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Efficient synthesis of novel glutamate homologues and investigation of their affinity and selectivity profile at ionotropic glutamate receptors

Andrea Pinto[a,*], Lucia Tamborini[a], Federica Mastronardi[a], Roberta Ettari[a], Diego Romano[b], Birgitte Nielsen[c], Carlo De Micheli[a], and Paola Conti[a]

[a]*Dipartimento di Scienze Farmaceutiche DISFARM, Università degli Studi di Milano, Via Mangiagalli 25, 20133 Milano, Italy*
[b]*Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DEFENS), Università degli Studi di Milano, via Mangiagalli 25, 20133 Milano, Italy*
[c]*Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen OE, Denmark*

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ABSTRACT

A convenient synthesis of four new enantiomerically pure acidic amino acids is reported and their affinity at ionotropic glutamate receptors is analyzed. The new compounds are higher homologues of glutamic acid in which the molecular complexity has been increased by introducing an aromatic/heteroaromatic ring, i.e. a phenyl or a thiophene ring, that could give additional electronic interactions with the receptors. The results of the present investigation indicate that the insertion of an aromatic/heteroaromatic ring into the amino acid skeleton of glutamate higher homologues is well tolerated and this modification could be exploited to generate a new class of NMDA antagonists.

L-Glutamic acid (L-Glu, Figure 1) is the main excitatory neurotransmitter in the central nervous system (CNS), where it is involved in the modulation of many physiological processes such as learning, memory, and synaptic plasticity.[1] Once released from the presynaptic neurons into the glutamatergic synaptic cleft, L-Glu activates two main classes of receptors: G-protein-coupled metabotropic Glu receptors (mGluRs) and ligand-gated ionotropic Glu receptors (iGluRs). On the basis of the agonist selectivity, iGluRs have been named *N*-methyl-D-aspartic acid (NMDA) receptors, (*RS*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors, and kainic acid (KA) receptors.[1-5]

For many years now, we have been actively involved in the search for new selective NMDA antagonists, and we have successfully designed a number of ligands, some of which showed promising neuroprotective activity.[6-14] NMDA antagonists are typically characterized by an increase in the distance between the proximal and the distal acidic groups of Glu, e.g. 4-6 carbon atoms. The simplest example of a Glu higher homologue behaving as a NMDA antagonist is (*R*)-aminoadipic acid [(*R*)-AA, Figure 1]. The amino acid skeleton may also be incorporated into a cyclic structure to decrease the conformational freedom (Figure 1). It is worth pointing out that the eutomer of the majority of NMDA ligands possesses the R configuration of the α amino acidic stereogenic center, at variance with the endogenous ligand (L-Glu).

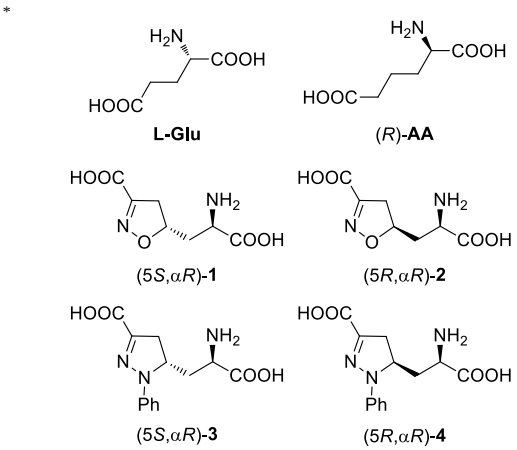


Figure 1. Structures of L-Glu, (*R*)-AA and some NMDA antagonists.

In the present paper, we exploited a Heck reaction to easily generate compounds (*R*)-**5** and (*R*)-**6** (Figure 2), and the corresponding saturated derivatives (*R*)-**7** and (*R*)-**8**, which are characterized by the *R* configuration at the amino acid

*Corresponding author. Tel.: +39-02-50319323; fax: +39-02-50319326; e-mail: andrea.pinto@unimi.it.

stereocenter and by a six-carbon-atom spacer between the proximal and the distal acidic groups, therefore matching the requirements needed to generate NMDA antagonists. The aromatic ring, i.e. a phenyl or a thiophene ring, can give additional electronic interactions with the binding pocket, which could strengthen the affinity for the target receptor. In addition, this moiety could be exploited to further decorate the molecule, in order to increase the binding affinity.

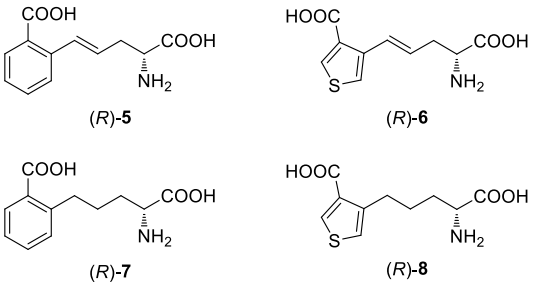
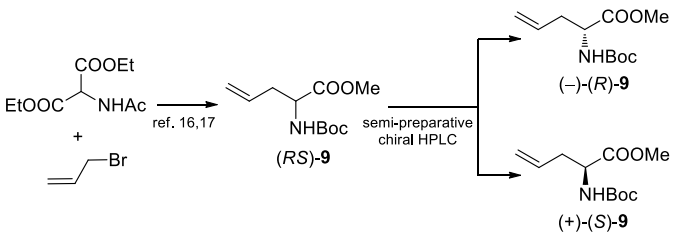


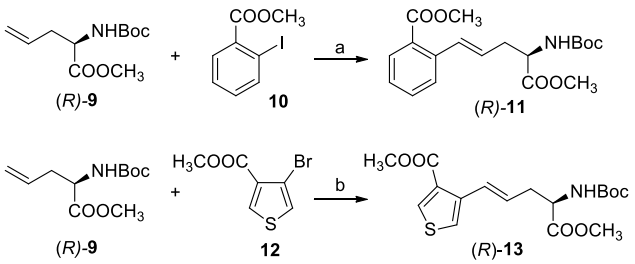
Figure 2. Structures of the synthesized compounds.

The key step to obtain the planned compounds is the Heck reaction between conveniently protected D-allylglycine (*R*)-**9** and the appropriate aryl halide, i.e. methyl 2-iodobenzoate **10** or methyl 4-bromothiophene-3-carboxylate **12**. D-allylglycine is commercially available but it is very expensive (about 700 euro/g). Therefore, we prepared the racemic alkene (*RS*)-**9**, with a conventional procedure, by reacting allyl bromide with diethyl acetamidomalonate,[16,17] and then we developed a suitable method for its chromatographic resolution with a semi-preparative chiral HPLC column (Scheme 1). An excellent enantiomeric separation (α = 4.2; Rs = 10.2) was achieved with a column containing *tris*-(2-methyl-5-chloro-phenyl)carbamoyl amylose as the stationary phase, affording (−)-**9** as the first fraction and (+)-**9** as the second fraction. The absolute configuration was assigned to each enantiomer on the basis of the optical activity, by comparison with that reported in the literature.[18] Intermediates **10** and **12** were easily obtained, in quantitative yield, by esterification of the corresponding commercially available carboxylic acid with methanol in the presence of $SOCl_2$.



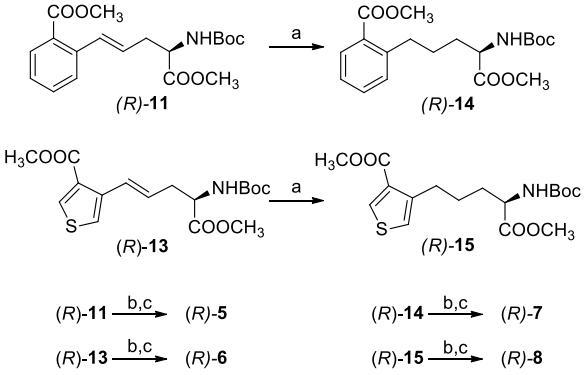
Scheme 1. Synthesis of the enantiopure alkenes (*R*)-**9**and (*S*)-**9**.

The Heck reaction was optimized, in terms of catalyst, base, solvent, temperature and reaction time, for each aryl halide. Using methyl 2-iodobenzoate **10**, the best yield was achieved performing the reaction in acetonitrile and using palladium acetate as a catalyst and diisopropylamine (DIPEA) as a base (Scheme 2). A good yield (75%) was obtained by refluxing the reaction mixture for 24 hours. Interestingly, a similar result can be obtained after only two hours, by heating the reaction mixture in a microwave reactor at 110 °C. As expected, we isolated only one stereoisomer to which we assigned the E configuration by taking into account the high value of the ^{1}H NMR coupling constant (*J* = 16.0 Hz) between the two olefinic protons. Unfortunately, by applying the same reaction conditions to methyl 4-bromothiophene-3-carboxylate **12**, we isolated the desired product (*R*)-**13** in only 15% yield. In this case, the use of Tetrakis(triphenylphosphine)-palladium and sodium acetate in DMF, under conventional heating at 110 °C for 18 hours, produced intermediate (*R*)-**13** in excellent yield (81%) (Scheme 2). The use of the microwave heating, even at lower temperature, resulted in a lower yield and purity.



Scheme 2. Reagents and conditions: a) $Pd(OAc)_2$, DIPEA, CH_3CN, µW, 130 °C, 2h; b) Tetrakis(triphenylphosphine)-palladium, NaOAc, DMF, 110 °C, 18 h.

Part of intermediates (*R*)-**11** and (*R*)-**13** were catalytically hydrogenated in the presence of 5% Pd/C to yield the saturated derivatives (*R*)-**14** and (*R*)-**15**, respectively (Scheme 3). Final amino acids (*R*)-**5**-**8** were obtained by alkaline hydrolysis of the ester functions followed by treatment with a 30% solution of trifluoroacetic acid in dichloromethane to remove the N-Boc protective group (Scheme 3).



Scheme 3. Reagents and conditions: a) H_2, Pd/C 5%, MeOH; b) NaOH 1N, MeOH; c) 30% TFA, CH_2Cl_2.

The new derivatives were submitted to in vitro assays, evaluating receptor binding in rat cortical membranes, using radioligands [^{3}H]CGP39653, [^{3}H]AMPA, and [^{3}H]KA for NMDA, AMPA, and KA receptors, respectively (Table 1).[19-21]

Table 1. Receptor binding affinities at native rat iGluRs.[a]

Compound	[^{3}H]AMPA IC_{50} (µM)	[^{3}H]KAINIC$_{50}$ (µM)	[^{3}H]CGP39653K$_i$ (µM)
(*R*)-**5**	> 100	> 100	> 100
(*R*)-**6**	> 100	> 100	52 [4.29±0.04]
(*R*)-**7**	> 100	> 100	32 [4.49±0.04]
(*R*)-**8**	> 100	> 100	14 [4.86±0.05]
(*R*)-**AA**[b]	nd	nd	13

[a] Data are given as mean [mean pK_i ± SEM] of three independent experiments. [b] Data from ref. 22

Three out of the four tested compounds bound to NMDA receptor, with (*R*)-**8** having the best affinity (K_i = 14 µM). Interestingly, the K_i value of (*R*)-**8** is almost identical to that of (*R*)-**AA**, our reference NMDA antagonist. The outcome of the present investigation indicates that a reduction in the

conformational flexibility of aminoadipic acid through the insertion of an aromatic/heteroaromatic ring is well tolerated by NMDA receptors. This result opens the possibility to further increase the interactions with the binding pocket through appropriate decorations of the aromatic/heteroaromatic ring thus generating novel NMDA antagonists provided with high potency and, hopefully, subtype selectivity. As an added value, the synthetic route leading to this new class of ligands is fast, cheap and versatile, since library of derivatives can be easily obtained by simply varying the nature of the aryl/heteroaryl halide.

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