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DIASTEREOSELECTIVE SYNTHESIS OF (2*S*,4*R*)-4-METHYLGLUTAMIC ACID (SYM 2081): A HIGH AFFINITY AND SELECTIVE LIGAND AT THE KAINATE SUBTYPE OF GLUTAMATE RECEPTORS

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Abstract: (2*S*,4*R*)-4-Methylglutamic acid, a high affinity (IC_{50} = 35 nM) and selective ligand for the kainate (KA) receptor subtype, has been synthesized in multi-gram quantities starting from (*S*)-1-*t*-butoxycarbonyl-5-*t*-butyldiphenylsiloxymethylpyrrolidine-2-one. The diastereoselective methylation step provides the required *trans* product in 47% isolated yield.

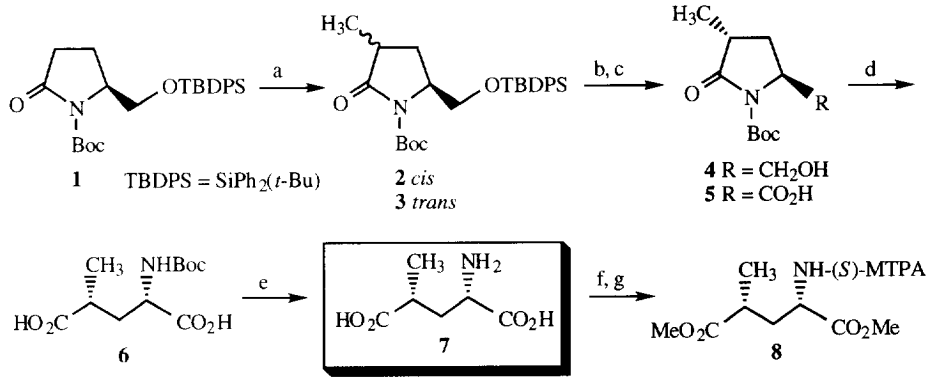
L-Glutamic acid is one of the major excitatory neurotransmitters in the mammalian brain, and mediates synaptic responses *via* ionotropic and metabotropic receptors.[1] The ionotropic glutamate receptors were traditionally classified as *N*-methyl-*D*-aspartate (NMDA) and non-NMDA receptors. The non-NMDA receptors have since been subdivided into 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) and kainic acid (KA) receptors.[2] Excessive stimulation of glutamate receptors has been associated with the pathology of a number of neurodegenerative and psychiatric disorders, such as stroke, epilepsy, Huntington's chorea, Alzheimer's, depression and anxiety.[2,3] Thus, selectively modulating excitatory neurotransmission at these receptors may have potential therapeutic use for treating neurodegenerative and psychiatric diseases. To date there has been little progress in neuropharmacological studies directed towards understanding the function of KA receptor subtypes, it is mainly due to the lack of a selective and high affinity ligand.

As part of our current efforts to develop agents capable of selectively modulating receptor subtypes of the glutamate receptors, we isolated (2*S*,4*R*)-4-methylglutamic acid (SYM 2081) by resolution of its racemate and have identified it as a high affinity, selective ligand at the KA receptor subtype (IC_{50} = 35 nM).[4] Even though, (2*S*,4*R*)-4-methylglutamic acid had been prepared through diastereomeric mixture via enzymatic synthesis,[5,6] or by using the nickel (II) complex as chiral auxiliary,[7] the scale in these preparations was very limited. We now report a diastereoselective synthesis of (2*S*,4*R*)-4-methylglutamic acid (**7**) on multi-gram scale (Scheme 1).

(*S*)-1-*t*-Butoxycarbonyl-5-*t*-butyldiphenylsilyloxymethylpyrrolidine-2-one (**1**)[8,9] was treated with one equivalent of lithium bis(trimethylsilyl)amide in THF at -78 °C to generate the corresponding enolate which was quenched with excess iodomethane to provide 4-methylated products **2** and **3** along with some unreacted starting materials. The desired *trans* isomer **3** was obtained as a predominant product (*cis*:*trans* is 1:6).[8] If more than one equivalent of base was used in order to drive the reaction to completion, varying amounts of dimethylated product were isolated. The predominant *trans* outcome of the reaction is a consequence of the electrophile approaching the face of the enolate opposite to the sterically demanding *t*-butyldiphenylsilyl (TBDPS) protecting group. The proton NMR spectrum exhibits that characteristic C(4) proton of the*trans* isomer **3** is at lower field (2.82 ppm) than that of the *cis* isomer **2** (2.56 ppm). This stereochemical assignment was confirmed by comparison of the chiral Mosher amide derivative to the previously prepared sample.[4]

The desired *trans* product **3** was purified by column chromatography and followed by crystallization from hexanes. The TBDPS protecting group was selectively removed by treatment of **3** with tetrabutylammonium fluoride to provide the primary alcohol **4**. Oxidation of the alcohol **4** by use of the Sharpless procedure[10] gave the corresponding acid **5**, which was hydrolyzed with lithium hydroxide in aqueous THF to afford the ring opening product **6**.[11] Finally, treatment of **6** with TFA in dichloromethane afforded (2*S*,4*R*)-4-methylglutamic acid (**7**).

Scheme 1



(a) $LiN(SiMe_3)_2$, THF, -78 °C, CH_3I; (b) n-Bu_4NF, HOAc, THF; (c) $NaIO_4$, $RuCl_3$, CH_3CN, CCl_4, H_2O; (d) LiOH, THF, H_2O; (e) TFA, CH_2Cl_2; (f) $SOCl_2$, MeOH; (g) (*S*)-(+)-α-Methoxy-α-(trifluoromethyl)-phenylacetyl chloride (MTPA-Cl), aqueous 20% Na_2CO_3, CH_2Cl_2.

The enantiomeric purity of **7** was ascertained after conversion to the corresponding (*S*)-N-α-methoxy-α-(trifluoromethyl)phenylacetyl dimethyl ester **8**. Mosher amide **8** was obtained by esterification of **7** with thionyl chloride in methanol followed by treatment with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride under Schotten-Baumann conditions.[12] Proton NMR analysis of the crude Mosher amide dimethyl ester **8** revealed identical patterns[13] to those of the Mosher amide sample (assigned as 2*S*,4*R* stereochemistry) prepared previously *via* chemical resolution,[4] characterized with the methyl ester singlets at 3.62 ppm and 3.76 ppm respectively, the methoxy group quartet at 3.54 ppm and amide proton doublet at 7.22 ppm. This indicates that no racemization had taken place during this synthetic sequence. In addition, compound **7** demonstrated an inhibition of [^{3}H]KA binding to cortical membranes[14] with an IC_{50} consistant with the previous value (IC_{50} = 35 nM), without any significant interaction with AMPA and NMDA receptors (IC_{50} greater than 7 μM for both). These data are in agreement with those obtained previously by using (2*S*,4*R*)-4-methylglutamic acid.[4]

In summary, (2*S*,4*R*)-4-methylglutamic acid was diastereoselectively synthesized from (*S*)-1-*t*-butoxycarbonyl-5-*t*-butyldiphenylsilyloxymethylpyrrolidine-2-one, which proved to be a workable route to prepare enantiomerically pure (2*S*,4*R*)-4-methylglutamic acid in multi-gram scale. Additional pharmacological and mechanistic studies are currently in progress. (2*S*,4*R*)-4-Methylglutamic acid (**7**) will be a valuable tool for elucidating the physiological and pharmacological functions of the KA receptor subtype of glutamate receptors.

Experimental Section

(3*R*,5*S*)-1-*t*-Butoxycarbonyl-5-*t*-butyldiphenylsiloxymethyl-3-methyl-pyrrolidine-2-one (3)

To a solution of **1** (15 g, 33 mmol) in THF (250 mL) at -78 °C was added $LiN(SiMe_3)_2$ (35 mL, 35 mmol). After stirring for 1 h, iodomethane (6.2 mL, 100 mmol) was added. The reaction mixture was stirred for another 2 h at -78 °C and then quenched with acetic acid. It was concentrated and diluted with water (200 mL) and extracted with EtOAc (100 mL x 3). The combined extracts were washed with brine and dried over $MgSO_4$. The solvent was evaporated and the residue was purified by flash column chromatography on silica gel, eluting with EtOAc:Hex (1:2.5). The desired (3*R*,5*S*)*trans* isomer **3** was eluted first from the column and crystallized from hexanes as white crystals (7.3 g, 47%), and the *cis* isomer **2** was obtained as an oil (0.9 g, 6%) along with the recovered starting material (4.5 g, 30%). The *trans* isomer **3**, mp 84-85 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.69-7.60 (m, 4H), 7.48-7.37 (m, 6H), 4.12 (m, 1H), 3.85 (dd, J = 10.3, 4.7 Hz, 1H), 3.72 (dd, J =10.3, 2.8 Hz, 1H), 2.82 (m, 1H), 2.31 (dd, J = 12.7, 8.9 Hz, 1H), 1.75 (ddd, J = 12.7, 11.5, 9.0 Hz, 1H), 1.42 (s, 9H), 1.20 (d, J = 7.1 Hz, 3H), 1.02 (s, 9H). MS (CI) *m/z* 468 (M + H), 452, 412, 368, 354, 290, 232, 212, 197, 170, 135. The *cis* isomer **2**, ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.67-7.57 (m, 4H), 7.43-7.32 (m, 6H), 4.09 (m, 1H), 3.90 (dd, J = 10.1, 5.4 Hz, 1H), 3.78 (dd, J = 10.1, 2.9 Hz, 1H), 2.56 (m, 1H), 2.35 (ddd, J = 12.8, 10.2, 8.1 Hz, 1H), 1.80 (ddd, J = 12.8, 8.3, 6.5 Hz, 1H), 1.42 (s, 9H), 1.25 (d, J = 7.1 Hz, 3H), 1.05 (s, 9H).

(3*R*,5*S*)-1-*t*-Butoxycarbonyl-5-hydroxymethyl-3-methylpyrrolidine-2-one (4)

To a solution of **3** (18.6 g, 40 mmol) and 20% acetic acid (25 mL, 88 mmol) in THF (15 mL) at 0 °C was added a 1.0 M solution of Bu_4NF in THF (160 mL, 160 mmol). The solution was stirred overnight at room temperature. EtOAc (500 mL) was added and the organic phase was extracted with 20% NH_4Cl solution (3 x 200 mL). The combined aqueous phases were extracted with EtOAc (200 mL). The combined organic phases were washed with brine (500 mL), dried over $MgSO_4$ and evaporated. The residue was purified by filtration through silica gel, eluted with EtOAc:Hex (7:3) to give 9.5 g of **4** as an oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 4.18 (m, 1H), 3.84 (dd, J = 11.3, 4.4 Hz, 1H), 3.74 (dd, J = 11.3, 4.4 Hz, 1H), 2.78 (m, 1H), 2.48 (br s, 1H), 2.20 (dd, J = 12.7, 8.7 Hz, 1H), 1.76 (ddd, J = 12.7, 11.5, 8.9 Hz, 1H), 1.52 (s, 9H), 1.18 (d, J = 7.1 Hz, 3H).

(3*R*,5*S*)-1-*t*-Butoxycarbonyl-5-carboxy-3-methylpyrrolidine-2-one (5)

To a solution of **4** (9.5 g) in a solvent mixture of CH_3CN:CCl_4:H_2O (2:2:3, 210 mL) was added $NaIO_4$ (26 g, 120 mmol) and $RuCl_3$ (2.2 mol%, 0.18 g). The solution was stirred for 3 h at room temperature and then diluted by the addition of CH_2Cl_2 (500 mL) and brine (200 mL). The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 200 mL). The combined organic phases were dried over $MgSO_4$ and concentrated. The residue was filtered through a pad of silica gel, eluted with EtOAc:Hex (8:2) containing 2% formic acid. The filtrate was concentrated, azeotroping with benzene. The residue was crystallized in EtOAc and hexane to give 8.7 g of the acid **5** (90% yield for two steps). mp 134-135 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.65 (br s, 1H), 4.60 (dd, J = 9.6, 1.2 Hz, 1H), 2.70 (m, 1H), 2.40 (ddd, J = 13.2, 8.5, 1.2 Hz, 1H), 2.0 (ddd, J = 13.2, 11.8, 9.7 Hz, 1H), 1.55 (s, 9H), 1.25 (d, J = 7.0 Hz, 3H).

(2*S*,4*R*)-N-*t*-Butoxycarbonyl-4-methylglutamic Acid (6)

A solution of **5** (6.2 g, 25.5 mmol) in THF (50 mL) was treated with LiOH (3.20 g, 80 mmol) and water (5 mL). After being stirred for 16 h at room temperature, THF was removed *in vacuo* and water (20 mL) was added. The pH was adjusted to 4 by the addition of acetic acid, ether (100 mL) was then added and the layers were separated. The aqueous phase was extracted with ether (100 mL x 3) and the combined organic phases were washed with brine, dried over Na_2SO_4. The solvent was evaporated followed by azeotroping with toluene. The residue was dried under high vacuum to give 6.58 g of the product **6** as a white foam which was carried on to the next step without further purification, ^{1}H NMR (200 MHz, D_2O): δ 3.95 (dd, J = 10.4, 4.6 Hz, 1H), 2.45 (m, 1H), 2.0 (m, 1H), 1.60 (ddd, J = 14.3, 10.3, 4.2 Hz, 1H), 1.25 (s, 9H), 1.1 (d, J = 7.1 Hz, 3H).

(2*S*,4*R*)-4-Methylglutamic Acid (7)

The N-Boc protected diacid **5** was stirred in 100 mL of TFA and CH_2Cl_2 (40:60) for 3 h at room temperature. The reaction mixture was concentrated *in vacuo* and the residue was azeotroped with toluene (50 mL). The residue was dissolved in 150 mL of water and extracted with a 5% solution of trioctylamine in chloroform (3 x 200 mL), followed by chloroform 3 x 200 mL) The combined aqueous phases were lyophilyzed for 48 h to give the product **7** (4.6 g, 66% in two steps) as a white foam which was recrystallized from acetone and water. mp 169-170 °C, $[\alpha]_D^{25}$ = +24° (c 0.96, 6 N HCl) [Lit., $[\alpha]_D^{20}$ = +23° (c 2.5, 6 N HCl)[5] and $[\alpha]_D^{25}$ = +24° (c 0.013, 6 N HCl)[6]]. ^{1}H NMR (200 MHz, D_2O): δ 3.61 (dd, J = 7.7, 5.9 Hz, 1H), 2.50 (dqd, J = 8.6, 7.0, 5.4 Hz, 1H), 2.08 (ddd, J = 14.7, 8.6, 5.9 Hz, 1H), 1.70 (ddd, J = 14.7, 7.7, 5.4 Hz, 1H), 1.05 (d, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, D_2O): 181.3, 174.8, 54.0, 37.3, 34.9, 18.0. MS (CI) m/z 162 (m+H), 144, 128, 116, 98. Anal. Calcd for $C_6H_{11}NO_4$: C, 44.71; H, 6.88; N, 8.69. Found: C, 44.59; H, 6.85; N, 8.61.

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