SUPPLEMENTARY NOTE 1

Discovery of a chemical probe for the L3MBTL3 methyl-lysine reader domain

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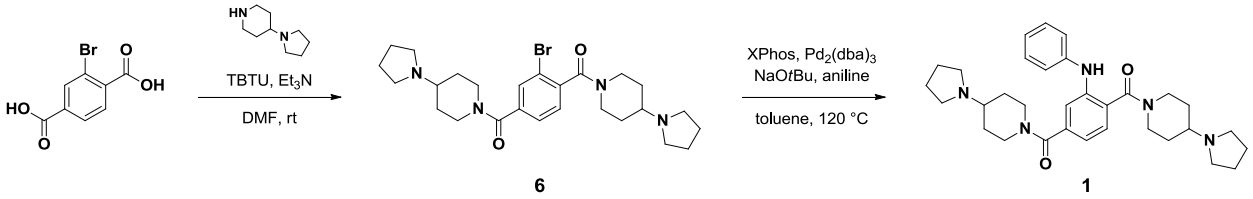
Supplementary Methods

1. Synthesis of UNC1215, UNC1021, and UNC1079

General procedures. Analytical LCMS data for all compounds were acquired using an Agilent 6110 Series system with the UV detector set to 220 nm. Samples were injected (<10 µL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 µm, C18 column at room temperature. Mobile phases A (H_2O + 0.1% acetic acid) and B (MeOH + 0.1% acetic acid) were used with a linear gradient from 10% to 100% B in 5.0 min, followed by a flush at 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury spectrometer at 400 MHz for proton (^{1}H NMR) and 100 MHz for carbon (^{13}C NMR); chemical shifts are reported in ppm (δ) relative to residual protons in deuterated solvent peaks. Preparative HPLC was performed using an Agilent Prep 1200 series with the UV detector set to 220 nm and 254 nm. Samples were injected onto a Phenomenex Luna 250 × 30 mm, 5 µm, C18 column at room temperature. Mobile phases of A (H_2O + 0.1% TFA) and B (MeOH) were used with a flow rate of 40 mL/min. A general gradient of 0-5 minutes at 5% B, 5-20 minutes increasing from 5 to 40% B, and 20-25 minutes increasing from 40 to 80% B was used, followed by a 100% B flush for another 3 minutes. Small variations in this purification method were made as needed to achieve ideal separation for each compound. Analytical LCMS (at 220 nm) and NMR were used to establish the purity of targeted compounds. All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by ^{1}HNMR and LC-MS.

Supplementary Scheme 1. Synthesis of UNC1215 (1), route 1.

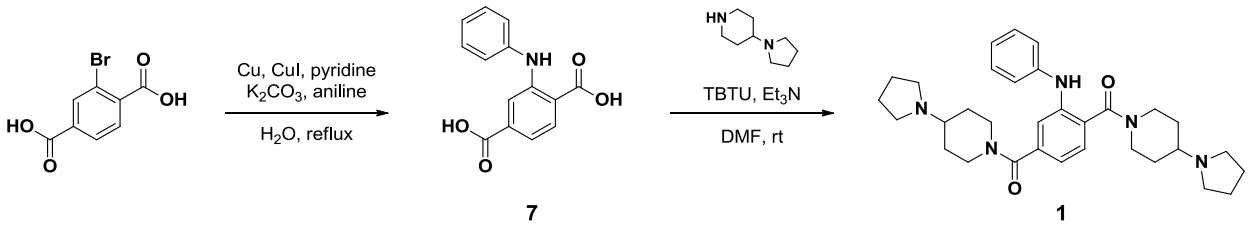


2-Bromo-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (**6**): To a mixture of 2-bromoterephthalic acid (85 mg, 0.35 mmol) and TBTU (290 mg, 0.90 mmol) in DMF (1 mL), a solution of 4-(1-pyrrolidinyl)piperidine (129 mg, 0.83 mmol) and triethylamine (290 µL, 2.08 mmol) in DMF (0.5 mL)

was added. The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3×). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 255 mg (98%) of the TFA salt of **6** as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.77 (dd, *J* = 7.0 Hz, 1.2 Hz, 1H), δ 7.55 – 7.50 (m, 1H), δ 7.50 – 7.40 (m, 1H), δ 4.90 – 4.71 (m, 2H), δ 3.95 – 3.75 (m, 1H), δ 3.75 – 3.59 (m, 4H), δ 3.59 – 3.50 (m, 1H), δ 3.50 – 3.39 (m, 2H), δ 3.28 – 3.07 (m, 6H), δ 3.02 – 2.84 (m, 2H), δ 2.38 – 1.95 (m, 12H), δ 1.93 – 1.55 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD, multiple rotamers observed) δ 169.7, 168.9, 168.7, 140.3, 140.1, 139.4, 139.3, 132.5, 132.4, 129.3, 127.7, 127.6, 120.3, 120.2, 63.1, 62.9, 62.9, 52.9, 52.9, 46.5, 45.9, 41.0, 40.9, 30.5, 29.9, 29.5, 29.3, 23.9, 23.8. MS (ESI+): 517.2 + 519.2 $[M+H]^+$; HPLC: 100%, t_R: 0.72 min.

2-phenylamino-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (**UNC1215**, **1**): A mixture of the TFA salt of **6** (65 mg, 0.087 mmol), 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (21 mg, 50 mol%), tris(dibenzylideneacetone)dipalladium(0) (20 mg, 25 mol%), and sodium *tert*-butoxide (20 mg, 0.30 mmol) in anhydrous toluene (1 mL) was added to a sealable reaction tube. The solution was degassed with N_2 for 20 minutes and aniline (11 μL, 0.12 mmol) was added subsequently. The reaction tube was tightly sealed and the reaction was stirred at 120 °C for 15 hours. The reaction was cooled to room temperature, diluted with CH_2Cl_2, and filtered over a thick pad of celite which was subsequently washed with CH_2Cl_2. The CH_2Cl_2 was removed by rotary evaporation. The crude mixture was brought up in a small amount of a methanol-water mixture and filtered. The resulting filtrate was purified by reverse phase HPLC and the solvents were removed to afford 44 mg (68%) of the TFA salt of **1** as a tan solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.35 (d, *J* = 7.8 Hz, 1H), δ 7.32 – 7.25 (m, 2H), δ 7.23 (d, *J* = 1.2 Hz, 1H), δ 7.13 – 7.07 (m, 2H), δ 7.02 – 6.96 (m, 2H), δ 4.82 – 4.53 (m, 2H), δ 4.10 – 3.74 (m, 2H), δ 3.72 – 3.50 (m, 4H), δ 3.47 – 3.35 (m, 2H), δ 3.28 – 2.99 (m, 6H), δ 2.99 – 2.69 (m, 2H), δ 2.37 – 1.88 (m, 12H), δ 1.76 – 1.44 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 171.6, 170.3, 143.6, 143.4, 138.8, 130.6, 130.1, 127.1, 123.4, 120.6, 119.4, 116.9, 63.0, 62.9, 52.9, 52.7, 46.9, 41.4, 30.4, 29.4, 23.9, 23.9. MS (ESI+): 530.4 $[M+H]^+$; HPLC: 100%, t_R: 2.67 min. HRMS calcd. for $C_{32}H_{43}N_5O_2$ + H: 530.35; found: 530.3473 $[M+H]^+$. HRMS calcd. for $C_{32}H_{43}N_5O_2$ - H: 528.33; found: 528.3305 $[M-H]^-$.

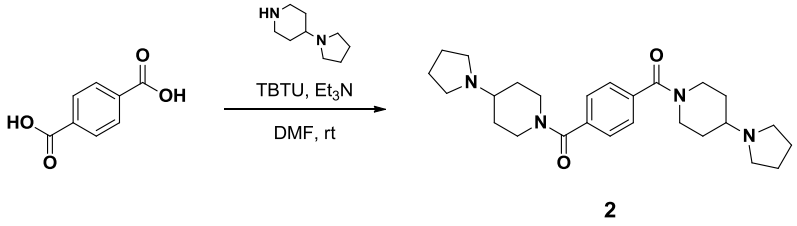
Supplementary Scheme 2. Synthesis of UNC1215 (1), route 2.



2-(phenylamino)terephthalic acid (5): Compound **7** was prepared according to a reported procedure.[1] In brief, potassium carbonate (1.7 g, 12.2 mmol) was dissolved in 40 mL of distilled, degassed water, followed by addition of 2-bromoterephthalic acid (2.0 g, 8.2 mmol). Next, aniline (1.5 g, 16.3 mmol), pyridine (120 µL, 1.5 mmol), copper(0) (40 mg), and copper(I) iodide (40 mg) were added. The reaction mixture was stirred under reflux for 4 hours and then cooled. Enough 1 M NaOH was added to completely dissolve the product. The mixture was then filtered through a thick pad of celite to remove the copper catalyst and the resulting filtrate was acidified with 3 M HCl to yield a yellow precipitate. The crude product was carried foward without further purification.

2-phenylamino-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (**UNC1215**, **1**): To a mixture of **7** (60 mg, 0.233 mmol) and TBTU (195 mg, 0.61 mmol) in DMF (1 mL), a solution of 4-(1-pyrrolidinyl)piperidine (86 mg, 0.56 mmol) and triethylamine (195 µL, 1.4 mmol) in DMF (1 mL) was added. The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3×). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 75 mg (43% over two steps) of the TFA salt of **1** as a tan solid. ^{1}H NMR, ^{13}C NMR, and HRMS same as above.

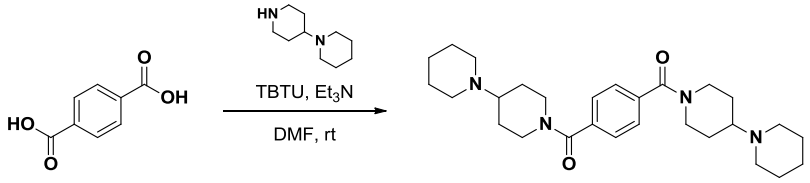
Supplementary Scheme 3. Synthesis of UNC1021 (2).



1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (**2**): To a mixture of terephthalic acid (250 mg, 1.51 mmol) and TBTU (1.26 g, 3.91 mmol) in DMF (2 mL) was added a solution of 4-(1-

pyrrolidinyl)piperidine (567 mg, 3.61 mmol) and triethylamine (1.26 mL, 9.03 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 640 mg (64%) of the TFA salt of **2** as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (s, 4H), δ 4.87 – 4.67 (m, 2H), δ 4.00-3.76 (m, 2H), δ 3.76 – 3.56 (m, 4H), δ 3.45 (tt, *J* = 11.7, 4.1 Hz, 2H), δ 3.29 – 3.06 (m, 6H), δ 3.06 – 2.79 (m, 2H), δ 2.41 – 1.93 (m, 12H), δ 1.80 – 1.52 (m, 4H). ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 138.3, 128.42, 63.0, 52.9, 46.9, 41.6, 30.2, 29.5, 23.9. MS (ESI+): 439.35 $[M+H]^+$; HPLC: 100%, t_R: 0.57 min.

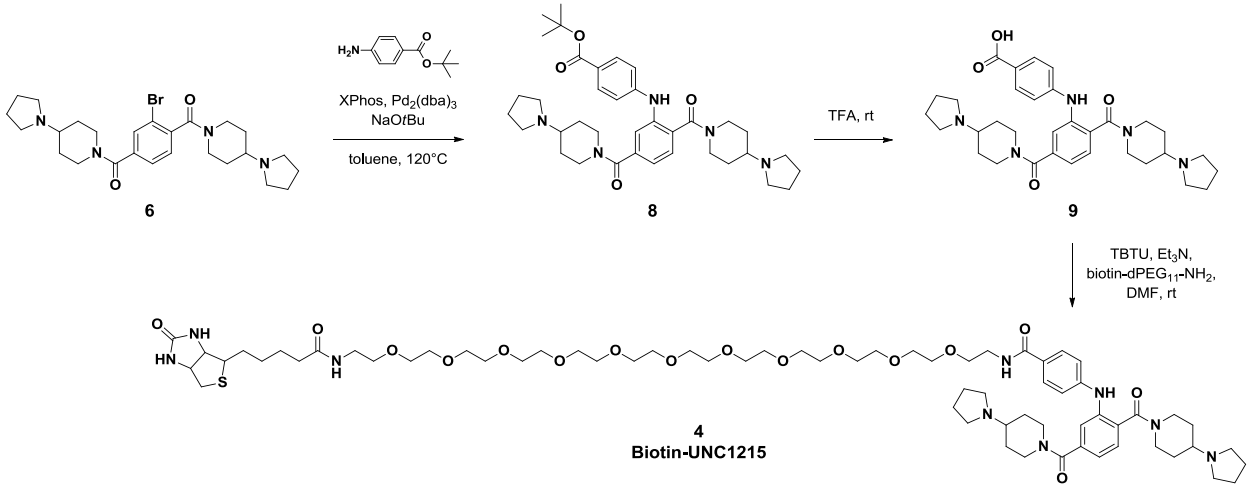
Supplementary Scheme 4. Synthesis of UNC1079 (3).



3

1,4-Bis(4-(piperidinyl)piperidinyl)benzamide (**3**): To a mixture of terephthalic acid (75 mg, 0.45 mmol) and TBTU (377 mg, 1.17 mmol) in DMF (1 mL), a solution of 4-piperidinopiperidine (182 mg, 1.08 mmol) and triethylamine (377 µL, 2.71 mmol) in DMF (0.5 mL) was added. The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 281 mg (90%) of the TFA salt of **3** as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (s, 4H), δ 4.90 – 4.64 (m, 2H), δ 4.07-3.73 (m, 2H), δ 3.61 – 3.43 (m, 6H), δ 3.31 – 3.11 (m, 2H), δ 3.10 – 2.97 (m, 4H), δ 2.97 – 2.78 (m, 2H), δ 2.36 – 1.92 (m, 8H), δ 1.92 – 1.64 (m, 10H), δ 1.60 – 1.44 (m, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ 171.4, 138.3, 128.42, 64.8, 51.3, 47.3, 41.8, 28.0, 27.1, 24.5, 22.9. MS (ESI+): 467.4 $[M+H]^+$; HPLC: 100%, t_R: 1.11 min.

Supplementary Scheme 5. Synthesis of Biotin-UNC1215 (4).

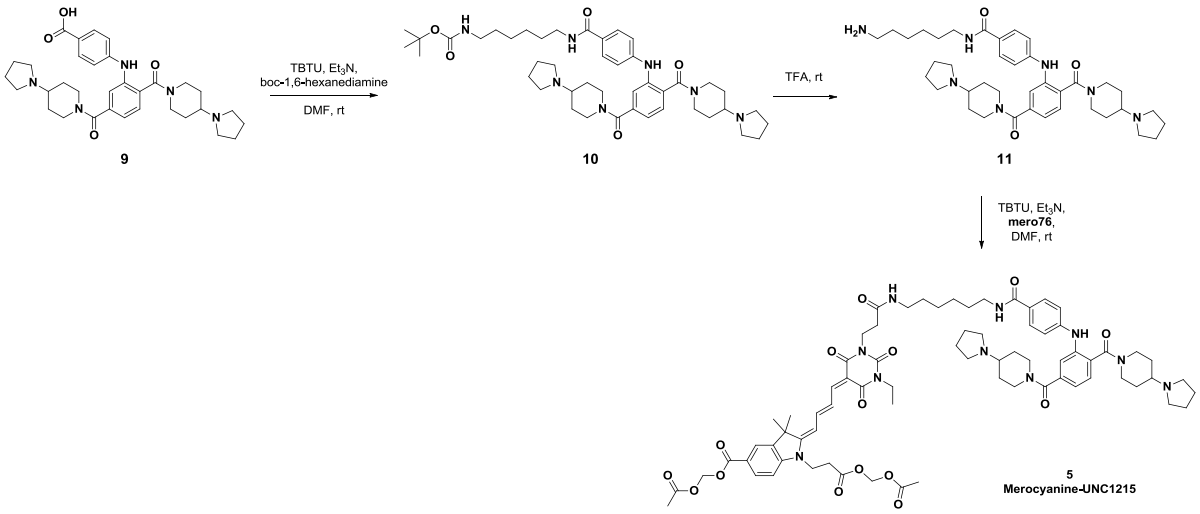


2-(*tert* butyl-4-aminobenzoate)-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (8): Compound **8** was prepared similarly to **1** in route 1. A mixture of **6** (75 mg, 0.14 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (35 mg, 50 mol%), tris(dibenzylideneacetone)dipalladium(0) (33 mg, 25 mol%), and sodium *tert*-butoxide (21 mg, 0.22 mmol) in anhydrous toluene (1 mL) was added to a sealable reaction tube. The solution was degassed for 20 minutes and *tert*-butyl-4-aminobenzoate (39 mg, 0.20 mmol) was added subsequently. The reaction tube was tightly sealed and the reaction was stirred at 120 °C for 15 hours. The reaction was cooled to room temperature, diluted with CH_2Cl_2, and filtered over a pad of celite. The CH_2Cl_2 was removed by rotary evaporation. The crude mixture was carried forward without further purification.

2-(4-aminobenzoic acid)-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (9): A crude mixture of compound **8** was dissolved in CH_2Cl_2 (2 mL) and TFA (1 mL) and the solution was stirred at room temperature for 4 hours. The CH_2Cl_2 and TFA were removed by rotary evaporation. The crude mixture was brought up in a small amount of a methanol-water mixture and filtered. The resulting filtrate was purified by reverse phase HPLC and the solvents were removed to afford 51 mg (44% over 2 steps) of the TFA salt of **9** as a tan solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93- 7.88 (m, 2H), δ 7.45 (d, *J* = 7.8 Hz, 1H), δ 7.41 (d, *J* = 1.2 Hz, 1H), δ 7.19 (dd, *J* = 7.8, 1.5 Hz, 1H), δ 7.10 – 7.04 (m, 2H), δ 4.83 – 4.57 (m, 2H), δ 4.08 – 3.71 (m, 2H), δ 3.71 – 3.60 (m, 2H), 3.60 – 3.49 (m, 2H), δ 3.47 – 3.34 (m, 2H), δ 3.28 – 2.98 (m, 6H), δ 2.98 – 2.68 (m, 2H), δ 2.38 – 1.88 (m, 12H), δ 1.75 – 1.37 (m, 4H). MS (ESI+): 574.3 $[M+H]^+$; HPLC: 100%, t_R: 1.49 min.

2-(4-biotin-dPEG$_{11}$-aminobenzoate)-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (4): To a mixture of **9** (43 mg, 0.05 mmol) and TBTU (22 mg, 0.070 mmol) in DMF (0.5 mL), a solution of biotin-dPEG$_{11}$-NH$_2$ (50 mg, 0.064 mmol) and triethylamine (45 µL, 0.32 mmol) in DMF (0.5 mL) was added. The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 51 mg (61%) of the TFA salt of **4** as a colorless solid. MS (ESI+): 1326.7 $[M+H]^+$, 664.0 $[M+2H]^{2+}$, 443.0 $[M+3H]^{3+}$; HPLC: 100%, t_R: 3.21 min.

Supplementary Scheme 6. Synthesis of Merocyanine-UNC1215 (5).



2-(*tert* butyl-4-amino-*N*-(6-aminohexyl)benzamide)-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (10): To a mixture of the TFA salt of **9** (28 mg, 0.036 mmol) and TBTU (45 mg, 0.14 mmol) in DMF (0.5 mL), a solution of boc-1,6-hexanediamine (28 mg, 0.13 mmol) and triethylamine (45 µL, 0.32 mmol) in DMF (0.5 mL) was added. The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was carried forward without further purification.

2-(4-amino-*N*-(6-aminohexyl)benzamide)-1,4-Bis(4-(pyrrolidinyl)piperidinyl)benzamide (11): A crude mixture of compound **10** was dissolved in CH_2Cl_2 (2 mL) and TFA (1 mL) and the solution was

stirred at room temperature for 4 hours. The CH_2Cl_2 and TFA were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 25 mg (69% over two steps) of the TFA salt of **11** as a clear, light tan solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.77- 7.71 (m, 2H), δ 7.43 (d, *J* = 7.8 Hz, 1H), δ 7.37 (d, *J* = 1.0 Hz, 1H), δ 7.15 (dd, *J* = 7.8, 1.3 Hz, 1H), δ 7.10 – 7.05 (m, 2H), δ 4.83 – 4.57 (m, 2H), δ 4.08 – 3.73 (m, 2H), δ 3.73 – 3.50 (m, 4H), 3.50 – 3.33 (m, 4H), δ 3.28 – 3.00 (m, 6H), δ 2.97 – 2.88 (m, 3H), δ 2.86 – 2.66 (m, 1H), δ 2.38 – 1.88 (m, 12H), δ 1.77 – 1.37 (m, 12H). MS (ESI+): 672.4 $[M+H]^+$; HPLC: 98%, t_R: 1.01 min.

Merocyanine-UNC1215 (5): To a mixture of the TFA salt of **11** (10.7 mg, 10.5 µmol) and TBTU (4.2 mg, 13.0 µmol) in DMF (0.5 mL) was added merocyanine dye **mero76** (6.0 mg, 8.8 µmol) and triethylamine (4.6 µL, 26 µmol). The mixture was stirred at room temperature for 16 hours. The reaction mixture was loaded neat onto a 250 x 21.2 mm, 15 micron Phenomenex C18 preparative column and eluted at 8 mL/min with a gradient of 10% solvent B (H_2O/ACN 5:95, TFA 0.05%) 90% solvent A (H_2O/ACN 95:5, TFA 0.05%) for 2 min, increasing to 100% solvent B over 30 min and held for a total of 45 min. The product fractions were combined, concentrated, and lyophilized to give 9.8 mg (83%) of dark purple powdery solid. MS (ESI+): 1337.0 $[M+H]^+$.

[1] Goodell, J. R., Madhok, A. A., Hiasa, H. & Ferguson, D. M. Synthesis and evaluation of acridine- and acridone-based anti-herpes agents with topoisomerase activity. *Bioorg. Med. Chem.* **14**, 5467-5480 (2006).