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3-Carboxy-4-phosphonocyclopentane amino acids: New metabotropic glutamate receptor ligands

A.-S. Bessis[1], **G. Vadesne**[1], **E. Bourrat**[1], **G. Bertho**[1], **J.-P. Pin**[2], and **F. C. Acher**[1]

[1] Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR8601-CNRS, Université René Descartes-Paris V, Paris, France
[2] Centre INSERM-CNRS de Pharmacologie-Endocrinologie, UPR 9023-CNRS, Montpellier, France

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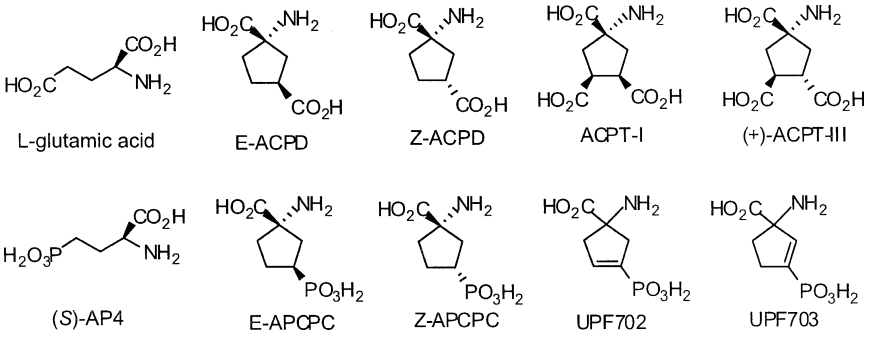
Summary. Two glutamic acid analogs (1*SR*,3*RS*,4*RS*)- and (1*SR*,3*SR*,4*SR*)-1-amino-4-phosphono cyclopentane-1,3-dicarboxylic acids (APCPD) have been synthesized. Pure E-(diethoxy-phosphoryl)-acrylic acid ethyl ester was obtained from ethyl propiolate, phenol and triethylphosphite. It was used as dienophile in a Diels-Alder reaction. Oxidation and cyclization afforded 3-(ethoxy-carbonyl)-4-(diethoxy-phosphoryl)-cyclopentanone. Bucherer-Bergs reaction and hydrolysis yielded APCPD-III and -IV which are inactive on mGlu1a receptor and antagonists on mGlu2 and mGlu8a receptors.

Keywords: Cyclopentane glutamate analog – ACPT – L-AP4 – Metabotropic glutamate receptors

Introduction

Glutamic acid (Glu, Scheme 1) is the major excitatory neurotransmitter in the brain. However, disturbance of glutamate synaptic transmission plays an important part in several neuropathologies such as epilepsy, stroke damage, Parkinson's disease, pain, anxiety, drug withdrawal symptoms . . . Thus, glutamate receptors are attractive therapeutic targets. These are of two types: the channel-gated type known as ionotropic glutamate receptors (iGluR) (Dingledine et al., 1999) and the G-protein coupled type known as metabotropic glutamate receptors (mGluR). Eight mGluR subtypes have been identified. They are classified in three groups (I–III) according to their sequence similarity, transduction mechanism and pharmacological profile (Conn and Pin, 1997; Pin et al., 1999; Schoepp et al., 1999; Bräuner-Osborne et al., 2000; Pin and Acher, 2002). Group I receptors (mGlu1,5R) activate phospholipase C while Group II (mGlu2,3R) and Group III (mGlu4,6,7,8R) inhibit adenylyl cyclase when expressed in heterologous systems (Conn and Pin, 1997). Among the known competitive ligands only a limited number display submicromolar potencies and few are subtype specific. Agonist pharmacophore models have shown that glutamate binds to the receptors in a common extended conformation, and revealed several group specific interaction regions (Bessis et al., 1999; Jullian et al., 1999; De Colle et al., 2000). These data were later confirmed and refined with tridimensional models and crystallographic structure of the glutamate binding site (Bessis et al., 2000; Kunishima et al., 2000; Malherbe et al., 2001; Rosemond et al., 2001; Bertrand et al., 2002). Our interest has focused on group III agonists among which are found (*S*)-AP4, ACPT-I, (+)-ACPT-III, E- and Z-APCPC, UPF702, UPF703 (Scheme 1) (Johansen et al., 1995; Acher et al., 1997; Schoepp et al., 1999; Amori et al., 2000; De Colle et al., 2000). Interestingly these compounds contain a glutamate motif that is an α-amino acid moiety and a distal acidic function, as well as an additional acidic function located in the vicinity of this distal function. The interactions of this third acidic function with specific residues afford a major affinity increase: while efficacy of E- or Z-ACPD (Scheme 1) lies in the mM range, those of ACPT-I, (+)-ACPT-III, E- and Z-APCPC drop to the micromolar range. In fact, the agonist pharmacophore models of mGlu4/8R revealed two specific polar subsites (Fig. 1A) which fit the highly



Scheme 1

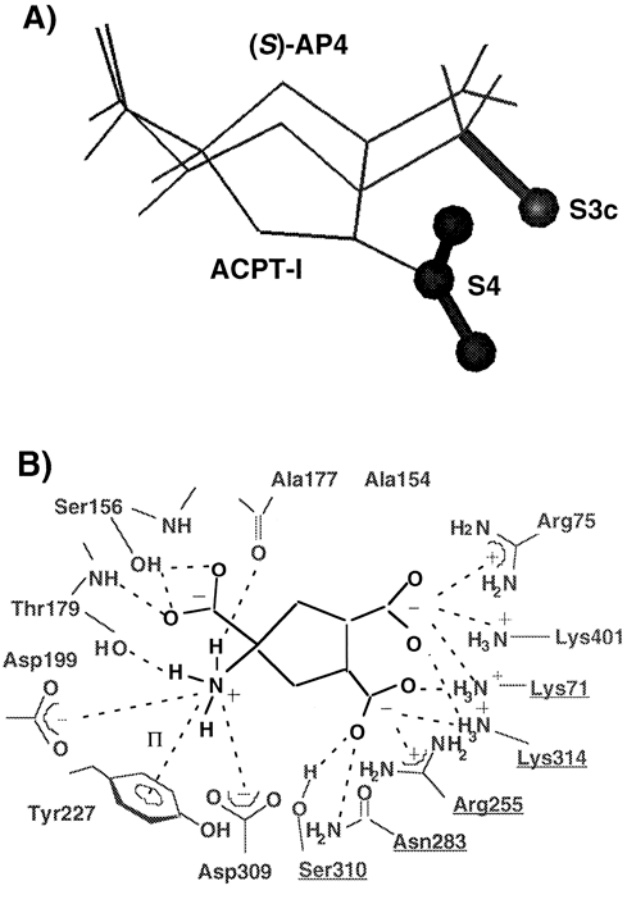
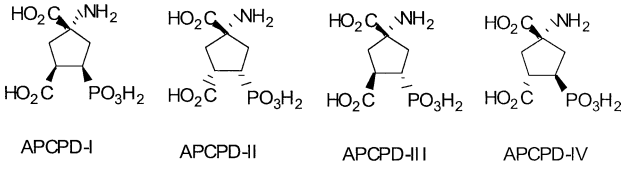


Fig. 1. **A** Superimposition of (*S*)-AP4 and ACPT-I as in the mGlu4/8R pharmacophore models. The two group III selective sites S3c and S4 described in Bessis et al. 1999 are highlighted. **B** Docking of ACPT-I at mGlu8R binding site. Hydrogen bonds and ionic interactions are displayed as dotted lines. Group III selective polar residues are underlined

basic environment disclosed in the homology model of the proteins (Fig. 1B) (Bessis et al., 1999; De Colle et al., 2000; Bessis et al., 2000; Bessis et al., 2001; Bertrand et al., 2002). In order to combine these two sites in a single molecule, we designed the APCPD



Scheme 2

structure for which four possible diastereomers are found (Scheme 2). Among the eight enantiomers of APCPD, it is difficult to predict which might be agonist or antagonist, as it was shown with ACPTs that both situations are met (Acher et al., 1997). Thus all isomers should be synthesized and tested. In the present study two diastereoisomers have been prepared and their biological activity evaluated on cloned mGlu receptors.

Material and methods

Chemistry

All chemicals and solvents were of the best quality available from commercial suppliers and used without further purification. Melting points were obtained using a Büchi capillary melting point apparatus and are uncorrected. ^{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) or external references (3-(trimethylsilyl)[2,2,3,3-$^{2}H_4$] propionic acid sodium salt in D_2O or H_3PO_4 95%). Gas chromatography (GC) was performed on a Hewlett-Packard 4890A chromatograph equipped with a flame ionization 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.). GC coupled to a mass spectrometer (GC/MS) was performed on a Hewlett-Packard 5890-II chromatograph equipped with a HP 5972 mass selective detector and a HP ultra-2 5 MS capillary column (25 m × 0.2 mm). Five column temperature programs were used: A) 80°C (2 min), 80–240°C (6°C/min), 240°C (5 min); B) 100°C–240°C (8°C/min); C) 100°C (2 min), 100–240°C (8°C/min), 240°C (5 min); D) 110°C (2 min), 110–270°C (8°C/min), 270°C (5 min); E) 140°C (2 min), 140–300°C (10°C/min), 300°C (5 min). Injector and detector temperature were set to 250°C for A) and B) and to 250°C/280°C for C) D) and E). TLC was performed

on Merck 60F_{254} precoated silica gel plates. Products were visualized by UV light (254 nm), alkaline 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 (Von Arx et al., 1976). Merck 60H silica gel (230–400 mesh) was used for flash chromatography.

E- and Z-3-(diethoxyphosphoryl)acrylic acid ethyl ester: **E-2** and **Z-2**

Procedure I: Triethylphosphite (1.4 mL, 8.1 mmol, 1 eq) was added dropwise under stirring to propiolic acid **1a** (8.1 mmol, 1 eq) in ether (18 mL). Reaction was followed by TLC and ^{31}P NMR. After 2 days, solvent was removed in vacuo and the residue purified by flash chromatography to afford **E-2** (0.35 g, 1.48 mmol, 18.3%) and **Z-2** (0.16 g, 0.68 mmol, 8.4%) eluted with CH_2Cl_2/AcOEt 7:3, and **3** eluted with CH_2Cl_2/3% MeOH.

Procedure II: Triethylphosphite (8 mL, 46 mmol, 1 eq) was added dropwise over 10 min to a solution of phenol (4.8 g, 51 mmol, 1.1 eq) and ethyl propiolate **1b** (49 mmol, 1 eq) in CH_2Cl_2 (50 mL). The mixture was stirred for 5–6 h at rt, evaporated, diluted with CH_2Cl_2 (300 mL) and washed with a saturated aqueous $NaHCO_3$ solution (2 × 300 mL). The aqueous phase was extracted with CH_2Cl_2 (300 mL), the combined organic phases were washed with water (300 mL), dried over anhydrous Na_2SO_4 and evaporated. Pure **E-2** (6.6 g, 28 mmol, 61%) was obtained after flash chromatography (CH_2Cl_2 and CH_2Cl_2/AcOEt 1:1).

E-2: TLC (CH_2Cl_2/AcOEt 7:3) R_f 0.34, (AcOEt) R_f 0.40; GC (A) t_R 10.5 min; GC/MS (C) t_R 15.3 min, m/z 236 (1%, M), 191 (33%, M-OEt), 163 (100%, M-CO_2Et), 135 (100%), 107 (24%), 81 (37%, PO_3H_2), 65 (20%); ^{1}H NMR ($CDCl_3$) δ 6.86 (dd, 1H, $^3J_{HHtrans}$ 17.2 Hz, $^2J_{HP}$ 18.5 Hz, H_3), 6.67 (dd, 1H, $^3J_{HHtrans}$ 17.2 Hz, $^3J_{HP}$ 20.5 Hz, H_2), 4.18 (q, 2H, $^3J_{HH}$ 7.2 Hz, $CO_2C\underline{H}_2$), 4.06 (m, 4H, $^3J_{HH}$ 7.2 Hz, $POC\underline{H}_2$), 1.24 (m, 9H, $^3J_{HH}$ 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.5 (d, $^3J_{CP}$ 28 Hz, C_1), 137.3 (d, $^2J_{CP}$ 6.7 Hz, C_2), 131.9 (d, $^1J_{CP}$ 184 Hz, C_3), 62.5 (d, $^2J_{CP}$ 5.4 Hz, $PO\underline{C}H_2$), 61.4 (s, $CO_2\underline{C}H_2$), 16.3 (d, $^3J_{CP}$ 6.2 Hz, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 15.2.

Z-2: TLC (CH_2Cl_2/AcOEt 7:3) R_f 0.20, (AcOEt) R_f 0.28; GC (A) t_R 10.0 min; GC/MS (C) t_R 13.9 min, m/z 191 (11%, M-OEt), 163 (38%, M-CO_2Et), 135 (100%), 107 (9%), 81 (31%), 65 (11%); ^{1}H NMR ($CDCl_3$) δ 6.43 (dd, 1H, $^3J_{HHcis}$ 13.6 Hz, $^3J_{HP}$ 46.7 Hz, H_2), 6.05 (dd, 1H, $^3J_{HHcis}$ 13.6 Hz, $^2J_{HP}$ 14.8 Hz, H_3), 4.12 (q, 2H, $^3J_{HH}$ 7.2 Hz, $CO_2C\underline{H}_2$), 4.02 (m, 4H, $^3J_{HH}$ 7.2 Hz, $POC\underline{H}_2$), 1.18 (t, 6H, $^3J_{HH}$ 7.2 Hz, $POCH_2C\underline{H}_3$), 1.17 (t, 3H, $^3J_{HH}$ 7.2 Hz, $CO_2CH_2C\underline{H}_3$); ^{13}C NMR ($CDCl_3$) δ 164.4 (d, $^3J_{CP}$ 10.5 Hz, C_1), 137.2 (s, C_2), 129.4 (d, $^1J_{CP}$ 185 Hz, C_3), 62.2 (d, $^2J_{CP}$ 5.8 Hz, $PO\underline{C}H_2$), 61.3 (s, $CO_2\underline{C}H_2$), 16.2 (d, $^3J_{CP}$ 6.3 Hz, $POCH_2\underline{C}H_3$), 13.9 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 12.8.

2,3-Bis-(diethoxyphosphoryl)propionic acid ethyl ester: **3**

Compound **3** was identified in Procedure I described above.

TLC (AcOEt) R_f 0.07; GC (A) t_R = 18.8 min; GC/MS (D) t_R = 20.0 min, m/z 329 (62%, M-OEt), 301 (20%, M-CO_2Et), 273 (11%), 245 (13%), 237 (100%, M-PO_3Et_2), 217 (17%), 199 (20%), 165 (39%), 137 (42%), 109 (72%), 91 (34%), 81 (65%, PO_3H_2), 55 (47%); ^{1}H NMR (CD_3OD) δ 4.28–4.04 (m, 10H, OCH_2), 3.35–3.14 (m, 1H, H_2), 2.61–2.42 (m, 1H, H_3), 2.29–2.09 (m, 1H, H_3), 1.39–1.28 (m, 15H, CH_3); ^{13}C NMR (CD_3OD) δ 170.1 (dd, $^2J_{CP}$ 1.6 Hz, $^3J_{CP}$ 5.3 Hz, C_1), 66.8 (dd, $^2J_{CP}$ 8.4 Hz, $^2J'_{CP}$ 6.8 Hz, $PO\underline{C}H_2$), 65.7 (dd, $^2J_{CP}$ 6.3 Hz, $^2J'_{CP}$ 4.7 Hz, $PO\underline{C}H_2$), 65.0 (s, $CO_2\underline{C}H_2$), 42.8 (dd, $^1J_{CP}$ 131 Hz, $^2J_{CP}$ 4.8 Hz, C_2), 25.5 (dd, $^1J_{CP}$ 144 Hz, $^2J_{CP}$ 5.3 Hz, C_3), 18.5 (d, $^3J_{CP}$ 5.8 Hz, $POCH_2\underline{C}H_3$), 16.3 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR (CD_3OD) δ 29.3 (d, $^3J_{PP}$ 71.2 Hz, P_2), 22.8 (d, $^3J_{PP}$ 71.2 Hz, P_3).

E-6-(Diethoxyphosphoryl)cyclohex-3-enecarboxylic acid ethyl ester: **E-4**

E-6-(Ethoxyhydroxyphosphoryl)cyclohex-3-enecarboxylic acid ethyl ester: **E-5**

A solution of olefin **E-2** (3.6 g, 15.2 mmol, 1 eq), 3-sulfolene (1.8 g, 15.2 mmol, 1 eq) and hydroquinone (30 mg) in ethanol (2 mL) was introduced in a pyrex screw-cap tube placed in a pressure reaction vessel. The vessel was heated at 127°C for 24 h then cooled to rt. The mixture was transferred and evaporated to give an oily residue that was taken up in cyclohexane (80 mL) and washed with a saturated aqueous $NaHCO_3$ solution (80 mL). The aqueous phase was extracted with cyclohexane (2 × 40 mL), the combined organic phases were washed with water (160 ml), dried over anhydrous Na_2SO_4 and evaporated. **E-4** (2.8 g, 9.6 mmol, 63.5%) was recovered. The combined aqueous phases were acidified to pH 1 and extracted with CH_2Cl_2 (2 × 400 mL). Theses organic layers were dried over anhydrous Na_2SO_4 and evaporated to yield crude phosphonic acid **E-5**. It was esterified back to **E-4** by refluxing an ethanol (10 mL) solution with boron trifloride diethyl etherate (1 mL). The mixture was evaporated after 3 d, dissolved in AcOEt (80 mL), washed with a saturated aqueous $NaHCO_3$ solution (3 × 80 mL), brine (80 mL), dried over anhydrous Na_2SO_4 and evaporated to give **E-4** (0.88 g, 3 mmol) with a total yield of 83% from **E-2**.

E-4: TLC (AcOEt) R_f 0.28; GC (A) t_R 14.4 min; GC/MS (C) t_R 19.1 min, m/z 290 (17%, M), 245 (19%, M-OEt), 244 (22%), 217 (12%, M-CO_2Et), 138 (42%), 111 (30%), 79 (100%), 77 (60%); ^{1}H NMR ($CDCl_3$) δ 5.67 (s, 2H, H_3, H_4), 4.16–4.04 (m, 6H, OCH_2), 2.80 (m, 1H, H_1), 2.45–2.25 (m, 5H, H_2, H_5, H_6), 1.27 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.5 (d, $^3J_{CP}$ 9.2 Hz, CO), 124.6 (s, C_3, C_4), 61.9 (d, $^2J_{CP}$ 6.5 Hz, $PO\underline{C}H_2$), 61.6 (d, $^2J_{CP}$ 6.5 Hz, $PO\underline{C}H_2$), 60.7 (s, $CO_2\underline{C}H_2$), 39.3 (d, $^2J_{CP}$ 2.6 Hz, C_1), 32.6 (d, $^1J_{CP}$ 143.9 Hz, C_6), 27.5 (d, $^3J_{CP}$ 10.2 Hz, C_2), 23.7 (d, $^2J_{CP}$ 2 Hz, C_5), 16.4 (d, $^3J_{CP}$ 5.5 Hz, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 31.0.

E-5: ^{1}H NMR ($CDCl_3$) δ 10.22 (s, 1H, OH), 5.66 (s, 2H, H_3, H_4), 4.06 (m, 4H, OCH_2), 2.79 (m, 1H, H_1), 2.47–2.20 (m, 5H, H_2, H_5, H_6), 1.26 (m, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 174.5 (d, $^3J_{CP}$ 9.2 Hz, CO), 124.6 (s, C_3, C_4), 61.4 (d, $^2J_{CP}$ 6.5 Hz, $PO\underline{C}H_2$), 60.7 (s, $CO_2\underline{C}H_2$), 39.1 (s, C_1), 32.6 (d, $^1J_{CP}$ 143.9 Hz, C_6), 27.3 (d, $^3J_{CP}$ 10.2 Hz, C_2), 23.3 (s, C_5), 16.3 (d, $^3J_{CP}$ 5.8 Hz, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 33.3.

Z-6-(Diethoxyhydroxyphosphoryl)cyclohex-3-enecarboxylic acid ethyl ester: **Z-4**

A solution of **Z-2** (0.215 g, 0.91 mmol, 1 eq), 3-sulfolene (0.107 g, 0.91 mmol, 1 eq) and hydroquinone (5 mg) were reacted in the same conditions as **E-2**. The crude product contained a mixture of **Z-** and **E-4** (88:12).

Z-4: TLC (AcOEt) R_f 0.23; GC (A) t_R 14.6 min; GC/MS (C) t_R 19.2 min, m/z 290 (12%, M), 245 (16%, M-OEt), 244 (16%), 217 (7%, M-CO_2Et), 138 (49%), 111 (34%), 79 (100%), 77 (60%); ^{1}H NMR ($CDCl_3$) δ 5.65 (s, 2H, H_3, H_4), 4.14–3.99 (m, 6H, OCH_2), 2.90 (md, 1H, $^3J_{HP}$ 22.7 Hz, H_1), 2.71–2.17 (m, 5H, H_2, H_5, H_6), 1.27 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$) δ 173 (s, CO), 125.2 (d, $^3J_{CP}$ 8.9 Hz, C_4), 124.8 (s, C_3), 61.9 (d, $^2J_{CP}$ 6.8 Hz, $PO\underline{C}H_2$), 61.7 (d, $^2J_{CP}$ 6.8 Hz, $PO\underline{C}H_2$), 60.4 (s, $CO_2\underline{C}H_2$), 38.1 (d, $^2J_{CP}$ 2.6 Hz, C_1), 33.2 (d, $^1J_{CP}$ 145.0 Hz, C_6), 26.4 (d, $^3J_{CP}$ 8.2 Hz, C_2), 23.9 (s, C_5), 16.4 (d, $^3J_{CP}$ 5.2 Hz, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 30.9.

E-4-(diethoxyphosphoryl)-3-ethoxycarbonylhexanedioic acid diethyl ester: **E-6**

A solution of 1 M $KMnO_4$ (54 mL, 3 eq) was added to a cooled solution of **E-6** (5.2 g, 17.9 mmol, 1 eq) in acetone (25 mL) under

vigorous stirring. Stirring was carried on for 5–6 h. The excess of $KMnO_4$ was then reduced by a slow addition of sodium bisulfite ($Na_2S_2O_5$, 6.2 g, 2.2 eq). After 20 min, the solution was acidified to pH 2 with a concentrated HCl aqueous solution and extracted with THF/AcOEt 1:1 (3 × 200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and evaporated. The oily residue (6.4 g) was used as such in the next step. A sample (0.334 g, 1.15 mmol) was purified by flash chromatography (CH_2Cl_2/AcOEt 1:1) then (AcOEt/1% AcOH) to give pure **E-6** (0.174 g, 0.49 mmol) as an oil. TLC (AcOEt / AcOH 1%) R_f 0.13; ^{1}H NMR ($CDCl_3$) δ 10.34 (s, 2H, CO_2H) 4.07 (m, 6H, OCH_2), 3.40 (m, 1H, H_3), 2.87–2.30 (m, 5H, H_4, CH_2), 1.26 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$) δ 176.0 (s, C_1), 174.9 (d, $^3J_{CP}$ 11.8 Hz, CO), 172.4 (d, $^3J_{CP}$ 22.6 Hz, CO), 63.1 (d, $^2J_{CP}$ 6.8 Hz, PO$\underline{C}H_2$), 62.9 (d, $^2J_{CP}$ 6.9 Hz, PO$\underline{C}H_2$), 61.4 (s, O$\underline{C}H_2$), 39.4 (s, C_3), 33.5 (d, $^1J_{CP}$ 152 Hz, C_4), 32.3, 30.5 (s, C_2, C_5), 16.2 (d, $^3J_{CP}$ 4.6 Hz, $POCH_2\underline{C}H_3$), 13.9 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 29.4. GC was performed on the pentaethyl ester from an esterified (HCl/EtOH, 100°C) sample of **E-6**. GC/MS (D) t_R 21.9 min, m/z 410 (0%, M), 365 (100%, M-OEt), 337 (33%, M-CO_2Et), 291 (33%), 263 (38%), 238 (33%), 227 (35%), 189 (48%), 165 (34%), 109 (50%), 81 (50%).

E-3-(Ethoxycarbonyl)-4-(diethoxyphosphoryl)cyclopentanone: **E-7**

A solution of crude **E-6** (6 g) in acetic anhydride (115 mL) and sodium acetate was refluxed for 2 h with stirring, then cooled to 0°C, diluted with ethanol (150 mL), neutralized with a saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (2 × 1 L). The organic phase was dried over anhydrous Na_2SO_4 and evaporated. The oily residue (4 g) was purified by flash chromatography (CH_2Cl_2/AcOEt 6:4) to afford **E-7** as an oil (2.6 g, 8.9 mmol, 53% from **E-4**). TLC (AcOEt/CH_2Cl_2 4:6) R_f 0.33; GC (B) t_R = 16.3 min; GC/MS (C) t_R = 20.3 min, m/z 292 (7%, M), 247 (20%, M-OEt), 246 (20%), 219 (45%, M-CO_2Et), 191 (32%, M-CO_2Et-CO), 163 (48%), 109 (55%), 81 (100%), 53 (67%); ^{1}H NMR ($CDCl_3$) δ 4.18–4.00 (m, 6H, O-CH_2), 3.35–3.20 (m, 1H, H_3), 2.96–2.79 (m, 1H, H_4), 2.68–2.34 (m, 4H, H_2, H_5), 1.24 (m, 9H, $^3J_{HH}$ 7.2 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 213.0 (d, $^3J_{CP}$ 12.6 Hz, $\underline{C}O_2$), 173.2 (d, $^3J_{CP}$ 7.4 Hz, C_1), 62.4 (d, $^2J_{CP}$ 3.7 Hz, PO$\underline{C}H_2$), 61.5 (s, $CO_2\underline{C}H_2$), 41.5 (d, $^3J_{CP}$ 5.3 Hz, C_2), 41.2 (s, C_3), 37.9 (s, C_5), 35.2 (d, $^1J_{CP}$ 151 Hz, C_4), 16.4 (d, $^3J_{CP}$ 5.8 Hz, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 29.2.

Ethyl-1-spiro-5′-hydantoin-4-(diethoxyphosphoryl)cyclopentane-3-carboxylic acid ester: (1SR, 3RS, 4SR)-**8** and (1SR, 3SR, 4RS)-**9**

KCN (0.118 g, 1.9 mmol, 1.3 eq) was added to a solution of **E-7** (0.416 g, 1.4 mmol, 1 eq) and ammonium carbonate (0.682 g, 7.1 mmol, 5 eq) in EtOH (8 mL) and water (8 mL). The resulting mixture was heated with a reflux condenser at 60°C for 27 h. The solution was cooled, concentrated, acidified to pH 1 with concentrated HCl, stirred for 2 h under a well ventilated hood (toxic hazard), and extracted with CH_2Cl_2 (2 × 60 mL). Combined organic phases were dried over anhydrous Na_2SO_4 and evaporated. The oily residue (0. 442 g) was purified by flash chromatography (CH_2Cl_2/6% MeOH). The major isomer **8** (0.119 g, 0.33 mmol) was eluted first, followed by a mixed fraction (0.032 g, 0.088 mmol) and lastly by the minor isomer **9** (0.032 g, 0.088 mmol) with a total yield of 36%.
8: TLC (CH_2Cl_2/6% MeOH) R_f 0.33; ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 1H, $NH_{5'}$), 4.21–4.03 (m, 6H, O-CH_2), 3.39–3.21 (m, 1H, H_3), 3.07–2.92 (m, 1H, H_4), 2.58 (ddd, 1H, $^3J_{HP}$ 25.1 Hz, $^3J_{HH}$ 11.2 Hz, $^2J_{HH}$ 14 Hz, H_{5a}), 2.45 (dd, 1H, $^2J_{HH}$ 13.3 Hz, $^3J_{HH}$10.3 Hz, H_2), 2.31 (dd, 1H, $^2J_{HH}$ 13.3 Hz, $^3J_{HH}$ 8.3 Hz, H_2), 2.20–2.05 (m, 1H, H_{5b}), 1.34–1.22 (m, 9, CH_3); ^{13}C NMR ($CDCl_3$) δ 175.8 (s, $C_{4'}$), 172.5 (d, $^3J_{CP}$ 6.3 Hz, $\underline{C}O_2$), 155.6 (s, $C_{2'}$), 69.5 (d, $^3J_{CP}$ 3.2 Hz, C_1), 62.8, 62.7 (s, PO$\underline{C}H_2$), 61.5 (s, $CO_2\underline{C}H_2$), 44.0 (s, C_3), 42.1 (d, $^3J_{CP}$ 7.4 Hz, C_2), 37.0 (s, C_5), 36.4 (d, $^1J_{CP}$ 147 Hz, C_4), 16.4, 16.3 (s, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 32.6.
9: TLC (CH_2Cl_2/6% MeOH) R_f 0.24; ^{1}H NMR ($CDCl_3$) δ 6.57 (s, 1H, $NH_{5'}$), 4.22–4.04 (m, 6H, O-CH_2), 3.31 (m, 1H, $^3J_{H3H2a}$ 10.2 Hz, $^3J_{H3H4}$ 7.3 Hz, $^3J_{H3H2b}$ 2.5 Hz, H_3), 2.81 (m, 1H, $^3J_{HH}$ 11.9 Hz, $^3J_{H4H3}$ 7.3 Hz, H_4), 2.57 (dd, 1H, $^2J_{HH}$ 14.0 Hz, $^3J_{H2aH3}$ 10.2 Hz, H_{2a}), 2.41 (m, 1H, $^2J_{HH}$ 13.2 Hz, H_{5a}), 2.24–2.16 (m, 1H, $^2J_{HH}$ 13.2 Hz, $^3J_{HP}$ 8.0 Hz, $^3J_{HH}$ 1.8 Hz, H_{5b})), 2.20–2.05 (m, 1H, $^1J_{HHgem}$ 14.0 Hz, $^3J_{H2bH3}$ 2.5 Hz, H_{2b}), 1.34–1.22 (m, 9H, CH_3); ^{13}C NMR ($CDCl_3$) δ 176.0 (d, $^3J_{CP}$ 5.8 Hz, $\underline{C}O_2$), 174.9 (s, $C_{4'}$), 155.3 (s, $C_{2'}$), 69.9 (d, $^3J_{CP}$ 13.7 Hz, C_1), 62.4, 62.3 (s, PO$\underline{C}H_2$), 61.9 (s, $CO_2\underline{C}H_2$), 43.8 (s, C_3), 41.0 (d, $^3J_{CP}$ 8.4 Hz, C_2), 39.2 (s, C_5), 38.5 (d, $^1J_{CP}$ 151 Hz, C_4), 16.5, 16.4 (s, $POCH_2\underline{C}H_3$), 14.1 (s, $CO_2CH_2\underline{C}H_3$); ^{31}P NMR ($CDCl_3$) δ 29.1.

1-Amino-4-phosphonocyclopentane-1,3-dicarboxylic acid: (1*SR*, 3*RS*, 4*SR*)-**10** and (1*SR*, 3*SR*, 4*RS*)-**11**

A solution of hydantoin 8 or 9 (0.119 g, 0.32 mmol) in 6N HCl (6 mL) was heated 3 d at 110°C in a screw-cap bottle and evaporated. The residue was dried under vacuum over KOH dissolved in water (150 mL) (pH 3–4) and deposited on a AG50X4 column (H^+, 50–100 mesh). The zwitterion of **8** or **9** (0.071 g, 0.28 mmol, 88%) was eluted with water and dried under vacuum.
10: TLC (nBuOH/AcOH/H_2O 2:1:1) R_f 0.16; ^{1}H NMR (D_2O) δ 3.39 (m, 1H, H_3), 3.00–2.72 (m, 3H, H_4, H_2, H_5), 2.55 (dd, 1H, J_{HH} 14.2 Hz, $^3J_{HH}$ 9.1H, 0, H_2), 2.33–2.22 (m, 1H, H_5); ^{13}C NMR (D_2O) δ 179.9 (d, $^3J_{CP}$ 5.6 Hz, CO_2H en C_3), 176.7 (s, CO_2H en C_1), 67.9 (d, $^3J_{CP}$ 8.2 Hz, C_1), 47.8 (d, $^3J_{CP}$ 0.9 Hz, C_3), 42.9 (d, $^1J_{CP}$ 138 Hz, C_4), 42.3 (d, $^3J_{CP}$ 8.4 Hz, C_2), 40.3 (s, C_5); ^{31}P NMR (D_2O) δ 26.5. Anal. Calc. C33.21%, H4.78%, N5.53%, Obs. C33.11%, H4.91%, N5.41%.
11: TLC (nBuOH/AcOH/H_2O (2:1:1) R_f 0.06; ^{1}H NMR (D_2O) δ 3.39 (m, 1H, H_3), 2.86 (ddd, 2H, $^2J_{HH}$ 14.5 Hz, $^3J_{HH}$ 8.9 Hz, J_{HP} 1.8 Hz, H_2, H_5), 2.66 (m, 1H, H_4), 2.53–2.41 (m, 1H, $^2J_{HH}$ 14.5 Hz, J_{HH} 8.3 Hz, H_5), 2.35 (dd, 1H, $^2J_{HH}$ 14.5 Hz, $^3J_{HH}$ 7.8 Hz, H_2); ^{13}C NMR (D_2O) δ 180.6 (d, $^3J_{CP}$ 3.9 Hz, CO_2H en C_3), 176.5 (s, CO_2H en C_1), 67.8 (d, $^3J_{CP}$ 13.2 Hz, C_1), 48.8 (d, $^2J_{CP}$ 3.8 Hz, C_3), 43.3 (d, $^1J_{CP}$ 141 Hz, C_4), 43.1 (d, $^3J_{CP}$ 11.9 Hz, C_2), 40.6 (s, C_5); ^{31}P NMR (D_2O) δ 22.5. GC was performed on derivatized samples [1) trifluoroacetic anhydride 50°C 15 min; 2) trimethyl orthoformate 110°C 2 h, (Kudzin and Luczak, 1995)], both trifluoroacetyl and formyl derivatives have been detected but are identical for **10** and **11**. GC (B) t_R 20.8 and 25.3 min; GC/MS (E) t_R 17.3 min, m/z 259 (3%), 241 (4%), 147 (8%), 129 (42%), 112 (13%), 101 (9%), 83 (16%), 70 (28%), 57 (100%) and t_R 18.7 min, m/z 279 (15%), 167 (24%), 149 (100%), 104 (22%), 76 (15%), 70 (17%), 57 (65%).

Pharmacology

Culture and transfection of HEK 293 cells

HEK 293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and transfected by electroporation as previously described, with 10 μg carrier DNA (pRK), plasmid DNA containing mGlu1a (1 μg), mGlu2 (4 μg) or mGlu8a (4 μg) receptor coding sequences and 10 × 10^6 cells (Brabet et al., 1998). To enable coupling of mGlu2 and mGlu8a receptors to PLC, the receptors were co-expressed with the chimeric G-protein α subunit Gqi9 (2 μg) (Conklin et al., 1993; Gomeza et al., 1996; Parmentier et al., 1998).

Determination of accumulation of inositol phosphates (IP)

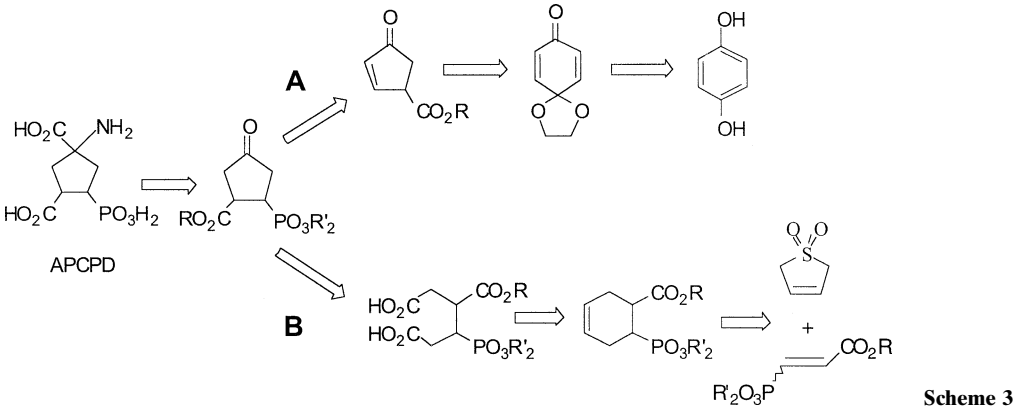
Determination of IP accumulation in transfected cells was performed after labeling the cells overnight with [^{3}H]-myo-inositol (23.4 Ci/mol) as previously described (Blahos et al., 2001; Brabet et al., 1998). The cells were pre-incubated for 1 hour with the Glu

degrading enzyme glutamate pyruvate transaminase (1 U/ml) and 2 mM pyruvate to avoid the possible action of Glu released from the cells. The stimulation was then conducted for 30 min in a medium containing 10 mM LiCl, glutamate pyruvate transaminase (plus pyruvate) or the indicated concentration of agonist. Results are expressed as the % amount of IP produced over the radioactivity present in the membranes.

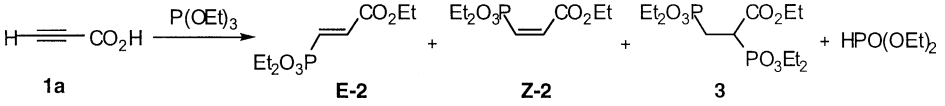
Results

Chemistry

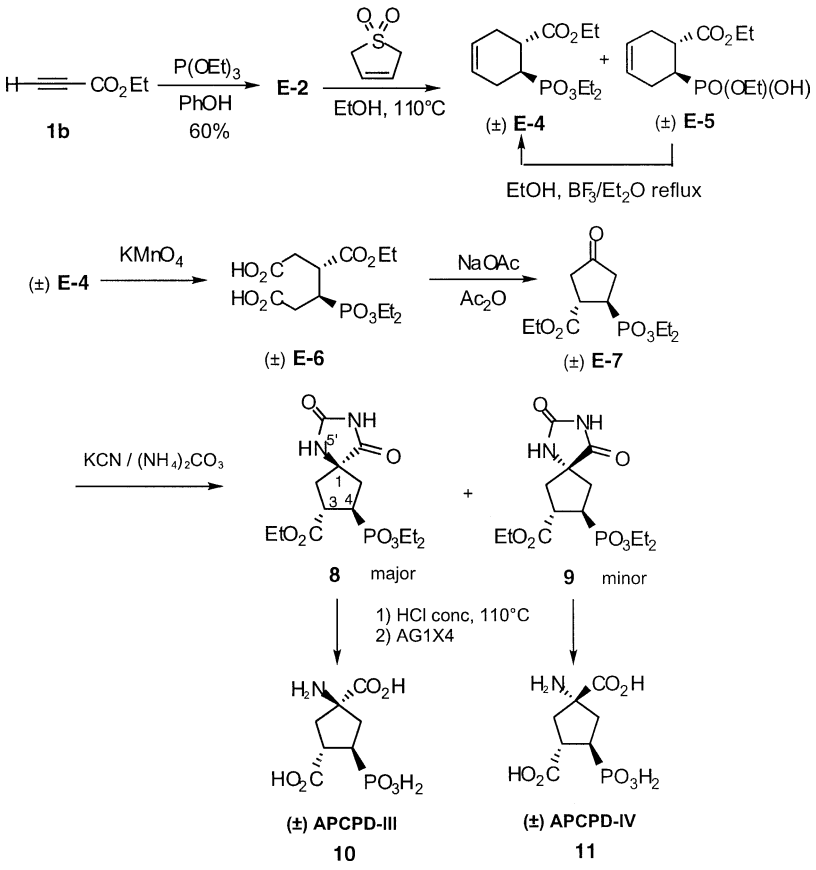
Theoretically, all isomers of APCPD (Scheme 2) can be obtained from the 3-phosphono-4-carboxycyclopentanone by a Bucherer-Bergs or Strecker synthesis. Two strategies were considered for its preparation (Scheme 3). The first one (path A) was based on hydrophosphinylation of the carboxy-cyclopentenone that was described by M. Pirrung (Scheme 3) (Crooks et al., 1986; Harvey, 1966; Pirrung and Nunn, 1996). The second strategy (path B) was based on a similar synthetic scheme as for ACPTs starting with carboxy-vinyl phosphonate (Acher et al., 1997). We initially favored the first strategy because the key carboxy-cyclopentenone could allow the addition of various nucleophilic substituents leading to a series of new cyclopentyl glutamate analogs. First steps of path A proceeded as described (Pirrung and Nunn, 1996). However the critical photochemical rearrangement failed in our hands probably because of inadequate equipment. We thus turn to the second procedure. Syntheses of E, Z-mixtures of carboxyvinyl phosphonates such as **2** have been described (Coover Jr. et al., 1957; Kirillova and Kukhtin, 1965; Meppelder and Beck, 1975; van der Host et al., 1974). We first attempted to reproduce the procedure of Kirillova et al. (Kirillova and Kukhtin, 1965) by reacting propiolic acid **1a** and triethylphosphite (Procedure I). However **2** was obtained with a poor yield together with the bis-phosphonate **3** and diethylphosphonate (Scheme 4). Decomposition of triethylphosphite occurred because of the strong acidity of propiolic acid (Olah and McFarland, 1971). This side reaction was greatly reduced by using ethyl propiolate **1b** and phenol as a proton donor (Harvey, 1966) (Scheme 5). Moreover **E-2** was obtained as the only stereoisomer with a 60% yield (Procedure II). It was subsequently used in a Diels-Alder reaction with sulfolene. Cyclohexene triester **E-4** was recovered as well as some phosphonic acid **E-5**. Hydrolysis of the phosphonate ester may be due to sulfur dioxide resulting from thermal decomposition of sulfolene. Phosphonic acid was re-sterified to **E-4** with boron trifluoride etherate and ethanol (Kadaba, 1971). The *trans* stereochemistry between the carboxyester and the phosphonate groups was preserved at this stage. However when the same Diels-Alder reaction was run with **Z-2**, a mixture of **Z-** and **E-4** isomers (88:12) was obtained. Oxidation of cyclohexene triester **E-4** afforded **E-6** which could be cyclized to the target cyclopentanone **E-7** which in turn was converted to hydantoins **8** and **9** by a Bucherer-Bergs reaction. Stereochemistry was assigned by NOE (Nuclear Overhauser Effect) NMR experiments on the separated diastereoisomers. Thus,



Scheme 3



Scheme 4



Scheme 5

with the phosphonate and carboxy ester groups in a *trans* relation, NOE were detected between H-5′ and H-3 for **8** and H-4 for **9**. Acid hydrolysis allowed complete deprotection of amino acids APCPD-III **10** and APCPD-IV **11**.

Pharmacology

The effect of APCPD-III **10** and APCPD-IV **11** were examined on representative members of the three mGluR groups, rat mGlu1a, mGlu2 and mGlu8a receptors transiently expressed in HEK 293 cells as previously described (Brabet et al., 1998). None of the receptors were activated by **10** or **11** at 1 mM concentration (Fig. 2A). Glutamate induced response at 10 μM was not inhibited in mGlu1a expressing cells with 1 mM **10** and **11**. However, the glutamate effect on mGlu2 and mGlu8a at 20 μM was antagonized by these amino acids at 1 mM concentration (Fig. 2B, $IC_{50} \geq 300\mu M$ dose-response curves not shown). The strongest antagonist effect was observed with APCPD-IV **11** on mGlu8a receptor ($IC_{50} \approx 300\mu M$).

Discussion

Synthesis of APCPD-III and IV was achieved following a procedure analogous to that of ACPTs (Acher et al., 1997). They are the most stable diastereoisomers of the series because of the *trans* relation between the carboxy and the phosphonate on C-3 and C-4 respectively. This relative *trans* stereochemistry results from olefin **E-2** and is maintained throughout the synthesis (Scheme 5). Experimental conditions were found that afford only the **E-2** isomer. Thus, separation of diastereoisomers of 2 could be avoided (Scheme 4). With the less stable isomer **Z-2**, epimerization may occur more easily as shown with the Diels-Alder reaction. Synthesis of pure **Z-2** and APCPD-I and -II are under current study, using mild reaction conditions as for ACPT-I and -II (Acher et al., 1997).

APCPD-III and -IV are inactive on mGlu1aR and antagonists on mGlu2 and 8a receptors. Although APCPDs are glutamate/ACPD as well as AP4/APCPC analogs (Scheme 1), the effect of isomers III

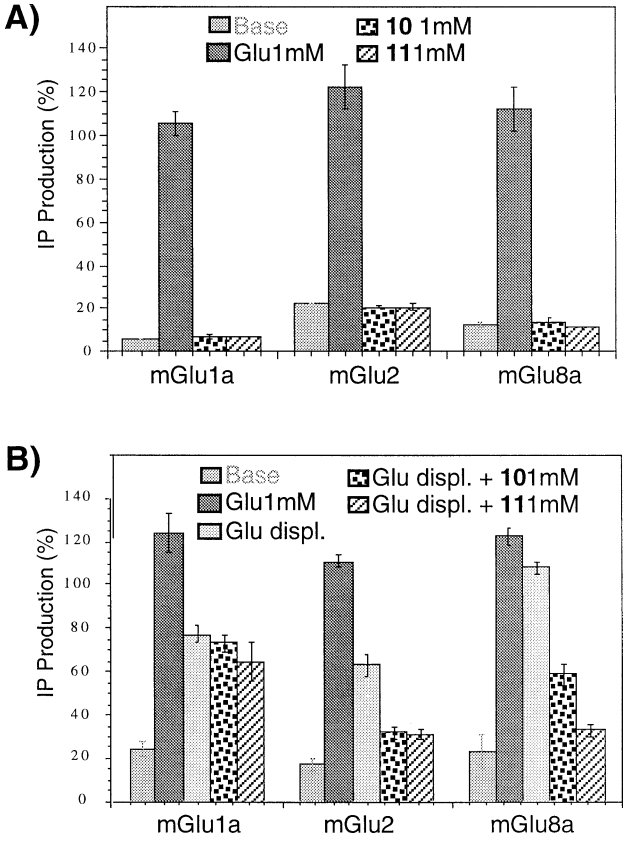
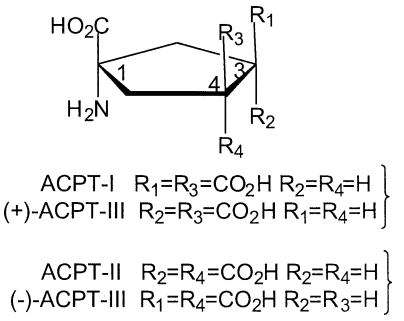


Fig. 2. Agonist (**A**) and antagonist (**B**) activities of APCPD-III and -IV. IP formation was determined in cells expressing mGlu1a, mGlu2 and mGlu8a receptors under control conditions (*Base*), after stimulation with 1 mM Glu/APCPD-III/ APCPD-IV (**A**) or Glu in combination with or without APCPD (**B**). Glutamate concentrations (*Glu displ.*) that were displaced in panel B were 10 μM for mGlu1aR expressing cells and 20 μM for mGlu2 and 8aR expressing cells. Data are means of 3 independent experiments



Scheme 6

and IV are quite different from these agonists. Cyclopentane amino acids carrying an acidic group at the 3-position, are mGluR agonists. Changing from a carboxylate (ACPD) to a phosphonate (APCPC) substituent affords increased selectivity and potency at group III receptors (De Colle et al., 2000). Adding another carboxylic group at C-4 of ACPDs induces variable effects at these receptors, according to the stereochemistry of this carbon. In fact, in the ACPT's series, agonists are observed with $R_3 = CO_2H$, $R_4 = H$ while antagonists are found with $R_3 = H$, $R_4 = CO_2H$ whatever the stereochemistry at C-3 (Scheme 6). Interestingly, the (±)-ACPT-III mixture activates mGlu4/8 receptors because the (+) isomer is a more potent agonist than the (−) isomer is as an antagonist. Similarly we hypothesized that if one of the enantiomers of APCPD-III or -IV would be a potent agonist, the racemic mixture would activate mGlu8 receptor. Since it is not the case, we conclude that none of the four isomers are potent agonists of this receptor. The tridimensional structures of mGluR binding sites (Bessis et al., 2000; Kunishima et al., 2000; Malherbe et al., 2001; Rosemond et al., 2001; Tsuchiya et al., 2002; Bertrand et al., 2002) allow to better understand the subtle switch between agonists and competitive antagonists (Bessis et al., 2002). The additional acidic group of APCPD-III and -IV compared to ACPD or APCPC may prevent the ligand binding domain (LBD) to adopt a conformation that triggers receptor activation. Yet a different orientation of this group in APCPD-I and -II may allow the LBD active conformation to be reached.

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Authors' address: Dr Francine C. Acher, Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, UMR8601-CNRS, Université René Descartes-Paris V, 45 rue des Saints-Pères, 75270 Paris Cedex 06, France, Fax: (33) 1 42 86 83 87,
E-mail: acher@biomedicale.univ-paris5.fr