Synthesis and Pharmacological Characterization of Aminocyclopentanetricarboxylic Acids: New Tools to Discriminate between Metabotropic Glutamate Receptor Subtypes

Francine C. Acher,[†] Frédérique J. Tellier,[†,‡] Robert Azerad,*[,†] Isabelle N. Brabet,[§] Laurent Fagni,[§] and Jean-Philippe R. Pin[§]

Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400 CNRS, Université René Descartes-Paris V, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France, and Centre INSERM-CNRS de Pharmacologie-Endocrinologie, UPR 9023, rue de la Cardonille, 34094 Montpellier Cedex 5, France

Received March 27, 1997[⊗]

The four stereoisomers of 1-aminocyclopentane-1,3,4-tricarboxylic acid {ACPT-I (**18**) and -II (**19**), (3*R*,4*R*)-III [(−)-**20**], and (3*S*,4*S*)-III [(+)-**20**]} have been synthesized and evaluated for their effects at glutamate receptors subtypes. ACPTs are ACPD analogues in which a third carboxylic group has been added at position 4 in the cyclopentane ring. None of the ACPT isomers showed a significant effect on ionotropic NMDA, KA, and AMPA receptors. On the other hand, ACPT-II (**19**) was found to be a general competitive antagonist for metabotropic receptors (mGluRs) and exhibited a similar affinity for mGluR1a ($K_B = 115 \pm 2$ μM), mGluR2 ($K_B = 88 \pm 21$ μM), and mGluR4a ($K_B = 77 \pm 9$ μM), the representative members of group I, II and III mGluRs, respectively. Two other isomers, ACPT-I (**18**) and (+)-(3*S*,4*S*)-ACPT-III [(+)-**20**], were potent agonists at the group III receptor mGluR4a ($EC_{50} = 7.2 \pm 2.3$ and 8.8 ± 3.2 μM) and competitive antagonists with low affinity for mGluR1a and mGluR2 ($K_B > 300$ μM). Finally, (−)-(3*R*,4*R*)-ACPT-III [(−)-**20**] was a competitive antagonist with poor but significant affinity for mGluR4a ($K_B = 220$ μM). These results demonstrate that the addition of a third carboxylic group to ACPD can change its activity (from agonist to antagonist) and either increase or decrease its selectivity and/or affinity for the various mGluR subtypes.

Introduction

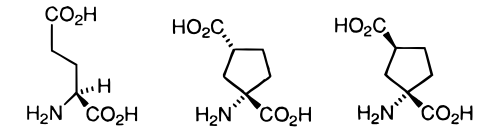
Most of the fast excitatory synapses in the brain use the common amino acid glutamate (Glu) as a transmitter. Accordingly, Glu is involved in many brain functions such as motor control, vision, or central control of heart for example. Glu synapses are also extremely plastic, *i.e.* the synaptic efficiency can be modified in a long term range, a property that is thought to be essential for brain development, learning, and memory.[1−4] Glu is also the main endogenous neurotoxin, being responsible for the neuronal death observed after ischemia, hypoxia, epileptic seizures, or brain trauma.[5,6] It is therefore not surprising that Glu is also supposed to be involved in several brain disorders such as epilepsy, Parkinson's or Alzeimer's disease, and Huntington's chorea.[6]

Numerous potential therapeutic applications are therefore expected from drugs modulating the different effects of Glu. Two main types of Glu receptors have been characterized: the ionotropic and metabotropic receptors.[7,8] The ionotropic receptors (iGluRs) are Glu-gated cationic channels directly responsible for the fast depolarization of postsynaptic cells. They are constituted of different subunits and classified into three groups based on their pharmacology and functional properties: the *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate receptors. The metabotropic Glu receptors (mGluRs) regulate the activity of ionic channels or enzymes producing second messengers via GTP-binding proteins.[9−11] Although generally not directly involved in the fast synaptic transmission, these receptors modulate the efficiency of these synapses either by modulating postsynaptic ionic channels and receptors or by regulating the release of Glu.[9−11] mGluRs constitute therefore an excellent target for drugs designed to modulate the action of Glu in the brain.[12−14]

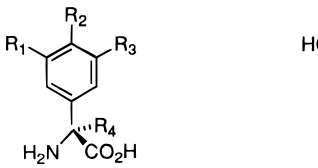
Eight cloned mGluRs have been characterized and classified into three groups based on sequence homology, pharmacology, and transduction mechanism.[9−11] Group I includes mGluR1 and mGluR5 and their splice variants (1a, 1b, 1c, 1d, 5a, and 5b) that all activate phospholipase C (PLC). Group II contains mGluR2 and mGluR3 that both inhibit adenylyl cyclase. Group III includes mGluR4, mGluR6, mGluR7, and mGluR8 and their splice variants (4a, 4b, 7a, 7b, 8a, and 8b), all being also negatively coupled to adenylyl cyclase when expressed in heterologous expression systems. Among the compounds acting specifically on mGluRs, some ACPD stereoisomers have been the first selective mGluR agonists characterized:[15,16] (1*S*,3*R*)-ACPD [(−)-**1**] behaves as an agonist at both group I and group II mGluRs, and as a low-affinity agonist at group III; (1*S*,3*S*)-ACPD [(+)-**2**] is more potent on group II mGluRs than on the members of the other groups.[10,17,18] Later several more specific ligands were described.[11,12,19] It was shown that group I mGluRs are selectively activated by (3,5-dihydroxyphenyl)glycine (3,5-DHPG, **4**), while (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (2*R*,4*R*-APDC, **7**), (2*S*,1′*S*,2′*S*)-2-(carboxycyclopropyl)-glycine (L-CCG-I, **8**), (2*S*,2′*R*,3′*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV, **9**), and 2-aminobicyclo[3.1.0]-hexane-2,6-dicarboxylic acid (LY354740, **13**)[20] are selective agonists for group II mGluRs and 2-amino-4-

[†] URA 400 CNRS, Paris.
[‡] Current address: Unité de Phytopharmacie et des Médiateurs Chimiques, INRA, route de Saint-Cyr, 78026 Versailles, France.
[§] UPR 9023 CNRS, Montpellier.
[⊗] Abstract published in *Advance ACS Abstracts,* August 15, 1997.

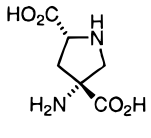
S0022-2623(97)00207-0 CCC: $14.00



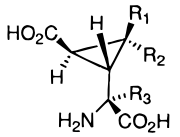
L-Glutamic acid (-)-**1** (+)-**2**



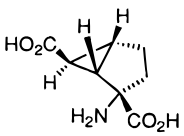
3: R_1 = OH, R_2, R_3, R_4 = H
4: R_1, R_3 = OH, R_2, R_4 = H
5: R_1 = OH, R_2 = CO_2H, R_3, R_4 = H
6: R_1, R_3 = H, R_2 = CO_2H, R_4 = Me



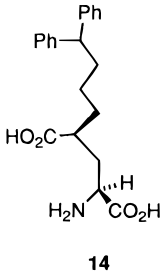
7



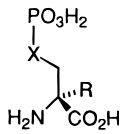
8: R_1, R_2, R_3 = H
9: R_1, R_3 = H, R_2 = CO_2H
10: R_1, R_3 = H, R_2 = Phenyl
11: R_1, R_2 = H, R_3 = Me
12: R_1, R_2 = H, R_3 = 9'-methylxanthene



13



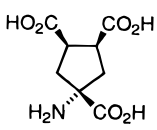
14



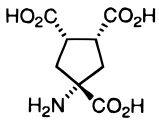
15: X = CH_2, R = H
16: X = O, R = H
17: X = CH_2, R = Me

phosphonobutyrate (L-AP4, **15**) and L-serine *O*-phosphate (L-SOP, **16**) for group III mGluRs. Antagonists[11,12,19] include (4-carboxy-3-hydroxyphenyl)glycine (4C3HPG, **5**), α-methyl(4-carboxyphenyl)glycine (MCPG, **6**) for group I mGluRs, (2*S*,1′*S*,2′*S*,3′*R*)-2-(2′-carboxy-3′-phenylcyclopropyl)glycine (PCCG4, **10**), (2*S*,1′*S*,2′*S*)-2-(9′-methylxanthene)-2-(carboxycyclopropyl)glycine (LY341495, **12**),[21] and (2*S*,4*S*)-2-amino-4-(4,4′-diphenylbut-1-yl)pentane-1,5-dioic acid (LY307452, **14**) for group II mGluRs and 2-amino-2-methyl-4-phosphonobutyrate (MAP4, **17**) for group III mGluRs. With the discovery of LY341495 (**12**) and LY354740 (**13**), a large increase in affinity was gained. However, there are still needs for more selective and potent drugs acting on mGluRs that could help define the specific physiological roles of the different mGluR subtypes. One possible approach is to introduce additional functional groups, possibly forming new ionic or hydrogen bonds with the receptor, on already characterized effectors. For example the addition of a second hydroxyl group on (3-hydroxyphenyl)glycine (3-HPG, **3**) generates 3,5-DHPG (**4**) and results in an increased affinity for group I mGluRs.[22−24] Similarly, selectivity for group II mGluRs was increased when adding a third carboxylic group on L-CCG-I (**8**), which generates DCG-IV (**9**),[25] or when replacing C-4 of (1*S*,3*R*)-ACPD [(−)-**1**] by a nitrogen atom, which generates (2*R*,4*R*)-APDC (**7**).[26] Other tricarboxylic cycloamino acids, derived from iGluR agonists, have occasionally been described.[27−30] Their affinities, compared to the corresponding dicarboxylic analogues, were generally decreased; however no activity on metabotropic receptors has been reported.

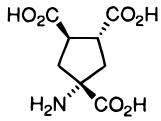
As a continuation of our efforts to obtain new conformationally constrained cyclic analogues of glutamic acid,[31] in order to provide new and useful data for the elaboration of the pharmacophores of metabotropic receptors, we have prepared the four stereoisomers of 1-aminocyclopentane-1,3,4-tricarboxylic acid (ACPT) (**18**−**20**) and determined their *in vitro* affinities for glutamate receptors (iGluR and mGluR).[32] We expected that the introduction of a third carboxylic group on ACPD may possibly increase its affinity and/or selectivity for some mGluRs, as observed for DCG-IV (**9**) compared to L-CCG-I (**8**) (see above). In addition, we were fascinated by the fact that, depending on the relative stereochemistry of the 3,4-dicarboxy groups of ACPTs, we might be dealing in each case with an ambivalent molecule, related simultaneously either to a 1:1 mixture of D and L *cis* or *trans* ACPD units, for the *meso cis* ACPT-I (**18**) or the *meso trans* ACPT-II (**19**), respectively, or to a 1:1 mixture of D or L *cis* and *trans* ACPD units, for each enantiomer of the *trans* ACPT-III (**20**). Such features made their biological study particularly exciting.



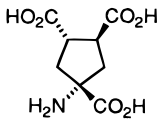
18
(1*S*,3*R*,4*S*)
ACPT-I



19
(1*R*,3*R*,4*S*)
ACPT-II



(-)-20
(3*R*,4*R*)
(-)-ACPT-III



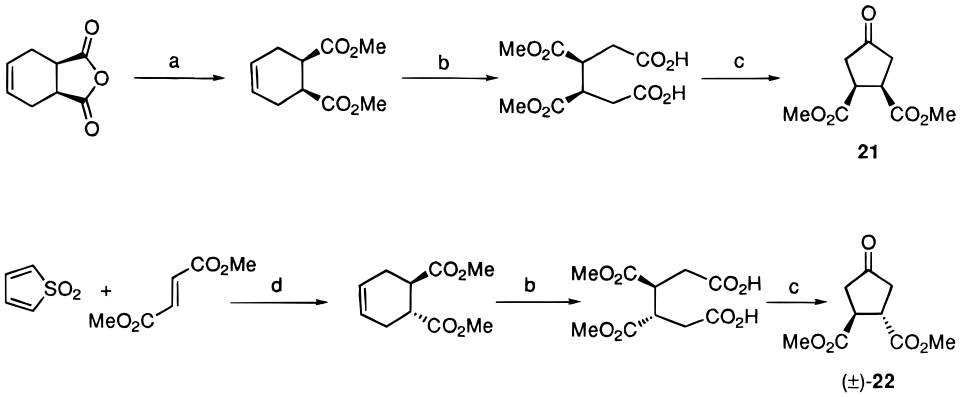
(+)-20
(3*S*,4*S*)
(+)-ACPT-III

Chemistry

ACPT-I (**18**), ACPT-II (**19**), (±)-ACPT-III (**20**), (3*R*,4*R*)-ACPT-III [(−)-**20**], and (3*S*,4*S*)-ACPT-III [(+)-**20**] have been synthesized *via* Bucherer-Bergs or Strecker reactions starting from the known keto diesters **21** or **22**, followed by hydrolysis of the resulting spirohydantoins and separation of isomeric amino acids by ion exchange chromatography.

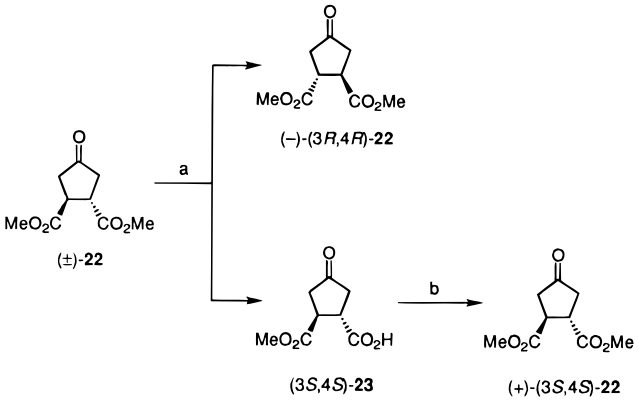
Keto diesters **21** and (±)-**22** were prepared following literature procedures[33−36] as depicted in Scheme 1 and their diastereomeric purities (dr > 99.8:0.2) checked by GC. Pig liver esterase (PLE) resolution of (±)-**22** according to Rosenquist et al.[36] afforded (−)-(3*R*,4*R*)-**22** and (+)-(3*S*,4*S*)-**22** (Scheme 2). When the reaction was stopped at 48% hydrolysis, these authors rose the ee of the enriched keto diester by crystallizing the racemate and recovering the mother liquors. We obtained variable ee's by this method, and as a moderate enantiomeric ratio (E = 40)[37] was measured, we chose to carry out hydrolysis up to 55% and then recycle the reesterified enriched monoacid (see the Experimental

Scheme 1[a]



[a] (a) H_2SO_4/MeOH; (b) $KMNO_4$; (c) NaOAc/Ac_2O, 140 °C; (d) sealed tube, 100 °C.

Scheme 2[a]

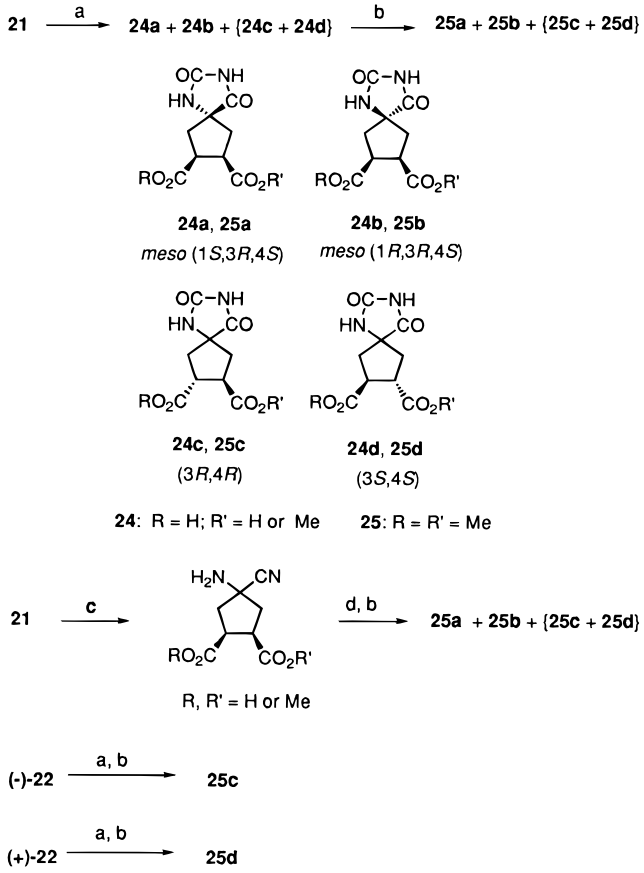


[a] (a) PLE, pH 7.0; (b) CH_2N_2/CH_2Cl_2.

Section). Thus, both enantiomers (−)-**22** and (+)-**22** had ee > 98%, as deduced from the enantiomeric excesses of the corresponding monoacids **23**, measured by GC after derivatization to their (*R*)-α-methylbenzylamides.[38] At this step, Pybrop was preferred to isobutyl chloroformate[38] as a coupling reagent to ensure a complete reaction and an adequate measurement of enantiomeric purity.

Hydantoins **24** and **25**[39] were prepared from keto diesters **21** or **22** by the Bucherer-Bergs reaction (Scheme 3, Table 1). Assuming that the first step of the mechanism was an addition of cyanide on the imine derived from **22**,[40,41] attack on either face of the ring must lead to a single isomer **25c** and/or **25d**. However, the same addition on the imine derived from **21** afforded, as expected, two *meso* isomers **25a** and **25b**. Attack on the less hindered face was favored and gave a major spirohydantoin **25b** with a carbonyl group at C-1 in a *trans* position to the preexisting carboxylates, as previously described for *cis*-3,4-dimethylcyclopentanone.[41] Conversely, in the Strecker reaction, the formation of the opposite *meso* isomer **25a** was favored,[41] when avoiding an alkaline equilibration.[42] In addition we observed that compounds exhibiting a *cis* relation between C-3 and C-4 substituents (**21**, **24a**, **24b**, **18**, and **19**) were easily epimerized to the corresponding isomers with a *trans* relation (**22**, **24c**, **24d**, and **20**). Thus besides hydantoins **24a** and **24b**, racemate (**24c** + **24d**) was also formed starting from **21** (Table 1). Purification was achieved at the amino acid stage, so hydantoin proportions were estimated from those of amino acids obtained under mild hydrolytic conditions,

Scheme 3[a]



[a] (a) $(NH_4)_2CO_3$, KCN, 55 °C; (b) HCl/MeOH 60 °C; (c) NH_4Cl, KCN, rt; (d) $(NH_4)_2CO_3$, rt.

assuming that a minimal epimerization had occured at this step (see below).

Hydantoins **24** and **25** could be hydrolyzed in strong acidic (6 N HCl, 110 °C, 5 d) or alkaline (BaO, 120 °C, 2 d) conditions (Scheme 4, Table 1). The resulting ACPTs were purified and completely separated by ion exchange chromatography to afford **18** (ACPT-I), **19** (ACPT-II), and (±)-**20** (ACPT-III) in about the same equilibrated proportions (5:15:80) in all cases (see the Experimental Section). Epimerization of **18** and/or **19** gave the most stable epimer (±)-**20**. When **18** and/or **19** were the desired compounds, a milder hydrolysis of hydantoins was required. Thus hydrolysis of intermediate diBoc-hydantoins **26** by LiOH at room temperature (Scheme 4), as previously described,[43,44] significantly

Table 1. ACPT (**18**:**19**:**20**) Ratios Obtained under Different Chemical Conditions[a]

keto diester	hydantoin **24** synthesis[b]	hydantoin **24** obtained	hydantoin **24** hydrolysis	**18**:**19**:**20** ratio (%)
21	B	**24a** + **24b** + (**24c** + **24d**)	HCl	6:19:75
	B		BaO	5:8:87
	B		mild	19:65:16
	S		mild	32:50:18
(±)-**22**	B	(**24c** + **24d**)	HCl	8:11:81
(−)-**22**	B	**24c**	mild	0.6:1.9:97.5[c,d]
(+)-**22**	B	**24d**	mild	0.3:1.8:97.9[e,d]

[a] See the Experimental Section. [b] B stands for Bucher-Bergs synthesis and S for Strecker synthesis. [c] (−)-**20** isomer. [d] Ratio measured by GC after derivatization. [e] (+)-**20** isomer.

reduced epimerization at C-3 and C-4. When **21** was treated by the Bucherer-Bergs or Strecker procedure, followed by mild hydrolysis then separation, the isomer ratios **18**:**19**:**20** were respectively 19:65:16 or 32:50:18, with 36% or 39% total overall yields (Table 1). As mentioned above, Bucherer-Bergs conditions favored the *meso* isomer **19** (65%), whereas Strecker conditions increased the *meso* isomer **18** proportion from 19 to 32%, next to some unavoidable (±)-**20** (16−18%). Although double epimerization at C-3 and C-4 was less probable, mild hydrolytic conditions were also applied to **25c** and **25d** to ensure chiral integrity (ee > 98%) and higher yields of (−)- and (+)-**20** (50% and 49% from **22**, respectively).

Absolute Configuration Assignments

The relative stereochemistry of functional groups in **18** and **19** was determined by NOE analysis on their *N*-*t*-Boc trimethyl esters derivatives **27** and **28** (Scheme 5). Significant NOEs were observed between the N-H proton and H-3 and H-4 of **27**, when no such effect was noted with **28**. Absolute configurations of (+)- and (−)-**20** were deduced from known configurations of (+)- and (−)-**22**.[34,36]

Pharmacological Results

We first examined whether the ACPTs could activate or antagonize ionotropic receptors. For that reason, the different ACPT molecules were applied alone (at 1 mM) or in combination with the ionotropic glutamate receptor agonists, NMDA or kainate (both at 100 μM), on cerebellar granule neurons maintained in culture for 7−9 days;[45] at this concentration, kainate activates both AMPA and kainate receptors. Currents evoked by these molecules were recorded using the whole cell configuration of the patch-clamp technique, at a membrane potential of −60 mV, as previously described.[15] None of the ACPT isomers induced currents (data not shown). On the same patches both NMDA and kainate induced inward currents as expected from activation of the NMDA and non-NMDA receptors. The antagonistic effects of ACPT-I (**18**), -II (**19**) and -III (**20**) were also examined on NMDA- or kainate-induced responses. No significant inhibition was observed with ACPT-II (**19**), and ACPT-III (**20**) (data not shown). A small inhibition (10%) of both NMDA and kainate responses was however observed with ACPT-I (**18**) (data not shown).

The effect of the different ACPTs was then analyzed on representative members of group I, II, and III mGluRs, mGluR1a, mGluR2, and mGluR4a, respectively, transiently expressed in HEK 293 cells.[18] The level of activation of each receptor subtype was then determined by measuring total inositol phosphate accumulation. Although mGluR2 and mGluR4a do not normally couple to this transduction cascade, this was made possible by co-expressing these receptors with the chimeric G-proteins Gqo5 and Gqi9.[18] We previously reported that the pharmacological profiles of these receptors was not altered using this assay.[18] None of the ACPT molecules activated mGluR1a or mGluR2 (Figure 1a). However, both ACPT-I (**18**) and ACPT-III (**20**) activated mGluR4a in a dose-dependent manner (Figure 1b) with EC_{50} values of 7.2 ± 2.3 and 40 ± 8 μM (n = 3), respectively. Among the enantiomers of ACPT-III (**20**), only (+)-ACPT-III [(+)-**20**] was an agonist at mGluR4a with an EC_{50} of 8.8 ± 3.2 μM (n = 2) (Figure 1c).

ACPT-I (**18**), -II (**19**), and -III (**20**) inhibited Glu-induced IP formation in cells expressing mGluR1a (Figure 2), and all induced a shift to the right of the Glu dose−response curve as expected for competitive antagonists (Figure 3 and data not shown). ACPT-II (**19**) and -III (**20**), but not -I (**18**) also inhibited Glu-induced responses in mGluR2 expressing cells, ACPT-II (**19**) being more potent than ACPT-III (**20**). ACPT-II (**19**) also antagonized the Glu effect on mGluR4a (Figure 2), and appeared therefore to be a general mGluR antagonist. A Shild plot analysis indicated that ACPT-II (**19**) has a similar potency on mGluR1a, mGluR2, and mGluR4a, and that in each case the slope is close to unity as expected for a competitive antagonist (Figure 3). The respective K_B values of ACPT-II (**19**) were 115 ± 2, 88 ± 21, 77 ± 9 μM (n = 3) for mGluR1a, mGluR2, and mGluR4a, respectively.

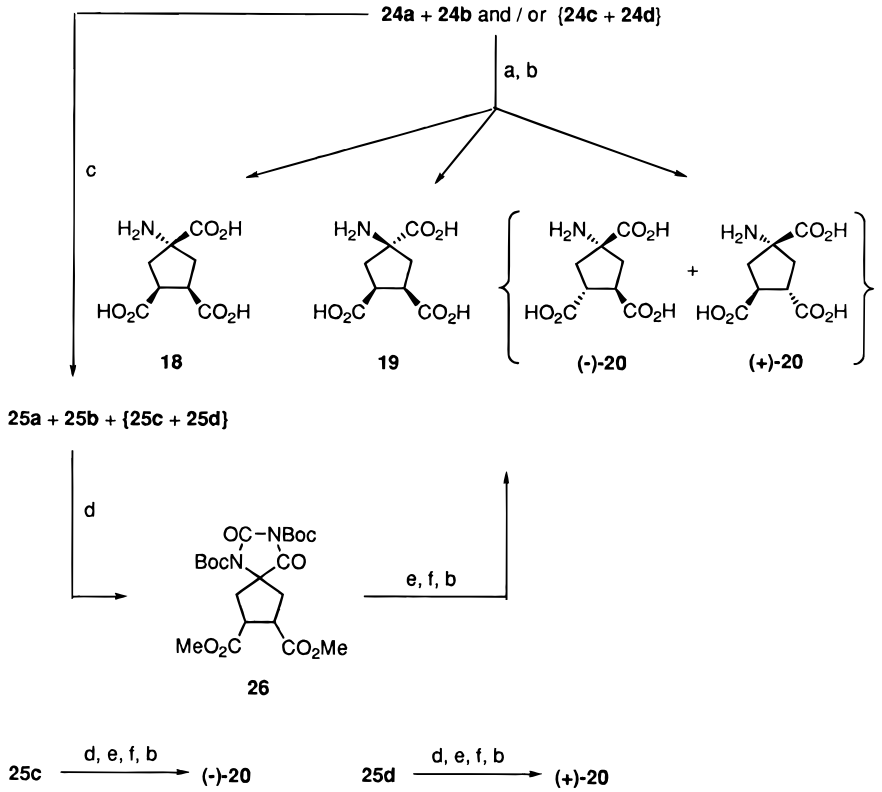
Since ACPT-III (**20**) that consists of two enantiomers antagonized the effect of Glu on mGluR1a and mGluR2, the effect of both (+)- and (−)-ACPT-III (**20**) were examined. Both (+)- and (−)-ACPT-III (**20**) inhibited with a low potency Glu responses in cells expressing mGluR1a (Figure 4a) or mGluR2 (Figure 4b). We also examined the possible antagonist effect of (−)-ACPT-III [(−)-**20**] on mGluR4a because, as described above (Figure 1c), only the (+) enantiomer was found to be an agonist on this receptor. (−)-ACPT-III [(−)-**20**] antagonized the effect of Glu on mGluR4a (Figure 4c) at high concentration, and the Shild plot analysis indicated a competitive inhibition (slope = 0.87) with a K_B value of 220 μM (data not shown).

Discussion

In the present study we described the synthesis of the four isomers of ACPT and their activity at Glu receptor subtypes, both ionotropic and metabotropic (Table 2). These compounds are analogues of ACPDs, the first described selective agonists for mGluRs, in which an additional carboxylic group has been introduced at position 4 in the cyclopentane ring.

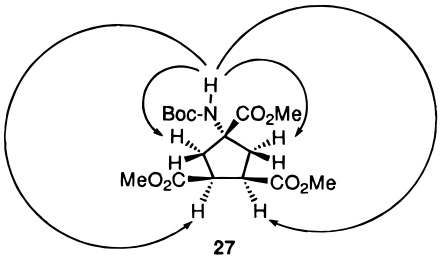
None of these compounds showed potent activity at the NMDA and non-NMDA receptors. Among these, only ACPT-I (**18**) slightly decreased kainate responses at high concentration. However, the effect was too small to be analyzed in details so that nonspecific effect cannot be excluded. Among the four isomers of ACPD, only the (1*R*,3*R*) is an agonist at the NMDA receptor subtype,[46,47] and ACPT-I (**18**) and (+)-ACPT-III [(+)-**20**] correspond to (1*R*,3*R*)-ACPD [(−)-**2**] with an additional carboxyl group at position 4 (Table 3). Therefore, this additional

Scheme 4[a]



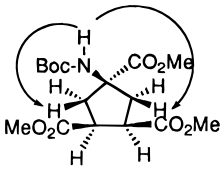
[a] (a) 6 N HCl, 110 °C or BaO, 120 °C; (b) ion-exchange chromatography; (c) HCl/MeOH 60 °C; (d) Boc_2O, DMAP, CH_3CN; (e) 1 N LiOH/CH_3CN; (f) 2 N HCl/AcOH.

Scheme 5



27

(1*S*,3*R*,4*S*)



28

(1*R*,3*R*,4*S*)

group dramatically affects the activity and affinity of this compound on ionotropic Glu receptors, as already described for a similar modification of well-characterized iGluR agonists.[27−30]

All ACPT isomers display interesting properties on mGluRs (Table 2). ACPT-II (**19**) is a nonselective mGluR antagonist, acting with a similar affinity on the representative members of each group of mGluRs. Since ACPT-II (**19**) is devoid of activity at ionotropic Glu receptors, this compound will be useful to examine the properties of the glutamatergic transmission in the total absence of mGluR activation. It will also be useful to determine whether a mGluR rather than an ionotropic glutamate receptor is involved in a specific physiological function, avoiding the use in a first set of experiments of a battery of selective antagonists.

ACPT-II (**19**) corresponds to (1*R*,3*S*)- or (1*S*,3*R*)-ACPD [(+)-**1** or (−)-**1**] with an additional carboxylic group at position 4 in a *cis* relationship with the 3-carboxylate (Table 3). Comparing the effect of ACPT-II (**19**) on mGluR1 and mGluR2 to its parent ACPD's effect, we note that agonists are turned into antagonists and affinity maintained or decreased. On mGluR4 the same comparison shows that affinity is markedly increased. We suspect that the additional group lies in a sterically unfavorable location at mGluR binding site. Accordingly, this steric hindrance would be associated with a decrease in affinity of the ligand. A steric hindrance has already been speculated for the change of agonist to antagonist properties of L-CCG-I (**8**) and L-AP4 (**15**) resulting from the addition of a methyl group on the alpha carbon [MCCG-I (**11**) and MAP4 (**17**), respectively]. This was also associated with a decrease in the apparent affinity of the compounds.[18] It can be also noticed that since ACPT-II (**19**) and (1*R*,3*S*)-ACPD [(+)-**1**] (D configuration) exhibit a similar affinity at mGluR1 and mGluR2 (K_B 77−115 μM *vs* EC_{50} 110−127 μM, Table 2) it cannot be excluded that ACPT-II (**19**) binds to the receptor as (1*R*,3*S*)-ACPD [(+)-**1**] does. In the case of mGluR4, the sterical reason for ACPT-II (**19**) to be an antagonist has to be modulated by an additional factor since the added carboxylic group increases the affinity of the ligand compared to that of its parent ACPD molecules. As discussed below, this strongly suggests that this additional group promotes additional interactions (likely hydrogen bonds) with the receptor.

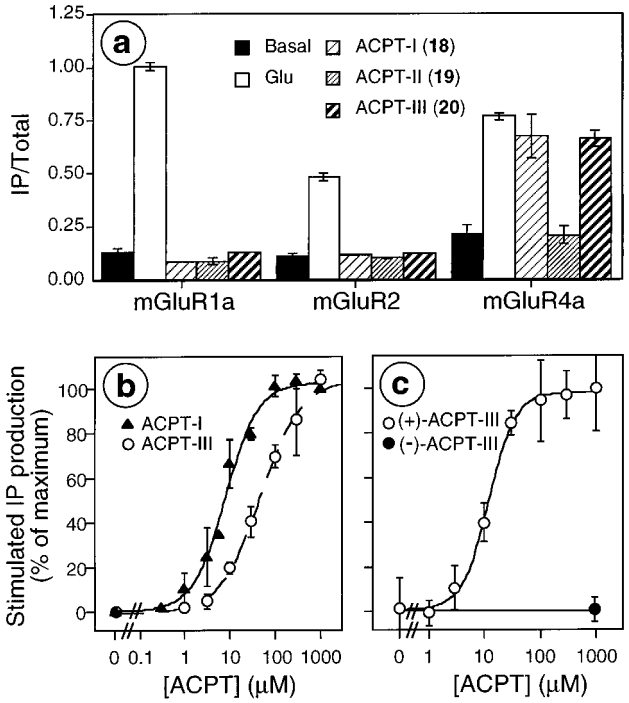


Figure 1. Agonist activity of the different ACPT isomers. (a) IP formation was determined in cells expressing mGluR1a, mGluR2 (plus Gqi9), or mGluR4a (plus Gqo5) under control condition (Basal) or after 30 min stimulation with Glu (1 mM) or ACPT (3 mM), as indicated on the figure. (b) Agonist dose−response curves of ACPT-I (**18**) (closed triangles) and ACPT-III (**20**) (open circles) on mGluR4a-expressing cells. IP formation was determined after a 30 min stimulation of cells expressing mGluR4a and Gqo5 with the indicated concentrations of ACPT-I (**18**) and -III (**20**). (c) Agonist dose−response curves of (+)-ACPT-III [(+)-(**20**)] (open circles) and (−)-ACPT-III [(−)-(**20**)] (closed circles) on mGluR4a-expressing cells. Data are expressed as the IP formation over radioactivity remaining in the membranes (a), or as the percentage of the maximal ACPT-induced IP formation (b and c). Data are means ± SEM of three or four independent experiments performed in triplicates (a and b), or means ± SEM of triplicate determination from a typical experiment (c).

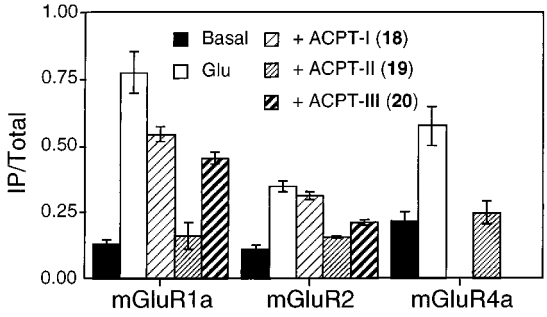


Figure 2. Antagonist activity of the different ACPT isomers. IP formation was determined in cells expressing mGluR1a, mGluR2 (plus Gqi9), or mGluR4a (plus Gqo5) under control condition (Basal) or after 30 min stimulation with Glu alone or in combination with 1 mM ACPT, as indicated on the figure. The glutamate concentration used was 3 μM for mGluR1a-expressing cells, and 30 μM for mGluR2- and mGluR4a-expressing cells. Data are means ± SEM of three or four independent experiments performed in triplicates.

Similarly, the high affinity of PCCG4 (**10**), LY341495 (**12**), and LY307452 (**14**) mGluR2 antagonists could be explained by a stabilization of the ligand−receptor complex resulting from a positive interaction between the added aromatic groups and protein residues. In all cases the steric bulk of an additional group does not prevent the ligand from being adequately positioned but prevents further events leading to the activation of the receptor.

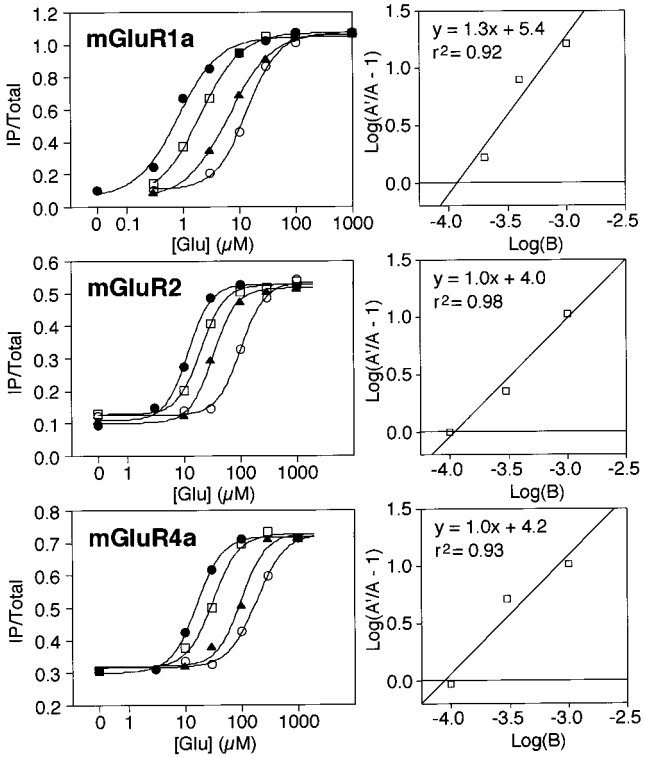


Figure 3. Antagonist activity of ACPT-II (**18**) on mGluR1a, mGluR2, and mGluR4a. Graphs on the left represent the IP formation in cells expressing mGluR1a (top), mGluR2 and Gqi9 (middle), or mGluR4a and Gqo5 (bottom) induced by different concentrations of Glu applied alone (closed circles) or in combination with ACPT-II (**18**) at a concentration of 200 (mGluR1a) or 100 (mGluR2 or mGluR4a) μM (open squares); 400 (mGluR1a) or 300 (mGluR2 or mGluR4a) μM (closed triangles); 1000 μM (open circles). The EC_{50} value for Glu applied alone (A) or in combination with a fixed concentration (B) of ACPT-II (**18**) (A′) were determined as described in the text. The Shild plots obtained from these values are presented on the right. Data are means of triplicate determinations from typical experiments.

ACPT-I (**18**) and (+)-ACPT-III [(+)-**20**] represent a new series of specific agonists for mGluR4 (and possibly other group III mGluRs), in addition to the phosphono and phosphate derivatives L-AP4 (**15**) and L-SOP (**16**) that are inactive on group I and group II mGluRs. They display weak antagonist properties (except ACPT-I (**18**) which is devoid of activity on mGluR2 in the range of concentration examined) on mGluR1a and mGluR2. Compounds with agonist properties at one mGluR subtype and with antagonist properties on the members of another group of mGluRs have already been described. One of these, (*S*)-4-carboxy-3-hydroxyphenylglycine (4C3HPG) (**5**) is a potent competitive antagonist at mGluR1 and a potent agonist at mGluR2. Such a property has been proposed to be of special interest[13,14] since group I mGluRs like mGluR1a often potentiate fast excitatory transmission, whereas group II mGluRs often inhibit glutamatergic transmission.[10] Accordingly, 4C3HPG (**5**) can decrease an excess of Glu excitation by both inhibiting mGluR1 and activating mGluR2. Such a property makes therefore 4C3HPG (**5**) a good candidate for protection against neurotoxicity and epileptic seizure, two properties that have been verified experimentally.[48,49] Since L-AP4-sensitive group III

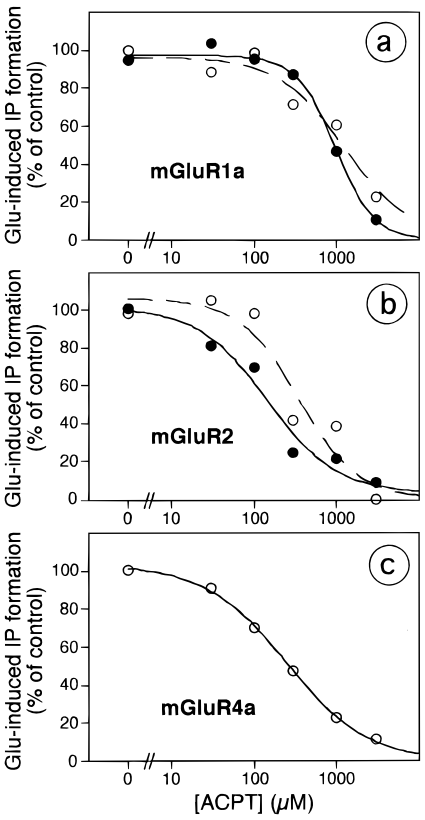


Figure 4. Antagonist activity of (+)-ACPT-III [(+)-(**20**)] and (−)-ACPT-III [(−)-(**20**)] on mGluR1a, mGluR2, and mGluR4a. Cells expressing mGluR1a (a), mGluR2 (plus Gqi9) (b), or mGluR4a (plus Gqo5) (c) were stimulated with Glu (3 μM in a and b, 20 μM in c) and various concentrations of (+)-ACPT-III [(+)-(**20**)] (closed circles) or (−)-ACPT-III [(−)-(**20**)] (open circles). Data are means of triplicate determinations from typical experiments.

mGluRs, especially mGluR4, mGluR7, and mGluR8 are also mostly presynaptic, inhibiting Glu release at many synapses,[10] the properties of ACPT-I (**18**) and (+)-ACPT-III [(+)-**20**] on mGluRs make them good candidates for neuroprotective and anticonvulsant agents.

Whereas the racemic mixture of ACPT-III (**20**) has agonist properties at mGluR4a, the (+) enantiomer is an agonist and the (−) enantiomer is a competitive antagonist. Similarly, the (*S*) enantiomer of 2-amino-3-(3-hydroxy-5-phenyl-4-isoxazolyl)propionic acid (APPA) is a full AMPA receptor agonist, whereas the (*R*) enantiomer is a competitive antagonist with a similar affinity,[50] and this explained why the racemic mixture (*RS*)-APPA behaved as a partial agonist. Since then it has been demonstrated that the mixture of a full agonist and a competitive antagonist behaves as a partial agonist, the maximal response obtained being dependent on the affinity of the two molecules and on the molar ratio used.[51] Accordingly, it is not surprising that on mGluR4a, (±)-ACPT-III (**20**) behaves as a full agonist since the agonist enantiomer is about 20 times more potent than the enantiomer with antagonist property.

It is interesting to compare the activities of the different ACPD isomers with those of ACPT, since these later correspond to the formers in which a new carboxylic group has been introduced at position 4 on the cyclopentane ring (Table 3). When a polar group has been introduced in other conformationally constrained mGluR agonists, variable effects have resulted. The addition of a carboxyl group on L-CCG-I (**8**) to obtain DCG-IV (**9**) results in an increased selectivity of the compound at group II mGluRs (but no increase in affinity), and the addition of a second hydroxyl group on 3-HPG (**3**) to obtain 3,5-DHPG (**4**) increases its affinity on group I mGluRs. However, the addition of a carboxylic group on 3-HPG (**3**) to generate 4C3HPG (**5**) does not modify the affinity of the compound on mGluR1, but changes its property from an agonist to an antagonist. On mGluR2, this additional acidic group dramatically increases the affinity of the compound since 3-HPG (**3**) has no effect on this receptor subtype, but 4C3HPG (**5**) is a potent agonist of this receptor.

On mGluR1a and mGluR2, this additional carboxylic group changes in all cases the agonist properties of ACPD into antagonist properties and results in most cases in a decrease in affinity which could be due to steric or ionic effects, as discussed above for ACPT-II (**19**). In contrast, on mGluR4a, this additional carboxylic group results in an increased affinity in most cases, with a conserved agonist property in some cases {ACPT-I (**18**) and (+)-ACPT-III [(+)-**20**]} and a switch from agonist to antagonist in others {ACPT-II (**19**) and (−)-ACPT-III [(−)-**20**]}. Moreover, it has been shown that replacement of the carboxylic group at position 3 on *trans*-ACPD by a phosphono group increases the affinity of this cyclopentane derivatives.[52] These observations suggest that stabilizing interaction (probably hydrogen bonds) between oxygen atoms of the additional carboxylate or of the phosphonate function and protein residues could be involved. This hypothesis is reinforced by the large decrease of affinity of 2-amino-4-methylphosphinobutanoic acid compared to L-AP4 (**15**).[52]

An examination of the concept that ACPTs may represent ambivalent di-ACPD molecules leads to a similar conclusion to those evoked above, considering a simple addition of a third carboxylic group on ACPD. None of the results observed with ACPTs reflects any effect originating from the superposition of the constitutive ACPD isomers. Further studies on other ACPD analogues as well as molecular modeling are under current investigations.

Summary

The four epimers of 1-aminocyclopentane-1,3,4-tricarboxylic acid have been prepared and evaluated as agonist or antagonist of glutamate receptor subtypes. We have shown that all ACPTs are inactive on ionotropic glutamate receptors, that both ACPT-II (**19**) and (3*R*,4*R*)-ACPT-III [(−)-**20**] are general antagonists of mGluR1, mGluR2, and mGluR4, the representative members of group I, group II, and group III, respectively, and that ACPT-I (**18**) and (3*S*,4*S*)-ACPT-III [(+)-**20**] are potent agonists at mGluR4 and antagonists at the representative members of group I and II mGluRs.

Experimental Section

Chemistry. General Procedures. Melting points were obtained using a Büchi capillary melting point apparatus and are uncorrected. ^{1}H (250.13 MHz) and ^{13}C (62.9 MHz) NMR spectra were recorded on an ARX 250 Bruker spectrometer. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane. NOE experiments were performed on an ARX 500 Bruker spectrometer. Optical rotations were measured at the sodium D line (589 nm), at room temperature (rt), with a Perkin-Elmer 241 polarimeter using a 1-dm pathlength cell. Gas chromatography (GC) was performed on Varian 3700 or 3400 chromatographs equipped with a flame

Table 2. Activities of the Different Isomers of ACPT at mGluR1a, mGluR2, and mGluR4a, and Comparison with ACPD Isomers[a]

	mGluR1a		mGluR2		mGluR4a	
	EC_{50}	K_B	EC_{50}	K_B	EC_{50}	K_B
ACPT-I (**18**)	ne[b]	>1000	ne[b]	ne[b]	7.2 ± 2.3	
ACPT-II (**19**)	ne[b]	115 ± 2	ne[b]	88 ± 21	ne[b]	77 ± 9
(±)-ACPT-III [(±)-**20**]	ne[b]	>300	ne[b]	>300	40 ± 8	
(+)-ACPT-III [(+)-**20**]	ne[b]	nd[c]	ne[b]	nd[c]	8.8 ± 3.2	
(−)-ACPT-III [(−)-**20**]	ne[b]	nd[c]	ne[b]	nd[c]	ne[b]	220
(1*S*,3*R*-)-ACPD [(−)-**1**]	9.3 ± 2.0[d]		18 ± 1[e]		>1000[e]	
(1*R*,3*S*)-ACPD [(+)-**1**]	127 ± 15[d]		110 ± 10[e]		>1000[e]	
(1*S*,3*S*)-ACPD [(+)-**2**]	>300[d]		13 ± 3[e]		48 ± 3[e]	
(1*R*,3*R*)-ACPD [(−)-**2**]	>1000[d]		≥1000[e]		ne[b,e]	nd[c]

[a] Means (μM) ± SEM of the EC_{50} (for the agonist activity) or K_B (for the antagonist activity) values determined as described in the text. [b] ne: no effect at 1 mM. [c] nd: not determined. [d] Values from ref 18. [e] Values from ref 22.

Table 3. Stereochemical Relationship between ACPT and ACPD Isomers from Which the Formers can be Derived by Stereospecific Addition of a Third Carboxylic Group at C-4

	ACPT			
	(1*S*,3*R*,4*S*)-I (**18**)	(1*R*,3*R*,4*S*)-II (**19**)	(3*R*,4*R*)-III [(−)-**20**]	(3*S*,4*S*)-III [(+)-**20**]
ACPD	(1*R*,3*R*) (−)-**2** (1*S*,3*S*) (+)-**2**	(1*R*,3*S*) (+)-**1** (1*S*,3*R*) (−)-**1**	(1*R*,3*S*) (+)-**1** (1*S*,3*S*) (+)-**2**	(1*R*,3*R*) (−)-**2** (1*S*,3*R*) (−)-**1**

ionisation detector, using helium (1 bar) as carrier gas and fitted with a Flexibond OV-1701 capillary column (15 m × 0.25 mm, Pierce Chemical Co.) or a Chirasil-Val capillary column (50 m × 0.32 mm, Alltech). GC of amino acids was performed after derivatization to *N*-(trifluoroacetyl)-*O*-isopropyl esters.[53] Mass spectra were recorded on a Hewlett-Packard 5890-II/5972 instrument equipped with a GC−mass coupling (30 m × 0.2 mm capillary Ultra-2 column): injection temperature 250 °C; detection temperature 280 °C. Method A: column temperature 90 °C (2 min), 90−250 °C (8 °C/min), 250 °C (5 min). Method B: column temperature 120 °C (2 min), 120−300 °C (10 °C/min), 300 °C (5 min). TLC was performed on Merck $60F_{254}$ precoated silica gel plates (0.2 mm thick, 8 cm migration) with the indicated solvent systems. Products were visualized by UV light (254 nm), alcaline potassium permanganate solution [$KMnO_4$ (1 g), K_2CO_3 (5 g), KOH (0.5 g) in 100 mL of H_2O], 2% (w/v) ninhydrin in ethanol and TDM reagent.[54] Merck 60H silica gel (230−400 mesh) was used for flash chromatography. Pig liver esterase (PLE, EC 3.1.1.1, 260 units mg^{-1}) was purchased from Sigma.

***cis*-Dimethyl Cyclopentanone-3,4-dicarboxylate (21).** Heating *cis*-1,2,3,6-tetrahydrophthalic anhydride (45.6 g, 0.3 mol) in H_2SO_4/MeOH afforded its dimethyl ester [53.4 g, 0.27 mmol, 90.0%, GC (OV-1701, 150 °C) t_R 4.51 min] which was oxidized by $KMnO_4$ (59.1 g, 0.225 mol, 83.7%, mp 141−142 °C, lit.[35] mp 147 °C), then cyclized to **21** (22.9 g, 0.114 mol, 50.7%, **21**:**22** dr > 99.8:0.2) as previously described:[35] mp 55−57 °C (lit.[35] mp 58 °C); TLC (CH_2Cl_2/EtOAc, 3:2) R_f 0.58; GC (OV-1701, 120−240 °C at 6 °C/min) t_R 9.5 min.

***trans*-Dimethyl Cyclopentanone-3,4-dicarboxylate [(±)-22].** *trans*-1,2,3,6-tetrahydrophthalic dimethyl ester [7 g, 0.035 mol, 71%, GC (OV-1701, 150 °C) t_R 4.72 min] was prepared from 3-sulfolene (6 g, 0.05 mol) and dimethyl fumarate (7.21 g, 0.05 mol) as previously described.[33] Oxidation with $KMnO_4$ [6.6 g, 0.025 mol, 72%, mp 164 °C (lit.[36] mp 168−169 °C)] and then cyclization as described[35] for **21** afforded (±)-**22** (2.6 g, 0.013 mol, 52%, **22**:**21** dr > 99.8:0.2): mp 63−64 °C (lit.[36] mp 64−65 °C); TLC (CH_2Cl_2/EtOAc, 3:2) R_f 0.66; GC (OV-1701, 120−240 °C at 6 °C/min) t_R 8.4 min.

***trans*-Dimethyl Cyclopentanone-3,4-dicarboxylate [(−)-22] and [(+)-22].** To a solution of (±)-**22** (0.4 g, 2 mmol) in a 0.01 M pH 7.0 phosphate buffer (200 mL) was added PLE (15 μL, 11 mg·mL^{-1}) at rt.[36] A solution of 0.1 N NaOH was added with a pH-stat to maintain the pH at 7.0 until 0.55 equiv of base (11 mL) had been consumed. Hydrolysis was stopped by cooling to 0 °C, acidification to pH 2 with 1 N HCl, and saturation with NaCl. The mixture was extracted with EtOAc (3 × 60 mL). The combined organic layers were dried and evaporated, and the residue was purified by flash chromatography. Elution with increasing amounts of EtOAc (0−10%) in CH_2Cl_2 afforded (3*R*,4*R*)-**22** (0.172 g, 0.859 mmol, ee > 98%), and further elution with CH_2Cl_2/EtOAc/AcOH (60:40:0.3) afforded (3*S*,4*S*)-**23** (0.195 g, 1.05 mmol, 79% ee). Monoacid (3*S*,4*S*)-**23** was esterified with CH_2N_2. Crystallization in ether/pentane of the resulting diester (3*S*,4*S*)-**22** gave variable amounts of (±)-**22** crystals, raising the ee of the mother liquor from 79% to 83−98%.[36] When (3*S*,4*S*)-**22** (0.284 g, 1.42 mmol, 88.6% ee) was reprocessed with PLE as described above and hydrolysis stopped at 65% (9.1 mL 0.1 N NaOH added), (3*R*,4*R*)-**22** (0.089 g, 0.445 mmol) and (3*S*,4*S*)-**23** (0.160 g, 0.86 mmol, ee > 99.8%) were obtained, TLC (CH_2Cl_2/EtOAc/AcOH, 70:30:1) R_f (**22**) 0.68, (**23**) 0.25. (3*R*,4*R*)-**22** (ee > 98%): $[\alpha]_D$ −135.7° (*c* 0.56, $CHCl_3$) (lit.[34] $[\alpha]_D$ −133.3°). (3*S*,4*S*)-**22** (ee > 99.8%): $[\alpha]_D$ + 130.1° (*c* 0.69, $CHCl_3$) (lit. $[\alpha]_D$ +134.4°,[34] + 136°[55]).

Determination of Optical Purity of 22 and 23. A solution of diester **22** (3 mg, 0.015 mmol) in 0.02 N NaOH (0.75 mL, 1 equiv) was stirred for 1.5 h, acidified to pH 4 with 1 N HCl (7.5 μL), evaporated, and dried under vacuum to yield **23**.

To a solution of 3 mg (0.016 mmol) of **23** in THF (0.5 mL) was added Et_3N (5 μL, 2.2 equiv), (*R*)-(+)-α-methylbenzylamine (4 μL, 2 equiv, ee > 99.6%), a few crystals of DMAP and PyBrop (9 mg, 1.2 equiv). After being stirred overnight at rt, the solvent was removed and the residue partitioned between CH_2Cl_2 (2 mL) and saturated aqueous NaCl (1.5 mL) to which were added crystals of $KHSO_4$ (pH 3). The emulsion was stirred for 15 min, and then the organic phase was separated, washed with saturated aqueous NaCl (2 × 1 mL), and dried on Na_2SO_4. GC (OV-1701, 120−240 °C at 6 °C/min) t_R (3*R*,4*R*) 26.5 min and (3*S*,4*S*) 27.5 min; (Ultra-2, method A) t_R 23.4 and 23.8 min; GC MS *m*/*z* (%) 289 $[M]^+$ (7), 274 $[M - CH_3]^+$ (3), 258 $[M - OCH_3]^+$ (0.9), 214 (7), 120 (100), 105 (86.4).

General Procedure for the Preparation of Hydantoins by the Bucherer-Bergs Synthesis. KCN (0.154 g, 1.2 equiv) was added to a solution of **21** or **22** (0.4 g, 2 mmol) and $(NH_4)_2CO_3$ (0.96 g, 5 equiv) in MeOH (4 mL) and water (3 mL). The resulting mixture was heated with a reflux condenser at 55−60 °C for 2.5 h with **21** or 5 h with **22**. The solution was cooled, acidified to pH 3−4 with dilute HCl, stirred for 15 min, and concentrated or evaporated and dried under vacuum for reesterification. When reaction lasted over 2.5 h the partially hydrolyzed mixture was reesterified with a solution of dry HCl in MeOH in a screw-cap bottle at 60 °C for 3 h and evaporated. In both cases, the crude mixture was extracted with EtOAc (100 mL), the organic phase washed with saturated NaCl solution (20 mL) to which solid $NaHCO_3$ was added, if needed, to maintain neutral pH, washed again with brine (20 mL), dried over Na_2SO_4, and evaporated to afford **25** (76−82% yield), TLC (CH_2Cl_2/EtOAc, 3:2) R_f (**25a** or **25b**) 0.20, (**25c**) 0.15.

General Procedure for the Preparation of Hydantoins by the Strecker Synthesis. NH_4Cl (1.08 g, 10 equiv) and

KCN (0.13 g, 1 equiv) were added to a solution of **21** (0.4 g, 2 mmol) dissolved in water (5 mL). After 3 days of stirring at rt, $(NH_4)_2CO_3$ (0.211 g, 1.1 equiv) was added, and the mixture was stirred for an additional 30 h and then acidified to pH 4 with 1 N HCl, evaporated, and dried under vacuum. The residue was esterified with HCl/MeOH and purified as described in the Bucherer-Bergs synthesis to afford **25** (70% yield from **21**).

General Procedure for the Hydrolysis of Hydantoins with HCl or BaO. A mixture of hydantoins **24** or **25** was obtained by reaction of **21** or **22** (0.2 g, 1 mmol) with KCN/$(NH_4)_2CO_3$ as described previously. Partial purification was achieved by further heating the mixture 1 h at 80 °C without a reflux condenser, cooling, acidifying to pH 3, evaporating to dryness, and extracting the resulting residue with MeOH. After removal of the solvent, the crude mixture of hydantoins was dissolved in 6 N HCl (20 mL) or in a suspension of BaO (1.5 g) in water (20 mL), transferred to a screw-cap bottle and heated 5 days at 110 °C or 2 days at 120 °C, respectively. HCl was removed by evaporation, and BaO by neutralization with dilute H_2SO_4 aqueous solution and filtration through a Celite pad. Purification of the ACPT mixture was carried out as described below. Using HCl hydrolysis, **18** (0.01 g), **19** (0.03 g), (±)-**20** (0.12 g) in 64.1% overall yield were obtained from **21**, and **18** (0.015 g), **19** (0.02 g), (±)-**20** (0.15 g) in 74.2% overall yield from (±)-**22**. Using BaO hydrolysis, **18** (0.009 g), **19** (0.016 g), (±)-**20** (0.162 g) in 75.1% overall yield were obtained from **21**.

General Procedure for Mild Hydrolysis of Hydantoins. To the mixture of hydantoin esters **25** prepared from **21** or **22** (2 mmol) following the Bucherer-Bergs or Strecker procedure and dissolved in CH_3CN (20 mL) were added di-*tert*-butyl dicarbonate (1.15 mL, 3 equiv) and DMAP (0.015 g, 0.08 equiv).[43,44] Solvent was removed after 1.5 h. The residue was then purified on a short silica gel column (2 × 13 cm) by rapid elution with CH_2Cl_2 (60 mL) and CH_2Cl_2/EtOAc (4:1, 150 mL). Fractions of di-Boc diester **26** were detected by TLC {-(CH_2Cl_2/EtOAc, 9:1) R_f (*meso*-**26**) 0.59 and [(*RS*,*RS*)-**26**] 0.51} with TDM reagent and evaporated to give an oily residue (76−81% yield) which was dissolved in CH_3CN (3 mL) and 1 N LiOH (12 mL). The mixture was stirred for 5 h at rt, cooled, neutralized with 1 N HCl (12 mL), evaporated, and dried under vacuum. The residue was treated with a solution of dry 2 N HCl in AcOH for 0.5 h and evaporated to dryness. Purification of the resulting ACPTs mixture was carried out as described below.

Starting from **21** and using the Bucherer-Bergs procedure, **18** (0.035 g), **19** (0.116 g), and (±)-**20** (0.029 g) in 36.1% overall yield were obtained. Starting from **21** and using the Strecker procedure, **18** (0.062 g), **19** (0.096 g), and (±)-**20** (0.035 g) in 38.8% overall yield were obtained. Starting from (−) or (+)-**22** and using the Bucherer-Bergs procedure, (−)- and (+)-**20** in 50% and 49% overall yield were respectively obtained (see below).

General Procedure for Purification of ACPT-I (18), ACPT-II (19), and ACPT-III (20). The mixture of ACPTs obtained from **21** or **22** (2 mmol) by the Bucherer-Bergs or Strecker procedure and by acidic, alkaline, or mild hydrolysis of resulting hydantoins was dissolved in water (up to 1.2 L in the case of mild hydrolysis). The solution was adjusted at pH 4 and deposited on a Dowex 50 × 4 column (H^+, 20−50 mesh, 3 × 17 cm). The resin was rinsed with water (0.3 L) and the mixture of ACPTs eluted with 0.5 M aqueous NH_4OH. Ninhydrin positive fractions were pooled and evaporated. The residue was dissolved in boiled water (0.4 L), pH adjusted at 9, and the solution deposited on a AG1×4 (AcO^-, 200−400 mesh, 3 × 19 cm). The resin was rinsed with boiled water, and ACPT-I (**18**), ACPT-II (**19**), and ACPT-III (**20**) were respectively eluted with 0.5, 0.8, and 1 M AcOH.

(1*S*,3*R*,4*S*)-1-Aminocyclopentane-1,3,4-tricarboxylic Acid (18). Compound **18** was prepared by the Bucherer-Bergs procedure followed by HCl hydrolysis (4% yield from **21** and 6% yield from **22**) or BaO hydrolysis (3.6% yield from **21**) or mild hydrolysis (7% yield from **21**). When it was prepared by the Strecker procedure followed by mild hydrolysis, a 12.4% yield was obtained from **21**. Purification was carried out as described above: TLC (*n*-BuOH/AcOH/H_2O, 4:1:1) R_f 0.15; ^{1}H NMR (D_2O, NH_4^+ salt) δ 3.20 (m, 2H, H-3, H-4), 2.63 (m, 2H, H-2, H-5), 2.24 (m, 2H, H-2, H-5); ^{13}C NMR (D_2O, NH_4^+ salt) δ 183.8 and 179.0 (CO), 70.0 (C-1), 50.8 (C-3, C-4), 42.1 (C-2, C-5); GC (OV-1701, 190 °C) t_R 19.2 min, (Ultra 2, method B) t_R 13.9 min, (Chirasil-Val, 190 °C) t_R 22.4 min; GC MS *m*/*z* 352 [M − CO_2iPr]$^+$ (4), 296 (30), 250 (26), 222 (100). Anal. ($C_8H_{11}NO_6$·1.8H_2O) C, H, N.

(1*R*,3*R*,4*S*)-1-Aminocyclopentane-1,3,4-tricarboxylic Acid (19). Compound **19** was prepared by the Bucherer-Bergs procedure followed by HCl hydrolysis (12% yield from **21** and 8% yield from **22**) or BaO hydrolysis (6.4% yield from **21**) or mild hydrolysis (23.2% yield from **21**). When it was prepared by the Strecker procedure followed by mild hydrolysis, a 19.2% yield was obtained from **21**. Purification was carried out as described above. TLC (*n*-BuOH/AcOH/H_2O, 4:1:1) R_f 0.15; ^{1}H NMR (D_2O, NH_4^+ salt) δ 3.33 (m, 2H, H-3, H-4), 2.52 (m, 2H, H-2, H-5), 2.32 (m, 2H, H-2, H-5); ^{13}C NMR (D_2O, NH_4^+ salt) δ 183.6 and 180.4 (CO), 69.2 (C-1), 53.7 (C-3, C-4), 42.3 (C-2, C-5); GC (OV-1701, 190 °C) t_R 16.4 min, (Ultra 2, method B) t_R 14.2 min, (Chirasil-Val, 190 °C) t_R 18.3 min; GC MS *m*/*z* 380 [M − OiPr]$^+$ (1), 352 [M − CO_2iPr]$^+$ (12), 296 (21), 250 (30), 222 (100). Anal. ($C_8H_{11}NO_6$·1.8H_2O) C, H, N.

(3*RS*,4*RS*)-1-Aminocyclopentane-1,3,4-tricarboxylic Acid [(±)-20]. Compound (±)-**20** was prepared by the Bucherer-Bergs procedure followed by HCl hydrolysis (48.1% yield from **21** and 60.1% yield from **22**), BaO hydrolysis (65% yield from **21**), or mild hydrolysis (5.8% yield from **21**). When it was prepared by the Strecker procedure followed by mild hydrolysis, a 7% yield was obtained from **21**. Purification was carried out as described above. TLC (*n*-BuOH/AcOH/H_2O, 4:1:1) R_f 0.15; ^{1}H NMR (D_2O, NH_4^+ salt) δ 3.19 (m, 2H, H-3, H-4), 2.59 (m, 1H), 2.42 (m, 2H), 2.10 (m, 1H); ^{13}C NMR (D_2O, NH_4^+ salt) δ 185.9, 183.9 and 179.2 (CO), 69.9 (C-1), 53.2 (C-3, C-4), 43.3 and 42.8 (C-2, C-5); GC (OV-1701, 190 °C) t_R 13.5 min, (Ultra 2, method B) t_R 13.7 min, (Chirasil-Val, 190 °C) t_R (3*S*,-4*S*) 15.0 min and (3*R*,4*R*) 15.1 min; GC MS *m*/*z* 440 [M + H]$^+$ (0.01), 380 [M − OiPr]$^+$ (2), 352 [M − CO_2iPr]$^+$ (15), 296 (12), 250 (20), 222 (100). Anal. ($C_8H_{11}NO_6$·1.8H_2O) C, H, N.

(3*R*,4*R*)-1-Aminocyclopentane-1,3,4-tricarboxylic Acid [(−)-20]. Compound (−)-**20** was prepared from (−)-**22** (0.134 g, 0.67 mmol, ee > 98%) by the Bucherer-Bergs procedure followed by mild hydrolysis (**26**, 0.209 g, 0.445 mmol) and purification as described above. After cation exchange chromatography a mixture (0.091g, 0.362 mmol) containing mostly (−)-**20** (97.5%) and small amounts of **18** (0.6%) and **19** (1.9%) as diammonium salts was recovered. Pure (−)-**20** (0.083 g, 0.333 mmol, 49.8% overall yield, ee 99.2%) was obtained after anion exchange chromatography: GC (Chirasil-Val, 150 °C) t_R 86.1 min; $[\alpha]_D$ −32.3° (*c* 0.73, H_2O).

(3*S*,4*S*)-1-Aminocyclopentane-1,3,4-tricarboxylic Acid [(+)-20]. Compound (+)-**20** was prepared from (+)-**22** (0.172 g, 0.858 mmol, ee > 99.8%) by the Bucherer-Bergs procedure followed by mild hydrolysis (**26**, 0.235 g, 0.5 mmol) and purification as described above. After cation exchange chromatography a mixture (0.109 g, 0.434 mmol) containing mostly (+)-**20** (98.2%) and small amounts of **18** (< 0.4%) and **19** (1.8%) as diammonium salts were recovered. Pure (+)-**20** (0.105 g, 0.421 mmol, 49.1% overall yield, ee 98.3%) was obtained after anion exchange chromatography: GC (Chirasil-Val, 150 °C) t_R 84.7 min; $[\alpha]_D$ +26.4° (*c* 0.64, H_2O).

(1*S*,3*R*,4*S*)-Trimethyl *N*-*t*-Boc-1-aminocyclopentane-1,3,4-tricarboxylic Ester (27). ACPT-I (**18**, 10 mg, 0.046 mmol) was suspended in dry 3 M HCl in MeOH (5 mL), heated 5 h at 70 °C in a screw-cap bottle, evaporated, and kept overnight under vacuum on KOH. To the resulting triester in CH_2Cl_2 (2 mL) was added Et_3N (10 μL, 2 equiv), DMAP (7 mg, 1.2 equiv), and Boc-on (23 mg, 2 equiv), and the solution was stirred 2 months at rt. Purification by flash chromatography (19 × 1 cm, EtOAc/cyclohexane, 3:7) afforded **27** (8.2 mg, 0.023 mmol) in 50% overall yield: TLC (CH_2Cl_2/MeOH, 95:5) R_f 0.31; ^{1}H NMR ($CDCl_3$) δ 4.90 (br s, 1H, NH), 3.71 (s, 3H, OCH_3), 3.66 (s, 6H, 2 × OCH_3), 3.36 (m, 2H, H-3, H-4), 2.67 and 2.66 (2 dd, 2H, J = 6.4, 14.0 Hz, H-2, H-5), 2.34 (br dd, 2H, J = 6.4, 14.0 Hz, H-2, H-5), 1.41 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$) δ 173.1 (CO), 155.1 (NCO), 80.5 [*C*(CH_3)$_3$], 64.9

(C-1), 52.7 and 52.0 (C-3, C-4), 45.2 (3 × OCH_3), 39.6 (C-2, C-5), 28.2 [C(CH_3)$_3$].

(1*R*,3*R*,4*S*)-Trimethyl *N*-*t*-Boc-1-aminocyclopentane-1,3,4-tricarboxylic Ester (28). ACPT-II (**19**), 12.3 mg, 0.061 mmol) was esterified, N-protected (3 weeks), and purified as described above to afford **28** (11.0 mg, 0.031 mmol) in 54% overall yield: TLC (CH_2Cl_2/MeOH, 95:5) R_f 0.27; ^{1}H NMR ($CDCl_3$) δ 5.21 (br s, 1H, NH), 3.71 (s, 3H, OCH_3), 3.66 (s, 6H, 2 × OCH_3), 3.26 (m, 2H, H-3, H-4), 2.65 and 2.64 (2 dd, 2H, J = 6.0, 14.0 Hz, H-2, H-5), 2.29 (br dd, 2H, J = 6.0, 14.0 Hz, H-2, H-5), 1.39 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR ($CDCl_3$) δ 174.3 and 173.4 (CO), 155.0 (NCO), 80.2 [*C*(CH_3)$_3$], 64.3 (C-1), 52.7 and 52.0 (C-3, C-4), 45.5 (3 × OCH_3), 39.7 (C-2, C-5), 28.2 [C(*C*H_3)$_3$].

Biological Assays. 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.[18] Electroporation was carried out in a total volume of 300 μL with 10 μg of carrier DNA, plasmid DNA containing mGluR1a (0.3 μg), mGluR2 (2 μg), or mGluR4a (5 μg), and 10 million cells. To allow mGluR2 and mGluR4a to activate PLC, an effect easier to measure than the inhibition of cAMP production, these receptors were co-expressed with the chimeric G-proteins Gqo5 and Gqi9 as previously described.[18] We previously reported that the pharmacological profiles of these two receptors was 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/mol, NEN, France).[18] 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 $y = (y_{max} - y_{min})/[1 + (x/EC50)^n] + y_{min}$ and the kaleidagraph program.

Culture and Recordings of Cerebellar Granule Neurons. Cerebellar granule cells were cultured from 7 days old mice as previously described.[45] Neurons were recorded using the patch-clamp technique in the whole cell configuration, and drugs were applied using a fast application technique as previously described.[15]

Acknowledgment. We are indebted to Odile Convert (Université Pierre et Marie Curie, Paris) for NOE experiments and their interpretation. This work was supported by grants from the French Ministry of Education, Research and Professional Insertion (ACC-SV5, no. 9505077), the European community Biomed2 (BMH4-CT96-0228) and Biotech2 (BIO4-CT96-0049) programs, and the Fondation pour la Recherche Médicale.

References

(1) Bliss, T. V. P.; Collingridge, G. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **1993**, *361*, 31−39.
(2) Linden, D. J. Long-term synaptic depression in the mammalian brain. *Neuron* **1994**, *12*, 457−472.
(3) Siegelbaum, S. A.; Kandel, E. R. Learning related synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* **1991**, *1*, 113−120.
(4) Ito, M. Long-term depression. *Annu. Rev. Neurosci.* **1989**, *12*, 85−102.
(5) Choi, D. W. Cerebral hypoxia: some new approaches and unanswered questions. *J. Neurosci.* **1990**, *10*, 2493−2501.
(6) Choi, D. W. Glutamate neurotoxicity and diseases of the nervous system. *Neuron* **1988**, *1*, 623−634.
(7) Nakanishi, S. Molecular diversity of glutamate receptors and implications for brain function. *Science* **1992**, *258*, 597−603.
(8) Hollmann, M.; Heinemann, S. Cloned glutamate receptors. *Annu. Rev. Neurosci.* **1994**, *17*, 31−108.
(9) Nakanishi, S. Metabotropic glutamate receptors: synaptic transmission, modulation, and plasticity. *Neuron* **1994**, *13*, 1031−1037.
(10) Pin, J.-P.; Duvoisin, R. The metabotropic glutamate receptors: Structure and functions. *Neuropharmacology* **1995**, *34*, 1−26.
(11) Conn, P.; Pin, J.-P. Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205−237.
(12) Knöpfel, T.; Kuhn, R.; Allgeier, H. Metabotropic glutamate receptors: novel targets for drug development. *J. Med. Chem.* **1995**, *38*, 1417−1426.
(13) Nicoletti, F.; Bruno, V.; Copani, A.; Casabona, G.; Knopfel, T. Metabotropic glutamate receptors: A new target for the therapy of neurodegenerative disorders? *Trends Neurosci.* **1996**, *19*, 267−271.
(14) Riedel, G. Function of metabotropic glutamate receptors in learning and memory. *Trends Neurosci.* **1996**, *19*, 219−224.
(15) Manzoni, O.; Fagni, L.; Pin, J. P.; Rassendren, F.; Poulat, F.; Sladeczek, F.; Bockaert, J. (Trans)-1-amino-cyclopentyl-1,3-dicarboxylate stimulates quisqualate phosphoinositide-coupled receptors but not ionotropic glutamate receptors in striatal neurons and *Xenopus* oocytes. *Mol. Pharmacol.* **1990**, *38*, 1−6.
(16) Palmer, E.; Monaghan, D. T.; Cotman, C. W. *trans*-ACPD, a selective agonist of the phosphoinositide coupled excitatory amino acid receptor. *Eur. J. Pharmacol.* **1989**, *166*, 585−587.
(17) Joly, C.; Gomeza, J.; Brabet, I.; Curry, K.; Bockaert, J.; Pin, J.-P. Molecular, functional and pharmacological characterization of the metabotropic glutamate receptor type 5 splice variants: comparison with mGluR1. *J. Neurosci.* **1995**, *15*, 3970−3981.
(18) 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.
(19) Roberts, P. J. Pharmacological tools for the investigation of metabotropic glutamate receptors (mGluRs): Phenylglycine derivatives and other selective antagonists—an update. *Neuropharmacology* **1995**, *34*, 813−819.
(20) Monn, J. A.; Valli, M. J.; Massey, S. M.; Wright, R. A.; Salhoff, C. R.; Johnson, B. G.; Howe, T.; Alt, C. A.; Rhodes, G. A.; Robey, R. L.; Griffey, K. R.; Tizzano, J. P.; Kallman, M. J.; Helton, D. R.; Schoepp, D. D. Design, synthesis, and pharmacological characterization of (+)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740): a potent, selective, and orally active group 2 metabotropic glutamate receptor agonist possessing anticonvulsant and anxiolytic properties. *J. Med. Chem.* **1997**, *40*, 528−537.
(21) Ornstein, P. L.; Bleisch, T. J.; Arnold, M. B.; Wright, R. A.; Johnson, B. G.; Tizzano, J. P.; Helton, D. R.; Kallman, M. J.; Schoepp, D. D. Novel antagonists for metabotropic glutamate receptors. *Neuropharmacology* **1996**, *35*, A22.
(22) Brabet, I.; Mary, S.; Bockaert, J.; Pin, J.-P. Phenylglycine derivatives discriminate between mGluR1 and mGluR5 mediated responses. *Neuropharmacology* **1995**, *34*, 895−903.
(23) Ito, I.; Kohda, A.; Tanabe, S.; Hirose, E.; Hayashi, M.; Mitsunaga, S.; Sugiyama, H. 3,5-Dihydroxyphenylglycine: a potent agonist of metabotropic glutamate receptors. *NeuroReport* **1992**, *3*, 1013−1016.
(24) Schoepp, D. D.; Goldsworthy, J.; Johnson, B. G.; Salhoff, C. R.; Baker, S. R. 3,5-Dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic glutamate receptors in the rat hippocampus. *J. Neurochem.* **1994**, *63*, 769−772.
(25) Hayashi, Y.; Momiyama, A.; Takahashi, T.; Ohishi, H.; Ogawa-Meguro, R.; Shigemoto, R.; Mizuno, N.; Nakanishi, S. Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. *Nature* **1993**, *366*, 687−690.
(26) Monn, J. A.; Valli, M. J.; Johnson, B. G.; Salhoff, C. R.; Wright, R. A.; Howe, T.; Bond, A.; Lodge, D.; Spangle, L. A.; Paschal, J. W.; Campbell, J. B.; Griffey, K.; Tizzano, J. P.; Schoepp, D. D. Synthesis of the four Isomers of 4-Aminopyrrolidine-2,4-dicarboxylate: Identification of a Potent, Highly Selective, and Systemically-Active Agonist for Metabotropic Glutamate Receptors Negatively Coupled to Adenylate Cyclase. *J. Med. Chem.* **1996**, *39*, 2990−3000.
(27) Anand, H.; Roberts, P. J.; Badman, G.; Dixon, A. J.; Collins, J. F. Novel kainic acid analogues: effects on cyclic GMP content of adult rat cerebellar slices. *Biochem. Pharmacol.* **1986**, *35*, 409−415.
(28) Allan, R. D.; Hanrahan, J. R.; Hambley, T. W.; Johnston, G. A. R.; Mewet, K. N.; Mitrovic, A. D. Synthesis and activity of a potent *N*-methyl-D-aspartic acid agonist, trans-1-aminocyclobutane-1,3-dicarboxylic acid, and related phosphonic and carboxylic acids. *J. Med. Chem.* **1990**, *33*, 2905−2915.
(29) Gaoni, Y.; Chapman, A. G.; Parvez, N.; Pook, P. C.-K.; Jane, D. E.; Watkins, J. C. Synthesis, NMDA receptor antagonist activity, and anticonvulsant action of 1-aminocyclobutanecarboxylic acid derivatives. *J. Med. Chem.* **1994**, *37*, 4288−4296.
(30) Allan, R. D.; Apostopoulos, C.; Hambley, T. W. The synthesis and structure of a cyclobutane analogue of glutamic acid with an acetic acid side chain. *Aust. J. Chem.* **1995**, *48*, 919−928.

(31) Tellier, F.; Acher, F.; Brabet, I.; Pin, J. P.; Bockaert, J.; Azerad, R. Synthesis of conformationally-constrained stereospecific analogs of glutamic acid as antagonists of metabotropic receptors. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2627−2632.

(32) Azerad, R.; Acher, F.; Pin, J.-P. Composés constituant notamment des effecteurs de récepteurs du système nerveux central sensibles aux amino-acides neuroexcitateurs, leur préparation et leur applications biologiques. French patent app. FR97/01573, 1997.

(33) Sample, T. E.; Hatch, L. F. Diethyl *trans*-Δ^4-tetrahydrophthalate. *Organic Synthyses*; Wiley: New York, 1988; Collect. Vol. VI, pp 454−458.

(34) Suemune, H.; Tanaka, M.; Obaishi, H.; Sakai, K. Enzymatic procedure for the synthesis of prostaglandin A2. *Chem. Pharm. Bull.* **1988**, *36*, 15−21.

(35) Gais, H.-J.; Bülow, G.; Zatorski, A.; Jentsch, M.; Maidonis, P.; Hemmerle, H. Enzyme-catalyzed asymmetric synthesis. 8. Enantioselectivity of pig liver esterase catalyzed hydrolysis of 4-substituted meso cyclopentane 1,2-diesters. *J. Org. Chem.* **1989**, *54*, 5115−5122.

(36) Rosenquist, A.; Kvarnström, I.; Svensson, S.; Classon, B.; Samuelsson, B. Synthesis of enantiomerically pure *trans*-3,4-substituted cyclopentanols by enzymatic resolution. *Acta Chem. Scand.* **1992**, *46*, 1127−1129.

(37) Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. Quantitative analyses of biochemical kinetic resolutions of enantiomers. *J. Am. Chem. Soc.* **1982**, *104*, 7294−7299.

(38) Ornstein, P. L.; Arnold, M. B.; Augenstein, N. K.; Paschal, J. W. Synthesis of 6-oxodecahydroisoquinoline-3-carboxylates. Useful intermediates for the preparation of conformationally defined excitatory amino acid antagonists. *J. Org. Chem.* **1991**, *56*, 4388−4392.

(39) As mentioned in the experimental part, hydantoin esters were generally not obtained as pure diesters, because of partial hydrolysis resulting from the use of alkaline reaction conditions.

(40) Edward, T.; Jitrangsri, C. Stereochemistry of the Bucherer-Bergs and Strecker reactions of 4-*tert*-butylcyclohexanone. *Can. J. Chem.* **1975**, *53*, 3339−3350.

(41) Trigo, G. G.; Avenadaño, C.; Santos, E. Stereochemistry of the Bucherer-Bergs and Strecker reactions of tropinone, *cis*-bicyclo-[3.3.0]octan-3-one and *cis*-3,4-dimethylcyclopentanone. *Can. J. Chem.* **1979**, *57*, 1456−1461.

(42) Sufrin, J. R.; Coulter, A. W.; Talalay, P. Structural and conformational analogues of L-Methionine as inhibitors of the enzymatic synthesis of S-adenosyl-L-methionine. IV. Further mono-, bi- and tricyclic amino acids. *Molecular Pharmacol.* **1979**, *15*, 661−677.

(43) Kübick, S.; Meissner, R. S.; Rebeck, J. J. Synthesis of α,α-dialkylated amino acids with adenine or thymine residues. A new mild and facile hydrolysis of hydantoins. *Tetrahedron Lett.* **1994**, *35*, 6635−6638.

(44) Wysong, C. L.; Yokum, T. S.; Morales, G. A.; Gundry, R. L.; Mc Laughlin, M. L.; Hammer, R. P. 4-Aminopiperidine-4-carboxylic acid: a cyclic α,α-disubstituted amino acid for preparation of water-soluble highly helical peptides. *J. Org. Chem.* **1996**, *61*, 7650−7651.

(45) Van-Vliet, B. J.; Sebben, M.; Dumuis, A.; Gabrion, J.; Bockaert, J.; Pin, J.-P. Endogenous amino acid release from cultured cerebellar neuronal cells: effect of tetanus toxin on glutamate release. *J. Neurochem.* **1989**, *52*, 1229−1239.

(46) Schoepp, D. D.; Johnson, B. G.; Salhoff, C. R.; Mcdonald, J. W.; Johnston, M. V. *In vitro* and *in vivo* pharmacology of trans and cis-(±)-1-amino-1,3-cyclopentanedicarboxylic acid - dissociation of metabotropic and ionotropic excitatory amino acid receptor effects. *J. Neurochem.* **1991**, *56*, 1789−1796.

(47) Curry, K.; Peet, M. J.; Magnuson, D. S. K.; McLennan, H. Synthesis, resolution, and absolute configuration of the isomers of the neuronal excitant 1-amino-1,3-cyclopentanedicarboxylic acid. *J. Med. Chem.* **1988**, *31*, 864−867.

(48) Buisson, A.; Choi, D. W. The inhibitory mGluR agonist, S-4-carboxy-3-hydroxy-phenylglycine selectively attenuates NMDA neurotoxicity and oxygen glucose deprivation-induced neuronal death. *Neuropharmacology* **1995**, *34*, 1081−1087.

(49) Thomsen, C.; Klitgaard, H.; Sheardown, M.; Jackson, H. C.; Eskesen, K.; Jacobsen, P.; Treppendahl, S.; Suzdak, P. D. (S)-4-carboxy-3-hydroxyphenylglycine, an antagonist of metabotropic glutamate receptor (mGluR)1a and an agonist of mGluR2, protects against audiogenic seizures in DBA/2 mice. *J. Neurochem.* **1994**, *62*, 2492−2495.

(50) Ebert, B.; Lenz, S. M.; Brehm, L.; Bregnedal, P.; Hansen, J. J.; Frederiksen, K.; Bøgesø, K. P.; Krogsgaard-Larsen, P. Resolution, absolute stereochemistry and pharmacology of the S-(+)- and R-(−)-isomers of the apparent partial AMPA receptor agonist (R,S)-2-amino-3-(3-hydroxy-5-phenylisoxazol-4-yl)propionic acid [(R,S)-APPA]. *J. Med. Chem.* **1994**, *37*, 878−884.

(51) Ebert, B.; Madsen, U.; Søby, K. K.; Krogsgaard-Larsen, P. Functional partial agonism at ionotropic excitatory amino acid receptors. *Neurochem. Int.* **1996**, *29*, 309−316.

(52) 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 differentiation from the L-(+)-2-amino-4-phosphonobutanoic acid-sensitive receptor in the lateral perforant pathway in rats. *Mol. Pharmacol.* **1995**, *48*, 140−149.

(53) Maurs, M.; Ducrocq, C.; Righini-Tapie, A.; Azerad, R. Gas chromatographic resolution of substituted glutamic acid enantiomers. *J. Chromatogr.* **1985**, *25*, 444−449.

(54) Von Arx, E.; Faupel, M.; Brugger, M. Das 4,4′-Tetramethyldiamino-diphenylmethan Reagens (TDM). Eine Modifikation der Chlor-o-Tolidin Farbereaktion für die Dünnschicht-chromatographie. (The 4,4′-Tetramethyldiaminodiphenylmethane reagent (TMD). A modification of the Chlorine-o-Tolidine color reaction for thin layer chromatography). *J. Chromatogr.* **1976**, *120*, 224−228.

(55) Solladié, G.; Lohse, O. New reagent for the optical resolution of ketones: (−) (1R,2R,5R)-5-methyl-2-(1-mercapto-1-methyl)-cyclohexanol. Application to trans dimethyl cyclopentanone-3,4-dicarboxylate. *Tetrahedron: Asymmetry* **1993**, *4*, 1547−1552.

JM970207B