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Synthesis of (2S,2'R,3'R)-2-(1'-[^{3}H],2',3'-Dicarboxylcyclopropyl)-glycine ([^{3}H]-DCG-IV)

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SUMMARY

The conformationally restricted analog of L-glutamic acid (L-Glu, **1**), (2S,2'R,3'R)-2-(2',3'-dicarboxylcyclopropyl)-glycine (DCG-IV, **2**) is a potent group II mGluR agonist. In order to study the distribution of group IImGluRs in the brain and to establish a radioligand binding assay we have developed a synthesis of [^{3}H]-DCG-IV (**2a**). The key intermediate, α-bromo aldehyde **7**, was prepared in four steps starting from (-)-Feist's acid (**3**). The incorporation of tritium was performed by reaction of **7** with tri-n-butyltin tritide to give **8**, which was transformed in two steps into **2a**.

Key Words: m-Glu receptors, amino acids, DCG-IV, tri-n-butyltin tritide

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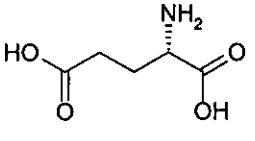
INTRODUCTION

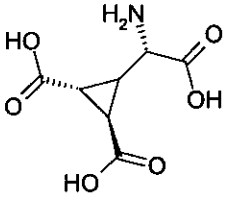
L-Glutamic acid (L-Glu, **1**) is widely recognized as the primary excitatory neurotransmitter in the mammalian central nervous system (CNS) (1). Glutamate receptors have been classified into two major classes, ionotropic glutamate receptors (iGluR) and metabotropic glutamate receptors (mGluRs) (2 - 4). The ion channel linked iGluRs are further subdivided into N-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainic acid (KA) receptors according to their responses to exogenous exitatory amino acids (EAAs) (5).

The G-protein coupled mGluRs consist of a family of at least eight receptors, grouped according to primary amino acid sequence homology, agonist pharmacology and signal transduction mechanism (6 - 7). The first group includes mGluR1 and mGluR5, which are negatively coupled to IP3/Ca2+ signal transduction *via* activation of phospholipase C, whereas the members of group II, mGluR2 and mGluR3, as well as those of group III, mGluR4, mGluR6, mGluR7 and mGluR8, are negatively linked to adenylate cyclase.

The conformationally restricted analog of L-glutamic acid (**1**), (2S,2'R,3'R)-2-(2',3'-dicarboxylcyclopropyl)-glycine (DCG-IV, **2**) (8), has revealed to be a particularly interesting compound, being a potent group II mGluR agonist with neuroprotective properties, also active as an agonist at the NMDA receptor (9 - 10).



L-Glu **1**



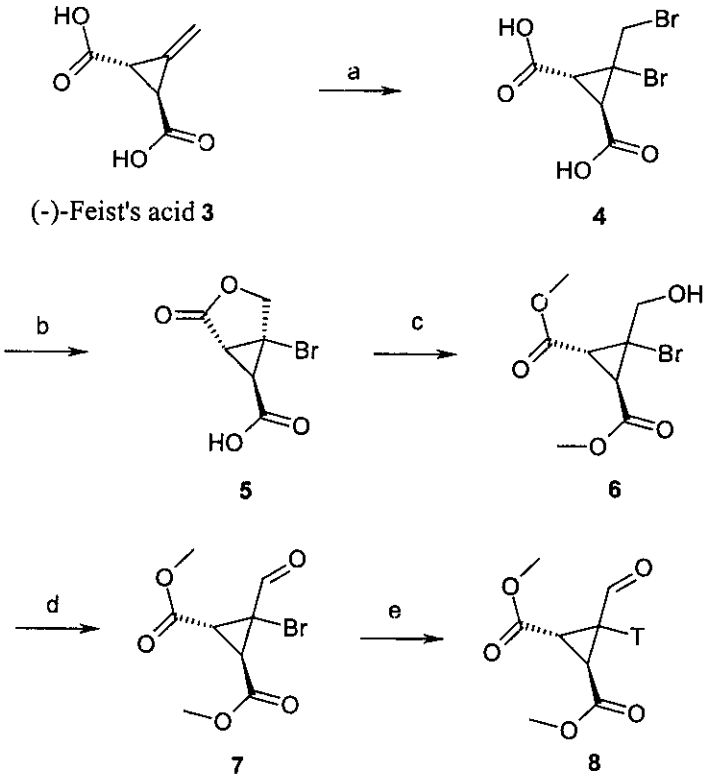
DCG-IV **2**

Figure 1. Structures of L-glutamic acid and DCG-IV

In order to study the distribution of groupII mGluRs in the brain and to establish a radioligand binding assay we have developed a synthesis of [^{3}H]-DCG-IV (**2a**).

RESULTS AND DISCUSSION

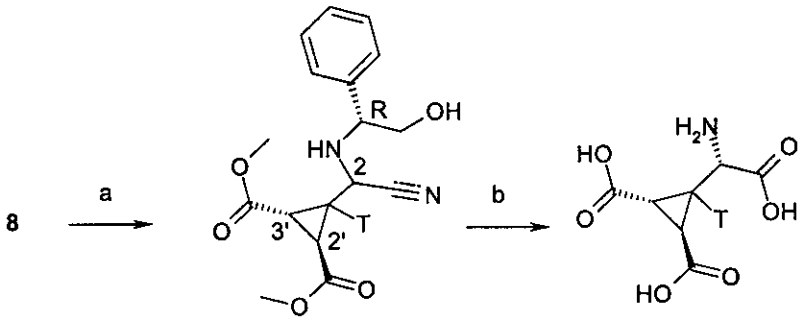
The key intermediate of the synthesis is the α-bromo aldehyde **7**, which was prepared in four steps starting from (-)-Feist's acid (**3**) (11 - 14). Bromination of **3** yielded 64% of the dibromo derivative **4**, which was heated in water to give 61% of the lactone **5**. Opening of **5** and esterification was performed in 97% yield with sulfuric acid in methanol to the alcohol **6**, which was oxidized with PCC to give 69% of the α-bromo aldehyde **7**.



Scheme 1. Synthesis of **8**. a) Br_2 in diethyl ether, RT, 16 h, 64%; b) H_2O, reflux, 4 h, 61%; c) MeOH, conc. sulfuric acid, RT, 2 h, 97%; d) PCC, $MeCl_2$, RT, 16 h, 69%; e) Bu_3SnT, AIBN, cyclohexane-THF, 50°C, 18 h.

For the incorporation of tritium a radical reaction was used. The α-bromo aldehyde **7** was treated with tri-n-butyltin tritide (15) and AIBN in a mixture of cyclohexane-THF (1:1) at 50°C to give **8**.

The key step of the synthesis is a diastereoselective Strecker reaction involving the nucleophilic addition of cyanide to the Schiff base formed by condensation of **8** with (R)-α-phenylglycinol to induce preferentially the (S)-chirality at the newly formed stereogenic center (16 - 17).



9a: 2S,2'R,3'R
9b: 2R,2'R,3'R

[^{3}H]-DCG-IV **2a**

Scheme 2. Synthesis of **2a**. a) 1. (R)-α-phenylglycinol, MeOH, RT, 6 h; 2. TMSCN, RT, 15h; b) 1. lead tetraacetate, MeOH-dichloromethane 1:1, 0°C, 15 min; 2. 6N HCl, reflux, 15 h.

Reaction of **8** with (R)-α-phenylglycinol in methanol at room temperature for 6 hours, followed by treatment with trimethylsilyl cyanide for 15 hours at room temperature yielded a mixture of the expected two α-amino nitriles **9a-b** in a ratio of 4:1. Column chromatography of the mixture yielded **9a**, which was then submitted to oxidative cleavage with lead tetraacetate, acidic hydrolysis (6N HCl), ion exchange resin chromatography and HPLC-purification to afford **2a**.

In summary, we have developed a synthesis of [^{3}H]-DCG-IV (**2a**) starting from (-)-Feist's acid (**3**) with the α-bromo aldehyde **7** as key intermediate. [^{3}H]-DCG-IV (**2a**) was used to establish a radioligand binding assay and to study the distribution of group II mGluRs in the brain (18 - 19).

EXPERIMENTAL

General.

Reagent grade solvents were used without further purification. Evaporation means removal of the solvent by use of a Büchi rotary evaporator at 30-40° C in vacuo. Silica gel used for column chromatography was Kieselgel-60 (70-230 mesh) supplied by E. Merck AG, Darmstadt. TLC plates coated with silica gel 60 F_{254} (Merck) were used. Melting points were determined with a Büchi 510 apparatus and are uncorrected. Proton NMR spectra were recorded on a Bruker AC 250 spectrometer, δ values in ppm relative to internal TMS (coupling constant *J* in Hz; multiplicity: s = singlett, d = doublet, t = triplet, q = quartet, m = multiplet) are given. Optical rotations were determined with a Perkin-Elmer 241polarimeter, c in g/100 ml. Mass spectra were recorded with a MS 9 apparatus updated with a Finnigan MAT data system SS 200.

Chemistry.

(1R,2R)-3-Bromo-3-bromomethyl-cyclopropane-1,2-dicarboxylic Acid (4).

To a cooled (0°C) and stirred solution of (1S,2S)-3-methylene-cyclopropane-1,2-dicarboxylic acid (**3**) (11 - 14) (4.0 g, 28.2 mmol) in diethyl ether (250 ml) was added bromine (2 ml) and stirring was continued over a period of 16 h at room temperature. Filtration, evaporation of the solvent and crystallization of the crude product from dichloromethane/hexane yielded **4** (5.45 g, 64%) as a pale brown solid. mp 233°C (dec.); $[\alpha]^{20}_{D}$ = +80° (c = 0.25 in MeOH); ^{1}H NMR ([D_6]DMSO): δ = 2.58 (d, *J* = 6.5 Hz, 1 H), 2.63 (d, *J* = 6.5 Hz, 1 H), 4.07 (d, *J* = 11 Hz, 1 H), 4.17 (d, *J* = 11 Hz, 1 H); MS (FAB) *m/z* = 299, 301, 303 [M-H$^+$].

(1RS,5R,6R)-1-Bromo-4-oxa-bicyclo[3.1.0]hexane-6-carboxylic Acid (5).

A solution of (1R,2R)-3-bromo-3-bromomethyl-cyclopropane-1,2-dicarboxylic acid (**4**) (5.4 g, 17.9 mmol) in water (100 ml) was boiled under reflux conditions over a period of 4 h. Filtration, evaporation of the solvent and column chromatography of the crude product (dichloromethane/methanol 9:1) gave **5** (2.75 g) as a solid.

Further crystallization from dichloromethane/hexane yielded 7 (2.41 g/ 61%) as a light yellow solid. mp 196-198°C; $[\alpha]^{20}_D$ = -36° (c = 0.25 in MeOH); ^{1}H NMR ([D_6]DMSO): δ = 2.64 (d, *J* = 3 Hz, 1 H), 2.93 (d, *J* = 3 Hz, 1 H), 4.56 (d, *J* = 10 Hz, 1 H), 4.71 (d, *J* = 10 Hz, 1 H); MS (EI) *m/z* = 220, 222 [M^+], 202, 204 (48) [M^+ -H_2O], 123 (100) [M^+ -H_2O, -Br], 97 (98).

(1R,2R)-3-Bromo-1,2-dicarbomethoxy-3-hydroxymethyl-cyclopropane (6).

To a stirred solution of (1RS,5R,6R)-1-bromo-4-oxa-bicyclo[3.1.0]hexane-6-carboxylic acid (**5**) (2.41 g, 10.9 mmol) in methanol (25 ml) was added sulfuric acid (conc., 2.5 ml) and stirring was continued over a period of 2 h. The reaction mixture was poured into ice/water (100 ml) and extracted with two100 ml portions of ethyl acetate. The combined organic layers were washed with water (50 ml) and two 50 ml portions of saturated sodium hydrogen carbonate solution, dried ($MgSO_4$) and evaporated to yield **6** (2.85 g, 97%) as a light yellow oil. $[\alpha]^{20}_D$ = +98.4° (c = 0.25 in MeOH); ^{1}H NMR ($CDCl_3$): δ = 2.66 (dd, *J* = 6, 8 Hz, 1 H), 2.70 (d, *J* = 6.5 Hz, 1 H), 2.87 (d, *J* = 6.5 Hz, 1 H), 3.77 (s, 3 H), 3.80 (s, 3 H), 3.99 (dd, *J* = 8, 12.5 Hz, 1 H), 4.16 (dd, *J* = 6, 12.5 Hz, 1 H); MS (EI) *m/z* = 267, 269 [M+H^+], 249, 251 (3) [M^+ -OH], 235, 237 (14) [M^+ -OMe], 207, 209 (94), 175, 177 (82), 169 (83), 155 (46), 113 (100), 59 (76).

(1R,2R)-3-Bromo-1,2-dicarbomethoxy-3-formyl-cyclopropane (7).

To a stirred solution of (1R, 2R)-3-bromo-1,2-dicarbomethoxy-3-hydroxymethyl-cyclopropane (**6**) (2.8 g, 10.5 mmol) in dichloromethane (120 ml) was added pyridinium chlorochromate (3.35 g, 15.7 mmol) and stirring was continued over a period of 16 h. Diethyl ether (120 ml) was added to the reaction mixture, which was then filtered with the aid of a whatman glass microfibre filter and evaporated. Column chromatography of the crude product (diethyl ether/hexane 1:1) yielded7 (1.93 g, 69%) as a white solid. mp 52°C; $[\alpha]^{20}_D$ = +108° (c = 0.25 in MeOH); ^{1}H NMR ($CDCl_3$): δ = 3.16 (d, *J* = 6.5 Hz, 1 H), 3.27 (d, *J* = 6.5 Hz, 1 H), 3.76 (s, 3 H), 3.83 (s, 3 H), 9.26 (s, 1 H); MS (EI) *m/z* = 265, 267 [M+H^+], 233, 235 (24) [M^+ -OMe], 204, 206 (24), 176, 178 (44), 153 (100), 125 (95), 59 (69).

Radiochemistry.

Preparation of tri-n-butyltin tritide

A 10 ml two-necked flask was attached to the tritiation apparatus and flushed with argon. n-BuLi (250 µl of a 1.39M solution, 0.347 mmol) and TMEDA (65 µl, 0.434 mmol) were added and the side neck was sealed with a 10 mm three layer septum from *Hamilton AG, CH-7402 Bonaduz*. The solution was degassed by repetitive (three times) freezing, evacuating and thawing using liquid nitrogen as coolant.

Then tritium gas was introduced (initial pressure 678 mbar) and the solution was stirred for 2.5 h, while the tritium pressure decreased to 610 mbar. A white precipitate of LiT was observed. The reaction mixture was cooled to −190°C and excess tritium gas was reabsorbed onto the uranium bed. The volatile components, including [^{3}H]-n-butane, were lyophilyzed into another flask and the vacuum was relieved using dry nitrogen. The solid LiT was suspended in THF (440 µl, distilled from $LiAlH_4$). Triethylborane (340 µl of a 1M solution) was added and after stirring for 15 min a clear solution was obtained. Then tri-n-butyltin chloride (94µl, 0.346 mmol) was added. After stirring for 15 min the solvent was lyophilyzed into another flask and the vacuum was relieved with dry nitrogen.

The two-necked flask was removed from the tritiation apparatus and the residue was suspended in n-heptane (2 ml). The total activity was 6Ci. The suspension was applied onto a Chromabond cartridge, 0.5 g silica gel, *Machery-Nagel* (Cat. No. 730073). The cartridge was rinsed with n-heptane (3 x 1ml). The total activity of the eluate was 5.03 Ci. The solvent was removed by lyophilization and the residual tri-n-butyltin tritide was immediately used in the next step.

3-[^{3}H]-(1R,2R)-1,2-Dicarbomethoxy-3-formyl-cyclopropane (8).

Tri-n-butyltin tritide (5.03 Ci) was dissolved in cyclohexane-tetrahydrofuran 1:1 (1 ml) under argon, (1R,2R)-3-bromo-1,2-dicarbomethoxy-3-formylcyclopropane (7) (48 mg, 0.181 mmol) and 2,2'-azobisisobutyronitrile (AIBN) (1.2 mg) were added and the reaction mixture was stirred for 18 h at 50°C. Then saturated potassium fluoride solution (1 ml) was added and stirring was continued for 1 h at RT.

Partitioning between diethylether and ice/water, washing the organic layers with saturated sodium chloride solution and drying over anhydrous sodium sulfate yielded crude **8** with total ^{3}H-activity of 4.84 Ci. Column chromatography on 9 g LichroprepSi60 15-25 µm (Merck #1.09336) with hexane-diethylether 1:1 afforded 2.18 Ci of **8**. The radiochemical purity was 96% according to TLC (20).

*(2S,2'R,3'R)-N-[(R)-α-Phenylglycinyl]-2-(1'-[^{3}H],2',3'-dicarbo-methoxy-cyclopropyl)-glycinonitrile (**9a**)*

A solution of 3-[^{3}H]-(1R,2R)-1,2-dicarbomethoxy-3-formyl-cyclopropane (**8**) (1.3 Ci) and (R)-α-phenyglycinol (6.8 mg, 0.05 mmol) in dry methanol (0.45 ml) was stirred for 6 h at RT. Trimethylsilyl cyanide (12 µl, 0.096 mmol) was added at 0°C and stirring was continued for 15 h at RT. Column chromatography on 7 g LichroprepSi60 15-25 µm with hexane/ethyl acetate 1:1 afforded 456 mCi ^{3}H-acivity of **9**.

*(2S,2'R,3'R)-2-(1'-[^{3}H],2',3'-Dicarboxylcyclopropyl)-glycine ([^{3}H]-DCG-1/4) (**2a**).*

To a solution of **9a** (4.2 mg, 0.0126 mmol; total ^{3}H-activity 220 mCi) in dichloromethane - methanol 1:1 (0.5 ml) was added leadtetraacetate (6.7 mg, 0.015 mmol) at 0°C under argon and the mixture was stirred for 15 min at this temperature. The solvents were evaporated, 6N hydrochloric acid (1.5 ml) was added and the reaction mixture was stirred under reflux conditions for 15 h.

The crude product was partitioned between dichloromethane and water and the aqueous phase was lyophilized. The residue was dissolved in 5 ml of water and this solution was applied onto a small cation exchange column (5x40mm, 0.6 g Dowex 50Wx4 H^{+}-form). After rinsing with water (15 ml) the product was eluted with 2N ammonium hydroxide solution (20 ml). HPLC-purification (21) of 35 mCi of this sample afforded 14.2 mCi of **2a**. To get rid of the orthophosphoric acid the solution of [^{3}H]-DCG-1/4 (**2a**) was applied onto a SP-Sephadex cation exchange column (H^{+}-form, 10x100 mm). After washing with two 10 ml aliquots of water the product was eluted with 2N ammonium hydroxide solution using a fraction collector. Total

^{3}H-activity of **2a** was 11.7 mCi. The radiochemical purity was 98.3% according to TLC (silica gel60, n-butanol - acetic acid - water 3 : 1 : 1). The specific activity determined by HPLC was 17.5 Ci/mmol.

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REFERENCES AND NOTES

1. G. L. Collinridge, R. A. Lester - *Pharmacol. Rev.* **40**: 143 – 210 (1989).
2. M.D. Cunningham, J. W. Ferkany, S. J. Enna - *Life Sci.* **54**: 135 – 148 (1994).
3. M. Hollmann, S. Heinemann - *Annu. Rev. Neurosci.* **17**: 31 – 108 (1994).
4. S. Nakanishi, M. Masu - *Annu. Rev. Biophys. Biomol. Struct.* **23**: 319 – 348 (1994).
5. J. C. Watkins, P. Kroogsgaard-Laarsen, T. Honore - *Trends Pharmacol. Sci.* **11**: 25 – 33 (1990).
6. S. Nakanishi - *Science* **258**: 597 – 603 (1992).
7. J. P. Pin, R. Duvoisin - *Neuropharmacology* **34**: 1 – 26 (1995).
8. M. Ishida, T. Saitoh, K. Shimamoto, Y. Ohfune, H. Shinozaki - *Br. J. Pharmacol.* **109**: 1169 – 1177 (1994).
9. V. Bruno, A. Copani, G. Battaglia, R. Raffaele, H. Shinozaki, F. Nicoletti - *Eur. J. Pharmacol.* **256**: 109 – 112 (1994).
10. Y. Ohfune, K. Shimamoto, M. Ishida, H. Shinozaki - *Bioorg. Med. Chem. Lett.* **3**: 15 – 18 (1993).

11. F. Feist - *Liebigs Ann.* **436**: 125 – 153 (1924).

12. F. R. Goss, C. K. Ingold, J. F. Thorpe - *J. Chem. Soc.* 127: 460 – 469 (1925).

13. A. T. Blomquist, D. T. Longone - *J. Am. Chem. Soc.* 81: 2012 –2017 (1959).

14. W. V. E. Doering, H. D. Roth - *Tetrahedron* 26: 2825 – 2835 (1970).

15. H. Andres, H. Morimoto and P. G. Williams - *Synthesis and Applications of Isotopically Labelled Compounds 1991, Proc. Int. Symp.* 4th: 40 – 45 (1992). Ed.: E. Buncel and G.W. Kabalka, Elsevier Science Publishers, Amsterdam.

16. T.K. Chakraborty, G.V. Reddy, K.A. Hussain - *Tetrahedron Lett.* **32**: 7597 – 7600 (1991).

17. R. Pellicciari, M. Marinozzi, B. Natalini, G. Costantino, R. Luneia, G. Giorgi, F. Moroni, C. Thomsen - *J. Med. Chem.* **39**: 2259 – 2269 (1996).

18. J. Cartmell, G. Adam, S. Chaboz, R. Henningsen, J. A. Kemp, A. Klingelschmidt, V. Metzler, F. Monsma, H. Schaffhauser, J. Wichmann, V. Mutel - *Brit. J. Pharmacol.* **123**: 497 – 504 (1998).

19. V. Mutel, G. Adam, S. Chaboz, J. A. Kemp, A. Klingelschmidt, J. Messer, J. Wichmann, T. Woltering, J. G. Richards - *J. Neurochem.* **71**: 2558 – 2564 (1998).

20. The loss of ^{3}H-activity during chromatography was probably due to hydrogen exchange catalyzed by the slightly acidic silica gel. It is therefore recommended to omit chromatography at this stage of the synthesis.

21. HPLC-conditions: (a) column: LiChrocart Superspher RP-18e 5µm 4x250 mm Merck #16858; (b) mobile phase: 20 mM orthophosphoric acid; (c) flow rate: 0.5 ml/min, UV-detection at 205 nm.

