Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Data for

(+)-Fluorenylethylchloroformate (FLEC) – improved synthesis for application in chiral analysis and peptidomimetic synthesis.

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Materials and Methods

Materials

(*R*)-(-)-2-(2-Isoindolinyl)butan-1-ol was purchased from Acros Organics and (*S*)-(−)-2-(diphenylhydroxymethyl)pyrrolidine purchased from Bioscientific. Fmoc-protected amino acids, piperidine, diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), 1H-benzotriazolium 1-[bis(dimethylamino)methylene]-hexafluorophosphate (1-),3-oxide(HBTU), 1H-benzotriazolium 1-[bis(dimethylamino)methylene]-5-chloro-hexafluorophosphate (1-),3-oxide (HCTU), Rink-amide resin and Wang-resin were obtained from Auspep (Melbourne, Australia). All other chemicals were purchased from Sigma Aldrich.

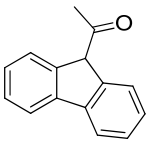
General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded one of two spectrometers and are specified by field strength (300, 400 MHz), (1) Bruker 300.23 MHz Widebore Spectrometer with Avance console, (2) Varian 599.77 MHz Spectrometer. ^{1}H and ^{13}C NMR spectra are reported as follows: shift in ppm (integration, splitting, *J* couplings in Hertz) where s = singlet, br. s = broad singlet, d = doublet, dd = doublet of doublets and t = triplet. Infra-red spectra were recorded on a Scimitar Series Varian 800 FT-IR fitted with a PIKE Technologies MIRacle ATR and running Varian Resolutions software package version 4.0. Ionisable compounds were subjected to *low resolution mass spectrometry*. *Low resolution mass spectrometry* (ESI-MS) was performed using a Micromass Platform II single quadrupole mass spectrometer equipped with an atmospheric pressure (ESI/APCI) ion source. Sample management was facilitated by an Agilent 1100 series HPLC system. *All final compounds were analysed by high-resolution mass spectrometry (HRMS).* HRMS was performed using a Bruker Apex-II Fourier Transform Ion Cyclotron Resonance Mass spectrometer fitted with an electrospray (ESI) ion source. Chiral high performance liquid chromatography for the analysis of 1-(9-fluorenyl)ethanol were performed using petroleum benzine : *i*-PrOH : AcOH, 500 : 56 : 0.56 over 15 min at a flow rate of 1 mL/min on a

Phenomenex Lux 5u cellulose-1 column (150 × 4.60 mm). The eluent was monitored at 254 nm. Optical rotations were measured using a Jasco P-2000 polarimeter at ambient temperature. Analytical reverse-phase high performance liquid chromatography-mass spectrometry of peptides and Feoc-amino acids was performed using the Shimadzu 2020 LCMS system, incorporating a photodiode array detector (254 nm) coupled directly to an electrospray ionization source and a single quadrupole mass analyser. Standard RP-HPLC was carried out at room temperature. Standard RP-HPLC of peptides was carried out at room temperature employing a Phenomenex Luna C8 (100 x 2.0 mm I.D.) column eluting with a gradient of 0-80 % CH_3CN in 0.05% aqueous trifluoroacetic acid, over 10 min at a flow rate of 0.2 ml/min. Mass spectra were acquired in positive ion mode with a scan range of 200-2000 *m/z*. Column and solvent conditions for the individual Feoc-amino acids are specified per compound in the body of the experimental.

Unusual Boc-amino acids were synthesised from commercially available starting materials following literature methods. Boc-**(±)-** β^2-leucine was synthesised following the methods of Zoltán et al.[1] and Boc-*exo*-7-azabicyclo[2.2.1]heptane-2-carboxylic acid and Boc-*endo*-7-azabicyclo[2.2.1]heptane-2-carboxylic acid were synthesised following the methods developed by Zhang et al.[2]

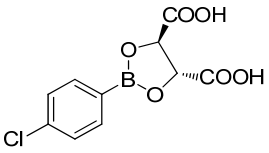
9-Acetylflourene (2)



Chemical Formula: $C_{15}H_{12}O$
Molecular Weight: 208.26

To an oven dried round-bottom flask under N_2 equipped with stirrer bar and condenser was added fluorene (20.0 g, 0.120 mol) and potassium *t*-butoxide (20.3 g, 0.180 mol). The powdered solids were thoroughly mixed (20 min.) and dry EtOAc (30.0 mL, 0.300 mol) in dry ether (400 mL) was added drop-wise. The resulting mixture was refluxed for 7.5 h, with consumption of the starting material confirmed by TLC (50 % EtOAc in hexane). The mixture was cooled and treated with saturated NH_4Cl (100 mL). The aqueous phase was extracted with ether (3 × 100 mL) and the combined organic phases dried (Na_2SO_4)and the solvent was removed *in vacuo* to give a yellow solid (21.81 g, 87 %). R_f 0.56 (14 % EtOAc in hexane). ^{1}H-NMR: ($CDCl_3$, 300 MHz) δ 7.87 (2H, d, J = 7.6 Hz), 7.57 (2H, d, J = 7.6 Hz), 7.50 (2H, t, J= 7.5 Hz), 7.41 (2H, dd, J = 7.4 Hz and 0.8 Hz), 4.86 (1H, s), 1.69 (3H, s). The product was reacted on without further purification. The compound was stored under vacuum and away from light to minimise degradation.

(4*R*, 5*R*)-2-(4-chlorophenyl)-1,3,2-dioxaborolane-4,5-dicarboxylic acid

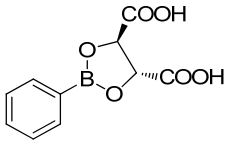


Chemical Formula: $C_{10}H_8BClO_6$
Molecular Weight: 270.43

To an oven dried round-bottomed flask under N_2 equipped with stirrer bar and condenser was added 4-chlorophenyl-boronic acid (0.938 g, 6.00 mmol), (L)-tartaric acid (0.900 g, 6.00 mmol) and Ca_2H (0.505 g, 12.0 mmol). Anhydrous THF (10 mL) was added *via* syringe, and the resulting suspension refluxed for 1 h. The reaction mixture was cooled to room temperature before being filtered under N_2 into an oven dried round-bottomed flask that was then sealed with a septum. Conversion to product was quantitative as measured by ^{1}H-NMR, and the filtrate was used as a 6 mmol/10 mL solution without further purification. ^{1}H-NMR: (^{d8}THF, 300 MHz) δ 7.77 (2H, d, J = 8.2 Hz); 7.37 (2H, d, J = 8.1 Hz);

5.02 (2H, s). ^{1}H NMR: ($CDCl_3$, 300 MHz) δ 7.80 (2H, d, J = 8.3 Hz), 7.33 (2H, d, J = 8.2 Hz), 5.08 (2H, s).

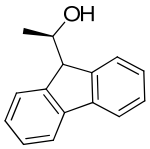
(4*R*, 5*R*)-2-phenyl-1,3,2-dioxaborolane-4,5-dicarboxylic acid



Chemical Formula: $C_{10}H_9BO_6$
Molecular Weight: 235.99

Phenylboronic acid was treated in the same fashion as 4-chlorophenyl-boronic acid described above. ^{1}H-NMR: (^{d8}THF, 300 MHz) δ 7.79 (2H, d, J = 6.7 Hz), 7.46-7.41 (2H, m), 7.32 (1H, t, J = 7.6 Hz), 5.00 (2H, s).

***(R)*-(+)-1-(9-fluorenyl)ethanol (3) *via* chiral reduction using $NaBH_4$ and (L)-Tar-B-X**

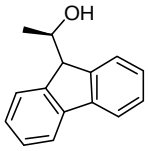


Chemical Formula: $C_{15}H_{14}O$
Molecular Weight: 210.27

To a 100 mL oven dried round-bottomed flask under N_2 equipped with stirrer bar was added 9-acetylfluorene (1.1 g, 5.3 mmol) and Tar-B-X (20 mL of a 0.5 M solution in THF, 10 mmol). The ketone and Tar-B-X were stirred for 15 min after which $NaBH_4$ (0.15 g, 3.9 mmol) was added directly to the solution. The mixture was stirred for 1 h at room temperature and then quenched with 1 M HCl (CAUTION: H_2 evolution). The mixture was basified to pH 12 with 2 M NaOH and stirred for 30 min. The solution was extracted with hexane (3 × 10 mL), dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (10 – 25 % EtOAc in hexane) to give the title compound as a white solid. ^{1}H NMR: ($CDCl_3$, 300 MHz) δ 7.77 (2H, d, J = 7.6 Hz), 7.73 (1H, d, J =

7.5 Hz), 7.53 (1H, d, *J* = 7.4 Hz), 7.43-7.37 (2H, m), 7.35-7.29 (1H, m), 4.56-4.52 (1H, m), 4.16 (1H, d, *J* = 4.3 Hz), 3.97 (1H, br. s) 0.93 (3H, d, *J* = 6.4 Hz). When X = 4-Cl-phenyl, (0.13 g, 15 % yield, 19 % *ee,* HPLC); When X = phenyl, (1.9 g, 88 % yield, 35 % *ee*, HPLC).

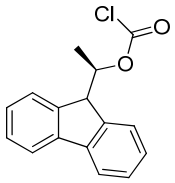
General procedure for the synthesis of (*R*)-(+)-1-(9-fluorenyl)ethanol (3), *via* the Corey-Bakshi-Shibata reduction



Chemical Formula: $C_{15}H_{14}O$
Molecular Weight: 210.27

To a round bottomed flask charged with freshly distilled MTBE (50 mL) was added *(S)*-α, α-diphenyl-2-pyrrolidinemethanol (2.00 g, 7.85 mmol) and DEANB (22.3 mL, 126 mmol). The solution was warmed to 45°C and while maintaining the temperature, a solution of 9-acetylflourene (21.8 g, 105 mmol) in MTBE (400 mL) was added drop-wise, *via* cannular. The reaction was complete at the end of ketone addition. The reaction was cooled to 0-10°C under a rapid flow of nitrogen, and MeOH (100 mL) was added over 20 min. (CAUTION: H_2 evolution). Once addition was complete the solution was warmed to room temperature and stirred for 30 min, or until H_2 evolution ceased. The solvents were removed at 65°C and at 500 mbar to azeotropically distill the MeOH and to further quench the reaction. The residue was diluted with MTBE (200 mL) and washed with 1M HCl (100 mL). The organic layer was removed and set aside while the aqueous layer was back-extracted with MTBE (50 mL). The organic layers were combined and washed with 1M HCl (120 mL), H_2O (120 mL) and 10 % brine (120 mL). The organic solution was dried over Na_2SO_4, the solvent was removed *in vacuo*. The crude residue was purified by flash chromatography (0-15 % EtOAc in hexane) to give the title compound as a white solid (60 %, 67 % *ee*). This was recrystallised from petroleum spirits (40°C-60°C) to give white needle-like crystals (4.84 g, 22 %, > 99 % *ee*, HPLC). ^{1}H-NMR was consistent to previously reported values.

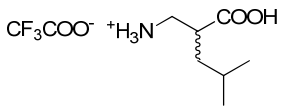
(*R*)-(+)-1,9-fluorenylethylchloroformate (4)



Chemical Formula: $C_{16}H_{13}ClO_2$
Molecular Weight: 272.73

To a stirred solution of triphosgene (bis(trichloromethyl)carbonate) (2.91 g, 7.71 mmol) dissolved in dry DCM (200 mL) and cooled to 0°C was added 1-(9-fluorenyl)ethanol (4.84 g, 23.0 mmol), followed by a solution of pyridine (1.86 mL, 23.0 mmol) in dry DCM (20 mL) added over 30 min. During the addition, the temperature was maintained between 0-5°C. After the addition was complete, the mixture was allowed to rise slowly to room temperature, and stirring was continued for 2 h. The resulting mixture was washed with H_2O (3 × 20 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the title compound as a brown oil (5.67 g, 90 %). $[\alpha]_D^{24.3}$ +52.6 (c = 1, 77 % *ee* c.f. lit[3] +67.9, CH_2Cl_2). ^{1}H-NMR: ($CDCl_3$, 400 MHz) δ 7.78 (2H, dd, J = 7.5, 4.2 Hz); 7.69 (1H, d, J = 7.6 Hz); 7.52 (1H, dd, J = 7.5, 0.7 Hz); 7.48 – 7.40 (2H, m); 7.39-7.32 (2H, m); 5.71-5.65 (1H, m), 4.41 (1H, d, J = 4.4 Hz), 0.85 (d, J = 6.4 Hz, 3H).

(±)- β^2-leucine· trifluoroacetate

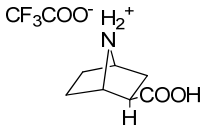


Chemical Formula: $C_9H_{16}F_3NO_4$
Molecular Weight: 259.22

To Boc-(±)- β^2-leucine (0.081 g, 0.35 mmol) was added 50 % TFA in DCM (8 mL). The solution was stirred at room temperature for 20 min. The solvent was removed *in vacuo* and the residue diluted with 50 % CH_3CN in H_2O and lyophilised (× 2) to give the title compound asa pale oil (56 mg, 65 %). ^{1}H-

NMR: (CD_3OD, 300 MHz) 2.93-2.76 (2H, m); 2.58-2.45 (1H, m); 1.50-1.35 (2H, m); 1.17-1.09 (1H, m); 0.71 (6H, s). ESI-MS (-): *m/z* = 144.2 $[M-H]^-$.

Exo-7-azabicyclo[2.2.1]heptane-2-carboxylic acid· trifluoroacetate



Chemical Formula: $C_9H_{12}F_3NO_4$
Molecular Weight: 255.19

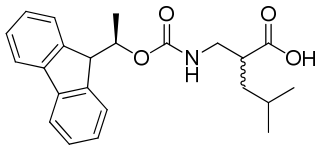
To Boc-*exo*-7-azabicyclo[2.2.1]heptane-2-carboxylic acid (0.053 g, 0.22 mmol) was added 50 % TFA in DCM (8 mL). The solution was stirred at room temperature for 20 min. The solvent was removed *in vacuo* and the residue diluted with 50 % CH_3CN in H_2O and lyophilised (× 2) to give the title compound as a brown solid (73 mg, > 99 %). ^{1}H-NMR: (D_2O, 300 MHz) δ4.58 – 4.56 (1H, m), 4.38 (1H, t, *J* = 4.1 Hz), 3.08 (1H, dd, *J* = 9.7, 5.7 Hz), 2.34 – 2.15 (2H, m), 2.10 – 2.02 (2H, m), 1.92 – 1.80 (2H, m). ESI-MS (+): *m/z* = 242.4 $[M+H]^+$.

_(R)_-(+)-Feoc-(D,L)-phenylalanine (5)

To a solution of (L)-phenylalanine (0.10 g, 0.59 mmol) in 1 M sodium borate buffer, pH 6.9 (6 mL) was added was added a solution of FLEC (0.19 g, 0.71 mmol) in 25 % CH_3CN in acetone (3 mL). The reaction was stirred at room temperature for 1 h. The mixture was washed with hexane (3 × 20 mL) and the aqueous layer acidified to pH 2 with 10 % citric acid (10 mL) and extracted with ethyl acetate (2 × 20 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed *in vacuo* to give the title compound as a clear oil (0.23 g, 97 %). R_f 0.69 (1 % CH_3COOH in EtOAc). IR: 1712 cm^{-1}. ^{1}H-NMR: ($CDCl_3$, 400 MHz) δ 7.78-7.73 (2H, m); 7.56-7.53 (2H, m); 7.41-7.36 (2H, m); 7.33-7.30 (5H, m); 7.21

(2H, t, *J* = 8.0 Hz); 5.59-5.54 (1H, m); 4.33 (1H, dd, *J* = 3.8, 13.1 Hz); 3.29 (1H, dd, *J* = 5.4, 13.9 Hz); 3.21-3.13 (1H, m); 3.10-3.00 (1H, m); 0.68 (3H, dd, *J* = 6.4, 18.6 Hz). ESI-MS (+): *m/z* = 402.4 $[M+H]^+$, 424.4 $[M+Na]^+$. Diastereomers were separated by analytical RP-HPLC when the eluent is 0-64 % CH_3CN in 0.1 % aqueous TFA over 10 min at a flow rate of 1 mL/min on a Luna C8 (150 × 4 mm I.D.) column: **Diastereomer 1:** R_t = 12.66 min, **Diastereomer 2:** R_t = 13.24 min.

***(R)*- (+)-Feoc-(±)-β^2-leucine (6)**

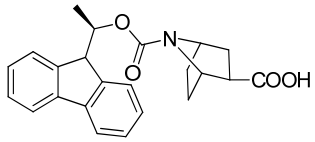


Chemical Formula: $C_{23}H_{27}NO_4$
Molecular Weight: 381.46

To a solution of β^2-leucine·TFA (0.11 g, 0.41 mmol) in anhydrous DCM (20 mL), under N_2, was added TMS-Cl (0.10 mL, 0.82 mmol). The reaction was stirred at room temperature for 1 h and then cooled to 0°C. DIPEA (0.11 mL , 0.62 mmol) was added drop-wise followed by a solution of FLEC (0.11 g, 0.41 mmol) in anhydrous DCM (1 mL). The reaction was allowed to warm to room temperature and stirred for 16 h. The solvent was evaporated *in vacuo*, and the residue was taken up in 50:50 CH_3CN:H_2O and lyophilised. The product was purified by dry column vacuum chromatography (25 % EtOAc in hexane then 0.1 % acetic acid in EtOAc) to give the title compound as a clear oil (75 mg, 48 %). IR: 1707 cm^{-1}. ^{1}H-NMR: (CD_3OD , 300 MHz) δ 7.74 (2H, m); 7.71 (1H, d, *J* = 7.6 Hz); 7.54 (1H, d, *J* = 7.3 Hz); 7.37-7.24 (4H, m); 5.48-5.39 (1H, m); 4.27 (1H, d, *J* = 3.5 Hz); 3.40-3.14 (2H, obscured by CD_3OD, m); 2.76-2.67 (1H, m); 1.68-1.50 (2H, m); 1.34-1.28 (2H, m); 0.90 (6H, d, *J* = 6.4 Hz); 0.59 (3H, d, *J* = 6.3 Hz). ^{13}C-NMR: (CD_3Cl_3, 400 MHz) δ 180.53, 156.40, 143.53, 143.37, 142.20, 141.63, 127.65, 127.58, 127.26, 126.94, 124.69, 120.02, 119.94, 73.43, 51.86, 43.81, 38.77, 38.70, 26.98, 22.56, 14.38, 14.28. ESI-MS (+): *m/z* = 382.25 $[M+H]^+$, ESI-MS (-): 379.97 $[M-H]^-$, 761.31 $[2M-H]^-$. HRMS: Calc 382.2013, Found

382.2027 ± 3.7 ppm. Diastereomers were separated analytically by RP-HPLC when the eluent is 40 % CH_3CN in 0.1 % TFA over 60 min at a flow rate of 1 mL/min on a Synergi Hydro-RP C18 with polar cap, 4 µm (250 × 4.60 mm I.D.) column: **Diastereomer 1:** R_t = 24.73 min, **Diastereomer 2:** R_t = 26.77 min.

***(R)*-(+)-Feoc-(±)-(*exo*)-azabicyclo[2.2.1]heptan-2-oate (7)**



Chemical Formula: $C_{23}H_{23}NO_4$
Molecular Weight: 377.43

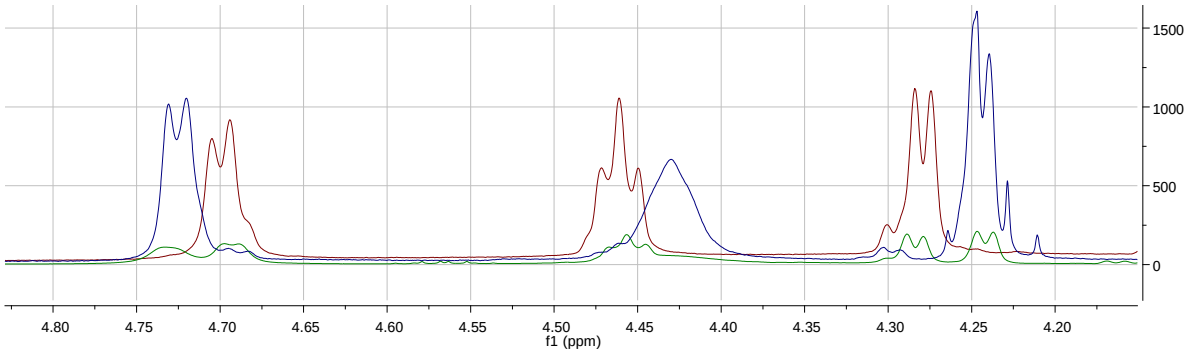
To a solution of (±)- (*Exo*)-azabicyclo[2.2.1]heptan-2-oate ·TFA (166 mg, 0.650 mmol) in dry DCM (25 mL), under N_2, was added TMS-Cl (167 µL, 1.30 mmol). The reaction was refluxed for 1 h and then cooled to 0°C. DIPEA (170 µL, 0.975 mmol) was added drop-wise followed by a solution of FLEC (1 eq) in dry DCM (1 mL). The reaction was allowed to warm to room temperature and stirred for 16 h. The solvent was evaporated *in vacuo*, and the residue taken up in 50:50 CH_3CN:H_2O and lyophilised. The product was purified by dry column vacuum chromatography (25 % EtOAc in hexane then 0.1 % acetic acid in EtOAc) to yield a pale yellow oil (0.111 g, 45 %). IR: 1734, 1710 cm^{-1}. Diastereomers were separated analytically by RP-HPLC when the eluent was 32 % CH_3CN in 0.1 % TFA over 40 min at a flow rate of 1 mL/minon a Luna C8, 100 Å, 5 µm, (50 x 4.60 mm I.D.) column: **Diastereomer 1:**R_t = 23.02 min, **Diastereomer 2:** R_t = 24.45 min. Diastereomers were separated by preparatory RP-HPLC when the eluent was 36 % CH_3CN in 0.1 % TFA over 80 min at a flow rate of 8 mL/min on a Phenomenex Luna C8 (150 × 4.6 mm I.D.) column. Purity was > 95 %.

Diastereomer 1: white solid (20 mg). ^{1}H-NMR: *(*$CDCl_3$, 400 MHz) δ 7.72 (2H, t, J = 6.4 Hz), 7.61 (1H, d, J = 7.5 Hz), 7.52 (1H, d, J = 7.4 Hz), 7.37 (2H, q, J = 7.4 Hz); 7.31-7.28 (2H, m); 5.52 (2H, m, 5.55-5.50); 4.70 (1H, d, J = 4.2 Hz); 4.46 (1H, t, J = 4.3 Hz); 4.27 (1H, d, J = 3.8 Hz); 2.63 (1H, dd, J = 8.8,

4.9 Hz); 2.26 – 2.23 (1H, m); 1.93 – 1.78 (2H, m); 1.71 (1H, dd, *J* = 12.4, 9.0 Hz); 1.51 (2H, ddd, *J* = 20.4, 12.1, 3.3 Hz); 0.66 (3H, d, *J* = 6.4 Hz). ^{13}C-NMR: ($CDCl_3$, 400 MHz) δ 143.6, 143.4, 142.2, 141.6, 127.7, 127.6, 127.2, 127.0, 126.0, 124.8, 120.0, 119.9, 73.9, 59.4, 56.1, 52.0, 47.3, 33.8, 29.0, 14.3. HRMS: Calc $[M+H]^+$ 378.1700, Found 378.1701 ± 0.3 ppm. Mp: 218°C (dec.).

Diastereomer 2: white solid (4 mg). ^{1}H-NMR: ($CDCl_3$, 400 MHz) δ 7.72 (2H, t, *J* = 8.0 Hz), 7.64 (1H, d, *J* = 7.4 Hz), 7.53 (1H, d, *J* = 7.4 Hz), 7.36 (2H, q, *J* = 7.0 Hz), 7.32-7.28 (2H, m), 5.56-5.52 (1H, m), 4.71 (1H, d, *J* = 3.7 Hz); 4.42 (1H, m); 4.23 (1H, d, *J* = 3.8 Hz); 2.60-2.57 (1H, m); 2.24-2.21 (1H, m); 1.85-1.82 (2H, m); 1.68 (1H, dd, *J* = 9.2, 12.2); 1.54-1.42 (2H, m); 0.67-0.64 (3H, m). ^{13}C-NMR: ($CDCl_3$, 400 MHz) δ 155.0, 143.6, 143.5, 142.2, 141.6, 127.6, 127.6, 127.2, 127.0, 126.1, 124.8, 120.0, 119.9, 73.5, 59.3, 56.0, 51.9, 47.3, 33.8, 29.5, 29.0, 14.3. HRMS: Calc $[M+H]^+$ 378.1700, Found 378.1702 ± 0.6 ppm.

Superimposed aliphatic regions of the ^{1}H-NMR spectra of the (+)-Feoc-(±)-Abh$_{exo}$diastereomers – Mixture of diastereomers (green) Diastereomer 1 (red) Diastereomer 2 (blue), showing the shift in resonances corresponding to the bridgehead protons of azabicyclohexane moiety H1″ and H4″.



Synthesis of cyclo[Abh^{exo}-Met-Gln-Trp-Phe-Gly] diastereomers

Synthesis of linear precursor H_2N-Met-Gln-Trp(Boc)-Phe-Gly-WANG

The precursor resin H_2N-Met-Gln-Trp (Boc)-Phe-Gly-WANG was synthesised using 0.1 mmol of Fmoc-Gly-WANG resin (0.75 mmol/g) on a CEM Liberty Microwave Peptide Synthesizer. Couplings were performed using the default instrument protocol: 5 molar equivalents of Fmoc amino acid and HCTU with activation using diisopropylethylamine in dimethylformamide over 5 minutes at 75°C (25 W microwave power). Fmoc deprotection was performed using the default instrument protocol: 20 % piperidine in DMF (1 × 30 s, 1 × 3 min) at 75°C (35 W microwave power).

Synthesis of H_2N-Abh$_{exo1}$-Met-Gln-Trp-Phe-Gly-CO_2H (11a)

To the H_2N-Met-Gln-Trp (Boc)-Phe-Gly-WANG resin (0.05 mmol) was added, (*R*)- (+)-Feoc-Abh$_{exo1}$ **7a** (18 mg, 0.05 mmol), DIPEA (26 µL, 0.050 mmol) and HCTU (20 mg, 0.050 mmol). The reaction was agitated under nitrogen for 2 hours and the coupling was monitored by the Kaiser test. The resin was washed (3 × DMF) and the FLEC group removed by treatment with 20 % piperidine in DMF (5 mL). The peptide-resin was washed and dried (DMF, MeOH, Et_2O), and the peptide was cleaved from the resin using a 5 mL solution of 95 % TFA, 2.5 % triisopropylsilane and 2.5 % H_2O. The crude peptide was precipitated out by the addition of ice-cold ether and collected by gravity filtration. The crude peptide was dissolved in 15 mL 50 % CH_3CN in H_2O and lyophilised. The peptide was purified by RP-HPLC to give the purified peptide as a white fluffy powder (12 mg, 30 % yield). LC-MS: R_t = 12.70 min, *m/z* = 791.5 $[M+H]^+$.

Synthesis of linear precursor, H_2N-Abh$_{exo1}$-Met-Gln-Trp-Phe-Gly-CO_2H (11b)

To the H_2N-Met-Gln-Trp (Boc)-Phe-Gly-WANG resin (0.050 mmol) was added, (*R*)-(+)-Feoc-Abh$_{exo2}$ **7b** (8.0 mg, 0.020 mmol), DIPEA (26 µL, 0.050 mmol) and HCTU (20 mg, 0.050 mmol). The reaction was agitated under nitrogen for 2 hours and the coupling monitored by the Kaiser test. The resin was washed

(3 × DMF) and the FLEC group removed by treatment with 20 % piperidine in DMF (5 mL). The peptide-resin was washed (DMF, MeOH, Et_2O), dried and cleaved from the resin using a 5 mL solution of 95 % TFA, 2.5 % triisopropylsilane and 2.5 % H_2O. The crude peptide was precipitated out by the addition of ice-cold ether and filtered under gravity. The crude peptide was dissolved in 15 mL 50 % CH_3CN in H_2O and lyophilised. The peptide was purified by RP-HPLC to give the purified peptide as a white fluffy powder (4.0 mg, 25 % yield). LC-MS: R_t = 12.70 min, *m/z* = 791.5 $[M+H]^+$.

General procedure for head-to-tail cyclisation

To the linear peptide (1 eq) in DMF (1 mg/mL) was added DIPEA (6 eq) and BOP (6 eq). The reaction mixture was stirred for 16 h at room temperature and monitored to completion by LC-MS. The reaction mixture was diluted with 50 % CH_3CN in H_2O (25 mL) and lyophilised. The product was purified by preparatory RP-HPLC on a Phenomenex Luna C8 (150 × 4.6 mm I.D) column.

cyclo[Abh$_{exo1}$-Met-Gln-Trp-Phe-Gly] 12a

Linear precursor 1, HN-Abh$_{exo1}$-Met-Gln-Trp-Phe-Gly-COOH (12 mg, 0.015 mmol) was reacted following the general procedure for head-to-tail cyclisation to give a white fluffy powder (1.6 mg, 17 %). LC-MS: R_t = 14.66 min, *m/z* = 773.50 $[M+H]^+$.

Neurokinin A agonist cyclo[Abh$_{exo2}$-Met-Gln-Trp-Phe-Gly] 12b

Linear precursor 2, HN-Abh$_{exo2}$-Met-Gln-Trp-Phe-Gly-COOH (2.0 mg, 0.0025 mmol) was reacted following the general procedure for head-to-tail cyclisation to give a white fluffy powder (1.4 mg, 72 %). LC-MS: R_t = 14.40 min, *m/z* = 773.50 $[M+H]^+$.

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