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Glutamate transporter blockers: enantiomerically pure (2*S*,3*S*)- and (2*S*,3*R*)-3-methyl glutamic acids

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Abstract—A short four-step synthesis of (2*S*,3*R*)- and (2*S*,3*S*)-3-methyl glutamic acids is reported; the (2*S*,3*R*) isomer presented a significant inhibitory effect on glutamate transport.

1. Introduction

L-Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. Excitatory neurotransmission is initiated by the presynaptic release of glutamate into the synaptic cleft where glutamate activates ionotropic (i-GluRs) and metabotropic (m-GluRs) receptors; glutamate can be removed by glutamate transporter proteins which use the electrochemical gradients across the plasma membranes as driving forces for neurons. The glutamate uptake system[1] consists of at least five different transporter proteins called excitatory amino acid transporters ($EAAT_1$, $EAAT_2$, $EAAT_3$, $EAAT_4$ and $EAAT_5$). Prolonged exposure of neurons to elevated glutamate concentrations can lead to excitotoxicity which has been implicated in the pathogenesis of a number of neurological disorders such as ischemia after a stroke.[2]

The presence of distinct EAAT subtypes leads to questions regarding the possibility of subtype specific roles and the need for selective inhibitors.

2. Results

It has been reported[3,4] that *threo*-3-methyl glutamate (T3MG) as a racemic mixture of (2*S*,3*R*)- and (2*R*,3*S*)-isomers blocked glutamate transport by $EAAT_2$ (K_i = 18 μM), the *erythro* form being inactive. By analogy with (*R*)-glutamate which was inactive, we supposed that the (2*R*)-isomers of 3-methyl glutamate were inactive and we were therefore interested in developing a short and efficient synthesis of the (2*S*,3*S*)- and (2*S*,3*R*)-isomers in diastereomerically pure form to examine the precise interaction between a blocker and the transporters.

In the literature several syntheses of 3-methyl glutamic acid by asymmetric Michael addition, with ethyl crotonate have been reported. Gani et al.[5] used Schöllkopf's auxiliary but purification of the amino ester after cleavage of the chiral auxiliary was tedious; Soloshonok et al.[6] used Ni complexes of Schiff bases for the same synthesis but the yield was very poor (7%). An enantiospecific synthesis involving Arndt Eistert homologation[5] of (2*S*,3*S*)-3-methyl aspartic acid provided (2*S*,3*R*)-3-methyl glutamic acid in eight steps. Herein, we describe our results in the figure below using commercially available and inexpensive (1*R*,2*R*,5*R*)-2-hydroxypinan-3-one as a chiral auxiliary to give the (2*S*)-amino esters (Scheme 1).[7,8]

We have previously studied the asymmetric Michael addition of methyl glycinate Schiff base with ethyl crotonate and we obtained three diastereomers; having established that in the alkylation reactions of such Schiff bases[9] the nature of the ester was important to the diastereomeric ratio of the product, we replaced the methyl ester with a *tert*-butyl ester. If lithium diisopropylamide (LDA) is used as the base, the alkylation of the Li enolate of Schiff base at −80°C in THF yielded the addition compound and polycondensation products. In the presence of 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) instead of LDA as base at −30°C, the reaction of the Schiff Base **1** gave two diastereomers **2a** and **2b** in 75% yield, with a diastereomeric ratio (d.r.) of 56:44 as determined by

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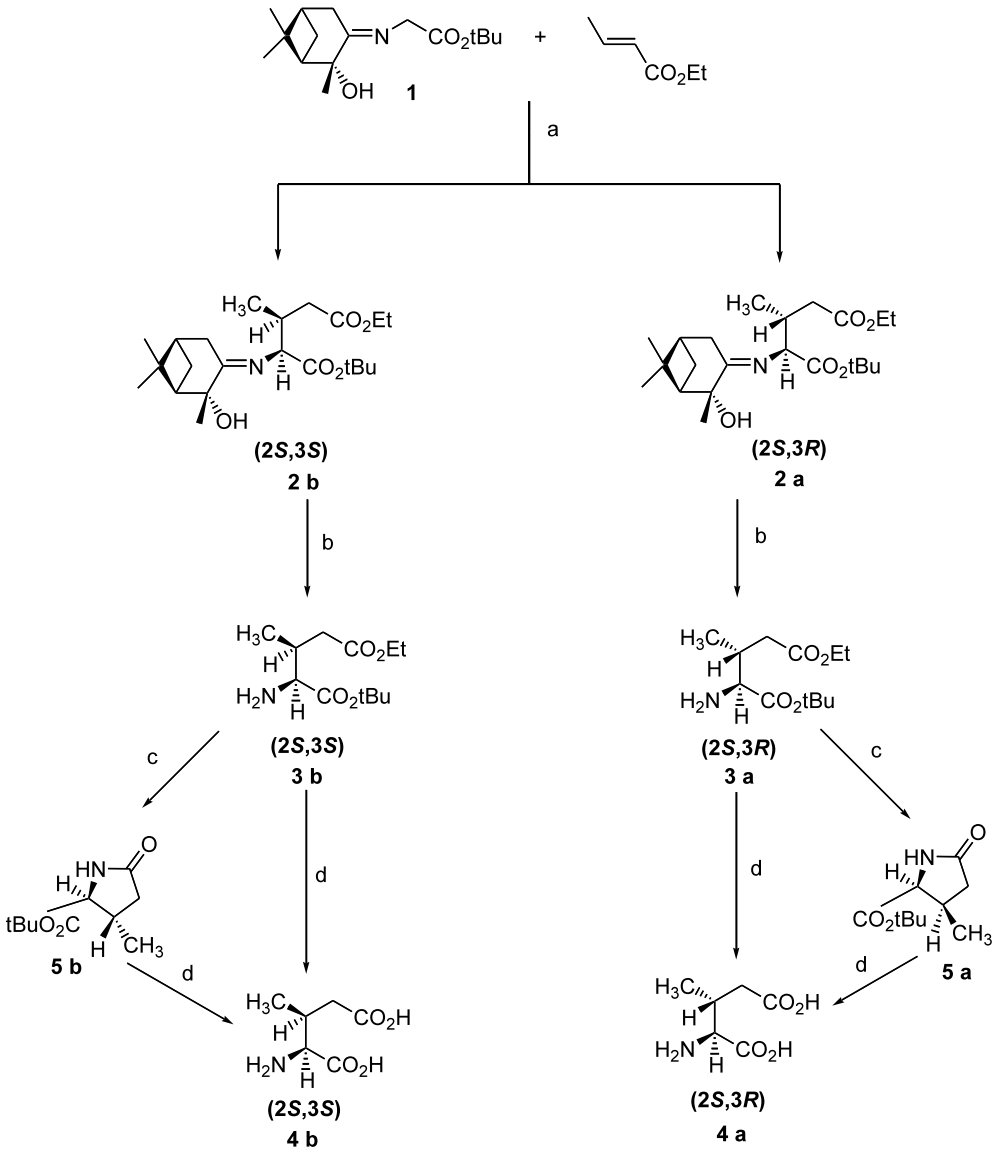

Scheme 1. *Reagents and conditions*: (a) (1) CH_3MgBr (1.3 equiv., 3 M in Et_2O), THF, −30°C, 10 min, (2) DBU (1.1 equiv., −30°C, 1 h, $Y_{(2a)}=42\%$, $Y_{(2b)}=33\%$; (b) (1) 15% citric acid (1.2 equiv.), rt, 3 days, (2) Na_2CO_3 ,$Y_{(3a)}=50\%$, $Y_{(3b)}=67\%$; (c) cyclisation at rt; (d) (1) 6N HCl, reflux, 3 h, (2) propylene oxide/MeOH, $Y_{(4a)}$ and $Y_{(4b)}=95\%$.

HPLC, without any polycondensation product. CH_3MgBr was found to chelate the hydroxy group of the chiral auxiliary.

The two diastereomers were easily separated by silica gel column chromatography and the synthesis was continued with each diastereomer. Cleavage of the chiral auxiliary using 15% citric acid at room temperature followed by neutralisation with Na_2CO_3 afforded the two amino esters **3a** and **3b** in 82% and 73% yields respectively which, after treatment with 6N HCl at 80°C provided the corresponding amino acid hydrochloride salts in 80% yield. The hydrochloride salts were then treated with propylene oxide in methanol to furnish the amino acids **4a** and **4b** in 90% yield.

The cyclic *tert*-butyl-3-methyl pyroglutamates **5a**, **5b** were obtained quantitatively if the amino esters were kept at room temperature for 3 h. Determination of the configuration at C-3 was based on coupling constants between Hα and Hβ of **5a** and **5b**. For **5a** $J_{H\alpha H\beta}=7.55$ Hz which corresponds to a *cis* product and for **5b** $J_{H\alpha H\beta}=5.71$ Hz corresponding to a *trans* product. These results permitted the assignment of (2*S*,3*R*) configuration to the major compound **4a** and (2*S*,3*S*) configuration to the minor compound **4b**. These configurations were confirmed by comparison with the specific rotations reported in the literature.

The first results concerning the biological activity, synaptic activity evaluated by Ca^{++} current measurement, studied in the laboratory of Neuroplasticity in our University showed that (2*S*,3*R*)-3-methyl glutamic acid exhibited an important inhibitory effect on glutamate transport, whereas the (2*S*,3*S*) isomer presented a modest inhibitory effect (ca. 20% of the inhibitory activity of (2*S*,3*R*)-3-methyl glutamic acid).

3. Conclusion

We can conclude that for the *threo* compounds, the (2*S*,3*R*) isomer is effectively the active isomer and also that the stereogenicity of the β carbon is important, the (2*S*,3*S*) enantiomer being less active than the (2*S*,3*R*) isomer.

4. Experimental

Melting points were obtained using a Büchi 510 capillary apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 250 MHz using Brücker AC250 instrument. For ^{1}H NMR spectra recorded in $CDCl_3$ chemical shifts are quoted in parts per million and are referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants are reported in hertz (Hz). Low resolution mass spectra were recorded on micromass electrospray instrument with only molecular ion and other major peaks being reported. Flash chromatography was carried out using E-Merck silica gel (Kieselgel 60, 230–400 mesh) as stationary phase. Thin layer chromatography was carried out on aluminium plates pre-coated with Merck silica gel 60F254 which were visualized by quenching of UV fluorescence or by staining with a 10% methanol phosphomolybdic acid solution followed by heat. Preparative HPLC were performed on a Waters delta 4000 apparatus equipped with a Delta-Pack C18 column (15 Mm, 40×100 nm) and a UV detector, using a linear gradient of CH_3CN in H_2O with 0.1% TFA from 0 to 100% in 15 min. THF was distilled from sodium/benzophenone ketyl. Reagents were supplied from commercial sources (Aldrich, Fluka). The Schiff base **1** was prepared as previously described.[8]

4.1. Synthesis of (2*S*,3*R*)-2a and (2*S*,3*S*)-2b

A solution of CH_3MgBr in Et_2O (3.07 ml, 9.24 mmol) was added at −30°C to chiral Schiff base **1** (2 g, 7.1 mmol) dissolved in anhydrous THF (15 ml); DBU (1.4 ml, 9.24 mmol) was added and the mixture stirred during 20 min after which ethyl crotonate (1.06 ml, 8.52 mmol) was introduced and the reaction followed by TLC. After 30 min a NH_4Cl saturated solution (5 ml) was added and the aqueous phase extracted with EtOAc. The combined extracts were dried (Mg SO_4), evaporated under reduced pressure and the residue purified by silica gel column chromatography (Et_2O/CH_2Cl_2/petroleum ether 3/7/2). Two diastereomers were separated, the (2*S*,3*R*) isomer (major, 42% yield) and the (2*S*,3*S*) isomer (minor, 33% yield) as yellow oils.

(2*S*,3*R*)-2a (major isomer): R_f=0.48 (EtOAc/petroleum ether, 1/1); ^{1}H NMR (250 MHz, C_6D_6): δ 0.82 (s, 3H); 1.05 (t, 3H, J=7.2 Hz), 1.23 (s, 3H), 1.30 (d, 3H, J=6.8 Hz), 1.43 (s, 9H), 1.67 (s, 3H), 1.88 (m, 1H), 1.91 (d, 1H, J=4.4 Hz), 2.25 (t, 1H, J=5.9 Hz), 2.35 (m, 1H), 2.6 (m, 3H), 2.84 (m, 2H), 3.1 (m, 1H), 4.08 (q, 2H, J=7.1 Hz), 4.2 (d, 1H, J=6.2 Hz); MS (ESI) m/z: 396.3 $(M+H)^+$, 813.5 $(2M+Na^+)$.

(2*S*,3*S*)-2b (minor isomer): R_f=0.41 (EtOAc/petroleum ether, 1/1); ^{1}H NMR (250 MHz, C_6D_6): δ 0.8 (s, 3H), 1.08 (t, 3H, J=7.1 Hz), 1.25 (s, 3H), 1.35 (d, 3H, J=6.8 Hz), 1.45 (s, 9H), 1.7 (s, 3H), 1.85 (m, 1H), 1.97 (d, 1H, J=10.1 Hz), 2.25 (t, 1H, J=5.9 Hz), 2.39 (m, 3H), 2.52 (m, 2H), 2.88 (s, 1H), 3.12 (m, 1H), 4.06 (q, 2H, J=7.1 Hz), 4.41 (d, 1H, J=6.2 Hz); MS (ESI) m/z: 396.3 $(M+H)^+$, 813.5 $(2M+Na^+)$.

4.2. Synthesis of (2S,3*R*)-3a and (2*S*,3*S*)-3b

Citric acid solution (15%, 4.4 ml, 2.1 mmol) was added to a solution of Schiff base (0.8 g, 2 mmol) in THF (5.8 ml). The mixture was stirred at room temperature for 4 days. After evaporation of the solvent, the residue was dissolved in H_2O (10 ml) and washed with Et_2O (3×15 ml). The aqueous phase was adjusted to pH 7 using Na_2CO_3 and the amino ester was extracted with Et_2O (3×5 ml). The organic phase was dried ($MgSO_4$) and evaporated under reduced pressure to give a yellow oil.

(2*S*,3*R*)-3a: Yield 50% R_f=0.25 (EtOAc/petroleum ether, 1/1); ^{1}H NMR (250 MHz, C_6D_6): δ 1.01 (d, 3H, J=7 Hz), 1.08 (t, 3H, J=7.1 Hz), 1.42 (s, 9H), 2.34 (dd, 1H, J=14.3 Hz, 6.8 Hz), 2.63 (dd, 1H, J=14.3 Hz, 6.5 Hz), 2.69 (m, 1H), 3.43 (d, 1H, J=3.4 Hz), 4.06 (q, 2H, J=7.1 Hz); MS (ESI) m/z: 246.2 $(M+H)^+$, 190.1 $(M-{}^t\mathrm{Bu}+H)^+$.

(2*S*,3*S*)-3b: Yield 67% R_f=0.1 (EtOAc/petroleum ether, 1/1); ^{1}H NMR (250 MHz, C_6D_6): δ 1.09 (t, 3H, J=7.1 Hz), 1.1 (d, 3H, J=6.4 Hz), 1.45 (s, 9H), 2.26 (dd, 1H, J=14.9 Hz, 8.7 Hz), 2.5 (m, 1H), 2.67 (dd, 1H, J=14.9 Hz, 4.5 Hz), 3.17 (d, 1H, J=5.7 Hz), 4.06 (q, 2H, J=7.1 Hz); MS (ESI) m/z: 246.2 $(M+H)^+$, 190.1 $(M-{}^t\mathrm{Bu}+H)^+$.

4.3. Synthesis of (2*S*,3*R*) 5a and (2*S*,3*S*) 5b *tert*-butyl-3-methylpyroglutamates

On standing at room temperature for 12 h, **3a** and **3b** cyclised to afford the corresponding pyroglutamates in quantitative yield.

(2*S*,3*R*)-5a: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.05 (d, 3H, J=7 Hz), 1.42 (s, 9H), 2 (dd, 1H, J=16.5 Hz, 6.8 Hz), 2.41 (d.d, 1H, J=16.5 Hz, 8.1 Hz), 2.73 (m, 1H), 4.05 (d, 1H, J=7.5 Hz), 6.2 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 16.03, 28.5, 33.1, 38.28, 60.86, 82.91, 170.27, 178.06; MS (FAB$^+$) m/z: 200 $(M+H)^+$, 399 $(2M+H)^+$. HRMS: m/z calcd for $C_{10}H_{18}NO_3$ 200.1287, found 200.1284.

(2*S*,3*R*)-5b: ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21 (d, 3H, J=6.1 Hz), 1.4 (s, 9H), 1.97 (m, 1H), 2.48 (m, 2H), 3.65 (d, 1H, J=5.7 Hz), 6.4 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 20.38, 38.37, 34.64, 38.6, 63.5, 82.69, 171.15, 177.35; MS (FAB$^+$) m/z: 200 $(M+H)^+$, 399 $(2M+H)^+$. HRMS: m/z calcd for $C_{10}H_{18}NO_3$ 200.1287, found 200.1284.

4.4. Synthesis of (2*S*,3*R*)-4a and (2*S*,3*S*)-4b 3-methyl glutamic acids

The amino ester **3a** (or **3b**) was dissolved in 3N HCl solution (30 equiv.) and the mixture heated under reflux for 3 h. After evaporation under reduced pressure, the hydrochloride was dissolved in MeOH and propylene oxide was added. A precipitate appeared after 15 min and was filtered; the solid was washed three times with Et_2O and dried to afford the amino acid in 95% yield.

(2*S*,3*R*)-4a: mp 171–172°C; ^{1}H NMR (400 MHz, D_2O): δ 0.73 (d, 3H, $J=5.8$ Hz), 2.05 (m, 1H), 2.3 (m, 2H), 3.45 (d, 1H, $J=4.2$ Hz); ^{13}C NMR (400 MHz), D_2O): δ 14.85, 31.3, 38.21, 58.81, 173.56, 177.03; MS (FAB$^+$) $m/z=162$ (M+H)$^+$, 323 (2M+H)$^+$; HRMS: m/z calcd for $C_6H_{13}NO_4$ 162.0767, found 162.0775; $[\alpha]_D=+22.3$ (C1, 6N HCl) $[\alpha]_D$ lit.[5] $=+22.6$ (c 1.03, 6N HCl).

(2*S*,3*S*)-4b: mp 169–170°C; ^{1}H NMR (400 MHz, D_2O): δ 0.95 (d, 3H, $J=6.8$ Hz), 2.3 (m, 1H), 2.39 (m, 1H), 2.5 (m, 1H), 3.7 (d, 1H), $J=3.7$ Hz); ^{13}C NMR (400 MHz), D_2O): δ 14.73, 31.28, 38.2, 59.56, 173.18, 177.22; MS (FAB$^+$) $m/z=162$ (M+H)$^+$; HRMS: m/z calcd for $C_6H_{13}NO_4$ 162.0767, found 162.0760; $[\alpha]_D=+36.3$ (c 1, 6N HCl) $[\alpha]_D$ lit.[5] $=36.8$ (c 1.03, 6N HCl).

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