Synthesis and Pharmacology of Highly Selective Carboxy and Phosphono Isoxazole Amino Acid AMPA Receptor Antagonists

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Received November 8, 1995[⊗]

(*RS*)-2-Amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA, **5**) and the selective AMPA receptor antagonist (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]propionic acid (AMOA, **7**) have been used as leads for the design and synthesis of a number of potential AMPA receptor antagonists. Two parallel series of AMOA analogs were synthesized, containing either a distal carboxylic acid (compounds **8b**−**g** and **11b**) or a phosphonic acid (compounds **9a**−**g**, **10c**, and **11c**). Pharmacological characterization of the synthesized compounds was carried out using a series of receptor binding assays and by in vitro electrophysiological experiments using the rat cortical slice model. The two analogs with a *tert*-butyl substituent, (*RS*)-2-amino-3-[5-*tert*-butyl-3-(carboxymethoxy)-4-isoxazolyl]propionic acid (ATOA, **8b**) and the corresponding phosphonic acid analog ATPO (**9b**), were the most potent and selective AMPA antagonists within each series. ATOA and ATPO showed IC_{50} values of 150 and 28 μM, respectively, toward AMPA-induced depolarizations in the cortical slice model compared to $IC_{50} = 320$ μM for the parent compound, AMOA. These two new competitive AMPA antagonists were significantly more selective than AMOA, showing no antagonism (up to 1 mM) toward NMDA-induced responses, whereas AMOA (at 1 mM) showed weak (19%) inhibition toward NMDA-induced responses. The structure−activity relationships for the two series of compounds revealed considerable differences with respect to the substituents effects, and the phosphonic acid analogs generally exhibited significantly higher potencies compared to the carboxylic acid analogs.

Introduction

(*S*)-Glutamic acid (Glu, **1**) is the major excitatory amino acid (EAA) neurotransmitter in the central nervous system.[1−3] The *N*-methyl-D-aspartic acid (NMDA, **2**) and (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl-)propionic acid (AMPA, **5**) subtypes of EAA receptors are the subject of extensive exploration as potential targets for drug intervention in different neurodegenerative diseases.[1,4,5] The availability of a broad spectrum of phosphono amino acids showing potent and highly selective competitive antagonism of NMDA receptor function has played a crucial role in the characterization of this subtype of EAA receptors.[6,7] The pharmacology of the NMDA receptors is closely associated with this particular class of amino acids, notably, (*R*)-2-amino-5-phosphonovaleric acid[8] (AP5, **3**) and (*R*)-[3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid[9] (CPP, **4**).

AMPA (**5**) and a number of structurally related isoxazole amino acids, including (*RS*)-2-amino-3-(5-*tert*-butyl-3-hydroxy-4-isoxazolyl)propionic acid (ATPA, **6b**), are potent and selective AMPA receptor agonists.[3,10,11] Although ATPA (**6b**) is somewhat weaker than AMPA as an AMPA agonist, the observation that ATPA is active after systemic administration to animals makes this compound an important tool for studies of the pharmacology of AMPA receptors.[12,13] Structure−activity studies on ATPA (**6b**) and other AMPA analogs with different substituents in the 5-position of the isoxazole ring, including the phenyl analog **6f**, have given rise to the hypothesis that the AMPA receptors contain a lipophilic cavity capable of accommodating substituents of a certain size.[14−16]

AMPA has previously been converted into the AMPA receptor antagonist (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]propionic acid (AMOA, **7**), which shows neuroprotective properties.[17,18] AMOA is a relatively weak antagonist at AMPA receptors and also shows very weak antagonist activity toward NMDA-induced responses.[17] In order to investigate the importance of the substituent in the 5-position of the isoxazole ring, a number of AMOA analogs, compounds **8b**−**g**, have been synthesized and pharmacologically characterized.

Since a distal phosphonic acid is an important, though not essential, structural element of competitive NMDA antagonists,[6,7] and since many AMPA receptor ligands, including AMOA (**7**), contain an isoxazole nucleus, we designed "structural hybrids" containing both of these elements as novel EAA receptor antagonists. Thus, in parallel with the series of AMOA analogs (compounds **8b**−**g**), we describe the synthesis and pharmacological characterization of compounds **9a**−**g** containing a distal phosphonic acid.

The potent and selective AMPA agonist (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid[19] (**10a**) has previously been converted into the carboxymethyl analog **10b**, which quite surprisingly was shown to be an NMDA antagonist.[20] We here

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⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1996.

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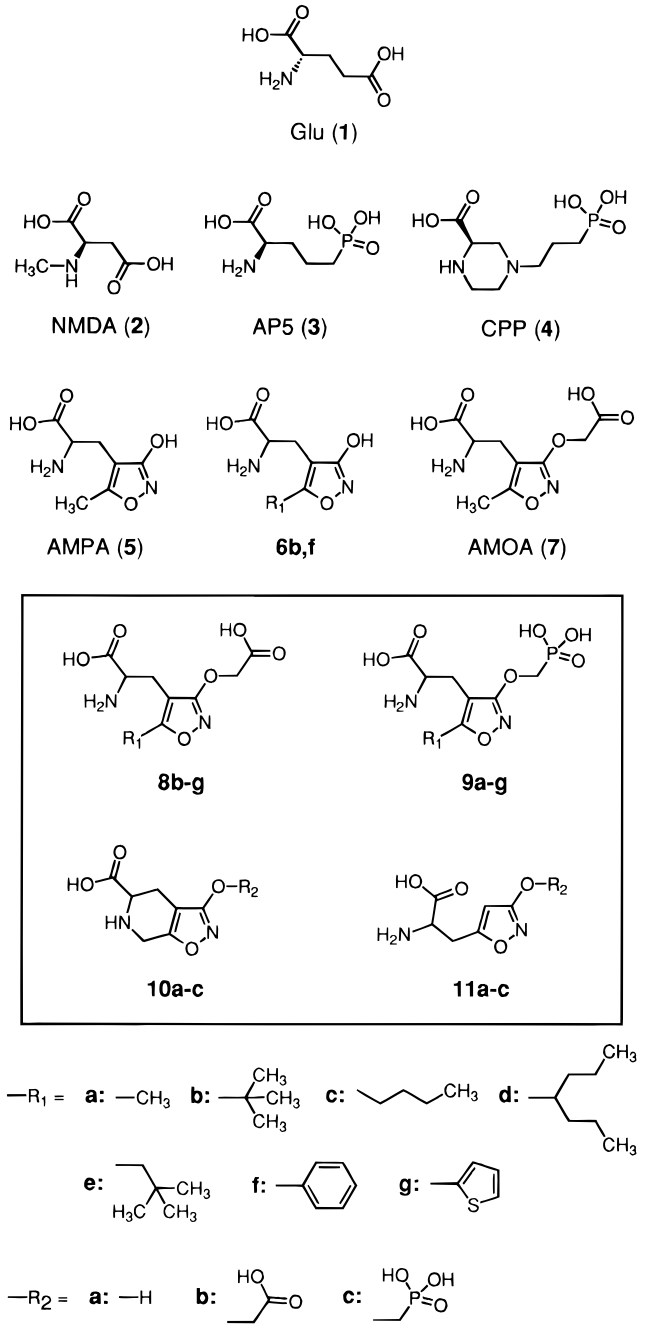


Figure 1. Structures of Glu (**1**) and ligands at either NMDA receptors (**2**−**4**) or AMPA receptors (**5**−**7**). The compounds synthesized (**8b**−**g**, **9a**−**g**, **10c**, and **11b**,**c**) are depicted in the box.

describe the synthesis and pharmacology of the phosphonic acid analog of **10b**, compound **10c**, and compounds **11b**,**c**, derived from the AMPA agonist homoibotenic acid (**11a**).[10]

Results

Chemistry. Two different strategies were used for the syntheses of the target compounds (Scheme 1). Alkylation of the hydroxy group at the 3-position of the isoxazole ring of **12a**,**c**−**e**[21,22] with either chloroacetic acid ester or {[(*p*-tolylsulfonyl)oxy]methyl}phosphonate ester followed by full deprotection using 1 M hydrochloric acid gave the desired carboxy or phosphono amino acids, compounds **8c**−**e** and **9a**,**c**−**e**, respectively. For the synthesis of compounds **8b**,**g** and **9b**,**f**,**g**, the protected distal acid functionalities were introduced at an earlier step of the synthetic sequences. This procedure provided, in most cases, a better yield as compared to the alkylation of the acetamidomalonate derivatives **12a**,**c**−**e**, as described above. The low yields (15−59%) of the alkylation reactions probably reflect the formation and subsequent decomposition of an *N*-alkylated product in analogy with earlier observations.[17,23] Introduction of the acetamidomalonate group in compounds **16b**,**g** and **17b**,**f**,**g** was performed via regioselective NBS bromination under free radical conditions followed by a Sorensen synthesis. Different variants of the Sorensen synthesis were performed, including the use of dimethyl or diethyl acetamidomalonate and sodium hydride, potassium *tert*-butoxide (Scheme 1), or sodium ethoxide (Scheme 2) as the base. For the synthesis of compound **8b**, it proved essential to use the Boc-protected intermediate **20b** in order to produce the final product **8b** in reasonable yield and with satisfactory purity. Compound **20b** was synthesized from **18b** using diethyl [*N*-(*tert*-butyloxycarbonyl)amino]malonate[24] and sodium hydride. Final deprotection was carried out in NaOH followed by HCl under reflux.

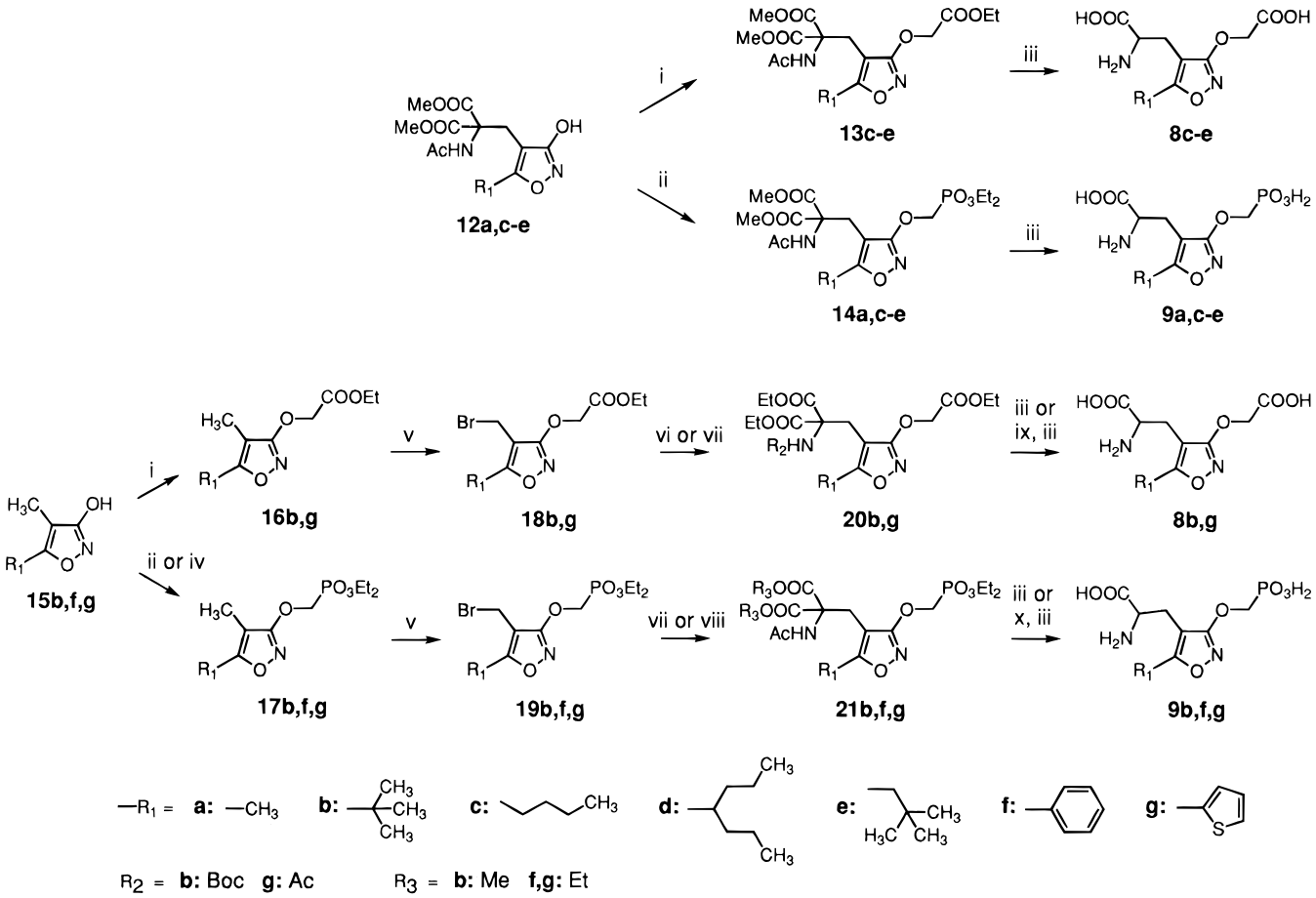
For the synthesis of compounds **8f**, **10c**, and **11b**,**c**, modified versions of the above strategies were used (Scheme 2). Protection of the amino acid moiety of compound **6f**[15] followed by *O*-alkylation gave the intermediate **23**, which was deprotected in two steps. For the synthesis of compound **10c**, a fully protected intermediate, compound **25**,[25] was NBS brominated, and subsequent cyclization was accomplished with sodium hydride.

Compounds **11b**,**c** were synthesized from 5-methyl-3-isoxazolol (tachigaren, **29**)[26] by *O*-alkylation followed by NBS bromination, Sorensen synthesis, and deprotection, in analogy with the preparation of compounds **8b**,**g** and **9b**,**f**,**g**.

In Vitro Pharmacology. The compounds **8b**−**g**, **9a**−**g**, **10c**, and **11b**,**c** were studied in different receptor binding assays. For the determination of affinity for AMPA receptors, the ligands [^{3}H]AMPA[27] and [^{3}H]-6-cyano-7-nitroquinoxaline-2,3-dione ([^{3}H]CNQX)[28] were used (Table 1). None of the compounds described showed significant affinity in the [^{3}H]kainic acid binding assay[29] (IC_{50} > 100 μM), and only compounds **10b**,[20]**c** showed significant affinity for [^{3}H]CPP binding sites[9] (Table 1).

All new compounds were studied electrophysiologically, using the rat cortical slice model.[32] The phosphonic acid analog of AMOA, (*RS*)-2-amino-3-[5-methyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid (AMPO, **9a**), showed markedly increased antagonist potency toward AMPA-induced responses, as compared to AMOA (**7**) (Table 1). The *tert*-butyl analog of AMOA, (*RS*)-2-amino-3-[5-*tert*-butyl-3-(carboxymethoxy)-4-isoxazolyl]-propionic acid (ATOA, **8b**), also exhibited a more potent antagonist effect than AMOA. (*RS*)-2-Amino-3-[5-*tert*-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic acid (ATPO, **9b**) was the most potent compound, showing more than 10-fold increase in potency as compared to AMOA as an AMPA antagonist. All compounds listed in Table 1 with significant AMPA antagonist potencies (IC_{50} < 1 mM) gave parallel rightward shifts of the AMPA dose−response curve, as illustrated in Figure 2 for ATPO (**9b**). The antagonist profiles of ATOA (**8b**),

Scheme 1[a]



[a] (i) $ClCH_2COOEt$, K_2CO_3; (ii) $CH_3(C_6H_4)SO_3CH_2PO_3Et_2$, NaH; (iii) 1 M HCl; (iv) $CH_3SO_3CH_2PO_3Et_2$, K_2CO_3; (v) NBS; (vi) $BocNHCH(COOEt)_2$, NaH; (vii) $AcNHCH(COOEt)_2$, $(CH_3)_3COK$; (viii) $AcNHCH(COOMe)_2$, NaH; (ix) 2 M NaOH; (x) $(CH_3)_3SiBr$.

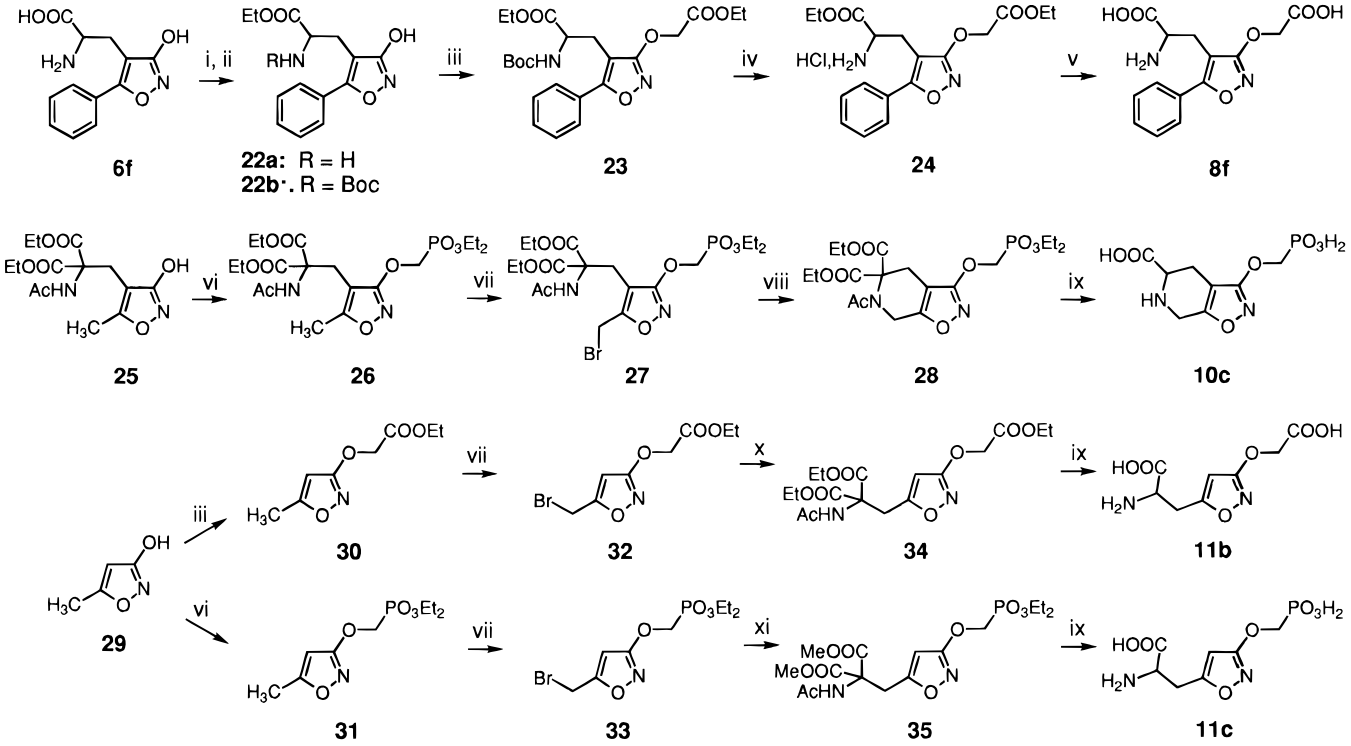
AMPO (**9a**), and ATPO (**9b**) are compared with that of AMOA (**7**) in Figure 3. Not only are these compounds more potent as AMPA antagonists, as compared to AMOA, but they also show a higher degree of selectivity. In the case of ATOA (**8b**) and ATPO (**9b**) (Figure 3), no significant antagonism of NMDA responses are observed even at concentrations of 1 mM, whereas AMOA (**7**) and AMPO (**9a**) did show a weak inhibitory effect at 1 mM toward NMDA-induced responses (19 ± 7% and 31 ± 4% inhibition, respectively) (see Figure 3). For compounds showing a significant AMPA receptor antagonist effect (IC_{50} < 1 mM), a weak antagonism of kainic acid responses was generally observed at 1 mM concentrations of the antagonist (Figure 3). This probably reflects that kainic acid shows weak AMPA receptor agonist effects in addition to its agonism at kainic acid receptors.[3] The bicyclic compound **10c** showed a selective NMDA antagonist effect comparable with that described for the carboxylic acid analog **10b**[20] (Table 1). The two compounds derived from the AMPA agonist homoibotenic acid (**11a**), compounds **11b**,**c**, were completely inactive in the studies performed (Table 1).

Discussion

A series of "structural hybrids" of the AMPA receptor antagonist AMOA (**7**) and the NMDA antagonists AP5 (**3**) and CPP (**4**) were synthesized and characterized pharmacologically in vitro. These analogs contain different substituents in the 5-position of the isoxazole ring and either a carboxylic acid or a phosphonic acid as the distal acidic moiety (Figure 1). In electrophysiological experiments antagonist potencies of the new compounds varied considerably, and all compounds showing AMPA antagonist activity (IC_{50} < 1 mM) gave parallel rightward shifts of the AMPA dose−response curves, indicative of competitive antagonism. The results of [^{3}H]-AMPA binding studies showed weak inhibition by the more potent antagonists AMOA (**7**), ATOA (**8b**), AMPO (**9a**), and ATPO (**9b**) with IC_{50} values ranging from 31 to 90 μM. Using the [^{3}H]CNQX binding assay, a better correlation between the electrophysiological data and the binding data was demonstrated, although AMOA does show some anomaly. The fairly low affinities observed in the [^{3}H]AMPA and [^{3}H]CNQX binding assays for the antagonists described may reflect a difference in binding mode for these antagonists as compared to the binding mode for the agonist AMPA as well as for the quinoxalinedione antagonist CNQX. The observed affinities may also reflect dissimilar binding to different subtypes of AMPA receptors.

Among the carboxylic acid analogs, only ATOA (**8b**) showed higher AMPA antagonist potency than AMOA (**7**). All of the analogs **8c**−**g** were virtually inactive (IC_{50} > 1 mM). Generally, the phosphonic acid analogs **9a**−**g** showed higher potency than the corresponding carboxylic acid analogs (**7** and **8b**−**g**), ATPO (**9b**) being the most potent AMPA antagonist. Concomitantly with the potency increase, ATOA (**8b**) and ATPO (**9b**) also showed improved selectivity toward AMPA receptors, as compared with AMOA (**7**). In contrast to AMOA,

Scheme 2[a]



[a] (i) HCl/EtOH; (ii) BocOBoc, TEA; (iii) $ClCH_2COOEt$, K_2CO_3; (iv) HCl/EtOAc; (v) TEA; (vi) $CH_3(C_6H_4)SO_3CH_2PO_3Et_2$, NaH; (vii) NBS; (viii) NaH; (ix) 1 M HCl; (x) $AcNHCH(COOEt)_2$, C_2H_5ONa; (xi) $AcNHCH(COOMe)_2$, NaH.

Table 1. Receptor Binding and Electrophysiological Data (Mean ± SEM, $n = 3-6$)

	IC_{50} (μM)				
compd	[^{3}H]AMPA	[^{3}H]CNQX	[^{3}H]CPP	[^{3}H]KAIN	electrophysiology[a]
AMOA[b] (**7**)	90 ± 14	8.0 ± 0.7	>100	>100	320 ± 25
ATOA (**8b**)	33 ± 6	12 ± 5	>100	>100	150 ± 14
8c	>100	87 ± 20	>100	>100	>1000[c]
8d	>100	63 ± 18	>100	>100	>1000[d]
8e	>100	>100	>100	>100	>1000
8f	>100	>100	>100	>100	>1000
8g	>100	>100	>100	>100	>1000
AMPO (**9a**)	31 ± 3	6.9 ± 2.6	>100	>100	60 ± 7
ATPO (**9b**)	35 ± 3	5.7 ± 3.2	>100	>100	28 ± 3
9c	>100	18 ± 6	>100	>100	140 ± 14
9d	>100	nt	>100	>100	350 ± 38
9e	>100	35 ± 10	>100	>100	>1000[e]
9f	>100	>100	>100	>100	>1000
9g	>100	>100	>100	>100	360 ± 64
10b[f]	>100	nt	16.4	>100	170 ± 10[g]
10c	>100	>100	22 ± 6	>100	135 ± 18[g]
11b	>100	>100	>100	>100	>1000
11c	>100	>100	>100	>100	>1000
CNQX	0.37 ± 0.04	0.038 ± 0.004	25[h]	1.5[h]	0.6[i]
CPP (**4**)	>100	nt	0.050 ± 0.025	nt	1.6[i]
KAIN	4.0 ± 0.9	100[j]	>100	0.016 ± 0.004	agonist

[a] Antagonism of AMPA-induced responses. [b] Reference 17. [c] 29 ± 7% inhibition at 1000 μM **8c**. [d] 45 ± 4% inhibition at 1000 μM **8d**. [e] 43 ± 3% inhibition at 1000 μM **9e**. [f] Reference 20. [g] Antagonism of NMDA-induced responses. [h] Reference 30. [i] K_i value. [j] Reference 31. nt, not tested.

neither ATOA nor ATPO showed any antagonist activity at 1 mM concentrations toward NMDA-induced responses (Figure 3).

The increased antagonist potency for the two *tert*-butyl analogs **8b** and **9b** is interesting, as the opposite effect on agonist activity is observed for AMPA (**5**) and the AMPA analog with a *tert*-butyl substituent, ATPA[11,33] (**6b**), showing EC_{50} values of 3.5 and 48 μM, respectively. Such discrepancies in structure−activity relationships for AMPA agonists versus antagonists may indicate a difference in binding mode, possibly reflecting that these two categories of compounds bind to different sites at the AMPA receptors and/or different conformations of the receptors.

For the bicyclic phosphonic acid analog **10c**, which is an analog of compound **10b**, previously shown to be a selective NMDA antagonist,[20] equal NMDA antagonist effects were observed. The comparable potency of **10b**,**c** as NMDA antagonists is somewhat surprising, since conversion of NMDA antagonists containing a distal carboxylic acid into the corresponding phosphonic acid analogs generally results in a significant increase in antagonist potency.[6,7] AMOA (**7**), **8b**−**g**, and **9a**−**g**, as well as **10b**,**c**, all have the same chain length as the

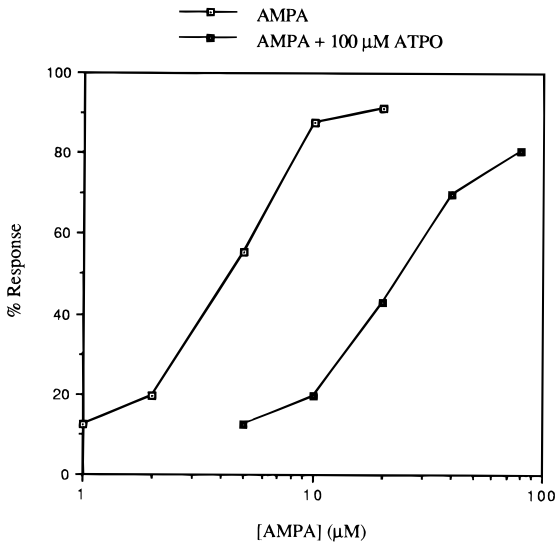


Figure 2. Dose−response curves as determined in the rat cortical slice preparation for AMPA (**5**) alone and AMPA coapplied with 100 μM ATPO (**9b**).

potent and selective NMDA antagonist CPP (**4**). The pharmacological selectivities of the compounds under study seem to indicate that only compounds **10b**,**c** can adopt conformations recognizable by the "antagonist conformation" of the NMDA receptor. For the other analogs, the AMPA structural element may mediate the AMPA receptor selectivity. The two compounds **11b**,**c**, derived from the weak AMPA agonist homoibotenic acid (**11a**),[10] were inactive, indicating that the introduction of a carboxymethyl or a phosphonomethyl group on the 3-hydroxyisoxazole moiety of AMPA agonists is not a generally applicable principle for the design of AMPA receptor antagonists.

In conclusion, a number of AMPA receptor antagonists, containing either a distal carboxylic acid or a phosphonic acid moiety, has been synthesized. The antagonist effects of the compounds were highly dependent on the substituents on the molecules as well as on the nature of the distal acidic moiety, phosphonic acids generally being more potent than the corresponding carboxylic acids. The phosphonic acid analog ATPO (**9b**) was markedly more potent and selective than the parent compound, AMOA (**7**), as an AMPA receptor antagonist.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and are uncorrected. Column chromatography (CC) was performed on Merck silica gel 60 (70−230 mesh, ASTM) and flash chromatography on Merck silica gel 60 H. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Denmark, Mr. P. Hansen, Department of General and Organic Chemistry, University of Copenhagen, or Analytical Research Department, H. Lundbeck A/S, Denmark, and are within ±0.4% of the calculated values unless otherwise stated. ^{1}H NMR spectra were recorded on a Varian EM 360L (60 MHz), a Bruker AC-200F (200 MHz), or a Bruker AC 250 (250 MHz) instrument using TMS or 1,4-dioxane, unless otherwise stated, as internal standard for spectra recorded in organic or aqueous solvents, respectively. Compounds containing the 3-hydroxyisoxazole moiety were visualized on TLC plates using UV light and a $FeCl_3$ spraying reagent (yellow colors). 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. Drying of organic phases was performed with $MgSO_4$, and evaporations were performed under vacuum on a rotary evaporator at 15 mmHg. Tachigaren (**29**) was kindly supplied by Cheminova A/S, Denmark.

General Procedure A: Preparation of 3-[(Ethoxycarbonyl)methoxy]isoxazoles (Compounds 13c−e, 16g, 23, and 30). A mixture of 3-hydroxyisoxazole **12c**−**e**, **15g**, **22b**, or **29**, K_2CO_3 (2 equiv), and acetone was stirred for 30 min at 60 °C. Ethyl chloroacetate (3 equiv) was added and stirring continued for 5 h at 60 °C. After cooling the reaction mixture was filtered and evaporated.

General Procedure B: Preparation of 3-[(Diethoxyphosphoryl)methoxy]isoxazoles (Compounds 14a,c−e, 17b, 26, and 31). Sodium hydride (1.1 equiv) was suspended in DMF under nitrogen, and 3-hydroxyisoxazole **12a**,**c**−**e**, **15b**, **25**, or **29** dissolved in DMF was added dropwise. Diethyl {[(*p*-tolylsulfonyl)oxy]methyl}phosphonate (1.5 equiv) dissolved in DMF was added, and the reaction mixture was stirred at room temperature for 3 days and then evaporated to dryness (l mmHg). H_2O was added to the residue and extracted with EtOAc. The EtOAc phase was dried and evaporated.

General Procedure C: Preparation of ω-Carboxy or ω-Phosphono α-Amino Acids (Compounds 8c−e,g, 9a−g, 10c, and 11b,c). The protected intermediate **13c**−**e**, **20g**, **14a**,**c**−**e**, ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[5-phenyl-3-(phosphonomethoxy)-4-isoxazolyl]propionate, ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[3-(phosphonomethoxy)-5-(2-thienyl)-4-isoxazolyl]propionate, or **34** was boiled under reflux in 1 M HCl for 12−72 h (monitored by TLC and ninhydrin spray reagent) and then evaporated and reevaporated twice from H_2O.

General Procedure D: Preparation of Isoxazole Bromomethyl Derivatives (Compounds 18b,g, 19b,f,g, 27, 32, and 33). A solution of starting material **16b**,**g**, **17b**,**f**,**g**, **26**, **30**, or **31** in CCl_4 was treated under reflux with NBS (1.05 equiv) and benzoyl peroxide (0.1 equiv) over a period of 4−8 h. NBS and benzoyl peroxide were added in four equal portions at 1−2 h intervals. After cooling of the reaction mixture, filtration and evaporation gave crude brominated product.

Methyl 2-Acetamido-3-{5-butyl-[3-(ethoxycarbonyl)-methoxy]-4-isoxazolyl}-2-(methoxycarbonyl)propionate (13c). 13c was prepared from **12c**[14] according to general procedure A and purified by CC [tol−EtOAc (3:1)] and recrystallization (EtOAc−light petroleum), which gave **13c** as colorless crystals (400 mg, 31%): mp 67−68 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.03 (s, 1H), 4.76 (s, 2H), 4.26 (q, 2H, J = 7 Hz), 3.79 (s, 6H), 3.41 (s, 2H), 2.51 (t, 2H, J = 7 Hz), 1.99 (s, 3H), 1.60 (m, 2H), 1.4−1.2 (m, 5H), 0.91 (t, 3H, J = 7.2 Hz). Anal. ($C_{19}H_{28}N_2O_9$) C, H, N.

Methyl 2-Acetamido-3-{3-[(ethoxycarbonyl)methoxy]-5-(1-propylbutyl)-4-isoxazolyl}-2-(methoxycarbonyl)propionate (13d). 13d was prepared from **12d**[22] according to general procedure A and purified by CC [tol−EtOAc (2:1) containing 1% AcOH] and recrystallization (tol−light petroleum), which gave **13d** as colorless crystals (660 mg, 47%): mp 95−96.5 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.75 (s, 2H), 4.25 (q, 2H, J = 7 Hz), 3.75 (s, 6H), 3.45 (s, 2H), 2.7 (m, 1H), 1.95 (s, 3H), 1.55 (m, 4H), 1.30 (t, 3H, J = 7 Hz), 1.20 (m, 4H), 0.9 (t, 6H, J = 7 Hz). Anal. ($C_{22}H_{34}N_2O_9$) C, H, N.

Methyl 2-Acetamido-3-{3-[(ethoxycarbonyl)methoxy]-5-(2,2-dimethylpropyl)-4-isoxazolyl}-2-(methoxycarbonyl)propionate (13e). 13e was prepared from **12e**[22] according to general procedure A, purified by CC [tol−EtOAc (2:1) containing 1% AcOH], and recrystallized (tol−light petroleum) to give **13e** as colorless crystals (34 mg, 48%): mp 127−128 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.75 (s, 2H), 4.25 (q, 2H, J = 7 Hz), 3.8 (s, 6H), 3.45 (s, 2H), 2.4 (s, 2H), 2.0 (s, 3H), 1.3 (t, 3H, J = 7 Hz), 0.95 (s, 9H). Anal. ($C_{20}H_{30}N_2O_9$) C, H, N.

Methyl 2-Acetamido-3-{3-[(diethoxyphosphoryl)methoxy]-5-methyl-4-isoxazolyl}-2-(methoxycarbonyl)propionate (14a). 14a was prepared from **12a**[21] according to general procedure B, purified by CC [tol−EtOAc (1:3) containing 1% AcOH], and recrystallized (EtOAc−light petroleum) to give **14a** as a colorless powder (410 mg, 15%): mp 96.5−98.0 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.81 (s, 1H), 4.53 (d, 2H, J =

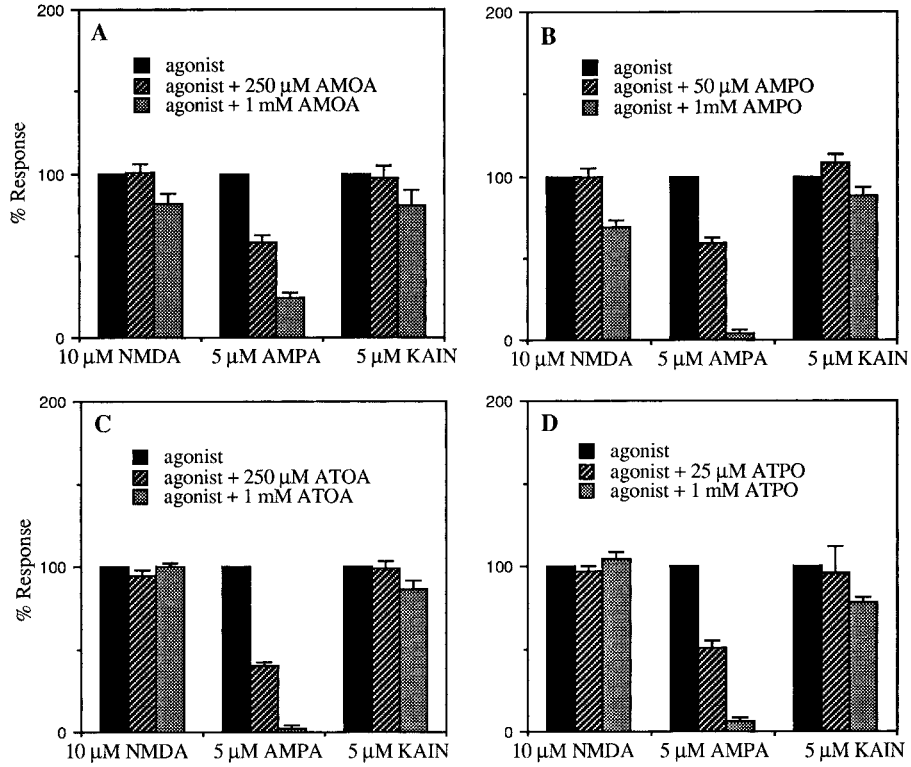


Figure 3. Effects of AMOA (**7**) (A), AMPO (**9a**) (B), ATOA (**8b**) (C), and ATPO (**9b**) (D) on rat cortical depolarizations induced by NMDA, AMPA, or kainic acid (KAIN). Normalized responses ± SEM; $n = 3-6$.

8.6 Hz), 4.22 (q, 2H, $J = 7.0$ Hz), 4.18 (q, 2H, $J = 7.0$ Hz), 3.82 (s, 6H), 3.37 (s, 2H), 2.21 (s, 3H), 2.04 (s, 3H), 1.35 (t, 6H, $J =$ 7.0 Hz). Anal. ($C_{17}H_{27}N_2O_{10}P$) C, H, N.

Methyl 2-Acetamido-3-{5-butyl-3-[(diethoxyphosphoryl)methoxy]-4-isoxazolyl}-2-(methoxycarbonyl)propionate (14c). 14c was prepared from **12c**[22] according to general procedure B, purified by CC [tol−EtOAc (1:1) containing 1% AcOH], and recrystallized (EtOAc−light petroleum) to give **14c** as colorless crystals (310 mg, 17%): mp 94−95 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.86 (s, 1H), 4.54 (d, 2H, $J = 8.5$ Hz), 4.22 (q, 2H, $J = 7.0$ Hz), 4.18 (q, 2H, $J = 7.1$ Hz), 3.81 (s, 6H), 3.37 (s, 2H), 2.52 (t, 2H, $J = 7.6$ Hz), 2.04 (s, 3H), 1.56 (m, 2H), 1.42−1.25 (m, 2H), 1.35 (t, 6H, $J = 7.1$ Hz), 0.91 (t, 3H, $J = 7.2$ Hz). Anal. ($C_{20}H_{33}N_2O_{10}P$) C, H, N.

Methyl 2-Acetamido-3-{3-[(diethoxyphosphoryl)methoxy]-5-(1-propylbutyl)-4-isoxazolyl}-2-(methoxycarbonyl)propionate (14d). 14d was prepared from **12d**[22] according to general procedure B, purified by CC [tol−EtOAc (1:1)], and recrystallized to give **14d** as a colorless powder (164 mg, 24%): mp 92−93 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.83 (s, 1H), 4.54 (d, 2H, $J = 8.2$ Hz), 4.22 (q, 2H, $J = 7.1$ Hz), 4.18 (q, 2H, $J = 7.1$ Hz), 3.80 (s, 6H), 3.40 (s, 2H), 2.65 (m, 1H), 2.02 (s, 3H), 1.6 (m, 4H), 1.4−1.2 (m, 4H), 1.35 (t, 6H, $J = 7.1$ Hz), 0.87 (t, 6H, $J = 7.0$ Hz). Anal. ($C_{23}H_{39}N_2O_{10}P$) C, H, N.

Methyl 2-Acetamido-3-{3-[(diethoxyphosphoryl)methoxy]-5-(2,2-dimethylpropyl)-4-isoxazolyl}-2-(methoxycarbonyl)propionate (14e). 14e was prepared from **12e**[22] according to general procedure B, purified by CC [tol−EtOAc (1:1) containing 1% AcOH], and recrystallized (EtOAc−light petroleum) to give **14e** as colorless crystals (310 mg, 31%): mp 163−164 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.95 (s, 1H), 4.55 (d, 2H, $J = 8.4$ Hz), 4.22 (q, 2H, $J = 7.1$ Hz), 4.19 (q, 2H, $J =$ 7.1 Hz), 3.80 (s, 6H), 3.40 (s, 2H), 2.43 (s, 2H), 2.03 (s, 3H), 1.35 (t, 6H, $J = 7.1$ Hz), 0.96 (s, 9H). Anal. ($C_{21}H_{35}N_2O_{10}P$) C, H, N.

(*RS*)-2-Amino-3-[5-butyl-3-(carboxymethoxy)-4-isoxazolyl]propionic Acid Zwitterion (8c) Dihydrate. 8c was prepared from **13c** according to general procedure C. The residue was dissolved in H_2O and EtOH and the pH adjusted to ca. 3 by addition of TEA. Recrystallization of the precipitate, first from AcOH and then from H_2O, afforded **13c** (102 mg, 50%): mp 215−217 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 3.9 (m, 1H), 2.9 (m, 2H), 2.6 (t, 2H, $J = 7$ Hz), 1.5 (m, 2H), 1.2 (m, 2H), 0.76 (t, 3H, $J = 7$ Hz). Anal. ($C_{12}H_{18}N_2O_6 \cdot 2H_2O$) C, N; H: calcd, 6.87; found, 6.25.

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-(1-propylbutyl)-4-isoxazolyl]propionic Acid Zwitterion (8d). 8d was prepared from **13d** according to general procedure C. Recrystallization (H_2O) gave **8d** (104 mg, 60%): mp 214−216 °C dec; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 4.65 (s, 2H), 3.6 (t, 1H, $J =$ 7 Hz), 2.9−2.7 (m, 3H), 1.6 (m, 4H), 1.25 (m, 4H), 0.9 (t, 6H, $J = 7$ Hz). Anal. ($C_{15}H_{24}N_2O_6$) H, N; C: calcd, 54.87; found, 54.42.

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-(2,2-dimethylpropyl)-4-isoxazolyl]propionic Acid Zwitterion (8e). 8e was prepared from **13e** according to general procedure C. Recrystallization (H_2O) gave **8e** (11.5 mg, 56%): mp 228−231 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.65 (s, 2H), 3.9 (dd, 1H, J = 7, 5.5 Hz), 2.9 (m, 2H), 2.5 (d, 1H, $J = 15$ Hz), 2.4 (d, 1H, J = 15 Hz), 0.85 (s, 9H). Anal. ($C_{13}H_{20}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-[5-methyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic Acid Zwitterion (9a). 9a was prepared from **14a** according to general procedure C. Recrystallization (H_2O) afforded **9a** (220 mg, 75%): mp 225−230 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.29 (d, 2H, $J = 9.6$ Hz), 4.05 (t, 1H, $J = 6.4$ Hz), 2.95 (d, 2H, $J = 6.4$ Hz), 2.24 (s, 3H). Anal. ($C_8H_{13}N_2O_7P$) C, H, N.

(*RS*)-2-Amino-3-[5-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic Acid Zwitterion (9c) Hydrate. 9c was prepared from **14c** according to general procedure C. The residue was dissolved in H_2O−EtOH (1:2) and the pH adjusted to ca. 2.5. Recrystallization (H_2O) of the obtained precipitate afforded **9c** (44 mg, 22%): mp 177−180 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.25 (d, 2H, $J = 9$ Hz), 4.05 (t, 1H, $J = 6.3$ Hz), 2.95 (d, 2H, $J = 6.3$ Hz), 2.55 (t, 2H, $J = 7$ Hz), 1.5 (quintet, 2H, $J = 7$ Hz), 1.2 (sextet, 2H, $J = 7$ Hz), 0.75 (t, 3H, $J = 7$ Hz). Anal. ($C_{11}H_{19}N_2O_7P \cdot 1.5H_2O$) C, H, N.

(*RS*)-2-Amino-3-[3-(phosphonomethoxy)-5-(1-propylbutyl)-4-isoxazolyl]propionic Acid Zwitterion (9d) Monohydrate. 9d was prepared from **14d** according to general procedure C. The residue was dissolved in H_2O−EtOH (1:2) and the pH adjusted to ca. 2.5. Recrystallization (H_2O) of the precipitate gave **9d** (30 mg, 29%): mp 218−220 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.25 (d, 2H, $J = 9$ Hz), 3.95 (br t, 1H,

J = 7 Hz), 2.95−2.75 (m, 3H), 1.5 (m, 4H), 1.1 (m, 4H), 0.75 (t, 6H, J = 7 Hz). Anal. ($C_{14}H_{25}N_2O_7P\cdot H_2O$) C, H, N.

(*RS*)-2-Amino-3-[5-(2,2-dimethylpropyl)-3-(phosphonomethoxy)-4-isoxazolyl]propionic Acid Zwitterion (9e) Hydrate. 9e was prepared from **14e** according to general procedure C. The residue was dissolved in H_2O−EtOH (1:2) and the pH adjusted to ca. 2.5. Recrystallization (H_2O) of the precipitate gave **9e** (45 mg, 25%): mp 217−220 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.26 (d, 2H, J = 9.2 Hz), 4.13 (t, 1H, J = 6.6 Hz), 3.00 (m, 2H), 2.53 (d, 1H, J = 14.7 Hz), 2.47 (d, 1H, J = 14.7 Hz), 0.86 (s, 9H). Anal. ($C_{12}H_{21}N_2O_7P\cdot 0.75H_2O$) C, H, N.

Ethyl {[4-Methyl-5-(2-thienyl)-3-isoxazolyl]oxy}acetate (16g). 16g was prepared from **15g**[34] according to general procedure A and purified by flash chromatography [*n*-heptane−EtOAc−MeOH (20:10:1)] to give an oil, which crystallized upon standing (8.7 g, 59%). A small sample was recrystallized (1-propanol) to give **16g** as colorless crystals: mp 69−71 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.50−7.43 (m, 2H), 7.14 (dd, 1H, J = 4.8, 3.9 Hz), 4.86 (s, 2H), 4.28 (q, 2H, J = 7.1 Hz), 2.15 (s, 3H), 1.31 (t, 3H, J = 7.1 Hz). Anal. ($C_{12}H_{13}NO_4S$) C, H, N.

Diethyl {[(5-*tert*-Butyl-4-methyl-3-isoxazolyl)oxy]methyl}phosphonate (17b). 17b was prepared from **15b**[11] according to general procedure B and purified by CC [tol−EtOAc (1:1) containing 1% AcOH] to give a colorless oil (350 mg, 59 mg). Distillation (15 mmHg, 230−240 °C) of a small sample gave **17b**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.55 (d, 2H, J = 8.7 Hz), 4.25 (q, 2H, J = 7.1 Hz), 4.21 (q, 2H, J = 7.1 Hz), 1.95 (s, 3H), 1.36 (t, 6H, J = 7.1 Hz), 1.34 (s, 9H). Anal. ($C_{13}H_{24}NO_5P$) C, H, N.

Diethyl {[(4-Methyl-5-phenyl-4-isoxazolyl)oxy]methyl}phosphonate (17f). A mixture of **15f**[15] (5.0 g, 29 mmol), K_2CO_3 (7.9 g, 57 mmol), and DMF (100 mL) was stirred at 80 °C for 30 min. Diethyl {[(methylsulfonyl)oxy]methyl}phosphonate (14 g, 57 mmol) dissolved in DMF (25 mL) was added and the reaction mixture stirred at 80 °C for 3.5 h. After cooling, the mixture was poured into H_2O (400 mL) at 0 °C and extracted with ether. The organic phase was dried and evaporated. Flash chromatography [*n*-heptane−EtOAc−TEA (10:10:1)] afforded **17f** as a colorless oil (2.6 g, 28%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.76−7.65 (m, 2H), 7.55−7.40 (m, 3H), 4.64 (d, 2H, J = 8.6 Hz), 4.35−4.15 (m, 4H), 2.13 (s, 3H), 1.37 (t, 6H, J = 7.1 Hz). Anal. ($C_{15}H_{20}NO_5P$) H, N; C: calcd, 55.38; found, 54.74.

Diethyl {{[4-Methyl-5-(2-thienyl)-3-isoxazolyl]oxy}methyl}phosphonate (17g). 17g was prepared from **15g**[34] (5.0 g, 28 mmol) by the method described for the synthesis of **17f**. Flash chromatography [*n*-heptane−EtOAc (1:2)] gave **17g** (3.0 g, 33%) as a yellow oil, which was used in the next step without further characterization: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.50−7.44 (m, 2H), 7.15 (dd, 1H, J = 4.8, 3.8 Hz), 4.63 (d, 2H, J = 8.6 Hz), 4.26 (q, 2H, J = 7.1 Hz), 4.23 (q, 2H, J = 7.1 Hz), 2.11 (s, 3H), 1.37 (t, 6H, J = 7.1 Hz).

Ethyl {[4-(Bromomethyl)-5-*tert*-butyl-3-isoxazolyl]oxy}acetate (18b). 18b was prepared from **16b**[23] according to general procedure D, yielding crude **18b** (12.0 g, 100%). A small sample was distilled (128−131 °C, 0.2 mmHg) to give **18b**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.85 (s, 2H), 4.38 (s, 2H), 4.27 (q, 2H, J = 7.1 Hz), 1.41 (s, 9H), 1.30 (t, 3H, J = 7.1 Hz). Anal. ($C_{12}H_{18}BrNO_4$) C, H, N.

Ethyl {[4-(Bromomethyl)-5-(2-thienyl)-3-isoxazolyl]oxy}acetate (18g). 18g was prepared from **16g** according to general procedure D. Crude **18g** (2.8 g, 88%) crystallized upon standing: mp 79−84 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.62 (dd, 1H, J = 3.8, 1.1 Hz), 7.57 (dd, 1H, J = 5.1, 1.1 Hz), 7.19 (dd, 1H, J = 5.1, 3.8 Hz), 4.90 (s, 2H), 4.47 (s, 2H), 4.27 (q, 2H, J = 7.1 Hz), 1.30 (t, 3H, J = 7.1 Hz). The crude product was used for the next step without further characterization.

Diethyl {{[4-(Bromomethyl)-5-*tert*-butyl-3-isoxazolyl]oxy}methyl}phosphonate (19b). 19b was prepared from **17b** according to general procedure D and purified by CC [cyclohexane−EtOAc (1:1)] to give crude **19b** (1.1 g, 87%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.59 (d, 2H, J = 9 Hz), 4.34 (s, 2H), 4.27 (q, 2H, J = 7 Hz), 4.23 (q, 2H, J = 7 Hz), 1.41 (s, 9H), 1.35 (t, 6H, J = 7 Hz). The crude product was used in the next step without further characterization.

Diethyl {{[4-(Bromomethyl)-5-phenyl-3-isoxazolyl]oxy}methyl}phosphonate (19f). 19f was prepared from **17f** according to general procedure D. Crude product (3.0 g, 100%) was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.85−7.74 (m, 2H), 7.60−7.50 (m, 3H), 4.69 (d, 2H, J = 8.8 Hz), 4.42 (s, 2H), 4.29 (q, 2H, J = 7.1 Hz), 4.26 (q, 2H, J = 7.1 Hz), 1.39 (t, 6H, J = 7.1 Hz).

Diethyl {{[4-(Bromomethyl)-5-(2-thienyl)-3-isoxazolyl]oxy}methyl}phosphonate (19g). 19g was prepared from **17g** according to general procedure D. Crude product (3.7 g, 100%): ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.65−7.58 (m, 2H), 7.21 (dd, 1H, J = 5.0, 3.8 Hz), 4.67 (d, 2H, J = 8.8 Hz), 4.44 (s, 2H), 4.28 (q, 2H, J = 7.1 Hz), 4.25 (q, 2H, J = 7.1 Hz), 1.39 (t, 6H, J = 7.1 Hz). The crude product was used in the next step without further purification.

Ethyl 3-{5-*tert*-Butyl-3-[(ethoxycarbonyl)methoxy]-4-isoxazolyl}-2-[*N*-(*tert*-butyloxycarbonyl)amino]-2-(ethoxycarbonyl)propionate (20b). To a suspension of NaH (1.6 g, 60% suspension, 41 mmol) in DMF (37 mL) was added a solution of diethyl [*N*-(*tert*-butyloxycarbonyl)amino]malonate[24] (11.2 g, 41 mmol) in DMF (37 mL). After stirring for 15 min, a solution of **18b** (11.8 g, 37 mmol) in DMF (15 mL) was added. The reaction mixture was stirred for 18 h at room temperature and evaporated. An ice-cold solution of the residue in $CHCl_3$ (300 mL) was washed with ice-cold H_2O (100 mL), dried, and evaporated. CC (tol containing 0−50% EtOAc) of the residue gave crude **20b** as an oil (16 g, 84%): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.22 (s, 1H), 4.72 (s, 2H), 4.40−4.05 (m, 6H), 3.52 (s, 2H), 1.41 (s, 9H), 1.34 (s, 9H), 1.25 (t, 9H, J = 7.2 Hz). The crude product was used in the next step without further characterization.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-{3-[(ethoxycarbonyl)methoxy]-5-(2-thienyl)-4-isoxazolyl}propionate (20g). A mixture of diethyl acetamidomalonate (3.5 g, 16.2 mmol) and potassium *tert*-butoxide (1.9 g, 17 mmol) in *N*-methylpyrrolidone (30 mL) was stirred at room temperature for 30 min. Compound **18g** (2.8 g, 8.1 mmol) in *N*-methylpyrrolidone (5 mL) was added, and the resulting mixture was stirred for 1 h and then poured into H_2O (250 mL) at 0 °C. The aqueous phase was extracted with ether (3 × 200 mL), and the combined organic phases were washed with brine (200 mL), dried, and evaporated. Flash chromatography [*n*-heptane−EtOAc−MeOH (20:10:1)] followed by another flash chromatography [CH_2Cl_2−EtOAc (7:1)] gave crude **20g** as a greenish oil (2.3 g, 59%). A small sample was recrystallized (EtOH) to give **20g**: mp 92−94 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.51 (dd, 1H, J = 3.7, 1.0 Hz), 7.47 (dd, 1H, J = 5.1, 1.0 Hz), 7.13 (dd, 1H, J = 5.1, 3.7 Hz), 7.07 (br s, 1H), 4.81 (s, 2H), 4.29 (q, 2H, J = 7.1 Hz), 4.29−3.90 (m, 4H), 3.73 (s, 2H), 1.81 (s, 3H), 1.32 (t, 3H, J = 7.1 Hz), 1.17 (t, 6H, J = 7.1 Hz). Anal. ($C_{21}H_{26}N_2O_9S$) C, H, N.

Methyl 2-Acetamido-3-{5-*tert*-butyl-3-[(diethoxyphosphoryl)methoxy]-4-isoxazolyl}-2-(methoxycarbonyl)propionate (21b). To a suspension of NaH (32 mg, 60% dispersion, 0.78 mmol) in DMF (10 mL) under nitrogen was added a solution of dimethyl acetamidomalonate (135 mg, 0.84 mmol) in DMF (2 mL). After stirring for 15 min a solution of **19b** (250 mg, 0.71 mmol) in DMF (3 mL) was added and stirring continued for 18 h. The reaction mixture was evaporated (1 mmHg), H_2O added, and extraction performed with EtOAc. The organic phase was dried and evaporated and the residue purified by CC [tol−EtOAc (1:4)] and recrystallized (EtOAc−light petroleum) to give **21b** (185 mg, 53%): mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.52 (s, 1H), 4.53 (d, 2H, J = 8.1 Hz), 4.24 (q, 2H, J = 7.1 Hz), 4.20 (q, 2H, J = 7.1 Hz), 3.75 (s, 6H), 3.61 (s, 2H), 2.03 (s, 3H), 1.36 (t, 6H, J = 7.1 Hz), 1.35 (s, 9H). Anal. ($C_{20}H_{33}N_2O_{10}P$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-{3-[(diethoxyphosphoryl)methoxy]-5-phenyl-4-isoxazolyl}propionate (21f). 21f was prepared from **19f** (3.0 g, 7.4 mmol) by the procedure described for compound **20g**. Flash chromatography [*n*-heptane−EtOAc (1:4)] and recrystallization (EtOAc−*n*-heptane) gave **21f** (1.6 g, 40%) as colorless crystals: mp 89−91 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.70−7.60

(m, 2H), 7.53−7.42 (m, 3H), 6.67 (br s, 1H), 4.61 (d, 2H, J = 8.5 Hz), 4.32−4.09 (m, 6H), 3.99−3.84 (m, 2H), 3.70 (s, 2H), 1.64 (s, 3H), 1.37 (t, 6H, J = 7.1 Hz), 1.15 (t, 6H, J = 7.1 Hz). Anal. ($C_{24}H_{33}N_2O_{10}P$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-{3-[(diethoxyphosphonyl)methoxy]-5-(2-thienyl)-4-isoxazolyl}propionate (21g). **21g** was prepared from **19g** (3.7 g, 9.1 mmol) by the procedure described for compound **20g**. Flash chromatography [*n*-heptane−EtOAc (1:4) followed by EtOAc] gave crude **21g** (2.8 g, 60%). A small sample was recrystallized (2-propanol) to give **21g**: mp 104−105 °C; ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.55−7.46 (m, 2H), 7.14 (dd, 1H, J = 5.1, 3.8 Hz), 6.91 (br s, 1H), 4.60 (d, 2H, J = 8.4 Hz), 4.31−3.95 (m, 8H), 3.70 (s, 2H), 1.82 (s, 3H), 1.37 (t, 6H, J = 7.1 Hz), 1.17 (t, 6H, J = 7.1 Hz). Anal. ($C_{22}H_{31}N_2O_{10}PS$) C, H, N.

(*RS*)-2-Amino-3-[5-*tert*-butyl-3-(carboxymethoxy)-4-isoxazolyl]propionic Acid Zwitterion (8b) Hemihydrate. To a solution of **20b** (16 g, 31 mmol) in MeOH (110 mL) was added 2 M NaOH (5.55 mL), and the mixture was boiled under reflux for 3 h and then evaporated. The residue was dissolved in 1 M HCl (148 mL), boiled under reflux for 30 min, and evaporated. The residue was dissolved in H_2O and the pH adjusted to ca. 3.5 by addition of TEA. Recrystallization (H_2O) of the obtained precipitate afforded **8b** (6.2 g, 68%): mp 233−235 °C dec; ^{1}H NMR (D_2O, CF_3COOD, 200 MHz) δ 4.87 (s, 2H), 4.26 (br t, 1H, J = 7.4 Hz), 3.24 (dd, 1H, J = 15.5, 6.7 Hz), 3.08 (dd, 1H, J = 15.5, 7.9 Hz), 1.30 (s, 9H). Anal. ($C_{12}H_{18}N_2O_6 \cdot 0.5H_2O$) C, H, N.

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-(2-thienyl)-4-isoxazolyl]propionic Acid Zwitterion (8g) Hydrate. **8g** was prepared from **20g** according to general procedure C. Treatment of the residue with H_2O gave **8g** as colorless crystals (1.0 g, 71%): mp 228−230 °C dec; ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.86 (d, 1H, J = 5.0 Hz), 7.69 (d, 1H, J = 3.8 Hz), 7.26 (dd, 1H, J = 5.0, 3.8 Hz), 4.69 (s, 2H), 3.79 (t, 1H, J = 7 Hz), 3.15 (dd, 1H, J = 15.2, 5.7 Hz), 2.97 (dd, 1H, J = 15.2, 8.2 Hz). Anal. ($C_{12}H_{12}N_2O_6S \cdot 1.25H_2O$) C, H, N.

(*RS*)-2-Amino-3-[5-*tert*-butyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic Acid Zwitterion (9b) Monohydrate. **9b** was prepared from **14b** according to general procedure C. The residue was dissolved in H_2O−EtOH (1:2) and the pH adjusted to ca. 2.5. Recrystallization (H_2O) of the obtained precipitate gave **9b** as a colorless powder (99 mg, 50%): mp 218−220 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.25 (d, 2H, J = 9 Hz), 4.1 (t, 1H, J = 7 Hz), 3.15 (dd, 1H, J = 15, 7 Hz), 2.9 (dd, 1H, J = 15, 7 Hz), 1.25 (s, 9H). Anal. ($C_{11}H_{19}N_2O_7P \cdot H_2O$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-[5-phenyl-3-(phosphonomethoxy)-4-isoxazolyl]propionate. A solution of compound **21f** (1.2 g, 2.2 mmol) and trimethylsilyl bromide (1.5 mL, 11 mmol) in CH_3CN (25 mL) was stirred at room temperature for 24 h. The mixture was boiled under reflux for 30 min and then evaporated to dryness. After addition of H_2O (25 mL) and acetone (30 mL), the mixture was stirred at room temperature for 1 h and the acetone was evaporated. The aqueous phase was extracted with EtOAc and the organic phase washed with brine. After drying and evaporation, ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[5-phenyl-3-(phosphonomethoxy)-4-isoxazolyl]propionate (1.07 g, 99%) was obtained: mp 167−169 °C dec; ^{1}H NMR ($CDCl_3$, DMSO-d_6, 250 MHz) δ 7.70−7.57 (m, 2H), 7.53−7.41 (m, 3H), 6.96 (br s, 1H), 4.56 (d, 2H, J = 9.2 Hz), 4.18−4.02 (m, 2H), 3.91−3.75 (m, 2H), 3.69 (s, 2H), 1.74 (s, 3H), 1.11 (t, 6H, J = 7.1 Hz). Anal. ($C_{20}H_{25}N_2O_{10}P$) C, H, N.

(*RS*)-2-Amino-3-[5-phenyl-3-(phosphonomethoxy)-4-isoxazolyl]propionic Acid Zwitterion (9f) Monohydrate. **9f** was prepared from ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[5-phenyl-3-(phosphonomethoxy)-4-isoxazolyl]propionate according to general procedure C. The residue was dissolved in H_2O and the pH adjusted to ca. 3 by addition of 0.1 M NaOH, which afforded **9f** (500 mg, 63%) as a colorless precipitate: mp 231−232 °C dec; ^{1}H NMR (DMSO-d_6, CF_3COOH, 250 MHz) δ 7.77−7.66 (m, 2H), 7.64−7.51 (m, 3H), 4.45 (dd, 2H, J = 8.8, 1.1 Hz), 4.14 (br s, 1H), 3.25−3.00 (m, 2H). Anal. ($C_{13}H_{15}N_2O_7P \cdot H_2O$) C, H, N.

(*RS*)-2-Amino-3-[3-(phosphonomethoxy)-5-(2-thienyl)-4-isoxazolyl]propionic Acid Zwitterion (9g) Monohydrate. **9g** was prepared from compound **21g** (1.0 g, 1.9 mmol) by the procedure described for ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[5-phenyl-3-(phosphonomethoxy)-4-isoxazolyl]propionate followed by general procedure C. The residue was dissolved in H_2O and the pH adjusted to ca. 2.5 with 0.1 M NaOH. Treatment of the precipitate with H_2O gave **9g** (420 mg, 66%): mp 220−221 °C dec; ^{1}H NMR (DMSO-d_6, CF_3COOH, 250 MHz) δ 7.90 (d, 1H, J = 4.8 Hz), 7.65 (d, 1H, J = 3.3 Hz), 7.29 (t, 1H, J = 4.3 Hz), 4.44 (d, 2H, J = 8.6 Hz), 4.11 (br s, 1H), 3.28−2.99 (m, 2H). Anal. ($C_{11}H_{13}N_2O_7PS \cdot H_2O$) C, H, N.

Ethyl (*RS*)-2-Amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)-propionate Hydrochloride (22a). To a solution of acetyl chloride (26 mL, 366 mmol) in EtOH (130 mL) was added **6f**[23] (1.40 g, 5.64 mmol). The reaction mixture was boiled under reflux for 1.5 h, evaporated, and reevaporated twice from tol. Recrystallization (EtOH) gave **22a** (1.46 g, 83%): mp 200−201 °C dec; ^{1}H NMR (D_2O, 200 MHz, CH_3CN δ 2.0) δ 7.65−7.45 (5H, m), 4.28 (t, 1H, J = 13.1 Hz), 4.1−3.8 (2H, m), 3.25 (dd, 1H, J = 6.2, 15.5 Hz), 3.16 (dd, 1H, J = 6.9, 15.5 Hz), 1.02 (t, 3H, J = 7.2 Hz). Anal. ($C_{14}H_{17}ClN_2O_4$) C, H, N.

Ethyl (*RS*)-2-[*N*-(*tert*-Butyloxycarbonyl)amino]-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionate (22b). To an ice-cold solution of **22a** (700 mg, 2.24 mmol) in EtOH (30 mL) were added TEA (937 μL, 6.72 mmol) and a solution of di-*tert*-butyl dicarboxylate (BocOBoc) (1.30 mL, 5.57 mmol) in EtOH (5 mL), and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was evaporated, and H_2O (40 mL) and EtOAc (40 mL) were added. The mixture was cooled on ice and, while stirring, acidified with AcOH. The phases were separated, and the aqueous phase was extracted with EtOAc (2 × 40 mL). The combined and dried organic phases were filtered and evaporated. Recrystallization (EtOAc) gave TLC-pure **22b** (640 mg, 76%). A small sample was recrystallized (EtOAc) to give **22b**: mp 128.5−129.0 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.71 (m, 2H), 7.49 (m, 3H), 6.38 (br s, 1H), 5.44 (br d, 1H, J = 8 Hz), 4.55 (m, 1H), 4.25−3.90 (m, 2H), 3.11 (m, 2H), 1.40 (s, 9H), 1.18 (t, 3H, J = 7 Hz). Anal. ($C_{19}H_{24}N_2O_6$) C, H, N.

Ethyl (*RS*)-2-[*N*-(*tert*-Butyloxycarbonyl)amino]-3-{3-[(ethoxycarbonyl)methoxy]-5-phenyl-4-isoxazolyl}propionate (23). **23** was prepared from **22b** according to general procedure A and purified by CC [tol−EtOAc (2:1)] to give a colorless solid (330 mg, 54%). A small sample was recrystallized (EtOAc−light petroleum) to give **23** as colorless crystals: mp 72.5−73.5 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.69 (m, 2H), 7.48 (m, 3H), 5.5 (br d, 1H, J = 8.5 Hz), 4.95 (d, 1H, J = 16 Hz), 4.84 (d, 1H, J = 16 Hz) 4.58 (m, 1H), 4.29 (q, 2H, J = 7.15 Hz), 4.20−3.85 (m, 2H), 3.12 (d, 2H, J = 6.3 Hz), 1.37 (s, 9H), 1.32 (t, 3H, J = 7.15 Hz), 1.16 (t, 3H, J = 7.15 Hz). Anal. ($C_{23}H_{30}N_2O_8$) C, H, N.

Ethyl (*RS*)-2-Amino-3-{3-[(ethoxycarbonyl)methoxy]-5-phenyl-4-isoxazolyl}propionate Hydrochloride (24). To an ice-cold solution of HCl in EtOAc (10 mL, 2.7 M) was added **23** (240 mg, 0.52 mmol), and the mixture was stirred at 0 °C for 2 h. The reaction mixture was evaporated, reevaporated twice from tol, and dried. The crude crystalline product was recrystallized (EtOAc) to give **24** (156 mg, 75%): mp 132.0−132.5 °C; ^{1}H NMR (D_2O, 200 MHz, DOH δ 4.7) δ 7.60 (m, 2H), 7.50 (m, 3H), 4.91 (s, 2H), 4.29 (t, 1H, J = 6.4 Hz), 4.22 (q, 2H, J = 7.2 Hz), 4.00−3.65 (m, 2H), 3.27 (m, 2H), 1.20 (t, 3H, J = 7.2 Hz), 0.95 (t, 3H, J = 7.2 Hz). Anal. ($C_{18}H_{23}ClN_2O_6$) C, H, N.

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-phenyl-4-isoxazolyl]propionic Acid Zwitterion (8f). A mixture of **24** (150 mg, 0.38 mmol), TEA (300 μL, 2.15 mmol), and H_2O (5 mL) was stirred at room temperature overnight. The reaction mixture was evaporated and reevaporated twice from tol. The residue was dissolved in H_2O, the pH was adjusted to ca. 3.5 with 0.1 M HCl, and the solution was left at 5 °C. Crude **8f** (80 mg, 69%) precipitated, and recrystallization (H_2O) afforded **8f**: mp 236−238 °C dec; ^{1}H NMR (D_2O, NaOD, 200 MHz, CH_3CN δ 2.00) δ 7.70 (m, 2H), 7.50 (m, 3H), 4.61 (s, 2H), 3.46 (dd, 1H, J = 6.0, 8.5 Hz), 2.93 (dd, 1H, J = 6.0, 14.7 Hz), 2.73 (dd, 1H, J = 8.5, 14.7 Hz). Anal. ($C_{14}H_{14}N_2O_6$) C, H, N.

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-{3-[(diethoxyphosphoryl)methoxy]-5-methyl-4-isoxazolyl}propionate (26). **26** was prepared from compound **25**[25] (2.0 g, 6.1 mmol) according to general procedure B, purified by CC [tol−EtOAc (1:4)], and recrystallized (EtOAc−light petroleum) to give **26** (0.8 g, 27%) as colorless crystals: mp 68−70 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.8 (br s, 1H), 4.53 (d, 2H, J = 8.5 Hz), 4.35−4.10 (m, 8H), 3.36 (s, 2H), 2.21 (s, 3H), 2.04 (s, 3H), 1.34 (t, 6H, J = 7.1 Hz), 1.26 (t, 6H, J = 7.1 Hz). Anal. ($C_{19}H_{31}N_2O_{10}P$) H, N; C: calcd, 47.69; found, 48.11.

Ethyl 2-Acetamido-3-{5-(bromomethyl)-3-[(diethoxyphosphoryl)methoxy]-4-isoxazolyl}-2-(ethoxycarbonyl)-propionate (27). **27** was prepared from **26** according to general procedure D, purified by CC [tol−EtOAc (4:1)], and recrystallized (EtOAc−light petroleum) to give **27** (360 mg, 41%): mp 66−67 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.9 (s, 1H), 4.55 (d, 2H, J = 8.4 Hz), 4.29 (s, 2H), 4.35−4.12 (m, 8H), 3.46 (s, 2H), 2.06 (s, 3H), 1.35 (t, 6H, J = 7.1 Hz), 1.26 (t, 6H, J = 7.1 Hz). Anal. ($C_{19}H_{30}BrN_2O_{10}P$) C, H, N.

Ethyl 6-Acetyl-5-(ethoxycarbonyl)-3-[(diethoxyphosphoryl)methoxy]-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylate (28). A solution of **27** (360 mg, 0.65 mmol) in CH_3CN (5 mL) was added to a suspension of NaH (51 mg, 60% dispersion, 1.3 mmol) in CH_3CN at 0 °C. The mixture was stirred at room temperature for 1 h and then acidified with AcOH and evaporated to dryness. H_2O was added and extraction performed with EtOAc. After drying, evaporation, and CC [tol−EtOAc (4:1)], recrystallization (EtOAc−light petroleum) afforded **25** (213 mg, 69%): mp 71−72 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.66 (br s, 2H), 4.56 (d, 2H, J = 8.9 Hz), 4.3−4.1 (m, 8H), 3.24 (br s, 2H), 2.24 (s, 3H), 1.36 (t, 6H, J = 7.1 Hz), 1.26 (t, 6H, J = 7.1 Hz). Anal. ($C_{19}H_{29}N_2O_{10}P$) C, H, N.

(*RS*)-3-(Phosphonomethoxy)-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic Acid Zwitterion (10c). **10c** was prepared from **28** according to general procedure C. The residue was dissolved in H_2O−EtOH (1:2) and the pH adjusted to ca. 2.5. Recrystallization (H_2O) of the precipitate gave **10c** (34 mg, 29%): mp 228−230 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 4.4 (d, 1H, J = 16 Hz), 4.25 (m, 3H), 4.12 (dd, 1H, J = 10.5, 5 Hz), 3.05 (dd, 1H, J = 16, 5 Hz), 2.75 (dd, 1H, J = 16, 10.5 Hz). Anal. ($C_8H_{11}N_2O_7P$) C, H, N.

Ethyl [(5-Methyl-3-isoxazolyl)oxy]acetate (30). **30** was prepared from tachigaren[26] (**29**) according to general procedure A and purified by flash chromatography [tol−EtOAc (19:1)] to give an oil (2.8 g, 30%). A small sample was distilled (0.2 mmHg, 85−86 °C) to give **30** as a colorless oil: ^{1}H NMR (CCl_4, 60 MHz) δ 5.6 (s, 1H), 4.7 (s, 2H), 4.2 (q, 2H, J = 7 Hz), 2.35 (s, 3H), 1.3 (t, 3H, J = 7 Hz). Anal. ($C_8H_{11}NO_4$) H, N; C: calcd, 51.88; found, 51.47.

Diethyl {[(5-Methyl-3-isoxazolyl)oxy]methyl}phosphonate (31). **31** was prepared from tachigaren[26] (**29**) according to general procedure B and purified by CC [CH_2Cl_2−EtOAc (6:1)] to give **31** (3.1 g, 25%) as a pale yellow oil, which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.70 (s, 1H), 4.54 (d, 2H, J = 8.9 Hz), 4.24 (q, 2H, J = 7.1 Hz), 4.20 (q, 2H, J = 7.1 Hz), 2.34 (s, 3H), 1.36 (t, 6H, J = 7.1 Hz).

Diethyl {{[5-(Bromomethyl)-3-isoxazolyl]oxy}methyl}-phosphonate (33). **33** was prepared from **31** according to general procedure D and purified by CC [CH_2Cl_2−EtOAc (6:1)] to give crude **33** (1.0 g, 27%), which was used in the next step without further purification: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.04 (s, 1H), 4.56 (d, 2H, J = 8.9 Hz), 4.34 (s, 2H), 4.24 (q, 2H, J = 7.1 Hz), 4.20 (q, 2H, J = 7.1 Hz), 1.36 (t, 6H, J = 7.1 Hz).

Ethyl 2-Acetamido-2-(ethoxycarbonyl)-3-{3-[(ethoxycarbonyl)methoxy]-5-isoxazolyl}propionate (34). From **30** was, according to general procedure D, prepared crude ethyl {[5-(bromomethyl)-3-isoxazolyl]oxy}acetate (**32**) (3.0 g, 100%). To a solution of sodium ethoxide prepared from Na (265 mg, 11.5 mmol) and EtOH (30 mL) was added diethyl acetamidomalonate (2.5 g, 1.5 mmol). To this mixture was added a solution of **32** (3.0 g, 11.5 mmol) in EtOH (20 mL) followed by reflux for 4 h. After evaporation, H_2O was added and extraction performed with CH_2Cl_2. The organic phase was extracted with ice-cold 1 M NaOH, dried, and evaporated. Flash chromatography [tol−EtOAc (3:1)] gave, after recrystallization (tol−cyclohexane), **31** (666 mg, 15%): mp 85−86 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 6.8 (br s, 1H), 5.7 (s, 1H), 4.8 (s, 2H), 4.2 (q, 6H, J = 7 Hz), 3.8 (s, 2H), 2.0 (s, 3H), 1.3 (t, 9H, J = 7 Hz). Anal. ($C_{17}H_{24}N_2O_9$) C, H, N.

Methyl 2-Acetamido-3-{3-[(diethoxyphosphoryl)methoxy]-5-isoxazolyl}-2-(methoxycarbonyl)propionate (35). **35** was prepared from **33** (1.0 g, 3 mmol) by the method described for compound **21b**. Recrystallization (2-propanol) afforded **35** (802 mg, 61%): mp 106−107 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.77 (s, 1H), 5.72 (s, 1H), 4.51 (d, 2H, J = 9.0 Hz), 4.24 (q, 2H, J = 7.1 Hz), 4.20 (q, 2H, J = 7.1 Hz), 3.84 (s, 6H), 3.79 (s, 2H), 2.05 (s, 3H), 1.36 (t, 6H, J = 7.1 Hz). Anal. ($C_{16}H_{25}N_2O_{10}P$) C, H, N.

(*RS*)-2-Amino-3-[3-(carboxymethoxy)-5-isoxazolyl]propionic Acid Zwitterion (11b). **11b** was prepared from **34** according to general procedure C. Recrystallization (H_2O) afforded **11b** (125 mg, 69%): mp 252−255 °C dec; ^{1}H NMR (D_2O, NaOD, 60 MHz) δ 6.2 (s, 1H), 4.7 (s, 2H), 3.7 (dd, 1H, J = 7.5, 5.5 Hz), 3.1 (m, 2H). Anal. ($C_8H_{10}N_2O_6$) C, H, N.

(*RS*)-2-Amino-3-[3-(phosphonomethoxy)-5-isoxazolyl]-propionic Acid Zwitterion (11c). **11c** was prepared from **35** according to general procedure C. The residue was dissolved in H_2O−EtOH (1:2) and the pH adjusted to ca. 2.5. Recrystallization (H_2O) of the precipitate gave **11c** (89 mg, 35%): mp 209−210 °C dec; ^{1}H NMR (D_2O, 200 MHz) δ 6.05 (s, 1H), 4.25 (m, 1H), 4.20 (d, 2H, J = 8.9 Hz), 3.31 (br d, 1H, J = 6.1 Hz). Anal. ($C_7H_{11}N_2O_7P$) C, H, N.

Receptor Binding Assays. Affinity for AMPA receptors was determined using the ligands [^{3}H]AMPA[27] and [^{3}H]-CNQX,[28] and for determination of NMDA and kainic acid receptor affinities, [^{3}H]CPP[9] and [^{3}H]kainic acid,[29] respectively, were used. The membrane preparations used in all the receptor binding experiments were prepared according to the method of Ransom and Stec.[35]

In Vitro Electrophysiology. A rat cortical slice preparation for determination of excitatory amino acid-evoked depolarizations described by Harrison and Simmonds[32] was used in a slightly modified version. 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 Ag/AgCl pellet electrode. The cortex and corpus callosum parts were constantly superfused with a Mg^{2+}-free oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium, and the potential difference between the electrodes was recorded on a chart recorder. For determination of IC_{50} values, inhibition curves were constructed. The depolarization induced by 5 μM AMPA was inhibited with increasing concentrations of the antagonist in question, and for compounds **10b**,**c**, the depolarizations induced by 10 μM NMDA were inhibited. Application of agonists were done for 90 s, and for antagonist experiments, the antagonists were applied alone for 90 s followed by coapplication of the agonist and antagonist for another 90 s.

Acknowledgment. This work was supported by grants from the Lundbeck Foundation, EEC (BIO2-CT93-0243), and The Danish State Biotechnology Programme (1991−1995). The technical assistance of Ulla Geneser, Jørgen S. Johansen, and Brian Rosenberg is gratefully acknowledged.

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JM950826P