

## Resolution and Regioselective Protection of Glutamic Acid Analogues. II- Synthesis, Resolution and Configuration Assignment of (+)- $\alpha$ -Methyl-4-Carboxyphenylglycine (M4CPG)

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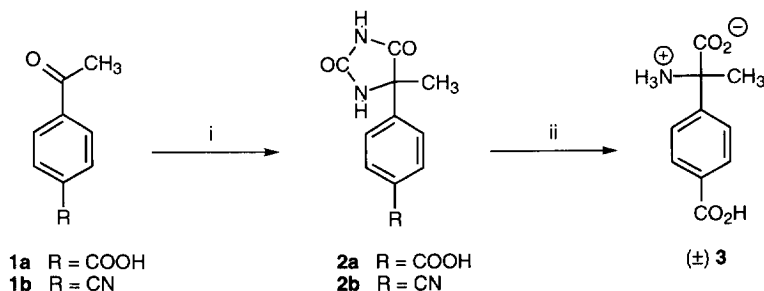
**Abstract:** Diastereomeric dipeptides (S)-Leu-(S)-M4CPG and (S)-Leu-(R)-M4CPG have been synthesized from ( $\pm$ )-M4CPG and separated by anion exchange chromatography. Absolute configuration of (S)-(+)-M4CPG was assigned by X-Ray crystallography of (S)-Leu-(S)-M4CPG.  
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No potent and selective antagonist of metabotropic glutamate receptors <sup>1</sup> was known until the recent discovery of the substituted phenylglycine family <sup>2,3</sup>. Among the numerous members of this family that have been tested <sup>4</sup>, (+)-M4CPG ( $\alpha$ -methyl-4-carboxyphenylglycine) appears to be the only potent competitive antagonist at both mGluR1 and mGluR2 receptors <sup>5,6</sup>. This compound was originally prepared by resolution of the racemic mixture through fractional crystallization of diastereomeric salts and no absolute configuration could be ascribed <sup>2</sup>. Recently, several other antagonists of either mGluR1 <sup>7</sup> or mGluR2 <sup>8</sup> receptors have been described, but (+)-M4CPG remains the only potent antagonist at both types and is therefore of major interest as a pharmacological tool. In this paper we describe the synthesis and resolution of ( $\pm$ )-M4CPG, and the absolute configuration assignment of (+)-M4CPG.

### RESULTS AND DISCUSSION

#### Synthesis of ( $\pm$ )-M4CPG.

Optically active unnatural amino acids can be prepared by enantioselective synthesis or by resolution of a racemic mixture <sup>9</sup>. Following the latter procedure, we first prepared ( $\pm$ )-M4CPG in quantitative yield using the Bücherer-Bergs' synthesis <sup>10</sup> and subsequent acidic hydrolysis (Scheme 1). Two different starting ketones have been used: 4-acetylbenzoic acid **1a**, or 4-acetylbenzotrile **1b** (the latter being much cheaper).



i : KCN, (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, 60 °C, 3/7 days; ii : 6N HCl, 110 °C

Scheme 1

### Analytical resolution of ( $\pm$ )-M4CPG.

Several liquid or gas chromatographic methods have been described for the analytical separation of D- and L-isomers of amino acids. However, very few methods are available for the separation of  $\alpha,\alpha$ -dialkylated amino acids <sup>11</sup>. No resolution was obtained by HPLC, when free ( $\pm$ )-M4CPG was injected on a Crownpack CR(+) column <sup>12</sup>, or its N-Boc dimethylester on a Pirkle column <sup>13</sup>, or using Marfey's reagent <sup>14</sup> or *o*-phthalaldehyde-N-acetylcysteine derivatization <sup>11e</sup> on a Nucleosil C18 column. GC, using a Chirasil-Val column with appropriate derivatization of the amino acid <sup>15</sup>, was similarly unsuccessful. Finally, a satisfactory analytical resolution was achieved by GC on an OV-1701 column, after derivatization to (S)-O-acetyllactyl-M4CPG diastereomers <sup>16</sup>, or better, by ligand exchange HPLC on a chiral column <sup>17,18</sup>. The second method was preferred as non derivatized amino acids are injected, and as an excellent separation is observed (Fig. 1A).

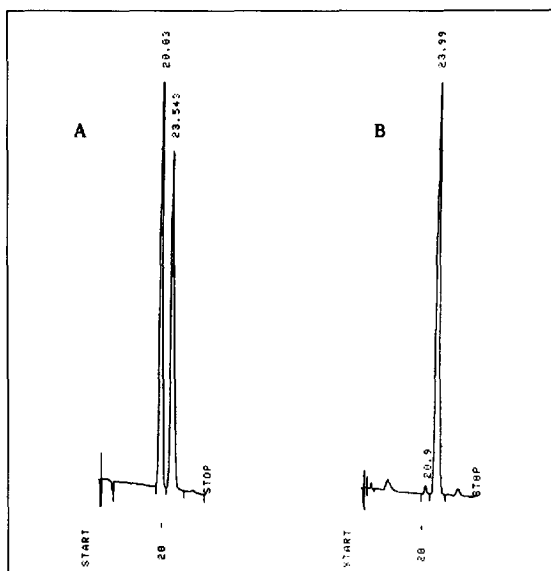
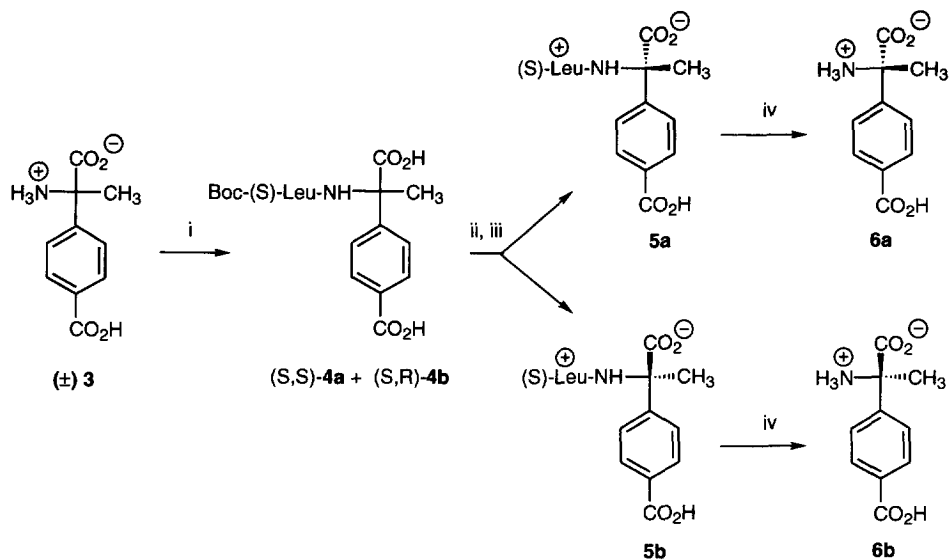


Fig. 1. Chiral ligand exchange HPLC of M4CPG: **A**, racemic mixture; **B**, (S)-M4CPG. Chiralpak MA(+) column (50 x 4.6 mm); eluent: 0.25 mM aqueous  $\text{CuSO}_4\text{-CH}_3\text{CN}$  (85:15), 0.8 mL.min<sup>-1</sup>; detection at 243 nm.

### Preparative resolution of ( $\pm$ )-M4CPG.

Preparative chemical resolution of ( $\pm$ )-M4CPG has been previously achieved through fractional crystallization <sup>5</sup>. We have recently reported a chemical resolution of glutamate analogues <sup>19</sup>, and we have tried to extend this method to the resolution of ( $\pm$ )-M4CPG. However, the use of boroxazolidinone complexes of M4CPG was not carried on because of the difficulties experienced in the coupling with a chiral amine, and the poor stability of the resulting diastereomers. Nevertheless, another method <sup>20</sup>, based on the preparation of (S)-leucine dipeptides was fully successful. Although coupling ( $\pm$ )-M4CPG with Boc-leucine was difficult, as previously noted for  $\alpha,\alpha'$ -dialkyl amino acids <sup>20</sup>, it was achieved as described in Scheme 2 with excellent yield. However, much care must be taken as not to racemize Boc-leucine, which would cause an impairment of the resolution process. Other non-racemizing coupling methods using HOBt/EDC <sup>20a</sup> or Brop reagent <sup>21</sup> gave poor yields. The use of trimethylsilyl chloride as a coupling reagent <sup>22</sup> should be preferred in such case.



i: Boc-(S)-Leu-OSu, Et<sub>3</sub>N, DMSO; ii: 2N HCl/AcOH; iii: AG1X4 (AcO<sup>-</sup>), AcOH (0.18M-0.25M); iv: 6N HCl, 110°C

Scheme 2

The resulting crude diastereomeric dipeptides **4a** + **4b** were deprotected in acidic media and separated by elution with aqueous acetic acid (0.18 M - 0.25 M) from an anion exchange column. Diastereomeric ratio (*dr*) was determined by HPLC (Fig. 2A). (S)-Leu-(S)-M4CPG **5a** was first eluted in a pure form (35% yield, *dr* >99.5 %, Fig. 2B), followed by a mixture of isomeric dipeptides (62% yield, **5a**:**5b** ratio = 2:8 ), then pure **5b** (3%, *dr* >99.5 %). An additional chromatography of the middle fraction would lead to a larger amount of pure **5b**. This was not achieved because we were mostly interested in the (S,S) dipeptide.

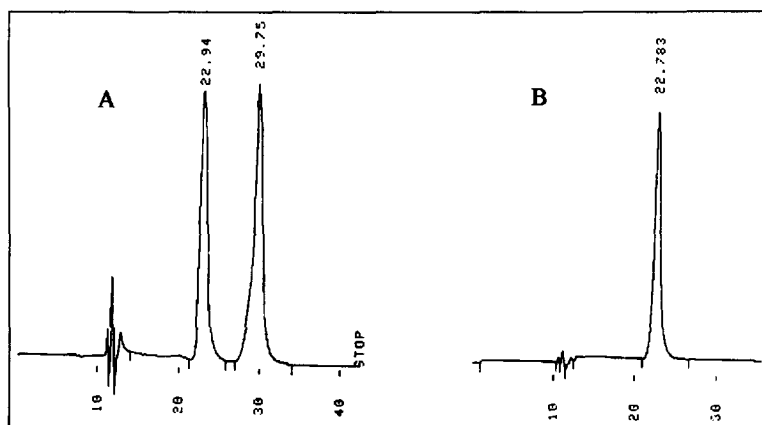


Fig. 2. Reverse phase HPLC of diastereomeric Leu-M4CPG dipeptides: **A**, diastereomeric mixture, after coupling and deprotection; **B**, (S)-Leu-(S)-M4CPG (first eluted from the ion exchange column). Nucleosil 5C18 (250 x 4.6 mm); eluent: 0.1M Na phosphate buffer, pH 3.0-MeOH (8:2), 0.4 mL.min<sup>-1</sup>; detection at 254 nm.

The pure dipeptide (S)-Leu-(S)-M4CPG **5a** was hydrolyzed. (S)-Leucine and (S)-M4CPG were purified by anion exchange chromatography and their optical purity checked by GC for (S)-Leu<sup>15</sup> or by HPLC as described above for (S)-M4CPG (**6a**, ee 97%, Fig. 1B). The recovered leucine exhibited a 97.5% enantiomeric excess, showing that almost no racemization had occurred during the coupling step.

#### Absolute configuration assignment of (+)-M4CPG.

Our first intention was to determine the absolute configuration of (+)-M4CPG by nOe experiments performed on the diketopiperazine resulting from cyclization of dipeptides **5a** and **5b**, for which one stereocenter is known. An analogous assignment has been previously described<sup>23</sup>. However, since we were unable to cyclize either dipeptide, we turned to the crystallographic method. The pure dipeptide **5a** was crystallized in a toluene-water mixture, and the X-Ray analysis of this compound revealed that the unknown center possessed the S absolute configuration (Fig. 3). This result is in agreement with the pharmacologists' assumption, which was made by analogy with related metabotropic receptors effectors of known S configuration<sup>5,24</sup>.

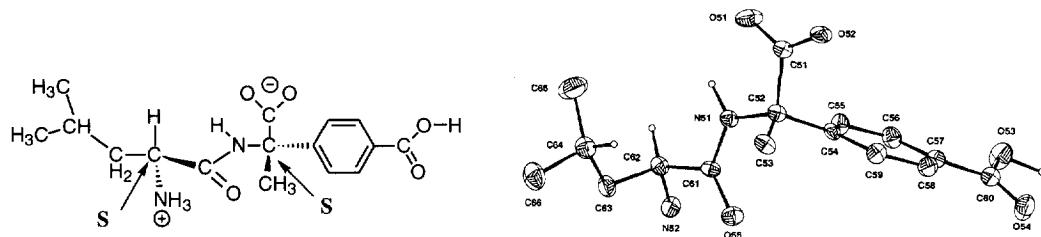


Fig. 3. Absolute stereostructure and ORTEP view of (S)-leucyl-(S)- $\alpha$ -methyl-4-carboxyphenylglycine.

In this study, we have thus described a new method for the preparation and enantiomeric purity measurement of (+)-M4CPG and have established its absolute configuration to be (S).

## EXPERIMENTAL

### Instrumentation

Melting points were determined with a Büchi capillary tube melting point apparatus. <sup>1</sup>H- (250.13 MHz) and <sup>13</sup>C- (62.9 MHz) NMR spectra were recorded on a ARX 250 Bruker Spectrometer. Chemical shifts ( $\delta$  in ppm) are given with reference to TMS, using residual <sup>1</sup>H, or <sup>13</sup>C for deuterated solvents (CD<sub>3</sub>OD, 3.30, 49.0; DMSO-D<sub>6</sub>, 2.49, 30.7), and internal 3-(trimethylsilyl)-[2,2,3,3,2H<sub>4</sub>]-propionic acid, sodium salt (sealed capillary) for D<sub>2</sub>O. Multiplicities (J Hz) are reported as br (broad), s (singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). TLC was performed on Merck 60F<sub>254</sub> precoated silicagel plates (0.2 mm thick, 10 cm migration). Products were visualized by UV light (254 nm), 2% (w/v) ninhydrin in ethanol and TDM reagent<sup>24</sup>. HPLC of dipeptides was performed with an Altex Chromatem 380 pump, a Rheodyne 7125 valve (20  $\mu$ l loop), a Pye-Unicam LC-UV detector set at 254 nm, and a Shimadzu CR-3A integrator, using a Nucleosil 5C<sub>18</sub> (25 cm x 4.6 mm I.D.) column equilibrated with 0.1 M NaH<sub>2</sub>PO<sub>4</sub> buffer pH 3-MeOH (8:2) at a 0.4 mL min<sup>-1</sup> flow rate. The resolution of ( $\pm$ )-M4CPG was effected by HPLC using a Chiralpak MA(+) column (5 x

0.46 cm I.D., Daicel) equilibrated with 0.25 mM aqueous  $\text{CuSO}_4\text{-CH}_3\text{CN}$  (85:15), at a  $0.8 \text{ mL}\cdot\text{min}^{-1}$  flow rate, with detection at 243 nm. GC was performed on Varian 3700 or 3400 chromatographs equipped with a flame ionisation detector, using helium (1 bar) as carrier gas. Resolution of ( $\pm$ )-M4CPG by GC was performed after derivatization to (S)-O-acetyllactylamide **16** on a Flexibond™ OV-1701 capillary column (15 m x 0.25 mm, Pierce Chemical Co.) run at 170°C (5 min), then 170°C to 195°C (4°C/min). Optical purity of (S)-leucine was determined on a Chirasil-Val capillary column (50 m x 0.32 mm, Alltech) run at 130°C. Mass spectra were recorded on a Hewlett-Packard 5890-II / 5972 fitted with a GC-mass coupling (30 m capillary HP1 column); column temp., 120°C (2 min), 120°C to 300°C (8°C/min), then 300°C (5 min). Optical rotations were measured with a Perkin Elmer 241 polarimeter using a 1-dm cell. X-Ray analysis was carried out by S. Halut in the Laboratoire de Chimie des Métaux de Transition, Université P. et M. Curie, Paris.

### ( $\pm$ )-M4CPG **3**.

Ammonium carbonate (6.84 g, 40 mmol) and potassium cyanide (0.616 g, 9.6 mmol) were added by portions to a suspension of the ketone **1b** (1.16 g, 8 mmol) in ethanol-water (1:1, 24 mL). The mixture was heated with magnetic stirring at 60°C for 3 days. The temperature was then raised to 90°C for 90 min to eliminate excess ammonium carbonate. After cooling and acidification with concentrated HCl, the crystalline hydantoin **2b** was filtered, washed with acidic water, dried under vacuum to afford a white solid in quantitative yield. **<sup>1</sup>H NMR** (DMSO- $\text{D}_6$ ) 10.96 (s, 1H, NH), 8.77 (s, 1H, NH), 7.94 (d, 2H, ArCH, J=8.6), 7.54 (d, 2H, ArCH, J=8.6), 1.73 (s, 3H,  $\text{CH}_3$ ); **<sup>13</sup>C NMR** ( $\text{CD}_3\text{OD}$ ) 177.1 and 156.8 (2xCO), 146.2 and 119.3 (2xArC quat), 133.6 and 127.6 (2xArCH), 113.1 (CN), 66.0 (C- $\text{CH}_3$ ), 25.6 (C- $\text{CH}_3$ ).

Hydantoin **2a** was obtained following the same procedure and heating the mixture for 7 days at 60°C. **<sup>1</sup>H NMR** (DMSO- $\text{D}_6$ ) 12.95 (s, 1H, COOH), 10.83 (s, 1H, NH), 8.69 (s, 1H, NH), 7.94 (d, 2H, ArCH, J=8.1), 7.59 (d, 2H, ArCH, J=8.1), 1.66 (s, 3H,  $\text{CH}_3$ ); **<sup>13</sup>C NMR** (DMSO- $\text{D}_6$ ) 167.1 (COOH), 176.7 and 156.3 (2xCO), 144.8 and 130.5 (2xArC quat), 129.7 and 125.9 (2x2ArCH), 64.2 (C- $\text{CH}_3$ ), 25.2 (C- $\text{CH}_3$ ).

To hydantoin **2a** or **2b** (1.72 g, 8 mmol) was added 6N HCl (20 mL) in a tightly closed bottle, and the mixture was stirred at 110°C for 8 days. The solvent was evaporated under reduced pressure and the resulting residue was dissolved into water (750 mL) and deposited directly on a AG50X4 ( $\text{H}^+$ ) column (20-50 mesh, 19 x 2.8 cm). After washing with water (300 mL), the aminoacid was eluted with 0.5 M  $\text{NH}_4\text{OH}$  (500 mL), and the UV and ninhydrin reacting fractions evaporated and dried under vacuum. Pure ( $\pm$ )-M4CPG **3** (ammonium salt) was obtained as a white solid (1.792 g, 7.93 mmol, 99.1%). **M.p.** 282°C ; **<sup>1</sup>H NMR** ( $\text{D}_2\text{O}$ ) 7.94 (d, 2H, ArH, J=8.3), 7.61 (d, 2H, ArH, J=8.3), 1.99 (s, 3H,  $\text{CH}_3$ ); **<sup>13</sup>C NMR** ( $\text{D}_2\text{O}$ ) 178.7 and 177.8 (2xCO), 143.2 et 139.7 (2xArC quat), 132.3 and 128.7 (2x2ArCH), 65.7 (C- $\text{CH}_3$ ), 24.2 (C- $\text{CH}_3$ ); **GC**:  $R_t$  = 57.42 and 58.62 min; **HPLC**:  $R_t$  = 20.0 and 23.5 min (Fig. 1A); **GC/MS** of N-trifluoroacetyl-O,O'-diisopropylester:  $R_t$  = 13.63 min,  $m/z$  (%) 330 (16) [ $\text{M}^+\text{-OiPr}$ ], 303 (28), 302 (100) [ $\text{M}^+\text{-CO}_2\text{iPr}$ ], 260 (32), 147 (28), 102 (15), 69 (8) [ $\text{CF}_3^+$ ], 59 (2) [ $\text{OiPr}^+$ ].

### Resolution of ( $\pm$ )-M4CPG **3** using Boc-(S)-Leu.

**Synthesis of (S)-Leu-M4CPG **5a** and **5b**.** To a stirred solution of ( $\pm$ )-M4CPG **3** (150 mg, 0.66 mmol) in DMSO (3.5 mL) was added triethylamine (465  $\mu\text{L}$ , 3.32 mmol), then Boc-Leu-OSu (436 mg, 1.32 mmol). The mixture was stirred at room temperature for 4 days, 1 equivalent of Boc-Leu-OSu was added and the solution was stirred for 4 more days. The solution was then diluted with water (20 mL) and saturated aqueous  $\text{NaHCO}_3$

(10 mL). After washing with EtOAc (3 x 20 mL), the solution was cautiously acidified to pH 3 with  $\text{KH}_2\text{SO}_4$ , saturated with NaCl, and extracted with EtOAc. The combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ) and evaporated to give quantitatively the crude diastereomeric mixture (1:1) of **4a** + **4b** as a colorless oil. A sample was purified by flash chromatography (silicagel H60, iPrOH-20%  $\text{NH}_4\text{OH}$  in water, 9:3).  **$^1\text{H}$  NMR** ( $\text{CD}_3\text{OD}$ ) 7.86 (d, 2H, ArH,  $J=8.4$ ), 7.51 and 7.48 (2d, 2H, ArH,  $J=8.4$ ), 3.97 (m, 1H, N-CH-CO), 1.96 (br, 3H, C- $\text{CH}_3$ ), 1.40-1.80 (m, 3H,  $\text{CH}_2+\text{CH}$ ), 1.51 and 1.49 (2s, 9H,  $(\text{CH}_3)_3\text{C}$ ), 0.96, 0.95 and 0.91 (3d, 6H,  $2\times\text{CH}_3$ ,  $J=6.4$ );  **$^{13}\text{C}$  NMR** ( $\text{CD}_3\text{OD}$ ) 178.2, 174.0 and 173.5 ( $3\times\text{CO}$ ), 158.1 (O-CO-NH), 148.2 and 134.3 ( $2\times\text{ArC}$  quat), 129.9, 126.9 and 126.8 ( $2\times\text{ArCH}$ ), 80.7 ( $(\text{CH}_3)_3\text{C}$ ), 64.0 ( $\text{C}_\alpha$  M4CPG), 55.6 and 55.4 ( $\text{C}_\alpha$  Leu), 41.7 and 41.4 ( $\text{CH}_2$  Leu), 28.8 ( $(\text{CH}_3)_3\text{C}$ ), 26.0 (CH Leu), 23.5, 22.6, 22.4 and 21.7 ( $3\times\text{CH}_3$ ); **HPLC**:  $R_t = 22.9$  and 29.7 min (Fig. 2A).

Deprotection was effected by dissolution of crude **4a** + **4b** in a 2N solution of dry HCl in AcOH (3mL) kept at room temperature for 30 min. The solvent was evaporated under reduced pressure; the residue was dissolved in water (250 mL) and the resulting solution was adjusted to pH 8-9, then deposited on a Dowex AG1X4 (200-400 mesh,  $\text{AcO}^-$ ) (5 x 56 cm). After washing with water (400 mL), the dipeptides were eluted with 0.18 M AcOH (2 L), 0.2 M AcOH (2 L), 0.22 M AcOH (2 L), and 0.25 M AcOH (3 L), and localized by UV and ninhydrin. Fractions in the three last liters of 0.25 M AcOH were combined according to their purity (checked by HPLC) and evaporated to give **5a** + **5b** (210 mg, 0.65 mmol, 98.5 %), as white solids. Four fractions were obtained: (S)-Leu-(S)-M4CPG **5a** (74 mg,  $dr > 99.5\%$ ), (S)-Leu-(S)-M4CPG (19 mg,  $dr = 90\%$ ), (S)-Leu-(R)-M4CPG (112 mg,  $dr = 95\%$ ), (S)-Leu-(R)-M4CPG **5b** (5 mg,  $dr > 99.5\%$ ).

(S)-Leu-(S)-M4CPG **5a**. **M.p.** above  $300^\circ\text{C}$  (dec.);  $[\alpha]_{\text{D}}^{22} = +46.8$  (c 1, 6N HCl);  **$^1\text{H}$  NMR** ( $\text{D}_2\text{O}$ ) 8.10 (d, 2H, ArH,  $J=8.6$ ), 7.72 (d, 2H, ArH,  $J=8.6$ ), 4.18 (t, 1H, N-CH-CO,  $J=6.4$ ), 2.02 (s, 3H, C- $\text{CH}_3$ ), 1.70-1.95 (m, 3H,  $\text{CH}_2+\text{CH}$ ), 1.07 and 1.04 (2d, 6H,  $2\times\text{CH}_3$ ,  $J=6.8$ ).  **$^{13}\text{C}$  NMR** ( $\text{CD}_3\text{OD}$ ) 174.9, 169.8 and 169.4 ( $3\times\text{CO}$ ), 146.5 and 131.6 ( $2\times\text{ArC}$  quat), 130.8 and 127.6 ( $2\times\text{ArCH}$ ), 63.6 ( $\text{C}_\alpha$  M4CPG), 53.0 ( $\text{C}_\alpha$  Leu), 41.9 ( $\text{CH}_2$  Leu), 25.4 (CH Leu), 23.7, 22.9 and 22.3 ( $3\times\text{CH}_3$ ); **Crystal data** <sup>26</sup> at 295 K:  $\text{C}_{32}\text{H}_{47}\text{ClN}_4\text{O}_{11}$ , triclinic system, space group P1,  $a = 8.858(1)$  Å,  $b = 9.093(1)$  Å,  $c = 11.985(2)$  Å,  $\alpha = 68.92(1)^\circ$ ,  $\beta = 86.15(2)^\circ$ ,  $\gamma = 73.68(1)^\circ$ ,  $V = 864(1)$  Å<sup>3</sup>,  $Z = 1$ ,  $d_{\text{calc}} = 1.34$  g.cm<sup>-3</sup>, nb of variables 436,  $R = 0.037$ ,  $R_w = 0.039$ ; intensity data were collected on an Enraf-Nonius CAD4 diffractometer in  $\omega/2\theta$  scan mode using  $\text{MoK}\alpha$  ( $\lambda = 0.71069$  Å).

(S)-Leu-(R)-M4CPG **5b**.  **$^1\text{H}$  NMR** ( $\text{D}_2\text{O}$ ) 8.08 (d, 2H, ArH,  $J=8.3$ ), 7.64 (d, 2H, ArH,  $J=8.3$ ), 4.18 (t, 1H, N-CH-CO), 2.01 (s, 3H, C- $\text{CH}_3$ ), 1.60-1.90 (m, 3H,  $\text{CH}_2+\text{CH}$ ), 1.05 and 1.00 (2d, 6H,  $2\times\text{CH}_3$ ,  $J=6.1$ );  **$^{13}\text{C}$  NMR** ( $\text{CD}_3\text{OD}$ ) 174.9, 169.7 and 169.4 ( $3\times\text{CO}$ ), 146.6 and 131.3 ( $2\times\text{ArC}$  quat), 130.6 and 127.6 ( $2\times\text{ArCH}$ ), 63.7 ( $\text{C}_\alpha$  M4CPG), 52.9 ( $\text{C}_\alpha$  Leu), 41.6 ( $\text{CH}_2$  Leu), 25.4 (CH Leu), 23.7, 23.0 and 22.2 ( $3\times\text{CH}_3$ ).

#### (S)(+)-M4CPG **6a**.

(S)-Leu-(S)-M4CPG **5a** (20 mg) was refluxed in 6N HCl (10 mL) during 17 h. After evaporation to dryness under reduced pressure, the residue was repeatedly dissolved in water and evaporated. The solution of the residual white solid in water (100 mL) was adjusted to pH 8-9, then deposited on a small column (1.5 x 11 cm) of Dowex 1X4 (200-400 mesh,  $\text{AcO}^-$ ). After washing with water (100 mL), the column was eluted with 0.2 M AcOH (150 mL) and 0.6 M AcOH (250 mL), in 10 mL fractions. (S)-Leu (7 mg) was eluted as soon as the pH of the eluent became acidic (fraction 2), while pure (S)-M4CPG **6a** (11 mg) was eluted in fractions 17-22.

$[\alpha]_D^{22} = + 84.3$  (c 0.28, 6N HCl) (lit. <sup>2</sup> + 93); enantiomeric excess determined by HPLC: 97% (Fig. 1B); <sup>1</sup>H and <sup>13</sup>C NMR data identical to the racemic compound.

The enantiomeric purity of recovered Leu was checked by chiral GC (130°C) after derivatization <sup>15</sup>, and showed an enantiomeric excess of 97.5%.

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