Design of Cyclopentaisoxazoline Amino Acids as Conformationally Constrained Agonists at Glutamate Receptors

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We have prepared four isomeric 3-hydroxycyclopentaisoxazoline amino acids **12**–**15**, which represent analogues of glutamic acid having restricted conformations, through a strategy based on the 1,3-dipolar cycloaddition of bromonitrile oxide to a suitably protected 1-aminocyclopent-2-enecarboxylic acid. These target compounds proved to be inactive when assayed at ionotropic and metabotropic glutamate receptors, except for **12** which is an agonist primarily at mGluR5 (EC_{50} = 79 μM), but is less active at mGluR2 and only marginally active at mGluR1. The biological data are accounted for through comparison of the conformational profiles of the test compounds with that of reference agonists, i.e., *N*-methyl-D-aspartate (NMDA, **2**), and 1-aminocyclopentane-1,3-dicarboxylic acids [*trans*-ACPD, **10**; *cis*-ACPD, **11**].

Introduction

L-Glutamic acid [(*S*)-Glu, **1**; Scheme 1], the major excitatory neurotransmitter in the mammalian central nervous system (CNS), acts at several receptor subtypes, which can be grouped into two heterogeneous families: the ionotropic and the metabotropic glutamate receptors (iGluRs and mGluRs, respectively).[1−5] The fast excitatory effects of Glu are mediated by the three subclasses of iGluRs, which are named after their archetypal agonists, i.e., *N*-methyl-D-aspartate (NMDA, **2**), 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propanoic acid (AMPA, **3**), and kainic acid (KA, **4**).[2,3] The mGluRs are G-protein-coupled receptors, which control the activity of membrane-bound enzymes and/or modulate the opening of ion channels.[1,6] So far, eight different clones of mGluRs have been identified: mGluR1−8. Based on their pharmacology, sequence homology, and signal transduction pathways, the mGlu receptors are subclassified into three groups: Group I (mGluR1 and mGluR5), Group II (mGluR2 and mGluR3), and Group III (mGluR4, mGluR6, mGluR7, and mGluR8).[1]

The activation of both iGluRs and mGluRs gives rise to a variety of pivotal physiological functions, such as learning, memory, and developmental plasticity.[7−9] On the other hand, an overactivation of the same receptors, caused by an excessive release of endogenous Glu, is implicated in the pathogenesis of several acute and chronic diseases.[9]

A prerequisite for the identification of the physio-pathological role played by the subgroups of iGluRs and mGluRs is the availability of highly selective agonists and/or antagonists. Since Glu is a highly flexible molecule, a number of constrained analogues have been designed and tested with the aim of uncovering the conformational requirements needed to activate the different Glu receptor subtypes. In general, an extended conformation is required for a fruitful interaction with mGlu receptors, whereas a folded conformation is necessary to fit the binding sites of the iGluRs.[10] Nevertheless, not all the extended conformations are capable to bind efficiently to mGluRs. The same holds true for the folded conformations with respect to the different iGluRs. As an example, whereas CIP-A (**5**, Scheme 1), a (3-carboxyisoxazolo)proline, in which a folded conformation of Glu is locked into a bicyclic structure, is a potent AMPA/KA receptor agonist,[11] its (3-carboxyisoxazolo)cyclopentane analogues **6** and **7** are totally inactive at the same receptors.[12] Similarly, the stereoisomers **8** and **9**, which embed two different extended conformations (homologues) of Glu, are inactive as both agonists and antagonists when tested at the different mGluRs.[12] The inactivity of **8** and **9** at mGluRs has been rationalized by calculating the distances between the pharmacophoric groups, i.e., ω-COOH/α-COOH and ω-COOH/α-NH_2; these values are significantly higher than those calculated for the Glu analogue *trans*-1-aminocyclopentane-1,3-dicarboxylic acid [*trans*-

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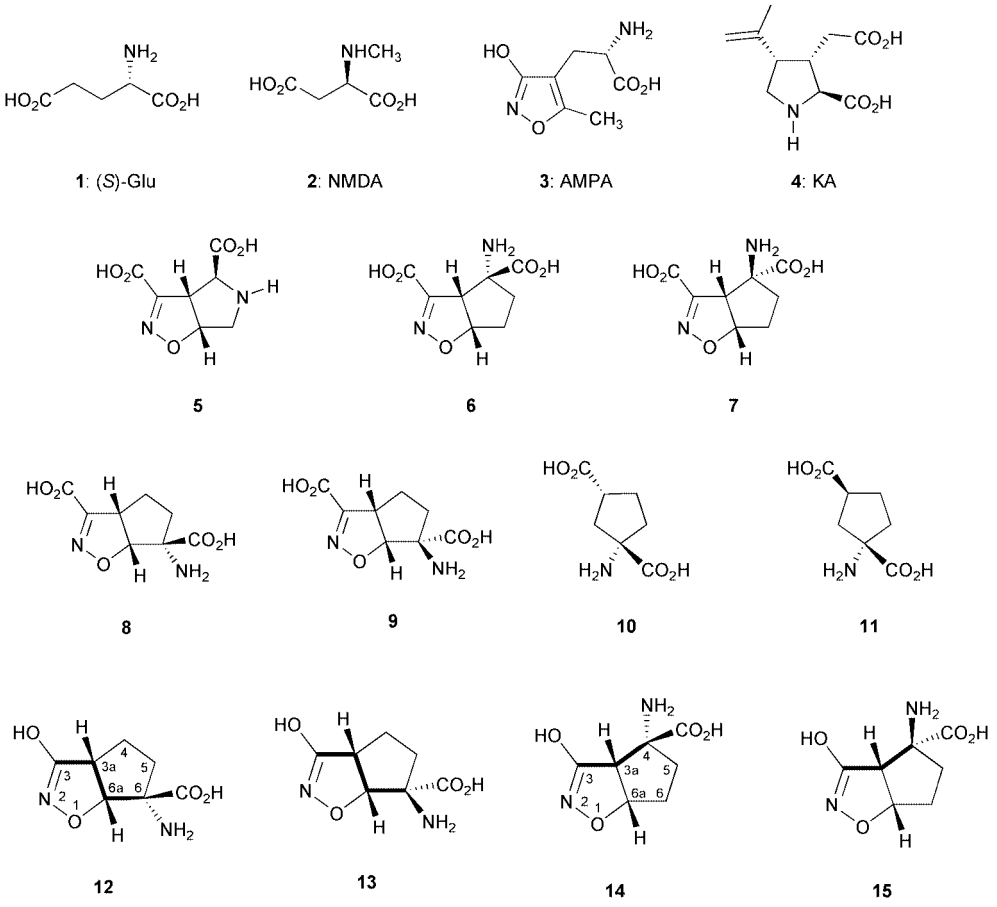


Scheme 1. Structures of reference compounds **1**−**11** and new compounds **12**−**15**

ACPD, **10**], which is an unselective mGluRs agonist that is usually taken as the reference compound.

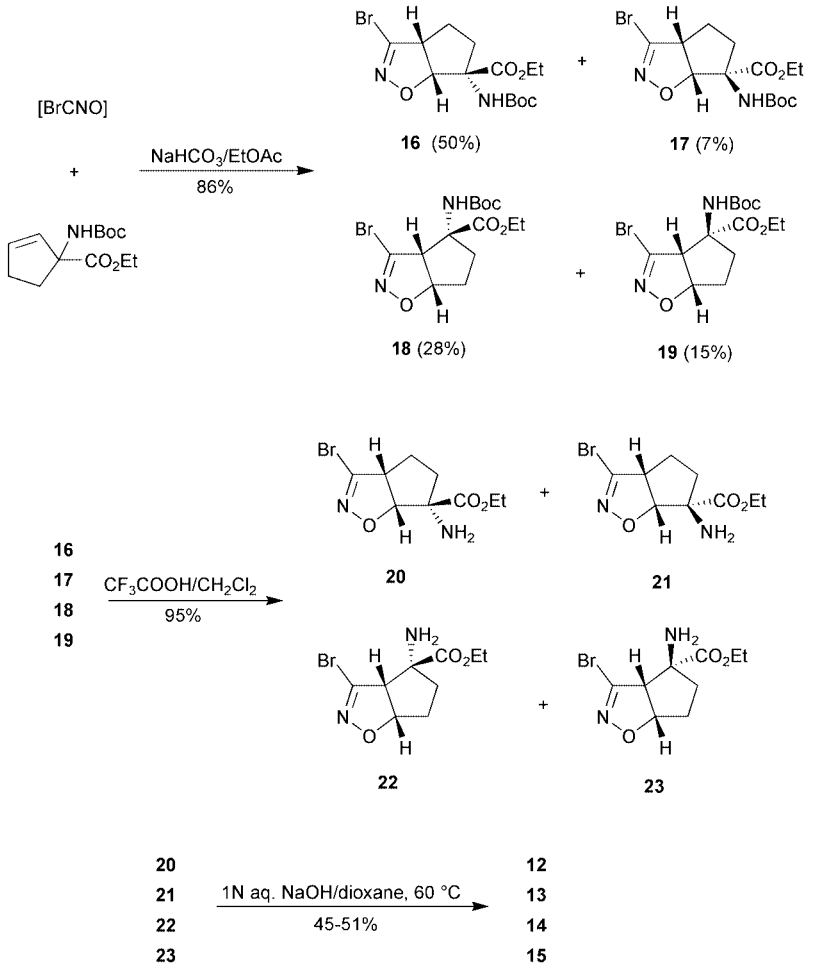
To match the distances of the pharmacophoric groups mentioned above with those of **10**, we designed the racemic cyclopentaisoxazoline amino acids **12** and **13** (Scheme 1), which resemble extended conformations of Glu. In addition, the applied synthetic strategy gave the regioisomers **14** and **15** (Scheme 1), which contain two locked conformations of aspartic acid. Thus, we investigated and compared their structural features with those of NMDA (**2**).

This paper deals with the synthesis of the four stereoisomers **12**−**15** and evaluation of their activity at iGluRs, as well as at representative subtypes of mGluRs. Furthermore, we have compared the results of conformational analyses performed on the new compounds with the conformations of NMDA (**2**), *trans*-ACPD (**10**), and *cis*-ACPD (**11**); the latter compound is an agonist at NMDA and mGlu receptors.

Results and Discussion

The 1,3-dipolar cycloaddition of bromonitrile oxide to ethyl 1-[(*tert*-butoxycarbonyl)amino]cyclopent-2-enecarboxylate yielded a racemic mixture of bicyclic cyclopentaisoxazoline derivatives **16**−**19** (Scheme 2). It is worth noting that the pericyclic reaction produces all four of the possible stereoisomers **16**, **17**, **18**, and **19** in an 86% yield and a relative ratio of 50:7:28:15. As previously observed in a related cycloaddition,[12] the major stereoisomer **16** derives from a transition state that is stabilized by a hydrogen bond between the NHBoc group of the dipolarophile and the negatively charged oxygen atom of the nitrile oxide. The separation of the four cycloadducts was achieved only after conversion into their corresponding primary amines **20**−**23**. As Scheme 2 displays, treatment of the crude reaction mixture of **16**−**19** with 30% trifluoroacetic acid in dichloromethane afforded a mixture of the amino esters **20**−**23** (95% yield), which were separated by column chromatography on silica gel. The final cyclopentaisoxazoline amino acids **12**, **13**, **14**, and **15** were obtained from intermediates **20**−**23** in 45−51% yield by alkaline hydrolysis of their ester groups and concomitant nucleophilic displacement of the 3-bromo moiety, followed by purification by cation-exchange column chromatography.

For analytical purposes, we reconverted the bicyclic amino esters **20**−**23** separately into their corresponding NBoc derivatives. The relative percentages of the stereoisomers **16**−**19**, reported in Scheme 2, were obtained by HPLC analysis of the crude reaction mixture.



Scheme 2

The assignment of the structures of all the synthesized compounds is based on the ^{1}H NMR spectra of the amino esters **20**−**23**. The multiplicity of the signal of 6a-H, which is the most deshielded proton, is diagnostic for the assignment of the regiochemistry because it resonates as a doublet in the cycloadducts **20** and **21**, and as a multiplet in the cycloadducts **22** and **23**. The structures of the stereoisomeric pairs **20**/**21** and **22**/**23** were assigned by taking into account the upfield shifts of the signals of protons 6a-H and 3a-H, respectively, observed in compounds **21** and **23**. Such a shielding effect, reported in the literature as the *syn*-upfield rule,[13] is explained by the spatial proximity of the amino group. As a matter of fact, 3a-H of **23** resonates at $\delta = 3.42$ ppm (cf. $\delta = 4.03$ ppm observed for derivative **22**). The same considerations hold true when comparing the chemical shifts of 6a-H in derivatives **21** and **20** ($\delta = 4.73$ and 5.08 ppm, respectively). To further support our assignment, we note that stereoisomer **20** derives from cycloadduct **16**, the major component of the 1,3-dipolar cycloaddition, whose transition state is stabilized by an intermolecular hydrogen bond.[12]

The four isomeric amino acid derivatives **12**−**15** were tested in vitro at iGlu and mGlu receptors by means of receptor binding techniques, electrophysiological activity in the rat cortical wedge preparation, and second messenger assays. Their receptor affinities for NMDA, AMPA, and KA were determined using the radio-ligands 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid ([^{3}H]CPP), [^{3}H]AMPA, and [^{3}H]KA, respectively.[14−16] As shown in Table 1, none of the compounds under study, evaluated at up to 0.1 mM in binding assays and up to 1.0 mM in the

Table 1. Receptor binding and electrophysiological data

Compound[a]	Receptor binding IC_{50} [μM]			Electrophysiology EC_{50} [μM]
	[^{3}H]AMPA	[^{3}H]KA	[^{3}H]CPP	
12	> 100	> 100	> 100	> 1000
13	> 100	> 100	> 100	> 1000
14	> 100	> 100	> 100	> 1000
15	> 100	> 100	> 100	> 1000
AMPA	0.040 ± 0.014	> 100	> 100	3.5 ± 0.2
KA	4.0 ± 1.2	0.007 ± 0.002	> 100	25 ± 3
NMDA				11 ± 3

[a] The data are mean values ± SEM, $n = 3-4$.

electrophysiological test, displayed significant activity at the above-mentioned iGluRs as either agonists or antagonists.

We also assayed the same compounds at group-I mGluRs (mGluR1 and mGluR5), and at mGluR2 and mGluR4 as representatives for groups II and III, respectively.[17] The data reported in Table 2 show that, while amino acids **13**−**15** are inactive at all the receptors tested, the stereoisomer **12** behaves as an mGluR5 agonist, with lower activity at mGluR2 and only marginal activity at mGluR1. Thus, **12** distinguishes between the two group-I mGluRs, being more than tenfold selective for mGluR5 over mGluR1. The overall pharmacological profile is significantly different from that of the structural analogues *trans*-ACPD (**10**) and *cis*-ACPD (**11**).[18]

Table 2. Potency and subtype selectivity at cloned mGlu receptors expressed in CHO cells

Compound	EC_{50} [μM]			
	mGluR1	mGluR5	mGluR2	mGluR4
(*S*)-Glu (**1**)[a]	4.9	3.1	0.29	9.8
(±)-*trans*-ACPD (**10**)[a]	15	23	2	ca. 800
(±)-*cis*-ACPD (**11**)[b]	> 300	> 300	13	50
12	ca. 1000[c]	79±32[d]	240±37[e]	> 1000
13	> 1000	> 1000	> 1000	> 1000
14	> 1000	> 1000	> 1000	> 1000
15	> 1000	> 1000	> 1000	> 1000

[a] Ref.[23] [b] Ref.[1] [c] 42 ± 9% at 1 mM. [d] Maximum response: 67 ± 5%. [e] Maximum response: 84 ± 2%.

where N-α and C-α correspond to the central atoms of each amino acid's amino and carboxylate functions, respectively, and C-ω corresponds to the C-3 carbon atom of the 3-hydroxyisoxazoline moiety (Table 3). Indeed, based on the results obtained previously by our research group,[22] the 3-hydroxyisoxazoline moiety can act as a surrogate for the acidity of the 3-hydroxyisoxazole nucleus of AMPA and both are bioisosteres of the distal carboxylate function of Glu.

Table 3. Molecular parameters describing the pharmacophoric distances d_1 ($d_{N\text{-}\alpha,C\text{-}\omega}$) and d_2 ($d_{C\text{-}\alpha,C\text{-}\omega}$) of compounds **12**−**15**

Conformation	d_1 [Å]	d_2 [Å]
12A	4.20	4.63
12B	3.76	4.91
13A	4.60	4.18
13B	4.75	3.66
14A	2.99	3.92
14B	2.89	3.74
15A	3.81	2.94
15B	3.67	2.91
2A[a]	3.84	3.00
5A[b]	4.18	4.40

[a] Ref.[25] [b] Ref.[11]

To account for the data reported above, we carried out a conformational analysis on the derivatives **12**−**15** by means of theoretical calculations at the STO-3G level.[19,20] After localization of all the minima, the energies of the optimized conformations were recalculated in a polarizable conductor-like solvation model (C-PCM)[21] to obtain values that are compatible with water solutions.

All compounds showed two populated conformations, labeled **A** and **B** in Figure 1. In fact, because of the presence of the isoxazoline moiety in their bicyclic skeletons, the cyclopentane ring can only assume the E_5 and ^5E conformations. The conformations of compounds **12**−**15** were analyzed preliminary by examining the interatomic distances among the three ionizable centers, i.e., N-α, C-α, and C-ω,

None of the compounds **12**−**15** displays activity for the AMPA, KA, and NMDA receptors, even though conformation **12A** reproduces almost exactly the d_1 and d_2 distances [d_1 = 4.20 Å; d_2 = 4.63 Å (Table 3)] of the suggested active conformation of CIP-A (**5**) (**5A**: d_1 = 4.18 Å; d_2 = 4.40 Å),[11,23] a potent AMPA and KA agonist. If **12A** and **5A** are superimposed however, through an rms fit of the three proposed pharmacophoric groups (Figure 2, a), the steric volumes occupied between C-α and C-ω are very different. This outcome suggests that the steric interaction present in **12A** could, at least in part, account for the inactivity of derivative **12**. We also superimposed conformations **12B**, **13A**, and **13B** with that of conformation **5A**, but the superimpositions are poor (figures not shown), which confirms

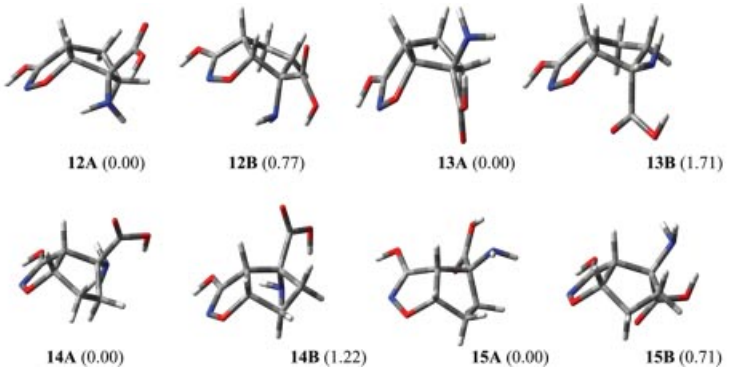


Figure 1. Three-dimensional plots of the populated conformations of compounds **12**−**15**; in parentheses are the relative energies [kcal/mol]

the significant differences found in the d_1 and d_2 parameters.

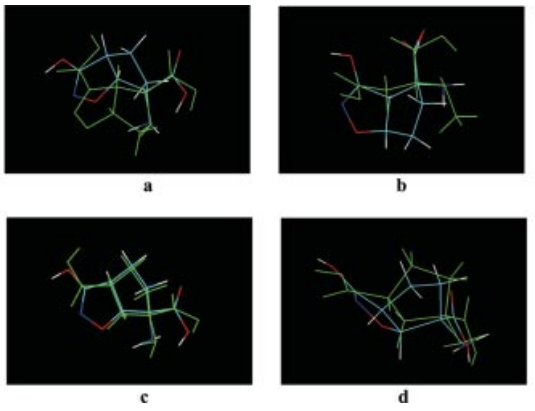


Figure 2. Superimpositions of populated conformations: (**a**) **12A** vs. **5A** (green); (**b**) **15A** vs. **2A** (green); (**c**) **12A** vs. **10A** (green); (**d**) **13A** vs. **11A** (green)

As far as the NMDA receptor is concerned, a folded conformation of Glu has been suggested as the bioactive one.[24,25] Moreover, the active conformation of **2** (**2A**, Figure 2, b) possesses N-α positioned *anti* to C-ω, while the C-α and C-ω carboxylate groups are in a relative *gauche* arrangement. This situation corresponds to d_1 and d_2 distances of ca. 3.9 and 3.0 Å, respectively. Two of our compounds, namely **14** and **15**, have a through-bond path between C-α and C-ω that matches the one displayed by reference compound **2**. Nevertheless, the data reported in Table 3 clearly show that, whereas in both conformations of compound **15** (in particular **15A**) the d_1 and d_2 parameters are similar to those of **2A**, the corresponding values for derivative **14** are very different. As a consequence, derivative **15** possesses a putative correct arrangement of its three ionizable groups and, from such a viewpoint, should be active at NMDA receptors. The overall molecular size, however, of **15** is significantly greater than that of **2** and a large portion of **15** extends outside the molecular volume of **2** (Figure 2, b), in spite of a good superimposition of the aspartic acid backbone.

Finally, we tried to rationalize the pharmacology of the test compounds at metabotropic glutamate receptors. Two conclusions have been drawn in the literature[26,27] from a study of mGluR1, -2 and -4 pharmacophore models. Glu activates all mGluRs in a similar extended conformation (d_1, $d_2 > 4.5$ Å), and selectivity would result only from the different protein environments in the binding sites. As far as the conformations of **12A** and **13A** (Table 3) are considered, one distance (d_2 for **12A** and d_1 for **13A**) is longer than 4.5 Å and the other one (d_1 for **12A** and d_2 for **13A**) is close to this limit value. All the other conformations are too much folded and, consequently, their parameters are far from the ideal ones. Compounds **12** and **13** can be viewed as conformationally constrained analogues of *trans*-ACPD (**10**) and *cis*-ACPD (**11**), respectively, with the 3-carboxylic group of the lead compound replaced by the hydroxyimino function and constrained into the isoxazoline ring. Compounds **10** and **11** are rather flexible molecules and can adopt several conformations that mimic the conformations of Glu. The overlap of **12A** and **13A**, the most populated conformations of **12** and **13**, respectively, with the extended conformations of **10** (**10A**, Figure 2, c) and **11** (**11A**, Figure 2, d), respectively, turned out to be optimal with the pair **12A/10A** (Figure 2, c) and less satisfactory with the pair **13A/11A** (Figure 2, d). As shown in Table 2, compound **12** is the only one that possesses some affinity for the mGluRs. It is inactive at mGluR4, it displays activity at mGluR5, it is less active at mGluR2, and only marginally active at mGluR1. In general, **12** is less potent than **10**, but with a significantly different pharmacological profile. These outcomes further support previous results[18,26] that indicate that conformational parameters similar to those of the potent lead compounds are necessary, but not sufficient, requirements to confer activity at the target receptors, because additional steric interactions may be unfavorable.

Experimental Section

Materials and Methods: ^{1}H NMR spectra were recorded using 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 Hz. TLC analyses were performed on commercial silica gel 60 F_{254} aluminum sheets; spots were detected by spraying with dilute alkaline potassium permanganate solution or ninhydrin. Melting points were determined with a Büchi apparatus and are uncorrected. Elemental analyses (C, H, N) of new compounds were performed by Redox s.n.c. (Milan); all analyses agree with theoretical values (± 0.4%).

1,3-Dipolar Cycloaddition of Bromonitrile Oxide to Ethyl 1-[(*tert*-Butoxycarbonyl)amino]cyclopent-2-enecarboxylate: Dibromoformaldoxime (1.9 g, 9.4 mmol) and solid $NaHCO_3$ (3.5 g) were added to a solution of ethyl 1-[(*tert*-butoxycarbonyl)amino]cyclopent-2-enecarboxylate[12] (1.2 g, 4.7 mmol) in EtOAc (15 mL). The mixture was stirred vigorously for 3 d. The progress of the reaction was monitored by TLC (petroleum ether/EtOAc, 4:1). Water (10 mL) was added to the reaction mixture and the organic layer was separated and dried with anhydrous Na_2SO_4. The crude material, obtained after evaporation of the solvent, was purified by column chromatography on silica gel (eluent: petroleum ether/EtOAc, 85:15) to give an inseparable mixture of the cycloadducts **16**−**19** (1.52 g, 86% yield).

Synthesis of Cyclopentaisoxazoline Amino Esters 20−23: The mixture of the cycloadducts **16**−**19** (1.52 g, 4.03 mmol) was treated with 30% trifluoroacetic acid in CH_2Cl_2 (10.3 mL) at 0 °C. The reaction mixture was stirred at room temperature until disappearance of the starting material was observed (3 h). The volatile components were evaporated under vacuum and the residue was treated with 10% K_2CO_3 solution (25 mL) and extracted with EtOAc (4 × 10 mL). The pooled organic extracts were dried with anhydrous Na_2SO_4 and concentrated under vacuum. The residue was chromatographed on a column of silica gel (eluent: petroleum ether/EtOAc, 1:1) to give four fractions (95% overall yield). The compounds were eluted in the following order: **22** (298 mg), **20** (530 mg), **21** (75 mg), and **23** (160 mg).

Ethyl (±)-(3a*R*,6*S*,6a*R*)-6-Amino-3-bromo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-carboxylate (20): R_f = 0.65 (cyclohexane/

EtOAc, 1:4). ^{1}H NMR ($CDCl_3$): δ = 1.26 (t, *J* = 7.3 Hz, 3 H), 1.58 (td, *J* = 8.42, 8.42 and 12.82 Hz, 1 H) 1.85 (br. s, 2 H), 1.94−2.05 (m, 2 H), 2.11 (td, *J* = 5.49 and 7.33 Hz, 1 H), 3.84 (td, *J* = 5.49, 5.49 and 9.5 Hz, 1 H), 4.18 (q, *J* = 7.3 Hz, 2 H), 5.08 (d, *J* = 9.5 Hz, 1 H) ppm.

Ethyl (±)-(3a*R*,6*R*,6a*R*)-6-Amino-3-bromo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-carboxylate (21): R_f = 0.52 (cyclohexane/EtOAc, 1:4). ^{1}H NMR ($CDCl_3$): δ = 1.30 (t, *J* = 7.4 Hz, 3 H), 1.58 (br. s, 2 H), 1.58−1.80 (m, 1 H), 2.00−2.38 (m, 3 H), 3.89 (dd, *J* = 8.2, 8.6 Hz, 1 H), 4.23 (q, *J* = 7.4 Hz, 2 H), 4.73 (d, *J* = 8.6 Hz, 1 H) ppm.

Ethyl (±)-(3a*R*,4*R*,6a*S*)-4-Amino-3-bromo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-carboxylate (22): R_f = 0.80 (cyclohexane/EtOAc, 1:4). ^{1}H NMR ($CDCl_3$): δ = 1.31 (t, *J* = 7.4 Hz, 3 H), 1.77 (br. s, 2 H), 1.77−1.98 (m, 1 H), 2.10−2.28 (m, 3 H), 4.03 (d, *J* = 9.6 Hz, 1 H), 4.23 (q, *J* = 7.4 Hz, 2 H), 5.20−5.37 (m, 1 H) ppm.

Ethyl (±)-(3a*R*,4*S*,6a*S*)-4-Amino-3-bromo-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-carboxylate (23): R_f = 0.41 (cyclohexane/EtOAc, 1:4). ^{1}H NMR ($CDCl_3$): δ = 1.29 (t, *J* = 7.4 Hz, 3 H), 1.55−1.93 (m, 3 H), 2.15−2.52 (m, 3 H), 3.42 (d, *J* = 8.2 Hz, 1 H), 4.20 (q, *J* = 7.4 Hz, 2 H), 5.29−5.38 (m, 1 H) ppm.

Conversion of 20−23 into the Corresponding Derivatives 16−19: Each amino ester **20**−**23** (20 mg, 0.072 mmol) was treated with TEA (10.92 mg, 0.108 mmol) and di-*tert*-butyl dicarbonate (23.57 mg, 0.108 mmol) in CH_2Cl_2 (2 mL) to give the corresponding NBoc derivatives **16**−**19**, which were not purified but instead used directly as standards for HPLC analysis (column: LiChrospher Si 60 Merck; eluent: petroleum ether/EtOAc, 85:15; flow: 0.5 mL/min; λ = 254 nm). **16:** R_t = 11.65 min; **18:** R_t = 13.23 min; **19:** R_t = 16.05 min; **17:** R_t = 18.15 min.

(±)-(3a*R*,6*S*,6a*R*)-6-Amino-3-hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-carboxylic Acid (12): Aqueous NaOH solution (1 N, 4 mL) was added to a solution of **20** (277 mg, 1 mmol) in dioxane (5 mL). The reaction mixture was stirred at room temperature for 2 h and then heated at 60 °C for 15 h. The residue was dissolved in 2 N HCl and the solution was submitted to cation-exchange chromatography using Amberlite IR-120 plus. The acidic solution was slowly eluted onto the resin, and then the column was washed with water until the pH was neutral. 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 recrystallized from water/MeOH, filtered, washed sequentially with MeOH and Et_2O, and then dried in vacuo at 50 °C to give the amino acid **12** (84 mg, 45 %) as white prisms. $C_7H_{10}N_2O_4·H_2O$: calcd. C 41.18, H 5.92, N 13.72; found C 41.37, H 6.09, N 13.69. R_f = 0.37 (*n*BuOH/water/AcOH, 60:25:15). M.p. > 210 °C (dec.). ^{1}H NMR (D_2O): δ = 1.75−1.89 (m, 1 H, 5-H), 1.97−2.21 (m, 3 H, 4-H, 4-H′, 5-H′), 3.53 (td, *J* = 2.5, 8.1, 8.1 Hz, 1 H, 3a-H), 5.07 (d, *J* = 8.1 Hz, 1 H, 6a-H) ppm.

(±)-(3a*R*,6*R*,6a*R*)-6-Amino-3-hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-6-carboxylic Acid (13): Compound **21** (277 mg, 1 mmol), submitted to the procedure described for compound **20**, afforded **13** (95 mg, 51%) as white prisms. $C_7H_{10}N_2O_4$ (186.17): calcd. C 45.16, H, 5.41, N 15.05; found C 44.96, H 5.65, N 14.87. R_f = 0.32 (*n*BuOH/water/AcOH, 60:25:15). M.p. > 200 °C (dec.). ^{1}H NMR (D_2O): δ = 1.80−1.89 (m, 1 H, 5-H), 1.94−2.07 (m, 1 H, 5-H′), 2.11−2.34 (m, 2 H, 4-H, 4-H′), 3.52 (td, *J* = 2.6, 9.0, 9.0 Hz, 1 H, 3a-H), 4.93 (d, *J* = 9.0 Hz, 1 H, 6a-H) ppm.

(±)-(3a*R*,4*R*,6a*S*)-4-Amino-3-hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-carboxylic Acid (14): Compound **22** (277 mg, 1 mmol), submitted to the procedure described for compound **20**, afforded **14** (86 mg, 46%) as white prisms. $C_7H_{10}N_2O_4·0.2H_2O$ (189.77): calcd. C 44.30, H 5.52; N 14.76; found C 44.50, H 5.37, N 14.71. R_f = 0.41 (*n*BuOH/water/AcOH, 60:25:15). M.p. > 220 °C (dec.). ^{1}H NMR (D_2O): δ = 1.91−2.22 (m, 4 H, 5-H, 5-H′, 6-H, 6-H′), 3.58 (d, *J* = 8.1 Hz, 1 H, 3a-H), 5.20 (td, *J* = 1.6, 8.1, 8.1 Hz, 1 H, 6a-H) ppm.

(±)-(3a*R*,4*S*,6a*S*)-4-Amino-3-hydroxy-4,5,6,6a-tetrahydro-3a*H*-cyclopenta[*d*]isoxazole-4-carboxylic Acid (15): Compound **23** (277 mg, 1 mmol), submitted to the procedure described for compound **20**, afforded **15** (93 mg, 50 %) as white prisms. $C_7H_{10}N_2O_4$ (186.17): calcd. C 45.16, H 5.41, N 15.05; found C 44.91, H 5.59, N 15.10. R_f = 0.30 (*n*BuOH/water/AcOH, 60:25:15. M.p. > 210 °C (dec.). ^{1}H NMR (D_2O): δ = 1.92−2.06 (m, 2 H, 5-H, 5-H′), 2.09−2.20 (m, 1 H, 6-H), 2.50−2.62 (m, 1 H, 6-H′), 3.22 (d, *J* = 7.3 Hz, 1 H, 3a-H), 5.14−5.20 (m, 1 H, 6a-H) ppm.

Pharmacology

Receptor Binding: The affinities for NMDA, AMPA, and KA receptors were determined using the ligands [^{3}H]CPP,[14] [^{3}H]AMPA,[15] and [^{3}H]KA,[16] respectively. The membrane preparations used in all the receptor-binding experiments were prepared according to the method of Ransom and Stec.[28]

In Vitro Electrophysiology: A rat cortical slice preparation for determination of excitatory amino acid evoked depolarizations, as described by Harrison and Simmonds,[29] was used in a slightly modified version. Wedges (500 μm thick) of rat brain, containing cerebral cortex and corpus callosum, were placed through a grease barrier for electrical isolation with each part in contact with a Dri-Ref-5SH (World Precision Instruments) electrode. The cortex and corpus callosum parts were superfused constantly with an Mg^{2+}-free (and Ca^{2+}-free for the corpus callosum) oxygenated Krebs buffer at room temperature. The test compounds were added to the cortex superfusion medium and the potential difference between the electrodes recorded on a chart recorder. Applications of agonists were made for 90 s at each concentration tested, typically at 15 min intervals. In experiments designed to detect antagonist effects, the potential antagonist was applied alone for 90 s followed by co-application of agonist (NMDA, AMPA or KA) and the potential antagonist for another 90 s.

Metabotropic Testing: Four metabotropic subtypes (mGluR1a, mGluR2, mGluR4a, or mGluR5) were expressed in Chinese hamster ovary cell lines and maintained and assayed as described previously.[17]

Theoretical Calculations: The conformational space of all the compounds analyzed was explored with the Hartree−Fock approach at the STO-3G level[19,20] as implemented in Gaussian98. Although the amino acids **12**−**15** exist as ions under physiological conditions, calculations were performed on the uncharged species to avoid strong intramolecular electrostatic interactions that would have led to unrealistic geometries during optimizations in vacuo; such a method provides geometries that compare satisfactorily with those of the corresponding solvated ionized species.[23] We fully optimized all the starting geometries deriving from the pseudorotational path of the five-membered carbocyclic ring and from rotation around the exocyclic single bonds. The energies of the confor-

mations were recalculated in a polarizable conductor-like solvation model (C-PCM)[21] to obtain values compatible with water solutions.

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