Supporting information

Non-coordinating anions assemble cyanine amphiphiles into ultra-small fluorescent nanoparticles

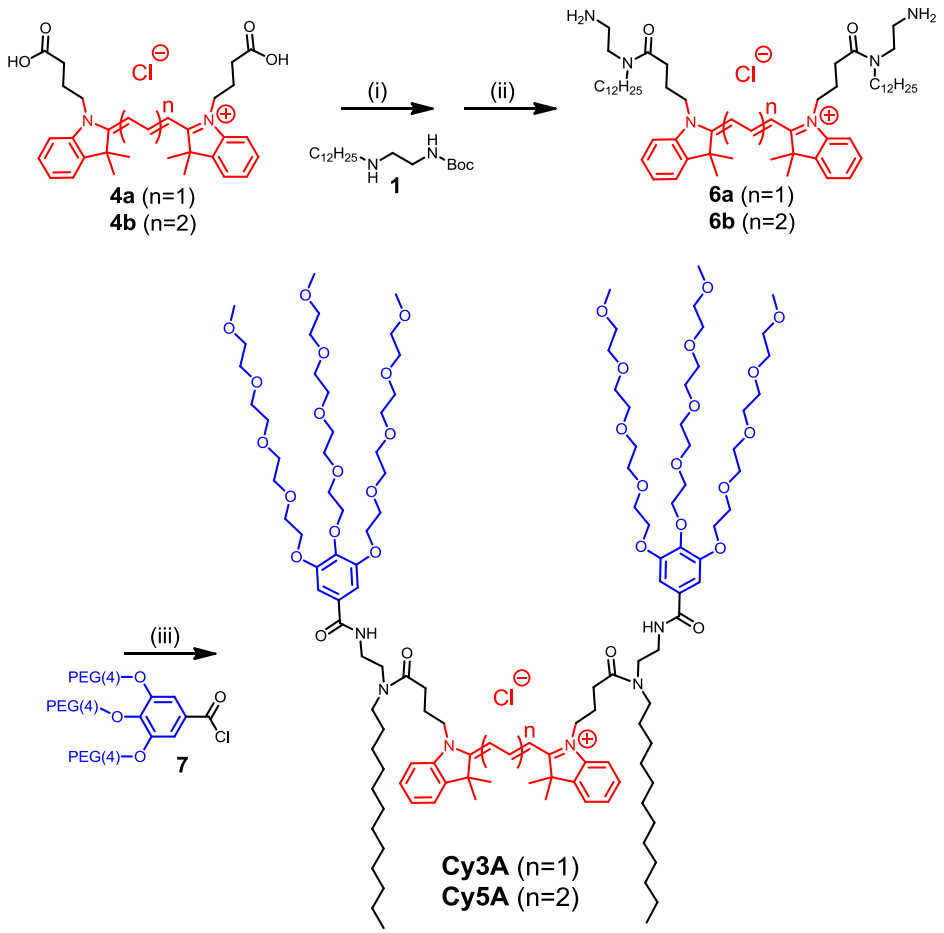
Ievgen Shulov, Youri Arntz, Yves Mély, Vasyl G. Pivovarenko and Andrey S. Klymchenko

Materials and methods

Sodium tetraphenylborate (≥99.5 %), lithium tetrakis(pentafluorophenyl)borate ethyl etherate, acetonitrile were purchased from Sigma-Aldrich and used as received. Sodium phosphate monobasic (>99.0 %, Sigma-Aldrich) and sodium phosphate dibasic dihydrate (>99.0 %, Sigma-Aldrich) were used to prepare 20 mM phosphate buffer solutions at pH 7.4. MilliQ-water (Millipore) was used in all experiments. All starting materials for synthesis were purchased from Alfa Aesar and Sigma Aldrich or TCI Europe and used as received unless stated otherwise. NMR spectra were recorded on a Bruker Avance III 400 MHz spectrometer. Mass spectra were obtained using an Agilent Q-TOF 6520 mass spectrometer. Matrix assisted laser desorption/ionization time of flight mass spectra (MALDI-TOF-MS) were measured on a Bruker Autoflex spectrometer. M/z values are given in gram/mol. Synthesis of alkyl-rhodamine B dyes is described in supporting information.

Synthesis of dye amphiphiles

To obtain final Cy3A and Cy5A amphiphiles, firstly, we prepared Cy3 and Cy5 derivatives bearing two carboxylic groups, **4a** and **4b**, respectively (Scheme S1). They were coupled with hydrophobic mono-boc-protected diamine **1** (prepared from 1-iodododecane and N-boc-ethylenediamine) to give compounds **5a** and **5b**, respectively, which after boc removal gave corresponding dye diamines **6a** and **6b**. The latter were then reacted with PEGylated gallic acid chloride **7** to afford final amphiphiles **Cy3A** and **Cy5A**, respectively.



Scheme S1. Synthesis and chemical structure of Cy3A and Cy5A.

***tert*-Butyl N-[2-(dodecylamino)ethyl]carbamate (1).** 1-iodododecane (1 eq., 3.08 g, 2.57 mL, 10.4 mmol) and N-boc-ethylenediamine (3 eq., 5 g, 31.2 mmol) were placed in a reaction flask. Anhydrous acetonitrile (40 mL) was added via syringe. The reaction mixture was stirred under reflux for 6h. Solvent was removed under reduced pressure. The crude product was purified by column chromatography (SiO_2, DCM/MeOH, 95:5). An additional purification step was done using recrystallization from acetonitrile, which furnished 2.53 g (yield 74%) of the title compound **1** as white solid. **^{1}H NMR (400 MHz, Chloroform-*d*) δ** 7.59 (br. s, 2H), 5.61 (t, *J* = 5.6 Hz, 1H), 3.59 (q, *J* = 5.3 Hz, 2H), 3.22 (t, *J* = 5.0 Hz, 2H), 3.08 – 2.97 (m, 2H), 1.85 (p, *J* = 7.8 Hz, 2H), 1.44 (s, 9H), 1.40 – 1.33 (m, 2H), 1.33 – 1.17 (m, 16H), 0.86 (t, *J* = 6.7 Hz, 3H). **^{13}C NMR (101 MHz, Chloroform-*d*) δ** 157.78, 81.18, 49.05, 48.39, 37.72, 32.01, 29.72, 29.71, 29.61, 29.50, 29.43, 29.10, 28.49, 26.73, 26.17, 22.78, 14.21. **HRMS (*m/z*):** $[M+H]^+$ calcd. for $C_{19}H_{41}N_2O_2$, 329.3163; found, 329.3162.

1-(4-Ethoxy-4-oxobutyl)-2,3,3-trimethyl-3H-indol-1-ium bromide (2). 2,3,3-trimethylindolenine (1 eq., 2.97 g, 3 mL, 18.7 mmol) and ethyl 4-bromobutyrate (3 eq., 10.9 g, 8.03 mL, 56 mmol) were placed in a reaction flask. Anhydrous acetonitrile (40 mL) was added via syringe. The reaction mixture was stirred under reflux for 48 hours. After cooling to room temperature, diethyl ether (40 ml) was added and formed slightly red precipitate was removed by filtration, and washed several times with diethyl ether. The product was purified by recrystallization from acetonitrile, which furnished 4.3 g (yield 65%) of the title compound **2** as pink crystals. **^{1}H NMR (400 MHz, Chloroform-*d*) δ** 8.06 –

7.96 (m, 1H), 7.61 – 7.48 (m, 3H), 4.92 – 4.83 (m, 2H), 4.05 (q, *J* = 7.1 Hz, 2H), 3.17 (s, 3H), 2.70 (t, *J* = 6.2 Hz, 2H), 2.29 – 2.17 (m, 2H), 1.61 (s, 6H), 1.19 (t, *J* = 7.1 Hz, 3H). **^{13}C NMR (101 MHz, Chloroform-*d*) δ** 196.52, 172.78, 141.63, 141.27, 130.00, 129.65, 123.13, 115.93, 60.97, 54.67, 48.59, 30.56, 23.12, 22.94, 16.32, 14.16. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{17}H_{24}NO_2$, 274.1802; found, 274.1810.

Compound 3a (for structure – see SI file). Compound **2** (1 eq., 3 g, 8.47 mmol) was placed in a reaction flask. Anhydrous pyridine (40 mL) was added via syringe. Obtained mixture was preheated to 110 °C until complete dissolving of indoleninium salt, then triethyl orthoformate (1.5 eq., 1.88 g, 2.12 mL, 12.7 mmol) was quickly added dropwise to the boiling solution of indoleninium salt using syringe. The reaction mixture was stirred under reflux for 4 hours. Then, solvent was removed under reduced pressure. The residue was redissolved in DCM (100 mL), washed with 1M aq. solution of hydrochloric acid (3 × 100 mL), once with brine, and dried over sodium sulphate. After solvent evaporation, the product was purified by gradient column chromatography (SiO_2, DCM/MeOH, 98:2 to 90:10), which furnished 3.83 g (yield 71%) of title compound **3a** as a red solid. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.60 (t, *J* = 13.4 Hz, 1H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.53 – 7.45 (m, 4H), 7.39 – 7.31 (m, 2H), 6.64 (d, *J* = 13.6 Hz, 2H), 4.27 (t, *J* = 7.7 Hz, 4H), 4.15 (q, *J* = 7.1 Hz, 4H), 2.63 (t, *J* = 6.7 Hz, 4H), 2.16 (p, *J* = 7.0 Hz, 4H), 1.81 (s, 12H), 1.27 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR (101 MHz, Methanol-d_4) δ** 176.10, 174.36, 152.32, 143.30, 142.19, 129.98, 126.78, 123.55, 112.45, 103.96, 61.78, 50.66, 44.53, 31.63, 28.31, 23.33, 14.54. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{35}H_{45}N_2O_4$, 557.3374; found, 557.3360.

Compound 3b (for structure – see SI file)**.** 1-(4-ethoxy-4-oxobutyl)-2,3,3-trimethyl-3H-indol-1-ium bromide (1 eq., 2.09 g, 5.9 mmol) were placed in a reaction flask. Anhydrous pyridine (40 mL) was added via syringe. The mixture was preheated to 110 °C until complete dissolving of the indoleninium salt, then 1,1,3,3-tetramethoxypropane (1.5 eq., 1.45 g, 1.47 mL, 8.85 mmol) was quickly added dropwise to the boiling solution of indoleninium salt. Reaction mixture was stirred under reflux for 4 hours. Then, solvent was removed under reduced pressure. The residue was redissolved in DCM (100 mL), washed three times with 1M aq. solution of hydrochloric acid (3 × 100 mL), once with brine, and dried over sodium sulphate. After solvent evaporation, crude product was purified by gradient column chromatography (SiO_2, DCM/MeOH, 95:5 to 9:1), which furnished 2.55 g (yield 65 %) of the title compound **3b** as a blue solid with metallic luster. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.30 (t, *J* = 13.1 Hz, 2H), 7.49 (dd, *J* = 7.5, 1.1 Hz, 2H), 7.41 (ddd, *J* = 8.4, 7.3, 1.2 Hz, 2H), 7.35 (d, *J* = 7.9 Hz, 2H), 7.26 (td, *J* = 7.4, 1.1 Hz, 2H), 6.64 (t, *J* = 12.4 Hz, 1H), 6.38 (d, *J* = 13.7 Hz, 2H), 4.23 – 4.08 (m, 8H), 2.56 (t, *J* = 6.6 Hz, 4H), 2.13 – 2.02 (m, 4H), 1.72 (s, 12H), 1.26 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR (101 MHz, Methanol-d_4) δ** 174.74, 174.44, 155.65, 143.45, 142.55, 129.72, 126.87, 126.26, 123.43, 111.96, 104.43, 61.79, 50.57, 44.25, 31.44, 27.94, 23.21, 14.56. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{37}H_{47}N_2O_4$, 583.3530; found, 583.3536.

Compound 4a. Compound **3a** (1 eq., 3 g, 4.7 mmol) was hydrolyzed using 30% aq. solution of hydrobromic acid (25 mL) while stirring at 110 °C for about 4 h (control by TLC). Then, solvent was removed under reduced pressure. The residue was redissolved in DCM (100 mL), and washed with 10% aq. solution of sodium carbonate (3 × 100 mL), once with brine, dried over sodium sulphate,

filtered, and the filtrate evaporated to dryness in vacuum. The crude product **4a** had a purity of >90% and was used directly for the next step without further purification. Yield 1.59 g (58%) as a red solid. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 12.50 (br s, 2H), 8.36 (t, *J* = 13.4 Hz, 1H), 7.64 (d, *J* = 7.4 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 7.45 (td, *J* = 8.0, 1.2 Hz, 2H), 7.30 (t, *J* = 7.4 Hz, 2H), 6.57 (d, *J* = 13.4 Hz, 2H), 4.15 (t, *J* = 7.7 Hz, 4H), 2.42 (t, *J* = 7.1 Hz, 4H), 1.96 (p, *J* = 7.3 Hz, 4H), 1.70 (s, 12H). **^{13}C NMR (101 MHz, DMSO-d_6) δ** 173.95, 173.75, 149.93, 141.86, 140.61, 128.59, 125.17, 122.49, 111.36, 102.68, 48.89, 43.18, 30.78, 27.41, 22.41. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{31}H_{37}N_2O_4$, 501.2748; found, 501.2756.

Compound 4b. The compound **3b** was hydrolyzed using 30% aq. solution of hydrobromic acid (50 mL) while stirring at 110 °C for about 4 h (control by TLC). Then, solvent was removed under reduced pressure. The residue was redissolved in DCM (100 mL), and washed with 10% aq. solution of sodium carbonate (3 × 100mL), once with brine, dried over sodium sulphate, filtered and the filtrate evaporated to dryness in vacuum. The crude product **4b** had a purity of >90% and was used directly for the next step without further purification. Yield 1.51 g (55%) as a dark blue solid. **^{1}H NMR (400 MHz, DMSO-d_6) δ** 8.35 (t, *J* = 13.1 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.46 – 7.33 (m, 4H), 7.24 (td, *J* = 7.1, 1.7 Hz, 2H), 6.54 (t, *J* = 12.3 Hz, 1H), 6.37 (d, *J* = 13.8 Hz, 2H), 4.11 (t, *J* = 7.7 Hz, 4H), 2.42 (t, *J* = 7.0 Hz, 4H), 1.95 – 1.83 (m, 4H), 1.68 (s, 12H). **^{13}C NMR (101 MHz, DMSO-d_6) δ** 173.87, 172.70, 154.14, 141.93, 141.07, 128.36, 125.50, 124.64, 122.40, 110.89, 103.14, 48.89, 42.76, 30.49, 27.10, 22.19. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{33}H_{39}N_2O_4$, 527.2904; found, 527.2915.

Compound 5a. Compound **4a** (1 eq., 500 mg, 0.86 mmol), HBTU (2.2 eq., 717 mg, 1.89 mmol), and HOBt (3 eq., 348 mg, 2.58 mmol) were placed in a reaction flask. DMF (5mL) and DIPEA (10 eq., 1.42 mL, 8.6 mmol) were added via syringe. After stirring the reaction mixture for 30 min, tert-butyl N-[2-(dodecylamino)ethyl]carbamate (2 eq., 564 mg, 1.72 mmol) dissolved in anhydrous DCM (2 mL) was added dropwise. The reaction mixture was stirred at ambient temperature for 24 h. Solvent was removed under reduced pressure. The residue was redissolved in DCM (50 mL), washed with saturated brine solution (3 ×100 mL), dried over sodium sulphate, filtered, and the filtrate evaporated to dryness in vacuum. The crude product was purified by flash column chromatography (SiO_2, DCM/MeOH, 98:2), which furnished 796 mg (yield 77%) of the title compound **5a** as a red solid. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.60 (t, *J* = 13.4 Hz, 1H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.55 – 7.45 (m, 4H), 7.35 (t, *J* = 7.3 Hz, 2H), 6.68 – 6.53 (m, 2H), 4.29 – 4.18 (m, 4H), 3.52 – 3.44 (m, 4H), 3.45 – 3.37 (m, 4H), 3.32 – 3.22 (m, 4H), 2.77 – 2.69 (m, 2H), 2.69 – 2.60 (m, 2H), 2.20 – 2.11 (m, 4H), 1.82 (s, 12H), 1.63 – 1.55 (m, 4H), 1.46 (s, 9H), 1.45 (s, 9H), 1.41 – 1.29 (m, 36H), 0.98 – 0.89 (m, 6H). **^{13}C NMR (101 MHz, Methanol-d_4) δ** 176.09, 176.01, 174.13, 174.07, 173.85, 173.82, 158.37, 158.31, 152.32, 152.29, 143.37, 143.33, 142.21, 142.18, 130.01, 126.75, 123.48, 112.71, 112.53, 103.98, 103.93, 103.84, 103.82, 80.29, 80.05, 61.76, 50.65, 50.63, 50.16, 47.61, 47.23, 44.81, 44.68, 39.75, 39.53, 38.88, 33.06, 33.05, 30.76, 30.73, 30.54, 30.50, 30.46, 30.28, 29.82, 28.83, 28.82, 28.61, 28.41, 28.39, 28.37, 28.35, 28.09, 27.93, 27.92, 23.72, 23.71, 14.47, 14.45. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{69}H_{113}N_6O_6$, 1121.8716; found, 1121.8698.

Compound 5b. The compound **5b** prepared using the same procedure as described above for **5a**. Yield 748 mg (74%) as blue solid. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.28 (td, *J* = 13.2, 4.7 Hz, 2H), 7.50 (d, *J* = 7.4 Hz, 2H), 7.45 – 7.36 (m, 4H), 7.27 (t, *J* = 7.0 Hz, 2H), 6.73 (t, *J* = 12.4 Hz, 1H), 6.46 – 6.35 (m, 2H), 4.25 – 4.12 (m, 4H), 3.53 – 3.45 (m, 4H), 3.45 – 3.40 (m, 2H), 3.39 – 3.32 (m, 2H), 3.31 – 3.23 (m, 4H), 2.69 (t, *J* = 7.0 Hz, 2H), 2.61 (t, *J* = 7.1 Hz, 2H), 2.19 – 2.07 (m, 4H), 1.74 (s, 12H), 1.65 – 1.54 (m, 4H), 1.46 (s, 9H), 1.45 (s, 9H), 1.38 – 1.27 (m, 36H), 0.92 (t, *J* = 6.3 Hz, 6H). **^{13}C NMR (101 MHz, Methanol-d_4) δ** 174.54, 174.50, 173.94, 173.91, 173.70, 173.68, 167.33, 158.25, 155.42, 143.50, 142.54, 129.71, 127.27, 126.18, 123.34, 112.14, 112.02, 104.62, 104.57, 104.49, 104.43, 80.20, 79.95, 50.53, 50.50, 50.06, 48.18, 47.44, 47.11, 44.62, 44.39, 39.70, 39.58, 38.88, 33.02, 30.77, 30.75, 30.73, 30.71, 30.58, 30.56, 30.45, 30.42, 30.25, 29.78, 28.86, 28.84, 28.70, 28.63, 28.07, 28.05, 27.99, 27.88, 23.69, 23.64, 14.52, 14.49. **HRMS (*m/z*):** $[M]^+$ calcd. for $C_{71}H_{115}N_6O_6$, 1147.8873; found, 1147.8870.

Compound 6a. Compound **6a** (1 eq., 500 mg, 0.416 mmol) was dissolved in DCM (3 mL). TFA (100 eq., 3.18 mL, 41.6 mmol) was added dropwise and the reaction mixture was stirred at ambient temperature for 4 h. Solvent was removed under reduced pressure. To remove the remained traces of TFA, the residue was co-evaporated with methanol (3 × 50 mL). After removal of the Boc protecting group the crude product was used directly for the next step without further purification. Yield 426 mg (81%) as red viscous oil. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.42 (t, *J* = 13.3 Hz, 1H), 7.43 – 7.37 (m, 2H), 7.35 – 7.27 (m, 4H), 7.21 – 7.12 (m, 2H), 6.49 – 6.32 (m, 2H), 4.15 – 4.03 (m, 4H), 3.58 – 3.47 (m, 4H), 3.20 – 3.13 (m, 4H), 3.00 (t, *J* = 6.0 Hz, 4H), 2.54 – 2.44 (m, 4H), 2.06 – 1.96 (m, 4H), 1.64 (s, 12H), 1.43 – 1.32 (m, 4H), 1.19 – 1.08 (m, 36H), 0.74 (t, *J* = 6.6 Hz, 6H). **^{13}C NMR** spectrum was difficult to measure due to the sample aggregation. **HRMS (*m/z*):** $[M]^{3+}/3$ calcd. for $C_{59}H_{99}N_6O_2$, 307.9271; found, 307.9260.

Compound 6b. The compound prepared using the same procedure as described above for **6a**. After removal of the Boc protecting group the crude product **6b** was used directly in the next step without further purification. Yield 415 mg (79%) as blue viscous oil. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.07 (t, *J* = 13.1 Hz, 2H), 7.29 (d, *J* = 7.6 Hz, 2H), 7.22 – 7.16 (m, 4H), 7.09 – 7.03 (m, 2H), 6.45 (t, *J* = 12.4 Hz, 1H), 6.19 (d, *J* = 13.7 Hz, 2H), 4.00 (t, *J* = 7.6 Hz, 4H), 3.45 (t, *J* = 6.0 Hz, 4H), 3.13 – 3.08 (m, 4H), 2.94 (t, *J* = 6.0 Hz, 4H), 2.40 (t, *J* = 6.7 Hz, 4H), 1.98 – 1.88 (m, 4H), 1.54 (s, 12H), 1.35 – 1.24 (m, 4H), 1.10 – 1.05 (m, 36H), 0.69 (t, *J* = 6.9 Hz, 6H). **^{13}C NMR** spectrum was difficult to measure due to the sample aggregation. **HRMS (*m/z*):** $[M]^{3+}/3$ calcd. for $C_{61}H_{101}N_6O_2$, 316.5990; found, 316.5998.

Compound Cy3A. Aliphatic amine derivative of cyanine **6a** and triethyl amine (10 eq.) were dissolved in anhydrous DMF (1 mL), and 3,4,5-tris(tetraethyleneoxy)benzoyl chloride **7** (4 eq.) (prepared in situ from corresponding acid and oxalyl chloride)[1] was added dropwise as a solution in anhydrous DMF at 0 °C using ice bath. After stirring at 0 °C for 2h, the reaction mixture was heated to 60 °C for another 2h until completion. After solvent evaporation in vacuum, the residue was redissolved in DCM (50 mL), washed with saturated brine solution (3 ×100 mL), dried over sodium sulphate, filtered, and the filtrate evaporated to dryness in vacuum. The crude product was purified by flash column

chromatography (SiO_2, DCM/MeOH, 95:5 to 90:10), which furnished 10 mg (yield 20%) of **Cy3A** as a red oil. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.70 – 8.44 (m, 1H), 7.63 (d, *J* = 7.4 Hz, 2H), 7.56 – 7.44 (m, 4H), 7.40 (t, *J* = 7.3 Hz, 2H), 7.31 (s, 1H), 7.25 (s, 1H), 6.80 (s, 1H), 6.79 (s, 1H), 6.71 – 6.42 (m, 2H), 4.31 – 4.18 (m, 12H), 4.17 – 4.05 (m, 4H), 3.99 – 3.85 (m, 6H), 3.85 – 3.79 (m, 6H), 3.79 – 3.72 (m, 12H), 3.71 – 3.62 (m, 52H), 3.62 – 3.57 (m, 12H), 3.56 – 3.45 (m, 4H), 3.41 (s, 18H), 3.39 – 3.31 (m, 4H), 2.75 – 2.63 (m, 2H), 2.62 – 2.41 (m, 2H), 2.22 – 2.09 (m, 4H), 1.85 (s, 12H), 1.73 – 1.54 (m, 4H), 1.44 – 1.18 (m, 36H), 0.97 (t, *J* = 7.4 Hz, 6H). **^{13}C NMR** spectrum was difficult to measure due to the sample aggregation. **HRMS ESI (*m/z*):** $[M+1]^+$ calcd. for $C_{127}H_{213}N_6O_{34}$, 2367.5151, found 2367.5054; **MALDI TOF MS (*m/z*):** calc. [M+] 2366.512, found 2366.589.

Compound Cy5A. The compound prepared using the same procedure as described above for **Cy3A**. The crude product was purified by flash column chromatography (SiO_2, DCM/MeOH, 95:5 to 90:10). Yield 15 mg (18%) as blue oil. **^{1}H NMR (400 MHz, Methanol-d_4) δ** 8.37 – 8.20 (m, 2H), 7.54 (d, *J* = 7.5 Hz, 2H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.38 (d, *J* = 7.9 Hz, 2H), 7.34 – 7.30 (m, 2H), 7.29 (s, 1H), 7.28 (s, 1H), 7.23 (s, 1H), 7.22 (s, 1H), 6.72 – 6.52 (m, 1H), 6.38 (d, *J* = 13.5 Hz, 2H), 4.29 – 4.12 (m, 12H), 4.11 – 3.97 (m, 4H), 3.88 (t, *J* = 4.6 Hz, 4H), 3.83 (t, *J* = 4.7 Hz, 4H), 3.81 – 3.75 (m, 4H), 3.74 – 3.69 (m, 12H), 3.69 – 3.60 (m, 48H), 3.59 – 3.52 (m, 12H), 3.41 (t, *J* = 8.0 Hz, 4H), 3.37 (s, 18H), 3.36 – 3.33 (m, 8H), 2.79 – 2.67 (m, 2H), 2.66 – 2.56 (m, 2H), 2.18 – 2.02 (m, 4H), 1.77 (s, 12H), 1.69 – 1.55 (m, 4H), 1.45 – 1.24 (m, 36H), 0.98 – 0.88 (m, 6H). **^{13}C NMR** spectrum was difficult to measure due to an aggregation of the sample. **HRMS ESI (*m/z*):** $[M+1]^+$ calcd. for $C_{129}H_{215}N_6O_{34}$, 2393.5307; found, 2393.5314; **MALDI TOF MS (*m/z*):** calc. [M+] 2392.527, found 2392.53.

Preparation of fluorescent NPs

The cyanine (Cy3A or Cy5A) derivative was dissolved at 1 mM in DMSO. The concentration was measured by photometry using the molar extinction coefficient of Cy3 and Cy5 derivatives, 150,000 and 250,000 M^{-1} cm^{-1} in methanol, respectively. To prepare 1 µM of nanoparticle solution, 2 µL of 1 mM cyanine (Cy3A or Cy5A) derivative stock solution was added quickly under stirring (shaking) using a micropipette to 1.98 mL of Milli-Q® water (Millipore). Then, to the obtained solution a 10-fold excess of the corresponding borate solution (20 µL of 1 mM stock solution) was added quickly under stirring, using a micropipette.

For the DLS measurements of nanoparticle suspension, 2 µM dye concentration was used to obtain sufficient signal. To this end, 4 µL of 1 mM cyanine (Cy3A or Cy5A) derivative stock solution was added quickly under stirring using a micropipette to a 1.96 mL of Milli-Q® water (Millipore). Then to the obtained solution, a 10-fold excess of the corresponding borate solution (40 µL of 1 mM stock solution) was added quickly under stirring using a micropipette. Zeta potential measurements were performed using 5 µM dye concentration.

For the DLS and AFM measurements, to remove possible aggregates the obtained solution of prepared nanoparticles was additionally filtered through a 0.1 µm PVDF NS "Ultrafree®-CL" centrifugal filter unit (Merck Millipore).

Optical spectroscopy

Absorption and emission spectra were recorded on a Cary 400 Scan UV-visible spectrophotometer (Varian) and a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon) equipped with a thermostated cell compartment, respectively. For standard recording of fluorescence spectra, the excitation wavelength was set to 520 nm. The fluorescence spectra were corrected for detector response and lamp fluctuations. Fluorescence quantum yields of Cy3A were measured using rhodamine B in water (QY = 31%)[2] as a reference. Excitation wavelength was 520 nm. Fluorescence quantum yields of Cy5A were measured using DiD in methanol as a reference (QY = 33%).[3] Excitation wavelength was 600 nm. Hydrodynamic diameter and zeta-potential measurements were performed on a Zetasizer Nano ZSP (Malvern Instruments S.A.) with a laser source at 633 nm.

AFM measurements

AFM measurements were performed using a Solver-Pro-M (NT-MDT) instrument. The measurements were in liquid phase. Cantilevers were NSG03 (NT-MDT) with a tip curvature radius of 10 nm or OTR4 (Bruker) with a tip curvature radius of 15 nm. The NPs were prepared as described before. To deposit NPs on the mica surface, 100 µL of 10-100 mM calcium chloride solution (depends on the counterion) was first incubated for 30 min. Then, the solution was removed with a filter paper and 100 µL of an undiluted suspension of NPs was deposited on the mica surface. After 30 min, the solution was removed using a filter paper and then replaced with 100 µL of a 10-100 mM solution of calcium chloride. The obtained sample was imaged in liquid phase, using the tapping mode (~37 kHz).

Fluorescence correlation spectroscopy (FCS) and data analysis

FCS measurements were performed on a two-photon platform including an Olympus IX70 inverted microscope. Two-photon excitation at 760 nm (1–5 mW laser output power) was provided using an InSight DeepSee laser (Spectra Physics). The measurements were carried out in a 96-well plate, using a 200 µL volume per well. The focal spot was set about 20 µm above the cover-slip. The normalized autocorrelation function, $G(\tau)$ was calculated online by using an ALV-5000E correlator (ALV, Germany) from the fluorescence fluctuations, $\delta F(t)$, by $G(\tau) = \langle \delta F(t) \delta F(t+\tau) \rangle / \langle F(t) \rangle^2$, where $\langle F(t) \rangle$ is the mean fluorescence signal, and τ is the lag time. Assuming that fluorescent NPs diffuse freely in a Gaussian excitation volume, the correlation function, $G(\tau)$, calculated from the fluorescence fluctuations was fitted according to Thompson:[4]

$$G(\tau) = \frac{1}{N}\left(1 + \frac{\tau}{\tau_{\mathrm{d}}}\right)^{-1}\left(1 + \frac{1}{S^2}\frac{\tau}{\tau_{\mathrm{d}}}\right)^{-1/2}$$

where τ_{d} is the diffusion time, N is the mean number of fluorescent species within the two-photon excitation volume, and S is the ratio between the axial and lateral radii of the excitation volume. The

excitation volume is about 0.34 fL and S is about 3 to 4. Typical data recording time was 5 min, using freshly prepared NPs without further dilution. The measurements were done with respect to a reference 5(6)-carboxytetramethylrhodamine (TMR, from Sigma-Aldrich) in water. The hydrodynamic diameter, *d*, of NPs was calculated as: $d_{NPs} = \tau_{d(NPs)} / \tau_{d(TMR)}) \times d_{TMR}$, where d_{TMR} is a hydrodynamic diameter of TMR (1.0 nm). The concentration of NPs was calculated from the number of species by: $C_{NPs} = N_{NPs}/N_{TMR} \times C_{TMR}$, using a TMR concentration of 50 nM.

Antenna effect

The antenna effect is expressed as $AE = (n_D \cdot \varepsilon_D \cdot E)/(n_A \cdot \varepsilon_A)$, where n_D and n_A are the numbers of donors and acceptors, respectively, per particle, ε_D and ε_A are the extinction coefficients of donor and acceptor, respectively, and E is the FRET efficiency.

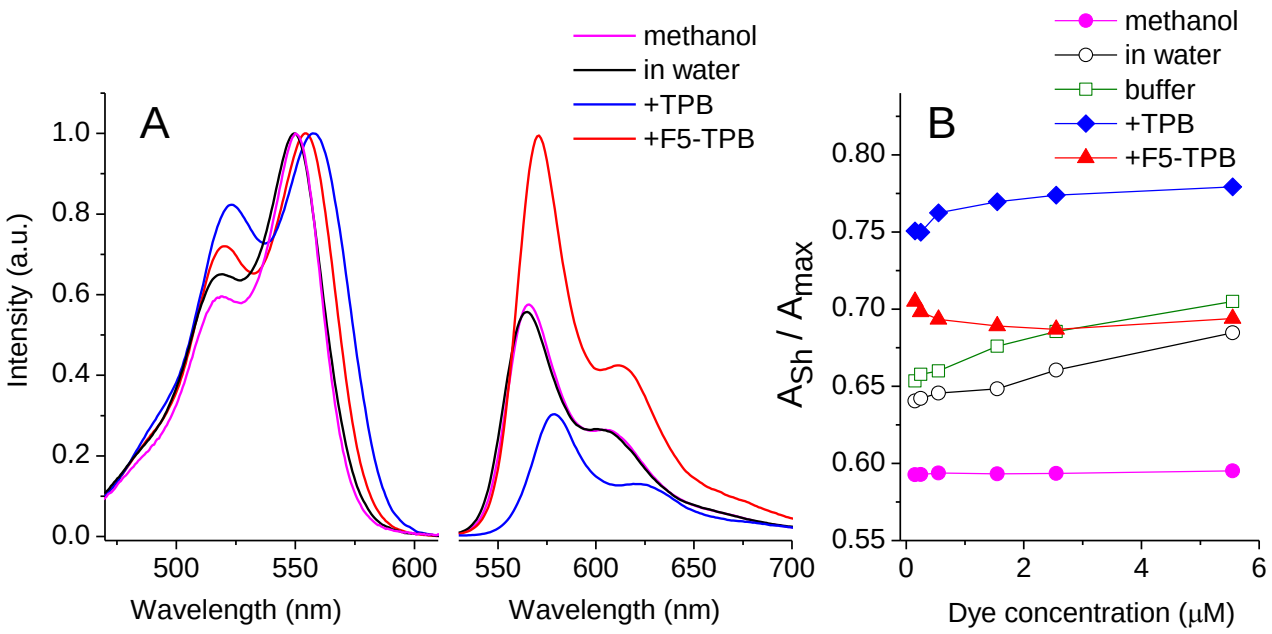


Figure S1. (A) Absorption (normalized) and fluorescence spectra of Cy3A (1 μM) and (B) dependence of the ratio of the absorption bands A_{sh}/A_{max} on dye concentration in methanol and aqueous media in the presence of different counterions (10 mol. eq.). Emission spectra were normalized to the same absorbance at 520 nm. λ_{ex} = 520 nm.

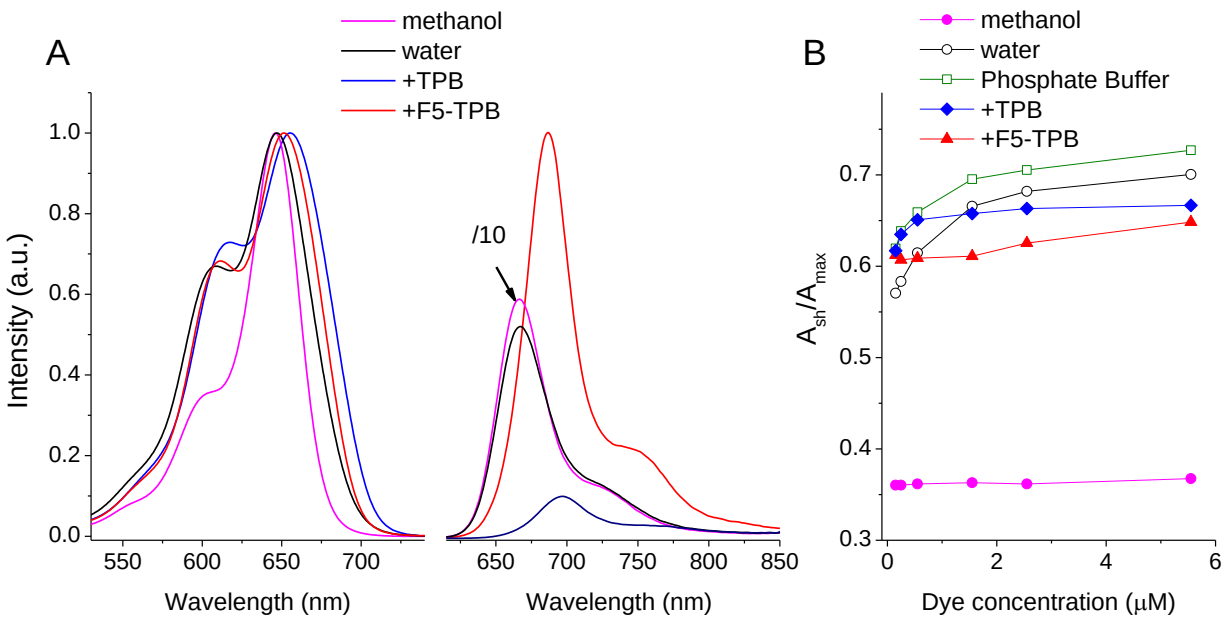


Figure S2. (A) Absorption (normalized) and fluorescence spectra of Cy5A (1 μM) and (B) dependence of the ratio of the absorption bands A_{sh}/A_{max} on dye concentration in methanol and aqueous media in the presence of different counterions (10 mol. eq.). λ_{ex} = 600 nm.

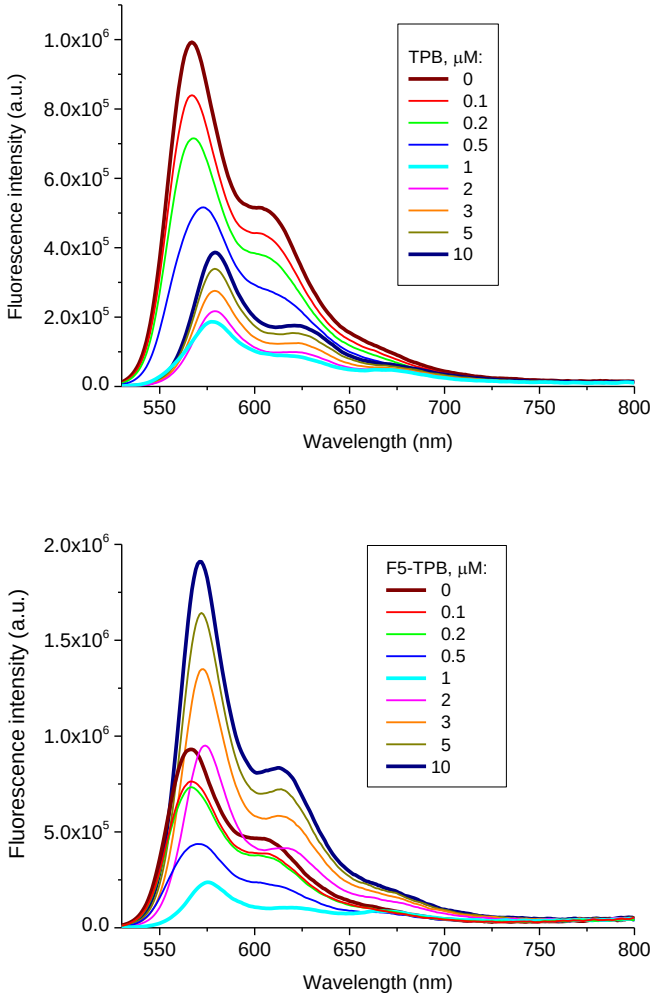


Figure S3. Fluorescence spectra of 1 μM Cy3A in water at increasing concentrations of TPB (top) and F5-TPB (bottom). λ_{ex} = 520 nm.

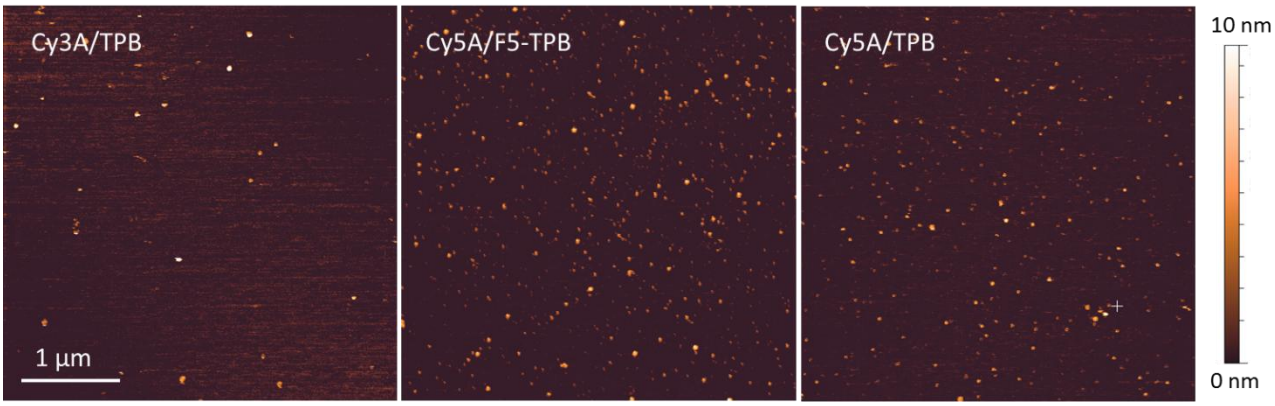
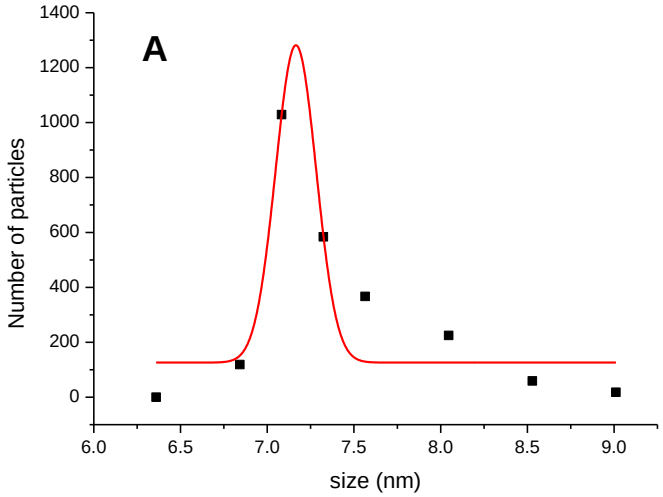
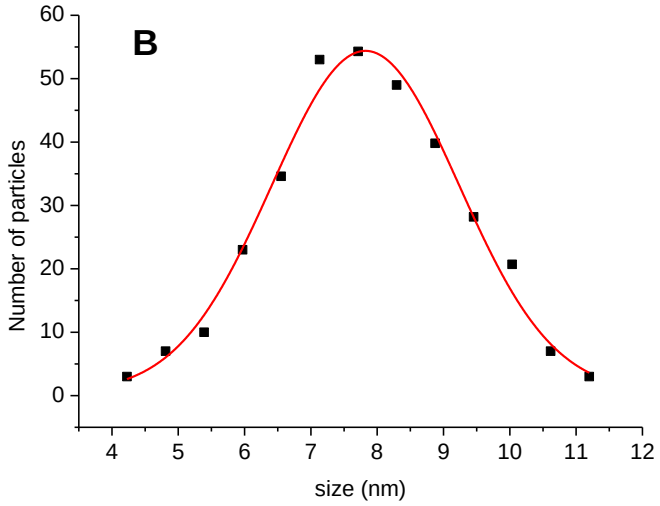


Figure S4. Atomic force microscopy of nanostructures composed of Cy3A/TPB, Cy5A/F5-TPB and Cy5A/TPB.

AFM size statistics





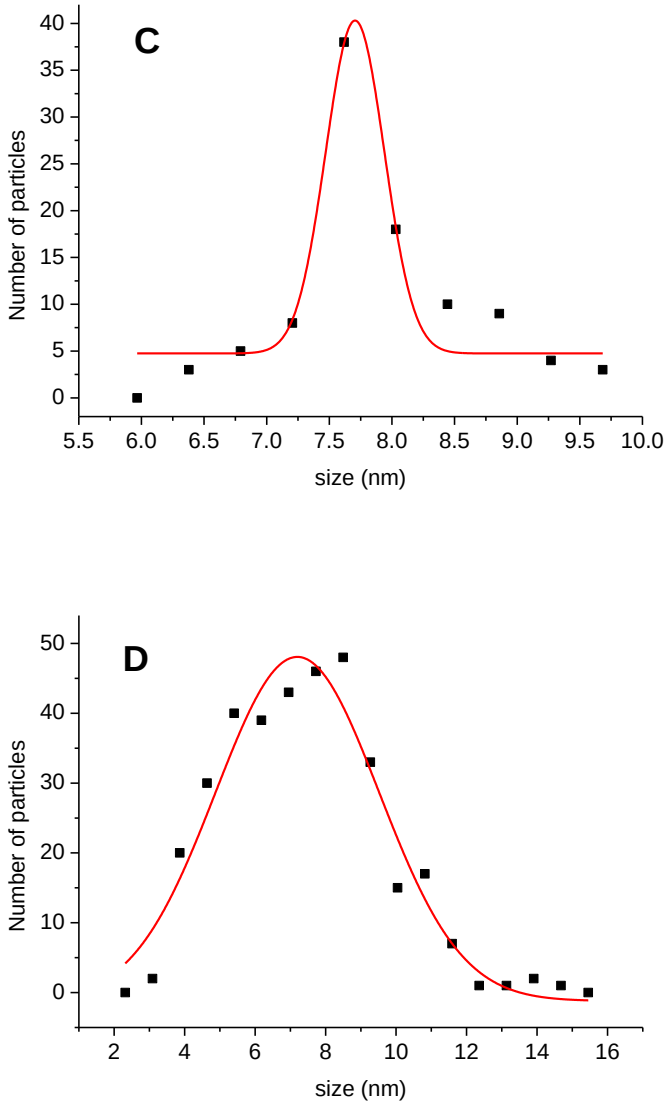
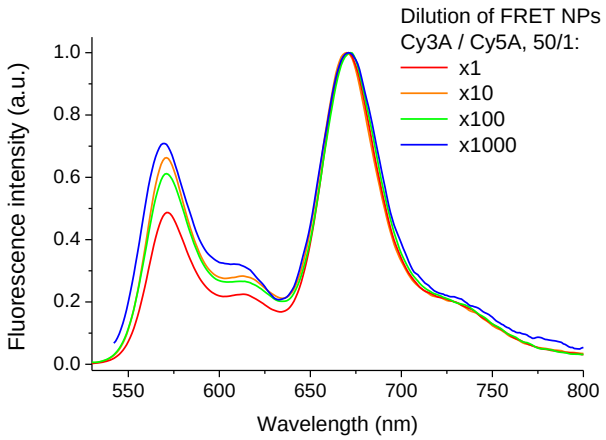


Figure S5. AFM size statistics of micellar NPs prepared by counterion-induced self-assembly of Cy3A and Cy5A amphiphiles by tetraphenylborate counterions: (A) **Cy3A ─ F5-TPB**, average size **7 nm**; (B) **Cy3A ─ TPB**, average size **8 nm**; (C) **Cy5A ─ F5-TPB**, average size **8 nm**; (D) **Cy5A ─ TPB**, average size **7 nm**. Particle diameter was estimated from the height measurements. Gaussian model was applied for fitting the size distribution.

Table S1. Two-photon fluorescence correlation spectroscopy (FCS) data of Cy3A/F5-TPB NPs in comparison to tetramethyl-rhodamine dye (TMR).[a]

	TMR	Cy3A/F5-TPB
τ_{corr} , ms	0.033±0.002	0.19±0.02
N	13±2	18±2
Bri, kHz	0.25±0.07	1.9±0.4
Size, nm	1	6.5±0.7
Bri/TMR	1	5.9±0.3
Bri/Cy3A	6.8	41±5
Conc., nM	50	80±2
Dye/NP	1	25±1

[a] τ_{corr} – correlation time, N – number of emissive species per excitation volume, Bri – brightness per particle, Size – hydrodynamic diameter measured based on τ_{corr}; Bri/TMR and Bri/Cy3A is brightness with respect to TMR and Cy3A, calculated based on literature data on two-photon absorption cross-section of cyanine 3 (25 GM)[5] and quantum yield of Cy3A in micelles (0.2); Conc. – concentration of species; Dye/NP number of dyes per emissive species estimated from N.



Figures S6. Normalized fluorescence spectra of FRET NPs at different dilutions. Initial concentration of Cy3A, Cy5A and F5-TPB in non-diluted (x1) solution were 1, 0.02 and 10 μM, respectively. NPs were diluted to different extent in milliQ water.

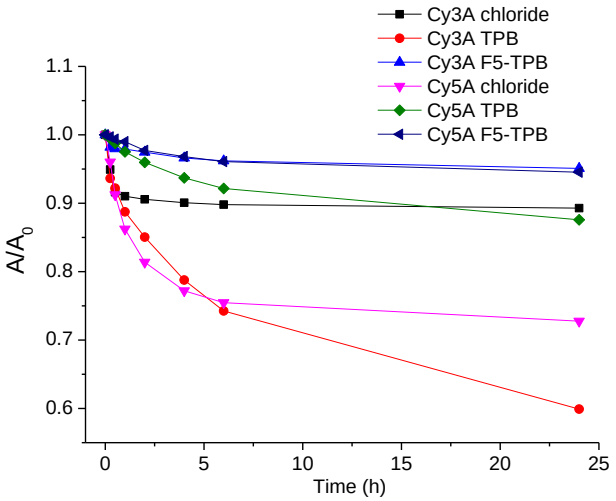
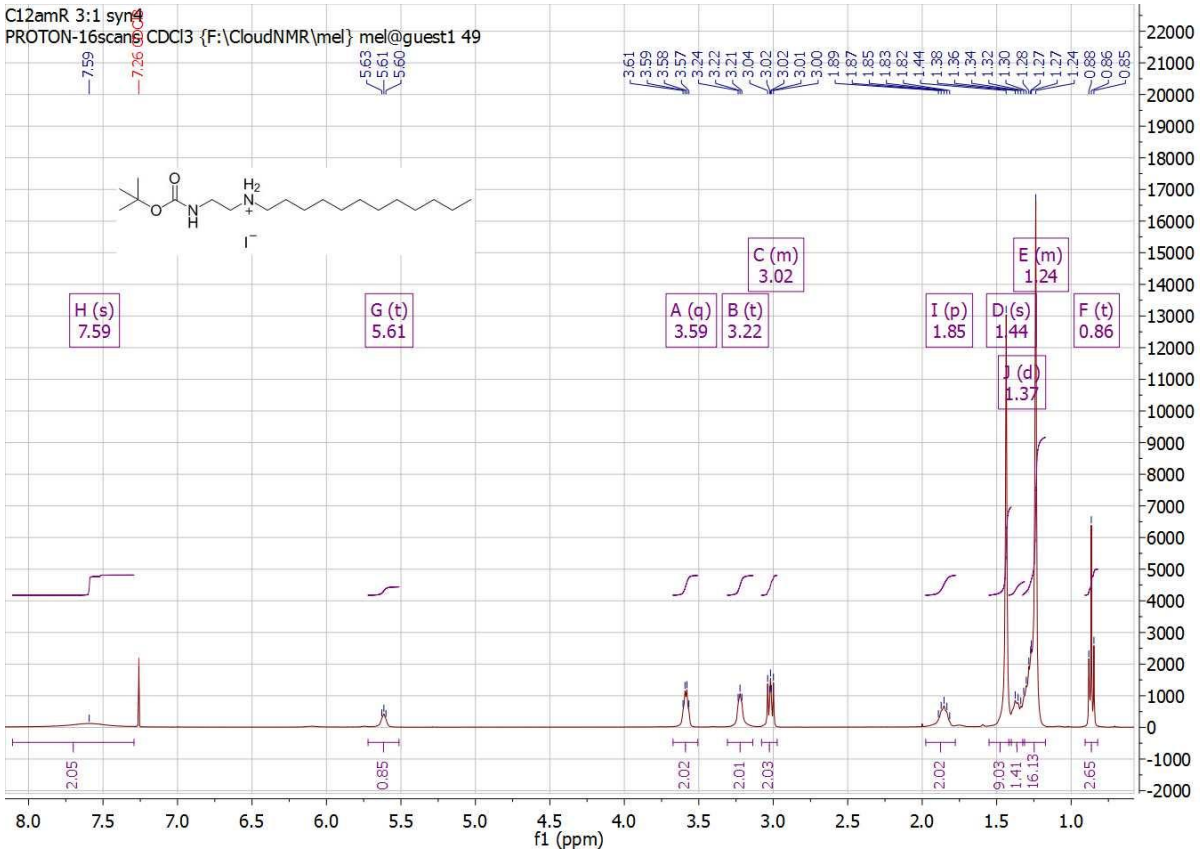


Figure S7. Time-dependence of the maximum absorbance of Cy3A and Cy5A dye amphiphiles with different borate counterions in plastic cuvettes. Concentration of Cy3A and Cy5A dyes were 1 μM, while concentration of the borate counterions was 10 μM.



Figure

Figure S8. ^{1}H NMR spectrum of compound **1**.

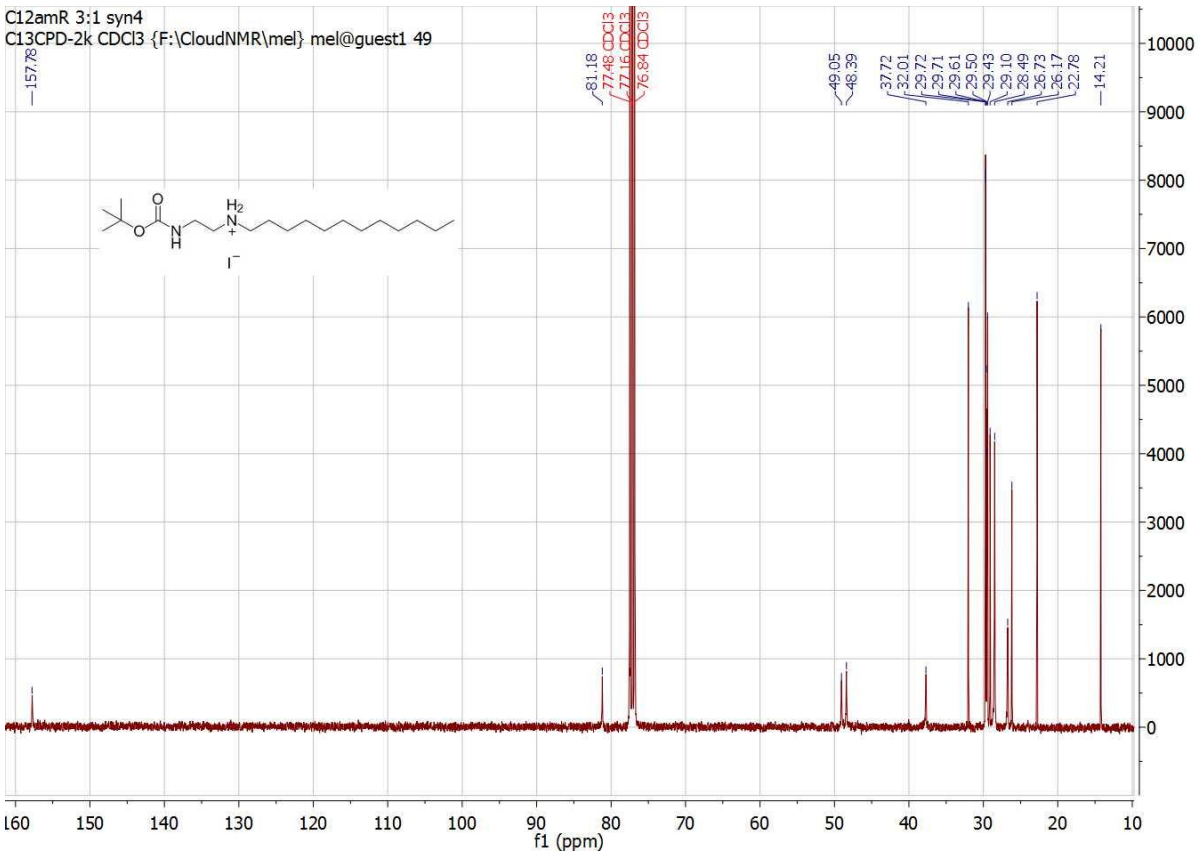


Figure S9. ^{13}C NMR spectrum of compound **1**.

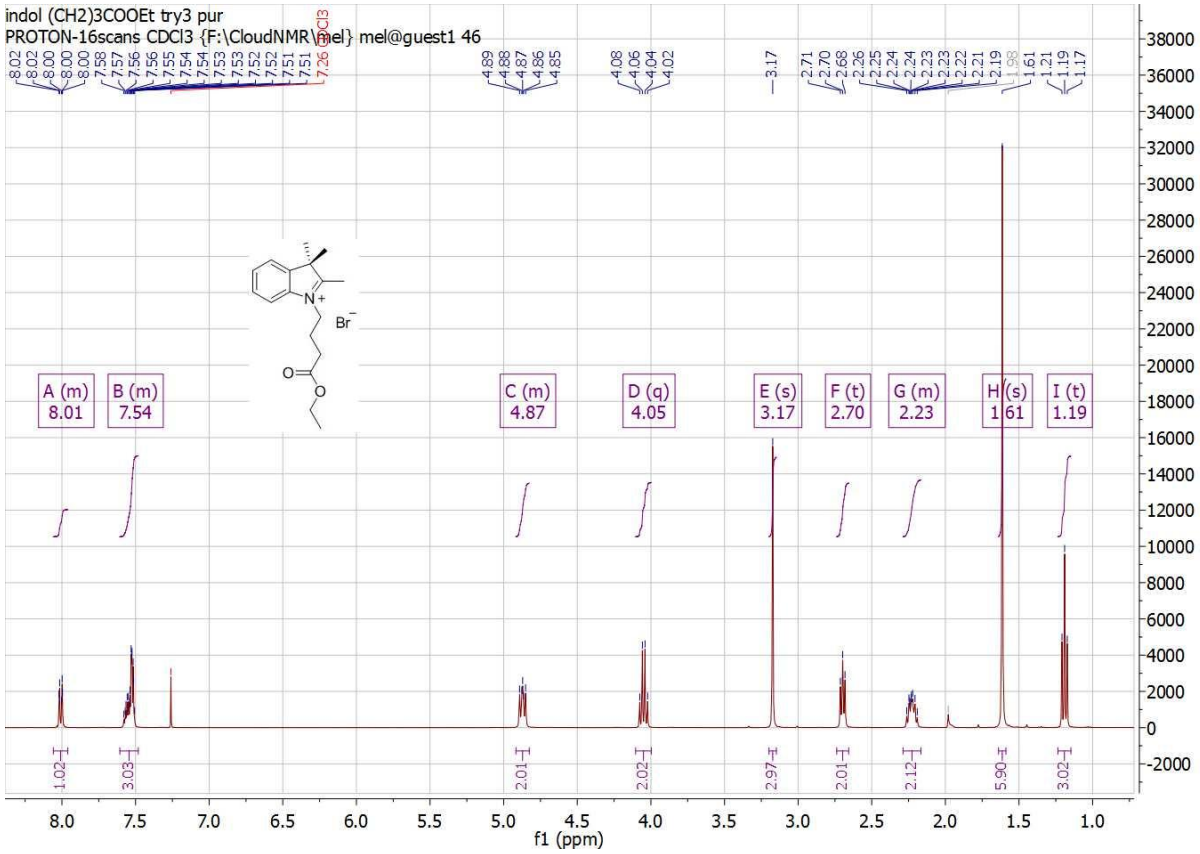


Figure S10. ^{1}H NMR spectrum of compound **2**.

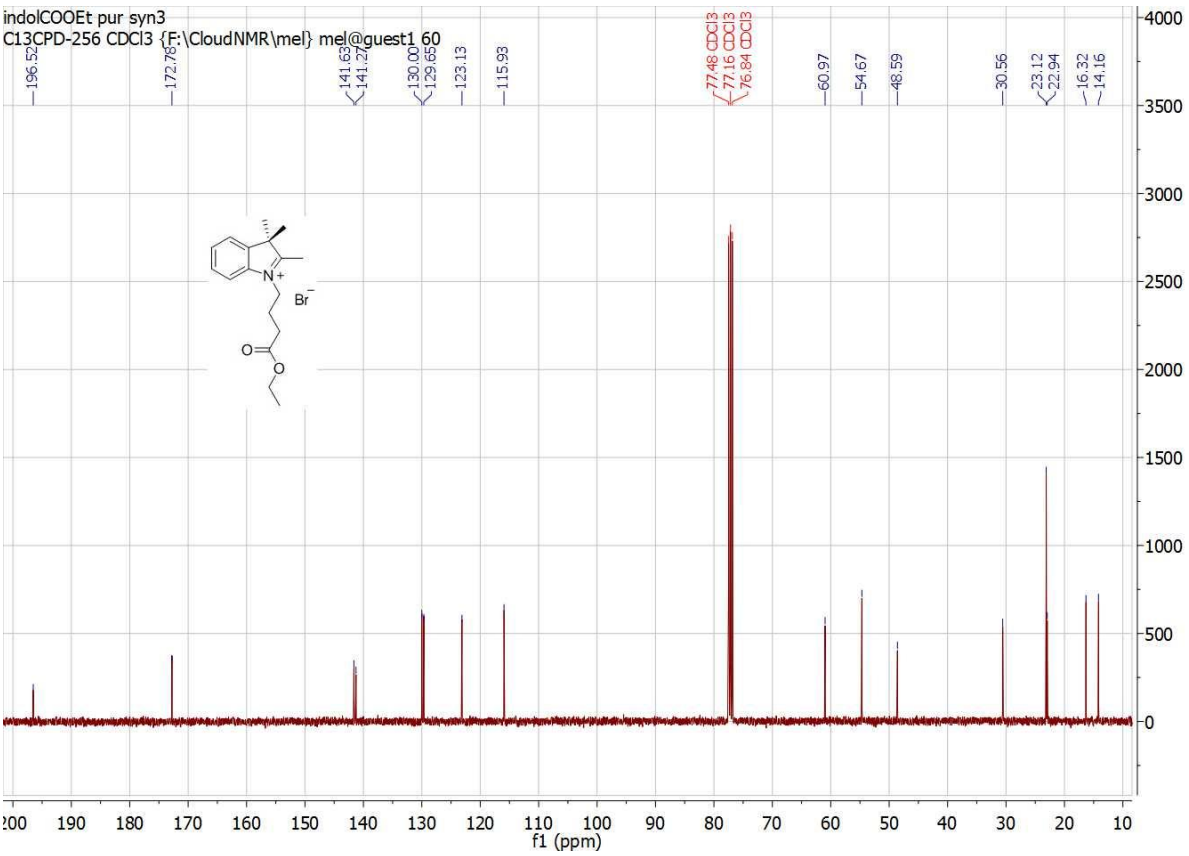


Figure S11. ^{13}C NMR spectrum of compound **2**.

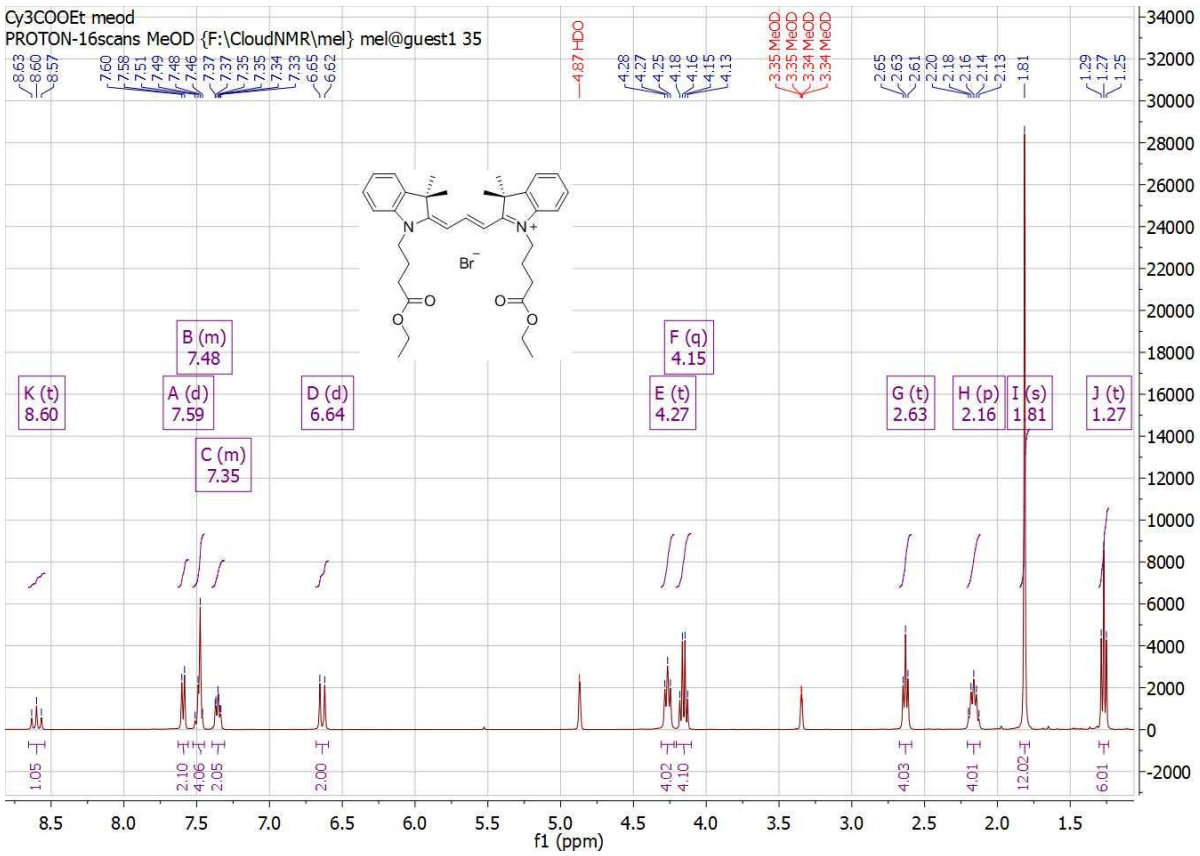


Figure S12. ^{1}H NMR spectrum of compound **3a**.

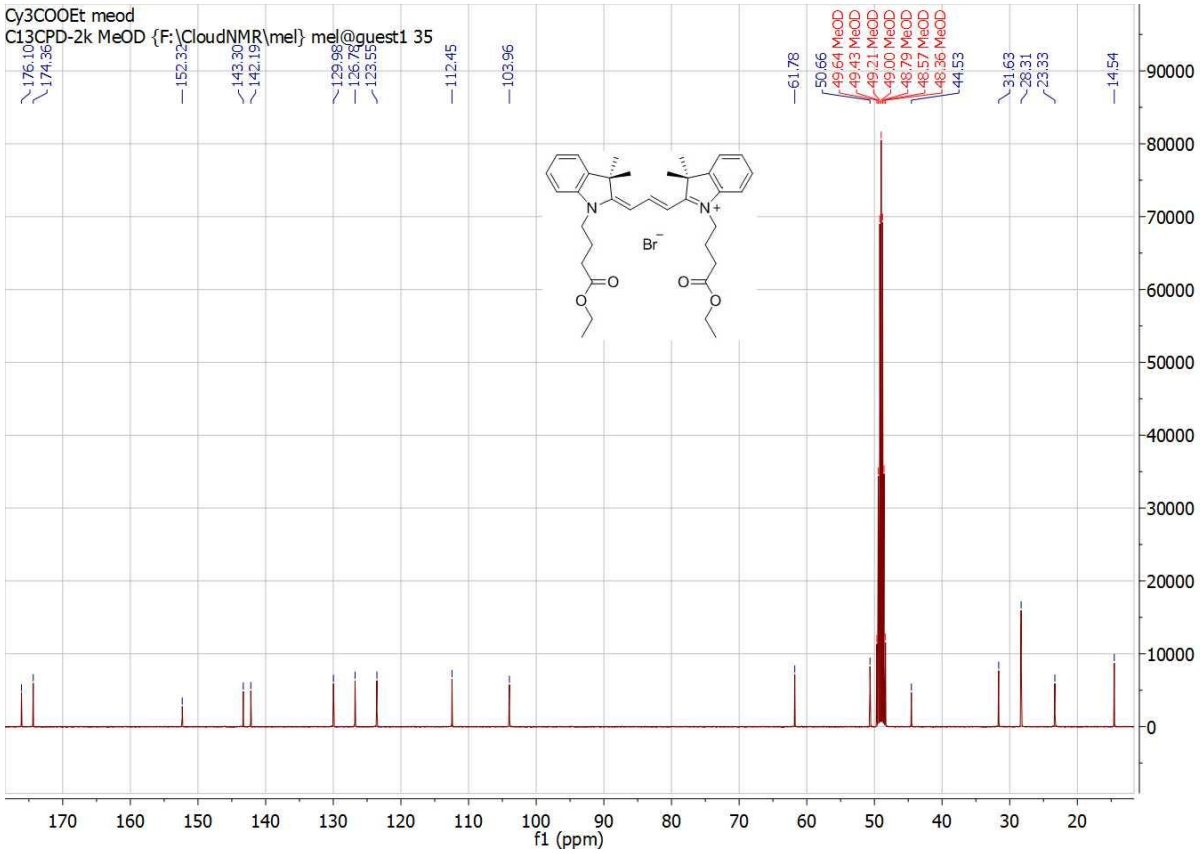


Figure S13. ^{13}C NMR spectrum of compound **3a**.

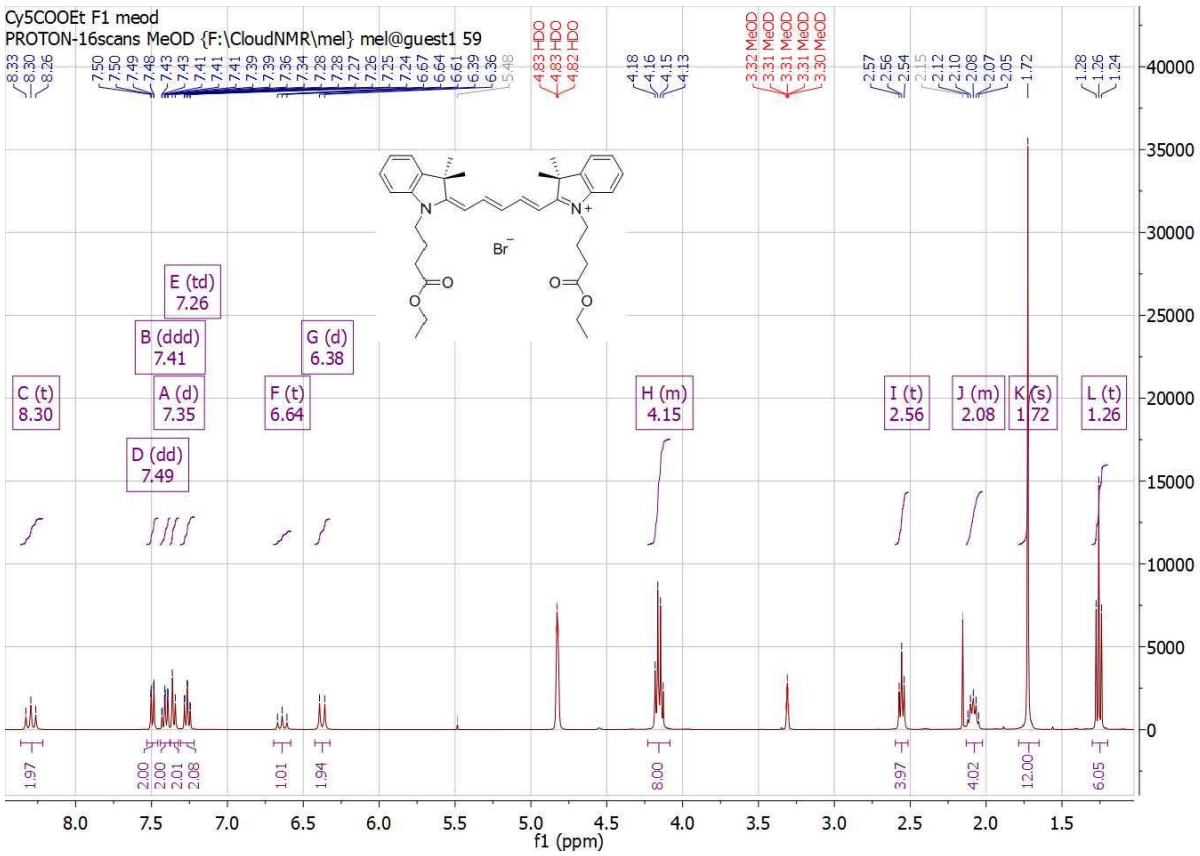


Figure S14. ^{1}H NMR spectrum of compound **3b**.

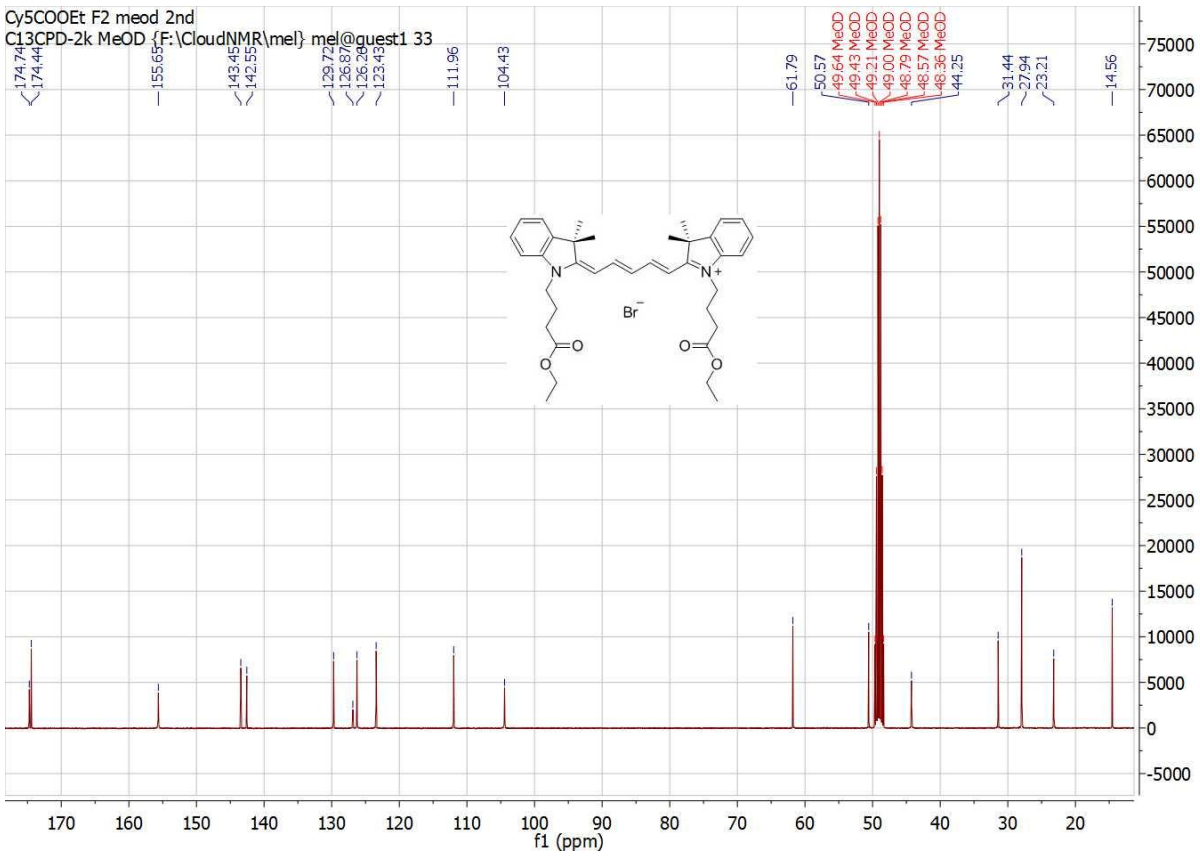


Figure S15. ^{13}C NMR spectrum of compound **3b**.

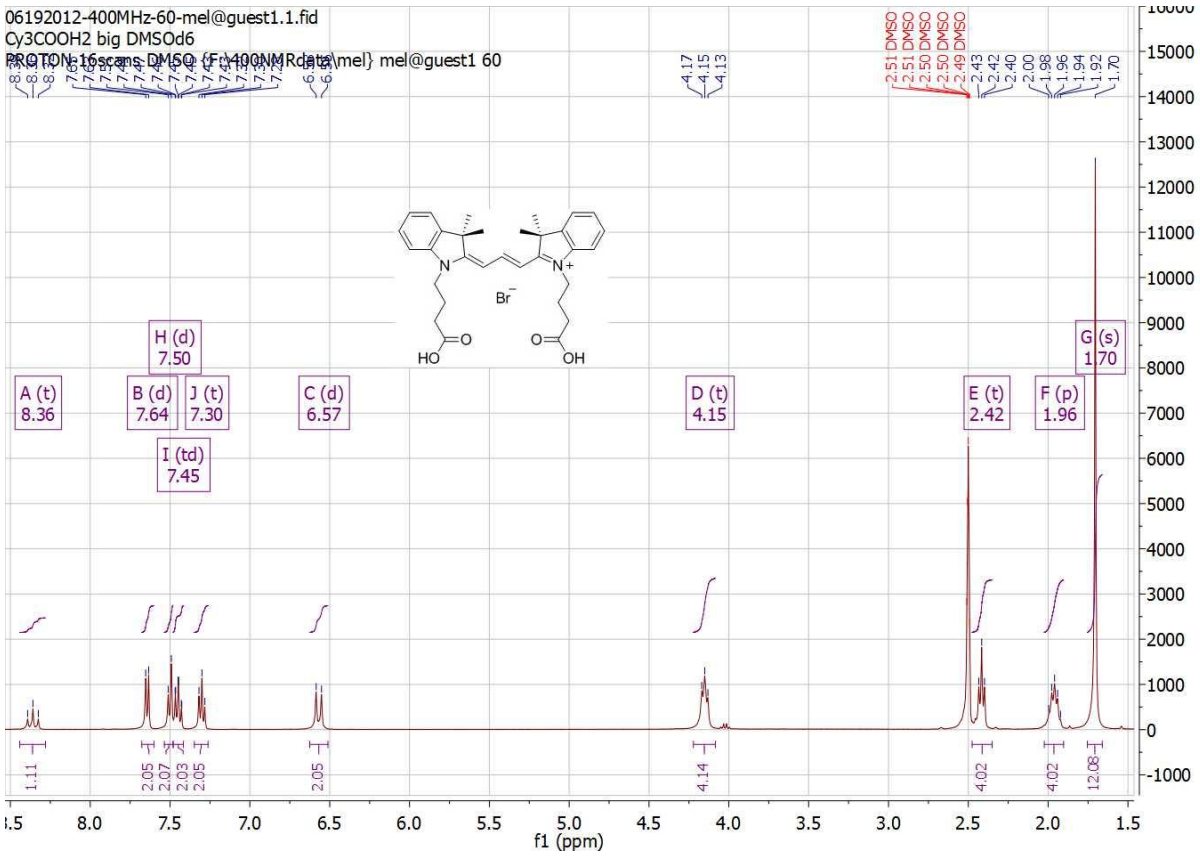


Figure S16. ^{1}H NMR spectrum of compound **4a**.

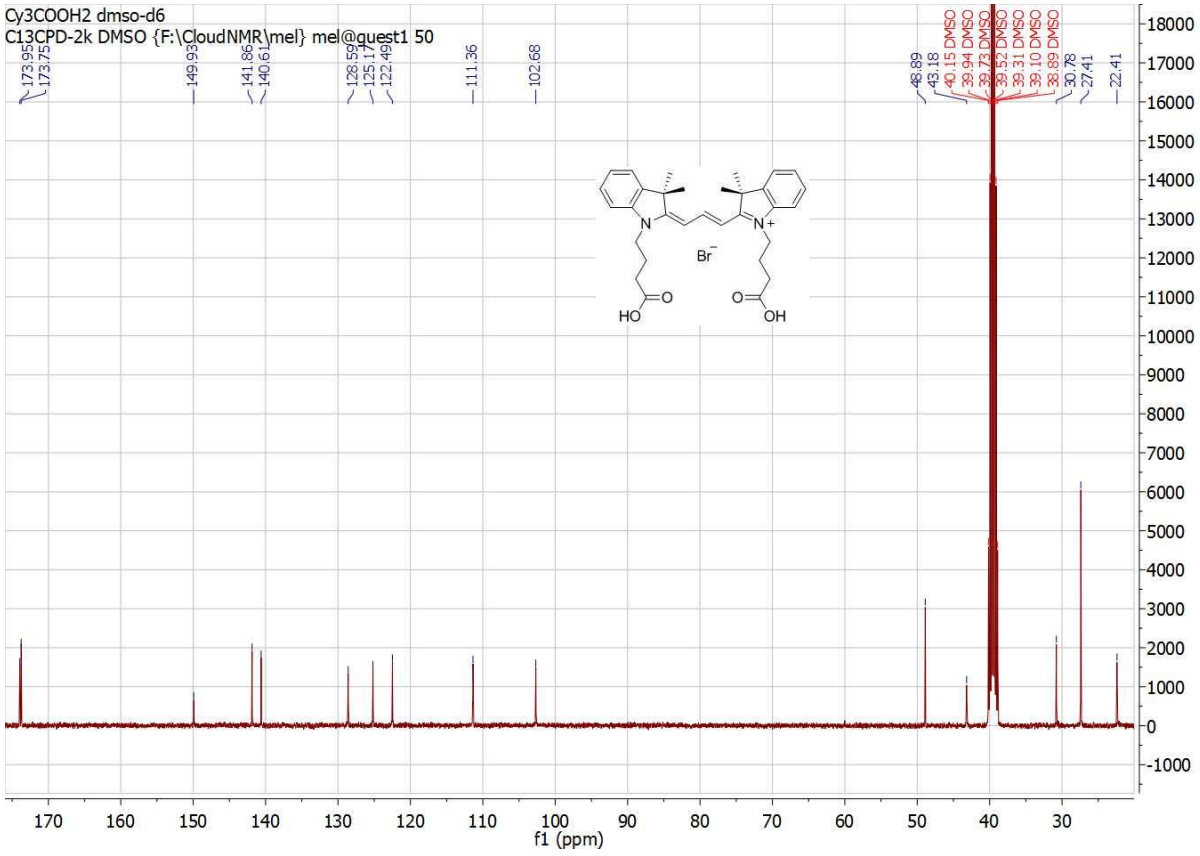


Figure S17. ^{13}C NMR spectrum of compound **4a**.

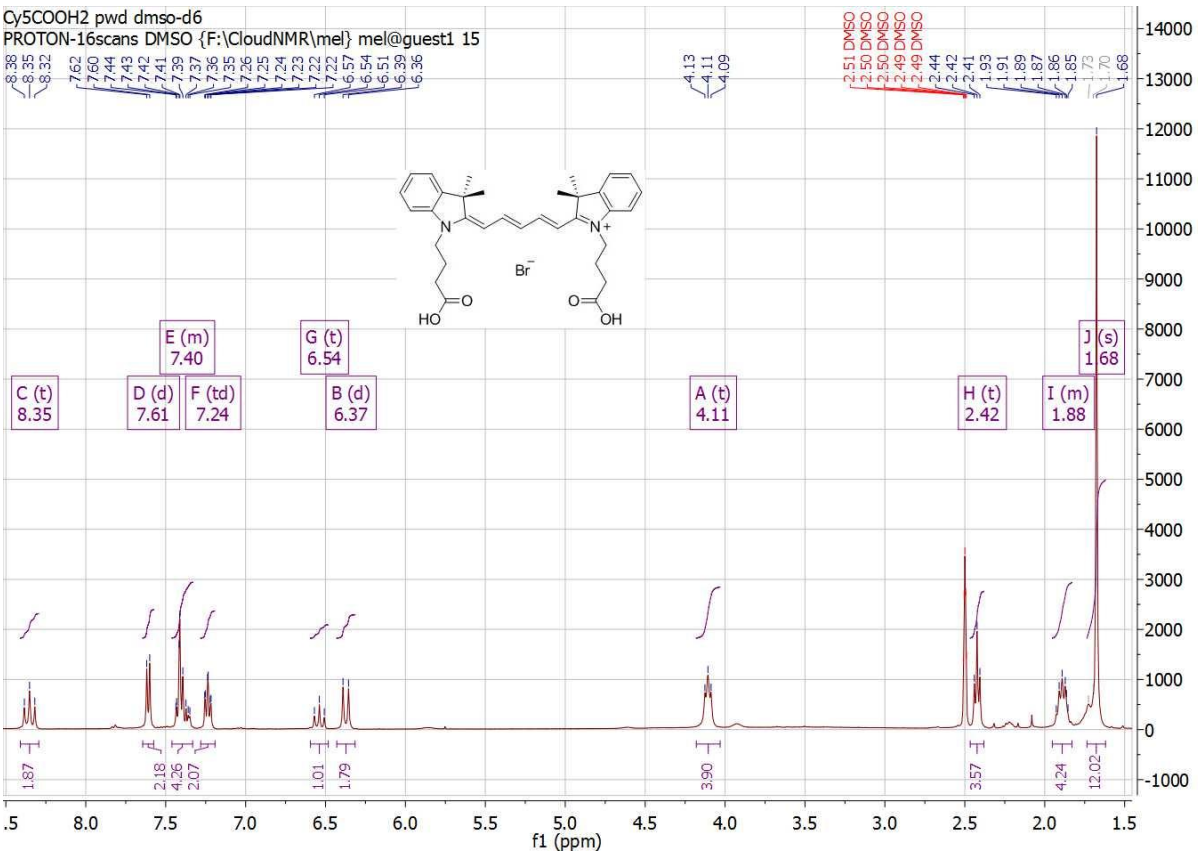


Figure S18. ^{1}H NMR spectrum of compound **4b**.

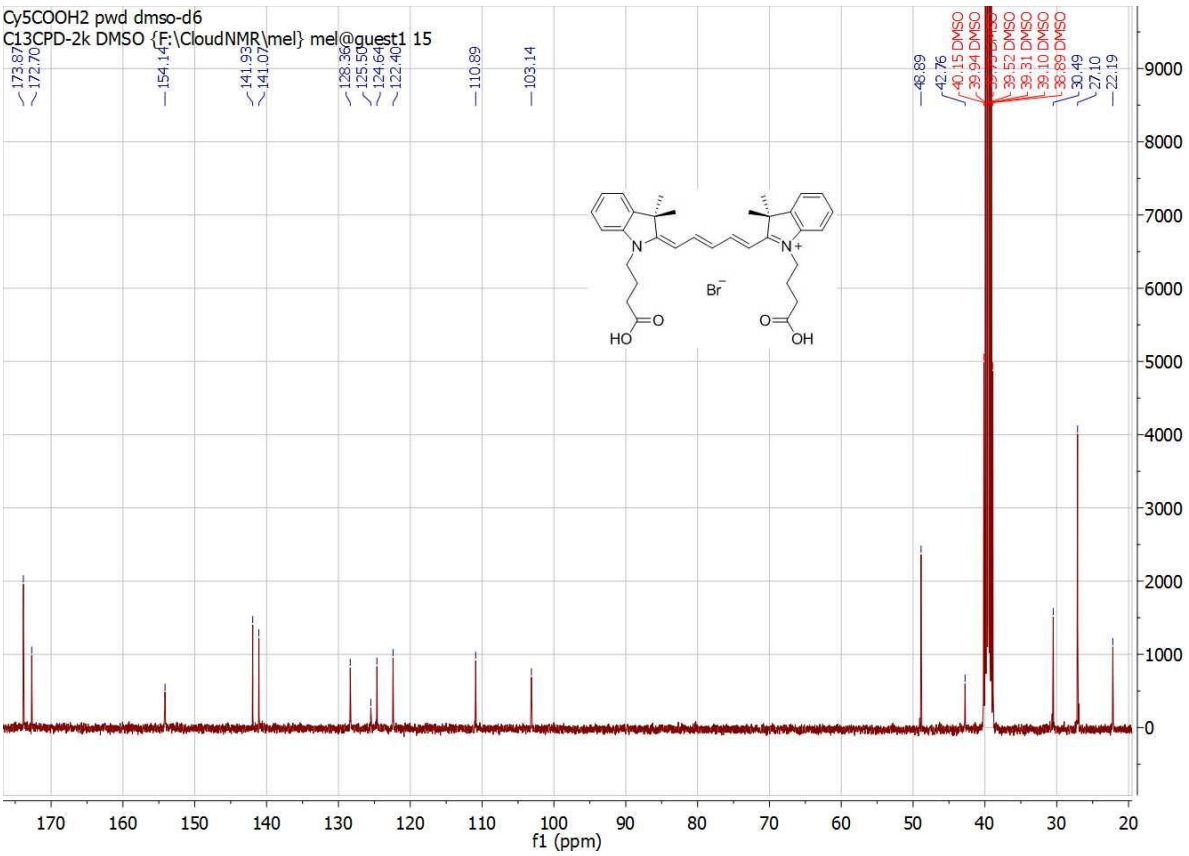


Figure S19. ^{13}C NMR spectrum of compound **4b**.

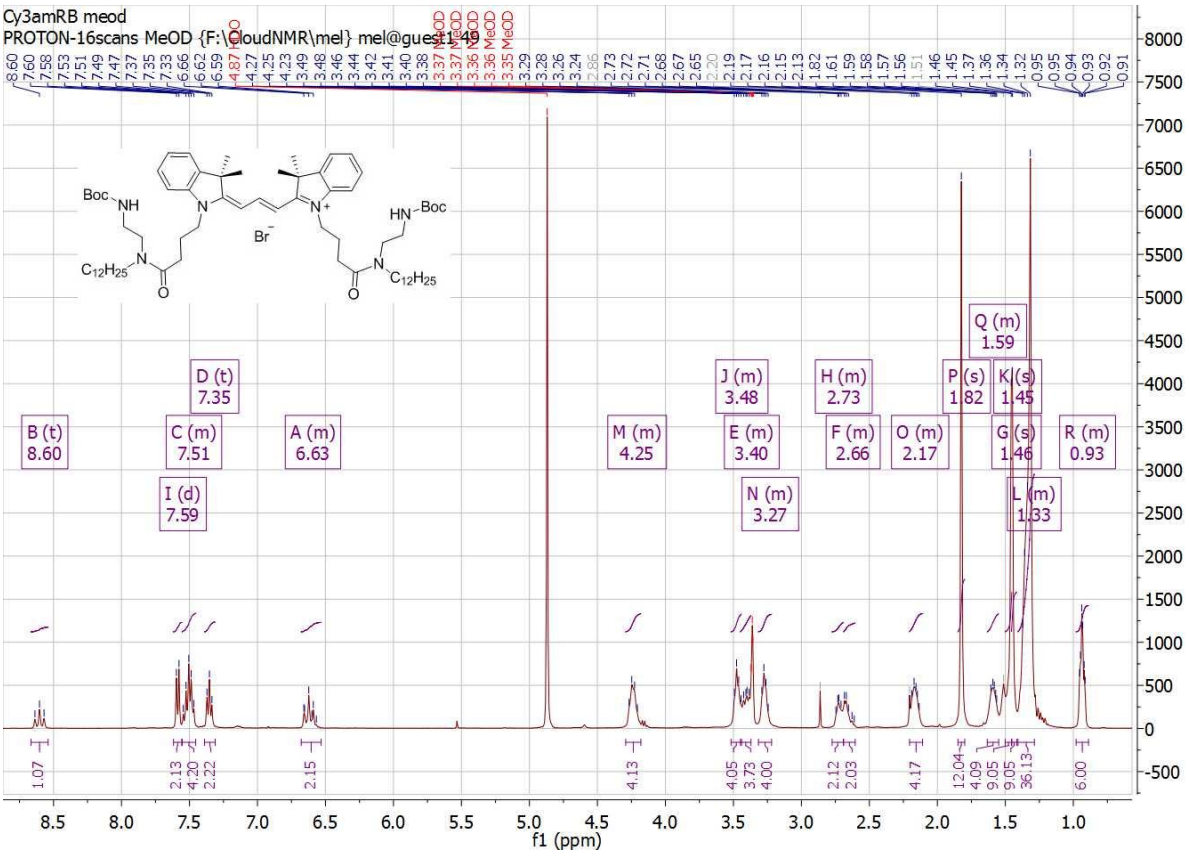


Figure S20. ^{1}H NMR spectrum of compound **5a**.

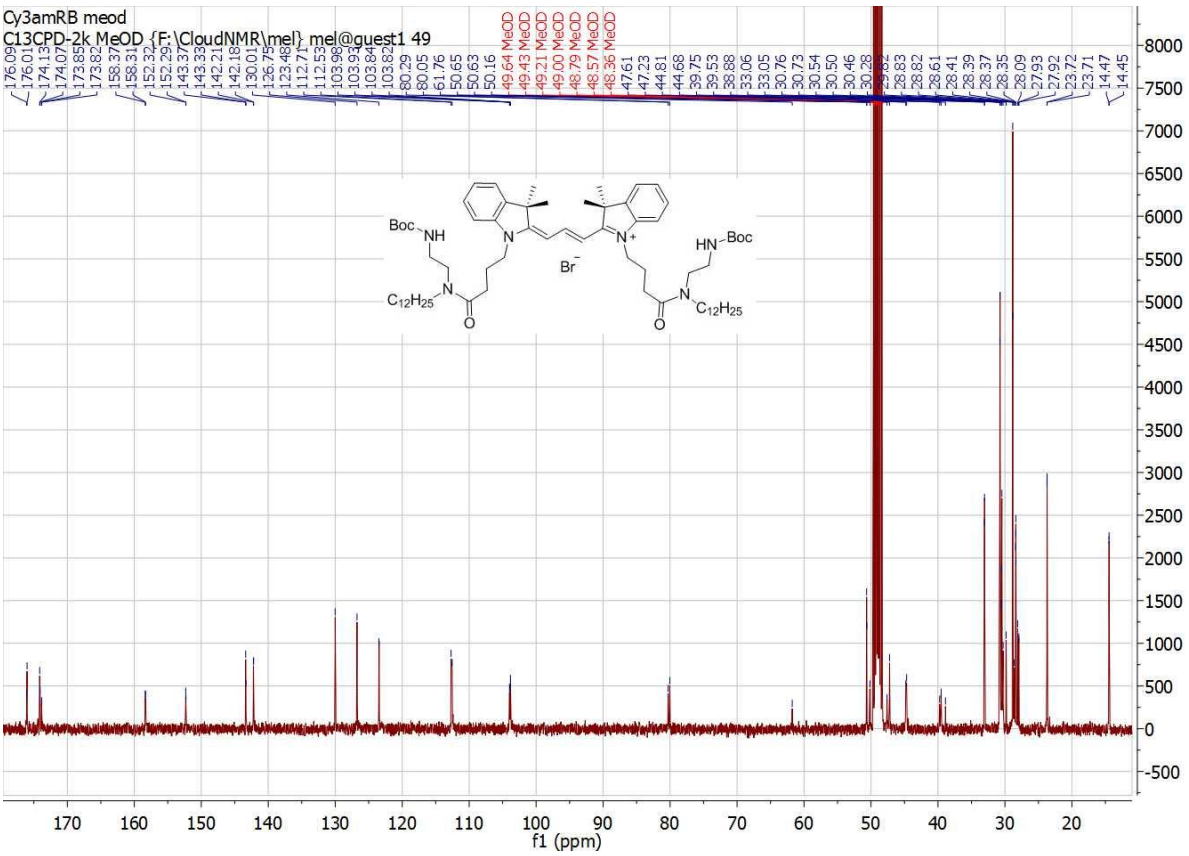


Figure S21. ^{13}C NMR spectrum of compound **5a**.

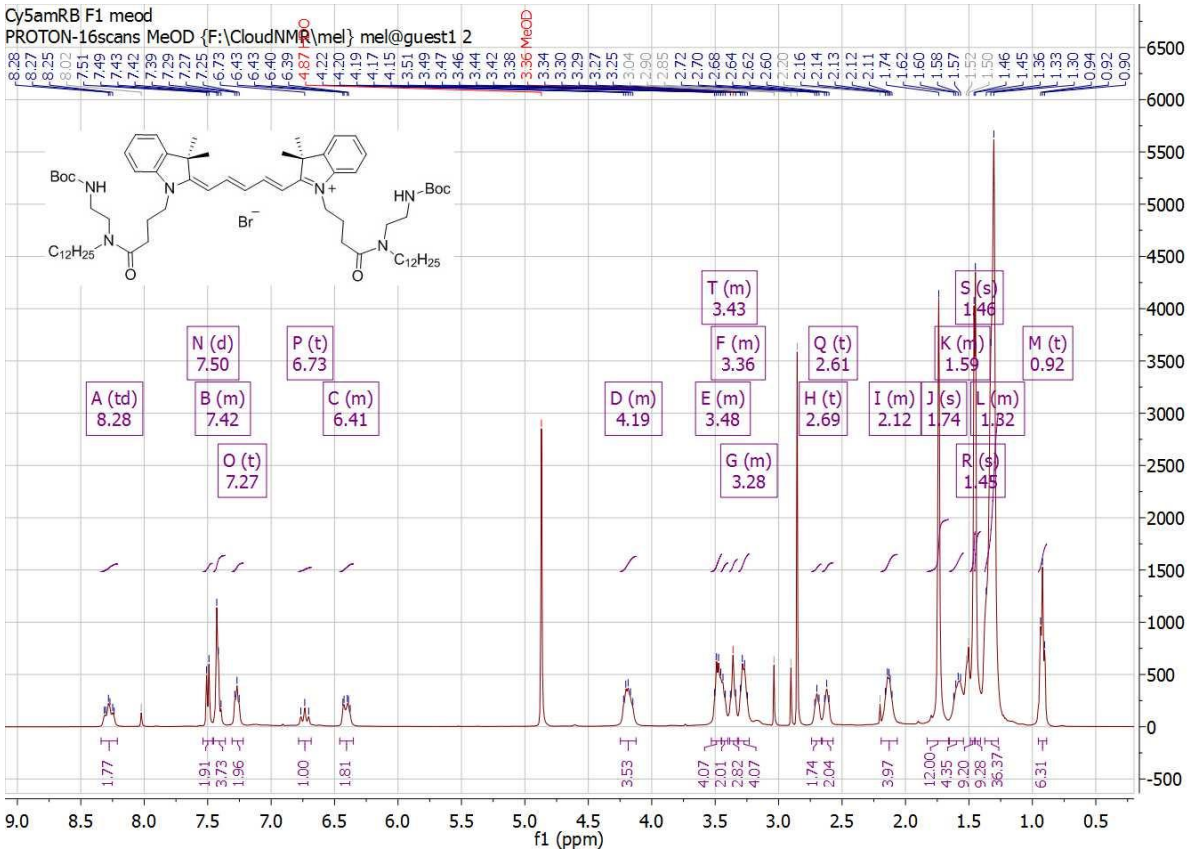


Figure S22. ^{1}H NMR spectrum of compound **5b**.

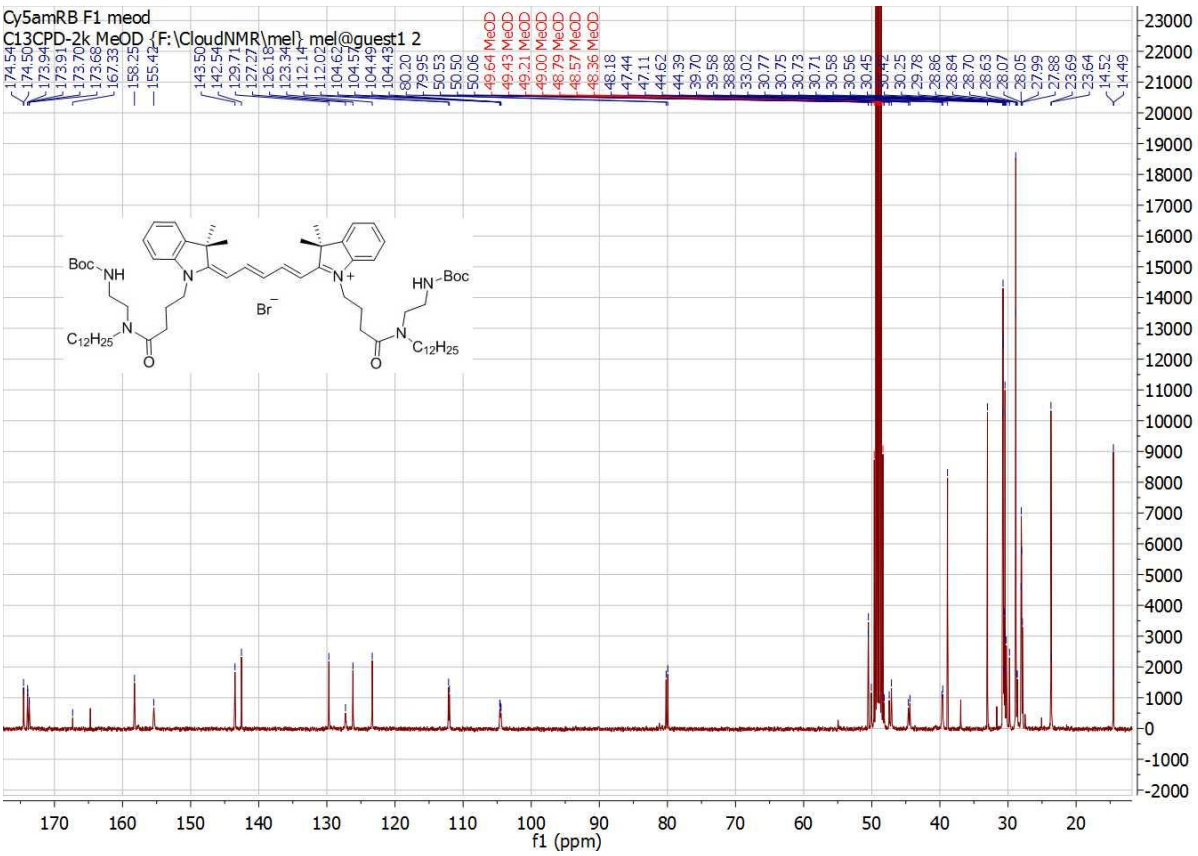


Figure S23. ^{13}C NMR spectrum of compound **5b**.

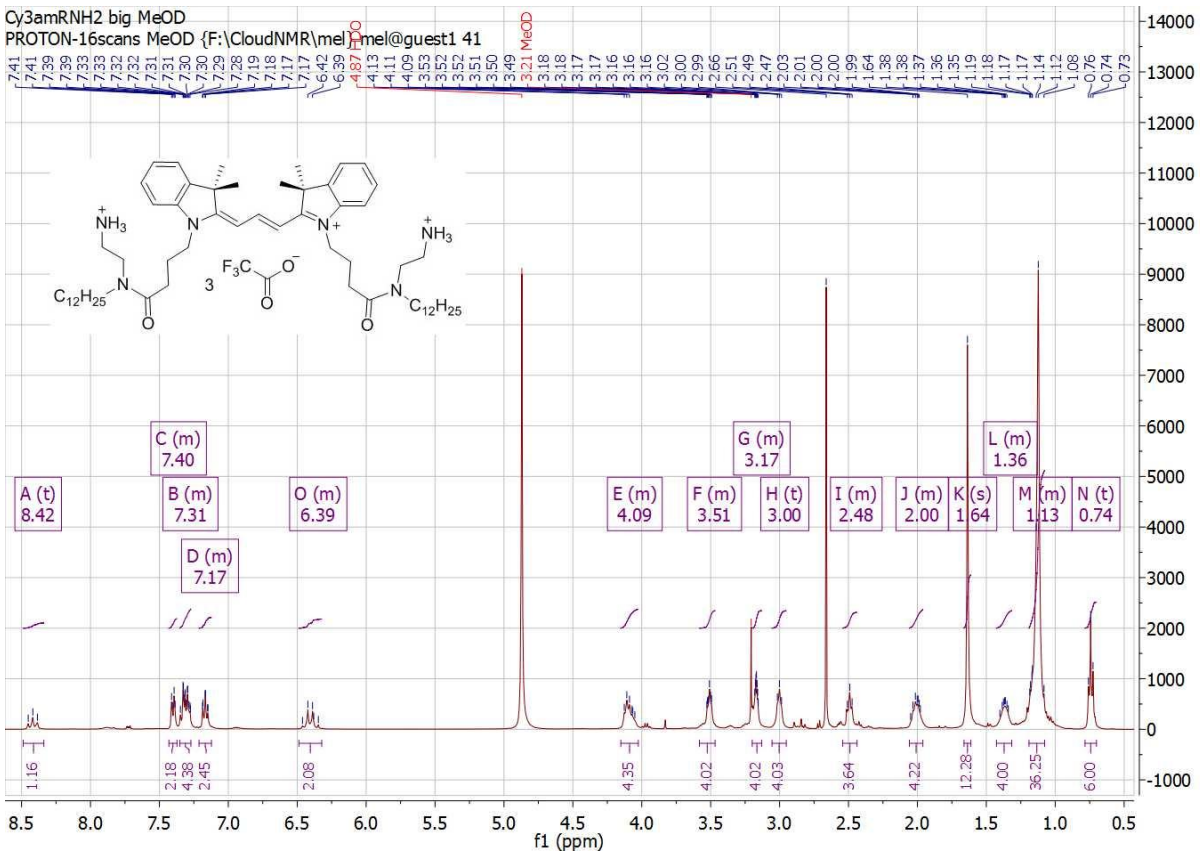


Figure S24. ^{1}H NMR spectrum of compound **6a**.

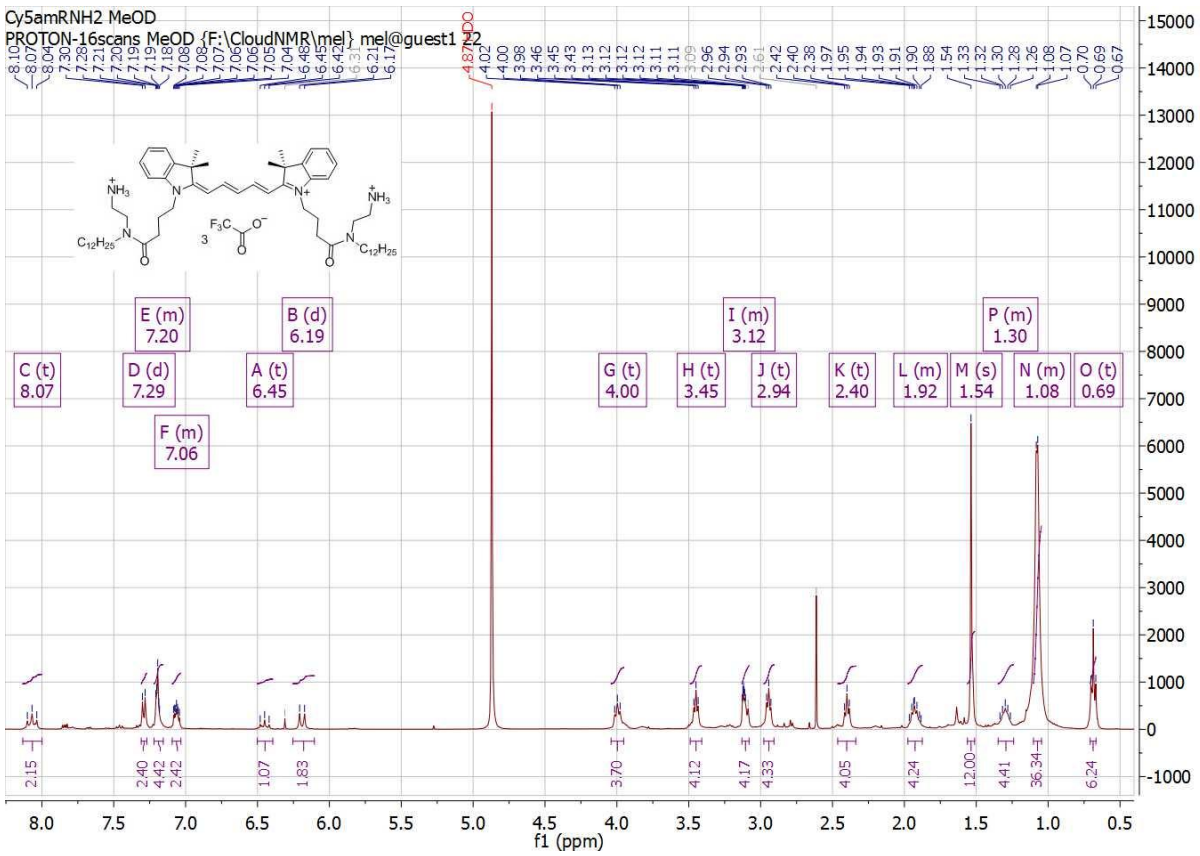


Figure S25. ^{1}H NMR spectrum of compound **6b**.

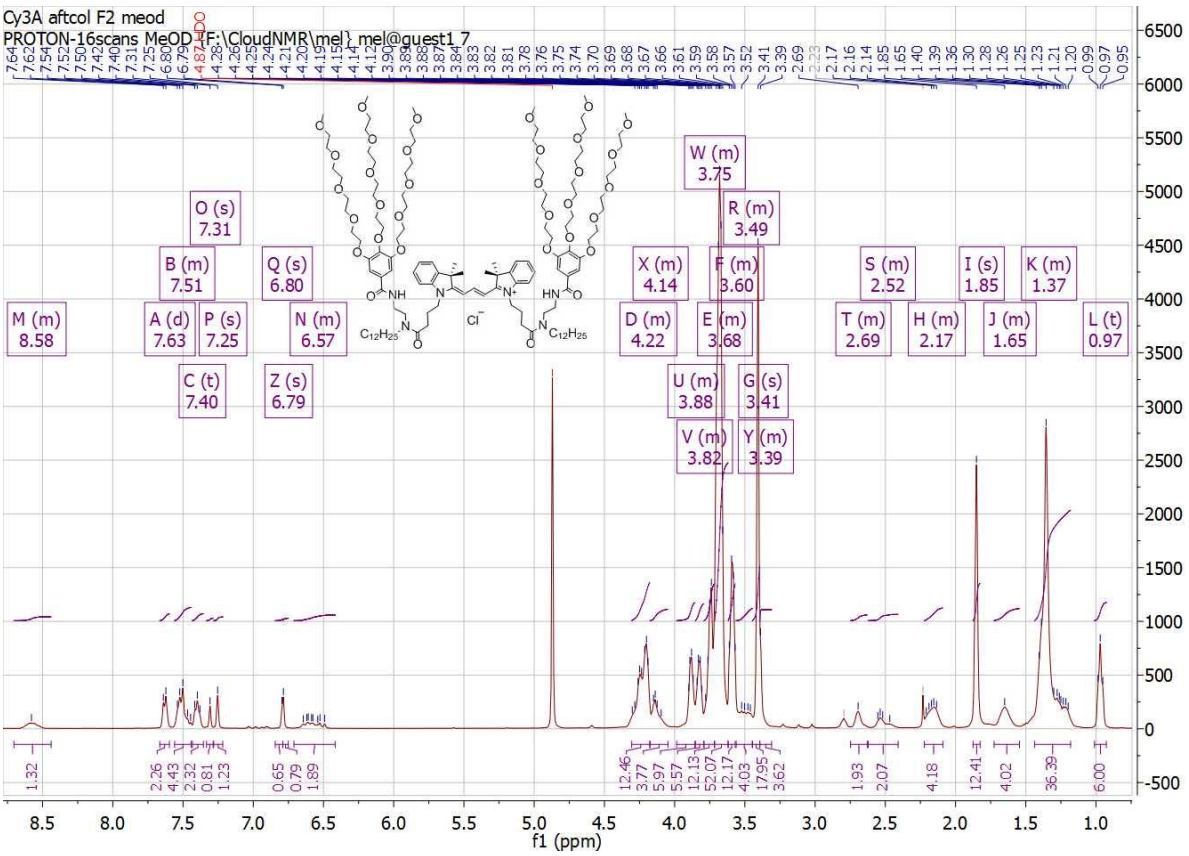


Figure S26. ^{1}H NMR spectrum of compound **Cy3A**. Partial aggregation of the dye may limit the resolution of this spectrum.

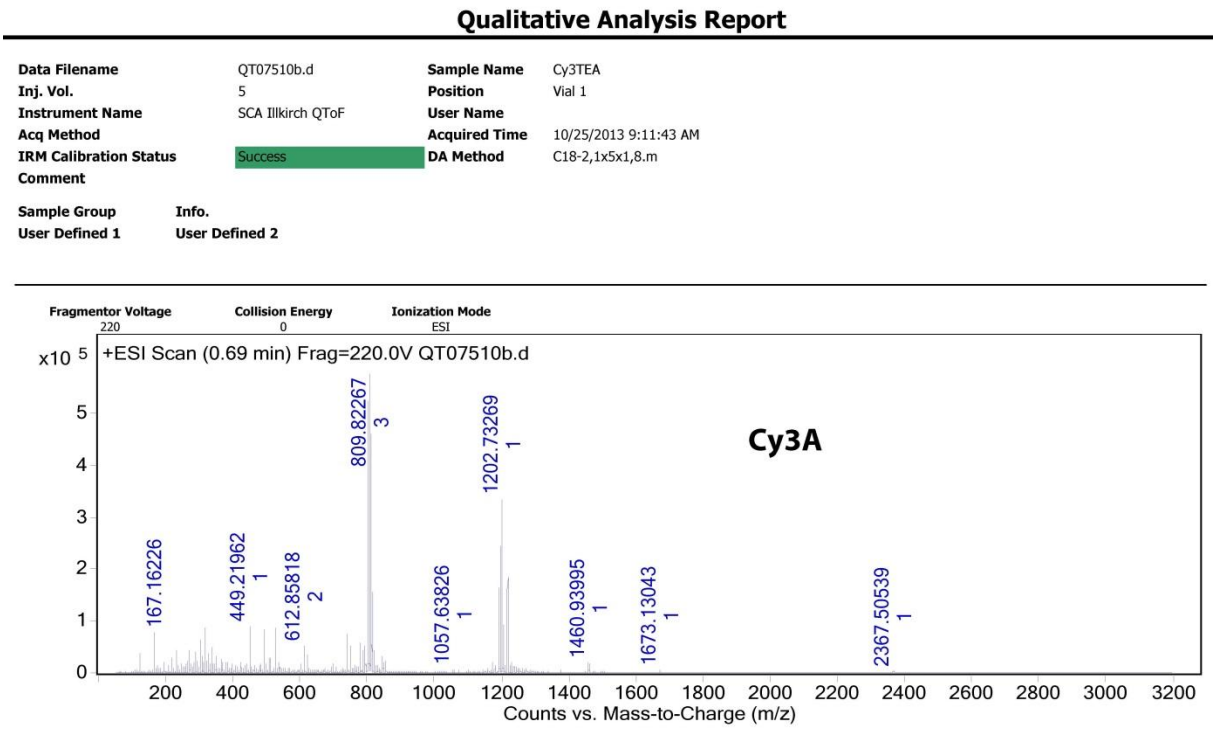


Figure S27. HRMS data of compound **Cy3A**.

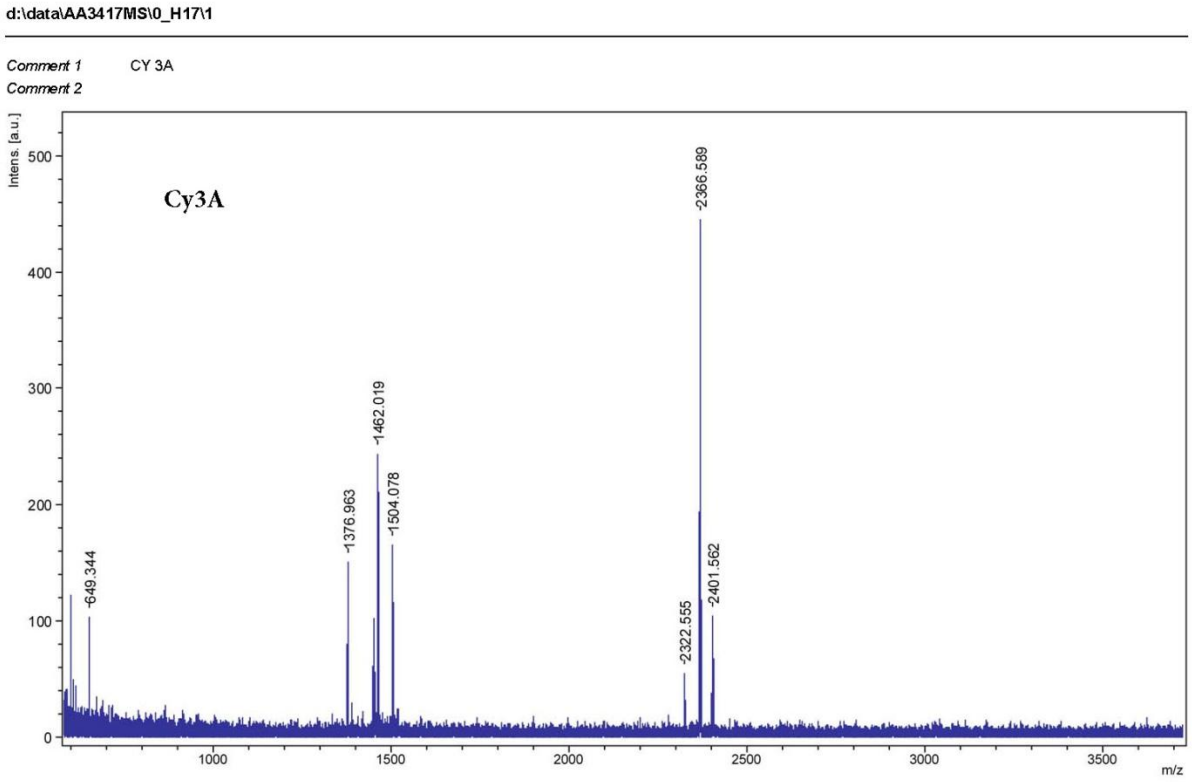


Figure S28. MALDI-TOF MS data of compound **Cy3A**.

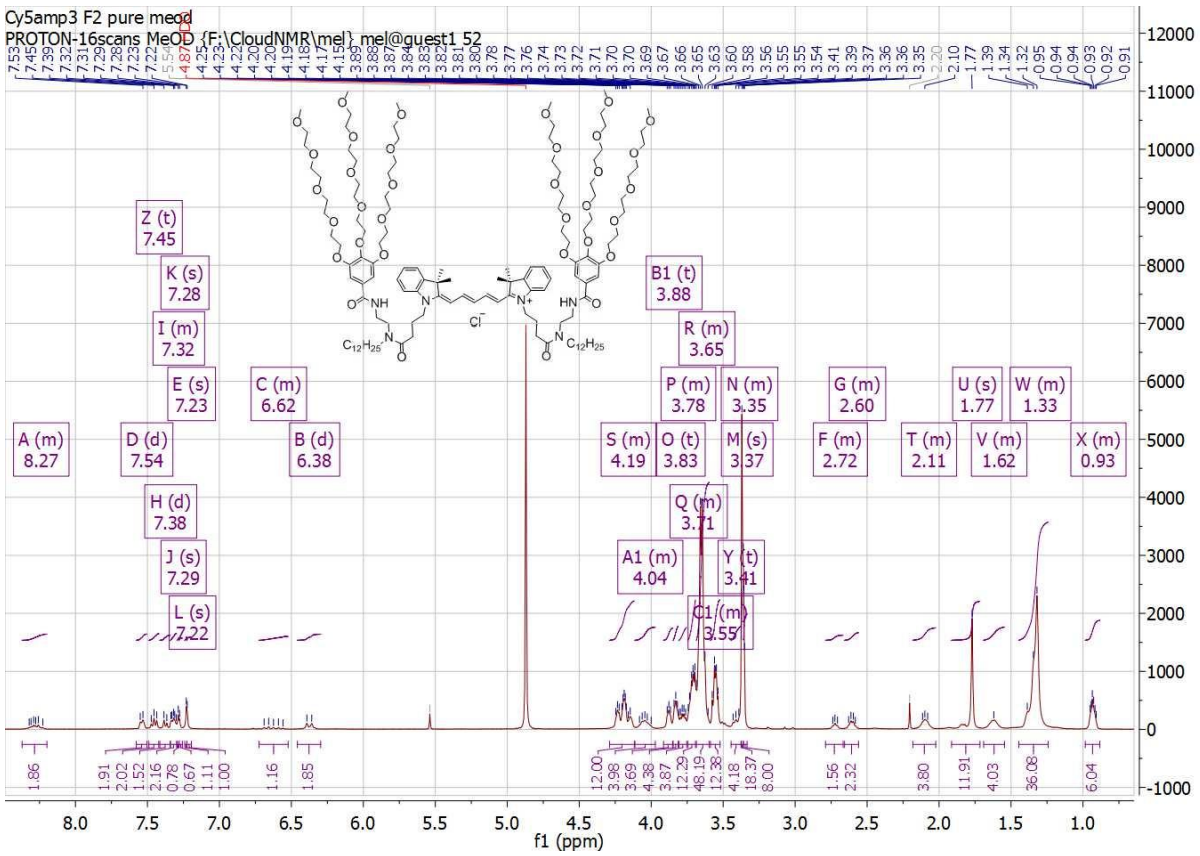


Figure S29. ^{1}H NMR spectrum of compound **Cy5A**. Partial aggregation of the dye may limit the resolution of this spectrum.

Qualitative Analysis Report

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Comment			

Sample Group	**Info.**
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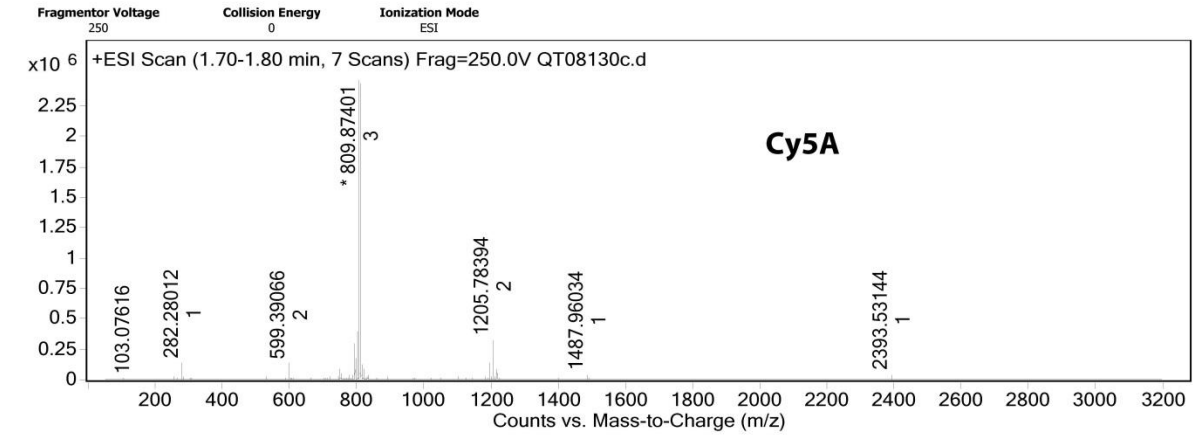

Figure S30. HRMS data of compound **Cy5A**.

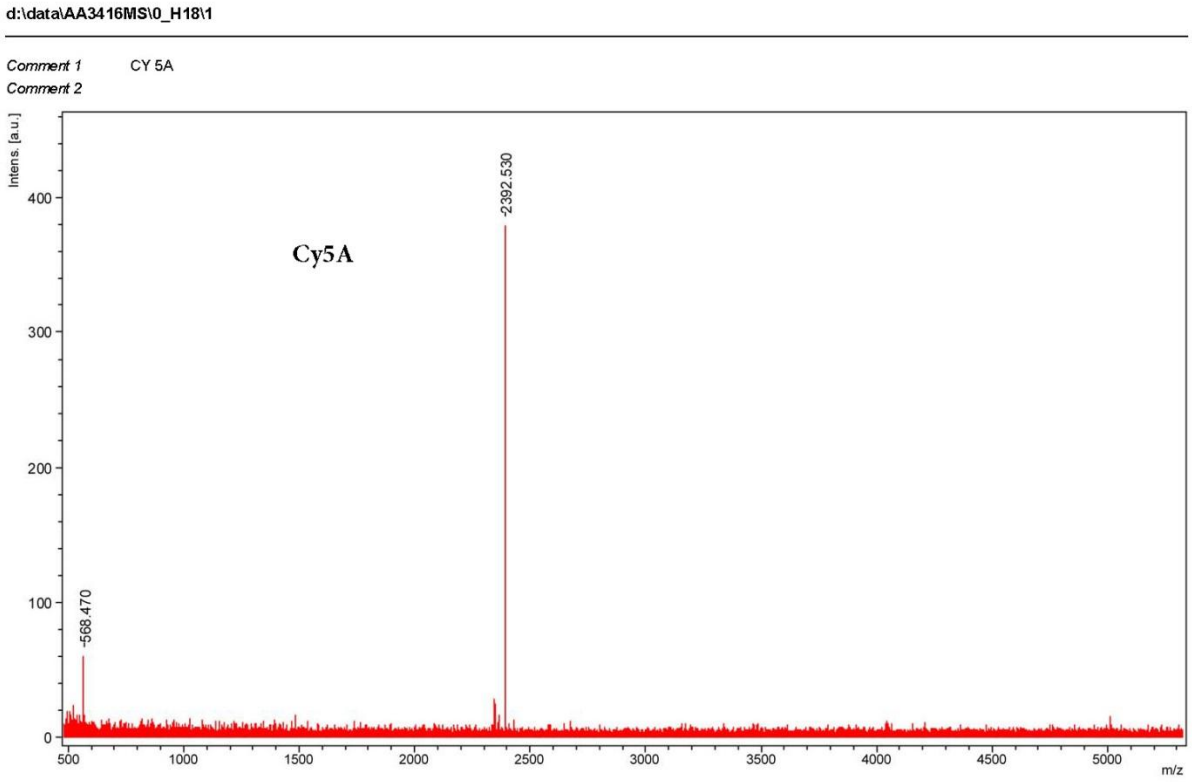

Figure S31. MALDI-TOF MS data of compound **Cy5A**.

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