Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 119–131

BIOORGANIC & MEDICINAL CHEMISTRY

# Aryl and cycloalkyl analogues of AMPA: synthetic, pharmacological and stereochemical aspects

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Received 22 July 1997; accepted 9 September 1997

**Abstract**

We have previously shown that (*RS*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid (APPA, **2**) is a functional partial agonist at the (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) subtype of excitatory amino acid receptors, reflecting that (*S*)-APPA is a full agonist and (*R*)-APPA a competitive antagonist at AMPA receptors. We have now synthesized and pharmacologically characterized (*RS*)-2-amino-3-[3-hydroxy-5-(2-fluorophenyl)isoxazol-4-yl]propionic acid (2-F-APPA, **5a**), 3-F-APPA (**5b**), 4-F-APPA (**5c**), (*S*)-4-F-APPA (**6**), (*R*)-4-F-APPA (**7**), and the fully and partially, respectively, saturated APPA (**2**) analogues, (*RS*)-2-amino-3-(3-hydroxy-5-cyclohexylisoxazol-4-yl)propionic acid (**5d**) and compound **5e** containing a 1-cyclohexenyl ring. The absolute stereochemistry of **6** and **7** was established on the basis of comparative circular dichroism studies on **6**, **7**, and (*S*)- and (*R*)-APPA. 4-F-APPA (**5c**), (*S*)-4-F-APPA (**6**), **5d**, and **5e** were shown to selectively inhibit [$^3$H]AMPA binding and to activate AMPA receptors. Whereas (*S*)-4-F-APPA (**6**) showed full AMPA receptor agonism, (*R*)-4-F-APPA (**7**) was an AMPA receptor antagonist. Co-administration of (*S*)- and (*R*)-4-F-APPA to the rat cortical wedge preparation produced functional partial AMPA receptor agonism. Semi empirical calculations showed that the magnitude of the torsional angle of the bond connecting the two rings in the series of nonannulated bicyclic AMPA analogues appears to be of importance for the potency and efficacy of these compounds. 

*Key words*: Excitatory amino acid receptor, AMPA agonist, AMPA antagonist, functional partial agonism, conformational analysis.

## 1. Introduction

(*S*)-Glutamic acid [(*S*)-Glu, Fig. 1], 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 [1–4], a heterogeneous class of metabotropic receptors (mGluRs) has been shown to have important functions in neurotransmission processes in the CNS [5]. It is now generally agreed that all subtypes of these receptors are potential targets for therapeutic intervention in a number of CNS diseases [6,7].

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 [8–11]. There also is growing evidence of an implication of EAA receptors in schizophrenia [12,13]. As in Alzheimer's disease (AD), the role of these receptors in the etiology and the clinical

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0968-0896/98/$19.00 
*PII*: S0968-0896(97)10017-7

manifestations of schizophrenia is still very incompletely understood, but there is evidence to suggest that hypoactivity at EAA receptors also is a factor of importance in the latter CNS disorder [13–15]. Thus, in AD as well as schizophrenia, compounds capable of activating EAA receptors as agonists or, perhaps more likely, partial or functional partial agonists may have therapeutic interest [15].

During the past years, a number of agonist and antagonist ligands for pharmacological characterization of subtypes of EAA receptors have been developed [16,17]. Whereas tritiated NMDA, the classical NMDA receptor agonist, is not useful for radioligand receptor binding [18], the tritiated form of glycine, which is a co-agonist at the NMDA receptor [19], the competitive antagonist [$^3$H]-(*RS*)-[3-(2-carboxypiperazin-4-yl)propyl]-phosphonic acid ([$^3$H]CPP) [20], and the noncompetitive antagonist [$^3$H]-(*RS*)-5,10-epimino-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cycloheptene ([$^3$H]MK-801) [21], have been extensively used as tools for the pharmacological characterization of the NMDA receptors. [$^3$H]Kainic acid is the standard ligand for studies of kainic acid receptors [22], and [$^3$H]AMPA [23] and [$^3$H]-6-cyano-7-nitro-quinoxaline-2,3-dione ([$^3$H]CNQX) [24] are effective agonist and antagonist ligands, respectively, for AMPA receptor characterization.

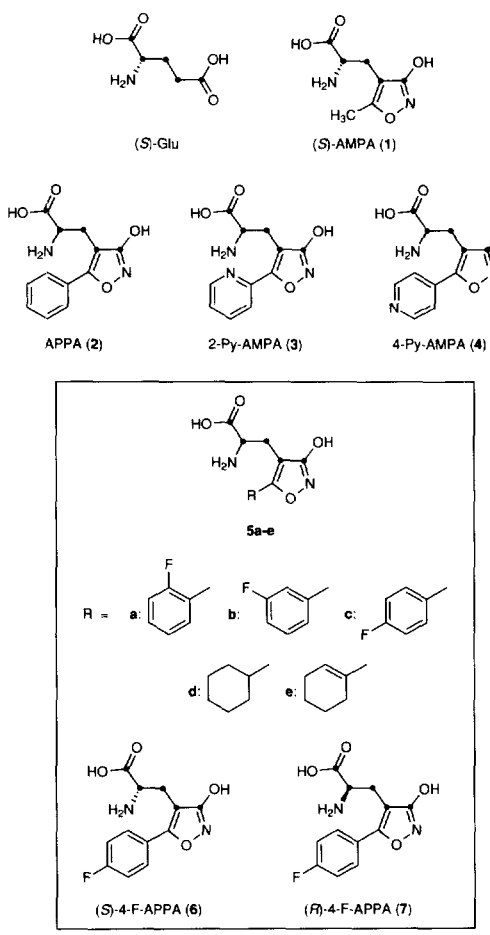



Fig. 1. Structures of (*S*)-glutamic acid [(*S*)-Glu], (*S*)-AMPA and a number of nonannulated bicyclic AMPA analogues.

A large number of agonists, including (*S*)-AMPA (**1**), and antagonists for AMPA receptors have been described [1,16,17], but so far only one compound, (*RS*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid (APPA, **2**) (Fig. 1), shows partial AMPA receptor agonism [25]. This effect is, however, only apparent and reflects that (*S*)-APPA is a full AMPA agonist and (*R*)-APPA a competitive antagonist of comparable potencies [26]. This interaction between full agonist and competitive antagonist underlies the pharmacological concept, functional partial agonism [27,28], which may have therapeutic interest [29].

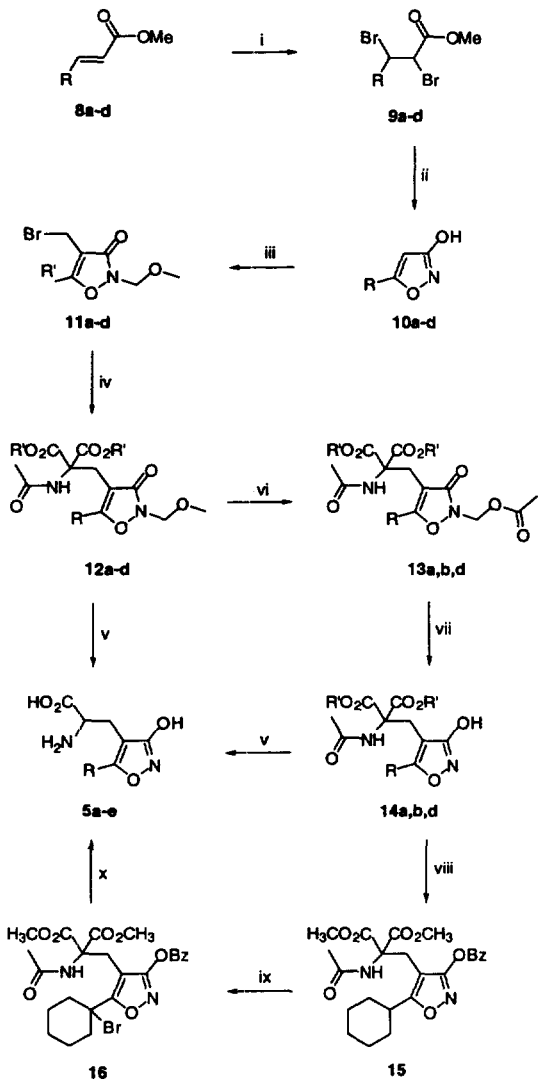
In agreement with the findings for (*S*)- and (*R*)-APPA, the (*S*)-form of (*RS*)-2-amino-3-[3-hydroxy-5-(2-pyridyl)isoxazol-4-yl]propionic acid (2-Py-AMPA, **3**), which is an AMPA agonist, and the competitive AMPA antagonist, (*R*)-2-Py-AMPA, also interact in a functional partial agonistic manner, whereas (3)- and 4-Py-AMPA (**4**), do not interact significantly with AMPA receptors [30]. In order to further explore this pharmacological concept, we now report the synthesis and pharmacological characterization of the (*S*)-form (**6**) and (*R*)-form (**7**) of (*RS*)-2-amino-3-[5-(4-fluorophenyl)-3-hydroxyisoxazol-4-yl]propionic acid (4-F-APPA, **5c**), the 2-fluorophenyl (**5a**) and 3-fluorophenyl (**5b**) isomers and the fully (**5d**) and partially (**5e**) saturated analogues of APPA (**2**) (Fig. 1).

## 2. Synthesis

### 2.1 Chemistry

The 3-substituted 2,3-dibromopropanoates (**9a–d**), were easily obtained by bromination of the corresponding propenoates (**8a–d**) (Scheme 1). The 5-aryl-3-isoxazolols (**10a–c**) were prepared in high yields by treatment of **9a–c** with hydroxylamine in alkaline media using an improved version of a previously reported method [31]. Under similar conditions **9d** gave a complex mixture from which **10d** was isolated in a very low yield. By substituting *N*-hydroxyurea for hydroxylamine, compound **10d** was obtained in a yield of 61%. We have previously shown that 3-aryl-2,3-dibromopropanoates easily undergo base-catalyzed dehydrobromination to give the corresponding 2-bromopropenoates [32]. It is assumed that the reaction of these intermediates with anionic *N*-hydroxyurea is initiated by a conjugated addition reaction followed by cyclization and dehydrobromination reactions and, finally,

elimination of the carboxamide group, from N-2 of the oxazoline ring formed, as an isocyanate ion [32,33]. Reaction of **10a–d** with 1,3,5-trioxane in aqueous hydrobromic acid (62%) and subsequent treatment of the intermediate 2,4-bis(bromomethyl)isoxazolin-3-ones with methanol under previously described conditions [34] gave **11a–d** (Scheme 1). A Sorensen reaction




a: R = 2-F-phenyl, R' = Me
b: R = 3-F-phenyl, R' = Me
c: R = 4-F-phenyl, R' = Et
d: R = cyclohexyl, R' = Me
e: R = 1-cyclohexenyl, R' = Me

Scheme 1. (i) $Br_2$; (ii) $NH_2OH$ or $H_2NCONHOH$, NaOH; (iii) $(CH_2O)_3$, 62% aq HBr, MeOH; (iv) $AcNHCH(COOR')_2$, NaH; (v) $CF_3CO_2H$ (1 M); (vi) $BF_3{\cdot}OEt_2$, $(Ac)_2O$; (vii) MeONa; (viii) BzCl; (ix) NBS; (x) HBr (1 M).

converted these compounds into **12a–d**. Compounds **12c** and **12d** were deprotected to give **5c** and **5d** using aqueous trifluoroacetic acid. Deprotection of **12a** and **12b** under the same reaction conditions resulted in low yields of **5a** and **5b**. Therefore, **12a** and **12b** were transformed into the 2-acetoxymethyl compounds **13a** and **13b** by treatment with acetic anhydride in the presence of boron trifluoride etherate [34]. Selective deprotection with sodium methoxide gave the 3-isoxazolols **14a** and **14b**, which were converted into the isoxazole amino acids **5a** and **5b** by treatment with aqueous trifluoroacetic acid. An attempt to introduce bromine at C-1 of the cyclohexyl group of **12d** was unsuccessful. Thus, **12d** was also selectively deprotected to give **14d** via **13d** using the reaction conditions described for the transformation of **12a,b** into **14a,b**. *O*-Benzoylation of **14d** followed by bromination of intermediate **15** using NBS gave **16**, which was dehydrohalogenated, deprotected, and decarboxylated by reflux in aqueous hydrobromic acid (1 M) to give **5e**. Compound **5e** was isolated as the zwitterion.

## 3. Resolution and stereochemistry

### *3.1 Resolution of 4-F-APPA (5c)*

The chemical resolution of zwitterionic 4-F-APPA (**5c**) to give (S)-(+)-4-F-APPA (**6**) and (*R*)-(−)-4-F-APPA (**7**) was achieved via diastereomeric salt formation using the (*S*)-(+)- and (*R*)-(−)-forms of 1-phenylethylamine (PEA), respectively. Compounds **6** and **7** were obtained with enantiomeric excess (*ee*) of 99.8% and 99.6%, respectively. The enantiomeric purity of the enantiomer **7** was determined by using a chiral crown ether column [Crownpak CR(−)]. The stereochemical purity of the enantiomer **6** was determined by ligand exchange HPLC on a chiral stationary phase consisting of (*S*)-pipecolic acid bound to silica and chelated with $Cu^{++}$ [35]. In agreement with earlier observations using these types of columns for the separation of α-amino acids of known absolute stereochemistry [26,36,37], (*S*)-(+)-4-F-APPA (**6**) eluted before the corresponding (*R*)-form (**7**) using the crown ether column, and after the (*R*)-form (**7**) using (*S*)-pipecolic acid derivatized column material.

The CD spectrum of the (*S*)-(+)-form of APPA (**2**) (Fig. 2), the configuration of which previously has been determined by an X-ray analysis [26], shows a positive Cotton effect at 210 nm ($\Delta\varepsilon = +0.3\,m^2/mol$) and a negative band at about 240 nm. As expected, the CD spectrum of the (*R*)-(−)-form of APPA (**2**) is a mirror image of the (*S*)-(+)-enantiomer in the $\Delta\varepsilon = 0\,m^2/mol$ axis. The positive Cotton effect of (*S*)-(+)-APPA at 210 nm is in agreement with the empirical correlation between the absolute configuration and the CD spectra

of (*S*)-amino acids in acidic solution, stating that (*S*)-amino acids show a positive Cotton effect near 220 nm, most likely due to a forbidden $n \rightarrow \pi^*$ transition of the $\alpha$-carboxylic acid.

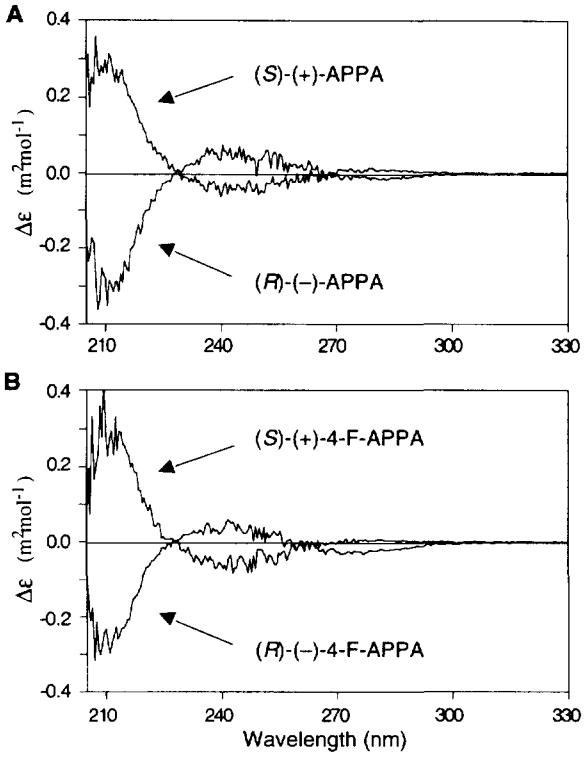



Fig. 2. Circular dichroism spectra of (*S*)-(+)-APPA and *R*-(−)-APPA (**A**) and (*S*)-(+)-4-F-APPA (**6**) and (*R*)-(−)-4-F-APPA (**7**) (**B**).

The CD spectra of (+)- and (−)-4-F-APPA (Fig. 2) clearly illustrates that the two compounds are enantiomers. Furthermore, the CD spectrum of (+)-4-F-APPA has almost the exact same location and magnitude of the positive (210 nm) as well as the negative (240 nm) Cotton effect as that of (*S*)-(+)-APPA, supporting the assignment of (+)-4-F-APPA (**6**) as having the (*S*)-configuration and (−)-4-F-APPA (**7**) as having the (*R*)-configuration.

## 4. In vitro pharmacology

Receptor binding studies were performed using [$^3$H]AMPA, [$^3$H]kainic acid, and [$^3$H]CNQX in the presence or absence of KSCN, and the NMDA receptor complex ligands [$^3$H]CPP, [$^3$H]MK-801 and [$^3$H]glycine. The tested compounds were only active in receptor binding assays evaluating AMPA receptor activities (Table 1). In [$^3$H]AMPA binding, compounds **5c**, **6**, **5d**, and **5e** were rather weak inhibitors, whereas **7** was inactive ($IC_{50} > 100\,\mu M$). In the rat cortical wedge, 4-F-APPA (**5c**) showed weak partial AMPA receptor agonism of approximately 65% relative efficacy and with an $EC_{50}$ value of 310 $\mu$M (Fig. 3 and Table 1). The response to **5c** (100 $\mu$M) could be reduced by the AMPA receptor antagonist, 6-nitro-7-sulfamoylbenzo-(*f*)quinoxaline-2,3-dione (NBQX) (10 $\mu$M) (data not shown). Compounds **5a** and **5b** were much weaker than **5c**. Full dose response curves could not be obtained due to the limited solubility of the compounds (data not shown). Both compounds were tested at concentrations up to 4 mM. Assuming that **5a** and **5b** both produce dose response curves with a maximum response of 100% (relative to AMPA), $EC_{50}$ values of 1300 $\mu$M and

Table 1
$pK_a$ Values and in vitro radioligand binding and electrophysiological data (mean ± SEM, $n$ = 3–5)

| | | $IC_{50}$, $\mu$M | | | $EC_{50}$, $\mu$M | $K_i$, $\mu$M |
|---|---|---|---|---|---|---|
| Compound | $pK_a$ Values | [$^3$H]AMPA | [$^3$H]CNQX | [$^3$H]CNQX + KSCN | Electrophysiology | |
| AMPA | 2.1; 5.2; 10.1 | 0.04 ± 0.01 | 18 ± 5 | 0.4 ± 0.1 | 3.5 ± 0.5 | |
| APPA | 2.0; 4.9; 10.4 | 35 ± 10 | 190 ± 20[a] | 86 ± 10 | 390 ± 60 | |
| (*R*)-APPA | | > 100 | 66 ± 8 | 320 ± 40[a] | | 290 ± 24[b] |
| (*S*)-APPA | | 6 ± 2 | 310 ± 20[a] | 84 ± 15 | 230 ± 12 | |
| 2-F-APPA (**5a**) | 2.1; 4.6; 9.9 | 93 ± 12 | > 100 | > 100 | > 1000 (1300) | |
| 3-F-APPA (**5b**) | 1.6; 4.6; 9.8 | > 100 | > 100 | > 100 | > 1000 (2000) | |
| 4-F-APPA (**5c**) | 1.5; 5.0; 10.1 | 34 ± 17 | > 100 | > 100 | 310 ± 120 | |
| (*S*)-4-F-APPA (**6**) | | 16 ± 6 | > 100 | > 100 | 150 ± 18 | |
| (*R*)-4-F-APPA (**7**) | | > 100 | > 100 | > 100 | | 340 ± 23[b] |
| **5d** | 2.2; 5.1; 10.0 | 26 ± 14 | 100 ± 19 | 108 ± 33 | 640 ± 40 | |
| **5e** | < 2; 4.8; > 10 | 6.4 ± 0.4 | 68 ± 11 | 76 ± 7 | 44 ± 4 | |

[a]$IC_{50}$ values above 100 $\mu$M in binding assays are calculated using the equation: % Inhibition = 100×[Inhibitor]/$IC_{50}$ + [Inhibitior], assuming competitive interaction and one binding site.
[b]Antagonist, $K_i$ value against AMPA. All compounds were inactive ($IC_{50} > 100\,\mu M$) in [$^3$H]MK-801 (baseline and fully stimulated), [$^3$H]CPP, [$^3$H]kainic acid, and [$^3$H]glycine binding assays.

2000 $\mu$M, respectively, could be estimated. Compound **5d** was only slightly more potent than compound **5a** with an $EC_{50}$ value of 640 $\mu$M. However, compound **5e** turned out to be a fairly potent and full agonist with an $EC_{50}$ value of 44 $\mu$M. The responses to **5a**, **5b**, **5d**, and **5e** could all be antagonized by NBQX (5 $\mu$M or 10 $\mu$M), whereas CPP (5 $\mu$M) did not affect the responses (data not shown). Thus, in accordance with the binding assays (Table 1), **5a**, **5b** and **5d** are weak, but selective, AMPA receptor agonists, whereas **5e** is a selective and fairly potent AMPA receptor agonist.

Compound **6** is a full AMPA receptor agonist (Fig. 3). Analysis of the dose response curves resulted in an $EC_{50}$ value of 150 $\mu$M, and a Hill slope close to 2. The response to **6** could be completely antagonized by co-application of 5 $\mu$M NBQX, whereas 5 $\mu$M CPP was unable to antagonize the response to **6**.

Since (*S*)-4-F-APPA (**6**) shows the characteristics of a full AMPA receptor agonist and since (*R*)-4-F-APPA (**7**) does not provoke any excitatory effect even when administered at a concentration of 1 mM, the partial agonism observed for the racemate, **5c** (Fig. 3), obviously only is apparent. The effects of **7** on responses by **6** were examined using a fixed concentration of **6** (200 $\mu$M) and varying concentrations of **7** (1 to 2000 $\mu$M). An analysis of these data using the method of Lazareno and Birdsall [38] disclosed that **7** acts as a competitive AMPA receptor antagonist with a $K_i$ value of 340 $\mu$M. Co-administration of **6** and **7** at fixed ratios produced excitatory effects of different relative efficacies as exemplified in Fig. 4. Thus, co-administration of this pair of enantiomers at a 1:1 ratio gave a dose–response curve indistinguishable from that of **5c**, whereas co-administration of **6** and **7** at a 1:5 ratio produced excitatory effects of approximately 10% relative efficacy (Fig. 4). These dose–response relationships indicate that **6** and **7** show interactive effects at the AMPA receptor previously named functional partial agonism [26–28].

## 5. Conformational analysis

The torsional drive calculation was performed on model compounds relevant to the bicyclic AMPA analogues **2–4** and **5a–e**. The energy barrier ($E(\omega)$) for a 360° rotation of the substituent in the 5-position of the isoxazole ring was performed on the 5-substituted 4-methyl-3-isoxazolol moiety in 20° steps, which generated 18 low-energy conformations for each structure (Table 2). The low-energy conformation for each of these structures was further energy minimized using the AM1 force field in the Spartan package. For all of the structures shown in Table 2, the lowest-energy conformation turned out to prefer a rather co-planar conformation between the two ring systems, except **5d**, which prefered a conformation, which has a dihedral angle of approximately 90°. Performing the torsional drive for compound **5d**, the saturated ring is in a chair conformation with the isoxazole ring in an equatorial position.

## 6. Discussion and conclusion

There is evidence to suggest that hypoactivity at central EAA receptors is a factor of importance in the clinical manifestations of AD [8–10] and schizophrenia [12–15]. Stimulation of EAA receptors, including AMPA receptors, by full agonists may, however, not be accomplished without concomitant excitotoxicity making therapeutic use of such compounds quite unlikely.

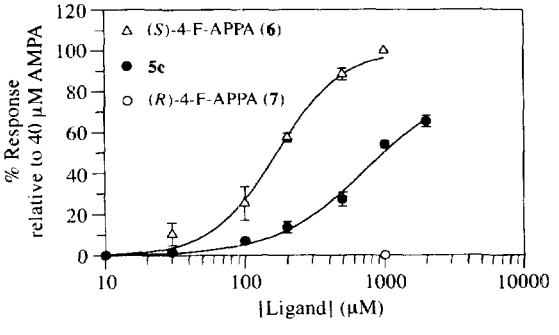



Fig. 3. Dose–response curves from the rat cortical wedge preparation. (*S*)-4-F-APPA (**6**) (△), 4-F-APPA (**5c**) (●) and (*R*)-4-F-APPA (**7**) (○). The percentage response values are mean values ± SEM relative to the maximum AMPA response and are plotted as a function of the concentration of the ligand (at least three experiments).

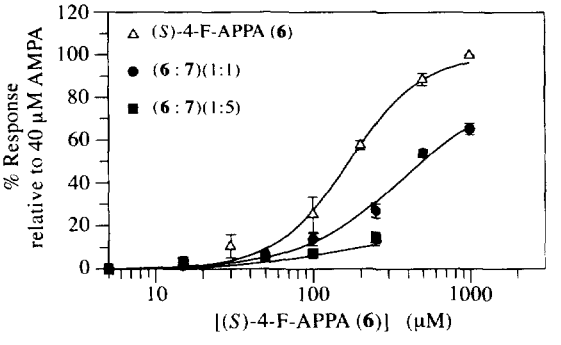
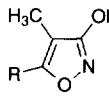
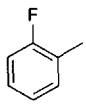
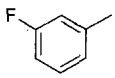
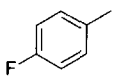



Fig. 4. Dose–response curves from the rat cortical wedge preparation. (*S*)-4-F-APPA (**6**) (△), and fixed molar ratios of (*S*)-4-F-APPA (**6**) and (*R*)-4-F-APPA (**7**) of (1:1) (●) and (1:5) (■). The percentage response values are mean values ± SEM relative to the maximum AMPA response and are plotted as a function of the concentration of (*S*)-4-F-APPA (**6**) (at least three experiments).

Table 2
Calculated approximate energy barriers for rotation about the bond connecting the two rings of model compounds relevant to compounds **2–4**, **5a–e**

| Compound |  | $E(\omega)$ kcal/mol |
|---|---|---|
| **2** |  | 3.46 |
| **3** |  | 3.80 |
| **4** |  | 3.74 |
| **5a** |  | 2.08 |
| **5b** |  | 3.65 |
| **5c** |  | 3.56 |
| **5d** |  | 0.76 |
| **5e** |  | 3.17 |

Partial agonists showing an appropriately adjusted agonist/antagonist profile may, on the other hand, be capable of restoring, in a nontoxic manner, activity in brain areas suffering from glutamatergic hypoactivity.

With the exception of APPA (**2**) [26,27], compounds showing partial AMPA receptor agonism have not been described. The partial agonism of APPA (**2**) is, however, only apparent, since (*S*)-APPA turned out to be a full AMPA receptor agonist and (*R*)-APPA a competitive antagonist [26]. In continuation of these studies, it was demonstrated that partial agonism at any desired level of relative efficacy could be achieved by co-administration of (*S*)- and (*R*)-APPA at appropriate fixed ratios, and this pharmacological principle of potential therapeutic interest was termed functional partial agonism [27–29].

Within the series of fluoro-substituted analogues of APPA, compounds **5a–c** (Fig. 1), only 4-F-APPA (**5c**) was active at AMPA receptors (Table 1), showing the characteristics of a partial agonist (Fig. 3). As in the case of APPA [26–28], this effect is only apparent, and pharmacological studies on enantiomerically pure (*S*)-4-F-APPA (**6**) and (*R*)-4-F-APPA (**7**) revealed that this pair of enantiomers produces functional partial agonism (Fig. 4).

Prompted by these observations, we also synthesized the cyclohexyl and 1-cyclohexenyl analogues of APPA, compounds **5d** and **5e**, respectively (Fig. 1 and Scheme 1). Whereas **5d** was some threefold weaker than (*S*)-APPA as an AMPA receptor agonist, **5e** was markedly more potent (Table 1), and **5e** was shown to be a full agonist at AMPA receptors. Thus, the potency of this class of AMPA agonists is strongly dependent on the structure of the 6-membered ring in the 5-position of the 3-isoxazolol unit.

We have previously postulated that the agonist conformation of nonannulated bicyclic analogues of AMPA containing a heterocyclic ring in the 5-position of the 3-isoxazolol unit may be rather planar and stabilized by a hydrogen bond between the ammonium group of the amino acid and an "ortho" positioned heteroatom of the heterocyclic substituent [39]. Thus, 2-Py-AMPA was markedly more potent than 4-Py-AMPA (Fig. 1) as an AMPA agonist [30]. In order to shed some light on the factors of importance in the structure–activity relationships of the present compounds, we have calculated approximate energy barriers for rotation about the bond connecting the two rings of model compounds relevant to compounds **2–4** and **5a–e** (Table 2). Whereas **2–4** and **5b,c,e** were estimated to show comparable approximate rotational energy barriers, this barrier appeared to be lower for **5a** and, in particular, **5d**, and in contrast to all of the other compounds, **5d** seemed, on the basis of the energy minimization, to prefer a nonplanar conformation. This latter observation may contribute to the very low agonist potency of **5d**, whereas unfavourable steric effects of the fluorine atoms of **5a,b** may contribute to the virtual inactivity of these compounds (Table 1). It is postulated that the coplanarity of the two rings and the possible interaction between the ammonium group and the double bond of the cyclohexenyl group may contribute to the high potency of **5e**.

## 7. Experimental

### *7.1 Chemistry*

#### *7.1.1 General procedures*

A number of synthetic steps required the use of anhydrous solvents. $CH_2Cl_2$ was dried over NaH. DMF was dried as described [40]. Organic phases were dried using $MgSO_4$. Solvents were removed under reduced pressure by rotary evaporation. Flash and Column chromatography (CC) were performed on silica gel 60, 0.063–0.200 mm (Merck) following described procedures [41].

Preparative thin-layer chromatography (TLC) was perfomed using silica gel 60 $PF_{254}$. All new compounds were colourless, unless otherwise stated. Melting points were determined in capillary tubes and are uncorrected. Elemental analyses were performed by Mr G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Denmark, Mr P. Hansen, Department of General and Organic Chemistry, University of Copenhagen, or the Analytical Research Department, H. Lundbeck A/S, Denmark, and are within ±0.4% of the calculated values unless otherwise stated. $^1H$ and $^{13}C$ NMR spectra were recorded at 200 and 50.32 MHz, respectively, on a Bruker AC-200 F instrument, unless otherwise stated. $^1H$ NMR Spectra at 60 MHz were recorded on a Varian EM-360-L spectrometer. Signal positions for the $^1H$ NMR spectra are given as $\delta$ values relative to TMS, when $CDCl_3$ was used as a solvent, and relative to 1,4-dioxane ($\delta$ 3.70) when $D_2O$ was used. $^{13}C$ NMR signals are given as $\delta$ values using the $CDCl_3$ peak ($\delta$ 76.93) as an internal standard. Coupling constants are given in Hz. Optical rotations were measured in thermostated cuvettes on a Perkin–Elmer 241 polarimeter. CD spectra of the enantiomers of APPA (**2**) and of (*S*)-4-F-APPA (**6**) and (*R*)-4-F-APPA (**7**) ($c = 0.136$ mM, 0.1 M HCl) were recorded in 1.0 cm cuvettes at room temperature on a Jasco J-720 spectropolarimeter.

*7.1.2 Determination of stereochemical purity*

Chiral HPLC was performed on a 150×4 mm Crownpak CR(−) column (Daicel) thermostated at 38°C with a Hetofrig thermostat and eluted with 0.4 ml/min of aqueous $HClO_4$, pH 1.5. The instrumentation used consisted of a Jasco 880-PU pump, a Rheodyne 7125 injector, and a Waters 480 UV detector set at 200 nm connected to a Hitachi Chromato-Integrator D-2000. Ligand exchange chiral HPLC was performed on a 120×4.6 mm column, containing a silica-based packing material with immobilized (*S*)-pipecolic acid and chelated $Cu^{++}$, prepared according to directions in the literature [35]. The column was thermostated at 50°C with an LKB 2155 HPLC column oven and eluted with 1.0 ml/min of 50 mM potassium dihydrogen phosphate containing 0.07 mM $CuSO_4$, pH 4.6, with Waters instrumentation consisting of an M510 pump connected to a U6K injector and a Waters 990 Photodiode Array Detector. The enantiomeric purity was determined from peak areas.

*7.1.3 Determination of stoichiometric $pK_a$ values*

$pK_a$ value determinations were performed on an interconnected automatic TitriLab® titrator system consisting of a burette station ABU 93 Triburette, a controller unit VIT90 Video Titrator and a sample station SAM90 from the Analytical Instruments Division of Radiometer A/S, DK-2400 Copenhagen NV, Denmark, using the following Radiometer electrodes: Glas electrode (pHG 201), reference electrode (reference 201, Ag/AgCl). Titration curves were fitted by a weighted least squares method.

*7.1.3.1 Methyl (E)-3-(4-fluorophenyl)-2-propenoate (**8c**).* Concentrated $H_2SO_4$ (14 ml) was added to a solution of (*E*)-3-(4-fluorophenyl)-2-propenoic acid (20.0 g, 120 mmol) in $CH_3OH$ (240 ml). The solution was heated under reflux for 2 h. $H_2O$ was azeotroped off with $CH_3OH$ (200 ml), $CH_3OH$ (200 ml) was added, and the mixture was heated under reflux overnight. The mixture was evaporated to dryness, dissolved in ether (200 ml), and washed with iced $H_2O$ (2×200 ml) and iced aqueous $Na_2CO_3$ (2×200 ml, 1 M). The organic phase was dried and evaporated to give **8c** (20.5 g, 95%): mp 39–40°C (lit. [42]: bp 114–116°C/0.6 Pa). $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 3.81 (s, 3H), 6.55 (d, $J = 15$, 1H), 7.04–7.08 (m, 2H), 7.48–7.55 (m, 2H), 7.82 (d, $J = 15$, 1H).

*7.1.3.2 Methyl (E)-3-(3-fluorophenyl)-2-propenoate (**8b**).* Synthesized, as described for **8c**, by use of (*E*)-3-(3-fluorophenyl)-2-propenoic acid (5.0 g, 30.1 mmol), $CH_3OH$ (60 ml) and concentrated $H_2SO_4$ (3.5 ml). Crude **8b** was obtained as a yellow oil (5.16 g, 95%) (lit. [42]: bp 68–70°C/6.0 Pa). $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 3.70 (s, 3H), 6.20 (d, $J = 15$, 1H), 6.70–7.20 (m, 4H), 7.40 (d, $J = 15$, 1H).

*7.1.3.3 Methyl (E)-3-(2-fluorophenyl)-2-propenoate (**8a**).* Compound **8a** was synthesized, as described for **8c**, from (*E*)-3-(2-fluorophenyl)-2-propenoic acid (5.0 g, 30 mmol), $CH_3OH$ (60 ml) and concentrated $H_2SO_4$ (3.5 ml). Crude **8a** was obtained as an oil (4.45 g, 82%) (lit. [42]: bp 122–123°C/1.2 Pa). $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 3.82 (s, 3H), 6.55 (d, $J = 16$, 1H), 7.0–7.23 (m, 2H), 7.2–7.42 (m, 1H), 7.47–7.60 (m, 1H), 7.82 (d, $J = 16$, 1H).

*7.1.3.4 Methyl 2,3-dibromo-3-(4-fluorophenyl)propanoate (**9c**).* A solution of $Br_2$ (1.46 ml, 28.4 mmol) in $CCl_4$ (5 ml) was added to an ice-cooled suspension of **8c** (5.10 g, 28.3 mmol) in $CCl_4$ (5 ml). The reaction mixture was stirred at rt overnight and then evaporated. The resulting crystalline product was triturated with light petroleum with stirring for 2 h. The crystals were collected and dried in vacuo to give **9c** (8.11 g, 84%). A sample was recrystallized (light petroleum): mp 63–65°C. $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 3.90 (s, 3H), 4.80 (d, $J = 10$, 1H), 5.40 (d, $J = 10$, 1H), 7.00–7.80 (m, 4H). Anal. ($C_{10}H_9Br_2FO_2$) C, H, Br.

*7.1.3.5 Methyl 2,3-dibromo-3-(3-fluorophenyl)propanoate (**9b**).* Synthesized, as described for **9c**, from **8b** (5.1 g, 28.3 mmol) and $Br_2$ (1.45 ml, 28.3 mmol). The crude **9b** was purified by flash chromotography and recrystallized (light petroleum) to give **9b** (6.51 g, 68%): mp 65–70°C. $^1H$ NMR (60 MHz, $CDCl_3$) $\delta$ 3.75 (s, 3H),

4.50 (d, $J = 10$, 1H), 5.15 (d, $J = 10$, 1H), 7.00–7.48 (m, 4H). Anal. ($C_{10}H_9Br_2FO_2$) C, H, Br.

*7.1.3.6 Methyl 2,3-dibromo-3-(2-fluorophenyl)propanoate (**9a**).* Compound **9a** was synthesized, as described for **9c**, from **8a** (4.90 g, 27.2 mmol) and $Br_2$ (1.41 ml, 27.5 mmol) to give crude **9a** (8.43 g, 91%). A sample was recrystallized (light petroleum): mp 55–60°C. $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 3.90 (s, 3H), 5.00 (d, $J = 12$, 1H), 5.60 (d, $J = 12$, 1H), 7.02–7.49 (m, 4H). Anal. ($C_{10}H_9Br_2FO_2$) C, H, Br.

*7.1.3.7 5-(4-Fluorophenyl)-3-hydroxyisoxazole (**10c**).* NaOH (9.88 g, 247 mmol) was added to an ice-cold solution of $NH_2OH$, HCl (6.13 g, 88.3 mmol) in $CH_3OH$ (175 ml), and the mixture was stirred for 10 min. To this solution was portionwise added **9c** (12.0 g, 35.3 mmol) during 1 h, and stirring was continued at 0°C for an additional 1 h. The mixture was refluxed for 6 h and evaporated. The residue was dissolved in $H_2O$ (70 ml), and the solution was acidified with HCl (4 M) at 0°C and stirred at 0°C for 30 min. The resulting crystals were collected, washed with $H_2O$ and dried in vacuo to give **10c** (4.6 g, 73%). A sample was recrystallized (EtOAc–heptane): mp 207–208°C. $^1$H NMR ($CDCl_3$) $\delta$ 6.19 (s, 1H), 7.10–7.25 (m, 2H), 7.68–7.82 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 90.0, 114.6, 115.0, 123.1, 126.2, 126.4, 159.7, 167.3, 170.0. Anal. ($C_9H_3FNO_2$) C, H, N.

*7.1.3.8 5-(3-Fluorophenyl)-3-hydroxyisoxazole (**10b**).* Synthesized, as described for **10c**, from **9b** (1.70 g, 5.00 mmol), $NH_2OH$, HCl (0.87 g, 12.5 mmol) and NaOH (1.40 g, 35.0 mmol). Crude **10b** was extracted with hot EtOAc (2×15 ml), and the combined organic extracts were dried and evaporated to give **10b** (760 mg, 85%) as a yellowish solid. Two recrystallizations (EtOAc–light petroleum) gave **10b** (440 mg, 49%): mp 185–189°C. $^1$H NMR (60 MHz, $CDCl_3$) $\delta$ 6.20 (s, 1H), 7.05–7.21 (m, 1H), 7.36–7.59 (m, 3H). Anal. ($C_9H_6FNO_2$) H, N; C: calcd, 60.34; found, 59.98.

*7.1.3.9 5-(2-Fluorophenyl)-3-hydroxyisoxazole (**10a**).* Compound **10a** was synthesized, as described for **10c**, from **9a** (1.70 g, 5.00 mmol), $NH_2OH$, HCl (0.87 g, 12.5 mmol) and NaOH (1.40 g, 35.0 mmol). Crude **10a** was subjected to CC [eluent: tol containing AcOH (1%)] to give 0.67 g (75%) of **10a**. A sample was recrystallized (EtOAc): mp 170–174°C. $^1$H NMR ($CDCl_3$) $\delta$ 6.32 (d, $J = 4.5$, 1H), 7.08–7.51 (m, 3H), 7.82–8.00 (m, 1H). Anal. ($C_9H_6FNO_2$) C, H, N.

*7.1.3.10 5-Cyclohexyl-3-hydroxyisoxazole (**10d**).* *N*-Hydroxyurea (570 mg, 7.5 mmol) was added to a solution of NaOH (700 mg, 17.5 mmol) in $CH_3OH$ (15 ml). A solution of **9d** [43] (1.64 g, 5.0 mmol) in $CH_3OH$ (5 ml) was added dropwise, and the mixture was stirred at rt for 6 h and then refluxed for 20 h. After evaporation, $H_2O$ (15 ml) was added to the residue. The mixture was cooled in an ice-bath and acidified with concentrated HCl to precipitate crude **10d**. The product was extracted with boiling heptane, and the extracts were concentrated to give crystalline **10d** (506 mg, 61%): mp 118–119°C (lit. [44]: mp 123°C; lit. [45]: mp 125°C). $^1$H NMR ($CDCl_3$) $\delta$ 1.00–2.27 (m, 10H), 2.32–2.95 (m, 1H), 5.47 (s, 1H). Anal. ($C_9H_{13}NO_2$) C, H, N.

*7.1.3.11 4-(Bromomethyl)-5-(4-fluorophenyl)-2-(methoxymethyl)isoxazolin-3-one (**11c**).* A mixture of **10c** (200 mg, 1.10 mmol), 1,3,5-trioxane (149 mg, 1.66 mmol) and aqueous HBr (4 ml, 62%) in a sealed ampoule was placed in an oil bath at 60°C for 20 h. The reaction mixture was extracted with $CH_2Cl_2$ (3×25 ml) followed by addition of $CH_3OH$ (50 ml) and then vigorous stirring for 2 h at rt. $CH_2Cl_2$ (50 ml) was added and the organic phase was washed with $H_2O$ (3×75 ml), dried and evaporated to give **11c** (344 mg, 99%): mp 197–199°C. $^1$H NMR ($CDCl_3$) $\delta$ 3.47 (s, 3H), 4.40 (s, 2H), 5.30 (s, 2H), 7.20–7.32 (m, 2H), 7.80–7.92 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 20.3, 57.3, 75.6, 106.4, 116.4, 116.9, 122.4, 129.6, 129.8, 162.0, 166.0, 167.1. Anal. ($C_{12}H_{11}BrFNO_3$) C, H, N.

*7.1.3.12 4-(Bromomethyl)-5-(3-fluorophenyl)-2-(methoxymethyl)isoxazolin-3-one (**11b**).* Synthesized, as described for **11c**, from **10b** (500 mg, 2.79 mmol), 1,3,5-trioxane (373 mg, 4.14 mmol), and aqueous HBr (4 ml, 62%). Compound **11b** was obtained as a semicrystalline solid (845 mg, 96%). A sample was recrystallized (EtOAc): mp 190–192°C. $^1$H NMR ($CDCl_3$) $\delta$ 3.48 (s, 3H), 4.39 (s, 2H), 5.29 (s, 2H), 7.22–7.36 (m, 1H), 7.48–7.69 (m, 3H). Anal. ($C_{12}H_{11}BrFNO_3$) C, H, N.

*7.1.3.13 4-(Bromomethyl)-5-(2-fluorophenyl)-2-(methoxymethyl)isoxazolin-3-one (**11a**).* Synthesized, as described for **11c**, from **10a** (1.65 g, 9.20 mmol), 1,3,5-trioxane (1.20 g, 13.3 mmol) and aqueous HBr (13 ml, 62%). Compound **11a** was obtained as an oil (2.48 g, 85%) $^1$H NMR ($CDCl_3$) $\delta$ 3.49 (s, 3H), 4.31 (s, 2H), 5.30 (s, 2H), 7.20–7.42 (m, 2H), 7.53–7.69 (m, 2H). Anal. ($C_{12}H_{11}BrFNO_3$) C, H, N.

*7.1.3.14 4-(Bromomethyl)-5-cyclohexyl-2-(methoxymethyl)isoxazolin-3-one (**11d**).* Synthesized, as described for **11c**, from **10d** (1.45 g, 8.67 mmol), 1,3,5-trioxane (1.17 g, 1.30 mmol) and aqueous HBr (12 ml, 62%). Compound **11d** was obtained as a semicrystalline solid (2.14 g, 81%). A sample was recrystallized (EtOAc): mp 199–201°C. $^1$H NMR ($CDCl_3$) $\delta$ 1.20–2.04 (m, 10H), 2.81 (tt, $J = 3.3$, $J = 11.7$, 1H), 3.38 (s, 3H), 4.22 (s, 2H), 5.16 (s, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 18.8, 25.2, 25.4 (2C), 29.1 (2C), 36.9, 57.0, 75.3, 105.4, 166.1, 175.1. Anal. ($C_{12}H_{18}BrNO_3$) C, H, N.

*7.1.4 Ethyl 2-acetamido-2-(ethoxycarbonyl)-3-[5-(4-fluorophenyl)-2-(methoxymethyl)-3-oxoisoxazolin-4-yl]propanoate (**12c**)*

A suspension of NaH in mineral oil (241 mg, 60%, 6.02 mmol) was added during 30 min at 0°C to a solution of diethyl acetamidomalonate (1.13 g, 6.02 mmol) in dry DMF (25 ml). After stirring for further 30 min, a solution of **11c** (1.73 g, 5.47 mmol) in dry DMF (10 ml) was added during 15 min. After stirring at rt for 20 h, the mixture was evaporated, dissolved in $CH_2Cl_2$ (75 ml) and washed with ice-cold NaOH (80 ml, 1 M) and with ice-cold $H_2O$ (2×100 ml). The organic phase was dried and evaporated. CC of the residue [eluent: tol:EtOAc (1:1)] gave **12c** (1.71 g, 70%) as an oil. $^1H$ NMR ($CDCl_3$) $\delta$ 1.24 (t, $J = 7$, 6H), 1.54 (s, 3H), 3.42 (s, 3H), 3.66 (s, 2H), 4.05–4.35 (m, 4H), 5.23 (s, 2H), 6.82 (br s, 1H), 7.10–7.25 (m, 2H), 7.65–7.75 (m, 2H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 13.6 (2C), 20.6 (2C), 21.9, 57.1, 62.7, 75.1, 101.2, 114.2, 114.8, 124.4, 128.4, 130.0, 130.5, 159.8, 164.6, 167.2, 169.3, 170.8, 174.0. Anal. ($C_{21}H_{25}FN_2O_8$) C, H, N.

*7.1.5 Methyl 2-acetamido-3-[5-(3-fluorophenyl)-2-(methoxymethyl)-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propanoate (**12b**)*

Synthesized, as described for **12c**, from **11b** (5.0 g, 15.8 mmol), a suspension of NaH in mineral oil (530 mg, 80%, 17.7 mmol) and dimethyl acetamidomalonate (3.30 g, 17.4 mmol). After stirring at rt for 20 h the reaction mixture was neutralized with AcOH and evaporated. The residue was dissolved in EtOAc (50 ml) and washed with ice-cold $H_2O$ (2×100 ml). The organic solution was dried and evaporated. CC of the residue [eluent: tol:EtOAc (2:1), AcOH (1%)] produced **12b** as an oil (3.20 g, 47.5%). $^1H$ NMR ($CDCl_3$) $\delta$ 1.52 (s, 3H), 3.42 (s, 3H), 3.65 (s, 2H), 3.73 (s, 6H) 5.23 (s, 2H), 6.80 (br s, 1H), 7.18–7.30 (m, 1H), 7.34–7.56 (m, 3H). Anal. ($C_{19}H_{21}FN_2O_8$) C, H, N.

*7.1.5.1 Methyl 2-acetamido-3-[5-(2-fluorophenyl)-2-(methoxymethyl)-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propanoate (**12a**).* Synthesized, as described for **12c**, from **11a** (2.40 g, 7.59 mmol), a suspension of NaH in mineral oil (332 mg, 60%, 8.30 mmol) and dimethyl acetamidomalonate (1.60 g, 8.30 mmol). CC of the residue [eluent: tol:EtOAc (1:1)] gave **12a** (2.39 g, 74%) as a semicrystalline solid. A sample was recrystallized (EtOAc): mp 203.5–204.5°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.55 (s, 3H), 3.46 (s, 3H), 3.55 (s, 2H), 3.70 (s, 6H), 5.23 (s, 2H), 6.80 (br s, 1H), 7.12–7.36 (m, 3H), 7.45–7.61 (m, 1H). Anal. ($C_{19}H_{21}FN_2O_8$) C, H, N.

*7.1.5.2 Methyl 2-acetamido-3-[5-cyclohexyl-2-(methoxymethyl)-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propanoate (**12d**).* Synthesized, as described for **12c**, from **11d** (7.02 g, 23.1 mmol), a suspension of NaH in mineral oil (1.0 g, 60%, 25 mmol), and dimethyl acetamidomalonate (4.8 g, 25 mmol). CC of the residue [eluent: tol:EtOAc (2:1)] gave **12d** (9.49 g, 99%) as a semicrystalline solid. A sample was recrystallized (tol–light petroleum): mp 115.5–116.5°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.1–1.9 (m, 10H), 2.02 (s, 3H), 2.6–2.7 (m, 1H), 3.30 (s, 2H), 3.36 (s, 3H), 3.82 (s, 6H), 5.10 (s, 2H), 7.24 (s, 1H). Anal. ($C_{19}H_{28}N_2O_8$) C, H, N.

*7.1.5.3 Methyl 2-acetamido-3-[2-(acetoxymethyl)-5-(3-fluorophenyl)-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propanoate (**13b**).* $BF_3{\cdot}Et_2O$ (800 $\mu$L, 6.36 mmol) was added to a mixture of **12b** (2.0 g, 4.71 mmol), $CHCl_3$ (30 ml) and $(Ac)_2O$ (30 ml). After stirring overnight at rt, water (75 ml) was carefully added at 0°C, and stirring was continued for 1 h at rt. The phases were separated and the aqueous phase was extracted with $CHCl_3$ (3×70 ml). The combined organic phases were dried and evaporated. The crude reaction product was subjected to CC [eluent: tol:EtOAc (1:1)] to give TLC pure **13b** (1.54 g, 72%). $^1H$ NMR ($CDCl_3$) $\delta$ 1.52 (s, 3H), 2.10 (s, 3H), 3.65 (s, 2H), 3.72 (s, 6H), 5.86 (s, 2H), 6.72 (br s, 1H), 7.15–7.33 (m, 3H), 7.44–7.62 (m, 1H).

*7.1.5.4 Methyl 2-acetamido-3-[2-(acetoxymethyl)-5-(2-fluorophenyl)-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propanoate (**13a**).* Synthesized, as described for **13b**, from **12a** (1.29 g, 3.04 mmol), $BF_3{\cdot}Et_2O$ (520 $\mu$L, 4.14 mmol), $CHCl_3$ (20 ml), and $(Ac)_2O$ (20 ml). The crude reaction product was subjected to CC [eluent: tol:EtOAc (1:2)] to give TLC pure **13a** (784 mg, 57%). $^1H$ NMR ($CDCl_3$) $\delta$ 1.52 (s, 3H), 2.12 (s, 3H), 3.51 (s, 2H), 3.70 (s, 6H), 5.86 (s, 2H), 6.72 (br s, 1H), 7.13–7.37 (m, 3H), 7.44–7.62 (m, 1H).

*7.1.5.5 Methyl 2-acetamido-3-[2-(acetoxymethyl)-5-cyclohexyl-3-oxoisoxazolin-4-yl]-2-(methoxycarbonyl)propanoate (**13d**).* Synthesized, as described for **13b**, from **12d** (4.0 g, 9.70 mmol), $BF_3{\cdot}Et_2O$ (1.34 ml, 10.7 mmol), $CH_2Cl_2$ (50 ml), and $(Ac)_2O$ (2.0 ml). CC of the residue [eluent: tol:EtOAc (2:1)] gave **13d** (3.0 g, 70%) as a semicrystalline solid. A sample was recrystallized (EtOAc): mp 153.5–154.0°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.1–1.9 (m, 10H), 2.02 (s, 3H), 2.08 (s, 3H), 2.5–2.8 (m, 1H), 3.31 (s, 2H), 3.82 (s, 6H), 5.73 (s, 2H), 7.08 (s, 1H). Anal. ($C_{20}H_{28}N_2O_9$) C, H, N.

*7.1.5.6 Methyl 2-acetamido-3-[5-(3-fluorophenyl)-3-hydroxyisoxazol-4-yl]-2-(methoxycarbonyl)propanoate (**14b**).* A mixture of Na (114 mg, 4.56 mg-atom) in $CH_3OH$ (80 ml) and **13b** (1.54 g, 3.31 mmol) was refluxed for 3 h. The reaction mixture was cooled, acidified with AcOH, evaporated and re-evaporated with tol. $H_2O$ (75 ml) was added, and the water phase was extracted with $CH_2Cl_2$ (3×75 ml). The combined

organic phases were dried and evaporated. The crude product was subjected to CC [eluent: tol:EtOAc (1:1), AcOH (1%)] to give **14b** (1.03 g, 82%). A sample was recrystallized [$CH_3OH$:ether:light petroleum (3:1:1)]: mp 206–208°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.62 (s, 3H), 3.62 (s, 6H), 3.72 (s, 2H), 6.58 (br s, 1H), 7.13–7.35 (m, 3H), 7.44–7.61 (m, 1H). Anal. ($C_{17}H_{17}FN_2O_7$) C, H, N.

*7.1.5.7 Methyl 2-acetamido-3-[5-(2-fluorophenyl)-3-hydroxyisoxazol-4-yl]-2-(methoxycarbonyl)propanoate (**14a**).* Synthesized, as described for **14b**, from Na (97 mg, 4.22 mg-atom), $CH_3OH$ (70 ml) and **13a** (1.27 g, 2.81 mmol) to give **14a** (640 mg, 60%). A sample was recrystallized (2-propanol): mp 203.5–204.5°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.58 (s, 3H), 3.64 (s, 6H), 3.72 (s, 2H), 6.60 (s, 1H), 7.13–7.35 (m, 3H), 7.44–7.61 (m, 1H). Anal. ($C_{17}H_{17}FN_2O_7$) C, H, N.

*7.1.5.8 Methyl 2-acetamido-3-(5-cyclohexyl-3-hydroxyisoxazol-4-yl)-2-(methoxycarbonyl)propanoate (**14d**).* Synthesized, as described for **14b**, from Na (230 mg, 10 mg-atom), $CH_3OH$ (100 ml) and **13d** (3.3 g, 7.5 mmol). The crude product, which contained about 20% of a compound proposed to be the 2-hydroxymethyl derivative of **14d** [$^1H$ NMR ($CDCl_3$) $\delta$ 5.26 (s, $CH_2OH$)], was refluxed in $CH_3OH$ (100 ml) for 3 h, evaporated and dried to give $^1H$ NMR pure **14d** (2.21 g, 79%). A sample was recrystallized (EtOAc): mp 205.0–205.5°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.1–1.9 (m, 10H), 2.03 (s, 3H), 2.4–2.7 (m, 1H), 3.39 (s, 2H), 3.82 (s, 6H), 6.77 (s, 1H). Anal. ($C_{17}H_{24}N_2O_7$) C, H, N.

*7.1.5.9 Methyl 2-acetamido-3-[3-(benzoyloxy)-5-cyclohexylisoxazol-4-yl]-2-(methoxycarbonyl)propanoate (**15**).* To a solution of **14d** (2.08 g, 5.65 mmol) in $CH_2Cl_2$ (50 ml) was added $Et_3N$ (787 $\mu$l, 5.65 mmol) and benzoyl chloride (656 $\mu$l, 5.65 mmol) and the resulting solution was stirred at rt for 1 h. Evaporation, followed by CC [tol:EtOAc (3:2)] gave **15** (2.34 g, 88%). A sample was recrystallized (tol–light petroleum): mp 120.5–121.0°C. $^1H$ NMR ($CDCl_3$) $\delta$ 1.22–1.38 (m, 6H), 1.4–2.0 (m, 5H), 1.84 (s, 3H), 3.50 (s, 2H), 3.68 (s, 6H), 6.68 (s, 1H), 7.52 (m, 2H), 7.67 (m, 1H), 8.16 (m, 2H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 22.6, 25.1 (2C), 25.2 (2C), 25.7, 30.6 (2C), 35.8, 53.4, 65.6, 100.7, 127.6, 128.5 (2C), 130.3 (2C), 134.2, 162.6, 165.8, 167.6 (2C), 169.5, 177.4 Anal. ($C_{24}H_{28}N_2O_8$) C, H, N.

*7.1.5.10 Methyl 2-acetamido-3-[3-(benzoyloxy)-5-(1-bromocyclohexyl)isoxazol-4-yl]-2-(methoxycarbonyl)-propanoate (**16**).* A solution of **15** (3.15 g, 6.67 mmol) in $CCl_4$ (125 ml) was treated under reflux with NBS (a total of 1.88 g, 10.53 mmol) and benzoylperoxide (a total of 173 mg, 0.71 mmol) for 48 h. Filtration and evaporation to dryness followed by CC [$CH_2Cl_2$:ether (19:1)] gave **16** as crystals (1.29 g, 35.1%). $^1H$ NMR ($CDCl_3$) $\delta$ 1.51–1.85 (m, 6H), 1.88 (s, 3H), 2.29–2.53 (m, 4H), 3.67 (s, 8H), 6.86 (s, 1H), 7.48–7.71 (m, 3H), 8.06–8.21 (m, 2H); $^{13}C$ NMR ($CDCl_3$) $\delta$ 23.0, 23.1 (2C), 24.5 (2C), 26.3, 29.4, 38.4, 53.7, 60.9, 65.3, 103.1, 127.4, 128.7 (2C), 130.5 (2C), 134.5, 162.6, 166.2, 167.5 (2C), 169.9, 172.0.

*7.1.5.11 (RS)-2-Amino-3-(5-cyclohexenyl-3-hydroxyisoxazol-4-yl)propanoic acid (**5e**).* A mixture of **16** (1.16 g, 2.10 mmol) and aqueous HBr (100 ml, 0.20 mol, 2 M) was refluxed for 7 h. The reaction mixture was filtered and evaporated twice from $H_2O$ (2×10 ml). The residue was dissolved in $H_2O$ (40 ml). The solution was washed with EtOAc (3×40 ml) followed by evaporation to dryness. The resulting crude product was dissolved in $H_2O$ (2 ml), and pH of the solution was adjusted to 3 using NaOH (1 M). The resulting crystals were collected and dried and recrystallised twice ($H_2O$) to give **5e** (205 mg, 39%): mp 223–224°C (decomp.). $^1H$ NMR (DMSO-$d_6$) $\delta$ 1.69–1.90 (m, 4H), 2.28–2.51 (m, 4H), 2.68 (m, 2H), 3.70–3.75 (m, 1H), 6.31–6.37 (m, 1H); $^{13}C$ NMR (DMSO) $\delta$ 23.0, 23.5, 25.0, 26.7, 26.8, 55.8, 101.9, 127.8, 135.9, 167.5, 174.8, 182.3. Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

*7.1.5.12 (RS)-2-Amino-3-[5-(4-fluorophenyl)-3-hydroxyisoxazol-4-yl]propanoic Acid (4-F-APPA) (**5c**).* A mixture of **12c** (812 mg, 1.91 mmol) and aqueous $CF_3COOH$ (10 ml, 10 mmol, 1 M) was refluxed for 12 h. The reaction mixture was evaporated and re-evaporated twice from $H_2O$ and subsequently twice from tol. The dried residue was subjected to preparative TLC [eluent: $CH_3CN$:$H_2O$:AcOH (8:1:1); *Rf* 0.31]. The crude product was recrystallized twice from $H_2O$ to give **5c** (331 mg, 65%): mp 245–247°C (decomp). p$K_a$-values [10.1 ± 0.3 (3$\sigma$), 5.0 ± 0.3 (3$\sigma$), 1.5 ± 0.3 (3$\sigma$)]. $^1H$ NMR ($D_2O$) $\delta$ 3.20 (d, $J$ = *6.55*, 2H), 4.27 (t, $J$ = *6.55*, 1H), 7.15–7.28 (m, 2H), 7.60–7.68 (m, 2H); $^{13}C$ NMR ($D_2O$) $\delta$ 23.4, 52.7, 102.4, 113.9, 116.9, 117.3, 119.6, 130.3, 130.4, 169.2, 172.8, 173.1. Anal. ($C_{12}H_{11}FN_2O_4 \cdot 1/4H_2O$) C, H, N.

*7.1.5.13 (RS)-2-Amino-3-[5-(3-fluorophenyl)-3-hydroxyisoxazol-4-yl]propanoic acid (3-F-APPA) (**5b**).* Compound **14b** (840 mg, 2.21 mmol) was refluxed in aqueous $CF_3COOH$ (50 ml, 100 mmol, 2 M) for 20 h. The reaction mixture was evaporated and re-evaporated from $H_2O$ and twice from tol. The residue was dissolved in $H_2O$ (3 ml) and an aqueous solution of $Et_3N$ in $H_2O$ (0.45 M) was added to pH 3. The resulting crude product was dissolved in $H_2O$ (100 ml), and the solution washed with EtOAc (50 ml), concentrated to 80 ml and left at 5°C. The resulting crystals were collected and recrystallized ($H_2O$) to give **5b**·$H_2O$ (204 mg, 32%): mp 226–227°C (decomp.). p$K_a$-values [9.8 ± 0.2 (3$\sigma$), 4.6 ± 0.2 (3$\sigma$), 1.6 ± 0.2 (3$\sigma$)]. $^1H$ NMR ($D_2O$) $\delta$ 2.57 (dd, $J$ = 9.3, $J$ = 14.7, 1H), 2.82 (dd, $J$ = 4.9, $J$ = 14.7, 1H),

3.39 (dd, $J = 4.9$, $J = 9.3$, 1H), 7.09–7.26 (m, 1H), 7.32–7.55 (m, 3H). Anal. ($C_{12}H_{11}FN_2O_4 \cdot H_2O$) C, H, N.

*7.1.5.14 (RS)-2-Amino-3-[5-(2-fluorophenyl)-3-hydroxyisoxazol-4-yl]propanoic acid (2-F-APPA) (**5a**).* Synthesized, as described for **5b**, from **14a** (580 mg, 1.52 mmol) and aqueous $CF_3COOH$ (50 ml, 100 mmol, 2 M). The residue was dissolved in $H_2O$ (8 ml) and an aqueous solution of $Et_3N$ in $H_2O$ (1.4 M) was added to pH 3. The resulting crystals were washed with EtOAc and recrystallized twice from $H_2O$ to give **5a** (77 mg, 19%): mp 212–214°C (decomp.). p$K_a$-values [$9.9 \pm 0.1$ ($3\sigma$), $4.6 \pm 0.1$ ($3\sigma$), $2.1 \pm 0.1$ ($3\sigma$)]. $^1$H NMR ($D_2O$) $\delta$ 2.41 (dd, $J = 9.4$, $J = 14.9$, 1H), 2.74 (dd, $J = 4.6$, $J = 14.9$, 1H), 3.31 (dd, $J = 4.6$, $J = 9.4$, 1H), 7.15–7.36 (m, 2H), 7.40–7.60 (m, 2H). Anal. ($C_{12}H_{11}FN_2O_4$) C, H, N.

*7.1.5.15 (RS)-2-Amino-3-(5-cyclohexyl-3-hydroxyisoxazol-4-yl)propanoic acid (**5d**).* Synthesized, as described for **5c**, from **12d** (514 mg, 1.17 mmol) and aqueous $CF_3COOH$ (10 ml, 10 mmol, 1 M). The crude product was purified by preparative TLC [eluent: $CH_3CN$:$H_2O$:AcOH (8:1:1), *Rf* 0.35]. Recrystallization ($H_2O$) gave **5d** (176 mg, 59%): mp 220–222°C (decomp). p$K_a$-values [$10.0 \pm 0.2$ ($3\sigma$), $5.1 \pm 0.2$ ($3\sigma$), $2.2 \pm 0.2$ ($3\sigma$)]. $^1$H NMR ($D_2O$) $\delta$ 1.20–1.90 (m, 10H), 2.67 (tt, $J = 3.3$, $J = 11.3$, 1H), 3.10 (d, $J = 6.9$, 2H), 4.28 (t, $J = 6.9$, 1H). Anal ($C_{12}H_{18}N_2O_4$) C, H, N.

*7.1.5.16 (R)-(−)-2-Amino-3-[5-(4-fluorophenyl)-3-hydroxyisoxazol-4-yl]propanoic acid [(R)-(−)-4-F-APPA] (7).* To a suspension of 4-F-APPA (**5c**) (620 mg, 2.33 mmol) in $C_2H_5OH$ (35 ml) was added (*S*)-(−)-PEA (590 $\mu$L, 4.66 mmol). The mixture was heated to reflux, and the resulting solution was filtered and cooled. Ether (30 ml) was added, and after standing for 24 h at 5°C the jelly-like precipitate was collected, washed with ether, and dried in vacuo. The salt of (*R*)-(−)-4-F-APPA and (*S*)-(−)-PEA was recrystallized three times from $C_2H_5OH$:ether containing ca 100% excess of (*S*)-(−)-PEA. The salt [141 mg, de 99.9%, Crownpak CR(−)] was dissolved in $H_2O$ (10 ml) and the solution was acidified with AcOH to pH 3. Recrystallization from $H_2O$ gave 67 mg (22%) of **7**: mp 247°C (decomp.); $[\alpha]_D^{25}$ −38.5° (*c* 0.54, HCl 1 M); *ee* 99.6%. $^1$H NMR ($D_2O$/NaOD) $\delta$ 2.56 (dd, $J = 9.5$, $J = 14.9$, 1H), 2.82

Elemental analysis

| | | Calcd(%) | | | | Found(%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C | H | N | Br | C | H | N | Br |
| **9c** | $C_{10}H_9Br_2FO_2$ | 35.32 | 2.67 | | 47.00 | 35.55 | 2.76 | | 46.87 |
| **9b** | $C_{10}H_9Br_2FO_2$ | 35.32 | 2.67 | | 47.00 | 35.49 | 2.74 | | 47.34 |
| **9a** | $C_{10}H_9Br_2FO_2$ | 35.32 | 2.67 | | 47.00 | 35.52 | 2.62 | | 46.72 |
| **10c** | $C_9H_6FNO_2$ | 60.34 | 3.38 | 7.82 | | 59.97 | 3.35 | 7.88 | |
| **10b** | $C_9H_6FNO_2$ | 60.34 | 3.38 | 7.82 | | 59.77 | 3.45 | 7.81 | |
| **10a** | $C_9H_6FNO_2$ | 60.34 | 3.38 | 7.82 | | 60.23 | 3.37 | 7.83 | |
| **10d** | $C_9H_{13}NO_2$ | 65.65 | 7.84 | 8.38 | | 64.47 | 7.81 | 8.44 | |
| **11c** | $C_{12}H_{11}BrFNO_3$ | 45.59 | 3.51 | 4.43 | | 45.19 | 3.17 | 4.15 | |
| **11b** | $C_{12}H_{11}BrFNO_3$ | 45.59 | 3.51 | 4.43 | | 45.84 | 3.54 | 4.44 | |
| **11a** | $C_{12}H_{11}BrFNO_3$ | 45.59 | 3.51 | 4.43 | | 45.30 | 3.40 | 4.47 | |
| **11d** | $C_{12}H_{18}BrNO_3$ | 47.38 | 5.96 | 4.60 | | 47.51 | 5.73 | 4.78 | |
| **12c** | $C_{21}H_{25}FN_2O_8$ | 55.75 | 5.57 | 6.19 | | 56.03 | 5.51 | 6.23 | |
| **12b** | $C_{19}H_{21}FN_2O_8$ | 53.77 | 4.99 | 6.60 | | 53.94 | 4.78 | 6.63 | |
| **12a** | $C_{19}H_{21}FN_2O_8$ | 53.77 | 4.99 | 6.60 | | 54.04 | 4.94 | 6.71 | |
| **12d** | $C_{19}H_{28}N_2O_8$ | 55.33 | 6.84 | 6.79 | | 55.55 | 6.94 | 6.69 | |
| **13b** | $C_{20}H_{21}FN_2O_9$ | 53.16 | 4.68 | 6.19 | | 53.35 | 4.52 | 6.19 | |
| **13a** | $C_{20}H_{21}FN_2O_9$ | 53.16 | 4.68 | 6.19 | | 53.46 | 4.62 | 6.27 | |
| **13d** | $C_{20}H_{28}N_2O_9$ | 54.54 | 6.41 | 6.36 | | 54.40 | 6.54 | 6.45 | |
| **14b** | $C_{17}H_{17}FN_2O_7$ | 53.69 | 4.51 | 7.37 | | 53.73 | 4.50 | 7.17 | |
| **14a** | $C_{17}H_{17}FN_2O_7$ | 53.69 | 4.51 | 7.37 | | 53.65 | 4.56 | 7.39 | |
| **14d** | $C_{17}H_{24}N_2O_7$ | 55.43 | 6.57 | 7.60 | | 55.88 | 6.54 | 7.54 | |
| **15** | $C_{24}H_{28}N_2O_8$ | 61.01 | 5.97 | 5.93 | | 60.73 | 5.98 | 6.13 | |
| **5e** | $C_{12}H_{16}N_2O_4$ | 57.13 | 6.39 | 11.10 | | 57.45 | 6.37 | 11.19 | |
| **5c** | $C_{12}H_{11}FN_2O_4,1/4H_2O$ | 52.35 | 4.39 | 10.22 | | 52.09 | 4.11 | 9.84 | |
| **5b** | $C_{12}H_{11}FN_2O_4,H_2O$ | 50.69 | 4.61 | 9.89 | | 50.85 | 4.36 | 9.84 | |
| **5a** | $C_{12}H_{11}FN_2O_4$ | 54.14 | 4.16 | 10.52 | | 53.78 | 4.29 | 10.34 | |
| **5d** | $C_{12}H_{18}N_2O_4$ | 56.68 | 7.13 | 11.01 | | 56.73 | 7.03 | 10.89 | |
| **6** | $C_{12}H_{11}FN_2O_4$ | 54.14 | 4.16 | 10.52 | | 54.16 | 4.12 | 10.48 | |
| **7** | $C_{12}H_{11}FN_2O_4$ | 54.14 | 4.16 | 10.52 | | 54.12 | 4.06 | 10.45 | |

(dd, $J = 4.9$, $J = 14.9$, 1H), 3.40 (dd, $J = 4.9$, $J = 9.5$, 1H), 7.22 (dd, $J = 5.6$, $J = 8.7$, 2H), 7.67 (dd, $J = 5.6$, $J = 8.7$, 2H). Anal ($C_{12}H_{11}FN_2O_4$) C, H, N.

*7.1.5.17 (S)-(+)-2-Amino-3-[5-(4-fluorophenyl)-3-hydroxyisoxazol-4-yl]propanoic acid [(S)-(+)-4-F-APPA] (6).* The mother liquors from the two first crystallizations of the salt of (*R*)-(−)-4-F-APPA and (*S*)-(−)-PEA were evaporated. The residue consisted of a mixture of the diastereomeric salts with a diastereomeric excess of the salt of (*S*)-(+)-4-F-APPA and (*S*)-(−)-PEA (de 65%, Crownpak CR(−)). The mixture of the diastereomeric salts was dissolved in $H_2O$ (10 ml) and the solution was acidified with AcOH to pH 3. The resulting crystals were collected and dried in vacuo. The partly resolved **6** (225 mg, 0.85 mmol) was processed, as described above for **7**, using (*R*)-(+)-PEA (215 mg, 1.69 mmol). The salt of (*S*)-(+)-4-F-APPA and (*R*)-(+)-PEA was recrystallized three times. The salt [125 mg, de 99.8%, Crownpak CR(−)] was dissolved in $H_2O$ (10 ml) and the solution was acidified with AcOH to pH 3. Recrystallization from water gave 32 mg (14%) of **6**: mp 246°C decomp.; $[\alpha]_D^{25}$ +37.9° (*c* 0.25, HCl 1 M); *ee* 99.8%. The $^1$H NMR spectrum of **6** was identical with that of **7**. Anal ($C_{12}H_{11}FN_2O_4$) C, H, N.

*7.1.6 Radioligand binding assays*

The membrane preparation used in the [$^3$H]AMPA, [$^3$H]kainic acid, [$^3$H]CPP, [$^3$H]MK-801, [$^3$H]glycine and [$^3$H]CNQX binding assays was prepared as described [21]. [$^3$H]AMPA [23], [$^3$H]kainic acid [22], [$^3$H]CPP [20] and [$^3$H]CNQX [24,27] binding were performed following published procedures. [$^3$H]MK-801 binding to fully stimulated membranes was performed essentially as described earlier [46]. [$^3$H]Glycine binding was carried out by a modified version of the method described [47], using filtration through Whatman GF/B filters instead of centrifugation to isolate bound ligand.

*7.1.7 In vitro electrophysiology*

A rat cortical wedge preparation for testing the depolarizing activity of EAAs described by Harrison and Simmonds [48] was used in a modified version. Wedges (500 $\mu$m thick) of rat brain containing cerebral cortex and corpus callosum were placed with the cortex part between two layers of absorbent fiber and the corpus callosum part between two other layers of absorbent fiber. The two halves were electrically insulated from each other with a grease gap. The cortical part was constantly perfused with a $Mg^{2+}$-free, oxygenated Krebs buffer to which the compounds tested were added, whereas the corpus callosum part was perfused with a $Mg^{2+}$- and $Ca^{2+}$-free Krebs buffer. The two parts were each in contact with an Ag/AgCl electrode through which DC potentials were measured and plotted on a chart recorder.

*7.1.8 Computational methods*

Semiempirical calculations were performed by the use of SPARTAN 4.1.1. [49].

## Acknowledgements

This work was supported by grants from the Lundbeck Foundation and The Danish State Biotechnology Programme (1991–1995). The technical assistance of Karen Jørgensen, Ulla Geneser, Tina Lindgreen and Jørgen S. Johansen and the secretarial assistance of Anne Nordly are gratefully acknowledged.

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