Synthesis and Anticonvulsant Activity of Novel Bicyclic Acidic Amino Acids

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Bicyclic acidic amino acids (±)-**6** and (±)-**7**, which are conformationally constrained homologues of glutamic acid, were prepared via a strategy based on a 1,3-dipolar cycloaddition. The new amino acids were tested toward ionotropic and metabotropic glutamate receptor subtypes; both of them behaved as antagonists at mGluR1,5 and as agonists at mGluR2. Furthermore, whereas (±)-**6** was inactive at all ionotropic glutamate receptors, (±)-**7** displayed a quite potent antagonism at the NMDA receptors. In the in vivo tests on DBA/2 mice, the compounds displayed an anticonvulsant activity. The interesting pharmacological profile of (±)-**7** qualifies it as a lead of novel neuroprotective agents.

Introduction

(*S*)-Glutamic acid (Glu, **1**) (Figure 1) is the main excitatory neurotransmitter in the central nervous system, where it is involved in the physiological regulation of processes such as learning and memory.[1−3] On the other hand, glutamatergic hyperactivity leads to neurotoxicity typical of some acute and chronic neurodegenerative diseases, i.e., cerebral ischemia, epilepsy, amyotrophic lateral sclerosis, and Parkinson's and Alzheimer's diseases.[1−3]

Glu interacts with specific receptors belonging to two families: the ionotropic receptors (iGluRs) and the metabotropic receptors (mGluRs).[1−3] Both families are composed of different receptor classes and subtypes. On the basis of the specific interaction with selective ligands, iGluRs have been pharmacologically classified into *N*-methyl-D-aspartic acid (NMDA) receptors, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and kainic acid (KA) receptors.[4,5] The iGluRs are transmembrane cation channels, and their ligand-induced opening promotes fast excitatory transmission at the synaptic level.

Conversely, mGluRs are G-protein-coupled receptors whose activation produces metabolic changes in the postsynaptic cells, due to the stimulation/inhibition of the release of second messengers. As a consequence, they mainly modulate the fast excitatory effects of Glu, and they do not produce rapid and large changes in neuronal membrane conductances. To date, eight subtypes of mGluRs have been cloned[6] and, subsequently, categorized into three groups (I−III) according to their sequence homology, coupling with the second messenger, and pharmacology. In particular, activation of mGluR1,5 (group I) stimulates phosphoinositide hydrolysis leading to inositol-1,4,5-triphosphate (IP_3) formation and consequent mobilization of intracellular calcium, whereas activation of mGluR2,3 (group II) and mGluR4,6,7,8 (group III), due to a negative coupling to adenylyl cyclase, induces a decrease in the concentration of cAMP.

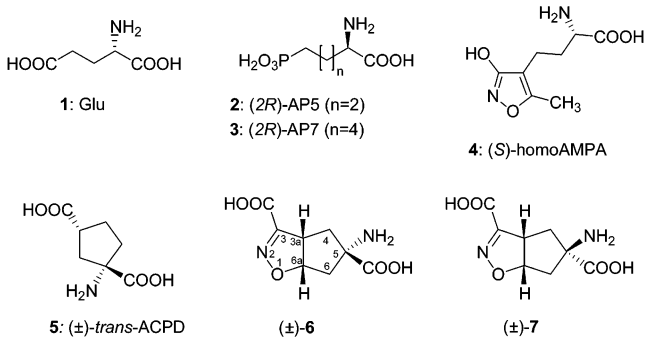

Figure 1. Structure of model and tested compounds.

The physiological and pharmacological characterization of the different Glu receptors is of primary interest, since the modulation of the glutamatergic pathways may represent a relevant therapeutic approach in the treatment of a number of neurodegenerative pathologies and neuropsychiatric diseases, as well as learning and memory impairments. Experimental evidence suggests the potential therapeutic application of NMDA antagonists in the treatment of cerebral ischemia and other neurodegenerative disorders.[7,8] So far, a large number of competitive and noncompetitive NMDA antagonists have been tested as drug candidates. Unfortunately, almost all of them have shown unacceptable adverse effects in humans, i.e., psychotomimetic and cardiovascular side effects.[9] However, the recent approval of a noncompetitive NMDA antagonist for treatment of Alzheimer's disease show that a clinical window can be achieved.

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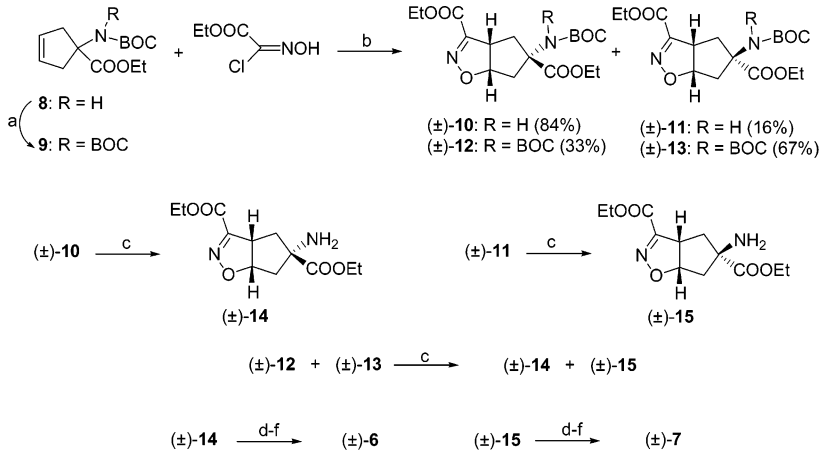
Scheme 1[a]



[a] Reagents and conditions: (a) BOC_2O, DMAP, THF, Δ; (b) $NaHCO_3$, AcOEt; (c) 30% CF_3COOH, CH_2Cl_2; (d) 1 N NaOH, EtOH; (e) 2 N HCl; (f) Amberlite IR-120plus, 10% pyridine (aq).

On the other hand, it has been suggested that both group I antagonists as well as group II and III agonists may represent potentially useful tools for neuroprotection.[10,11] To better characterize the roles played by mGluRs in physiological processes, numerous efforts have been devoted to identify novel, high affinity ligands that are family and subtype selective.[6]

From a therapeutic perspective, neuroprotective agents could also be ligands which interact simultaneously with different ionotropic and metabotropic glutamate receptors, i.e., antagonists at iGluRs and group I mGluRs or agonists at both group II and group III mGluRs giving rise to synergistic effects.[12] In such a way the therapeutic activity could be optimized and the adverse side-effects significantly reduced.

A survey of literature reports on the structure–activity relationship of ligands interacting with NMDA receptors[13] puts in evidence that an increase of the distance between the proximal and the distal acidic groups of Glu leads to NMDA antagonists. In general, the most potent NMDA antagonists bear a chain of four or six carbon atoms linking the two acidic groups, i.e., (2*R*)-2-amino-5-phosphonopentanoic acid [(2*R*)-AP5, **2**] and (2*R*)-2-amino-7-phosphonoeptanoic acid [(2*R*)-AP7, **3**] (Figure 1). It is worth pointing out that the eutomer of the majority of NMDA ligands possesses an absolute configuration of the stereogenic center at the amino acidic moiety opposite to that of natural Glu, the endogenous neurotransmitter.

Homologation of the Glu backbone embedded in selective ligands may lead to a significant modification of their pharmacological profile. For instance, the homo-derivative of (*S*)-AMPA [(*S*)-Homo-AMPA, **4**] (Figure 1) behaves as a selective agonist at mGluR6 (mGluRIII subtype) and completely loses the activity at AMPA receptors, which characterizes the reference compound.[14]

NMDA antagonists as well as mGluRs ligands have also been extensively studied from a conformational point of view. Taking into account a number of amino acids whose conformation is constrained into cyclic structures, it has been found that the majority of NMDA antagonists usually adopt a folded conformation with the proximal and the distal acidic functionalities oriented toward the same face of the molecule.[13,15] On the contrary, it was ascertained that Glu binds to the different types of mGluRs in a fully extended conformation, i.e., the extended conformation represented by (±)-*trans*-ACPD (**5**) (Figure 1). The selectivity among the mGluR subtypes can be achieved through the introduction of specific functionalities, able to establish additional interactions with amino acids of the binding site.[16,17]

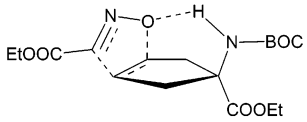


Figure 2. Transition state stabilized by an intermolecular hydrogen bond.

To deepen the investigation on the conformational requirements needed for the selective interaction with NMDA and mGluRs, we have designed novel homologues of Glu, i.e., 5-amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic acids (±)-**6** and (±)-**7**, characterized by an extended conformation locked in a bicyclic structure.

Chemistry

The key step in the synthesis of target compounds (±)-**6** and (±)-**7** is represented by the 1,3-dipolar cycloaddition of ethoxycarbonylformonitrile oxide, generated in situ by treatment of ethyl 2-chloro-2-(hydroxyimino)-acetate with a base, to a suitably protected 1-amino-cyclopent-3-enecarboxylic acid (**8** and **9**) (Scheme 1). Dipolarophile **8** was prepared according to the procedure reported in the literature,[18] whereas **9** was synthesized by refluxing a THF solution of **8** with excess di-*tert*-butyl dicarbonate in the presence of 4-(dimethylamino)pyridine. The cycloaddition step, carried out on dipolarophile **8**, produced a mixture of the two stereoisomers (±)-**10** and (±)-**11** in a 84:16 ratio. As reported in the literature,[19–22] the outcome of the nitrile oxide cycloaddition to alkenes bearing hydroxy, amido, and carbamate groups is influenced by the transition state depicted in Figure 2, which is stabilized by an intermolecular hydrogen bond. Accordingly, the relative configurations to stereoisomers (±)-**10** and (±)-**11** were preliminarly assigned on the basis of their relative abundances in the reaction mixture and subsequently

Table 1. Rat Cortical Membrane Receptor Binding and in Vitro Electrophysiological Data

	receptor binding, IC_{50} (μM)			electrophysiology	
compd	[^{3}H]CPP	[^{3}H]AMPA	[^{3}H]KA	agonism: EC_{50} (μM)	antagonism: IC_{50}(μM)
(±)-**6**	>100	>100	>100	>1000	>1000[a]
(±)-**7**	0.49 ± 0.08	>100	>100	>1000	14 ± 2[b]
(±)-AP5	0.29 ± 0.048[c]				
(*R*) -AP5	0.88 ± 0.45[c,d]				
(*S*)-AP5	29 ± 9[c,d]				
(*R*)-CPPene	0.040 ± 0.01[c]				

[a] Antagonism of NMDA (10 μM), AMPA (5 μM), or KA (5 μM) induced responses. [b] Antagonism of NMDA (10 μM) induced responses. [c] Data from literature, ref 13. [d] [^{3}H]Glu NMDA sensitive binding. Data are a mean of at least three individual determinations ± SEM.

Table 2. Activities at Cloned Rat mGlu Receptors Expressed in CHO Cells[a]

	mGluR1		mGluR5		mGluR2		mGluR4	
compd	EC_{50} (μM)	K_i (μM)	EC_{50} (μM)	K_i (μM)	EC_{50} (μM)	K_i (μM)	EC_{50} (μM)	K_i (μM)
(±)-**6**		27 ± 7		440 ± 70	16 ± 3[b]		>1000	>1000
(±)-**7**		94 ± 16		640 ± 140	c		>1000	>1000
(±)-**5**	15 ± 2.2		23 ± 2.6		2.0 ± 0.3		~800	

[a] Data are given as mean ± SEM of at least three independent experiments. [b] Max. = 59 ± 7%. [c] 1 mM concentration produces a 36 ± 7% activation.

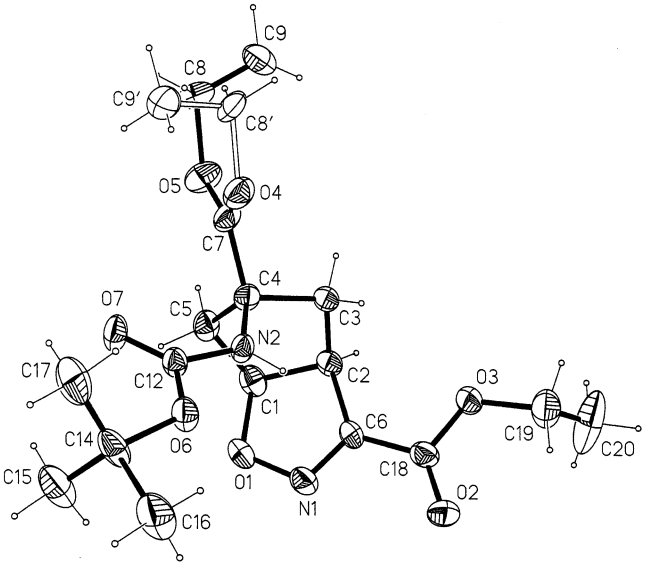


Figure 3. Perspective view of molecular structure with numbering scheme for **10**. Displacement ellipsoids are drawn at 50% of probability level while the size of the hydrogen atom is arbitrary. Empty bonds indicate a different position of the disordered C8−C9 fragment.

established by an X-ray analysis carried out on cycloadduct (±)-**10**; Figure 3 shows the crystallographic structure of **10**. The geometry of **10**, optimized at the B3LYP/6-31+G(d,p) level, is in good agreement with the X-ray data and put in evidence that the above-mentioned hydrogen bond (N−H···O), which characterizes the transition state leading to (±)-**10**, is also present in the solid state of the same cycloadduct.

To reverse the stereopreference observed with dipolarophile **8**, we carried out the corresponding 1,3-dipolar cycloaddition of ethoxycarbonylformonitrile oxide to alkene **9**. In this case, we caused both an increase in the bulkiness of the substituents at nitrogen and the removal of the hydrogen bond interaction. As expected, the cycloaddition proceeded with a reversed diastereoselectivity [(±)-**12**/(±)-**13** ratio 33: 67].

Stereoisomers (±)-**10** and (±)-**11** were separated by a silica gel column chromatography, whereas (±)-**12** and (±)-**13** turned out to be inseparable. Treatment of the mixture of (±)-**12** and (±)-**13** with excess trifluoroacetic acid afforded the corresponding primary amines (±)-**14** and (±)-**15**, which were separated by a silica gel column chromatography. Final amino acids (±)-**6** and (±)-**7** were obtained by reacting intermediate amino esters with 1 N sodium hydroxide followed by treatment with hydrochloric acid and cation exchange column chromatography.

Results and Discussion

The two stereoisomeric amino acids (±)-**6** and (±)-**7** were assayed in vitro by means of receptor binding techniques, second messenger assays and electrophysiological studies (Tables 1 and 2). The receptor affinity for NMDA, AMPA, and KA receptors was determined by using the radioligands [^{3}H]CPP, [^{3}H]AMPA, and [^{3}H]KA, respectively.[23−25] Compounds (±)-**6** and (±)-**7** showed no significant affinity for AMPA and KA receptors (IC_{50} > 100 μM). Whereas compound (±)-**6** showed no affinity for the NMDA receptors (IC_{50} > 100 μM), its stereoisomer (±)-**7** displayed a noticeable affinity for the NMDA receptors (IC_{50} = 0.49 μM), which is comparable to that reported for (±)-AP5 (IC_{50} = 0.29 μM)[13] and 10 times lower than that displayed by (*R*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonene [(*R*)-CPPene], one of the most potent NMDA antagonists[13] (Table 1). The above-reported pharmacological data were confirmed in the rat cortical slice model[26] where (±)-**6**, tested up to 1000 μM concentration, was inactive both as an agonist and as an antagonist, while (±)-**7** was able to antagonize the responses induced by 10 μM NMDA (IC_{50} = 14 μM). Moreover, in the same test (±)-**7** proved to be a weak antagonist of the AMPA receptors. As a matter of fact, 1 mM (±)-**7** produced approximately a 32% inhibition of the response induced by 5 μM AMPA but did not affect the response induced by 5 μM KA.

The activity of (±)-**6** and (±)-**7** at rat mGluRs was evaluated at mGluR1 and mGluR5 (group I), at mGluR2 (representative of group II) and at mGluR4 (representative of group III); all the receptors were expressed in CHO cells.[27] As shown in Table 2, the two amino acids (±)-**6** and (±)-**7** displayed pharmacological profiles quite different from that reported for (±)-*trans*-ACPD (**5**), the

Table 3. ED_{50} Values of Test Compounds against Audiogenic Seizures, Audiogenic Seizures after Pretreatment with D-Serine, and NMDA- or 3,5-DHPG-Induced Seizures in DBA/2 Mice[a]

	audiogenic seizures, μmol/kg		audiogenic seizures after pretreatment with D-serine, μmol/kg		NMDA-induced seizures, μmol/kg		3,5-DHPG-induced seizures, μmol/kg	
compd	clonus	tonus	clonus	tonus	clonus	tonus	clonus	tonus
(±)-**6**[b]	42	25	41	24	>100	91	>100	84
	30−59	16−37	26−63	18.32		66−126		60−117
(±)-**7**[b]	28	22	33	27	54	39	70	45
	19−41	14−35	23−45	20−36	42−69	29−54	55−89	35−56
(±)-CPPene[b]	1.8	0.79	2.5	1.2	17	11	8.6	6.8
	1.2−2.6	0.44−1.43	1.9−3.5	0.7−1.7	13−22	8.5−14	5.9−12	5.0−9.2
5,7-DCKA[c]	2.4	2.2	12	8.5	26	22	14	11
	1.2−5.0	1.2−3.9	7.6−18	5.2−14	17−39	15−31	9.4−22	7.3−18
PPG[c]	3.5	1.3	3.6	1.7	28	19	5.3	3.7
	2.1−5.6	0.7−2.6	2.4−5.2	1.0−2.8	20−41	17−22	3.2−8.7	2.0−6.9
felbamate	204	97	674	328	122	51		
	149−282	51−185	357−1275	189−569	62−240	28−92		
valproate	258	187	275	205	878	498		
	196−341	127−273	216−351	124−339	645−1120	319−777		
phenytoin	9.1	7.3	11	8.7	31	2.2		
	5.6−14.9	5.8−9.1	7.9−15	6.1−12.4	23−42	1.5−3.3		

[a] All data, calculated according to the method reported in ref 35, represent the 95% confidence limits of the CD_{50} values. At least 32 animals were used to calculate each ED_{50} value. [b] The values are expressed as μmol/kg for ip administration. [c] The values are expressed as nmol/mouse for icv administration.

prototype of a nonselective mGluRs agonist. In fact, both compounds behaved as antagonists at mGluR1,5 and as a weak [compound (±)-**7**] or a partial [compound (±)-**6**] agonist, respectively, at mGluR2. This latter difference in activity is based on the observation that (±)-**7** (at 1 mM) did not, in contrast to (±)-**6**, inhibit the response to 30 μM Glu. They did not significantly activate nor inhibit mGluR4 receptors (Table 2).

Even though (±)-**6** and (±)-**7** are nonselective mGluR ligands, and not particularly potent, it is of significance that a concomitant inhibition of group I and activation of group II may lead to a synergistic effect in terms of reduction of intracellular calcium concentrations by means of two different biochemical pathways, i.e., phospholipase C (PLC) inhibition and reduction of the cAMP concentration. Moreover, the NMDA antagonism observed with (±)-**7** could further increase the potential neuroprotective effect of such a compound. On the basis of these considerations, we decided to evaluate the in vivo anticonvulsant activity of (±)-**6** and (±)-**7**. We measured the potency (ED_{50} values) of (±)-**6** and (±)-**7** to block seizures induced in DBA/2 mice by audiogenic stimuli (Table 3). The ED_{50} values found for both (±)-**6** and (±)-**7** are lower than those reported for well-known anticonvulsant drugs, i.e., felbamate and valproate (Table 3), whereas (±)-**6** and (±)-**7** are less effective to block audiogenic seizures when compared to ligands acting exclusively through NMDA receptors such as (±)-CPPene and 5,7-dichlorokynurenic acid (5,7-DCKA). The potency remained almost unaffected when the compounds were tested against audiogenic seizures induced in DBA/2 mice after a pretreatment with D-serine. This result indicates that (±)-**6** and (±)-**7** do not interact with the glycine binding site of the NMDA receptors, at variance with the outcome of the tests carried out with 5,7-DCKA, where the ED_{50} values turned out to be significantly increased.

The efficacy of (±)-**6** and (±)-**7** to block seizures induced by NMDA and 3,5-dihydroxyphenylglycine was also evaluated (Table 3). Whereas (±)-**6** proved to be inefficacious, amino acid (±)-**7** was active in both tests, thus confirming the ability of such a compound to interact with both NMDA and mGlu receptors, as already observed in the in vitro assays.

In summary, we herein report the synthesis of two new homologues of Glu which behaved as anticonvulsant agents. Furthermore, if the concomitant activity at NMDA and mGlu receptors would produce synergistic effects, amino acid(±)-**7** could become a promising lead of neuroprotective agents; investigations along this line are underway. We are now working on the synthesis of the two enantiomers of (±)-**7** since most often NMDA and mGlu receptors require an opposite absolute configuration at the amino acid stereogenic center. This will enable us to evaluate if the unique pharmacological profile of (±)-**7** is due to one active enantiomer or is the sum of one enantiomer blockade of NMDA receptors plus the other enantiomer interaction with the different mGluRs.

Experimental Section

Material and Methods. Ethyl 2-chloro-2-(hydroxyimino)-acetate[28] and ethyl *N*-(*tert*-butoxycarbonyl)-1-amino-cyclopent-3-enecarboxylate **8**[18] were prepared according to literature procedures. ^1H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury 300 (300 MHz) spectrometer in $CDCl_3$ or D_2O solution at 20 °C. Chemical shifts (δ) are expressed in ppm and coupling constants (J) in hertz. HPLC analyses were performed with a Jasco PU-980 pump equipped with a UV−vis detector Jasco UV-975. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were further evidenced by spraying with a dilute alkaline potassium permanganate solution or with ninhydrin. Melting points were determined on a Büchi apparatus and are uncorrected. Microanalyses of new compounds agreed with theoretical values ±0.3%.

Ethyl *N*,*N*-(Di-*tert*-butoxycarbonyl)-1-amino-cyclopent-3-enecarboxylate (9). To a stirred solution of **8**[18] (1.25 g, 4.9 mmol) in THF (20 mL) were sequentially added 4-(dimethylamino)pyridine (60 mg, 0.49 mmol) and a THF solution (10 mL) of di-*tert*-butyl dicarbonate (1.6 g, 7.35 mmol). The mixture was refluxed for 6 days, and di-*tert*-butyl dicarbonate (0.5 equivalents) was added every day. The disappearance of the starting material was monitored by TLC (petroleum ether/AcOEt 9:1). The solvent was evaporated at reduced pressure, and the residue was subjected to column chromatography on silica gel (petroleum ether/AcOEt 95:5) to give **9** (1.48 g, 85%

yield) as yellow oil; R_f 0.49 (cyclohexane/AcOEt 9:1); ^{1}H NMR ($CDCl_3$): 1.25 (t, J = 7.2, 3H); 1.50 (s, 18H); 2.85 (d, J = 16.3, 2H); 3.09 (d, J = 16.3, 2H); 4.18 (q, J = 7.2, 2H); 5.64 (s, 2H).

Synthesis of (±)-(3a*S*,5*S*,6a*S*)-5-*tert*-Butoxycarbonylamino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid Diethyl Ester and (±)-(3a*S*,5*R*,6a*S*)-5-*tert*-Butoxycarbonylamino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid Diethyl Ester [(±)-10 and (±)-11]. To a solution of **8** (1.8 g, 7.06 mmol) in AcOEt (40 mL) was added ethyl 2-chloro-2-(hydroxyimino)-acetate (2.14 g, 14.12 mmol) and $NaHCO_3$ (5 g). The mixture was vigorously stirred for 3 days; the progress of the reaction was monitored by TLC (petroleum ether/AcOEt 7:3). Water was added to the reaction mixture, and the organic layer was separated and dried over anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was chromatographed on silica gel (petroleum ether/AcOEt 4:1) to give 1.850 g of (±)-**10** and 0.292 g of (±)-**11**. Overall yield: 82%.

(±)-(3a*S*,5*S*,6a*S*)-5-*tert*-Butoxycarbonylamino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic acid diethyl ester (±)-**10**: colorless needles from isopropyl ether, mp 138−139 °C; R_f 0.25 (cyclohexane/AcOEt 7:3); HPLC retention time: 20.18 min. (column: LiChrospher Si 60 Merck; eluant: petroleum ether/AcOEt 80:20; flow: 1 mL/min; λ = 254 nm); ^{1}H NMR ($CDCl_3$, 50 °C) 1.26 (t, J = 7.0, 3H); 1.35 (t, J = 7.0, 3H); 1.40 (s, 9H); 2.38 (bd, J = 15.0, 1H); 2.45 (dd, J = 15.0, 6.2, 1H); 2.64 (dd, J = 14.6, 9.5, 1H); 2.75 (bd, J = 14.6, 1H); 4.00 (ddd, J = 9.5, 9.5, 2.9, 1H); 4.19 (m, 2H); 4.32 (m, 2H); 4.89 (bs, 1H); 5.33 (ddd, J = 9.5, 6.2, 1.8, 1H); ^{13}C NMR ($CDCl_3$) 14.52; 14.55; 28.53; 39.46; 45.63; 50.99; 62.17; 62.40; 65.77; 80.42; 88.92; 154.46; 154.86; 160.38; 172.63; Anal. ($C_{17}H_{26}N_2O_7$) C, H, N.

(±)-(3a*S*,5*R*,6a*S*)-5-*tert*-Butoxycarbonylamino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic acid diethyl ester (±)-**11**: colorless needles from isopropyl ether, mp 118−119 °C; R_f 0.28 (cyclohexane/AcOEt 7:3); HPLC retention time: 18.42 min. (column: LiChrospher Si 60 Merck; eluant: petroleum ether/AcOEt 80:20; flow: 1 mL/min; λ = 254 nm); ^{1}H NMR ($CDCl_3$, 50°C) 1.25 (t, J = 7.3, 3H); 1.37 (t, J = 7.0, 3H); 1.44 (s, 9H); 2.48 (dd, J = 14.6, 5.0, 1H); 2.57 (d, J = 7.7, 2H); 2.67 (dd, J = 14.6, 7.0, 1H); 4.05 (ddd, J = 10.3, 7.7, 7.7, 1H); 4.18 (q, J = 7.0, 2H); 4.35 (q, J = 7.3, 2H); 4.98 (bs, 1H); 5.37 (ddd, J = 10.3, 7.0, 5.0, 1H); ^{13}C NMR ($CDCl_3$) 14.41; 14.57; 28.63; 41.30; 45.16; 51.28; 62.37; 62.43; 66.18; 80.94; 89.02; 154.13; 155.18; 160.62; 172.30; Anal. ($C_{17}H_{26}N_2O_7$) C, H, N.

Synthesis of (±)-(3a*S*,5*S*,6a*S*)-5-*N*,*N*-(Di-*tert*-butoxycarbonyl)amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]-isoxazole-3,5-dicarboxylic Acid Diethyl Ester and (±)-(3a*S*,5*R*,6a*S*)-5-*N*,*N*-(Di-*tert*-butoxycarbonyl)amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid Diethyl Ester [(±)-12 and (±)-13]. To a solution of **9** (1.48 g, 4.17 mmol) in AcOEt (40 mL) were added ethyl 2-chloro-2-(hydroxyimino)acetate (1.26 g, 8.34 mmol) and $NaHCO_3$ (3 g). The mixture was vigorously stirred for 7 days; during this time 4 equiv (2.52 g, 16.68 mmol) of ethyl 2-chloro-2-(hydroxyimino)acetate was further added to the mixture. The progress of the reaction was monitored by TLC (petroleum ether/AcOEt 9:1). Water was added to the reaction mixture, and the organic layer was separated and dried over anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was chromatographed on silica gel (petroleum ether/AcOEt 9:1) to give an inseparable mixture of (±)-**12** and (±)-**13** (1.12 g, 57% yield).

Synthesis of (±)-(3a*S*,5*S*,6a*S*)-5-Amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid Diethyl Ester and (±)-(3a*S*,5*R*,6a*S*)-5-Amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid Diethyl Ester [(±)-14 and (±)-15]. The mixture of (±)-**12** and (±)-**13** (1.12 g, 2.38 mmol) was treated with a 30% dichloromethane solution of trifluoroacetic acid (6 mL) at 0 °C. The solution was stirred at room temperature until disappearance of the starting material (3 h). The volatiles were removed under vacuum, and the residue was treated with a 20% $NaHCO_3$ solution (20 mL) and extracted with AcOEt (3 × 10 mL). The pooled organic extracts were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was purified by a silica gel column chromatography (petroleum ether/AcOEt 1:2) to give 0.190 g of (±)-**14** and 0.298 g of (±)-**15** as yellowish oils in 76% overall yield.

(±)-(3a*S*,5*S*,6a*S*)-5-Amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic acid diethyl ester (±)-**14**: R_f 0.44 (cyclohexane/AcOEt 3:7); ^{1}H NMR ($CDCl_3$) 1.26 (t, J = 7.3, 3H); 1.36 (t, J = 7.0, 3H); 1.57 (bs, 2H); 2.16 (m, 2H); 2.38 (dd, J = 14.3, 9.2, 1H); 2.53 (dd, J = 14.6, 7.0, 1H); 3.99 (dd, J = 9.2, 9.2, 1H); 4.16 (q, J = 7.0, 2H); 4.33 (m, 2H); 5.36 (dd, J = 9.2, 7.0, 1H); Anal. ($C_{12}H_{18}N_2O_5$) C, H, N.

(±)-(3a*S*,5*R*,6a*S*)-5-Amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic acid diethyl ester (±)-**15**: R_f 0.19 (cyclohexane/AcOEt 3:7); ^{1}H NMR ($CDCl_3$) 1.26 (t, J = 7.3, 3H); 1.37 (t, J = 7.0, 3H); 1.59 (bs, 2H); 2.12−2.40 (m, 4H); 4.08−4.20 (m, 3H); 4.35 (q, J = 7.0, 2H); 5.41−5.46 (ddd, J = 10.0, 6.3, 6.3, 1H); Anal. ($C_{12}H_{18}N_2O_5$) C, H, N.

The same treatment with a 30% dichloromethane solution of trifluoroacetic acid, carried out on cycloadducts (±)-**10** and (±)-**11**, gave the above-reported amino esters in comparable yields.

(±)-(3a*S*,5*S*,6a*S*)-5-Amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid (±)-6. (±)-**14** (0.19 g, 0.7 mmol) was treated with 1 N NaOH (2.1 mL) at room temperature overnight. The solution was made acid with 2 N HCl, then submitted to a cation exchange chromatography, using Amberlite IR-120 plus. The acidic solution was slowly placed on the resin, and then the column was washed with water until neutral pH. The compound was then eluted off the resin with 10% aqueous pyridine, and the product-containing fractions (detected with ninhydrin on a TLC plate) were combined and concentrated under vacuum. The residue was crystallized from water/methanol, filtered, washed sequentially with methanol and ethyl ether, and dried in vacuo at 50°C to give amino acid (±)-**6** as white prisms (0.102 g, 63% yield).

(±)-**6**: R_f 0.13 (*n*-butanol/water/acetic acid 4:2:1); decomposes in the range 154−230 °C; ^{1}H NMR (D_2O): 2.05 (dd, J = 14.6, 7.0, 1H); 2.06 (dd, J = 15.0, 4.8, 1H); 2.63 (ddd, J = 14.6, 9.9, 1.1, 1H); 2.72 (ddd, J = 15.0, 7.0, 1.1, 1H); 3.98 (ddd, J = 9.9, 9.9, 7.0, 1H); 5.30 (ddd, J = 9.9, 7.0, 4.8, 1H); ^{13}C NMR (D_2O) 39.14; 42.16; 50.50; 64.29; 89.79; 155.23; 162.28; 172.58; Anal. ($C_8H_{10}N_2O_5 \cdot H_2O$) C, H, N.

(±)-(3a*S*,5*R*,6a*S*)-5-Amino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid (±)-7. Amino ester (±)-**15** was treated under the same conditions reported for (±)-**14** to give amino acid (±)-**7** in 59% yield.

(±)-**7:** R_f 0.12 (*n*-butanol/water/acetic acid 4:2:1); decomposes in the range 173−225°C; ^{1}H NMR (D_2O): 2.10 (dd, J = 15.0, 10.3, 1H), 2.29 (dd, J = 15.8, 7.0, 1H); 2.44 (dd, J = 15.8, 2.6, 1H); 2.52 (dd, J = 15.0, 2.9, 1H); 3.87 (ddd, J = 10.3, 10.3, 2.9, 1H); 5.19 (ddd, J = 10.3, 7.0, 2.6, 1H); ^{13}C NMR (D_2O) 38.17; 43.45; 51.03; 63.98; 88.47; 154.50; 162.30; 172.08; Anal. ($C_8H_{10}N_2O_5$ x$1H_2O$) C, H, N.

Biological Testing. Receptor Binding and Electrophysiology Assays at iGluRs. The membrane preparations used in all the receptor-binding experiments were prepared according to Ransom and Stec[29] with slight modifications, as previously described.[30]

Affinity for AMPA,[24] KA,[25] and NMDA[26] receptor sites was determined using 5 nM [^{3}H]AMPA, 5 nM [^{3}H]kainic acid in the absence of $CaCl_2$, and 2 nM [^{3}H]CPP, respectively.

The binding assays were carried out with the modifications previously described.[30] The amount of bound radioactivity was determined using a Packard TOP-COUNT microplate scintillation counter.

The rat cortical wedge preparation, in a modified version, was used for the determination of the depolarizing effects of the compounds under study.[26,31] Tests for agonist activities of (±)-**6** and (±)-**7** were performed by their application for 90 s. Tests for antagonist activities of (±)-**6** and (±)-**7** were performed by their preapplication for 90 s followed by co-application of the compound under study with a standard

agonist, AMPA (5 μM), NMDA (10 μM), or KA (5 μM), for further 90 s.

Data Analysis. Binding data were analyzed by the nonlinear curve fitting program GRAFIT 3.0.[32] Data were fitted to the following equation: $B = 100 - (100 \times [\text{inhibition}]^n)/(IC_{50}^n + [\text{inhibitor}]^n)$, where B is the binding, expressed as a percentage of total specific binding, and n the Hill coefficient.

Metabotropic Testing. The mGluR subtypes mGluR1α, mGluR2, mGluR4a, and mGluR5a were expressed in Chinese hamster ovary (CHO) cell lines. Cell were maintained and tested as previously described.[33]

In Vivo Pharmacology. Male DBA/2 mice (6−12 g; 22−26 days old) were used. The animals were housed in groups of 10 in PVC cages (260 mm × 440 mm × 120 mm) at a temperature of 21−23 °C and a relative humidity of 57 ± 2%; a 12 h light/dark cycle was applied (light on in the interval 07:00 a.m. to 07:00 p.m.). Food and water were available ad libitum.

Procedure. DBA/2 mice were exposed to a loud sound stimulus which produced a whole-body clonus.[34] Drug (or vehicle, water) was administered intraperitoneally (ip) in a volume of 100 μL/10 g body weight. Drug (or vehicle) was also administered intracerebroventricularly (icv) (1 mm anterior to the bregma, 1 mm lateral to the midline, and 3 mm depth) under light ethyl ether anaesthesia using a Hamilton syringe with a 25 short-gauge butterfly needle for delivering a volume of 10 μL. Following drug (or vehicle) injection, the mice were maintained at a body temperature of 36−38 °C by applying heating lamps, when required. Prior to the test for sound-induced seizures, mice were observed for drug related abnormal motor behavior effects. Anticonvulsant tests were carried out on individual mice 45 min after drug (or vehicle) administration under a Perspex dome (58 cm in diameter) fitted with a doorbell generating a sound of 110 dB for a period of 60s or until the onset of a clonic seizure. The sound stimulus produced a sequential seizure response, consisting of a wild running phase (score 1), clonic seizures (score 2), tonic extension (score 3), and occasionally respiratory arrest (score 4) as previously reported.[34] The compounds were dissolved in DMSO and the pH adjusted to 7.3−7.7 with a phosphate buffer solution for either icv or ip injection.

The anticonvulsant activity of test compounds was evaluated after administration of the convulsant. In particular, DBA/2 mice (n = 10 per group) received 18.7 nmol/mouse of NMDA for tonic extension seizures or 1.9 nmol/mouse of NMDA for clonic seizures. The occurrence of seizure-related activity was then observed for the following 60 min.[34] In another set of experiments, the anticonvulsant agent (10−100 μmol/kg, ip) was administered to DBA/2 mice (n = 10 per group) 15 min prior the icv injection of (*R*,*S*)-3,5-dihydroxyphenylglycine (DHPG, 1.5 μmol), and the occurrence of seizure-related activity was observed for the following 90 min.

Interaction with the glycine recognition site of the NMDA receptor complex. DBA/2 mice were pretreated with D-serine (300 nmol/mouse, icv), 30 min before the administration of a glycine receptor site agonist. Our previous studies have shown that D-serine, administered to DBA/2 mice directly into the lateral cerebral ventricle (300 nmol/mouse), is not by itself a convulsant.[34]

X-ray Analysis of (±)-(3a*S*,5*S*,6a*S*)-5-*tert*-Butoxycarbonylamino-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-3,5-dicarboxylic Acid Diethyl Ester (±)-10. Diffraction data were collected at room temperature from a colorless 0.8 × 0.7 × 0.6 mm^3 prismatic crystal sample (obtained from diethyl ether) by using a Siemens P4 automated four-circle single-crystal diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Lattice parameters were obtained from least-squares refinement of the setting angles of 39 reflections within $3 \le 2\theta \le 19°$ range. 6295 reflections were measured by the fixed speed ω scan technique up to $2\theta = 55°$. No crystal decay was evidenced by the check reflections monitored every 97 measurements. Intensities were evaluated by profile fitting of a 96-steps peak scan among 2θ shells procedure[36] and then corrected for Lorentz polarization effects. Because of the large dimension of the sample, absorption correction was applied by Gaussian integration of the indexed crystal shape. Data-collection and reduction has been performed by XSCANS[37] and SHELXTL package.[38] Structure was solved by a combination of standard Direct Methods[39] and Fourier synthesis, and refined by minimizing the function $\sum w(F_o^2 - F_c^2)^2$ with the full matrix least-squares technique based on all 1865 independent F^2 [R(int) = 0.0186], by using SHELXL97.[40] All non hydrogen atoms were refined anisotropically. Hydrogen atoms were located on the difference Fourier maps and included in the model refinement among the "riding model" method with the X−H bond geometry and isotropic displacement parameter depending on the parent atom X. An empirical extinction parameter was included in the last refinement cycles [0.0040(7)]. The last difference map showed no significant electron density residuals (max and min value = 0.194 and −0.126 Å^{-3}).

The final geometrical calculations and drawings were carried out with the PARST program[41] and the XPW utility of the Siemens package, respectively. Further details of crystal structure of compound **10** can be obtained, free of charge, on application to CCDC (189745), 12 Union Road, Cambridge CB2 lEZ UK

Ab initio calculations, molecular modeling, and geometry optimization have been carried out by using the Gaussian98[42] series of programs. Because of the large computational costs, geometry optimization of derivative **10** was only performed at HF/6-31+G(d,p) and B3LYP/6-31+G(d,p) level. Both levels of calculation predict a conformational energy difference of 2.85 kcalmol^{-1} for the couple of diastereomers **10** and **11**.

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Supporting Information Available: Seven tables containing the X-ray crystallographic crystal data of derivative **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Wheal, H. V.; Thomson, A. M., Eds. *Excitatory Amino Acids and Synaptic Transmissions*; Academic Press: London, 1995.
(2) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. Ligands for glutamate receptors: Design and therapeutic prospects. *J. Med. Chem*. **2000**, *43*, 2609−2645.
(3) Ozawa, S.; Kamiya, H.; Tsuzuki, K. Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol*. **1998**, *54*, 581−618.
(4) Monaghan, D. T.; Wenthold, R. J., Eds. *The Ionotropic Glutamate Receptors*; Humana Press: Totowa, NJ, 1997.
(5) Jonas, P.; Monyer, H. Eds. *Handbook of Experimental Pharmacology, Ionotropic Glutamate Receptors in the CNS*; Springer-Verlag: Berlin, 1999; vol. 141.
(6) Conn, P. J.; Pin, J. P. Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol*. **1997**, *37*, 205−237.
(7) Arias, R. L.; Tasse, J. R. P.; Bowlby, M. R. Neuroprotective interaction effects of NMDA and AMPA receptor antagonists in an in vivo model of cerebral ischemia. *Brain Res*. **1999**, *816*, 299−308.
(8) Palmer, G. C. Neuroprotection by NMDA receptor antagonists in a variety of neuropathologies. *Current Drug Targets* **2001**, *2*, 241−271.
(9) Herling, P. L. *Excitatory Amino Acids − Clinical Results with Antagonists*; Academic Press: London, 1997.
(10) Pellicciari, R.; Costantino, G. Metabotropic G-protein-coupled receptors as therapeutic targets. *Curr. Opin. Chem. Biol*. **1999**, *3*, 433−440.
(11) Monn, J. A.; Valli, M. J., Massey, S. M.; Wright, R. A.; Salhoff, C. R.; Johnoson, B. G.; Howe, T., Alt, C. A.; Rhodes, G. A.; Robey, R. L.; Griffey, K. R.; Tizzano, J. P.; Kallman, M. J.; Helton, D. R.; Schoepp, D. D. Design, synthesis, and pharmacological characterization of (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicar-

boxylic acid (LY354740): a potent, selective, and orally active group 2 metabotropic glutamate receptor agonist possessing anticonvulsant and anxiolytic properties. *J. Med. Chem.* **1997**, *40*, 528−537.
(12) Weiser, T. AMPA Receptor Antagonists with Additional Mechanisms of Action: New Opportunities for Neuroprotective Drugs? *Curr. Pharm. Design* **2002**, *8*, 941−951
(13) Johnson G.; Ornstein, P. L. Competitive NMDA antagonists-A comprehensive analysis of molecular biological, structure activity and molecular modeling relationships. *Curr. Pharm. Design* **1996**, *2*, 331−356.
(14) Ahmadian, H.; Nielsen, B.; Bräuner-Osborne, H.; Johansen, T. N.; Stensbøl, T. B.; Sløk, F. A.; Sekiyama, N.; Nakanishi, S.; Krogsgaard-Larsen, P.; Madsen, U. (*S*)-Homo-AMPA, a specific agonist at the mGlu6 subtype of metabotropic glutamic acid receptors. *J. Med. Chem.* **1997**, *40*, 3700−3705.
(15) Hutchison, A. J.; Williams, M.; Angst, C.; De Jesus, R.; Blanchard, L.; Jackson, R. H.; Wilusz, E. J.; Murphy, D. E.; Bernard, P. S.; Schneider, J.; Campbell, T.; Guida, W.; Sills, M. A. 4-(Phosphonoalkyl)- and 4-(phosphonoalkenyl)-2-piperidinecarboxylic acids: synthesis, activity at *N*-methyl-D-aspartic acid receptors and anticonvulsant activity. *J. Med. Chem.* **1989**, *32*, 2171−2178.
(16) Kozikowski, A. P.; Steensma, D.; Araldi, G. L.; Tückmantel, W.; Wang, S.; Pshenichkin, S.; Surina, E.; Wroblewski, J. T. Synthesis and biology of the conformationally restricted ACPD analogue, 2-aminobicyclo[2.1.1]hexane-2,5-dicarboxylic acid-I, a potent mGluR agonist. *J. Med. Chem.* **1998**, *41*, 1641−1650.
(17) Jullian, N.; Brabet, I.; Pin, J. P.; Acher, F. C. Agonist selectivity of mGluR1 and mGluR2 metabotropic receptors: a different environment but similar recognition of an extended glutamate conformation. *J. Med. Chem.* **1999**, *42*, 1546−1555.
(18) Hodgson, D. M.; Thompson, A. J.; Wadman, S. Carbamate-directed hydroboration: enantioselective synthesis of the excitatory amino acid 1-aminocyclopentane-1,3-dicarboxylic acid. *Tetrahedron Lett.* **1998**, *39*, 3357−3358.
(19) Caramella, P.; Cellerino, G. Selectivity in cycloadditions. II. Polar and steric control in the 1,3-dipolar cycloaddition of benzonitrile oxide to 3-substituted cyclopentenes. *Tetrahedron Lett.* **1974**, *2*, 229−232.
(20) Curran, D. P.; Choi, S. M.; Gothe, S. A.; Lin, F.-T. Directed nitrile oxide cycloaddition reactions. The use of hydrogen bonding to direct regio- and stereochemistry in nitrile oxide cycloadditions with cyclopentenylamides. *J. Org. Chem.* **1990**, *55*, 3710−3712.
(21) Curran, D. P.; Gothe, S. A.; Choi, S. M. Hydrogen bond directed nitrile oxide cycloaddition reactions of allylic 2°-amides. *Heterocycles* **1993**, *35*, 1371−1395.
(22) Park, K.-H.; Kurth, M. J. Diastereoselective synthesis of hydantoin- and isoxazoline-substituted dispirocyclobutanoids. *J. Org. Chem.* **2000**, *65*, 3520−3524.
(23) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, M. Binding of [^{3}H]2-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid to rat brain membranes. A selective high-affinity ligand for N-methyl-D-aspartate receptors. *J. Pharmacol. Ther.* **1987**, *240*, 778−784.
(24) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosc. Lett.* **1985**, *54*, 27−32.
(25) 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.
(26) Harrison, N. L.; Simmonds, M. A. Quantitative studies on some agonists of N-methyl-D-aspartate in slices of rat cerebral cortex. *Br. J. Pharmacol.* **1985**, *84*, 381−391.
(27) Bräuner-Osborne, H.; Sløk, F. A.; Skjærbæk, N.; Ebert, B.; Sekiyama, N.; Nakanishi, S.; Krogsgaard-Larsen, P. A new highly selective metabotropic excitatory amino acid agonist: 2-amino-4-(3-hydroxy-5-methylisoxazol-4-yl)butyric acid. *J. Med. Chem.* **1996**, *39*, 3188−3194.
(28) Kozikowski, A. P.; Adamczyk, M. Methods for the stereoselective cis-cyanohydroxylation and -carboxyhydroxylation of olefins. *J. Org. Chem.* **1983**, *48*, 366−372.
(29) Ransom, 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.
(30) Stensbøl, T. B.; Johansen, T. N.; Egebjerg, J.; Ebert, B.; Madsen, U.; Krogsgaard-Larsen, P. Resolution, absolute stereochemistry and molecular pharmacology of the enantiomers of ATPA. *Eur. J. Pharmacol.* **1999**, *380*, 153−162.
(31) Conti, P.; De Amici, M.; De Sarro, G.; Rizzo, M.; Stensbøl, T. B.; Bräuner-Osborne, H.; Madsen, U.; Toma, L.; De Micheli. C. Synthesis and Enantiopharmacology of New AMPA-Kainate Receptor Agonists. *J. Med. Chem.* **1999**, *42*, 4099−4107.
(32) Leatherbarrow, R. J. *GraFit version 3.0*. Erithacus Software: Staines, UK, 1992.
(33) Clausen, R. P.; Bräuner-Osborne, H.; Greenwood, J. R.; Hermit, M. B.; Stensbøl, T. B.; Nielsen, B.; Krogsgaard-Larsen, P. 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. *J. Med. Chem.* **2002**, 45, 4240−4245.
(34) De Sarro, G.; Ongini, E.; Bertorelli, R.; Aguglia, U.; De Sarro, A. Excitatory amino acid neurotransmission through both NMDA and non-NMDA receptors is involved in the anticonvulsant activity of felbamate in the DBA/2 mice. *Eur. J. Pharmacol.* **1994**, *262*, 11−19.
(35) Litchfield, J. T.; Wilcoxon, F. A Simplified Method of Evaluating Dose−Effects Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.
(36) Diamond, R. Profile analysis in single-crystal diffractometry. *Acta Crystallogr., Sect. A* **1969**, *25*, 43−55.
(37) Bruker. XSCANS, release 2.31. Bruker AXS Inc., Madison Wisconsin, 1999.
(38) Sheldrick, G. M. SHELXTL, VMS version 5.05, Siemens Analytical X-ray Instruments Inc., Madison WI, 1991.
(39) Altomare, A.; Cascarano, O.; Giacovazzo, C.; Guagliardi, C.; Burla, M. C.; Polidori, G.; Camalli, M. SIRPOW 92 − a program for automatic solution of crystal structures by direct methods optimized for powder data. *J. Appl. Crystallogr.* **1994**, *27*, 435−436.
(40) Sheldrick, G. M. SHELXL97. *Program for Crystal Structure Refinement*; University of Göttingen: Germany, 1997.
(41) Nardelli, M. PARST: a system of FORTRAN routines for calculating molecular structure parameters from results of crystal structure analyses. *Comput. Chem.* **1983**, *7*, 95−98 (version locally modified).
(42) Gaussian 98, Revision A.9, Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr., Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1998.

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