Supporting Information

A PEGylated Water-soluble Fluorescent and Colorimetric Probe for Carbon Monoxide Detection

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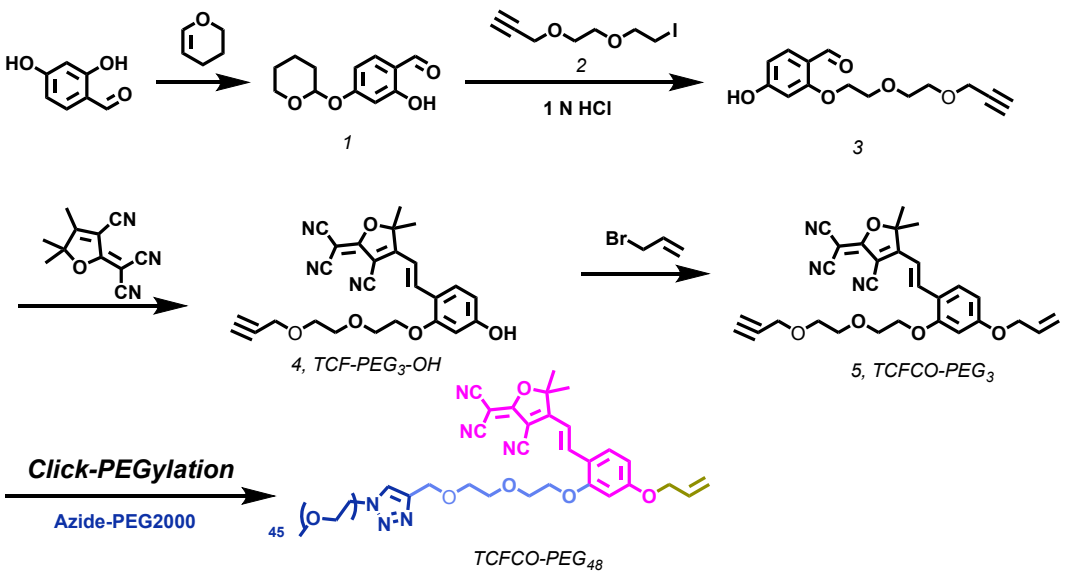
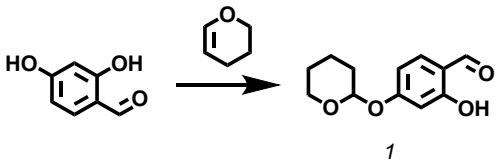
1. Materials and methods

2,4-Dihydroxybenzaldehyde (98%), 2,4-dihydro-2H-pyran (98%), copper sulfate pentahydrate (98%), Imidazole (98%), Triphenylphosphine (99%), were purchased from Energy Chemical. Pyridinium para-toluenesulfonate (98%), L-ascorbic acid (99%), Iodine (99.99%), Propynol Ethoxylate (98%), were purchased from Innochem. TCF, Cesium carbonate (99.9%), Allyl Bromide (99%), Azide Poly (ethylene glycol) 2000 (Azido PEG, Mn = 2.0 kg/mol) were purchased from Sigma-Aldrich. Gases including carbon monoxide (CO), hydrogen sulfide (H_2S), hydrogen (H_2), oxygen (O_2), carbon dioxide (CO_2) and sulfur dioxide (SO_2) were purchased from Dalian Special Gases Co., Ltd. All other chemicals and solvents were purchased from Energy Chemical or Innochem. Milli-Q water with a resistivity of 18.2 MΩ·cm was used in this study. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker instrument (400 MHz and 100 MHz, respectively) and internally referenced to tetramethylsilane signal or residual protio solvent signals. Data for ^{1}H NMR are recorded as follows: chemical shift (δ, ppm), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), intergration, coupling constant (Hz). Data for ^{13}C NMR are reported in terms of chemical shift (δ, ppm). High-resolution Mass spectrometric data was detected on a time-of-flight mass spectrometer (Agilent 1260-6230). Absorption spectra were measured on Spark® multimode microplate reader. Fluorescence spectra were performed on a F-4700 Fluorescence Spectrophotometer. Excitation and emission slit widths (slit: 10/10 nm) were modified to adjust the fluorescence intensity to a suitable range. Infrared spectra (IR) were recorded on a Nicolet NEXUS 470 FT-IR Spectrometer in ambient air at room temperature. The IR spectra were collected in KBr pellets from 400 to 4000 cm^{-1} with 4 cm^{-1} resolution and averaged 10 times.

2. Synthesis of probe TCFCO-PEG$_{48}$



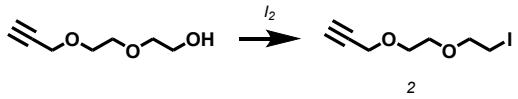
Scheme S1. Route for the synthesis of probe TCFCO-PEG$_{48}$.



Synthesis of Compound 1. 2,4-Dihydroxybenzaldehyde (2.0 g, 14.50 mmol) and 2,4-dihydro-2H-pyran (1.91 g, 22.73 mmol) were dissolved in dry CH_2Cl_2 (150 mL). Under stirring, pyridinium para-toluenesulfonate (PPTS, 3.64 g, 14.50 mmol) was added to the solution. After 4 h at ambient temperature, the reaction mixture was diluted with sodium phosphate buffer pH 6.8 (2 x 100 mL) and extracted with EtOAc. The organic layer was washed with water (2 x 100 mL) and brine (1 x 100 mL), dried over anhydrous Na_2SO_4, and filtered. After evaporated, the crude product was purified by silica gel column chromatography to afford compound 1 as yellow oil (1.69 g, yield 52%).

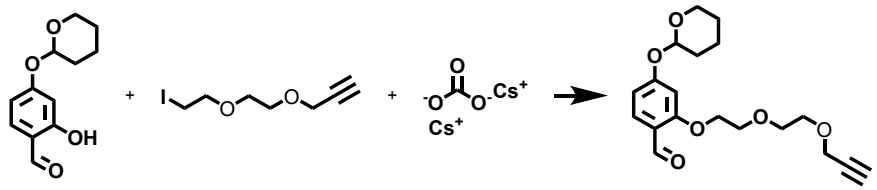
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61-2.04 (m, 6H); 3.63-3.84 (m, 2H); 5.51 (m, 1H); 6.65 (m, 2H); 7.44 (d, J = 8.6 Hz, 1H); 9.73 (s, 1H); 11.36 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$):

δ 194.54, 164.30, 164.11, 135.26, 115.72, 109.38, 103.63, 96.20, 62.16, 29.93, 24.94, 18.41. HRMS (ESI/[M+Na]$^+$) Calcd. for: $C_{12}H_{14}NaO_4$: 245.0790. Found: 245.0788.



Synthesis of Compound 2. I_2 (6.84 g, 27 mmol) was added to a solution of Triphenylphosphine (7.07 g, 27 mmol) and imidazole (1.84 g, 27 mmol) in dry CH_2Cl_2 (10 mL) at 0°C. Under stirring, Propynol Ethoxylate (3.0 g, 20.77 mmol) in CH_2Cl_2 (1 mL) was added, and then the reaction mixture was stirring from 0°C to RT for 5-10 h until the substrate was consumed (monitoring via TLC). Then, the formed precipitate was removed by filtering through kieselguhr, washed with CH_2Cl_2 and removed from the filtrate under reduced pressure. The crude product was purified by silica gel column chromatography. Compound 2 (3.0 g, 56.8 %) was obtained as colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 2.44 (t, J = 2.4 Hz, 1H), 3.27 (t, J = 6.8 Hz, 2H), 3.73 (t, J = 6.8 Hz, 6H), 4.22 (d, J = 2.4 Hz, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 76.03, 73.63, 70.98, 68.99, 68.04, 57.47, 1.72. HRMS (ESI/[M+Na]$^+$) Calcd. for: $C_7H_{11}INaO_2$: 276.9701. Found: 276.9691.

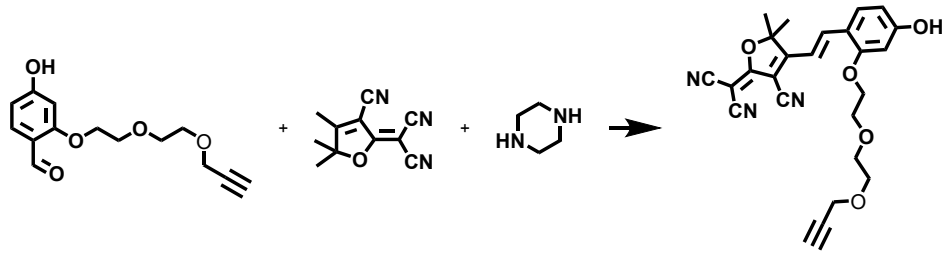


Synthesis of Compound 3. Compound 1 (900 mg, 4 mmol) and propargyl-MEG iodide (1.08 g, 4,28 mmol) were dissolved in dry DMF (10 mL). Cesium carbonate (1.69 g, 5.2 mmol) was added, then the reaction mixture was stirred at RT overnight. The reaction was quenched by water (150 mL) and extracted with EtOAc (3 x 100 mL). The combined

organic layers were washed with brine (1 x 100 mL), dried over anhydrous Na_2SO_4, filtrated, and evaporated to afford a yellow oil without further purification.

The oil (1.09 g, 3.1 mmol) was dissolved in MeOH (20 mL). After addition of 1 M HCl (8 mL), the reaction mixture was stirred at RT for 5 h. After the extracted with EtOAc (3 x 50 mL), the organic layers were concentrated under reduced pressure, the crude product was purified by silica gel column chromatography to afford compound 3 as yellow oil (485 mg, 78 %).

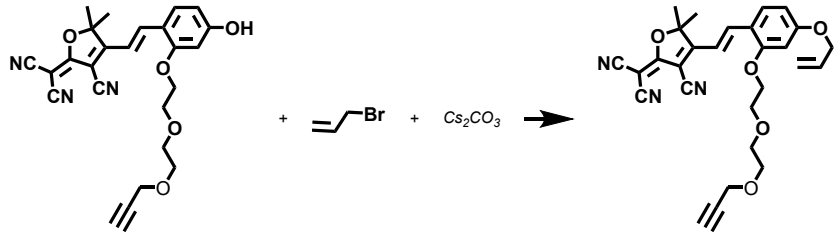
^{1}H NMR (400 MHz, $CDCl_3$): δ 10.19 (s, 1H), 7.68 (d, J = 8.6 Hz, 1H), 6.50 (dd, J = 8.6, 2.0 Hz, 1H), 6.39 (d, J = 2.0 Hz, 1H), 4.23 - 4.10 (m, 4H), 3.88 (dd, J = 5.6, 3.7 Hz, 2H), 3.80 - 3.70 (m, 4H), 2.48 (t, J = 2.4 Hz, 1H).^{13}C NMR (101 MHz, $CDCl_3$): δ 189.24, 164.74, 163.55, 130.85, 117.97, 109.13, 99.73, 79.23, 75.13, 70.54, 69.45, 69.08, 67.94, 58.40. HRMS (ESI/$[M+H]^+$) Calcd. for: $C_{14}H_{16}O_5$: 265.1076. Found: 265.1068.



Synthesis of compound TCF-PEG$_3$-OH. A piece of piperazine was added to a solution of 2-Dicyanomethylene-3-cyano-4,5,5-trimethyl-2,5-dihydrofuran (TCF, 340 mg, 1.70 mmol) and Compound 3 (450 mg 1.70 mmol) in EtOH (16 mL). After 8h at RT, EtOH was remove under reduced pressure, the crude product was purified by silica gel column chromatography to obtain compound 4 as a red solid (500 mg, 66%).

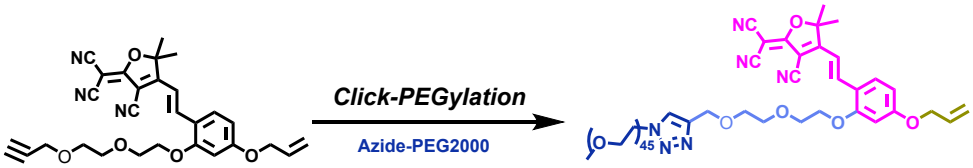
^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20 (d, J = 16.2 Hz, 1H), 7.87 (d, J = 8.4 Hz, 1H), 7.26

(d, J = 16.2 Hz, 1H), 6.63 - 6.54 (m, 2H), 4.28 - 4.16 (m, 4H), 3.89 (dd, J = 5.4, 3.4 Hz, 2H), 3.74 - 3.60 (m, 4H), 1.80 (s, 6H).^{13}C NMR (101 MHz, DMSO): δ 177.94, 177.13, 165.02, 161.66, 144.88, 133.79, 115.37, 113.61, 112.76, 112.28, 112.14, 110.07, 100.55, 99.06, 94.94, 80.76, 77.54, 70.11, 69.21, 68.97, 68.60, 57.95, 52.95, 25.86. HRMS (ESI/[M+H]$^+$) Calcd. for: $C_{25}H_{24}N_3O_5$: 446.1716. Found: 446.1712.

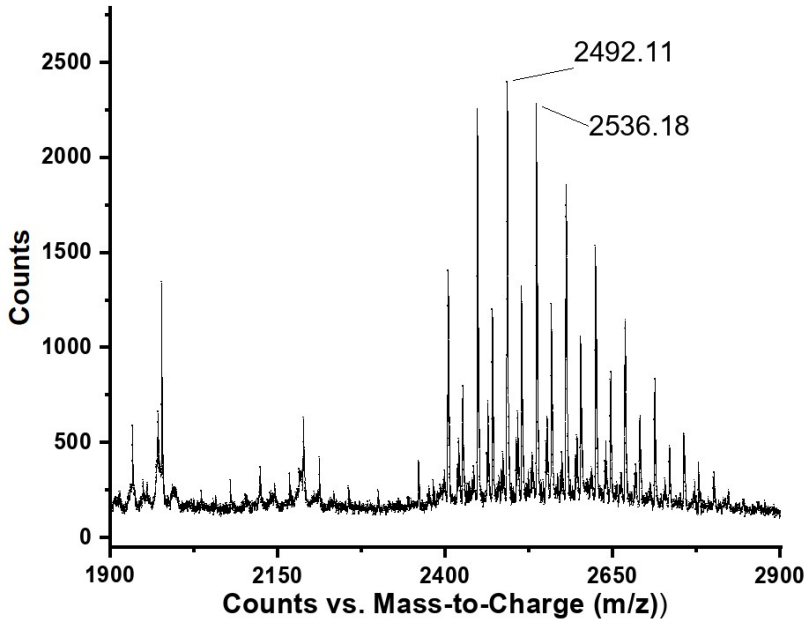


Synthesis of TCFCO-PEG$_3$. Cesium Carbonate (193 mg, 0.6 mmol) were added to the solution of Compound 4 (88 mg, 0.2 mmol) and Allyl Bromide (72 mg, 0.6 mmol) in dichloromethane (CH_3CN, 9 mL). After 5 h at RT, CH_3CN was removed under reduced pressure, the crude product was purified by silica gel column chromatography to obtain the pure yellow product (68 mg, 70 %).

^{1}H NMR (400 MHz, DMSO-d_6): δ 8.21 (d, J = 16.4 Hz, 1H), 7.96 (d, J = 8.5 Hz, 1H), 7.33 (d, J = 16.4 Hz, 1H), 6.78 (d, J = 9.2 Hz, 2H), 6.12 (ddt, J = 16.2, 10.5, 5.3 Hz, 1H), 5.55 - 5.41 (m, 1H), 5.37 (d, J = 10.5 Hz, 1H), 4.77 (d, J = 5.3 Hz, 2H), 4.32 (t, J = 4.3 Hz, 2H), 4.18 (d, J = 2.5 Hz, 2H), 3.89 (t, J = 4.4 Hz, 2H), 3.74 - 3.60 (m, 4H), 1.81 (s, 6H), 1.30 (d, J = 8.6 Hz, 2H).^{13}C NMR (101 MHz, DMSO): δ 177.87, 176.96, 164.41, 161.15, 144.25, 133.41, 118.61, 116.70, 113.48, 112.63, 111.98, 108.77, 100.31, 99.26, 96.03, 80.75, 77.53, 70.10, 69.32, 69.21, 68.97, 68.91, 57.95, 53.48, 29.54, 25.76, 14.41. HRMS (ESI/[M+Na]$^+$) Calcd. for: $C_{28}H_{27}N_3NaO_5$: 508.1848. Found: 508.1853.



Synthesis of probe TCFCO-PEG$_{48}$. Azido PEG2000 (87 mg, 0.04 mmol, 1.05 eqv.), $CuCl_2$ (3 mg, 0.016 mmol, 0.4 eqv.), and L-ascorbic acid (3.6 mg, 0.02 mmol, 0.5 eqv.) were dissolved in Milli-Q water (2 mL). Compound 5 (20 mg, 0.04 mmol, 1 eqv.) dissolved in DMF was added dropwise into the solution. The reaction mixture was stirred at RT until compound 5 was completely reacted (monitoring via TLC). After the reaction, the mixture was diluted with DCM (10 mL) and extracted with water. The organic phase was dried with anhydrous $MgSO_4$ and the solution was evaporated under reduced pressure. The product was purified by column chromatography to afford product as red powder (80 mg, 80%). The MS of the compound was measured as below.



3. Sensing property of probe TCFCO-PEG$_{48}$

To examine the selectity of the probe to CO, the potential property of the probe for sensing

anions was stuided. Probe 1 was dissolved in 3 mL distilled water to obtain the probe aqueous solutions (5 μM), and then Na_xA (A_x= Cl^-, Br^-, HCO_3^-, CO_3^{2-}, HS^-, SO_3^{2-}, $S_2O_3^{2-}$, SO_4^{2-}, NO_2^-, NH_4^+, BH_4^-) and H_2O_2, tert-butyl hydroperoxide was added to above probe aqueous solutions, respectively, and the concentration of anions was 1 mM. Meanwhile, the absoption and fluorescent emission spectra of the probe-anions were measured. $PdCl_2$ solution was prepared as following: NaCl (final concentration 10 mM) was added to $PdCl_2$ (final concentration 1mM) suspension in ethanol, following by gentle heating with stirring to transparent solution. And then, the solution was filtered out using a hydrophilic 0.2 μm PTFE membrane filter.

4. Determination of the detection limit

The detection limit was calculated based on the method reported in previous literature literatureliterature. The fluorescence emission spectrum of probe TCFCO-PEG_{48} was measured by three times and the standard deviation of blank measurement was achieved. The fluorescnece intensity at 630 nm was plotted as a concentration of CORM-2. The detection limit was calculated by using detection limt = 3σ/k: where σ is standard deviation of blank measurement, k is the slop between the fuoresnce intensity versus CORM-2 concentration.

5. The preparation for test paper of probe TCFCO-PEG_{48}.

For practical applications, we made the probe TCFCO-PEG_{48} into a text paper. Firstly, we cut the filter paper into a test paper strip. Then, the test paper was put into the prepared probe solution (water, 5 mM, 50 μL). Noted that each test paper contains the same amount of probe. The test paper was then dried in the drying oven at 60 ^oC. Finally, the test paper was obtained in reserve.

6. Cell Culture and Imaging.

H9c2 cells were cultured and propagated in serum-containing RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin – streptomycin solution with incubation in a humidified atmosphere containing 5% CO_2 at 37 ℃ according to the manufacturer's specifications. It was seeded at a density of 1×10^4 cells/cm^2. One or two days prior to imaging, the cells were passaged and plated in phenol red-free medium Confocal Dish, and allowed to grow to 50-70% confluence and washed with cell culture medium twice. Microscopy was performed with a Leica SP8 laser scanning confocal microscope.

7. MTT assay

H9c2 cells were seed in 96-well plates at density of 5000 cells/well. Plates were maintained at 37 °C in a 5% CO_2 / 95% air incubator for 24 hours. Then the culture medium in each wells were replaced by fresh medium containing different concentration of TCFCO-or TCFCO-PEG_{48}. After 4, 12 or 24 hours treatment, into each well, 15 μL MTT solution (5 mg/mL in phosphate buffer solution) were added. After 4 hours incubation at 37 °C, the absorbance of each wells at 490 nm was recorded by the Spark® multimode microplate reader.

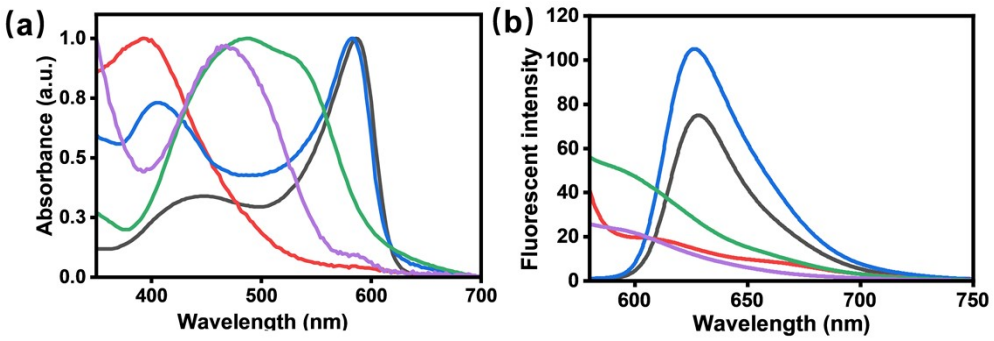


Figure S1. Absorption (a) and fluorescence spectra (b) of TCFCO-PEG_3 (green line), TCFCO-PEG_{48} (purpule line), TCF-PEG_3-OH (bule line), TCF-OH (black line) and TCFCO (red line) in PBS buffer (pH=7.4, 20%DMSO) (λ_{Ex}=560 nm).

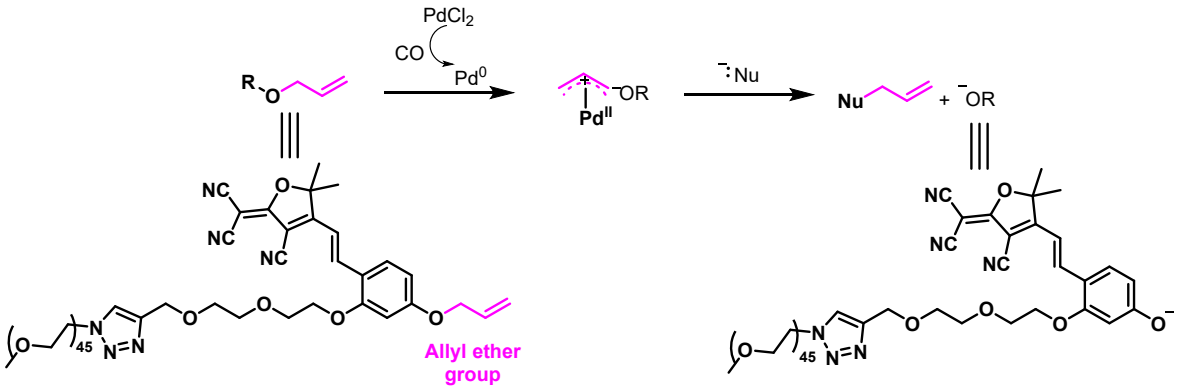


Figure S2. Proposed mechanism for Pd0-mediated Tsuji-Trost reaction.

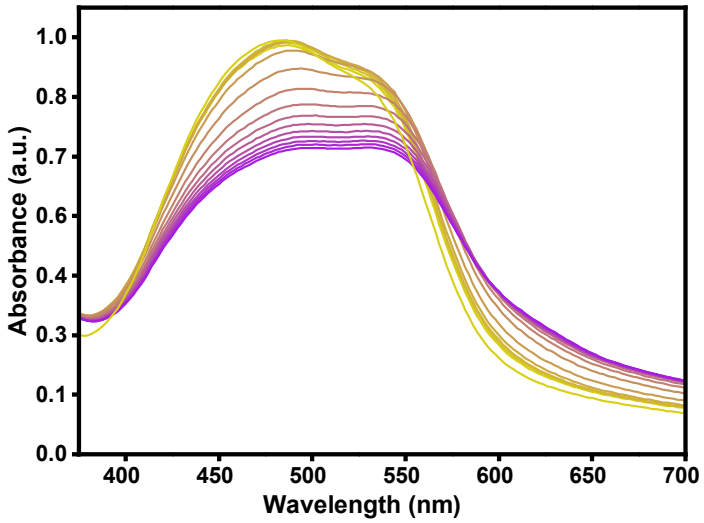


Figure S3. Absorption spectra of TCFCO-PEG_3 (10 μM) with 20 μM $PdCl_2$ upon treatment with CORM-2 (20 μM,) in PBS-DMSO (8 : 2, V/V, pH=7.4).

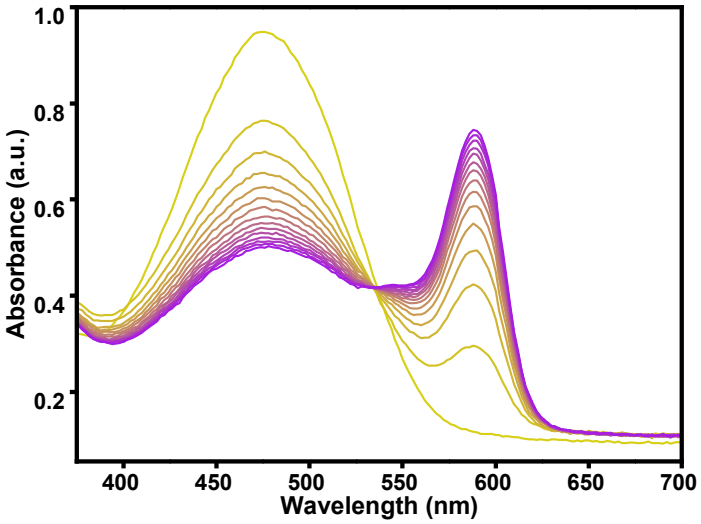


Figure S4. Absorption spectra of TCFCO-PEG_3 (10 μM) with 20 μM $PdCl_2$ upon treatment with CORM-2 (20 μM) in PBS-DMSO (4 : 6, V / V pH=7.4).

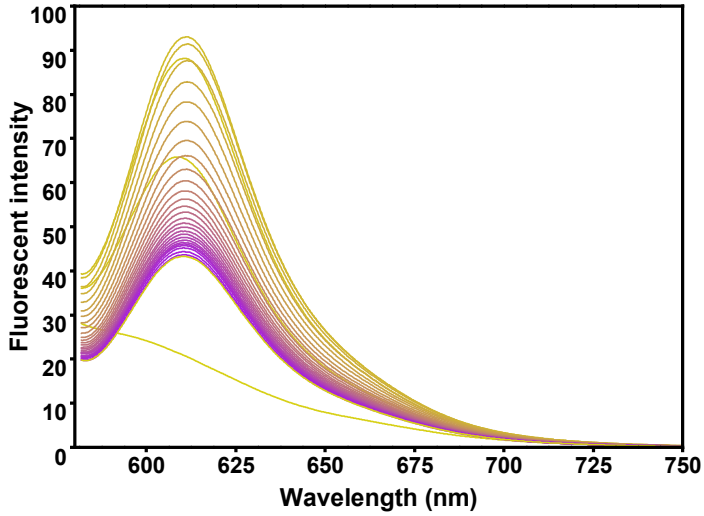


Figure S5. Fluorescence spectra of TCFCO-PEG_3 (10 μM) with 20 μM $PdCl_2$ upon treatment with CORM-2(20 μM) in PBS-DMSO (8 : 2, V / V pH=7.4).

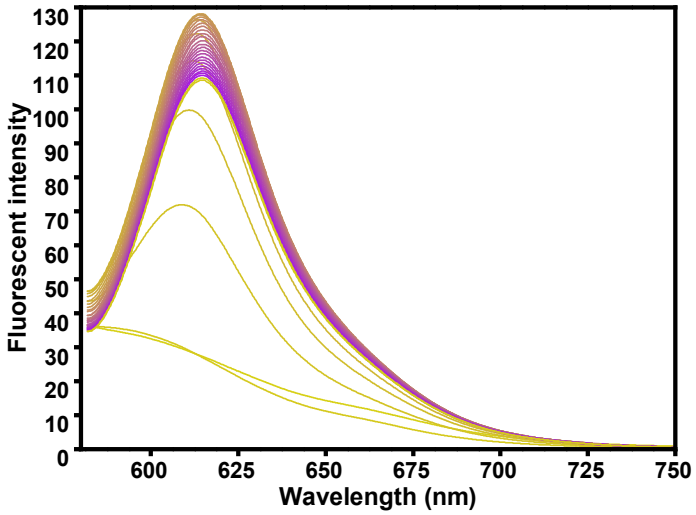


Figure S6. Fluorescence spectra of TCFCO-PEG_3 (10 μM) with 20 μM $PdCl_2$ upon treatment with CORM-2 (20 μM) in PBS-DMSO (7 : 3, V / V pH=7.4).

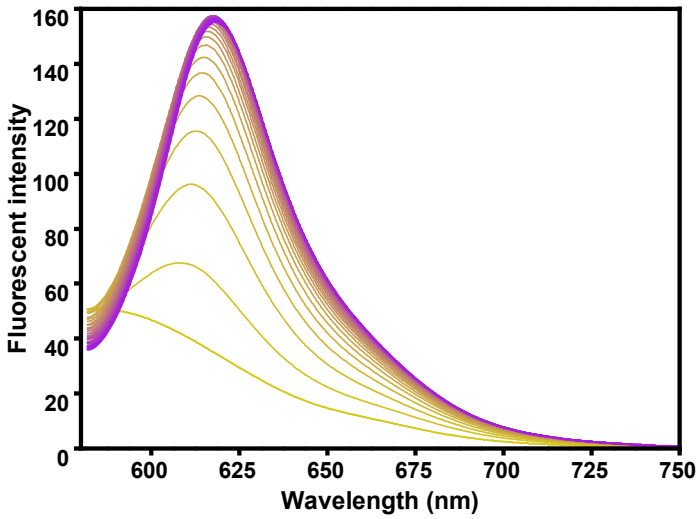


Figure S7. Fluorescence spectra of TCFCO-PEG_3 (10 μM) with 20 μM $PdCl_2$ upon treatment with CORM-2(20 μM,) in PBS-DMSO (6 : 4,V / V pH=7.4).

Figure S8. Color pictures of probe TCFCO-PEG_{48} (20 μM) with 20 μM $PdCl_2$ after reacting with different concentrations of CORM-2 (0, 0.2, 1.8, 1.5 and 10 eqv. of the probe) in PBS (pH=7.4).

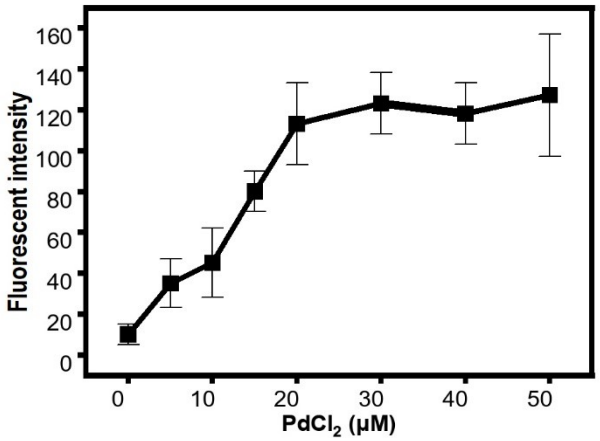


Figure S9. Fluorescence responses of probe TCFCO-PEG_{48} (10 μM) with a variety concentration of $PdCl_2$ on CORM-2(20 μM).

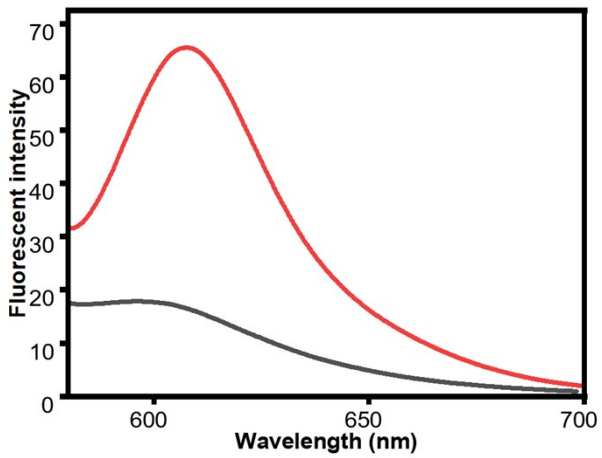


Figure S10. The fluorescence of probe system (TCFCO-PEG_{48} (10 μM) $PdCl_2$ (20 μM)) was measured before and after exposure to the atmosphere of 250 ppm CO for 15 minutes (λ_{Ex}=560 nm).

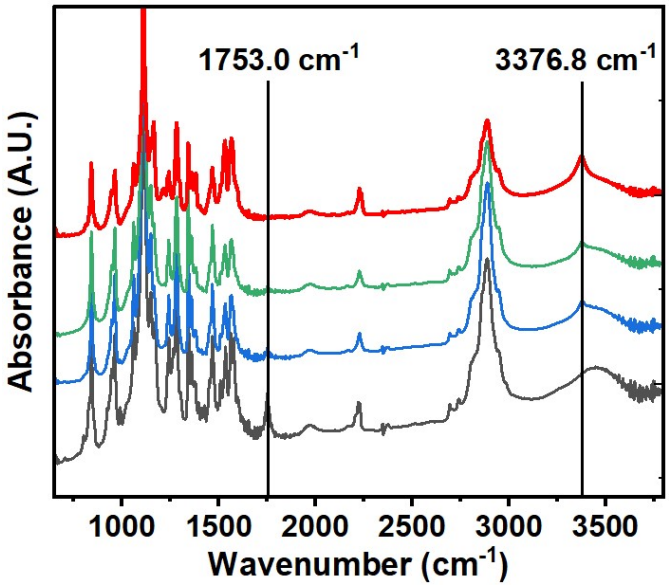
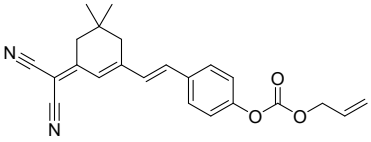
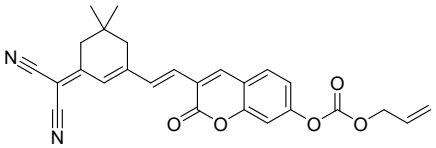
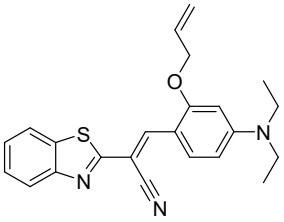
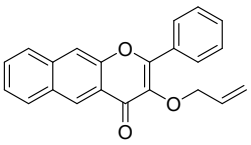
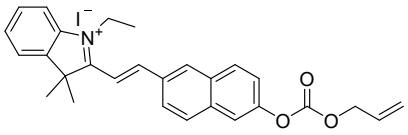
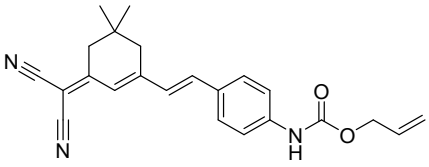
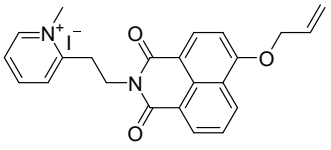
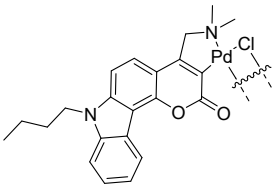


Figure S11. IR spectra of TCFCO-PEG_{48} (black line), TCFCO-PEG_{48}-OH (red line), and of the products of TCFCO-PEG_{48} after reacting with CORM-2 for 10 min (blue line) and 20 min (green line).

Table S1. Comparison of TCFCO-PEG_{48} with known CO sensing fluorescent probes

Reference	Probe structure	Emission (nm)	Solvent	Detection application
Talanta **2019**, 201, 40–45		λ_{ex}=550 nm λ_{em}=671 nm	10 mM PBS buffer, 20 % DMSO, v/v, pH 7.4	HeLa cells
Dyes Pigm. **2019**, 170, 107634		λ_{ex}=510 nm λ_{em}=710 nm	10 mM PBS buffer, 5 % DMSO, v/v, pH 7.4	Living HeLa cells and zebrafish
Anal. Chem. **2019**, 91, 9388-9392		λ_{ex}=465 nm λ_{em}=546 nm (Single emission) λ_{em}=546 nm &710 nm	5% DMSO / PBS mixed Solution, pH 7.4	In vivo detection of CO in living mice and living cells
ACS Omega **2020**, 5, 10021-10033		λ_{ex}=411 nm/λ_{em}=600 nm	8 % DMSO in DMEM, pH =7.4	HUVECs cells
New J. Chem. **2020**, 44, 12107-12112		λ_{ex} =410 nm λ_{em}=515 nm &600 nm	10 mM HEPES, 20 % DMSO, pH 7.4	HeLa, RAW264.7 cells, and zebrafish
Spectrochim. Acta Part A **2020**, 227, 117657		λ_{ex} =440 nm λ_{em}=592 nm &655 nm	10 mM PBS buffer, with 20% DMSO, v/v, pH 7.4	HeLa cells
Dyes Pigm. **2020**, 173, 107861		λ_{ex} =430 nm λ_{em}=466 nm &566 nm	20 mM PBS buffer, 10 % DMSO, pH =7.4	HepG2 cells
Chem. Sci. **2014**, 5, 3439-3448.		λ_{ex} =374 nm λ_{em}=477 nm (one-photon) λ_{ex},TPM =740 nm	PBS buffer-DMSO (9 : 1, v/v) pH 7.4	HeLa, MCF-7 and MKN-28

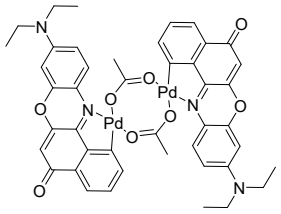
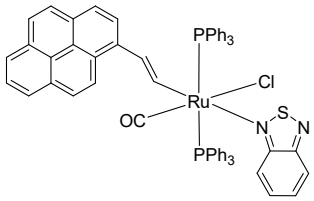
Angew. Chem. Int. Ed. **2017**, 56, 13489–13492		λ_{ex} =580 nm λ_{em} 660 nm	10 mM PBS buffer, 5 % DMSO, v/v, pH 7.4	Raw 264.7, Zebrafish embryos and living mice
J. Am. Chem. Soc. **2014**, 136, 11930−11933		λ_{ex} =355 nm λ_{em}=500 nm	Methonal	Silica gel

Table S2. The rate for the reaction between the probe TCFCO-PEG_3 and CORM-2 under the catalyst of $PdCl_2$ in PBS buffer (pH=7.4).

TCFCO-PEG_3 (μM)	CORM-2 (μM)	Initial rate (μM/min)
5 μM	20 μM	0.013
10 μM	20 μM	0.016
20 μM	20 μM	0.012
30 μM	20 μM	0.014
20 μM	5 μM	0.013
20 μM	10 μM	0.015
20 μM	20 μM	0.014
20 μM	30 μM	0.017

According to chemical reaction rate equation, rate=k[TCFCO-PEG_3]^x[CORM-2]^y, reaction order were calculated as below: x=0 y=0. According to integrated rate law of zero-order reaction, [TCFCO-PEG_3] = [TCFCO-PEG_3]$_0$-kt, the apparent reaction rate constant for probe TCFCO-PEG_3 was calculated as 0.0138 μM/min.

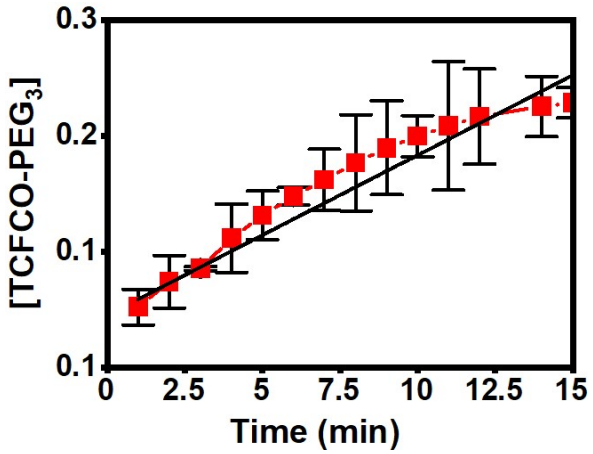


Figure S12. Curve fitting using kinetic models for the reaction between TCFCO-PEG_3 and CORM-2.

Table S3. The rate for the reaction between probe TCFCO-PEG_{48} and CORM-2 under the catalyst of $PdCl_2$ in PBS buffer (pH=7.4).

TCFCO-PEG48 (μM)	CORM-2 (μM)	Initial rate (μM/min)
5 μM	20 μM	0.16
10 μM	20 μM	0.28
20 μM	20 μM	0.63
30 μM	20 μM	0.94
20 μM	5 μM	0.21
20 μM	10 μM	0.32
20 μM	20 μM	0.62
20 μM	30 μM	0.95

According to chemical reaction rate equation, rate = $k[\text{TCFCO-PEG48}]^x[\text{CORM-2}]^y$, the reaction order was calculated: x = 1, y =1 . According to integrated rate law of second-order reaction, $1/[\text{TCFCO-PEG}_{48}] = 1/[\text{TCFCO-PEG}_{48}]+kt$, the apparent reaction rate constant for probe TCFCO-PEG_{48} was calculated as 0.00203 μM/min.

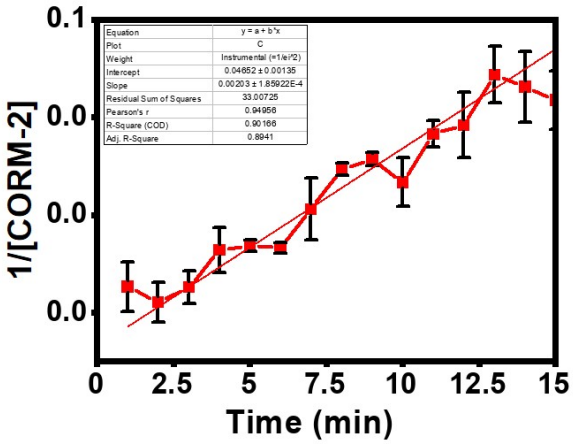


Figure S13. Curve fitting using kinetic models for the reaction between TCFCO-PEG$_{48}$ and CORM-2.

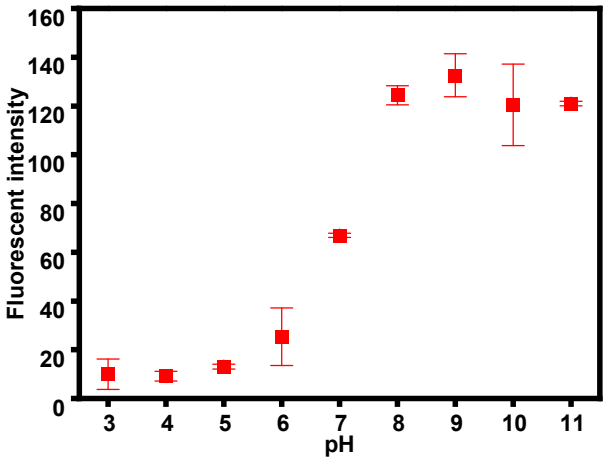


Figure S14. Fluorescence responses of probe TCFCO-PEG$_{48}$ (10 μM) with 20 μM $PdCl_2$ and CORM-2 (20 μM) to various pH in PBS solution. The solution with different pH values were prepared by adding 1 M NaOH or 1M HCl solution using the pH meter.

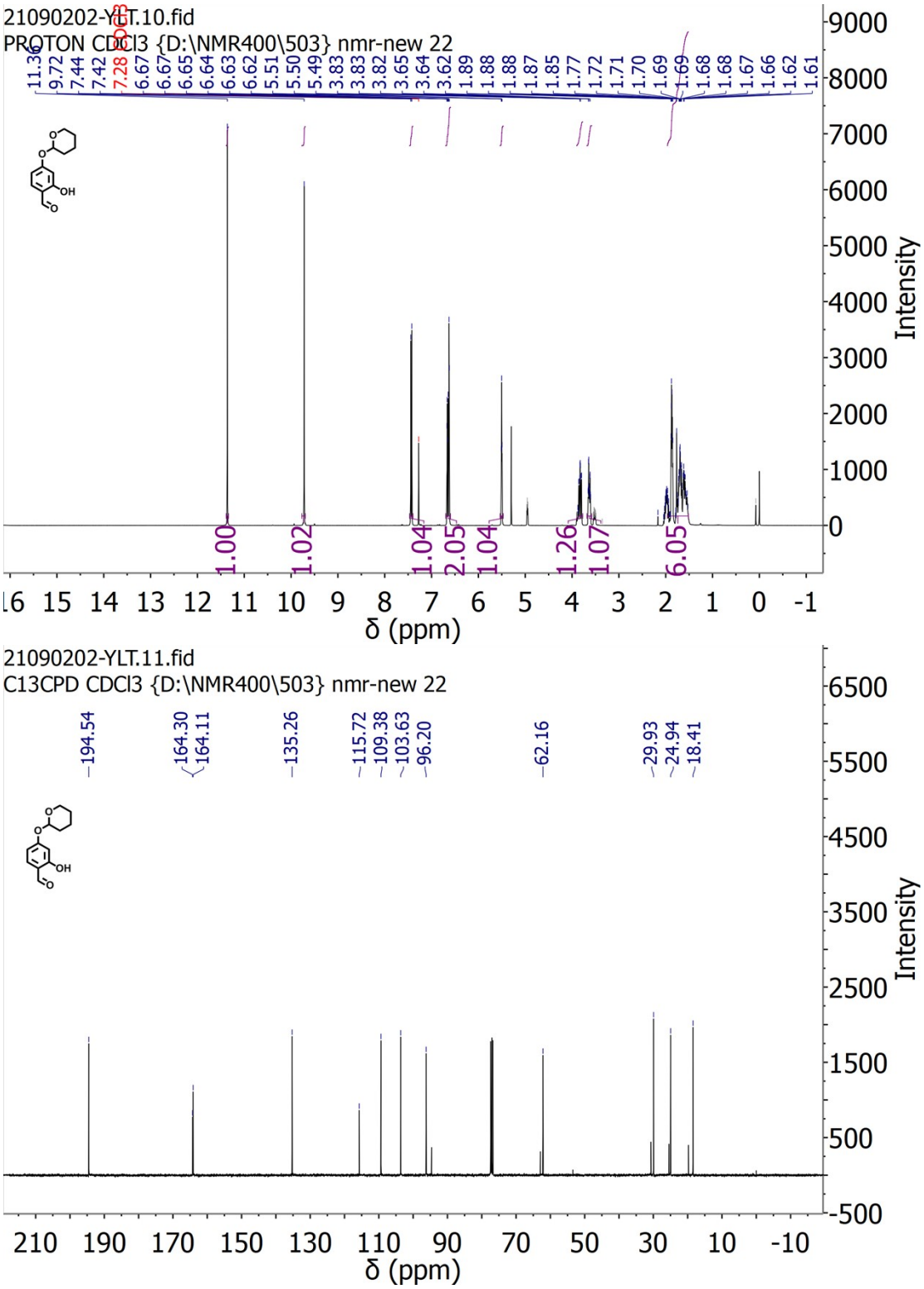


Figure S15. ^{1}H NMR and ^{13}C NMR spectra of Compound 1.

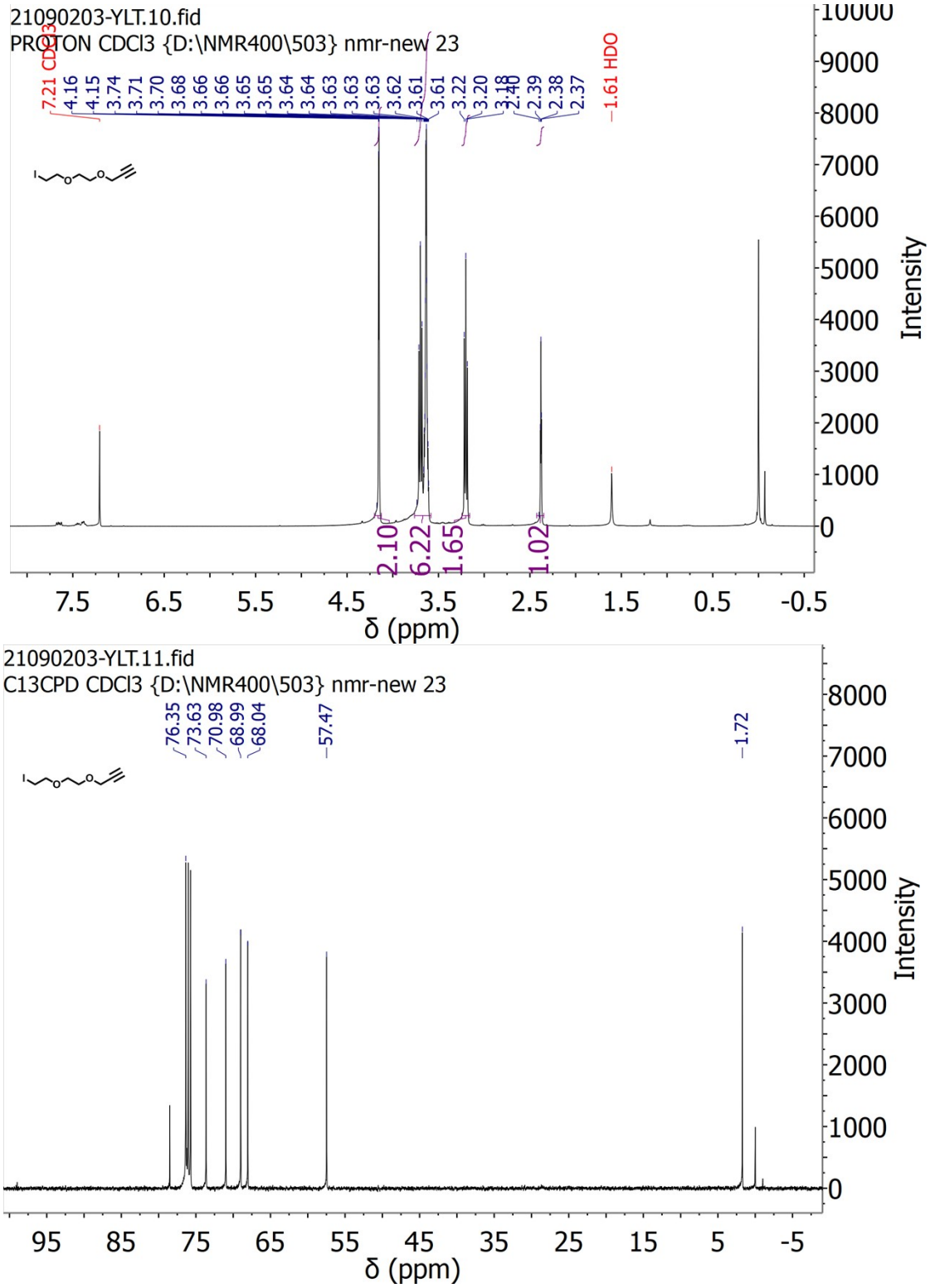


Figure S16. ^{1}H NMR and ^{13}C NMR spectra of Compound 2.

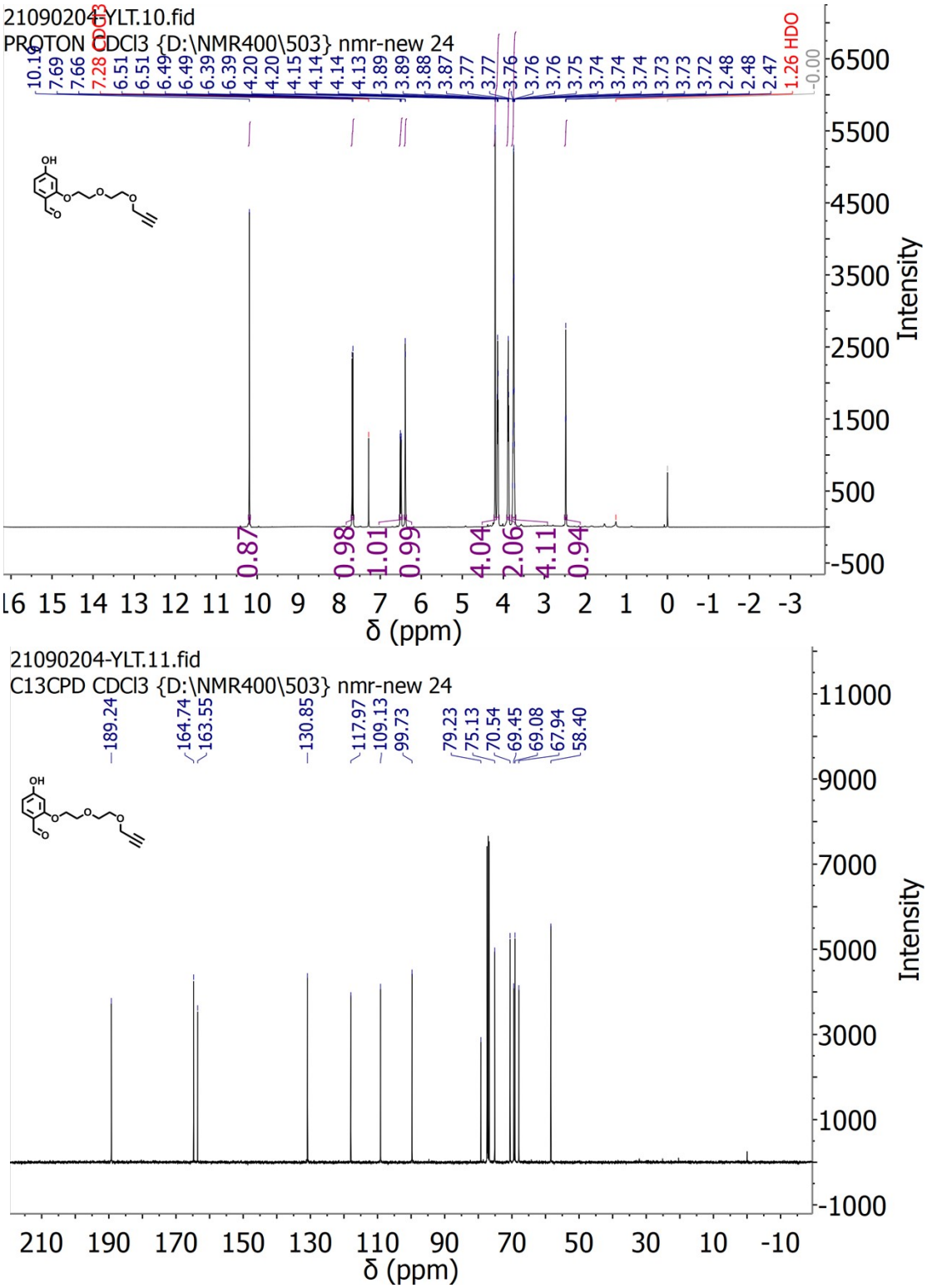


Figure S17. ^{1}H NMR and ^{13}C NMR spectra of Compound 3.

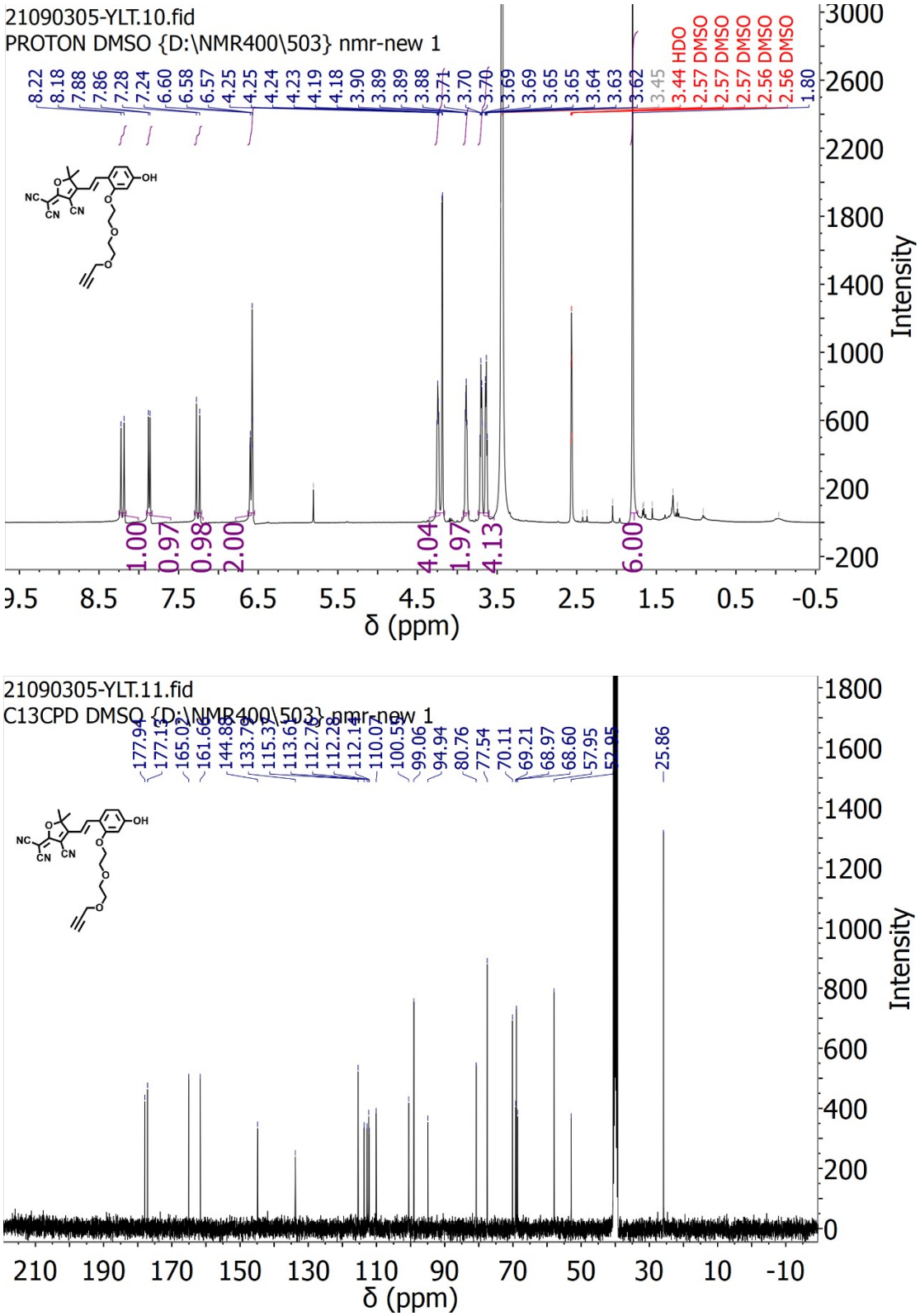


Figure S18. ^{1}H NMR and ^{13}C NMR spectra of TCF-PEG$_3$-OH.

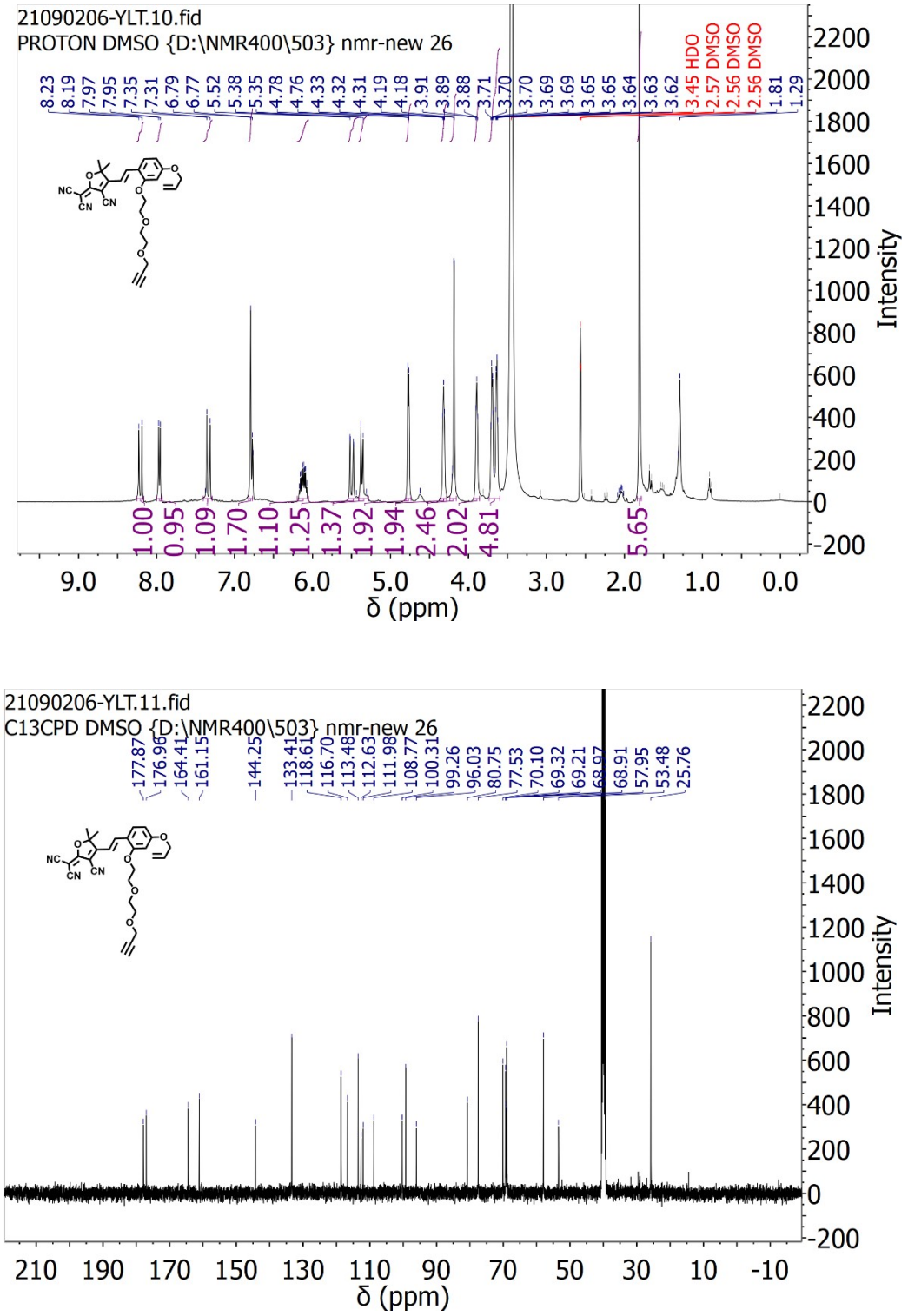


Figure S19. ^{1}H NMR and ^{13}C NMR spectra of TCFCO-PEG$_3$.