Communications to the Editor

Synthesis, Resolution, and Biological Evaluation of the Four Stereoisomers of 4-Methylglutamic Acid: Selective Probes of Kainate Receptors

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Introduction. Excitatory amino acids (EAAs), such as glutamic acid, play a critical role in both the developing and mature central nervous system (CNS).[1] However, excessive release of glutamic acid can result in neuronal cell death, a phenomenon which has been termed excitotoxicity.[2] Converging lines of evidence indicate that excitotoxic cell death substantially contributes to the pathophysiology of both acute (e.g. stroke) and chronic (e.g. Alzheimer's) neurodegenerative disorders in the CNS.[3] Thus, the potential therapeutic use of compounds capable of modulating glutamate receptor function has been recognized for more than a decade.[4]

Recent molecular biological studies have demonstrated a remarkable degree of heterogeneity among ionotropic glutamate receptors[5] which can be broadly subdivided into kainic acid (KA), *N*-methyl-D-aspartate (NMDA), and α-amino-3-hydroxy-5-methylisoxazolepropionic acid (AMPA) receptors based on their respective affinities for these exogenous unnatural amino acids.[6] While there are a number of compounds which exhibit high specificity and selectivity at the NMDA receptor,[7] there has been a lack of selective and high-affinity ligands for the kainate receptor.

Herein, we report the preparation of the four possible stereoisomers of 4-methylglutamic acid. As assessed by radioligand binding studies, these single isomers exhibit a variable degree of selectivity for KA receptors depending upon their stereochemical configuration. Among them, the (2*S*,4*R*)-isomer has been identified as having exceptional selectivity for the KA receptor with an IC_{50} for inhibition of [^{3}H]KA binding of 35 nM comparable to Kainic acid itself (IC_{50} of ~11 nM).

Chemistry. The *threo* isomers (2*S*,4*S* and 2*R*,4*R*) were prepared by diastereoselective alkylation of protected D- or L-glutamic acid.[8] Thus, *N*-(4-nitrobenzoyl)-D-glutamic acid dimethyl ester (**2**) was prepared by treating D-glutamic acid with thionyl chloride in methanol, followed by reaction with 4-nitrobenzoyl chloride under Schotten–Baumann conditions. Compound **2** was treated with lithium bis(trimethylsilyl)amide (2.2 equiv) in anhydrous tetrahydrofuran at −78 °C to generate the γ-enolate, which was then reacted with iodomethane (3 equiv) to give the corresponding 4-methylated product **3** (Scheme 1). TLC analysis of the reaction mixture in different solvent systems indicated the presence of a single product. The proton and carbon-13 NMR analysis of this crude reaction mixture showed that the product seemed to be a single isomer along with various amounts of unreacted starting material. Furthermore, a mixture of the *threo* and *erythro* isomers (four stereoisomers of 4-methylglutamic acid) was prepared *via* literature procedure[9] and then converted to the corresponding *N*-(*p*-nitrobenzoyl)-4-methylglutamic acid dimethyl ester. Comparison of these NMR spectra revealed that the alkylation product **3** matched one NMR pattern of this diasteroisomeric mixture of *N*-(*p*-nitrobenzoyl)-4-methylglutamic acid dimethyl ester, indicating only one isomer obtained in the alkylation step. Acidic hydrolysis of compound **3** provided the fully deprotected (2*R*,4*R*)-4-methylglutamic acid (**4**) which was confirmed as the *threo* isomer by comparison with literature spectrocopic data.[10] This highly diastereoselective alkylation is presumably due to the formation of a chelated transition state which was approached by an electrophile almost exclusively from one face, leading to a single stereoisomer product. The newly formed 4-position chiral center seems to be influenced by the stereocenter at the α-carbon. For the (2*S*,4*S*)-isomer, the commercially available *N*-(4-nitrobenzoyl)-L-glutamic acid diethyl ester was methylated using the procedure described above to provide the corresponding methylated isomer **6** (Scheme 2) as a single product. Compound **6** was subsequently deprotected under acidic conditions to afford the corresponding (2*S*,4*S*)-4-methylglutamic acid (**7**). The two C-3 methylene protons of **7** exhibited characteristic chemical shifts (same as **4**) of the *threo* isomers (1.85–2.16 ppm) in contrast to the *erythro* isomers (1.62–1.76 and 2.05–2.20 ppm). However, approximately 3% of epimerization at C-4 was observed by proton NMR at the acidic hydrolysis step. This observation is similar to literature reports.[10] The minor diastereoisomer, however, was easily removed in the final crystallization step. The spectroscopic data of the two *threo* isomers (**4** and **7**) are in agreement with literature data.[10,11]

The mixture of (2*S*,4*R*)- and (2*R*,4*S*)-4-methylglutamic acids **9** (*erythro* enantiomers) was obtained by fractional crystallization from a mixture of the racemates of two diastereoisomers of **8**, which was prepared according to a reported literature procedure.[9] The purity of the mixture of *erythro* enantiomers **9** was monitored by proton NMR since the chemical shift patterns of C-3 methylene protons of the *erythro* isomers is different from *threo* isomers.[10] The mixture of *erythro* enantiomers **9** was then converted into a pair of diastereomers **10** through esterification with thionyl chloride and methanol followed by treatment with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (*S*-MTPA). The resulting diastereomeric Mosher amides **10** were then separated by HPLC on a preparative silica gel column to give the single isomers **11** and **12**. The proton NMR analysis showed that the methoxy, methyl

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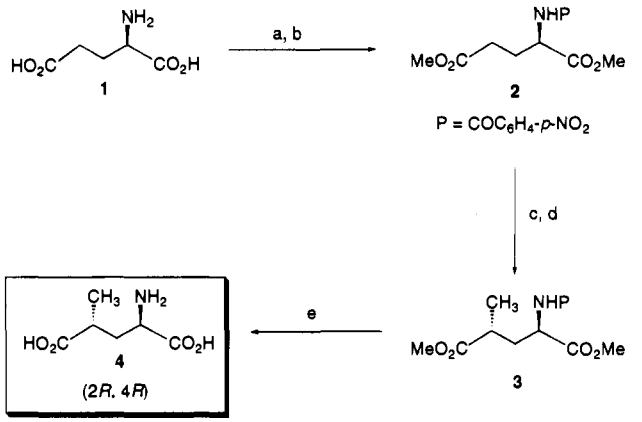
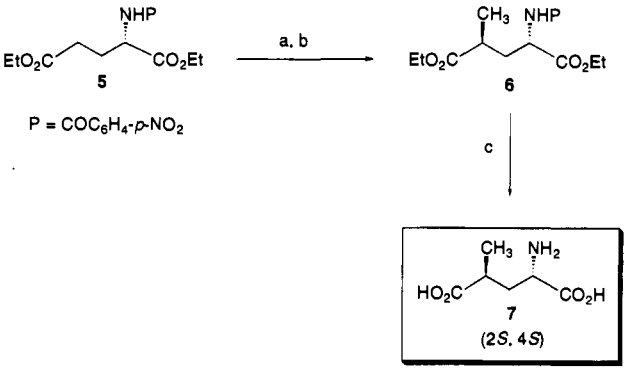
Scheme 1[a]



[a] (a) $SOCl_2$, MeOH, 0 °C to room temperature; (b) p-NO_2-C_6H_4COCl, aqueous 20% Na_2CO_3, CH_2Cl_2; (c) $LiN(SiMe_3)_2$, THF, −78 °C; (d) CH_3I; (e) 6 N HCl, reflux.

Scheme 2[a]



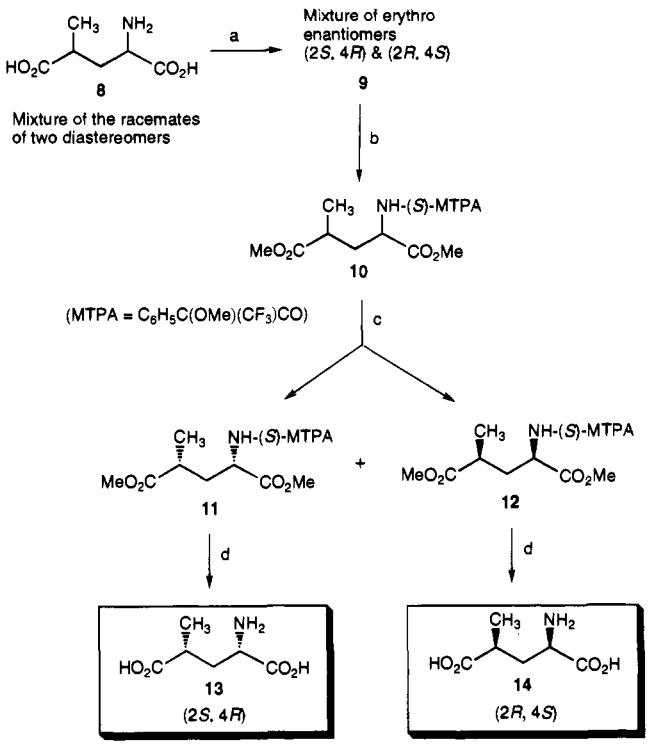
[a] (a) $LiN(SiMe_3)_2$, THF, −78 °C; (b) CH_3I; (c) 6 N HCl, reflux.

esters, and amide protons gave characteristic chemical shifts as follows: for **11**, 3.55, 3.62, 3.75, and 7.22 ppm; for **12**, 3.38, 3.70, 3.75, and 7.55 ppm. The absolute configurations of **11** and **12** were assigned as 2*S*,4*R* and 2*R*,4*S*, respectively, by using the modified Mosher's method.[12] Compounds **11** and **12** were subsequently hydrolyzed under acidic conditions followed by crystallization as described above to afford (2*S*,4*R*)-4-methylglutamic acid (**13**) and (2*R*,4*S*)-4-methylglutamic acid (**14**), respectively (Scheme 3).

Results and Discussion. 4-Methylglutamic acid as the D,L mixture has been reported to have weak affinity at the NMDA receptor by inhibiting binding of [^{3}H]-D-2-amino-5-phosphonopentanoate to rat brain membranes.[13] Single diastereoisomers of 4-methylglutamic acid have been prepared through enzymatic synthesis followed by chromatographic separation,[10,11] as well as by use of a chiral auxiliary derived from proline through an enantiospecific synthesis.[14] *erythro*-4-Methyl-L-glutamic acid has been reported as a glutamate uptake inhibitor.[15] In preliminary studies, however, we found that the mixture of the racemates of two diastereomers **8** of 4-methylglutamic acid inhibited [^{3}H]KA binding to rat forebrains with moderate affinity but was far less potent at either NMDA or AMPA receptors (data not shown). This interesting finding prompted us to prepare the four individual diastereoisomers of 4-methylglutamic acid and evaluate their affinties at the KA receptor subtype.

The inhibition of [^{3}H]KA binding to rat cortical membranes[16] by the four stereoisomers along with

Scheme 3[a]



[a] (a) Fractional crystallization from acetone and water; (b) $SOCl_2$, MeOH, (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA), aqueous 20% Na_2CO_3, CH_2Cl_2; (c) HPLC separation; (d) 6 N HCl, reflux.

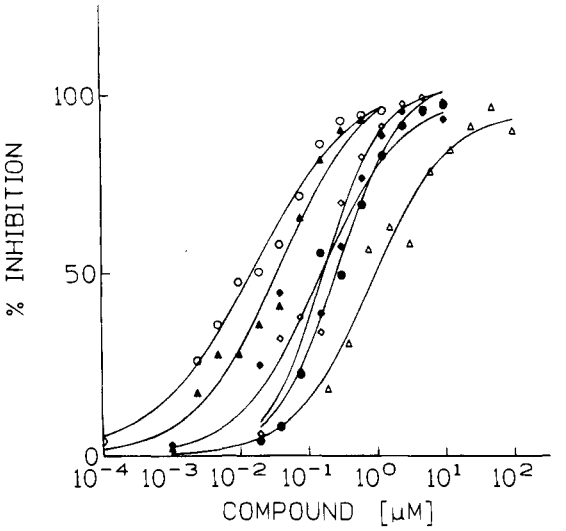


Figure 1. Inhibition of [^{3}H]KA binding to rat cortical membranes by the four stereoisomers of 4-methylglutamic acid, kainic acid, and L-glutamic acid. Various concentrations of the compounds were applied to incubation medium in the presence of 2−5 nM [^{3}H]KA. The experiment was repeated with at least three separate determinations done in triplicate, and similar results were obtained. KA (open circles), (2*S*,4*R*)-isomer 13 (filled triangles), (2*S*,4*S*)-isomer 7 (open diamonds), (2*R*,4*R*)-isomer 4 (filled diamonds), L-glutamic acid (filled circles), (2*R*,4*S*)-isomer 14 (open triangles).

kainic acid and L-glutamic acid are shown in Figure 1. While the *threo* isomers (2*S*,4*S* and 2*R*,4*R*) of 4-methylglutamic acid were moderately potent inhibitors of [^{3}H]KA binding (IC_{50}'s of 0.31 and 0.33 μM, respectively), there was no significant affinity difference between them. Nonetheless, the IC_{50} to inhibit [^{3}H]-AMPA binding to AMPA receptors and [^{3}H]CGS19755

binding to NMDA receptors was greater than 10 μM (data not shown), demonstrating a marked degree of selectivity for the KA receptor subtype. In contrast, the *erythro* isomers (2*S*,4*R* and 2*R*,4*S*) exhibited a different profile of effects at glutamate receptors. Thus, while maintaining an exquisite selectivity for kainate compared with AMPA and NMDA receptors (IC_{50}'s at AMPA is >100 μM and IC_{50}'s at NMDA is >7 μM, data not shown), the potency of the (2*S*,4*R*)-isomer to inhibit [^{3}H]-KA binding (IC_{50} of ~35 nM) was comparable to kainic acid (IC_{50} of ~11 nM). The (2*R*,4*S*)-isomer was more than 20-fold less potent (IC_{50} of ~820 nM). Both *erythro* isomers, however, were almost equally potent as inhibitors of radioligand binding to AMPA and NMDA receptors (IC_{50}'s are greater than 7 μM, data not shown).

Since L-glutamic acid is a conformationally flexible molecule, it is reasonable to assume that each subtype of the glutamate receptors might exhibit different conformational preferences for glutamic acid. Introduction of a methyl group at the 4-position of glutamic acid results in marked selectivity for the KA receptor subtype, suggesting that the resulting conformational changes are not favored by AMPA and NMDA receptor subtypes. Especially noteworthy is (2*S*,4*R*)-4-methyl glutamic acid (SYM 2081), which was shown to be the most selective KA receptor ligand with an affinity comparable to kainic acid itself. Moreover, the 4-methyl group also adds a steric component to the overall picture of the molecule. The steric bulk may also contribute to the selectivity in addition to the specific conformation. These results demonstrate that the NMDA, AMPA, and KA receptors have subtle but different preferences for glutamic acid binding conformations. It implies that 4-methyl substitution on glutamic acid plays a far more critical role in selectivity for the KA receptor than it does for the AMPA and NMDA receptor subtypes.

In summary, the four stereoisomers of 4-methylglutamic acid were prepared *via* diastereoselective alkylation or chemical resolution. Introduction of a methyl group at the 4-position, resulting in conformational changes of the molecule, enhances selectivity at the KA receptor subtype. (2*S*,4*R*)-4-Methylglutamic acid was identified as having exceptional selectivity for KA receptor subtype with an IC_{50} for inhibition of [^{3}H]-KA binding comparable to kainic acid itself. This is the most selective ligand for the KA receptor subtype without significant cross reactivity with AMPA or NMDA receptors described to date. While additional electrophysiological and functional studies will be required to determine whether these compounds possess agonist or antagonist properties, they will undoubtedly be valuable for elucidating the physiological and pharmacological functions of the KA receptor subtype of glutamate receptors.

Supporting Information Available: Experimental details for the preparation of compounds in this paper and their physical properties data (5 pages). Ordering information is given on any current masthead page.

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