Electronic Supplementary Information

BODIPY-Cucurbituril Complexes: Supramolecular Approach toward Improvement of Photodynamic Activity

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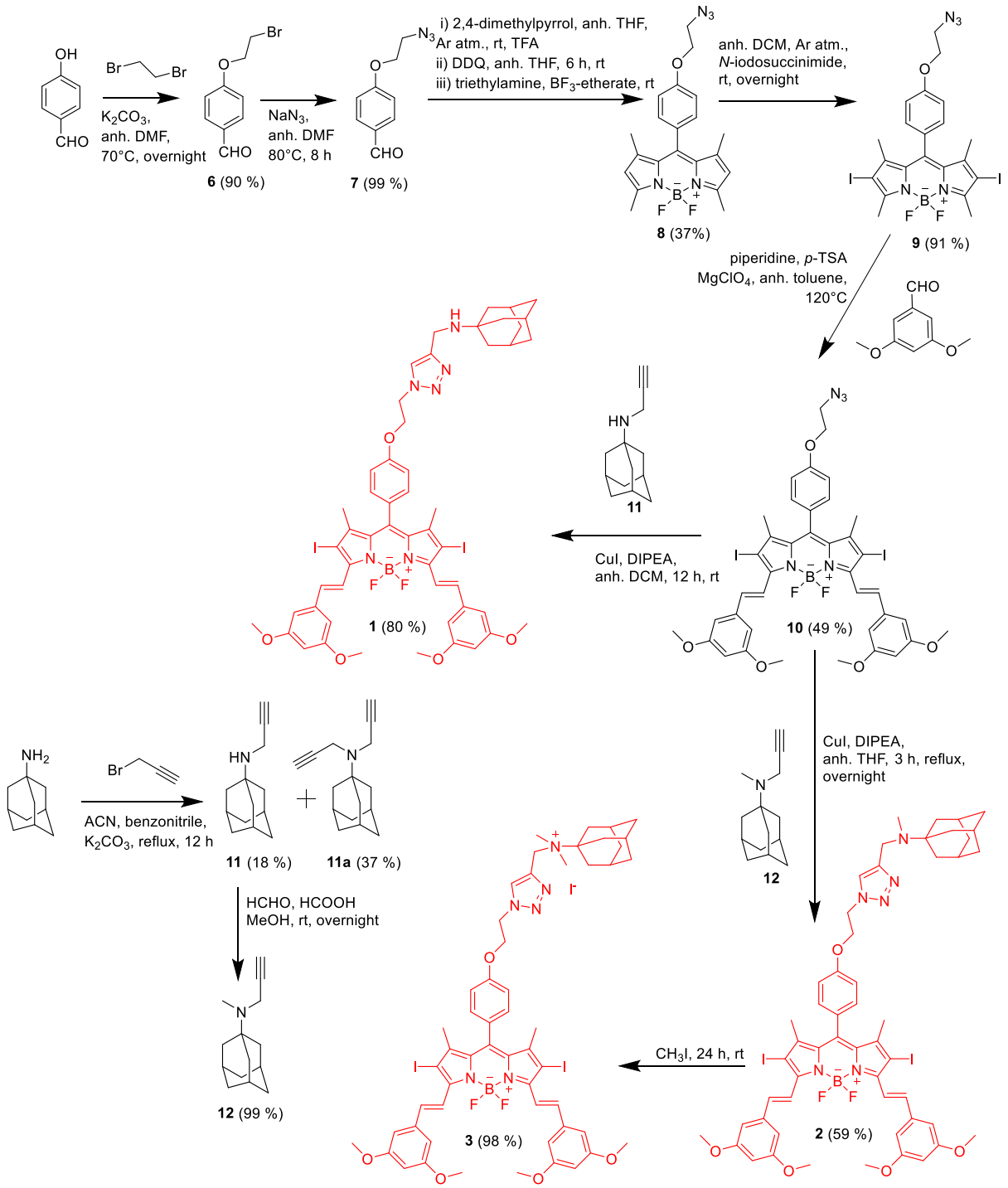
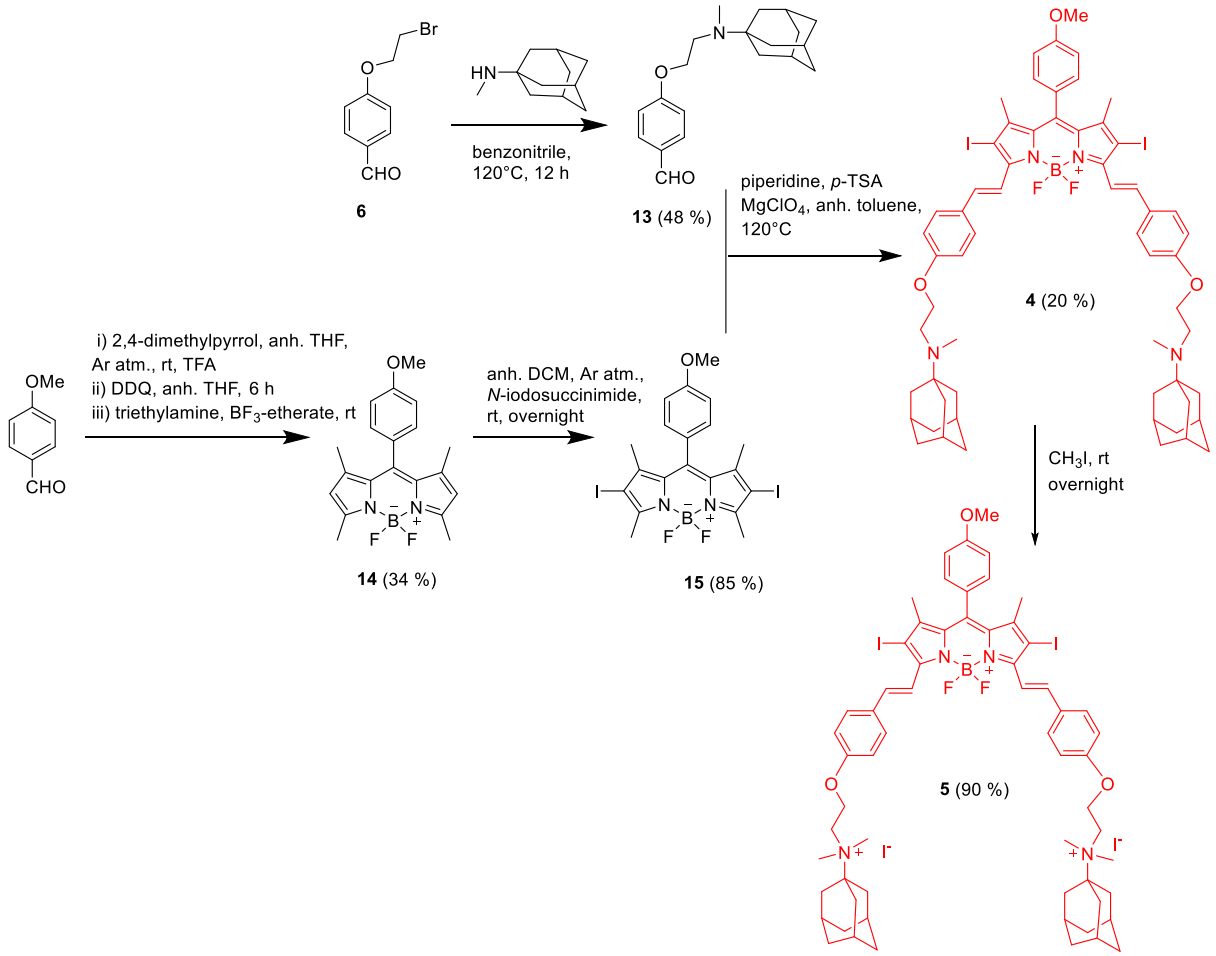
Content



Scheme S1. Synthesis of BODIPYs **1**-**3**.



Scheme S2. Synthesis of BODIPYs **4** and **5**.

General

All organic solvents were of analytical grade. All other chemicals for the syntheses were purchased from certified suppliers (i.e., TCI Europe, Acros, and Merck) and used as received. TLC was performed on Merck aluminum sheets coated with silica gel 60 F254. Merck Kieselgel 60 (0.040−0.063 mm) was used for column chromatography. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian VNMR S500 NMR spectrometer (Agilent Technologies, Santa Clara, USA) or Jeol JNM-ECZ600R (Jeol, Akishima, Japan). The chemical shifts are reported as δ values in ppm and are indirectly referenced to $Si(CH_3)_4$ *via* the signal from the solvent. *J* values are given in Hz. The UV−Vis spectra were recorded using a Shimadzu UV-2600 spectrophotometer (Shimadzu, Kyoto, Japan). The fluorescence spectra were measured using an FS5 Spectrofluorometer and FLS-1000 Photoluminescence Spectrometer (Edinburg Instruments, Edinburg, United Kingdom). HRMS spectra were measured at UHPLC system Acquity UPLC I-class (Waters, Millford, USA) coupled to high resolution mass spectrometer (HRMS) Synapt G2Si (Waters, Manchester, UK) based on Q-TOF were used for HRMS spectra measurement. Chromatography was carried out using Acquity UPLC Protein BEH C4 (2.1 x 50mm, 1.7 µm, 300 Å) column using gradient elution with ACN and 0.1% formic acid at flow-rate 0.4 ml/min. Electrospray ionization was operated in positive ion mode. The ESI spectra were recorded in the range 50 - 5000 m/z using leucine-enkefaline as a lock mass reference and sodium iodide for external calibration or in the range 50 - 1200 m/z using leucine-enkefaline as a lock mass reference and sodium formate for

external calibration. Low resolution MS spectra were measured on Expression® Compact Mass Spectrometer (Advion, USA) that works with single-quad detector.

Synthesis of 8-(4-(2-(4-(adamantan-1-yl-aminomethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy) phenyl)-3,5-bis(3,5-dimethoxystyryl)-1,7-dimethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**1**)

Under an argon atmosphere, BODIPY **10** (60 mg, 0.062 mmol) and compound **11** (12.0 mg, 0.068 mmol) were reacted in the presence of DIPEA (21 µl, 0.125 mmol) and CuI (12 mg, 0.065 mmol) in anhydrous DCM for 12 hours. After reaction completion, the solvent was evaporated and purification was done with silica gel chromatography with $CHCl_3$:MeOH (99:1, v/v) to separate a green-colored product **1**. Yield 57 mg, 80 %. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 8.08-8.05 (d, 2 H), 7.74 (s, 1 H), 7.67- 7.65 (d, 2H), 7.20-7.19 (d, 2H), 7.03-7.02 (d, 2H), 6.79 (s, 4H), 6.47 (s, 2H), 4.80 (s, 2 H), 4.44 (s, 2 H), 3.85 (s, 12H), 2.11 (m, 3 H), 1.74-1.69 (m, 13 H), 1.49 (s, 6 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 161.18, 159.05, 150.59, 146.21, 139.63, 138.78, 133.56, 129.91, 128.27, 123.34, 119.48, 115.57, 105.74, 101.96, 83.43, 66.64, 55.58, 49.76, 42.74, 36.82, 29.74, 29.68, 17.94. UV-vis (DMF) λ_{max} (log ε) 642 (4.87), 360 (4.62). HRMS (ESI): *m/z* calculated for $C_{52}H_{55}BF_2I_2N_6O_5$: $[M+H]^+$: 1147.2457; found: 1147.2457.

Synthesis of 8-(4-(2-(4-(adamantan-1-yl-methylaminomethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)phenyl)-3,5-bis(3,5-dimethoxystyryl)-1,7-dimethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**2**)

BODIPY **10** (117 mg, 0.122 mmol), compound **12** (50 mg, 0.246 mmol) and CuI (7 mg, 0.037 mmol) were dissolved in anhydrous THF and DIPEA (50 µL, 0.291 mmol)) was added afterwards. Reaction mixture was refluxed overnight. After reaction completion, the solvents were evaporated and the crude was purified by silica gel chromatography in MeOH:$CHCl_3$ (1:250, v/v) to obtain a green-colored solid. Yield 83 mg, 59 %. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.05 (d, *J* = 16.6 Hz, 2H), 7.77 (bs, 1H), 7.65 (d, *J* = 16.6 Hz, 2H), 7.20 – 7.15 (m, AA', BB', 2H), 7.03 – 6.99 (m, AA', BB', 2H), 6.77 (d, *J* = 2.3 Hz, 4H), 6.46 (t, *J* = 2.2 Hz, 2H), 4.79 (t, *J* = 5.1 Hz, 2H), 4.45 (t, *J* = 5.2 Hz, 2H), 3.84 (s, 14H), 2.30 (s, 3H), 2.13 (s, 3H), 1.83 (s, 6H), 1.71 – 1.61 (m, 6H), 1.47 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.11, 159.02, 150.52, 146.12, 139.57, 139.31, 138.72, 133.49, 129.84, 128.21, 119.42, 115.56, 105.68, 101.90, 83.36, 66.56, 55.53, 49.73, 44.76, 38.62, 36.76, 33.72, 29.72, 17.85. UV-vis (DMF) λ_{max} (log ε) 643 (4.98), 359 (4.71). HRMS (ESI): m/z calculated for $C_{53}H_{57}BF_2I_2N_6O_5$: $[M+H]^+$: 1161.2614; found: 1161.2615.

Synthesis of 8-(4-(2-(4-(adamantan-1-yl-dimethylammoniomethyl)-1*H*-1,2,3-triazol-1-yl)ethoxy)phenyl)-3,5-bis(3,5-dimethoxystyryl)-1,7-dimethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene iodide (**3**)

A mixture of BODIPY **2** (30 mg, 0.025 mmol) and CH_3I (1.5 mL) was stirred 24 h at room temperature in well-closed flask. Then, precipitate was collected and carefully washed with diethyl ether to obtain compound **3** as green solid. Yield 33 mg, 98%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 9.38 (s, 1H), 8.07-8.04 (s, 2H), 7.66 - 7.64 (d, 2 H), 7.17-7.18 (d, 2H), 7.13-7.12 (d, 2H), 6.78-6.77 (d, 4 H), 6.47-6.46 (d, 2 H), 4.93-4.91 (t, 2 H), 4.53-4.51 (t, 2H), 3.85 (s, 12H), 3.71 (s, 2H), 2.99 (s, 6H), 2.16 (s, 3H), 1.79-1.67 (m, 12H), 1.48 (s, 6 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 161.16, 159.06, 150.48, 146.26, 139.64, 138.79, 133.56, 131.28, 129.79, 128.10, 119.49, 115.91, 105.70, 101.92, 83.41, 66.03, 50.20, 44.63, 35.86, 35.20, 30.54, 17.96. UV-vis (DMF) λ_{max} (log ε) 643 (5.01), 358 (4.72).

Synthesis of 8-(4-methoxyphenyl)-3,5-bis(4-(2-(4-adamantan-1-yl-methylaminomethyl) ethoxy)styryl)-1,7-dimethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**4**)

Aldehyde **13** (75 mg, 0.24 mmol), and BODIPY **15** (80 mg, 0.12 mmol) were dissolved in anhydrous toluene. To this mixture, 0.5 ml piperidine and a catalytic amount of $Mg(ClO_4)_2$ and *p*-TSA were added

and kept heated at 120°C using the Dean-Stark trap. The reaction completion was confirmed by TLC and purification was done with silica gel chromatography to separate the green-colored fraction with EtOAc:MeOH:triethylamine (95:4:1, v/v/v) as eluent. Yield 32 mg, 20%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.13 (d, *J* = 16.7 Hz, 2H), 7.61 – 7.57 (m, 6H), 7.18 – 7.16 (m, 2H), 7.05 – 7.03 (m, 2H), 6.96 – 6.93 (m, 4H), 4.06 (t, *J* = 6.9 Hz, 4H), 3.90 (s, 3H), 2.89 (t, *J* = 6.8 Hz, 4H), 2.38 (s, 6H), 2.11 (s, 6H), 1.73 (d, *J* = 3.2 Hz, 12H), 1.69 – 1.65 (m, 6H), 1.63 – 1.60 (m, 6H), 1.50 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.62, 160.23, 150.52, 145.79, 139.23, 138.59, 133.38, 129.78, 129.41, 127.45, 116.75, 114.94, 82.74, 68.12, 55.54, 54.54, 48.65, 42.86, 38.73, 36.92, 35.22, 29.71, 17.82. UV-vis (DMF) λ_{max} (log ε) 663 (4.87), 453 (4.20), 381 (4.58). HRMS (ESI): m/z calculated for $C_{60}H_{69}BF_2I_2N_4O_3$: $[M+H]^+$: 1197.3593; found: 1197.3591.

Synthesis of 8-(4-methoxyphenyl)-3,5-bis(4-(2-(4-adamantan-1-yl-dimethyl ammoniomethyl)ethoxy)styryl)-1,7-dimethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene diiodide(**5**)

A mixture of BODIPY **4** (20 mg, 0.016 mmol) and CH_3I (1.5 mL) was stirred 24 h in a well-closed flask. Then, precipitate was collected and carefully washed with diethyl ether to obtain compound **5** as green solid. Yield 22 mg, 90%. ^{1}H NMR (600 MHz, DMSO-D_6) δ 8.07 (d, *J* = 16.6 Hz, 2H), 7.64 (d, *J* = 8.4 Hz, 4H), 7.44 (d, *J* = 16.5 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.16 (dd, *J* = 8.4, 6.2 Hz, 6H), 4.57 (t, *J* = 5.1 Hz, 4H), 3.86 (s, 3H), 3.72 (t, *J* = 5.1 Hz, 4H), 3.01 (s, 12H), 2.26 (s, 6H), 2.10 – 2.06 (m, 12H), 1.68 – 1.63 (m, 12H), 1.48 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 160.83, 159.24, 150.34, 146.31, 139.03, 133.55, 130.20, 130.01, 129.50, 126.64, 117.07, 116.13, 115.78, 115.51, 85.05, 75.62, 62.75, 56.85, 55.91, 44.82, 40.62, 35.08, 34.23, 30.40, 17.84. UV-vis (DMF) λ_{max} (log ε) 656 (4.89), 439 (4.17), 374 (4.62).

General procedure for conversion BODIPYs to corresponding hydrochlorides.

Free base BODIPY was dissolved in THF (2 mL) and few drops of concentrated HCl were added. Mixture was stirred for 10 minutes and then evaporated under reduced pressure. Solid residue was washed by diethyl ether and dried under vacuo.

Synthesis of 4-(2-bromoethoxy)benzaldehyde (**6**)

A mixture of 1,2-dibromoethane (8.2 mL, 0.094 mol), 4-hydroxybenzaldehyde (4.0 g, 0.032 mol), and anhydrous K_2CO_3 (9.0 g, 0.065 mol) in anhydrous DMF (15 mL) was stirred overnight at 70°C. The reaction mixture was poured into 100 ml distilled water and extracted with DCM. The solvent was removed under reduced pressure after drying the organic phase with anhydrous $MgSO_4$. The crude product was purified by silica gel chromatography hexane:EtOAc (8:2, v/v) to afford benzaldehyde **6** as a colorless oil. Yield 6.68 g, 90%. ^{1}H-NMR (600 MHz, $CDCl_3$): 9.88 (s, 1 H), 7.84-7.82 (d, 2 H), 7.01-6.99 (d, 2 H), 4.37-4.35 (t, 2 H), 3.66-3.64 (t, 2 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 190.81, 163.14, 132.15, 130.64, 115.01, 68.08, 28.58.

Synthesis of 4-(2-azidoethoxy)benzaldehyde (**7**)

A mixture of aldehyde **6** (4.4 g, 0.019 mol) and NaN_3 (1.6 g, 0.024 mol) in anhydrous DMF (20 mL) was heated at 80°C for 8 hours. After reaction completion, excess DMF was evaporated under reduced pressure. The crude obtained was dissolved in DCM followed by extraction with water. The organic phase was dried with anhydrous $MgSO_4$ and removed under reduced pressure to obtain a colorless oil of azide **7**, no further purification was required as crude was used for subsequent steps. Yield 3.64 g, 99 %. ^{1}H-NMR (600 MHz, $CDCl_3$): 9.88 (s, 1H), 7.84−7.83 (d, 2H), 7.02−7.01 (d, 2H), 4.23-4.21 (t, 2H), 3.64-2.62 (t, 2H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 190.81, 163.22, 132.09, 130.58, 114.93, 67.31, 50.07.

Synthesis of 8-(4-(2-azidoethoxy)phenyl)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (8)

In 500 mL double-neck round-bottom flask, 2,4-dimethylpyrrole (2.3 mL, 22.13 mmol) was mixed in dry THF (150 mL) and degassed by argon for 30 min. Then, aldehyde **7** (1.91 g, 14.6 mmol) and 4-5 drops of trifluoroacetic acid (TFA) were added and the reaction mixture was kept for stirring overnight at room temperature. After overnight stirring, the reaction mixture turned light brown. To this mixture, 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) (2.24g, 10 mmol) dissolved in dry THF was added dropwise over a time of 1 hour and after which the reaction was allowed to stir for the next 6 hours (color changed to black). Triethylamine (52 mL, 368 mmol) was added dropwise over a time of 30 min and kept for stirring for the next 10 minutes at room temperature. After that, the reaction was kept under an ice bath for 20 minutes to cool down the mixture. Then, BF_3-etherate (51.8 mL, 412 mmol) was added dropwise and the reaction mixture was allowed to stir overnight at room temperature. THF was removed under vacuum and the crude obtained was dissolved in DCM, washed with brine, 0.5N $NaHCO_3$ solution and followed by water. The organic layer was dried over anhydrous $MgSO_4$ and silica gel column chromatography was done with hexane:EtOAc (8:2, v/v). BODIPY **8** was obtained as a crystalline red powder. Yield 1.55 g, 37 %. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 7.19 - 7.18 (d, 2H), 7.04 – 7.02 (d, 2H), 4.21-4.20 (d, 2H), 3.66-3.65 (d, 2H), 2.55 (s, 6H), 1.43 (s, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 158.94, 155.52, 143.26, 141.68, 131.93, 129.52, 127.96, 121.31, 115.30, 67.09, 50.37, 14.72. UV-vis (DMF) λ_{max} (log ε) 501 (4.85), 360 (3.79).

Synthesis of 8-(4-(2-azidoethoxy)phenyl)-1,3,5,7-tetramethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (9)

Compound **8** (210 mg, 0.51 mmol) and *N*-iodosuccinimide (250 mg, 1.11 mmol) were dissolved in anhydrous DCM (20 mL). The reaction was kept overnight at room temperature and the reaction completion was confirmed by TLC. The DCM was evaporated, and crude was purified by silica gel column chromatography in hexane:DCM (2:1, v/v) to obtain a pink-red colored solid. Yield 308 mg, 91 %. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 7.19 - 7.16 (m, 2H), 7.06 – 7.03 (m, 2H), 6.04 (s, 1H), 4.22-4.21 (d, 2H), 3.68-3.66 (t, 2H), 2.63 (s, 3H), 2.56 (s, 3H), 1.44 (s, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 159.29, 156.80, 145.41, 143.37, 141.32, 131.76, 129.36, 127.57, 115.55, 85.71, 67.12, 50.29, 17.28, 16.10. UV-vis (DMF) λ_{max} (log ε) 534 (4.90), 388 (4.09).

Synthesis of 8-(4-(2-azidoethoxy)phenyl)-3,5-bis(3,5-dimethoxystyryl)-1,7-dimethyl -2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (10)

BODIPY **9** (280 mg, 0.42 mmol), and 3,5-dimethoxybenzaldehyde (233 mg, 1.4 mmol) were dissolved in anhydrous toluene. To this mixture, 0.8 ml piperidine and a catalytic amount of $Mg(ClO_4)_2$ and *p*-TSA were added and kept heated at 120°C using the Dean-Stark trap. The reaction completion was confirmed by TLC and purification was done with silica gel chromatography with DCM as eluent to separate green-colored fraction. Yield 200 mg, 49%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 8.08-8.05 (d, 2 H), 7.68-7.65 (d, 2H), 7.21 - 7.20 (d, 2 H), 7.09 – 7.07 (m,2 H), 6.79 (s, 4 H), 6.48-6.47 (d, 2 H), 4.24-4.23 (d, 2H), 3.86 (S, 12 H), 3.69-3.68 (d, 2 H), 1.51 (s, 6 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 161.18, 159.43, 150.54, 146.25, 139.58, 138.81, 133.61, 129.82, 127.98, 119.51, 115.65, 105.73, 101.95, 83.41, 67.20, 55.60, 50.37, 17.88. UV-vis (DMF) λ_{max} (log ε) 643 (5.05), 359 (4.75).

Synthesis of *N*-(prop-2-yn-1-yl)adamantan-1-amine (11)

A mixture of propargyl bromide (595 µl, 4 mmol), 1-aminoadamantane (453 mg, 3 mmol), and K_2CO_3 (1.2 g, 9 mmol) was refluxed in acetonitrile (40 mL) and benzonitrile (10 mL) for 12 h. After reaction completion, the solvent was evaporated to obtain crude product. The crude thus obtained was dissolved in chloroform and washed with water, the organic layer was dried using anhydrous sodium

sulphate and purification was done with silica gel chromatography with chloroform as eluent to separate the disubstituted product **11a** first followed by amine **11** product as dense oil. Yield 170 mg, 18 %. Data for **11**: $R_{f\ (CHCl3)}$ = 0.18; ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 3.42-3.41 (d, 2 H), 2.19-2.18 (d, 1H), 1.68-1.59 (m, 13H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 83.80, 70.59, 51.00, 42.51, 36.56, 30.06, 29.48. MS (ESI) Calc. for $C_{13}H_{20}N$ $[M+H]^+$ 190.2 found 190.3. Di-substituted product *N,N*-di(prop-2-yn-1-yl)adamantan-1-amine (**11a**) was obtained in $CHCl_3$: hexane (8:2, v/v). Data for **11a**: Yield: 340 mg, 37%; $R_{f\ (CHCl3)}$ = 0.57; ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 3.67 (s, 4 H), 2.19-2.18 (d, 2H), 2.08 (s, 3H), 1.83-1.82 (d, 6H), 1.66-1.59 (m, 6H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 82.30, 72.17, 55.57, 40.12, 36.69, 35.18, 29.85.

Synthesis of *N,N*-(prop-2-yn-1-yl)methyladamantan-1-amine (12)

A mixture of compound **11** (30 mg, 0.158 mmol), formaldehyde (70 µL, 1.90 mmol, 5 eq) and formic acid (75 µL, 1.99 mmol) were stirred overnight in MeOH (10 mL) at room temperature. The methanol solvent was removed under reduced pressure and the crude obtained was mixed with chloroform. Then, the chloroform layer was washed with 10% NaOH solution, the organic layer was extracted, and dried with anhydrous sodium sulfate to obtain pure compound **12**. Yield 39.9 mg, 99%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 3.41 (s, 2 H), 2.41 (s, 3H), 2.18 (s, 1H), 2.08 (s, 3H), 1.74 (s, 6H), 1.66-1.58 (m, 6H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 82.87, 54.60, 39.11, 36.84, 33.79, 29.48.

Synthesis of 4-(2-(adamantan-1-yl-methylamino)ethoxy)benzaldehyde (13)

A mixture of aldehyde **7** (227 mg, 1 mmol), *N*-methyl-1-aminoadamantane (**12**) (165 mg,1 mmol) was stirred in benzonitrile (15 ml) for 12 h at 120°C. After reaction completion, the solvent was evaporated and purification was done with silica gel chromatography with EtOAc:MeOH (95:5, v/v) to separate the product **7** as off-white solid. Yield 150 mg, 48 %. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 9.87 (s, 1H), 7.83 - 7.80 (dd, 2 H), 7.01 - 6.98 (dd, 2 H), 4.08-4.06 (t, 2 H), 2.89-2.86 (t, 2 H), 2.36 (s, 3 H), 2.10 (s, 3H), 1.70-1.58 (m, 12 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 190.99, 164.21, 132.12, 129.95, 114.96, 68.48, 54.49, 48.53, 38.75, 36.88, 35.25, 29.68.

Synthesis of 8-(4-methoxyphenyl)-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (14)

In a 500 mL double-neck round-bottom flask, 2,4-dimethylpyrrole (2.3 mL, 22.13 mmol) was dissolved in dry THF (150 mL) and degassed by argon for 30 min. Then, 4-methoxybenzaldehyde (1.36 g, 10 mmol) and 4-5 drops of trifluoroacetic acid were added and the reaction mixture was kept for stirring overnight at room temperature. After overnight stirring, the reaction mixture turned light brown. To this mixture, DDQ (2.24 g, 10 mmol) dissolved in dry THF was added dropwise over a time of 1 hour and after which the reaction was allowed to stir for the next 6 hours (color changed to black). Triethylamine (51.4 mL, 366.8 mmol) was added dropwise over a time of 30 min and kept for stirring for the next 10 mins at room temperature. After that, the reaction was kept under an ice bath for 20 minutes to cool down the mixture. Then, BF_3-ethrate (51.8 mL, 412 mmol) was added dropwise and the reaction mixture was allowed to stir overnight at room temperature. THF was removed under vacuum and the crude obtained was dissolved in DCM, washed with brine, 0.5 N $NaHCO_3$ solution and followed by water. The organic layer was dried over anhydrous $MgSO_4$ and silicagel column chromatography was done with hexane:EtOAc (1:1, v/v). BODIPY **14** was obtained as a crystalline red powder. Yield 1.20 g, 34%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 7.18 - 7.16 (t, 2 H), 7.02 – 7.00 (t, 2 H), 5.97 (s, 2 H), 3.87 (s, 3H), 2.55 (s, 6 H), 1.43 (s, 6 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 160.28, 155.40, 143.30, 141.99, 132.00, 129.35, 127.20, 121.25, 114.67, 55.45, 14.69.

Synthesis of 8-(4-methoxyphenyl)-1,3,5,7-tetramethyl-2,6-diiodo-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**15**)

BODIPY **14** (200 mg, 0.56 mmol) and *N*-iodosuccinimide (275 mg, 1.2 mmol) were dissolved in anhydrous DCM (20 mL). The reaction was kept overnight, and the reaction completion was confirmed by TLC. The DCM was evaporated, and crude was purified by silica gel column chromatography with hexane: DCM (1:1, v/v) to obtain a pink-red colored product. Yield 290 mg, 85%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ = 7.15 - 7.12 (d, 2 H), 7.05 – 7.02 (d, 2 H), 3.89 (s, 3 H), 2.64 (s, 6 H), 1.44 (s, 6 H). ^{13}C-NMR (150 MHz, $CDCl_3$) δ = 160.68, 156.71, 145.51, 141.72, 131.88, 129.24, 126.84, 115.01, 85.68, 55.54, 17.31, 16.15.

Photophysical characterization

Fluorescence quantum yield and lifetime

Fluorescence quantum yields (Φ_F) were determined on a FS5 Spectrofluorometer (Edinburg Instruments) by the comparative method[1] using unsubstituted zinc(II) phthalocyanine (Φ_F = 0.32 (THF)[2]) for **1, 1·HCl**, **2**, **2·HCl**, **3**, **4**, **4·HCl**, **5** in DMF and rhodamine G6 (Φ_F = 0.94 (EtOH)[3]) for **8**, **9** in MeOH as reference compounds. Excitation wavelengths were 602 nm (for BODIPY **1, 1·HCl**, **2**, **2·HCl**, **3**, **4**, **4·HCl**, **5**), 480 nm (for BODIPY **8**) and 500 nm (for BODIPY **9**). The determination of Φ_F values was performed in triplicate, and the data represent the mean of these measurements. The estimated experimental error was ±10%. Absorption of the samples at the excitation wavelength was kept below 0.05 and at a Q band maximum below 0.1 to avoid the inner-filter effect. The results of Φ_F were corrected for the refractive indices of the solvents. Lifetimes were determined on FLS-1000 (Edinburg Instruments) with picosecond diode laser HPL-655 (λ_{ex} = 653.9 nm; 50 ns pulse period) as excitation source. For absorption, emission and excitation spectra see Figure S1.

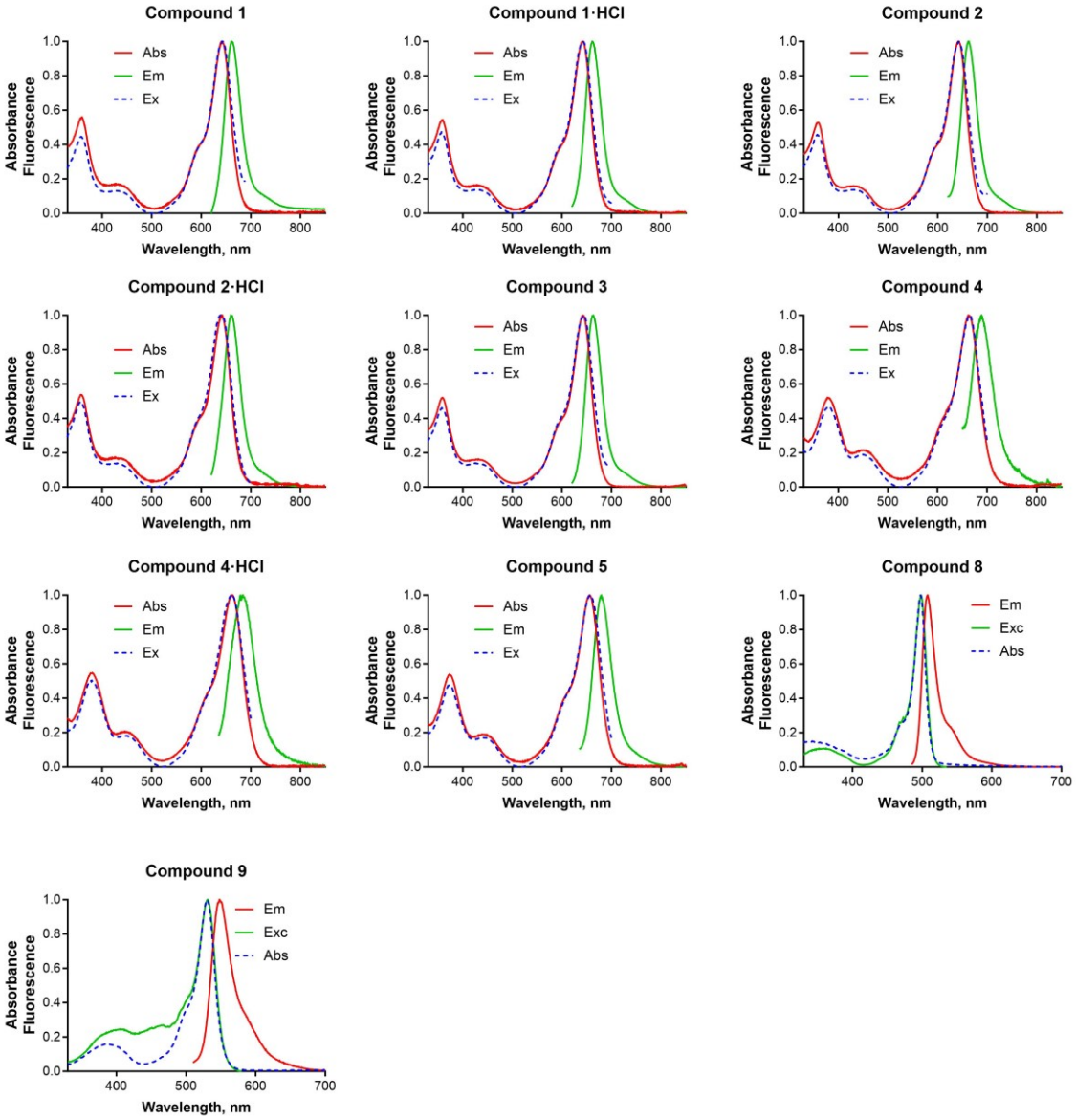


Figure S1. Normalized absorption (blue, dashed), emission (red) and excitation (green) spectra of compounds **1, 1·HCl**, **2**, **2·HCl**, **3**, **4**, **4·HCl**, **5** in DMF and **8** and **9** in MeOH.

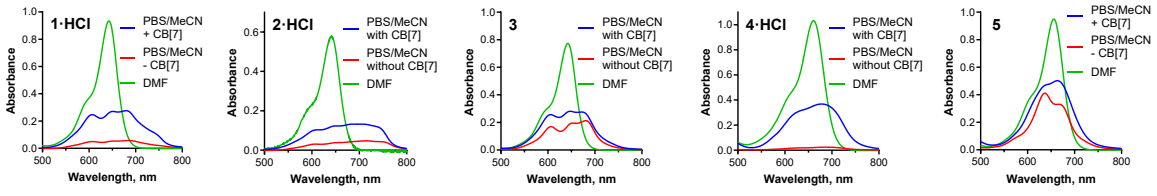


Figure S2. Absorption spectra (c = 10 µM) of BODIPYs in DMF (green) and in PBS containing 20% MeCN with (blue) or without (red) CB[7] (1 equiv *per* Ad).

Singlet oxygen quantum yields in DMF

Singlet oxygen quantum yield (Φ_Δ) were determined by the comparative method based on the decomposition of a chemical trap for singlet oxygen (1,3-diphenylisobenzofuran) and using

unsubstituted zinc(II) phthalocyanine (Φ_Δ = 0.56 (DMF)[4]) for **1, 1·HCl**, **2**, **2·HCl**, **3**, **4**, **4·HCl**, **5** in DMF or bengal rose (Φ_Δ = 0.76 (MeOH)[5]) for **8**, **9** in MeOH as reference compounds. During determination of Φ_Δ, irradiation was performed by 100 W, Xenon ozone-free lamp and 8 cm water filter (for all compounds), long-pass filter Newport 20CGA-455 (for **8**, **9**) and long-pass filter Newport FSQ-OG530 (for **1, 1·HCl**, **2**, **2·HCl**, **3**, **4**, **4·HCl**, **5**) were used to cut off undesirable wavelengths and heat. More details of the method are described elsewhere.[6] All the determinations were performed in triplicate, and the data represent the mean of the measurements. The estimated experimental error was ±10%.

Determination of singlet oxygen production in PBS

Stock solutions (100 μM) of BODIPYs were prepared in DMF. Stock solution (25 μM) of 9,10-anthracenedipropanoic acid (ADPA) was prepared in PBS buffer containing 20% MeCN. Stock solution of ADPA (2250 μL) was transfer into cuvette and solution of CB[7] in PBS was added in some experiments to reach one equivalent of CB[7] per Ad (*i.e.*, either 10 or 20 μM) and absorption spectrum was measured. Subsequently, dye stock solution (250 μL, final concentration of BODIPY was 10 μM) was added into cuvette and was stirred in dark for 1 hour and then absorption spectrum was measured. In the next step, the cuvette was irradiated (100 W, Xenon ozone-free lamp, 8 cm water filter, long-pass filter Newport FSQ-OG530, radiation flux = 62.1 mW) and absorption spectra were measured in selected intervals. For samples without CB[7], the step with its addition was skipped. Decay of ADPA was expressed as percentage of remaining ADPA based on its absorbance at 250 nm. All the determinations were performed in triplicate, and the data represent the mean of the measurements. The results are plotted in Fig. S3.

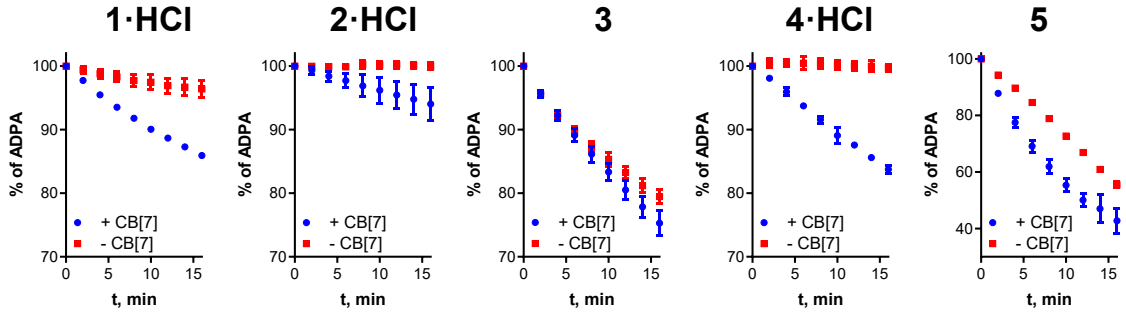


Figure S3. Determination of singlet oxygen production using decomposition of ADPA in PBS with 20% ACN in presence (blue dots) or absence (red squares) of CB[7].

Determination of log *P*

Stock solutions (100 μM) of final compounds were prepared in DMF. PBS buffer (400 μL) and *n*-octanol (400 μL) were put into plastic vial and stock solution of BODIPY (20 μL) was added. Vials were vortexed for 5 minutes and then centrifuged (MiniSpin®(Eppendorf), 10 000 rpm, 10 min). After this procedure, the octanol and water phases were separated (the middle part between the layers was discarded) and 50 μL of each layer was diluted into cuvette with DMF (2.5 mL) and fluorescence emission was measured. Log *P* was calculated by equation log P = (F_{OctOH}/F_{PBS}), where F_{OctOH} and F_{PBS} are values of fluorescence in octanol layer and PBS layer, respectively. Whole experiment was repeated six times for each compound. To determinate log *P* with addition of CB[7] the method was slightly changed: PBS buffer (400 μL), 2 equivalents of CB[7] (stock 1 mM) and stock solution of BODIPY (20 uL) were put into plastic vial and then covered with *n*-octanol (400 μL). Vials were vortexed and remaining procedure was the same as for determination without CB[7].

Titration of 4 with acid

Stock solution of BODIPY **4** (DMF, 100 μM) and aqueous hydrochloric acid (3.24 mM) were prepared. BODIPY **4** (25 μL) was added into cuvette with DMF (2.5 mL) to reach working concentration of 1 μM and absorption and emission spectra were measured. After that, hydrochloric acid (3 μL) was added into cuvette and both spectra were measured (Figure S4). The last step was repeated as long as the fluorescence was increasing.

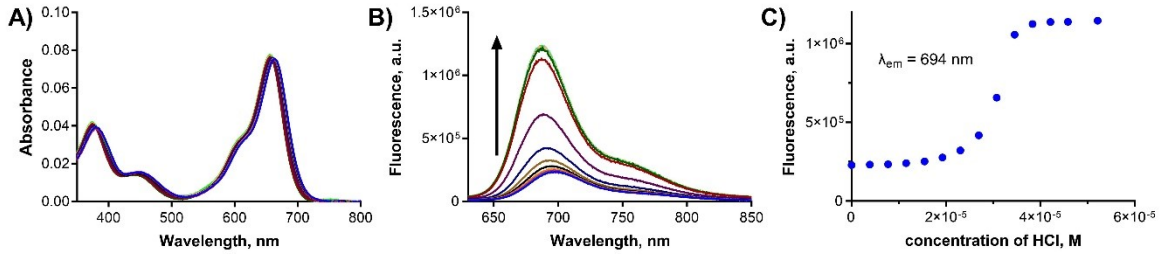


Figure S4. Changes in absorption (A) and emission (B) spectra of compound **4** (c = 1 μM) in DMF upon addition of HCl. C) Plot of the fluorescence intensity at 694 nm upon addition of HCl.

Interaction of BODIPYs with CB[7] studied by fluorescence

Stock solutions (100 μM) of BODIPYs were prepared in DMF. Solution of CB[7] (1 mM) was added to fluorescence cuvette with PBS with 20% MeCN (2.5 mL) to reach desired concentration of CB[7] in cuvette (0.25, 0.5, 1.0, 2.0 μM for **2 HCl** and **3**; or 0.5, 1.0, 2.0, 4.0 μM for **4·HCl** and **5**). Afterwards, solution of BODIPY (25 μL) was added to cuvette (final BODIPY concentration was 1 μM) and fluorescence was measured every 10 min. The results are plotted on Figure S5. Subsequently, the experiment was repeated with more equivalents (see Figure 3C in manuscript and Figure S6) and the fluorescence was measured only once after equilibration for 3 h (for **1 HCl, 2 HCl, 3** and **4 HCl**) or immediately after mixing (for **5**).

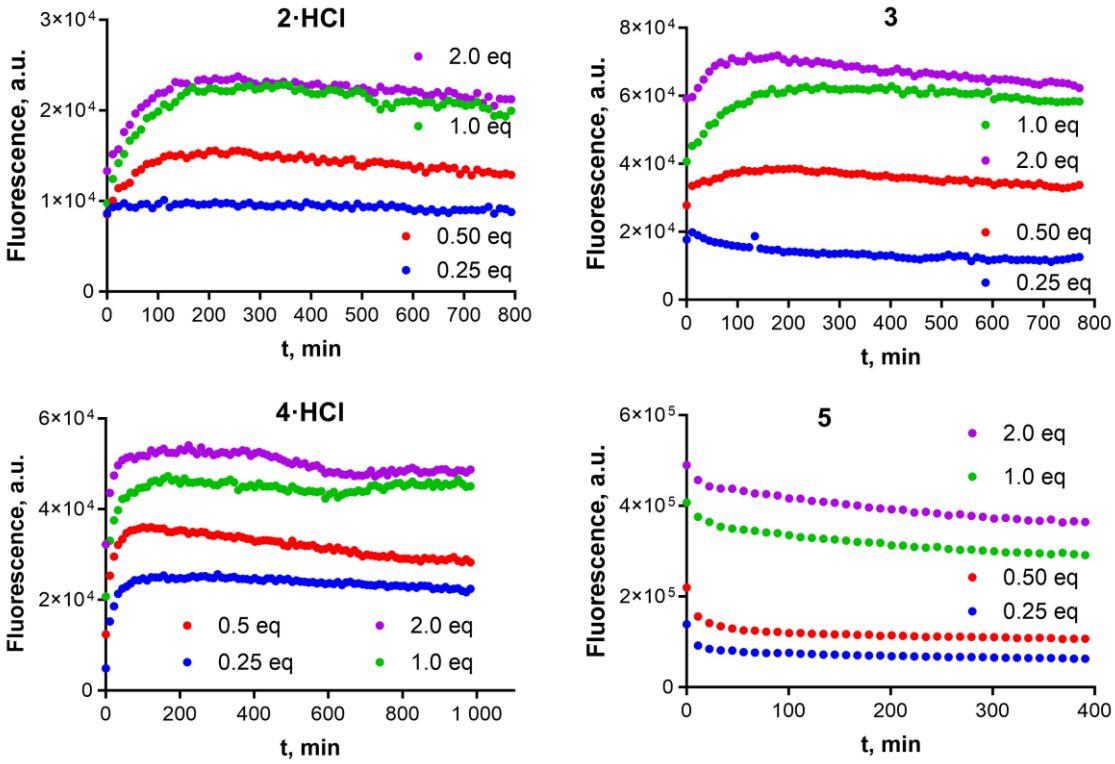


Figure S5. Monitoring of fluorescence intensity in fluorescence maximum of BODIPYs **2·HCl**, **3**, **4·HCl** and **5** (c = 1 μM) mixture upon addition into solution of CB[7] solution in PBS with 20% of MeCN. Amount of CB[7] is expressed as equivalents per one Ad unit. Decrease of intensity in case of **5** is due to slow adsorption of this bisquaternary compound onto the glass surface of the cuvette.

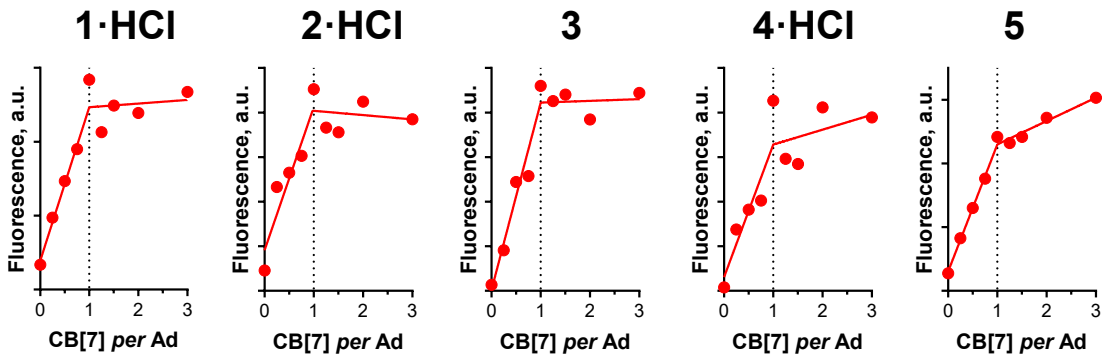


Figure S6. Changes in fluorescence intensity (dots) of BODIPYs (c = 1 μM) in PBS containing 20% MeCN upon addition of CB[7]. Corresponding lines represent segmental linear regression (calculated by Prism 10 for Windows, GraphPad).

Assessment of biological activity

Cell culture

All cell-based experiments were performer on HeLa cell line (human cervical carcinoma) obtained from American Type Culture Collection (USA). Cell culture was maintained in Dulbecco's Modified Eagle

Medium (without phenol red; Capricorn, Germany) supplemented with 10% foetal bovine serum (Capricorn), 1% Penicillin-Streptomycin solution (stock solution of 10,000 units penicillin and 10 mg streptomycin/mL; Sigma-Aldrich, Germany), 10 mM HEPES buffer (Lonza, USA), and 4 mM stable glutamine (L-Alanyl-L-Glutamine; Capricorn) under humidified atmosphere of 5% CO_2 at 37°C in an incubator. Cells were sub-cultured every 3-4 days.

Cytotoxicity experiments

Cytotoxicity experiments (photodynamic activity and toxicity without irradiation – further referred as "phototoxicity" and "dark toxicity", respectively) were performed on 96 well plates (TTP, Switzerland) with seeding concentration of 7,500 cells/well. Cells were left to grow for 24 h prior addition of PSs. Stock solution of studied BODIPYs were prepared in DMSO (10 mM) and CB[7] in ultra-pure water (1 mM; water was used in CB[7]-free experiments as well) and mixed prior each experiment in an appropriate ratio (1 equivalent of CB[7] *per* one Ad unit in the molecule) and further diluted to working concentrations in cell culture medium (0.03 µM - 30 µM). Incubation time with PSs was 12 or 24 h for phototoxicity or dark toxicity, respectively. Cells in phototoxicity experiments were subsequently washed and irradiated using Xe lamp (λ > 570 nm, 12.4 mW/cm^{-2}, 15 min, 11.2 J/cm^{-2}) and further incubated for 24 h. Viability was assessed with neutral red (NR) uptake assay. Stock solution of NR (Sigma-Aldrich) was diluted in cell culture medium to a final concentration 30 µg/ml and incubated with cells for 2 h. Cells were fixed (1% $CaCl_2$ in 0,5% formaldehyde), washed twice with PBS and lysed (1% glacial acetic acid in 50% ethanol). Absorbance in each well was measured using Tecan Infinite M200 (Tecan Group Ltd., Switzerland). Each group is expressed as a percentage of untreated control (100 %) after subtraction of positive control (cells treated with lethal concentration of hydrogen peroxide). Each experiment was performed at least three times in duplicate. EC_{50} values were calculated using GraphPad Prism 9.2.0 (GraphPad Software, USA) and statistical significance (p value) between EC_{50} values with/without CB[7] was determined for each compound with unpaired t-test with Holm-Sidak multiple comparison method.

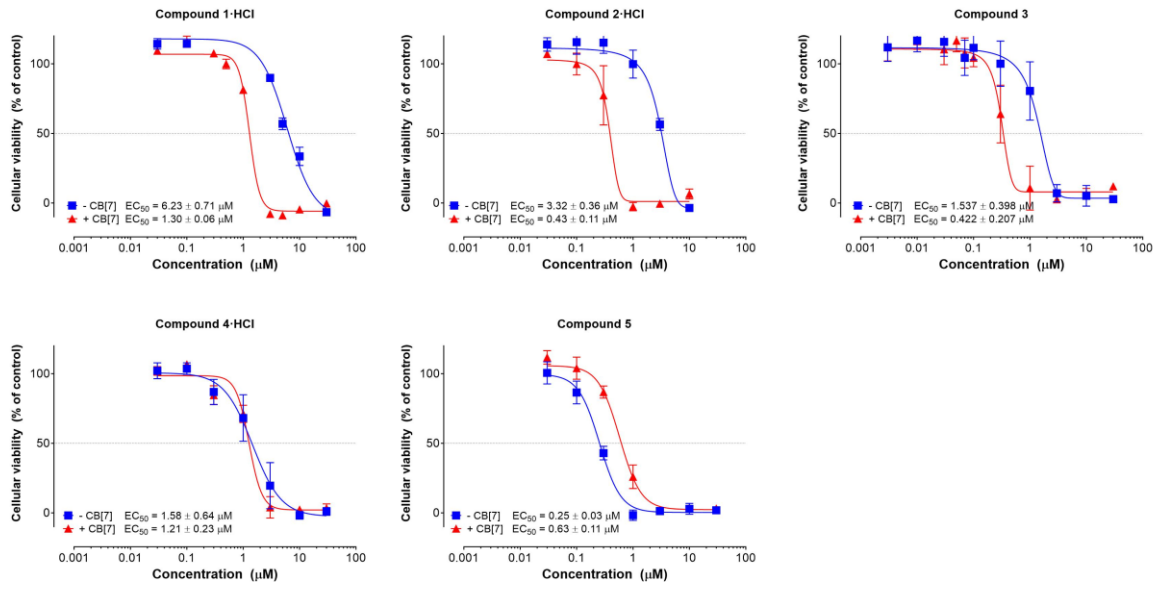


Figure S7. Photodynamic activity of studied BODIPYs against HeLa cells with (red triangle) or without (blue square) 1 equiv of CB[7] *per* one Ad moiety. Irradiation conditions: λ > 570 nm, 12.4 mW cm^{-2}, 15 min, 11.2 J cm^{-2}.

Subcellular localization and cellular uptake

Subcellular localisation was assessed on 3 cm glass-bottom Petri dishes suitable for confocal microscopy (WillCo Wells B.V., Netherlands). HeLa cells were incubated with 0.1 µM PSs for 12 h prior staining: 0.4 µM MitoTracker Green FM (Invitrogen, ThermoFisher Scientific, USA) and 0.4 µM LysoTracker Blue DND-22 (Invitrogen) for 20 min. Signal from lysosomal probe was very weak for **4·HCl** + CB[7] and **5** + CB[7]; therefore different staining was employed for further confirmation of the endolysosomal compartment as a subcellular localization: HeLa cells were co-incubated with 0.1 µM PSs and 0.5 mg/mL FITC-dextran conjugate (average MW 4,000 with FITC:Glucose = 1:250; Sigma-Aldrich) for 12 h. After both staining protocols: cells were washed twice with PBS, and fresh cell culture medium was added to the cells. Photomicrographs were acquired with NIS Elements AR 4.20.01 software (Laboratory Imaging s.r.o., Czech Republic) and Nikon Ti-E (Nikon, Japan) fluorescence microscope equipped with Nikon CFI Plan Apochromat Lambda 100× Oil objective lens, Andor Zyla 5.5 cooled sCMOS camera (Andor Technology, Oxford Instruments, Great Britain) and pE-300 CoolLED fluorescence source (CoolLED Ltd., Great Britain). FITC, Cy5 and DAPI filter cubes were used for acquisition of the signal from green (MitoTracker, FITC-dextran), deep-red (PS) and blue (LysoTracker) fluorescence channels, respectively.

Similar approach was used also for the determination of the changes in cellular uptake of BODIPYs with/without CB[7]. HeLa cells were incubated with 0.1 µM PSs for 12 h, subsequently washed twice with PBS and fresh cell culture medium was added. Images were taken with above-mentioned equipment only in Cy5 channel and using Nikon CFI Plan Apochromat Lambda 40× dry objective lens instead of 100× Oil objective lens to have larger field of view. The automatic exposure function in NIS Elements software was used to determine the optimal exposure time for image acquisition (n = 13 for each sample). Data are presented as reciprocal values of the exposure time.

In another approach, cells were seeded on 6 cm Petri dishes (TPP) and incubated with 4 µM PSs with/without CB[7] for 12 h. Cells were washed twice with PBS and scraped with cell scrapers (TPP) in PBS and centrifuged. Supernatant was removed and dry pellets were resuspended in 10% Triton X-100 and stored in -20°C until the fluorescence was measured using FS5 Spectrofluorometer. Integrated areas under the emission spectra of individual samples were then compared. Experiment was repeated twice.

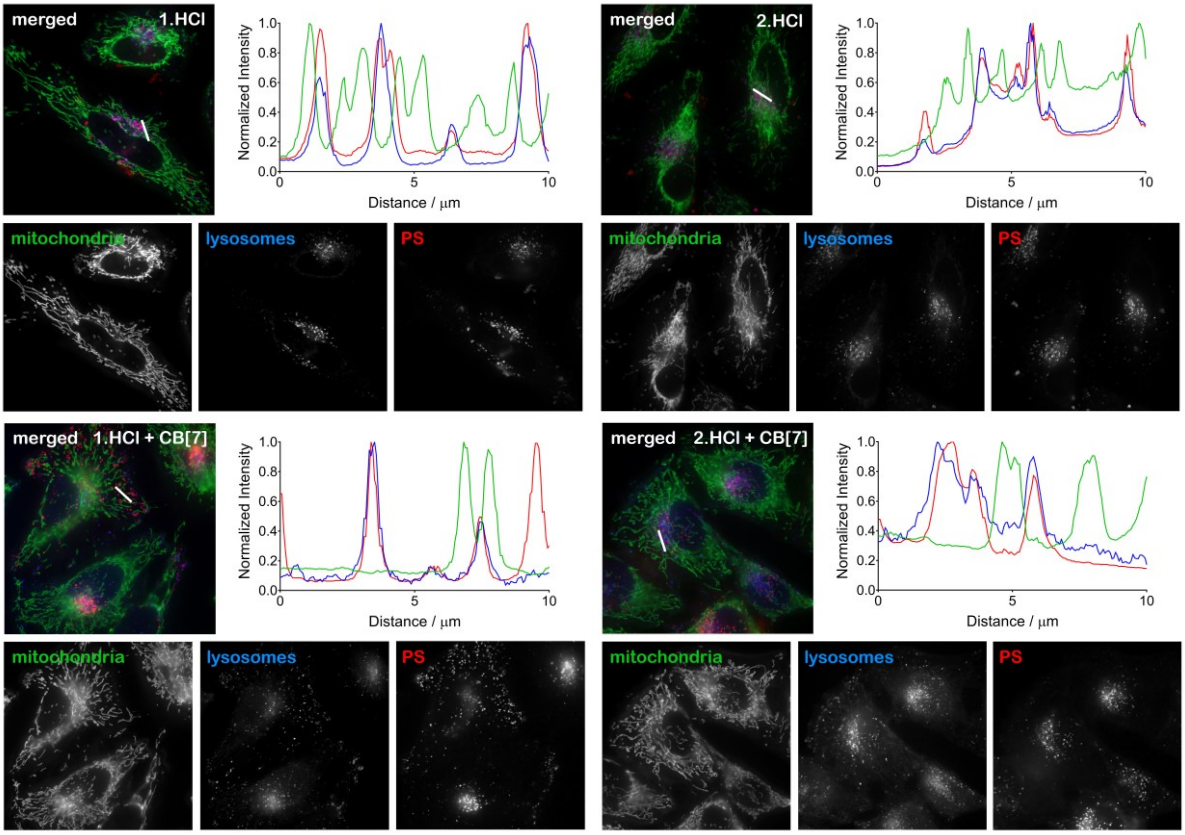


Figure S8. Subcellular localization of **1·HCl** and **2·HCl** (red) in HeLa cells determined by live-cell fluorescence imaging. HeLa cells were incubated with BODIPY or with BODIPY with 1 equiv of CB[7] *per* Ad unit and stained for lysosomes (blue) and mitochondria (green). The bars in the merge images indicate the measured part of an image for the intensity profile and represent 10 μm.

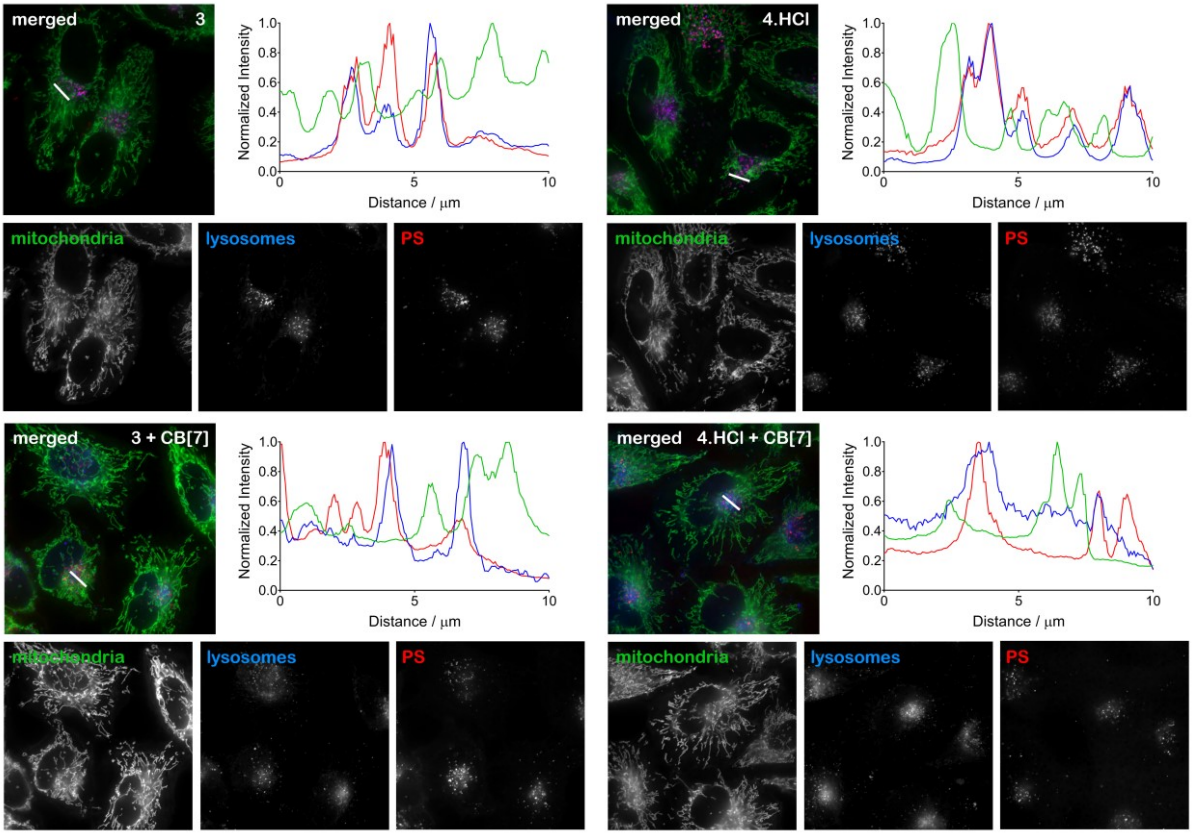


Figure S9. Subcellular localization of **3** and **4·HCl** (red) in HeLa cells determined by live-cell fluorescence imaging. HeLa cells were incubated with BODIPY or with BODIPY with 1 equiv of CB[7] *per* Ad unit and stained for lysosomes (blue) and mitochondria (green). The bars in the merge images indicate the measured part of an image for the intensity profile and represent 10 µm.

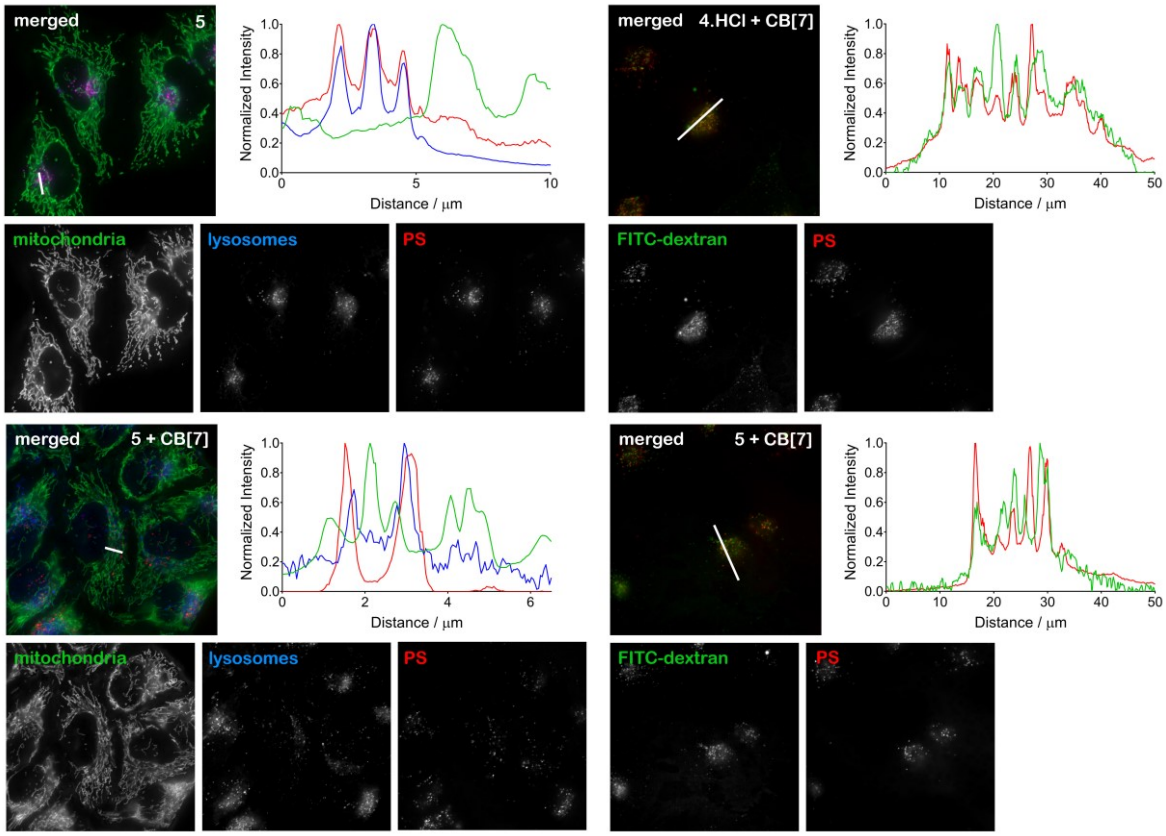


Figure S10. Subcellular localization of **5** (left figures, red) in HeLa cells determined by live-cell fluorescence imaging. HeLa cells were incubated with BODIPY or with BODIPY with 1 equiv of CB[7] *per* Ad unit and stained for lysosomes (blue) and mitochondria (green). The bars in the merge images indicate the measured part of an image for the intensity profile. The right figures shown subcellular localization of **4·HCl** and **5** (red) both with CB[7] determined with the use of alternative probe for vesicles of endolysosomal pathway (FITC-dextran, green).

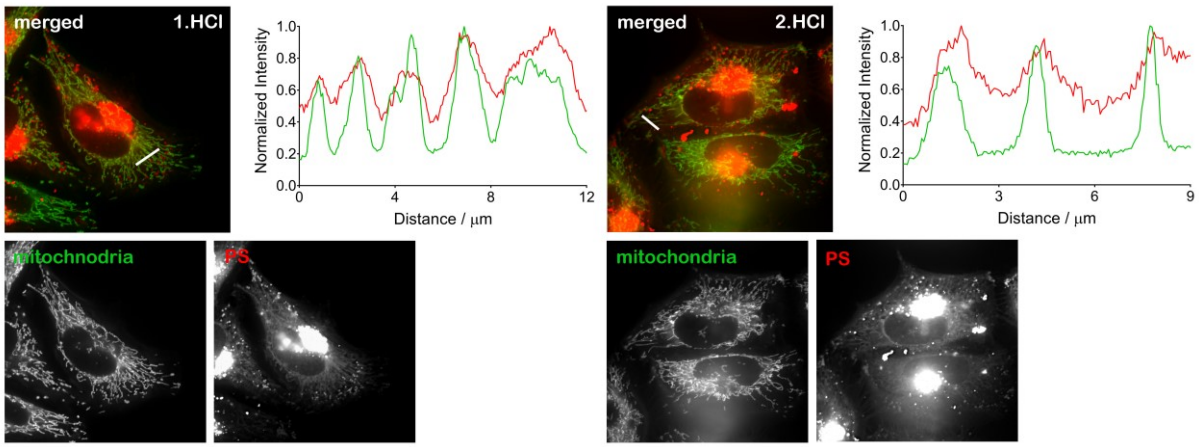


Figure S11. Subcellular localization of **1·HCl** and **2·HCl** (red) in HeLa cells determined by live-cell fluorescence imaging. HeLa cells were incubated with BODIPY and stained for mitochondria (green). The bars in the merge images indicate the measured part of an image for the intensity profile.

NMR spectra

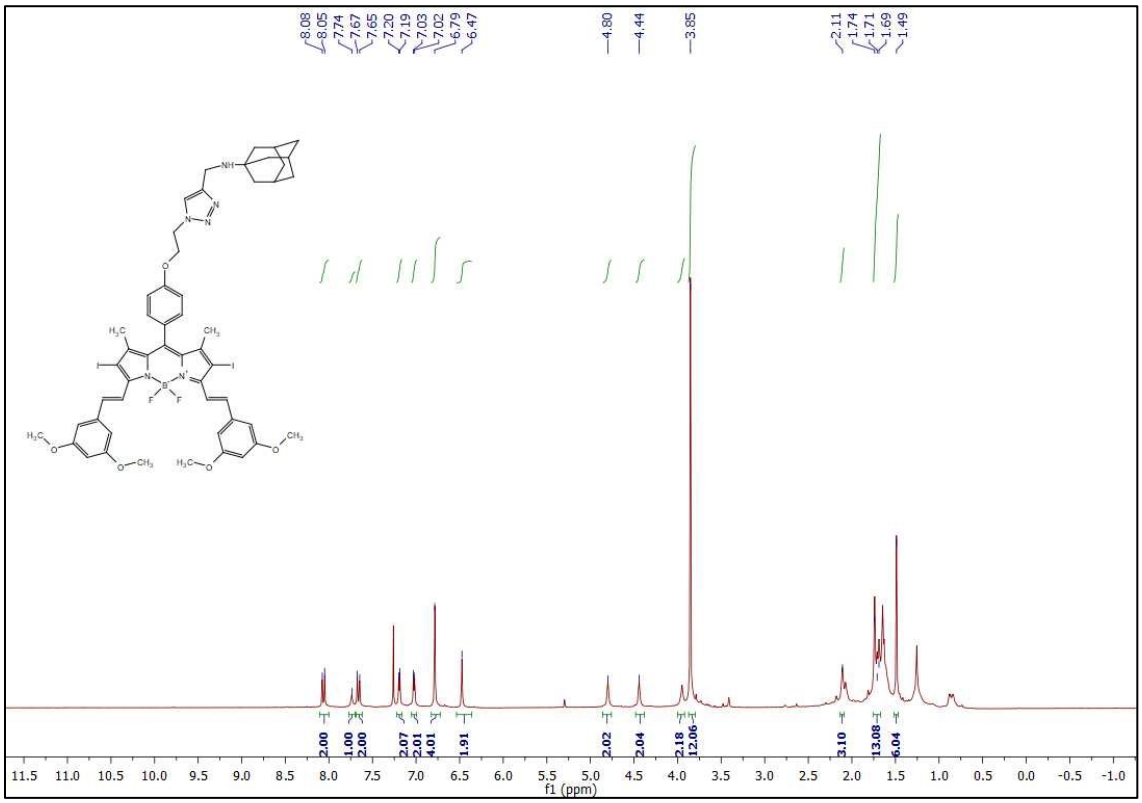


Figure S12: ^{1}H-NMR spectrum of **1** in $CDCl_3$.

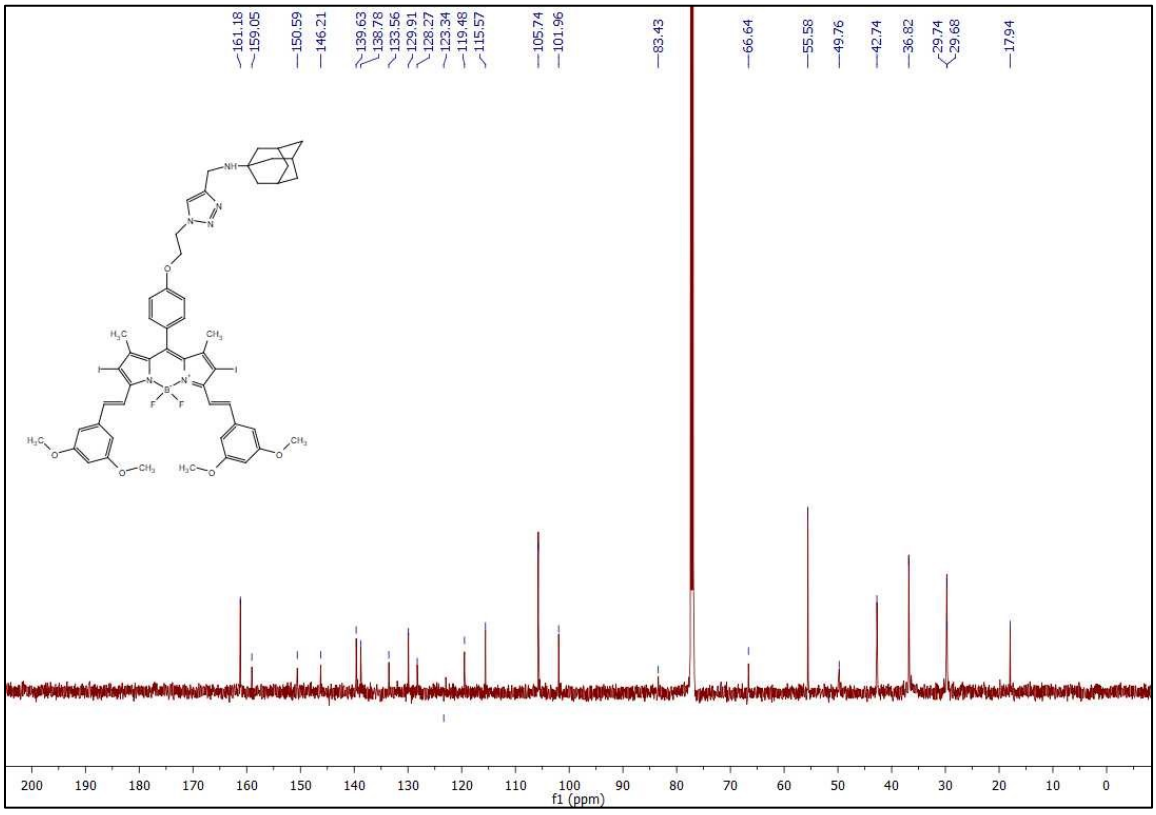


Figure S13: ^{13}C-NMR spectrum of **1** in $CDCl_3$.

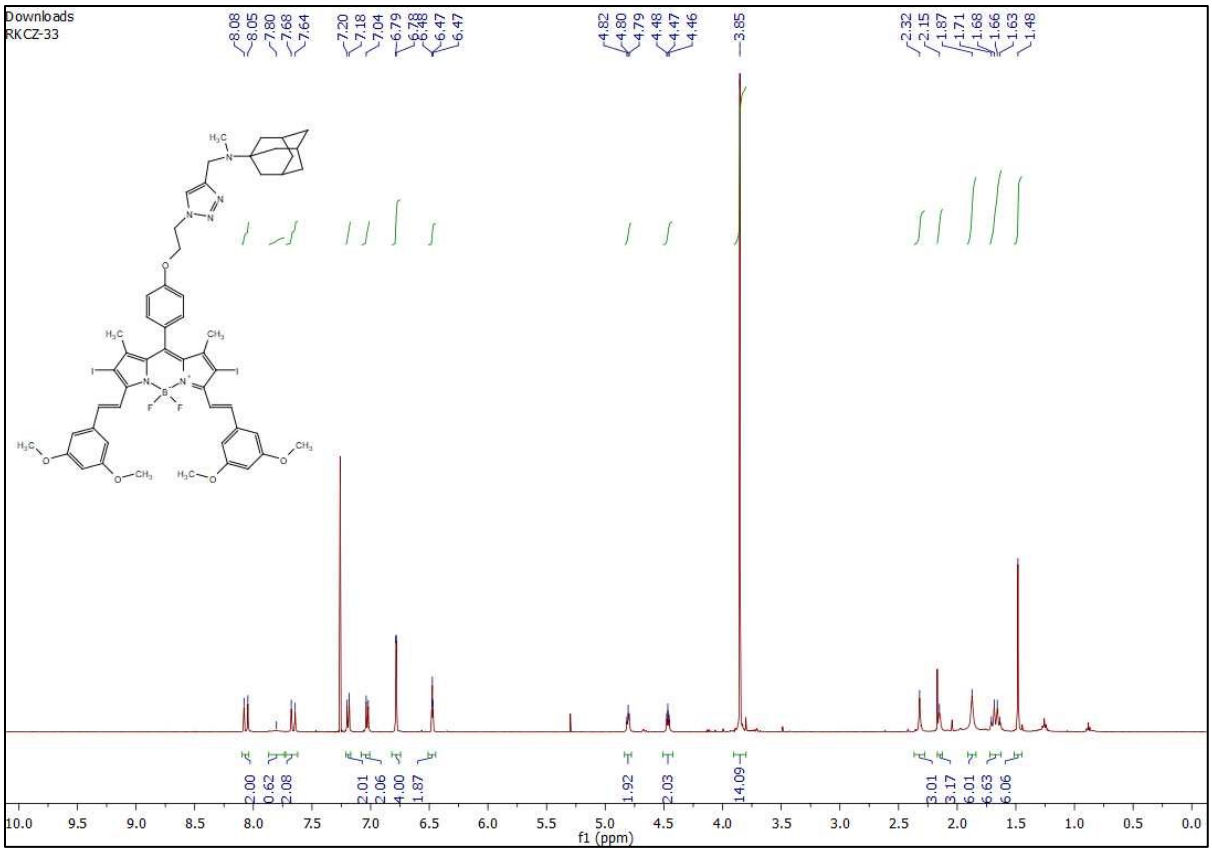


Figure S14: ^{1}H-NMR spectrum of **2** in $CDCl_3$.

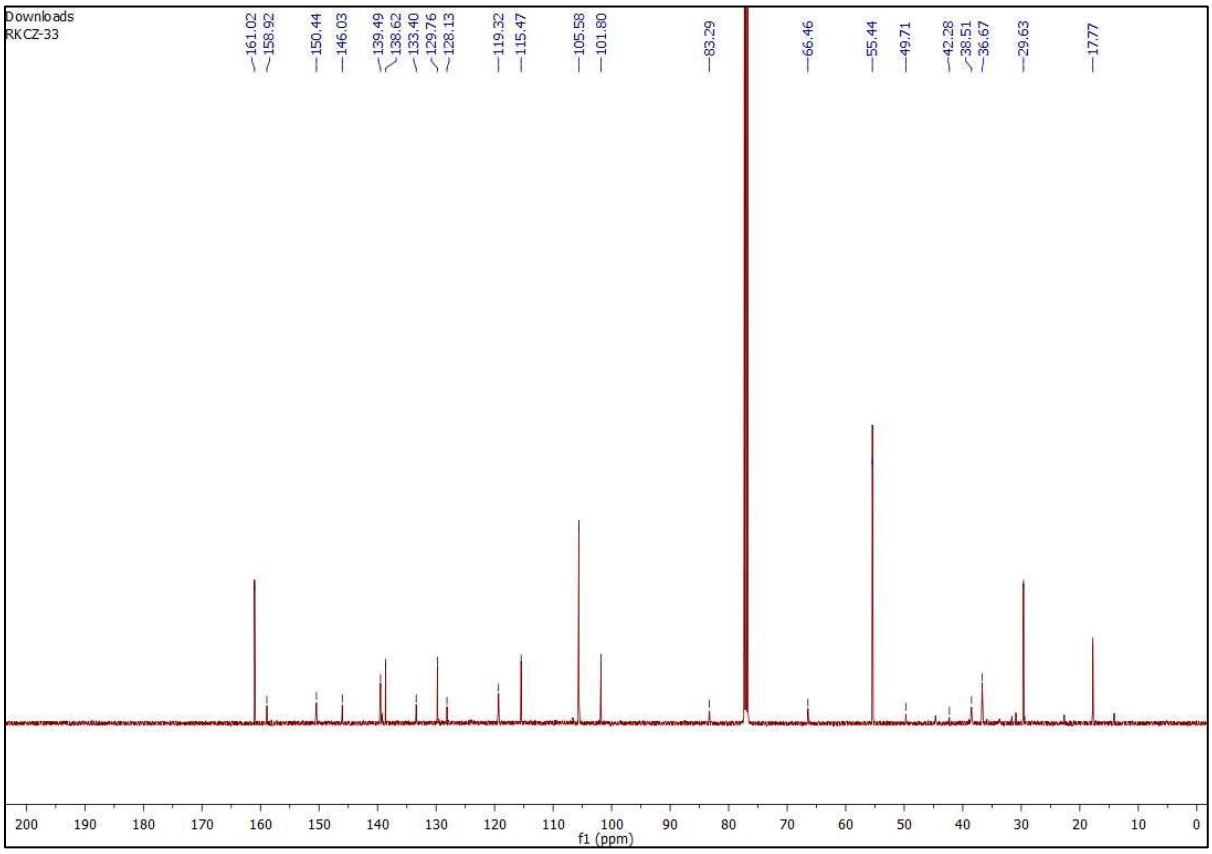


Figure S15: ^{13}C-NMR spectrum of **2** in $CDCl_3$.

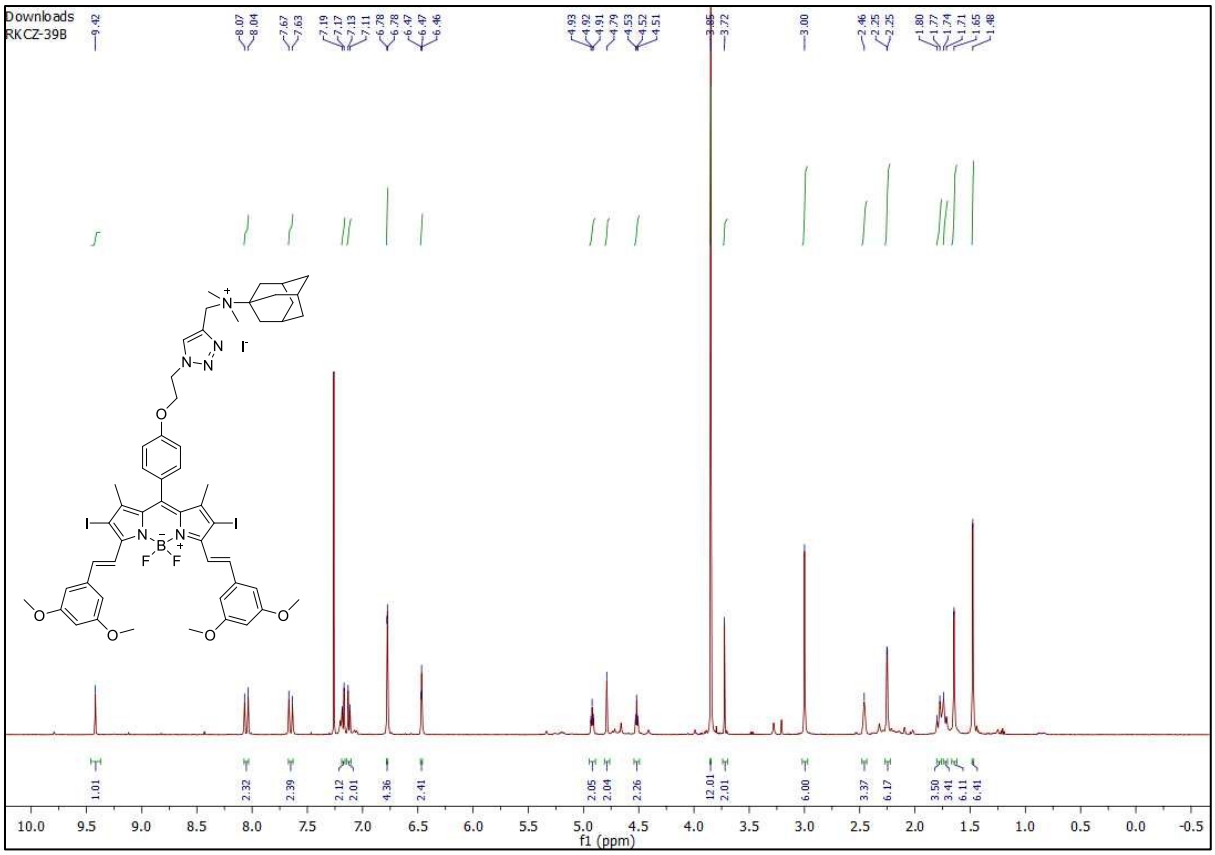


Figure S16: ^{1}H-NMR spectrum of **3** in $CDCl_3$.

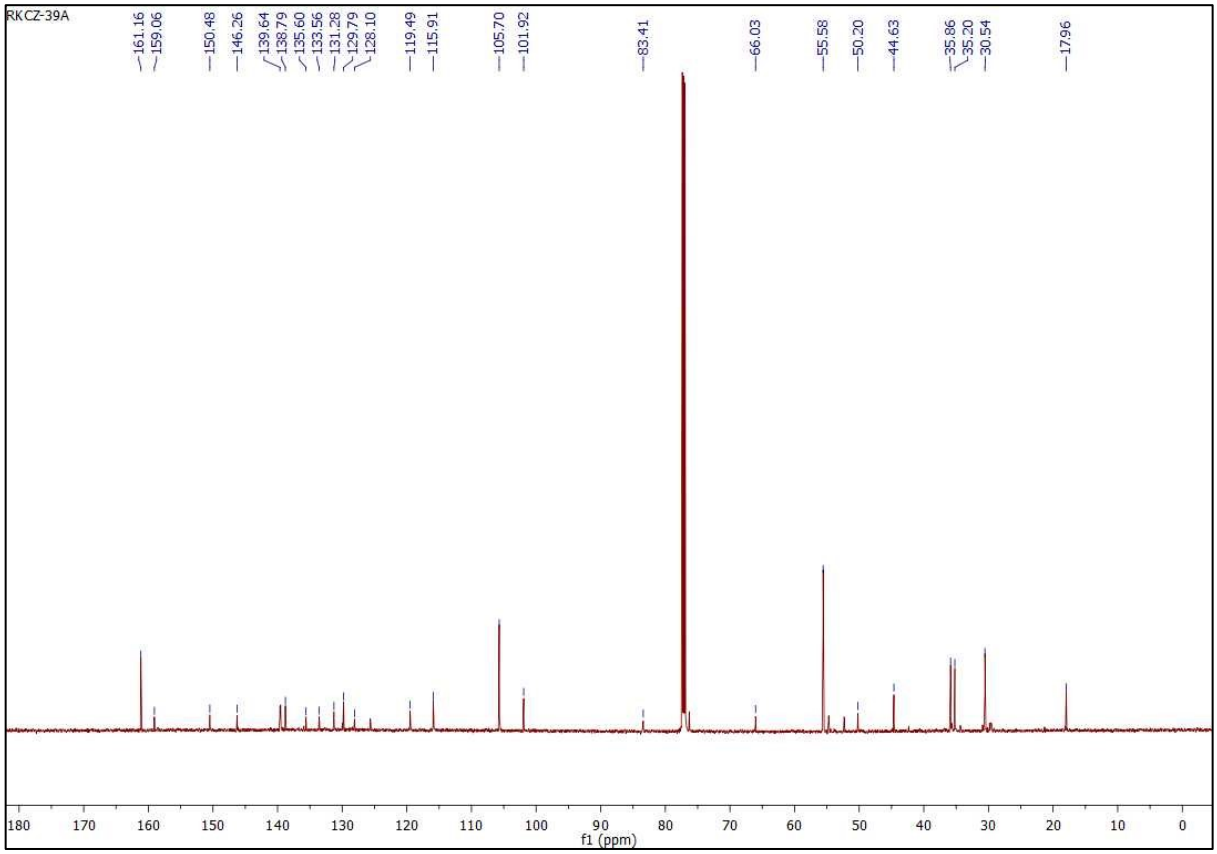


Figure S17: ^{13}C-NMR spectrum of **3** in $CDCl_3$.

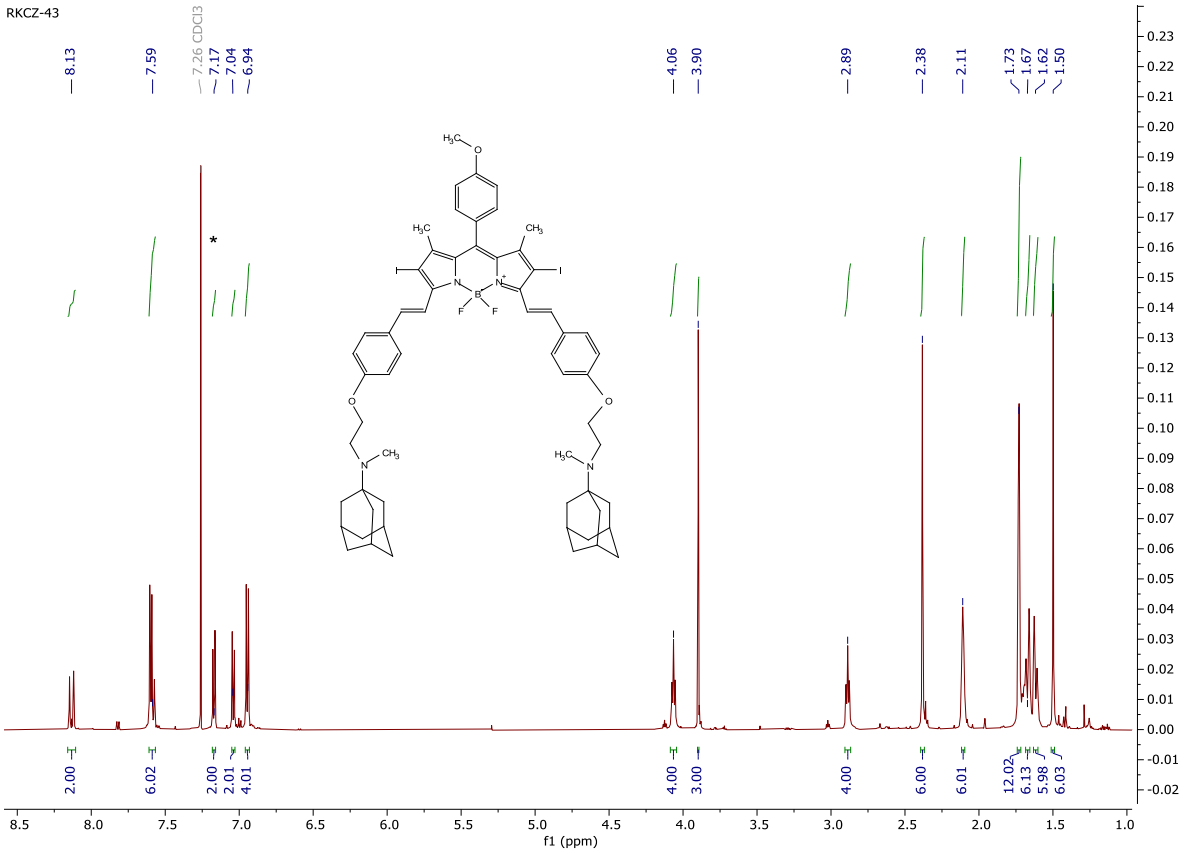


Figure S18: ^{1}H-NMR spectrum of **4** in $CDCl_3$.

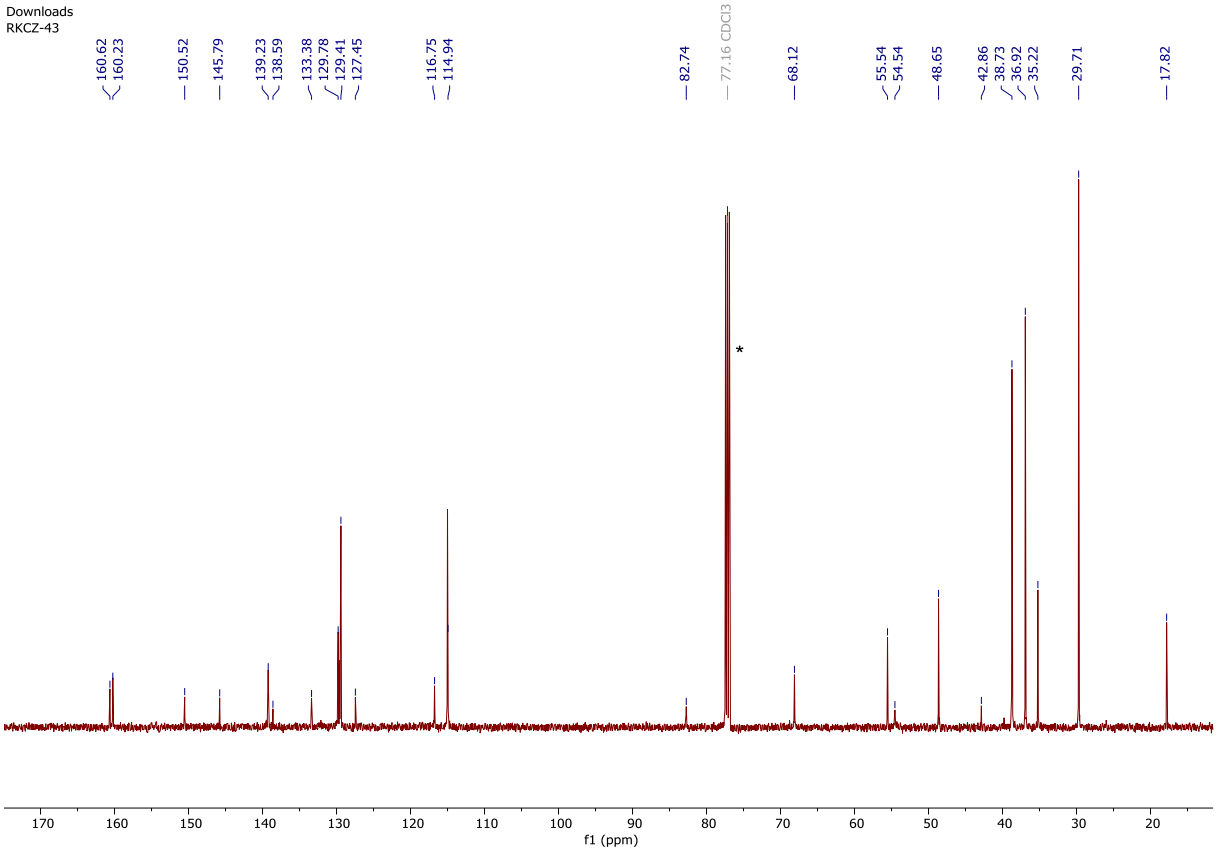


Figure S19: ^{13}C-NMR spectrum of **4** in $CDCl_3$.

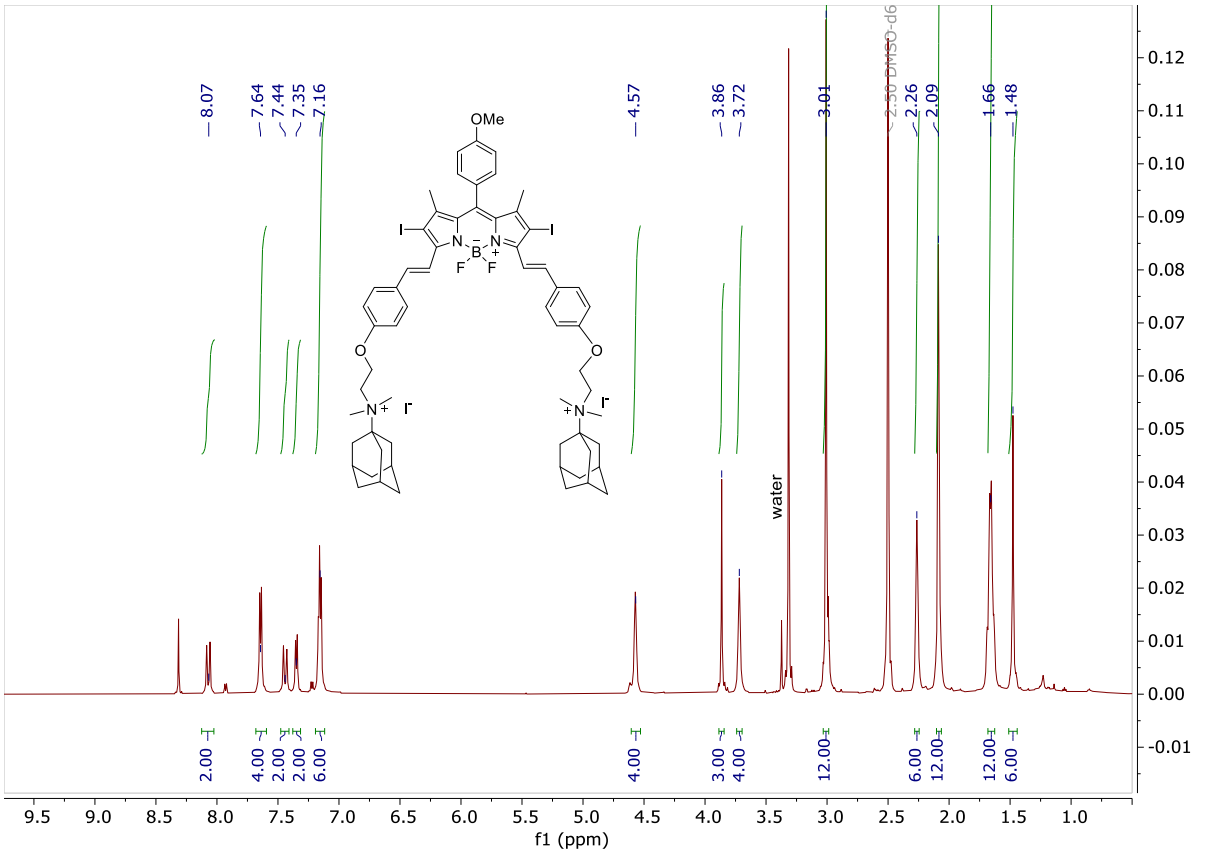


Figure S20: ^{1}H-NMR spectrum of **5** in DMSO-d_6.

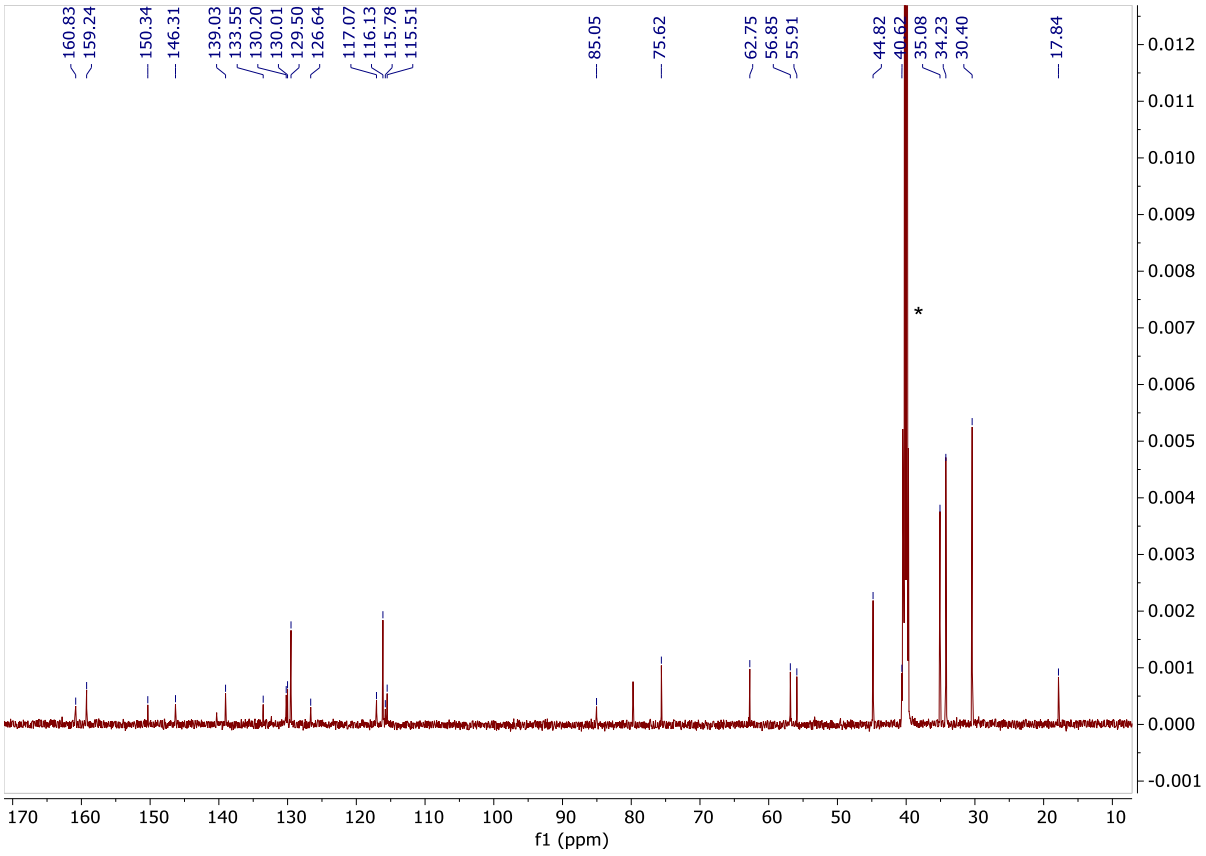


Figure S21: ^{13}C-NMR spectrum of **5** in DMSO-d_6.

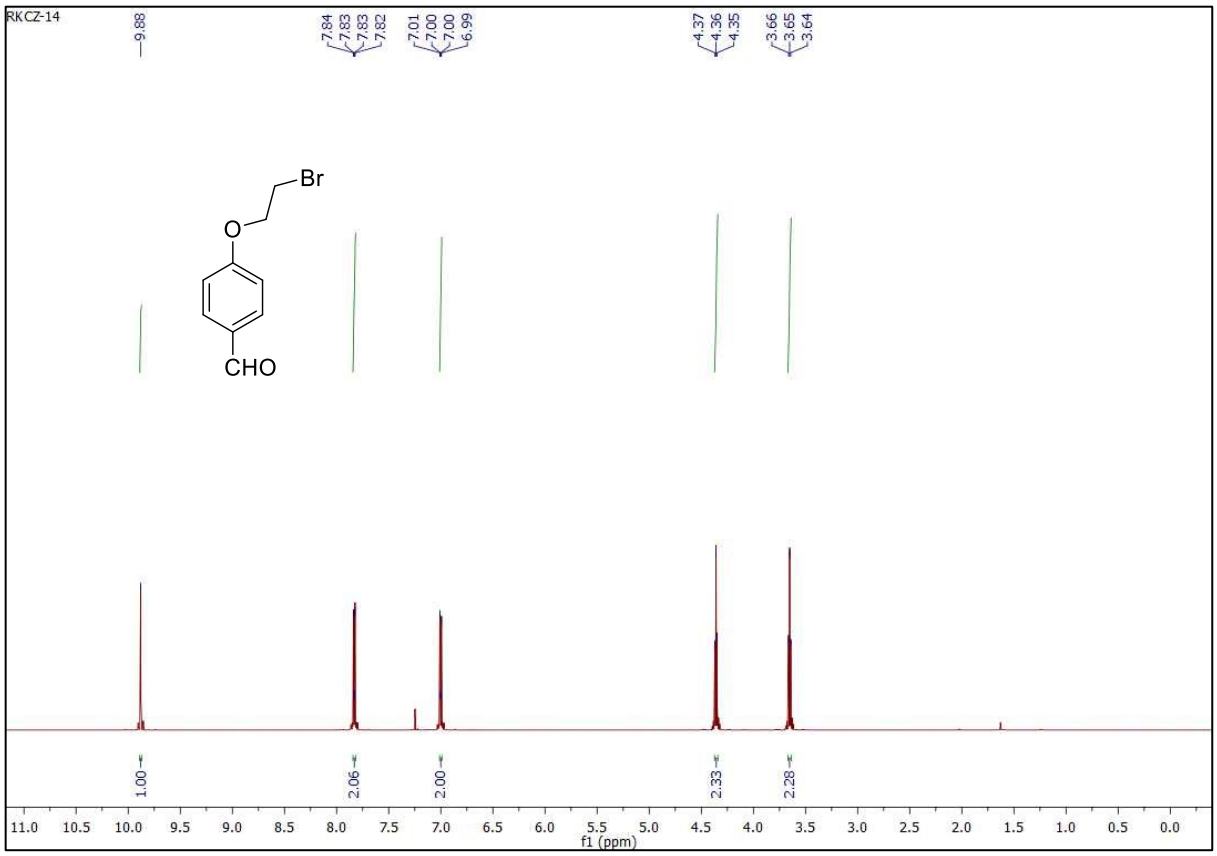


Figure S22: ^{1}H-NMR spectrum of **6** in $CDCl_3$.

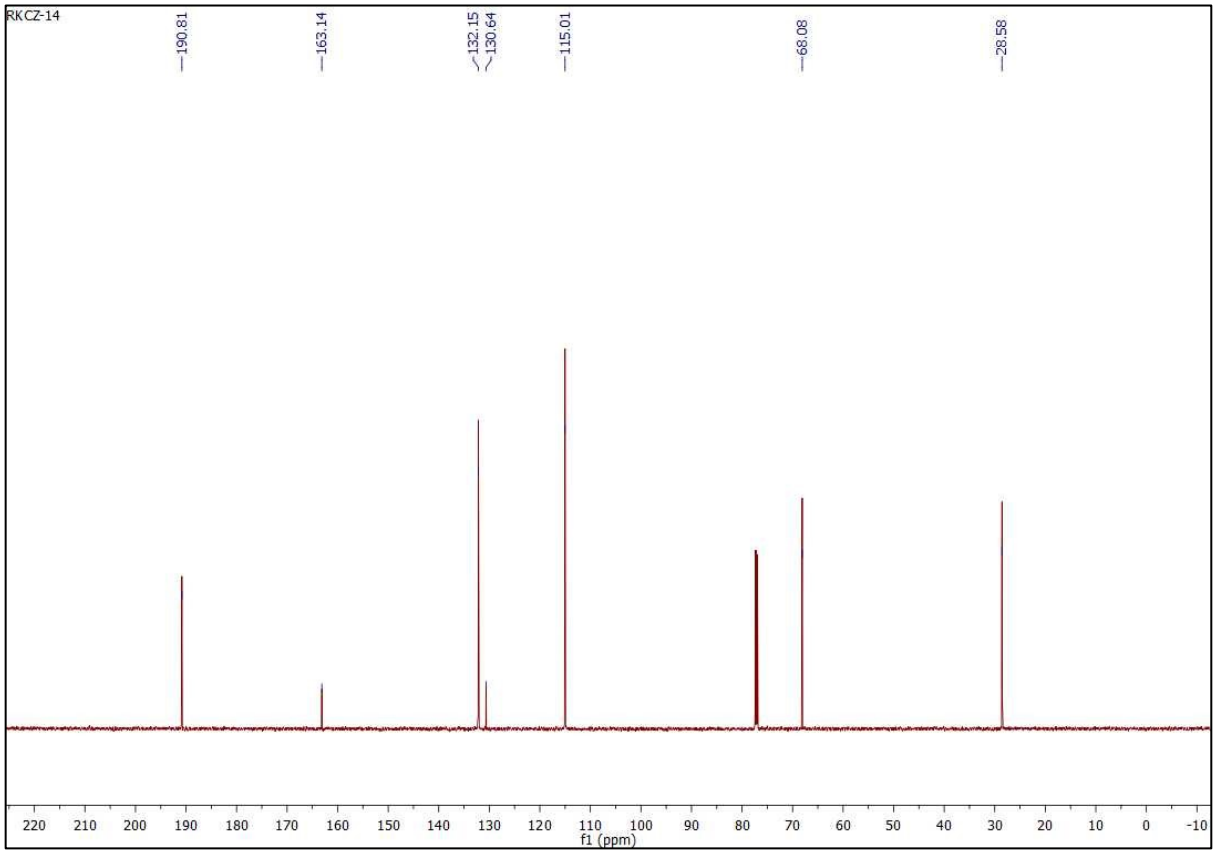


Figure S23: ^{13}C-NMR spectrum of **6** in $CDCl_3$.

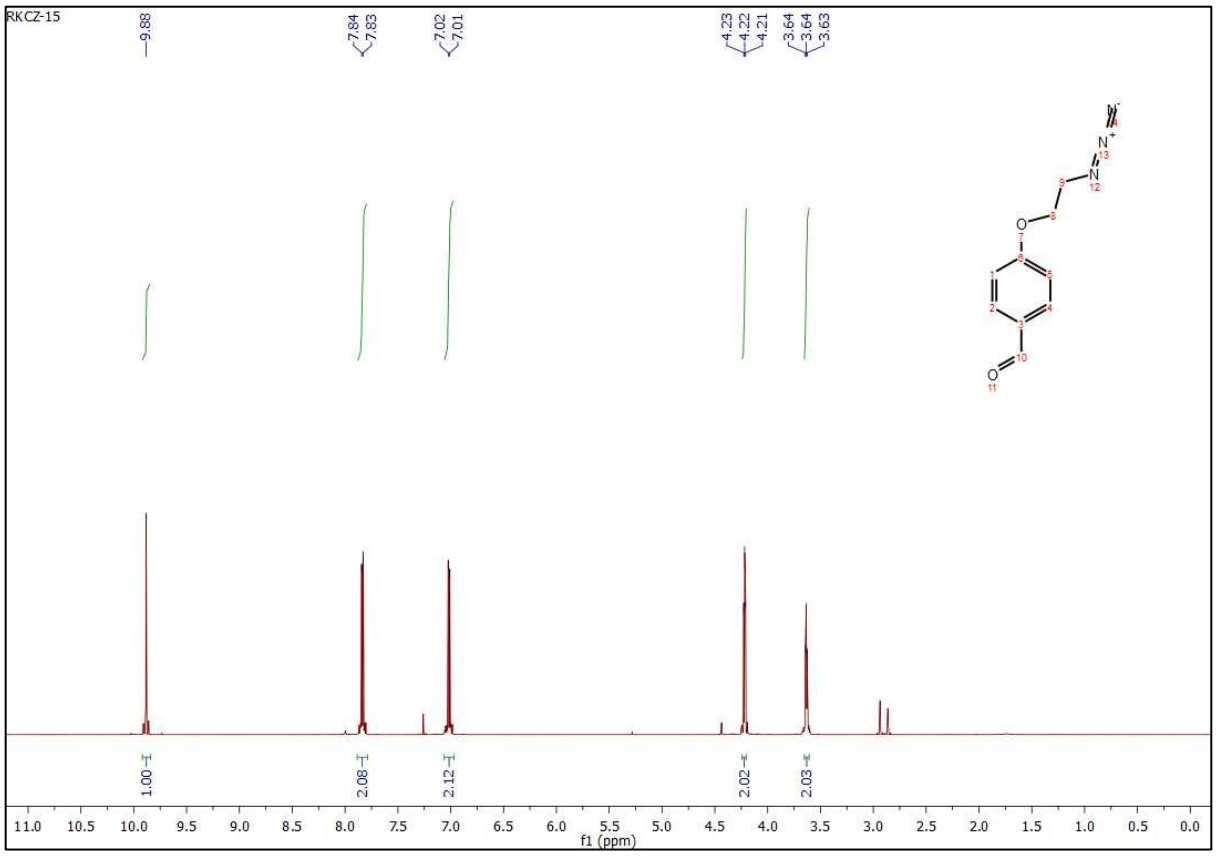


Figure S24: ^{1}H-NMR spectrum of **7** in $CDCl_3$.

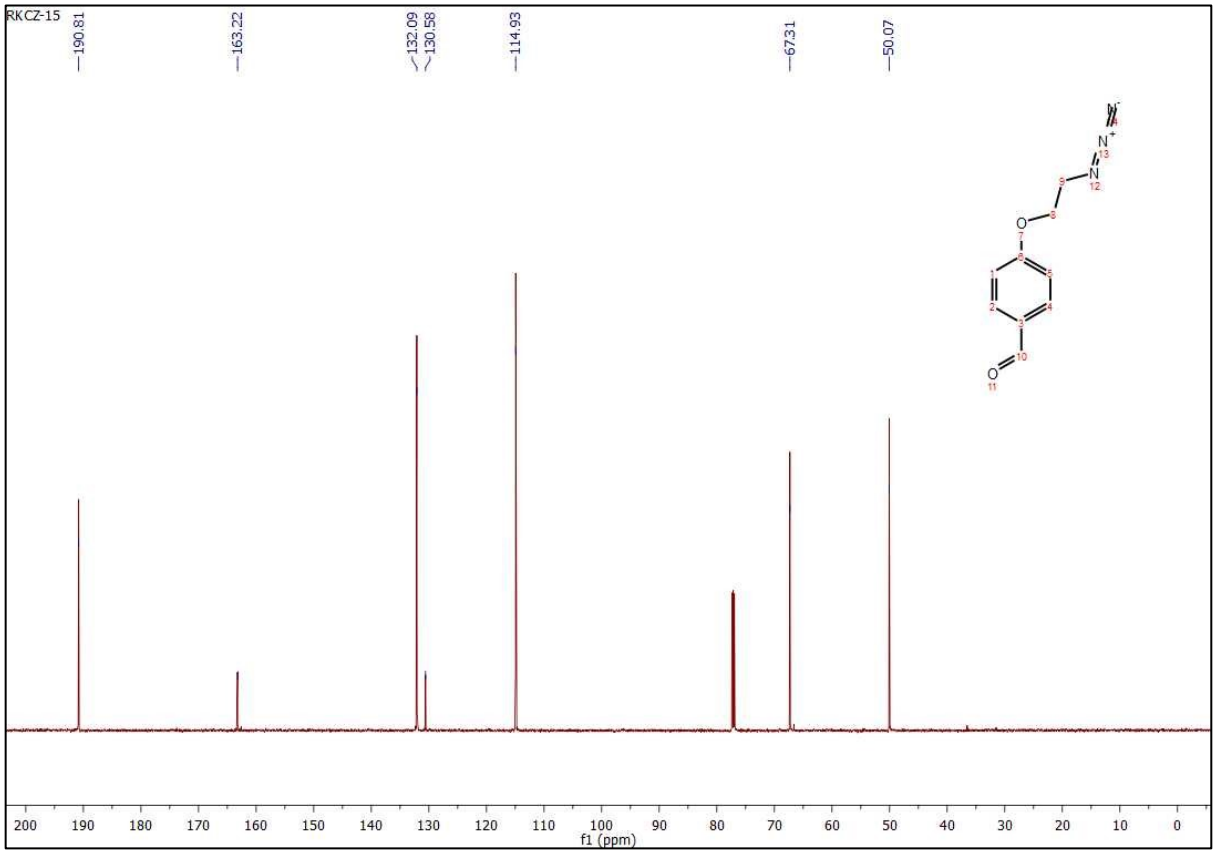


Figure S25: ^{13}C-NMR spectrum of **7** in $CDCl_3$.

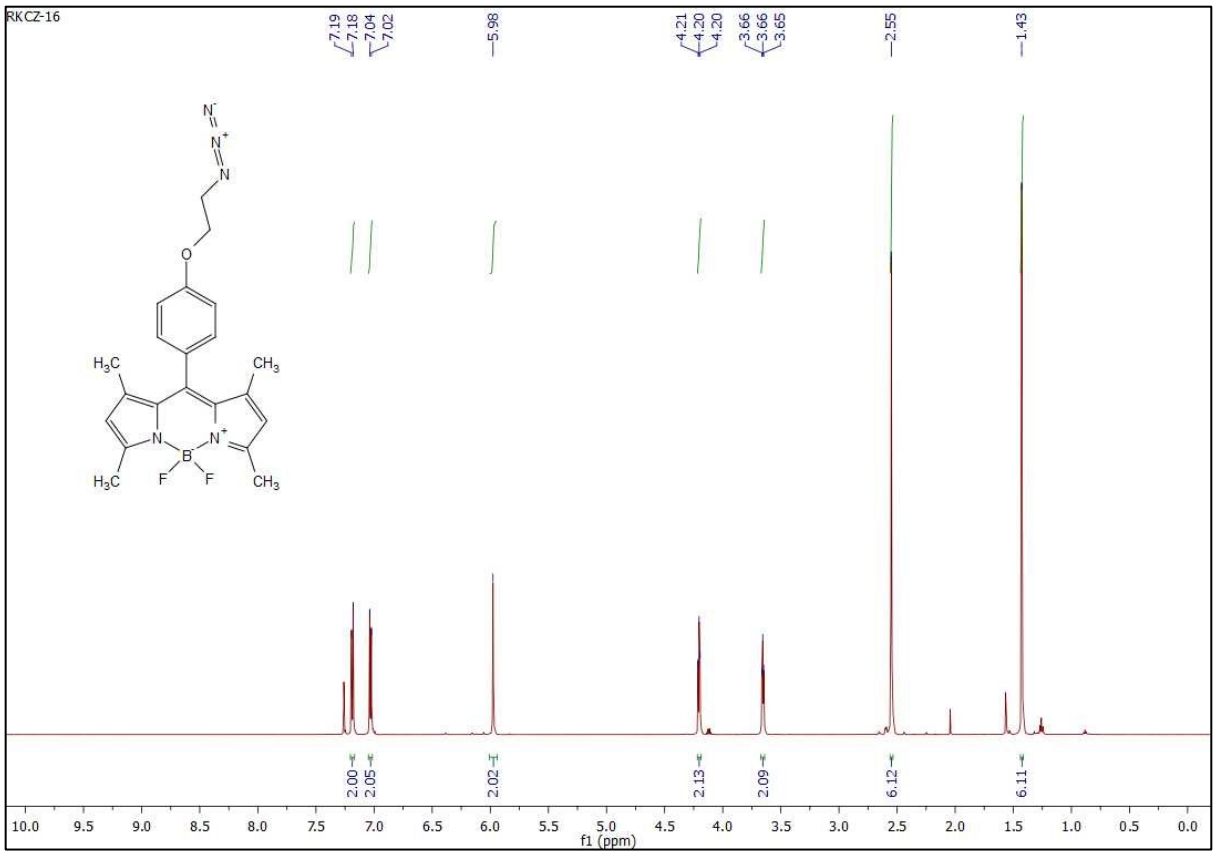


Figure S26: ^{1}H-NMR spectrum of **8** in $CDCl_3$.

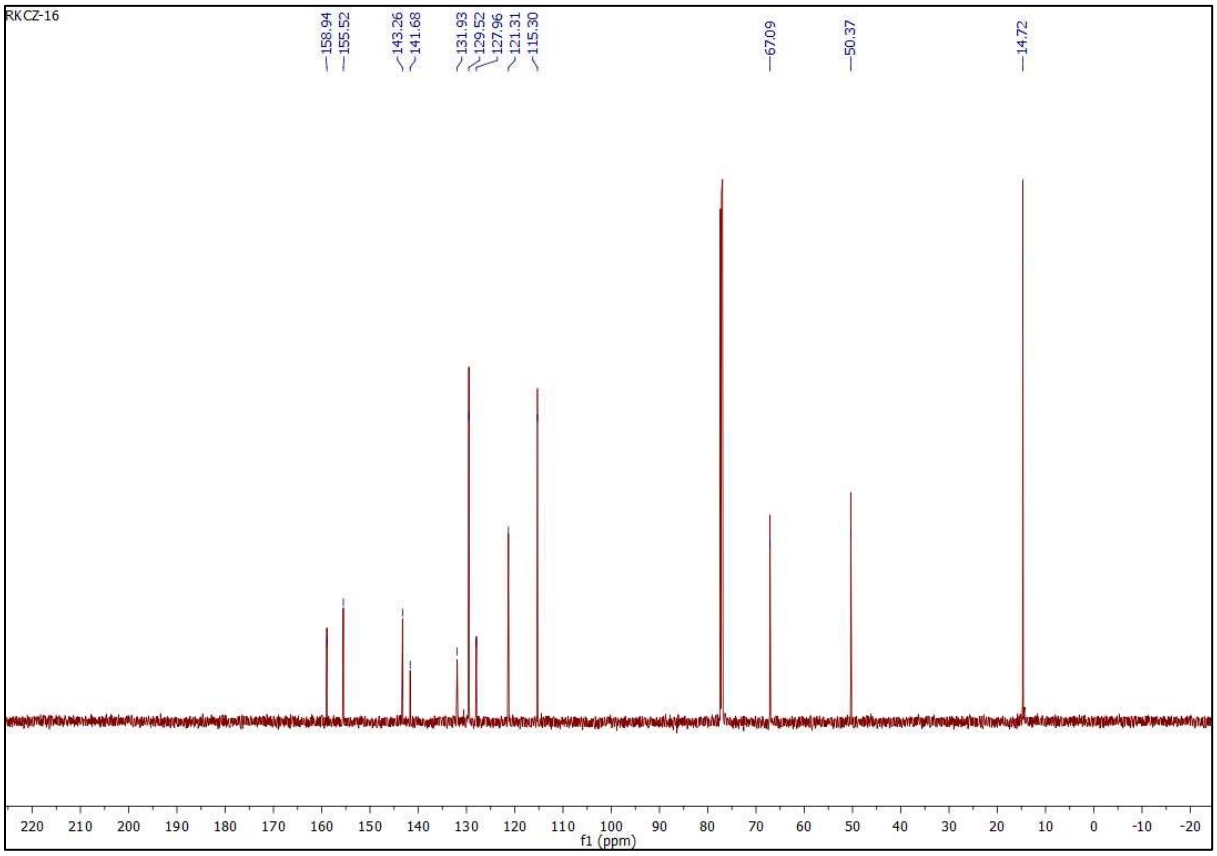


Figure S27: ^{13}C-NMR spectrum of **8** in $CDCl_3$.

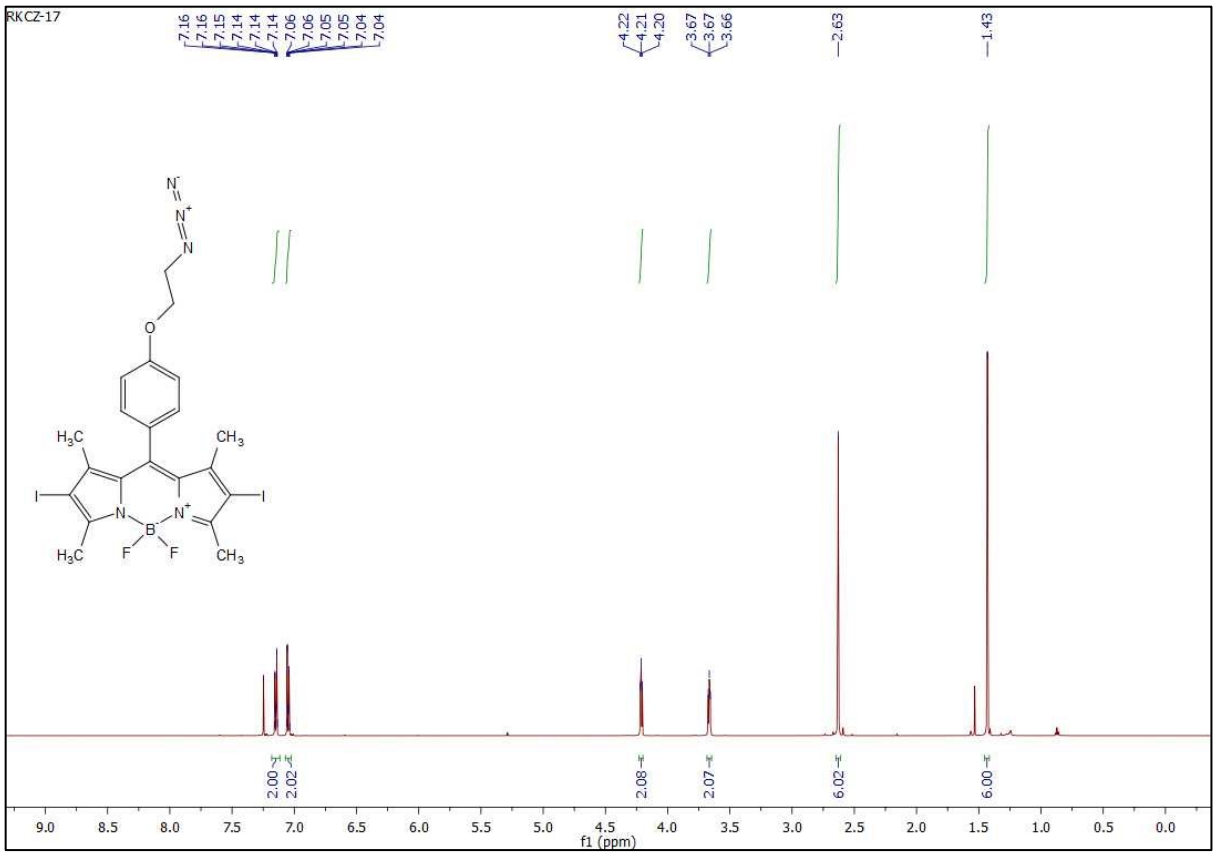


Figure S28: ^{1}H-NMR spectrum of **9** in $CDCl_3$.

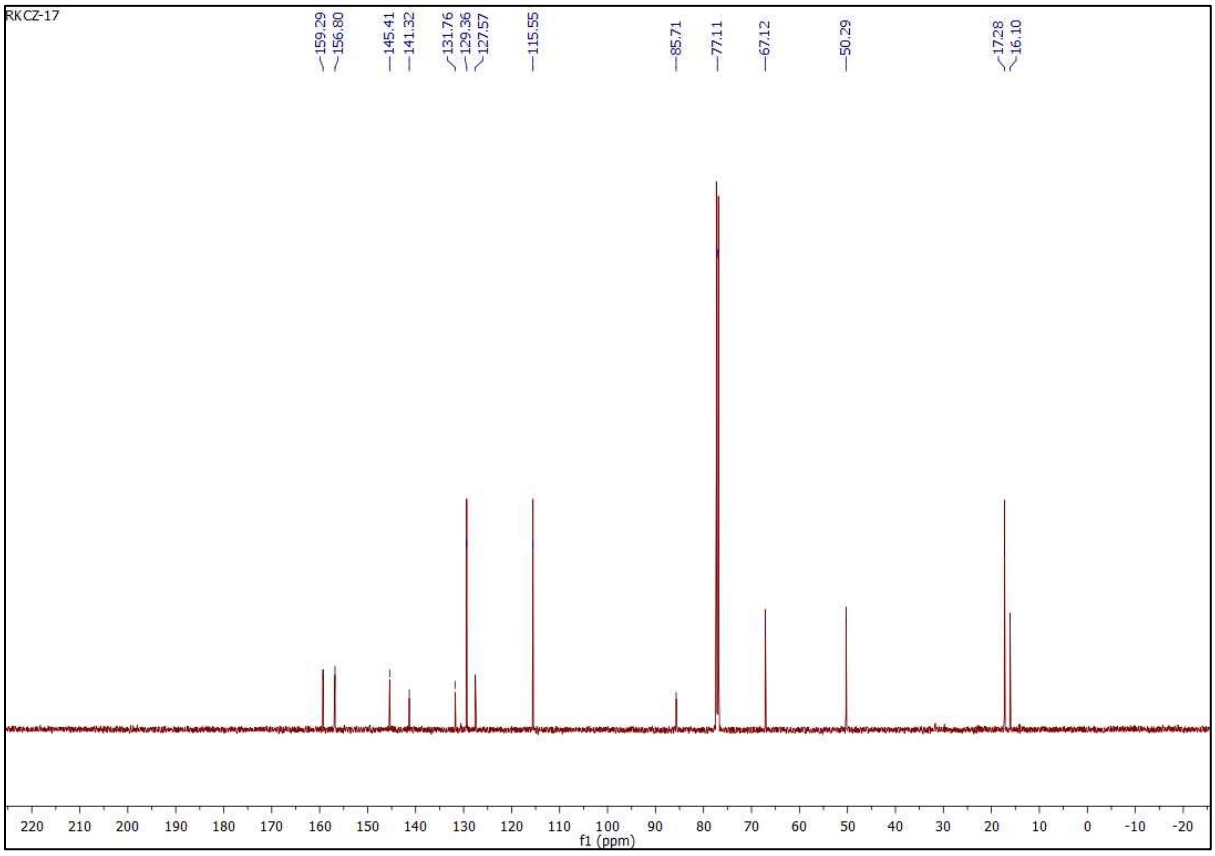


Figure S29: ^{13}C-NMR spectrum of **9** in $CDCl_3$.

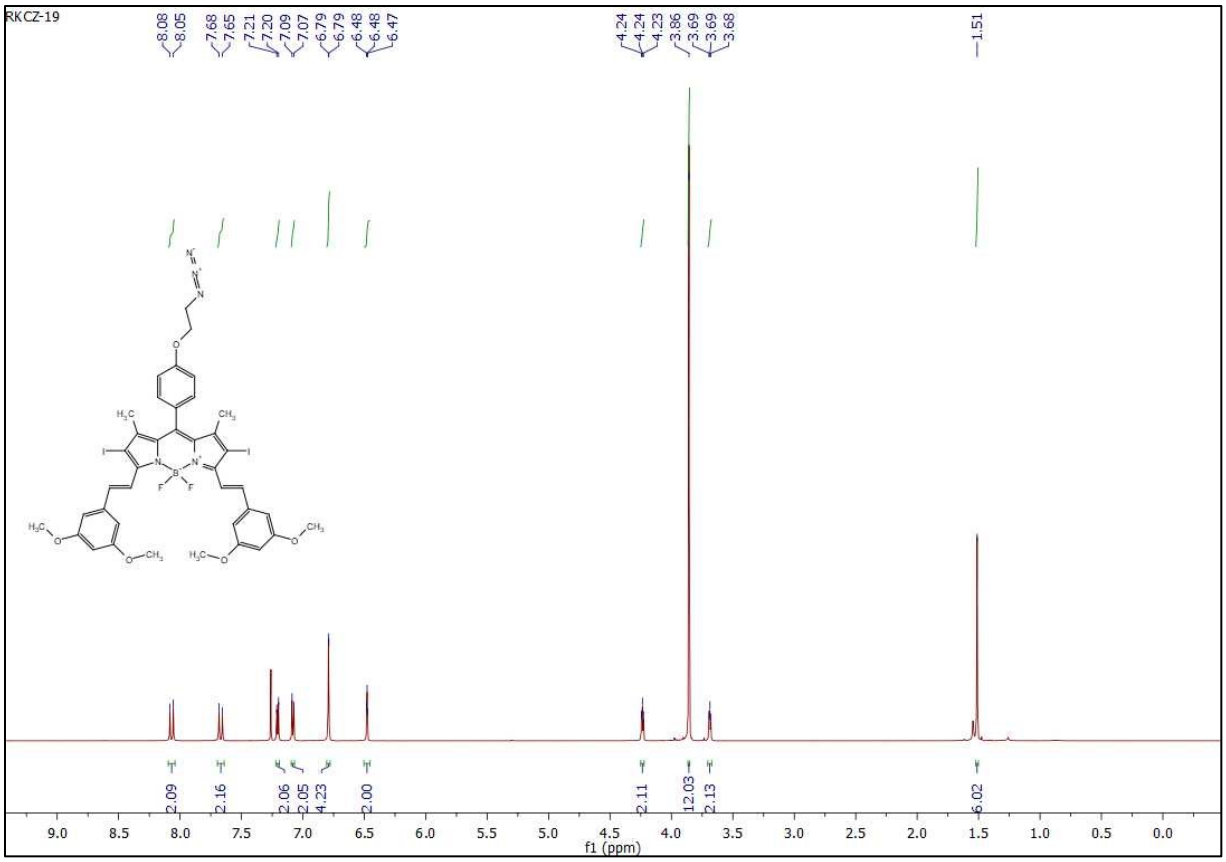


Figure S30: ^{1}H-NMR spectrum of **10** in $CDCl_3$.

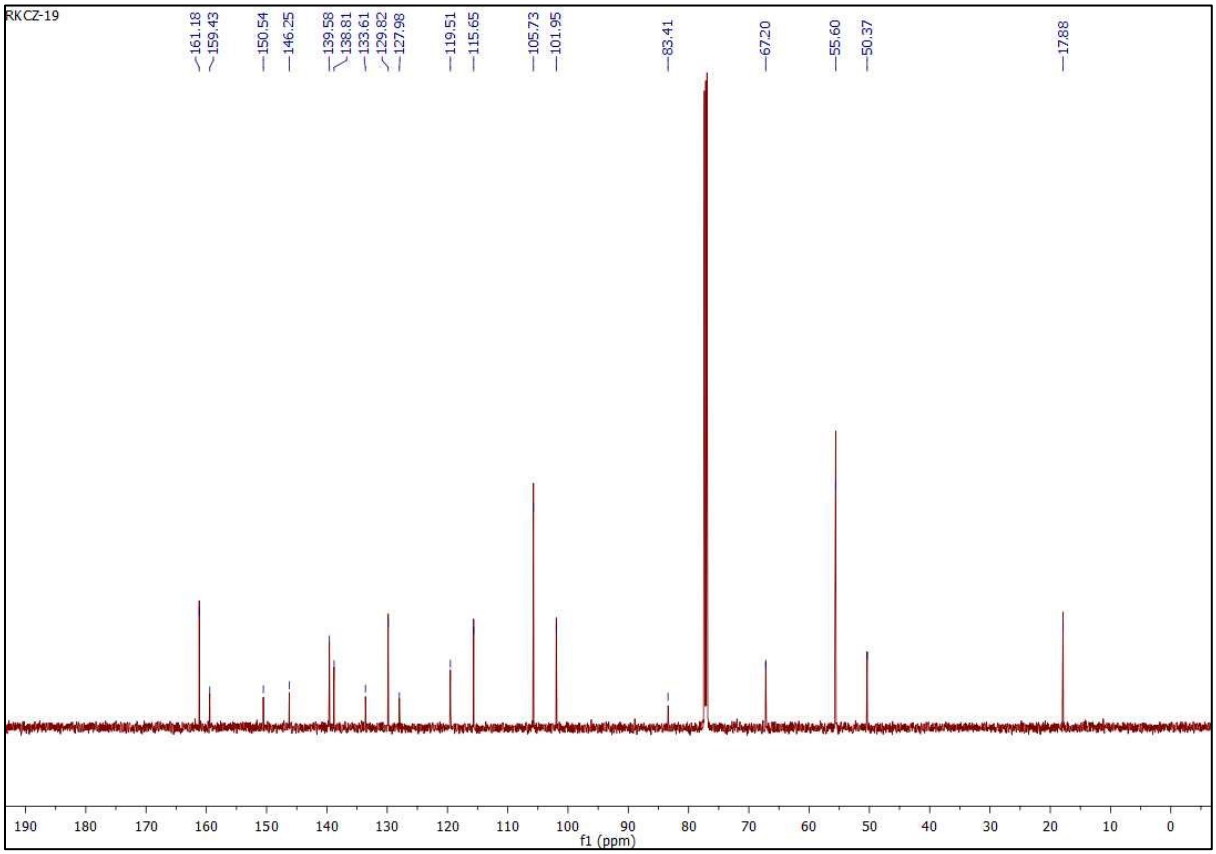


Figure S31: ^{13}C-NMR spectrum of **10** in $CDCl_3$.

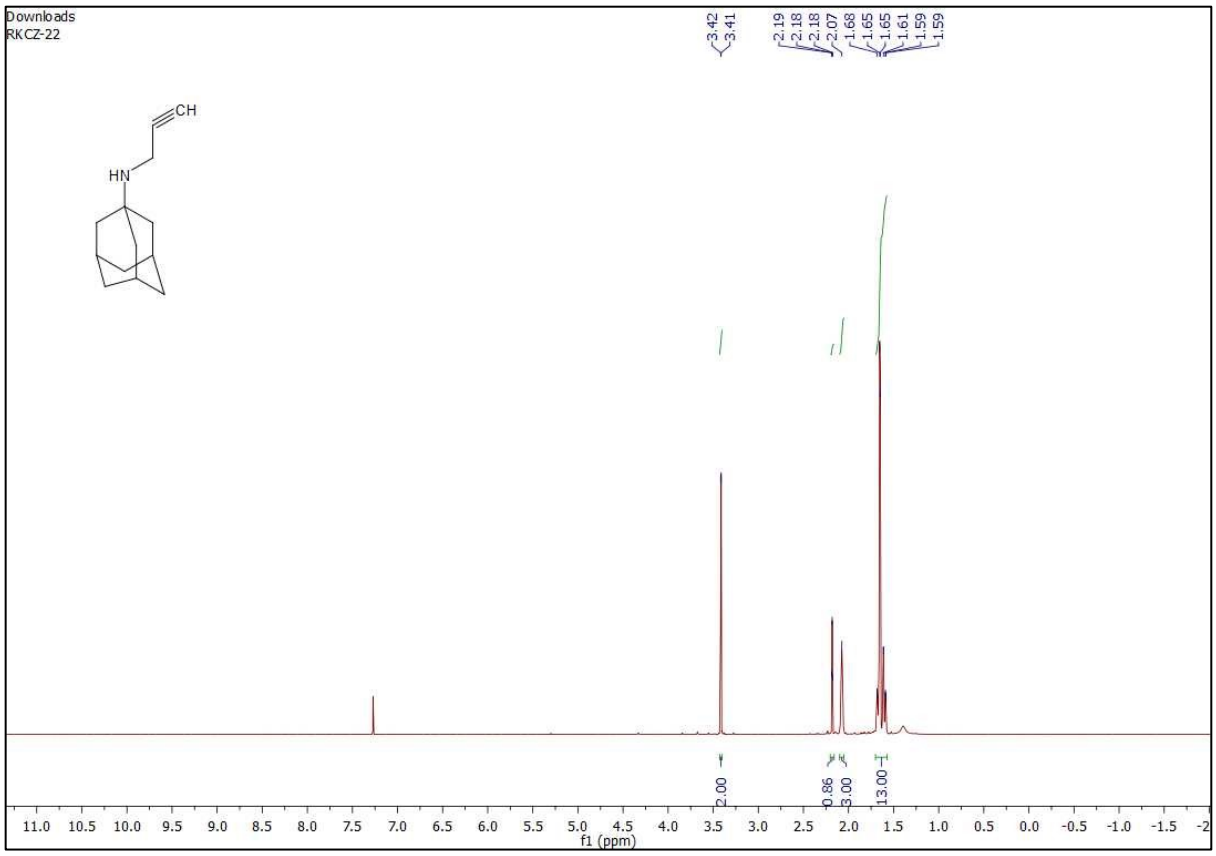


Figure S32: ^{1}H-NMR spectrum of **11** in $CDCl_3$.

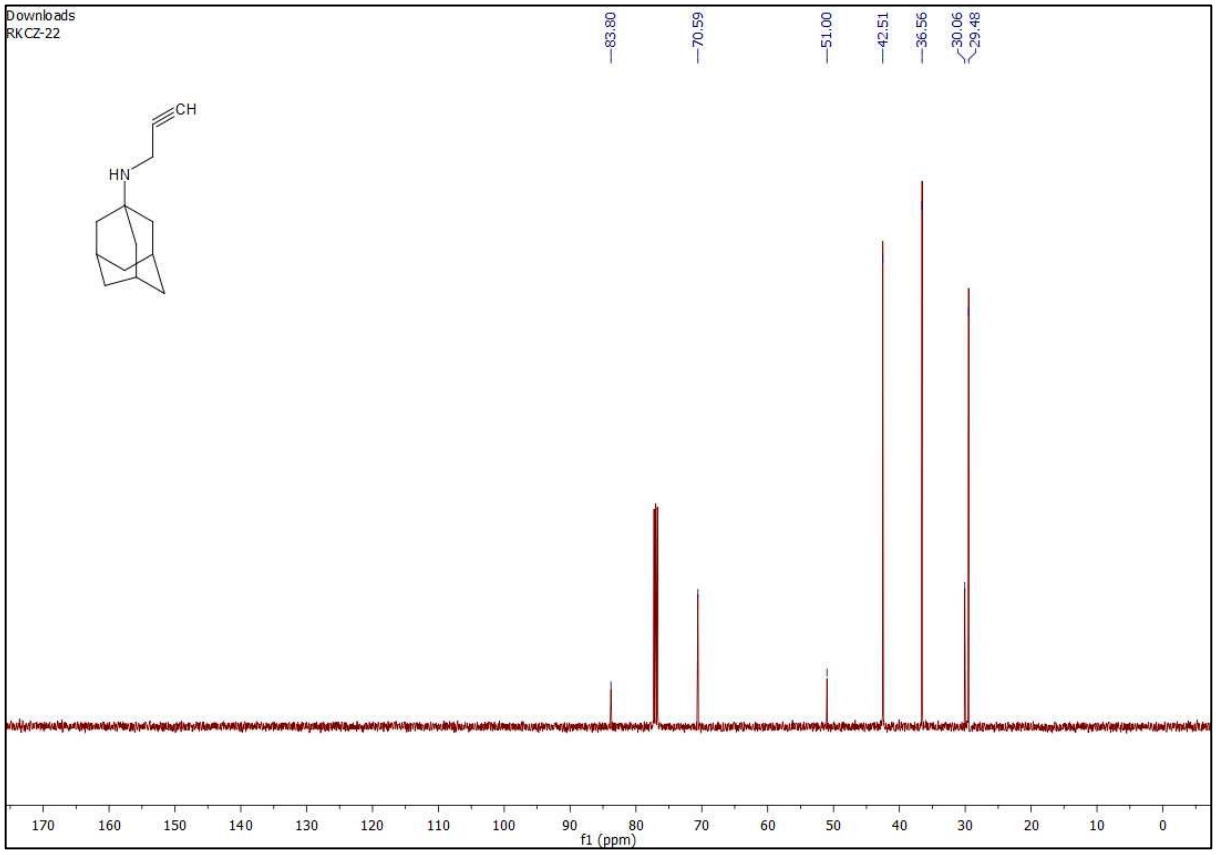


Figure S33: ^{13}C-NMR spectrum of **11** in $CDCl_3$.

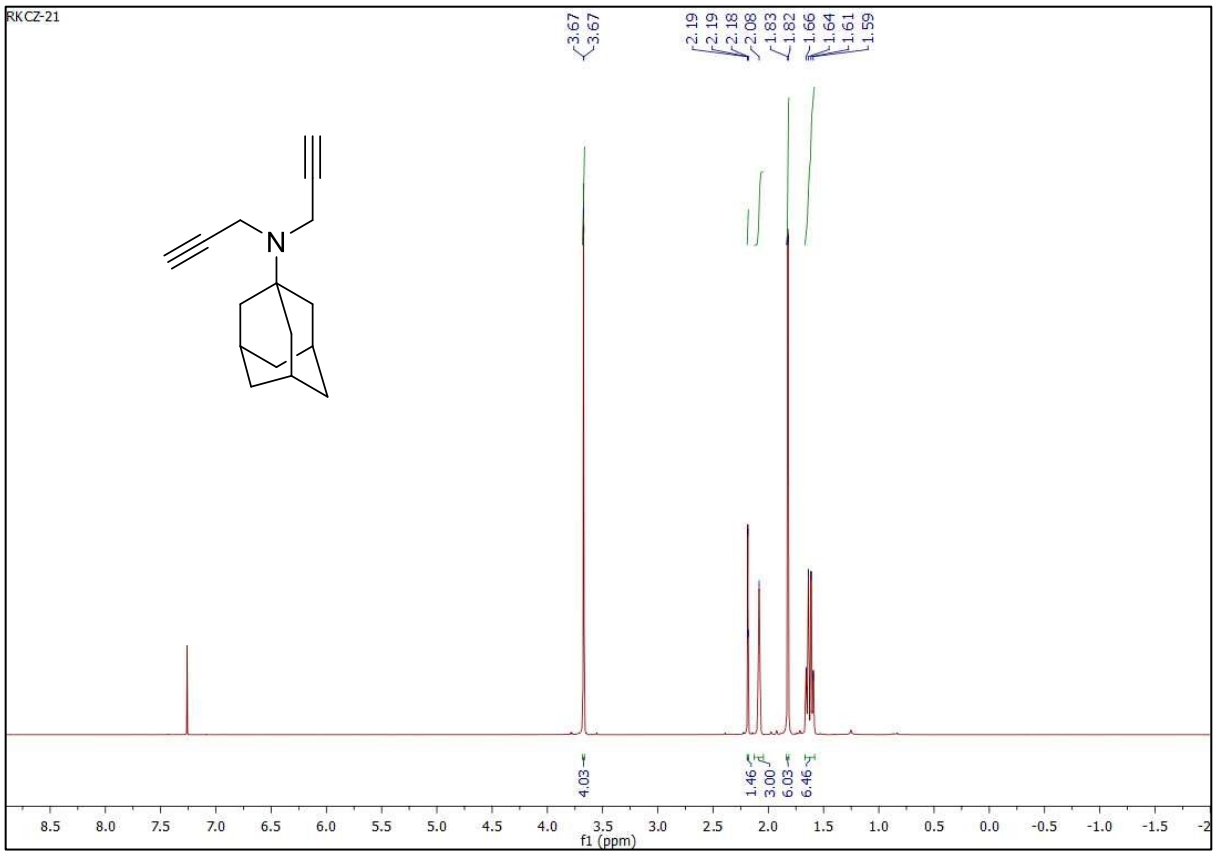


Figure S34: ^{1}H-NMR spectrum of **11a** in $CDCl_3$.

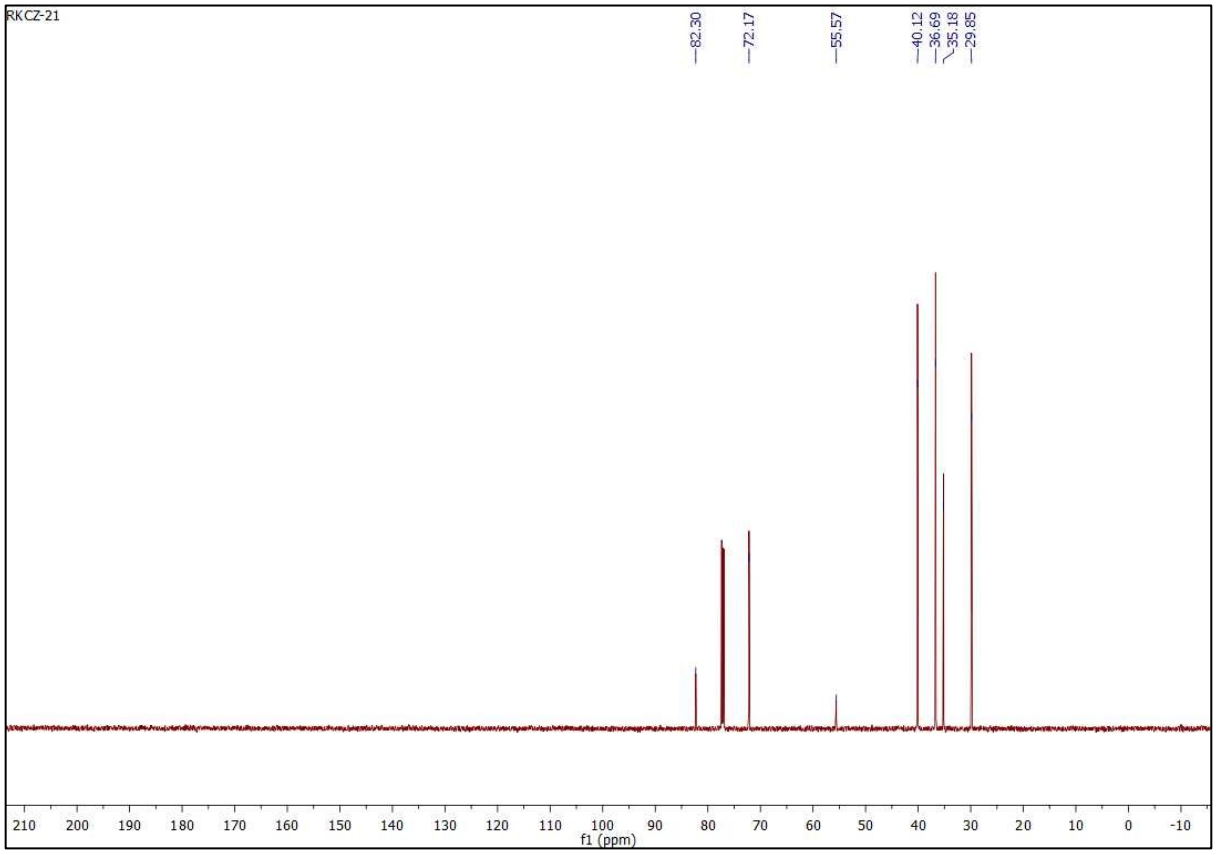


Figure S35: ^{13}C-NMR spectrum of **11a** in $CDCl_3$.

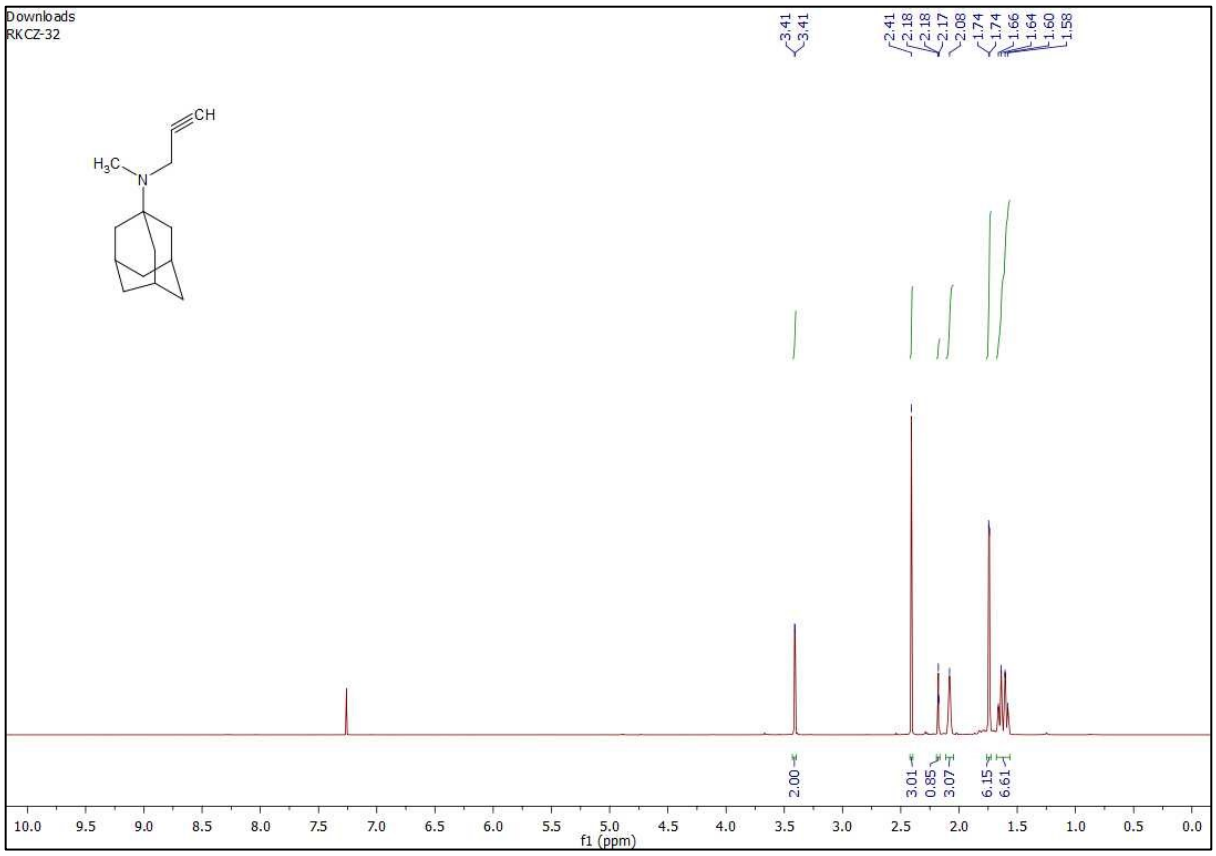


Figure S36: ^{1}H-NMR spectrum of **12** in $CDCl_3$.

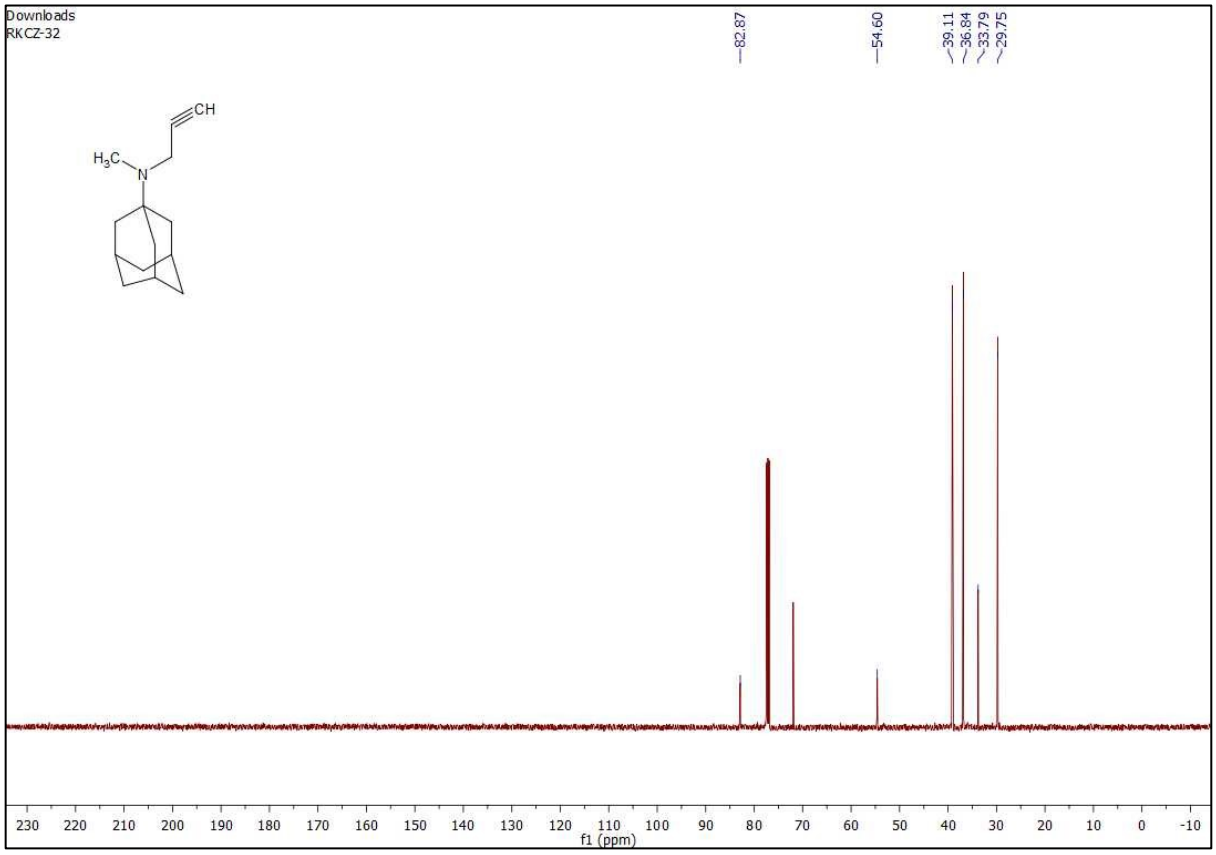


Figure S37: ^{13}C-NMR spectrum of **12** in $CDCl_3$.

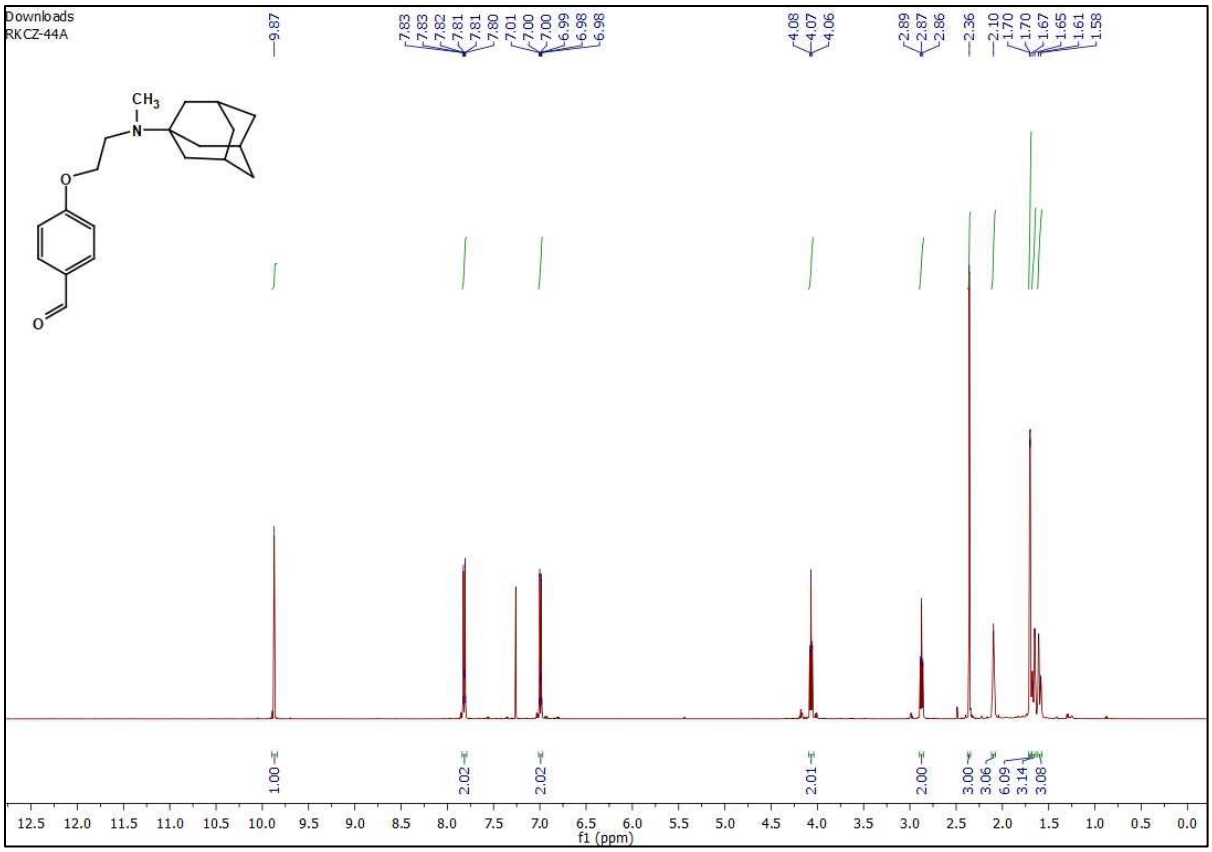


Figure S38: ^{1}H-NMR spectrum of **13** in $CDCl_3$.

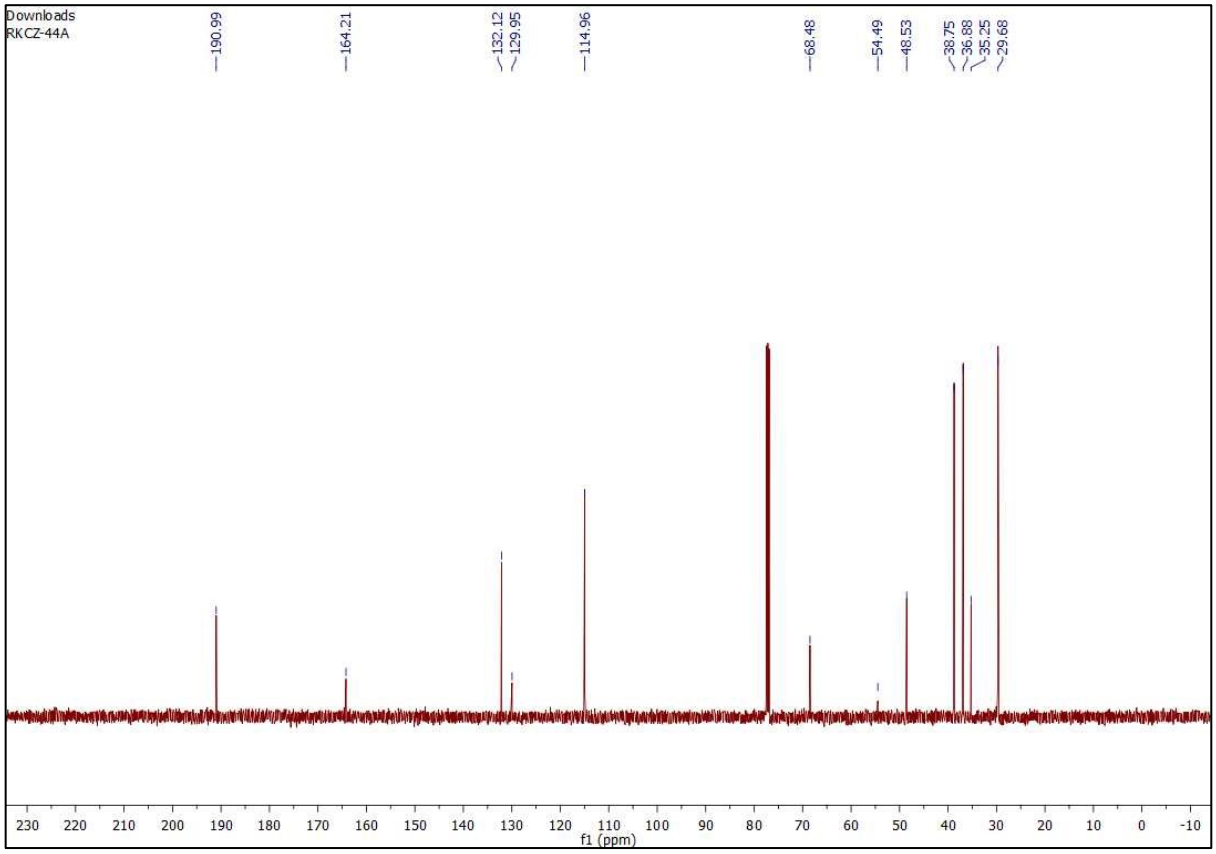


Figure S39: ^{13}C-NMR spectrum of **13** in $CDCl_3$.

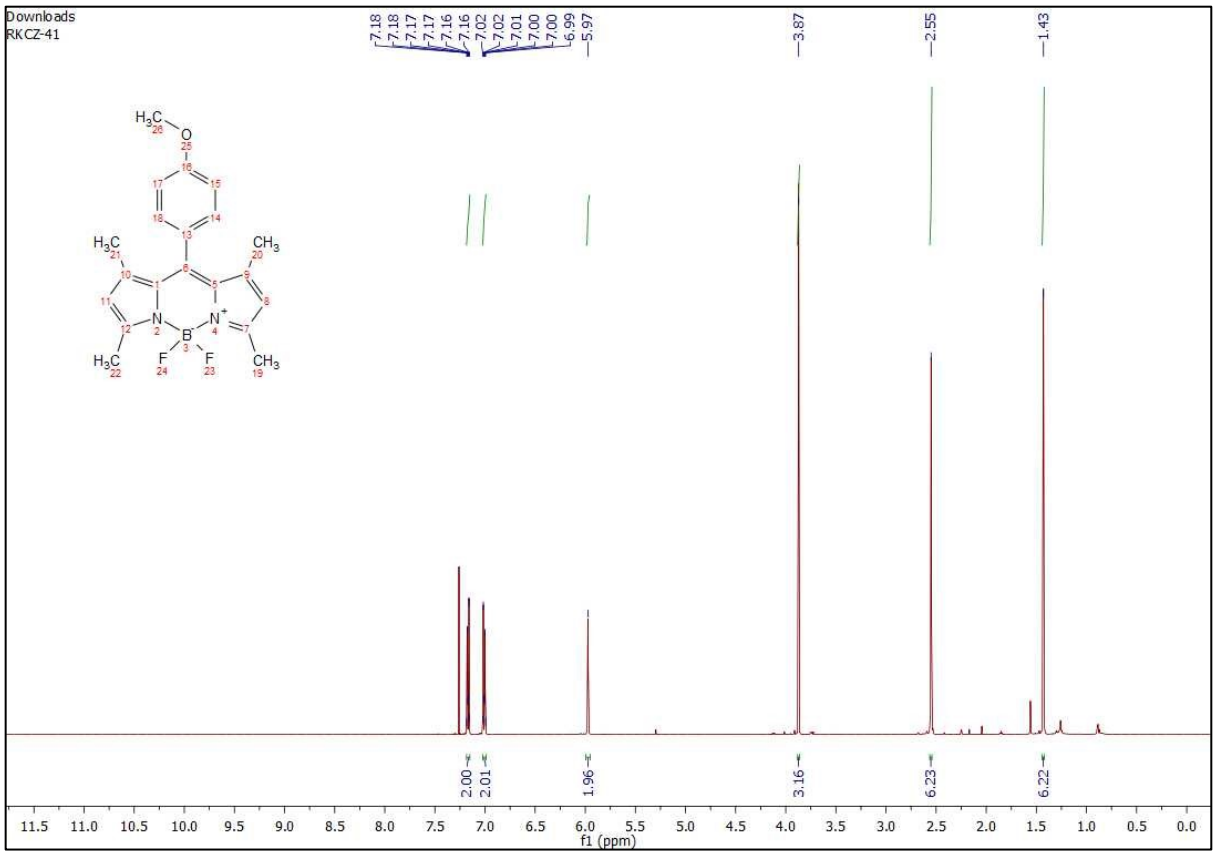


Figure S40: ^{1}H-NMR spectrum of **14** in $CDCl_3$.

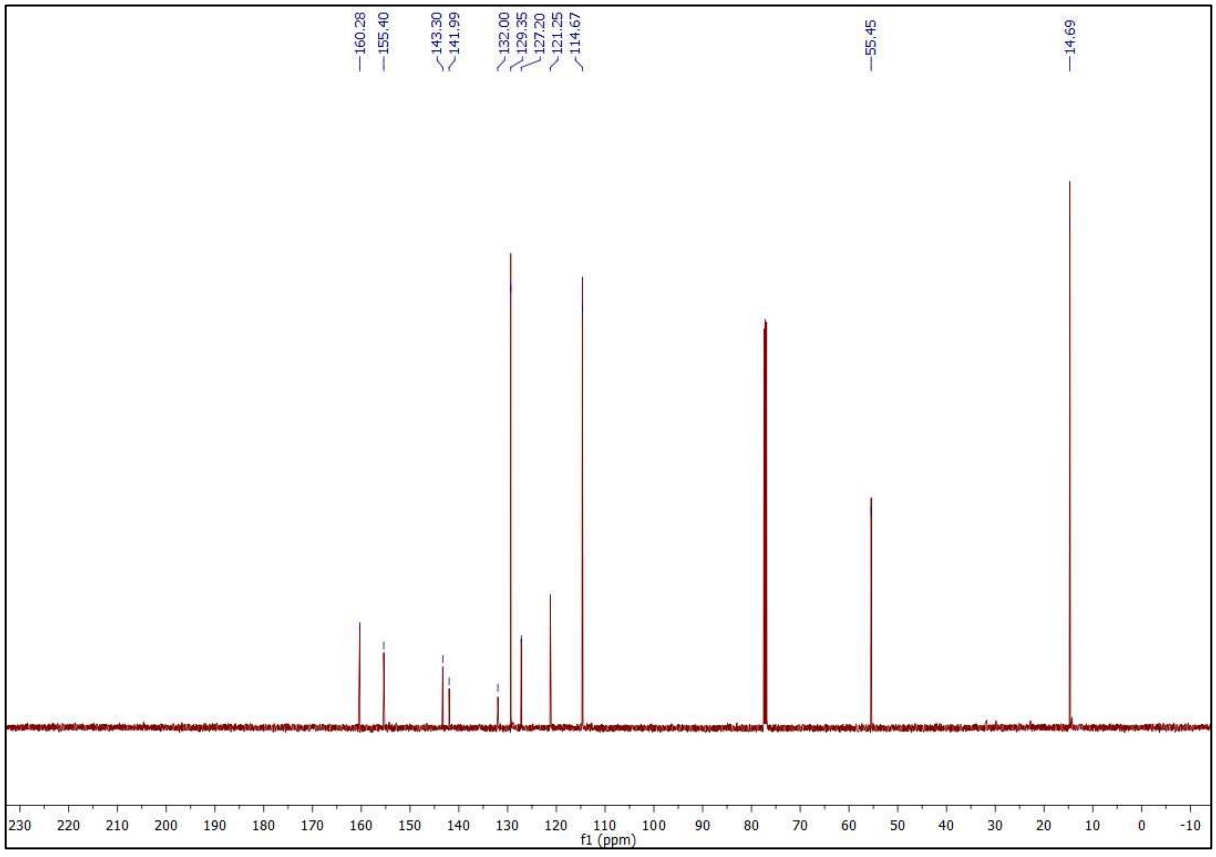


Figure S41: ^{13}C-NMR spectrum of **14** in $CDCl_3$.

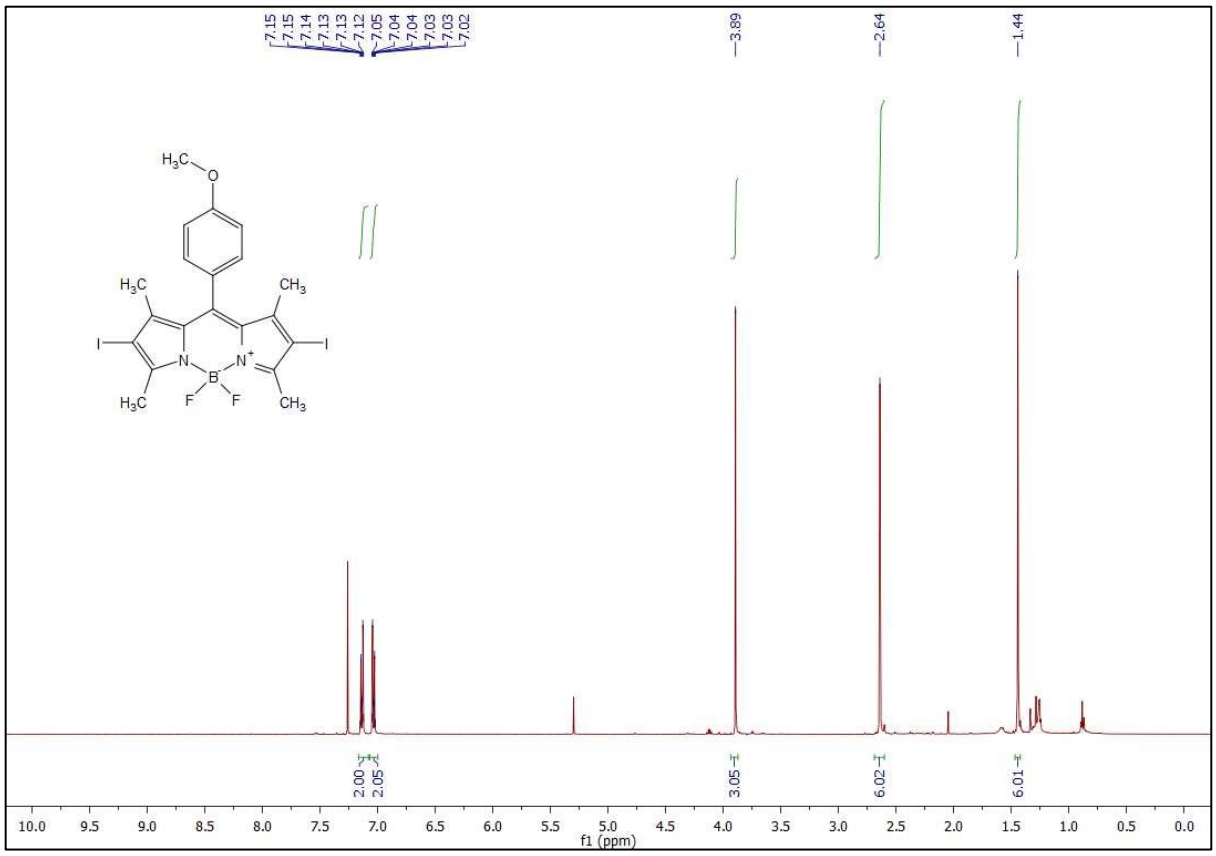


Figure S42: ^{1}H-NMR spectrum of **15** in $CDCl_3$.

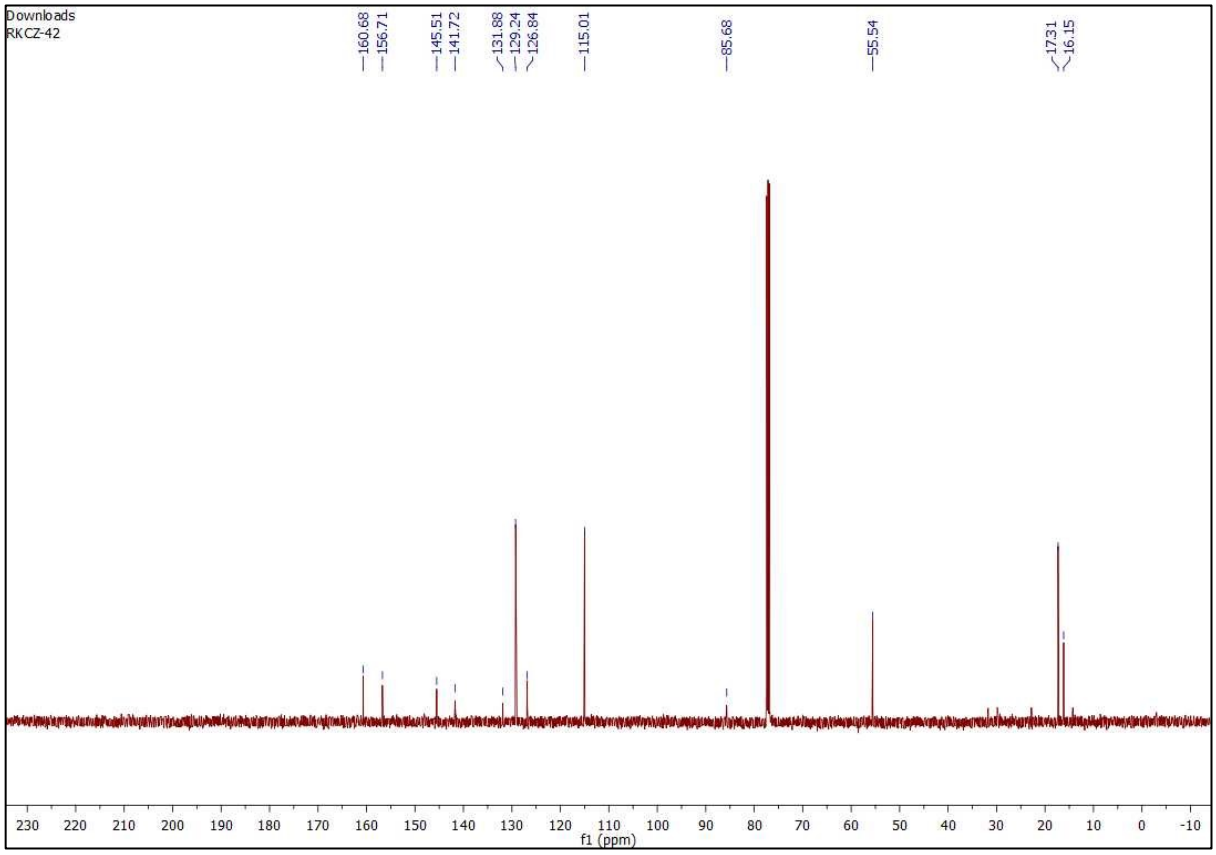


Figure S43: ^{13}C-NMR spectrum of **15** in $CDCl_3$.

HPLC

Assessment of purity by HPLC

The purity of the novel BODIPY compounds (**1·HCl**, **2·HCl**, **3**, **4·HCl,** and **5**) was evaluated by the LC 20A Prominence system (Shimadzu, Duisburg, Germany). The chromatograph consists of a DGU-20A3 degasser, LC-20 AD pumps, a SIL-20AC autosampler, a CTO-20AC column oven, an SPD-M20A photodiode array detector, and a CBM-20AC communication module. The data were processed using LabSolutions software, version 5.85.

All tested samples were analyzed on a Luna Omega PS C18 column (100 × 3.0 mm, particle size 5.0 μm; Phenomenex). The separation was performed using a gradient elution method using water as mobile phase A and methanol as mobile phase B for **1·HCl**, **2·HCl**, and **3**. The gradient time program was set as follows: 0–20 min 83→95% B; 20–30 min 95% B; 30–30.5 min 95→83% B, and the column was re-equilibrated for 4.5 min under the initial conditions. The column temperature was 40 °C, and the flow rate was 1 mL min^{-1}. The mobile phase had to be changed for **4·HCl** and **5**: water with 0.1% TFA as mobile phase A and acetonitrile with 0.1% TFA as mobile phase B. The gradient time program was set as follows: 0–20 min 50→80% B; 20–30 min 80% B; 30–30.5 min 80→50% B, and the column was re-equilibrated for 4.5 min under the initial conditions. The column temperature was 40 °C, and the flow rate was set at 1 mL min^{-1}.

All compounds were analyzed by a diode array detector, and the chromatograms (Figure S44) were recorded at wavelengths corresponding to the absorption maxima of the individual sample (**1·HCl,** **2·HCl**, and **3** at 640 nm; **4·HCl** and **5** at 660 nm).

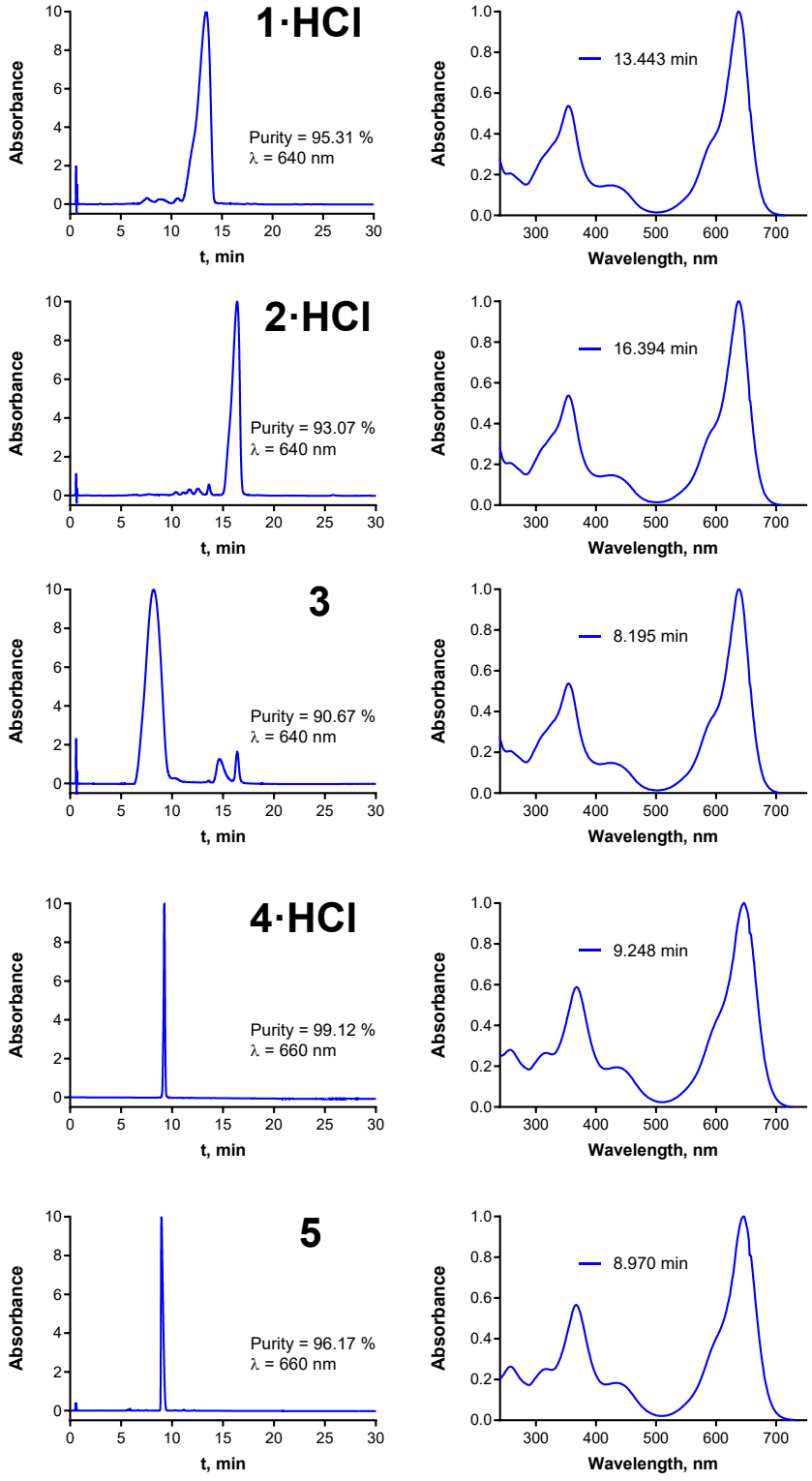


Figure S44. HPLC traces (left) and absorption spectrum in chromatographic peak (right) of BODIPYs **1·HCl, 2·HCl, 3, 4·HCl** and **5**.

References

1. V. Novakova, P. Zimcik, K. Kopecky, M. Miletin, J. Kuneš and K. Lang, *European Journal of Organic Chemistry*, 2008, **2008**, 3260-3263.
2. P. Zimcik, V. Novakova, K. Kopecky, M. Miletin, R. Z. Uslu Kobak, E. Svandrlikova, L. Váchová and K. Lang, *Inorganic Chemistry*, 2012, **51**, 4215-4223.
3. M. Fischer and J. Georges, *Chemical Physics Letters*, 1996, **260**, 115-118.
4. U. Michelsen, H. Kliesch, G. Schnurpfeil, A. K. Sobbi and D. Wohrle, *Photochemistry and Photobiology*, 1996, **64**, 694-701.
5. R. W. Redmond and J. N. Gamlin, *Photochemistry and Photobiology*, 1999, **70**, 391-475.
6. V. Novakova, M. Miletin, T. Filandrová, J. Lenčo, A. Růžička and P. Zimcik, *The Journal of Organic Chemistry*, 2014, **79**, 2082-2093.