Synthesis and Biological Evaluation of 1-Amino-2-Phosphonomethylcyclopropanecarboxylic Acids, New Group III Metabotropic Glutamate Receptor Agonists

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Stereoisomers of 1-amino-2-phosphonomethylcyclopropanecarboxylic acid (APCPr), conformationally restricted analogues of L-AP4 (2-amino-4-phosphonobutyric acid), have been prepared and evaluated at recombinant group III metabotropic glutamate receptors. They activate these receptors over a broad range of potencies. The most potent isomer (1*S*,2*R*)-APCPr displays a similar pharmacological profile as that of L-AP4 (EC_{50} 0.72, 1.95, >500, 0.34 μM at mGlu4, 6, 7, 8 receptors, respectively, and no effect at group I/II mGluRs). It was characterized on native receptors located in the basal ganglia (BG) where it induced a robust and reversible inhibition of synaptic transmission. It was tested *in vivo* in haloperidol-induced catalepsy, a model of Parkinsonian akinesia, by direct infusion in the globus pallidus of the BG. At a dose of 0.5 nmol/μL, catalepsy was significantly antagonized. This study reveals that (1*S*,2*R*)-APCPr is a potent group III mGluR agonist and confirms that these receptors may be considered as a therapeutic target in the Parkinson's disease.

Introduction

The acidic amino acid L-glutamic acid is the major excitatory neurotransmitter in the mammalian central nervous system (CNS). This excitatory amino acid activates both ionotropic and metabotropic receptors.[1] The ionotropic receptors are directly responsible for the fast depolarization of postsynaptic cells whereas the metabotropic receptors regulate the activity of ion channels or enzymes producing second messengers via GTP-binding proteins. So far, eight mGlu receptor subtypes have been cloned and classified into three groups, according to sequence similarity, transduction mechanism, and agonist pharmacology.[2] Group I includes mGlu1 and mGlu5 receptors, which are positively coupled to phosphatidyl inositol hydrolysis whereas group II (mGlu2, mGlu3) and group III receptors (mGlu4, mGlu6, mGlu7, and mGlu8) are both negatively coupled to adenylyl cyclase but are endowed with different pharmacology and neuronal localization.

With the discovery of potent and selective ligands, mGlu receptors have become valuable therapeutic targets.[3−7] However, while several subtype-selective ligands for group I and II receptors have been discovered, only few are known for group III. Yet recent evidence point out an important role for group III mGlu receptors for the treatment of psychiatric disorders such as anxiety and depression,[8] pain[9,10] and neurodegenerative disorders, such as Parkinson's disease (PD[a]).[5,11,12] The role of group III mGlu receptors[13,14] and especially mGlu4[15,16] in the normalization of the abnormal neurotransmission in the basal ganglia in animal models of PD has recently been emphasized suggesting that selective agonists of this receptor class could provide symptomatic benefits for Parkinsonian patients.

Most of the authors have used L-AP4 (L-2-amino-4-phosphonobutyric acid) in their experiments to validate mGlu4 receptor as a valuable target for PD.[15−18] L-AP4 is the prototype agonist of group III mGlu receptors but nonselective for the different group III mGluR subtypes. In addition, L-AP4 is also substrate of the Cl^- dependent/Na^+ independent cystine-glutamate exchanger that was previously identified as the inducer of quisqualate sensitization.[19,20] A classical approach to gain potency and selectivity is to structurally constrain the nonselective ligands. Indeed, several conformationally constrained analogues of L-AP4 have been synthesized, but all displayed weaker activity than L-AP4 at mGlu4 receptor (Chart 1). They include cyclopentyl derivatives (Cyclopentyl-AP4[21] and APCPe[22]), cyclobutyl derivatives (APCBu[22]), and cyclopropyl derivatives (APCPr initially named Cyclopropyl-AP4[21] and PCG[23]). Both (*Z*)-APCPr and (*E*)-APCPr have been synthesized[24] and tested on mGlu4 receptor as racemic mixtures,[21] yet the activity of

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[a] Abbreviations: APCPr, 1-amino-2-phosphonomethylcyclopropanecarboxylic acid; AP4, 2-amino-4-phosphonobutyric acid; AP5, 2-amino-5-phosphonopentanoic acid; NMDA, *N*-methyl D-aspartic acid; GABA, 4-aminobutyric acid; PHCCC, *N*-phenyl-7-(hydroxylimino)cyclopropa[*b*]-chromen-1a-carboxamide; mGluR, metabotropic glutamate receptor; GPCR, G-protein coupled receptor; PD, Parkinson's disease; BG, basal ganglia; GP, globus pallidus; STN, subthalamic nucleus; SNc, substantia nigra pars compacta; LPP, lateral perforant path; DG, dentate gyrus; ACSF, artificial cerebrospinal fluid.

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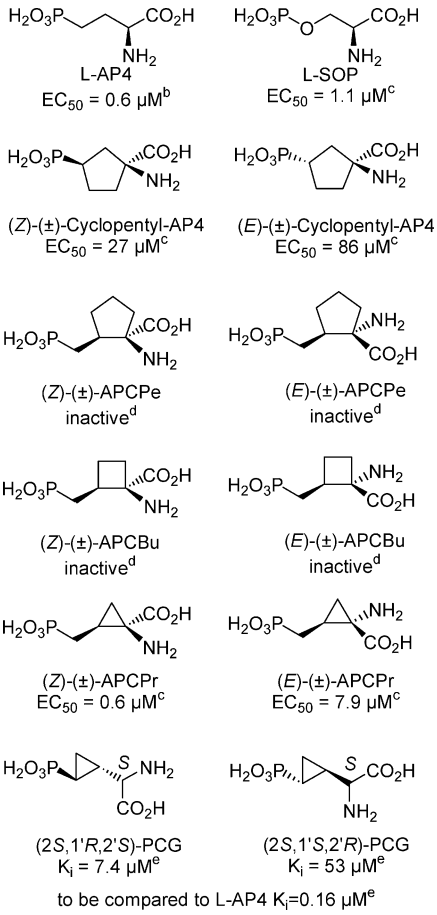
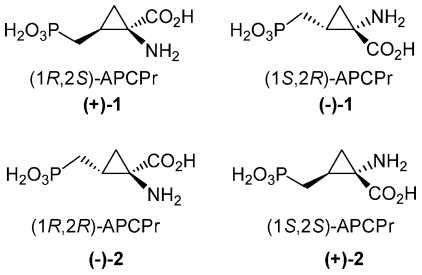
Chart 1. Cyclic Analogues of L-AP4:[a] Structures and EC_{50} Values at the mGlu4 Receptor



[a] Abbreviations: AP4, 2-amino-4-phosphonobutyric acid; SOP, serine-*O*-phosphate; cyclopentyl-AP4, 1-amino-3-phosphonocyclopentanecarboxylic acid; APCPe, 1-amino-2-phosphonomethylcyclopentanecarboxylic acid; APCBu, 1-amino-2-phosphonomethylcyclobutanecarboxylic acid; APCPr, 1-amino-2-phosphonomethylcyclopropanecarboxylic acid. [b] This study. [c] Reference 21. [d] Reference 22. [e] Reference 23.

Chart 2. The Four Stereoisomers of APCPr: **1** and **2** are the *Z* and *E* Diastereoisomers, Respectively[21]

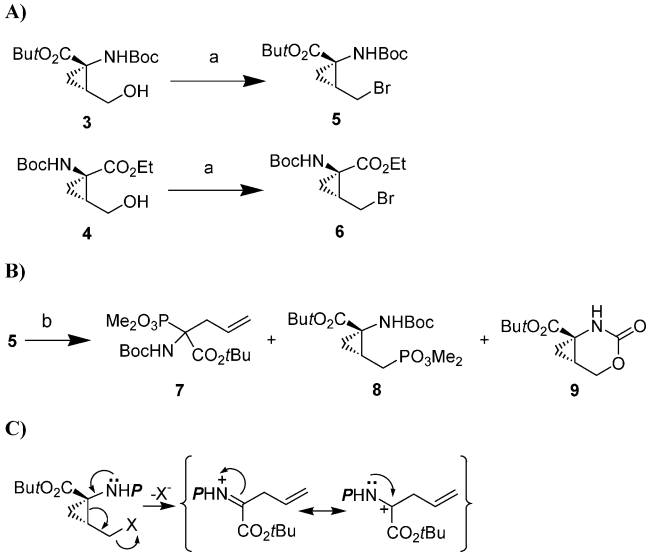


the pure enantiomers may differ from that of the racemates. In this study, we report on the synthesis of the four stereoisomers of 1-amino-2-phosphonomethylcyclopropanecarboxylic acid APCPr (**1** and **2**, Chart 2) and their functional activity on each group III mGlu receptor expressed in HEK 293 cells. The most potent stereoisomer was further characterized *in vitro* in brain slices and *in vivo* in a rat haloperidol-induced catalepsy model of Parkinsonian akinesia.

Results

Chemistry. *tert*-Butyl (1*S*,2*R*)-1-(*tert*-butoxycarbonylamino)-2-(hydroxymethyl)cyclopropane-1-carboxylate **3** and ethyl (1*R*,2*R*)-1-(*tert*-butoxycarbonylamino)-2-(hydroxymethyl)cyclopropane-1-carboxylate **4** were prepared from (*R*)-(+)-benzylglycerol according to literature references.[25−28] Those alcohols were then submitted to bromination using CBr_4 and polymer-bound PPh_3 in CH_2Cl_2 in the presence of triethylamine (Scheme 1A). The next step in the synthesis of APCPr, the introduction of the phosphonate functionality, was difficult to achieve. Bromide **5** was reacted with different phosphorus reactants under Arbuzov[29] or Michaelis−Becker conditions ($P(OMe)_3$, $P(OiPr)_3$, $HP(O)(OEt)_2$/NaH/toluene, $HP(O)(OPh)_2$/DBU/acetonitrile) but gave mostly the cyclopropane-cleavage product **7**, along with the phosphonate **8** and bicyclic product **9** in minor quantities (Scheme 1B). Replacing the bromide by a chloride or an iodide did not solve the problem, but decreasing the electrodonor capacity of the nitrogen of substrate **5** by changing the amine protective group (Boc) to a more electron-withdrawing group (trifluoroacetyl) afforded the phosphonate **12** with a significant decrease of cyclopropane cleavage and no other secondary product besides the alkene derivative. Indeed the intermediate carbocation resulting from the cyclopropane ring opening is less prone to be formed because, as the nitrogen free doublet is attracted by the trifluoroacetyl group, it is prevented from forming the iminium ion and stabilizing the carbocation (Scheme 1C). The protected APCPr **12** and the alkene **13** were separated by flash chromatography (Scheme 2). Acid hydrolysis allowed complete deprotection of **12** to give (−)-(1*S*,2*R*)-1-amino-2-phosphonomethylcyclopropanecarboxylic acid (1*S*,2*R*)-APCPr **(−)-1** which was purified by ionic chromatography. (−)-(1*R*,2*R*)-1-Amino-2-phosphonomethylcyclopropanecarboxylic acid **(−)-2** and enantiomers **(+)-1** and **(+)-2** were obtained in an identical manner (Scheme 2).

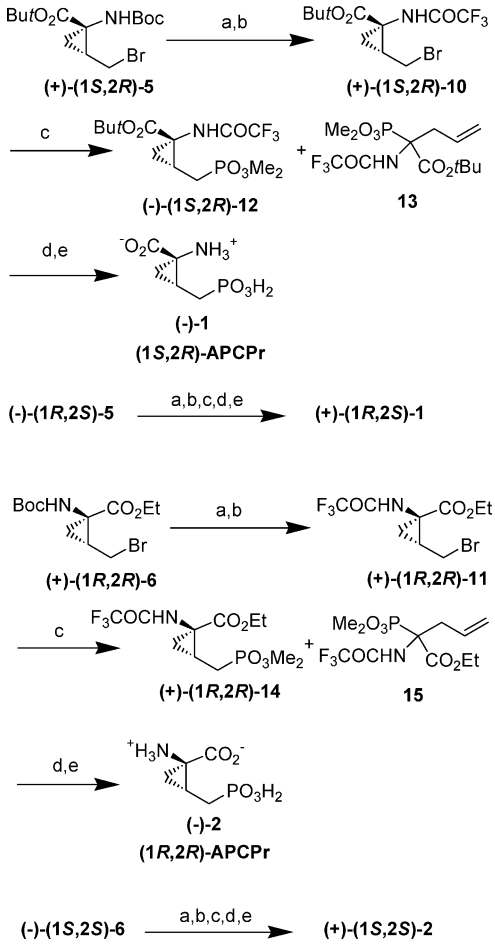
Scheme 1. Synthesis of Bromides **5** and **6**. Arbuzov and Side Reactions[a]



[a] Reagents and conditions: (a) polymer-bound PPh_3, CBr_4, NEt_3, CH_2Cl_2; (b) $P(OMe)_3$, reflux.

Pharmacological Activities of APCPrs at mGlu Receptors. The effects of the four stereoisomers of APCPr (Chart 2) were examined on all group III mGlu receptors (mGlu4, mGlu6, mGlu7, and mGlu8). These receptors were transiently expressed in HEK 293 cells as previously described,[30] and the total inositol phosphate production resulting from the receptor activation was determined. Since group III mGlu receptors are not normally coupled to PLC but rather inhibit adenylyl cyclase, this coupling was made possible by coexpressing this receptor with the chimeric G-protein alpha subunit Gαqi.[30,31] We previously reported that this assay gave more accurate results than the

Scheme 2. Synthesis of (1*S*,2*R*)-APCPr (−)-**1** and (1*R*,2*R*)-APCPr (−)-**2**[a]



[a] Reagents and conditions: (a) HCl/AcOEt; (b) TFAA, CH_2Cl_2; (c) $P(OMe)_3$, reflux; (d) 6 N HCl, reflux; (e) Dowex-H^+.

classical measurement of the inhibition of the forskolin-activated adenylyl cyclase activity and that the pharmacology of these receptors was not altered.[30]

APCPr studies are summarized in Table 1 which shows the EC_{50} values at all three group III mGlu receptor subtypes. The agonist dose−response curves for all four APCPr isomers on mGlu4 receptor are shown in Figure 1A. Among the tested compounds, (1*S*,2*R*)-APCPr **(−)-1** is the most potent in activating all group III mGlu receptors, with potencies comparable to that of the best group III mGlu receptors agonist L-AP4. The (1*S*,2*S*)-APCPr **(+)-2** isomer is somewhat less potent than the (1*S*,2*R*) isomer but mainly differs in that it is a partial agonist on all group III mGlu subtypes. As compared to the control activity induced by L-AP4 at 100 μM, the percentages of maximal activity of (1*S*,2*S*)-APCPr **(+)-2** are 75.3 ± 6.6%, 87 ± 6.4%, and 45.5 ± 13.4% on mGlu4, mGlu6, and mGlu8 receptors, respectively. In contrast, (1*R*,2*R*)-APCPr **(−)-2** shows weak activity at any of the tested mGlu receptors, except on mGlu8. In all cases, (1*R*) isomers are 20−100 times less potent than their enantiomers, as it is usually the case with mGlu receptors agonists. The agonist dose−response curves of the most active stereoisomer, (1*S*,2*R*)-APCPr **(−)-1**, on the different group III mGlu receptors are displayed in Figure 1B.

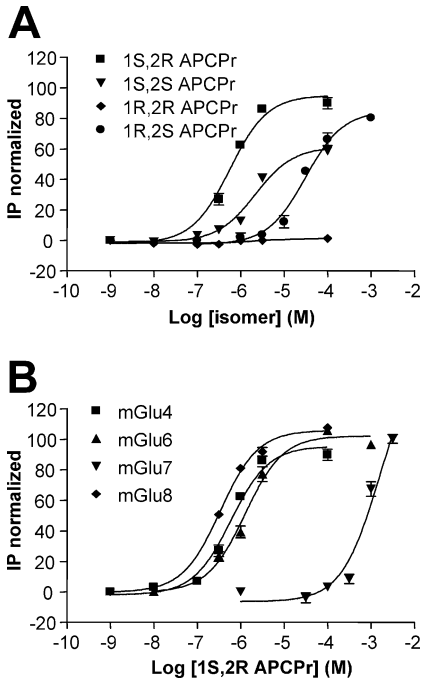


Figure 1. Activity of APCPr isomers on group III mGlu receptors transiently expressed in HEK 293 cells. (A) Dose−response curves of the four stereoisomers of APCPr on the mGlu4 receptor. (B) Dose−response curves of (1*S*,2*R*)-APCPr **(−)-1** on the different group III mGlu receptors. Receptor activation is determined by inositol phosphate production. Results are expressed as the percentage of the maximal activity induced by L-AP4. Present data correspond to a representative experiment from at least three different experiments.

Moreover potential activity of (1*S*,2*R*)-APCPr **(−)-1** was assessed on group I and group II mGlu receptors, as shown in Figure 2. A concentration of 100 μM (1*S*,2*R*)-APCPr **(−)-1** does not induce any activation of mGlu1, mGlu2, or mGlu5 receptors, their IP production remaining similar to their basal activity. No inhibition of the activity of mGlu1, mGlu2, or mGlu5 receptors induced by a submaximal dose of their specific agonist (quisqualic acid or DCG-IV) is observed in presence of 100 μM (1*S*,2*R*)-APCPr **(−)-1**. Altogether these data indicate that (1*S*,2*R*)-APCPr **(−)-1** is devoid of any nonspecific agonist or antagonist activity on group I and group II mGlu receptors.

Pharmacological Activity of (1*S*,2*R*)-APCPr (−)-1 at NMDA Receptors. Amino acids bearing a phosphonate in their side chain are well-known competitive antagonists of NMDA receptors,[32,33] their prototype being (*R*)-AP5 also named D-AP5.

Table 1. Activities of the Different Stereoisomers of APCPr at All Group III mGlu Receptors and Comparison with L-Glutamate and L-AP4. Data are Means ± SEM of Three Different Experiments at Least

agonists	mGlu4 EC_{50} (μM)	mGlu6 EC_{50} (μM)	mGlu7 EC_{50} (μM)	mGlu8 EC_{50} (μM)
Glu	16.4 ± 2	38.6 ± 6.6	2300 ± 100	8.2 ± 0.4
L-AP4	0.69 ± 0.27	nd[a]	800 ± 90[b]	0.56 ± 0.07
(1*S*,2*R*)-APCPr (−)-**1**	0.72 ± 0.21	1.95 ± 0.1	602 ± 312	0.34 ± 0.01
(1*R*,2*S*)-APCPr (+)-**1**	40.8 ± 13.6	111 ± 41	>3000	6.51 ± 0.74
(1*S*,2*S*)-APCPr (+)-**2**[c]	1.85 ± 0.76	3.41 ± 0.45	625 ± 82	2.15 ± 0.22
(1*R*,2*R*)-APCPr (−)-**2**	>1000	>1000	>1000	>300

[a] nd: not determined. [b] Data taken from reference 59. [c] Partial agonist: percentages of L-AP4 maximal response are 75.3.%, 87%, 45.5% at mGlu4, mGlu6, mGlu8 receptors, respectively.

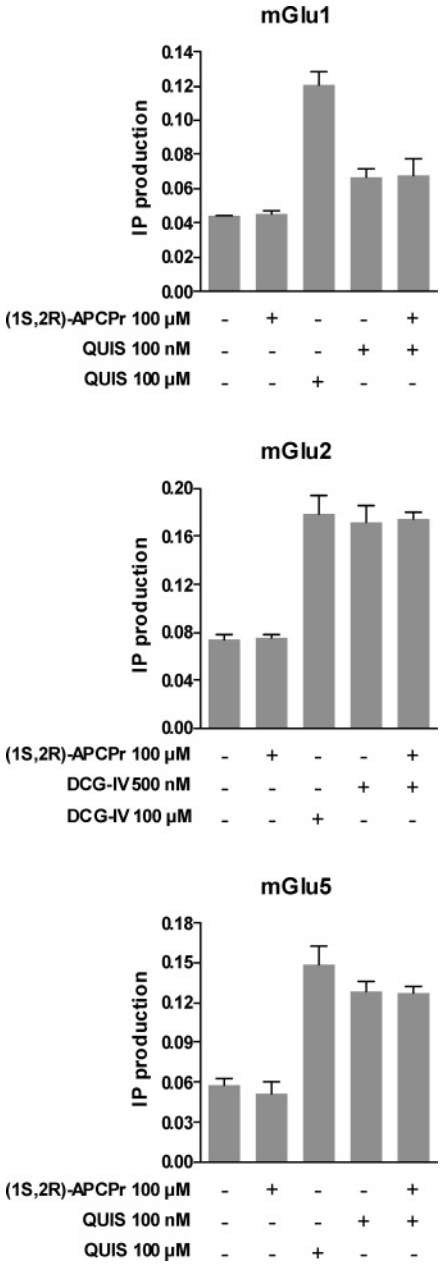


Figure 2. Lack of nonspecific agonist or antagonist activity of (1*S*,2*R*)-APCPr **(−)-1** on group I and group II mGlu receptors transiently expressed in HEK 293 cells. Receptor activations are determined by inositol phosphate production. Group I mGlu receptors (mGlu1 and mGlu5) are partially or fully activated by 100 nM or 100 μM of quisqualic acid (Quis), respectively. The group II mGlu2 receptor is partially or fully activated by 500 nM or 100 μM DCG-IV, respectively. No agonist or antagonist activity of (1*S*,2*R*)-APCPr **(−)-1** on these receptors is observed at 100 μM.

It was expected that the conformational restriction induced by the cyclopropyl ring on the $\alpha-\beta$ bond of APCPrs would favor conformations that differ from those that bind to the glutamate site of NMDA receptors which have been identified in the pharmacophore,[32] comparative models,[34] and the crystal structure.[35] Yet some flexibility remains in their side chain. We thus evaluated the ability of (1*S*,2*R*)-APCPr **(−)-1** to block recombinant NR1/NR2A NMDA receptors expressed in the *Xenopus* oocyte. As expected, (1*S*,2*R*)-APCPr **(−)-1** only weakly inhibited the responses evoked by the coagonists (glutamate and glycine applied both at their EC_{50}), with an IC_{50} equal to 315 $\pm$ 30 μM (nH 1.2 $\pm$ 0.1; n = 5; not shown). We may thus consider that (1*S*,2*R*)-APCPr **(−)-1** displays a selective effect at group III mGlu receptors at low micromolar concentrations.

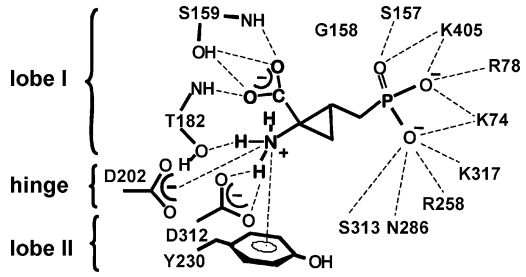


Figure 3. APCPr docked at the mGlu4 receptor binding site. Polar interactions between ligand and protein are represented by dotted lines.

Molecular Modeling. To gain further insights into the structural/conformational requirements for ligand potency, we have docked all four APCPrs in mGlu4 and mGlu8 receptor homology models.[36] We used the 3D-models of the active conformation of the amino-terminal domain of the receptors where the ligand binding site is located. This domain folds in two lobes connected by a hinge region allowing an open and a closed conformation similarly to a clamshell. The active conformation is the closed one that may be stabilized upon trapping an agonist in between the two lobes. Yet with poor agonists this conformation may not be reached. The conformational flexibility of each molecule was first investigated through a simulated annealing protocol as described in Jullian et al.[37] It appears that the APCPrs adopt mainly three conformations corresponding to the three positions of the phosphonate group. However, those three conformers exhibit very close total relative energy (energy window $\Delta E < 2$ kcal). During the minimization step of the docking procedure, all the conformers adopt eventually the same bound conformation. Each stereoisomer was docked into the active site of mGlu4 or mGlu8 receptor binding site model.[36] We have previously demonstrated that the rank order of potencies for a series of agonists of OR5.24 another class C GPCR is similar in functional and binding assays.[38] Thus, assuming that affinity and efficacy are comparable, agonist binding analysis may be qualitatively correlated with functional potencies, as outlined for mGlu4 receptor. Results for mGlu8 receptor are similar. Each stereoisomer but **(−)-2** (see below) reveals an identical binding network: the proximal functions are tied up to the conserved residues Ser159, Thr182, Asp202, Tyr230, and Asp312, the distal acidic functions are bound to Lys74, Arg78, Ser157, Arg258, Asn286, Ser313, Lys317, and Lys405. These interactions are classically observed in group III mGlu receptors agonists (Figure 3).[36] We then superimposed our models based on the Cα atoms of residues present at the binding site. The traces were quite similar but differed in one region, the one made of Ser157 and Gly158 in the mGlu4 receptor. Indeed the cyclopropyl ring is directed toward lobe II (Tyr182) for (1*S*) enantiomers whereas it is pointing to lobe I for (1*R*) enantiomers. In these latter enantiomers, it causes a steric clash with the Ser157-Gly158 peptidic bond that flipped ca. 180° (Figure 4). This kind of flip has been seen in crystallographic structures of various ligands in AMPA receptor.[39] The energy needed to flip this bond may be linked to the lower activities of (1*R*) enantiomers. A similar hindrance is found between the carbonyl group of the Ala154-Ala155 peptide bond and the (1*R*) ligands, in the mGlu8 receptor models. This hindrance is indeed independent of the nature of the side chains of the residues. Yet at mGlu8 receptor, the hydrophobic environment provided by Ala154, Ala155 and Ala177 around the cyclopropyl ring of (1*R*) ligands may partly compensate the

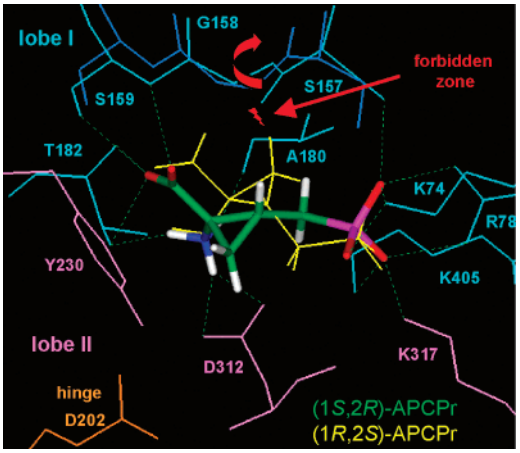


Figure 4. Superimposition of the two mGlu4 binding site models docked with (1*S*,2*R*)-APCPr **(−)-1** (sticks) and (1*R*,2*S*)-APCPr **(+)-1** (yellow lines). Residues of the (1*R*,2*S*)-APCPr **(+)-1** model (blue) are shown only in the forbidden zone, where the Ser157-Gly158 peptidic bond flipped. In the other regions, the traces and side chains are quite similar to those of the (1*S*,2*R*)-APCPr **(−)-1** model. Residues of lobe I are colored in cyan, those of lobe II in magenta, and those of the hinge in orange; carbon atoms of (1*S*,2*R*)-APCPr **(−)-1** in green, nitrogen in dark blue, oxygens in red, phosphorus in magenta, and hydrogens in white.

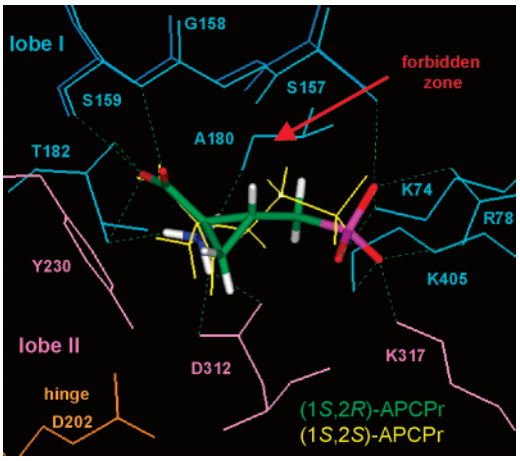


Figure 5. Superimposition of the two mGlu4 binding site models docked with (1*S*,2*R*)-APCPr **(−)-1** (sticks) and (1*S*,2*S*)-APCPr **(+)-2** (yellow lines). Residues of the (1*S*,2*S*)-APCPr **(+)-2** model (blue) are shown only in the forbidden zone. Colors are as indicated for Figure 4.

steric destabilization. It may thus explain a significanly lower EC_{50} at mGlu8 receptor than at mGlu4 receptor with these agonists (Table 1). Based on mGlu receptor pharmacophore models the region of the 1*R* cyclopropyl ring is sterically restricted,[40,41] this is in concordance with what we report here. It should also be noted that the active conformation of the binding domain of the receptor that is modeled in this study, may not be relevant for weak agonists such as (1*R*,2*R*)-APCPr **(−)-2** or partial agonists as (1*S*,2*S*)-APCPr **(+)-2**. Activities of the (1*S*) enantiomers are close to each other and indeed the traces are quite similar even in the Ser157-Gly158 region (Figure 5). Nevertheless, the methylphosphonate protons of the less potent (1*S*,2*S*)-APCPr **(+)-2** are directed to the same "forbidden zone" described above. This situation may be responsible for the partial agonism of this stereoisomer. A steric hindrance with the two homologous residues of mGlu8 receptor has been reported to be the cause of the partial agonism of FP429 at mGlu8 subtype.[42] In that previous study, FP429 was a full agonist at mGlu4 receptor and a partial agonist at mGlu8 receptor, we demonstrated that the side chains of Ala154-Ala155 were responsible for that property and suggested that hindrance with those side chains induced either a partial closing of the bilobate domain or a weaker stabilization of that domain resulting in partial agonism. In the present case, hindrance would occur both with Ser157 and/or Gly158 of the mGlu4 receptor and with Ala154 and/or Ala155 of the mGlu8 receptor, resulting also in a nonoptimal closed conformation of the two subtype binding domains. This effect being analogous with mGlu4 and mGlu8 receptors, mutagenesis experiments may not help to investigate the cause of partial agonism.

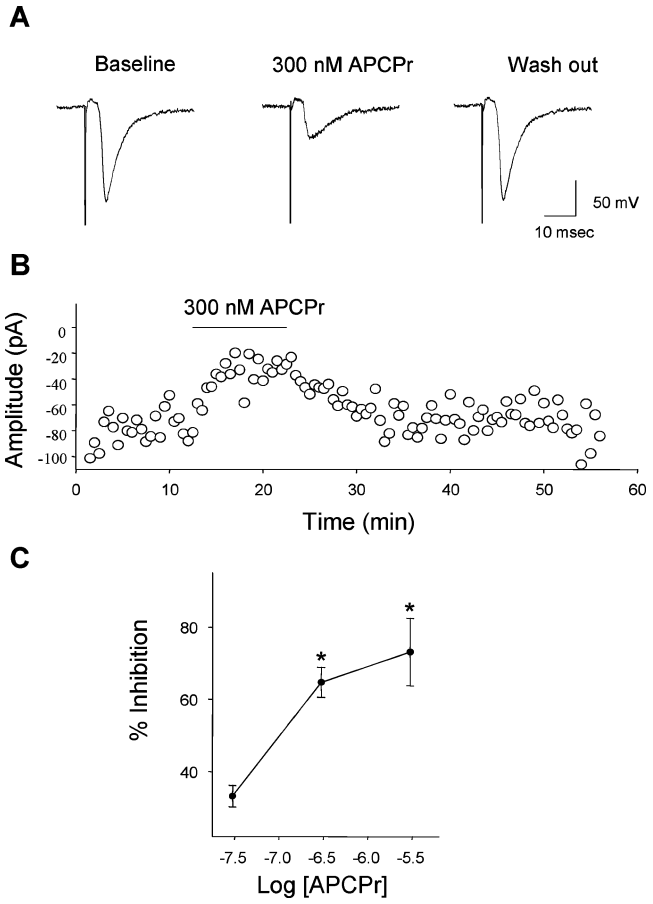


Figure 6. Effects of (1*S*,2*R*)-APCPr (−)-**1** on glutamatergic transmission at the STN-SNc synapse. (A) Representative traces of excitatory postsynaptic currents recorded in whole cell mode. Application of 300 nM (1*S*,2*R*)-APCPr **(−)-1** produced a marked inhibition of transmission which reversed when the compound was washed from the bath. (B−C) Concentration−response relationship of the (1*S*,2*R*)-APCPr **(−)-1**-induced inhibition of excitatory transmission at this synapse. Data represent mean ± SEM from 5 cells/point. $*p < 0.05$, paired *t*-test.

Electrophysiology. As (1*S*,2*R*)-APCPr **(−)-1** was shown to strongly activate recombinant group III mGlu receptors, we next chose to investigate the effect of this compound on native receptors. Previous studies have shown that group III mGlu receptors are presynaptically localized at glutamatergic and GABAergic nerve terminals and that activation of these receptors produces a marked reduction of Glu or GABA transmission. Therefore, (1*S*,2*R*)-APCPr **(−)-1** was tested *in vitro* for its ability to inhibit the excitatory transmission in the basal ganglia (BG) at the synapse between the subthalamic nucleus and the substantia nigra pars compacta (STN-SNc synapse) and at the hippocampal lateral perforant path-dentate gyrus synapse (LPP-DG). Group III modulation of transmission at the subthalamo-nigral synapse has been previously demonstrated to be solely

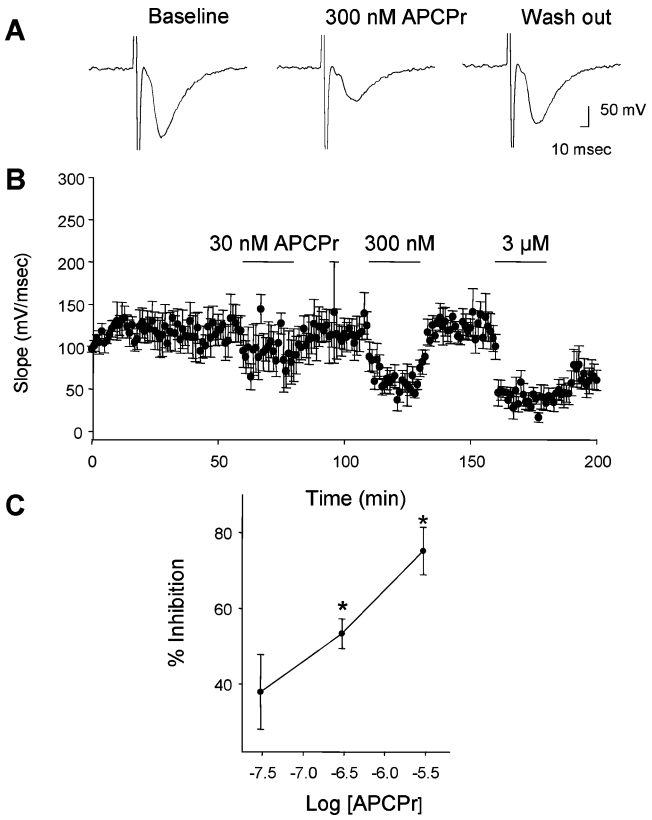


Figure 7. Effects of (1*S*,2*R*)-APCPr (−)-**1** on glutamatergic transmission at the LPP-DG synapse. (A) Representative traces of excitatory postsynaptic field potentials. Application of 300 nM (1*S*,2*R*)-APCPr **(−)-1** produced a marked inhibition of transmission which reversed when the compound was washed from the bath. (B−C) Concentration−response relationship of the (1*S*,2*R*)-APCPr **(−)-1**-induced inhibition of excitatory transmission at this synapse, Data represent mean ± SEM from eight independent experiments. *$p < 0.05$, paired *t*-test.

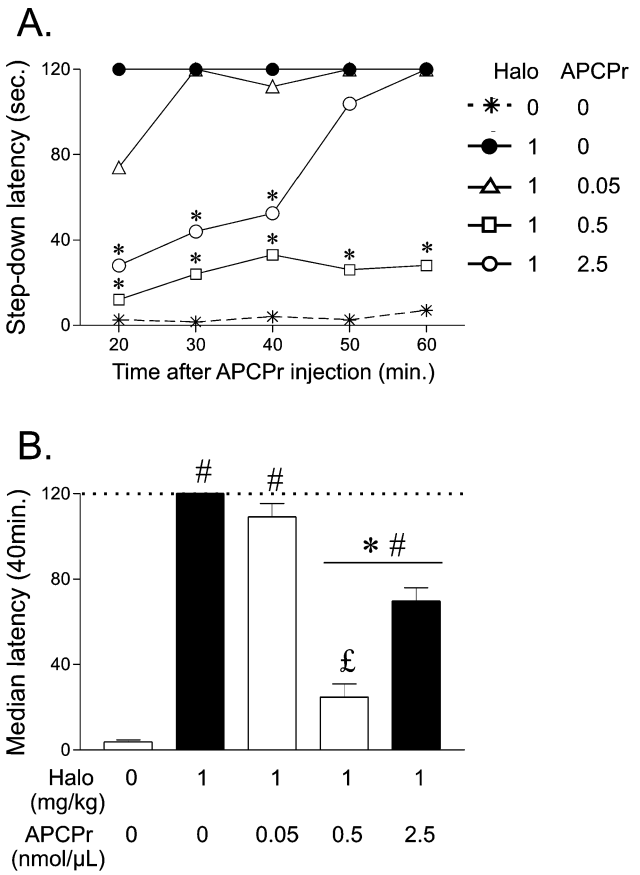


Figure 8. Effects of (1*S*,2*R*)-APCPr (−)-**1** injection into the globus pallidus on haloperidol-induced catalepsy. The animals were tested every 10 min, 20 min after bilateral APCPr **(−)-1** infusion (0, 0.05, 0.5, 2.5 nmol/μL) into the globus pallidus. (A) Time course effects of APCPr on the median latency to step off the rod after APCPr injection ($n = 7$ by dose of APCPr; $n = 11$ and $n = 7$ for haloperidol and control group, respectively). (B) Mean median latency ± SEM during the total duration of the test (40 min). #Significantly different from control ($p < 0.05$; significant Mann−Whitney U test). *Significantly different from haloperidol group ($p < 0.05$; significant Mann−Whitney U test). £Significantly different from APCPr 2.5 nmol/μL group ($p < 0.05$; significant Mann−Whitney U test).

meditated by mGlu4 receptors[43] while in the hippocampus, only the mGlu8 receptors are involved.[44]

Consistent with the observations in recombinant systems, we found that bath application of (1*S*,2*R*)-APCPr **(−)-1** induced a robust and reversible inhibition of synaptic transmission at both the STN-SNc synapse (Figure 6) and the LPP-DG synapse (Figure 7). The effects where dose dependent and at both synapses reached significance at the 300 nM dose. These data suggest that (1*S*,2*R*)-APCPr **(−)-1** activates native mGlu receptors in a manner consistent with our recombinant findings.

***In Vivo* Effect of (1*S*,2*R*)-APCPr (−)-1: Antiparkinsonian Effect When Injected into the CNS.** A growing number of animal studies suggest that the globus pallidus (GP) plays a key role in the pathophysiology of PD.[45] Increased GABA neurotransmission at the striato-pallidal synapse has been thought to underlie the alteration of GP firing activity, which is the hallmark of the pathophysiological dysfunction observed in PD. In particular, it was recently shown that activation of the mGlu4 receptor with the positive allosteric modulator PHCCC or L-AP4 decreases GABAergic transmission at the striato-pallidal synapse and reverses the akinesia produced by dopamine depletion in the reserpine model of PD.[12,15] This is consistent with a presynaptic inhibition of GABA transmission in the GP. We therefore tested the effects of (1*S*,2*R*)-APCPr **(−)-1** in haloperidol-induced catalepsy, a classical model of Parkinsonian akinesia, by directly infusing the compound into the GP. Systemic injection of the dopamine D1/D2 receptor antagonist, haloperidol (1 mg/kg i.p.), produced a profound cataleptic state measured as an increase in the median latency to step off a rod compared with controls ($p < 0.01$; Mann−Whitney U test after Kruskal−Wallis test; $H = 97.17$) (Figure 8).

While the lowest dose of (1*S*,2*R*)-APCPr **(−)-1** (0.05 nmol/μL) was ineffective, catalepsy induced by haloperidol was significantly antagonized by intrapallidal injection of (1*S*,2*R*)-APCPr **(−)-1** at the two highest doses tested ($p < 0.01$; Mann−Whitney U test for "Halo vs 0.5" and "Halo vs 2.5 nmol/μL"). (1*S*,2*R*)-APCPr **(−)-1** 2.5 nmol/μL reversed the haloperidol-induced catalepsy for 40 min, and then a progressive return to cataleptic state was observed (Figure 8A). In contrast, (1*S*,2*R*)-APCPr **(−)-1** 0.5 nmol/μL induced a marked decrease of the step-down latencies for the total duration of testing. This effect was significantly different from that produced by (1*S*,2*R*)-APCPr **(−)-1** 2.5 nmol/μL ($p < 0.01$; Mann−Whitney U test for "0.5 vs 2.5") (Figure 8B).

Discussion

Synthesis of the four isomers of APCPr was achieved following a procedure analogous to that of methanomethionine in the first steps with subsequent introduction of the phosphonate moiety.[25−28] They proved to be all group III mGlu receptor

agonists, the (1*S*) stereoisomers being more potent than the (1*R*) stereoisomers. (*Z*)-APCPr, which was previously described as an mGlu4 receptor agonist, is composed of (1*S*,2*R*)- and (1*R*,2*S*)-APCPr whereas (*E*)-APCPr from the same study is the racemic mixture of (1*R*,2*R*)- and (1*S*,2*S*)-APCPr.[21] The EC_{50} values of these racemates cannot be compared to those of the enantiomerically pure compounds because they were not tested in the same system. Nevertheless, they are concordant as the EC_{50}s of the most potent isomers of each racemate (1*S*,2*R*)-APCPr for (*Z*)-APCPr and (1*S*,2*S*)-APCPr for (*E*)-APCPr, and the EC_{50}s of racemates are found in the same range, respectively.

The present syntheses were performed in order to characterize the most potent enantiomer of the APCPr series and to improve the reported activity and selectivity. Unfortunately, selectivity was not found among the four isomers, although the best stereoisomer (1*S*,2*R*)-APCPr **(−)-1** is as potent as L-AP4. So, the additional cyclopropyl ring of conformationally restrained APCPr does not improve affinity, as the entropic gain is likely compensated by a loss of enthalpy; neither induces a better selectivity between group III mGlu receptors. However, (1*S*,2*R*)-APCPr **(−)-1** may be more selective with respect to other systems where L-AP4 plays a role[46] such as the cystine-glutamate exchanger previously identified as the quisqualate effect.[19,47,48] The large range of EC_{50}s covered by the four APCPr stereoisomers may be interpreted on the basis of homology models of the binding site in its active conformation (Figures 4 and 5). As we observed previously, contacts between agonists and lobe I are tight, particularly with the S157-G158 residues of the mGlu4 receptor (and homologous residues of other subtypes); consequently, steric hindrance in that region causes a large affinity decrease as determined for (1*R*,2*S*)-**(+)-1** and (1*R*,2*R*)-APCPr **(−)-2** or partial agonism as with (1*S*,2*S*)-APCPr **(+)-2**. A similar interpretation may explain the inactivity of APCPe and APCBu[22] (Chart 1).

As expected, (1*S*,2*R*)-APCPr **(−)-1** is inactive at group I and group II mGlu receptors and is only a weak inhibitor of NMDA receptors. It may thus be considered as inactive at these receptors at μM concentrations and may be used in further biological assays. Indeed it was shown to inhibit the excitatory transmission at the STN-SNc and the LPP-DG synapses. These results suggest that (1*S*,2*R*)-APCPr **(−)-1** activates native mGlu4 and mGlu8 receptors in a manner consistent with the recombinant data. Finally the effect of direct infusion of (1*S*,2*R*)-APCPr **(−)-1** into the GP was tested in an animal model of PD. The GP is known to be critically involved in motor control, and excessive striatal inhibition of the GP is believed to play a key role in the motor deficits observed in Parkinson's disease. Activation of the mGlu4 receptor decreases striatal inhibition of the GP and has previously been shown to exhibit antiparkinsonian effects.[12,15,49−51] Consistent with its potency at mGlu4 receptor, (1*S*,2*R*)-APCPr **(−)-1** was able to reverse haloperidol-induced akinesia. The finding of a somewhat biphasic effect in these studies may be explained by the activation of the mGlu7 receptor expected at higher doses. In fact, the inhibition of striato-pallidal transmission by L-AP4 exhibits an unusual biphasic concentration−response relationship that was interpreted to suggest an activation of mGlu7 receptor at concentrations above 30 μm.[15] The fact that the mGlu7 receptor is abundantly expressed in the GP[52] and does not yet play a significant role in the modulation of striato-pallidal transmission suggests that this receptor may be involved in modulating collateral inhibition at pallido-pallidal synapses. Therefore, activation of mGlu7 receptor in the GP may increase coherent activity across the nucleus and produce a proparkinsonian effect.[45]

Conclusion

The four stereoisomers of APCPrs have been synthesized and evaluated on cloned mGlu receptors. They all activate group III receptors over a large range of potency. The (1*S*,2*R*) isomer is the most active and shows similar potency as L-AP4. It was tested on brain slices and in an animal model of akinesia, a disabling symptom in Parkinson's disease. (1*S*,2*R*)-APCPr **(−)-1** was able to reverse the cataleptic effect. This result confirms that group III mGlu receptors may be considered as a therapeutic target for symptomatic treatment in the Parkinson's disease.

Experimental Section

Chemistry. General Procedures. All chemicals and solvents were of the best quality available from commercial suppliers and used without further purification. ^{1}H (250.13 MHz), ^{13}C (62.9 MHz), and ^{31}P (101.25 MHz) NMR spectra were recorded on an ARX 250 Bruker spectrometer. Chemical shifts (δ, ppm) are given with reference to residual ^{1}H or ^{13}C of deuterated solvents ($CDCl_3$ 7.24, 77.00; CD_3OD 3.30, 49.0; D_2O 4.80) or external reference (H_3PO_4 95%). TLC was performed on Merck $60F_{254}$ precoated silica gel plates. Products were visualized by 2% (w/v) ninhydrin in ethanol and TDM reagent.[53] Column chromatography was performed with a Biotage FLASH40i chromatography module (prepacked cartridge system). Optical rotations were measured at the sodium D line (589 nm) or at 546 nm at 20 °C, with a Perkin-Elmer 241 polarimeter using a 1 dm path length cell. High-pressure liquid chromatography (HPLC) was performed at 0 °C with an Altex Chromatem 380 pump, a Rheodyne 7125 valve (100 μL loop), a Pye-Unicam LC-UV detector set at 210 nm, and a Shimadzu CR-3A integrator, using a Crownpak CR(+) (150 mm * 4 mm i.d.) column equilibrated with pH 1.0 $HClO_4$ at a 0.1 mL/min flow rate.

(+)-*tert*-Butyl (1*S*,2*R*)-1-(*tert*-Butoxycarbonylamino)-2-(bromomethyl)cyclopropane-1-carboxylate 5. To a solution of **3** (2.2 g, 7.6 mmol, 1 equiv) in CH_2Cl_2 (180 mL) under argon at 0 °C were added CBr_4 (5.3 g, 15.9 mmol, 2 equiv) and NEt_3 (1.1 mL, 7.9 mmol, 1 equiv) followed by polymer-bound PPh_3 (5.0 g, 15.0 mmol, 2 equiv) in small portions. After being stirred at 25 °C for 5 h, the reaction mixture was filtered through a short plug of celite. After evaporation of the filtrate, the crude product was purified by flash chromatography. Elution with CH_2Cl_2 afforded **5** (1.5 g, 4.3 mmol, 56.4%) as a white solid: TLC (silica gel, CH_2Cl_2/EtOAc, 8:2, ninhydrin visualization) R_f 0.78; ^{1}H NMR ($CDCl_3$) δ 5.17 (br s, 1H), 3.52 (m, 1H), 3.41 (m, 1H), 2.15 (m, 1H), 1.69 (m, 1H), 1.44 (s, 9H), 1.43 (s, 9H), 1.08 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 170.8, 156.3, 81.8, 80.2, 41.2, 32.5, 29.2, 28.2, 27.9, 24.2; $[\alpha]^{20}_D$ 2.4 (*c* 1, $CHCl_3$).

(+)-Ethyl (1*R*,2*R*)-1-(*tert*-Butoxycarbonylamino)-2-(bromomethyl)cyclopropane-1-carboxylate 6. The (1*R*,2*R*) bromide **6** was synthesized from **4** in an identical manner to the (1*S*,2*R*) bromide **5** described above: TLC (silica gel, CH_2Cl_2/EtOAc, 9:1, ninhydrin visualization) R_f 0.64; ^{1}H NMR ($CDCl_3$) δ 5.20 (br s, 1H), 4.19 (m, 2H), 3.72 (dd, J = 6.8, 10.4 Hz, 1H), 3.54 (t, J = 10.0 Hz, 1H), 1.95 (m, 1H), 1.70 (dd, J = 6.0, 12.8 Hz, 1H), 1.52 (m, 1H), 1.45 (s, 9H), 1.30 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.6, 155.7, 80.2, 61.8, 41.0, 32.9, 30.6, 28.3, 25.5, 14.2; $[\alpha]^{20}_D$ 53.1 (*c* 1, $CHCl_3$).

***tert*-Butyl 1-[*N*-(*tert*-Butoxycarbonyl)amino]-1-[(dimethoxyphosphoryl)methyl]-but-3-enecarboxylate 7.** To **5** (64 mg, 0.18 mmol, 1 equiv) under Ar was added $P(OMe)_3$ (25 μL, 0.21 mmol, 1.2 equiv). The resulting mixture was heated under reflux for 4 h. The solution was cooled and evaporated. The crude mixture was extracted with EtOAc (200 mL), and the organic phase was washed with H_2O (100 mL), saturated $NaHCO_3$ solution (2 × 100 mL), and brine (3 × 100 mL), dried over $MgSO_4$, and evaporated. The residue was purified by flash chromatography. Elution with increasing amounts of EtOAc (10−100%) in CH_2Cl_2 afforded **7** (21 mg, 0.06 mmol, 30.8%) as a colorless oil: TLC (silica gel, EtOAc, TDM visualization) R_f 0.31; ^{1}H NMR ($CDCl_3$) δ 5.61 (m, 2H), 5.12 (m, 2H), 3.79 (t, J = 10.4 Hz, 6H), 3.37 (m, 1H), 2.92

(m, 1H), 1.47 (s, 9H), 1.41 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 167.4, 153.5, 131.6 (d, J = 11.3 Hz), 119.6, 83.6, 79.6, 63.9 (d, J = 147.7 Hz), 54.3 (d, J = 7.0 Hz), 53.8 (d, J = 7.0 Hz), 35.2, 28.2, 27.7; ^{31}P NMR ($CDCl_3$) δ 22.2.

***tert*-Butyl (1*S*,2*R*)-1-[*N*-(*tert*-Butoxycarbonyl)amino]-2-[(dimethoxyphosphoryl)methyl]cyclopropanecarboxylate 8. 8** (4 mg, 0.01 mmol, 5.8%) was obtained as a colorless oil from the previous reaction: TLC (silica gel, EtOAc, TDM visualization) R_f 0.28; ^{1}H NMR ($CDCl_3$) δ 5.86 (br s, 1H), 3.78 (d, J = 6.4 Hz, 3H), 3.74 (d, J = 6.4 Hz, 3H), 2.24 (m, 1H), 1.85 (m, 1H), 1.73 (m, 1H), 1.43 (s, 9H), 1.41 (s, 9H), 1.29 (m, 1H), 0.88 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 171.9, 156.6, 81.2, 79.4, 51.9 (d, J = 6.5 Hz), 51.5 (d, J = 6.5 Hz), 38.2, 29.7, 28.3, 27.9, 24.4 (d, J = 151.2 Hz), 21.1; ^{31}P NMR ($CDCl_3$) δ 28.4.

***tert*-Butyl (1*S*,6*R*)-3-Oxo-4-oxa-2-azabicyclo[4.1.0]heptane-1-carboxylate 9. 9** (9 mg, 0.04 mmol, 23.5%) was obtained from the previous reaction as colorless crystals: TLC (silica gel, CH_2Cl_2/EtOAc 80:20, TDM visualization) R_f 0.32; ^{1}H NMR ($CDCl_3$) δ 5.87 (s, 1H), 4.53 (m, 1H), 4.14 (m, 1H), 2.11 (m, 1H), 1.59 (m, 1H), 1.46 (s, 9H), 1.34 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 168.6, 152.9, 83.3, 66.5, 38.7, 28.0, 21.7, 18.9.

(+)-*tert*-Butyl (1*S*,2*R*)-1-[*N*-(Trifluoroacetyl)amino]-2-(bromomethyl)cyclopropane-1-carboxylate 10. To **5** (1.5 g, 4.3 mmol, 1 equiv) was added a solution of 2 M HCl/AcOEt (130 mL). After the mixture was stirred at 25 °C for 2h, the HCl/AcOEt was evaporated and the residue was dried under vacuum. The crude amine hydrochloride was used without further purification: TLC (silica gel, CH_2Cl_2/CH_3OH/NH_4OH, 85:17:2.5, ninhydrin visualization) R_f 0.65; ^{1}H NMR (CD_3OD) δ 4.87 (br s, 1H), 3.78 (dd, J = 7.8, 11.1 Hz, 1H), 3.61 (m, 1H), 2.32 (m, 1H), 1.81 (dd, J = 6.5, 9.6 Hz, 1H), 1.52 (s, 9H), 1.39 (dd, J = 6.9, 14.1 Hz, 1H); ^{13}C NMR (CD_3OD) δ 170.3, 87.5, 43.8, 31.0, 30.9, 29.8, 22.8.

The amine hydrochloride was suspended in CH_2Cl_2 (130 mL) under argon. Trifluoroacetic anhydride TFAA (2.3 mL, 16.3 mmol, 4 equiv) was added dropwise. The mixture was stirred for 1 h. After evaporation, **10** was obtained as a pale yellow oil (1.41 g, 4.1 mmol, 95.3%): TLC (silica gel, CH_2Cl_2/EtOAc, 8:2, ninhydrin visualization) R_f 0.75; ^{1}H NMR ($CDCl_3$) δ 6.98 (br s, 1H), 3.58 (dd, J = 6.8, 10.8 Hz, 1H), 3.58 (t, J = 9.6 Hz, 1H), 2.29 (m, 1H), 1.93 (dd, J = 5.9, 9.2 Hz, 1H), 1.42 (s, 9H), 1.23 (t, J = 6.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 168.3, 158.8 (q, J = 37.4 Hz), 115.5 (q, J = 288.5 Hz), 83.3, 39.9, 31.3, 28.7, 27.8, 23.9.

(+)-Ethyl (1*R*,2*R*)-1-[*N*-(Trifluoroacetyl)amino]-2-(bromomethyl)cyclopropane-1-carboxylate 11. The (1*R*,2*R*) bromide **11** was synthesized from **6** in an identical manner to the (1*S*,2*R*) bromide **10** described above.

Amine hydrochloride: TLC (silica gel, CH_2Cl_2/CH_3OH/NH_4OH, 85:17:2.5, ninhydrin visualization) R_f 0.75; ^{1}H NMR (CD_3OD) δ 4.36 (q, J = 7.2 Hz, 2H), 3.88 (dd, J = 6.0, 10.8 Hz, 1H), 3.54 (t, J = 10.4 Hz, 1H), 2.31 (m, 1H), 1.80 (m, 2H), 1.37 (t, J = 7.2 Hz, 3H); ^{13}C NMR (CD_3OD) δ 170.0, 66.1, 42.8, 32.6, 31.5, 24.2, 16.4.

11: TLC (silica gel, CH_2Cl_2/EtOAc, 8:2, ninhydrin visualization) R_f 0.73; ^{1}H NMR ($CDCl_3$) δ 8.01 (br s, 1H), 4.24 (q, J = 7.2 Hz, 2H), 3.75 (dd, J = 6.8, 10.4 Hz, 1H), 3.54 (t, J = 10.0 Hz, 1H), 2.08 (m, 1H), 1.89 (dd, J = 6.4, 7.6 Hz, 1H), 1.67 (dd, J = 6.4, 9.2 Hz, 1H), 1.27 (t, J = 7.2 Hz, 3H); ^{13}C NMR δ 173.9, 158.6 (q, J = 37.9 Hz), 115.2 (q, J = 287.3 Hz), 62.8, 39.8, 32.4, 29.3, 24.8, 13.7.

(−)-*tert*-Butyl (1*S*,2*R*)-1-[*N*-(Trifluoroacetyl)amino]-2-[(dimethoxyphosphoryl))methyl]cyclopropane-1-carboxylate 12. To **10** (1.41 g, 4.1 mmol, 1 equiv) under argon was added $P(OMe)_3$ (2.0 mL, 16.9 mmol, 4 equiv). The resulting mixture was heated under reflux for 8 h. The solution was cooled and evaporated. The crude mixture was extracted with EtOAc (200 mL), and the organic phase was washed with H_2O (100 mL), saturated $NaHCO_3$ solution (2 × 100 mL), and brine (3 × 100 mL), dried over $MgSO_4$, and evaporated. The residue was purified by flash-chromatography. Elution with increasing amounts of EtOAc (10−100%) in CH_2Cl_2 afforded **12** (782 mg, 2.1 mmol, 50.9%): TLC (silica gel, EtOAc, TDM visualization) R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 8.83 (br s, 1H), 3.76 (d, J = 6.5 Hz, 3H), 3.72 (d, J = 6.5 Hz, 3H), 2.30 (ddd, J = 4.4, 15.6, 19.2 Hz, 1H), 1.88 (m, 1H), 1.77 (m, 1H), 1.51 (m, 1H), 1.31 (s, 9H), 1.01 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 168.9, 158.6 (q, J = 36.3 Hz), 115.6 (q, J = 288.3 Hz), 81.9, 52.7 (d, J = 6.5 Hz), 52.4 (d, J = 6.5 Hz), 37.0 (d, J = 8.9 Hz), 27.3, 24.2 (d, J = 142.0 Hz), 20.0 (d, J = 12.1 Hz), 19.3 (d, J = 4.2 Hz); ^{31}P NMR ($CDCl_3$) δ 33.0; $[\alpha]^{20}_D$ −51.6 (*c* 1, CH_3OH).

***tert*-Butyl 1-[*N*-(Trifluoroacetyl)amino]-1-(dimethoxyphosphoryl)-3-butenecarboxylate 13.** This compound was isolated as the major secondary product of the previous Arbuzov reaction (13.1% yield). TLC (silica gel, EtOAc, TDM visualization) R_f 0.39; ^{1}H NMR ($CDCl_3$) δ 7.31 (br s, 1H), 5.44 (m, 1H), 5.15 (d, J = 5.6 Hz, 1H), 5.09 (s, 1H), 3.81 (d, J = 11.0 Hz, 3H), 3.74 (d, J = 11.0 Hz, 3H), 3.49 (m, 1H), 2.97 (m, 1H), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 166.3, 155.4 (q, J = 37.0 Hz), 129.7 (d, J = 13.1 Hz), 121.9, 115.4 (q, J = 288.7 Hz), 85.3, 64.6 (d, J = 147.8 Hz), 54.8 (d, J = 7.0 Hz), 53.8 (d, J = 7.0 Hz), 34.0, 27.6; ^{31}P NMR ($CDCl_3$) δ 19.4.

(+)-Ethyl (1*R*,2*R*)-1-[*N*-(Trifluoroacetyl)amino]-2-[(dimethoxyphosphoryl))methyl]cyclopropane-1-carboxylate 14. The (1*R*,2*R*) phosphonate **14** was synthesized from **11** in an identical manner to the (1*S*,2*R*) phosphonate **12** described above. The recovery was lower with a 17.5% yield: TLC (silica gel, EtOAc, TDM visualization) R_f 0.16; ^{1}H NMR ($CDCl_3$) δ 8.19 (br s, 1H), 4.17 (m, 2H), 3.72 (d, J = 4.8 Hz, 3H), 3.68 (d, J = 4.8 Hz, 3H), 2.05 (ddd, J = 0.8, 7.2, 18.4 Hz, 2H), 1.82 (m, 1H), 1.66 (dd, J = 5.8, 8.0 Hz, 1H), 1.47 (dd, J = 5.8, 9.2 Hz, 1H), 1.22 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.2, 158.1 (q, J = 37.4 Hz), 115.7 (q, J = 287.8 Hz), 61.8, 52.4 (d, J = 6.3 Hz), 52.3 (d, J = 6.3 Hz), 37.2 (d, J = 10.5 Hz), 23.2 (d, J = 3.2 Hz), 22.5, 21.8 (d, J = 104.9 Hz), 13.9; ^{31}P NMR ($CDCl_3$) δ 32.6; $[\alpha]^{20}_D$ 8.9 (*c* 1, $CHCl_3$).

Ethyl 1-[*N*-(Trifluoroacetyl)amino]-1-(dimethoxyphosphoryl)-3-butenecarboxylate 15. This compound is the major secondary product of the previous Arbuzov reaction (27.1% yield). TLC (silica gel, EtOAc, TDM visualization) R_f 0.17; ^{1}H NMR ($CDCl_3$) δ 7.30 (br s, 1H), 5.49 (m, 1H), 5.19 (d, J = 4.4 Hz, 1H), 5.13 (s, 1H), 4.35 (q, J = 7.2 Hz, 2H), 3.86 (d, J = 10.8 Hz, 3H), 3.79 (d, J = 11.2 Hz, 3H), 3.55 (c, J = 7.2 Hz, 1H), 3.04 (c, J = 7.2 Hz, 1H), 1.33 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 167.5, 155.5 (q, J = 35.8 Hz), 129.7 (d, J = 12.6 Hz), 121.0, 115.3 (q, J = 288.3 Hz), 64.3 (d, J = 14.7 Hz), 63.2, 55.0 (d, J = 6.8 Hz), 54.0 (d, J = 7.4 Hz), 34.1, 13.9; ^{31}P NMR ($CDCl_3$) δ 19.0.

(−)-(1*S*,2*R*)-1-Amino-2-phosphonomethylcyclopropanecarboxylic Acid 1. Compound **12** (440 mg, 1.17 mmol) was refluxed in 6 N HCl (10 mL) for 22 h. The solvent was evaporated, and the remaining yellow oil was diluted in 700 mL H_2O (pH 3), applied to an AG50-X4 cation-exchange column. The column was eluted with H_2O. Evaporation of the combined ninhydrin positive fractions containing the desired material gave a white solid (219 mg, 1.12 mmol, 96.0%): HPLC t_R 15.9 min; ^{1}H NMR (D_2O) δ 1.82−2.10 (br m, 3H), 1.55−1.69 (m, 2H); ^{13}C NMR (D_2O) δ 174.7, 41.3 (d, J = 11.0 Hz), 28.0 (d, J = 133.6 Hz), 24.7 (d, J = 3.7 Hz), 21.3 (d, J = 8.4 Hz); ^{31}P NMR (D_2O) δ 22.0; $[\alpha]^{20}_D$ −51.5 (*c* 1, H_2O); Anal. ($C_5H_{10}NO_5P$•2/3H_2O) C, N. H: calcd; 5.52; found, 5.25.

(−)-(1*R*,2*R*)-1-Amino-2-phosphonomethylcyclopropanecarboxylic Acid 2. The (1*R*,2*R*) phosphonate **2** was synthesized from **14** in an identical manner to the (1*S*,2*R*) phosphonic acid **1** described above. HPLC t_R 18.1 min; ^{1}H NMR (D_2O) δ 1.72−2.09 (br m, 4H), 1.34 (t, J = 7.0 Hz, 1H); ^{13}C NMR (D_2O) δ 175.9, 40.6, 28.5 (d, J = 133.1 Hz), 23.3 (d, J = 3.6 Hz), 22.3 (d, J = 7.0 Hz); ^{31}P NMR (D_2O) δ 23.5; $[\alpha]^{20}_{546}$ −9.8 (*c* 1, H_2O); Anal. ($C_5H_{10}NO_5P$•2/3H_2O) C, N. H: calcd, 5.52; found, 5.11.

The enantiomers were prepared in an identical fashion.

(−)-*tert*-Butyl (1*R*,2*S*)-1-(*tert*-butoxycarbonylamino)-2-(bromomethyl)cyclopropane-1-carboxylate ((−)-5): $[\alpha]^{20}_D$ −2.8 (*c* 1, $CHCl_3$). **(−)-Ethyl (1*S*,2*S*)-1-(*tert*-butoxycarbonylamino)-2-(bromomethyl)cyclopropane-1-carboxylate ((−)-6):** $[\alpha]^{20}_D$ −46.5 (*c* 1, $CHCl_3$). **(+)-*tert*-Butyl (1*R*,2*S*)-1-[*N*-(trifluoroacetyl)amino]-2-[(dimethoxyphosphoryl))methyl]cyclopropane-1-carboxylate ((+)-12):** $[\alpha]^{20}_D$ 54.0° (*c* 1, CH_3OH). **(−)-Ethyl (1*S*,2*S*)-1-[*N*-**

(trifluoroacetyl)amino]-2-[(dimethoxyphosphoryl))methyl]cyclopropane-1-carboxylate ((−)-14): $[\alpha]^{20}_D$ −7.8 (*c* 1, $CHCl_3$). **(+)-(1*R*,2*S*)-1-Amino-2-phosphonomethylcyclopropanecarboxylic acid ((+)-1):** $[\alpha]^{20}_D$ 52.3 (*c* 1, H_2O). **(+)-(1*S*,2*S*)-1-amino-2-phosphonomethylcyclopropanecarboxylic acid ((+)-2):** HPLC t_R 19.9 min; $[\alpha]^{20}_{546}$ 8.2 (*c* 1, H_2O).

Molecular Modeling. Conformational Analyses. All molecules were protonated on the α-amino group and deprotonated on the α-carboxylic and distal phosphonic groups. They were subjected to a conformational search using Discover 3.00 (Insight II Accelrys, San Diego, CA). The flexibility of each molecule was investigated through a simulated annealing protocol using the CVFF forcefield (Accelrys) and setting the dielectric constant to 80, as already described.[37,40] After initial minimization, the temperature of the system was raised up to 900 K for 1 ps and cooled down to 300 K during 5 ps. The resulting conformation was minimized again, using a combination of steepest-descent (until derivative less than 5 kcal/mol) and conjugated-gradient (derivative less than 0.05 kcal/mol) methods. This procedure was repeated 100 times for each molecule, and each final minimized conformation was archived. In order to classify them into families, the 100 conformations were compared through a clustering analysis based on two distances, *d1* the α-amine/phosphonate distance and *d2* the α-carboxylate/phosphonate distance.

Docking. Each stereoisomer was docked into the active site of mGlu4 or mGlu8 receptor ATD (homology modeling model[36,54]). The ligands were first manually positioned by superimposing the α-carboxylate, the amine, and the γ-acidic group on those of glutamate. The obtained protein−ligand complex was therefore subjected to energy minimization (steepest-descent convergence, 1 kcal. mol^{-1}. $Å^{-1}$; conjugate-gradient convergence, 0.1 kcal. mol^{-1}. $Å^{-1}$). This was performed using the Discover 3.00 calculation engine with the CFF force field (Insight II, Accelrys). Nonbond cutoff method and dielectric constant were set to cell-multipode and distance dependent ($\epsilon = 1R$), respectively. In order to obtain optimal proximal bindings between ligand and protein,[36] three distances between the α-carboxylic and amino groups of the ligand and three residues of the proximal pocket (S159, A180, T182) were constrained using a quadratic potential. During the minimization procedure, the force constant was gradually decreased from 1000 to 100 kcal mol^{-1}.$Å^{-2}$. Additionally, in order to compensate for some forcefield discrepancies and to maintain the correct geometry of the cyclopropyl ring bearing charged substituents on the α-carbon, distances and dihedral angles of this rigid part of the ligand were tethered using a decreasing force contant from 1000 to 500 kcal mol^{-1}.$Å^{-2}$.

Pharmacology. Culture and Transfection of HEK 293 Cells. HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal calf serum and transfected by electroporation as previously described.[30] Electroporation was carried out in a total volume of 300 μL with 10 μg of carrier DNA, plasmid DNA containing mGlu4a, mGlu6, mGlu7, or mGlu8a receptor (5 μg), and 10 million cells. To allow those receptors to activate PLC, an effect easier to measure than the inhibition of cAMP production, these receptors were coexpressed with the chimeric G-protein Gqi9 as previously described.[30] We previously reported that the pharmacological profiles of these receptors were identical to that characterized by measuring the inhibition of cAMP formation.

Determination of Inositol Phosphates (IP) Accumulation. Determination of IP accumulation in transfected cells was performed as previously described after labeling the cells overnight with [^{3}H]myoinositol (23.4 Ci/mmol, NEN, France).[30] The stimulation was conducted for 30 min in a medium containing 10 mM LiCl and the agonist at the indicated concentration. The basal IP formation was determined after 30 min incubation in the presence of 10 mM LiCl and the Glu-degrading enzyme glutamate pyruvate transaminase (1 unit/mL) and 2 mM pyruvate to avoid the possible action of Glu released from the cells. Results are expressed as the amount of IP produced over the radioactivity present in the membranes. The dose−response curves were fitted using the equation $y = (y_{max} - y_{min})/[1 + (x/EC_{50})^n] + y_{min}$ and the kaleidagraph program.

Pharmacological Assay on Recombinant NMDA Receptors. Recombinant NR1/NR2A NMDA receptors were expressed in *Xenopus laevis* oocytes after nuclear coinjection of the corresponding pcDNA3-based expression plasmids containing either the rat NR1-1a or the rat NR2A insert. Oocytes were prepared, injected, voltage-clamped, and superfused as described previously.[55] The external solution contained (in mM) 100 NaCl, 2.5 KCl, 0.3 $BaCl_2$, 5 HEPES, and 10 Tricine. The pH was adjusted to 7.3 with NaOH. Tricine was used to remove traces of contaminant Zn, which acts as a very potent allosteric inhibitor of NR1/NR2A NMDA receptors (see ref 55). NMDA currents were induced by simultaneous application of L-glutamate and glycine and recorded at −60 mV and at room temperature (20−24 °C). Their inhibition by (1*S*,2*R*)-APCPr **(−)-1** was measured by adding various amounts of the compound during a response to glutamate and glycine, both applied at their EC_{50} (5 μM for glutamate and 2 μM for glycine, respectively). Inhibition dose−response curves were fitted with the following Hill equation: $I_{APCPr}/I_{control} = 1 - 1/(1 + (IC_{50}/[APCPr])^{nH})$, where *nH* is the Hill coefficient and IC_{50} is the concentration of APCPr producing 50% current inhibition.

Electrophysiology. All patch clamp experiments were performed on slices from 15 to 20-d-old Sprague Dawley rats (Taconic, Germantown, NY). Animals were killed by decapitation, and brains were rapidly removed and submerged in an ice-cold solution containing (in mM): choline chloride 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $MgSO_4$ 8, glucose 10, and $NaHCO_3$ 26, equilibrated with 95% O_2/5% CO_2.[43] The brain was glued to the chuck of a vibrating blade microtome (Leica Microsystems, Nussloch GmbH), and parasagittal slices (250 μm thick) were obtained. Slices were immediately transferred to a 500 mL holding chamber containing artificial cerebrospinal fluid (in mM): NaCl 124, KCl 2.5, $MgSO_4$ 1.3, NaH_2PO_4 1.0, $CaCl_2$ 2, glucose 20, and $NaHCO_3$ 26, equilibrated with 95% O_2/5% CO_2. In all experiments 5 μM glutathione, 500 μM pyruvate, and 250 μM kynurenic acid were included in the choline chloride buffer and in the holding chamber ACSF. Whole-cell patch-clamp recordings were obtained as described previously.[15] During recording, slices were maintained fully submerged on the stage of a 1 mL brain slice chamber at 32 °C and perfused continuously with equilibrated ACSF (2−3 mL/min). Neurons were visualized using a differential interference contrast microscope and an infrared video system. Patch electrodes were pulled from borosilicate glass on a two-stage puller and had resistances in the range of 3−7 MΩ when filled with the following internal solution: (in mM): 140 cesium methane sulfonate, 16 HEPES, 10 NaCl, 2 EGTA, 2 MgATP, 0.2 mM Na3-GTP. Excitatory postsynaptic currents (EPSCs) were evoked by electrically stimulation, in the presence of blockers of GABAA (25 μM bicuculline methobromide) and GABAB (100 nM CPG 55845) receptors. Bipolar tungsten stimulation electrodes were placed locally in the SNc ∼100 μm rostral to the recording site. EPSCs were evoked from a holding potential of −70 mV by single pulses that ranged from 5 to 25 V, 200−400 μs, delivered once every 30 s. All field recording experiments were performed on hippocampal slices from adult (100−160 g) male Sprague Dawley rats (Taconic, Germantown, NY). Slices were obtained using previously described methods.[44,56,57] For hippocampal field recordings a patch electrode filled with ACSF was placed in the dendritic region of the dentate gyrus. Field excitatory postsynaptic potentials (fEPSPs) were isolated and characterized as previously described.[56,57] Compounds were applied to the bath using a three-way stopcock and were always applied for 10−20 min in order to achieve a plateau concentration.

Behavior Assays. Animals. Male Wistar rats (Charles River, L'Arbresle, France), were housed in groups of two per cage and maintained in temperature-controlled conditions with a 12 h light/dark cycle (7.00 A.M. to 7.00 P.M., lights off). Water and food were provided *ad libitum*. All procedures were conducted in accordance with the requirements of the French "Ministère de l'Agriculture et de la Pêche" Décret no. 87−848, October 19, 1987, and to the

European Communities council directive of November 24, 1986 (86/609/EEC).

Catalepsy Measurement. Male Wistar rats ($n = 39$, 280−300 g, Charles River, France) were used for this experiment. At period of 85 min after haloperidol injection (1 mg/kg intraperitoneal injection i.p.), APCPr **(−)-1** (0, 0.05, 0.5, and 2.5 nmol/μL; $n = 11$, 7, 7, and 7, respectively) was infused bilaterally into the globus pallidus, and the animals were tested in the horizontal bar test immediately afterward. Each animal was gently placed with its forepaws on a metal rod suspended 9 cm above the floor, and the time elapsing before it climbed down from the bar was recorded in seconds (with a cutoff time of 120 s) each 10 min for the 60-min testing. In addition, a control group ($n = 7$) receiving both vehicle of haloperidol and APCPr **(−)-1** solutions was used in this experiment.

Surgery. The animals were anesthetized by systemic injection of xylazine (15 mg/kg) and ketamine (100 mg/kg) and placed in a stereotaxic instrument (David Kopf, Tujunga, CA) with the incisor bar positioned −3.0 mm under the interaural line for surgical procedures based on the stereotaxic coordinates.[58] All the animals were implanted with 10 mm bilateral stainless steel guide cannulae (23 gauge) positioned 3 mm above the injection site in the globus pallidus at the following coordinates: (AP) −0.92 mm, (L) ±3.0 mm, and (DV) −4 mm from bregma. The guide cannulae were then anchored to the skull with four stainless steel screws and dental ciment. Stainless steel wire inlet cannulae (10 mm) were placed inside to prevent occlusion.

Injection Procedure. The bilateral intrapallidal injections were performed with stainless steel injector needles (13 mm, 30 gauge) inserted inside the implanted guide cannulae and fitted so that they protruded 3 mm below, within the globus pallidus. The injectors were connected via a polyethylene catheter (Tygon, i.d. 0.25 mm) to Hamilton microsyringes (10 μL) fitted to a micropump (CMA/100, Stockholm, Sweden). The flow delivered by the pump was set at 0.166 μL/min for a volume of 0.5 μL/side. At the end of injection, injector needles were left in place for an additional 3 min to allow the diffusion of the solution. Immediately afterward, step-down latencies were recorded.

Drugs. The mixed D1/D2 dopaminergic receptor antagonist haloperidol (Haldol injectable solution 5 mg/mL; Janssen, Boulogne, France) was dissolved in physiological 0.9% saline solution and injected systemically at a dose of 1 mg/kg. The selective group III metabotropic glutamate receptors agonist APCPr **(−)-1** was freshly dissolved in distilled water. The APCPr **(−)-1** solution was adjusted to a pH of 6.5−7.5 with 0.1 N NaOH.

Data and Statistical Analysis. Catalepsy data were analyzed by using a multiple Kruskal-Wallis "H" test. The median latency was calculated for each dose and for each 10 min period. Individual comparisons were performed using the nonparametric Mann−Whitney U test.

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Supporting Information Available: Chemical schemes for the preparation of *tert*-butyl (1*S*,2*R*)-1-(*tert*-butoxycarbonylamino)-2-(hydroxymethyl)cyclopropane-1-carboxylate **3** and ethyl (1*R*,2*R*)-1-(*tert*-butoxycarbonylamino)-2-(hydroxymethyl)cyclopropane-1-carboxylate **4**. Table of ratios of **8** over **7** derivatives obtained with various leaving groups or protecting groups of **5**. Combustion analysis data of **1** and **2**. ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR spectra of **1**. Superimposition of mGlu8 receptor binding site docked with **(−)-1** and **(+)-1**, and with **(−)-1** and **(+)-2**. This material is available free of charge via the Internet at http://pubs.acs.org

References

(1) Ozawa, S.; Kamiya, H.; Tsuzuki, K. Glutamate receptors in the mammalian central nervous system. *Prog. Neurobiol.* **1998**, *54*, 581−618.

(2) Pin, J.-P.; Acher, F. The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. *Curr. Drug Targets-CNS Neur. Dis.* **2002**, *1*, 297−317.

(3) Spooren, W. P. M.; Gasparini, F.; Salt, T. E.; Kuhn, R. Novel allosteric antagonists shed light on mGlu5 receptors and CNS disorders. *Trends Pharmacol. Sci.* **2001**, *22*, 331−337.

(4) Niswender, C. M.; Jones, C. K.; Conn, P. J. New therapeutic frontiers for metabotropic glutamate receptors. *Curr. Top. Med. Chem.* **2005**, *5*, 847−857.

(5) Conn, P. J.; Battaglia, G.; Marino, M. J.; Nicoletti, F. Metabotropic glutamate receptors in the basal ganglia motor circuit. *Nature Rev. Neurosci.* **2005**, *6*, 787−798.

(6) Marino, M. J.; Hess, J. F.; Liverton, N. Targeting the metabotropic glutamate receptor mGluR4 for the treatment of diseases of the central nervous system. *Curr. Top. Med. Chem.* **2005**, *5*, 885−895.

(7) Marino, M. J.; Conn, P. J. Glutamate-based therapeutic approaches: allosteric modulators of metabotropic glutamate receptors. *Curr. Opin. Pharmacol.* **2006**, *6*, 98−102.

(8) Swanson, C. J.; Bures, M.; Johnson, M. P.; Linden, A. M.; Monn, J. A.; Schoepp, D. D. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. *Nat. Rev. Drug Discovery* **2005**, *4*, 131−144.

(9) Neugebauer, V. Metabotropic glutamate receptors−important modulators of nociception and pain behavior. *Pain* **2002**, *98*, 1−8.

(10) Li, W.; Neugebauer, V. Differential changes of group II and group III mGluR function in central amygdala neurons in a model of arthritic pain. *J. Neurophysiol.* **2006**, *96*, 1803−1815.

(11) Kearney, J. A. F.; Albin, R. L. mGluRs: a target for pharmacotherapy in Parkinson disease. *Exp. Neurol.* **2003**, *184*, S30−S36.

(12) Marino, M. J.; Williams, D. L. J.; O'Brien, J. A.; Valenti, O.; McDonald, T. P.; Clements, M. K.; Wang, R.; DiLella, A. G.; Hess, J. F.; Kinney, G. G.; Conn, P. J. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13668−13673.

(13) Marino, M. J.; Valenti, O.; Conn, P. J. Glutamate receptors and Parkinson's disease. *Drugs Aging* **2003**, *20*, 377−397.

(14) Rouse, S. T.; Marino, M. J.; Bradley, S. R.; Awad, H.; Wittmann, M.; Conn, P. J. Distribution and roles of metabotropic glutamate receptors in the basal ganglia motor circuit: implications for treatment of Parkinson's disease and related disorders. *Pharmacol. Ther.* **2000**, *88*, 427−435.

(15) Valenti, O.; Marino, M. J.; Wittmann, M.; Lis, E.; DiLella, A. G.; Kinney, G. G.; Conn, P. J. Group III Metabotropic Glutamate Receptor-Mediated Modulation of the Striatopallidal Synapse. *J. Neurosci.* **2003**, *23*, 7218−7226.

(16) Matsui, T.; Kita, H. Activation of group III metabotropic glutamate receptors presynaptically reduces both gabaergic and glutamatergic transmission in the rat globus pallidus. *Neuroscience* **2003**, *122*, 727−737.

(17) Awad-Granko, H.; Conn, P. J. Activation of groups I or III metabotropic glutamate receptors inhibits excitatory transmission in the rat subthalamic nucleus. *Neuropharmacology* **2001**, *41*, 32−41.

(18) Zhang, C.; Albin, R. L. Increased response to intrastriatal L(1)-2-amino-4-phosphonobutyrate (L-AP4) in unilateral 6-hydroxydopamine-lesioned Rats. *Exp. Neurol.* **2000**, *165*, 278−284.

(19) Chase, L. A.; Roon, R. J.; Wellman, L.; Beitz, A. J.; Koerner, J. F. L-quisqualic acid transport into hippocampal neurons by a cystine sensitive carrier is required for the induction of quisqualate sensitization. *Neuroscience* **2001**, *106*, 287−301.

(20) Vestergaard, H. T.; Vogensen, S. B.; Madsen, U.; Ebert, B. Analogues of homoibotenic acid show potent and selective activity following sensitisation by quisqualic acid. *Eur. J. Pharmacol.* **2004**, *488*, 101−109.

(21) Johansen, P. A.; Chase, L. A.; Sinor, A. D.; Koerner, J. F.; Johnson, R. L.; Robinson, M. B. Type 4a metabotropic glutamate receptor: identification of new potent agonists and differenciation from the L-(+)-2-amino-4-phosphonobutanoic acid-sensitive receptor in the lateral perforant pathway in rats. *Mol. Pharmacol.* **1995**, *48*, 140−149.

(22) Johnson, R. L.; Rao, K. S. 2,3-Ethylene- and 2,3-trimethylene-bridged analogues of the group III metabotropic glutamate receptor ligand 2-amino-4-phosphonobutanoic acid. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 57−60.

(23) Amori, L.; Serpi, M.; Marinozzi, M.; Costantino, G.; Diaz, M. G.; Hermit, M. B.; Thomsen, C.; Pellicciari, R. Synthesis and preliminary biological evaluation of (2S,1′R,2′S)- and (2S,1′S,2′R)-2-(2′-phosphonocyclopropyl)glycines, two novel conformationally constrained l-AP4 analogues. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 196−199.

(24) Kroona, H. B.; Peterson, N. L.; Koerner, J. F.; Johnson, R. L. Synthesis of the 2-amino-4-phosphonobutanoic acid analogues (*E*)- and (*Z*)-2-amino-2,3-methano-4-phosphonobutanoic acid and their evaluation as inhibitors of hippocampal excitatory neurotransmission. *J. Med. Chem.* **1991**, *34*, 1692−1699.
(25) Burgess, K.; Ho, K.-K.; Ke, C.-Y. Synthesis of a valuable cyclopropyl chiron for preparation of 2,3-methanoamino acids. *J. Org. Chem.* **1993**, *58*, 3767−3768.
(26) Burgess, K.; Ho, K.-K.; Moye-Sherman, D. Asymmetric synthesis of 2,3-methanoamino acids. *Synlett* **1994**, 575−583.
(27) Burgess, K.; Lim, D. Y. Asymmetric syntheses of the stereoisomers of protected 2,3-methanoglutamine. *Tetrahedron Lett.* **1995**, *36*, 7815−7818.
(28) Burgess, K.; Ke, C.-Y. Large scale syntheses of N-protected 2,3-methanomethionine strereoisomers. *Synthesis* **1996**, 1463−1467.
(29) Bhattacharya, A. K.; Thyagarayan, G. The Michaelis-Arbuzov rearrangement. *Chem. Rev.* **1981**, *81*, 415−430.
(30) Gomeza, J.; Mary, S.; Brabet, I.; Parmentier, M.-L.; Restituito, S.; Bockaert, J.; Pin, J.-P. Coupling of mGluR2 and mGluR4 to Gα15, Gα16 and chimeric Gαq/i proteins: characterization of new antagonists. *Mol. Pharmacol.* **1996**, *50*, 923−930.
(31) Conklin, B. R.; Farfel, Z.; Lustig, K. D.; Julius, D.; Bourne, H. R. Substitution of three amino acids switches receptor specificity of Gqα to that of Giα. *Nature* **1993**, *363*, 274−276.
(32) Ortwine, D. F.; Malone, T. C.; Bigge, C. F.; Drummond, J. T.; Humblet, C.; Johnson, G.; Pinter, G. W. Generation of N-methyl-D-aspartate agonist and competitive antagonist pharmacophore models. Design and synthesis of phosphonoalkyl-substituted tetrahydroisoquinolines as novel antagonists. *J. Med. Chem.* **1992**, *35*, 1345−1370.
(33) Whitten, J. P.; Harrison, B. L.; Weintraub, H. J.; McDonald, I. A. Modeling of competitive phosphono amino acid NMDA receptor antagonists. *J. Med. Chem.* **1992**, *35*, 1509−1514.
(34) Laube, B.; Schemm, R.; Betz, H. Molecular determinants of ligand discrimination in the glutamate-binding pocket of the NMDA receptor. *Neuropharmacology* **2004**, *47*, 994−1007.
(35) Furukawa, H.; Singh, S. K.; Mancusso, R.; Gouaux, E. Subunit arrangement and function in NMDA receptors. *Nature* **2005**, *438*, 185−192.
(36) Bertrand, H.-O.; Bessis, A.-S.; Pin, J.-P.; Acher, F. C. Common and selective molecular determinants involved in metabotropic glutamate receptor agonist activity. *J. Med. Chem.* **2002**, *45*, 3171−3183.
(37) Jullian, N.; Brabet, I.; Pin, J.-P.; Acher, F. C. Agonist selectivity of mGluR1 and mGluR2 metabotropic receptors: a different environment but similar recognition of an extended glutamate conformation. *J. Med. Chem.* **1999**, *42*, 1546−1555.
(38) Luu, P.; Acher, F.; Bertrand, H.-O.; Fan, J.; Ngai, J. Molecular determinants of ligand selectivity in a vertebrate odorant receptor. *J. Neurosci.* **2004**, *24*, 10128−10137.
(39) Hogner, A.; Kastrup, J. S.; Jin, R.; Liljefors, T.; Mayer, M. L.; Egebjerg, J.; Larsen, I. K.; Gouaux, E. Structural basis for AMPA receptor avtivation and ligand selectivity: crystal structures of five agonist complexex with the GluR2 ligand-binding core. *J. Mol. Biol.* **2002**, *322*, 93−109.
(40) Bessis, A.-S.; Jullian, N.; Coudert, E.; Pin, J.-P.; Acher, F. C. Extended glutamate activates metabotropic receptor types 1, 2 and 4: selective features at mGluR4 binding site. *Neuropharmacology* **1999**, *38*, 1543−1551.
(41) Pin, J.-P.; De Colle, C.; Bessis, A.-S.; Acher, F. New perspective in the development of selective metabotropic glutamate receptor ligands. *Eur. J. Pharmacol.* **1999**, *375*, 277−294.
(42) Frauli, M.; Hubert, N.; Schann, S.; Triballeau, N.; Bertrand, H.-O.; Acher, F. C.; Neuville, P.; Pin, J.-P.; Prézeau, L. Amino-pyrrolidine tricarboxylic acids (APTCs) give new insight into group III metabotropic glutamate receptor activation mechanism. *Mol. Pharmacol.* **2007**, *71*, 704−712.
(43) Valenti, O.; Mannaioni, G.; Seabrook, G. R.; Conn, P. J.; Marino, M. J. Group III metabotropic glutamate-receptor-mediated modulation of excitatory transmission in rodent substantia nigra pars compacta dopamine neurons. *J. Pharmacol. Exp. Ther.* **2005**, *313*, 1296−1304.
(44) Cai, Z.; Saugstad, J. A.; Sorensen, S. D.; Ciombor, K. J.; Zhang, C.; Schaffhauser, H.; Hubalek, F.; Pohl, J.; Duvoisin, R. M.; Conn, P. J. Cyclic AMP-dependent protein kinase phosphorylates group III metabotropic glutamate receptors and inhibits their function as presynaptic receptors. *J. Neurochem.* **2001**, *78*, 756−766.
(45) Bergman, H.; Feingold, A.; Nini, A.; Raz, A.; Slovin, H.; Abeles, M.; Vaadia, E. Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. *Trends Neurosci.* **1998**, *21*, 32−38.
(46) Natale, N. R.; Magnusson, K. R.; Nelson, J. K. Can selective ligands for glutamate binding proteins be rationally designed? *Curr. Top. Med. Chem.* **2006**, *6*, 823−847.
(47) Robinson, M. B.; Whittemore, E. R.; Marks, R. L.; Koerner, J. F. Exposure of hippocampal slices to quisqualate sensitizes synaptic responses to phosphonate-containing analogues of glutamate. *Brain Res.* **1986**, *381*, 187−190.
(48) Harris, E. W.; Stevens, D. R.; Cotman, C. W. Hippocampal cells primed with quisqualate are depolarized by AP4 and AP6, ligands for a putative glutamate uptake site. *Brain Res.* **1987**, *418*, 361−365.
(49) MacInnes, N.; Messenger, M. J.; Duty, S. Activation of group III metabotropic glutamate receptors in selected regions of the basal ganglia alleviates akinesia in the reserpine-treated rat. *Br. J. Pharmacol.* **2004**, *141*, 15−22.
(50) Lopez, S.; Turle-Lorenzo, N.; Amalric, M. Intrapallidal injections of group III metabotropic glutamate receptors agonist reduce akinetic deficits in parkinsonian rats. *Behav. Pharmacol.* **2005**, *16*, B13.
(51) Ossowska, K.; Konieczny, J.; Wardas, J.; Pietraszek, M.; Kuter, K;, Wolfarth, S.; Pilc, A. An influence of ligands of metabotropic glutamate receptor subtypes on parkinsonian-like symptoms and the striatopallidal pathway in rats. *Amino Acids* **2007**, *32*, 179−188.
(52) Kosinski, C. M. S. R. B.; Conn, P. J.; Levey, A. I.; Landwehrmeyer, G. B.; Penney, J. B. J.; Young, A. B.; Standaert, D. G. Localization of metabotropic glutamate receptor 7 mRNA and mGluR7a protein in the rat basal ganglia. *J. Comp. Neurol.* **1999**, *415*, 266−284.
(53) Von Arx, E.; Faupel, M.; Brugger, M. Das 4, 4′-Tetramethyldiaminodiphenylmethan Reagens (TDM). Eine Modifikation der Chlor-o-Tolidin Farbereaktion für die Dünnschicht-chromatographie. *J. Chromatogr.* **1976**, *120*, 224−228.
(54) Bessis, A.-S.; Bertrand, H.-O.; Galvez, T.; De Colle, C.; Pin, J.-P.; Acher, F. C. Three-dimensional model of the extracellular domain of the type 4a metabotropic glutamate receptor: new insights into the activation process. *Protein Sci.* **2000**, *9*, 2200−2209.
(55) Paoletti, P.; Ascher, P.; Neyton, J. High-affinity zinc inhibition of NMDA NR1-NR2A receptors. *J. Neurosci.* **1997**, *17*, 5711−5725.
(56) Gereau, R. W.; Conn, P. J. Multiple presynaptic metabotropic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area CA1. *J. Neurosci.* **1995**, *15*, 6879−6889.
(57) Macek, T. A.; Winder, D. G.; Gereau, R. W.; Ladd, C. O.; Conn, P. J. Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. *J. Neurophysiol.* **1996**, *76*, 3798−3806.
(58) Paxinos, G. C. W. *The Rat Brain in Stereotaxic Coordinates*; Academic Press: Sydney, 2005.
(59) Corti, C.; Restituito, S.; Rimland, J. M.; Brabet, I.; Corso, M.; Pin, J.-P.; Ferraguti, F. Cloning and characterisation of alternative mRNA forms for the rat metabotropic glutamate receptors mGluR7 and mGluR8. *Eur. J. Neurosci.* **1998**, *10*, 3629−3641.

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