Multi-Organelle Imaging with Dye Combinations: Targeting the ER, Mitochondria, and Plasma Membrane

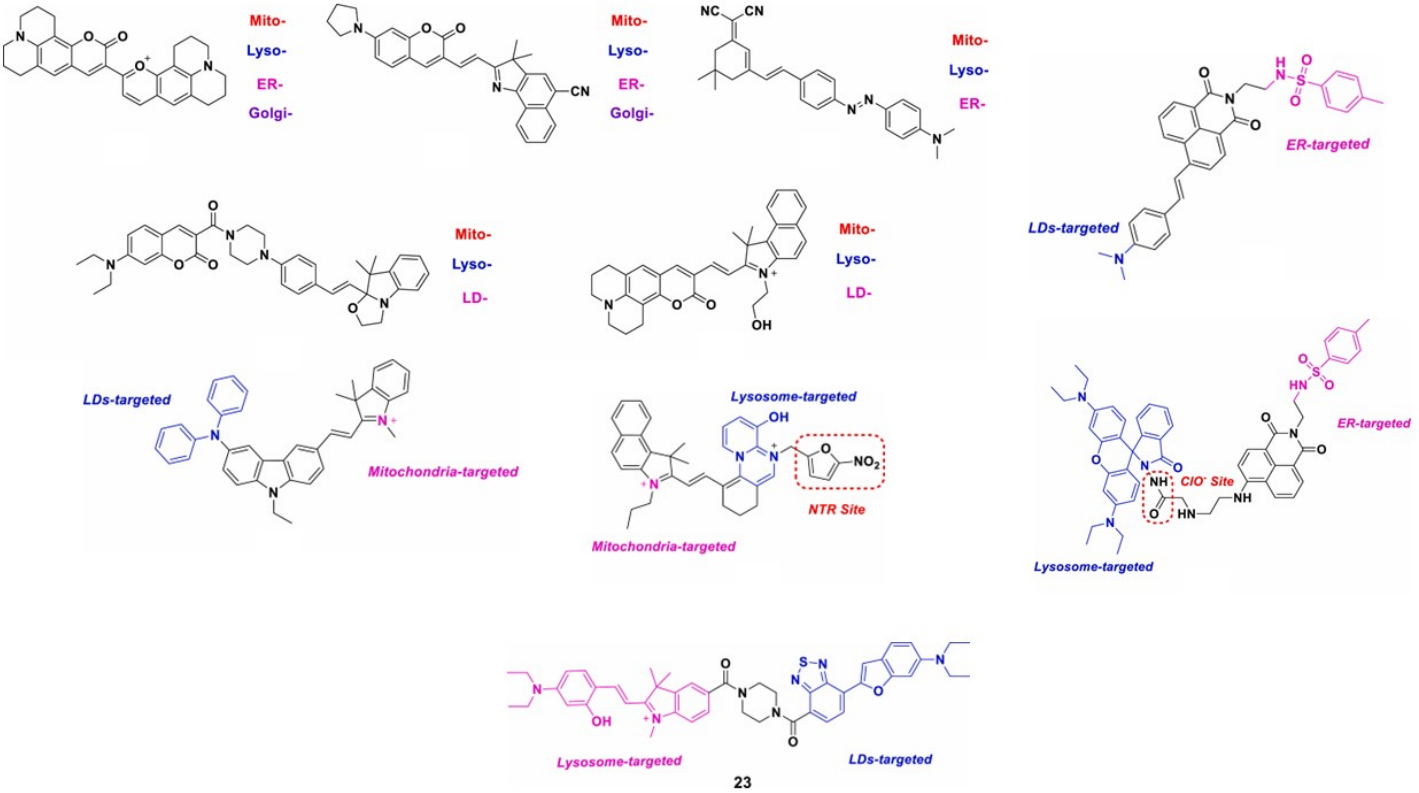
Yogesh Dubey and Sriram Kanvah*

[a]Department of Chemistry, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar

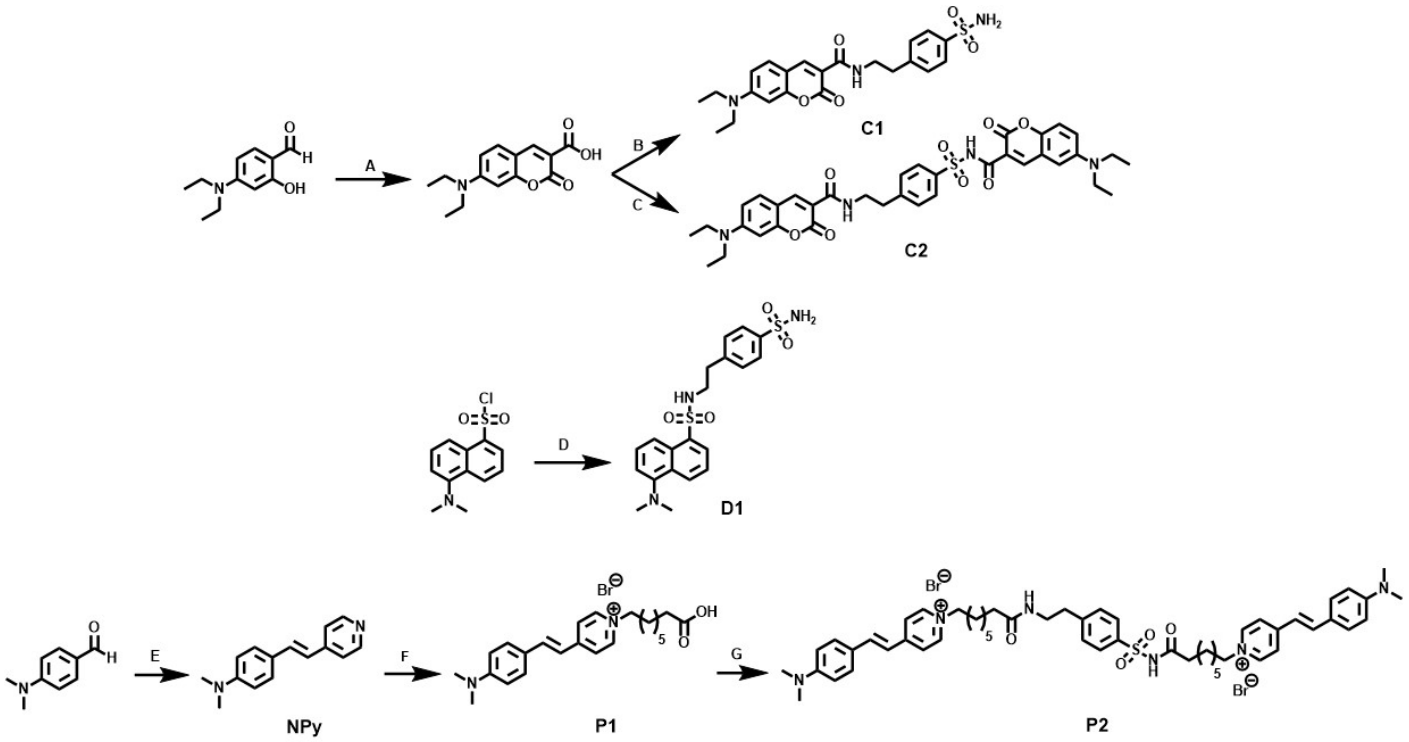
e-mail: sriram@iitgn.ac.in

Table of Contents

Figure S1. A few examples of reported fluorophores for multi-organelle imaging



S2. Synthetic procedures



A. Diethylmalonate, piperidine, NaOH, HCl B. 4-(2-Aminoethyl)benzenesulfonamide, HATU, EDC.HCl, DMAP, Et_3N, DMF C. 4-(2-Aminoethyl)benzenesulfonamide, HATU, EDC.HCl, DMAP, Et_3N, DMF D. 4-(2-Aminoethyl)benzenesulfonamide, Et_3N, DCM E. 4-Picoline, KOH F. 8-bromoctanoic acid, EtOH G. 4-(2-Aminoethyl)benzenesulfonamide, HATU, EDC.HCl, DMAP, Et_3N, DMF

7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid (Route A)[1]

In a round bottom flask equipped with magnetic bead, 4-(Diethylamino)-2-hydroxybenzaldehyde (1 equiv) was dissolved in ethanol (0.1 M), followed by the addition of diethyl malonate (1 equiv) and piperidine (2 equiv). The reaction mixture was refluxed for 16 h, then quenched with 3N HCl and extracted with brine and dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated using rotary evaporator. The resulting crude product was dissolved in ethanol (0.1 M), and 12N NaOH was added dropwise. The reaction was refluxed for 24 h and subsequently quenched with 12N HCl on ice. The resulting orange precipitate was filtered, washed with water, and purified by silica gel column chromatography using 60% ethyl acetate in hexanes, yielding the pure product as a yellow solid in 75% yield.

7-(diethylamino)-2-oxo-N-(4-sulfamoylphenethyl)-2H-chromene-3-carboxamide (C1) (Route B)

In a round-bottom flask equipped with a magnetic stir bar, Cou-COOH (1 equiv), 4-(2-aminoethyl)benzenesulfonamide (1 equiv), HATU (1.1 equiv), EDC·HCl (1.1 equiv), and DMAP (1 equiv) were added and purged with argon gas to ensure an inert atmosphere. Under this atmosphere, dry DMF (0.1 M) was added to dissolve the reagents, followed by the addition of triethylamine (2 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 24 h. Completion of the reaction was confirmed by TLC using 1% MeOH in methylene chloride (DCM). The crude product was concentrated using rotary evaporator and purified by silica gel column chromatography with a gradient elution from 1% to 10% methanol in methylene chloride, yielding the pure product as a bright yellow solid in 45% yield.

7-(diethylamino)-N-(4-(N-(6-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)sulfamoyl)phenethyl)-2-oxo-2H-chromene-3-carboxamide (C2) (Route C)

Following the general procedure from Samantha et. al with slight modifications.[2] In a round-bottom flask equipped with a magnetic stir bar, 4-(2-aminoethyl)benzenesulfonamide (1 equiv), Cou-COOH (2.2 equiv), HATU (1.5 equiv), EDC·HCl (1.5 equiv), and DMAP (1 equiv) were added and purged with argon gas to ensure an inert atmosphere. Under this atmosphere, dry DMF (0.1 M) was added to dissolve the reagents, followed by the addition of triethylamine (5 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The completion of the reaction was confirmed by TLC using 2% MeOH in DCM. The crude product was concentrated using rotary evaporator and purified by silica gel column chromatography with a gradient elution from 1% to 10% methanol in DCM, yielding the pure product as a bright yellow solid in 41% yield.

5-(dimethylamino)-N-(4-sulfamoylphenethyl)naphthalene-1-sulfonamide (D1) (Route D)

In a round-bottom flask equipped with a magnetic stir bar, 4-(2-aminoethyl)benzenesulfonamide (1 equiv) and triethylamine (4 equiv) were dissolved in DCM and stirred for 20 minutes. To this solution, 5-(dimethylamino)naphthalene-1-sulfonyl chloride (2 equiv) was added, and the reaction was stirred at room temperature for 12 hours, with progress monitored by TLC. The reaction was allowed to proceed until the starting material was fully consumed. The crude reaction mixture was then concentrated using rotary evaporation and purified by silica gel column chromatography with isocratic elution using 1% methanol in DCM, yielding the pure product as a colorless solid in 91% yield.

(E)-N,N-dimethyl-4-(2-(pyridin-4-yl)vinyl)aniline (NPy) (Route E)[3]

In a round-bottom flask equipped with a magnetic stir bar, 4-(dimethylamino)benzaldehyde (1 equiv), 4-picoline (5 equiv), and potassium hydroxide (5 equiv) were heated at 80 °C for 12 h. After the reaction mixture cooled to room temperature, ice-cold water was added. The resulting orange precipitate was filtered, washed with water, and yielded the product with a 22% yield.

(E)-1-(7-carboxyheptyl)-4-(4-(dimethylamino)styryl)pyridin-1-ium bromide (P1) (Route F)

In a round-bottom flask equipped with a magnetic stir bar, NPy (1 equiv) and 8-bromooctanoic acid (1.2 equiv) were dissolved in ethanol (0.1 M) and refluxed for 24 hours. The reaction mixture was then completely dried using a rotary evaporator. A minimal amount of dichloromethane (DCM) was added, followed by sonication. The resulting red precipitate was filtered, washed with water, and yielded the product with a 51% yield.

4-((E)-4-(dimethylamino)styryl)-1-(8-((4-(2-(8-(4-((E)-4-(dimethylamino)styryl)pyridin-1-ium-1-yl)octanamido)ethyl)phenyl)sulfonamido)-8-oxooctyl)pyridin-1-ium bromide (P2) (Route G)

Following the general procedure from Samantha et. al with slight modifications.[2] In a round-bottom flask equipped with a magnetic stir bar, 4-(2-aminoethyl)benzenesulfonamide (1 equiv), 8C-COOH (2.2 equiv), HATU (1.5 equiv), EDC·HCl (1.5 equiv), and DMAP (1 equiv) were added and purged with argon gas to ensure an inert atmosphere. Under this atmosphere, dry DMF (0.1 M) was added to dissolve the reagents, followed by the addition of triethylamine (5 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 24 hours. Completion of the reaction was confirmed by TLC using 2% MeOH in DCM. The crude product was concentrated using rotary evaporation and purified by silica gel column chromatography with a gradient elution from 1% to 12% methanol in DCM, yielding the pure product as a red solid in 41% yield.

Figure S3. Absorption spectral data for **C1**, **C2**, **D1**, **P1** and **P2** in different solvents at concentration of 20 μM

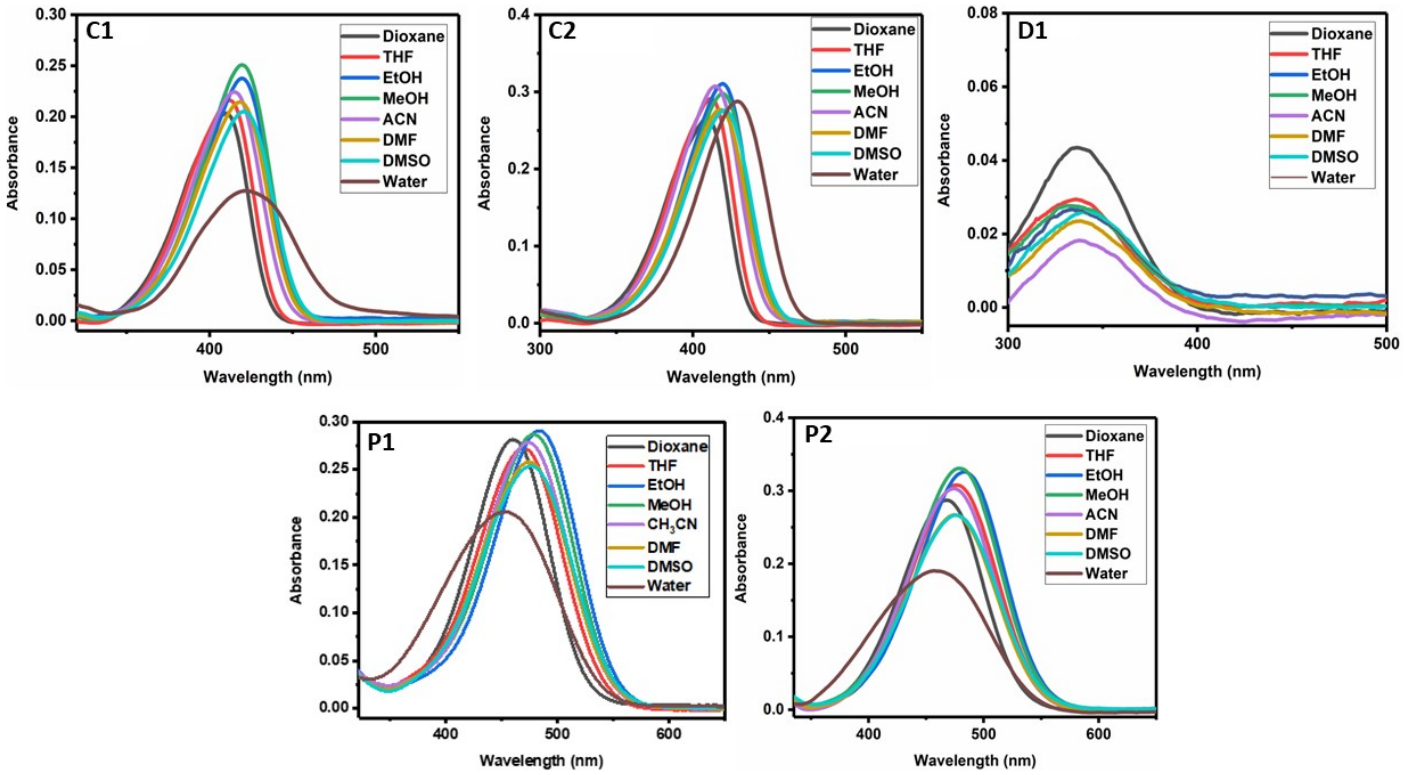


Figure S4. Emission spectral data for **C1**, **C2**, **D1**, **P1** and **P2** in different solvents at concentration of 20 μM

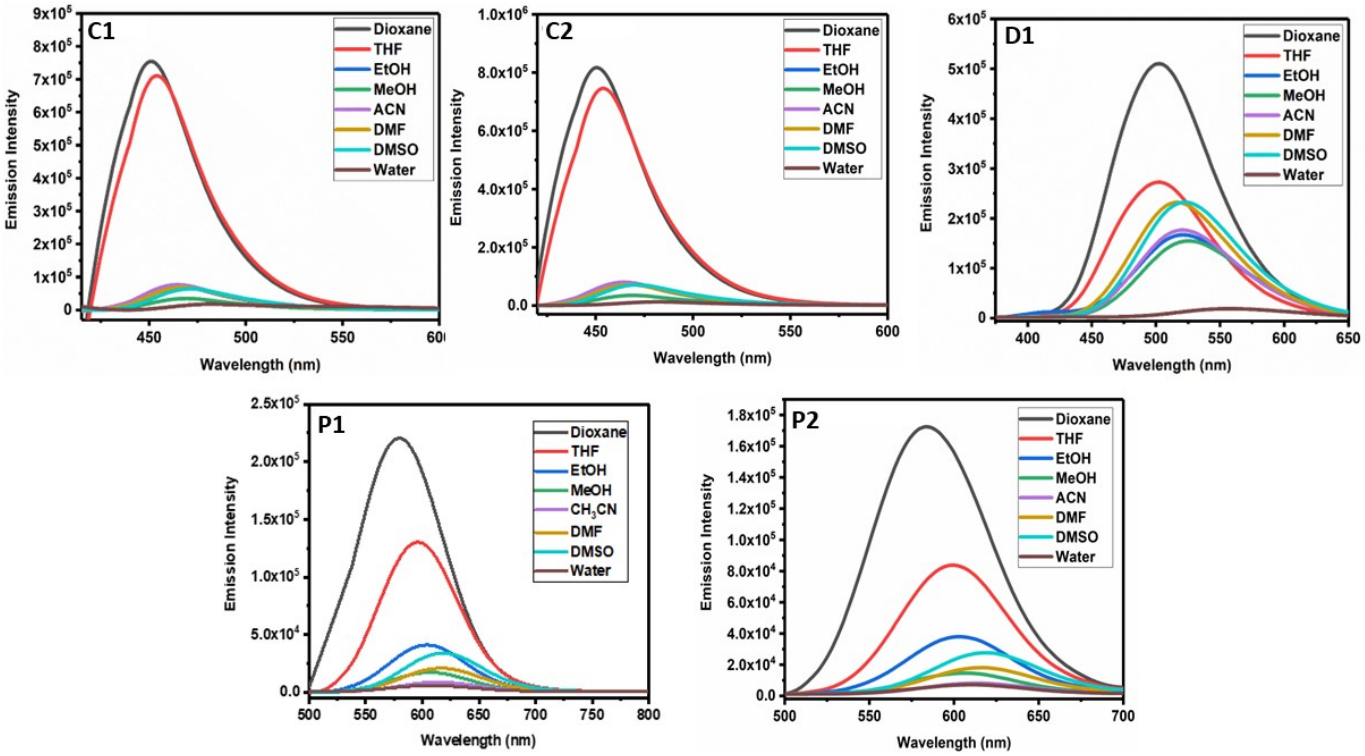


Table S1. Molar extinction coefficient [ε ($M^{-1}cm^{-1}$) × 10^3] for **C1**, **C2**, **D1**, **P1** and **P2** in different solvents

Solvent	C1	C2	D1	P1	P2
	ε	ε	ε	ε	ε
Dioxane	10.2	13.3	2.2	14.1	24.8
THF	10.8	14.6	1.5	13.7	15.4
EtOH	11.9	15.5	1.4	14.6	16.3
CH_3OH	12.6	14.9	1.4	14.3	16.5
CH_3CN	11.2	15.4	0.9	13.9	15.1
DMF	10.7	13.9	1.2	12.9	13.4
DMSO	10.3	13.8	1.4	12.7	13.3
H_2O	6.4	14.4	0.6	10.3	9.5

Table S2. Relative quantum yield for **C1**, **C2**, **P1** and **P2** with reference to quinine sulfate

Molecule	Φ (MeOH) % yield
C1	1.24
C2	1.21
P1	0.51
P2	0.58

Figure S5. Normalized spectral overlap between **C1/C2**-emission and the **P2**-excitation

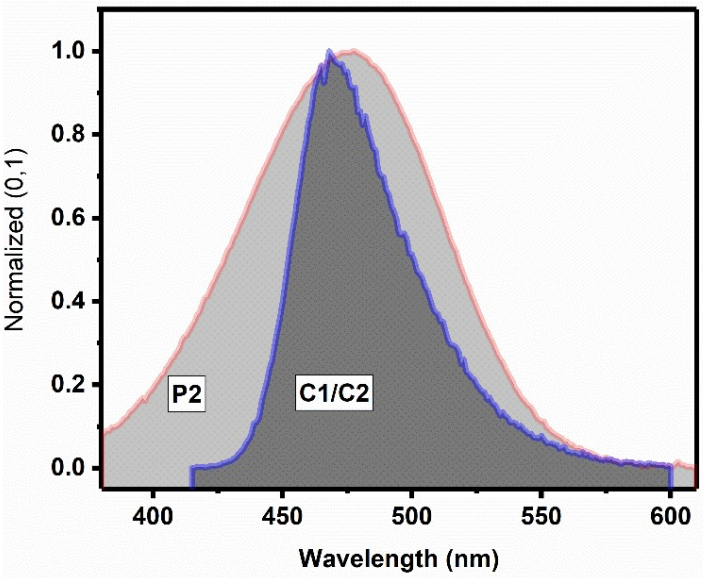
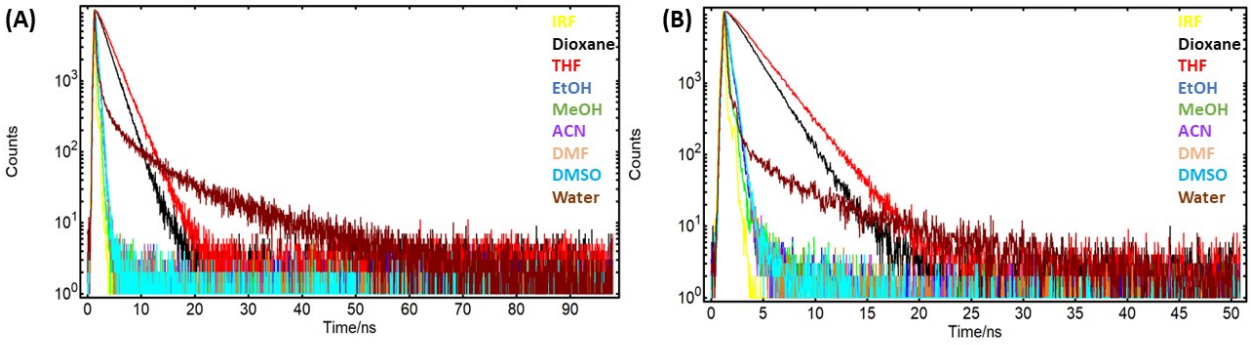


Figure S6. Lifetime decay profiles for **C1** (A) and **C2** (B) in various solvents



Tabulated lifetime decay data for **C1** and **C2**

Compound	Solvent	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	χ 2
C1	Dioxane	1.86	-	-	1.051
	THF	2.30	-	-	1.038
	EtOH	0.28	0.66	-	0.979
	MeOH	0.14	0.73	-	1.028
	ACN	0.31	-	-	1.020
	DMF	0.32	-	-	1.111
	DMSO	0.34	1.74	-	0.948
	Water	0.10	1.83	11.56	1.245
C2	Dioxane	1.89	-	-	0.991
	THF	2.35	-	-	1.056
	EtOH	0.29	1.12	-	1.237
	MeOH	0.14	1.22	-	1.181
	ACN	0.32	1.12	-	1.007
	DMF	0.32	-	-	1.287
	DMSO	0.31	1.21		0.991
	Water	0.08	1.52	8.24	1.015

Lifetime decay profiles for **C1** (left panel) and **C1+P2** (right panel) in DMSO**:**

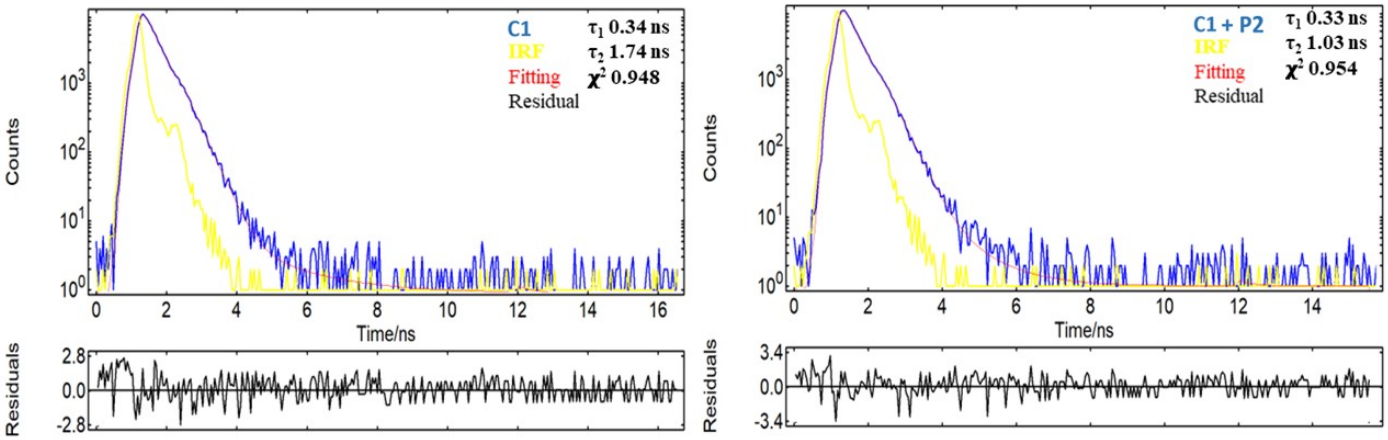


Figure S7. MTT Assay for **C1**, **C2**, **D1**, **P1** and **P2** in COS-7, HeLa and A549 cell lines at bioimaging concentration of 1 μM

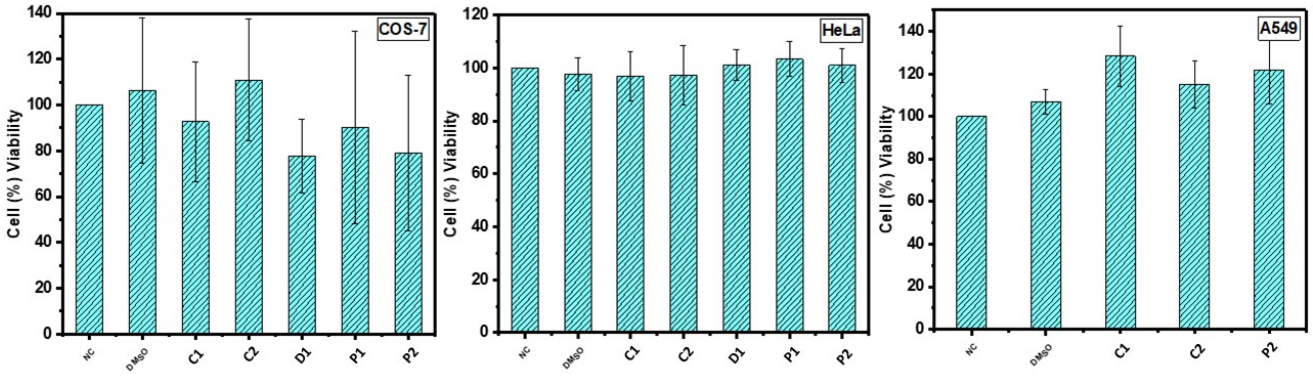


Figure S8. Preliminary Imaging for **C2**, **D1** and **P2** in COS-7. Scale bar: 10 μm

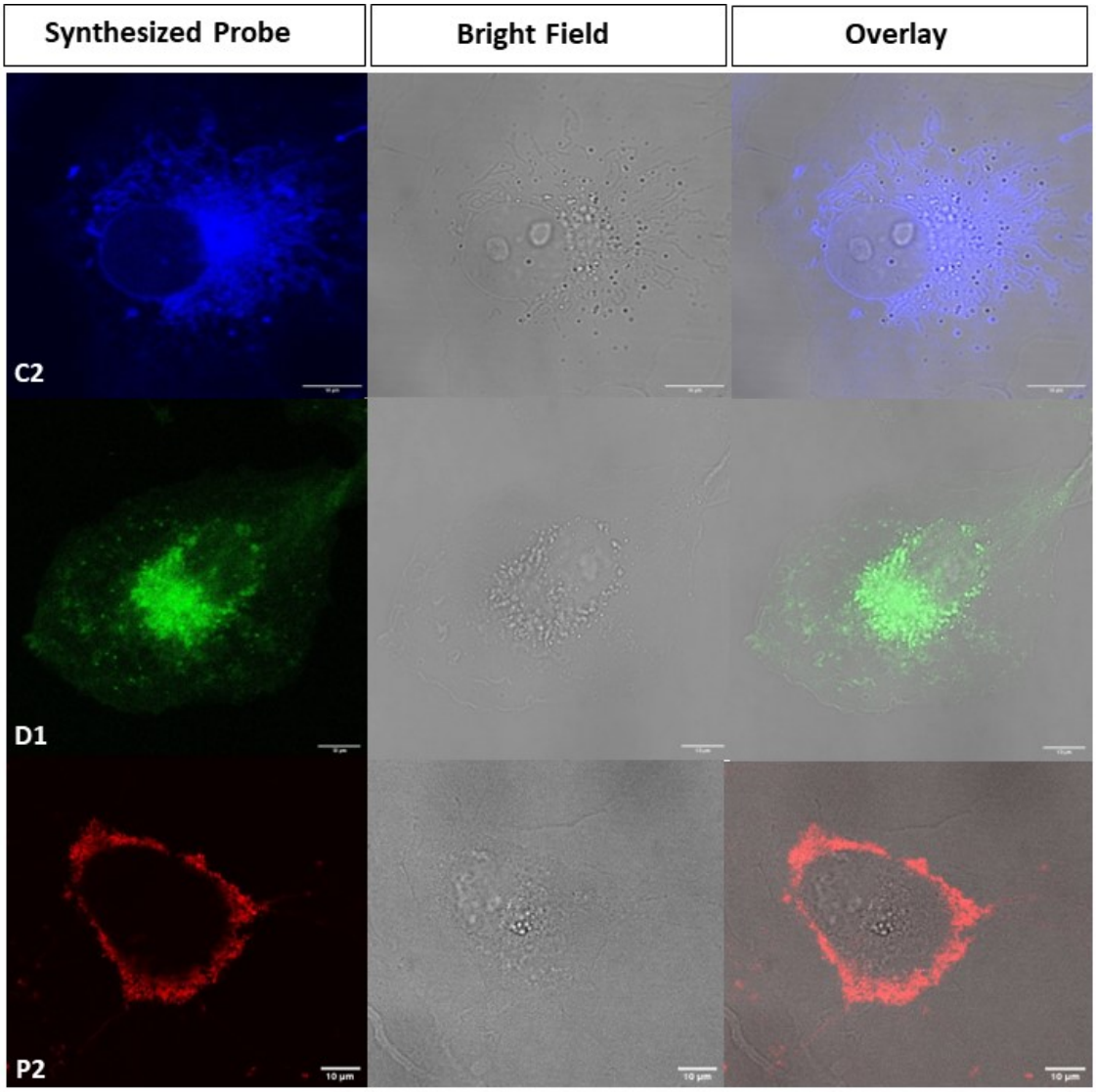


Figure S9. Preliminary Imaging for **C1**, **C2** and **D1** in HeLa Cells. Scale bar: 10 μm

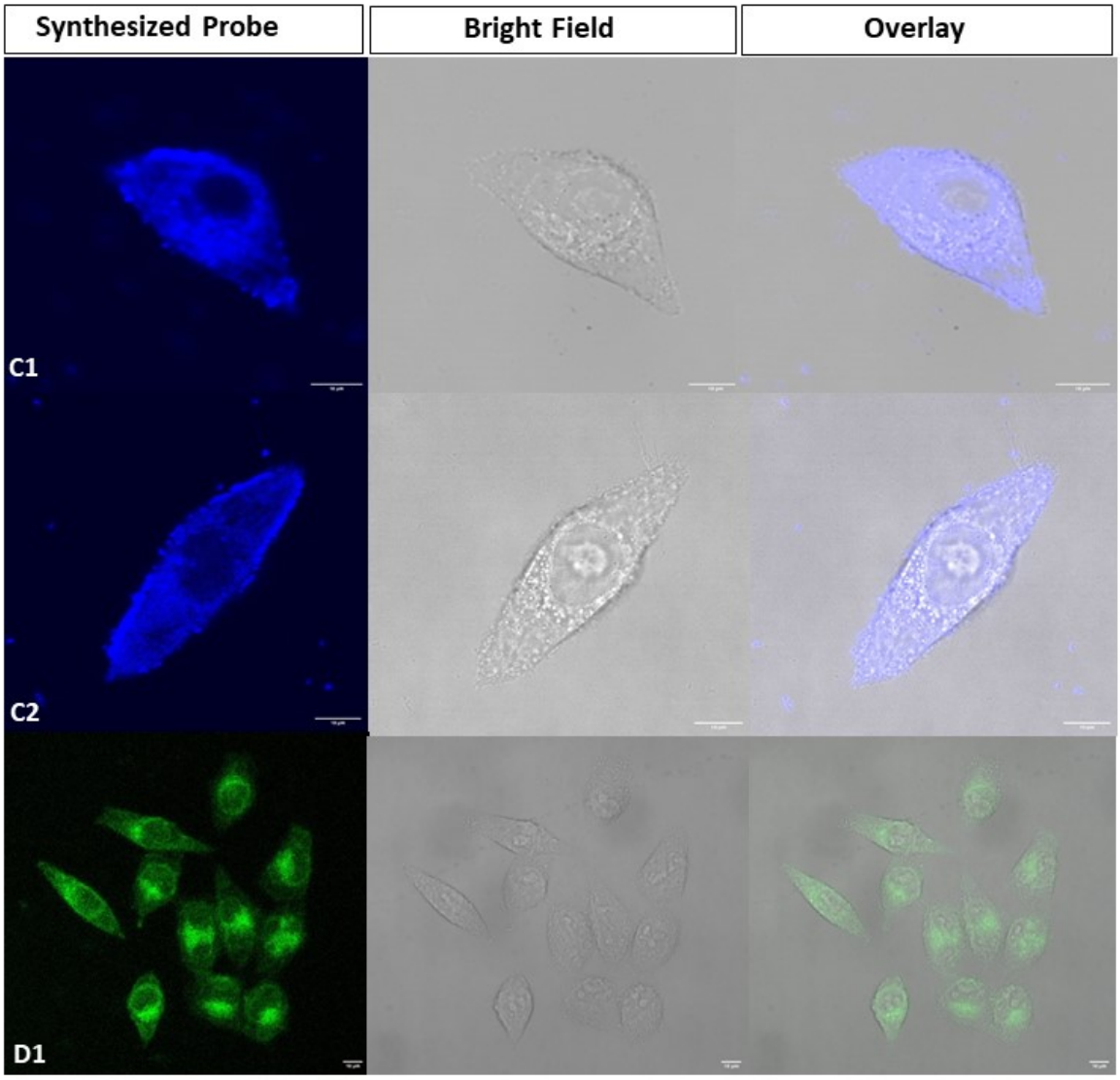


Figure S10. Preliminary Imaging for **C1**, **C2** and **P2** (**P2*** - At saturation of mitochondrial fluorescence) in A549. Scale bar: 10 µm

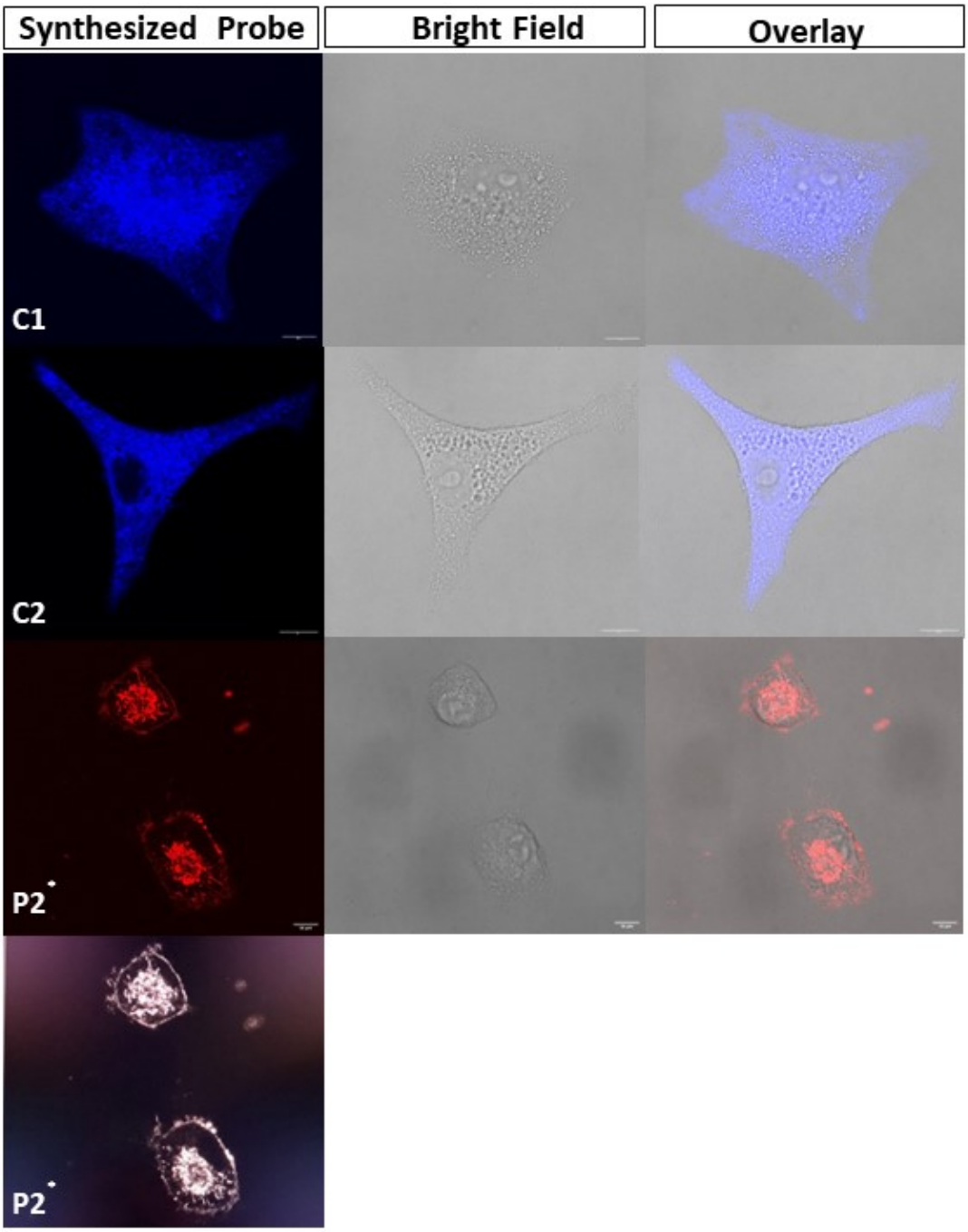


Figure S11. Colocalization for **C2**, **D1** and **P2** in COS-7 cells. Scale bar: 10 μm

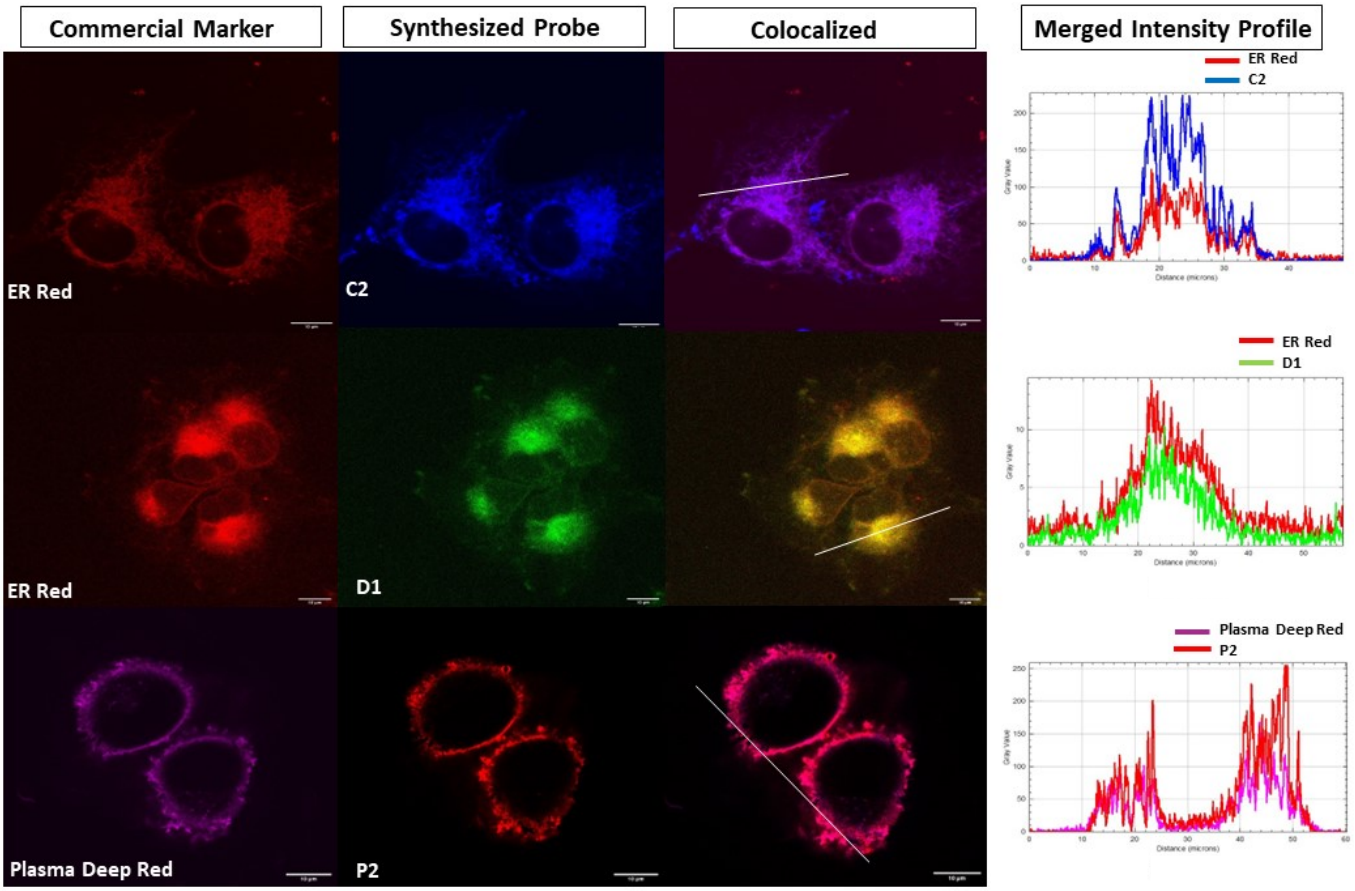


Figure S12. Colocalization for **C1**, **C2** and **D1** in HeLa cells. Scale bar: 10 μm

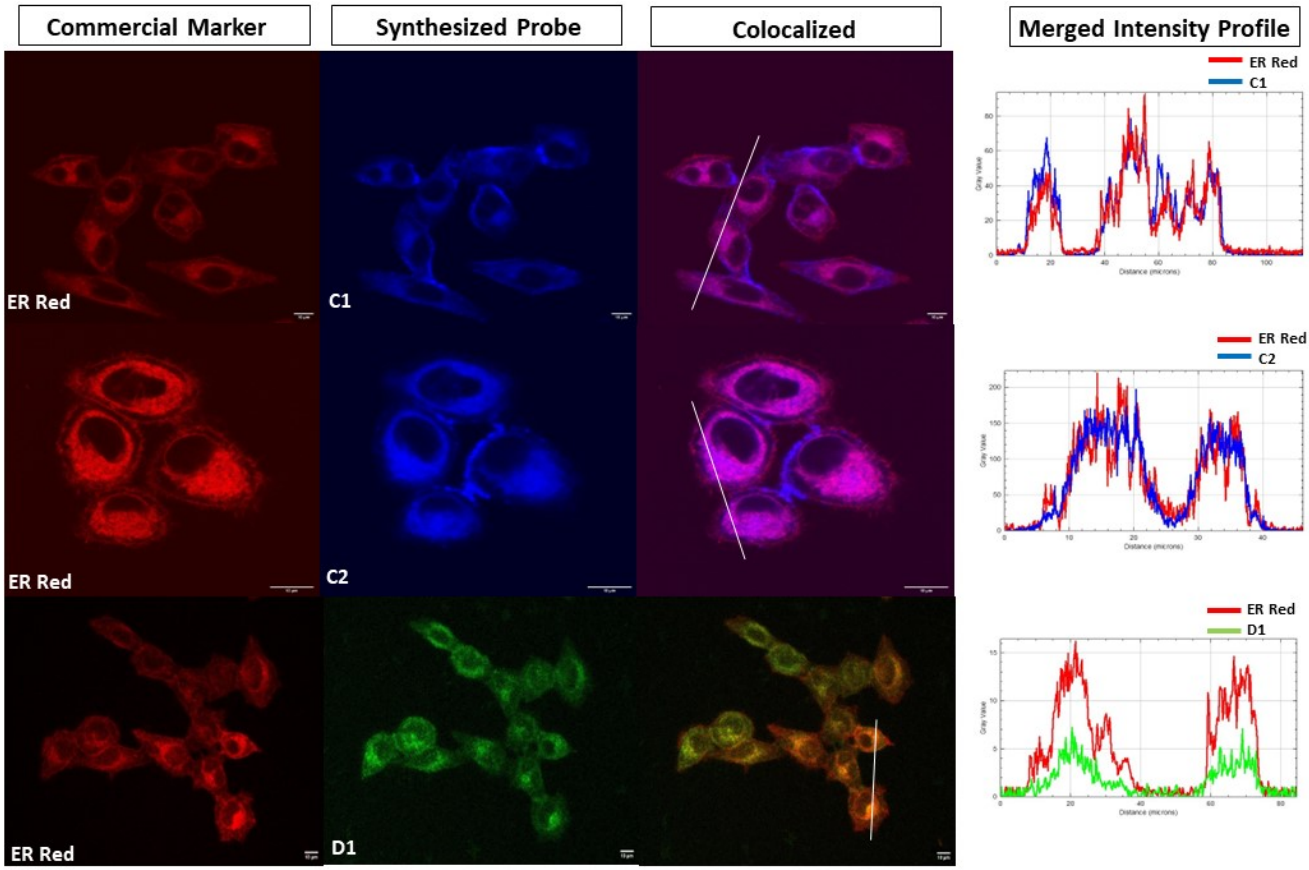


Figure S13. Colocalization for **C1** and **C2** in A549 cells. Scale bar: 10 μm

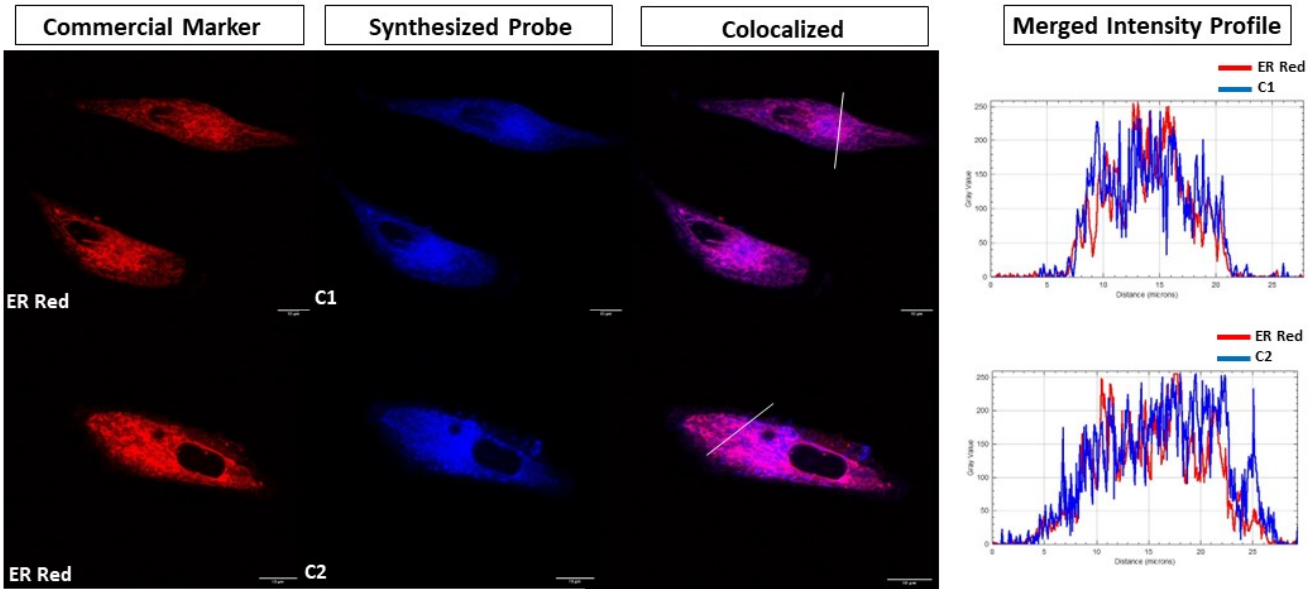


Figure S14. Visualization of rough and smooth endoplasmic reticulum using **C1**, **C2**, and **D1** in COS-7. Scale bar: 10 μm

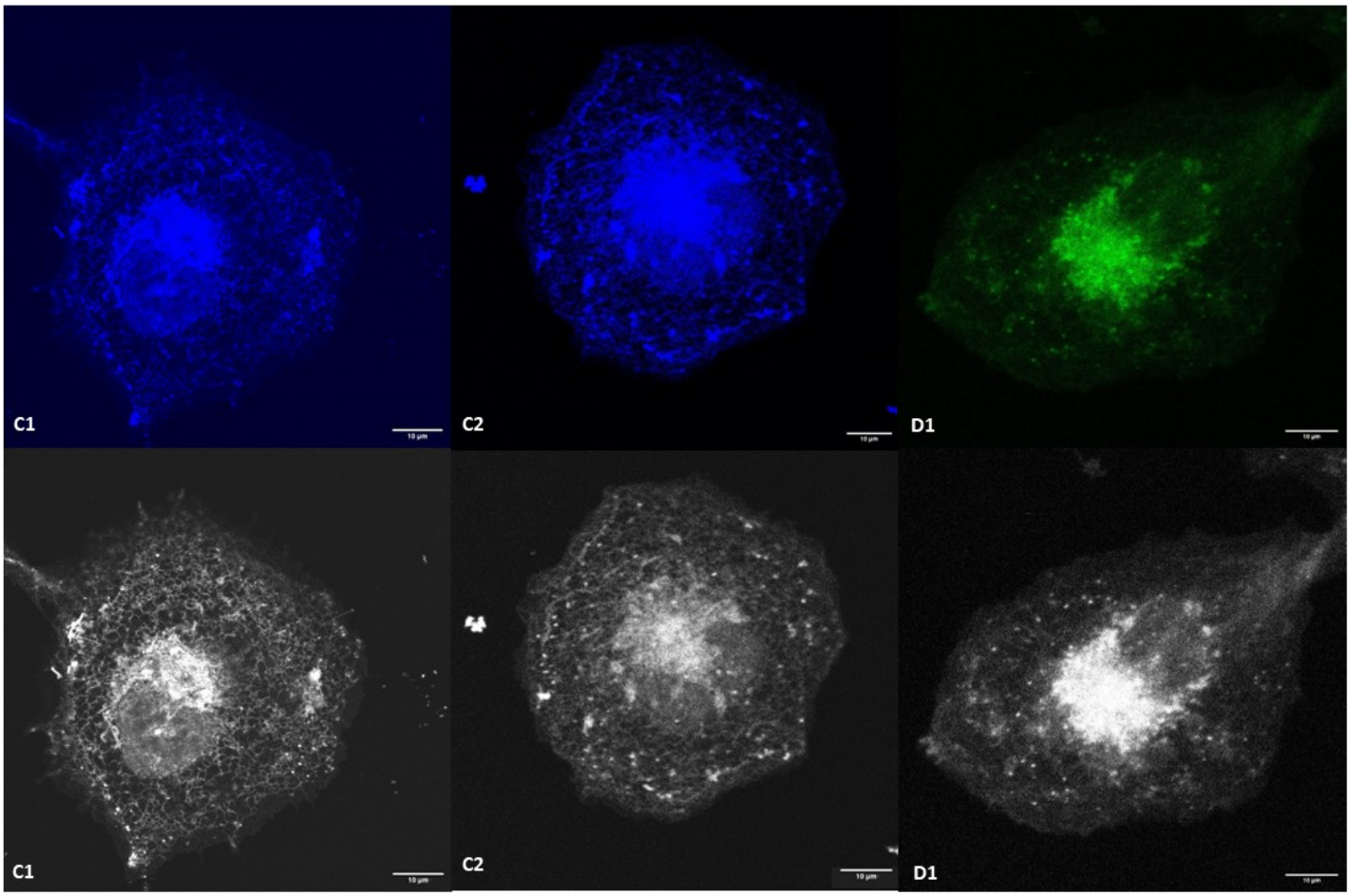


Figure S15. Compatibility with trypsinization **C1**, **C2, D1**, **P1** and **P2** in HeLa Cells. Scale bar: 10 μm

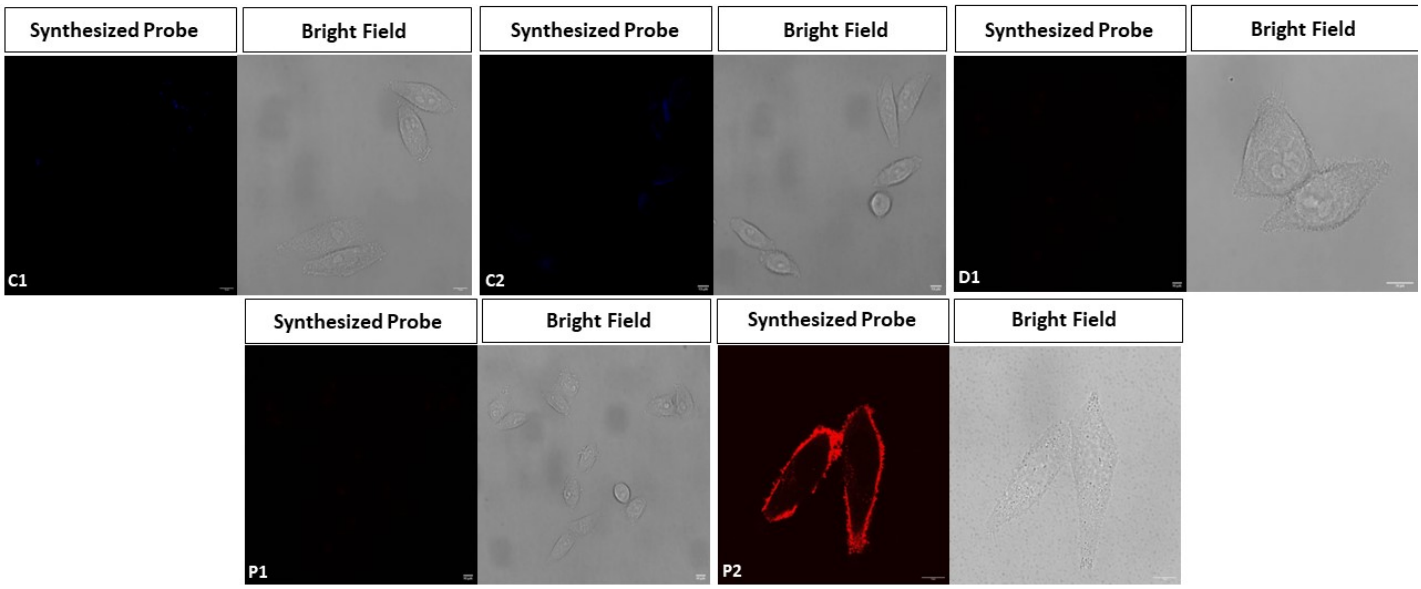


Figure S16. Standardized condition for multi-organelle imaging (HeLa cells-upper panel and A549 cells-lower panel)

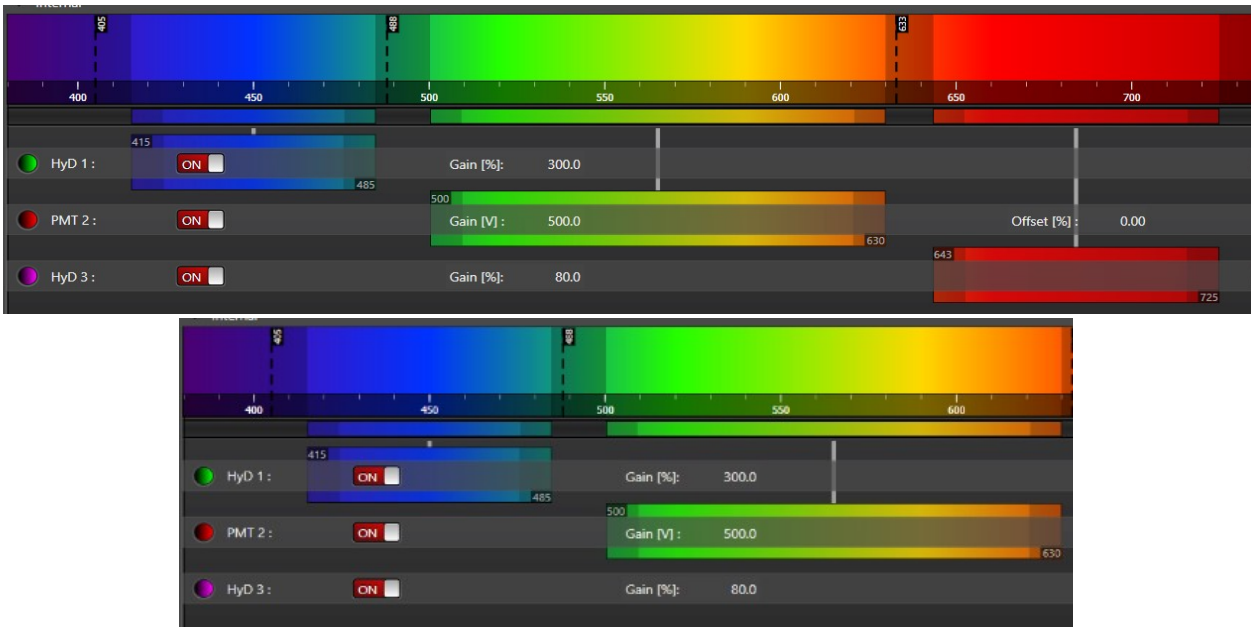


Figure S17. No Spillover experiment with (A549 cells) Scale bar: 10 µm

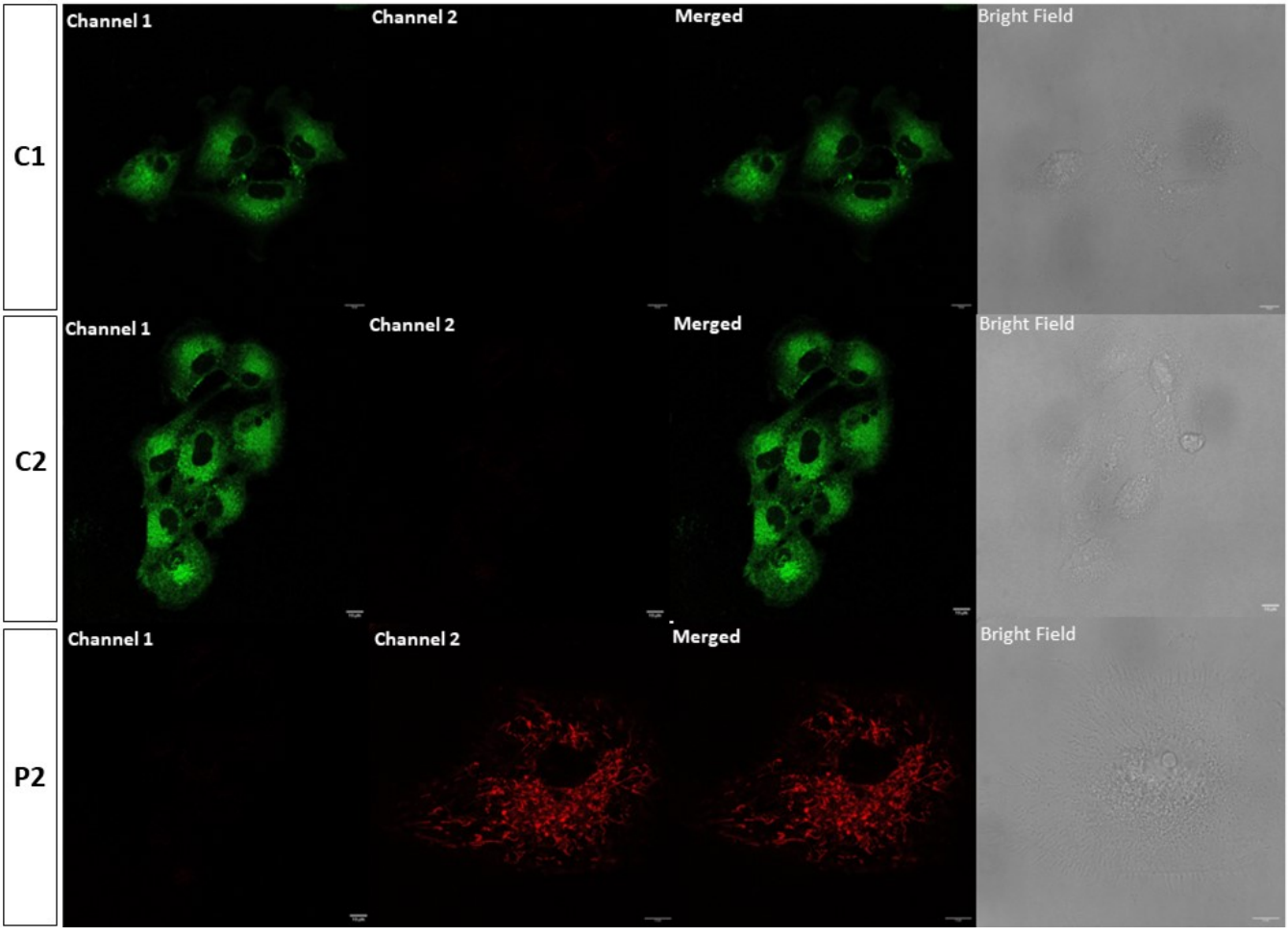


Figure S18. Triple Imaging using **C1/C2** and **P2** in HeLa cells. Scale bar: 10 µm

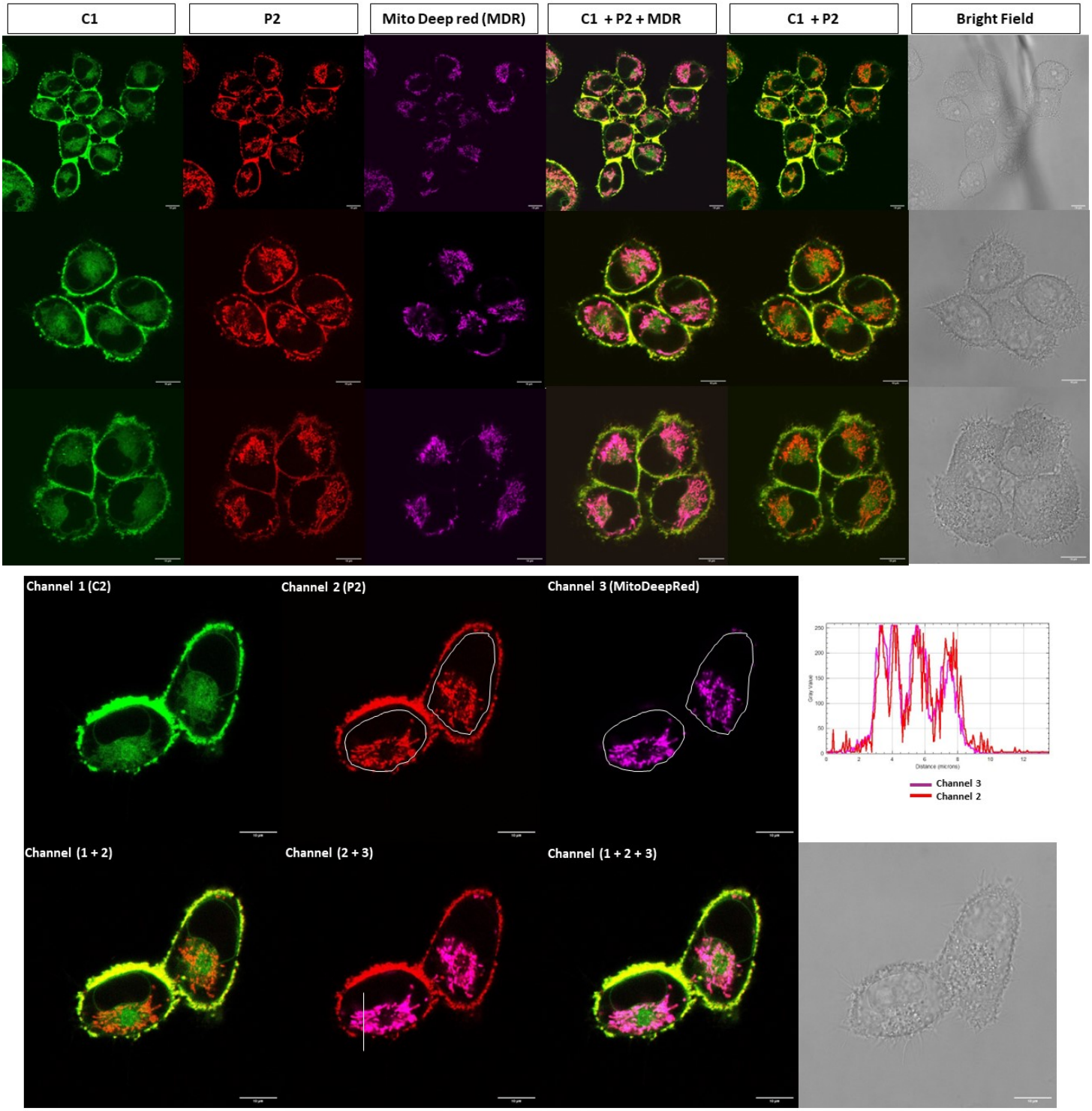


Figure S19. Dual Imaging using **C2** and **P2** in A549 cells. Scale bar: 10 µm

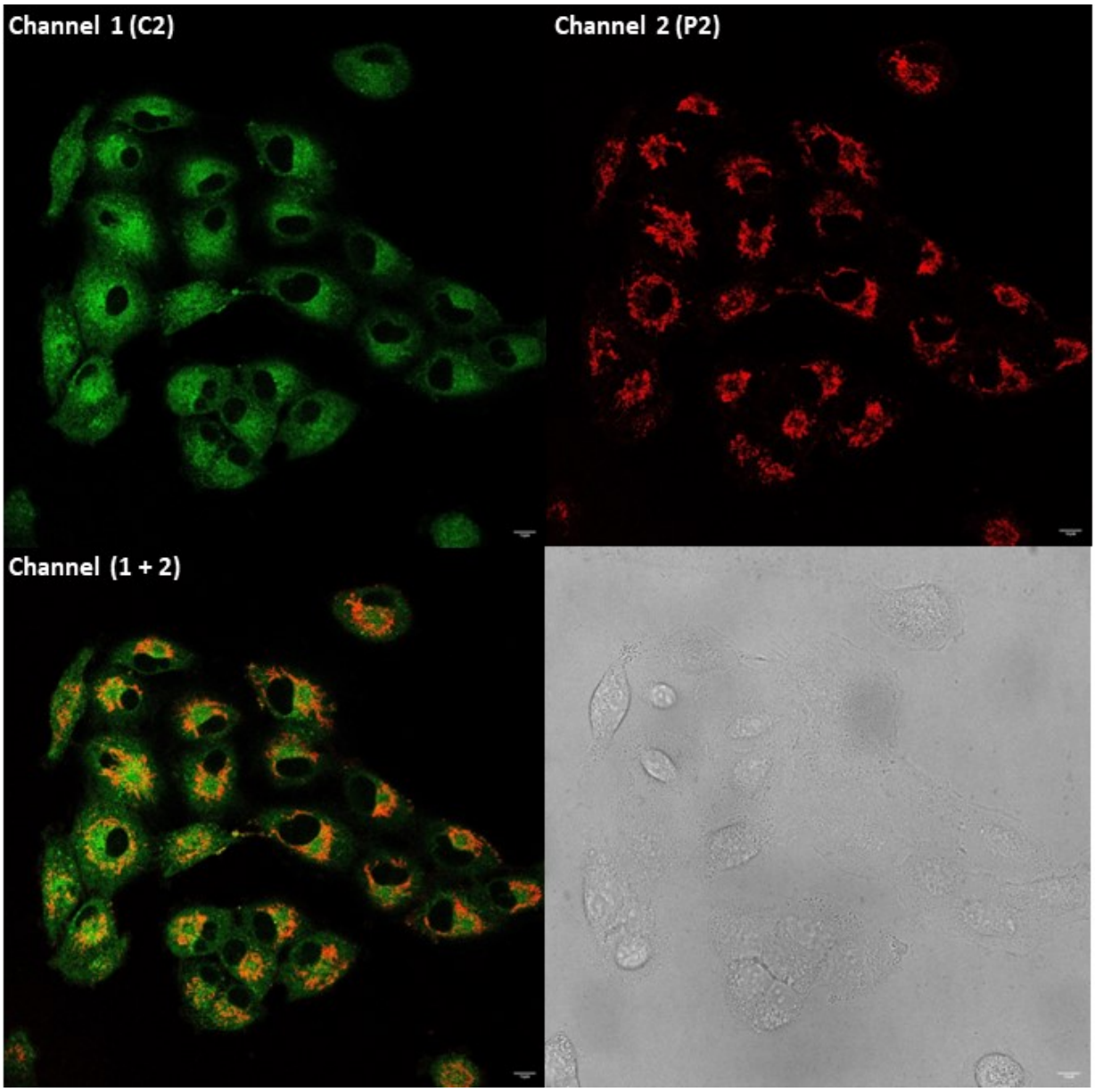
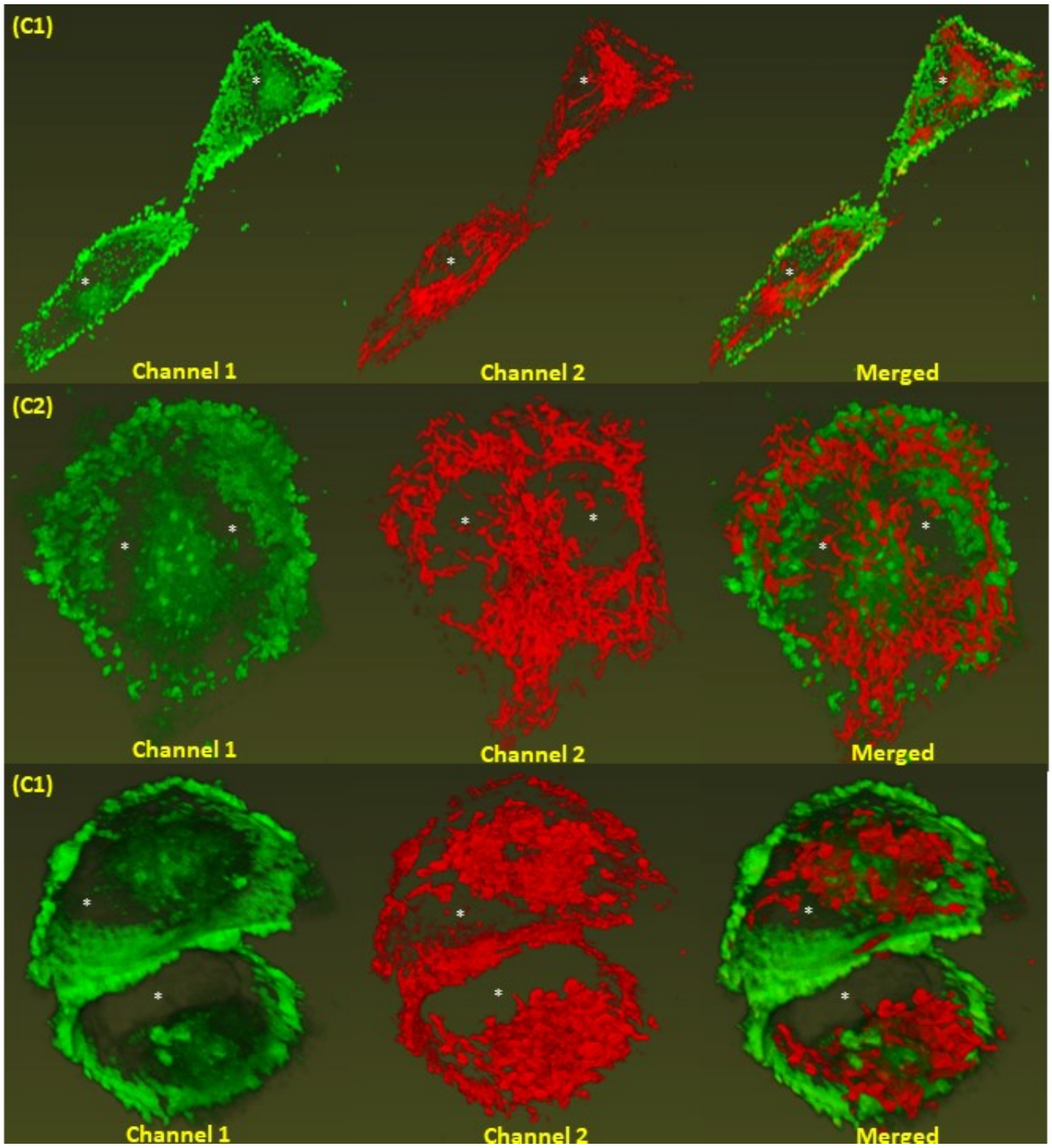


Figure S20. 3D Projection (Upper panel – HeLa and Lower panel – A549)



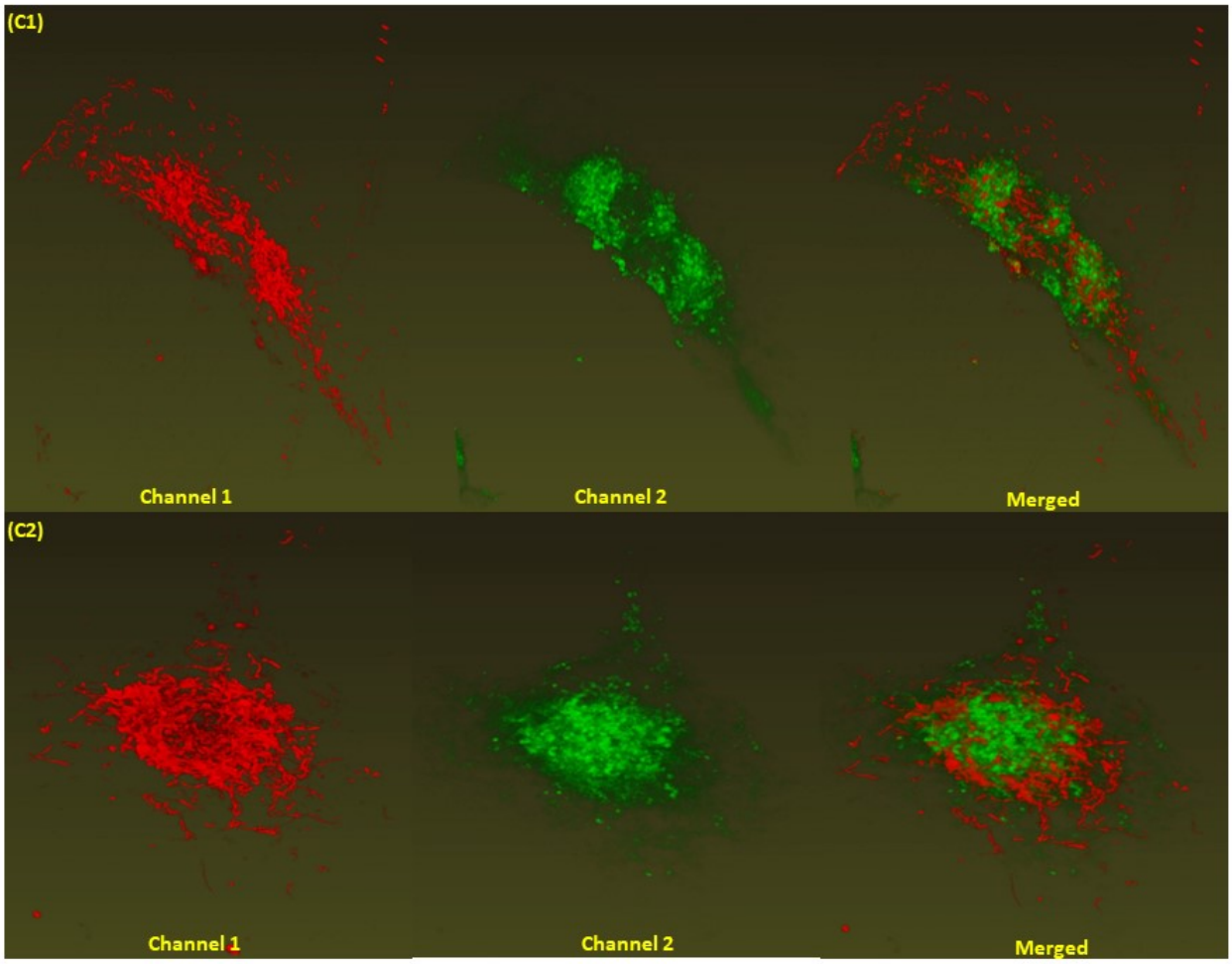

(C1)
Channel 1
Channel 2
Merged
(C2)
Channel 1
Channel 2
Merged

Figure S21. Response to pH: The dye (10 μM) was dissolved in water of varying pH levels (1, 3, 5, 7, 9, 11, 13) and excited at the respective λ_{max} of the probe in water.

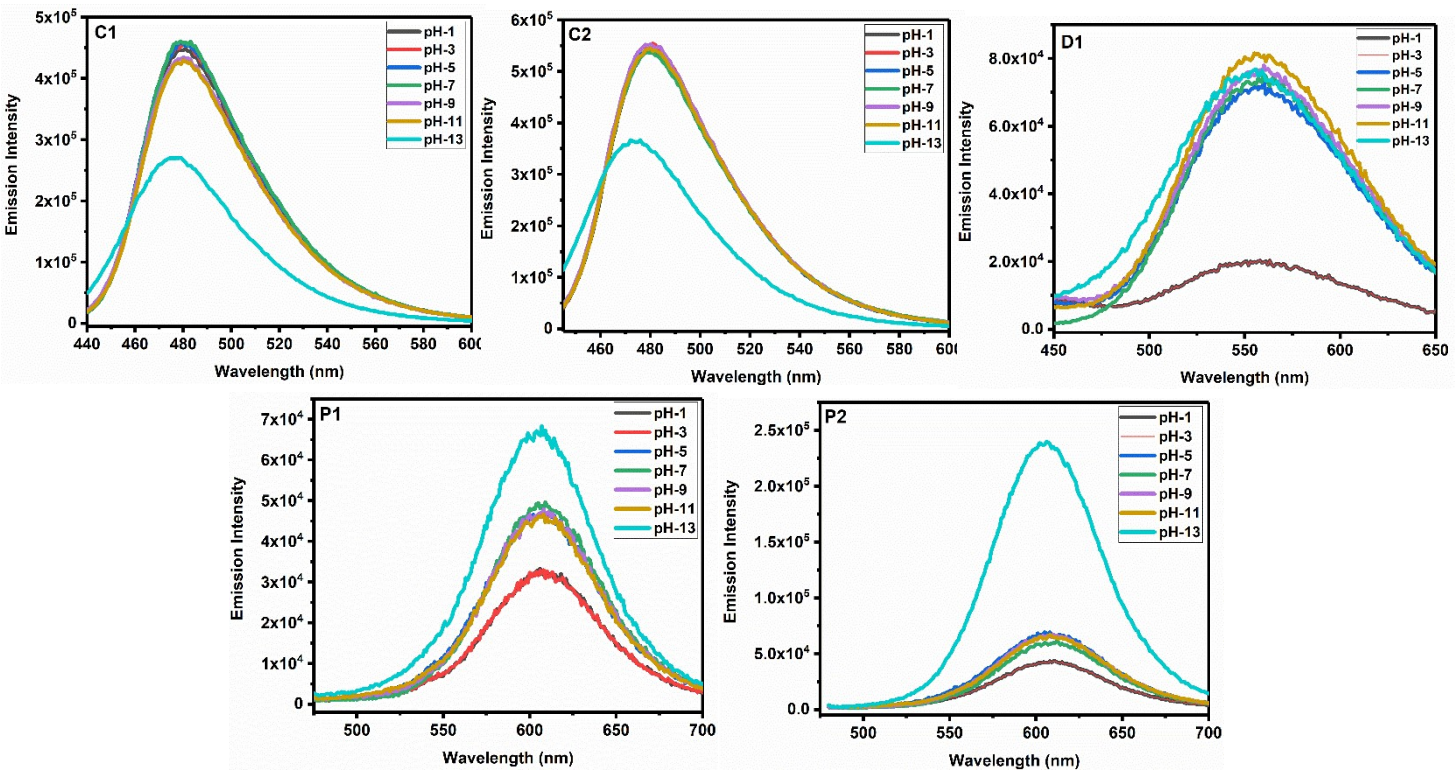
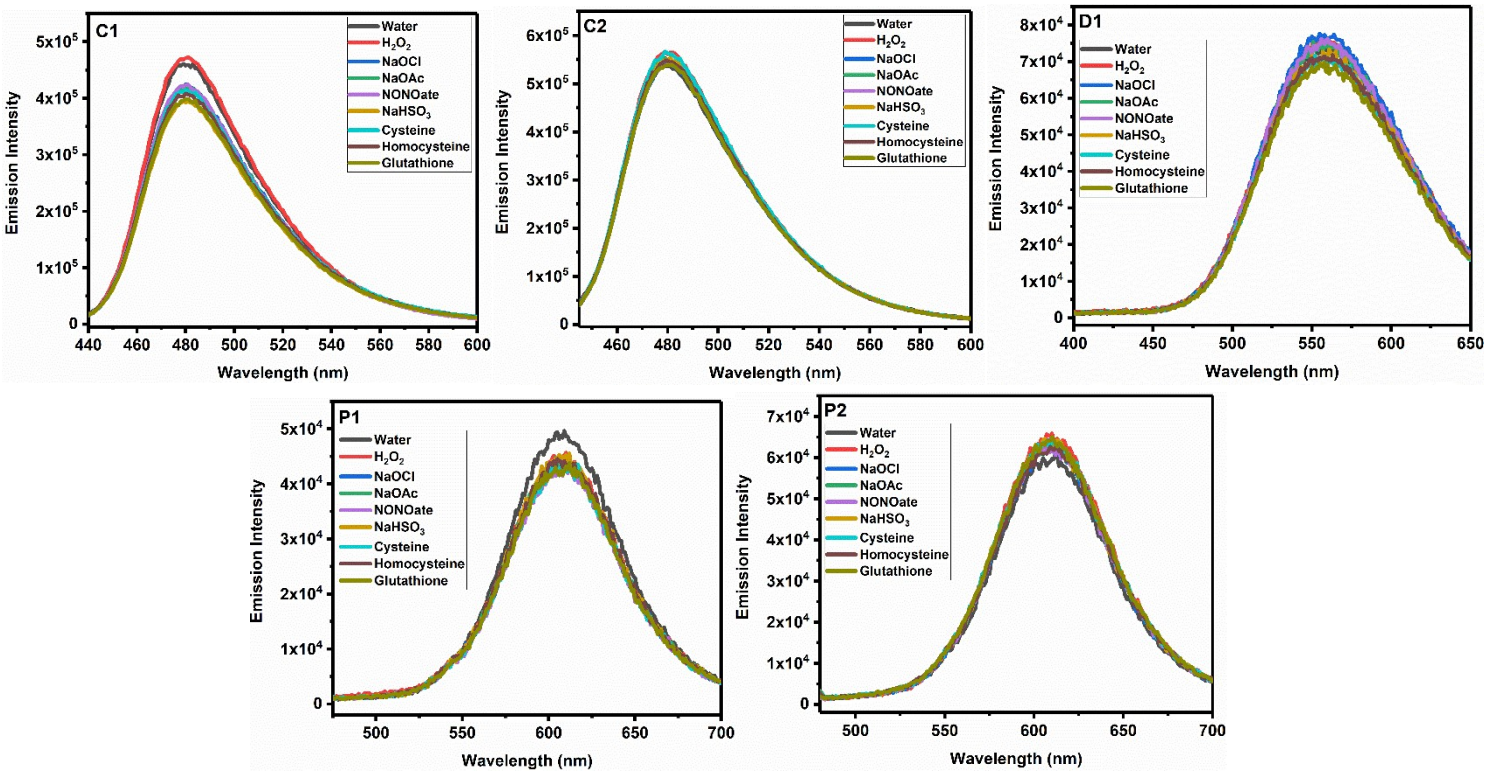


Figure S22. Response to analyte - The dye (10 μM) and analyte (10 μM) were mixed in water, incubated for 15 minutes, and excited at the respective λ_{max} of the probe in water.



S23 . Characterization Data

7-(diethylamino)-2-oxo-2H-chromene-3-carboxylic acid

^{1}H NMR (500 MHz, DMSO-d6): δ (ppm) 12.52 (s, 1H), 8.60 (s, 1H), 7.65 (d, J = 10.0 Hz, 1H), 6.82-6.79 (dd, J_1 = 5.0 Hz, J_2 = 5.0 Hz, 1H), 6.58 (s, 1H), 6.49 (q, J = 5.0 Hz, 4H), 1.14 (t, J = 5.0 Hz, 6H). **^{13}C NMR** (126 MHz; DMSO-d6) δ (ppm) 164.98, 159.96, 158.38, 153.38, 149.94, 132.33, 110.53, 107.84, 107.63, 96.39, 44.86, 12.79. **HRMS** (ESI) m/z: calculated for $C_{14}H_{15}NO_4Na^+$ $[M+Na]^+$: 284.0893; Found: 284.0901

7-(diethylamino)-2-oxo-N-(4-sulfamoylphenethyl)-2H-chromene-3-carboxamide (C1)

^{1}H NMR (500 MHz, DMSO-d6): δ (ppm) 8.73 (t, J = 5.0 Hz, 1H), 8.65 (s, 1H), 7.76 (d, J = 10.0 Hz, 2H), 7.68 (d, J = 10.0 Hz, 1H), 7.45 (d, J = 10.0 Hz, 2H), 7.31 (s, 2H), 6.80 (dd, 1H), 6.60 (d, J = 5.0 Hz, 1H), 3.58 (q, J = 5.0 Hz, 2H), 3.48 (q, J = 5.0 Hz, 4H), 2.92 (t, J = 5.0 Hz, 2H), 1.14 (t, J = 5.0 Hz, 6H). **^{13}C NMR** (126 MHz; DMSO-d6) δ (ppm) 162.66, 162.17, 157.69, 152.91, 148.21, 144.06, 142.59, 132.07, 129.60, 126.22, 110.61, 109.69, 108.12, 96.30, 44.80, 35.38, 12.78. **HRMS** (ESI) m/z: calculated for $C_{22}H_{26}N_3O_5S^+$ $[M+H]^+$: 444.1588; Found: 444.1595

7-(diethylamino)-N-(4-(N-(6-(diethylamino)-2-oxo-2H-chromene-3-carbonyl)sulfamoyl)phenethyl)-2-oxo-2H-chromene-3-carboxamide (C2)

^{1}H NMR (500 MHz, DMSO-d6): δ (ppm) 8.73 (t, J = 5.0 Hz, 1H), 8.66 (s, 1H), 7.76 (d, J = 10.0 Hz, 2H), 7.68 (d, J = 10.0 Hz, 1H), 7.52 (s, 4H), 7.45 (d, J = 10.0 Hz, 2H), 7.32 (s, 2H), 6.80 (dd, 1H), 6.60 (s, 1H), 4.47 (s, 1H), 3.58 (q, J = 5.0 Hz, 2H), 3.48 (q, J_1 = 5.0 Hz, J_2 = 10.0 Hz, 4H), 3.16 (q, J = 5.0 Hz, 4H), 2.92 (t, J = 5.0 Hz, 2H), 1.31 (t, J = 5.0 Hz, 6H), 1.14 (t, J = 5.0 Hz, 6H). **^{13}C NMR** (126 MHz; DMSO-d6) δ (ppm) 162.67, 162.17, 157.69, 152.92, 148.20, 144.07, 142.59, 133.00, 132.07, 130.72, 129.60, 129.54, 128.29, 126.22, 110.61, 109.69, 108.12, 96.30, 59.90, 55.38, 52.44, 44.80, 35.38, 12.77, 7.94. **HRMS** (ESI) m/z: calculated for $C_{36}H_{39}N_4O_8S^+$ $[M+H]^+$: 685.2332; Found: 685.2029

5-(dimethylamino)-N-(4-sulfamoylphenethyl)naphthalene-1-sulfonamide (D1)

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.46 (d, J = 10.0 Hz, 1H), 8.27 (d, J = 10.0 Hz, 1H), 8.09 (dd, 1H), 8.05 (t, J = 5.0 Hz, 1H), 7.66 (d, J = 10.0 Hz, 2H), 7.63-7.56 (m, 2H), 7.27 (t, J = 5.0 Hz, 5H), 3.05 (q, J = 5.0 Hz, 2H), 2.83 (s, 6H), 2.71 (t, J = 5.0 Hz, 2H). **^{13}C NMR** (126 MHz;

$CDCl_3$) δ (ppm) 151.81, 143.32, 142.58, 136.40, 129.90, 129.59, 129.53, 128.71, 128.31, 126.04, 124.07, 119.60, 115.64, 45.54, 43.94, 35.55. **HRMS** (ESI) m/z: calculated for $C_{20}H_{24}N_3O_4S_2^+$ $[M+H]^+$: 434.1203; Found: 434.1209

(E)-N,N-dimethyl-4-(2-(pyridin-4-yl)vinyl)aniline (NPy)

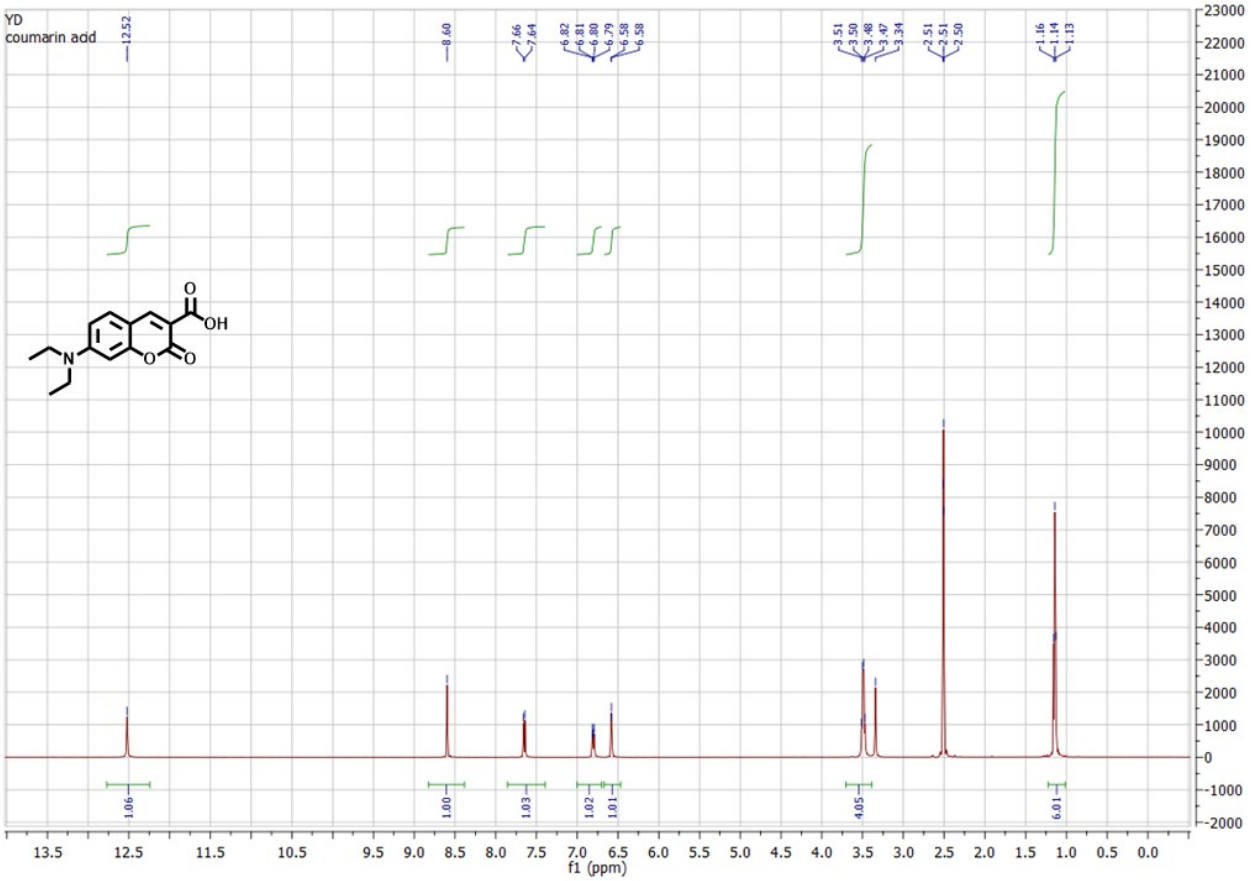
^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 8.51 (d, *J* = 5.0 Hz, 2H), 7.44 (d, *J* = 10.0 Hz, 2H), 7.32 (d, *J* = 5.0 Hz, 2H), 7.22 (s, 1H), 6.79 (d, *J* = 15.0 Hz, 1H), 6.71 (d, *J* = 10.0 Hz, 2H), 3.01 (s, 6H). **^{13}C NMR** (126 MHz; $CDCl_3$) δ (ppm) 150.78, 150.01, 145.57, 133.35, 128.32, 124.26, 121.17, 120.40, 112.19, 40.32. **HRMS** (ESI) m/z: calculated for $C_{15}H_{17}N_2^+$ $[M+H]^+$: 225.1386; Found: 225.1379

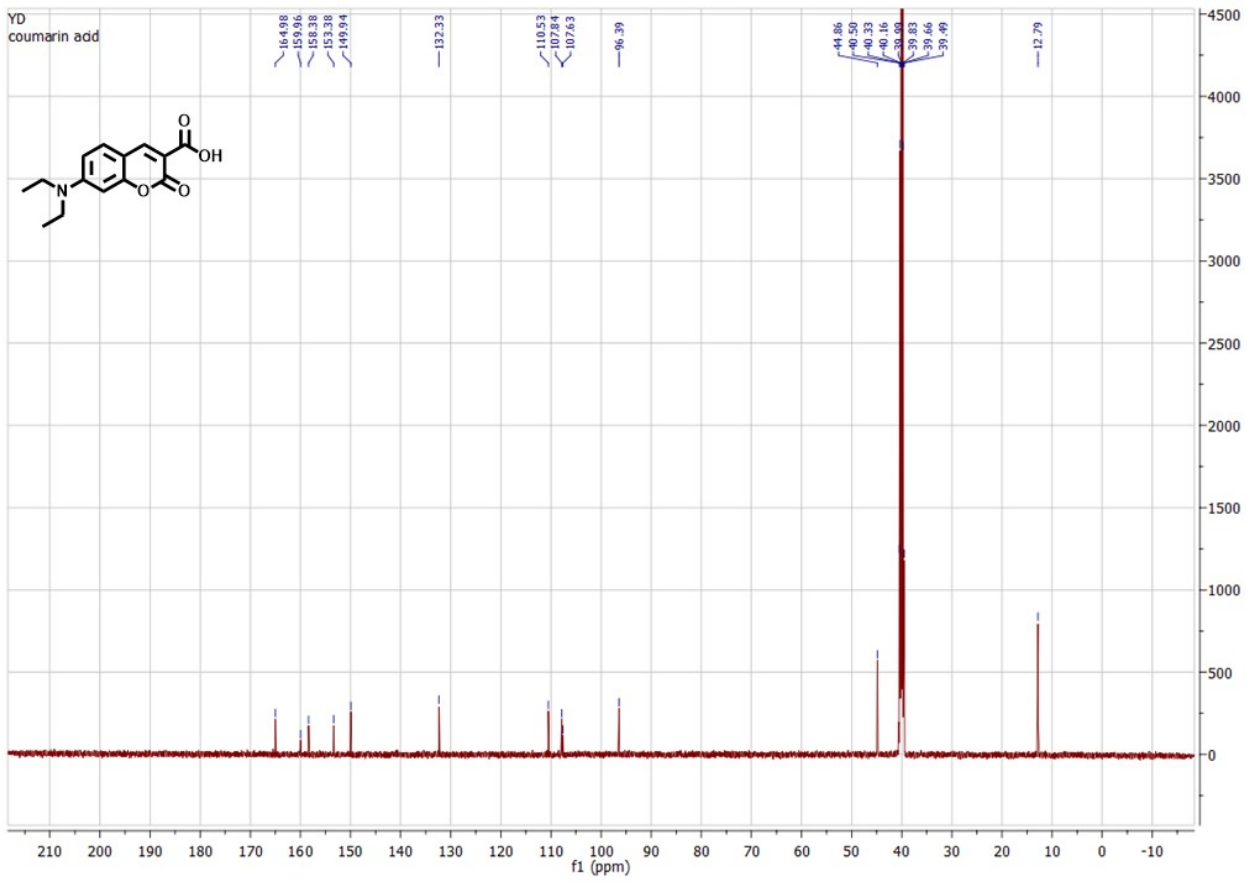
(E)-1-(7-carboxyheptyl)-4-(4-(dimethylamino)styryl)pyridin-1-ium bromide (P1)

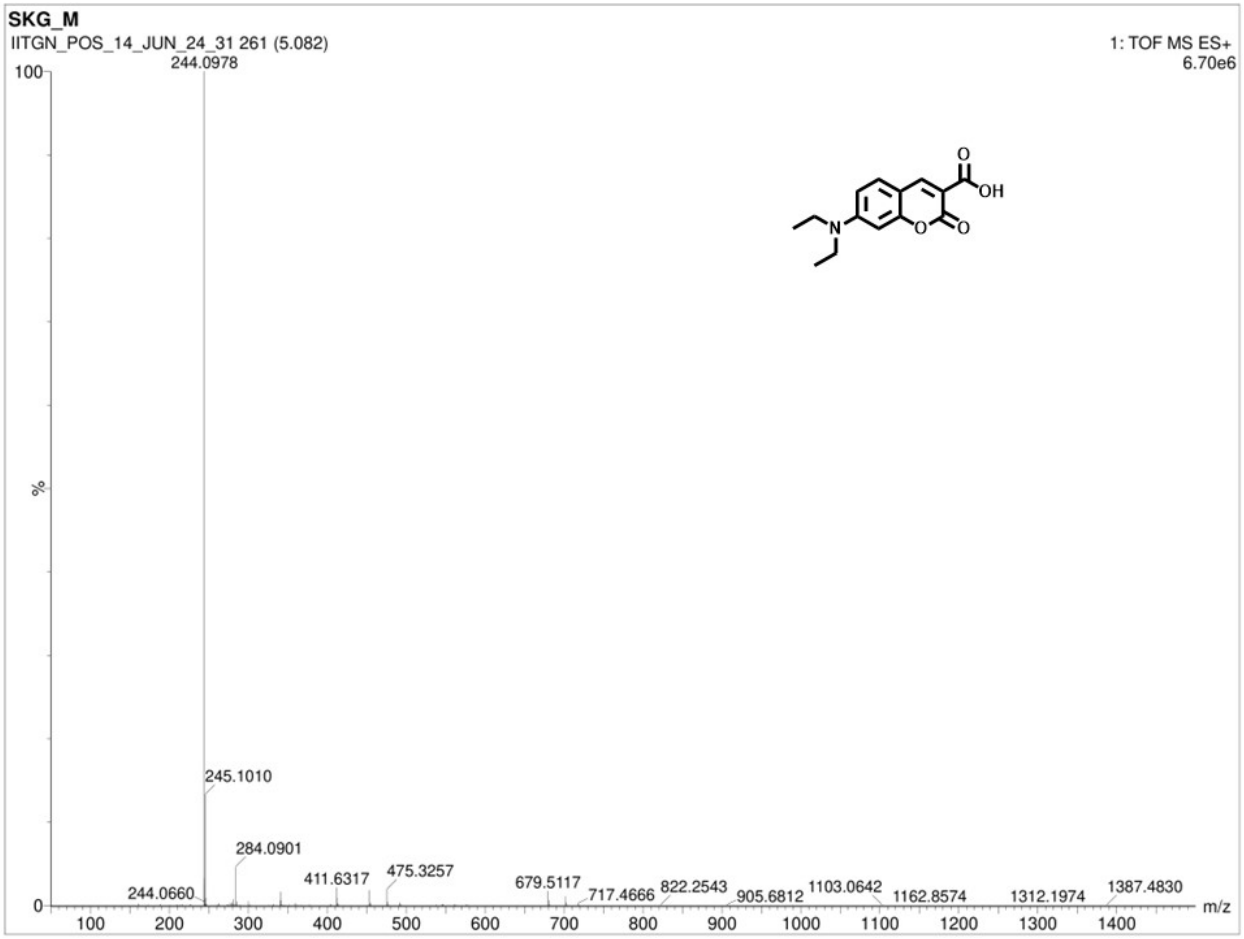
^{1}H NMR (500 MHz, DMSO-d6): δ (ppm) 12.00 (s, 1H), 8.80 (d, *J* = 10.0 Hz, 2H), 8.08 (d, *J* = 5.0 Hz, 2H), 7.94 (d, *J* = 15.0 Hz, 1H), 7.61 (d, *J* = 5.0 Hz, 2H), 7.19 (d, *J* = 15.0 Hz, 1H), 6.79 (d, *J* = 10.0 Hz, 2H), 4.42 (t, *J* = 5.0 Hz, 2H), 3.03 (s, 6H), 2.19 (t, *J* = 5.0 Hz, 2H), 1.87 (t, *J* = 5.0 Hz, 2H), 1.48 (t, *J* = 5.0 Hz, 2H), 1.28 (t, *J* = 5.0 Hz, 6H). **^{13}C NMR** (126 MHz; DMSO-d6) δ (ppm) 174.92, 154.20, 152.40, 143.96, 142.65, 130.66, 122.97, 122.88, 117.61, 112.43, 59.55, 34.05, 30.91, 28.75, 28.55, 25.76, 24.80. **HRMS** (ESI) m/z: calculated for $C_{23}H_{31}N_2O_2^+$ $[M]^+$: 367.2380; Found: 367.2390

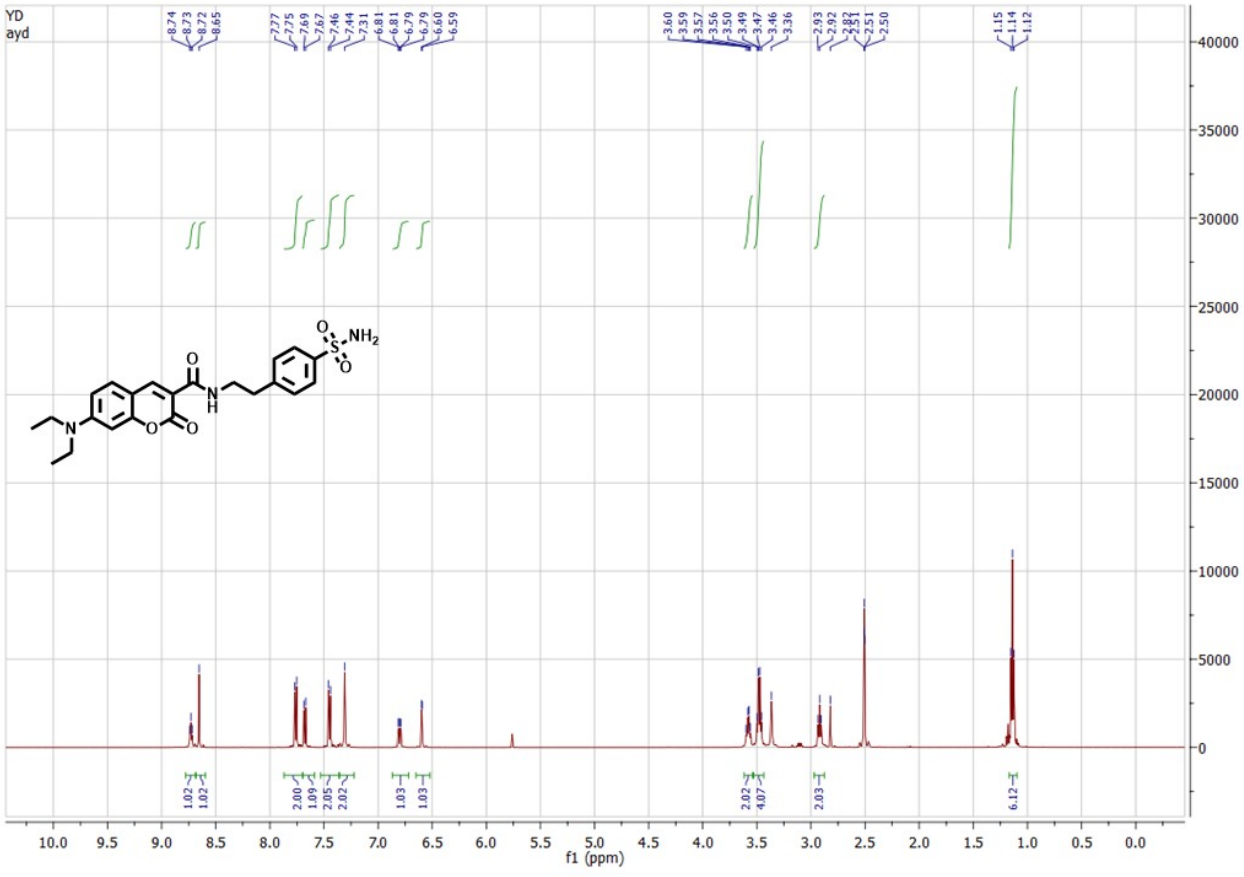
4-((E)-4-(dimethylamino)styryl)-1-(8-((4-(2-(8-(4-((E)-4-(dimethylamino)styryl)pyridin-1-ium-1-yl)octanamido)ethyl)phenyl)sulfonamido)-8-oxooctyl)pyridin-1-ium bromide (P2)

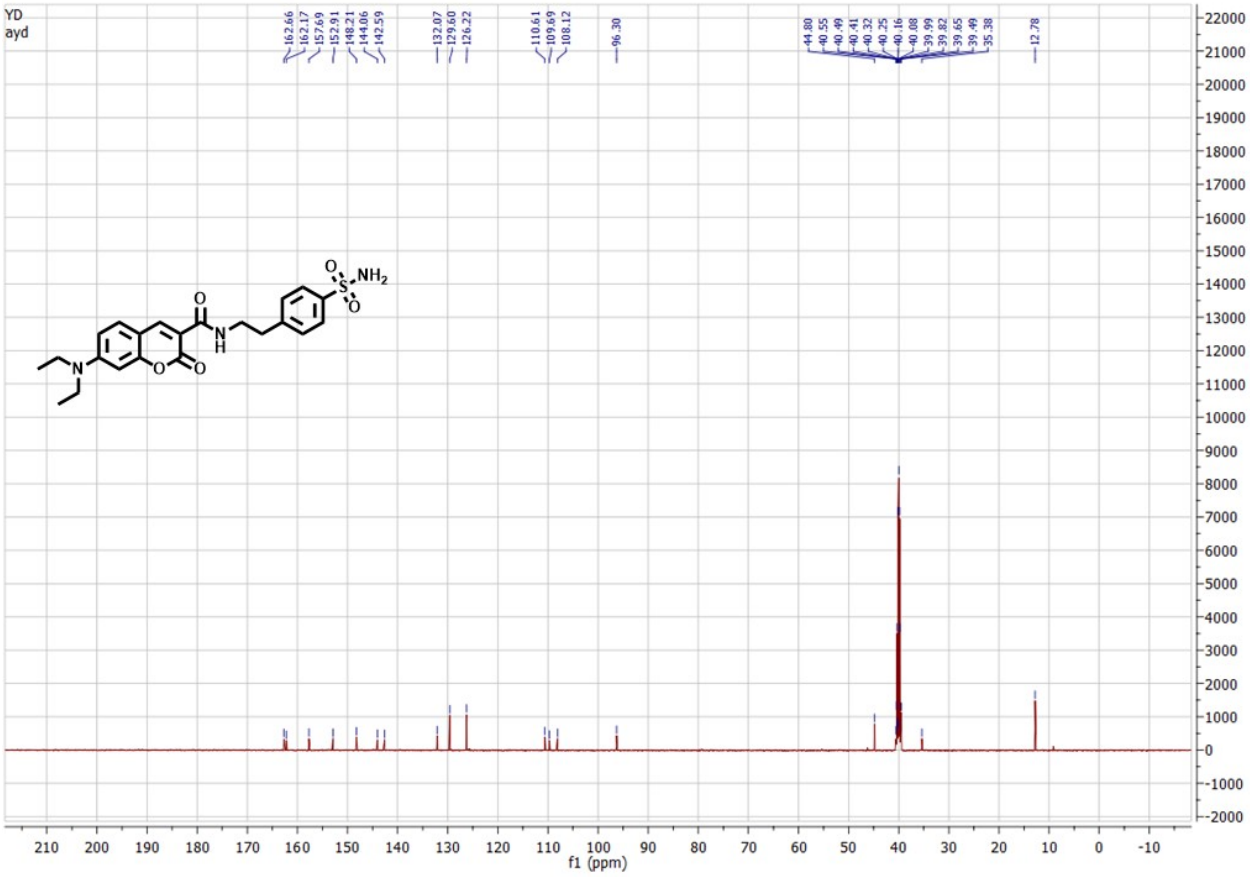
^{1}H NMR (500 MHz, DMSO-d6): δ (ppm) 8.79 (d, *J* = 10.0 Hz, 2H), 8.05 (d, *J* = 10.0 Hz, 2H), 7.95-7.90 (m, 2H), 7.74 (d, *J* = 10.0 Hz, 1H), 7.66 (d, *J* = 10.0 Hz, 1H), 7.60 (d, *J* = 10.0 Hz, 2H), 7.53 (s, 4H), 7.38 (d, *J* = 10.0 Hz, 1H), 7.34 (s, 1H), 7.30 (d, *J* = 10.0 Hz, 1H), 7.18 (d, *J* = 15.0 Hz, 1H), 6.79 (d, *J* = 10.0 Hz, 2H), 4.48 (s, 1H), 4.42 (d, *J* = 5.0 Hz, 2H), 3.29 (d, *J* = 5.0 Hz, 2H), 3.16 (q, J_1 = 5.0 Hz, J_2 = 10.0 Hz, 4H), 3.02 (s, 6H), 2.83 (s, 6H), 2.79-2.75 (m, 1H), 2.02 (q, *J* = 5.0 Hz, 2H), 1.87 (d, *J* = 10.0 Hz, 2H), 1.44 (d, *J* = 5.0 Hz, 2H), 1.32-1.18 (m, 19H). **^{13}C NMR** (126 MHz; DMSO-d6) δ (ppm) 172.54, 172.48, 161.54, 154.20, 152.40, 144.25, 143.93, 142.65, 142.47, 133.01, 130.71, 130.66, 129.56, 129.53, 129.24, 128.31, 126.10, 125.57, 122.96, 122.87, 117.59, 112.43, 59.93, 59.59, 52.46, 35.76, 30.93, 28.61, 25.78, 25.56, 7.94. **HRMS** (ESI) m/z: calculated for $C_{54}H_{70}Br_2N_6O_4S^+$ $[M]^+$: 1056.3546; Found: 1056.2958

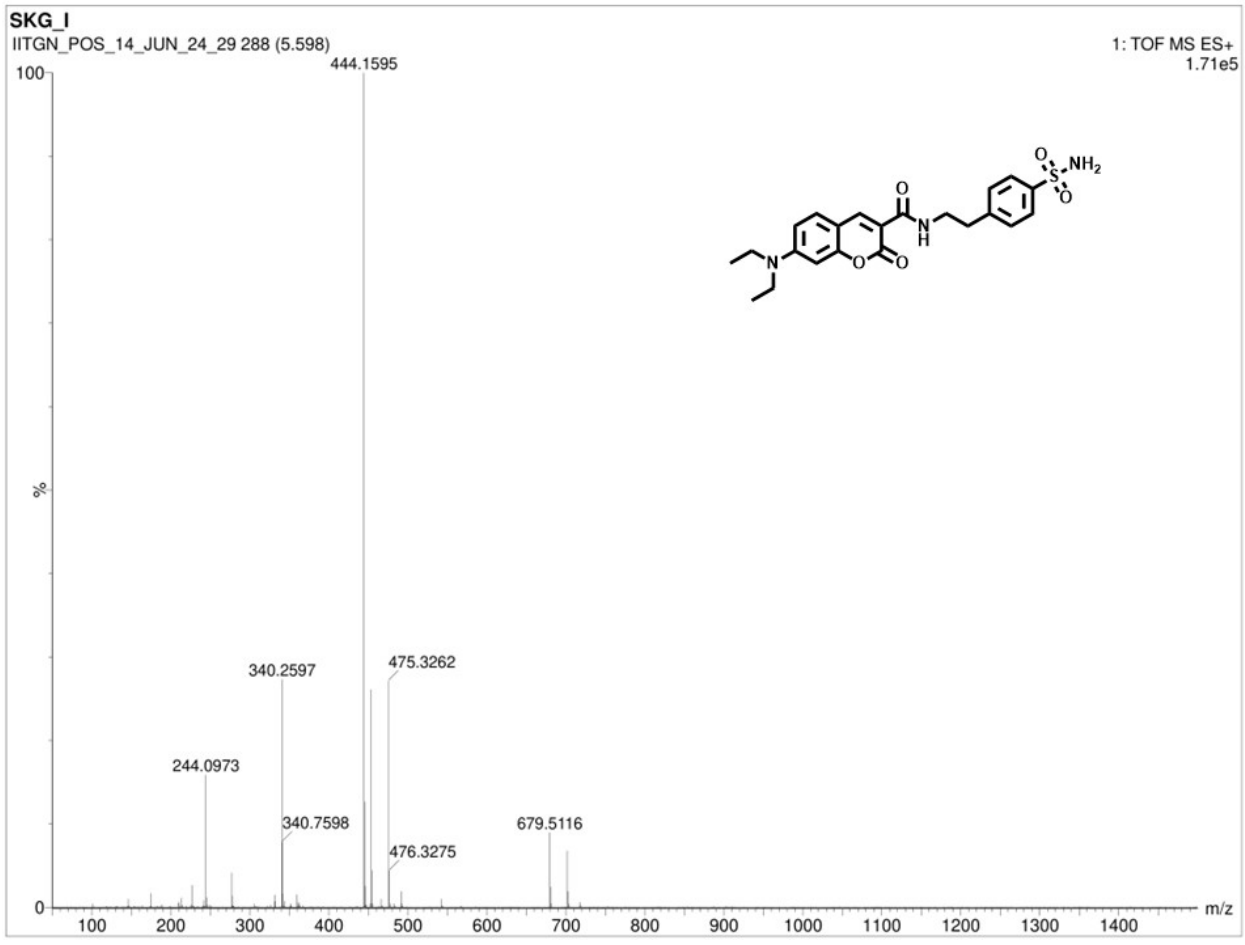

YD
coumarin acid
12.52
8.60
7.66
7.64
6.82
6.81
6.80
6.79
6.58
6.58
3.51
3.50
3.48
3.47
3.34
2.51
2.51
2.50
1.16
1.14
1.13
1.06
1.00
1.03
1.02
1.01
4.05
6.01
f1 (ppm)

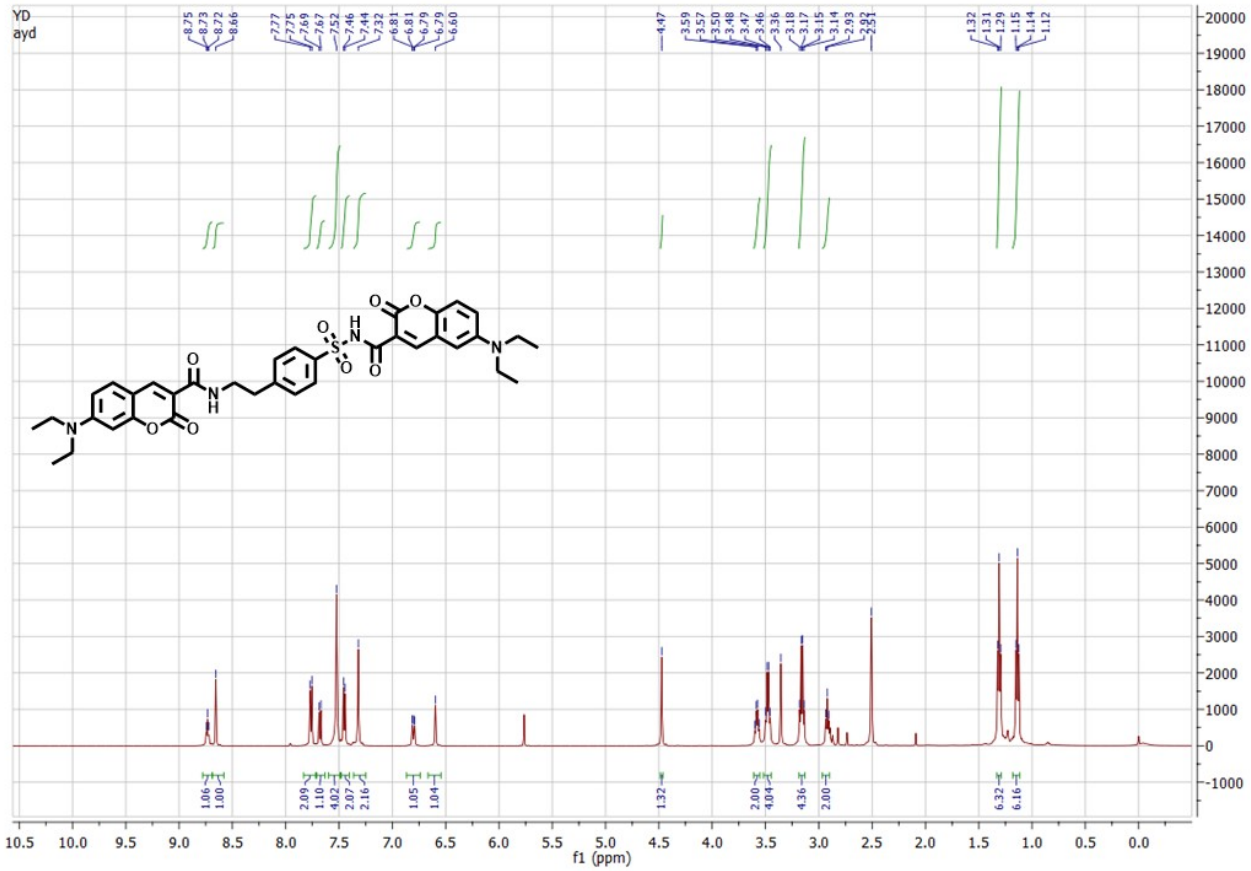

YD
coumarin acid
164.98
159.96
158.38
153.38
149.94
132.33
110.53
107.84
107.63
96.39
44.86
40.50
40.33
40.16
39.99
39.83
39.66
39.49
12.79
f1 (ppm)

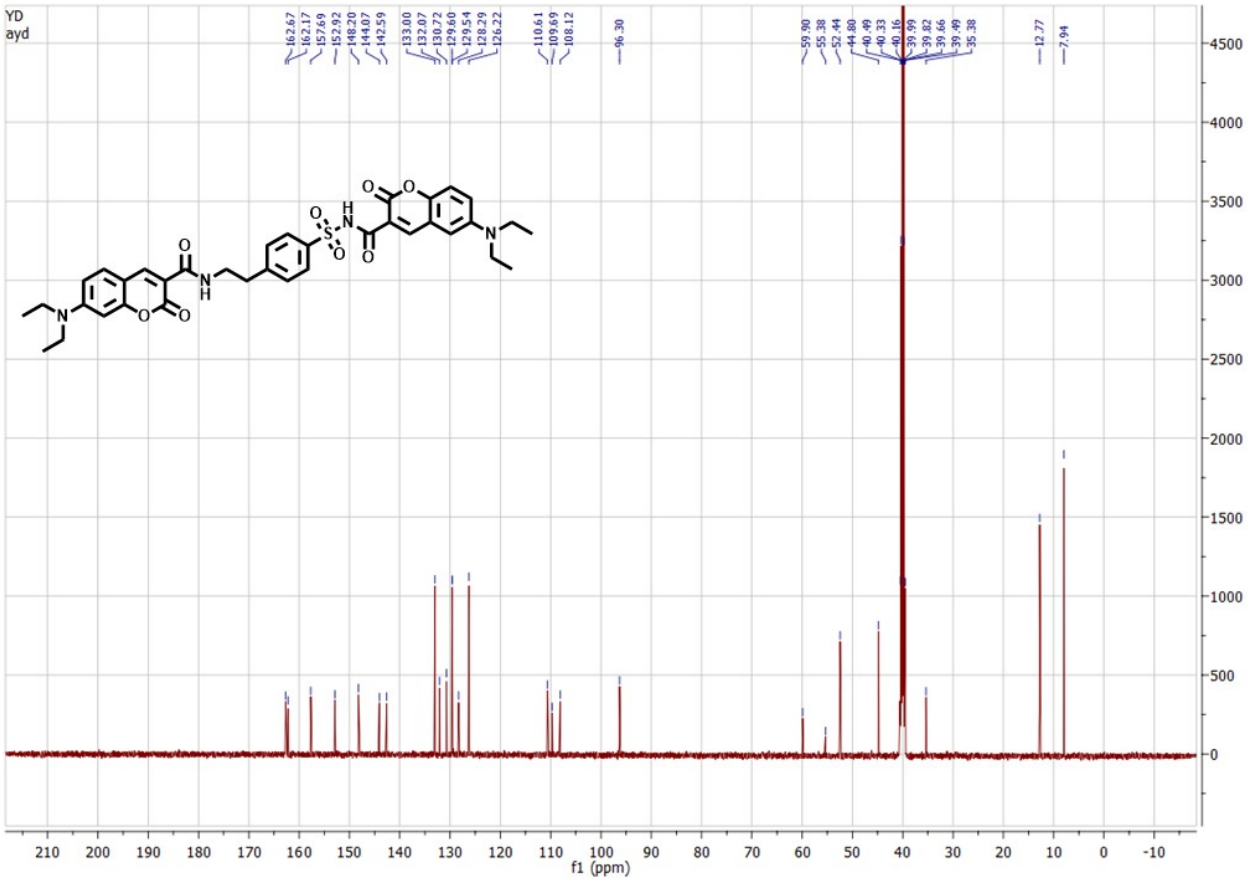

SKG_M
IITGN_POS_14_JUN_24_31 261 (5.082)
1: TOF MS ES+
6.70e6
244.0978
245.1010
284.0901
244.0660
411.6317
475.3257
679.5117
717.4666
822.2543
905.6812
1103.0642
1162.8574
1312.1974
1387.4830
%
m/z

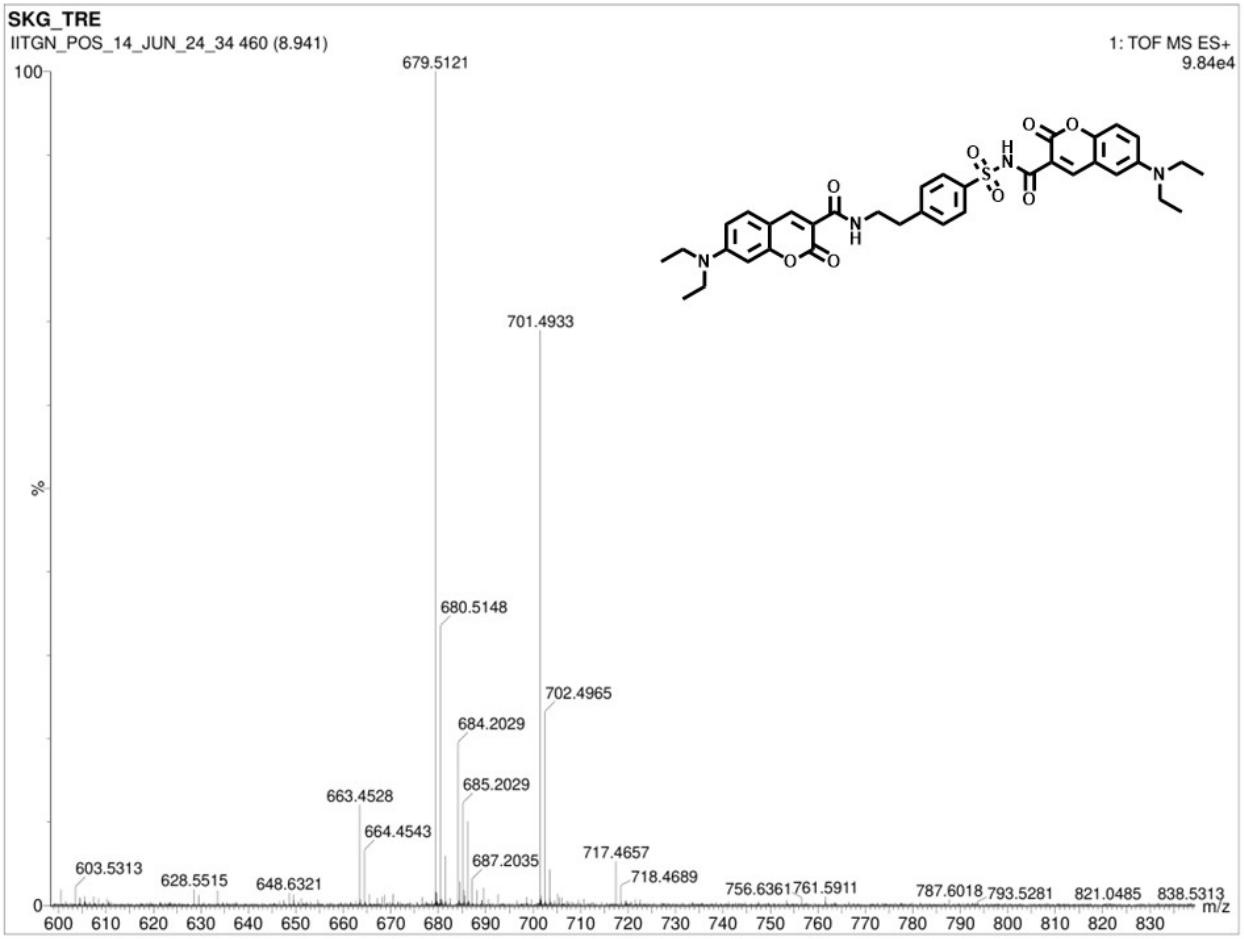

YD
ayd
f1 (ppm)

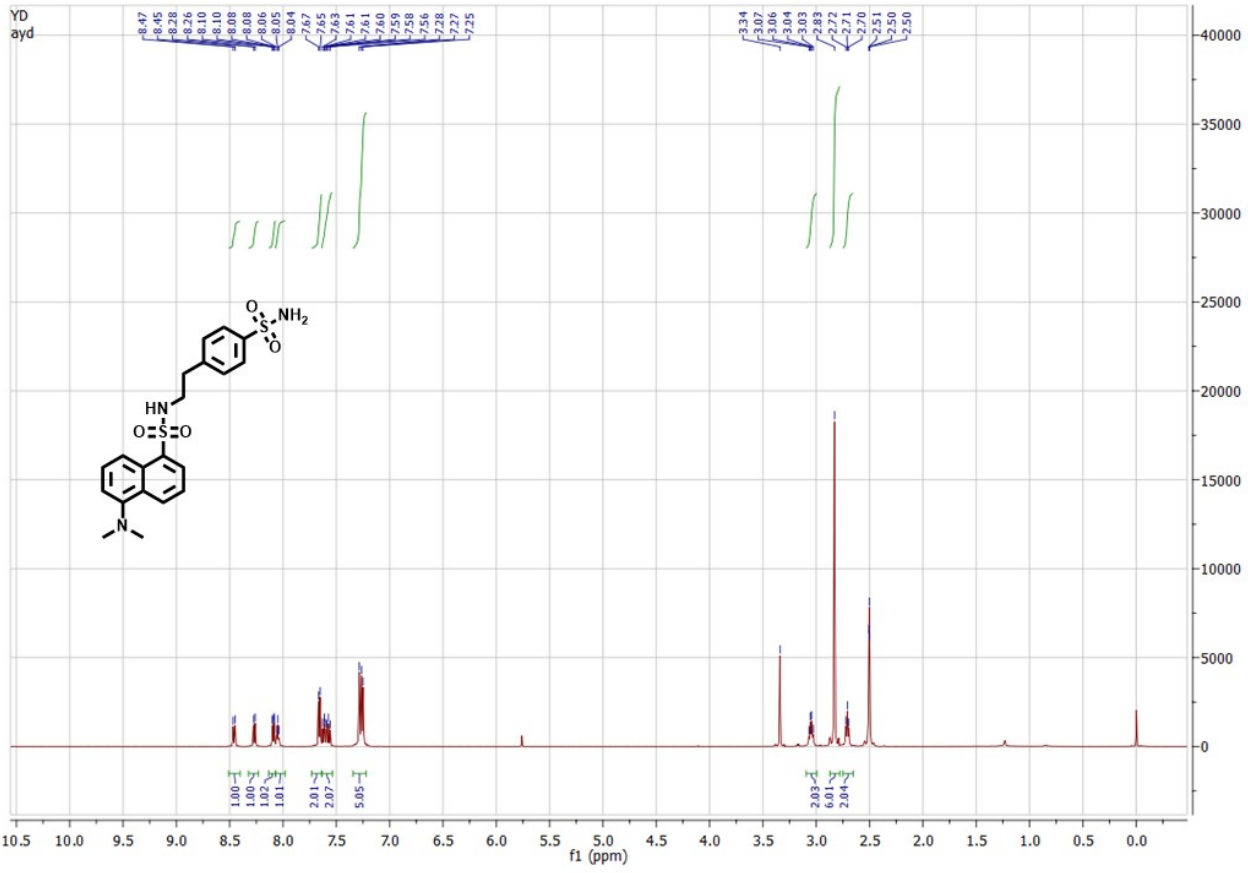

YD
ayd
162.66
162.17
157.69
152.91
148.21
144.06
142.59
132.07
129.60
126.22
110.61
109.69
108.12
96.30
44.80
40.55
40.49
40.41
40.32
40.25
40.16
40.08
39.99
39.82
39.65
39.49
39.32
12.78
O
S
NH2
O
O
N
H
O
O
N
f1 (ppm)

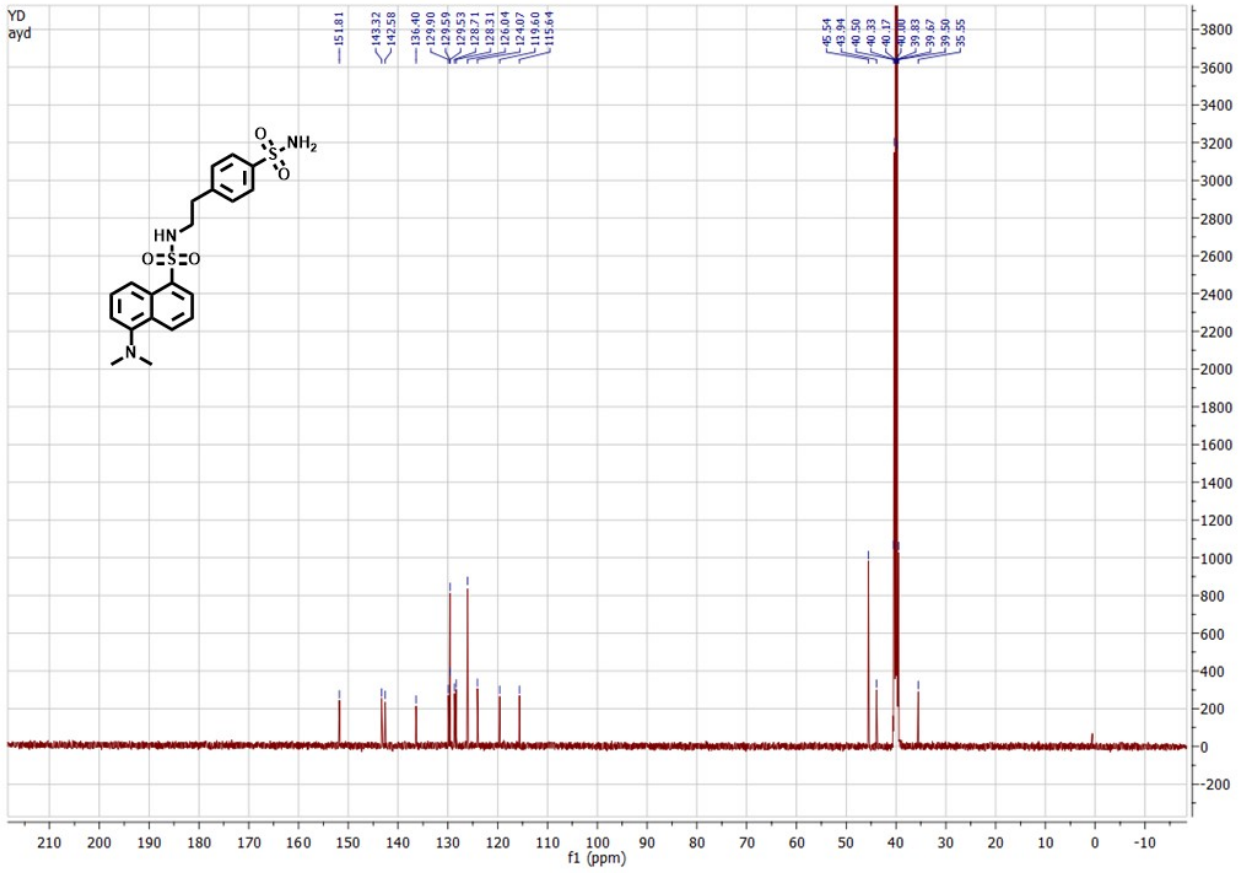

SKG_I
IITGN_POS_14_JUN_24_29 288 (5.598)
1: TOF MS ES+
1.71e5
444.1595
475.3262
340.2597
244.0973
340.7598
679.5116
476.3275
%
m/z
O
S
NH2
O
O
N
H
O
O
N

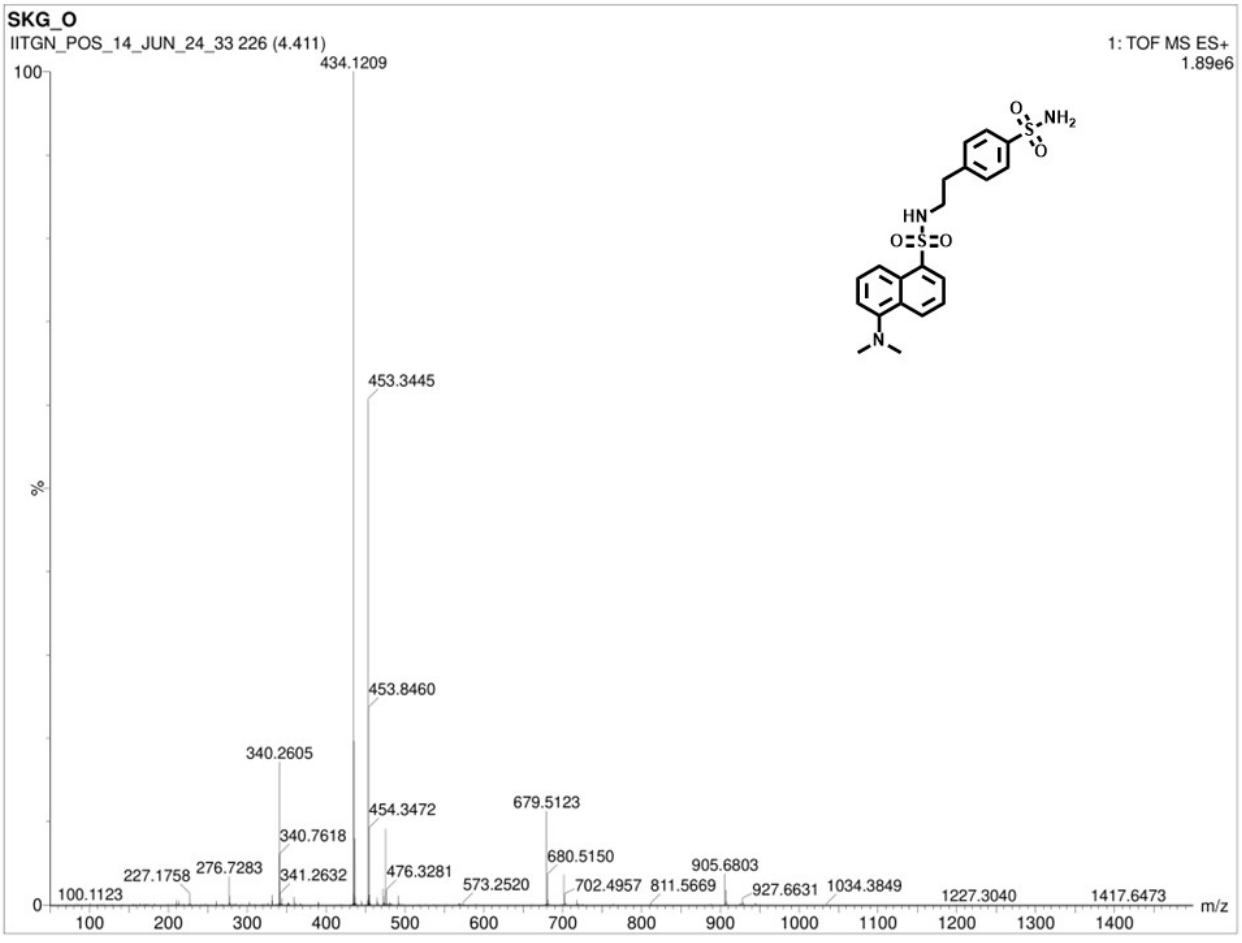

YD
ayd
f1 (ppm)

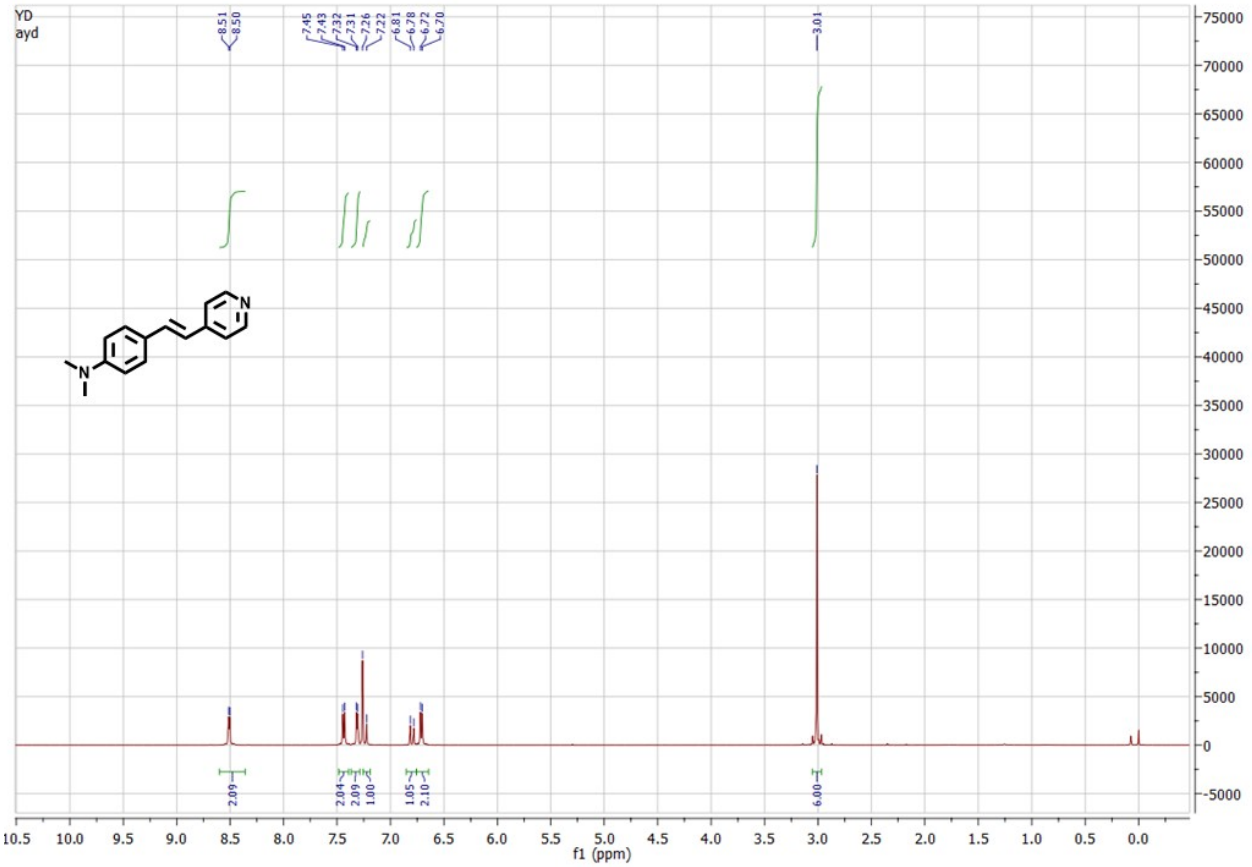

YD
ayd
f1 (ppm)

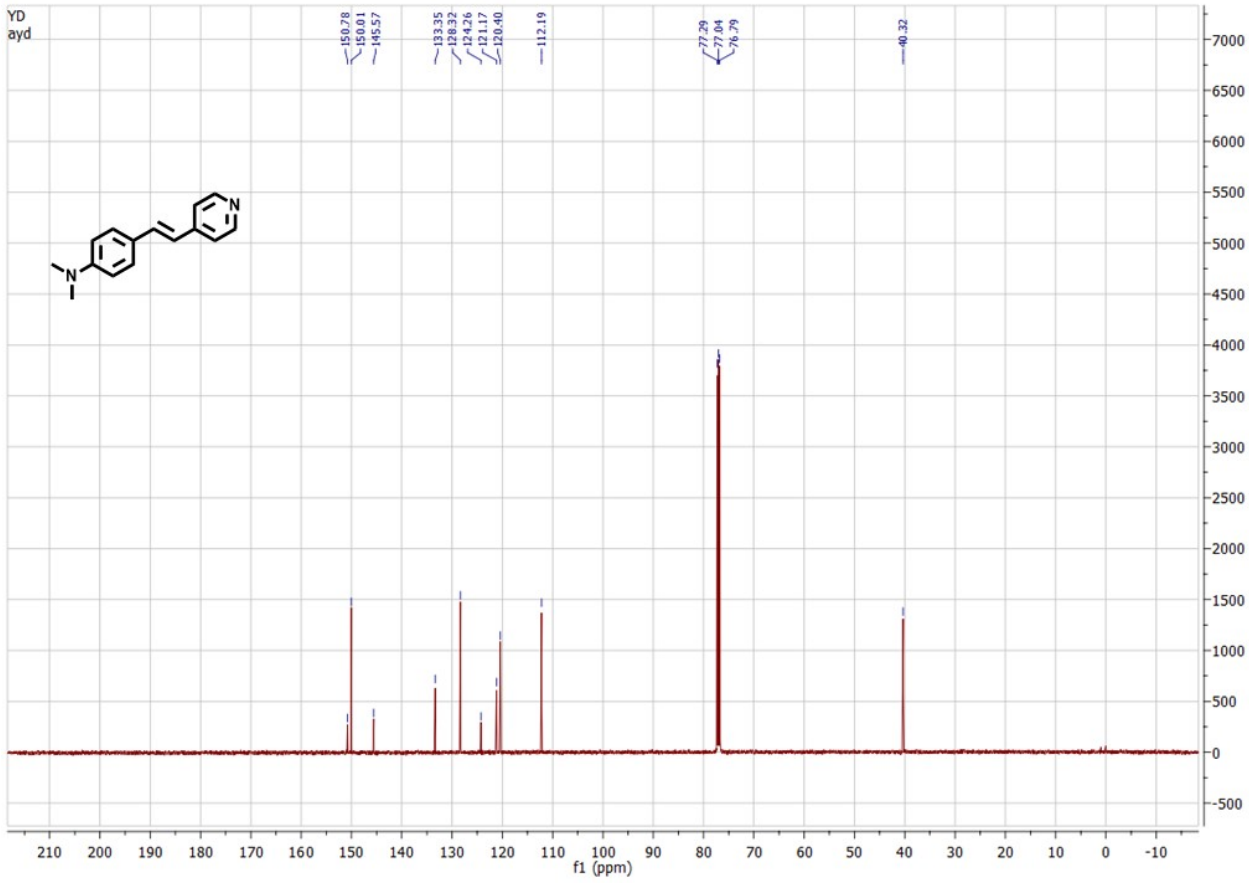

SKG_TRE
IITGN_POS_14_JUN_24_34 460 (8.941)
1: TOF MS ES+
9.84e4
679.5121
701.4933
680.5148
702.4965
684.2029
685.2029
663.4528
664.4543
687.2035
717.4657
718.4689
603.5313
628.5515
648.6321
756.6361
761.5911
787.6018
793.5281
821.0485
838.5313
%
m/z

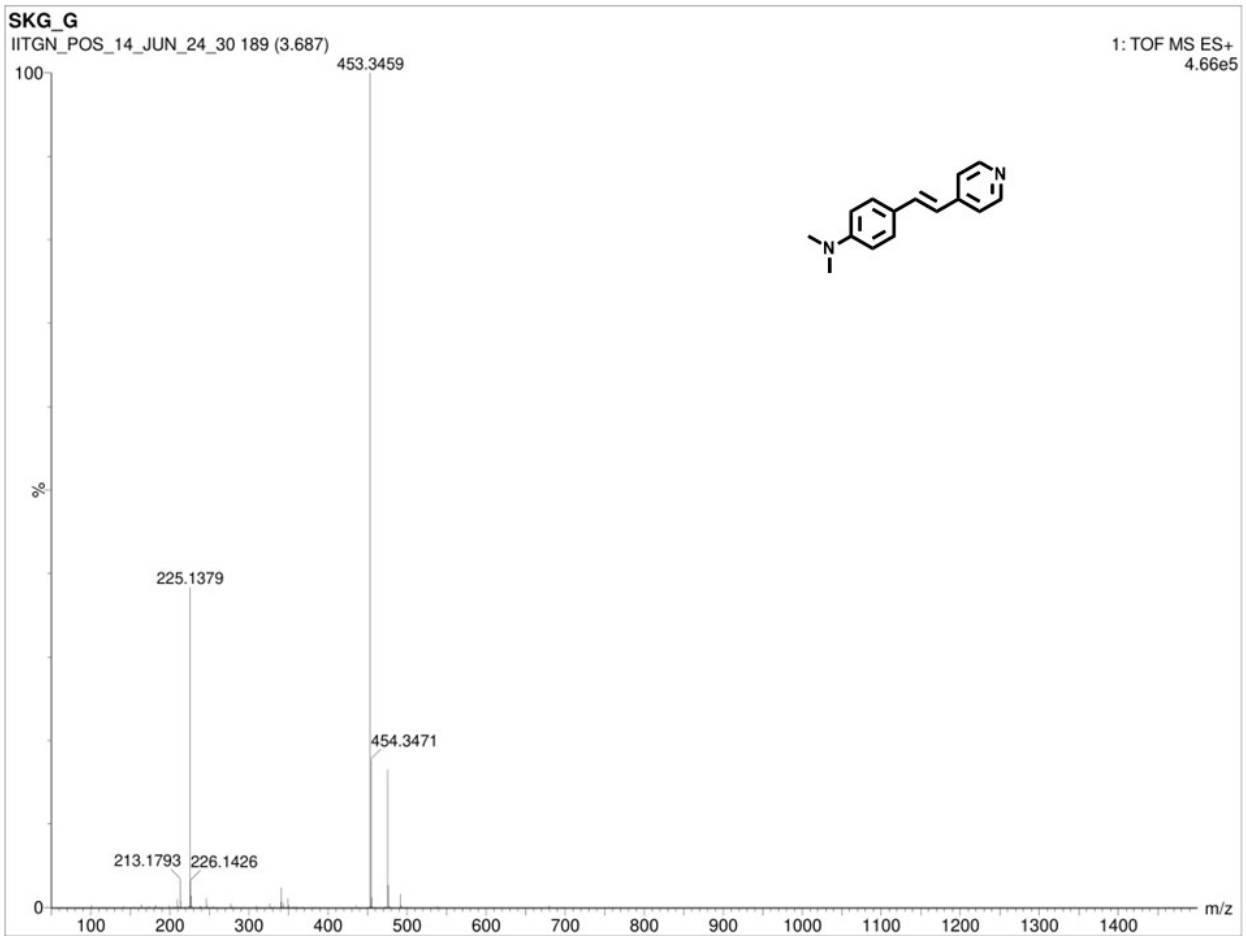

YD
ayd
f1 (ppm)

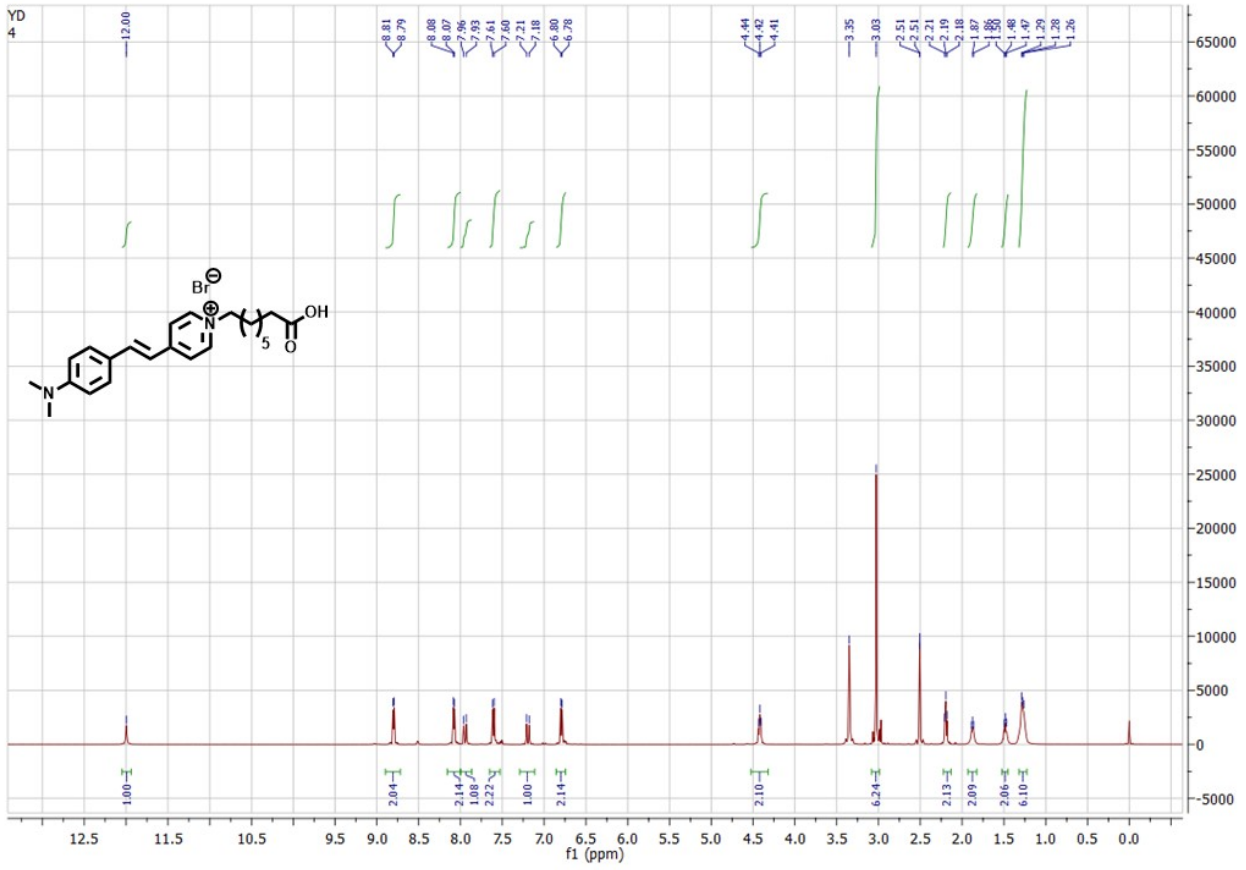

YD
ayd
151.81
143.32
142.58
136.40
129.90
129.59
129.53
128.71
128.31
126.04
124.07
119.60
115.64
45.54
43.94
40.50
40.33
40.17
40.00
39.83
39.67
39.50
35.55
f1 (ppm)

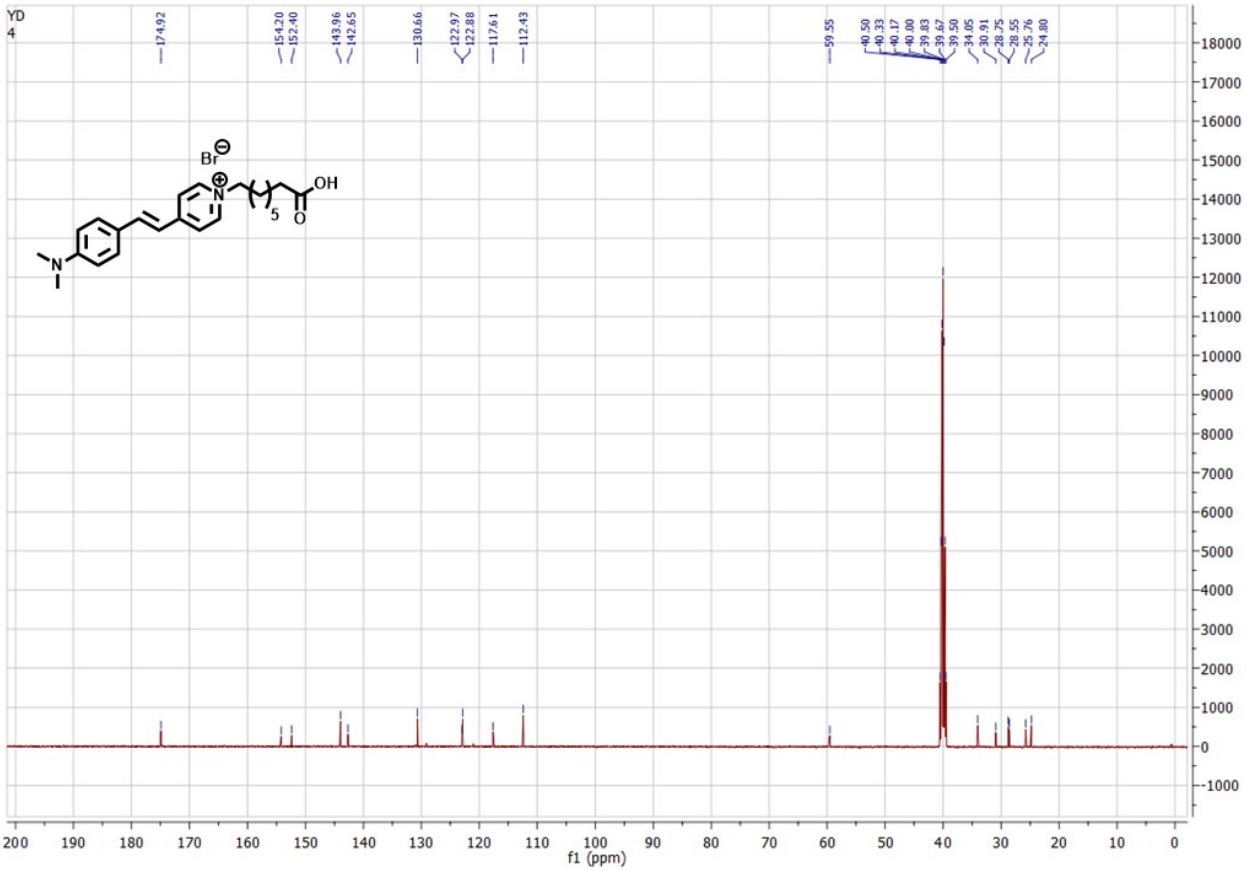

SKG_O
IITGN_POS_14_JUN_24_33 226 (4.411)
1: TOF MS ES+
1.89e6
434.1209
453.3445
453.8460
340.2605
454.3472
679.5123
340.7618
680.5150
905.6803
227.1758
276.7283
341.2632
476.3281
573.2520
702.4957
811.5669
927.6631
1034.3849
1227.3040
1417.6473
100.1123
%
m/z

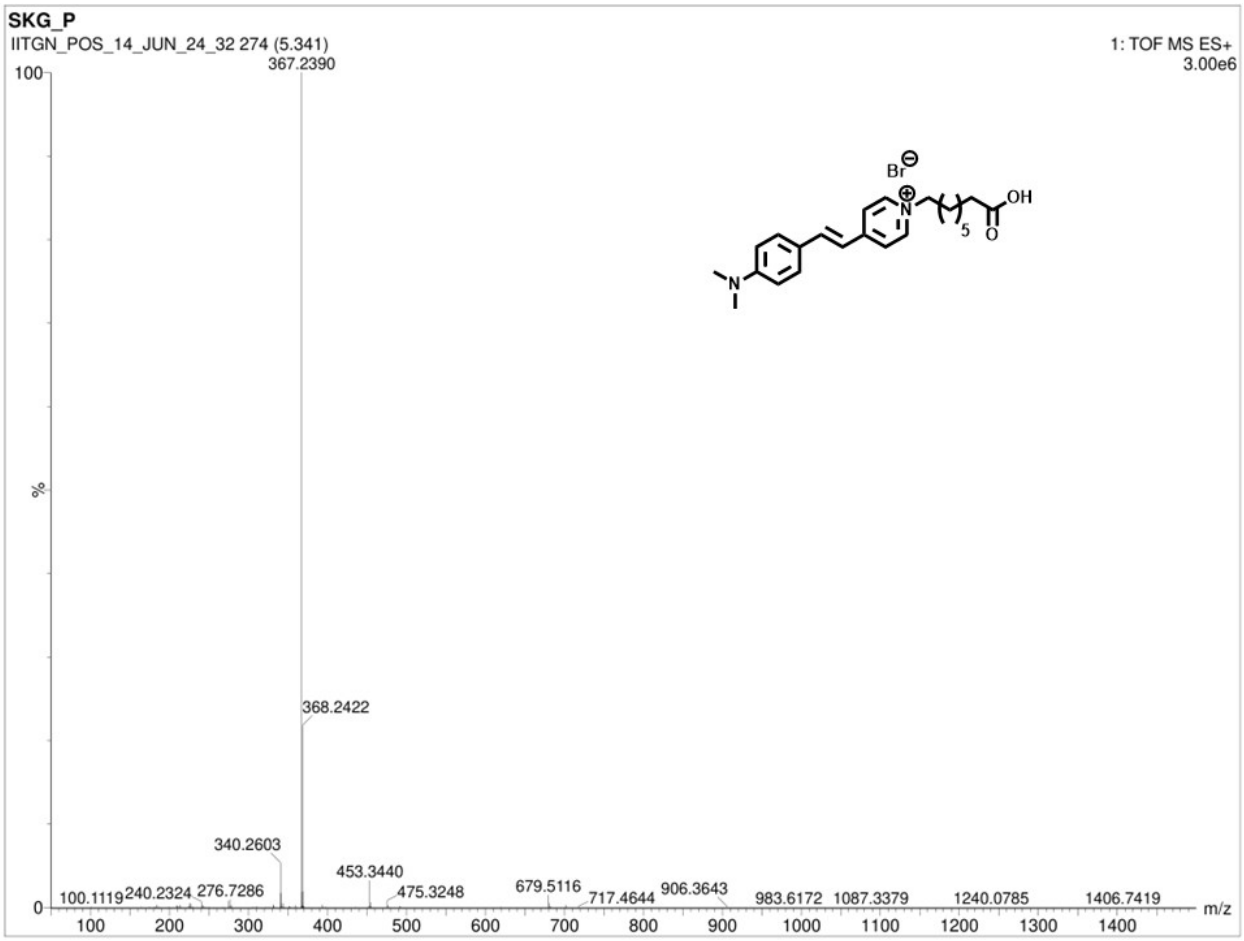

YD
ayd
8.51
8.50
7.45
7.43
7.32
7.31
7.26
7.22
6.81
6.78
6.72
6.70
3.01
2.09
2.04
2.09
1.00
1.05
2.10
6.00
f1 (ppm)

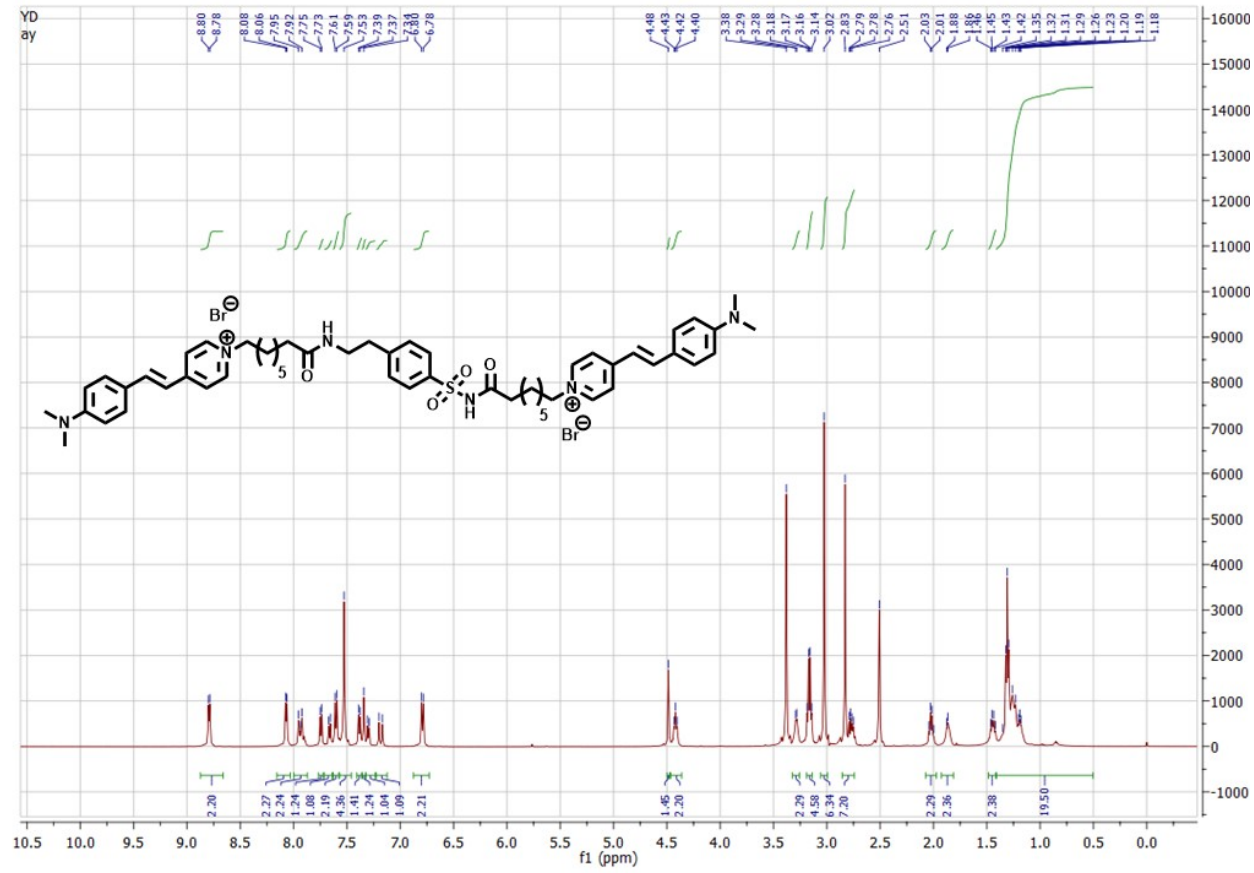

YD
ayd
150.78
150.01
145.57
133.35
128.32
124.26
121.17
120.40
112.19
77.29
77.04
76.79
40.32
f1 (ppm)

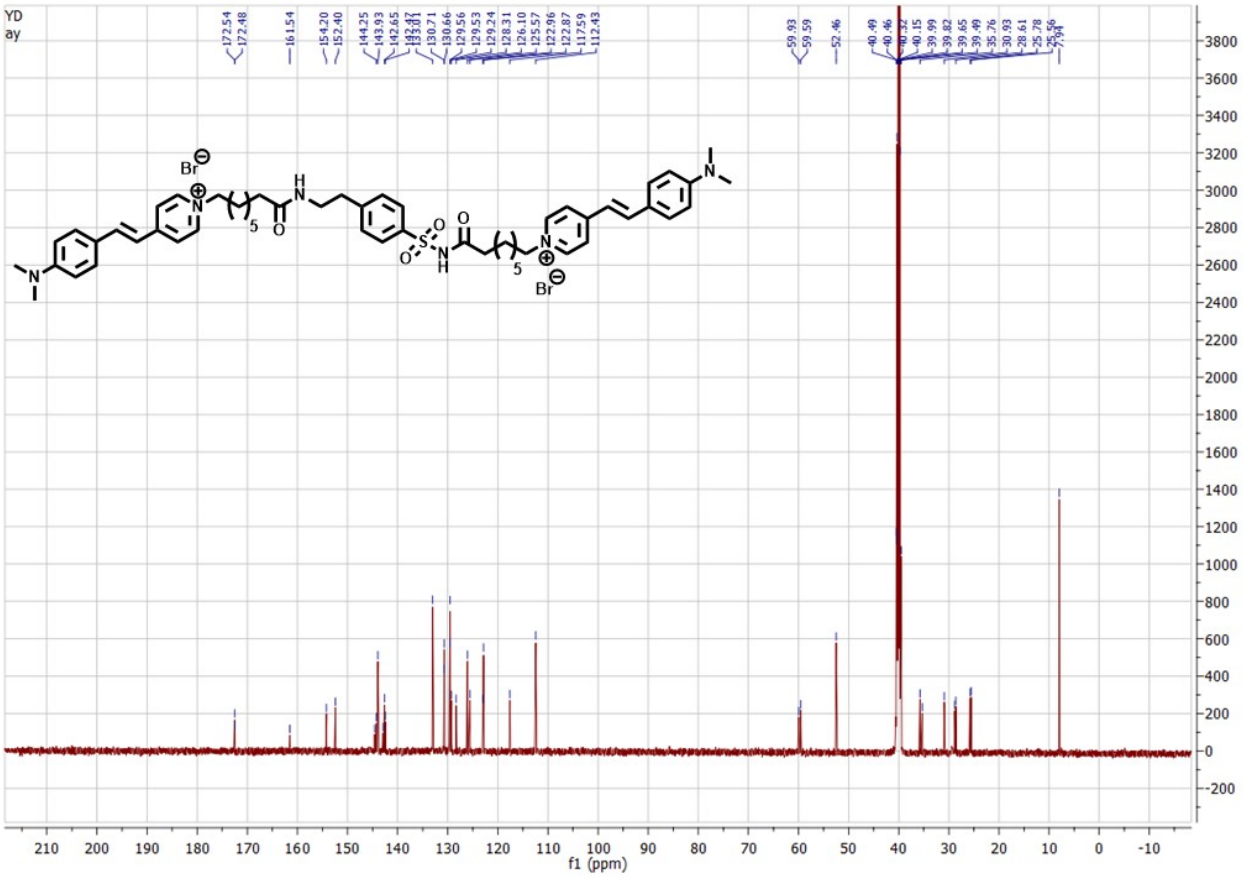

SKG_G
IITGN_POS_14_JUN_24_30 189 (3.687)
1: TOF MS ES+
4.66e5
453.3459
454.3471
225.1379
213.1793
226.1426
%
m/z

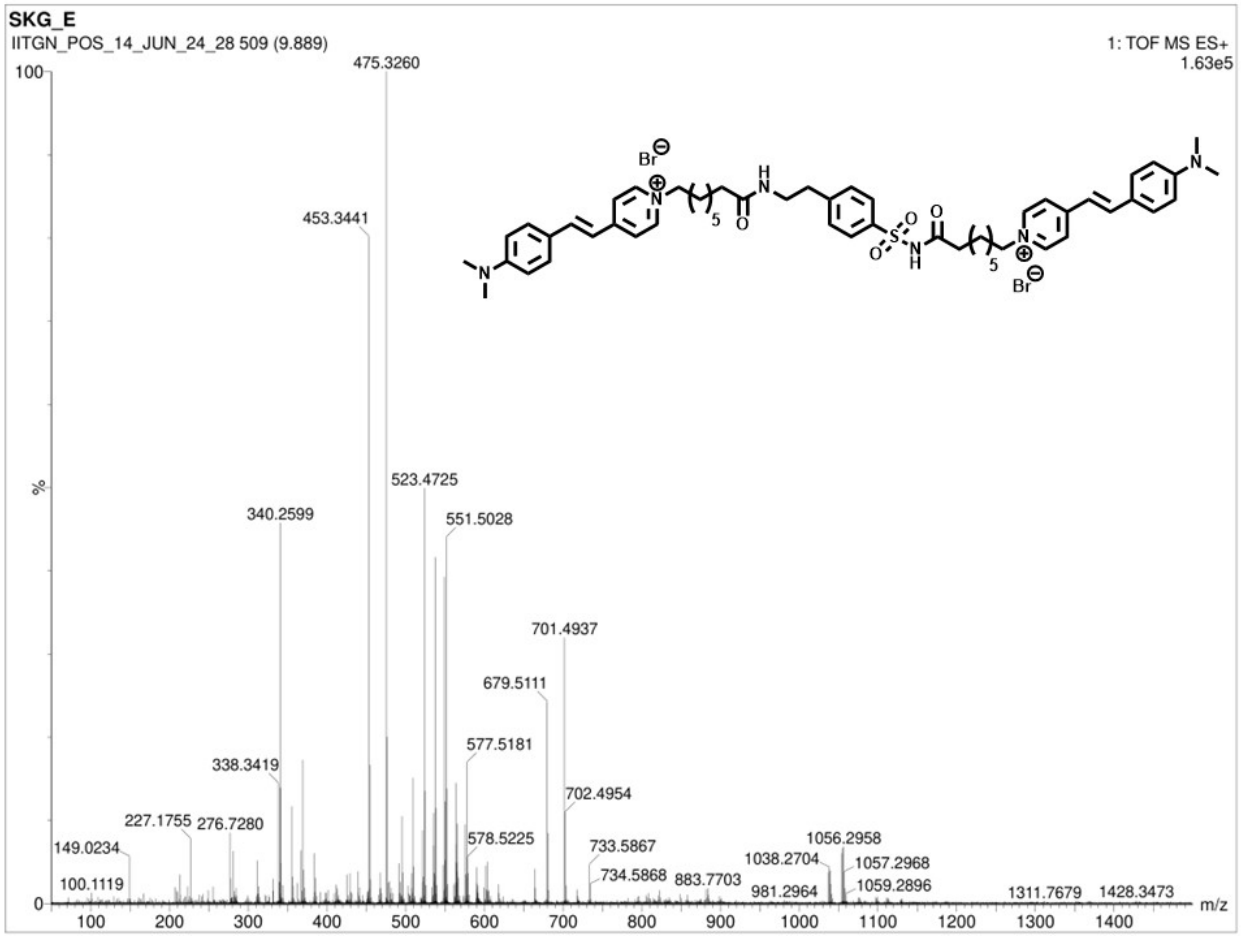

YD
4
f1 (ppm)


YD
4
174.92
154.20
152.40
143.96
142.65
130.66
122.97
122.88
117.61
112.43
59.55
40.50
40.33
40.17
40.00
39.83
39.67
39.50
34.05
30.91
28.75
28.55
25.76
24.80
Br
OH
O
5
N
f1 (ppm)


SKG_P
IITGN_POS_14_JUN_24_32 274 (5.341)
1: TOF MS ES+
3.00e6
367.2390
368.2422
340.2603
453.3440
100.1119
240.2324
276.7286
475.3248
679.5116
717.4644
906.3643
983.6172
1087.3379
1240.0785
1406.7419
%
m/z
Br
OH
O
5
N


YD
ay
f1 (ppm)


YD
ay
f1 (ppm)



References

1. Nelson, Grady L., Conor T. Ronayne, Lucas N. Solano, Sravan K. Jonnalagadda, Shirisha Jonnalagadda, Tanner J. Schumacher, Zachary S. Gardner et al, *Bioorganic & Medicinal Chemistry Letters*, 2021, **52**, 128411.
2. James, Samantha N., and Mark J. Coster, *Tetrahedron Letters*, 2015, **56**, 2059-2061.
3. Mukherjee, Tarushyam, M. Aravintha Siva, Komal Bajaj, Virupakshi Soppina, and Sriram Kanvah, *Journal of Photochemistry and Photobiology B: Biology*, 2020, **203**, 111732.