Electronic Supplementary Material (ESI) for Chemical Science.

Tetrazine as a General Phototrigger to Turn on Fluorophores

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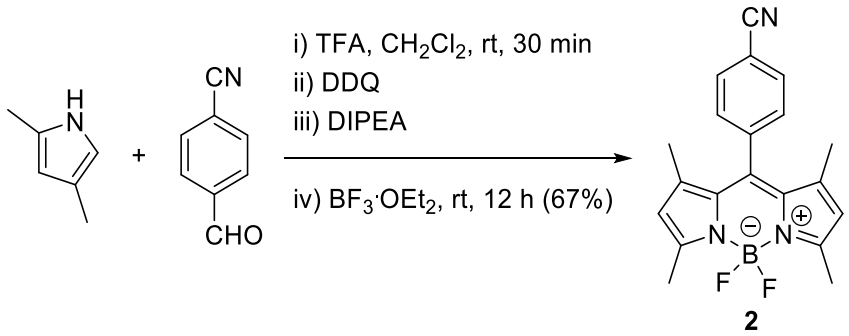
1. Materials and Methods

Reagents were purchased from Sigma-Aldrich, Oakwood Chemical and Biotium and used without further purification. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AVANCE III HD 600 MHz spectrometer. UV-vis absorption spectra were measured on a Thermo Scientific Evolution 220 UV-Vis Spectrophotometer. Fluorescence measurements were conducted on a Horiba FluoroMax-4 spectrofluorometer. ESI mass spectroscopy was performed on a Bruker MicroToF ESI LC-MS System.

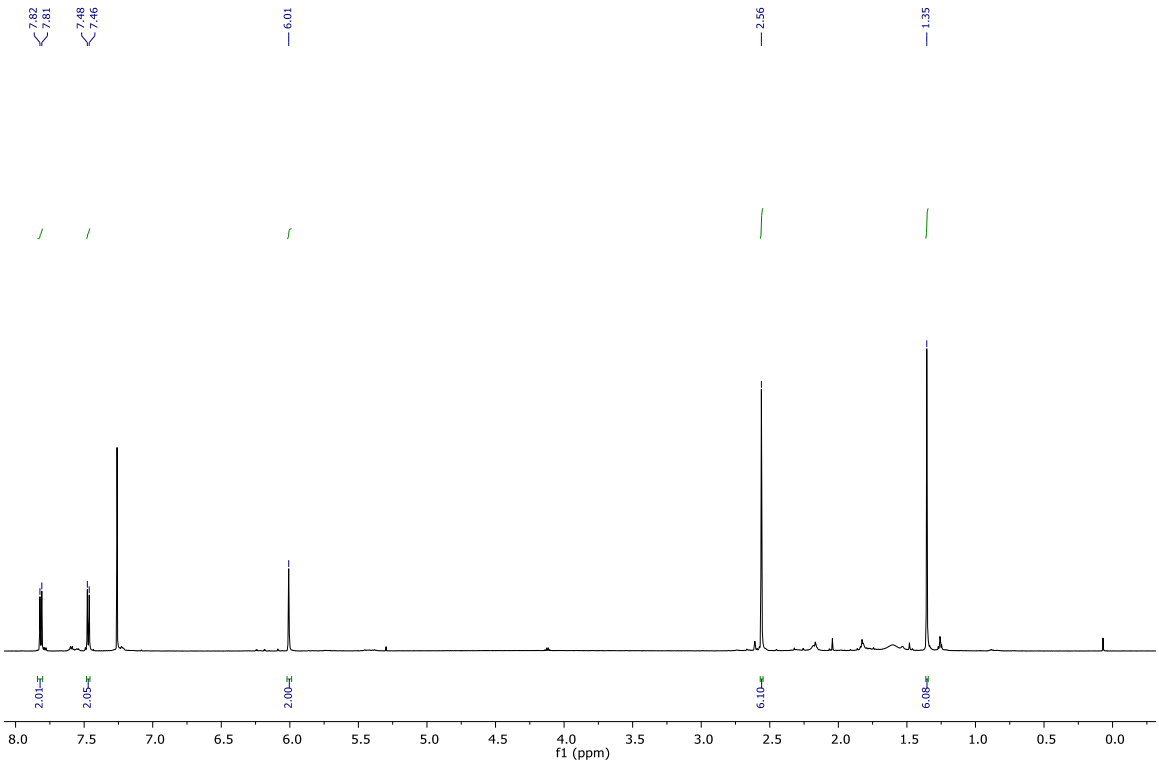
Fluorescence assays

Stock solutions of 1 mM in acetonitrile were diluted into 3 mL, 1 uM in the corresponding solvent in a 1 cm x 1 cm quartz cuvette. Measurements of emission spectra were recorded before light irradiation. Activation ratios were calculated from the peak emission intensity of the photolyzed tetrazine and the pre-irradiation intensity. Light irradiation was stopped at the corresponding time points to record emission spectra and continued until a plateau was reached. Control experiments using IEDDA activations were performed using excess BCN (1000 eq.) for fast fluorescence turn-on.

2. Synthesis and Characterization



Synthesis of CN-BODIPY (**2**)**.** 4-Formylbenzonitrile (0.5 g, 3.8 mmol) and 2,4-dimethylpyrrole (0.85 mL, 8.25 mmol) were dissolved in dichloromethane (100 mL) under nitrogen in a 500 mL flask, 4 drops of TFA were added and stirred at room temperature for 30 minutes. DDQ (865 mg, 3.8 mmol) in dichloromethane (100 mL) was added to the reaction, followed by DIPEA (7.75 mmol, 44.5 mmol)and boron trifluoride diethyl ether complex (7.75 mL, 45% BF_3 content). The reaction mixture was stirred for additional 12 h. 60 mL of water was added and the solution was extracted with dichloromethane, dried with anhydrous sodium sulfate and filtered, the solvent was removed under reduced pressure and then purified by column chromatography (20% ethyl acetate in hexane). CN-BODIPY (**2**) (303 mg, 67% yield) was obtained as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (d, J = 8.1 Hz, 2H), 7.47 (d, J = 8.2 Hz, 2H), 6.01 (s, 2H), 2.56 (s, 6H), 1.35 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 156.74, 142.72, 140.24, 138.83, 133.03, 130.89, 129.53, 121.96, 118.24, 113.45, 14.79, 14.74. ESIMS $[M+H]^+$ calcd. for $C_{20}H_{18}BF_2N_3$ 350.16 , found 350.16.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2.00
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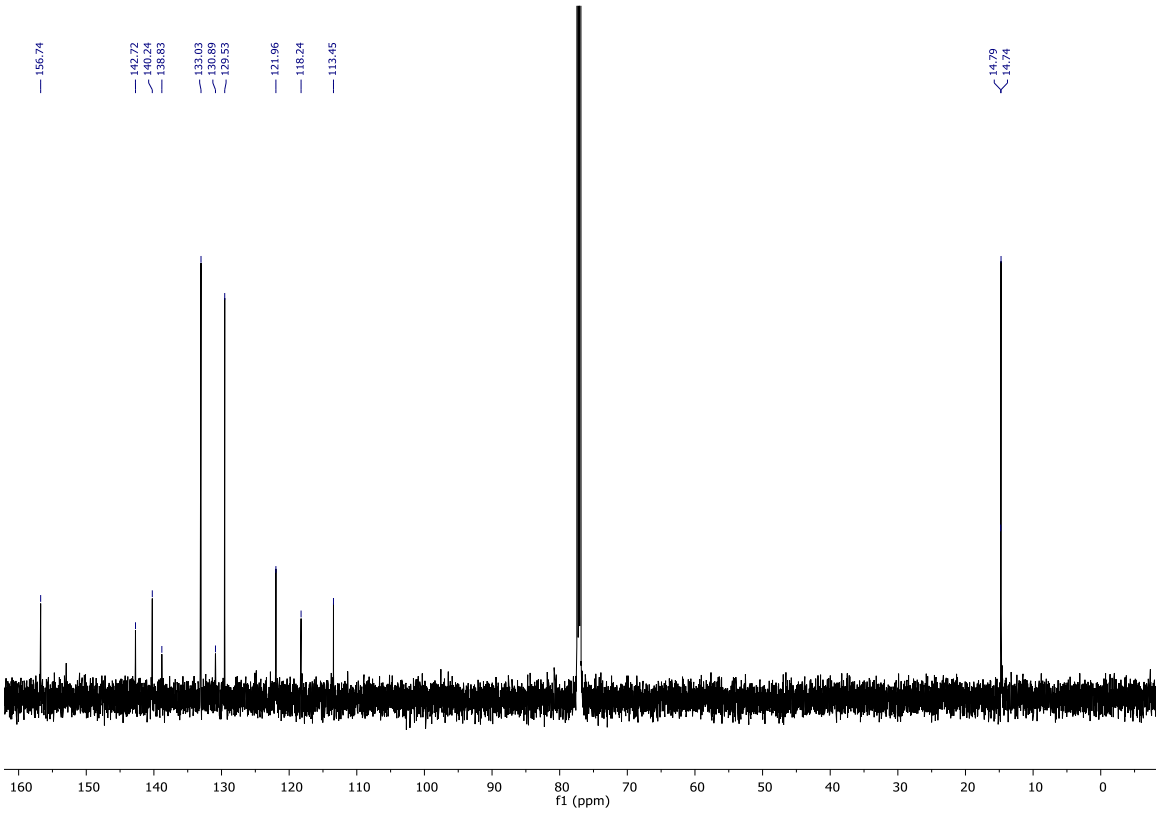
6.08
f1 (ppm)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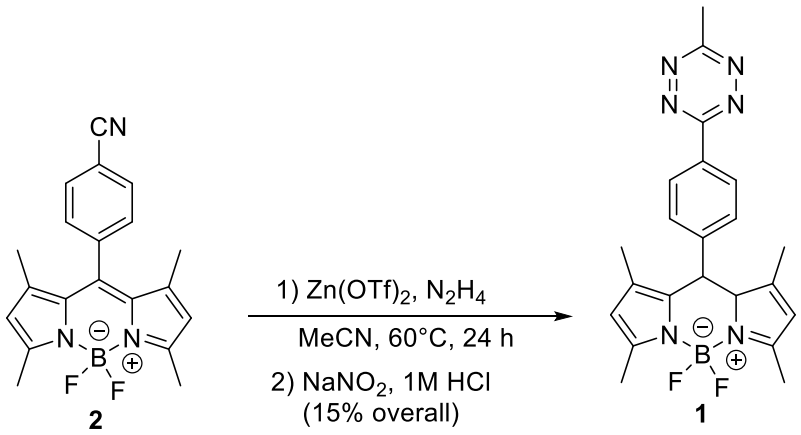
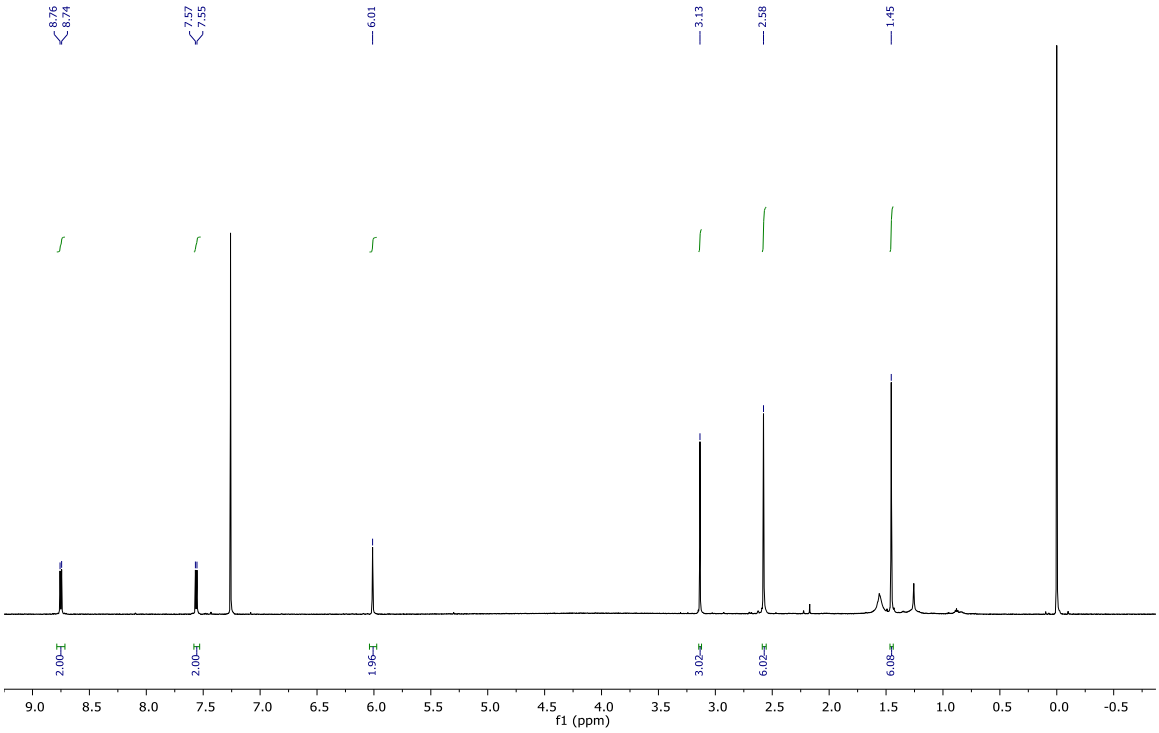
f1 (ppm)



Synthesis of Tz-BODIPY (**1**)**.** CN-BODIPY (**2**) (66 mg, 0.19 mmol) and Zinc trifluoromethanesulfonate (33 mg, 0.09 mmol) were loaded into a Schlenk tube, flushed three times with nitrogen, added acetonitrile (100 μL, 1.9 mmol) and anhydrous hydrazine (300 uL, 9.45 mmol). The reaction was stirred for 24 h at 60 °C, then, cooled to 0 °C, and sodium nitrite (201 mg, 2.91 mmol) in 10 mL of water was added and the pH was adjusted to 3.0 with 1M HCl, the organic phase was extracted with dichloromethane, dried with anhydrous sodium sulfate and filtered, the solvent was removed under reduced pressure and then purified by column chromatography (50% ethyl acetate in hexane). Tz-BODIPY (**1**) (12 mg, 15% yield) was obtained ad a dark red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.74 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 7.5 Hz, 2H), 5.99 (s, 2H), 3.13 (s, 3H), 2.62 (s, 6H), 1.44 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ167.75, 163.79, 156.19, 143.03, 140.29, 139.63, 132.67, 131.13, 129.37, 128.73, 121.66, 29.85, 21.40, 14.81. ESIMS $[M+H]^+$ calcd. for $C_{22}H_{21}BF_2N_6$ 418.19, found 418.19.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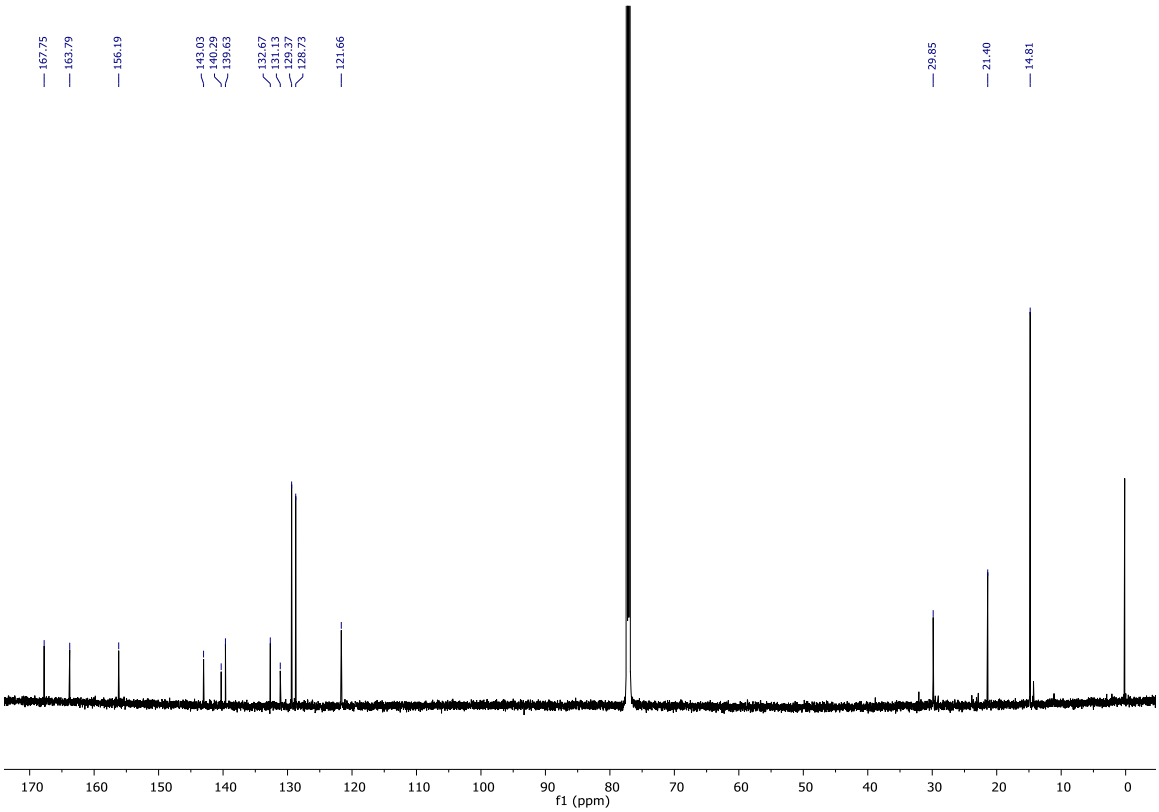
6.09
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)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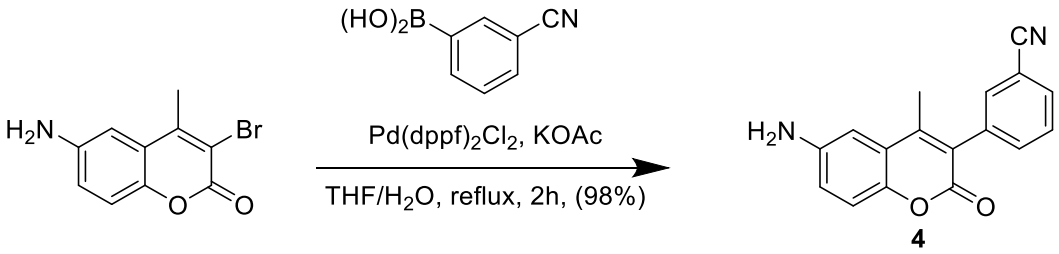
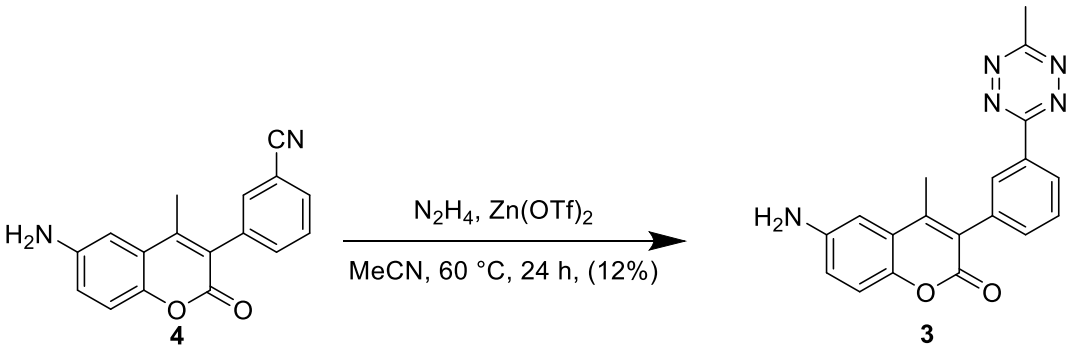
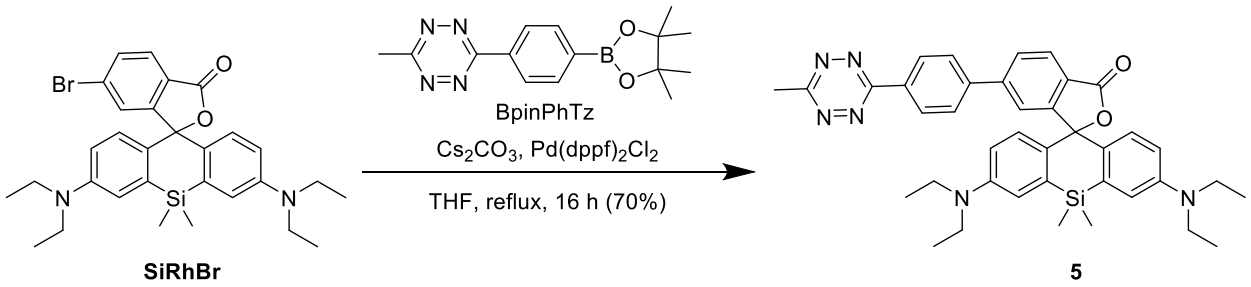


4

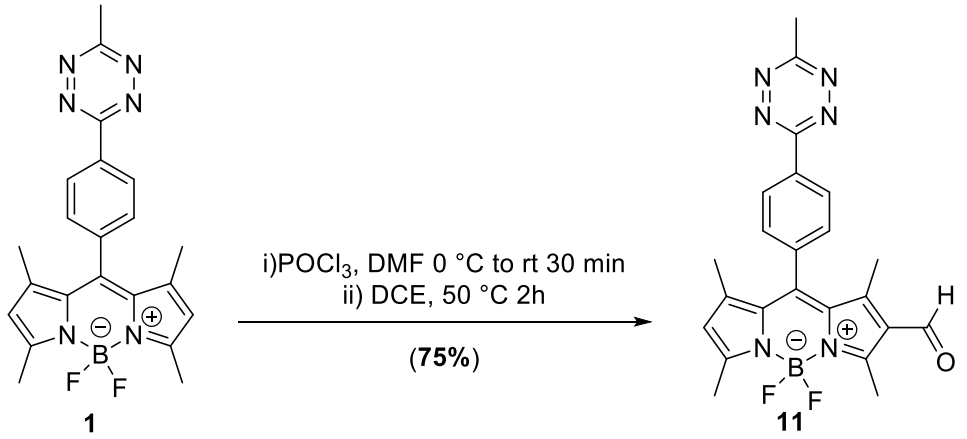
Synthesis of compound (**4**). Bromocoumarin (525mg, 2.1 mmol), 3-cyanophenylboronicacid (456 mg, 3.1 mmol, $Pd(dppf)_2Cl_2$ (80 mg, 0.1 mmol) and potassium carbonate (571 mg, 4.1 mmol) were dissolved in dioxane/water (20mL, v/v = 3:1) in a sealed tube, under nitrogen atmosphere. The solution was stirred under reflux for 8 h. Then, the reaction was cooled to room temperature, and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (50 mL), and diluted with water (50 mL), extracted three times with dichloromethane; dried over anhydrous sodium sulfate, removed the solvent under reduced pressure. The residue was purified by column chromatography (DCM: MeOH 10:0.05) to afford compound **4** as a faint yellow solid (600mg, 98% yield). ^{1}H NMR (600 MHz, DMSO-d_6) δ 7.83 (m, , 1H), 7.79 – 7.76 (m, 1H), 7.64 (m, 2H), 7.52 (d, J = 8.7 Hz, 1H), 6.61 (d, J = 8.7 Hz, 1H), 6.46 (d, J = 2.1 Hz, 1H), 6.21 (s, 2H), 2.15 (s, 3H). ^{13}C NMR (150 MHz, DMSO) δ 160.52, 154.69, 153.24, 149.79, 136.81, 135.83, 134.28, 131.28, 129.42, 127.14, 118.84, 117.67, 111.65, 111.24, 108.96, 98.31, 16.31. ESIMS $[M+H]^+$ calcd. for $C_{17}H_{13}N_2O_2$ 277.09, found 277.12.



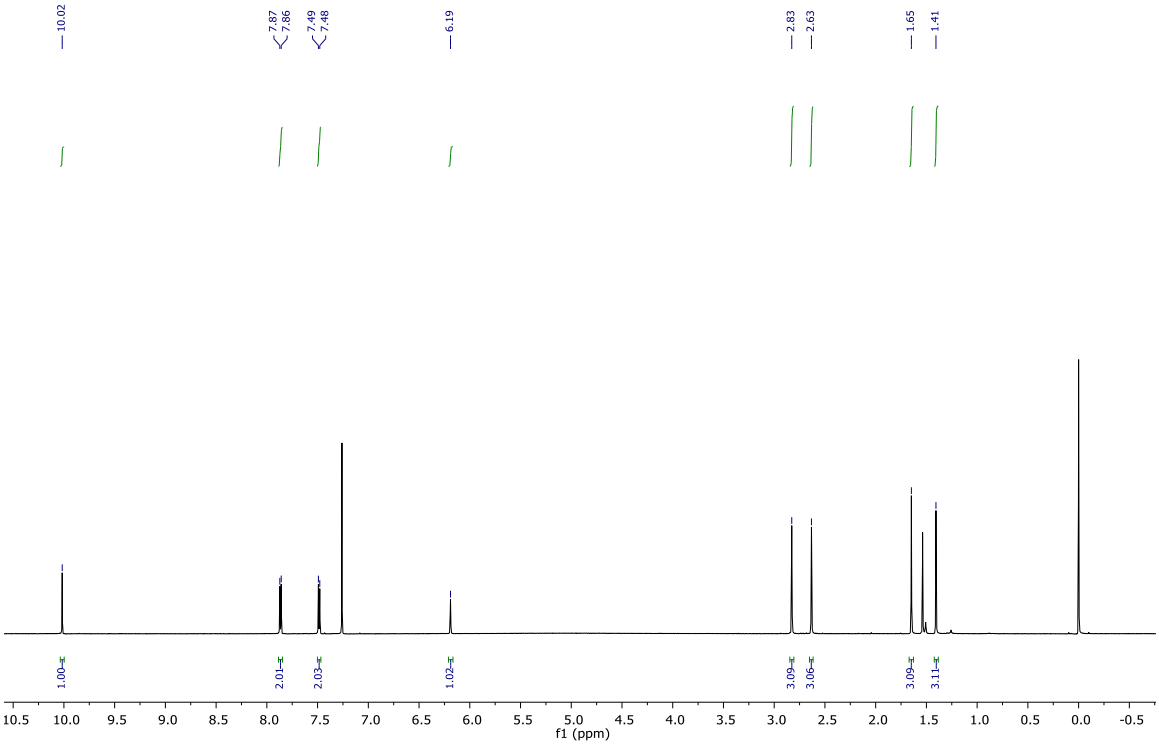
Synthesis of compound (**3**). Compounds **3** and **4** were synthetized from the procedure reported in ref. 43. Compound **4** (68 mg, 0.28 mmol), zinc (II) triflate (52 mg, 0.07 mmol), acetonitrile (0.146 mL, 2.8 mmol), anhydrous hydrazine (0.44 mL, 14 mmol) were loaded in a sealed glass tube, the resulting solution was stirred at 60 °C for 24 h. After the reaction, the solution was cooled to 0 °C, and the tube was opened slowly, sodium nitrite dissolved in water was added, and the pH was adjusted to 3.0 with HCl 1 M. The solution was extracted three times with dichloromethane, the solution was dried over sodium sulfate, and the solvent was removed under reduced pressure. The residue was purified by column chromatography (50% EA in hexanes) to afford compound **3** as a red solid (12mg, 12% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (d, J = 7.6 Hz, 1H) , 8.35 (s, 1H), 7.74 (t, J = 8 Hz, 1H), 7.62 (d, J = 7.6 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 6.63 (d, J = 8.5 Hz, 1H), 6.50 (s, 1H), 6.20 (s, 2H), 3.10(s, 3H), 2.31 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.2, 163.4, 160.3, 154.9, 152.9, 149.7, 136.4, 134.4, 130.9, 129.7, 129.5, 127.3, 126.6, 119.2, 111.2, 109.5, 98.5, 21.1, 16.8. ESIMS $[M+H]^+$ calcd. for $C_{19}H_{16}N_5O_2$ 346.13, found 346.09.



Synthesis of compound (**5**). Compounds **5**, SiRhBr, and BpinPhTz, were synthetized from the procedure reported in ref. 45. BpinPhTz (29 mg, 0.093 mmol), SiRhBr (50 mg, 0.085 mmol), cesium carbonate (84 mg, 0.25 mmol), and $Pd(dppf)_2Cl_2$ (14 mg, 0.017 mmol) were dissolved in THF (4 mL) in a sealed tube under nitrogen. The solution was stirred under reflux for 16 h. The reaction was cooled to room temperature, the solvent was removed under reduced pressure. The residue was purified by column chromatography (25% ethyl acetate in hexane) to afford a red solid (40 mg, 70% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.58 (d, J = 8.3 Hz, 1H), 8.02 (d, J = 8.2 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.57 (s, 1H), 7.43 (d, J = 16.2 Hz, 1H), 6.89 (s, 2H), 6.73 (d, J = 8.9 Hz, 2H), 6.52 (d, J = 6.5 Hz, 2H), 3.36 (q, J = 7.1 Hz, 8H), 3.11 (s, 3H), 1.16 (t, J = 7.1 Hz, 12H), 0.6 (s, 3H), 0.61 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 166.2, 163.8, 146.1, 139.8, 138.7, 136.5, 129.8, 127.9, 127.8, 127.7, 125.6, 123.3, 123.3, 115.3, 112.3, 43.8, 20.7, 12.02, -1.9. ESIMS $[M+H]^+$ calcd. for $C_{35}H_{41}N_6O_2Si$ 655.31, found 655.28.



Synthesis of compound (**11**). 2 mL of phosphoryl chloride and 2 mL of DMF were stirred in a 50 mL round bottom flask for 30 minutes at 0 °C. Then a solution of compound (**1**) (15.6 mg, 0.037 mmol) in 10 mL of 1,2-Dichloroethane, were added. The reaction mixture was warmed to 50 °C and stirred for additional 2 hours, and then the reaction was cooled to room temperature and poured slowly to a saturated solution of sodium bicarbonate at 0 °C. The organic layer was extracted three times with DCM, dried over sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (10% ethyl acetate in hexane) to afford compound (**11**) (12.2 mg, 75% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.02 (s, 1H), 8.80 (d, *J* = 8.3 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H), 6.19 (s, 1H), 3.15 (s, 3H), 2.84 (s, 3H), 2.64 (s, 3H), 1.74 (s, 3H), 1.51 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 185.95, 167.91, 163.65, 162.46, 157.17, 147.08, 142.86, 142.24, 138.70, 133.91, 133.36, 129.55, 129.19, 129.02, 126.66, 124.52, 29.85, 21.38, 15.27, 13.18, 12.05. ESIMS $[M+H]^+$ calcd. for $C_{23}H_{21}BF_2N_6O$ 447.18, found 447.18.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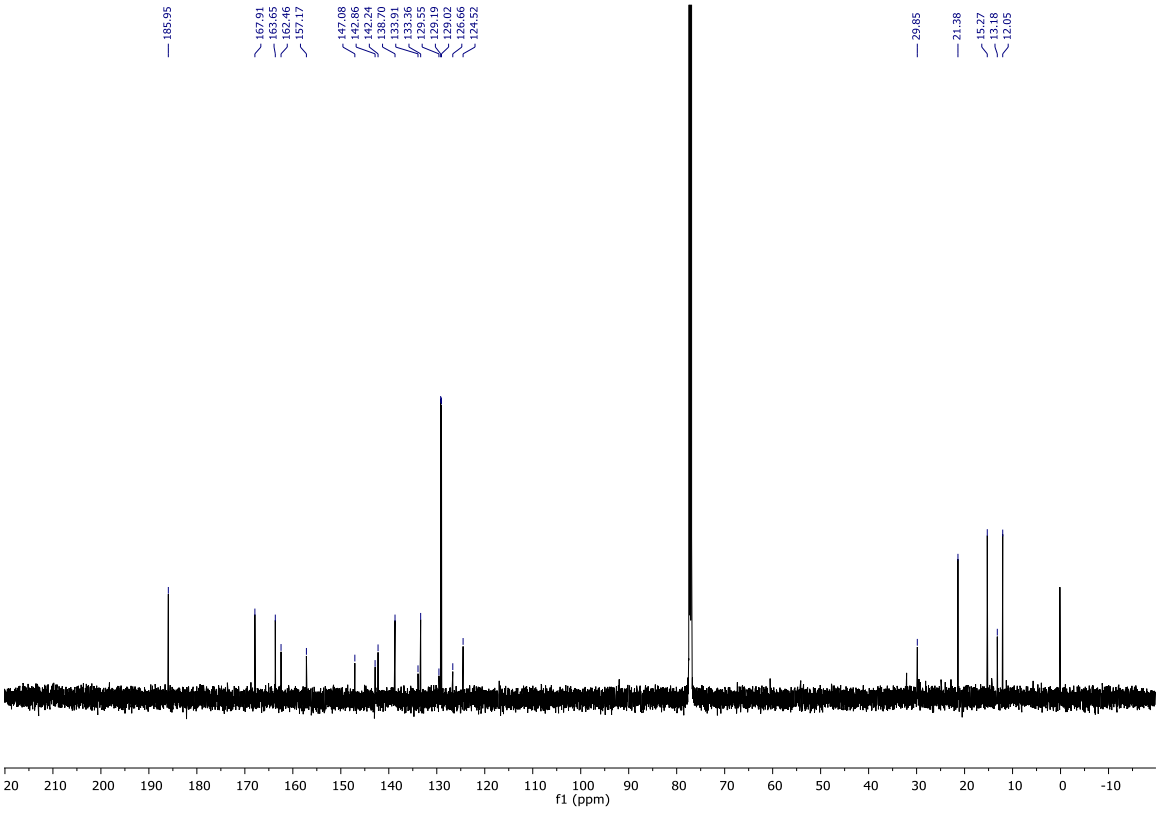
3.09
3.11
f1 (ppm)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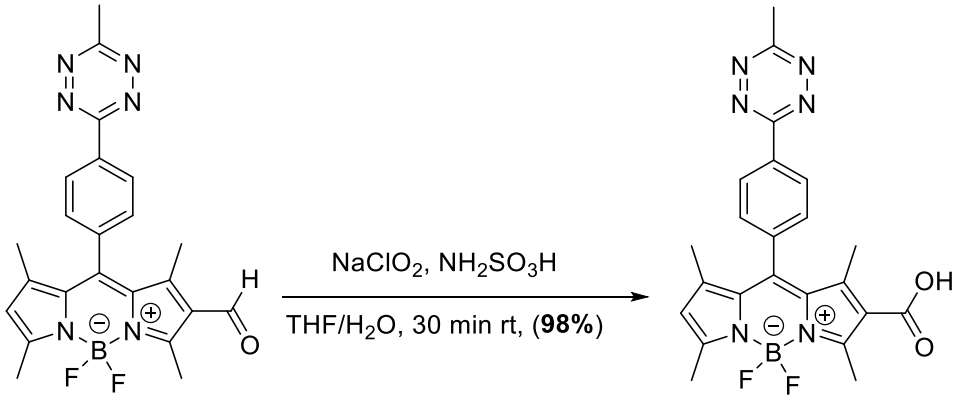
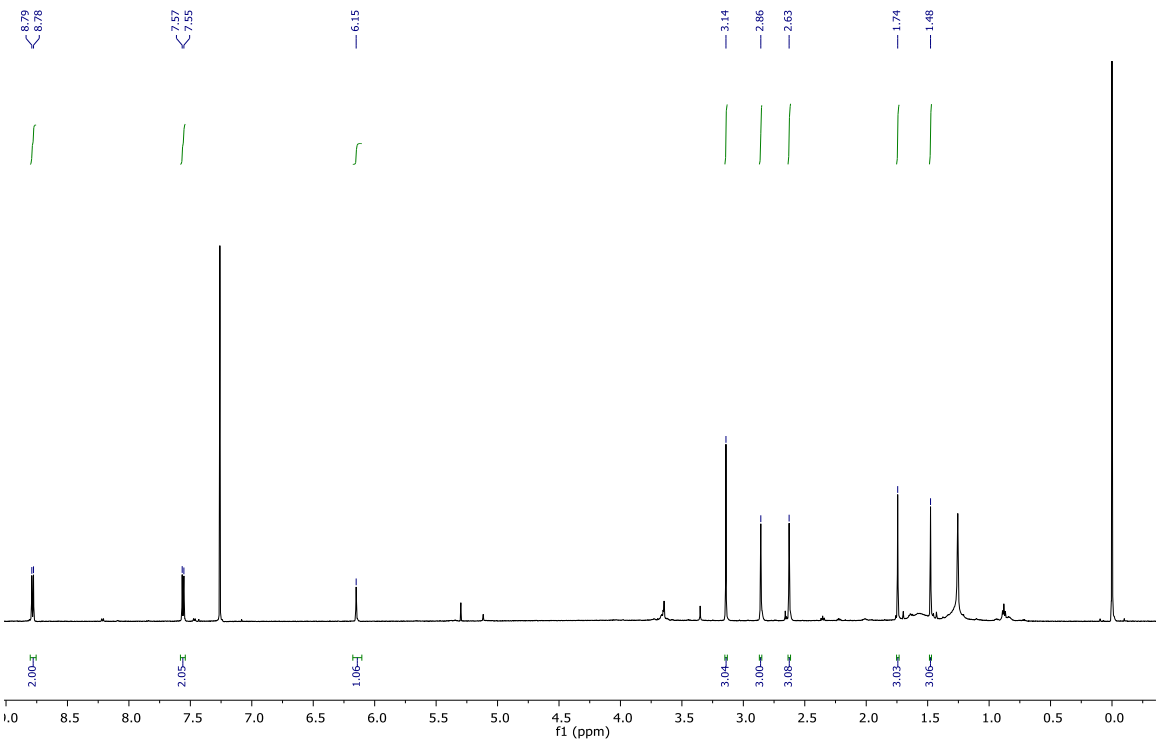
f1 (ppm)



11 **12**

Synthesis of Compound (**12**). Compound (**11**) (9 mg, 0.02 mmol) was dissolved in THF (6 mL) and H_2O (2 mL) in a 50 mL round bottom flask, sulfamic acid (3.8 mg, 0.04 mmol, 2 eq.) and sodium chlorite (3.6 mg, 0.04 mmol) were added and stirred at room temperature for 30 minutes. The reaction was diluted with ethyl acetate and poured into a solution of sodium thiosulfate. The organic layer was extracted three times with ethyl acetate, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (3% methanol in dichloromethane) to afford Tz-BODIPY-CO_2H (**12**) (9.1 mg, 98% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.78 (d, J = 8.3 Hz, 2H), 7.56 (d, J = 8.3 Hz, 2H), 6.15 (s, 1H), 3.14 (s, 3H), 2.86 (s, 3H), 2.63 (s, 3H), 1.74 (s, 3H), 1.48 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 169.38, 167.84, 163.66, 161.19, 157.73, 146.47, 144.85, 142.04, 139.06, 133.10, 129.23, 128.95, 124.08, 29.85, 21.40, 15.27, 15.23, 13.70. ESIMS $[M-H]^-$ calcd. for $C_{23}H_{20}BF_2N_6O_2$ 461.18, found 461.21.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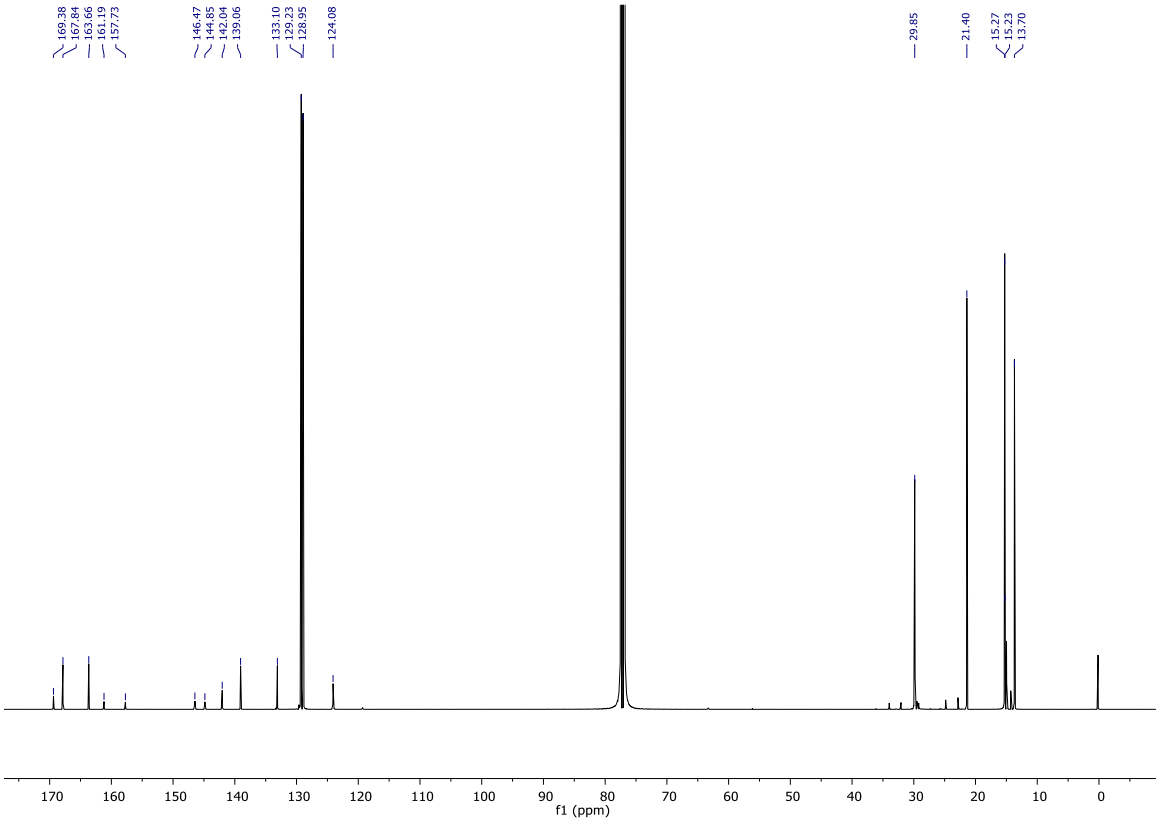
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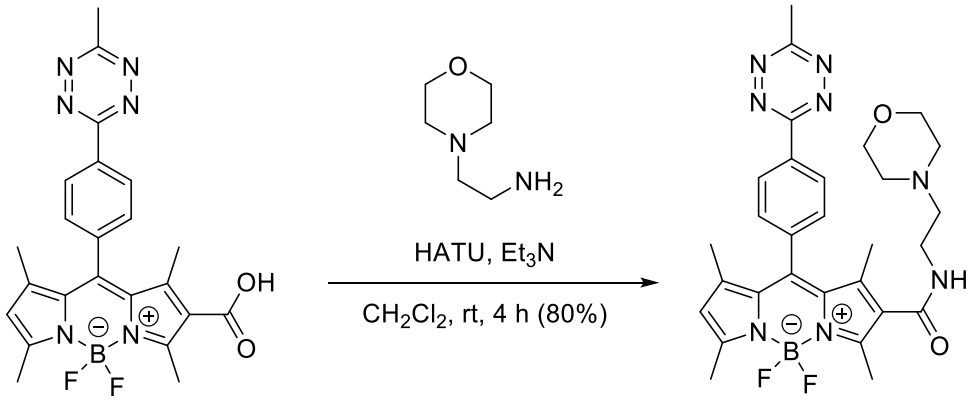
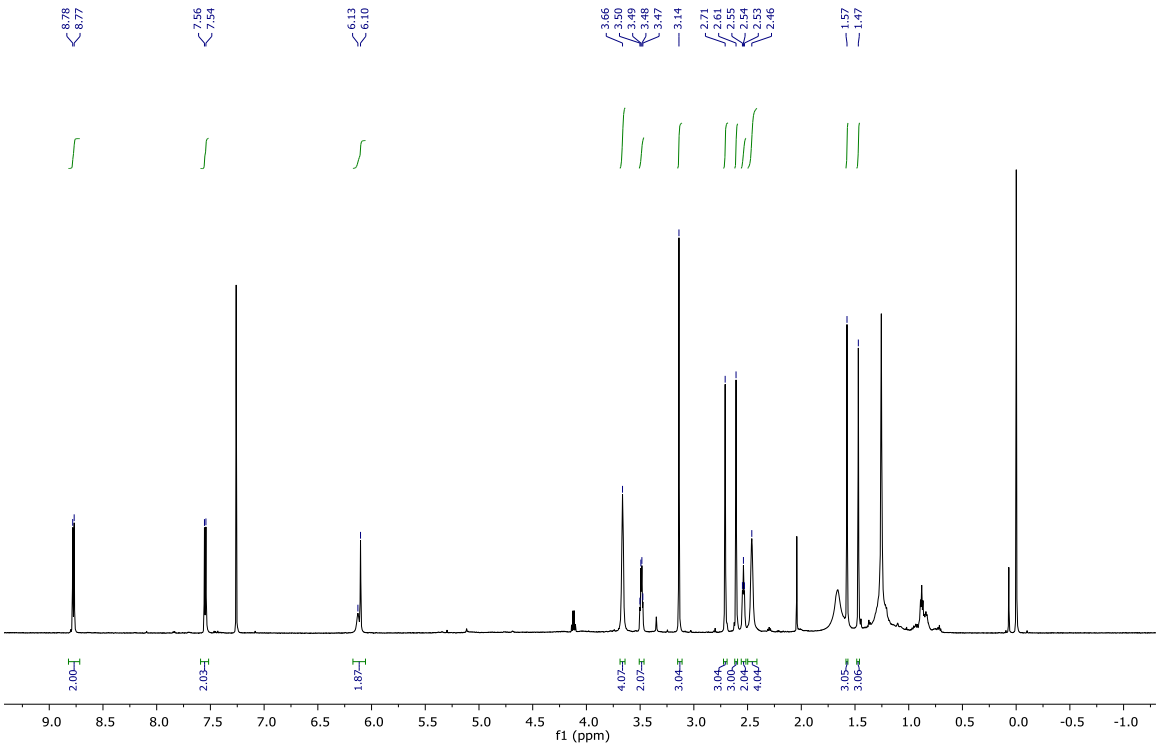


12 7

Synthesis of compound (**7**). A solution of compound (**12**) (5 mg, 0.011 mmol), HATU (4.5 mg 0.012 mmol, 1.1 eq) and triethylamine (5 μL, 0.033 mmol, 3 eq.) in DCM (5 mL) was stirred for 10 minutes under nitrogen in a 50 mL round bottom flask, before the addition of 4-(2-Aminoethyl)morpholine (2.8 mg, 0.022 mmol, 2 eq.). The mixture was stirred for additional 4 h at room temperature. The reaction was washed with a saturated solution of sodium bicarbonate, dried with sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (100% ethyl acetate) to afford 5 mg of compound (**7**) (80% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.78 (d, J = 8.1 Hz, 2H), 7.55 (d, J = 8.1 Hz, 2H), 6.11 (d, J = 13.4 Hz, 2H), 3.66 (m, 4H), 3.51 – 3.47 (m, 2H), 3.14 (s, 3H), 2.71 (s, 3H), 2.61 (s, 3H), 2.56 – 2.51 (m, 2H), 2.50 – 2.42 (s, 4H), 1.57 (s, 3H), 1.47 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.84, 165.01, 163.71, 159.77, 152.66, 141.58, 139.52, 139.18, 133.11, 129.75, 129.26, 128.93, 123.25, 67.04, 57.13, 53.41, 35.81, 29.85, 21.37, 15.06, 13.74, 13.03. ESIMS $[M+H]^+$ calcd. for $C_{29}H_{33}BF_2N_8O_2$ 575.28, found 575.31.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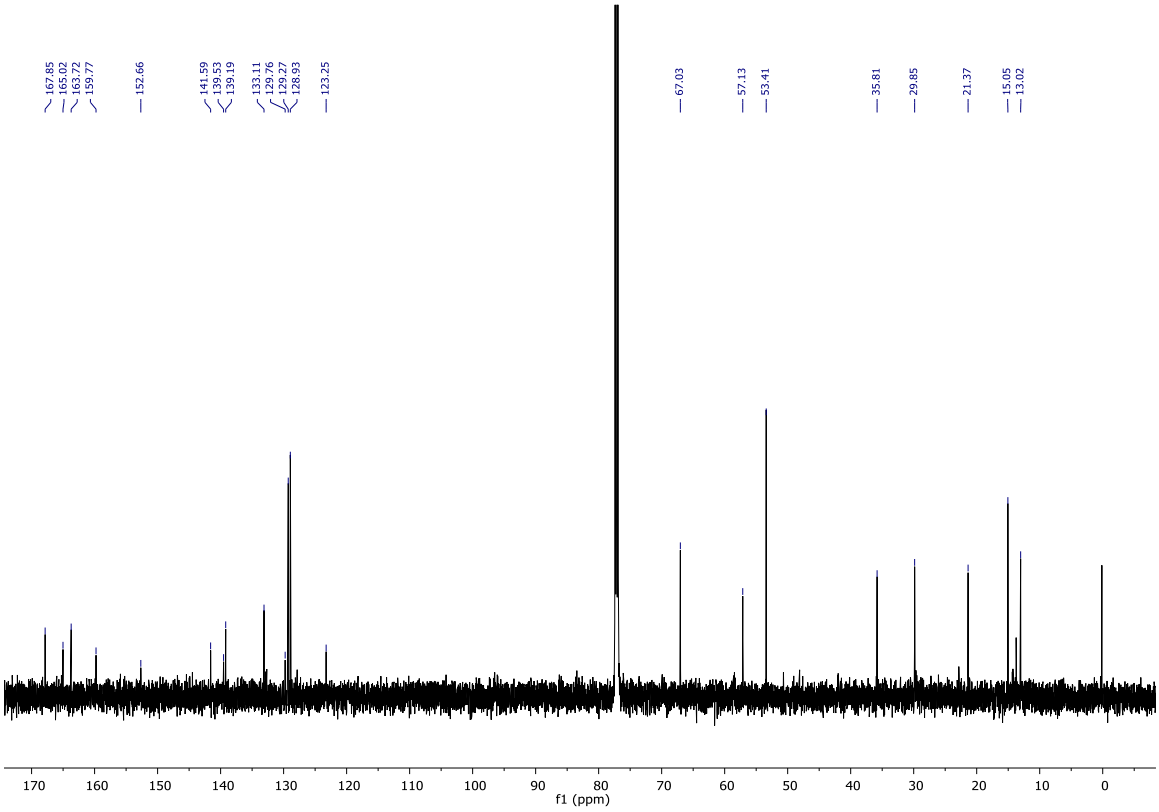
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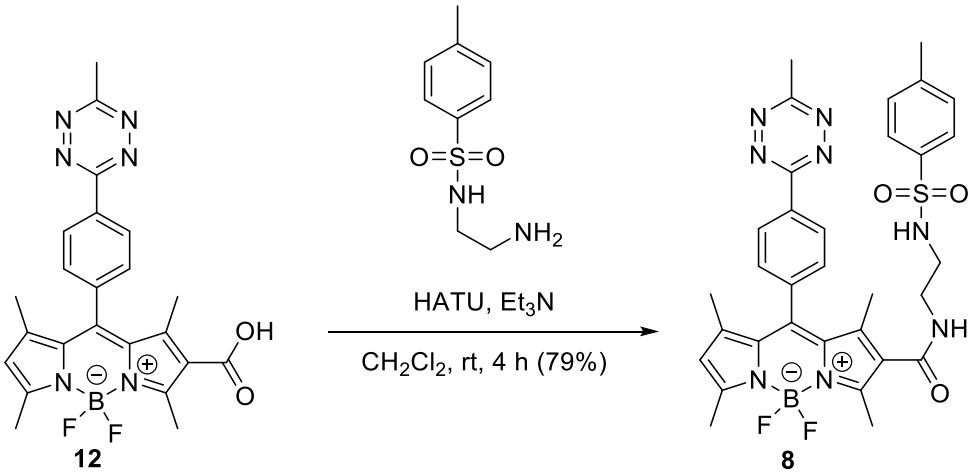
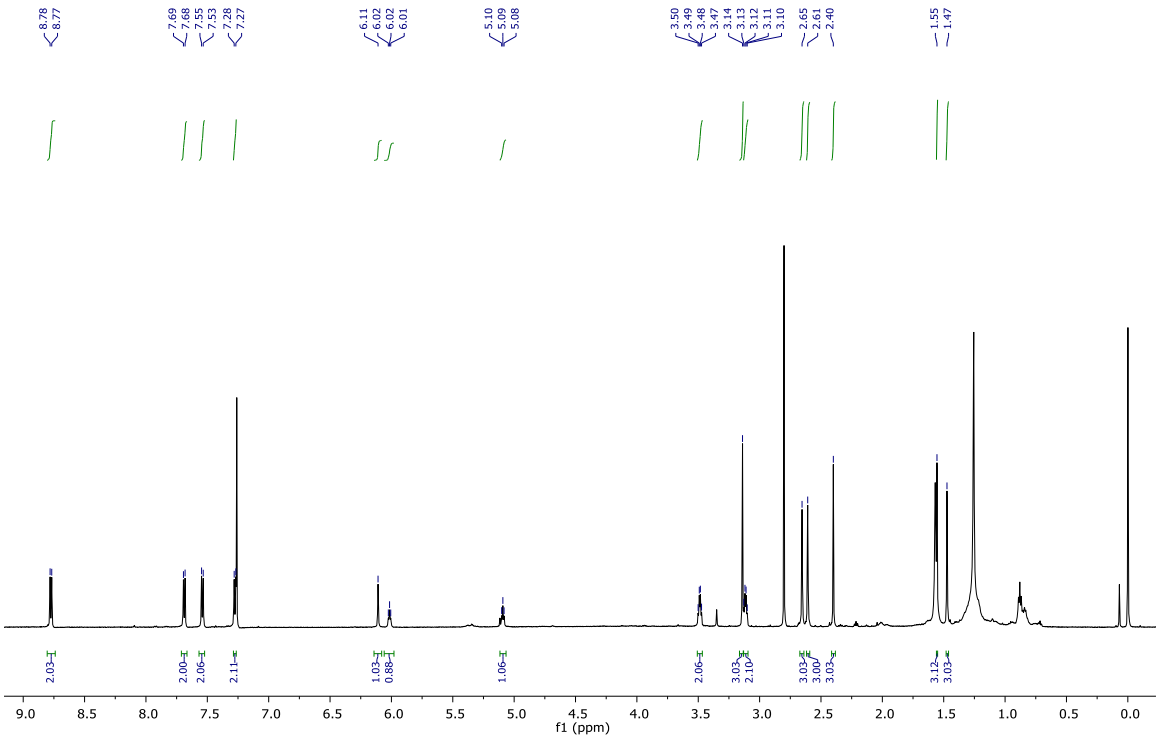


Synthesis of compound (**8**). A solution of compound (**12**) (3.5 mg ,0.0075 mmol), HATU (3.2 mg, 0.0083 mmol, 1.1 eq.) and triethylamine (3.1 μL, 0.0225 mmol, 3 eq.) in DCM (5 mL), were stirred for 10 minutes under nitrogen in a 50 mL round bottom flask, before the addition of 3.2 mg of N-(2-Aminoethyl)-4-methylbenzenesulfonamide (3.2 mg, 0.015 mmol, 2 eq.). The reaction was stirred for additional 4 h at room temperature. The reaction was washed with a saturated solution of sodium bicarbonate, dried with sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (100% ethyl acetate) to afford 3.9 mg of compound (8) (79% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (d, *J* = 8.0 Hz, 2H), 7.69 (d, *J* = 8.1 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 8.2 Hz, 2H), 6.11 (s, 1H), 6.02 (t, *J* = 5.8 Hz, 1H), 5.09 (t, *J* = 5.9 Hz, 1H), 3.49 (q, *J* = 5.7 Hz, 2H), 3.14 (s, 3H), 3.11 (q, *J* = 5.7 Hz, 2H), 2.65 (s, 3H), 2.61 (s, 3H), 2.40 (s, 3H), 1.55 (s, 3H), 1.47 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.82, 166.16, 163.69, 157.43, 143.84, 141.65, 139.10, 136.75, 133.13, 129.96, 129.25, 128.96, 127.16, 123.37, 43.58, 39.65, 38.77, 29.86, 21.64, 21.37, 15.09, 13.22. ESIMS $[M+H]^+$ calcd. for $C_{32}H_{33}BF_2N_8O_3S$ 659.25, found 659.28.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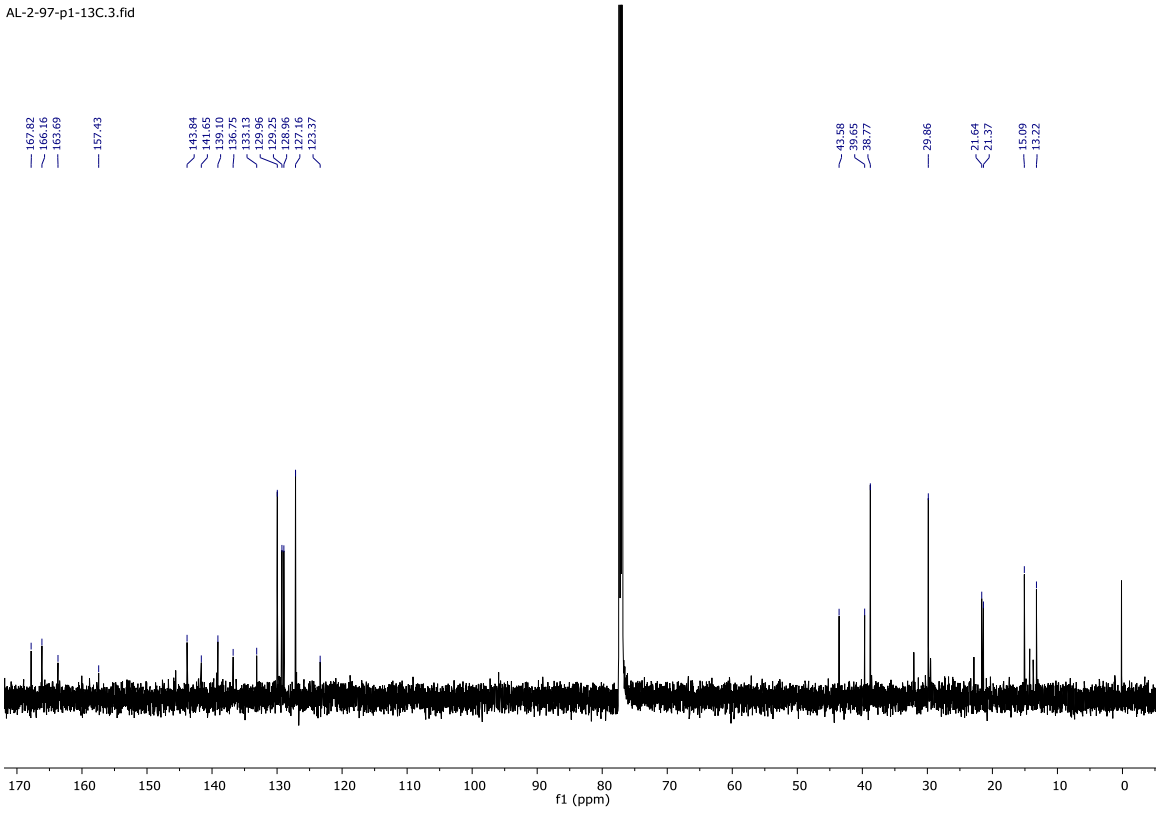
3.11
3.10
2.65
2.61
2.40
1.55
1.47
2.03
2.00
2.06
2.11
1.03
0.88
1.06
2.06
3.03
2.10
3.03
3.00
3.05
3.12
3.03
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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13.22
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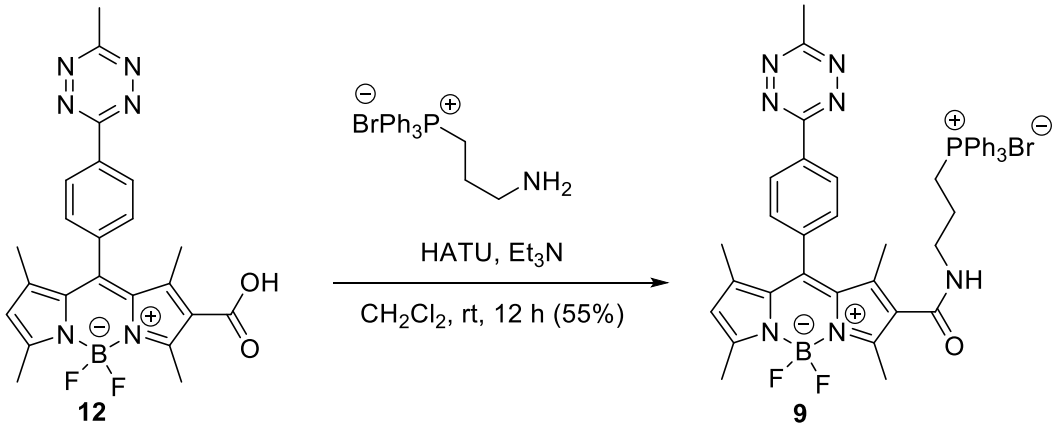
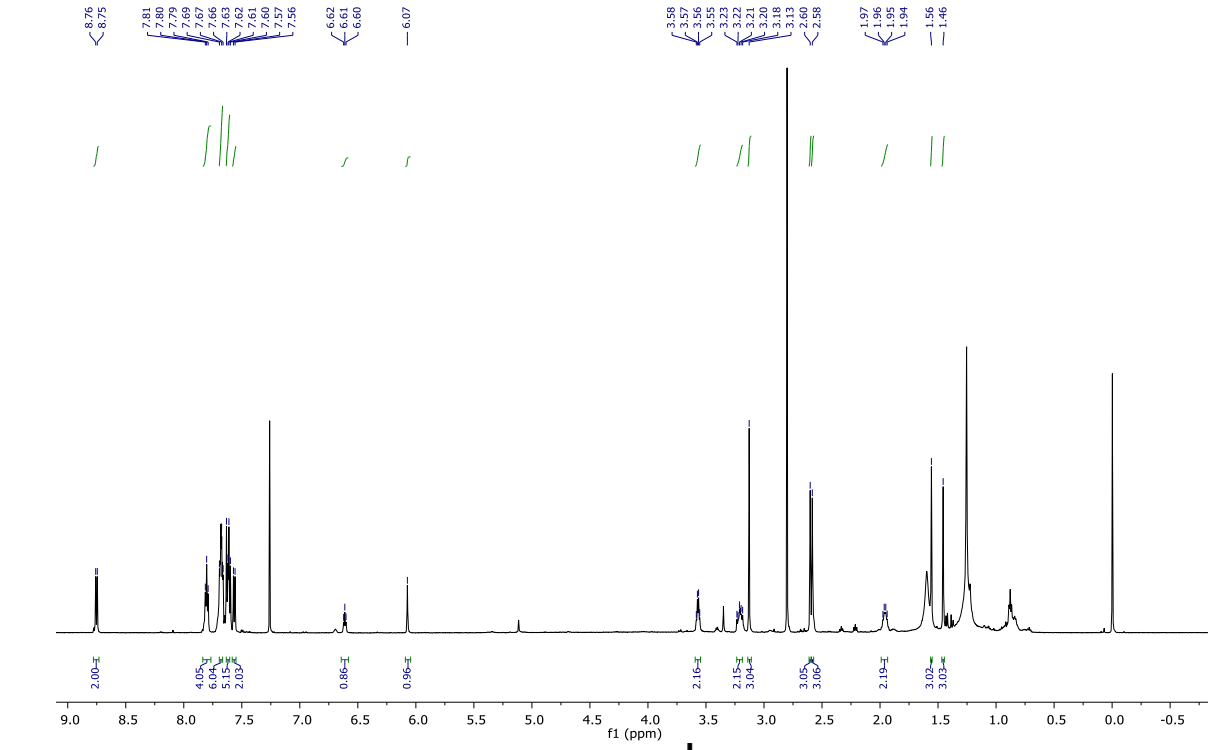
f1 (ppm)



Synthesis of compound (**9**). A solution of compound (**12**) (8 mg, 0.017 mmol), HATU (7.2mg, 0.019 mmol, 1.1 eq), and triethylamine (7.1 μL, 0.047 mmol, 3 eq.), in DCM (5 mL) was stirred for 10 minutes under nitrogen, then, (3-aminopropyl)triphenylphosphonium bromide (13.4 mg, 0.034 mmol, 2 eq.). The reaction was stirred for additional 12 h at room temperature. The reaction was washed with a saturated solution of sodium bicarbonate, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (3% Methanol in DCM) to afford compound (**9**) (8 mg, 55% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.75 (d, *J* = 8.2 Hz, 2H), 7.84 – 7.77 (m, 4H), 7.69 – 7.67 (m, 6H), 7.63 – 7.60 (m, 5H), 7.57 (d, *J* = 8.2 Hz, 2H), 6.61 (t, *J* = 6.1 Hz, 1H), 6.07 (s, 1H), 3.59 – 3.55 (m, 2H), 3.23 – 3.19 (m, 2H), 3.13 (s, 3H), 2.60 (s, 3H), 2.58 (s, 3H), 1.99 – 1.93 (m, 2H), 1.56 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.68, 166.10, 163.84, 159.05, 153.94, 145.15, 141.94, 139.64, 139.06, 135.55, 135.53, 133.05, 130.87, 130.78, 129.38, 129.01, 122.97, 118.25, 117.68, 38.76, 32.07, 29.84, 21.34, 15.04, 13.30. ESIMS $[M+H]^+$ calcd. for $C_{44}H_{42}BF_2N_7OP$ 764.32, found 764.35.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2.00
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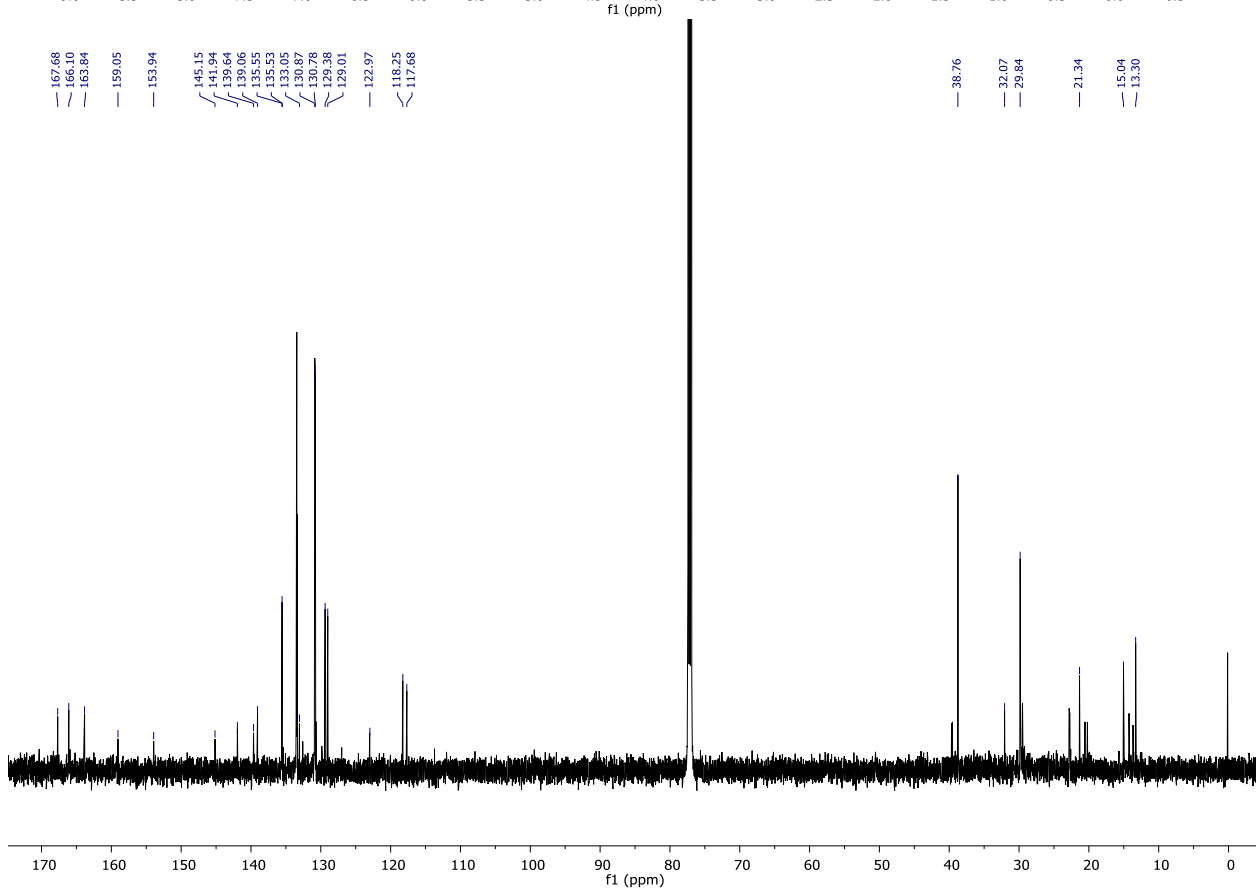
f1 (ppm)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32.07
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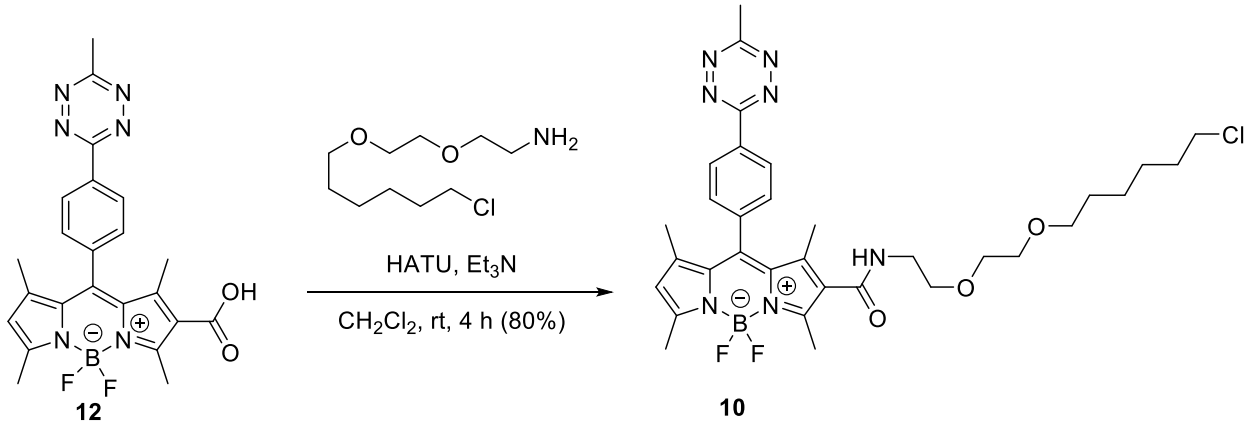
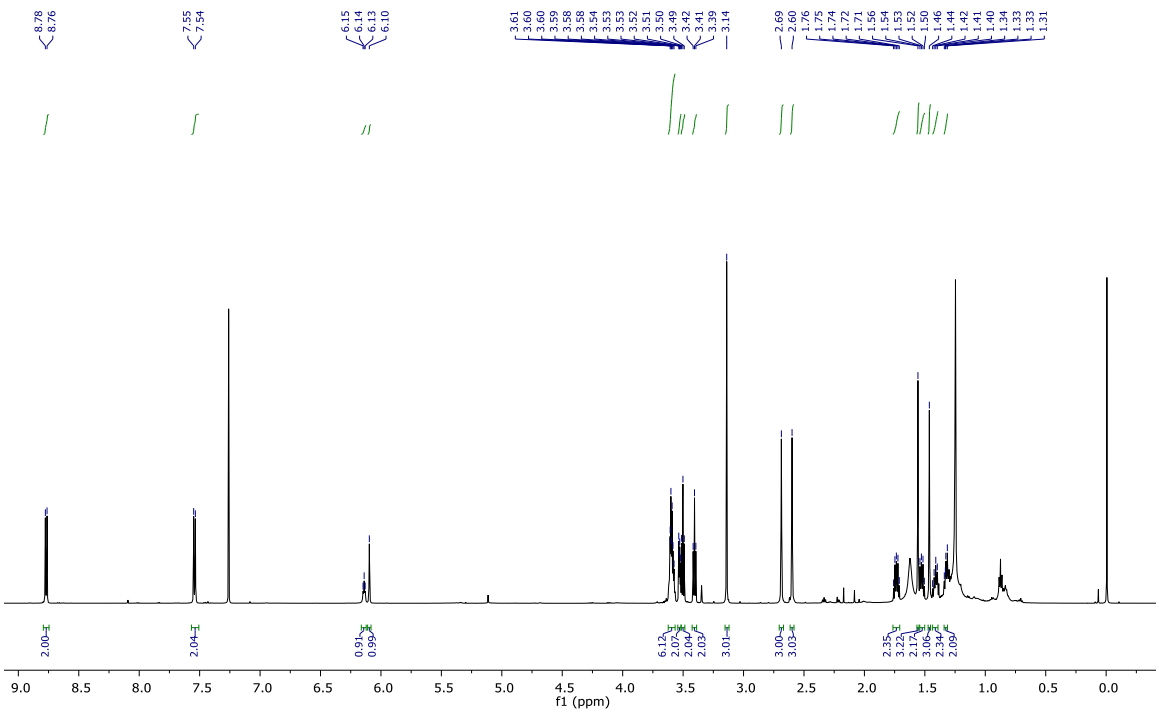
21.34
15.04
13.30
f1 (ppm)



12 10

Synthesis of compound (**10**). A solution of compound (**12**) (10 mg, 0.022 mmol), HATU (9.1 mg, 0.024 mmol, 1.1 eq.), and triethylamine (9.2 μL, 0.061 mmol, 3 eq) in DCM (5 mL) was stirred for 10 minutes under nitrogen in a 50 mL round bottom flask, then, 2-[2-[(6-chlorohexyl)oxy] ethoxy]-ethanamine (13.4 mg, 0.044 mmol, 2 eq.) was added to the solution. The reaction was stirred for additional 4 h at room temperature. The reaction was washed with a saturated solution of sodium bicarbonate, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The product was purified by column chromatography (40% ethyl acetate in hexanes) to afford compound (**10**) (11.8 mg, 80% yield) as a red solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.77 (d, J = 8.3 Hz, 2H), 7.54 (d, J = 8.3 Hz, 2H), 6.13 (t, J = 4.3 Hz, 1H), 6.10 (s, 1H), 3.62 – 3.56 (m, 6H), 3.55 – 3.52 (m, 2H), 3.50 (t, J = 6.7 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 3.14 (s, 3H), 2.69 (s, 3H), 2.60 (s, 3H), 1.74 (dt, J = 14.5, 6.8 Hz, 2H), 1.56 (s, 3H), 1.54 – 1.50 (m, 2H), 1.46 (s, 3H), 1.43 – 1.39 (m, 2H), 1.34 – 1.31 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.82, 165.09, 163.67, 159.57, 152.71, 145.31, 141.49, 139.56, 139.16, 132.98, 132.54, 129.71, 129.20, 128.89, 127.90, 123.17, 71.41, 70.34, 70.11, 69.89, 45.21, 39.36, 32.63, 29.84, 29.49, 26.78, 25.51, 21.39, 15.06, 13.65, 13.05. ESIMS $[M+H]^+$ calcd. for $C_{33}H_{41}BClF_2N_7O_3$ 668.30, found 668.29.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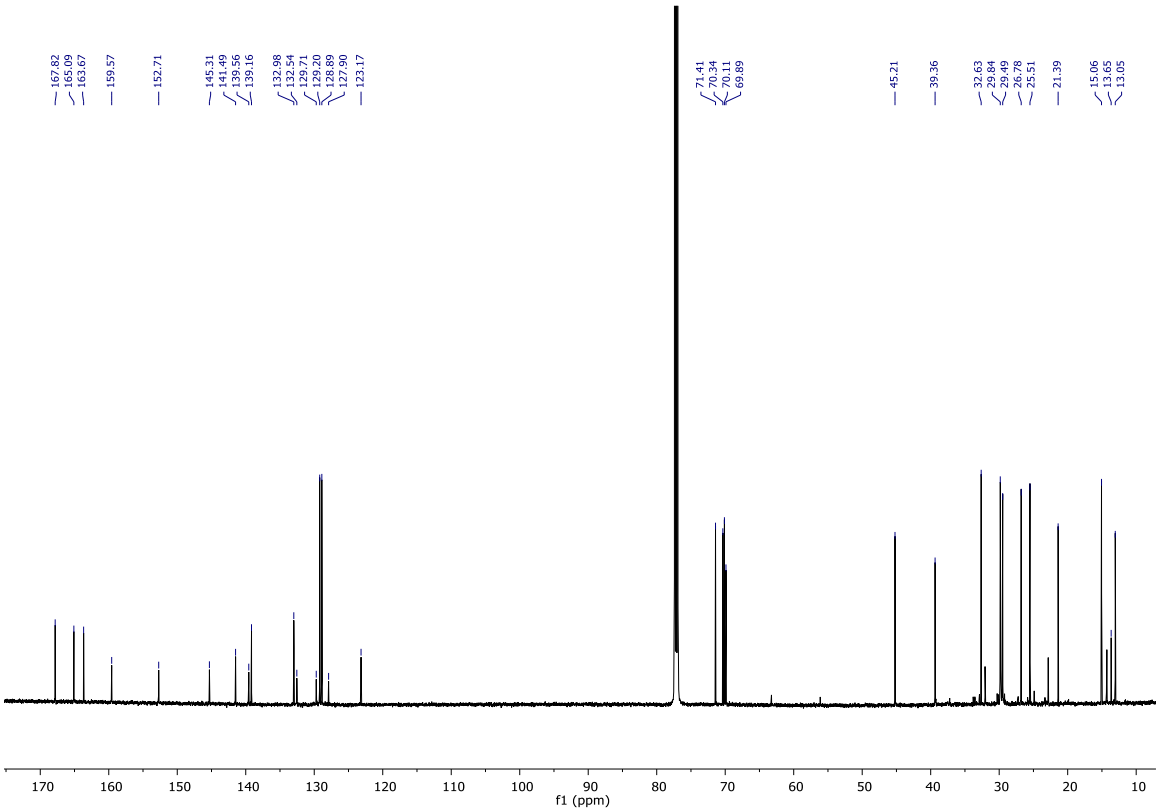
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)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69.89
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29.84
29.49
26.78
25.51
21.39
15.06
13.65
13.05
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

3. Quantum yield determination

Quantum yields measurements were determined using fluorescein (fluorescence quantum yield of 0.92 in 0.1 M NaOH); Quinine sulfate (fluorescence quantum yield of 0.546 in H_2SO_4 0.5 M); and Rhodamine B (fluorescence quantum yield of 0.31 in water) as a standard.

The fluorescence quantum yield, Φ_f (sample), were calculated according to equation as following:

$$\frac{\Phi_{f,sample}}{\Phi_{f,ref}} = \frac{OD_{ref} \cdot I_{sample} \cdot d_{sample}^2}{OD_{sample} \cdot I_{ref} \cdot d_{ref}^2}$$

Φ_f: quantum yield of fluorescence;
I: integrated emission intensity;
OD: optical density at the excitation wavelength;
d: refractive index of solvents, d_{CH3CN}=1.34; $d_{ethanol}$=1.36; d_{water}=1.33.

4. Cell culture

A-431 cells were incubated in complete medium Dulbecco's modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37°C in atmosphere containing 5% CO_2. CHO-K1 cells were incubated in complete medium (F-12K supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin) at 37°C in atmosphere containing 5% CO_2.

5. Co-localization assay

A stock solution of organelle targeting Tz-BODIPYs in chromatographic grade, anhydrous DMSO was prepared to a concentration of 2 mM. The solution was diluted to a final concentration of 2 μM by complete growth medium. Commercial MitoView™ 633, ER-Tracker™ Blue-White DPX, and LysoView™ 633 were prepared to a concentration of 1mM, and the stock solution was diluted

to the working concentration in complete medium (100 nM). After incubation of Tz-BODIPY-MOR, Tz-BODIPY-Ts, and Tz-BODIPY-TPP for 30 minutes under dark conditions, cells were washed with PBS (pH 7.4) twice and turned to confocal laser scanning microscope (CLSM). Cell imaging was performed with a Nikon Instruments A1 Confocal Laser Microscope. BODIPY dyes were activated using 405 nm light at 100% laser intensity. Differential interference contrast (DIC) and fluorescent images were processed and analyzed using ImageJ. The Pearson's Coefficient was calculated by ImageJ.

6. H2B-HaloTag expression

Plasmids was purchased from addgene (Plasmid #91564). Transfection of plasmid was done in CHO-K1 cells by X-tremeGENE™ Transfection Reagents (Sigma) according to their protocol.

7. PALM image acquisition and analysis

Just prior to PALM imaging, cells were washed and incubated with 2uM Tz-BODIPY-Halo in DMEM complete media for 30 minutes at room temperature in the dark before washing and subsequent imaging in 1x PBS (pH 7.4).

Imaging was performed at room temperature on the Nikon n-STORM system, featuring a CFI HP Apo TIRF AC 100x oil objective (NA 1.49) on an inverted Nikon Ti Eclipse microscope with a quad cube filter (Chroma, zt405/488/561/640 m-TRF), piezo stage, and Perfect Focus System (Nikon) for Z-stability. Lasers used in this study: 50 mW 405 nm diode laser and 200mW 561 nm solid-state lasers within an agilent MLC400B laser combiner with AOTF modulation.

PALM imaging was controlled with NIS-Elements Ar software and captured by an Andor iXON DU 897 EMCCD camera (EM gain setting=100, pixel size = 160 nm, 512x512 pixel field) with a

cylindrical lens inserted in the light path (to introduce astigmatism and improve singal-to-noise). 405nm laser power was used to activate/photobleach fluorescence and acquisition frames were collected in the 488 nm channel upon observing spontaneous reactivation fluorescent events within nucleus. Imaging frames were collected for a total of 20,000 – 30,000 frames.

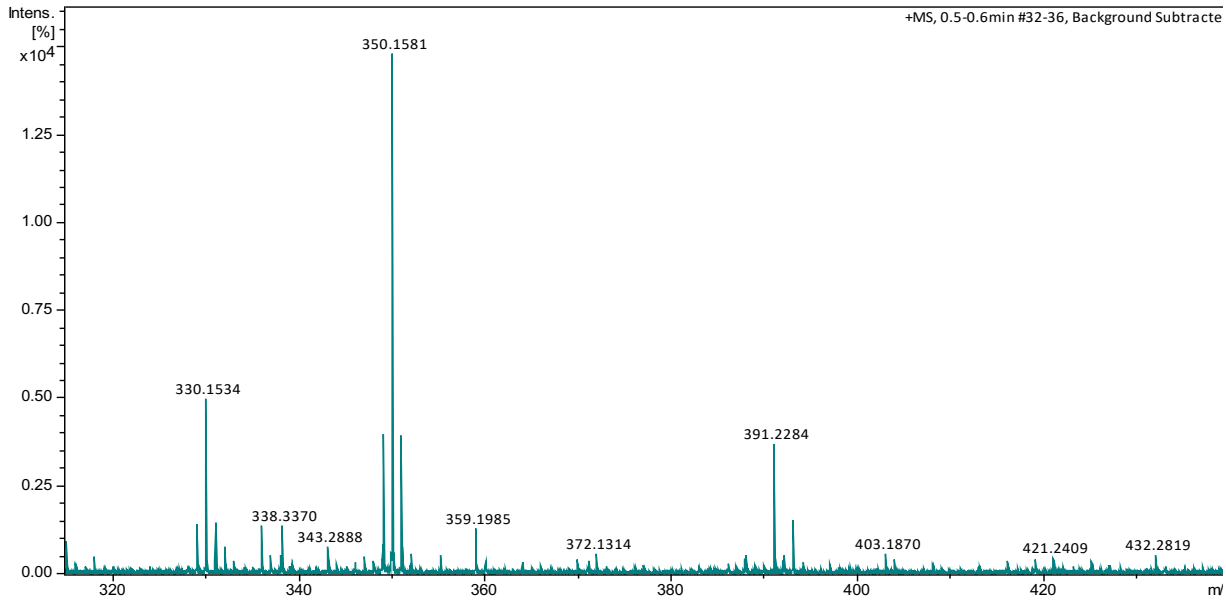

Figure S1. ESI-MS analysis of Tz-BODIPY after 20 minutes of light irradiation at 254 nm of a 1 μM solution, showing the evolution of CN-BODIPY $[M+H]^+$ calcd. for $C_{20}H_{18}BF_2N_3$ 350.16, found 350.16.

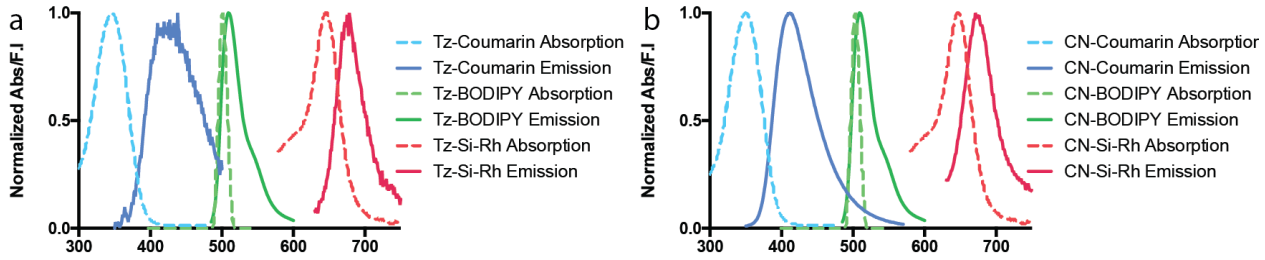

Figure S2. a) Normalized absorbance spectra (dashed lines) of Tetrazine fluorophore dyes; emission spectra (solid lines). b) Normalized absorbance spectra (dashed lines) of nitrile fluorophore dyes; emission spectra (solid lines).

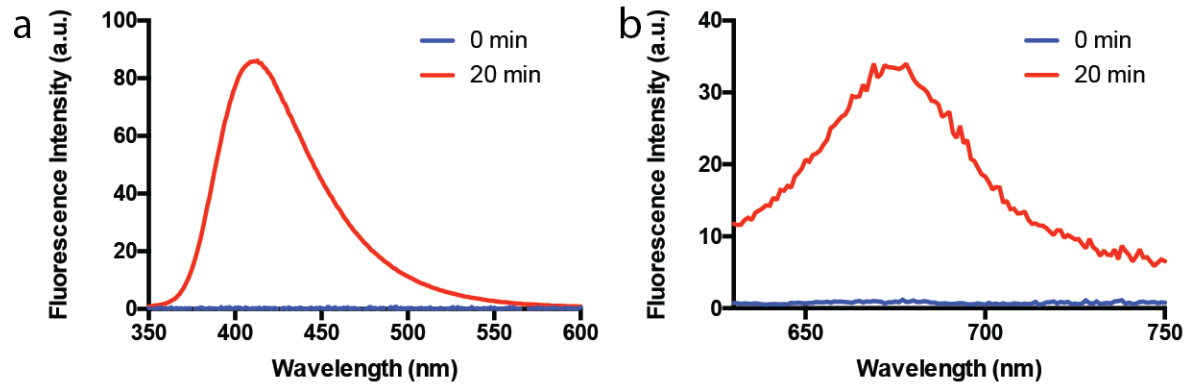

Figure S3. a) Emission spectra of Tz-Coumarin before and after activation with 254 nm light b) Emission spectra of Tz-Si-Rhodamine before and after activation with 254 nm light.

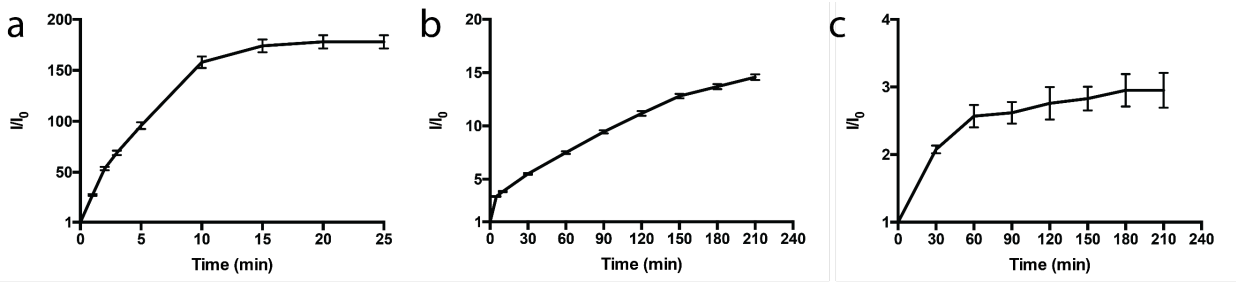

Figure S4. Fluorescence turn-on fold signal at 509 nm, from 1 uM solution of Tz-BODIPY dissolved in acetonitrile a) Photoactivation of Tz-BODIPY using a 254 nm handheld lamp. b) Photoactivation of Tz-BODIPY using a 365 nm handheld lamp. c) Photoactivation of Tz-BODIPY using 405 nm light.

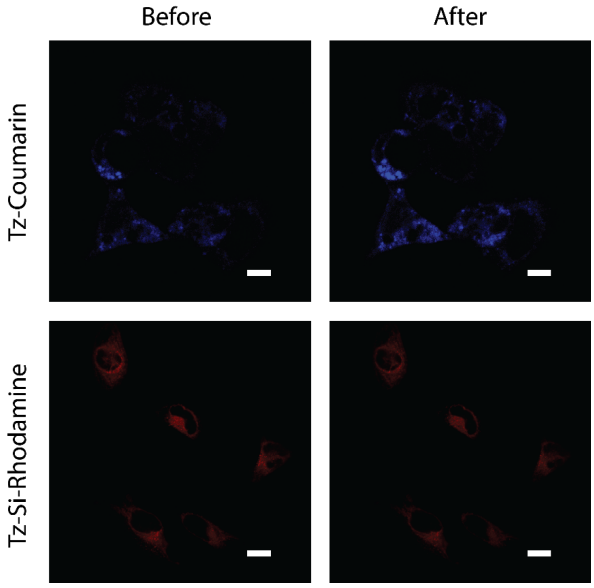

Figure S5. Photoactivation of Tz-Coumarin and Tz-Si-Rhodamine incubated in A431 cells using 405 nm light. Confocal images were taken before and after irradiation. Scale bar = 20 µm.

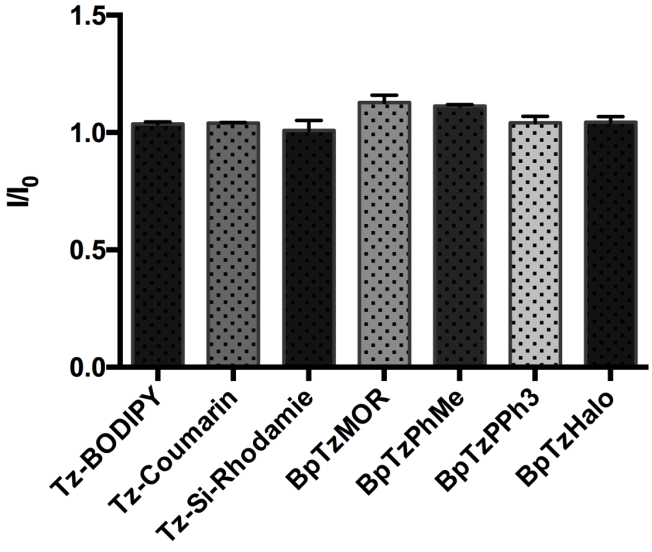

Figure S6. Relative fluorescence intensity of synthesized Fluorophore-Tetrazine compounds after 3 hours in dark conditions, dissolved in PBS buffer. 1 μM solutions in acetonitrile.

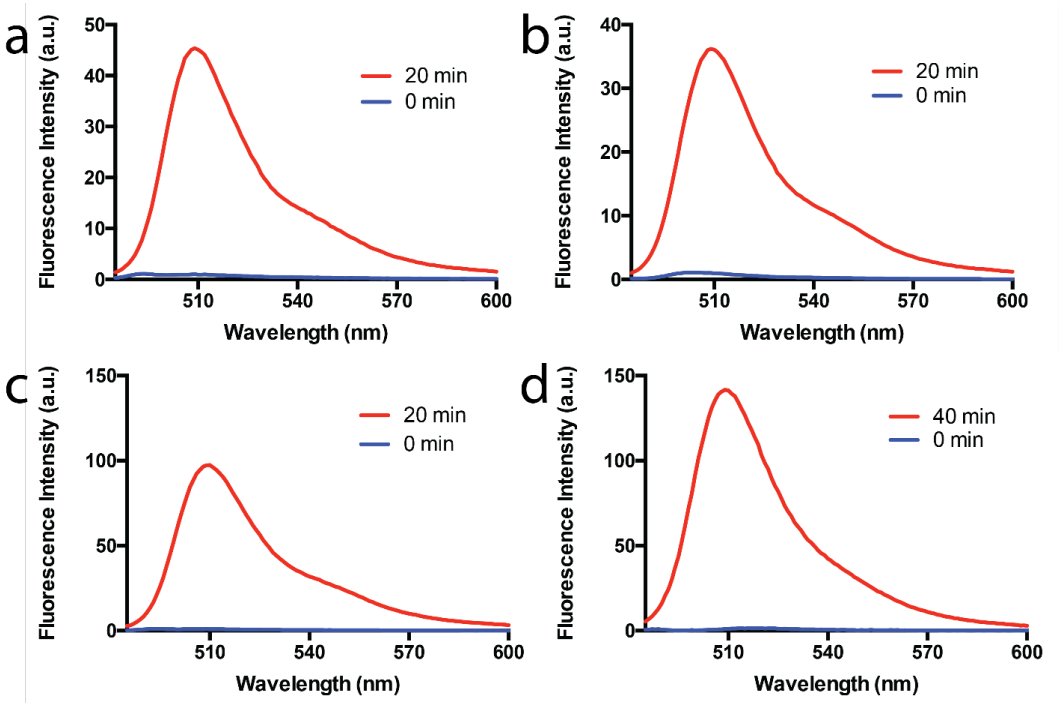

Figure S7. Emmision spectra of the photoactivation of BODIPY targeting probes, 1 μM in acetonitrile a) Tz-BODIPY-MOR b) Tz-BODIPY-Ts c) Tz-BODIPY-TPP d) Tz-BODIPY-Halo.

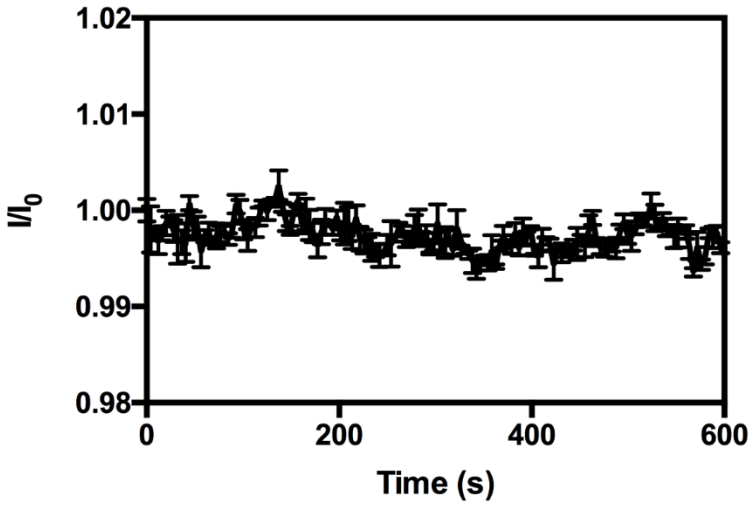

Figure S8. Fluorescence signal from Tz-BODIPY-Halo in CHO K1 cells, with no irradiation from 405 nm light.

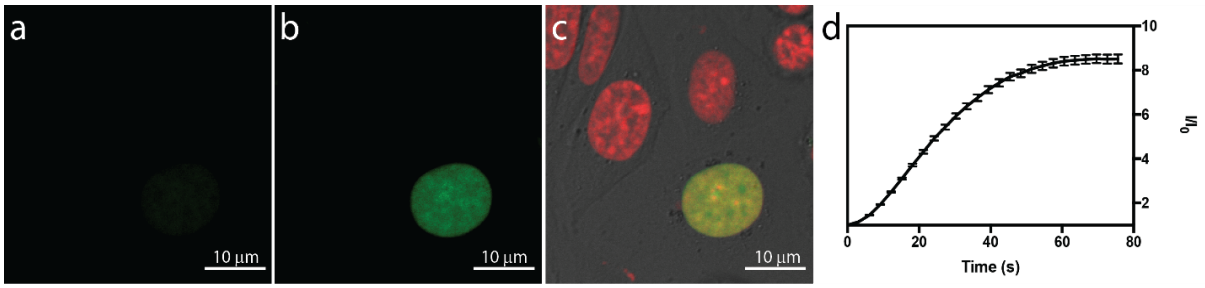

Figure S9. Confocal images of Tz-BODIPY-Halo in H2B-HaloTag-expressed CHO-K1 cells. a) Fluorescence image before activation b) Fluorescence image after 60 second irradiation using 405 nm laser c) Merged image of activated Tz-BODIPY-Halo, DRAQ5 and DIC d) Fluorescence intensity signal from the photoactivation of Tz-BODIPY-Halo.