SUPPORTING MATERIALS

QuatCy: A Quaternary Ammonium Heptamethine Cyanine Modification With Improved Characteristics

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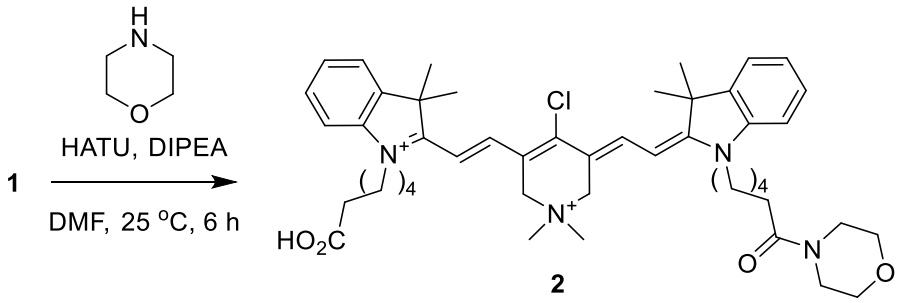
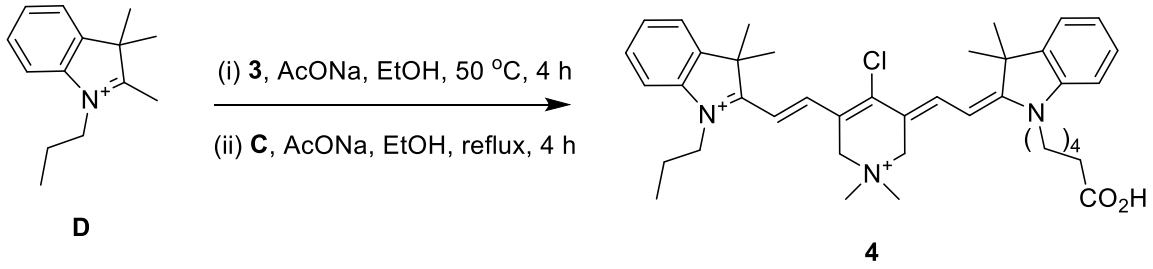
A. General Experimental Procedures

All reagents and compound **A** were purchased at a high commercial quality (typically 97 % or higher) and used without further purification. Compound **B** was synthesized according to the literature procedure with some modification.[1, 2] High field NMR spectra were recorded at normal temperature (298 K) with Bruker Avance III at 400 MHz for ^{1}H, and 100 MHz for ^{13}C and were calibrated using residual non-deuterated solvent as an internal reference ($CDCl_3$: ^{1}H NMR = 7.24, ^{13}C NMR = 77.0, MeOD: ^{1}H NMR = 3.30, ^{13}C NMR = 49.0, DMSO-d_6: ^{1}H NMR = 2.50, ^{13}C NMR = 39.5). The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, dd = double doublet, dt = double triplet, dq = double quartet, m = multiplet, br = broad. Electrospray ionization mass spectrometry (ESI-MS) data were collected on triple-stage quadrupole instrument in a positive mode. LC-MS analyses were collected from Agilent 1260 Infinity Quaternary LC and Agilent 6120 Quadrupole LC/MS modules using Poroshell 120 EC-C18 2.7 µM (4.6 x 50 mm) column in 5-95% CH_3CN/H_2O gradient with 0.1% formic acid over 10 minutes. Prep HPLC was performed on Agilent 1260 Infinity in 30-90% (compound **1** and **5**), 40-70% (compound **2**) and 30-80% (compound **4**) CH_3CN/H_2O gradient with 0.1% TFA over 20 mins. All statistical analyses were carried out by Graphpad Prism version 6.0 (Graphpad Software, La Jolla, CA, USA).

B. Synthetic Scheme



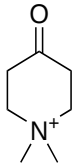
Scheme S1. Preparation of compound **2**.



Scheme S2. Preparation of compound **4**.

C. Synthetic Procedure and Characterizations

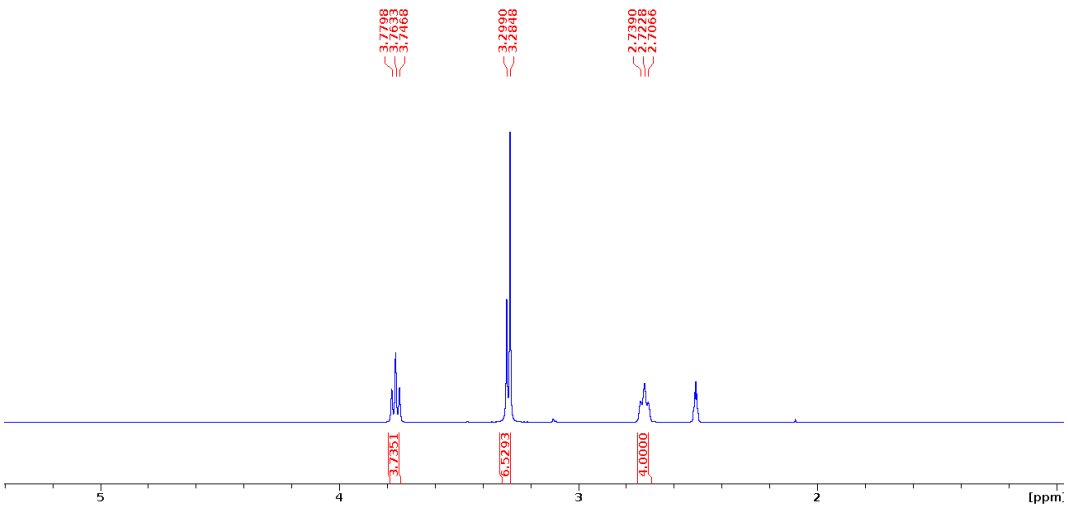
Synthesis of 1,1-dimethyl-4-oxopiperidin-1-ium iodide



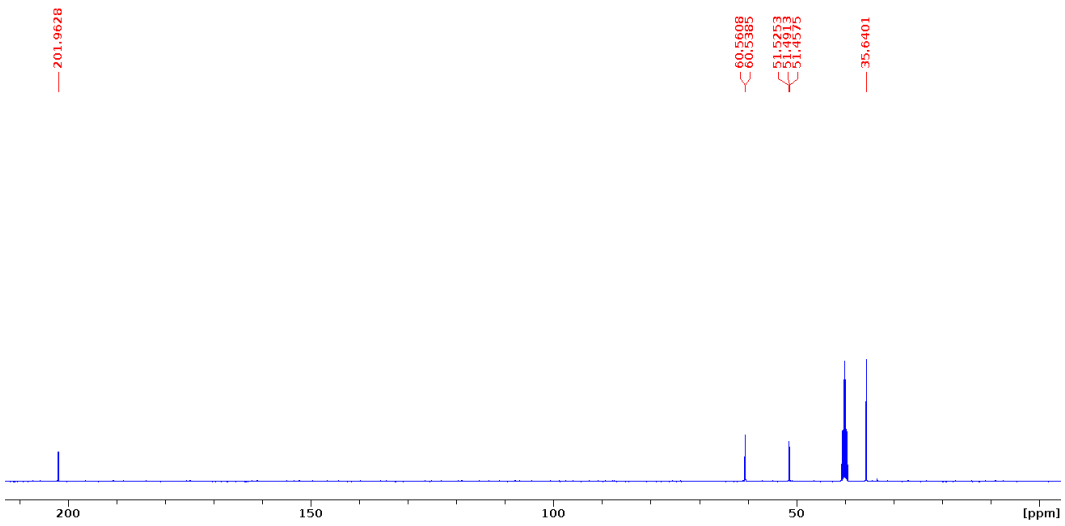
Ammonium salt compound was synthesized according to the previously reported procedure.[3] 1-methyl-4-piperidone (50.0 g, 0.44 mol) was dissolved in acetone (700 mL) and cooled down to 0 °C. Methyl iodide (60.0 mL, 0.96 mol) was slowly added into the solution over 10 minutes. Then, the mixture was stirred at room temperature for 4 h. Subsequently, the white precipitate was filtered and washed with cold acetone (200 mL) to give the white solid (110 g, 98% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ 2.72 (t, *J* = 6.5 Hz, 4H), 3.30 (s, 6H), 3.76 (t, *J* = 6.6 Hz, 4H); ^{13}C NMR (100 MHz, DMSO-d6) δ 202.0, 60.6 (2C), 51.5(3C), 35.6.

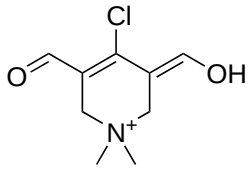
^{1}H NMR spectrum:



^{13}C NMR spectrum:



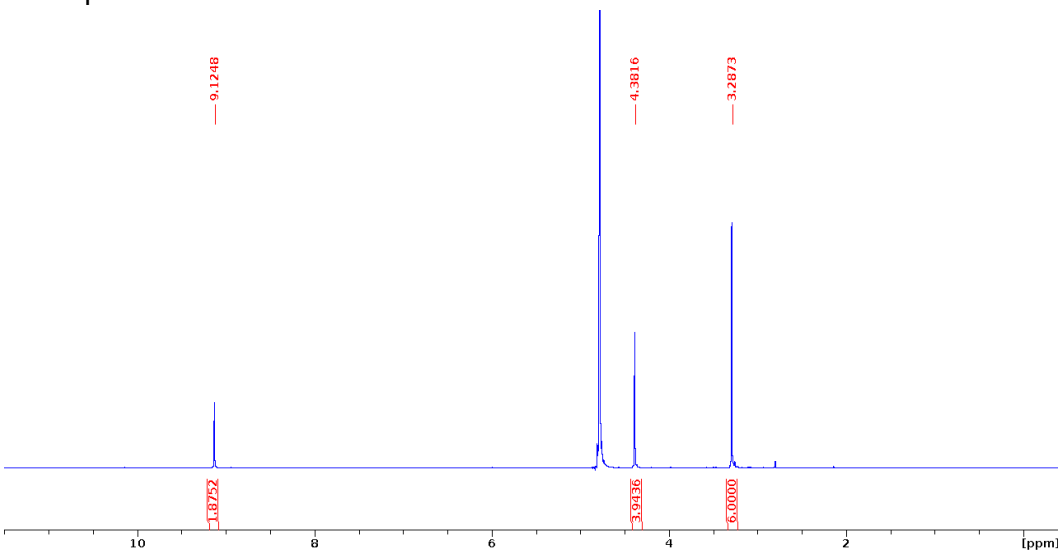
Synthesis of (*E*)-4-chloro-5-formyl-3-(hydroxymethylene)-1,1-dimethyl-1,2,3,6-tetrahydropyridin-1-ium (**3**)



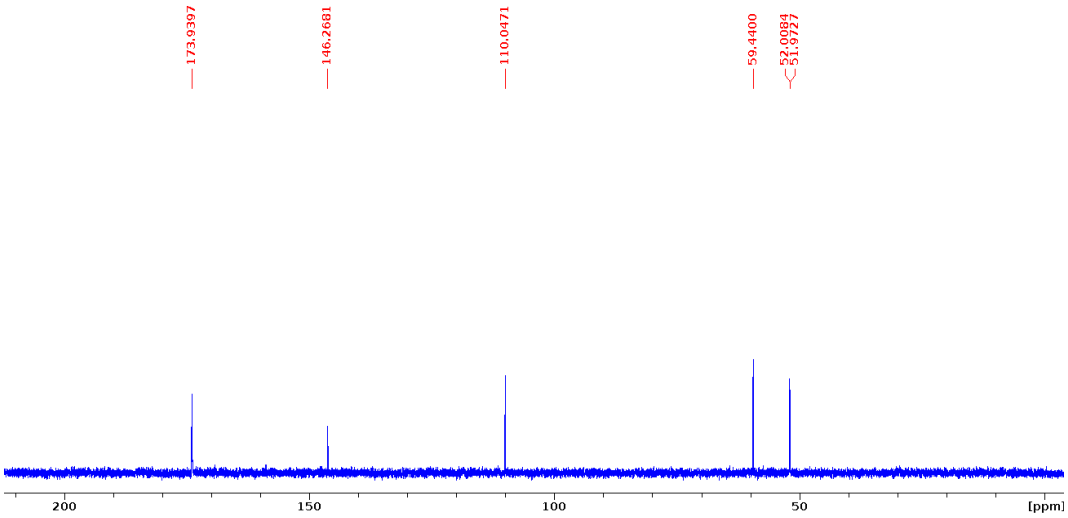
Compound **3** was prepared according to the literature method with some modifications.[1] DMF (9.00 mL, 0.12 mol) was cooled down at 0 ºC. Then, $POCl_3$ (5.50 mL, 0.06 mol) was slowly added and stirred at 0 ºC for 30 min. The 1,1-dimethyl-4-oxopiperidin-1-ium iodide (5.00 g, 0.02 mol) was added. The mixture solution was heated at 80 ºC for 3 h. After cooling down, the hydrochloric acid solution (20% v/v, 30.0 mL) was dropwise added and stirred at room temperature for 1 h. After that the mixture solution was sonicated for 20 min and allowed to stand in freezer overnight (-20 ºC). Consequently, the product was precipitated and filtered to obtain the dark yellow solid (1.45 g, 31% yield).

^{1}H NMR (400 MHz, D_2O) δ 9.12 (s, 2H), 4.38 (s, 4H), 3.29 (s, 6H); ^{13}C NMR (100 MHz, D_2O) δ 173.9, 146.3, 110.1, 59.4, 52.0, 52.0; HRMS (ESI) m/z for $C_9H_{13}ClNO_2^+$ calculated: 202.0629; found 202.0627.

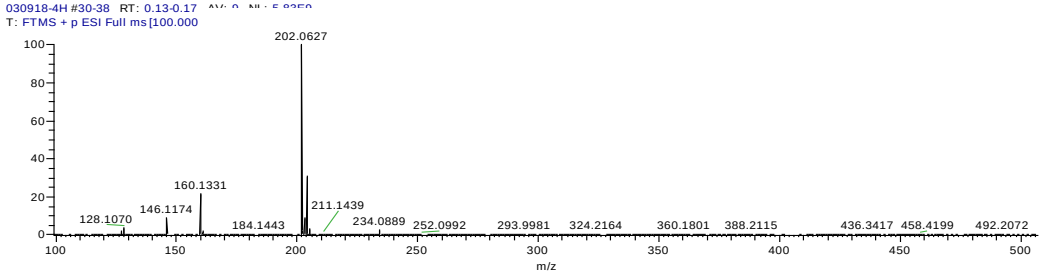
^{1}H NMR spectrum:



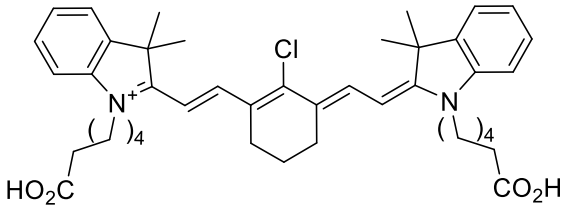
^{13}C NMR spectrum:



HRMS (ESI)



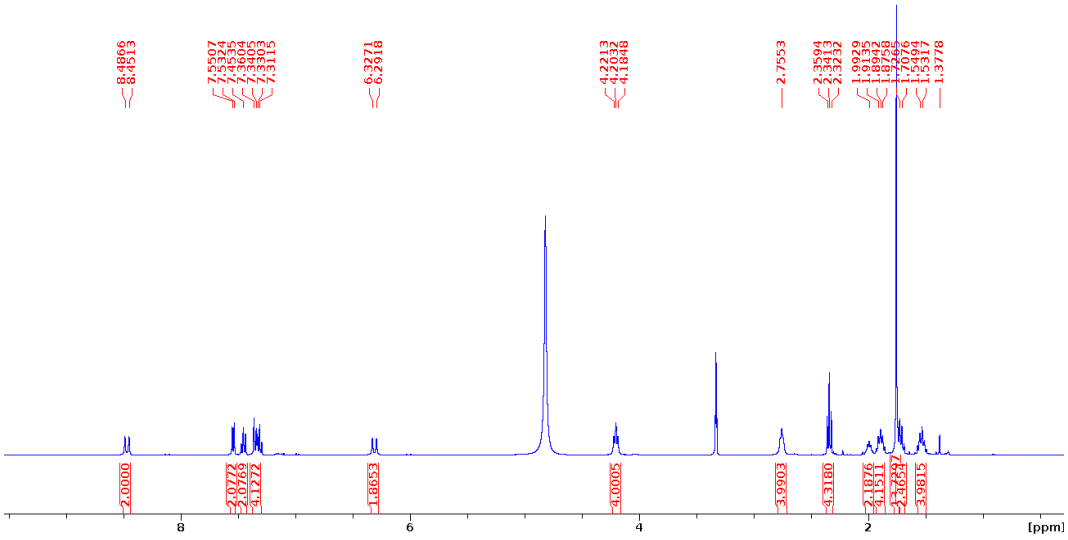
Synthesis of 1-(5-carboxypentyl)-2-((*E*)-2-((*E*)-3-(2-((*E*)-1-(5-carboxypentyl)-3,3-dimethylindolin-2-ylidene)ethylidene)-2-chlorocyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-3*H*-indol-1-ium (**B**)



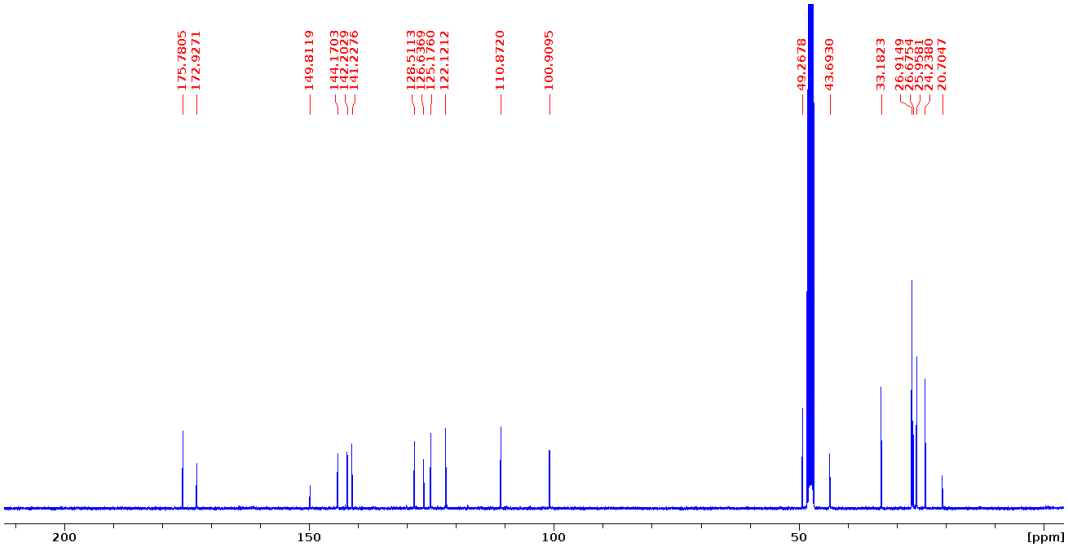
Compound **B** (1.5 g, 38%) was synthesized according to the literature procedure with some modification.[1, 2] The overall yield in three steps of **B** was 24%.

^{1}H NMR (400 MHz, MeOD) δ 8.47 (d, *J* = 14.1 Hz, 2H), 7.54 (d, *J* = 7.3 Hz, 2H), 7.48 – 7.42 (m, 2H), 7.37 – 7.28 (m, 4H), 6.31 (d, *J* = 14.1 Hz, 2H), 4.20 (t, *J* = 7.3 Hz, 3H), 2.75 (d, *J* = 5.5 Hz, 4H), 2.34 (t, *J* = 7.2 Hz, 4H), 2.04 – 1.96 (m, 2H), 1.95 – 1.84 (m, 4H), 1.76 (s, 12H), 1.74 – 1.68 (m, 3H), 1.59 – 1.49 (m, 4H); ^{13}C NMR (100 MHz, MeOD) δ 175.78, 172.93, 149.82, 144.17, 142.21, 141.23, 128.51, 126.64, 125.18, 122.12, 110.88, 100.91, 49.27, 43.70, 33.19, 26.92, 26.68, 25.96, 24.24, 20.71.

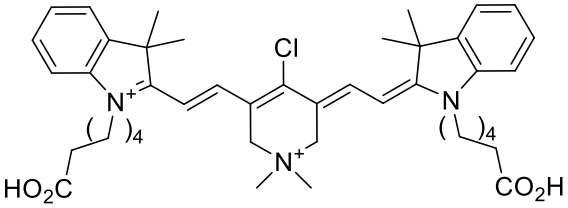
^{1}H NMR spectrum:



^{13}C NMR spectrum:



Synthesis of 1-(2-carboxyethyl)-2-((*E*)-2-((*E*)-5-(2-((*E*)-1-(2-carboxyethyl)-3,3-dimethylindolin-2-ylidene)ethylidene)-4-chloro-1,1-dimethyl-1,2,5,6-tetrahydropyridin-1-ium-3-yl)vinyl)-3,3-dimethyl-3*H*-indol-1-ium (**1**)

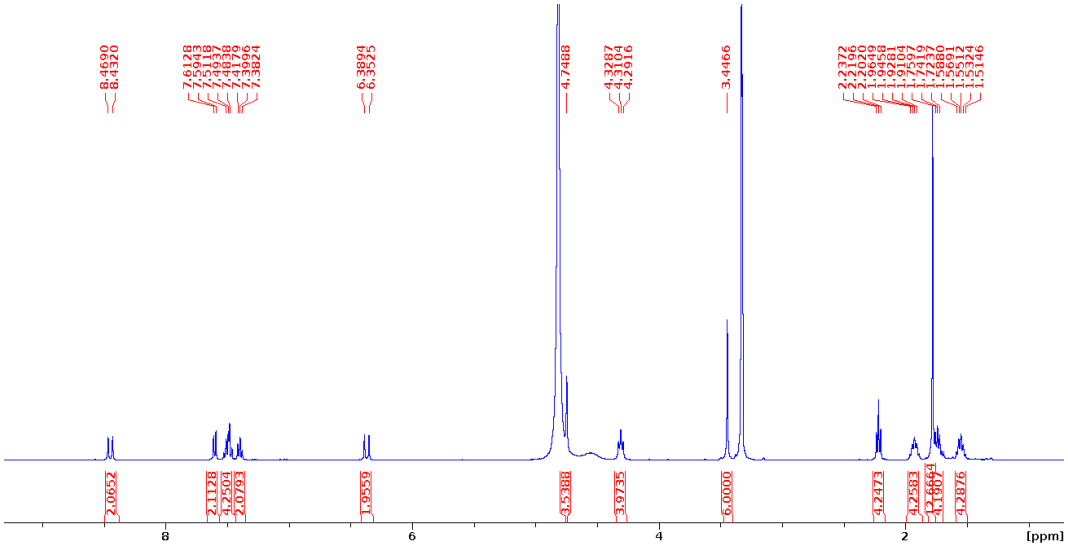


Compounds **C** (2.2 g, 6.23 mmol) which was synthesized according to the previously reported procedure[2] and sodium acetate (0.51 g, 6.22 mmol) were dissolved in absolute ethanol (100 mL) and stirred at room temperature for 30 min. Compound **3** (0.70 g, 2.94 mmol) was added to the solution. The solution was refluxed for 5 h. The solvent was removed by using a rotovap. The crude material was purified by preparative reverse-phase HPLC (30-90% CH_3CN/H_2O containing 0.1% TFA). The purified product was lyophilized to yield the green solid (1.60 g, 76% yield). The overall yield of **1** in three steps was 23%.

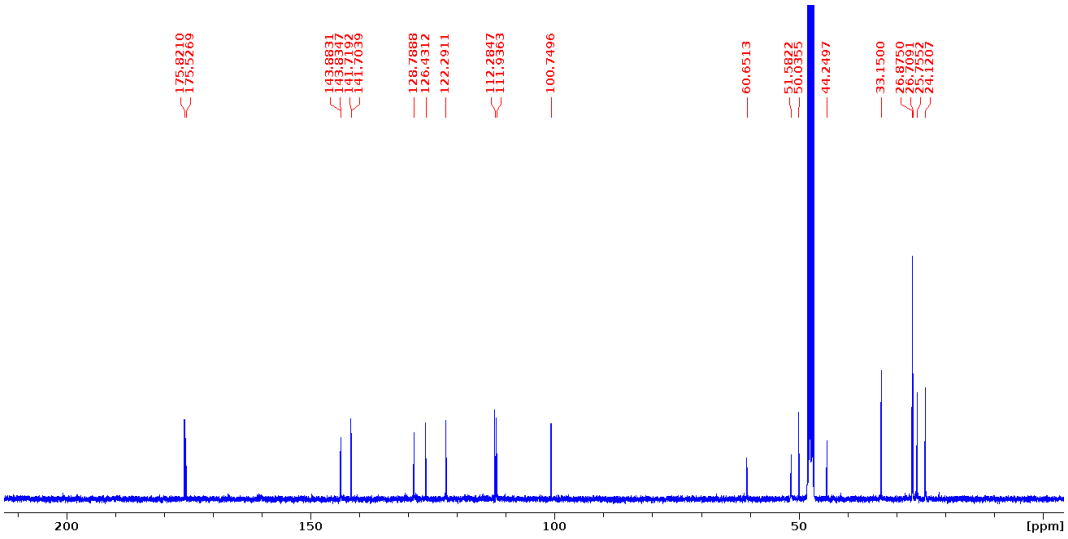
^{1}H NMR (400 MHz, MeOD) δ 8.45 (d, *J* = 14.8 Hz, 2H), 7.60 (d, *J* = 7.4 Hz, 2H), 7.51-7.48 (m, 4H), 7.40 (t, *J* = 7.1 Hz, 2H), 6.37 (d, *J* = 14.8 Hz, 2H), 4.75 (s, 4H), 4.31 (t, *J* = 7.4 Hz, 4H), 3.45 (s, 6H), 2.22 (t, *J* = 7.0 Hz, 4H), 1.96-1.91 (m, 4H), 1.78 (s, 12H), 1.76-1.72 (m, 4H), 1.59-1.51 (m, 4H); ^{13}C NMR (100 MHz, MeOD) δ 175.8, 175.5, 143.9, 143.8, 141.7 (2C), 128.8, 126.4, 122.3, 112.3, 111.9, 100.7, 60.7, 51.6, 50.0, 44.2, 33.2,

26.9, 26.7, 25.8, 24.1; HRMS (ESI) m/z for $C_{43}H_{56}ClN_3O_4^{2+}$ calculated: 356.6974; found 356.6968.

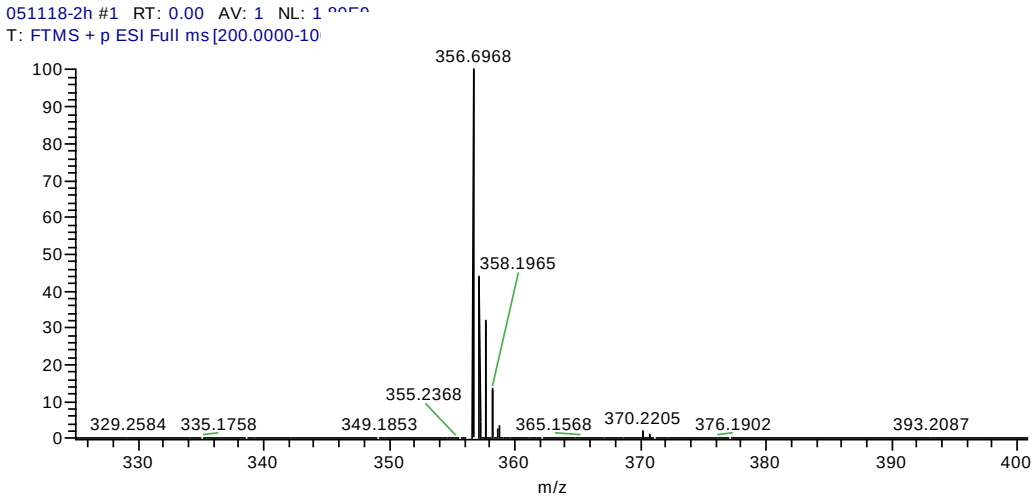
^{1}H NMR spectrum:



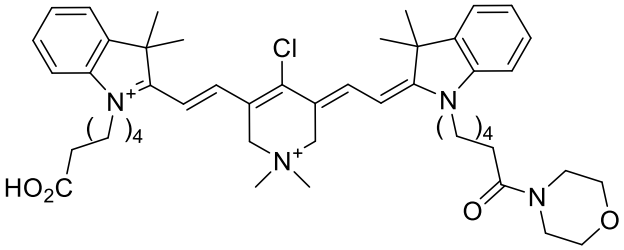
^{13}C NMR spectrum:



HRMS (ESI)



Synthesis of 1-(5-carboxypentyl)-2-((*E*)-2-((*E*)-4-chloro-5-(2-((*E*)-3,3-dimethyl-1-(6-morpholino-6-oxohexyl)indolin-2-ylidene)ethylidene)-1,1-dimethyl-1,2,5,6-tetrahydropyridin-1-ium-3-yl)vinyl)-3,3-dimethyl-3*H*-indol-1-ium (**2**)

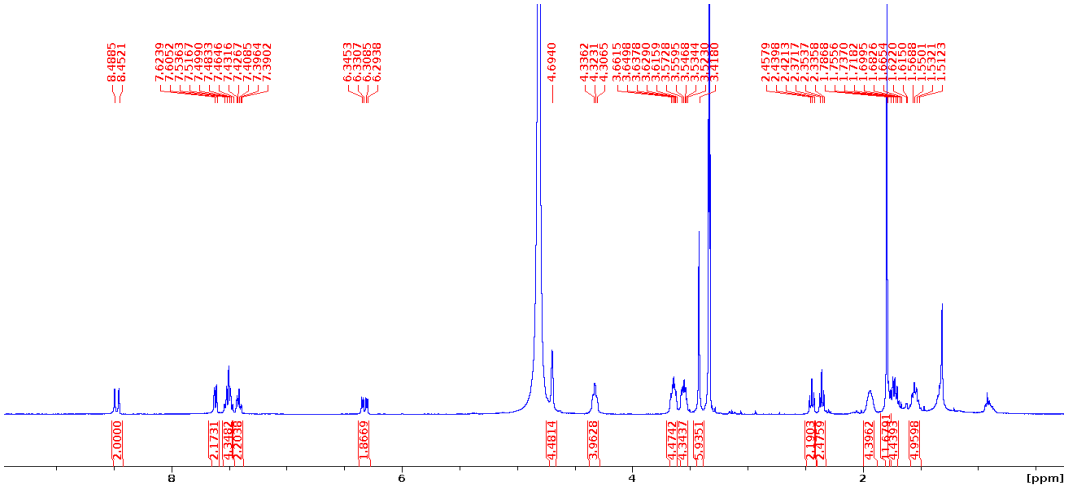


Compound **1** (50.0 mg, 0.053 mmol), DIPEA (8.00 µL, 0.046 mmol) and HATU (24.0 mg, 0.063 mmol) were added in 1 mL DMF and stirred for 30 min. Morpholine (4.50 µL, 0.051 mmol) was added afterwards and stirred for 6 h under argon environment. Solvent was removed and the crude was purified by preparative reverse-phase HPLC (40-70% CH_3CN/H_2O containing 0.1% TFA) to get the desired product as green solid (6 mg, 11% yield).

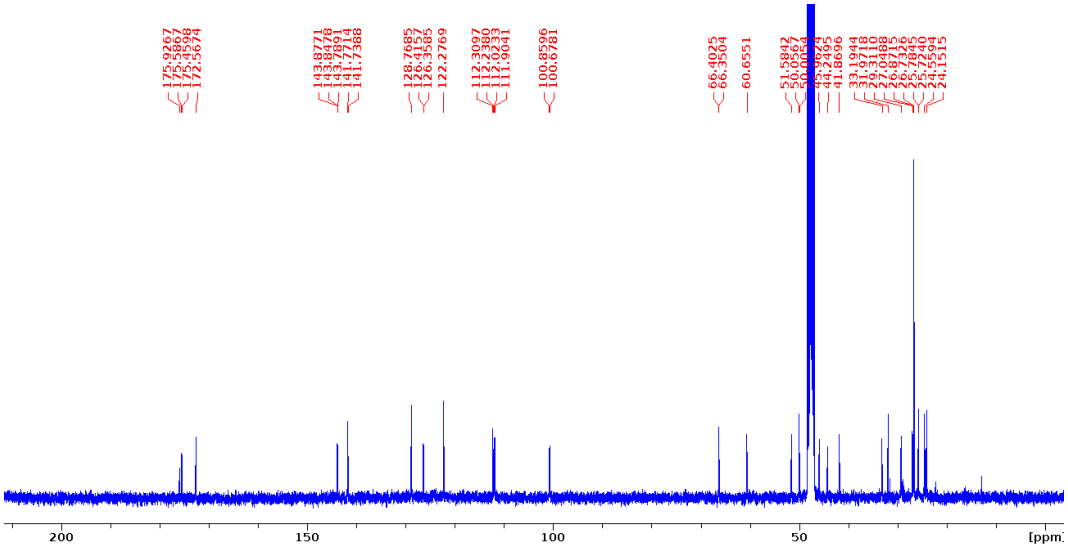
^{1}H NMR (400 MHz, MeOD) δ 8.47 (d, *J* = 14.6 Hz, 2H), 7.61 (d, *J* = 7.5 Hz, 2H), 7.53 – 7.46 (m, 4H), 7.43 – 7.39 (m, 2H), 6.32 (dd, *J* = 14.8, 5.9 Hz, 2H), 4.69 (s, 4H), 4.32 (t, *J* = 5.9 Hz, 4H), 3.66 – 3.62 (m, 4H), 3.78 – 3.44 (m, 4H), 3.42 (s, 6H), 2.44 (t, *J* = 7.3 Hz, 2H), 2.35 (t, *J* = 7.2 Hz, 2H), 1.84 (bs, 4H), 1.79 (s, 12H), 1.75 – 1.67 (m, 4H), 1.57 – 1.51 (m, 4H); ^{13}C NMR (100 MHz, MeOD) δ 175.9, 175.6, 175.5, 172.6, 143.9 (2C), 143.8, 141.8, 141.7, 128.8, 126.4 (2C), 122.3, 112.3, 112.2, 112.0, 111.9, 100.9, 100.7, 66.4 (2C), 60.7, 51.6, 50.1, 50.0, 46.0, 44.2, 41.9, 33.2, 32.0, 29.3, 27.1, 26.9, 26.7,

25.8, 25.7, 24.6, 24.2; HRMS (ESI) m/z for $C_{47}H_{63}ClN_4O_4^{2+}$ calculated: 391.2263; found 391.2260.

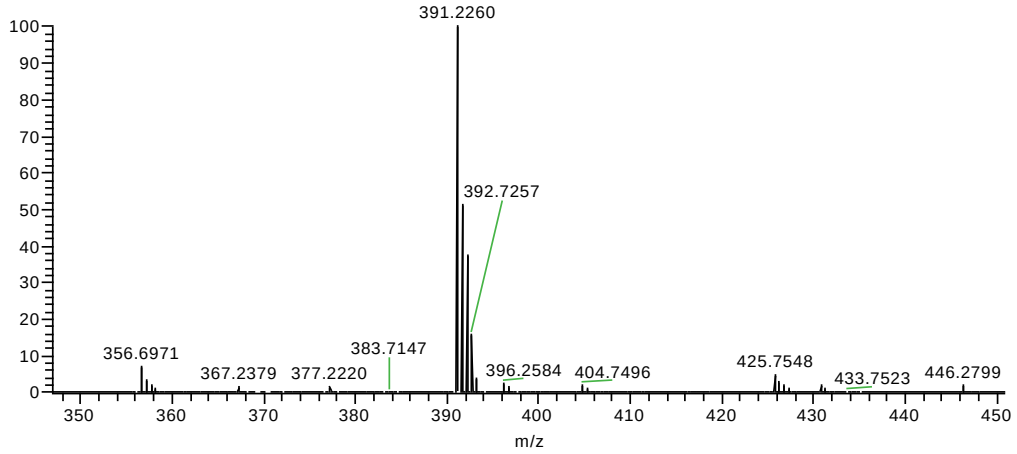
^{1}H NMR spectrum:



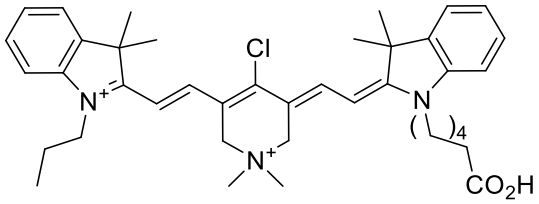
^{13}C NMR spectrum:



HRMS (ESI)



Synthesis of 2-((*E*)-2-((*E*)-5-(2-((*E*)-1-(5-carboxypentyl)-3,3-dimethylindolin-2-ylidene)ethylidene)-4-chloro-1,1-dimethyl-1,2,5,6-tetrahydropyridin-1-ium-3-yl)vinyl)-3,3-dimethyl-1-propyl-3*H*-indol-1-ium (**4**)

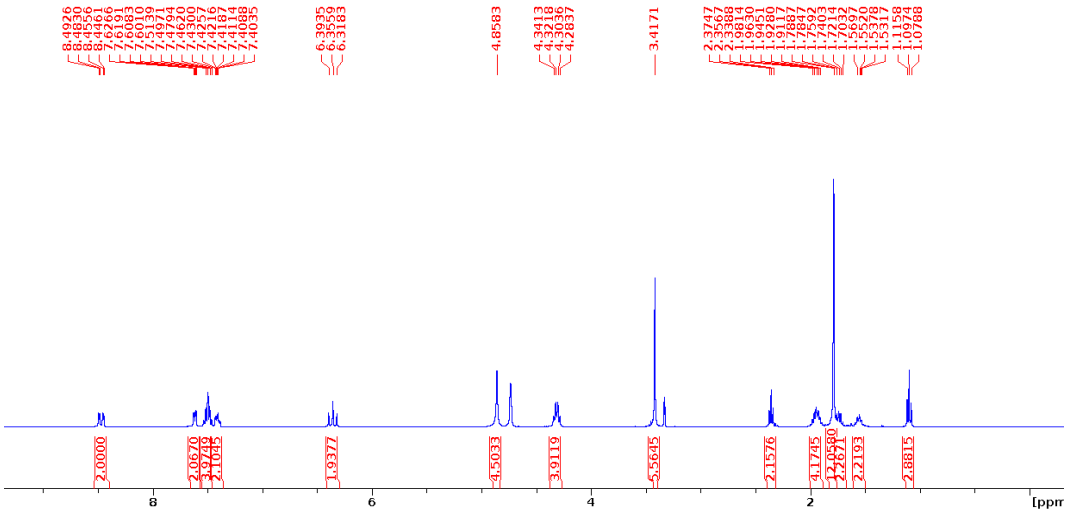


Compounds **D** (0.20 g, 0.99 mmol) which was synthesized according to the previously reported procedure[4] and sodium acetate (50.0 mg, 0.61 mmol) were dissolved in absolute ethanol (20 mL) and stirred at room temperature for 15 min. Compound **3** (0.22 g, 0.67 mmol) was added to the solution. The solution was heat at 50 ^{0}C for 4 h. The solvent was removed by using a rotovap and lyophilizer. The crude material was used next step without purification. Compounds **C** (0.18 g, 0.66 mmol) and sodium acetate (45.0 mg, 0.55 mmol) were dissolved in absolute ethanol (10 mL). The crude material was added into the mixture and refluxed for 4 h. The solvent was removed by using a rotovap and purified by preparative reverse-phase HPLC (30-80% CH_3CN/H_2O containing 0.1% TFA). The purified product was lyophilized to yield the green solid (75 mg, 17% yield).

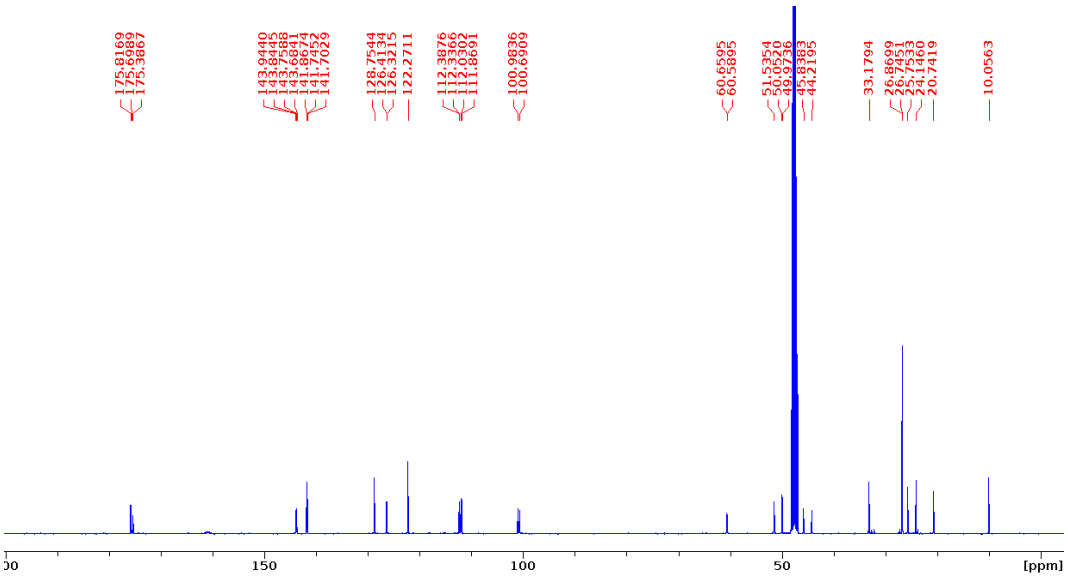
^{1}H NMR (400 MHz, MeOD) δ 8.45 (d, *J* = 14.8 Hz, 2H), 7.98 (d, *J* = 1.5 Hz, 2H), 7.85 (dd, *J* = 8.4, 1.6 Hz, 2H), 7.28 (d, *J* = 8.4 Hz, 2H), 6.32 (d, *J* = 14.7 Hz, 2H), 4.72 (s, 4H), 4.28 (t, *J* = 7.4 Hz, 4H), 3.41 (s, 6H), 2.36 (t, *J* = 7.2 Hz, 4H), 1.93 –1.80 (m, 4H), 7.43 – 7.40 (m, 2H), 6.36 (t, *J* = 15.0 Hz, 2H), 4.86 (s, 4H), 4.31 (q, *J* = 7.7 Hz, 4H), 3.42 (s,

6H), 2.36 (t, *J* = 7.2 Hz, 2H), 1.98-1.76 (m, 4H), 1.74 (s, 12H), 1.72-1.70 (m, 2H), 1.57-1.53 (m, 2H), 1.10 (t, *J* = 7.4 Hz, 3H; ^{13}C NMR (100 MHz, MeOD) δ 175.8, 175.7, 175.4, 143.9 (2C), 143.8, 143.7, 141.9, 141.7 (2C), 128.8, 126.4, 126.3, 122.3, 112.4, 112.3, 112.0, 111.9, 101.0, 100.7, 60.7, 60.6, 51.5, 50.1, 50.0, 45.8, 44.2, 33.2, 26.9, 26.7, 25.8, 24.1, 20.7, 10.1; HRMS (ESI) m/z for $C_{40}H_{52}ClN_3O_2^{2+}$ calculated: 321.1885; found 321.1892.

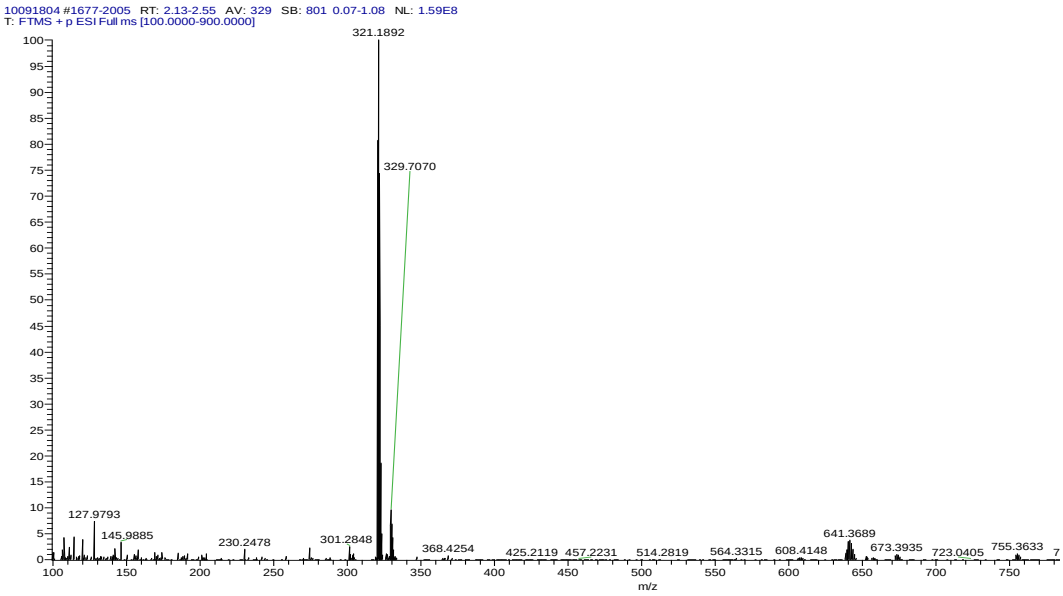
^{1}H NMR spectrum:



^{13}C NMR spectrum:



HRMS (ESI)



D. General Procedure For X-Ray Structure Determination

A suitable crystal was selected and mounted on a Kapton loop and transferred to a Bruker Micro-Source CMOS diffractometer. The crystal was kept at 100.0 K during data collection. Employing Olex2[5], the structure was solved with the ShelXT[6] structure solution program using Intrinsic Phasing and refined with the XL[7] refinement package using Least Squares minimization. Disorder in both Trifluoro acetic acid (TFA) molecules resulted in a poorly modeled anions, which in turn resulted in a high wR2 and R value. Also, one of the two hexanoic acid terminal arms was disordered between two positions. It is possible that the disorder hexanoic acid would lead to a deprotonated carboxyl group, this in turn would reduce the number of TFA anions per cation from two to one, with possible inclusion of solvent (Figure S1). The crystal may also be a mixture of the di and mono cations. Exclusion of the TFA (SQUEEZE) results in a void of 245A^3 containing approximately 107 electrons, which is equal to two TFA molecules.

E. Table and Figures

Table S1. Comparative photophysical properties of **A**, **B** and **1**

compound	solvent	λ_{abs} (nm)	λ_{em} (nm)	$\Delta\lambda^a$	ε_{max} ($cm^{-1}M^{-1}$)
A	H_2O	779	811	32	172560
	MeOH	783	817	34	237100
B	H_2O	775	795	20	57540
	MeOH	781	807	26	232260
1	H_2O	743	765	22	152000
	MeOH	753	781	28	233680

[a]Stokes' shifts of **A**, **B** and **1**.

Table S2. Calculated structural properties i.e. logP, logD, Polar surface area and hydrogen bond donors and acceptors were calculated using ChemAxon's Marvin and JChem plugins. These values were calculated at pH 7.4.

Compound	logP	logD	polarizibility	polar surface area (Å)	hydrogen bond donor/acceptor
1	1.10	2.64	80.36	87.71	1 / 4
B	6.57	3.63	77.05	87.71	1 / 4
A	5.26	9.15	86.30	121.85	1 / 6

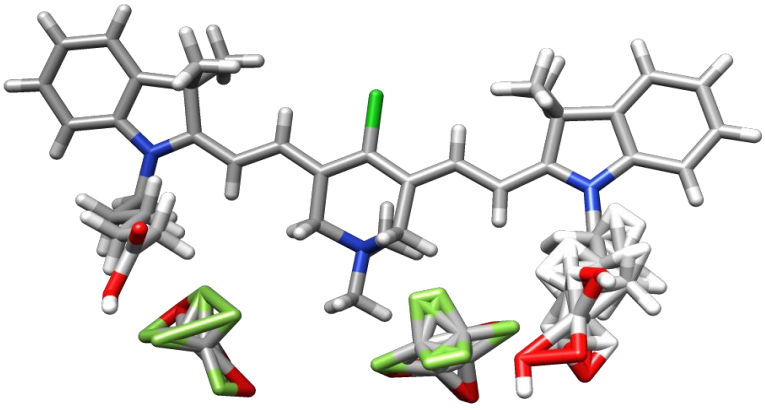
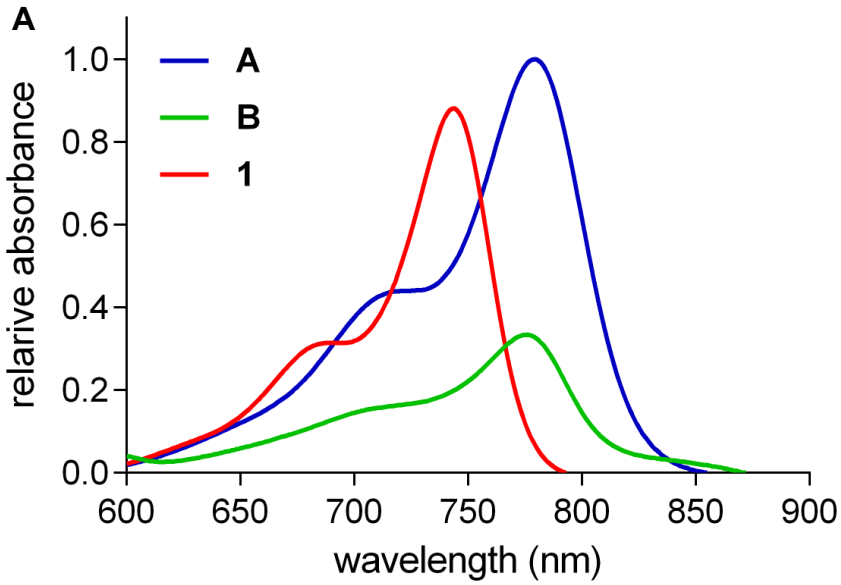
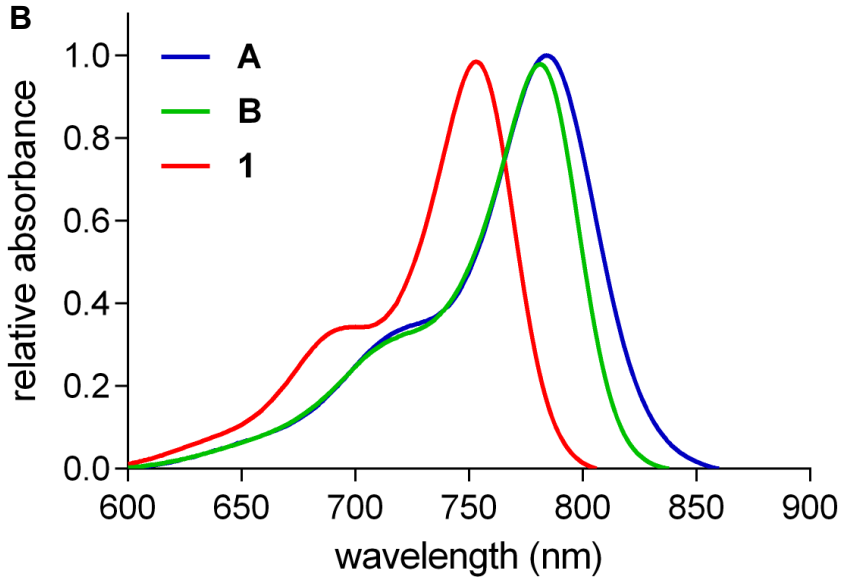


Figure S1. Representation of **1** from coordinates collected in a single crystal X-ray analysis.


A
relarive absorbance
1.0
0.8
0.6
0.4
0.2
0.0
A
B
1
600
650
700
750
800
850
900
wavelength (nm)


B
relative absorbance
1.0
0.8
0.6
0.4
0.2
0.0
A
B
1
600
650
700
750
800
850
900
wavelength (nm)

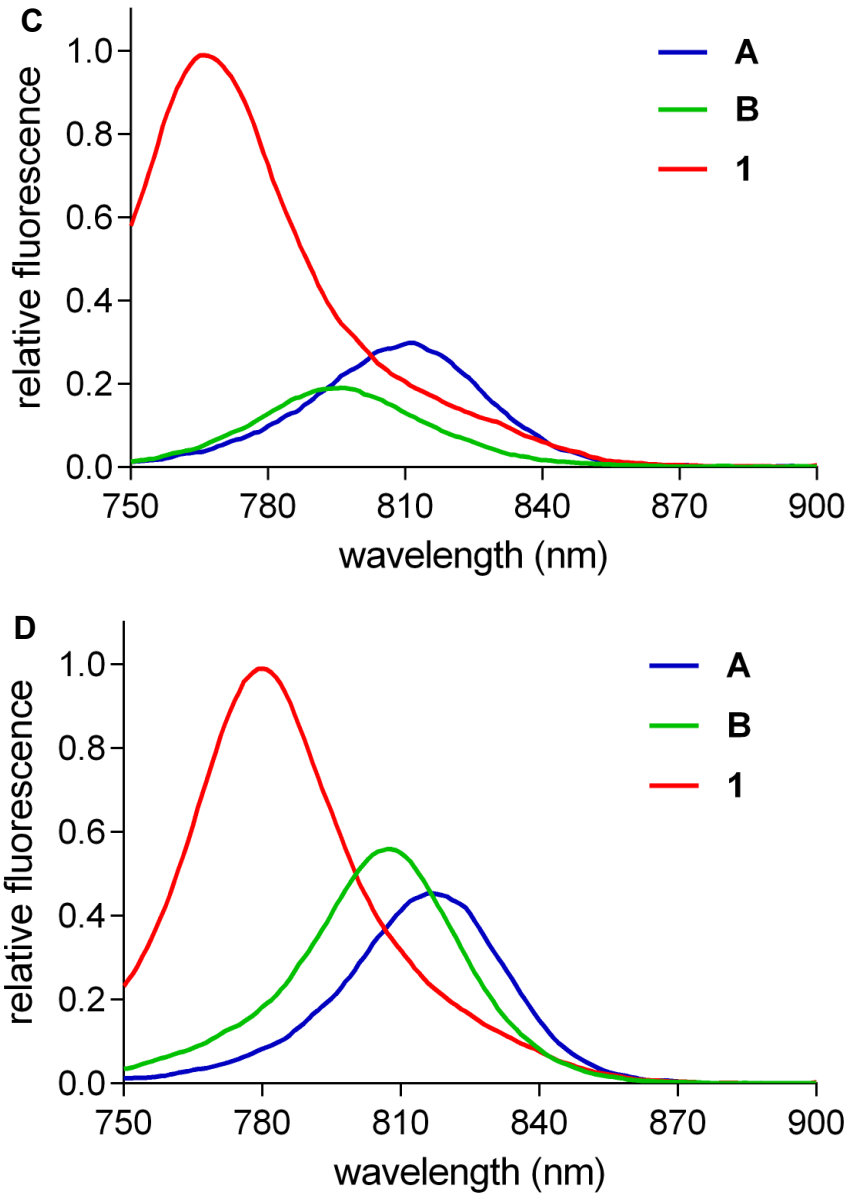


Figure S2. Relative absorbance of **A**, **B** and **1** (5 µM) in (A), H_2O; (B) MeOH and relative fluorescence in (C) H_2O; (D) MeOH.

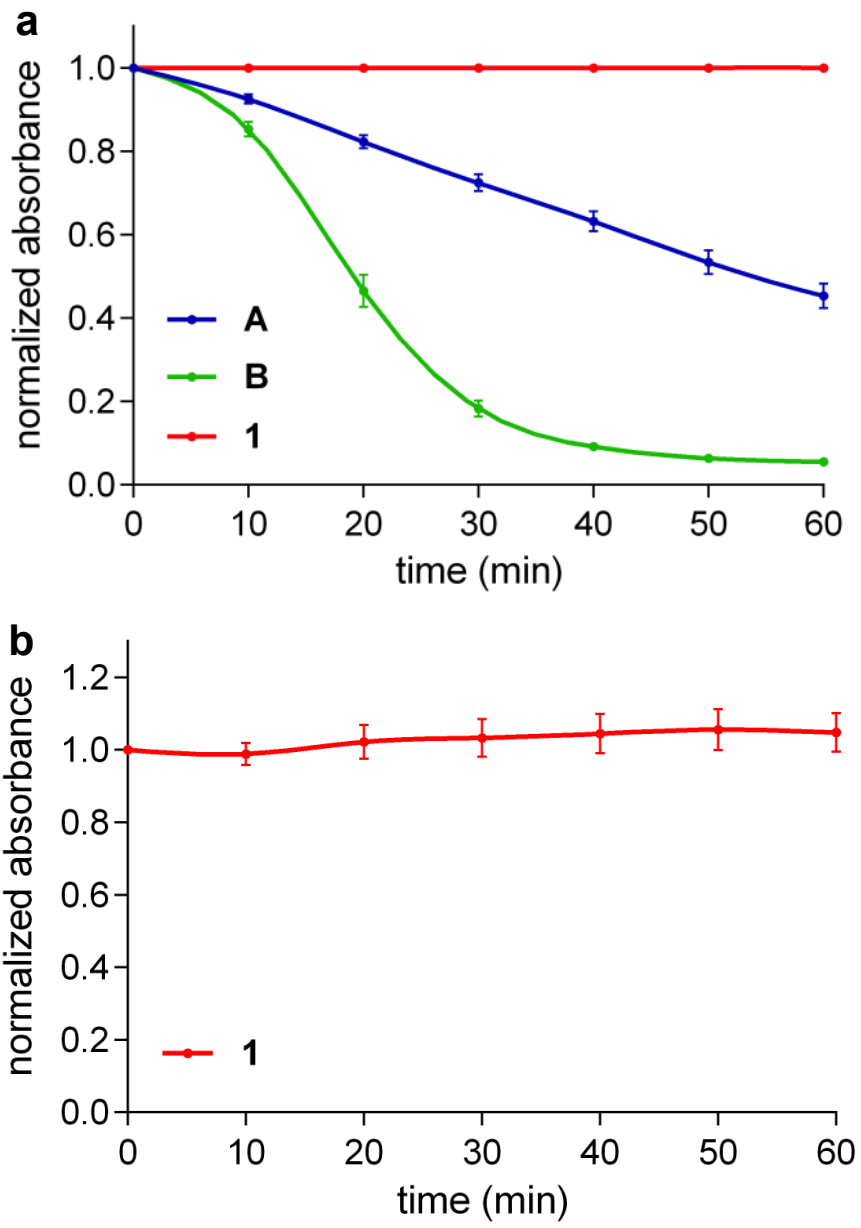


Figure S3. Photostabilities in 10 mM pH 7.4 PBS of a) **A**, **B** and **1** (20 μM) at 780 nm (Thor Lab, LED780E); b) **1** (20 μM) at 750 nm (Thor Lab, LED750L). Compound **1** was the most photostable whereas **B** was the least stable and decomposed. (n = 3, mean ± SD)

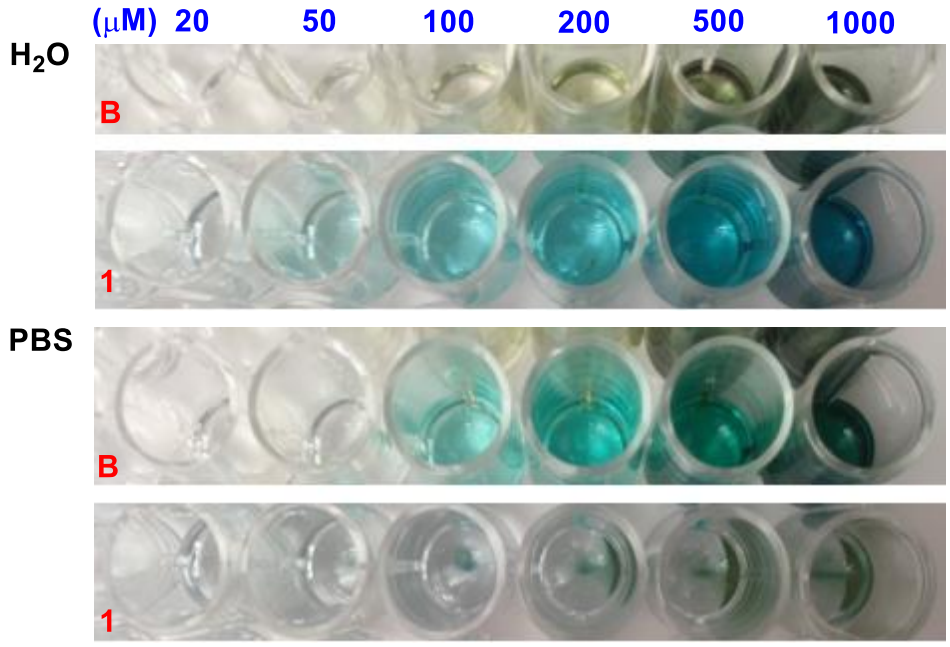


Figure S4. Solubility in 24 well-plate (at 25 °C) of **B** and **1** in H_2O and 10 mM pH 7.4 PBS buffer.

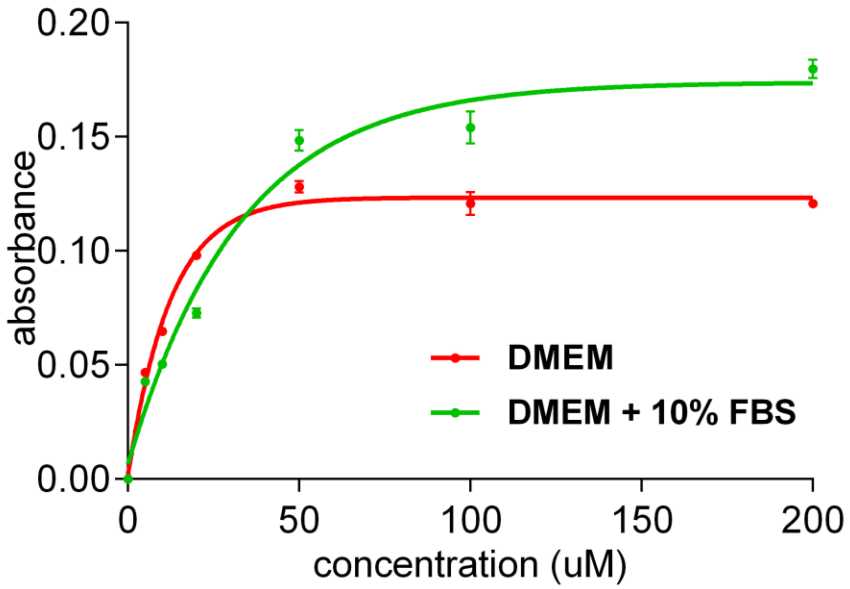


Figure S5. Solubility (at 25 °C) of QuatCy **1** in DMEM (Dulbecco's Modified Eagle's medium) and DMEM mixed with 10% FBS (Fetal bovine serum). QuatCy proved more soluble in DMEM mixed with 10% FBS. (n = 3, mean ± SD)

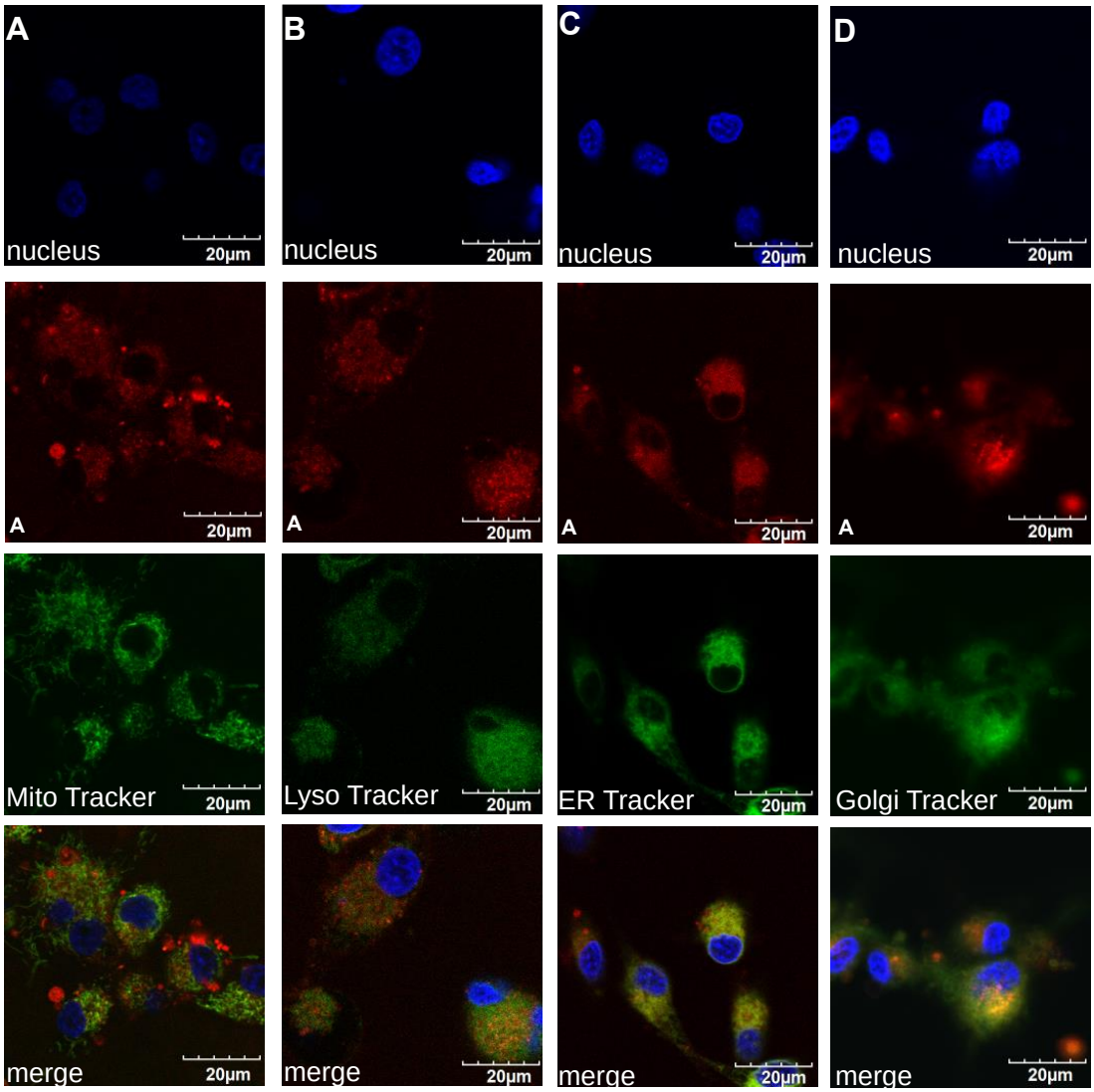


Figure S6. Localization of compound **A** (20 μM) after 30 min incubation at 37 °C with U87-MG cells (A) Mitochondria (B) Lysosome (C) ER Tracker and (D) Golgi in DMEM/F12 supplemented by 10% FBS pH 7.4.

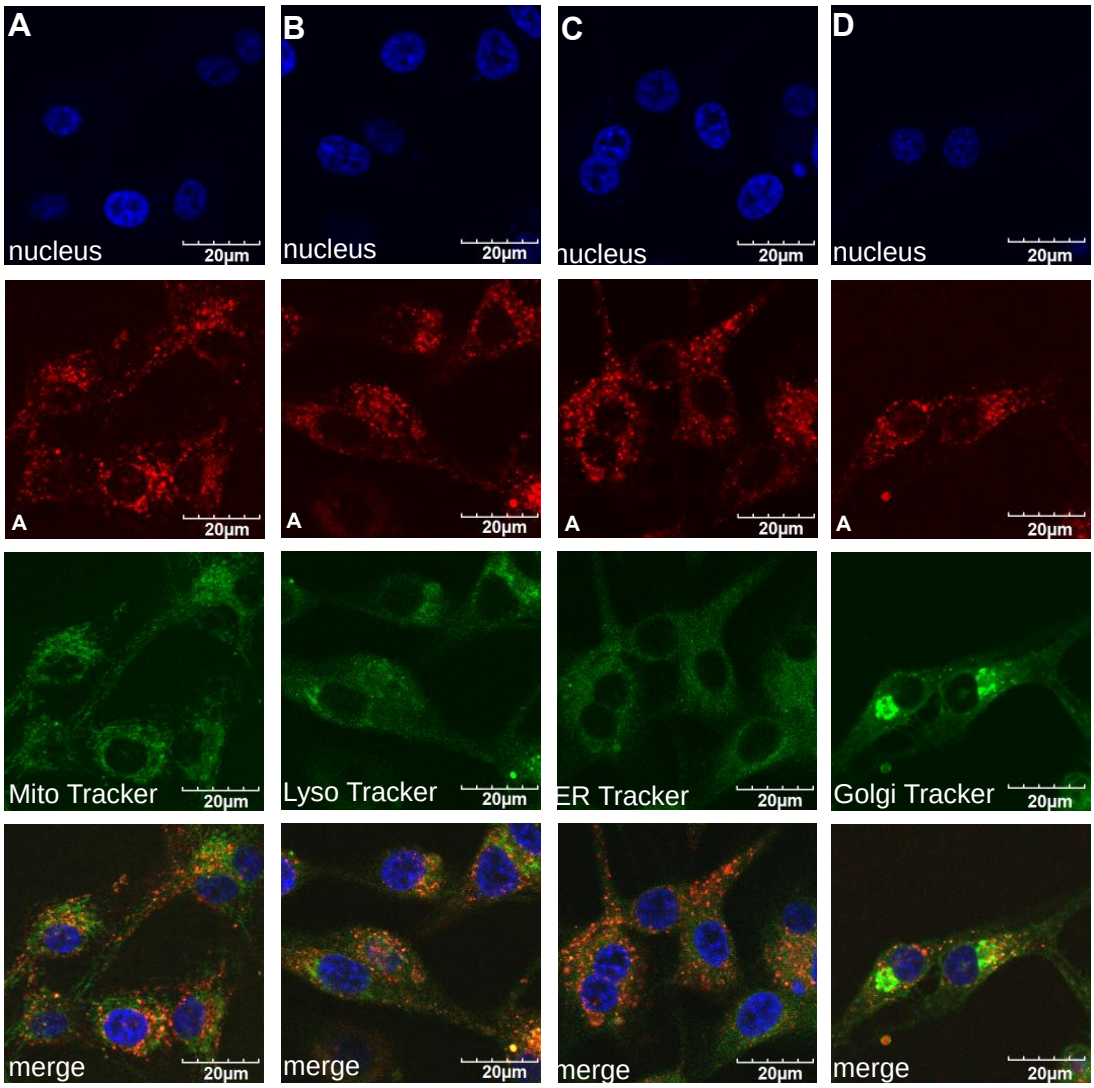


Figure S7. Localization of compound **A** (20 μM) after 24 h incubation at 37 °C with U87-MG cells(A) Mitochondria (B) Lysosome (C) ER Tracker and (D) Golgi in DMEM/F12 supplemented by 10% FBS pH 7.4.

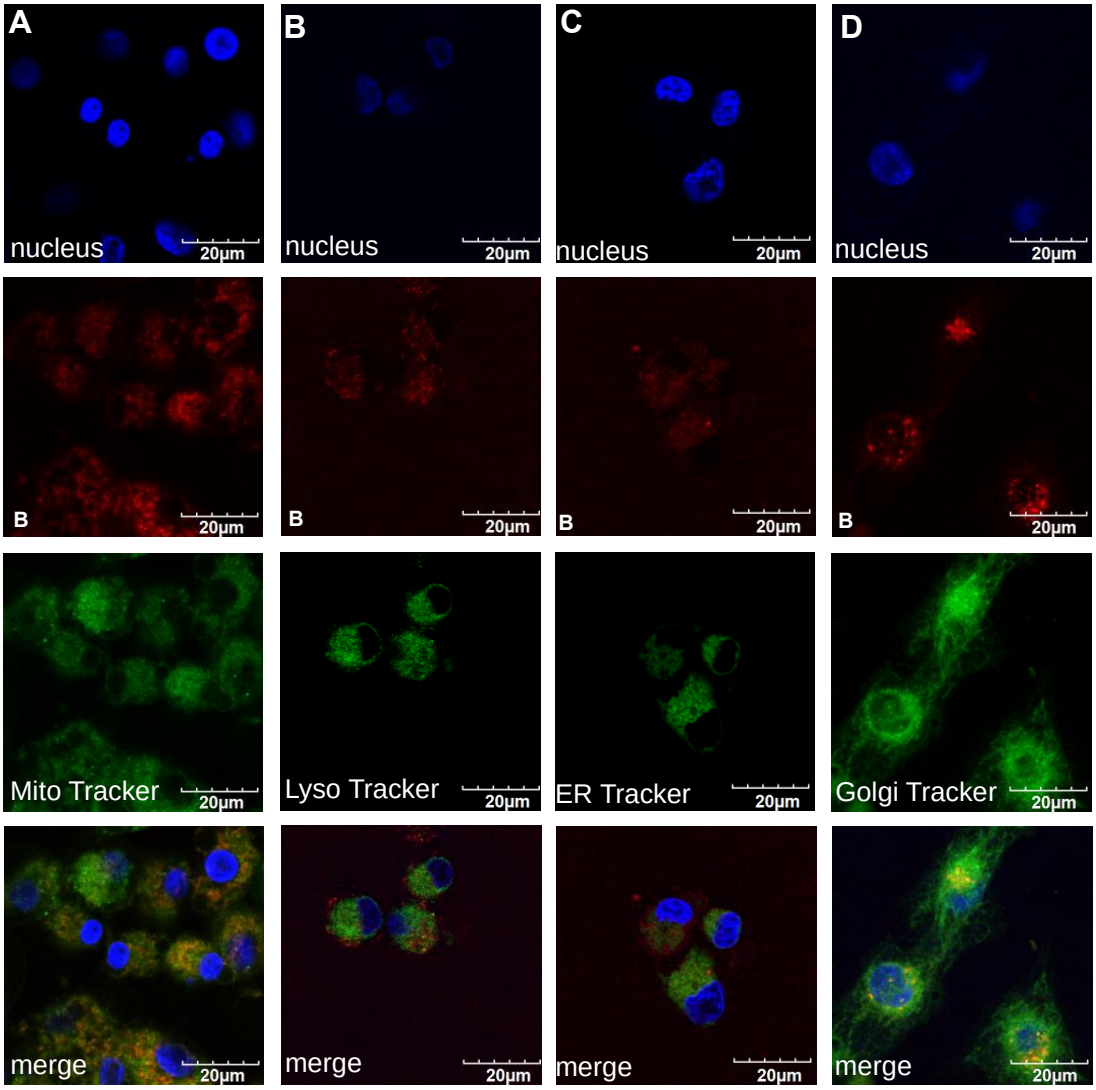


Figure S8. Localization of compound **B** (20 μM) after 30 min incubation at 37 °C with U87-MG cells (A) Mitochondria (B) Lysosome (C) ER Tracker and (D) Golgi in DMEM/F12 supplemented by 10% FBS pH 7.4.

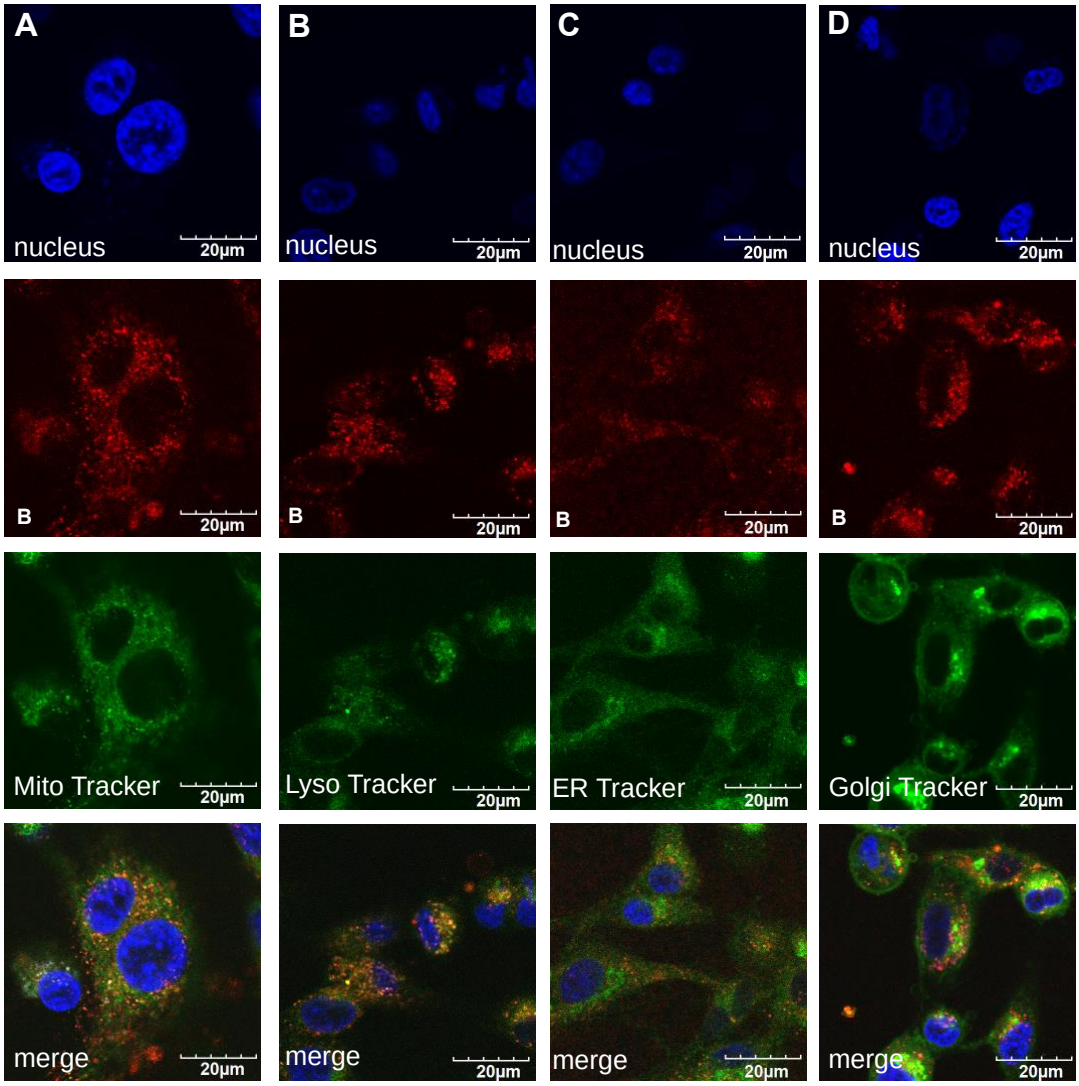


Figure S9. Localization of compound **B** (20 μM) after 24 h incubation at 37 °C with U87-MG cells (A) Mitochondria (B) Lysosome (C) ER Tracker and (D) Golgi in DMEM/F12 supplemented by 10% FBS pH 7.4.

F. References

1. Tan X, Luo S, Wang D, Su Y, Cheng T, Shi C. A NIR Heptamethine Dye With Intrinsic Cancer Targeting, Imaging and Photosensitizing Properties. Biomaterials. 2012; 33: 2230-9.
2. Henary M, Pannu V, Owens EA, Aneja R. Near infrared active heptacyanine dyes with unique cancer-imaging and cytotoxic properties. Bioorg Med Chem Lett. 2012; 22: 1242-6.
3. Stoll RS, Peters MV, Kuhn A, Heiles S, Goddard R, Buhl M, et al. Photoswitchable Catalysts: Correlating Structure and Conformational Dynamics with Reactivity by a Combined Experimental and Computational Approach. J Am Chem Soc. 2009; 131: 357-67.
4. Usama SM, Thavornpradit S, Burgess K. Optimized Heptamethine Cyanines for Photodynamic Therapy. ACS Applied Bio Materials. 2018; 1: 1195-205.
5. Dolomanov OV, Bourhis LJ, Gildea RJ, Howard JAK, Puschmann H. OLEX2: a complete structure solution, refinement and analysis program. J Appl Crystallogr. 2009; 42: 339-41.
6. Sheldrick GM. SHELXT - Integrated space-group and crystal-structure determination. Acta Crystallogr, Sect A Found Adv. 2015; 71: 3-8.
7. Sheldrick GM. A short history of SHELX. Acta Crystallogr, Sect A Found Crystallogr. 2008; 64: 112-22.