Supporting Information for

A Reaction-Based Fluorescent Probe for Selective Imaging of Carbon Monoxide in Living Cells Using a Palladium-Mediated Carbonylation

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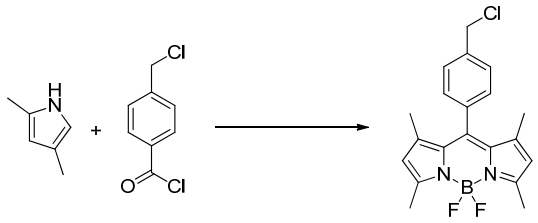
General Methods

All reactions utilizing air- or moisture-sensitive reagents were performed in dried glassware under an atmosphere of dry N_2. When dry solvent was used the solvent was passed over activated alumina. Other reagents were used without further purification. Silica gel P60 (SiliCycle) was used for column chromatography and SiliCycle 60 F254 silica gel (precoated sheets, 0.25 mm thick) was used for analytical thin layer chromatography and visualized by fluorescence quenching under UV light. Ruthenium hexacarbonyldi-μ-chlorodimer was purchased from Strem, 4-(chloromethyl)benzoyl chloride was purchased from TCI America, carbon monoxide gas was purchased from Praxair, and all other reagents were purchased from Sigma-Aldrich. CORM-3 [$Ru(CO)_3$(glycinate)] was prepared according to the literature procedure and spectral properties were in accordance with those reported.[1] ^1H and ^{13}C NMR spectra for characterization of new compounds were collected in $CDCl_3$ (Cambridge Isotope Laboratories) at 25 °C at the reported frequency at the College of Chemistry NMR Facility at the University of California, Berkeley. All chemical shifts are reported in parts per million and referenced to the residual solvent peak from $CHCl_3$ at 7.27 ppm. Splitting patterns are indicated as follows: br, broad; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dd, doublet of doublets; dt, doublet of triplets. Low-resolution mass spectral analyses were carried out using a LC-MS (Agilent Technology 6130, Quadrupole LC/MS). Fluorescence spectra were obtained using a Quanta Master 4 L-format scanning spectrofluorometer equipped with an LPS-220B 75 W xenon lamp and power supply, A-1010B lamp housing with an integrated igniter, switchable 814 photon-counting/analog photomultiplier detection unit, and MD5020 motor driver (Photon Technology International, Inc.) and UV spectra were aquired using a Cary Bio50 UV spectrophotometer (Varian). Samples for emission and absorption measurements were contained in 1 cm × 0.1 cm quartz cuvette (Starna).

Probe Synthesis and New Compound Characterization

Compound **2**:



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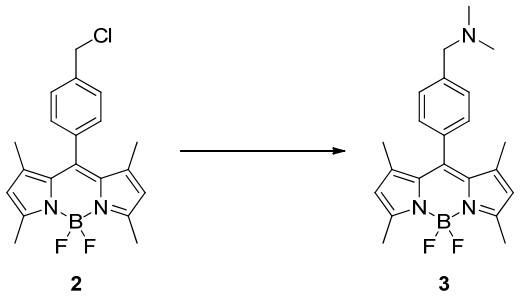
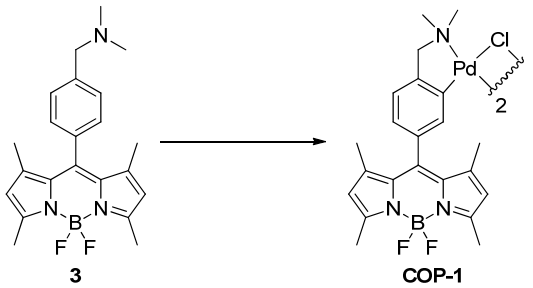
To an oven dried 250 mL 3-necked round bottomed flask with a magnetic stir bar and equipped with a water cooled condenser was charged dry CH_2Cl_2 (100 mL) followed by 2,4-dimethylpyrrole (3.12 mL, 30.3 mmol, 2.1 equiv.). Under flow of nitrogen, 4-(chloromethyl)benzoyl chloride (2.864 g, 14.36 mmol, 1.0 equiv.) was added in approximately 200 mg portions. The mixture was then heated in an oil bath at 50 °C for 80 min and then subsequently allowed to cool to room temperature. The solution was transferred to an oven dried 500 mL round bottomed flask and the majority of solvent was removed in vacuo until 10-15 mL of CH_2Cl_2 remained. Dry toluene (180 mL) was then added to the flask and the solution was put under an atmosphere of nitrogen. Dry triethylamine (8.5 mL) was charged to the flask and the mixture was allowed to stir at room temperature for 15 min, at which point BF_3•Et_2O (9.5 mL) was added to the flask in a dropwise fashion. The flask was then equipped with a water cooled condenser and heated to 50 °C for 1 h. The mixture was then allowed to cool to room temperature and the solvent was removed in vacuo. The resulting residue was dissolved in CH_2Cl_2 (100 mL) and transferred to a separatory funnel. The organic layer was washed with water (3 × 50 mL) and then dried over Na_2SO_4, filtered and concentrated. The residue was dry loaded onto 75 mL of silica and subsequently purified by flash chromatography on silica gel CH_2Cl_2:Hexanes (50:50 → 60:40 → 75:25) to provide **2** (1.47 g, 3.95 mmol) as an orange solid in 27% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 5.99 (s, 2H), 4.57 (s, 2H), 2.56 (s, 6H), 1.38 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.8, 143.2, 141.1, 138.8, 135.2, 131.5, 129.4, 128.6, 121.5, 45.8, 14.8, 14.7. HRMS calcd for $C_{20}H_{21}BClF_2N_2$ ($M+H^+$) 373.1449; found 373.1440.

Compound **3**:



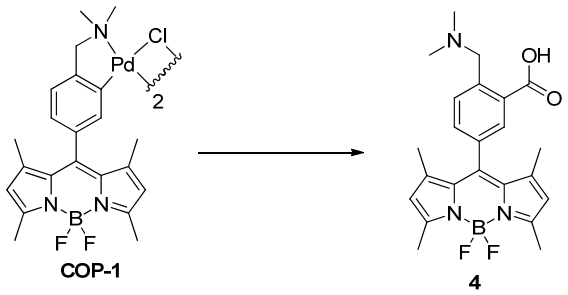
To a 10 mL microwave vial (CEM corp.) was weighed **2** (376 mg, 1.01 mmol, 1.0 equiv.), K_2CO_3 (276 mg, 2.0 mmol, 2.0 equiv.), and KI (336 mg, 2.02 mmol, 2.0 equiv.). A magnetic stir bar was added and the vial was subsequently charged with CH_3CN (5.0 mL) and Me_2NH (2.3 mL aq. 40 wt%, 20 mmol, 20 equiv.). The microwave vial cap was affixed onto the vial and the reaction mixture was heated at 80 °C (100 W) for 40 min followed by 20 min at 100 °C. After the vessel was cooled back to room temperature the mixture was diluted with CH_2Cl_2 (60 mL), transferred to a separatory funnel and washed with water (2 × 30 mL) and Brine (1 × 45 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting residue was purified by flash chromatography eluting with CHCl3 → 66% $CHCl_3$, 30% ethyl acetate, 3% methanol, 0.5% triethylamine →45% $CHCl_3$, 45% ethyl acetate, 9% methanol, 1% triethylamine. The fractions containing pure product as indicated by fluorescence visualization of TLC were combined and concentrated to provide **3** (326 mg, 0.855 mmol) as an orange solid in 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43 (d, *J* = 7.9 Hz, 2H), 7.24 (d, *J* = 7.9 Hz, 2H), 5.98 (s, 2H), 3.50 (s, 2H), 2.55 (s, 6H), 2.26 (s, 6H), 1.39 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.53, 143.27, 141.95, 139.96, 133.98, 131.66, 130.15, 128.06, 121.36, 64.25, 45.45, 14.78, 14.54. HRMS calcd for $C_{22}H_{27}BF_2N_3$ ($M+H^+$) 382.2261; found 382.2258.

COP-1:



To a 20 mL vial was weighed **3** (254 mg, 0.667 mmol, 1.05 equiv.) and $Pd(OAc)_2$ (143 mg, 0.636 mmol, 1.00 equiv.). A magnetic stir bar was added to the vial along with benzene (12 mL). The mixture was sonicated for 1 minute, then nitrogen atmosphere was established. The vial was then wrapped in foil to protect from light and placed in a 50 °C oil bath and the mixture was stirred for 14 h. The reaction mixture was then cooled to room temperature and hexanes (6 mL) was added which caused an immediate precipitation of an orange solid. The solid was collected via filtration to provide the acetate bridged dimer (340 mg) that co-crystallized with one benzene relative to two dimers (1 benzene : 4 Pd-complex). Some of this material was retained for evaluation and the remaining (264 mg, 0.483 mmol) was dissolved in acetone that had been saturated with LiCl. This mixture was stirred at room temperature protected from light for 4 h, at which point the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2 (20 mL) and passed through a pad of celite. The pad of celite was further washed with CH_2Cl_2 (75 mL) and the combined eluent was concentrated in vacuo to provide the chloride dimer **COP-1** (251 mg, 0.481 mmol) as an orange solid in 97% yield over both steps. The ^{1}H NMR spectrum indicates that the dimer exist as a mixture of isomers which coalesce upon heating of the sample. While we hypothesize that the isomers are rotomers about the BODIPY-Aryl bond, it is difficult to assign the predominant solution structure. Therefore, the reported peaks correspond to the predominant isomer. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.04 (s, 1H), 6.98 (d, J = 7.6 Hz, 1H), 6.88 (d, J = 7.5 Hz, 1H), 5.98 (s, 2H), 3.98 (s, 2H), 2.85 (s, 6H), 2.55 (s, 6H), 1.47 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1. 147.8, 144.2, 143.6, 143.0, 131.9, 131.7, 131.2, 124.2, 122.0, 121.1, 73.2, 53.1, 14.76. Elemental Analysis calcd for $C_{44}H_{50}B_2Cl_2F_4N_6Pd_2$: C, 50.61; H, 4.83; N, 8.05; Found: C, 50.64; H, 5.01; N, 7.80.

Compound **4**:



To a 50 mL round bottomed flask was weighed COP-1 (35.0 mg, 0.067 mmol). A magnetic stir bar was added along with CH_2Cl_2 (15 mL) and water (0.5 mL). The flask was fitted with an air condenser and an atmosphere of CO was established via flushing the apparatus from a balloon of CO. The mixture was heated in an oil bath at 31 C overnight (14 h) while maintaining an atmosphere of CO. The reaction mixture was cooled to room temperature, transferred to a separatory funnel and diluted with CH_2Cl_2 (50 mL). The solution was washed with water (1 × 20 mL), dried over Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography by eluting with a copious amount of a mixed solvent (~1.5 L) of methanol in $CHCl_3$ (6% → 12% → 15% methanol). The product containing fractions were concentrated and when transferring to a vial the residue was dissolved in $CHCl_3$ and passed through a short plug of celite in a pipette to remove any residual silica. The product **4** (27.3 mg, 0.064 mmol) was isolated as an orange solid in 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14 (s, 1H), 7.39 (d, *J* = 7.7 Hz, 1H), 7.35 (d, *J* = 7.7 Hz, 1H), 5.98 (s, 2H), 3.98 (s, 2H), 2.57 (s, 6H), 2.55 (s, 6H), 1.37 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): 174.8, 156.2, 142.8, 139.8, 136.7, 133.4, 132.7, 131.9, 131.3, 130.9, 121.7, 62.4, 42.5, 14.9, 14.8. MS calcd for $C_{23}H_{27}BF_2N_3O_2$ ($M+H^+$) 426.2159; found 426.2157. For *in vitro* evaluations of the carbonylation reaction with CO gas (1 atm) the identity of the product formed from COP-1 was confirmed to be the acid **4** via comparison of LC-MS for the observed and authentic product (Figure S1).

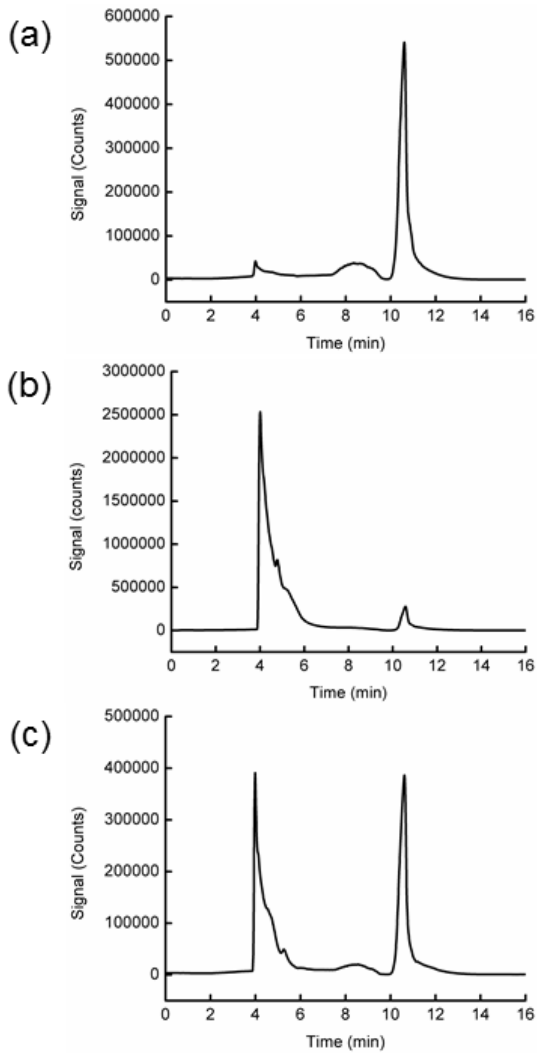


Figure S1. HPLC-MS traces in scanning ion mode (**4**+H, m/z = 426; COP-1-$(CH_3CN)_2$, m/z = 568). HPLC conditions: Gradient from 80% water, 20% CH_3CN to 100% CH_3CN over 8 min using an Agilent 300extend-C18, 3.5 μm, 4.6 × 100mm column. (a) **COP-1** dissolved in acetonitrile; (b) Compound **4**; (c) A reaction was performed with 10 μM **COP-1** in 100 mL of H_2O under an atmosphere of CO gas and at 37 °C.

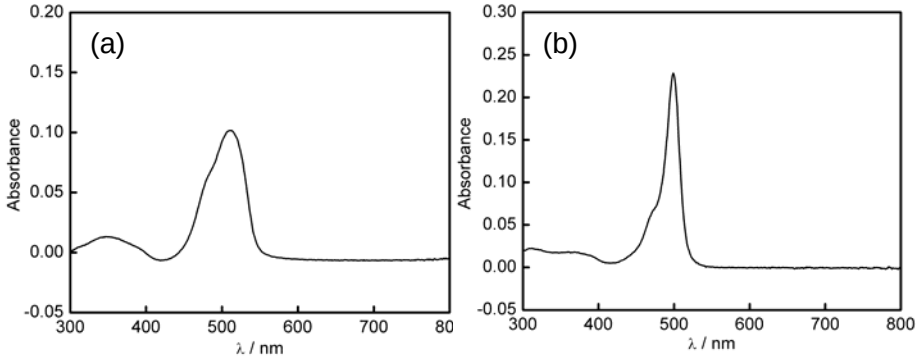


Figure S2. Absorbance spectra of (a) 4 μM COP-1 (b) 10 μM **4**.

Quantum Yields

Quantum yields were determined according to the literature procedure using fluorescein as the standard. Absorption and emission spectra for COP-1, the carbonylation product **4**, and fluorescein were obtained over a range of concentrations (100 nM to 10 uM) where a linear correlation between concentration and absorption was observed and the absorbance was within 0.01 to 0.1. The quantum yield was calculated according to the equation $\Phi_{sample} = \Phi_{standard}$ $(Grad_{sample}/ Gad_{standard})(\eta_{sample}/\eta_{standard})$; where Φ is quantum yield, $\Phi_{standard}$ = 0.925 in 0.1 M NaOH, Grad is the slope of the plot of absorbance versus integrated emission intensity, and η is the refractive index of the solvent.

COP-1 Fluorescence Responses to CO

A 1.0 μM solution of COP-1 in DPBS (-Ca, -Mg) buffer was prepared in a cuvette from a 500 μM stock solution of COP-1 in DMSO. The cuvette was placed in a water bath set to 37 °C. A t=0 spectrum was acquired and 10 μL of a 5 mM stock solution of CORM-3 in Millipore water was added to the cuvette to bring the concentration of the CORM-3 in solution to 50 μM. Emission spectra were recorded by quickly removing the cuvette from the water bath, obtaining the spectrum and returning the cuvette to the bath. Spectra were taken at t = 0, 5, 15, 30, 45, and 60 min. See Figure 1 in the main text. For *in vitro* evaluations of the carbonylation reaction with either CORM-3 or CO gas (1 atm) the identity of the product formed from COP-1 was confirmed to be the acid **4** via comparison of LC-MS for the observed and authentic product.

In Vitro CORM-3 Dose Dependence

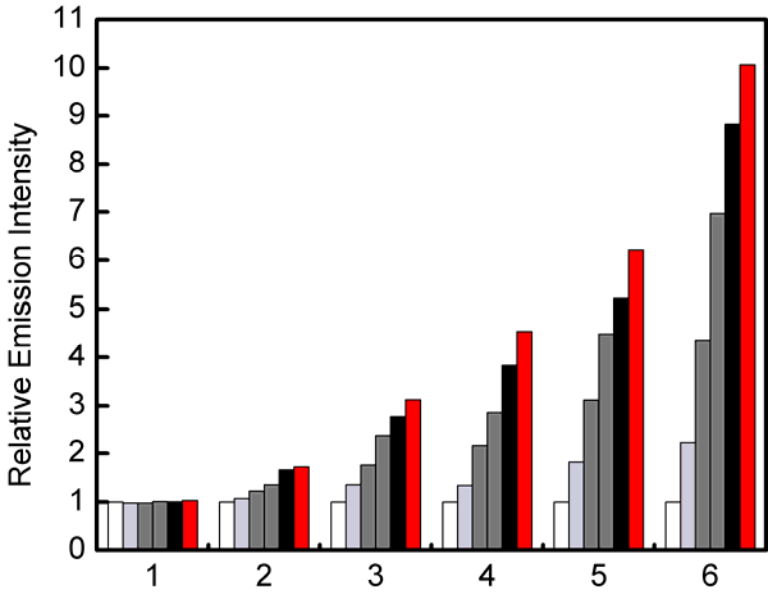


Figure S3. Relative integrated emission intensities of the turn-on response of 1.0 μM COP-1 in PBS at 37 °C to various levels of CORM-3 as observed at 0, 5, 15, 30, 45 and 60 minutes. Legend: (1) Vehicle control; (2) 1.0 μM CORM-3; (3) 5 μM CORM-3; (4) 10 μM CORM-3; (5) 20 μM CORM-3; (6) 50 μM CORM-3.

A 1.0 μM solution of COP-1 in DPBS (-Ca, -Mg) buffer was prepared in a cuvette from a 500 μM stock solution of COP-1 in DMSO. The cuvette was placed in a water bath set to 37 °C. A t=0 spectrum was acquired then CORM-3 was added from a 5 mM stock solution in Millipore water was added to the cuvette to bring the solution to the desired concentration of CORM-3. Emission spectra were recorded by quickly removing the cuvette from the water bath, obtaining the spectrum and returning the cuvette to the bath. Spectra were taken at t = 0, 5, 15, 30, 45, and 60 min.

Selectivity Tests

A 1.0 μM solution of COP-1 in DPBS (-Ca, -Mg) buffer was prepared in a cuvette from a 500 μM stock solution of COP-1 in DMSO. The cuvette was placed in a water bath set to 37 °C. A t=0 spectrum was acquired then the analyte of interest was added to the vial to bring the concentration of analyte to 50 μM. Emission spectra were recorded by quickly removing the cuvette from the water bath, obtaining the spectrum and returning the cuvette to the bath. Spectra were taken at t = 0, 5, 15, 30, 45, and 60 min. See Figure 1b in the main text.

H_2O_2: 5 μL of 10 mM stock solution of H_2O_2 in Millipore water was added to 995 μL of 1.0 μM solution of COP-1 in DPBS.

TBHP: 5 μL of 10 mM stock solution of TBHP in Millipore water was added to 995 μL of 1.0 μM solution of COP-1 in DPBS.

NaOCl: 5 μL of 10 mM stock solution of NaOCl in Millipore water was added to 995 μL of 1.0 μM solution of COP-1 in DPBS.

$O_2^{\bullet-}$: 5 μL of ~10 mM saturated solution of KO_2 in Millipore water was added to 995 μL of 1.0 μM solution of COP-1 in DPBS.

NO: 3.85 μL of 6.5 mM Prolin-NONOATE (13 mM NO equiv.) was added to 996 μL of 1.0 μM solution of COP-1 in DPBS. Note: All solutions degassed prior to reaction.

$ONOO^-$: 0.56 μL of 89 mM $ONOO^-$ solution in Millipore water was added to 999 μL of 1.0 μM solution of COP-1 in DPBS.

H_2S: 5 μL of a 10 mM Na2S solution in Millipore water was added to 995 μL of 1.0 μM solution of COP-1 in DPBS.

Cell Culture and Labeling Procedures

HEK 293T cells were maintained in exponential growth as a monolayer in Dulbecco's Modified Eagle Mediaum (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone), and incubated at 37 °C in 5% CO_2. One or two days before imaging, the cells were passaged and plated in phenol red-free medium on a 4-well Lab Tek borosilicate chambered cogerglass slides (Nunc) and allowed to grow to 60-80% convluence. For all experiments, solutions of COP-1 were prepared in DMSO (500 μM) and diluted in DPBS (+Ca, +Mg) to 1.0 μM. CORM-3 solutions were prepared in Millipore water to 5 mM and then diluted in DPBS immediately prior to application to the cells. The DMEM media was removed from the chambers containing cells and the well was washed with DPBS. The buffer was then replaced with buffer that was 50 μM in CORM-3 or a vehicle control and incubated at 37 °C for 15 minutes at which point 100 μL of the buffer was removed from the well, mixed with COP-1 and returned to the cell culture to bring the concentration of probe to 1.0 μM. The cells were then incubated at 37

°C for 30 min prior to imaging. For nuclear staining studies, cells were incubated with 1 μM Hoechst 33342 at 37 °C for 45 min prior to imaging.

Confocal Fluorescence Imaging Experiments

Confocal fluorescence imaging studies were performed with a Zeiss laser scanning microscope 710 with a 40× water objective lens, with Zen 2009 software (Carl Zeiss). COP-1 was excited using a 488 nm Ar laser, and emission collected using a META detector between 500 and 650 nm. Hoechst 33342 was excited with a 405 nm diode laser, and emission collected using a META detector between 450 and 500 nm. The cells were imaged at 37 ºC and 5 % CO2 throughout the course of the experiment. Image analysis was performed using ImageJ (National Institute of Health).

WST Cell Proliferation Assay

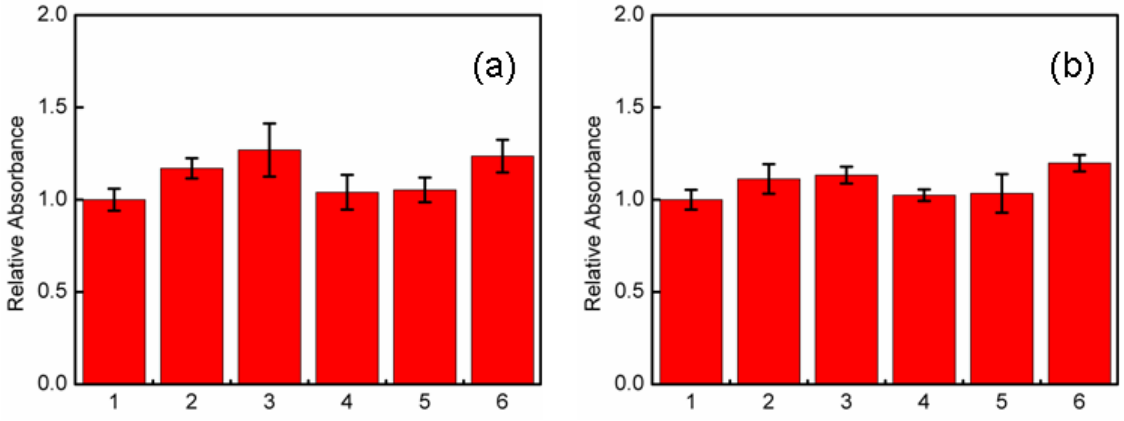


Figure S4. WST assay results for HEK293T cells. Each bar represents a normalized average of at least three and up to six wells of a 96-well plate. (a) HEK293T cells incubates with COP-1 or vehicle control. Legend: (1) Vehicle control; (2) 500 nM COP-1; (3) 1 μM COP-1; (4) 2 μM COP-1; (5) 5 μM COP-1; 10 μM COP-1. (b) HEK293T cells incubated with COP-1 and 50 μM CORM-3 or vehichle control. Legend: (1) Vehicle Control and 50 μM CORM-3; (2) 500 nM COP-1 and 50 μM CORM-3; (3) 1 μM COP-1 and 50 μM CORM-3; (4) 2 μM COP-1 and 50 μM CORM-3; (5) 5 μM COP-1 and 50 μM CORM-3; 10 μM COP-1 and 50 μM CORM-3.

HEK293T cells were maintained in exponential growth as a monolayer in Dulbecco's Modified Eagle Mediaum (DMEM, Invitrogen) supplemented with 10% fetal bovine serum (FBS, Hyclone), and incubated at 37 °C in 5% CO_2. One or two days before imaging, the cells were passaged and plated in phenol red-free medium in a poly-Lysine coated 96-well plate (Corning polylysine coated clear bottomed 96-well plate) and allowed to grow to ~80% confluence. Solutions of COP-1 in DPBS (+Ca, +Mg) buffer were prepared from a 500 μM stock solution in DMSO. The DMEM was removed and

cells were covered in DPBS buffer containing COP-1 at various concentrations (0.5-10 μM) or a vehicle control. The cells were incubated for 15 min at 37 °C and then 10 μL of 1 mM CORM-3 was added to half of the wells to bring the concentration to ~100 μM. The cells were then incubated for 30 min at 37 °C at which point 10 μL of the WST reagent solution (Roche) was added to all wells. Thee cells were incubated at 37 °C and absorbance readings were taken at 55, 75, and 100 min using a Spectramax M2 plate reader.

References

1) Clark, J. E.; Naughton, P.; Shurey, S.; Green, C. J.; Johnson, T. R.; Mann, B. E.; Foresti, R.; Motterlini, R. *Circulation Research* **2003**, *93*, e2-e8.

NMR Spectra

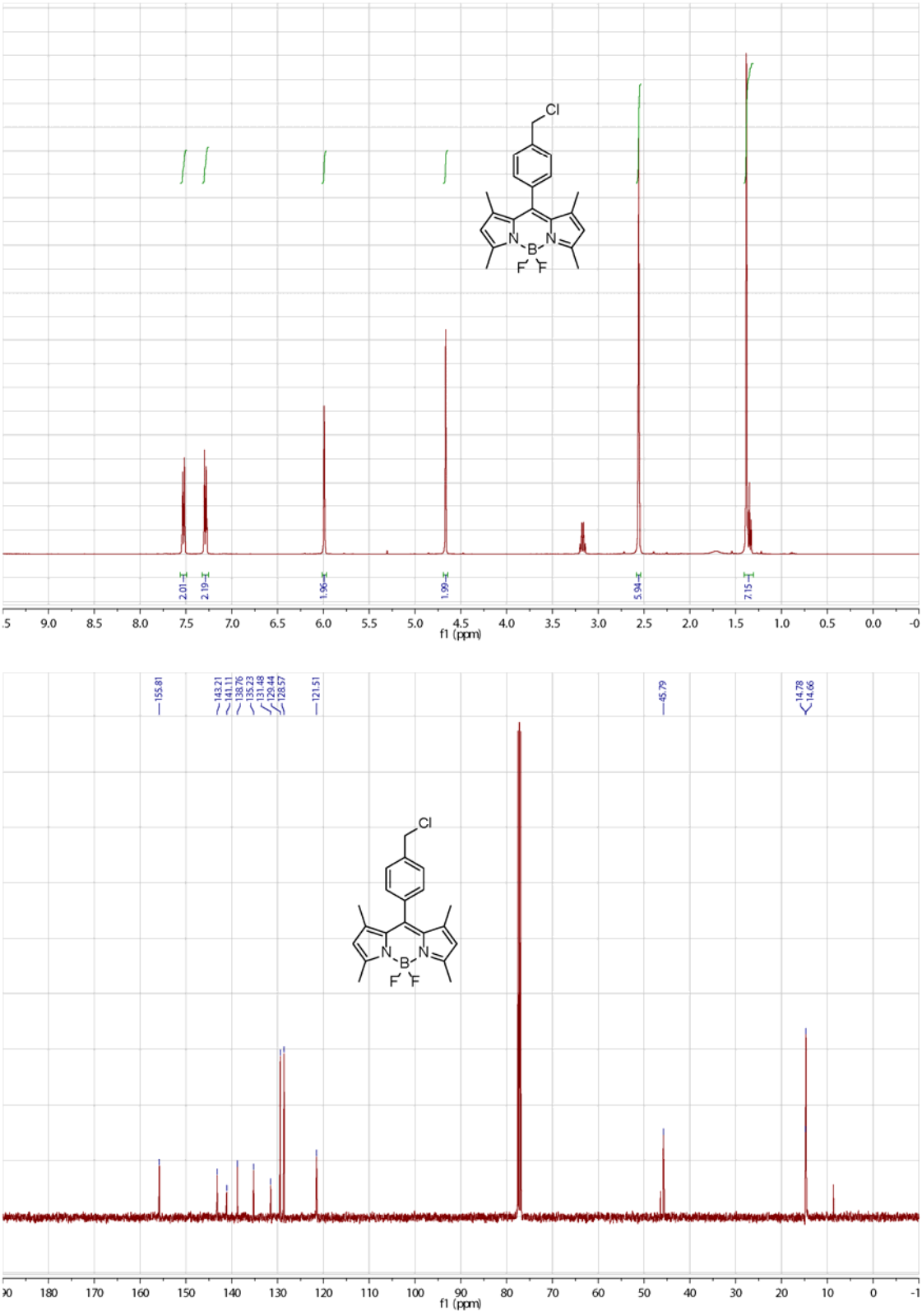


Figure S5. ^{1}H and ^{13}C NMR spectra of compound **2**.

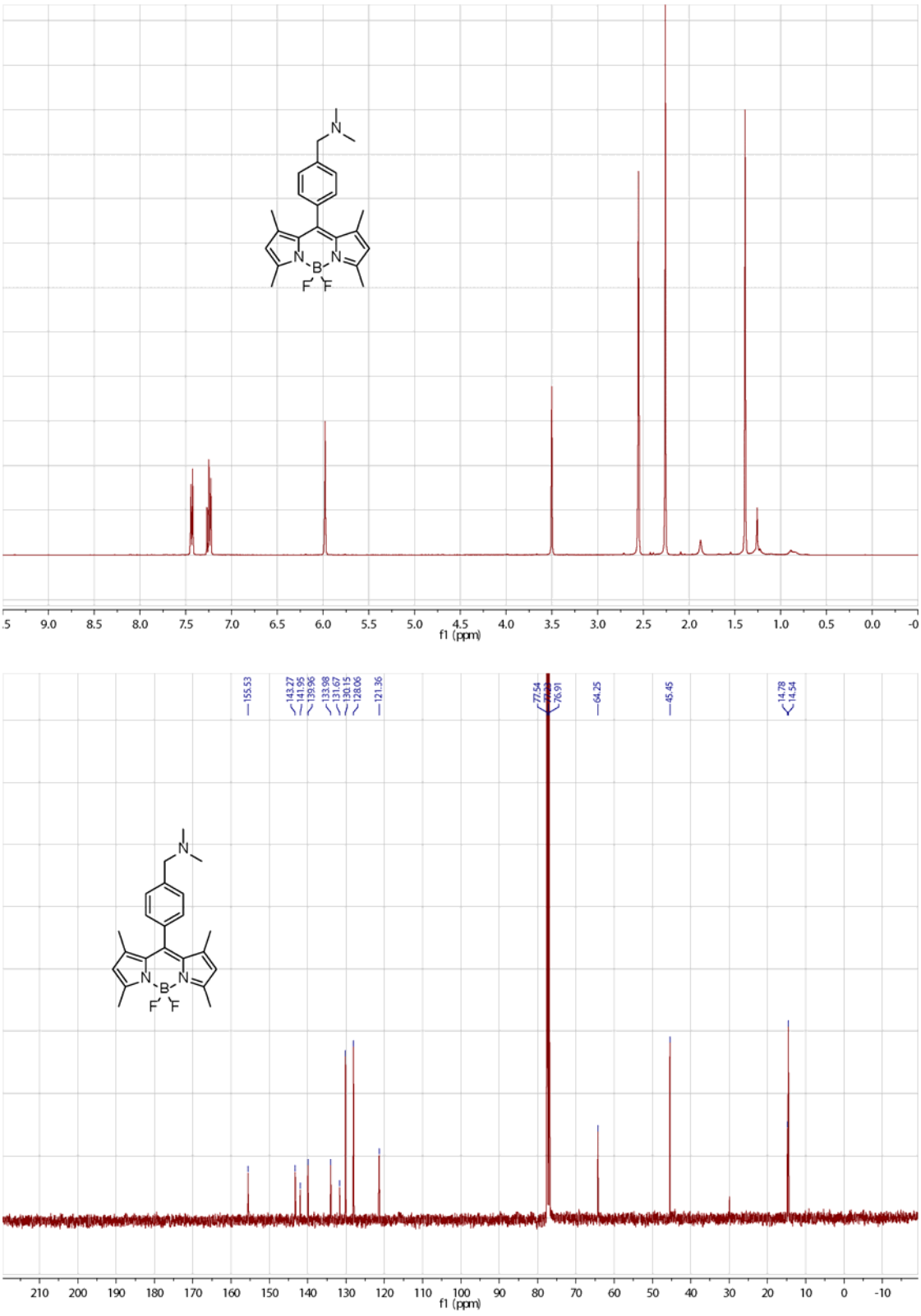


Figure S6. ^{1}H and ^{13}C NMR spectra of compound **3**.

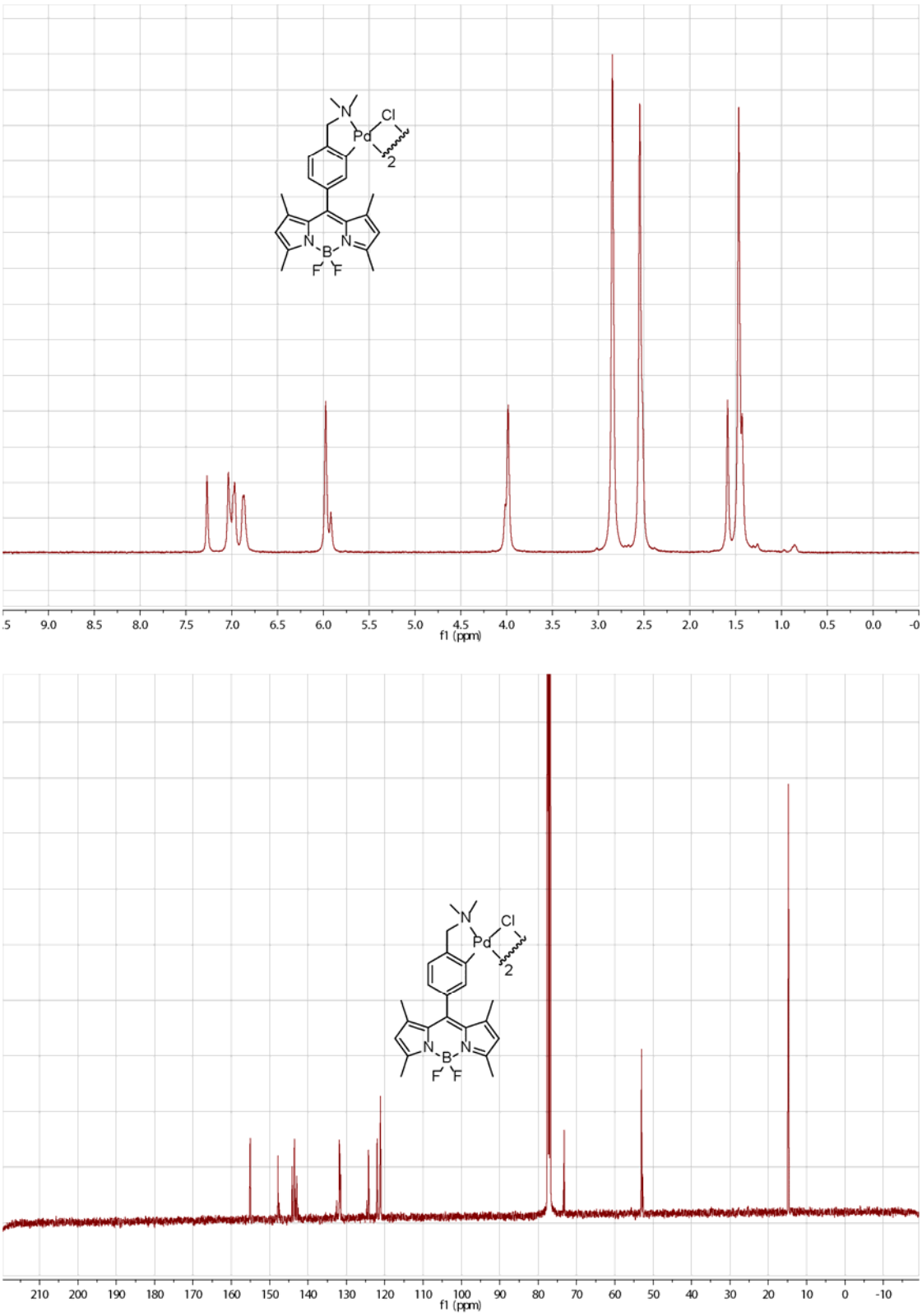


Figure S7. ^{1}H and ^{13}C NMR spectra of COP-1.

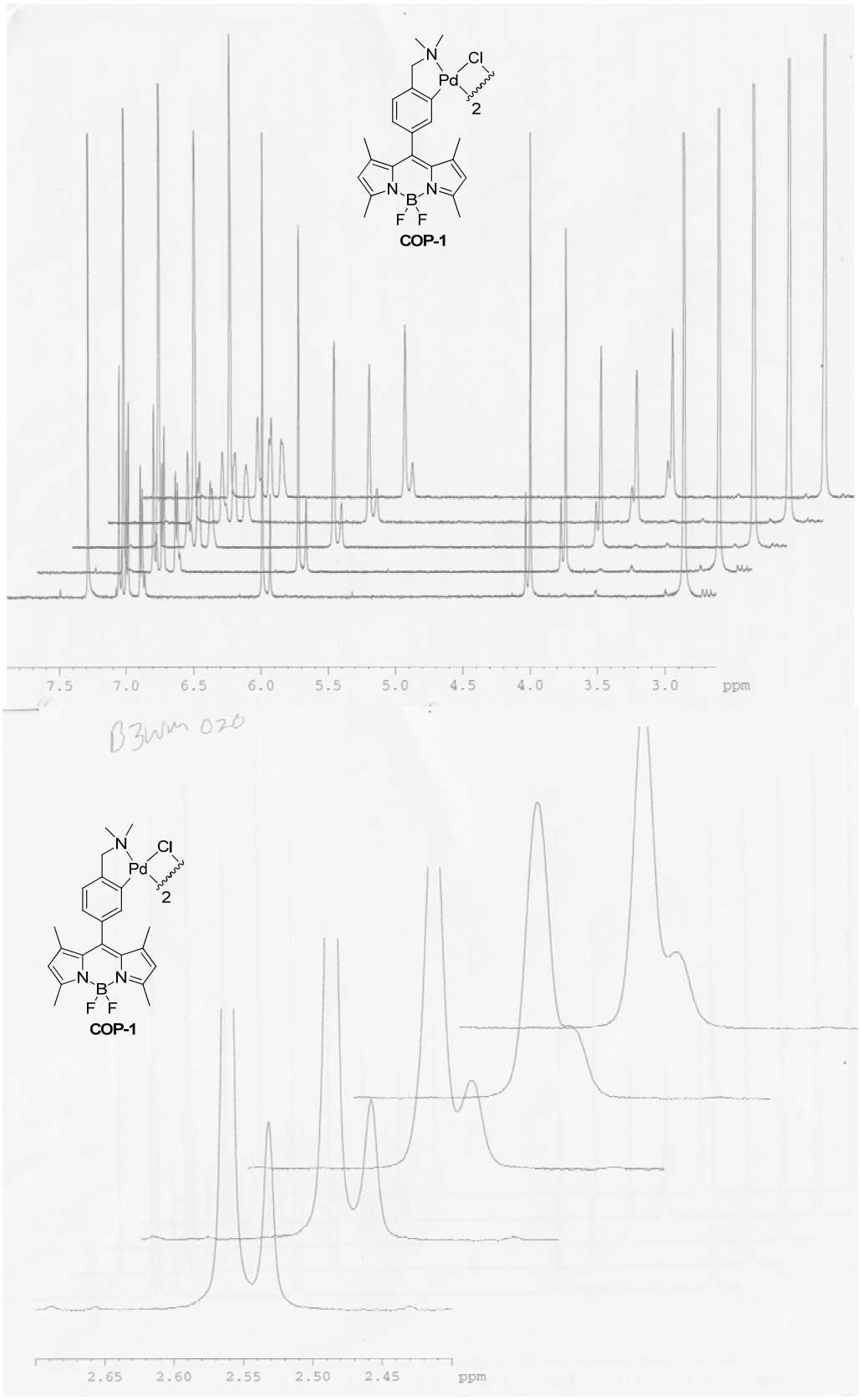


Figure S8. [1]H NMR spectra of COP-1 at 20 °C, 30 °C, 40 °C, 48 °C, 51.5 °C.

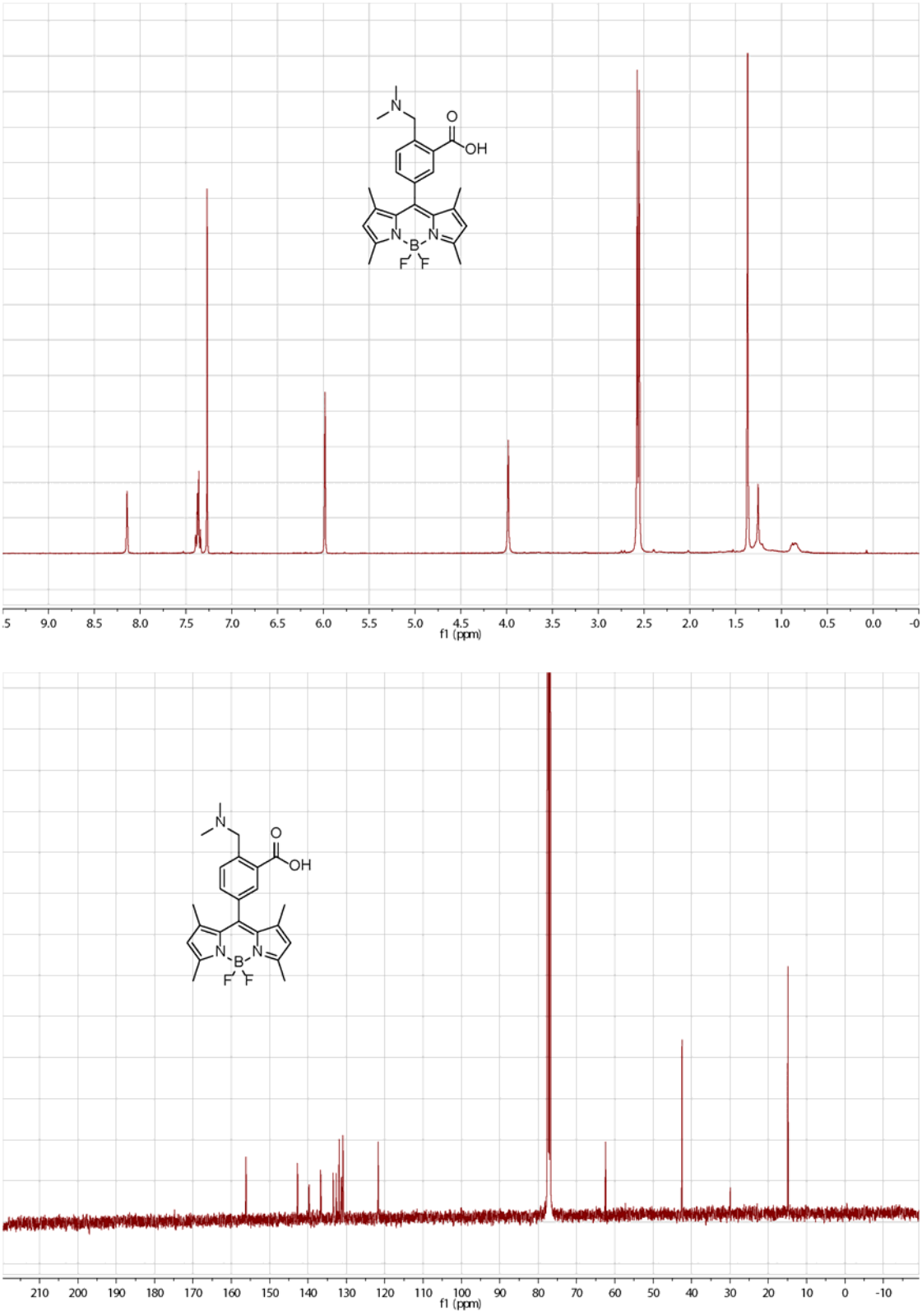


Figure S9. ^{1}H and ^{13}C NMR spectra of compound **4**.