Ibotenic Acid Analogues. Synthesis, Molecular Flexibility, and in Vitro Activity of Agonists and Antagonists at Central Glutamic Acid Receptors

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The syntheses of (*RS*)-α-amino-3-hydroxy-5-*tert*-butyl-4-isoxazolepropionic acid (**9**, ATPA), (α-*RS*,β-*RS*)-α-amino-β-methyl-3-hydroxy-5-isoxazolepropionic acid (**8**), (*RS*)-α-amino-3-hydroxy-5-isoxazolebutyric acid (**15a**), and (*RS*)-α-amino-3-hydroxy-5-isoxazolevaleric acid (**15b**) are described. The compounds were tested in vitro together with (*RS*)-α-amino-3-hydroxy-5-(bromomethyl)-4-isoxazolepropionic acid (ABPA) as inhibitors of the binding of radioactive-labeled (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) to rat brain synaptic membranes. These data were compared with the earlier reported effects of the compounds on single neurons in the feline spinal cord obtained by microelectrophoretic techniques. The three compounds AMPA, ATPA, and ABPA are agonists at the class of receptors assumed to represent a subtype of physiological (*S*)-glutamic acid (Glu) receptors. Inhibition of [^{3}H]AMPA binding by ATPA was 1 order of magnitude weaker than that of AMPA, in agreement with the relative potency of these compounds in vivo. ABPA proved to be equipotent with AMPA both as an inhibitor of AMPA binding and as a neuronal excitant. The compounds **8**, **15a**, and **15b** have no effect as inhibitors of AMPA binding, in agreement with in vivo studies that have shown that **8** does not affect the firing of central neurons whereas **15a** and **15b** are antagonists at NMDA receptors, a subpopulation of excitatory receptors not affected by AMPA. Molecular mechanical calculations on AMPA, ATPA, and ABPA using the program MM2 showed that conformations of AMPA, ABPA, and especially ATPA by rotation of the amino acid side chain have energy barriers. A possible receptor-active conformation is suggested.

The central excitatory amino acids (*S*)-glutamic acid (Glu) and (*S*)-aspartic acid (Asp) seem to activate various classes of receptors, which have been subdivided into three groups: the quisqualic acid (QUIS), the (*R*)-*N*-methyl-aspartic acid (NMDA), and the kainic acid receptors.[1] These classes seem to be distinct in the central nervous system (CNS).[2] The QUIS receptors seem to represent primarily the physiological postsynaptic Glu receptors,[3-6] and consequently there is a particular interest in selective agonists and antagonists for these receptors. While the NMDA receptors may represent physiological ASP receptors, the physiological significance of the kainic acid receptors is unknown. This paper describes the synthesis and in vitro activity of some QUIS receptor agonists and of two NMDA receptor antagonists derived from the naturally occurring Glu analogue (*R*,*S*)-3-hydroxy-5-isoxazoleglycine (ibotenic acid).

Earlier structure–activity studies on the very potent QUIS receptor agonist (*RS*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and related compounds[7] have indicated that the substituents present on the isoxazole ring might determine the preferred conformations of the amino acid side chain and thereby force it into a receptor-active conformation. This assumption prompted the synthesis and in vitro testing of analogues of AMPA, in which the methyl group was replaced by more bulky groups, namely, (*RS*)-α-amino-3-hydroxy-5-*tert*-butyl-4-isoxazolepropionic acid (**9**, ATPA) and (*RS*)-α-amino-3-hydroxy-5-(bromomethyl)-4-isoxazolepropionic acid (ABPA).[11] Furthermore, the homologues of ibotenic acid (*RS*)-α-amino-3-hydroxy-5-isoxazolebutyric acid (**15a**), (*RS*)-α-amino-3-hydroxy-5-isoxazolevaleric acid (**15b**), and *RS*,*RS*)-α-amino-β-methyl-3-hydroxy-4-methyl-5-isoxazolepropionic acid (**8**) were synthesized (Chart I). All of the compounds were tested in vitro (Table I).

In an attempt to elucidate the molecular mobility of AMPA, ATPA, and ABPA, molecular mechanical calculations using Allinger's MM2 program[8,9] were performed on these compounds. The results of these studies are compared with the effects of the compounds on cat spinal neurons[10,11] and their ability to inhibit the binding of [^{3}H]AMPA to rat brain synaptic membranes.

Chart I. Structures of (*S*)-AMPA, Ibotenic Acid, and Analogues

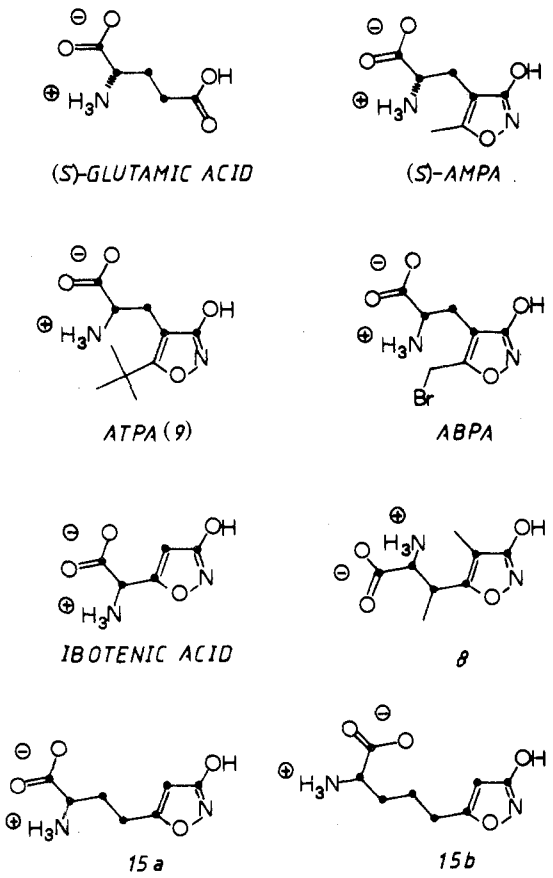

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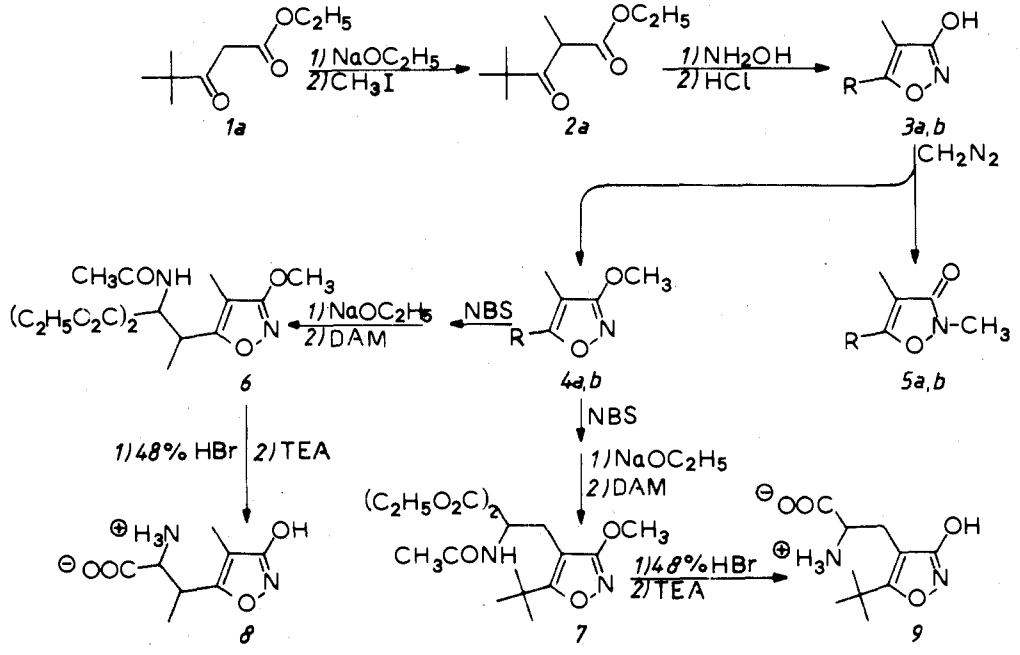
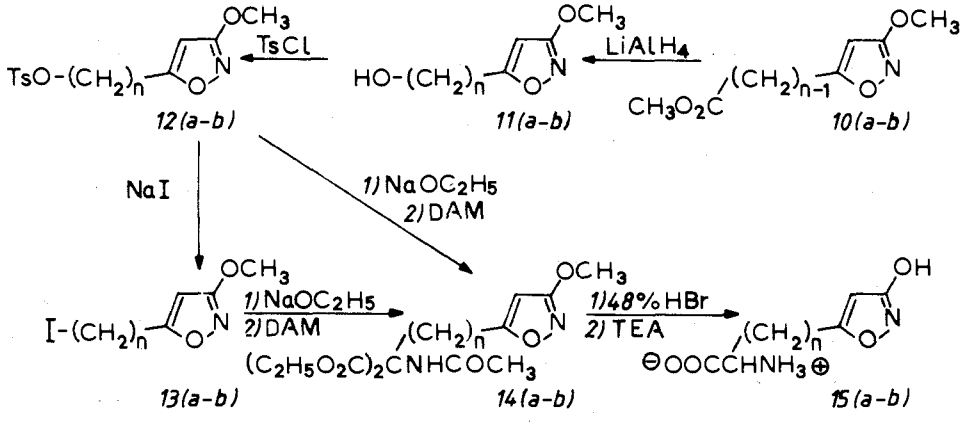
Chart II. Route of Synthesis of 8 and 9[a]



[a] a, R = $C(CH_3)_3$; b, R = C_2H_5. DAM = $CH_3CONHCH(CO_2C_2H_5)_2$.

Chart III. Route of Synthesis of 15a and 15b[a]



[a] a, n = 2; b, n = 3. DAM = $CH_3CONHCH(CO_2C_2H_5)_2$.

Synthesis and Spectroscopy. The 3-hydroxyisoxazole **3a** was synthesized by treatment of **2a** with hydroxylamine under basic conditions (Chart II). In agreement with earlier findings,[12,13] **3a** and **3b** reacted with diazomethane to give separable mixtures of O- and N-methylated products of which the former compounds were isolated and purified. The compounds **6** and **7** were synthesized by treatment of **4a,b** with *N*-bromosuccinimide (NBS) followed by reaction of the crude products with diethyl (acetylamino)sodiomalonate. ^1H NMR spectroscopy of the former reaction products showed selective bromination of the C(4)-methyl group in **4a** and of the methylene group

Table I. Comparison of the Potencies of a Number of Isoxazole Amino Acids as Neuronal Excitants and as Inhibitors of the Binding of [^3H]AMPA

compd	microelectrophoresis: GDEE-sensitive neuronal excitant (rel potency)[a-c]	microelectrophoresis: NMDA antagonist act.[d]	ligand binding, inhibition of (*RS*)-[^3H]AMPA binding, IC_{50} ± SEM, μM
(*S*)-Glu	++	na	1.3 ± 0.6[e]
(*R,S*)-AMPA	++++++	na	0.8 ± 0.3
(*S*)-AMPA	++++++	na	0.6 ± 0.1
(*R*)-AMPA	+(+)	na	4.8 ± 0.8
ATPA (**9**)	+++	na	10.6 ± 0.5
ABPA	+++++	na	0.6 ± 0.1
8	0	na	>100
15a	na	yes	64.0 ± 4.0
15b	na	yes	>100

[a] From ref 7. [b] From ref 10. [c] From ref 11. [d] From ref 16. [e] From ref 15, na = no activity.

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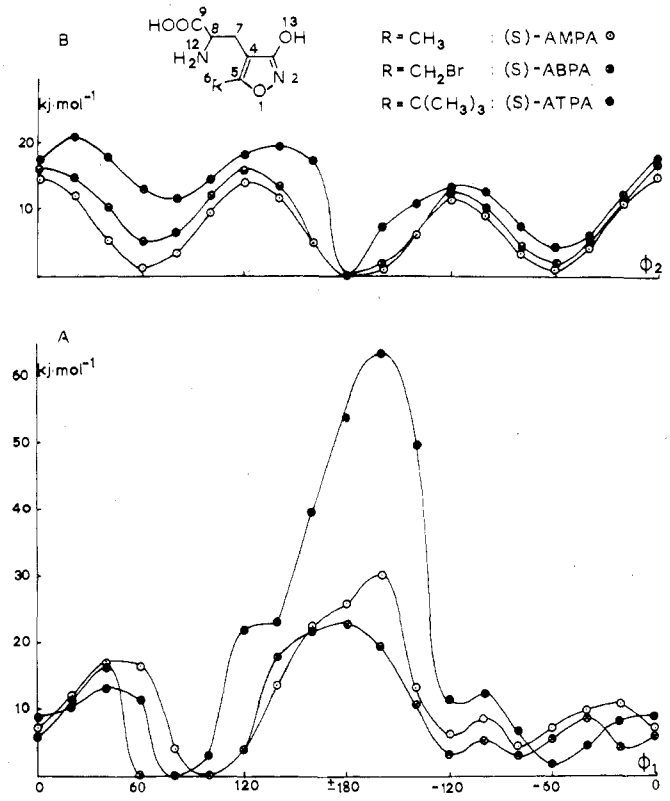

Figure 1. The effect of varying the torsion angles ϕ_1 = C(3)–C(4)–C(7)–C(8) (A) or ϕ_2 = C(4)–C(7)–C(8)–N(12) (B) in steps of 20° on the energy of (*S*)-AMPA, (*S*)-ABPA, and (*S*)-ATPA, respectively. Ordinate axis: the difference between the energy of each molecule at a specific value of the torsion angle and the minimum energy found for the molecule. Abscissa axis: values of the torsion angles ϕ_1 (A) and ϕ_2 (B).

in **4b**. The syntheses of **8** and **9** followed published procedures.[13,14] In the synthesis of **15a,b** (Chart III) treatment of the tosylate **12b** or the corresponding iodide **13b** gave **14b** in yields of 20% and 60%, respectively. For all of the new compounds **2a**, **3a**, **4a,b**, **6**, **7**, **8–9**, and **11a,b–15a,b** the ^{1}H NMR and IR spectroscopic data were consistent with the proposed structures. The structural determinations were supported by elemental analyses. For the final products **8**, **9**, **15a**, and **15b** the spectroscopic data are reported.

Conformational Analyses. A conformational study of the three related molecules shown in Figure 1 has been performed by using Allinger's molecular mechanics 2 (MM2) program. The MM2 program uses an empirical force field approach and is designed to give optimum moleuclar geometry for molecules in conformational energy minima. The steric energy of a particular conformation is calculated as the sum of terms representing bond stretching or compressing, bond angle bending, and torsinal and nonbonded potential energies.[8] Since the present version of the MM2 method is unable to handle charged species properly, we have chosen to perform the calculations on the uncharged species. The MM2 program does not contain parameters for the isoxazole nucleus; therefore, the calculations were performed with a furan nucleus instead. The errors associated with this replacement are thought to be small, especially because both nuclei are planar and rigid. Full energy minimization was performed with respect to all internal coordinates.

To gain insight into the effects of the C(5) substituents on the conformational behavior of the C(4) amino acid side chain, we have studied rotation about the C(4)–C(7) and the C(7)–C(8) bonds. The effects of variation of the torsion angle ϕ_1 = C(3)–C(4)–C(7)–C(8) on the energy of (*S*)-AMPA, (*S*)-ABPA, and (*S*)-ATPA are illustrated in Figure 1A, which shows the relative energy of each molecule as a function of the ϕ_1 torsional angle. Only the energy deriving from the rotation of the side chain is considered, excluding the basic energy of the molecule.

For AMPA two broad minima of similar energy are found at ϕ_1 = ca. −80° and ca. 100° (Figure 1), the former corresponding to the conformation of (*S*)-AMPA observed in the crystalline state (ϕ_1 = −77.1°).[13] Replacing the C(5)-methyl group in AMPA by a bromomethyl group as in ABPA results in energy variations similar to those of AMPA. In ATPA, where the C(5)-methyl group in AMPA has been replaced by a tertiary butyl group, two broad minima of nearly equal energy are found at ϕ_1 = ca. 80° and ca. −60°, reflecting the increase in size of the C(5)-alkyl group. This means that the three molecules have similar minimum-energy conformations with respect to ϕ_1 (Figure 1A).

The energy barriers arising from close contact developing between the side chain in the C(4) position and the C-(3)-hydroxyl group (ϕ_1 = ca. 40°) are similar (13–17 kJ mol^{-1}) for AMPA, ABPA, and ATPA. The energy barriers arising from close contact between the side chain in the C(4) position and the group in the C(5) position (ϕ_1 = ca. −160°) are similar for AMPA (30 kJ mol^{-1}) and ABPA (23 kJ mol^{-1}) but more than twice this value for ATPA (63 kJ mol^{-1}) (Figure 1A).

The effects of varying the torsion angle ϕ_2 = C(4)–C-(7)–C(8)–N(12) on the energy of AMPA, ABPA, and ATPA are shown in Figure 1B. Starting with the conformation of AMPA observed in the crystalline state (ϕ_2 = −65.9°),[13] the energy variations are similar for all of the three molecules, the maximum barrier being 20 kJ mol^{-1}.

Structure–Activity Discussion

The affinities of ABPA, **8**, **9**, and **15a,b** for [^{3}H]AMPA-binding sites on synaptic membranes prepared from rat brains were studied as described earlier.[15] Table I shows the biological potencies of the described compounds measured both in vivo[10,11,16] and in vitro. ABPA is as potent as AMPA as an inhibitor of [^{3}H]AMPA binding (Table I) and as an agonist at QUIS receptors.[11] These recent studies have shown that ABPA actually is more potent than reported earlier.[10] From the results of the MM2 calculations, ABPA was anticipated to be as potent as AMPA. ATPA is less potent (5–10-fold) than AMPA, both by excitation of feline spinal neurons[10] and as an inhibitor of [^{3}H]AMPA binding to rat brain membranes. The difference in activity between ATPA and AMPA may be due to the decreased molecular mobility (Figure 1) and/or steric effects of the bulky tertiary butyl groups at the binding sites. The results (Table I) of ATPA being more potent than (*R*)-AMPA in vivo but less potent in vitro may be due to the proposed partially agonist/antagonist activity of (*R*)-AMPA.[11] It might be expected from the MM2 calculations for ATPA to show a tendency toward a semifolded conformation, with a gauche arrangement about the C(4)–C(7) bond. This implies that

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if the receptor-active conformations of the compounds demand the torsion angle ϕ_1 to be close to 180°,[17] then, other factors being equal, ATPA should be less potent than AMPA because of the larger rotational barrier. Actually, as mentioned ATPA is somewhat less potent than AMPA. Furthermore, the proposal of ϕ_1 being close to 180° in the receptor-active conformation of AMPA and ATPA seems to be in accordance with the results of recent studies on the bicyclic analogue of ibotenic acid, (*R*,*S*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-7-carboxylic acid (7-HPCA), and (*R*,*S*)-3-hydroxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-5-carboxylic acid (5-HPCA).[11]

In agreement with the reported antagonistic effects of **15a** and **15b** on NMDA-induced excitations of feline spinal neuron,[16] their inhibitory effect on [^{3}H]AMPA binding was negligible (Table I). Furthermore, the receptor does not accept branching in the β-position of the amino acid side chain; compound **8**, in which a methyl group is placed in this position of the rather potent compund (*RS*)-α-amino-3-hydroxy-4-methyl-5-isoxazolpropionic acid,[15] was totally inactive in both biological test systems.

Experimental Section

Chemistry. General Procedures. Melting points, determined in capillary tubes, are corrected. Analyses, indicated by elemental symbols, were wihtin ±0.4% of the theoretical values and were performed by P. Hansen, Chemical Laboratory II, University of Copenhagen.

Thin-layer chromatography (TLC) and column chromatography (CC) were accomplished with silica gel F_{254} plates (Merck) and silica gel (Woelm, 0.063–0.100 mm), respectively. All evaporations were performed at ca. 2000 Pa with a rotatory evaporator. The pK_a values were determined following a published procedure,[18] the number in parentheses being the estimated standard deviation on the last significant digit.

A Perkin-Elmer grating infrared spectrophotometer (Model 247) and a JEOL JMN-C-60HL (60 MHz) ^{1}H NMR instrument were used. ^{1}H NMR spectra were recorded with Me_4Si as an internal standard. Compounds dissolved in D_2O were referred to sodium 3-(trimethylsilyl) propanesulfonate.

(*RS*)-Ethyl 2,4,4-Trimethyl-3-oxovalerate (2a). To a solution of sodium ethoxide in ethanol prepared from ethanol (30 mL) and sodium (1.23 g, 53.5 mmol) was added **1a**[19] (9.20 g, 52.5 mmol). After the mixture was stirred at room temperature for 2 h, methyl iodide (7.60 g, 53.5 mmol) was added. After the mixture was heated at 60 °C for 3 h, the solution was evaporated. The residue was mixed with water (30 mL) and extracted with ether (3 × 30 mL). The combined organic phases were dried (Na_2SO_4), filtered, and evaporated. Ball-tube distillation [190 °C (2000 Pa)] gave **2a** (7.10 g, 71%) as an oil. Anal. ($C_{10}H_{18}O_3$) C, H.

3-Hydroxy-4-methyl-5-*tert*-butylisoxazole (3a). To a solution of hydroxylammonium chloride (2.64 g, 38 mmol) in water (20 mL) was added at 0 °C sodium hydroxide (38 mL, 2 N, 76 mmol). While **2a** (7.10 g, 38 mmol) was added slowly, the mixture was stirred vigorously (ca. 100 min at −5 °C). After addition of iced concentrated hydrochloric acid (11.4 mL, ~114 mmol), the mixture was placed in a refrigerator to crystallize for 24 h. Filtration of the solution gave **3a** (4.51 g, 75%), mp 87–88 °C. Anal. ($C_8H_{13}NO_2 \cdot {}^1/_8H_2O$) C, H, N.

3-Methoxy-4-methyl-5-*tert*-butylisoxazole (4a). Diazomethane in excess was added to a suspension of **3a** (4.51 g, 29.0 mmol) in ether (50 mL). After the mixture was stirred for 2 h, the excess diazomethane was destroyed by adding concentrated formic acid in excess. The solution was neutralized by sodium carbonate (1 M) and washed with water (2 × 50 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated in vacuo. CC of the residue [silica gel, 120 g; eluents: toluene containing ethyl acetate (14%)] gave an oil [R_f 0.78; eluent: toluene–ethyl acetate (6:1)]. Distillation [97–100 °C (2000 Pa)] gave 4a (1.19 g, 24.2%) as an oil. Anal. ($C_9H_{15}NO_2$) C, H, N.

3-Methoxy-4-methyl-5-ethylisoxazole (4b). Diazomethane in excess was added to a suspension of **3b**[20] (6.0 g, 47.2 mmol) in ether (50 mL) and treated as described for **3a**. CC of the crude product [silica gel, 120 g; eluents: toluene containing ethyl acetate (20%)] gave an oil [R_f 0.61; eluent: toluene–ethyl acetate (4:1)], which by distillation [78–79 °C (1500 Pa)] gave **4b** (2.64 g, 39.7%) as an oil. Anal. ($C_7H_{11}NO_2$) C, H, N.

(*RS*)-Ethyl α-(Ethoxycarbonyl)-α-(acetylamino)-β-methyl-3-methoxy-4-methylisoxazole-5-propionate (6). A mixture of **4b** (1.88 g, 13.3 mmol), *N*-bromosuccinimide (NBS) (2.4 g, 13.3 mmol), and benzoyl peroxide (50 mg) in carbon tetrachloride (20 mL) was refluxed for 80 min. Every 20 min, NBS and benzoyl peroxide were added in quarter portions. After cooling, the mixture was filtered and evaporated in vacuo to give an oil. The oil was dissolved in ethanol (10 mL). This solution was added to a solution of diethyl (acetylamino)sodiomalonate, prepared from sodium (306 mg, 13.3 mmol), ethanol (30 mL), and diethyl (acetylamino)malonate (DAM) (2.90 g, 13.3 mmol). This reaction mixture was refluxed for 5 h, cooled, and evaporated in vacuo. To the resiude was added water (90 mL) and the mixture was extracted with ether (3 × 90 mL). The combined ether phases were dried (Na_2SO_4) and filtered. Evaporation in vacuo and CC [silica gel, 100 g; eluents: toluene containing ethyl acetate (50%)] gave an oil [R_f 0.36; eluents: toluene–ethyl acetate (1:1)], which was crystallized (ethyl acetate–light petroleum) to give **6** (960 mg, 20.3%), mp 107–108 °C. Anal. ($C_{16}H_{24}N_2O_7$) C, H, N.

Ethyl α-(Ethoxycarbonyl)-α-(acetylamino)-3-methoxy-5-*tert*-butylisoxazole-4-propionate (7). A mixture of 4a (850 mg, 5 mmol) in carbon tetrachloride (15 mL) was treated as described for **4b**. CC of the product [silica gel, 80 g; eluents: toluene containing ethyl acetate (50%)] gave an oil [R_f 0.46; eluents: toluene–ethyl acetate (1:1)], which was crystallized (ether–light petroleum) to give **7** (710 mg, 36.9%), mp 104–104.5 °C. Anal. ($C_{18}H_{28}N_2O_7$) C, H, N.

(α-*RS*,β-*RS*)-α-Amino-β-methyl-3-hydroxy-4-methylisoxazole-5-propionic Acid (8). A solution of **6** (535 mg, 1.5 mmol) in aqueous hydrobromic acid (40 mL, 48%) was refluxed at 140 °C for 15 min. Then the solution was evaporated in vacuo. The residue was dissolved in water (5 mL) and added a solution of triethylamine (150 mg, 1.5 mmol) in ethanol (5 mL) to give **8** (220 mg, 73.3%): mp 236.5–237 °C; IR (KBr) 3400–2300 (s, broad signal), 1660–1600 (s, broad signal), 1520 (s) cm^{-1}; ^{1}H NMR (60 MHz, D_2O + CF_3COOD) δ 4.34 (1 H, d, J = 4.8 Hz), 3.95–3.62 (1 H, m), 1.83 (3 H, s), 1.42 (3 H, d, J = 7.8 Hz); pK_a values (H_2O, 24 °C), 2.24 (±0.12), 5.41 (±0.02), 8.95 (±0.01). Anal. ($C_8H_{12}N_2O_4$) C, H, N.

(*RS*)-α-Amino-3-hydroxy-5-*tert*-butylisoxazole-4-propionic Acid (9). A solution of **7** (385 mg, 1 mmol) in aqueous hydrobromic acid (30 mL, 48%) was refluxed at 140 °C for 15 min. The solution was evaporated in vacuo. The residue was dissolved in water (3 mL) and a solution of triethylamine (100 mg, 1 mmol) in ethanol (3 mL) was added. Crystallization gave **9** (190 mg, 77%): mp 246–247 °C dec; IR (KBr) 3450 (m, broad signal), 3040 (m), 2950 (m), 1680 (m), 1630 (s), 1520 (m) 1490 (m) cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ 3.03 (1 H, t, J = 7.1 Hz), 1.84 (2 H, d, J = 7.1 Hz), 1.35 (9 H, s); pK_a values (H_2O, 24 °C), 2.46 (±0.04), 5.26 (±0.01), 10.24 (±0.04). Anal. ($C_{10}H_{16}N_2O_4 \cdot H_2O$) C, H, N.

3-Methoxy-5-(2-hydroxyethyl)isoxazole (11a). Lithium aluminum hydride ($LiAlH_4$; (450 mg, 12 mmol) in small portions was added to a mixture of **10a**[21] (1.48 g, 8 mmol) in dry ether (50 mL), and the mixture was stirred for $4^1/_2$ h at room temperature. Hydrochloric acid (8 mL, 4 M) was added, the organic phase was separated, and the aqueous phase was extracted with ether (3 × 20 mL). The combined ether phases were dried (Na_2SO_4) and evaporated. Ball-tube distillation [170 °C (30 P)] of the residue

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gave 11a (810 mg, 71%) as an oil. Anal. ($C_6H_9NO_3$) C, H, N.

3-Methoxy-5-(3-hydroxypropyl)isoxazole (11b). $LiAlH_4$ (1.4 g, 37.5 mmol) was added to a mixture of **10b**[22] (5 g, 25 mmol) dissolved in dry ether (150 mL). After the mixture was stirred for 6 h, hydrochloric acid (25 mL, 4 M) was slowly added to the mixture. The mixture was extracted with ether (3 × 50 mL), and the combined organic phases were dried (Na_2SO_4), filtered, and evaporated in vacuo. Distillation [105–106 °C (40 Pa)] gave **11b** (3.62 g, 92%) as an oil. Anal. ($C_7H_{11}NO_3$) C, H, N.

3-Methoxy-5-[2-[(*p*-tolylsulfonyl)oxy]ethyl]isoxazole (12a). Pyridine (500 mg, 6.5 mmol) and 11a (720 mg, 5 mmol) were added to a solution of *p*-toluenesulfonyl chloride (960 mg, 5 mmol) in toluene (3 mL). The clear solution was kept at 5 °C for 48 h. After filtration the organic phases were washed with hydrochloric acid (5 mL, 10%) and ice-cooled water (4 × 5 mL), dried (Na_2SO_4), evaporated, and recrystallized (ether–light petroleum) to give **12a** (790 mg, 53%). Anal. ($C_{13}H_{15}NO_5S$) C, H, N, S.

3-Methoxy-5-[3-[(*p*-tolylsulfonyl)oxy]propyl]isoxazole (12b). Pyridine (6.6 g, 85.9 mmol) and 11b (9.42 g, 60 mmol) were added to a solution of *p*-toluenesulfonyl chloride (12.6 g, 66 mmol) in toluene (36 mL). The clear solution was stored at 5 °C for 72 h. After filtration the organic phase was washed with hydrochloric acid (60 mL, 3 M) and ice-cooled water (4 × 60 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was crystallized (ether–light petroleum) to give **12b** (12.98 g, 69.5%), mp 37.2–38.7 °C. Anal. ($C_{14}H_{17}NO_5S$) C, H, N, S.

3-Methoxy-5-(2-iodoethyl)isoxazole (13a). A solution of dried (140 °C, 24 h) sodium iodide (600 mg, 4 mmol) and 12a (590 mg, 2 mmol) in acetone (6 mL) was stirred for 24 h at room temperature. After filtration and evaporation, the residue was dissolved in ether (10 mL), and the organic phase was washed with aqueous sodium thiosulfate (2 × 5 mL, 10%), dried (Na_2SO_4), filtered, and evaporated in vacuo to give **13a** (450 mg, 89%) as an oil. Anal. Calcd for $C_6H_8NO_2I$: C, 28.48; H, 3.19; N, 5.54; I, 50.15. Found: C, 30.20; I, 47.80.

3-Methoxy-5-(3-iodopropyl)isoxazole (13b). A solution of dried (140 °C, 24 h) sodium iodide (10.49 g, 70 mmol) and **12b** in acetone was stirred at room temperature for 20 h. After filtration and evaporation, the residue was dissolved in ether (100 mL) and washed with aqueous sodium thiosulfate (2 × 50 mL, 10%). The organic phase was dried (Na_2SO_4), filtered, and evaporated in vacuo to give **13b** (8.84 g, 95%) as an oil. Anal. Calcd for $C_7H_{10}NO_2I$: C, 31.48; H, 3.77; N, 5.24; I, 47.52. Found: C, 32.05; I, 46.60.

Ethyl α-(Ethoxycarbonyl)-α-(acetylamino)-3-methoxyisoxazole-5-butyrate (14a). DAM (780 mg, 3.6 mmol) was added to a solution of sodium (82 mg, 3.6 mmol) in ethanol (7 mL). After the mixture was stirred for 15 min, a solution of 13a (900 mg, 3.6 mmol) in ethanol (7 mL) was added and the mixture was refluxed for 4 h. After evaporation in vacuo the residue was dissolved in water (10 mL) and extracted with ether (3 × 20 mL). The organic phases were dried (Na_2SO_4), filtered, and evaporated. Crystallization (ethyl acetate–light petroleum) gave **14a** (560 mg, 45%), mp 103.7–105.7 °C. Anal. ($C_{15}H_{22}N_2O_7$) C, H, N.

Ethyl α-(Ethoxycarbonyl)-α-(acetylamino)-3-methoxyisoxazole-5-valerate (14b). DAM (7.16 g, 33 mmol) was added to a solution of sodium (760 mg, 33 mmol) dissolved in ethanol (75 mL). After stirring for 15 min, a solution of **13b** (8.84 g, 33 mmol) in ethanol (75 mL) was added. The mixture was refluxed for 4 h and evaporated in vacuo. Water (100 mL) was added to the residue and extracted with ether (3 × 200 mL). The combined ether phases were dried ($NaSO_4$), filtered, and evaporated in vacuo. Crystallization (ethyl acetate–light petroleum) gave **14b** (7.37 g, 63%), mp 78.5–80 °C. Anal. ($C_{16}H_{24}N_2O_7$) C, H, N.

***(RS)*-α-Amino-3-hydroxyisoxazole-5-butyric Acid (15a).** A solution of 14a (250 mg, 0.7 mmol) in aqueous hydrobromic acid (15 mL, 48%) was refluxed at 140 °C for 15 min. The solution was evaporated in vacuo and the residue was dissolved in water (1 mL). A solution of triethylamine in ethanol (2.3 mL, 0.3 mmol/mL) was added. Crystallization gave **15a** (70 mg, 54%): mp ~250 °C dec; IR (KBr) 3400 (s, broad signal), 3100–2500 (s, broad signal), 1620 (s), 1590 (s), 1500 (s), 1450 (w), 1415 (s), 1365 (m) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 5.88 (1 H, s), 3.57–3.18 (1 H, m), 3.06–2.45 (2 H, m), 2.27–1.64 (2 H, m). Anal. ($C_7H_{10}N_2O_4 \cdot H_2O$) C, H, N.

***(RS)*-α-Amino-3-hydroxyisoxazole-5-valeric Acid (15b).** Compound 14b (560 mg, 1.6 mmol) was dissolved in aqueous hydrobromic acid (60 mL, 48%) and refluxed at 140 °C for 15 min. The mixture was evaporated in vacuo. The residue was dissolved in water (6 mL) and added to triethylamine (170 mg, 1.7 mmol) in ethanol (6 mL). Crystallization gave **15b** (230 mg, 72%): mp ~260 °C dec; IR (KBr) 3400 (w, broad signal), 3200–2500 (s, broad signal), 1640 (s), 1615 (s), 1500 (s, broad signal), 1450 (w), 1420 (s), 1375 (s) cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 5.84 (1 H, s) 4.16–3.78 (1 H, m) 2.82–2.45 (2 H, m), 2.15–1.52 (4 H, m). Anal. ($C_8H_{12}N_2O_4 \cdot {}^1/_4\ H_2O$) C, H, N.

Binding Studies. Membrane preparations and binding assays were performed essentially as detailed previously[15] with racemic [^3H]AMPA (New England Nuclear, Boston, MA) as the labeled ligand. The assays consisted of a suspension of rat brain membranes (protein concentration 0.1–0.6 mg/mL in 0.05 M Tris-citrate buffer (pH 7.4), incubated in triplicate at 2 °C for 30 min with 49 nM [^3H]AMPA (spact. 15.3 Ci/mmol) and varying concentratins of the test compound (final volume 2 mL).

Bound radioactivity was separated from free radioactivity by centrifugation at 48000*g* for 10 min followed by superficial rinsing of the pellet with water (5 mL, twice) and was measured by conventional techniques.

Specific [^3H]AMPA binding (1880 cpm) was defined as the difference between binding in the absence (5550 cpm) and in the presence (3670 cpm) of 1 mM Glu. IC_{50} values (the concentration of test compound giving 50% inhibition of specific [^3H]AMPA binding) were estimated from at least four different concentrations of test compound by computerized log–probit analyses.

Conformational Studies. Allinger and Yuh's "Operating Instructions for MM2 and MMP2 Programs"[23] was used for the studies. Besides the parameters already placed in the program, the following parameters were read in, using the symbols from the operating instructions: torsion angle, N(12)–C(8)–C(9)–O(11), $V_1 = 0.0$, $V_2 = 0.0$, $V_3 = 0.0$; bending angles, C(5)–O(1)–C(2), $K_s = 0.77$, angle = 108.0°; C(3)–O(13)–H, $K_s = 0.35$, angle = 106.9°; C(5)–C(6)–Br, $K_s = 0.63$,angle = 109.5°. The starting coordinates were taken from the X-ray structure of AMPA.[13]

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