(2*S*,4*S*)-2-Amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic Acid: A Potent and Selective Antagonist for Metabotropic Glutamate Receptors Negatively Linked to Adenylate Cyclase

Camille G. Wermuth,[†] André Mann,[†] Angèle Schoenfelder,[†] Rebecca A. Wright,[‡] Bryan G. Johnson,[‡] J. Paul Burnett,[‡] Nancy G. Mayne,[‡] and Darryle D. Schoepp*,[‡]

UPR 421, Centre de Neurochimie du CNRS, Strasbourg, France, and Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285

Received November 2, 1995

Metabotropic glutamate receptors (mGluRs) are a heterogeneous and novel family of G-protein linked receptors that couple to multiple second messengers, including inhibition of adenylate cyclase, activation of phosphoinositide-specific phospholipase C, and modulation of ion channels currents.[1−4] Subtypes of mGluRs have been cloned and fall into three groups. Group 1 mGluRs couple to phosphoinositide hydrolysis when expressed and include mGluR1 (α, *β*, and c splice variants) and mGluR5 (a and b splice variants). Group 2 mGluRs are coupled to inhibition of cyclic adenosine 5′-monophosphate (cAMP) formation and include mGluR2 and mGluR3. Both group 1 and group 2 mGluRs can be selectively activated by compound (1*S*,3*R*)-aminocyclopentane-1,3-dicarboxylic acid (ACPD). Group 3 mGluRs (mGluR4, mGluR6, mGluR7, and mGluR8) also couple negatively to cAMP. But unlike the others, group 3 mGluRs are insensitive to ACPD and potently activated by L-2-amino-4-phosphonobutanoic acid (L-AP4).

Studies of metabotropic glutamate receptor function are greatly hampered by the lack of potent and selective antagonists. The first antagonist described for mGluRs was L-2-amino-3-phosphonopropanoic acid (L-AP3). L-AP3 will block ACPD-stimulated phosphoinositide hydrolysis in brain slices[5] and cells expressing group 1 mGluRs,[6] but requires high micromolar to millimolar concentrations. A series of phenylglycine derivatives which act as competitive agonists/antagonists have more recently been described.[7] The most accepted mGluR antagonist of this series appears to be (+)-α-methyl-4-carboxyphenylglycine (MCPG). However, the usefulness of MCPG is limited by its low potency (high micro- to millimolar concentrations are required) and lack of selectivity for group 1 versus group 2 mGluRs.[8]

In this paper we provide preliminary studies on a structurally novel and highly stereoselective antagonist for mGluRs which binds selectively to mGluRs at low micromolar concentrations and potently antagonized a group 2 mGluR (human mGluR2), but with no effects on group 1 mGluRs (human mGluR1α and mGluR5a).

Chemistry. As shown in Scheme 1, the synthesis of compound **1** began with the known aldehyde **4**, which was obtained from (*R*)-serine.[9] The Wittig−Horner reaction of **4** and triethyl phosphonoacetate under protic conditions gave a 95:5 mixture of the corresponding *E* and *Z* enoates **5**, which were reduced via catalytic hydrogenation to ester **6**. After some experimentation it was found that alkylation next to the carboxylate with 4,4-diphenyl-1-bromobutane[10] occurred smoothly in THF/HMPT, with 2 equiv of LDA, and gave rise to **7** as an inseparable mixture of diastereomers. To get the fully protected glutamate **9**, the restoration of the amino acid function was performed as follows:[11] (i) the oxazolidine ring was opened in refluxing ethanol in the presence of

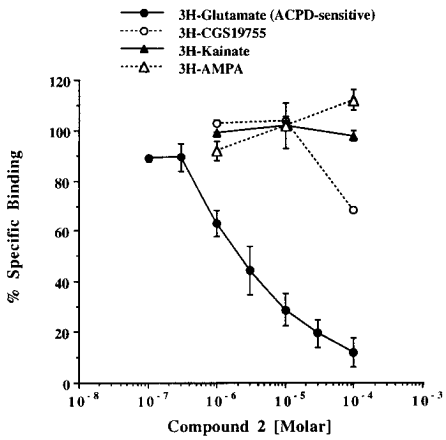


Figure 1. Displacement of ligand binding to glutamate receptors in membranes of the rat forebrain by compound **2**. Data represent mean ± SE of three experiments performed in triplicate.

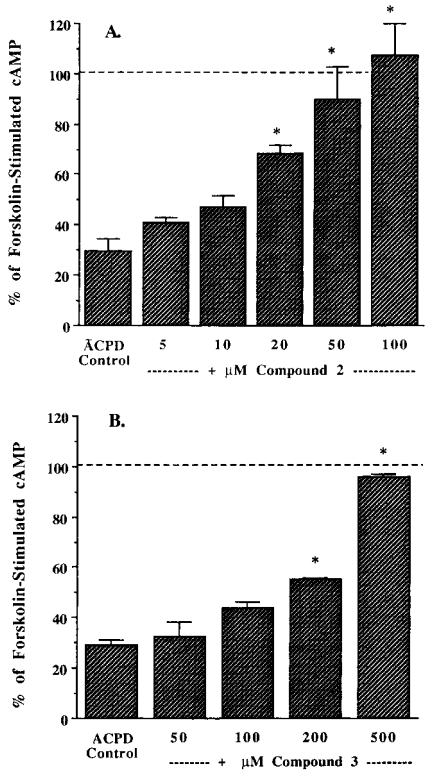


Figure 2. Concentration-dependent reversal by compounds **2** (panel A) and **3** (panel B) of ACPD (20 μM) inhibition of forskolin (15 μM)-stimulated cAMP formation in human mGluR2 expressing non-neuronal cells. Data are mean ± SE of three experiments performed in triplicate. An asterisk (*) indicates significantly different when compared to the ACPD control ($p < 0.05$, Duncan's Procedure).

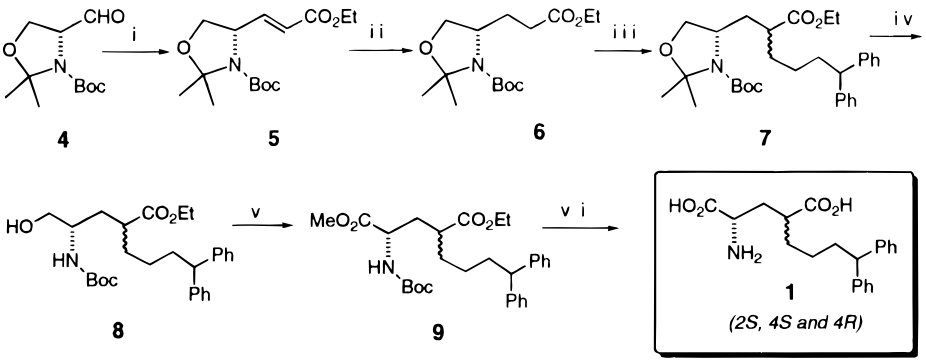
* Address correspondence to: CNS Research, drop 0510, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285. Phone (317) 276-6316. FAX (317) 276-5546. E-mail address: SCHOEPP_DARRYLE_D@Lilly.Com.

[†] Centre de Neurochimie du CNRS.

[‡] Lilly Research Laboratories.

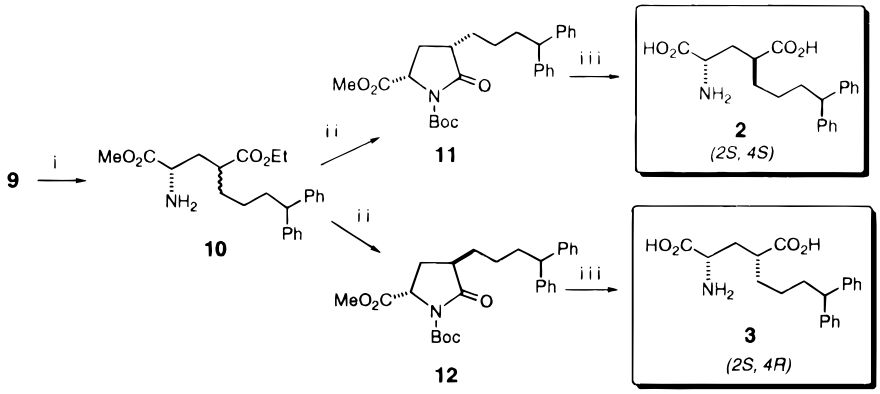
0022-2623/96/1839-0814$12.00/0

Scheme 1[a]



[a] Reagents, conditions, and yields: (i) $(EtO)_2POCH_2CO_2Et$, aqueous K_2CO_3 (3M), *n*-Bu4N$^+$I$^-$, 12 h (88%); (ii) H_2, Pd/C (100%); (iii) LDA, THF/HMPT, 10/1, −78 °C, then $Br(CH_2)_3CHPh_2$ (91%); (iv) *p*-PTOH, EtOH (63%); (v) pyridinium dichromate, DMF, 12 h, then CH_2N_2 in Et_2O (65% for two steps); (vi) LiOH in dimethoxyethane, then HCl(g) in AcOEt, 12 h (56%).

Scheme 2[a]



[a] Reagents, conditions, and yields: (i) TFA, then K_2CO_3, then toluene, 110 °C; then Boc_2O, TEA, DMAP, CH_2Cl_2 (80%); (ii) Boc_2O, TEA, DMAP, CH_2Cl_2 (80%), then separation by column chromatography of the mixture of **11** + **12** (40% for **11**, 35% for **12**); (iii) LiOH in dimethoxyethane, then AcOH in HCl (86% for **2**, 75% for **3**).

pPTSA to afford alcohol **8**; (ii) the primary alcohol was oxidized to the carboxylic acid using PDC in DMF; and (iii) diazomethane esterification was used to produce the methyl ester **9**. Finally, compound **1** was obtained as a mixture of epimers at carbon C-4 after hydrolysis.

In order to obtain the two enantiomerically pure compounds **2** and **3** the following sequence was developed (Scheme 2). The Boc protection of compound **9** was removed by a brief treatment with TFA. The corresponding base **10** was lactamized in refluxing toluene. At this stage the two epimeric lactams could not be separated by column chromatography. The nitrogen was Boc-protected, and only then a separable mixture of **11** and **12** was obtained. The assignment of their relative stereochemistry, respectively *cis* for **11** and *trans* for **12**, was performed by NOE experiments and correlation with literature data.[12,13] Finally, removal of the N- and O-protecting groups, under successively basic and acidic conditions, gave the fully deprotected γ-glutamates **2** (2*S*,4*S*) and **3** (2*S*,4*R*) with diastereomer excesses greater than 95% as determined by HPLC.[14]

Results. Amino acid **1**, as a mixture of 2*S*,4*S* and 2*S*,4*R* diastereomers, was initially evaluated for its ability to displace metabotropic glutamate receptor binding to rat brain membranes (as measured by displacement of ACPD-sensitive [^{3}H]glutamate binding).[15] **1** was relatively potent in displacing this binding with an $IC_{50} = 4.2 \pm 1.2$ μM ($n = 3$). The metabotropic glutamate receptor binding affinity of **1** (Figure 1) resided in the 2*S*,4*S* isomer (**2**, $IC_{50} = 4.1 \pm 1.7$ μM), with the 2*S*,4*R* isomer (**3**) being much less potent ($IC_{50} = 55.3 \pm 10.6$ μM, $n = 3$). **2** was also evaluated for affinity at ionotropic glutamate receptors (NMDA, AMPA, and kainate) using radioligand binding assays. No appreciable displacement of [^{3}H]AMPA,[16] [^{3}H]kainate,[17] or [^{3}H]CGS 19755 (NMDA receptor ligand)[18] binding was observed at concentrations which displaced ACPD-sensitive [^{3}H]glutamate binding (IC_{50}s >100 μM) (Figure 1). **2** and **3** were each evaluated in a functional assay of a group 2 mGluR. Both compounds produced concentration-dependent reversal of ACPD-induced inhibition of forskolin-stimulated cAMP formation in human mGluR2 expressing non-neuronal cells.[19]. The 2*S*,4*S* isomer (**2**) fully reversed ACPD at a concentration of 50 μM with an $IC_{50} = 18.1 \pm 1.7$ μM (Figure 2). The 2*S*,4*R* isomer (**3**) was much less potent ($IC_{50} = 171 \pm 9$ μM), and 500 μM was needed to fully reverse ACPD. Compound **2** was further examined for activity at phosphoinositide coupled mGluRs using cells expressing human mGluR1α and human mGluR5a receptors.[20,21] When tested in either cell line, compound **2** (tested at 300 μM) did not stimulate phosphoinositide hydrolysis or significantly antagonize stimulation by ACPD (100 μM) (Figure 3).

Discussion. The initial activity of **1** was discovered by the observation that this compound displaced [^{3}H]-glutamate binding to membranes of the rat forebrain. This assay was performed under conditions where [^{3}H]-glutamate binding is ACPD-sensitive, but insensitive to the mGluR1 and -5 (group 1) agonist quisqualate or

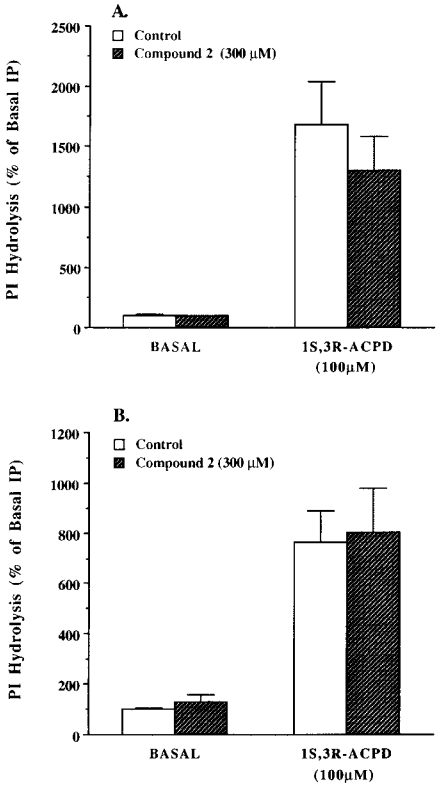


Figure 3. Lack of effect of compound **2** (300 μM) on basal and ACPD (100 μM)-stimulated phosphoinositide hydrolysis in human mGluR1α (panel A) and human mGluR5a (panel B) expressing non-neuronal cells. Data were expressed as a percentage of basal [^{3}H]inositol monophosphates (IP) in each experiment. Values are mean $\pm$ SE of three experiments performed in triplicate.

the mGluR4, -6, -7, and -8 (group 3) agonist L-AP4.[15] This mGluR affinity of **1** resided in the 2*S*,4*S* isomer (**2**), and further ligand binding studies showed that **2** has no appreciable affinity for ion-channel-linked glutamate receptors. Concentrations of **2** which displaced mGluR binding also were demonstrated to antagonize activation of a group 2 mGluR (human mGluR2) expressed in non-neuronal cells. As in mGluR binding, the 2*S*,4*R* isomer (**3**) was much less potent. Although **2** potently antagonized mGluR2 receptors, it had no agonist or antagonist activities at group 1 phosphoinositide-coupled mGluRs. These data with a structurally novel antagonist provide further evidence that ACPD-sensitive [^{3}H]glutamate binding represents binding to group 2 mGluRs (including mGluR2) and represents a way of predicting the relative affinity of compounds for group 2 mGluRs.

Compound **2** has a novel mGluR antagonist profile when compared to other known antagonists. This includes no ionotropic glutamate receptor affinity, but selective antagonism of negatively-coupled cAMP-linked mGluRs without effects on phosphoinositide coupled mGluRs. This makes **2** a useful new pharmacological tool for investigating mGluRs. Additional studies are in progress to characterize the activity of **2** at other cloned mGluRs coupled to cAMP (i.e., group 3) and in other functional assays for mGluRs.

Supporting Information Available: Experimental details for the compounds in this paper (6 pages). Ordering information is given on any current masthead page.

References

(1) Nakanishi, S. Molecular diversity of glutamate receptors and implications for brain function. *Science* **1992**, *258*, 597−603.
(2) Schoepp, D. D.; Conn, P. J. Metabotropic glutamate receptors in brain function and pathology. *Trends Pharmacol. Sci.* **1993**, *14*, 13−20.
(3) Knopfel, T.; Kuhn, R.; Allgeier, H. Metabotropic glutamate receptors: novel targets for drug development. *J. Med. Chem.* **1995**, *38*, 1417−1426.
(4) Pin, J.-P.; Duvoisin, R. The metabotropic glutamate receptors: Structure and functions. *Neuropharmacology* **1995**, *34*, 1−26.
(5) Schoepp, D. D.; Johnson, B. G.; Smith, E. C. R.; McQuaid, L. A. Stereoselectivity and mode of inhibition of phosphoinositide coupled excitatory amino acid receptors by 2-amino-3-phosphonopropionic acid. *Mol. Pharmacol.* **1990**, *38*, 222−228.
(6) Saugstad, J. A.; Segerson, T. P.; Westbrook, G. L. L-2-amino-3-phosphonopropionic acid competitively antagonizes metabotropic glutamate receptors 1a and 5 in *Xenopus oocytes*. *Eur. J. Pharmacol.—Mol. Pharmacol. Sect.* **1995**, *289*, 395−397.
(7) Watkins, J.; Collingridge, G. Phenylglycine derivatives as antagonists of metabotropic glutamate receptors. *Trends Pharmacol. Sci.* **1994**, *15*, 333−342.
(8) Thomsen, C.; Boel, E.; Suzdek, P. D. Actions of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family. *Eur. J. Pharmacol.—Mol. Pharmacol. Sect.* **1994**, *267*, 77−84.
(9) Garner, P.; Park, J. M. The synthesis and configurational stability of differentially protected β-hydroxy-α-amino aldehydes. *J. Org. Chem.* **1988**, *52*, 2361−2364.
(10) N'Goka, V.; Schlewer, G.; Linget, J. M.; Chambon, J. P.; Wermuth, C. G. GABA-uptake inhibitors: Construction of a general pharmacophore model and successful prediction of a new representative. *J. Med. Chem.* **1991**, *34*, 2547−2557.
(11) Jako, I.; Uiber, P.; Mann, A.; Wermuth, C. G.; Boulanger, Th.; Norberg, B.; Evrard, G.; Durant, F. Stereoselective synthesis of 3-alkylated glutamic acids: Application to the synthesis of secokainic acid. *J. Org. Chem.* **1991**, *56*, 5729−5733.
(12) Dikshit, D. K.; Panay, S. K. Aldol reactions of pyroglutamates: Chiral synthesis of 4α(S)- and 4β-(arylmethyl)pyroglutamates. *J. Org. Chem.* **1992**, *57*, 1920−1924.
(13) Ezquerra, J.; Pedregal, C.; Yruretagoyena, R.; Rubio, A.; Carreno, M. C.; Escribano, A.; Garcia Ruano, J. L. Synthesis of enantiomerically pure 4-substituted glutamic acids and prolines: General aldol reaction of pyroglutamate lactam lithium enolate mediated by $Et_2O{\cdot}BF_3$. *J. Org. Chem.* **1995**, *60*, 2925−2930.
(14) Donzanti, B. A.; Yamamoto, B. K. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. *Life Sci.* **1988**, *43*, 913−922.
(15) Wright, R. A.; McDonald, J. W.; Schoepp, D. D. Distribution and ontogeny of 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid-sensitive and quisqualate-insensitive [^{3}H]glutamate binding sites in the rat brain. *J. Neurochem.* **1994**, *63*, 938−945.
(16) Nielsen, E. O.; Madsen, U.; Schaumburg, K.; Brehm, L.; Krogsgaard-Larsen, P. Studies on receptor-active conformations of excitatory amino acid agonists and antagonists. *Eur. J. Med. Chem.—Chim. Ther.* **1986**, *21*, 433−437.
(17) Simon, J. R.; Contrera, J. F.; Kuhar, M. J. Binding of [^{3}H]kainic acid, an analogue of L-glutamate, to brain membranes. *J. Neurochem.* **1976**, *26*, 141−147.
(18) Murphy, D. E.; Hutchison, A. J.; Hurt, S. D.; Williams, M.; Sills, M. A. Characterization of the binding of [^{3}H]-CGS 19755: a novel N-methyl-D-aspartate antagonist with nanomolar affinity in rat brain. *Br. J. Pharmacol.* **1988**, *95*, 932−938.
(19) Schoepp, D. D.; Johnson, B. G.; Salhoff, C. R.; Valli, M. J.; Desai, M. A.; Monn, J. A. Selective inhibition of forskolin-stimulated cyclic AMP formation in rat hippocampus by a novel mGluR agonist, 2*R*,4*R*-4-aminopyrrolidine-2,4-dicarboxylate (2,*R*-4*R*-APDC). *Neuropharmacology* **1995**, *34*, 843−850.
(20) Kingston, A. E.; Burnett, J. P.; Mayne, N. G.; Lodge, D. Pharmacological analysis of 4-carboxyphenylglycine derivatives: Comparison of effects on mGluR1a and mGluR5a subtypes. *Neuropharmacology* **1995**, *34*, 887−894.
(21) Desai, M. A.; Burnett, J. P.; Mayne, N. G.; Schoepp, D. D. Cloning and expression of a human mGluR1α: Enhanced coupling upon co-transfection with a glutamate transporter. *Mol. Pharmacol.* **1995**, *48*, 648−657.

JM9508144