

## SYNTHESIS AND BIOLOGICAL ACTIVITY OF GLUTAMIC ACID DERIVATIVES

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**Abstract:** In order to develop new specific glutamate analogues at metabotropic glutamate receptors, Diels-Alder, 1-4 ionic and radical reactions were performed starting from (2*S*)-4-methyleneglutamic acid. Preliminary pharmacological evaluation by measuring IP accumulation using rat forebrain synaptoneurosomes has shown that (2*S*)-4-(2-phthalimidoethyl)glutamic acid (**3a**), (2*S*)-4-(4-phthalimidobutyl)glutamic acid (**3b**) and 1-[(*S*)-2-amino-2-carboxyethyl]-3,4-dimethylcyclohex-3-ene-1-carboxylic acid (**8**) presented moderate antagonist activities. © 1998 Elsevier Science Ltd. All rights reserved.

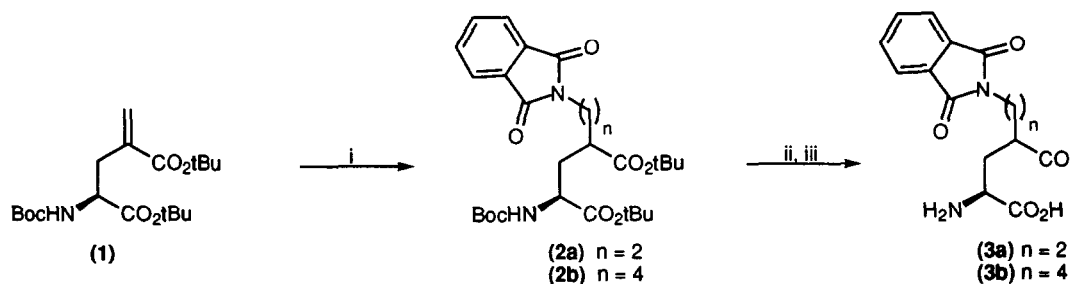
Glutamate (Glu), the main central nervous system excitatory neurotransmitter, acts on two classes of receptors. The first class includes glutamate ion channel-receptors, which are subdivided into three subtypes according to selective agonists: NMDA (N-methyl-D-aspartate), AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionate) and KA (kainate) receptors<sup>1,2</sup>. Glu also activates metabotropic receptors (mGluRs) linked, via a G-protein, to phospholipase C (PLC)<sup>3,5</sup>, or adenylate cyclase<sup>2,6</sup>. Molecular cloning<sup>7,9</sup> has revealed the existence of at least 8 subtypes of mGluRs. Sequence alignments, agonist selectivities and coupling properties have allowed to classify mGluRs into three sub-groups termed Group I (mGluR1 and 5), Group II (mGluR2 and 3) and Group III (mGluR4, 6, 7 and 8). In transfected cells, Group II and Group III mGluRs are coupled to adenylate cyclase inhibition<sup>10-14</sup>, while Group I mGluRs are linked to PLC stimulation<sup>2,7-9,12,15-18</sup>. The activation of PLC-coupled mGluRs leads to the hydrolysis of membrane phosphatidylinositol-4,5-bisphosphate into diacylglycerol and inositol-1,4,5-trisphosphate (IP<sub>3</sub>). In turn, diacylglycerol is an activator of protein kinase C<sup>19</sup>, whereas IP<sub>3</sub> mobilizes calcium from intracellular stores<sup>20</sup>. IP<sub>3</sub> is then catabolized into various inositol phosphates (IP).

PLC-coupled mGluRs are likely involved in the molecular mechanisms underlying brain synaptic plasticity phenomena such as those occurring in learning and memory processes<sup>21-23</sup>, in post lesional compensatory events<sup>24-26</sup> and in nervous system development<sup>27-29</sup>. Nevertheless, the precise role of these PLC-linked mGluRs has not yet been clearly elucidated, mainly because of the lack of specific agonists and antagonists. Our aim was to develop new compounds to obtain pharmacological tools for studying mGluRs. Considering that design of both the pocket binding sites of the receptor and the ligand conformation could not yet be determined with sufficient accuracy, we have chosen to synthesize molecules that are structurally related to glutamate, the main natural endogenous ligand. Biological properties of 4-substituted glutamic acid derivatives being not well documented, we have considered the synthesis of various 4-substituted glutamate analogues using optically pure 4-methylene glutamic acid as a chiral building block<sup>30-32</sup>. Thus, phthalimide (**3a**), (**3b**), phosphonic (**5**) and cyclohexenic (**8**) glutamate derivatives, containing a bulky environment around 4 position, were prepared and evaluated in rat forebrain synaptoneurosomes for potential metabotropic modulatory activity.

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### Synthesis of (2*S*)-4-(2-phthalimidoethyl)glutamic acid (3a) and (2*S*)-4-(4-phthalimidobutyl) glutamic acid (3b)

Starting from *N*-Boc-*tert*-butyl-4-methylene glutamate (1) previously obtained in a three short step synthesis, radical reactions were performed following Ferritto procedure<sup>33</sup> (Scheme 1). Thus, slow addition (1h 30 min.) of a mixture containing 1 eq. of (1), 1.5 eq. of Bu<sub>3</sub>SnH and a catalytic amount of AIBN in benzene to a solution of iodomethyl or iodopropyl phthalimide in benzene, at 80°C, yielded (2a) or (2b) (50% yield) with a moderate to good diastereoisomeric excess respectively (36 to 74% detected by <sup>1</sup>H NMR in C<sub>6</sub>D<sub>6</sub>). Treatment of (2a) or (2b) in acidic conditions followed by addition of a large excess of propylene oxide in methanol afforded (3a) or (3b) as a white powder in a good yield (70%).

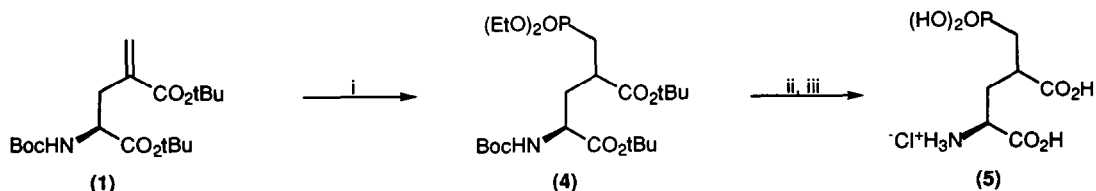


(i) iodomethyl or iodopropylphthalimide, Bu<sub>3</sub>SnH, AIBN, benzene, Argon, 80°C, 2 h. yield = 50-55%, (2a): de = 36%, (2b) = 74% (ii) 3N HCl/THF, rt, 24 to 72 h. yield = 100% (iii) propylene oxide/MeOH. yield = 65-75%

### Scheme 1. Preparation of (2*S*)-4-(2-phthalimidoethyl)glutamic acid

### Synthesis of (2*S*)-4-(phosphonomethyl)glutamic acid hydrochloride (5)

Arbuzov reaction<sup>34,35</sup> of triethylphosphite (1.5 eq.) with (1) in phenol, at 110°C gave (4) in a 90% yield with a moderate diastereoisomeric excess (42% detected by <sup>1</sup>H and <sup>31</sup>P NMR in C<sub>6</sub>D<sub>6</sub>). Removal of the diethyl group was carried out at room temperature, in the presence of 3 eq. of BrSiMe<sub>3</sub> in acetonitrile, for three days<sup>36</sup>. In these conditions, the Boc protecting group was also removed. It is noticeable that warming the mixture up to only 40°C in order to decrease the reaction time led to the loss of the  $\gamma$ -carboxylic group. Finally, cleaving the *tert*-butyl esters in 3N HCl/THF, at room temperature for two days resulted in compound (5) as its hydrochloride salt (scheme 2). Isolation of the free (2*S*)-4-(phosphonomethyl)glutamic acid by adding propylene oxide could not be performed because of reaction of the phosphonic group with propylene oxide.

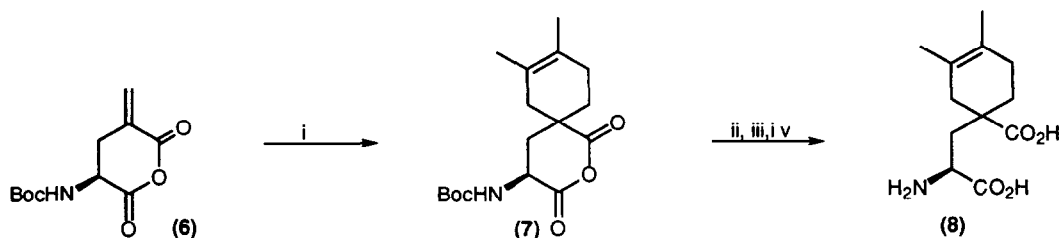


(i) triethylphosphite, phenol, Argon, 110°C, 24 h. yield = 90%, de = 42% (ii) BrSiMe<sub>3</sub>, acetonitrile, Argon, rt, 72 h. yield = 91% (iii) 3N HCl/THF, rt, 48 h. yield = 100%

### Scheme 2. Preparation of (2*S*)-4-(phosphonomethyl)glutamic acid hydrochloride

### Synthesis of 1-[(S)-2-amino-2-carboxyethyl]-3,4-dimethylcyclohex-3-ene-1-carboxylic acid (8)

Synthesis of compound (8) was first carried out using compound (1) as starting material. Reaction of (1) in the presence of 2,3-dimethylbutadiene led to the corresponding cycloadduct only under thermal conditions. After screening different solvents at different temperatures, the best yield was obtained using 5 eq. of 2,3-dimethylbutadiene, in orthodichlorobenzene, at 170°C for 24 hours. Nevertheless, although yield was good, it was difficult to purify the cycloadduct by silica-gel chromatography because of formation of numerous by-products. Thus, we have chosen to modify the starting material in order to increase its reactivity (scheme 3). Reaction of (6), easily obtained from (2S)-4-methylene glutamic acid, in acetic anhydride, at 60°C for 15 minutes resulted in the corresponding (2S)-4-methylene glutamic anhydride which was transformed to the spiro molecule (7) in a quantitative yield using 5 eq. of 2,3-dimethylbutadiene in benzene, at 70°C, thus showing the better reactivity of the anhydride. A low diastereoisomeric excess was detected ( $^1\text{H}$  NMR in  $\text{C}_6\text{D}_6$ ). Free amino acid (8) was isolated in a 35% overall yield after hydrolysis of (7) in a mixture water/THF, removal of the Boc protecting group using mild conditions (1N HCl gas in ethyl acetate as described by Rapoport<sup>37</sup>) followed by addition of a large excess of propylene oxide in methanol.



(i) 2,3-dimethylbutadiene, benzene, Argon, 70°C, 24 h. yield = 100%, de = 10% (ii)  $\text{H}_2\text{O}/\text{THF}$ , rt, 18 h. yield = 90% (iii) 1N HCl gas/AcOEt, Argon, rt, 18 h. yield = 60% (iv) propylene oxide/MeOH. yield = 65%

**Scheme 3.** Preparation of 1-[(S)-2-amino-2-carboxyethyl]-3,4-dimethylcyclohex-3-ene-1-carboxylic acid

### Pharmacological evaluation of compounds (3a), (3b), (5) and (8) in rat brain synaptoneurosomes

In order to test the action of compounds (3a), (3b), (5) and (8) on group I metabotropic receptors, we have measured their effects on basal and mGluR agonists-stimulated IP formation in 8 day old rat brain synaptoneurosomes. Synaptoneurosomes are preparations of nerve terminals containing both the pre- and post-synaptic parts which are resealed and attached. In this experimental model, we have previously shown that (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate (ACPD) and quisqualate (QA) stimulated IP formation via PLC-coupled mGluR receptor activation<sup>5,38</sup>. We have thus measured the accumulation of [ $^3\text{H}$ ]-inositol mono- and bis-phosphates ( $\text{IP}_1$  and  $\text{IP}_2$ , respectively) in [ $^3\text{H}$ ]-inositol pre-labelled synaptoneurosomes as previously described<sup>39</sup>. In a first set of experiments, the four compounds [(3a), (3b), (5) and (8)] were tested at 1 mM, either alone or in the presence of ACPD (30 and 1000  $\mu\text{M}$ ) or QA (0.3 and 10  $\mu\text{M}$ ). For each agonist, the low concentration used was close to the  $\text{EC}_{50}$  value (concentration evoking half-maximal response), and the high one induced the maximal response ( $\text{E}_{\text{max}}$ ). None of the four compounds tested had effect *per se* (data not shown), indicating no agonist activity for any of them. However, when tested in the presence of agonist some of the compounds tested (3a, 3b and 8) have slight but significant inhibitory effects (Figure 1). These effects were more pronounced on agonist-induced  $\text{IP}_2$  formations.

In order to better characterize these antagonist effects, we compared the action of (3b) which seems to be slightly more efficient than the other compounds tested, with that of (S)- $\alpha$ -methyl-4-carboxyphenylglycine (MCPG). This latter compound is considered to date, as one of the most potent group I mGluR antagonist, while being not selective, since it is also a good antagonist for group II mGluRs<sup>40,41</sup>. We thus performed dose-

response curves of ACPD- and QA-induced IP<sub>1</sub> and IP<sub>2</sub> formations, either in the absence or in the presence of MCPG or (3b) (Figure 2). The increase of EC<sub>50</sub> values of both ACPD and QA responses (Table 1) indicates that MCPG is a competitive antagonist of group I mGluRs, as previously described<sup>40,41</sup>. Conversely, compound (3b) has more complex effects on ACPD- or QA-evoked response. This effect seemed to be non-competitive versus QA response, since it mainly affected the E<sub>max</sub> value (Table 1 and Figure 2). Concerning the effect of compound (3b) on ACPD-induced IP formation, both EC<sub>50</sub> and E<sub>max</sub> values seemed to be modified. These data suggested that ACPD and QA differently interact on mGluR binding site and that interaction of compound (3b) with mGluR is also different from that of MCPG.

**Table 1: Effects of 1 mM MCPG and (3b) on EC<sub>50</sub> and E<sub>max</sub> of ACPD- and QA-induced IP<sub>1</sub> and IP<sub>2</sub> formations.**

	IP <sub>1</sub> formation		IP <sub>2</sub> formation	
	EC <sub>50</sub> (μM)	E <sub>max</sub> (%)	EC <sub>50</sub> (μM)	E <sub>max</sub> (%)
ACPD	17±1	284±17	38±6	700±94
ACPD + MCPG	109±5*	269±10*	224±34*	643±41
ACPD + (3b)	37±6*	264±18	79±11*	543±78*
QA	0.10±0.02	240±22	0.32±0.08	524±110
QA + MCPG	0.63±0.07*	225±40	1.34±0.21*	358±38
QA + (3b)	0.14±0.03	216±23*	0.27±0.07	359±65

Data are means ± S.E.M. of EC<sub>50</sub> and E<sub>max</sub> values determined from at least 3 independent dose-response curves, each performed in triplicate. Statistical differences between values either determined in the absence or in the presence of antagonist (MCPG or (3b)) were calculated using paired Student's t-test (\* p<0.05).

Finally, the synthesis of new derivatives of glutamate, starting from (2S) 4-methylene glutamic acid, could lead to the development of a new family of mGluR antagonists, different from the family of phenylglycine derivatives, the yet single class of substances that has emerged in this field.

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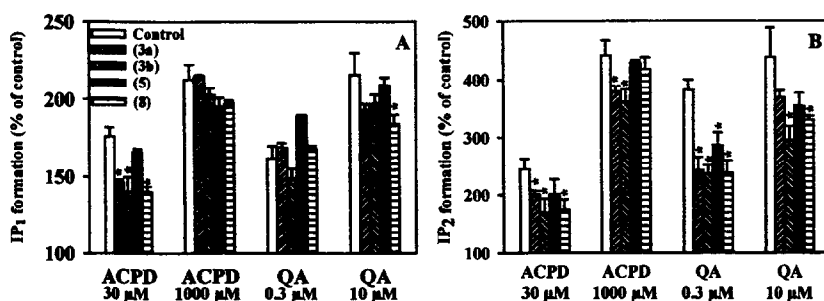


Figure 1: Effects of 1 mM (3a), (3b), (5) or (8) on ACPD- and QA-induced  $IP_1$  (A) and  $IP_2$  (B) formations in rat brain synaptoneurosomes. Synaptoneurosomes were labelled for 1h with [ $^3H$ ]inositol. After two washes, synaptoneurosomes were stimulated with 10 mM LiCl for 13 min, then agonists (or buffer, for control) were added for a further 20 min. The various compounds tested were added at the same time as LiCl.  $IP_1$  and  $IP_2$  were extracted, separated and quantified as previously described<sup>39</sup>. The data are expressed as percentages of control value (obtained in the absence of any agonist or antagonist). The figure represents the results (means  $\pm$  S.E.M.) of one experiment performed in triplicates and representative of three independent ones. Statistical significance between agonist stimulations obtained in the absence and in the presence of tested compound, were calculated using Student's t-test (\*  $p < 0.05$ ).

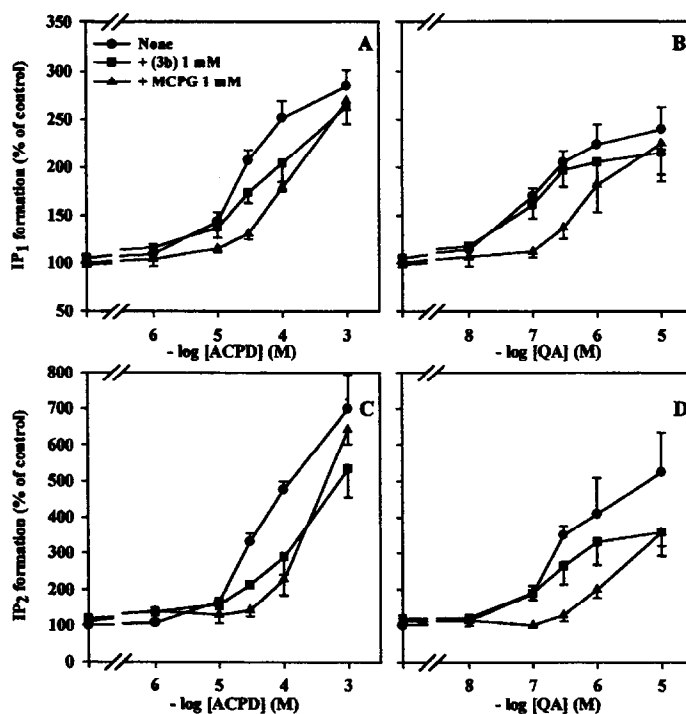


Figure 2: Dose-response curves of ACPD (A and C)- and QA (B and D)-induced  $IP_1$  (A and B) and  $IP_2$  (C and D) formations performed either in the absence or in the presence of 1 mM (3b) or MCPG. Stimulations were performed as described in Figure 1. Data, expressed as percentages of control values, were means  $\pm$  S.E.M. of at least three independent experiments, performed in triplicates.

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