J. Med. Chem. **1998,** *41,* 930−939

Excitatory Amino Acid Receptor Ligands: Resolution, Absolute Stereochemistry, and Enantiopharmacology of 2-Amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic Acid

Tommy N. Johansen, Bjarke Ebert, Hans Bräuner-Osborne, Michael Didriksen,† Inge T. Christensen, Karina K. Søby,† Ulf Madsen, Povl Krogsgaard-Larsen,* and Lotte Brehm

PharmaBiotec Research Center, Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark, and Pharmacological Research, H. Lundbeck A/S, 9 Ottiliavej, DK-2500 Valby-Copenhagen, Denmark

Received October 6, 1997

(*RS*)-2-Amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid (Bu-HIBO, **6**) has previously been shown to be an agonist at (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptors and an inhibitor of $CaCl_2$-dependent [^{3}H]-(*S*)-glutamic acid binding (*J. Med. Chem.* **1992**, *35*, 3512−3519). To elucidate the pharmacological significance of this latter binding affinity, which is also shown by quisqualic acid (**3**) but not by AMPA, we have now resolved Bu-HIBO via diastereomeric salt formation using the diprotected Bu-HIBO derivative **11** and the enantiomers of 1-phenylethylamine (PEA). The absolute stereochemistry of (*S*)-Bu-HIBO (**7**) (ee = 99.0%) and (*R*)-Bu-HIBO (**8**) (ee > 99.6%) were established by an X-ray crystallographic analysis of compound **15**, a salt of (*R*)-PEA, and diprotected **8**. Circular dichroism spectra of **7** and **8** were recorded. Whereas **7** (IC_{50} = 0.64 μM) and **8** (IC_{50} = 0.57 μM) were equipotent as inhibitors of $CaCl_2$-dependent [^{3}H]-(*S*)-glutamic acid binding, neither enantiomer showed significant affinity for the synaptosomal (*S*)-glutamic acid uptake system(s). AMPA receptor affinity (IC_{50} = 0.48 μM) and agonism (EC_{50} = 17 μM) were shown to reside exclusively in the *S*-enantiomer, **7**. Compounds **7** and **8** did not interact detectably with kainic acid or *N*-methyl-D-aspartic acid (NMDA) receptor sites. Neither **7** nor **8** affected the function of the metabotropic (*S*)-glutamic acid receptors $mGlu_2$ and $mGlu_{4a}$, expressed in CHO cells. Compound **8** was shown also to be inactive at $mGlu_{1\alpha}$, whereas **7** was determined to be a moderately potent antagonist at $mGlu_{1\alpha}$ (K_i = 110 μM) and $mGlu_{5a}$ (K_i = 97 μM). Using the rat cortical wedge preparation, the AMPA receptor agonist effect of **7** was markedly potentiated by coadministration of **8** at 21 °C, but not at 2−4 °C. These observations together indicate that the potentiation of the AMPA receptor agonism of **7** by **8** is not mediated by metabotropic (*S*)-glutamate receptors but rather by the $CaCl_2$-dependent (*S*)-glutamic acid binding system, which shows the characteristics of a transport mechanism. After intravenous administration in mice, **7** (ED_{50} = 44 μmol/kg) was slightly more potent than AMPA (**1**) (ED_{50} = 55 μmol/kg) and twice as potent as Bu-HIBO (**6**) (ED_{50} = 94 μmol/kg) as a convulsant, whereas **8** was inactive. After subcutaneous administration in mice, Bu-HIBO (ED_{50} = 110 μmol/kg) was twice as potent as AMPA (ED_{50} = 220 μmol/kg) as a convulsant. Since **7** and Bu-HIBO (EC_{50} = 37 μM) are much weaker than AMPA (EC_{50} = 3.5 μM) as AMPA receptor agonists in vitro, the presence of a butyl group in the molecules of Bu-HIBO and **7** seems to facilitate the penetration of these compounds through the blood−brain barrier.

Introduction

(*S*)-Glutamic acid, which is the main excitatory neurotransmitter in the central nervous system (CNS), and other excitatory amino acids (EAAs) operate through four different classes of receptors. In addition to the three heterogeneous classes of ionotropic EAA (iGlu) receptors, named *N*-methyl-D-aspartic acid (NMDA), (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA), and kainic acid receptors,[1−3] a heterogeneous class of metabotropic EAA (mGlu) receptors has been shown to have important functions in the central excitatory neurotransmission processes.[4] It is now generally agreed that iGlu as well as mGlu receptors play important roles in the healthy as well as the diseased CNS and that all subtypes of these receptors are potential targets for therapeutic intervention in a number of diseases.[5,6]

EAA receptors are involved in the mechanisms of long-term potentiation, which is believed to play an important role in learning and memory functions, and the deficits of these functions in Alzheimer patients may, to some extent, be caused by hypoactivity at iGlu and/or mGlu receptors in the brain.[7−10] There also is growing evidence of an implication of EAA receptors in schizophrenia.[11,12] As in Alzheimer's disease (AD), the role of these receptors in the etiology and the clinical manifestations of schizophrenia is still very incompletely understood, but there is evidence to suggest that hypoactivity at EAA receptors also is a factor of impor-

* Corresponding author. Phone: (+45) 35370850, ext. 511. Fax: (+45) 35372209.
† H. Lundbeck A/S.

S0022-2623(97)00673-0 CCC: $15.00
Published on Web 02/24/1998

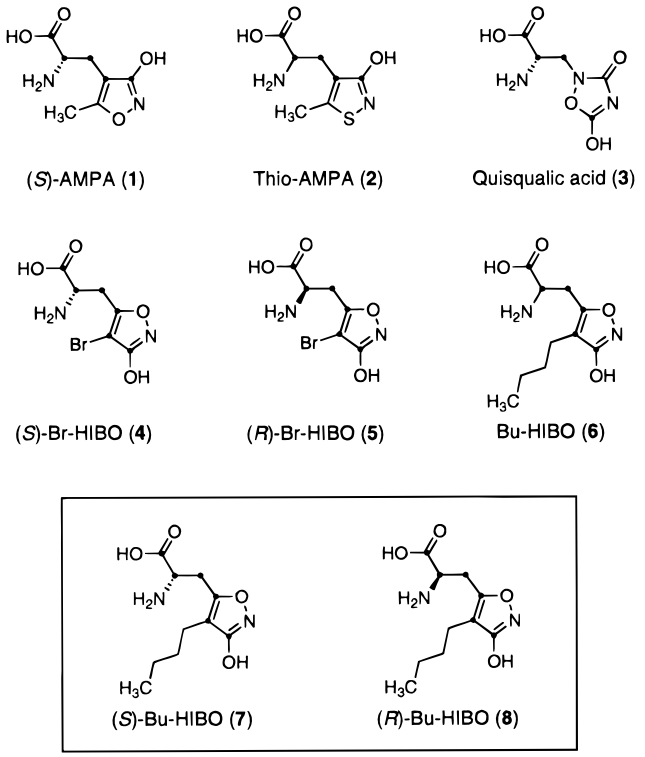


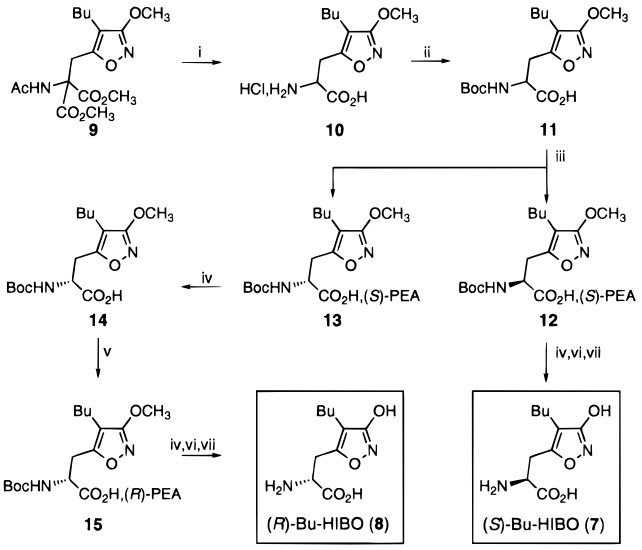
Figure 1. Structures of a number of heterocyclic analogues and homologues of glutamic acid.

tance in the latter CNS disorder.[12−14] In AD as well as schizophrenia, agonists or partial agonists at EAA receptors, including AMPA receptors, may have therapeutic interest, and furthermore, compounds capable of enhancing EAA receptor activation are potential drugs in these disorders.[15]

A large number of specific and full AMPA receptor agonists have been developed,[16,17] including (*S*)-AMPA (**1**)[18,19] and (*RS*)-2-amino-3-(3-hydroxy-5-methylisothiazol-4-yl)propionic acid (Thio-AMPA, **2**)[20] (Figure 1). The naturally occurring amino acid, quisqualic acid (**3**) having the *S*-configuration, is an equally potent AMPA agonist, but this amino acid also interacts with other iGlu receptors,[21] a number of mGlu receptor subtypes,[22] and transport mechanisms associated with EAA-operated synapses.[23] An equally complex pharmacological profile is shown by (*S*)-2-amino-3-(4-bromo-3-hydroxyisoxazol-5-yl)propionic acid [(*S*)-Br-HIBO, **4**], which is an agonist at AMPA receptors[24] and an agonist/antagonist at receptors coupled to phosphoinositide (PI) hydrolysis.[25,26] The AMPA receptor agonism of **4** is enhanced in vitro[27] and in vivo[24] by (*R*)-Br-HIBO (**5**), which is inactive at iGlu and mGlu receptors[24,26] but is equipotent with **4** as an inhibitor of $CaCl_2$-dependent glutamic acid binding.[24] The mechanisms underlying this enhancement effect, of potential therapeutic interest, are very incompletely understood.[24,27]

Since Bu-HIBO (**6**) shows an in vitro pharmacological profile[28] qualitatively and quantitatively very similar to that of Br-HIBO,[21,29] and since the lipophilic butyl group of **6** may facilitate the penetration of this compound through the blood−brain barrier (BBB), the enantiomers of **6** were chosen as model compounds for studies of the mechanisms involved in the enhancement effect of **5** described above. We here report the resolution, determination of absolute stereochemistry, and molecular and in vivo pharmacology of (*S*)-Bu-HIBO (**7**) and (*R*)-Bu-HIBO (**8**).

Scheme 1[a]



[a] Reagents: (i) 2 M HCl; (ii) $(Boc)_2O$, Et_3N; (iii) (*S*)-PEA, EtOH−Et_2O; (iv) extraction; (v) (*R*)-PEA, EtOH−Et_2O; (vi) 48% HBr; (vii) Et_3N.

Results

Resolution. The resolution of Bu-HIBO (**6**) was performed through a diastereomeric salt formation using the diprotected derivative **11**. This key intermediate was prepared from methyl 2-acetamido-3-(4-butyl-3-methoxyisoxazol-5-yl)-2-(methoxycarbonyl)propionate (**9**)[28] through a selective deprotection of the dimethyl acetamidomalonate moiety followed by Boc protection of the primary amino group using di-*tert*-butyl dicarbonate (Scheme 1). A salt primarily consisting of compound **12** was obtained by adding (*S*)-1-phenylethylamine [(*S*)-PEA] to a solution of **11**. After three recrystallizations of the crude product, no change in the optical rotation of **12** was observed, and no sign of the diastereomeric impurity, compound **13**, could be detected in the ^1H NMR spectrum of compound **12** (δ_{OMe} of **12**, 3.90 ppm; δ_{OMe} of **13**, 3.86 ppm). To partly resolved **14**, obtained from the mother liquor of the first precipitation of compound **12**, was then added (*R*)-PEA following a procedure similar to that used for the preparation of **12**. Crude **15** precipitated, and after two recrystallizations no change in optical rotation was seen.

To establish the absolute stereochemistry of **7** and **8**, an X-ray crystallographic analysis of compound **15** was performed. Furthermore, circular dichroism (CD) spectra of **7** and **8** were recorded and compared to those of the respective Br-HIBO enantiomers, **4** and **5**,[24] and to those of the *S*- and *R*-forms of 2-amino-3-(3-hydroxyisoxazol-5-yl)propionic acid (HIBO).[30] The CD spectra of **7** and **8** (Figure 2) are mirror images, demonstrating that the two compounds are enantiomers. In agreement with earlier observations in acidic solution,[31−33] the *S*-form **7** shows a relatively strong positive Cotton effect at 220 nm ($\Delta\epsilon = +0.2$ m^2/mol). The structurally related 3-isoxazolol amino acids, (*S*)-Br-HIBO[24] and (*S*)-HIBO,[30] also show a positive Cotton effect at 220 nm of approximately the same magnitude (not illustrated).

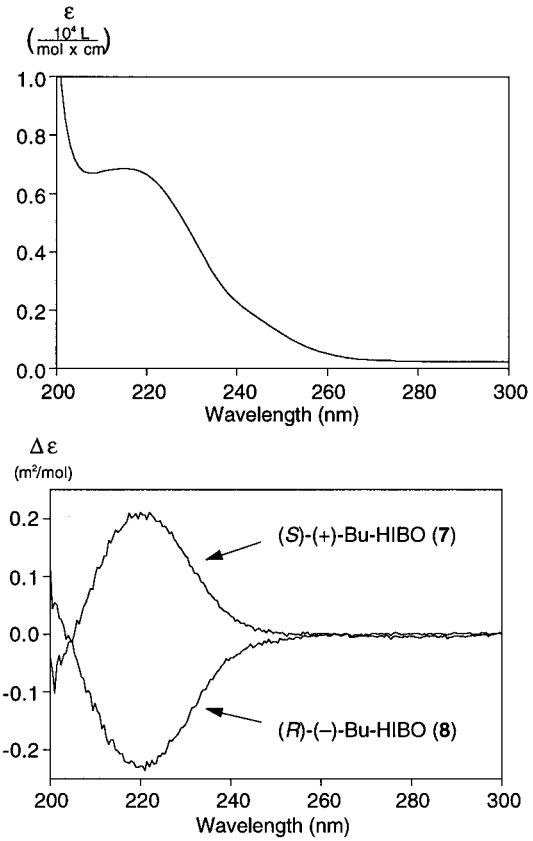


Figure 2. UV spectrum of (*S*)-Bu-HIBO (**7**) (top) and CD spectra of **7** and (*R*)-Bu-HIBO (**8**) (bottom) (c = 0.16 mM, 0.1 M HCl).

Chiral HPLC Analysis. The enantiomeric excess (ee) of (*S*)-Bu-HIBO (**7**) was determined to 99.0% using an (*S*)-proline ligand-exchange column. In agreement with earlier observations using this type of column,[34] (*R*)-Bu-HIBO (**8**) elutes prior to the *S*-enantiomer ensuring accurate quantification of the minor peak. For determination of the stereochemical purity of **8**, the Crownpak CR(−) column was used. As expected,[35] the elution order of the Bu-HIBO enantiomers on this chiral column was reversed as compared to that determined using the (*S*)-proline column. When analyzing a sample of **8** on the Crownpak CR(−) column, no trace of **7** could be seen. However, after spiking the sample with a small amount of Bu-HIBO, the *S*-enantiomer was detectable. The ee of the spiked sample of **8** was 99.6%, indicating that the isolated **8** had a stereochemial purity > 99.6% ee.

X-ray Crystallographic Analysis of Compound 15. The X-ray analysis established the absolute configuration of the N-protected α-amino carboxylate moiety of **15** to be *R*, determined relative to (*R*)-PEA used in the diastereomeric salt formation. A perspective drawing of the molecular structure of **15** with atomic labeling is shown in Figure 3. The molecular packing of **15** is depicted in Figure 4.

Bond lengths and angles of both the anion and cation of **15** are all within expected ranges,[37] the geometry of the 3-methoxyisoxazole moiety being consistent with the general pattern of 3-alkoxy- and 3-hydroxyisoxazoles (ref 38 and references therein). The isoxazole ring is planar within the limits of experimental error. The exocyclic oxygen and carbon atoms O2, C6, and C13 are

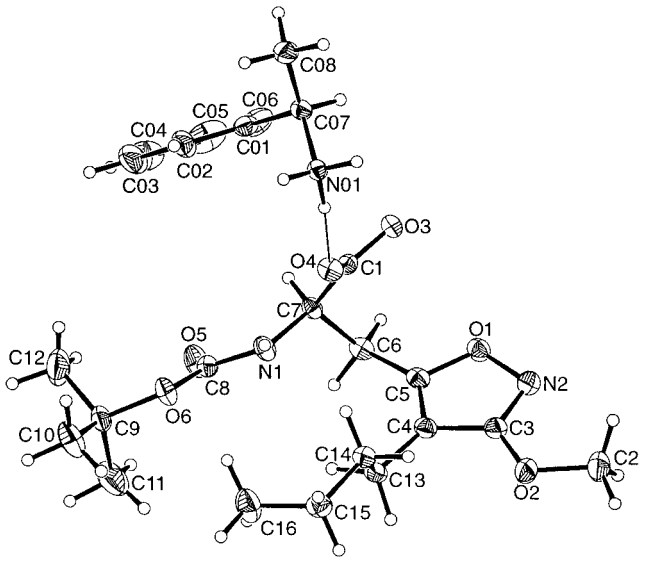


Figure 3. Perspective drawing[36] of compound **15** with atomic labeling of non-hydrogen atoms. Displacement ellipsoids of non-hydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size. A hydrogen bond between the cation and anion is indicated with a thin line.

Table 1. Selected Torsion Angles (deg) for (*R*)-1-Phenylethylammonium (*R*)-3-(4-Butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionate (**15**)[a]

	Anion		
N2−C3−O2−C2	−4.3(2)	C7−N1−C8−O5	14.0(2)
C3−O2−C2−H2$_A$	−175.0(1)	C7−N1−C8−O6	167.3(1)
C4−C5−C6−C7	72.0(2)	N1−C8−O6−C9	−176.2(1)
C1−C7−C6−C5	42.2(2)	O5−C8−O6−C9	2.5(2)
N1−C7−C6−C5	−80.3(2)	C8−O6−C9−C10	−178.0(9)
C5−C6−C7−H7	158.0(1)	C8−O6−C9−C11	64.7(2)
O4−C1−C7−C6	−125.6(1)	C8−O6−C9−C12	−60.4(2)
N1−C7−C1−O4	−0.8(2)	C3−C4−C13−C14	71.0(2)
N1−C7−C1−O3	178.7(1)	C4−C13−C14−C15	−174.1(1)
C6−C7−N1−C8	−88.2(2)	C13−C14−C15−C16	−65.5(2)
C1−C7−N1−C8	147.2(1)		
	Cation		
C06−C01−C07 −N01	162.2(2)	C02−C01−C07−H07	−2.0(1)
C06−C01−C07−C08	−60.0(2)		

[a] Estimated standard derivations are given in parentheses.

at distances of −0.022(2), −0.046(3), and 0.023(2) Å, respectively, from this plane. The conformation of compound **15** is described by selected torsion angles listed in Table 1. An intramolecular hydrogen bond is found in the anion between the amide and carboxylate groups (N1−H1···O4) (Table 2).

The crystal packing (Figure 4 and Table 2) can be described as a collection of infinite anion/cation/anion "sandwiches" along the *a* axis. Within each "sandwich" the ammonium group of a cation is hydrogen-bonded to the carboxylate groups of three symmetry-related anions. Furthermore a few close contacts [C−H···O(N)], which may be classified as hydrogen bonds,[39] are observed within each "sandwich". Between the "sandwiches" only one close contact (C−H···O=C) is observed.

In Vitro Binding Affinities. The affinities of (*S*)-Bu-HIBO (**7**) and (*R*)-Bu-HIBO (**8**) at different recognition sites have been studied using [^{3}H][3-(2-carboxypiperazin-4-yl)propyl]phosphonic acid ([^{3}H]CPP),[40] [^{3}H]-glycine,[41] [^{3}H]AMPA,[42] [^{3}H]kainic acid,[43] and [^{3}H]-(*S*)-glutamic acid[44] (Table 3). Neither **7** nor **8** showed any detectable affinity (IC_{50} > 100 μM) in the [^{3}H]CPP, [^{3}H]-

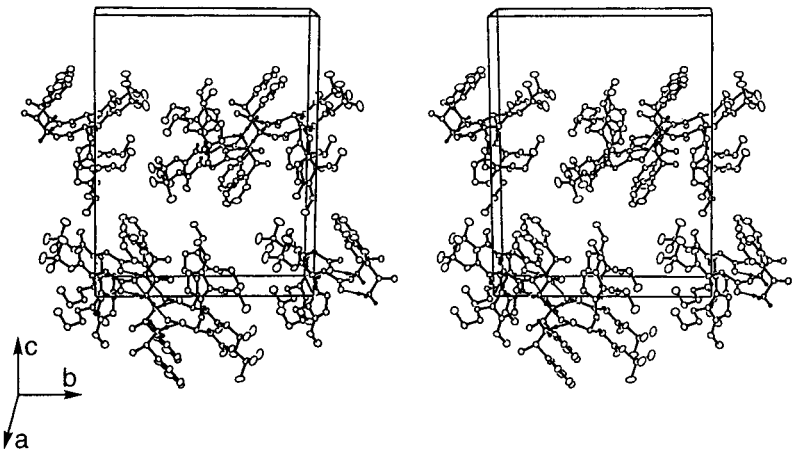


Figure 4. Stereoscopic view[36] of the packing of compound **15**. Displacement ellipsoids of non-hydrogen atoms are shown at the 50% probability level. For clarity only hydrogen atoms (represented by spheres of arbitrary size) bonded to nitrogen atoms are shown. Intermolecular hydrogen bonds are indicated by thin lines.

Table 2. Hydrogen Bond Geometries and Close Contacts (Å, deg) for (*R*)-1-Phenylethylammonium (*R*)-3-(4-Butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionate (**15**)[a,b]

D−H···A	D−H	H···A	D···A	DHA
		Intramolecular		
N1−H1···O4^{i}	0.91(2)	2.13(2)	2.578(2)	109(2)
		Intermolecular		
N01−H01$_A$···O4^{i}	0.98(2)	1.74(2)	2.718(2)	173(2)
N01−H01$_B$···O3^{ii}	0.92(2)	1.89(2)	2.802(2)	171(2)
N01−H01$_C$···O3^{iii}	0.91(2)	1.91(2)	2.808(2)	167(2)
C2−H2$_A$···O5^{iv}	1.02(2)	2.51(2)	3.054(2)	113(2)
C10−H10$_B$···O5^{ii}	0.98(2)	2.52(2)	3.463(3)	162(2)
C07−H07···N2^{iii}	0.97(2)	2.54(2)	3.392(2)	147(1)
C08−H08$_B$···O4^{iii}	0.98(2)	2.54(2)	3.444(2)	153(2)
C06−H06···O3^{iii}	1.08(2)	2.55(2)	3.471(2)	143(2)
O1·····O4^{v}			2.996(2)	

[a] Symmetry codes: (i) x, y, z; (ii) $1 + x, y, z$; (iii) $^1/_2 + x, ^1/_2 - y, -z$; (iv) $^3/_2 - x, -y, -^1/_2 + z$; (v) $-1 + x, y, z$. [b] Estimated standard deviations are given in parentheses.

Table 3. Receptor Binding and Electrophysiological Data [pIC$_{50}$ and pEC$_{50}$ values ± SEM ($n = 3$) in parentheses]

	IC$_{50}$ (μM)		EC$_{50}$ (μM)
compound	[^{3}H]AMPA	[^{3}H]-(*S*)-glutamic acid[a]	cortical wedge
AMPA	0.04[b] (7.41 ± 0.02)	>100	3.5[b] (5.48 ± 0.03)
quisqualic acid (**3**)	0.08 (7.10 ± 0.04)	0.025 (7.60 ± 0.05)	7.3 (5.15 ± 0.09)
Br-HIBO	0.60[c]	0.20[c]	23 (4.65 ± 0.07)
(*S*)-Br-HIBO (**4**)	0.34[c]	0.22[c]	21 (4.67 ± 0.024)
(*R*)-Br-HIBO (**5**)	32[c]	0.15[c]	>1000
Bu-HIBO (**6**)	1.8 (5.76 ± 0.05)	0.63 (6.20 ± 0.05)	37 (4.43 ± 0.02)
(*S*)-Bu-HIBO (**7**)	0.48 (6.33 ± 0.04)	0.64 (6.19 ± 0.05)	17 (4.77 ± 0.024)
(*R*)-Bu-HIBO (**8**)	>1000	0.57 (6.25 ± 0.01)	>1000

[a] In the presence of 2.5 mM $CaCl_2$. [b] Reference 20. [c] Reference 24.

glycine, or [^{3}H]kainic acid binding experiments. **8** did not show any affinity in [^{3}H]AMPA binding (IC$_{50}$ > 100 μM), whereas **7** (IC$_{50}$ = 0.48 μM) was slightly more effective than Bu-HIBO (**6**) (IC$_{50}$ = 1.8 μM) as a displacer of [^{3}H]AMPA binding. In the $CaCl_2$-dependent [^{3}H]-(*S*)-glutamic acid binding assay,[44] **7** and **8** were approximately equipotent (Table 3), thus displaying no stereoselectivity for this site. The effects of AMPA and **6**−**8** on synaptosomal (*S*)-glutamic acid uptake system(s) using [^{3}H]-(*R*)-aspartic acid as a nonmetabolizable substrate were studied following an earlier described procedure.[45] In agreement with published data, the following IC$_{50}$ values for a series of reference compounds were determined: (*S*)-aspartic acid (10 μM), (*R*)-aspartic acid (13 μM), (*S*)-glutamic acid (18 μM), and (*R*)-glutamic acid (>300 μM). Under these test conditions, none of the compounds under study, AMPA and **6**−**8**, showed detectable inhibitory effects (IC$_{50}$ > 300 μM).

In Vitro Electrophysiology. Electrophysiological experiments were performed using the rat cortical wedge preparation.[46,47] In accordance with the affinities in [^{3}H]AMPA receptor binding, **7** was a selective and potent AMPA receptor agonist (EC$_{50}$ = 17 μM) (Figure 5A), whereas **8** was inactive (EC$_{50}$ > 1000 μM) when administered alone (Table 3). The depolarization induced by **7** was completely blocked by the AMPA receptor antagonist 6-nitro-7-sulfamoylbenzo[*f*]quinoxaline-2,3-dione (NBQX) (5 μM) and was insensitive to the NMDA antagonist CPP (5 μM). Coapplication of **8** (100 μM) and **7** at 21 °C resulted in a leftward parallel shift of the dose−response curve to **7** (Figure 5B) yielding an EC$_{50}$ value of 5 μM (pEC$_{50}$ = 5.27 ± 0.077) as compared to 17 μM for **7** alone [pEC$_{50}$ = 4.77 ± 0.024; paired *t*-test, $p < 1\%$ compared with **8** (100 μM) and **7**]. When these experiments were carried out at 2−4 °C, rather than at 21 °C, this enhancement of the depolarizing effect of **7** by coadministration of **8** was markedly reduced (Figure 5C). Thus, at 2−4 °C an EC$_{50}$ value of 15 μM (pEC$_{50}$ = 4.82 ± 0.084) for the depolarization of **7**, administered alone, was determined, whereas this value was only reduced to 12 μM (pEC$_{50}$ = 4.92 ± 0.032; paired *t*-test, nonsignificant) when **7** was coadministered with **8** (100 μM).

Application of **8** (100 μM), 10−15 min after termination of an application of **7** (50 μM), resulted in an NBQX-sensitive and CPP-insensitive response approximately 35% of the maximum response to AMPA. This priming of **8** (100 μM) was stronger when quisqualic acid (100 μM) was used as an agonist instead of **7**. Thus, pretreatment of the slices with 100 μM quisqualic acid resulted in an NBQX-sensitive and CPP-insensitive

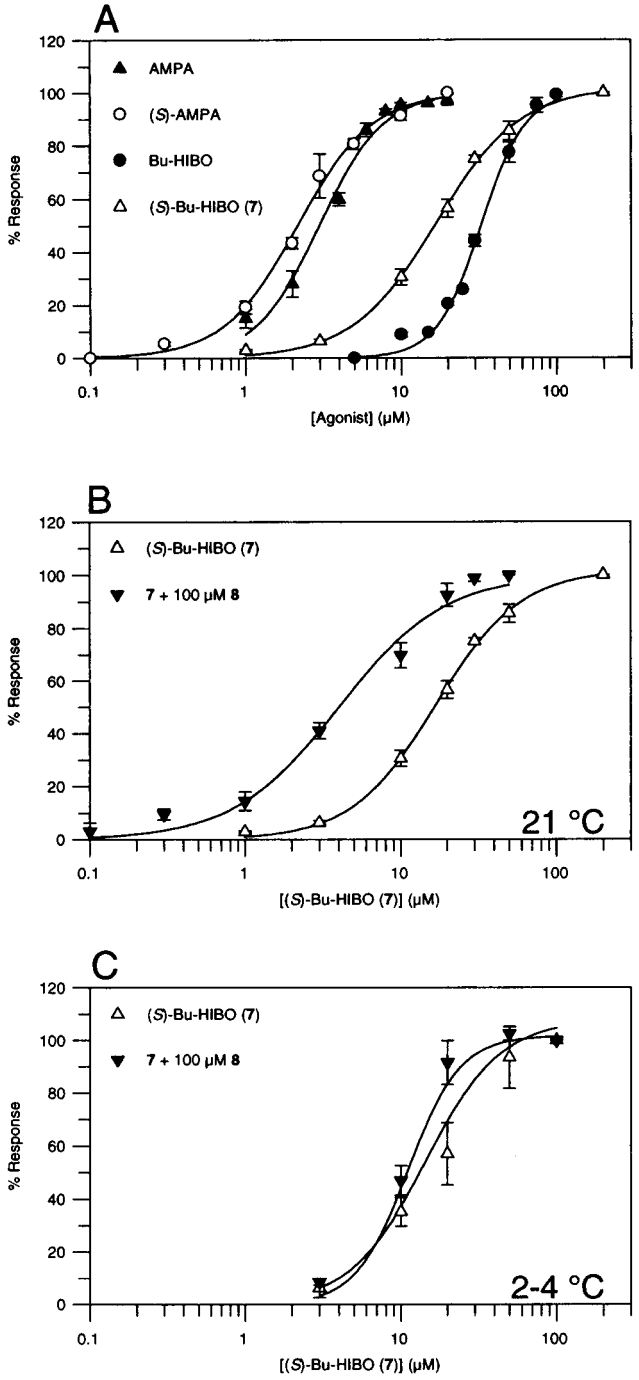


Figure 5. Dose−response curves obtained from the rat cortical wedge preparation for AMPA, (*S*)-AMPA (**1**), Bu-HIBO (**6**), and (*S*)-Bu-HIBO (**7**) at 21 °C (A) and for **7** alone and in the presence of a fixed concentration of (*R*)-Bu-HIBO (**8**) (100 μM) at 21 °C (B) and at 2−4 °C (C).

response close to the maximum response to AMPA (not illustrated).

Activity at mGlu Receptors in Vitro. Based on pharmacology, signal transduction pathway, and sequence homology, the eight mGlu receptor subtypes have been divided into three groups: $mGlu_1/mGlu_5$, $mGlu_2/mGlu_3$, and $mGlu_4/mGlu_6/mGlu_7/mGlu_8$.[4,22] In this study, $mGlu_1$, $mGlu_2$, and $mGlu_4$ were chosen as representative subtypes for these three groups. Initially, we tested **7** and **8** alone and in the presence of (*S*)-glutamic acid (1 mM). As seen in Figure 6, none of the compounds displayed any activity as agonists or

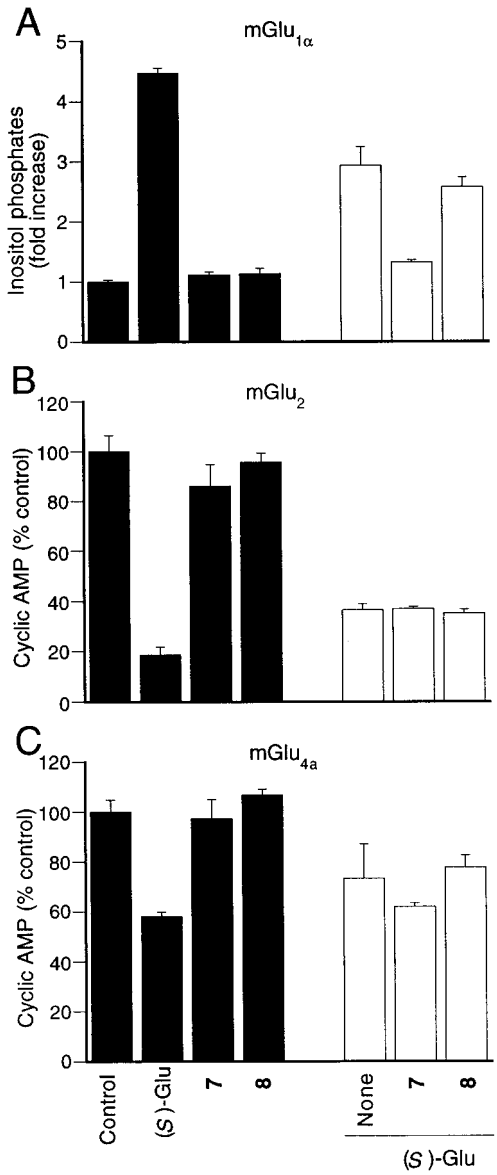


Figure 6. Agonist and antagonist activities at CHO cells expressing $mGlu_{1\alpha}$, $mGlu_2$, and $mGlu_{4a}$. A: In agonist assays, $mGlu_{1\alpha}$-expressing cells were incubated with ligands at a concentration of 1 mM for 20 min. For antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 20 min in the presence of 20 μM (*S*)-Glu. Total IP formation was determined by ion-exchange assay and the fold increase in IP level calculated, compared to control cells (incubated in buffer only). B and C: In agonist assays, $mGlu_2$- and $mGlu_{4a}$-expressing cells were incubated with ligands (1 mM) for 10 min in the presence of 10 μM forskolin. In antagonist assays, cells were preincubated with ligand (1 mM) for 20 min and then incubated with ligand (1 mM) for 10 min in the presence of 20 μM ($mGlu_2$) or 50 μM ($mGlu_{4a}$) (*S*)-Glu. Cyclic AMP levels were measured by an RIA assay (Amersham) and set to 100% in control cells (incubated in buffer only). Data represent means (±SD) of representative experiments performed in triplicate.

antagonists at cell lines expressing $mGlu_2$ or $mGlu_{4a}$. Furthermore, **7** as well as **8** were inactive as agonists at the $mGlu_1$ receptor subtype. When tested as antagonists at the $mGlu_{1\alpha}$-expressing cell line, **7** inhibited the response induced by (*S*)-glutamic acid, whereas **8** had no effect (Figure 6A). As seen in Figure 7, **7** was able to antagonize the response of (*S*)-glutamic acid in a dose-dependent manner with an IC_{50} value of 245 μM

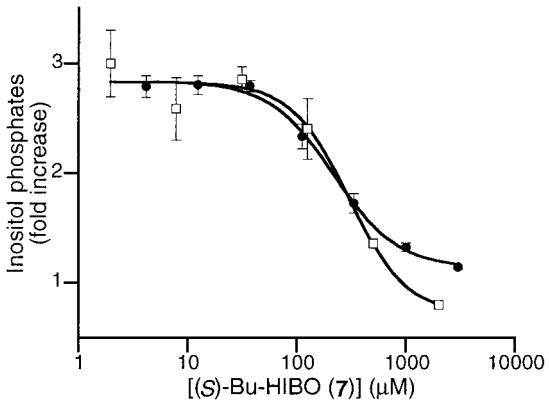


Figure 7. Concentration−response curves for the antagonist (*S*)-Bu-HIBO (**7**) in the presence of 20 or 10 μM (*S*)-Glu at CHO cells expressing $mGlu_{1\alpha}$ (●) or $mGlu_{5a}$ (□), respectively. For further details, see Figure 6.

Table 4. Convulsive Potencies in Mice

compound	ED_{50} (μmol/kg)	
	subcutaneous	intravenous
AMPA	220[a]	55
Bu-HIBO (**6**)	110	94
(*S*)-Bu-HIBO (**7**)	nt	44
(*R*)-Bu-HIBO (**8**)	nt	>350

[a] Reference 20. nt, not tested.

corresponding to a K_i value of 110 μM ($pK_i = 3.96 \pm 0.01$). **7** was also tested for antagonistic effect at $mGlu_{5a}$, which along with $mGlu_{1\alpha}$ forms group I of mGlu receptors. As shown in Figure 7, **7** displayed very similar antagonistic effects at $mGlu_{5a}$ as seen for $mGlu_{1\alpha}$ with an IC_{50} value of 305 μM corresponding to a K_i value of 97 μM ($pK_i = 4.02 \pm 0.04$).

In Vivo Pharmacology. The convulsant activities of AMPA, **6**, and enantiomerically pure **7** and **8** were studied in mice after subcutaneous and/or intravenous administration (Table 4). As compared to AMPA, **6** was the more potent convulsant after subcutaneous administration and the less potent convulsant after intravenous administration. The convulsive potency of **6** was unaffected by the route of adminstration, whereas AMPA was 4 times more potent when given intravenously than when given subcutaneously. Due to limited amounts of compounds, **7** and **8** were only tested intravenously. After this adminstration, **7** was approximately twice as potent as **6**, whereas **8** did not affect the animals even at the highest dose tested (80 mg/kg).

Discussion

The symptoms of Alzheimer and schizophrenic patients may, to some extent, reflect hypoactivity at iGlu and, perhaps, mGlu receptors.[7−14] Rapid agonist-induced desensitization of iGlu receptors, notably AMPA receptors,[48−50] and possible excitotoxic effects[51,52] in vivo of full iGlu receptor agonists may make it difficult to administer such compounds safely to patients.[10,15] On the other hand, compounds capable of sensitizing or of blocking desensitization of iGlu receptors may have therapeutic interest.[53]

Whereas AMPA[29] and a number of AMPA analogues[16,17] specifically activate AMPA receptors as full agonists, other structurally related AMPA agonists derived from the ibotenic acid homologue, HIBO,[16] show more complex pharmacological profiles. Thus, like (*S*)-AMPA (**1**),[18] (*S*)-Br-HIBO (**4**)[24] (Figure 1) is a potent AMPA receptor agonist, but **4** also interacts with mGlu receptors coupled to PI hydrolysis.[26] Whereas (*R*)-AMPA is inactive,[18,19] **4** and (*R*)-Br-HIBO (**5**) are equipotent inhibitors of $CaCl_2$-dependent [^{3}H]-(*S*)-glutamic acid binding (Table 3), and **5** has been shown to enhance excitation produced by **4** and quisqualic acid (**3**), but not that by AMPA.[24,27]

We have now resolved Bu-HIBO (**6**) and characterized the pharmacology of (*S*)-Bu-HIBO (**7**) and (*R*)-Bu-HIBO (**8**) (Figure 1) in vitro and in vivo. No stereoselectivity for $CaCl_2$-dependent [^{3}H]-(*S*)-glutamic acid binding is observed for **7** and **8**. This is in analogy to the results previously obtained for **4** and **5**, both of which show slightly higher affinity for this binding site.[27] Enhancement of **7**-induced depolarizations of rat cortical neurons by the enantiomer **8**, showing no depolarizing effect per se, was observed, and this effect was subjected to further characterization. The pronounced enhancement effect was demonstrated using a fixed concentration of **8** (100 μM), which increased the potency of **7** more than 3-fold (Figure 5B). Comparison of the dose−response curves for **7** and the racemic compound **6** reveals that the enhancement effect is concentration-dependent, since at low concentrations **7** was more potent than the racemate **6**, whereas **6** and **7** became approximately equipotent at higher concentrations (Figure 5A). Furthermore, this enhancement effect of **8** turned out to be temperature-dependent, as lowering of the test temperature from 21 °C (Figure 5B) to 2−4 °C (Figure 5C) virtually eliminated the enhancement. This latter observation is in agreement with the general behavior of uptake/transport mechanisms.

Whereas the degree of selectivity of the interaction of (*S*)-Br-HIBO (**4**) with mGlu receptors coupled to PI hydrolysis is unclear,[25,26] **7** was shown to antagonize exclusively the group I mGlu receptor, $mGlu_{1\alpha}$, without affecting detectably the group II and III mGlu receptors, $mGlu_2$ and $mGlu_{4a}$, respectively (Figure 6A). Like Br-HIBO,[21] neither **7** nor **8** showed significant effects on the synaptosomal (*S*)-glutamic acid uptake system, which is labeled by [^{3}H]-(*R*)-aspartic acid.

Since **8** does not interact detectably with the synaptosomal uptake system(s) generally accepted to be responsible for the removal of neurotransmitter (*S*)-glutamic acid from the synaptic cleft[45,54] or with any of the iGlu or mGlu receptors studied, it seems unlikely that these synaptic mechanisms are involved in the enhancement phenomenon observed for **8** (Figure 5B). As tentatively proposed for the enantiomers of Br-HIBO,[27] it may be concluded that the $CaCl_2$-dependent (*S*)-glutamic acid binding system mediates this enhancement and that this system represents an energy-requiring presynaptic transport mechanism of as yet unknown physiological relevance. We envisage that quisqualic acid (**3**) and both enantiomers of Br-HIBO and Bu-HIBO are substrates for this transport mechanism. The AMPA receptor-active compounds **3**,[16] (*S*)-Br-HIBO (**4**),[24] and (*S*)-Bu-HIBO (**7**) (Table 3) may be taken up in this transport system and/or associated vesicular structures and released by subsequent treatment with the receptor-inactive enantiomers (*R*)-Br-

HIBO (**5**) and (*R*)-Bu-HIBO (**8**). Such mechanisms may explain the priming effects of **3** and **7**, whereas **8** showed excitatory effects after pretreatment with **3** or **7**. This effect of **8** was more pronounced after pretreatment with **3**, which shows higher affinity for the $CaCl_2$-dependent [^{3}H]-(*S*)-glutamic acid binding site as compared to **7** (Table 3).

We have previously shown that Thio-AMPA (**2**) is more active than AMPA after systemic administration in mice.[20] This observation probably reflects that **2** penetrates the BBB more easily due to the lipophilic character of the sulfur atom and the higher pK_a value of the isothiazol-3-ol nucleus of **2** (7.00) than that of the 3-isoxazolol nucleus of AMPA (5.12).[20] We anticipated that the presence of a butyl group in the molecules of **6**−**8** would facilitate the penetration of these compounds through the BBB. We have shown that although **6** and **7** are markedly weaker than AMPA as AMPA agonists (Table 3), **7** is slightly more potent and **6** slightly weaker than AMPA as convulsants after intravenous administration in mice. Compound **8**, which is assumed to penetrate the BBB to approximately the same extent as **6** and **7**, was inactive in this animal model (Table 4). In light of the proposed mechanism of action of **8**, this compound may, however, be able to produce gentle facilitation of the central (*S*)-glutamic acid neurotransmission system, but more detailed animal behavioral studies of compounds **7** and **8** must await the synthesis of further quantities of compound.

Experimental Section

Chemistry. General Procedures. Thin-layer chromatography (TLC) was performed on silica gel F_{254} plates (Merck). Evaporations were performed under vacuum on a rotary evaporator at approximately 15 mmHg. Light petroleum was the fraction boiling at 40−60 °C. Melting points were determined in capillary tubes and are not corrected. Elemental analyses were performed by Mr. G. Cornali, Microanalytical Laboratory, LEO Pharmaceutical Products, Copenhagen, or by Mr. P. Hansen, Department of General and Organic Chemistry, University of Copenhagen, and are within ±0.4% of the theoretical values. ^{1}H NMR spectra were recorded on a Bruker AC-200 F spectrometer. TMS or 1,4-dioxane (δ 3.70) was used as internal standard for spectra recorded in $CDCl_3$ or D_2O, respectively. *J* values are given in Hz. Optical rotations were measured in thermostated cuvettes on a Perkin-Elmer 241 polarimeter. UV and CD spectra were recorded at room temperature in 1.0-cm cuvettes on a Perkin-Elmer Lambda 2 spectrophotometer and on a Jasco J-720 spectropolarimeter, respectively.

(*RS*)-2-Amino-3-(4-butyl-3-methoxyisoxazol-5-yl)propionic Acid Hydrochloride (10). A suspension of methyl 2-acetamido-3-(4-butyl-3-methoxyisoxazol-5-yl)-2-(methoxycarbonyl)propionate (**9**)[28] (2.3 g, 6.46 mmol) in aqueous hydrochloric acid (100 mL, 2 M) was refluxed for 24 h. The resulting solution was evaporated and reevaporated from toluene (tol). The residue was crystallized (AcOH−Et_2O) to give **10** (1.55 g, 86%, hygroscopic): mp 146−148 °C dec; ^{1}H NMR (D_2O) δ 4.28 (t, *J* = 6.1, 1H), 3.89 (s, 3H), 3.31 (d, *J* = 6.1, 2H), 2.25 (t, *J* = 7.4, 2H), 1.3−1.5 (m, 2H), 1.1−1.3 (m, 2H), 0.82 (t, *J* = 7.2, 3H). Anal. ($C_{11}H_{19}ClN_2O_4\cdot 0.75H_2O$) C, H, Cl, N.

(*RS*)-3-(4-Butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionic Acid (11). To a solution of **10** (1.0 g, 3.59 mmol) and Et_3N (1.75 mL, 12.6 mmol) in H_2O (30 mL) was added a solution of di-*tert*-butyl dicarbonate (1.05 mL, 4.56 mmol) in THF (30 mL). After the mixture was stirred for 72 h at room temperature, THF was evaporated, and the aqueous mixture was cooled, acidified with hydrochloric acid (pH ∼ 3), and immediately extracted with AcOEt (3 × 60 mL). The combined organic phases were dried, filtered, and evaporated. The residue was recrystallized (AcOEt−light petroleum) to give **11** (697 mg). The mother liquor furnished an additional 336 mg of recrystallized (cyclohexane) **11**. Total amount of **11** 1.03 g (84%): mp 88−91 °C; ^{1}H NMR ($CDCl_3$) δ 5.38 (d, *J* = 8.0, 1H), 4.59 (m, 1H), 3.96 (s, 3H), 3.24 (m, 2H), 2.22 (t, *J* = 7.4, 2H), 1.55−1.15 and 1.43 (m, 4H and s, 9H), 0.90 (t, *J* = 7.0, 3H). Anal. ($C_{16}H_{26}N_2O_6$) C, H, N.

(*S*)-1-Phenylethylammonium (*S*)-3-(4-Butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionate (12). To a solution of **11** (1.51 g, 4.41 mmol) in EtOH (5 mL) were added (*S*)-(−)-PEA (589 μL, 4.62 mmol) and subsequently Et_2O (5 mL). On standing at room temperature, **12** precipitated. The crystals formed were collected and dried in vacuo. The salt was recrystallized three times from a mixture of EtOH and Et_2O (1:1) to give **12** (690 mg, 34%): mp 174−176 °C; $[\alpha]^{25}_D$ +22.4° (*c* = 1.04, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.32 (m, 5H), 5.69 (d, *J* = 6.1, 1H), 4.28 (q, *J* = 6.8, 1H), 4.02 (q, *J* = 6, 1H), 3.90 (s, 3H), 3.07 (dd, *J* = 6, 14, 1H), 2.61 (dd, *J* = 6, 14, 1H), 2.19 (t, *J* = 7.1, 2H), 1.48 (d, *J* = 6.8, 3H), 1.45−1.15 and 1.40 (m, 4H and s, 9H), 0.90 (t, *J* = 6.9, 3H). Anal. ($C_{24}H_{37}N_3O_6$) C, H, N.

(*R*)-1-Phenylethylammonium (*R*)-3-(4-Butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionate (15). The mother liquor from a first crystallization of a batch of **12** was evaporated. The residue consisted of a 4:1 mixture of the diastereomeric salts **13** and **12**, respectively: ^{1}H NMR ($CHCl_3$) δ_{OMe} 3.86 (compound **13**), 3.90 (compound **12**). The mixture of the diastereomeric salts (437 mg, 0.94 mmol) was dissolved in $CHCl_3$ (40 mL), and H_2O (25 mL) and AcOH (3 mL) were added. The phases were separated, and the aqueous phase was extracted with $CHCl_3$ (40 mL). The combined organic phases were dried, filtered, evaporated, and reevaporated from tol. The partly resolved **14** crystallized by addition of light petroleum. The resulting crystals of **14** (238 mg, 0.69 mmol) were collected by filtration, dried in vacuo, and processed as described above for **12** using (*R*)-(+)-PEA (100 μL, 0.78 mmol). Crude **15** was recrystallized twice from EtOH and Et_2O (1:1) to give 180 mg of **15** (0.39 mmol, 57%): mp 175−176 °C; $[\alpha]^{25}_D$ −22.0° (*c* = 1.00, MeOH). The ^{1}H NMR spectrum of **15** was virtually identical with that of **12**. Anal. ($C_{24}H_{37}N_3O_6$) C, H, N.

(*S*)-(+)-2-Amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic Acid Hemihydrate [(*S*)-Bu-HIBO, 7]. The salt **12** (650 mg, 1.40 mmol) was dissolved in $CHCl_3$ (50 mL) and treated twice with a mixture of H_2O (25 mL) and AcOH (4 mL). The organic phase was dried, filtered, evaporated, and reevaporated from tol. The residue was dissolved in 48% aqueous hydrobromic acid (10 mL) and refluxed for 30 min. After evaporation and reevaporation (twice) from H_2O, the residue was dissolved in H_2O (3 mL), and Et_3N was added until pH ∼ 3−4. Compound **7** (131 mg, 39%) was precipitated by addition of EtOH (10 mL). Recrystallization from H_2O gave the hemihydrate of **7** (85 mg, 25%): mp 227 °C dec; ee = 99.0%; $[\alpha]^{25}_D$ +19.0° (*c* = 0.58, 1 M HCl); $\Delta\epsilon$ (220 nm) = +0.2 m^2/mol; ^{1}H NMR [D_2O−CF_3COOH (19:1)] δ 4.41 (t, *J* = 6.3, 1H), 3.34 (d, *J* = 6.3, 2H), 2.26 (t, *J* = 7.7, 2H), 1.41 (m, 2H), 1.26 (m, 2H), 0.83 (t, *J* = 7.2, 3H). Anal. ($C_{10}H_{16}N_2O_4\cdot 0.5H_2O$) C, H, N.

(*R*)-(−)-2-Amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic Acid Hemihydrate [(*R*)-Bu-HIBO, 8]. Compound **8** was obtained from **15** (480 mg, 1.04 mmol) by a method analogous to that used for the preparation of **7**. Precipitated **8** (121 mg, 51%) was recrystallized from H_2O to give the hemihydrate of **8** (90 mg, 37%): mp 236 °C dec; ee > 99.6%; $[\alpha]^{25}_D$ −21.1° (*c* = 0.52, 1 M HCl); $\Delta\epsilon$ (220 nm) = −0.2 m^2/mol. The ^{1}H NMR spectrum of **8** was virtually identical with that of **7**. Anal. ($C_{10}H_{16}N_2O_4\cdot 0.5H_2O$) C, H, N.

Determination of Enantiomeric Purity. Chiral HPLC using a Crownpak CR(−) column (4.0 × 150 mm; Daicel) was performed at 37 °C. The column was eluted with 0.4 mL/min aqueous $HClO_4$ (pH 2.0). The HPLC instrumentation used consisted of a Jasco 880 pump, a Rheodyne 7125 injector equipped with a 20 μL loop, and a Waters M480 UV detector,

set at 210 nm, attached to a Merck-Hitachi D-2000 chromato-integrator. Chiral ligand-exchange HPLC was performed on a column (4.6 × 120 mm) containing Cu^{2+}-complexed (*S*)-proline as chiral selector. The silica-based packing material was synthesized following a published procedure.[34] The column was connected to an HPLC instrumentation consisting of a Waters M510 pump, a Waters UK6 injector, and a Waters M480 UV detector, set at 210 nm, attached to a Merck-Hitachi D-2000 chromato-integrator. The column was thermostated at 50 °C and eluted at 1.0 mL/min with aqueous KH_2PO_4 (50 mM, pH 4.6)/2-propanol (4:1). All compounds injected were dissolved in H_2O (approximately 0.2 mg/mL).

X-ray Analysis of (*R*)-1-Phenylethylammonium (*R*)-3-(4-Butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionate (15). Crystal data: $C_{24}H_{37}N_3O_6$, M_r = 463.57; colorless needles (2-propanol); orthorhombic, space group $P2_12_12_1$ (No. 19); a = 6.446(2) Å, b = 17.506(3) Å, c = 22.465(6) Å; V = 2535(1) Å^3, Z = 4, D_c = 1.215 Mg m^{-3}; $F(000)$ = 1000, μ(Cu $K\alpha$) = 0.714 mm^{-1}, T = 122 K; crystal size 0.11 × 0.13 × 0.30 mm.

Data Collection and Processing. Diffraction data were collected at low temperature (122 ± 0.5 K) on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated Cu $K\alpha$ radiation (λ = 1.541 84 Å).[55] Unit cell dimensions were determined by least-squares refinement of the setting angles of 20 reflections (θ: 37−42°). Intensity data were collected in the $\omega/2\theta$ scan mode ($\theta \leq 75°$, hkl, $h-k-l$, and $-h-k-l$). The intensities of five reflections monitored every 10^4 s showed an average decrease in the intensities of 8%. The orientation of the crystal was checked every 600 reflections. Data were reduced using the data reduction package DREADD.[56,57] The data were corrected for Lorentz and polarization effects and decay, but no absorption correction was made. A total of 8331 reflections were averaged (R_{int} = 0.016) according to the point-group symmetry 222, resulting in 5196 unique reflections. The number of reflections with $F^2 > 2\sigma(F^2)$ is 4801.

Structure Determination and Refinement. The structure was solved by direct methods using SHELXS-86.[58] Full-matrix least-squares refinements (SHELXL-93)[59] were performed on F^2 minimizing $\sum w(F_o^2 - F_c^2)^2$ with anisotropic displacement parameters for the non-H atoms. The positions of all the H atoms were located in subsequent difference electron density maps. Positional and isotropic displacement parameters were refined for all the H atoms, except for the methyl H atoms of the *tert*-butyl group, since a couple of these H atoms tended to give unreasonable geometries. The H atoms of the *tert*-butyl group were included in idealized positions (riding model) with fixed isotropic displacement parameters equal to 1.5 U_{eq} for the parent C atom.

The refinement (5196 all unique data, 413 parameters) converged at R_F = 0.0381, wR_{F^2} = 0.0837; $w^{-1} = \sigma^2(F_o^2) + (0.0421P)^2 + 0.3141P$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.033. The final shift/esd was 0.000. Min and max values in the final difference electron density map were −0.20 and 0.19 e Å^{-3}. The *R*-configuration of the protected α-amino acid moiety was determined relative to the known (*R*)-PEA. The absolute configuration was supported by refinement of the Flack absolute structure parameter ϵ in the final structure factor calculation ϵ = 0.00(14).[60] The large standard deviation of ϵ is due to the atoms present in the compound (C, H, N, O), which produce minor anomalous scattering. Complex neutral-atom scattering factors were taken from ref 61 and used as contained in SHELXL-93.[59]

Receptor Binding. Affinity for NMDA, AMPA, and kainic acid receptor sites was determined using the ligands [^{3}H]-CPP,[40] [^{3}H]glycine,[41] [^{3}H]AMPA,[42] and [^{3}H]kainic acid,[43] respectively. The membrane preparations used in all four receptor binding experiments were prepared according to the method of Ransom and Stec.[62] [^{3}H]-(*S*)-Glutamic acid receptor binding was performed as described previously.[44]

In Vitro Electrophysiology. A rat cortical wedge preparation for determination of EAA-evoked depolarizations described by Harrison and Simmonds[46] was used in a modified version, as described previously.[47]

Cell Culture. The Chinese hamster ovary (CHO) cell lines expressing $mGlu_{1\alpha}$, $mGlu_2$, $mGlu_{4a}$, and $mGlu_{5a}$ were maintained as described previously.[63−66] The cell lines were grown in a humidified 5% CO_2/95% air atmosphere at 37 °C in DMEM containing a reduced concentration of (*S*)-glutamine (2 mM), 1% (*S*)-proline, penicillin (100 U/mL), streptomycin (100 mg/mL), and 10% dialyzed fetal calf serum (all GIBCO, Paisley, Scotland). Two days before assay 1.8 × 10^6 cells were divided into the wells of 24-well plates.

Measurement of PI Hydrolysis and Cyclic AMP Formation. PI hydrolysis was measured essentially as described previously.[67,68] Briefly, the cells were labeled with [^{3}H]inositol (2 μCi/mL) 24 h prior to the assay. For agonist assay, the cells were incubated with ligand dissolved in PBS−LiCl for 20 min, and agonist activity was determined by measurement of ^{3}H-labeled mono-, bis-, and trisinositol phosphates by ion-exchange chromatography. For antagonist assay, the cells were preincubated with the ligand dissolved in PBS−LiCl for 20 min prior to incubation with ligand and 20 μM ($mGlu_{1\alpha}$) or 10 μM ($mGlu_{5a}$) (*S*)-glutamic acid. The antagonist effect was then determined as the inhibitory effect of the (*S*)-glutamic acid-mediated response. K_i values were calculated from IC_{50} values by the use of the Cheng−Prusoff equation.[69] The assay of cyclic AMP formation was performed as described previously.[67,68] Briefly, the cells were incubated for 10 min in PBS containing the ligand, 10 μM forskolin, and 1 mM 3-isobutyl-1-methylxanthine (IBMX) (both Sigma Chemicals, St. Louis, MO). The agonist activity was then determined as the inhibitory effect of the forskolin-induced cyclic AMP formation. For antagonist assay, the cells were preincubated with ligand dissolved in PBS containing 1 mM IBMX for 20 min prior to a 10-min incubation in PBS containing the ligand (1 mM), (*S*)-glutamic acid [20 μM ($mGlu_2$) or 50 μM ($mGlu_{4a}$)], forskolin (10 μM), and IBMX (1 mM). All experiments were performed at least twice in triplicate.

Synaptosomal (*S*)-Glutamic Acid Uptake. The experiments were carried out essentially as described.[45] In short: Rat cortical synaptosomes were prepared from male Sprague−Dawley rats (200−300 g) using a previously described method.[70] The final synaptosomal pellet was suspended in assay buffer at a final concentration of 2 mg of original tissue/mL. Uptake of [^{3}H]-(*R*)-aspartic acid was measured in a buffer containing 128 mM NaCl, 10 mM glucose, 5 mM KCl, 1.5 mM NaH_2PO_4, 1.77 mM $CaCl_2$, 1 mM $MgSO_4$, and 10 mM Tris (pH 7.4). Following a 5-min preincubation of the synaptosomes, the assay was initiated by adding 200 μL of membrane suspension to microtiter plates containing 25 μL of test substance and 25 μL of 3 μM [^{3}H]-(*R*)-aspartic acid [3 μM nonradioactive (*R*)-aspartic acid plus 30 nM [^{3}H]-(*R*)-aspartic acid]. Total binding was determined in the presence of buffer, and nonspecific binding was determined in the presence of 1 mM (*R*)-aspartic acid. Following 2 min of incubation, the reaction mixture was filtered through a 96-well Whatman GF/B filter using the Packard filtermate system and rinsed with 3 × 250 μL of ice-cold buffer. Radioactivity was determined using the Packard topcount system.

In Vivo Convulsant Activity. Convulsant activity was determined in male mice (NMRI/BOM, SPF, Bomholtgaard, Denmark) weighing 24−26 g. The mice were kept in groups of 15 in plastic cages (40 × 25 × 15 cm). The compounds were dissolved in saline and administered subcutaneously or intravenously. AMPA was given intravenously as a hydrobromide salt in doses of 1.25, 5, and 20 mg/kg. Bu-HIBO (**6**), also as a hydrobromide salt,[28] was given in doses of 10, 20, and 40 mg/kg (subcutaneous) or 1.25, 5, 20, and 40 mg/kg (intravenous). (*R*)-Bu-HIBO (**8**) was given in doses of 40 and 80 mg/kg (intravenous) and (*S*)-Bu-HIBO (**7**) in doses of 5, 20, and 40 mg/kg (intravenous). Each group consisted of 5−10 mice. The animals were observed for 30 min for the presence or absence of clonic/tonic convulsions. ED_{50} values were calculated by log−probit analysis.

Acknowledgment. This work was supported by grants from the Danish State Biotechnology Program

(1991−1995), the Lundbeck Foundation, H. Lundbeck A/S, the Alfred Benzon Foundation, and the Danish Natural Science Research Council. Professor Shigetada Nakanishi, Kyoto University, Japan, is gratefully acknowledged for his kind gift of cell lines expressing mGlu receptors. The technical assistance of Mr. Flemming Hansen, Mrs. Karen Jørgensen (both University of Copenhagen), Mr. Jørgen S. Johansen, and Ms. Heidi Petersen and the secretarial assistance of Mrs. Anne-Mette Nielsen and Mrs. Anne Nordly are gratefully acknowledged.

Supporting Information Available: For (*R*)-1-phenylethylammonium (*R*)-3-(4-butyl-3-methoxyisoxazol-5-yl)-2-[(*tert*-butyloxycarbonyl)amino]propionate (**15**), tables listing final atomic coordinates, equivalent isotropic (non-H atoms) or isotropic (H atoms) displacement parameters, anisotropic displacement parameters for the non-H atoms, and selected bond lengths and angles (6 pages); a list of structure factors (10 pages). Ordering information is given on any current masthead page.

References

(1) Krogsgaard-Larsen, P., Hansen, J. J., Eds. *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*; Ellis Horwood: Chichester, 1992.
(2) Collingridge, G. L., Watkins, J. C., Eds. *The NMDA Receptor*; Oxford University Press: Oxford, 1994.
(3) Wheal, H. V., Thomson, A. M., Eds. *Excitatory Amino Acids and Synaptic Transmission*; Academic Press: London, 1995.
(4) Conn, P. J., Patel, J., Eds. *The Metabotropic Glutamate Receptors*; Humana Press: Totowa, New Jersey, 1994.
(5) Danysz, W.; Parsons, C. G.; Bresink, I.; Quack, G. Glutamate in CNS disorders. *Drug News Perspect.* **1995**, *8*, 261−277.
(6) Knöpfel, T.; Kuhn, R.; Allgeier, H. Metabotropic glutamate receptors: Novel targets for drug development. *J. Med. Chem.* **1995**, *38*, 1417−1426.
(7) Deutsch, S. I.; Morihisa, J. M. Glutamatergic abnormalities in Alzheimer's disease and a rationale for clinical trials with L-glutamate. *Clin. Neuropharmacol.* **1988**, *11*, 18−35.
(8) Greenamyre, J. T.; Young, A. B. Excitatory amino acids and Alzheimer's disease. *Neurobiol. Ag.* **1989**, *10*, 593−602.
(9) Bowen, D. M. Treatment of Alzheimer's disease. Molecular pathology versus neurotransmitter-based therapy. *Br. J. Psychiat.* **1990**, *157*, 327−330.
(10) Madsen, U.; Ebert, B.; Krogsgaard-Larsen, P. Modulation of AMPA receptor function in relation to glutamatergic abnormalities in Alzheimer's disease. *Biomed. Pharmacother.* **1994**, *48*, 305−311.
(11) Carlsson, M.; Carlsson, A. Interactions between glutamatergic and monoaminergic systems within the basal ganglia − implications for schizophrenia and Parkinson's disease. *Trends Neurosci.* **1990**, *13*, 272−276.
(12) Ulas, J.; Cotman, C. W. Excitatory amino acid receptors in schizophrenia. *Schizophrenia Bull.* **1993**, *19*, 105−117.
(13) Deutsch, S. I.; Mastropaolo, J.; Schwartz, B. L.; Rosse, R. B.; Morihisa, J. M. A "glutamatergic hypothesis" of schizophrenia. Rationale for pharmacotherapy with glycine. *Clin. Neuropharmacol.* **1989**, *12*, 1−13.
(14) Drejer, J. Therapeutic opportunities in modulators of excitatory amino acid-mediated neurotransmission. In *Excitatory Amino Acid Receptors: Design of Agonists and Antagonists*; Krogsgaard-Larsen, P., Hansen, J. J., Eds.; Ellis Horwood: Chichester, 1992; pp 352−375.
(15) Ebert, B.; Søby, K. K.; Madsen, U.; Krogsgaard-Larsen, P. Glutamic acid receptors in schizophrenia and Alzheimer's disease: Functional partial agonism and receptor modulation as potential therapeutic approaches. In *Schizophrenia − An Integrated View*; Fog, R., Gerlach, J., Hemmingsen, R., Eds.; Munksgaard: Copenhagen, 1995; pp 379−398.
(16) Krogsgaard-Larsen, P.; Ebert, B.; Lund, T. M.; Bräuner-Osborne, H.; Sløk, F. A.; Johansen, T. N.; Brehm, L.; Madsen, U. Design of excitatory amino acid receptor agonists, partial agonists and antagonists: Ibotenic acid as a key lead structure. *Eur. J. Med. Chem.* **1996**, *31*, 515−537.
(17) Sløk, F. A.; Ebert, B.; Lang, Y.; Krogsgaard-Larsen, P.; Lenz, S. M.; Madsen, U. Excitatory amino acid receptor agonists. Synthesis and pharmacology of analogues of 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid. *Eur. J. Med. Chem.* **1997**, *32*, 329−338.
(18) Hansen, J. J.; Lauridsen, J.; Nielsen, E.; Krogsgaard-Larsen, P. Enzymic resolution and binding to rat brain membranes of the glutamic acid agonist, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. *J. Med. Chem.* **1983**, *26*, 901−903.
(19) Hansen, J. J. Stereoselectivity of excitatory amino acid receptors: Enzymatic resolution of AMPA receptor agonists. *Curr. Top. Med. Chem.* **1993**, *1*, 377−385.
(20) Matzen, L.; Engesgaard, A.; Ebert, B.; Didriksen, M.; Frølund, B.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. AMPA receptor agonists: Synthesis, protolytic properties, and pharmacology of 3-isothiazolol bioisosteres of glutamic acid. *J. Med. Chem.* **1997**, *40*, 520−527.
(21) Nielsen, E. Ø.; Madsen, U.; Schaumburg, K.; Brehm, L.; Krogsgaard-Larsen, P. Studies on receptor-active conformations of excitatory amino acid agonists and antagonists. *Eur. J. Med. Chem. − Chim. Ther.* **1986**, *21*, 433−437.
(22) Conn, P. J.; Pin, J.-P. Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205−237.
(23) Hansen, J. J.; Krogsgaard-Larsen, P. Structural, conformational, and stereochemical requirements of central excitatory amino acid receptors. *Med. Res. Rev.* **1990**, *10*, 55−94.
(24) Hansen, J. J.; Nielsen, B.; Krogsgaard-Larsen, P.; Brehm, L.; Nielsen, E. Ø.; Curtis, D. R. Excitatory amino acid agonists. Enzymic resolution, X-ray structure, and enantioselective activities of (*R*)- and (*S*)-bromohomoibotenic acid. *J. Med. Chem.* **1989**, *32*, 2254−2260.
(25) Chung, D. S.; Winder, D. G.; Conn, P. J. 4-Bromohomoibotenic acid selectively activates a 1-aminocyclopentane-1*S*,3*R*-dicarboxylic acid-insensitive metabotropic glutamate receptor coupled to phosphoinositide hydrolysis in rat cortical slices. *J. Neurochem.* **1994**, *63*, 133−139.
(26) Thomsen, C.; Bau, A.; Faarup, P.; Foged, C.; Kanstrup, A.; Suzdak, P. D. Effects of bromohomoibotenate on metabotropic glutamate receptors. *NeuroReport* **1994**, *5*, 2417−2420.
(27) Madsen, U.; Ebert, B.; Hansen, J. J.; Krogsgaard-Larsen, P. The nondepolarizing D-form of bromohomoibotenic acid (Br-HIBO) enhances depolarizations by L-Br-HIBO or quisqualate. *Eur. J. Pharmacol.* **1993**, *230*, 383−386.
(28) Christensen, I. T.; Ebert, B.; Madsen, U.; Nielsen, B.; Brehm, L.; Krogsgaard-Larsen, P. Excitatory amino acid receptor ligands. Synthesis and biological activity of 3-isoxazolol amino acids structurally related to homoibotenic acid. *J. Med. Chem.* **1992**, *35*, 3512−3519.
(29) Krogsgaard-Larsen, P.; Honore, T.; Hansen, J. J.; Curtis, D. R.; Lodge, D. New class of glutamate agonist structurally related to ibotenic acid. *Nature (London)* **1980**, *284*, 64−66.
(30) Bischoff, F.; Johansen, T. N.; Ebert, B.; Krogsgaard-Larsen, P.; Madsen, U. Excitatory amino acid receptor ligands: Asymmetric synthesis, absolute stereochemistry and pharmacology of (*R*)- and (*S*)-homoibotenic acid. *Bioorg. Med. Chem.* **1995**, *3*, 553−558.
(31) Dirkx, I. P.; Sixma, F. L. J. Spectropolarimetry II: The optical rotatory dispersion of α-amino-acids and α-hydroxy-acids. *Recl. Trav. Chim. Pay-Bas* **1964**, *83*, 522−534.
(32) Jennings, J. P.; Klyne, W.; Scopes, P. M. Optical rotatory dispersion. Part X. Amino acids. *J. Chem. Soc.* **1965**, 294−297.
(33) Legrand, M.; Rougier, M. J. Application of the optical activity to stereochemical determinations. In *Stereochemistry. Fundamentals and Methods. Volume 2: Determination of Configurations by Dipole Moment, CD or ORD*; Kaagan, H. B., Ed.; Georg Thieme Publishers: Stuttgart, 1977; pp 33−183.
(34) Gübitz, G.; Jellenz, W.; Santi, W. Resolution of the optical isomers of underivatized amino acids on chemically bound chiral phases by ligand exchange chromatography. *J. Liq. Chromatogr.* **1981**, *4*, 701−712.
(35) Shinbo, T.; Yamagushi, T.; Nishimura, K.; Sugiura, M. Chromatographic separation of racemic amino acids by use of chiral crown ether-coated reversed-phase packings. *J. Chromatogr.* **1987**, *405*, 145−153.
(36) Johnson, C. K. *ORTEP II. A fortran thermal-ellipsoid plot program for crystal structure illustrations;* ORNL-5138; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.
(37) Allen, F. H.; Kennard, O.; Watson, D. G. Tables of bond lengths determined by X-ray and neutron diffraction. Part 1. Bond lengths in organic compounds. *J. Chem. Soc., Perkin Trans. 2* **1987**, S1−S19.
(38) Brehm, L.; Frydenvang, K.; Krogsgaard-Larsen, P.; Liljefors, T. Crystal structure and quantum chemical ab initio calculations of ibotenic acid, an excitatory amino acid receptor agonist. *J. Mol. Struct.* **1997**, in press.
(39) Jeffrey, G. A.; Saenger, W. *Hydrogen Bonding in Biological Structures;* Springer-Verlag: Berlin, 1991.
(40) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, K. Binding of [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat membranes. A selective high affinity ligand for *N*-methyl-D-aspartate receptors. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 778−783.

(41) Kemp, J. A.; Foster, A. C.; Leeson, P. D.; Priestly, T.; Tridgett, R.; Iversen, L. L.; Woodruff, G. N. 7-Chlorkynurenic acid is a selective antagonist at the glycine modulatory site of the *N*-methyl-D-aspartate receptor complex. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6547−6550.
(42) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **1985**, *54*, 27−32.
(43) Braitman, D. J.; Coyle, J. T. Inhibition of [^{3}H]kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. *Neuropharmacology* **1987**, *26*, 1247−1251.
(44) Honoré, T.; Drejer, J.; Nielsen, M.; Braestrup, C. Differentiation of Cl^-/Ca^{2+}-dependent and sodium-dependent ^{3}H-glutamate binding to cortical membranes from rat brain by high energy radiation inactivation analysis. *J. Neurol. Transm.* **1986**, *65*, 93−101.
(45) Willis, C. L.; Humphrey, J. M.; Koch, H. P.; Hart, J. A.; Blakely, T.; Ralston, L.; Baker, C. A.; Shim, S.; Kadri, M.; Chamberlin, A. R.; Bridges, R. J. L-*trans*-2,3-Pyrrolidine dicarboxylate: Characterization of a novel excitotoxin. *Neuropharmacology* **1996**, *35*, 531−539.
(46) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some antagonists of *N*-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381−391.
(47) Ebert, B.; Lenz, S. M.; Brehm, L.; Bregnedal, P.; Hansen, J. J.; Frederiksen, K.; Bøgesø, K. P.; Krogsgaard-Larsen, P. Resolution, absolute stereochemistry, and pharmacology of the *S*-(+)- and *R*-(−)-isomers of the apparent partial AMPA receptor agonist (*R,S*)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid [(*R,S*)-APPA]. *J. Med. Chem.* **1994**, *37*, 878−884.
(48) Sommer, B.; Seeburg, P. H. Glutamate receptor channels: Novel properties and new clones. *Trends Pharmacol. Sci.* **1992**, *13*, 291−296.
(49) Trussell, L. O.; Zhang, S.; Raman, I. M. Desensitization of AMPA receptors upon multiquantal neurotransmitter release. *Neuron* **1993**, *10*, 1185−1196.
(50) Lerma, J.; Morales, M.; Ibarz, J. M.; Somohano, F. Rectification properties and Ca^{2+} permeability of glutamate receptor channels in hippocampal cells. *Eur. J. Neurosci.* **1994**, *6*, 1080−1088.
(51) May, P. C.; Robison, P. M. Cyclothiazide treatment unmasks AMPA excitotoxicity in rat primary hippocampal cultures. *J. Neurochem.* **1993**, *60*, 1171−1174.
(52) Sauer, D. EAA receptors and excitotoxicity. *Drug News Perspect.* **1993**, *6*, 88−94.
(53) Vyklicky, L., Jr.; Patneau, D. K.; Mayer, M. L. Modulation of excitatory synaptic transmission by drugs that reduce desensitization at AMPA/kainate receptors. *Neuron* **1991**, *7*, 971−984.
(54) Kanner, B. I. Sodium−coupled GABA and glutamate transporters. In *Neurotransmitter Transporters: Structure, Function, and Regulation*; Reith, M. E. A., Ed.; Humana Press: Totowa, N.J., 1997; pp 151−213.
(55) Enraf-Nonius. *CAD-4 Software. Version 5.0;* Enraf-Nonius: Delft, The Netherlands, 1989.
(56) Blessing, R. H. Data reduction and error analysis for accurate single crystal diffraction intensities. *Crystallogr. Rev.* **1987**, *1*, 3−58.
(57) Blessing, R. H. DREADD − data reduction and error analysis for single-crystal diffractometer data. *J. Appl. Crystallogr.* **1989**, *22*, 396−397.
(58) Sheldrick, G. M. Phase annealing in SHELX-90: Direct methods for larger structures. *Acta Crystallogr.* **1990**, *A46*, 467−473.
(59) Sheldrick, G. M. *SHELXL-93. Program for the refinement of crystal structures;* University of Göttingen: Göttingen, Germany, 1993.
(60) Flack, H. D. On enantiomorph-polarity estimation. *Acta Crystallogr.* **1983**, *A39*, 876−881.
(61) *International Tables for Crystallography*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1992; Vol. C, Tables 4.2.6.8 and 6.1.1.4.
(62) Ramson, R. W.; Stec, N. L. Cooperative modulation of [^{3}H]MK-801 to the *N*-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.
(63) Aramori, I.; Nakanishi, S. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. *Neuron* **1992**, *8*, 757−765.
(64) Tanabe, Y.; Masu, M.; Ishii, T.; Shigemoto, R.; Nakanishi, S. A family of metabotropic glutamate receptors. *Neuron* **1992**, *8*, 169−179.
(65) Tanabe, Y.; Nomura, A.; Masu, M.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. *J. Neurosci.* **1993**, *13*, 1372−1378.
(66) Abe, T.; Sugihara, H.; Nawa, H.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate Ca^{2+} signal transduction. *J. Biol. Chem.* **1992**, *267*, 13361−13368.
(67) Hayashi, Y.; Tanabe, Y.; Aramori, I.; Masu, M.; Shigemoto, R.; Ohfune, Y.; Nakanishi, S. Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.* **1992**, *107*, 539−543.
(68) Hayashi, Y.; Sekiyama, N.; Nakanishi, S.; Jane, D. E.; Sunter, D. C.; Birse, E. F.; Udvarhelyi, P. M.; Watkins, J. C. Analysis of agonist and antagonist activities of phenylglycine derivatives of different cloned metabotropic glutamate receptor subtypes. *J. Neurosci.* **1994**, *14*, 3370−3377.
(69) Cheng, Y.-C.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50% inhibition (I_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(70) Falch, E.; Jacobsen, P.; Krogsgaard-Larsen, P.; Curtis, D. R. GABA-mimetic activity and effects on diazepam binding of aminosulfonic acids structurally related to piperidine-4-sulfonic acid. *J. Neurochem.* **1985**, *44*, 68−75.

JM9706731