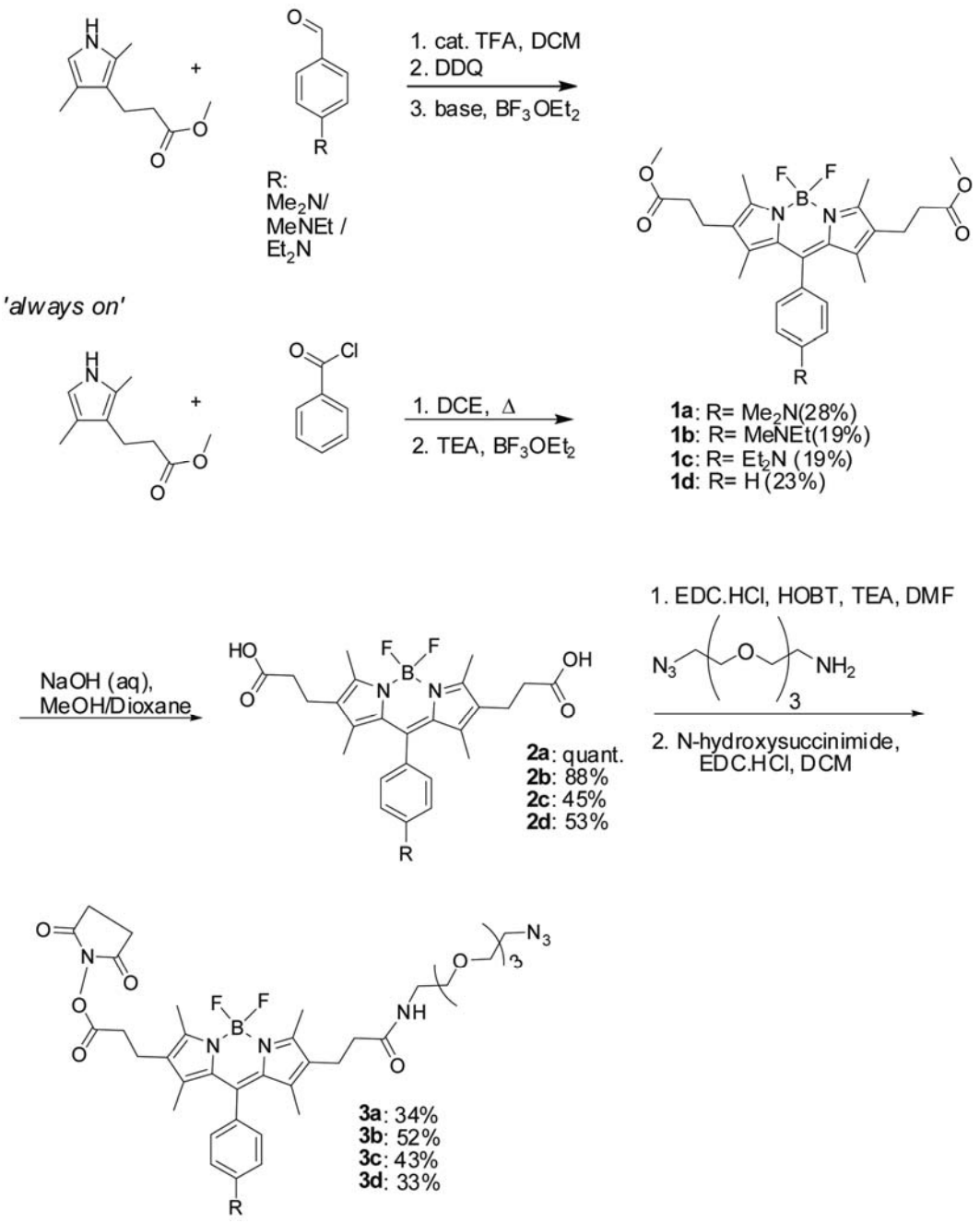
Supporting Information

Supplemental Scheme and Figures



Scheme S1. Synthesis of bifunctional BODIPY dyes.

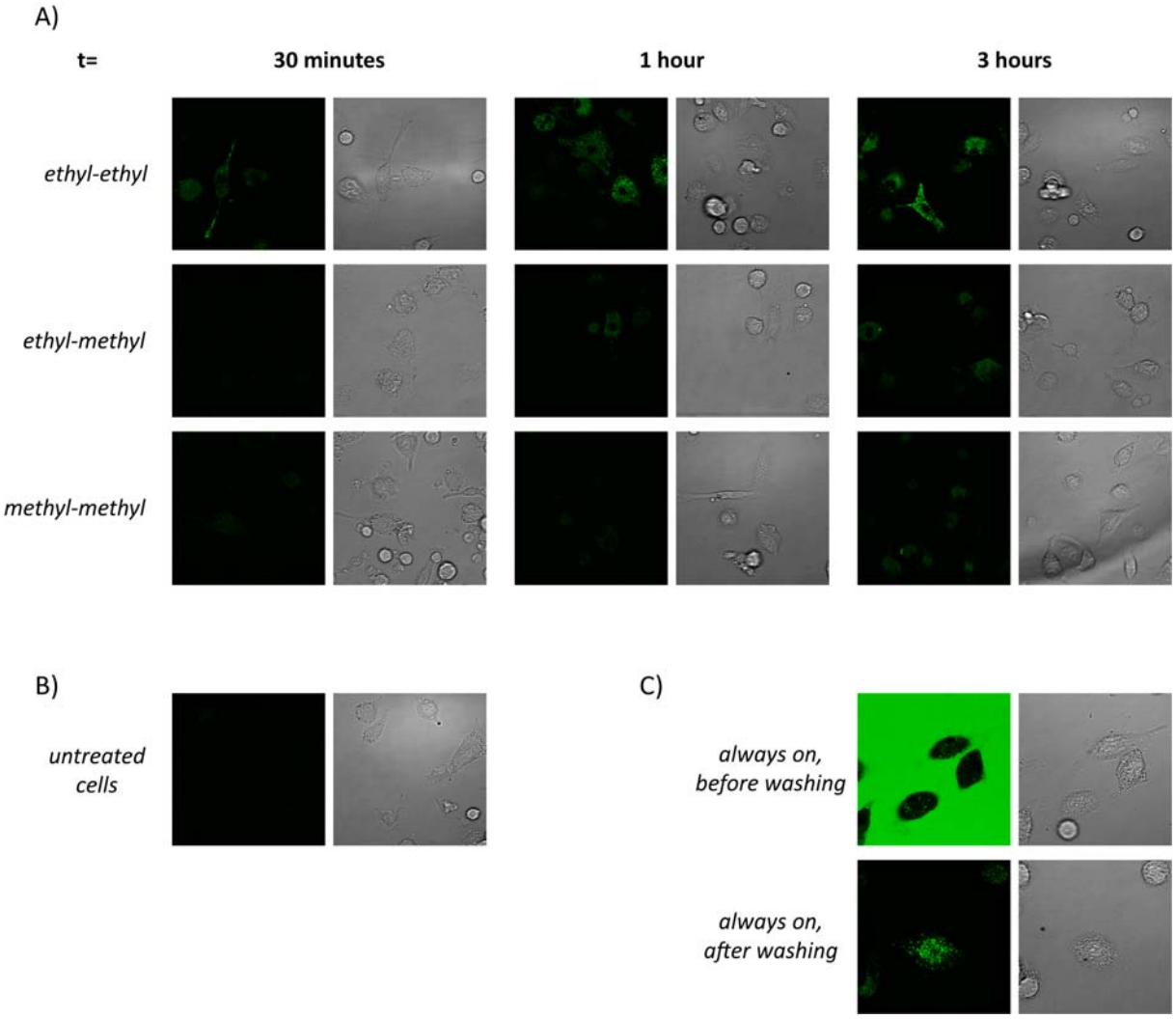


Figure S1. Fluorescence (left) and bright field phase contrast images (right) of immature dendritic cells. A) Fluorescence microscopy time-course experiment, showing that the fluorescence signal increases over time. Times indicated are incubation times at 37 °C, 5% CO_2. B) Untreated cells are completely dark with the laser settings used. C) The “always on“ control probe cannot be used without washing due to a large amount of background.

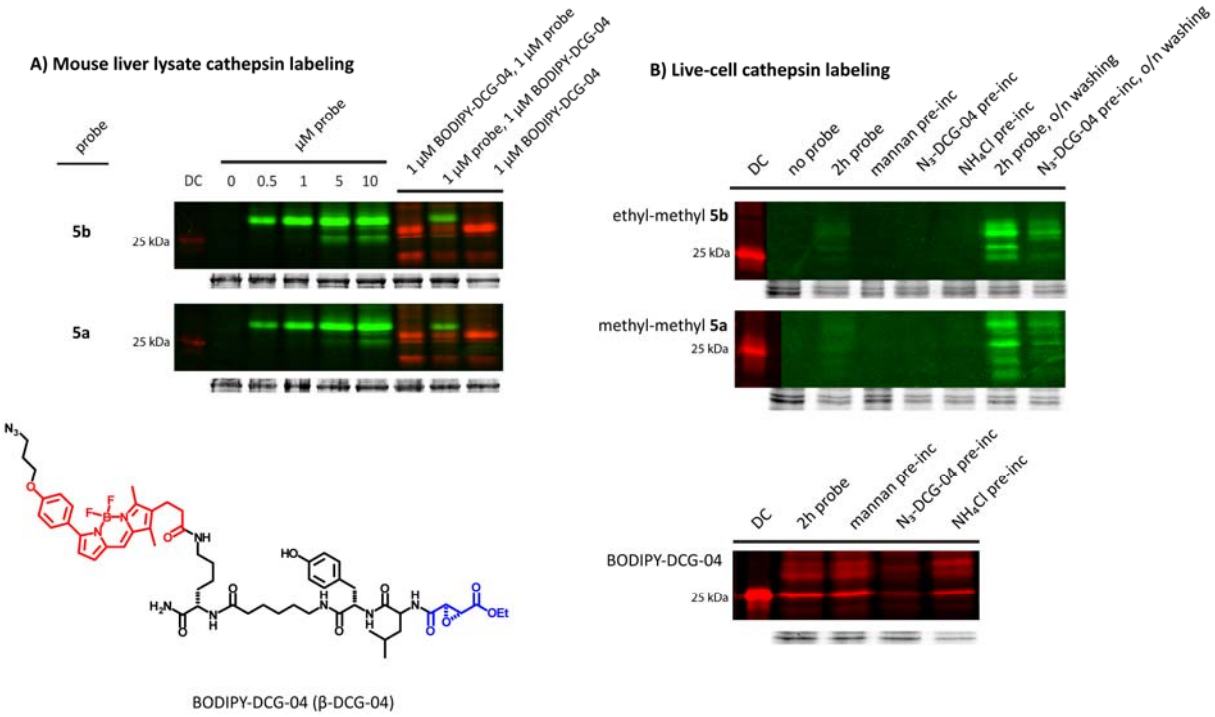


Figure S2. Cell lysate (A) and Live cell (B) labeling experiment with probes **5a** and **5b** and the cell-permeable cathepsin inhibitor BODIPY-DCG-04.[1]

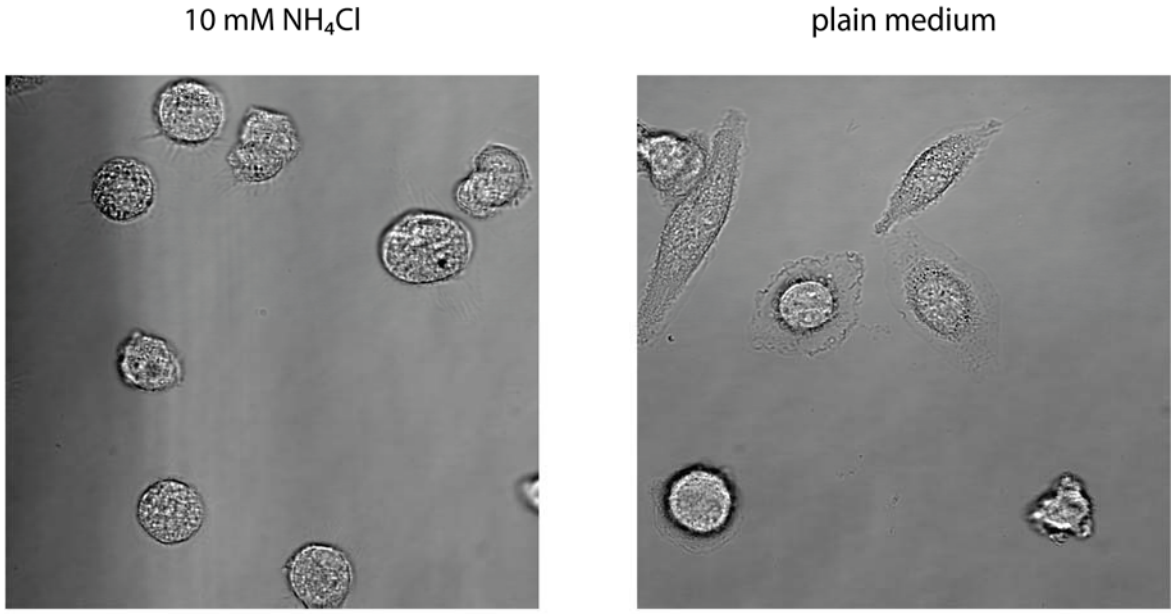


Figure S3. Morphology changes induced by ammonium chloride treatment of dendritic cells are reversible. Left: cells in the presence of 10 mM NH_4Cl were rounded up and showed extensive vacuolisation. Right: Washing in normal medium after NH_4Cl treatment restores the normal morphology of the cells.

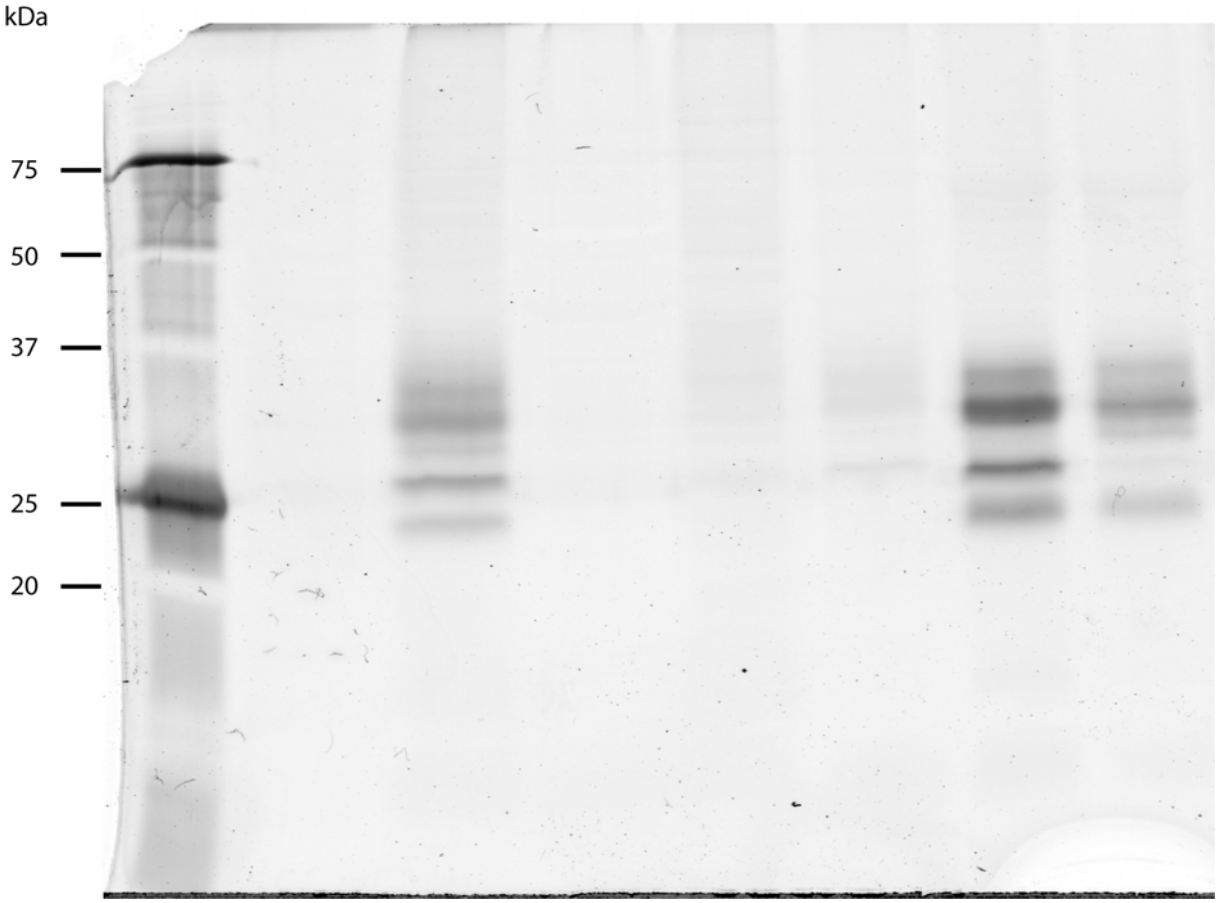


Figure S4. Representative gel image (TAMRA settings, λ_{ex} 532 nm; λ_{em} 580 nm), corresponding to Figure 3C (probe **5c**), showing the complete molecular weight marker.

Materials and Methods

Synthesis

General

All reagents were of commercial grade and used as received unless stated otherwise. Reaction solvents were of analytical grade and when used under anhydrous conditions stored over flame-dried 3 Å molecular sieves. Dichloromethane was distilled over CaH_2 prior to use. Solvents used for column chromatography were of technical grade and distilled before use. Flash chromatography was performed on silica gel (Screening Devices BV, 40-63 µm, 60 Å). Reactions were routinely monitored by TLC analysis on DC-alufolien (Merck, Kieselgel60, F254) with detection by UV-absorption (254/366 nm) where applicable and spraying with a solution of $(NH_4)_6Mo_7O_{24}\cdot H_2O$ (25 g/l) and $(NH_4)_4Ce(SO_4)_4\cdot 2H_2O$ (10 g/l) in 10% sulfuric acid in water followed by charring at ~150°C. ^{1}H and ^{13}C NMR spectra were recorded on a Brüker AV-400 (400 MHz) or Brüker DMX-600 (600 MHz). Chemical shifts are given in ppm (δ) relative to the residual solvent peak or TMS (0 ppm) as internal standard. Coupling constants are given in Hz. LC-MS measurements were conducted on a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer (ESI^+) coupled to a Surveyor HPLC system (Thermo Finnigan) equipped with a standard C_{18} (Gemini, 4.6 mmD x 50 mmL, 5µ particle size, Phenomenex) analytical column and buffers A: H_2O, B: ACN, C: 0.1% aq. TFA. High resolution mass spectra were recorded on a LTQ Orbitrap (Thermo Finnigan) mass spectrometer equipped with an electronspray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10 mL min^{-1}, capillary temperature 250 °C) with resolution R=60000 at *m/z* 400 (mass range *m/z*=150-2000) and dioctylphtalate (*m/z* = 391.28428) as a "lock mass". The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). For reversed-phase HPLC purification of the final compounds an automated HPLC system equipped with a C_{18} semiprep column (Gemini C_{18}, 250x10 mm, 5 µ particle size, Phenomenex) was used.

1,3,5,7-Tetramethyl-2,6-bis-(2-methoxycarbonylethyl)-8-[4-(*N,N*-dimethylamino)phenyl]-4,4-difluoro- 4-bora-3a,4a-diaza-*s*-indacene (1a)

Methyl 2,4-dimethyl-3-pyrrolepropionate (1.43 g, 7.9 mmol, 2.2 eq) and N,N-dimethylaminobenzaldehyde (0.54 g, 3.6 mmol, 1 eq) were dissolved in dry dichloromethane (100 mL) followed by addition of a catalytic amount of TFA. The reaction mixture was stirred overnight at room temperature under an argon atmosphere. 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 0.98 g, 4.3 mmol, 1.2 eq) was added and stirring was continued for 1 h. The reaction was concentrated *in vacuo*, dissolved in dichloroethane (100 mL) followed by addition of diisopropylethylamine (DIPEA, 2.5 mL, 14.4 mmol, 4 eq) and BF_3OEt_2 (2.5 mL, 20 mmol, 5.5 eq). The mixture was stirred for 2h, washed with water (2 x 50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by silica column chromatography (1 → 13% EtOAc in toluene) gave the product as an orange powder (0.53 g, 1 mmol, 28%). R_f= 0.6 (4:1 toluene: EtOAc)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (d, *J* = 8.7 Hz, 2H, 2 x C*H*$_{ar}$), 6.80 (d, *J* = 8.7 Hz, 2H, 2 x C*H*$_{ar}$), 3.68 (s, 6H, 2 x C*H*$_3$), 3.05 (s, 6H, 2 x C*H*$_3$), 2.71 – 2.63 (m, 4H, 2 x C*H*$_2$), 2.56 (s, 6H, 2 x C*H*$_3$), 2.42 – 2.33 (m, 4H, 2 x C*H*$_2$), 1.43 (s, 6H, 2 x C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.30, 153.41, 150.81, 142.49, 139.74, 131.78, 128.96, 128.84, 122.69, 112.51, 51.78, 40.48, 34.42,

19.50, 12.66, 12.27. ESI-HRMS (m/z): calcd. for $[C_{29}H_{36}BF_2N_3O_4 + H]^+$ 540.28449; obsd. 540.28420

1,3,5,7-Tetramethyl-2,6-bis-(2-methoxycarbonylethyl)-8-[4-(*N,N*-ethylmethylamino)phenyl]-4,4-difluoro- 4-bora-3a,4a-diaza-*s*-indacene (1b)

Methyl 2,4-dimethyl-3-pyrrolepropionate (1.23 g, 6.8 mmol, 2.2 eq) and N,N-ethylmethylaminobenzaldehyde (0.49 g, 3.0 mmol, 1 eq) were dissolved in dry dichloromethane (100 mL) followed by addition of a catalytic amount of TFA. The reaction mixture was stirred overnight at room temperature under an argon atmosphere. DDQ (0.82 g, 3.6 mmol, 1.2 eq) was added and stirring was continued for 1 h. The reaction was concentrated *in vacuo*, dissolved in dichloroethane (100 mL) followed by addition of TEA (1.25 mL, 9 mmol, 3 eq) and BF_3OEt_2 (1.9 mL, 15 mmol, 5 eq). The mixture was refluxed for 40 min, cooled to room temperature, washed with water (2 x 50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by repeated silica column chromatography (1 → 25% EtOAc in toluene/ 1 → 25% EtOAc in PE + 1% AcOH) gave the product as orange crystals (0.31 g, 0.56 mmol, 19%). R_f= 0.6 (4:1 toluene: EtOAc)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.02 (d, *J* = 8.6 Hz, 2H, 2 x CH_{ar}), 6.77 (d, *J* = 8.6 Hz, 2H, 2 x CH_{ar}), 3.65 (s, 6H, 2 x CH_3), 3.46 (q, *J* = 7.0 Hz, 2H, CH_2), 2.97 (s, 3H, CH_3), 2.68 – 2.60 (m, 4H, 2 x CH_2), 2.53 (s, 6H, 2 x CH_3), 2.39 – 2.32 (m, 4H, 2 x CH_2), 1.42 (s, 6H, 2 x CH_3), 1.16 (t, *J* = 7.0 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.28, 153.39, 149.57, 142.62, 139.75, 131.84, 129.11, 128.86, 122.39, 112.60, 51.75, 46.96, 37.55, 34.45, 19.55, 12.66, 12.27, 11.15. ESI-HRMS (m/z): calcd. for $[C_{30}H_{38}BF_2N_3O_4 + H]^+$ 554.30015; obsd. 554.29979

1,3,5,7-Tetramethyl-2,6-bis-(2-methoxycarbonylethyl)-8-[4-(*N,N*-diethylamino)phenyl]-4,4-difluoro- 4-bora-3a,4a-diaza-*s*-indacene (1c)

Methyl 2,4-dimethyl-3-pyrrolepropionate (1.81 g, 10 mmol, 2.2 eq) and N,N-diethylaminobenzaldehyde (0.83 g, 4.7 mmol, 1 eq) were dissolved in dry DCM (100 mL) followed by addition of a catalytic amount of TFA. The reaction mixture was stirred overnight at room temperature under an argon atmosphere. DDQ (1.18 g, 5.2 mmol, 1.1 eq) was added and stirring was continued for 1 h. The reaction was concentrated *in vacuo*, dissolved in dichloroethane (100 mL) followed by addition of TEA (1.96 mL, 14.1 mmol, 3 eq) and BF_3OEt_2 (2.95 mL, 23.5 mmol, 5 eq). The mixture was refluxed for 40 min, cooled to room temperature, washed with water (2 x 50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by repeated silica column chromatography (1 → 25% EtOAc in PE + 1% AcOH) gave the product as orange crystals (0.50 g, 0.87 mmol, 19%). R_f= 0.75 (3:1 toluene: EtOAc)
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.74 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 3.65 (s, 6H, 2 x CH_3), 3.41 (q, *J* = 7.0 Hz, 4H, 2 x CH_2), 2.72 – 2.57 (m, 4H, 2 x CH_2), 2.53 (s, 6H, 2 x CH_3), 2.41 – 2.29 (m, 4H, 2 x CH_2), 1.44 (s, 6H, 2 x CH_3), 1.20 (t, *J* = 7.0 Hz, 6H, 2 x CH_3).
^{13}C NMR (101 MHz, $CDCl_3$) δ 173.26, 153.19, 148.24, 142.73, 139.71, 131.78, 129.09, 128.73, 121.62, 112.09, 51.73, 44.45, 34.38, 19.46, 12.60, 12.44, 12.28. ESI-HRMS (m/z): calcd. for $[C_{31}H_{40}BF_2N_3O_4 + H]^+$ 568.31528; obsd. 568.31547

1,3,5,7-Tetramethyl-2,6-bis-(2-methoxycarbonylethyl)-8-phenyl-4,4-difluoro- 4-bora-3a,4a-diaza-*s*-indacene (1d)

Methyl 2,4-dimethyl-3-pyrrolepropionate (0.9 g, 5 mmol, 2.2 eq) was dissolved in dichloroethane (20 mL). Benzoyl chloride (0.29 mL, 2.5 mmol, 1 eq) was added and the

mixture was heated to reflux for 3h. After cooling to room temperature, TEA (1.04 mL, 7.5 mmol, 3 eq) and BF_3OEt_2 (1.57 mL, 12.5 mmol, 5 eq) were added and the reaction mixture was stirred for 16 h. The mixture was washed with water (2 x 50 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure. Purification by repeated silica column chromatography (1 → 12% EtOAc in PE) gave the product as an orange powder (0.29 g, 0.58 mmol, 23%). R_f= 0.8 (1:1 toluene: EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 – 7.45 (m, 3H, 3 x CH_{ar}), 7.28 – 7.23 (m, 2H, 2 x CH_{ar}), 3.65 (s, 6H, 2 x CH_3), 2.67 – 2.57 (m, 4H, 2 x CH_2), 2.55 (s, 6H, 2 x CH_3), 2.39 – 2.32 (m, 4H, 2 x CH_2), 1.29 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.20, 154.16, 141.02, 139.59, 135.54, 131.02, 129.30, 129.25, 129.08, 128.19, 51.81, 34.35, 19.45, 12.74, 11.95. ESI-HRMS (m/z): calcd. for $[C_{27}H_{31}BF_2N_2O_4 + H]^+$ 497.24225; obsd. 497.24199

1,3,5,7-Tetramethyl-2,6-bis-(2-carboxyethyl)-8-[4-(*N,N*-dimethylamino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (2a)

1a (230 mg, 0.43 mmol) was dissolved in methanol/dioxane (1:1, 20 mL). NaOH (aq, 1M, 5 mL) was added and the mixture was heated to 40 °C for 50 min. The mixture was neutralized with HCl (aq, 1M, 5.5 mL), followed by addition of water and extraction with EtOAc. The mixture was dried ($MgSO_4$), filtered and concentrated *in vacuo* to give the product as an orange powder in quantitative yield (217 mg, 0.43 mmol, quant.). R_f= 0.3 (2:1 toluene: EtOAc + AcOH)

^{1}H NMR (400 MHz, MeOD/$CDCl_3$) δ 7.06 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.85 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 3.03 (s, 6H, 2 x CH_3), 2.65 (t, *J* = 7.8 Hz, 4H, 2 x CH_2), 2.51 (s, 6H, 2 x CH_3), 2.34 (t, *J* = 7.8 Hz, 4H, 2 x CH_2), 1.45 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, MeOD/$CDCl_3$) δ 176.28, 154.10, 151.98, 143.53, 140.61, 132.48, 129.95, 129.68, 123.38, 113.37, 40.60, 35.15, 20.16, 12.69, 12.51. ESI-HRMS (m/z): calcd. for $[C_{27}H_{32}BF_2N_3O_4 + H]^+$ 512.25315; obsd. 512.25291

1,3,5,7-Tetramethyl-2,6-bis-(2-carboxyethyl)-8-[4-(*N,N*-ethylmethylamino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (2b)

1b (37 mg, 0.067 mmol) was dissolved in methanol/dioxane (1:1, 3 mL). NaOH (aq, 1M, 0.84 mL) was added and the mixture was heated to 40 °C for 1h. The mixture was neutralized with HCl (aq, 1M, 1 mL), followed by addition of water and extraction with EtOAc. The mixture was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Pure product (31 mg, 0.059 mmol, 88%) was obtained by silica column chromatography (10 → 30% EtOAc in toluene + 1% AcOH). R_f= 0.3 (2:1 toluene: EtOAc + AcOH)

^{1}H NMR (400 MHz, MeOD/$CDCl_3$) δ 7.00 (d, *J* = 8.4 Hz, 2H, 2 x CH_{ar}), 6.83 (d, *J* = 8.5 Hz, 2H, 2 x CH_{ar}), 3.47 (q, *J* = 6.8 Hz, 2H, CH_2), 2.96 (s, 3H, CH_3), 2.64 (t, *J* = 7.6 Hz, 4H, 2 x CH_2), 2.49 (s, 6H, 2 x CH_3), 2.33 (t, *J* = 7.6 Hz, 4H, 2 x CH_2), 1.45 (s, 6H, 2 x CH_3), 1.14 (t, *J* = 6.9 Hz, 3H, CH_3). ^{13}C NMR (101 MHz, MeOD/$CDCl_3$) δ 176.35, 154.18, 150.67, 143.76, 140.66, 132.58, 130.06, 129.92, 123.03, 113.41, 47.49, 37.73, 35.20, 20.21, 12.68, 12.52, 11.24. ESI-HRMS (m/z): calcd. for $[C_{28}H_{34}BF_2N_3O_4 + H]^+$ 526.26882; obsd. 526.26792

1,3,5,7-Tetramethyl-2,6-bis-(2-carboxyethyl)-8-[4-(*N,N*-diethylamino)phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (2c)

1c (0.45 g, 0.8 mmol) was dissolved in methanol/dioxane (1:1, 20 mL). NaOH (aq, 4M, 2.4 mL) was added and the mixture was heated to 40 °C for 1h. The mixture was neutralized with HCl (aq, 1M, 10 mL), followed by addition of water and extraction with EtOAc. The mixture was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Pure product (0.2 g, 0.36

mmol, 45%) was obtained by silica column chromatography (10 → 20% EtOAc in toluene + 1% AcOH). R_f= 0.3 (2:1 toluene: EtOAc + AcOH)
^{1}H NMR (400 MHz, MeOD/$CDCl_3$) δ 6.99 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.79 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 3.47 – 3.36 (m, 4H, 2 x CH_2), 2.65 (t, *J* = 7.9 Hz, 4H, 2 x CH_2), 2.52 (s, 6H, 2 x CH_3), 2.35 (t, *J* = 7.9 Hz, 4H, 2 x CH_2), 1.47 (s, 6H, 2 x CH_3), 1.20 (t, *J* = 7.0 Hz, 6H, 2 x CH_3).
^{13}C NMR (101 MHz, MeOD/$CDCl_3$) δ 176.08, 153.75, 148.95, 143.50, 140.46, 132.32, 129.69, 129.66, 122.19, 112.83, 45.01, 34.97, 19.98, 12.68, 12.58, 12.54. ESI-HRMS (m/z): calcd. for $[C_{29}H_{36}BF_2N_3O_4 + H]^+$ 540.28449; obsd. 540.28417

1,3,5,7-Tetramethyl-2,6-bis-(2-carboxyethyl)-8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (2d)
1d (30 mg, 0.06 mmol) was dissolved in methanol/dioxane (1:1, 4 mL). NaOH (aq, 1M, 0.84 mL) was added and the mixture was heated to 40 °C for 15 min. The mixture was neutralized with HCl (aq, 1M, 1 mL), followed by addition of water and extraction with EtOAc. The mixture was dried ($MgSO_4$), filtered and concentrated *in vacuo.* Pure product (15 mg, 0.032 mmol, 53%) was obtained by silica column chromatography (10 → 20% EtOAc in toluene + 1% AcOH). R_f= 0.45 (1:1 toluene: EtOAc + AcOH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 – 7.46 (m, 3H, 3 x CH_{ar}), 7.35 – 7.21 (m, 2H, 2 x CH_{ar}), 2.65 (t, *J* = 7.8 Hz, 4H, 2 x CH_2), 2.53 (s, 6H, 2 x CH_3), 2.35 (t, *J* = 7.8 Hz, 4H, 2 x CH_2), 1.34 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.05, 168.66, 154.79, 141.95, 140.41, 136.19, 131.65, 130.25, 129.97, 129.80, 128.90, 96.94, 34.98, 20.03, 12.75, 12.17. ESI-HRMS (m/z): calcd. for $[C_{25}H_{27}BF_2N_2O_4 + H]^+$ 469.21092; obsd. 469.21079

1,3,5,7-Tetramethyl-2-(2-(1-azidotetraethyleneglycol)aminocarbonylethyl)-6-(2-succinimidyloxycarbonylethyl)-8-[4-(*N,N*-dimethylamino)- phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (3a)
2a (97 mg, 0.19 mmol, 1 eq) was dissolved in *N,N*-dimethylformamide (DMF, 10 mL). 11-azidotetraethyleneglycolamine[2] (43 mg, 0.17 mmol, 0.9 eq), triethylamine (40 µL, 0.29 mmol, 1.5 eq) and 1-hydroxybenzotriazole (HOBt, 23 mg, 0.17 mmol, 0.9 eq) were added, the mixture was cooled to 0 °C and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl (EDC, 32 mg, 0.17 mmol, 0.9 eq) was added. After stirring for 16 h at ambient temperature, DMF was evaporated and the solids were dissolved in dichloromethane (DCM, 20 mL). N-hydroxysuccinimide (175 mg, 1.52 mmol, 8 eq) and EDC-HCl (291 mg, 1.52 mmol, 8 eq) were added and after 3h stirring at room temperature, the reaction was finished according to TLC. The mixture was washed with HCl (aq, pH 3) and water, dried ($MgSO_4$), filtered and concentrated. Pure compound (53 mg, 0.065 mmol) was obtained by silica column chromatography (0 → 1% MeOH in DCM) in 34% yield. R_f=0.75 (10:1 DCM: MeOH)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.78 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.30 (s, 1H, N*H*), 3.73 – 3.56 (m, 10H, 5 x CH_2), 3.53 (t, *J* = 5.0 Hz, 2H, CH_2), 3.41 (dd, *J* = 10.1, 5.1 Hz, 2H, CH_2), 3.39 – 3.34 (m, 2H, CH_2), 3.03 (s, 6H, 2 x CH_3), 2.83 (d, *J* = 8.4 Hz, 4H, 2 x CH_2), 2.79 – 2.70 (m, 2H, CH_2), 2.71 – 2.61 (m, 4H, 2 x CH_2), 2.53 (s, 6H, 2 x CH_3), 2.30 – 2.18 (m, 2H, CH_2), 1.42 (s, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.43, 171.94, 169.21, 167.85, 154.42, 152.37, 150.80, 142.64, 140.33, 139.36, 132.00, 131.49, 129.75, 128.88, 127.27, 122.43, 112.50, 70.70, 70.65, 70.52, 70.28, 70.06, 69.92, 50.72, 40.45, 39.45, 36.70, 31.32, 25.67, 25.55, 25.47, 20.02, 19.13, 12.74, 12.62, 12.33, 12.29. ESI-HRMS (m/z): calcd. for $[C_{39}H_{52}BF_2N_8O_8 + Na]^+$ 831.37900; obsd. 831.37902

1,3,5,7-Tetramethyl-2-(2-(1-azidotetraethyleneglycol)aminocarbonylethyl)-6-(2-succinimidyloxycarbonylethyl)-8-[4-(*N,N*-ethylmethylamino)- phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (3b)

2b (63 mg, 0.12 mmol, 1 eq) was dissolved in *N,N*-dimethylformamide (DMF, 5 mL). 11-azidotetraethyleneglycolamine[2] (28 mg, 0.11 mmol, 0.9 eq), TEA (15 µL, 0.11 mmol, 0.9 eq) and HOBt (15 mg, 0.11 mmol, 0.9 eq) were added, the mixture was cooled to 0 °C and EDC-HCl (21 mg, 0.11 mmol, 0.9 eq) was added. After stirring for 16 h at ambient temperature, DMF was evaporated and the solids were dissolved in DCM (10 mL). N-hydroxysuccinimide (115 mg, 0.96 mmol, 8 eq) and EDC-HCl (184 mg, 0.96 mmol, 8 eq) were added and after 2h stirring at room temperature, the reaction was finished according to TLC. The mixture was washed with HCl (aq, pH 3) and water, dried ($MgSO_4$), filtered and concentrated. Pure compound (51 mg, 0.062 mmol) was obtained by silica column chromatography (0 → 1% MeOH in DCM) in 52% yield. R_f=0.6 (15:1 DCM: MeOH)

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.02 (s, 2H, 2 x C*H*$_{ar}$), 6.78 (s, 2H, 2 x C*H*$_{ar}$), 5.99 (t, *J* = 5.2 Hz, 1H, N*H*), 3.66 – 3.56 (m, 10H, 5 x C*H*$_2$), 3.52 (t, *J* = 5.1 Hz, 2H, C*H*$_2$), 3.47 (dd, *J* = 13.8, 6.8 Hz, 2H, C*H*$_2$), 3.44 – 3.39 (m, 2H, C*H*$_2$), 3.38 – 3.33 (m, 2H, C*H*$_2$), 2.98 (s, 3H, C*H*$_3$), 2.83 (s, 4H, 2 x C*H*$_2$), 2.78 – 2.71 (m, 2H, C*H*$_2$), 2.70 – 2.59 (m, 4H, 2 x C*H*$_2$), 2.52 (d, *J* = 16.9 Hz, 6H, 2 x C*H*$_3$), 2.24 – 2.17 (m, 2H, C*H*$_2$), 1.43 (s, 6H, 2 x C*H*$_3$), 1.17 (t, *J* = 6.9 Hz, 3H, C*H*$_3$).

^{13}C NMR (151 MHz, $CDCl_3$) δ 172.04, 169.15, 167.88, 154.41, 152.45, 149.57, 142.78, 140.29, 139.39, 132.07, 131.57, 129.84, 129.09, 127.34, 122.15, 112.58, 70.82, 70.77, 70.65, 70.42, 70.17, 69.97, 50.79, 39.46, 36.82, 31.39, 25.72, 20.01, 19.21, 12.81, 12.67, 12.38, 12.33, 11.16. ESI-HRMS (m/z): calcd. for $[C_{40}H_{53}BF_2N_8O_8 + Na]^+$ 845.39466; obsd. 845.39463

1,3,5,7-Tetramethyl-2-(2-(1-azidotetraethyleneglycol)aminocarbonylethyl)-6-(2-succinimidyloxycarbonylethyl)-8-[4-(*N,N*-diethylamino)- phenyl]-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (3c)

2c (35 mg, 0.064 mmol, 1 eq) was dissolved in *N,N*-dimethylformamide (DMF, 3 mL). 11-azidotetraethyleneglycolamine[2] (17 mg, 0.06 mmol, 0.9 eq), TEA (8 µL, 0.06 mmol, 0.9 eq) and HOBt (8 mg, 0.06 mmol, 0.9 eq) were added, the mixture was cooled to 0 °C and EDC-HCl (12 mg, 0.06 mmol, 0.9 eq) was added. After stirring for 16 h at ambient temperature, DMF was evaporated and the solids were dissolved in DCM (10 mL). N-hydroxysuccinimide (37 mg, 0.32 mmol, 5 eq) and EDC-HCl (61 mg, 0.32 mmol, 5 eq) were added and after 2 h stirring at room temperature, the reaction was finished according to TLC. The mixture was washed with HCl (aq, pH 3) and water, dried ($MgSO_4$), filtered and concentrated. Pure compound (23 mg, 0.027 mmol) was obtained by silica column chromatography (0 → 2% MeOH in DCM) in 43% yield. R_f=0.8 (15:1 DCM: MeOH)

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.6 Hz, 2H, 2 x C*H*$_{ar}$), 6.74 (d, *J* = 8.7 Hz, 2H, 2 x C*H*$_{ar}$), 6.09 (s, 1H, N*H*), 3.72 – 3.57 (m, 10H, 5 x C*H*$_2$), 3.52 (t, *J* = 4.9 Hz, 2H, C*H*$_2$), 3.47 – 3.34 (m, 8H, 4 x C*H*$_2$), 2.89 – 2.79 (m, 4H, 2 x C*H*$_2$), 2.79 – 2.72 (m, 2H, C*H*$_2$), 2.71 – 2.60 (m, 4H, 2 x C*H*$_2$), 2.54 (s, 6H, 2 x C*H*$_3$), 2.22 (t, *J* = 7.7 Hz, 2H, C*H*$_2$), 1.45 (s, 6H, 2 x C*H*$_3$), 1.20 (t, *J* = 7.0 Hz, 6H, 2 x C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.99, 169.15, 169.07, 167.76, 154.17, 152.15, 148.20, 142.82, 140.21, 139.28, 131.98, 131.47, 129.63, 128.98, 127.12, 121.35, 112.00, 70.66, 70.62, 70.49, 70.25, 70.02, 69.85, 50.64, 44.38, 39.31, 36.68, 31.25, 25.59, 19.90, 19.08, 12.38, 12.32, 12.26. ESI-HRMS (m/z): calcd. for $[C_{41}H_{55}BF_2N_8O_8 + Na]^+$ 859.41033; obsd. 859.41055

1,3,5,7-Tetramethyl-2-(2-(1-azidotetraethyleneglycol)aminocarbonylethyl)-6-(2-succinimidyloxycarbonylethyl)-8-phenyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (3d)

2d (27 mg, 0.06 mmol, 1 eq) was dissolved in *N,N*-dimethylformamide (DMF, 2 mL). 11-azidotetraethyleneglycolamine[2] (16 mg, 0.054 mmol, 0.9 eq), TEA (7.5 µL, 0.054 mmol, 0.9 eq) and HOBt (7 mg, 0.054 mmol, 0.9 eq) were added, the mixture was cooled to 0 °C and EDC-HCl (10.5 mg, 0.054 mmol, 0.9 eq) was added. After stirring for 24 h at ambient temperature, DMF was evaporated and the solids were dissolved in DCM (5 mL). N-hydroxysuccinimide (55 mg, 0.48 mmol, 8 eq) and EDC-HCl (92 mg, 0.48 mmol, 8 eq) were added and after 2 h stirring at room temperature, the reaction was finished according to TLC. The mixture was washed with HCl (aq, pH 3) and water, dried ($MgSO_4$), filtered and concentrated. Pure compound (15 mg, 0.02 mmol) was obtained by silica column chromatography (0 → 1% MeOH in DCM) in 33% yield. R_f=0.7 (15:1 DCM: MeOH)
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 – 7.44 (m, 3H, 3 x C*H*$_{ar}$), 7.35 – 7.23 (m, 2H, 2 x C*H*$_{ar}$), 6.02 (s, 1H, N*H*), 3.70 – 3.56 (m, 10H, 5 x C*H*$_2$), 3.53 (t, *J* = 5.0 Hz, 2H, C*H*$_2$), 3.45 – 3.40 (m, 2H, C*H*$_2$), 3.39 – 3.31 (m, 2H, C*H*$_2$), 2.84 (s, 4H, 2 x C*H*$_2$), 2.77 – 2.70 (m, 2H, C*H*$_2$), 2.70 – 2.58 (m, 4H, 2 x C*H*$_2$), 2.55 (s, 6H, 2 x C*H*$_3$), 2.20 (t, *J* = 7.7 Hz, 2H, C*H*$_2$), 1.30 (s, 6H, 2 x C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.90, 169.01, 167.68, 155.10, 153.07, 141.06, 140.02, 139.15, 135.28, 132.06, 130.66, 130.11, 129.22, 129.05, 128.03, 127.56, 70.67, 70.62, 70.50, 70.26, 70.04, 69.81, 50.64, 39.34, 36.56, 31.19, 29.71, 25.59, 19.82, 19.01, 12.74, 12.61, 11.92, 11.87. ESI-HRMS (m/z): calcd. for $[C_{37}H_{46}BF_2N_7O_8 + Na]^+$ 788.33677; obsd. 788.33660

General procedure for BODIPY-DCG-04 derivatives (4a-d)

BODIPY **3a-d** (~20 µmol, 1 eq) was dissolved in DMF (0.5 mL) and DIPEA (1 eq) was added. A solution of DCG-04 amine[3] (0.95 eq) in DMF was dropwise added. The mixture was stirred for 3h at room temperature, concentrated and isolated using silica column chromatography (3 → 7% MeOH in DCM). The product was lyophilised from t-butanol/H_2O to give a hygroscopic orange powder.

N,N-dimethylamino BODIPY-DCG-04 (**4a**)

Yield: 10 mg (7.3 µmol, 37%). R_f=0.45 (10:1 DCM:MeOH)
^{1}H NMR (600 MHz, MeOD) δ 7.05 (d, *J* = 8.4 Hz, 2H, 2 x C*H*$_{ar}$), 7.01 (d, *J* = 8.3 Hz, 2H, 2 x C*H*$_{ar}$), 6.88 (d, *J* = 8.8 Hz, 2H, 2 x C*H*$_{ar}$), 6.69 (d, *J* = 8.2 Hz, 2H, 2 x C*H*$_{ar}$), 4.45 (t, *J* = 7.6 Hz, 1H, C*H*), 4.41 – 4.36 (m, 1H, C*H*), 4.31 – 4.21 (m, 3H, C*H*$_2$ + C*H*), 3.68 – 3.51 (m, 12H, 5 x C*H*$_2$ + 2 x C*H*), 3.47 (t, *J* = 5.5 Hz, 2H, C*H*$_2$), 3.34 – 3.32 (m, 4H, 2 x C*H*$_2$), 3.17 – 3.07 (m, 3H, C*H*$_2$ + C*H*$_2$-H1), 3.08 – 2.99 (m, 7H, 2 x C*H*$_3$ + C*H*$_2$-H2), 2.99 – 2.93 (m, 1H, C*H*$_2$-H1), 2.85 – 2.78 (m, 1H, C*H*$_2$-H2), 2.65 (dd, *J* = 13.5, 7.2 Hz, 4H, 2 x C*H*$_2$), 2.49 (s, 6H, 2 x C*H*$_3$), 2.28 – 2.17 (m, 6H, 3 x C*H*$_2$), 1.81 – 1.73 (m, 1H, C*H*$_2$-H1), 1.67 – 1.60 (m, 1H, C*H*$_2$-H2), 1.60 – 1.32 (m, 18H, 6 x C*H*$_2$ + 2 x C*H*$_3$), 1.30 (t, *J* = 7.2 Hz, 3H, C*H*$_3$), 1.25 – 1.16 (m, 2H, C*H*$_2$), 0.93 (d, *J* = 6.4 Hz, 3H, C*H*$_3$), 0.89 (d, *J* = 6.4 Hz, 3H, C*H*$_3$). ^{13}C NMR (151 MHz, MeOD) δ 177.16, 176.12, 175.14, 174.97, 173.67, 173.08, 169.22, 168.35, 157.31, 154.81, 154.57, 152.56, 143.88, 141.04, 140.85, 132.79, 132.73, 131.36, 130.55, 130.44, 130.10, 128.90, 123.71, 116.24, 113.68, 71.61, 71.56, 71.47, 71.31, 71.11, 70.60, 63.22, 56.46, 54.41, 54.24, 53.40, 53.04, 51.75, 41.65, 40.57, 40.52, 40.20, 40.18, 38.20, 37.44, 37.27, 36.66, 32.79, 30.01, 29.84, 27.41, 26.50, 25.88, 24.34, 23.31, 22.03, 21.28, 21.18, 14.37, 12.79, 12.56, 12.53. ESI-HRMS (m/z): calcd. for $[C_{68}H_{98}BF_2N_{13}O_{14} + H]^+$ 1370.75011; obsd. 1370.75147

N,N-ethylmethylamino BODIPY-DCG-04 (4b)

Yield: 9 mg (6.5 µmol, 43%). R_f=0.45 (10:1 DCM:MeOH)

^{1}H NMR (600 MHz, MeOD) δ 7.06 – 6.98 (m, 4H, 4 x CH_{ar}), 6.87 (d, *J* = 8.8 Hz, 2H, 2 x CH_{ar}), 6.69 (d, *J* = 8.5 Hz, 2H, 2 x CH_{ar}), 4.45 (t, *J* = 7.6 Hz, 1H, C*H*), 4.39 (dd, *J* = 9.1, 5.9 Hz, 1H, C*H*), 4.31 – 4.19 (m, 3H, CH_2 + C*H*), 3.67 – 3.56 (m, 10H, 4 x CH_2 + 2 x C*H*), 3.56 – 3.52 (m, 2H, CH_2), 3.52 – 3.44 (m, 4H, 2 x CH_2), 3.34 – 3.32 (m, 4H, 2 x CH_2), 3.18 – 3.08 (m, 3H, CH_2 + CH_2-H1), 3.07 – 3.01 (m, 1H, CH_2-H2), 2.99 – 2.93 (m, 4H, CH_3 + CH_2-H1), 2.85 – 2.79 (m, 1H, CH_2-H2), 2.66 (dd, *J* = 13.8, 7.2 Hz, 4H, 2 x CH_2), 2.47 (s, 6H, 2 x CH_3), 2.28 – 2.19 (m, 6H, 3 x CH_2), 1.81 – 1.73 (m, 1H, CH_2-H1), 1.67 – 1.60 (m, 1H, CH_2-H2), 1.59 – 1.32 (m, 18H, 6 x CH_2 + 2 x CH_3), 1.30 (t, *J* = 7.1 Hz, 3H, CH_3), 1.23 – 1.17 (m, 2H, CH_2), 1.15 (t, *J* = 7.0 Hz, 3H, CH_3), 0.93 (d, *J* = 6.4 Hz, 3H, CH_3), 0.89 (d, *J* = 6.4 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, MeOD) δ 177.15, 176.12, 175.15, 174.97, 173.68, 173.08, 168.73, 168.41, 157.31, 154.76, 154.51, 151.09, 143.97, 141.03, 140.85, 132.82, 132.76, 131.36, 130.53, 130.42, 130.23, 128.90, 123.28, 116.24, 113.64, 71.61, 71.56, 71.48, 71.32, 71.11, 70.61, 63.21, 56.48, 54.38, 54.24, 53.39, 53.18, 51.75, 47.62, 41.65, 40.52, 40.20, 40.18, 38.20, 37.76, 37.44, 37.27, 36.66, 32.79, 30.01, 29.84, 27.42, 26.50, 25.89, 24.34, 23.31, 22.02, 21.29, 21.19, 14.36, 12.78, 12.56, 12.53, 11.24. ESI-HRMS (m/z): calcd. for $[C_{69}H_{100}BF_2N_{13}O_{14} + H]^+$ 1384.76578; obsd. 1384.76725

N,N-diethylamino BODIPY-DCG-04 (4c)

Yield: 12 mg (8.6 µmol, 31%). R_f=0.3 (10:1 DCM:MeOH)

^{1}H NMR (600 MHz, MeOD/$CDCl_3$) δ 7.03 – 6.95 (m, 4H, 4 x CH_{ar}), 6.77 (d, *J* = 8.7 Hz, 2H, 2 x CH_{ar}), 6.74 – 6.69 (m, 2H, 2 x CH_{ar}), 4.45 (t, *J* = 7.5 Hz, 1H, C*H*), 4.40 (t, *J* = 7.3 Hz, 1H, C*H*), 4.33 – 4.23 (m, 3H, CH_2 + C*H*), 3.67 – 3.60 (m, 9H, 4 x CH_2 + C*H*), 3.60 – 3.56 (m, 2H, CH_2), 3.55 (d, *J* = 1.8 Hz, 1H, C*H*), 3.50 (t, *J* = 5.3 Hz, 2H, CH_2), 3.42 (q, *J* = 7.0 Hz, 4H, 2 x CH_2), 3.39 – 3.32 (m, 4H, 2 x CH_2), 3.20 – 3.08 (m, 3H, CH_2 + CH_2-H1), 3.05 – 2.98 (m, 1H, CH_2-H2), 2.94 (dd, *J* = 13.7, 7.8 Hz, 1H, CH_2-H1), 2.89 – 2.81 (m, 1H, CH_2-H2), 2.64 (dd, *J* = 15.2, 7.0 Hz, 4H, 2 x CH_2), 2.50 (s, 6H, 2 x CH_3), 2.26 – 2.17 (m, 6H, 3 x CH_2), 1.81 – 1.73 (m, 1H, CH_2-H1), 1.66 – 1.58 (m, 1H, CH_2-H2), 1.58 – 1.49 (m, 6H, 3 x CH_2), 1.46 (s, 6H, 2 x CH_3), 1.40 – 1.29 (m, 7H, 2 x CH_2 + CH_3), 1.22 – 1.17 (m, 6H, 2 x CH_3), 1.17 – 1.10 (m, 2H, CH_2), 0.91 (d, *J* = 5.9 Hz, 3H, CH_3), 0.88 (d, *J* = 5.8 Hz, 3H, CH_3). ^{13}C NMR (151 MHz, MeOD/$CDCl_3$) δ 176.00, 175.08, 174.02, 173.68, 172.45, 171.84, 167.90, 167.20, 156.22, 153.71, 153.62, 148.82, 143.21, 140.42, 140.33, 132.14, 130.74, 129.73, 129.66, 129.46, 129.37, 127.93, 121.89, 115.78, 115.72, 112.62, 70.99, 70.89, 70.54, 70.44, 70.04, 62.88, 55.50, 53.96, 53.20, 52.82, 52.29, 51.06, 44.84, 41.11, 39.79, 39.54, 39.49, 37.76, 36.93, 36.84, 36.18, 32.05, 29.19, 29.00, 26.60, 25.74, 25.69, 25.19, 23.40, 23.07, 21.85, 20.68, 20.57, 14.19, 12.62, 12.53, 12.44, 12.40. ESI-HRMS (m/z): calcd. for $[C_{70}H_{102}BF_2N_{13}O_{14} + H]^+$ 1398.78144; obsd. 1398.78302

phenyl BODIPY-DCG-04 (4d)

Yield: 8.4 mg (6 µmol, 31%). R_f=0.3 (10:1 DCM:MeOH)

^{1}H NMR (400 MHz, MeOD/$CDCl_3$) δ 7.54 – 7.47 (m, 3H, 3 x CH_{ar}), 7.27 – 7.22 (m, 2H, 2 x CH_{ar}), 7.01 (d, *J* = 8.5 Hz, 2H, 2 x CH_{ar}), 6.73 (d, *J* = 8.5 Hz, 2H2 x CH_{ar}), 4.49 – 4.39 (m, 2H,2 x C*H*), 4.39 – 4.23 (m, 3H, CH_2 + C*H*), 3.69 – 3.64 (m, 9H, 4 x CH_2 + C*H*), 3.61 – 3.56 (m, 2H, CH_2), 3.54 – 3.49 (m, 3H, CH_2 + C*H*), 3.39 – 3.33 (m, 4H, 2 x CH_2), 3.24 – 3.06 (m, 3H, CH_2 + CH_2-H1), 3.05 – 2.97 (m, 1H, CH_2-H2), 2.96 – 2.82 (m, 2H, CH_2), 2.69 – 2.60 (m, 4H, 2 x CH_2), 2.53 (s, 6H, 2 x CH_3), 2.27 – 2.12 (m, 6H, 3 x CH_2), 1.85 – 1.73 (m, 1H, CH_2-H1), 1.69 – 1.58 (m, 1H, CH_2-H2), 1.57 – 1.41 (m, 6H, 3 x CH_2), 1.36 – 1.28 (m, 13H, 3 x CH_3 + 2 x CH_2), 1.16 – 1.05 (m,

2H, CH_2), 0.96 – 0.86 (m, 6H, 2 x CH_3). ^{13}C NMR (101 MHz, MeOD/$CDCl_3$) δ 175.56, 174.67, 173.46, 171.97, 171.43, 167.49, 166.70, 155.91, 141.15, 139.96, 139.86, 135.49, 131.08, 130.49, 129.97, 129.83, 129.43, 129.29, 128.22, 127.61, 115.53, 70.77, 70.68, 70.64, 70.26, 70.19, 69.87, 62.69, 55.25, 53.80, 52.78, 52.71, 51.82, 50.83, 40.99, 39.50, 39.34, 39.18, 38.13, 37.62, 36.64, 36.52, 35.98, 31.79, 29.33, 28.90, 28.69, 26.24, 25.37, 24.96, 23.03, 22.91, 21.74, 20.33, 20.21, 14.05, 12.54, 11.86. ESI-HRMS (m/z): calcd. for $[C_{66}H_{93}BF_2N_{12}O_{14} + H]^+$ 1327.70789; obsd. 1327.70956

General procedure for mannose cluster-BODIPY-DCG-04 derivatives (5a-d)

BODIPY-DCG-04 derivative **4a-d** (~5 µmol, 1 eq) dissolved in DMF (degassed, 1 mL) was added to mannose cluster **6**[4] (0.9 eq) in H_2O (degassed, 1 mL) under an argon atmosphere. Sodium ascorbate (1 eq) and $CuSO_4$ (10 mol%), as solution in water (degassed), were added to the mixture followed by heating to 80 °C for 12 h. The reaction was monitored by LC-MS. When needed, additional sodium ascorbate and $CuSO_4$ were added. Upon prolonged heating (>48h), decomposition of starting materials was observed, and so the reaction was terminated by concentration under reduced pressure and semi-preparative HPLC (C_{18} column, solvent A: 0.2% TFA in H_2O; solvent B: acetonitrile) purification. HPLC fractions containing the product were combined and lyophilised, yielding the compound as a hygroscopic orange/reddish powder.

Mannose cluster-N,N-dimethylamino BODIPY-DCG-04 (5a)

Hydrolysis of the ethyl ester of DCG-04 was observed and this compound was isolated besides the title compound. HPLC (27-41% B in 12', 450 µL injection) yielded 13% (2.5 mg, 0.55 µmol) product and 10% (1.9 mg, 0.42 µmol) hydrolysed product. ESI-HRMS (m/z): calcd. for $[C_{199}H_{312}BF_2N_{45}O_{66} + 3H]^{3+}$ 1480.42552; obsd. 1480.42662

Mannose cluster-N,N-ethylmethylamino BODIPY-DCG-04 (5b)

HPLC (27-41% B in 12', 450 µL injection) yielded 29% (6.2 mg, 1.4 µmol) product. ESI-HRMS (m/z): calcd. for $[C_{200}H_{314}BF_2N_{45}O_{66} + 3H]^{3+}$ 1485.43157; obsd. 1485.43328

Mannose cluster-N,N-diethylamino BODIPY-DCG-04 (5c)

This reaction proceeded very sluggishly, and was terminated after 24 h to prevent decomposition of the formed product as well as remaining starting materials.
HPLC (21-36% B in 12', 450 µL injection) yielded 4% (0.56 mg, 0.13 µmol) product. ESI-HRMS (m/z): calcd. for $[C_{201}H_{316}BF_2N_{45}O_{66} + 3H]^{3+}$ 1490.10345; obsd. 1490.10513

Mannose cluster-phenyl BODIPY-DCG-04 (5d)

HPLC (27-41% B in 12', 450 µL injection) yielded 20% (3.3 mg, 0.75 µmol) product. ESI-HRMS (m/z): calcd. for $[C_{197}H_{307}BF_2N_{44}O_{66} + 3H]^{3+}$ 1466.41228; obsd. 1466.41367

Absorption and Fluorescence Spectroscopy

All spectroscopic experiments were performed in citrate/phosphate buffer (McIlvaine)[5] at a concentration of ca. 1 µM (absorption) or 100 nM (fluorescence) of **5a-d**. Measurements were conducted on a Shimadzu UV1700 pharmaspec UV-VIS spectrophotometer (absorbance) and a Shimadzu RF-5301PC spectrofluorometer (fluorescence). For pH-fluorescence curves, the pH was set using different ratios of citric acid (0.1 M stock solution) vs phosphate buffer (0.2 M stock solution). The excitation wavelength was set equal to the

maximum of the corresponding absorption spectrum and the emission spectrum recorded. Experiments were conducted at a concentration of 25 nM in a total volume of 2 mL and performed in quadruplicate. SDS was added to a final concentration of 0.2% from a 10% stock solution in water.

Cell culture of primary cells

Immature dendritic cells were obtained from the bone marrow of C75BL/6 mice. The use of animals was approved by the ethics committee of Leiden University. Mice were sedated, bone marrow of tibiae and femurs was flushed out and washed with PBS. Cells were grown in dendritic cell selection medium (IMDM containing granulocyte-macrophage colony stimulating factor (GM-CSF) 2:1 vol/vol) containing 8% FCS, penicillin/streptomycin (100 units/mL), glutamax (2 mM) and beta-mercaptoethanol (20 µM). Cells were selected for 10 days (37 °C; 5% CO_2) after which they were either used directly or frozen and stored at -80°C until further use.

Live-cell microscopy

Experiments were conducted on a Leica TCS SPE confocal microscope, using GFP or dsRed filter settings (λ_{ex} 488 or 532 nm). Immature dendritic cells (DCs, 30-75 x 10^4 cells/well) were seeded onto Labtek II, sterile, 4- or 8-chamber borosilicate coverglass systems (Fisher Emergo). Stock solutions of probes and other reagents were prepared in conditioned DC medium. For time-course experiments cells were treated with 10 µM of probe **5a-d**, incubated for the indicated time (37 °C; 5% CO_2) and imaged. Removal of the probe-containing medium followed by addition of fresh cell culture medium was required for the "always on" control probe **5d**. Experiments that required pre-treatment were conducted as follows: cells were incubated (37 °C; 5% CO_2) with mannan (3 mg/mL), NH_4Cl (10 mM), or N_3-DCG-04 (10 µM) for 2 h. Probes **5a-d** (1 µM) were added directly to the medium, and incubation was continued for 2h, after which the medium was refreshed and cells imaged. Alternatively, cells were washed with PBS (2 x 0.5 mL) incubated overnight in medium and imaged after 16 h. All experiments were performed at least in duplicate.

SDS-PAGE analysis

Labeling of cathepsins in lysate

Mouse liver lysate (40 µg) in 25 mM MES buffer pH 5.0 was incubated with 0, 0.5, 1 , 5 or 10 µM of probes **5a-d** for 1h at 37 °C. For competition experiments, lysate was pre-incubated with BODIPY-DCG-04 (1 µM) for 1 h at 37 °C, followed by incubation with probes **5a-d** (1 µM). Subsequently, samples were boiled with 4 x Laemli's sample buffer under reducing conditions and resolved on a 12.5% SDS-PAGE gel. To visualize the pH-dependent probes, gels were fixed overnight in MeOH/H_2O/acetic acid (50/40/10). After washing with H_2O/acetic acid (90/10), gels were imaged with a Typhoon 2000 imager (GE Healthcare) using the Cy2 (λ_{ex} 532 nm; λ_{em} 526 nm) and TAMRA (λ_{ex} 532 nm; λ_{em} 580 nm) settings. After imaging, gels were stained with coomassie brilliant blue to visualize total protein loaded. Gel images were coloured with ImageJ.

Labeling of cathepsins in live-cells

Immature mouse dendritric cells were seeded onto tissue-coated 24-wells plates for same-day experiments or on borosilicate 4-chamber microscope slides for overnight experiments

(2 x 10^5 cells/well). Stock solutions of probes and other reagents were prepared in conditioned DC medium. For labeling experiments cells were treated with 1 µM of probe **5a-d**, incubated for 2h (37 °C; 5% CO_2), washed with ice-cold PBS (2x 0.5 mL) and lysed (35 µL Invitrogen complete cell extraction buffer). Experiments that required pre-treatment were conducted as follows: cells were incubated (37 °C; 5% CO_2) with mannan (3 mg/mL), NH_4Cl (10 mM), or N_3-DCG-04 (10 µM) for 2 h. Probes **5a-d** (1 µM) were added directly to the medium, and incubation was continued for 2h. Cells were washed with ice-cold PBS (2x 0.5 mL) and lysed in 35 µL lysis buffer. For the overnight time-point, cells were incubated in fresh medium after the washing step for 16 h before lysis. Lysates were collected, centrifuged (4 °C, 14.000 rpm, 10 min) and stored at –80 °C until further use. Lysates were thawed and the protein concentration was determined by a DC (detergent-compatible) protein assay (Biorad). Samples (20 µg protein/well) were boiled with 4 x Laemli's sample buffer under reducing conditions and resolved on a 12.5% SDS-PAGE gel. To visualize the pH-dependent probes, gels were fixed overnight in MeOH/H_2O/acetic acid (50/40/10). After washing with H_2O/acetic acid (90/10), gels were imaged with a Typhoon 2000 imager (GE Healthcare) using the Cy2 settings (λ_{ex} 532 nm; λ_{em} 526 nm). After imaging, gels were stained with coomassie brilliant blue to visualize total protein loaded. Gel images were coloured with ImageJ.

References

1. Verdoes, M.; Florea, B. I.; Hillaert, U.; Willems, L. I.; van der Linden, W. A.; Sae-Heng, M.; Filippov, D. V.; Kisselev, A. F.; van der Marel, G. A.; Overkleeft, H. S., *ChemBioChem* **2008,** *9*, 1735
2. Schwabacher, A. W.; Lane, J. W.; Schiesher, M. W.; Leigh, K. M.; Johnson, C. W., *J. Org. Chem.* **1998,** *63*, 1727
3. Greenbaum, D.; Medzihradszky, K. F.; Burlingame, A.; Bogyo, M., *Chem. Biol.* **2000,** *7*, 569
4. Hillaert, U.; Verdoes, M.; Florea, B. I.; Saragliadis, A.; Habets, K. L.; Kuiper, J.; Van Calenbergh, S.; Ossendorp, F.; van der Marel, G. A.; Driessen, C.; Overkleeft, H. S., *Angew Chem Int Ed Engl* **2009,** *48*, 1629
5. McIlvaine, T.C., *J. Biol. Chem.* **1921**, *49*, 183

Spectra

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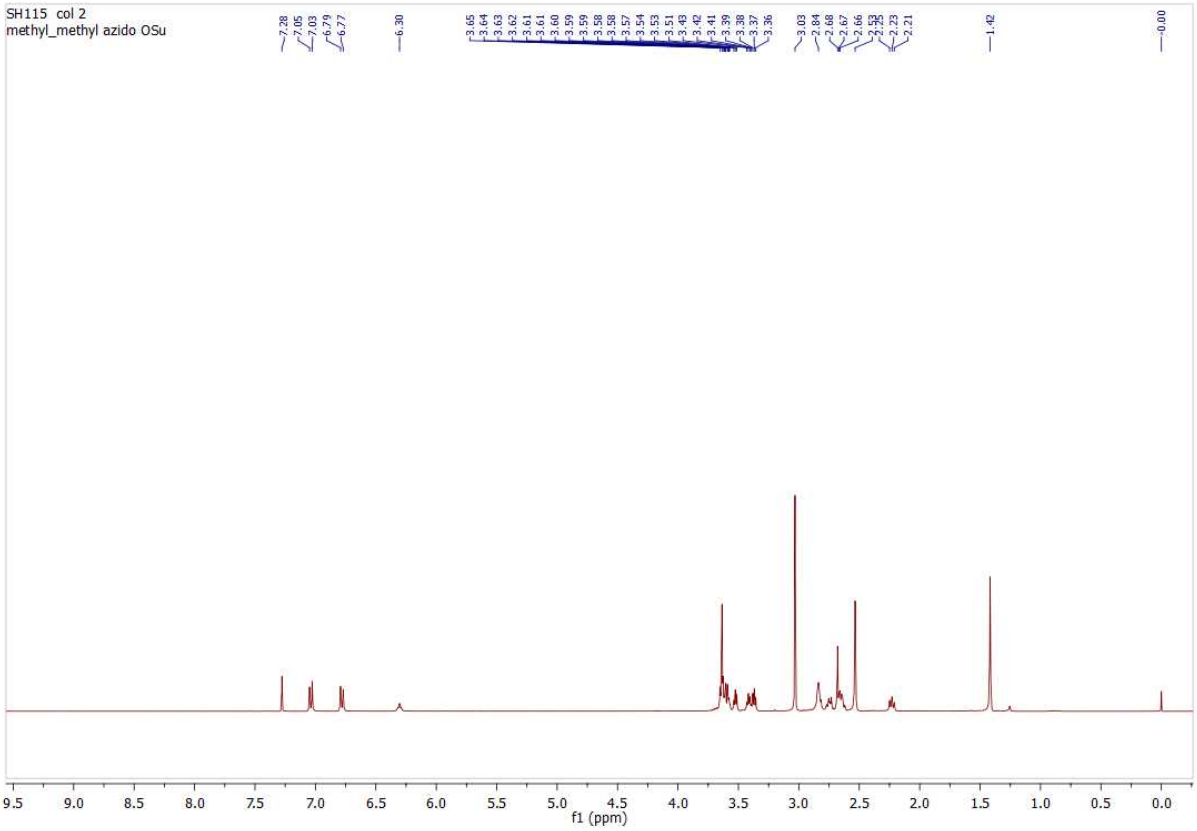

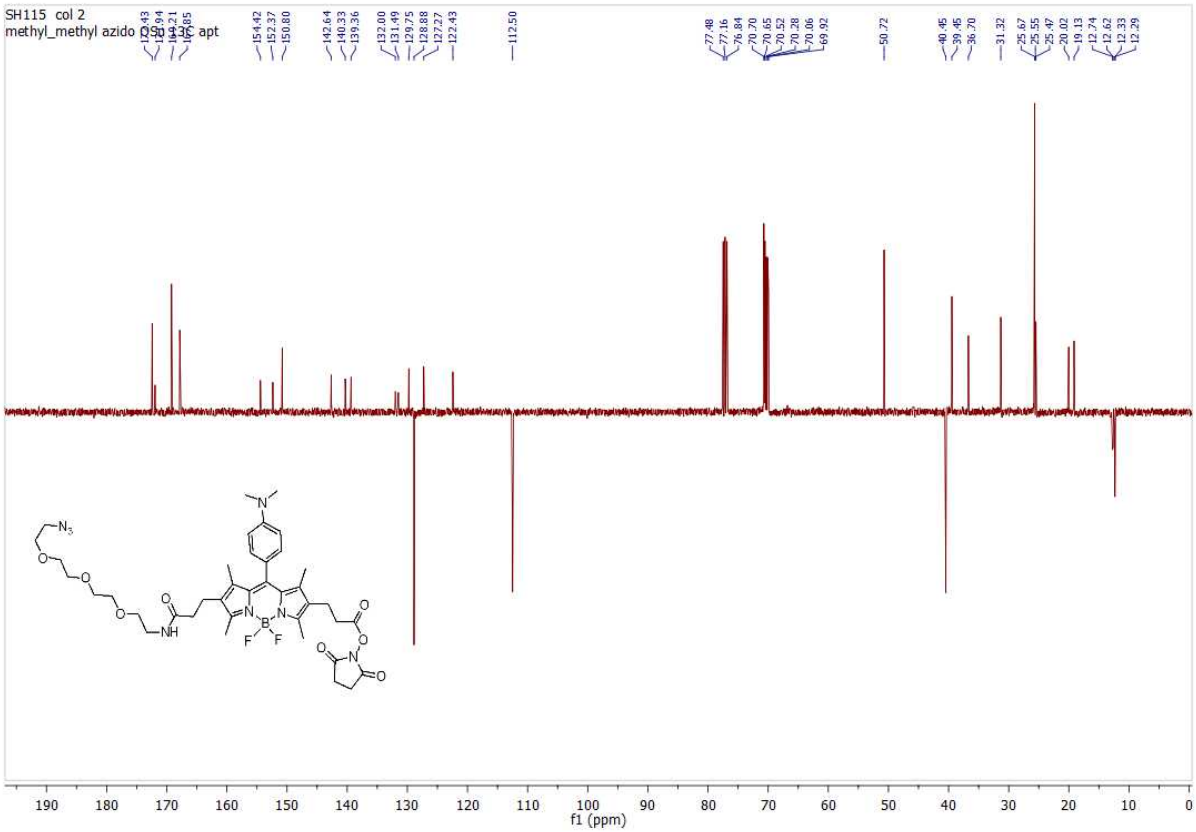

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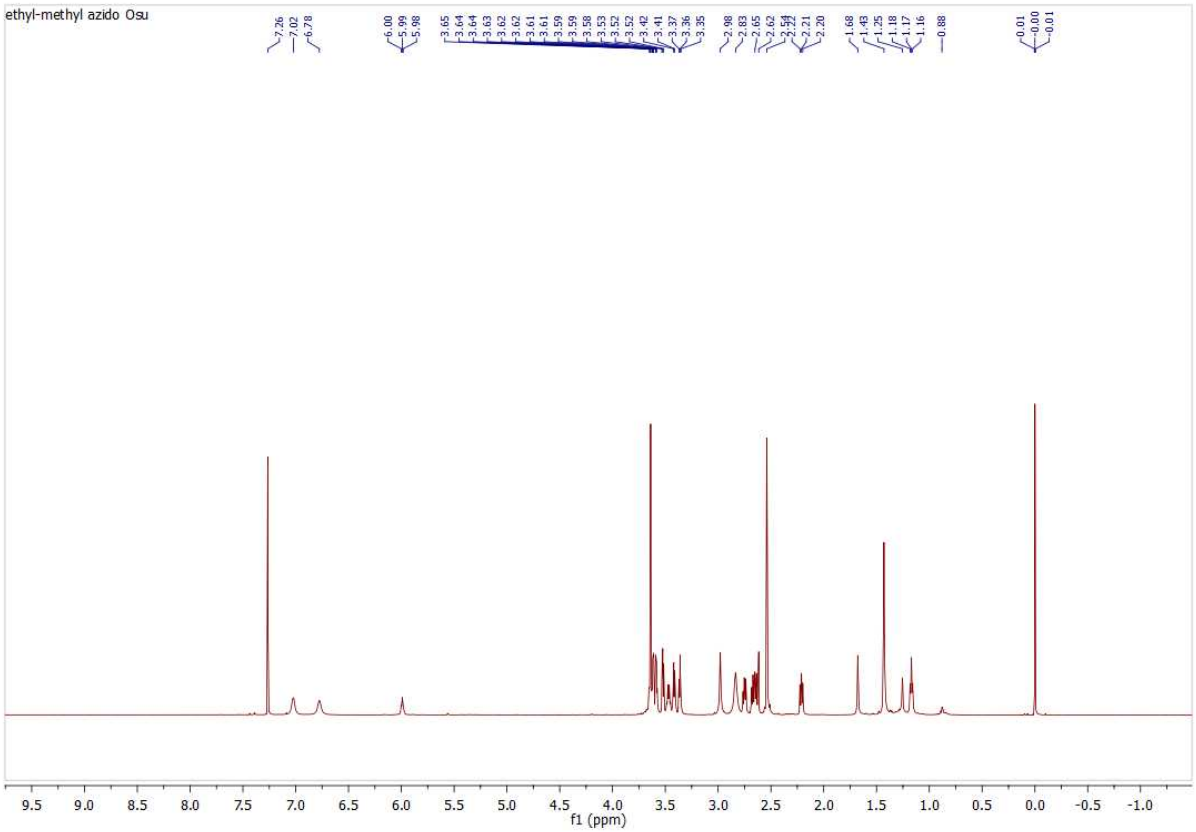
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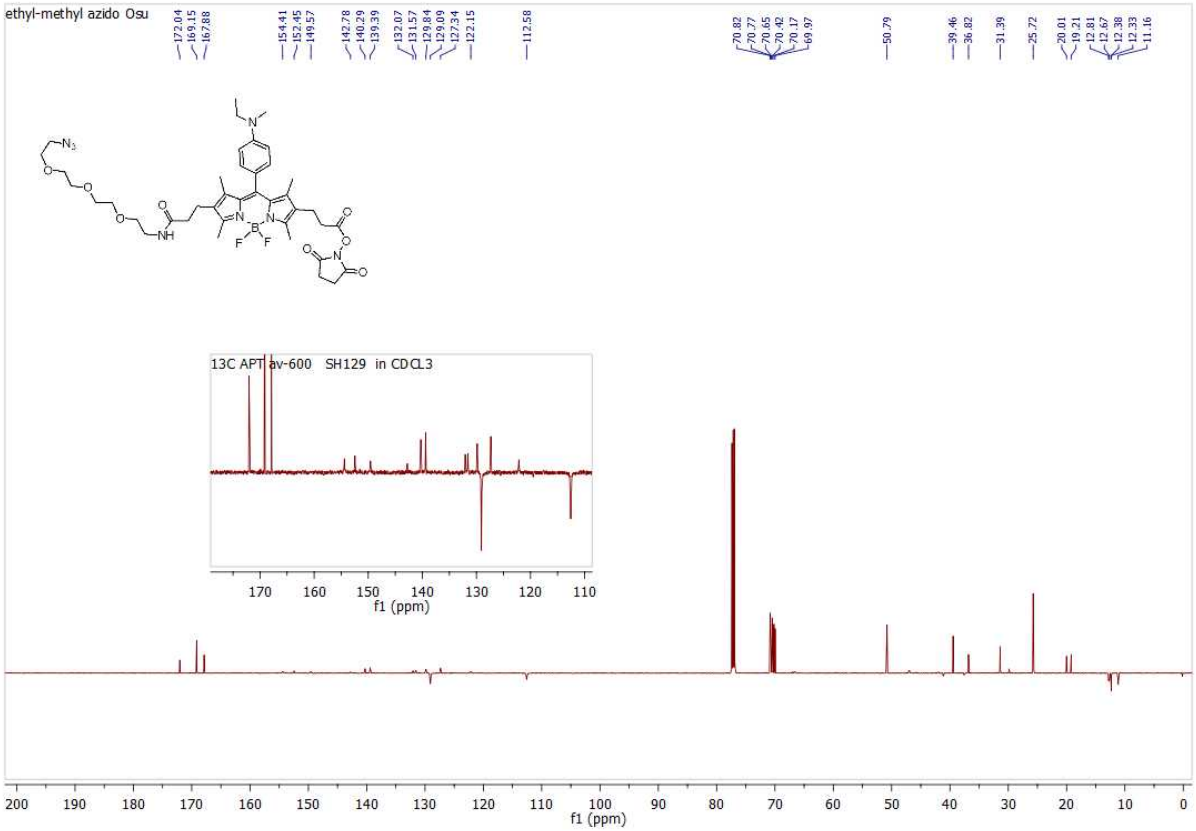
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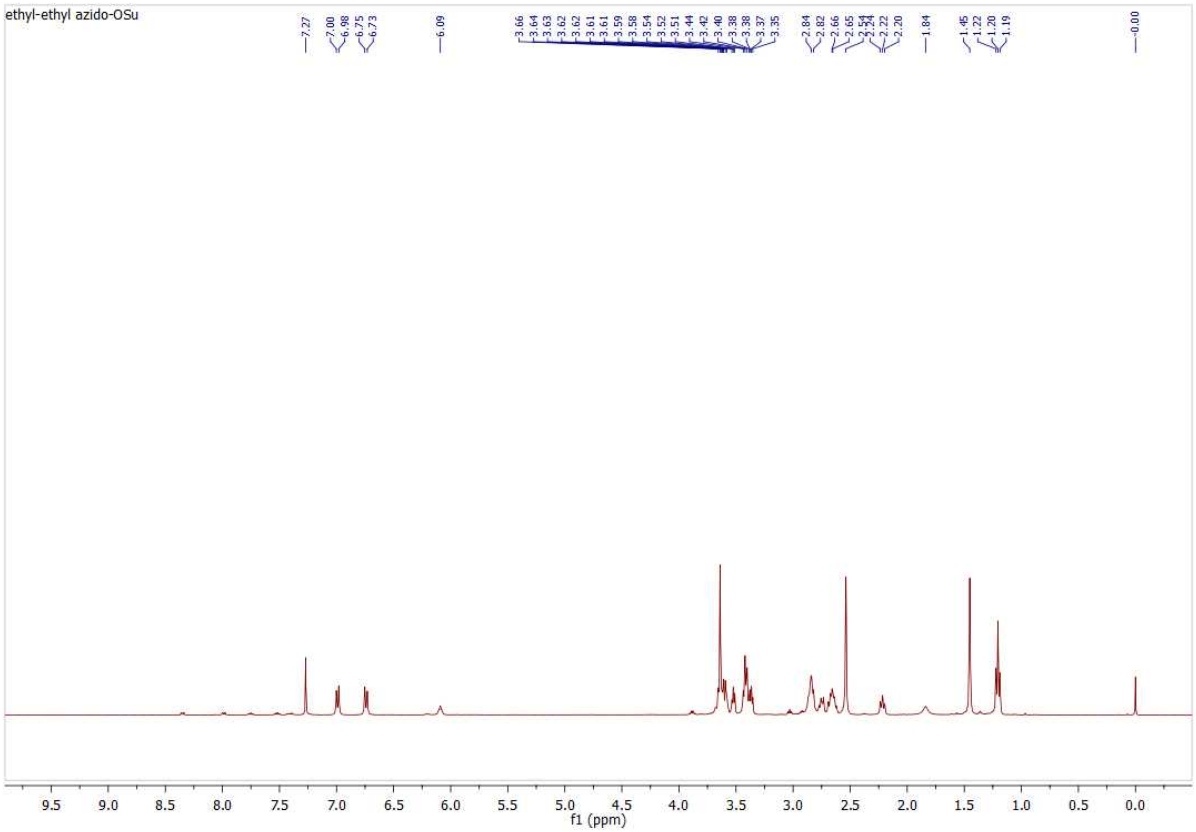

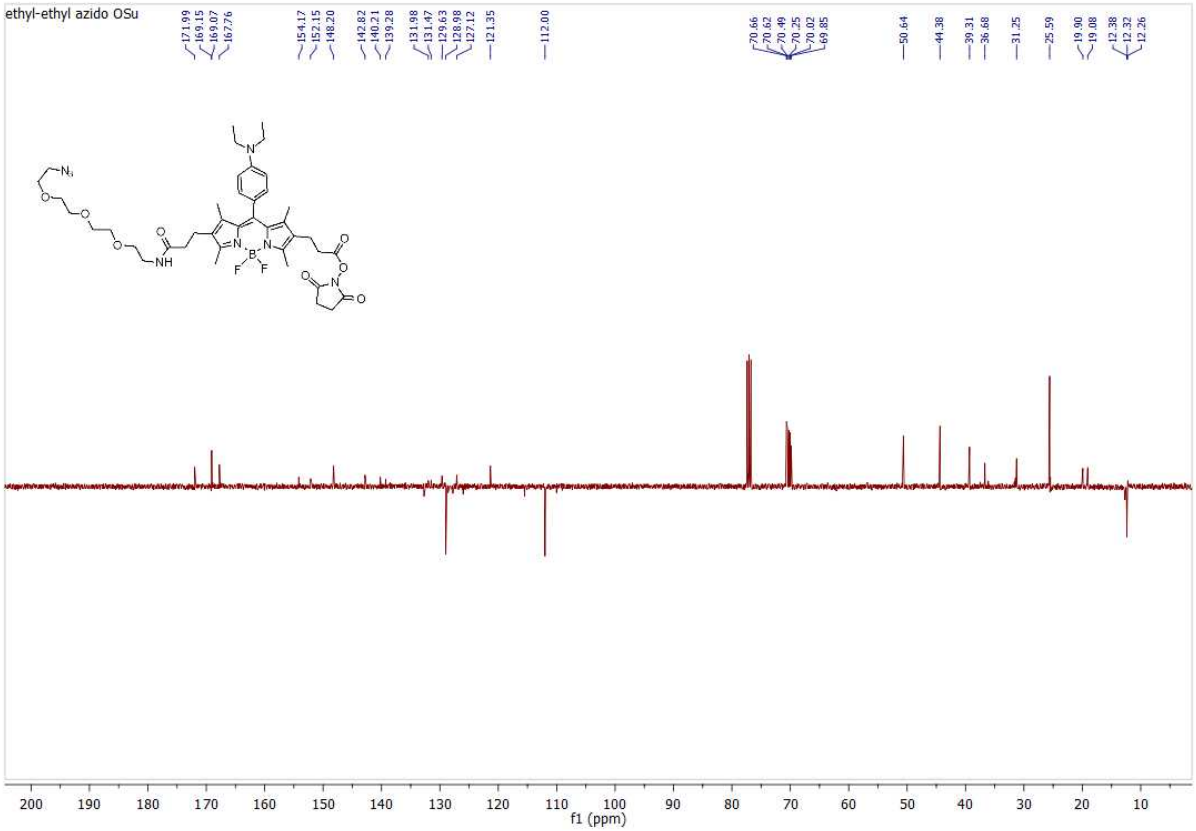

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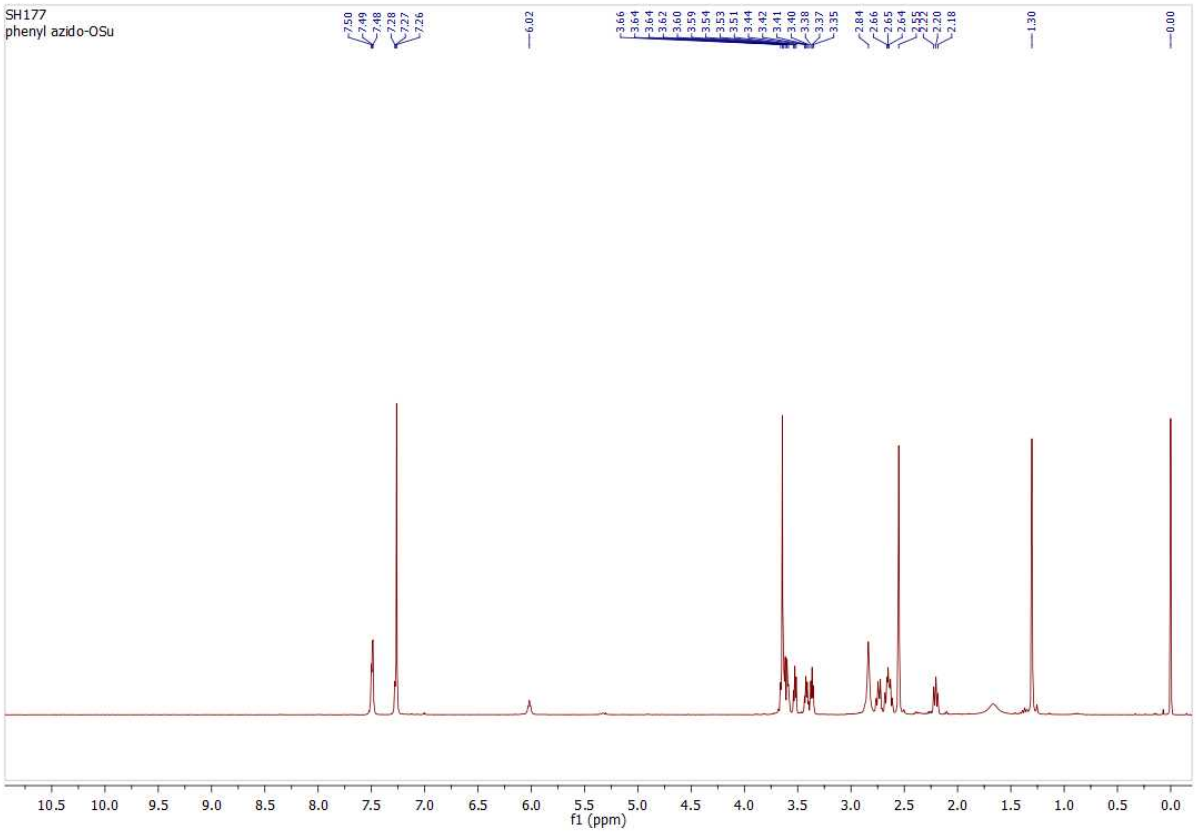

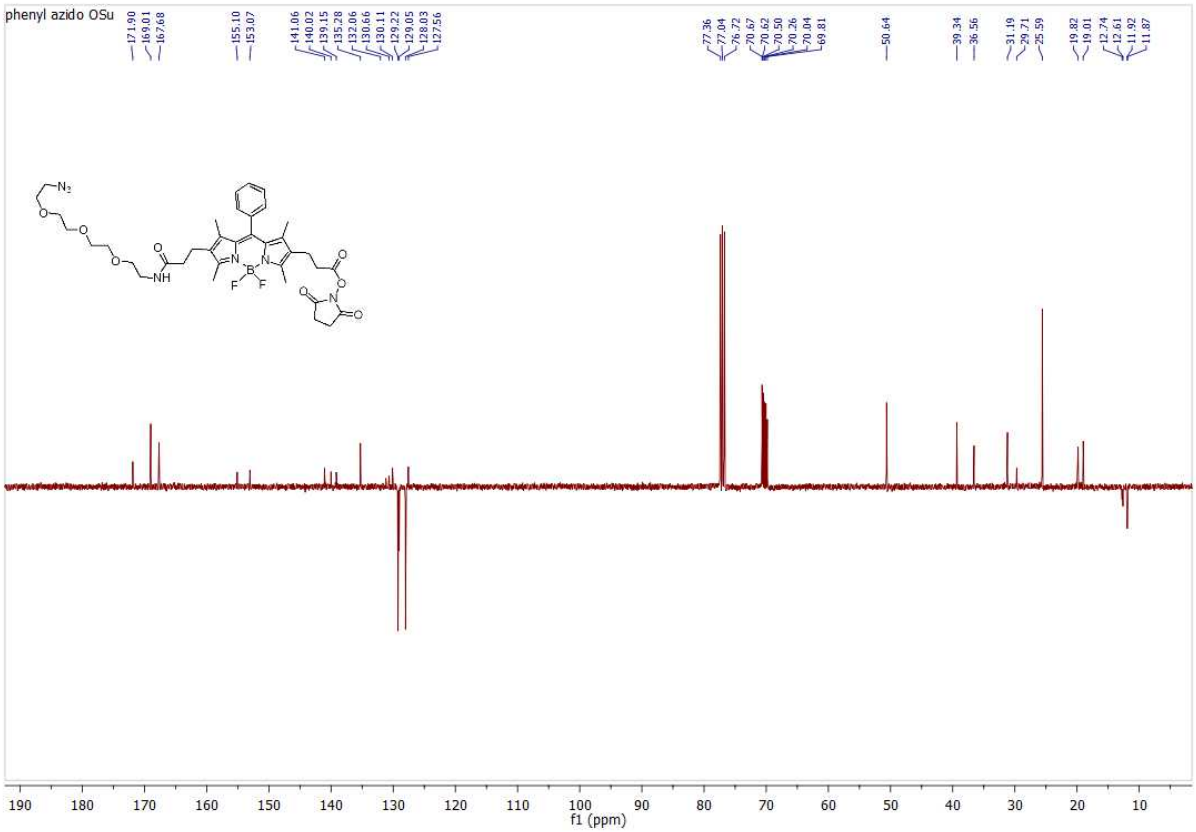

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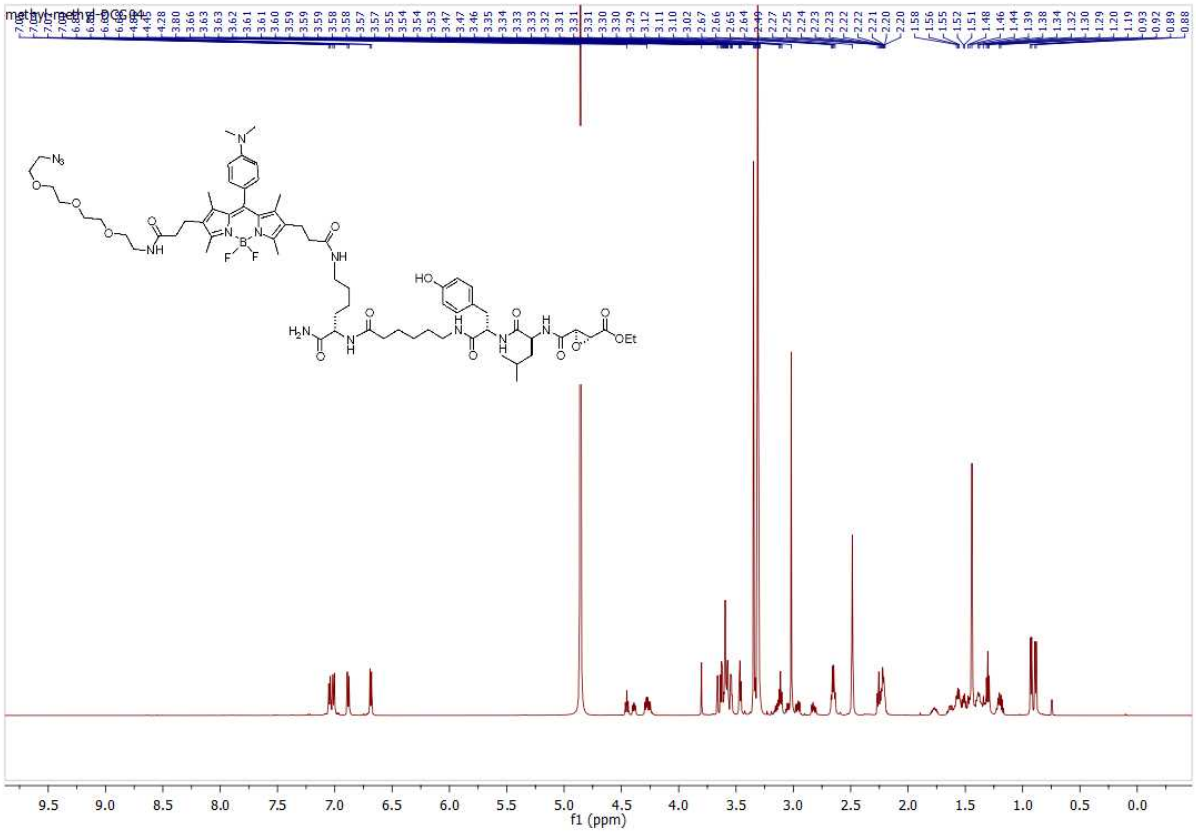

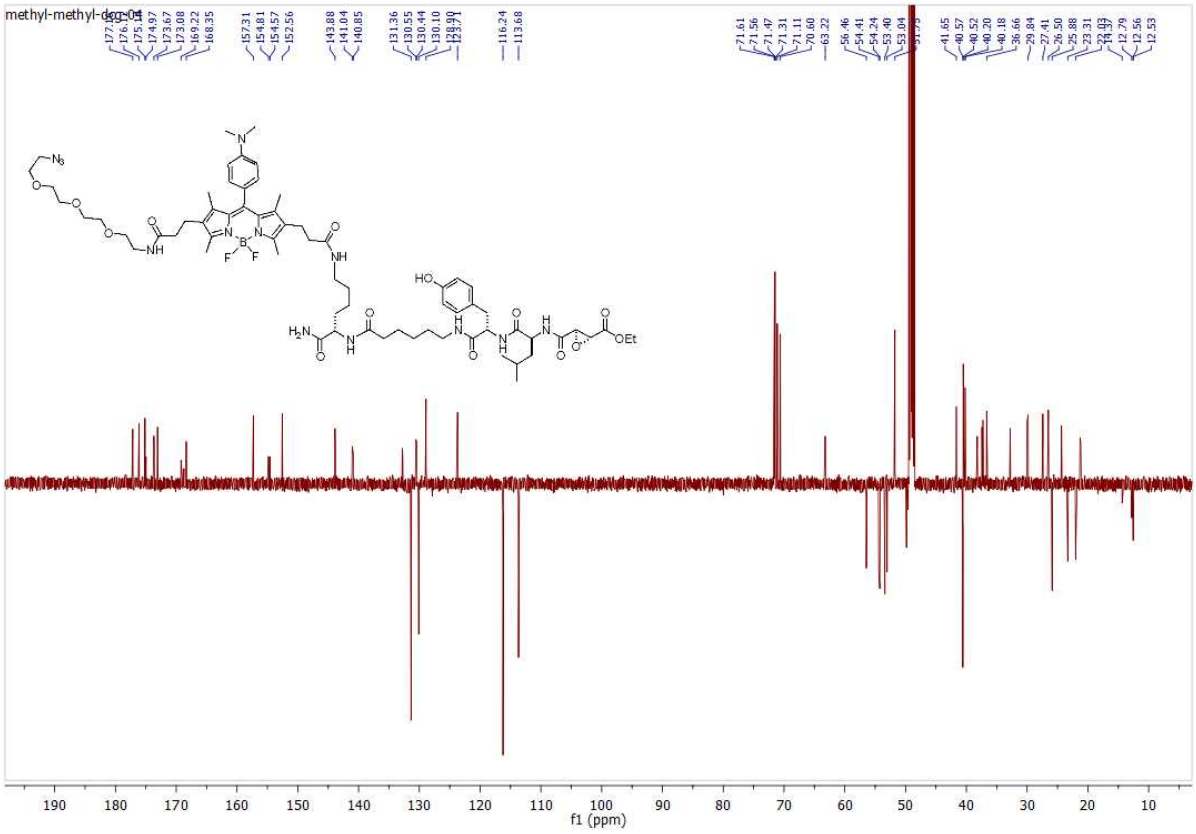

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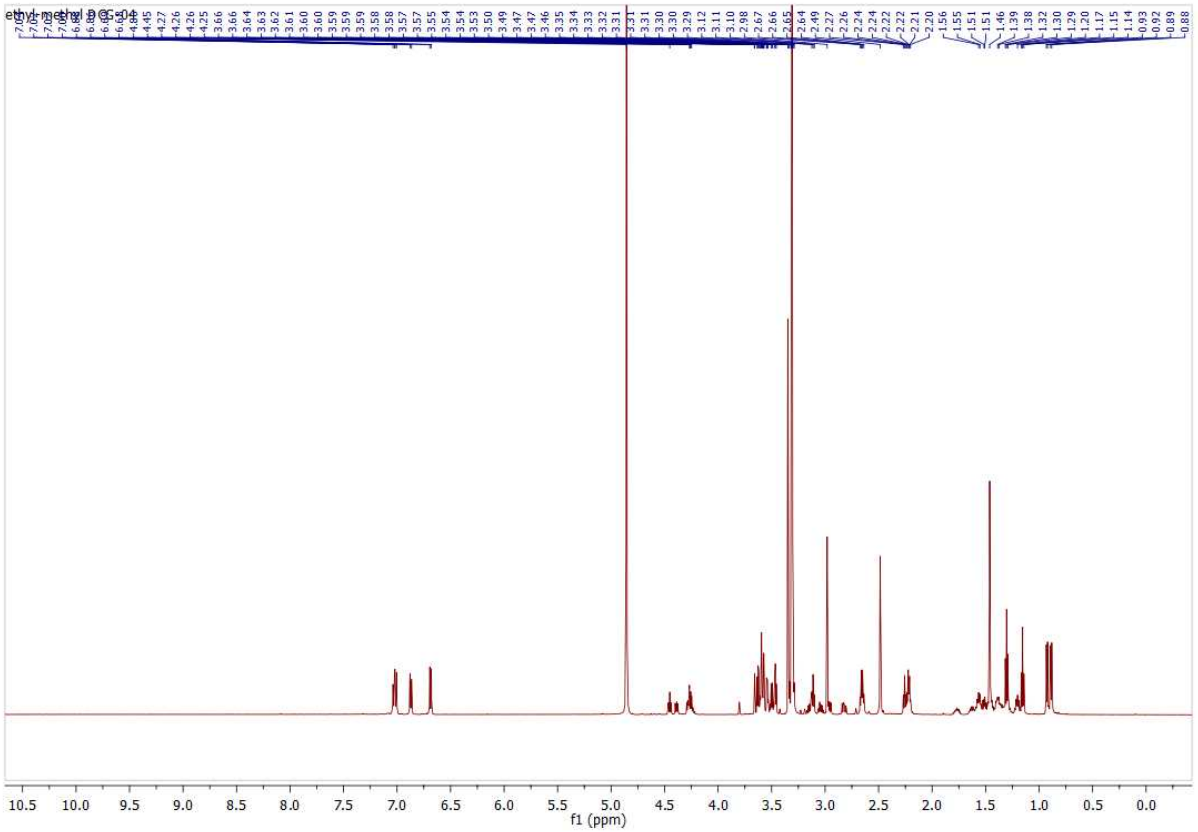

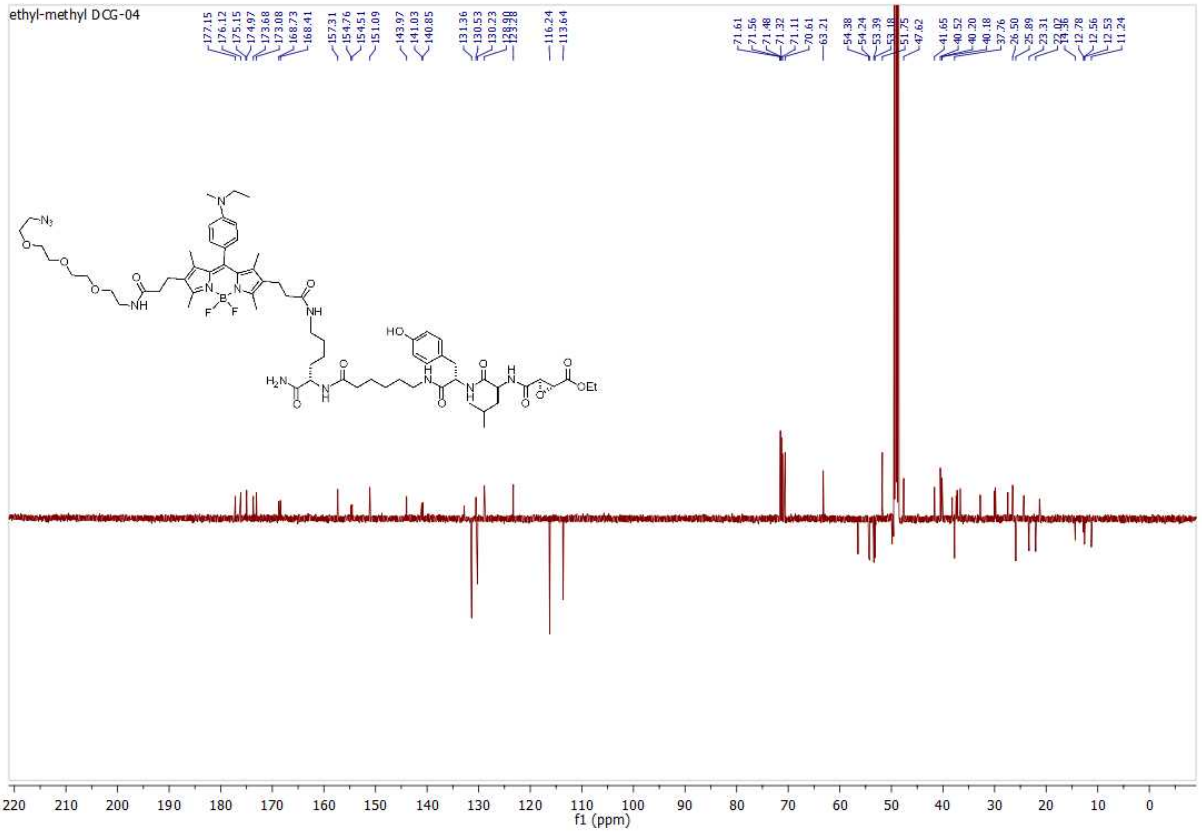

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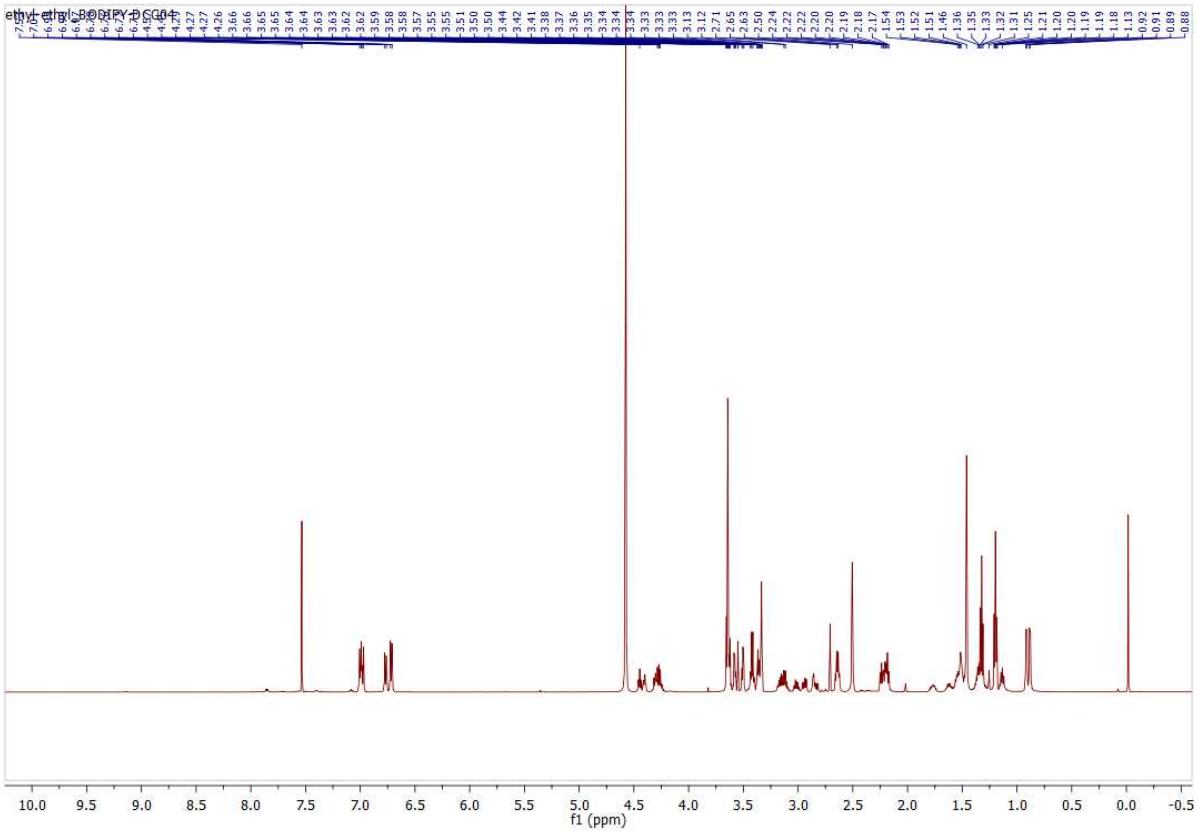

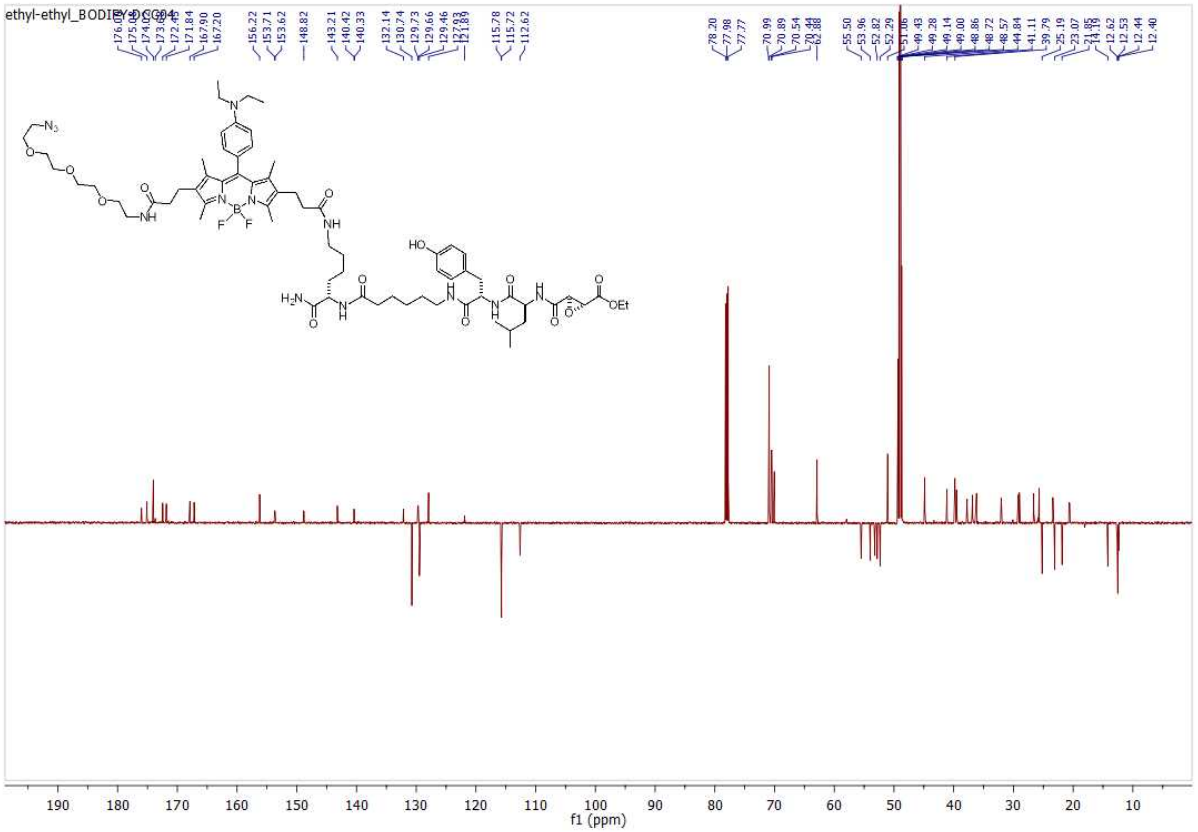

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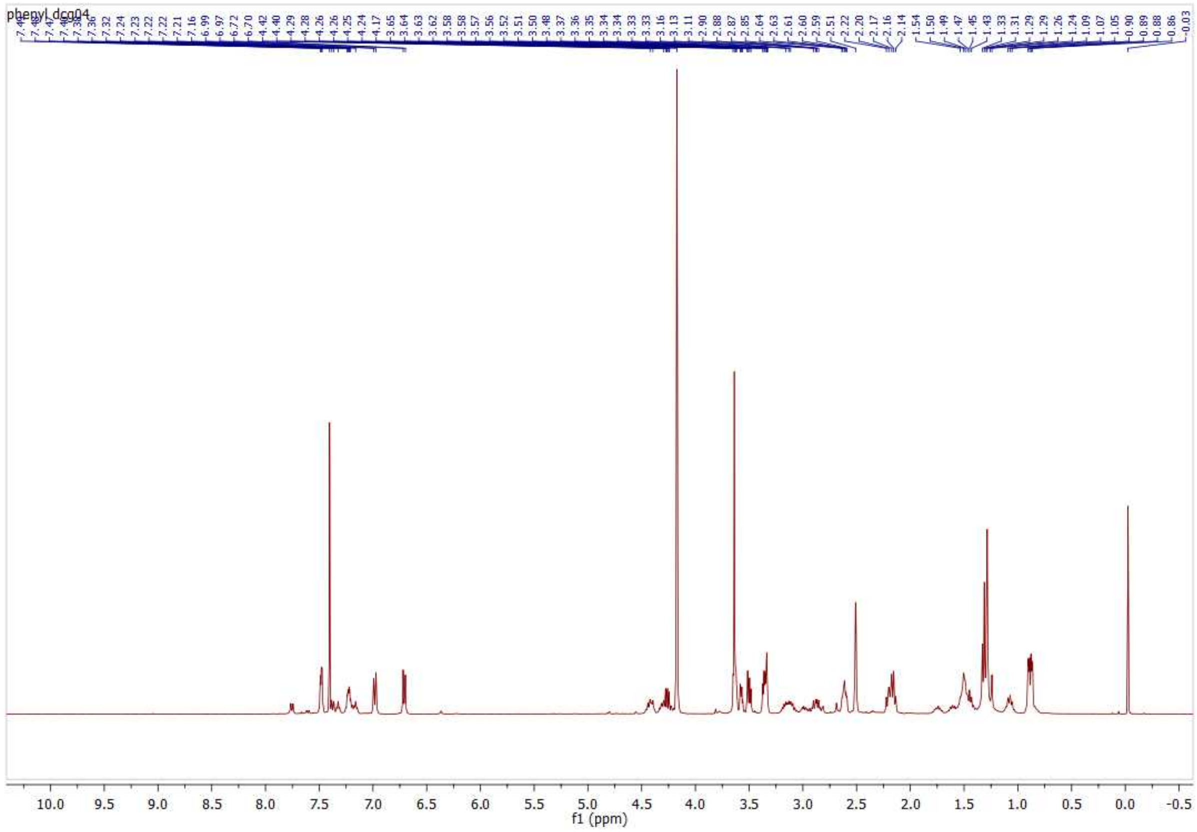

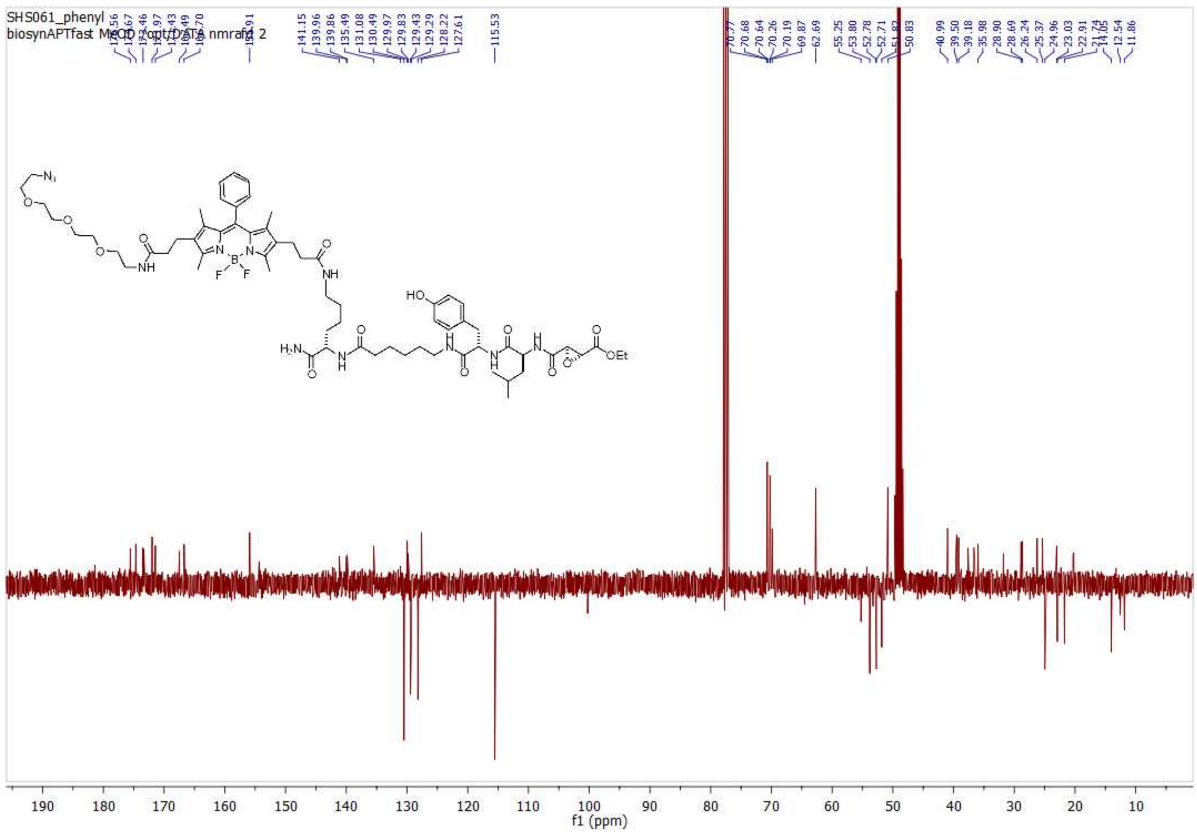

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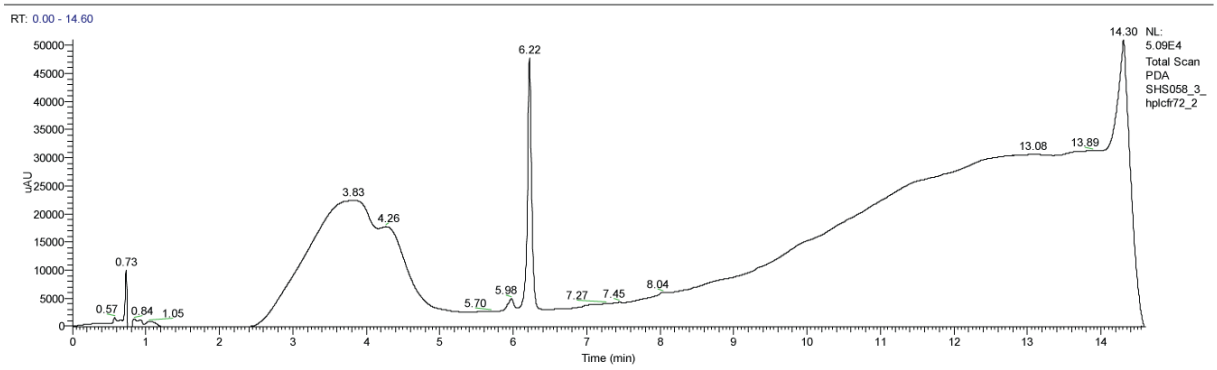

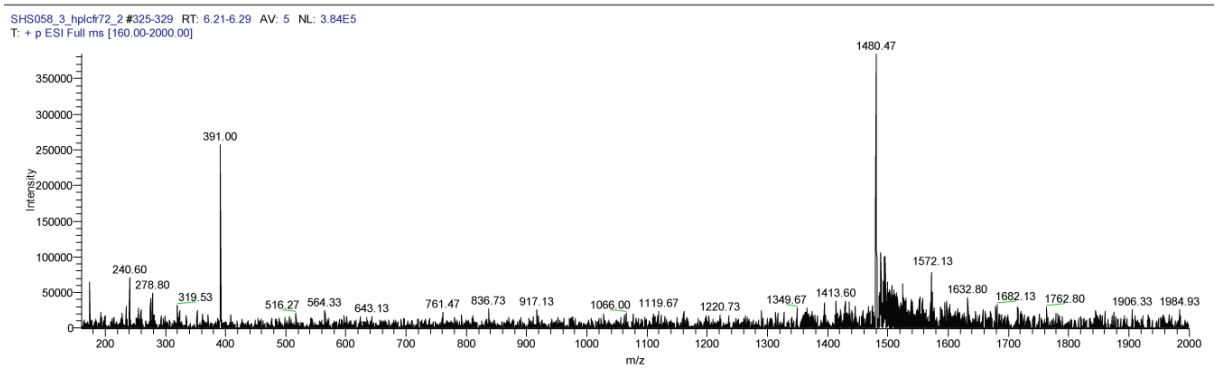

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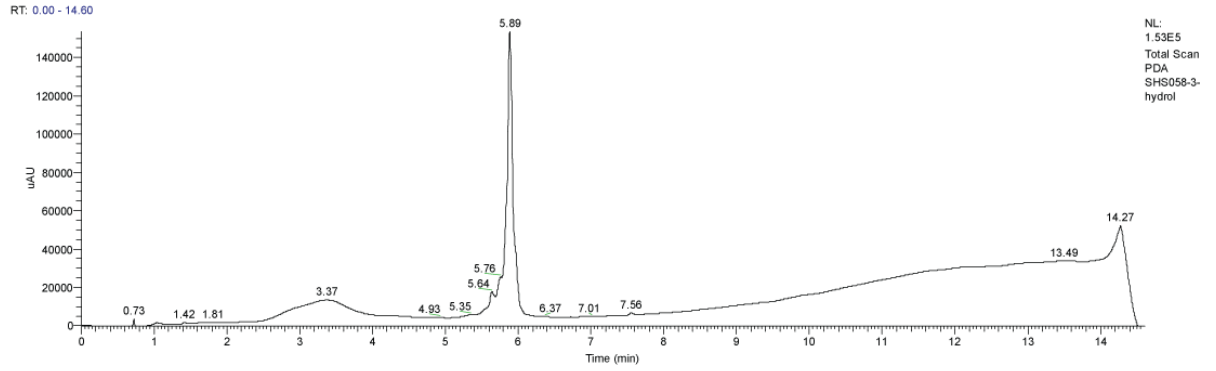

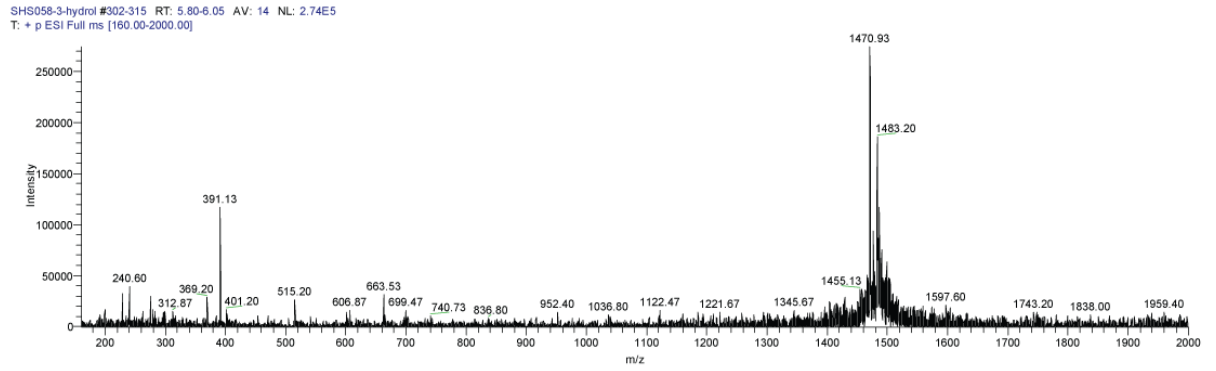

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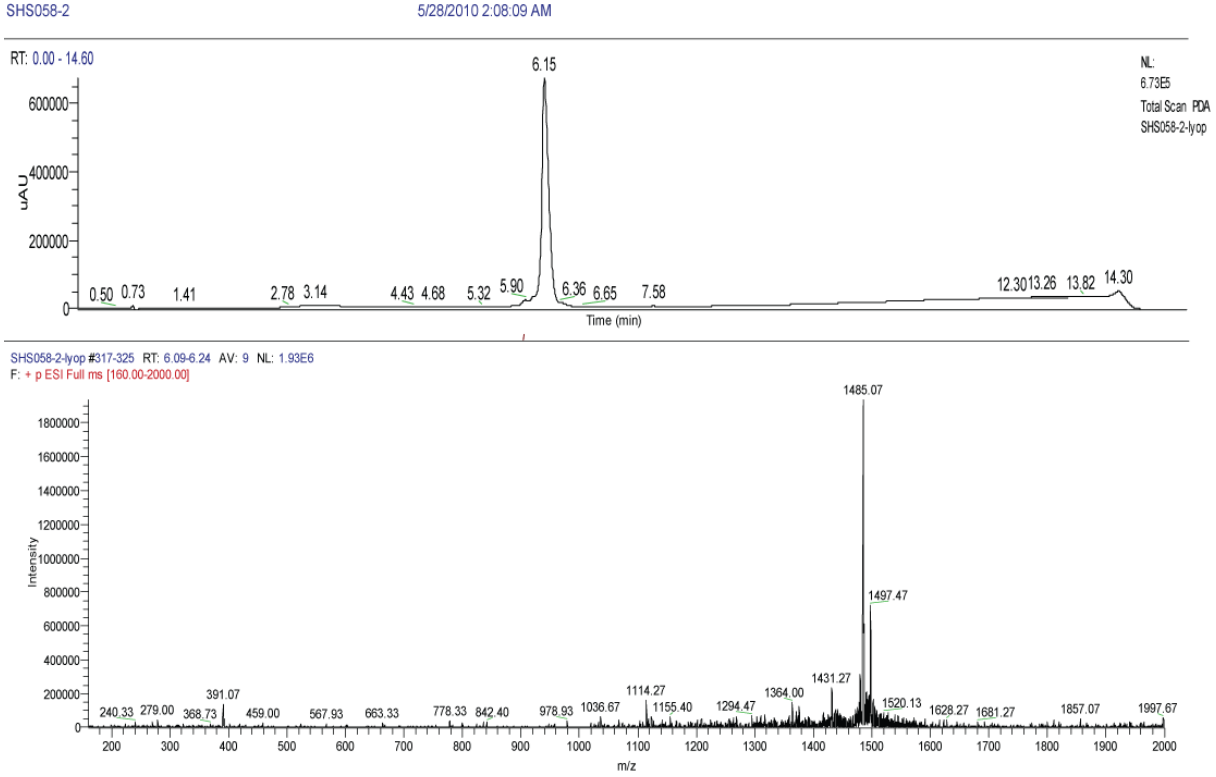

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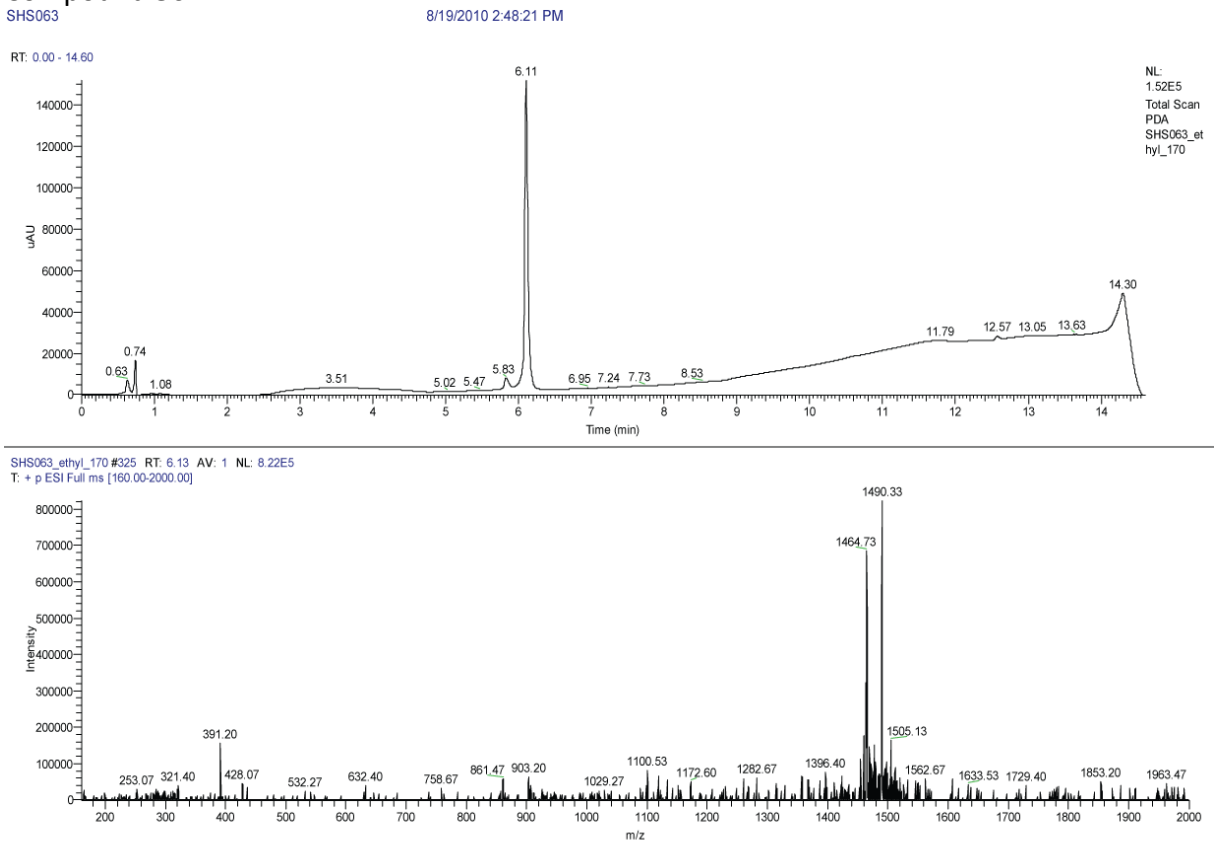

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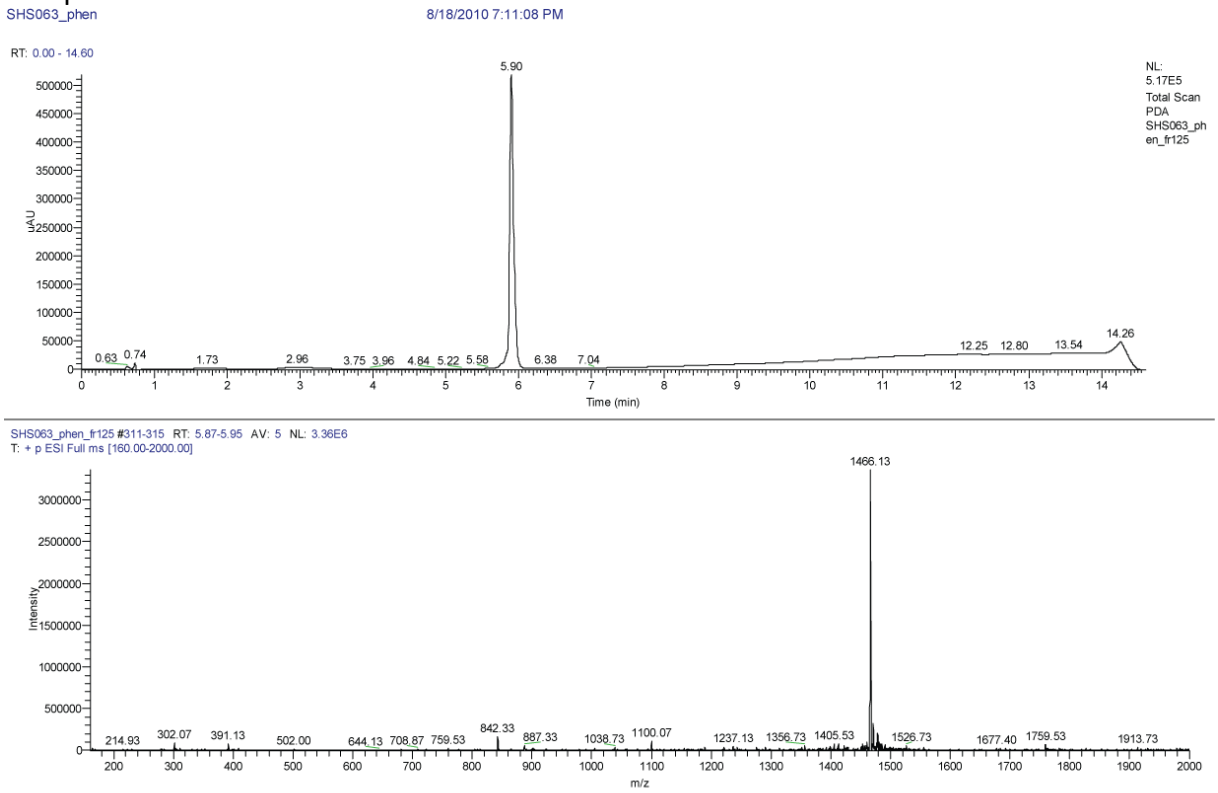
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