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Stereospecific synthesis and absolute configuration of the (2*S*,3*S*,4*S*)-isomer of 2-methyl-2-(carboxycyclopropyl)glycine (MCCG)

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Abstract—The conformationally restricted metabotropic glutamate receptor antagonist (2*S*,3*S*,4*S*)-2-methyl-2-(carboxycyclopropyl)glycine **1** (MCCG) has been synthesized in a stereoselective manner (>99% ee) with the (2*S*,3*S*,4*S*) absolute configuration of this molecule being confirmed by X-ray crystallographic analysis. Subsequent physico–chemical studies were undertaken and the data are at odds with those of the commercially available product.

1. Introduction

Many rigid or conformationally restricted analogs of the excitatory neurotransmitter L-glutamate **2** (L-Glu, Fig. 1) have now been synthesized for the elucidation of the pharmacology of glutamate receptors located in the central nervous systems of many species.[1–3] Glutamate receptors are now divided into two broad categories, those associated with the neurotransmission of excitatory responses mediated through ion channel-coupled receptors (ionotropic glutamate receptors (iGluRs) and those associated with neuroregulatory processes mediated through G-protein-coupled receptors, known as metabotropic glutamate receptors (mGluRs). L-Glu analogs containing pharmacophoric functional groups of known geometry and stereochemistry have also been used to differentiate between mGluRs and iGluRs and then to further differentiate the receptor sub-types contained within each family. To date, eight distinct metabotropic glutamate receptor proteins (mGluR1–8) have been identified and divided into three subgroups according to sequence homology, signal transduction mechanism and pharmacology.[4,5] Group I is linked to the activation of phospholipase C that subsequently increases the hydrolysis of phosphatidyl inositol (PI), while groups II and III are both negatively coupled to adenylate cyclase activity. The mGluRs have been implicated in both normal and pathological neurotransmission in the mammalian central nervous system and are thus considered novel targets for therapeutic intervention.

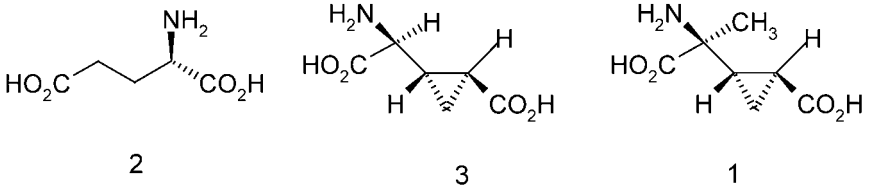

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Figure 1.

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The use of cyclopropyl glycine derivatives in pharmacological studies on mGluRs is well documented[7–9] and examples within this family of compounds can be found which interact with both individual and groups of iGluRs and mGluRs.[9] One particular compound, 2-(carboxycyclopropyl)glycine (CCG), has been synthesized in its eight individual isomeric forms and subsequently tested in pharmacological systems using mGluRs.[6] A number of the analogs of this compound show pharmacological activity, but of particular importance to this current study is the observation that the (2*S*,3*S*,4*S*)-isomer of CCG (L-CCG-I) **3** is a potent group II mGluR agonist.[9] Replacement of the α-amino acid proton of **3** with a methyl group produces MCCG **1**, transforming this molecule from an agonist into an antagonist.[7] The original communication concerning the synthesis (without X-ray crystallography validation), assigned the configurations of the (2*S*,3*S*,4*S*)- and (2*S*,3*R*,4*R*)-isomers with some detail of physical data.[10] Since that time the widespread use of the supposed (2*S*,3*S*,4*S*)-isomer has become standard in the field both as a pharmacological tool and as a model for continuing SAR studies and new compound synthesis. In our continuing program to elucidate mGluR function we had occasion to synthesise **1** by a route slightly different from the published procedure.[10] We were puzzled to find that NMR and chiroptical data agree with the published figures of the supposed (2*S*,3*R*,4*R*)-isomer and proceeded to produce crystals for X-ray determination of configuration. This analysis confirmed that our compound has the (2*S*,3*S*,4*S*)-configuration. Using expressed mGluR2 receptors we have compared the actions of our compound with that of the commercially available product. Both are antagonists, however our compound is about three times more potent than the commercially available one.

2. Chemistry

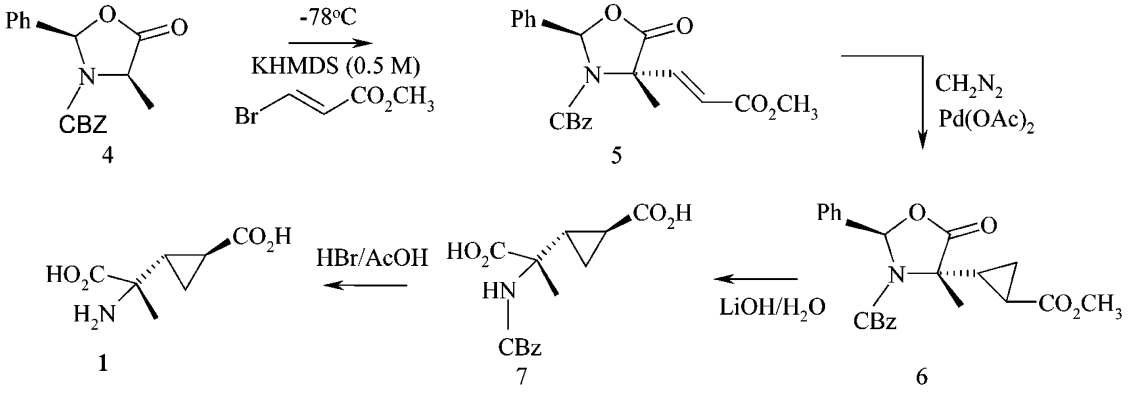
The route used for the synthesis of (2*S*,3*S*,4*S*)-MCCG **1** (Scheme 1) was developed in order to avoid a complex cyclopropanation step in the published method.[10] Starting with the known[11] (2*R*,4*R*) oxazolidinone {$[\alpha]^{20}=+27.0$} **4**, alkylation with methyl-(*E*)-3-bromopropenoate took place stereospecifically to give the unsaturated adduct **5** in 62% yield. In this case the alkyl group reacted at the *Re* face with high selectivity. This process has been called 'self-reproduction of chirality' by Seebach.[13] This compound can then be cyclopropanated with diazomethane in the presence of palladium acetate, to give one isomer only of **6**. Two subsequent deprotection and hydrolysis steps yield **1** as a single isomer $[\alpha]_D=+77$ (*c* 0.5, H_2O) {commercial MCCG, $[\alpha]_D=-54.5$ (*c* 0.12, H_2O)} and in an enantiomeric excess of 99% as judged by chiral HPLC (Chirex column, D-penicillamine stationary phase, 1 mM $CuSO_4$ mobile phase).

3. X-Ray studies

A crystal of **1** with dimensions 0.15×0.10×0.12 mm was mounted on a glass fiber. Room temperature data were collected on a Rigaku/ADSC CCD area detector in two sets of scans ($\phi=0.0$ to 190.0°, $\chi=0°$; and $\omega=-18.0$ to 23.0°, $\chi=-90°$) using 1.00° oscillations with 35.0-s exposures. The crystal-to-detector distance was 39.94(3) mm with a detector swing angle of −5°. The data was processed using the d*TREK program and corrected for Lorentz and polarization effects.

A primitive orthorhombic unit cell was found, while the space group was determined on the basis of systematic absences. The determination of the configuration has been performed as a relative configurational assigment in relation to the absolute configuration[12] at C(2) and was found to exist as zwitterionic and contain an additional water molecule in the lattice. The absolute configurations of C(4) and C(6) determined to be *S* (Fig. 2). All non-hydrogen atoms were refined anisotropically, while all hydrogens involved in hydrogen-bonding were refined isotropically. All other hydrogens were included in calculated positions. A list of crystallographic data appears in Table 1. All calculations were performed using the teXsan[12] crystallographic software package of Molecular Structure Corporation.

Comparative analyses (specific rotation value, ^1H NMR) were performed on the commercial and synthe-



Scheme 1.

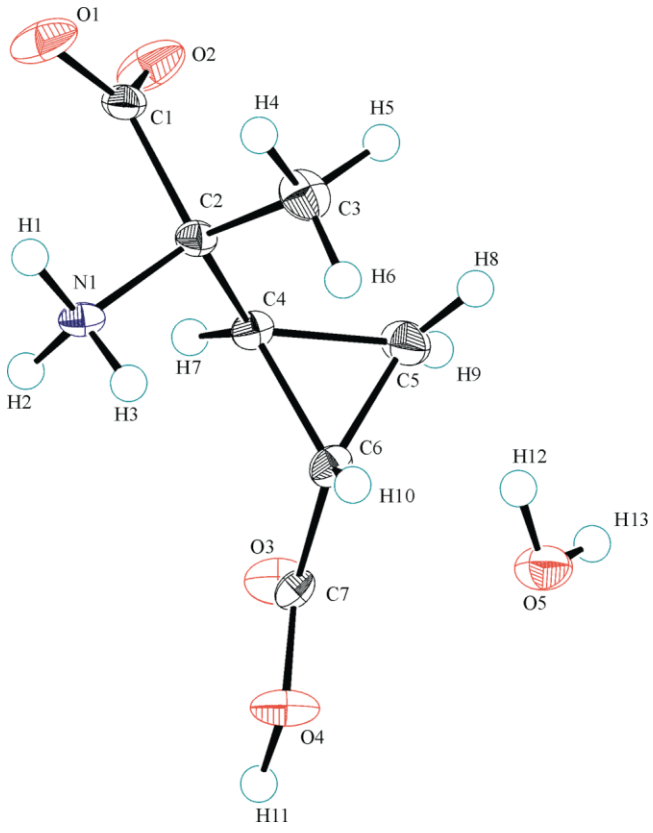


Figure 2. X-Ray crystal structure of (2*S*,3*S*,4*S*)-MCCG.

Table 1. Crystallographic data

Formula	$C_7H_{13}NO_5$
F_w	191.18
Colour, habit	Clear, block
Crystal size (mm)	$0.15\times0.12\times0.10$
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	7.0802(9)
b (Å)	10.0398(8)
c (Å)	12.170(2)
α (°)	90
β (°)	90
γ (°)	90
V (Å^3)	865.1(2)
Z	4
D_{calcd} (g/cm^3)	1.468
$F(000)$	408.00
Radiation	Mo Kα
μ (mm^{-1})	0.125
Transmission factors	0.993–1.000
φ Oscillation range ($\chi=0.0$) (°)	0.0–190.0
ω Oscillation range ($\chi=90.0$) (°)	−18.0–23.0
$2\theta_{max}$ (°)	50.2
Total reflections	6164
Unique reflections	1535
No. of variables	142
R_{merge}	0.089
R, R_w (on F^2, all data)	0.076, 0.106
Goodness-of-fit	0.88
Max Δ/σ (final cycle)	0.02
Residual density (e/Å^3)	0.40, −0.35

$R=\Sigma||F_o^2|-|F_c^2||/\Sigma|F_o^2|$, $R_w=\Sigma((F_o^2-F_c^2)^2/\Sigma w(F_o^2)^2)^{0.5}$.

sized MCCG 1 and the data clearly indicates that our finding are consistent with the compound in the previously published investigation,[10] which is assigned the (2*S*,3*S*,4*S*)-configuration. The discrepancies between our study and the published synthesis[10] suggest a transposition of the two MCCG isomers in the originally published structure and what is assigned as the (2*S*,3*S*,4*S*)-configuration is in fact the (2*S*,3*R*,4*R*)-configuration.

Many of the receptor systems associated with glutamic acid demonstrate a clear and strong dependence on both geometry and stereochemistry. We also believe this to be the case for the isomers of MCCG. Using the well documented isomers of LCCG as a template we can see that the mGluR2 receptor demonstrates a clear preference for the (*S*)-configuration at the α-amino acid carbon and also a 50–100 times preference for the (2*S*,3*S*,4*S*)-isomer over the (2*S*,3*R*,4*R*)-isomer.[9] In this case the stereochemistry of the ring is crucial and indicates that the orientation determines the agonist properties the two isomers exhibit. With the two MCCG isomers the difference in apparent IC_{50} is only about 3× between the commercial product and the (2*S*,3*S*,4*S*)-isomer. In this case the rings must be able to attain a conformation which favors antagonist activity in both cases. It is surprising that commercial MCCG with the (2*S*,3*R*,4*R*)-configuration has substantial antagonist activity at mGluR2, since the parent compound LCCG-I does not have the same tolerance towards inversion of chiral centers at the cyclopropane ring. Of greater importance perhaps is the fact that much of the design work for next generation group II agonists and antagonists is based on the erroneous assumption that the absolute stereochemistry of the ligand is known. Our work has demonstrated the dangers of making such assumptions and the importance of careful assignment of absolute configuration.

4. Experimental

4.1. General

Melting points were obtained with a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured using a Optical Activity AA-1000 polarimeter. ^{1}H and ^{13}C spectra were recorded on a Bruker AC-200 spectrometer with $SiMe_4$ as internal standard and using $CDCl_3$ as solvent. Elemental analysis were performed by the Canadian Microanalytical Service Ltd. HPLC were determined with a Varian 9012 pump and Varian 9050 UV–vis detector. Column chromatography was performed using silica gel 60 of 230–400 mesh.

4.2. (2*R*,4*S*)-2-Phenyl-3-(carbobenzyloxy)-4-methyl-4-[(3-methoxy)carbonyl-(1*E*)-propenyl]oxazolidin-5-one, 5

To a solution of potassium hexamethyldisilazide (0.5 M in toluene 26.7 mL, 13.36 mmol) at −78°C was added a solution of **4** (3.78 g, 12.15 mmol) in THF (20 mL) dropwise. After the mixture was stirred for 45 min at

−78°C a solution of methyl (*E*)-3-bromopropenoate (2.20 g, 13.36 mmol) in THF (5 mL) at −78°C. The resultant mixture was stirred for a further 45 min and then quenched with sat. NH_4Cl (20 mL). The aqueous layer was extracted with Et_2O (2×30 mL) and the combined organic layers were dried over $MgSO_4$ and evaporated to give the crude product as an oil. The oil was chromatographed on silica (hexanes:ethyl acetate 4:1) to give 3.0 g (62%) of **5**. $[\alpha]_D^{25}=+149$ (*c* 0.18 $CHCl_3$); ^1H NMR, ($CDCl_3$) δ 1.98 (s, 3H), 3.78 (s, 3H), 4.95–5.20 (br. m, 2H), 6.1 (d, $J=15.9$ Hz, 1H), 6.5 (s, 1H), 7.0 (d, $J=15.9$ Hz, 1H), 7.2–7.4 (m, 10H). Anal. calcd for $C_{22}H_{21}NO_6$: C, 66.83; H, 5.35; N, 3.54. Found: C, 66.80; H, 5.36; N, 3.54%.

4.3. Cyclopropanation adduct, 6

To a solution of **5** (2.0 g, 5.06 mmol), $Pd(OAc)_2$ (57 mg, 0.25 mmol) and ether (65 mL) was added in a dropwise manner, a solution of CH_2N_2 in ether (100 mL) at 0°C. The mixture was stirred at rt. overnight, filtered and evaporated to give an oily residue which was chromatographed on silica (hexanes:ethyl acetate 4:1) to give pure **6** as a solid (1.4 g, 67%). mp 125–127°C. $[\alpha]_D^{25}=+84$ (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.95 (ddd, $J=8.7$, 6.7, 5.1 Hz, 1H), 1.2 (dt, $J=9.7$, 5.1 Hz, 1H), 1.81 (s, 3H), 2.18 (dt, $J=8.7$, 4.8 Hz, 1H), 2.4 (br. s, 1H), 3.67 (s, 3H), 5.1 (br. s, 2H), 6.43 (s, 1H), 7.2–7.43 (m, 10H). Anal. calcd for $C_{23}H_{23}NO_6$: C, 67.47; H, 5.66; N, 3.42. Found: C, 67.44; H, 5.66; N, 3.45%.

4.4. (2*S*,3*S*,4*S*)-*N*-Carbobenzyloxy-2-methyl-2-(carboxycyclopropyl)glycine, 7

To a stirred solution of **6** (1.18 g, 2.88 mmol) in THF/H_2O (3:1, 40 mL) was added $LiOH\cdot H_2O$ (484 mg, 11.52 mmol) at 4°C. The mixture was stirred overnight at 25°C and then 5% aqueous $NaHCO_3$ added (200 mL). The aqueous layer was washed with hexanes and acidified to pH 2 with 2 N HCl. The resulting solution was extracted with EtOAc (3×50 mL) and the organic solution dried over $MgSO_4$ and evaporated to give **7** as an oil (0.89 g, quant.); ^1H NMR ($CDCl_3$) δ 1.02 (m, 1H), 1.18 (dt, $J=9.7$, 5.1 Hz, 1H), 1.4 (s, 3H), 1.88 (br. m, 1H), 2.0 (br. m, 1H), 5.0 (s, 2H).

4.5. (2*S*,3*S*,4*S*)-2-Methyl-2-(carboxycyclopropyl)glycine, 1

To a solution of **7** (0.87 g, 2.83 mmol) in CH_2Cl_2 (3 mL) was added 32% HBr in AcOH (5 mL). The resulting solution was stirred at rt for 1.5 h. The solvent was evaporated and the residue redissolved in H_2O and evaporated again. The residue was dissolved in anhydrous ethanol (10 mL) and propylene oxide added (5 ml). The resulting mixture was briefly heated under reflux and the solvents removed in vacuo. The residue was dissolved in a small amount of H_2O and passed through a reversed-phase C18 column using H_2O as eluent. The product was recrystallized from H_2O to give pure **1** (420 mg, 85%). $[\alpha]_D^{25}=+77$ (*c* 0.7, H_2O); ^1H NMR (D_2O) δ 1.0–1.09 (m, 1H), 1.11–1.12 (m, 1H), 1.24 (s, 3H), 1.62–1.7 (m, 1H), 1.78–1.83 (m, 1H). Anal. calcd for $C_7H_{11}NO_4\cdot H_2O$: C, 43.98; H, 6.85; N, 7.33. Found: C, 43.97; H, 6.83; N, 7.35%.

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