A Facile Approach to Tryptophan Derivatives for the Total Synthesis of Argyrin Analogues

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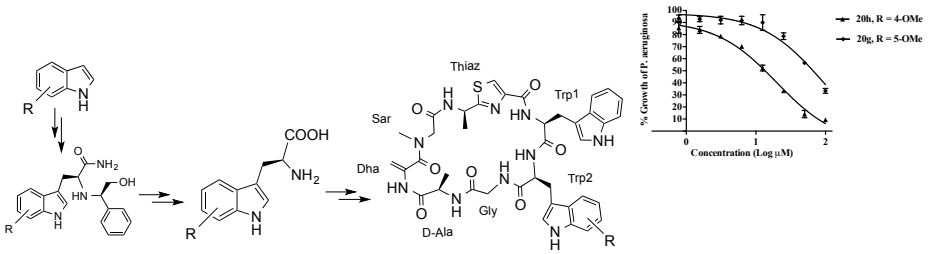


Electronic Supplementary Information

1. General information

Chemicals and solvents were purchased from standard suppliers and used without further purification. Deuterated solvents were purchased from Goss and Aldrich Chemical Co. Anhydrous solvents were purchased from Fluka and Acros. All other solvents were used as supplied (Analytical or HPLC grade) without further purification. All reactions requiring anhydrous conditions were performed using flame- or oven-dried apparatus under nitrogen atmosphere. Reactions were monitored by analytical thin-layer chromatography on commercially available pre-coated aluminium backed plates (Merck Kieselgel 60 F254). Visualization of the silica plates was achieved using either a UV lamp (λ = 254 nm) or by staining with diluted potassium permanganate solution. Organic solvents were evaporated under reduced pressure at *ca.* 35 °C (water bath temperature).

Melting points were recorded on a Gallenkamp melting point apparatus. Infrared spectra were measured on an Avatar 360 Nicolet FT-IR spectrophotometer in the range of 4000–500 cm^{-1} using KBr discs. Absorption maxima (υ_{max}) are reported in wavenumbers (cm^{-1}). Only signals representing functional groups are reported. Absorptions from the fingerprint region are not listed.

Optical rotation was measured on an ADP220 polarimeter (Bellingham & Stanley Ltd). $[\alpha]_D$ values are reported in 10^{-1} deg cm^{-2} g^{-1}, and concentration (*c*) is in gram per 100 mL. High-resolution mass spectra were recorded on a Waters 2795 separation module/micromass LCT platform.

^{1}H and ^{13}C NMR spectra were recorded at 20 °C on a Bruker AV400 operating at 400.13 MHz and 101.62 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm), referenced to $CDCl_3$ (^{1}H, 7.28 ppm; ^{13}C, 77.1 ppm), DMSO-d_6 (^{1}H, 2.50 ppm; ^{13}C, 39.51 ppm) or CD_3OD (^{1}H, 3.31 ppm; ^{13}C, 77.23 ppm). Coupling constants (*J*) are recorded in Hz and significant multiplicities described by singlet (s), doublet (d), triplet (t), quadruplet (q), broad (br), multiplet (m) or doublet of doublets (dd). Spectra were assigned using appropriate COSY, DEPT and HSQC.

Analytical reverse-phase high performance liquid chromatography (RP-HPLC) was performed on an Onyx monolithic-C18 column (100 x 4.6 mm) and a linear gradient of 10–60% B in 12.0 min at a flow rate of 3.0 mL min^{-1}. Eluent detection was monitored by UV absorbance at 214 nm. Solvent A was 0.06 % TFA in water and solvent B was 0.06 % TFA in 90% aqueous acetonitrile. Preparative RP-HPLC was performed on an Onyx monolithic-C18 semi-preparative column (100 x 10 mm) at a flow rate of 9.0 mL min^{-1}.

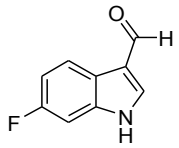
Solid-phase peptide synthesis (SPPS) was carried out in a glass column on a continuous flow manual peptide synthesiser NOVASYN® GEM. Acylation with activated Fmoc-amino acids were typically accomplished with a four-fold excess of Fmoc-amino acid, 3.9 equivalents of HATU or PyOxim and eight equivalents of DIEA. Coupling cycles were carried out for a minimum of 4 h. The washing and Fmoc-deprotection cycles were performed at a flow rate of 2.8 mL min^{-1} with DMF and 20% piperidine in DMF, respectively. The Fmoc-deprotection was monitored using an in-line UV detector and monitoring at 344 nm.

2. Synthetic procedures

2.1 General procedure for the preparation of indole-3-carbaldehyde **2**

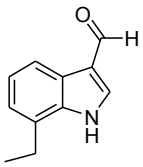
Phosphoryl chloride (1.5 eq) was added dropwise to dry DMF (8–10 mL) at 0 °C. The mixture was stirred for 10 min. Then the solution of indole (1.0 eq) in DMF was added dropwise to the mixture. The solution was heated to 45 °C and was stirred for a further 2 h. The reaction was poured into ice water and extracted with Et_2O (2 x 30 mL). The aqueous layer was treated with 1 M aq NaOH to pH 9 and extracted with EtOAc (3 x 30 mL). The organic extracts were combined, washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to yield the indole-3-carbaldehyde.

6-Fluoro-1*H*-indole-3-carbaldehyde (2d)



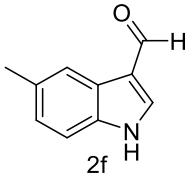
The synthesis was carried out using phosphoryl chloride (1.2 mL, 13.4 mmol), 5-fluoroindole (1.2 g, 9.0 mmol) in DMF (8 mL) to yield **2d**[1] as yellow crystals (0.90 g, 66 % yield): m.p. 174–178 °C; TLC R_f = 0.2 (Hexane–EtOAc, 2:1); IR (KBr): ν = 2933, 1641, 1530, 1448, 1149 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 7.08 (td, J = 8 and 2 Hz, 1H, H-5 or H-7), 7.31 (dd, J = 8 and 2 Hz, 1H, H-5 or H-7), 8.22 (dd, J = 8 and 2 Hz, 1H, H-4), 8.24 (s, 1H, H-2), 10.03 (s, 1H, CHO), 11.28 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 98.5 (d, J = 26 Hz), 110.4 (d, J = 24 Hz), 119.1, 121.2, 122.4 (d, J = 10 Hz), 137.9, 159.18, 161.5, 184.4; MS: *m/z* (+ESI) calcd for $C_9H_7FNO^+$ 164.0512, found 164.0430 [MH^+].

7-Ethyl-1*H*-indole-3-carbaldehyde (2e)



The synthesis was carried out using phosphoryl chloride (1.0 mL, 10.9 mmol), 7-ethylindole (1.0 mL, 7.3 mmol) and DMF (8 mL) to afford **2e**[2] as a brown powder (1.1 g, 95 % yield): m.p. 92–94 °C; TLC R_f = 0.2 (Hexane–EtOAc, 2:1). IR (KBr): ν = 3166, 3054, 1628, 1456, 1228 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.38 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.98 (q, J = 7.5 Hz, 2H, CH_2CH_3), 7.18 (d, J = 7 Hz, 1H, H-5 or H-6), 7.29 (t, J = 7 Hz, 1H, H-5 or H-6), 7.90 (d, J = 1 Hz, 1H, H-2), 8.19 (d, J = 7 Hz, 1H, H-4), 9.95 (br, 1H, NH), 10.05 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 24.0, 119.3, 119.70, 123.1, 123.4, 127.6, 135.91, 136.3, 146.5, 185.7. MS: *m/z* (+ESI) calcd for $C_{11}H_{12}NO^+$ 174.0919, found 174.0805 [MH^+]

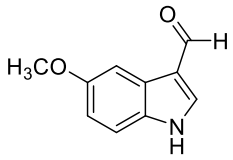
5-Methyl-1*H*-indole-3-carbaldehyde (2f)



2f

Using phosphoryl chloride (1.0 mL, 11.3 mmol), 5-methylindole (1.0 g, 7.58 mmol) and DMF (10 mL) the title compound **2f**[3] was obtained as yellow crystals (0.8 g, 65 % yield): m.p. 150–151 °C; TLC R_f = 0.2 (Hexane–EtOAc, 2:1). IR (KBr): ν = 3214, 1636, 1437, 1388 cm^{-1}. ^{1}H NMR (400 MHz, acetone-d_6): δ 2.45 (s, 3H, CH_3), 7.12 (dd, J = 8 and 2 Hz, 1H, H-6 or H-7), 7.44 (dd, J = 8 and 2 Hz, 1H, H-6 or H-7), 8.06 (d, J = 2 Hz, 1H, H-4), 8.15 (s, 1H, H-2), 10.02 (s, 1H, CHO), 11.08 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 20.7, 111.7, 118.9, 121.1, 124.9, 125.12, 131.5, 135.72, 137.2, 184.4. MS: *m/z* (+ESI) calcd for $C_{10}H_{10}NO^+$ 160.0762, found 160.0667 [MH^+].

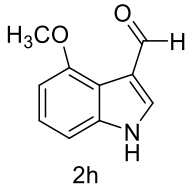
5-Methoxy-1*H*-indole-3-carbaldehyde (2g)



2g

Using phosphoryl chloride (2.87 mL, 30.6 mmol), 5-methoxyindole (3 g, 20.4 mmol) and DMF (10 mL), the title compound **2g** was obtained as yellow crystals (1.9 g, 54 % yield): m.p. 179–183 °C; TLC R_f = 0.2 (Hexane–EtOAc, 2:1) IR (KBr): ν = 3170, 1641, 1432, 1263, 790 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (s, 3H, OCH_3), 6.95 (dd, J = 7 and 2 Hz, 1H, H-6), 7.34 (d, J = 7 Hz, 1H, H-7), 7.78 (s, 1H, H-2), 7.79 (d, J = 2 Hz, 1H, H-4), 9.99 (s, 1H, CHO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 55.8, 103.1, 112.5, 114.8, 119.4, 125.2, 131.6, 136.3, 156.57, 185.2. MS: *m/z* (+ESI) calcd for $C_{10}H_{10}NO_2^+$ 176.0712, found 176.0650 [MH^+].

4-Methoxy-1*H*-indole-3-carbaldehyde (2h)

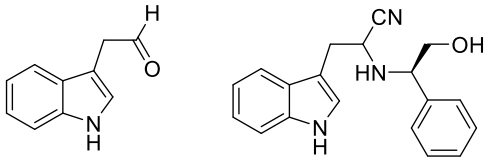


2h

The synthesis was carried out using phosphoryl chloride (0.9 mL, 10.2 mmol), 4-methoxylindole (1 g, 6.8 mmol) and DMF (10 mL) to afford **2h** as a yellow solid (0.8 g, 70 % yield): m.p. 148–150 °C; TLC R_f = 0.2 (Hexane–EtOAc, 2:1). IR (KBr): ν = 3250, 1642, 1361, 1323, 790 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.01 (s, 3H, OCH_3), 6.77 (dd, J = 7, 2 Hz, 1H, H-6), 7.18 (d, J = 7 Hz, 1H, H-7), 7.19 (s, 1H, H-5), 8.04 (s, 1H, H-2), 10.51 (s, 1H, CHO). ^{13}C NMR (100 MHz, acetone-d_6): δ 54.8, 102.1, 105.7, 116.2, 119.1, 123.7, 128.5, 138.2, 154.4, 186.7. MS: *m/z* (+ESI) calcd for $C_{10}H_{10}NO_2^+$ 176.0712, found 176.0672 [MH^+].

2.2 General procedure for the preparation of 2-(2-hydroxy-1-phenylethylamino)-3-(1*H*-indol-3-yl)propanenitrile **4**

2-(2-Hydroxy-1-phenylethylamino)-3-(1*H*-indol-3-yl)propanenitrile (4a)

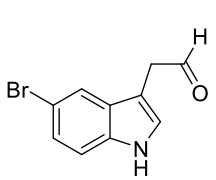


3a **4a**

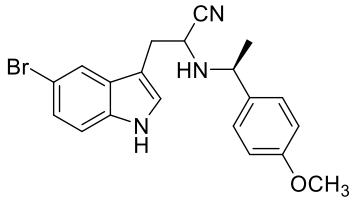
A suspension of (methoxymethyl)triphenylphosphonium chloride (2.3 g, 6.9 mmol) in dry THF (18 mL) cooled to 0 °C under nitrogen was treated with 2.5 M butyllithium solution in THF (3.3 mL, 8.28 mmol). The mixture was stirred for 15 min, then **2a** (500 mg, 3.45 mmol) in dry THF (15 mL) was added and stirred at room temperature for a further 2 h. Excess butyllithium was quenched by adding a few drops of MeOH. The mixture was neutralized with 3 M aq HCl. The solvent was removed *in vacuo*. The residual material was partitioned between EtOAc (100 mL) and brine (100 mL). The organic extract was dried over $MgSO_4$, concentrated, and was subjected to column chromatography (hexane–EtOAc, 1:1) to afford the enol ether intermediate. The enol ether intermediate was subsequently treated with a mixture of THF (18 mL) and 1 M aq HCl (12 mL). After being heated under reflux for 3 h, the solution was cooled and partitioned between Et_2O (80 mL) and brine (80 mL). The organic extract was dried and concentrated to afford the unstable **3a**, which was taken to the next step without further purification.

(*R*)-2-phenylglycinol (449 mg, 3.3 mmol) and acetic acid (372 μL, 6.57 mmol) was added to a solution of **3a** (435 mg, 2.74 mmol) in MeOH (20 mL) at 0 °C. Sodium cyanide (160 mg, 3.28 mmol) was added to the solution immediately. The mixture was allowed to warm to room temperature and stirred for 16 h. The solvent was removed under reduced pressure, and the residual material was partitioned between CH_2Cl_2 (2 x 40 mL) and brine (80 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated *in vacuo*, followed by purification using column chromatography ($CHCl_3$–MeOH, 30:1) to afford **4a** as an amorphous orange solid (307 mg, 29 % yield from **2a**): TLC R_f = 0.3 (Hexane–EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, t_R 5.7 min for (*R,R*) isomer and 6.7 min for (*S,R*) isomer; IR (KBr): ν = 3413, 2924, 2873, 1455, 1356, 744 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.53 (br, 1 H, NH), 3.12 (d, J = 6 Hz, 1H, CH_AH$_B$), 3.21 (d, J = 5 Hz, 1H, CH$_A$$H_B$), 3.49 (dd, J = 11 and 9 Hz, 1H, CH_AH$_B$OH), 3.65 (dd, J = 6 and 4 Hz, 1H, α-H), 3.67 (dd, J = 6 and 4 Hz, 1H, CH$_A$$H_B$OH), 4.03 (dd, J = 9 and 4 Hz, 1H, CHCH$_2$OH), 7.11 (m, 2H, Ar Hs), 7.20 (m, 1H, Ar H) 7.27–7.34 (m, 6 H, Ar Hs), 7.53 (d, J = 8 Hz, 1H, Ar H), 8.28 (br, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.5, 49.1, 63.1, 67.3, 109.2, 111.5, 118.6, 119.7, 120.6, 122.3, 123.8, 127.3, 127.7, 128.3, 128.9, 136.2, 138.3, 139.6. MS: *m/z* (+ESI) calcd for $C_{19}H_{20}N_3O^+$ 306.1606, found 306.1680 [MH^+].

3-(5-Bromo-1*H*-indol-3-yl)-2-[1-(4-methoxyphenyl)ethylamino]propanenitrile (S1)



3b

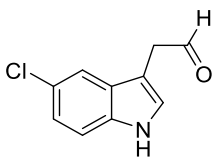


S1

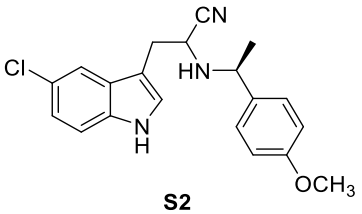
The reagents (methoxymethyl)triphenylphosphonium chloride (2.14 g, 6.3 mmol), 2.5 M butyllithium solution (3 mL, 7.5 mmol) and **2b** (700 mg, 3.1 mmol) were used to yield the unstable **3b**, which was taken to the next step without further purification.

Synthesis was then carried out using **3b** (486 mg, 2.1 mmol), (*S*)-4-methoxy-α-methylbenzylamine (362 µL, 2.5 mmol), acetic acid (277 µL, 4.9 mmol) and sodium cyanide (120 mg, 2.5 mmol) to afford **S1** as a yellow oil (442 mg, 39 % yield from **2b**): TLC R_f = 0.4 (Hexane–EtOAc, 2:1); RP-HPLC 10–60 % B in 12 min, t_R 7.0 min for (*R*,*R*) isomer and 7.6 min for (*S*,*R*) isomer; IR (KBr): ν = 3418, 2960, 1459, 1244, 755 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (d, J = 7 Hz, 3H, CH_3), 1.66 (br, 1H, NH), 3.11 (d, J = 6 Hz, 2H, CH_2), 3.54 (t, J = 6 Hz, 1H, α-H), 3.83 (s, 3H, OCH_3), 4.00 (q, J = 6 Hz, 1H, C*H*CH_3), 6.90 (d, J = 9 Hz, 2H, Ar Hs), 7.13 (d, J = 2.5 Hz, 1H, Ar H) 7.19–7.25 (m, 3H, Ar Hs), 7.28 (d, J = 2 Hz, 1H, Ar H), 7.66 (d, J = 2 Hz, 1H, Ar H), 8.40 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.9, 28.7, 49.3, 55.4, 56.0, 109.2, 112.9, 113.0, 114.2, 120.6, 121.3, 124.8, 125.2, 127.9, 129.1, 134.8, 135.1, 159.1.

3-(5-Chloro-1*H*-indol-3-yl)-2-[1-(4-methoxyphenyl)ethylamino]propanenitrile (S2)



3c

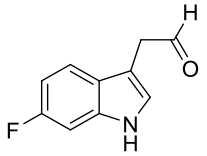


S2

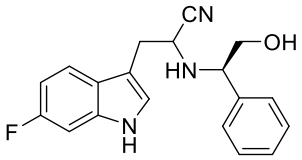
Synthesis was carried out according to the procedure described under 2.2 using (methoxymethyl)triphenylphosphonium chloride (2.68 g, 7.8 mmol), 2.5 M butyllithium solution (3.7 mL, 9.4 mmol) and **2c** (700 mg, 3.9 mmol) to afford the unstable **3c** which was taken to the next step without further purification.

Using **3c** (754 mg, 3.9 mmol), (*S*)-4-methoxy-α-methylbenzylamine (690 µL, 4.7 mmol) and acetic acid (530 µL, 9.4 mmol) and sodium cyanide (229 mg, 4.7 mmol), the title compound **S2** was obtained as a yellow oil (611 mg, 45 % yield from **2c**): TLC R_f = 0.4 (Hexane–EtOAc, 2:1); RP-HPLC 10–60 % B in 12 min, t_R 7.0 min for (*R*,*R*) isomer and 7.6 min for (*S*,*R*) isomer; IR (KBr): ν = 3419, 2963, 1609, 1457, 756 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.32 (d, J = 6 Hz, 3H, CH_3), 1.68 (br, 1H, NH), 3.11 (d, J = 6 Hz, 2H, CH_2), 3.53 (t, J = 6 Hz, 1H, α-H), 3.82 (s, 3H, OCH_3), 4.00 (q, J = 6 Hz, 1H, C*H*CH_3), 6.87-6.89 (m, 2H, Ar Hs), 7.12-7.19 (m, 2H, Ar Hs), 7.23-7.30 (m, 3H, Ar Hs), 7.47 (d, J = 2 Hz, 1H, Ar H), 8.38 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.9, 29.6, 49.3, 55.4, 56.0, 109.3, 112.5, 114.2, 118.3, 120.7, 122.7, 125.0, 125.5, 127.9, 128.6, 134.6, 135.1, 159.1.

3-(6-Fluoro-1*H*-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (4d)



3d

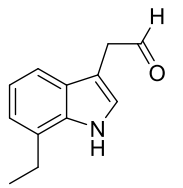


4d

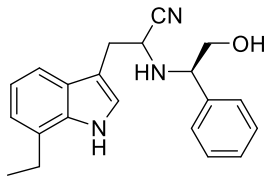
Synthesis carried out according to the procedure described under section 2.2 using (methoxymethyl)triphenylphosphonium chloride (2.9 g, 8.6 mmol), 2.5 M butyllithium solution (4 mL, 10.2 mmol) and **2d** (700 mg, 4.3 mmol) to afford the unstable **3d** which was taken to the next step without further purification.

Compound **3d** (759 mg, 4.3 mmol), (*R*)-2-phenylglycinol (704 mg, 5.1 mmol), acetic acid (581 µL, 10.3 mmol) and sodium cyanide (251 mg, 5.1 mmol) were used to give **4d** as a yellow amorphous solid (614 mg, 44 % yield from **2d**): TLC R_f = 0.3 (Hexane–EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, t_R 6.0 min for (*R*,*R*) isomer and 7.2 min for (*S*,*R*) isomer; IR (KBr): ν = 3425, 3025, 2925, 1627, 758 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.50 (br, 1 H, NH), 3.14 (d, J = 4 Hz, 1H, CH_AH$_B$), 3.16 (d, J = 4 Hz, 1H, CH$_A$$H_B$), 3.49 (dd, J = 12 and 8 Hz, 1H, CH_AH$_B$OH), 3.62 (t, J = 6 Hz, 1H, α-H), 3.67 (dd, J = 11 and 4 Hz, 1H, CH$_A$$H_B$OH), 4.0 (dd, J = 9 and 4 Hz, 1H, CHCH$_2$OH), 76.81-6.88 (m, 1H, Ar H), 6.98 (dd, J = 4 and 9 Hz, 1H, Ar H), 7.04-7.08 (m, 1H, Ar H), 7.24-7.32 (m, 5 H, Ar Hs), 7.40 (dd, J = 9 and 5 Hz, 1H, Ar H), 8.27 (br, 1 H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.5, 49.0, 63.1, 67.3, 97.7 (d, J = 26 Hz), 108.6 (d, J = 24 Hz), 109.4, 119.5 (d, J = 11 Hz), 120.5, 123.9, 124.0 (d, J = 3 Hz), 127.6, 128.4, 129.0, 136.2 (d, J = 12 Hz), 138.2, 160.1 (d, J = 237 Hz). MS: *m/z* (+ESI) calcd for $C_{19}H_{19}FN_3O^+$ 324.1512, found 324.1248 [MH$^+$].

3-(7-Ethyl-1*H*-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (4e)



3e



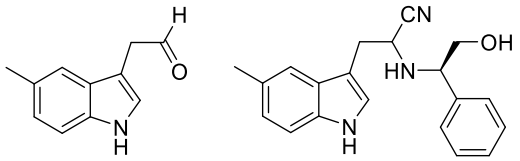
4e

Synthesis was carried out according to the procedure described under section 2.2 using (methoxymethyl)triphenylphosphonium chloride (2.7 g, 8.0 mmol), 2.5 M butyllithium solution (3.8 mL, 9.7 mmol) and **2e** (700 mg, 4.04 mmol) to afford the unstable **3e** which was taken to the next step without further purification.

Compound **3e** (700 mg, 3.74 mmol), (*R*)-2-phenylglycinol (613 mg, 4.5 mmol), acetic acid (507 µL, 8.97 mmol) and sodium cyanide (219 mg, 4.48 mmol) were reacted to afford **4e** as a brown amorphous solid (444 mg, 33 % yield from **2e**): TLC R_f = 0.3 (Hexane–EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, t_R 7.0 min for (*R*,*R*) isomer and 8.1 min for (*S*,*R*) isomer; IR (KBr): ν = 3415, 2964, 2930, 1453, 753 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (t, J = 8 Hz, 3H, CH$_2$CH_3), 2.49 (br, 1 H, NH), 2.85 (q, J = 8 Hz, 2H, CH_2CH$_3$), 3.25 (d, J = 6 Hz, 1H, CH_AH$_B$), 3.27 (d, J = 4 Hz, 1H, CH$_A$$H_B$), 3.56 (dd, J = 12 and 9 Hz, 1H, CH_AH$_B$OH), 3.70 (dd, J = 8 and 6 Hz, 1H, α-H), 4.09 (dd, J = 11 and 4 Hz, 1H, CH$_A$$H_B$OH), 4.09 (dd, J = 8, 4 Hz, 1H, CHCH$_2$OH), 7.06-7.13 (m, 2H, Ar Hs), 7.17 (d, J = 3 Hz, 1H, Ar H), 7.30-7.38 (m, 5H, Ar Hs), 7.41 (m, 1H, Ar H), 8.27 (br, 1 H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.8, 24.0, 29.7, 49.2, 63.1, 67.3, 109.7, 116.3, 120.0, 120.5, 120.8, 123.3, 126.9,

127.1, 127.6, 128.3, 128.9, 135.1, 138.4. MS: *m/z* (+ESI) calcd for $C_{21}H_{24}N_3O^+$ 334.1919, found 334.1843 [MH^+].

3-(5-Methyl-1*H*-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (4f)

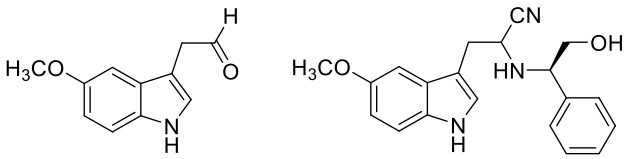


3f **4f**

Synthesis was carried out using (methoxymethyl)triphenylphosphonium chloride (3 g, 8.8 mmol), 2.5 M butyllithium solution (4.2 mL, 10.5 mmol) and **2f** (700 mg, 4.4 mmol) to afford the unstable **3f** which was taken to the next step without further purification.

Compound **3f** (600 mg, 3.5 mmol), (*R*)-2-phenylglycinol (570 mg, 4.2 mmol), acetic acid (470 µL, 8.3 mmol) and sodium cyanide (203 mg, 4.2 mmol) were reacted to yield **4f** as a brown oil (496 mg, 35 % yield from **2f**): TLC R_f = 0.4 (Hexane–EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, t_R 6.5 min for (*R*,*R*) isomer and 7.2 min for (*S*,*R*) isomer; IR (KBr): ν = 3405, 3017, 2922, 1450, 756 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.33 (br, 1 H, NH), 2.44 (s, 3H, CH_3), 3.21 (d, J = 4 Hz, 1H, CH_AH_B), 3.23 (d, J = 3 Hz, 1H , CH_AH_B), 3.55 (dd, J = 12 and 9 Hz, 1H, CH_AH_BOH), 3.68 (dd, J = 12 and 6 Hz, 1H, α-H), 3.73 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 4.08 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 7.04 (m, 1H, Ar H), 1.2 (m, 1H, Ar H), 7.24 (s, 1H, Ar H), 7.26 (s, 1H, Ar H), 7.29-7.36 (m, 5 H, Ar Hs), 8.12 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.5, 29.7, 48.9, 63.1, 67.3, 108.8, 111.1, 118.2, 120.5, 123.7, 123.9, 127.4, 127.6, 128.2, 128.8, 128.9, 134.5, 138.3. MS: *m/z* (+ESI) calcd for $C_{20}H_{22}N_3O^+$ 320.1763, found 320.1711 [MH^+].

3-(5-Methoxy-1*H*-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (4g)

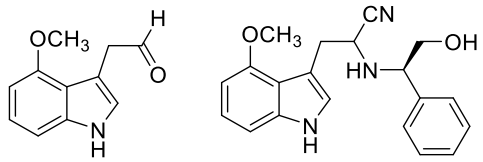


3g **4g**

(Methoxymethyl)triphenylphosphonium chloride (2.74 g, 8 mmol), 2.5 M butyllithium solution (3.8 mL, 9.6 mmol) and **2g** (700 mg, 4 mmol) were used to afford the unstable **3g** which was taken to the next step without further purification.

Compound **3g** (500 mg, 2.6 mmol), (*R*)-2-phenylglycinol (434 mg, 3.1 mmol), acetic acid (359 µL, 6.3 mmol) and sodium cyanide (155 mg, 3.2 mmol) were reacted to yield **4g** as a brown oil (177 mg, 13 % yield from **2g**): TLC R_f = 0.5 (Hexane–EtOAc, 1:1), RP-HPLC 10–60 % B in 12 min, t_R 5.5 min for (*R*,*R*) isomer and 6.4 min for (*S*,*R*) isomer; IR (KBr): ν = 3405, 3011, 2938, 1480, 757 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.05 (br, 1 H, NH), 3.18 (d, J = 2 Hz, 1H , CH_AH_B), 3.20 (s, 1H , CH_AH_B), 3.52 (dd, J = 11 and 9 Hz, 1H, CH_AH_BOH), 3.64 (t, J = 6 Hz, 1H, α-H), 3.72 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.78 (s, 3H, OCH_3), 4.05 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.85 (dd, J = 9 and 2 Hz, 1H, Ar H), 6.92 (d, J = 2.5 Hz, 1H, Ar H), 7.12 (d, J = 3 Hz, 1H, Ar H), 7.22 (d, J = 11 and 4 Hz, 1H, Ar H), 7.27–7.35 (m, 5 H, Ar H), 8.09 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.7, 48.9, 55.9, 63.1, 67.3, 100.2, 109.1, 112.1, 112.8, 120.4, 124.3, 127.6, 128.2, 128.8, 131.3, 138.3, 154.2. MS: *m/z* (+ESI) calcd for $C_{20}H_{22}N_3O_2^+$ 336.1712, found 336.1786 [MH^+].

3-(4-Methoxy-1*H*-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (4h)



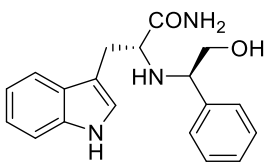
3h **4h**

(Methoxymethyl)triphenylphosphonium chloride (2.74 g, 8 mmol), 2.5 M butyllithium solution (3.8 mL, 9.6 mmol) and **2h** (700 mg, 4 mmol) were reacted to afford the unstable **3h** which was taken to the next step without further purification.

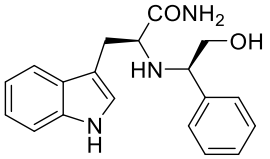
Using **3h** (756 mg, 4.0 mmol), (*R*)-2-phenylglycinol (657 mg, 4.8 mmol), acetic acid (543 μL, 9.6 mmol) and sodium cyanide (235 mg, 4.8 mmol), the title compound **4h** was obtained as a brown oil (258 mg, 19 % yield from **2h**): TLC R_f = 0.5 (Hexane–EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, t_R 5.8 min for (*R*,*R*) isomer and 6.2 min for (*S*,*R*) isomer; IR (KBr): ν = 3401, 2924, 1357, 1255, 733 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 2.51 (br, 1 H, NH), 3.18 (dd, J = 14 and 9 Hz, 1H , CH_AH_B), 3.47 (dd, J = 14 and 7 Hz,1H , CH_AH_B), 3.58 (dd, J = 11 and 9 Hz, 1H, CH_AH_BOH), 3.65 (s, 3H, OCH_3), 3.77 (dd, J = 11 and 4 Hz, 1H, α-H), 3.93 (dd, J = 9 and 6 Hz, 1H, CH_AH_BOH), 4.14 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.41 (d, J = 8 Hz, 1 H, Ar H), 6.96 (d, J = 8 Hz, 1H, Ar H), 7.00 (d, J = 2.5 Hz, 1H, Ar H), 7.09 (t, J = 7 Hz, 1H, Ar H), 7.26 –7.28 (m, 5 H, Ar Hs), 8.25 (br, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.6, 50.4, 54.8, 63.1, 67.3, 99.4, 104.7, 109.9, 116.9, 120.9, 122.7, 123.1, 127.6, 128.0, 128.7, 138.1, 138.6, 154.2. MS: *m/z* (+ESI) calcd for $C_{20}H_{22}N_3O_2^+$ 336.1712, found 336.1656 [MH^+].

2.3 General procedure for the preparation of (*S*)-2-((*R*)-2-hydroxy-1-phenylethyl amino)-3-(1*H*-indol-3-yl)propanamide **6**

(*S*)-2-((*R*)-2-hydroxy-1-phenylethylamino)-3-(1*H*-indol-3-yl)propanamide ((*S*,*R*)-6a)



(*R*,*R*)-**5a**



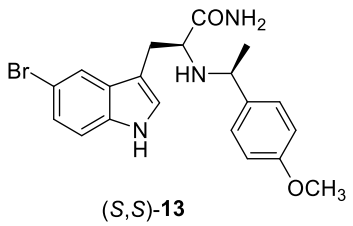
(*S*,*R*)-**6a**

To a solution of α-aminonitrile **4a** (257 mg, 0.8 mmol) in DMSO (3.5 mL), K_2CO_3 (138 mg, 1.3 mmol) and 30 % H_2O_2 (0.6 mL, 6.3 mmol) were added at 20 °C. After stirring at room temperature for 2 h, another portion of 30 % H_2O_2 (0.4 mL, 4.7 mmol) was added and stirred for a further hour. The resultant mixture was extracted with Et_2O (3 x 50 mL) and brine (50 mL). The organic extracts were combined, dried over $MgSO_4$ and concentrated. Purification of the residual material by column chromatography ($CHCl_3$–MeOH, 10:1) gave (*R*,*R*)-**5a** as yellow oil (first eluting diastereoisomer, 35 mg, 13 % yield) and (*S*,*R*)-**6a** as a white foam (second eluting diastereoisomer, 101 mg, 38 % yield).

(*R*,*R*)-**5a** TLC R_f = 0.5 ($CHCl_3$–MeOH, 6:1), IR (KBr): ν = 3412, 2917, 1662, 744 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 3.10 (br, 1 H, NH), 3.24 (dd, J = 14 and 6 Hz, 1H, CH_AH_B), 3.20 (d, J = 6 Hz, 1H, CH_AH_B), 3.36 (dd, J = 9 and 3 Hz, 1H, α-H), 3.51 (dd, J = 10 and 8 Hz, 1H, CH_AH_BOH), 3.60 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.96 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.40 (br, 1H, NH), 7.00 (m, 1H, Ar H) 7.10 (m, 1H, Ar H), 7.22–7.33 (m, 5H, Ar Hs), 7.36-7.41 (m, 2H, Ar Hs), 7.69 (d, J = 8 Hz 1H, Ar H), 10.16 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 26.6, 59.5, 63.6, 66.7, 110.4, 111.1, 118.5, 119.2, 121.2, 124.3, 127.3, 128.0, 128.0, 128.2, 136.8, 141.3, 176.3. MS: *m/z* (+ESI) calcd for $C_{19}H_{22}N_3O_2^+$ 324.1712, found 324.1638 [MH^+].

(*S*,*R*)-**6a** TLC R_f = 0.4 ($CHCl_3$–MeOH, 6:1), IR (KBr): ν = 3412, 2917, 1662, 744 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.97 (dd, J = 15 and 8 Hz, 1H, CH_AH_B), 3.22 (dd, J = 14 and 5 Hz, 1H, CH_AH_B), 3.31 (dd, J = 9 and 5 Hz, 1H, α-H), 3.46 (dd, J = 11 and 8 Hz, 1H, CH_AH_BOH), 3.60 (dd, J = 11 and 5 Hz, 1H, CH_AH_BOH), 3.77 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.71 (br, 1H, NH), 6.92–7.13 (m, 7H, Ar Hs), 7.14 (d, J = 2 Hz, 1H, Ar H), 7.45 (m, 3H, Ar H), 10.17 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 30.0, 60.4, 63.5, 67.2, 111.1, 111.3, 118.6, 121.3, 123.6, 126.8, 127.2, 127.8, 127.9, 136.8, 140.9, 177.1. MS: *m/z* (+ESI) calcd for $C_{19}H_{22}N_3O_2^+$ 324.1712, found 324.1365 [MH^+].

(*S*)-3-(5-Bromo-1*H*-indol-3-yl)-2-((*S*)-1-(4-methoxyphenyl)ethylamino)propanamide ((*S*,*S*)-13)

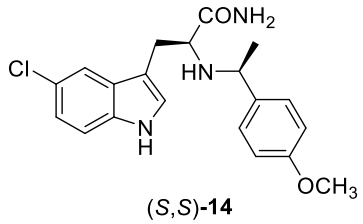


(*S*,*S*)-**13**

Synthesis was carried out according to the procedure described under section 2.3 using α-aminonitrile **S1** (356 mg, 0.9 mmol) in DMSO (3.5 mL), K_2CO_3 (172 mg, 1.2 mmol) and 30 % H_2O_2 (0.8 mL, 7.3 mmol), followed by a further 30 % H_2O_2 (0.4 mL, 2.3 mmol). The synthesized compound was

purified column chromatography (Hexane–EtOAc, 1 : 4) followed by RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) gave (*S*,*S*)-**13** as a yellow foam (196 mg, 53 %): TLC R_f = 0.2 (Hexane–EtOAc, 1: 4); IR (KBr): ν = 3267, 3006, 2961, 1669, 1244, 755 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.24 (d, J = 7 Hz, 3H, CH_3), 1.83 (br, 1H, NH), 2.79 (dd, J = 14 and 9 Hz, 1H, CH_AH_B), 3.22 (m, 2H, CH_AH_B, α-H), 3.55 (q, J = 7 Hz, 1H, C*H*CH_3), 3.76 (s, 3H, OCH_3), 6.10 (br, 1H, NH), 6.53 (d, J = 9 Hz, 2H, Ar Hs), 6.63 (d, J = 8 Hz, 2H, Ar Hs), 6.90 (d, J = 2 Hz, 1H, Ar H), 7.21 (s, 1H, Ar H), 7.23 (d, J = 2 Hz, 1H, Ar H), 7.48 (d, J = 2 Hz, 1H, Ar H), 8.75 (br, 1H, NH). ^{13}C NMR (100 MHz $CDCl_3$): δ 24.1, 29.6, 55.2, 56.4, 59.8, 110.9, 112.7, 112.8, 113.5, 121.5, 124.3, 125.0, 127.0, 128.9 135.1, 135.7, 158.4, 178.2. MS: *m/z* (+ESI) calcd for $C_{20}H_{23}BrN_3O_2^+$ 416.0974, found 416.0773 [MH^+].

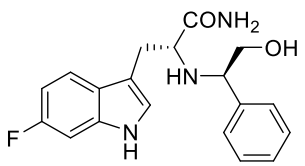
(*S*)-3-(5-Chloro-1*H*-indol-3-yl)-2-((*S*)-1-(4-methoxyphenyl)ethylamino)propanamide ((*S*,*S*)-14)



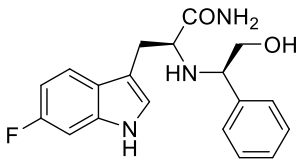
(*S*,*S*)-**14**

Synthesis was carried out according to the procedure described under section 2.3 using α-aminonitrile **S2** (542 mg, 1.5 mmol) in DMSO (3.5 mL), K_2CO_3 (275 mg, 2.0 mmol) and 30 % H_2O_2 (1.1 mL, 11.6 mmol), followed by a further portion of 30 % H_2O_2 (0.4 mL, 2.3 mmol). Purification was achieved by column chromatography (Hexane–EtOAc, 1:4) followed by RP-HPLC (Onyx Monolithic C18, 100 x 10 mm) gave (*S*,*S*)-**14** as a yellow foam (406 mg, 71 %): TLC R_f = 0.4 (Hexane–EtOAc, 2:1); IR (KBr): ν = 3269, 2961, 2928, 1670, 1279, 755 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.24 (d, J = 7 Hz, 3H, CH_3), 1.85 (br, 1H, NH), 2.79 (dd, J = 14 and 9 Hz, 1H, CH_AH_B), 3.22 (m, 2H, CH_AH_B, α-H), 3.54 (q, J = 6 Hz, 1H, C*H*CH_3), 3.75 (s, 3H, OCH_3), 6.12 (br, 1H, NH), 6.52 (d, J = 8 Hz, 2H, Ar Hs), 6.63 (d, J = 8 Hz, 2H, Ar Hs), 6.91 (d, J = 2 Hz, 1H, Ar H), 7.05–7.18 (m, 2H, Ar Hs), 7.31 (d, J = 2 Hz, 1H, Ar H), 8.75 (br, 1H, NH). ^{13}C NMR (100 MHz $CDCl_3$): δ 24.1, 29.6, 55.1, 56.4, 59.8, 110.9, 112.2, 113.5, 118.4, 121.5, 124.5, 125.2, 127.0, 128.3, 134.8, 135.8, 158.4, 178.2. MS: *m/z* (+ESI) calcd for $C_{20}H_{23}ClN_3O_2^+$ 372.1479, found 372.1255 [MH^+].

(*S*)-3-(6-Fluoro-1*H*-indol-3-yl)-2-((*R*)-2-hydroxy-1-phenylethylamino)propanamide ((*S*,*R*)-6d)



(*R*,*R*)-**5d**



(*S*,*R*)-**6d**

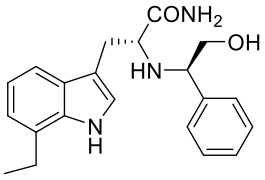
The α-aminonitrile **4d** (557 mg, 1.7 mmol) in DMSO (4 mL), K_2CO_3 (308 mg, 2.2 mmol) and 30 % H_2O_2 (1.3 mL, 13 mmol) were reacted, followed by an additional 30 % H_2O_2 (0.4 mL, 4.7 mmol) to afford (*R*,*R*)-**5d** as a yellow foam (first eluting diastereoisomer, 71 mg, 12 % yield) and (*S*,*R*)-**6d** as white foam (second eluting diastereoisomer, 207 mg, 35 % yield).

(*R*,*R*)-**5d** TLC R_f = 0.5 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3423, 3311, 2919, 1664, 759 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 3.23 (dd, J = 14 and 5 Hz, 1H, CH_AH_B), 3.32 (m, 2H, CH_AH_B, α-H), 3.52 (dd, J = 11 and 9 Hz, 1H, C$H_A$$H_B$OH), 3.62 (dd, J = 11 and 4 Hz, 1H, C$H_A$$H_B$OH), 3.97 (dd, J = 9 and 4 Hz, 1H, C*H*CH_2OH), 6.58 (br, 1H, NH), 6.80 (td, J = 9 and 2 Hz, 1H, Ar H), 7.14 (dd, J = 10 and 2 Hz, 1H, Ar H), 7.21–7.43 (m, 6H, Ar Hs), 7.66 (dd, J = 9 and 5 Hz, 1H, Ar H), 10.29 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 26.5, 59.3, 63.6, 66.9, 97.1 (d, J = 26 Hz), 106.9 (d, J = 24

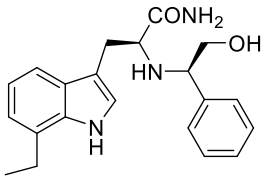
Hz), 110.6, 120.2 (d, J = 10 Hz), 124.8, 124.9 (d, J = 3 Hz), 127.3, 127.9, 128.3, 136.7 (d, J = 13 Hz), 141.3, 159.7 (d, J = 233 Hz), 176.8. MS: *m/z* (+ESI) calcd for $C_{19}H_{21}FN_3O_2^+$ 342.1618, found 342.1321 [MH^+].

(*S*,*R*)-**6d** TLC R_f = 0.4 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3423, 3311, 2919, 1664, 759 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.95 (dd, J = 16 and 8 Hz, 1H, CH_AH_B), 3.21 (dd, J = 14 and 5 Hz, 1H, CH_AH_B), 3.30 (dd, J = 9 and 5 Hz, 1H, α-H), 3.48 (dd, J = 11 and 8 Hz, 1H, CH_AH_BOH), 3.62 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.77 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.73 (td, J = 9 and 2 Hz, 1H, Ar H), 6.84 (br, 1H, NH), 6.92–7.18 (m, 7H, Ar Hs), 7.41 (dd, J = 9 and 6 Hz, 1H, Ar H), 7.50 (br, 1H, NH), 10.29 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 28.0, 60.2, 63.5, 67.1, 97.2 (d, J = 26 Hz), 107.0 (d, J = 24 Hz), 111.3, 119.6 (d, J = 10 Hz), 124.3 (d, J = 3 Hz), 124.5, 126.9, 127.2, 128.0, 136.7 (d, J = 13 Hz), 140.9, 159.7 (d, J = 233 Hz), 177.4. MS: *m/z* (+ESI) calcd for $C_{19}H_{21}FN_3O_2^+$ 342.1618, found 342.1298 [MH^+].

(*S*)-3-(7-Ethyl-1*H*-indol-3-yl)-2-((*R*)-2-hydroxy-1-phenylethylamino)propanamide ((*S*,*R*)-6e)



(*R*,*R*)-**5e**



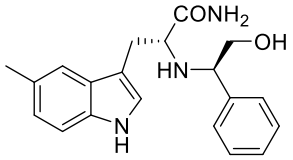
(*S*,*R*)-**6e**

Synthesis was carried out using α-aminonitrile **4e** (396 mg, 1.1 mmol) in DMSO (3 mL), K_2CO_3 (172 mg, 1.4 mmol) and 30 % H_2O_2 (0.8 mL, 8.4 mmol), followed by a further portion of 30 % H_2O_2 (0.4 mL, 4.7 mmol) to give (*R*,*R*)-**5e** as a yellow oil (first eluting diastereoisomer, 71 mg, 17 % yield) and (*S*,*R*)-**6e** as a yellow foam (second eluting diastereoisomer, 196 mg, 47 % yield).

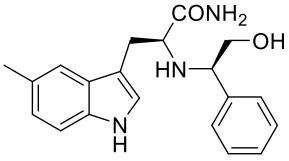
(*R*,*R*)-**5e** TLC R_f = 0.5 ($CHCl_3$–MeOH, 6:1), IR (KBr): ν = 3417, 3311, 2929, 1665, 749 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 1.32 (t, J = 8 Hz, 3H, CH_2CH_3), 2.91 (q, J = 8 Hz, 2H, CH_2CH_3), 3.24 (dd, J = 14 and 5 Hz, 1H, CH_AH_B), 3.23 (d, J = 7 Hz, 1H, CH_AH_B), 3.34 (d, J = 6 Hz, 1H, α-H), 3.51 (dd, J = 12 and 6 Hz, 1H, CH_AH_BOH), 3.61 (dd, J = 11 and 5 Hz, 1H, CH_AH_BOH), 3.97 (dd, J = 9 and 5 Hz, 1H, $CHCH_2OH$), 6.45 (br, 1H, NH), 6.96 (d, J = 5 Hz, 2H, Ar Hs), 7.21–7.23 (m, 4H, Ar Hs), 7.36-7.41 (m, 2H, Ar Hs), 7.55 (t, J = 5 Hz, 1H, Ar H), 10.18 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.8, 23.9, 26.7, 59.5, 63.6, 66.9, 110.8, 116.9, 118.9, 119.9, 123.9, 126.6, 127.3, 128.0, 128.0, 128.2, 135.4, 141.4, 176.6. MS: *m/z* (+ESI) calcd for $C_{21}H_{26}N_3O_2^+$ 352.1947, found 352.1683 [MH^+].

(*S*,*R*)-**6e** TLC R_f = 0.4 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3417, 3311, 2929, 1665, 749 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 1.35 (t, J = 8 Hz, 3H, CH_2CH_3), 2.94 (q, J = 8 Hz, 2H, CH_2CH_3), 3.21 (dd, J = 15 and 5 Hz, 1H, CH_AH_B), 3.23 (dd, J = 16 and 8 Hz, 1H, CH_AH_B), 3.30 (dd, J = 9 and 5 Hz, 1H, α-H), 3.45 (dd, J = 11 and 8 Hz, 1H, CH_AH_BOH), 3.60 (dd, J = 12 and 4 Hz, 1H, CH_AH_BOH), 3.76 (dd, J = 8 and 4 Hz, 1H, $CHCH_2OH$), 6.73 (br, 1H, NH), 6.89–7.10 (m, 6H, Ar Hs), 7.12 (d, J = 2 Hz, 1H, Ar H), 7.32 (d, J = 8 Hz, 1H, Ar H), 7.45 (br, 1H, NH), 10.15 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.9, 24.0, 30.1, 60.5, 63.6, 67.2, 111.5, 116.4, 119.0, 120.0, 123.2, 126.8, 127.2, 127.8, 127.9, 135.5, 141.0, 177.2. MS: *m/z* (+ESI) calcd for $C_{21}H_{26}N_3O_2^+$ 352.1947, found 352.1595 [MH^+].

(*S*)-3-(5-Methyl-*1*H-indol-3-yl)-2-((*R*)-2-hydroxy-1-phenylethylamino)propanamide ((*S*,*R*)-6f)



(*R*,*R*)-**5f**



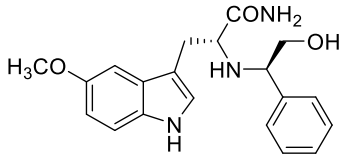
(*S*,*R*)-**6f**

The α-aminonitrile **4f** (468 mg, 1.5 mmol) in DMSO (3 mL), K_2CO_3 (261 mg, 1.9 mmol) and 30 % H_2O_2 (1.3 mL, 11.1 mmol), and a further 30 % H_2O_2 (0.4 mL, 4.7 mmol) were reacted to give (*R*,*R*)-**5f** as a yellow oil (first eluting diastereoisomer, 12 mg, 2 % yield) and (*S*,*R*)-**6f** as a yellow foam (second eluting diastereoisomer, 48 mg, 10 % yield).

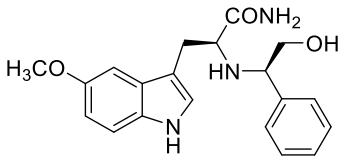
(*R*,*R*)-**5f** TLC R_f = 0.6 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3310, 2919, 1662, 756 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.39 (s, 3H, CH_3), 3.22 (dd, J = 6 and 4 Hz, 2H, CH_AH_B), 3.32 (t, J = 6 Hz, 1H, α-H), 3.49 (dd, J = 11 and 9 Hz, 1H, CH_AH_BOH), 3.58 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.93 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.31 (br, 1H, NH), 6.94 (dd, J = 8 and 2 Hz, 1H, Ar H), 7.22–7.33 (m, 5H, Ar Hs), 7.35-7.39 (m, 2H, Ar Hs), 7.44 (s, 1H, Ar H), 9.97 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 20.8, 26.8, 59.5, 63.6, 66.8, 110.1, 110.8, 118.8, 122.8, 124.2, 127.2, 127.2, 127.9, 128.2, 128.3, 135.2, 141.5, 176.3. MS: *m/z* (+ESI) calcd for $C_{20}H_{24}N_3O_2^+$ 338.1869, found 338.1823 [MH^+].

(*S*,*R*)-**6f** TLC R_f = 0.5 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3310, 2919, 1662, 756 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.33 (s, 3H, CH_3), 2.91 (dd, J = 15 and 9 Hz, 1H, CH_AH_B), 3.18 (dd, J = 14 and 4 Hz, 1H, CH_AH_B), 3.29 (dd, J = 9 and 5 Hz, 1H, α-H), 3.45 (dd, J = 12 and 8 Hz, 1H, CH_AH_BOH), 3.59 (dd, J = 12 and 8 Hz, 1H, CH_AH_BOH), 3.75 (dd, J = 8 and 4 Hz, 1H, $CHCH_2OH$), 6.61 (br, 1H, NH), 6.90–6.95 (m, 2H, Ar Hs), 7.00-7.05 (m, 2H, Ar Hs), 7.07-7.12 (m, 2H, Ar Hs), 7.20 (s, 1H, Ar H), 7.37 (br, 1H, NH), 10.00 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 20.8, 30.1, 60.2, 63.5, 67.1, 110.5, 110.9, 118.3, 122.9, 123.7, 126.8, 127.2, 127.3, 127.9, 128.0, 135.3, 141.0, 177.0. MS: *m/z* (+ESI) calcd for $C_{20}H_{24}N_3O_2^+$ 338.1869, found 338.1942 [MH^+].

(*S*)-3-(5-Methoxy-1*H*-indol-3-yl)-2-((*R*)-2-hydroxy-1-phenylethylamino)propanamide ((*S*,*R*)-6g)



(*R*,*R*)-**5g**



(*S*,*R*)-**6g**

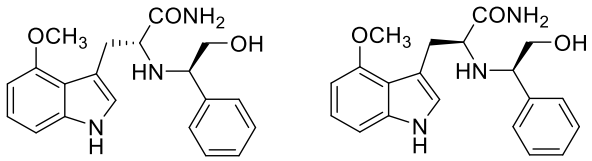
The α-aminonitrile **4g** (266 mg, 0.8 mmol) in DMSO (3 mL), K_2CO_3 (141 mg, 1.0 mmol) and 30 % H_2O_2 (0.6 mL, 5.9 mmol), followed by a further 30 % H_2O_2 (0.4 mL, 4.7 mmol) were reacted to yield (*R*,*R*)-**5g** as a yellow oil (first eluting diastereoisomer, 17 mg, 6 % yield) and (*S*,*R*)-**6g** as a yellow foam (second eluting diastereoisomer, 30 mg, 10 % yield).

(*R*,*R*)-**5g** TLC R_f = 0.6 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3331, 2931, 1661, 1481, 1216, 756 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.93 (br, 1H, NH), 3.18 (dd, J = 15 and 7 Hz, 1H, CH_AH_B), 3.30 (m, 2H, CH_AH_B, α-H), 3.50 (dd, J = 11 and 9 Hz, 1H, CH_AH_BOH), 3.60 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.78 (s, 3H, OCH_3), 3.98 (dd, J = 8 and 4 Hz, 1H, $CHCH_2OH$), 6.31 (br, 1H, NH), 6.75 (dd, J = 2 and 9 Hz, 1H, Ar H), 7.21 (d, J = 2 Hz, 1H, Ar H), 7.24–7.7.23 (m, 3H, Ar Hs), 7.28-7.33 (m, 2H, Ar Hs), 7.40 (m, 2H, Ar Hs), 9.95 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 26.5,

54.9, 59.2, 63.5, 66.1, 100.8, 110.2, 111.6, 111.7, 123.0, 127.3, 127.9, 128.2, 128.4, 131.9, 141.4, 153.8, 176.2. MS: *m/z* (+ESI) calcd for $C_{20}H_{24}N_3O_3^+$ 354.1818, found 354.1886 [MH^+].

(*S*,*R*)-**6g** TLC R_f = 0.4 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3331, 2931, 1661, 1481, 1216, 756 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.93 (dd, J = 14 and 9 Hz, 1H, CH_AH_B), 3.15 (dd, J = 14 and 5 Hz, 1H, CH_AH_B), 3.28 (dd, J = 9 and 5 Hz, 1H, α-H), 3.45 (dd, J = 11 and 8 Hz, 1H, CH_AH_BOH), 3.59 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.70 (s, 3H, OCH_3) 3.75 (dd, J = 9 and 4 Hz, 1H, $CHCH_2OH$), 6.56 (br, 1H, NH), 6.76 (dd, J = 9 and 2 Hz, 1H, Ar H), 6.95 (m, 2H, Ar Hs), 6.97 (s, 1H, Ar H), 7.03-7.11 (m, 4H, Ar Hs), 7.29 (d, J = 9 Hz, 1H, Ar H), 7.33 (br, 1H, NH), 9.97 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 30.1, 54.9, 60.1, 63.6, 67.2, 100.3, 110.8, 111.7, 111.9, 124.3, 126.8, 127.3, 127.9, 128.0, 132.0, 141.1, 153.8, 176.8. MS: *m/z* (+ESI) calcd for $C_{20}H_{24}N_3O_3^+$ 354.1818 found 354.1914 [MH^+].

(*S*)-3-(4-Methoxy-1*H*-indol-3-yl)-2-((*R*)-2-hydroxy-1-phenylethylamino)propanamide ((*S*,*R*)-6h)



(*R*,*R*)-**5h** (*S*,*R*)-**6h**

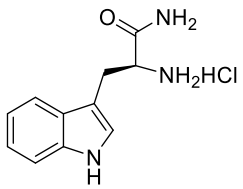
The α-aminonitrile **4h** (213 mg, 0.6 mmol) in DMSO (3 mL), K_2CO_3 (113 mg, 0.8 mmol) and 30 % H_2O_2 (0.5 mL, 4.7 mmol followed by a further 0.4 mL, 4.7 mmol) were reacted to give (*R*,*R*)-**5h** as a yellow oil (first eluting diastereoisomer, 16 mg, 7 % yield) and (*S*,*R*)-**6h** as a yellow foam (second eluting diastereoisomer, 25 mg, 11 % yield).

(*R*,*R*)-**5h** TLC R_f = 0.6 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3317, 2932, 1665, 1086, 755 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.90 (br, 2H, NH), 3.16 (dd, J = 10 and 6 Hz, 1H, CH_AH_B), 3.46 (m, 4 H, CH_AH_B, α-H, CH_AH_BOH, CH_AH_BOH), 3.69 (dd, J = 8 and 4 Hz, 1H), 3.86 (s, 3H, OCH_3), 6.22 (br, 1H, NH), 6.49 (dd, J = 7 and 2 Hz, 1H, Ar H), 7.00–7.02 (m, 2H, Ar Hs), 7.06 (d, J = 2 Hz, 1H, Ar H), 7.11 (br, 1H, NH), 7.22 (dd, J = 8 and 4 Hz, 1H, Ar H), 7.27 (m, 4H, Ar Hs), 10.07 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 29.9, 54.34, 61.0, 63.6, 66.4, 98.8, 104.9, 111.6, 117.7, 122.0, 122.5, 127.0, 127.9, 128.0, 138.4, 141.9, 154.7, 176.5. MS: *m/z* (+ESI) calcd for $C_{20}H_{24}N_3O_3^+$ 354.1818, found 354.1598 [MH^+].

(*S*,*R*)-**6h** TLC R_f = 0.5 ($CHCl_3$–MeOH, 6:1); IR (KBr): ν = 3317, 2932, 1665, 1086, 755 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 2.90 (br, 1H, NH), 3.02 (dd, J = 14 and 8 Hz, 1H, CH_AH_B), 3.32 (dd, J = 14 and 5 Hz, 1H, CH_AH_B), 3.37 (dd, J = 8 and 5 Hz, 1H, α-H), 3.45 (dd, J = 12 and 8 Hz, 1H, CH_AH_BOH), 3.59 (dd, J = 11 and 4 Hz, 1H, CH_AH_BOH), 3.70 (s, 3H, OCH_3), 3.71 (dd, J = 8 and 4 Hz, 1H, $CHCH_2OH$), 6.40 (dd, J = 6 and 2 Hz, 1H, Ar H), 6.51 (br, 1H, NH), 6.92–6.97 (m, 2H, Ar Hs), 6.99-7.02 (m, 4H, Ar Hs), 7.07-7.13 (m, 1 H, Ar H), 7.22 (br, 1H, NH), 10.10 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 31.4, 54.2, 61.2, 63.6, 67.2, 99.0, 104.8, 111.5, 117.7, 122.1, 122.6, 126.7, 127.2, 127.8, 138.6, 141.3, 154.6, 177.2. MS: *m/z* (+ESI) calcd for $C_{20}H_{24}N_3O_3^+$ 354.1818, found 354.1441 [MH^+].

2.4 General procedure for the preparation of (*S*)-2-amino-3-(1*H*-indol-3 yl) propanamide **7**

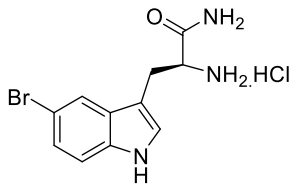
(*S*)-2-Amino-3-(1*H*-indol-3-yl)propanamide (7a)



7a

To a stirred solution of **6a** (101 mg, 0.3 mmol), 10 % Pd/C (50 mg, 50 % *w/w*) in MeOH (10 mL) and ammonium formate (98 mg, 1.56 mmol) were added under nitrogen. The resulting mixture was stirred at reflux for 4 h. The catalyst was removed by filtration through Celite and washed with MeOH (5 mL). The filtrate was dried and concentrated. Purification of the residual material by column chromatography (CH_2Cl_2–MeOH, 4:1) yielded the amino amide which was acidified with 1M HCl to give **7a**[4] hydrochloric salt as a white solid (30 mg, 41 %): m.p. 256–257 °C (Lit[4] m.p. 254-255 °C); TLC R_f = 0.2 ($CHCl_3$–MeOH, 4:1). IR (KBr): ν = 3388, 3247, 2914, 1693, 1492, 751 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 3.23 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.30 (dd, J = 15 and 7 Hz, 1H, CH$_A$$H_B$), 4.20 (t, J = 7 Hz, 1H, α-H), 7.08 (t, J = 8 Hz, 1H, Ar H), 7.16 (t, J = 8 Hz, 1H, Ar H), 7.20 (s, 1H, C2H), 7.41 (d, J = 8 Hz, 1H, Ar H), 7.58 (d, J = 8 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 26.8, 53.2, 106.3, 112.0, 118.2, 119.5, 122.2, 125.4, 126.5, 136.2, 171.8. MS: *m/z* (+ESI) calcd for $C_{11}H_{14}N_3O^+$ 204.1137, found 204.1016 [MH^+].

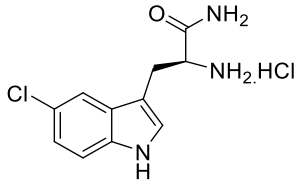
(*S*)-2-Amino-3-(5-bromo-1*H*-indol-3-yl)propanamide (7b)



7b

A solution of **13** (510 mg, 1.2 mmol) and iPr_3SiH (252 µL, 1.2 mmol) in trifluoroacetic acid (4 mL) was stirred at 60 °C for 42 h. The mixture was evaporated to dryness *in vacuo*, and then triturated with cold diethyl ether. The crude **7b** was used directly to the next acid hydrolysis step.

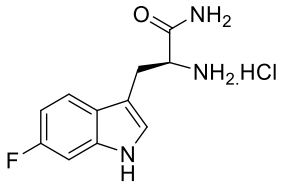
(*S*)-2-Amino-3-(5-chloro-1*H*-indol-3-yl)propanamide (7c)



7c

A solution of **14** (446 mg, 1.2 mmol) and iPr_3SiH (246 µL, 1.2 mmol) in trifluoroacetic acid (4 mL) was stirred at 60 °C for 40 h. The reaction mixture was evaporated to dryness *in vacuo* and then triturated with cold diethyl ether. The crude **7c** was used directly for the next acid hydrolysis step.

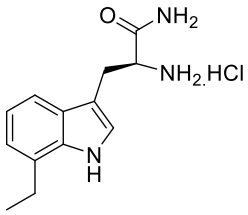
(*S*)-2-Amino-3-(6-fluoro-1*H*-indol-3-yl)-propanamide (7d)



7d

The compound **6d** (207 mg, 0.6 mmol) was treated with 10 % Pd/C (62 mg, 35 % *w/w*) in MeOH (8 mL) and ammonium formate (190 mg, 3.0 mmol) to afford **7d** hydrochloric as a pale orange solid (102 mg, 65 %): m.p. 232–233 °C; TLC R_f = 0.2 ($CHCl_3$–MeOH, 4:1); IR (KBr): ν = 3472, 3271, 2983, 1658, 1451 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 3.13 (dd, J = 14 and 7 Hz, 1H, CH_AH_B), 3.20 (dd, J = 14 and 7 Hz, 1H, CH_AH_B), 4.17 (t, J = 7 Hz, 1H, α-H), 6.81 (ddd, J = 11, 2 and 1 Hz, 1H, Ar H), 7.04 (dd, J = 10 and 2 Hz, 1H, Ar H), 7.12 (s, 1H, C2H), 7.43 (dd, J = 9 and 4 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 26.8, 53.1, 97.8 (d, J = 26 Hz), 106.3, 107.9 (d, J = 25 Hz), 119.0 (d, J = 10 Hz), 123.2, 125.5 (d, J = 3 Hz), 136.1 (d, J = 12 Hz), 159.6 (d, J = 233 Hz), 171.8. MS: *m/z* (+ESI) calcd for $C_{11}H_{12}FN_3O^+$ 222.1043, found 222.0912 [MH^+].

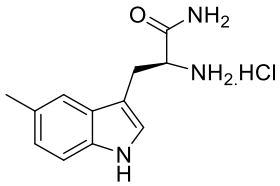
(*S*)-2-Amino-3-(7-ethyl-1*H*-indol-3-yl)propanamide (7e)



7e

The compound **6e** (275 mg, 0.8 mmol) was treated with 10 % Pd/C (90 mg, 35 % *w/w*) in MeOH (10 mL) and ammonium formate (246 mg, 3.9 mmol) to give **7e** hydrochloric salt as a pale purple solid (102 mg, 65 %): m.p. 160-161 °C; R_f = 0.2 ($CHCl_3$–MeOH, 4:1); IR (KBr): ν = 3411, 3154, 3045, 1687, 1413 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 1.13 (t, J = 8 Hz, 3H, CH_2CH_3), 2.69 (q, J = 8 Hz, 2H, CH_2CH_3), 3.16 (dd, J = 15 and 7 Hz, 1H, CH_AH_B), 3.22 (dd, J = 16 and 7 Hz, 1H, CH_AH_B), 4.18 (t, J = 7 Hz, 1H, α-H), 6.88 (d, J = 6 Hz, 1H, Ar H), 6.98 (t, J = 7 Hz, 1H, Ar H), 7.18 (s, 1H, C2H), 7.40 (d, J = 8 Hz, 1H, Ar.). ^{13}C NMR (100 MHz, D_2O): δ 13.5, 23.6, 26.9, 53.2, 106.7, 116.0, 119.9, 120.5, 125.0, 126.5, 128.2, 134.9, 171.8. MS: *m/z* (+ESI) calcd for $C_{13}H_{18}N_3O^+$ 232.1450, found 232.1390 [MH^+].

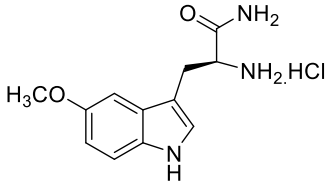
(*S*)-2-Amino-3-(5-methyl-1*H*-indol-3-yl)propanamide (7f)



7f

The compound **6f** (94 mg, 0.3 mmol) was treated with10 % Pd/C (32 mg, 35 % *w/w*) in MeOH (6 mL), and ammonium formate (88 mg, 1.4 mmol) to yield **7f** hydrochloric salt as a colourless solid (26 mg, 37 %): m.p. 82–84 °C; TLC R_f = 0.2 ($CHCl_3$–MeOH, 4:1); IR (KBr): v = 3402, 2916, 1668, 1431 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 2.33 (s, 3H, CH_3), 3.05 (dd, J = 15 and 7 Hz, 1H, CH_AH_B), 3.12 (dd, J = 16 and 7 Hz, 1H, CH_AH_B), 3.84 (t, J = 6 Hz, 1H, α-H), 7.00 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.12 (s, 1H, C-2H), 7.30 (d, J = 8 Hz, 1H, Ar H), 7.38 (s, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 20.4, 28.6, 54.1, 107.5, 111.7, 117.7, 123.5, 125.0, 127.0, 129.0, 134.5, 176.4. MS: *m/z* (+ESI) calcd for $C_{12}H_{16}N_3O^+$ 218.1293, found 218.1220 [MH^+].

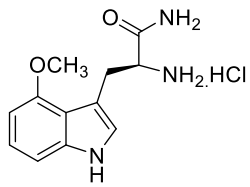
(*S*)-2-Amino-3-(5-methoxy-1*H*-indol-3-yl)propanamide (7g)



7g

The compound **6g** (80 mg, 0.2 mmol) was treated with 10 % Pd/C (30 mg, 35 % *w/w*) in MeOH (8 mL), and ammonium formate (71 mg, 1.1 mmol) to give **7g** hydrochloric salt as a pale purple solid (38 mg, 64 %): m.p. 247–249 °C; TLC R_f = 0.2 ($CHCl_3$–MeOH, 4:1). IR (KBr): v = 3354, 3167, 2954, 1687, 1462 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 3.17 (dd, J = 15 and 8 Hz, 1H, CH_AH_B), 3.23 (dd, J = 15 and 7 Hz, 1H, CH_AH_B), 3.75 (s, 3H, OCH_3), 4.18 (t, J = 7 Hz, 1H, α-H), 6.78 (dd, J = 8 and 2 Hz, 1H, C6H), 7.05 (d, J = 2 Hz, 1H, C4H), 7.17 (s, 1H, C2H), 7.29 (d, J = 8 Hz, 1H, C7H). ^{13}C NMR (100 MHz, D_2O): δ 26.8, 53.1, 56.0, 100.6, 106.0, 111.7, 112.8, 126.2, 126.9, 131.6, 152.9, 171.9. MS: *m/z* (+ESI) calcd for $C_{12}H_{16}N_3O_2^+$ 234.1243 found 234.1187 [MH^+].

(*S*)-2-Amino-3-(4-methoxy-1*H*-indol-3-yl)propanamide (7h)



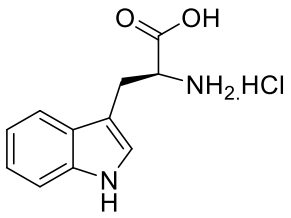
7h

Synthesis was carried out according to the procedure described under section 2.4 using **6h** (55 mg, 0.2 mmol), 10 % Pd/C (20 mg, 35 % *w/w*) in MeOH (8 mL), and ammonium formate (49 mg, 0.8 mmol) to give **7h** hydrochloric salt as a pale yellow solid. The crude **7h** was used directly for the next acid hydrolysis step.

(28 mg, 68 %): m.p. 231–233 °C; TLC R_f = 0.2 ($CHCl_3$–MeOH, 4:1). IR (KBr): v = 3354, 3168, 2955, 1687, 1463 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 3.22 (dd, J = 15 and 7 Hz, 1H, CH_AH_B), 3.35 (dd, J = 15 and 7 Hz, 1H, CH_AH_B), 3.84 (s, 3H, OCH_3), 4.24 (t, J = 7 Hz, 1H, α-H), 6.53 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.02 (dd, J = 8, 1 Hz, 1H, Ar H), 7.04 (s, 1H, C2H), 7.06 (d, J = 8 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 28.2, 54.4, 55.1, 99.7, 105.4, 106.3, 116.2, 123.1, 124.2, 138.0, 153.5, 171.9. MS: *m/z* (+ESI) calcd for $C_{12}H_{16}N_3O_2^+$ 234.1243 found 234.1096 [MH^+]

2.5 General procedure for the preparation of (*S*)-tryptophans **8**

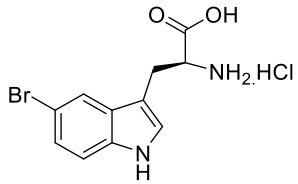
(*S*)-Tryptophan•HCl (8a)



8a

To a two-neck round bottom flask containing the α-aminoamide **7a** (86 mg, 0.4 mmol) was added a solution of 1 M aq HCl (6 mL). The reaction mixture was heated under reflux for 5 h and then cooled to room temperature. The residual was lyophilized to give **8a**[5] as a white solid (100 mg, 83 % yield): m.p. 246–247 °C; IR (KBr): ν = 3386, 2941, 1735 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 3.23 (dd, J = 16, 7 Hz, 1H, CH_AH$_B$), 3.37 (dd, J = 15 and 6 Hz, 1H, CH$_A$$H_B$), 4.23 (dd, J = 8 and 6 Hz, 1H, α-H), 7.07 (td, J = 8 and 1 Hz, 1H, Ar H), 7.17 (td, J = 8 and 1 Hz, 1H, Ar H), 7.20 (s, 1H, C-2H), 7.42 (d, J = 8 Hz, 1H, Ar H), 7.55 (d, J = 8 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 25.7, 53.2, 106.2, 112.0, 118.2, 119.5, 122.1, 125.3, 126.4, 136.2, 171.8. MS: *m/z* (+ESI) calcd for $C_{11}H_{13}N_2O_2^+$ 205.0977, found 205.0953 [MH$^+$].

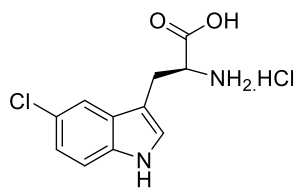
(*S*)-5-Bromotryptophan•HCl (8b)



8b

To a two-neck round bottom flask containing α-aminoamide **7b** (90 mg, 0.2 mmol) was added a solution of 1 M aq HCl (5 mL). The reaction mixture was heated under reflux for 18 h, cooled to room temperature and lyophilized to give **8b**[6] as a yellow solid (78 mg, 99 % yield): m.p. 220–221 °C; ^{1}H NMR (400 MHz, D_2O): δ 3.20 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.28 (dd, J = 15 and 5 Hz, 1H, CH$_A$$H_B$), 4.18 (dd, J = 7 and 5 Hz, 1H, α-H), 7.16 (dd, J = 8 and 2 Hz, 1H, Ar H), 7.17 (s, 1H, C-2H), 7.25 (d, J = 8 Hz, 1H, Ar H), 7.63 (d, J = 2 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 25.6, 53.3, 106.0, 112.0, 113.5, 120.5, 124.6, 126.5, 128.2, 134.2, 172.0. MS: *m/z* (+ESI) calcd for $C_{11}H_{12}BrN_2O_2^+$ 283.0082, found 283.0150 [MH$^+$].

(*S*)-5-Chlorotryptophan•HCl (8c)

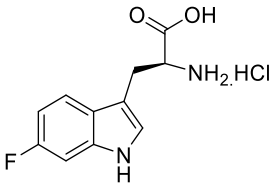


8c

To a two-neck round bottom flask containing α-aminoamide **7c** (62 mg, 0.3 mmol) was added a solution of 1 M aq HCl (5 mL). The reaction mixture was heated under reflux for 18 h, cooled to room temperature and lyophilized to give **8c**[6] as a yellow solid (59 mg, 95 % yield): m.p. 230–233 °C; IR (KBr): ν = 3456, 3138, 3037, 1730, 1407 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 3.16 (dd, J = 15 and 7

Hz, 1H, CH_AH$_B$), 3.23 (dd, J = 15 and 5 Hz, 1H, CH$_A$$H_B$), 4.16 (dd, J = 7 and 5 Hz, 1H, α-H), 7.00 (dd, J = 8 and 2 Hz, 1H, Ar H), 7.16 (s, 1H, C-2H), 7.25 (d, J = 8 Hz, 1H, Ar H), 7.41 (d, J = 2 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 25.6, 53.2, 106.0, 113.0, 117.3, 122.0, 124.4, 126.6, 127.5, 134.6, 171.9 ppm. MS: *m/z* (+ESI) calcd for $C_{11}H_{12}ClN_2O_2^+$ 239.0587, found 239.0587 [MH^+].

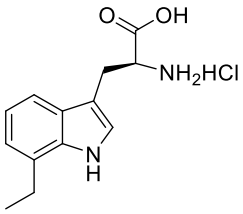
(*S*)-6-Fluorotryptophan•HCl (8d)



8d

To a two-neck round bottom flask containing α-aminoamide **7d** (102 mg, 0.4 mmol) was added 1 M aq HCl (6 mL). The reaction mixture was heated under reflux for 5 h, cooled to room temperature and lyophilized to give **8d**[6] as a white solid (92 mg, 90 % yield): m.p. 218–220 °C; IR (KBr): ν = 3458, 3015, 1732, 1412 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 3.26 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.33 (dd, J = 15 and 5 Hz, 1H, CH$_A$$H_B$), 4.22 (dd, J = 7 and 5 Hz, 1H, α-H), 6.84 (ddd, J = 10, 9 and 2 Hz, 1H, Ar H), 7.10 (dd, J = 10 and 2 Hz, 1H, Ar H), 7.15 (s, 1H, C-2H), 7.45 (dd, J = 9 and 5 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 25.7, 53.2, 97.8 (d, J = 26 Hz), 106.4, 108.0 (d, J = 25 Hz), 119.0 (d, J = 10 Hz), 123.1, 125.5 (d, J = 3 Hz), 136.2 (d, J = 13 Hz), 159.6 (d, J = 234 Hz), 171.9 ppm. MS: *m/z* (+ESI) calcd for $C_{11}H_{12}FN_2O_2^+$ 223.0883, found 223.0752 [MH^+].

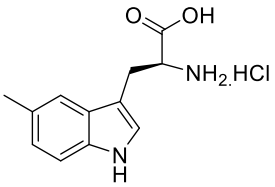
(*S*)-7-Ethyltryptophan•HCl (8e)



8e

To a two-neck round bottom flask containing α-aminoamide **7e** (151 mg, 0.6 mmol) was added 1 M aq HCl (8 mL). The reaction mixture was heated under reflux for 5 h, cooled to room temperature and lyophilized to give **8e** as a white solid (151 mg, 99 % yield): m.p. 216–218 °C; IR (KBr): ν = 3393, 3137, 3041, 1728, 1407 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 1.14 (t, J = 8 Hz, 3H, CH$_2$CH_3), 2.71 (q, J = 8 Hz, 2H, CH_2CH$_3$), 3.22 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.31 (dd, J = 15 and 5 Hz, 1H, CH$_A$$H_B$), 4.20 (dd, J = 7 and 5 Hz, 1H, α-H), 6.93 (t, J = 8 Hz, 1H, Ar H), 6.98 (t, J = 8 Hz, 1H, Ar H), 7.17 (s, 1H, C-2H), 7.36 (d, J = 8 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 13.4, 23.7, 25.8, 53.3, 106.7, 115.9, 119.9, 120.6, 125.0, 126.4, 128.3, 134.9, 171.9. MS: *m/z* (+ESI) calcd for $C_{13}H_{17}N_2O_2^+$ 233.1290, found 233.1174 [MH^+].

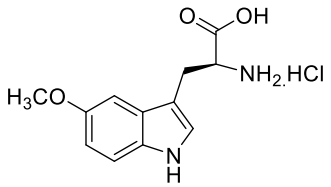
(*S*)-5-Methyltryptophan•HCl (8f)



8f

To a two-neck round bottom flask containing α-aminoamide **7f** (28 mg, 0.1 mmol) was added 1 M aq HCl (5 mL). The reaction mixture was heated under reflux for 16 h, cooled to room temperature and lyophilized to give **8f** as a white solid (27 mg, 96 % yield): m.p. 112–114 °C; IR (KBr): ν = 3406, 2918, 1736, 1485 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 2.32 (s, 3H, CH_3), 3.25 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.35 (dd, J = 15 and 5 Hz, 1H, CH$_A$$H_B$), 4.21 (dd, J = 7 and 5 Hz, 1H, α-H), 7.00 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.16 (s, 1H, Ar H), 7.30 (d, J = 8 Hz, 1H, Ar H), 7.36 (s, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 20.4, 25.8, 53.3, 105.7, 111.8, 117.6, 123.7, 125.5, 126.7, 129.2, 134.6, 172.0 ppm. MS: *m/z* (+ESI) calcd for $C_{12}H_{15}N_2O_2^+$ 219.1134, found 219.1036 [MH$^+$].

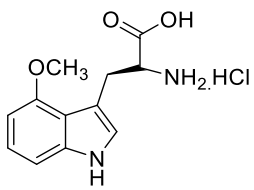
(*S*)-5-Methoxytryptophan•HCl (8g)



8g

A mixture of α-aminoamide **7g** (38 mg, 0.1 mmol) and 1 M aq HCl (4 mL) was heated under reflux for 5 h, cooled to room temperature and lyophilized to give **8g**[6] as a white solid (38 mg, 99 % yield): m.p. 224–225 °C; IR (KBr): ν = 3358, 2909, 1728, 1478, 1214 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 3.22 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.30 (dd, J = 15 and 5 Hz, 1H, CH$_A$$H_B$), 3.74 (s, 3H, OCH_3), 4.21 (dd, J = 7 and 5 Hz, 1H, α-H), 6.78 (dd, J = 8 and 2 Hz, 1H, Ar H), 7.02 (d, J = 2 Hz, 1H, Ar H), 7.15 (s, 1H, C-2H), 7.28 (d, J = 8 Hz, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 25.7, 53.2, 56.0, 100.4, 106.0, 111.8, 112.8, 126.1, 126.8, 131.7, 152.9, 172.0. MS: *m/z* (+ESI) calcd for $C_{12}H_{15}N_2O_3^+$ 235.1083, found 235.1068 [MH$^+$]

(*S*)-4-Methoxytryptophan•HCl (8h)

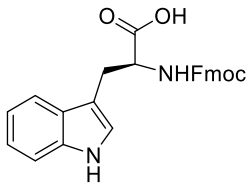


8h

A mixture of α-aminoamide **7h** (28 mg, 0.1 mmol) and 1 M aq HCl (4 mL) was heated under reflux for 5 h, cooled to room temperature and lyophilized to give **8h** as a white solid (36 mg, 99 % yield): m.p. 220–222 °C; IR (KBr): ν = 3358, 2906, 1728, 1480, 1215 cm^{-1}. ^{1}H NMR (400 MHz, D_2O): δ 3.24 (dd, J = 15 and 7 Hz, 1H, CH_AH$_B$), 3.37 (dd, J = 15 and 7 Hz, 1H, CH$_A$$H_B$), 3.86 (s, 3H, OCH_3), 4.28 (t, J = 7 Hz, 1H, α-H), 6.55 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.04 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.06 (s, 1H, C2H), 7.08 (d, J = 8 Hz, 1H, Ar H). ^{13}C NMR (100 MHz, D_2O): δ 28.2, 54.4, 55.1, 99.7, 105.4, 106.3, 116.2, 123.1, 124.2, 138.0, 153.6, 171.8 ppm. MS: *m/z* (+ESI) calcd for $C_{12}H_{15}N_2O_3^+$ 235.1083, found 235.1068 [MH$^+$].

2.6 General procedure for the preparation of (*S*)-*N*-(fluoren-9-ylmethoxycarbonyl)-tryptophans **9**

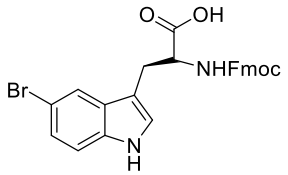
(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-tryptophan (9a)



9a

Tryptophan hydrochloric salt **8a** (100 mg, 0.42 mmol) was added to a 10 mL aqueous solution of sodium carbonate (89 mg, 0.84 mmol) followed by 9-fluorenylmethyl succinimidyl carbonate (141 mg, 0.42 mmol) in THF (3 mL). The mixture was stirred for 2 h at room temperature. THF was removed under *vacuo* and the crude mixture was poured into water (15 mL) and extracted with Et_2O (2 x 15 mL). The pH of the aqueous layer was adjusted to 2 using 3 M aq HCl and was extracted with CH_2Cl_2 (2 x 20 mL). The organic extracts were combined and washed with brine, dried over $MgSO_4$ and concentrated *in vacuo* to give **9a**[7] as a pale yellow solid (114 mg, 44 % yield): m.p. 182–185 °C (Lit.[6] m.p. 170–172 °C); $[\alpha]^{24}_D = -29.0$ (*c* = 1, MeOH) (Lit.[8] $[\alpha]^{25}_D = -29.5$ (*c* = 1, DMF)) ; IR (KBr): *v* = 3416, 3057, 2950, 1710, 743 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 3.31 (dd, *J* = 15 and 8 Hz, 1H, CH_AH$_B$), 3.46 (dd, *J* = 15 and 5 Hz, 1H, CH_AH$_B$), 4.20 (t, *J* = 8 Hz, 1H, Fmoc CH), 4.31 (m, 2H, Fmoc CH_2), 4.69 (m, 1H, α-H), 6.68 (d, *J* = 8 Hz, 1H, NH), 7.07 (t, *J* = 7 Hz, 1H, Ar H), 7.14 (t, *J* = 7 Hz, 1H, Ar H), 7.28 (s, 1H, C-2H), 7.30 (m, 2H, Ar Hs), 7.41 (m, 3H, Ar H), 7.69 (m, 3H, Ar Hs), 7.84 (d, *J* = 7 Hz, 2H, Ar Hs), 10.10 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 27.5, 47.1, 54.8, 66.4, 110.3, 111.4, 118.4, 118.9, 119.9, 121.4, 123.6, 125.3, 125.3, 127.1, 127.6, 127.8, 136.8, 141.2, 144.1, 144.2, 156.1, 173.0 ppm. MS: m/z (+ESI) calcd for $C_{26}H_{23}N_2O_4^+$ 427.1658, found 427.1743 [MH^+].

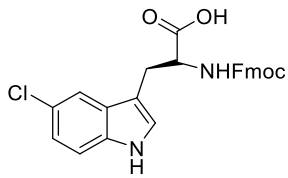
(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-5-bromo-tryptophan (9b)



9b

Compound **8b** (66 mg, 0.21 mmol), Na_2CO_3 (65 mg, 0.62 mmol) and 9-fluorenylmethyl succinimidyl carbonate (71 mg, 0.21 mmol) were reacted to give **9b** as a white solid (23 mg, 20 % yield): m.p. 130–132 °C; $[\alpha]^{24}_D$ = –23.0 (*c* = 1, MeOH); IR (KBr): *v* = 3411, 3060, 2950, 1709, 737 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 3.25 (dd, *J* = 16 and 8 Hz, 1H, CH_AH$_B$), 3.41 (dd, *J* = 16 and 5 Hz, 1H, CH_AH$_B$), 4.20 (t, *J* = 8 Hz, 1H, Fmoc CH), 4.30 (m, 2H, Fmoc CH_2), 4.60 (m, 1H, α-H), 6.70 (d, *J* = 8 Hz, 1H, NH), 7.23 (dd, *J* = 8 and 2 Hz, 1H, Ar H), 7.30 (m, 3H, Ar Hs), 7.40 (m, 3H, Ar Hs), 7.66 (dd, *J* = 8 and 2 Hz, 2H, Ar Hs), 7.86 (m, 3H, Ar Hs), 10.34 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 27.1, 47.1, 54.7, 66.3, 110.3, 111.8, 113.2, 119.9, 120.9, 123.9, 125.1, 125.3, 125.3, 127.1, 127.6, 129.7, 135.2, 141.2, 141.2, 144.1, 144.2, 155.9, 172.6. MS: m/z (+ESI) calcd for $C_{26}H_{22}BrN_2O_4^+$ 505.0763, found 505.0978 [MH^+].

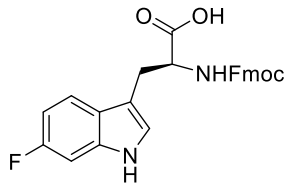
(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-5-chloro-tryptophan (9c)



9c

Compound **8c** (59 mg, 0.25 mmol), Na_2CO_3 (79 mg, 0.75 mmol) and 9-fluorenylmethyl succinimidyl carbonate (84 mg, 0.25 mmol) were reacted to give **9c** as a white solid (23 mg, 20 % yield): m.p. 98–99°C; $[\alpha]^{24}_D$ = –16.0 (*c* = 1, MeOH); IR (KBr): *v* = 3414, 3058, 2953, 1710, 748 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 3.26 (dd, *J* = 15 and 8 Hz, 1H, CH_AH$_B$), 3.41 (m, 1H, CH_AH$_B$), 4.20 (t, *J* = 8 Hz, 1H, Fmoc CH), 4.30 (m, 2H, Fmoc CH_2), 4.61 (m, 1H, α-H), 6.71 (d, *J* = 8 Hz, 1H, NH), 7.11 (dd, *J* = 8 and 2 Hz, 1H, Ar H), 7.27–7.42 (m, 6H, Ar Hs), 7.66 (dd, *J* = 8 and 4 Hz, 2H, Ar Hs), 7.70 (m, 1H, Ar H), 7.85 (d, *J* = 8 Hz, 2H, Ar Hs), 10.31 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 27.2, 47.1, 54.7, 66.3, 100.3, 112.7, 117.8, 119.9, 119.9, 121.4, 124.2, 125.2, 125.3, 127.1, 127.6, 129.0, 134.9, 141.2, 141.2, 144.1, 144.1, 156.0, 172.7 MS: m/z (+ESI) calcd for $C_{26}H_{22}ClN_2O_4^+$ 461.1268, found 461.1317 [MH^+].

(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-6-fluoro-tryptophan (9d)

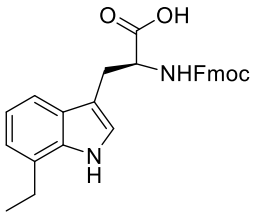


9d

Compound **8d** (90 mg, 0.35 mmol), sodium carbonate (74 mg, 0.70 mmol) and 9-fluorenylmethyl succinimidyl carbonate (117 mg, 0.35 mmol) were reacted to afford **9d** as a white solid (77 mg, 50 % yield): m.p. 102–104 °C; $[\alpha]^{24}_D$ = –19.0 (*c* = 1, MeOH); IR (KBr): *v* = 3419, 3064, 2952, 1710, 745 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): δ 3.26 (dd, *J* = 14 and 8 Hz, 1H, CH_AH$_B$), 3.41 (dd, *J* = 14 and 5 Hz, 1H, CH$_A$$H_B$), 4.21 (t, *J* = 8 Hz, 1H, Fmoc CH), 4.31 (m, 2H, Fmoc CH_2), 4.64 (m, 1H, α-H),

6.68 (d, J = 8 Hz, NH), 6.87 (dt, J = 10 and 2 Hz, 1H, Ar H), 7.15 (dd, J = 10 and 2 Hz, 1H, Ar H), 7.27 (s, 1H, C-2H), 7.30 (m, 2H, Ar Hs), 7.41 (t, J = 7 Hz, 2H, Ar Hs), 7.66 (m, 3H, Ar Hs), 7.85 (d, J = 8 Hz, 2H, Ar Hs), 10.19 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 27.4, 47.1, 54.7, 66.3, 97.3 (d, J = 26 Hz), 107.2 (d, J = 25 Hz), 110.6, 119.9, 119.4 (d, J = 10 Hz), 124.2 (d, J = 3 Hz), 124.6, 125.3, 125.3, 127.0, 127.6, 136.6 (d, J = 13 Hz), 141.2, 144.1, 144.2, 156.0, 159.7 (d, J = 233 Hz), 172.8. MS: m/z (+ESI) calcd for $C_{26}H_{22}FN_2O_4^+$ 445.1564, found 445.1790 [MH^+].

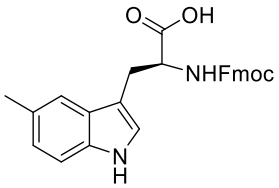
(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-7-ethyl-tryptophan (9e)



9e

Compound **8e** (102 mg, 0.38 mmol), Na_2CO_3 (80 mg, 0.76 mmol) and 9-fluorenylmethyl succinimidyl carbonate (128 mg, 0.38 mmol) were used to yield **9e** as a white solid (83 mg, 50 % yield): m.p. 146–148 °C; $[\alpha]^{24}_D$ = –26.0 (c = 1, MeOH); IR (KBr): ν = 3422, 3057, 2964, 1711, 746 cm^{-1}. ^{1}H NMR (400 MHz, acetone-d_6): δ 1.31 (t, J = 8 Hz, 3H, CH_2CH_3), 2.91 (q, J = 8 Hz, 2H, CH_2CH_3), 3.27 (dd, J = 15 and 8 Hz, 1H, CH_AH_B), 3.43 (dd, J = 15 and 5 Hz, 1H, CH_AH_B), 4.20 (t, J = 7 Hz, 1H, Fmoc CH), 4.28 (m, 2H, Fmoc CH_2), 4.67 (m, 1H, α-H), 6.65 (d, J = 8 Hz, 1H, NH), 7.01 (m, 2H, Ar Hs), 7.27 (s, 1H, C-2H), 7.30 (m, 2H, Ar Hs), 7.40 (t, J = 7 Hz, 2H, Ar Hs), 7.54 (d, J = 7 Hz, 1H, Ar H), 7.66 (m, 2H, Ar Hs), 7.85 (d, J = 7 Hz, 2H, Ar Hs), 10.08 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 13.7, 23.8, 27.6, 47.1, 54.8, 66.3, 110.7, 116.2, 119.3, 119.9, 120.1, 123.2, 125.3, 125.3, 126.9, 127.1, 127.6, 135.4, 141.2, 144.1, 156.0, 173.0. MS: m/z (+ESI) calcd for $C_{28}H_{27}N_2O_4^+$ 455.1971, found 455.2142 [MH^+].

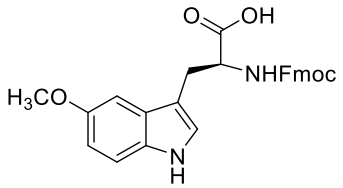
(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-5-methyl-tryptophan (9f)



9f

Compound **8f** (48 mg, 0.19 mmol), Na_2CO_3 (40 mg, 0.38 mmol) and 9-fluorenylmethyl succinimidyl carbonate (64 mg, 0.19 mmol) were used to yield **9f** as a colourless film (55 mg, 66 % yield): m.p. 178 °C; $[\alpha]^{24}_D$ = –15 (c = 1, MeOH); IR (KBr): ν = 3424, 3038, 2920, 1708, 1448 cm^{-1}. ^{1}H NMR (400 MHz, acetone-d_6): δ 2.42 (s, 3H, CH_3), 3.25 (dd, J = 15 and 8 Hz, 1H, CH_AH_B), 3.42 (dd, J = 15 and 5 Hz, 1H, CH_AH_B), 4.20 (t, J = 7 Hz, 1H, Fmoc CH), 4.29 (m, 2H, Fmoc CH_2), 4.65 (m, 1H, α-H), 6.65 (d, J = 8 Hz, 1H, NH), 6.97 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.23 (d, J = 2 Hz, 1H, Ar H), 7.28 (s, 1H, C-2H), 7.30 (m, 2H, Ar Hs), 7.40 (t, J = 7 Hz, 2H, Ar Hs), 7.48 (s, 1H, Ar H), 7.66 (d, J = 7 Hz, 2H, Ar Hs), 7.85 (d, J = 7 Hz, 2H, Ar Hs), 9.97 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): δ 20.9, 27.5, 47.1, 54.8, 66.3, 109.8, 111.1, 118.0, 119.9, 123.0, 123.7, 125.3, 127.1, 127.6, 127.6, 128.0, 135.1, 141.2, 144.1, 156.0, 173.0. MS: m/z (+ESI) calcd for $C_{27}H_{25}N_2O_4^+$ 441.1814, found 441.2083 [MH^+].

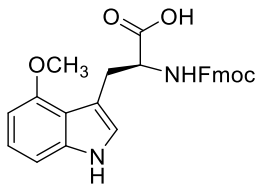
(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-5-methoxy-tryptophan (9g)



9g

Compound **8g** (300 mg, 1.28 mmol), Na_2CO_3 (271 mg, 2.56 mmol) and 9-fluorenylmethyl succinimidyl carbonate (431 mg, 1.28 mmol) were used to yield **9g**[5] as a white powder (479 mg, 82 % yield): m.p. 198 °C; $[\alpha]^{24}_D$ = –23 (*c* = 1, MeOH) (Lit[6] $[\alpha]^{24}_D$ = –24.5 (*c* = 1, MeOH)); IR (KBr): *v* = 3415, 2949, 1703, 1493, 1218 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): *δ* 3.25 (dd, *J* = 15 and 8 Hz, 1H, C*H*$_A$H$_B$), 3.39 (dd, *J* = 15 and 5 Hz, 1H, C*H*$_A$H$_B$), 3.82 (s, 3H, OCH_3), 4.20 (t, *J* = 7 Hz, 1H, Fmoc CH), 4.29 (m, 2H, Fmoc CH_2), 4.61 (m, 1H, α-H), 6.63 (d, *J* = 8 Hz, 1H, NH), 6.79 (dd, *J* = 8 and 1 Hz, 1H, Ar H), 7.21 (m, 1H, Ar H), 7.30 (m, 4H, Ar Hs), 7.40 (t, *J* = 7 Hz, 2H, Ar Hs), 7.66 (d, *J* = 7 Hz, 2H, Ar H), 7.85 (d, *J* = 7 Hz, 2H, Ar H), 9.96 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): *δ* 27.5, 47.1, 54.7, 55.0, 66.3, 100.2, 110.0, 111.6, 111.9, 119.9, 124.1, 125.3, 125.3, 127.1, 127.6, 128.1, 131.7, 141.2, 144.1, 144.2, 154.0, 155.9, 172.8. MS: m/z (+ESI) calcd for $C_{27}H_{25}N_2O_5^+$ 457.1763, found 457.1913 [MH^+].

(*S*)-*N*-(Fluoren-9-ylmethoxycarbonyl)-4-methoxy-tryptophan (9h)



9h

Compound **8h** (38 mg, 0.14 mmol), Na_2CO_3 (29 mg, 0.28 mmol) and 9-fluorenylmethyl succinimidyl carbonate (47 mg, 0.14 mmol) were used to afford **9h** as a white powder (39 mg, 62 % yield): m.p. 168 °C; $[\alpha]^{24}_D$ = –27 (*c* = 1, MeOH); IR (KBr): *v* = 3415, 2949, 1703, 1494, 1218 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): *δ* 3.20 (dd, *J* = 15 and 9 Hz, 1H, C*H*$_A$H$_B$), 3.34 (dd, *J* = 15 and 5 Hz, 1H, C*H*$_A$H$_B$), 3.76 (s, 3H, OCH_3), 4.15 (t, *J* = 7 Hz, 1H, Fmoc CH), 4.24 (m, 2H, Fmoc CH_2), 4.57 (m, 1H, α-H), 6.57 (d, *J* = 9 Hz, 1H, NH), 6.73 (dd, *J* = 9 and 1 Hz, 1H, Ar H), 7.16 (m, 2H, Ar Hs), 7.24 (m, 3H, Ar Hs), 7.35 (t, *J* = 8 Hz, 2H, Ar Hs), 7.61 (d, *J* = 7 Hz, 2H, Ar Hs), 7.80 (d, *J* = 8 Hz, 2H, Ar Hs), 9.90 (br, 1H, NH). ^{13}C NMR (100 MHz, acetone-d_6): *δ* 28.4, 48.0, 55.7, 55.9, 67.2, 101.1, 111.0, 112.5, 112.8, 112.84, 120.8, 125.0, 125.1, 126.1, 127.9, 127.93, 128.5, 129.1, 132.7, 142.0, 145.0, 145.05, 154.9, 156.9. MS: m/z (+ESI) calcd for $C_{27}H_{25}N_2O_5^+$ 457.1763, found 457.1781 [MH^+].

2.7 Standard protocol for the preparation of linear argyrin analogues **18** (by Fmoc/tBu solid-phase peptide synthesis)

2-Chlorotrityl chloride polystyrene resin (1.0 g, 1.2 mmol, theoretical loading 1.2 mmol g^{-1}) was swollen in CH_2Cl_2 (6 mL) for 1 h. A solution of Fmoc-sarcosine (373 mg, 1.2 mmol) and DIPEA (418 μL, 2.4 mmol) in CH_2Cl_2 (2 mL) was added to the resin suspension. The reaction mixture was gently stirred at room temperature for 2 h. MeOH (500 μL) was added and the suspension was stirred for a further 15 min. The derivatized resin was collected in a Buchner funnel, washed with DMF (10 mL), CH_2Cl_2 (15 mL) and hexane (5 mL), and dried *in vacuo* to give the resin-bound Fmoc-sarcosine (1.363 g, Fmoc substitution 0.84 mmol g^{-1}, 70 %).

The resin-bound Fmoc-sarcosine (1 eq.) was placed in a reaction column, swollen with DMF–CH_2Cl_2 (1 mL) for 12 h, and Fmoc-deprotection was carried out on a continuous flow of 20 % *v/v* piperidine in DMF (2.8 mL min^{-1}, 10 min) using NOVASYN® GEM manual peptide synthesiser. The reaction was monitored post-column at 344 nm. The resin was then washed with DMF (2.8 mL min^{-1}, 5 min), and the peptide sequence D-Ala-thiazole-Trp-Trp(R)-Gly-D-Ala-Ph(Se)-Sar (**17**) was assembled manually using NOVASYN® GEM manual peptide synthesiser.

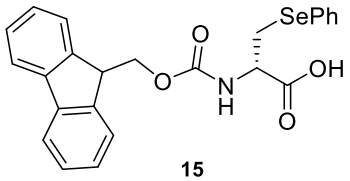
Sequential acylation reactions were carried out at ambient temperature for 4 h using appropriate *N*-Fmoc-protected amino acids (4 eq.) [i.e. Fmoc-Ph(Se)-OH (**15**), Fmoc-D-Ala-OH, Fmoc-Gly-OH, Fmoc-Trp(R)-OH (**9a-h**), Fmoc-Trp-OH, Boc-D-Ala-thiazole-OH (**16**)] and carboxyl-activating reagent, PyOxim (4 eq.) or HATU (3.9 eq.) and DIPEA (8 eq.) in DMF (1.0–1.5 mL). Sequential Fmoc-deprotection was achieved using 20 % *v/v* piperidine in DMF (2.8 mL min^{-1}, 10 min).

After final acylation reaction, the peptidyl-resin was filtered, washed successively with DMF, CH_2Cl_2 and hexane, and dried *in vacuo*.

The resin product was suspended in a mixture of water (0.25 mL), iPr_3SiH (0.25 mL) and CH_2Cl_2 (5 mL), followed by the addition of TFA (5 mL). The reaction mixture was allowed to stand at ambient temperature for 1 h. The suspension was filtered, washed with CH_2Cl_2 (3 mL), and the filtrate was evaporated to dryness *in vacuo*.

The residual material was triturated with diethyl ether (2 mL) to afford the linear peptides **18a-h** as buff solids, which were dissolved in water (2–5 mL) and lyophilized overnight.

(*S*)-*N*-(9-Fluorenylmethoxycarbonyl)-l-β-phenylselenocysteine (15)

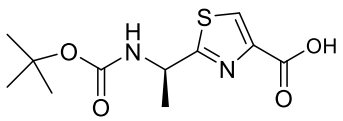


15

(*S*)-Phenylselenocysteine was obtained from *N*-Boc-L-serine using established protocols.[9]

9-Fluorenylmethyl succinimidyl carbonate (2.77 g, 8.2 mmol) was dissolved in THF (20 mL) and added to a stirred solution of (*S*)-phenyselenocysteine (2.87 g, 8 mmol) and $NaHCO_3$ (2.3 g, 28 mmol) in water (25 mL) over 10 min. The reaction mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*. The residue was dissolved in H_2O (40 mL), acidified with saturated aqueous $KHSO_4$ (30 mL), extracted with ethyl acetate (3 × 40 mL), washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The oily residue was purified by column chromatography ($CHCl_3$–MeOH, 19:1) to afford Fmoc-phenylselenocysteine **15** as a white solid (3.49 g, 93 % yield); m.p. 98–99°C; TLC R_f = 0.5 ($CHCl_3$–MeOH, 9:1 + 1 % AcOH). IR (KBr): ν = 3358, 3063, 1727, 1711, 1682, 1522, 1449, 1248 and 758 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 3.37 (dd, J = 4 and 16 Hz, 1H, CH_AH$_B$), 3.45 (dd, J = 4 and 16 Hz, 1H, CH_AH$_B$), 4.20 (t, J = 8 Hz, 1H, OCH$_2$CH), 4.36 (m, 2H, OCH_2CH), 4.77 (m, 1H, α-H), 5.58 (d, J = 8 Hz, 1H, NH), 7.26 (m, 4H, Ar Hs), 7.34 (m, 2H, Ar Hs), 7.43 (m, 2H, Ar Hs), 7.59 (m, 4H, Ar Hs), 7.79 (d, J = 8 Hz, 2H, Ar Hs). ^{13}C NMR (100MHz, $CDCl_3$): δ 29.7, 47.1, 53.9, 67.4, 120.0, 125.1, 127.1, 127.8, 127.9, 128.6, 129.3, 133.8, 141.3, 143.7, 143.7, 155.8, 174.9. MS: *m/z* (+ESI) calcd for $C_{24}H_{22}NO_4Se^+$ 468.0636 found 468.0135 [MH^+].

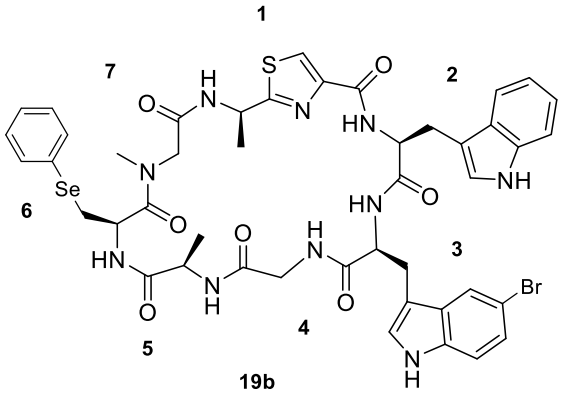
(*R*)-2-(1-tert-Butoxycarbonylaminoethyl)thiazole-4-carboxylic acid (16)



16

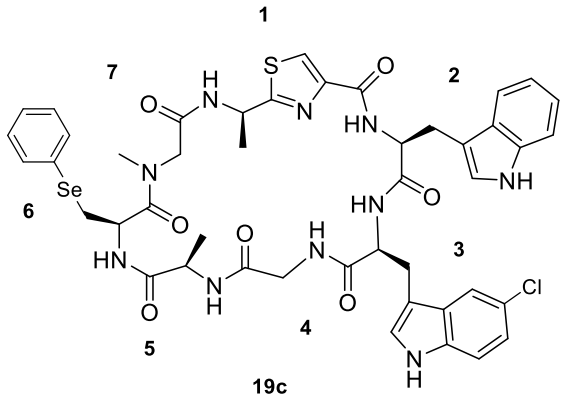
Boc-D-Ala-thiazole-OEt (1.24 g, 4.1 mmol), obtained from *N*-Boc-D-alanine using established protocols,[10] was dissolved in solution of THF–MeOH–H_2O, 9:6:6 v/v. Lithium hydroxide (118 mg, 4.9 mmol) in water (2 mL) was added at 0 °C. The mixture was warmed to room temperature and stirred overnight. The solvent was removed *in vacuo*. The residue was dissolved in H_2O (40 mL) and extracted with EtOAc (2 x 20 mL). The aqueous layer was acidified with saturated aqueous $KHSO_4$ to pH 2 and extracted with CH_2Cl_2 (3 x 40 mL). The organic layers were washed with brine, dried and concentrated. The residue was recrystallized with CH_2Cl_2–Hexane to give the carboxylic acid **16** as a pale yellow solid (986 mg, 88 % yield), m.p. 58–61 °C; $[\alpha]^{26}_D$ = +31 (c =1.0, $CHCl_3$). IR (Solid): ν = 3362, 3105, 2979, 1692, 1517, 1366, 1242, 1058 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.47 (s, 9H, C(CH_3)$_3$), 1.66 (d, J = 6 Hz, 3H, CHCH_3), 5.10 (br, s, 1H, α-CH), 5.30 (br, s, 1H, α-NH), 8.23 (s, 1H, C5H). ^{13}C NMR (100 MHz, $CDCl_3$): 21.7, 28.4, 60.5, 80.2, 128.7, 146.6, 155.0, 164.1, 171.4. MS: *m/z* (+ESI) calcd for $C_{11}H_{17}N_2O_4SNa^+$ 295.0562, found 295.0352 [MNa^+].

Cyclo[D-Ala-thiazole-(5-Br-Trp)-Trp-Gly-D-Ala-Ph(Se)-Sar] (19b)



Diisopropylethylamine (92 µL, 0.53 mmol), PyBOP (88 mg, 0.17 mmol) and HOBt (22 mg, 0.1 mmol) were added successively to a solution of the linear peptide **18b** (62 mg, 0.06 mmol) in CH_2Cl_2 (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) to afford the cyclic peptide **19b** as a white powder (10 mg, 17 % yield): RP-HPLC 10–60 % B in 12 min, t_R 9.9 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.65 (d, J = 7 Hz, 3H, 5-CH_3), 1.56 (d, J = 7 Hz, 3H, 1-CH_3 (i.e. dipeptide residue 1 CH_3)), 3.13 (dd, J = 14 and 8 Hz, 1H, 3-CH_AH$_B$), 3.22 (s, 3H. 7-CH_3), 3.24 (m, 1H, 3-CH$_A$$H_B$), 3.31 (m, 1H, 4-C$H_AH_B$), 3.34 (m, 1H, 6-C$H_AH_B$), 3.45 (m, 1H, 6-CH$_A$$H_B$), 3.49 (m, 1H, 7-C$H_AH_B$), 3.50 (m, 1H, 2-C$H_AH_B$), 3.67 (m, 1H, 2- CH$_A$$H_B$), 3.74 (m, 1H, 4-CH$_A$$H_B$), 4.13 (q, J = 7 Hz, 1H, 5-α-CH), 4.18 (t, J = 8 Hz, 1H, 3-α-CH), 4.35 (d, J = 17 Hz, 1H, 7-CH$_A$$H_B$), 4.60 (td, J = 7 and 4 Hz, 1H, 6-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 5.32 (m, 1H, 1-α-CH), 7.01-7.32 (m, 12H, Ar Hs), 7.43 (d, J = 3 Hz, 6-NH), 7.56 (m, 2H, Ar Hs), 7.58 (d, J = 7 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-C^{5}H), 8.06 (d, J = 9 Hz, 1H, 5-NH), 8.30 (s, 1H, 3-NH), 8.64 (d, J = 9 Hz, 1H, 2-NH), 8.88 (t, J = 5 Hz, 1H, 4-NH), 10.81 (d, J = 2 Hz, indole-NH), 11.16 (d, J = 2 Hz, indole-NH) ppm. ES-MS *m/z* calcd for $C_{45}H_{48}N_{10}O_7SSe^+$ 1031.1777, found 1033.1757 [MH$^+$].

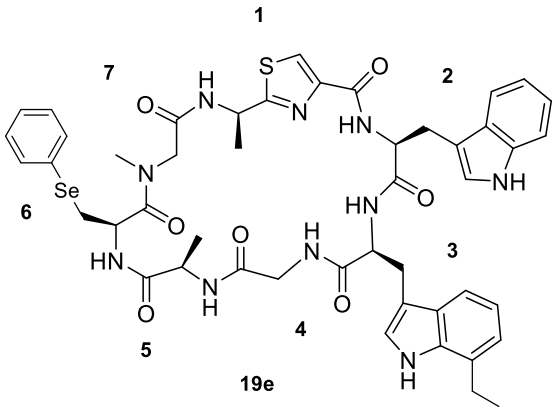
Cyclo[D-Ala-thiazole-Trp-(5-Cl-Trp)-Gly-D-Ala-Ph(Se)-Sar] (19c)



Diisopropylethylamine (87 µL, 0.50 mmol), PyBOP (86 mg, 0.17 mmol) and HOBt (22 mg, 0.17 mmol) were added successively to a solution of the linear peptide **18c** (56 mg, 0.05 mmol) in CH_2Cl_2 (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) to afford the cyclic peptide **19c** as a white powder (11 mg, 20 % yield): RP-HPLC 10–60 % B in 12 min, t_R 9.5 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.65 (d, J = 7 Hz, 3H, 5-CH_3), 1.56 (d, J = 7 Hz, 3H, 1-CH_3), 3.12 (dd, J = 14 and 8 Hz, 1H, 3-CH_AH$_B$), 3.22 (s, 3H. 7-CH_3), 3.24 (m, 1H, 3-CH$_A$$H_B$), 3.31 (m, 1H, 4-C$H_AH_B$), 3.34 (m, 1H, 6-C$H_AH_B$), 3.45 (m, 1H, 6-CH$_A$$H_B$), 3.49 (m, 1H, 7-C$H_AH_B$), 3.50 (m, 1H, 2- C$H_AH_B$), 3.67 (m, 1H, 2-CH$_A$$H_B$), 3.74 (m, 1H, 4-CH$_A$$H_B$), 4.13 (q, J = 7 Hz, 1H, 5-α-CH), 4.18 (t, J = 8 Hz, 1H, 3-α-CH),

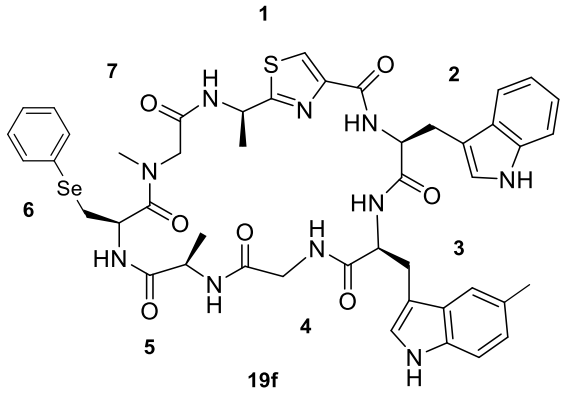
4.35 (d, J = 17 Hz, 1H, 7-CH_AH_B), 4.60 (td, J = 7 and 4 Hz, 1H, 6-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 5.32 (m, 1H, 1-α-CH), 6.97–7.32 (m, 12H, Ar Hs), 7.43 (d, J = 3 Hz, 6-NH), 7.56 (m, 2H, Ar Hs), 7.58 (d, J = 7 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-C^5H), 8.07 (d, J = 9 Hz, 1H, 5-NH), 8.33 (s, 1H, 3-NH), 8.64 (d, J = 9 Hz, 1H, 2-NH), 8.88 (t, J = 5 Hz, 1H, 4-NH), 10.81 (d, J = 2 Hz, indole-NH), 11.17 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{45}H_{48}N_{10}O_7SSe^+$ 987.2282, found 987.2104 [MH^+].

Cyclo[D-Ala-thiazole-Trp-(7-Et-Trp)-Gly-D-Ala-Ph(Se)-Sar] (19e)



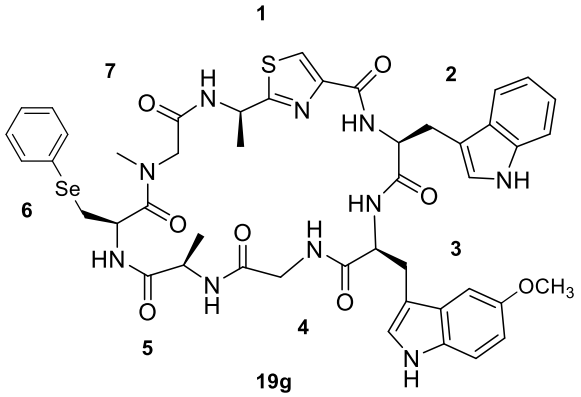
Diisopropylethylamine (80 μL, 0.46 mmol), PyBOP (81 mg, 0.17 mmol) and HOBt (21 mg, 0.16 mmol) were added successively to a solution of the linear peptide **18e** (52 mg, 0.05 mmol) in CH_2Cl_2 (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) to afford the cyclic peptide **19e** as a white powder (14 mg, 28 % yield): RP-HPLC 10–60 % B in 12 min, t_R 9.9 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.65 (d, J = 7 Hz, 3H, 5-CH_3), 1.27 (t, J = 7 Hz, 3H, 3-CH_2CH_3), 1.56 (d, J = 7 Hz, 3H, 1-CH_3), 2.86 (q, J = 7 Hz, 2H, 3-CH_2CH_3), 3.12 (dd, J = 14 and 8 Hz, 1H, 3-CH_AH_B), 3.22 (s, 3H, 7-CH_3), 3.26 (m, 1H, 3-CH_AH_B), 3.31 (m, 1H, 4-CH_AH_B), 3.34 (m, 1H, 6-CH_AH_B), 3.47 (m, 1H, 6-CH_AH_B), 3.49 (m, 1H, 7-CH_AH_B), 3.50 (m, 1H, 2- CH_AH_B), 3.67 (m, 1H, 2- CH_AH_B), 3.74 (m, 1H, 4-CH_AH_B), 4.14 (q, J = 7 Hz, 1H, 5-α-CH), 4.22 (t, J = 8 Hz, 1H, 3-α-CH), 4.35 (d, J = 17 Hz, 1H, 7-CH_AH_B), 4.60 (td, J = 7 and 4 Hz, 1H, 6-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 5.32 (m, 1H, 1-α-CH), 6.97–7.32 (m, 12H, Ar Hs), 7.44 (d, J = 3 Hz, 6-NH), 7.56 (m, 2H, Ar Hs), 7.58 (d, J = 7 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-C^5H), 8.11 (d, J = 9 Hz, 1H, 5-NH), 8.35 (s, 1H, 3-NH), 8.65 (d, J = 9 Hz, 1H, 2-NH), 8.92 (t, J = 5 Hz, 1H, 4-NH), 10.83 (d, J = 2 Hz, indole-NH), 10.95 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{47}H_{53}N_{10}O_7SSe^+$ 981.2985, found 981.2607 [MH^+].

Cyclo[D-Ala-thiazole-Trp-(5-Me-Trp)-Gly-D-Ala-Ph(Se)-Sar] (19f)



Diisopropylethylamine (89 µL, 0.51 mmol), PyBOP (90 mg, 0.17 mmol) and HOBt (22 mg, 0.17 mmol) were added successively to a solution of the linear peptide **18f** (57 mg, 0.06 mmol) and in CH_2Cl_2 (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) to afford the cyclic peptide **19f** as a white powder (15 mg, 27 % yield): RP-HPLC 10–60 % B in 12 min, t_R 9.4 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.65 (d, J = 7 Hz, 3H, 5-CH_3), 1.56 (d, J = 7 Hz, 3H, 1-CH_3), 2.41 (s, 3H, 3-CH_3), 3.11 (dd, J = 14 and 8 Hz, 1H, 3-CH_AH$_B$), 3.22 (s, 3H, 7-CH_3), 3.27 (m, 1H, 3-CH$_A$$H_B$), 3.31 (m, 1H, 4-C$H_AH_B$), 3.34 (m, 1H, 6-C$H_AH_B$), 3.45 (m, 1H, 6-CH$_A$$H_B$), 3.49 (m, 1H, 7-C$H_AH_B$), 3.67 (dd, J = 14 and 4 Hz, 1H, 2-CH_AH$_B$), 3.75 (dd, J = 17 and 4 Hz, 1H, 2-CH$_A$$H_B$), 3.76 (m, 1H, 4-CH$_A$$H_B$), 4.13 (q, J = 7 Hz, 1H, 5-α-CH), 4.20 (t, J = 8 Hz, 1H, 3-α-CH), 4.35 (d, J = 17 Hz, 1H, 7-CH$_A$$H_B$), 4.60 (td, J = 7 and 4 Hz, 1H, 6-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 5.32 (m, 1H, 1-α-CH), 6.94–7.56 (m, 12H, Ar Hs), 7.44 (d, J = 3 Hz, 6-NH), 7.56 (m, 2H, Ar Hs), 7.85 (d, J = 7 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-C^{5}H), 8.09 (d, J = 9 Hz, 1H, 5-NH), 8.28 (s, 1H, 3-NH), 8.64 (d, J = 9 Hz, 1H, 2-NH), 8.85 (t, J = 5 Hz, 1H, 4-NH), 10.78 (d, J = 2 Hz, indole-NH), 10.81 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{46}H_{51}N_{10}O_7SSe^+$ 967.2828, found 967.2723 [MH^+].

Cyclo[D-Ala-thiazole-Trp-(5-OMe-Trp)-Gly-D-Ala-Ph(Se)-Sar] (19g)

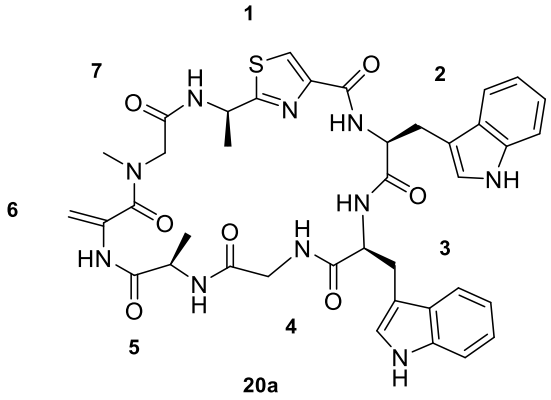


Diisopropylethylamine (186 µL, 1.0 mmol), PyBOP and (185 mg, 0.36 mmol) HOBt (48 mg, 0.36 mmol) were added successively to a solution of the linear peptide **18g** (119 mg, 0.12 mmol) and in CH_2Cl_2 (220 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) to afford the cyclic peptide **19g** as a white powder (12 mg, 11 % yield): RP-HPLC 10–60 % B in 12 min, t_R 8.5 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.65 (d, J = 7 Hz, 3H, 5-CH_3), 1.56 (d, J = 7 Hz, 3H, 1-CH_3), 3.12 (dd, J = 14 and 8 Hz, 1H, 3-CH_AH$_B$), 3.22 (s, 3H. 7-CH_3), 3.24 (m, 1H, 3-CH$_A$$H_B$), 3.31 (m, 1H, 4-C$H_AH_B$), 3.34 (m, 1H, 6-C$H_AH_B$), 3.45 (m, 1H, 6-CH$_A$$H_B$), 3.49 (m, 1H, 7-C$H_AH_B$), 3.50 (m, 1H, 2-C$H_AH_B$), 3.67 (m, 1H, 2-CH$_A$$H_B$), 3.74 (m, 1H, 4-CH$_A$$H_B$), 3.80 (s, 3H, 3-$OCH_3$), 4.13 (q, J = 7 Hz, 1H, 5-α-

CH), 4.20 (t, J = 8 Hz, 1H, 3-α-CH), 4.37 (d, J = 17 Hz, 1H, 7-CH_AH_B), 4.60 (td, J = 7 and 4 Hz, 1H, 6-α-CH), 4.79 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 5.32 (m, 1H, 1-α-CH), 6.75–7.28 (m, 12H, Ar Hs), 7.42 (d, J = 3 Hz, 6-NH), 7.56 (m, 2H, Ar Hs), 7.58 (d, J = 7 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-C^5H), 8.11 (d, J = 9 Hz, 1H, 5-NH), 8.36 (s, 1H, 3-NH), 8.65 (d, J = 9 Hz, 1H, 2-NH), 8.85 (t, J = 5 Hz, 1H, 4-NH), 10.79 (d, J = 2 Hz, indole-NH), 10.82 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{46}H_{51}N_{10}O_8SSe^+$ 983.2777, found 983.2693 [MH^+].

2.8 Preparation of argyrin analogues **20** (an oxidation–elimination reaction to reveal the dehydroalanine residue)

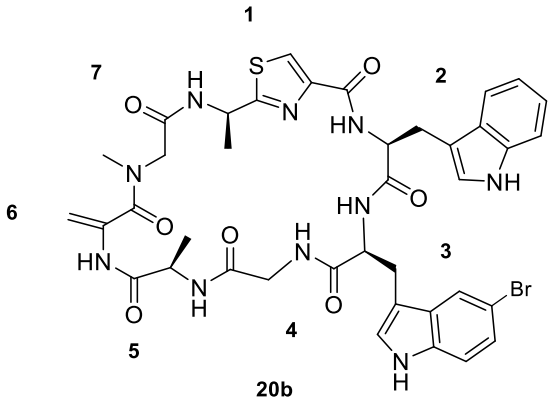
Cyclo[D-Ala-thiazole^{1}-Trp^{2}-Trp^{3}-Gly^{4}-D-Ala^{5}-Dha^{6}-Sar^{7}], Argyrin E (20a)



20a

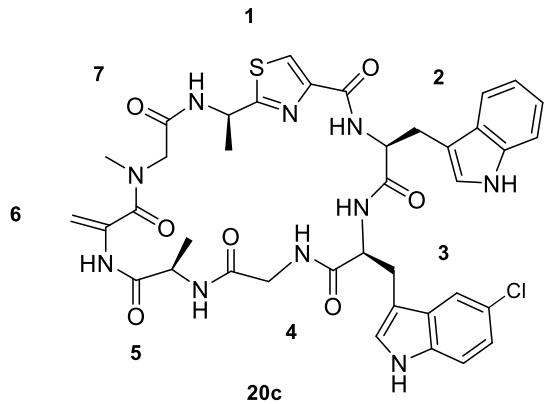
Sodium periodate (3.5 mg, 16 μmol) was added to a solution of cyclic peptide **19a** (4 mg, 4 μmol) in water (2 mL) and acetonitrile (2 mL) at room temperature. The solution was stirred for 2 h. The solvent was removed and the residue was partitioned between water (2 mL) and CH_2Cl_2–iPrOH (7:2; 2 x 10 mL). The organic extracts were combined and concentrated to dryness *in vacuo*. The residue was dissolved in acetonitile (4 mL), and water (2 mL) and saturated aqueous Na_2CO_3 (2 mL) were added successively. The reaction mixture was stirred for 2 days, diluted with water (4 mL) and extracted with CH_2Cl_2–iPrOH (7:2; 2 x 10 mL). The organic extracts were combined, washed with water (5 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by preparative RP-HPLC (Onyx Monolithic C_{18}, 100 x 10 mm) to afford the argyrin **20a**[11] as a pale yellow powder (2 mg, 66 % yield): RP-HPLC 10– 60 % B in 12 min, t_R 6.8 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (d, J = 7 Hz, 3H, 5-CH_3), 1.54 (d, J = 7 Hz, 3H, 1-CH_3), 3.08 (s, 3H, 7-NCH_3), 3.15 (m, 2H, 3-CH_2), 3.21 (m, 1H, 2-CH_AH$_B$), 3.22(m, 1H, 7-CH_AH$_B$), 3.39 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.80 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.88 (dd, J = 16 and 8 Hz, 4-CH$_A$$H_B$), 4.24 (m, 1H, 3-α-CH), 4.35 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.89 (s, 1H, 6-CH_AH$_B$), 5.20 (s, 1H, 6-CH$_A$$H_B$), 5.39 (m, 1H, 1-α-CH), 6.90– 7.75 (m, 10H, Ar Hs), 8.03 (s, 1H, 1-C^{5}H), 8.12 (d, J = 9 Hz, 1H, 5-NH), 8.29 (d, J = 9 Hz, 1H, 1-NH), 8.52 (d, J = 9 Hz, 1H, 2-NH), 8.57 (s, 1H, 3-NH), 8.79 (t, J = 4 Hz, 1H, 4-NH), 9.39 (s, 1H, 6-NH), 10.84 (d, J = 2 Hz, indole-NH), 11.05 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{39}H_{43}N_{10}O_7S^+$ 795.3037, found 795.2959 [MH^+].

Cyclo[D-Ala-thiazole-Trp-(5-Br-Trp)-Gly-D-Ala-Dha-Sar] (20b)



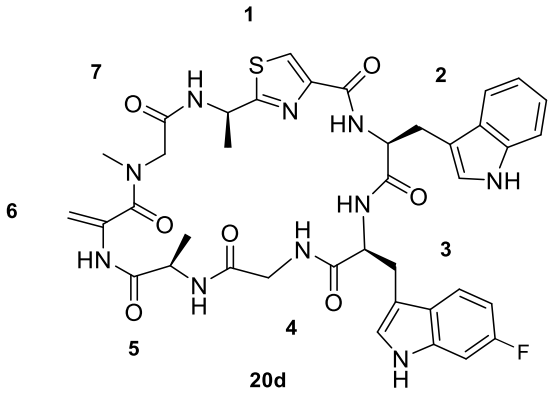
The masked cyclic peptide **19b** (4 mg, 4 μmol) was treated as above to afford argyrin **20b** as a buff solid (2 mg, 58 % yield): RP-HPLC 10–60 % B in 12 min, t_R 8.0 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (d, J = 7 Hz, 3H, 5-CH_3), 1.54 (d, J = 7 Hz, 3H, 1-CH_3), 3.13 (s, 3H, 7-NCH_3), 3.15 (m, 2H, 3-CH_2), 3.21 (m, 1H, 2-CH_AH$_B$), 3.22 (m, 1H, 7-CH_AH$_B$), 3.39 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.80 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.88 (dd, J = 16 and 8 Hz, 4-CH$_A$$H_B$), 4.24 (m, 1H, 3-α-CH), 4.35 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.92 (s, 1H, 6-CH_AH$_B$), 5.16 (s, 1H, 6-CH$_A$$H_B$), 5.39 (m, 1H, 1-α-CH), 6.95–7.77 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C^{5}H), 8.09 (d, J = 9 Hz, 1H, 5-NH), 8.27 (d, J = 9 Hz, 1H, 1-NH), 8.40 (s, 1H, 3-NH), 8.56 (d, J = 9 Hz, 1H, 2-NH), 8.73 (t, J = 4 Hz, 1H, 4-NH), 9.22 (s, 1H, 6-NH), 10.76 (d, J = 2 Hz, indole-NH), 11.15 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{39}H_{42}N_{10}BrO_7S^+$ 875.2122, found 875.1877 [MH^+].

Cyclo[D-Ala-thiazole-Trp-(5-Cl-Trp)-Gly-D-Ala-Dha-Sar] (20c)



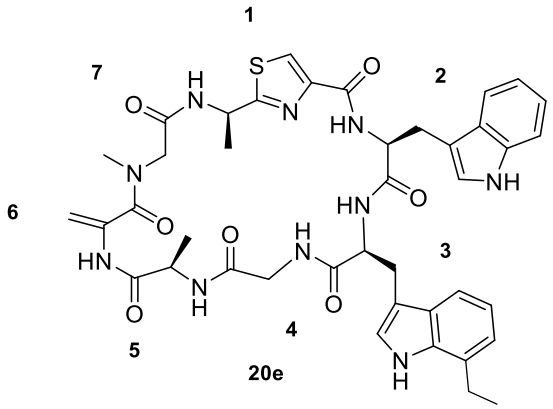
The masked cyclic peptide **19c** (5 mg, 5 μmol) was used to afford argyrin **20c** as a buff solid (3 mg, 67 % yield): RP-HPLC 10–60 % B in 12 min, t_R 7.5 min. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (d, J = 7 Hz, 3H, 5-CH_3), 1.54 (d, J = 7 Hz, 3H, 1-CH_3), 3.13 (s, 3H, 7-NCH_3), 3.15 (m, 2H, 3-CH_2), 3.21 (m, 1H, 2-CH_AH$_B$), 3.22 (m, 1H, 7-CH_AH$_B$), 3.39 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.85 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.88 (dd, J = 16 and 8 Hz, 4-CH$_A$$H_B$), 4.21 (m, 1H, 3-α-CH), 4.34 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.92 (s, 1H, 6-CH_AH$_B$), 5.16 (s, 1H, 6-CH$_A$$H_B$), 5.39 (m, 1H, 1-α-CH), 6.92–7.77 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C^{5}H), 8.09 (d, J = 9 Hz, 1H, 5-NH), 8.27 (d, J = 9 Hz, 1H, 1-NH), 8.40 (s, 1H, 3-NH), 8.56 (d, J = 9 Hz, 1H, 2-NH), 8.74 (t, J = 4 Hz, 1H, 4-NH), 9.24 (s, 1H, 6-NH), 10.76 (d, J = 2 Hz, indole-NH), 11.15 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{39}H_{42}ClN_{10}O_7S^+$ 829.2647, found 829.2347 [MH^+].

Cyclo[D-Ala-thiazole-Trp-(6-F-Trp)-Gly-D-Ala-Dha-Sar] (20d)



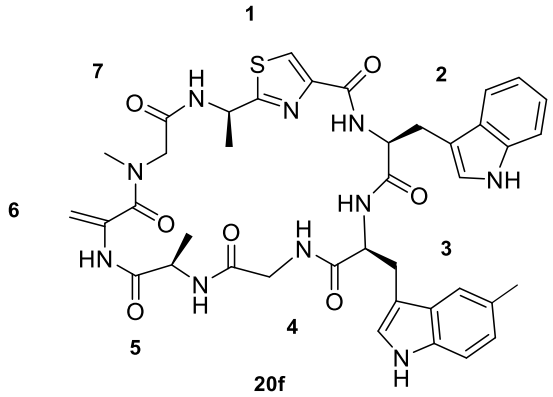
The masked cyclic peptide **19d** (5 mg, 5 μmol) was used to afford argyrin **20d** as a buff solid (3 mg): RP-HPLC 10–60 % B in 12 min, t_R 7.0 min. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 0.87 (d, J = 7 Hz, 3H, 5-CH$_3$), 1.54 (d, J = 7 Hz, 3H, 1-CH$_3$), 3.12 (s, 3H, 7-NCH$_3$), 3.15 (m, 2H, 3-CH$_2$), 3.21 (m, 1H, 2-CH_AH$_B$), 3.22 (m, 1H, 7-CH_AH$_B$), 3.39 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.80 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.88 (dd, J = 16 and 8 Hz, 4-CH$_A$$H_B$), 4.21 (m, 1H, 3-α-CH), 4.35 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.92 (s, 1H, 6-CH_AH$_B$), 5.16 (s, 1H, 6-CH$_A$$H_B$), 5.40 (m, 1H, 1-α-CH), 6.86–7.77 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C^{5}H), 8.09 (d, J = 9 Hz, 1H, 5-NH), 8.27 (d, J = 9 Hz, 1H, 1-NH), 8.40 (s, 1H, 3-NH), 8.56 (d, J = 9 Hz, 1H, 2-NH), 8.69 (t, J = 4 Hz, 1H, 4-NH), 9.21 (s, 1H, 6-NH), 10.74 (d, J = 2 Hz, indole-NH), 10.99 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{39}H_{42}FN_{10}O_7S^+$ 813.2943, found 813.3016 [MH$^+$].

Cyclo[D-Ala-thiazole-Trp-(7-Et-Trp)-Gly-D-Ala-Dha-Sar] (20e)



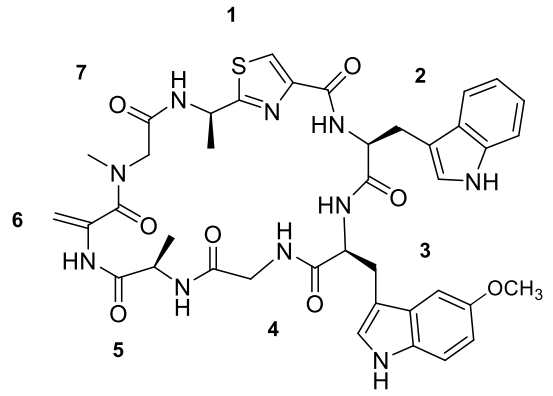
The masked cyclic peptide **19e** (4 mg, 4 μmol) was used to afford argyrin **20e** as a buff solid (2 mg, 52 % yield): RP-HPLC 10–60 % B in 12 min, t_R 7.9 min. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 0.87 (d, J = 7 Hz, 3H, 5-CH$_3$), 1.27 (t, J = 7 Hz, 3H, 3-CH$_2$CH_3), 1.55 (d, J = 7 Hz, 3H, 1-CH$_3$), 2.85 (q, J = 7 Hz, 2H, 3-CH_2CH$_3$), 3.12 (s, 3H, 7-NCH$_3$), 3.15 (m, 2H, 3-CH$_2$), 3.21 (m, 1H, 2-CH_AH$_B$), 3.24 (m, 1H, 7-CH_AH$_B$), 3.39 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.80 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.88 (dd, J = 16, 8 Hz, 4-CH$_A$$H_B$), 4.24 (td, J = 12 and 4 Hz, 1H, 3-α-CH), 4.35 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.92 (s, 1H, 6-CH_AH$_B$), 5.16 (s, 1H, 6-CH$_A$$H_B$), 5.40 (m, 1H, 1-α-CH), 6.93–7.76 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C^{5}H), 8.12 (d, J = 9 Hz, 1H, 5-NH), 8.27 (d, J = 9 Hz, 1H, 1-NH), 8.41 (d, J = 2 Hz, 1H, 3-NH), 8.55 (d, J = 9 Hz, 1H, 2-NH), 8.72 (t, J = 4 Hz, 1H, 4-NH), 9.23 (s, 1H, 6-NH), 10.74 (d, J = 2 Hz, indole-NH), 10.89 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{41}H_{47}N_{10}O_7S^+$ 823.3350, found 823.3311 [MH$^+$].

Cyclo[D-Ala-thiazole-Trp-(5-Me-Trp)-Gly-D-Ala-Dha-Sar] (20f)



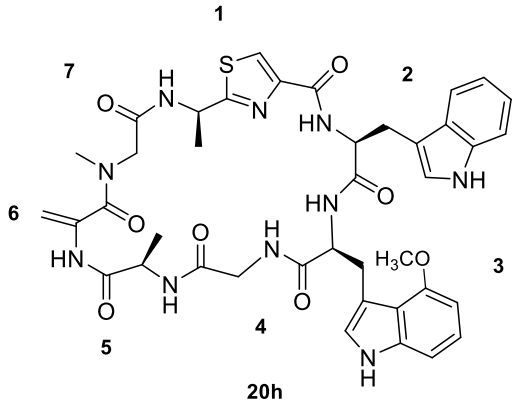
The masked cyclic peptide **19f** (5 mg, 5 μmol) was used to yield argyrin **20f** as a buff solid (3 mg, 66 % yield): RP-HPLC 10–60 % B in 12 min, t_R 7.3 min. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 0.87 (d, J = 7 Hz, 3H, 5-CH$_3$), 1.54 (d, J = 7 Hz, 3H, 1-CH$_3$), 2.40 (s, 3H, 3-CH$_3$), 3.12 (s, 3H, 7-NCH$_3$), 3.15 (m, 2H, 3-CH$_2$), 3.20 (m, 1H, 2-CH_AH$_B$), 3.24 (m, 1H, 7-CH_AH$_B$), 3.38 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.80 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.88 (dd, J = 16 and 8 Hz, 4-CH$_A$$H_B$), 4.22 (m, 1H, 3-α-CH), 4.35 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.92 (s, 1H, 6-CH_AH$_B$), 5.16 (s, 1H, 6-CH$_A$$H_B$), 5.40 (m, 1H, 1-α-CH), 6.91–7.76 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C^{5}H), 8.09 (d, J = 9 Hz, 1H, 5-NH), 8.27 (d, J = 9 Hz, 1H, 1-NH), 8.37 (s, 1H, 3-NH), 8.56 (d, J = 9 Hz, 1H, 2-NH), 8.70 (t, J = 4 Hz, 1H, 4-NH), 9.22 (s, 1H, 6-NH), 10.75 (d, J = 2 Hz, indole-NH), 10.76 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{40}H_{45}N_{10}O_8S^+$ 809.3193, found 809.3016 [MH$^+$].

Cyclo[D-Ala-thiazole-Trp-(5-OMe-Trp)-Gly-D-Ala-Dha-Sar] (20g)



The masked cyclic peptide **19g** (4 mg, 4 μmol) was used to yield argyrin **20g** as a buff solid (2 mg, 58 % yield): RP-HPLC 10–60 % B in 12 min, t_R 6.5 min. ^{1}H NMR (400 MHz, DMSO-d$_6$): δ 0.68 (d, J = 7 Hz, 3H, 5-CH$_3$), 1.55 (d, J = 7 Hz, 3H, 1-CH$_3$), 3.13 (s, 3H, 7-NCH$_3$), 3.15 (m, 2H, 3-CH$_2$), 3.21 (m, 1H, 2-CH_AH$_B$), 3.22 (m, 1H, 7-CH_AH$_B$), 3.40 (m, 1H, 4-CH_AH$_B$), 3.44 (m, 1H, 2-CH$_A$$H_B$), 3.79 (s, 3H, 3-OCH$_3$), 3.80 (d, J = 16 Hz, 1H, 7-CH$_A$$H_B$), 3.86 (dd, J = 16 and 8 Hz, 4-CH$_A$$H_B$), 4.22 (m, 1H, 3-α-CH), 4.34 (m, 1H, 5-α-CH), 4.78 (td, J = 12 and 4 Hz, 1H, 2-α-CH), 4.93 (s, 1H, 6-CH_AH$_B$), 5.16 (s, 1H, 6-CH$_A$$H_B$), 5.39 (m, 1H, 1-α-CH), 6.90–7.70 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C^{5}H), 8.12 (d, J = 9 Hz, 1H, 5-NH), 8.27 (d, J = 9 Hz, 1H, 1-NH), 8.42 (s, 1H, 3-NH), 8.56 (d, J = 9 Hz, 1H, 2-NH), 8.70 (t, J = 4 Hz, 1H, 4-NH), 9.24 (s, 1H, 6-NH), 10.75 (d, J = 2 Hz, indole-NH), 10.76 (d, J = 2 Hz, indole-NH). ES-MS *m/z* calcd for $C_{40}H_{45}N_{10}O_8S^+$ 825.3143, found 825.3056 [MH$^+$].

Cyclo[D-Ala-thiazole[1]-Trp[2]-(4-OMe-Trp)[3]-Gly[4]-D-Ala[5]-Dha[6]-Sar[7]], Argyrin A (20h)



The masked cyclic peptide **19h** (4 mg, 4 μmol) was used to afford argyrin **20**[11] as a buff solid (2 mg): RP-HPLC 10–60 % B in 12 min, t_R 6.6 min. ES-MS *m/z* calcd for $C_{40}H_{45}N_{10}O_8S^+$ 825.3143, found 825.3092 [MH^+].

3. Antibacterial activity

The MIC_{50} of selected compounds against bacterial strains *Pseudomonas aeruginosa* PAO1 and *Proteus mirabilis* Hauser 1885 were determined in Muller–Hinton broth. Bacteria were grown overnight in Muller–Hinton broth with shaking (200 rpm) at 37 ºC. The bacterial sample was then diluted with fresh broth to give an $OD_{610\ nm}$ of 0.05. To 990 µL of the freshly prepared bacterial sample was added 10 µL of a 10 mM solution of the test compound in DMSO. This sample was then serially diluted with freshly prepared $OD_{610\ nm}$ 0.05 bacterial broth containing 1% DMSO.
A volume of 200 µL of the prepared samples, containing bacterial culture and the desired concentration of each compound, were then dispensed into a 96-well microtiter plate and incubated at 37 ºC over 15 h. The MIC_{50} were determined by measurement of $OD_{610\ nm}$ at 13 h. Each compound was evaluated in duplicate at concentrations from 0 to 100 µM, and each experiment was repeated at least three times.

Table S1. MIC_{50} of selected argyrin analogues evaluated against *Pseudomonas aeruginosa* PAO1 and *Proteus mirabilis* Hauser 1885.

Compound	**MIC_{50} (µM)**	
	P. aeruginosa PAO1	*Proteus mirabilis* Hauser 1885
Argyrin A **(20h)**	19.8 ± 1.6	19.2 ± 1.7
Argyrin E **(20a)**	>100	>100
20c	>100	>100
20f	>100	>100
20g	90.7 ± 3.7	100

Figure S1. The effects of argyrin A **20h** and analogue **20g** on the growth of *Pseudomonas aeruginosa* PAO1 in Muller–Hinton broth at 13 h.

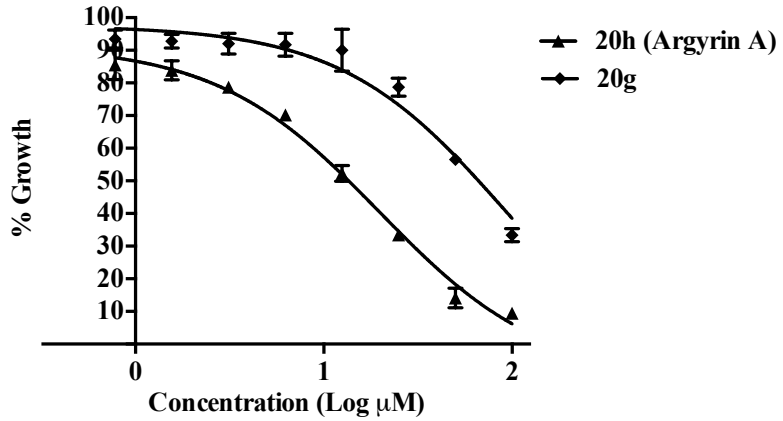
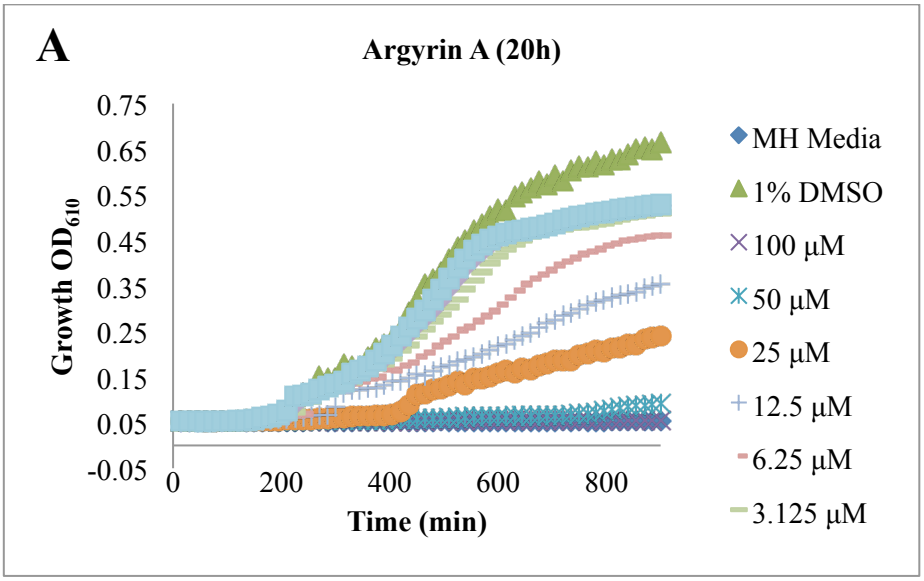


Figure S2. Growth curves of (**A**) *P. aeruginosa* PAO1 and (**B**) *Proteus mirabilis* Hauser 1885. Growth of bacteria was measured in the absence (1% DMSO control) and presence of argyrin A (**20h**) at concentration range of 100–3.125 µM. MH Media without bacteria is also used as a control.



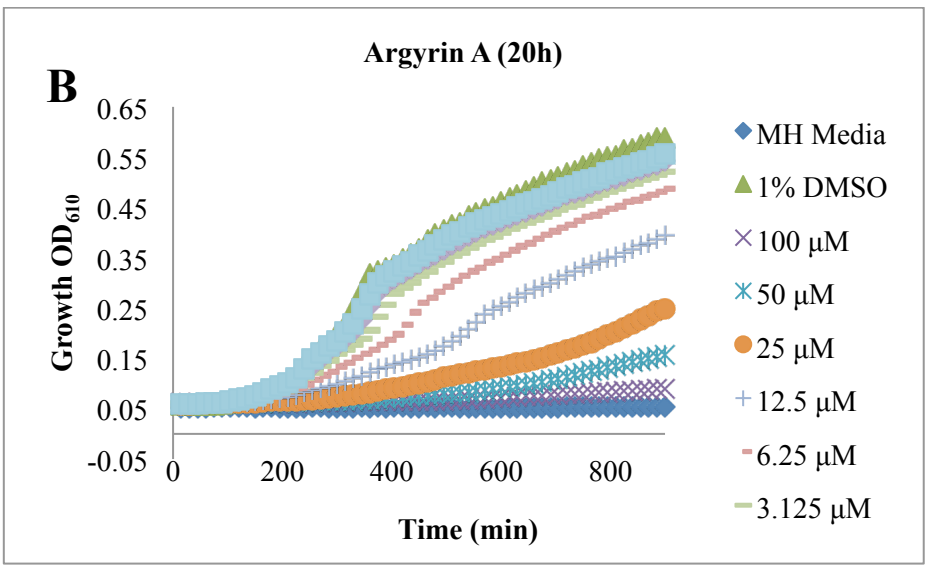


Figure S3. Growth curves of (**A**) *P. aeruginosa* PAO1 and (**B**) *Proteus mirabilis* Hauser 1885. Growth of bacteria was measured in the absence (1% DMSO control) and presence of argyrin E (**20a**) at concentration range of 100–3.125 µM. MH Media without bacteria is also used as a control.

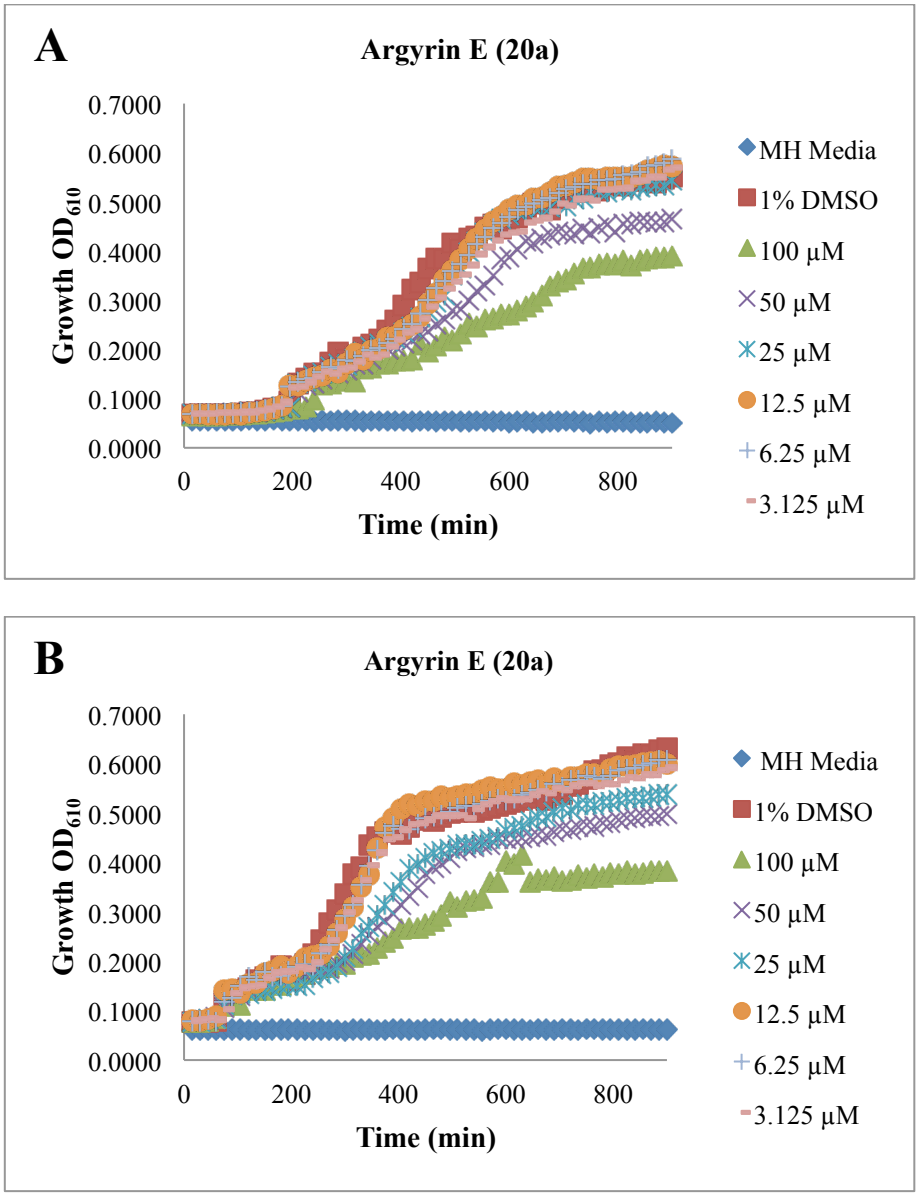
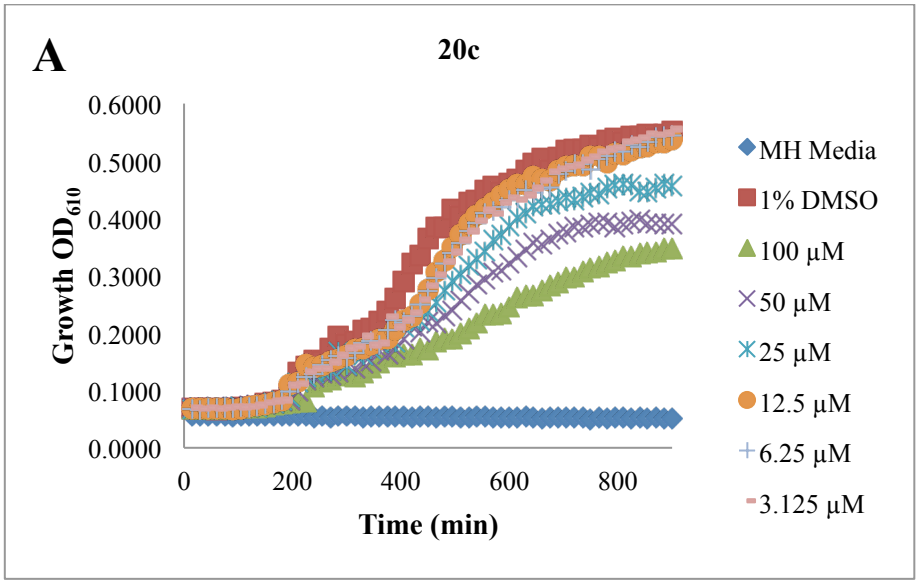


Figure S4. Growth curves of (**A**) *P. aeruginosa* PAO1 and (**B**) *Proteus mirabilis* Hauser 1885. Growth of bacteria was measured in the absence (1% DMSO control) and presence of **20c** at concentration range of 100–3.125 µM. MH Media without bacteria is also used as a control.



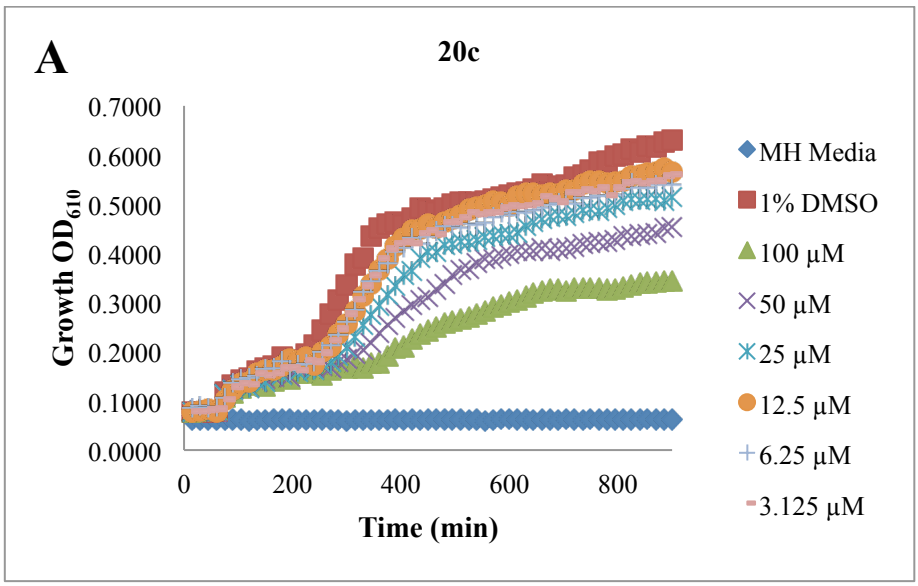


Figure S5. Growth curves of (**A**) *P. aeruginosa* PAO1 and (**B**) *Proteus mirabilis* Hauser 1885. Growth of bacteria was measured in the absence (1% DMSO control) and presence of **20f** at concentration range of 100–3.125 µM. MH Media without bacteria is also used as a control.

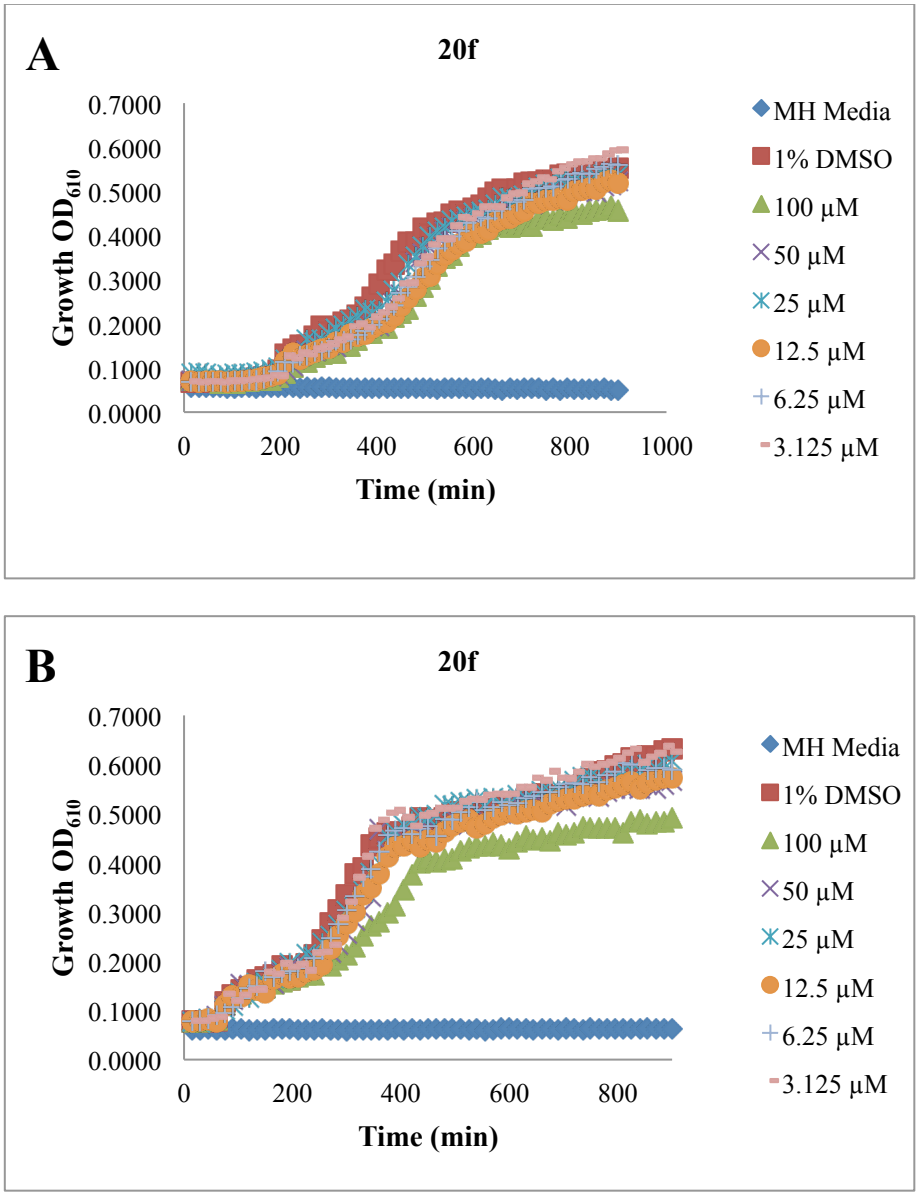
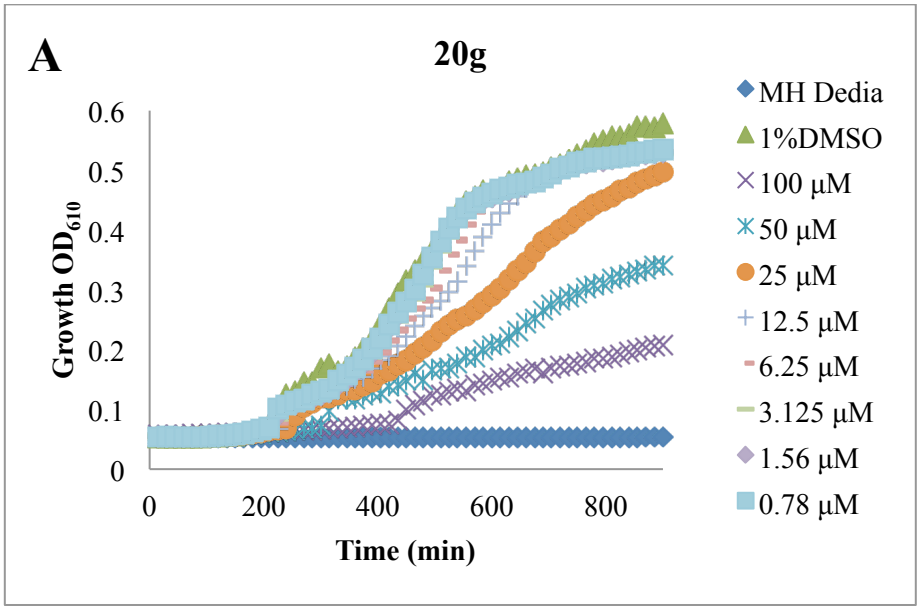
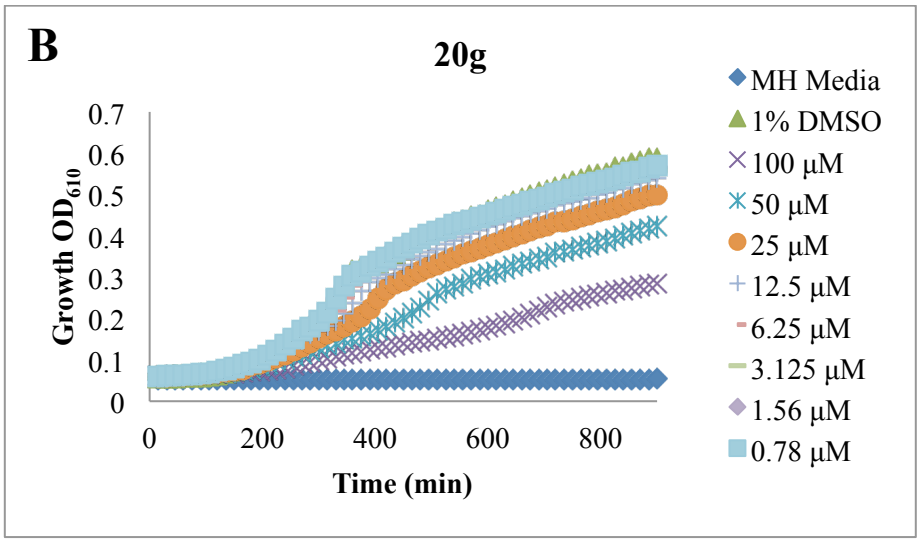
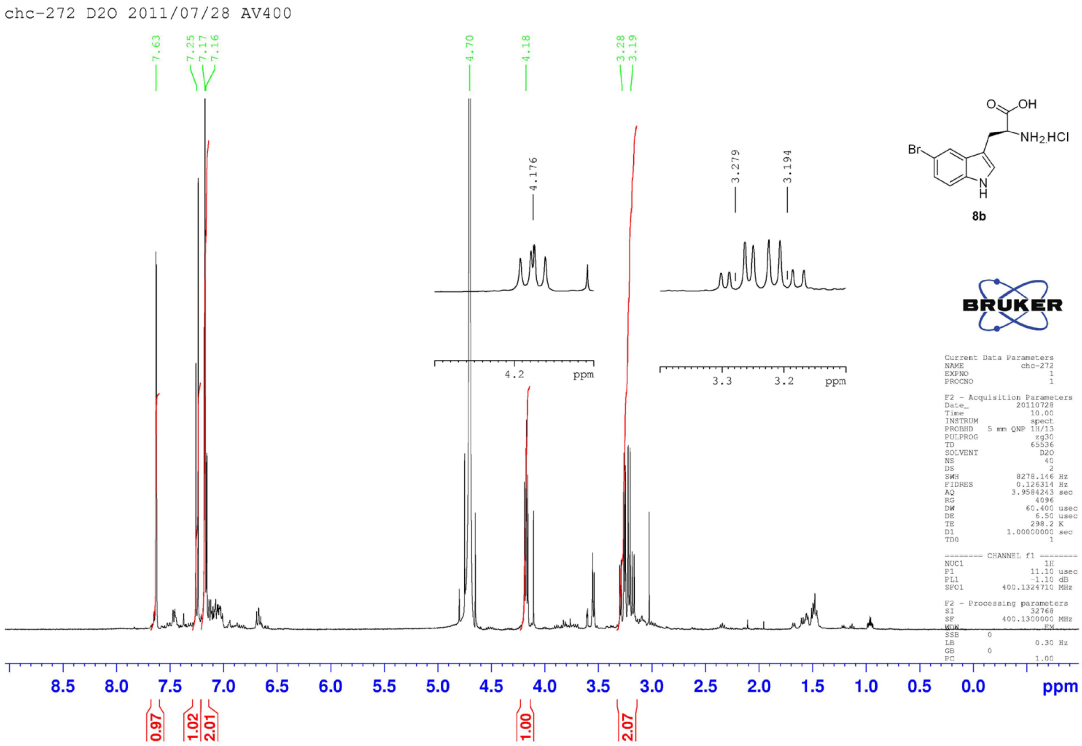


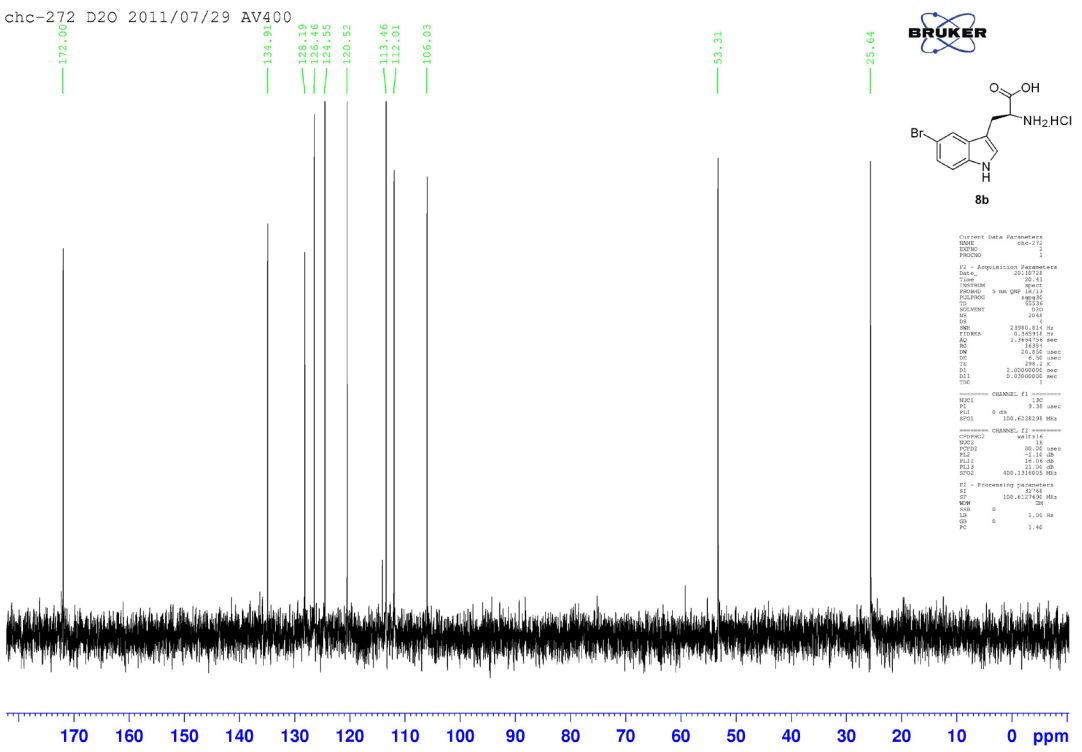
Figure S6. Growth curves of (**A**) *P. aeruginosa* PAO1 and (**B**) *Proteus mirabilis* Hauser 1885. Growth of bacteria was measured in the absence (1% DMSO control) and presence of **20g** at concentration range of 100–3.125 µM. MH Media without bacteria is also used as a control.





4. ^{1}H and ^{13}C NMR spectra of (*S*)-tryptophan analogues





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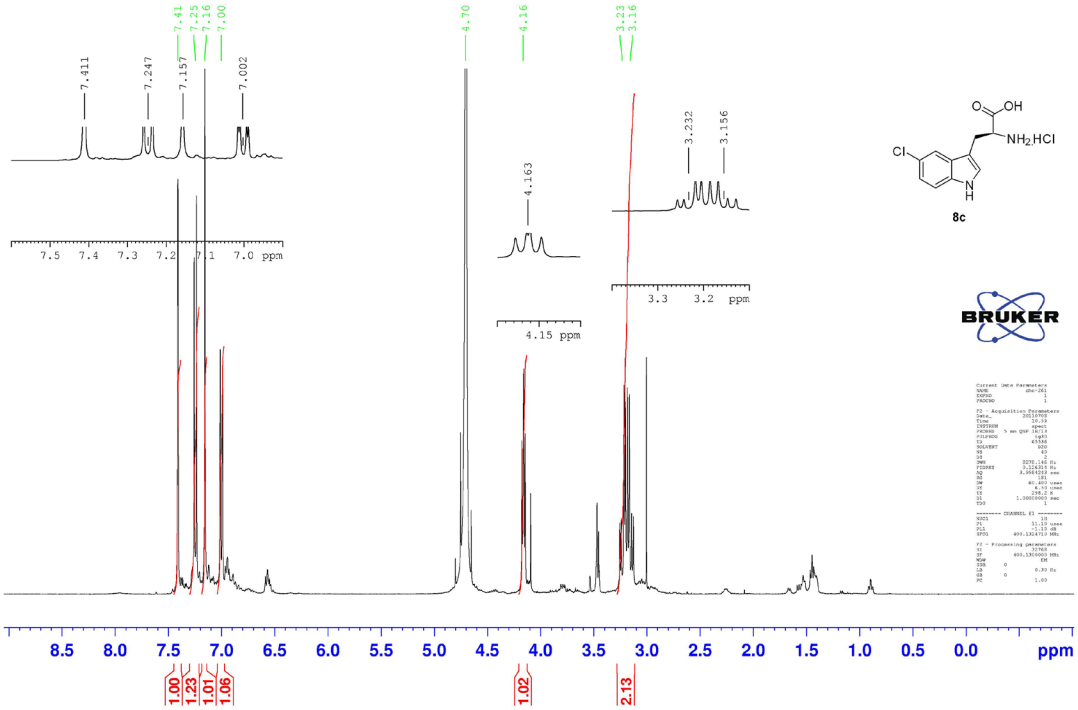

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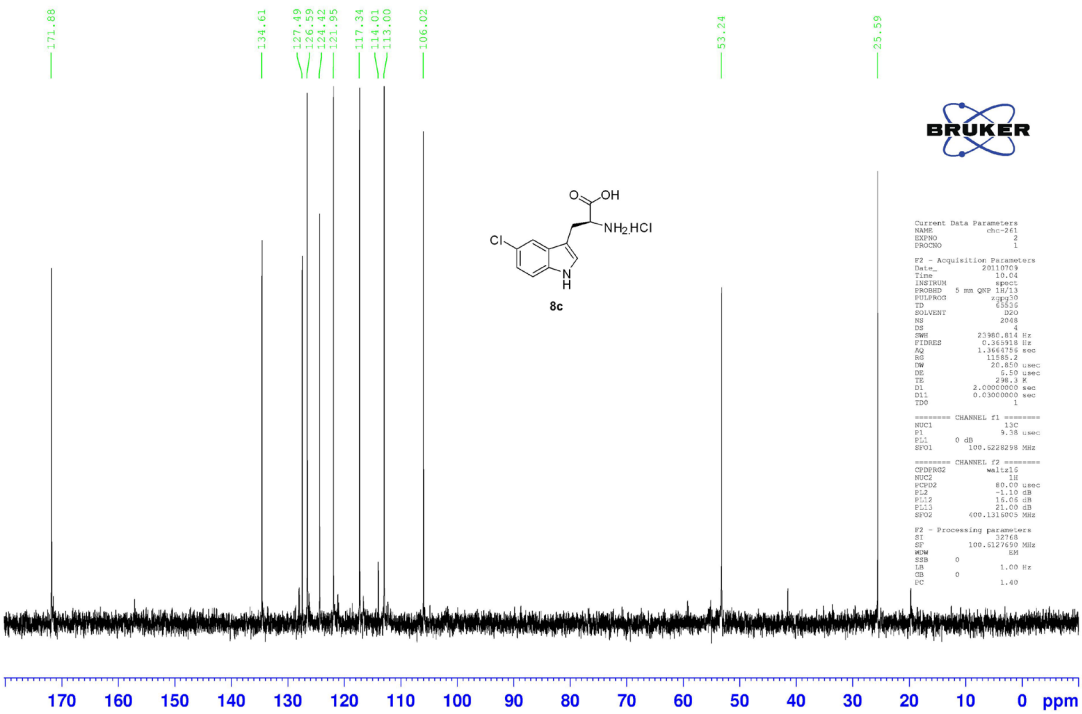

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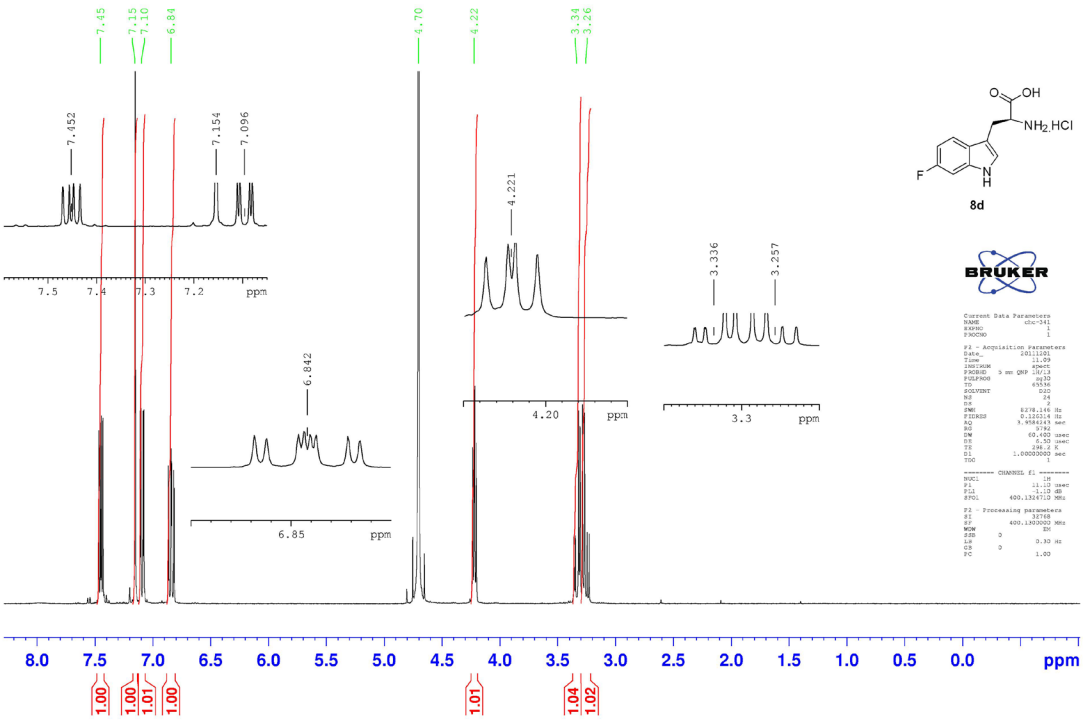

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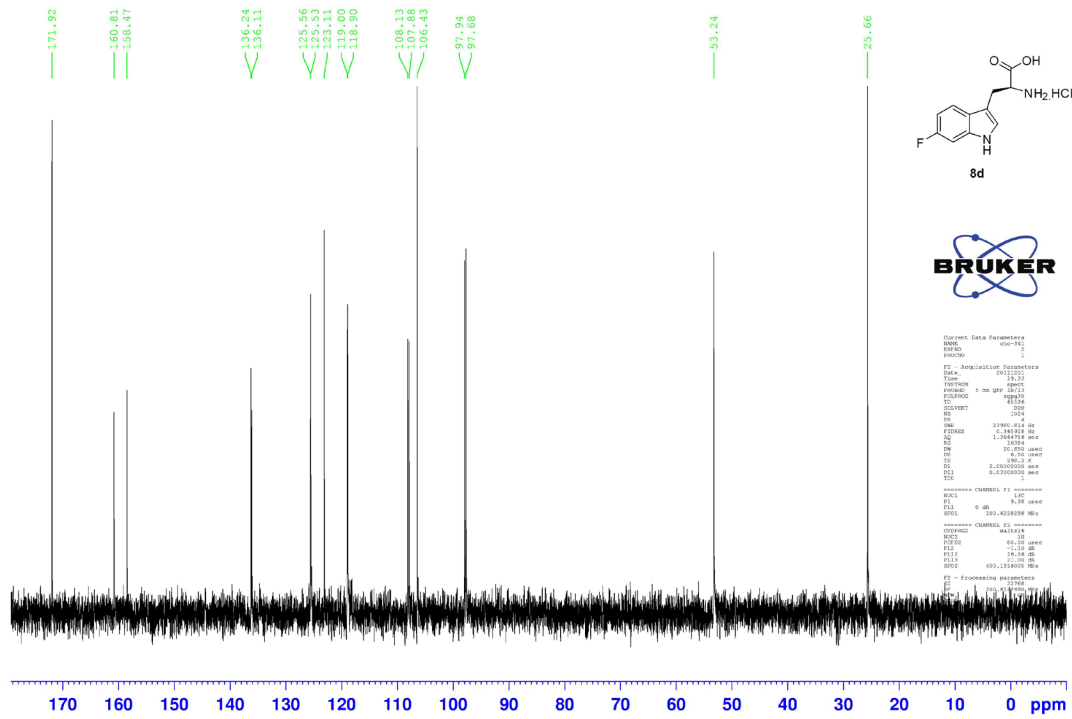

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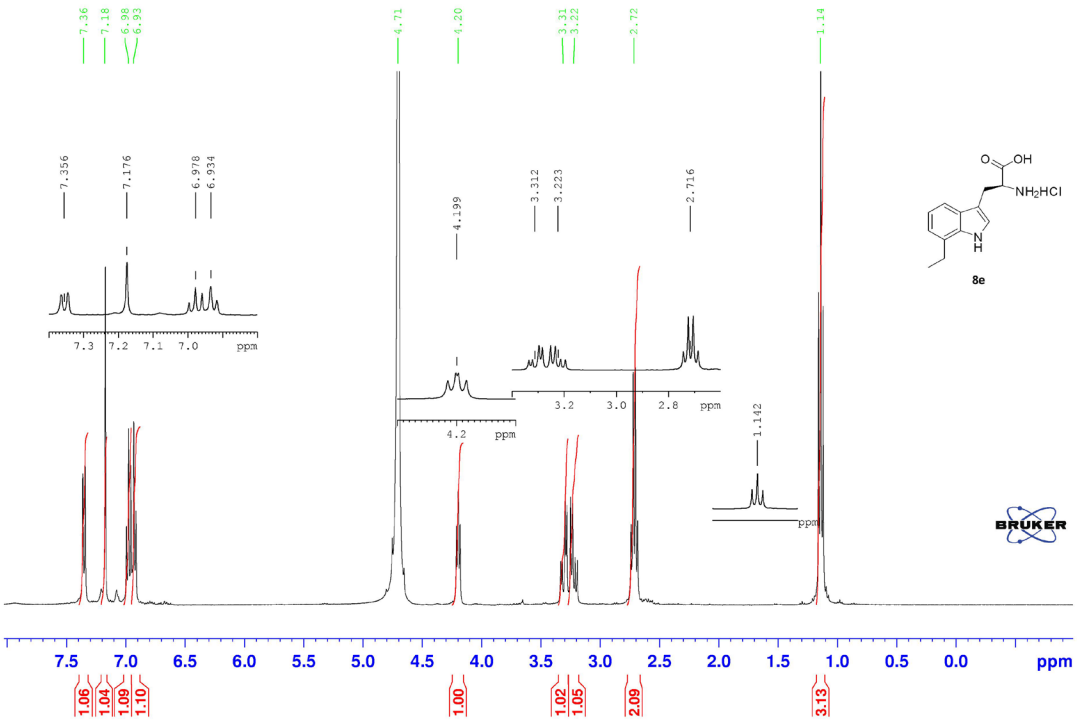

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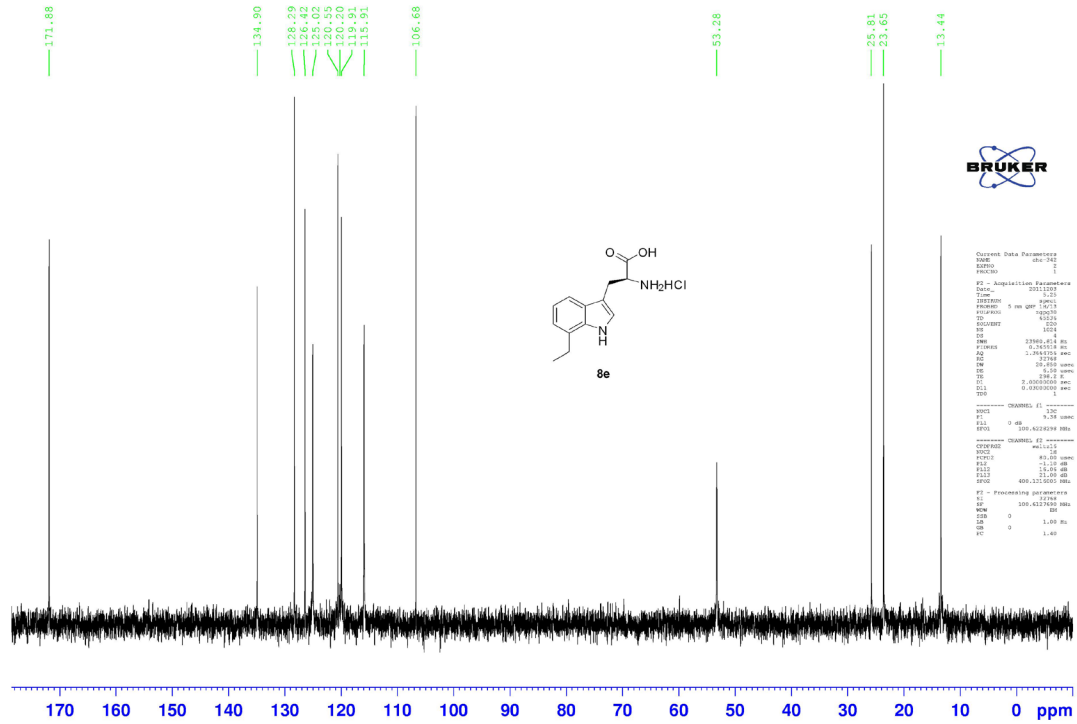

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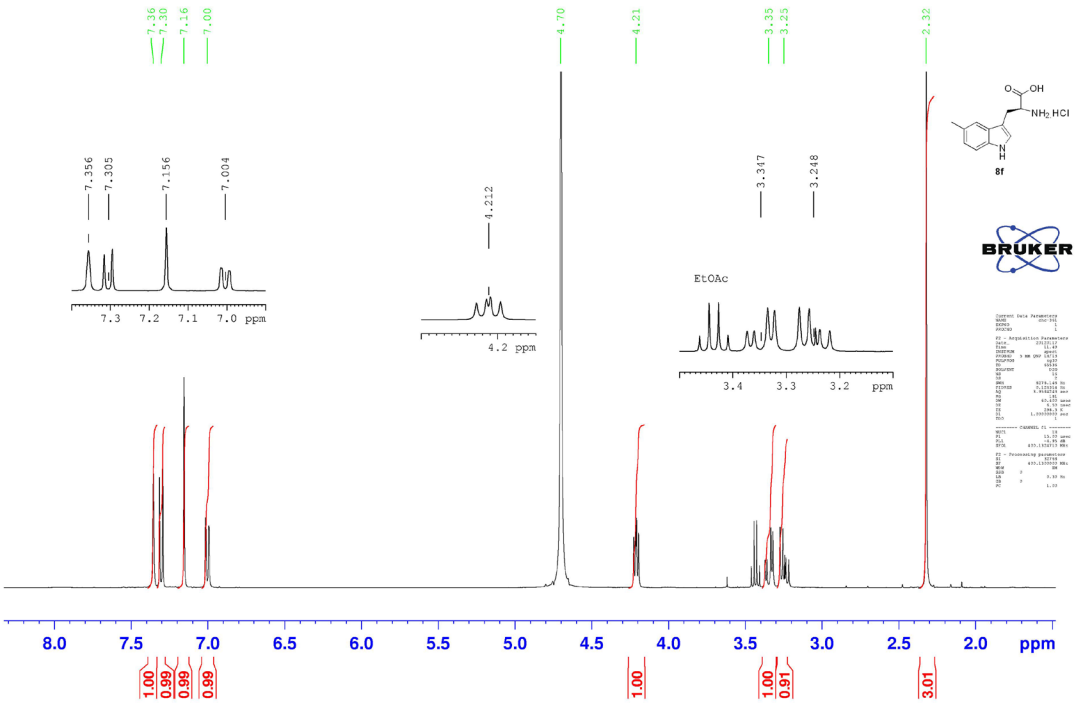

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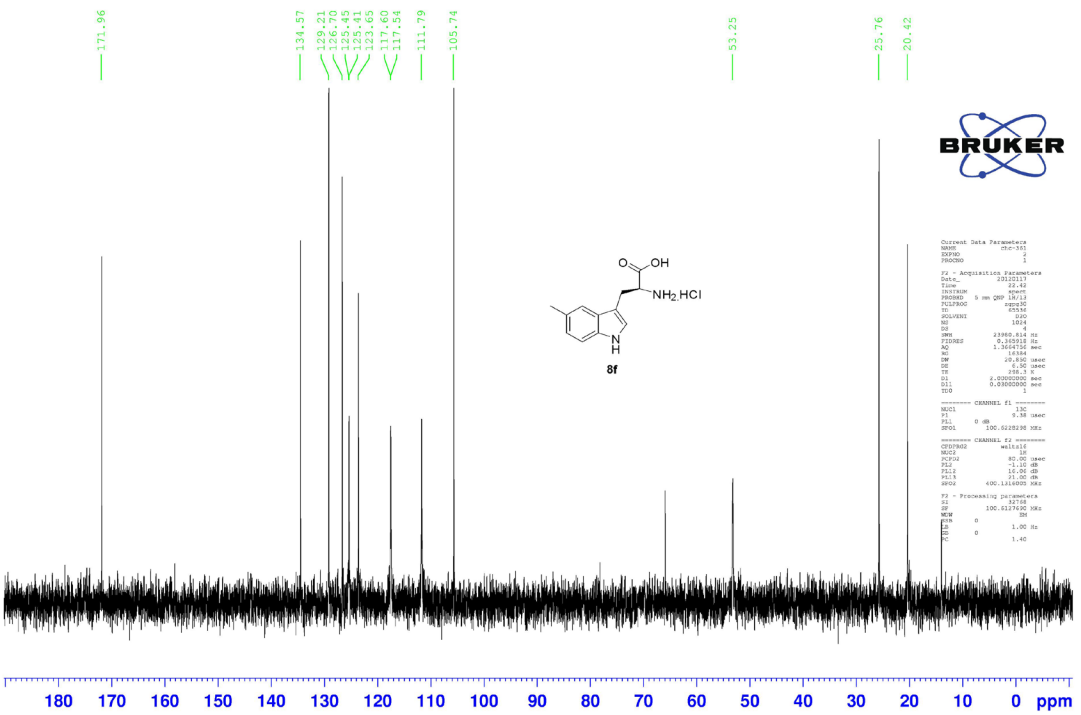

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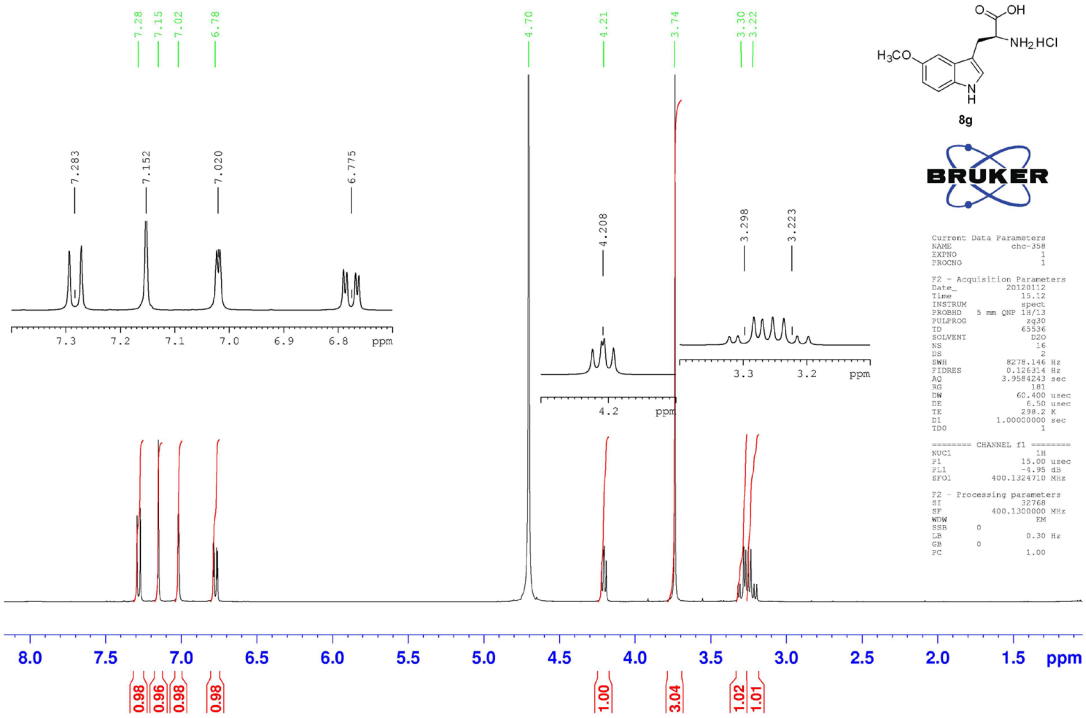

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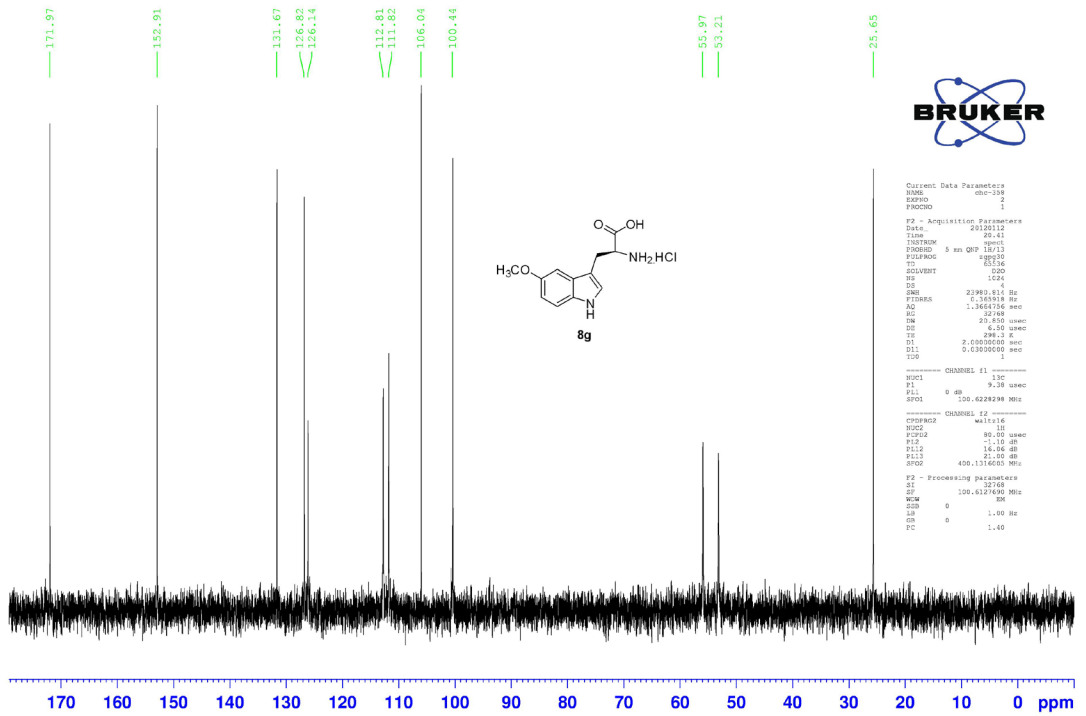

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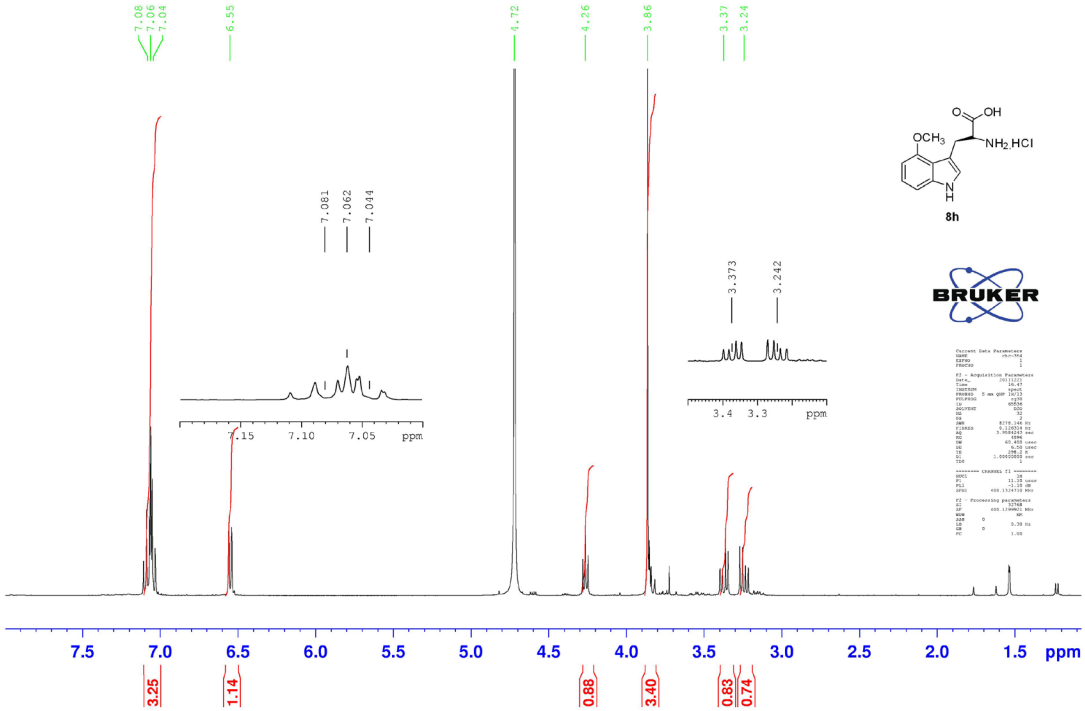

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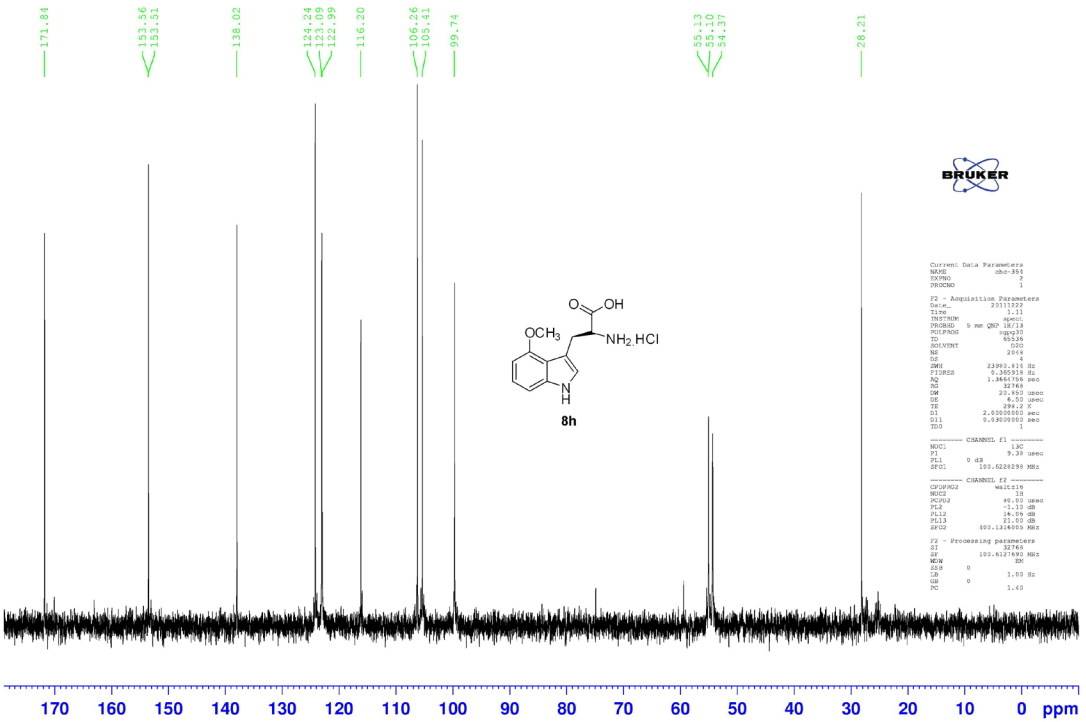

5. References

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