(*S*)-(+)-2-(3′-Carboxybicyclo[1.1.1]pentyl)-glycine, a Structurally New Group I Metabotropic Glutamate Receptor Antagonist

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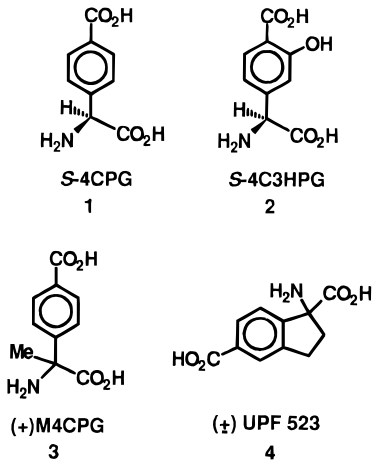
Introduction. Metabotropic glutamate receptors (mGluRs) are involved in important central nervous system (CNS) functions such as long term potentiation (LTP) and long term depression (LTD) of synaptic transmission and have attracted considerable attention because of their therapeutic potential for the treatment of a range of CNS disorders such as stroke, ischemia, head trauma, and Alzheimer's disease.[1]

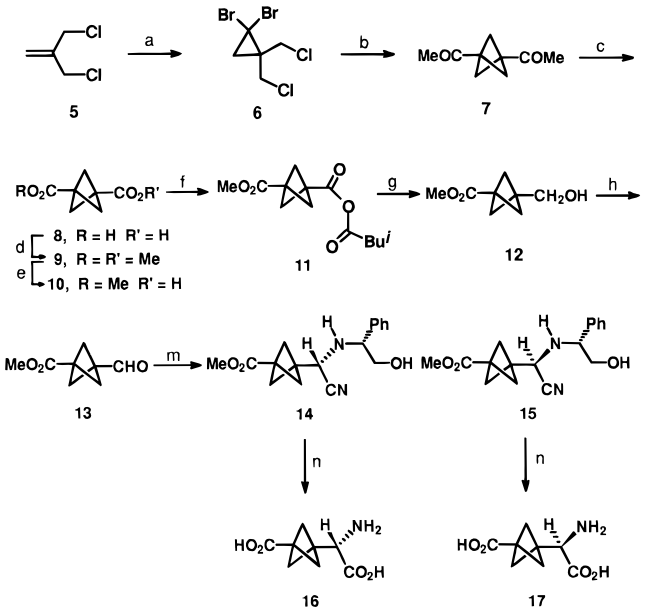
Cloning and expression studies[2] carried out so far have shown that this receptor family is composed of eight mGluR subtypes, further subdivided in three groups according to sequence homology, pharmacology, and signal transduction mechanisms. When expressed in transfected cells, group I mGluRs (mGluR1 and mGluR5) use inositol triphosphate/diacylglycerol as second messengers, while group II (mGluR2 and mGluR3) as well as group III (mGluR4, mGluR6, mGluR7, and mGluR8) are negatively coupled to the activity of adenylyl cyclase.

Among the ligands which have been utilized for the pharmacological and physiological characterization of mGluRs, an important role has been played by (carboxyphenyl)glycine (CPG) derivatives, first reported by Watkins *et al.* in 1993.[3] Among the members of this class, which are known to produce different and often opposite effects on signal transduction processes, (*S*)-(4-carboxyphenyl)glycine [(*S*)-4CPG, **1**], (*S*)-(4-carboxy-3-hydroxyphenyl)glycine [(*S*)-4C3HPG, **2**], and (+)-α-methyl(4-carboxyphenyl)glycine (M4CPG, **3**) have exhibited interesting properties as mGluR1 antagonists albeit with activity also at mGluR2 receptor subtypes.[4] With the aim of finding new more potent and selective mGluR antagonists, we have addressed ourselves to the task of defining relevant structural features of this class of compounds. In this connection, we have previously reported that (±)-1-aminoindan-1,5-dicarboxylic acid (UPF 523, **4**),[5] in which the (carboxyphenyl)glycine core is inserted into a partially rigidified bicyclic structure, is endowed with an increased potency and selectivity as a mGluR1 subtype antagonist compared with (*S*)-4CPG (**1**) and M4CPG (**3**). The pharmacological profile of UPF 523 (**4**) demonstrates that conformational tuning is required in CPGs for achieving selectivity toward particular mGluR subtypes. In the present paper we address the still unexplored role played by the phenyl moiety of CPGs. Thus, while the coplanarity between the α-amino acidic and the *ω*-carboxy functionalities is generally accepted to be a crucial feature of the CPG class of compounds, no reports dealing with the specific role of the aromatic moiety have yet appeared. With the aim of clarifying this point, we now report the synthesis and preliminary biological evaluation of (*S*)-(+)- and (*R*)-(−)-2-(3′-carboxybyciclo[1.1.1]pentyl)-glycine (CBPG). The bicyclo[1.1.1]pentane moiety, which serves as a spacer in these compounds, has a different stereoelectronic profile to the phenyl ring but, analogously to the latter, is able to keep the *ω*-carboxylate and the α-amino acidic moieties in the coplanar disposition crucial for activity.

Chart 1



Scheme 1[a]



[a] (a) NaOH, $CHBr_3$, Me_4NBr, EtOH, CH_2Cl_2, rt; (b) i. MeLi, pentane, −78 °C, ii. biacetyl, *hν*, 0 °C; (c) Br_2, NaOH, 0 °C, then 50 °C; (d) i. $SOCl_2$, reflux, ii. MeOH, refllux; (e) NaOH, MeOH, reflux; (f) $ClCO_2iBu$, 4-Me-morpholine, THF, −10 °C, then rt; (g) $NaBH_4$, rt; (h) PCC, CH_2Cl_2, rt; (m) i. (*R*)-2-phenylglycinol, MeOH, rt, ii. TMSCN, 0 °C, then rt, iii. flash chromatography; (n) i. Pb(OAc)4, CH_2Cl_2−MeOH (1:1, v/v), 0 °C, ii. 6, N HCl, reflux, iii. Dowex 50X2-200, 10% Py.

Chemistry. The preparation of (*S*)-(+)- and (*R*)-(−)-2-(3′-carboxybicyclo[1.1.1]pentyl)glycine (**16** and **17**) involves the key intermediate bicyclo[1.1.1]pentane-1,3-dicarboxylic acid (**8**; Scheme 1), obtained essentially according to the procedure developed by Michl.[6] Accordingly, the bis(dibromocarbene) adduct **6**, obtained

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Table 1. Effects of **16** and **17** on Second-Messenger Formation in BHK Cells Expressing mGluR1a, mGluR2, mGluR4a, or mGluR5a

	(*S*)-(+)-CBPG (**16**)		(*R*)-(−)-CBPG (**17**)	
	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)
mGluR1a	>300	25 ± 6	>300	>300
mGluR2	>300	>300	>300	>300
mGluR4a	>300	>300	>300	>300
mGluR5a	>300[a] (103 ± 33)	>300	>300	>300

[a] **16** stimulated basal PI hydrolysis in BHK cells expressing mGluR5a, but the half-maximal concentration was not reached at a 300 μM concentration. The calculated maximal response to **16** was 54 ± 4% of the maximal response to glutamate, and the half-maximal concentration of **16** for obtaining this effect was 103 ± 33 μM. Measurements of PI hydrolysis (mGluR1a and mGluR5a) or cAMP formation (mGluR2 and mGluR4a) were performed as described in the methods section, and test compounds were applied alone (agonist effect) or prior to glutamate (antagonist effect) (10 μM for mGluR1a, 5 μM for mGluR5a, and 50 μM for mGluR2 and mGluR4a) to cells expressing individual mGluR subtypes. The values are the mean of two to four experiments which were performed in duplicate or triplicate.

from **5**, undergoes ring closure by treatment with methyllithium to give [1.1.1]propellane[6] which is then submitted to reaction with biacetyl, as the acetyl radical source, followed by sodium hypobromite oxidation of the 1,3-diacetylbicyclo[1.1.1]pentane (**7**) thus obtained. Esterification of **8** *via* the acyl chloride afforded the diester **9** which was hydrolyzed to the monoester **10**.[6] This compound was converted to alcohol **12** by sodium borohydride reduction of the mixed anhydride **11**, and PCC oxidation of **12** provided the aldehyde **13** in 16% overall yield from **9**. Aldehyde **13** was then reacted with (*R*)-α-phenylglycinol (MeOH, room temperature, 2 h)[7] followed by treatment of the resulting Schiff base with TMSCN for 10 h to give a 2:1 (^{1}H-NMR) mixture of the two expected α-aminonitrile derivatives **14** and **15** (34%) which were separated by medium pressure chromatography (MPC) in 22% and 12% yields, respectively.

It is known[8] that when employed in diastereoselective Strecker synthesis, the inductive effect of chiral α-phenylglycinols is such that the newly formed chiral center is opposite in sign to that of the α-phenylglycinol employed. Accordingly, an *S* configuration was tentatively assigned to the more abundant product, the N-substituted α-aminonitrile **14**, and *R* configuration to the less abundant isomer, the N-substituted α-aminonitrile **15**. The two α-aminonitriles **14** and **15** were finally submitted to oxidative cleavage with lead tetraacetate,[9] acidic (6 N HCl) hydrolysis, and ion exchange chromatography on Dowex 50 × 2-200 resin to afford respectively (*S*)-(+)-2-(3′-carboxybicyclo[1.1.1]pentyl)-glycine (**16**) and (*R*)-(−)-2-(3′-carboxybicyclo[1.1.1]pentyl)-glycine (**17**) in 76% and 79% yields.[10]

Results And Discussion. The two stereoisomers of 2-(3′-carboxybicyclo[1.1.1.]pentyl)glycine [(*S*)-(+)-CBPG, **16**, and (*R*)-(−)-CBPG, **17**] were evaluated in an assay employing baby hamster kidney (BHK) cells individually expressing mGluR1a, mGluR2, mGluR4, or mGluR5 (Table 1).[4b] At concentrations up to 300 μM, (*R*)-(−)-CBPG (**17**) was inactive at all the mGluR subtypes examined. Conversely, (*S*)-(+)-CBPG (**16**) dose-dependently antagonized glutamate-evoked PI hydrolysis in cells expressing mGluR1a with an IC_{50} = 25 μM, showing an almost complete inhibition at 300 μM. In this assay, 1 mM M4CPG (**3**) completely blocked PI

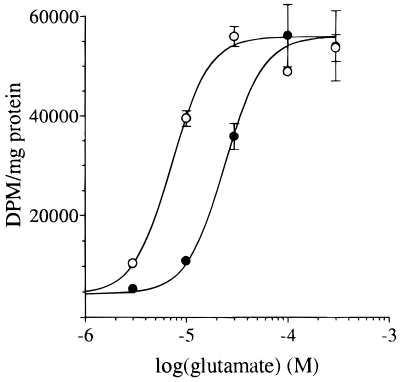

Figure 1. Dose−response curves for stimulation of PI hydrolysis in BHK cells expressing mGluR1a as stimulated by glutamate alone (○) or by glutamate in the presence of 100 μM (*S*)-(+)-CBPG (**16**) (●). Values are mean ± SEM of two individual experiments performed in duplicate (basal PI hydrolysis 4650 ± 350 dpm/mg of protein). In the presence of 100 μM (*S*)-(+)-CBPG (**16**), the levels of PI hydrolysis were 4710 ± 460 dpm/mg of protein.

hydrolysis in mGluR1a cells as stimulated by 10 μM glutamate (to 103 ± 2% of the basal levels; see Figure 1). Furthermore, the dose−response curve for glutamate was shifted to the right by 100 μM (*S*)-(+)-CBPG (**16**) in a parallel manner, suggesting that **16** is a competitive antagonist of mGluR1a. Inactive in concentrations up to 300 μM at mGluR2 and mGluR4, **16** was a weak partial agonist at mGluR5, showing a maximal effect which was about one-half of that obtained with a concentration of glutamate (30 μM) which was optimal for stimulating PI hydrolysis in these cells. The present results indicate that this compound is a new potent mGluR1 antagonist with mixed activity as a mGluR5 partial agonist. (*S*)-(+)-CBPG (**16**) was selective for mGluRs over ionotropic glutamate receptors since (*S*)-(+)-CBPG (**16**) did not displace α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainate (KA), or [3-(2-carboxypiperazin-4-yl)prop-1-ylphosphonic acid (CPP, a competitive NMDA antagonist) binding from rat cerebral cortical membranes at concentrations up to 300 μM (data not shown).

(Carboxyphenyl)glycine derivatives such as (*S*)-4CPG (**1**) or (*S*)-4C3HPG (**2**) also show a mixed activity as mGluR1 antagonists and mGluR2 agonists, but it should be pointed out that (*S*)-(+)-CBPG (**16**) is the first example of a mixed antagonist/partial agonist at members of the same group (group I) of mGluRs. Since the first reports by Watkins *et al.*,[3] (carboxyphenyl)glycines have been widely used as lead structures on route toward more potent and selective mGluR antagonists. Structural elaborations on the (carboxyphenyl)glycine skeleton have included α-alkylation of the amino acidic moiety,[11] homologation and bioisosteric replacement of the distal carboxy moiety,[4d] and conformational constraining of the α-amino acidic side chain.[5a] The interesting pharmacological properties displayed by **16** broaden the existing structure−activity relationship. In particular, two interesting features emerge from our results. First, the presence of an aromatic moiety is not a requirement for mGluR1 antagonism, providing that a suitable spacer is able to dispose the pharmacophoric groups in a well-defined, coplanar orientation. This implies that the phenyl moiety of (carboxyphenyl)glycine derivatives neither is involved in specific ligand−receptor interactions nor plays a role in dispersing the

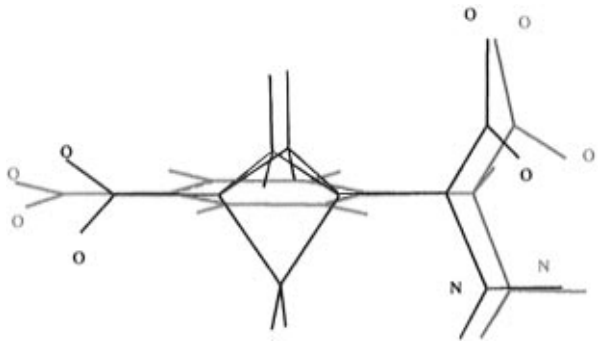

Figure 2. Superimposition of (*S*)-(+)-CBPG (**16**) (green) and (*S*)-4CPG (**1**) (red). The α-amino acidic and the distal carboxylate moieties of **16** retain the coplanar disposition of **1**, but the relative distance is shorter by about 0.8 Å.

charges of the α-amino acidic moiety and the distalcarboxylate group across the whole structure. Second, previous structure−activity relationship studies have determined the distance between the α-amino acidic moiety and the distal carboxy group of α-methyl(4-carboxyphenyl)glycine to be optimal for mGluR1 antagonism.[4d] In this regard, **16** is endowed with a distance between the α-amino acidic group and the distal carboxylate shorter than that of α-methyl(4-carboxyphenyl)glycine (of about 0.8 Å; Figure 2) but still retains potency as a mGluR1 antagonist in the same order of magnitude as classical (carboxyphenyl)glycines, thus indicating that a certain degree of tolerance is allowed for receptor recognition. These observations can certainly be of help in designing new, structurally diverse mGluR1 antagonists with increased potency and selectivity.

In summary, we have reported that (*S*)-(+)-CBPG (**16**) is a structurally novel mGluR1 antagonist endowed with good potency and selectivity toward the mGluR1 receptor subtype with no effect on group II and III mGluRs.

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Supporting Information Available: Experimental procedures (5 pages). Ordering information is given on any current masthead page.

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(10) Selected spectroscopic data for compounds **16** and **17** are as follows. **16**: mp 260 °C dec; ^{1}H-NMR (D_2O) δ 2.00 (6H, s, 3 × CH_2), 3.75 (1H, s, CH); ^{13}C-NMR (D_2O) δ 35.75, 47.47, 49.00, 53.22, 169.82, 173.09; $[\alpha]^{20}_D = +7.8°$ (*c* 1, H_2O). **17**: mp 260 °C dec; $[\alpha]^{20}_D = -12.1°$ (*c* 1, H_2O).

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