Selective Agonists at Group II Metabotropic Glutamate Receptors: Synthesis, Stereochemistry, and Molecular Pharmacology of (*S*)- and (*R*)-2-Amino-4-(4-hydroxy[1,2,5]thiadiazol-3-yl)butyric Acid

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Received March 19, 2002

Homologation of analogues of the central excitatory neurotransmitter glutamic acid (Glu), in which the distal carboxy group has been bioisosterically replaced by acidic heterocyclic units, has previously provided subtype selective ligands for metabotropic Glu receptors (mGluRs). The (*S*)-form of the 1,2,5-thiadiazol-3-ol Glu analogue, 2-amino-3-(4-hydroxy[1,2,5]thiadiazol-3-yl)propionic acid (TDPA, **6**), is an 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptor agonist, which in addition stereospecifically activates group I mGluRs. We have now synthesized the (*S*)- and (*R*)-forms of 2-amino-4-(4-hydroxy[1,2,5]thiadiazol-3-yl)-butyric acid (homo-TDPA, **7**) and shown that whereas neither enantiomer interacts with AMPA receptors, (*S*)- and (*R*)-**7** appear to be selective and equipotent agonists at group II mGluRs as represented by the mGluR2 subtype. The activities of (*S*)- and (*R*)-**7** are rationalized by conformational analysis, comparison with the potent and specific group II mGluR agonist (−)-LY379268 [(−)-**12**], and docking to a homology model of mGluR2.

Introduction

Glutamic acid (Glu) exerts its functions as the major central excitatory neurotransmitter via two classes of receptors: the ionotropic Glu receptors (iGluRs) and the metabotropic receptors (mGluRs). Whereas the iGluRs, which are ligand-gated ion channels, are subdivided into *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainic acid receptors, the mGluRs belong to the family of G-protein-coupled receptors and comprise mGluR1−8.[1−3] Group I and group II mGluRs include mGluR1,5 and mGluR2,3 respectively, whereas mGluR4,6−8 constitute group III mGluRs.[2−4] The complex roles of Glu receptors in the healthy and the diseased brain are far from being mapped out in detail, but all subtypes of iGluRs and mGluRs are considered potential therapeutic targets for a number of neurologic and psychiatric diseases.[1−6]

In light of the therapeutic interest in mGluR ligands, different rational and semirational approaches to the design of mGluR agonists and antagonists have been reported.[7−11] During recent years, we have shown that homologation of Glu and heterocyclic bioisosteres of Glu is a useful tool for the conversion of potent iGluR agonists into subtype selective mGluR agonists or antagonists. Thus, the homologue of Glu, (*S*)-**1**, stereospecifically activates mGluR2,6 (groups II and III),[12,13] whereas the (*S*)-form of 2-amino-4-(3-hydroxy-5-methyl-4-isoxazolyl)butyric acid (homo-AMPA, **5**) is a stereospecific agonist at mGluR6 (Figure 1).[12] On the other hand, the (*S*)-enantiomer of homoibotenic acid (**3**), derived from the nonselective Glu receptor agonist ibotenic acid (**2**),[14,15] shows stereospecific antagonist effects at mGluR1,5 (group I).[15]

We have recently reported the synthesis and pharmacological characterization of the (*S*)- and (*R*)-forms of 2-amino-3-(4-hydroxy[1,2,5]thiadiazol-3-yl)propionic acid (TDPA, **6**).[16] (*S*)-**6** was shown to stereoselectively activate AMPA receptors and to be a stereospecific agonist at mGluR1,5 (group I).[16] We report here the synthesis and molecular pharmacology at mGluRs of the (*S*)- and (*R*)-enantiomers of 2-amino-4-(4-hydroxy[1,2,5]-thiadiazol-3-yl)butyric acid (homo-TDPA, **7**).

Results

Chemistry. The stereocontrolled synthesis of (*S*)-**7** was based on aldehyde **8**[17] as the starting material (Scheme 1). Compound **8** was converted into amino nitrile **9** by treatment with ammonium cyanide. Without isolation of **9**, the nitrile group of **9** was hydrolyzed under Radziszewski conditions to provide amino amide **10** as a 1:1 mixture of diastereomers as established by ^{13}C NMR spectroscopy. The reported method[18] for conversion of α-amino amides into 1,2,5-thiadiazol-3-ols by reaction with sulfur monochloride in dimethylformamide (DMF) was not useful for the conversion of **10** into (*S*)-**11**, probably due to decomposition reactions caused by the hydrogen chloride formed during the reaction. To accomplish the ring closure under milder conditions, **10** was treated with thionyl chloride in the presence of triethylamine. This method provided compound (*S*)-**11** in 46% yield and without formation of elemental sulfur as a byproduct, which characterizes the previously published synthetic procedure.[18] The protected 1,2,5-thiadiazol-3-ol amino acid (*S*)-**11** was converted into target compound (*S*)-**7** by treatment with trifluoroacetic acid (TFA) in DCM. (*S*)-**7** was however

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10.1021/jm020122x CCC: $22.00
Published on Web 08/13/2002

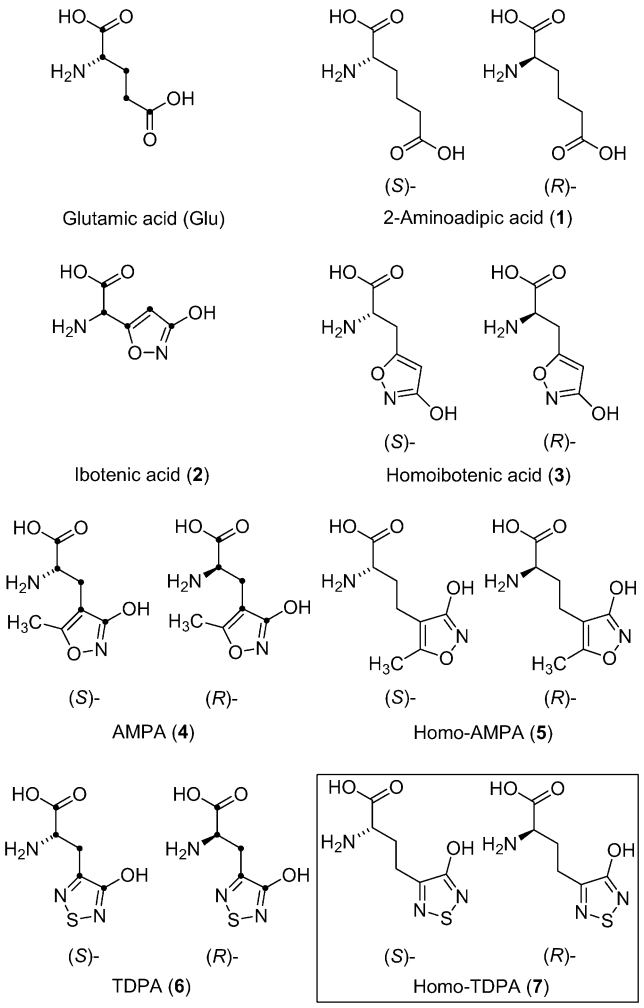
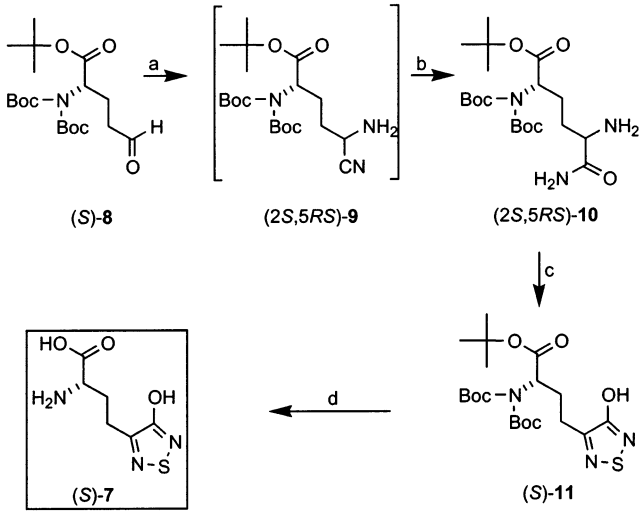


Figure 1. Structures of Glu, ibotenic acid, (*S*)- and (*R*)-AMPA, (*S*)- and (*R*)-TDPA, and the (*S*)- and (*R*)-enantiomers of the homologues of these excitatory amino acids.

Scheme 1[a]



[a] Reagents: (a) NH_4Cl, KCN, NH_3(aq), MeOH. (b) H_2O_2, NaOH(aq), THF, EtOH. (c) $SOCl_2$, NEt_3, CH_2Cl_2. (d) TFA, CH_2Cl_2, HCl(aq), propylene oxide.

obtained in a quite extensively racemized form with an enantiomeric excess (ee) of 50−60%, as determined by analytical high-performance liquid chromatography (HPLC). Both (*S*)- and (*R*)-**7** were effectively separated and isolated on a Crownpak HPLC column with enantiomeric purities of ee = 99.4% and ee > 99.9%, respectively. Because the racemization processes observed during the reaction sequences exemplified in Scheme 1 were not complete, the absolute stereochemistry of (*S*)- and (*R*)-**7** could be derived from the known configuration of (*S*)-**8**.[17]

Table 1. Pharmacology at Cloned mGlu Receptors of Glu, the Homologous Amino Acid (*S*)-**1**, and the Enantiomers of **3**, **5**, and **7** in which the Distal Carboxyl Group of **1** Has Been Bioisosterically Replaced by Different Acidic Heterocyclic Units

	EC_{50} or K_i values (μM)[a]			
compd	mGluR1α	mGluR5a	mGluR2	mGluR4a
Glu[b]	10 ± 2	4.4 ± 0.8	4.0 ± 0.5	12 ± 2
(*S*)-**1**[b,c]	>1000	>1000	35 ± 1	>3000
(*S*)-**3**[c]	**250 ± 27**	**490 ± 8**	>1000	>1000
(*R*)-**3**[c]	>1000	>1000	>1000	>1000
(*S*)-**5**[d]	>1000	>1000	>1000	>1000
(*R*)-**5**[d]	>1000	>1000	>1000	>1000
(*S*)-**7**	>1000	190 ± 80	40 ± 3	>1000
(*R*)-**7**	>1000	>1000	37 ± 3	>1000

[a] Data in plain or bold numbers are agonist (EC_{50}) and antagonist (K_i) potencies, respectively. [b] Data from ref 25. [c] Data from ref 15. [d] Data from ref 12.

In Vitro Pharmacology. The new compounds (*S*)- and (*R*)-**7** were studied in receptor binding assays in order to determine their affinity for the iGluRs. The radioligands [^{3}H]-(*RS*)-2-amino-4-methyl-5-phosphono-3-(*E*)-pentenoic acid ([^{3}H]CGP 39653),[19] [^{3}H]AMPA,[20] and [^{3}H]kainic acid[21] were used to determine affinity for NMDA, AMPA, and kainic acid receptors, respectively. With the exception of (*R*)-**7**, which showed vanishingly low affinity for NMDA receptor sites (IC_{50} > 100 μM), neither enantiomer exhibited detectable affinity for the iGluRs. This NMDA receptor affinity of (*R*)-**7** was shown to reflect a weak NMDA antagonist effect (IC_{50} = 287 ± 17 μM) as determined electrophysiologically using the rat cortical wedge model.[22,23] The NMDA antagonist effect of (*R*)-**7** may be compared with that of the classical NMDA antagonist, (*R*)-**1** (Figure 1).[24]

The enantiomers of **7** were also tested at mGluR1α,-5a, mGluR2, and mGluR4a as representatives of groups I, II, and III mGluRs, respectively (Table 1), using established second messenger assay systems.[25] Neither enantiomer showed detectable effects at mGluR4a. Whereas (*S*)-**7**, but not (*R*)-**7**, turned out to be a weak agonist at mGluR5a, neither compound showed effect at the other group I receptor, mGluR1α. Not only (*S*)-**7** but also (*R*)-**7** proved to be agonists at mGluR2 comparable in potency with (*S*)-**1**. The lack of stereoselectivity of these agonist effects of (*S*)- and (*R*)-**7** at mGluR2 is hitherto unique; (*R*)-**1** for example does not interact detectably with mGluR2.[12]

Molecular Modeling. A homology model of the closed state of the mGluR2 extracellular binding domain was built from the crystal structure of the closed ligand binding domain of mGluR1 in complex with glutamate (code 1EWK),[26] using SWISSMODEL.[27] The binding site was analyzed with methyl and water probes using GRID 18.[28] The grid maps were used as a guide for reintroducing the ligand (Glu) and binding site water molecules taken from the crystal structure. The protein structure was prepared and refined as recommended using Impact 1.8/OPLS-AA force field,[29] and docking studies were

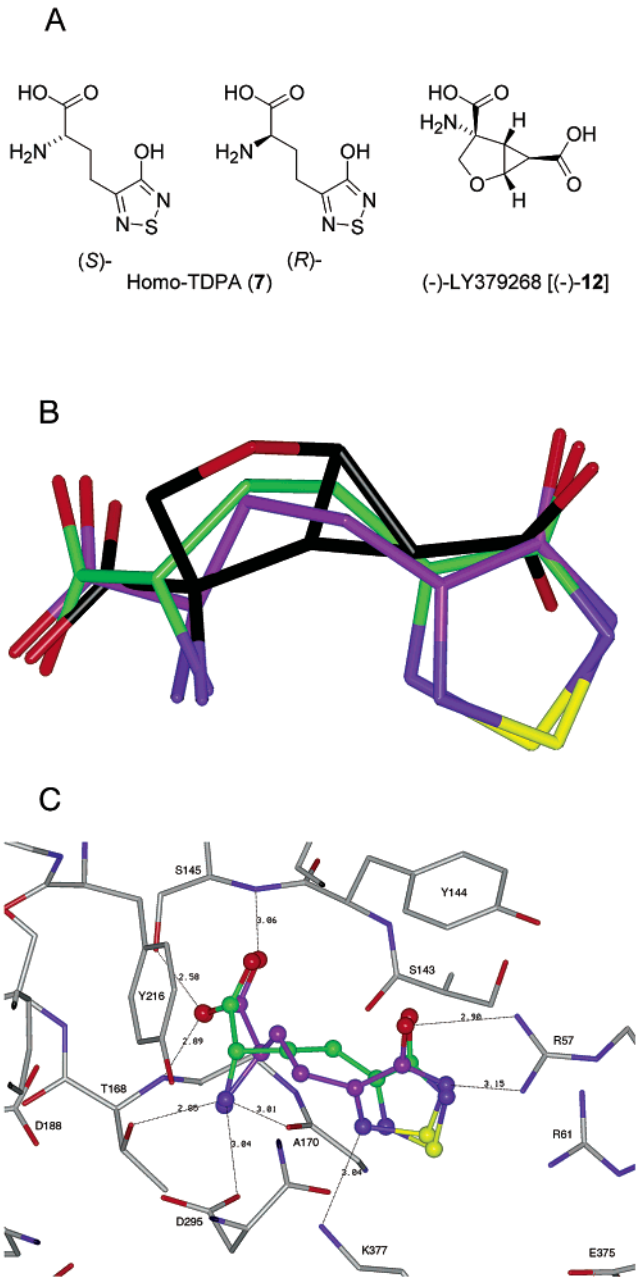


Figure 2. (A) Structures of (*S*)- and (*R*)-2-amino-4-(4-hydroxy-[1,2,5]thiadiazol-3-yl)butyric acid [(*S*)- and (*R*)-Homo-TDPA] and (−)-LY379268. (B) Superimposition of (−)-**12** (black), low energy conformations of (*R*)- and (*S*)-**7** (purple and green, respectively). (C) Superimposed final models of (*R*)- and (*S*)-**7** bound to a homology model of the binding domain of mGluR2. Protein shown is minimized with (*R*)-**7** bound (protein with (*S*)-**7** is similar). Water molecules removed for clarity. Hydrogen bonding between (*R*)-**7** and the protein indicated.

carried out on (*R*)- and (*S*)-**1**, (*R*)- and (*S*)-**5**, (*R*)- and (*S*)-**7**, and the active (−) and inactive (+) enantiomers of the potent specific mGluR2 agonist (−)-**12** (Figure 2),[30] using Glide.[29] Tri-ionized ligands (overall charge −1) were used throughout. Docked poses were ranked according to Glide's E-model.[29] High-ranking binding modes presenting similar pharmacophore geometries to that of the extended receptor-bound Glu were found for (−)-**12** and both (*R*)- and (*S*)-**7** but not for (*R*)- or (*S*)-**5** nor (+)-**12**. Because **1** is highly flexible, it was possible to dock both (*R*)- and (*S*)-**1** in reasonable binding modes.

Monte Carlo conformational analysis of (*R*)- and (*S*)-**7** in water using the MMFFs force field with GB-SA continuum solvation model in Macromodel 7.2[29] revealed that the global minimum of (*S*)-**7** and a low energy local minimum of (*R*)-**7** ($\Delta E = 1$ kcal/mol) corresponded closely to the predicted binding mode of Glu. According to a parallel analysis, the enantiomers of **5** were unable to achieve the same conformations as **7** at a reasonable energy due to the steric effect of the 5-methyl group. In addition, a superimposition based on the ionized groups (Figure 2B) shows that the extended conformation of Glu, and the proposed binding conformations of (*R*)- and (*S*)-**7** follow the envelope of (−)-**12** but not (+)-**12**. Although conformations of both (*R*)- and (*S*)-**1** were found that could be superimposed with Glu and (−)-**12**, ΔE for the active conformations of the enantiomers was 2.2 kcal/mol, and the active conformation of (*R*)-**1** was 4.5 kcal/mol above the global minimum.

Both (*R*)- and (*S*)-**7** in their predicted binding modes were reintroduced to the homology-built protein. The two complexes were again refined by the protein preparation procedure in Impact 1.8/OPLS-AA. Finally, an extra minimization of the bound ligand and binding site protons was performed with the MMFFs force field to refine the ligand geometry and hydrogen bond network. Only slight differences were noted between the final complexes, other than the conformations of the enantiomeric ligands. The homology-built models and binding modes of (*R*)- and (*S*)-**7** were similar to the published model of mGluR2 and the binding mode of (−)-**12** therein.[31]

Discussion

Homologation of Glu and heterocyclic Glu analogues (Figure 1) has proved to be a useful principle for the conversion of Glu receptor ligands showing predominant iGluR agonist activity into subtype selective or specific mGluR ligands. The stereostructure−activity studies so far reported for this class of GluR ligands have not revealed compounds with *R*-configuration showing agonist or antagonist effects at mGluRs. The *S*-forms of these compounds on the other hand show a broad spectrum of pharmacological effects, ranging from the mGluR2,6 agonist effects of (*S*)-**1**[13] to the specific mGluR6 agonist effect of (*S*)-**5**[12] and the mGluR1,5 antagonist effect of (*S*)-**3**.[15] In light of these previous results, the equipotent agonist effects of (*S*)- and (*R*)-**7** at mGluR2 are noteworthy (Table 1).

In the iGluR field, it has been possible to cocrystallize the recombinant S1−S2 binding domain of iGluR2 subtype of AMPA receptors with a number of specific AMPA agonists,[32,33] as well as the atypical AMPA agonist, kainic acid.[34] Whereas the (*S*)-α-amino acid parts of these AMPA agonists show essentially identical binding modes, the substituted 3-isoxazolol units of these compounds interact very differently with amino acid residues and protein backbone components at the receptor recognition site.[32,33]

Analogous studies on active enantiomers of the mGluR ligands **1**, **3**, **5**, and **7** remain to be carried out. However, because a recombinant binding domain of mGluR1 has been crystallized and cocrystallized with Glu,[26] such studies may be performed in the future and may disclose different binding modes for the acidic heterocyclic units of these mGluR ligands.

The docking studies indicate that low energy conformations of (*S*)- and (*R*)-**7** are able to bind to mGluR2 in a similar mode to that predicted for Glu and the specific agonist (−)-**12** (Figure 2C).[31] Because of the geometry of the 1,2,5-thiadiazol-3-ol ring, and in particular the unsubstituted sp^2-hybridized 5-position ring nitrogen, not only (*S*)-**7** but also (*R*)-**7** are able to fold into conformations that are superimposable with (−)-**12**. It is also interesting that this N(5) is able to directly accept a hydrogen bond from the side chain of K377 according to the model. Neither (*S*)-**5** nor (*R*)-**5** are able to achieve these mGluR2 binding conformations at a reasonable energy due to the steric hindrance of the 5-methyl substituent of **5**, although, in a slightly more extended conformation, (*S*)-**5** but not (*R*)-**5** may bind to mGluR6. While the fully flexible (*S*)- and (*R*)-**1** can be docked to the mGluR2 model and can fold to binding conformations similar to those of Glu, (−)-**12**, and **7**, the conformational penalty for folding (*R*)-**1** is significantly greater than for (*S*)-**1** or (*R*)-**7**. This may explain the measurable activity of (*R*)-**7**, but not of (*R*)-**1**, at mGluR2. It is worth noting that the α-amino acid group of (−)-**12** is α,α-disubstituted, indicating that the binding cavity of mGluR2 has space for binding both (*R*)- and (*S*)-α-amino acids, so long as the distal acidic moiety is also capable of achieving a favorable orientation.

It can be concluded that enantiopharmacological studies on homologues of iGluR agonists continue to provide mGluR ligands showing very different pharmacological effects. In particular, homo-AMPA (**5**) has become a standard agonist for the pharmacological characterization of mGluR6.[35]

The results so far indicate that homo-TDPA (**7**) is a group II selective agonist. Although very few ligands tested to date have shown significant selectivity between the subtypes within the three groups of mGluRs, further studies are underway to test these homologues at all mGluR subtypes.

Experimental Section

Chemistry. Thin-layer chromatography (TLC) was performed on silica gel F_{254} plates (Merck). All compounds were detected using UV-light, $KMnO_4$, or ceric sulfate/ammonium molybdate spraying reagents. Amino compounds were developed using ninhydrin. The 1,2,5-thiadiazol-3-ol compounds were developed using a solution of $FeCl_3$ in diluted H_2SO_4. ^{1}H NMR and ^{13}C NMR were recorded on a 300 MHz Varian Gemini spectrometer using $CDCl_3$ or D_2O as solvent with $CDCl_3$ (δ 7.25/77.0) or dioxane (δ 3.75/67.2), respectively, as internal standards. Chemical shifts are given in parts per million (δ), and coupling constants (*J*) are given in Hertz. Column chromatography (CC) was performed using Merck silica gel 60 (0.045−0.063 mm). All solvents and reagents were obtained from Fluka or Aldrich and used as purchased. Elemental analyses were performed at the Analytical Research Department, H. Lundbeck A/S, Denmark, and are within ±0.4 of the theoretical values. Analytical HPLC was performed using a Crownpak CR(−) column (150 mm × 4 mm) column with $HClO_4$ (pH 1.5, 38 °C, 270 nm) as the mobile phase. Preparative HPLC was performed with a CR(+) Crownpak column (150 mm × 10 mm) using 0.1% aqueous TFA as eluent at room temperature. Optical rotations were determined on a Perkin-Elmer 241 Polarimeter.

***tert*-Butyl (2*S*,5*RS*)-5-Amino-2-di(*tert*-butoxycarbonyl)-amino-5-carbamoylpentanoate [(2*S*,5*RS*)-10].** A solution containing potassium cyanide (0.50 g, 7.7 mmol) and ammonium chloride (0.50 g, 9.3 mmol) in 25% aqueous ammonia (7.5 mL) was prepared. *tert*-Butyl (*S*)-2-di(*tert*-butoxycarbonyl)-amino-5-oxopentanoate[17] (2.00 g, 5.16 mmol) in MeOH (10 mL) was added, and the solution was stirred overnight. The product was extracted with Et_2O, and the organic phase was washed with brine followed by evaporation. Crude (2*S*,5*RS*)-**9** was dissolved in tetrahydrofuran (THF)/EtOH (1:3, 20 mL), and to this solution was slowly added a mixture of 35% hydrogen peroxide (1 mL) in 1 M NaOH (30 mL) at 0 °C. The mixture was stirred for 2 h and then extracted with Et_2O. The organic phase was washed with brine and evaporated. Gradient CC on silica using MeOH/DCM (1:20) as the first eluent and NEt_3/MeOH/DCM (1:2:37) as the second eluent gave a (1:1) diastereomeric mixture of (2*S*,5*RS*)-**10** (1.92 g, 86%) as an oil. ^{1}H NMR ($CDCl_3$): δ 7.09/6.94 (1H, 2s (diastereomers)), 6.00 (1H, s), 4.78−4.63 (1H, m), 3.36−3.27 (1H, m), 2.22−2.08 (1H, m), 1.96−1.74 (2H, m), 1.64−1.35 (3H, m), 1.44 (18H, s), 1.38 (9H, s). ^{13}C NMR ($CDCl_3$): δ 178.10, 178.07, 169.78, 169.76, 152.61, 152.53, 82.89, 82.87, 81.3, 58.7, 58.3, 54.8, 54.3, 32.0, 31.6, 27.8, 27.7, 25.8, 25.1.

***tert*-Butyl (*S*)-2-Di(*tert*-butoxycarbonyl)amino-4-(4-hydroxy[1,2,5]thiadiazol-3-yl)butyrate [(*S*)-11].** To a solution of (2*S*,5*RS*)-**10** (1.90 g, 4.9 mmol) in CH_2Cl_2 (50 mL) were added NEt_3 (1.5 mL, 1.09 g, 11 mmol) and thionyl chloride (0.39 mL, 0.64 g, 5.4 mmol) at −78 °C. The reaction was slowly heated to room temperature. After it was stirred for 7 h, Et_2O, H_2O and 1 M TFA were added to the reaction until pH 4. The aqueous phase was extracted with Et_2O. The organic phases were washed with brine, dried (Na_2SO_4), and evaporated. Gradient CC on silica using pentane/Et_2O (1:1 to 1:2) as eluent gave (*S*)-**11** (0.94 g, 46%) as an oil. ^{1}H NMR ($CDCl_3$): δ 4.83 (1H, dd, *J* = 5.2 and 9.5 Hz), 2.93−2.74 (2H, m), 2.63−2.50 (1H, m), 2.37−2.22 (1H, m), 1.47 (18H, s), 1.43 (9H, s). ^{13}C NMR ($CDCl_3$): δ 169.6, 162.7, 152.4, 151.7, 82.9, 81.4, 58.1, 27.8, 27.8, 26.5, 25.7.

(*S*)-2-Amino-4-(4-hydroxy[1,2,5]thiadiazol-3-yl)butyric Acid [(*S*)-7] and (*R*)-2-Amino-4-(4-hydroxy[1,2,5]-thiadiazol-3-yl)butyric Acid [(*R*)-7]. To a solution of (*S*)-**11** (0.88 g, 1.9 mmol) in CH_2Cl_2 (15 mL) was added TFA (15 mL), and the solution was stirred for 2 h. The solution was evaporated, and the residue was evaporated from 1 M HCl twice. HPLC analysis (Crownpak CR(−) 150 mm × 4 mm) revealed a 50% ee of (*S*)-**7**. The white powder was dissolved in H_2O (15 mL). To this solution was added propylene oxide (0.5 mL), and after it was stirred for 30 min, the reaction mixture was filtered. The crystals were washed with H_2O, 2-propanol, and Et_2O. Further crops were gained, and all precipitations were separated by HPLC (Crownpak CR(+) 150 mm × 10 mm) using 0.1% TFA in H_2O as eluent at room temperature. The first eluting TFA salts were pooled, evaporated from 1 M HCl twice, and crystallized from H_2O (1 mL) by adding propylene oxide (0.25 mL), giving a total yield of 30 mg of (*R*)-**7**; $[\alpha]_D^{25}$ −38.1°, $[\alpha]_{578}^{25}$ −39.6° (*c* 0.407, 0.1 M HCl). Anal. ($C_6H_9N_3O_3S$) C, H, N; ee > 99.9% (HPLC, spiked). The second eluting TFA salts were pooled, evaporated from 1 M HCl twice, and crystallized from H_2O (2 mL) by adding 0.5 mL of propylene oxide (0.5 mL) giving a total yield of 125 mg of (*S*)-**7**; $[\alpha]_D^{25}$ +38.2°, $[\alpha]_{578}^{25}$ −40.0° (*c* 0.390, 0.1 M HCl). Anal. ($C_6H_9N_3O_3S$) C, H, N; ee = 99.4% (HPLC). Overall yield of (*R*)- and (*S*)-**7** was 155 mg (40%); mp > 200 °C. NMR data for the HCl salt of **7**: ^{1}H NMR (D_2O): δ 4.19 (1H, t, *J* = 6.6 Hz), 2.82−2.66 (2H, m), 2.30−2.11 (2H, m). ^{13}C NMR (D_2O): δ 172.4, 162.3, 151.1, 52.8, 27.7, 24.6.

Cell Culture. The Chinese hamster ovary (CHO) cells expressing mGluR1α, mGluR2, mGluR4a, and mGluR5a were maintained as described previously.[36−38] The cell lines were grown in a humidified 5% CO_2/95% air atmosphere at 37 °C in Dulbecco's modified Eagle's medium containing a reduced concentration of (*S*)-glutamine (100 mg/mL) and 10% dialyzed fetal calf serum (all GIBCO, Paisley, Scotland). Two days before the inositol phosphate assay, 1.8×10^6 cells were divided into the wells of 48 well plates, and 2 days before the cyclic adenosine 5′-monophosphate (AMP) assay, 1.0×10^6 cells were divided into the wells of 96 well plates.

Measurement of PI Hydrolysis and Cyclic AMP Formation. The mGluR subtypes mGluR1α, mGluR2, mGluR4a,

and mGluR5a were expressed in CHO cell lines. All compounds were tested for agonist and antagonist activity at 1 mM concentrations unless otherwise stated, by the method previously described.[15]

Receptor Binding Assays. Affinities for NMDA, AMPA, and kainic acid receptors were determined using [^{3}H]CGP,[19] [^{3}H]AMPA,[20] and [^{3}H]kainic acid[21] with the modifications previously described.[23] The membrane preparation used in all of the receptor binding experiments was prepared according to the method described by Ransom and Stec.[39] The amount of bound radioactivity was determined using a Packard TOPCOUNT, microplate scintillation counter. Data were analyzed using Grafit 3.0, Leatherbrow software. Data were fitted with the equation: $B = 100 - (100 \times [\text{inhibitor}]^n)/(IC_{50}n + [\text{inhibitor}]^n)$, where B is the binding as percentage of a total specific binding and n is the Hill coefficient.

In Vitro Electrophysiology. A rat cortical preparation[22] in a modified version[23] was used for the determination of the depolarizing effects of the excitatory amino acids analogues under study. Agonists were applied for 90 s. Receptor selectivity was determined by antagonizing responses, approximately corresponding to the EC_{50} values of the compounds in question, 5 μM (*RS*)-(*E*)-2-amino-4-propyl-5-phosphono-3-pentenoic acid (CGP 39653) and 5 and 20 μM 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(*f*)quinoxaline (NBQX) for NMDA, AMPA, and Kainate receptors, respectively. Antagonists were applied for 90 s, followed by a coapplication of agonist and antagonist for 90 s.

Acknowledgment. This work was supported by grants from the Lundbeck Foundation, The Novo Nordisk Foundation, and The Danish Medical Research Council. The secretarial assistance of Anne Nordly is gratefully acknowledged. Professor Shigetada Nakanishi (Kyoto University) is gratefully acknowledged for providing the mGluR expression cell lines.

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JM020122X