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

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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 (\(\nu_{\text{max}}\)) 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 (\(\delta\)) 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\(_3\)OD (\(^1\)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\textsuperscript{®} 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 Et₂O (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 MgSO₄ and concentrated *in vacuo* to yield the indole-3-carbaldehyde.

6-Fluoro-1H-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 as yellow crystals (0.90 g, 66 % yield): m.p. 174–178 °C; TLC *R*ₐ = 0.2 (Hexane–EtOAc, 2:1); IR (KBr): *ν* = 2933, 1641, 1530, 1448, 1149 cm⁻¹. ¹H NMR (400 MHz, acetone-d₆): 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), 7.11 (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). ¹³C NMR (100 MHz, acetone-d₆): δ 98.5 (d, *J* = 26 Hz), 110.4 (d, *J* = 26 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 C₉H₇FNO⁺ 164.0512, found 164.0430 [MH⁺].

7-Ethyl-1H-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 as a brown powder (1.1 g, 95 % yield): m.p. 92–94 °C; TLC *R*ₐ = 0.2 (Hexane–EtOAc, 2:1). IR (KBr): *ν* = 3166, 3054, 1628, 1456, 1228 cm⁻¹. ¹H NMR (400 MHz, CDCl₃): δ 1.38 (t, *J* = 7.5 Hz, 3H, CH₂CH₃), 2.98 (q, *J* = 7.5 Hz, 2H, CH₂CH₃), 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). ¹³C NMR (100 MHz, CDCl₃): 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 C₁₁H₁₂NO⁺ 174.0919, found 174.0805 [MH⁺].
5-Methyl-1H-indole-3-carbaldehyde (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 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): $\nu = 3214, 1636, 1437, 1388$ cm$^{-1}$. $^1$H NMR (400 MHz, acetone-d$_6$): $\delta$ 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$_6$): $\delta$ 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-1H-indole-3-carbaldehyde (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): $\nu = 3170, 1641, 1432, 790$ cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 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$_3$): $\delta$ 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-1H-indole-3-carbaldehyde (2h)

The synthesis was carried out using phosphoryl chloride (0.9 mL, 10.2 mmol), 4-methoxyindole (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): $\nu = 3250, 1642, 1361, 1323, 790$ cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 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$_6$): $\delta$ 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-(1H-indol-3-yl)propanenitrile 4

2-(2-Hydroxy-1-phenylethylamino)-3-(1H-indol-3-yl)propanenitrile (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 MgSO₄, 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 Et₂O (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 CH₂Cl₂ (2 x 40 mL) and brine (80 mL). The organic extracts were combined, dried over MgSO₄ and concentrated in vacuo, followed by purification using column chromatography (CHCl₃–MeOH, 30:1) to afford 4a as an amorphous orange solid (307 mg, 29 % yield from 2a): TLC Rf = 0.3 (Hexane–EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, fR 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, CDCl₃): δ 2.53 (br, 1 H, NH), 3.12 (d, J = 6 Hz, 1H, CH₂A), 3.21 (d, J = 5 Hz, 1H, CH₂B), 3.49 (dd, J = 11 and 9 Hz, 1H, CH₂BOH), 3.65 (dd, J = 6 and 4 Hz, 1H, α-H), 3.67 (dd, J = 6 and 4 Hz, 1H, CH₂BOH), 4.03 (dd, J = 9 and 4 Hz, 1H, CH₂OH), 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. ¹³C NMR (100 MHz, CDCl₃): 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 C₁₉H₂₀N₃O⁺ 306.1606, found 306.1680 [MH⁺].
3-(5-Bromo-1H-indol-3-yl)-2-[1-(4-methoxyphenyl)ethylamino]propanenitrile (S1)

![Structure of S1](image)

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): \nu = 3418, 2960, 1459, 1244, 755 cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 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, \(\alpha\)-H), 3.83 (s, 3H, OCH\(_3\)), 4.00 (q, \(J = 6\) Hz, 1H, CH\(_3\)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\(_3\)): \(\delta\) 24.9, 28.9, 49.2, 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-1H-indol-3-yl)-2-[1-(4-methoxyphenyl)ethylamino]propanenitrile (S2)

![Structure of S2](image)

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): \nu = 3419, 2963, 1609, 1457, 756 cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 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, \(\alpha\)-H), 3.82 (s, 3H, OCH\(_3\)), 4.00 (q, \(J = 6\) Hz, 1H, CH\(_3\)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\(_3\)): \(\delta\) 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-1H-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (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 Rf = 0.3 (Hexane –EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, tR 6.0 min for (R,R) isomer and 7.2 min for (S,R) isomer; IR (KBr): v = 3425, 3025, 2925, 1627, 758 cm⁻¹. 1H NMR (400 MHz, CDCl₃): δ 2.50 (br, 1 H, NH), 3.14  (d, J = 4 Hz, 1H, CH₂B), 3.16 (d, J = 4 Hz, 1H, CH₂A), 3.49 (dd, J = 12 and 8 Hz, 1H, CH₂BOH), 3.62 (t, J = 6 Hz, 1H, α-H), 3.67 (dd, J = 11 and 4 Hz, 1H, CH₂AHBOH), 4.0 (dd, J = 9 and 4 Hz, 1H, CH₂CH₂OH), 7.68-6.88 (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. 13C NMR (100 MHz, CDCl₃): δ 29.5, 49.0, 63.1, 67.3, 97.7 (d, J = 26 Hz), 109.7, 116.3, 120.0, 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 C₁₉H₁₉FN₃O+ 324.1512, found 324.1248 [MH +].

3-(7-Ethyl-1H-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (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 Rf = 0.3 (Hexane –EtOAc, 1:1); RP-HPLC 10–60 % B in 12 min, tR 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. 1H NMR (400 MHz, CDCl₃): δ 1.37 (t, J = 8 Hz, 3H, CH₂C₃H₃), 2.49 (br, 1 H, NH), 2.85 (q, J = 8 Hz, 2H, CH₂CH₃), 3.25 (d, J = 6 Hz, 1H, CH₃CH₂O), 3.27 (d, J = 4 Hz, 1H, CH₃H₂), 3.56 (dd, J = 12 and 9 Hz, 1H, CH₂H₂OH), 3.70 (dd, J = 8 and 6 Hz, 1H, α-H), 4.09 (dd, J = 11 and 4 Hz, 1H, CH₂H₂OH), 4.09 (dd, J = 8, 4 Hz, 1H, CH₂CH₂OH), 7.06-7.13 (m, 1H, 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). 13C NMR (100 MHz, CDCl₃): 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, 160.1 ppm. MS: m/z (+ESI) calcd for C₁₉H₂₁FN₃O⁺ 336.1638, found 336.1373 [MH +].

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127.1, 127.6, 128.3, 128.9, 135.1, 138.4. MS: m/z (+ESI) calcd for C_{21}H_{24}N_{3}O^+ 334.1919, found 334.1843 [MH^+].

3-(5-Methyl-1H-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (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, CDCl₃): δ 2.33 (br, 1 H, NH), 2.44 (s, 3H, CH₃), 3.21 (d, J = 4 Hz, 1H, CH₂CH₂OH), 3.23 (d, J = 3 Hz, 1H, CH₃H₃), 3.55 (dd, J = 12 and 9 Hz, 1H, CH₂CH₂OH), 3.68 (dd, J = 12 and 6 Hz, 1H, α-H), 3.73 (dd, J = 11 and 4 Hz, 1H, CH₂CH₂OH), 4.08 (dd, J = 9 and 4 Hz, 1H, CH₃H₃OH), 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). ^13C NMR (100 MHz, CDCl₃): 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_{3}O^{+} 320.1763, found 320.1711 [MH^+].

3-(5-Methoxy-1H-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (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, 3017, 2938, 1480, 757 cm⁻¹. ^1H NMR (400 MHz, CDCl₃): δ 2.05 (br, 1 H, NH), 3.18 (d, J = 2 Hz, 1H, CH₃H₃), 3.20 (s, 1H, CH₃H₃), 3.52 (dd, J = 11 and 9 Hz, 1H, CH₂CH₂OH), 3.64 (t, J = 6 Hz, 1H, α-H), 3.72 (dd, J = 11 and 4 Hz, 1H, CH₃H₃OH), 3.78 (s, 3H, OCH₃), 4.05 (dd, J = 9 and 4 Hz, 1H, CHCH₂OH), 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). ^13C NMR (100 MHz, CDCl₃): δ 29.7, 48.9, 55.9, 63.1, 67.3, 100.2, 109.1, 112.1, 112, 120.4, 124.3, 127.6, 128.2, 128.8, 131.3, 138.3, 154.2. MS: m/z (+ESI) calcd for C_{20}H₂₃N₂O₂^{+} 336.1712, found 336.1786 [MH^+].
3-(4-Methoxy-1H-indol-3-yl)-2-(2-hydroxy-1-phenylethylamino)propanenitrile (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 $R_\text{f} = 0.5$ (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): $\nu = 3401, 2924, 1357, 1255, 733$ cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 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, $\alpha$-H), 3.93 (dd, $J = 9$ and 6 Hz, 1H, $CH_AH_{BOH}$), 4.14 (dd, $J = 9$ and 4 Hz, 1H, $CHCH_{BOH}$), 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$_3$): $\delta$ 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$_3$O$_2^+$ 336.1712, found 336.1656 [MH$^+$].

S9
2.3 General procedure for the preparation of \((S)-2-((R)-2\text{-hydroxy-1-phenylethylamino})-3-(1H\text{-indol-3-yl})\text{propanamide} \ 6

\((S)-2-((R)-2\text{-hydroxy-1-phenylethylamino})-3-(1H\text{-indol-3-yl})\text{propanamide} \ ((S,R)-6a)

To a solution of \(\alpha\)-aminonitrile \(4a\) (257 mg, 0.8 mmol) in DMSO (3.5 mL), K\(_2\)CO\(_3\) (138 mg, 1.3 mmol) and 30 \% H\(_2\)O\(_2\) (0.6 mL, 6.3 mmol) were added at 20 °C. After stirring at room temperature for 2 h, another portion of 30 \% H\(_2\)O\(_2\) (0.4 mL, 4.7 mmol) was added and stirred for a further hour. The resultant mixture was extracted with Et\(_2\)O (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): \(\nu = 3412, 2917, 1662, 744 \text{ cm}^{-1}\). \(^1\)H NMR (400 MHz, acetone-\(d_6\)): \(\delta 3.10 \text{ (br, 1 H, NH)}, 3.24 \text{ (dd, } J = 14 \text{ and } 6 \text{ Hz, 1H, CH}_A\text{H}_B\), 3.20 \text{ (d, } J = 6 \text{ Hz, 1H, CH}_A\text{H}_B\), 3.36 \text{ (dd, } J = 9 \text{ and } 3 \text{ Hz, 1H, } \alpha\text{-H}), 3.51 \text{ (dd, } J = 10 \text{ and } 8 \text{ Hz, 1H, CH}_A\text{H}_B\text{OH}), 3.60 \text{ (dd, } J = 11 \text{ and } 4 \text{ Hz, 1H, CH}_A\text{H}_B\text{OH}), 3.96 \text{ (dd, } J = 9 \text{ and } 4 \text{ Hz, 1H, CHCH}_2\text{OH}), 7.00 \text{ (m, 1H, Ar H), 7.10 \text{ (m, 1H, Ar H), 7.22–7.33 \text{ (m, 5H, Ar Hs), 7.36-7.41 \text{ (m, 2H, Ar Hs), 7.69 \text{ (d, } J = 8 \text{ Hz 1H, Ar H), 10.16 \text{ (br, 1H, NH)}})}\). \(^{13}\)C NMR (100 MHz, acetone-\(d_6\)): \(\delta 26.6, 59.5, 63.6, 66.7, 110.4, 111.1, 118.5, 119.2, 121.2, 124.3, 127.3, 128.0, 128.0, 128.2, 136.8, 141.3, 176.3\). MS: \(m/z\) (+ESI) calcd for C\(_{19}\)H\(_{22}\)N\(_3\)O\(_2^+\) 324.1712, found 324.1638 [MH\(^+\)].

\((S,R)-6a\) TLC \(R_f = 0.4\) (CHCl\(_3\)–MeOH, 6:1), IR (KBr): \(\nu = 3412, 2917, 1662, 744 \text{ cm}^{-1}\). \(^1\)H NMR (400 MHz, acetone-\(d_6\)): \(\delta 2.97 \text{ (dd, } J = 15 \text{ and } 8 \text{ Hz, 1H, CH}_A\text{H}_B\), 3.22 \text{ (dd, } J = 14 \text{ and } 5 \text{ Hz, 1H, CH}_A\text{H}_B\), 3.31 \text{ (dd, } J = 9 \text{ and } 5 \text{ Hz, 1H, } \alpha\text{-H}), 3.46 \text{ (dd, } J = 11 \text{ and } 8 \text{ Hz, 1H, CH}_A\text{H}_B\text{OH}), 3.56 \text{ (dd, } J = 11 \text{ and } 5 \text{ Hz, 1H, CHCH}_2\text{OH}), 3.77 \text{ (dd, } J = 9 \text{ and } 4 \text{ Hz, 1H, CHCH}_2\text{OH}), 6.71 \text{ (br, 1H, NH), 6.92–7.13 \text{ (m, 7H, Ar Hs), 7.14 \text{ (d, } J = 2 \text{ Hz, 1H, Ar H), 7.45 \text{ (m, 3H, Ar H), 10.17 \text{ (br, 1H, NH)}})}\). \(^{13}\)C NMR (100 MHz, acetone-\(d_6\)): \(\delta 30.0, 60.4, 63.5, 67.2, 111.1, 111.3, 118.6, 121.3, 123.6, 126.8, 127.2, 127.8, 127.9, 136.8, 140.9, 177.1\). MS: \(m/z\) (+ESI) calcd for C\(_{19}\)H\(_{22}\)N\(_3\)O\(_2^+\) 324.1712, found 324.1365 [MH\(^+\)].

\((S)-3-(5\text{-Bromo-1H\text{-indol-3-yl})-2-((S)-1-(4\text{-methoxyphenyl}ethy lamino)propanamide (}((S,S)-13)

Synthesis was carried out according to the procedure described under section 2.3 using \(\alpha\)-aminonitrile \(S1\) (356 mg, 0.9 mmol) in DMSO (3.5 mL), K\(_2\)CO\(_3\) (172 mg, 1.2 mmol) and 30 \% H\(_2\)O\(_2\) (0.8 mL, 7.3 mmol), followed by a further 30 \% H\(_2\)O\(_2\) (0.4 mL, 2.3 mmol). The synthesized compound was
purified column chromatography (Hexane–EtOAc, 1 : 4) followed by RP-HPLC (Onyx Monolithic C18, 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): \( \nu = 3267, 3006, 2961, 1669, 1244, 755 \) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.24 (d, \( J = 7 \) Hz, 3H, CH\(_3\)), 1.83 (br, 1H, NH), 2.79 (dd, \( J = 14 \) and 9 Hz, 1H, CH\(_2\)H\(_3\)), 3.22 (m, 2H, CH\(_2\)H\(_3\), \( \alpha\)-H), 3.55 (q, \( J = 7 \) Hz, 1H, CHCH\(_3\)), 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). \(^1\)C NMR (100 MHz CDCl\(_3\)): \( \delta \) 24.1, 29.6, 55.2, 56.4, 59.8, 110.9, 112.7, 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\(_3\)O\(_2\) \(+ 416.0974\), found 416.0773 \([\text{MH}^+\])

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

Synthesis was carried out according to the procedure described under section 2.3 using \( \alpha\)-aminonitrile S\(_2\) (542 mg, 1.5 mmol) in DMSO (3.5 mL), K\(_2\)CO\(_3\) (275 mg, 2.0 mmol) and 30 % H\(_2\)O\(_2\) (1.1 mL, 11.6 mmol), followed by a further portion of 30 % H\(_2\)O\(_2\) (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): \( \nu = 3269, 2961, 2928, 1670, 1279, 755 \) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta \) 1.24 (d, \( J = 7 \) Hz, 3H, CH\(_3\)), 1.85 (br, 1H, NH), 2.79 (dd, \( J = 14 \) and 9 Hz, 1H, CH\(_2\)H\(_3\)), 3.22 (m, 2H, CH\(_2\)H\(_3\), \( \alpha\)-H), 3.54 (q, \( J = 6 \) Hz, 1H, CHCH\(_3\)), 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, 2 H, Ar Hs), 7.31 (d, \( J = 2 \) Hz, 1H, Ar H), 8.75 (br, 1H, NH). \(^1\)C NMR (100 MHz CDCl\(_3\)): \( \delta \) 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\(_3\)O\(_2\) \(+ 372.1479\), found 372.1255 \([\text{MH}^+\])

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

The \( \alpha\)-aminonitrile 4d (557 mg, 1.7 mmol) in DMSO (4 mL), K\(_2\)CO\(_3\) (308 mg, 2.2 mmol) and 30 % H\(_2\)O\(_2\) (1.3 mL, 13 mmol) were reacted, followed by an additional 30 % H\(_2\)O\(_2\) (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): \( \nu = 3423, 3311, 2919, 1664, 759 \) cm\(^{-1}\). \(^1\)H NMR (400 MHz, acetone-d\(_6\)): \( \delta \) 3.23 (dd, \( J = 14 \) and 5 Hz, 1H, CH\(_2\)H\(_3\)), 3.32 (m, 2H, CH\(_2\)H\(_3\), \( \alpha\)-H), 3.52 (dd, \( J = 11 \) and 9 Hz, 1H, CH\(_2\)H\(_3\)OH), 3.62 (dd, \( J = 11 \) and 4 Hz, 1H, CH\(_2\)H\(_3\)OH), 3.97 (dd, \( J = 9 \) and 4 Hz, 1H, CHCH\(_2\)OH), 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). \(^1\)C NMR (100 MHz, acetone-d\(_6\)): \( \delta \) 26.5, 59.3, 63.6, 66.9, 97.1 (d, \( J = 26 \) Hz), 106.9 (d, \( J = 24 \) Hz).
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_{3}O_{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}. \(^1\)H NMR (400 MHz, acetone-d_{6}): δ 2.95 (dd, J = 16 and 8 Hz, 1H, CH_{A}H_{B}), 3.21 (dd, J = 14 and 5 Hz, 1H, CH_{A}H_{B}), 3.30 (dd, J = 9 and 5 Hz, 1H, CH_{A}H_{B}), 3.48 (dd, J = 11 and 8 Hz, 1H, CH_{A}H_{B}), 3.62 (dd, J = 11 and 4 Hz, 1H, CH_{A}H_{B}), 3.77 (dd, J = 9 and 4 Hz, 1H, CH_{A}H_{B}), 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). \(^1\)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, 128.3, 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_{3}O_{2}^{+} 342.1618, found 342.1298 [MH^{+}].

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

Synthesis was carried out using α-amino-nitrile 4e (396 mg, 1.1 mmol) in DMSO (3 mL), K_{2}CO_{3} (172 mg, 1.4 mmol) and 30 % H_{2}O_{2} (0.8 mL, 8.4 mmol), followed by a further portion of 30 % H_{2}O_{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}. \(^1\)H NMR (400 MHz, acetone-d_{6}): δ 1.32 (t, J = 8 Hz, 3H, CH_{2}C_{3}H_{5}), 2.91 (q, J = 8 Hz, 2H, CH_{2}CH_{3}), 3.24 (dd, J = 14 and 5 Hz, 1H, CH_{A}H_{B}), 3.23 (d, J = 7 Hz, 1H, CH_{A}H_{B}), 3.34 (d, J = 6 Hz, 1H, α-H), 3.51 (dd, J = 12 and 5 Hz, 1H, CH_{A}H_{B}), 3.61 (dd, J = 11 and 5 Hz, 1H, CH_{A}H_{B}), 3.97 (dd, J = 9 and 5 Hz, 1H, CH_{A}H_{B}), 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). \(^1\)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, 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_{3}O_{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}. \(^1\)H NMR (400 MHz, acetone-d_{6}): δ 1.35 (t, J = 8 Hz, 3H, CH_{2}C_{3}H_{5}), 2.94 (q, J = 8 Hz, 2H, CH_{2}CH_{3}), 3.21 (dd, J = 15 and 5 Hz, 1H, CH_{A}H_{B}), 3.23 (dd, J = 16 and 8 Hz, 1H, CH_{A}H_{B}), 3.30 (dd, J = 9 and 5 Hz, 1H, α-H), 3.45 (dd, J = 11 and 8 Hz, 1H, CH_{A}H_{B}), 3.60 (dd, J = 12 and 4 Hz, 1H, CH_{A}H_{B}), 3.76 (dd, J = 8 and 4 Hz, 1H, CH_{A}H_{B}), 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). \(^1\)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_{3}O_{2}^{+} 352.1947, found 352.1595 [MH^{+}].
The α-aminonitrile 4f (468 mg, 1.5 mmol) in DMSO (3 mL), K₂CO₃ (261 mg, 1.9 mmol) and 30 % H₂O₂ (1.3 mL, 11.1 mmol), and a further 30 % H₂O₂ (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 Rₓ = 0.6 (CHCl₃–MeOH, 6:1); IR (KBr): ν = 3310, 2919, 1662, 756 cm⁻¹. ¹H NMR (400 MHz, acetone-d₆): δ 2.39 (s, 3H, CH₃), 3.22 (dd, J = 6 and 4 Hz, 2H, CH₂Hₓ), 3.32 (t, J = 6 Hz, α-H), 3.49 (dd, J = 11 and 9 Hz, 1H, CHₓH₀H), 3.58 (dd, J = 11 and 4 Hz, 1H, CHₓH₀H), 3.93 (dd, J = 9 and 4 Hz, 1H, CHₓH₀H), 6.31 (br, 1H, NH), 6.94 (dd, J = 8 and 2 Hz, 1H, Ar H), 7.22–7.33 (m, 5H, Ar Hs), 7.34-7.39 (m, 2H, Ar Hs), 7.44 (s, 1H, Ar H), 9.97 (br, 1H, NH). ¹³C NMR (100 MHz, acetone-d₆): δ 20.8, 26.8, 59.5, 63.6, 66.8, 110.1, 110.8, 118.8, 122.8, 124.2, 127.2, 127.9, 128.2, 128.3, 135.2, 141.5, 176.3. MS: m/z (+ESI) calcd for C₂₀H₂₄N₃O₂⁺ 338.1869, found 338.1823 [MH⁺].

(S,R)-6f TLC Rₓ = 0.5 (CHCl₃–MeOH, 6:1); IR (KBr): ν = 3310, 2919, 1662, 756 cm⁻¹. ¹H NMR (400 MHz, acetone-d₆): δ 2.33 (s, 3H, CH₃), 2.91 (dd, J = 15 and 9 Hz, 1H, CHₓHₓ), 3.18 (dd, J = 14 and 4 Hz, 1H, CHₓHₓ), 3.29 (dd, J = 9 and 5 Hz, 1H, α-H), 3.45 (dd, J = 12 and 8 Hz, 1H, CHₓH₀H), 3.59 (dd, J = 12 and 8 Hz, 1H, CHₓH₀H), 3.75 (dd, J = 8 and 4 Hz, 1H, CHₓH₀H), 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). ¹³C NMR (100 MHz, acetone-d₆): 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 C₂₀H₂₄N₃O₂⁺ 338.1869, found 338.1942 [MH⁺].

The α-aminonitrile 4g (266 mg, 0.8 mmol) in DMSO (3 mL), K₂CO₃ (141 mg, 1.0 mmol) and 30 % H₂O₂ (0.6 mL, 5.9 mmol), followed by a further 30 % H₂O₂ (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 Rₓ = 0.6 (CHCl₃–MeOH, 6:1); IR (KBr): ν = 3331, 2931, 1661, 1481, 1216, 756 cm⁻¹. ¹H NMR (400 MHz, acetone-d₆): δ 2.93 (br, 1H, NH), 3.18 (dd, J = 15 and 7 Hz, 1H, CHₓHₓ), 3.30 (m, 2H, CHₓHₓ, α-H), 3.50 (dd, J = 11 and 9 Hz, 1H, CHₓH₀H), 3.60 (dd, J = 11 and 4 Hz, 1H, CHₓH₀H), 3.78 (s, 3H, OCH₃), 3.98 (dd, J = 8 and 4 Hz, 1H, CHₓH₀H), 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). ¹³C NMR (100 MHz, acetone-d₆): 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): v = 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\_A\_\text{H}_\text{B}), 3.15 (dd, J = 14 and 5 Hz, 1H, CH\_A\_\text{H}_\text{B}), 3.28 (dd, J = 9 and 5 Hz, 1H, α-H), 3.45 (dd, J = 11 and 8 Hz, 1H, CH\_A\_\text{H}_\text{B}), 3.59 (dd, J = 11 and 4 Hz, 1H, CH\_A\_\text{H}_\text{B}), 3.70 (s, 3H, OCH_3) 3.75 (dd, J = 9 and 4 Hz, 1H, CH\_A\_\text{H}_\text{B}), 4.56 (br, 1H, NH), 6.56 (br, 1H, NH), 6.76 (dd, J = 9 and 2 Hz, 1H, Ar H), 6.95 (d, J = 9 Hz, 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). ^13C 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 354.1914 [MH^+].

(S)-3-(4-Methoxy-1H-indol-3-yl)-2-((R)-2-hydroxy-1-phenylethylamino)propanamide ((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): v = 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\_A\_\text{H}_\text{B}), 3.46 (m, 4 H, CH\_A\_\text{H}_\text{B}, α-H, CH\_A\_\text{H}_\text{B}, CH\_A\_\text{H}_\text{B}, CH\_A\_\text{H}_\text{B}), 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). ^13C NMR (100 MHz, acetone-d_6): δ 29.9, 54.3, 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 = 0.6 (CHCl_3–MeOH, 6:1); IR (KBr): v = 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\_A\_\text{H}_\text{B}), 3.32 (dd, J = 14 and 5 Hz, 1H, CH\_A\_\text{H}_\text{B}), 3.37 (dd, J = 8 and 5 Hz, 1H, α-H), 3.45 (dd, J = 12 and 8 Hz, 1H, CH\_A\_\text{H}_\text{B}), 3.59 (dd, J = 11 and 4 Hz, 1H, CH\_A\_\text{H}_\text{B}), 3.70 (s, 3H, OCH_3), 3.71 (dd, J = 8 and 4 Hz, 1H, CH\_A\_\text{H}_\text{B}), 4.60 (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, 1H, Ar H), 7.22 (br, 1H, NH), 10.10 (br, 1H, NH). ^13C 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-(1H-indol-3-yl)propanamide 7

(S)-2-Amino-3-(1H-indol-3-yl)propanamide (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 (CH₂Cl₂–MeOH, 4:1) yielded the amino amide which was acidified with 1M HCl to give 7a hydrochloric salt as a white solid (30 mg, 41 %): m.p. 256–257 °C (Lit: m.p. 254-255 °C); TLC Rt = 0.2 (CHCl₃–MeOH, 4:1). IR (KBr): ν = 3388, 3247, 2914, 1693, 1492, 751 cm⁻¹. ¹H NMR (400 MHz, D₂O): δ 3.23 (dd, J = 15 and 7 Hz, 1H, C₆H₄A B), 3.30 (dd, J = 15 and 7 Hz, 1H, C₆H₄A 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). ¹³C NMR (100 MHz, D₂O): 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) calced for C₁₁H₁₄N₃O⁺ 204.1137, found 204.1016 [MH⁺].

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

A solution of 13 (510 mg, 1.2 mmol) and iPr₂SiH (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-1H-indol-3-yl)propanamide (7c)

![Chemical Structure of 7c]

A solution of 14 (446 mg, 1.2 mmol) and iPr$_3$SiH (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-1H-indol-3-yl)propanamide (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): $v = 3472, 3271, 2983, 1658, 1451$ cm$^{-1}$. $^1$H NMR (400 MHz, D$_2$O): $\delta$ 3.13 (dd, $J = 14$ and 7 Hz, 1H, CH$_A$), 3.20 (dd, $J = 14$ and 7 Hz, 1H, CH$_B$), 4.17 (t, $J = 7$ Hz, 1H, CH$_A$), 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$_2$O): $\delta$ 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$_3$O$^+$ 222.1043, found 222.0912 [MH$^+$].

(S)-2-Amino-3-(7-ethyl-1H-indol-3-yl)propanamide (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; TLC $R_f = 0.2$ (CHCl$_3$–MeOH, 4:1); IR (KBr): $v = 3411, 3154, 3045, 1687, 1413$ cm$^{-1}$. $^1$H NMR (400 MHz, D$_2$O): $\delta$ 1.13 (t, $J = 8$ Hz, 3H, CH$_3$), 2.69 (q, $J = 8$ Hz, 2H, CH$_2$CH$_3$), 3.16 (dd, $J = 15$ and 7 Hz, 1H, CH$_A$H$_B$), 3.22 (dd, $J = 16$ and 7 Hz, 1H, CH$_A$H$_B$), 4.18 (t, $J = 7$ Hz, 1H, CH$_B$), 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$_2$O): $\delta$ 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$_3$O$^+$ 232.1450, found 232.1390 [MH$^+$].
(S)-2-Amino-3-(5-methyl-1H-indol-3-yl)propanamide (7f)

The compound 6f (94 mg, 0.3 mmol) was treated with 10 % 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 Rf = 0.2 (CHCl3–MeOH, 4:1); IR (KBr): ν = 3402, 2916, 1668, 1431 cm⁻¹. ¹H NMR (400 MHz, D₂O): δ 2.33 (s, 3H, CH₃), 3.05 (dd, J = 15 and 7 Hz, 1H, CH₃H₆), 3.12 (dd, J = 16 and 7 Hz, 1H, CH₃H₇), 3.84 (t, J = 6 Hz, 1H, α-H), 6.90 (dd, J = 8 and 1 Hz, 1H, Ar H), 7.12 (s, 1H, C₂H), 7.30 (d, J = 8 Hz, 1H, Ar H), 7.38 (s, 1H, Ar H). ¹³C NMR (100 MHz, D₂O): 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 C₁₂H₁₆N₃O⁺ 218.1293, found 218.1220 [MH⁺].

(S)-2-Amino-3-(5-methoxy-1H-indol-3-yl)propanamide (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 Rf = 0.2 (CHCl₃–MeOH, 4:1). IR (KBr): ν = 3354, 3167, 2954, 1687, 1462 cm⁻¹. ¹H NMR (400 MHz, D₂O): δ 3.17 (dd, J = 15 and 8 Hz, 1H, CH₃H₆), 3.23 (dd, J = 15 and 7 Hz, 1H, CH₃H₇), 3.75 (s, 3H, OCH₃), 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). ¹³C NMR (100 MHz, D₂O): 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 C₁₂H₁₆N₃O₂⁺ 234.1243 found 234.1187 [MH⁺].

(S)-2-Amino-3-(4-methoxy-1H-indol-3-yl)propanamide (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 Rf = 0.2 (CHCl3–MeOH, 4:1). IR (KBr): ν = 3354, 3168, 2955, 1687, 1463 cm⁻¹. 1H NMR (400 MHz, D₂O): δ 3.22 (dd, J = 15 and 7 Hz, 1H, CH₂H₆), 3.35 (dd, J = 15 and 7 Hz, 1H, CH₂H₆), 3.84 (s, 3H, OCH₃), 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). 13C NMR (100 MHz, D₂O): 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 C₁₂H₁₆N₃O₂⁺ 234.1243 found 234.1096 [MH⁺]
2.5 General procedure for the preparation of (S)-tryptophans 8

(S)-Tryptophan•HCl (8a)

![Structure of 8a](image)

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 as a white solid (100 mg, 83 % yield): m.p. 246–247 °C; IR (KBr): \(v = 3386, 2941, 1735 \text{ cm}^{-1}\); \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 3.23\) (dd, \(J = 16, 7 \text{ Hz}, 1\text{H}, \text{CH}_2\text{NH})\), 3.37 (dd, \(J = 15\) and 6 Hz, 1H, \(\text{CH}_3\text{H}_9\)), 4.23 (dd, \(J = 8\) and 6 Hz, 1H, \(\alpha\)-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\(_2\)O): \(\delta = 25.7, 53.2, 106.2, 112.0, 118.2, 119.5, 122.1, 125.3, 126.4, 136.2, 171.8\). MS: \(m/z\) (+ESI) calcd for C\(_{11}\)H\(_{13}\)N\(_2\)O\(_2\)\(^+\) 205.0977, found 205.0953 [MH\(^+\)].

(S)-5-Bromotryptophan•HCl (8b)

![Structure of 8b](image)

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 as a yellow solid (78 mg, 99 % yield): m.p. 220–221 °C; \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 3.20\) (dd, \(J = 15\) and 7 Hz, 1H, \(\text{CH}_2\text{NH})\), 3.28 (dd, \(J = 15\) and 5 Hz, 1H, \(\text{CH}_3\text{H}_9\)), 4.18 (dd, \(J = 7\) and 5 Hz, 1H, \(\alpha\)-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\(_2\)O): \(\delta = 25.6, 53.3, 106.0, 112.0, 113.5, 120.5, 124.6, 126.5, 128.2, 134.2, 172.0\). MS: \(m/z\) (+ESI) calcd for C\(_{11}\)H\(_{12}\)BrN\(_2\)O\(_2\)\(^+\) 283.0082, found 283.0150 [MH\(^+\)].

(S)-5-Chlorotryptophan•HCl (8c)

![Structure of 8c](image)

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 as a yellow solid (59 mg, 95 % yield): m.p. 230–233 °C; IR (KBr): \(v = 3456, 3138, 3037, 1730, 1407 \text{ cm}^{-1}\); \(^1\)H NMR (400 MHz, D\(_2\)O): \(\delta = 3.16\) (dd, \(J = 15\) and 7
Hz, 1H, CH₃H), 3.23 (dd, J = 15 and 5 Hz, 1H, CH₃H), 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). ¹³C NMR (100 MHz, D₂O): δ 25.6, 53.2, 106.0, 117.3, 122.0, 124.4, 126.6, 127.5, 134.6, 171.9 ppm. MS: m/z (+ESI) calced for C₁₁H₁₂ClN₂O₂⁺ 239.0587, found 239.0587 [MH⁺].

(S)-6-Fluorotryptophan•HCl (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 as a white solid (92 mg, 90% yield): m.p. 218–220 °C; IR (KBr): ν = 3458, 3015, 1732, 1412 cm⁻¹. ¹H NMR (400 MHz, D₂O): δ 3.26 (dd, J = 15 and 7 Hz, 1H, CH₂H), 3.33 (dd, J = 15 and 5 Hz, 1H, CH₂H), 4.22 (dd, J = 15 and 7 Hz, 1H, CH₂H), 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). ¹³C NMR (100 MHz, D₂O): 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) calced for C₁₁H₁₂FN₂O₂⁺ 223.0883, found 223.0752 [MH⁺].

(S)-7-Ethyltryptophan•HCl (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. ¹H NMR (400 MHz, D₂O): δ 1.14 (t, J = 8 Hz, 3H, CH₂CH₃), 2.71 (q, J = 8 Hz, 2H, CH₂CH₃), 3.22 (dd, J = 15 and 7 Hz, 1H, CH₃CH₃), 3.31 (dd, J = 15 and 5 Hz, 1H, CH₃CH₃), 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). ¹³C NMR (100 MHz, D₂O): δ 13.4, 23.7, 25.8, 53.3, 106.7, 115.9, 119.9, 120.6, 125.0, 128.3, 134.9, 171.9. MS: m/z (+ESI) calced for C₁₃H₁₇N₂O₂⁺ 233.1290, found 233.1174 [MH⁺].
(S)-5-Methyltryptophan•HCl (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. ¹H NMR (400 MHz, D₂O): δ 2.32 (s, 3H, CH₃), 3.25 (dd, J = 15 and 7 Hz, 1H, CH₂H₃), 3.35 (dd, J = 15 and 5 Hz, 1H, CH₂H₄), 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). ¹³C NMR (100 MHz, D₂O): δ 20.4, 25.8, 53.3, 105.7, 111.8, 117.6, 123.7, 125.5, 126.7, 134.6, 172.0 ppm. MS: m/z (+ESI) calcd for C₁₂H₁₅N₂O₂⁺ 219.1134, found 219.1036 [MH⁺].

(S)-5-Methoxytryptophan•HCl (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 as a white solid (38 mg, 99 % yield): m.p. 224–225 °C; IR (KBr): ν = 3358, 2909, 1728, 1478, 1214 cm⁻¹. ¹H NMR (400 MHz, D₂O): δ 3.22 (dd, J = 15 and 7 Hz, 1H, CH₂H₃), 3.30 (dd, J = 15 and 5 Hz, 1H, CH₂H₄), 3.74 (s, 3H, OCH₃), 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, C₂H), 7.28 (d, J = 8 Hz, Ar H). ¹³C NMR (100 MHz, D₂O): 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 C₁₂H₁₅N₂O₃⁺ 235.1083, found 235.1068 [MH⁺].

(S)-4-Methoxytryptophan•HCl (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. ¹H NMR (400 MHz, D₂O): δ 3.24 (dd, J = 15 and 7 Hz, 1H, CH₂H₃), 3.37 (dd, J = 15 and 7 Hz, 1H, CH₂H₄), 3.86 (s, 3H, OCH₃), 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). ¹³C NMR (100 MHz, D₂O): 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 C₁₂H₁₅N₂O₃⁺ 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)

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 Et₂O (2 x 15 mL). The pH of the aqueous layer was adjusted to 2 using 3 M aq HCl and was extracted with CH₂Cl₂ (2 x 20 mL). The organic extracts were combined and washed with brine, dried over MgSO₄ and concentrated in vacuo to give 9a as a pale yellow solid (114 mg, 44 % yield): m.p. 182–185 °C (Lit. m.p. 170–172 °C); [α]_24^D = −29.0 (c = 1, MeOH) (Lit. [α]_25^D = −29.5 (c = 1, DMF)); IR (KBr): ν = 3416, 3057, 2950, 1710, 743 cm⁻¹. ¹H NMR (400 MHz, acetone-d₆): δ 3.31 (dd, J = 15 and 8 Hz, 1H, C₆H₄CH), 3.46 (dd, J = 15 and 5 Hz, 1H, C₆H₄CH), 4.20 (t, J = 8 Hz, 1H, Fmoc CH), 4.31 (m, 2H, Fmoc CH₂), 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). ¹³C NMR (100 MHz, acetone-d₆): 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, 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 C₂₆H₂₃N₂O₄⁺ 427.1658, found 427.1743 [MH⁺].
(S)-N-(Fluoren-9-ylmethoxycarbonyl)-5-bromo-tryptophan (9b)

8b (66 mg, 0.21 mmol), Na2CO3 (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; [α]24D = –23.0 (c = 1, MeOH); IR (KBr): ν = 3411, 3060, 2950, 1709, 737 cm⁻¹. 1H NMR (400 MHz, acetone-d₆): δ 3.25 (dd, J = 16 and 8 Hz, 1H, CH₁₆H₉₀), 3.41 (dd, J = 16 and 5 Hz, 1H, CH₁₁H₂₀), 4.20 (t, J = 8 Hz, 1H, Fmoc CH), 4.30 (m, 2H, Fmoc CH₂), 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). 13C NMR (100 MHz, acetone-d₆): 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, 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 C₂₆H₂₂BrN₂O₄⁺ 505.0763, found 505.0978 [MH⁺].

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

8c (59 mg, 0.25 mmol), Na₂CO₃ (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; [α]24D = –16.0 (c = 1, MeOH); IR (KBr): ν = 3414, 3058, 2953, 1710, 748 cm⁻¹. 1H NMR (400 MHz, acetone-d₆): δ 3.26 (dd, J = 15 and 8 Hz, 1H, CH₁₆H₉₀), 3.41 (m, 1H, CH₁₁H₂₀), 4.20 (t, J = 8 Hz, 1H, Fmoc CH), 4.30 (m, 2H, Fmoc CH₂), 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). 13C NMR (100 MHz, acetone-d₆): 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, 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 C₂₆H₂₂ClN₂O₄⁺ 461.1268, found 461.1317 [MH⁺].

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

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; [α]24D = –19.0 (c = 1, MeOH); IR (KBr): ν = 3419, 3064, 2952, 1710, 745 cm⁻¹. 1H NMR (400 MHz, acetone-d₆): δ 3.26 (dd, J = 14 and 8 Hz, 1H, CH₁₆H₉₀), 3.41 (dd, J = 14 and 5 Hz, 1H, CH₁₁H₂₀), 4.21 (t, J = 8 Hz, 1H, Fmoc CH), 4.31 (m, 2H, Fmoc CH₂), 4.64 (m, 1H, α-H),...
6.68 (d, $J = 8$ Hz, NH), 6.87 (dt, $J = 8$ 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$_6$): $\delta$ 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, 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$_2$O$_4$ $^+ 445.1564$, found 445.1790 [MH$^+]$.

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

![9e](image)

Compound 8e (102 mg, 0.38 mmol), Na$_2$CO$_3$ (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): $\nu$ = 3422, 3057, 2964, 1711, 746 cm$^{-1}$. $^1$H NMR (400 MHz, acetone-d$_6$): $\delta$ 1.31 (t, $J = 8$ Hz, 3H, CH$_2$C$_3$H$_7$), 2.91 (q, $J = 8$ Hz, 2H, C$_2$H$_2$CH$_3$), 3.27 (m, $J = 8$ 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$_6$): $\delta$ 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$_2$O$_4$ $^+ 455.1971$, found 455.2142 [MH$^+]$.

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

Compound 8f (48 mg, 0.19 mmol), Na$_2$CO$_3$ (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): $\nu$ = 3424, 3038, 2920, 1708, 1448 cm$^{-1}$. $^1$H NMR (400 MHz, acetone-d$_6$): $\delta$ 2.42 (s, 3H, CH$_3$), 3.25 (m, $J = 8$ Hz, 1H, CH$_3$CH$_2$), 4.20 (t, $J = 7$ Hz, 1H, Fmoc CH), 4.29 (m, 2H, Fmoc CH$_2$), 4.65 (m, 1H, $\alpha$-H), 6.65 (d, $J = 8$ Hz, 1H, NH), 6.70 (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), 8.15 (d, $J = 7$ Hz, 2H, Ar Hs), 9.97 (br, 1H, NH). $^{13}$C NMR (100 MHz, acetone-d$_6$): $\delta$ 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$_2$O$_4$ $^+ 441.1814$, found 441.2083 [MH$^+]$. 

S24
(S)-N-(Fluoren-9-ylmethoxycarbonyl)-5-methoxy-tryptophan (9g)

[Chemical structure diagram]

Compound 8g (300 mg, 1.28 mmol), Na2CO3 (271 mg, 2.56 mmol) and 9-fluorenylmethyl succinimidyl carbonate (431 mg, 1.28 mmol) were used to yield 9g as a white powder (479 mg, 82 % yield): m.p. 198 °C; [α]24D = –23 (c = 1, MeOH) (Lit6 [α]24D = –24.5 (c = 1, MeOH)); IR (KBr): ν = 3415, 2949, 1703, 1493, 1218 cm−1. 1H NMR (400 MHz, acetone-d6): δ 3.25 (dd, J = 15 and 8 Hz, 1H, CHAB), 3.39 (dd, J = 15 and 5 Hz, 1H, CHAB), 3.82 (s, 3H, OCH3), 4.20 (t, J = 7 Hz, 1H, Fmoc CH), 4.29 (m, 2H, Fmoc CH2), 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 (m, 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). 13C NMR (100 MHz, acetone-d6): 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 C27H25N2O5+ 457.1763, found 457.1913 [MH+].

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

[Chemical structure diagram]

Compound 8h (38 mg, 0.14 mmol), Na2CO3 (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; [α]24D = –27 (c = 1, MeOH); IR (KBr): ν = 3415, 2949, 1703, 1494, 1218 cm−1. 1H NMR (400 MHz, acetone-d6): δ 3.20 (dd, J = 15 and 9 Hz, 1H, CHAB), 3.34 (dd, J = 15 and 5 Hz, 1H, CHAB), 3.76 (s, 3H, OCH3), 4.15 (t, J = 7 Hz, 1H, Fmoc CH), 4.24 (m, 2H, Fmoc CH2), 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). 13C NMR (100 MHz, acetone-d6): 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 C27H25N2O5+ 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\(_2\)Cl\(_2\) (6 mL) for 1 h. A solution of Fmoc-sarcosine (373 mg, 1.2 mmol) and DIPEA (418 µL, 2.4 mmol) in CH\(_2\)Cl\(_2\) (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\(_2\)Cl\(_2\) (15 mL) and hexane (5 mL), and dried \textit{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\(_2\)Cl\(_2\) (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\(_2\)Cl\(_2\) and hexane, and dried \textit{in vacuo}.

The resin product was suspended in a mixture of water (0.25 mL), iPr\(_3\)SiH (0.25 mL) and CH\(_2\)Cl\(_2\) (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\(_2\)Cl\(_2\) (3 mL), and the filtrate was evaporated to dryness \textit{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)

(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)-phenylselenocysteine (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\(_2\)O (40 mL), acidified with saturated aqueous KHSO\(_4\) (30 mL), extracted with ethyl acetate (3 x 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): \(v = 3358, 3063, 1727, 1711, 1682, 1449, 1248\) and 758 cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 3.37\) (dd, \(J = 4\) and 16 Hz, 1H, CH\(_2\)), 3.45 (dd, \(J = 4\) and 16 Hz, 1H, CH\(_3\)), 4.20 (t, \(J = 8\) Hz, 1H, OCH\(_2\)), 4.36 (m, 2H, OC\(_2\)H), 4.77 (m, 1H, \(\alpha\)-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\(_3\)): \(\delta = 29.7, 47.1, 53.9, 120.0, 125.1, 127.1, 127.8, 127.9, 128.6, 129.3, 133.8, 141.3, 143.7, 143.7, 155.8, 174.9\). MS: \(m/z\) (+ESI) calcd for C\(_{24}\)H\(_{22}\)NO\(_4\)Se\(^+\) 468.0636 found 468.0135 [MH\(^+\)].

(R)-2-(1-tert-Butoxycarbonylaminoethyl)thiazole-4-carboxylic acid (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\(_2\)O, 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\(_2\)O (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\(_2\)Cl\(_2\) (3 x 40 mL). The organic layers were washed with brine, dried and concentrated. The residue was recrystallized with CH\(_2\)Cl\(_2\)-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): \(v = 3362, 3105, 2979, 1692, 1517, 1366, 1242, 1058\) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 1.47\) (s, 9H, C(CH\(_3\))\(_3\)), 1.66 (d, \(J = 6\) Hz, 3H, CHCH\(_3\)), 5.10 (br, s, 1H, \(\alpha\)-CH), 5.30 (br, s, 1H, \(\alpha\)-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\(_2\)O\(_4\)Na\(^+\) 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 CH₂Cl₂ (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C₁₈, 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, tᵣ 9.9 min. ¹H NMR (400 MHz, DMSO-d₆): δ 0.65 (d, J = 7 Hz, 3H, 5-CH₃), 1.56 (d, J = 7 Hz, 3H, 1-CH₃ (i.e. dipeptide residue 1 CH₃)), 3.13 (dd, J = 14 and 8 Hz, 1H, 3-CH₂H₄), 3.22 (s, 3H, 7-CH₃), 3.24 (m, 1H, 3-CH₃H₆), 3.31 (m, 1H, 4-CH₂H₅), 3.34 (m, 1H, 6-CH₂H₅), 3.45 (m, 1H, 6-CH₃H₅), 3.49 (m, 1H, 7-CH₃H₅), 3.50 (m, 1H, 2-CH₃H₆), 3.67 (m, 1H, 2-CH₃H₅), 3.74 (m, 1H, 4-CH₃H₅), 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-CH₃H₅), 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 = 3 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-CH₃H), 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 caled for C₄₅H₄₈N₁₀O₇SSe+ 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 CH₂Cl₂ (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C₁₈, 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, tᵣ 9.5 min. ¹H NMR (400 MHz, DMSO-d₆): δ 0.65 (d, J = 7 Hz, 3H, 5-CH₃), 1.56 (d, J = 7 Hz, 3H, 1-CH₃), 3.13 (dd, J = 14 and 8 Hz, 1H, 3-CH₂H₄), 3.22 (s, 3H, 7-CH₃), 3.24 (m, 1H, 3-CH₃H₆), 3.31 (m, 1H, 4-CH₂H₅), 3.34 (m, 1H, 6-CH₂H₅), 3.45 (m, 1H, 6-CH₃H₅), 3.49 (m, 1H, 7-CH₃H₅), 3.50 (m, 1H, 2-CH₃H₆), 3.67 (m, 1H, 2-CH₃H₅), 3.74 (m, 1H, 4-CH₃H₅), 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-CH₃H₅), 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 = 3 Hz, 1H, 1-NH), 7.95 (s, 1H, 1-CH₃H), 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 caled for C₄₅H₄₈N₁₀O₇SSe⁺ 1031.1777, found 1033.1757 [MH⁺].
4.35 (d, J = 17 Hz, 1H, 7-CHA\(_B\)), 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\(^3\)H), 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\(_7\)SSe\(^+\) 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\(_2\)Cl\(_2\) (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\(_6\)): \(\delta\) 0.65 (d, J = 7 Hz, 3H, 5-CH\(_3\)), 1.27 (t, J = 7 Hz, 3H, 3-CH\(_2\)C\(_3\)H\(_3\)), 1.56 (d, J = 7 Hz, 3H, 1-CH\(_3\)), 2.86 (q, J = 7 Hz, 2H, 3-CH\(_2\)CH\(_3\)), 3.12 (dd, J = 14 and 8 Hz, 1H, 3-CHA\(_BH\)), 3.22 (s, 3H, 7-CH\(_3\)), 3.26 (m, 1H, 3-CHA\(_BH\)), 3.31 (m, 1H, 4-CHA\(_BH\)), 3.34 (m, 1H, 6-CHA\(_BH\)), 3.47 (m, 1H, 6-CHA\(_BH\)), 3.49 (m, 1H, 7-CHA\(_BH\)), 3.50 (m, 1H, 2-CHA\(_BH\)), 3.67 (m, 1H, 2-CHA\(_BH\)), 3.74 (m, 1H, 4-CHA\(_BH\)), 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-CHA\(_BH\)), 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\(^3\)H), 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\(_7\)SSe\(^+\) 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 CH₂Cl₂ (120 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C₁₈, 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, tᵣ 9.4 min. ¹H NMR (400 MHz, DMSO-d₆): δ 0.65 (d, J = 7 Hz, 3H, 5-CH₃), 1.56 (d, J = 7 Hz, 3H, 1-CH₃), 3.11 (dd, J = 14 and 8 Hz, 1H, 3-CH₃H₉), 3.22 (s, 3H, 7-CH₃), 3.27 (m, 1H, 3-CH₅H₉), 3.31 (m, 1H, 4-CH₅H₉), 3.34 (m, 1H, 6-CH₅H₉), 3.45 (m, 1H, 6-CH₅H₉), 3.49 (m, 1H, 7-CH₅H₉), 3.67 (dd, J = 14 and 4 Hz, 1H, 2-CH₅H₉), 3.75 (dd, J = 17 and 4 Hz, 1H, 2-CH₅H₉), 3.76 (m, 1H, 4-CH₅H₉), 4.13 (q, J = 7 Hz, 1H, 5-α-CH), 4.20 (t, J = 8 Hz, 1H, 6-α-CH), 4.35 (d, J = 17 Hz, 1H, 7-CH₅H₉), 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-C₅H), 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 caleed for C₄₆H₅₁N₁₀O₇SSe⁺ 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 CH₂Cl₂ (220 mL) at room temperature. The reaction mixture was stirred for 3 days, concentrated and purified by preparative RP-HPLC (Onyx Monolithic C₁₈, 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, tᵣ 8.5 min. ¹H NMR (400 MHz, DMSO-d₆): δ 0.65 (d, J = 7 Hz, 3H, 5-CH₃), 1.56 (d, J = 7 Hz, 3H, 1-CH₃), 3.12 (dd, J = 14 and 8 Hz, 1H, 3-CH₅H₉), 3.22 (s, 3H, 7-CH₃), 3.24 (m, 1H, 3-CH₅H₉), 3.31 (m, 1H, 4-CH₅H₉), 3.34 (m, 1H, 6-CH₅H₉), 3.45 (m, 1H, 6-CH₅H₉), 3.49 (m, 1H, 7-CH₅H₉), 3.50 (m, 1H, 2-CH₅H₉), 3.67 (m, 1H, 2-CH₅H₉), 3.74 (m, 1H, 4-CH₅H₉), 3.80 (s, 3H, 3-OCH₃), 4.13 (q, J = 7 Hz, 1H, 5-α-
4.20 (t, $J = 8$ Hz, 1H, 3-CH), 4.37 (d, $J = 17$ Hz, 1H, 7-CH$_3$H), 4.60 (td, $J = 7$ and 4 Hz, 1H, 6-CH), 4.79 (td, $J = 12$ and 4 Hz, 1H, 2-CH), 5.32 (m, 1H, 1-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$_5$H), 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$_8$Se $^+$ 983.2777, found 983.2693 [MH$^+$].
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)

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\(_2\)Cl\(_2\)-iPrOH (7:2; 2 x 10 mL). The organic extracts were combined and dried over MgSO\(_4\) and concentrated in vacuo. The residue was dissolved in acetonitrile (4 mL), and water (2 mL) and saturated aqueous Na\(_2\)CO\(_3\) (2 mL) were added successively. The reaction mixture was stirred for 2 days, diluted with water (4 mL) and extracted with CH\(_2\)Cl\(_2\)-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\(_6\)): \(\delta\) 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\(_2\)H\(_3\)), 3.22 (m, 1H, 7-CH\(_2\)H\(_3\)), 3.39 (m, 1H, 4-CH\(_2\)H\(_3\)), 3.44 (m, 1H, 2-CH\(_2\)H\(_3\)), 3.80 (d, \(J = 16\) Hz, 1H, 7-CH\(_2\)H\(_3\)), 3.88 (dd, \(J = 16\) and 8 Hz, 4-CH\(_2\)H\(_3\)), 4.24 (m, 1H, 3-CH\(_3\)), 4.35 (m, 1H, 5-CH\(_3\)), 4.78 (td, \(J = 12\) and 4 Hz, 1H, 2-CH\(_3\)), 4.89 (s, 1H, 6-CH\(_2\)H\(_3\)), 5.20 (s, 1H, 6-CH\(_2\)H\(_3\)), 5.39 (m, 1H, 1-CH\(_3\)), 5.89–7.75 (m, 10H, Ar Hs), 8.03 (s, 1H, 1-C\(_3\)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\(_7\)S\(^+\) 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_3H_b), 3.22 (m, 1H, 7-CH_2H_b), 3.39 (m, 1H, 4-CH_2H_b), 3.44 (m, 1H, 2-CH_3H_b), 3.80 (d, J = 16 Hz, 1H, 7-CH_3H_b), 3.88 (dd, J = 16 and 8 Hz, 4-CH_3H_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_3H_b), 5.16 (s, 1H, 6-CH_2H_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 calcld for C_{39}H_{42}N_{10}BrO_{7}S^+ 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_3H_b), 3.22 (m, 1H, 7-CH_2H_b), 3.39 (m, 1H, 4-CH_2H_b), 3.44 (m, 1H, 2-CH_3H_b), 3.85 (d, J = 16 Hz, 1H, 7-CH_2H_b), 3.88 (dd, J = 16 and 8 Hz, 4-CH_3H_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_3H_b), 5.16 (s, 1H, 6-CH_2H_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 calcld for C_{39}H_{42}ClN_{10}O_{7}S^+ 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. 1H NMR (400 MHz, DMSO-d6): δ 0.87 (d, J = 7 Hz, 3H, 5-CH3), 1.54 (d, J = 7 Hz, 3H, 1-CH3), 3.12 (s, 3H, 7-NCH3), 3.15 (m, 2H, 3-CH2), 3.21 (m, 1H, 2-CHAHz), 3.22 (m, 1H, 7-CHAHz), 3.39 (m, 1H, 4-CHAHz), 3.44 (m, 1H, 2-CHAHz), 3.80 (d, J = 16 Hz, 1H, 7-CHAHz), 3.88 (dd, J = 16 and 8 Hz, 4-CHAHz), 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-CHAHz), 5.16 (s, 1H, 6-CHAHz), 5.40 (m, 1H, 1-α-CH), 6.86–7.77 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C5H), 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 C39H42FN10O7S+ 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. 1H NMR (400 MHz, DMSO-d6): δ 0.87 (d, J = 7 Hz, 3H, 5-CH3), 1.27 (t, J = 7 Hz, 3H, 3-CH2CH3), 1.55 (d, J = 7 Hz, 3H, 1-CH3), 2.85 (q, J = 7 Hz, 2H, 3-CH2CH3), 3.12 (s, 3H, 7-NCH3), 3.39 (m, 2H, 3-CH2), 3.21 (m, 1H, 2-CHAHz), 3.24 (m, 1H, 7-CHAHz), 3.44 (m, 1H, 4-CHAHz), 3.80 (d, J = 16 Hz, 1H, 7-CHAHz), 3.88 (dd, J = 16 and 8 Hz, 4-CHAHz), 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-CHAHz), 5.16 (s, 1H, 6-CHAHz), 5.40 (m, 1H, 1-α-CH), 6.93–7.76 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C5H), 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), 10.99 (d, J = 2 Hz, indole-NH). ES-MS m/z calcd for C41H47N10O7S+ 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-\(d_6\)): \( \delta \) 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-CHA\(_B\)), 3.24 (m, 1H, 7-CHA\(_B\)), 3.38 (m, 1H, 4-CHA\(_B\)), 3.44 (m, 1H, 2-CHA\(_B\)), 3.80 (d, \( J = 16 \) Hz, 1H, 7-CH\(_A\)H\(_B\)), 3.82 (d, \( J = 16 \) and 8 Hz, 4-CH\(_A\)H\(_B\)), 4.22 (m, 1H, 3-\( \alpha \)-CH), 4.35 (m, 1H, 5-\( \alpha \)-CH), 4.78 (td, \( J = 12 \) and 4 Hz, 1H, 2-\( \alpha \)-CH), 4.92 (s, 1H, 6-CH\(_A\)H\(_B\)), 5.16 (s, 1H, 6-CH\(_A\)H\(_B\)), 5.40 (m, 1H, 1-\( \alpha \)-CH), 6.91–7.76 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C\(^3\)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\(_8\)S\(^+\) 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-\(d_6\)): \( \delta \) 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-CHA\(_B\)), 3.22 (m, 1H, 7-CHA\(_B\)), 3.40 (m, 1H, 4-CHA\(_B\)), 3.44 (m, 1H, 2-CHA\(_B\)), 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-\( \alpha \)-CH), 4.34 (m, 1H, 5-\( \alpha \)-CH), 4.78 (td, \( J = 12 \) and 4 Hz, 1H, 2-\( \alpha \)-CH), 4.93 (s, 1H, 6-CH\(_A\)H\(_B\)), 5.16 (s, 1H, 6-CH\(_A\)H\(_B\)), 5.39 (m, 1H, 1-\( \alpha \)-CH), 6.90–7.70 (m, 9H, Ar Hs), 8.00 (s, 1H, 1-C\(^3\)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\(_8\)S\(^+\) 825.3143, found 825.3056 [MH\(^+\)].
The masked cyclic peptide 19h (4 mg, 4 μmol) was used to afford argyrin 2011 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_{8}S\(^+\) 825.3143, found 825.3092 [MH\(^+\)].
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 °C. 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 °C 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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. aeruginosa</em> PAO1</td>
</tr>
<tr>
<td>Argyrin A (20h)</td>
<td>19.8 ± 1.6</td>
</tr>
<tr>
<td>Argyrin E (20a)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>20c</td>
<td>&gt;100</td>
</tr>
<tr>
<td>20f</td>
<td>&gt;100</td>
</tr>
<tr>
<td>20g</td>
<td>90.7 ± 3.7</td>
</tr>
</tbody>
</table>

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
5. References