Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1241–1244

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

(+)-4-Phosphonophenylglycine (PPG) A New Group III Selective Metabotropic Glutamate Receptor Agonist

Fabrizio Gasparini,[a,*] Werner Inderbitzin,[a] Eric Francotte,[b] Gabrielle Lecis,[b] Paul Richert,[b] Zorica Dragic,[a] Rainer Kuhn[a] and Peter J. Flor[a]

[a]*Nervous System Research, Novartis Pharma AG, 4002 Basel, Switzerland*
[b]*Core Technologies, Novartis Pharma AG, 4002 Basel, Switzerland*

Received 28 December 1999; accepted 29 March 2000

Abstract—A new synthesis of (*R*,*S*)-PPG (4-phosphonophenylglycine) and the separation of the protected enantiomers leading after deprotection to (+)- and (−)-PPG are described. Pharmacological characterization at the group III metabotropic glutamate receptors hmGluR4a and hmGluR7b revealed (+)-PPG as the active enantiomer.

L-Glutamate is the major excitatory neurotransmitter in the mammalian brain and plays a pivotal role in numerous processes in the nervous system as well as in pathophysiological disorders such as neurodegenerative processes and epilepsies. The action of glutamate is mediated through a heterogeneous family of two major types of receptors: ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs). Ionotropic glutamate receptors are ligand gated channel whereas metabotropic receptors are G-protein coupled receptors linked to second messenger pathways. To date eight metabotropic receptor subtypes have been identified and classified into three groups according to their amino acid identities, second messenger coupling and pharmacology.[1] Group I mGluRs (1 and 5) are coupled to the phosphoinositide/Ca^{2+} cascade. Group II mGluRs (2 and 3) and group III mGluRs (4, 6, 7 and 8) are negatively coupled to adenylate cyclase.

L-AP4 (**1**) and L-SOP (**2**) are the prototypic agonists for group III mGluRs[2] (Fig. 1). Up to now, the only known selective agonists for group III mGluRs are cyclic analogues of L-AP4 or L-AP5 described by Johansen et al.,[3] Z-cyclopropyl-AP4 (**3**) ($EC_{50}=0.5$ μM, rmGluR4) and the cyclobutylene-AP-5 derivative (**4**) ($EC_{50}=4.5$ μM, rmGluR4).

As a part of our effort aimed at the discovery of novel agents acting at group III mGluRs, we directed our attention to a series of phosphono-substituted amino acids containing a phenyl spacer between the amino acid moiety and the phosphonic acid. This series of compounds were synthesized and characterized by Bigge et al.[4] in a program aiming to identify new NMDA receptor competitive antagonists. By varying the distance between the phosphono and the amino acid function it was demonstrated that the most active compound of the series was (**5**) with a K_D of 3 μM at NMDA whereas (*R*,*S*)-PPG (**6**) ((±)-4-phosphonophenylglycine) which has the shortest possible distance between the two moieties has no affinity for the NMDA receptor using [^{3}H]-CPP binding up to 100 μM.

The structural analogy of (*R*,*S*)-PPG with known mGluR ligands like the 4-carboxyphenylglycine[5] (**7**) or even closer phosphono subtituted phenyl glycines like MPPG (**8**) (α-methyl-4-phosphonophenyl glycine)[6] or CPPG (**9**) (α-cyclopropylphosphophonophenyl glycine)[7] (Fig. 2) prompted us to synthesize and to pharmacologically characterize it. To achieve that goal we developed a new synthesis leading to (*R*,*S*)-PPG and a method for the enantiomeric separation of it.

Chemistry

The synthesis of (*R*,*S*)-PPG starts with 4-hydroxy-benzaldehyde (**10**) as described in Scheme 1. The starting material was first esterified with trifluoromethanesulfonic anhydride. The triflate (**11**) was then converted to the corresponding phosphonate (**12**) using a palladium

*Corresponding author. Fax: +41-61-696-8676;
e-mail: fabrizio.gasparini@pharma.novartis.com

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00197-9

catalyzed coupling reaction.[8] Conversion of the aldehyde to the amino nitrile, acetylation of the amine and subsequent acid hydrolysis performed in concentrated HCl gave (*R,S*)-PPG (**6**) with an overall yield of about 45%. Although, two steps longer than the published synthesis by Bigge et al.,[4] the overall yield is significantly higher 45% compared to only 0.6%.

The preparation of enantiomeric pure non-natural amino acids can be achieved using a variety of methods. Most frequently used are the fractional crystallization of diastereoisomeric mixtures, the enantioselective synthesis and the chromatographic separation using a chiral support.[9,10] Our tentatives of fractional crystallization did not allow us to access the enantiomers of (*R,S*)-PPG.

We first succeeded in separating the enantiomers of (*R,S*)-PPG in a analytical scale using a Chrompak CR + column. However, a scale-up to a preparative scale could not be achieved using this type of column. Therefore, we decided to use a different type of stationary phase, which necessitates the conversion of the amino acid and phosphonate functionalities to protected less hydrophilic groups allowing an elution with organic solvents. The protection of (*R,S*)-PPG was achieved in three steps: (1) protecting the amine with benzyl chloroformate; (2) converting the phosphonic acid to a dimethyl phosphonate; (3) esterification of the carboxylic acid to a benzyl ester (Scheme 2).

This fully protected (*R,S*)-PPG ((±)-**16**) derivative could be resolved on a gram scale on a Chiralcel OJ column.[11] The two enantiomers were obtained in very high enantiomeric purity (>99.9%, HPLC).[12] The deprotection sequence was done in two steps: (1) hydrolysis of the phosphonate ester using trimethylsilyliodide (2)

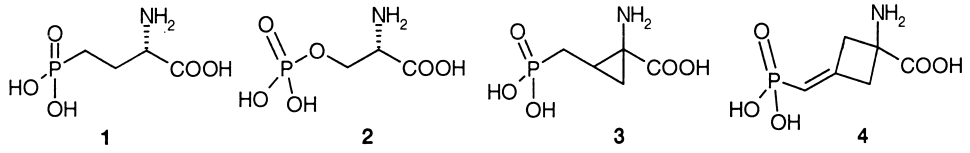


Figure 1. Structures of group III mGluRs agonists.

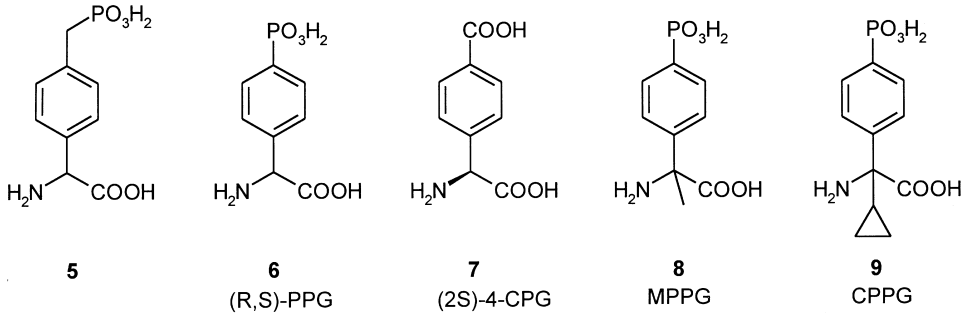
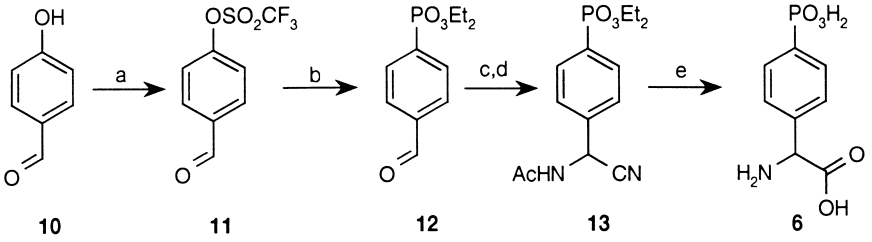
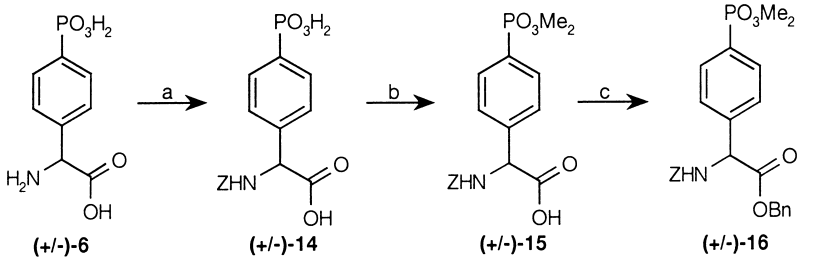


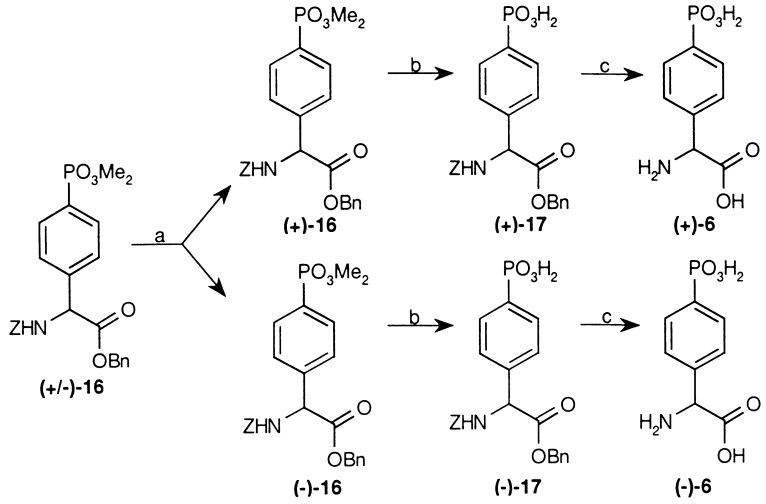
Figure 2. Structures of phenyl glycines derivatives.



Scheme 1. Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, 90%; (b) $Pd(OAc)_2$, PO_3Et_2, 85%; (c) Me_3SiCN, NH_3, ZnI_2; (d) Ac_2O, pyridine, 70%; (e) HCl 20%, 100 °C, 3 h, 85%.



Scheme 2. Reagents and conditions: (a) BnOCOCl, NaOH, Et_2O/H_2O, 53%; (b) $CH(OMe)_3$, TsOH, 57%; (c) BnCl, Na_2CO_3, DMF, 71%.



Scheme 3. Reagents and conditions: (a) Separation on Chiralcel OJ;[10] (b) TMSI, CH_2Cl_2, −20 °C, 60%; (c) PdO, H_2, 1 bar, 90%.

hydrogenolysis using a Pd catalysed hydrogenation (Scheme 3). Using these mild deprotection conditions the two enantiomers (+)-**6** and (−)-**6** were isolated with an enantiomeric purity of 99.0 and 99.2% (HPLC),[13] respectively.

Results and Discussion

We have previously shown that (*R,S*)-PPG is a selective group III mGluRs agonist with no appreciable activity, up to 300 μM, at hmGluR1, −2, −3, and −5 and at the cloned ionotropic receptor subtypes NMDA1A/2A,-2B, GluR3, GluR6.[14] To determine the functional activity of (+)-PPG and (−)-PPG the enantiomers were tested on the human mGluR4a stably expressed in CHO cells using a cAMP radioimmunoassay. The (+)-enantiomer was found to have an EC_{50} of 3.2 μM, whereas the (−)-enantiomer was found inactive up to 2000 μM (Fig. 3). This very high stereoselective recognition of the glutamate binding site for the metabotropic glutamate receptors has been previously demonstrated for mGluR2 and mGluR3 receptors with the group II agonist LY354740[15] and for the mGluR4 receptor with L-AP4.[3]

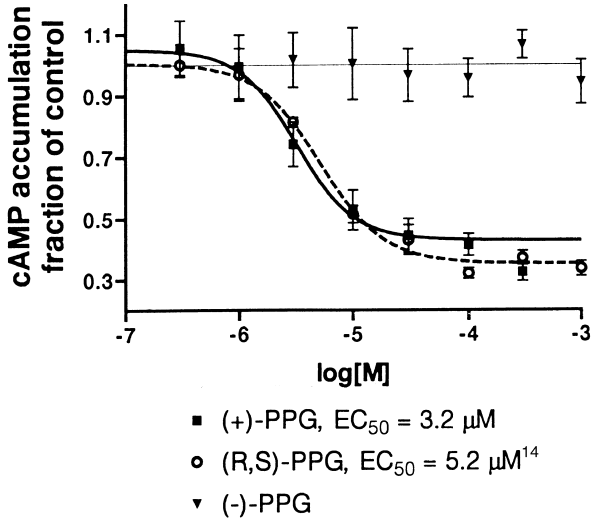


Figure 3. Concentration response curves of (*R,S*)-PPG (open circles), (+)-PPG (black squares) and (−)-PPG (black triangles). Inhibition of forskolin-stimulated cAMP accumulation in CHO cells expressing hmGluR4a. Forskolin (10 μM) stimulated cAMP formation by about 40-fold (taken as control). All values are given as fraction of control.

In addition, using the same cAMP assay both enantiomers were tested on the human mGluR7b and in analogy to the mGluR4 subtype, (+)-PPG was found active with an EC_{50} of 48 μM ((*R,S*)-PPG, EC_{50} = 185 μM[14]), whereas (−)-PPG was devoid of activity up to 500 μM (data not shown).

In conclusion, we have reported the synthesis of the (+)- and (−)-enantiomers of PPG with high enantiomeric purity using chromatographic separation. The pharmacological characterization at the hmGlu4 receptor demonstrates that (+)-PPG is the active isomer with a very high stereoselective recognition.

Acknowledgements

The authors would like to thank T. Leonhardt and S. Lukic for excellent technical assistance.

References and Notes

1. Conn, P. J.; Pin, J. P. *Annu. Rev. Pharmacol. Toxicol.* **1997**, *37*, 205.
2. Thomsen, C.; Kristensen, P.; Mulvihill, E.; Haldeman, B.; Suzdak, P. D. *Eur. J. Pharmacol.* **1992**, *227*, 361.
3. Johansen, P. A.; Chase, L. A.; Sinor, A. D.; Koerner, J. F.; Johnson, R. L.; Robinson, M. B. *Mol. Pharmacol.* **1995**, *48*, 140.
4. Bigge, C. F.; Drummond, J. T.; Johnson, G.; Malone, T.; Probert, A. W.; Marcoux, F. W.; Coughenour, L. L.; Brahce, L. J. *J. Med. Chem.* **1989**, *32*, 1580 (published erratum appears in *J. Med. Chem.* **1989**, *32*, 2583).
5. Bedingfield, J. S.; Kemp, M. C.; Jane, D. E.; Tse, H.-W.; Roberts, P. J.; Watkins, J. C. *Br. J. Pharmacol.* **1995**, *116*, 3323.

6. Jane, D. E.; Pittaway, K.; Sunter, D. C.; Thomas, N. K.; Watkins, J. C. *Neuropharmacology* **1995**, *34*, 851.
7. Tom, N. J.; Jane, D. E.; Kemp, M. C.; Bedingfield, J. S.; Roberts, P. J. *Br. J. Pharmacol.* **1996**, *119*, 851.
8. Thurieau, C.; Simonet, S.; Paladino, J.; Prost, J. F.; Verbeuren, T.; Fauchère, J. L. *J. Med. Chem.* **1994**, *37*, 625.
9. (a) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon Press: Oxford, 1989. (b) Duthaler, R. O. *Tetrahedron*, **1994**, *50*, 1539.
10. Francotte E. In *The Impact of Stereochemistry on Drug Development and Use*; H. Y. Aboul-Enein and I. W. Wainer (Eds.); Chemical Analysis Series Vol. 142; John Wiley & Sons; New York, 1997; Chapter 23.
11. The preparative HPLC was performed with a Shimadzu modular liquid chromatograph (Burckard Instrumente, Zürich, Switzerland) composed of an LC-8A Pump, a multi-wavelength UV–vis detector model SPD-10A. The UV signal (210 nm) was recorded and processed by an Epson Microcomputer, using the Class LC-10 chromatographic software (Shimadzu, Burckard Instrumente, Switzerland). 6.9 g of racemate were dissolved in 50 mL of hexane:2-propanol 40:60 (v/v) and injected via the pump on a 10 cm (i.d.) by 50 cm Chiralcel OJ column (Daicel Chemical Industries, Japan). The flow-rate was 150 mL/min. The mobile phase consisted of a mixture of hexane:2-propanol 40:60 (v/v). Under the applied chromatographic conditons, the (+) enantiomer was isolated from a first fraction collected between 75 and 110 min, and the (−) enantiomer from a second fraction collected between 125 and 200 min. Evaporation of the collected fraction allowed the isolation of 3.14 g of (+) enantiomer and 3.4 g of (−) enantiomer, both with an enantiomeric purity greater than 99.9%.
12. The enantiomeric purity was determined on an analytical Chiralcel OJ column (0.46×25 cm); mobile phase, hexane:2-propanol 50:50 (v/v); separation factor, 1.90.
13. The enantiomeric purity was determined on an analytical Chrompak CR+ column, mobile phase, 0.48% perchloric acid in water, separation factor 3.8.
14. Gasparini, F.; Bruno, V.; Battaglia, G.; Lukic, S.; Leonhardt, T.; Inderbitzin, W.; Laurie, D.; Sommer, B.; Varney, M. A.; Hess, S. D.; Johnson, E. C.; Kuhn, R.; Urwyler, S.; Sauer, D.; Portet, C.; Schmutz, M.; Nicoletti, F.; Flor, P. J. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 1678.
15. 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. *J. Med. Chem.* **1997**, *40*, 528.