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Synthesis and preliminary biological evaluation of (2*S*,1′*R*,2′*S*)- and (2*S*,1′*S*,2′*R*)-2-(2′-phosphonocyclopropyl)glycines, two novel conformationally constrained L-AP4 analogues

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Abstract—Two novel constrained L-AP4 analogues, (2*S*,1′*R*,2′*S*)- and (2*S*,1′*S*,2′*R*)-2-(2′-phosphonocyclopropyl)glycines (**7**) and (**8**), were synthesized and evaluated as mGluR ligands. Compound **7** showed to be a group III mGluRs agonist with micromolar activity.

Among the strategies aimed at finding new potent and selective ligands for glutamate receptors, the bioisosteric replacement of L-glutamate ω-carboxylate function has been a particularly fruitful one.[1] The substitution of this moiety for the phosphonate one in compounds characterized by an appropriate intermediate chain and by the D-configuration at the amino acidic moiety, such as in D-AP5 (**1**, Chart 1) and D-AP7 (**2**), has given access to the first generation of potent and selective NMDA competitive antagonists.[2] More recently, another phosphonate analogue of L-glutamate, L-AP4 (**3**), has proved to be a potent agonist for the group III family of metabotropic glutamate receptors (mGluRs) and as such an important tool for its pharmacological characterization.[3] The lack of selectivity of L-AP4 (**3**) towards the array of group III subtypes (mGluR4, 6, 7, and 8), however, gives interest to the search for new potent, subtype selective group III ligands. Conformational freezing of L-AP4 (**3**), in this regard, is a route that has been followed with some success.

(+)-PPG (**4**), an L-AP4 analogue in which the phosphonate and the glycine moieties are kept in a collinear

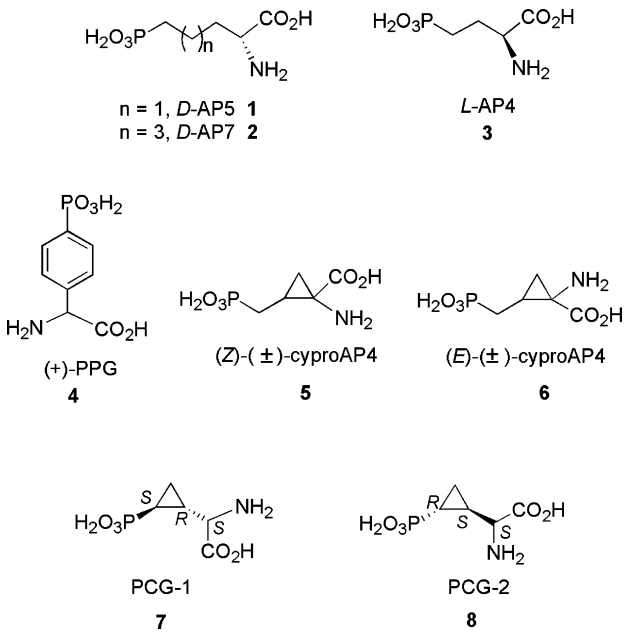

Chart 1. Selected L-Glu analogs as excitatory amino acid receptors ligands.

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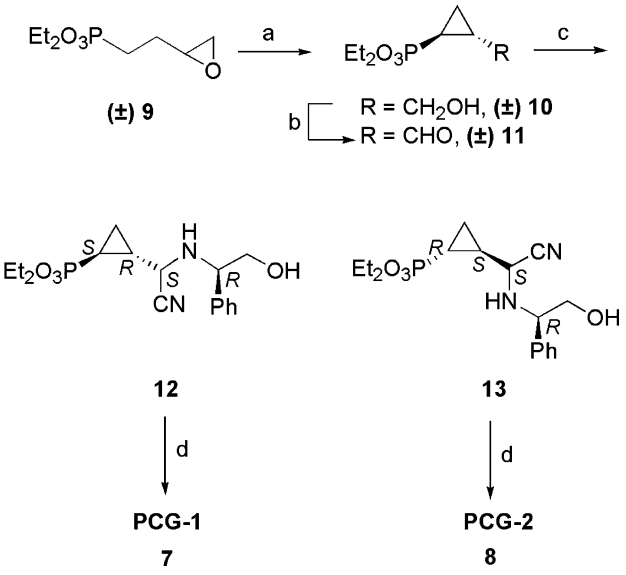
fashion by the phenyl spacer, is a potent mGluR4 agonist (EC_{50} = 3.2 μM), also active at mGluR7 (EC_{50} = 48 μM),[4] reported to be endowed with neuroprotective properties[5] and anxiolytic- and antidepressant-like effects.[6] The introduction of a cyclopropyl ring in 2,3 position of the skeleton of L-AP4 (**3**) as in (*Z*)- and (*E*)-(±)-2-amino-2,3-methano-4-phosphonobutanoic acids (**5** and **6**, respectively) has been shown to give group III agonists in which the potency of the parent is retained.[7] Compound **5** with an EC_{50} of 0.58 μM is the most potent mGluR4a agonist so far reported. Although these derivatives have been instrumental in producing pharmacophore models for group III agonists,[8,9] a clear picture of the steric and conformational requirements for the selective interaction of phosphonate analogue with individual group III mGluR subtypes is still missing.

In the frame of our continuing interest in the development of chemical tools for the characterization of glutamatergic pathways, we have undertaken the synthesis of two novel conformationally constrained analogues of L-AP4 (**3**), namely *trans*-(2*S*,1′*R*,2′*S*)- and (2*S*,1′*S*,2′*R*)-2-(2′-phosphonocyclopropyl) glycines (PCG-1, **7**) and (PCG-2, **8**) in which the pharmacophoric groups, the glycine moiety and the phosphonate group, are partially rigidified into extended disposition according to the pharmacophore models for group III agonists.[8,9] The two new compounds are phosphono analogs of carboxycyclopropylglycines (L-CCGs), conformationally constrained analogs of L-glutamate. (2*S*,1′*S*,2′*S*)-L-CCG-I (**15**), in particular, is a potent mGluR agonist with significant activity also at group III subtypes. It must be mentioned that compounds **7** and **8** have previously and independently been prepared with a different chemistry at Novartis (Basel) and tested at the human mGlu4 receptor. The biological activity is consistent with our present results. The Novartis's data are still unpublished.[18]

The synthesis and the preliminary biological properties of **7** and **8** are reported herein (Scheme 1). *trans*-Diethyl 2-(hydroxymethyl) cyclopropyl phosphonate (**10**) was obtained from the readily available diethyl γ,δ-epoxybutylphosphonate[10] (**9**) via a basic catalyzed intramolecular epoxide opening reaction.[11] While in the original report *n*BuLi was used as the base, in our hands LDA (1.1 equiv in THF, −78 °C) proved to be the base of choice affording the desired **10** in 59% yield. Oxidation of **10** by PCC in dichloromethane at room temperature led in 63% yield to the racemic aldehyde **11** which was submitted to the condensation with *R*-(−)-α-phenylglycinol (MeOH, rt, 24 h). The subsequent addition of trimethylsilyl cyanide to the Schiff base thus formed (TMSCN, 0 °C then rt, 24 h) afforded the mixture of (2*S*,1′*R*,2′*S*)- and (2*S*,1′*S*,2′*R*)-aminonitriles **12** and **13** along with minor amounts of the (2*R*,1′*R*,2′*S*)- and (2*R*,1′*S*,2′*R*)-diastereoisomers (2*S*/2*R* = 75:25 by HPLC).[12] Separation of the two more abundant α-aminonitriles by flash chromatography (ethyl acetate/methanol, 95:5) and subsequent mplc (acetone/*n*-hexane, 70:30) afforded the desired isomers **12** and **13** in 10% and 15% yields, respectively. The two (2*S*)-α-aminonitriles



Scheme 1. Reagents and conditions: (a) LDA, THF, −78–0 °C; (b) PCC, CH_2Cl_2, rt; (c) (i) *R*-(−)-α-phenylglycinol, MeOH, rt; (ii) TMSCN; (iii) mplc; (d) (i) $Pb(OAc)_4$, CH_2Cl_2-MeOH, 0 °C; (ii) 6 N HCl, reflux; (iii) Dowex 1X8-200.

12 and **13** thus obtained were then submitted to oxidative cleavage with lead tetraacetate (CH_2Cl_2–MeOH, 0 °C, 20 min), acidic hydrolysis (6 N HCl), and ion-exchange resin chromatography (Dowex 1X8-200, 1.5 N acetic acid) to afford the corresponding amino acids (2*S*,1′*R*,2′*S*)-PCG-1 (**7**) and (2*S*,1′*S*,2′*R*)-PCG-2 (**8**) in 42% and 46% yields, respectively.[13]

The absolute stereochemistry of the (phosphonocyclopropyl)glycines **7** and **8** was tentatively assigned on the basis of NMR analyses. ^1H–^1H coupling constants ($J_{2\text{-}1'}$), which are similar in both distereoisomers **7** and **8** (*J* = 9.86 Hz and *J* = 9.98 Hz, respectively), suggested that the relative orientation of H(2)–H(1′) was antiperi-

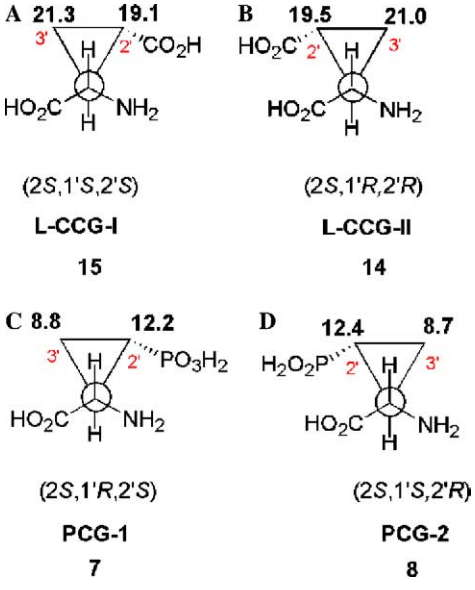


Figure 1. Newman projections of the proposed preferred conformations of L-CCG-I (**15**), L-CCG-II (**14**), PCG-1 (**7**) and PCG-2 (**8**).

Table 1. EC_{50} (μM) values for PCG-1 (**7**) and PCG-2 (**8**)

Compound	Group I		Group II		Group III	
	mGluR1	mGluR5	mGluR2	mGluR4	mGluR6	mGluR7
PCG-1 (**7**)	>1000	>1000	>1000	9.4 ± 3.0	13 ± 3	700 ± 140
PCG-2 (**8**)	>1000	>1000	>1000	49 ± 15	46 ± 23	>1000
L-Glutamate	7.3 ± 1.0	1.2 ± 0.3	26 ± 2	6.1 ± 1.5	7.6 ± 0.5	>1000
L-AP4 (**3**)	>1000	>1000	>1000	0.51 ± 0.14	0.34 ± 0.07	188 ± 79

Functional data were obtained from CHO cells expressing rmGluR1, rmGluR5 or rmGluR6 or from BHK cells expressing hmGluR2, hmGluR7 or rmGluR4 using previously described methods.[3,12b,16] Potencies were determined by: (i) measuring intracellular calcium concentration (group I mGluRs), (ii) GTP(γ)^{35}S stimulation assay (mGluR2) and (iii) measuring cAMP formation (group III mGluRs). The data shown are means ± SEM of at least three independent experiments performed in triplicate.

Table 2. Binding affinity values expressed as K_i (μM), for PCG-1 (**7**) and PCG-2 (**8**)

Compound	mGluR4	mGluR8
PCG-1 (**7**)	7.4 ± 2.7	63 ± 16
PCG-2 (**8**)	53 ± 15	54 ± 4
L-Glutamate	3.4 ± 0.5	3.4 ± 1.1
L-AP4 (**3**)	0.16 ± 0.02	1.9 ± 0.3

[^{3}H]L-AP4 and [^{3}H]LY341495[17] binding experiments were performed on rmGluR4 and rmGluR8 receptor-expressing cell membranes (BHK cells) using a SPA assay.[16] Data are means ± SEM of at least three independent experiments performed in triplicate.

planar in both diastereoisomers. This evidence was also confirmed by NOESY experiments, which did not show any NOE between these two protons in both diastereoisomers **7** and **8**. Taking into account the structural analogy between the (phosphonocyclopropyl)glycines **7** and **8**, and the two *trans*-2-(2′-carboxycyclopropyl)glycine diastereoisomers [L-CCG-II (**14**) and L-CCG-I (**15**)],[14] whose absolute configurations were previously unambiguously defined,[15] a comparative study on the evaluation of '*γ-gauche*' effects played by the amino- and carboxylic groups, on C(2′) and C(3′) in the 2-(2′-carboxycyclopropyl)glycines (**14** and **15**) and 2-(2′-phosphonocyclopropyl)glycines (**7** and **8**), may be instrumental in disclosing the absolute stereochemistries of our new compounds. In particular, assuming also in the case of **14** and **15**, an antiperiplanar disposition between H(2) and H(1′) and considering that an amino group has a larger shielding or '*γ-gauche*' effect on the corresponding carbon atom than does the carboxylic group,[15] the diastereoisomer showing the C(2′) chemical shift more shielded is endowed with 2*S*,1′*S*,2′*S* configuration as depicted in Figure 1A. On the contrary, the 2*S*,1′*R*,2′*R*-isomer, as depicted in Figure 1B, having the amino group in a gauche relationship with C(3′), shows the chemical shift of this carbon atom more shielded than in the other isomer. Analogously, the diastereoisomer **7**, showing C(2′) up-fielded and C(3′) down-fielded in comparison with compound **8**, can be assigned with the 2*S*,1′*R*,2′*S*-configuration (Fig. 1C), in analogy with L-CCG-I (**15**). On the contrary, PCG-2 (**8**) will be endowed with 2*S*,1′*S*,2′*R*-configuration (Fig. 1D).

The new derivatives **7** and **8** were evaluated as potential mGluRs ligands by functional assays (rmGluR1, rmGluR5, hmGluR2, rmGluR4, and hmGluR7) and binding experiments (rmGluR4 and rmGluR8).[16] As summarized in Tables 1 and 2, the new derivatives **7** and **8** show activity as group III mGluRs agonists albeit with significant differences in potency and subtype. In particular, PCG-1 (**7**) able to displace labeled L-AP4 from rat mGluR4 with a K_i of 7.4 μM can be considered an acceptably potent agonist for this receptor subtype. Reduction of the conformational flexibility of L-AP4 (**3**) through insertion of a cyclopropyl ring in 3,4 positions yielded the two *trans*-cyclopropyl derivatives **7** and **8**. Evaluated for their ability to interact with a set of glutamate receptors, both derivatives **7** and **8** showed to be inactive at ionotropic glutamate receptors (NMDA, KA, and AMPA) and at prototypic group I and group II mGluR subtypes. On the contrary, both compounds activated the four group III mGluR subtypes with different potencies, with PCG-1 (**7**) always more potent than PCG-2 (**8**). The most active isomer PCG-1 (**7**), however, is about 10-fold less potent than the parent L-AP4 (**3**) or the other cyclopropyl-AP4 derivative **5** at all the group III mGluR subtypes. While confirming the need for an extended disposition of pharmacophoric moieties for group III recognition, our results indicated that the cyclopropyl ring in 3,4 position of the L-AP4 skeleton, induces a conformational disposition which is accepted but not optimal for interacting with the conserved group III mGluR binding pockets. It can also be speculated that there is a need for the phosphonate moiety of a certain degree of conformational flexibility for a proper interaction with group III receptors. This evidence is in contrast with the experience gained for other glutamate receptors (NMDA-Rs and group II mGluRs, in particular), where restriction of the L-glutamate flexibility by insertion of cyclopropyl moiety yielded very potent and highly selective compounds.

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13. Analytical data of the final compounds **7** and **8**. Compound **7**: ^{1}H NMR (D_2O, 200 MHz) δ 0.99 (3H, m, 2′-CH and 3′-CH_2), 1.43 (1H, m, 1′-CH), 3.37 (1H, d, J = 9.86 Hz, 2-CH); ^{13}C NMR (D_2O, 50 MHz) δ 8.9 (d, J_{C-P} = 5.0 Hz), 12.2 (d, J_{C-P} = 183.5 Hz), 16.9 (d, J_{C-P} = 3.5 Hz), 56.3, 171.1; ^{31}P NMR (D_2O, 50 MHz) δ 24.6; $[\alpha]_D^{20}$ +81.9 (*c* 1.0, H_2O).
Compound **8**: ^{1}H NMR (D_2O, 200 MHz) δ 0.94 (2H, m, 3′-CH_2), 1.16 (1H, m, 2′-CH), 1.55 (1H, m, 1′-CH), 3.37 (1H, d, J = 9.98 Hz, 2-CH); ^{13}C NMR (D_2O, 50 MHz) δ 8.7, 12.4 (d, J_{C-P} = 184.9 Hz), 16.7, 56.0, 170.7; ^{31}P NMR (D_2O, 50 MHz) δ 25.28; $[\alpha]_D^{20}$ +11.8 (*c* 1.0, H_2O).
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