Synthesis and Pharmacology of 3-Isoxazolol Amino Acids as Selective Antagonists at Group I Metabotropic Glutamic Acid Receptors

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Using ibotenic acid (**2**) as a lead, two series of 3-isoxazolol amino acid ligands for (*S*)-glutamic acid (Glu, **1**) receptors have been developed. Whereas analogues of (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid [AMPA, (*RS*)-**3**] interact selectively with ionotropic Glu receptors (iGluRs), the few analogues of (*RS*)-2-amino-3-(3-hydroxy-5-isoxazolyl)propionic acid [HIBO, (*RS*)-**4**] so far known typically interact with iGluRs as well as metabotropic Glu receptors (mGluRs). We here report the synthesis and pharmacology of a series of 4-substituted analogues of HIBO. The hexyl analogue **9** was shown to be an antagonist at group I mGluRs. The effects of **9** were shown to reside exclusively in (*S*)-**9** (K_b = 30 μM at mGlu$_1$ and K_b = 61 μM at mGlu$_5$). The lower homologue of **9**, compound **8**, showed comparable effects at mGluRs, but **8** also was a weak agonist at the AMPA subtype of iGluRs. Like **9**, the higher homologue, compound **10**, did not interact with iGluRs, but **10** selectively antagonized mGlu$_1$ (K_b = 160 μM) showing very weak antagonist effect at mGlu$_5$ (K_b = 990 μM). The phenyl analogue **11** turned out to be an AMPA agonist and an antagonist at mGlu$_1$ and mGlu$_5$, and these effects were shown to originate in (*S*)-**11** (EC$_{50}$ = 395 μM, K_b = 86 and 90 μM, respectively). Compound **9**, administered icv, but not sc, was shown to protect mice against convulsions induced by *N*-methyl-D-aspartic acid (NMDA). Compounds **9** and **11** were resolved using chiral HPLC, and the configurational assignments of the enantiomers were based on X-ray crystallographic analyses.

Introduction

The main excitatory amino acid neurotransmitter in the central nervous system (CNS), (*S*)-glutamic acid (Glu, **1**), operates through two main groups of receptors: the ionotropic Glu receptors (iGluRs) and the metabotropic Glu receptors (mGluRs).[1−3] Each of these groups of receptors is divided into three subgroups: the iGluRs comprise the *N*-methyl-D-aspartic acid (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and kainic acid (KA) receptors, and the mGluR subgroups are named I, II, and III. The iGluR subgroups each consists of a number of subtypes forming homo- or heteromeric receptors in tetra- or pentameric configurations.[4,5] The mGluR groups consist of the following individual subtypes: group I, mGlu$_{1,5}$; group II, mGlu$_{2,3}$; and group III, mGlu$_{4,6−8}$.[6,7] It is generally agreed that iGluRs as well as mGluRs are important in the healthy as well as the diseased CNS, and all subtypes of these receptors are thus potential targets for therapeutic intervention in a number of neurologic and psychiatric diseases.[8−10]

A number of selective ligands have been developed for iGluRs, notably for NMDA and AMPA receptors, whereas relatively few selective ligands have been reported for mGluRs.[9,11] Ibotenic acid (**2**) has been used as a lead in the search for selective agents at different subtypes of GluRs, and these lines of research have, for
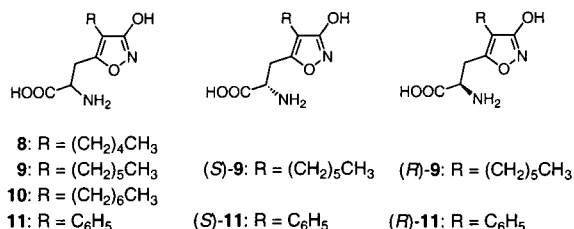

Glu (**1**)   Ibotenic acid (**2**)   (*S*)-AMPA (**3**)

4: R = H [(*S*)-HIBO]
5: R = CH$_3$
6: R = (CH$_2$)$_3$CH$_3$

7
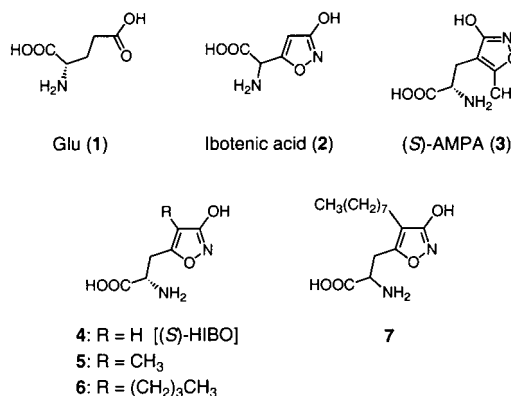
example, led to (*S*)-AMPA (**3**) and (*S*)-homoibotenic acid [(*S*)-HIBO, **4**].[12−14] (*S*)-HIBO and the 4-alkyl-substituted analogues **5** and **6** have previously been shown to be agonists at AMPA receptors and antagonists at group I mGluRs.[15−17] On the other hand, no activity was observed for the 4-octyl-substituted analogue of HIBO (**7**) at iGluRs[18] or mGluRs (results of this work).

These observations prompted us to synthesize compounds **8**−**11** in order to study in detail the relationship between the structure of the 4-substituents and the pharmacology of these compounds. Two of these analogues, compounds **9** and **11**, were resolved by chiral HPLC, and the absolute configuration was assigned on the basis of X-ray crystallographic analyses. All compounds synthesized have been tested pharmacologically in receptor binding assays for iGluR activity, in an in vitro electrophysiological model, and in second-mes-

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senger assays for mGlu$_1$, mGlu$_2$, mGlu$_4$, and mGlu$_5$ receptor activity. In addition compound **9** has been tested for activity against NMDA-induced convulsions in mice.

8: R = (CH$_2$)$_4$CH$_3$
9: R = (CH$_2$)$_5$CH$_3$ (*S*)-**9**: R = (CH$_2$)$_5$CH$_3$ (*R*)-**9**: R = (CH$_2$)$_5$CH$_3$
10: R = (CH$_2$)$_6$CH$_3$
11: R = C$_6$H$_5$ (*S*)-**11**: R = C$_6$H$_5$ (*R*)-**11**: R = C$_6$H$_5$
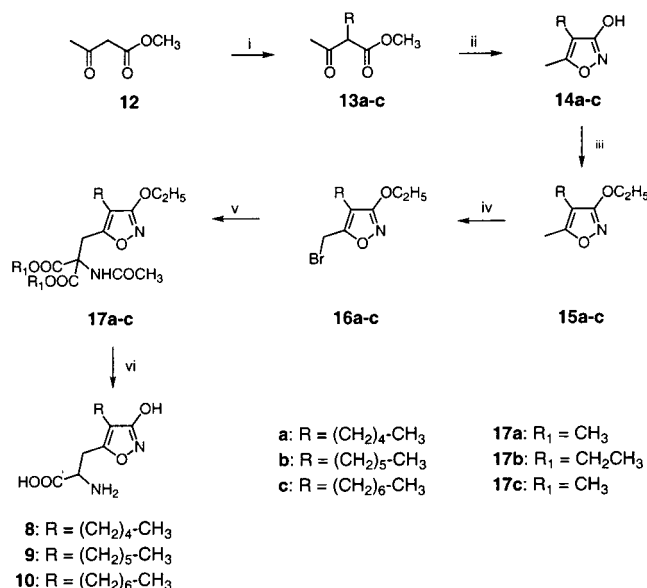
Results

Synthesis. The synthesis of all of the 4-alkyl-HIBO analogues **8**−**10** started from methyl acetoacetate (**12**) (Scheme 1). The alkyl substituents were introduced using sodium methoxide and alkylation with the appropriate alkyl bromide. Cyclization with hydroxylamine afforded the 4-substituted 3-isoxazolols **14a**−**c**, which were subsequently converted into the *O*-ethyl-protected analogues **15a**−**c**. Deprotections were accomplished using concentrated hydrobromic acid. The final products **8**−**10** all crystallized as zwitterions directly after evaporation of the hydrobromic acid solutions and subsequent re-evaporation with water. Compounds **8**−**10** were all highly insoluble in water.

Compound **11** was synthesized from 5-methyl-4-phenyl-3-isoxazolol (**18**)[19] (Scheme 2), which in analogy to the syntheses described above was *O*-ethyl protected and then converted into the target compound **11** via intermediates **20** and **21**. However, the bromination of **19** was carried out with NBS, and the final product was obtained as a zwitterion after adjustment of a solution of the hydrobromide salt to pH 3−4 using triethylamine.

Chromatographic Resolution. Compounds **9** and **11** were resolved by chiral HPLC using the Chirobiotic T column,[20] which shows good enantioseparation for

Scheme 1[a]

a: R = (CH$_2$)$_4$-CH$_3$ **17a**: R$_1$ = CH$_3$
b: R = (CH$_2$)$_5$-CH$_3$ **17b**: R$_1$ = CH$_2$CH$_3$
c: R = (CH$_2$)$_6$-CH$_3$ **17c**: R$_1$ = CH$_3$

8: R = (CH$_2$)$_4$-CH$_3$
9: R = (CH$_2$)$_5$-CH$_3$
10: R = (CH$_2$)$_6$-CH$_3$

[a] Reagents: (i) CH$_3$ONa/CH$_3$OH, alkyl bromide; (ii) NH$_2$OH; (iii) NaH, ethyl bromide; (iv) Br$_2$/AcOH; (v) NaH/(R$_1$OOC)$_2$CHNHCOCH$_3$; (vi) 48% HBr.
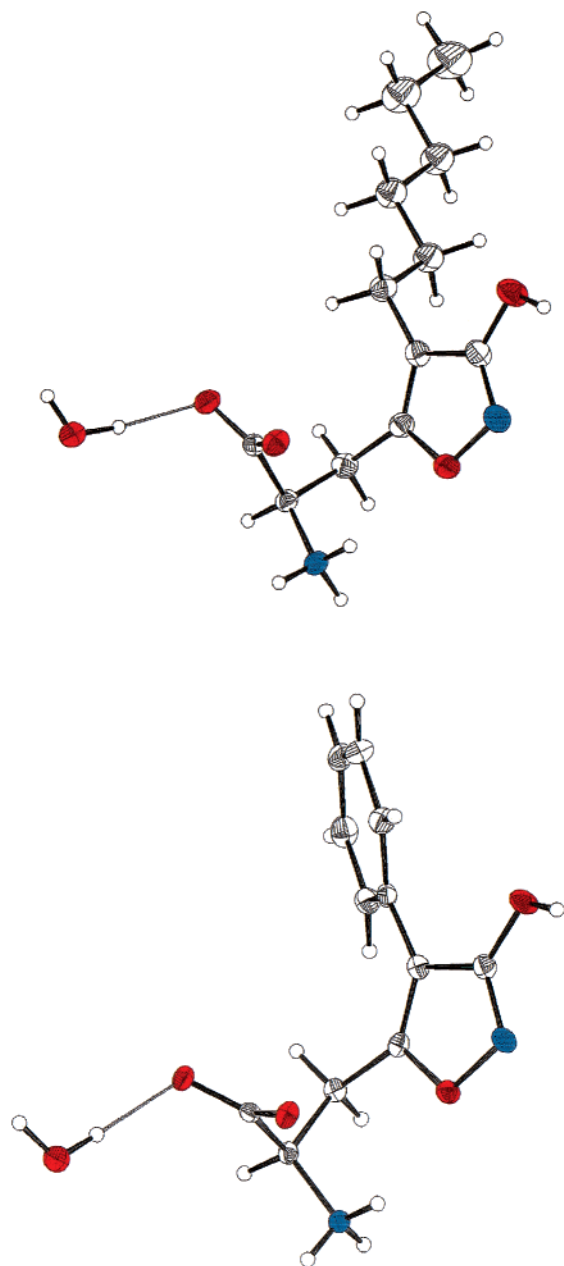
Scheme 2[a]
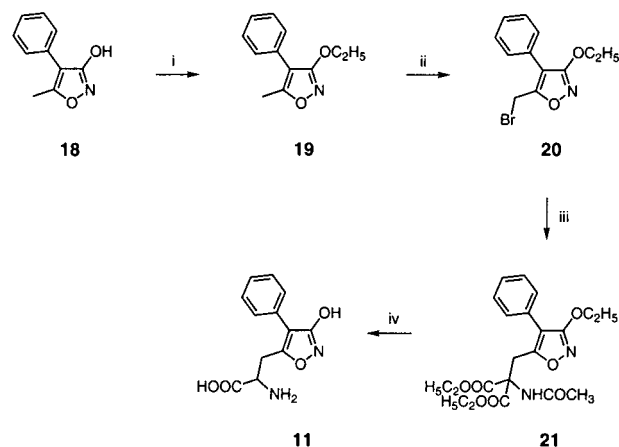
[a] Reagents: (i) K$_2$CO$_3$, ethyl bromide; (ii) NBS; (iii) NaH/(H$_5$C$_2$OOC)$_2$CHNHCOCH$_3$; (iv) 48% HBr.

naturally occurring amino acids as well as 3-isoxazolol acidic amino acids.[21,22] In both chromatographic resolutions, volatile mobile phases could be used, thus facilitating the workup procedure of the pure enantiomers. Upon crystallization, all four enantiomers were obtained with high enantiomeric excess (ee ≥ 98.7%). Determination of the enantiomeric excess of the late eluting (−)-enantiomers on the Chirobiotic T column, (−)-**9** and (−)-**11**, was performed on an analytical column. To precisely determine the enantiomeric excess of the two (+)-enantiomers, (+)-**9** and (+)-**11**, an (*S*)-pipecolic acid ligand-exchange column and a Crownpak CR(+) column, respectively, were used. In general, both of these columns show opposite elution order as compared to that of the Chirobiotic T column.[23,24]

X-ray Crystallographic Analyses. Perspective drawings[25] of the molecular structures of zwitterionic monohydrates of (*R*)-(−)-**9** and (*R*)-(−)-**11** with atomic labeling are depicted in Figure 1. The molecular geometry of the two compounds is comparable, and only minor differences are observed in the molecules due to the different substituents on carbon atom C4. The orientation of the amino acid side chain is slightly different [(−)-**9**: O1−C5−C6−C7 = 67.0(2)°; (−)-**11**: O1−C5−C6−C7 = 47.8(2)°], the hexyl moiety of (−)-**9** is observed in an extended conformation, and the interplanar angle between the phenyl ring and the isoxazole ring of (−)-**11** is 38.94(7)°.

The unit cell and the crystal packing characteristics of the two compounds are comparable. The crystal packings of the two compounds are observed with alternating hydrophobic and hydrophilic layers. The hexyl and the phenyl moieties of **9** and **11**, respectively, are packed in the hydrophobic layers of the crystal packing. Due to the difference in the size of these substituents, the unit cell of (−)-**9** is elongated along the *c*-axis [(−)-**9**: *c* = 35.331(5) Å; (−)-**11**: *c* = 28.603(4) Å]. In the hydrophilic layers the amino acid moieties, the 3-isoxazolol rings, and the water molecules are found.

Configurational Assignment. For (−)-**9** the Flack absolute structure[26,27] parameter for the *R*-configuration was calculated to *x* = 0.00(18) and for the *S*-configuration to *x* = 0.96(16). For (−)-**11** the Flack absolute structure parameter for the *R*-configuration was calcu-

Table 1. Receptor Binding Affinities and Electrophysiological Data from the Cortical Slice Model

	IC$_{50}$ (μM)			EC$_{50}$ (μM)
compd	[³H]AMPA	[³H]CPP	[³H]KA	electrophysiology
(*S*)-HIBO (**4**)^a	0.8 ± 0.4	>100	>100	330 ± 45
5^b	0.32	>100	>100	18
6^c	0.48	>100	>100	17 ± 1
7^d	>100	>100	>100	>500*
8	11 ± 4	>100	>100	630 ± 80
9	>100	>100	>100	>1000
(*S*)-**9**	>100	>100	>100	>1000
(*R*)-**9**	>100	>100	>100	>1000
10	>100	>100	>100	>500*
11	39 ± 3	>100	>100	590 ± 75
(*S*)-**11**	23 ± 5	>100	>100	395 ± 45
(*R*)-**11**	>100	31 ± 4	>100	>1000

^{a} Ref 14. ^{b} Ref 15. ^{c} Ref 17. ^{d} Ref 18. Data represent the mean ± SEM of at least three independent results. *Estimated value. Due to very low solubility compounds **7** and **10** were only tested at 200 μM, at which concentration no significant responses were observed.

types of chiral columns,[17,21–24,28,29] the observed elution orders for the two pairs of enantiomers under investigation are in accordance with the assignments based on the crystallographic analyses.

Additional information on the configuration of the isolated isomers was obtained from the circular dichroism (CD) spectra. In analogy to what has been shown for a number of structurally similar compounds, namely (*S*)-(+)-HIBO (**4**), (*S*)-(+)-Br-HIBO, (*S*)-(+)-Bu-HIBO (**6**), and (*S*)-(+)-2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)-propionic acid [(*S*)-(+)-APPA] (compounds with known absolute configuration from heavy atom method X-ray crystallographic analyses),[14,17,28,30] compounds (+)-**9** and (+)-**11** show a positive Cotton effect at 215–220 nm in acidic solution, strongly supporting the assignment of both these compounds as having the *S*-configuration.

Receptor Binding Affinities. The affinities for iGluRs were determined using [³H]AMPA,[31] [³H]-3-(2-carboxy-4-piperazinyl)propyl-1-phosphonic acid[32] ([³H]-CPP), and [³H]KA[33] as radioligands for AMPA, NMDA, and KA receptors, respectively. (*S*)-HIBO (**4**), **5**, and **6** have previously been shown to possess moderate affinity for the [³H]AMPA binding site (Table 1). Compounds **8** and **11** showed weak affinity for the [³H]AMPA binding site, the latter with the *S*-form as the active one. None of the other compounds synthesized showed significant affinity for the [³H]AMPA binding site. Only (*R*)-**11** showed detectable affinity for the [³H]CPP binding site, whereas all other compounds showed no affinity (IC$_{50}$ > 100 μM) for this binding site or for the [³H]KA binding site.

In Vitro Electrophysiology. The excitatory activity of all compounds were investigated in the rat cortical slice model[34] (Table 1). Compounds **5**[15] and **6**[17] have previously been shown to express potent agonist activity at AMPA receptors, whereas (*S*)-HIBO (**4**) is a weak agonist[14] and **7** is inactive.[18] However, due to very low solubility in the buffer solution, **7** could only be tested up to 200 μM, at which concentration no significant activity was observed. Compounds **8**, **11**, and (*S*)-**11** were found to be weak agonists at AMPA receptors, whereas **9**, (*S*)-**9**, (*R*)-**9**, **10**, and (*R*)-**11** were found to be inactive (EC$_{50}$ > 1 mM). In analogy with **7**, compound **10** was, due to low solubility, tested at concentrations up to 200 μM. All compounds showing no agonist

Figure 1. Perspective drawings[25] of (*R*)-**9** (top) and (*R*)-**11** (bottom). Displacement ellipsoids enclose 50% probability. Hydrogen atoms are represented by spheres of arbitrary size. The hydrogen bonds observed between the compounds and the water molecules are indicated by thin lines.

lated to x = 0.00(17) and for the *S*-configuration to x = 0.97(16). These results strongly suggest that (−)-**9** and (−)-**11** both possess the *R*-configuration. However, a distinct statement is not possible due to the high standard deviation of the Flack parameter.

To support the configurational assignment based on the X-ray analyses, the elution orders of the isomers were studied on three analytical HPLC columns relying on three different modes of chiral recognition. On the Chirobiotic T and Crownpak CR(−) columns the two (+)-enantiomers, compounds (+)-**9** and (+)-**11**, elute before the corresponding (−)-enantiomers, whereas opposite elution orders were seen on the (*S*)-pipecolic acid ligand-exchange column. On the basis of the elution orders for a large number of natural α-amino acids and 3-isoxazolol-containing α-amino acids studied on these three

Table 2. Pharmacological Effects at Cloned mGluRs Expressed in Chinese Hamster Ovary Cells

	K_b (μM)			
compd	$mGlu_{1\alpha}$	$mGlu_{5a}$	$mGlu_2$	$mGlu_{4a}$
(S)-HIBO (4)[a]	250 ± 27	490 ± 8	>1000	>1000
5[a]	190 ± 18	180 ± 37	>1000	>1000
6[b]	110 ± 3	97 ± 9	>1000	>1000
7	>100	nd	>100	>100
8	140 ± 17	190 ± 51	>1000	>1000
9	140 ± 40	110 ± 16	>1000	>1000
(S)-9	30 ± 5	61 ± 11	>1000	>1000
(R)-9	>1000	>1000	>1000	>1000
10	160 ± 19	990 ± 290*	>1000	>1000
11	160 ± 10	nd	>1000	>1000
(S)-11	86 ± 2	90 ± 15	>1000	>1000
(R)-11	>1000	>1000	>1000	>1000

[a] Ref 16. [b] Ref 17. nd, not determined. Data represent the mean ± SEM of at least two independent results. *Estimated from partial concentration−response curves.

activity were also tested for antagonist activity (at 1 mM; compounds **7** and **10** at 100 μM) toward AMPA (5 μM)-, NMDA (10 μM)-, and KA (5 μM)-induced responses, and no significant antagonist effects were observed for any of these compounds.

Effects at mGluRs. The interaction of the compounds with cloned mGluRs was measured using second-messenger assays.[16] The activities were determined at group I mGluRs ($mGlu_{1\alpha}$ and $mGlu_{5a}$) and at $mGlu_2$ and $mGlu_{4a}$ as representatives of groups II and III, respectively. (S)-HIBO (**4**), **5**, and **6** have previously been shown to exhibit antagonist effects at $mGlu_1$ and $mGlu_5$[16,17] (Table 2). Similar antagonist effects at group I mGluRs were found for **8**, **9**, (S)-**9**, **11**, and (S)-**11**, the most potent antagonist being (S)-**9** (K_b = 30 and 61 μM at $mGlu_1$ and $mGlu_5$, respectively). In contrast, compound **10** was found to be a selective antagonist at $mGlu_1$, showing a very weak effect at $mGlu_5$. None of the compounds tested showed any agonist or antagonist activity at $mGlu_2$ or $mGlu_{4a}$. The R-forms of **9** and **11** were inactive at all receptor subtypes as both agonists and antagonists (Table 2).

Anticonvulsant Activity. Compound **9** was tested for anticonvulsant activity against NMDA-induced convulsions in mice. NMDA was given iv 20 mg/kg, and compound **9**, administered 15 min before NMDA, antagonized the convulsions completely at 12.5 and 6.3 μg/mouse when given icv. Compound **9** did not induce any behavioral changes at these doses. When administered sc (up to 50 mg/kg) 30 min before NMDA, no anticonvulsant activity of **9** was observed toward the NMDA-induced convulsions.

Discussion

There is accumulating evidence to suggest that mGluR ligands may have therapeutic potential in certain neurologic and psychiatric diseases.[8,9] Notably, antagonists at group I mGluRs have shown anticonvulsant activity, neuroprotective properties, or analgesic effects in animal models.[35−41] Thus, selective antagonists for group I mGluRs have considerable pharmacological interest.

HIBO [(RS)-**4**] and a few 4-alkyl-substituted HIBO analogues have previously been synthesized and shown to be agonists at AMPA receptors and antagonists at group I mGluRs. Compounds **5** and **6** were both potent AMPA receptor agonists and antagonists at group I
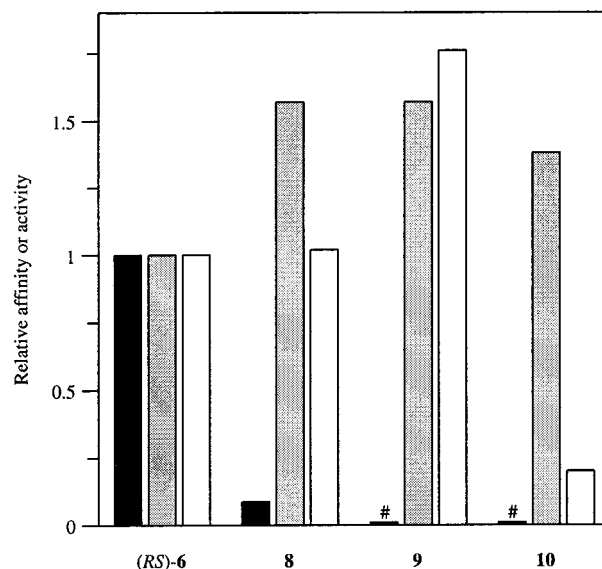
Figure 2. Histograms illustrating the differences in affinity toward [^{3}H]AMPA (black bars) binding and activities at $mGlu_1$ (gray bars) and $mGlu_5$ (white bars) for (RS)-**6**, **8**, **9**, and **10**. The respective affinity and activities of (RS)-**6** are estimated to be twice the values given in Tables 1 and 2 for the S-form (**6**), and these values are set to 1.0; the values for **8**−**10** are normalized relative to this. # < 0.05.

mGluRs (Tables 1 and 2), whereas no activity at iGluRs was observed for the octyl analogue **7**. This indicates that the tolerance for the size of substituents in the 4-position of HIBO analogues is limited. In the search for selective antagonists at group I mGluRs, a new series of HIBO analogues has now been synthesized, namely the pentyl-, hexyl-, heptyl-, and phenyl-substituted analogues, compounds **8**−**11**, respectively. For compounds **8** and **11** a dramatic loss in activity at AMPA receptors compared to **6**[17] was observed, and **9** and **10** did not show detectable AMPA receptor activity. In contrast, these analogues showed antagonist activity at group I mGluRs. Compounds **8**, **9**, and **11** showed approximately equipotent effects at $mGlu_1$ and $mGlu_5$ receptors.

Resolution of **9** and **11** by chiral HPLC afforded the enantiomers (ee ≥ 98.7%) of the two compounds. The absolute configurations were established by X-ray crystallographic analyses, elution orders in three chiral HPLC systems, and CD spectra. The antagonist effects were shown to reside in the S-forms, and (S)-**9** was found to be the most potent $mGlu_1$ and $mGlu_5$ antagonist of the compounds tested. Compound **10** showed a different and quite interesting pharmacological profile, being an antagonist at $mGlu_1$, equipotent with **8** and **9**, but with very low effect at $mGlu_5$. Thus, **10** is a rather selective antagonist at $mGlu_1$, showing weak or no effect at other mGluRs or iGluRs.

A comparison of the affinity for AMPA receptors and the activity at $mGlu_1$ and $mGlu_5$ receptors of (RS)-**6**, **8**, **9**, and **10** is illustrated in Figure 2. The affinities for AMPA receptors disappear with increasing length of the alkyl side chains. The effects at $mGlu_1$ are of the same order of magnitude for the four compounds, whereas at $mGlu_5$ the activity peaks for **9** and is almost lost for **10**.

Compound **10** showed limited solubility in water and buffer solutions in agreement with what was previously observed for compound **7**.[18] However, the solubility of **9**

was high enough to enable testing at concentrations up to 1 mM, and it was decided to study **9** for anticonvulsant activity. Compound **9** antagonized NMDA-induced convulsions when given icv, whereas no activity was observed when **9** was given sc. This indicates that **9** is not capable of penetrating the blood−brain barrier and/or that **9** is metabolized in vivo to an extent that prevents sufficient concentrations to be reached in the CNS.

In conclusion, new selective group I mGluR antagonists have been synthesized and characterized. (*S*)-**9** is the most potent antagonist in this series at mGlu$_1$ and mGlu$_5$, whereas **10** was selective at mGlu$_1$, showing very low effect at mGlu$_5$ and no activity at other GluRs. The results of the present structure−activity studies seem to suggest that the group I mGlu$_1$ and mGlu$_5$ receptor recognition sites contain lipophilic pockets of slightly different sizes, which may be exploited in the attempts to design mGlu$_1$- and mGlu$_5$-selective antagonists.

Experimental Section

Chemistry. General Procedures. Compounds were visualized on TLC plates (Merck silica gel 60 F$_{254}$) using UV light or a KMnO$_4$ spraying reagent. Column chromatography (CC) was performed using silica gel C60-H (230−400 mesh, Rhône-Poulenc). Compounds containing amino groups were visualized using a ninhydrin spraying reagent. Melting points were determined in capillary tubes and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC-200F (200 MHz) or Varian Gemini-2000 BB spectrometer (300 MHz). *J* values are given in hertz (Hz). Chemical shifts (δ) are given in ppm relative to TMS for compounds dissolved in CDCl$_3$. Elemental analyses were performed at Analytical Research Department, H. Lundbeck A/S, Denmark, or at Microanalytical Laboratory at the Department of Physical Chemistry, University of Vienna, Austria, and were within ±0.4% of the theoretical values unless otherwise stated. IR spectra were recorded from KBr disks on a Perkin-Elmer 781 grating infrared spectrophotometer. Optical rotations were measured in thermostated cuvettes on a Perkin-Elmer 241 polarimeter. Circular dichroism (CD) spectra were recorded in 0.1 M HCl in 1.0-cm cuvettes at room temperature on a Jasco J-720 spectropolarimeter.

Methyl (*RS*)-2-Acetylheptanoate (13a). Sodium (4.3 g, 187 mmol) was dissolved in MeOH (100 mL) and methyl acetoacetate (40 mL, 373 mmol) added over 15 min. The solution was cooled to 0 °C and *n*-pentyl bromide (23.5 mL, 186 mmol) added over 10 min at 0 °C. The reaction mixture was stirred overnight at 50 °C and then evaporated. Water (100 mL) was added and extracted with CH$_2$Cl$_2$ (4 × 100 mL). The organic phases were dried (MgSO$_4$) and evaporated, and the residue was distilled (0.3 mmHg, 78−92 °C) using a vigreaux column, which gave **13a** (20.0 g, 58%) as an oil: ^{1}H NMR (CDCl$_3$) δ 3.7 (s, 3H), 3.4 (t, *J* = 7 Hz, 1H), 2.2 (s, 3H), 1.8 (m, 2H), 1.3 (m, 6H), 0.9 (t, *J* = 7 Hz, 3H).

Methyl (*RS*)-2-Acetyloctanoate (13b). Prepared according to the procedure described for compound **13a**. **13b** (12.0 g, 32%) was obtained after distillation (0.2 mmHg, 62−74 °C): ^{1}H NMR (CDCl$_3$) δ 3.7 (s, 3H), 3.4 (t, *J* = 7.3 Hz, 1H), 2.2 (s, 3H), 1.85 (m, 2H), 1.3 (m, 8H), 0.9 (t, *J* = 7.1 Hz, 3H).

Methyl (*RS*)-2-Acetylnonanoate (13c). Prepared according to the procedure described for compound **13a**. **13c** (22.3 g, 56%) was obtained after distillation (0.3 mmHg, 94−95 °C): ^{1}H NMR (CDCl$_3$) δ 3.7 (s, 3H), 3.4 (t, *J* = 7 Hz, 1H), 2.2 (s, 3H), 1.8 (m, 2H), 1.3 (m, 10H), 0.9 (t, *J* = 7 Hz, 3H).

5-Methyl-4-pentyl-3-isoxazolol (14a). To a solution of NH$_2$OH·HCl (2.9 g, 42 mmol) in MeOH (10 mL) heated to 60 °C was added a solution of NaOH (1.7 g, 42 mmol) in water (1 mL) and MeOH (10 mL). The mixture was cooled to −50 °C. To a solution of **13a** (3.72 g, 20 mmol) in MeOH (4 mL) was

added a solution of NaOH (0.84 g, 21 mmol) in water (0.5 mL) and MeOH (4 mL). This mixture was cooled to −50 °C, stirred for 10 min, and added to the above NH$_2$OH solution. The reaction mixture was stirred at −50 °C for 2.5 h and acetone (2.1 g) was added. The reaction mixture was then quickly added to 4 M HCl (24 mL) heated to 85 °C and stirred for 45 min at 85 °C. The MeOH was evaporated and CC (toluene−AcOEt−AcOH 20:2:1) gave **14a** (1.2 g, 65%) as an oil: ^{1}H NMR (CDCl$_3$) δ 2.26 (t, *J* = 7.1 Hz, 2H), 2.24 (s, 3H), 1.52 (quintet, *J* = 7.1 Hz, 2H), 1.30 (m, 4H), 0.89 (t, *J* = 7.1 Hz, 3H).

4-Hexyl-5-methyl-3-isoxazolol (14b). Prepared according to the procedure described for compound **14a**. **14b** (3.7 g, 58%) was obtained as an oil after CC: ^{1}H NMR (CDCl$_3$) δ 2.27 (t, *J* = 7.1 Hz, 2H), 2.24 (s, 3H), 1.52 (quintet, *J* = 7.1 Hz, 2H), 1.29 (m, 6H), 0.88 (t, *J* = 7.1 Hz, 3H).

4-Heptyl-5-methyl-3-isoxazolol (14c). Prepared according to the procedure described for compound **14a**. **14c** (3.1 g, 75%) was obtained as an oil after CC: ^{1}H NMR (CDCl$_3$) δ 2.27 (t, *J* = 7.1 Hz, 2H), 2.24 (s, 3H), 1.52 (quintet, *J* = 7.1 Hz, 2H), 1.30 (m, 8H), 0.89 (t, *J* = 7.1 Hz, 3H).

3-Ethoxy-5-methyl-4-pentylisoxazole (15a). NaH (620 mg, 60%, 15.4 mmol) was added to a solution of **14a** (2.18 g, 12.9 mmol) in dry DMF (50 mL) and the mixture stirred for 45 min. Ethyl bromide (1.6 mL, 20.9 mmol) was added over a period of 10 min and the reaction mixture stirred at room temperature overnight. After evaporation and addition of water (100 mL) the mixture was extracted with AcOEt (3 × 120 mL). The organic phases were dried (MgSO$_4$), evaporated and CC (toluene−AcOEt 5:1) of the residue gave **15a** (1.2 g, 47%) as an oil: ^{1}H NMR (CDCl$_3$) δ 4.25 (q, *J* = 7 Hz, 2H), 2.22 (s, 3H), 2.20 (t, *J* = 7 Hz, 2H), 1.47 (m, 2H), 1.40 (t, *J* = 7 Hz, 3H), 1.27 (m, 4H), 0.87 (t, *J* = 7 Hz, 3H).

3-Ethoxy-4-hexyl-5-methylisoxazole (15b). Prepared according to the procedure described for compound **15a**. **15b** (1.6 g, 45%) was obtained as an oil after CC: ^{1}H NMR (CDCl$_3$) δ 4.25 (q, *J* = 7 Hz, 2H), 2.22 (s, 3H), 2.20 (t, *J* = 7 Hz, 2H), 1.45 (m, 2H), 1.40 (t, *J* = 7 Hz, 3H), 1.24 (m, 6H), 0.87 (t, *J* = 7 Hz, 3H).

3-Ethoxy-4-heptyl-5-methylisoxazole (15c). Prepared according to the procedure described for compound **15a**. **15c** (2.1 g, 59%) was obtained as an oil after CC: ^{1}H NMR (CDCl$_3$) δ 4.27 (q, *J* = 7 Hz, 2H), 2.22 (s, 3H), 2.20 (t, *J* = 7 Hz, 2H), 1.48 (m, 2H), 1.37 (t, *J* = 7 Hz, 3H), 1.27 (m, 8H), 0.87 (t, *J* = 7 Hz, 3H).

5-Bromomethyl-3-ethoxy-4-pentylisoxazole (16a). To a solution of **15a** (1.20 g, 6.1 mmol) in CCl$_4$ (5 mL) and AcOH (2 mL) was added bromine (2 g, 13 mmol). The reaction mixture was left stirring at room temperature for 6 days. Water (150 mL) was added and NaHSO$_3$ until no bromine color was left. The solution was extracted with CH$_2$Cl$_2$ (3 × 150 mL), dried and evaporated. CC (toluene−AcOEt 20:1) gave **16a** (440 mg, 26%) as an oil: ^{1}H NMR (CDCl$_3$) δ 4.33 (s, 2H), 4.32 (q, *J* = 7 Hz, 2H), 2.22 (t, *J* = 7 Hz, 2H), 1.48 (quintet, *J* = 7 Hz, 2H), 1.40 (t, *J* = 7 Hz, 3H), 1.28 (m, 4H), 0.89 (t, *J* = 7 Hz, 3H).

5-Bromomethyl-3-ethoxy-4-hexylisoxazole (16b). Prepared according to the procedure described for compound **16a**. **16b** (1.5 g, 73%) was obtained after CC as an oil: ^{1}H NMR (CDCl$_3$) δ 4.33 (s, 2H), 4.32 (q, *J* = 7 Hz, 2H), 2.29 (t, *J* = 7 Hz, 2H), 1.54 (m, 2H), 1.41 (t, *J* = 7 Hz, 3H), 1.30 (m, 6H), 0.89 (t, *J* = 7 Hz, 3H).

5-Bromomethyl-3-ethoxy-4-heptylisoxazole (16c). Prepared according to the procedure described for compound **16a**. **16c** (300 mg, 69%) was obtained after CC as an oil: ^{1}H NMR (CDCl$_3$) δ 4.33 (s, 2H), 4.32 (q, *J* = 7 Hz, 2H), 2.30 (t, *J* = 7 Hz, 2H), 1.53 (quintet, *J* = 7 Hz, 2H), 1.40 (t, *J* = 7 Hz, 3H), 1.28 (m, 8H), 0.89 (t, *J* = 7 Hz, 3H).

Methyl 2-Acetamido-3-(3-ethoxy-4-pentyl-5-isoxazolyl)-2-(methoxycarbonyl)propionate (17a). To a solution of dimethyl acetamidomalonate (650 mg, 1.8 mmol) in dry DMF (5 mL) was added NaH (75 mg, 60%, 1.8 mmol). After stirring for 30 min a solution of **16a** (440 mg, 1.6 mmol) in dry DMF (5 mL) was added. The reaction mixture was stirred at room temperature overnight, evaporated and the residue added water (25 mL) and extracted with AcOEt (3 × 25 mL). The

organic phases were extracted with 1 M NaOH (25 mL), water (2 × 25 mL), dried (MgSO₄) and evaporated. CC (toluene–AcOEt 6:1) gave after recrystallization (toluene–light petroleum) **17a** (180 mg, 29%) as colorless crystals: mp 105.5–106.5 °C; ¹H NMR (CDCl₃) δ 6.73 (s, 1H), 4.25 (q, *J* = 7 Hz, 2H), 3.82 (s, 6H), 3.70 (s, 2H), 2.13 (t, *J* = 7 Hz, 2H), 2.00 (s, 3H), 1.42 (m, 2H), 1.40 (t, *J* = 7 Hz, 3H), 1.28 (m, 4H), 0.89 (t, *J* = 7 Hz, 3H). Anal. ($C_{18}H_{28}N_2O_7$) C, H, N.

Ethyl 2-Acetamido-2-ethoxycarbonyl-3-(3-ethoxy-4-hexyl-5-isoxazolyl)propionate (17b). Prepared according to the procedure described for compound **17a** using diethyl acetamidomalonate. **17b** (600 mg, 45%) was obtained after CC and recrystallization as colorless crystals: mp 54.0–56.5 °C; ¹H NMR (CDCl₃) δ 6.75 (s, 1H), 4.29 (q, *J* = 7 Hz, 4H), 4.25 (q, *J* = 7 Hz, 2H), 3.71 (s, 2H), 2.15 (t, *J* = 7 Hz, 2H), 1.99 (s, 3H), 1.40 (m, 2H), 1.36 (t, *J* = 7 Hz, 3H), 1.29 (t, *J* = 7 Hz, 6H), 1.26 (m, 6H), 0.87 (t, *J* = 7 Hz, 3H). Anal. ($C_{21}H_{34}N_2O_7$) C, H, N.

Methyl 2-Acetamido-3-(3-ethoxy-4-heptyl-5-isoxazolyl)-2-(methoxycarbonyl)propionate (17c). Prepared according to the procedure described for compound **17a**. **17c** (310 mg, 52%) was obtained after CC and recrystallization as colorless crystals: mp 84.0–86.0 °C; ¹H NMR (CDCl₃) δ 6.75 (s, 1H), 4.26 (q, *J* = 7 Hz, 2H), 3.83 (s, 6H), 3.71 (s, 2H), 2.13 (t, *J* = 7 Hz, 2H), 2.00 (s, 3H), 1.41 (m, 2H), 1.39 (t, *J* = 7 Hz, 3H), 1.25 (m, 8H), 0.87 (t, *J* = 7 Hz, 3H). Anal. ($C_{20}H_{32}N_2O_7$) C, H, N.

(RS)-2-Amino-3-(3-hydroxy-4-pentyl-5-isoxazolyl)propionic Acid (8). A mixture of **17a** (180 mg, 0.47 mmol) in concentrated hydrobromic acid (48%, 10 mL) was refluxed for 1 h. The reaction mixture was evaporated to dryness and reevaporated after addition of water (10 mL). The crystalline residue was washed with hot AcOEt and subsequently with hot water to give **8** (76 mg, 67%) as colorless crystals: mp 221–226 °C dec; ¹H NMR (NaOD, D₂O) δ 3.48 (dd, *J* = 5.1 and 7.8 Hz, 1H), 2.88 (dd, *J* = 5.1 and 14.7 Hz, 1H), 2.76 (dd, *J* = 7.8 and 14.7 Hz, 1H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.40 (quintet, *J* = 7.3 Hz, 2H), 1.23 (m, 4H), 0.82 (t, *J* = 6.9 Hz, 3H). Anal. ($C_{11}H_{18}N_2O_4$) C, H, N.

(RS)-2-Amino-3-(4-hexyl-3-hydroxy-5-isoxazolyl)propionic Acid (9). Prepared according to the procedure described for compound **8**. **9** (290 mg, 80%) was obtained as colorles crystals: mp 207–219 °C dec; ¹H NMR (NaOD, D₂O) δ 3.55 (dd, *J* = 4.8 and 8.4 Hz, 1H), 2.97 (dd, *J* = 4.8 and 14.7 Hz, 1H), 2.81 (dd, *J* = 8.4 and 14.7 Hz, 1H), 2.24 (t, *J* = 7.3 Hz, 2H), 1.48 (m, 2H), 1.32 (m, 6H), 0.90 (t, *J* = 6.8 Hz, 3H). Anal. ($C_{12}H_{20}N_2O_4$) C, H, N.

(RS)-2-Amino-3-(4-heptyl-3-hydroxy-5-isoxazolyl)propionic Acid (10). Prepared according to the procedure described for compound **8**. **10** (52 mg, 70%) was obtained as colorles crystals: mp 221–227 °C dec; ¹H NMR (NaOD, D₂O) δ 3.48 (dd, *J* = 5.4 and 7.8 Hz, 1H), 2.88 (dd, *J* = 5.4 and 14.7 Hz, 1H), 2.76 (dd, *J* = 7.8 and 14.7 Hz, 1H), 2.15 (t, *J* = 7.3 Hz, 2H), 1.40 (m, 2H), 1.23 (m, 8H), 0.81 (t, *J* = 6.8 Hz, 3H). Anal. ($C_{13}H_{22}N_2O_4 \cdot 0.5H_2O$) C, H, N.

3-Ethoxy-5-methyl-4-phenylisoxazole (19). To a solution of 5-methyl-4-phenyl-3-isoxazolol[19] (**18**) (1.8 g, 10.27 mmol) in acetone (60 mL) was added potassium carbonate (2.8 g, 20.54 mmol) and the mixture was stirred at 60 °C for 30 min. Ethyl bromide (1.68 g, 15.4 mmol) was added in quarter portions at 15-min intervals. The reaction mixture was refluxed for 4 h and then cooled, filtered and evaporated. CC (toluene–AcOEt 19:1) gave **19** (1.4 g, 67%) as an oil: ¹H NMR (CDCl₃) δ 7.7–7.5 (m, 5H), 4.37 (q, *J* = 7.1 Hz, 2H), 2.44 (s, 3H), 1.43 (t, *J* = 7.1 Hz, 3H).

5-Bromomethyl-3-ethoxy-4-phenylisoxazole (20). To a solution **19** (1.1 g, 5.41 mmol) in CCl₄ (25 mL) were added, in quarter portions at 90-min intervals, dibenzoyl peroxide (100 mg) and NBS (1.06 g, 5.95 mmol). The mixture was refluxed for 15 h and cooled. Light petroleum (25 mL) was added and the solution was filtered and evaporated. CC (toluene) gave **20** (1.1 g, 72%) as an oil: ¹H NMR (CDCl₃) δ 7.3–7.6 (m, 5H), 4.45 (s, 2H), 4.40 (q, *J* = 7.1 Hz, 2H), 1.44 (t, *J* = 7.1 Hz, 3H).

Ethyl 2-Acetamido-2-ethoxycarbonyl-3-(3-ethoxy-4-phenyl-5-isoxazolyl)propionate (21). To a stirred suspension of NaH (57 mg, 80%, 1.98 mmol) in dry DMF (6 mL), was added, at room temperature, diethyl acetamidomalonate (430 mg, 1.98 mmol), and this solution was stirred for 15 min. **20** (560 mg, 1.98 mmol) dissolved in dry DMF (2 mL) was dropwise added and the mixture was stirred at room temperature for 24 h. The resulting solution was evaporated, diluted with water and extracted with AcOEt. The organic layer was dried (MgSO₄), evaporated and CC (toluene–AcOEt 4:1 and 1% AcOH) gave **21** (500 mg, 60%) after recrystallization (AcOEt–light petroleum): mp 129–130 °C; ¹H NMR (CDCl₃) δ 7.2–7.5 (m, 5H), 6.62 (br s, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 4.0–4.2 (m, 4H), 3.96 (s, 2H), 1.65 (s, 3H), 1.40 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 6H). Anal. ($C_{21}H_{26}N_2O_7$) C, H, N.

(RS)-2-Amino-3-(3-hydroxy-4-phenyl-5-isoxazolyl)propionic Acid (11). **21** (450 mg, 1.07 mmol) was dissolved in 48% hydrobromic acid (15 mL) and refluxed at 140 °C for 30 min. The solution was rapidly cooled and evaporated, then twice dissolved in water and re-evaporated. After drying in vacuo (over KOH) the crude hydrobromide salt was dissolved in water (500 mL) and EtOH (300 mL) and pH adjusted to 3–4 with a solution of triethylamine in EtOH. The precipitate was recrystallized from water to give zwitterionic **11** (226 mg, 85%): mp 220 °C dec; ¹H NMR (D₂O) δ 7.1–7.5 (m, 5H), 3.40 (dd, *J* = 5.6 and 8.0 Hz, 1H), 2.90 (dd, *J* = 5.6 and 14.7 Hz, 1H), 2.73 (dd, *J* = 8.0 and 14.7 Hz, 1H). Anal. ($C_{12}H_{12}N_2O_4 \cdot 0.25H_2O$) C, H, N.

Chiral Liquid Chromatography. Analytical ligand-exchange HPLC was performed on a column (120 × 4.6 mm) containing (*S*)-pipecolic acid chemically bound following a procedure used for attachment of (*S*)-proline to a silica-based packing material.[42] The column was connected to an HPLC instrumentation consisting of a Waters M510 pump, a Waters U6K injector, and a Waters 991 photodiode array detector with a detection wavelength set at 220 nm. The column was thermostated at 50 °C using a LKB 2155 HPLC column oven and eluted at 1.0 mL/min with aqueous KH₂PO₄ (50 mM, pH 4.5) containing CuSO₄ (0.12 mM). Analytical HPLC using the Chirobiotic T column (150 × 4.6 mm) was performed at room temperature. The column was connected to the same HPLC instrumentation as described above for the ligand-exchange column and was eluted at 0.5 mL/min. For (*R*)-**9** the mobile phase consisted of an aqueous NH₄OAc/AcOH buffer (15 mM, pH 4.0) and EtOH (70:30), whereas for (*R*)-**11** aqueous AcOH (pH 4.0) and EtOH (60:40) was used. For the determination of the elution order of the enantiomers of **9** and **11** on the Crownpak CR(−) column (150 × 4 mm) the column was eluted at 0.4 mL/min with aqueous HClO₄ (pH 3.9) at 50 °C or water at 45 °C, respectively. Analytical HPLC using the Crownpak CR(+) column (150 × 10 mm) was performed at 45 °C eluting with aqueous trifluoroacetic acid (pH 3) at 2 mL/min. The HPLC instrumentation used for both Crownpak CR columns consisted of a Jasco 880-PU pump, a Rheodyne injector model 7125 equipped with a 5.0-mL loop, and a Waters model 480 detector set at 210 nm. Preparative chiral chromatographic separations were performed at room temperature on a Chirobiotic T column (500 × 10 mm) equipped with a guard column (50 × 4.6 mm). The column was connected to the same HPLC instrumentation as described above for the Crownpak CR columns.

(S)-(+)-2-Amino-3-(4-hexyl-3-hydroxy-5-isoxazolyl)propionic Acid [(S)-9] and (R)-(−)-2-Amino-3-(4-hexyl-3-hydroxy-5-isoxazolyl)propionic Acid [(R)-9]. Compound **9** (140 mg, 0.55 mmol) was dissolved in a mixture of an aqueous NH₄OAc/AcOH buffer (300 mM, pH 6.0) and EtOH (30:70) (110 mL), passed through a Millex HV filter (45 μm, Millipore), and resolved on the Chirobiotic T column (500 × 10 mm) in approximately 6 mg injections eluting the column with a mixture of an aqueous NH₄OAc/AcOH buffer (100 mM, pH 4.0) and EtOH (80:20) at 1.1 mL/min with a detection wavelength at 210 nm. Each run was divided into two main fractions. The combined fractions of each of the resolved enantiomers were evaporated, reevaporated three times from water and finally

dried in vacuo. Recrystallization (water) of the first eluting (+)-enantiomer afforded compound (*S*)-**9** (42.2 mg, 57%): mp 212−220 °C dec; ee = 99.7%; $[\alpha]^{25}_{589}$ = +18.9 (*c* = 0.173, 1.0 M HCl); $\Delta\epsilon$ (220 nm) = +0.20 m²/mol. Anal. ($C_{12}H_{20}N_2O_4$·0.75H_2O) C, N; H: calcd, 8.03; found, 7.57. Recrystallization (water) of the second eluting (−)-enantiomer gave compound (*R*)-**9** (36.5 mg, 48%): mp 212−220 °C dec; ee = 98.7%; $[\alpha]^{25}_{589}$ = −16.5 (*c* = 0.194, 1.0 M HCl); $\Delta\epsilon$ (220 nm) = −0.20 m²/mol. Anal. ($C_{12}H_{20}N_2O_4$·H_2O) C, N; H: calcd, 8.08; found, 7.59.

(*S*)-(+)-2-Amino-3-(3-hydroxy-4-phenyl-5-isoxazolyl)-propionic Acid [(*S*)-11] and (*R*)-(−)-2-Amino-3-(3-hydroxy-4-phenyl-5-isoxazolyl)propionic Acid [(*R*)-11]. Compound **11** (124 mg, 0.489 mmol) was dissolved in aqueous ammonia (10 mM) in a concentration of 2 mg/mL, filtered through a Millex HV filter (0.45 mm, Millipore) and resolved in 4 mg injections on the Chirobiotic T column. The column was eluted with a mixture of aqueous AcOH (pH 4) and EtOH (60:40) at 1.5 mL/min with a detection wavelength at 254 nm. The collected fractions of the first peak were pooled, evaporated, and reevaporated twice from water. After drying in vacuo, the crystalline residue was recrystallized (water) to give (*S*)-**11** (51.9 mg, 80%): mp 223−224 °C dec; ee = 99.9%; $[\alpha]^{25}_{589}$ = +28.2° (*c* = 0.39, 0.1 M HCl); $\Delta\epsilon$ (214 nm) = +0.23 m²/mol; IR 3450 (m), 3220 (m), 3100−2600 (multiple, m), 1600 (s), 1520 (s) cm^{-1}. Anal. ($C_{12}H_{12}N_2O_4$·H_2O) C, H, N. The collected fractions of the second peak were treated as described for the first eluting enantiomer to give (*R*)-**11** (50.1 mg, 77%): mp 226−227 °C dec; ee = 99.7%; $[\alpha]^{25}_{589}$ = −29.3° (*c* = 0.39, 0.1 M HCl); $\Delta\epsilon$ (214 nm) = −0.25 m²/mol; IR spectrum of (*R*)-**11** was identical with that of (*S*)-**11**.

X-ray Crystallographic Analysis of (*R*)-(−)-9 Monohydrate. Colorless single crystals were obtained from a solution in water. Crystal data: $C_{12}H_{20}N_2O_4$·H_2O, M_r = 274.32, orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 5.3916(9) Å, *b* = 7.8160(10) Å, *c* = 35.331(5) Å, *V* = 1488.9(4) Å³, *Z* = 4, D_c = 1.224 Mg m^{-3}, *F*(000) = 592, μ(Cu Kα) = 0.795 mm^{-1}, *T* = 122.0(5) K, crystal dimensions = 0.16 × 0.09 × 0.08 mm.

Data Collection and Processing. Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu Kα radiation (λ = 1.54184 Å).[43] Intensities were collected using the $\omega/2\theta$ scan mode. Unit cell dimensions were determined by least-squares refinement of 24 reflections (θ range 31.65−37.18°).[43] The reflections were measured in the range −6 < *h* < 4, −9 < *k* < 9, −43 < *l* < 44 (2.50° < θ < 74.85°). Data were reduced using the programs of Blessing (DREADD).[44,45] The intensities of five standard reflections were monitored every 10⁴ s (decay 3.1%, corrected). Absorption correction was applied using the program ABSORB (T_{min} = 0.908; T_{max} = 0.945).[46] A total of 11327 reflections were averaged according to the point group symmetry 222 resulting in 3067 unique reflections (R_{int} = 0.0353 on F_o^2).

Structure Solution and Refinement. The structure was solved by the direct method using the program SHELXS97[47,48] and refined using the program SHELXL97.[27] Full matrix least-squares refinement on F^2 was performed, minimizing $\Sigma w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with fixed isotropic displacement parameters. Correction for extinction was applied (coefficient: 0.0022(2)). The refinement (240 parameters, 3067 reflections) with the molecule having the *R*-configuration converged at R_F = 0.0279, wR_F^2 = 0.0656 for 2772 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma^2(F_o^2) + (0.0289P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.053. In the final difference Fourier map maximum and minimum electron densities were 0.167 and −0.144 e Å^{-3}, respectively. Refinement of the Flack absolute structure factor *x* in the final refinement gave *x* = 0.00(18).[26,27] The large standard deviation of *x* is due to the atom types present in the compound (C, H, N, and O), which produce minor anomalous scattering. Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.[27,49]

X-ray Crystallographic Analysis of (*R*)-(−)-11 Monohydrate. Colorless single crystals were obtained from a solution in water. Crystal data: $C_{12}H_{12}N_2O_4$·H_2O, M_r = 266.25, orthorhombic, space group $P2_12_12_1$ (No. 19), *a* = 5.4161(7) Å, *b* = 8.0495(9) Å, *c* = 28.603(4) Å, *V* = 1247.0(3) Å³, *Z* = 4, D_c = 1.418 Mg m^{-3}, *F*(000) = 560, μ(Cu Kα) = 0.948 mm^{-1}, *T* = 122.0(5) K, crystal dimensions = 0.28 × 0.10 × 0.04 mm.

Data Collection and Processing. Diffraction data were collected on an Enraf-Nonius CAD-4 diffractometer using graphite monochromated Cu Kα radiation (λ = 1.54184 Å).[43] Intensities were collected using the $\omega/2\theta$ scan mode. Unit cell dimensions were determined by least-squares refinement of 20 reflections (θ range 38.81−40.26°).[43] The reflections were measured in the range −6 < *h* < 6, −10 < *k* < 10, −35 < *l* < 35 (3.09° < θ < 74.86°). Data were reduced using the programs of Blessing (DREADD).[44,45] The intensities of five standard reflections were monitored every 10⁴ s (decay 4.1%, corrected). Absorption correction was applied using the program ABSORB (T_{min} = 0.860; T_{max} = 0.965).[46] A total of 6715 reflections were averaged according to the point group symmetry 222 resulting in 2560 unique reflections (R_{int} = 0.0158 on F_o^2).

Structure Solution and Refinement. The structure was solved by the direct method using the program SHELXS97[47,48] and refined using the program SHELXL97.[27] Full matrix least-squares refinement on F^2 was performed, minimizing $\Sigma w(F_o^2 - F_c^2)^2$, with anisotropic displacement parameters for the non-hydrogen atoms. The positions of the hydrogen atoms were located on intermediate difference electron density maps and refined with fixed isotropic displacement parameters. Correction for extinction was applied (coefficient: 0.0041(4)). The refinement (216 parameters, 2560 reflections) with the molecule having the *R*-configuration converged at R_F = 0.0270, wR_F^2 = 0.0633 for 2383 reflections with $F_o > 4\sigma(F_o)$; $w = 1/[\sigma^2(F_o^2) + (0.0330P)^2 + 0.0745 P]$, where $P = (F_o^2 + 2F_c^2)/3$; S = 1.064. In the final difference Fourier map maximum and minimum electron densities were 0.221 and −0.157 e Å^{-3}, respectively. Refinement of the Flack absolute structure factor *x* in the final refinement gave *x* = 0.00(17).[26,27] The large standard deviation of *x* is due to the atom types present in the compound (C, H, N, and O), which produce minor anomalous scattering. Complex atomic scattering factors for neutral atoms were as incorporated in SHELXL97.[27,49]

Receptor Binding. Affinity for AMPA, KA, and NMDA receptors was determined using the ligands [³H]AMPA, [³H]-KA, and [³H]CPP, respectively,[31−33] with the modifications previously described.[50] The membrane preparations used in all the receptor binding experiments were prepared according to the method of Ransom and Stec.[51]

In Vitro Electrophysiology. The rat cortical slice preparation for determination of excitatory amino acid-evoked depolarizations described by Harrison and Simmonds[34] was used in a modified version.[52] Application of agonists were made for 90 s at each concentration tested. The receptor selectivity of agonists was tested using the AMPA receptor antagonist NBQX or the NMDA receptor antagonist CPP. In antagonist experiments, the antagonists were applied alone for 90 s followed by co-application with agonists for another 90 s.

Second-Messenger Assays. The mGluR subtypes mGlu$_{1\alpha}$, mGlu₂, mGlu$_{4a}$, and mGlu$_{5a}$ were expressed in Chinese hamster ovary cell lines. All compounds were tested for agonist and antagonist activity at 1 mM concentrations, unless otherwise stated, by the method previously described.[16]

Anticonvulsant Activity. Male mice (NMRI/BOM, SPF, Bomholtgård, Denmark) were used for the studies of anticonvulsant effects. The mice were kept in groups of 10 in plastic cages (35 × 30 × 12 cm) under a 12-h day/night cycle (lights on 6 a.m.). They had free access to food and water. Each dose group consisted of 5−10 mice. Compound **9** was administered sc (10 mL/kg) 30 min or icv (5 μL/mouse) 15 min before NMDA (140 μmol/kg = 20 mg/kg) was given iv (10 mL/kg). The icv administration was given into the third ventricle by free hand injection as previously described.[53] The mice were observed for behavioral changes before NMDA and subsequently for up to 1 h for presence of clonic/tonic convulsions.

Supporting Information Available: Tables for compounds (*R*)-**9** and (*R*)-**11**, listing final atomic coordinates, equivalent isotropic displacement parameters, anisotropic displacement parameters for non-hydrogen atoms, and a full list of bond lengths, bond angles, torsion angles, hydrogen bond dimensions; an illustration of the crystal packings; lists of structure factors. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Hollmann, M.; Heinemann, S. Cloned glutamate receptors. *Annu. Rev. Neurosci.* **1994**, *17*, 31−108.

(2) Nakanishi, S.; Masu, M. Molecular diversity and functions of glutamate receptors. *Annu. Rev. Biophys. Biomol. Struct.* **1994**, *23*, 319−348.

(3) Wheal, H. V., Thomson, A. M., Eds. *Excitatory Amino Acids and Synaptic Transmission*; Academic Press: London, 1995.

(4) Monaghan, D. T., Wenthold, R. J., Eds. *The Ionotropic Glutamate Receptors*; Humana Press: Totowa, NJ, 1997.

(5) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev.* **1999**, *51*, 7−61.

(6) Conn, P. J., Patel, J., Eds. *The Metabotropic Glutamate Receptors*; Humana Press: Totowa, NJ, 1994.

(7) Moroni, F., Nicoletti, F., Pellegrini-Giampietro, D. E., Eds. *Metabotropic Glutamate Receptors and Brain Function*; Portland Press: London, 1998.

(8) Parsons, C. G.; Danysz, W.; Quack, G. Glutamate in CNS disorders as a target for drug development: an update. *Drug News Perspect.* **1998**, *11*, 523−569.

(9) 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

(10) Lees, G. J. Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. *Drugs* **2000**, *59*, 33−78.

(11) Schoepp, D. D.; Jane, D. E.; Monn, J. A. Pharmacological agents acting at subtypes of metabotropic glutamate receptors. *Neuropharmacology* **1999**, *38*, 1431−1476.

(12) Krogsgaard-Larsen, P.; Honoré, T.; Hansen, J. J.; Curtis, D. R.; Lodge, D. New class of glutamate agonist structurally related to ibotenic acid. *Nature* **1980**, *284*, 64−66.

(13) 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.

(14) 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.

(15) Hansen, J. J.; Jørgensen, F. S.; Lund, T. M.; Nielsen, B.; Reinhardt, A.; Breum, I.; Brehm, L.; Krogsgaard-Larsen, P. AMPA receptor agonists: structural, conformational and stereochemical aspects. In *Excitatory amino acid receptors. Design of agonists and antagonists*; Krogsgaard-Larsen, P., Hansen, J. J., Eds.; Ellis Horwood: Chichester, 1992; pp 216−245.

(16) Bräuner-Osborne, H.; Nielsen, B.; Krogsgaard-Larsen, P. Molecular pharmacology of homologues of ibotenic acid at cloned metabotropic glutamic acid receptors. *Eur. J. Pharmacol.* **1998**, *350*, 311−316.

(17) Johansen, T. N.; Ebert, B.; Bräuner-Osborne, H.; Didriksen, M.; Christensen, I. T.; Søby, K. K.; Madsen, U.; Krogsgaard-Larsen, P.; Brehm, L. Excitatory amino acid receptor ligands: resolution, absolute stereochemistry, and enantiopharmacology of 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl)propionic acid. *J. Med. Chem.* **1998**, *41*, 930−939.

(18) 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.

(19) Nakamura, N.; Tajima, Y.; Sakai, K. Direct phenylation of isoxazoles using palladium catalysts synthesis of 4-phenylmuscimol. *Heterocycles* **1982**, *17*, 235−245.

(20) Armstrong, D. W.; Liu, Y.; Ekborgott, K. H. A covalently bonded teicoplanin chiral stationary phase for HPLC enantioseparations. *Chirality* **1995**, *7*, 474−497.

(21) Berthod, A.; Liu, Y.; Bagwill, C.; Armstrong, D. W. Facile liquid chromatographic enantioresolution of native amino acids and peptides using a teicoplanin chiral stationary phase. *J. Chromatogr.* **1996**, *A731*, 123−137.

(22) Johansen, T. N.; Ebert, B.; Falch, E.; Krogsgaard-Larsen, P. AMPA receptor agonists: Resolution, configurational assignment, and pharmacology of (+)-(*S*)- and (−)-(*R*)-2-amino-3-[3-hydroxy-5-(2-pyridyl)isoxazol-4-yl]propionic acid (2-Py-AMPA). *Chirality* **1997**, *9*, 274−280.

(23) Gübitz, G. Direct separation of enantiomers by high performance ligand exchange chromatography on chemically bonded chiral phases. *J. Liq. Chromatogr.* **1986**, *9*, 519−535.

(24) 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.

(25) Johnson, C. K., Ed. *ORTEP II Report ORNL-5138. A Fortran Thermal-Ellipsoid Plot Program for Crystal Structure Illustrations*; Oak Ridge National Laboratory: Oak Ridge, TN, 1976.

(26) Flack, H. D. On enantiomorph-polarity estimation. *Acta Crystallogr.* **1983**, *A39*, 876−881.

(27) Sheldrick, G. M., Eds. *SHELXL-97. Program for crystal structure refinement*; University of Göttingen: Göttingen, Germany, 1997.

(28) 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.

(29) Falch, E.; Brehm, L.; Mikkelsen, I.; Johansen, T. N.; Skjærbæk, N.; Nielsen, B.; Stensbøl, T. B.; Ebert, B.; Krogsgaard-Larsen, P. Heteroaryl analogues of AMPA. 2. Synthesis, absolute stereochemistry, photochemistry, and structure−activity relationships. *J. Med. Chem.* **1998**, *41*, 2513−2523.

(30) 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*, 2252−2260.

(31) Honoré, T.; Nielsen, M. Complex structure of quisqualate-sensitive glutamate receptors in rat cortex. *Neurosci. Lett.* **1985**, *54*, 27−32.

(32) Murphy, D. E.; Schneider, J.; Boehm, C.; Lehmann, J.; Williams, K. Binding of [³H]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.

(33) Braitman, D. J.; Coyle, J. T. Inhibition of [³H]kainic acid receptor binding by divalent cations correlates with ion affinity for the calcium channel. *Neuropharmacology* **1987**, *26*, 1247−1251.

(34) 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.

(35) Knöpfel, T.; Kuhn, R.; Allgeier, H. Metabotropic glutamate receptors: novel targets for drug development. *J. Med. Chem.* **1995**, *38*, 1417−1426.

(36) Dalby, N. O.; Thomsen, C. Modulation of seizure activity in mice by metabotropic glutamate receptor ligands. *J. Pharmacol. Exp. Ther.* **1996**, *276*, 516−522.

(37) Thomsen, C.; Dalby, N. O. Roles of metabotropic glutamate receptor subtypes in modulation of pentylenetetrazole-induced seizure activity in mice. *Neuropharmacology* **1998**, *37*, 1465−1473.

(38) Chapman, A. G.; Yip, P. K.; Yap, J. S.; Quinn, L. P.; Tang, E.; Harris, J. R.; Meldrum, B. S. Anticonvulsant actions of LY 367385 ((+)-2-methyl-4-carboxyphenylglycine) and AIDA ((*R*,*S*)-1-aminoindan-1,5-dicarboxylic acid). *Eur. J. Pharmacol.* **1999**, *368*, 17−24.

(39) Pellegrini-Giampietro, D. E.; Peruginelli, F.; Meli, E.; Cozzi, A.; Albani-Torregrossa, S.; Pellicciari, R.; Moroni, F. Protection with metabotropic glutamate 1 receptor antagonists in models of ischemic neuronal death: time course and mechanisms. *Neuropharmacology* **1999**, *38*, 1607−1619.

(40) Chapman, A. G. Glutamate and epilepsy. *J. Nutr.* **2000**, *130*, 1043S−1045S.

(41) Chen, Y.; Bacon, G.; Sher, E.; Clark, B. P.; Kallman, M. J.; Wright, R. A.; Johnson, B. G.; Schoepp, D. D.; Kingston, A. E. Evaluation of the activity of a novel metabotropic glutamate receptor antagonist (±)-2-amino-2-(3-cis and trans)-carboxycyclobutyl-3-(9-thioxanthyl acid) in the in vitro neonatal spinal cord and in an in vivo pain model. *Neuroscience* **2000**, *95*, 787−793.

(42) Gübitz, G.; Jellenz, W.; Santi, W. Resolution of the optical isomers of underivatisated amino acids on chemically bonded chiral phases by ligand exchange chromatography. *J. Liq. Chromatogr.* **1981**, *4*, 701−712.

(43) *CAD-4 Software,* version 5.0; Enraf-Nonius: Delft, The Netherlands, 1989.

(44) Blessing, R. H. Data reduction and error analysis for accurate single-crystal diffraction intensities. *Crystallogr. Rev.* **1987**, *1*, 3−58.

(45) Blessing, R. H. DREADD − data reduction and error analysis for single-crystal diffractometer data. *J. Appl. Crystallogr.* **1989**, *22*, 396−397.

(46) DeTitta, G. T. ABSORB: an absorption correction programme for crystals enclosed in capillaries with trapped mother liquor. *J. Appl. Crystallogr.* **1985**, *18*, 75−79.

(47) Sheldrick, G. M., Ed. *SHELXS-97. Program for the solution of crystal structures*; University of Göttingen: Göttingen, Germany, 1997.

(48) Sheldrick, G. M. Phase annealing in SHELX-90: Direct methods for large structures. *Acta Crystallogr.* **1990**, *A46*, 467−473.

(49) Wilson, A. J. C., Ed. *International Tables for Crystallography, Vol. C. Tables 6.1.1.4 and 4.2.6.8*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1995.

(50) Stensbøl, T. B.; Borre, L.; Johansen, T. N.; Egebjerg, J.; Madsen, U.; Ebert, B.; Krogsgaard-Larsen, P. Resolution, absolute stereochemistry and molecular pharmacology of the enantiomers of ATPA. *Eur. J. Pharmacol.* **1999**, *380*, 153−162.

(51) Ransom, R. W.; Stec, N. L. Cooperative modulation of [³H]MK-801 binding to the *N*-methyl-D-aspartate receptor ion channel complex by L-glutamate, glycine and polyamines. *J. Neurochem.* **1988**, *51*, 830−836.

(52) Madsen, U.; Frølund, B.; Lund, T. M.; Ebert, B.; Krogsgaard-Larsen, P. Design, synthesis and pharmacology of model compounds for indirect elucidation of the topography of AMPA receptor sites. *Eur. J. Med. Chem.* **1993**, *28*, 791−800.

(53) Sánchez, C.; Meier, E. Central and peripheral mediation of hypothermia, tremor and salivation induced by muscarinic agonists in mice. *Pharmacol. Toxicol.* **1993**, *72*, 262−267.