# Buchwald Hartwig diversification of unprotected halotryptophans, halotryptophan containing tripeptides and the natural product barettin in aqueous conditions

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## **General Experimental**

All reagents were purchased from commercial suppliers and were used without further purification unless otherwise stated.

Proton NMR (<sup>1</sup>H), carbon NMR (<sup>13</sup>C) and fluorine NMR (<sup>19</sup>F) were recorded on a Bruker Ascend 500 (500 MHz), Bruker 500 UltraShield (500 MHz), Bruker Ascend 700 (700 MHz), Bruker 400 UltraShield (400 MHz) or a Bruker UltraShield (300 MHz) spectrometer. Fluorine NMR were also recorded as proton decoupled (<sup>19</sup>F{<sup>1</sup>H}). Using an HSQC experiment with multiplicity editing, the <sup>13</sup>C NMR signals were assigned to CH<sub>3</sub>, CH<sub>2</sub>, CH and C. The NMR experiments were carried out in deuterated chloroform (CDCl<sub>3</sub>), deuterated DMSO (DMSO*d*<sub>6</sub>) or deuterated methanol (CD<sub>3</sub>OD). The chemical shifts ( $\delta$ ) are quoted in parts per million (ppm). Multiplicities are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad for the <sup>1</sup>H NMR. Coupling constants are reported in Hertz (Hz). Quoted purity was determined by <sup>1</sup>H NMR.

Thin layer chromatography (TLC) was performed using aluminium sheets of silica gel 60 F254 and was visualised under a Mineralight model UVGL-58 lamp (254 nm).

High and low resolution mass spectra were recorded at the University of St Andrews on a Thermo Orbitrap Velos Pro system equipped with a Phenomenex Kinetix evo C18 3.5  $\mu$ m 2.1  $\times$  100 mm column, held at 40 °C.

Microwave reactions were carried out on a Biotage Initiator+.

5-Bromotryptophan, 6-bromotryptophan, 7-bromotryptophan were prepared as described previously.<sup>1</sup>

The IUPAC names of some compounds were obtained using ChemDraw Professional (version 16.0.1.4(77)).

#### **Standard Reaction Procedures:**

#### Standard reaction procedure of 5-bromoindole (Method A):

[Pd('Bu-XPhos)G<sub>1</sub>] (2 mol%, 2.7 mg) was placed in a vial, to this was added, under inert atmosphere (Argon), 5-bromoindole (0.2 mmol, 1.0 eq.) followed by THF (0.2 mL), aniline (0.4 mmol, 2 eq) and finally a solution of  $K_2CO_3$  (0.24 mmol, 1.2 eq in 0.2 mL of water). The vial was closed and stirred at 65 °C for 16 h. The solution was allowed to cool to room temperature and was diluted with 5 mL water followed by extraction with ethyl acetate, the organic phase was then dried over MgSO<sub>4</sub>, filtered and solvent evaporated to afford the crude product. The crude product was purified by automated chromatography on a Biotage Isolera Four, using a 10 g silica cartridge, eluant mixture *n*-hexane/dichloromethane: 0% dichloromethane for 2CV, gradient to 20% dichloromethane for 2CV, 20% dichloromethane for 9CV, gradient to 50% dichloromethane for 5CV, 50% dichloromethane for 11CV, gradient to 100% dichloromethane over 3CV, 100% dichloromethane for 4CV, flow 12 mL/min, room temperature, monitored at 254 and 280 nm.

#### Microwave reaction procedure for halotryptophans (Method B):

[Pd(<sup>t</sup>Bu-XPhos)G<sub>1</sub>] (5 mol%, 1.7 mg) was placed in a vial, to this was added, under inert atmosphere (Argon), halotryptophan (0.05 mmol, 1.0 eq.), followed by 1,4-dioxane (0.3 mL), aniline (0.1 mmol, 2.0 eq.) and finally a solution of KOH (0.2 mmol, 11.2 mg in 0.3 mL of water). The vial was sealed and then stirred and heated in the microwave at 100 °C for 8 min. The solution was allowed to cool to room temperature and then diluted with 1.5 mL water. The solution was acidified to pH = 1-3 with a 0.1 M HCl solution. The resulting solution was washed  $4 \times 5$  mL diethylether and then the aqueous was evaporated under reduced pressure. The crude product was purified by automated reverse phase chromatography on a Biotage Isolera Four, see purification methods.

#### Microwave reaction procedure for peptides (Method C):

[Pd(<sup>t</sup>Bu-XPhos)G<sub>1</sub>] (15 mol%, 1.0 mg) was placed in a vial, to this was added, under inert atmosphere (Argon), halotryptophan-tripeptide (0.01 mmol, 1.0 eq.), followed by 1,4-dioxane (0.3 mL), aniline (0.02 mmol, 2.0 eq.) and finally a solution of KOH (0.04 mmol, 2.2 mg in 0.3 mL of water). The vial was sealed and then stirred and heated in the microwave at 100 °C for the appropriate time. The solution was allowed to cool to room temperature and then diluted with 1.5 mL water. The solution was acidified to pH = 2-3 with a 0.1 M HCl solution and

evaporated to dryness under reduced pressure. The crude product was purified by automated reverse phase chromatography on a Biotage Isolera Four, see purification methods.

## **Standard Purification Procedures:**

## **Reverse phase purification for tryptophans (Purification Method A):**

The purification of the desired BHA product of halotryptophans was performed on an automatic reverse phase column chromatography using Biotage Isolera Four. The crude sample is dissolved in 0.1 M HCl (2-6 mL) and then introduced to a KP C-18 12 g cartidge. Reverse phase chromatography conditions, eluant mixture methanol/water: 2% methanol for 1CV, gradient to 15% methanol over 1.1CV, held at 15% methanol for 5.7CV, gradient to 56% methanol over 7.0CV, held at 56% methanol for 3CV, gradient to 95% methanol over 6.7CV, held at 95% methanol for 4CV, flow 12 mL/min, room temperature, monitored at 254 and 280 nm.

## **Reverse phase purification for peptides (Purification Method B):**

The purification of the desired BHA product of halotryptophans was performed on an automatic reverse phase column chromatography using Biotage Isolera Four. The crude sample is dissolved in water (2-3 mL) with a few drops of methanol as required and then introduced to a KP C-18 12 g cartidge. Reverse phase chromatography conditions, eluant mix methanol/water: 2% methanol for 1CV, gradient to 15% methanol over 1.1CV, held at 15% methanol for 5.7CV, gradient to 56% methanol over 7CV, held at 56% methanol for 3CV, gradient to 71% methanol over 2.8CV, held at 71% methanol for 4CV, gradient to 86% methanol for 4CV, flow 12 mL/min, room temperature, monitored at 254 and 280 nm.

#### N-phenyl-1H-indol-5-amine (5)



Synthesis as per Method A, 10.0 mg, yield 85%, purity >95%.

*δ* H (400 MHz, CDCl<sub>3</sub>) 8.12 (1 H, s, NH), 7.46 (1 H, d, *J* 2.0, ArH), 7.33 (1 H, d, *J* 8.6, ArH), 7.25 – 7.16 (3 H, m, ArH), 7.06 (1 H, dd, *J* 8.6, 2.0, ArH), 7.01 – 6.95 (2 H, m, ArH), 6.84 (1 H, t, *J* 7.3, ArH), 6.49 (1 H, m, ArH).

δ C (100 MHz, CDCl<sub>3</sub>), 146.2 (C), 135.2 (C), 132.8 (C), 129.4 (CH), 128.7 (C), 125.1 (CH), 119.3 (CH), 118.4 (CH), 115.5 (CH), 113.3 (CH), 111.8 (CH), 102.5 (CH).

(S)-2-amino-3-(5-(phenylamino)-1H-indol-3-yl)propanoic acid (7)



Synthesi as per Method B, purification Method A, 33.6 mg, yield 75%, purity >95%.

*δ* H (500 MHz, CD<sub>3</sub>OD) 7.37 (1 H, d, *J* 1.9, ArH), 7.27 (1 H, d, *J* 8.6, ArH), 7.13 (1 H, s, ArH), 7.11 – 7.05 (2 H, m, ArH), 6.96 (1 H, dd, *J* 8.6, 1.9, ArH), 6.89 (2 H, d, *J* 7.5 ArH), 6.65 (1 H, t, *J* 7.5 ArH), 3.96 (1 H, dd, *J* 8.9, 4.3, CH), 3.42 (1 H, dd, *J* 15.2, 4.3, CH<sub>A</sub>H<sub>B</sub>), 3.14 (1 H, dd, *J* 15.2, 8.9, CH<sub>A</sub>H<sub>B</sub>).

*δ* C (126 MHz, CD<sub>3</sub>OD) 173.3 (CO<sub>2</sub>H), 148.2 (C), 136.8 (C), 134.9 (C), 130.0 (CH), 129.0 (C), 125.9 (CH), 119.3 (CH), 118.9 (CH), 116.0 (CH), 113.0 (CH), 110.9 (CH), 108.4 (C), 55.2 (CH), 28.1 (CH<sub>2</sub>).

LCMS: r.t. 3.87 min (ESI) 279 (98), 296 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for C<sub>17</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 296.1399; found: 296.1390.

## (S)-2-amino-3-(6-(phenylamino)-1H-indol-3-yl)propanoic acid (8)



Synthesi as per Method B, purification Method A, 22.8 mg, yield 51%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.51 (1 H, d, *J* 8.5, ArH), 7.16 – 7.09 (3 H, m, ArH), 7.03 (1 H, s, ArH), 6.98 (2 H, d, *J* 7.7, ArH), 6.85 (1 H, dd, *J* 8.5, 1.9, ArH), 6.72 (1 H, t, *J* 7.3, ArH), 3.97 (1 H, dd, *J* 9.0, 4.1, CH), 3.44 (1 H, dd, *J* 15.1, 4.1, CH<sub>A</sub>H<sub>B</sub>), 3.14 (1 H, dd, *J* 15.2, 9.0, CH<sub>A</sub>H<sub>B</sub>).  $\delta$  C (126 MHz, CD<sub>3</sub>OD) 173.4 (CO<sub>2</sub>H), 146.9 (C), 140.1 (C), 139.3 (C), 130.0 (CH), 124.2 (CH), 123.6 (C), 120.1 (CH), 119.7 (CH), 117.1 (CH), 114.8(CH), 109.0 (C), 102.0 (CH), 55.8 (CH), 28.3 (CH<sub>2</sub>).

LCMS: r.t. 3.86 min (ESI) 221 (20), 237 (22), 279 (56), 296 (100) [M+H]<sup>+</sup>; HRMS: *m*/*z* calcd for C<sub>17</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 296.1399; found: 296.1387.

## (S)-2-amino-3-(7-(phenylamino)-1H-indol-3-yl)propanoic acid (9)



Synthesi as per Method B, purification Method A, 10.1 mg, yield 68%, purity >95%.

*δ* H (500 MHz, DMSO- *d*<sub>6</sub>) 11.04 (1H, s, b), 8.24 (1 H, s, NH), 7.23-7.16 (3 H, m, ArH), 7.05 (2H, d, *J* 7.6 ArH), 7.02 (1 H, d, *J* 2.4, ArH), 6.96 (1 H, d, *J* 7.4, ArH), 6.91 (1 H, t, *J* 7.6, ArH), 6.75 (1 H, t, *J* 7.2, ArH), 3.52 (1 H, dd, *J* 8.1, 4.4, CH), 3.28 (1 H, dd, *J* 15.1, 4.4, CH<sub>A</sub>*H*<sub>B</sub>), 3.02 (1 H, dd, *J* 15.1, 8.1, C*H*<sub>A</sub>H<sub>B</sub>).

*δ* C (126 MHz, DMSO- *d*<sub>6</sub>) 170.5 (CO<sub>2</sub>H), 144.8 (C), 129.2 (C), 129.0 (CH), 128.8 (C), 128.2 (C), 123.7 (CH), 118.9 (CH), 118.7 (CH), 115.8 (CH), 111.8 (CH), 109.8 (C), 109.4 (CH), 54.8 (CH), 27.2 (CH<sub>2</sub>).

LCMS: r.t. 3.91 min (ESI) 279 (80), 296 (100) [M+H]<sup>+</sup>; HRMS: *m*/*z* calcd for C<sub>17</sub>H<sub>18</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 296.1399; found: 296.1388.

## (S)-2-amino-3-(5-(p-tolylamino)-1H-indol-3-yl)propanoic acid (10a)



Synthesi as per Method B, purification Method A, 9.1 mg, yield 59%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.36 (1 H, d, *J* 2.0, ArH), 7.24 (1 H, d, *J* 8.6, ArH), 7.12 (1 H, s, ArH), 6.97 – 6.90 (3 H, m, ArH), 6.88 – 6.79 (2 H, m, ArH), 3.79 (1 H, dd, *J* 9.5, 4.0, CH), 3.42 (1 H, dd, *J* 15.2, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.05 (1 H, dd, *J* 15.2, 9.5, CH<sub>A</sub>H<sub>B</sub>), 2.18 (3 H, s, CH<sub>3</sub>).

*δ* C (126 MHz, DMSO- *d*<sub>6</sub>) 174.5 (CO<sub>2</sub>H), 145.7 (C), 137.5 (C), 134.7 (C), 130.5 (CH), 129.1 (C), 128.8 (C), 125.7 (CH), 118.3 (CH), 116.7 (CH), 112.8 (CH), 110.2 (CH), 109.1 (C), 56.6 (CH), 28.5 (CH<sub>2</sub>), 20.6 (CH<sub>3</sub>).

LCMS: r.t. 4.21 min (ESI) 204 (10), 235 (22), 293 (70), 310 (100) [M+H]<sup>+</sup>; HRMS: *m*/*z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 310.1556; found: 310.1544.

(S)-2-amino-3-(7-(p-tolylamino)-1H-indol-3-yl)propanoic acid (10b)



Syntheis as per Method B, purification Method A, 8.2 mg, yield 53%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.32 (1 H, dd, *J* 7.3, 1.6, ArH), 7.12 (1 H, s, ArH), 6.98 (2 H, d, *J* 8.4, ArH), 6.95-6.91 (2 H, m, ArH), 6.84 (2 H, d, *J* 8.4, ArH), 3.83 (1 H, dd, *J* 9.5, 4.0, CH), 3.48 (1 H, dd, *J* 15.0, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.10 (2 H, dd, *J* 15.0, 9.5, CH<sub>A</sub>H<sub>B</sub>), 2.22 (3 H, s, CH<sub>3</sub>).

*δ* C (126 MHz, DMSO- *d*<sub>6</sub>) 170.7 (CO<sub>2</sub>H), 141.8 (C), 129.5 (CH), 129.1 (C), 128.6 (C), 128.6 (C), 127.7 (C), 123.6 (CH), 118.9 (CH), 116.7 (CH), 110.9 (CH), 109.7 (C), 107.8 (CH), 54.8 (CH), 27.2 (CH<sub>2</sub>), 20.3 (CH<sub>3</sub>).

LCMS: r.t. 4.50 min (ESI) 235 (16), 293 (65), 310 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 310.1556; found: 310.1544.

## (S)-2-amino-3-(5-(o-tolylamino)-1H-indol-3-yl)propanoic acid (11a)



Synthesi as per Method B, purification Method A, 29.1 mg, yield 63%, purity >95%.

 $\delta$  H (400 MHz, CD<sub>3</sub>OD) 7.34 (1 H, d, *J* 1.8, ArH), 7.27 (1 H, d, *J* 8.6, ArH), 7.14 (1 H, s, ArH), 7.05 (1 H, d, *J* 7.4, ArH), 6.96 – 6.87 (3 H, m, ArH), 6.72 – 6.62 (1 H, m, ArH), 3.81 (1 H, dd, *J* 9.2, 4.0, CH), 3.42 (1 H, dd, *J* 15.2, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.08 (1 H, dd, *J* 15.2, 9.2, CH<sub>A</sub>H<sub>B</sub>), 2.24 (3 H, s, CH<sub>3</sub>).

δ C (100 MHz, CD<sub>3</sub>OD) 174.4 (CO<sub>2</sub>H), 146.4 (C), 137.4 (C), 134.9 (C), 131.4 (CH), 129.2 (C), 127.4 (CH), 126.5 (C), 125.8 (CH), 120.1 (CH), 119.1 (CH), 115.9 (CH), 112.8 (CH), 111.5 (CH), 109.1 (C), 56.5 (CH), 28.5 (CH<sub>2</sub>), 18.1 (CH<sub>3</sub>).

LCMS: r.t. 4.25 min (ESI) 235 (10), 293 (70), 310 (100)  $[M+H]^+$ ; HRMS: m/z calcd for  $C_{18}H_{20}N_3O_2 [M+H]^+$ : 310.1556; found: 310.1543.

## (S)-2-amino-3-(7-(o-tolylamino)-1H-indol-3-yl)propanoic acid (11b)



Syntheis as per Method B, purification Method A, 9.6 mg, yield 62%, purity >95%.  $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.33 (1 H, d, *J* 7.9, ArH), 7.16-7.11 (2 H, m, ArH), 7.00 – 6.90 (2 H, m, ArH), 6.84 – 6.75 (2 H, m, ArH), 6.71 (1 H, d, *J* 7.5, ArH), 3.87 (1 H, dd, *J* 9.4, 4.0, CH), 3.49 (1 H, dd, *J* 15.2, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.12 (1 H, dd, *J* 15.2, 9.4, CH<sub>A</sub>H<sub>B</sub>), 2.28 (3 H, s, CH<sub>3</sub>).  $\delta$  C (126 MHz, CD<sub>3</sub>OD) 174.4 (CO<sub>2</sub>H), 144.0 (C), 131.7 (CH), 131.2 (C), 130.7 (C), 129.9 (C), 128.7 (C), 127.5 (CH), 124.7 (CH), 121.8 (CH), 120.9 (CH), 118.8 (CH), 113.1 (CH), 112.5 (CH), 110.2 (C), 56.7 (CH), 28.6 (CH<sub>2</sub>), 18.1 (CH<sub>3</sub>). LCMS: r.t. 4.42 min (ESI) 235 (6), 293 (58), 310 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for

 $C_{18}H_{20}N_{3}O_{2} [M+H]^{+}: 310.1556; found: 310.1541.$ 

## (S)-2-amino-3-(7-(methyl(phenyl)amino)-1H-indol-3-yl)propanoic acid (13b)



Syntheis as per Method B, purification Method A, 0.6 mg, yield 4%, purity 75%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.59 (1 H, dd, *J* 8.0, 0.9, ArH), 7.11 (1 H, s, ArH), 7.10 – 7.04 (3 H, m, ArH), 6.93 (1 H, dd, *J* 7.5, 0.9, ArH), 6.65 (1 H, dt, *J* 7.3, 1.0, ArH), 6.64 – 6.60 (2 H, m, ArH), 4.60 (3 H, s, NCH<sub>3</sub>), 3.84 (1 H, dd, *J* 9.3, 4.0, CH), 3.49 (1 H, dd, *J* 15.1, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.13 (1 H, dd, *J* 15.1, 9.3, CH<sub>A</sub>H<sub>B</sub>), 2.12 (2 H, s, CH<sub>3</sub>).

LCMS: r.t. 4.44 min (ESI) 235 (10), 293 (50), 310 (100)  $[M+H]^+$ ; HRMS: *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>  $[M+H]^+$ : 310.1556; found: 310.1546.

(S)-2-amino-3-(5-((4-(trifluoromethyl)phenyl)amino)-1H-indol-3-yl)propanoic acid (14a)



Syntheis as per Method B, purification Method A, 3.9 mg, yield 7%, purity 80%.

*δ* H (500 MHz, CD<sub>3</sub>OD) 7.50 (1 H, d, *J* 2.0, ArH), 7.32 (3 H, d, *J* 8.6, ArH), 7.17 (1 H, s, ArH), 6.99 (1 H, dd, *J* 8.6, 2.0, ArH), 6.91 (2 H, d, *J* 8.6, ArH), 3.79 (1 H, dd, *J* 9.2, 4.0, CH), 3.42 (1 H, dd, *J* 15.3, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.10 (1 H, dd, *J* 15.3, 9.2, CH<sub>A</sub>H<sub>B</sub>).

δ C (126 MHz, DMSO- *d*<sub>6</sub>) 166.2 (CO<sub>2</sub>H), 150.3 (C), 133.4 (C), 132.2 (C), 127.9 (C), 126.4 (d, *J*<sub>CF</sub> 3.8, CH), 125.0 (CH), 117.5 (CH), 112.6 (CH), 112.0 (CH), 111.7 (CH), 109.5 (C), 54.8 (CH), 27.1 (CH<sub>2</sub>), CF<sub>3</sub> and the attached aromatic carbon were not observed.

δ F (470 MHz, DMSO- *d*<sub>6</sub>) -59.1 (CF<sub>3</sub>).

LCMS: r.t. 4.94 min (ESI) 344 (30), 347 (68), 364 (100)  $[M+H]^+$ ; HRMS: *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub>  $[M+H]^+$ : 364.1273; found: 364.1258.

## (S)-2-amino-3-(7-((4-(trifluoromethyl)phenyl)amino)-1H-indol-3-yl)propanoic acid (14b)



Syntheis as per Method B, purification Method A, 2.8 mg, yield 15%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.51 (1 H, dd, *J* 6.9, 2.0, ArH), 7.35 (2 H, d, *J* 8.6, ArH), 7.14 (1 H, s, ArH), 7.06 – 7.00 (2 H, m, ArH), 6.84 (2 H, d, *J* 8.6, ArH), 3.84 (1 H, dd, *J* 9.3, 4.0, CH), 3.48 (1 H, dd, *J* 15.1, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.13 (1 H, dd, *J* 15.1, 9.3, CH<sub>A</sub>H<sub>B</sub>).

δ C (126 MHz, DMSO- *d*<sub>6</sub>) 170.3 (CO<sub>2</sub>H), 149.2 (C), 130.6 (C), 129.1 (C), 126.4 (d, *J*<sub>CF</sub> 3.7, CH), 125.8 (C), 124.2 (CH), 118.8 (CH), 117.4 (q, *J*<sub>CF</sub> 32, C), 114.2 (CH), 113.8 (CH), 113.5 (CH), 110.0 (C), 54.7 (CH), 27.1 (CH<sub>2</sub>), CF<sub>3</sub> not observed.

δ F (470 MHz, DMSO- *d*<sub>6</sub>) -59.2 (CF<sub>3</sub>).

LCMS: r.t. 5.03 min (ESI) 347 (68), 364 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 364.1273; found: 364.1257.

(S)-2-amino-3-(5-((3,4,5-trimethoxyphenyl)amino)-1H-indol-3-yl)propanoic acid (15a)



Synthesi as per Method B, purification Method A, 38.6 mg, yield 67%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.43 (1 H, d, *J* 2.0, ArH), 7.27 (1 H, d, *J* 8.6, ArH), 7.14 (1 H, s, ArH), 6.96 (1 H, dd, *J* 8.6, 2.0, ArH), 6.22 (2 H, s, ArH), 3.79 (1 H, dd, *J* 9.5, 4.1, CH), 3.69 (6 H, s, CH<sub>3</sub>), 3.64 (3 H, s, CH<sub>3</sub>), 3.43 (1 H, dd, *J* 15.2, 4.1, CH<sub>A</sub>H<sub>B</sub>), 3.06 (1 H, dd, *J* 15.2, 9.5, CH<sub>A</sub>H<sub>B</sub>).  $\delta$  C (126 MHz, CD<sub>3</sub>OD) 174.4 (CO<sub>2</sub>H), 154.9 (C), 145.4 (C), 136.6 (C), 135.0 (C), 131.3 (C), 129.1 (C), 125.9 (CH), 119.0 (CH), 112.9 (CH), 111.5 (CH), 109.2 (C), 93.5 (CH), 61.3 (CH<sub>3</sub>), 56.6 (CH), 56.3 (CH<sub>3</sub>), 28.5 (CH<sub>2</sub>).

LCMS: r.t. 3.74 min (ESI) 170 (15), 193 (15), 370 (18), 386 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 386.1716; found: 386.1699.

(S)-2-amino-3-(7-((3,4,5-trimethoxyphenyl)amino)-1H-indol-3-yl)propanoic acid (15b)



Synthesi as per Method B, purification Method A, 11.3 mg, yield 59%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.39 – 7.33 (1 H, m, ArH), 7.14 (1 H, s, ArH), 7.00 – 6.94 (2 H, m, ArH), 6.21 (2 H, s, ArH), 3.84 (1 H, dt, *J* 9.2, 4.0, CH), 3.68 (6 H, s, CH<sub>3</sub>), 3.65 (3 H, s, CH<sub>3</sub>), 3.46 (1 H, dd, *J* 15.2, 4.0, CH<sub>A</sub>H<sub>B</sub>), 3.11 (1 H, dd, *J* 15.2, 9.2, CH<sub>A</sub>H<sub>B</sub>).

*δ* C (126 MHz, CD<sub>3</sub>OD) 173.0 (CO<sub>2</sub>H), 153.6 (C), 141.9 (C), 130.6 (C), 130.2 (C), 128.7 (C), 128.0 (C), 123.5 (CH), 119.4 (CH), 112.5 (CH), 112.2 (CH), 108.8 (C), 93.3 (CH), 59.9 (CH<sub>3</sub>), 55.3 (CH), 54.9 (CH<sub>3</sub>), 27.1(CH<sub>2</sub>).

LCMS: r.t. 3.92 min (ESI) 369 (34), 386 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for C<sub>18</sub>H<sub>20</sub>N<sub>3</sub>O<sub>2</sub> [M+H]<sup>+</sup>: 386.1716; found: 386.1698.

((S)-2-((S)-2-aminopropanamido)-3-(7-(p-tolylamino)-1H-indol-3-yl)propanoyl)-Lphenylalanine (19)



Syntheis as per Method C with microwave reaction time of 24 mins, purification Method B, 2.8 mg, yield 44%, purity >80%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.23 (1 H, dd, *J* 6.3, 2.5, ArH), 7.21 – 7.16 (2 H, m, ArH), 7.15 – 7.10 (3 H, m, ArH), 7.03 (1 H, s, ArH), 6.97 (2 H, d, *J* 8.2, ArH), 6.93 – 6.88 (2 H, m, ArH), 6.84 (2 H, d, *J* 8.2, ArH), 4.69 (1 H, dd, *J* 8.6, 5.6, CH), 4.56 – 4.50 (1 H, m, b, CH), 3.76 (1 H, q, *J* 7.1, CH), 3.24 (1H, dd, b, *J* 5.6, CH<sub>A</sub>*H*<sub>B</sub>(Trp)), 3.15 (1 H, dd, *J* 13.9, 4.7, CH<sub>A</sub>*H*<sub>B</sub>(Ph)), 3.04 (1 H, dd, *J* 14.8, 8.6, C*H*<sub>A</sub>H<sub>B</sub>(Trp)), 2.97 (1 H, dd, *J* 13.8, 7.4, C*H*<sub>A</sub>H<sub>B</sub>(Ph)), 2.21 (3 H, s, CH<sub>3</sub>), 1.37 (3 H, d, *J* 7.1, CH<sub>3</sub>).

LCMS: r.t. 4.90 min (ESI) 265 (80), 528 (100) [M+H]<sup>+</sup>; HRMS: *m*/*z* calcd for C<sub>30</sub>H<sub>34</sub>N<sub>5</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 528.2611; found: 528.2592.

((S)-2-amino-3-(7-(*p*-tolylamino)-1H-indol-3-yl)propanoyl)-L-alanyl-L-phenylalanine (20)



Syntheis as per Method C with microwave reaction time of 48 mins, purification Method B, 10.2 mg, yield 99%, purity >95%.

 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.25 (1 H, p, *J* 4.0, ArH), 7.22 – 7.15 (4 H, m, ArH), 7.13-7.09 (1 H, m, ArH), 7.03 (1 H, s, ArH), 6.98 (2 H, d, *J* 8.2, ArH), 6.92 – 6.89 (2 H, m, ArH), 6.84 (2 H, m, d, *J* 8.2, ArH), 4.57 (1 H, t, b, *J* 5.2, CH), 4.37 (1 H, q, *J* 7.1, CH), 4.08 (1 H, dd, *J* 8.7, 5.7, CH), 3.30 (1 H, dd, *J* 15.0, 5.7, CH<sub>A</sub>*H*<sub>B</sub>(Trp)), 3.14 (1 H, dd, *J* 13.9, 5.2, CH<sub>A</sub>*H*<sub>B</sub>(Ph)), 3.08 (1

H, dd, *J* 15.0, 8.7, *CH*<sub>A</sub>H<sub>B</sub>(Trp)), 2.96 (1 H, dd, *J* 13.9, 8.1, *CH*<sub>A</sub>H<sub>B</sub>(Ph)), 2.21 (3 H, s, CH<sub>3</sub>), 1.31 (3 H, d, *J* 7.1, CH<sub>3</sub>).

δ C (126 MHz, CD<sub>3</sub>OD) 174.0 (CO<sub>2</sub>H), 169.7 (CO), 143.4 (C), 138.4 (C), 131.0 (C), 130.6 (CH), 130.5 (CH), 130.4 (C), 130.1 (C), 129.6 (C), 129.4 (CH), 127.8 (CH), 125.1 (CH), 121.0 (CH), 118.0 (CH), 112.7 (CH), 111.9 (CH), 108.6 (C), 55.4 (CH), 54.8 (CH), 50.5 (CH), 38.3 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>), 20.7 (CH<sub>3</sub>), 18.3 (CH<sub>3</sub>).

LCMS: r.t. 4.80 min (ESI) 265 (18), 363 (14), 511 (14), 528 (100) [M+H]<sup>+</sup>; HRMS: *m/z* calcd for C<sub>30</sub>H<sub>34</sub>N<sub>5</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 528.2611; found: 528.2590.

#### Solid phase synthesis of tripeptide:

# (S)-4-amino-5-(((S)-6-amino-1-(((S)-2-(7-bromo-1H-indol-3-yl)-1-carboxyethyl)amino)-1-oxohexan-2-yl)amino)-5-oxopentanoic acid (NH<sub>2</sub>-Glu-Lys-(7-Br)-Trp-OH) (18)

2-Chlorotrityl chloride resin (0.5 g, 0.8-3 mmol/g, Fluorochem) was washed and swollen in anhydrous dichloromethane (5 mL) for 1 h. A solution of Fmoc-7-bromotryptophan (108 mg, 0.2 mmol) and *N*,*N*-diisopropylethylamine (175  $\mu$ L, 1 mmol) in dry dichloromethane (5 mL) was added and the resin was shaken overnight at room temperature. The solution was drained and resin washed with dichloromethane (3 × 5 mL). The resin was capped using a mixture of dichloromethane (4.5 mL), methanol (0.5 mL) and *N*,*N*-diisopropylethylamine (0.25 mL) for 30 min. Finally, the resin was washed with dichloromethane (3 × 5 mL) and dried under a flow of argon. Fmoc deprotection was carried out for 30 min using 10% piperidine in dimethylformamide (3 mL × 2). The resin was washed successively each with dimethylformamide and dichloromethane (3 × 5 mL) and dried under a flow of argon. Loading was estimated to be 0.15 mmol for 0.5 g of resin. Half of this resin was used for tripeptide synthesis.

Fmoc-Lys(Boc)-OH (140 mg, 0.3 mmol, 4 eq.) in dimethylformamide (2.5 mL) and 2,4,6collidine (0.5 mL) was cooled in an ice bath and activated for 10 min with HATU (115 mg, 0.3 mmol). This solution was added to the loaded resin (~275 mg) and shaken for 3 h at room temperature. Resin was washed successively each with dimethylformamide and dichloromethane ( $3 \times 5$  mL). Fmoc deprotection was performed and coupling was repeated with Boc-Glu(O<sup>t</sup>Bu)-OH (90 mg, 0.3 mmol, 4 eq.) as described above. Partially protected tripeptide was cleaved from resin using 20% hexafluoroisopropanol in dichloromethane (3 mL  $\times$  2). Combined filtrate was concentrated. Boc/<sup>t</sup>BuO deprotection was carried out using a mixture of trifluoroacetic acid-water-tri-isopropylsilane (95 : 2.5 : 2.5, 5 mL). Purification by reverse phase chromatography (Biotage C-18, 12 g column), using a 5 - 95% linear gradient of acetonitrile in water (containing 0.1% TFA) afforded the product as a pale yellow solid (52 mg), yield 90% (based on bis-TFA salt), purity >95%.



 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 8.12 (1 H, d, J 7.9, NH), 7.44 (1 H, dd, *J* 8.0, 0.9, ArH), 7.14 – 7.09 (2 H, m, ArH), 6.80 (1 H, t, *J* 7.7, ArH), 4.62 (1 H, td, *J* 5.3, 2.6, CH), 4.23 (1 H, dd, *J* 8.0, 6.1, CH), 3.83 (1 H, t, *J* 6.4, CH), 3.21 (1 H, dd, *J* 15.0, 5.0, CH<sub>A</sub>H<sub>B</sub>(Trp)), 3.07 (1 H, dd, *J* 15.0, 7.9, CH<sub>A</sub>H<sub>B</sub>(Trp)), 2.74 (2 H, t, *J* 7.7, CH<sub>2</sub>), 2.39 – 2.30 (2 H, m, CH<sub>2</sub>), 1.94 (2 H, q, *J* 7.2, CH<sub>2</sub>), 1.64 (1 H, ddd, *J* 9.7, 7.2, 4.9, CH<sub>A</sub>H<sub>B</sub>(Lys)), 1.56 – 1.46 (3 H, m, CH<sub>2</sub> & CH<sub>A</sub>H<sub>B</sub>(Lys)), 1.32 – 1.22 (2 H, m, CH<sub>2</sub>).

δ C (126 MHz, CD<sub>3</sub>OD) 176.1 (C=O), 174.8 (C=O), 173.3 (C=O), 169.7 (C=O), 136.3 (C), 130.4 (C), 125.9 (CH), 125.0 (CH), 121.1 (CH), 118.9 (CH), 112.3 (C), 105.5 (C-Br), 54.6 (CH), 54.4 (CH), 53.5 (CH), 40.4 (CH<sub>2</sub>), 32.5 (CH<sub>2</sub>), 30.2 (CH<sub>2</sub>), 28.5 (CH<sub>2</sub>), 28.1 (CH<sub>2</sub>), 27.8 (CH<sub>2</sub>), 23.5 (CH<sub>2</sub>).

LCMS: r.t. 2.50 min; MS (ESI) 238.5 (15), 262.5 (70), 271.6 (100)  $[M(^{81}Br)+2H]^{2+}$ , 411.1 (20), 542.2 (90)  $[M(^{81}Br)+H]^+$ ; HRMS: m/z calcd for  $C_{22}H_{31}Br_1N_5O_6$   $[M(^{81}Br)+H]^+$ : 542.1432; found: 542.1422.

# (S)-4-amino-5-(((S)-6-amino-1-(((S)-1-carboxy-2-(7-(p-tolylamino)-1H-indol-3-yl)ethyl)amino)-1-oxohexan-2-yl)amino)-5-oxopentanoic acid (21)

[Pd(<sup>t</sup>Bu-XPhos)G<sub>1</sub>] (5 mol%, 0.55 mg) and tripeptide NH<sub>2</sub>-Glu-Lys-(7-Br)-Trp-OH (0.016 mmol, 1.0 eq.) were placed in a vial, to this was added, under inert atmosphere (Argon), *p*-toluidine solution (0.032 mmol, 3.4 mg, in 1,4-dioxane 0.3 mL) and a solution of KOH (0.08 mmol, 2.2 mg in water 0.3 mL). The vial was sealed and then stirred and heated in the microwave at 100 °C for 20 mins. The solution was allowed to cool to room temperature and then diluted with 1.5 mL water. The solution was acidified to pH = 5-6 with a 0.1 M HCl solution and solvent removed by evaporation. The resulting brown amorphous solid was

purified by reverse phase chromatography (Biotage C-18, 12 g column), using a 2-95% linear gradient of acetonitrile in water (containing 0.1% TFA) afforded the product as a light brown solid (5.2 mg), yield 41% purity >90%.



 $\delta$  H (500 MHz, CD<sub>3</sub>OD) 7.27 – 7.22 (1 H, m, ArH), 7.05 (1 H, s, ArH), 6.98 (2 H, d, *J* 8.1, ArH), 6.92 – 6.85 (4 H, m, ArH), 4.56 (1 H, dd, *J* 7.9, 4.4, CH), 4.30 (1 H, t, *J* 6.9, CH), 3.57 (1 H, dd, *J* 7.4, 5.0, CH), 3.37 (1 H, dd, *J* 14.6, 4.4, CH<sub>A</sub>H<sub>B</sub>(Trp)), 3.10 (1 H, dd, *J* 14.6, 7.9, CH<sub>A</sub>H<sub>B</sub>(Trp)), 2.83 (2 H, t, *J* 7.0, CH<sub>2</sub>), 2.33 – 2.25 (2 H, m, CH<sub>2</sub>), 2.23 (3 H, s), 1.98 – 1.89 (1 H, m, CH<sub>A</sub>H<sub>B</sub>(Glu)), 1.83 – 1.70 (2 H, m, CH<sub>A</sub>H<sub>B</sub>(Glu) & CH<sub>A</sub>H<sub>B</sub>(Lys)), 1.64 – 1.53 (3 H, m, CH<sub>2</sub> & CH<sub>A</sub>H<sub>B</sub>(Lys)), 1.42 – 1.33 (2 H, m, CH<sub>2</sub>).

δ C (126 MHz, CD<sub>3</sub>OD) 181.5 (C=O), 181.1 (C=O), 178.7 (C=O), 172.4 (C=O), 143.67 (C), 130.5 (CH), 129.7 (C), 123.8 (CH), 120.0 (CH), 117.9 (CH), 113.3 (CH), 112.8 (C), 110.8 (CH), 56.9 (CH), 55.2 (CH), 54.5 (CH), 40.3 (CH<sub>2</sub>), 35.3 (CH<sub>2</sub>), 32.3 (CH<sub>2</sub>), 30.9 (CH<sub>2</sub>), 29.3 (CH<sub>2</sub>), 27.8 (CH<sub>2</sub>), 23.2 (CH<sub>2</sub>), 20.7 (CH<sub>3</sub>)

LCMS: r.t. 3.52 min; MS (ESI) 275.1 (40), 284.1 (100)  $[M+2H]^{2+}$ , 377.8 (10), 567.3 (30)  $[M+H]^+$ ; HRMS: m/z calcd for C<sub>29</sub>H<sub>39</sub>N<sub>6</sub>O<sub>6</sub>  $[M+H]^+$ : 567.2926; found: 567.2922.

(*S*,*Z*)-1-(3-(3,6-dioxo-5-((6-(*p*-tolylamino)-1H-indol-3-yl)methylene)piperazin-2yl)propyl)guanidine (23)



[Pd('Bu-XPhos)G<sub>1</sub>] (10 mol%, 0.98 mg) was placed in a vial, to this was added, under inert atmosphere (Argon), barettin (0.014 mmol, 1.0 eq.), followed by a solution of p-toluidine in 1,4-dioxane (0.028 mmol, 3.07 mg in 0.3 mL), and finally a solution of KOH (0.057 mmol, 3.2 mg in 0.3 mL of water). The vial was sealed and then stirred and heated in the microwave at 100°C for 8 mins. The solution was allowed to cool to room temperature and then the palladium was sequestered by the addition of pL-Dithiothreitol (DTT) (0.35 mmol, 54 mg). After stirring with the DTT for 30 mins the reaction mixture was transferred to two eppendorf tubes and spun down in a centrifuge, the supernatant liquid was purified by HPLC on a Kinetics Evo C18 column 10 mm ID, particle size  $4\mu$ m, eluting methanol containing 0.1% Formic acid/water, gradient: 5%-40% methanol over 40 mins, increasing to 95% methanol over 20 mins and returned to 5% methanol. Product eluted at 41 mins, 1.0 mg, yield 16%, purity >95%.

*δ* H (700 MHz, CD<sub>3</sub>OD) 8.52 (4 H, s, NH), 7.59 (1 H, s, ArH), 7.51 (1 H, d, *J* 8.5, ArH), 7.20 (1 H, s, ArH), 7.12 (1 H, d, *J* 2.0, ArH), 7.02 (2 H, d, *J* 8.3, ArH), 6.98 (2 H, d, *J* 8.3, ArH), 6.90 (1 H, dd, *J* 8.5, 2.0, ArH), 3.21 (2 H, t, *J* 7.1, CH), 2.25 (3 H, s, CH<sub>3</sub>), 2.01 – 1.93 (1 H, m), 1.93 – 1.85 (1 H, m), 1.78-1.65 (2 H, m).

LCMS: r.t. 4.35 min (ESI) 446 (100) [M+H]<sup>+</sup>; HRMS: *m*/*z* calcd for C<sub>30</sub>H<sub>34</sub>N<sub>5</sub>O<sub>4</sub> [M+H]<sup>+</sup>: 446.2299; found: 446.2299.

 D. R. M. Smith, T. Willemse, D. S. Gkotsi, W. Schepens, B. U. W. Maes, S. Ballet and R. J. M. Goss, *Org. Lett.* 2014, 16, 2622-2625. *N*-phenyl-1H-indol-5-amine (5)



(S)-2-amino-3-(5-(phenylamino)-1H-indol-3-yl)propanoic acid (7)



# COSY NMR (500 MHz, CD<sub>3</sub>OD) (7)



HSQC NMR (500 MHz, CD<sub>3</sub>OD) (7)





(S)-2-amino-3-(6-(phenylamino)-1H-indol-3-yl)propanoic acid (8)





# <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD) (8)



# HSQC NMR (500 MHz, CD<sub>3</sub>OD) (8)



HMBC NMR (500 MHz, CD<sub>3</sub>OD) (8)



## (S)-2-amino-3-(7-(phenylamino)-1H-indol-3-yl)propanoic acid (9)





## (S)-2-amino-3-(5-(p-tolylamino)-1H-indol-3-yl)propanoic acid (10a)



# <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) (10a)



## <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD) (10a)



COSY NMR (500 MHz, CD3OD) (10a)



HSQC NMR (500 MHz, CD<sub>3</sub>OD) (10a)





(S)-2-amino-3-(7-(p-tolylamino)-1H-indol-3-yl)propanoic acid (10b)







COSY NMR (500 MHz, DMSO-d<sub>6</sub>) (10b)





#### (S)-2-amino-3-(5-(o-tolylamino)-1H-indol-3-yl)propanoic acid (11a)







HSQC NMR (400 MHz, CD<sub>3</sub>OD) (11a)





(S)-2-amino-3-(7-(o-tolylamino)-1H-indol-3-yl)propanoic acid (11b)







COSY NMR (500 MHz, CD<sub>3</sub>OD) (11b)





HMBC NMR (500 MHz, CD<sub>3</sub>OD) (11b)



# (S)-2-amino-3-(7-(methyl(phenyl)amino)-1H-indol-3-yl)propanoic acid (13b)



## <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) (13b)





#### Fig. S1. Mass spectrometry analysis of compound 13b

TIC of product (A background peak is oserved at 6.66 min), **B**) EIC for product **C**) Extracted mass spectrum showing the mass of the desired product (m/z = 310.1543)

# (S)-2-amino-3-(5-((4-(trifluoromethyl)phenyl)amino)-1H-indol-3-yl)propanoic acid (14a)





<sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>) (14a)



COSY NMR (500 MHz, DMSO-d<sub>6</sub>) (14a)



HSQC NMR (500 MHz, DMSO-d<sub>6</sub>) (14a)



# HMBC NMR (500 MHz, DMSO-d<sub>6</sub>) (14a)



<sup>19</sup>F NMR (470 MHz, DMSO-*d*<sub>6</sub>) (14a)



(S)-2-amino-3-(7-((4-(trifluoromethyl)phenyl)amino)-1H-indol-3-yl)propanoic acid (14b)





COSY NMR (500 MHz, DMSO-d<sub>6</sub>) (14b)



<sup>19</sup>F NMR (470 MHz, DMSO-*d*<sub>6</sub>) (14b)



(S)-2-amino-3-(5-((3,4,5-trimethoxyphenyl)amino)-1H-indol-3-yl)propanoic acid (15a)







(S)-2-amino-3-(7-((3,4,5-trimethoxyphenyl)amino)-1H-indol-3-yl)propanoic acid (15b)





# <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD) (15b)



COSY NMR (500 MHz, CD<sub>3</sub>OD) (15b)





(S)-4-amino-5-(((S)-6-amino-1-(((S)-2-(7-bromo-1H-indol-3-yl)-1-carboxyethyl)amino)-1-oxohexan-2-yl)amino)-5-oxopentanoic acid (NH<sub>2</sub>-Glu-Lys-(7-Br)-Trp-OH) (18)





# <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD) (18)



HSQC NMR (500 MHz, CD<sub>3</sub>OD) (18)



HMBC NMR (500 MHz, CD<sub>3</sub>OD) (18)





Fig. S2. Mass spectrometry analysis of the tripeptide (18)

TIC of product(A background peak is oserved at 6.83 min), **B**) EIC for product **C**) Extracted mass spectrum showing the mass of the desired product (m/z = 542.1422)

((S)-2-((S)-2-aminopropanamido)-3-(7-(p-tolylamino)-1H-indol-3-yl)propanoyl)-Lphenylalanine (19)





Fig. S3. Mass spectrometry analysis of the BHA tripeptide product (19) TIC of product (A background peak is oserved at 6.71 min), B) EIC for product C) Extracted mass spectrum showing the mass of the desired product (m/z= 528.2592)

((S)-2-amino-3-(7-(p-tolylamino)-1H-indol-3-yl)propanoyl)-L-alanyl-L-phenylalanine(20)







Fig. S4. Mass spectrometry analysis of the BHA tripeptide product (20)

A) TIC of product (A background peak is oserved at 6.66 min), B) EIC for product C) Extracted mass spectrum showing the mass of the desired product (m/z= 528.2591)

(S)-4-amino-5-(((S)-6-amino-1-(((S)-1-carboxy-2-(7-(p-tolylamino)-1H-indol-3-yl)ethyl)amino)-1-oxohexan-2-yl)amino)-5-oxopentanoic acid (21)



## <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) (21)



# <sup>13</sup>C NMR (126 MHz, CD<sub>3</sub>OD) (21)



# COSY NMR (500 MHz, CD<sub>3</sub>OD) (21)



#### HMBC NMR (500 MHz, CD<sub>3</sub>OD) (21)





A) TIC of product (A background peak is oserved at 6.83 min), B) EIC for product C) Extracted mass spectrum showing the mass of the desired product (m/z= 567.2922m)

(S,Z)-1-(3-(3,6-dioxo-5-((6-(p-tolylamino)-1H-indol-3-yl)methylene)piperazin-2-yl)propyl)guanidine (23)



# <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) (23)





Fig. S6. Mass spectrometry analysis: BHA product (23) of *p*-toluidine (10) and barettin
B) TIC of product (A background peak is oserved at 6.78 min), B) EIC for product C) Extracted mass spectrum showing the mass of the desired product (*m*/*z*= 446.2299)