Heteroaryl Analogues of AMPA. 2. Synthesis, Absolute Stereochemistry, Photochemistry, and Structure−Activity Relationships

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We have previously shown that (*S*)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionic acid [(*S*)-APPA, **2**] is a weak agonist at (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors, specifically activated by (*S*)-AMPA (**1**), whereas (*S*)-2-amino-3-[3-hydroxy-5-(2-pyridyl)-4-isoxazolyl]propionic acid [(*S*)-2-Py-AMPA, **5**] and (*RS*)-2-amino-3-[3-hydroxy-5-(2-thiazolyl)-4-isoxazolyl]propionic acid (**4**) are potent AMPA agonists. On the other hand, (*R*)-APPA (**3**) and (*R*)-2-Py-AMPA (**6**) have been shown to be weak AMPA antagonists. We now report the synthesis of 2-Py-AMPA (**7a**) and the isomeric compounds 3-Py-AMPA (**7b**) and 4-Py-AMPA (**7c**) as well as the **7a** analogues, (*RS*)-2-amino-3-[3-hydroxy-5-(6-methyl-2-pyridyl)-4-isoxazolyl]propionic acid (**7d**) and (*RS*)-2-amino-3-[3-hydroxy-5-(2-quinolinyl)-4-isoxazolyl]propionic acid (**7e**). Furthermore, (*RS*)-2-amino-3-[3-hydroxy-5-(2-furyl)-4-isoxazolyl]-propionic acid (2-Fu-AMPA, **7f**) and its 5-bromo-2-furyl derivative (**7g**) were synthesized, and (*S*)-2-Fu-AMPA (**8**) and (*R*)-2-Fu-AMPA (**9**) were prepared by semipreparative chiral HPLC resolution of **7f**. HPLC analyses and circular dichroism spectroscopy indicated the absolute stereochemistry of **8** and **9** to be *S* and *R*, respectively. This was confirmed by an X-ray crystallographic analysis of **9**·HCl. In receptor binding (IC_{50} values) and rat cortical wedge electrophysiological (EC_{50} values) studies, **7c** ($IC_{50} = 5.5 \pm 0.6$ μM; $EC_{50} = 96 \pm 5$ μM) was shown to be markedly weaker than **7a** ($IC_{50} = 0.57 \pm 0.16$ μM; $EC_{50} = 7.4 \pm 0.2$ μM) as an AMPA agonist, whereas **7b**,**d**,**e** were inactive. The very potent AMPA agonist effect of **7f** ($IC_{50} = 0.15 \pm 0.03$ μM; $EC_{50} = 1.7 \pm 0.2$ μM) was shown to reside exclusively in **8** ($IC_{50} = 0.11 \pm 0.01$ μM; $EC_{50} = 0.71 \pm 0.11$ μM), whereas **9** did not interact significantly with AMPA receptors, either as an agonist or as an antagonist. **8** was shown to be photochemically active and is a potential photoaffinity label for the recognition site of the AMPA receptors. Compound **7g** turned out to be a very weak AMPA receptor agonist ($IC_{50} = 12 \pm 0.7$ μM; $EC_{50} = 160 \pm 15$ μM). None of these new compounds showed detectable effects at *N*-methyl-D-aspartic acid (NMDA) or kainic acid receptors in vitro. The present studies have emphasized that the presence of a heteroatom in the 2-position of the heteroaryl 5-substituent greatly facilitates AMPA receptor agonist activity.

Introduction

The central excitatory amino acid (EAA) neurotransmitter (*S*)-glutamic acid [(*S*)-Glu] operates through three heterogeneous classes of ionotropic receptors named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainic acid receptors[1−4] and a number of subtypes of metabotropic receptors.[5] These or perhaps distinct subtypes of these receptors have been associated with certain central nervous system (CNS) diseases and are potential therapeutic targets in such diseases.[6,7]

Our projects on the design of therapeutically useful EAA receptor ligands have recently been focused on aryl and heteroaryl analogues of AMPA. This line of research was initiated by the synthesis of (*RS*)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionic acid (APPA)[8] and the observation that (*S*)-APPA (**2**) (Figure 1), like (*S*)-AMPA (**1**),[9] shows AMPA receptor agonism, whereas (*R*)-APPA (**3**) is an AMPA antagonist.[10] When administered together, **2** and **3** produced functional partial agonism.[10−12] Like (*RS*)-2-amino-3-[3-hydroxy-5-(2-thiazolyl)-4-isoxazolyl]propionic acid (**4**),[13,14] (*S*)-2-amino-3-[3-hydroxy-5-(2-pyridyl)-4-isoxazolyl]propionic acid [(*S*)-2-Py-AMPA, **5**] (Figure 1) is markedly more potent than **2** as an AMPA agonist, whereas (*R*)-2-Py-AMPA (**6**) and **3** are equipotent as AMPA antagonists.[15] The enantiomers of 2-Py-AMPA[15] as well as the *S*- and *R*-forms of the 4-fluorophenyl analogue of APPA[16] also produce functional partial agonism after coadministration.

On the basis of these observations and structure−activity studies on these heteroaryl analogues of AMPA containing five-membered heterocyclic rings, we concluded that the presence of a 2-positioned heteroatom of the ring substituent at the 5-position greatly enhances AMPA receptor binding affinity and agonist potency.[14]

To further substantiate this correlation between structural, stereochemical, and electrostatic characteristics of AMPA analogues and their AMPA agonist potencies, and as an attempt to develop analogous

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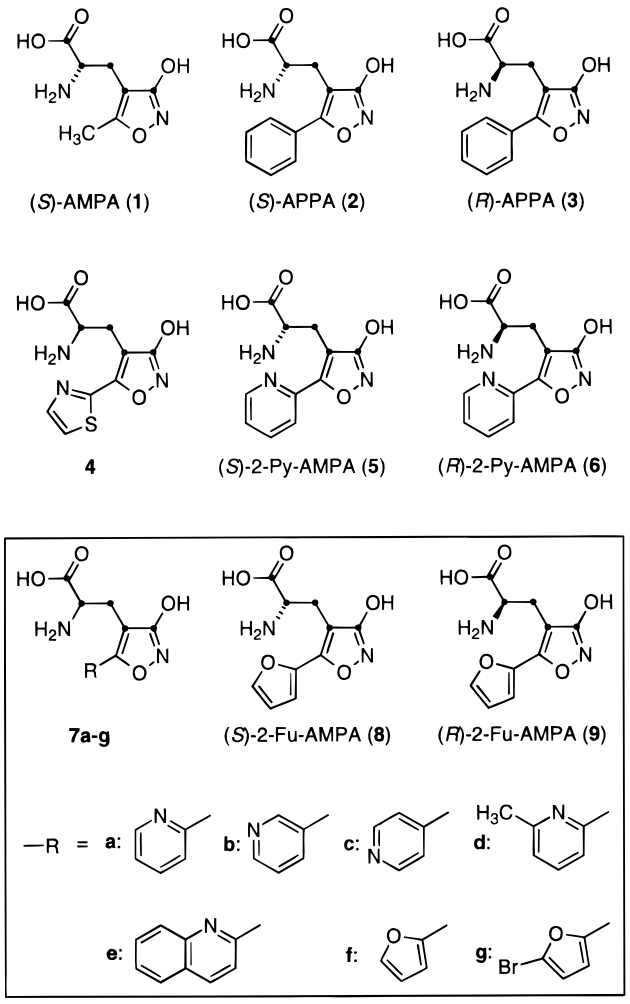


Figure 1. Structures of (*S*)-AMPA (**1**), (*S*)-APPA (**2**), (*R*)-APPA (**3**), and a number of AMPA analogues containing heteroaromatic 5-substituents.

correlations for structurally related AMPA antagonists, we have now synthesized and pharmacologically characterized the Py-AMPA analogues **7a**−**e**, (*RS*)-2-amino-3-[3-hydroxy-5-(2-furyl)-4-isoxazolyl]propionic acid (2-Fu-AMPA, **7f**), and its bromo-substituted analogue **7g** and optical isomers, (*S*)-2-Fu-AMPA (**8**) and (*R*)-2-Fu-AMPA (**9**) (Figure 1). A further aim of this work was to develop a potent and photochemically active AMPA agonist for future radio- and photolabeling of AMPA receptor recognition site(s).

Results

Chemistry. It has been reported[17] that addition of bromine to 3-pyridylpropenoates is accompanied by precipitation of perbromide adducts, and a similar observation was made in connection with the conversion of **10a**−**e** into **11a**−**e** (Scheme 1). Compounds **11a**−**e** were, however, obtained as hydrobromides in high yields by addition of bromine to solutions of the hydrobromides of **10a**−**e** in glacial acetic acid, followed by heating (60 °C) of the suspensions of precipitated products. Treatment of some 2,3-dihalogenopropionates with hydroxylamine has previously been reported[18] to give 3-isoxazololes. Following this procedure, compounds **11a**−**e** were converted into the 3-isoxazolols **12a**−**e** in good yields. Methylation of compounds **12a**−**e** gave mixtures of O-methylated (**13a**−**e**) and N-methylated (**14a**−**e**) products. Reaction of the sodium salts of **12a**−**e** with dimethyl sulfate in dry DMF was shown to give the best obtainable yields and the highest ratios between **13a**−**e** and the byproducts **14a**−**e**.

Deprotonation of the isoxazole rings of **13a**−**e** using butyllithium and subsequent treatment of the lithium salts formed by excess of paraformaldehyde gave the hydroxymethyl analogues, **16a**−**e**, in varying yields. The 4-pyridyl analogue, **16c**, was obtained in the highest yield, whereas **13b**, containing a 3-pyridyl group, under the same reaction conditions gave a complex reaction mixture, from which **16b** could not be isolated. The brominated derivative of **13b**, compound **15b**, was, however, readily lithiated and subsequently converted into **16b** by treatment with paraformaldehyde (Scheme 1).

Compounds **16a**−**e** were transformed into the corresponding dimethyl acetamidomalonates **18a**−**e** via **17a**−**e**, and deprotection of **18a**−**e** by treatment with hydrobromic acid gave the target compounds, **7a**−**e**, isolated in the zwitterionic forms.

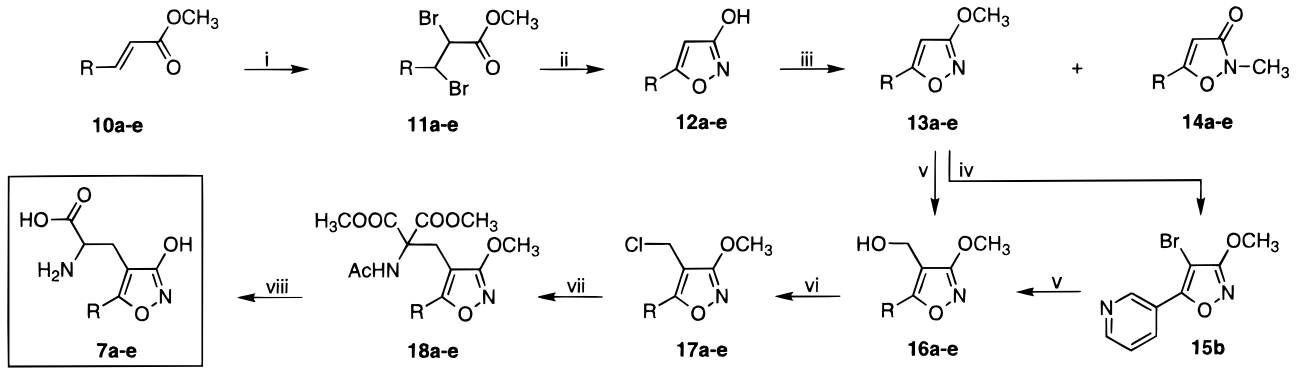
The synthesis of **7f** is outlined in Scheme 2. The key intermediate in this reaction sequence, 5-(2-furyl)-4-methyl-3-isoxazolol (**21**), was synthesized from the β-oxo ester **19** and hydroxylamine. According to the literature,[19,20] this synthesis can be accomplished with concomitant formation of only minor amounts of the isomeric 3-isoxazolin-5-one **20**. Using the procedure described by Sato et al.,[19] where the sodium salt of **19** was treated with hydroxylamine at −35 °C, compound **21** was formed selectively and could be isolated in 68% yield. Treatment of **19** with hydroxylamine under the conditions described by Jacobsen et al.[20] gave the isomer **20** as the only product.

The hydroxy group of **21** was protected as the (pivaloyloxy)methyl ether, a protecting group which can be removed under mild acidic or basic conditions. Bromination of this protected intermediate **22** with NBS in the absence of benzoyl peroxide catalyst gave **24**, which was converted into the Boc-protected diethyl aminomalonate **25**. Compound **25** was stepwise deprotected using aqueous base and subsequently aqueous acid to give **7f**.

As an attempt to synthesize the 3-isoxazolin-5-one analogue of **7f**, compound **30**, intermediate **20** was N-benzoylated to give **27** and substantial amounts of the isomeric compound **26**. NBS bromination of **27** gave **28**, which was converted into the Boc-protected diethyl aminomalonate **29** with concomitant loss of the benzoyl group. Attempts to deprotect **29** to the target compound **30** were, however, unsuccessful.

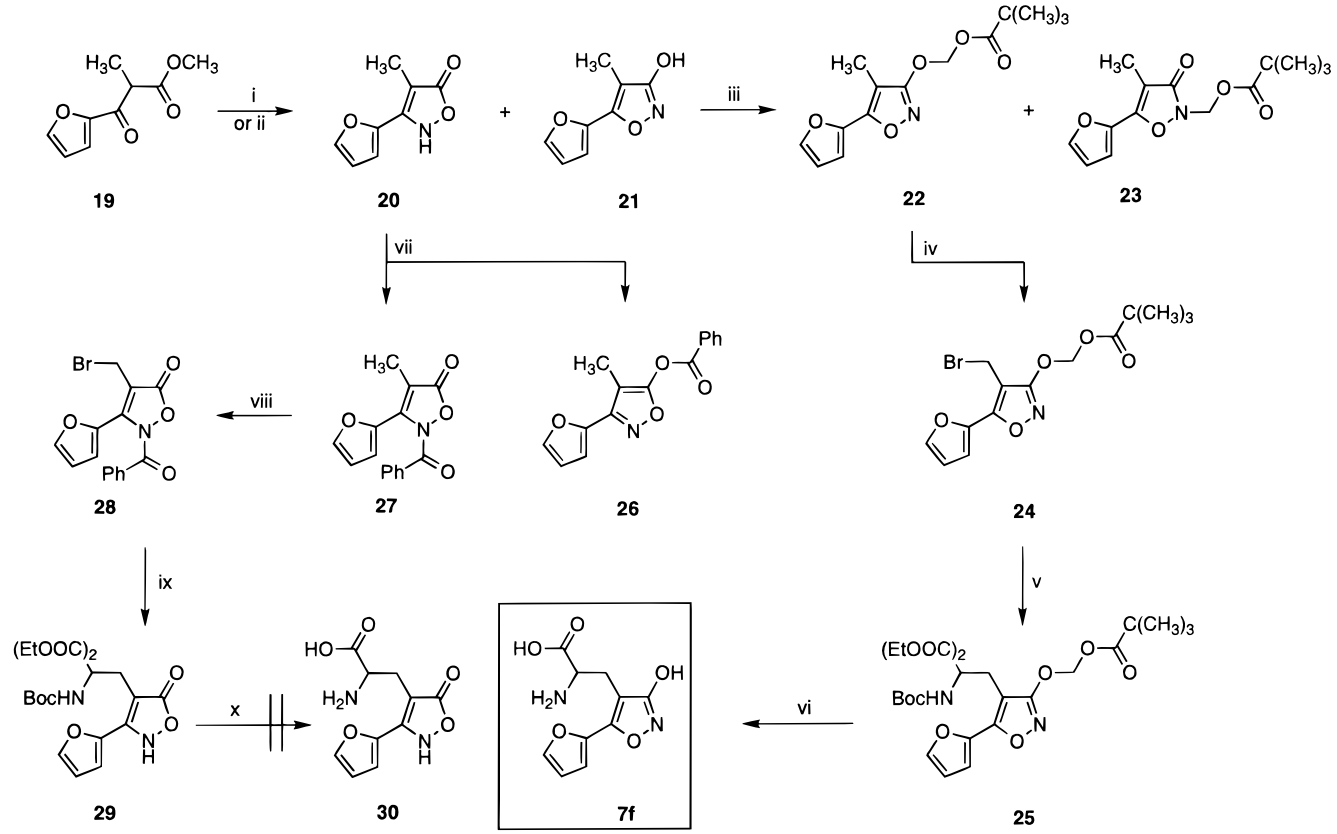
Bromination of compound **22** in the presence of benzoyl peroxide gave a mixture of **24** and a compound shown by NMR spectroscopy to be the 5-bromofuryl analogue of **24**. Separation of this 5-bromofuryl compound, a potential intermediate for the synthesis of **7g**, from **24** failed, and an alternative route for the synthesis of **7g** was developed (Scheme 3). Treatment of **21** with pivaloyl chloride gave **31** in high yield, and in the absence of benzoyl peroxide catalyst, NBS bromination of **31** produced **32**. In the presence of benzoyl peroxide and by using an excess of NBS, an inseparable 1:2 mixture of **32** and **33** was formed. Treatment of this

Scheme 1[a]



[a] (i) Br_2; (ii) NH_2OH, NaOH; (iii) Me_2SO_4, NaOH; (iv) Br_2; (v) *n*-BuLi, $(CH_2O)_n$; (vi) $SOCl_2$; (vii) AcNHCH $(COOMe)_2$, NaH; (viii) 48% aq HBr.

Scheme 2[a]



[a] (i) NH_2OH, NaOH, pH 10; (ii) NH_2OH, NaOH, −35 °C; (iii) $(CH_3)_3CCOOCH_2I$, K_2CO_3; (iv) NBS; (v) BocNHCH$(COOEt)_2$, NaH; (vi) NaOH, EtOH, 4 M HCl; (vii) PhCOCl, TEA; (viii) NBS; (ix) BocNHCH$(COOEt)_2$, NaH; (x) NaOH, EtOH, 4 M HCl.

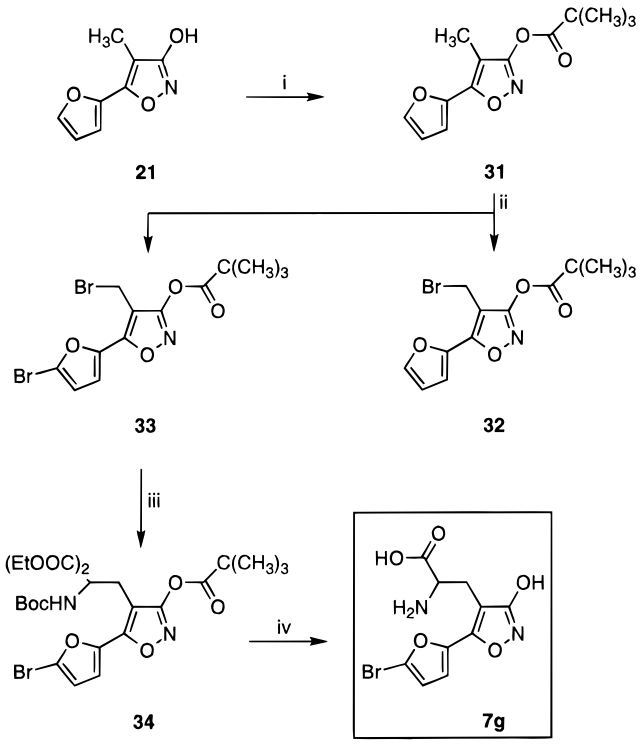
mixture with Boc-protected diethyl aminomalonate gave a mixture of **34** and its nonbrominated analogue, from which **34** was isolated chromatographically and deprotected to **7g** under conditions similar to those used for the conversion of **25** into **7f** (Scheme 2).

A HPLC analysis of **7g** did, however, disclose the presence of approximately 0.25% of **7f**. Prior to receptor binding and electrophysiological studies on **7g**, a sample of this compound was further purified by preparative HPLC, resulting in a sample of **7g** containing less than 0.05% of **7f** as determined by analytical reverse-phase HPLC.

Chiral HPLC Resolution of 7f. Compound **7f** was resolved by chiral HPLC using a Chirobiotic T column, which contains the macrocyclic glycopeptide Teicoplanin as chiral selector.[21] In analogy with previous reports on resolution of α-amino acids,[15,22] the *S*-enantiomer, compound **8**, was less retained than the *R*-enantiomer, compound **9**, on this chiral HPLC column. Compounds **8** and **9** were isolated having enantiomeric excess (ee) > 99.8%. Due to unsatisfactory separation of the enantiomers on the Chirobiotic T column, only the enantiomeric excess of the late-eluting enantiomer, **9**, could be precisely determined using this column. For the analysis of **8**, a ligand-exchange (*S*)-pipecolic acid column, which showed the reverse elution order for the enantiomers, was chosen.

The UV spectrum of **8**, which was identical to that of **9**, only showed one broad absorption band (250−300 nm) with a maximum at 278 nm. This relatively strong UV

Scheme 3[a]



[a] (i) $(CH_3)_3CCOCl$, TEA; (ii) NBS, $(PhCO)_2O_2$; (iii) $BocNHCH(COOEt)_2$, NaH; (iv) NaOH, EtOH, 4 M HCl.

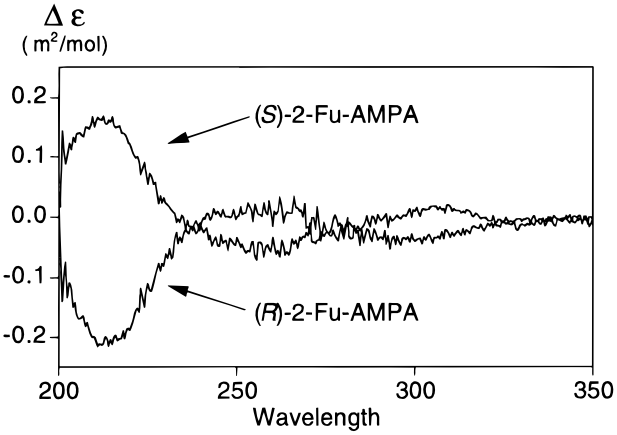


Figure 2. Circular dichroism spectra of **8** and **9**.

absorption is most likely due to the conjugated aromatic bicyclic ring system of **8** and **9**. In the circular dichroism (CD) spectra of **8** and **9** (Figure 2) only minor absorption bands were found in the same region, indicating that the contribution of the bicyclic ring system to the CD spectra is small. The CD spectrum of **8** showed a positive Cotton effect ($\Delta\epsilon = +0.18$ m²/mol) at 210−220 nm, which is in agreement with what is generally observed for α-amino acids having *S*-configuration.[15,23]

Photochemical Stability of 7f. The photochemical stability of compound **7f** in aqueous solution was investigated using a 125-W UV lamp and was compared to that of AMPA. The time course of the degradation process is shown in Figure 3. Under the conditions used, **7f** was relatively quickly decomposed, $t_{1/2}$ being less than 30 min, whereas no detectable decomposition was observed for AMPA even after UV light exposure for 2 h. The UV light treatment of **7f** resulted in

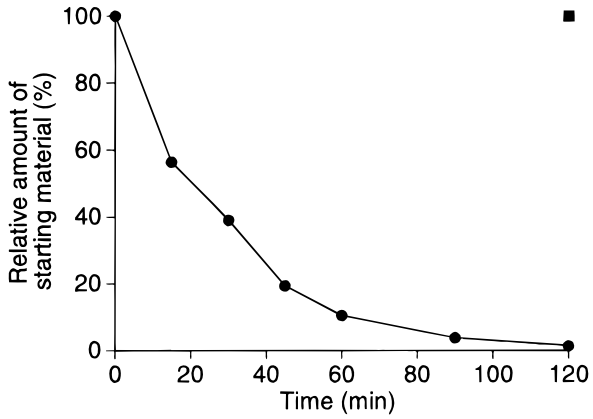


Figure 3. Time course of the photochemical degradation of **7f** (●) and AMPA (■), both dissolved in water, using a 125-W UV lamp. Each data point represents the relative amount of compound left in the solution determined by HPLC analysis and originates from single injections.

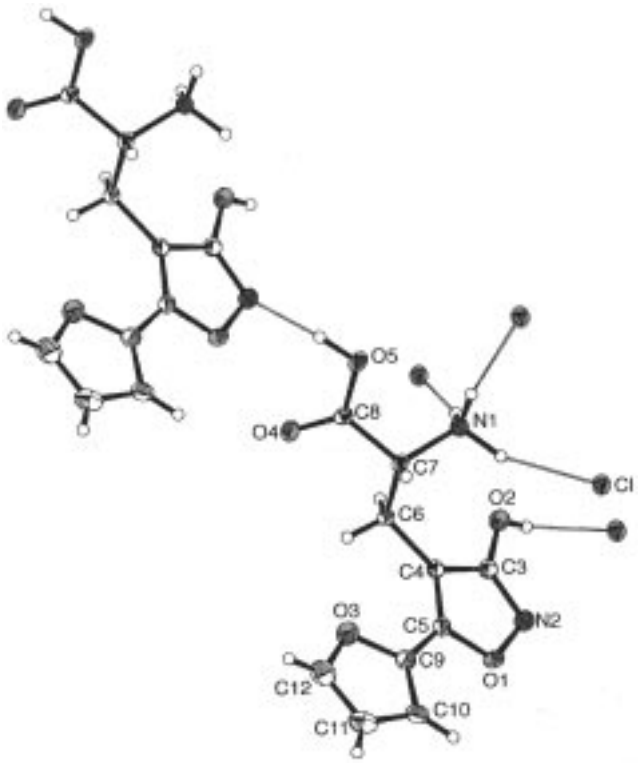


Figure 4. Hydrogen-bonding pattern in crystals of **9**·HCl (Table 2). Two cations and four chloride anions are shown in perspective drawing.[25] Displacement ellipsoids of the non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size. Hydrogen bonds are indicated by thin lines.

formation of a number of products, all of which were more polar than the starting material. However, no attempts were made to isolate or characterize any of the degradation products formed.

X-ray Crystallographic Analysis of Compound 9·HCl. A perspective drawing of the molecular structure of **9**·HCl with atomic labeling is depicted in Figure 4. The absolute configuration was established to be *R* according to the procedure of Flack.[26,27] The bond lengths and angles are consistent with the general pattern of related compounds.[10,28−30] The isoxazole and furan rings are both planar within the limits of experimental errors, the *inter*planar angle being 10.4(1)°. The

Table 1. Selected Torsion Angles (deg) for (*R*)-2-Amino-3-[5-(2-furyl)-3-hydroxy-4-isoxazolyl]propionic Acid (**9**) Hydrochloride[a]

torsion angles			
O3−C9−C5−C4	10.0(2)	C4−C6−C7−C8	−176.6(1)
O1−C5−C9−C10	9.6(2)	C6−C7−C8−O4	16.8(2)
C3−C4−C6−C7	−89.7(2)	N1−C7−C8−O5	−42.8(1)
C5−C4−C6−C7	95.4(2)	O4−C8−O5−H5	6(2)
C4−C6−C7−N1	62.0(1)	N2−C3−O2−H2	−3(1)

[a] Estimated standard deviations are given in parentheses.

Table 2. Hydrogen Bonds and Close Contacts[24] Geometries (Å, deg) in Crystals of (*R*)-2-Amino-3-[5-(2-furyl)-3-hydroxy-4-isoxazolyl]propionic Acid (**9**) Hydrochloride[a,b]

D−H···A	D−H	H···A	D···A	< DHA
		Intramolecular		
N1−H1C···O5^{i}	0.92(2)	2.478(2)	2.709(2)	94(1)
		Intermolecular		
O5−H5···N2^{ii}	0.93(3)	1.80(3)	2.718(2)	170(3)
O2−H2···Cl^{iii}	0.86(2)	2.10(2)	2.961(1)	173(2)
N1−H1A···Cl^{i}	0.98(2)	2.21(2)	3.160(1)	164(2)
N1−H1B···Cl^{iv}	0.94(2)	2.23(2)	3.148(1)	163(2)
N1−H1C···Cl^{v}	0.92(2)	2.33(2)	3.238(1)	169(2)
C7−H7···O4^{vi}	0.98(2)	2.46(2)	3.346(2)	150(1)
C12−H12···O4^{vii}	0.87(2)	2.55(2)	3.254(2)	138(2)
C6−H6B···O1^{iv}	0.98(2)	2.60(1)	3.287(2)	127(1)

[a] Symmetry code: (i) x, y, z; (ii) $-1+x, -1+y, z$; (iii) $1-x, 1/2+y, 1/2-z$; (iv) $-1+x, y, z$; (v) $1-x, -1/2+y, 1/2-z$; (vi) $1+x, y, z$; (vii) $1/2+x, 3/2-y, -z$. [b] Estimated standard deviations are given in parentheses.

conformation of the cation is described by the selected torsion angles given in Table 1. A close *intra*molecular contact is observed between the ammonium group and the hydroxyl oxygen atom of the carboxyl group (Table 2). This contact may be classified as the minor component of a three-center hydrogen bond.[31] All of the five hydrogen atoms bonded to oxygen and nitrogen atoms of the cation are utilized in the formation of hydrogen bonds (Figure 4, Table 2).

In Vitro Pharmacology. All 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 EAA receptor ligands. Whereas none of the new compounds showed significant affinity for [^{3}H]-kainic acid binding sites[32] or NMDA receptor sites, labeled by tritiated (*RS*)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid ([^{3}H]CPP),[33] compounds **5**, **7a**,**c**,**f**, and **8** were shown to be potent inhibitors of [^{3}H]-AMPA receptor binding[34] (Table 3). Compounds **5**, **7a**,**c**,**f**, and **8** turned out to be AMPA receptor agonists showing full agonism (data not shown) and relative potencies in agreement with their relative receptor affinities (Figure 5). Thus, the AMPA agonist activities of **7a**,**f** have been shown to reside exclusively in the *S*-enantiomers, compounds **5**[15] and **8**, respectively. Whereas the *R*-enantiomer of **7a**, compound **6**, previously has been shown to be a weak AMPA receptor antagonist;[15] the *R*-form of **7f**, compound **9**, was inactive as an agonist as well as an antagonist at AMPA receptors.

Discussion

On the basis of quite extensive structure−activity studies on analogues of AMPA containing different alkyl groups in the 5-position of the isoxazole ring, a model has been proposed for the AMPA receptor recognition site containing a lipophilic cavity of limited size in addition to binding sites recognizing stereospecifically the charged groups of AMPA analogues.[35−38] Enantiopharmacological studies on APPA (Figure 1) accordingly disclosed weak AMPA agonist effects of **2**, whereas **3** showed competitive AMPA receptor antagonism of comparable potency.[10] An analysis of these effects led to the development of the concept of functional partial agonism stating that partial agonism at any desired level of relative efficacy can be established by coadministration of an agonist of a competitive antagonist at appropriate concentrations.[10−12]

Replacement of the phenyl group of APPA by the isosteric 2-thienyl group quite surprisingly provided an AMPA receptor agonist much more potent than **2** and approximately equipotent with AMPA.[13] This observation was suggested to reflect an electrostatic interaction with the receptor protein and/or a stabilization of a receptor-active conformation of the agonist by the heteroatom in the 2-position of the 5-substituent of the isoxazole ring and prompted us to synthesize and pharmacologically characterize a series of structurally related compounds.[14] Some of these analogues, including **4** (Figure 1), all of which contained sulfur and/or nitrogen in the 2-position of the ring, also contained ring methyl substituents. A few of these compounds, notably **4**, showed very potent AMPA receptor agonism.[14] The structure−activity analysis revealed highly regioselective steric effects of the methyl groups and, furthermore, vanishingly low AMPA agonist effects of analogues containing heterocyclic substituents carrying partial positive or negative charges.[14]

To further elucidate the structural factors determining the pharmacology of this class of AMPA analogues, we have now synthesized (Scheme 1) the 2-, 3-, and 4-pyridyl analogues of APPA, compounds **7a**−**c**, and **7d,e** which, like **7a**, contain a nitrogen atom in the 2-position of the 5-substituent (Figure 1). Within this group of compounds, only **7a** showed potent AMPA receptor agonism (Table 3). Whereas **7c** was a weak AMPA agonist, **7b,d,e** were inactive, emphasizing the critical importance of a 2-positioned heteroatom for effective interaction with the AMPA receptor and the sensitivity of this interaction to steric effects.

Pharmacological studies on the enantiomers of 2-Py-AMPA, obtained using chiral HPLC techniques, have identified **5** as a potent AMPA agonist and **6** as a weak AMPA antagonist equipotent with **3**.[15] In light of these observations, we have now synthesized the 2-furyl analogue of **7a**, compound **7f** (Scheme 2). In agreement with previous structure−activity studies on heteroaryl analogues of AMPA,[13,14,16] **7f** turned out to be a very potent AMPA agonist (Table 3). This, in turn, prompted us to subject **7f** to a chiral HPLC resolution and to establish the absolute stereochemistry of the enantiomers by an X-ray crystallographic analysis on **9**·HCl (Figure 4). Circular dichroism (CD) spectra of the enantiomers of Fu-AMPA (Figure 2) were in agreement with the stereochemical assignment.

As predicted, the AMPA agonist effect of **7f** was shown to reside specifically in the *S*-enantiomer (**8**), but whereas **3**[10] as well as **6**[15] showed competitive AMPA receptor antagonism, **9** turned out to be pharmacologi-

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

	receptor binding IC_{50} (μM)			electropharmacology	
compound	[^{3}H]AMPA	[^{3}H]kainic acid	[^{3}H]CPP	agonism EC_{50} (μM)	antagonism K_i (μM)
(*S*)-AMPA (**1**)	0.040 ± 0.014	>100	>100	3.5 ± 0.2	
2	5.5 ± 2.2[b]	>100[b]	>100[b]	230 ± 12[b]	
3	>100[b]	>100[b]	>100[b]		286 ± 24[b]
4	0.094[a]	4.9[a]	>100[a]	2.3[a]	
5	0.19 ± 0.06[c]	>100[c]	>100[c]	4.5 ± 0.3[c]	
6	>100[c]	>100[c]	>100[c]		270 ± 50[c]
7a	0.57 ± 0.16	>100	>100	7.4 ± 0.2	
7b	>100	>100	>100	>1000	
7c	5.5 ± 0.6	>100	>100	96 ± 5	
7d	>100	>100	>100	>1000	
7e	>100	>100	>100	>1000	
7f	0.15 ± 0.03	>100	>100	1.7 ± 0.2	
7g	12 ± 1	>100	>100	160 ± 15	
8	0.11 ± 0.01	>100	>100	0.71 ± 0.11	
9	100	>100	>100	>1000	
kainic acid	4.0 ± 1.2	0.007 ± 0.002	>100	Nd	

[a] Reference 14. [b] Reference 10. [c] Reference 15. Nd, not determined.

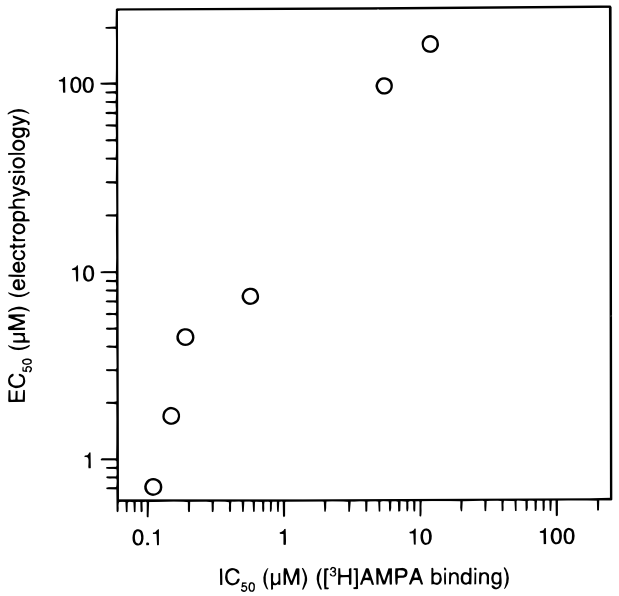


Figure 5. Comparison of [^{3}H]AMPA receptor binding with electrophysiological data for compounds **8**, **7f**, **5**, **7a**, **7c**, and **7g** showing a decreasing order of affinity and potency. Data from Table 3.

cally inactive (Table 3). Thus, within this class of compounds, the AMPA receptors impose different and strict stereochemical and structural demands on agonists and antagonists. This conclusion is supported by the observation that the bromo analogue of **7f**, compound **7g**, is some 2 orders of magnitude weaker than the parent compound as an AMPA agonist (Table 3).

The synthesis of **7f** (Scheme 2) was seriously impeded by the sensitivity of the furan ring to acidic reaction conditions. Furthermore, **7f** turned out to be highly sensitive to light. This photosensitivity was confirmed in a series of photochemical decomposition studies as illustrated in Figure 3. Considering the high AMPA receptor affinity and agonist potency of **8**, this photochemical sensitivity is interesting, and we have initiated the characterization of **8** as a potentially useful photolabel for native and recombinant AMPA receptors. The results on these studies are in progress and will be reported separately.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, LEO Pharmaceutical Products, Denmark, and are within ±0.4% of the calculated values. Flash chromatography (FC) was performed on Merck silica gel 60 H (5−40 μm) and column chromatography (CC) on Merck silica gel 60 (70−230 mesh, ASTM). Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel F_{254} plates. Compounds containing the 3-isoxazolol moiety were visualized using a $FeCl_3$ spraying reagent, and compounds containing amino groups were visualized using a ninhydrin spraying reagent. All compounds under study were also detected on TLC plates using UV light and a $KMnO_4$ spraying reagent. ^{1}H NMR spectra were recorded on a Bruker AC 200F (200-MHz) or a Varian 360L (60-MHz) spectrometer. DMF was dried over CaH_2 and distilled. IR spectra were recorded from KBr disks on a Perkin-Elmer 781 grating infrared spectrophotometer. CD and UV spectra of **8** and **9** (0.06 mM, 0.1 M HCl) were recorded at ambient temperature in 1.0-cm cuvettes on a Jasco J-720 spectropolarimeter and on a Perkin-Elmer Lambda 2 spectrophotometer, respectively. Optical rotations were measured in thermostated cuvettes on a Perkin-Elmer 241 polarimeter.

High-Performance Liquid Chromatography. The HPLC instrumentation used for the Chirobiotic T column (10 × 500 mm, ASTEC) consisted of a Jasco 880PU pump, a Rheodyne 7125 injector equipped with a 5.0-mL loop, and a Waters M480 detector set at 254 nm connected to a Merck-Hitachi Chromato-Integrator D-2000. The column was eluted at 1.2 mL/min with 10 mM aqueous NH_4OAc/EtOH (20:80). Chiral HPLC with the analytical Chirobiotic T column (4.6 × 150 mm, ASTEC) was performed using a Waters M510 pump connected to a Waters U6K injector and a Waters 991 photodiode array detector. The column was eluted at ambient temperature with 0.5 mL/min 10 mM NH_4OAc (pH 4.0)/EtOH (20:80). Chiral ligand-exchange HPLC was performed on a column (4.6 × 120 mm) containing (*S*)-pipecolic acid chemically bound to a silica-based packing material following a procedure used for attachment of (*S*)-proline.[39] The column (50 °C) was eluted with 1.0 mL/min 50 mM KH_2PO_4 (pH 4.6) containing 0.1 mM $CuSO_4$ and was connected to the same HPLC instrumentation used for the analytical Chirobiotic T column. Reverse-phase HPLC with a Knauer RP18 (20 × 250 mm) column was performed using a Shimadzu LC-6A pump, a Rheodyne injector equipped with a 5.0-mL loop, and a Shimadzu SPD-6A detector set at 254 nm. The column was eluted at 5.0 mL/min with 100 mM aqueous HOAc/MeOH (60:40). Analytical reverse-phase HPLC was performed on a Supelco LC-18-DB column (4.6 × 150 mm). The column was eluted at ambient temperature with 1.0 mL/min 15 mM aqueous HOAc/MeOH (70:30) and was connected to the HPLC instrumentation used for the analytical Chiro-

biotic T column. The ee values for **8** and **9** and the chemical purity of compound **7g** were based on peak areas at 280 nm.

Syntheses of 10a−e, 11c, and 12c. The methyl 2-propenoates (**10a−e**) were prepared in analogy with the preparation of ethyl 3-(3-pyridyl)propenoate[40] from the acids corresponding to **10a**,[41] **10b**,[40] **10d**,[42] and **10e**.[43] The syntheses of compounds **11c** and **12c** have previously been reported.[44]

General Procedure for the Preparation of (2*RS*,3*SR*)-Methyl 2,3-Dibromopropionate Hydrobromides 11a,b,d,e. To a solution of **10a**,**b**,**d**, or **e** (25 mmol) in HOAc (35 mL) was added HBr in HOAc (33%, 25 mmol) followed by a solution of Br_2 (25 mmol) in HOAc (35 mL). The mixture was stirred at 60 °C for 4 h and left at room temperature overnight. The precipitate was collected, washed with HOAc and Et_2O, and recrystallized from MeOH−Et_2O.

11a·HBr: yield 91%; mp 164−167 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 8.75 (1H, dd, J = 2 and 6 Hz), 8.1−7.85 (2H, m), 7.7−7.4 (1H, m), 5.85 (1H, d, J = 11 Hz), 5.40 (1H, d, J = 11 Hz), 3.85 (3H, s). Anal. ($C_9H_9Br_2NO_2$·HBr) C, H, Br, N.

11b·HBr: yield 87%; mp 182−184 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 9.05 (1H, br s), 8.9−8.6 (2H, m), 8.00 (1H, dd, J = 1.5 and 8 Hz), 5.75 (1H, d, J = 11 Hz), 5.40 (1H, d, J = 11 Hz), 3.80 (3H, s). Anal. ($C_9H_9Br_2NO_2$·HBr) C, H, Br, N.

11d·HBr: yield 94%; mp 189−190 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 8.05 (1H, d, J = 9 Hz), 7.85 (1H, dd, J = 2 and 9 Hz), 7.55 (1H, dd, J = 2 and 8 Hz), 5.80 (1H, d, J = 11 Hz), 5.45 (1H, d, J = 11 Hz), 3.85 (3H, s), 2.60 (3H, s). Anal. ($C_{10}H_{11}Br_2NO_2$·HBr) C, H, N.

11e·HBr: yield 94%; mp 171−174 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 8.55 (1H, d, J = 9 Hz), 8.2−7.65 (5H, m), 5.80 (1H, d, J = 11 Hz), 5.55 (1H, d, J = 11 Hz), 3.85 (3H, s). Anal. ($C_{13}H_{11}Br_2NO_2$·HBr) C, H, Br, N.

General Procedure for the Preparation of 5-Substituted 3-Isoxazolols 12a,b,d,e. To an ice-cooled solution of NaOH (140 mmol) in MeOH (100 mL) was added hydroxylamine hydrochloride (50 mmol). The mixture was stirred for 10 min, and **11a**,**b**,**d**, or **e** (20 mmol) was added in portions during a period of 1 h. After 1 h at 0 °C, the mixture was refluxed for 2 h and evaporated. H_2O (50 mL) was added to the residue, and the pH of the solution was adjusted to 4 with concentrated HCl. The precipitate was collected and washed with H_2O.

12a: yield 63%; mp > 225 °C (MeOH−H_2O); ^{1}H NMR [60 MHz, $CDCl_3$−DMSO-d_6 (1:1)] δ 8.70 (1H, dt, J = 1.5 and 6 Hz), 8.0−7.75 (2H, m), 7.55−7.25 (1H, m), 6.55 (1H, s). Anal. ($C_8H_6N_2O_2$) C, H, N.

12b: yield 56%; mp 211−215 °C dec (EtOH−H_2O); ^{1}H NMR (60 MHz, DMSO-d_6) δ 9.10 (1H, d, J = 3 Hz), 8.75 (1H, dd, J = 2 and 5 Hz), 8.25 (1H, dt, J = 2 and 9 Hz), 7.60 (1H, dd, J = 5 and 9 Hz), 6.75 (1H, s). Anal. ($C_8H_6N_2O_2$) C, H, N.

12d: yield 58%; mp 206−208 °C (EtOH−H_2O); ^{1}H NMR (60 MHz, $CDCl_3$−DMSO-d_6 [9:1]) δ 8.0−7.6 (2H, m), 7.25 (1H, dd, J = 2 and 7 Hz), 6.55 (1H, s), 2.55 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

12e: The crude product was extracted with boiling EtOAc, and the extracts were submitted to FC [tol−EtOAc (0−25%)]; yield 43%; mp 207−209 °C (EtOAc); ^{1}H NMR (60 MHz, DMSO-d_6) δ 8.45 (1H, d, J = 9 Hz), 8.25−7.6 (5H, m), 6.75 (1H, s). Anal. ($C_{12}H_8N_2O_2$) C, H, N.

General Procedure for the Preparation of 5-Substituted 3-Methoxyisoxazoles 13 and 5-Substituted 2-Methylisoxazolin-3-ones 14. A solution of **12** (10 mmol) and NaOH (10 mmol) in H_2O (15 mL) and EtOH (15 mL) was evaporated to dryness and further dried for 2 h at 2 Pa. The residue was suspended in dry DMF (15 mL) and cooled to −10 °C. Me_2SO_4 (11 mmol) was added dropwise, and the mixture was stirred at −10 °C for 1 h and then at room temperature for 15 h. The resulting solution was evaporated, and H_2O (25 mL) was added to the residue. Extraction with CH_2Cl_2 (3 × 50 mL), drying, and evaporation gave a mixture of **13** and **14**, which was submitted to FC [tol−EtOAc (1:1)]. The first fractions contained **13** and the later fractions **14**.

13a: yield 59%; mp 45−47 °C (heptane); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.70 (1H, dt, J = 2 and 7 Hz), 7.95−7.75 (2H, m), 7.35 (1H, dd, J = 5 and 7 Hz), 6.55 (1H, s), 4.05 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

14a: yield 14%; mp 137−139 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.75 (1H, dt, J = 2 and 6 Hz), 8.0−7.6 (2H, m), 7.50−7.30 (1H, m), 6.35 (1H, s), 3.65 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

13b: yield 47%; mp 80−81 °C (heptane); ^{1}H NMR (60 MHz, $CDCl_3$) δ 9.05 (1H, d, J = 3 Hz), 8.70 (1H, dd, J = 2 and 5 Hz), 8.10 (1H, dt, J = 2 and 9 Hz), 7.40 (1H, dd, J = 5 and 9 Hz), 6.25 (1H, s), 4.05 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

14b: yield 10%; mp 98−99.5 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 9.05 (1H, d, J = 3 Hz), 8.85 (1H, dd, J = 2 and 5 Hz), 8.05 (1H, dt, J = 2 and 9 Hz), 7.55 (1H, dd, J = 5 and 9 Hz), 6.25 (1H, s), 3.65 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

13c: yield 60%; mp 126−128 °C (heptane); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.55 (2H, d, J = 5 Hz), 7.40 (2H, dd, J = 1 and 5 Hz), 6.10 (1H, s), 3.90 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

14c: yield 19%; mp 134−137 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.65 (2H, dd, J = 1 and 5 Hz), 7.45 (2H, dd, J = 1 and 5 Hz), 6.15 (1H, s), 3.55 (3H, s). Anal. ($C_9H_8N_2O_2$) C, H, N.

13d: yield 51%; mp 58−60 °C (heptane); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.85−7.75 (2H, m), 7.35 (1H, dd, J = 5 and 10 Hz), 6.60 (1H, s), 4.05 (3H, s), 2.55 (3H, s). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

14d: yield 17%; mp 77−79 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.0−7.25 (3H, m), 6.45 (1H, s), 3.70 (3H, s), 2.65 (3H, s). Anal. ($C_{10}H_{10}N_2O_2$) C, H, N.

13e: yield 59%; mp 93−94 °C (heptane); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.4−7.5 (6H, m), 6.70 (1H, s), 4.10 (3H, s). Anal. ($C_{13}H_{10}N_2O_2$) C, H, N.

14e: yield 17%; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.45−7.40 (6H, m), 6.60 (1H, s), 3.60 (3H, s).

3-Methoxy-4-bromo-5-(3-pyridyl)isoxazole (15b). Br_2 (0.76 mL, 15 mmol) in HOAc (13 mL) was added to a solution of **13b** (2.36 g, 13.6 mmol) in HOAc (13 mL). The mixture was stirred at 60 °C for 2 days and cooled. The precipitate was collected and added to $NaHCO_3$ (5%, 15 mL). The mixture was extracted with CH_2Cl_2 (2 × 35 mL), and the combined extracts were washed with $NaHSO_3$ (10%, 20 mL), dried, and evaporated. FC of the residue [tol−EtOAc (0−30%)] gave **15b** (1.67 g, 49%): mp 111−113 °C (heptane); ^{1}H NMR (60 MHz, $CDCl_3$) δ 9.30 (1H, d, J = 3 Hz), 8.75 (1H, dd, J = 2 and 5 Hz), 8.25 (1H, dt, J = 2 and 8 Hz), 7.45 (1H, dd, J = 5 and 8 Hz), 4.10 (3H, s). Anal. ($C_9H_7BrN_2O_2$) C, H, Br, N.

General Procedure for the Preparation of 5-Substituted 3-Methoxy-4-(hydroxymethyl)isoxazoles 16. A solution of **13a**,**c**−**e** or **15b** (5 mmol) in dry THF (35 mL) was cooled to −78 °C, and a solution of *n*-butyllithium in hexane (1.6 M, 7.5 mmol) was added during 10 min. Paraformaldehyde (45 mmol) was added, and the mixture was stirred at −78 °C for 30 min and then at room temperature for 2 h. The reaction mixture was evaporated, and H_2O (20 mL) and CH_2Cl_2 (30 mL) were added to the residue. The pH was adjusted to 6 with 4 M HCl, and the phases were separated. The aqueous phase was extracted with CH_2Cl_2 (2 × 30 mL), and the combined organic phases were dried and evaporated. Compounds **16** were isolated by FC [tol−EtOAc (10−50%)] and crystallized from EtOAc−light petroleum.

16a: yield 62%; mp 90−92 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.70 (1H, dt, J = 1 and 5 Hz), 7.90 (2H, m), 7.35 (1H, m), 4.60 (2H, s), 4.05 (3H, s). Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

16b: from **15b**; yield 58%; mp 136−139 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 9.10 (1H, d, J = 3 Hz), 8.70 (1H, dd, J = 2 and 5 Hz), 8.20 (1H, dt, J = 2 and 8 Hz), 7.45 (1H, dd, J = 5 and 8 Hz), 4.65 (2H, s), 4.10 (3H, s). Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

16c: yield 76%; mp 135−137 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.70 (2H, m), 7.75 (2H, dd, J = 2 and 6 Hz), 4.60 (2H, s), 4.05 (3H, s). Anal. ($C_{10}H_{10}N_2O_3$) C, H, N.

16d: yield 51%; mp 119−121 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.90 (2H, d, J = 5 Hz), 7.35 (1H, m), 4.65 (2H, s), 4.05 (3H, s), 2.65 (3H, s). Anal. ($C_{11}H_{12}N_2O_3$) C, H, N.

16e: yield 52%; mp 143−145 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.42 (1H, d, J = 9 Hz), 8.2−7.4 (5H, m), 4.76 (2H, s), 4.12 (3H, s). Anal. ($C_{14}H_{12}N_2O_3$) C, H, N.

General Procedure for the Preparation of 5-Substituted 3-Methoxy-4-(chloromethyl)isoxazoles 17. A mixture of **16** (2 mmol) and thionyl chloride (10 mL) was refluxed for 2 h and evaporated. Crystallization of the respective residues gave **17b**·HCl and **17c**·HCl. Otherwise, $NaHCO_3$ (5%, 15 mL) was added to the residue, and the mixture was extracted with CH_2Cl_2 (2 × 25 mL). The combined extracts were dried, evaporated, and crystallized.

17a: yield 83%; mp 88−89 °C (Et_2O−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.75 (1H, dt, J = 1.5 and 5 Hz), 7.85 (2H, m), 7.30 (1H, m), 5.05 (2H, s), 4.15 (3H, s). Anal. ($C_{10}H_9ClN_2O_2$) C, H, Cl, N.

17b·HCl: yield 84%; mp 212−215 °C (MeOH−Et_2O); ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.96 (1H, s), 8.72 (1H, d, J = 5 Hz), 8.48 (1H, d, J = 8 Hz), 7.88 (1H, dd, J = 5 and 8 Hz), 4.39 (2H, s), 3.85 (3H, s). Anal. ($C_{10}H_9ClN_2O_2$·HCl) C, H, Cl, N.

17c·HCl: yield 63%; mp > 225 °C (MeOH−Et_2O); ^{1}H NMR (60 MHz, D_2O) δ 9.30 (2H, dd, J = 2 and 7 Hz), 8.55 (2H, dd, J = 2 and 7 Hz), 4.90 (2H, s), 4.20 (3H, s). Anal. ($C_{10}H_9ClN_2O_2$·HCl) C, H, Cl, N.

17d: yield 87%; mp 102−104 °C (Et_2O−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.90−7.75 (2H, m), 7.45−7.25 (1H, m), 5.15 (2H, s), 4.20 (3H, s), 3.65 (3H, s). Anal. ($C_{11}H_{11}ClN_2O_2$) C, H, N.

17e: yield 64%; mp 123−126 °C (EtOAc−Et_2O−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.4−7.55 (6H, m), 5.15 (2H, s), 4.15 (3H, s). Anal. ($C_{14}H_{11}ClN_2O_2$) C, H, Cl, N.

General Procedure for the Preparation of Methyl 2-Acetamido-2-(methoxycarbonyl)-3-(3-methoxy-5-substituted-4-isoxazolyl)propionates 18. Sodium hydride (60% in mineral oil, 2.5 mmol) was added in small portions to a solution of dimethyl acetaminomalonate (2.2 mmol) in dry DMF (5 mL). The mixture was stirred at room temperature for 1 h. A solution of **17** (2.0 mmol) in dry DMF (5 mL) was added, and stirring was continued for 6−18 h. The mixture was evaporated, and H_2O (20 mL) was added to the residue. Extraction with CH_2Cl_2 (3 × 25 mL), drying, and FC of the residue [tol−EtOAc (10−80%)] gave compounds **18**.

18a: yield 76%; oil; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.70 (1H, dt, J = 2 and 5 Hz), 7.95−7.80 (2H, m), 7.35 (1H, m), 4.05 (3H, s), 3.85 (2H, s), 3.70 (6H, s), 1.70 (3H, s).

18b: yield 70%; oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.93 (1H, d, J = 2 Hz), 8.69 (1H, d, J = 5 Hz), 7.95 (1H, dt, J = 2 and 8 Hz), 7.43 (1H, dd, J = 5 and 8 Hz), 4.01 (3H, s), 3.69 (2H, s), 3.60 (6H, s), 1.63 (3H, s).

18c: yield 58%; mp 174−175 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.80 (2H, dd, J = 2 and 5 Hz), 7.65 (2H, dd, J = 2 and 5 Hz), 4.05 (3H, s), 3.75 (2H, s), 3.65 (6H, s), 1.60 (3H, s). Anal. ($C_{17}H_{19}N_3O_7$) C, H, N.

18d: yield 75%; mp 118−120 °C (light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.75−8.60 (1H, m), 7.4−7.1 (2H, m), 3.95 (3H, s), 3.75 (2H, s), 3.65 (6H, s), 2.55 (3H, s), 1.55 (3H, s). Anal. ($C_{18}H_{21}N_3O_7$) C, H, N.

18e: yield 36%; mp 167−168 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.5−7.4 (6H, m), 4.05 (3H, s), 3.80 (2H, s), 3.55 (6H, s), 1.55 (3H, s). Anal. ($C_{21}H_{21}N_3O_7$) C, H, N.

General Procedure for the Preparation of (*RS*)-2-Amino-3-(3-hydroxy-5-substituted-4-isoxazolyl)propionic Acids 7a−e. A solution of **18** in aqueous HBr (48%, 20 mL) was refluxed for 1 h. The reaction mixture was evaporated, and the residue was dissolved in H_2O (10 mL). The solution was treated with charcoal, and **7** was precipitated by addition of 1 M Na_2CO_3 to pH 3.

7a: yield 76%; mp > 225 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.68 (1H, d, J = 5 Hz), 7.98 (1H, t, J = 7 Hz), 7.85 (1H, d, J = 7 Hz), 7.47 (1H, t, J = 5 Hz), 3.74 (1H, m), 3.41 (1H, dd, J = 3 and 12 Hz), 3.15 (1H, dd, J = 3 and 12 Hz). Anal. ($C_{11}H_{11}N_3O_4$·0.25H_2O) C, H, N.

7b: yield 69%; mp > 225 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.84 (1H, d, J = 1.4 Hz), 8.67 (1H, dd, J = 1.4 and 4 Hz), 8.08 (1H, d, J = 8 Hz), 7.55 (1H, dd, J = 4 and 8 Hz), 3.68 (1H, m), 2.93 (1H, dd, J = 7 and 15 Hz), 2.76 (1H, dd, J = 4 and 15 Hz). Anal. ($C_{11}H_{11}N_3O_4$·0.33H_2O) C, H, N.

7c: yield 76%; mp > 225 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.72 (2H, d, J = 6 Hz), 7.63 (2H, d, J = 6 Hz), 3.73 (1H, dd, J = 3.6 and 6 Hz), 3.07−2.81 (2H, m). Anal. ($C_{11}H_{11}N_3O_4$·H_2O) C, H, N.

7d: yield 61%; mp 215−217 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.87 (1H, t, J = 8 Hz), 7.62 (1H, d, J = 7 Hz), 7.36 (1H, d, J = 8 Hz), 3.75 (1H, br s), 3.45 (1H, dd, J = 4.5 and 15 Hz), 3.11 (1H, dd, J = 7 and 15 Hz), 2.55 (3H, s). Anal. ($C_{12}H_{13}N_3O_4$·H_2O) C, H, N.

7e: yield 32%; mp > 225 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.55 (1H, d, J = 8 Hz), 8.19 (1H, d, J = 8 Hz), 8.07−7.68 (4H, m), 3.84 (1H, br s), 3.63 (1H, d, J = 14 Hz), 3.34 (1H, dd, J = 3 and 14 Hz). Anal. ($C_{15}H_{13}N_3O_4$·H_2O) C, H, N.

3-(2-Furyl)-4-methylisoxazolin-5-one (20). A solution of hydroxylamine hydrochloride (1.0 g, 14 mmol) in NaOH (0.5 M, 20 mL) at 2 °C was adjusted to pH 10 with 0.5 M NaOH. The pH of the reaction mixture was kept at 10.0 ± 0.2 by using a pH-state (TTT80 combined with an ABU80 autoburet; both from Radiometer, Copenhagen) while **19**[45] (2.0 g, 11 mmol) was added dropwise over a period of 30 min. The mixture was stirred at room temperature for 2 h, and concentrated HCl (10 mL) was added in one portion. The mixture was left at 5 °C for 20 h, and the precipitate (1.42 g, 71%) was collected. A sample was recrystallized (EtOAc−light petroleum) to give **20**: mp 134−136 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.48 (1H, dd, J = 0.6 and 1.8 Hz), 6.73 (1H, dd, J = 0.6 and 3.5 Hz), 6.43 (1H, dd, J = 1.8 and 3.5 Hz), 1.88 (3H, s). Anal. ($C_8H_7NO_3$) C, H, N.

5-(2-Furyl)-4-methylisoxazol-3-ol (21). Hydroxylamine hydrochloride (2.45 g, 35 mmol) and NaOH (1.44 g, 36 mmol) were suspended in MeOH (25 mL) and water (2.5 mL), and the mixture was cooled in an ice bath. After stirring for 10 min, the mixture was filtered. The filtrate was dropwise added to a solution of **19**[45] (3.20 g, 17.6 mmol) and NaOH (0.70 g, 17.6 mmol) in MeOH (25 mL) and H_2O (2.5 mL) at −35 °C. The mixture was stirred at −35 °C for 3 h and then at 5 °C for 30 min. Concentrated HCl (35 mL) was added in one portion, and the mixture was evaporated. H_2O (35 mL) was added to the residue, and the mixture was left at 5 °C for 20 h. The precipitate (1.99 g, 68%) (mp 160−163 °C) was almost pure **21** contaminated with **20**. FC [tol−EtOAc (10:1)] followed by recrystallization (EtOAc−light petroleum) gave pure **21**: mp 164−166 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.45 (1H, dd, J = 0.6 and 1.8 Hz), 6.64 (1H, d, J = 3.4 Hz), 6.42 (1H, dd, J = 1.8 and 3.4 Hz), 2.01 (3H, s). Anal. ($C_8H_7NO_3$) C, H, N.

3-[(Pivaloyloxy)methyloxy]-4-methyl-5-(2-furyl)isoxazole (22) and 2-[(Pivaloyloxy)methyl]-4-methyl-5-(2-furyl)isoxazolin-3-one (23). Chloromethyl pivalate (5.80 mL, 40 mmol) was added to a solution of NaI (6.0 g, 40 mmol) in acetone (50 mL), and the mixture was stirred at room temperature for 2 h. After filtration, the filtrate was evaporated and the iodomethyl pivalate was dissolved in DMF (20 mL). A mixture of **21** (2.23 g, 13.5 mmol) and K_2CO_3 (3.73 g, 27 mmol) in DMF (60 mL) was stirred at 40 °C for 1 h, and the solution of iodomethyl pivalate was added. Stirring was continued for 20 h at 40 °C, and the mixture was evaporated. Water (50 mL) was added, and the mixture was extracted with EtOAc (3 × 75 mL). The combined organic extracts were dried and evaporated to give a mixture of **22** and **23**. The compounds were separated by FC (tol). The first fractions contained **22** (oil, 2.55 g, 68%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.90 (1H, d, J = 2 Hz), 7.10 (1H, d, J = 3.5 Hz), 6.85 (1H, dd, J = 2 and 3.5 Hz), 6.25 (2H, s), 2.25 (3H, s), 1.25 (9H, s). The latter fractions contained **23** (0.80 g, 21%): mp 91−92 °C (Et_2O−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.85 (1H, d, J = 2 Hz), 7.10 (1H, d, J = 3.5 Hz), 6.75 (1H, dd, J = 2 and 3.5 Hz), 6.00 (2H, s), 2.20 (3H, s), 1.20 (9H, s). Anal. ($C_{14}H_{17}NO_5$) C, H, N.

3-[(Pivaloyloxy)methyloxy]-4-(bromomethyl)-5-(2-furyl)-isoxazole (24). A mixture of **22** (4.62 g, 16.5 mmol) and *N*-bromosuccinimide (NBS) (3.50 g, 19.8 mmol) in CCl_4 (200 mL) was refluxed for 20 h. The reaction mixture was filtered, and the filtrate was evaporated. FC (tol) of the residue gave **24** (3.70 g, 63%) as an oil: ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.85 (1H, d, J = 2 Hz), 7.20 (1H, d, J = 3.5 Hz), 6.80 (1H, dd, J = 2 and 3.5 Hz), 6.15 (2H, s), 4.50 (2H, s), 1.25 (9H, s).

Ethyl 2-[*N*-(*tert*-Butyloxycarbonyl)amino]-2-(ethoxycarbonyl)-3-[3-[(pivaloyloxy)methyloxy]-5-(2-furyl)-4-isoxazolyl]propionate (25). Sodium hydride (60% in mineral oil, 0.64 g, 16 mmol) was added to a solution of diethyl *N*-(*tert*-butyloxycarbonyl)aminomalonate[46] (3.32 g, 12 mmol) in DMF (20 mL). The mixture was stirred at room temperature for 1 h. A solution of **24** (3.58 g, 10 mmol) in DMF (20 mL) was added, and stirring was continued for 20 h. After evaporation, H_2O (40 mL) was added and the mixture was extracted with EtOAc (3 × 50 mL). The extracts were dried and evaporated. FC [tol−EtOAc (0−10%)] of the residue afforded 3.70 g (67%) of almost pure **25** as an oil. CC (tol−EtOAc) of the oil followed by recrystallization (Et_2O−light petroleum) gave pure **25**: mp 96−98 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.54 (1H, d, J = 1.8 Hz), 6.92 (1H, d, J = 3.4 Hz), 6.47 (1H, dd, J = 1.8 and 3.4 Hz), 5.88 (2H, s), 4.4−4.2 (2H, m), 4.2−4.0 (2H, m), 3.66 (2H, br s), 1.34 (9H, s), 1.23 (9H, s), 1.5−1.2 (6H, m). Anal. ($C_{26}H_{36}N_2O_{11}$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(2-furyl)-4-isoxazolyl]propionic Acid (7f). A mixture of **25** (1.16 g, 2.10 mmol), NaOH (2 M, 10 mL), and EtOH (4 mL) was refluxed for 1.5 h and evaporated. The residue was dissolved in H_2O (20 mL), and the aqueous solution was washed with EtOAc (2 × 20 mL). To the aqueous solution was added HCl (4 M, 8 mL), and the mixture was refluxed for 1 h, cooled, and washed with EtOAc (2 × 25 mL). The aqueous solution was treated with charcoal and evaporated. The residue was dissolved in H_2O (6 mL) at 80 °C, and 1 M Na_2CO_3 was added to pH 3. After 20 h at 5 °C, the precipitate (435 mg, 81%) was collected. Recrystallization ($EtOH-H_2O$) afforded **7f**: mp > 240 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.90 (1H, d, J = 1.1 Hz), 7.00 (1H, d, J = 3.4 Hz), 6.70 (1H, dd, J = 1.1 and 3.4 Hz), 3.67 (1H, m), 2.98 (2H, m). Anal. ($C_{10}H_{10}N_2O_5 \cdot H_2O$) C, H, N.

3-(2-Furyl)-4-methyl-5-(benzoyloxy)isoxazole (26) and 2-Benzoyl-3-(2-furyl)-4-methylisoxazolin-5-one (27). Triethylamine (4.67 mL, 33.5 mmol) was added to a mixture of **20** (4.61 g, 27.9 mmol) and benzoyl chloride (3.40 mL, 29.3 mmol) in tol (110 mL). After stirring for 20 h at room temperature, the reaction mixture was washed with $NaHCO_3$ (5%, 100 mL) and H_2O (100 mL), dried, and evaporated. FC (tol) of the residue first eluted **26** (1.68 g, 22%) and subsequently **27** (3.91 g, 52%). Both compounds were recrystallized from EtOAc−light petroleum.

26: mp 88−90 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.35 (2H, dd, J = 1.5 and 8 Hz), 7.85−7.60 (4H, m), 7.10 (1H, d, J = 3.5 Hz), 6.70 (1H, dd, J = 1.5 and 3.5 Hz), 2.10 (3H, s). Anal. ($C_{15}H_{11}NO_4$) C, H, N.

27: mp 149−150 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.15 (2H, m), 7.85−7.60 (4H, m), 7.10 (1H, d, J = 3.5 Hz), 6.75 (1H, dd, J = 1.5 and 3.5 Hz), 2.20 (3H, s). Anal. ($C_{15}H_{11}NO_4$) C, H, N.

2-Benzoyl-3-(2-furyl)-4-(bromomethyl)isoxazolin-5-one (28). Compound **28** was prepared from **27** by the method described for the synthesis of **24**. **28**: yield 79%; mp 131−133 °C (EtOAc−light petroleum); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.40−7.65 (6H, m), 7.45 (1H, d, J = 3.5 Hz), 6.85 (1H, dd, J = 1.5 and 3.5 Hz), 4.60 (2H, s). Anal. ($C_{15}H_{10}BrNO_4$) C, H, N.

Ethyl 2-[*N*-(*tert*-Butyloxycarbonyl)amino]-2-(ethoxycarbonyl)-3-[3-(2-furyl)-5-oxo-4-isoxazolinyl]propionate (29). Compound **29** was prepared from **28** by the method described for the synthesis of **25**. FC (tol) and recrystallization (EtOAc) gave **29** (67%): mp 155−157 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.75 (1H, br s), 7.35 (1H, d, J = 3.5 Hz), 6.70 (1H, dd, J = 1.5 and 3.5 Hz), 4.40 (4H, q, J = 8 Hz), 3.65 (2H, s), 1.40 (9H, s), 1.30 (6H, t, J = 8 Hz). Anal. ($C_{20}H_{26}N_2O_9$) C, H, N.

3-(Pivaloyloxy)-4-methyl-5-(2-furyl)isoxazole (31). Compound **31** was prepared from **21** by the method described for the synthesis of **27**. FC (tol) and recrystallization (EtOAc−light petroleum) gave **31** (87%): mp 73−74 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.65 (1H, d, J = 1.5 Hz), 6.85 (1H, d, J = 3.5 Hz), 6.55 (1H, dd, J = 1.5 and 3.5 Hz), 2.05 (3H, s), 1.40 (9H, s). Anal. ($C_{13}H_{15}NO_4$) C, H, N.

3-(Pivaloyloxy)-4-(bromomethyl)-5-(2-furyl)isoxazole (32). Compound **32** was prepared from **31** by the method described for the synthesis of **24**. FC (tol) and recrystallization (light petroleum) gave **32** (76%): mp 66−67 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.60 (1H, d, J = 1.5 Hz), 7.00 (1H, d, J = 3.5 Hz), 6.55 (1H, dd, J = 1.5 and 3.5 Hz), 4.40 (2H, s), 1.40 (9H, s). Anal. ($C_{13}H_{14}BrNO_4$) C, H, N.

(*RS*)-2-Amino-3-[3-hydroxy-5-(5-bromo-2-furyl)-4-isoxazolyl]propionic Acid (7g). A mixture of **31** (2.04 g, 8.18 mmol), NBS (1.74 g, 9.77 mmol), and benzoyl peroxide (50 mg, 0.2 mmol) in CCl_4 (100 mL) was refluxed for 20 h. Another portion of NBS (1.74 g) and benzoyl peroxide (50 mg) were added, and the mixture was refluxed for an additional 20 h. Filtration and evaporation followed by FC (tol) gave 2.44 g of a 1:2 mixture of **32** and **33**, respectively. This mixture was reacted with diethyl *N*-(*tert*-butyloxycarbonyl)aminomalonate by the method described for the synthesis of **25**. FC (tol) gave **34** (oil, 2.1 g): ^{1}H NMR (60 MHz, $CDCl_3$) δ 6.85 (1H, d, J = 3.5 Hz), 6.40 (1H, d, J = 3.5 Hz), 4.4−3.9 (4H, m), 3.45 (2H, br s), 1.45 (9H, s), 1.40 (9H, s), 1.6−1.1 (6H, m).

Compound **34** was deprotected by the method described for the synthesis of **7f**. Crude **7g** was dissolved in dilute HCl, treated with charcoal, and reprecipitated by addition of Na_2CO_3 (1 M) to give **7g** (0.48 g, 46%): mp > 230 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.02 (1H, d, J = 3.5 Hz), 6.84 (1H, d, J = 3.5 Hz), 3.68 (1H, t, J = 5.1 Hz), 2.91 (2H, d, J = 5.1 Hz). Anal. ($C_{10}H_9BrN_2O_5$) C, H, N.

The isolated **7g** was shown to contain 0.25% of **7f** as determined on the Supelco-LC-18-DB column. Therefore, **7g** was submitted to preparative reverse-phase HPLC using the Knauer RP18 column (20 × 250 mm). Appropriate fractions containing **7g** were combined and evaporated. Recrystallization of the crystalline residue (H_2O) gave **7g** with a content of **7f** < 0.05% as determined by analytical reverse-phase HPLC.

(*S*)-2-Amino-3-[3-hydroxy-5-(2-furyl)-4-isoxazolyl]propionic Acid (8) and (*R*)-2-Amino-3-[3-hydroxy-5-(2-furyl)-4-isoxazolyl]propionic Acid (9). Compound **7f** (250 mg, 0.98 mmol) was dissolved in H_2O (30 mL) and resolved on the Chirobiotic T column (10 × 500 mm) in 12 injections each of 2.5 mL. Fractions containing the first enantiomer, compound **8**, were pooled and evaporated, reevaporated from H_2O, and finally lyophilized from H_2O. Recrystallization of the crude product (H_2O) gave **8** (64 mg, 55%): mp > 240 °C; ^{1}H NMR (200 MHz, DMSO-d_6 containing several drops of D_2O) δ 7.72 (1H, d, J = 1.5 Hz), 6.94 (1H, d, J = 3.6 Hz), 6.61 (1H, dd, J = 1.5 and 3.6 Hz), 3.69 (1H, d, J = 1.5 Hz), 2.98 (2H, m); ee = 99.9%; $[\alpha]^{22.9}_D$ = +22.5 (c = 0.36, 1 M HCl); λ_{max} (log ϵ) = 278 nm (4.3); $\Delta\epsilon$(210 nm) = +0.18 m^2/mol. Anal. ($C_{10}H_{10}N_2O_5$) C, H, N. The combined fractions containing the second enantiomer, compound **9**, were processed as described above for **8** affording **9** (65 mg, 56%): mp > 240 °C; ee > 99.8%; $[\alpha]^{22.9}_D$ = −22.4 (c = 0.39, 1 M HCl); λ_{max} (log ϵ) = 278 nm (4.3); $\Delta\epsilon$(210 nm) = −0.20 m^2/mol; ^{1}H NMR and IR spectra were identical with those of compound **8.**

X-ray Crystallographic Analysis of (*R*)-2-Amino-3-[3-hydroxy-5-(2-furyl)-4-isoxazolyl]propionic Acid, Hydrochloride (9·HCl). Single crystals of **9**·HCl suitable for X-ray crystallographic analysis were obtained from a solution of compound **9** (4.5 mg, 19 μmol) in aqueous 4 M HCl (30 μL) and HOAc (200 μL). Crystal data: $C_{10}H_{10}N_2O_5 \cdot HCl$, M_r = 274.66, colorless elongated prisms, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 5.2616(9) Å, b = 7.7536(11) Å, c = 28.326(4) Å, V = 1155.6(3) Å^3, Z = 4, D_c = 1.579 Mg m^{-3}, F(000) = 568, μ(Cu Kα) = 3.12 mm^{-1}, T = 122 ± 0.5 K, crystal dimensions = 0.14 × 0.23 × 0.42 mm.

Data Collection and Processing. Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer,[47] using

graphite monochromated Cu Kα radiation ($\lambda = 1.54184$ Å) and the $\omega - 2\theta$ scan mode ($\theta = 3-75°$, $\pm h$, $\pm k$, $\pm l$). Unit cell dimensions were determined by least-squares refinement of 20 reflections (θ range 41.31−42.61°). Data were reduced using the programs of Blessing (DREADD).[48,49] Five standard reflections were monitored every 10^4 s, and appropiate scaling was performed using the program SCALE3.[48,49] Absorption correction was applied using the program ABSORB ($T_{min} = 0.442$, $T_{max} = 0.683$).[48−50] A total of 9565 reflections were averaged ($R_{int} = 0.028$ on F_o^2) according to the point group symmetry 222 resulting in 2381 unique reflections.

Structure Solution and Refinement. The structure was solved by direct methods using the program SHELXS-97.[51] Full-matrix least-squares refinement (SHELXL-97)[26] was performed on F^2 for all unique reflections, minimizing $\sum w(F_o^2 - F_c^2)$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with isotropic displacement parameters. Extinction correction was applied, extinction coefficient: 0.0074(5).[26] The refinement (208 parameters, 2381 reflections) with the molecule having the *R* configuration converged at $R_F = 0.0221$, $Rw_F^2 = 0.0603$ for 2367 reflections with $F_o > 4\sigma(F_o)$; $w^{-1} = \sigma(F_o^2) + (0.0294P)^2 + 0.2351P$, where $P = (F_o^2 + 2F_c^2)/3$; $S = 1.093$. The Flack absolute structure factor: 0.00(1).[26,27] Maximum and minimum electron density in the final difference Fourier map was 0.29 and −0.27 e Å^{-3}, respectively (both near the Cl^- atom). Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL-97.[26,52]

Receptor Binding Assays. Affinity for AMPA receptors was determined using the ligand [^{3}H]AMPA,[34] and for determination of NMDA and kainic acid receptor affinities, [^{3}H]-CPP[33] and [^{3}H]kainic acid,[32] respectively, were used. The membrane preparations used in all of the receptor binding experiments were prepared according to the method of Ransom and Stec.[53]

In Vitro Electrophysiology. A rat cortical wedge preparation for determination of EAA-evoked depolarizations described by Harrison and Simmonds[54] 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 a Ag/AgCl pellet electrode. The cortex was superfused with Mg^{2+}-free Krebs buffer containing 25 mM $CaCl_2$, 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 containing 2.5 mM $CaCl_2$, 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 AMPA analogues at 1 mM concentrations, the compounds were applied alone for 90 s followed by coapplication of agonist (AMPA, 5 μM) and potential antagonist for another 90 s.

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Supporting Information Available: For (*R*)-2-amino-3-[5-(2-furyl)-3-hydroxy-4-isoxazolyl]propionic acid (**9**) hydrochloride, tables listing final atomic coordinates, equivalent isotropic (non-hydrogen atoms) or isotropic (hydrogen atoms) displacement parameters, anisotropic displacement parameters of the non-hydrogen atoms, and selected bond lengths and angles (3 pages). Ordering information is given on any current masthead page.

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