Synthesis and Pharmacological Characterization at Glutamate Receptors of the Four Enantiopure Isomers of Tricholomic Acid

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The two enantiomeric pairs of *erythro*- and *threo*-amino-(3′-hydroxy-4′,5′-dihydro-isoxazol-5′-yl)-acetic acids were synthesized via the 1,3-dipolar cycloaddition of bromonitrile oxide to (*R*)- or (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine. The pharmacological profiles of the studied amino acids reflect the relationship between the activity/selectivity and the stereochemistry of the two stereogenic centers: while the (2*S*,5′*S*) stereoisomer is an agonist at the AMPA and KA receptors, its (2*R*,5′*R*) enantiomer interacts selectively with the NMDA receptors; the (2*S*,5′*R*) stereoisomer is the only one capable to activate the mGluRs.

Introduction

The acidic amino acid neurotransmitter L-glutamate (Glu[a]) plays a pivotal role in the excitatory pathways of the mammalian central nervous system (CNS).[1,2] Once released from the presynaptic neurons into the glutamatergic synaptic cleft, Glu activates two main classes of receptors: G-protein-coupled metabotropic Glu receptors (mGluRs) and ligand-gated ionotropic Glu receptors (iGluRs). The mGluRs exert modulatory effects on neuronal excitability and synaptic transmission. So far, eight mGluR subtypes (mGluR1–8) have been cloned from mammalian brain.[3] On the other hand, the iGluRs are the major players in the fast neuronal signaling and represent a potential therapeutic target for the treatment of a number of neurological and psychiatric disorders, for example, chronic pain, stroke, epilepsy, drug addiction, schizophrenia, and Parkinson's disease.[4,5] On the basis of agonist selectivity, iGluRs have been subclassified into *N*-methyl-D-aspartate (NMDA) receptors, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionate (AMPA) receptors, and kainate (KA) receptors.[1,2] The functional ion channel is composed of four subunits, which can assemble either homomerically or heteromerically. A total of seven NMDA subunits (NR1, NR2A-D, NR3A,B), four AMPA subunits (GluR1–4), and five KA subunits (GluR5–7 and KA1,2) have been cloned and characterized.

Although an extensive preclinical literature suggests a therapeutic potential for a number of NMDA, AMPA, KA, and metabotropic receptor–ligands, only NMDA antagonists have been clinically investigated in detail. At present, Memantine is the only drug acting at NMDA receptors as an uncompetitive, low-affinity, open-channel blocker that has been approved by the EMEA and the FDA for the treatment of Alzheimer's disease.[6]

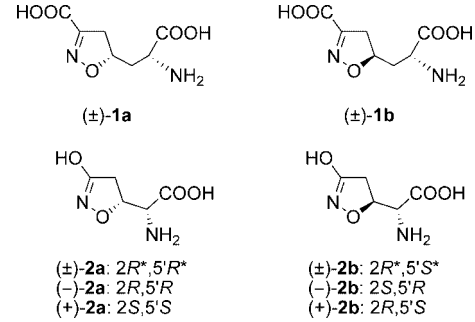

Figure 1. Structures of reference [(±)-**1a**, (±)-**1b**, (±)-**2a**, and (±)-**2b**] and target compounds [(−)-**2a**, (+)-**2a**, (−)-**2b**, and (+)-**2b**].

In a recent paper[7] we reported the synthesis and the pharmacological characterization at Glu receptors of the racemic form of the two acidic amino acids, 5-(2-amino-2-carboxyethyl)-4,5-dihydroisoxazole-3-carboxylic acids (±)-**1a** and (±)-**1b** (Figure 1). Later on, we prepared and tested their lower homologues (±)-**2a** and (±)-**2b**,[8] which are Glu analogues in a partially locked conformation. While both (±)-**1a** and (±)-**1b** were endowed with a remarkable NMDA antagonist activity and were devoid of any activity at mGluRs, amino acids (±)-**2a** and (±)-**2b** activated all three iGluRs and, marginally, some mGluR subtypes. Therefore, they were classified as nonselective agonists of the Glu receptors. Furthermore, the *erythro* isomer (±)-**2a** was roughly 10 times more active at iGluRs than its *threo* counterpart (±)-**2b**, whereas the opposite held true concerning mGluRs activity. It is worth pointing out that the (2*S*,5′*S*)-diastereomer [(+)-**2a**] is a naturally occurring amino acid, termed tricholomic acid. It is a flycidal substance isolated from different species of mushrooms such as *Tricholoma muscarium*,[9] *Amanita strobiliformis*,[10] and *Ustilago maydis*.[11] Its biological activity, evaluated on rat cortical neurones[12] and on giant neurons of an African giant snail (*Achatina fulica*, Férrusac),[13,14] turned out to be similar to that displayed by Glu. Because at that time the classification of the different Glu

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[a] Abbreviations: Glu, L-glutamate; mGluRs, metabotropic glutamate receptors; iGluRs, ionotropic glutamate receptors; NMDA, *N*-methyl-D-aspartate; AMPA, (*S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionic acid; KA, kainate; EMEA, European Agency for the Evaluation of Medicinal Products; FDA, Food and Drug Administration; HEK, Human Embryonic Kidney; CHO, Chinese Hamster Ovary.

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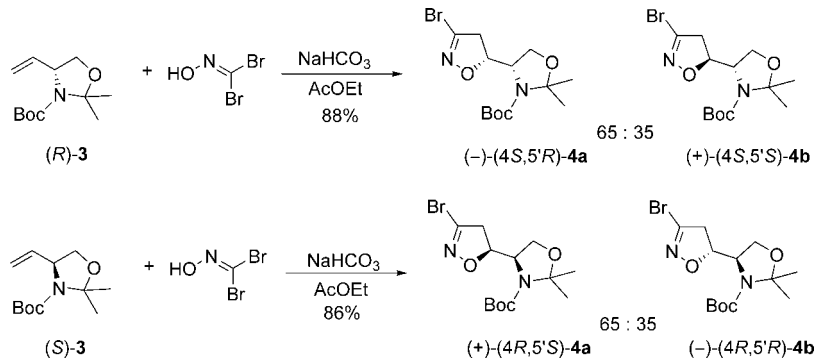
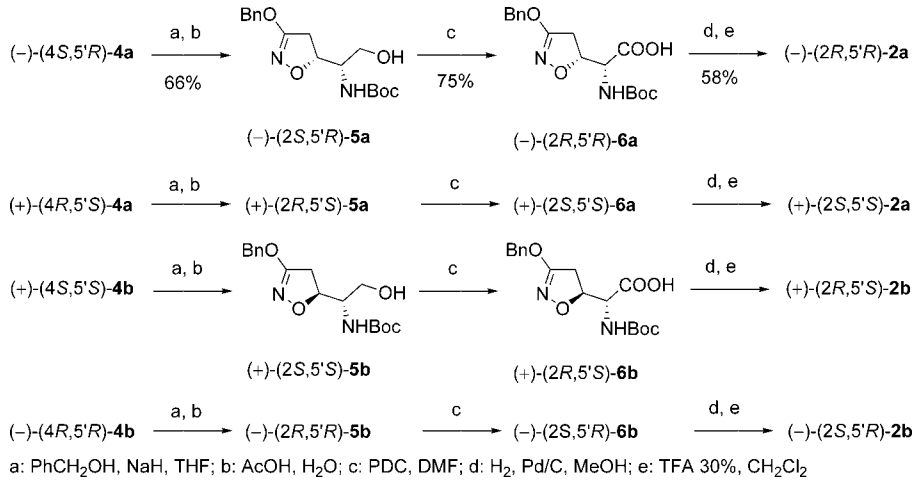
Scheme 1. Cycloaddition of Bromonitrile Oxide to (*R*)- or (*S*)-3-(*tert*-Butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine



Scheme 2. Synthesis of the Target Amino Acids



receptors was unknown, the pharmacological profile of tricholomic acid was not ascertained and it is, at present, undefined.

To investigate in depth their pharmacological profile, we now report a simplified and versatile synthesis[15,16] of the enantiomers of *erythro*-[(−)-**2a**, -(+)-**2a**] and *threo*-[(−)-**2b**, -(+)-**2b**] tricholomic acid along with their characterization at iGluRs performed by in vitro binding to rat cortical membranes and calcium imaging assays on HEK cells expressing the different iGluR1–6 cloned subtypes. The activity of the test compounds at representative mGluRs was ascertained by second messenger assays at cloned receptors, expressed in Chinese hamster ovary (CHO) cells.

Results and Discussion

The key step in the synthesis of target amino acids (−)-**2a**, (+)-**2a**, (−)-**2b**, and (+)-**2b** is the 1,3-dipolar cycloaddition of bromonitrile oxide to either (*R*)- or (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine [(*R*)-**3** or (*S*)-**3**; Scheme 1]. Dipolarophiles (*R*)-**3** and (*S*)-**3** were prepared from (*S*)- and (*R*)-Garner's aldehyde,[17] respectively, following the procedure reported in the literature.[18] The two pairs of diastereomeric cycloadducts *erythro*-(−)-**4a**/*threo*-(+)-**4b** and *erythro*-(+)-**4a**/*threo*-(−)-**4b**, obtained in a 65:35 ratio[19] (Scheme 1), were separated by silica gel column chromatography and then separately submitted to the reaction sequences depicted in Scheme 2. Nucleophilic displacement of the 3-bromo moiety by the benzyloxy anion gave the expected benzyloxy-substituted intermediates in high yield (93%), whereas the subsequent opening of the oxazolidine ring to yield amino alcohols **5** turned out to be rather problematic. Indeed, among the different methodologies reported in the literature to achieve this transformation, the desired isomeric amino alcohols (−)-**5a**/(+)-**5a** and (+)-**5b**/(−)-**5b** were obtained in acceptable average yield (71%) only by treatment of the intermediates with a 5:1 acetic acid/water mixture.[20] Derivatives **5** were then transformed into final amino acids *erythro*-(−)-**2a**/(+)-**2a** and *threo*-(−)-**2b**/(+)-**2b** via the sequential oxidation of the primary alcohols to the corresponding carboxylic acids **6** followed by hydrogenolysis of the benzyl group and treatment with a dichloromethane solution of trifluoroacetic acid.

Because the relative stereochemistry of the corresponding racemates (±)-**2a** and (±)-**2b** had been previously assigned by ^{1}H NMR spectroscopic data,[8] the absolute configuration at the stereogenic centers of the pairs of amino acids (−)-**2a**/(+)-**2b** and (+)-**2a**/(−)-**2b** was attributed by chemical correlation with the enantiopure dipolarophiles (*R*)-**3** and (*S*)-**3**, respectively. Chiral HPLC analyses, carried out on final amino acids (+)-**2b** and (−)-**2b**, gave a value of enantiomeric excess higher than 99%. Conversely, chromatographic resolution of (−)-**2a**/(+)-**2a** was not achieved.

The two pairs of enantiomeric acidic amino acids (−)-**2a**/(+)-**2a** and (−)-**2b**/(+)-**2b** were tested in vitro by means of receptor binding techniques, calcium imaging assays, and second messenger assays. The receptor affinities for NMDA, AMPA, and KA receptors were determined by use of the radioligands [^{3}H]CGP39653, [^{3}H]AMPA, and [^{3}H]KA, respectively,[21–23] while the activity at cloned rat iGluR1–6 subtypes, expressed in HEK cells, was evaluated with a calcium imaging assay.[24] The mGluRs activities of the new compounds were measured at rat mGluR1a (representative of group I), mGluR2 (representative of group II), and mGluR4 (representative of group III) expressed in CHO cells.[25]

Table 1. Affinity for iGluRs Using Rat Cortical Membranes[a,b]

cmpd	[^{3}H]AMPA IC_{50} (μM)	[^{3}H]KA IC_{50} (μM)	[^{3}H]CGP K_i (μM)
(±)-**2a**[c]	1.4 [5.86 ± 0.04]	0.76 [6.14 ± 0.08]	1.5 [5.82 ± 0.05]
(−)-(2*R*,5′*R*)-**2a**	>100	>100	0.67 [6.19 ± 0.05]
(+)-(2*S*,5′*S*)-**2a**	0.95 [6.02 ± 0.01]	0.29 [6.55 ± 0.06]	41 [4.40 ± 0.07]
(±)-**2b**[c]	19 [4.73 ± 0.04]	6.0 [5.22 ± 0.03]	73 [4.14 ± 0.06]
(+)-(2*R*,5′*S*)-**2b**	12 [4.92 ± 0.03]	11 [4.95 ± 0.04]	75 [4.13 ± 0.03]
(−)-(2*S*,5′*R*)-**2b**	>100	36 [4.45 ± 0.04]	95 [4.03 ± 0.05]
ibotenic acid[d]	>100	22 [4.66 ± 0.07]	5.3 [5.28 ± 0.04]

[a] Data are given as mean [mean pIC_{50} ± SEM or mean pK_i ± SEM] of at least three individual experiments. [b] The membrane preparations used in all the receptor-binding experiments were prepared according to the method of Ransom and Stec (ref 26). [c] Data from ref 8. [d] Data from ref 27.

Table 2. Calcium Imaging Assay (Fluo-4) Using HEK-Cells for Expression of Cloned Rat iGluR1–6 Subtypes[a]

	iGluR1$_i$		iGluR2(Q)$_i$		iGluR3$_i$		iGluR4$_i$		iGluR5(Q)		iGluR6(Q)	
cmpd	EC_{50}(μM)	%	EC_{50}(μM)	%	EC_{50}(μM)	%	EC_{50}(μM)	%	EC_{50}(μM)	%	EC_{50}(μM)	%
L-Glu	35 [4.49 ± 0.12]	100	140 [3.87 ± 0.05]	100	100 [3.99 ± 0.05]	100	17 [4.78 ± 0.03]	100	130 [3.93 ± 0.13]	100	66 [4.23 ± 0.15]	100
ibotenic acid	>1000	6	>1000	2	>1000	1	>1000	32	>1000	8	>1000	96
(−)-(2*R*,5′*R*)-**2a**	>1000	15	>1000	13	>1000	10	>1000	9	>1000	10	>1000	11
(+)-(2*S*,5′*S*)-**2a**	120 [3.94 ± 0.02]	90	480 [3.35 ± 0.11]	74	170 [3.81 ± 0.15]	88	30 [4.53 ± 0.06]	91	280 [3.60 ± 0.13]	91	82 [4.09 ± 0.04]	91
(+)-(2*R*,5′*S*)-**2b**	>1000	13	>1000	14	>1000	10	>1000	7	>1000	8	>1000	15
(−)- (2*S*,5′*R*)-**2b**	>1000	35	>1000	20	>1000	23	>1000	90	>1000	12	>1000	11

[a] Numbers in square brackets are pEC_{50} ± SEM. Numbers in the right column are maximal response as % of the Glu response. Testing performed as duplicates and repeated three times, except for ibotenic acid ($n = 2$ due to limited amount of compound).

Table 3. Activities at Cloned Rat mGlu Receptors Expressed in CHO Cells[a]

cmpd	mGluR1a EC_{50}(μM)	mGluR2 EC_{50}(μM)	mGluR4 EC_{50}(μM)
(±)-**2a**[b]	790 [3.1 ± 0.1]	>1000	>1000
(−)-(2*R*,5′*R*)-**2a**	>1000	>1000	>1000
(+)-(2*S*,5′*S*)-**2a**	610 [3.24 ± 0.11]	>1000	>1000
(±)-**2b**[b]	110 [4.1 ± 0.3]	100 [4.0 ± 0.1]	>1000
(+)-(2*R*,5′*S*)-**2b**	>1000	>1000	>1000
(−)-(2*S*,5′*R*)-**2b**	82 [4.10 ± 0.08]	84 [4.08 ± 0.07]	>1000
ibotenic acid[c]	43 [4.37 ± 0.01]	110 [3.97 ± 0.04]	>1000

[a] Data are given as mean [mean pEC_{50} ± SEM] of at least three independent experiments. [b] Data from ref 8. [c] Data from ref 27.

The racemic form of tricholomic acid (±)-**2a** binds efficiently to all three iGluRs with similar IC_{50} values (Table 1). When comparing the data obtained for the two enantiomers (−)-**2a** and (+)-**2a**, we observe that while the (−)-form is a ligand highly selective for the NMDA receptors, the (+)-form possesses affinity for the AMPA and KA receptors. Thus, the lack of selectivity for iGluRs, previously determined for the racemate (±)-**2a**, is due to the overlap of selective activities of the individual enantiomers. The picture is less clear for the corresponding stereoisomers (+)-**2b** and (−)-**2b**, generally showing somewhat lower affinities, (+)-**2b** with some selectivity for the AMPA and KA receptors and (−)-**2b** with a slight preference for the KA receptors. The results of these binding studies are confirmed by the data obtained on cloned rat AMPA (iGluR1–4) and KA (iGluR5,6) receptor subtypes (Table 2). Amino acid (−)-**2a** does not activate any of the six subtypes, whereas its enantiomer (+)-**2a** is an agonist at all subtypes, slightly weaker than Glu but with a similar profile. On the other hand, both amino acids (+)-**2b** and (−)-**2b** are inactive at all the tested subtypes.

The data reported in Table 3 reveal that the activity at mGluRs, previously observed for the two racemates (±)-**2a** and (±)-**2b**, is due in both cases to a single enantiomer, (+)-**2a** and (−)-**2b**, respectively. It is worth noting that the potency of (−)-**2b** is close to that of ibotenic acid[27] and that (−)-**2b** has an overall pharmacology similar to that of ibotenic acid.

In summary, we herein report the synthesis of the four enantiopure isomers of tricholomic acid and the evaluation of their activity at iGluRs and mGluRs. Particularly interesting are the pharmacological profiles of enantiomers (−)-**2a** and (+)-**2a** at iGluRs. While the first one interacts exclusively with the NMDA receptors, its mirror image is an agonist of the AMPA and KA receptors. As a consequence, it can be deduced that the natural amino acid (+)-**2a**, which is found in a number of mushrooms, is most likely contributing to the poisonous activity by activation of the AMPA and KA receptors present in the mammalian nervous system.

Experimental Section

Material and Methods. All reagents were purchased from Sigma. Dibromoformaldoxime[28] was prepared according to a literature procedure. Dipolarophiles (*R*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine (*R*)-**3** and (*S*)-3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4-vinyloxazolidine (*S*)-**3** were prepared from (*S*)- and (*R*)-Garner's aldehyde,[17] respectively, following the procedure reported in the literature.[18] ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded with a Varian Mercury 300 spectrometer in DMSO-d_6 or D_2O. Analysis of the crude mixture of cycloadducts **4a/4b** was carried out using an Agilent 1100 HPLC with diode array detector coupled with an ion trap Bruker Esquire 3000+ with ESI+ interface. HPLC analyses of final amino acids were carried out using a Merck Hitachi L7100 HPLC with a HP 1050 DAD. Specific optical rotations were determined with a Jasco J-810 polarimeter coupled with a Haake N3-B thermostat. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were visualized by spraying with a dilute alkaline potassium permanganate solution or with ninhydrin. Melting points were determined on a model B 540 Büchi apparatus and are uncorrected. Microanalyses (C, H, N) of new compounds agreed with the theoretical value within ±0.4%.

(4*S*,5′*R*)-4-(3′-Bromo-4′,5′-dihydro-isoxazol-5′-yl)-2,2-dimethyl-*N*-Boc-oxazolidine [(−)-4a] and (4*S*,5′*S*)-4-(3′-Bromo-4′,5′-dihydro-isoxazol-5′-yl)-2,2-dimethyl-*N*-Boc-oxazolidine [(+)-4b]. To a solution of (*R*)-**3** (5.0 g, 22 mmol) in AcOEt (100 mL) was added dibromoformaldoxime (6.75 g, 33 mmol) and $NaHCO_3$ (9.2 g). The mixture was vigorously stirred for 24 h at room temperature; the progress of the reaction was monitored by TLC (petroleum ether/AcOEt 95:5). Water was added 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 95:5) to give (−)-**4a** (4.37 g) and (+)-**4b** (2.35 g). Overall yield: 88%. Compound (−)-**4a**: crystallized from hexane as white needles; mp 82–84 °C; R_f 0.4 (petroleum ether/AcOEt 9:1); $[\alpha]^{20}_{365} = -405.3$ ($c = 1.104$, $CHCl_3$); HPLC retention time, 6.21 min; Anal. ($C_{13}H_{21}BrN_2O_4$) C, H, N. Compound (+)-**4b**: crystallized from diisopropyl ether as white needles; mp 131–132 °C; R_f 0.23 (petroleum ether/AcOEt 9:1); $[\alpha]^{20}_{365} = +35.1$ ($c = 1.112$, $CHCl_3$); HPLC retention time, 5.92 min; Anal. ($C_{13}H_{21}BrN_2O_4$) C, H, N.

(4*R*,5′*S*)-4-(3′-Bromo-4′,5′-dihydro-isoxazol-5′-yl)-2,2-dimethyl-*N*-Boc-oxazolidine [(+)-4a] and (4*R*,5′*R*)-4-(3′-Bromo-4′,5′-dihydro-isoxazol-5′-yl)-2,2-dimethyl-*N*-Boc-oxazolidine [(−)-4b]. The procedure described above was applied to dipolarophile (*S*)-**3** to yield cycloadducts (+)-**4a** and (−)-**4b** in identical ratio. Compound (+)-**4a**: crystallized from hexane as white needles; mp 83–85 °C; R_f 0.4 (petroleum ether/AcOEt 9:1); $[\alpha]^{20}_{365} = +403.0$ ($c = 1.12$, $CHCl_3$); Anal. ($C_{13}H_{21}BrN_2O_4$) C, H, N. Compound (−)-**4b**: crystallized from diisopropyl ether as white needles; mp 130–131 °C; R_f 0.23 (petroleum ether/AcOEt 9:1); $[\alpha]^{20}_{365} = -35.8$ ($c = 1.0$, $CHCl_3$); Anal. ($C_{13}H_{21}BrN_2O_4$) C, H, N.

(2*S*,5′*R*)-*N*-Boc-2-amino-2-(3′-benzyloxy-4′,5′-dihydro-isoxazol-5′-yl)ethanol [(−)-5a]. To a solution of benzyl alcohol (7.75 mL, 75 mmol) in dry THF (50 mL) was added in small portions NaH (0.9 g, 37.5 mmol), and the mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. A solution of (−)-**4a** (4.37 g, 12.5 mmol) in dry THF (20 mL) was then added with a syringe, and the mixture was refluxed for 2 h. HCl (2 N) was added to the reaction mixture, and after evaporation of the solvent, the aqueous layer was extracted with Et_2O. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated. Excess benzyl alcohol was removed by Kugelrohr distillation under reduced pressure. The residue was then purified by column chromatography (petroleum ether/AcOEt 9:1) to give 4.4 g of a yellow oil which was directly treated with 100 mL of a mixture of water and acetic acid (1:5 v/v). After stirring for 48 h, the solution was evaporated under reduced pressure and the residue was purified by column chromatography (petroleum ether/AcOEt 7:3) to give (−)-**5a** (2.77 g, overall yield 66%). Compound (−)-**5a**: white prisms from diisopropyl ether; mp 75–76 °C; R_f 0.43 (petroleum ether/AcOEt 1:1); $[\alpha]^{20}_D = -54.02$ ($c = 1.004$, $CHCl_3$); Anal. ($C_{17}H_{24}N_2O_5$) C, H, N.

The above-described methodology was applied to (+)−**4a**, (+)-**4b**, and (−)-**4b** to give derivatives (+)-**5a**, (+)-**5b**, and (−)-**5b**, respectively, in comparable yield. Compound (+)-**5a**: white prisms from diisopropyl ether; mp 75–76 °C; R_f 0.43 (petroleum ether/AcOEt 1:1); $[\alpha]^{20}_D = +53.83$ ($c = 1.002$, $CHCl_3$); Anal. ($C_{17}H_{24}N_2O_5$) C, H, N. Compound (+)-**5b**: white prisms from diisopropyl ether; mp 96–98 °C; R_f 0.33 (petroleum ether/AcOEt 1:1); $[\alpha]^{20}_D = +53.55$ ($c = 1.004$, $CHCl_3$); Anal. ($C_{17}H_{24}N_2O_5$) C, H, N. Compound (−)-**5b**: white prisms from diisopropyl ether; mp 94–95 °C; $[\alpha]^{20}_D = -56.57$ ($c = 1.0$, $CHCl_3$); Anal. ($C_{17}H_{24}N_2O_5$) C, H, N.

(2*R*,5′*R*)-*N*-Boc-2-amino-2-(3′-benzyloxy-4′,5′-dihydro-isoxazol-5′-yl) Acetic Acid [(−)-6a]. To a solution of (−)-**5a** (2.77 g, 8.2 mmol) in DMF (45 mL), pyridinium dichromate (45.7 g, 123 mmol) was added, and the mixture was stirred at room temperature for 6 h. The progress of the reaction was monitored by TLC ($CHCl_3$/MeOH 9:1 + 1% acetic acid). Water (150 mL) was added, and the mixture was extracted with AcOEt (3 × 100 mL). The pooled organic layers were then extracted with a 1 N NaOH solution (4 × 80 mL), the aqueous phase was made acidic with 2 N HCl, and extracted with AcOEt (3 × 100 mL). The organic extracts were washed with brine, dried over anhydrous Na_2SO_4, and the solvent evaporated to give (−)-**6a** (2.15 g, yield 75%). Compound (−)-**6a**: colorless prisms; mp 143–146 °C; $[\alpha]^{20}_D = -126.12$ ($c = 1.0$, $CHCl_3$); Anal. ($C_{17}H_{22}N_2O_6$) C, H, N.

The above-described methodology was also applied to (+)−**5a**, (+)-**5b**, and (−)-**5b** to give derivatives (+)-**6a**, (+)-**6b**, and (−)-**6b**, respectively, in comparable yield. Compound (+)-**6a**: colorless prisms; mp 143–145 °C; $[\alpha]^{20}_D = +125.23$ ($c = 1.0$, $CHCl_3$); Anal. ($C_{17}H_{22}N_2O_6$) C, H, N. Compound (+)-**6b**: colorless oil; $[\alpha]^{20}_D = +62.04$ ($c = 1.004$, $CHCl_3$); Anal. ($C_{17}H_{22}N_2O_6$) C, H, N. Compound (−)-**6b**: colorless oil; $[\alpha]^{20}_D = -63.13$ ($c = 1.006$, $CHCl_3$); Anal. ($C_{17}H_{22}N_2O_6$) C, H, N.

(2*R*,5′*R*)-Amino-(3′-hydroxy-4′,5′-dihydro-isoxazol-5′-yl)-acetic Acid [(−)-2a]. To a solution of (−)-**6a** (2.15 g, 6.1 mmol) in MeOH (100 mL), 5% palladium on carbon powder (Engelhard cod.5011) was added and the mixture was stirred in a hydrogen atmosphere at room temperature for 15 min. The progress of the reaction was monitored by TLC ($CHCl_3$/MeOH 9:1 + 1% CH_3COOH). The mixture was filtered, and the solvent was evaporated to give 1.4 g of a white solid, which was directly treated with 15 mL of a 30% CH_2Cl_2 solution of trifluoroacetic acid at 0 °C. The solution was stirred at room temperature for 5 h. The volatiles were removed under vacuum and the residue was taken up with MeOH, filtered, washed with MeOH and Et_2O, and dried under vacuum to give amino acid (−)-**2a** (570 mg, yield 58%). Compound (−)-**2a**: crystallized from water/ethanol as white prisms; mp 188–190 °C dec.; $[\alpha]^{20}_D = -105.0$ ($c = 0.204$, H_2O); $[\alpha]^{20}_D$ lit.[15] = −101 ($c = 0.2$, H_2O); HPLC retention time, 5.6 min; Anal. ($C_5H_8N_2O_4$) C, H, N.

The above-described methodology was also applied to (+)-**6a**, (+)-**6b**, and (−)-**6b** to give derivatives (+)-**2a**, (+)-**2b**, and (−)-**2b**, respectively, in comparable yield. Compound (+)-**2a**: crystallized from water/ethanol as white prisms; mp 187–189 °C dec.; $[\alpha]^{20}_D = +100.6$ ($c = 0.202$, H_2O); $[\alpha]^{20}_D$ lit.[15] = +103 ($c = 0.2$, H_2O); HPLC retention time, 5.6 min; Anal. ($C_5H_8N_2O_4$) C, H, N. Compound (+)-**2b**: crystallized from methanol/diethyl ether as white prisms; mp > 180 °C dec.; $[\alpha]^{20}_D = +52.5$ ($c = 0.2$, H_2O); $[\alpha]^{20}_D$ lit.[15] = +63 ($c = 0.2$, H_2O); HPLC retention time, 7.1 min.; ee > 99%; Anal. ($C_5H_8N_2O_4$) C, H, N. Compound (−)-**2b**: crystallized from methanol/diethyl ether as white prisms; mp > 180 °C dec.; $[\alpha]^{20}_D = -54.5$ ($c = 0.202$, H_2O); $[\alpha]^{20}_D$ lit.[15] = −65 ($c = 0.2$, H_2O); HPLC retention time, 4.6 min.; ee > 99%; Anal. ($C_5H_8N_2O_4$) C, H, N.

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Supporting Information Available: Elemental Analyses; ^1H- and ^{13}C NMR data; HPLC and LC-MS experimental conditions; information on the binding and in vitro functional assays at cloned iGlu and mGlu receptor subtypes. This material is available free of charge via the Internet at http://pubs.asc.org.

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