Supporting Information for

Near-Infrared Fluorogenic Imaging of Carbon Monoxide in Live Cells Using Palladium-Mediated Carbonylation

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Materials and Instruments

Lipopolysaccharide (LPS), Zinc protoporphyrin (ZnPP), hemin and (OC-6-44)-tricarbonylchloro (glycinato) ruthenium (CORM-3) were purchased from Sigma-Aldrich. Ultrapure water was obtained from a Milli-Q system. HepG2 cells were supplied by the cell bank of Central Laboratory at Xiangya Hospital (Changsha, China). All other chemical reagents were purchased from commercial suppliers without further purification unless otherwise stated.

Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 (Qingdao Ocean Chemicals), and column chromatography was performed on silica gel (mesh 200-300, Qingdao Ocean Chemicals). Mass spectra (MS) were recorded on an LTQ Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) and an LCQ Advantage ion trap mass spectrometry (Thermo Finnigan). ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker DRX-400 NMR spectrometer (Bruker) using tetramethylsilane (TMS) as an internal standard. UV-vis absorption spectra were recorded on a UV-1800 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were recorded with an FS5 spectrofluorometer (Edinburgh, UK) with excitation and emission slits of 6.0 nm. Confocal fluorescence images were obtained on a Nikon A1+ confocal microscope (Japan).

In vitro characterizations

The probes including NIR-COP1 and NIR-COP2 were dissolved in DMSO (1 mM) and stored at 4 °C for future use. NIR-COP1 (1.0 μM, PBS/DMSO = 19:1, v/v) was incubated with different concentrations of CORM-3 at 37 °C 1 h. Fluorescence spectra were obtained with both excitation and emission slit widths of 6 nm. The samples were excited at 630 nm and fluorescence emission was collected from 645 nm to 850 nm. The detection limit was calculated according to Equation 1:

$$DL = 3\sigma/k \qquad (1)$$

DL: detection limit; σ: standard deviation of triplicate experiments; k: slope for the linear fitting curves of fluorescence intensities of NIR-COP1 toward the concentrations of CORM-3.

For selectivity assays, all solutions of various possible interfering species were prepared with ultrapure water, and NIR-COP1 (1.0 μM) was incubated with each substance: (0) Blank; (1) H_2O_2 (50 μM); (2) HClO (50 μM); (3) PBS; (4) Cu^{2+} (0.5 mM); (5) Fe^{3+} (50 μM); (6) Zn^{2+} (50 μM); (7) H_2S (50 μM); (8) NO_2^- (50 μM); (9) Tert-butyl hydroperoxide (TBHP) (50 μM); (10) L-Ascorbic acid sodium salt (AA) (50.0 μM) or (11) CORM-3 (50 μM) at 37 °C for 1.0 h. For studying the response of NIR-COP2 to H_2S, NIR-COP2 (1.0 μM) was incubated with H_2S (50 μM) at 37 °C for 1.0 h.

The response time of NIR-COP1 toward CO was investigated via time-dependent fluorescence measurements. NIR-COP1 (1.0 μM) was incubated with CORM-3 (50 μM) at 37 °C. Fluorescence spectra were obtained at different time periods (0, 5, 15, 25, 35, 45, 55 and 65 min).

Cell culture and confocal fluorescence imaging

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 U/mL) and incubated at 37 °C in a humidified incubator containing 5% (v/v) CO_2.

CCK-8 assay

The cytotoxicity of NIR-COP1 against HepG2 cells were studied using a standard MTT assay. HepG2 cells were seeded into a 96-well plate with 5×10^3 cells/well in 100 μL culture medium. The cells were grown at 37 °C for 24 h. Then, the cells were then incubated with a fresh culture medium containing NIR-COP1 (2 μM) with or without CORM-3 (50 μM) for 0-8 h. Afterwards, the culture medium was removed and the cells were washed with cold PBS for three times. The cells were incubated with CCK-8 reagent at 37 °C for 4 h. DMSO (100 μL/well) was then added for 10 min to dissolve the precipitated formazan violet crystals. The absorbance at 450 nm was recorded using an ELx800 microplate reader.

NIR Fluorescence Imaging in Cells.

HepG2 cells were incubated with 1 mL of fresh culture medium containing NIR-COP1 (1 μM) at 37 °C for 30 min. The cells were treated with different concentrations of CORM-3 (15-100 μM) for 30 min. The cells were then washed with cold PBS for three times.

For investigating the ability of NIR-COP1 to image endogenous CO, HepG2 cells were treated with heme (20 μM) for 8 h and then incubated with NIR-COP1 (1 μM) for 30 min. For imaging CO in a cellular model of inflammation, HepG2 cells were stimulated with LPS (2 mg/mL) for 24 h and then incubated with NIR-COP1 (1 μM) for 30 min. For imaging CO in a cellular model of oxidative stress, HepG2 cells were treated with APAP (400 μM) for 12 h, treated with or without ZnPP (10 μM) for 12 h, and incubated with NIR-COP1 (1 μM) for 30 min. The control group was only treated with NIR-COP1 (1 μM). For imaging the dynamic responses of the probe toward CO in live cells, the cells were pretreated with CORM-3 (50 μM) and then treated with NIR-COP1 (2 μM), fluorescence images were obtained at different time intervals.

Confocal fluorescence images were acquired with a Nikon A1+ confocal microscope with an excitation wavelength of 640 nm and an emission collection range of 663 - 738 nm.

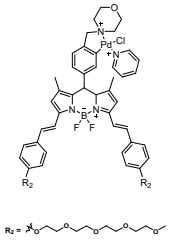
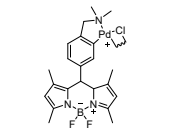
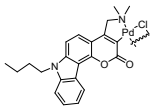
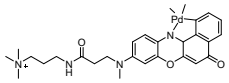
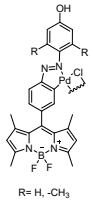
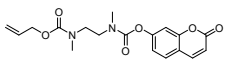
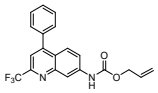
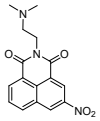
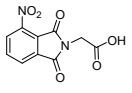
Flow cytometric analysis.

HepG2 cells cultured for 24 h were first incubated with NIR-COP1 for 30 min, and then incubated with different concentrations of CORM-3 for 1 h. The cells were washed three times with PBS, detached with trypsin and washed twice with PBS. The cells were suspended in PBS for flow cytometry analysis on a CytoFLEX flow cytometer. Cells treated with NIR-COP1 only were used as a control. The mean fluorescence was determined by counting 20,000 events on the CytoFLEX flow cytometer. Flow cytometry data were analysed using FlowJo 7.6.

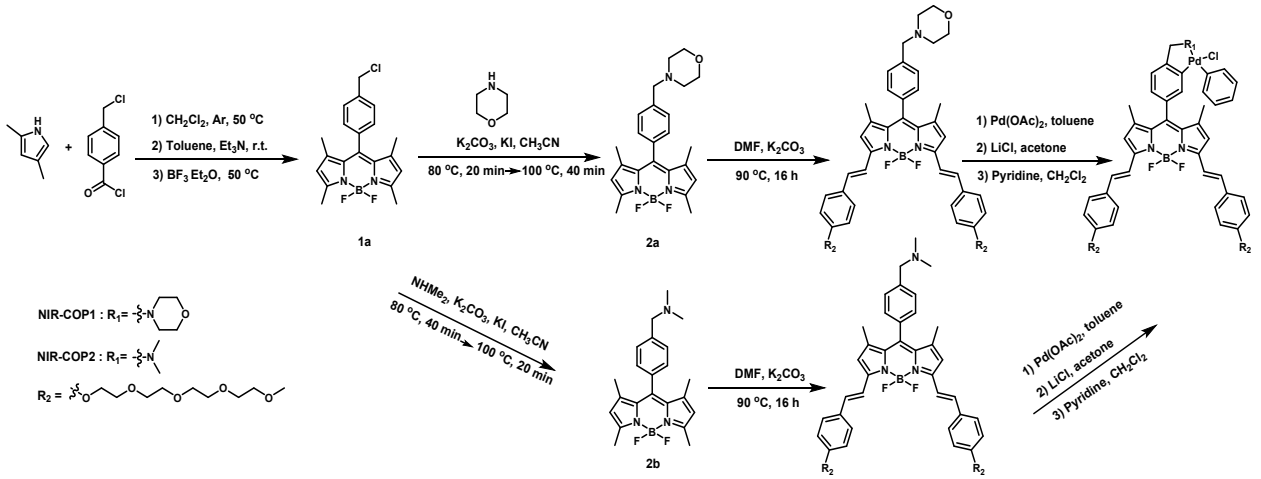
Subcellular colocalization assay.

HepG2 cells were incubated with 1 mL fresh culture medium containing NIR-COP1 (5 μM) at 37 C for 30 min. The cells were then incubated with Hoechst 33342 (0.5 $\mu g \cdot mL^{-1}$), Mito-Tracker Green (0.5 μM) or Lyso-Tracker Green (100 nM) for 30 min and washed with cold PBS twice. To obtain fluorescence signals from Lyso-Tracker Green and Mito-Tracker Green, the emission wavelength was collected from 500 nm to 540 nm with an excitation wavelength of 488 nm. Fluorescence signals for Hoechst 33342 were collected from 440 nm to 480 nm with an excitation wavelength of 405 nm.

Table S1. Analytical characteristics of typical fluorescent probes for CO.

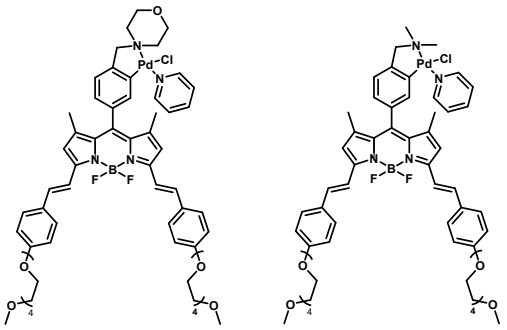
Probe structure	Excitation (nm)	Emission (nm)	Response ratio (CO)	Response ratio (H_2S)	LOD	Ref.
	630	665	12	<2.8	0.3 μM	This work
	499	573	10	3.5	N/A	J.Am.Chem.Soc.2012, 134, 15668−15671
	378	477	11	<2.8	0.65 μM	Chem. Sci., 2014, 5, 3439–3448.
	543	650	25	3 (GSH 5.5)	0.23 μM	Chem. Sci., 2019, 10, 320–325
	498	512	9	1	0.72 μM	Anal. Chem. 2016,88, 11154−11159
	340	460	150	1	7.77 nM	Chem. Commun., 2015, 51, 4410--4413
	425	520	<11	NO	41 nM	Sens. Actuators, B, 2021, 347, 130631.
	405	550	280	NO	19.8 nM	Sens. Actuators, B, 2021, 344:130245.
	390	503	10	NO	16 nM	Anal. Chem., 2019, 91, 8602 —8606

Scheme S1. Synthesis routes (a) and structures (b) for NIR-COP1 and NIR-COP2.

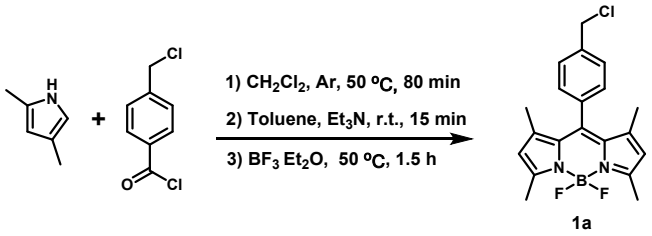


(a)

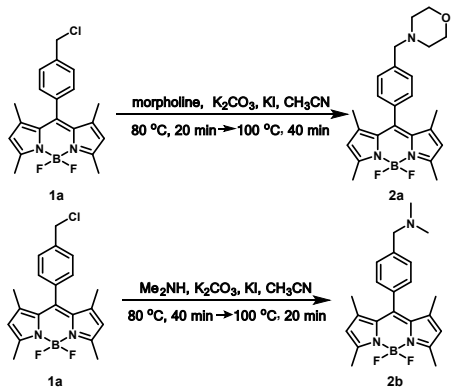
(b)



Synthetic procedures for intermediates and final compounds



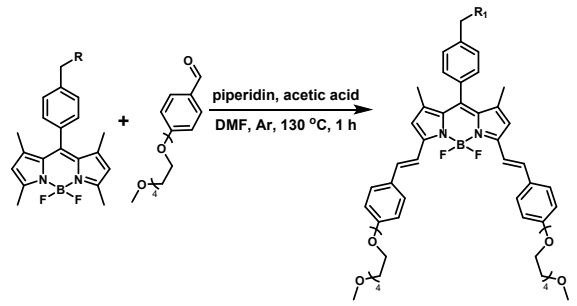
Compound 1a: Compound 1a was prepared according to literature with slight modifications.[1] Briefly, 2,4-dimethylpyrrole were (2.1 eq) was refluxed with 4-(chloromethyl) benzoylchloride (1.0 eq) in dichloromethane (DCM) for 2 h under nitrogen protection. Then the reaction was rotavaped. Toluene and DCM were added to dissolve the product followed by addition of triethylamine (4.26 eq). The mixture was stirred at room temperature for 30 min. Boron trifluoride etherate (5.4 eq) was added at 50 °C and stirred for another 1 h. The mixture was purified by column chromatography using hexanes and dichloromethane as the elution solvent (gradient from 1:1 to 3:1 (v/v)). Compound 1a was obtained as an orange powder with an overall yield of 21%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.51 (d, *J* = 7.2 Hz, 2H), 7.27 (d, *J* = 7.5 Hz, 2H), 5.98 (s, 2H), 4.65 (s, 2H), 2.55 (s, 6H), 1.37 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-d) δ 155.80, 143.17, 141.07, 138.73, 135.23, 131.46, 129.39, 128.55, 121.48, 45.74, 29.84, 14.61.



Compound 2a and 2b: Compound 2a and 2b were prepared according to literature with slight modifications.[1]

Compound 2a: Compound 2 (1.0 eq, 376 mg), K_2CO_3 (2.0 eq, 276 mg) and KI (2.0 eq, 336 mg) were added to a flask (25 mL). Then, CH_3CN (5.0 mL) and morpholine (4 eq, 520 mg) were added. The reaction mixture was heated at 80 °C for 20 min and then 100 °C for 40 min. After cooling, the mixture was diluted with CH_2Cl_2 (20 mL), transferred to a separatory funnel and washed with water (2 mL) and brine (1.5 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel using CH_2Cl_2 : hexanes (1:10→1:8→ 1:4 (1% triethylamine)) to afford compound 2a (232 mg, 0.55 mmol) as an orange solid (yield: 55%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.44 (d, *J* = 7.8 Hz, 2H), 7.21 (d, *J* = 6.4 Hz, 2H), 5.96 (s, 2H), 3.72 (t, *J* = 4.7 Hz, 4H), 3.57 (s, 2H), 2.53 (s, 6H), 2.44 (t, *J* = 4.7 Hz, 4H), 1.36 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-d) δ 155.44, 143.08, 141.77, 138.78, 133.88, 129.89, 127.97, 121.27, 67.04, 63.11, 53.62, 14.63, 14.41.

Compound 2b: Compound 2 (1.0 eq, 376 mg), K_2CO_3 (2.0 eq, 276 mg) and KI (2.0 eq, 336 mg) were added to a flask (10 mL). Then, CH_3CN (5.0 mL) and dimethylamine (20 eq, 2.3 mL aq. 40 wt%, 20 mmol) were added. The reaction mixture was heated at 80 °C for 40 min and then 100 °C for 20 min. After cooling, the mixture was diluted with CH_2Cl_2 (20 mL), transferred to a separatory funnel and washed with water (2 mL) and brine (1.5 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting residue was purified by flash chromatography on silica gel using CH_2Cl_2 : hexanes (1:10→1:8→ 1:4 (1% triethylamine)) to afford compound 2b (186.7 mg, 0.49 mmol) as an orange solid (yield: 49%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.38 (d, *J* = 7.7 Hz, 2H), 7.15 (d, *J* = 7.7 Hz, 2H), 5.92 (s, 2H), 3.45 (s, 2H), 2.49 (s, 6H), 2.21 (s, 6H), 1.34 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-d) δ 155.40, 143.16, 141.86, 139.91, 133.83, 131.55, 130.02, 127.93, 121.26, 64.12, 45.33, 14.63, 14.40.

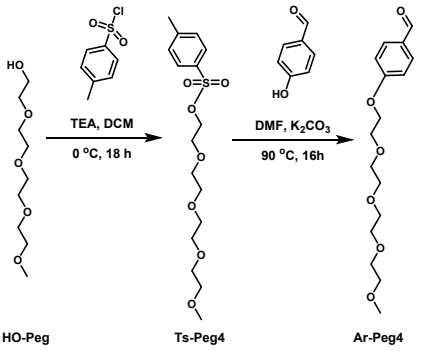


Ar-Peg4

3a: R_1=morpholine
3b: R_1=NMe_2

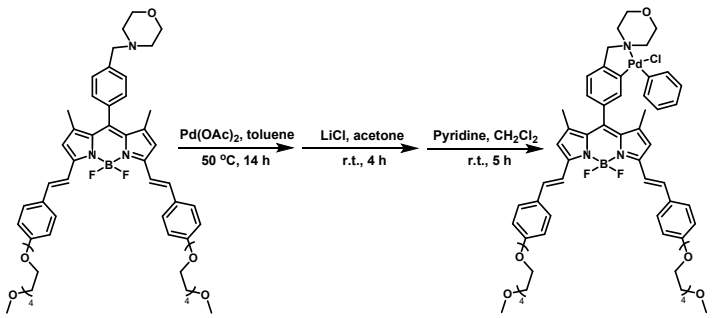
Compound 3a: Compound 2a (1eq, 84.6 mg) and 4-((2,5,8,11-tetraoxatridecan-13-yl)oxy)benzaldehyde (4eq, 249.9 mg) were dissolved in DMF. Pyrrolidine (0.026 eq, 0.3 mL) and acetic acid (0.013eq, 0.3 mL) were added via stirring. The reaction mixture was refluxed for 1.5 h. After cooling to room temperature, ethyl acetate and H_2O were added. The water layer was extracted with ethyl acetate and the combined organic layers were washed with saturated bicarbonate solution (3×), H_2O (3×) and brine (1×). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The resulting residue was purified by flash chromatography on silica gel using CH_2Cl_2 : Hexanes : Triethylamine (5:50:1→10:40:1→ 25:25:1) to afford Compound 3b (54.6 mg, 0.054 mmol) as a blue viscous solid (yield: 27%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (q, *J* = 12.7, 8.1 Hz, 6H), 7.53 (d, *J* = 7.2 Hz, 2H), 7.33 (d, *J* = 7.5 Hz, 3H), 7.27 (s, 1H), 7.02 (d, *J* = 8.0 Hz, 4H), 6.69 (d, *J* = 6.3 Hz, 2H), 4.25 (s, 4H), 3.97 (s, 4H), 3.80 (d, *J* = 22.6 Hz, 26H), 3.64 (s, 4H), 3.46 (d, *J* = 7.2 Hz, 6H), 2.56 (s, 4H), 1.50 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-d) δ 159.66, 152.67, 141.67, 138.04, 135.80, 134.17, 133.20, 129.81, 129.65, 129.02, 128.52, 117.55, 117.21, 114.95, 71.95, 70.86, 70.65, 70.63, 70.53, 69.68, 67.53, 66.93, 65.86, 63.03, 59.05, 53.52, 14.63.

Compound 3b: Compound 3b, prepared similarly as Compound 3a using Compound 2b, was obtained as a blue viscous solid with a yield of 27%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.61 – 7.50 (m, 6H), 7.40 (s, 2H), 7.19 (t, *J* = 16.6 Hz, 4H), 6.91 (d, *J* = 6.9 Hz, 4H), 6.57 (s, 2H), 4.12 (s, 4H), 3.84 (s, 4H), 3.67 (d, *J* = 23.8 Hz, 20H), 3.52 (s, 6H), 3.34 (s, 6H), 2.24 (s, 6H), 1.40 (s, 6H). ^{13}C NMR (100 MHz, Chloroform-d) δ 159.32, 152.33, 141.39, 138.62, 137.72, 135.44, 133.91, 132.86, 129.72, 129.35, 128.68, 128.19, 117.23, 116.90, 114.62, 71.61, 70.52, 70.30, 70.27, 70.18, 69.34, 67.20, 63.36, 58.69, 44.54, 14.27, 8.59.



Ts-Peg4: Compound HO-Peg (1.0 eq, 104.1 mg), 4-hydroxybenzaldehyde (1.0 eq, 190 mg) and TEA (1.9 eq, 0.26 mL) were dissolved in DCM, and the mixture was stirred at 0 °C for 18 h. After reaction, DCM and water were added and the water layer was extracted with DCM. The combined organic layers were washed with HCl (3×), saturated bicarbonate (3×) and brine (1×). The organic layer was dried over $MgSO_4$, and removed by evaporation under reduced pressure to obtain Ts-Peg4 as a yellow liquid (yield: 92%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.45 (d, *J* = 8.0 Hz, 2H), 7.05 (d, *J* = 8.4 Hz, 2H), 3.83 (q, *J* = 7.2, 4.6 Hz, 2H), 3.35 – 3.16 (m, 14H), 2.98 (s, 3H), 2.10 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-d) δ 143.87, 132.17, 128.99, 126.86, 70.84, 69.45, 69.41, 69.37, 69.30, 68.64, 67.51, 57.61, 20.37.

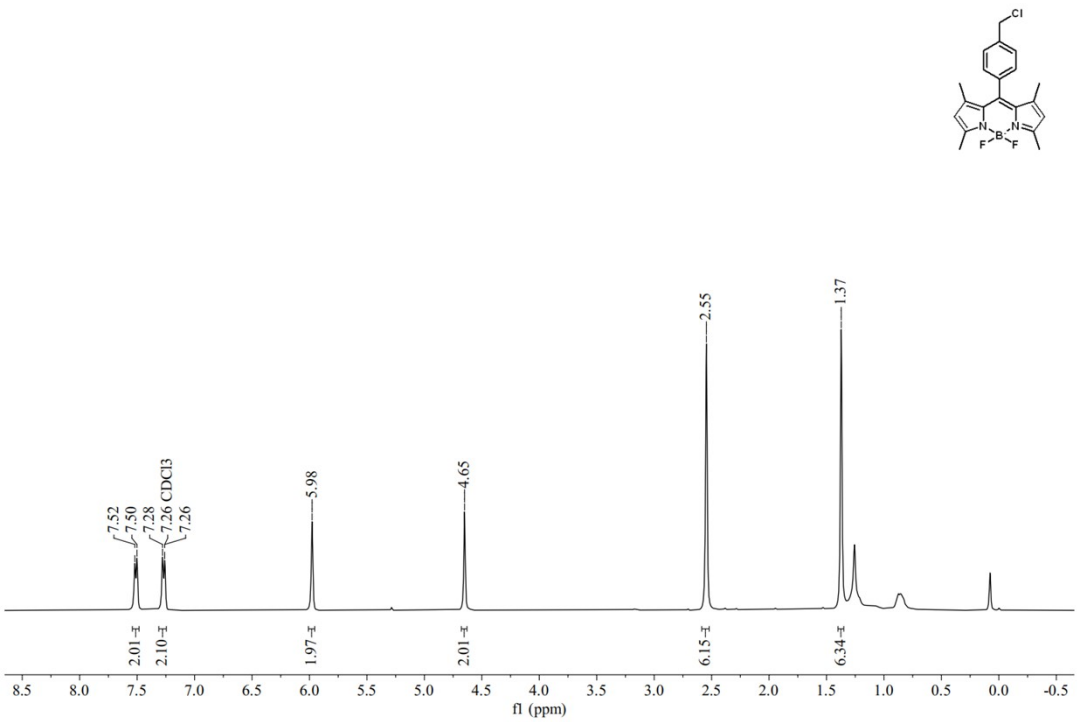
Ar-Peg4: Compound Ts-Peg (1eq, 181.2 mg), 4-hydroxybenzaldehyde (1.1eq, 67.2 mg) and K_2CO_3 (2eq, 138.2 mg) were dissolved in DMF, and the mixture was stirred at 90 °C for 16 h. After cooling to room temperature, ethyl acetate and water were added. The water layer was extracted with ethyl acetate and the combined organic layers were washed with saturated bicarbonate (3×), water (3×) and brine (1×). The organic layer was dried over $MgSO_4$, and removed by evaporation under reduced pressure to obtain Ar-Peg4 as a light-yellow liquid (yield: 90%). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.83 (s, 1H), 7.78 (d, J = 8.3 Hz, 2H), 6.97 (d, J = 8.2 Hz, 2H), 4.17 (s, 2H), 3.84 (s, 2H), 3.68 (s, 2H), 3.62 (s, 8H), 3.49 (s, 2H), 3.32 (s, 3H). ^{13}C NMR (100 MHz, Chloroform-d) δ 190.78, 163.85, 131.92, 130.01, 114.87, 71.89, 70.86, 70.58, 70.56, 70.47, 69.43, 67.75, 58.98.



NIR-COP1: Compound 3a (1 eq, 153.7 mg) and $Pd(OAc)_2$ (0.95eq, 34.1 mg) were dissolved in toluene. The mixture was stirred at 50 °C for 14 h in the dark. After reaction, CH_2Cl_2 was added, filtered over Celite and concentrated in vacuo. A saturated solution of LiCl in acetone (6 mL) was added and stirred at room temperature for 4 h. The mixture was concentrated in vacuo, dissolved in CH_2Cl_2, and filtered over Celite to remove excess LiCl. Hexanes were then added and the precipitate was collected with a funnel. The precipitate was dissolved in CH_2Cl_2. Pyridine (2.2 eq, 1.3 μL) was added, and the solution was stirred for 4 h. Hexanes were added, and the precipitate was collected with a funnel to afford NIR-COP1 as a dark blue solid (yield: 55%). 1H NMR (400 MHz, Chloroform-d) δ 8.81 (d, J = 5.0 Hz, 1H), 8.74 (d, J = 6.4 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.52 (d, J = 8.6 Hz, 5H), 7.31 (t, J = 6.3 Hz, 3H), 7.19 – 7.09 (m, 3H), 6.91 (d, J = 8.5 Hz, 4H), 6.59 (s, 1H), 6.54 (s, 1H), 5.87 (s, 1H), 4.42 (s, 1H), 4.11 (d, J = 30.5 Hz, 7H), 3.85 (s, 6H), 3.75 – 3.57 (m, 24H), 3.52 (t, J = 4.7 Hz, 4H), 3.35 (s, 6H), 1.43 (s, 6H).^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.70, 153.38, 153.20, 152.45, 141.50, 138.70, 138.61, 135.72, 133.13, 132.40, 131.37, 129.66, 129.01, 125.58, 125.05, 124.80, 117.37, 117.19, 115.00, 71.97, 70.89, 70.67, 70.64, 70.55, 69.71, 67.57, 62.48, 59.07, 58.91, 14.60.

NIR-COP2: NIR-COP2, prepared similarly as NIR-COP1, was obtained Similarly to as a dark blue solid with a yield of 55%. ^{1}H NMR (400 MHz, Chloroform-*d*) 1H NMR (400 MHz, Chloroform-d) δ 8.89 (s, 1H), 8.85 (s, 1H), 7.84 (s, 1H), 7.61 (d, J = 6.4 Hz, 6H), 7.39 (s, 3H), 7.24 (d, J = 16.0 Hz, 2H), 7.14 (s, 1H), 7.01 (s, 4H), 6.64 (s, 1H), 6.01 (s, 1H), 4.24 (s, 4H), 4.13 (d, J = 3.0 Hz, 1H), 3.95 (s, 4H), 3.80 (s, 4H), 3.74 (s, 18H), 3.62 (s, 4H), 3.44 (s, 6H), 2.94 (d, J = 49.2 Hz, 6H), 1.54 (s, 6H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 159.62, 153.31, 153.18, 152.32, 148.06, 141.61, 139.22, 138.67, 138.46, 135.59, 133.13, 131.51, 129.62, 128.94, 125.50, 125.00,121.95, 117.31, 114.95, 71.92, 70.83, 70.61, 70.59, 70.49, 69.65, 67.52, 59.02, 52.69, 14.53.

Figure S1. ^{1}H NMR and ^{13}C NMR spectra for Compound 1a.



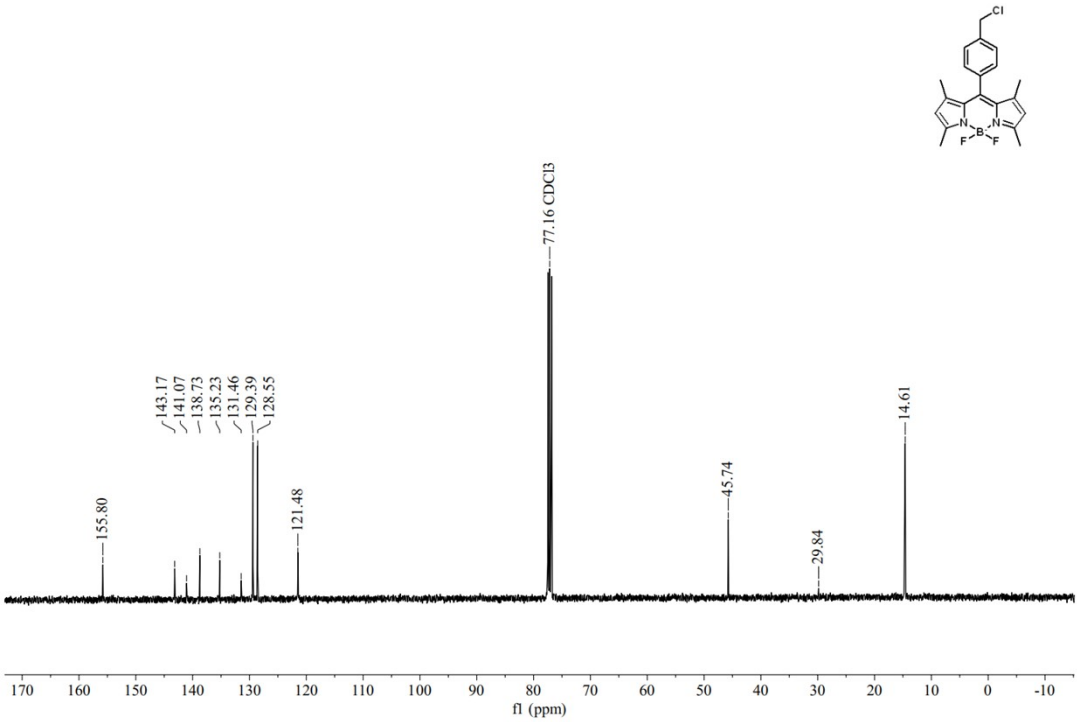


Figure S2. ^{1}H NMR and ^{13}C NMR spectra for compound 2a.

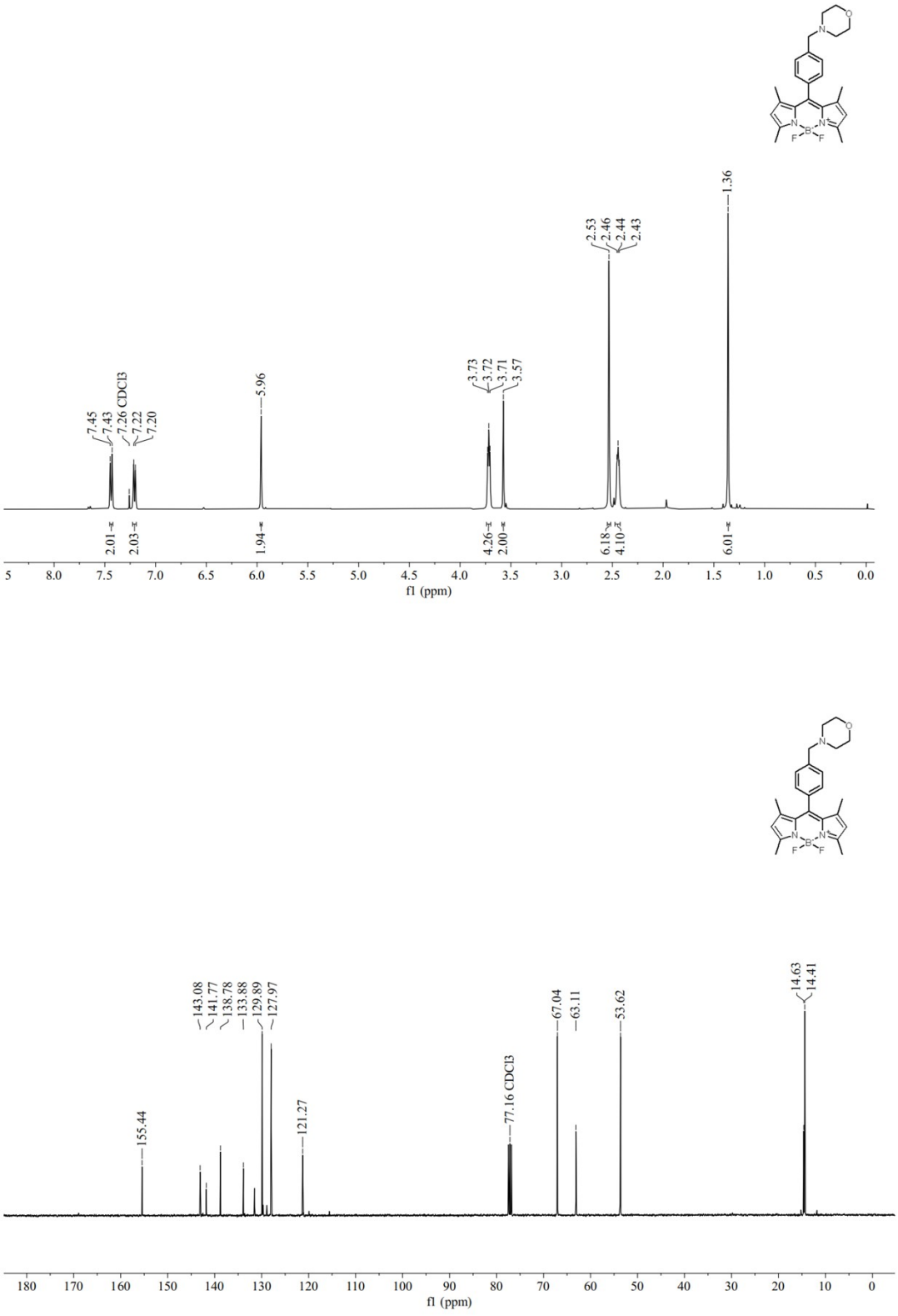
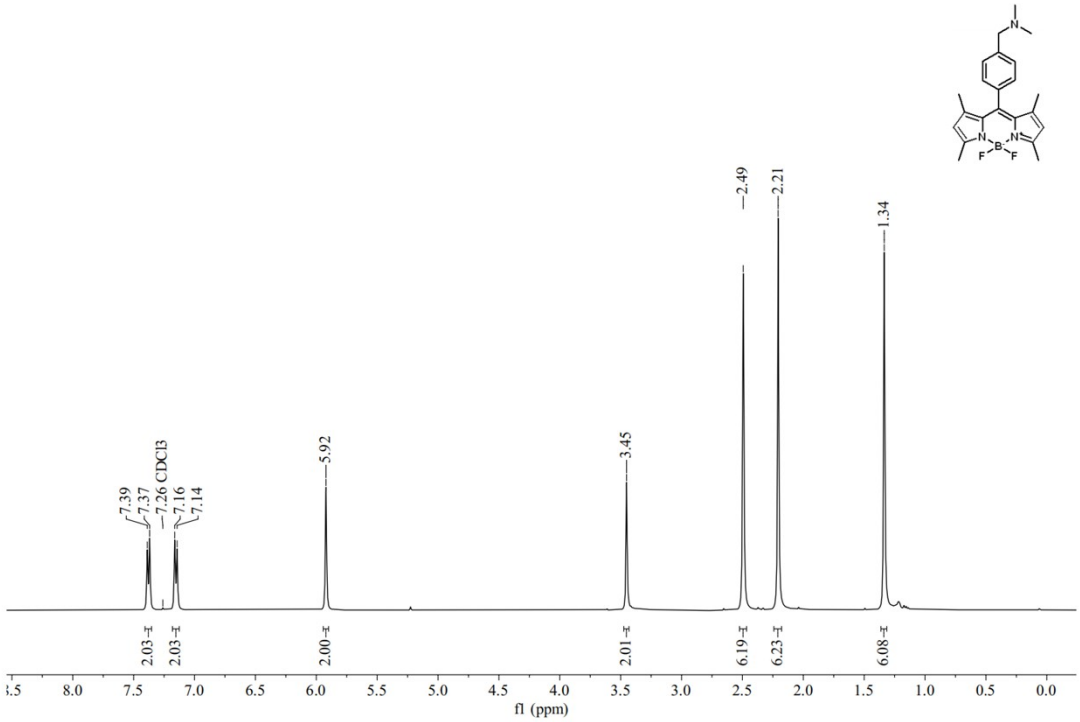


Figure S3. ^{1}H NMR and ^{13}C NMR spectra for Compound 2b.



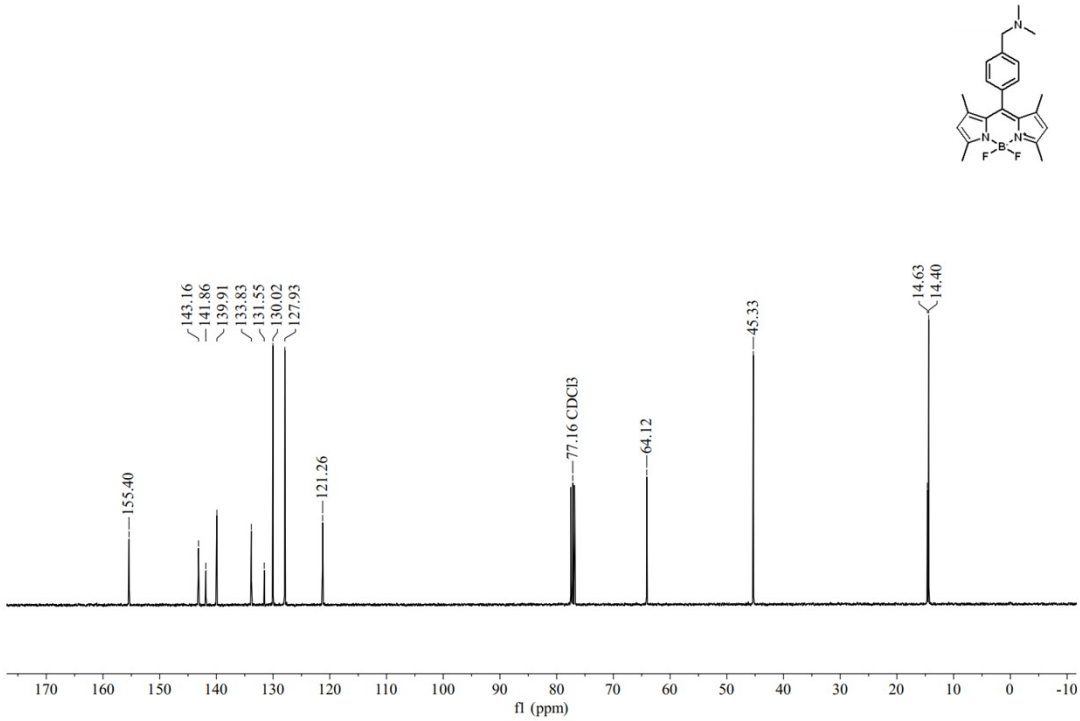
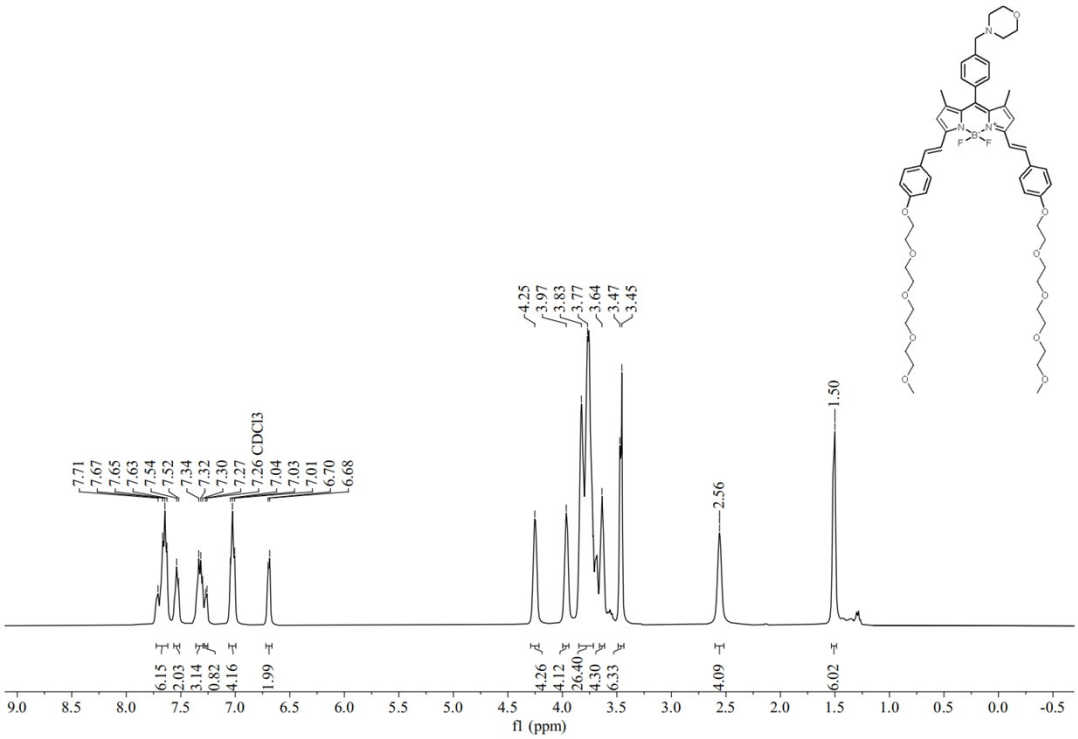


Figure S4. ^{1}H NMR and ^{13}C NMR spectra for compound 3b.



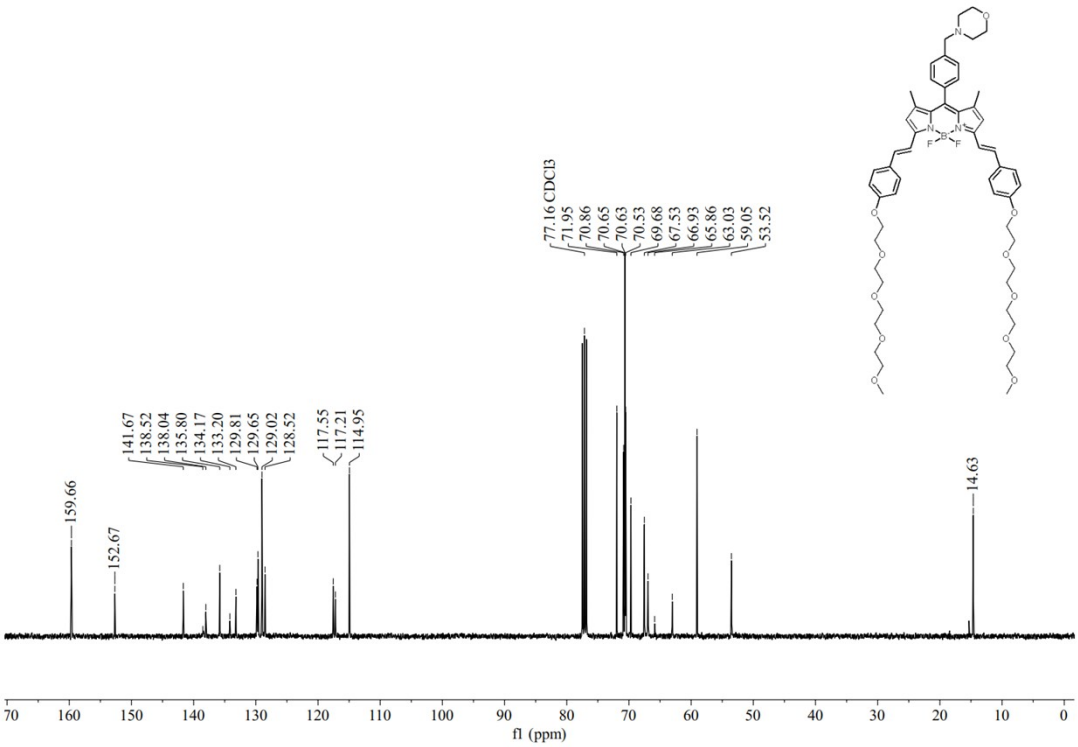


Figure S5. ^{1}H NMR and ^{13}C NMR spectra for compound 3b.

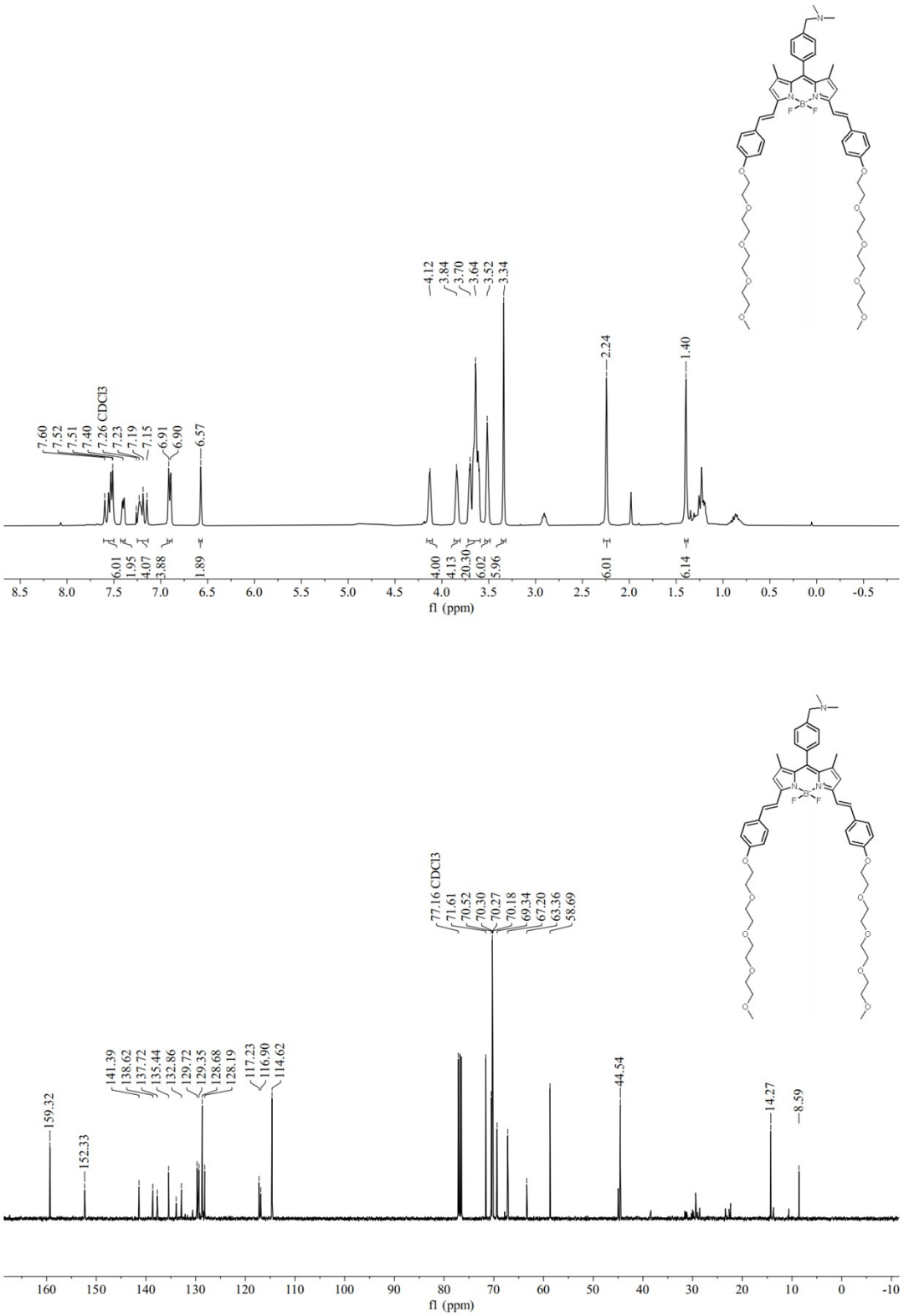
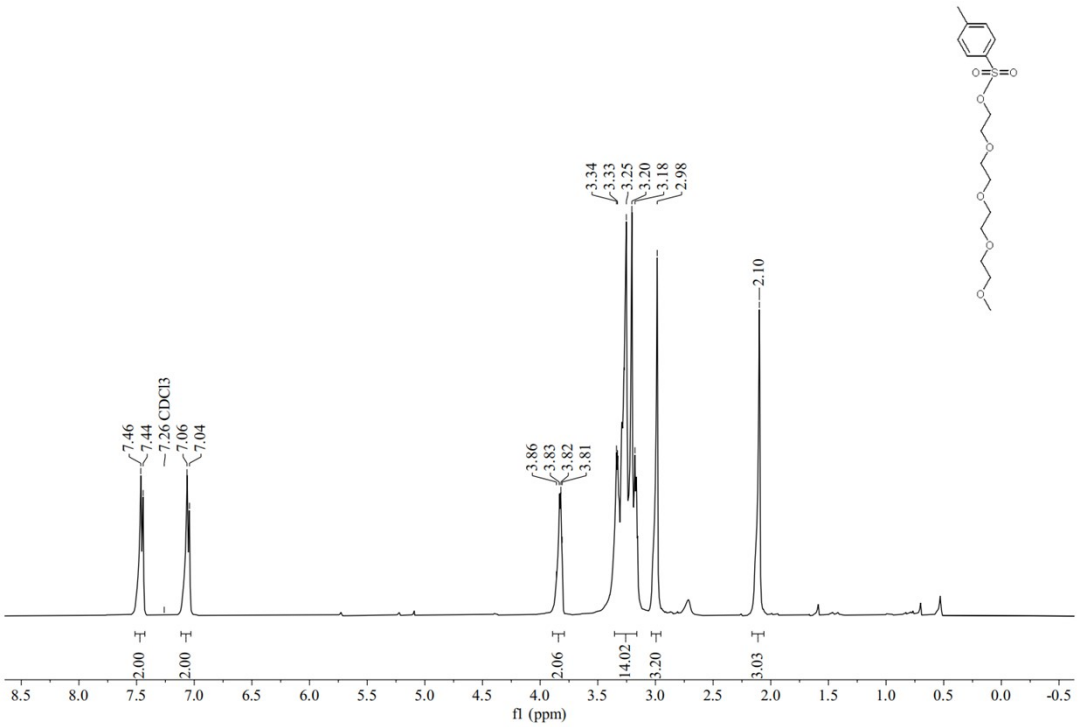


Figure S6. ^{1}H NMR and ^{13}C NMR spectra for compound Ts-Peg4.



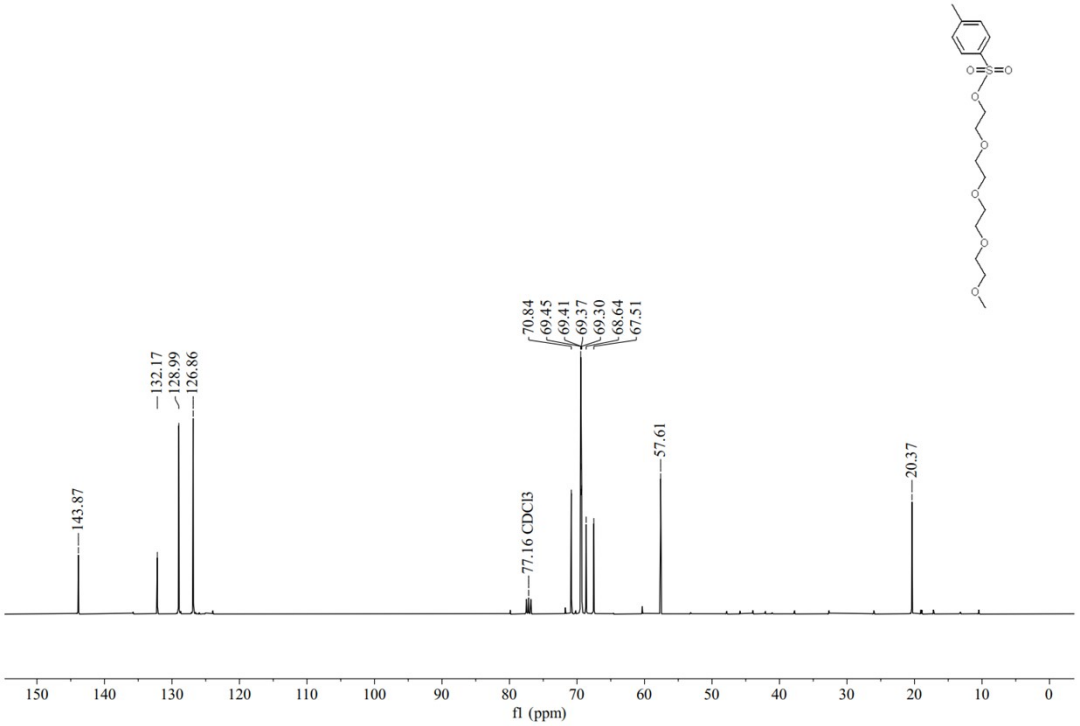


Figure S7. ^{1}H NMR and ^{13}C NMR spectra for compound Ar-Peg4.

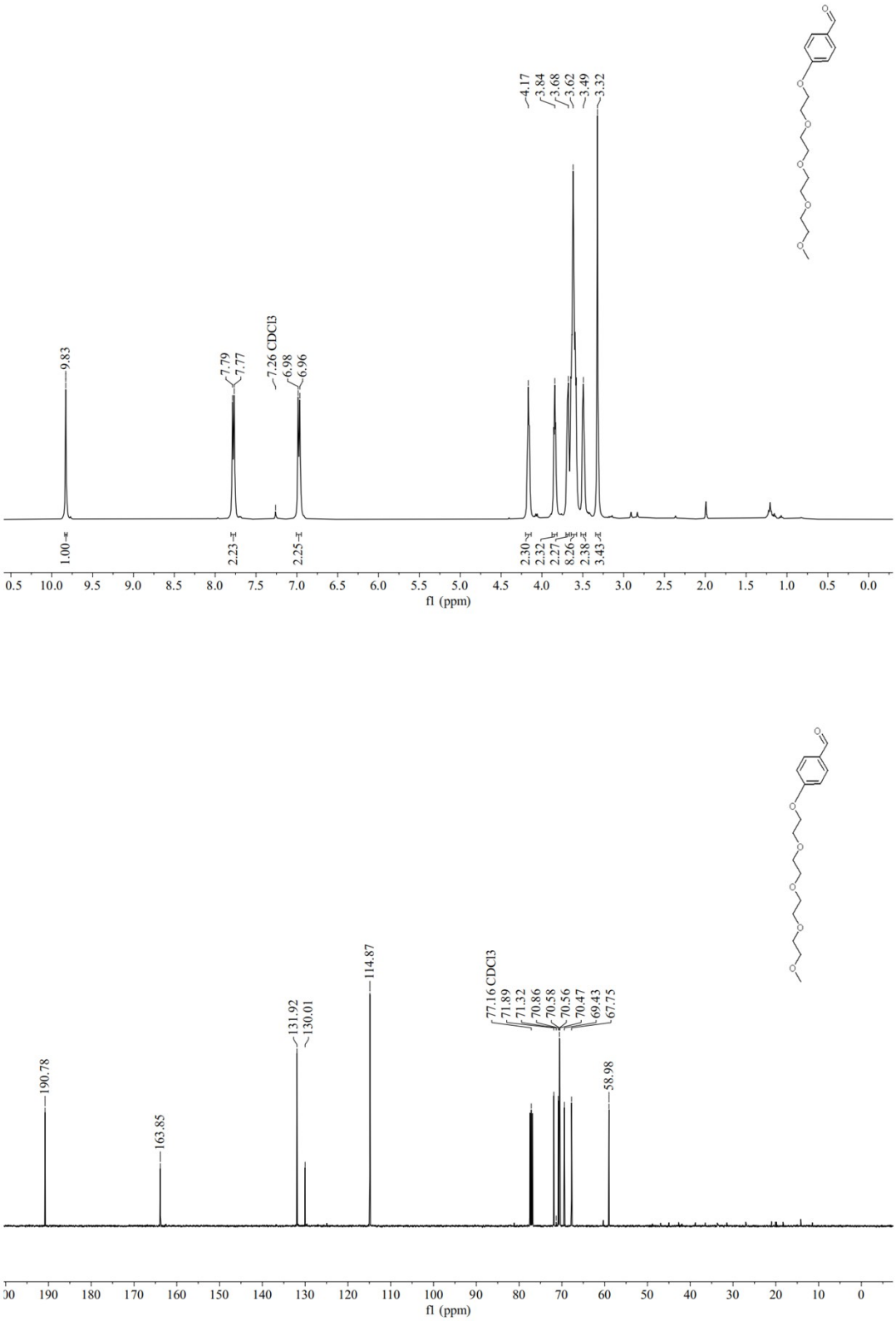
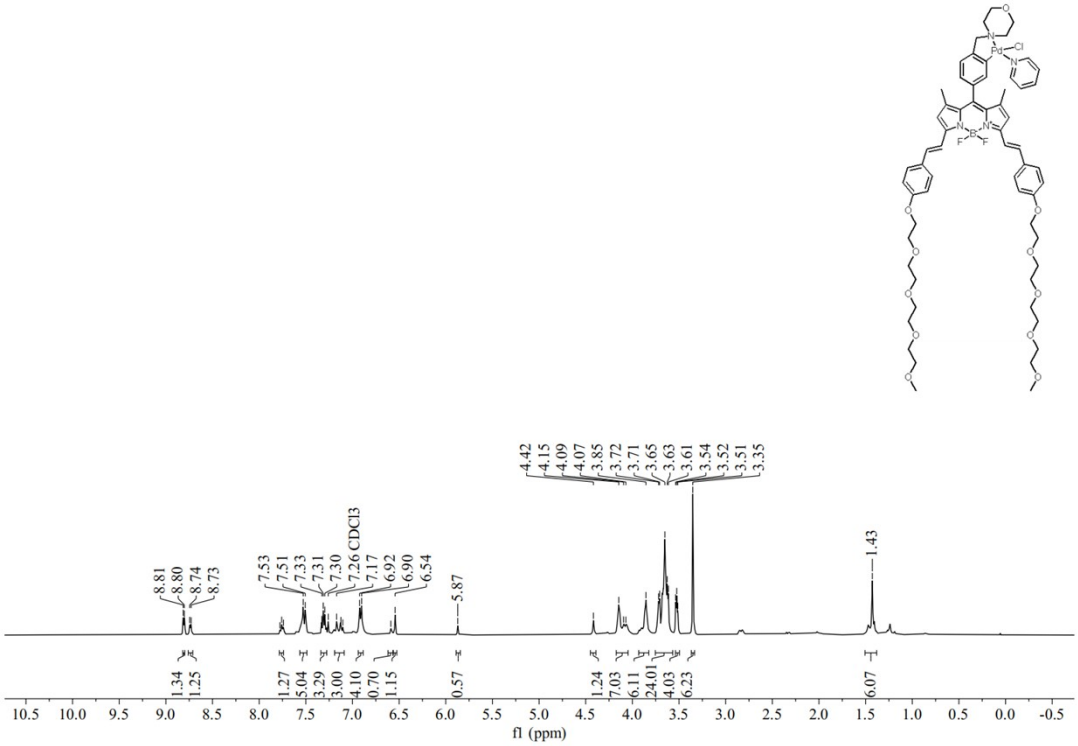


Figure S8. ^{1}H NMR and ^{13}C NMR spectra for compound NIR-COP1.



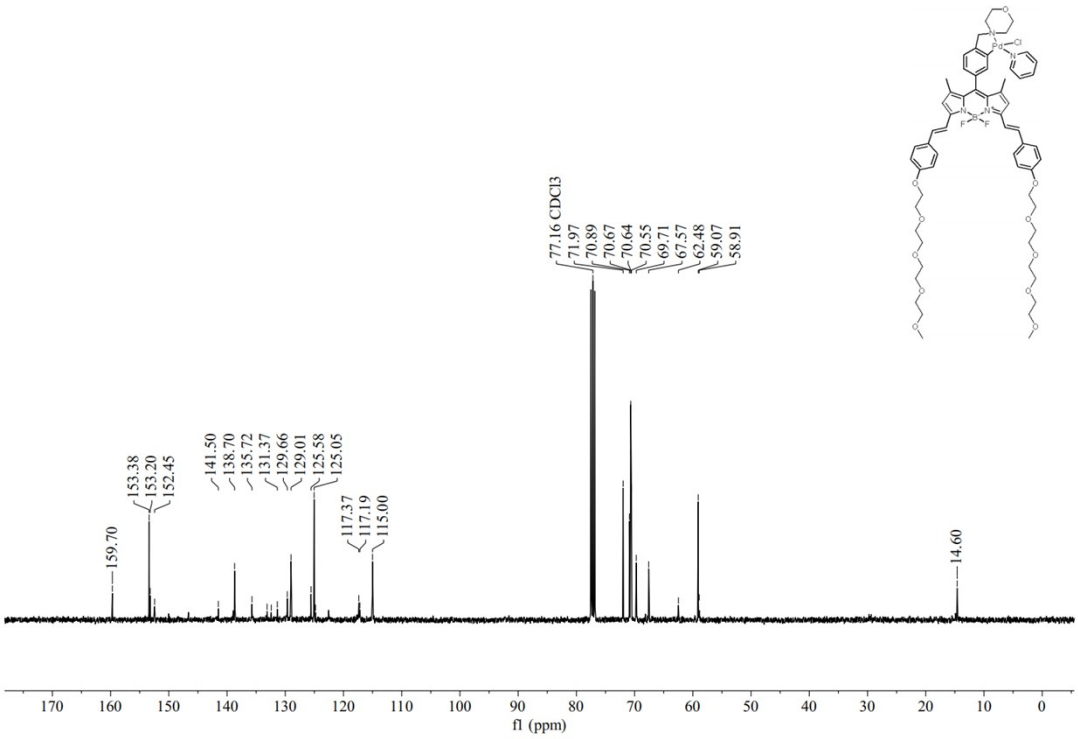
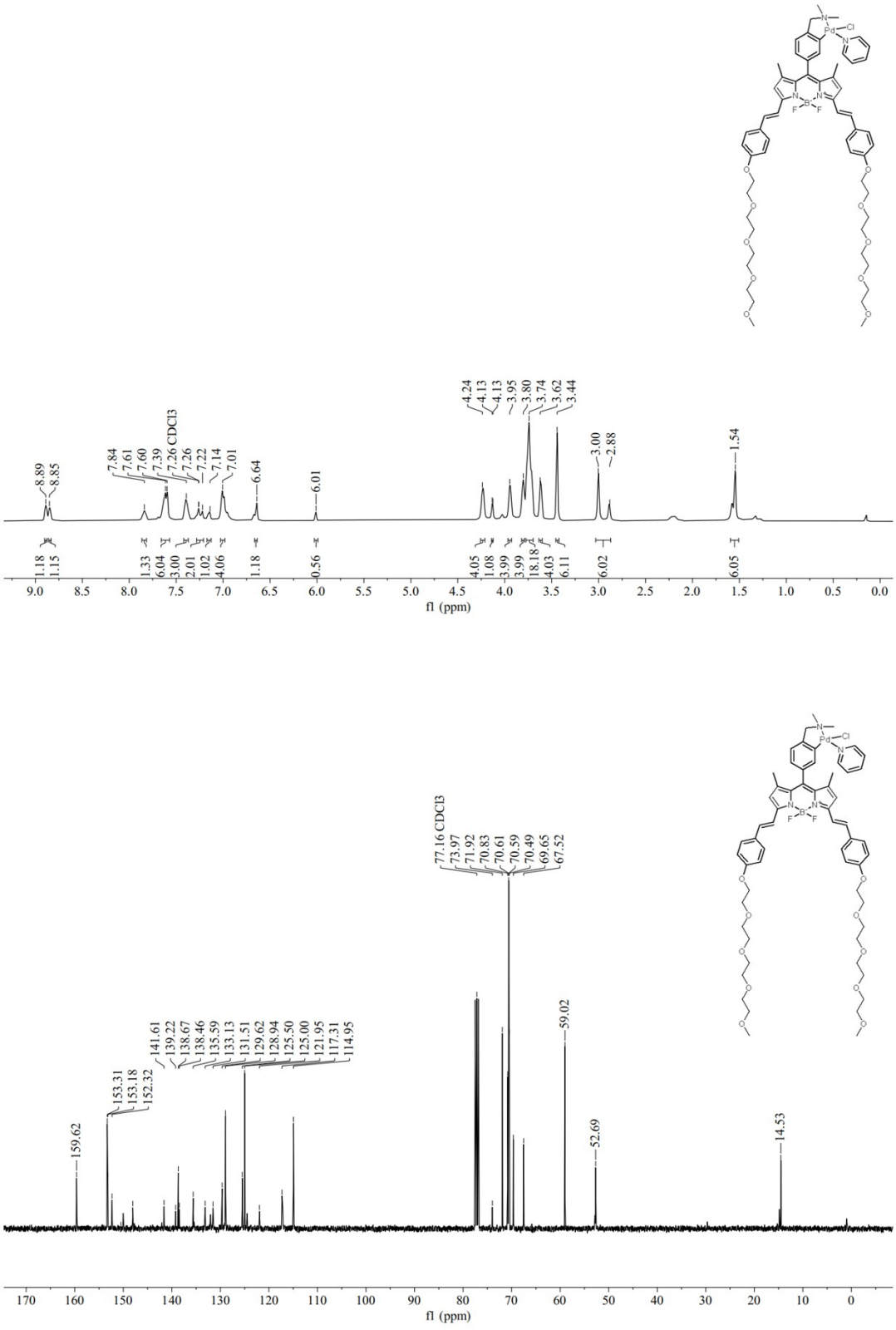


Figure S9. ^{1}H NMR and ^{13}C NMR spectra compound NIR-COP2.



(1) Michel, B. W.; Lippert, A. R.; Chang, C. J. *J. Am. Chem. Soc.* **2012**, *134*, 15668-15671.

Figure S10. (a) Absorbance spectra for NIR-COP1 (2 μM) in the abscence or presence of CORM-3 (50 μM). (b) Absorbance spectra for NIR-COP2 (2 μM) in the abscence or presence of CORM-3 (50 μM).

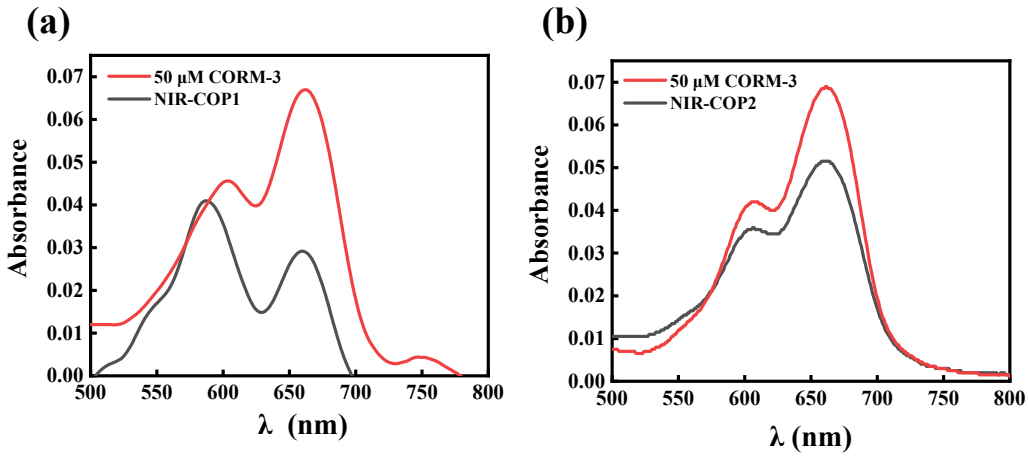


Figure S11. Fluorescence responses of NIR-COP1 (a) and NIR-COP2 (b) to H2S.

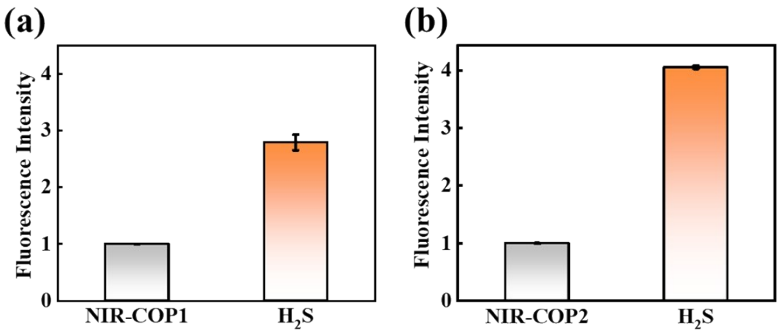


Figure S12. the fluorescence for NIR-COP1 incubated with 50 μM of CORM-3 upon excitation at 640 nm.

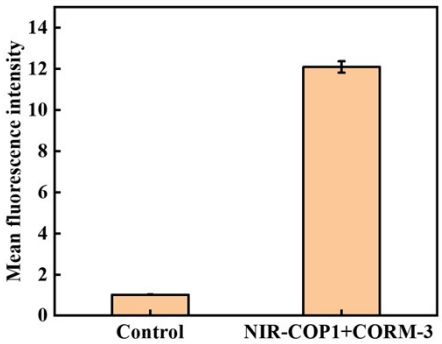


Figure S13. ESI-MS spectrum for NIR-COP1 (1 μM) treated with excessive CORM-3. There emerged a new peak with a m/z charge of 1079.02 upon incubating probe NIR-COP1 with CORM-3, corresponding to CO-induced formation of carbonylation product.

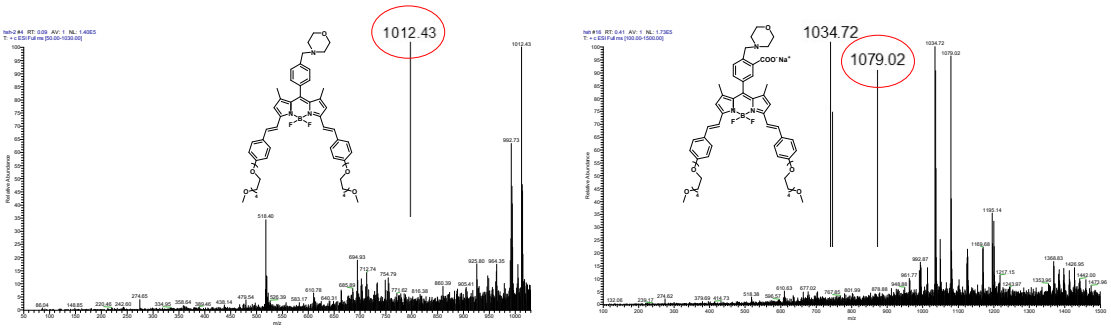


Figure S14. (a)NIR-COP1 and its responses to CO in pH. (b) The responsiveness of NIR-COP1 to CORM-3 in HepG2 cell lysates.

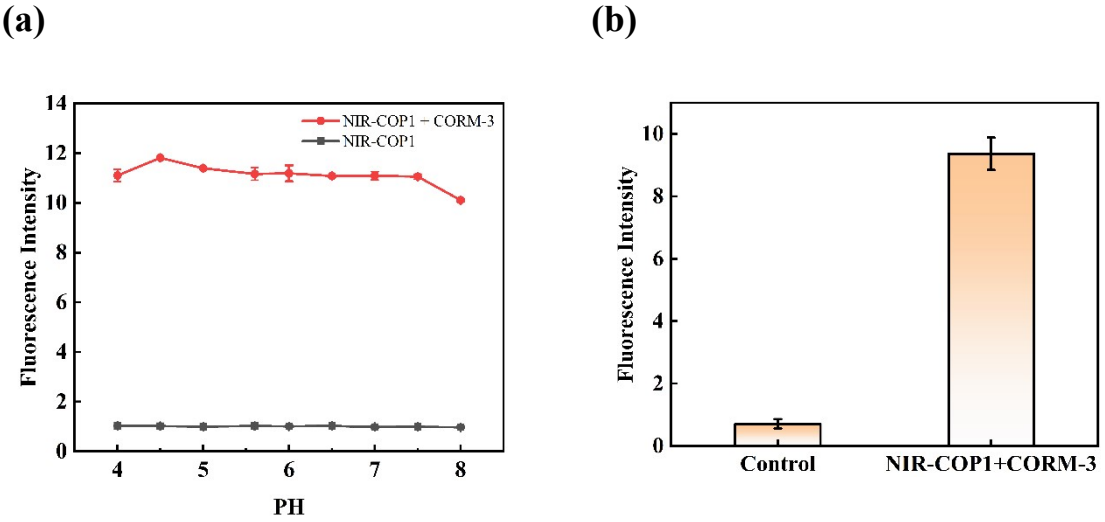


Figure S15. Cell viability of HeLa cells treated with different concentrations of NIR-COP1 with or without addition of CORM-3 for different time intervals.

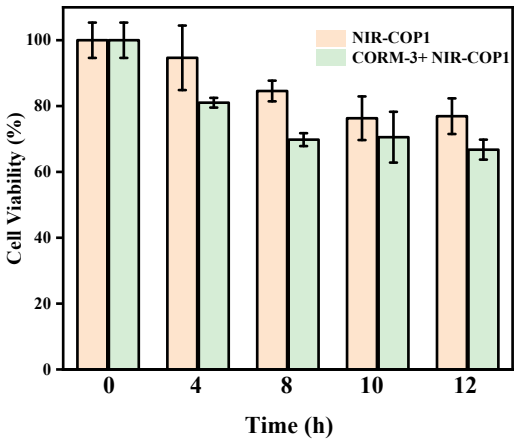


Figure S16. (a) Time-dependent fluorescence imaging of HepG2 cells pretreated with CORM-3 and incubated with NIR-COP1. (b) Relative fluorescence intensities for cells in (a).

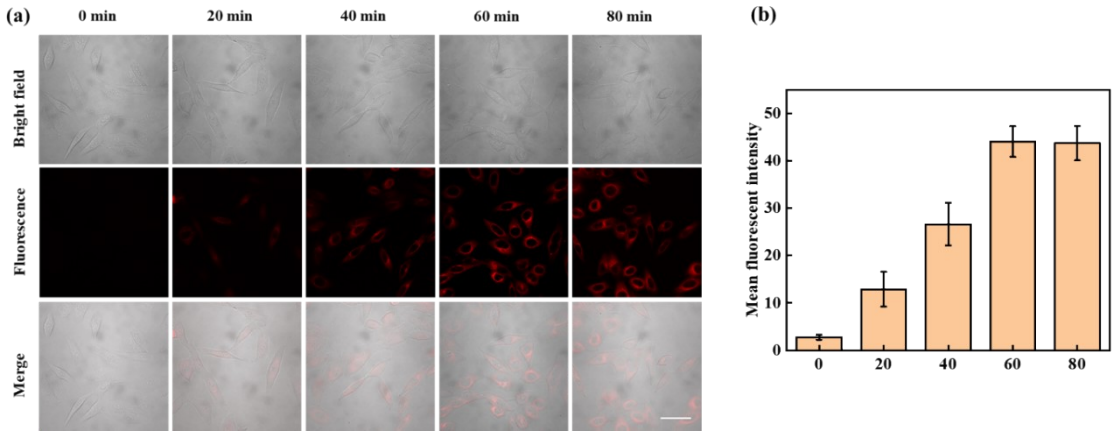


Figure S17. Subcelllular localization of NIR-COP1. HepG2 cells were incubated with NIR-COP1 (5 μM) for 30 min, and then treated with Hoechst 33342 (a), Lyso-Tracker Green (b) or MitoTracker Green for 30 min. The NIR fluorescence from NIR-COP1 did not exhibit substantial overlap with the fluorescence signals from Hoechst 13142, Mio-tracker green and Lyso-tracker green, with Pearson's correlation coefficients (PCC) of 0.12, 0.62 and 0.45, respectively. Scale bar = 25 μm.

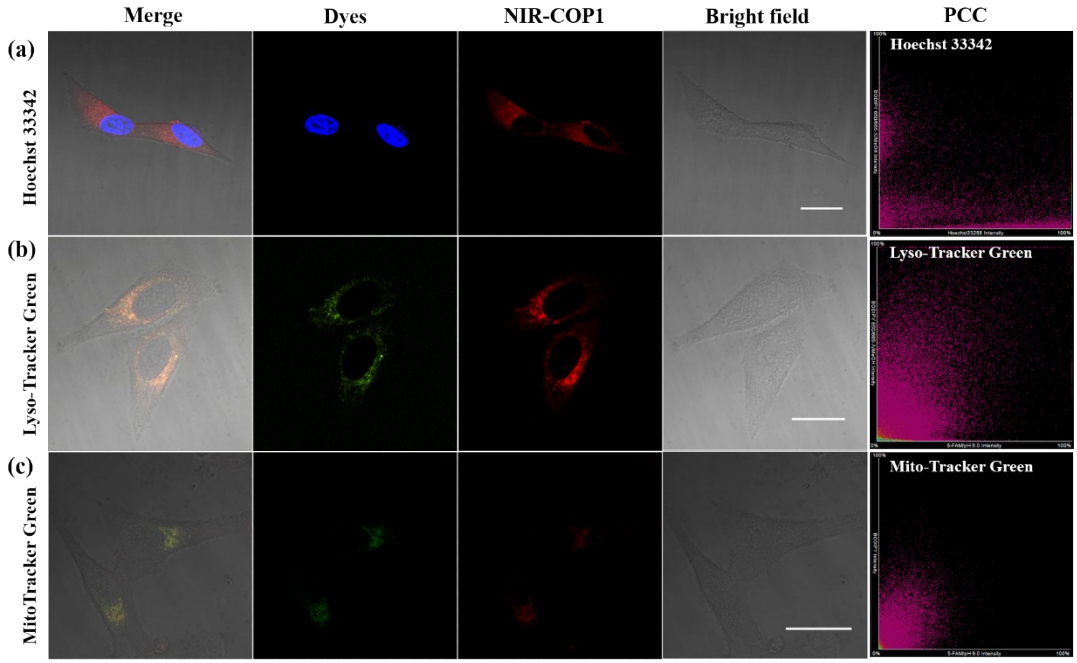


Figure S18. (a) Confocal fluorescence images for HepG2 cells incubated with NIR-COP1 (1 μM) with or without pretreatments with heme (20 μM). Scale bar = 50 μm. (b) Mean fluorescence intensities for cells in (a). Values are mean ± s.d. from at least 20 cells. λ_{ex} = 640 nm, λ_{em} = 663–738 nm.