Amino Acids (1995) 9: 391–395

Amino
Acids

Neuroexcitatory amino acids: 4-methylene glutamic acid derivatives

Short Communication

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Accepted June 30, 1995

Summary. A short synthesis of 4-methylene glutamic acid was achieved. Under thermal conditions the corresponding anhydride reacted with 2,3 dimethylbutadiene to afford the corresponding DIELS-ALDER adduct in good yield. L-4-methylene glutamic acid essentially acts on glutamate metabotropic receptors and is as potent as L-Glu in producing IPs.

Keywords: Amino acids – Diels-Alder reaction – Excitatory amino acids – Metabotropic receptor – 4-Methylene glutamic acid

Introduction

At most excitatory synapses in the mammalian brain, the major neurotransmitter is the amino acid L-Glutamate (L-Glu) which binds and activates a variety of receptors. The first class includes glutamate ion channel-receptors, which are subdivided into three subtypes: NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and KA (kainate) (Sommer, 1992; Nakanishi, 1992). Glu also activates metabotropic receptors (mGluR) linked, *via* a G-protein, to phospholipase C (PLC) (Sladeczek, 1985; Sugiyama, 1987; Récasens, 1988) or adenylate cyclase (Nakanishi, 1992; Schoepp, 1993). Molecular cloning (Houamed, 1991; Masu, 1991) has revealed the existence of at least 8 subtypes of mGluRs. mGluR2, mGluR3, mGluR4, mGluR6, mGluR7 and mGluR8 are coupled to adenylate cyclase inhibition (Tanabe, 1992, 1993; Nakajima, 1993; Okamoto, 1994) while mGluR1α, mGluR1β, mGluRlγ, mGluR5a and mGluR5b (Nakanishi, 1992; Tanabe, 1992; Abe, 1992; Minakami, 1993; Pin, 1992) are linked to PLC stimulation. PLC-coupled mGluRs are likely involved in the molecular mechanisms underlying brain synaptic plasticity phenomena such as those occuring in learning and memory processes (Kano, 1987; Otani, 1991; Zheng, 1992; Bortolotto, 1994; Shigemoto, 1994), in postlesional compensatory

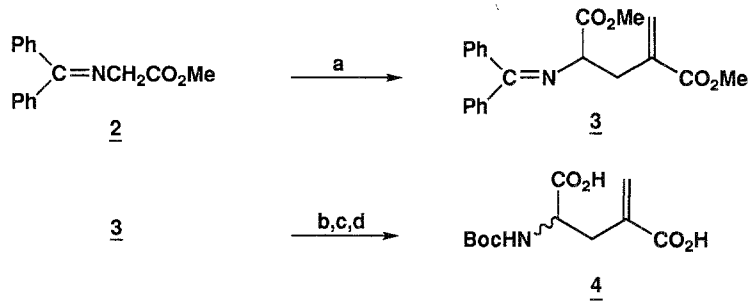
events (Nicoletti, 1987; Seren, 1989; Mayat, 1994) and in nervous system development (Nicoletti, 1986; Dudek, 1989; Guiramand, 1989; Palmer, 1990; Mayat, 1994). These receptors could also serve to prevent neuronal apoptosis in granule cell cultures (Copani, 1995). Despite this tremendous potential implications in brain physiology and pathophysiology, the precise role of these PLC-linked mGLUR has not yet been clearly elucidated, largely because of the lack of specific agonists or antagonists. Our aim was to develop new substances to obtain pharmacological tools for studying mGLURs.

Methods and results

We prepared 4-methylene glutamic acid in a short four step synthesis; we consider this compound as a good precursor for the obtention of numerous aminodiacids using different reactions on the double bond. We describe here the DIELS-ALDER reaction. N-protected 4-methylene glutamate dimethyl ester **3** was easily obtained by alkylation (using LDA) of the N-(diphenyl methylene)-glycine methyl ester **2** with methyl 2-bromomethyl acrylate **1** prepared by esterification of commercial 2-bromomethyl acrylic acid using trimethylchlorosilane in methanol. Treatment of **3** with 1N HCl afforded the aminodiester which was N-protected by reaction with diterbutyldicarbonate; after saponification with 2N NaOH and acidification the N-Boc 4-methylene glutamic acid **4** was obtained in 66% overall yield. The two enantiomers were synthesized using the same strategy, the key step beeing the diastereoselective alkylation (de > 98%, detected by ^{1}H NMR) of the Schiff base prepared from (R,R,R) or (S,S,S) 2-hydroxypinan-3-one (Tabcheh, 1991) and tert-butylglycinate. **4** was quantitatively transformed into the anhydride **5**. DIELS-ALDER reaction with dimethylbutadiene was tested on the N-Boc 4-methylene glutamate dimethyl ester and on the anhydride **5** using Lewis acids or thermal conditions. Lewis acids (BF_3, Et_2O; $ZnCl_2$; $TiCl_4$) were ineffective, only polymerisation products of the diene were obtained at room temperature. After screening several reaction conditions, the better result was obtained starting from the anhydride **5** which afforded the cycloadduct **6** in a quantitative yield after reaction at 70°C in benzene during 24h; the N-Boc aminodiester needed a higher temperature (170°C in 1,2-dichlorobenzene during 24h) to give the cycloadduct in 55% yield after purification. Successive treatments of **6** by 2N NaOH, HCl and propylene oxide gave **7** in good yield.

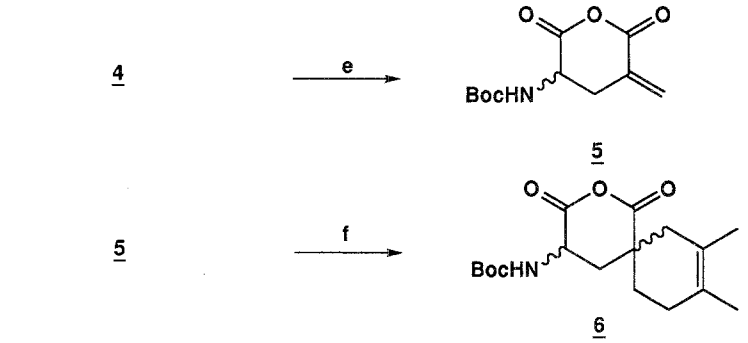
In vitro affinity

Racemic 4-methylene glutamic acid and the two enantiomers were tested by Professor RECASENS' team (Université Montpellier 2). In vitro affinity were determined by measuring IP accumulation in the presence of lithium chloride using rat forebrain synaptoneurosomes or hippocampal neurons in primary culture (Récasens, 1988; Blanc, 1995) previously labelled with ^{3}H-myo-inositol. DL-Met-Glu enhances IP formation in 8 day-old rat for brain

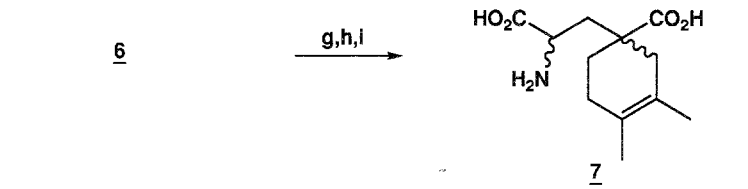


(a) LDA/(1) -80°C 18h Yield=97% (b)1N HCl 24h Yield=100%

(c) 1)NH_3gas 15min. 2)Boc_2O 15h Yield=90% (d) 1)2N NaOH 2h 2)1N HCl Yield=76%



(e)$(CH_3CO)_2O$ 45°C 10min. Yield=100% (f) 2,3 dimethylbutadiene 70°C 24h Yield=100%



(g) 1)2N NaOH 2h 2)1N HCl Yield=87% (h) HClgas -20°C 2h Yield=90%

(i) Propylene oxide/MeOH 10 min. Yield=80%

synaptoneurosomes L-Met-Glu also stimulates efficiently IPs accumulation in synaptoneurosomes with an apparent affinity 20 times higher than that of D-Met-Glu. L-Met-Glu is about as potent as L-Glu in producing IPs. The effect of L-Met-Glu is neither blocked by APV (1mM) or DNQX (0.1mM)alone nor by a combination of these two compounds. This result indicates that-L-Met-Glu essentially act on glutamate metabotropic receptors in synaptoneurosomes. Diastereoisomers **7** are actually tested in vitro as described above.

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Received February 26, 1995

Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. – Herausgeber: Prof. Dr. Gert Lubec, Universitäts-Kinderklinik, Währinger Gürtel 18–20, A-1090 Wien. – Redaktion: Sachsenplatz 4–6, A-1201 Wien. – Hersteller: Satz und Umbruch: Best-Set Typesetter Ltd., Hong Kong; Offsetdruck: Novographic, Ing. W. Schmid, Maurer-Lange-Gasse 64, A-1238 Wien. – Verlagsort: Wien – Herstellungsort: Wien. – Printed in Austria.