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EXCITATORY AMINO ACID RECEPTOR ANTAGONISTS: SYNTHESIS AND PHARMACOLOGY OF 3-(CARBOXYMETHOXY)ISOXAZOLES DERIVED FROM AMPA

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Abstract: Three acidic amino acids were synthesized using the non-NMDA antagonist, (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]propionic acid (AMOA), as a lead structure. (*RS*)-3-(carboxymethoxy)-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (**1**) was found to be a selective NMDA antagonist, whereas (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-trifluoromethyl-4-isoxazolyl]propionic acid (**2**) was shown to be a non-NMDA antagonist.

Antagonists for excitatory amino acid (EAA) receptors have interest as pharmacological tools and as potential therapeutic agents in a number of neurodegenerative disorders, including brain damages following ischaemic insults[1,2]. Three ionotropic EAA receptor subtypes have been identified[3,4]: *N*-Methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) and kainic acid (KAIN) receptors. Distinction between the latter two, often named non-NMDA receptors, has been, and continues to be, hampered by lack of specific antagonists. However, in recent years some progress has been made. Certain quinoxalinediones and AMPA derivatives, such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline (NBQX)[5] and (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]propionic acid (AMOA)[6], respectively, have shown selective antagonist effects at AMPA receptors.

In order to investigate the versatility of the 3-carboxymethoxy substituent, present in AMOA, in the design of AMPA receptor antagonists, we synthesized three new analogues as shown in Fig. 1. Two selective AMPA receptor agonists were chosen as lead structures. The bicyclic AMPA analogue, (*RS*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (5-HPCA), is a conformationally rigid compound with potent agonist activity at AMPA receptors[7]. More recently (*RS*)-2-amino-3-(3-hydroxy-5-trifluoromethyl-4-isoxazolyl)propionic acid (Trifluoro-AMPA) has also proven to be a very potent AMPA receptor agonist, slightly

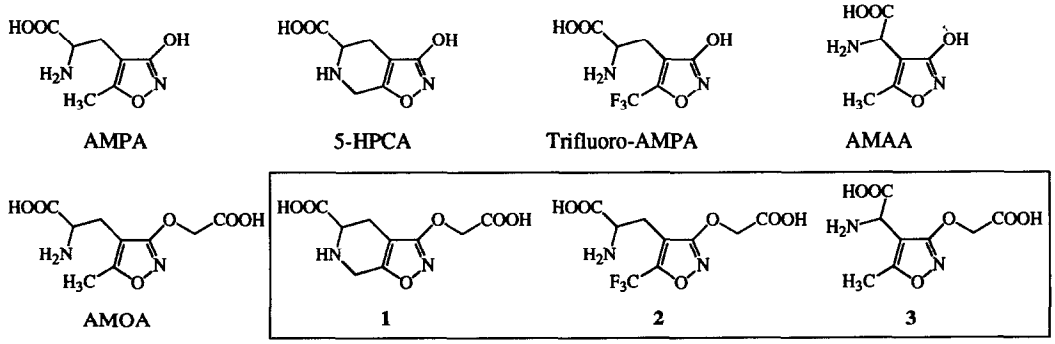


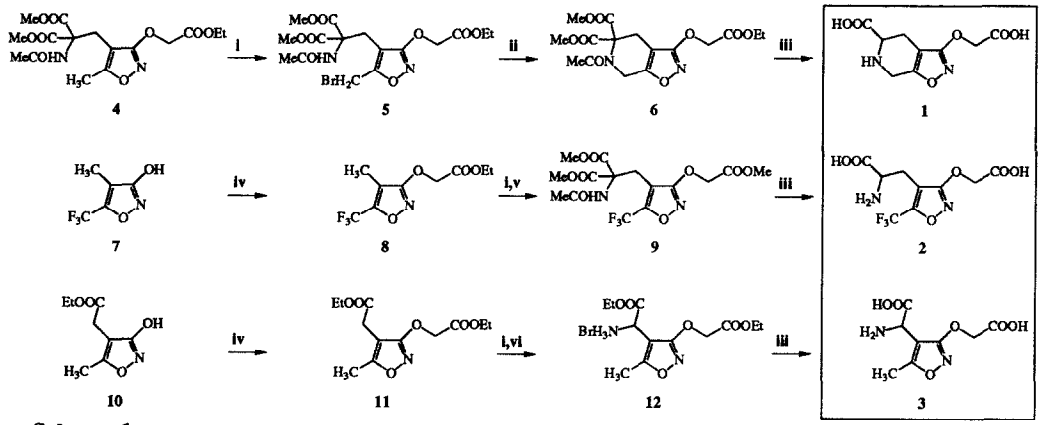
Figure 1. A comparison of the structures of some EAA agonists (top) and the corresponding carboxymethoxy analogues (bottom): the antagonist AMOA and three new compounds, **1**, **2** and **3**.

more potent than AMPA itself[8]. Based on these structures, the two compounds (*RS*)-3-(carboxymethoxy)-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (**1**) and (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-trifluoromethyl-4-isoxazolyl]propionic acid (**2**) were synthesized. A similar approach was used for the design of (*RS*)-2-amino-3-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]acetic acid (**3**) as a potential EAA antagonist, using the potent and specific NMDA agonist (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)acetic acid (AMAA)[9] as a lead structure.

Chemistry. The bicyclic acidic amino acid **1** (Scheme 1) was synthesized from methyl 2-acetamido-2-methoxycarbonyl-3-[3-(ethoxycarbonylmethoxy)-5-methyl-4-isoxazolyl]propionate[6] (**4**). Bromination of compound **4** was carried out using *N*-bromosuccinimide (NBS) and benzoylperoxide to give the intermediate **5**. *N*-deprotonation of compound **5** using sodium hydride as a base and subsequent cyclization gave **6**. Deprotection and monodecarboxylation to give product **1** was accomplished by reflux in 1 M hydrochloric acid.

Compound **2** was synthesized from 3-hydroxy-4-methyl-5-(trifluoromethyl)isoxazole[8] (**7**). Reaction with potassium carbonate and ethyl chloroacetate in acetone afforded the *O*-alkylated product **8** in 44 % yield, whereas no *N*-alkylated product could be isolated. The lack of *N*-alkylated product, which is presumed to be formed transiently under these reaction conditions, probably reflects instability of such a product, in analogy with previous observations for this alkylation procedure[6,10]. Bromination of compound **8** using NBS followed by a Sørensen synthesis afforded the intermediate **9**. Deprotection was carried out by reflux in 1 M hydrochloric acid to give compound **2**.

Compound **3** was synthesized using ethyl (3-hydroxy-5-methyl-4-isoxazolyl)acetate[11] (**10**) as the starting material. *O*-alkylation with potassium carbonate and ethyl chloroacetate gave **11** in 47 % yield, without detection or isolation of an *N*-alkylated product. NBS-bromination of compound **11** followed by reaction with liquid ammonia gave compound **12** isolated as the hydrobromide salt. Reflux in 1 M hydrochloric acid afforded compound **3**.



Scheme 1. i) NBS; ii) NaH; iii) HCl; iv) K_2CO_3, $ClCH_2COOEt$; v) $MeCONHCNa(COOEt)_2$, MeOH; vi) NH_3 (liq)

In vitro pharmacology. The affinity for different EAA receptor sites was investigated in binding assays using the tritiated ligands [^{3}H]-(*RS*)-3-(2-carboxy-4-piperazinyl)propyl-1-phosphonic acid ([^{3}H]CPP)[12], [^{3}H]AMPA[13]

Table 1. IC_{50} values in μM from binding studies, ±SEM, n = 3. *40 % inhibition at 100 μM

	[3H]CPP	[3H]AMPA	[3H]KAIN
AMAA	4.5 ±1.6	> 100	> 100
AMPA	> 100	0.04 ±0.02	> 100
KAIN	> 100	4.0 ±0.9	0.016 ±0.004
AMOA	> 100	90 ±14	> 100
1	16 ±4	> 100	> 100
2	> 100	> 100*	> 100
3	> 100	> 100	> 100

and [3H]KAIN[14] as markers for NMDA, AMPA and KAIN receptors, respectively. The results obtained are shown in Table 1. Compound **1** shows weak inhibitory effect on [3H]CPP binding, whereas both compound **2** and **3** have IC_{50} values above 100 μM in all three binding assays. These studies were supplemented by electrophysiological experiments using the rat cortical slice preparation[15]. Fig. 2 illustrates the antagonist profile of compound **1** and **2** compared to the results obtained for AMOA. Compound **1** is seen to reduce the NMDA response to approximately 30 % at 250 μM, whereas complete blockade of the NMDA response is seen at 1 mM. The antagonist profile of compound **2** is very similar to what has previously been demonstrated for the antagonist AMOA[6] (Fig. 2). Thus, compound **2** shows significant antagonist activity towards AMPA responses at 250 μM, whereas only marginal effects are observed on NMDA and KAIN responses at 1 mM. Dose-response curves obtained in this cortical slice model with NMDA and AMPA are shifted rightwards in a parallel manner with compound **1** and **2**, respectively, suggesting that these compounds are competitive antagonists (not illustrated). Compound **3** (250 μM) did not affect excitation induced by NMDA (10 μM), AMPA (5 μM) or KAIN (5 μM).

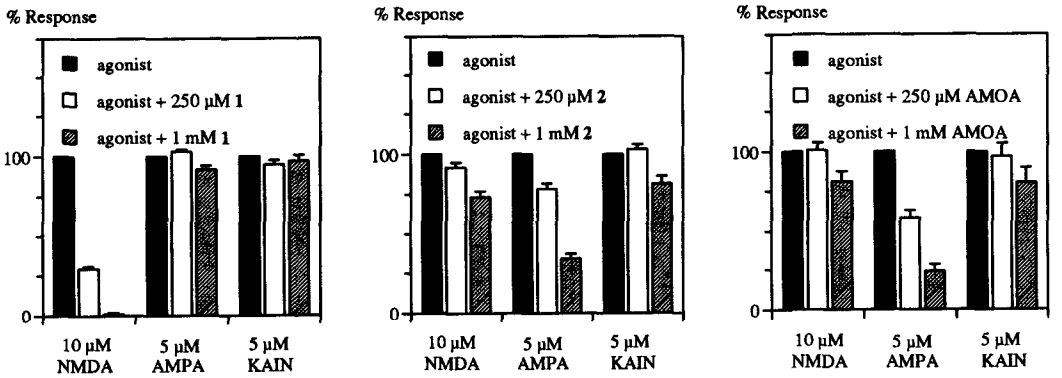


Figure 2. Effects of compounds **1**, **2** and AMOA on cortical depolarizations induced by NMDA, AMPA or KAIN. % Response (normalized), obtained from the rat cortical slice preparation, ±SEM, n = 3-6.

Discussion. Modification of the structure of the potent and specific AMPA agonist, AMPA, by introduction of an acetic acid moiety onto the 3-hydroxy group has previously led to the non-NMDA antagonist AMOA[6] (Fig. 1). This approach has been further exploited in this paper, where the carboxymethoxy substituent has been incorporated into the molecules of the AMPA agonists, 5-HPCA and Trifluoro-AMPA to give compound **1** and **2**, respectively. A similar structural modification of the potent NMDA agonist AMAA afforded compound **3** (Fig. 1).

In contrast to the selective AMPA antagonist profile of the AMPA derivative AMOA, compound **1** was, quite surprisingly, found to be an NMDA receptor antagonist devoid of effect at non-NMDA receptors. This effect of **1**

was shown by the weak affinity in the [^{3}H]CPP binding assay (Table 1) and by antagonist effects towards NMDA-induced depolarization in the rat cortical slice preparation (Fig. 2). On the other hand, compound **2**, derived from Trifluoro-AMPA, was shown to have an antagonist profile very similar to that of AMOA, showing relatively selective non-NMDA receptor antagonism (Fig. 2). In light of the relatively effective AMPA receptor antagonism by AMOA[6] and **2** (Fig. 2) the very weak effects of these compounds on [^{3}H]AMPA binding (Table 1) are unexpected and quite enigmatic.

Both the NMDA receptor antagonism by compound **1** and the AMPA receptor antagonism by compound **2** were shown to be competitive. In an earlier study, the AMPA receptor agonist Trifluoro-AMPA was found to have slightly greater agonist potency than AMPA itself[8]. This increased agonist effect was ascribed to the enhanced acidity of the 3-hydroxy moiety in Trifluoro-AMPA as compared to AMPA, rather than a change in preferred conformation of the alanine side-chain due to the 5-trifluoromethyl substituent[8]. For compound **2**, the acidity of the terminal carboxyl group is predicted to be similar to that of the equivalent group in AMOA.

It is noteworthy that compound **1** and **2**, as well as AMOA, have the same chain length between the two carboxyl groups, as have 2-aminosuberic acid (2AS) (Fig.3) and a number of phosphonate analogues like D-2-amino-7-phosphonoheptanoic acid (APH) and CPP, all of which are potent and selective NMDA antagonists[16,17]. The AMPA antagonist profiles of compound **2** and AMOA probably reflect that the AMPA moiety of these two molecules are recognized by the AMPA receptors. In light of this it was unexpected, that compound **1**, the bicyclic analogue of AMOA and carboxymethoxy derivative of the AMPA agonist 5-HPCA, turned out to be a selective NMDA receptor antagonist. This suggests that compound **1** does not reflect the receptor-recognizable conformations of compound **2** or AMOA. The preferred conformation(s) of compound **1** apparently are closer to the conformation(s) required for NMDA receptor antagonists with the same chain length, such as 2AS, APH and CPP. The different antagonist profiles of AMOA and **2** (AMPA receptor antagonists) and **1** (NMDA receptor antagonist) indicate that the presence of an α-amino acid moiety and a second acidic moiety separated by a certain chain length are not the only factors of importance for selective receptor blockade.

Compound **3**, the carboxymethyl analogue of AMAA, was found to be inactive. Thus, although AMAA shows potent agonist effect at NMDA receptors, these receptors do not recognize compound **3**. Compound **3** has a chain length comparable to that of 2-aminopimelic acid (2AP) (Fig. 3), which, in contrast to 2-aminoadipic acid (2AA), is a very weak NMDA receptor antagonist[16].

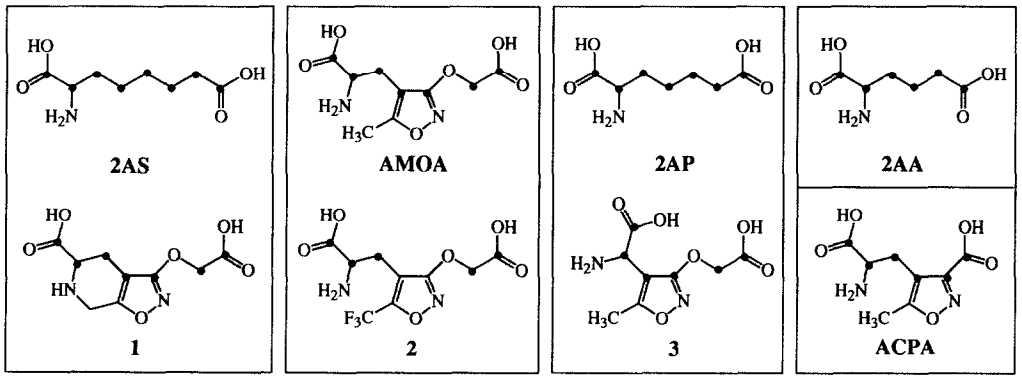


Figure 3. A comparison of the structures of the NMDA antagonists 2AS and **1**, the AMPA antagonists AMOA and **2**, the virtually inactive compounds 2AP and **3**, the NMDA antagonist 2AA and the AMPA agonist ACPA.

Compound **1** does not reflect the conformations of AMOA or **2** interacting with the AMPA receptors. It is obviously difficult from these three structures, containing the 3-(carboxymethoxy)isoxazole moiety, to derive the conformations necessary for obtaining antagonist activity at NMDA *versus* AMPA receptors. Interestingly, the compound (*RS*)-2-amino-3-(3-carboxy-5-methyl-4-isoxazolyl)propionic acid (ACPA), which is an analogue of 2AA (Fig. 3), has been shown to be an extremely potent AMPA receptor agonist[18].

AMOA, the carboxymethoxy analogue of AMPA, is an effective non-NMDA antagonist showing neuroprotective effects[19,20]. However, conversion of the acidic heterocyclic hydroxy group of 3-isoxazolol amino acids, with specific EAA receptor agonist actions, into carboxymethoxy groups does not represent a generally useful principle for conversion of an EAA agonist into an antagonist for the same EAA receptor. Thus, the corresponding analogues of the specific AMPA agonists 5-HPCA and Trifluoro-AMPA and the specific NMDA agonist AMAA, compounds **1**, **2** and **3**, respectively, show different pharmacological patterns. Whereas **1** is an NMDA antagonist, **2** shows non-NMDA receptor blockade, and **3** is inactive.

Experimental protocols

Methyl 6-acetyl-3-[(ethoxycarbonyl)methoxy]-5-(methoxycarbonyl)-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-5-carboxylate (**6**). A suspension of NBS (0.7 g; 3.9 mmol), benzoylperoxide (80 mg) and **4**[6] (1.0 g; 2.6 mmol) in CCl_4 (60 mL) was refluxed for 6 h. NBS and benzoylperoxide were added in quarter portions at 90-min intervals. After cooling and filtration the reaction mixture containing crude **5** (1.2 g) was evaporated. Crude **5** (1.2 g) was dissolved in dry DMF (3 mL) and over a period of 1 min added to a suspension of NaH (156 mg, 80 %; 5.2 mmol) in DMF (5 mL) kept at -5 °C. The reaction mixture was stirred at -5 °C for 30 min, at room temp for another 30 min and then evaporated. After addition of H_2O and extraction with CH_2Cl_2, the dried and evaporated organic phase was subjected to column chromatography (toluene - ethyl acetate 3:1, containing 1 % glacial acetic acid), which gave **6** (100 mg; 10 %) after recrystallization (ethyl acetate). Mp. 154-155 °C. ^{1}H NMR: δ 4.8 (2H, s), 4.6 (2H, br s), 4.2 (2H, q, J=7 Hz), 3.8 (6H, s), 3.3 (2H, br s), 2.2 (3H, s), 1.25 (3H, t, J=7 Hz). Analysis ($C_{16}H_{20}N_2O_9$) C, H, N.

(RS)-3-(Carboxymethoxy)-4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridine-5-carboxylic Acid (**1**) *Hydrate*. A mixture of **6** (85 mg; 0.22 mmol) and 1 M HCl (8 mL) was refluxed for 3.5 h. After cooling the reaction mixture was extracted with CH_2Cl_2 and the aqueous phase evaporated. The residue was dissolved in 2 drops of H_2O, added 2 mL EtOH and pH adjusted to *ca* 4 by addition of triethylamine, after which crude **1** precipitated. Recrystallization (H_2O) twice gave **1** (25 mg; 43 %) as a monohydrate. Mp. 247-250 °C (decomp). ^{1}H NMR (D_2O): δ 4.8 (2H, s), 4.45 (1H, d, J=15 Hz), 4.3 (1H, d, J=15 Hz), 4.2 (1H, dd, J=9 and 6 Hz), 3.1 (1H, dd, J=15 and 6 Hz), 2.75 (1H, dd, J=15 and 9 Hz). Analysis ($C_9H_{10}N_2O_6,H_2O$) C, H, N.

Ethyl (4-methyl-5-trifluoromethyl-3-isoxazolyloxy)acetate (**8**). A suspension of **7**[8] (3.3 g; 20 mmol) and K_2CO_3 (6.8 g; 49 mmol) in acetone (100 mL) was stirred at room temp for 30 min. Upon addition of ethyl chloroacetate (6.3 mL; 60 mmol) the reaction mixture was refluxed for 3 h. The reaction mixture was cooled, filtered and subjected to column chromatography (toluene) to give crude **8** (2.2 g; 44 %). Kugelrohr distillation (130 °C, 0.15 mmHg) of an analytical sample gave **8** as a colourless oil. ^{1}H NMR: δ 4.9 (2H, s), 4.3 (2H, q, J=7 Hz), 2.1 (3H, q, J=1.6 Hz), 1.3 (3H, t, J=7 Hz). Analysis ($C_9H_{10}NO_4F_3$) C, H, N.

Methyl 2-acetamido-2-methoxycarbonyl-3-[3-[(ethoxycarbonyl)methoxy]-5-trifluoromethyl-4-isoxazolyl]-propionate (**9**). A suspension of NBS (6.8 g; 38 mmol), benzoylperoxide (700 mg) and **8** (3.2 g; 12.6 mmol) in CCl_4 (200 mL) was refluxed for 72 hours. NBS and benzoylperoxide were added in quarter portions at 24-hour intervals. After cooling, filtration and evaporation the residue was subjected to column chromatography (toluene) to give crude 4-bromomethyl-3-[(ethoxycarbonyl)methoxy]-5-(trifluoromethyl)isoxazole (1.0 g). The crude product was dissolved in THF (15 mL) and at 0 °C added dropwise to the sodiumsalt of dimethyl acetamidomalonate prepared from sodium (72 mg; 3.2 mmol) and diethyl acetamidomalonate (684 mg; 3.2 mmol) in CH_3OH (4 mL). The reaction mixture was refluxed for 75 min, cooled and evaporated. After addition of H_2O, the mixture was extracted with CH_2Cl_2 and the combined organic phases dried and evaporated to give **9** (510 mg; 24 %) after recrystallization (toluene - light petroleum). Mp. 117-118 °C. ^{1}H NMR: δ 6.9 (1H, br s), 4.8 (2H, s), 3.85 (3H, s), 3.8 (6H, s), 3.6 (2H, br s), 2.0 (3H, s). Analysis ($C_{15}H_{17}N_2O_9F_3$) C, H, N.

(RS)-2-Amino-3-[3-(carboxymethoxy)-5-trifluoromethyl-4-isoxazolyl]propionic Acid (**2**). A suspension of **9** (380 mg; 0.89 mmol) in 1 M HCl (20 mL) was refluxed for 24 h. The reaction mixture was cooled and extracted with ethyl acetate. The organic phase was extracted with H_2O and the combined aqueous phases evaporated to give **2** (200 mg; 75 %) after recrystallization (H_2O). Mp. 210-212 °C (decomp). ^1H NMR (D_2O): δ 4.85 (2H, s), 4.1 (1H, t, J=7 Hz), 3.2 (2H, br t, J=7 Hz). Analysis ($C_9H_9N_2O_6F_3$) C, H, N.

Ethyl [3-[(ethoxycarbonyl)methoxy]-5-methyl-4-isoxazolyl]acetate (**11**). A suspension of **10**[11] (1.3 g; 7 mmol) and K_2CO_3 (1.9 g; 14 mmol) in acetone (125 mL) was stirred at 60 °C for 30 min. Ethyl chloroacetate (1.5 mL; 14 mmol) was added and the mixture was stirred at 60 °C for 2 h followed by addition of ethyl chloroacetate (1.5 mL; 14 mmol) and stirring at 60 °C for another 2 h. The reaction mixture was filtered, evaporated and subjected to column chromatography [toluene containing ethyl acetate (12.5-33 %)] to give **11** (890 mg; 47 %) after recrystallization (toluene - light petroleum). Mp. 53-53.5 °C. ^1H NMR: δ 4.95 (2H, s), 4.4 (2H, q, J=7 Hz), 4.25 (2H, q, J=7 Hz), 3.4 (2H, s), 2.4 (3H, s), 1.3 (6H, t, J=7 Hz). Analysis ($C_{12}H_{17}NO_6$) C, H, N.

(RS)-2-Amino-2-[3-(carboxymethoxy)-5-methyl-4-isoxazolyl]acetic acid (**3**). A suspension of NBS (268 mg; 1.5 mmol), benzoylperoxide (40 mg) and **11** (400 mg; 1.5 mmol) in CCl_4 (15 mL) was refluxed for 4 h. NBS and benzoylperoxide were added in quarter portions at 60-min intervals. After cooling, filtration and evaporation the residue was subjected to column chromatography [toluene containing ethyl acetate (5-10 %)] to give crude ethyl (*RS*)-2-bromo-2-[3(ethoxycarbonyl)methoxy-5-methyl-4-isoxazolyl]acetate (190 mg). The crude product (190 mg) was dissolved in ether and added to liquid ammonia (10 mL). After stirring for 5 min the reaction mixture was carefully evaporated, H_2O added and extracted with ether. The aqueous phase was evaporated to give crude **12** (90 mg). A mixture of 1 M HCl (2 mL) and crude **12** (90 mg) was refluxed for 3.5 h and evaporated. The residue was transferred to a basic ion exchange column (IRA-400) and eluted with acetic acid (1 M) to give **3** (39 mg; 11.5 % from **11**) after recrystallization (H_2O). Mp. 179-180 °C (decomp.). ^1H NMR: δ 5.0 (1H, s), 4.8 (2H, s), 2.4 (3H, s). Analysis ($C_8H_{10}N_2O_6$,1/4H_2O) C, H, N.

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