Electronic Supplementary Material (ESI)

Photoactivable Prodrug for Simultaneous Release of Mertansine, and CO along with a BODIPY Derivative as a Luminescent Marker in Mitochondria: A Proof of Concept for NIR Image-Guided Cancer Therapy

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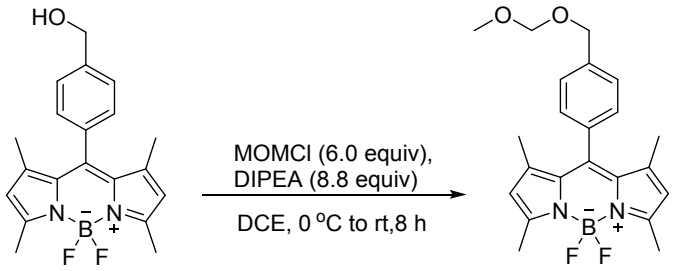
1. Experimental Procedure:

Experimental procedure for the preparation of compound 2:



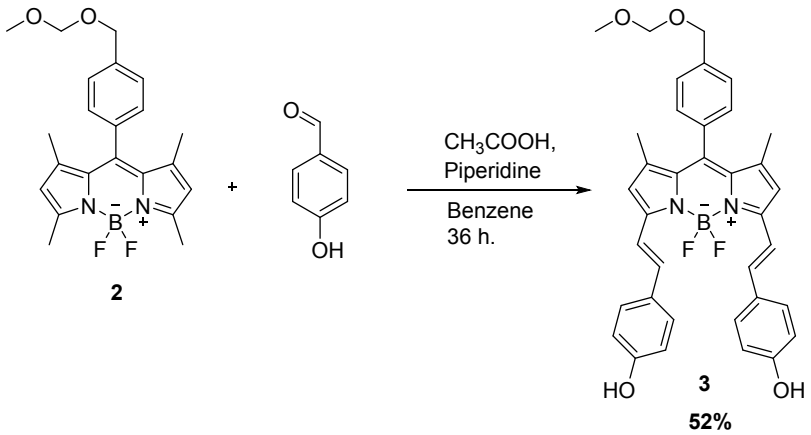
1 **2** **(92%)**

To a Schlenk tube containing compound **1** (200 mg, 1.0 equiv) in DCE (5.0 mL) was added DIPEA (0.850 mL, 8.8 equiv) under nitrogen atmosphere and resulting solution was stirred for 5 minute at 0 °C. To this solution, the solution of chloromethyl methyl ether (0.272 mg, 6.0 equiv) in DCE (2 mL) was added dropwise at 0 °C. The Schlenk tube containing the reaction mixture was placed in preheated oil bath at 55 °C with constant stirring for 8 h. After completion, the reaction mixture

was cooled to ambient temperature and quenched with ice cold water and extracted with DCM. Organic layer was dried over anhydrous Na_2SO_4 and evaporated under *vacuo* to dryness followed by purification using flash silica column chromatography to afford the corresponding product **2** (207 mg) in 92% yield.

Reaction Time: 8 h; R*f***:** 0.5 (6:1, Pet. Ether: EtOAc); yellow solid; mp = 119-121 ^oC; 207 mg, 92 % yield. **^{1}H NMR (400 MHz, $CDCl_3$)** δ 7.50(d, J = 7.93 Hz, 2H), 7.28 (d, J = 7.93 Hz, 2H), 5.99 (s, 2H), 4.76 (s, 2H), 4.70 (s, 2H), 3.45 (s, 3H), 2.56 (s, 6H), 1.39 (s, 6H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 155.4, 143.0, 141.5, 138.9, 134.2, 131.4, 128.3, 128.0, 121.2, 95.9, 68.8, 55.5, 14.6, 14.5; **ESI HRMS:** calcd. For $C_{22}H_{26}BF_2N_2O_2$ $[M+H]^+$: 399.2050, found: 399.2049.

Experimental procedure for the preparation of compound 3:

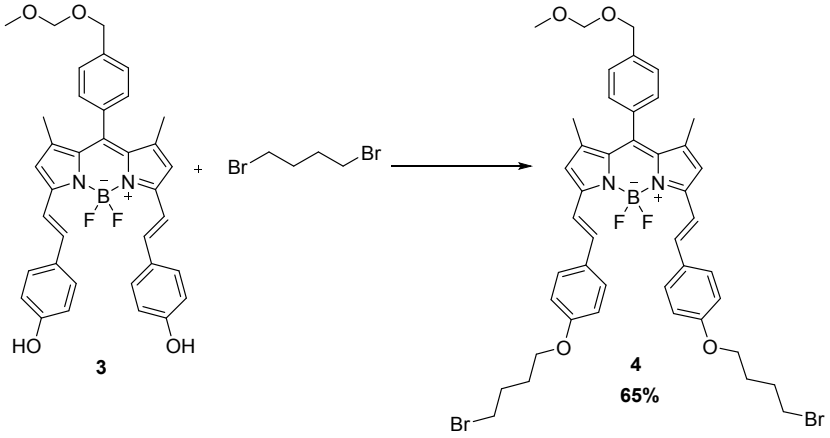


To a two neck round bottom flask containing compound **2** (200 mg, 1.0 equiv) and 4-hydroxy benzaldehyde (245 mg, 4.0 equiv) equipped with a Dean-Stark apparatus and a reflux condenser was added dry benzene (120 mL), piperidine (640 μL), and acetic acid (640 μL) under nitrogen atmosphere. The resulting solution was heated to reflux for 36 h. After complete consumption of aldehyde (monitored by TLC), the solvent was evaporated under *vacuo*. The resulting reaction mixture was dissolved in the ethyl acetate (100 mL) and the organic layer was washed with water

(50 mL x 3), dried over Na_2SO_4 before concentration. The crude product obtained was subjected for the purification using flash silica gel column chromatography to afford the desired product **3** (160 mg) as blue solid in 52% yield.

Reaction Time: 36 h; R*f***:** 0.5 (1:1, Pet. Ether : EtOAc); blue solid**;** mp = 212-214 ^oC; 160 mg, 52 % yield. **^{1}H NMR (400 MHz, DMSO-*d*$_6$)** δ 9.99 (s, 2H), 7.58-7.28 (m, 12H), 6.98-6.82 (m, 6H), 4.70 (s, 2H), 4.64 (s, 2H), 3.31 (s, 3H), 1.40 (s, 6H); **^{13}C NMR (100 MHz, DMSO-*d*$_6$)** δ 159.0, 152.2, 141.2, 139.2, 137.5, 136.9, 133.4, 132.3, 129.0, 128.4, 128.3, 127.3, 117.9, 116.0, 115.0, 95.4, 68.3, 54.9, 14.3; **ESI HRMS:** calcd for $C_{36}H_{33}BF_2N_2O_4$ [M + Na]: 629.2394, found: 629.2389.

Experimental procedure for the preparation of compound 4:

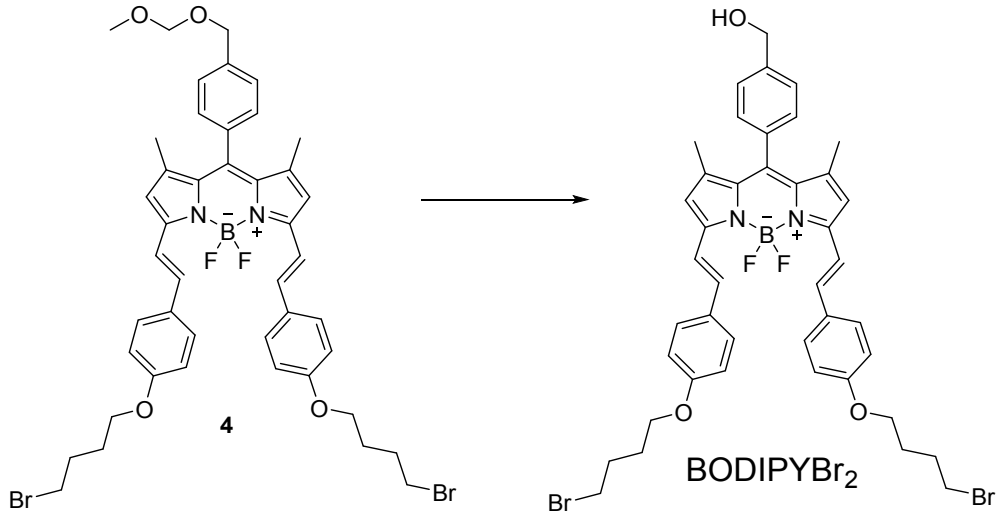


To a two neck round bottom flask containing compound **3** (400 mg, 1.0 equiv) in DMF (5.0 ml) was added potassium carbonate (0.227 mg, 2.5 equiv) and the resulting slurry was stirred under nitrogen gas followed by the addition of 1,4-dibromobutane (0.712 mg, 5.0 equiv) at room temperature. The reaction mixture was further stirred for 24 h at same temperature. After complete consumption of the staring material (24 h, monitored by TLC) the reaction was quenched with ice cold water followed by extraction with ethyl acetate (100 mL x 2). The organic layer was dried

over anhydrous Na_2SO_4 and evaporated under *vacuo* to dryness. The residue was purified by silica gel column chromatography to afford the corresponding product **4** (380 mg) in 65 % yield.

Reaction Time: 24 h; R*f*: 0.4 (2:1, Pet. Ether: EtOAc); dark green solid; mp = 205-207 ^oC; 380 mg, 65% yield**. ^{1}H NMR (400MHz, $CDCl_3$)** δ 7.62 (d, *J* = 16.02 Hz, 2H), 7.57 (d, *J* = 8.39 Hz, 4H), 7.50 (d, *J* = 8.01 Hz, 2H), 7.31 (d, *J* = 7.63 Hz, 2H), 7.22 (d, *J* = 16.40 Hz, 2H), 6.91 (s, J = 8.394 H), 6.62 (s, 2H), 4.77 (s, 2H), 4.71 (s, 2H), 4.04 (t, *J* = 5.9 Hz, 4H), 3.51 (t, *J* = 6.6 Hz, 4H), 3.45 (s, 3H), 2.12-2.07 (m, 4H), 2.00-1.96 (m, 4H), 1.45 (6H, s); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 159.7, 152.7, 141.7, 138.9, 137.9, 135.7, 134.5, 133.2, 129.6, 129.0, 128.6, 128.2, 117.5, 117.2, 114.7, 95.9, 68.9, 66.9, 55.5, 33.4, 29.4, 27.8, 14.7; **ESI HRMS:** calcd for $C_{44}H_{48}BBr_2F_2N_2O_4$ (M+1) : 875.2036, found: 875.1990.

Experimental procedure for the synthesis of $BODIPYBr_2$:

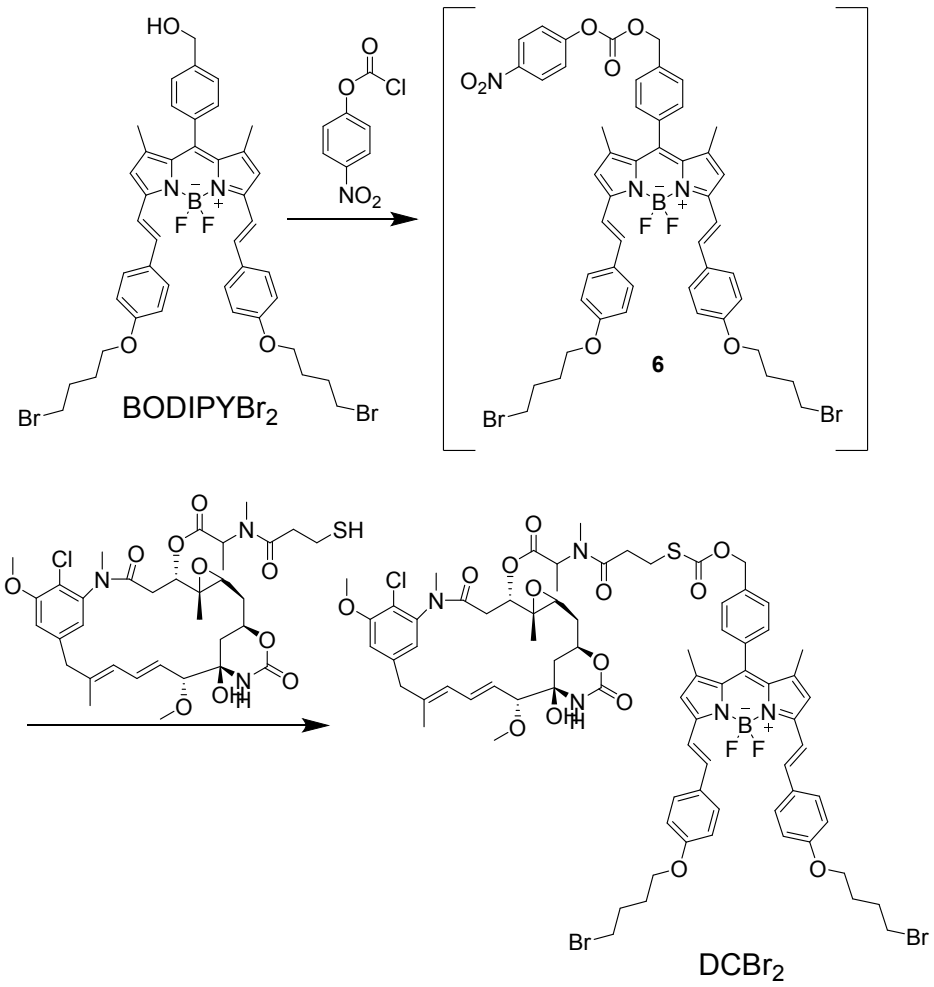


To a round bottom flask, containing compound **4** (50 mg) in 1,4-dioxane (50 ml) under nitrogen atmosphere was added three fold diluted 4N HCl in 1,4-dioxane (2.0 ml) dropwise below 10 ^oC over 30 min. After complete addition, the reaction mixture was allowed to attain room temperature and stirred further for 24 h. After completion of the reaction, the excess HCl was quenched by the

addition of K_2CO_3 (1.0 equiv). The resulting reaction mixture was filtered through cotton plug and the filtrate was concentrated under *vacuo*. The crude residue was purified by column chromatographic purification to afford the corresponding product BODIPYBr_2 in 32% yield.

Reaction Time: 24 h; R*f*: 0.5 (2:3, Pet. Ether: EtOAc); green solid; mp = 197-199 ^oC; 15.2 mg, 32% yield**. ^{1}H NMR (400MHz, DMSO-*d*$_6$)** δ 7.78-7.24 (m, 12H), 7.04 (d, J = 7.32 Hz, 4H), 6.93 (s, 2H), 4.63 (s, 2H), 4.08 (t, J = 5.79, 4H), 3.63 (t, J = 5.79 Hz, 4H), 2.03-1.94 (m, 4H), 1.90-1.83 (m, 4H), 1.42 (s, 6H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 159.7, 152.0, 143.7, 141.4, 138.3, 136.5, 132.5, 132.4, 130.3, 128.8, 128.0, 126.8, 118.0, 115.9, 115.1, 66.8, 62.4, 34.8, 29.0, 27.3, 14.3 ; **ESI HRMS:** calcd for $C_{42}H_{44}BBr_2F_2N_2O_3$ (M+1): 831.1774, found: 831.1725.

Experimental procedure for the synthesis of $DCBr_2$:



A 20 mL Schlenk flask equipped with a magnetic stir-bar was charged a solution of $BODIPYBr_2$ (3.36 g, 12.5 mmol, 100 mol %), 4-nitrophenyl chloroformate (16mg , 0.08 mmol, 1.4 equiv.) and diisopropyl ethyl amine (21 µl , 0.12mmol , 2.0 equiv.) in 10 mL DCM. The solution was sparged with Ar for 20 min and stirred for overnight. After 12 hrs, mertansine (53 mg, 0.072 mmol, 1.3 equiv.) was added and stirred further for 12 hours. Purification by silica gel column chromatography using Dichloromethane-Methanol as the eluent.

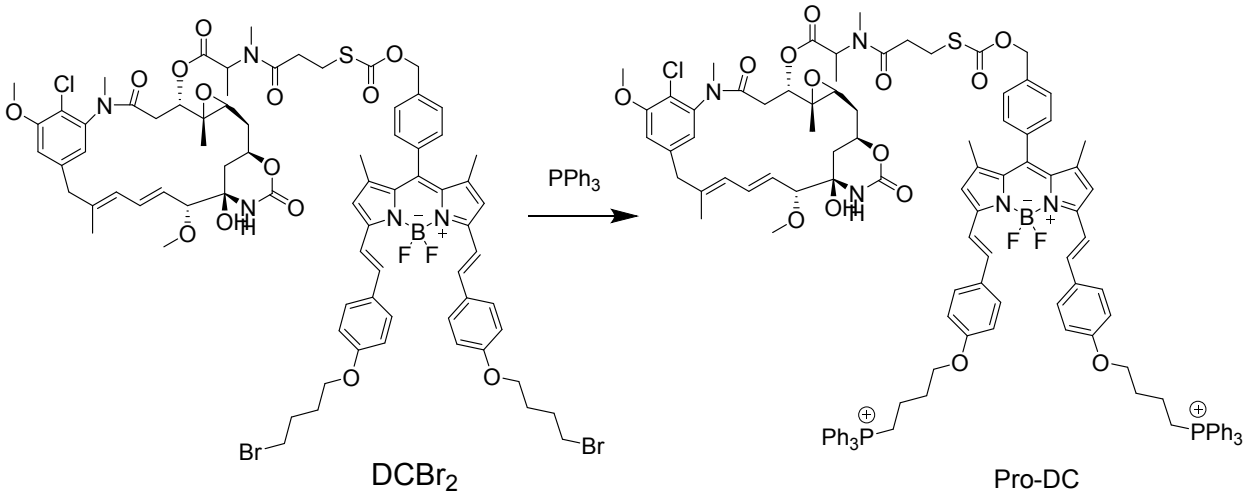
Reaction Time: 24 h; R*f*: 0.3 (20:1, DCM : MeOH); green solid; mp = 255-258 ^{o}C; 61 % yield**.**

^{1}H NMR (500 MHz, $CDCl_3$-*d_3*) δ 7.61 (s, 1H), 7.57 (d, J = 5 Hz, 3H), 7.50 (d, J = 15 Hz, 1H), 7.32 (d, J = 20 Hz, 1H), 7.21 (s, 1H), 6.91 (d, J = 15 Hz, 3H), 6.81 (d, J = 5 Hz, 2H), 6.71 (d, J = 20 Hz, 2H), 6.66 (d, J = 5 Hz, 2H), 6.60 (s, 1H), 6.45 - 6.42 (t, J = 10 Hz, 2H), 6.24 (s, 2H), 5.66

(q, J = 10 Hz, 2H), 5.41 (q, J = 10 Hz, 2H), 4.82 (s, 1H), 4.77 (d, J = 3 Hz, 1H), 4.74 (d, J = 3 Hz, 1H), 4.30-4.25 (m, 2H), 4.04 (t, J = 7.5 Hz, 3H), 3.98 (s, 3H), 3.68-3.65 (m, 3H), 3.52-3.45 (m, 3H), 3.35 (s, 3H), 3.34 (s, 1H), 3.2 (s, 3H), 3.11 (d, J = 7.5 Hz, 2H), 3.02 (t, J = 5 Hz, 2H), 2.84 (s, 6H), 2.69-2.72 (m, 2H), 2.61-2.57 (m, 3 H), 2.18 (d, J = 10 Hz, 2H), 2.10-2.08 (m, 2H), 1.97-1.93(m, 2H), 1.68 (t, J = 5 Hz, 3H), 1.56(d, J = 7.5 Hz, 3H), 1.43 (s, 1H), 1.31-1.20 (m, 8H), 0.88-0.79 (m, 5H); **^{13}C NMR (500 MHz, $CDCl_3$)** δ 172.13, 170.19, 157.37, 153.71, 143.71, 143.51, 142.33, 140.86, 134.74, 128.95, 126.62, 123.65, 120.19, 116.19, 114.56, 89.90, 82.25, 79.59, 75.52, 68.63, 61.39, 57.98, 53.76, 47.98, 40.27, 39.04, 37.53, 36.90, 33.80, 32.11, 31.11, 29.27, 21.27, 21.19, 16.93, 15.98, 14.82, 13.48.

ESI HRMS: calcd for $C_{77}H_{87}BBr_2ClF_2N_5O_{14}S$ (M+1): 1580.4087, found: 1580.4072.

Experimental procedure for the synthesis of Pro-DC:



A mixture of compound $DCBr_2$ (18 mg, 0.0113 mmol, 1 equiv.) and triphenylphosphine (6 mg, 0.024 mmol, 2.2 equiv.) in anhydrous toluene (10 mL) was refluxed for 2 days under nitrogen gas. The reaction mixture was then cooled to room temperature, and the precipitating product (Pro-DC) was isolated by filtration under reduced pressure and washed three times with diethyl ether.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70 (d, J = 10 Hz, 1H), 7.61 (d, J = 10 Hz, 3H), 7.51-7.49 (m, *2*H), 7.37-7.25 (m, 30H), 7.29 (s, 1H), 7.23 (d, J = 20 Hz, 1H), 6.94 (d, J = 10 Hz, 2H), 6.85 (d, J = 5 Hz, 2H), 6.74 (d, J = 15 Hz, 2H), 6.70 (s, 2H), 6.64 (s, 1H), 6.46 - 6.42 (t, J = 10 Hz, 2H), 6.33

(s, 2H), 5.66 (q, J = 10 Hz, 2H), 5.42 (q, J = 5 Hz, 2H), 4.85 (s, 1H), 4.79 (d, J = 5 Hz, 2H), 4.32 (t, J = 10 Hz, 2H), 4.08 (t, J = 7.5 Hz, 3H), 3.96 (s, 3H), 3.53 (d, J = 10 Hz, 3H), 3.43 (d, J = 10 Hz, 1H), 3.15 (d, J = 10 Hz, 2H), 3.06 (d, J = 10 Hz, 2H), 2.87 (s, 6H), 2.79-2.73 (m, 2H), 2.67-2.56 (m, 3 H), 2.21 (d, J = 10 Hz, 2H), 2.07-1.98 (m, 4H), 1.59(d, J = 7.5 Hz, 3H), 1.35-1.24 (m, 7H), 0.92-0.79 (m, 5H); **^{13}C NMR (500 MHz, $CDCl_3$)** δ 172.16, 170.19, 157.37, 153.72, 143.51, 142.34, 140.85, 138.62, 135.07, 133.47, 129.95, 128.93, 126.63, 123.66, 120.20, 116.19, 114.57, 89.91, 82.25, 79.59, 75.53, 68.64, 61.35, 57.98, 53.76, 47.98, 40.27, 39.10, 37.55, 36.91, 34.87, 33.81, 32.11, 31.11, 21.20, 19.93, 15.99, 13.49.

ESI MS: calcd for $C_{113}H_{117}BClF_2N_5O_{14}P_2S$ (M+1): 1945.75, found: 1945.69.

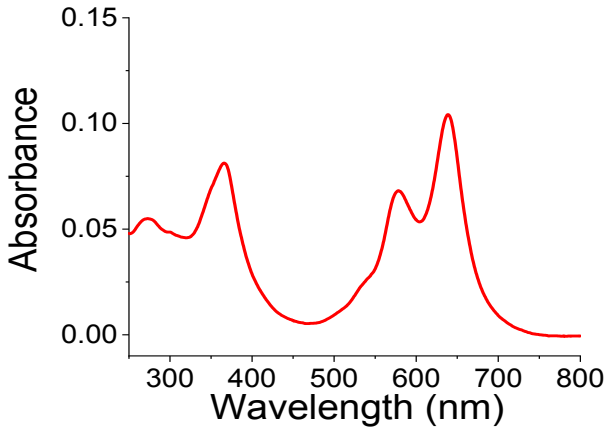


Figure S1. UV-Vis absorption spectra of Pro-DC.

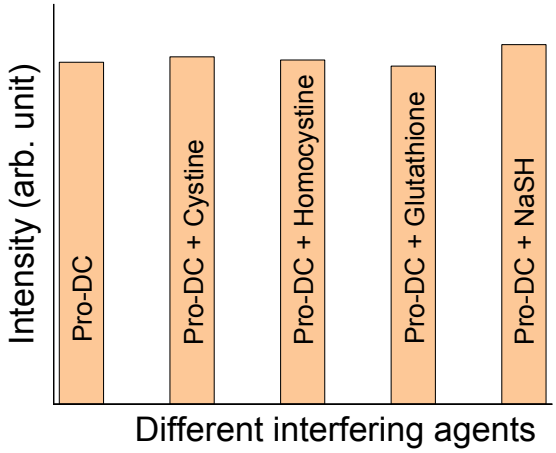


Figure S2. Interfering studies. The interfering studies was done by cysteine, homocysteine, glutathione and NaSH.

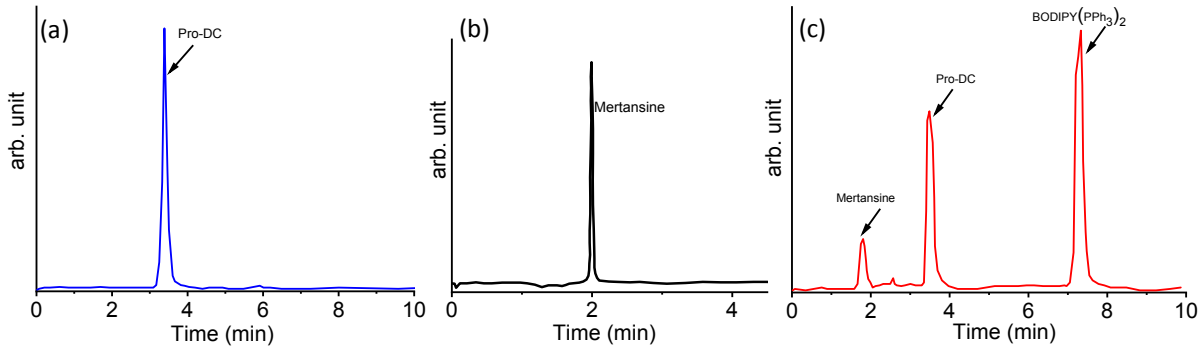


Figure S3. HPLC studies: (a) Pro-DC; (b) Mertansine; (c) HPLC chromatogram of light exposed Pro-DC.

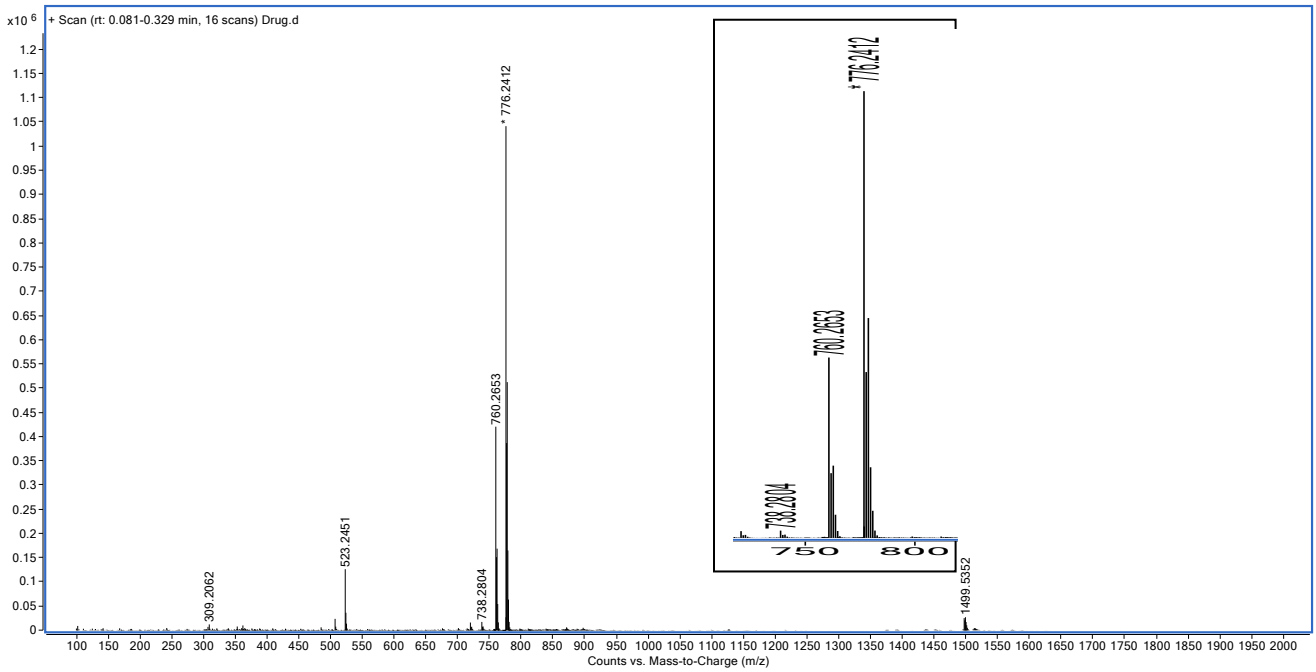


Figure S4. HRMS data of HPLC peak of mertansine.

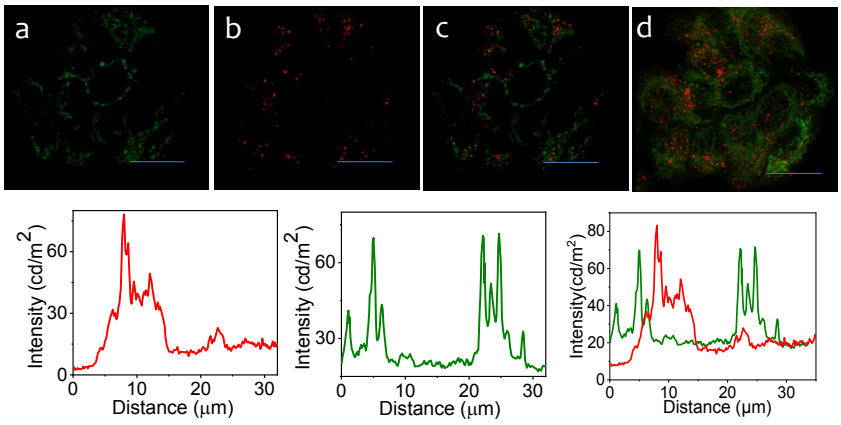


Figure S5. Colocalization experiments (Confocal Microscopy) of intracellular localization of Pro-DC using lysotraker probes: Wide field microscopy images of in cellulo-emission of Pro-DC (panel a) with intensity along traced line shown underneath. Emission from lysotraker green (panel b) and intensity along the same line shown below. The overlap of the intensity is shown in panel c and d (3D view). panel c and d shows there is no overlap of the green and red fluorescence, indicating the Pro-DC are not localised over lysosome.

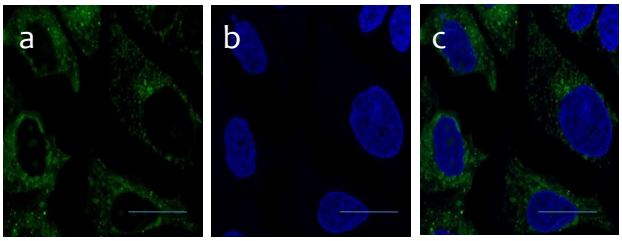


Figure S6 Colocalization experiments of intracellular localization of Pro-DC using DAPI: Confocal Laser Scanning Microscopy images of cellular emission of Pro-DC (panel a). Emission from DAPI (panel b). Panel c shows the overlap of the green and blue fluorescence, indicating no localization of Pro-DC at mitochondria. Scale bar 10 μm.

Phototoxic Index

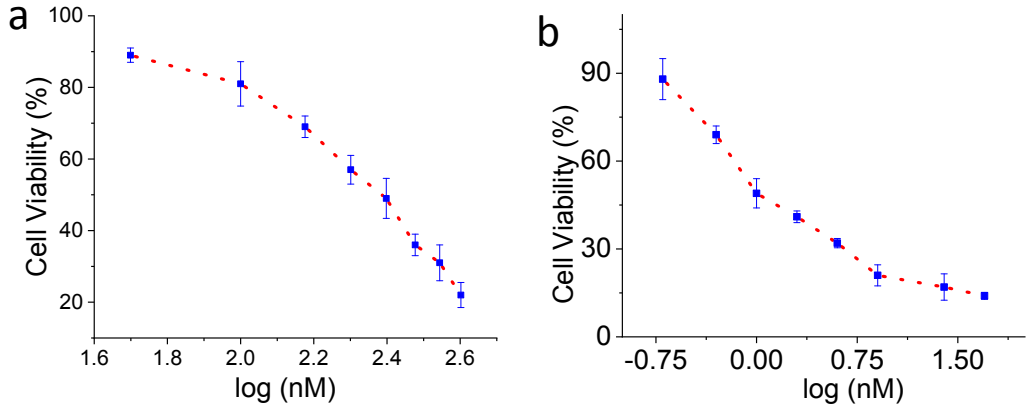


Figure S7. Logarithmic fitting curve for cell viability of Pro-DC (a) without light and (b) with light.

The phototoxic index (PI) of Pro-DC was calculated, which denotes the ratio of the dark and light-exposed IC50 values. The Pro-DC without light (Figure S7a) revealed IC_{50} 249nM. Whereas in presence of light irradiation the IC_{50} is significantly lower and around 0.9 (Figure S7b). We believe this higher value was based on our molecular design, where the multiple molecular components contribute synergistically to the observed elevated cellular toxicity.

Experiment for evolution of CO gas:

$PdCl_2$ (100 mg) was dissolved in two drops of conc. HCl and diluted with 05 mL of distilled water. Saturated solution of cold phosphomolybdic acid in water was prepared separately. Two solutions were then mixed in a glass vial in 1:2 (phosphomolybdic acid : $PdCl_2$) ratio. One piece of filter papers were then dipped in this phosphomolybdic acid-$PdCl_2$ solution and then this was dried at room temperature for 2 hour. In a glass vial, Pro-Dc (1, 0.4 mmol) were taken and 8.0 mL of MeOH was added into this reaction mixture. Then, one strip of the above dried filter paper was dipped inside the glass vial and the solution was irradiated under blue light for 30 min. It was observed that the yellow colour of the strip was changed to dark-blue color, indicating the evolution of CO gas from the solution.

Figure S8. Experiment for CO elimination.

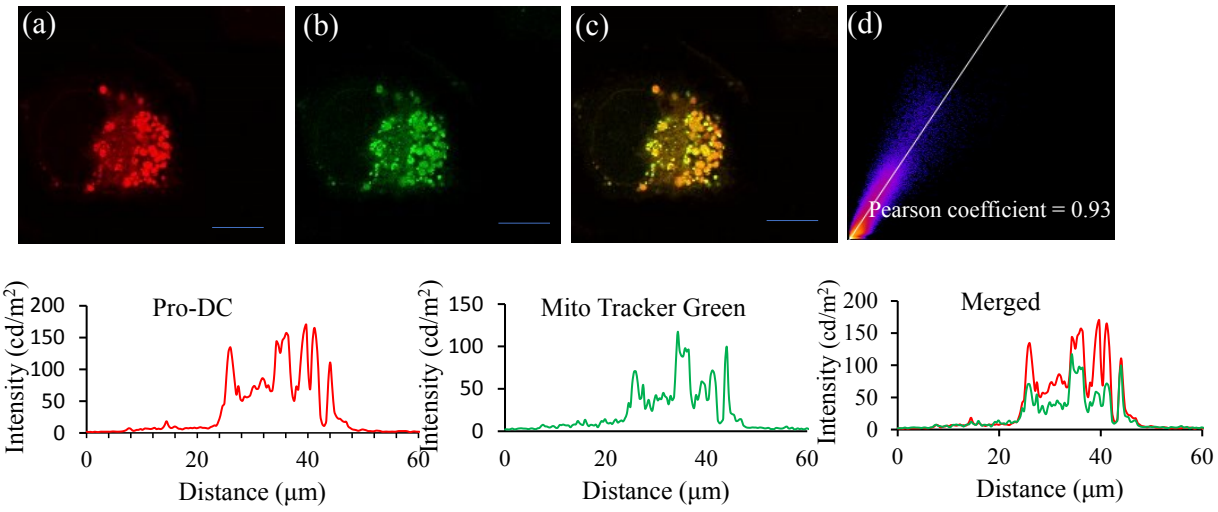


Figure S9. Colocalization studies of intracellular localization of Pro-DC using MitoTracker probes: Widefield microscopy images of intracellular emission of Pro-DC (panel a) with intensity along the traced line shown underneath. Emission from Mito Tracker Green (panel b) and intensity along the same line shown below. The co-localisation of the intensity is shown in panel c. Panel c shows the overlap of the green and red fluorescence, indicating mitochondria localization of Pro-DC. Panel d shows the Pearson co-efficient = 0.93. Scale bar 10 μm.

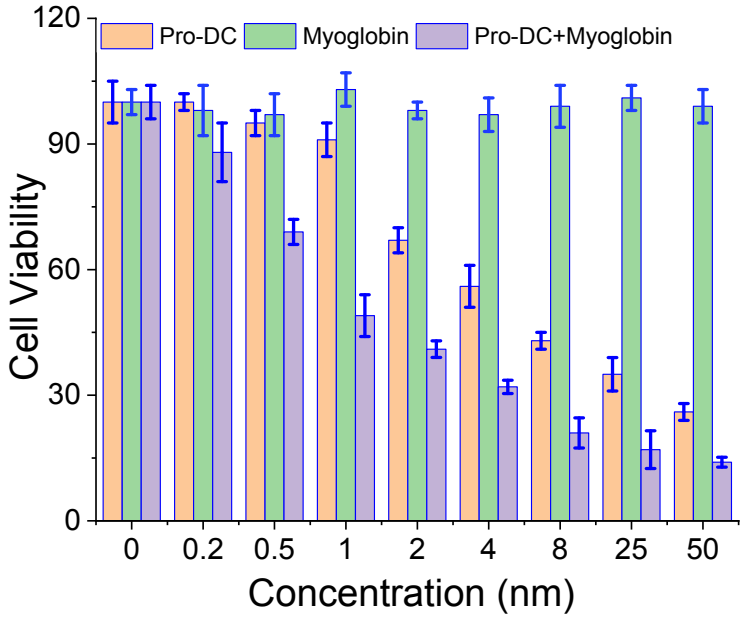


Figure S10. Viability of MCF-7 cells upon treatment with different concentrations of Pro-DC, Myoglobin and Pro-DC+Myoglobin under blue light irradiation.

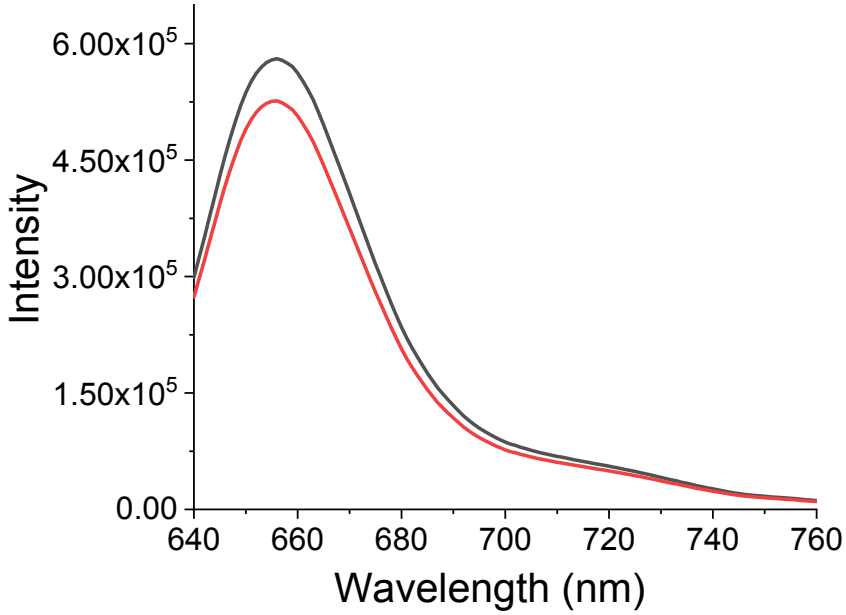
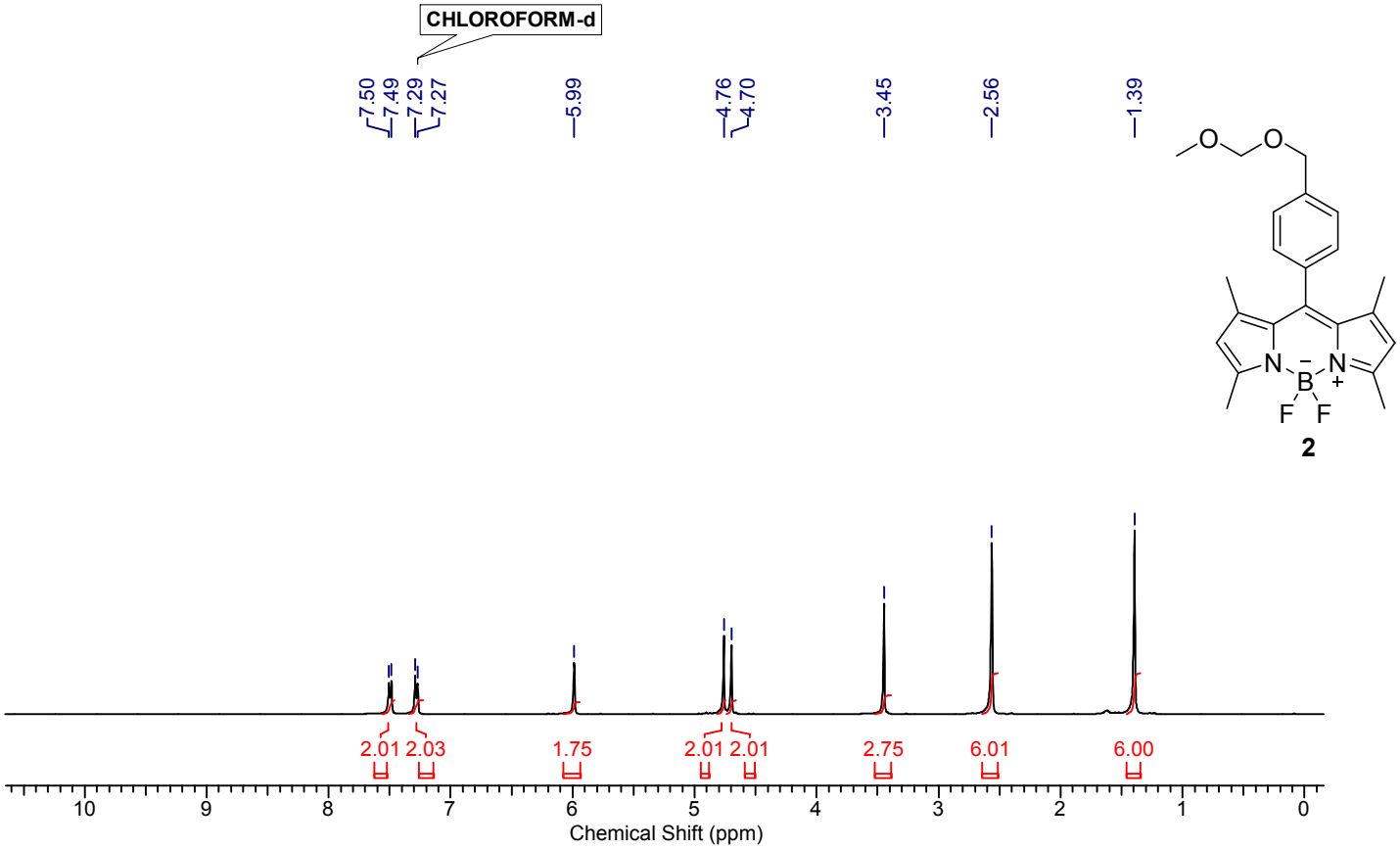
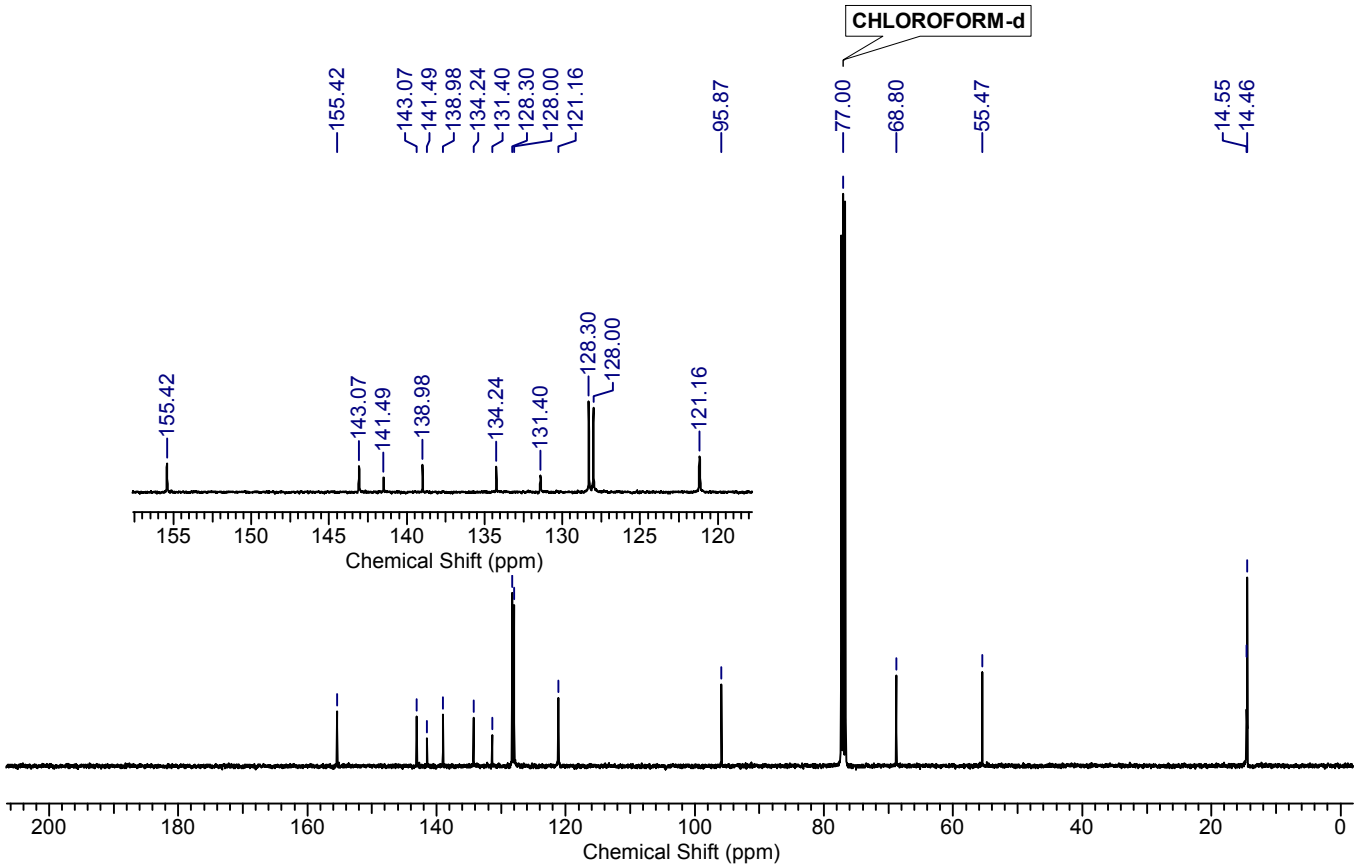


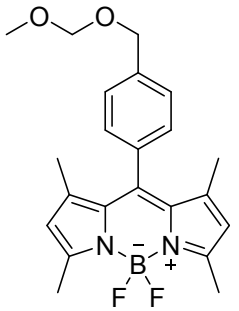
Figure S11. Emission spectra of Pro-DC (1 μM) in aq. HEPES buffer solution (10 mM, pH 7.4) upon irradiation with a blue LED lamp (λ_{irr} = 400 nm, 1.0 mW/cm^2). The gray line is after 80 minutes and the red line is after 2.5 hours.

^{1}H NMR (400 MHz, $CDCl_3$)



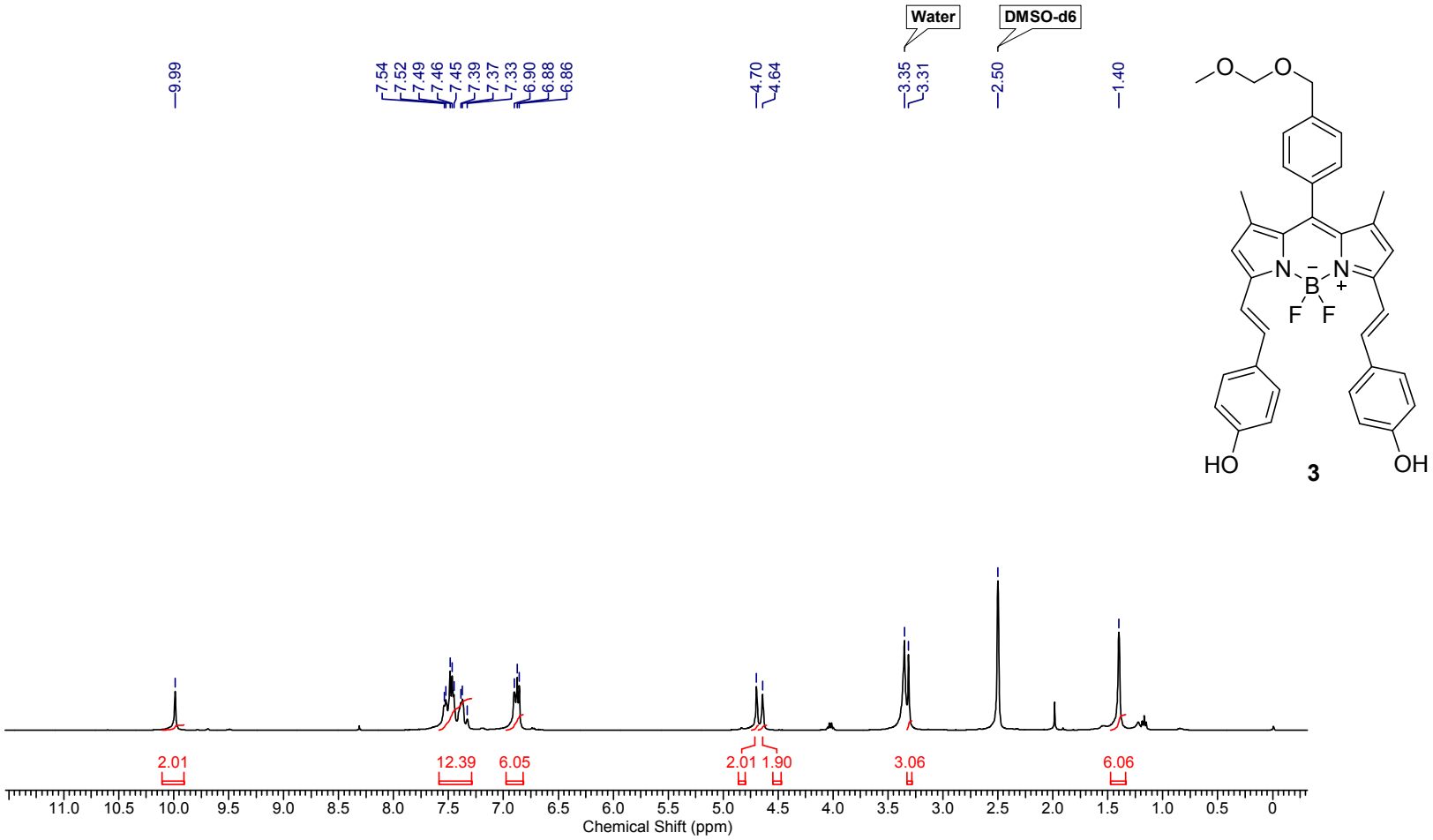
^{13}C NMR (100 MHz, $CDCl_3$)



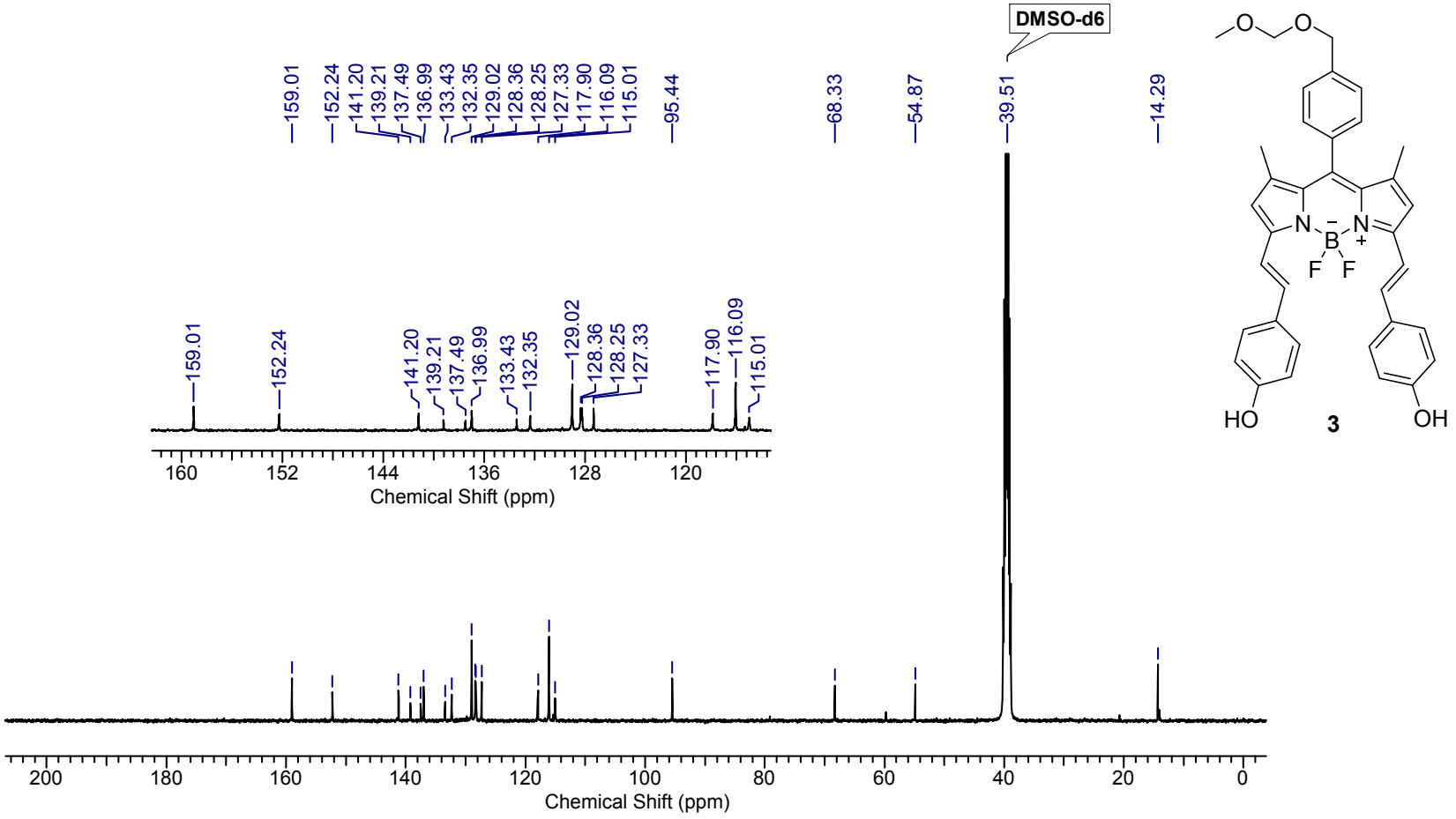


2

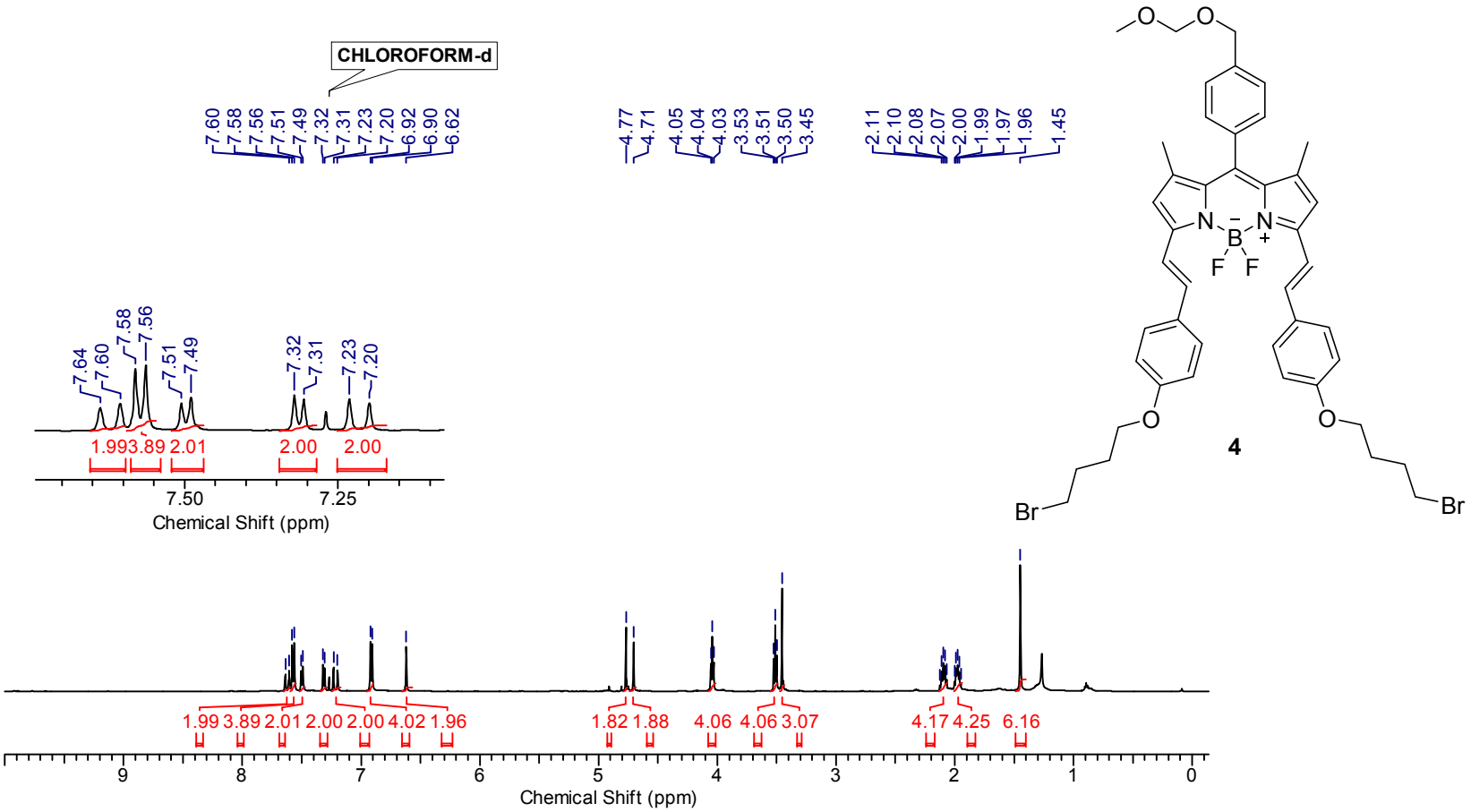
^{1}H NMR (400 MHz, DMSO-d_6)



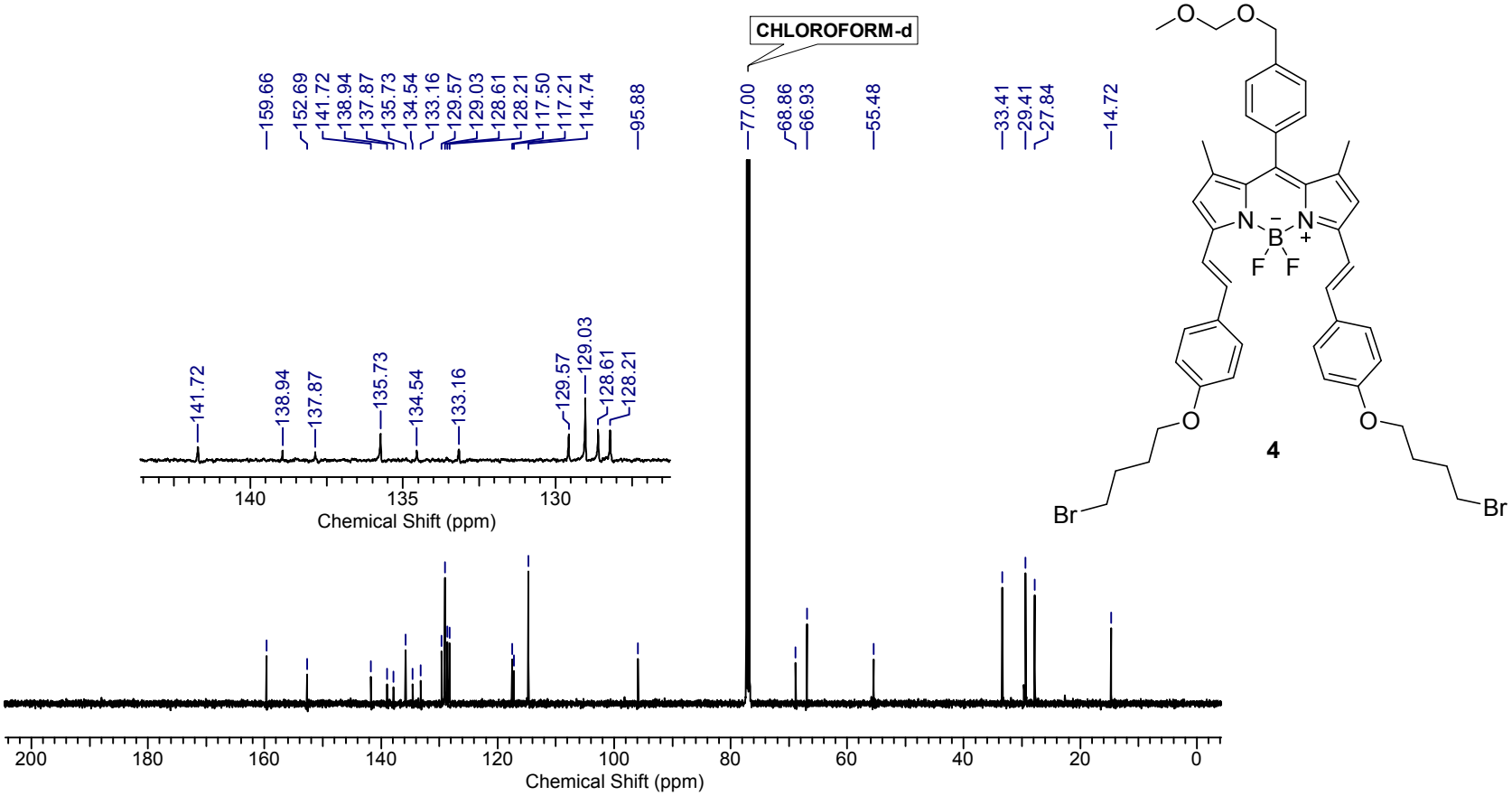
^{13}C NMR (100 MHz, DMSO-d_6)



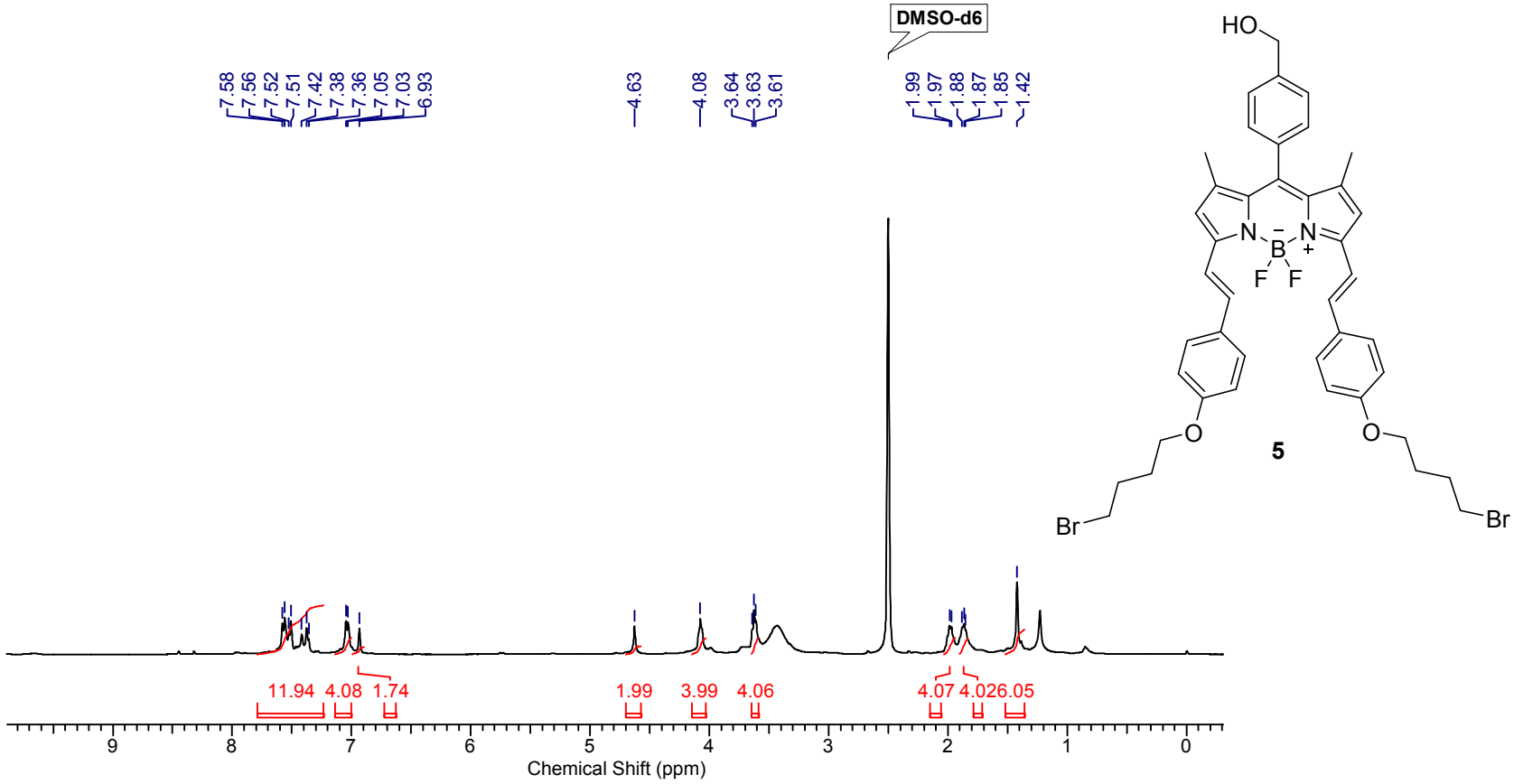
^{1}H NMR (400 MHz, $CDCl_3$)



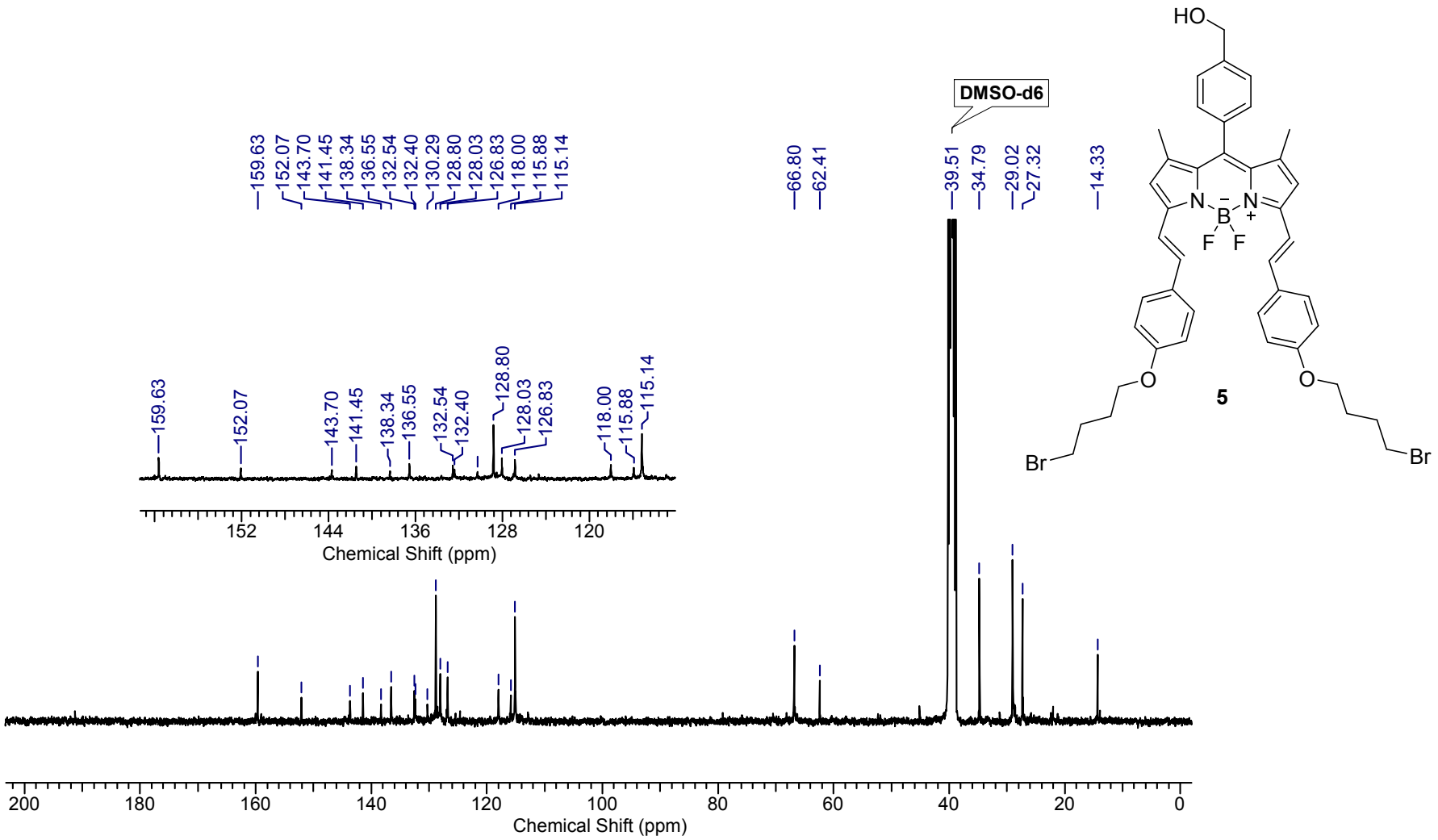
^{13}C NMR (100 MHz, $CDCl_3$)



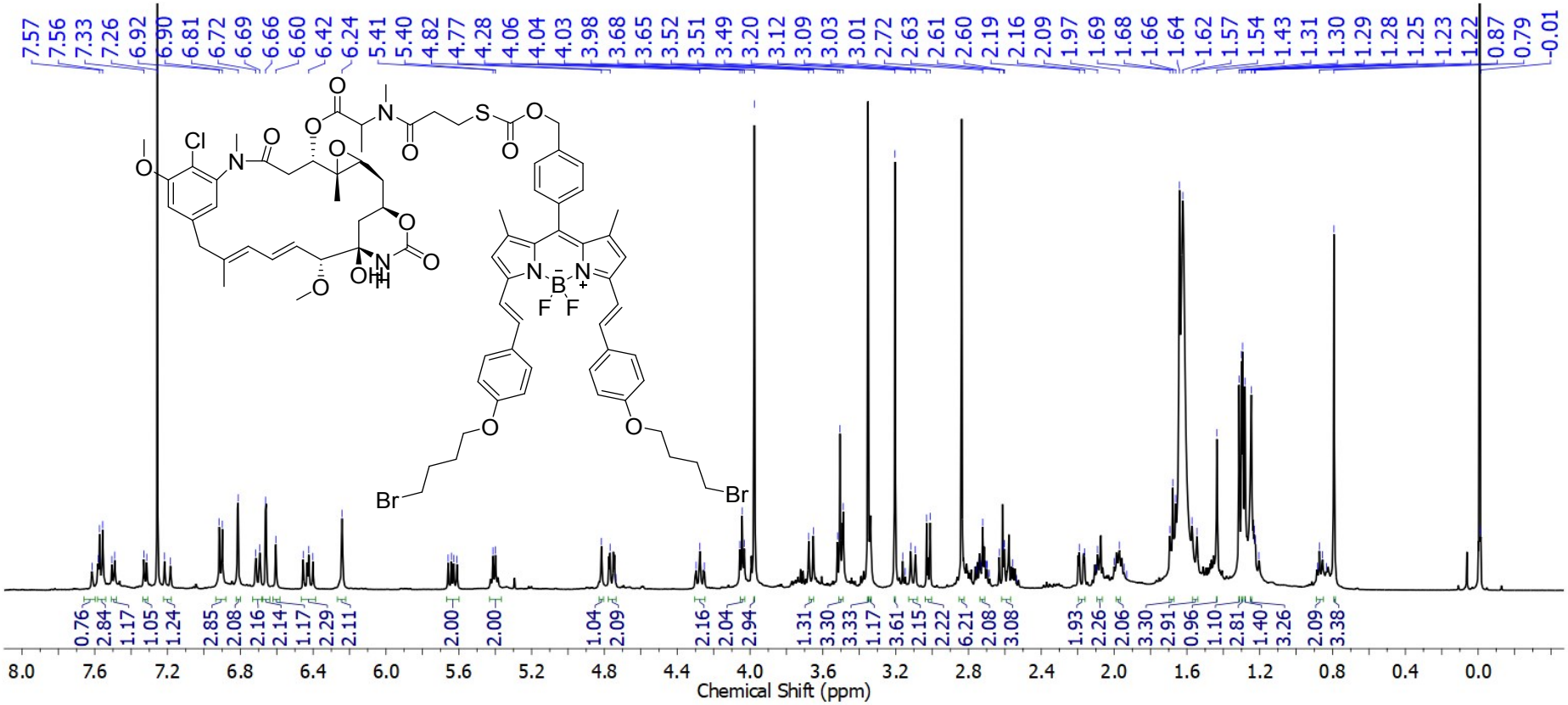
^{1}H NMR (400 MHz, DMSO-d_6)



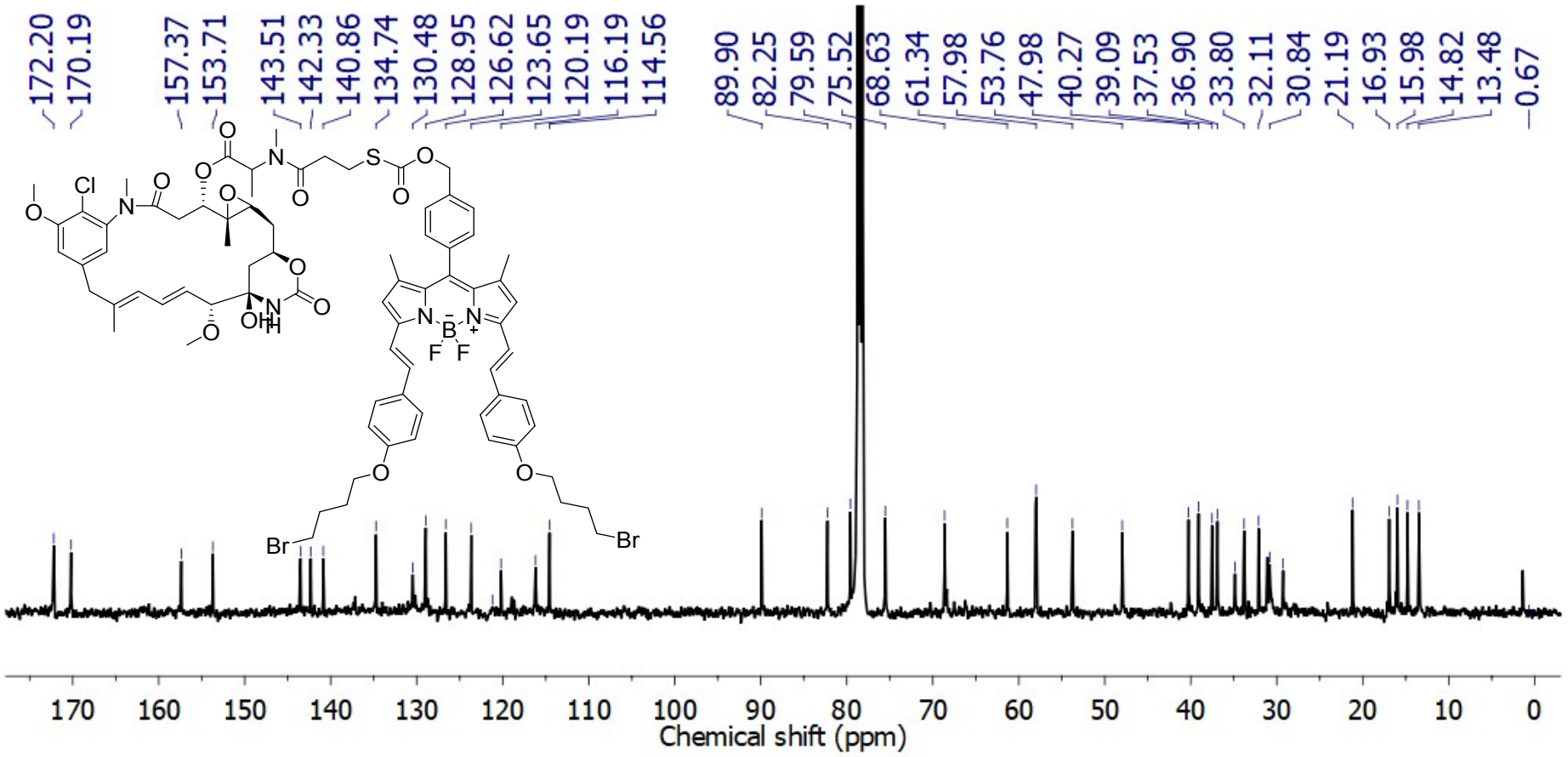
^{13}C NMR (100 MHz, DMSO-d_6)



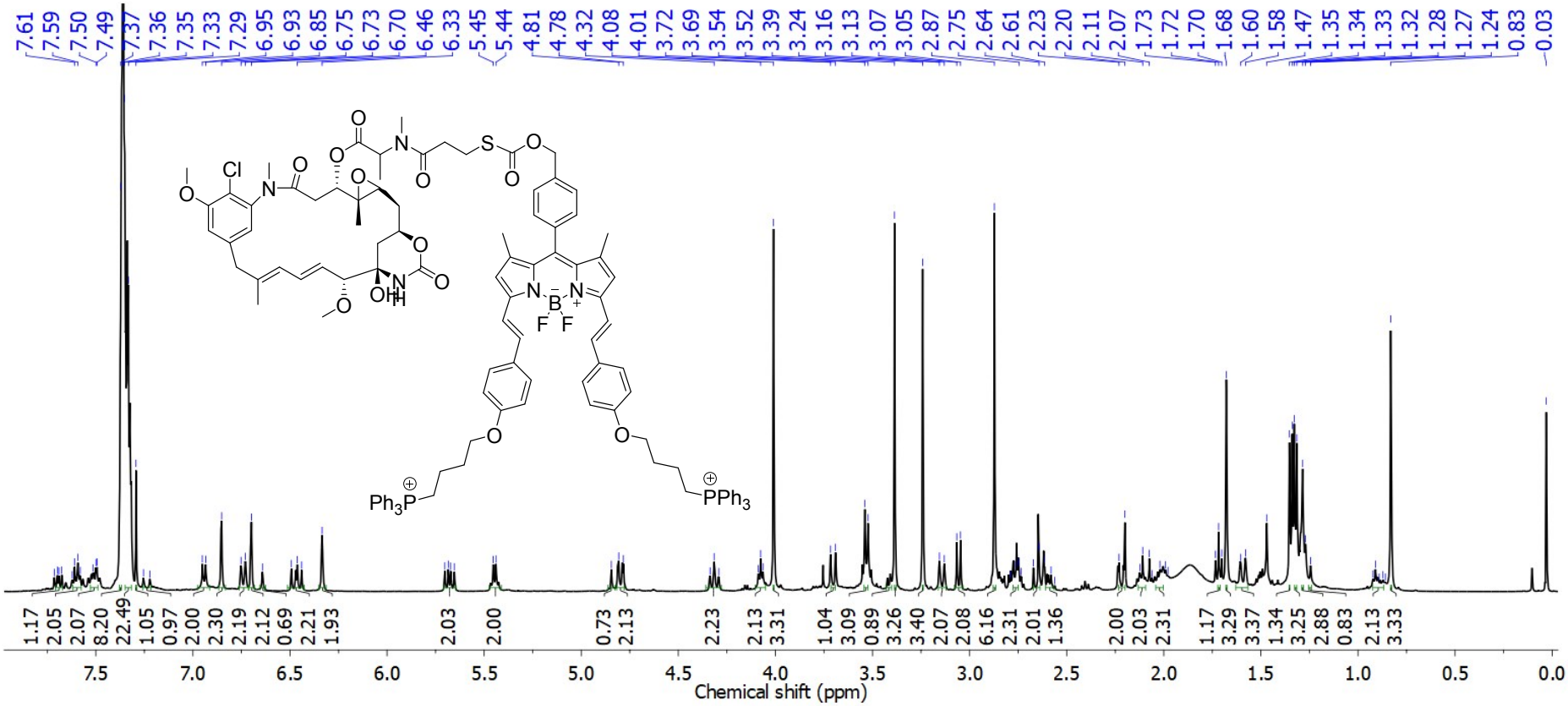
^{1}H NMR (500 MHz, $CDCl_3$-d_3)



^{13}C NMR (125 MHz, DMSO-d_6)



^{1}H NMR (500 MHz, $CDCl_3$-d_3)



^{13}C NMR (125 MHz, DMSO-d_6)