dd, 1H), 3.01 (dd, 1H), 3.85−3.96 (m, 1H), 3.90 (s, 3H); MS ((M + H)$^+$) *m*/*z* 231. Anal. ($C_8H_{10}N_2O_6$·0.25H_2O) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-3-ethoxy-4-isoxazolyl)propionic Acid (3b). A suspension of **15b** (2.5 g, 6 mmol) in 1 M HCl (200 mL) was heated under reflux for 20 h. The reaction mixture was cooled to room temperature, washed with Et_2O and concentrated. The residue was triturated with Et_2O followed by the addition of water (10 mL). Compound **3b** was obtained as colorless crystals (1.0 g, 68%): mp 238−240 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.34 (t, 3H), 2.90 (dd, 1H), 3.03 (dd, 1H), 3.96 (dd, 1H), 4.23 (q, 2H); MS ((M + H)$^+$) *m*/*z* 245. Anal. ($C_9H_{12}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-3-isopropoxy-4-isoxazolyl)-propionic Acid (3d). A suspension of **15d** (1.15 g, 2.7 mmol) in 1 M HCl (100 mL) was heated under reflux for 24 h. The mixture was cooled to room temperature and washed with EtOAc (3 × 100 mL). The aqueous phase was filtered and concentrated. The residue was dissolved in water (10 mL), and the precipitate that formed was stirred at room temperature for 16 h affording **3d** (0.46 g, 66%): mp 242−243 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.32 (dd, 6H), 2.88 (dd, 1H), 3.01 (dd, 1H), 3.96 (dd, 1H), 4.79 (h, 1H); MS ((M + H)$^+$) *m*/*z* 259. Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-3-hydroxy-4-isoxazolyl)propionic Acid Hydrate (3j). A suspension of **15b** (1.1 g, 2.7 mmol) in 47% HBr (aq) (5 mL) was heated under reflux for 1 h. The solution was cooled to room temperature and washed with CH_2Cl_2 (3 × 30 mL), concentrated and triturated with Et_2O. The residue was dissolved in water, and pH of the solution was adjusted to about 3.5 by addition of 0.1 M NaOH. Compound **3j** was obtained as colorless crystals (0.34 g, 57%): mp 175−177 °C; ^{1}H NMR (DMSO-d_6) δ 3.00 (d, 2H), 3.88 (t, 1H); MS ((M + H)$^+$) *m*/*z* 217. Anal. ($C_7H_8N_2O_6$·0.25H_2O) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-2,3-dihydro-2-methyl-3-oxo-4-isoxazolyl)propionic Acid Hydrate (4a). A suspension of **16a** (0.05 g, 0.1 mmol), **17a** (0.30 g, 0.8 mmol), and 1.0 M HCl (55 mL) was heated under reflux for 16 h. The mixture was cooled to room temperature, washed with EtOAc (3 × 50 mL), and concentrated. The residue was dissolved in water (4 drops) and EtOH (5 mL), and pH of the solution was adjusted to about 3 by addition of TEA. The precipitate that formed was collected by filtration to give **4a** as colorless crystals (0.07 g, 72%): mp 211−212 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.87 (dd, 1H), 2.97 (dd, 1H), 3.43 (s, 3H), 3.92 (dd, 1H); MS ((M + H)$^+$) *m*/*z* 231. Anal. ($C_8H_{10}N_2O_6$·0.25H_2O) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-2-ethyl-2,3-dihydro-3-oxo-4-isoxazolyl)propionic Acid Monohydrate (4b). A suspension of **16b** (60 mg, 0.1 mmol) and 1.0 M HCl (30 mL) was boiled under reflux for 18 h. The mixture was cooled to room temperature, washed with EtOAc (3 × 30 mL), and concentrated. The residue was dissolved in water (2 drops) and EtOH (1 mL), and pH of the solution was adjusted to about 3 by addition of TEA. The resulting mixture was concentrated, and the residue recrystallized twice (EtOH) to yield **4b** as colorless crystals (5 mg, 13%): ^{1}H NMR (D_2O, 1,4-dioxane δ 3.70) δ 1.28 (t, 3H), 3.19 (d, 2H), 4.01 (q, 2H), 4.18 (t, 1H); MS ((M + H)$^+$) *m*/*z* 245. Anal. ($C_9H_{12}N_2O_6$·H_2O) C, N; H: calcd, 5.38; found, 4.74.

Ethyl (*RS*)-2-Amino-3-[5-(ethoxycarbonyl)-3-hydroxy-4-isoxazolyl]propionate (18). A mixture of **3j** (3.5 g, 11.8 mmol) and a saturated solution of HCl in EtOH (50 mL) was heated under reflux for 2.5 h and evaporated to dryness to give crude **18** (4.15 g, 100%): ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3H), 1.34 (t, 3H), 3.20 (dd, 1H), 3.32 (dd, 1H), 4.18 (q, 2H), 4.36−4.42 (m, 3H). The crude product was used in the next step without further purification.

Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[5-(ethoxycarbonyl)-3-hydroxy-4-isoxazolyl]propionate (19). To a solution of **18** (19.0 g, 70.0 mmol) in THF (175 mL) and water (175 mL) was added a solution of $(Boc)_2O$ (19.5 g, 89.2 mmol) and TEA (34 mL, 243 mmol) in THF (50 mL). The resulting solution was stirred at room temperature for 3 days and then concentrated. To the residue was added water (250 mL), and pH of the aqueous phase was adjusted to 3 with 1 M HCl. The aqueous phase was extracted with EtOAc (3 × 400 mL), and the combined organic phase was dried ($MgSO_4$) and evaporated to dryness. The residue was subjected to flash chromatography [silica gel, eluent: EtOAc/heptane/HOAc (1:5:1%)] to give crude **19** (20.7 g, 79%). A sample was recrystal-

lized (heptane/EtOAc): mp 91−93 °C; ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H), 1.40 (s, 9H), 1.42 (t, 3H), 3.02 (dd, 1H), 3.25 (dd, 1H), 4.14−4.25 (m, 2H), 4.43 (q, 2H), 4.40−4.50 (m, 1H), 5.40 (br s, 1H). Anal. ($C_{16}H_{24}N_2O_8$) C, H, N. The crude product was used in the next step without further purification.

General Procedure for the Preparation of Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[3-alkoxy-5-(ethoxycarbonyl)-4-isoxazolyl]propionates 20c,e−h. A solution of **19** and K_2CO_3 in DMF (40 mL) was stirred at 40 °C for 1 h followed by addition of alkyl bromide, and the resulting mixture was stirred at 40 °C for an additional 4 h. The mixture was then poured onto an ice/water mixture (100 mL), and the aqueous phase was extracted with Et_2O (3 × 100 mL). The combined organic phase was washed with water (2 × 50 mL) and brine (50 mL), dried ($MgSO_4$), and concentrated. Flash chromatography [silica gel, eluent: EtOAc/heptane (1:7)] gave compounds **20c**,**e**−**h** as oils, which were used in the next step without further purification.

Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[5-(ethoxycarbonyl)-3-propoxy-4-isoxazolyl]propionate (20c). Compound **19** (1.4 g, 3.8 mmol), K_2CO_3 (1.0 g, 7.5 mmol), and propyl bromide (0.69 g, 5.6 mmol) were used to prepare **20c** (1.4 g, 92%): ^{1}H NMR ($CDCl_3$) δ 1.04 (t, 3H), 1.26 (t, 3H), 1.38 (s, 9H), 1.42 (t, 3H), 1.85 (sextet, 2H), 2.95 (dd, 1H), 3.13 (dd, 1H), 4.17 (q, 2H), 4.29 (t, 2H), 4.43 (q, 2H), 4.40−4.50 (m, 1H), 5.25 (br d, 1H).

Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[3-allyloxy-5-(ethoxycarbonyl)-4-isoxazolyl]propionate (20e). Compound **19** (1.0 g, 2.7 mmol), K_2CO_3 (0.75 g, 5.4 mmol), and allyl bromide (0.49 g, 4.0 mmol) were used to prepare **20e** (1.0 g, 90%): ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H), 1.38 (s, 9H), 1.42 (t, 3H), 2.98 (dd, 1H), 3.15 (dd, 1H), 4.18 (q, 2H), 4.43 (q, 2H), 4.40−4.50 (m, 1H), 4.82 (dt, 2H), 5.22 (br d, 1H), 5.32 (ddd, 1H), 5.48 (ddd, 1H), 6.09 (tdd, 1H).

Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[3-butoxy-5-(ethoxycarbonyl)-4-isoxazolyl]propionate (20f). Compound **19** (1.0 g, 2.8 mmol), K_2CO_3 (0.74 g, 5.4 mmol), and butyl bromide (0.55 g, 4.0 mmol) were used to prepare **20f** (1.1 g, 95%): ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3H), 1.27 (t, 3H), 1.35 (s, 9H), 1.42 (t, 3H), 1.50 (sextet, 2H), 1.82 (quintet, 2H), 2.98 (dd, 1H), 3.12 (dd, 1H), 4.18 (q, 2H), 4.35 (t, 2H), 4.43 (q, 2H), 4.45−4.51 (m, 1H), 5.22 (br d, 1H).

Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[5-(ethoxycarbonyl)-3-(*trans*-2-butenyloxy)-4-isoxazolyl]-propionate (20g). Compound **19** (1.4 g, 3.8 mmol), K_2CO_3 (1.0 g, 7.5 mmol), and crotyl bromide (0.90 g, 5.6 mmol) were used to prepare **20g** (0.80 g, 50%): ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3H), 1.38 (s, 9H), 1.42 (t, 3H), 1.76 (d, 3H), 2.97 (dd, 1H), 3.14 (dd, 1H), 4.18 (q, 2H), 4.43 (q, 2H), 4.40−4.50 (m, 1H), 4.75 (d, 2H), 5.22 (br d, 1H), 5.76 (dt, 1H), 5.89 (dq, 1H).

Ethyl (*RS*)-2-[(*tert*-Butyloxycarbonyl)amino]-3-[5-(ethoxycarbonyl)-3-pentyloxy-4-isoxazolyl]propionate (20h). **19** (1.7 g, 4.6 mmol), K_2CO_3 (1.3 g, 9.3 mmol), and pentyl bromide (1.1 g, 7.0 mmol) were used to prepared **20h** (1.8 g, 76%): ^{1}H NMR ($CDCl_3$) δ 0.94 (t, 3H), 1.26 (t, 3H), 1.38 (s, 9H), 1.39−1.45 (m, 7H), 1.84 (quintet, 2H), 2.97 (dd, 1H), 3.11 (dd, 1H), 4.18 (q, 2H), 4.33 (t, 2H), 4.43 (q, 2H), 4.40−4.50 (m, 1H), 5.22 (br d, 1H).

Ethyl (*RS*)-2-[(*tert*-Butoxycarbonyl)amino]-3-[3-benzyloxy-5-(ethoxycarbonyl)-4-isoxazolyl]propionate (20i) and Ethyl (*RS*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(2-benzyl-5-ethoxycarbonyl-2,3-dihydro-3-oxo-4-isoxazolyl)propionate (21i). A mixture of **19** (3.2 g, 8.6 mmol), K_2CO_3 (2.4 g, 17.2 mmol), and acetone (40 mL) was heated to reflux temperature followed by addition of benzyl bromide (2.2 g, 12.9 mmol). The resulting mixture was heated under reflux for 1.5 h and concentrated. The residue was subjected to flash chromatography [silica gel, eluent: EtOAc/heptane (1:2)] to give **20i** (1.64 g, 41%): ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H), 1.39 (s, 9H), 1.43 (t, 3H), 2.96 (dd, 1H), 3.19 (dd, 1H), 4.12 (q, 2H), 4.35−4.60 (m, 1H), 4.45 (q, 2H), 5.20 (br d, 1H), 5.35 (d, 2H), 7.32−7.55 (m, 5H). And **21i** (0.7 g, 18%): ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H), 1.40 (s, 9H), 1.41 (t, 3H), 2.99−3.18 (m, 2H), 4.18 (q, 2H), 4.40 (q, 2H), 4.46−4.60 (m, 1H), 5.13 (d, 2H), 5.72 (br d, 1H), 7.35 (s, 5H).

(*RS*)-2-Amino-3-(5-carboxy-3-propoxy-4-isoxazolyl)propionic Acid (3c). A mixture of **20c** (1.0 g, 2.4 mmol) and 1 M HCl (100 mL) was heated under reflux for 5 h and then heated at 70 °C for 16 h. The mixture was cooled to room temperature, washed with EtOAc (3 × 100 mL), and concentrated. The residue was recrystallized (water) to give **3c** (0.32 g, 52%): mp 250−251 °C dec; ^{1}H NMR (D_2O, NaOD) δ 0.95 (t, 3H), 1.76 (sextet, 2H), 2.78 (dd, 1H), 2.90 (dd, 1H), 3.42 (dd, 1H), 4.17 (t, 2H); MS ($(M + H)^+$) *m*/*z* 259. Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

Compounds **3f**,**h** were prepared using methods analogous to that described for **3c**.

(*RS*)-2-Amino-3-(3-butoxy-5-carboxy-4-isoxazolyl)propionic Acid (3f). Yield: 0.33 g, 58%; mp 238−240 °C dec; ^{1}H NMR (D_2O, NaOD) δ 0.95 (t, 3H), 1.43 (sextet, 2H), 1.76 (quintet, 2H), 2.80 (dd, 1H), 2.91 (dd, 1H), 3.44 (dd, 1H), 4.25 (t, 2H); MS ($(M + H)^+$) *m*/*z* 273. Anal. ($C_{11}H_{16}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-(5-carboxy-3-pentyloxy-4-isoxazolyl)-propionic Acid (3h). Yield: 0.55 g, 70%; mp 244−245 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H), 1.32−1.40 (m, 4H), 1.73 (quintet, 2H), 2.90 (dd, 1H), 3.02 (dd, 1H), 3.95 (dd, 1H), 4.17 (t, 2H); MS ($(M + H)^+$) *m*/*z* 287. Anal. ($C_{12}H_{18}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-(3-allyloxy-5-carboxy-4-isoxazolyl)propionic Acid Hemihydrate Hydrochloride (3e). A mixture of **20e** (0.7 g, 1.69 mmol), 1 M NaOH (60 mL), and EtOH (30 mL) was heated at 90 °C for 16 h. The mixture was cooled to room temperature, acidified with 4 M HCl, heated at 40 °C for 4 h, and then concentrated. The residue was suspended in water (10 mL) and filtered, and the filtrate was subjected to ion-exchange chromatography (IRA-400, eluent: 1 M HOAc). Collection and evaporation of the ninhydrin-reactive fractions followed by recrystallization (water) gave **3e** as the hydrochloride salt (0.12 g, 28%): mp 243−244 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.93 (dd, 1H), 3.05 (dd, 1H), 3.99 (dd, 1H), 4.73 (d, 2H), 5.29 (dd, 1H), 5.44 (dd, 1H), 6.05 (tdd, 1H). Anal. ($C_{10}H_{12}N_2O_6 \cdot HCl \cdot 0.5H_2O$) C, H, N.

Compounds **3g**,**i** were prepared using methods analogous to that described for **3e.**

(*RS*)-2-Amino-3-[5-carboxy-3-(*trans*-2-butenyloxy)-4-isoxazolyl]propionic Acid (3g). Ion-exchange chromatography (IRA-400, eluent: 1 M HOAc) gave crude **3g** that was subjected to ion-exchange chromatography (Dowex-50, eluent: 1 M NH_3). Concentration of the ninhydrin-reactive fractions followed by recrystallization of the residue gave **3g** (40 mg, 13%): mp 203−204 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.71 (dd, 3H), 2.75 (dd, 1H), 2.90 (dd, 1H), 3.10 (dd, 1H), 4.65 (d, 2H), 5.73 (dt, 1H), 5.87 (dq, 1H); MS ($(M + H)^+$) *m*/*z* 271. Anal. ($C_{11}H_{14}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-(3-benzyloxy-5-carboxy-4-isoxazolyl)-propionic Acid (3i). A mixture of **20i** (0.65 g, 1.4 mmol) and 1 M NaOH (50 mL) was heated under reflux for 16 h. The mixture was cooled (5 °C), acidified with 4 M HCl, and concentrated. The residue was recrystallized from water to give **3i** (0.1 g, 23%): mp 209−211 °C dec; ^{1}H NMR (DMSO-d_6) δ 2.95 (dd, 1H), 3.05 (dd, 1H), 3.99 (t, 1H), 5.26 (s, 2H), 7.31−7.52 (m, 5H); MS ($(M + H)^+$) *m*/*z* 307. Anal. ($C_{14}H_{14}N_2O_6$) H, N; C: calcd, 54.90; found, 54.31.

(*RS*)-2-Amino-3-(2-benzyl-5-carboxy-2,3-dihydro-3-oxo-4-isoxazolyl)propionic Acid Monohydrate (4i). A mixture of **21i** (0.9 g, 1.9 mmol) and 1 M HCl was heated under reflux for 5 h. The mixture was concentrated to give **4i** (0.56 g, 80%): mp 146−148 °C dec; ^{1}H NMR (DMSO-d_6) δ 3.08 (dd, 1H), 3.19 (dd, 1H), 4.17 (br s, 1H), 5.16 (s, 2H), 7.24−7.45 (m, 5H); MS ($(M + H)^+$) *m*/*z* 307. Anal. ($C_{14}H_{14}N_2O_6 \cdot H_2O$) C, H, N.

(*RS*)-2-Amino-3-[3-ethoxy-5-(1*H*-tetrazol-5-yl)-4-isoxazolyl]propionic Acid (5). A suspension of **22**[16] (2.0 g, 4.9 mmol) in 1 M HCl (150 mL) was heated under reflux for 24 h. The mixture was concentrated, and the residue was dissolved in water. The pH of the solution was adjusted to about 3.5 by addition of NaOH (0.1 and 1 M), and **5** was collected by filtration (1.0 g, 76%): mp 273−275 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.38 (t, 3H), 3.08 (dd, 1H), 3.19 (dd, 1H), 4.23−4.36 (m,

3H); MS ((M + H)$^+$) *m*/*z* 269. Anal. ($C_9H_{12}N_6O_4$) C, H; N: calcd, 31.33; found, 30.72.

(*RS*)-2-Amino-3-[3-ethoxy-5-(1*H*-1,2,4-triazol-3-yl)-4-isoxazolyl]propionic Acid Hydrate (6). A suspension of **23**[16] (1.5 g, 2.3 mmol) in 1 M HCl (aq) (150 mL) was heated under reflux for 24 h. The solution was cooled to room temperature, washed with Et_2O (2 × 150 mL) and CH_2Cl_2 (150 mL), filtered, and concentrated. The residue was dissolved in water (5 mL), and pH of the solution was adjusted to about 3.5 by addition of NaOH (0.1 and 1 M) affording **6** (0.35 g, 56%): mp 225−227 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.38 (t, 3H), 2.94 (dd, 1H), 3.18 (dd, 1H), 3.58 (dd, 1H), 4.30 (q, 2H), 8.64 (s, 1H); MS ((M + H)$^+$) *m*/*z* 268. Anal. ($C_{10}H_{13}N_5O_4$·0.25H_2O) C, H, N.

Ethyl 3-Ethoxy-4-(phthalimidomethyl)isoxazole-5-carboxylate (24). A suspension of potassium phthalimide (3.6 g, 19.7 mmol) in DMF (125 mL) was heated to 90 °C followed by the addition of a solution of **13b** (5.0 g, 17.9 mmol) in DMF (85 mL). The mixture was stirred at 90 °C for 45 min, cooled, and concentrated. To the residue was added water (250 mL), and the aqueous phase was extracted with Et_2O (2 × 200 mL). The combined organic phase was dried ($MgSO_4$) and concentrated. The residue was recrystallized (EtOH) to give **24** (3.7 g, 60%): mp 93−94 °C; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H), 1.35 (t, 3H), 4.15 (q, 2H), 4.90 (s, 2H), 7.82−7.92 (m, 4H). Anal. ($C_{17}H_{16}N_2O_6$) C, H, N.

4-(Aminomethyl)-3-ethoxyisoxazole-5-carboxylic Acid Hydrochloride (25). A mixture of **24** (2.9 g, 8.4 mmol) and 1 M NaOH (330 mL) was heated under reflux for 45 min. The mixture was cooled on an ice bath, acidified with concentrated aqueous HCl, and extracted with Et_2O (3 × 400 mL). The combined organic phase was concentrated, and the residue was heated under reflux in 1 M HCl (600 mL) for 1 h. The mixture was cooled to room temperature, washed with Et_2O (3 × 600 mL), and concentrated. The residue was recrystallized (HOAc) to give **25** (1.5 g, 82%): mp 215−216 °C dec; ^{1}H NMR (D_2O) δ 1.38 (t, 3H), 4.13 (s, 2H), 4.33 (q, 2H). Anal. ($C_7H_{10}N_2O_4$·HCl) C, H, N.

4-{[(2-Amino-3,4-dioxo-1-cyclobuten-1-yl)amino]methyl}-3-ethoxyisoxazole-5-carboxylic Acid (7). A suspension of **25** (1.2 g, 5.3 mmol) and 3-amino-4-ethoxy-3-cyclobutene-1,2-dione (0.60 g, 5.9 mmol) in EtOH (300 mL) and 1 M NaOH (12 mL) was stirred at room temperature for 16 h and then concentrated. The residue was dissolved in water (100 mL) and washed with EtOAc (2 × 100 mL). The aqueous phase was evaporated to about 30 mL, and pH of the solution was adjusted to 3 with 1 M HCl. The precipitate that formed was recrystallized (water) to give **7** as a yellow powder (0.71 g, 47%): mp 236−238 °C dec; ^{1}H NMR (DMSO-d_6) δ 1.30 (t, 3H), 4.22 (q, 2H), 4.68 (br s, 2H); MS ((M + H)$^+$) *m*/*z* 282. Anal. ($C_{11}H_{11}N_3O_6$·2.25H_2O) C, H, N.

Receptor Binding Assays. Affinity for AMPA receptors was determined using the ligand [^{3}H]AMPA,[23] and for determination of NMDA and kainic acid receptor affinities, [^{3}H]-CPP[24] and [^{3}H]kainic acid,[22] respectively, were used. The membrane preparations used in all of the receptor binding experiments were prepared according to the method of Ransom and Stec.[26]

In Vitro Electropharmacology. A rat cortical wedge preparation for determination of excitatory amino acid-evoked depolarizations described by Harrison and Simmonds[27] was used in a slightly modified version.[17] Wedges (500 μm thick) of rat brain, containing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with an electrode. The cortex was superfused with Mg^{2+}-free Krebs buffer, whereas the corpus callosum part was superfused with Mg^{2+}- and Ca^{2+}-free Krebs buffer at 25 °C. The test compounds were added to the cortex superfusion medium, and the induced potential difference between the electrodes was recorded on a chart recorder. Agonists were applied for 90 s at each concentration tested. The sensitivity of agonist effects to the AMPA receptor antagonist, NBQX (5 μM), was tested at agonist concentrations producing at least 50% of maximal responses. Under these conditions, all of the recorded agonist responses were reversibly reduced by at least 70%. In experiments designed to detect antagonist effects of ACPA analogues at 1 mM concentrations, the compounds were applied alone for 90 s followed by coapplication of the appropriate agonist (5 μM AMPA, 10 μM NMDA, or 5 μM kainic acid) and potential antagonist for another 90 s.

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Supporting Information Available: Elemental analyses for **3a**−**j**, **4a**,**b**,**i**, **5**−**7**, **9**, **10**, **11b**, **12a**,**b**,**d**, **13b**, **15a**,**b**,**d**, **16a**,**b**, **17a**, **19**, **24**, and **25**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wheal, H. V., Thomson, A. M., Eds. *Excitatory Amino Acids and Synaptic Transmission*; Academic Press: London, U.K., 1995.
(2) Monaghan, D. T., Wenthold, R. J., Eds. *The Ionotropic Glutamate Receptors*; Humana Press: Totowa, NJ, 1997.
(3) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. Ligands for glutamate receptors: design and therapeutic prospects. *J. Med. Chem.* **2000**, *43*, 2609−2645.
(4) Conn, P. J., Patel, J., Eds. *The Metabotropic Glutamate Receptors*; Humana Press: Totowa, NJ, 1994.
(5) Parsons, C. G.; Danysz, W.; Quack, G. Glutamate in CNS disorders as a target for drug development: an update. *Drug News Perspect.* **1998**, *11*, 523−569.
(6) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev.* **1999**, *51*, 7−61.
(7) Mahanty, N. K.; Sah, P. Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. *Nature* **1998**, *394*, 683−687.
(8) Liu, S. Q.; Cull-Candy, S. G. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* **2000**, *405*, 454−457.
(9) Lee, H. K.; Barbarosie, M.; Kameyama, K.; Bear, M. F.; Huganir, R. L. Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* **2000**, *405*, 955−959.
(10) Staubli, U.; Rogers, G.; Lynch, G. Facilitation of glutamate receptors enhances memory. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 777−781.
(11) Lynch, G.; Granger, R.; Ambros-Ingerson, J.; Davis, C. M.; Kessler, M.; Schehr, R. Evidence that a positive modulator of AMPA-type glutamate receptors improves delayed recall in aged humans. *Exp. Neurol.* **1997**, *145*, 89−92.
(12) Amstrong, N.; Sun, Y.; Chen, G.-Q.; Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **1998**, *395*, 913−917.
(13) Armstrong, N.; Gouaux, E. Mechanisms for activation and antagonism of an AMPA-sensitive glutamate receptor: crystal structures of the GluR2 ligand binding core. *Neuron*, **2000**, *28*, 165−181.
(14) Sløk, F. A.; Ebert, B.; Lang, Y.; Krogsgaard-Larsen, P.; Lenz, S. M.; Madsen, U. Excitatory amino acid receptor agonists. Synthesis and pharmacology of analogues of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid. *Eur. J. Med. Chem.* **1997**, *32*, 329−338.
(15) Ebert, B.; Lenz, S. M.; Brehm, L.; Bregnedal, P.; Hansen, J. J.; Frederiksen, K.; Bøgesø, K. P.; Krogsgaard-Larsen, P. Resolution, absolute stereochemistry, and pharmacology of the *S*-(+)- and *R*-(−)-isomers of the apparent partial AMPA receptor agonist (*R*,*S*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid [(*R*,*S*)-APPA]. *J. Med. Chem.* **1994**, *37*, 878−884.
(16) (a) Bang-Andersen, B.; Lenz, S. M.; Skjærbæk, N.; Søby, K. K.; Hansen, H. O.; Ebert, B.; Bøgesø, K. P.; Krogsgaard-Larsen, P. Heteroaryl analogues of AMPA. Synthesis and quantitative structure−activity relationships. *J. Med. Chem.* **1997**, *40*, 2831−2842. (b) Bang-Andersen, B.; Bøgesø, K. P.; Krogsgaard-Larsen, P.; Lenz, S. M. 3-Alkoxyisoxazol-4-yl-substituted 2-amino carboxylic acid compounds. PCT Int. Application WO 9815542 A1, 1998; *Chem. Abstr.* **1998**, *128*, 295053. (c) Johansen, T. N.; Ebert, B.; Falch, E.; Krogsgaard-Larsen, P. AMPA receptor agonists: Resolution, configurational assignment, and pharmacology of (+)-(*S*)- and (−)-(*R*)-2-amino-3-[3-hydroxy-5-(2-pyridyl)isoxazol-4-yl]propionic acid (2-Py-AMPA). *Chirality* **1997**, *9*, 274−280.
(17) Falch, E.; Brehm, L.; Mikkelsen, I.; Johansen, T. N.; Skjærbæk, N.; Nielsen, B.; Stensbøl, T. B.; Ebert, B.; Krogsgaard-Larsen, P. Heteroaryl analogues of AMPA. 2. Synthesis, absolute ster-

eochemistry, photochemistry, and structure−activity relationships. *J. Med. Chem.* **1998**, *41*, 2513−2523.
(18) Vogensen, S. B.; Jensen, H. S.; Stensbøl, T. B.; Frydenvang, K.; Bang-Andersen, B.; Johansen, T. N.; Egebjerg, J.; Krogsgaard-Larsen, P. Resolution, configurational assignment and enantiopharmacology of 2-amino-3-[3-hydroxy-5-(2-methyl-2*H*-tetrazol-5-yl)isoxazol-4-yl]propionic acid, a potent GluR3 and GluR4 preferring AMPA receptor agonist. *Chirality* **2000**, *12*, 705−713.
(19) Madsen, U.; Frølund, B.; Lund, T. M.; Ebert, B.; Krogsgaard-Larsen, P. Design, synthesis and pharmacology of model compounds for indirect elucidation of the topography of AMPA receptor sites. *Eur. J. Med. Chem.* **1993**, *28*, 791−800.
(20) Madsen, U.; Wong, E. H. F. Heterocyclic excitatory amino acids. Synthesis and biological activity of novel analogues of AMPA. *J. Med. Chem.* **1992**, *35*, 107−111.
(21) Stensbøl, T. B.; Sløk, F. A.; Trometer, J.; Hurt, S.; Ebert, B.; Kjøller, C.; Egebjerg, J.; Madsen, U.; Diemer, N. H.; Krogsgaard-Larsen, P. Characterization of a new AMPA receptor radioligand, [^{3}H]2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid. *Eur. J. Pharmacol.* **1999**, *373*, 251−262.
(22) Braitman, D. J.; Coyle, J. T. Inhibition of [^{3}H]kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. *Neuropharmacology* **1987**, *26*, 1247−1251.
(23) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **1985**, *54*, 27−32.
(24) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, K. Binding of [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat membranes. A selective high affinity ligand for *N*-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778−783.
(25) Kinney, W. A.; Lee, N. E.; Garrison, D. T.; Podlesny, E. J.; Simmonds, J. T.; Bramlett, D.; Notvest, R.; Kowal, D. M.; Tasse, R. P. Bioisosteric replacement of the α-amino carboxylic acid functionality in 2-amino-5-phosphonopentanoic acid yields unique 3,4-diamino-3-cyclobutene-1,2-dione containing NMDA antagonists. *J. Med. Chem.* **1992**, *35*, 4720−4726.
(26) Ransom, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 binding to the *N*-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.
(27) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some antagonists of *N*-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381−391.

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