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## Diastereospecific synthesis of new 4-substituted L-theanine derivatives



Fatiha Sebih<sup>a,b</sup>, Salima Bellahouel<sup>b</sup>, Marc Rolland<sup>c</sup>, Aicha Derdour<sup>b</sup>, Jean Martinez<sup>a</sup>, Valérie Rolland<sup>a,\*</sup>

<sup>a</sup> IBMM-UMR 5247, CNRS, UM2, UM1, Place E. Bataillon, 34095 Montpellier Cédex 5, France

<sup>b</sup> Laboratoire de Synthèse Organique Appliquée LSOA, Université d'Oran, Département de Chimie, Bp 1524 El M'Naouer, Oran 31000, Algeria

<sup>c</sup> IEM-UMR5635, CNRS-UM2-ENSCM, Place E. Bataillon, 34095 Montpellier Cédex 5, France

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### ABSTRACT

Considering the biological activity of L-theanine as a potent agonist of NMDA receptors, impacting on glutamatergic synapse activity, we have developed an asymmetric synthesis of new enantiomerically pure 4-substituted L-theanine derivatives. The key step is a stereospecific alkylation on a previously synthesized and correctly protected (S)-pyroglutamate.

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### 1. Introduction

L-Theanine (5-N-ethylglutamine) is a non-proteinogenic amino acid<sup>1</sup> present in the range of 1–2.5% of green tea leaves essentially in *Camellia sinensis* and is the major flavour component of tea, that is, the 'umami taste'.<sup>2</sup>

In addition to its flavouring, L-theanine can modulate peripheral physiological parameters such as reducing blood pressure<sup>3</sup> and helping to manage allergic disorders.<sup>4</sup> Moreover, thanks to its great ability to cross the blood–brain barrier (BBB), L-theanine also displays a wide array of CNS actions resulting in beneficial behavioural effects such as anxiolytic and pro-mnesiant actions, likely via the acute modulation of neurotransmitter levels such as dopamine<sup>5</sup> and amino acids.<sup>6</sup> In addition, the long-term effects of L-theanine results in protective action against glutamate neurotoxicity while prevention of synaptic plasticity deficits under pathological conditions has also been identified. L-Theanine could therefore be an interesting structure to develop new agents that interact with the glutamatergic system, bearing in mind that it binds glutamatergic ionotropic receptors,<sup>7</sup> especially NMDA receptors, and that it crosses the BBB easily (Fig. 1).

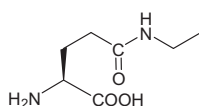


Figure 1. Structure of L-theanine (5-N-ethylglutamine).

During the search for new molecules capable of activating, blocking or modulating Glu receptor subtypes, such as ionotropic glutamate receptors (i-Glu Rs) according to the selective agonists, NMDA, AMPA and KA and the G-protein coupled metabotropic glutamate receptors (m-Glu Rs) and (or) excitatory aminoacid transporters (EAATs), we considered the synthesis of various 3 or 4-substituted glutamate analogues and various 3-substituted aspartate analogues (Fig. 2) and their biological activity evaluation.

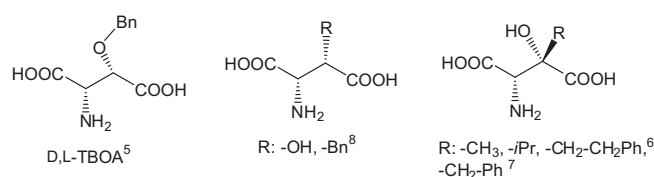
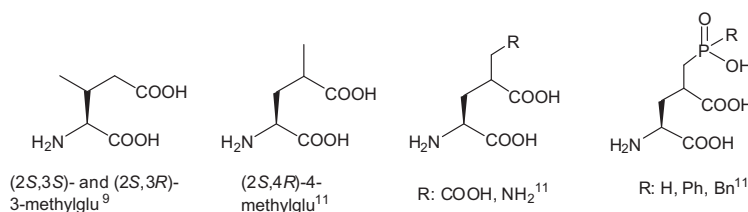


Figure 2.  $\beta$ -Substituted aspartate derivatives: inhibitors of EAATs.

DL-threo- $\beta$ -Benzyloxyaspartate (DL-TBOA) is the most potent blocker for the human EAAT1 and EAAT2.<sup>5</sup> Recently we reported on a concise, asymmetric synthesis of enantiomerically pure  $\beta$ -substituted  $\beta$ -hydroxy aspartates via an aldol reaction between a glycine enolate derived from an oxazinone intermediate (used as facial discriminator) and various  $\alpha$ -keto esters with excellent diastereoisomeric excess.<sup>6</sup> Enantiomerically pure  $\beta$ -methyl- $\beta$ -hydroxyaspartates,  $\beta$ -isopropyl- $\beta$ -hydroxyaspartates and  $\beta$ -phenylethyl- $\beta$ -hydroxyaspartates were prepared and characterized. These derivatives exhibited moderate inhibitory effects on glutamate transport as evaluated by electrophysiological measurements on cultured hippocampal neurons. Mekki et al. proposed two years

\* Corresponding author. Tel.: +33 04 67 14 49 36; fax: +33 04 67 14 48 66.

E-mail address: [Valerie.rolland@univ-montp2.fr](mailto:Valerie.rolland@univ-montp2.fr) (V. Rolland).



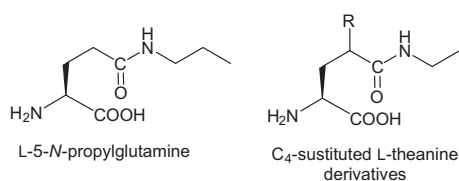
**Figure 3.** Some glutamic acid derivatives exhibiting biological activity on glutamatergic synapses.

ago a short, regiospecific and stereoselective synthesis of a new  $\text{L-}\beta\text{-threo-benzyl-}\beta\text{-hydroxyaspartate}$  (Fig. 2).<sup>7</sup> This new target mimics both the inhibitory EAATs effect of both  $\text{L-}\beta\text{-threo-OH-Asp}$  and of  $\text{L-}\beta\text{-threo-benzyl-Asp}$  which are the most potent synthetic EAATs blockers known to date.<sup>8</sup> The key step is a regiospecific and stereoselective Sharpless asymmetric aminohydroxylation (SAA) reaction on previously synthesized benzyl fumarate.<sup>7</sup>

The  $\text{C}_3$  or  $\text{C}_4$  alkylated glutamate analogue derivatives were also studied as EAAT blockers. Wehbe et al.<sup>9</sup> described a short four-step synthesis of (2S,3R)- and (2S,3S)-3-methyl glutamic acids: the Schiff base of *tert*-butyl glycinate and the inexpensive chiral auxiliary (2R,3R,4R)-2-hydroxypropan-3-one (HP) reacted with ethylcrotonate via an asymmetric Michael addition and led to both diastereoisomers (dr 56:44), which were easily separated by preparative HPLC. The biological activity of the glutamate transport blocker was evaluated by recording spontaneous excitatory transmissions, which showed that (2S,3R)-3-methyl glutamic acid exhibited important inhibitory effects on the glutamate transport (Fig. 3).<sup>9</sup>

One of the most studied inhibitors of glutamate transport is (2S,4R)-4-methylglutamic acid. Vandenberg et al. proved the real influence of the nature of the chemical group at the  $\text{C}_4$  position.<sup>10</sup> Ten years ago Wehbe et al. described the enantioselective synthesis of new 4-substituted glutamic acid derivatives with an acidic (carboxylic or phosphinic acid) or basic (amine) function. Some of them have moderate activity on glutamate transport.<sup>11</sup>

In continuation of our interests to find new tools for glutamatergic synapse studies, especially with regards to the positive modulator effects of  $\text{L-theanine}$  on NMDA receptors, we decided to apply our expertise in stereoselective syntheses to obtain  $\text{C}_4$  alkylated  $\text{L-theanine}$  derivatives. Synthetic  $\text{L-}$  and  $\text{D-theanine}$  and  $\text{L-}$  and  $\text{D-5-N-propylglutamine}$  have been previously synthesized by MAOS in an inexpensive and environmentally friendly method and these synthetic compounds were tested for their potential biological activity on cultured brain cells. The agonistic effect of synthetic  $\text{L-theanine}$  on NMDA receptors has been verified and confirmed on cultured hippocampal neurons by measuring the intracellular calcium changes with fura 2. The preliminary results indicate that enantiomerically pure  $\text{L-}$  and  $\text{D-5-N-propylglutamine}$  and  $\text{D-theanine}$  displayed greater positive modulator effects on NMDA-mediated responses than  $\text{L-theanine}$  and were not blockers of glutamate transport (Fig. 4).



**Figure 4.** New derivatives of  $\text{L-theanine}$ : potential NMDA receptors agonist or co-agonist.

## 2. Results and discussion

The direct  $\text{C}_4$  alkylation of glutamines has not been described in the literature and alkylation with different alkylating reagents on correctly protected  $\text{L-theanine}$  did not succeed. Del Bosco et al.<sup>12</sup> described a  $\text{C}_4$  alkylation using (S) protected glutamate as starting material. The alkylation reaction of the lithium enolate of  $\text{Z-Glu(O-Me)OtBu}$  with methyl iodide led to two diastereoisomers with a 3:1 ratio and 21% yield. Alkylation of the lithium enolate of  $\text{Z-Glu(OMe)OtBu}$  with benzyl bromide led to a single stereoisomer but in only 28% yield.

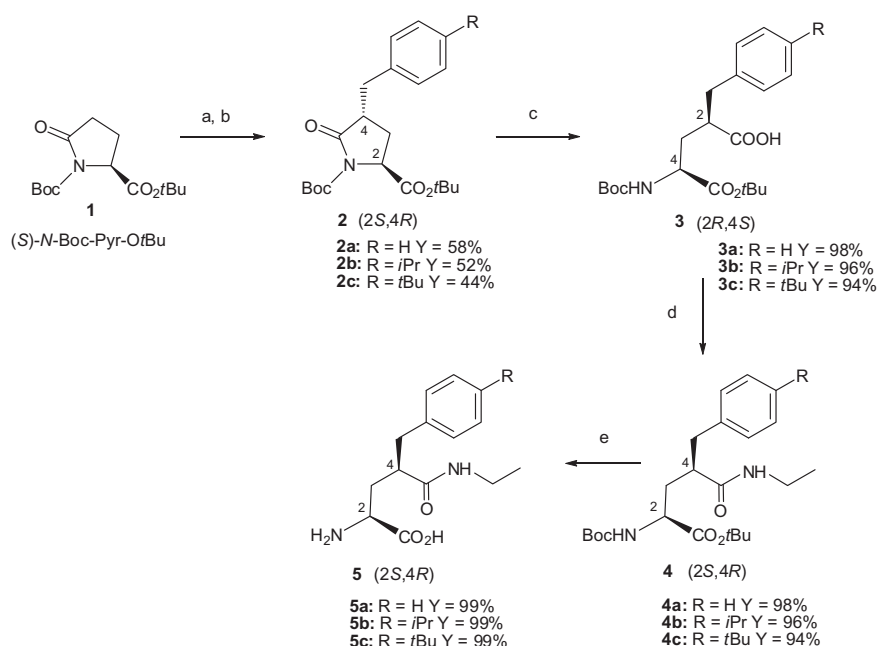
Considering that for the synthesis of theanine derivatives the last step is an amidification reaction on the lateral chain under basic conditions, the N and C protecting groups have to be stable. Alkylation of the lithium enolate of  $\text{Z-Glu(OtBu)OBn}$  with benzyl bromide or methyl bromide using Bosco's experimental conditions was unsuccessful because of the high steric hindrance of the *tert*-butyl protecting group compared to the methyl group, which led to  $\text{Z-Glu(OtBu)OBn}$  being completely recovered.

As described by Taver et al.,  $\text{L-pyroglutamic acid (L-Pyr-OH)}$  was chosen as the starting product.<sup>13</sup> The C-protecting group was first a benzyl ester ( $\text{L-Pyr-OBn}$ ). The stereoselective methylation using  $\text{LiHMDS}$  and methyl triflate proceeded with a *cis-trans* ratio of 1:4 in 34% yield over two steps. However, the lithium hydroxide ring opening did not proceed as well as planned and the benzyl ester was hydrolysed. A modification of the protecting group scheme was required and in our approach for the synthesis of theanine derivatives (Scheme 1), we investigated the use of a *tert*-butyl group instead of a benzyl moiety and we realized protection of commercially available (S)-pyroglutamate in (S)-*N*-Boc-Pyr-OtBu **1**.

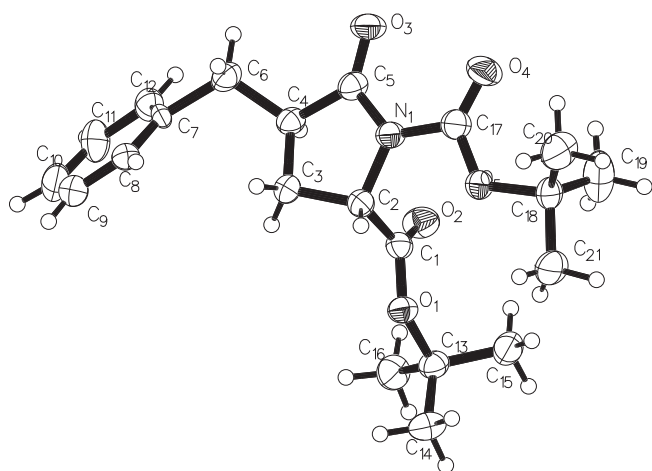
The key step was the alkylation reaction with methyl bromide, the alkylation step was carried out with a yield of 52% and led to dialkylation on  $\text{C}_2$  and  $\text{C}_4$  ( $\text{Y} = 15\%$ ) and a diastereomeric mixture (dr = 59:41) with  $\text{C}_4$  alkylation ( $\text{Y} = 37\%$ ), evaluated by LCMS analysis and  $^1\text{H}$  NMR characterization of the crude mixture. The low steric hindrance of methylbromide had an impact on the regioselectivity and stereoselectivity. The overall yield of the alkylation was higher than those reported by Del Bosco et al. due to the addition of HMPA (hexamethylphosphoramide) forming favourable aggregates.

With benzyl bromide, the alkylation step of the corresponding protected compound **1** led to  $\text{C}_4$ -benzyl derivatives (2S,4R)-4-benzyl-di-*tert*-butyl 5-oxopyrrolidine-1,2-dicarboxylate **2a**, (2S,4R)-4-(4-isopropylbenzyl)-di-*tert*-butyl 5-oxopyrrolidine-1,2-dicarboxylate **2b** and (2S,4R)-4-(4-*tert*-butylbenzyl)-di-*tert*-butyl 5-oxopyrrolidine-1,2-dicarboxylate **2c** with total stereospecificity (de >99%): in each case only one stereoisomer was isolated. The moderate yields (44–58%) were evaluated after separation by silica gel column chromatography. Even if moderate yields were observed, (S)-*N*-Boc-Pyr-OtBu **1** was easily recovered without any degradation or other protecting group hydrolysis.

The (2S,4R)-configuration of compound **2a** was confirmed by X-ray structure determination (Fig. 5) after recrystallization from an  $\text{AcOEt/cyclohexane}$  solvent system.



**Scheme 1.** General plan for the synthesis of enantiomerically pure C<sub>4</sub>-benzyl L-theanine derivatives from (*S*)-*N*-Boc-Pyr-OTBu, **1**. Reagent and conditions: (a) LiHMDS, HMPA, THF, −78 °C, 45 min then (b) 4-RBnBr, THF, −78 °C, 1 h; (c) LiOH/THF/H<sub>2</sub>O, −10 °C, 1 h; (d) EtNH<sub>2</sub>, BOP, TEA, THF, rt, 1 h; (e) TFA, DCM, rt, 90 min.



**Figure 5.** Ortep diagram of compound (2*S*,4*R*)-**2a**. Thermal ellipsoids are shown at 50% probability level.

The specific rotation measurements of enantiomerically pure **2a**, **2b** and **2c** were calculated as references. High resolution NMR spectral analyses (600 MHz) were compared with those in the literature for similar compounds (Table 1).<sup>11</sup>

For H<sub>6a</sub> and H<sub>6b</sub>, the dihedral angle was determined exactly from X-ray analysis but in solution this value was not significant because of possible rotational conformations.

The enantiomeric excess of **2a**, **2b** and **2c** were confirmed by chiral HPLC on two different columns: Chiralcel OD-RH column in water/acetonitrile as the solvent system and on a Chiralpak AD-H column in hexane/isopropanol as the solvent system.

The regioselectivity and stereoselectivity at C<sub>4</sub> were induced by the (*S*)-configuration of C<sub>2</sub>, while the high steric hindrance of different benzyl bromide alkylating reagents helped the *N*-Boc and *O**t*Bu protecting groups to behave as facial discriminators as shown in Scheme 2.

Compounds **2b** and **2c** did not crystallize but by using high resolution <sup>1</sup>H NMR analyses (600 MHz), we were able to confirm an (*R*)-absolute configuration for C<sub>4</sub>.

The following step was the ring opening. In the literature, when a pyrrolidone carboxylic acid or pyroglutamic acid is treated with 10 equiv of 33% aqueous solution of ethylamine EtNH<sub>2</sub> for 20 days at 37 °C in a sealed glass tube, the L-theanine is obtained in only 9% yield.<sup>14</sup> Yan et al. described lactam ring opening directly by EtNH<sub>2</sub> applying an enzymatic method with theanine synthetase. An equilibrium between L-Glu and L-theanine was observed and the yield did not exceed 20% of L-theanine after 7 days.<sup>15</sup>

In order to increase the yield and decrease the reaction time, we carried out the ring opening under basic conditions (NaOH or LiOH) while taking care of protecting group preservation, which was then followed by amide bond formation with EtNH<sub>2</sub> (Scheme 1). After ring opening according to IUPAC rules, C<sub>4</sub>-benzylated pyroglutamates starting compounds became C<sub>2</sub>-benzylated-5-oxopentanoic acids **3a**, **3b** and **3c**.

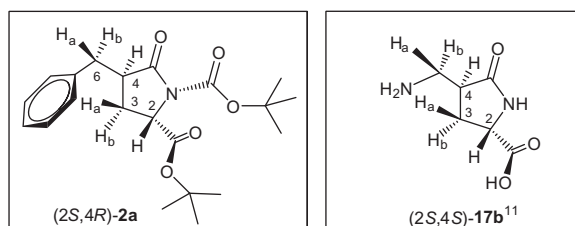
With NaOH or LiOH at RT, the *tert*-butyl ester was partially hydrolysed. The ring opening was optimized by separately stirring compounds **2a**, **2b** and **2c** at −10 °C over 10 min with an aqueous solution of LiOH (2 equiv) and THF and by increasing the temperature to −5 °C. The ring opening was followed by HPLC analysis and CCM. Compounds (2*R*,4*S*)-2-benzyl-5-((*tert*-butoxycarbonyl)amino)-5-oxopentanoic acid **3a**, (2*R*,4*S*)-2-(4-isopropylbenzyl)-5-*tert*-butoxy-4-((*tert*-butoxycarbonyl)amino)-5-oxopentanoic acid **3b**, and (2*R*,4*S*)-2-(4-*tert*-butylbenzyl)-5-*tert*-butoxy-4-((*tert*-butoxycarbonyl)amino)-5-oxopentanoic acid **3c** were obtained in 94–98% yield as colourless oils.

The amide bond formation was carried out in THF with a commercially available anhydrous solution of ethylamine in THF, using classic activation by BOP reagent [(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate] and triethylamine as the base. Compounds **4a**, **4b** and **4c** were obtained quantitatively after 1 h of stirring at rt. Finally, the *N*-Boc and *O**t*Bu protecting groups were removed by TFA in THF; the C<sub>4</sub> benzylated L-theanine derivatives **5a**, **5b** and **5c** were obtained as white solids in very good yields.

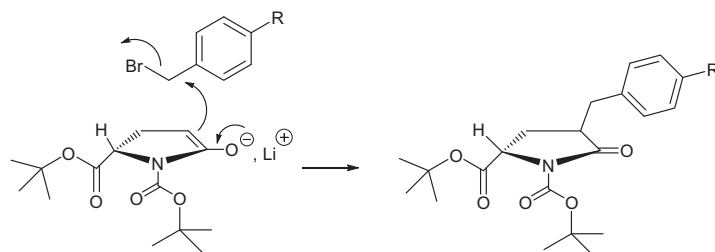
As described in the introduction, preliminary results indicated that enantiomerically pure L- and D-γ-N-propylglutamine and D-theanine displayed greater positive modulator effects on NMDA-mediated responses than L-theanine and interestingly were

**Table 1**

Chemical shifts (ppm/TMS) and coupling constants (Hz) of compounds **2a** (2*S*,4*R*) compared with those of **17b**<sup>11</sup>



	ppm (CDCl <sub>3</sub> ) <b>2a</b>	ppm (CD <sub>3</sub> OD) <b>17b</b> <sup>11</sup>	coupling constants (Hz)	<b>2a</b>	<b>17b</b> <sup>11</sup>	Dihedral angle <b>2a</b>	Dihedral angle <b>17b</b> <sup>11</sup>
$\delta$ (H <sub>2</sub> )	4.27	4.257	<sup>3</sup> J (H <sub>2</sub> -H <sub>3a</sub> )	2.1	1.7	24.06	19.0
$\delta$ (H <sub>3a</sub> )	2.01	2.282	<sup>3</sup> J (H <sub>2</sub> -H <sub>3b</sub> )	9.2	9.5	96.72	101.0
$\delta$ (H <sub>3b</sub> )	1.96	2.534	<sup>4</sup> J (H <sub>2</sub> -H <sub>4</sub> )	—	<0.5		
			<sup>2</sup> J (H <sub>3a</sub> -H <sub>3b</sub> )	13.2	13.3		
$\delta$ (H <sub>4</sub> )	2.91	2.872	<sup>3</sup> J (H <sub>3a</sub> -H <sub>4</sub> )	11.2	10.5	149.22	145.0
			<sup>3</sup> J (H <sub>3b</sub> -H <sub>4</sub> )	8.9	8.6	28.42	24.0
$\delta$ (H <sub>6a</sub> )	3.26	3.233	<sup>3</sup> J (H <sub>4</sub> -H <sub>6a</sub> )	9.3	8.3	169.54	
$\delta$ (H <sub>6b</sub> )	2.68	3.135	<sup>3</sup> J (H <sub>4</sub> -H <sub>6b</sub> )	4.3	6.9	63.0	
			<sup>2</sup> J (H <sub>6a</sub> -H <sub>6b</sub> )	14			



**Scheme 2.** Stereospecific alkylation of the lithium enolate of (*S*)-*N*-Boc-Pyr-OrBu, **1**.

not blockers of glutamate transport. The evaluation of the potential biological activity of C<sub>4</sub>-benzylated L-theanine derivatives **5a**, **5b** and **5c** is currently underway by measuring spontaneous synaptic transmissions on the intracellular calcium concentration ([Ca<sup>2+</sup>]<sub>i</sub>) of cultured hippocampal neurons with fura-2.<sup>16</sup>

### 3. Conclusion

A concise, asymmetric synthesis of enantiomerically pure C<sub>4</sub>-alkylated L-theanine derivatives as new co-agonists of NMDA receptors has been discussed. The key step is a stereospecific alkylation on a protected L-pyrroglutamate. The lactam ring opening procedure has also been optimized. We have confirmed the stereochemistry of C<sub>4</sub> by X ray analysis, <sup>1</sup>H NMR (600 MHz) and chiral HPLC data. This approach may be considered as a promising, short and efficient strategy to develop new tools, and to evaluate their impact on glutamatergic transmission in the central nervous system.

## 4. Experimental

### 4.1. General

Melting points were obtained using a Büchi 510 capillary apparatus and are uncorrected. <sup>1</sup>H NMR spectra were recorded at 300 MHz and 75 MHz using a Bruker AC300 instruments, at 600 MHz using a Bruker AC600 instrument. 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. Coupling constants are reported in Hertz (Hz). High resolution mass spectra (HRMS) were recorded on micromass electrospray instruments with only molecular ion

and other major peaks being reported. LC–MS identification was by electrospray on HPLC Waters Alliance 2690. Flash chromatography was carried out using E-Merck Silica Gel (Kieselgel 60, 230–400 mesh) as the stationary phase. Thin layer chromatography was carried out on aluminium plates pre-coated with Merck Silica-gel 60F254 and were visualized by quenching of Ultra-Violet fluorescence, or by staining with a 10% methanol phosphomolybdic acid solution followed by heating. Analytic HPLC were performed on a Waters apparatus 717 plus autosampler with Millennium<sup>32</sup> program on SymmetryShield™ RP<sub>18</sub> 3.5 μm 2.1 × 20 mm column and using linear gradient of ACN in H<sub>2</sub>O with 0.1% TFA in 5 min with 3 mL/min flow. Analytical chiral HPLC experiments were performed on a unit composed of a Merck D-7000 system manager, Merck-Lachrom L-7100 pump, Merck-Lachrom L-7360 oven, Merck-Lachrom L-7400 UV-detector and Jasco OR-1590 polarimeter using Beckman Coulter System Gold 126 Solvent Module HPLC machine with column Chiralcel OD-RH (250 × 4.6 mm) and water/acetonitrile solvent system or with Chiralpak AD-H (250 × 4.6 mm) and hexane/isopropanol solvent system. Both columns are from Chiral Technologies Europe (Illkirch, France). Optical rotations were determined on a Perkin–Elmer 341 polarimeter in an appropriate solvent (20 °C, sodium ray). THF was distilled from sodium/benzophenone ketyl. Reagents were supplied from commercial sources (ALDRICH, FLUKA).

### 4.2. Synthesis of (2*S*,4*R*)-4-benzyl-di-*tert*-butyl-5-oxopyrrolidine-1,2-dicarboxylate **2**: general procedure for the alkylation of (*S*)-*N*-Boc-Pyr-OrBu

A mixture of (*S*)-di-*tert*-butyl-5-oxopyrrolidine-1,2-dicarboxylate [(*S*)-*N*-Boc-Pyr-OrBu] **1** (1.2 g, 4.2 mmol) in anhydrous THF (15 mL) was stirred under argon and cooled to –78 °C. A solution

of LiHMDS 1 M in anhydrous THF (3.6 mL, 5 mmol, 1.2 equiv) was added dropwise under argon with a syringe, followed by HMPA (1.28 mL, 5 mmol, 1.2 equiv). After 45 min of stirring, aryl bromide (4.2 mmol, 1 equiv) was added dropwise with a syringe. After 1 h at  $-78^{\circ}\text{C}$ , the reaction was quenched by the slow addition of saturated  $\text{NH}_4\text{Cl}$  at rt. The solution was extracted 3 times with AcOEt. The combined organic layers were washed with water and brine, dried over  $\text{MgSO}_4$  and concentrated under reduced pressure. The crude products were purified by chromatography (silica gel, EtOAc/cyclohexane, 1:4) and led to products **2a**, **2b** and **2c** (Scheme 1). The separated starting compound **1**, (S) *N*-Boc-Pyr-OtBu, as also recovered.

#### 4.2.1. (2*S*,4*R*)-4-Benzyl-di-*tert*-butyl 5-oxopyrrolidine-1,2-dicarboxylate **2a**

This compound was obtained as a white solid in 58% yield after purification on silica gel chromatography. Compound **2a** was recrystallized from an AcOEt/cyclohexane mixture as the solvent system (X-ray analysis: deposition numbers 984,810). *de* >99%. *mp* 75–79  $^{\circ}\text{C}$ ;  $R_f$  = 0.3 (AcOEt/cyclohexane, 1:4).  $t_R$  = 2.64 min.  $[\alpha]_D^{20}$  =  $-4.0$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ). MS ( $\text{ES}^+$ )  $m/z$  376.4 ( $\text{M}+\text{H}$ ) $^+$ , 398.3 ( $\text{M}+\text{Na}$ ) $^+$ , 276.3 ( $\text{MH}-\text{Boc}$ ) $^+$ .  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 4.27 (dd, 1H,  $\text{H}_2$ ,  $^3J(\text{H}_2-\text{H}_{3a}) = 2.1$  Hz,  $^3J(\text{H}_2-\text{H}_{3b}) = 9.2$  Hz), 3.26 (dd, 1H,  $\text{H}_{6a}$ ,  $^3J(\text{H}_4-\text{H}_{6a}) = 9.3$  Hz,  $^2J(\text{H}_{6a}-\text{H}_{6b}) = 14.0$  Hz), 2.91 (dtd, 1H,  $\text{H}_4$ ,  $^3J(\text{H}_4-\text{H}_{6a}) = 9.3$  Hz,  $^3J(\text{H}_4-\text{H}_{6b}) = 4.3$  Hz,  $^3J(\text{H}_4-\text{H}_{3a}) = 11.2$  Hz,  $^3J(\text{H}_4-\text{H}_{3b}) = 8.9$  Hz), 2.68 (dd, 1H,  $\text{H}_{6b}$ ,  $^3J(\text{H}_4-\text{H}_{6b}) = 4.3$  Hz,  $^2J(\text{H}_{6a}-\text{H}_{6b}) = 14.0$  Hz), 2.01 (ddd, 1H,  $\text{H}_{3a}$ ,  $^3J(\text{H}_2-\text{H}_{3a}) = 2.1$  Hz,  $^3J(\text{H}_{3a}-\text{H}_4) = 11.2$  Hz,  $^2J(\text{H}_{3a}-\text{H}_{3b}) = 13.2$  Hz), 1.96 (ddd, 1H,  $\text{H}_{3b}$ ,  $^3J(\text{H}_{3b}-\text{H}_2) = 9.2$  Hz,  $^3J(\text{H}_{3b}-\text{H}_4) = 8.9$  Hz,  $^2J(\text{H}_{3a}-\text{H}_{3b}) = 13.2$  Hz), 1.51 (s, 1H,  $\text{NCO}_2\text{C}(\text{CH}_3)_3$ ), 1.44 (s, 9H,  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ), 7.17–7.29 (m, 5H, ArH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta_c$  (ppm) 27.57, 36.17, 43.26, 57.66, 82.10, 83.26, 126.38, 128.65, 129.03, 138.35, 149.35, 170.21, 174.43.

Chiralcel-OD-RH column; isocratic  $\text{H}_2\text{O}/\text{ACN}$  (80:20), 1 mL/min, 30 min, 214 nm,  $t_R$  = 21.18 min.

Chiralpak AD-H column; isocratic hexane/isopropanol (75:25), 1 mL/min, 45 min, 214 nm,  $t_R$  = 32.67 min.

#### 4.2.2. (2*S*,4*R*)-4-(4-Isopropylbenzyl)-di-*tert*-butyl 5-oxopyrrolidine-1,2-dicarboxylate **2b**

This compound was obtained as a white solid in 52% yield after purification by silica gel chromatography. *de* >99%. *mp* 62–65  $^{\circ}\text{C}$ .  $R_f$  = 0.52 (AcOEt/cyclohexane, 1:4).  $t_R$  = 3.02 min.  $[\alpha]_D^{20}$  =  $-3.4$  (c 1.0,  $\text{CH}_2\text{Cl}_2$ ). MS ( $\text{ES}^+$ )  $m/z$  418.3 ( $\text{M}+\text{H}$ ) $^+$ , 440.3 ( $\text{M}+\text{Na}$ ) $^+$ , 318.3 ( $\text{MH}-\text{Boc}$ ) $^+$ .  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ ):  $\delta$  (ppm) 4.34 (dd, 1H,  $\text{H}_2$ ,  $^3J(\text{H}_2-\text{H}_{3a}) = 2.1$  Hz,  $^3J(\text{H}_2-\text{H}_{3b}) = 9.3$  Hz), 3.22 (dd, 1H,  $\text{H}_{6a}$ ,  $^3J(\text{H}_4-\text{H}_{6a}) = 9.4$  Hz,  $^2J(\text{H}_{6a}-\text{H}_{6b}) = 14.1$  Hz), 2.9 (m, 1H,  $(\text{CH}_3)_2\text{-CH-Ph}$ ,  $^3J = 6.9$  Hz), 2.87 (dtd, 1H,  $\text{H}_4$ ,  $^3J(\text{H}_4-\text{H}_{6a}) = 9.4$  Hz,  $^3J(\text{H}_4-\text{H}_{6b}) = 4.3$  Hz,  $^3J(\text{H}_4-\text{H}_{3a}) = 11.1$  Hz), 2.65 (dd, 1H,  $\text{H}_{6b}$ ,  $^3J(\text{H}_4-\text{H}_{6b}) = 4.3$  Hz,  $^2J(\text{H}_{6b}-\text{H}_{6a}) = 14.0$  Hz), 2.03 (ddd, 1H,  $\text{H}_{3a}$ ,  $^3J(\text{H}_2-\text{H}_{3a}) = 2.1$  Hz,  $^3J(\text{H}_4-\text{H}_{3a}) = 11.1$  Hz,  $^2J(\text{H}_{3a}-\text{H}_{3b}) = 13.3$  Hz), 1.97 (ddd, 1H,  $\text{H}_{3b}$ ,  $^3J(\text{H}_2-\text{H}_{3b}) = 9.3$  Hz,  $^3J(\text{H}_4-\text{H}_{3b}) = 8.8$  Hz,  $^2J(\text{H}_{3a}-\text{H}_{3b}) = 13.3$  Hz), 1.51 (s, 9H,  $\text{NCO}_2\text{C}(\text{CH}_3)_3$ ), 1.44 (s, 9H,  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ), 1.23 (d, 6H,  $(\text{CH}_3)_2\text{-CH-Ph}$ ,  $^3J = 6.9$  Hz), 7.09–7.15 (2d, 4H, ArH,  $^3J = 8.1$  Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta_c$  (ppm) 23.99, 27.94, 33.68, 35.64, 43.32, 47.76, 82.25, 83.25, 126.66, 128.93, 135.55, 147.14, 149.43, 170.34, 174.64.

Chiralcel-OD-RH column;  $\text{H}_2\text{O}/\text{ACN}$  (65:35), 1 mL/min, 30 min, 214 nm,  $t_R$  = 14.87 min.

Chiralpak AD-H column; hexane/isopropanol (90:10), 1 mL/min, 30 min, 214 nm,  $t_R$  = 23.54 min.

#### 4.2.3. (2*S*,4*R*)-4-(4-*tert*-Butylbenzyl)-di-*tert*-butyl 5-oxopyrrolidine-1,2-dicarboxylate **2c**

This compound was obtained as a white solid in 44% yield after purification by silica gel chromatography. *de* >99%. *mp* 54–60  $^{\circ}\text{C}$ .

$R_f$  = 0.58 (AcOEt/cyclohexane, 1:4).  $t_R$  = 3.13 min.  $[\alpha]_D^{20}$  =  $-2.9$  (c 1.0 in  $\text{CH}_2\text{Cl}_2$ ). MS ( $\text{ES}^+$ )  $m/z$  432.3 ( $\text{M}+\text{H}$ ) $^+$ , 454.2 ( $\text{M}+\text{Na}$ ) $^+$ , 332.3 ( $\text{MH}-\text{Boc}$ ) $^+$ .  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  (ppm) 4.34 (dd, 1H,  $\text{H}_2$ ,  $^3J(\text{H}_2-\text{H}_{3a}) = 2.1$  Hz,  $^3J(\text{H}_2-\text{H}_{3b}) = 9.4$  Hz), 3.22 (dd, 1H,  $\text{H}_{6a}$ ,  $^3J(\text{H}_4-\text{H}_{6a}) = 9.1$  Hz,  $^2J(\text{H}_{6a}-\text{H}_{6b}) = 14.2$  Hz), 2.9 (dtd, 1H,  $\text{H}_4$ ,  $^3J(\text{H}_4-\text{H}_{6a}) = 4.3$  Hz,  $^3J(\text{H}_4-\text{H}_{6b}) = 9.1$  Hz,  $^3J(\text{H}_4-\text{H}_{3b}) = 8.8$  Hz),  $^3J(\text{H}_4-\text{H}_{3a}) = 11.1$  Hz), 2.65 (dd, 1H,  $\text{H}_{6b}$ ,  $^3J(\text{H}_4-\text{H}_{6b}) = 4.3$  Hz,  $^2J(\text{H}_{6b}-\text{H}_{6a}) = 14.2$  Hz), 2.04 (ddd, 1H,  $\text{H}_{3a}$ ,  $^3J(\text{H}_2-\text{H}_{3a}) = 2.1$  Hz,  $^3J(\text{H}_4-\text{H}_{3a}) = 11.1$  Hz,  $^2J(\text{H}_{3a}-\text{H}_{3b}) = 13.3$  Hz), 1.98 (ddd, 1H,  $\text{H}_{3b}$ ,  $^3J(\text{H}_2-\text{H}_{3b}) = 9.4$  Hz,  $^3J(\text{H}_4-\text{H}_{3b}) = 8.8$  Hz,  $^2J(\text{H}_{3a}-\text{H}_{3b}) = 13.3$  Hz), 1.50 (s, 9H,  $\text{NCO}_2\text{C}(\text{CH}_3)_3$ ), 1.43 (s, 9H,  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ), 1.3 (s, 9H,  $\text{Ph-C}(\text{CH}_3)_3$ ), 7.1–7.3 (2d, 4H, ArH,  $^3J = 8.2$  Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta_c$  (ppm) 27.91, 28.00, 31.32, 34.37, 35.79, 43.23, 57.72, 82.22, 83.22, 125.47, 128.62, 135.15, 149.40, 170.30, 174.60.

Chiralcel-OD-RH column;  $\text{H}_2\text{O}/\text{ACN}$  (65:35), 1 mL/min, 30 min, 214 nm,  $t_R$  = 12.43 min.

Chiralpak AD-H column; hexane/isopropanol (95:5), 1 mL/min, 45 min, 214 nm,  $t_R$  = 35.41 min.

#### 4.3. Synthesis of *C*<sub>4</sub>-benzylated glutamate derivatives: general procedure for the pyroglutamate ring opening

Pure **2a**, **2b** and **2c** (1 mmol, 1 equiv) were dissolved in an aqueous solution of LiOH (84 mg, 2 mmol, 2 equiv) with the minimum amount of THF and were stirred at  $-10^{\circ}\text{C}$  for 10 min and then for 1 h at  $-5^{\circ}\text{C}$ . The reaction was followed by HPLC until the disappearance of compounds **2a**, **2b** or **2c**. Compounds **3a**, **3b** or **3c** were extracted with AcOEt; the organic layers were washed 3 times with aq. citric acid, then with water. The organic layers were dried over  $\text{MgSO}_4$  and concentrated under reduced pressure; compounds **3a**, **3b** and **3c** were obtained in 94–98% yields as colourless oils.

##### 4.3.1. (2*R*,4*S*)-2-Benzyl-5-(*tert*-butoxy)-4-((*tert*-butoxy-carbonyl)-amino)-5-oxopentanoic acid **3a**

$t_R$  = 2.37 min.  $R_f$  = 0.43 (DCM/EtOH, 96:4).  $[\alpha]_D^{20}$  =  $-0.4$  (c 1.0, MeOH). MS ( $\text{ES}^+$ )  $m/z$  394.4 ( $\text{M}+\text{H}$ ) $^+$ , 406.4 ( $\text{M}+\text{Na}$ ) $^+$ , 294.4 ( $\text{MH}-\text{Boc}$ ) $^+$ .  $^1\text{H}$  NMR (300 MHz, MeOD)  $\delta$  (ppm) 4.0 (m, 1H,  $\text{H}_4$ ), 2.91 (m, 1H,  $\text{H}_2$ ), 2.79 (m, 2H,  $\text{CH}_2\text{-Ph}$ ), 2.10 (m, 1H,  $\text{H}_3$ ), 1.71 (m, 1H,  $\text{H}_3$ ), 1.42 (s, 18H,  $\text{NCO}_2\text{C}(\text{CH}_3)_3$ ,  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ), 7.16–7.25 (m, 5H, ArH).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta_c$  (ppm) 28.26, 28.77, 34.51, 39.79, 45.47, 54.33, 80.53, 82.74, 127.55, 129.47, 130.12, 140.24, 158.02, 173.35, 178.23.

##### 4.3.2. (2*R*,4*S*)-2-(4-Isopropylbenzyl)-5-*tert*-butoxy-4-((*tert*-butoxycarbonyl)-amino)-5-oxopentanoic acid **3b**

$t_R$  = 2.72 min.  $R_f$  = 0.29 (DCM/EtOH, 98:2).  $[\alpha]_D^{20}$  =  $-0.3$  (c 1.0, MeOH). MS ( $\text{ES}^+$ )  $m/z$  436.4 ( $\text{M}+\text{H}$ ) $^+$ , 458.3 ( $\text{M}+\text{Na}$ ) $^+$ , 336.2 ( $\text{MH}-\text{Boc}$ ) $^+$ .  $^1\text{H}$  NMR (300 MHz, MeOD)  $\delta$  (ppm) 4.03 (m, 1H,  $\text{H}_4$ ), 2.85 (m, 2H,  $\text{H}_2$  and  $\text{CH}(\text{CH}_3)_2$ ), 2.75 (m, 2H,  $\text{CH}_2\text{-Ph}$ ), 2.11 (m, 1H,  $\text{H}_{3a}$ ), 1.73 (m, 1H,  $\text{H}_{3b}$ ), 1.42 (s, 18H,  $\text{NCO}_2\text{C}(\text{CH}_3)_3$ ,  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ), 1.22 (d, 6H,  $\text{CH}(\text{CH}_3)_2$ ,  $^3J = 6.9$  Hz), 7.17–7.21 (2d, 4H, ArH,  $^3J = 7.9$  Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta_c$  (ppm) 24.60, 28.34, 28.84, 34.39, 35.05, 39.42, 45.47, 54.34, 80.54, 82.24, 127.49, 130.14, 137.54, 148.24, 157.96, 173.32, 178.41.

##### 4.3.3. (2*R*,4*S*)-2-(4-*tert*-Butylbenzyl)-5-*tert*-butoxy-4-((*tert*-butoxycarbonyl)-amino)-5-oxopentanoic acid **3c**

$t_R$  = 2.82 min.  $R_f$  = 0.35 (DCM/EtOH, 98:2).  $[\alpha]_D^{20}$  =  $-0.3$  (c 1.0, MeOH). MS ( $\text{ES}^+$ )  $m/z$  450.4 ( $\text{M}+\text{H}$ ) $^+$ , 472.3 ( $\text{M}+\text{Na}$ ) $^+$ , 350.4 ( $\text{MH}-\text{Boc}$ ) $^+$ .  $^1\text{H}$  NMR (300 MHz, MeOD)  $\delta$  (ppm) 4.02 (m, 1H,  $\text{H}_4$ ), 2.95 (m, 1H,  $\text{H}_2$ ), 2.75 (m, 2H,  $\text{CH}_2\text{-Ph}$ ), 2.10 (m, 1H,  $\text{H}_{3a}$ ), 1.90 (m, 1H,  $\text{H}_{3b}$ ), 1.43 (s, 18H,  $\text{NCO}_2\text{C}(\text{CH}_3)_3$ ,  $\text{CO}_2\text{C}(\text{CH}_3)_3$ ), 1.29 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ), 7.13–7.36 (2d, 4H, ArH,  $^3J = 7.9$  Hz).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta_c$  (ppm) 28.25, 28.64, 31.84, 34.29, 35.20, 45.35, 54.28, 80.01, 82.81, 126.33, 129.48, 136.71, 149.93, 173.10, 178.06.

#### 4.4. Synthesis of protected C<sub>4</sub>-benzylated L-theanine derivatives: general procedure for the amide bond formation on N<sub>5</sub>

Products **3a–c** (200 mg, 1 equiv) were suspended in dry THF (6 mL) under argon and stirred magnetically, after which the BOP reagent (1.2 equiv), triethylamine TEA (3 equiv) and ethylamine solution in anhydrous THF 2 M (1 equiv) were added. After 1 h at room temperature, the THF was evaporated under vacuum and the oily residue was dissolved in AcOEt, washed 3 times with aqueous citric acid solution (10%), 3 times with 1 M aqueous NaHCO<sub>3</sub> solution, saturated NH<sub>4</sub>Cl solution and finally with water. The organic layer was dried over MgSO<sub>4</sub>, filtered and concentrated under vacuum. Products **4a–c** were obtained in quantitative yields as white solids.

##### 4.4.1. (2S,4R)-tert-Butyl 4-benzyl-2-((tert-butoxycarbonyl)amino)-5-(ethylamino)-5-oxopentanoate or (2S,4R)-4-benzyl-N-Boc-theanine tert-butyl ester **4a**

$t_R = 2.32$  min.  $R_f = 0.48$  (cyclohexane/AcOEt, 6:4). Mp 75–79 °C.  $[\alpha]_D^{20} = +2.4$  (c 1.0, CHCl<sub>3</sub>). MS (ES<sup>+</sup>)  $m/z$  421.3 (M+H)<sup>+</sup>, 443.4 (M+Na)<sup>+</sup>, 321.3 (MH-Boc)<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 6.63 (s, 1H, NHBoc), 5.12 (s, 1H, NH, CH<sub>3</sub>CH<sub>2</sub>NH), 4.12 (m, 1H, H<sub>2</sub>), 3.22 (q, 2H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.28 Hz), 3.00 (dd, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph, <sup>3</sup>J (H<sub>4</sub>-H<sub>a</sub>) = 7.9 Hz, <sup>2</sup>J (H<sub>a</sub>-H<sub>b</sub>) = 4.3 Hz), 2.60 (dd, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph, <sup>3</sup>J (H<sub>4</sub>-H<sub>b</sub>) = 13.48 Hz, <sup>2</sup>J (H<sub>a</sub>-H<sub>b</sub>) = 4.3 Hz), 2.41 (m, 1H, H<sub>4</sub>), 2.17 (m, 1H, H<sub>3a</sub>), 1.59 (m, 1H, H<sub>3b</sub>), 1.42 (s, 18H, NCO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>, CO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>), 1.04 (d, 3H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.2 Hz), 7.20 (m, 5H, ArH); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta_c$  (ppm) 14.63, 27.95, 28.28, 29.57, 34.24, 37.28, 38.52, 44.89, 52.59, 80.11, 82.10, 126.23, 128.36, 128.93, 139.63, 156.62, 171.46, 173.62.

##### 4.4.2. (2S,4R)-tert-Butyl 4-(4-iso-propylbenzyl)-2-((tert-butoxycarbonyl)amino)-5-(ethylamino)-5-oxopentanoate or (2R,4S)-4-(4-isopropylbenzyl)-N-Boc-theanine tert-butyl ester **4b**

$t_R = 2.69$  min.  $R_f = 0.61$  (cyclohexane/AcOEt, 6:4). Mp 68–72 °C.  $[\alpha]_D^{20} = +2.3$  (c 1.0, CHCl<sub>3</sub>). MS (ES<sup>+</sup>)  $m/z$  463.5 (M+H)<sup>+</sup>, 485.4 (M+Na)<sup>+</sup>, 363.4 (MH-Boc)<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 6.52 (m, 1H, NHBoc), 5.12 (m, 1H, CH<sub>3</sub>CH<sub>2</sub>NH), 4.13 (m, 1H, H<sub>2</sub>), 3.21 (q, 2H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.28 Hz), 2.95 (dd, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph, <sup>3</sup>J (H<sub>4</sub>-H<sub>a</sub>) = 15.53 Hz, <sup>2</sup>J (H<sub>a</sub>-H<sub>b</sub>) = 6.8 Hz), 2.84 (m, 1H, -CH-(CH<sub>3</sub>)<sub>2</sub>), 2.58 (dd, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph, <sup>3</sup>J (H<sub>4</sub>-H<sub>b</sub>) = 13.37 Hz, <sup>2</sup>J (H<sub>a</sub>-H<sub>b</sub>) = 6.8 Hz), 2.3 (m, 1H, H<sub>4</sub>), 2.17 (m, 1H, H<sub>3a</sub>), 1.59 (m, 1H, H<sub>3b</sub>), 1.42 (s, 18H, NCO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>, CO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>), 1.22 (d, 6H, <sup>3</sup>J = 6.9 Hz), 1.02 (t, 3H, CH<sub>3</sub>-CH<sub>2</sub>NH, <sup>3</sup>J = 7.28 Hz), 7.17–7.21 (2d, 4H, ArH, <sup>3</sup>J = 7.9 Hz). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta_c$  (ppm) 14.63, 23.98, 24.05, 27.96, 33.7, 34.22, 45.18, 52.65, 80.06, 82.34, 126.40, 128.82, 136.83, 146.77, 156.23, 171.54, 173.76.

##### 4.4.3. (2S,4R)-tert-Butyl 4-(4-tert-butylbenzyl)-2-((tert-butoxycarbonyl)amino)-5-(ethylamino)-5-oxopentanoate or (2R,4S)-4-(4-tert-butylbenzyl)-N-Boc-theanine tert-butyl ester **4c**

$t_R = 2.77$  min.  $R_f = 0.67$  (cyclohexane/AcOEt, 6:4). Mp 60–68 °C.  $[\alpha]_D^{20} = +2.2$  (c 1.0, CHCl<sub>3</sub>). MS (ES<sup>+</sup>)  $m/z$  477.5 (M+H)<sup>+</sup>, 499.5 (M+Na)<sup>+</sup>, 377.3 (MH-Boc)<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 6.50 (m, 1H, NHBoc), 5.09 (m, 1H, CH<sub>3</sub>CH<sub>2</sub>NH), 4.09 (m, 1H, H<sub>2</sub>), 3.22 (q, 2H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.24 Hz), 2.96 (dd, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph, <sup>3</sup>J (H<sub>4</sub>-H<sub>a</sub>) = 15.58 Hz, <sup>2</sup>J (H<sub>a</sub>-H<sub>b</sub>) = 7.9 Hz), 2.59 (dd, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph, <sup>3</sup>J (H<sub>4</sub>-H<sub>b</sub>) = 13.74 Hz, <sup>2</sup>J (H<sub>a</sub>-H<sub>b</sub>) = 7.9 Hz), 2.37 (m, 1H, H<sub>4</sub>), 2.20 (m, 1H, H<sub>3a</sub>), 1.57 (m, 1H, H<sub>3b</sub>), 1.43 (s, 9H, NCO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>), 1.41 (s, 9H, CO<sub>2</sub>C(CH<sub>3</sub>)<sub>3</sub>), 1.27 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>), 1.02 (t, 3H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.24 Hz), 7.05–7.26 (2d, 4H, ArH, <sup>3</sup>J = 8.18 Hz). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta_c$  (ppm) 14.63, 27.96, 28.30, 31.37, 34.21, 34.36, 36.89, 38.14, 45.29, 51.28, 80.05, 82.31, 125.26, 128.55, 136.33, 149.01, 156.98, 171.56, 173.75.

#### 4.5. Synthesis of C<sub>4</sub>-benzylated L-theanine derivatives: general procedure for protecting group hydrolysis

The enantiomerically pure N- and C-protected **4a–c** (60 mg) were added to a solution of TFA/DCM (4 mL, 2 M). Reactions were stirred at room temperature. The protecting group hydrolysis was followed by CCM and analytical HPLC. After 2 h, the reaction mixtures were concentrated under reduced pressure. The residues were then washed with Et<sub>2</sub>O to afford products **5a–c** as white solids in quantitative yields.

##### 4.5.1. (2S,4R)-4-Benzyl-2-amino-5-(ethylamino)-5-oxopentanoic acid or (2S,4R)-4-benzyl-theanine **5a**

$t_R = 1.08$  min.  $R_f = 0.16$  (Et<sub>2</sub>O/AcOEt, 4/6). Mp 97–101 °C.  $[\alpha]_D^{20} = +1.3$  (c 1.0, CD<sub>3</sub>OD). MS (ES<sup>+</sup>)  $m/z$  265.3 (M+H)<sup>+</sup>, 287.3 (M+Na)<sup>+</sup>, 220.2 (M-CO<sub>2</sub>)<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  (ppm): 3.9 (m, 1H, H<sub>2</sub>), 3.10 (m, 2H, CH<sub>2</sub>-Ph), 2.92 (m, 3H, CH<sub>3</sub>CH<sub>2</sub>NH, H<sub>4</sub>), 2.33 (m, 1H, H<sub>3a</sub>), 1.96 (m, 1H, H<sub>3b</sub>), 0.97 (t, 3H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.3 Hz) 7.26 (m, 5H, ArH). <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD)  $\delta_c$  (ppm) 14.39, 33.68, 35.05, 39.24, 46.49, 52.48, 127.50, 129.34, 130.00, 139.73, 172.33, 175.73. HRMS calculated for C<sub>14</sub>H<sub>21</sub>N<sub>2</sub>O<sub>3</sub>  $m/z$  (M+H)<sup>+</sup> 265,1552; found 265,1548.

##### 4.5.2. (2S,4R)-4-(4-Iso-propylbenzyl)-2-amino-5-(ethylamino)-5-oxopentanoic acid or (2S,4R)-4-(4-iso-propylbenzyl)-theanine **5b**

$t_R = 1.58$  min.  $R_f = 0.24$  (Et<sub>2</sub>O/AcOEt, 4/6). Mp 82–87 °C.  $[\alpha]_D^{20} = +1.1$  (c 1.0, MeOH). MS (ES<sup>+</sup>)  $m/z$  307.3 (M+H)<sup>+</sup>, 329.4 (M+Na)<sup>+</sup>, 262.3 (M-CO<sub>2</sub>)<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, MeOD)  $\delta$  (ppm): 3.76 (m, 1H, H<sub>2</sub>), 3.17 (m, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph), 3.04 (m, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph), 2.9 (m, 4H, CH<sub>3</sub>CH<sub>2</sub>NH, CH-(CH<sub>3</sub>)<sub>2</sub>, H<sub>4</sub>), 2.24 (m, 1H, H<sub>3a</sub>), 1.97 (m, 1H, H<sub>3b</sub>), 1.20 (d, 6H, CH-(CH<sub>3</sub>)<sub>2</sub>, <sup>3</sup>J = 6.9 Hz), 0.89 (t, 3H, CH<sub>3</sub>-CH<sub>2</sub>NH, <sup>3</sup>J = 7.25 Hz), 7.12–7.18 (m, 4H, ArH). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta_c$  (ppm) 13.87, 23.79, 33.68, 34.35, 34.43, 38.21, 46.22, 53.05, 126.67, 129.44, 136.78, 147.59, 173.12, 175.98. HRMS calculated for C<sub>17</sub>H<sub>27</sub>N<sub>2</sub>O<sub>3</sub>  $m/z$  (M+H)<sup>+</sup> 307,2022; found 307,2025.

##### 4.5.3. (2S,4R)-4-(4-tert-Butylbenzyl)-2-amino-5-(ethylamino)-5-oxopentanoic acid or (2S,4R)-4-(4-tert-butylbenzyl) theanine **5c**

$t_R = 1.71$  min.  $R_f = 0.27$  (Et<sub>2</sub>O/AcOEt, 4/6). Mp 74–79 °C.  $[\alpha]_D^{20} = +0.9$  (c 1.0, CD<sub>3</sub>OD). MS (ES<sup>+</sup>)  $m/z$  321.3 (M+H)<sup>+</sup>, 343.5 (M+Na)<sup>+</sup>, 276.3 (MH-CO<sub>2</sub>)<sup>+</sup>. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 3.75 (m, 1H, H<sub>2</sub>), 3.12 (m, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph), 2.98 (m, 1H, CH<sub>3</sub>H<sub>b</sub>-Ph), 2.81 (m, 3H, CH<sub>3</sub>CH<sub>2</sub>NH, H<sub>4</sub>), 2.25 (m, 1H, H<sub>3a</sub>), 1.92 (m, 1H, H<sub>3b</sub>), 1.26 (s, 9H, C-(CH<sub>3</sub>)<sub>3</sub>), 0.87 (t, 3H, CH<sub>3</sub>CH<sub>2</sub>NH, <sup>3</sup>J = 7.25 Hz), 7.10–7.28 (2d, 4H, ArH, <sup>3</sup>J = 8.17 Hz). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta_c$  (ppm) 14.66, 14.71, 31.87, 33.88, 35.21, 35.25, 39.28, 46.58, 52.57, 126.38, 129.89, 136.77, 150.60, 172.22, 175.94. HRMS calculated for C<sub>18</sub>H<sub>29</sub>N<sub>2</sub>O<sub>3</sub>  $m/z$  (M+H)<sup>+</sup> 321,2178; found 321,2174.

#### 4.6. X-ray crystal-structure determination of **2a**

Suitable crystals of **2a** for X-ray diffraction studies were grown in AcOEt/cyclohexane as a colourless prism. The crystals of **1** (C<sub>21</sub>H<sub>29</sub>NO<sub>5</sub>) are monoclinic. At 187 K,  $a = 9.30881(19)$  Å,  $b = 10.5384(2)$  Å,  $c = 10.8965(2)$  Å,  $\beta = 104.491(2)^\circ$ ,  $V = 1034.93(4)$  Å<sup>3</sup>,  $M_r = 375.45$ ,  $Z = 2$ , Space group P2<sub>1</sub>,  $d_{\text{calcd}} = 1.205$  g/cm<sup>3</sup>,  $\mu(\text{Cu K}\alpha) = 0.70$  mm<sup>-1</sup>,  $F(000) = 404$ . Intensities of 19,131 reflections of which 3686 were unique ( $R_{\text{int}} = 0.052$ ) were measured with an Xcalibur, Sapphire 3, Gemini diffractometer (graphite monochromated CuK $\alpha$  radiation ( $k = 1.54184$  Å), CCD-detector,  $\omega$  scanning,  $2\theta_{\text{max}} = 67.8^\circ$ ).<sup>17</sup> Data reduction was performed with CrysAlisPro and an empirical absorption correction was applied.<sup>17</sup> Equivalent reflections, other than Friedel pairs (1716, 97% coverage), were merged.

The structures were solved by ab initio (charge-flipping) methods using SUPERFLIP<sup>18</sup> and refined using SHELXL.<sup>19</sup> H atoms were treated by a mixture of independent and constrained refinement. The carbon of the phenyl from the benzyl residue was disordered over two positions, which were used during the refinement. A view of the molecule is shown in Figure 5. Full-matrix least-squares refinement against  $F^2$  in anisotropic approximation for non-hydrogen atoms was converged to  $wR_2 = 0.112$  for 3686 reflections ( $R_1 = 0.043$  for 3529 reflections with  $F^2 > 2\sigma(F^2)$ ,  $S = 1.05$ ). Refinement of the absolute structure Flack parameter yielded a value of  $-0.04(13)$ ,<sup>20</sup> while the Hooft analysis<sup>21</sup> gave the absolute parameter  $\gamma = 0.07(9)$ ,  $P2(\text{true}) = 1.000$  and  $P3(\text{true}) = 1.000$ , which confirmed that the refined coordinates represent the true enantiomorph as the Parson parameter  $z = 0.08(8)$ . Final atomic coordinates, geometrical parameters and crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, 11 Union Road, Cambridge CB2 1EZ, UK (E-mail: deposit@ccdc.cam.ac.uk; fax: +44 1223 336033) and are available upon request by quoting the deposition numbers 984,810 for **2a**.

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## References

- Ekborg-Ott, K. H.; Taylor, A.; Armstrong, D. W. *J. Agric. Food Chem.* **1997**, *45*, 353–363.
- Vuong, O. V.; Bowyer, M. C.; Roach, P. D. *J. Sci. Food Agric.* **2011**, *91*, 1931–1939.
- Yokogoshi, H.; Kato, Y.; Sagesaka, Y.; Matsuura, T.; Kakuda, T.; Takeuchi, N. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 615–618.
- Kim, N. H.; Jeong, H. J.; Kim, H. M. *Amino Acids* **2012**, *42*, 1609–1618.
- Yamada, T.; Terashima, T.; Kawano, S.; Furuno, R.; Okubo, T.; Juneja, L. R.; Yokogoshi, H. *Amino Acids* **2009**, *36*, 21–27.
- Wehbe, J.; Kassem, T.; Rolland, V.; Rolland, M.; Tabcheh, M.; Roumestant, M. L.; Martinez, J. *Org. Biomol. Chem.* **2003**, *1*, 1938–1942.
- a Mekki, S.; Bellahouel, S.; Vanthuyne, N.; Rolland, M.; Derdour, A.; Martinez, J.; Rolland, V. *Tetrahedron: Asymmetry* **2012**, *23*, 94–99; b Mekki, S.; Rolland, V.; Bellahouel, S.; Van der Lee, A.; Rolland, M. *Acta Crystallogr.* **2011**, *C67*, o301–o305.
- Esslinger, C. S.; Agarwal, S.; Gerdes, J.; Wilson, P. A.; Davis, E. S.; Awes, A. N.; O'Brien, E.; Mavencamp, T.; Koch, H. P.; Poulsen, D. J.; Rhoderick, J. F.; Chamberlin, A. R.; Kavanaugh, M. P.; Bridges, R. J. *Neuropharmacology* **2005**, *49*, 850–861.
- Wehbe, J.; Rolland, V.; Roumestant, M. L.; Martinez, J. *Tetrahedron: Asymmetry* **2003**, *14*, 1123–1126.
- Vandenberg, R. J.; Mitrovic, A. D.; Chebib, M.; Balcar, V. J.; Johnston, G. A. R. *Mol. Pharmacol.* **1997**, *51*, 809.
- Wehbe, J.; Rolland, V.; Fruchier, A.; Roumestant, M. L.; Martinez, J. *Tetrahedron: Asymmetry* **2004**, *15*, 851–858.
- Del Bosco, M.; Johnstone, A. N. C.; Bazza, G.; Lopatriello, S.; North, M. *Tetrahedron* **1995**, *51*, 8545–8554.
- Tarver, J. E., Jr.; Terranova, K. M.; Joullie, M. M. *Tetrahedron* **2004**, *60*, 10277–10284.
- Lichtenstein, N. *J. Am. Chem. Soc.* **1942**, *64*, 1021–1022.
- Yan, S. H.; Dufour, J. P.; Meurens, M. J. *Tea Sci.* **2003**, *23*, 99–104.
- Crouzin, N.; de Jesus Ferreira, M. C.; Cohen-Solal, C.; Aimar, R. F.; Vignes, M.; Guiramand, J. *Free Radical Biol. Med.* **2007**, *42*(9), 1326–1337.
- CrysAlisPro, Version 1.171.35.21; Agilent Technologies: Yarnton, Oxfordshire, England, 2012.
- a Palatinus, L.; Chapuis, G. *J. Appl. Crystallogr.* **2007**, *40*, 786–790; b Palatinus, L.; Van der Lee, A. *J. Appl. Crystallogr.* **2008**, *41*, 975–984; c Palatinus, L.; Prathapa, S. J.; van Smaalen, S. *J. Appl. Crystallogr.* **2012**, *45*, 575–580.
- Sheldrick, G. M. *Acta Crystallogr., Sect. A* **2008**, *64*, 112–122.
- a Flack, H. D.; Bernardinelli, G. *Acta Crystallogr., Sect. A* **1999**, *55*, 908–915; b Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143–1148.
- a Hooft, R. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2008**, *41*, 96–103; b Hooft, R. W.; Straver, L. H.; Spek, A. L. *J. Appl. Crystallogr.* **2010**, *43*, 665–668.