

**Supplementary Information 1:** Polyamides were synthesized with Boc- $\beta$ -Ala-PAM resin (Peptides International) according to published synthesis protocols.<sup>22, 29</sup> Boc-4-Abu-OH was purchased from Novabiochem, (*R*)-2,4-Fmoc-Dbu(Boc)-OH was purchased from Bachem. (*R*)-3,4-CBZ-Dbu(Boc)-OH and (*S*)-3,4-CBZ-Dbu(Boc)-OH were purchased from Senn Chemicals AG. Generation of the hydroxyl and fluoro hairpin turns is described in Supplementary Fig. 3 and 4 and below.<sup>30, 31</sup> Enantiopurities of precursors were probed via analytical chiral HPLC assays of their tryptamide derivatives (Supplementary Fig. 6 and 7). All DNA oligomers were purchased from Integrated DNA Technologies. Polyamides were purified via preparative HPLC on an Agilent Technologies 1200 Series system using a Phenomenex Gemini column. Matrix-assisted, LASER desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on an Applied Biosystems Voyager DR Pro spectrometer.

Polyamide **1**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>60</sub>N<sub>17</sub>O<sub>8</sub><sup>+</sup> = 979.1, observed = 978.8.  
Polyamide **2R**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>62</sub>N<sub>18</sub>O<sub>8</sub><sup>+</sup> = 994.1, observed = 993.9.  
Polyamide **3R**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>48</sub>H<sub>63</sub>N<sub>18</sub>O<sub>9</sub><sup>+</sup> = 1036.1, observed = 1036.0.  
Polyamide **4R**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>62</sub>N<sub>18</sub>O<sub>8</sub><sup>+</sup> = 994.1, observed = 994.0.  
Polyamide **5R**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>48</sub>H<sub>63</sub>N<sub>18</sub>O<sub>9</sub><sup>+</sup> = 1036.1, observed = 1035.9.  
Polyamide **4S**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>62</sub>N<sub>18</sub>O<sub>8</sub><sup>+</sup> = 994.1, observed = 993.9.  
Polyamide **6R**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>60</sub>N<sub>17</sub>O<sub>9</sub><sup>+</sup> = 995.1, observed = 995.0.  
Polyamide **7R**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>59</sub>FN<sub>17</sub>O<sub>8</sub><sup>+</sup> = 996.5, observed = 996.7.  
Polyamide **6S**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>60</sub>N<sub>17</sub>O<sub>9</sub><sup>+</sup> = 995.1, observed = 995.1.  
Polyamide **7S**: MALDI-TOF [M+H]<sup>+</sup> calcd for C<sub>46</sub>H<sub>59</sub>FN<sub>17</sub>O<sub>8</sub><sup>+</sup> = 996.5, observed = 996.6.

Melting temperature analysis was performed as previously described<sup>22</sup> on a Varian Cary 100 spectrophotometer equipped with a thermo-controlled cell holder, using 10 mM sodium cacodylate buffer.

Plasmids were constructed as previously described<sup>18</sup> by annealing the following oligonucleotide pair: 5'-AGCTGCTGACTGACTATGGTTCTGACTGACTATGGTACTGACTGACTATGGTCCTGAC-TGACTATGGTGCTGACTGACTGCCGC-3' and 5'-GATCGCGGCAGTCAGTCAGCACCATAG-

TCAGTCAGGACCATAGTCAGTCAG-TACCATAGTCAGTCAGAACCATAGTCAGTCAGC-3'. 5' labeling of primers with  $^{32}\text{P}$  and subsequent DNase I footprinting titrations were performed as previously described.<sup>18, 25</sup>

### Synthesis of Fluoro and Hydroxyl Turn Precursors, and Tryptamide Derivatives

**General.** (S)-(-)-4-amino-2-hydroxybutyric acid (**14**), phthalic anhydride, solid sodium hydrogensulfonate ( $\text{NaHSO}_4$ ), 2M HCl in diethyl ether ( $\text{Et}_2\text{O}$ ), pyridine, diethylaminosulfur trifluoride (DAST), di-*tert*-butyldicarbonate ( $\text{Boc}_2\text{O}$ ), triphenyl phosphine ( $\text{PPh}_3$ ), 4-nitrobenzoic acid, diisopropylazodicarboxylate (DIAD), triethylamine, methyl iodide ( $\text{CH}_3\text{I}$ ), 1M aqueous HCl, anhydrous N,N-dimethylformamide (DMF) and tryptamine were purchased from Sigma-Aldrich. 1M aqueous NaOH, concentrated HCl, solid sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), and solid sodium bicarbonate ( $\text{NaHCO}_3$ ) were purchased from EMD Chemicals. Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) was purchased from Novabiochem. Solid citric acid hexahydrate was purchased from Mallinckrodt Chemicals. All NMR spectroscopy data was obtained on a Variant instrument operating at 500 MHz. HRMS were acquired using an Agilent 6200 Series TOF with an Agilent G1978A Multimode source in electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) or mixed (MM) ionization mode. Optical rotation measurements were measured on a Jasco P-1010 polarimeter at 589 nm in spectrophotometric grade solvents.

**(S)-methyl 4-(1,3-dioxoisindolin-2-yl)-2-hydroxy-butanoate (15).** (S)-(-)-4-amino-2-hydroxybutyric acid (4.00g, 33.6mmol) **14** was combined with phthalic anhydride (4.97g, 33.6mmol). The solids were ground, mixed, and heated to 140°C, at which point the phthalic anhydride melted and dissolved **14**. After 30 min, the reaction mixture was cooled to room temperature and re-dissolved in EtOAc (300mL). The solution was extracted with 1M  $\text{NaHSO}_4$  (100mL, pH3), washed with water (3x100mL), and brine

(100mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and concentrated in vacuo. To the crude mixture (8.3g) dissolved in anhydrous methanol (250mL) was slowly added 2M HCl in Et<sub>2</sub>O (250mL). The reaction was stirred at room temperature; TLC indicated completion after 3 h. The mixture was concentrated on rotovap and purified on a short silica gel column (9:1 DCM:EtOAc). The desired fractions were combined, concentrated and dried to give product **15** as a fine white powder (6.68g, 76% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 7.83 (m, 4H), 5.57 (d, *J* = 5.5 Hz, 1H), 4.11 (m, 1H), 3.68 (t, *J* = 7.25 Hz, 2H), 3.59 (s, 3H), 2.01 (m, 1H), 1.84 (m, 1H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 173.9, 167.8, 134.3, 131.7, 122.9, 68.0, 51.5, 34.3, 32.2; HRMS (MM: ESI-APCI) calcd for C<sub>13</sub>H<sub>13</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 286.0686, found 286.0684; [α]<sub>D</sub><sup>22</sup> +10.40 (c=0.5, CHCl<sub>3</sub>)

**(R)-methyl 4-(1,3-dioxoisindolin-2-yl)-2-fluorobutanoate (16)**. To hydroxy ester **15** (5.00g, 19.0mmol) in DCM (50mL) was added pyridine (1.923mL, 23.7mmol) at 0°C. The solution was purged with argon and DAST (3.11mL, 23.7mmol) was added drop-wise under argon pressure. The reaction was stirred at room temperature for 18 h and poured slowly into a cold saturated NaHCO<sub>3</sub> solution. The organic layer was extracted, washed with water (50mL), 0.1M HCl (50mL), water (50mL) and brine (50mL), and dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated in vacuo. The product was purified via silica gel column chromatography (DCM to 9:1 DCM:EtOAc). The desired fractions were combined, concentrated and dried to yield compound **16** as a pale yellow solid (2.21g, 44% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 7.85 (m, 4H), 5.24 (ddd, *J*<sub>H-H</sub> = 3.5 Hz, *J*<sub>H-H</sub> = 8.5 Hz, *J*<sub>H-F</sub> = 48 Hz, 2H), 3.72 (m, 2H), 3.65 (s, 3H), 2.09-2.29 (brm, 2H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 169.3 (<sup>2</sup>*J*<sub>C-F</sub> = 23.5 Hz), 167.7, 134.3, 131.7, 123.0, 86.7 (<sup>1</sup>*J*<sub>C-F</sub> = 181.0 Hz), 52.2, 33.1 (<sup>3</sup>*J*<sub>C-F</sub> = 3.3 Hz), 30.3 (<sup>2</sup>*J*<sub>C-F</sub> = 20.7 Hz); HRMS (MM: ESI-APCI) calcd for C<sub>13</sub>H<sub>12</sub>FNO<sub>4</sub> [M+H]<sup>+</sup> 266.0823, found 266.0830; [α]<sub>D</sub><sup>22</sup> -2.64 (c=0.5, CHCl<sub>3</sub>)

**(R)-4-amino-2-fluorobutanoic acid (17).** Ester **16** (300mg, 1.13mmol) was suspended in 6M HCl (3.0mL) and refluxed for 16 h under argon pressure. The mixture was cooled to room temperature, the phthalic acid precipitate was removed by filtration. The filtrate was concentrated in vacuo, and the product was purified by recrystallization in iPrOH and Et<sub>2</sub>O to afford **17** as white crystals (124mg, 91% yield). <sup>1</sup>H NMR [499.8 MHz, D<sub>2</sub>O]: δ 5.08 (ddd,  $J_{\text{H-H}} = 4.0$  Hz,  $J_{\text{H-H}} = 8.0$  Hz,  $J_{\text{H-F}} = 49.0$  Hz, 2H), 3.10 (m, 2H), 2.09-2.32 (brm, 2H); <sup>13</sup>C NMR [125.7 MHz, D<sub>2</sub>O]: δ 173.4 ( $^2J_{\text{C-F}} = 23.0$  Hz), 87.6 ( $^1J_{\text{C-F}} = 180.0$  Hz), 36.0 ( $^3J_{\text{C-F}} = 3.8$  Hz), 29.6 ( $^2J_{\text{C-F}} = 20.7$  Hz); HRMS (MM: ESI-APCI) calc'd for C<sub>4</sub>H<sub>8</sub>FNO<sub>4</sub> [M+H]<sup>+</sup> 122.0612, found 122.061; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +10.64 (c=0.5, H<sub>2</sub>O)

**(R)-4-(tert-butoxycarbonylamino)-2-fluorobutanoic acid (18).** To a solution of amino acid **17** (40mg, 0.33mmol) in THF (1mL) and water (1mL), solid NaHCO<sub>3</sub> (83mg, 0.99mmol) and Boc<sub>2</sub>O (87mg, 0.40mmol) was added at 0°C. After 30 min, the solution was slowly warmed to room temperature and stirred for 16 h, followed by extraction with Et<sub>2</sub>O (2x1.5mL) to remove generated *tert*-butanol and unreacted Boc<sub>2</sub>O. The aqueous layer was carefully acidified to pH2.5 with half-saturated citric acid at 0°C, and extracted with DCM (3x1.5mL). The combined organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated and dried to give product **18** as a colorless oil (52mg, 71% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 13.4 (brs, 1H), 6.91 (t,  $J = 5.5$  Hz, 1H), 4.95 (ddd,  $J_{\text{H-H}} = 3.5$  Hz,  $J_{\text{H-H}} = 8.5$  Hz,  $J_{\text{H-F}} = 49.0$  Hz, 2H), 3.04 (m, 2H), 1.75-2.01 (brm, 2H), 1.37 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 170.9 ( $^2J_{\text{C-F}} = 23.0$  Hz), 155.6, 86.6 ( $^1J_{\text{C-F}} = 180.5$  Hz), 77.7, 35.7, 32.4 ( $^2J_{\text{C-F}} = 20.2$  Hz), 28.2; HRMS (MM: ESI-APCI) calc'd for C<sub>9</sub>H<sub>16</sub>FNO<sub>4</sub> [M-H]<sup>-</sup> 220.0991, found 220.1018; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +5.32 (c=0.5, MeOH)

**(R)-methyl 4-(1,3-dioxoisindolin-2-yl)-2-hydroxy-butanoate (19).** To a solution of **15** (5.00g, 19.0mmol) in THF (125mL) was added PPh<sub>3</sub> (5.485g, 20.9mmol) followed by 4-nitrobenzoic acid

(9.53g, 57.0mmol). DIAD (4.06mL, 20.9mmol) was added dropwise to the solution at 0°C, and the reaction was stirred at room temperature. TLC showed reaction completion after 4 h, and the solution was concentrated in vacuo. The product was isolated via silica gel column chromatography (100% DCM), and the combined fractions were concentrated and dried in vacuo to afford the Mitsunobu intermediate (R)-4-(1,3-dioxisoindolin-2-yl)-1-methoxy-1-oxobutan-2-yl 4-nitrobenzoate as a pale yellow powder (7.3g, 93% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 8.30 (dd, *J* = 2.5 Hz, *J* = 9 Hz, 2H), 8.17 (dd, *J* = 2.5 Hz, *J* = 9 Hz, 2H), 7.78 (m, 4H), 5.33 (dd, *J* = 5 Hz, *J* = 7.5 Hz, 1H), 3.84 (m, 2H), 3.65 (s, 3H), 2.33 (m, 2H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 169.4, 167.9, 163.6, 150.4, 134.3, 134.0, 131.6, 130.7, 123.9, 123.0, 71.2, 52.5, 33.9, 30.0; HRMS (MM: ESI-APCI) calcd for C<sub>20</sub>H<sub>16</sub>N<sub>2</sub>O<sub>8</sub> [M+H]<sup>+</sup> 413.0979, found 413.0975; [α]<sub>D</sub><sup>22</sup> +28.6 (c=0.5, CHCl<sub>3</sub>).

To obtain the desired product **19**, triethylamine (9.93mL, 77.5mmol) was added to the intermediate (6.4g, 15.5mmol) in anhydrous methanol (480mL). The reaction was monitored by TLC and was complete after 4 h. The solution was then concentrated and purified via column chromatography (DCM to 9:1 DCM:EtOAc). The desired fractions were combined, concentrated and dried to afford compound **19** as a fine white powder (3.86g, 95% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 7.84 (m, 4H), 5.57 (d, *J* = 5.5 Hz, 1H), 4.12 (m, 1H), 3.68 (t, *J* = 7.5 Hz, 2H), 3.59 (s, 3H), 2.02 (m, 1H), 1.84 (m, 1H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 173.9, 167.8, 134.3, 131.7, 122.9, 67.9, 51.5, 34.3, 32.1; HRMS (MM: ESI-APCI) calcd for C<sub>13</sub>H<sub>13</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 286.0686, found 286.0688 [α]<sub>D</sub><sup>22</sup> -10.28 (c=0.5, CHCl<sub>3</sub>).

**(S)-methyl 4-(1,3-dioxisoindolin-2-yl)-2-fluorobutanoate (20)**. Synthesis was performed using R-enantiomer **19** (3.85g, 14.6mmol) in conjunction with the method for the synthesis of fluoroester **16**. The product **20** was synthesized and isolated as a pale yellow solid (1.60g, 41% yield). <sup>1</sup>H NMR [499.8

MHz, DMSO-d<sub>6</sub>):  $\delta$  7.85 (m, 4H), 5.24 (ddd,  $J_{\text{H-H}} = 3.5$  Hz,  $J_{\text{H-H}} = 8.5$  Hz,  $J_{\text{H-F}} = 48.0$  Hz, 2H), 3.72 (m, 2H), 3.65 (s, 3H), 2.09-2.29 (brm, 2H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>):  $\delta$  169.3 (<sup>2</sup> $J_{\text{C-F}} = 23.5$  Hz), 167.7, 134.3, 131.7, 123.0, 86.7 (<sup>1</sup> $J_{\text{C-F}} = 181.3$  Hz), 52.2, 33.1 (<sup>3</sup> $J_{\text{C-F}} = 3.8$  Hz), 30.3 (<sup>2</sup> $J_{\text{C-F}} = 20.2$  Hz); HRMS (MM: ESI-APCI) calc'd for C<sub>13</sub>H<sub>12</sub>FNO<sub>4</sub> [M+H]<sup>+</sup> 266.0823, found 266.0828;  $[\alpha]_{\text{D}}^{22} +2.4$  (c=0.5, CHCl<sub>3</sub>).

**(S)-4-amino-2-fluorobutanoic acid (21).** This compound was synthesized by following the method used for the synthesis of amino acid **17**, but using the S-enantiomer **20** (1.60g, 6.03mmol) as starting material. The product was synthesized and isolated as fluffy white crystals (725mg, 99% yield). <sup>1</sup>H NMR [499.8 MHz, D<sub>2</sub>O]:  $\delta$  5.08 (ddd,  $J_{\text{H-H}} = 4.0$  Hz,  $J_{\text{H-H}} = 8.0$  Hz,  $J_{\text{H-F}} = 49.0$  Hz, 2H), 3.09 (m, 2H), 2.08-2.32 (brm, 2H); <sup>13</sup>C NMR [125.7 MHz, D<sub>2</sub>O]:  $\delta$  173.4 (<sup>2</sup> $J_{\text{C-F}} = 23.0$  Hz), 87.7 (<sup>1</sup> $J_{\text{C-F}} = 180.0$  Hz), 36.0 (<sup>3</sup> $J_{\text{C-F}} = 3.3$  Hz), 29.6 (<sup>2</sup> $J_{\text{C-F}} = 20.7$  Hz); HRMS (MM: ESI-APCI) calcd for C<sub>4</sub>H<sub>8</sub>FNO<sub>4</sub> [M+H]<sup>+</sup> 122.0612, found 122.0613;  $[\alpha]_{\text{D}}^{22} -11.2$  (c=0.5, H<sub>2</sub>O).

**(S)-4-(tert-butoxycarbonylamino)-2-fluorobutanoic acid (22).** This compound was synthesized by following the method used for the synthesis of amino acid **18**, but using the S-enantiomer **21** (40mg, 0.33mmol) as starting material. The product **22** was synthesized and isolated as a colorless oil (51mg, 70% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>):  $\delta$  13.3 (brs, 1H), 6.91 (t,  $J = 5.5$  Hz, 1H), 4.95 (ddd,  $J_{\text{H-H}} = 3.5$  Hz,  $J_{\text{H-H}} = 8.5$  Hz,  $J_{\text{H-F}} = 49.0$  Hz, 2H), 3.04 (m, 2H), 1.75-2.01 (brm, 2H), 1.37 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>):  $\delta$  170.8 (<sup>2</sup> $J_{\text{C-F}} = 23.5$  Hz), 155.4, 86.4 (<sup>1</sup> $J_{\text{C-F}} = 180.5$  Hz), 77.6, 35.6, 32.3 (<sup>2</sup> $J_{\text{C-F}} = 19.9$  Hz), 28.1; HRMS (MM: ESI-APCI) calcd for C<sub>9</sub>H<sub>16</sub>FNO<sub>4</sub> [M-H]<sup>-</sup> 220.0991, found 220.1017;  $[\alpha]_{\text{D}}^{22} -5.04$  (c=0.5, MeOH).

**(S)-4-(tert-butoxycarbonylamino)-2-hydroxybutanoic acid (23).** To a solution of (S)-(-)-4-amino-2-hydroxybutyric acid **14** (2.00g, 16.8mmol) dissolved in water (50mL) and 1,4-dioxane (50mL), NaHCO<sub>3</sub> (1.55g, 18.5mmol) and added Boc<sub>2</sub>O (4.04g, 18.5mmol) was added at 0°C. After 15 min, the turbid solution was slowly warmed to room temperature and stirred for 16 h. The solution was extracted with Et<sub>2</sub>O (2x50mL) to rid of the generated *tert*-butanol and unreacted di-*tert*-butyldicarbonate. The aqueous layer was carefully acidified to pH2.5 with 1M HCl (~20mL) at 0°C, and extracted with DCM (3x50mL). The combined organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated and dried to give product **23** as a colorless oil (3.03g, 82% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 6.75 (t, *J* = 5.0 Hz, 1H), 3.93 (dd, *J* = 4.0 Hz, *J* = 8.5 Hz, 1H), 3.00 (m, 2H), 1.77 (m, 1H), 1.57 (m, 1H), 1.36 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 175.6, 155.5, 77.5, 67.7, 36.7, 34.1, 28.2; HRMS (MM: ESI-APCI) calcd for C<sub>9</sub>H<sub>17</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 242.0999, found 242.0997; [α]<sub>D</sub><sup>22</sup> -3.96 (c=0.5, MeOH).

**(S)-methyl 4-(tert-butoxycarbonylamino)-2-hydroxybutanoate (24).** To a solution of Boc-protected amino acid **23** (2.00g, 9.1mmol) dissolved in DMF (20mL) was added K<sub>2</sub>CO<sub>3</sub> (1.32g, 9.54mmol) at 0°C. After 15 min, CH<sub>3</sub>I (4.13g, 29.12mmol) was added slowly and the solution was stirred for 44 h at room temperature. The solution was then partitioned between H<sub>2</sub>O (100mL) and ethyl acetate (4 x 50mL). The organic fractions were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated and dried to give product **24** as a pale yellow oil (1.20g, 57% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 6.76 (t, *J* = 5.0 Hz, 1H), 5.39 (d, *J* = 6.0 Hz, 1H), 4.04 (m, 1H), 3.62 (s, 3H), 2.99 (m, 2H), 1.75 (m, 1H), 1.59 (m, 1H), 1.36 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 174.4, 155.5, 77.5, 67.8, 51.4, 36.4, 34.1, 28.2; HRMS (MM: ESI-APCI) calcd for C<sub>10</sub>H<sub>19</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 256.1155, found 256.1159; [α]<sub>D</sub><sup>22</sup> +4.56 (c=0.5, MeOH).

**(R)-methyl 4-(tert-butoxycarbonylamino)-2-hydroxybutanoate (25).** To a solution of ester **24** (650mg, 2.75mmol) in THF (13mL) was added PPh<sub>3</sub> (795g, 3.02mmol) followed by 4-nitrobenzoic acid (1.38g, 8.26mmol). DIAD (589μL, 3.03mmol) was added dropwise to the solution at 0°C, and the reaction was stirred at room temperature. TLC showed a completed reaction after 8 h, and the solution was concentrated in vacuo. The product was isolated via silica gel column chromatography (95:5 DCM:EtOAc to 9:1 DCM:EtOAc), and the combined fractions were concentrated and dried in vacuo to afford the Mitsunobu intermediate (*R*)-4-(tert-butoxycarbonylamino)-1-methoxy-1-oxobutan-2-yl 4-nitrobenzoate as a colorless oil (890mg, 84% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 8.38 (dd, *J* = 2.0 Hz, *J* = 9.0 Hz, 2H), 8.24 (dd, *J* = 2.0 Hz, *J* = 9.0 Hz, 2H), 6.99 (t, 5.5 Hz, 1H), 5.20 (m, 1H), 3.70 (s, 3H), 3.16 (m, 2H), 2.05 (m, 2H), 1.34 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 169.9, 163.8, 155.6, 150.5, 134.3, 130.8, 124.0, 77.7, 70.9, 52.4, 35.8, 30.8, 28.1; HRMS (MM: ESI-APCI) calcd for C<sub>17</sub>H<sub>2</sub>N<sub>2</sub>O<sub>8</sub> [M-Boc+2H]<sup>+</sup> 283.0925, found 283.0934; [α]<sup>22</sup><sub>D</sub> +16.64 (c=0.5, MeOH).

To obtain the desired product **25**, triethylamine (750μL, 5.38mmol) was added to the intermediate (410mg, 1.07mmol) in anhydrous methanol (30mL). The reaction was monitored by TLC and was complete after 1.5 h. The solution was then concentrated and run through a silica plug. DCM was run till the eluent was no longer UV active, at which point EtOAc was used to flush out the product. The EtOAc fractions were combined, concentrated and dried to afford compound **25** as a pale yellow oil (250mg, 99% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 6.76 (t, *J* = 5.0 Hz, 1H), 5.39 (d, *J* = 6.0 Hz, 1H), 4.04 (m, 1H), 3.62 (s, 3H), 2.99 (m, 2H), 1.76 (m, 1H), 1.60 (m, 1H), 1.36 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 174.4, 155.5, 77.5, 67.8, 51.4, 36.5, 34.1, 28.2; HRMS (MM: ESI-APCI) calcd for C<sub>10</sub>H<sub>19</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 256.1155, found 256.1158; [α]<sup>22</sup><sub>D</sub> -4.28 (c=0.5, MeOH).



**(R)-4-(tert-butoxycarbonylamino)-2-hydroxybutanoic acid (26).** To a solution of Boc-protected amino acid **25** in MeOH (2.5mL) was added dropwise 1M NaOH (2.5mL) at 0°C. The reaction mixture was warmed to room temperature and TLC showed a completed reaction after 30 min. The solution was cooled to 0°C and re-acidified to pH2 by dropwise addition of 1M HCl (2.5mL), and extracted with EtOAc (3 x 2.5mL). The organic fractions were then combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, concentrated and dried to give product **26** as a colorless oil (108mg, 93% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 6.75 (t, *J* = 5.0 Hz, 1H), 3.92 (dd, *J* = 4.0 Hz, *J* = 8.5 Hz, 1H), 3.00 (m, 2H), 1.77 (m, 1H), 1.56 (m, 1H), 1.36 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 175.6, 155.5, 77.4, 67.8, 36.7, 34.1, 28.2; HRMS (MM: ESI–APCI) calcd for C<sub>9</sub>H<sub>17</sub>NO<sub>5</sub> [M+Na]<sup>+</sup> 242.0999, found 242.0998; [α]<sup>22</sup><sub>D</sub> +4.12 (c=0.5, MeOH).

**(R)-tert-butyl 4-(2-(1H-indol-3-yl)ethylamino)-3-fluoro-4-oxobutylcarbamate (27).** To a solution of Boc-protected amino acid **18** (25mg, 0.11mmol) in anhydrous DMF (1.0mL) was added tryptamine (91mg, 0.55mmol), followed by PyBOP (65mg, 0.12mmol). The reaction was stirred at room temperature for 1.5 h, and subsequently partitioned between H<sub>2</sub>O (1mL) and EtOAc (3 x 1mL). The organic layers were then combined, washed with 10% citric acid (3 x 1mL), saturated NaHCO<sub>3</sub> (3 x 1mL), brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The product was purified on a silica gel column (9:1 Et<sub>2</sub>O:EtOAc), and the desired fractions were combined, concentrated and dried to afford the desired product **27** as a pale yellow oil (28mg, 68%). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]: δ 10.80 (s, 1H), 8.26 (t, *J* = 5.5 Hz, 1H), 7.53 (d, *J* = 8.0 Hz, 1H), 7.32 (app dt, *J* = 1.0 Hz, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 2.5 Hz, 1H), 7.05 (ddd, *J* = 1.0 Hz, *J* = 7.0 Hz, *J* = 7.0 Hz, 1H), 6.97 (ddd, *J* = 1.0 Hz, *J* = 7.0 Hz, *J* = 7.0 Hz, 1H), 6.87 (t, *J* = 5.5 Hz, 1H), 4.88 (ddd, *J* = 3.5 Hz, *J* = 8.5 Hz, *J* = 49.5 Hz, 1H), 3.38 (m, 2H), 3.03 (m, 2H), 2.84 (t, 7.5 Hz, 2H), 1.76-1.95 (m, 2H), 1.37 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]: δ 168.7

( $^2J_{C-F}$  = 19.9 Hz), 155.3, 136.2, 127.2, 122.6, 120.9, 118.3, 118.2, 111.6, 111.3, 89.3 ( $^1J_{C-F}$  = 181.9 Hz), 77.6, 35.7, 32.7 ( $^2J_{C-F}$  = 20.7 Hz), 28.2, 25.0; HRMS (MM: ESI-APCI) calcd for C<sub>19</sub>H<sub>26</sub>FN<sub>3</sub>O<sub>3</sub> [M+Na]<sup>+</sup> 386.1850, found 386.1855; [ $\alpha$ ]<sub>D</sub><sup>22</sup> +17.1 (c=0.5, MeOH).

**(S)-tert-butyl 4-(2-(1H-indol-3-yl)ethylamino)-3-fluoro-4-oxobutylcarbamate (28).** This compound was synthesized by following the method used for the synthesis of amide **27**, but instead using the S-enantiomer **23** (23mg, 0.10mmol) as starting material. The product **28** was synthesized and isolated as a pale yellow oil (25mg, 66% yield). <sup>1</sup>H NMR [499.8 MHz, DMSO-d<sub>6</sub>]:  $\delta$  10.80 (s, 1H), 8.26 (t,  $J$  = 5.5 Hz, 1H), 7.54 (d,  $J$  = 8.0 Hz, 1H), 7.32 (app dt,  $J$  = 1.0 Hz,  $J$  = 8.0 Hz, 1H), 7.14 (d,  $J$  = 2.0 Hz, 1H), 7.05 (ddd,  $J$  = 1.0 Hz,  $J$  = 7.0 Hz,  $J$  = 7.0 Hz, 1H), 6.97 (ddd,  $J$  = 1.0 Hz,  $J$  = 7.0 Hz,  $J$  = 7.0 Hz, 1H), 6.87 (t,  $J$  = 5.5 Hz, 1H), 4.88 (ddd,  $J$  = 3.5 Hz,  $J$  = 8.5 Hz,  $J$  = 49.5 Hz, 1H), 3.38 (m, 2H), 3.03 (m, 2H), 2.84 (t, 7.5 Hz, 2H), 1.76-1.95 (m, 2H), 1.37 (s, 9H); <sup>13</sup>C NMR [125.7 MHz, DMSO-d<sub>6</sub>]:  $\delta$  168.8 ( $^2J_{C-F}$  = 19.7 Hz), 155.6, 136.2, 127.2, 122.6, 120.9, 118.3, 118.2, 111.6, 111.4, 89.3 ( $^1J_{C-F}$  = 182.8 Hz), 77.7, 35.7, 32.7 ( $^2J_{C-F}$  = 19.9 Hz), 28.2, 25.0; HRMS (MM: ESI-APCI) calcd for C<sub>19</sub>H<sub>26</sub>FN<sub>3</sub>O<sub>3</sub> [M+Na]<sup>+</sup> 386.1850, found 386.1855; [ $\alpha$ ]<sub>D</sub><sup>22</sup> -16.6 (c=0.5, MeOH).

**(S)-tert-butyl 4-(2-(1H-indol-3-yl)ethylamino)-3-hydroxy-4-oxobutylcarbamate (29).** To a solution of Boc-protected amino acid **23** (24mg, 0.11mmol) in anhydrous DMF (1.0mL) was added tryptamine (89mg, 0.55mmol), followed by PyBOP (64mg, 0.12mmol). The reaction was stirred at room temperature for 1.5 h, and subsequently partitioned between H<sub>2</sub>O (1mL) and EtOAc (3 x 1mL). The organic layers were then combined, washed with 10% citric acid (3 x 1mL), saturated NaHCO<sub>3</sub> (3 x 1mL), brine, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, and concentrated. The product was purified on a silica gel column (9:1 Et<sub>2</sub>O:EtOAc), and the desired fractions were combined, concentrated and dried to afford the desired

product **29** as a pale yellow oil (25mg, 62%).  $^1\text{H}$  NMR [499.8 MHz, DMSO- $d_6$ ]:  $\delta$  10.8 (s, 1H), 7.80 (t,  $J = 6.0$  Hz, 1H), 7.56 (d,  $J = 8.0$  Hz, 1H), 7.32 (app dt,  $J = 1\text{Hz}$ ,  $J = 8.0\text{Hz}$ , 1H), 7.14 (d,  $J = 2.5$  Hz, 1H), 7.05 (ddd,  $J = 1.0$  Hz,  $J = 7.0$  Hz,  $J = 7.0$  Hz, 1H), 6.97 (ddd,  $J = 1.0$  Hz,  $J = 7.0$  Hz,  $J = 7.0$  Hz, 1H), 6.71 (t,  $J = 5.5$  Hz, 1H), 3.84 (m, 1H), 3.36 (m, 2H), 3.00 (m, 2H), 2.82 (t,  $J = 8.0$  Hz), 1.78 (m, 1H), 1.52 (m, 1H), 1.37 (s, 9H);  $^{13}\text{C}$  NMR [125.7 MHz, DMSO- $d_6$ ]:  $\delta$  173.5, 155.6, 136.3, 127.2, 122.6, 120.9, 118.3, 118.2, 111.7, 111.3, 77.5, 69.2, 36.8, 34.7, 28.3, 25.3; HRMS (MM: ESI-APCI) calcd for  $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_4$   $[\text{M}+\text{Na}]^+$  384.1894, found 384.1894;  $[\alpha]_D^{22}$  -14.4 (c=0.5, MeOH).

**(R)-tert-butyl 4-(2-(1H-indol-3-yl)ethylamino)-3-hydroxy-4-oxobutylcarbamate (30)**. This compound was synthesized by following the method used for the synthesis of amide **29**, but instead using the R-enantiomer **25** (10mg, 0.05mmol) as starting material. The product **30** was synthesized and isolated as a pale yellow oil (11mg, 66% yield).  $^1\text{H}$  NMR [499.8 MHz, DMSO- $d_6$ ]:  $\delta$  10.8 (s, 1H), 7.80 (t,  $J = 6.0$  Hz, 1H), 7.56 (d,  $J = 8.0$  Hz, 1H), 7.32 (app dt,  $J = 1\text{Hz}$ ,  $J = 8.0\text{Hz}$ , 1H), 7.14 (d,  $J = 2.5$  Hz, 1H), 7.05 (ddd,  $J = 1.0$  Hz,  $J = 7.0$  Hz,  $J = 7.0$  Hz, 1H), 6.97 (ddd,  $J = 1.0$  Hz,  $J = 7.0$  Hz,  $J = 7.0$  Hz, 1H), 6.71 (t,  $J = 5.5$  Hz, 1H), 3.84 (m, 1H), 3.36 (m, 2H), 3.00 (m, 2H), 2.82 (t,  $J = 8.0$  Hz), 1.78 (m, 1H), 1.52 (m, 1H), 1.37 (s, 9H); [125.7 MHz, DMSO- $d_6$ ]:  $\delta$  173.5, 155.6, 136.2, 127.2, 122.6, 120.9, 118.3, 118.2, 111.7, 111.3, 77.5, 69.2, 36.8, 34.7, 28.3, 25.3; HRMS (MM: ESI-APCI) calcd for  $\text{C}_{19}\text{H}_{27}\text{N}_3\text{O}_4$   $[\text{M}+\text{Na}]^+$  384.1894, found 384.1897;  $[\alpha]_D^{22}$  +15.0 (c=0.5, MeOH).

#### **Analytical chiral HPLC Assays for compounds 27-30.**

The enantiopurity of compounds **18**, **22**, **23** and **26** were determined by chiral HPLC assays performed on the UV active tryptamide derivatives **27-30**. The assays were taken on an Agilent 1100 Series HPLC utilizing a Chiralpak AD column (4.6 mm x 25cm) obtained from Daicel Chemical Industries, Ltd., and

the analytical data is included in Supplementary Fig. 6 and 7.