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Supplementary Information

Development of selective, fluorescent cannabinoid type 2 receptor ligands based on a 1,8-naphthyridin-2-(1*H*)-one-3-carboxamide scaffold

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clogP

cLogP of **2** and SR144528 were calculated in MarvinSketch 17.6 using the ChemAxon Method and electrolyte concentrations of 0.1 mol/dm^3 of Cl^- and Na^+ K^+.

Synthesis of all other compounds not reported within manuscript

6-Bromo-*N*-(4-methylcyclohexyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (1)

To a stirred solution of **5** (22 mg, 59.9 µmol) in DMF (1 mL) was added caesium carbonate (55 mg, 0.17 mmol). After stirring for 1 h, 4-(2-chloroethyl)morpholine hydrochloride (22 mg, 0.12 mmol) was added. The mixture was stirred at 50 °C for 12 h and upon cooling was evaporated under reduced pressure. Saturated aq. $NaHCO_3$ was added until pH 10-11 and then extracted with DCM (3 x 20 mL). The combined organics were washed with H_2O (1 x 60 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by crystallisation in ACN to yield **1** (10.5 mg, 22.0 µmol, 37%), as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (m, 1H, NH isomer A), 9.41 (m, 1H, NH isomer B) 8.80 (s, 1H, ArH), 8.69 (d, *J* = 2.3 Hz, 1H, ArH), 8.19 (t, *J* = 2.1 Hz, 1H, ArH), 5.04 – 4.58 (m, 2H, N1-CH_2), 4.32 – 3.91 (m, 1H, NHC<u>H</u> isomer A and NHC<u>H</u> isomer B), 3.92 – 3.55 (m, 4H, N-CH_2 morpholino), 3.40 – 2.22 (m, 6H, N1-$CH_2C\underline{H_2}$ & O-CH_2 morpholino), 2.12 – 0.88 (m, 12H, CH & CH_2 cyclohexyl, CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. Data matches the literature reference.[1] ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.50, 162.36, 161.26, 152.67, 152.61, 148.21, 141.02, 140.10, 124.54, 116.46, 55.51, 53.40, 49.06, 45.76, 33.99, 33.07, 32.11, 31.24, 30.25, 29.84, 29.74, 22.36, 21.80. Isomers A and B resolved as separate peaks in some instances, but are not assigned. HRMS-ESI calculated for $C_{22}H_{30}BrN_4O_3$ $[M+H]^+$ 477.1496, found *m/z* 477.1462. Analytical RP-HPLC R_t = 15.57 min (57%) and 15.84 min (43%).

Methyl 5-{6-bromo-3-[(4-methylcyclohexyl)carbamoyl]-2-oxo-1,2-dihydro-1,8-naphthyridin-1-yl}pentanoate (6)

To a stirred solution of **5**[1] (319 mg, 0.88 mmol) in DMF (4 mL), was added caesium carbonate (799 mg, 2.5 mmol). After stirring for 1 h, methyl 5-bromovalerate (0.25 mL, 1.8 mmol) was added. The mixture was stirred at 50 °C for 12 h and upon cooling was evaporated under reduced pressure. The solid was taken up in ACN and filtered. The filtrate was evaporated and then purified by recrystallisation in ACN to yield **6** (94 mg, 0.20 mmol,

22%), as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.93 (d, *J* = 7.8 Hz, 1H, NH isomer A), 9.55 (d, *J* = 8.1 Hz, 1H, NH isomer B), 8.79 (s, 1H, ArH), 8.70 (d, *J* = 2.4 Hz, 1H, ArH), 8.17 (dd, *J* = 3.5, 2.4 Hz, 1H, ArH), 4.65 – 4.46 (m, 2H, N-CH_2), 4.25 (m, 1H, NHC<u>H</u> isomer A), 3.9 (m, 1H, NHC<u>H</u> isomer B), 3.67 (s, 3H, O-CH_3), 2.47 – 2.34 (m, 2H, C<u>H$_2$</u>COOMe), 2.11 – 0.87 (m, 16H, N-CH_2(C<u>H$_2$</u>)$_2$, CH & CH_2 cyclohexyl & CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.92, 173.87, 162.34, 162.29, 161.55, 161.53, 152.77, 152.72, 148.34, 148.28, 140.76, 140.58, 139.83, 139.79, 124.47, 124.38, 116.25, 116.24, 114.27, 114.21, 51.71, 51.70, 49.03, 45.83, 41.80, 41.72, 34.01, 33.83, 33.82, 33.07, 32.12, 31.21, 30.30, 29.72, 27.48, 27.47, 22.47, 22.45, 22.36, 21.67. Isomers A and B resolved as separate peaks in some instances, but are not assigned. HRMS-ESI calculated for $C_{22}H_{28}BrN_3NaO_4$ $[M+Na]^+$ 500.1155, found *m/z* 500.1117. Analytical RP-HPLC R_t = 23.16 min (50%) and 24.15 min (50%).

Methyl 4-({6-bromo-3-[(4-methylcyclohexyl)carbamoyl]-2-oxo-1,2-dihydro-1,8-naphthyridin-1-yl}methyl)benzoate (7)

A solution of **5** (75 mg, 0.23 mmol), caesium carbonate (206 mg, 0.63 mmol) and methyl 4-bromomethylbenzoate (104 mg, 0.45 mmol) in DMF (1.1 mL) was reacted as described in the procedure for **6**. The crude residue was purified by flash silica gel column chromatography (99:1 DCM/MeOH) and a portion (6 mg) of the solid obtained (20 mg) was further purified by semi-preparative RP-HPLC to yield **7** (3.54 mg, 6.93 µmol), as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.92 – 9.75 (m, 1H, NH isomer A), 9.49 – 9.38 (m, 1H, NH isomer B), 8.84 (s, 1H, ArH), 8.73 – 8.65 (m, 1H, ArH), 8.22 – 8.16 (m, 1H, ArH), 8.00 – 7.89 (m, 2H, ArH Ph), 7.48 – 7.38 (m, 2H, ArH Ph), 5.81 (d, J = 8.6 Hz, 2H, N1-CH_2), 4.37 – 4.16 (m, 1H, NHC<u>H</u>), 2.11 – 0.89 (m, 15H, CH & CH_2 cyclohexyl, CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. HRMS-ESI calculated for $C_{25}H_{26}BrN_3NaO_4$ $[M+Na]^+$ 534.0999, found *m/z* 534.1008. Analytical RP-HPLC R_t = 24.16 min (50%) and 24.44 min (50%).

Methyl 5-[6-(4-methoxyphenyl)-3-[(4-methylcyclohexyl)carbamoyl]-2-oxo-1,2-dihydro-1,8-naphthyridin-1-yl]pentanoate (8)

Compound **6** (16 mg, 32.8 µmol), 4-methoxyphenylboronic acid (6.5 mg, 42.7 µmol) and Na_2CO_3 (9.1 mg, 85.4 µmol) were dissolved in a 1:4 *v*:*v* mixture of H_2O and DMF (1.5 mL). $Pd(OAc)_2$ (0.08 mg, 0.33 µmol) was added and the reaction mixture heated to 110 °C and

stirred for 3 h, then cooled to rt and diluted with H_2O (1.5 mL). The aqueous phase was extracted with EA (4 x 1.5 mL) and the combined organics washed with H_2O (3 x 6 mL) and sat. aq. NaCl (1 x 6 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was purified by flash silica gel column chromatography (2:1 hexane/EA) to yield the desired **8** (9.2 mg, 18.1 μmol, 55%), as a yellow solid (R_f 0.5, 1:1 hexane/EA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.04 (d, *J* = 7.8 Hz, 1H, NH isomer A), 9.67 (d, *J* = 8.0 Hz, 1H, NH, isomer B), 8.91 (s, 1H, ArH), 8.89 (d, *J* = 2.4 Hz, 1H, ArH), 8.19 – 8.12 (m, 1H, ArH), 7.63 – 7.50 (m, 2H, ArH MeOPh), 7.11 – 6.99 (m, 2H, ArH MeOPh), 4.73 – 4.54 (m, 2H, N-CH_2), 4.26 (m, 1H, NHCH isomer A), 3.93 (m, 1H, NHCH isomer B), 3.88 (s, 3H, PhO-CH_3), 3.67 (s, 3H, COOCH_3), 2.52 – 2.40 (m, 2H, CH_2COOMe), 2.36 (m, 1H, CHCH$_3$ isomer A), 2.09 (m, 1H, CHCH$_3$ isomer B), 1.90 – 0.87 (m, 15H, N-CH_2(CH_2)$_2$, CH_2 cyclohexyl, CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.04, 173.99, 162.56, 162.52, 162.10, 160.07, 160.06, 150.63, 150.58, 148.57, 148.51, 142.07, 141.89, 135.46, 135.43, 132.28, 132.22, 128.96, 128.92, 128.27, 123.56, 123.47, 114.93, 114.83, 55.58, 51.73, 51.70, 48.94, 45.80, 41.66, 41.57, 34.21, 34.05, 33.93, 33.91, 33.61, 33.11, 32.14, 31.20, 30.34, 29.75, 28.21, 27.61, 22.56, 22.53, 22.39, 21.65, 21.36. Isomers A and B resolved as separate peaks in some instances, but are not assigned. HRMS-ESI calculated for $C_{29}H_{35}N_3NaO_5$ $[M+Na]^+$ 528.2469, found *m/z* 528.2475. Analytical RP-HPLC R_t = 24.33 min (50%) and 25.08 min (50%).

5-{6-Bromo-3-[(4-methylcyclohexyl)carbamoyl]-2-oxo-1,2-dihydro-1,8-naphthyridin-1-yl}pentanoic acid (9)

A solution of **6** (60 mg, 0.13 mmol) and 10% NaOH (10 mL) was heated to 110 °C for 5 h. The mixture was cooled and conc. HCl added until pH 2-3. The precipitate was filtered and treated with diethyl ether to yield **9** (25 mg, 54.5 μmol, 43%) as a white solid (R_f 0.34, 1:1 hexane/EA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (d, *J* = 7.7 Hz, 1H, NH isomer A), 9.57 (d, *J* = 7.9 Hz, 1H, NH isomer B), 8.81 (s, 1H, ArH), 8.70 (d, *J* = 2.1 Hz, 1H, ArH), 8.18 (t, *J* = 2.7 Hz, 1H, ArH), 4.64 – 4.46 (m, 2H, N-CH_2), 4.25 (m, 1H, NHCH isomer A), 3.89 (m, 1H, NHCH isomer B), 2.56 – 2.35 (m, 2H, CH_2COOH), 2.15 – 0.87 (m, 16H, N-CH_2(CH_2)$_2$, CH & CH_2 cyclohexyl & CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.34, 178.28, 162.35, 162.30, 161.70, 161.69, 152.84, 152.80, 148.31, 148.26, 140.98, 140.81, 139.92, 139.88, 124.30, 124.21, 116.26, 116.25, 114.34, 114.28, 49.11, 45.91, 41.75, 41.67, 33.99, 33.65, 33.64, 33.04, 32.10, 31.20, 30.28, 29.84, 29.71, 27.40, 22.36, 22.21, 22.19, 21.68. Isomers A and B resolved as separate

peaks in some instances, but are not assigned. HRMS-ESI calculated for $C_{21}H_{26}BrN_3NaO_4$ $[M+Na]^+$ 486.0999, found *m/z* 486.1007.

***tert*-Butyl *N*-[8-(5-{6-bromo-3-[(4-methylcyclohexyl)carbamoyl]-2-oxo-1,2-dihydro-1,8-naphthyridin-1-yl}pentanamido)octyl]carbamate (10)**

To a solution of **9** (13 mg, 27.8 µmol) in anhydrous DMF (2 mL) were added DIPEA (14.5 µL, 85 µmol) and HATU (11 mg, 27.8 µmol). After stirring for 5 min, a solution of *tert*-butyl-*N*-(8-aminooctyl)carbamate (prepared according to a literature procedure)[2] (22 mg, 88.8 µmol) and DIPEA (14.5 µL, 85 µmol) in DMF (1.5 mL) was added. The reaction mixture was stirred for 14 hand then the solvent evaporated under reduced pressure. The crude residue was purified by flash silica gel column chromatography (1:1 hexane/EA) to yield **10** (14 mg, 19.6 µmol, 71%) as a grey solid (R_f 0.484 1:2 hexane/EA). ^{1}H NMR (400 MHz, MeOD-d_4) δ 10.33 (d, J = 7.9 Hz, 1H, NH isomer A), 9.89 (d, J = 7.9 Hz, 1H, NH isomer B), 8.83 – 8.75 (m, 2H, ArH), 8.52 (dd, J = 4.2, 2.4 Hz, 1H, ArH), 4.65 – 4.51 (m, 2H, N1-CH_2), 4.21 (m, 1H, NHC<u>H</u> isomer A), 3.81 (m, 1H, NHC<u>H</u> isomer B), 3.14 (t, J = 7.0 Hz, 2H, CH_2), 3.04 – 2.86 (m, 2H, CH_2), 2.32 – 2.20 (m, 2H, CH_2), 2.10 – 0.90 (m, 37H, CH, CH_2, CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. ^{13}C NMR (101 MHz, MeOD-d_4) δ 175.66, 163.62, 163.41, 154.11, 149.57, 142.26, 141.71, 124.66, 117.54, 117.51, 115.28, 115.24, 79.74, 50.33, 49.64, 49.43, 49.21, 49.00, 48.79, 48.57, 48.36, 46.69, 42.76, 42.66, 41.35, 40.76, 40.34, 40.23, 36.71, 36.67, 34.96, 33.82, 33.16, 32.69, 31.08, 30.95, 30.74, 30.35, 30.32, 30.09, 30.02, 28.80, 28.53, 28.39, 28.36, 27.91, 27.81, 27.77, 27.34, 24.43, 24.40, 22.56. Isomers A and B resolved as separate peaks in some instances, but are not assigned. HRMS-ESI calculated for $C_{34}H_{52}BrN_5NaO_5$ $[M+Na]^+$ 712.3044, found *m/z* 712.3065.

***tert*-Butyl *N*-(2-{2-[2-(5-{6-bromo-3-[(4-methylcyclohexyl)carbamoyl]-2-oxo-1,2-dihydro-1,8-naphthyridin-1-yl}pentanamido)ethoxy]ethoxy}ethyl)carbamate (11)**

Compound **9** (8.9 mg, 19.2 µmol), DIPEA (20.1 µL, 0.12 mmol), HATU (7.3 mg, 19.2 µmol) and *tert*-butyl *N*-{2-[2-(2-aminoethoxy)ethoxy]-ethyl}carbamate (prepared according to literature procedure)[3] (15 mg, 62.0 µmol) in DMF (1.8 mL) were used as described in the procedure for **10**. The crude product was purified by flash silica gel column chromatography (1:7 hexane/EA) to yield **11** (10 mg, 14.7 µmol, 77%) as a grey solid (R_f 0.19 1:2 hexane/EA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (d, J = 7.7 Hz, 1H, NH isomer A), 9.57 (d, J

= 8.0 Hz, 1H, NH isomer B), 8.76 (s, 1H, ArH), 8.69 (d, *J* = 2.1 Hz, 1H, ArH), 8.20 – 8.12 (m, 1H, ArH), 6.16 (s, 1H, NH), 4.98 (s, 1H, NH), 4.62 – 4.43 (m, 2H, N1-CH_2), 4.22 (m, 1H, NHC<u>H</u> isomer A), 3.87 (m, 1H, NHC<u>H</u> isomer B), 3.74 – 3.38 (m, 10H, CH_2), 3.36 – 3.20 (br m, 2H, CH_2), 2.36 – 2.21 (br m, 2H, CH_2), 2.10 – 0.85 (m, 25H, CH, CH_2, CH_3). Isomers A and B represent cis/trans (in no defined order) isomers. ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.31, 162.27, 161.65, 152.89, 152.86, 148.27, 140.83, 140.66, 139.86, 139.82, 124.33, 124.24, 116.24, 114.31, 114.25, 70.47, 70.36, 70.25, 70.10, 49.07, 45.88, 41.87, 39.60, 36.14, 33.99, 33.07, 32.11, 30.29, 29.71, 28.55, 27.53, 23.33, 22.36, 21.69. Isomers A and B resolved as separate peaks in some instances, but are not assigned. HRMS-ESI calculated for $C_{32}H_{49}BrN_5O_7$ $[M+H]^+$ 694.2810, found *m/z* 694.2870.

1-{4-[(8-Aminooctyl)carbamoyl]butyl}-6-bromo-*N*-(4-methylcyclohexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (12)

Compound **10** (11 mg, 16.1 μmol) was dissolved in DCM (0.9 mL) and TFA (0.9 mL) was added. After 1 h stirring, the reaction mixture was evaporated under N_2 stream, followed by reduced pressure. The crude was purified by semi-preparative RP-HPLC to yield the TFA salt of **12** (11.9 mg, 14.6 μmol, 91%) as a white solid. HRMS-ESI calculated for $C_{29}H_{45}BrN_5O_3$ $[M+H]^+$ 590.2700, found *m/z* 590.2703. Analytical RP-HPLC R_t = 18.12 min (40%) and 18.27 min (60%).

1-[4-({2-[2-(2-Aminoethoxy)ethoxy]ethyl}carbamoyl)butyl]-6-bromo-*N*-(4-methylcyclohexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (13)

A solution of **11** (8.0 mg, 11.5 μmol) and TFA (1.2 mL) in DCM (1.2 mL) was reacted as described in the procedure for **12**. The crude was purified by semi-preparative RP-HPLC to yield the TFA salt of **13** (8.1 mg, 9.8 μmol, 86%) as a white solid. HRMS-ESI calculated for $C_{27}H_{41}BrN_5O_5$ $[M+H]^+$ 594.2286, found *m/z* 594.2294. Analytical RP-HPLC R_t = 16.84 min (51%) and 17.01 min (49%).

6-Bromo-1-{4-[(8-acetamidooctyl)carbamoyl]butyl}-*N*-(4-methylcyclohexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (14)

To a solution of the TFA salt of **12** (2.4 mg, 2.9 μmol) and DIPEA (1.9 μL, 10.9 μmol, added as a 1:10 solution in DCM) in DCM (500 μL) was added acetic anhydride (0.28 μL, 3.0 μmol, added as a 1:10 solution in DCM) and the mixture stirred at rt for 1 h. The reaction

solvent was evaporated under N_2 stream. The product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **14** (1.76 mg, 2.8 μmol, 95%) as a white solid. HRMS-ESI calculated for $C_{31}H_{46}BrN_5NaO_4$ $[M+Na]^+$ 654.2625, found *m/z* 654.2612. Analytical RP-HPLC R_t = 21.04 min (40%) and 21.20 min (60%).

6-Bromo-1-[4-({2-[2-(2-acetamidoethoxy)ethoxy]ethyl}carbamoyl)butyl]-*N*-(4-methylcyclohexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (15)

The TFA salt of **13** (1.53 mg, 1.9 μmol), DIPEA (1.2 μL, 6.9 μmol), and acetic anhydride (0.18 μL, 1.9 μmol) in DCM (437 μL) were treated as in the procedure for **14**. The product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **15** (1.14 mg, 1.8 μmol, 96%) as a white solid. HRMS-ESI calculated for $C_{29}H_{42}BrN_5NaO_6$ $[M+Na]^+$ 658.2211, found *m/z* 658.2191. Analytical RP-HPLC R_t = 18.90 min (53%) and 19.11 min (47%).

6-Bromo-1-[4-({8-[6-(2-{4-[(*E*)-2-[2,2-difluoro-4-(thiophen-2-yl)-1λ4,3-diaza-2λ4-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-12-yl]ethenyl]phenoxy}acetamido)hexanamido]octyl}carbamoyl)butyl]-*N*-(4-methylcyclohexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (16)

To a solution of the TFA salt of **12** (4.9 mg, 6.0 μmol) in anhydrous DMF (500 μL), was added a solution of DIPEA (2.6 μL, 14.9 μmol) in anhydrous DMF (41.6 μL), followed by a solution of BODIPY 630/650-X-OSu (1.25 mg, 1.89 μmol) in anhydrous DMF (300 μL). The mixture was swirled, left standing for 12 h, then evaporated under reduced pressure. The crude product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **16** (1.98 mg, 1.7 μmol, 92%) as a bright blue solid. HRMS-ESI calculated for $C_{58}H_{70}BBrF_2N_8NaO_6S$ $[M+Na]^+$ 1157.4285, found *m/z* 1157.4175. Analytical RP-HPLC R_t = 25.44 min (cis trans isomers resolved as one peak in standard analytical method).

6-Bromo-1-(4-{[2-(2-{2-[6-(2-{4-[(*E*)-2-[2,2-difluoro-4-(thiophen-2-yl)-1λ4,3-diaza-2λ4-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-12-yl]ethenyl]phenoxy}acetamido)hexanamido]ethoxy}ethoxy)ethyl]carbamoyl}butyl)-*N*-(4-methylcyclohexyl)-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (17)

The TFA salt of **13** (5.32 mg, 6.5 µmol), DIPEA (2.8 µL, 15.9 µmol), BODIPY 630/650-X-OSu (1.25 mg, 1.89 µmol) and DMF (844 µL) were reacted as described in the procedure for **16**. The crude product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **17** (1.9 mg, 1.7 µmol, 88%) as a bright blue solid. HRMS-ESI calculated for $C_{56}H_{66}BBrF_2N_8NaO_8S$ $[M+Na]^+$ 1161.3870, found *m/z* 1161.3780. Analytical RP-HPLC R_t = 24.39 min (55%) and 24.50 min (45%).

Compounds **19**, **20** detailed in manuscript.

***trans*-*N*-(4-Hydroxycyclohexyl)-6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-carboxamide (21)**

To a stirred solution of **19** (800 mg, 2.0 mmol) in anhydrous DMF (40 mL) was added DIPEA (2 mL, 11.8 mmol) and HATU (743 mg, 2.0 mmol). The reaction mixture was stirred for 5 min, then *trans*-4-aminocyclohexanol hydrochloride (675 mg, 5.9 mmol) in DMF (40 mL) was added and the mixture stirred for 42 h. The DMF was evaporated under reduced pressure and the residue taken up in H_2O (50 mL) and extracted with DCM (3 x 50 mL). The combined organics were washed with H_2O (2 x 60 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude was purified by precipitation in ACN, yielding **21** (815 mg, 1.6 mmol, 82%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, *J* = 7.9 Hz, 1H, NH), 8.91 (s, 1H, ArH), 8.89 (d, *J* = 2.4 Hz, 1H, ArH), 8.17 (d, *J* = 2.4 Hz, 1H, ArH), 7.60 – 7.51 (m, 2H, ArH MeOPh), 7.10 – 7.00 (m, 2H, ArH MeOPh), 4.83 – 4.71 (m, 2H, N-CH_2), 4.07 – 3.93 (m, 1H, CH), 3.87 (s, 3H, O-CH_3), 3.75 – 3.64 (m, 5H, CH & O-CH_2 morpholino), 2.78 – 2.69 (m, 2H, N-CH_2), 2.68 – 2.57 (m, 4H, N-CH_2 morpholino), 2.18 – 2.09 (m, 2H, CH_2), 2.09 – 1.99 (m, 2H, CH_2), 1.54 – 1.36 (m, 4H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.57, 162.30, 160.11, 150.64, 148.65, 142.24, 135.44, 132.36, 128.85, 128.26, 123.24, 114.95, 114.78, 69.93, 67.15, 56.04, 55.58, 54.05, 47.94, 39.13, 34.04, 30.67. HRMS-ESI calculated for $C_{28}H_{35}N_4O_5$ $[M+H]^+$ 507.2602, found *m/z* 507.2571. Analytical RP-HPLC R_t = 13.85 min.

***tert*-Butyl *N*-[(1s,4s)-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl]carbamate (22)**

Compound **19** (experimental detailed in manuscript) (50 mg, 0.12 mmol), DIPEA (128 µL, 0.73 mmol), HATU (46 mg, 0.12 mmol) and 1-*N*-Boc-*cis*-1,4-cyclohexyldiamine (79 mg, 0.37 mmol) in DMF (2.5 mL) were used as described in the procedure for **10**. The reaction mixture was evaporated under reduced pressure and the residue taken up in EA (5 mL), washed with H_2O (3 x 5 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash silica gel column chromatography (4:1 EA/hexane) to yield **22** (53 mg, 87.2 µmol, 71%) as a yellow solid (R_f 0.34, 100% EA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.00 (d, *J* = 7.8 Hz, 1H, NH), 9.01 – 8.81 (m, 2H, ArH), 8.25 – 8.11 (m, 1H, ArH), 7.62 – 7.44 (m, 2H, ArH MeOPh), 7.09 – 6.95 (m, 2H, ArH MeOPh), 4.77 (t, *J* = 7.2 Hz, 2H, N1-CH_2), 4.72 – 4.59 (m, 1H, NH), 4.28 – 4.14 (m, 1H, CH), 3.86 (s, 3H, O-CH_3), 3.68 (t, *J* = 4.5 Hz, 5H, CH, N-CH_2 morpholino), 2.74 (t, *J* = 7.2 Hz, 2H, N1-CH_2CH_2), 2.64 (t, *J* = 4.4 Hz, 4H, O-CH_2 morpholino), 1.82 (d, *J* = 14.6 Hz, 6H, CH_2), 1.68 – 1.54 (m, 2H, CH_2), 1.45 (d, *J* = 1.4 Hz, 9H, *t*Bu CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.61, 162.11, 160.09, 155.28, 150.60, 148.63, 142.13, 135.39, 132.33, 128.81, 128.23, 123.26, 114.93, 114.76, 79.33, 67.12, 56.00, 55.55, 54.02, 47.41, 45.60, 39.09, 28.91, 28.64, 28.57. HRMS calculated for $C_{33}H_{44}N_5O_6$ $[M+H]^+$ 606.3286, found *m/z* 606.3246.

Compound **23** detailed in manuscript.

***trans*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-{[(*tert*-butoxy)carbonyl]amino}acetate (24)**

A stirred solution of Boc-glycine (10 mg, 59 µmol) and TFFH (16 mg, 59 µmol) in anhydrous DCM (1.6 mL) was cooled to 0 °C and Et_3N (41 µL, 0.30 mmol) was added. The mixture was warmed to rt and stirred for 30 min and then **21** (30 mg, 59 µmol) and DMAP (0.7 mg, 5.9 µmol) were added. The mixture was stirred for 14 h at rt and then evaporated under reduced pressure. The residue was taken up in EA (3 mL) and washed with H_2O (3 x 3 mL) and sat. aq. NaCl (1 x 3 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude residue was purified by flash silica column chromatography (100% EA) to yield **24** (8.5 mg, 12.8 µmol, 22%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.71 (d, *J* = 7.9 Hz, 1H, NH), 8.97 – 8.86 (m, 2H, ArH), 8.17 (d, *J* = 2.4, 1H, ArH), 7.61 – 7.52 (m, 2H, ArH MeOPh), 7.11 – 7.00 (m, 2H, ArH MeOPh), 5.05 – 4.98 (m, 1H, NH), 4.78 (t, *J*

= 7.1 Hz, 2H, N1-C$\underline{H_2}$), 4.51 – 4.33 (m, 1H, CH), 4.07 – 3.91 (m, 2H, CH_2), 3.87 (s, 3H, O-CH_3), 3.74 – 3.64 (m, 5H, CH, N-CH_2 morpholino), 2.76 (t, J = 7.2 Hz, 2H, N1-$CH_2C\underline{H_2}$), 2.66 (t, J = 4.5 Hz, 4H, O-CH_2 morpholino), 2.19 – 1.99 (m, 4H, CH_2), 1.70 – 1.06 (m, 13H, CH_2, *t*Bu CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.12, 162.56, 162.31, 160.13, 150.68, 148.61, 142.31, 135.48, 132.41, 128.84, 128.27, 123.22, 114.96, 114.80, 84.41, 69.94, 66.98, 55.91, 55.58, 53.90, 47.95, 47.47, 38.91, 34.03, 30.66, 28.47 (one quaternary carbon was not observed). HRMS-ESI calculated for $C_{35}H_{46}N_5O_8$ $[M+H]^+$ 664.3341, found 664.3339.

***cis*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-{[(*tert*-butoxy)carbonyl]amino}heptanoate (25)**

A solution of **20** (50 mg, 99 µmol), 7-{[(tert-butoxy)carbonyl]amino}heptanoic acid (48 mg, 0.20 mmol), TFFH (49 mg, 0.20 mmol), Et_3N (137 µL, 0.99 mmol) and DMAP (1.2 mg, 9.9 µmol) in DCM (4 mL) was reacted as described in the procedure for **24**. The crude residue was purified by flash silica column chromatography (1:2 hexane/EA) to yield **25** (48 mg, 66 µmol, 66%) as a yellow brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.85 (d, *J* = 7.8 Hz, 1H, NH), 8.97 – 8.88 (m, 2H, ArH), 8.17 (d, *J* = 2.4 Hz, 1H, ArH), 7.60 – 7.52 (m, 2H, ArH MeO$\underline{Ph}$), 7.09 – 7.01 (m, 2H, ArH MeO$\underline{Ph}$), 4.98 (s, 1H, NH), 4.80 (t, *J* = 7.1 Hz, 2H, N1-C$\underline{H_2}$), 4.68 – 4.49 (m, 1H, CH), 4.17 – 4.05 (m, 1H, CH), 3.88 (s, 3H, O-CH_3), 3.69 (t, *J* = 4.6 Hz, 4H, N-CH_2 morpholino), 3.11 (q, *J* = 6.7 Hz, 2H, CH_2), 2.76 (t, *J* = 7.1 Hz, 2H, N1-$CH_2C\underline{H_2}$), 2.70 – 2.52 (m, 4H, O-CH_2 morpholino), 2.33 (t, *J* = 7.5 Hz, 2H, CH_2), 1.93 – 1.60 (m, 10H, CH_2), 1.51 – 1.32 (m, 15H, CH_2 and *t*Bu CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.28, 162.66, 162.18, 160.14, 156.12, 150.67, 148.66, 142.30, 135.45, 132.41, 128.85, 128.27, 123.29, 114.97, 114.81, 69.19, 67.13, 56.06, 55.59, 54.06, 47.05, 40.68, 39.16, 34.74, 30.11, 29.84, 28.98, 28.66, 28.58, 27.84, 26.63, 25.14. HRMS-ESI calculated for $C_{40}H_{56}N_5O_8$ $[M+H]^+$ 734.4123, found *m/z* 734.4144.

***trans*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-{[(*tert*-butoxy)carbonyl]amino}heptanoate (26)**

A solution of **21** (28 mg, 55 µmol), 7-{[(tert-butoxy)carbonyl]amino}heptanoic acid (27 mg, 0.11 mmol), TFFH (27 mg, 0.11 mmol), Et_3N (77 µL, 0.55 mmol) and DMAP (0.7 mg, 5.5 µmol) in DCM (1 mL) was reacted as described in the procedure for **24**. The crude residue was purified by flash silica column chromatography (100% EA) to yield **26** (32 mg, 44 µmol, 80%) as a yellow brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.77 (d, *J* = 7.9 Hz, 1H, NH),

8.93 – 8.86 (m, 2H, ArH), 8.17 (d, *J* = 2.4 Hz, 1H, ArH), 7.61 – 7.50 (m, 2H, ArH MeOPh), 7.08 – 7.00 (m, 2H, ArH MeOPh), 4.84 – 4.71 (m, 3H, NH and N1-CH_2), 4.58 – 4.46 (m, 1H, CH), 4.12 – 3.97 (m, 1H, CH), 3.88 (s, 3H, O-CH_3), 3.69 (t, *J* = 4.6 Hz, 4H, N-CH_2 morpholino), 3.11 (q, *J* = 6.7 Hz, 2H, CH_2), 2.74 (t, *J* = 7.2 Hz, 2H, N1-CH_2CH_2), 2.69 – 2.58 (m, 4H, O-CH_2 morpholino), 2.29 (t, *J* = 7.5 Hz, 2H, CH_2), 2.20 – 1.96 (m, 4H, CH_2), 1.66 – 1.47 (m, 8H, CH_2), 1.44 (s, 9H, *t*Bu CH_3), 1.37 – 1.30 (m, 4H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.38, 162.58, 162.37, 160.14, 156.11, 150.69, 148.69, 142.26, 135.46, 132.39, 128.87, 128.28, 123.22, 114.97, 114.78, 71.78, 67.16, 56.05, 55.59, 54.07, 47.63, 40.66, 39.15, 34.67, 30.27, 30.07, 30.04, 28.92, 28.59, 26.60, 25.07 (one quaternary carbon was not observed). HRMS-ESI calculated for $C_{40}H_{55}N_5O_8$ $[M+H]^+$ 734.4123, found *m/z* 734.4072.

***tert*-Butyl *N*-({[(1s,4s)-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl]carbamoyl}methyl)carbamate (27)**

A solution of **22** (43 mg, 71.3 μmol) and TFA (0.3 mL) in DCM (3 mL) was reacted as described in the procedure for **12**, to yield the TFA salt of 6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-*N*-[(1s,4s)-4-aminocyclohexyl]-1,2-dihydro-1,8-naphthyridine-3-carboxamide (60 mg) as a yellow solid. HRMS calculated for $C_{28}H_{36}N_5O_4$ $[M+H]^+$ 506.2762, found *m/z* 506.2767. This TFA salt (55 mg, 65.3 μmol), DIPEA (68 μL, 0.39 mmol), HATU (25 mg, 65.3 μmol) and Boc-glycine (34 mg, 0.20 mmol) in DMF (2 mL) were used as described in the procedure for **10**. The reaction mixture was evaporated under reduced pressure and the residue taken up in EA (5 mL), washed with H_2O (3 x 5 mL), sat. aq. NaCl (3 mL) and sat. $NaHCO_3$ (1 mL), dried over $MgSO_4$, filtered and evaporated under reduced pressure. The crude product was purified by flash silica gel column chromatography (95:5 EA/MeOH) to yield **27** (13.4 mg, 20.3 μmol, 31%) as a yellow solid (R_f 0.10, 100% EA). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.01 (d, J = 7.5 Hz, 1H, NH), 8.94 – 8.87 (m, 2H, ArH), 8.17 (d, J = 2.4 Hz, 1H, ArH), 7.59 – 7.51 (m, 2H, ArH MeOPh), 7.08 – 7.01 (m, 2H, ArH MeOPh), 6.24 (d, J = 7.7 Hz, 1H, NH), 5.31 – 5.16 (m, 1H, NH), 4.79 (t, J = 6.8 Hz, 2H, N1-CH_2), 4.29 – 4.16 (m, 1H, CH), 4.03 – 3.90 (m, 1H, CH), 3.88 (s, 3H, O-CH_3), 3.78 (d, J = 5.8 Hz, 2H, COCH_2NH), 3.69 (t, J = 4.6 Hz, 4H, N-CH_2 morpholino), 2.82 – 2.72 (m, 2H, N1-CH_2CH_2), 2.71 – 2.57 (m, 4H, O-CH_2 morpholino), 1.90 – 1.75 (m, 6H, CH_2), 1.68 – 1.56 (m, 2H, CH_2), 1.47 (s, 9H, *t*Bu CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.81, 162.67, 162.22, 160.14, 156.30, 150.68, 148.65, 142.23, 135.46, 132.42, 128.82, 128.27, 123.24, 114.97,

114.80, 80.47, 67.14, 56.05, 55.58, 53.98, 46.50, 45.56, 44.85, 39.07, 29.83, 28.65, 28.49. HRMS calculated for $C_{35}H_{47}N_6O_7$ $[M+H]^+$ 663.3501, found *m/z* 663.3508.

cis-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-acetamidoacetate (28)

A solution of **23** (7.0 mg, 10.4 μmol) and TFA (0.2 mL) in DCM (0.8 mL) was reacted as described in the procedure for **12**. The crude residue was purified by semi-preparative RP-HPLC to yield the TFA salt of *cis*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-aminoacetate (4.3 mg, 4.75 μmol, 45%) as a yellow solid. Analytical RP-HPLC R_t = 12.83 min. This TFA salt (1.5 mg, 1.7 μmol), DIPEA (0.87 μL, 5.0 μmol), and acetic anhydride (0.17 μL, 1.8 μmol) in DCM (503 μL) were treated as in the procedure for **14**. The product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **28** (0.97 mg, 1.6 μmol, 96%) as a yellow solid. HRMS-ESI calculated for $C_{32}H_{40}N_5O_7$ $[M+H]^+$ 606.2922, found *m/z* 606.2933. Analytical RP-HPLC R_t = 14.73 min.

trans-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-acetamidoacetate (29)

A solution of **24** (4.1 mg, 6.2 μmol) and TFA (0.2 mL) in DCM (0.8 mL) was reacted as described in the procedure for **12**. The crude was purified by semi-preparative RP-HPLC to yield the TFA salt of *trans*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-aminoacetate (3.1 mg, 3.42 μmol, 55%) as a yellow solid. Analytical RP-HPLC R_t = 12.83 min. This TFA salt of (1.4 mg, 1.5 μmol), DIPEA (0.81 μL, 4.6 μmol), and acetic anhydride (0.16 μL, 1.7 μmol) in DCM (496 μL) were treated as in the procedure for **14**. The product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **29** (0.9 mg, 1.49 μmol, 96%) as a yellow solid. Low resolution MS-ESI calculated for $C_{32}H_{40}N_5O_7$ $[M+H]^+$ 606.2922, found 606.2. Analytical RP-HPLC R_t = 14.70 min.

cis-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-acetamidoheptanoate (30)

A solution of **25** (22 mg, 29.6 μmol) and TFA (0.25 mL) in DCM (1 mL) was reacted as described in the procedure for **12**. The crude was purified by semi-preparative RP-HPLC to

yield the TFA salt of *cis*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-aminoheptanoate (13 mg, 13.3 μmol, 45%) as a yellow solid. HRMS-ESI calculated for $C_{35}H_{48}N_5O_6$ $[M+H]^+$ 634.3599, found *m/z* 634.3595. Analytical RP-HPLC R_t = 14.41 min. This TFA salt (3.3 mg, 3.4 μmol), DIPEA (1.77 μL, 10.2 μmol), and acetic anhydride (0.35 μL, 3.7 μmol) in DCM (610 μL) were treated as in the procedure for **14**. The product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **30** (2.23 mg, 3.3 μmol, 98%) as a yellow solid. HRMS-ESI calculated for $C_{37}H_{50}N_5O_7$ $[M+H]^+$ 676.3705, found *m/z* 676.3710. Analytical RP-HPLC R_t = 16.76 min.

***trans*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-acetamidoheptanoate (31)**

A solution of **26** (15 mg, 20.8 μmol) and TFA (0.25 mL) in DCM (1 mL) was reacted as described in the procedure for **12**. The crude was purified by semi-preparative RP-HPLC to yield the TFA salt of *trans*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-aminoheptanoate (11.1 mg, 11.4 μmol, 55%) as a yellow solid. HRMS-ESI calculated for $C_{35}H_{48}N_5O_6$ $[M+H]^+$ 634.3599, found *m/z* 634.3583. Analytical RP-HPLC R_t = 14.42 min. This TFA salt (3.5 mg, 3.6 μmol), DIPEA (1.87 μL, 10.8 μmol), and acetic anhydride (0..7 μL, 4.0 μmol) in DCM (622 μL) were treated as in the procedure for **14**. The product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **31** (2.36 mg, 3.5 μmol, 97%) as a yellow solid. HRMS-ESI calculated for $C_{37}H_{50}N_5O_7$ $[M+H]^+$ 676.3705, found *m/z* 676.3737. Analytical RP-HPLC R_t = 16.81 min.

Compound **32** detailed in manuscript.

***trans*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-[6-(2-{4-[(E)-2-[2,2-difluoro-4-(thiophen-2-yl)-1λ4,3-diaza-2λ4-boratricyclo[7.3.0.03,7]dodeca-1(12),4,6,8,10-pentaen-12-yl]ethenyl]phenoxy}acetamido)hexanamido]acetate (33)**

The TFA salt of *trans*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 2-aminoacetate (1.7 mg, 1.9 μmol), DIPEA (1.3 μL, 7.6 μmol), BODIPY 630/650-X-OSu (1.25 mg, 1.89 μmol) and DMF (721 μL) were

reacted as described in the procedure for **16**. The crude product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **33** (1.51 mg, 1.36 μmol, 72%) as a bright blue solid. HRMS-ESI calculated for $C_{59}H_{64}BF_2N_8O_9S$ $[M+H]^+$ 1109.4582, found *m/z* 1109.4580. Analytical RP-HPLC R_t = 20.39 min.

***cis*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-[6-(2-{4-[(E)-2-[2,2-difluoro-4-(thiophen-2-yl)-1λ4,3-diaza-2λ4-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-12-yl]ethenyl]phenoxy}acetamido)hexanamido]heptanoate (34)**

A solution of **26** (15 mg, 20.8 μmol) and TFA (0.25 mL) in DCM (1 mL) was reacted as described in the procedure for **12**. The crude was purified by semi-preparative RP-HPLC to yield the TFA salt of *trans*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-aminoheptanoate (11.1 mg, 11.4 μmol, 55%) as a yellow solid. HRMS-ESI calculated for $C_{35}H_{48}N_5O_6$ $[M+H]^+$ 634.3599, found *m/z* 634.3583. Analytical RP-HPLC R_t = 14.42 min. This TFA salt (3.7 mg, 3.8 μmol), DIPEA (2.0 μL, 11.4 μmol), BODIPY 630/650-X-OSu (1.25 mg, 1.89 μmol) and DMF (732 μL) were reacted as described in the procedure for **16**. The crude product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **34** (1.74 mg, 1.48 μmol, 78%) as a bright blue solid. HRMS-ESI calculated for $C_{64}H_{74}BF_2N_8O_9S$ $[M+H]^+$ 1179.5366, found *m/z* 1179.5276. Analytical RP-HPLC R_t = 21.39 min.

***trans*-4-[6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-[6-(2-{4-[(E)-2-[2,2-difluoro-4-(thiophen-2-yl)-1λ4,3-diaza-2λ4-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-12-yl]ethenyl]phenoxy}acetamido)hexanamido]heptanoate (35)**

The TFA salt of *trans*-4-[6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-1,2-dihydro-1,8-naphthyridine-3-amido]cyclohexyl 7-aminoheptanoate (4.2 mg, 4.3 μmol), DIPEA (2.2 μL, 12.5 μmol), BODIPY 630/650-X-OSu (1.25 mg, 1.89 μmol) and DMF (735 μL) were reacted as described in the procedure for **16**. The crude product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **35** (2.16 mg, 1.83 μmol, 97%) as a bright blue solid. HRMS-ESI

calculated for $C_{64}H_{74}BF_2N_8O_9S$ $[M+H]^+$ 1179.5366, found *m/z* 1179.5275. Analytical RP-HPLC R_t = 21.43 min.

6-(4-Methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-*N*-[(1s,4s)-4-{2-[6-(2-{4-[(*E*)-2-[2,2-difluoro-4-(thiophen-2-yl)-1λ4,3-diaza-2λ4-boratricyclo[7.3.0.0^{3,7}]dodeca-1(12),4,6,8,10-pentaen-12-yl]ethenyl]phenoxy}acetamido)hexanamido]-acetamido}cyclohexyl]-1,2-dihydro-1,8-naphthyridine-3-carboxamide (36)

A solution of **27** (12 mg, 18.1 μmol) and TFA (0.4 mL) in DCM (1.6 mL) was reacted as described in the procedure for **12**. The crude was purified by semi-preparative RP-HPLC to yield the TFA salt of 6-(4-methoxyphenyl)-1-[2-(morpholin-4-yl)ethyl]-2-oxo-*N*-[(1s,4s)-4-(2-aminoacetamido)cyclohexyl]-1,2-dihydro-1,8-naphthyridine-3-carboxamide (9.27 mg, 10.3 μmol, 57%) as a yellow solid. HRMS calculated for $C_{30}H_{39}N_6O_5$ $[M+H]^+$ 563.2904, found *m/z* 563.2943. Analytical RP-HPLC R_t = 12.47 min. This TFA salt (3.39 mg, 3.8 μmol), DIPEA (1.85 μL, 10.6 μmol), BODIPY 630/650-X-OSu (1 mg, 1.51 μmol) and DMF (731 μL) were reacted as described in the procedure for **16**. The crude product was purified by semi-preparative RP-HPLC and passed through an Amberlyst A21 ion exchange resin to remove TFA, yielding **36** (2.29 mg, 2.07 μmol, quantitative yield*) as a bright blue solid. HRMS calculated for $C_{59}H_{65}BF_2N_9O_8S$ $[M+H]^+$ 1108.4742, found *m/z* 1108.4777. Analytical RP-HPLC R_t = 20.12 min. *The greater than 100% calculated yield for **36** (137%) was likely due to the commercially supplied container of BODIPY 630/650-X-OSu containing more than the stated 1 mg. Previous synthesis of dye compounds have used a larger container of BODIPY 630/650-X-OSu (e.g. 5 mg) split between three or four reactions, and therefore any excess in BODIPY 630/650-X-OSu would be spread across compounds.

cAMP assays in wild-type cells *not* expressing hCB_2

Linker-conjugate **30** and fluorescent ligand **32** invoked a small but significant response at 10 μM but not at 1 μM in the WT HEK-Flp line (control for CB_2 response; Supplementary Table 1). Fluorescent ligand **32** and smaller ligand **6** showed a significant response at 10 μM in the HEK293 WT cells (control for CB_1 response), but not at 1 μM. The potencies of **30** at CB_2 receptor and **32** at CB_2 and CB_1 receptor are too low to be able to exclude the 10 μM data point from the concentration response curve, therefore the potencies are an estimate and the E_{max} values will be slightly augmented by these non-specific effects. The response of **32** at 10 μM in the HEK293 WT cells (parental CB_1 line; 166.6% ± 11.1) was slightly greater than the 10 μM response in the HEK293-CB_1 cells (157% ± 2.1), therefore the E_{max} of **32** at 1 μM is reported for CB_1 receptor (Table 2). SR144528 was also screened for activity in WT cells and demonstrated no significant response in either cell line.

Supplementary Table 1: Forskolin stimulated cAMP response in wild type HEK293 cells.

	% response ± SEM, HEK-Flp WT line (for comparison with hCB_2 cAMP response)		% response ± SEM, HEK WT line (for comparison with hCB_1 cAMP response)	
Compound	10 μM	1 μM	10 μM	1 μM
1	109.5 ± 3.9	95.8 ± 4.7	-	-
6	100.6 ± 3.0	94.3 ± 2.2	121.5 ± 3.5 *	95.7 ± 3.6
8	97.4 ± 3.3	96.4 ± 1.9	107.2 ± 2.8	97.9 ± 2.6
20	111.9 ± 5.4	95.3 ± 1.4	-	-
28	109.8 ± 5.2	92.2 ± 1.1	-	-
30	122.9 ± 6.2 *	98.3 ± 2.5	-	-
32	139.5 ±10.0 *	100.5 ± 2.5	166.6 ± 11.1 *	105.4 ± 2.9
SR144528	97.4 ± 5.9	98.5 ± 3.8	94.5 ± 1.6	99.8 ± 2.2

cAMP levels measured in a BRET assay using a CAMYEL sensor. Data represent mean values ± SEM for at least three independent experiments conducted in duplicate. Values are normalised to basal (0%) and forskolin (100%) response. Data was analysed for normality in a D'Agostino & Pearson normality test and then analysed using a one sample *t* test for significant difference to forskolin only (100%) response and values marked with * demonstrated significant difference.

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