Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

for

Nitro Reduction-based Fluorescent Probes for Carbon Monoxide Require Reactivity Involving a Ruthenium Carbonyl Moiety

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[#]These two authors made equal contributions.

General Information

All reagents and solvents were of reagent grade from commercial suppliers (Sigma Aldrich and etc.). ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were performed on a Bruker-400 spectrometer. Mass spectral analyses were performed on an ABI API 3200 (ESI-Triple Quadruple) by the Georgia State University Mass Spectrometry Facilities. Fluorescence spectra were recorded on a Shimadzu RF-5301PC fluorometer. Absorption spectra were measured on Varian Cary 100 Bio UV-Visible spectrophotometer. **CORM-2**, **CORM-3**, **CORM-A1** and **CORM-401** were purchased from Sigma-Aldrich and were used without purification. **BW-CO-103**[1] and **COP-1**[2] were synthesized according to literature procedures. **iCORM-3** was prepared according to a literature procedure.[3] 2-Dicyanomethylen-3-cyano-4,5,5-trimethyl-2,5-dihydrofurane was synthesized according to the reported procedures.[4]

Synthesis

5-nitro-2-phenyl-1*H*-benzo[*de*]isoquinoline-1,3(2*H*)-dione (COFP) was synthesized according to a reported procedure.[5] 3-Nitro-1,8-naphthalic anhydride (0.62 mmol, 150 mg) and aniline (0.62 mmol, 58 mg) were refluxed in 4 ml acetic acid for 4 h. 20 ml water was added at room temperature and the formed yellow solid was filtered and washed with water. The crude product was purified by silica gel column chromatography to yield the pure product as white solid (103 mg, 52%). ^{1}H NMR (DMSO-d_6) δ 9.51 (s, 1H), 8.95 (s, 1H), 8.81 (d, *J* = 8.4 Hz, 1H), 8.68 (d, *J* = 7.2 Hz, 1H), 8.08 (t, *J* = 8.0 Hz, 1H), 7.56-7.48 (m, 3H), 7.41 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (DMSO-d_6) δ 163.2, 162.7, 145.9, 136.5, 135.7, 134.0, 131.1, 130.1, 129.9, 129.4, 129.1, 128.6, 124.7, 123.3, 122.9. HRMS calculated for $C_{18}H_{10}N_2O_4Na$ $[M+Na]^+$: m/z 341.0540, found 341.0538.

2-(2-morpholinoethyl)-5-nitro-*1H*-benzo[de]isoquinoline-1,3(*2H*)-dione (LysoFP-NO_2) was synthesized following a modified reported procedure.[6] To a solution of 3-nitro-1,8-naphthalic anhydride (0.33 mmol, 80 mg) in 20 ml ethanol was added 4-(2-aminoethyl) morpholine (0.39 mmol, 51 mg) dropwise in 3 ml ethanol. The solution was allowed to stir for 40 min at r.t. and then heated to reflux for another 2 h. The formed solid was filtered and washed with 5 ml cold ethanol. The crude product was purified by silica gel column chromatography to yield the pure product (42 mg, 36%). ^{1}H NMR ($CDCl_3$) δ 9.30 (d, *J* = 2.2 Hz, 1H), 9.13 (d, *J* = 2.2 Hz, 1H), 8.93-8.61 (m, 1H), 8.43 (d, *J* = 8.2 Hz, 1H), 8.07-7.77 (m, 1H), 4.36 (t, *J* = 6.7 Hz, 2H), 3.80-3.50 (m, 4H), 2.72 (t, *J* = 6.6 Hz, 2H), 2.58 (s, 4H). ^{13}C NMR ($CDCl_3$) δ 163.3, 162.6, 146.5, 135.7, 134.5, 131.1, 130.4, 129.3, 129.1, 124.8, 124.4, 123.3, 67.1, 56.2, 54.0, 37.7. HRMS calculated for $C_{18}H_{18}N_3O_5$ $[M+H]^+$: m/z 356.1237, found 356.1246.

***(E)*-2-(3-cyano-4-(3-hydroxy-4-nitrostyryl)-5,5-dimethylfuran-2(5H)-ylidene)malononitrile (NIR-CO)** was synthesized following a literature procedure.[7] To a solution of 3-hydroxy-4-nitrobenzaldehyde (105 mg, 0.63 mmol) in 5 ml ethanol was added piperidine (53 mg, 0.63 mmol) and 2-dicyanomethylen-3-cyano-4,5,5-trimethyl-2, 5-dihydrofurane (125 mg, 0.63 mmol). The mixture was allowed to stir at r.t. for 12 h and then filtered. The residue was purified by silica gel column chromatography to yield the pure product (80 mg, 36%). ^{1}H NMR (DMSO-d_6) δ 7.98 (d, *J* = 8.8 Hz, 1H), 7.81 (d, *J* = 16.8 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.24 (d, *J* = 16.4 Hz, 1H), 1.78 (s, 6H). ^{13}C NMR (DMSO-d_6) δ 177.0, 174.2, 152.31, 144.1, 140.4, 138.1, 126.1, 119.7, 119.3, 112.6, 111.9, 110.7, 102.2, 99.9, 55.6, 25.1. HRMS calculated for $C_{18}H_{11}N_4O_4$ $[M-H]^-$: m/z 347.0780, found 347.0774.

***cis*-$RuCl_2(DMSO)_4$ (Complex D)** was synthesized according to a reported procedure.[8] Briefly, 100 mg $RuCl_3.xH_2O$ was dissolved in 4 ml DMSO and stirred at reflux for 1 h. The color of the reaction mixture changed from deep brown to light yellow. After cooling to room temperature, the reaction mixture was cooled at -20 °C; the crystalized bright yellow solid was filtered and washed with acetone followed by drying in vacuum to afford 147 mg (63%) of a bright yellow solid. NMR shown the aqua species *cis,fac*-$[RuCl_2(dmso\text{-}S)_3(H_2O)]$ after dissolved in D_2O as suggested. ^{1}H NMR (D_2O) δ 3.48 (s, 6H), 3.46 (s, 6H), 3.37 (s, 6H), 2.70 (s, 6H). ^{13}C NMR (D_2O) δ 46.73, 45.70, 44.31, 38.67.

***N*-isobutyl-4-nitrobenzamide (PNB)** To a solution of 4-nitrobenzoic acid (200 mg, 1.2 mmol) in 5 ml DCM was added EDC (343 mg, 1.8 mmol), DMAP (219 mg, 1.8 mmol), triethylamine (181 mg, 1.8 mmol) and isobutylamine (87.6 mg, 1.2 mmol). The mixture was allowed to stir at r.t. for 12 h. The reaction solution was diluted with 15 ml DCM and washed with 10 ml 0.1 M HCl. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield the pure product as a white solid (205 mg, 77%). ^{1}H NMR ($CDCl_3$) δ 8.15 (d, *J* = 8.8 Hz, 2H), 7.90 (d, *J* = 8.4 Hz, 2H), 7.09 (s, 1H), 3.21 (t, *J* = 6.4 Hz, 2H), 1.87-1.84 (m, 1H), 0.90 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR ($CDCl_3$) δ 165.8, 149.6, 140.6, 128.2, 123.9, 47.78, 28.7 20.3. HRMS calcd for $C_{11}H_{14}N_2O_3$ $[M+H]^+$ 223.1083, found 223.1093.

4-amino-*N*-isobutylbenzamide (PAB) To a solution of **PNB** (45 mg, 0.18 mmol) in 1.8 ml acetone/water (5:1) was added zinc powder (117 mg, 1.8 mmol) and ammonium chloride (144 mg, 2.7 mmol). The mixture was vigorously shaken at room temperature for 1 min and then filtered. The filtrate was diluted with 10 ml ethyl acetate and washed with 5 ml saturated $NaHCO_3$ solution. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to yield the pure product as a white solid (23 mg, 67%). ^{1}H NMR (Methanol-d_4) δ 8.13 (s, 1H), 7.59 (d, *J* = 8.4 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 3.15 (t, *J* = 6.4 Hz, 2H), 1.92-1.86 (m, 1H), 0.94 (d, *J* = 6.8 Hz, 6H). ^{13}C NMR (Methanol-d_4) δ 170.6, 153.0, 129.8, 123.5, 114.7, 48.3, 29.9, 20.6. HRMS calcd for $C_{11}H_{16}N_2O$ $[M-H]^-$: m/z 191.1186, found 191.1184.

General procedures for spectral studies

Stock solutions preparation: COFP, **LysoFP-NO_2** and **NIR-CO** were dissolved in DMSO to afford a 500-μM stock solution; **COP-1** was dissolved in DMSO to afford a 200-μM stock solution. For BW-**CO-103**, **CORM-2**, **CORM-401** and **Complex D**, the compounds were freshly prepared in DMSO to afford 5-mM stock solutions. **CORM-3** and **CORM-A1** were dissolved in distilled water to afford 5-mM stock solutions. According to the literature procedure,[3] **CORM-3** was dissolved in PBS and incubated overnight to afford the 5-mM **iCORM-3** stock solution.

CO gas treatment: To a 6-ml headspace vial, 2 ml of the probe solution was added. 10 ml pure CO gas was directly bubbled into the solution by a headspace gas syringe. The resulting solution was further incubated at 37 °C for 60 min followed by the acquisition of the fluorescence spectra.

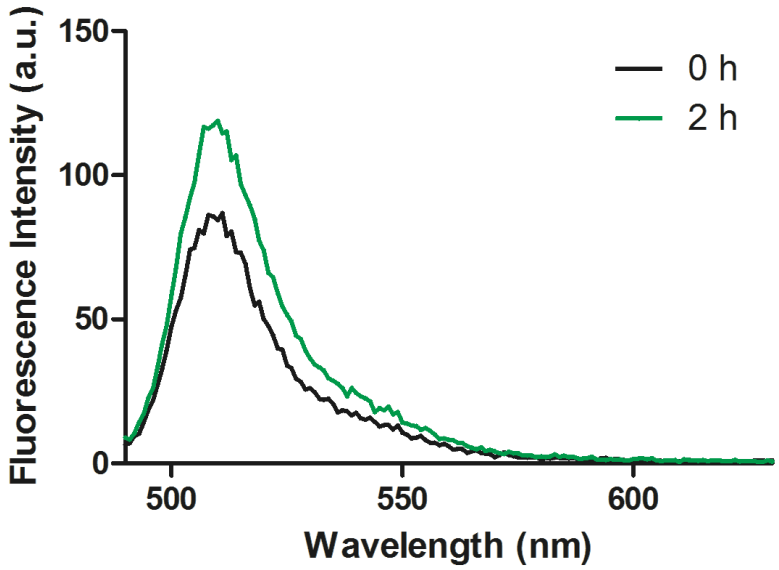


Figure S1. Fluorescence spectra of **COP-1** (1 μM) upon treatment with **CO-103** (100 μM) over 2 h in DMSO/PBS (pH = 7.4) 5:1 at 37 °C (λ_{ex} = 475 nm, slit widths: W_{ex} = 5 nm, W_{em} = 3 nm).

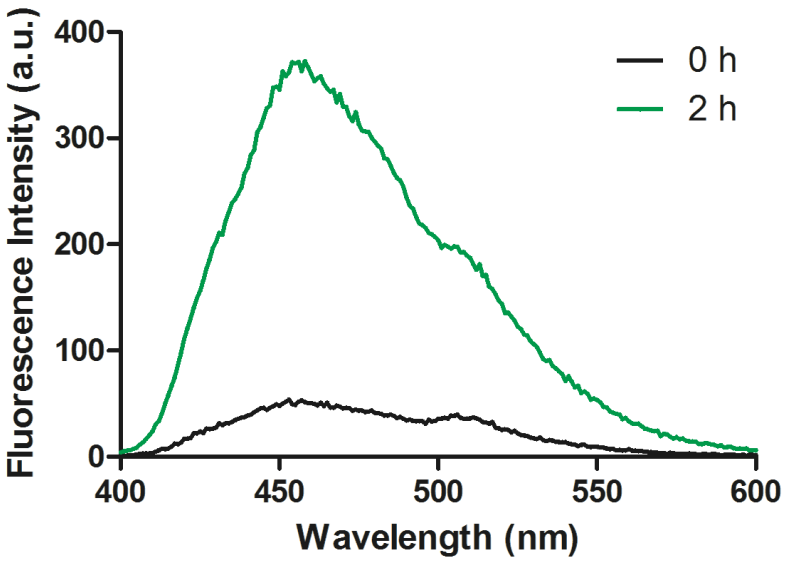


Figure S2. Fluorescence spectra of **CP-103** formation during incubation with **COP-1** (1 μM) in DMSO/PBS (pH = 7.4) 5:1 at 37 °C (λ_{ex} = 373 nm, slit widths: W_{ex} = W_{em} = 5 nm).

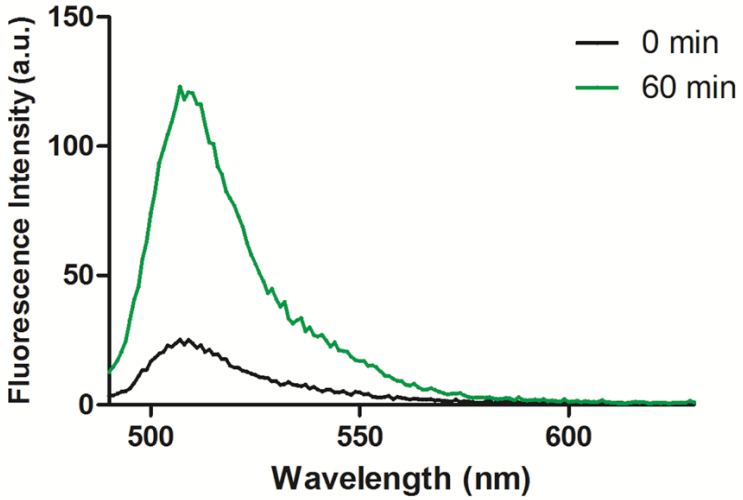


Figure S3. Fluorescent spectra of **COP-1** (1 μM) upon incubation with **CORM-A1** (100 μM) over 60 min in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex} = 475 nm, slit widths: W_{ex} = 5 nm, W_{em} = 3 nm).

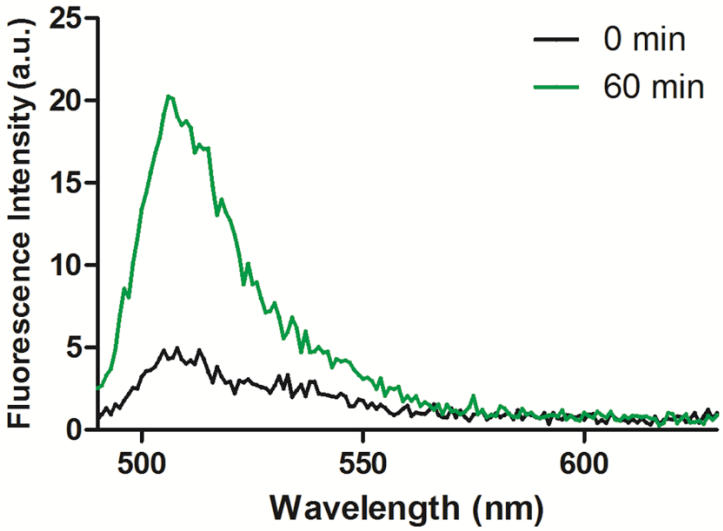


Figure S4. Fluorescent spectra of **COP-1** (1 μM) upon incubation with **CORM-401** (100 μM) over 60 min in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex} = 475 nm, slit widths: W_{ex} = 5 nm, W_{em} = 3 nm).

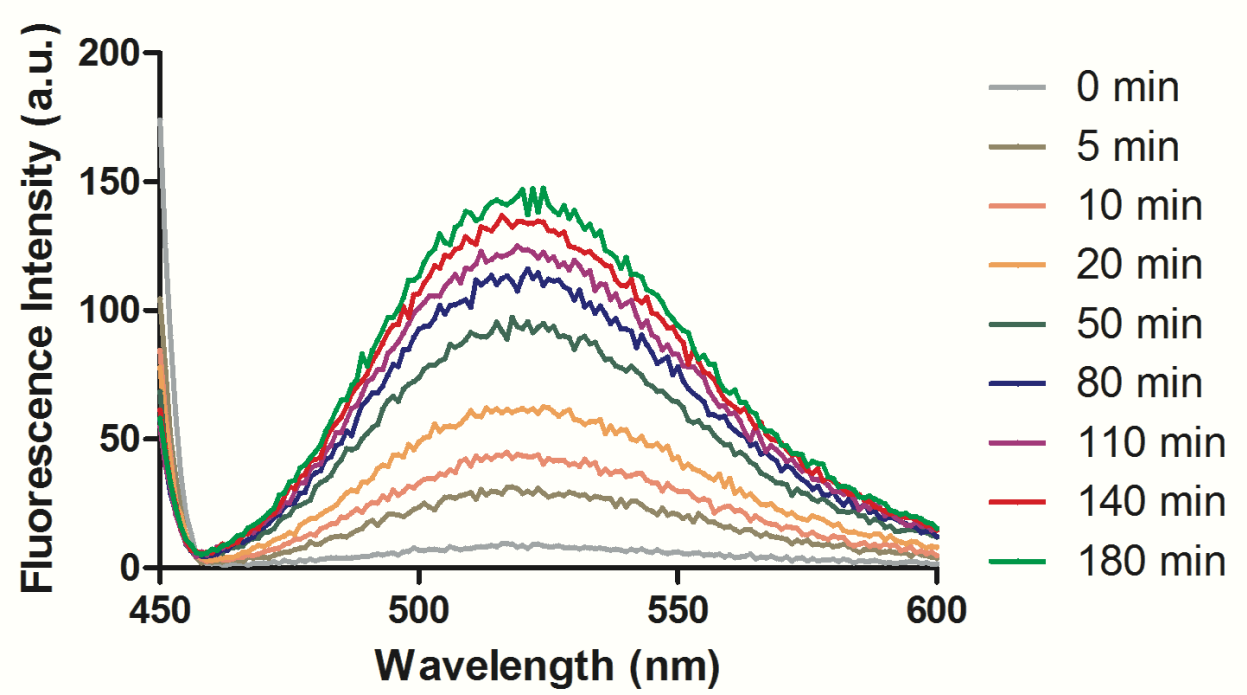


Figure S5. Fluorescent spectral changes of **COFP** (10 μM) upon incubation with **CORM-2** (100 μM) in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: W_{ex} = W_{em} = 10 nm)

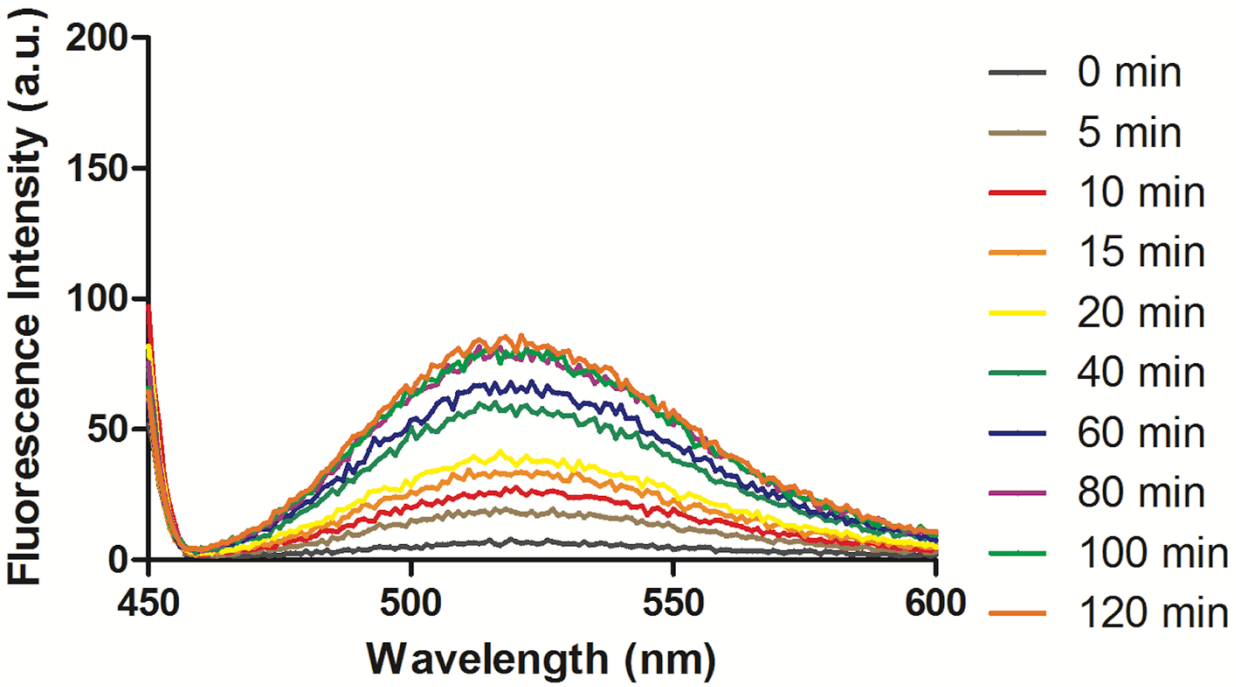


Figure S6. Fluorescent spectral changes of **COFP** (10 μM) upon incubation with reaction products between **CORM-2** and DMSO (100 μM) in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: $W_{ex} = W_{em} = 10$ nm)

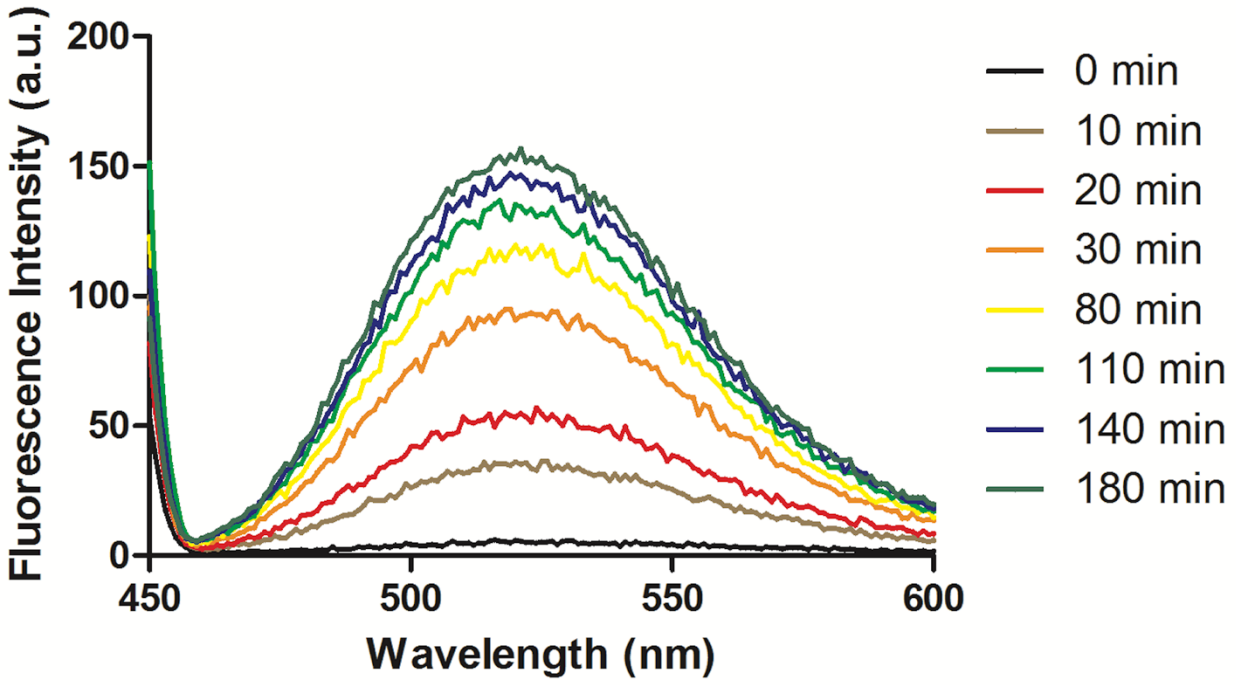


Figure S7. Fluorescent spectra of **COFP** (10 μM) upon incubation with **CORM-3** (100 μM) in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: $W_{ex} = W_{em} = 10$ nm)

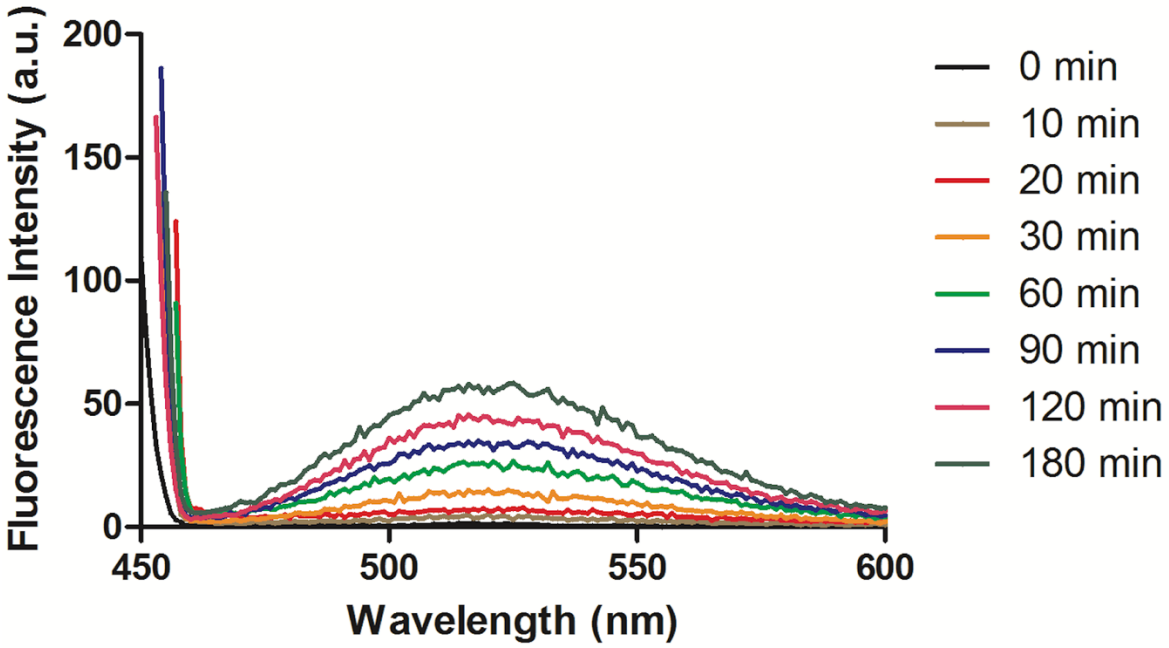


Figure S8. Fluorescent spectra of **COFP** (10 μM) upon incubation with **iCORM-3** (100 μM) in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: W_{ex} = W_{em} = 10 nm)

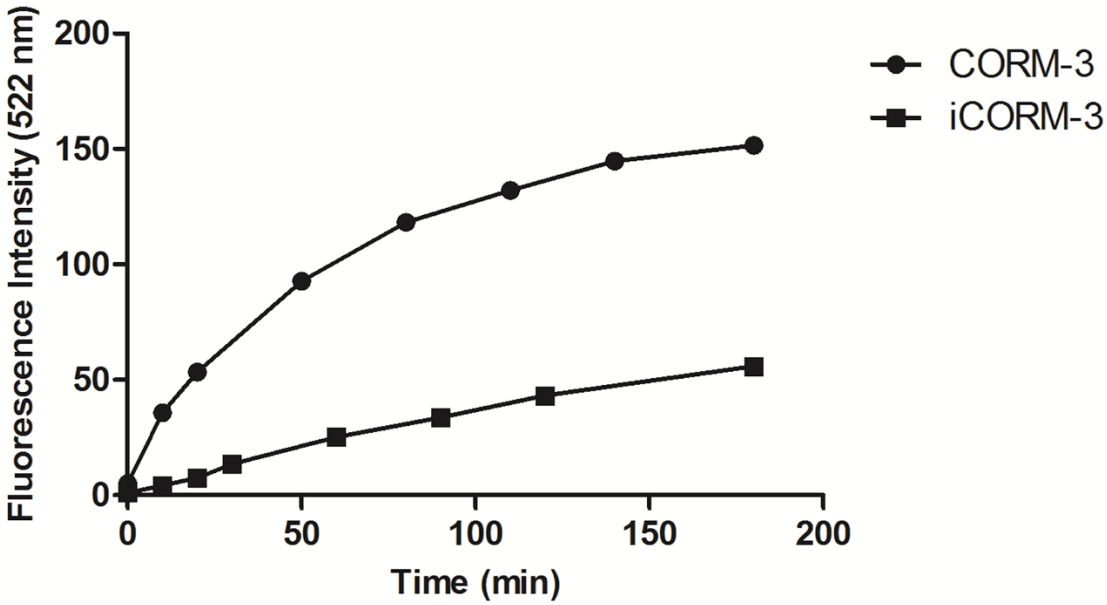


Figure S9. Fluorescence intensity changes from **COFP** (10 μM) upon treatment with **CORM-3** and **iCORM-3** in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, λ_{em}= 522 nm, slit widths: W_{ex} = W_{em} = 10 nm)

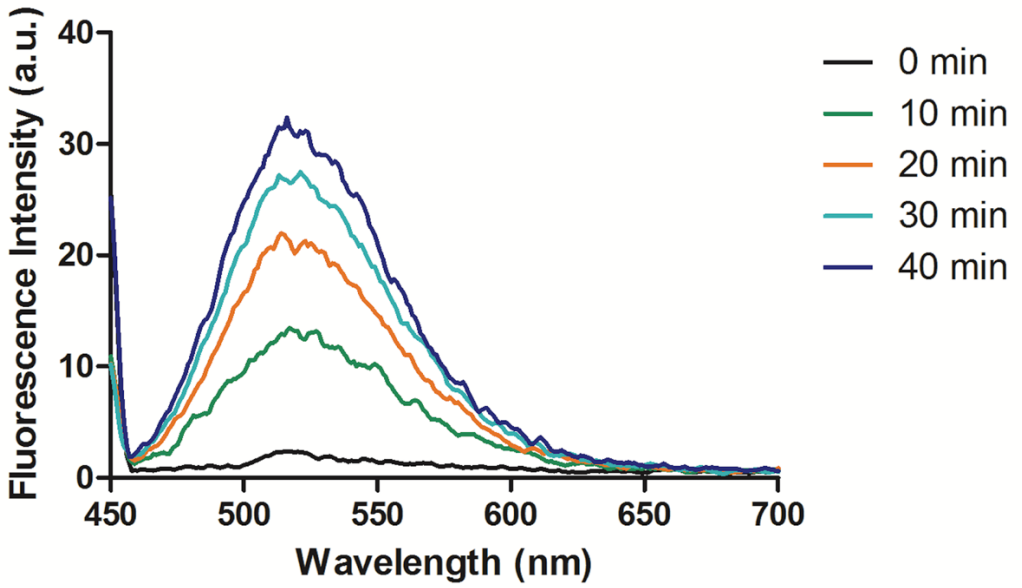


Figure S10. Fluorescent spectra of **LysoFP-NO_2** (10 μM) upon incubation with **CORM-3** (100 μM) in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: $W_{ex} = W_{em} = 10$ nm)

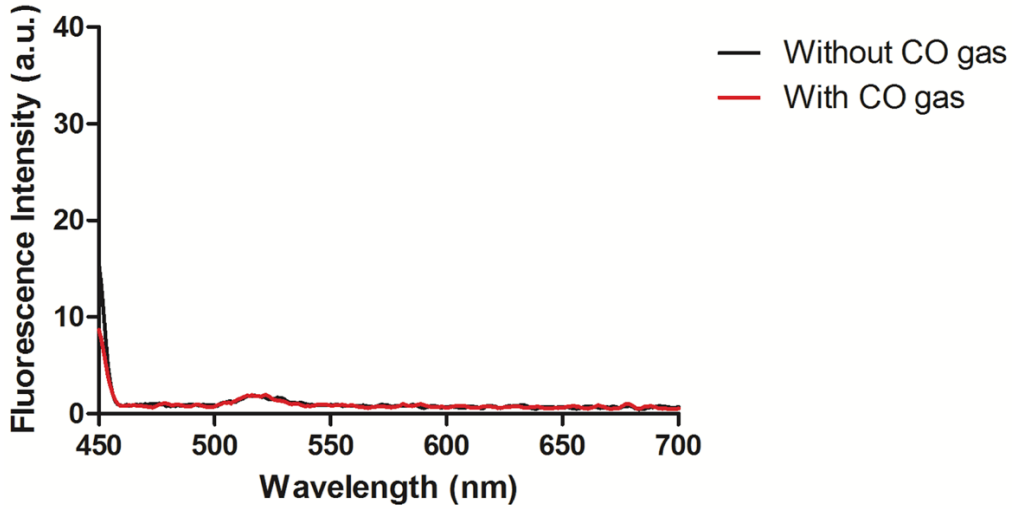


Figure S11. Fluorescent spectra of **LysoFP-NO_2** (10 μM) upon treatment with CO gas in PBS (2% DMSO) at 37 °C for 1 h. (λ_{ex}= 440 nm, slit widths: $W_{ex} = W_{em} = 10$ nm)

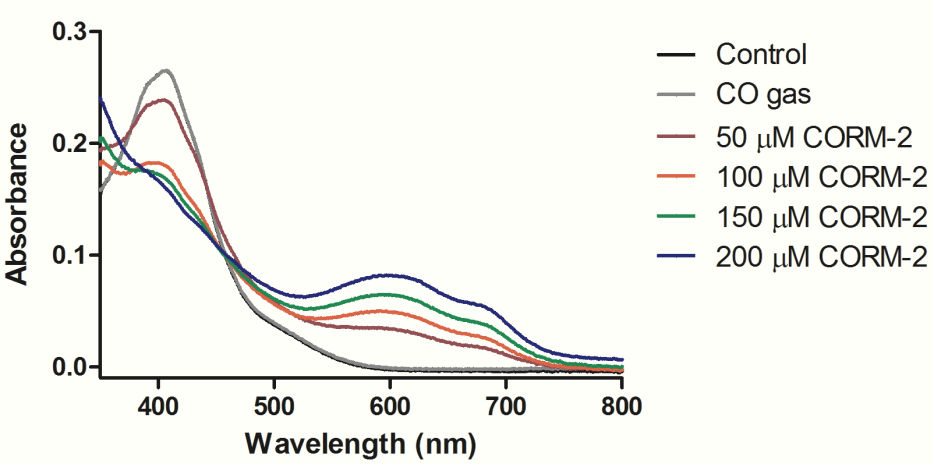


Figure S12. Absorption spectra of **NIR-CO** (10 μM) upon the treatment with CO gas and various concentrations of **CORM-2** in HEPES buffer (5 mM, pH = 7.4, 30% DMSO) at r.t. for 15 min.

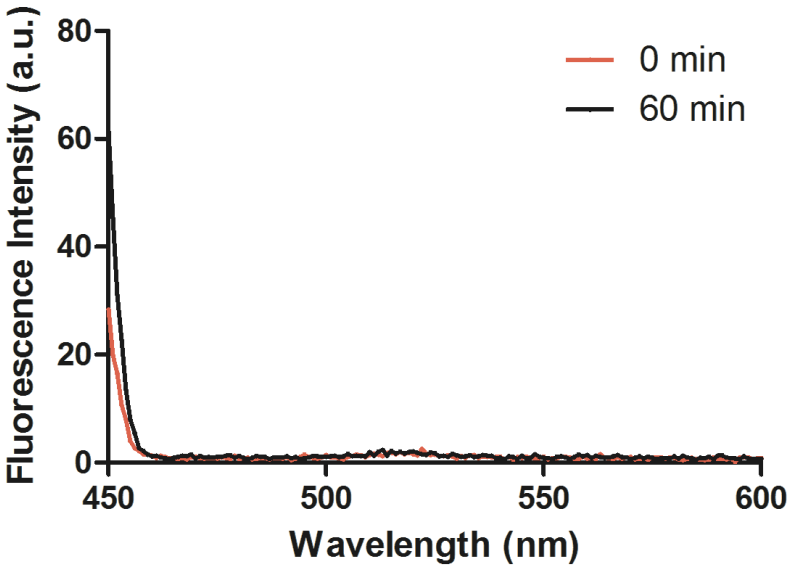


Figure S13. Fluorescent spectra of **COFP** (10 μM) upon incubation with **CORM-A1** (100 μM) over 60 min in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: W_{ex} = W_{em} = 10 nm)

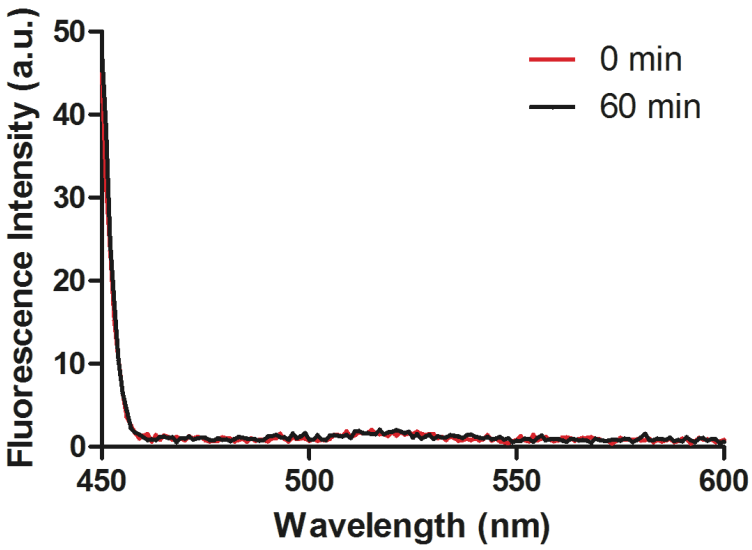


Figure S14. Fluorescent spectra of **COFP** (10 μM) upon incubation with **CORM-401** (100 μM) over 60 min in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: W_{ex} = W_{em} = 10 nm)

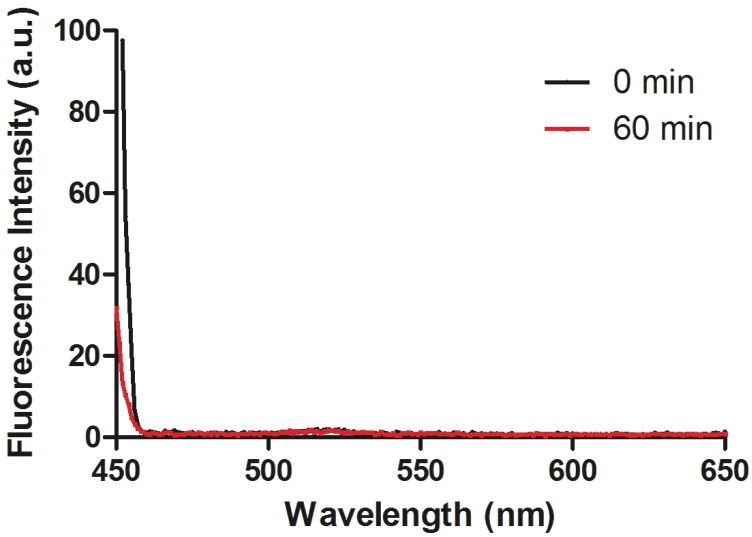
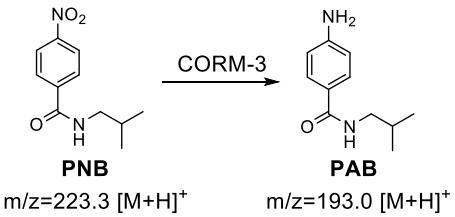


Figure S15. Fluorescent spectra of **COFP** (10 μM) upon incubation with **Complex D** (100 μM) for 60 min in PBS (pH = 7.4, 4% DMSO) at 37 °C. (λ_{ex}= 440 nm, slit widths: W_{ex} = W_{em} = 10 nm)

HPLC and LC-MS analysis of the reduction of PNB by CORM-3



Stock solution preparation: PNB was dissolved in DMSO to afford a 10 mM stock solution. **CORM-3** was dissolved in distilled water to afford a 100 mM stock solution.

20 µL of **PNB** stock solution was diluted with 360 µL PBS (pH 7.4), followed by addition of 20 µL of the CORM-3 stock solution. The final concentrations of PNB and CORM-3 were 500 µM and 5 mM respectively. The reaction mixture was incubated at 37°C and monitored by HPLC (gradient: ACN in water (0.1%TFA) 5-95% in 10 min, C18 4.6*150 mm column). For CO gas treatment, pure CO gas was bubbled through the **PNB** solution (500 µM in PBS, pH 7.4 with 5% DMSO) for 2 h at 37 °C. The formation of **PAB** was identified by HPLC (with **PAB** reference compound) and LC/MS.

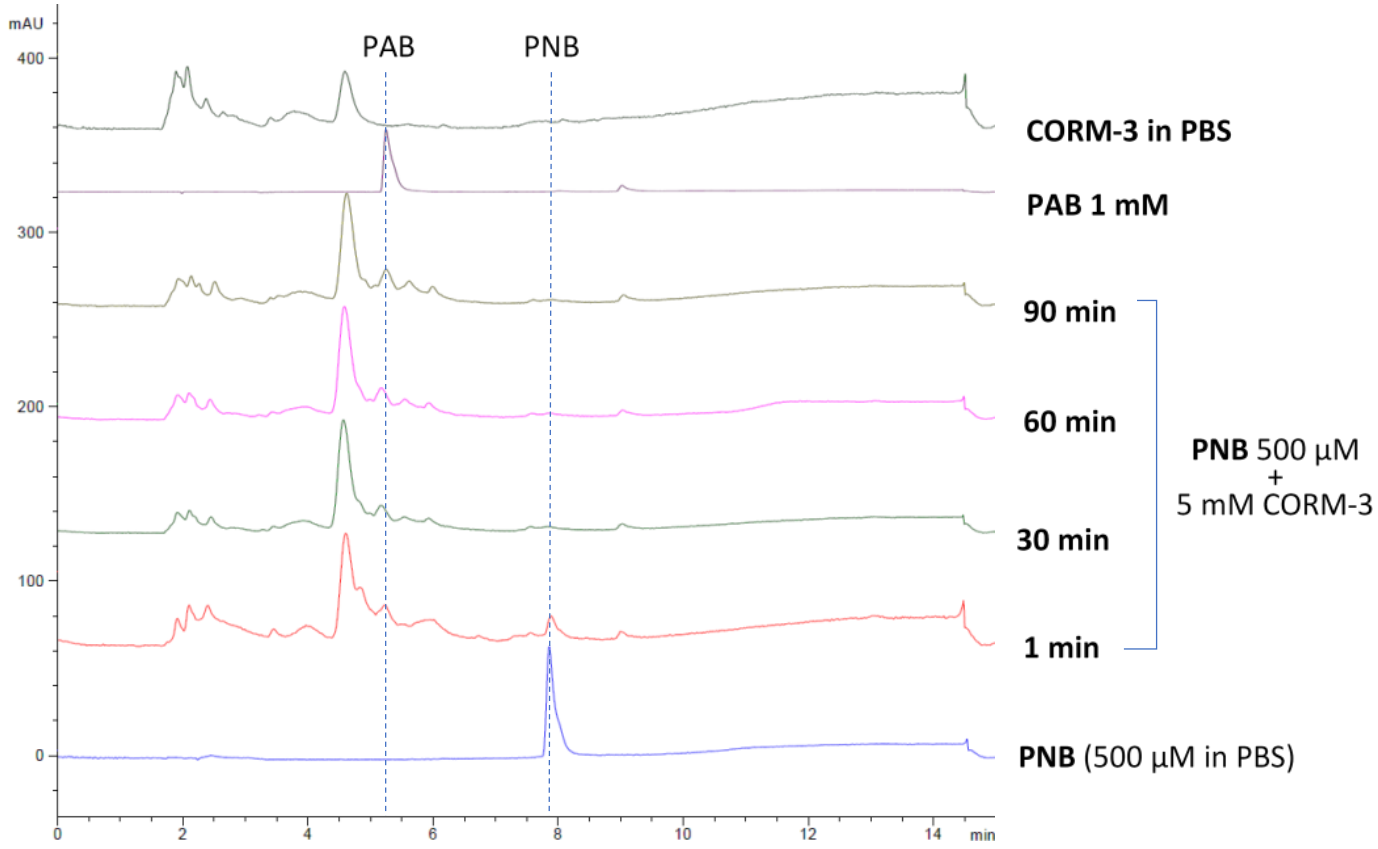
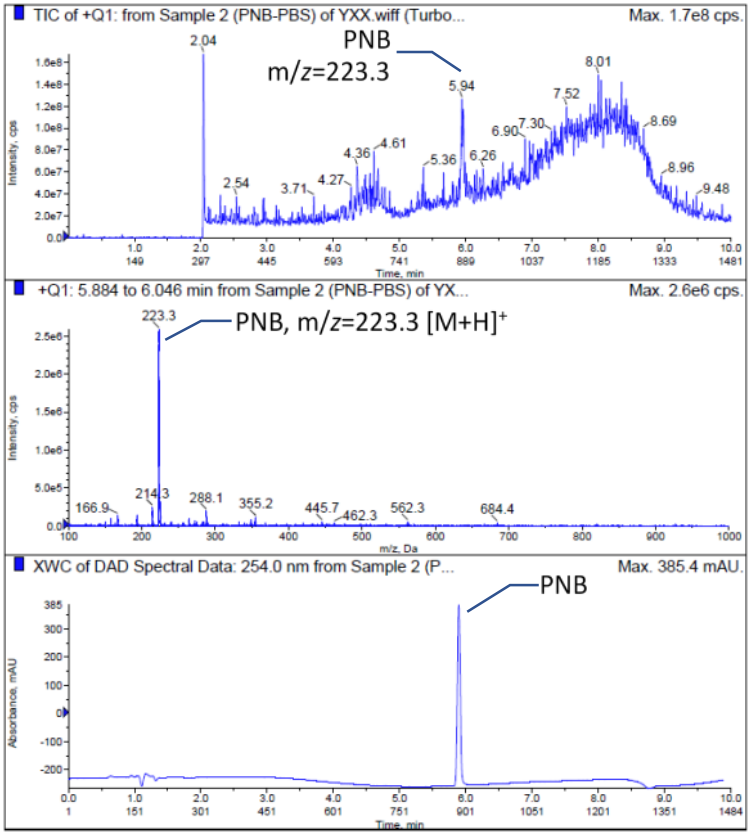
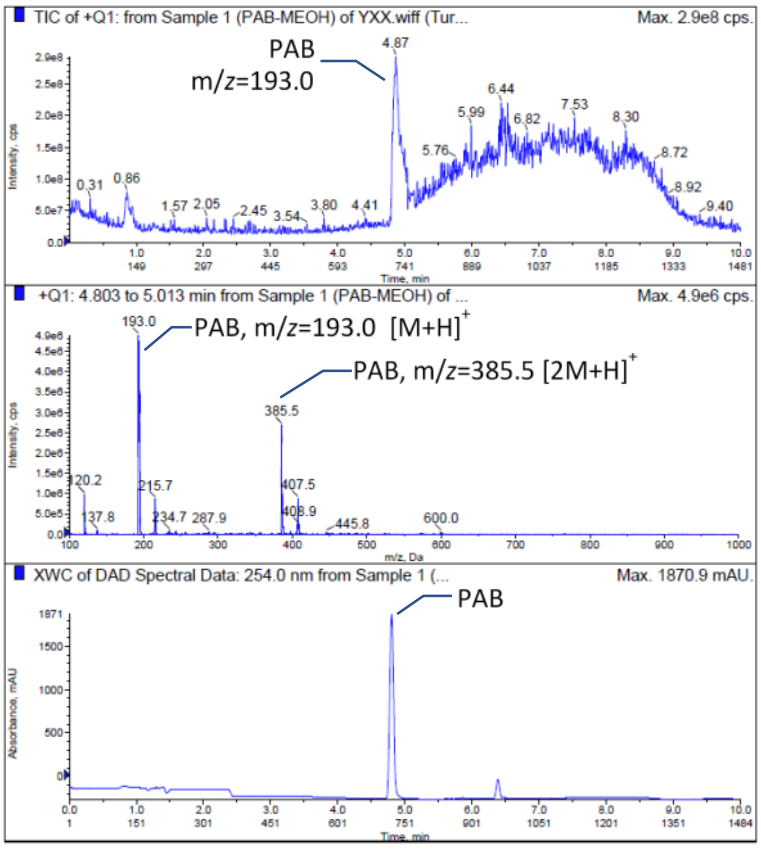


Figure S16. HPLC of the **PNB**/**CORM-3** reaction.

A


TIC of +Q1: from Sample 2 (PNB-PBS) of YXX.wiff (Turbo...
Max. 1.7e8 cps.
PNB
m/z=223.3
+Q1: 5.884 to 6.046 min from Sample 2 (PNB-PBS) of YX...
Max. 2.6e6 cps.
PNB, m/z=223.3 [M+H]+
XWC of DAD Spectral Data: 254.0 nm from Sample 2 (P...
Max. 385.4 mAU.
PNB
Intensity, cps
Absorbance, mAU
Time, min
m/z, Da

B


TIC of +Q1: from Sample 1 (PAB-MEOH) of YXX.wiff (Tur...
Max. 2.9e8 cps.
PAB
m/z=193.0
+Q1: 4.803 to 5.013 min from Sample 1 (PAB-MEOH) of ...
Max. 4.9e6 cps.
PAB, m/z=193.0 [M+H]+
PAB, m/z=385.5 [2M+H]+
XWC of DAD Spectral Data: 254.0 nm from Sample 1 (...
Max. 1870.9 mAU.
PAB
Intensity, cps
Absorbance, mAU
Time, min
m/z, Da

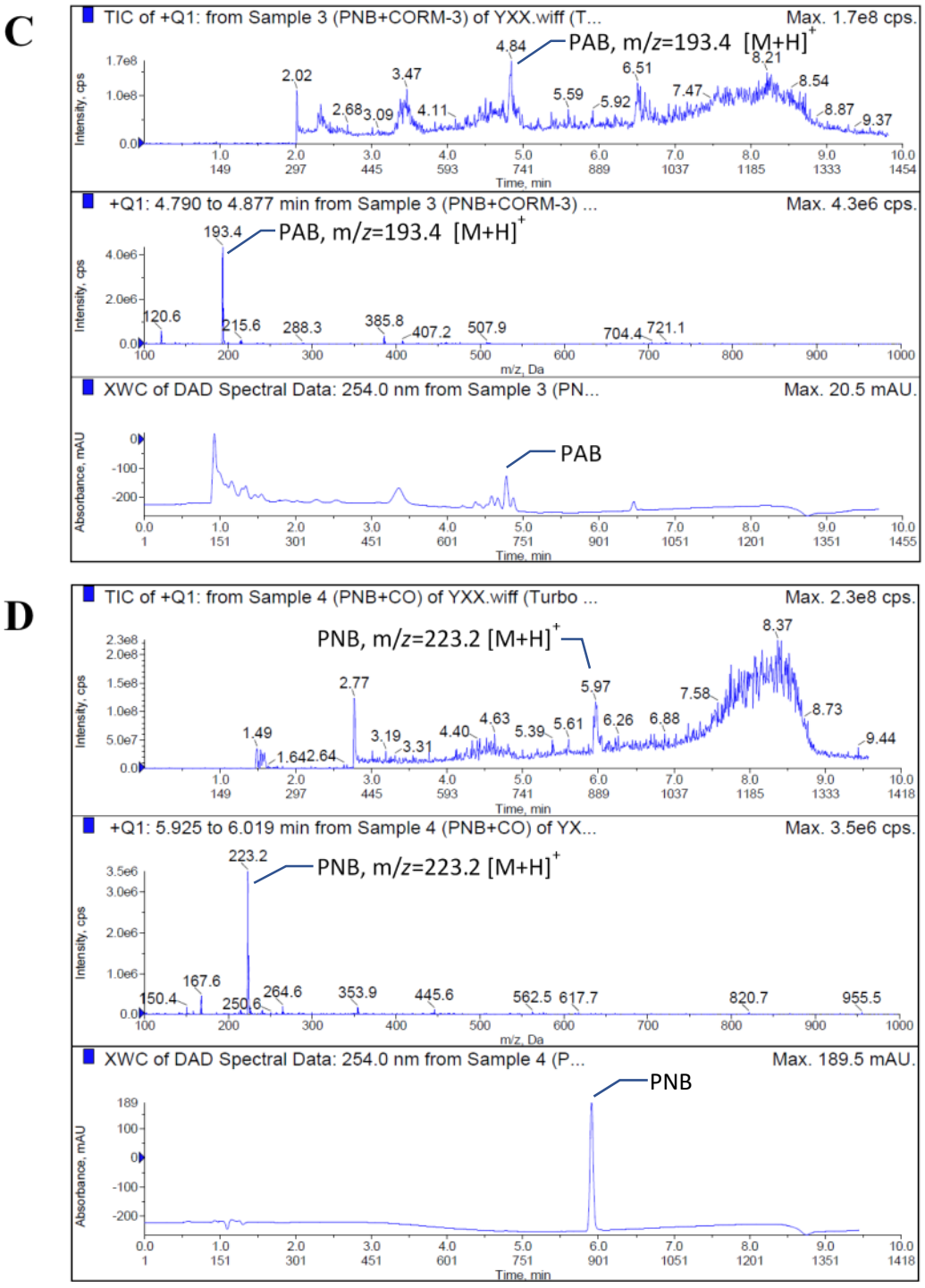


Figure S17. LC/MS analysis of the HPLC sample: (**A)** Before addition of CORM-3, PNB peak at 5.9 min with m/*z*=223.3 $[M+H]^+$; (**B**) **PAB** reference peak at 4.8 min with m/*z*=193.0 $[M+H]^+$; (**C**) Reaction mixture with CORM-3 showed **PAB** peak at 4.8 min with m/*z*=193.4 $[M+H]^+$; (**D**) Bubbling PNB with CO showed only **PNB** peak at 5.9 min with m/*z*=223.2 $[M+H]^+$

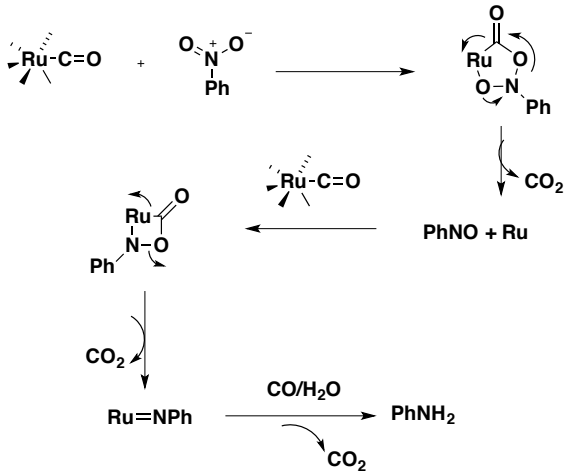


Figure S18. A literature proposed mechanism for the reduction of a nitro group to an amine by ruthenium-CO complexes.[9]

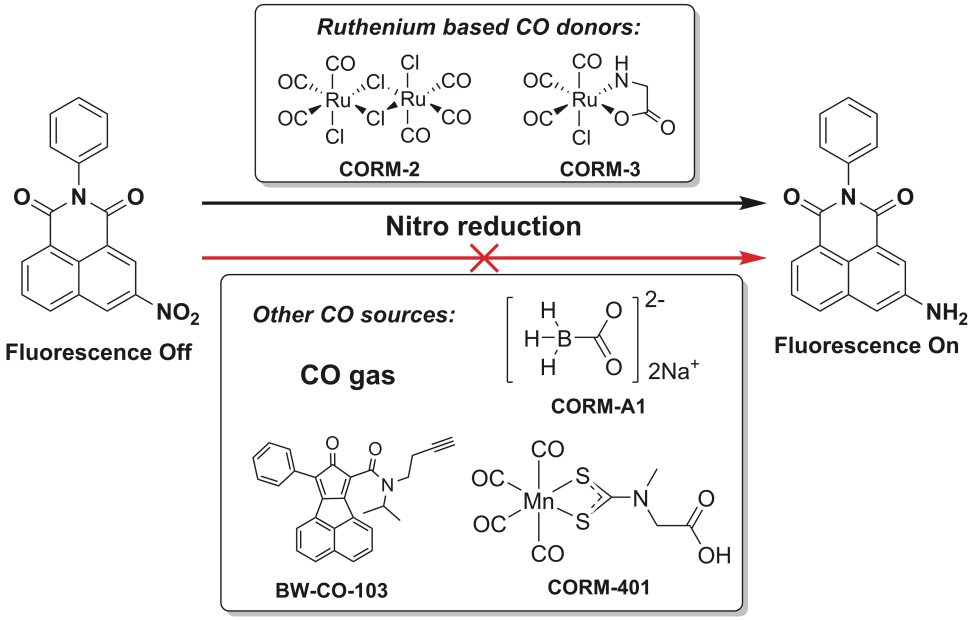
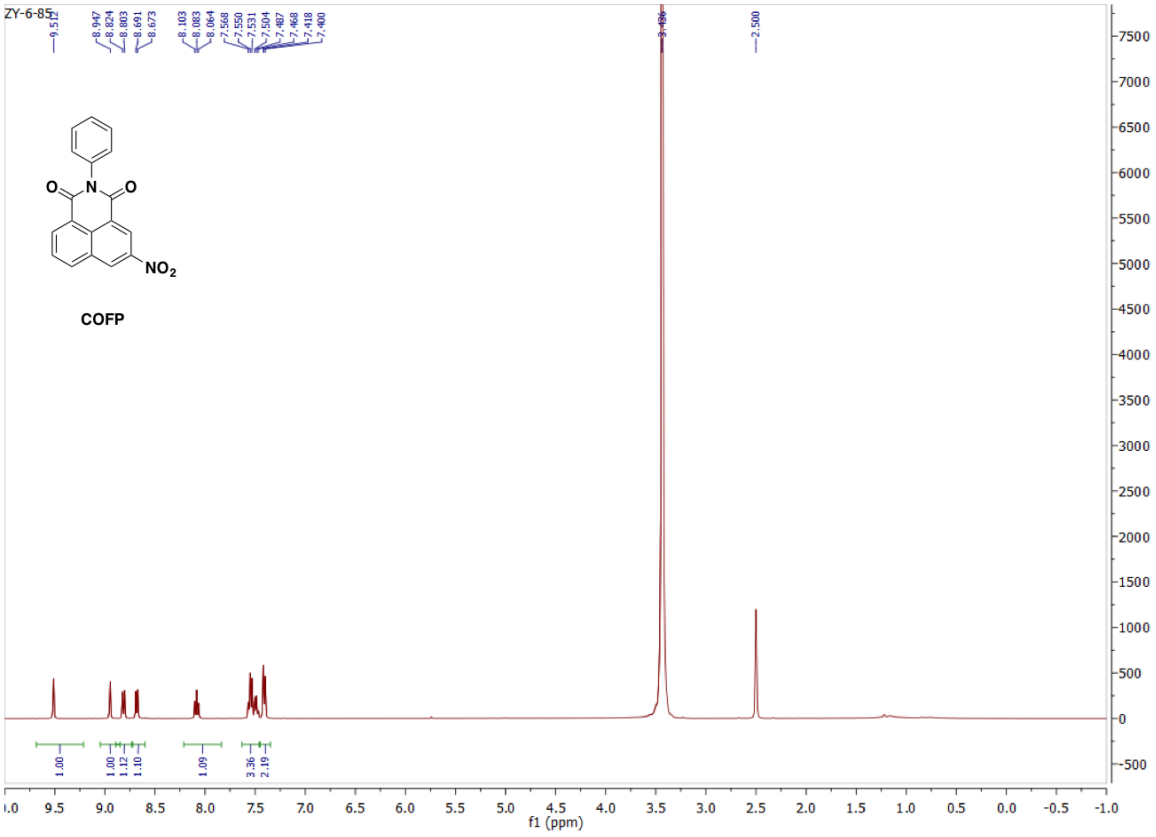


Figure S19. The requirement of the ruthenium carbonyl complex in CO sources for probe reduction.

Spectroscopic data of synthesized compounds


ZY-6-85
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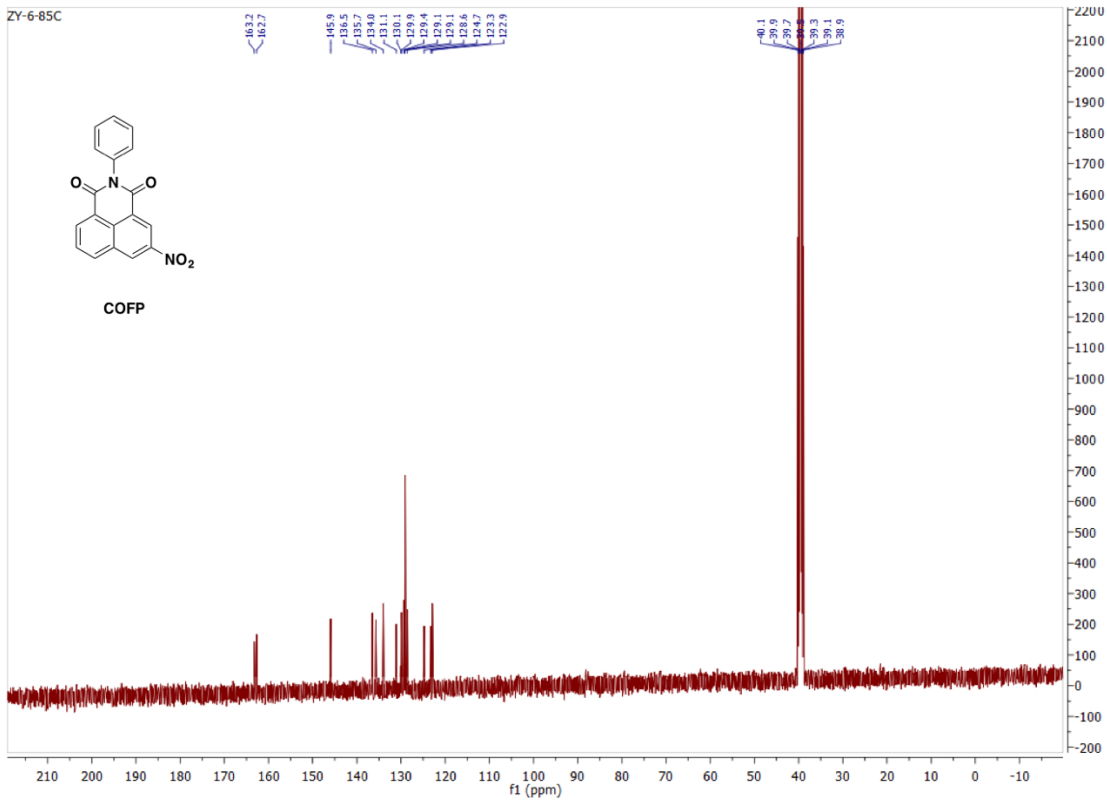
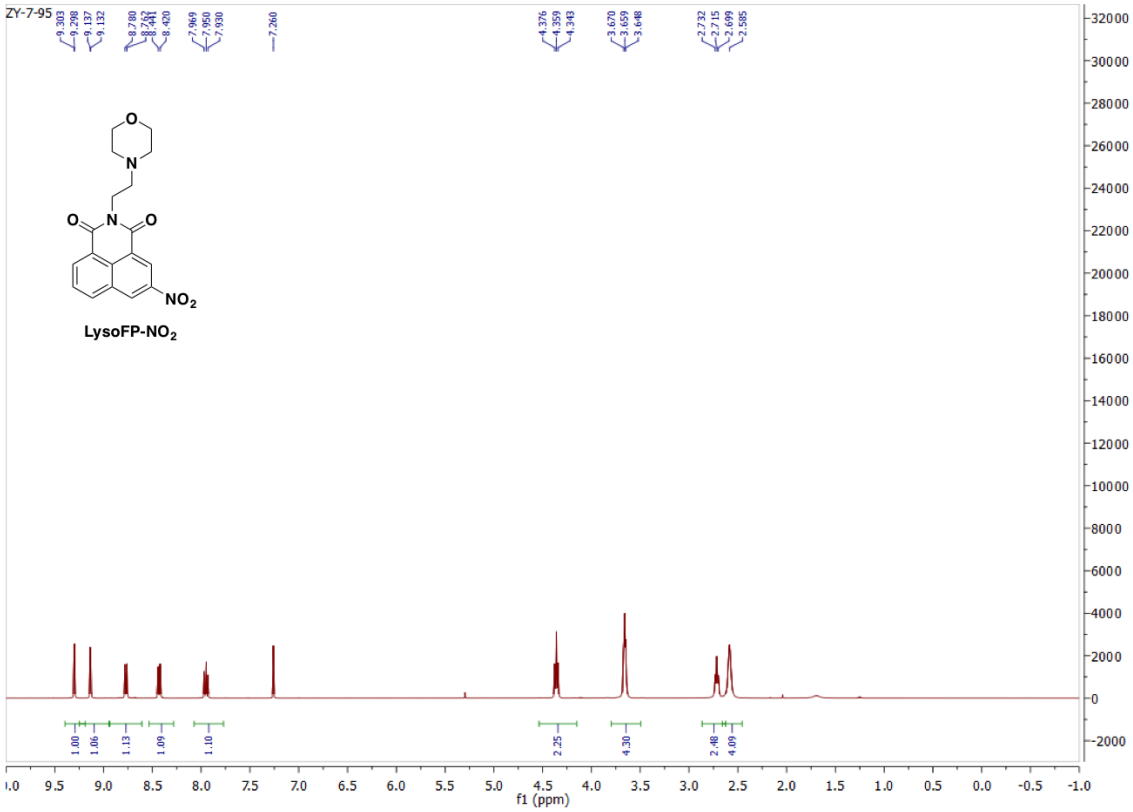
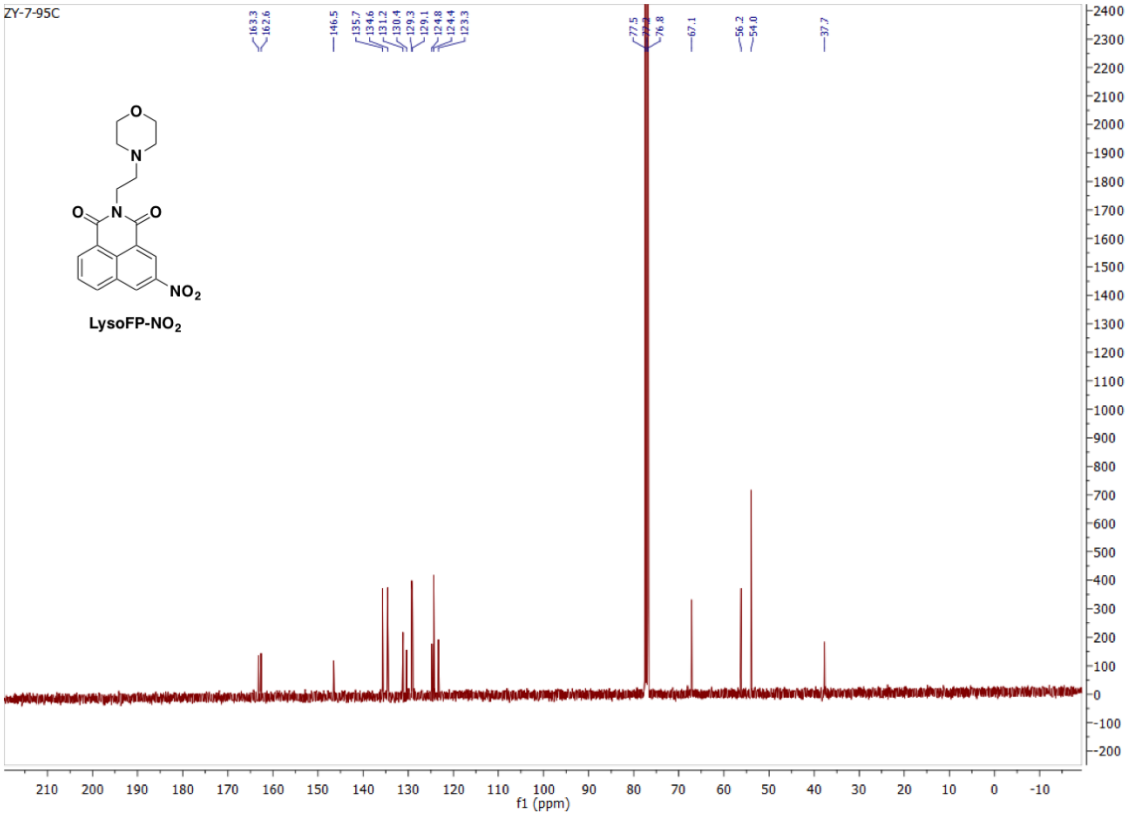
8.947
8.824
8.803
8.691
8.673
8.103
8.083
8.064
7.568
7.550
7.531
7.504
7.487
7.468
7.418
7.400
3.436
2.500
O
N
O
NO2
COFP
1.00
1.00
1.12
1.10
1.09
3.36
2.19
f1 (ppm)

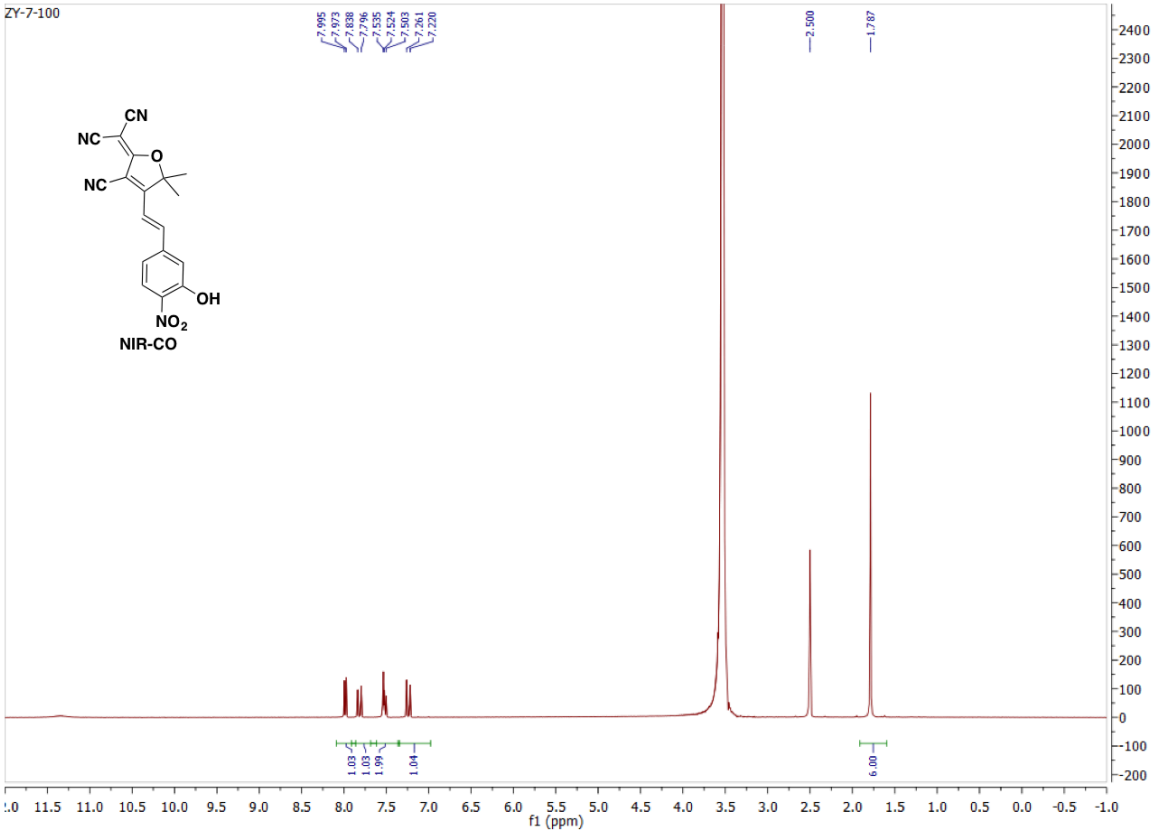

ZY-6-85C
163.2
162.7
145.9
136.5
135.7
134.0
131.1
130.1
129.9
129.4
129.1
128.6
124.7
123.3
122.9
40.1
39.9
39.7
39.5
39.3
39.1
38.9
O
N
O
NO2
COFP
f1 (ppm)

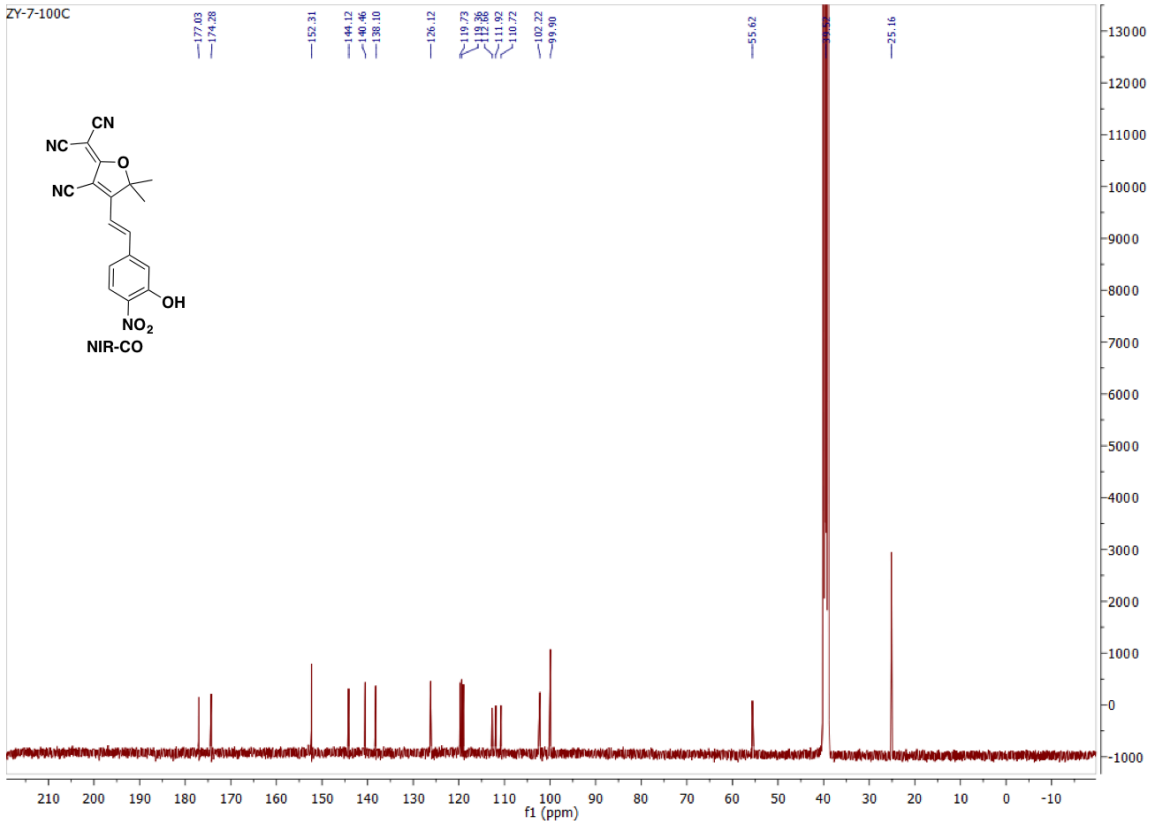


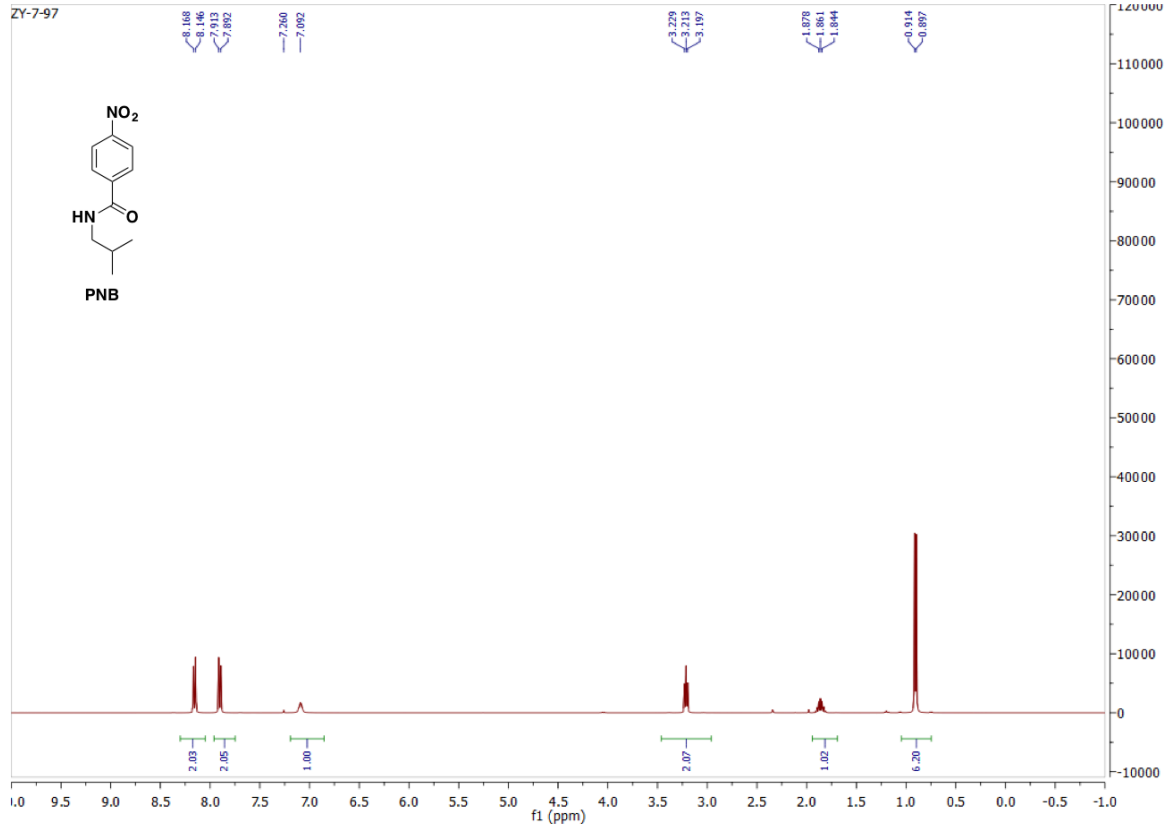
LysoFP-NO_2

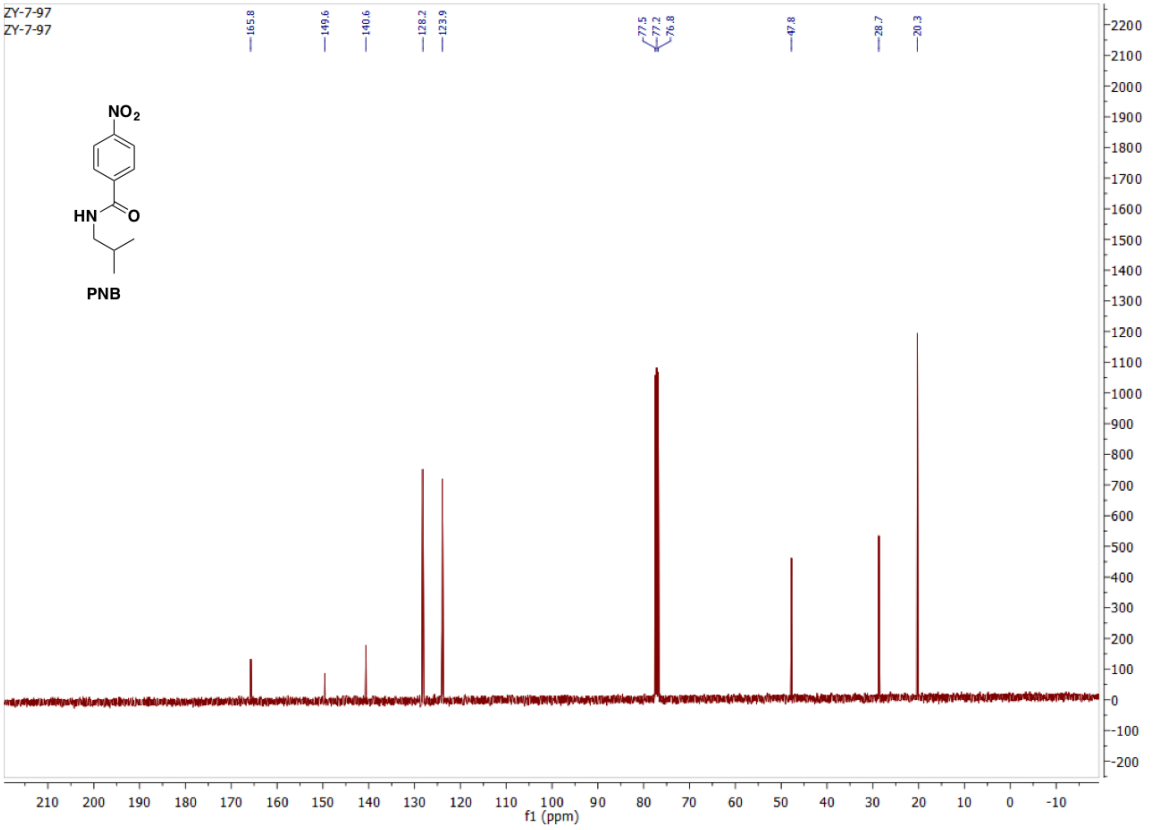


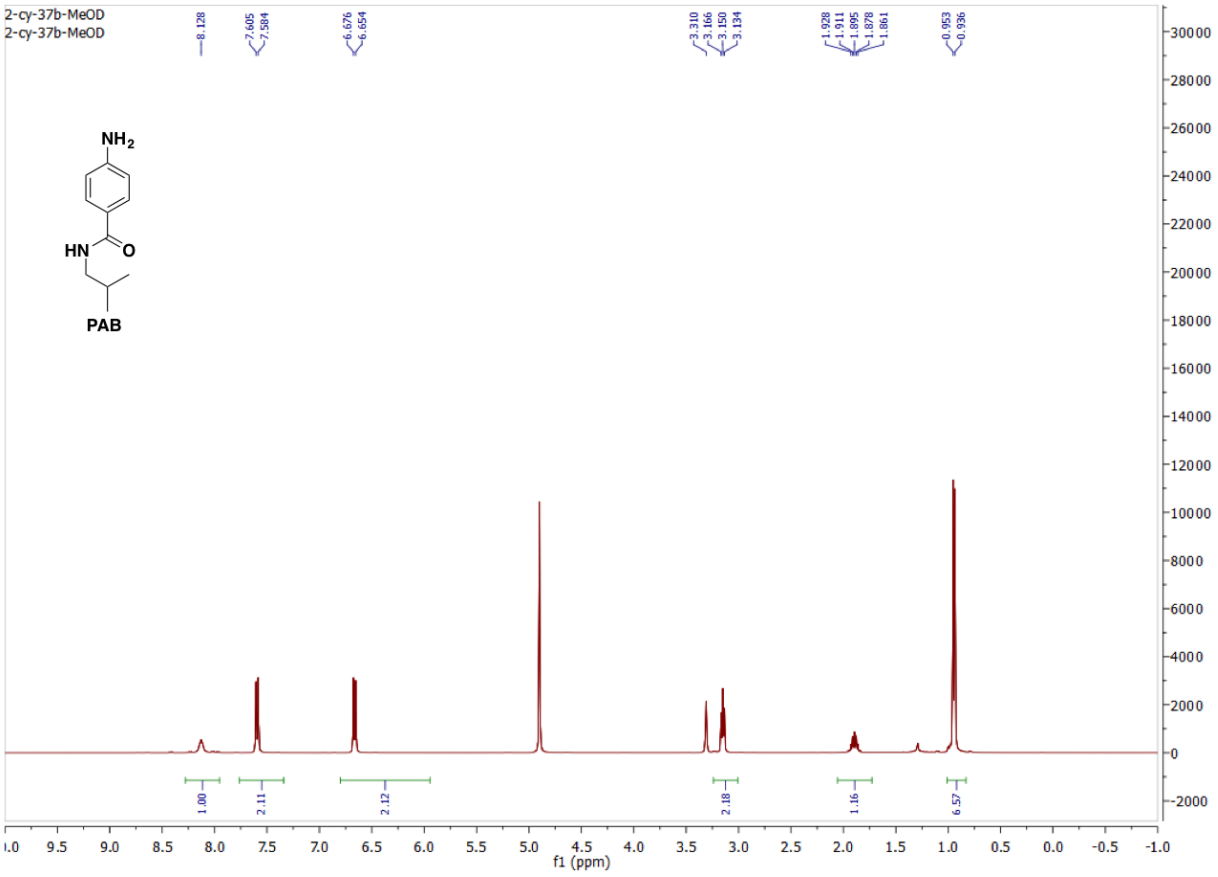
LysoFP-NO_2

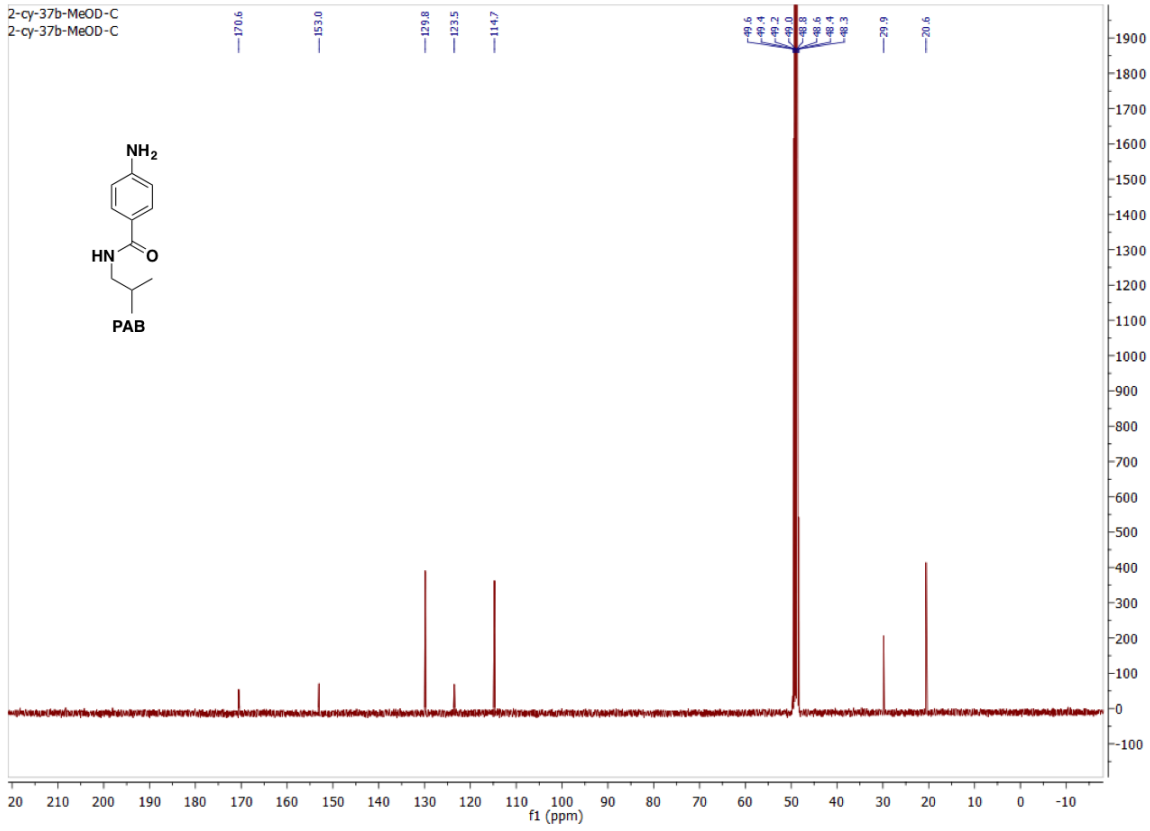

ZY-7-100
7.995
7.973
7.838
7.796
7.535
7.524
7.503
7.261
7.220
2.500
1.787
CN
NC
O
NC
OH
NO2
NIR-CO
1.03
1.03
1.99
1.04
6.00
f1 (ppm)

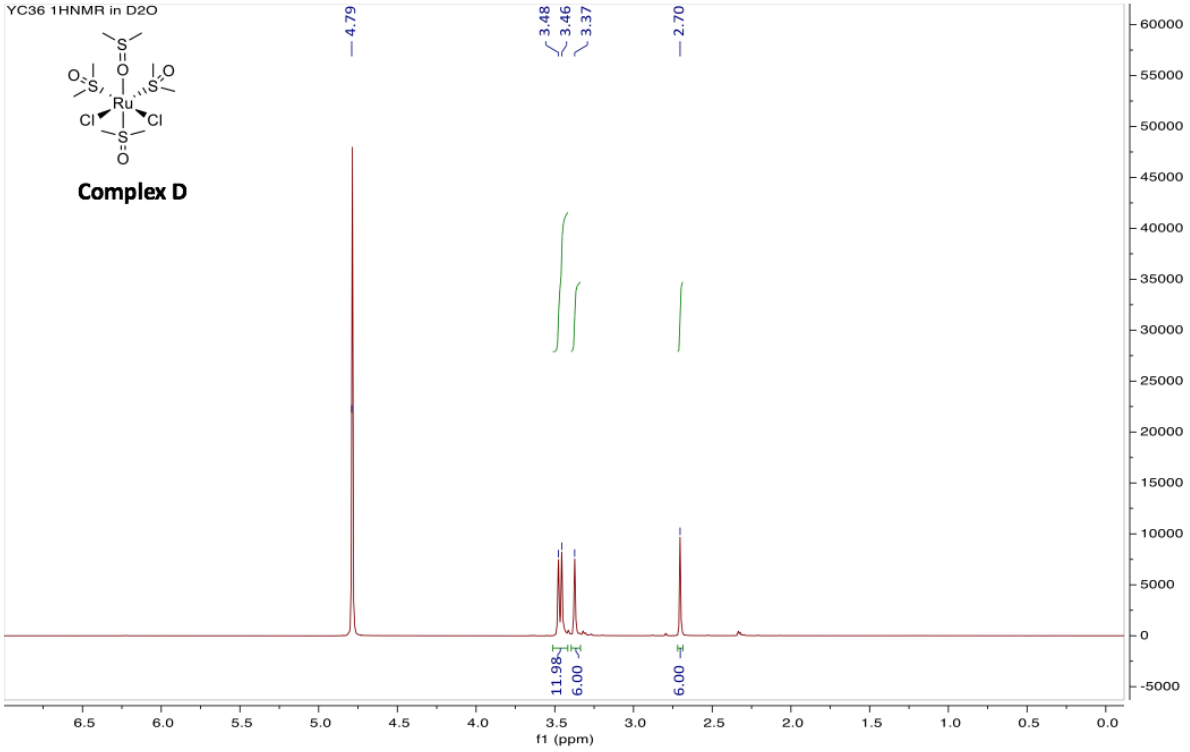

ZY-7-100C
177.03
174.28
152.31
144.12
140.46
138.10
126.12
119.73
111.92
110.72
102.22
99.90
55.62
39.52
25.16
CN
NC
O
NC
OH
NO2
NIR-CO
f1 (ppm)

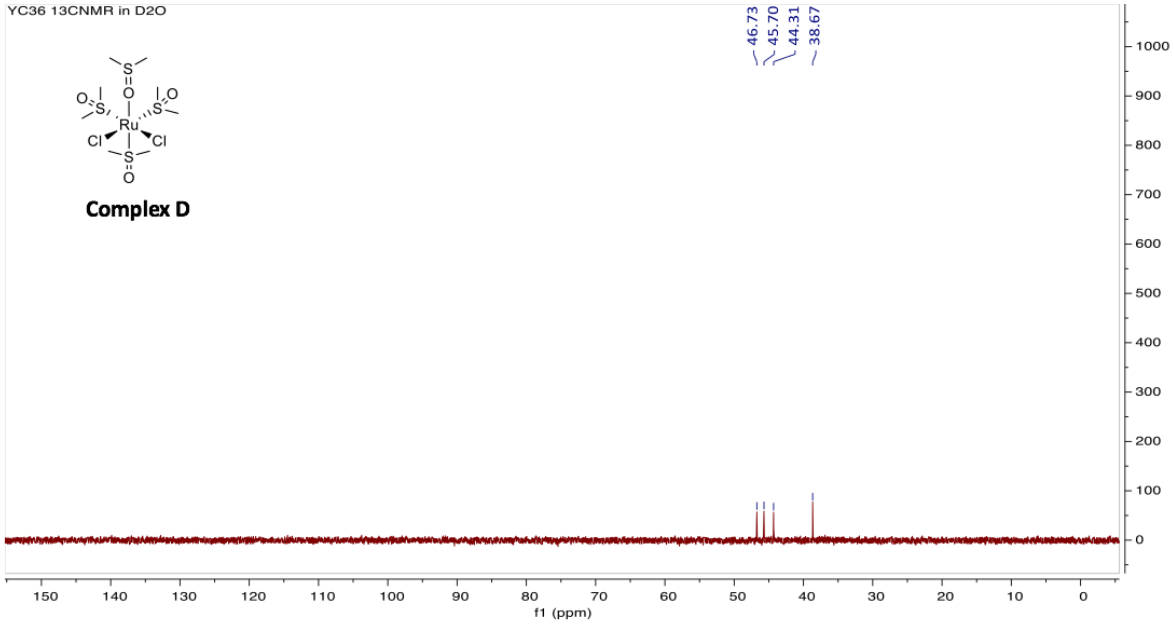

ZY-7-97
8.168
8.146
7.913
7.892
7.260
7.092
3.229
3.213
3.197
1.878
1.861
1.844
0.914
0.897
NO2
HN
O
PNB
2.03
2.05
1.00
2.07
1.02
6.20
f1 (ppm)


ZY-7-97
ZY-7-97
165.8
149.6
140.6
128.2
123.9
77.5
77.2
76.8
47.8
28.7
20.3
NO2
HN
O
PNB
f1 (ppm)


2-cy-37b-MeOD
2-cy-37b-MeOD
8.128
7.605
7.584
6.676
6.654
3.310
3.166
3.150
3.134
1.928
1.911
1.895
1.878
1.861
0.953
0.936
NH2
HN
O
PAB
1.00
2.11
2.12
2.18
1.16
6.57
f1 (ppm)


2-cy-37b-MeOD-C
2-cy-37b-MeOD-C
170.6
153.0
129.8
123.5
114.7
49.6
49.4
49.2
49.0
48.8
48.6
48.4
48.3
29.9
20.6
NH2
HN
O
PAB
f1 (ppm)





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