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Supporting Information

Organelle-Targeted BODIPY Photocages: Visible-Light-Mediated Subcellular Photorelease

*Dnyaneshwar Kand, Lorena Pizarro, Inbar Angel, Adi Avni, Dinorah Friedmann-Morvinski, and Roy Weinstain**

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Author Contributions

D.K. Conceptualization: Equal; Data curation: Equal; Formal analysis: Lead; Investigation: Lead; Methodology: Lead; Validation: Lead; Visualization: Equal; Writing—original draft: Supporting; Writing—review & editing: Supporting
L.P. Data curation: Supporting; Formal analysis: Supporting; Visualization: Equal; Writing—review & editing: Supporting
I.A. Data curation: Supporting; Investigation: Supporting; Methodology: Supporting; Writing—review & editing: Supporting
A.A. Funding acquisition: Supporting; Supervision: Supporting; Writing—review & editing: Supporting
D.F. Conceptualization: Supporting; Data curation: Supporting; Funding acquisition: Supporting; Investigation: Supporting; Supervision: Supporting; Visualization: Supporting; Writing—original draft: Supporting; Writing—review & editing: Supporting
R.W. Conceptualization: Equal; Data curation: Equal; Funding acquisition: Lead; Project administration: Lead; Supervision: Lead; Validation: Lead; Writing—original draft: Lead; Writing—review & editing: Equal.

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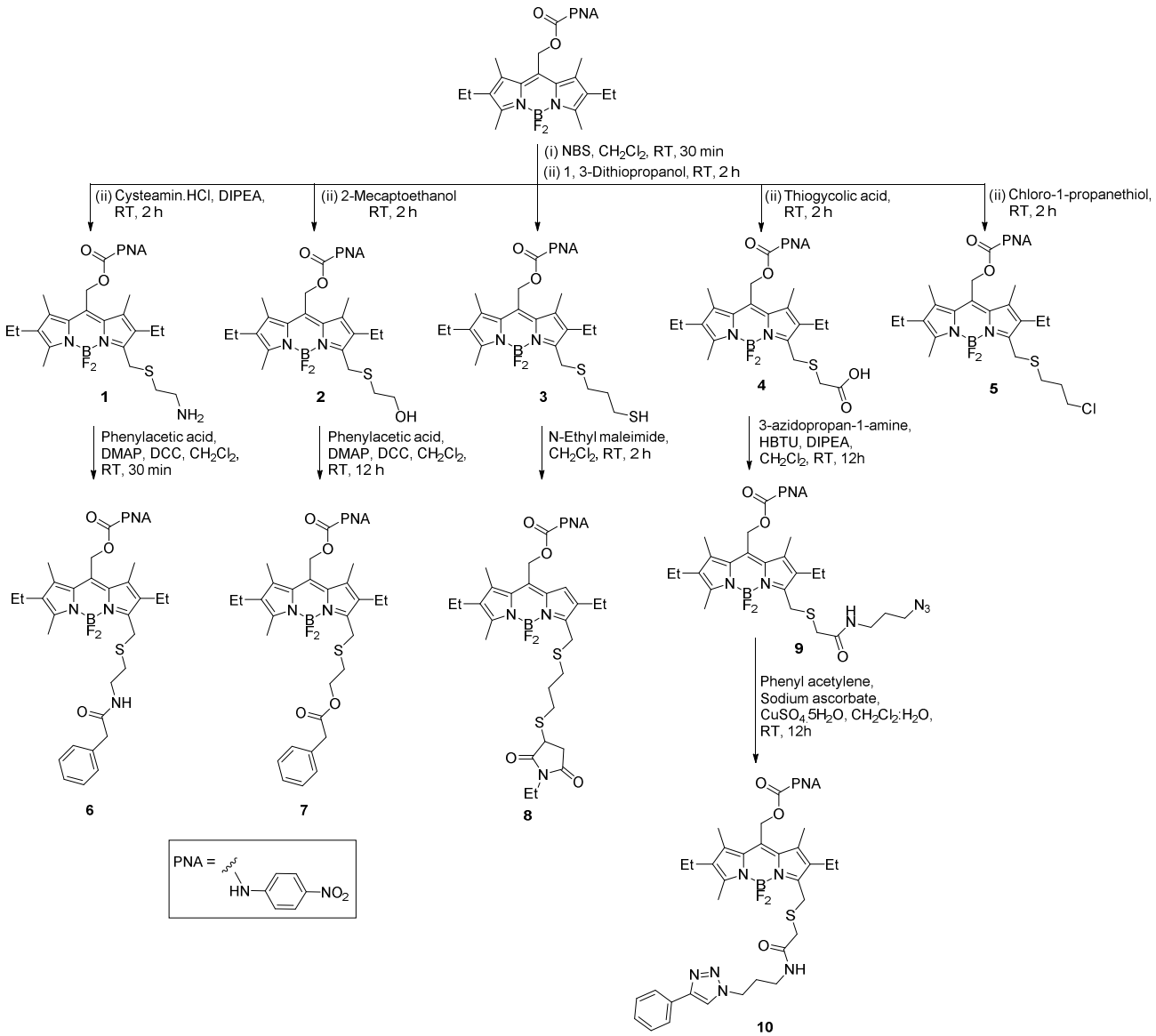


Figure S1. Synthetic scheme of compounds **1-10**.

Table S1: Spetroscopic properties of compounds **1-19**.

	absorption and emission			Photoreaction			
Compd	λ_{max} (nm)	λ_{em} (nm)	ε (M^{-1} cm^{-1})	$t_{1/2}$ (min)	Chemical yield (%)	Φ_r	$\varepsilon\,\Phi_r$
1	550	570	42980				
2	551	570	38710				
3	551	571	39710				
4	550	570	68610				
5	550	570	55487	10.23	88.	0.8×10^{-5}	4.88
6	550	570	55480	10.86	50	2.3×10^{-5}	1.26
7	550	569	48203	14.86	88	5.2×10^{-5}	2.50
8	550	568	37339	9.24	66	6.3×10^{-5}	2.34
	550	568	49663[a]				
10	550	570	60520	26.29	64	1.1×10^{-5}	0.65
11	550	568	30860	15.01	59	1.7×10^{-5}	1.00
	550	568	53319 [a]				
12	550	568	35700	16.27	58	1.6×10^{-5}	1.00
	550	568	55264 [a]				
13	550	567	53333	1.11	97	3.9×10^{-4}	21
	550	567	56639 [a]				
14	548	565	54470	1.02	79	3.4×10^{-4}	19
15	556	569	34940				
16	554	573	53100	0.42	78	8.1×10^{-4}	43
18	554	575	87291				
19	549	571	107516	17.12	75	2.0×10^{-5}	2.1

[a] Epsilon (ε) values as measured in acetonitrile.

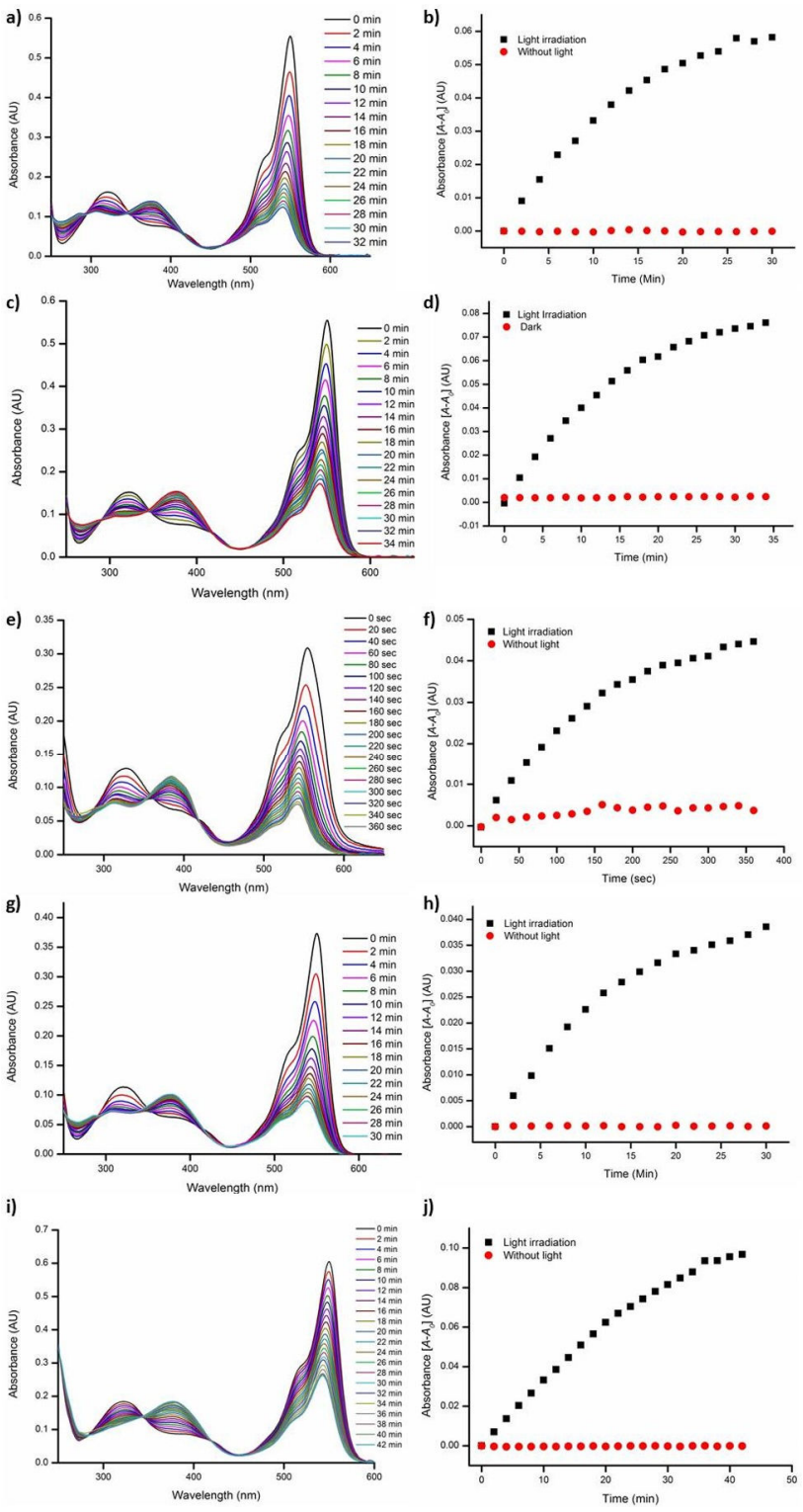


Figure S2. UV-Vis spectra of photorelease from BODIPYs **5-8** and **10** (10 μM in 7/3 acetonitrile/water) exciting at λ = 545 ± 40 nm: *Left panel:* Absorbance spectra (measured every 2 min), for BODIPY **5** (**a**), **6** (**c**), **7** (**e**), **8** (**g**) and **10** (**i**). *Right panel:* Absorbance at 380 nm *Vs* time is plotted, for BODIPY **5** (**b**), **6** (**d**), **7** (**f**), **8** (**h**) and **10** (**j**).

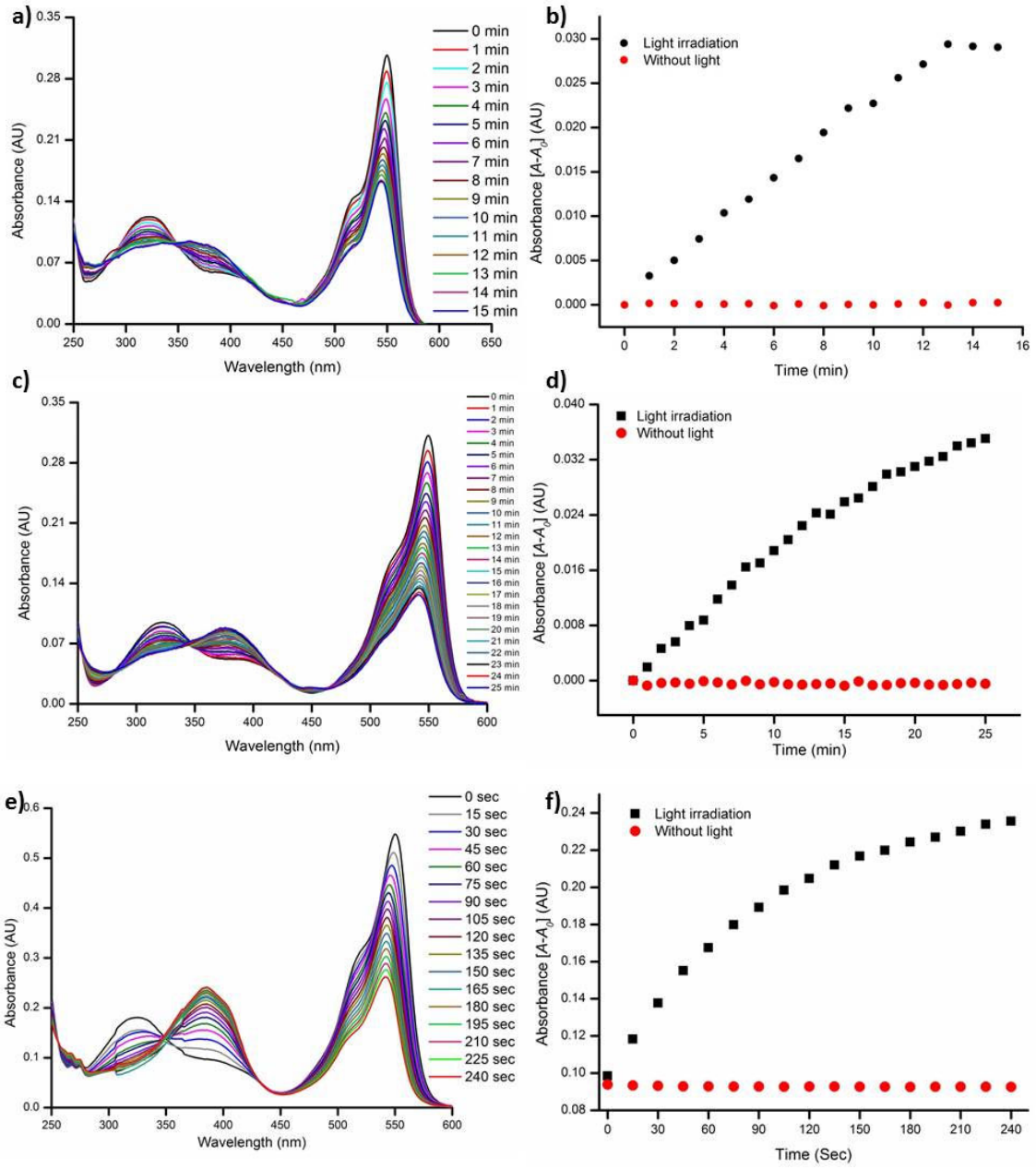


Figure S3. UV-Vis spectra of photorelease from BODIPYs **11-13** (10 μM in 7/3 acetonitrile/water), exciting at λ = 545 ± 40 nm: *Left panel:* Absorbance spectra (measured every 2 min) for BODIPY **11** (**a**), **12** (**c**) and **13** (**e**). *Right panel:* Absorbance at 380 nm *Vs* time is plotted, for BODIPY **11** (**b**), **12** (**d**) and **13** (**f**).

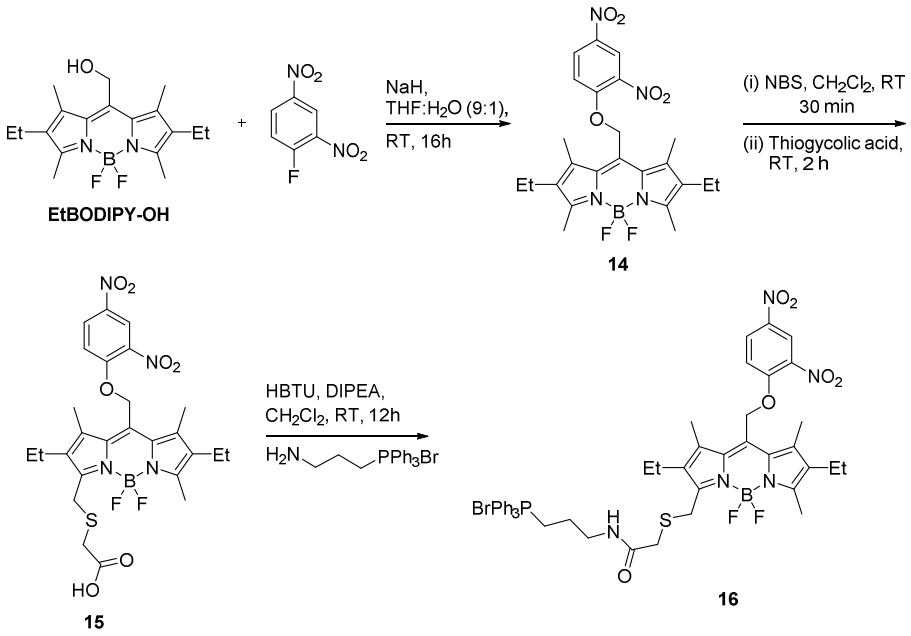


Figure S4. Synthetic scheme for compounds **14-16**.

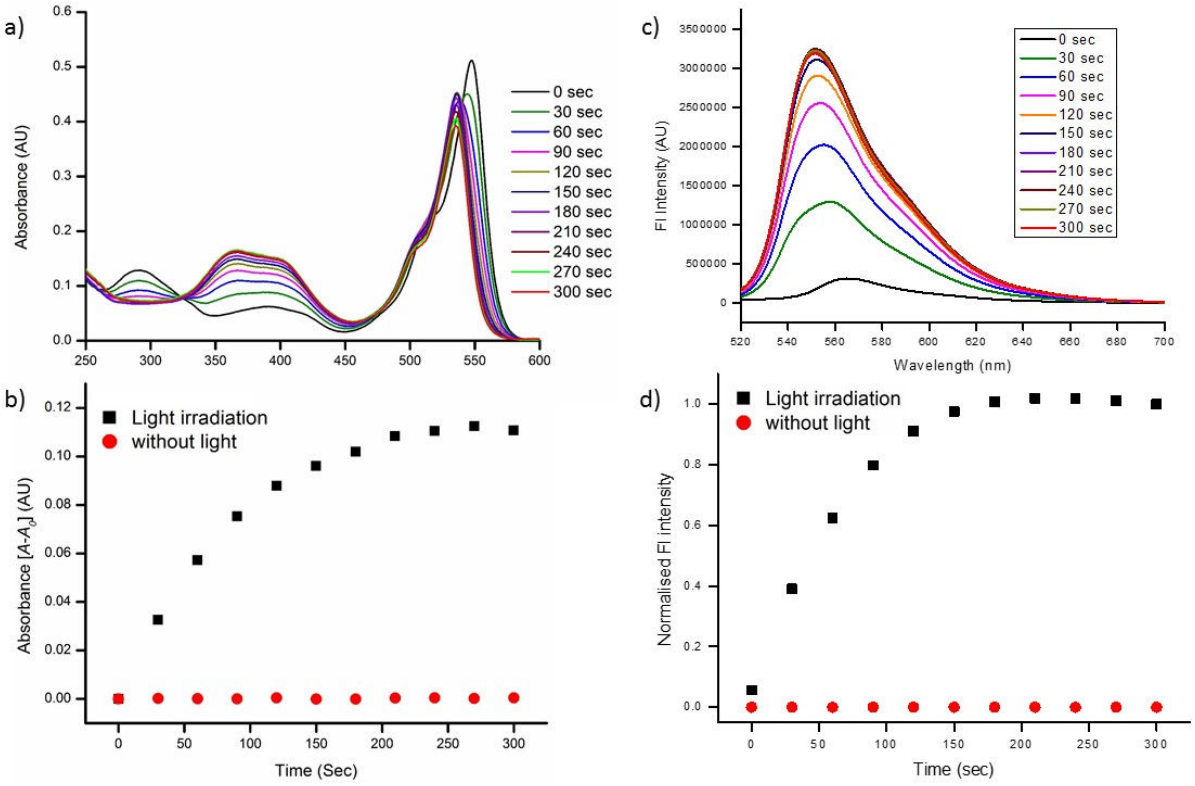


Figure S5. UV-Vis and emission spectra of photorelease from compound **14** (10 μM in 7/3 acetonitrile/water), exciting at λ = 545 ± 40 nm. (**a**) UV-Vis absorption spectra (measured every 30 sec). (**b**) Absorbance at 380 nm *Vs* time is plotted. (**c**) Fluorescence emission spectra (λ_{ex} = 510 nm, measured every 30 sec). (**d**) Normalized fluorescence at 560 nm *Vs* time is plotted.

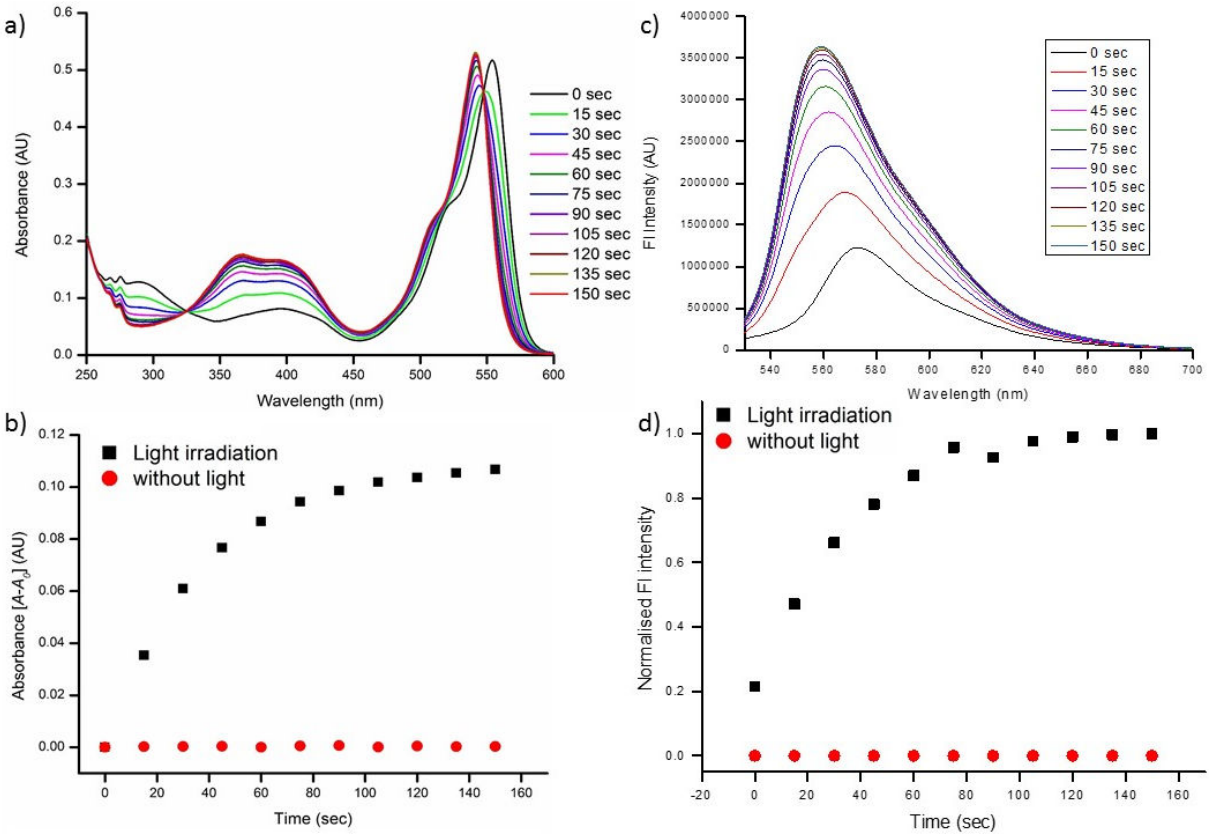


Figure S6. UV-Vis absorption and emission spectra of photorelease from compound **16** (10 μM in 7/3 acetonitrile/water), exciting at λ = 545 ± 40 nm. (**a**) UV-Vis absorption spectra (measured every 15 sec). (**b**) Absorbance at 380 nm *Vs* time is plotted. (**c**) Fluorescence emission spectra (λ_{ex} = 530 nm, measured every 15 sec). (**d**) Normalized fluorescence at 560 nm *Vs* time is plotted.

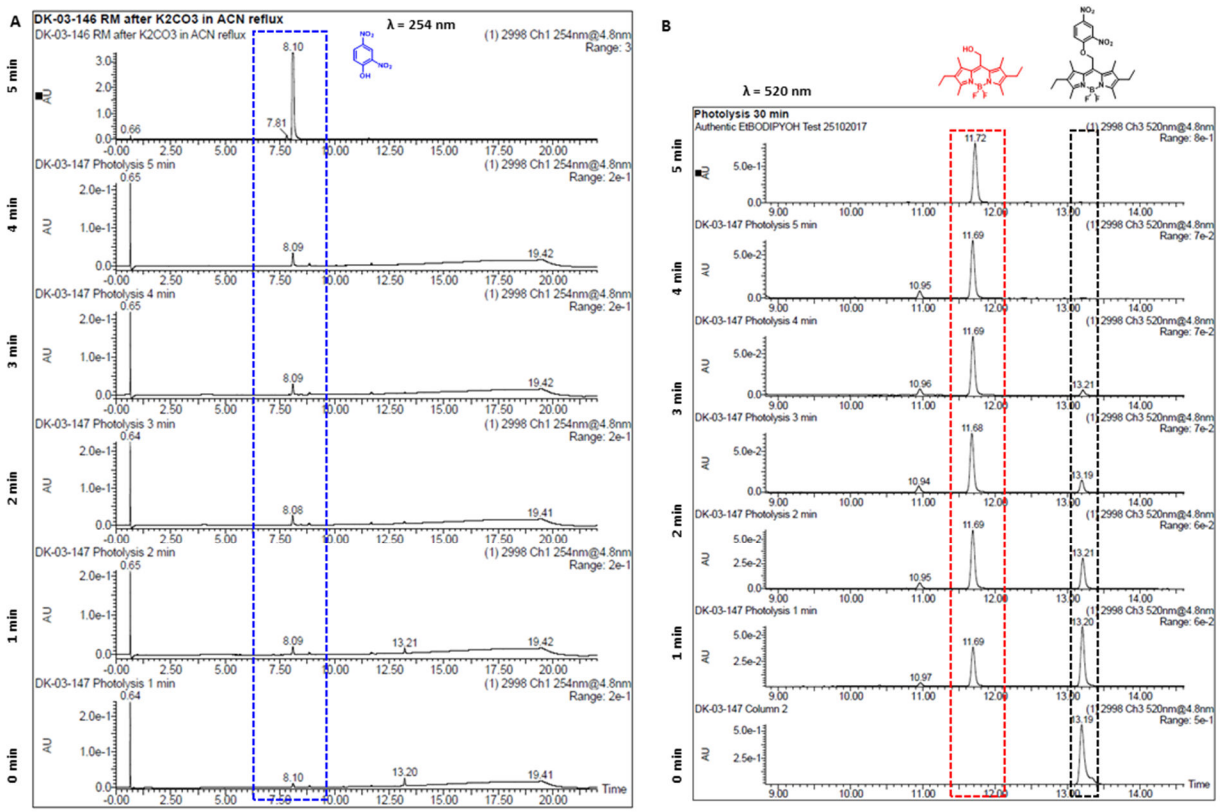


Figure S7. HPLC monitoring of photorelease from compound **14** (10 μM in 7/3 acetonitrile/water), exciting at λ = 545 ± 40 nm, monitoring at 254 nm (**A**) and 520 nm (**B**)

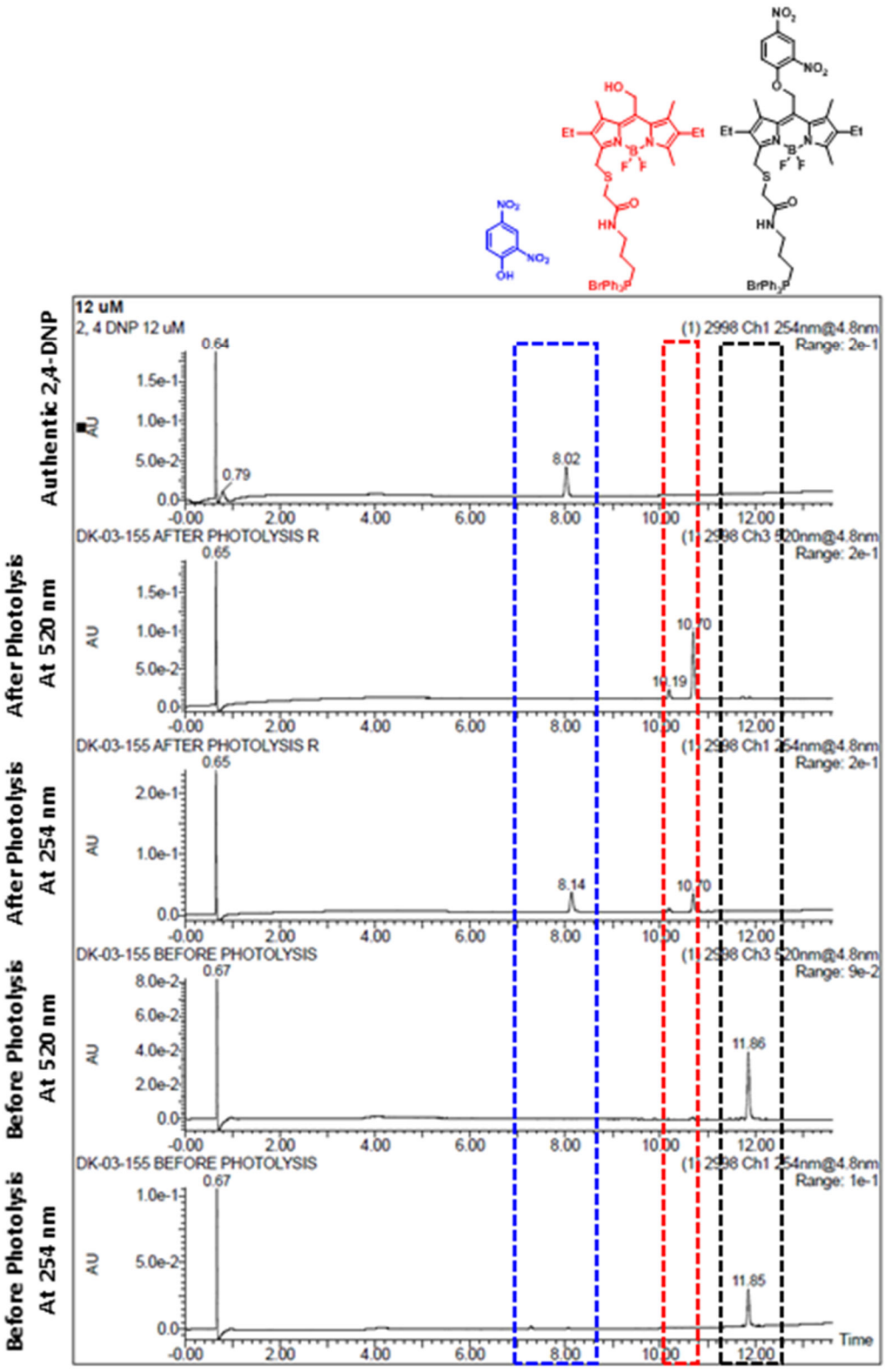


Figure S8. HPLC monitoring of photorelease from compound **16** (10 μM in 7/3 acetonitrile/water), exciting at λ = 545 ± 40 nm, monitoring at 520 and 254 nm.

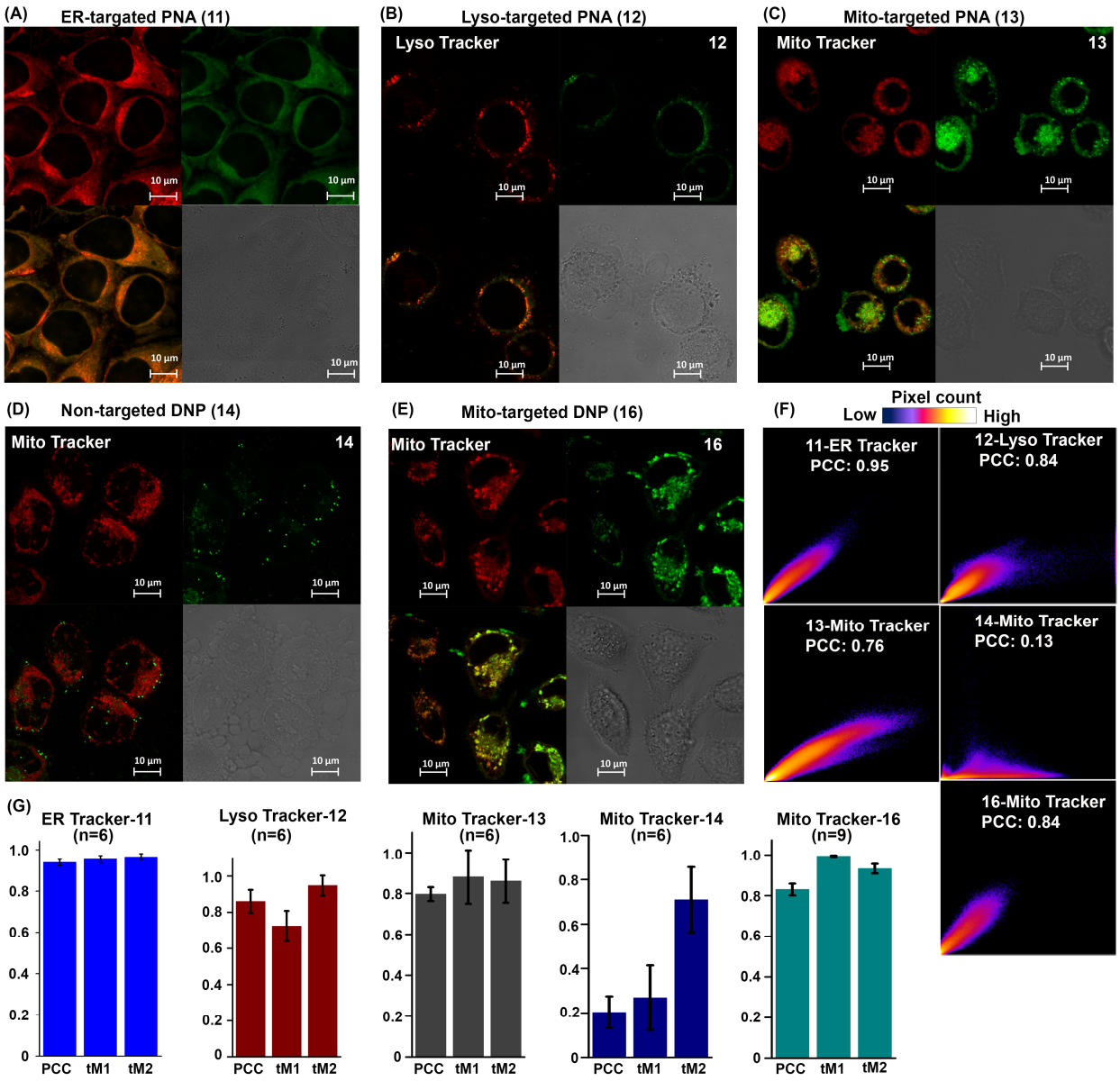


Figure S9. Distribution and co-localization of compounds **11-14** and **16**. Confocal fluorescence microscopic images of HeLa cells stained with (**A**) ER Tracker Blue white (2 μM) and **11** (10 μM, 30 min), (**B**) LysoTracker deep-red (2 μM) and **12** (10 μM, 30 min), (**C**) MitoTracker deep-red (2 μM), and **13** (10 μM, 30 min), (**D**) MitoTracker deep-red (2 μM), and **14** (10 μM, 30 min), (**E**) MitoTracker deep-red (2 μM), and **16** (10 μM, 30 min). (**F**) Quantification of colocalization between BODIPY photocages and organelle markers. 2D histograms of representative images showing correlation between pixel intensities in both channels. Source images for 2D histograms are displayed in Figure S10 and S11. (**G**) Bar graphs show Pearson correlation coefficients (PCC) of non-thresholded images and Manders correlation coefficients above the autothreshold for both channels, in every case derived from a minimum of six images.

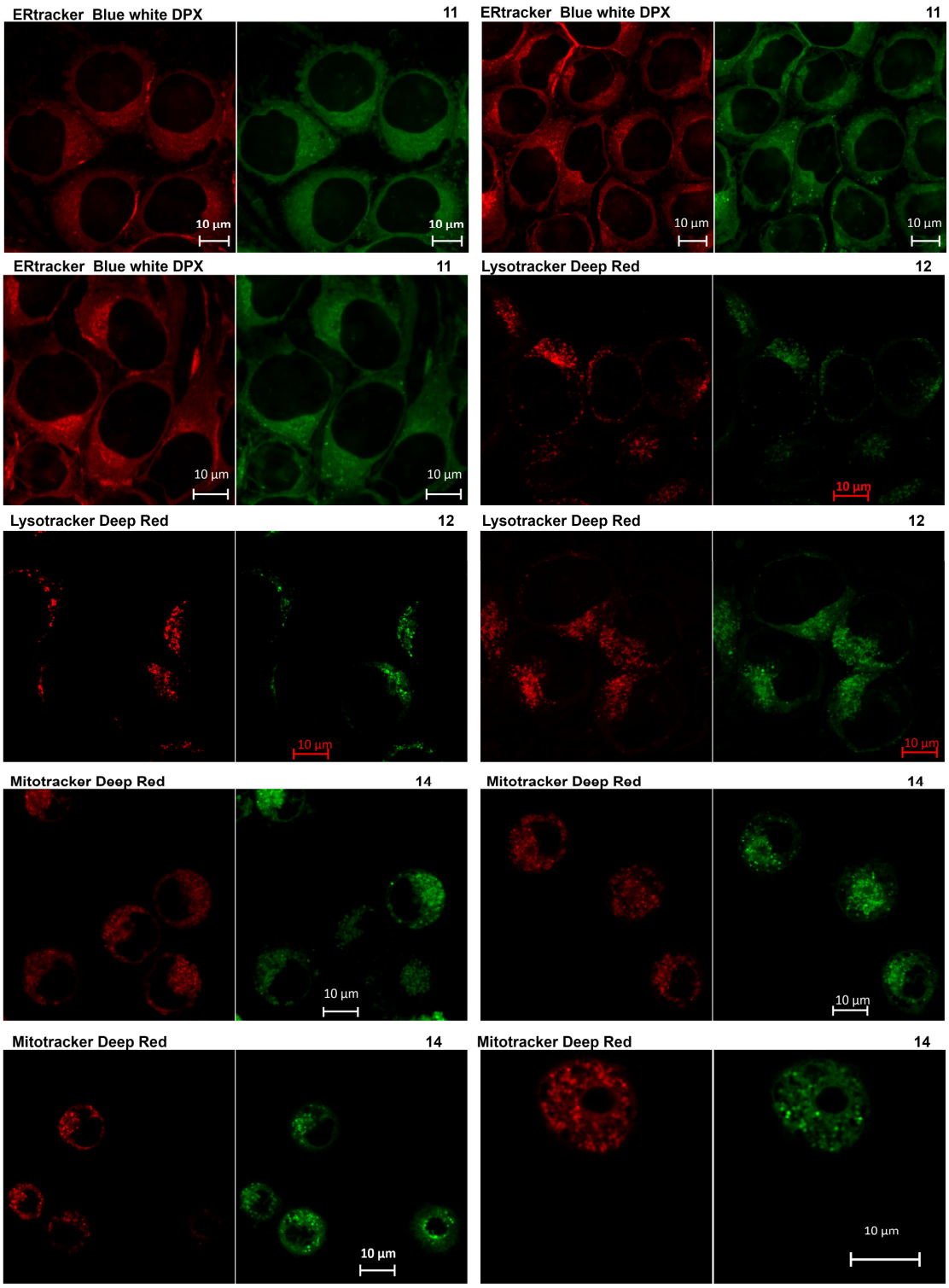


Figure S10. Source images for 2D histograms in Figure S9. *Left panels:* (Blue or red lookup-table) show respective organelle markers. *Right panels:* (green lookup-table) show the subcellular localization of organelle-targeted BODIPY photocages.

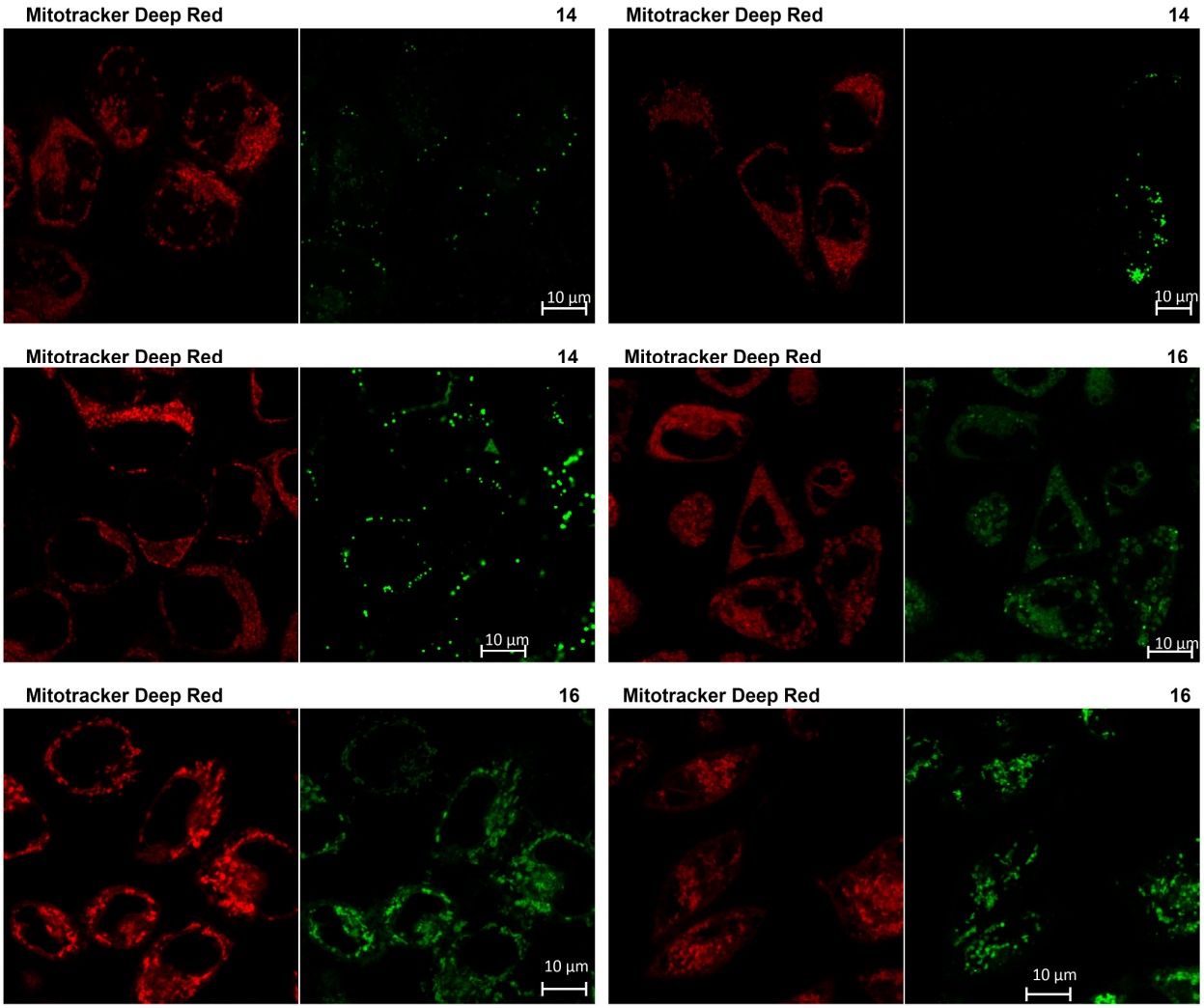


Figure S11. Source images for 2D histograms in Figure S9. Left panels (red lookup-table) show Mitotracker deep red, right panels (green lookup-table) show either absence of the subcellular localization of BODIPY photocage **14** or presence of mitochondria targeted BODIPY photocage **16**.

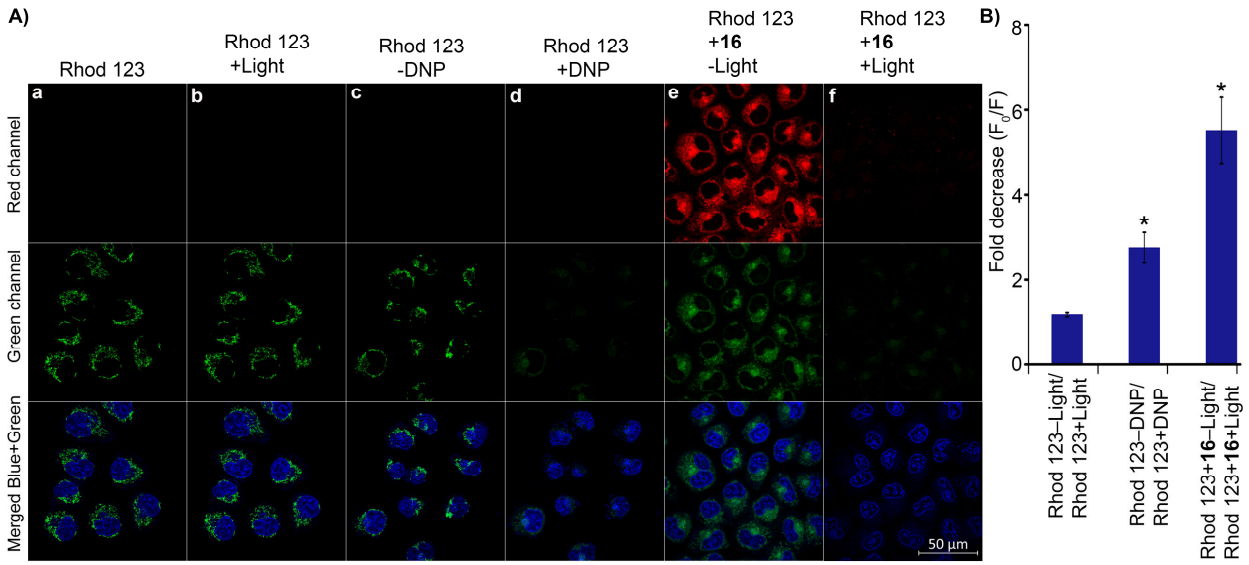


Figure S12. (**A**) Live-cell confocal fluorescence imaging of HeLa cells with Rhod123 and BODIPY **16** reveal that the mitochondrial membrane potential is changed after treatment with Light. Cells incubated with Rhodamine (26 µM, incubation time 15 min) and Hoechst dye (**a**) before light irradiation, (**b**) after light irradiation (**c**) before DNP treatment, (**d**) after incubation with 200 µM DNP, (**e**) cells incubated with Rhodamine (26 µM), compound **16** (25 µM) and Hoechst dye before light irradiation and (**f**) after light irradiation. (**B**) Bar graph presents decrease in fluorescence intensity of selected *ROI*s, where F_0 is fluorescence intensity before either light or DNP treatment and F is fluorescence intensity after either light or DNP treatment. .*Statistical significance (one-way ANOVA with Tukey correction, $p < 0.05$) from non-irradiated cells. Error bars represent standard error measurement (SE).

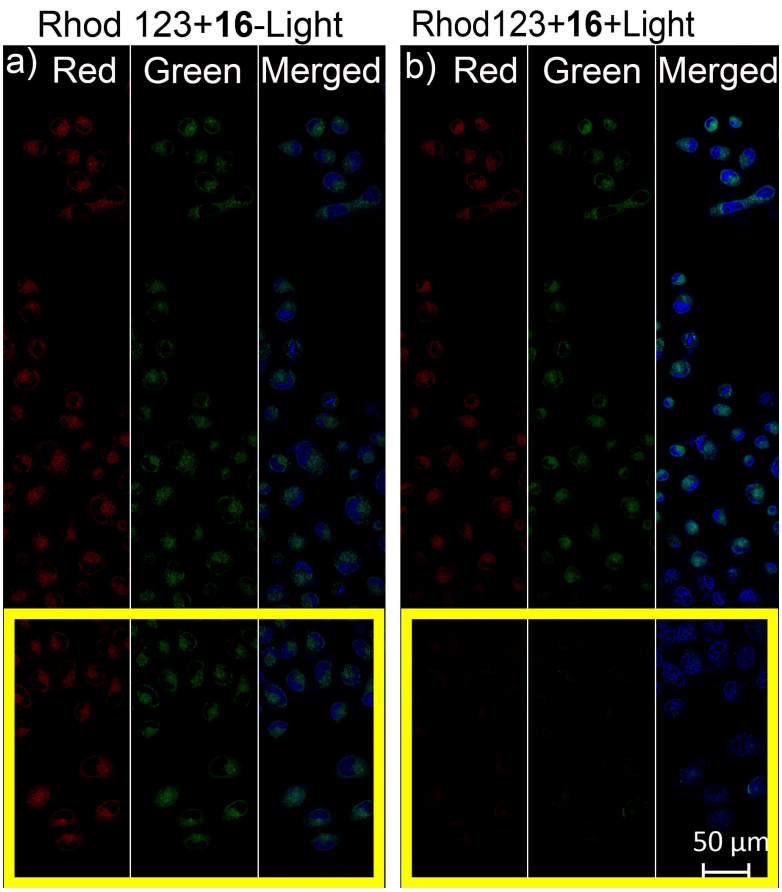


Figure S13. Confocal fluorescence image of HeLa cells incubated with Rhod123 (26 µM, 15 min), compound **16** (25 µM) and Hoechst dye (**a**) before light irradiation, (**b**) after light irradiation at selected region.

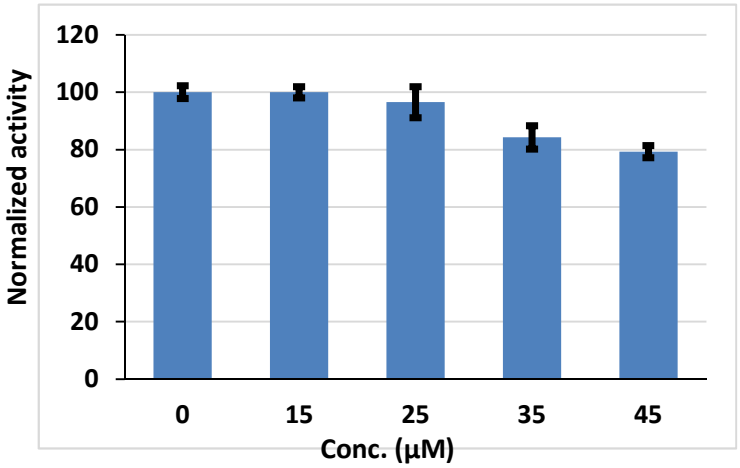


Figure S14. WST-1 cell viability assay. HeLa cells treated with BODIPY **16** (15, 25, 35 and 45 µM) were incubated at 37 °C for 24 h. Control experiments contained 0.1% DMSO. Error bars represent standard error (SE).

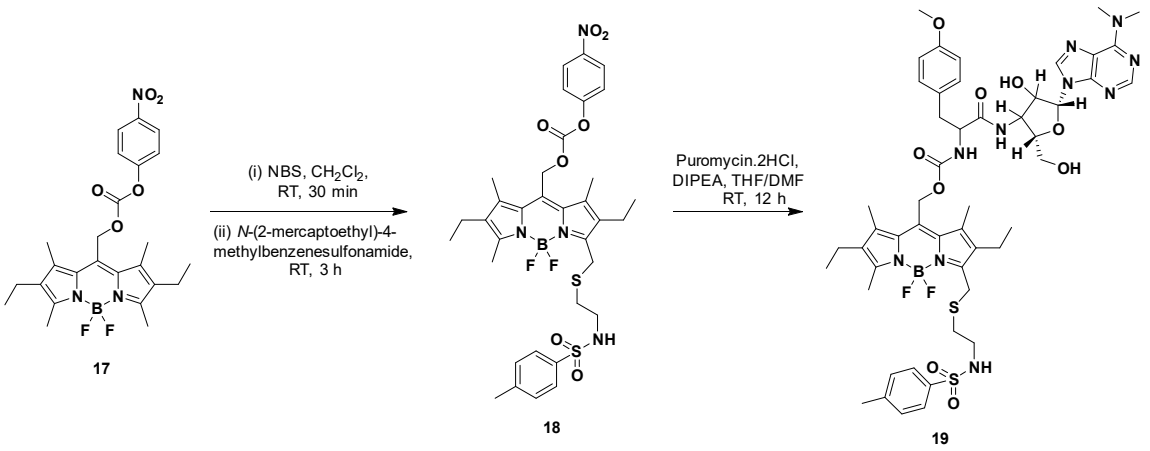


Figure S15. Synthetic scheme for BODIPY **19**.

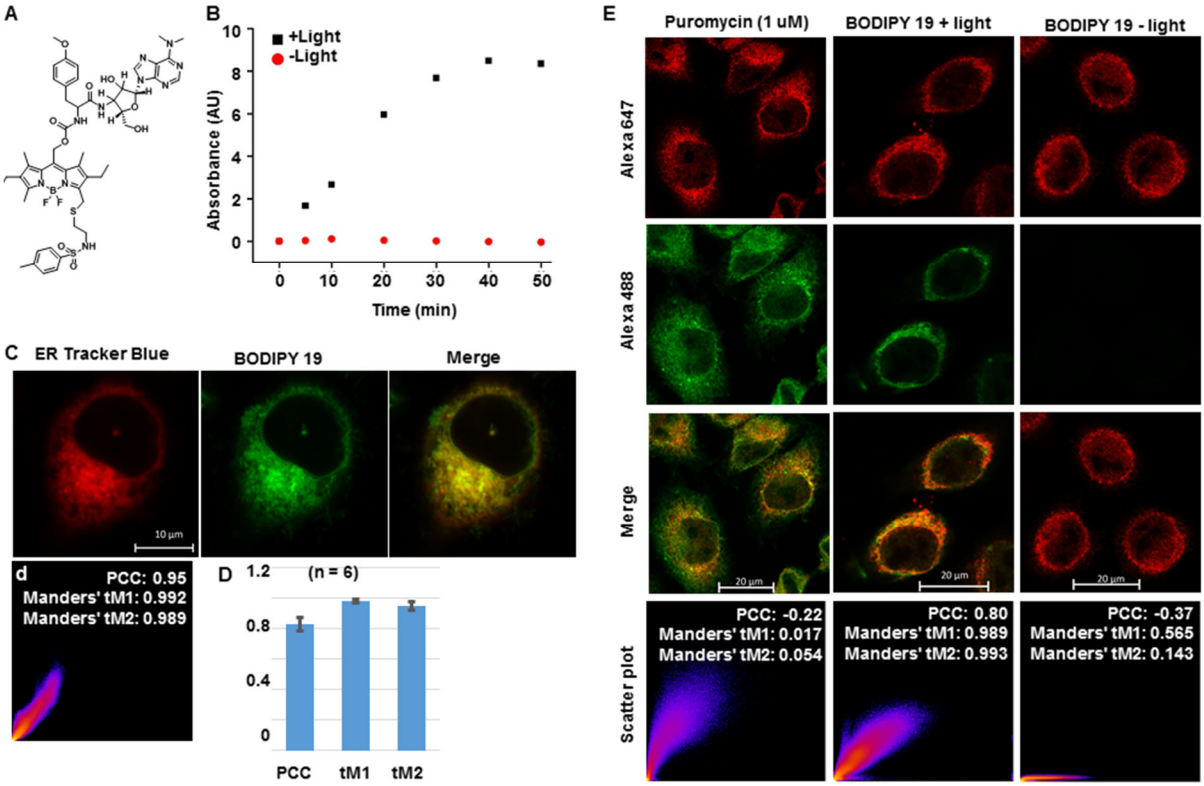


Figure S16. (**A**) Structure of BODIPY **19**. (**B**) *In vitro* monitoring (HPLC-MS) of light-mediated release of puromycin from **19** (100 µM in ACN/water 7/3) upon light irradiation (545/30 nm, 42 mW/cm^2) for the indicated times. Absorbance at 254 nm *Vs* time was plotted. (**C**) Distribution and co-localization of **19**. Confocal images of live HeLa cells treated with 2 µM of ER Blue white Tracker (**a**) and 5 µM of **19** (**b**) for 30 min. Areas of co-localization appear in yellow/orange in merged (**c**). Scatter plot (**d**) for colocalization of (**a** and **b**). (**D**) Bar graphs shows Pearson correlation coefficients (PCC) of non-thresholded images and Manders correlation coefficients above the autothreshold for both channels (n = 6). (**E**) Photorelease of puromycin in ER of HeLa cells. Confocal fluorescence microscopic images of HeLa cells treated with **19**

(20 μM) for 30 min and irradiated (545/30 nm, 42 mW/cm^2) for 5 min. Cells were then washed, fixed, stained with primary antibodies, anti-puromycin (3RH11, Kerafast; 1:500 v/v) and anti-calreticulin (PA3-900, Thermo Fisher Scientific; 1:100 v/v), subsequently stained with anti-mouse secondary antibody-Alexa Fluor 488 and anti-rabbit secondary antibody-Alexa Fluor 647 (1:500 v/v) and imaged. Scatter plots for co-localization were determined by ImageJ (CoLoc 2 plugin).

General synthetic and analytical methods

Pyrromethene605 was purchased from Exciton. Puromycin dihydrochloride was purchased from FERMENTEK. All other chemicals were purchased from Sigma-Aldrich and used as received unless otherwise stated. Anhydrous solvents and reagents (DCM, THF, DMSO and DMF) were obtained as Sureseal bottles from Sigma-Aldrich. Thin-layer chromatography and flash chromatography were performed using EMD pre-coated silica gel 60 F-254 plates and silica gel 60 (230-400 mesh), respectively. UV absorbance spectra were recorded on Agilent Cary 60 UV-Vis Spectrophotometer. Fluorescence spectra were recorded on Fluorolog 2 (Spex) fluorimeter. Low resolution ESI mass spectrometry was performed on LC/MS Acquity QDa detector coupled with Waters HPLC. High resolution ESI mass spectrometry was performed on a Waters SYNAPT system. ^{1}H- and ^{13}CNMR spectra were collected in $CDCl_3$, Acetone-d_6, DMSO-d_6 or CD_3OD (Cambridge Isotope Laboratories, Cambridge, MA) at 25 ^{o}C using a Bruker Advance III spectrometer at 400 MHz and 100 MHz respectively at the Department of Chemistry NMR Facility at Tel-Aviv University. All chemical shifts are reported in the standard δ notation of parts per million using the either TMS or residual solvent peak as an internal reference. Abbreviations: NBS: *N*-Bromosuccinimide, THF: tetrahydrofuran, DMF: dimethylformamide, DCM: dichloromethane, DIPEA: diisopropylethylamine, DMAP: dimethylaminopyridine, Et_3N: trimethylamine, PNA: *p*-nitroaniline, PNP: *p*-nitrophenol, EtOAc: ethyl acetate, Hex: n-hexane, RT: room temperature, ACN: acetonitrile, TFA: trifluoroacetic acid, DCC: N,N'-dicyclohexylcarbodiimide, HBTU: (2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate.

Preparative HPLC purification conditions.

Preparative HPLC was performed on Waters 2545 HPLC with XBridge C18 column (100 X 19 mm, 5 μm) using water (solvent A) and acetonitrile (solvent B) gradient of 0-100% solvent B in 20 minutes then 4 minutes at 100% solvent B at flow rate of 15 mL/min (solvent A and B both containing 0.1% TFA as an additive).

HPLC-MS Analysis conditions

HPLC-MS analysis was performed on Waters HPLC with XBridge C18 column (100 X 3 mm, 5 μm) using a water-acetonitrile gradient of 0% to 100% solvent B in 17 minutes then 3 minutes at

100% solvent B at flow rate of 1 mL/min (solvent A = water, solvent B = acetonitrile, both contain 0.1% TFA as an additive). Mass spectrometry was performed on LC/MS Acquity QDa detector coupled with Waters HPLC.

Molar absorption coefficient measurements

Molar absorption coefficients (ε) and maximum absorbance wavelengths (λ_{max}) were determined in 70% acetonitrile in water and/or 100% acetonitrile using Beer's law, from plots of absorbance vs. concentration. Recordings were performed in 10 mm path length quartz cuvettes at room temperature.

General photolysis and monitoring procedures

Compound sample (2 mL of 10 μM in 70% acetonitrile in water) was placed in 10x10x30 mm quartz cuvette (10 mm path) equipped with an internal magnetic stirrer. The cuvette was placed in front of a light source (Prizmatix UHP-T) equipped with a 545/30 nm filter (Chroma) and irradiated for the indicated times while constantly stirred. Light intensity at the cuvette was measured by light meter to be 42.1 ± 0.2 mW/cm^2 in all experiments. At each time point, samples were taken for analysis by UV-Vis spectrophotometer and/or HPLC-MS. Calibration curves for all tested and reference compounds were generated in each detection method. Photolysis half–lives ($t_{1/2}$) were calculated by monoexponential fitting in Origin 8.0 software.

General cell culture methods

In a glass-based dish, HeLa cells were grown in DMEM (Biological Industries) supplemented with 10% fetal bovine serum (Biological Industries), 2 mM glutamine (Biological Industries), 1 mM sodium pyruvate (Biological Industries), 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Cells were incubated in a humidified 37 ºC incubator with 5% CO_2 (LUMITRON).

Cell Viability Assay:

HeLa cells were seeded in 96-well plates at a density of 2×10^4 cells (100 μL of culture)$^{-1}$ well^{-1} except for the first and last columns to which only growth media was added. After a 24h adhering period, the cells were either left untreated or treated with different concentrations of BODIPY **16** (0, 15, 25, 35 and 45 μM). After a 24 h incubation period, the WST-1 tetrazolium salt colorimetric

proliferation assay was performed by adding 10 µL of dissolved WST-1 solution into each well and incubating for 3 h at 37 °C in a 5% CO_2 incubator. The absorbance of samples was determined using a microtiter plate reader at a wavelength of 450 nm against a background control; the reference wavelength was 690 nm. Each data point represents an average of 6 replicate wells.

Imaging instrumentation and experimental for photoactivation of BODIPY 16 and 19

For co-localization analysis and assessment of compound uptake cells were imaged with a laser scanning confocal microscope (Zeiss LSM 780 inverted microscope) equipped with a 63x 1.35 Oil objective lens. BODIPY compounds were excited with the 561 nm laser and emitted light was collected between 580 and 615 nm. All organelle markers were purchased from Thermo Fisher Scientific and following wavelengths were used for excitation and emission, ER Tracker Blue White: Excitation: 405 nm laser, Emission: 410-550 nm; Mito Tracker deep red Excitation: 633 nm laser, Emission: 647-684 nm; Lyso Tracker deep red Excitation: 633 nm laser, Emission: 647-697 nm. For Rhodamine 123 imaging: Excitation: 488 nm laser and emitted light was collected between 490-520 nm.

Photoactivation of BODIPY **16**: Photoactivation was done by irradiating cells with confocal lamp (RFP filter, excitation 545/25 nm, emission 605/70) at ~20 % lamp intensity for specified time durations and confocal images were acquired.

Photoactivation of BODIPY **19**: Photoactivation was done by irradiating cells with a light source (from Prizmatix) with a 545/30 nm filter (from Chroma) for 5 min and then cells were fixed, immunostained and confocal images were acquired.

Immunofluorescence staining

Cells were washed three times with Hanks' Balanced Salt solution (HBSS) and fixed with 4% paraformaldehyde at room temperature for 15 min. The cells were washed three times with HBSS and permeabilize with 0.1% Triton X-100 in HBSS for 10 min. Primary antibodies, anti-puromycin (3RH11, Kerafast; 1:500 v/v) and anti-calreticulin (PA3-900, Thermo Fisher Scientific; 1:100 v/v), were diluted in 0.1% Triton X-100 containing 10% FBS and incubated at 37°C for 30 min. Subsequently, the cells were washed three times with HBSS and stained with anti-mouse secondary antibody-Alexa Fluor 488 and anti-rabbit secondary antibody-Alexa

Fluor 647 (1:500 v/v) in HBSS containing 10% FBS and incubated at 37°C for 30 min. The cells were then washed with HBSS and stained with DAPI at room temperature for 10 min. Finally, the cells were washed three times with HBSS, mounted with Fluoromount G and imaged by confocal fluorescent microscopy.

Data Analysis

Image analysis and signal quantification were done using the measurement function in ZEN lite 2012 software.

Colocalization analysis

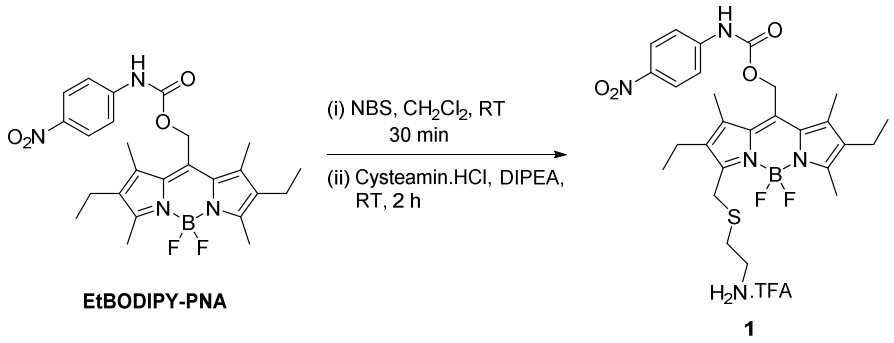
The colocalization plugin Coloc2 for the ImageJ (Fiji) software was used to generate 2D histograms and to determine Pearson correlation coefficients as well as Manders overlap coefficients. Non-corrected and non-thresholded images of BODIPY photocages and respective organelle markers were loaded into the plugin. Mean Pearson correlation coefficients for each BODIPY photocage and corresponding organelle marker pair were determined from the entire field of view ($n \geq 6$).

Statistical methods

Two-tailed student's t-test was performed whenever two groups were compared. Statistical significance was determined at $p < 0.05$. When more than two groups were compared, one-way ANOVA with Tukey correction was performed, and statistical significance was determined at $p < 0.05$.

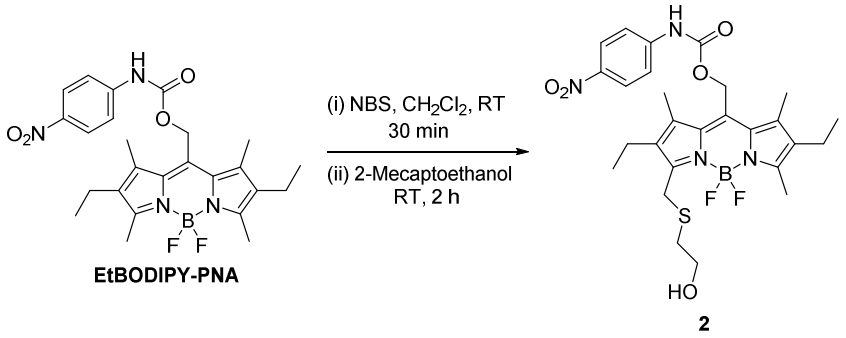
Synthetic procedures

a) Synthesis of 1:



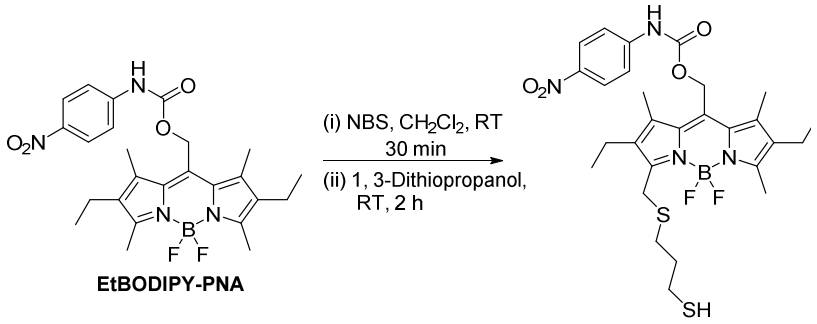
25 mg of **EtBODIPY-PNA** (0.05 mmol) were dissolved in dry CH_2Cl_2 (3 mL) at room temperature and 9 mg of N-Bromosuccinimide (0.05 mmol) were added. After stirring for 30 min at room temperature a solution of cysteamine hydrochloride (28 mg, 0.25 mmol) and DIPEA (9 uL, 0.05 mmol) in DMF (2 mL) was added to the reaction mixture and stirring was continued for 2 h at room temperature. The reaction mixture was evaporated under reduced pressure. The crude was purified by reverse phase prep-HPLC. Fractions containing the desired compound were immediately frozen and lyophilized to afford **1** (18 mg, 53%) as an orange solid. ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm): 8.25 (d, *J* = 9.3 Hz, 2H), 7.83 (d, *J* = 9.3 Hz, 2H), 5.57 (s, 2H), 4.14 (s, 2H), 3.97 (t, *J* = 6.8 Hz, 2H), 3.13 (t, *J* = 6.8 Hz, 2H), 2.51 - 2.58 (m, 7H), 2.43 (s, 3H), 2.39 (s, 3H), 1.12 (t, *J* = 7.5 Hz, 3H), 1.06 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, acetone-d_6) δ (ppm): 157.8, 152.6, 150.8, 145.0, 142.7, 139.0, 137.4, 134.9, 133.4, 133.2, 131.5, 124.9, 117.8, 58.4, 47.3, 26.8, 16.8, 16.6, 14.2, 13.9, 12.2, 11.8; LC/MS: Retention time 10.84 min, 574.37 $[M+H]^+$, HR-MS(ESI) calcd. for formula $C_{27}H_{34}BF_2N_5O_4SNa$ $[M+Na]^+$: 596.2290; Found: 596.2323.

b) Synthesis of 2:



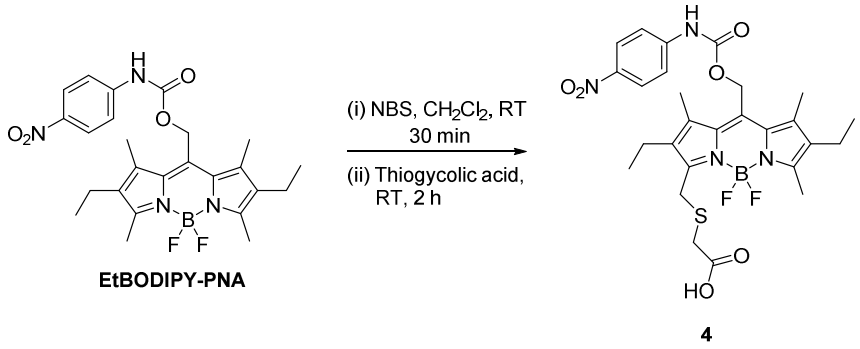
25 mg of **EtBODIPY-PNA** (0.05 mmol) were dissolved in dry DCM (3 mL) at room temperature and 9 mg of NBS (0.05 mmol) were added. After stirring for 30 min at room temperature *β*-mercaptoethanol (7 uL, 0.1 mmol) was added to the reaction mixture and stirring was continued for 2 h at room temperature. The reaction mixture was washed with saturated NH_4Cl solution (10 mL) and brine (10 mL), dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0.8-1% MeOH in DCM gradient) to furnish **2** as dark orange solid (11 mg, 38%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.29 (d, *J* = 7.2 Hz, 2H), 7.67 (d, *J* = 7.2 Hz, 2H), 7.46 (s, 1H), 5.57 (s, 2H), 4.10 (s, 2H), 3.81 (t, *J* = 5.7 Hz, 2H), 2.91 (t, *J* = 5.9 Hz, 2H), 2.57 - 2.62 (m, 5H), 2.61 (q, *J* = 7.6 Hz, 2H), 2.43 (s, 3H), 2.42 (s, 3H), 1.23 (t, *J* = 7.6 Hz, 3H), 1.15 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 158.5, 152.1, 151.4, 143.4, 143.3, 138.2, 136.6, 135.2, 133.7, 133.3, 131.6, 131.5, 126.3, 125.2, 117.9, 60.7, 59.0, 35.5, 26.7, 17.2, 17.1, 14.9, 14.5, 12.9, 12.7; LC/MS: Retention time 12.51 min, 597.21 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{27}H_{33}BF_2N_4O5SNa$ $[M+Na]^+$: 597.2130; Found: 597.2144.

c) Synthesis of 3:



25 mg of **EtBODIPY-PNA** (0.05 mmol) were dissolved in dry CH_2Cl_2 (3 mL) at room temperature and 9 mg of NBS (0.05 mmol) were added. After stirring for 30 min at room temperature 1,3-dithiopropanol (50 uL, 0.5 mmol) was added to the reaction mixture and stirring was continued for 2 h at room temperature. The reaction mixture was washed with saturated NH_4Cl solution (10 mL) and brine (10 mL), dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-10 % EtOAc in Hex gradient) to furnish **3** as dark orange solid (20 mg, 66%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.21 (d, *J* = 9.0 Hz, 2H), 7.57 (d, *J* = 9.0 Hz, 2H), 7.30 (s, 1H), 5.48 (s, 2H), 3.99 (s, 2H), 3.48 (s, 1H), 2.77 (t, *J* = 7.0 Hz, 2H), 2.61 (q, *J* = 7.3 Hz, 2H), 2.49 - 2.52 (m, 5H), 2.37 – 2.43 (m, 2H), 2.33 – 2.34 (m, 6H), 1.88 (quintet, *J* = 7.0 Hz, 2H), 1.13 (t, *J* = 7.4 Hz, 3H), 1.05 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 158.0, 152.2, 152.1, 143.3 (2), 137.8, 136.7, 134.9, 133.7, 133.2, 131.7, 131.3, 125.2, 117.9, 59.1, 33.5, 31.1, 27.3, 23.4, 17.3, 17.1, 14.8, 14.6, 13.0, 12.9, 12.7; LC/MS: Retention time 13.51 min, 627.38 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{28}H_{35}BF_2N_4O_4S_2Na$ $[M+Na]^+$: 627.2059; Found: 627.2065.

d) Synthesis of 4:

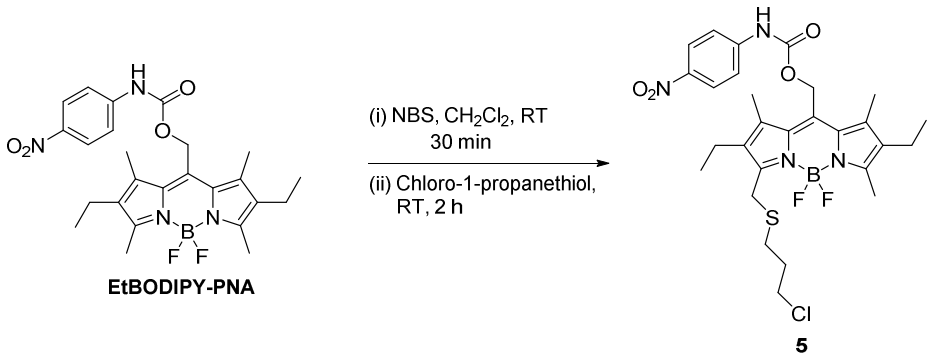


EtBODIPY-PNA

4

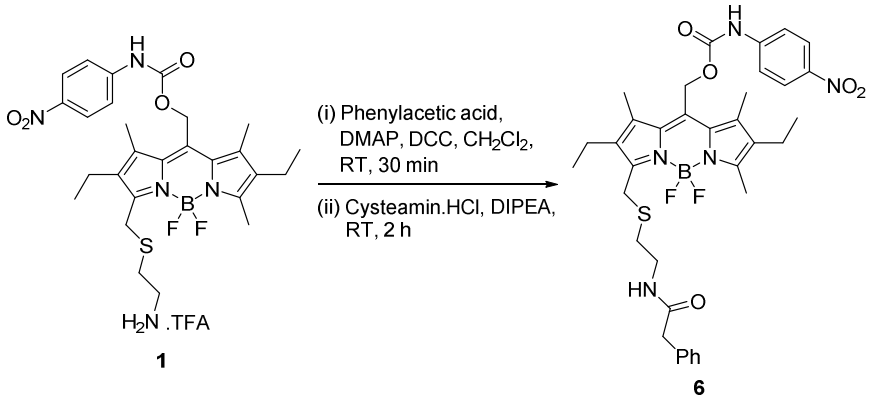
250 mg of **EtBODIPY-PNA** (0.5 mmol) were dissolved in dry DCM (25 mL) at room temperature and 89 mg of NBS (0.5 mmol) were added. After stirring for 30 min at room temperature thioglycolic acid (175 uL, 2.5 mmol) was added to the reaction mixture and stirring was continued for 2 h at room temperature. The reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-3% MeOH in DCM gradient) to furnish **4** as dark brown solid (150 mg, 51%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.14 (d, *J* = 9.2 Hz, 2H), 7.95 (s, 1H), 7.58 (d, *J* = 9.2 Hz, 2H), 5.45 (s, 2H), 4.10 (s, 2H), 3.43 (s, 2H), 2.45 - 2.50 (m, 5H), 2.41 (q, *J* = 7.6 Hz, 2H), 2.34 (s, 3H), 2.30 (s, 3H), 1.09 (t, *J* = 7.5 Hz, 3H), 1.04 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 174.5, 171.1, 156.9, 152.1, 149.4, 143.5, 143.0, 138.5, 136.3, 135.3, 133.5, 131.6 (2), 125.0, 121.9, 117.8, 58.8, 33.7, 27.6, 17.0 (2), 14.7, 14.4, 12.9 (2), 12.4; LC/MS: Retention time 12.27 min, 611.29 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{27}H_{30}BF_2N_4O_6S$ $[M-H]^+$: 587.1947; Found: 587.1989.

e) Synthesis of 5:



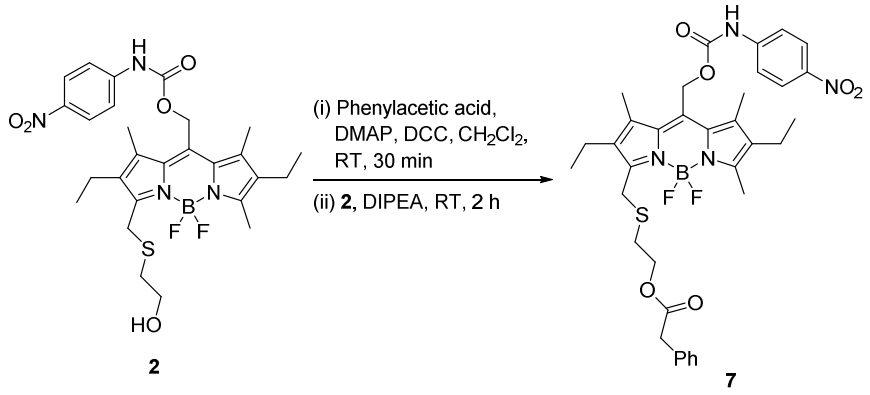
To a solution of **EtBODIPY-PNA** (20 mg, 0.04 mmol) in dry DCM (3 mL) at room temperature was added NBS (7 mg, 0.04 mmol) and the reaction mixture was stirred for 30 min at that temperature. Chloro-1-propanethiol (19 uL, 0.2 mmol) was added and stirring was continued for 2h at room temperature. The reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (Eluent: 0-12% EtOAc in Hex gradient) to furnish **5** as dark orange solid (14 mg, 58%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.22 (d, *J* = 9.1 Hz, 2H), 7.58 (d, *J* = 9.1 Hz, 2H), 7.17 (s, 1), 5.49 (s, 2H), 4.00 (s, 2H), 3.65 (t, *J* = 7.0 Hz, 2H), 2.80 (t, *J* = 7.0 Hz, 2H), 2.43 – 2.49 (m, 5H), 2.33 – 2.53 (m, 8H), 2.01 – 2.08 (m, 2H), 1.13 (t, *J* = 7.5 Hz, 3H), 1.05 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 158.0, 152.1, 152.0, 143.4, 143.2, 137.9, 136.7, 135.0, 133.7, 131.3, 125.2, 117.9, 59.1, 43.6, 32.2, 29.7, 27.2, 17.3, 17.1, 14.8, 14.6, 13.0, 12.9, 12.7; LC/MS: Retention time 14.35 min, 587.17 $[M\text{-}F]^+$, HR-MS(ESI) calcd. for formula $C_{28}H_{34}BF_2N_4O_4SClNa$ $[M+Na]^+$: 629.1948; Found: 629.1962.

f) Synthesis of 6:



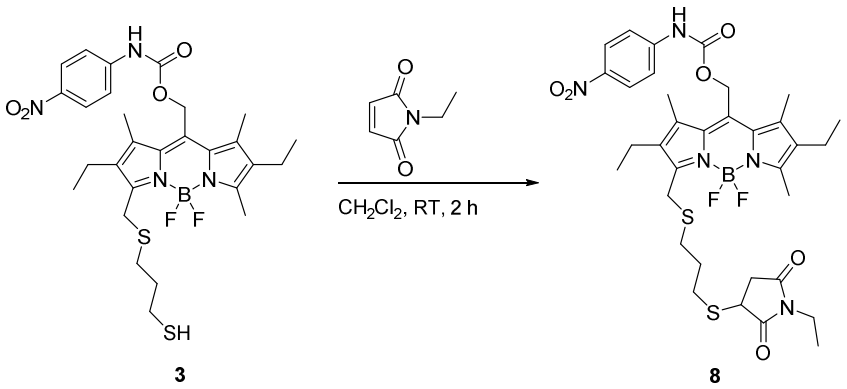
To a solution of phenylacetic acid (8 mg, 0.055 mmol) in dry DCM (3 mL), DCC (9 mg, 0.055 mmol) and DMAP (6 mg, 0.05 mmol) were added at room temperature and the reaction mixture was stirred for 30 min at that temperature. A solution of **1** (17 mg, 0.25 mmol) and DIPEA (45 uL, 0.25 mmol) in dry CH_2Cl_2 (3 mL) were added to the reaction mixture and stirring was continued for 4h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction was diluted with DCM (10 mL) and washed with saturated NH_4Cl solution (10 mL) and brine (10 mL), dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by reverse phase prep-HPLC. Fractions containing desired compound were immediately frozen and lyophilized to afford **6** (10 mg, 57%) as an orange solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.15 (d, *J* = 6.8 Hz, 2H), 8.12 (s, 1H), 7.30 (d, *J* = 6.8 Hz, 2H), 7.18 – 7.29 (m, 5H), 6.06 (s, 1H), 5.51 (s, 2H), 3.97 (s, 2H), 3.50 (s, 2H), 3.20 (q, *J* = 6.0 Hz, 2H), 2.76 (t, *J* = 6.4 Hz, 2H), 2.40 – 2.49 (m, 7H), 2.31 (s, 3H), 2.26 (s, 3H), 1.04 – 1.12 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 171.6, 158.5, 152.4, 151.0, 143.8, 143.1, 138.3, 136.7, 135.3, 134.5, 133.6, 133.2, 132.3, 131.5, 129.3, 128.8, 127.3, 125.1, 117.9, 58.7, 43.4, 38.7, 31.2, 26.9, 17.2, 17.1, 14.8, 14.6, 13.2, 13.0, 12.6; LC/MS: Retention time 13.21 min, 714.40 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{35}H_{40}BF_2N_5O_5SNa[M+Na]^+$: 714.2709; Found: 714.2759.

g) Synthesis of 7:



To a solution of phenylacetic acid (7 mg, 0.05 mmol) in dry DCM (5 mL), DCC (8 mg, 0.038 mmol) and DMAP (4 mg, 0.035 mmol) were added at room temperature and the reaction mixture was stirred for 30 min at that temperature. **2** (20 mg, 0.035 mmol) was then added and stirring was continued for 12h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction was diluted with DCM (10 mL) and washed with saturated NH_4Cl solution (10 mL) and brine (10 mL), dried with $MgSO_4$, filtered and solvents were removed under reduced pressure. The crude product was purified by silica gel column chromatography (Eluent: 0-10% EtOAc in Hex gradient) to furnish **7** as dark orange solid (14 mg, 58%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.15 (d, *J* = 7.2 Hz, 2H), 7.64 (s, 1H), 7.45 (d, *J* = 7.2 Hz, 2H), 7.19 – 7.28 (m, 5H), 5.51 (s, 2H), 4.02 (s, 2H), 3.94 (t, *J* = 6.5 Hz, 2H), 3.61 (s, 2H), 2.96 (t, *J* = 6.5 Hz, 2H), 2.47 - 2.53 (m, 5H), 2.38 - 2.44 (m, 5H), 2.31 (s, 3H), 1.13 (t, *J* = 7.5 Hz, 3H), 1.06 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 172.0, 158.3, 152.3, 151.7, 143.6, 143.1, 138.0, 136.8, 135.1, 133.8, 133.6, 133.0, 132.2, 131.4, 129.3, 128.6, 127.2, 125.1, 117.8, 64.2, 58.8, 41.1, 30.5, 29.7, 27.5, 17.3, 17.1, 14.7, 14.6, 13.2, 12.5; LC/MS: Retention time 13.74 min, 715.17 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{35}H_{39}BF_2N_4O_6SNa$ $[M+Na]^+$: 715.2549; Found: 715.2584.

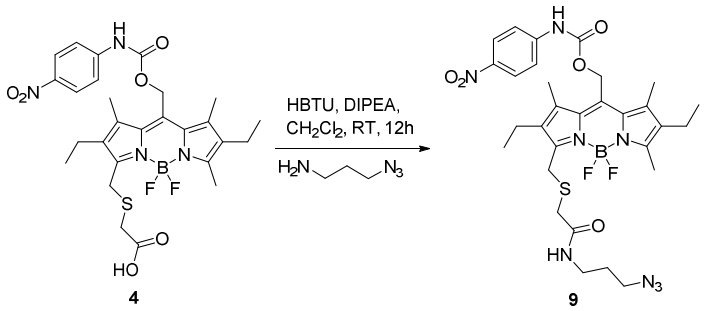
h) Synthesis of 8:



3 **8**

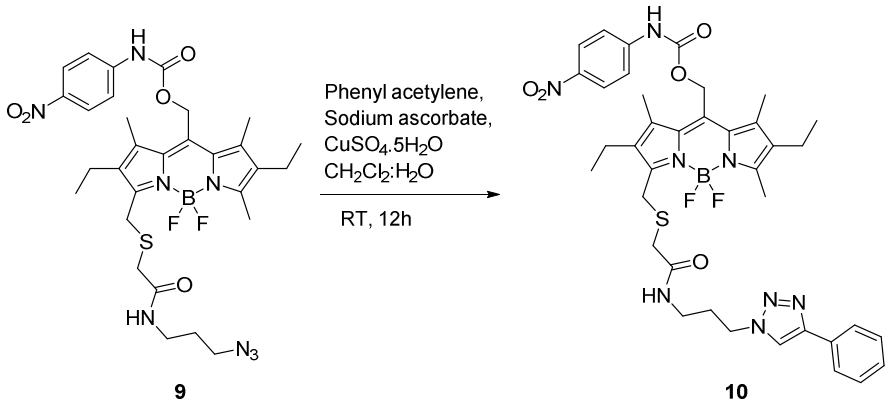
To a solution of **3** (8 mg, 0.013 mmol) in dry DCM (3 mL), N-ethyl maleimide (2 mg, 0.014 mmol) and DIPEA (3 uL, 0.017 mmol) were added at room temperature and stirring was continued for 30 min at that temperature. The reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (Eluent: 0-20% EtOAc in Hex gradient) to furnish **8** as dark orange solid (20 mg, 62%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.35 (s, 1H), 8.23 (d, J = 9.2 Hz, 2H), 7.69 (d, J = 9.2 Hz, 2H), 5.38 – 5.49 (m, 2H) 3.96 – 4.14 (m, 2H), 3.46 – 3.54 (m, 3H), 2.71 – 2.91 (m, 4H), 2.51 - 2.66 (m, 5H), 2.36 – 2.42 (m, 4H), 2.32 (s, 3H), 2.29 (s, 3H), 1.79 (quintet, J = 7.2 Hz, 2H), 1.06 – 1.14 (m, 6H), 1.05 (t, J = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 176.8, 175.5, 157.7, 153.6, 152.3, 143.9, 143.2, 137.6, 137.1, 135.0, 134.2, 132.8, 131.5, 131.3, 125.2, 117.9, 58.9, 38.6, 36.4, 34.1, 30.9, 30.2, 29.7, 29.6, 27.7, 17.3, 17.1, 14.6, 14.5, 12.8 (2), 12.7; LC/MS: Retention time 13.16 min, 752.31 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{34}H_{42}BF_2N_5O_6S_2Na$ $[M+Na]^+$: 752.2535; Found: 752.2578.

i) Synthesis of 9:



To a solution of **4** (20 mg, 0.034 mmol) in dry DCM (4 mL), HBTU (15 mg, 0.037 mmol) and DIPEA (7 uL, 0.037 mmol) were added at room temperature. 3-Azido-1-propylamine (4 mg, 0.037 mmol) was added to the reaction mixture and stirred for 16h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was diluted with CH_2Cl_2 (20 mL) and washed with saturated NH_4Cl solution (10 mL) and brine (10 mL). The organic layer was dried with $MgSO_4$, filtered and solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-50% EtOAc in Hex gradient) to furnish **9** as dark orange solid (14 mg, 61%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.22 (d, *J* = 9.1 Hz, 2H), 7.64 (d, *J* = 9.1 Hz, 2H), 7.46 (s, 1H), 5.47 (s, 2H) 4.00 (s, 2H), 3.23 – 3.45 (m, 5H), 2.53 (s, 3H), 2.39 - 2.50 (m, 6H), 2.36 (s, 3H), 2.33 (s, 3H), 1.78 (quintet, *J* = 6.8 Hz, 2H), 1.11 (t, *J* = 7.6 Hz, 3H), 1.06 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 168.8, 159.4, 152.3, 148.7, 143.7, 143.2, 138.9, 136.3, 135.7, 133.8 (2), 132.0, 131.6, 125.2, 117.9, 58.8, 49.2, 37.2, 36.3, 28.6, 28.2, 17.2, 17.1, 15.0, 14.5, 13.1, 12.6; LC/MS: Retention time 12.51 min, 693.49 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{30}H_{37}BF_2N_8O_5SNa$ $[M+Na]^+$: 693.2566; Found: 693.2617.

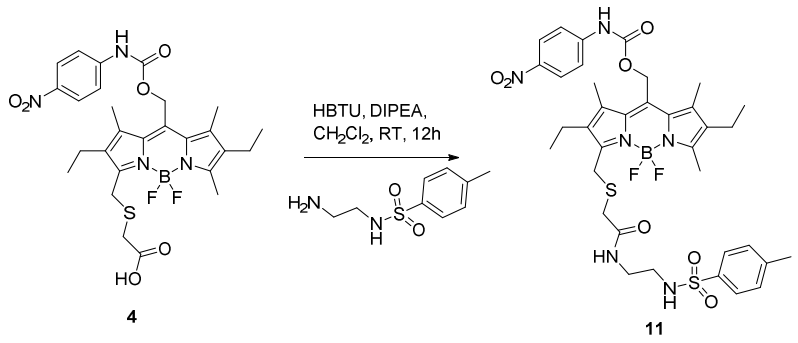
j) Synthesis of 10:



9 **10**

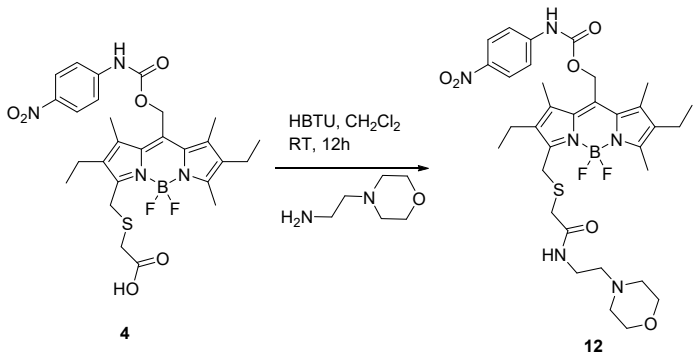
To a solution of **9** (22 mg, 0.033 mmol) in DCM:H_2O (1:1, 4 mL), phenylacetylene (3.6 uL, 0.033 mmol), sodium ascorbate (20 mg, 0.099 mmol) and $CuSO_4.H_2O$ (13 mg, 0.05 mmol) were added. The reaction mixture was stirred for 16h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was diluted with DCM (20 mL) and washed with saturated NH_4Cl solution (10 mL) and brine (10 mL). The organic layer was dried with $MgSO_4$, filtered and solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-100% EtOAc in Hex gradient) to furnish **10** as dark orange solid (16 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.41 (s, 1H), 8.23 (d, *J* = 9.2 Hz, 2H), 7.91 (s, 1H), 7.86 (d, *J* = 9.2 Hz, 2H), 7.75 (d, *J* = 7.1 Hz, 2H), 7.30 – 7.40 (m, 3H), 7.21 (br s, 1H), 5.38 (s, 2H), 4.16 (t, *J* = 6.2 Hz, 2H), 4.06 (s, 2H), 3.44 (s, 2H), 3.14 (q, *J* = 6.2 Hz, 2H), 2.46 - 2.52 (m, 5H), 2.28 (s, 3H), 2.20 – 2.24 (m, 5H), 1.89 (quintet, *J* = 6.3 Hz, 2H), 1.10 (t, *J* = 7.4 Hz, 3H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 169.6, 160.0, 152.6, 149.3, 147.9, 144.4, 143.0, 139.3, 136.3, 136.0, 134.0, 133.4, 131.8, 131.4, 130.1, 128.9, 128.3, 125.5, 125.1, 120.8, 118.2, 58.5, 47.1, 36.8, 36.5, 30.0, 28.7, 17.3, 17.0, 14.8, 13.1, 12.9, 12.6; LC/MS: Retention time 12.71 min, 795.51 $[M+Na]^+$,HR-MS(ESI) calcd. for formula $C_{38}H_{43}BF_2N_8O_5SNa$ $[M+Na]^+$: 795.3036; Found: 795.3083.

k) Synthesis of 11:



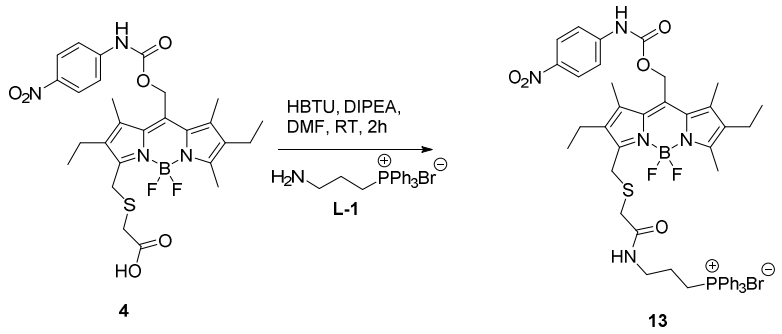
To a solution of **4** (40 mg, 0.068 mmol) in dry DCM (7 mL), HBTU (28 mg, 0.075 mmol) and DIPEA (12 uL, 0.068 mmol) were added at room temperature. N-(2-Aminoethyl)-4-methylbenzenesulfonamide (16 mg, 0.075 mmol) was added to the reaction mixture and stirred for 16h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: 0-50% EtOAc in Hex gradient) to furnish **11** as dark orange solid (28 mg, 52%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.46 (s, 1H), 8.20 (d, J = 9.1 Hz, 2H), 7.67 – 7.71 (m, 4H), 7.27 – 7.28 (m, 3H), 5.49 (s, 2H), 5.39 (s, 1H), 4.02 (s, 2H), 3.28 (s, 2H), 3.01 (q, J = 5.6 Hz, 2H), 2.94 (q, J = 5.6 Hz, 2H), 2.79 (s, 3H), 2.38 – 2.53 (m, 13H), 1.12 (t, J = 7.3 Hz, 3H), 1.07 (t, J = 7.3 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 170.2, 160.1, 152.6, 148.6, 144.0, 143.6, 143.1, 139.5, 137.1, 136.7, 136.0, 134.1, 133.5, 132.0, 131.7, 129.8, 126.9, 125.1, 118.0, 58.7, 43.7, 39.9, 38.6, 36.0, 28.1, 21.5, 17.3, 17.2, 14.8, 14.4, 13.2, 12.7; LC/MS: Retention time 12.79 min, 807.42 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{36}H_{43}BF_2N_6O_7S_2Na$ $[M+Na]^+$: 807.2593; Found: 807.2630.

l) Synthesis of 12:



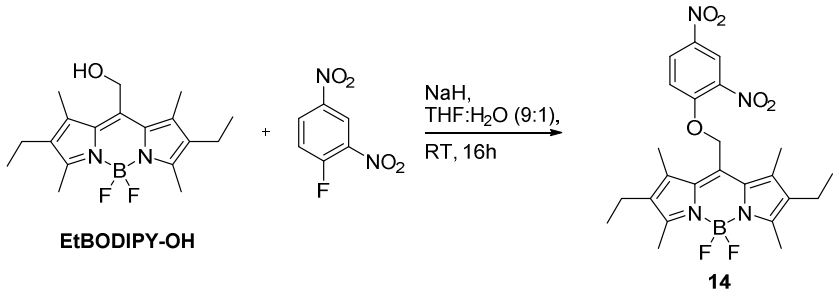
To a solution of **4** (10 mg, 0.017 mmol) in dry DCM (2 mL), HBTU (7 mg, 0.019 mmol) and 4-(2-aminoethyl)morpholine (2.5 uL, 0.019 mmol) were added at room temperature. The reaction mixture was stirred for 2h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: 0-3% MeOH in DCM gradient) to furnish **12** as dark orange solid (6 mg, 50%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.39 (s, 1H), 8.21 (d, *J* = 9.1 Hz, 2H), 7.63 (d, *J* = 9.1 Hz, 2H), 7.30 (s, 1H), 5.47 (s, 2H), 4.03 (s, 2H), 3.65 – 3.67 (m, 4H), 3.27 – 3.33 (m, 4H), 2.34 - 2.78 (m, 18H), 0.86 – 1.13 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 168.6, 159.1, 152.4, 149.8, 143.8, 143.2, 138.6, 136.4, 135.5, 133.7, 131.8, 131.7, 125.2, 117.9, 66.9, 58.9, 56.9, 53.2, 36.5, 36.3, 29.7, 28.2, 17.3, 17.1, 14.9, 14.5, 12.9, 12.7; LC/MS: Retention time 10.87 min, 701.39 $[M+H]^+$, HR-MS(ESI) calcd. for formula $C_{33}H_{44}BF_2N_6O_6S$ $[M+H]^+$: 701.3104; Found: 701.3135.

m)Synthesis of 13:



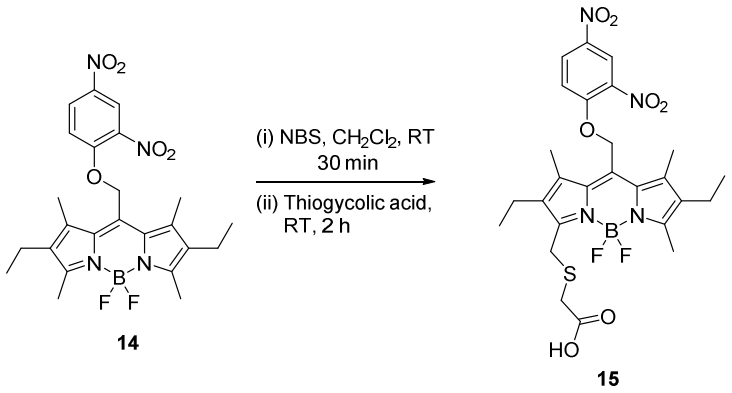
To a solution of **4** (30 mg, 0.051 mmol) in dry DMF (7 mL), HBTU (22 mg, 0.056 mmol) and DIPEA (9 uL, 0.051 mmol) were added at room temperature. Compound **L-1**[1] (23 mg, 0.056 mmol) was added to the reaction mixture and stirred for 2h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: 0–3% MeOH in DCM gradient) to furnish **13** as dark orange solid (25 mg, 55%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.15 (d, *J* = 9.2 Hz, 2H), 7.77 – 7.81 (m, 3H), 7.56 – 7.69 (m, 15H), 5.54 (s, 2H), 4.11 (s, 2H), 3.26 – 3.45 (m, 6H), 2.36 – 2.57 (m, 14H), 1.90 (m, 2H), 1.04 – 1.09 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): δ 171.6, 160.4, 160.0, 158.8, 152.9, 148.9, 144.6, 142.5, 139.1, 136.6, 133.8, 133.2, 133.1, 132.2, 131.4, 130.5, 130.3, 124.8, 118.0, 117.2, 117.0, 58.4, 39.6, 39.4, 35.6, 29.6, 27.7, 22.1, 20.1, 19.5, 16.9, 14.6, 14.2, 12.8, 12.4; LC/MS: Retention time 12.17 min, 890.34 $[M\text{-}Br]^+$, HR-MS(ESI) calcd. for formula $C_{48}H_{52}BF_2N_5O_5SP$ $[M\text{-}Br]^+$: 890.3488; Found: 890.3354.

n) Synthesis of 14:



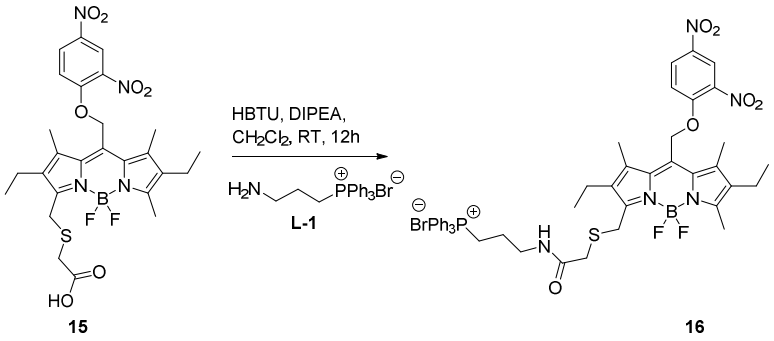
To a solution of **EtBODIPY-OH** (100 mg, 0.3 mmol) in THF/H_2O (9:1, 7 mL), sodium hydride (60% dispersion in mineral oil, 36 mg, 0.9 mmol) was added at room temperature. Then, 1-Fluoro-2, 4-dinitrobenzene (113 uL, 0.9 mmol) was added to the reaction mixture and stirred for 16h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was diluted with Ethyl acetate (30 mL), washed with saturated NH_4Cl solution (10 mL) and brine (10 mL). The organic layer was dried with $MgSO_4$, filtered and solvent was removed under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-100% EtOAc in Hex gradient) to furnish **14** as orange solid (55 mg, 55%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.76 (d, *J* = 2.8 Hz, 1H), 8.52 (dd, *J* = 9.2 and 2.8 Hz, 1H), 7.36 (d, *J* = 9.2 Hz, 1H), 5.42 (s, 2H), 2.53 (s, 6H), 2.39 (q, *J* = 7.6 Hz, 4H), 2.17 (s, 6H), 1.04 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 155.9, 155.3, 140.9, 139.2, 136.3, 134.1, 132.4, 129.2, 129.1, 128.6, 121.9, 113.8, 113.6, 63.8, 57.4, 29.7, 17.1, 14.6, 12.8, 12.3; LC/MS: Retention time 13.19 min, 501.27 $[M+H]^+$, HR-MS(ESI) calcd. for formula $C_{24}H_{27}BF_2N_4O_5Na$ $[M+Na]^+$: 523.1940; Found: 523.1923.

o) Synthesis of 15:



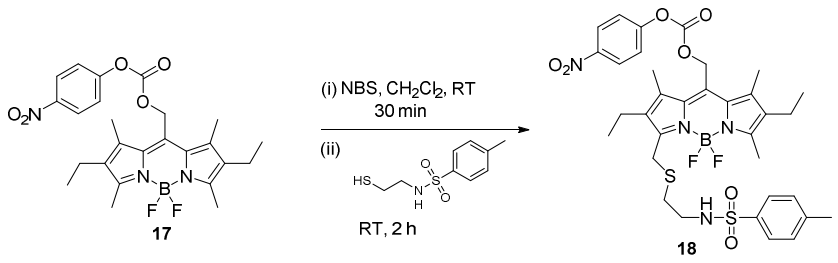
To a solution of **14** (100 mg, 0.2 mmol) in dry DCM (9 mL) at room temperature was added NBS (36 mg, 0.2 mmol) and the mixture was stirred 30 min at that temperature. Thioglycolic acid (42 uL, 0.6 mmol) was added to the reaction mixture and stirring was continued for 2h at room temperature. The reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-3% MeOH in DCM gradient) to furnish **15** as brown solid (70 mg, 59%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.75 (d, *J* = 2.8 Hz, 1H), 8.49 (dd, *J* = 9.2 and 2.8 Hz, 1H), 7.40 (d, *J* = 9.2 Hz, 1H), 5.41 (s, 2H), 4.15 (s, 2H), 3.48 (s, 2H), 2.53 (s, 3H), 2.49 (q, *J* = 7.5 Hz, 2H), 2.39 (q, *J* = 7.5 Hz, 2H), 2.17 (s, 3H), 2.16 (s, 3H), 1.10 (t, *J* = 7.5 Hz, 3H), 1.04 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 173.4, 171.1, 159.5, 155.1, 150.2, 140.9, 139.2, 138.0, 136.1, 135.6, 133.9, 133.9 (2), 129.4, 129.1, 121.9, 113.8, 63.7, 33.9, 27.7, 17.1, 17.0, 14.6, 14.3, 13.0, 12.4, 12.9. LC/MS: Retention time 11.85 min, 613.08 $[M+Na]^+$, HR-MS(ESI) calcd. for formula $C_{26}H_{28}BF_2N_4O_7S$ $[M-H]^+$: 589.1740; Found: 589.1765.

p) Synthesis of 16:



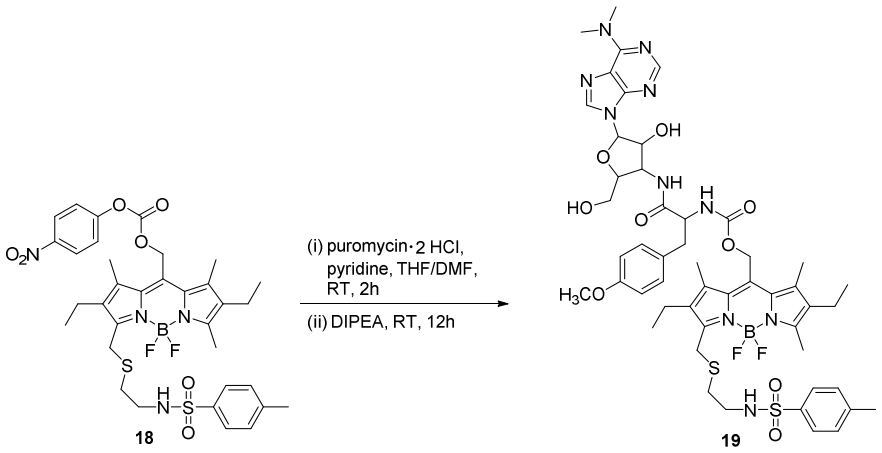
To a solution of **15** (50 mg, 0.085 mmol) in dry DCM (20 mL), HBTU (35 mg, 0.093 mmol) and DIPEA (17 uL, 0.093 mmol) were added at room temperature. Compound **L-1**[1] (37 mg, 0.093 mmol) was added to the reaction mixture and stirred for 12h at room temperature. Progress of the reaction was monitored by LCMS. Upon completion, the reaction mixture was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: 0–3% MeOH in DCM gradient) to furnish **16** as dark orange solid (30 mg, 40%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.81 (d, *J* = 2.8 Hz, 1H), 8.66 (d, *J* = 9.2 and 2.8 Hz, 1H), 7.86 – 7.89 (m, 4H), 7.65 – 7.77 (m, 12H), 6.94 (br s, 1H), 5.66 (s, 2H) 4.12 (s, 2H), 3.37 (s, 2H), 3.12 – 3.26 (m, 4H), 2.57 (s, 3H), 2.43 - 2.53(m, 4H), 2.29 (s, 3H), 2.26 (s, 3H), 1.88 (m, 2H), 1.12 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 170.2, 159.7, 155.5, 149.3, 140.8, 139.8, 138.8, 136.8, 135.6, 135.2 (2), 134.6, 133.4, 133.3, 132.0, 130.9, 130.6, 130.5, 130.0, 121.5, 118.2, 117.4, 115.3, 63.9, 38.6, 36.2, 28.4, 20.2, 19.7, 17.2, 17.1, 14.6, 14.4, 13.1, 12.3, 11.9; LC/MS: Retention time 11.82 min, 892.34 $[M\text{-}Br]^+$, HR-MS(ESI) calcd. for formula $C_{47}H_{50}BF_2N_5O_6PS$ $[M\text{-}Br]^+$: 892.3281; Found: 891.3259.

q) Synthesis of 18:



To a solution of **17**[2] (100 mg, 0.2 mmol) in dry DCM (15 mL) was added NBS (36 mg, 0.2 mmol) and the mixture was stirred for 30 min at room temperature. N-(2-mercaptoethyl)-4-methylbenzenesulfonamide[3] (92 mg, 0.4 mmol) was added to the reaction mixture and stirring was continued for 2h at room temperature. The reaction mixture was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (eluent: 0-25% EtOAc in Hex gradient) to furnish **18** as orange solid (70 mg, 48%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.39 (d, *J* = 9.1 Hz, 2H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 9.1 Hz, 2H), 7.38 (d, *J* = 8.2 Hz, 2H), 5.68 (s, 2H), 5.12 (t, *J* = 5.7 Hz, 1H), 3.98 (s, 2H), 3.25 (q, *J* = 6.4 Hz, 2H), 2.76 (t, *J* = 6.4 Hz, 2H), 2.62 (s, 3H), 2.44 - 2.58 (m, 13H), 1.15 – 1.21 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 158.9, 155.1, 152.1, 151.1, 145.5, 143.2, 138.2, 137.1, 136.6, 135.4, 133.6, 133.4, 131.5, 130.0, 129.6, 126.9, 125.3, 121.5, 61.8, 42.1, 31.7, 26.4, 21.4, 17.1, 17.1, 14.7, 14.4, 13.0, 12.9, 12.6. LC/MS: Retention time 12.90 min, 751.46 $[M+Na]^+$. HR-MS(ESI) calcd. for formula $C_{34}H_{39}BF_2N_4O_7S_2Na$ $[M+Na]^+$: 751.2219; Found: 751.2231.

r) Synthesis of 19:



Compound **18** (30 mg, 0.04 mmol) was dissolved in dry THF (3 mL) at room temperature under argon atmosphere. A solution of puromycin dihydrochloride (27 mg, 0.049 mmol) and pyridine (10 µL, 0.012 mmol) in DMF (1 mL) was added to reaction mixture and the resulting solution was stirred at room temperature. Three aliquots of DIPEA (9 µL each time) were sequentially added after 2h and stirring was continued for 12h at room temperature. The reaction mixture was evaporated under reduced pressure. The crude product was purified by reverse phase prep-HPLC. Fractions containing desired compound were immediately frozen and lyophilized to afford BODIPY **19** as a dark orange solid (15 mg, 34%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.49 (d, J = 9.6 Hz, 2H), 7.81 (dd, J = 15.6, 8.2 Hz, 2H), 7.37 (m, 3H), 7.18 (d, J = 8.2 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 6.76 (d, J = 6.6 Hz, 1H), 6.00 (s, 1H), 5.62 (d, J = 7.2 Hz, 1H), 5.36 (s, 2H), 5.17 (s, 1H), 4.60 – 4.41 (m, 3H), 4.20 – 4.01 (m, 3H), 3.95 (s, 3H), 3.91 – 3.70 (m, 6H), 3.32 (dd, J = 12.7, 6.3 Hz, 1H), 3.26 – 2.99 (m, 4H), 2.76 (dt, J = 18.7, 6.2 Hz, 3H), 2.63 – 2.43 (m, 11H), 2.31 (d, J = 13.8 Hz, 6H), 1.15 (dt, J = 12.5, 7.5 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 172.3, 171.4, 159.0, 158.4, 155.5, 154.8, 150.7, 147.1, 143.4, 138.6, 137.8, 137.4, 137.2, 135.4, 133.4, 132.6, 131.8, 130.3, 130.0, 129.8, 127.9, 127.2, 120.8, 114.5, 91.6, 84.5, 74.9, 61.0, 58.8, 56.7, 55.4, 49.3, 42.4, 41.8, 38.2, 37.6, 31.7, 26.6, 21.7, 17.3, 17.2, 14.9, 14.7, 13.1, 13.0, 12.6. LC/MS: Retention time 10.70 min, 1061.57 $[M+H]^+$. HR-MS(ESI) calcd. for formula $C_{50}H_{64}BF_2N_{10}O_9S_2$ $[M+H]^+$: 1061.4360; Found: 1061.4429.

Spectroscopic data

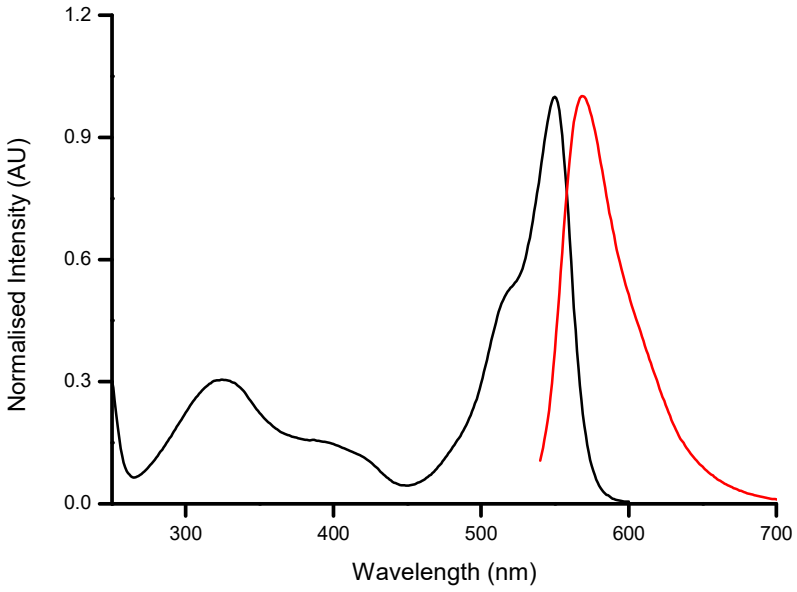


Figure S17. Normalized absorption (black line) and emission spectrum of **1** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c \sim 1 \times 10^{-5}$ M).

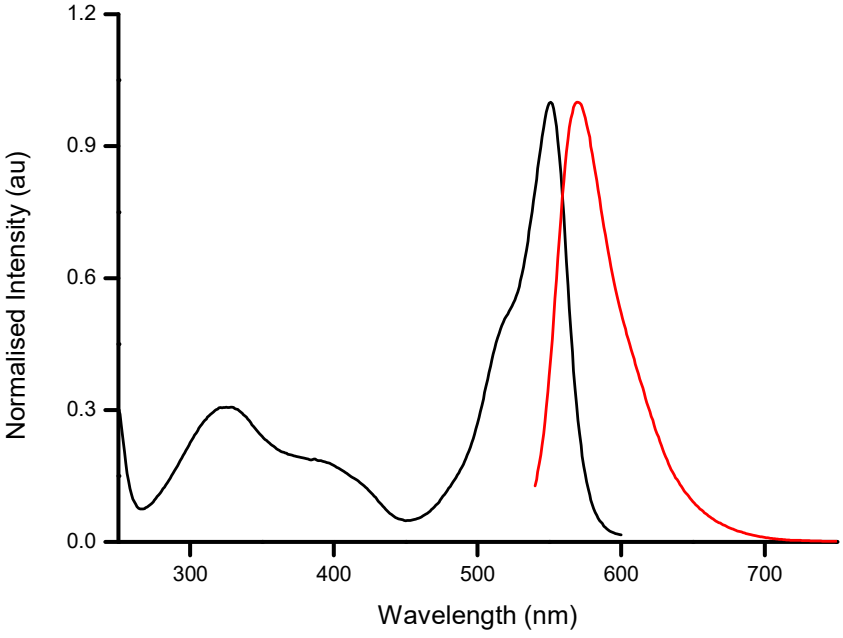


Figure S18. Normalized absorption (black line) and emission spectrum of **2** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

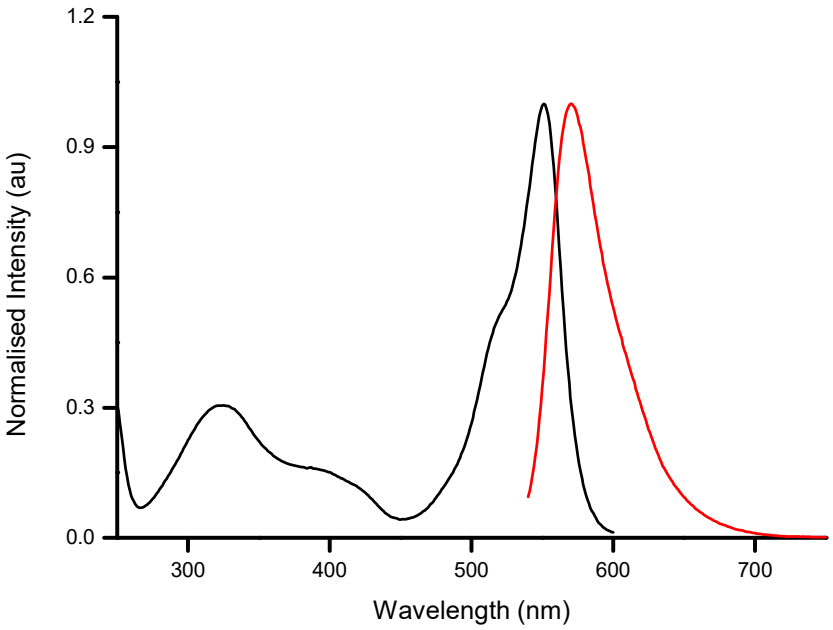


Figure S19. Normalized absorption (black line) and emission spectrum of **3** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

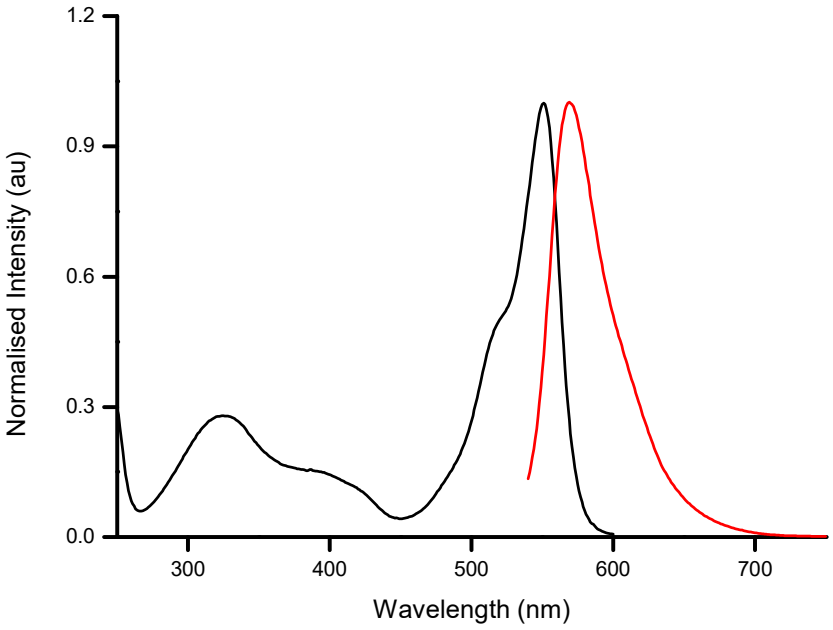


Figure S20. Normalized absorption (black line) and emission spectrum of **4** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

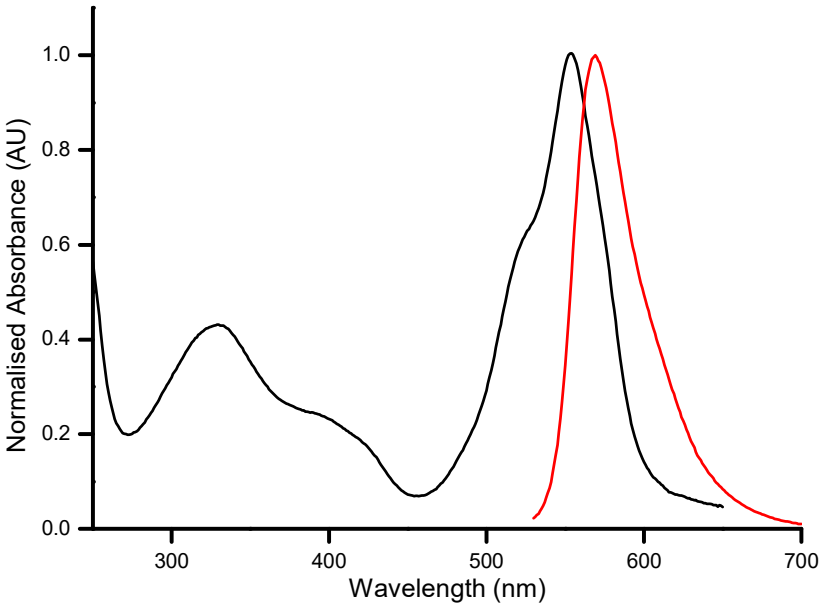


Figure S21. Normalized absorption (black line) and emission spectrum of **5** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, c = 1 ×10^{-5} M).

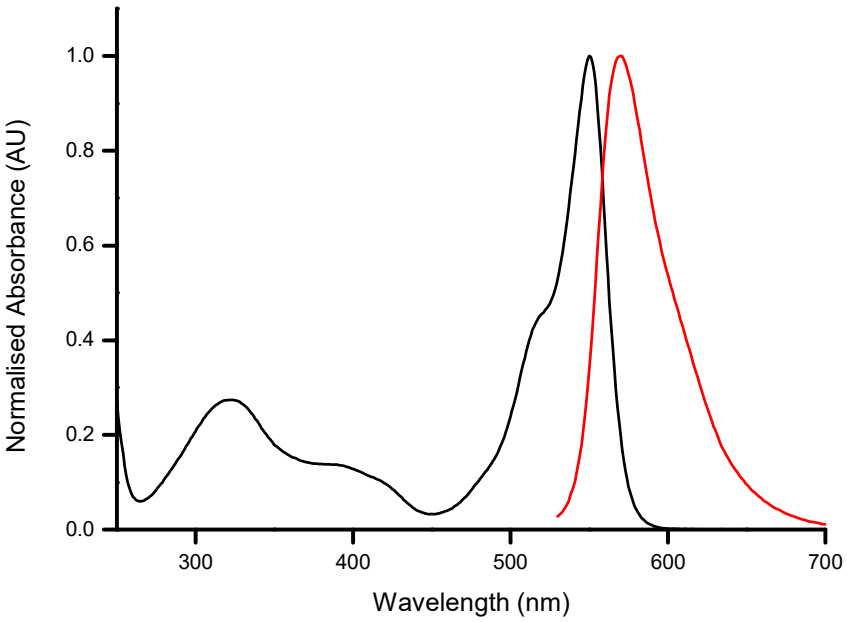


Figure S22. Normalized absorption (black line) and emission spectrum of **6** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, c = 1 ×10^{-5} M).

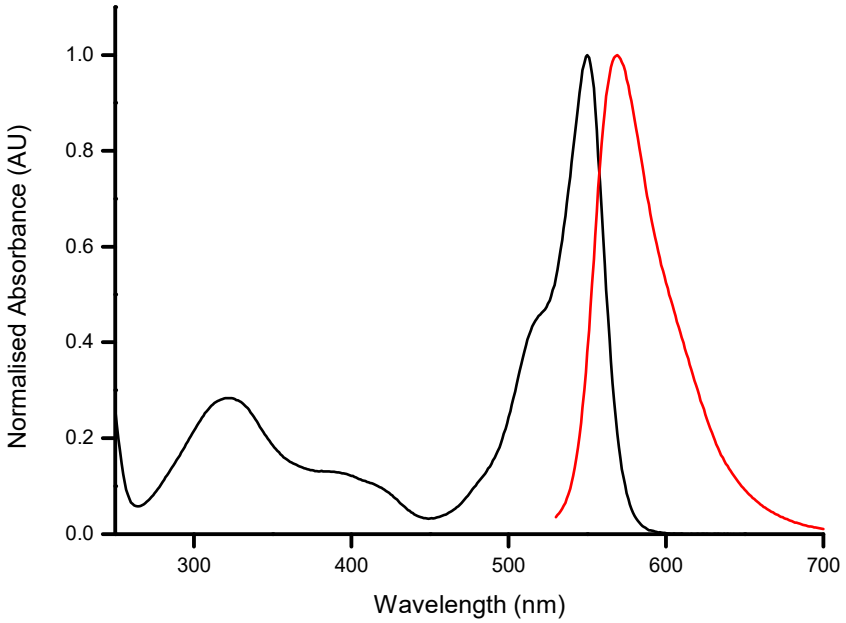


Figure S23. Normalized absorption (black line) and emission spectrum of **7** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, c = 1 ×10^{-5} M).

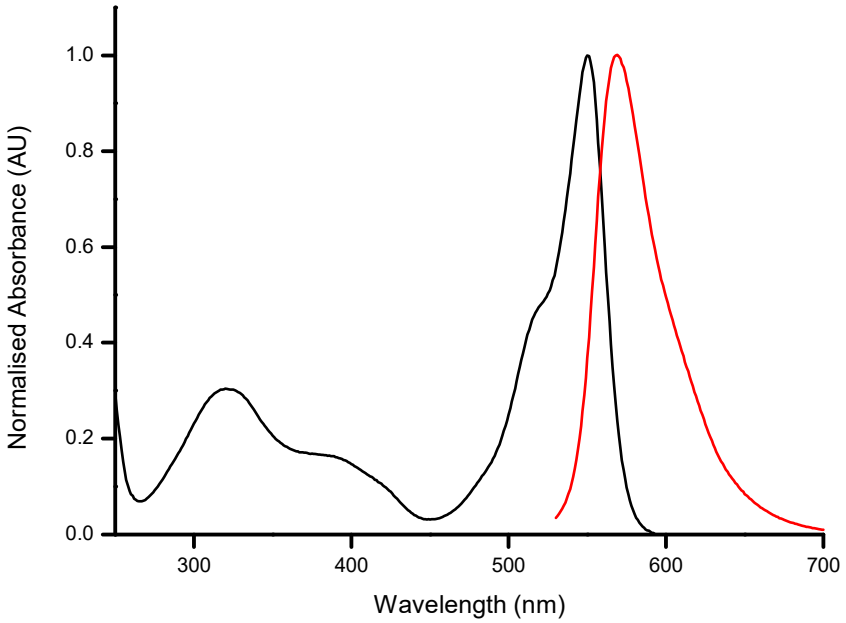


Figure S24. Normalized absorption (black line) and emission spectrum of **8** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, c = 1 ×10^{-5} M).

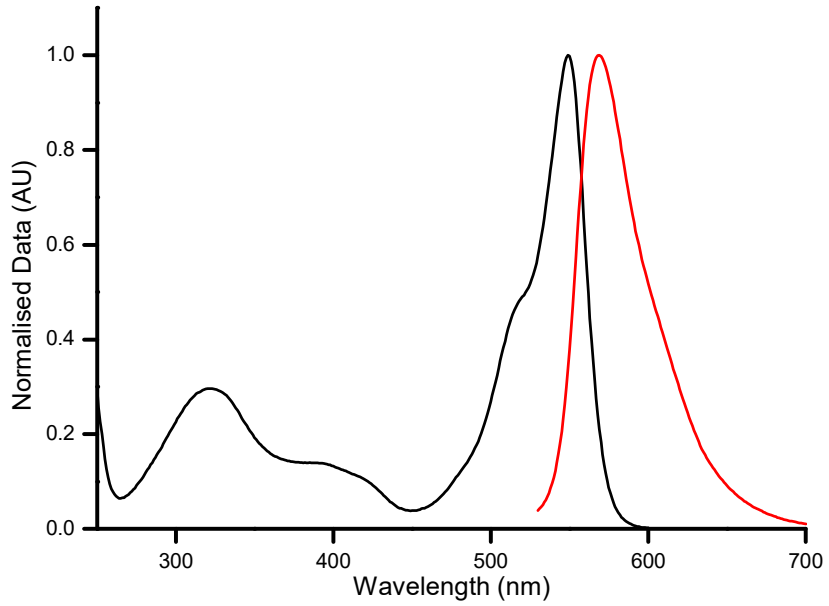


Figure S25. Normalized absorption (black line) and emission spectrum of **9** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

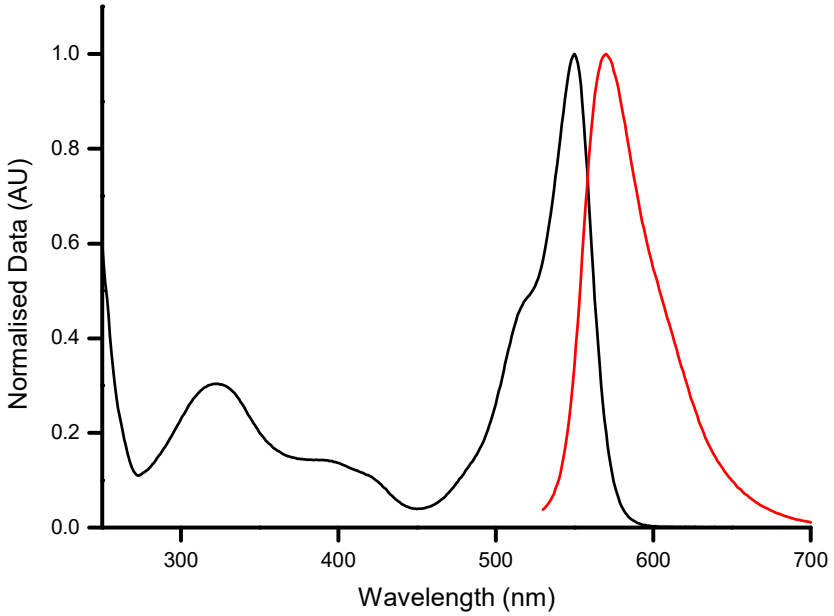


Figure S26. Normalized absorption (black line) and emission spectrum of **10** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

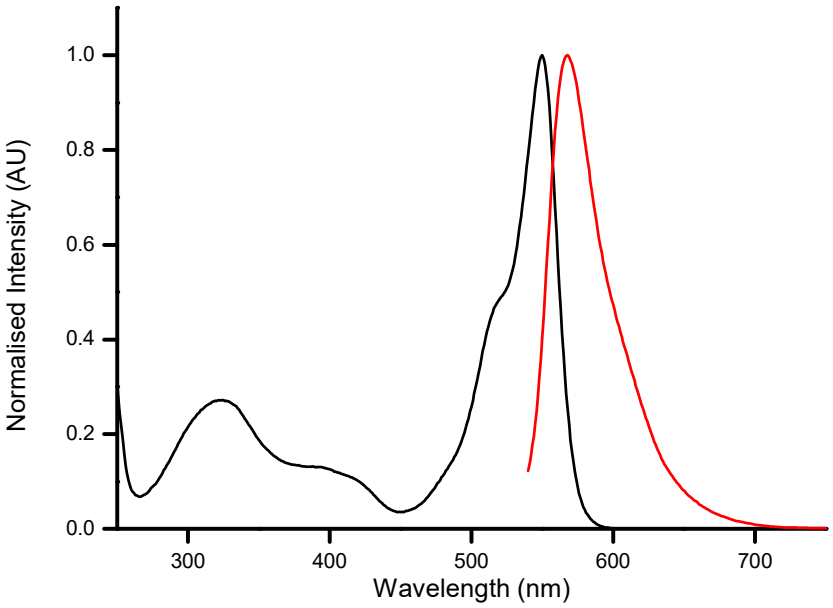


Figure S27. Normalized absorption (black line) and emission spectrum of **11** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

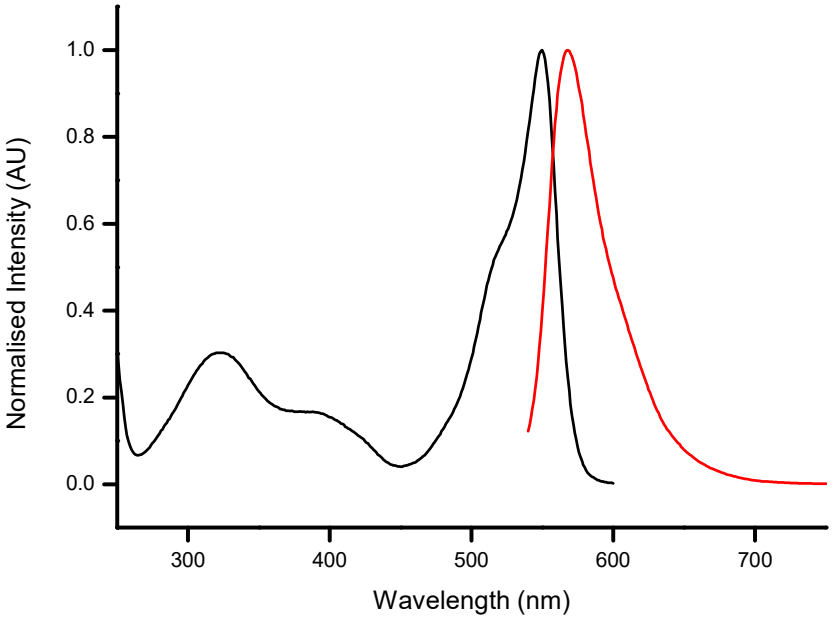


Figure S28. Normalized absorption (black line) and emission spectrum of **12** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

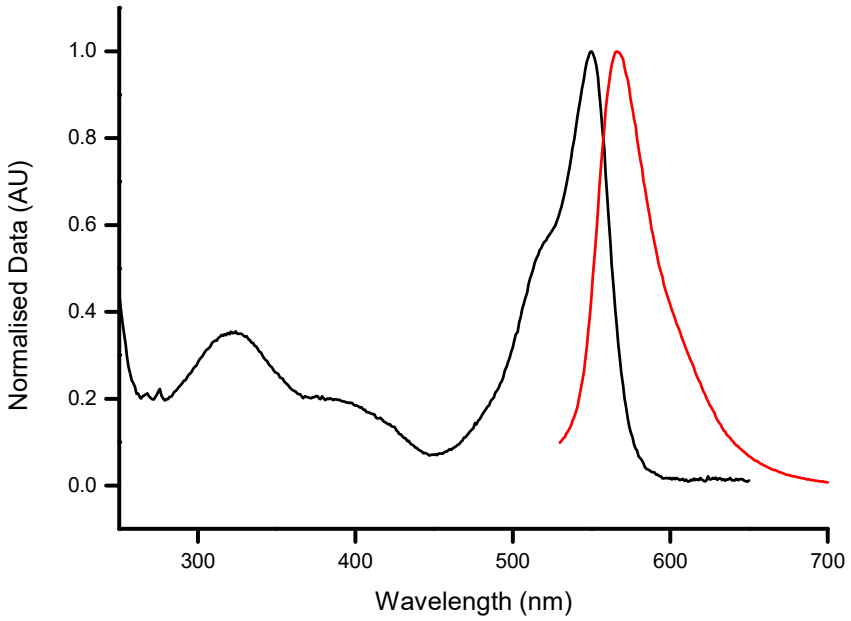


Figure S29. Normalized absorption (black line) and emission spectrum of **13** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

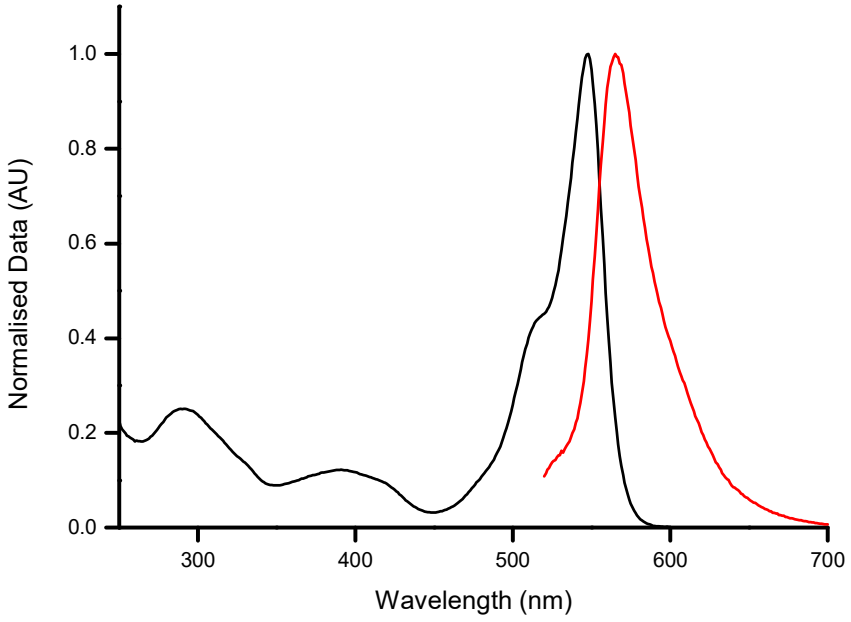


Figure S30. Normalized absorption (black line) and emission spectrum of **14** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

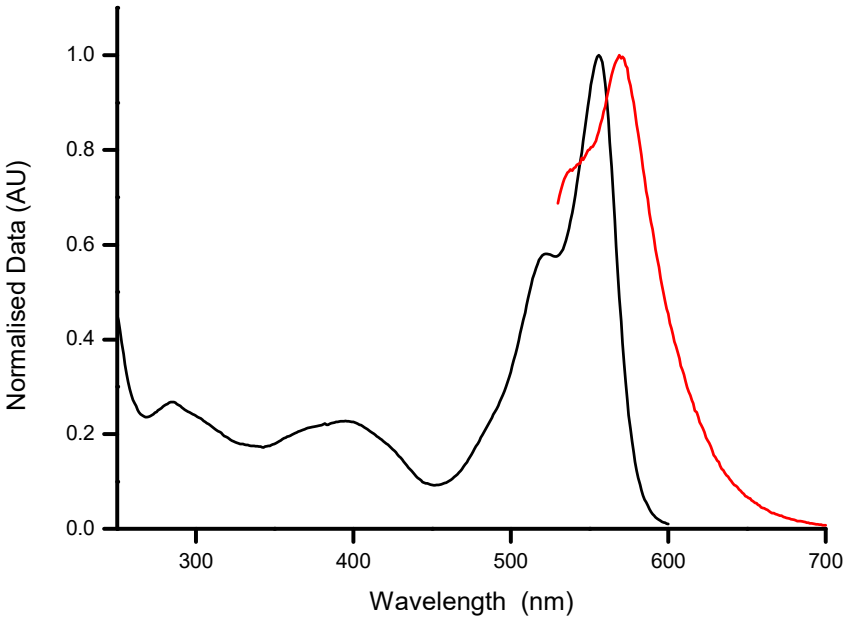


Figure S31. Normalized absorption (black line) and emission spectrum of **15** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, c = 1 ×10^{-5} M).

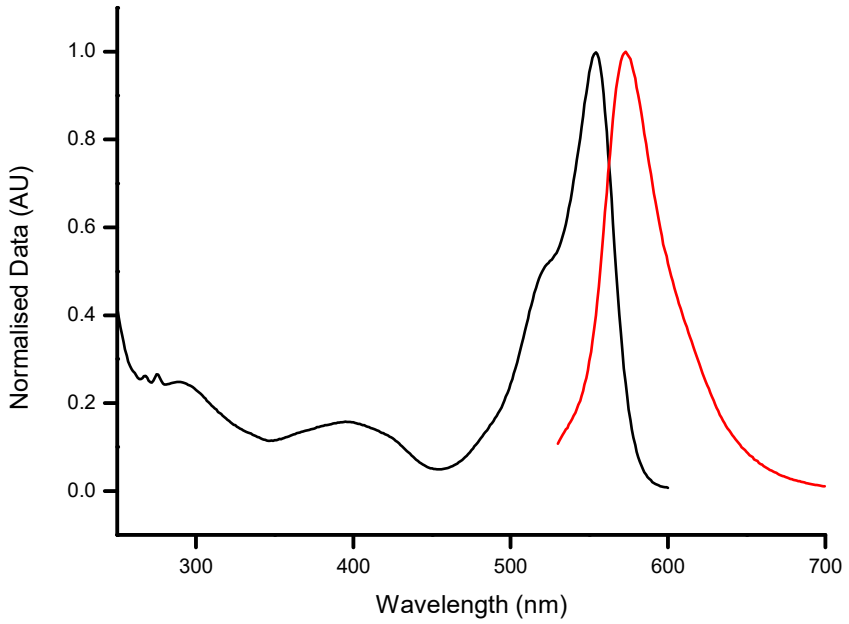


Figure S32. Normalized absorption (black line) and emission spectrum of **16** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, c = 1 ×10^{-5} M).

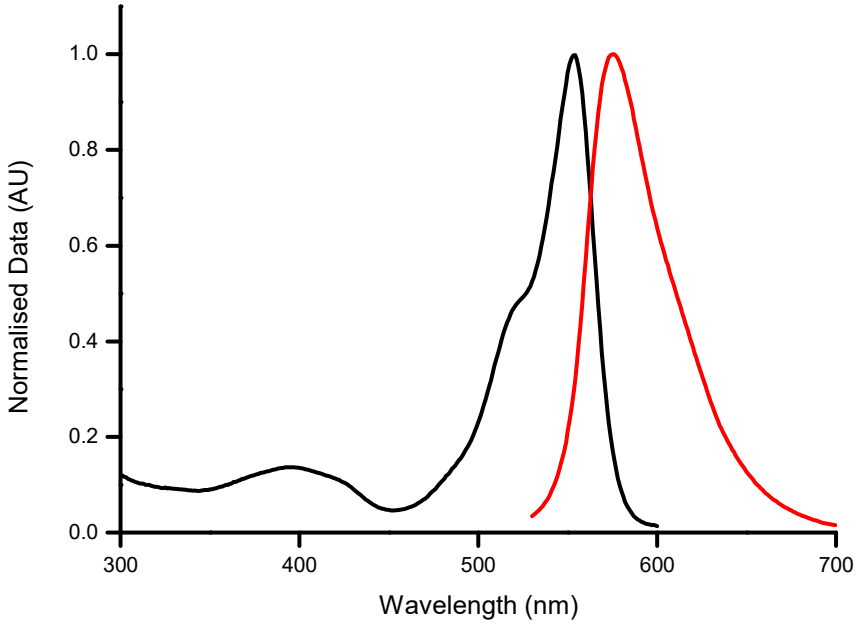


Figure S33. Normalized absorption (black line) and emission spectrum of **18** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

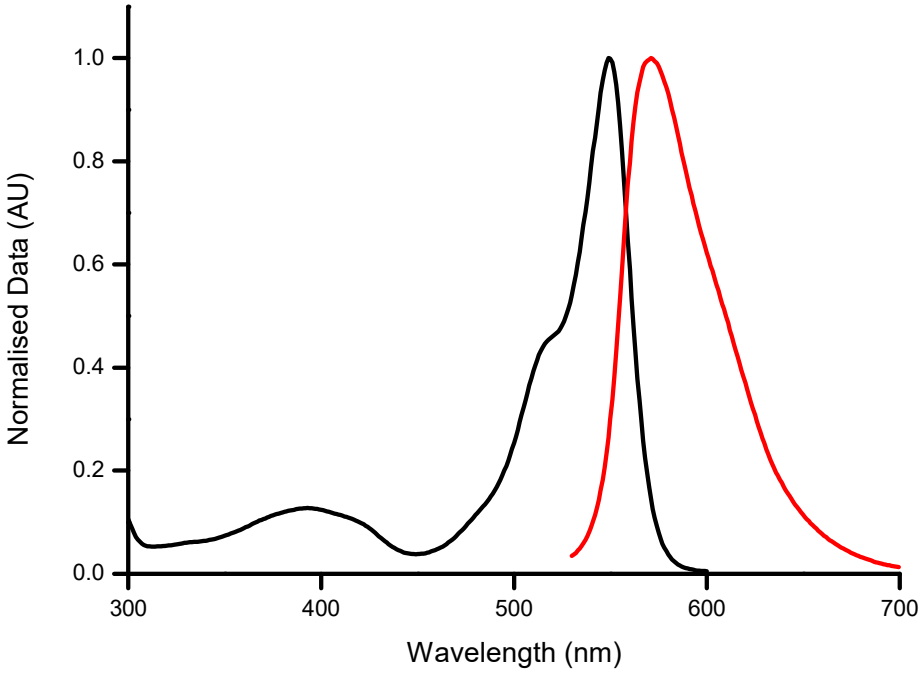


Figure S34. Normalized absorption (black line) and emission spectrum of **19** (red line; excitation wavelength: 520 nm) in acetonitrile/H_2O (7/3, $c = 1 \times 10^{-5}$ M).

^{1}H and ^{13}C NMR spectra

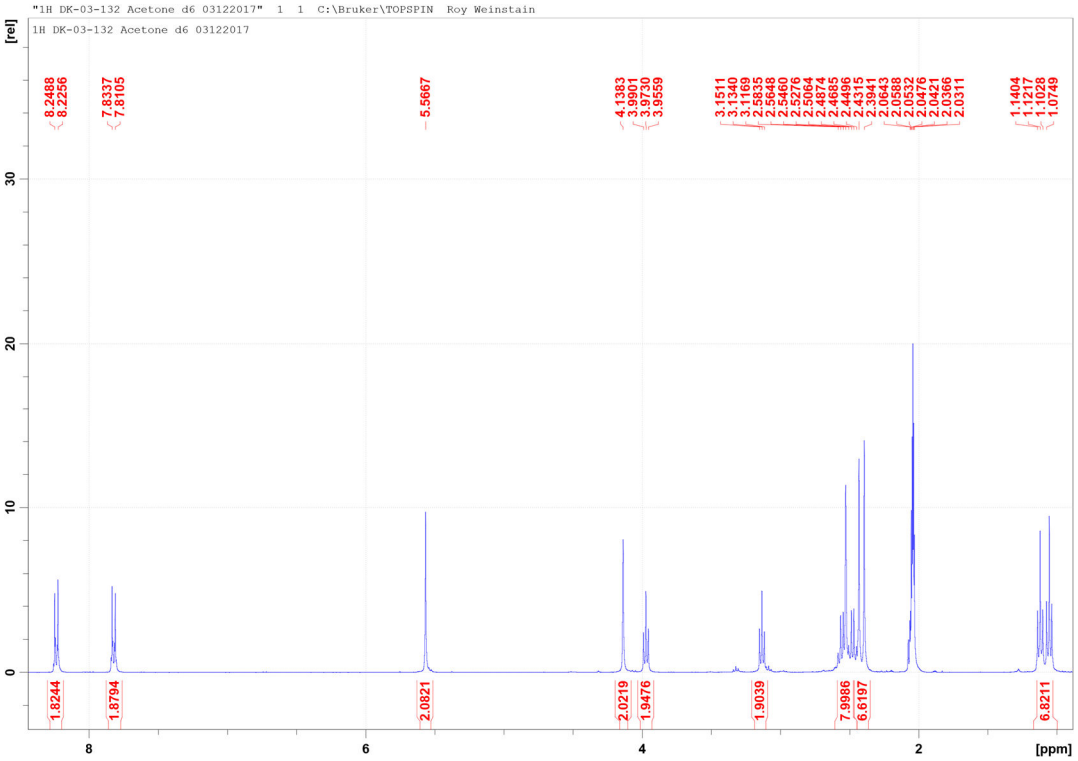


Figure S35. ^{1}H (400 MHz, Acetone-d_6): **1**

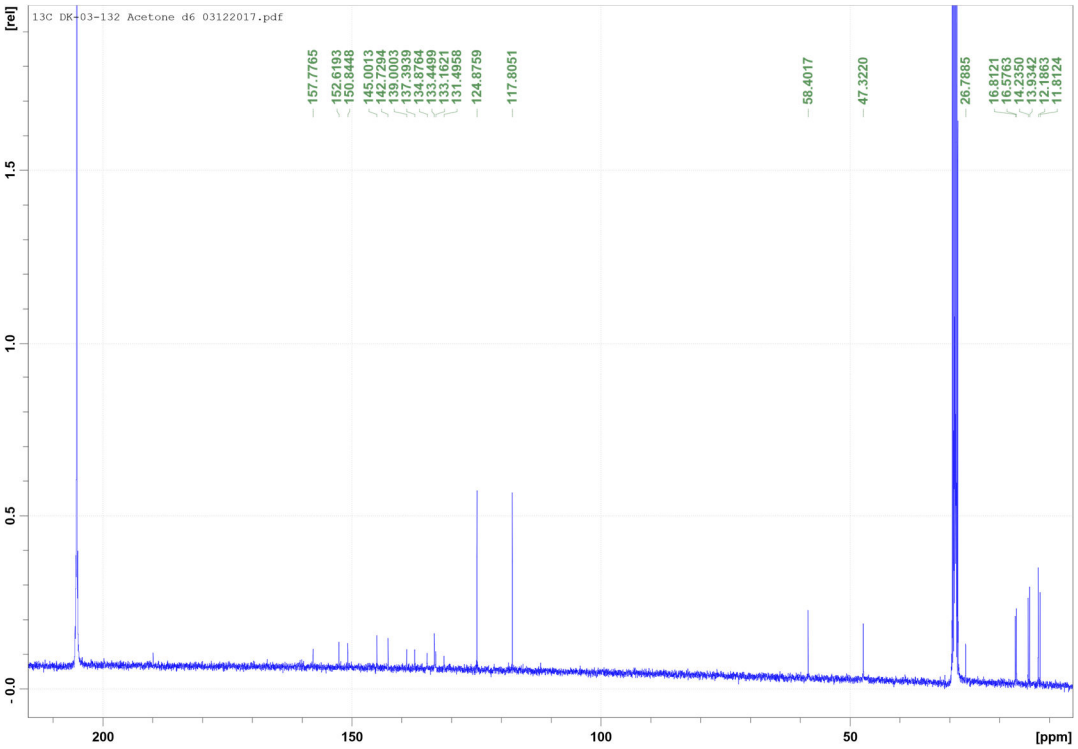


Figure S36. ^{13}C (101 MHz, Acetone-d_6): **1**

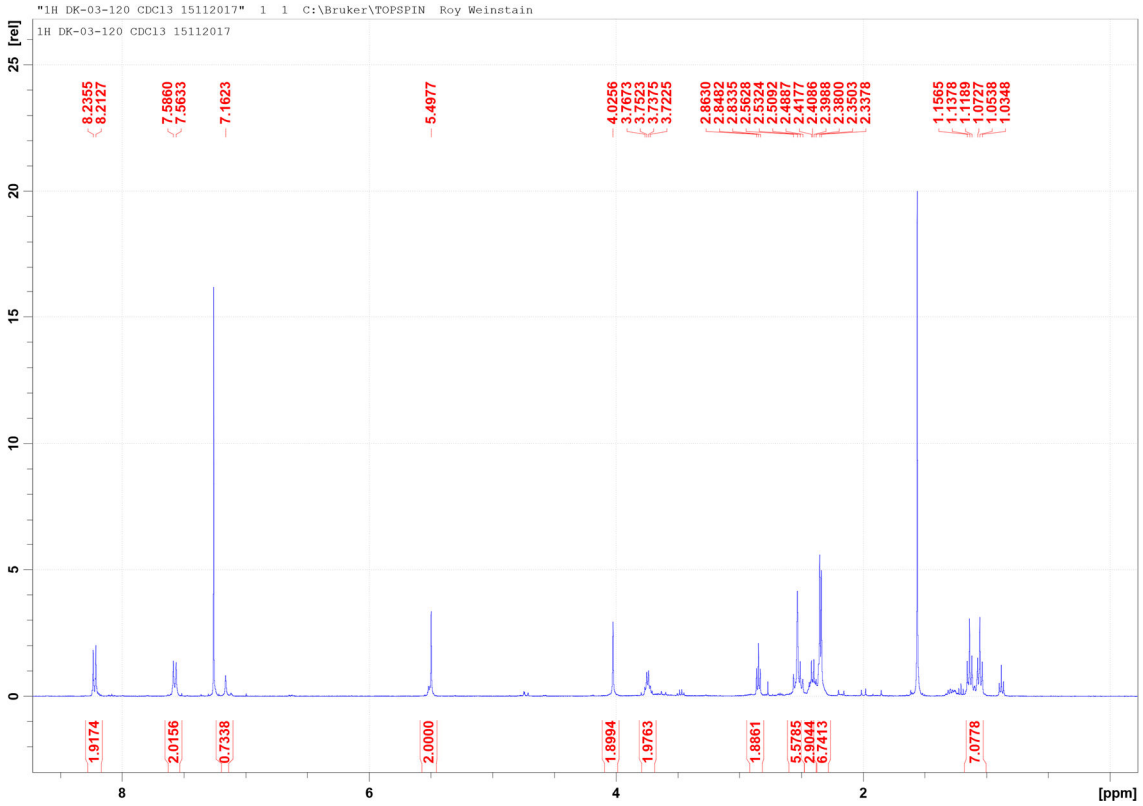


Figure S37. ^{1}H (400 MHz, $CDCl_3$): **2**

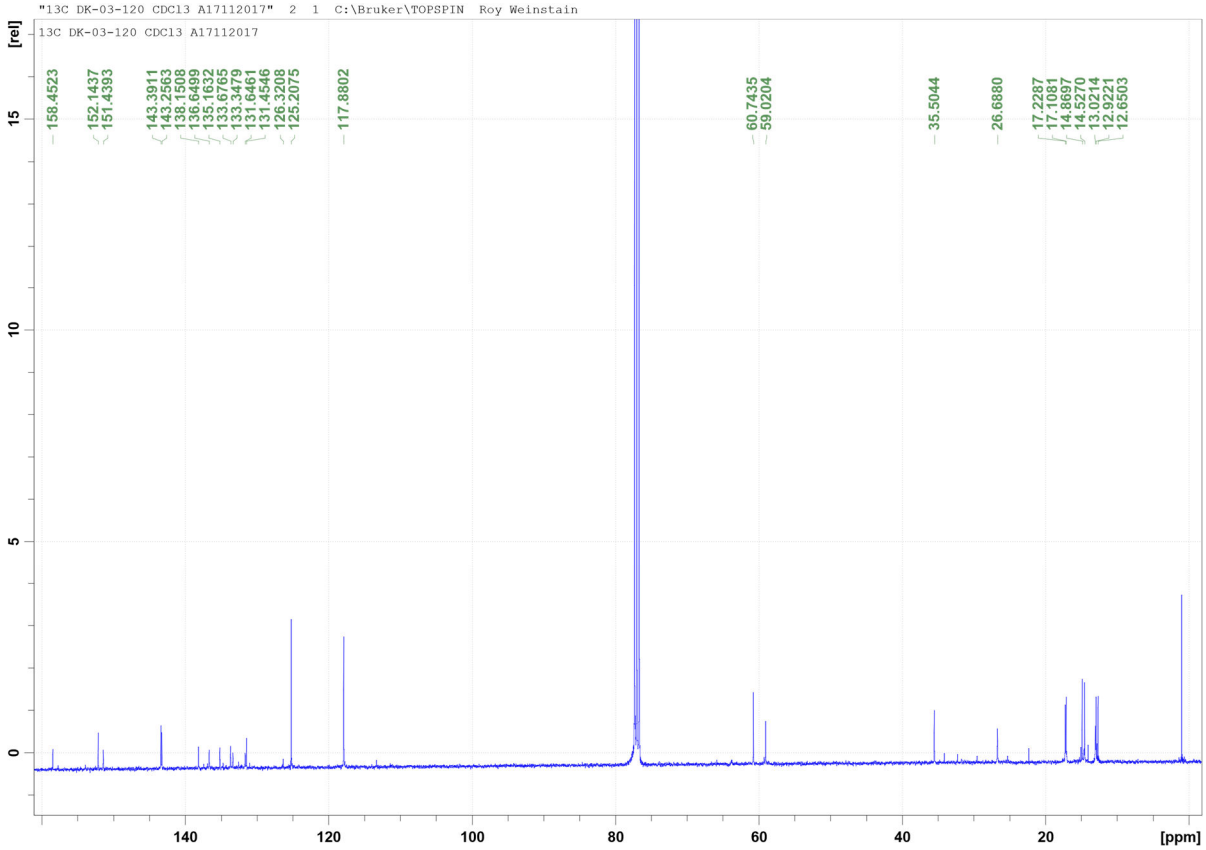


Figure S38. ^{13}C (101 MHz, $CDCl_3$): **2**

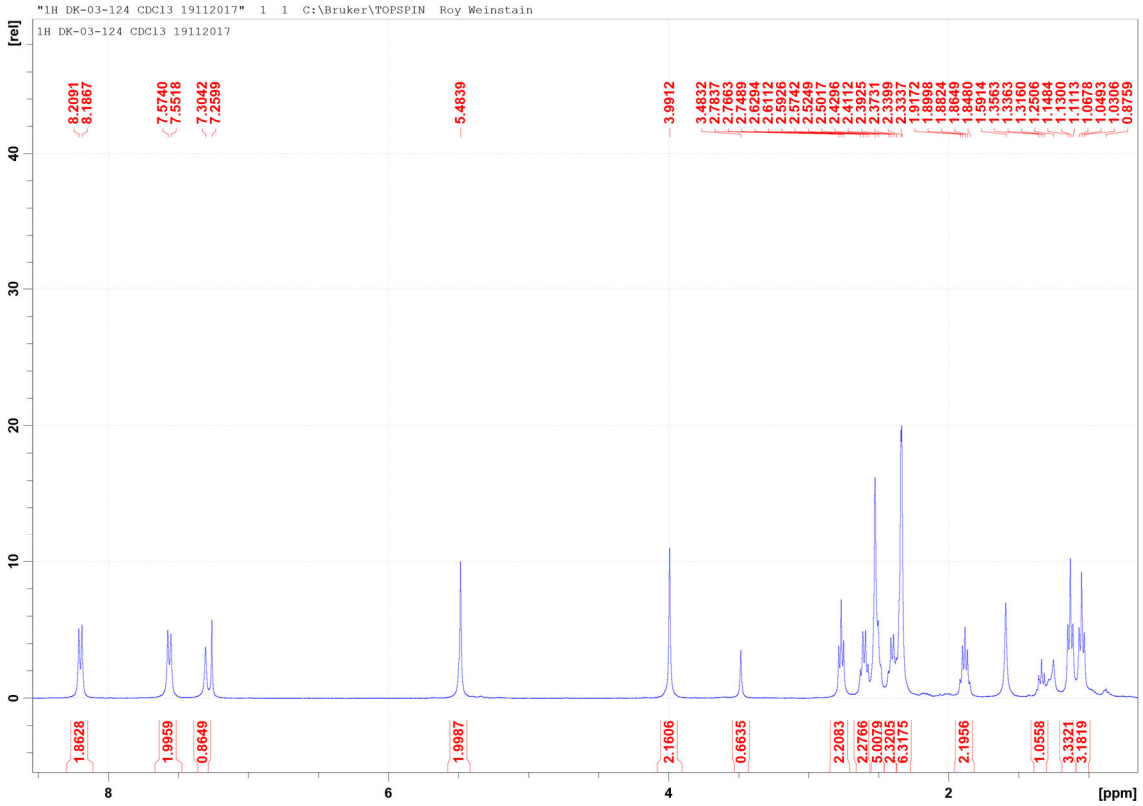


Figure S39. ^{1}H (400 MHz, $CDCl_3$): **3**

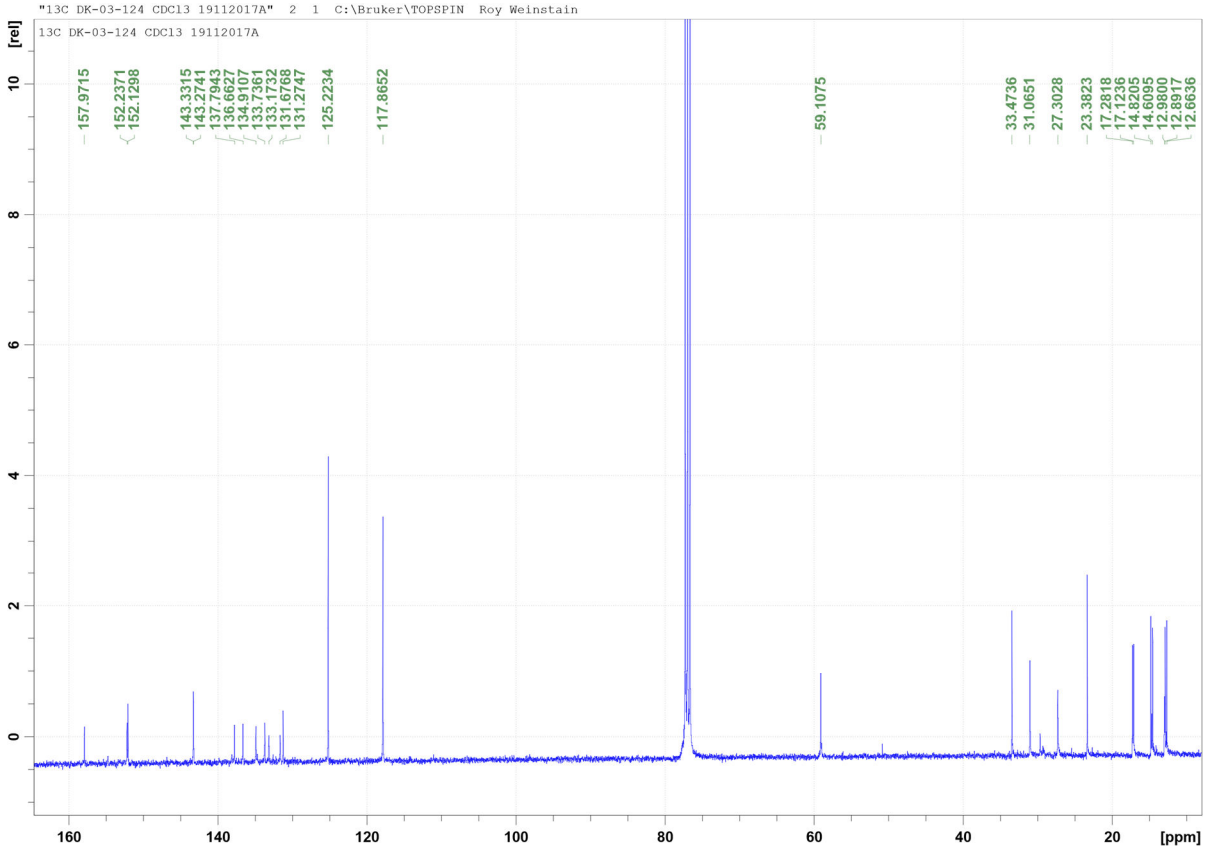


Figure S40. ^{13}C (101 MHz, $CDCl_3$): **3**

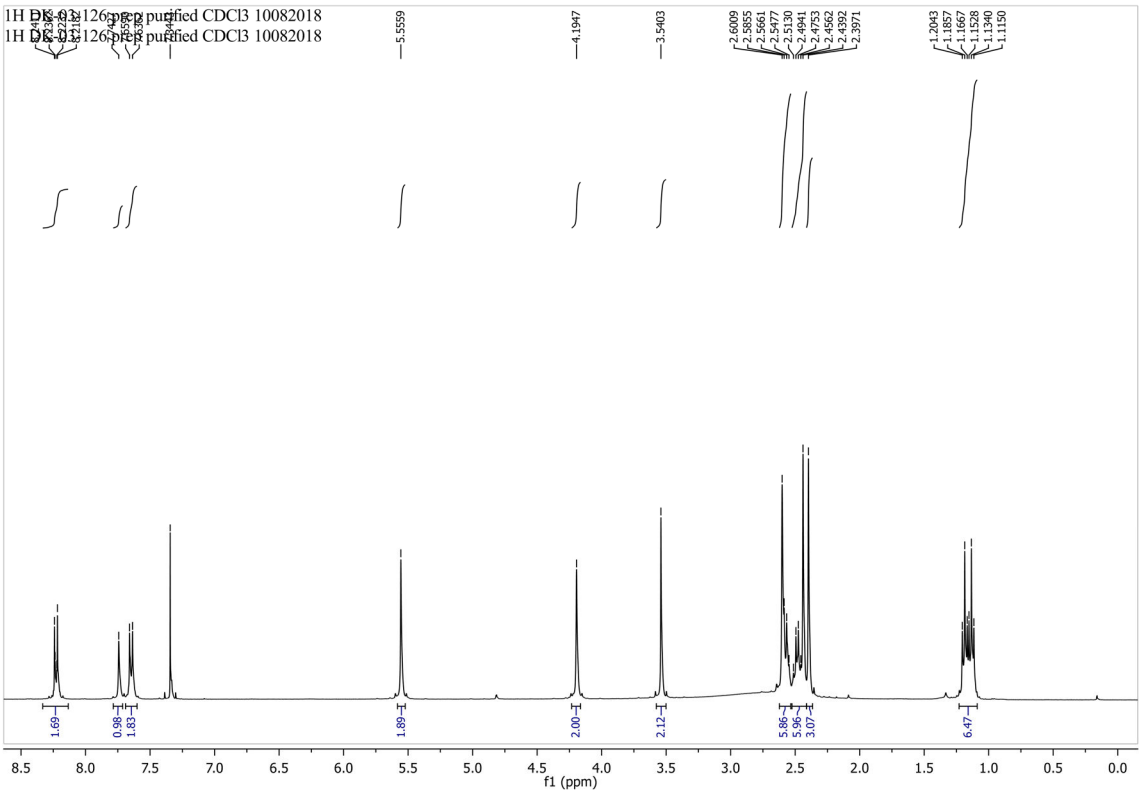


Figure S41. ^{1}H (400 MHz, $CDCl_3$): **4**

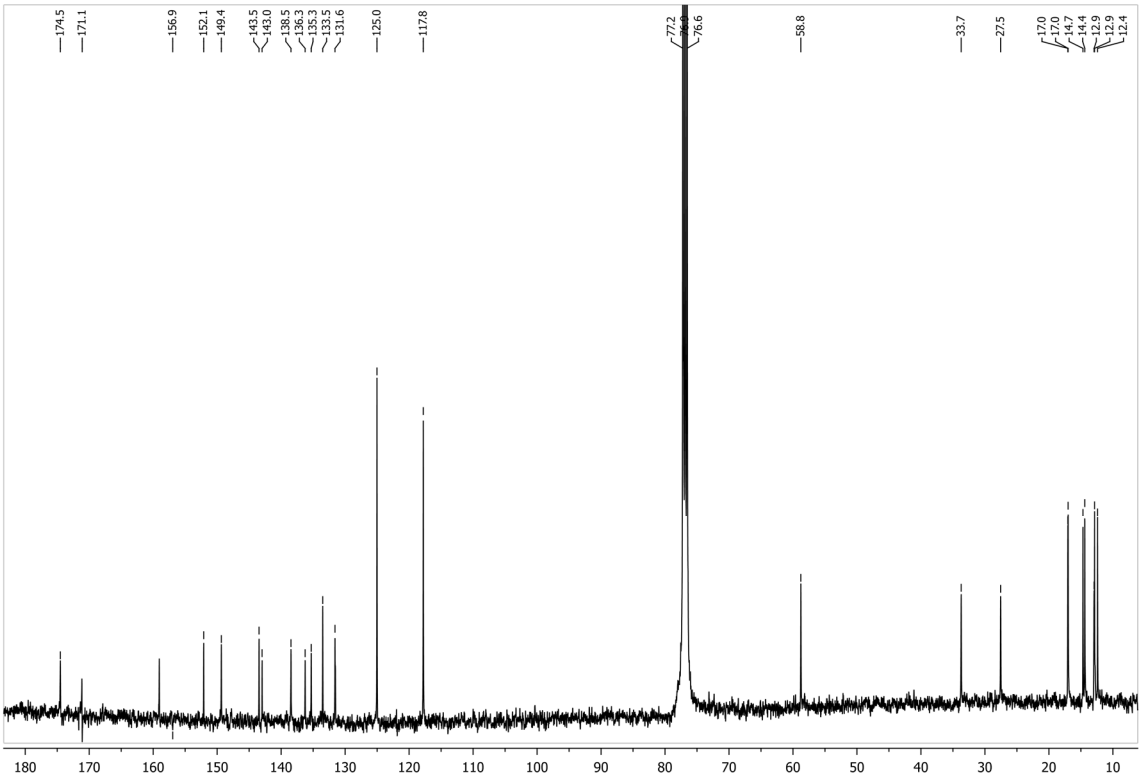


Figure S42. ^{13}C (101 MHz, $CDCl_3$): **4**

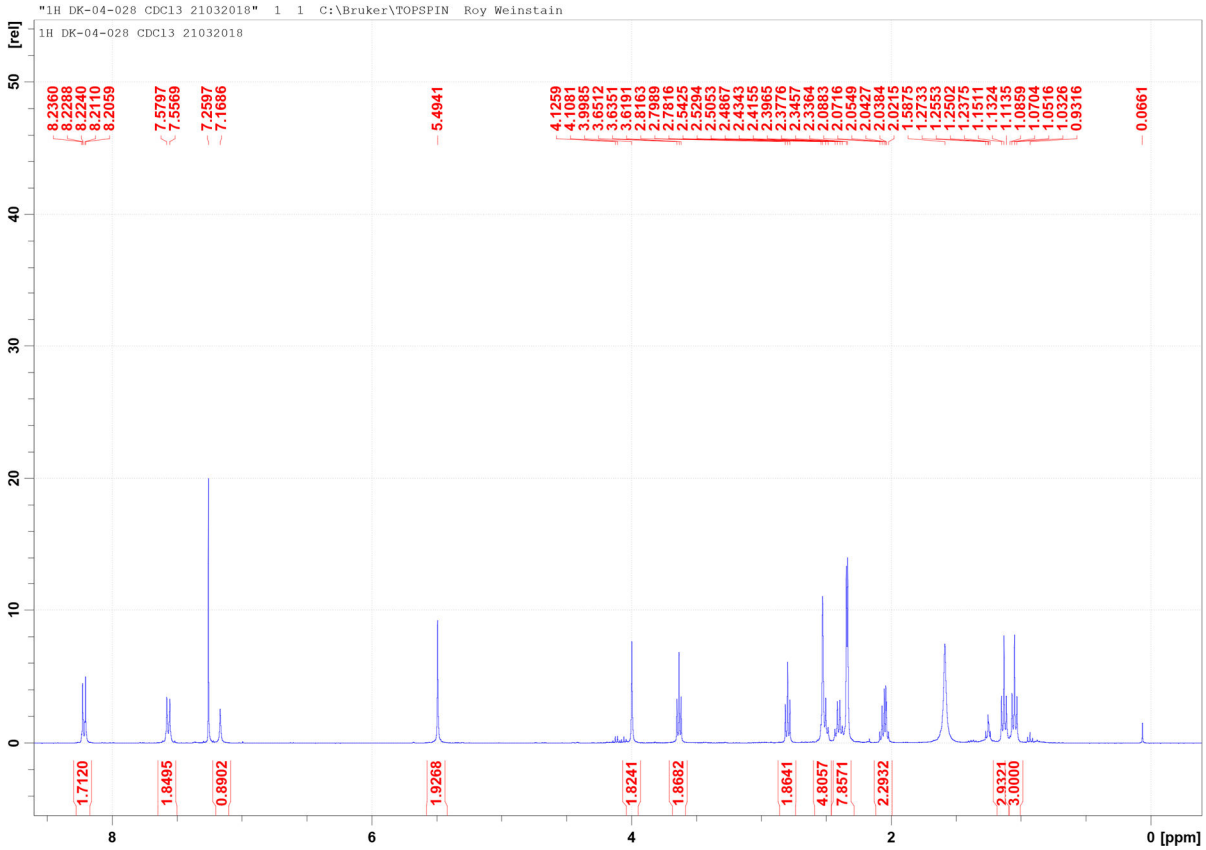


Figure S43. ^{1}H (400 MHz, $CDCl_3$): **5**

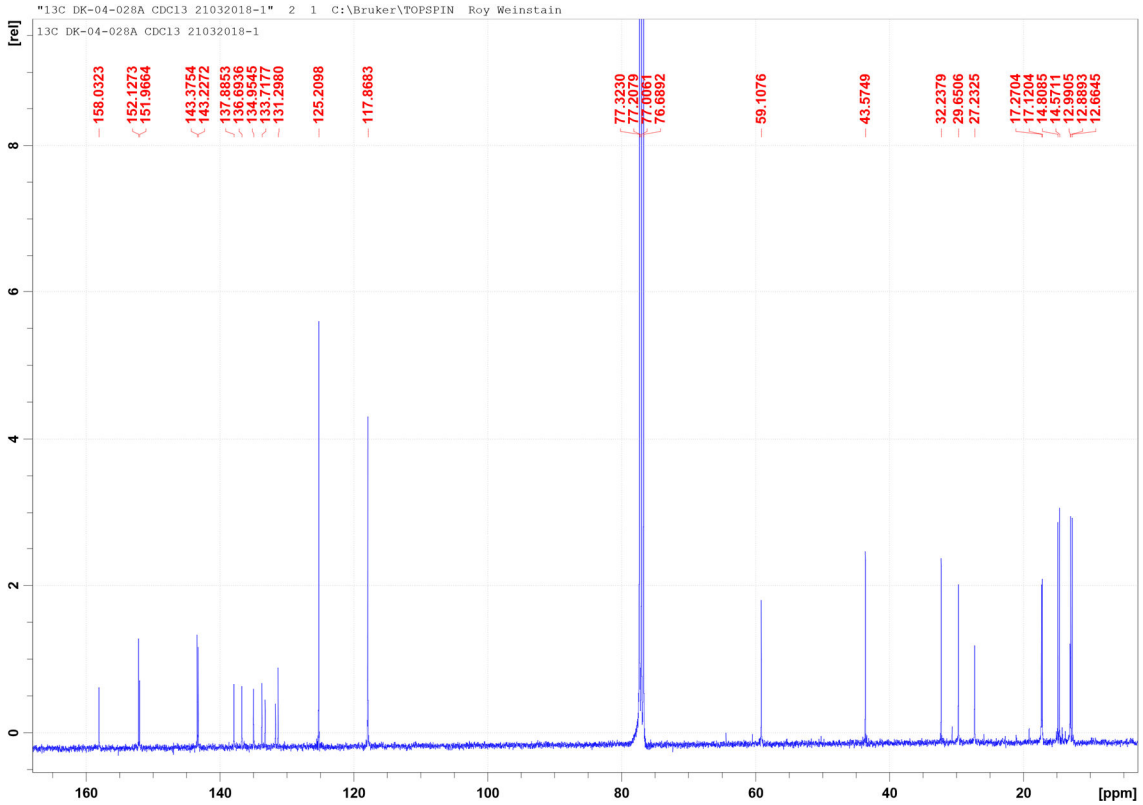


Figure S44. ^{13}C (101 MHz, $CDCl_3$): **5**

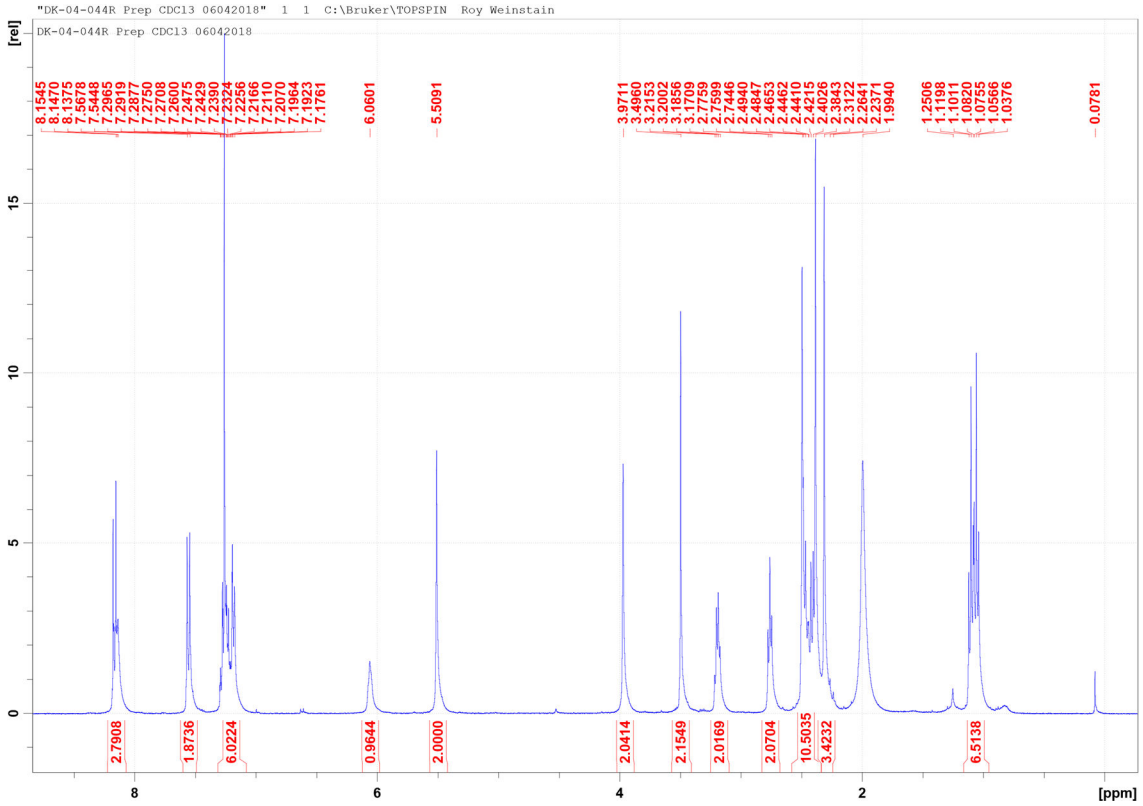


Figure S45. ^{1}H (400 MHz, $CDCl_3$): **6**

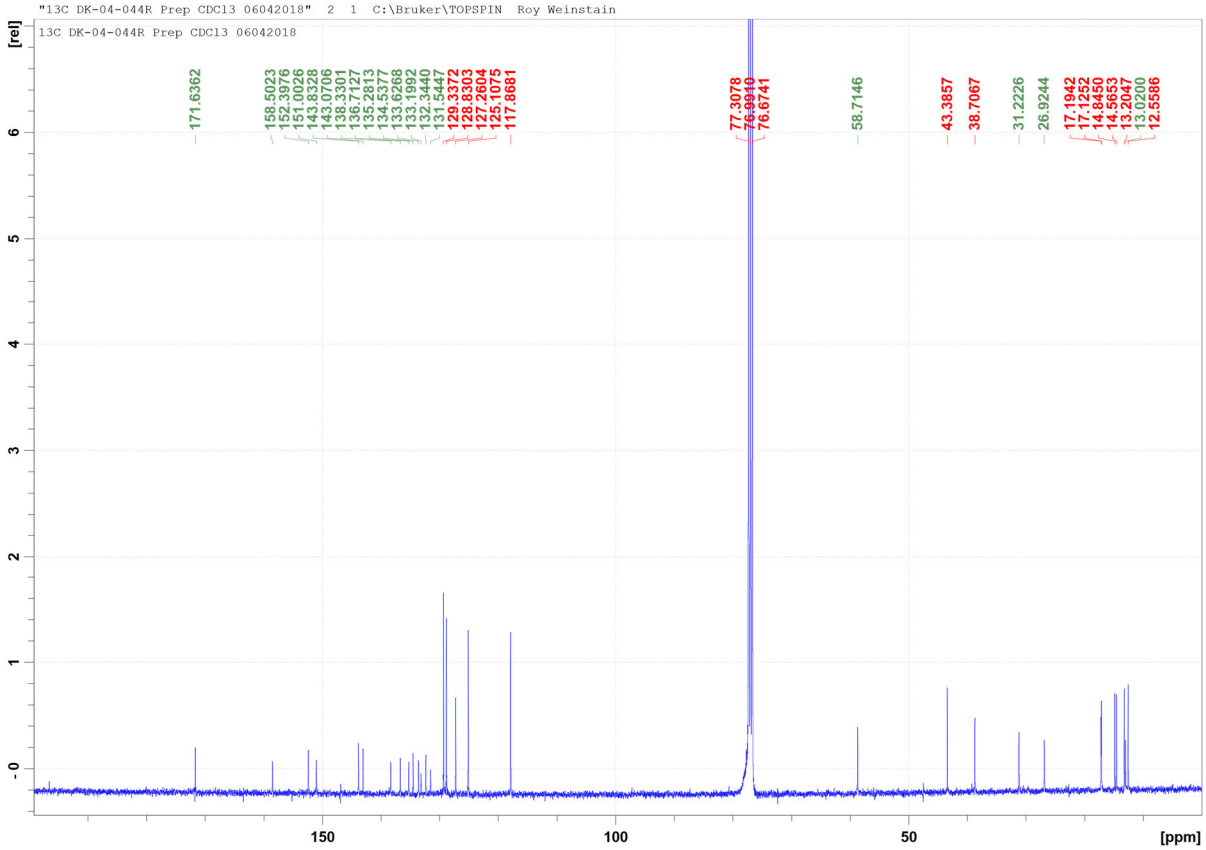


Figure S46. ^{13}C (101 MHz, $CDCl_3$): **6**

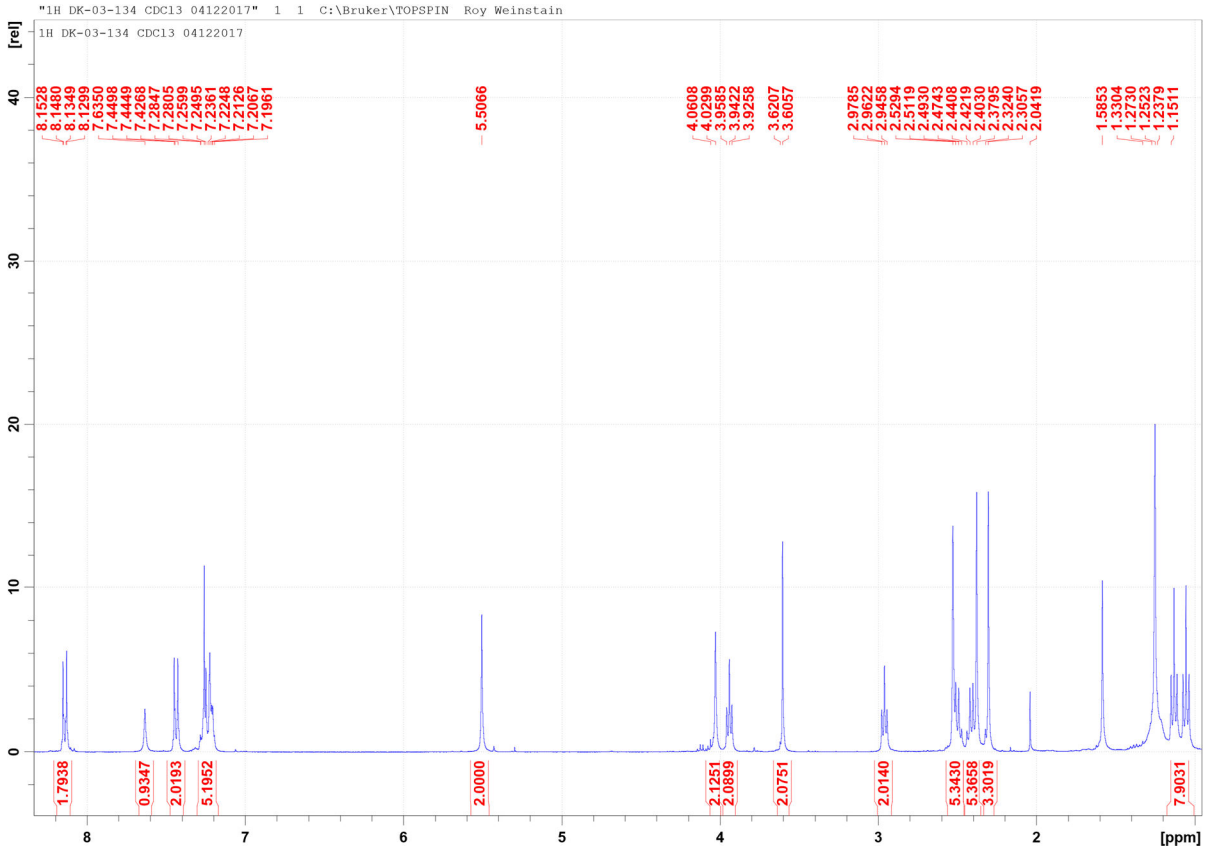


Figure S47. ^{1}H (400 MHz, $CDCl_3$): **7**

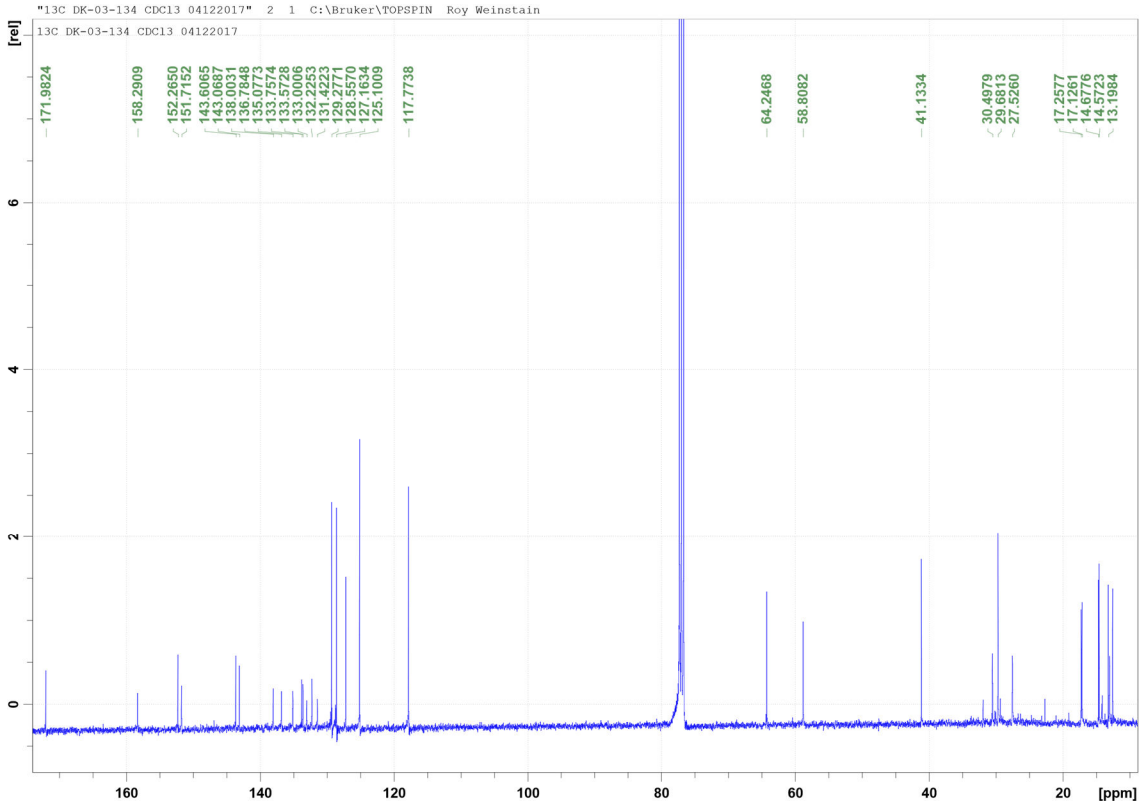


Figure S48. ^{13}C (101 MHz, $CDCl_3$): **7**

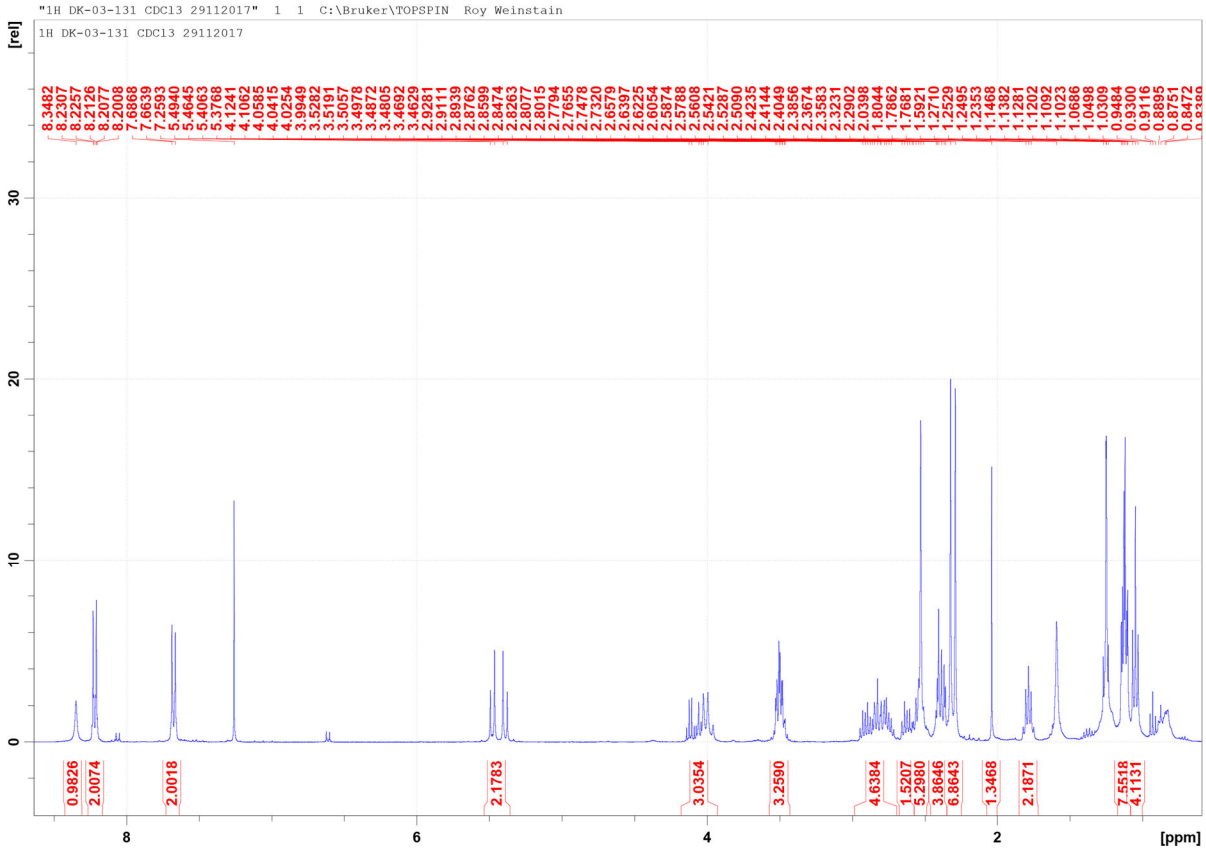


Figure S49. ^{1}H (400 MHz, $CDCl_3$): **8**

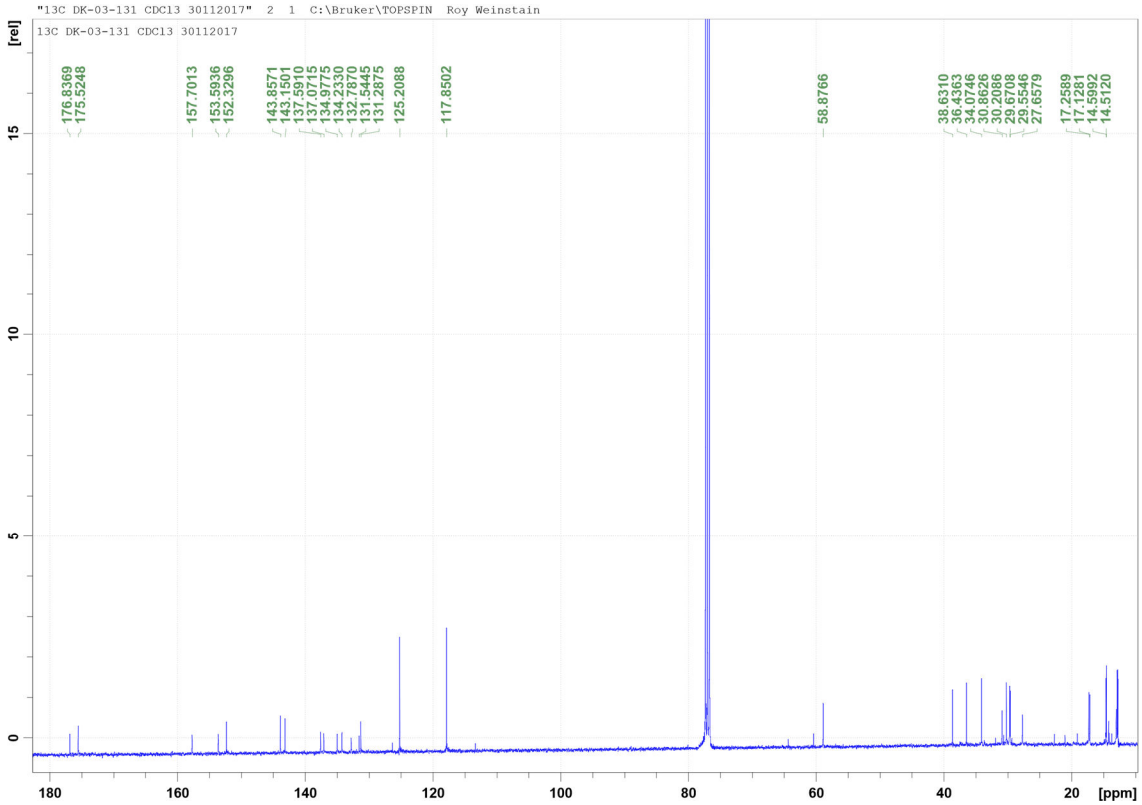


Figure S50. ^{13}C (101 MHz, $CDCl_3$): **8**

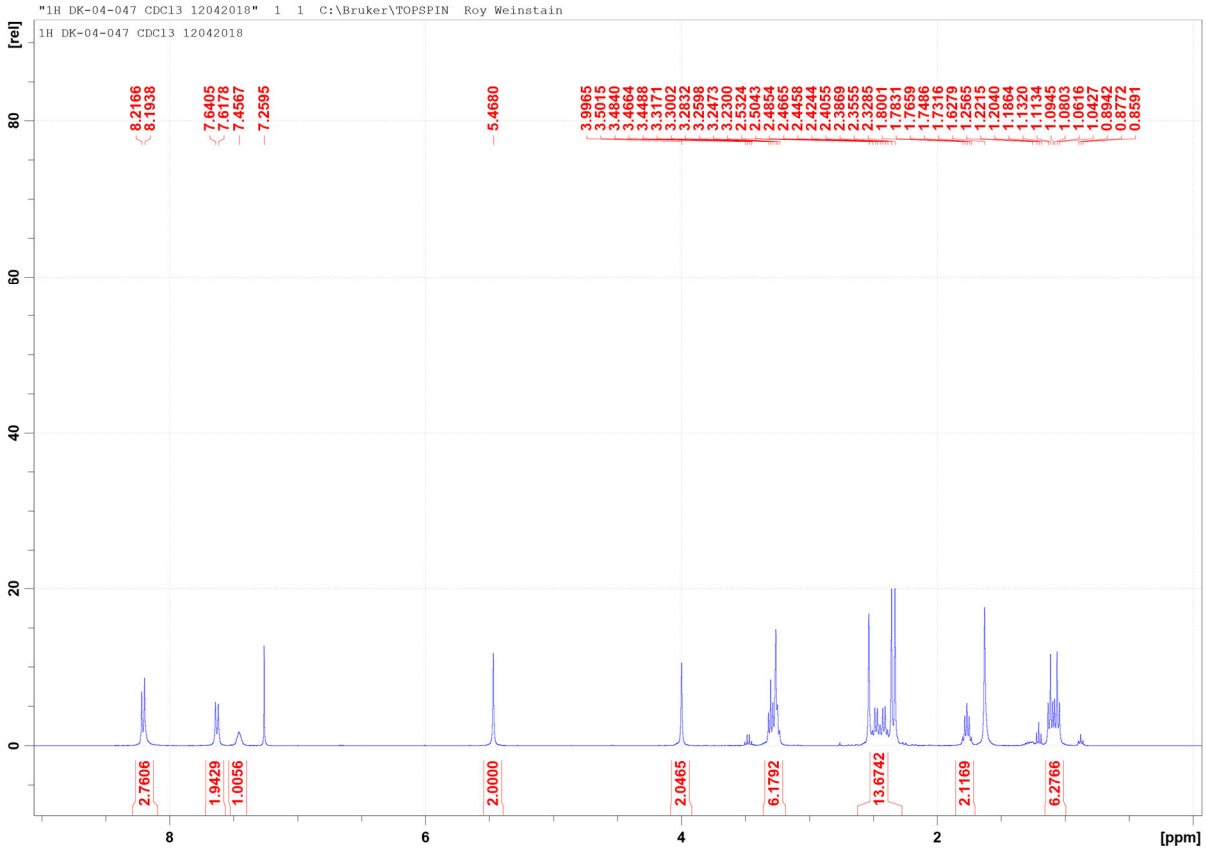


Figure S51. ^{1}H (400 MHz, $CDCl_3$): **9**

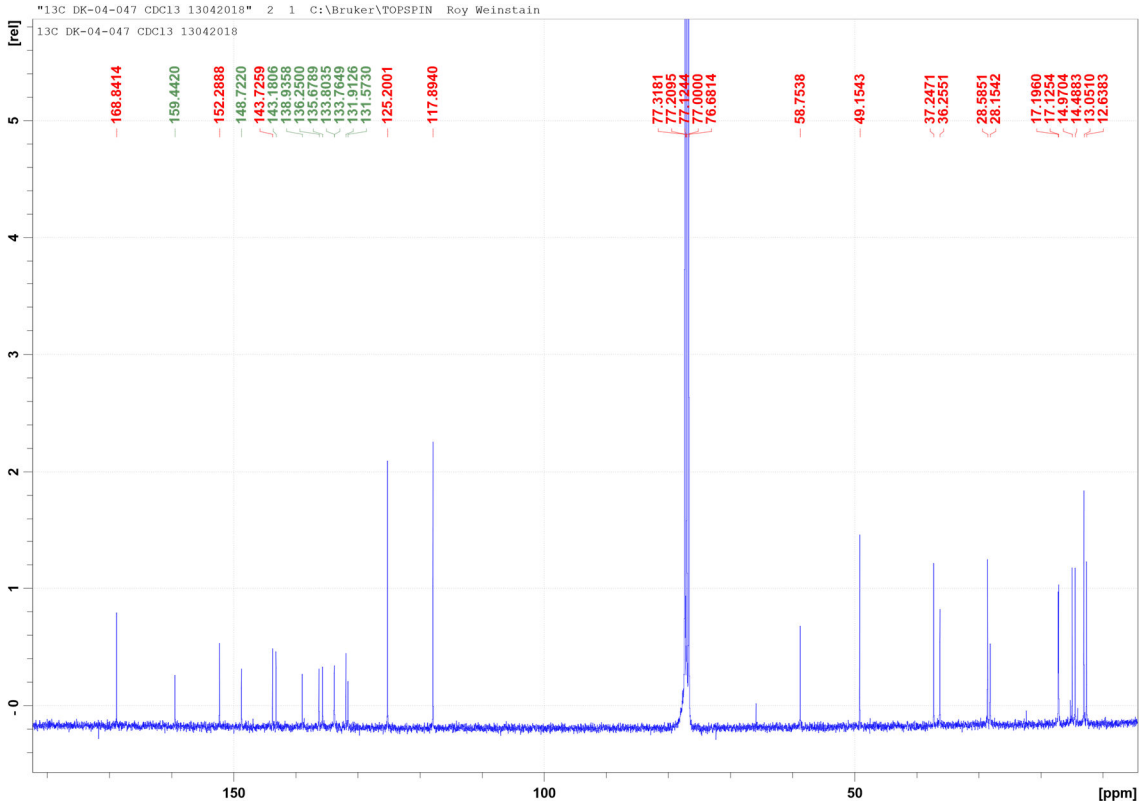


Figure S52. ^{13}C (101 MHz, $CDCl_3$): **9**

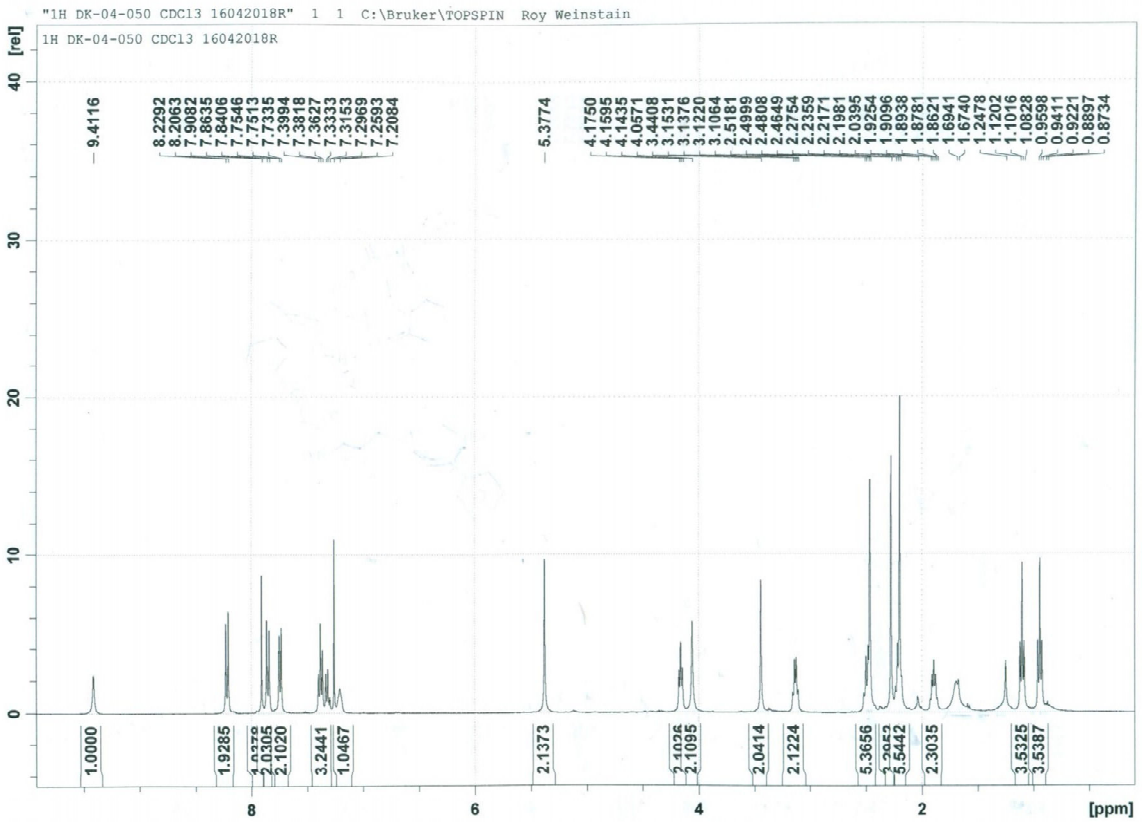


Figure S53. ^{1}H (400 MHz, $CDCl_3$): **10**

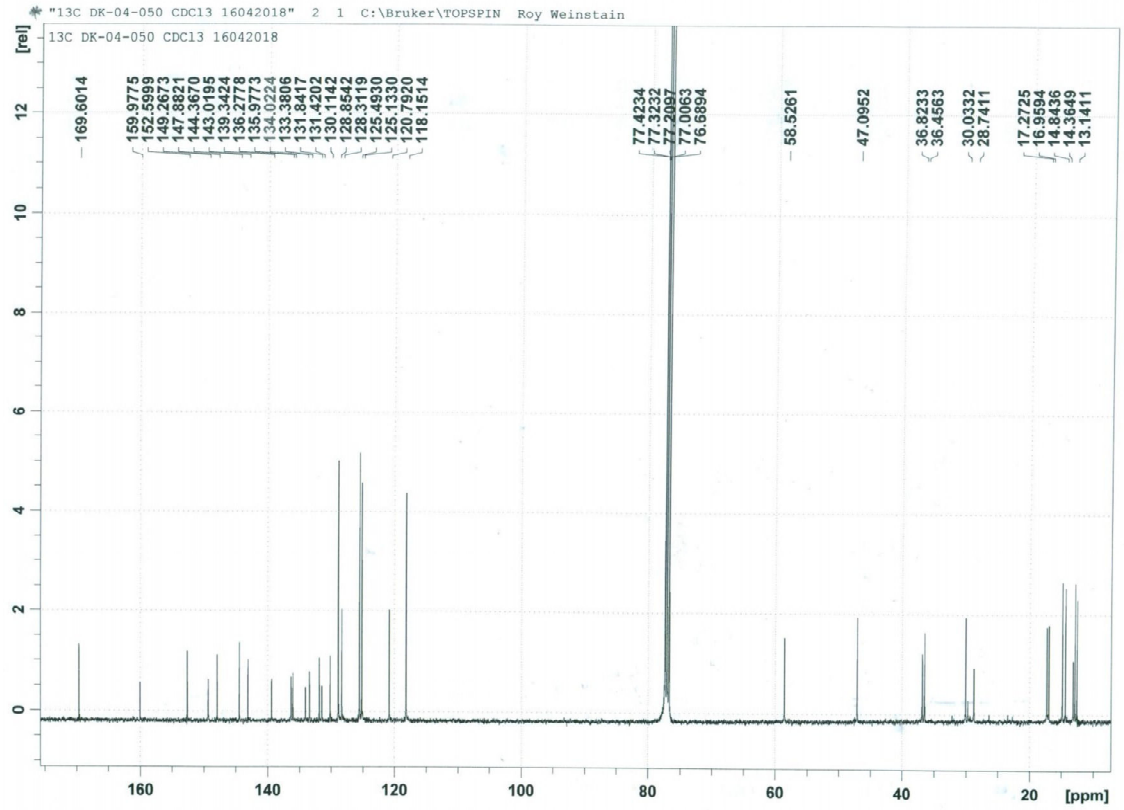


Figure S54. ^{13}C (101 MHz, $CDCl_3$): **10**

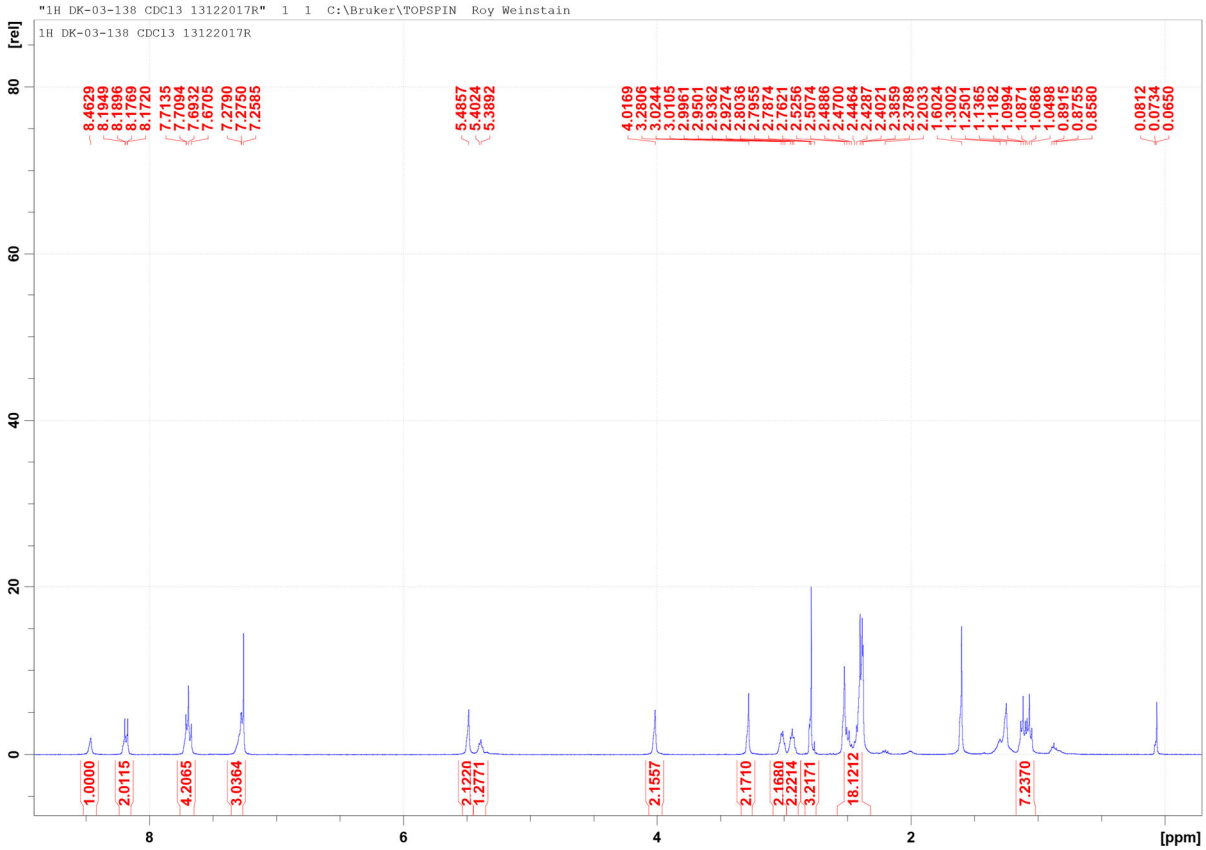


Figure S55. ^{1}H (400 MHz, $CDCl_3$): **11**

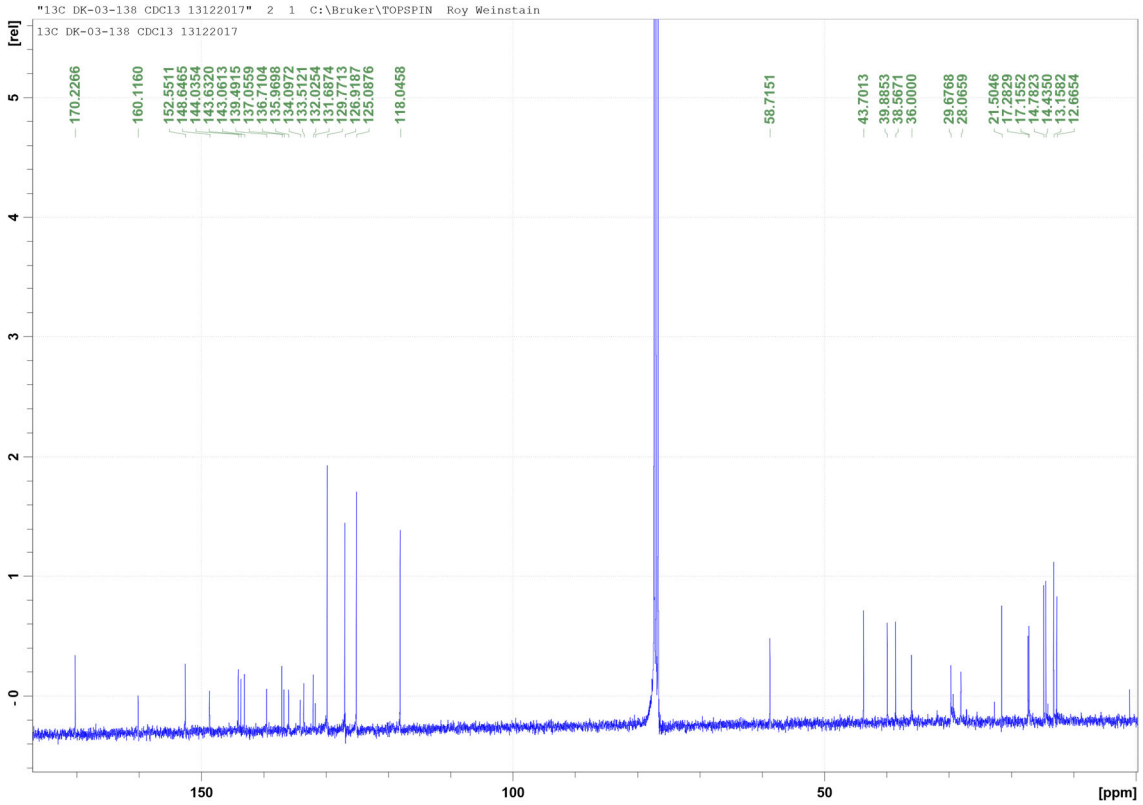


Figure S56. ^{13}C (101 MHz, $CDCl_3$): **11**

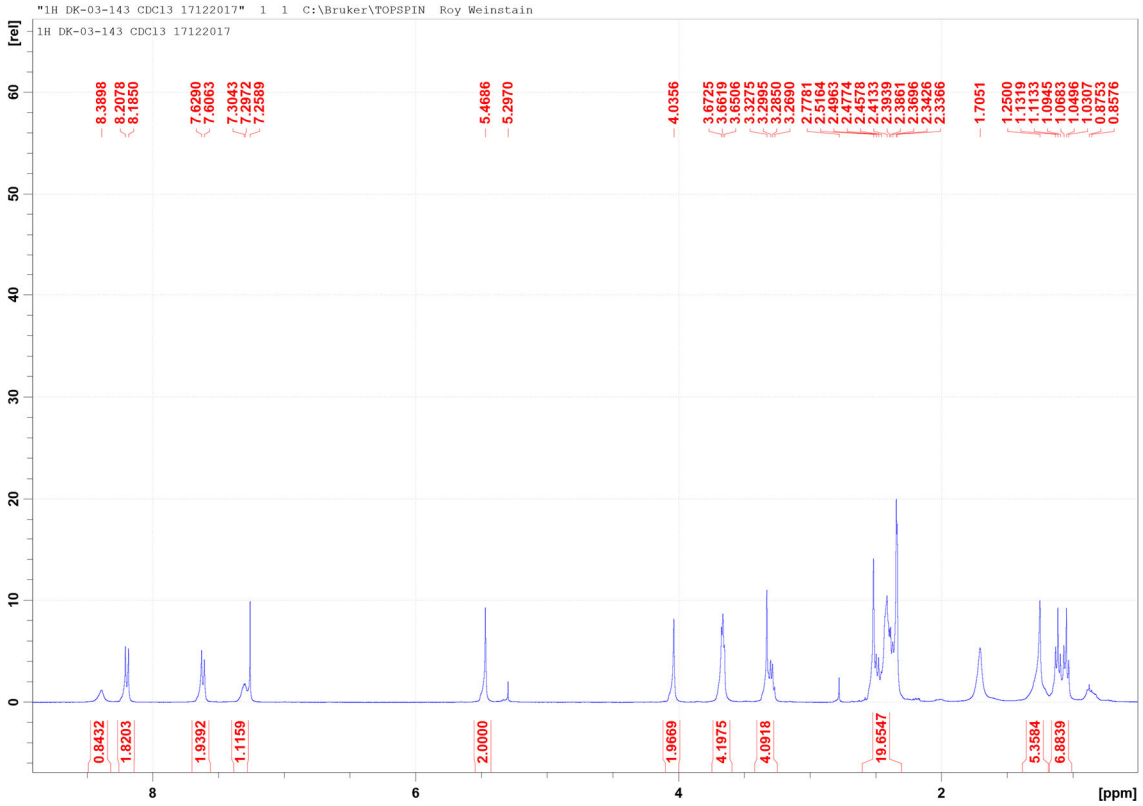


Figure S57. ^{1}H (400 MHz, $CDCl_3$): **12**

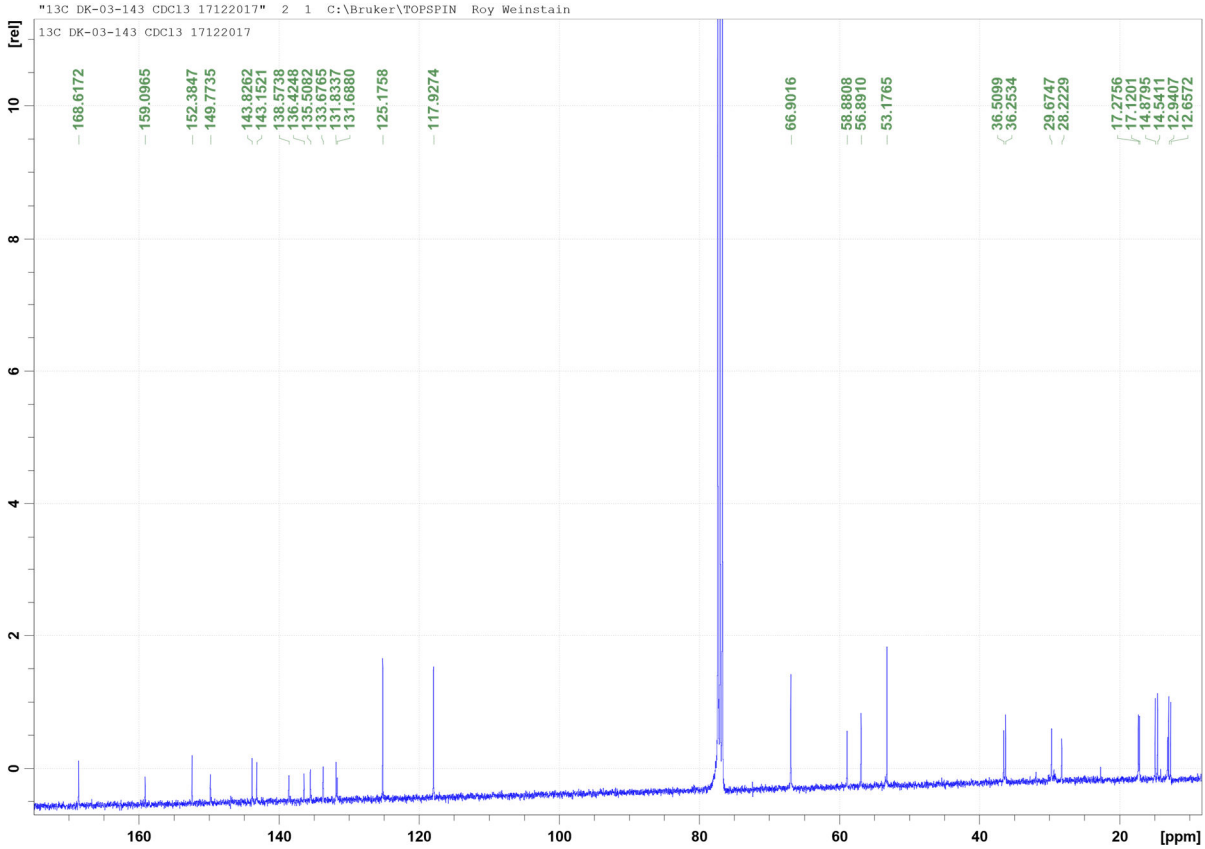


Figure S58. ^{13}C (101 MHz, $CDCl_3$): **12**

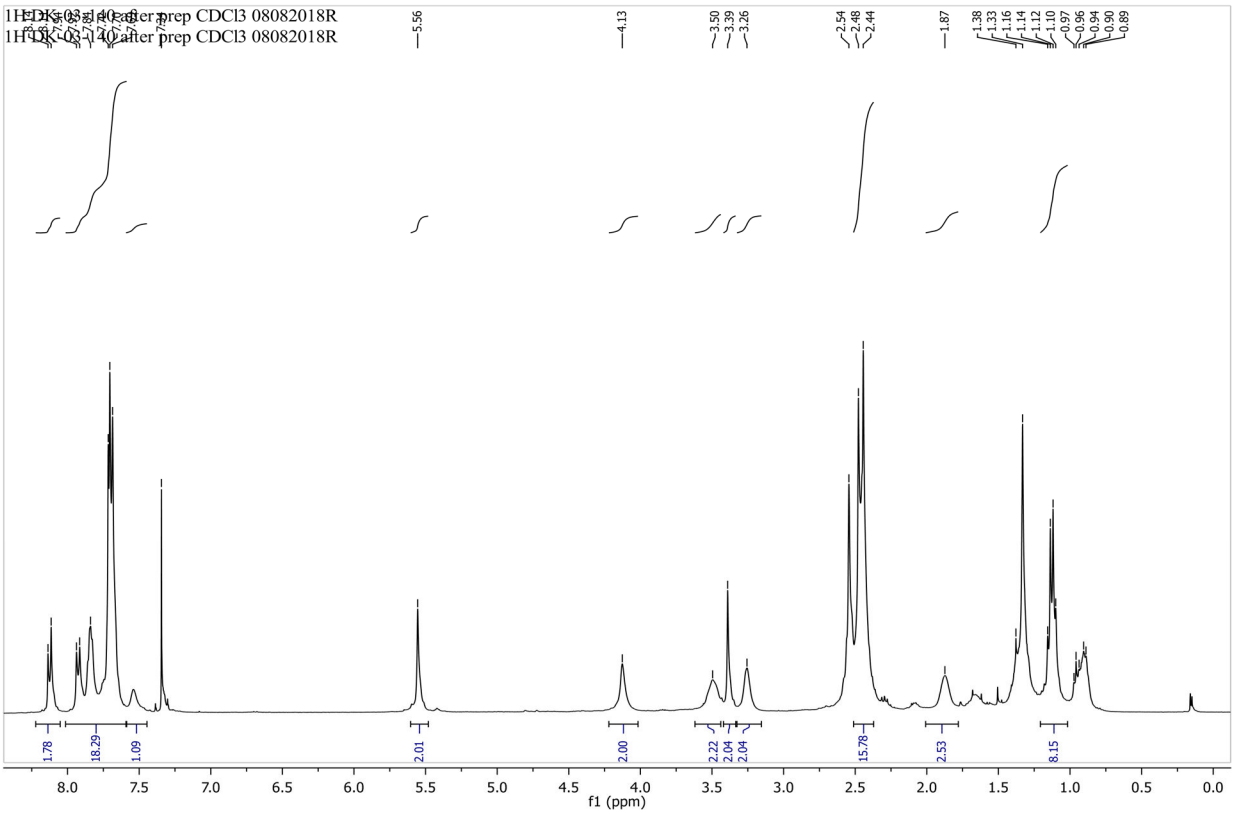


Figure S59. ^{1}H (100 MHz, $CDCl_3$): **13**

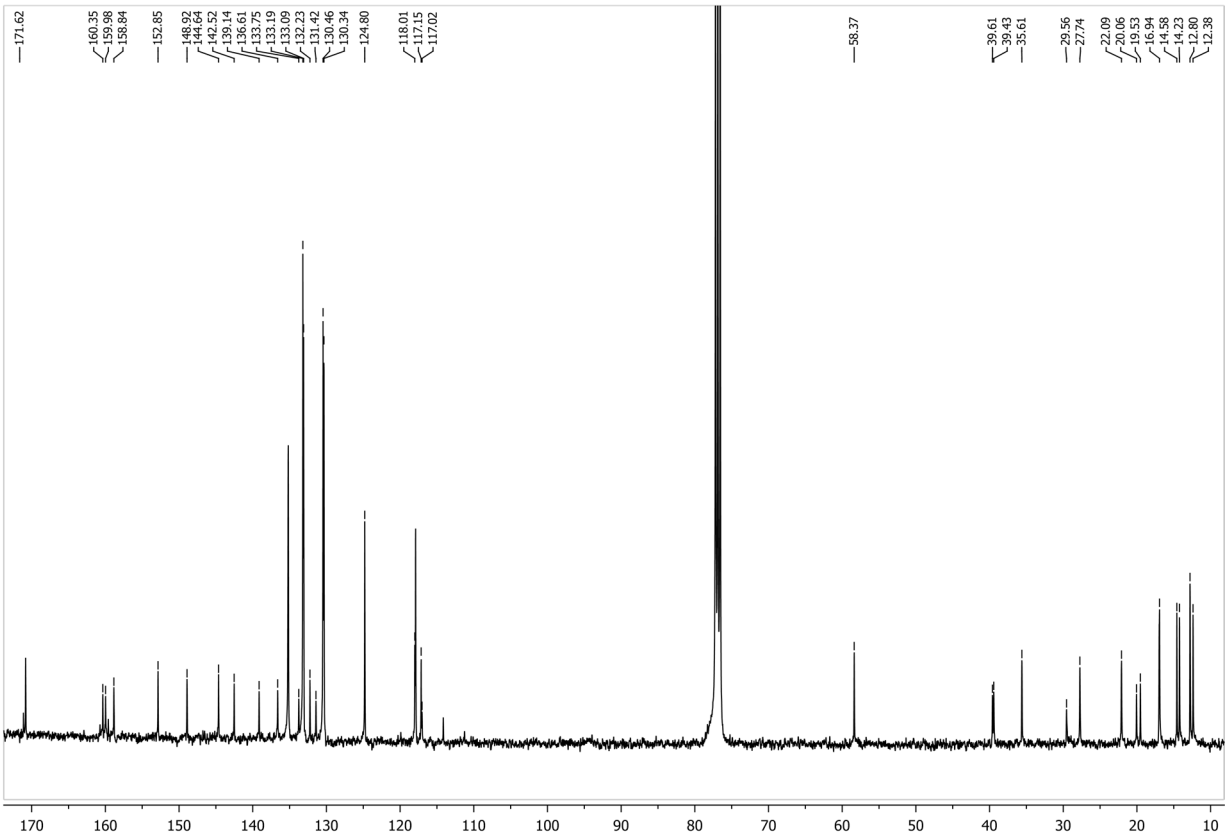


Figure S60. ^{13}C (101 MHz, $CDCl_3$): **13**

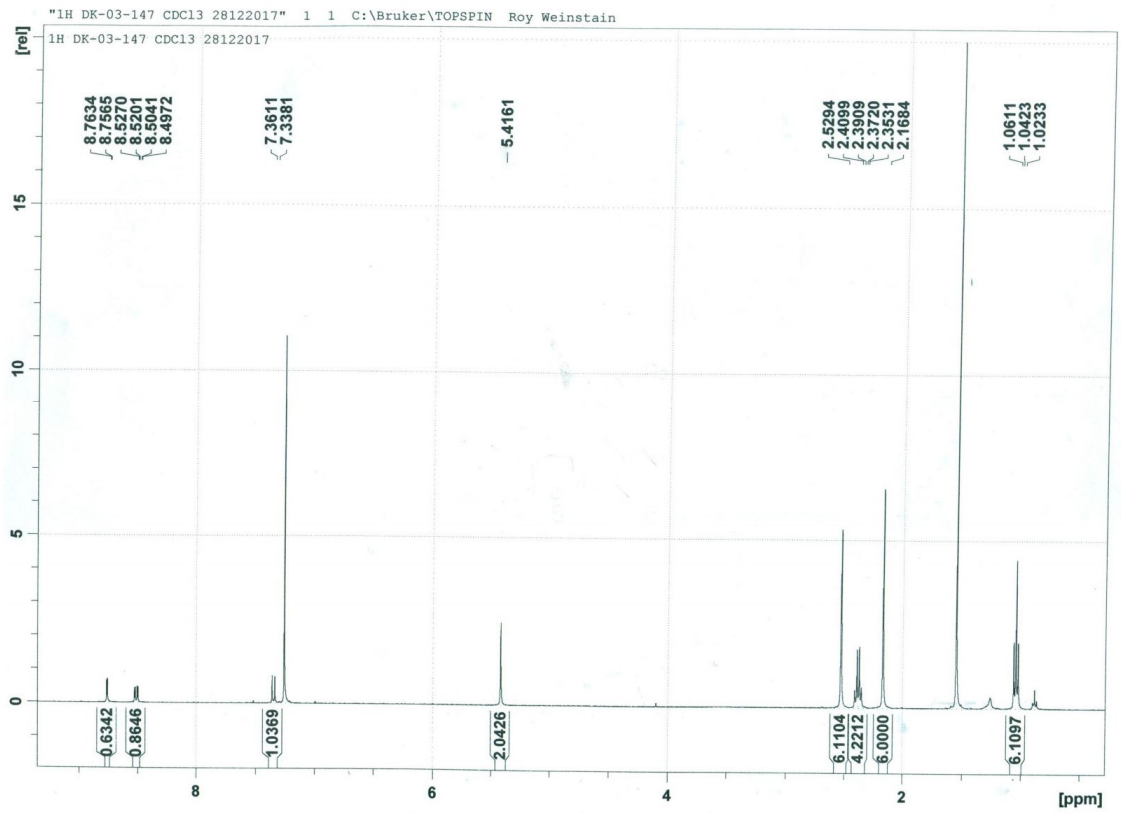


Figure S61. ^{1}H (400 MHz, $CDCl_3$): **14**

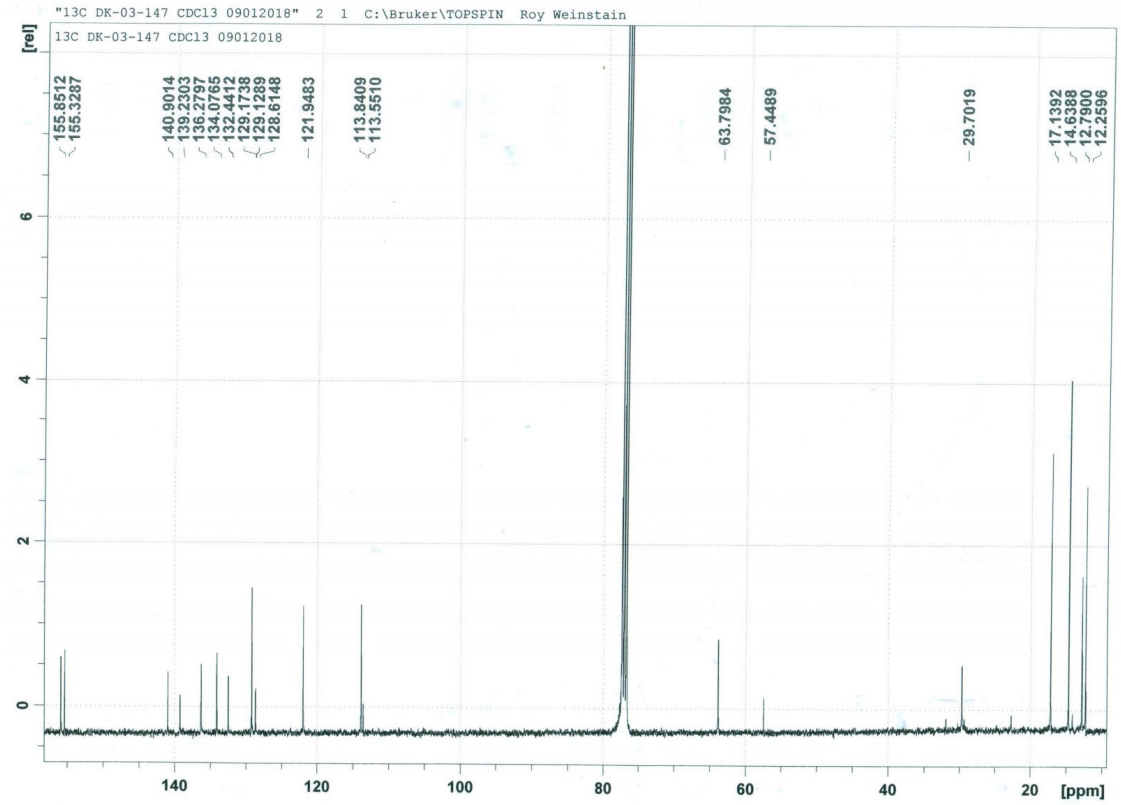


Figure S62. ^{13}C (101 MHz, $CDCl_3$): **14**

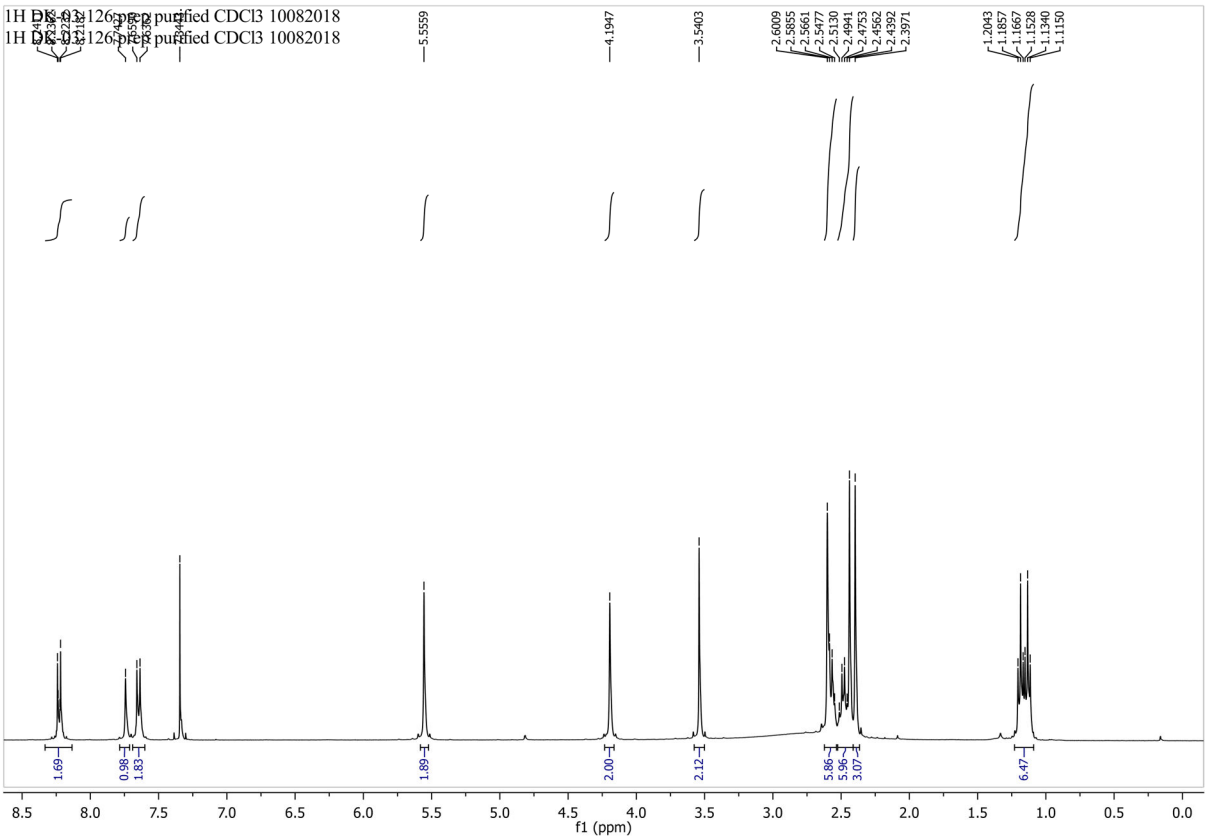


Figure S63. ^{1}H (400 MHz, $CDCl_3$): **15**

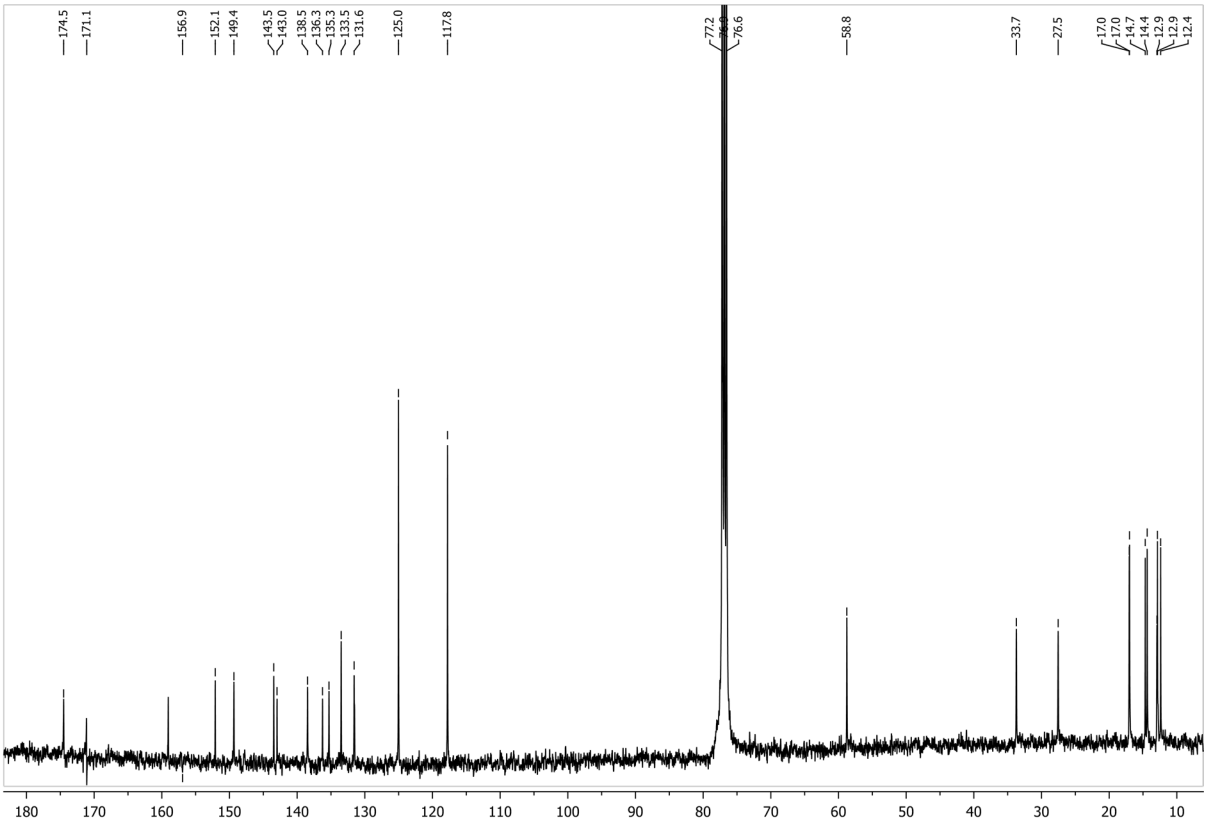


Figure S64. ^{13}C (101 MHz, $CDCl_3$): **15**

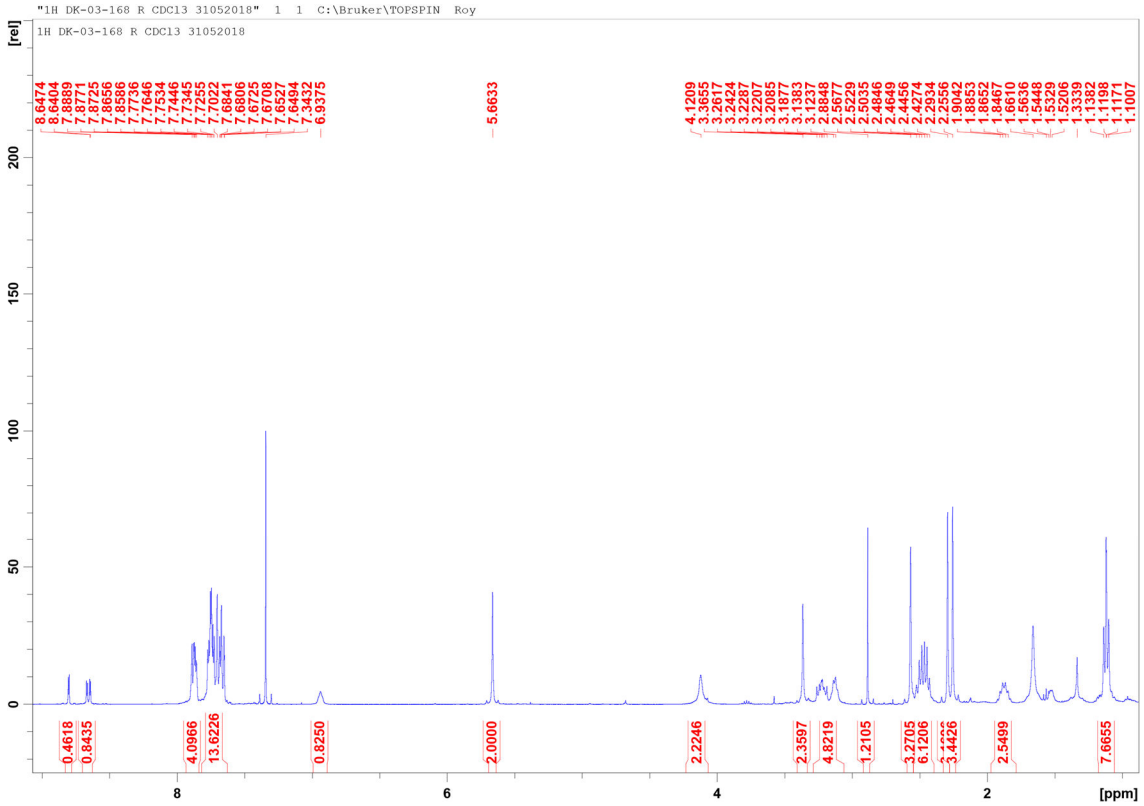


Figure S65. ^{1}H (400 MHz, $CDCl_3$): **16**

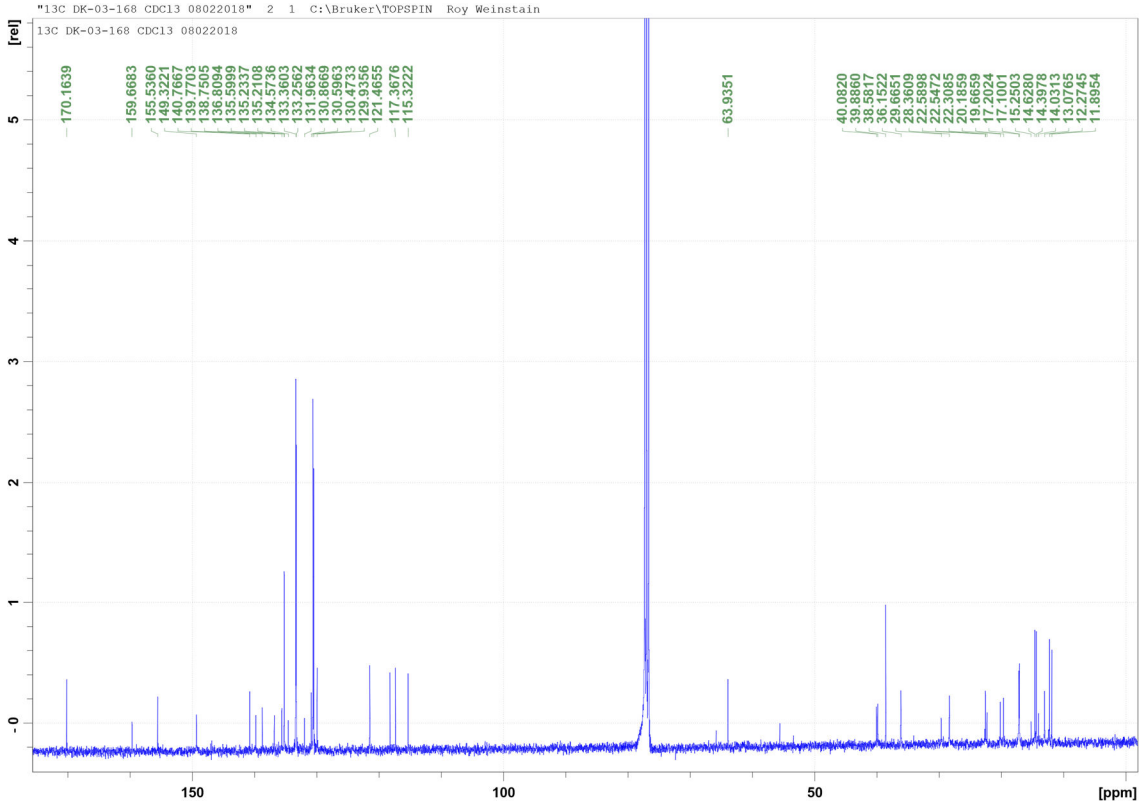


Figure S66. ^{13}C (101 MHz, $CDCl_3$): **16**

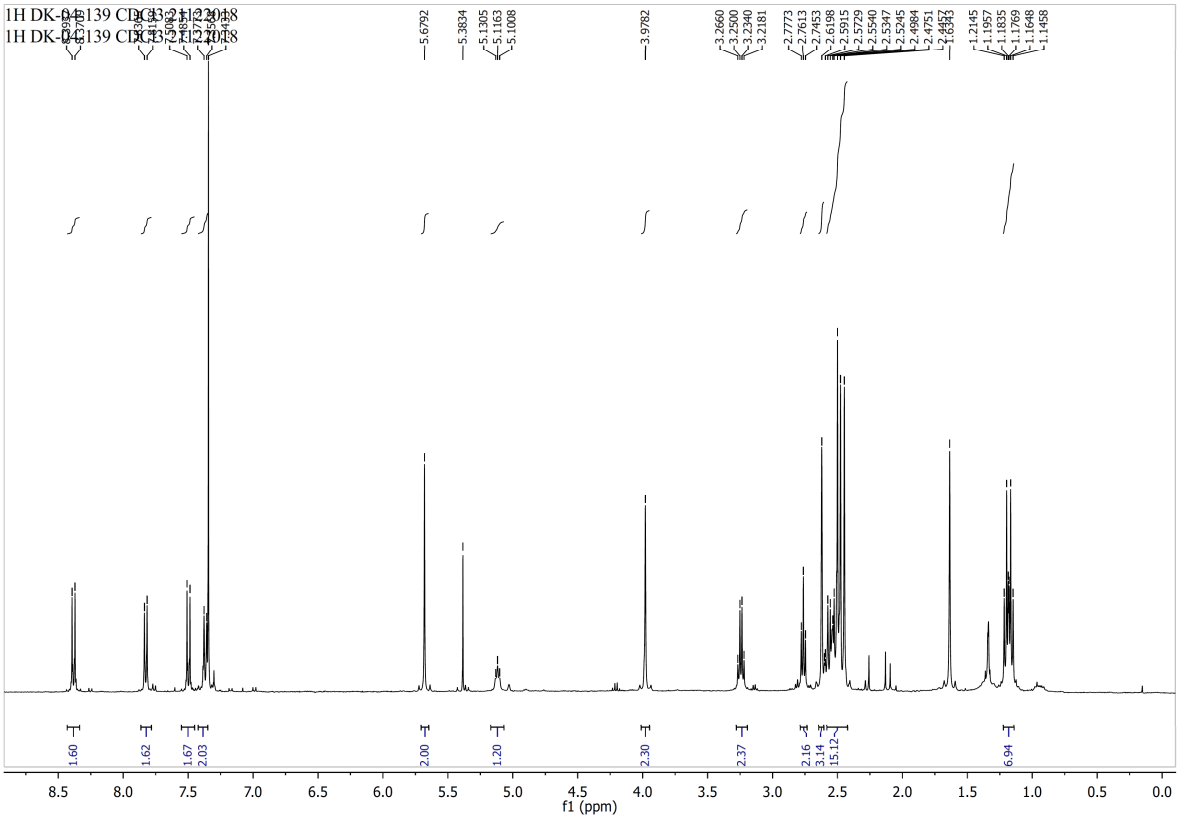


Figure S67. ^{1}H (400 MHz, $CDCl_3$): **18**

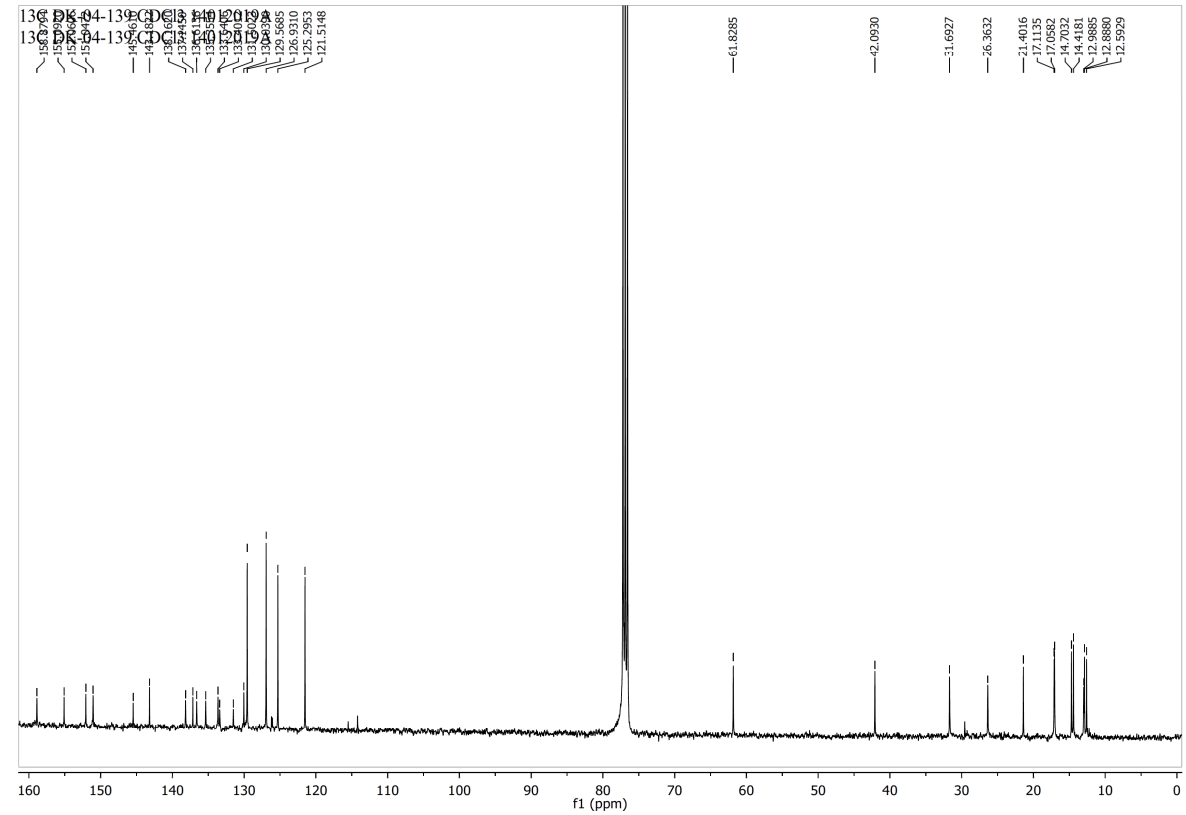


Figure S68. ^{13}C (101 MHz, $CDCl_3$): **18**

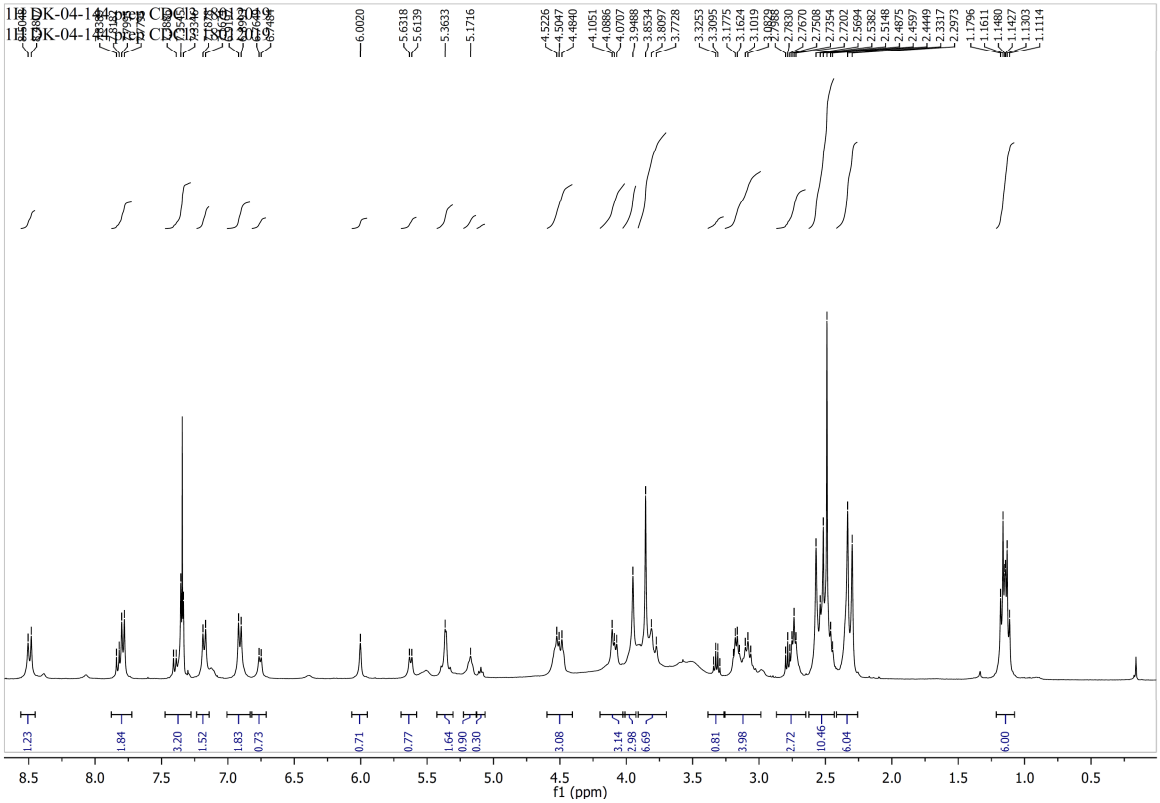


Figure S69. ^{1}H (400 MHz, $CDCl_3$): **19**

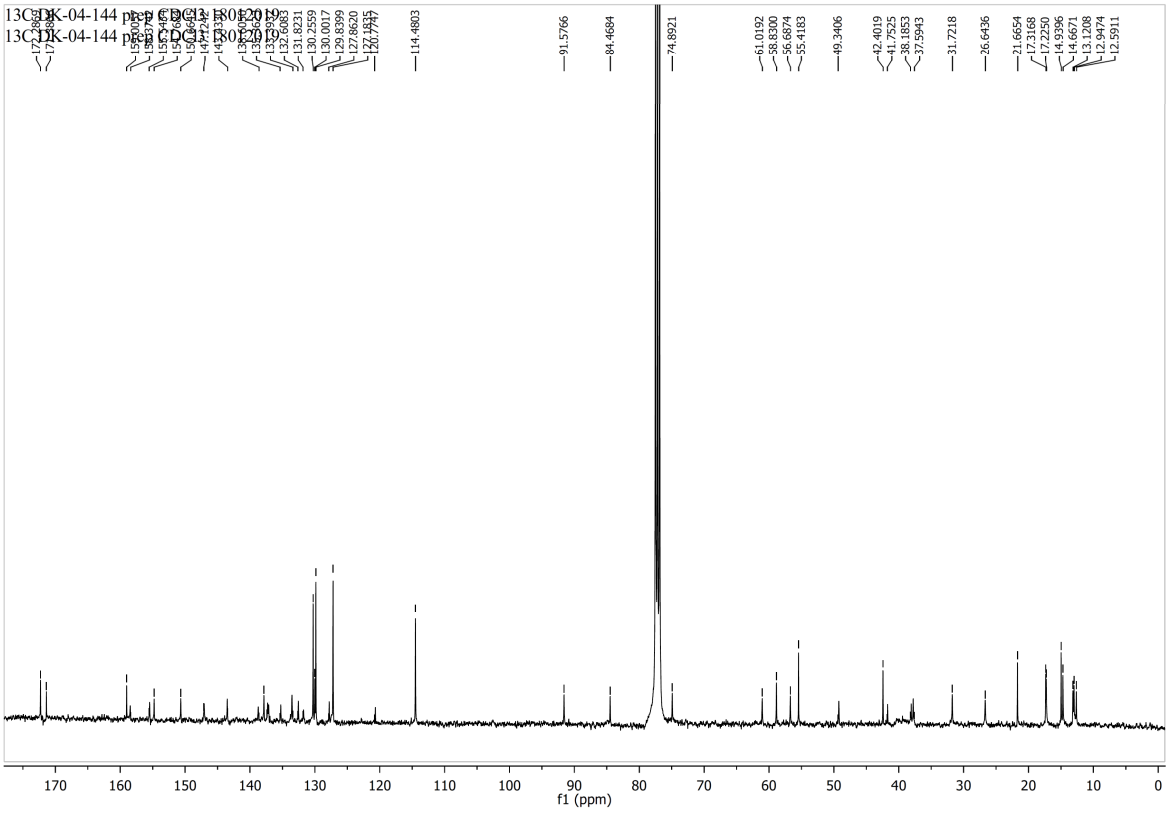


Figure S70. ^{13}C (101 MHz, $CDCl_3$): **19**

References

[1] C. J. Zhang, J. Wang, J. Zhang, Y. M. Lee, G. Feng, T. K. Lim, H. M. Shen, Q. Lin, B. Liu, *Angew. Chem. Int. Ed.* **2016**, *55*, 13770-13774.

[2] N. Rubinstein, P. Liu, E. W. Miller, R. Weinstain, *Chem. Commun. (Camb)* **2015**, *51*, 6369-6372.

[3] R. Ni, N. Mitsuda, T. Kashiwagi, K. Igawa, K. Tomooka, *Angew. Chem. Int. Ed.* **2015**, *54*, 1190-1194.