Eur J Med Chem (1997) 32, 329–338

Preliminary communication

Excitatory amino-acid receptor agonists. Synthesis and pharmacology of analogues of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid

FA Sløk[1], B Ebert[1], Y Lang[1], P Krogsgaard-Larsen[1]*, SM Lenz[2], U Madsen[1]

[1]*PharmaBiotec Research Center, Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen;*
[2]*H Lundbeck A/S, Department of Medicinal Chemistry, Ottiliavej 9, DK-2500 Valby, Denmark*

(Received 15 June 1996; accepted 26 September 1996)

Summary — We have previously proposed the existence of a lipophilic cavity of the 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptor recognition site capable of accommodating alkyl substituents of limited size in the 5-position of the isoxazole ring. In order to indirectly elucidate the approximate extent of this proposed cavity we have synthesized and pharmacologically characterized a number of AMPA analogues. For most of these AMPA analogues, a positive correlation between AMPA receptor affinity and agonist effect was observed. The only exception was demethyl-AMPA (**8a**), which showed relatively high AMPA receptor affinity (IC_{50} = 0.27 μM) but remarkably weak agonist potency (EC_{50} = 900 μM). Whereas the ethyl analogue of AMPA (Et-AMPA) (IC_{50} = 0.030 μM; EC_{50} = 2.3 μM) has previously been shown to be slightly more potent than AMPA (IC_{50} = 0.040 μM; EC_{50} = 3.5 μM), substitutions of a propyl or a butyl group for the methyl group of AMPA to give **8b** (IC_{50} = 0.090 μM; EC_{50} = 5.0 μM) or **8f** (IC_{50} = 1.0 μM; EC_{50} = 32 μM), respectively, result in progressive loss of the AMPA agonist effect. Analogues containing larger groups, such as isopentyl (**8e**), 1-propylbutyl (**8g**), 2,2-dimethylpropyl (**8h**), or benzyl (**14**) groups, were very weak or totally inactive as AMPA receptor ligands.

excitatory amino acid / AMPA receptor / AMPA agonist / structure–activity studies / receptor binding

Introduction

(*S*)-Glutamic acid ((*S*)-Glu), which is the main excitatory neurotransmitter in the central nervous system (CNS), and other excitatory amino acids (EAAs) operate through four different classes of receptors. In addition to the three heterogeneous classes of ionotropic EAA receptors (iGluRs), named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid receptors [1–3], a heterogeneous class of metabotropic EAA receptors (mGluRs) has been shown to have important functions in the central excitatory neurotransmission processes [4]. It is now generally agreed that iGluRs as well as mGluRs play important roles in the healthy as well as the diseased CNS, and that all subtypes of these receptors are potential targets for therapeutic intervention in a number of diseases [5, 6].

EAA receptors are involved in the mechanisms of long-term potentiation, which is believed to play an important role in learning and memory functions, and the deficits of these functions in Alzheimer patients may, to some extent, be caused by hypoactivity at iGluRs and/or mGluRs in the brain [7–10]. There is also growing evidence of an implication of EAA receptors in schizophrenia [11, 12]. As in Alzheimer's disease (AD), the role of these receptors in the etiology and the clinical manifestations of schizophrenia

*Correspondence and reprints

Abbreviations: AD: Alzheimer's disease; AMAA: (*RS*)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid; AMPA: (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid; APPA: (*RS*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid; ATPA: (*RS*)-2-amino-3-(5-*tert*-butyl-3-hydroxyisoxazol-4-yl)propionic acid; CPP: (*RS*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid; demethyl-AMPA: (*RS*)-2-amino-3-(3-hydroxyisoxazol-4-yl)propionic acid; EAA: excitatory amino acid; Et-AMPA: (*RS*)-2-amino-3-(5-ethyl-3-hydroxyisoxazol-4-yl)propionic acid; (*S*)-Glu: (*S*)-glutamic acid; iGluR: ionotropic glutamic acid receptor; mGluR: metabotropic glutamic acid receptor; Homo-AMPA: (*RS*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid; NBQX: 6-nitro-7-sulfamoylbenzo(*f*)quinoxaline-2,3-dione; NMDA: *N*- methyl-D-aspartic acid.

is still very incompletely understood, but there is evidence to suggest that hypoactivity at EAA receptors is also a factor of importance in the latter CNS disorder [12–14].

In AD as well as schizophrenia, EAA agonists and, in particular, partial agonists may have therapeutic interest [15]. We have previously described the syntheses and pharmacological characterization of homologues and analogues of AMPA, (*S*)-AMPA being the active enantiomer [15–17]. The lower homologue of AMPA, (*RS*)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid (AMAA) [18], or rather (*R*)-AMAA [19], is a highly selective NMDA agonist, whereas the higher homologue, (*RS*)-2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid (Homo-AMPA) is an equally selective agonist at mGluR6 [15, 20] (fig 1).

Analogues of AMPA with small alkyl substituents in the 5-position of the isoxazole ring, such as (*RS*)-2-amino-3-(5-ethyl-3-hydroxyisoxazol-4-yl)propionic acid (Et-AMPA) [21], are typically full AMPA agonists approximately equipotent with AMPA [15]. (*RS*)-2-Amino-3-(5-*tert*-butyl-3-hydroxyisoxazol-4-yl)propionic acid (ATPA) also is a full agonist at AMPA receptors, though markedly weaker than AMPA [22, 23]. (*RS*)-2-Amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid (APPA) (fig 1) shows the characteristics of a partial AMPA agonist [24] as the result of the interaction between the rather weak, but full, AMPA agonist, (*S*)-APPA, and the competitive AMPA antagonist, (*R*)-APPA [25, 26].

These structure–activity relationships suggest that the cavity, proposed to be present at the AMPA recognition site [27], has a limited size, just capable

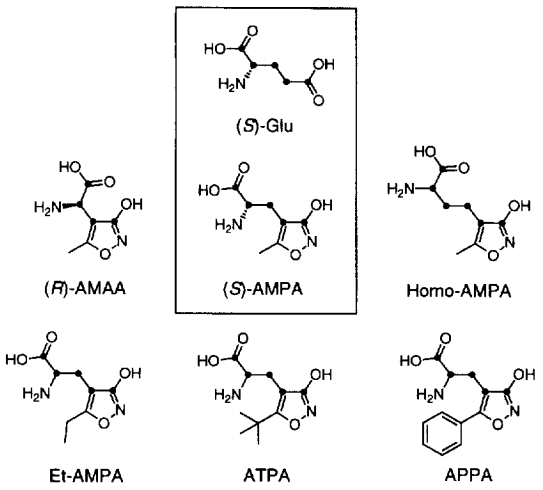
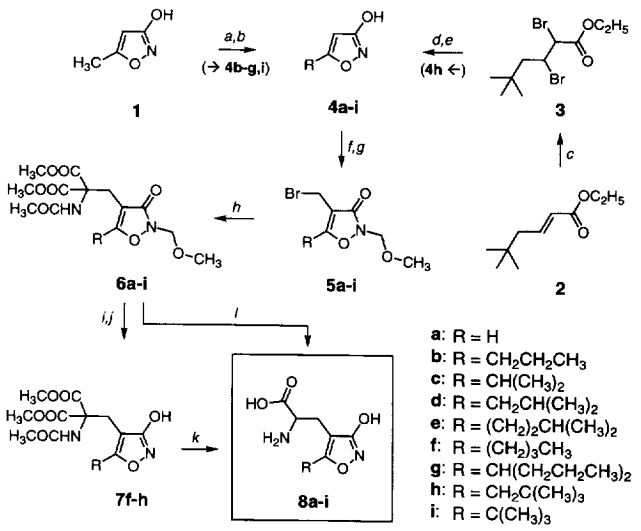


Fig 1. Structures of (*S*)-Glu, (*S*)-AMPA and some homologues and analogues of AMPA.

of accommodating the *tert*-butyl and phenyl groups of ATPA and APPA, respectively [15, 21]. In order to estimate the capacity of this proposed cavity more precisely and to study the pharmacological consequences of further increasing the size of substituents in the 5-position of the ring of AMPA, we have synthesized and pharmacologically characterized a series of AMPA analogues containing a range of substituents of different sizes.

Chemistry

All of the new 3-isoxazolol amino acids, **8a–h**, and the previously described analogue **8i** [22] were synthesized via the respective 3-isoxazolols, **4a–i** containing the desired substituent in the 5-position of the isoxazole ring (scheme 1). The syntheses of compounds **1** and **4a–c,i** [28, 29] have been reported previously. The 3-isoxazolols **4d–g** were all synthesized by alkylation of the deprotonated methyl group of the dianion of **1** using the appropriate alkyl halides. Alkylation with *n*-propyl chloride of the dianion of **1**, generated by using BuLi, gave a mixture of **4f** and **4g**, which could not be separated on a preparative scale. This mixture was transformed into a mixture of **5f** and **5g** and subsequently into a separable mixture of **6f**



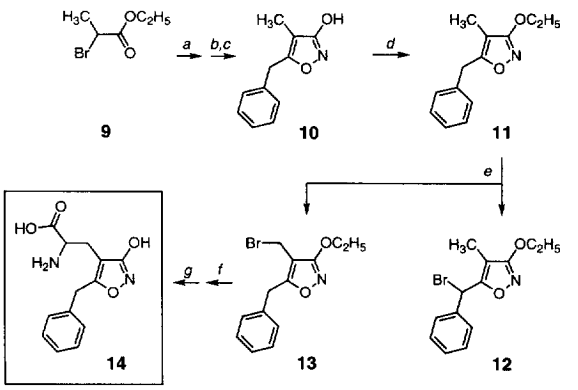
Scheme 1. (a) BuLi or LDA; (b) alkyl halide; (c) Br_2/AcOH; (d) $HONHCONH_2$, NaOH; (e) HCl; (f) 62% HBr, 1,3,5-trioxane; (g) CH_3OH; (h) $(H_3COOC)_2CNaNHCOCH_3$; (i) $(CH_3CO)_2O/BF_3OEt_2$; (j) CH_3ONa; (k) 1 M HCl; (l) CF_3COOH.

and **6g**, which were deprotected individually in two steps to give the final amino acids **8f** and **8g**, respectively. Monoalkylation of the 3-isoxazolols could be achieved using lithium diisopropylamide (LDA) as a base, and this method was used to synthesize **4d** and **4e** from the dianion of **1** and the appropriate alkyl halides.

Compound **4h** was synthesized using ethyl (*E*)-5,5-dimethyl-2-hexenoate (**2**), which was converted into the mixture of compounds, **3**, of unknown stereochemical composition. Treatment of **3** with hydroxyurea under basic conditions furnished 5-(2,2-dimethylpropyl)-3-isoxazolol (**4h**). The key step in the syntheses of the new 3-isoxazolol amino acids **8a–h** and the improved synthesis of ATPA (**8i**) is the transformation of compounds **4a–i** into the 5-alkyl-4-bromomethyl-2-(methoxymethyl)isoxazolin-3-ones **5a–i**. With the exception of **5a** (36% yield), these intermediates were typically produced in very high yields. This reaction step, which proceeds via the 2,4-bis(bromomethyl) analogues of **5a–i**, has been developed on the basis of a similar bromomethylation reaction described previously [30].

Conversion of intermediates **5a–i** into the target 3-isoxazolol amino acids, **8a–i**, proceeded through the dimethyl acetamidomalonates **6a–i**, which were subsequently deprotected, in one or two steps, to give the target 3-isoxazolol amino acids, **8a–i**, respectively (scheme 1). Thus, in the case of **6f–h**, the methoxymethyl groups were removed via transformation into acetoxymethyl groups, by reaction with boron trifluoride etherate and acetic anhydride, and subsequent treatment with sodium methoxide to give **7f–h**, respectively, following a previously described procedure [30]. Compounds **7f–h** were converted into **8f–h** by treatment with 1 M hydrochloric acid. Full deprotection of **6a–e,i** to give **8a–e,i** could be achieved by reflux in trifluoroacetic acid, without formation of detectable amounts of by-products. All of the target amino acids, **8a–i**, were isolated as zwitterions.

Compound **14** was synthesized following an alternative route (scheme 2). Reaction of phenylacetonitrile with (*RS*)-ethyl 2-bromopropionate (**9**) in the presence of $Zn/CuBr_2$ afforded (*RS*)-ethyl 2-methyl-3-oxo-4-phenylbutanoate, which was treated with hydroxylamine under basic conditions to give the appropriately substituted 3-isoxazolol, **10**. The *O*-protected derivative of **10**, compound **11**, was converted into 5-benzyl-4-bromomethyl-3-ethoxyisoxazole (**13**). The NBS bromination of **11** gave a complex reaction mixture, from which only **12** (8%) and **13** (7%) could be isolated using column chromatography. Compound **14** was obtained via a diethyl acetamidomalonate intermediate, which was fully deprotected by treatment with concentrated hydrobromic acid.



Scheme 2. (a) $PhCH_2CN$, $Zn/CuBr_2$; (b) NH_2OH, NaOH; (c) HCl; (d) EtBr, K_2CO_3; (e) NBS, benzoylperoxide; (f) $(EtOOC)_2CKNHCOCH_3$; (g) 48% HBr.

In vitro pharmacology

The new target compounds, **8a–h** and **14**, were characterized pharmacologically in iGluR binding assays using [^{3}H]-(*RS*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid ([^{3}H]CPP), a competitive NMDA antagonist ligand [31], [^{3}H]AMPA [32], and [^{3}H]kainic acid [33] as radioligands, and electrophysiologically using the rat cortical wedge preparation [34]. None of the compounds tested showed significant affinity in [^{3}H]CPP or [^{3}H]kainic acid binding assays (IC_{50} > 100 μM) (table I).

In the [^{3}H]AMPA receptor binding assay, **8b** showed receptor affinity comparable with those of AMPA and Et-AMPA, whereas **8c**, **8d**, and **8f** containing isopropyl, isobutyl, and butyl groups, respectively, in the 5-position of the isoxazole rings, showed progressively weaker AMPA receptor affinities. These receptor affinities of **8b–d,f** were in agreement with their depolarizing effects, which in all cases were sensitive to the AMPA receptor antagonist 6-nitro-7-sulfamoylbenzo(*f*)quinoxaline-2,3-dione (NBQX) [35], as recorded electrophysiologically (table I and fig 2). Similarly, the very low AMPA receptor affinities of **8e**, **8g**, **8h** and **14** correlate positively with the very weak (compound **8h**) or lack of depolarizing effect of these compounds. Demethyl-AMPA (**8a**) showed remarkably low agonist potency in the cortical wedge preparation in light of the effective binding of this compound to AMPA receptor sites. In all cases, the active compounds showed agonist efficacies not significantly different from that of AMPA (fig 2), indicating full agonism. None of the new compounds, showing vanishingly weak AMPA agonist effects (**8e**, **8g**, **8h**, and **14**) (table I), showed detectable antagonist effects (see *Experimental protocols*).

Table I. Receptor binding and in vitro electrophysiological data.

Compound	*Substituent in 5-position of ring (R)*	*IC50 (μM)*			*EC_{50} (μM)*
		[^{3}H]CPP	*[^{3}H]Kainic acid*	*[^{3}H]AMPA*	*Electrophysiology*
AMPA[a]	CH_3	> 100	> 100	0.040 ± 0.014	3.5 ± 0.2
Et-AMPA[a]	CH_2CH_3	> 100	> 100	0.030 ± 0.015	2.3 ± 0.2
ATPA (**8i**)[b]	$C(CH_3)_3$	> 100	> 100	11	48
APPA[c]	C_6H_5	> 100	> 100	35 ± 10	390 ± 60
8a	H	> 100	> 100	0.27 ± 0.06	900 ± 60
8b	$CH_2CH_2CH_3$	> 100	> 100	0.09 ± 0.01	5.0 ± 0.1
8c	$CH(CH_3)_2$	> 100	> 100	0.19 ± 0.02	9.0 ± 0.2
8d	$CH_2CH(CH_3)_2$	> 100	> 100	0.61 ± 0.045	23 ± 4.0
8e	$(CH_2)_2CH(CH_3)_2$	> 100	> 100	> 100	> 1000
8f	$(CH_2)_3CH_3$	> 100	> 100	1.0 ± 0.042	32 ± 3.0
8g	$CH(CH_2CH_2CH_3)_2$	> 100	> 100	99 ± 11	> 1000
8h	$CH_2C(CH_3)_3$	> 100	> 100	55 ± 10	420 ± 40
14	$CH_2C_6H_5$	> 100	> 100	> 100	> 1000
Kainic acid[a]	–	> 100	0.007 ± 0.002	4.0 ± 1.2	nt

IC_{50} and EC_{50} values ± SEM, n = 3–6; nt: not tested; [a]data from ref [21]; [b]data from ref [36]; [c]data from ref [25].

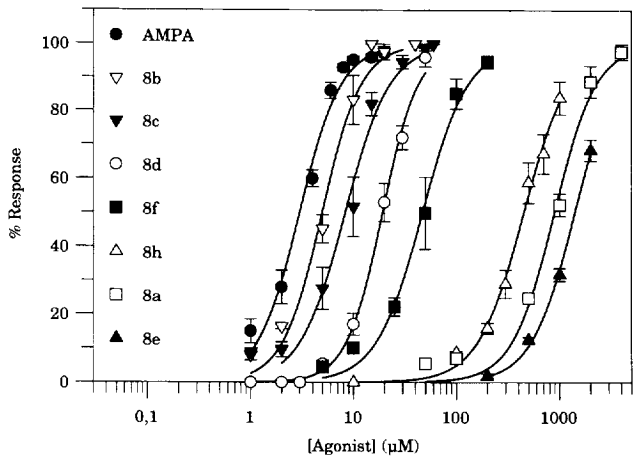


Fig 2. Dose–response curves as determined in the rat cortical wedge preparation for AMPA and analogues **8a–f,h**. Values are mean values ± SEM relative to the maximal AMPA response. Data were fitted to the equation: % response = MAX x $[Ago]^n$ / (EC_{50}^n + $[Ago]^n$), where MAX is the maximal response relative to the AMPA plateau response, [Ago] is the agonist concentration in μM, and n is the Hill slope, determined to be close to 2 for all compounds. A 100% response is determined as the maximal response for AMPA (for details see [26]).

Discussion

In AD as well as schizophrenia, partial agonists at EAA receptors may have therapeutic potential [10, 15, 36]. Such compounds may, at least in principle, be able to partially block excitatory activity in brain areas of EAA hyperactivity but still maintain a basic level of receptor stimulation. A partial agonist showing an appropriately adjusted agonist/antagonist profile may be capable of restoring activity in a nontoxic manner in brain areas suffering from EAA transmitter hypoactivity.

Previously synthesized AMPA analogues, notably Et-AMPA [21] and ATPA [22, 23, 37], show full AMPA receptor agonism, Et-AMPA being equipotent with AMPA and ATPA markedly weaker (table I). Previous attempts to identify partial AMPA agonists via resolution of AMPA agonists have failed [15]. Thus, the effect of AMPA as a full agonist resides in the (*S*)-enantiomer, whereas (*R*)-AMPA is virtually inactive [38], and the apparent partial agonism of APPA [24] has been shown to be the result of an interaction between the full AMPA agonist, (*S*)-APPA, and the competitive AMPA antagonist, (*R*)-APPA [25, 26].

We describe here the synthesis and relationship between AMPA receptor affinity, potency and relative efficacy of a series of analogues of AMPA containing

alkyl groups of systematically varied size in the 5-position of the isoxazole ring (table I). All of the active compounds within this series of 3-isoxazolol amino acids showed relative efficacies not significantly different from that of AMPA (fig 2), and, with the notable exception of demethyl-AMPA (**8a**), a positive correlation between AMPA receptor affinities (IC_{50} values) and NBQX-sensitive depolarizing activities (EC_{50} values). AMPA analogues containing propyl (**8b**) or isopropyl (**8c**) groups were only slightly weaker than AMPA, whereas introduction of butyl (**8f**) or isobutyl (**8d**) groups gave compounds showing potencies about an order of magnitude weaker than AMPA. Compounds containing isopentyl (**8e**) or more bulky (**8g**, **8h** and **14**) groups were very weak or inactive. Demethyl-AMPA (**8a**), which is a potent inhibitor of [^{3}H]AMPA binding (IC_{50} = 0.27 ± 0.06 μM), shows a vanishingly low agonist potency in the rat cortical wedge preparation (EC_{50} = 900 ± 60 μM) (table I).

On the basis of previous structure–activity studies on AMPA analogues, we have proposed the existence of a lipophilic cavity at the AMPA recognition site capable of accommodating alkyl substituents of limited size in the molecules of such analogues [15, 21, 27]. The present structure–activity analysis seems to support this simple model of the AMPA recognition site. Since none of the alkyl-substituted AMPA analogues so far synthesized and pharmacologically characterized show AMPA antagonist effects, these studies further suggest that the proposed cavity is present in the agonist rather than the antagonist conformation of the AMPA receptor. Since demethyl-AMPA (**8a**) shows rather high affinity for the AMPA receptor site but remarkably low agonist potency, we hypothesize that occupancy of this proposed cavity by an appropriately sized alkyl group contributes to the stabilization of the agonist conformation of the AMPA receptor. Structure–activity studies on structurally related AMPA receptor antagonists have indicated that larger alkyl groups, in the same position of the isoxazole nucleus of these compounds, are tolerated at the recognition site of the antagonist conformation of the AMPA receptor [39]. These attempts to indirectly elucidate the topography of the AMPA recognition site(s) may support the on-going attempts to localize this site using molecular biological techniques [40–42].

Whereas this approach has not led to partial AMPA receptor agonists, some of the compounds described, such as demethyl-AMPA (**8a**), may be useful tools for studies of AMPA receptor mechanisms. Electrophysiological studies at higher time resolution using patch-clamp techniques and recombinant AMPA receptors of different subunit combinations are being planned. Such studies may shed light on the apparent difference(s) in molecular mechanism of action of **8a** and AMPA analogues carrying different alkyl substituents in the 5-position of the ring.

Further attempts to design partial agonists at iGluRs on a systematic and rational basis are in progress.

Experimental protocols

Chemistry

Solvents were dried as described by Perrin et al [43] and flash chromatography was performed as described by Still et al [44]. Column chromatography (CC) was performed on silica gel C60-H (230-400 mesh, Rhône-Poulenc). Compounds containing the 3-isoxazolol unit were visualized on TLC plates (Merck silica gel 60 F_{254}) using UV light and $FeCl_3$ spray reagent. Compounds containing amino groups were visualized using a ninhydrin spray reagent. Solvents were removed in vacuo by rotary evaporation at 15 mmHg. Melting points were determined in capillary tubes and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker AC-200 or on a Bruker AC-250 MHz spectrometer. Chemical shifts are in parts per million (ppm) with respect to an internal standard. Elemental analyses were performed by G Cornali, microanalytical laboratory, Leo Pharmaceutical Products or by the analytical department, H Lundbeck A/S Denmark, and were within ±0.4% of the calculated values, unless otherwise stated.

*General bromomethylation procedure. Preparation of **5a–i** (10 mmol experiment)*
A mixture of 3-isoxazolol **4a–i** (10 mmol) and 1,3,5-trioxane (15 mmol) was placed in a sealed flask with aqueous HBr (10 mL; 62%) and heated to 60–80 °C for 16 h unless otherwise stated. The cooled reaction mixture was extracted with CH_2Cl_2 (5 x 10 mL) and CH_3OH (25 mL) was added. The reaction mixture was left standing for 2 h. After addition of further CH_2Cl_2 (50 mL), washing with water (3 x 100 mL) and drying ($MgSO_4$), filtration and evaporation of the organic phase, **5a–i** were obtained.

*General dimethyl acetamidomalonate alkylation procedure. Preparation of **6a–i** (10 mmol experiment)*
Dimethyl acetamidomalonate (1.89 g; 10 mmol) was dissolved in DMF (20 mL), and a suspension of sodium hydride in mineral oil (300 mg; 80%; 10 mmol) was added. The reaction mixture was stirred for 30 min at ambient temperature. A solution of 5-alkyl-4-bromomethyl-2-(methoxymethyl)isoxazolin-3-one **5a–i** (10 mmol) in DMF (20 mL) was added, and the reaction mixture was stirred for 14 h at ambient temperature. Glacial AcOH (3 mL) was added, and the reaction mixture was evaporated (0.1 mmHg; 60 °C). Addition of water (100 mL) and extraction with CH_2Cl_2 (1 x 200; 3 x 50 mL), drying ($MgSO_4$), filtration and evaporation afforded crude **6a–i**.

*General method for selective deprotection of methoxymethyl group. Preparation of **7f–h** (10 mmol experiment)*
To a solution of compound **6f–h** (10 mmol) in CH_2Cl_2 (30 mL) was added acetic anhydride (30 mL) and BF_3OEt_2 (625 μL; 5.0 mmol). The mixture was stirred for 48–72 h, and water (100 mL) added. After stirring for 1 h the mixture was extracted with CH_2Cl_2 and the extracts were washed with water. The organic phase was dried ($MgSO_4$) and evaporated. The crude product was re-evaporated twice from toluene, dissolved in CH_3OH (40 mL) and added to a solution of

sodium methoxide (200 mL; 0.1 M in CH_3OH). The mixture was refluxed for 15 min, cooled and acidified with glacial AcOH. After evaporation and addition of water, crude **7f–h** precipitated.

*General method for deprotection of protected amino acids. Preparation of **8b–e,i** (5.0 mmol experiment)*
Methyl 2-acetamido-3-[5-alkyl-2-(methoxymethyl)-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propionate **6b–e,i** (5.0 mmol) was boiled under reflux for 14 h in trifluoroacetic acid (50 mL; 1 M). The reaction mixture was evaporated and re-evaporated from water (3 x 25 mL). After the last evaporation, water was added (5 mL) and the solution was passed through a column containing Amberlite IRA-400 (50 mL; OH^- form). The column was washed with water until the eluent was neutral, then with AcOH (1 M) until all of the product was collected. The combined product fractions were evaporated and re-evaporated from water (2 x 25 mL) to give **8b–e,i** after recrystallization.

*Ethyl (±)-2,3-dibromo-5,5-dimethylhexanoate **3***
To a solution of compound **2** [45] (2.0 g; 12 mmol) in AcOH (10 mL), cooled to 0 °C and protected against light, was dropwise added a solution of bromine (675 µL; 13 mmol) in AcOH (10 mL). The mixture was stirred for 18 h in the dark at room temperature. Water (10 mL) and 1 M $Na_2S_2O_3$ (30 mL) was added and the mixture was extracted with light petroleum. The organic phase was dried ($MgSO_4$), evaporated and subjected to CC (toluene) to give **3**, which by TLC was shown to consist of two components (R_f = 0.75 and 0.50; toluene). Yield of the former compound: 1.1 g; 28%. Kugelrohr distillation (160 °C; 14 mmHg) of an analytical sample gave **3** as a slightly yellow oil. Anal $C_{10}H_{18}O_2Br_2$ (C, H). ^{1}H-NMR ($CDCl_3$, TMS): δ 4.5–4.2 (4H, m), 2.36 (1H, d, J = 15.7 Hz), 1.90 (1H, dt, J = 15.7, J = 7.8 Hz), 1.33 (3H, t, J = 7.1 Hz), 1.03 (9H, s). This compound was used in the next step. The second, probably diastereomeric, compound (R_f = 0.5) did not cyclize in the next reaction step and was discarded. ^{1}H-NMR ($CDCl_3$, TMS): δ 4.92 (1H, td, J = 6.0 Hz, J = 1.5 Hz), 4.37 (1H, d, J = 1.5 Hz), 4.20 (2H, q, J = 7.1 Hz), 3.10 (2H, d, J = 6.0 Hz), 1.30 (3H, t, J = 7.1 Hz), 1.15 (9H, s).

*5-Isobutyl-3-isoxazolol **4d***
A solution of diisopropylamine (7.1 mL; 50 mmol) in THF (70 mL) was cooled to –10 °C under N_2. BuLi (20 mL; 2.5 M in hexane; 50 mmol) was added and the reaction mixture was stirred for 10 min. Compound **1** [28, 29] (2.48 g; 25 mmol) dissolved in THF (20 mL) was added. After 1 h isopropylbromide (7.0 mL; 75 mmol) was added at –78 °C. After 3 h the temperature was allowed to increase slowly to –10 °C, and the reaction mixture was neutralized with AcOH. THF was evaporated, water (100 mL) was added and the mixture extracted with CH_2Cl_2 (3 x 75 mL). The organic phase was dried ($MgSO_4$), filtered and evaporated. CC (toluene/EtOAc 4:1, containing 1% AcOH) gave **4d** as a colourless oil, which solidified upon standing (3.11 g; 88%). Anal $C_7H_{11}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 12.06 (1H, s), 5.67 (1H, s), 2.51 (2H, d, J = 7.0 Hz), 2.00 (1H, septet, J = 6.7 Hz), 0.95 (6H, d, J = 6.6 Hz).

*5-Isopentyl-3-isoxazolol **4e***
Compound **4e** was synthesized as described for **4d** using **1** (2.48 g; 25 mmol) and isobutylbromide (8.2 mL; 75 mmol). The crude product was subjected to CC (toluene/EtOAc, 4:1) to give **4e** (2.69 g; 69%) as a crystalline product: mp 36–37 °C (toluene). Anal $C_8H_{13}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 11.63 (1H, s), 5.66 (1H, s), 2.63 (2H, t, J = 7.5 Hz), 1.57 (2H + 1H, m), 0.93 (6H, d, J = 6.2 Hz).

*5-Butyl-3-isoxazolol **4f** and 5-(1-propylbutyl)-3-isoxazolol **4g***
Compound **1** [28, 29] (8.0 g; 81 mmol) was dissolved in THF (100 mL) and the solution cooled to –78 °C. BuLi (67 mL; 2.5 M; 168 mmol) was added dropwise and the reaction mixture stirred at –78 °C for 30 min followed by dropwise addition of propylchloride (7.5 mL; 85 mmol). After stirring at –78 °C for 1 h, BuLi (33 mL; 82.5 mmol) was added again and after stirring for 30 min, a further amount of propylchloride (7.5 mL; 85 mmol) was added. After stirring for another 1 h the mixture was slowly brought to room temperature, poured on ice and acidified with HCl (4 M). The mixture was extracted with ether, dried ($MgSO_4$) and evaporated. After CC (toluene/EtOAc, 10:1 → 5:1, containing 2% AcOH) three fractions were obtained: (1) chromatographically pure **4g** (0.50 g; 3.4%); (2) mixture of **4f** and **4g** (3.52 g; 1:2 mixture of **4f** and **4g** based on ^{1}H-NMR); and (3) chromatographically pure **4f** (3.39 g; 29.7%). **4g**: ^{1}H-NMR ($CDCl_3$, TMS): δ 10.25 (1H, broad s), 5.64 (1H, s), 2.73 (1H, quintet, J = 7.5 Hz), 1.58 (2 x 2H, q, J = 7.5 Hz), 1.27 (2 x 2H, sextet, J = 7.5 Hz), 0.88 (2 x 3H, q, J = 7.5 Hz). **4f**: ^{1}H-NMR ($CDCl_3$, TMS): δ 10.25 (1H, broad s), 5.66 (1H, s), 2.64 (2H, t, J = 7.5 Hz), 1.65 (2H, quintet, J = 7.5 Hz), 1.39 (2H, sextet, J = 7.5 Hz), 0.94 (3H, t, J = 7.5 Hz). The crude mixture of **4f** and **4g** was used for the next reaction step without further purification.

*5-(2,2-Dimethylpropyl)-3-isoxazolol **4h***
A solution of NaOH (560 mg; 14 mmol) and hydroxyurea (450 mg; 4 mmol) in MeOH (15 mL) was stirred at room temperature for 30 min. Compound **3** (1.3 g; 4 mmol) dissolved in MeOH (5 mL) was then added dropwise and stirring continued for 6 h at room temperature, followed by reflux overnight. The reaction mixture was evaporated to dryness, water (10 mL) and conc HCl (3 mL) were added followed by stirring for 30 min. After extraction with ether, drying ($MgSO_4$), filtration and evaporation of the organic phase, CC (toluene/EtOAc, 1:1, containing 1% AcOH) gave **4h** (350 mg; 57%) as a colourless crystalline compound, mp 102–103 °C (toluene). Anal $C_8H_{13}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): 5.68 (1H, s), 2.52 (2H, s), 0.98 (9H, s).

*4-Bromomethyl-2-(methoxymethyl)isoxazolin-3-one **5a***
Compound **4a** [28] (2.55 g; 30 mmol) was bromomethylated according to the general procedure (reaction time; 8 days). The crude product was purified by CC (toluene/EtOAc, 2:1, containing 1% AcOH) affording **5a** (2.46 g; 36%) as a colourless oil. Anal $C_6H_8NO_3Br$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$, TMS): δ 8.00 (1H, s), 5.20 (2H, s), 4.18 (2H, s), 3.42 (3H, s).

*4-Bromomethyl-2-methoxymethyl-5-propylisoxazolin-3-one **5b***
Compound **4b** [29, 46] (2.55 g; 20 mmol) was bromomethylated according to the general procedure, to give **5b** (5.25 g; 99%) as a colourless oil. Anal $C_9H_{14}NO_3Br$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$, TMS): δ 5.16 (2H, s), 4.20 (2H, s), 3.40 (3H, s), 2.66 (2H, t, J = 7.7 Hz), 1.77 (2H, sextet, J = 7.5 Hz), 1.03 (3H, t, J = 7.3 Hz).

*4-Bromomethyl-5-isopropyl-2-(methoxymethyl)isoxazolin-3-one **5c***
Compound **4c** [29, 46] (2.54 g; 20.0 mmol) was bromomethylated according to the general procedure, to give **5c** (5.09 g; 96%) as a colourless oil. Anal $C_9H_{14}NO_3Br$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$, TMS): δ 5.10 (2H, s), 4.16 (2H, s), 3.34 (3H, s), 3.10 (1H, sextet, J = 7.0 Hz), 1.28 (6H, d, J = 7.0 Hz).

*4-Bromomethyl-5-isobutyl-2-(methoxymethyl)isoxazolin-3-one **5d***
Compound **4d** (2.17 g; 15.4 mmol) was bromomethylated according to the general procedure, to give **5d** (3.64 g; 85%) as

a colourless oil. Anal $C_{10}H_{16}NO_3Br$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$, TMS): δ 5.17 (2H, s), 4.19 (2H, s), 3.40 (3H, s), 2.54 (2H, d, J = 7.3 Hz), 2.14 (1H, septet, J = 6.9 Hz), 1.02 (6H, d, J = 6.6 Hz).

4-Bromomethyl-5-isopentyl-2-(methoxymethyl)isoxazolin-3-one ***5e***
Compound **4e** (2.30 g; 14.8 mmol) was bromomethylated according to the general procedure, to give **5e** (4.3 g; 99%) as a colourless oil. Anal $C_{11}H_{18}NO_3Br$ (C, H, N, Br). ^{1}H-NMR $CDCl_3$, TMS): δ 5.16 (2H, s), 4.19 (2H, s), 3.40 (3H, s), 2.67 (2H, t, J = 7.5 Hz), 1.63 (2H + 1H, m), 0.97 (6H, d, J = 6.2 Hz).

4-Bromomethyl-5-butyl-2-(methoxymethyl)isoxazolin-3-one ***5f*** *and 4-bromomethyl-2-methoxymethyl-5-(1-propylbutyl)isoxazolin-3-one* ***5g***
A mixture of **4f** and **4g** (3.5 g; ca 7.5 mmol **4f** and 15 mmol **4g**) was bromomethylated according to the general procedure, to give crude **5f** and **5g** (5.8 g; 90%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, TMS): δ 5.16 (1H + 2H, s), 4.19 (1H + 2H, s), 3.40 (3H, s), 3.38 (1.5H, s), 2.85 (1H, quintet, J = 7.5 Hz), 2.65 (1H, t, J = 7.5 Hz), 1.8–1.2 (2H + 8H, m), 0.95 (1.5H, t, J = 7.5 Hz), 0.9 (6H, t, J = 7.5 Hz).The crude mixture of **5f** and **5g** was used in the next step without further purification.

4-Bromomethyl-5-(2,2-dimethylpropyl)-2-(methoxymethyl)-isoxazolin-3-one ***5h***
Compound **4h** (1.15 g; 7.4 mmol) was bromomethylated according to the general procedure, to give **5h** (1.7 g; 79%) as a yellow oil. ^{1}H-NMR ($CDCl_3$, TMS): δ 5.18 (2H, s), 4.19 (2H, s), 3.40 (3H, s), 2.55 (2H, s), 1.05 (9H, s). The crude product was used in the next step without further purification.

4-Bromomethyl-5-tert-butyl-2-(methoxymethyl)isoxazolin-3-one ***5i***
Compound **4i** [29, 46] (423 mg; 3.0 mmol) was bromomethylated according to the general procedure, with a reaction temperature of 80 °C, to give **5i** (830 mg; 100%) as a colourless oil. Anal $C_{10}H_{16}NO_3Br$ (C, H, N, Br). ^{1}H-NMR ($CDCl_3$, TMS): δ 5.17 (2H, s), 4.34 (2H, s), 3.40 (3H, s), 1.43 (9H, s).

Methyl 2-acetamido-2-methoxycarbonyl-3-(2-methoxymethyl-3-oxoisoxazolin-4-yl)propionate ***6a***
4-Bromomethyl-2-(methoxymethyl)isoxazolin-3-one **5a** (1.70 g; 7.7 mmol) was treated as described for the general dimethyl acetamidomalonate alkylation procedure. The reaction mixture was purified by CC (toluene/EtOAc, 1:1, containing 1% AcOH) to give **6a** (1.65 g; 65%) as colourless crystals (ether/pentane): mp 125–127 °C. Anal $C_{13}H_{18}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.85 (1H, s), 7.23 (1H, broad s), 5.14 (2H, s), 3.80 (6H, s), 3.36 (3H, s), 3.34 (2H, s), 2.05 (3H, s).

Methyl 2-acetamido-2-methoxycarbonyl-3-(2-methoxymethyl-3-oxo-5-propylisoxazolin-4-yl)propionate ***6b***
Compound **5b** (4.78 g; 18.1 mmol) was treated as described for the general dimethyl acetamidomalonate alkylation procedure to give **6b** (4.98 g; 74%) as colourless crystals (ether/pentane): mp 87–88 °C. Anal $C_{16}H_{24}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.26 (1H, broad s), 5.11 (2H, s), 3.81 (6H, s), 3.36 (3H, s), 3.29 (2H, s), 2.49 (2H, t, J = 7.8 Hz), 2.03 (3H, s), 1.64 (2H, sextet, J = 7.6 Hz), 0.96 (3H, t, J = 7.4 Hz).

Methyl 2-acetamido-3-(5-isopropyl-2-methoxymethyl-3-oxoisoxazolin-4-yl)-2-(methoxycarbonyl)propionate ***6c***
Compound **5c** (4.96 g; 18.8 mmol) was treated as described for the general dimethyl acetamidomalonate alkylation procedure to give **6c** (5.1 g; 73%) as colourless crystals (ether/pentane): mp 126–126 °C. Anal $C_{16}H_{24}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.23 (1H, broad s), 5.12 (2H, s), 3.83 (6H, s), 3.38 (3H, s), 3.31 (2H, s), 3.01 (1H, septet, J = 7.0 Hz), 2.04 (3H, s), 1.22 (6H, d, J = 7.0 Hz).

Methyl 2-acetamido-3-(5-isobutyl-2-methoxymethyl-3-oxoisoxazolin-4-yl)-2-(methoxycarbonyl)propionate ***6d***
Compound **5d** (3.06 g; 11.0 mmol) was treated as described for the general dimethyl acetamidomalonate alkylation procedure to give **6d** (2.85 g; 67%) as colourless crystals (ether/pentane): mp 86.5–87 °C. Anal $C_{17}H_{26}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.2 (1H, s), 5.12 (2H, s), 3.82 (6H, s), 3.36 (3H, s), 3.29 (2H, s), 2.39 (2H, broad t, J = 7.3 Hz), 2.02 (3H + 1H, m), 0.94 (6H, d, J = 6.6 Hz).

Methyl 2-acetamido-3-(5-isopentyl-2-methoxymethyl-3-oxoisoxazolin-4-yl)-2-(methoxycarbonyl)propionate ***6e***
Compound **5e** (3.57 g; 12.2 mmol) was treated as described for the general dimethyl acetamidomalonate alkylation procedure to give **6e** (3.6 g; 74%) as colourless crystals (ether/pentane): mp 76.5–77.5 °C. Anal $C_{18}H_{28}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.20 (1H, s), 5.11 (2H, s), 3.82 (6H, s), 3.37 (3H, s), 3.30 (2H, s), 2.50 (2H, broad t, J = 8.0 Hz), 2.02 (3H, s), 1.49 (2H + 1H, m), 0.92 (6H, d, J = 6.2 Hz).

Methyl 2-acetamido-3-(5-butyl-2-methoxymethyl-3-oxoisoxazolin-4-yl)-2-(methoxycarbonyl)propionate ***6f*** *and methyl 2-acetamido-2-(methoxycarbonyl)-3-(2-methoxymethyl-3-oxo-5-propylbutyl)isoxazolin-4-yl)propionate* ***6g***
To a suspension of sodium hydride (0.88 g; 60% dispersion; 22 mmol) in DMF (50 mL), cooled to 0 °C, was added a solution of dimethyl acetamidomalonate (3.8 g; 20 mmol) in DMF (20 mL). After stirring at 0 °C for 30 min a solution of a mixture of **5f** and **5g** (5.8 g; ca 13 mmol **5f** and 7 mmol **5g**) in DMF (15 mL) was added at 0 °C, followed by stirring for 18 h at room temperature. The reaction mixture was evaporated to dryness, H_2O (100 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 x 100 mL). The organic phases were dried ($MgSO_4$) and evaporated. CC (toluene/EtOAc, 4:1 → 2:1) afforded **6g** (2.1 g; 33%) as colourless crystals (EtOAc/light petroleum): mp 89–90 °C. Anal $C_{20}H_{32}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_2$, TMS): δ 5.1 (2H, s), 3.8 (6H, s), 3.35 (3H, s), 3.3 (2H, s), 2.75 (1H, m), 2.0 (3H, s), 1.55 (4H, m), 1.25 (4H, m), 0.9 (6H, t, J = 7.1 Hz). Further elution gave **6f** (1.9 g; 66%) as colourless crystals (EtOAc/light petroleum): mp 73–74 °C. Anal $C_{17}H_{26}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 5.12 (2H, s), 3.83 (6H, s), 3.38 (3H, s), 3.30 (2H, s), 2.51 (2H, t, J = 7.5 Hz), 2.03 (3H, s), 1.35 (2H, m), 0.93 (3H, t, J = 7.2 Hz).

Methyl 2-acetamido-3-[5-(2,2-dimethylpropyl)-2-methoxymethyl-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propionate ***6h***
To a suspension of sodium hydride (260 mg; 60% dispersion; 6.4 mmol) in DMF (20 mL) under nitrogen at 0 °C was added a solution of dimethyl acetamidomalonate (1.2 g; 6.4 mmol) in DMF (5 mL). After stirring for 15 min at 0 °C, a solution of compound **5h** (1.7 g; 5.8 mmol) in DMF (5 mL) was added followed by stirring for 18 h at room temperature. The reaction mixture was evaporated to dryness, H_2O was added and the mixture extracted with EtOAc. The organic phase was dried ($MgSO_4$) and evaporated. CC (toluene/EtOAc, 1:1) gave **6h** (1.4 g; 60%): mp 104–105 °C (EtOAc/light petroleum). Anal $C_{18}H_{28}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): 5.12 (2H, s), 3.81 (6H, s), 3.36 (3H, s), 3.30 (2H, s), 2.40 (2H, s), 2.01 (3H, s), 0.98 (9H, s).

*Methyl 2-acetamido-3-(5-tert-butyl-2-methoxymethyl-3-oxoisoxazolin-4-yl)-2-(methoxycarbonyl)propionate **6i***
Compound **5i** (750 mg; 2.7 mmol) was treated as described for the general dimethyl acetamidomalonate alkylation procedure to give **6i** (720 mg; 69%) as colourless crystals: mp 123–125 °C (ether/pentane). Anal $C_{17}H_{26}N_2O_8$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.54 (1H, broad s), 5.12 (2H, s), 3.81 (6H, s), 3.47 (2H, s), 3.38 (3H, s), 2.01 (3H, s), 1.34 (9H, s).

*Methyl 2-acetamido-3-(5-butyl-3-hydroxyisoxazol-4-yl)-2-(methoxycarbonyl)propionate **7f***
Compound **6f** (4.4 g; 11.5 mmol) was deprotected as described for the general method for selective deprotection of the methoxymethyl group (reaction time: 72 h). Recrystallization of the crude product (EtOAc) afforded **7f** (3.2 g; 81%) as colourless crystals: mp 144–145 °C. Anal $C_{15}H_{22}N_2O_7$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 6.94 (1H, broad s), 3.81 (6H, s), 3.37 (2H, s), 2.45 (2H, t, J = 7.5 Hz), 2.01 (3H, s), 1.57 (2H, quintet, J = 7.3 Hz), 1.36 (2H, sextet, J = 7.3 Hz), 0.91 (3H, t, J = 7.2 Hz).

*Methyl 2-acetamido-3-[3-hydroxy-5-(1-propylbutyl)isoxazol-4-yl]-2-(methoxycarbonyl)propionate **7g***
Compound **6g** (1.0 g; 2.4 mmol) was deprotected as described for the general method for selective deprotection of the methoxymethyl group (reaction time: 48 h). Recrystallization of the crude product (EtOAc/light petroleum) afforded **7g** (0.6 g; 66%) as colourless crystals: mp 149–150 °C. Anal $C_{18}H_{28}N_2O_7$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 6.81 (1H, broad s), 3.82 (6H, s), 3.40 (2H, s), 2.65 (1H, m), 2.01 (3H, s), 1.55 (4H, m), 1.25 (4H, m), 0.88 (6H, t, J = 7 Hz).

*Methyl 2-acetamido-3-[5-(2,2-dimethylpropyl)-3-hydroxyisoxazol-4-yl]-2-(methoxycarbonyl)propionate **7h***
Compound **6h** (1.4 g; 3.5 mmol) was deprotected as described for the general method for selective deprotection of the methoxymethyl group (reaction time: 48 h). Recrystallization of the crude product (EtOAc/light petroleum) afforded **7h** (700 mg; 56%): mp 194–195 °C. Anal $C_{16}H_{24}N_2O_7$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): δ 6.75 (1H, s), 3.81 (6H, s), 3.41 (2H, s), 2.40 (2H, s), 2.02 (3H, s), 0.95 (9H, s).

*(RS)-2-Amino-3-(3-hydroxyisoxazol-4-yl)propionic acid zwitterion **8a***
A solution of compound **6a** (320 mg; 0.96 mmol) in trifluoroacetic acid (15 mL; 4 M) was heated at 80 °C for 48 h. The reaction mixture was evaporated, dissolved in H_2O (0.3 mL) and EtOH (3 mL). The pH of the solution was adjusted to 4 by adding a solution of triethylamine in EtOH to precipitate crude **8a** (166 mg). Recrystallization (EtOH/H_2O, 3:1) and three times from water gave **8a** (38 mg; 23%) as colourless crystals: mp 215–225 °C (decomp). Anal $C_6H_8N_2O_4$ (C, H, N), C: calcd: 41.86; found: 41.40. ^{1}H-NMR (D_2O, DSS, 37 °C): δ 8.36 (1H, s), 4.11 (1H, t, J = 6.5 Hz), 3.10 (2H, d, J = 6.5 Hz).

*(RS)-2-Amino-3-(3-hydroxy-5-propylisoxazol-4-yl)propionic acid zwitterion **8b***
Compound **6b** (1.86 g; 5.0 mmol) was deprotected as described in the general method to give **8b** (612 mg, 55%) as colourless crystals (H_2O): mp 200–205 °C (decomp). Anal $C_9H_{14}N_2O_4 \cdot 0.5\ H_2O$ (C, H, N). ^{1}H-NMR (D_2O, dioxane): δ 3.32 (1H, dd, J = 5.5 Hz, J = 8.7 Hz), 2.58 (1H, dd, J = 5.5 Hz, J = 14.4 Hz), 2.49 (2H, t, J = 7.5 Hz), 2.30 (1H, dd, J = 8.7 Hz, J = 14.4 Hz), 1.56 (2H, sextet, J = 7.4 Hz), 0.85 (3H, t, J = 7.3 Hz).

*(RS)-2-Amino-3-(3-hydroxy-5-isopropylisoxazol-4-yl)propionic acid zwitterion **8c***
Compound **6c** (1.49 g; 4.0 mmol) was deprotected as described in the general method to give **8c** (424 mg; 49%) as colourless crystals (H_2O): mp 210–220 °C (decomp). Anal $C_9H_{14}N_2O_4 \cdot 0.25\ H_2O$ (C, H, N). ^{1}H-NMR (D_2O, dioxane): δ 3.31 (1H, dd, J = 5.6 Hz, J = 8.5 Hz), 2.96 (1H, septet, J = 7.0 Hz), 2.57 (1H, dd, J = 5.6 Hz, J = 14.4 Hz), 2.29 (1H, dd, J = 8.5 Hz, J = 14.4 Hz), 1.14 (3H, d, J = 7.0 Hz), 1.12 (3H, d, J = 7.0 Hz).

*(RS)-2-Amino-3-(3-hydroxy-5-isobutylisoxazol-4-yl)propionic acid zwitterion **8d***
Compound **6d** (1.55 g; 4.0 mmol) was deprotected as described in the general method to give **8d** (519 mg; 57%) as colourless crystals (H_2O): mp 214–220 °C (decomp). Anal $C_{10}H_{16}N_2O_4$ (C, H, N). ^{1}H-NMR (D_2O, dioxane, 1 M CF_3COOD): δ 4.28 (1H, t, J = 6.1 Hz), 2.98 (2H, d, J = 6.1 Hz), 2.50 (2H, d, J = 7.3 Hz), 1.96 (1H, m), 0.86 (6H, d, J = 6.7 Hz).

*(RS)-2-Amino-3-(3-hydroxy-5-isopentylisoxazol-4-yl)propionic acid zwitterion **8e***
Compound **6e** (2.0 g; 5.0 mmol) was deprotected as described in the general method to give **8e** (640 mg; 51%) as colourless crystals (H_2O): mp 208–211 °C (decomp). Anal $C_{11}H_{18}N_2O_4 \cdot 0.5\ H_2O$ (C, H, N). ^{1}H-NMR (D_2O, dioxane): δ 3.30 (1H, dd, J = 5.4 Hz, J = 8.6 Hz), 2.57 (1H, dd, J = 5.4 Hz, J = 14.3 Hz), 2.51 (2H, t, J = 7.8 Hz), 2.32 (1H, dd, J = 8.6 Hz, J = 14.3 Hz), 1.44 (3H, m), 0.84 (6H, d, J = 6.1 Hz).

*(RS)-2-Amino-3-(5-butyl-3-hydroxyisoxazol-4-yl)propionic acid zwitterion **8f***
A solution of **7f** (200 mg; 0.58 mmol) in 1 M HCl (10 mL) was refluxed for 18 h and evaporated. The crude product was dissolved in H_2O (0.25 mL) and EtOH (2 mL) was added. Upon addition of triethylamine to pH 3.5 crude **8f** precipitated, which after recrystallization (H_2O) gave **8f** (60 mg; 45%) as colourless crystals: mp 189–190 °C (decomp). Anal $C_{10}H_{16}N_2O_4 \cdot 0.5\ H_2O$ (C, H, N). ^{1}H-NMR (D_2O): δ 3.8 (1H, t, J = 7 Hz), 2.8 (2H, d, J = 7 Hz), 2.55 (2H, t, J = 7.5 Hz), 1.5 (2H, quintet, J = 7.5 Hz), 1.25 (2H, sextet, J = 7.5 Hz), 0.8 (3H, t, J = 7.5 Hz).

*(RS)-2-Amino-3-[3-hydroxy-5-(1-propylbutyl)isoxazol-4-yl]-propionic acid zwitterion **8g***
Compound **7g** (100 mg; 0.26 mmol) was converted into **8g** (43 mg; 61%) by using the same method as described for compound **8f**: mp 199.5–201 °C (H_2O) (decomp). Anal $C_{13}H_{22}N_2O_4 \cdot 0.25H_2O$ (C, H, N). ^{1}H-NMR (D_2O): δ 3.8 (1H, dd, J = 5 Hz, J = 7.5 Hz), 2.7 (1H, m), 2.75 (2H, m), 1.1 (4H, m), 1.5 (4H, m), 0.75 (6H, t, J = 7.5 Hz).

*(RS)-2-Amino-3-[5-(2,2-dimethylpropyl)-3-hydroxyisoxazol-4-yl]propionic acid zwitterion **8h***
Compound **7h** (200 mg; 0.6 mmol) was converted into **8h** (95 mg; 65%) by using the same method as described for compound **8f**: mp 216–218 °C (H_2O) (decomp). Anal $C_{11}H_{18}N_2O_4 \cdot 0{,}25H_2O$ (C, H, N). ^{1}H-NMR (D_2O): δ 3.05 (1H, broad t, J = 6 Hz), 2.85 (2H, broad d, J = 6 Hz), 2.52 (1H, d, J = 15 Hz), 2.44 (1H, d, J = 15 Hz), 0.88 (9H, s).

*(RS)-2-Amino-3-(5-tert-butyl-3-hydroxyisoxazol-4-yl)propionic acid zwitterion **8i***
Compound **6i** (2.05 g; 5.3 mmol) was deprotected as described in the general method to give **8i** (370 mg; 30%) as colourless crystals: mp 208–210 °C (H_2O) (decomp). Anal $C_{10}H_{16}N_2O_4$ (C, H, N). ^{1}H-NMR (D_2O, dioxane) δ 3.78 (1H, t, J = 7 Hz), 2.94 (1H, dd, J = 15 Hz, J = 7 Hz), 2.84 (1H, dd, J = 15 Hz, J = 7 Hz), 1.25 (9 H, s).

5-Benzyl-4-methyl-3-isoxazolol ***10***
A mixture of phenylacetonitrile (157 g; 1.3 mol), activated zinc dust (100 g; 1.5 mol) and $CuBr_2$ (1 g; 4.4 mmol) in benzene (1000 mL) was heated to reflux temperature. (*RS*)-Ethyl 2-bromopropionate (**9**) (270 g; 1.5 mol) was added dropwise over a period of 1 h at reflux temperature, and the resulting mixture was boiled under reflux for 1 h. The reaction mixture was cooled to 0 °C, and 15% aqueous H_2SO_4 (500 mL; 0.84 mol) was added dropwise while the temperature was kept below 10 °C. The resulting solution was stirred for 2 h at room temperature. The solution was filtered and the phases separated. The organic phase was washed with water, dried ($MgSO_4$) and evaporated. Distillation of the resulting oil gave crude (*RS*)-ethyl 2-methyl-3-oxo-4-phenylbutanoate (165 g), bp 120–130 °C/0.8 mmHg. ^{1}H-NMR ($CDCl_3$, TMS): δ 7.39–7.16 (5H, m), 4.17 (2H, q, J = 6.9 Hz), 3.86 (2H, s), 3.62 (1H, q, J = 7.5 Hz), 1.31 (3H, d, J = 7.5 Hz), 1.27 (3H, q, J = 6.9 Hz). To a cold (–20 °C) solution of NaOH (31.5 g, 0.83 mol) in CH_3OH (600 mL) and water (37.5 mL) was added crude (*RS*)-ethyl 2-methyl-3-oxo-4-phenylbutanoate (165 g). The resulting solution was cooled to –30 °C and a solution of NH_2OH prepared as follows was added dropwise: a solution of NH_2OH, HCl (105 g; 1.5 mol) in hot water (105 mL) and CH_3OH (375 mL) was added to a solution of NaOH (61.5 g; 1.6 mol) in CH_3OH (600 mL) and water (37.5 mL) at 0 °C, and the resulting solution was filtered at 0 °C. The resulting reaction mixture was stirred for 1.5 h at –30 °C and for 1 h at 0 °C. The cold solution was then poured into an 80 °C aqueous solution of 8 M HCl (280 mL; 2.25 mol) over 1 h and the reaction mixture was boiled for further 1 h at 80 °C. The reaction mixture was cooled to 0 °C and the obtained precipitate was collected by filtration. The crystals were washed with water and dried in vacuo to give **10** (80 g; 43%), mp 134–136 °C. Anal $C_{11}H_{11}NO_2$ (C, H, N). ^{1}H-NMR ($CDCl_3$, TMS): 10.92 (1H, broad s), 7.38–7.13 (5H, m), 3.92 (2H, s), 1.83 (3H, s).

5-Benzyl-3-ethoxy-4-methylisoxazole ***11***
A suspension of **10** (220 g; 1.2 mol) and K_2CO_3 (248 g; 1.9 mol) in acetone (2.7 L) was stirred for 0.5 h at 20 °C. Ethyl bromide (578 g; 5.3 mol) was added, and the resulting reaction mixture was stirred for 24 h at 50 °C. The reaction mixture was cooled, filtered, evaporated and subjected to CC (EtOAc/heptane, 1:3) to give **11** (130 g; 60%) as an oil. ^{1}H-NMR ($CDCl_3$, TMS): δ 7.36–7.18 (5H, m), 4.29 (2H, q, J = 7.5 Hz), 3.93 (2H, s), 1.78 (3H, s), 1.41 (3H, t, J = 7.5 Hz). Crude **11** was used in the next step without further purification.

5-(α-Bromobenzyl)-3-ethoxy-4-methylisoxazole ***12*** *and 5-benzyl-4-bromomethyl-3-ethoxyisoxazole* ***13***
A mixture of **11** (10.0 g; 50 mmol) and *N*-bromosuccinimide (NBS) (8.6 g; 50 mmol) in CCl_4 (250 mL) was heated under reflux for 18 h. The reaction mixture was cooled, filtered and evaporated. The residue was subjected to CC (EtOAc/heptane/MeOH, 50:50:1) to give crude **12** as an oil (1.0 g; 7.5%). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.62–7.51 (2H, m), 7.43–7.29 (3H, m), 6.06 (1H, s), 4.31 (2H, q, J = 7.5 Hz), 1.83 (3H, s), 1.41 (3H, t, J = 7.5 Hz). Further elution gave crude **13** as an oil (0.9 g; 6.6%). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.43–7.20 (5H, m), 4.32 (2H, q, J = 7.5 Hz), 4.10 (2H, s), 4.02 (2H, s), 1.42 (3H, t, J = 7.5 Hz).

(RS)-2-Amino-3-(5-benzyl-3-hydroxyisoxazol-4-yl)propionic acid zwitterion hydrate ***14***
To a solution of diethyl acetamidomalonate (1.3 g; 6.0 mmol) and potassium *tert*-butoxide (0.68 g; 6.1 mmol) in *N*-methyl pyrrolidone (NMP) (10 mL) was added a solution of **13** (0.9 g; 3.0 mmol) in NMP (2 mL) at 22 °C. The reaction mixture was stirred for 1 h at 22 °C and then poured into an ice/water mixture. The aqueous phase was extracted with ether (3 x 100 mL) and the combined organic phases were washed with a saturated aqueous solution of NaCl. The organic phase was dried ($MgSO_4$), evaporated, and the residue subjected to CC (EtOAc/heptane, 7:3) to give crude ethyl 2-acetamido-3-(5-benzyl-3-ethoxyisoxazol-4-yl)-2-(ethoxycarbonyl)propionate (0.9 g). ^{1}H-NMR ($CDCl_3$, TMS): δ 7.33–7.14 (5H, m), 6.68 (1H, s), 4.38–4.09 (6H, m), 3.90 (2H, s), 3.45 (2H, s), 1.83 (3H, s), 1.39 (3H, t, J = 7.2 Hz), 1.28 (6H, t, J = 7.5 Hz). A mixture of crude ethyl 2-acetamido-3-(5-benzyl-3-ethoxyisoxazol-4-yl)-2-(ethoxycarbonyl)propionate (0.9 g; 2.1 mmol) and 48% HBr (10 mL) was heated under reflux for 1 h. The solution was evaporated and the residue was dissolved in water (20 mL). The solution was treated with active charcoal, and pH of the aqueous solution was adjusted to 3 using 4 M aqueous NaOH. The resulting crystals were collected by filtration and dried to give **14** as the zwitterion (0.2 g; 25%). Mp 198–201 °C (decom). Anal $C_{13}H_{14}N_2O_4 \cdot H_2O$ (C, H, N). ^{1}H-NMR (DMSO-d_6): δ 7.38–7.16 (5H, m), 3.99 (2H, s), 3.53 (1H, t, J = 5.0 Hz), 2.72 (2H, d, J = 5.0 Hz).

Receptor binding assays

Affinity for NMDA, AMPA and kainic acid receptors was determined using the ligands [^{3}H]CPP [31], [^{3}H]AMPA [32] and [^{3}H]kainic acid [33], respectively. The membrane preparations used in all the receptor binding experiments were prepared according to the method of Ransom and Stec [47].

In vitro electrophysiology

A rat cortical wedge preparation for determination of EAA-evoked depolarizations described by Harrison and Simmonds [34] 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 (and Ca^{2+}-free for the corpus callosum) oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium and the potential difference between the electrodes recorded on a chart recorder. Applications of agonists were made 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 co-application of agonist (AMPA, 5 μM) and potential antagonist for another 90 s.

Acknowledgments

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 P Bregnedal and O Rondeau and the secretarial assistance of A Nordly and AM Nielsen are gratefully acknowledged.

References

1 Krogsgaard-Larsen P, Hansen JJ (1992) *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*, Ellis Horwood, Chichester
2 Collingridge GL, Watkins JC (1994) *The NMDA Receptor*, Oxford University Press, Oxford
3 Wheal HV, Thomson AM (1995) *Excitatory Amino Acids and Synaptic Transmission*, Academic Press, London
4 Conn PJ, Patel J (1994) *The Metabotropic Glutamate Receptors*, Humana Press, New Jersey
5 Danysz W, Parsons CG, Bresink I, Quack G (1995) *Drug News Perspect* 8, 261–277
6 Knöpfel T, Kuhn R, Allgeier H (1995) *J Med Chem* 38, 1417–1426
7 Deutsch SI, Morihisa JM (1988) *Clin Neuropharmacol* 11, 18–35
8 Greenamyre JT, Young AB (1989) *Neurobiol Ag* 10, 593–602
9 Bowen DM (1990) *Br J Psychiatry* 157, 327–330
10 Madsen U, Ebert B, Krogsgaard-Larsen P (1994) *Biomed Pharmacother* 48, 305–311
11 Carlsson M, Carlsson A (1990) *Trends Neurosci* 13, 272–276
12 Ulas J, Cotman CW (1993) *Schizophr Bull* 19, 105–117
13 Deutsch SI, Mastropaolo J, Schwartz BL, Rosse RB, Morihisa JM (1989) *Clin Neuropharmacol* 12, 1–13
14 Drejer J (1992) *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists* (Krogsgaard-Larsen P, Hansen JJ, eds) Ellis Horwood, Chichester, 352–375
15 Krogsgaard-Larsen P, Ebert B, Lund TM et al (1996) *Eur J Med Chem* 31, 515–537
16 Hansen JJ, Lauridsen J, Nielsen E, Krogsgaard-Larsen P (1983) *J Med Chem* 26, 901–903
17 Krogsgaard-Larsen P, Johnston GAR, Lodge D, Curtis DR (1977) *Nature* 268, 53–55
18 Madsen U, Ferkany JW, Jones BE et al (1990) *Eur J Pharmacol Mol Pharmacol Sect* 189, 381–391
19 Madsen U, Frydenvang K, Ebert B, Johansen TN, Brehm L, Krogsgaard-Larsen P (1996) *J Med Chem* 39, 183–190
20 Bräuner-Osborne H, Sløk FA, Skjærbæk N et al (1996) *J Med Chem* 39, 3188–3194
21 Madsen U, Frølund B, Lund TM, Ebert B, Krogsgaard-Larsen P (1993) *Eur J Med Chem* 28, 791–800
22 Lauridsen J, Honoré T, Krogsgaard-Larsen P (1985) *J Med Chem* 28, 668–672
23 Krogsgaard-Larsen P, Hansen JJ, Lauridsen J, Peet MJ, Leah JD, Curtis DR (1982) *Neurosci Lett* 31, 313–317
24 Christensen IT, Reinhardt A, Nielsen B et al (1989) *Drug Des Dev* 5, 57–71
25 Ebert B, Lenz SM, Brehm L et al (1994) *J Med Chem* 37, 878–884
26 Ebert B, Madsen U, Lund TM, Lenz SM, Krogsgaard-Larsen P (1994) *Neurochem Int* 24, 507–515
27 Brehm L, Jørgensen FS, Hansen JJ, Krogsgaard-Larsen P (1988) *Drug News Perspect* 1, 138–144
28 Iwai I, Nakamura N (1966) *Chem Pharm Bull (Tokyo)* 14, 1277–1286
29 Jacobsen N, Kolind-Andersen H, Christensen J (1984) *Can J Chem* 62, 1940–1944
30 Begtrup M, Sløk FA (1993) *Synthesis* 9, 861–863
31 Murphy DE, Schneider J, Boehm C, Lehmann J, Williams K (1987) *J Pharmacol Exp Ther* 240, 778–783
32 Honoré T, Nielsen M (1985) *Neurosci Lett* 54, 27–32
33 Braitman DJ, Coyle JT (1987) *Neuropharmacology* 26, 1247–1251
34 Harrison NL, Simmonds MA (1985) *Br J Pharmacol* 84, 381–391
35 Sheardown MJ, Nielsen EØ, Hansen AJ, Jacobsen P, Honoré T (1990) *Science* 247, 571–574
36 Ebert B, Søby KK, Madsen U, Krogsgaard-Larsen P (1995) *Schizophrenia – An Integrated View* (Fog R, Gerlach J, Hemmingsen R, eds) Munksgaard, Copenhagen, 379–398
37 Ebert B, Madsen U, Lund TM, Holm T, Krogsgaard-Larsen P (1992) *Mol Neuropharmacol* 2, 47–49
38 Hansen JJ, Lauridsen J, Nielsen E, Krogsgaard-Larsen P (1983) *J Med Chem* 26, 901–903
39 Madsen U, Bang-Andersen B, Brehm L et al (1996) *J Med Chem* 39, 1682–1691
40 Hollmann M, Maron C, Heinemann S (1994) *Neuron* 13, 1331–1343
41 Stern-Bach Y, Bettler B, Hartley M, Sheppard PO, O'Hara PJ, Heinemann SF (1994) *Neuron* 13, 1345–1357
42 Kuusinen A, Arvola M, Keinanen K (1995) *EMBO J* 14, 6327–6332
43 Perrin DD, Armarego WLF, Perrin DR (1966) *Purification of Laboratory Chemicals*, Pergamon Press, Oxford
44 Still WC, Kahn M, Mitra A (1978) *J Org Chem* 43, 2923–2925
45 Marmor RS (1972) *J Org Chem* 37, 2901–2904
46 Sato K, Sugai S, Tomica K (1986) *Agric Biol Chem* 50, 1831–1837
47 Ransom RW, Stec NL (1988) *J Neurochem* 51, 830–836