# Synthesis and Glutamate-Agonistic Acitivity of (*S*)-2-Amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)-propanoic Acid Derivatives[1)]

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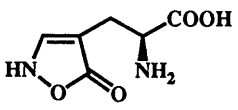
**(*S*)-2-Amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoic acid, (3a) and its analogs (3b—h) were prepared and evaluated for glutamate receptor-agonistic and antifungal activities. Several (*S*)- and (*R*)-2-amino-3-isoxazolylpropanoic acid derivatives (3a—d) were synthesized starting with (*S*)- and (*R*)-*N*-*tert*-butoxycarbonyaspartic acid α-methyl esters (4, $n=1$) by means of Masamune's chain extension reaction followed by isoxazolone formation with hydroxylamine and subsequent deprotection reactions. Furthermore, (*S*)- and (*R*)-*N*-*tert*-butoxycarbonylglutamic acid α-methyl esters (4, $n=2$) were converted to (*S*)- and (*R*)-2-amino-4-isoxazolylbutyric acid derivatives (3e—h) *via* the same sequence of reactions.**

**Keywords** isoxazol-5-one; isoxazolyl-L-alanine; asymmetric synthesis; glutamate receptor agonist; antifungal activity

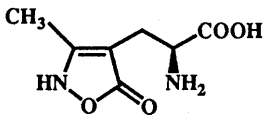
(*S*)-2-Amino-3-(2,5-dihydro-5-oxo-4-isoxazolyl)propanoic acid (**1**: TAN-950 A)[2a)] is a new antibiotic isolated from the culture filtrate of *Streptomyces platensis* A-136[2b)] in our research division. A biological study revealed that **1** has an affinity for glutamate receptors as well as antifungal activity. In the previous paper,[3)] we described the asymmetric synthesis of a variety of TAN-950 A-related analogs and their agonistic activities for glutamate receptors. In particular, (*S*)-2-amino-3-(2,5-dihydro-3-methyl-5-oxo-4-isoxazolyl)propanoic acid (**2**)[4)] was found to have a high affinity selective for quisqualate receptors with a strong agonistic activity for hippocampal neurons.[2b,5)]

On the other hand, Lambein *et al.* reported the isolation from sweet pea (*Lathyrus odoratus*) exudate of several isoxazol-5-one derivatives, which have an amino acid moiety at the N-2 position or the C-4 position of the isoxazol-5-one ring.[6)] Although the physiological role remains unclarified, they suggested that the isoxazolin-5-one-containing amino acids might play a role in promoting favorable microflora in the rhizosphere and in the protection of the roots against infection.[6)]

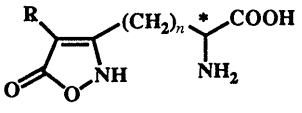
Thus, as a part of our research program on the structure–activity relationships of the isoxazolone-containing amino acids, we planned to synthesize (*S*)-2-amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoic acid (**3a**), (*S*)-2-amino-3-(2,5-dihydro-4-methyl-5-oxo-3-isoxazolyl)propanoic acid (**3c**) and their (*R*)-enantiomers (**3b, d**), which have an alanine moiety at the C-3 position of the isoxazolone nucleus. The isolation or the synthesis of isoxazolin-5-one derivatives which have an amino acid moiety at the C-3 position has not been reported.[6b)]



**1** (TAN-950 A)



**2**



**3**

| | R | *n* | configuration of C* |
|---|---|---|---|
| a | H | 1 | *S* |
| b | H | 1 | *R* |
| c | $CH_3$ | 1 | *S* |
| d | $CH_3$ | 1 | *R* |
| e | H | 2 | *S* |
| f | H | 2 | *R* |
| g | $CH_3$ | 2 | *S* |
| h | $CH_3$ | 2 | *R* |

Chart 1

Here we describe the asymmetric synthesis of isoxazolone-containing amino acid derivatives (**3**: Table I) starting with (*S*)- and (*R*)-*N*-*tert*-butoxycarbonyl (Boc) aspartic acid α-methyl esters (**4**, $n=1$)[7)] and (*S*)- and (*R*)-*N*-Boc-glutamic acid α-methyl esters (**4**, $n=2$).[8)] Their glutamate receptor-agonistic and antifungal activities were evaluated *in vitro* and the results are shown in Table II.

**Chemistry** The synthesis of isoxazolone-containing amino acids (**3**) was carried out by the method shown in Chart 2. The chain extension reaction of (*S*)-*N*-Boc-aspartic acid α-methyl ester [(*S*)-**4**, $n=1$][7)] to the oxoester (**6a**) was carried out by Masamune's method[9)] using *N*,*N*′-carbonyldiimidazole (CDI) and the magnesium salt of malonic acid mono-methyl ester **5** ($R=H$, $R^1=CH_3$). The reaction of **6a** with hydroxylamine effected the isoxazolone-ring closure,[10)] and produced the protected amino acid derivative **7a** in 46% yield. Hydrolysis of **7a** under basic conditions to **8a** followed by removal of the Boc group with hydrogen chloride afforded **3a** as a hydrochloride in 33% overall yield. The (*R*)-enantiomer (**3b**) was prepared by the same procedure, starting with (*R*)-**4** ($n=1$) in 28% overall yield.

Next, we carried out the synthesis of the 4-methyl derivative **3c**. The monoester [(*S*)-**4**, $n=1$][7)] was treated with CDI followed by the addition of the magnesium salt of the monomethyl ester of 2-methylmalonic acid (**5**, $R=R^1=CH_3$)[9)] to afford the oxo ester (**6c**) in 66% yield. Compound **6c** was converted to **3c** *via* **7c** and **8c** in a similar manner to the synthesis of **3a**, in 29% overall yield. The (*R*)-enantiomer (**3d**) was synthesized from (*R*)-**4** ($n=1$) *via* the same route as that used for **3c**.

The synthesis of the homologue **3e** was achieved by the use of (*S*)-*N*-Boc-glutamic acid α-methyl ester [(*S*)-**4**, $n=2$][8)] as the starting material. The chain extension reaction of (*S*)-**4** ($n=2$) to **6e** followed by treatment with hydroxylamine to form **7e**, and the subsequent deprotection

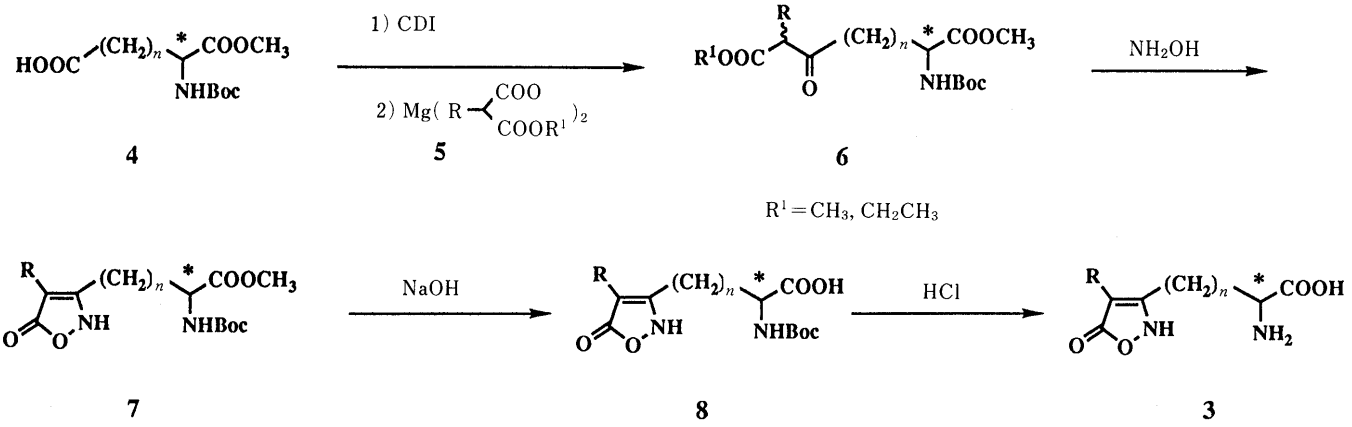
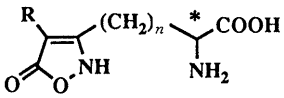



Chart 2

TABLE I. 2-Amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoic Acid Derivatives and 2-Amino-4-(2,5-dihydro-5-oxo-3-isoxazolyl)butyric Acid Derivatives (**3a—h**)

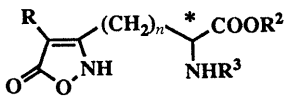



| No. | R | n | Configuration of C* | Yield (%) | Formula | Analysis (%) Calcd (Found) C | H | N | $^1$H-NMR $\delta$ | IR $\nu_{max}^{KBr}$ (cm$^{-1}$) | $[\alpha]_D$ (*c*) {temp. °C} in $H_2O$ | SI-MS (*m/z*) $(M+H)^+$ | HPLC retention time (min) | ee (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | H | 1 | *S* | 85 | $C_6H_8N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 33.12 (33.01 | 4.63 4.37 | 12.87 12.81) | 3.05 (2H, d, $J$=5.8 Hz), 3.87 (0.5H, s), 4.30 (1H, m), 5.18 (0.5H, s) (in DMSO-$d_6$) [3.18 (1H, dd, $J$=16.6, 6.7 Hz), 3.29 (1H, dd, $J$=16.6, 5.4 Hz), 4.42 (1H, dd, $J$=6.7, 5.4 Hz) (in $D_2O$)][a] | 3410, 2980, 1810, 1730, 1600, 1505 | +5.2 (0.1) {25} | 173 | 6.28 | 99.2[b] |
| **3b** | H | 1 | *R* | 80 | $C_6H_8N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 33.12 (32.72 | 4.63 4.90 | 12.87 12.54) | 3.05 (2H, d, $J$=5.8 Hz), 3.87 (0.5H, s), 4.30 (1H, m), 5.18 (0.5H, s) (in DMSO-$d_6$) [3.18 (1H, dd, $J$=16.6, 6.7 Hz), 3.29 (1H, dd, $J$=16.6, 5.4 Hz), 4.42 (1H, dd, $J$=6.7, 5.4 Hz) (in $D_2O$)][a] | 3410, 2980, 1810, 1730, 1600, 1505 | −5.0 (0.2) {22} | 173 | 3.42 | 99.0[b] |
| **3c** | $CH_3$ | 1 | *S* | 69 | $C_7H_{10}N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 36.30 (36.58 | 5.22 5.24 | 12.09 11.74) | 1.79 (3H, s), 3.22 (1H, dd, $J$=15.4, 6.6 Hz), 3.31 (1H, dd, $J$=15.4, 6.6 Hz), 4.28 (1H, t, $J$=6.6 Hz) (in $D_2O$) | 3450, 2980, 1720, 1640, 1500, 1440 | +9.6 (1.2) {25} | 187 | 6.68 | 98.8[b] |
| **3d** | $CH_3$ | 1 | *R* | 72 | $C_7H_{10}N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 36.30 (36.51 | 5.22 5.30 | 12.09 12.43) | 1.79 (3H, s), 3.22 (1H, dd, $J$=15.4, 6.6 Hz), 3.32 (1H, dd, $J$=15.4, 6.6 Hz), 4.35 (1H, t, $J$=6.6 Hz) (in $D_2O$) | 3450, 2980, 1720, 1640, 1500, 1440 | −10.1 (1.1) {25} | 187 | 4.28 | 99.4[b] |
| **3e** | H | 2 | *S* | 71 | $C_7H_{10}N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 36.30 (36.46 | 5.22 5.33 | 12.09 11.97) | 2.12 (2H, m), 2.61 (2H, m), 3.97 (1H, m), 3.85 (0.5H, s), 5.08 (0.5H, s) (in DMSO-$d_6$) [2.29 (2H, m), 2.75 (2H, dt, $J$=6.4, 3.0 Hz), 4.13 (1H, t, $J$=6.4 Hz) (in $D_2O$)][a] | 3410, 2980, 1800, 1730, 1600, 1505, 1440 | +22.8 (0.8) {25} | 187 | 13.96 | 98.2[c] |
| **3f** | H | 2 | *R* | 81 | $C_7H_{10}N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 36.30 (36.54 | 5.22 5.20 | 12.09 12.18) | 2.12 (2H, m), 2.61 (2H, m), 3.97 (1H, m), 3.85 (0.5H, s), 5.08 (0.5H, s) (in DMSO-$d_6$) [2.29 (2H, m), 2.75 (2H, dt, $J$=6.4, 3.0 Hz), 4.13 (1H, t, $J$=6.4 Hz) (in $D_2O$)][a] | 3410, 2980, 1800, 1730, 1600, 1505, 1440 | −24.4 (0.8) {25} | 187 | 8.28 | 98.8[c] |
| **3g** | $CH_3$ | 2 | *S* | 81 | $C_8H_{12}N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 39.11 (39.25 | 5.74 5.92 | 11.40 11.11) | 1.77 (3H, s), 2.26 (2H, m), 2.81 (2H, m), 4.08 (1H, m) (in $D_2O$) | 3350, 2900, 1745, 1630, 1560, 1500, 1210 | +26.8 (0.8) {25} | 201 | 18.31 | 99.4[c] |
| **3h** | $CH_3$ | 2 | *R* | 88 | $C_8H_{12}N_2O_4 \cdot HCl \cdot 1/2H_2O$ | 39.11 (39.37 | 5.74 6.08 | 11.40 11.46) | 1.77 (3H, s), 2.26 (2H, m), 2.81 (2H, m), 4.08 (1H, m) (in $D_2O$) | 3350, 2900, 1745, 1630, 1560, 1500, 1210 | −26.7 (0.9) {25} | 201 | 10.49 | 99.2[c] |

*a*) The signal of the proton at the 4-position of the isoxazolone ring overlapped the HDO signal at $\delta$ 4.8. *b*) Under condition B. *c*) Under condition A.

reactions gave **3e** in 5% overall yield. The same sequence of reactions starting with (*R*)-*N*-Boc-glutamic acid α-methyl ester [(*R*)-**4**, $n$=2][8] afforded **3f** in 19% overall yield.

Furthermore, the 4-methyl homologue **3g** and its (*R*)-enantiomer **3h** were synthesized in a similar manner to the synthesis of **3c** and **3d**, starting with (*S*)- and



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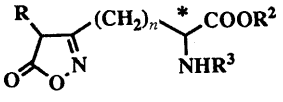

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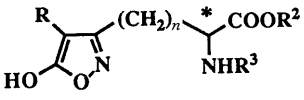


11

$R^2 = CH_3$, H
$R^3$ = Boc, H

Chart 3

(*R*)-*N*-Boc-glutamic acid α-methyl esters [(*S*)- and (*R*)-**4**, $n=2$], in 6% and 13% overall yields, respectively.

In the proton nuclear magnetic resonance ($^1$H-NMR) spectra in $CDCl_3$ of the synthetic intermediates **7a**, **b**, **e**, **f** and **8a**, **b**, **e**, **f**, the two protons corresponding to the C-4 methylene of the isoxazolone ring appear as a doublet of doublets or singlet at around $\delta$ 3.5, as shown in Tables IV and V. This result indicates that the 4-unsubstituted intermediates (**7a**, **b**, **e**, **f** and **8a**, **b**, **e**, **f**) exist as 4H-tautomers **10** (R=H; Chart 3) in $CDCl_3$.

On the other hand, in the case of the intermediates **7c**, **d**, **g**, **h** and **8c**, **d**, **g**, **h** which have a methyl group at the C-4 position, the $^1$H-NMR spectra indicated the existence of another tautomer **9**. Compounds **7g**, **h** and **8c**, **d**, **g**, **h** showed no C-4 methine proton in the $^1$H-NMR spectra in $CDCl_3$, and were considered to exist exclusively as 2H-tautomers (**9**). But compounds **7c**, **d** were observed to be in equilibrium between the 2H-tautomer **9** and the 4H-tautomer **10** (*ca.* 1:1) in $CDCl_3$. In addition, in the infrared spectra (IR) of **7a**—**f** and **8a**, **b**, **e**, **f**, an absorption near 1800 $cm^{-1}$ ($\nu_{max}C=O$) was observed and was taken as a proof of the existence of the 4H-tautomer **10**, while **7g**, **h** and **8c**, **d**, **g**, **h** showed absorption near 1720 $cm^{-1}$ ($\nu_{max}C=O$), which was assigned to the carbonyl group of the 2H-tautomer **9**.[11]

The $^1$H-NMR spectra of **3a**, **b**, **e**, **f** in DMSO-$d_6$ showed an equilibrium (1:1) between the 2H-tautomer **9** and the 4H-tautomer **10**, while in $D_2O$ only the 2H-tautomer **9** was observed. Furthermore, **3c**, **d**, **g**, **h** were observed to exist as the 2H-tautomer **9** in $D_2O$.

On the other hand, the occurrence of the OH-form **11** was not apparent in the NMR and IR spectra. Katritzky *et al.* reported that 4-unsubstituted isoxazolone derivatives exist as the 4H-tautomer in non-polar solvents and in the solid state, and a substituent on the C-4 position of isoxazolone tips the equilibrium towards the 2H-tautomer.[12] They also reported that the equilibrium with the OH-form **11** was observed only in the case of isoxazol-5-one derivatives having a C-4 substituent which can form a hydrogen bond with a 5-hydroxy group. Our experimental data (NMR and IR) are consistent with Katritzky's results.

From these results, amino acid derivatives **3** are considered to exist exclusively in form **9** under the conditions of the biological assay (aqueous solution, *vide post*).

**Determination of the Enantiomer Excess** The enantiomeric purities of the products **3** were measured by high-performance liquid chromatography (HPLC) on CROWNPAK CR (+)® under the conditions (A and B) described in the experimental section. The retention times of the enantiomers and the enantiomer excess (ee) of **3** are shown in Table I.

The pairs of enantiomers of the butanoic acid derivatives, **3e**, **f** and **3g**, **h**, were sufficiently separated on the chromatogram under condition A to allow calculation of the relative areas, and the ee values of **3e**, **f**, **g**, **h** were determined to be 98.2%, 98.8%, 99.4%, and 99.2%, respectively. On the other hand, the ee values of the propanoic acid derivatives **3a**, **b**, **c**, **d** were found to be 99.2%, 99.0%, 98.8%, 99.4%, respectively, under conditions B.

**Biological Activity** The isoxazolone derivatives **3** prepared in this report were tested for *in vitro* antifungal activity and *in vitro* glutamate receptor-agonistic activity, and the results are shown in Table II.

The antifungal activity was determined by a paper disc assay (measuring the diameter of the inhibition zone; 1000 μg/ml) on YNB agar (yeast nitrogen base; 28°C, 2 d). TAN-950 A (**1**) had inhibitory activity against the growth of *Candida albicans* and *Saccharomyces cerevisiae*. Compounds **3a**, **b**, **c** showed moderate activity against these organisms in this assay, but are less active than **1** (Table II). On the other hand, compounds **3d**, **e**, **f**, **g**, **h** are devoid of antifungal activity.

The glutamate receptor-binding assays were performed by using $^3$H-kainic acid (racemate),[13] $^3$H-AMPA,[13] [[$^3$H](*RS*)-α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid] and $^3$H-CPP [(*RS*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphoric acid][13] as labeled ligands for the three (kainate-, quisqualate- and *N*-methyl-D-aspartate (NMDA)-) subtypes of receptors on rat brain membrane preparations.[5] The affinities of compounds **3** to these receptors are expressed in terms of $IC_{50}$ (μM: the concentration of the test compound giving 50% inhibition of $^3$H-kainic acid-, $^3$H-AMPA-, or $^3$H-CPP-specific binding to the corresponding receptors). The excitatory potency of the compounds was determined by the microelectrophysiological method by using a rat hippocampus *in vitro*,[2] and is expressed as the minimum effective concentration to elicit firing of a neuron (MEC, μM).

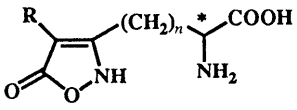
Compound **3a** had less affinity for the glutamate receptors than **1**, but the excitatory potency in the hippocampus was a little more potent (MEC; 100 μM) than those of **1** (MEC, 300 μM) and L-glutamic acid (MEC, 300 μM).

Compound **3b**, the enantioisomer of **3a**, and its methyl derivative **3d** showed increased selectivity for the NMDA-receptor compared with **3a**. But, unfortunately, their excitatory potencies are very weak.

In contrast, compound **3e**, a homologue of **3a**, was less selective for three subtype receptors, with moderate excitation potency (MEC, 300 μM). The non-selective character of **3e** as a glutamate agonist seems to be similar to that of **1** and glutamic acid.

These observations indicated that isoxazol-5-one deriva-

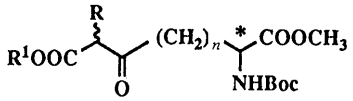
TABLE II. Antifungal Activity and Glutamate Receptor Binding of Isoxazol-5-one Derivatives (**3a—h**)



| No. | R | *n* | Configuration of C* | Antifungal activity Diameter of inhibition zone (mm) *C. albicans* (YNB) | *C. albicans* (YNB, pH 7) | *S. cerevisiae* (YNB) | Glutamate receptor binding[a] $IC_{50}$ ($\mu$M) *K* | *Q* | *N* | Excitation of neurons MEC ($\mu$M) |
|---|---|---|---|---|---|---|---|---|---|---|
| **3a** | H | 1 | *S* | 11 | 19 | 17 | =100 | 9.1 | 49 | 100 |
| **3b** | H | 1 | *R* | 0 | 14 | 12 | >100 | =100 | 5.0 | 1000 |
| **3c** | $CH_3$ | 1 | *S* | 0 | 0 | 21 | >100 | 12 | 34 | —[b] |
| **3d** | $CH_3$ | 1 | *R* | 0 | 0 | 0 | >100 | >100 | 18 | >1000 |
| **3e** | H | 2 | *S* | 0 | 0 | 0 | 14 | 18 | 5.3 | 300 |
| **3f** | H | 2 | *R* | 0 | 0 | 0 | >100 | =100 | 13 | —[b] |
| **3g** | $CH_3$ | 2 | *S* | 0 | 0 | 0 | 67 | =100 | 18 | —[b] |
| **3h** | $CH_3$ | 2 | *R* | 0 | 0 | 0 | >100 | >100 | >100 | —[b] |
| **1** | TAN-950A | | | 26 | 28 | 28 | 3.6 | 0.28 | 19 | 300 |
| | L-Glutamate | | | — | — | — | 0.11 | 0.24 | 0.98 | 300 |

*a*) "*K*", "*Q*", and "*N*" represent $IC_{50}$ values for displacement of $^3$H-kainate, $^3$H-AMPA, and $^3$H-CPP binding by the compound, respectively. *b*) Not measured.

TABLE III. Aspartic and Glutamic Acid Derivatives (**6a—h**)



| No. | R | $R^1$ | *n* | Configuration of C* | Yield (%) | $^1$H-NMR $\delta$ (in $CDCl_3$) | IR $\nu_{max}^{neat}$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|
| **6a** | H | $CH_3$ | 1 | *S* | 92 | 1.45 (9H, s), 3.10 (1H, dd, *J*=4.4, 18.4 Hz), 3.28 (1H, dd, *J*=4.4, 18.6 Hz), 3.49 (2H, s), 3.74 (3H, s), 3.75 (3H, s), 4.54 (1H, m), 5.47 (1H, m) mp 48—49 °C. *Anal.* Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.34; H, 7.12; N, 4.58. $[\alpha]_D^{24}$ −15.8° (*c*=0.6, MeOH) | 3390, 2990, 1750, 1720, 1680, 1510, 1440, 1370 (KBr) |
| **6b** | H | $CH_3$ | 1 | *R* | 79 | 1.45 (9H, s), 3.10 (1H, dd, *J*=4.4, 18.4 Hz), 3.28 (1H, dd, *J*=4.4, 18.4 Hz), 3.49 (2H, s), 3.74 (3H, s), 3.75 (3H, s), 4.54 (1H, m), 5.47 (1H, m) mp 48—49 °C. *Anal.* Calcd for $C_{13}H_{21}NO_7$: C, 51.48; H, 6.98; N, 4.62. Found: C, 51.38; H, 6.96; N, 4.65. $[\alpha]_D^{24}$ +14.2° (*c*=0.5, MeOH) | 3390, 2960, 1740, 1720, 1680, 1510, 1440, 1370 (KBr) |
| **6c** | $CH_3$ | $CH_3$ | 1 | *S* | 66 | 1.35 (3H, d, *J*=7.0 Hz), 1.45 (9H, s), 3.07 (0.34H, dd, *J*=18.2, 4.4 Hz), 3.14 (0.66H, dd, *J*=12.8, 4.4 Hz), 3.24 (0.66H, dd, *J*=10.8, 4.4 Hz), 3.29 (0.34H, dd, *J*=18, 4.4 Hz), 3.55 (1H, q, *J*=7.0 Hz), 3.74 (3H, s), 3.75 (3H, s), 4.53 (1H, m), 5.48 (1H, d, *J*=8.0 Hz) | 3370, 2980, 1750, 1720, 1520, 1440, 1370 |
| **6d** | $CH_3$ | $CH_3$ | 1 | *R* | 89 | 1.35 (3H, d, *J*=7.2 Hz), 1.44 (9H, s), 3.07 (0.34H, dd, *J*=18.2, 4.4 Hz), 3.15 (0.66H, dd, *J*=13, 4.4 Hz), 3.24 (0.66H, dd, *J*=10.6, 4.4 Hz), 3.29 (0.34H, dd, *J*=18, 4.4 Hz), 3.55 (1H, q, *J*=7.2 Hz), 3.74 (3H, s), 3.75 (3H, s), 4.53 (1H, m), 5.48 (1H, d, *J*=8.0 Hz) | 3370, 2980, 1750, 1720, 1520, 1440, 1370 |
| **6e** | H | $CH_3$ | 2 | *S* | 60 | 1.45 (9H, s), 2.05 (2H, m), 2.63 (2H, t, *J*=6.3 Hz), 3.44 (2H, s), 3.73 (6H, s), 4.22 (1H, m), 5.15 (1H, m) | 3390, 2990, 1750, 1720, 1520, 1440, 1370 |
| **6f** | H | $CH_3$ | 2 | *R* | 68 | 1.45 (9H, s), 2.05 (2H, m), 2.63 (2H, t, *J*=6.3 Hz), 3.44 (2H, s), 3.73 (6H, s), 4.22 (1H, m), 5.15 (1H, m) | 3390, 2990, 1750, 1720, 1520, 1440, 1370 |
| **6g** | $CH_3$ | $CH_3CH_2$ | 2 | *S* | 34 | 1.27 (3H, t, *J*=7.0 Hz), 1.34 (3H, d, *J*=7.2 Hz), 1.44 (9H, s), 1.80—2.90 (4H, m), 3.52 (1H, q, *J*=7.2 Hz), 3.75 (3H, s), 4.27 (1H, m), 5.11 (1H, m) | 3380, 2990, 1750, 1710, 1510, 1445, 1390, 1360 |
| **6h** | $CH_3$ | $CH_3CH_2$ | 2 | *R* | 63 | 1.27 (3H, t, *J*=7.0 Hz), 1.34 (3H, d, *J*=7.2 Hz), 1.44 (9H, s), 1.80—2.90 (4H, m), 3.52 (1H, q, *J*=7.2 Hz), 3.75 (3H, s), 4.27 (1H, m), 5.11 (1H, m) | 3380, 2990, 1750, 1710, 1510, 1445, 1390, 1360 |

tives (**3**) containing an amino acid moiety at the C-3 position are weak excitors of neurons compared with the TAN-950 A derivative **2**. The substitution position of an amino acid moiety on the isoxazolone ring is important in determining the selectivity for glutamate receptors and the excitation of neurons, as well as the antifungal activity.

## Experimental

IR spectra were measured with a Hitachi 215 spectrophotometer. $^1$H-NMR spectra were taken on a Varian Gemini-200 (200 MHz) with tetramethylsilane as an internal standard. Abbreviations are as follows: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; br=broad. The optical rotations were recorded with a JASCO DIP-181 digital polarimeter. The secondary ion mass spectra (SI-MS) were measured with a Hitachi M-80A mass spectrometer.

**Dimethyl (2*S*)-2-*tert*-Butoxycarbonylamino-4-oxohexan-1,6-dioate (6a,**

**Table III)** CDI (914 mg, 5.64 mmol) was added to a solution of α-methyl (*S*)-*N*-Boc-aspartate [(*S*)-**4**, $n=1$] (1.166 g, 4.72 mmol) in dry tetrahydrofuran (THF) (25 ml), and the mixture was stirred under an argon atmosphere for 6 h at room temperature. The magnesium salt of malonic acid monomethyl ester **5** ($R=H$, $R_1=CH_3$) (1.212 g, 4.70 mmol) was added to the mixture. The mixture was stirred at room temperature for 18 h, then the solvent was evaporated off under reduced pressure, and the residue was partitioned between AcOEt (20 ml) and water (10 ml). Insoluble materials were filtered off. The AcOEt layer was separated and the aqueous layer was extracted with AcOEt (10 ml × 3). The combined extract was washed successively with 1 N HCl, water, aqueous sodium bicarbonate, water and saturated aqueous NaCl. The extract was dried ($MgSO_4$) and evaporated *in vacuo*. The residue was subjected to chromatography on silica gel. Elution with AcOEt–hexane (1 : 3→1 : 2→1 : 1) gave **6a** (1.32 g, 92%) as colorless prisms.

Compounds **6b**, **e**, **f** (Table III) were prepared from (*R*)-**4** ($n=1$), (*S*)-**4** ($n=2$) and (*R*)-**4** ($n=2$), respectively, in a manner similar to that described for the synthesis of **6a**.

**Dimethyl (2*R*)-2-*tert*-Butoxycarbonylamino-5-methyl-4-oxohexan-1,6-dioate (6c, Table III)** CDI (2.12 g, 13.1 mmol) was added to a solution of α-methyl (*S*)-*N*-Boc-aspartate [(*S*)-**4**, $n=1$] (2.70 g, 10.9 mmol) in dry THF (60 ml), and the mixture was sttirred under an argon atmosphere for 6 h at room temperature. The magnesium salt of 2-methylmalonic acid monomethyl ester **5** ($R=R^1=CH_3$) (3.13 g, 10.9 mmol) was added. The reaction mixture was stirred at room temperature for 18 h, then the solvent was evaporated off under reduced pressure, and the residue was partitioned between AcOEt (50 ml) and water (20 ml). Insoluble materials were filtered off. The AcOEt layer was separated and the aqueous layer was extracted with AcOEt (15 ml × 3). The extract was combined, washed successively with 1 N HCl, water, aqueous sodium bicarbonate, water and saturated aqueous NaCl, dried ($MgSO_4$) and evaporated *in vacuo*. The residue was subjected to chromatography on silica gel. Elution with AcOEt–hexane (1 : 3→1 : 2→1 : 1) gave **6c** (2.28 g, 66%) as a colorless oil.

Compound **6d** (Table III) was prepared from (*R*)-**4** ($n=1$) in the same manner as that described for the preparation of **6c**. Compounds **6g**, **h** (Table III) were prepared from (*S*)-**4** ($n=2$) and (*R*)-**4** ($n=2$), respectively, in a manner similar to that described for the synthesis of **6c** by using the magnesium salt of 2-methylmalonic acid monoethyl ester **5** ($R=CH_3$, $R^1=C_2H_5$).

**Methyl (*S*)-2-*tert*-Butoxycarbonylamino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoate (7a, Table IV)** A mixture of **6a** (1.212 g, 4.0 mmol), hydroxylamine hydrochloride (278 mg, 4.0 mmol) and sodium carbonate (212 mg, 2.0 mmol) in EtOH (30 ml) was refluxed under an argon atmosphere for 1 h, then allowed to cool. Insoluble material was filtered off, and the filtrate was concentrated under reduced pressure. The concentrate was subjected to chromatography on silica gel. Elution with AcOEt–hexane (2 : 1)→AcOEt→AcOEt–MeOH (9 : 1) afforded **7a** (529 mg, 46%) as a pale yellow foam.

Compounds **7b**—**h** (Table IV) were prepared in a similar manner starting with **6b**—**h**, respectively.

**(*S*)-2-*tert*-Butoxycarbonylamino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoic Acid (8a, Table V)** A 0.5 N aqueous solution of NaOH (11.0 ml) was added to **7a** (529 mg, 1.85 mmol), and the mixture was stirred at room temperature for 2 h, then extracted with AcOEt. The aqueous layer was adjusted to pH 2.7 with 1 N aqueous HCl, saturated with NaCl and extracted with AcOEt (15 ml × 4). The extract was washed with saturated aqueous NaCl, dried ($MgSO_4$) and evaporated to give **8a** (465 mg, 92%) as a pale yellow foam.

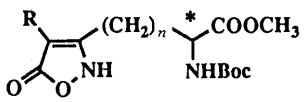
Compounds **8b**—**h** (Table V) were prepared in a similar manner starting with **7b**—**h**, respectively.

**(*S*)-2-Amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoic Acid Hydrochloride (3a·HCl, Table I)** A 4N aqueous HCl–dioxane solution (6 ml) was added to **8a** (465 mg, 1.71 mmol). The mixture was stirred at room temperature for 1 h. After evaporation of the solvent, $Et_2O$ was added to the residue, and the supernatant layer was removed by decantation (3 times). The resulting powder was dissolved in water (4 ml) and lyophilized to give the hydrochloride **3a**·HCl (318 mg, 85%) as an amorphous white powder.[14]

Compounds **3b**—**h** (Table I) were prepared in a similar manner starting with **8b**—**h**, respectively.

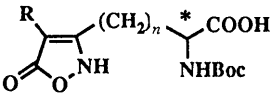
**Determination of the Enantiomer Excess** The enantiomeric purity of the products (**3**) was measured by HPLC on a CROWNPAK CR (+)® 0.4 × 15 cm column (Daicel Chemical Industries Ltd.) under the conditions described below. The spectrometric ultraviolet (UV) detector was set at 250 nm. The enantiomeric composition was determined from the peak areas. Condition A: mobile phase, aqueous $HClO_4$ (pH 2.0); temperature, room temperature (*ca.* 25 °C); flow rate, 0.2 ml/min. Condition B: mobile phase, aqueous $HClO_4$ (pH 1.0); temperature, 0 °C; flow rate; 0.7 ml/min.

TABLE IV. Methyl *N*-Boc-2-Amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoate and Methyl *N*-Boc-2-Amino-4-(2,5-dihydro-5-oxo-3-isoxazolyl)butyrate (**7a**—**h**)




| No. | R | *n* | Configuration of C* | Yield (%) | $^1$H-NMR δ (in $CDCl_3$) | IR $\nu_{max}^{neat}$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|
| **7a** | H | 1 | *S* | 46 | 1.44 (9H, s), 2.83 (1H, dd, *J* = 15, 7.8 Hz), 3.01 (1H, dd, *J* = 5.0, 15 Hz), 3.40 (1H, d, *J* = 23.4 Hz), 3.69 (1H, d, *J* = 23.4 Hz), 3.81 (3H, s), 4.61 (1H, m), 5.43 (1H, d, *J* = 7.8 Hz) | 3360, 2990, 1800, 1740, 1710, 1510, 1440, 1370 |
| **7b** | H | 1 | *R* | 47 | 1.44 (9H, s), 2.83 (1H, dd, *J* = 15, 7.8 Hz), 3.01 (1H, dd, *J* = 5.0, 15 Hz), 3.40 (1H, d, *J* = 23.4 Hz), 3.69 (1H, d, *J* = 23.4 Hz), 3.81 (3H, s), 4.61 (1H, m), 5.43 (1H, d, *J* = 7.8 Hz) | 3360, 2990, 1800, 1740, 1710, 1510, 1440, 1370 |
| **7c** | $CH_3$ | 1 | *S* | 42 | 1.38 (1.5H, d, *J* = 7.2 Hz), 1.45 (9H, s), 1.76 (1.5H, s), 3.59 (0.5H, q, *J* = 7.2 Hz), 3.81 (3H, s), 4.59 (1H, m), 5.43 (1H, b) | 3370, 2980, 1790, 1740, 1710, 1500, 1440, 1370 |
| **7d** | $CH_3$ | 1 | *R* | 51 | 1.38 (1.5H, d, *J* = 7.2 Hz), 1.45 (9H, s), 1.76 (1.5H, s), 3.59 (0.5H, q, *J* = 7.2 Hz), 3.81 (3H, s), 4.59 (1H, m), 5.43 (1H, b) | 3370, 2980, 1790, 1740, 1710, 1500, 1440, 1370 |
| **7e** | H | 2 | *S* | 14 | 1.45 (9H, s), 1.90—2.70 (4H, m), 3.39 (1H, d, *J* = 26.3 Hz), 3.52 (1H, d, *J* = 26.3 Hz), 3.73 (3H, s), 4.31 (1H, m), 5.30 (1H, d, *J* = 7.2 Hz) | 3390, 2990, 1800, 1740, 1710, 1520, 1450, 1370 |
| **7f** | H | 2 | *R* | 40 | 1.45 (9H, s), 1.90—2.70 (4H, m), 3.39 (1H, d, *J* = 26.3 Hz), 3.52 (1H, d, *J* = 26.3 Hz), 3.73 (3H, s), 4.31 (1H, m), 5.30 (1H, d, *J* = 7.2 Hz) | 3370, 2980, 1800, 1740, 1710, 1520, 1450, 1370 |
| **7g** | $CH_3$ | 2 | *S* | 24 | 1.45 (9H, s), 1.81 (3H, s), 1.80—2.80 (4H, m), 3.78 (3H, s), 4.21 (1H, m), 5.30 (1H, d, *J* = 7.4 Hz) | 3380, 2990, 1740, 1710, 1685, 1510, 1440, 1380 |
| **7h** | $CH_3$ | 2 | *R* | 30 | 1.45 (9H, s), 1.81 (3H, s), 1.80—2.80 (4H, m), 3.78 (3H, s), 4.2 (1H, m), 5.30 (1H, d, *J* = 7.4 Hz) | 3380, 2990, 1740, 1710, 1685, 1510, 1440, 1380 |

TABLE V. *N*-Boc-2-Amino-3-(2,5-dihydro-5-oxo-3-isoxazolyl)propanoic Acid and *N*-Boc-2-Amino-4-(2,5-dihydro-5-oxo-3-isoxazolyl)butyric Acid (**8a—h**)




| No. | R | *n* | Configuration of C* | Yield (%) | $^1$H-NMR $\delta$ (in $CDCl_3$) | IR $\nu_{max}^{KBr}$ ($cm^{-1}$) |
|---|---|---|---|---|---|---|
| **8a** | H | 1 | *S* | 92 | 1.44 (9H, s), 3.00 (2H, m), 3.44 (1H, d, *J*=23.4 Hz), 3.67 (1H, d, *J*=23.4 Hz), 4.64 (1H, m), 5.46 (1H, d, *J*=6.8 Hz) | 3350, 2990, 1800, 1700, 1520, 1370 |
| **8b** | H | 1 | *R* | 95 | 1.44 (9H, s), 3.00 (2H, m), 3.44 (1H, d, *J*=23.4 Hz), 3.67 (1H, d, *J*=23.4 Hz), 4.64 (1H, m), 5.48 (1H, d, *J*=6.8 Hz) | 3350, 2990, 1800, 1700, 1520, 1370 |
| **8c** | $CH_3$ | 1 | *S* | 86 | 1.36 (9H, s), 1.65 (3H, s), 2.85 (2H, d, *J*=6.5 Hz), 4.19 (1H, m), 7.21 (1H, d, *J*=8.6 Hz) | 3370, 3250, 2980, 1710, 1620, 1510, 1390 |
| **8d** | $CH_3$ | 1 | *R* | 63 | 1.36 (9H, s), 1.65 (3H, s), 2.85 (2H, d, *J*=6.5 Hz), 4.19 (1H, m), 7.21 (1H, d, *J*=8.6 Hz) | 3370, 3250, 2980, 1710, 1620, 1510, 1390 |
| **8e** | H | 2 | *S* | 92 | 1.45 (9H, s), 1.90—2.70 (4H, m), 3.43 (2H, s), 4.35 (1H, m), 5.23 (1H, d, *J*=6.8 Hz) | 3350, 2990, 1800, 1700, 1520, 1370 |
| **8f** | H | 2 | *R* | 88 | 1.45 (9H, s), 1.90—2.70 (4H, m), 3.43 (2H, s), 4.35 (1H, m), 5.23 (1H, d, *J*=6.8 Hz) | 3350, 2990, 1800, 1700, 1520, 1370 |
| **8g** | $CH_3$ | 2 | *S* | 98 | 1.46 (9H, s), 1.79 (3H, s), 1.90—2.70 (4H, m), 4.25 (1H, m), 5.54 (1H, d, *J*=7.2 Hz) | 3400, 2980, 1725, 1690, 1520, 1370, (neat) |
| **8h** | $CH_3$ | 2 | *R* | 81 | 1.46 (9H, s), 1.79 (3H, s), 1.90—2.70 (4H, m), 4.25 (1H, m), 5.54 (1H, d, *J*=7.2 Hz) | 3400, 2980, 1725, 1690, 1520, 1370, (neat) |

**Biological Activity** The antifungal activity of compounds **3** was evaluated by the following method: a sheet of filter paper disc (manufactured by Toyo Seisakusho, 8 mm in diameter) soaked in a 1000 $\mu$g/ml solution of a test compound (**3**) in methanol was placed on an agar plate, which was incubated at 28 °C for 2 d, and the diameter of the growth inhibition zone around the filter disc was measured. The following culture media were used: A, yeast nitrogen base agar medium; B, yeast nitrogen base agar medium (pH 7.0).

The binding assays and the determination of the excitatory potencies were carried out by the method described previously.[5]

**Acknowledgement** We thank Dr. S. Terao, Dr. A. Nagaoka, Dr. M. Suzuki and Dr. S. Kishimoto for their encouragement and fruitful suggestions throughout this work. We thank Dr. F. Lambein of the State University of Ghent, Belgium, for information concerning isoxazol-5-one-containing amino acids isolated from sweet pea (*Lathyrus odoratus*).

**References and Notes**

1) A part of this paper was presented at the 110th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1990; Abstracts of Papers, Vol. II, p. 140.
2) *a*) S. Tsubotani, Y. Funahashi, S. Hakoda, S. Harada, and M. Kondo, Abstracts of Papers, 110th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, August 1990, Vol. II, p. 203; *b*) S. Hakoda, S. Tsubotani, S. Harada, M. Suzuki, and T. Iwasa, Abstracts of Papers, The Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry, Fukuoka, 1990, p. 80.
3) N. Tamura, Y. Matsushita, T. Iwama, S. Harada, S. Kishimoto, and K. Itoh, *Chem. Pharm. Bull.*, **39**, 1199 (1991).
4) Compound **2** is less active than **1** as regards antifungal activity (unpublished data).
5) *a*) T. Iwama, Y. Nagai, S. Harada, K. Itoh, and A. Nagaoka, XIth International Congress of Pharmacology, Amsterdam, July 1990; Abstract Paper, *Eur. J. Pharmacol.*, **183**, 471 (1990); *b*) T. Iwama, Y. Nagai, S. Harada, N. Tamura, and A. Nagaoka, *ibid.*, **197**, 187 (1991).
6) *a*) F. Lambein, Y. Kuo, and R. Van Parijs, *Heterocycles*, **4**, 567 (1976) and references cited therein; *b*) Y. Kuo, F. Lambein, and R. Van Parijs, *Arch. Int. Physiol. Biochim.*, **84**, 169 (1976). The isolation of 2-amino-4-(2,5-dihydro-5-oxo-3-isoxazolyl)butanoic acid (**3e**) was described in this report, but the correct structure was 2-amino-4-(2,5-dihydro-5-oxo-2-isoxazolyl)butanoic acid; F. Lambein, personal communication.
7) E. Schröder and E. Klieger, *Justus Liebigs Ann. Chem.*, **674**, 208 (1964); H, Ishii, Y. Inoue, and R. Chujo, *Polymer J.*, **17**, 693 (1985).
8) E. Schröder and E. Klieger, *Justus Liebigs Ann. Chem.*, **673**, 196 (1964); E. Kato, K. Yamamoto, Y. Kawashima, T. Watanabe, M. Oya, T. Iso, and J. Iwao, *Chem. Pharm. Bull.*, **33**, 4836 (1985).
9) D. W. Brook, L. D.-L. Lu, and S. Masamune, *Angew. Chem., Int. Ed. Engl.*, **18**, 72 (1979).
10) A. R. Katritzky and S. Øksne, *Proc. Chem. Soc.*, **1961**, 387; N. Jacobsen, H. Kolind-Andersen, and J. Christensen, *Can. J. Chem.*, **62**, 1940 (1984); A. R. Katritzky, P. Barczynski, D. L. Ostercamp, and T. I. Yousaf, *J. Org. Chem.*, **51**, 4037 (1986).
11) R. Jacquier, C. Petrus, F. Petrus, and J. Verducci, *Bull. Soc. Chem. Fr.*, **1970**, 2690.
12) A. R. Katritzky, M. Karelson, and P. A. Harris, *Heterocycles*, **32**, 329 (1991) and references cited therein; A. R. Katritzky and A. J. Boulton, "The Tautomerism of Heterocycloes," Academic Press, New York, 1976, pp. 301—304, and refereces cited therein.
13) Purchased from New England Nuclear (Boston, U.S.A.).
14) Attempted crystallization of **3a—h**·HCl was unsuccessful.