

Electronic Supplementary Information *for*

A New Mitochondrion Targetable Fluorescence Probe for Carbon Monoxide-Specific Detection and Live Cell Imaging

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1. Experimental Details.

Materials and Reagents. Rhodamine B was purchased from Tokyo Chemical Industry (Tokyo, Japan). Hydrazine hydrate and benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate were bought from Saan Chemical Technology Company (Shanghai, China). 2-Pyridinecarboxaldehyde was purchased from Inoke Technology Company (Beijing, China). Heme was purchased from Haibo Biotechnology Company (Shanghai, China). Tricarbonylchloro(glycinato)ruthenium (CORM-3) as the CO releasing molecule was purchased from Selleck Chemicals (Houston, TX, USA). RPMI-1640 cell culture medium was purchased from Solaibao Technology Company (Beijing, China). A phosphate buffered saline (PBS) solution (10 mM, pH 7.4) was used as the buffer solution. Dimethyl sulfoxide (DMSO) was purchased from Saan Chemical Technology Company (Shanghai, China). Bruker AVANCE NEO 400 MHz spectrometer was used with deuterium reagents for ^1H NMR and ^{13}C NMR spectra. Electrospray ionization mass spectrum (ESI-MS) was tested on LC-MS 6410B instrument (Agilent Technologies, USA). High resolution electrospray ionization mass spectra (HR-ESI-MS) were measured at Thermo Scientific Q Exactive instrument (Bremen, Germany). FE20 pH meter (Mettler Toledo company) was used to pH measurements. Absorption spectra were performed with a T10CS spectrophotometer (Beijing, China). Fluorescence measurements were recorded on a F-7000 spectrofluorimeter. Fluorescence imaging were collected from a Leica TCS SP8 focused laser scanning microscope. The absorbance analysis for MTT were recorded on a Spectra Max M5 multifunctional microplate reader (America). FCM data were collected with BD FACS AriaIII.

General procedure for analysis. Unless otherwise specified, all the fluorescence measurements were operated in 10 mM PBS (pH 7.4) according to the following procedure. For CORM-3 detection, appropriate amount of PBS solution and probe added in to centrifugal tubes first, followed by addition of a proper volume of CORM-3 and PBS solution to obtain different concentrations. Then the mixed solution temperature was set to 37°C in a shaker for 30min through a gas bath thermostat. A portion of the reaction solution of 1 mL was transferred to a quartz cell measure UV-Vis absorbance and fluorescence spectra with $\lambda_{\text{ex/em}}=530/578$ nm with both excitation and emission slit widths of 5 nm. same conditions for comparison.

Synthesis of Compound 1. The raw material rhodamine B (91 mg, 0.38 mmol), hydrazine hydrate (0.25 mL, 3.8 mmol) and BOP [benzotriazol-1-yloxytris(dimethylamino) phosphonium hexafluorophosphate] reagent (180 mg, 0.4 mmol) were added to a 50 mL round bottom flask containing 10 mL of dichloromethane. The solution was stirred at room temperature for 6 hours. After initial purification via chromatography (silica gel, CH₂Cl₂: CH₃OH = 20: 1), the obtained yellow solid product was used directly for next reaction.

Synthesis of Compound RCO. Compound 1 (45.6 mg, 0.1 mmol) and 50 μ L 2-pyridinecarboxaldehyde were dissolved by 10 mL anhydrous methanol. The mixed solution was stirred for 10 h at room temperature. The solvent was removed by rotating evaporation. Then the yellow solid product was obtained after chromatography (silica gel, CH₂Cl₂:CH₃OH = 25:1). Yield: 39.2 mg (72 %). Molecular formula: C₃₄H₃₅ N₅O₂. ¹H NMR (400 MHz, CDCl₃): δ (ppm) 8.46 (d, J = 4.8 Hz, 1H), 8.36 (s, 1H), 8.01 (d, J = 7.6 Hz, 2H), 7.60 (t, J = 7.8 Hz, 1H), 7.51-7.41 (m, 2H), 7.14-7.10 (m, 2H), 6.54 (d, 8.8 Hz, 2H), 6.45 (d, J = 2.4 Hz, 2H), 6.24 (d, J = 2.4 Hz, 1H), 6.22 (d, J = 2.4 Hz, 1H), 3.31 (q, J = 7.1 Hz, 8H), 1.14 (t, J = 7.0 Hz, 12H); ¹³C NMR (100 MHz, CDCl₃): δ 165.5, 154.6, 152.9, 152.6, 149.0, 148.9, 145.8, 136.1, 133.8, 128.2, 128.0, 127.6, 123.7, 123.6, 123.5, 120.7, 106.0, 105.5, 96.3, 65.8, 44.3, 12.6; HRMS (ESI) m/z: [M + H]⁺, 546.2824 (calcd. for C₃₄H₃₅ N₅O₂, 546.2864).

Detecting CO in human serum samples. The human serum samples are provided by the Affiliated Hospital of Weifang Medical University. For the pre-treatment of serum, the human serum was centrifuged and filtered with 0.45 μ m Millipore filter, and known amounts of CO were added. 1.0 mL of the treated serum was diluted with PBS to afford the test sample. Then the CO concentrations are determined by standard addition method.

Cell Culture. The HepG2 cells were cultured in RPMI-1640 liquid medium containing 10% fetal bovine serum at 37 °C with 5% CO₂ incubator. The cells were passaged when the density reached 80% to 90%.

Cytotoxicity Test. In a 96-well plate, 7 $\times$ 10³ HepG2 cells were seeded into each well, and cultured for one day to completely adhere onto the plate surface. Different concentrations (0 to 50 μ M) of probes were configured in the gradient medium, and 100 μ L medium was draw separately to replace the original

medium and incubated at 37°C for 24 h. Then the medium was discarded and 100 μ L medium containing 0.5 mg/mL MTT was added to each well, and incubated at 37°C for 4 h. The medium was then replaced with 150 μ L DMSO to dissolve the blue-purple formamidinium crystals. After 5 minutes, the absorbance values of the wells were measured at 490 nm using a microplate reader. The cell viability (VR) of HepG2 cells was calculated by the following equation: $VR = A/A_0 \times 100\%$, where A is the absorbance of the experimental group, and A_0 is the control group. Cell viability from the control group was considered to be 100%.

Imaging Exogenous CO in Cells. The cells were divided into three groups equally. The first was treated with 10 μ M probe directly for imaging. The second and third groups were pretreated with different concentrations of (5 and 10 μ M) and then 10 μ M probe for 35 minutes. The stained cells were washed 3 times with PBS buffer solution prior to confocal imaging (λ_{ex} 515 nm; λ_{em} 570-650 nm).

Imaging Endogenous CO in Cells. The cells were equally divided into three groups. the control group was given no treatment, the other two groups were stimulated by respective 1 and 3 h heme (50 μ M) treatment and then treated with 10 μ M probe for 35 min. The stained cells were washed 3 times with PBS buffer solution. Add 10 μ M probe and incubate for 30minutes, the stained cells were washed 3 times with PBS buffer solution. Confocal imaging was performed on a laser scanning confocal microscope.

Flow cytometry. After treatment with exogenous or endogenous CO and probe, the stained cells were washed with PBS solution for 3 times. Then 300 μ L trypsin solution was added into each well to digest the cells for 2 min. Then the cells were washed 3 times again with 1 mL PBS every time via centrifugation in a speed of 3000 r/min every 5 min. Finally, 200 μ L PBS containing more 10000 cells was drawn for flow cytometry analysis. The cells were analyzed using a BD FACS AriaIII at exciting wavelength of 488 nm and filter of 575/26.

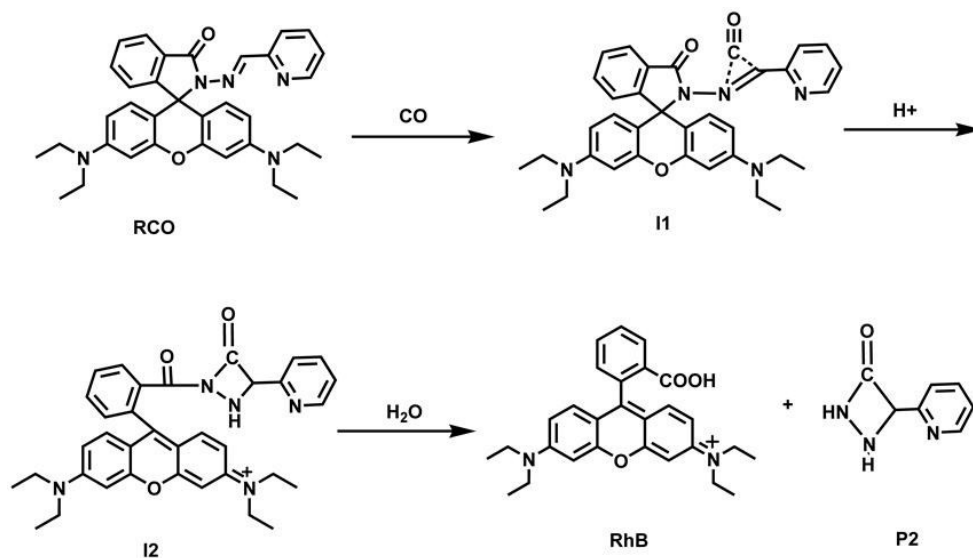
2. Computational Methods.

All the calculations were performed with density functional theory (DFT) implemented in Gaussian 09 program package.¹ The ground state equilibrium geometries and the normal modes of vibration of all compounds were computed using density functional theory (DFT)

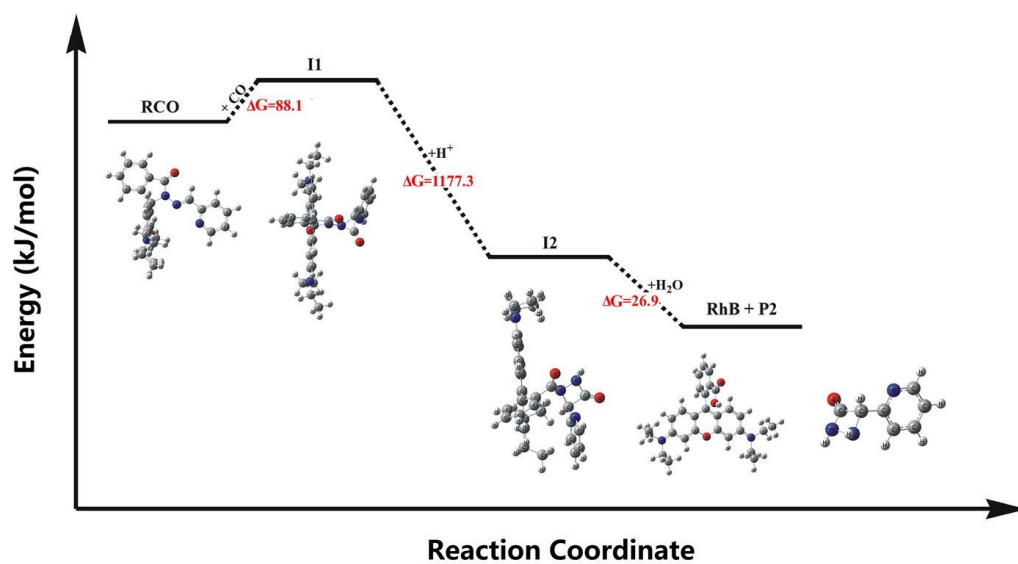
with the hybrid B3LYP functional² at 6-31+G(d,p) level. Bulk solvent effects have been included using polarizable continuum model (PCM).³

References

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- 2) A. D. Becke, *J. Chem. Phys.*, **1993**, *98*, 5648–5652.
- 3) J. Tomasi, B. Mennucci and R. Cammi, *Chem. Rev.*, **2005**, *105*, 2999–3094.



Scheme S1. Proposed Reaction Mechanism of RCO reacting with CO.



Scheme S2. Energy Profiles of the Proposed Reaction Route and Optimized Structures.

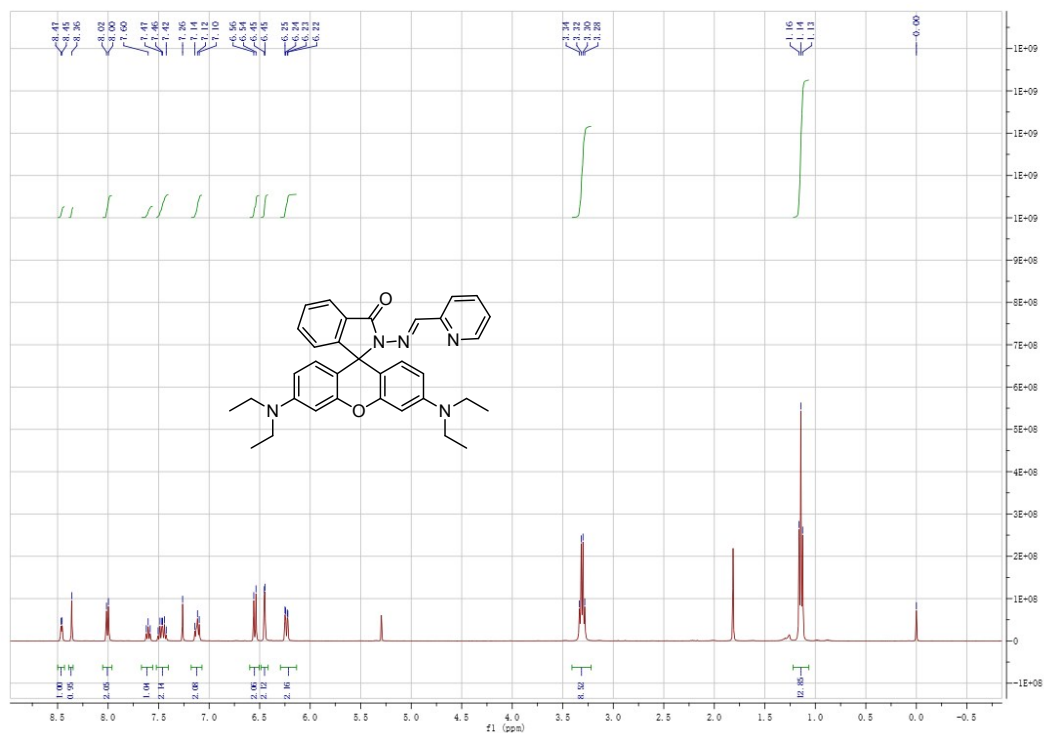


Figure S1. ¹H NMR spectrum of RCO in CDCl₃.

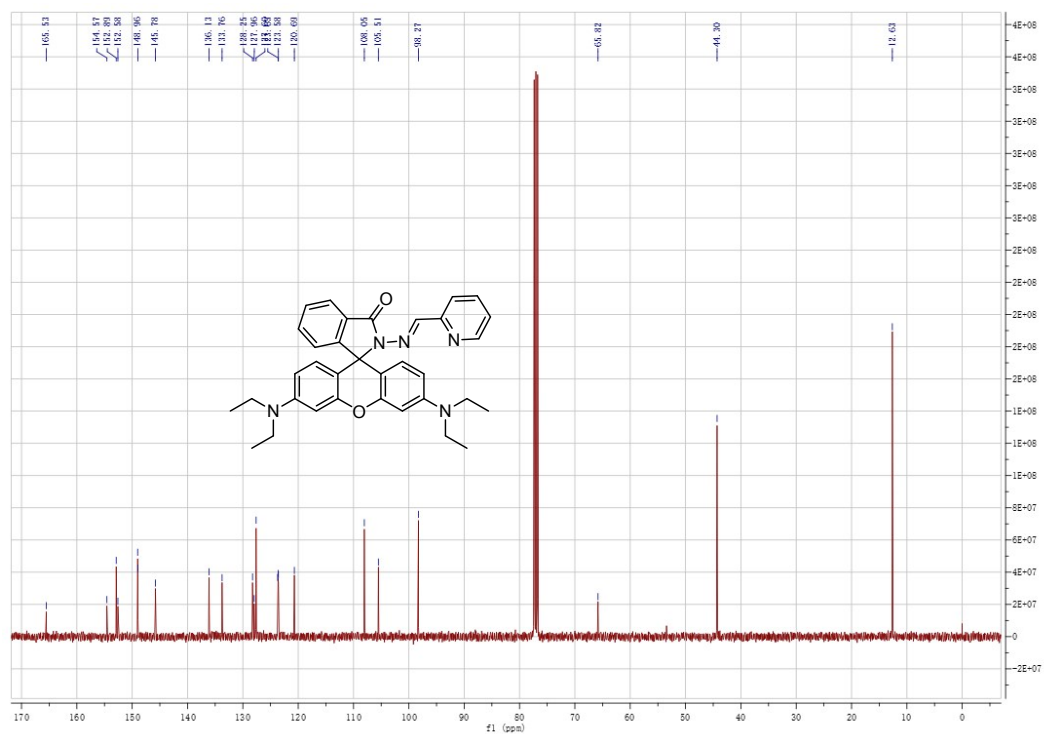


Figure S2. ¹³C NMR spectrum of RCO in CDCl₃.

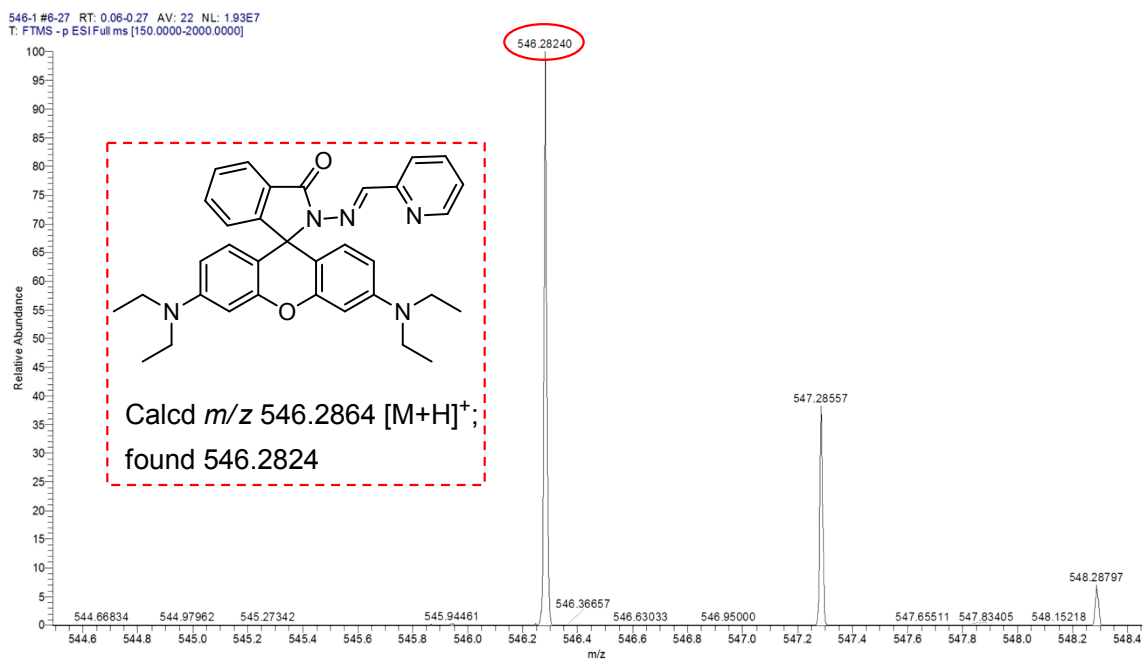


Figure S3. High-resolution mass spectrum of **RCO**.

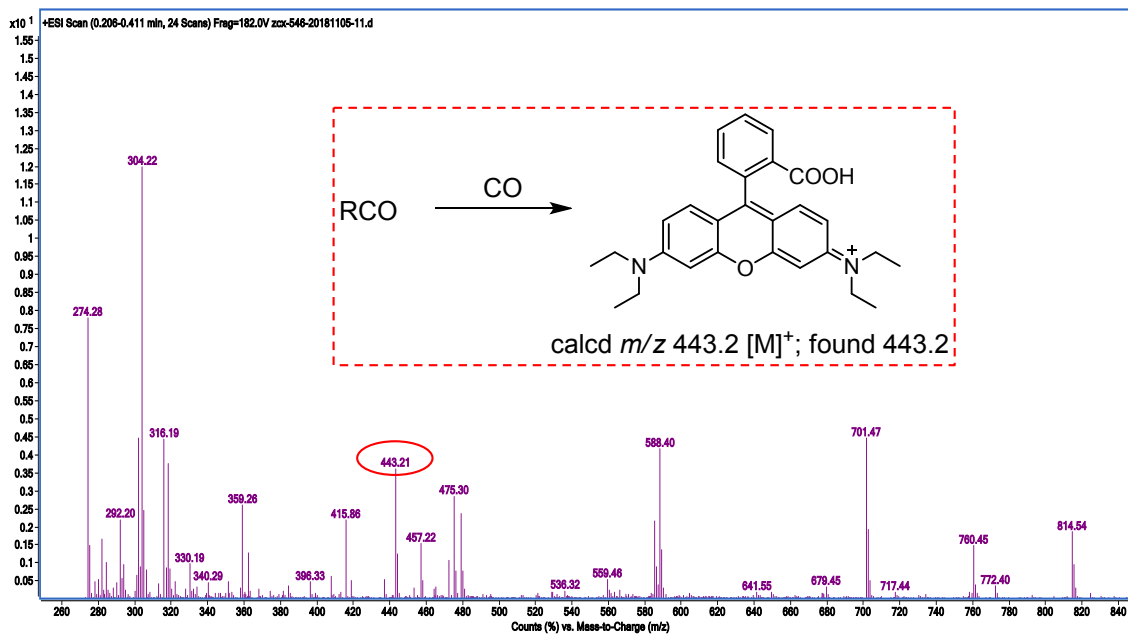


Figure S4. ESI mass spectrum of **RCO** (10 μ M) reacting with CO (CORM-3 as donor) (10 μ M) for 20 min.

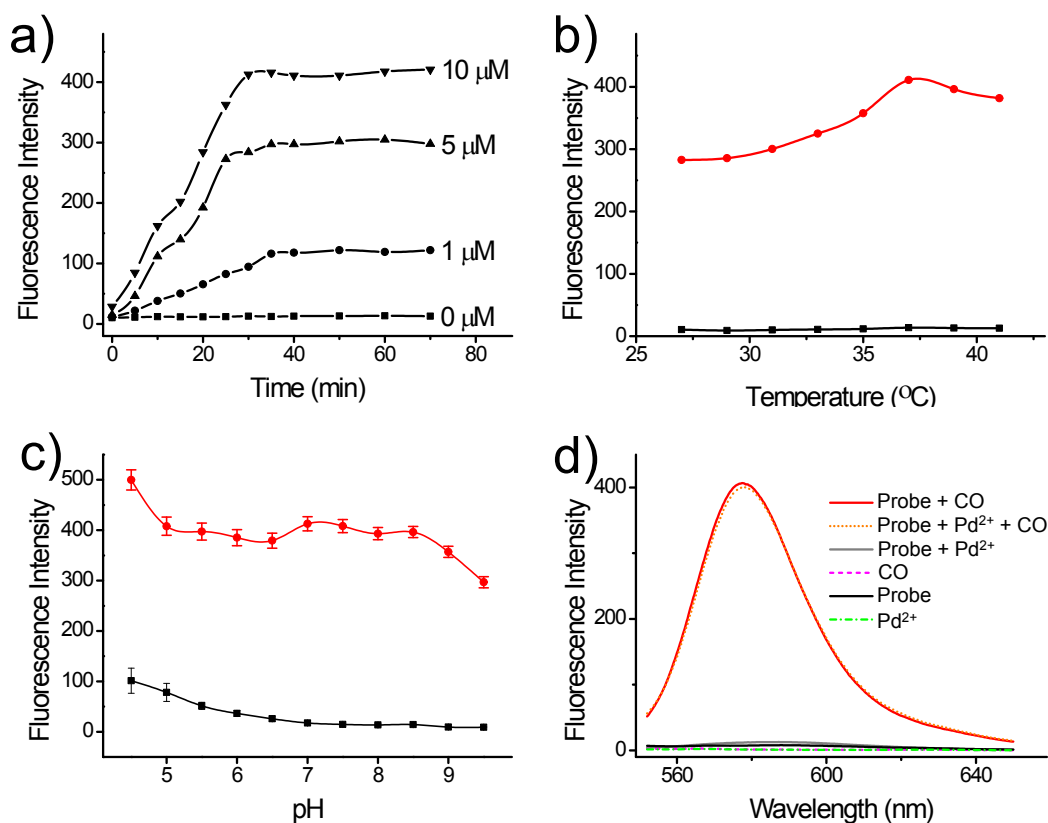


Figure S5. (a) Plots of fluorescence intensity of **RCO** (10 μM) vs. the reaction time in the presence of varied concentrations of CO (CORM-3 as donor) (0-10 μM). The measurements were performed at 37 $^{\circ}\text{C}$ in PBS (pH 7.4). (b) Effects of temperature on the fluorescence of **RCO** (10 μM) with (red) and without (black) 10 μM CO. (c) Effects of pH on the fluorescence of **RCO** (10 μM) with (red) and without (black) 10 μM CO. (d) Fluorescence emission spectra of different reaction systems (10 μM probe, 10 μM Pd^{2+} , 10 μM CO). $\lambda_{\text{ex/em}} = 525/578$ nm.

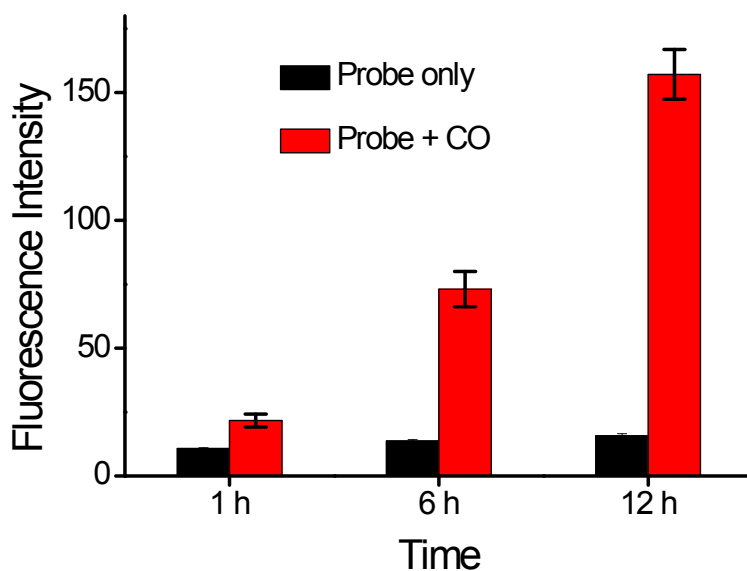


Figure S6. Fluorescence intensity changes of **RCO** (10 μM) solution after continuous CO gas ventilation. $\lambda_{\text{ex/em}} = 525/578$ nm.

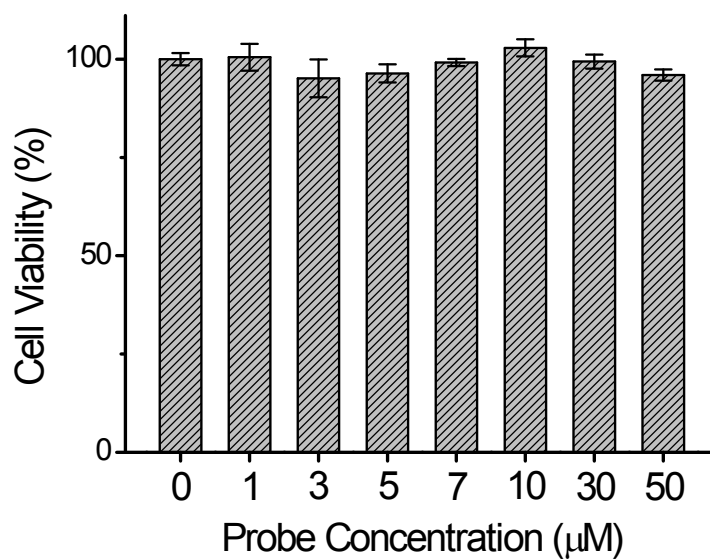
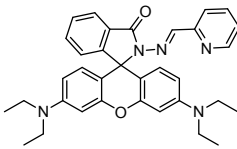
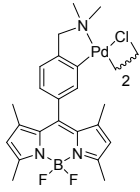
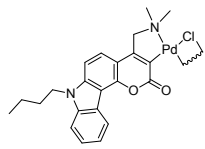
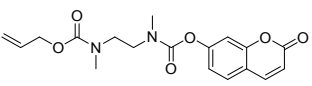
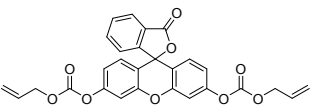
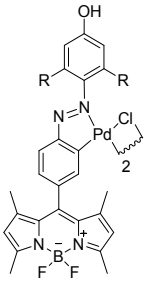
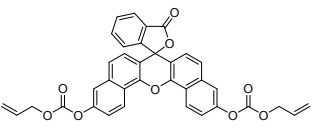
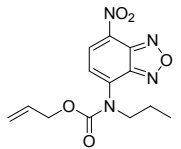
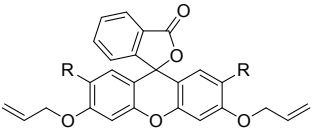
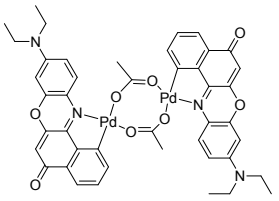
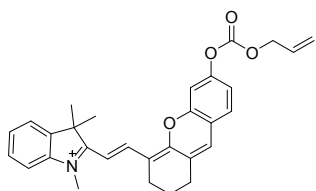


Figure S7. Effects of **RCO** at varied concentrations (0–50 μM) on the viability of HepG2 cells. The viability of cells without **RCO** is defined as 100%. The results are the mean $\pm$ standard deviation of five separate measurements. As is seen, less than 50 μM of **RCO** hardly affects the cell viability, showing low cytotoxicity and good biocompatibility.

Table S1. Comparison of **RCO** with other CO probes

probe	Detection limit (nM)	$\lambda_{\text{ex/em}}$ (nm)	Reaction equilibrium time (min)	Palladium -free	Subcellular localization
 this work	10	530/578	30	yes	mitochondria
 Chang et al, <i>J. Am. Chem. Soc.</i> 2012, 134, 15668	unknown	475/503	>60	no	no
 Lin et al, <i>Chem. Sci.</i> 2014, 5, 3439	653	370/477	40	no	no
 Pal et al, <i>Chem. Commun.</i> 2015, 51, 4410	7.77	340/460	>30	no	no
 Feng et al, <i>Anal. Chem.</i> 2016, 88, 10648	37	490/520	>15	no	no

	720 nM	498/512	20	no	no
Tang et al, <i>Anal. Chem.</i> 2016, 88, 11154					
	127	620/670	45	no	no
Zhang et al., <i>RSC Adv.</i> 2016, 6, 65373					
	26.3	480/549	30	no	no
Zhang et al., <i>Tetrahedron Lett.</i> 2016, 57, 2927					
	46 (R=H) 29 (R=Cl)	490/516 493/527	>20	no	no
	50	580/660	200	no	no
Lin et al., <i>Angew. Chem. Int. Ed.</i> 2017, 56, 13489					



3.2

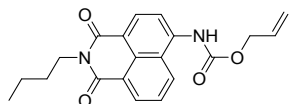
670/714

3

no

no

Feng et al., *Sens. Actuators B* **2018**, 255, 2314



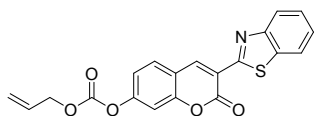
58

420/(545 vs 472)

no

no

Feng et al., *Sens. Actuators B* **2017**, 251, 389



25

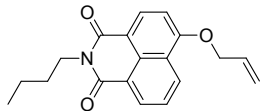
462/490

15

no

no

Feng et al., *Sens. Actuators B* **2017**, 240, 625



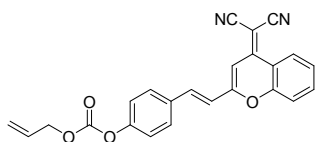
17.9

430/(545 vs 455)

no

no

Zhu et al., *New J. Chem.* **2018**, 42, 14417



57

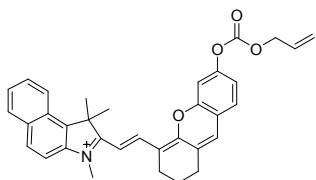
565/685

40

no

no

Qi et al., *Spectrochim. Acta A* **2018**, 202, 284



170

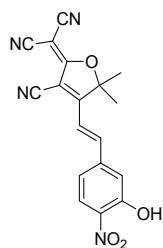
690/736

18

no

mitochondria

Li et al., *Talanta* **2018**, 188, 691



6.1

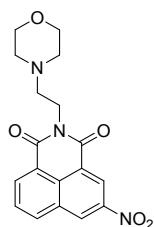
580/665

>40

yes

no

Wang et al., *Sens. Actuators B* **2019**, 291, 329



600

440/525

>45

yes

lysosomes

Dhara et al., *Anal. Chem.* **2018**, 90, 2933

Table S2. Detection of CO in serum samples (100-fold diluted).

Sample	CORM-3 Added (μM)	CO Found (μM)	Recovery (%)
1	0	0.50	-
	0.2	0.69 ± 0.0088	95.0 ± 4.4
	0.4	0.89 ± 0.0216	99.2 ± 5.4
2	0	0.55	-
	0.2	0.75 ± 0.0096	101.7 ± 4.8
	0.4	0.88 ± 0.0104	94.2 ± 2.6

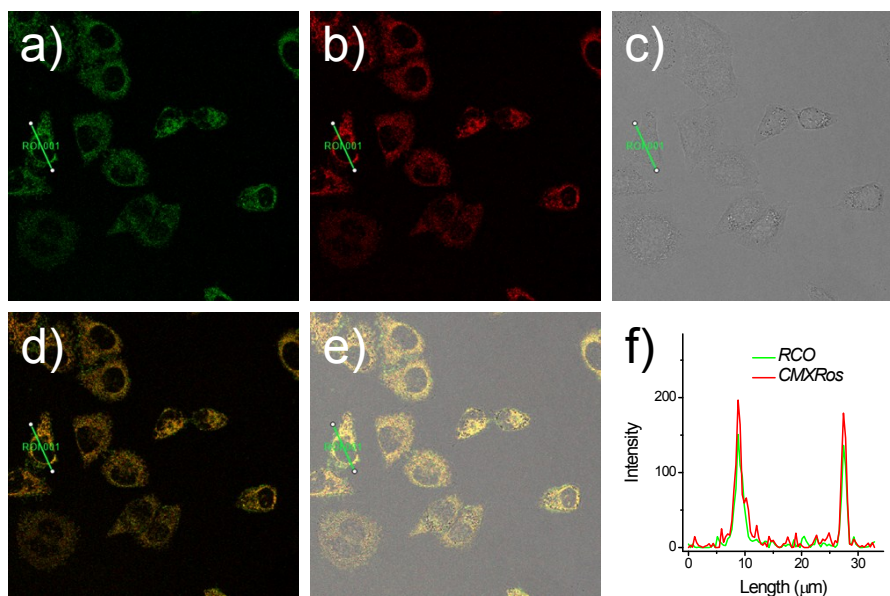


Figure S8. Colocalization of **RCO** (pseudocolor green) and Mito-tracker Red CMXRos (pseudocolor red) in HepG2 cells. Cells were co-stained with **RCO** (10 μM) and CMXRos (50 nM), and then treated with CORM-3 (10 μM) at 37 °C for 25 min. a) Fluorescence image from **RCO** channel ($\lambda_{\text{ex}} = 515$ nm, $\lambda_{\text{em}} = 520\text{--}555$ nm). b) Fluorescence image from CMXRos channel ($\lambda_{\text{ex}} = 561$ nm, $\lambda_{\text{em}} = 590\text{--}700$ nm). c) Corresponding differential interference contrast (DIC) image. d) Merged image of images (a) and (b). e) Merged image of images (a), (b) and (c). f) Intensity profiles of **RCO** and CMXRos within the linear ROI 1 (green lines in Fig. S7a and 7b) across the HepG2 cell.

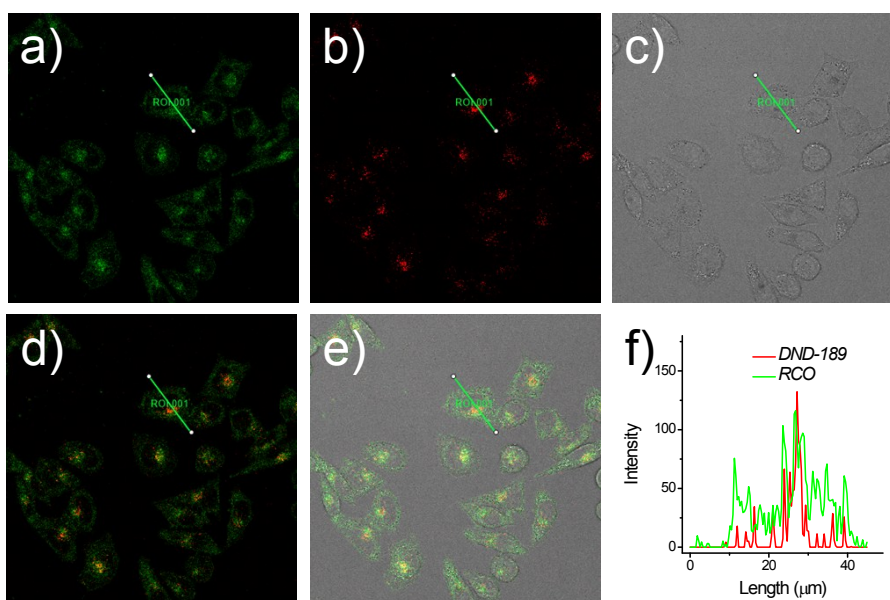


Figure S9. Colocalization of **RCO** (pseudocolor green) and Lyso Sensor DND-189 (pseudocolor red) in HepG2 cells. Cells were co-stained with **RCO** (10 μM) and DND-189

(30 nM), and then treated with CORM-3 (10 μ M) at 37 °C for 25 min. a) Fluorescence image from **RCO** channel ($\lambda_{\text{ex}} = 515$ nm, $\lambda_{\text{em}} = 520\text{--}555$ nm). b) Fluorescence image from DND-189 channel ($\lambda_{\text{ex}} = 442$ nm, $\lambda_{\text{em}} = 460\text{--}510$ nm). c) Corresponding differential interference contrast (DIC) image. d) Merged image of images (a) and (b). e) Merged image of images (a), (b) and (c). f) Intensity profiles of **RCO** and DND-189 within the linear ROI 1 (green lines in Fig. S8a and 8b) across the HepG2 cell.

Cartesian Coordinates

RCO

C	-6.43803900	-0.99007300	-0.06354700
C	-5.14981200	-1.09844500	-0.56838000
C	-4.22278800	-0.04883900	-0.51056500
C	-4.66555100	1.13487700	0.08555300
C	-5.95153500	1.27164400	0.60875900
C	-6.87333700	0.20875800	0.55967300
C	-2.64378600	2.26368200	-0.40619700
C	-2.09553200	1.14093900	-1.03125500
C	-0.83862400	1.31577900	-1.62652500
H	-0.37848300	0.47565900	-2.13931200
C	-0.15766200	2.52472300	-1.60127100
C	-0.70994600	3.65453700	-0.94174700
C	-1.97962200	3.48960200	-0.35554700
H	-7.10406400	-1.83427400	-0.18027700
H	-4.85620300	-2.03434700	-1.03562800
H	-6.19001000	2.21420200	1.08331200
H	0.79165600	2.58484800	-2.11601600
H	-2.47076300	4.28877500	0.18337600
O	-3.87380000	2.24954300	0.20147000
C	-2.79164000	-0.20724300	-0.99023000
C	-2.68564800	-0.97131100	-2.30373700
C	-1.97803400	-2.15945700	-2.12997900
C	-3.18449300	-0.62636300	-3.55696700
C	-1.74354700	-3.04020200	-3.18679600
C	-2.95607800	-1.50080300	-4.62483100
H	-3.73644800	0.29737700	-3.70258400
C	-2.24271700	-2.69751500	-4.44450000
H	-1.18860600	-3.95817900	-3.01999300
H	-3.33718000	-1.25041500	-5.61081800

H	-2.08036400	-3.35780300	-5.29130000
C	-1.55706200	-2.29139200	-0.71449500
O	-0.91894700	-3.20633700	-0.19979600
N	-2.03793500	-1.15321000	-0.07566700
N	-1.94388900	-0.77076800	1.21960100
C	-1.31855600	-1.49781000	2.08160000
H	-0.84134800	-2.43804800	1.81586100
C	-1.22565600	-1.07109200	3.48927900
C	-0.51261300	-1.89264300	4.38156500
C	-0.40751900	-1.51546100	5.71891300
H	-0.05248400	-2.80867700	4.02265200
C	-1.70675000	0.42081800	5.16767900
C	-1.01756100	-0.33039800	6.12843500
H	0.13868700	-2.13456800	6.42488200
H	-2.19647000	1.35130200	5.45092200
H	-0.96659100	0.00842800	7.15849200
N	-1.81574700	0.07406700	3.88506500
N	-0.04615100	4.87961600	-0.86117100
N	-8.14897100	0.35250200	1.10884000
C	1.28712000	5.04266900	-1.44117600
C	2.17469100	6.03988100	-0.68903000
H	1.21753900	5.33936600	-2.50320700
H	1.79021900	4.07629100	-1.41241400
H	3.16289900	6.06687400	-1.16079500
H	1.77722000	7.05892300	-0.70751600
H	2.30109000	5.73814300	0.35549100
C	-0.83089100	6.10540800	-0.67631400
C	-0.90976300	6.60418200	0.77299500
H	-1.84148300	5.94075800	-1.06736700
H	-0.39032000	6.88692400	-1.30723900
H	-1.53168500	7.50567500	0.82798900
H	-1.34590300	5.84727600	1.43123900
H	0.08228500	6.84889400	1.16251600
C	-9.08351700	-0.77316500	1.10607700
C	-10.05976400	-0.77354700	2.28681100
H	-8.50285700	-1.69409000	1.16568000
H	-9.65124400	-0.80962600	0.15868400
H	-10.68804900	-1.66914800	2.23190300
H	-9.52033700	-0.79087700	3.23910400
H	-10.72775500	0.09284100	2.28523100
C	-8.74018800	1.69348200	1.18977300

C	-8.62981100	2.36334200	2.56613400
H	-9.79733800	1.61484800	0.90729200
H	-8.27388200	2.33066600	0.42968800
H	-9.07746000	3.36392200	2.53442000
H	-9.14797200	1.78149700	3.33340600
H	-7.58617200	2.46602400	2.87743900

II

C	-4.91104000	-3.77908200	1.17897100
C	-3.99032400	-2.93678300	0.57297500
C	-4.26028000	-1.58858300	0.29770800
C	-5.52614600	-1.12510200	0.66524100
C	-6.47133100	-1.94652500	1.28282400
C	-6.19020800	-3.29688100	1.56426900
C	-5.09937800	1.04217600	-0.19523400
C	-3.81413900	0.69220700	-0.61441100
C	-3.07958200	1.69642000	-1.26172600
H	-2.07674500	1.47012300	-1.61305700
C	-3.58118200	2.97036600	-1.47860000
C	-4.88227500	3.32511200	-1.03203800
C	-5.63115500	2.31631300	-0.39544200
H	-4.63780300	-4.81586500	1.32100900
H	-3.02610500	-3.34762200	0.28601300
H	-7.41024200	-1.48700100	1.56209100
H	-2.95915800	3.67789000	-2.00959200
H	-6.62187300	2.49968700	-0.00178900
O	-5.93857600	0.16508500	0.45064300
C	-3.22241300	-0.67689000	-0.33566000
C	-2.55827900	-1.30688900	-1.56303100
C	-1.19549900	-1.52062400	-1.34775500
C	-3.14213500	-1.66527000	-2.77470400
C	-0.37567800	-2.09215800	-2.32034200
C	-2.32936200	-2.23893400	-3.75866900
H	-4.20106900	-1.50411300	-2.95356100
C	-0.95932900	-2.45167600	-3.53688200
H	0.68112400	-2.24457900	-2.12440800
H	-2.76593000	-2.52412900	-4.71162500
H	-0.35203200	-2.89788800	-4.31874100
C	-0.83049400	-1.05328000	0.01311000
O	0.24878700	-1.08694000	0.59165000
N	-2.00771500	-0.55956800	0.54403600

N	-2.09995500	0.00218800	1.79768900
C	-1.56504600	-0.54245700	3.12631700
H	-0.77872000	-1.29311400	3.04613500
C	-2.53864800	-0.73560000	4.23867700
C	-2.44351000	-1.86779100	5.05977800
C	-3.34996600	-2.01635100	6.10947200
H	-1.67587000	-2.61346500	4.87534600
C	-4.33523800	0.05817000	5.42640000
C	-4.32171700	-1.03467100	6.29939600
H	-3.29901400	-2.88319300	6.76217900
H	-5.07445900	0.84812300	5.54437100
H	-5.04888800	-1.10490400	7.10221100
N	-3.46691600	0.21684700	4.42410100
N	-5.41283100	4.60325000	-1.19704000
N	-7.13968500	-4.10201600	2.19520900
C	-4.61916600	5.67195700	-1.80503000
C	-4.91007700	7.06316600	-1.23289400
H	-4.76070700	5.68656200	-2.90037400
H	-3.56613600	5.46002100	-1.62180800
H	-4.24447800	7.79203700	-1.70774000
H	-5.93717700	7.39124000	-1.41829200
H	-4.72943400	7.08615200	-0.15366600
C	-6.86911900	4.77583400	-1.22083800
C	-7.49179800	5.19983600	0.11673500
H	-7.32894200	3.84182400	-1.56411700
H	-7.10648600	5.52512200	-1.98577700
H	-8.58137200	5.27324900	0.01713700
H	-7.26890700	4.47785900	0.90751500
H	-7.11266600	6.17260900	0.44180500
C	-6.82729000	-5.49273500	2.52657000
C	-7.54660200	-6.00970000	3.77659300
H	-5.75606500	-5.56339600	2.71651200
H	-7.04877100	-6.15763200	1.67251300
H	-7.22410100	-7.03717900	3.97641500
H	-7.30081300	-5.39638900	4.64925900
H	-8.63444600	-6.02638900	3.66324600
C	-8.56226700	-3.77063400	2.04813000
C	-9.17077400	-3.00691300	3.23191700
H	-9.11329900	-4.70697900	1.89701600
H	-8.69606400	-3.19287200	1.12662400
H	-10.22410000	-2.77840200	3.03051700

H	-9.11913000	-3.59444700	4.15285900
H	-8.64530900	-2.06413900	3.41032400
C	-1.19314600	0.75651100	2.47709800
O	-0.53080700	1.75648200	2.44574300

I2

C	-6.61454200	-3.62137600	0.43951400
C	-5.45465200	-2.91248600	0.29080600
C	-5.44622800	-1.49020700	0.16257900
C	-6.72884200	-0.86373000	0.17618000
C	-7.91096600	-1.55719400	0.32920500
C	-7.89969700	-2.97174800	0.46379900
C	-5.74326400	1.27005200	-0.15838600
C	-4.43846400	0.69821300	-0.17074000
C	-3.36544400	1.62106000	-0.34285100
H	-2.34877300	1.24448100	-0.33771600
C	-3.57799900	2.96461800	-0.51344800
C	-4.90605900	3.52153700	-0.53084800
C	-5.98249900	2.62117100	-0.32125900
H	-6.54548400	-4.69439200	0.54747600
H	-4.51309900	-3.44751700	0.27949600
H	-8.82687900	-0.98397800	0.34263700
H	-2.71798700	3.60935500	-0.63077600
H	-7.01069500	2.95127700	-0.28326600
O	-6.84057800	0.48746400	0.03227200
C	-4.28765500	-0.69857200	0.01153800
C	-2.93582200	-1.33126800	-0.02832400
C	-2.36869800	-1.98399400	1.08808300
C	-2.24270800	-1.35055300	-1.25167700
C	-1.15007000	-2.66199900	0.95074100
C	-1.02113800	-2.01215500	-1.37140100
H	-2.68390000	-0.86995100	-2.11989600
C	-0.47445900	-2.67283600	-0.26786900
H	-0.74171800	-3.17462900	1.81474800
H	-0.50670000	-2.02311000	-2.32724700
H	0.47097900	-3.19829600	-0.35812600
C	-3.06209400	-2.05963100	2.41882900
O	-3.37779200	-3.12889400	2.93717300
N	-3.45614200	-0.86738400	2.96955900
N	-4.07676300	-0.87813000	4.26665000
C	-2.73450800	0.40788000	3.26988500

H	-3.10363300	1.24730800	2.67667600
C	-1.22659200	0.37143600	3.32859900
C	-0.48333000	1.51551800	3.02400800
C	0.90173300	1.47473600	3.19720400
H	-0.97608200	2.41734700	2.67393200
C	0.65612800	-0.79512900	3.92907000
C	1.48657200	0.29746500	3.66037800
H	1.50960400	2.34684900	2.97652500
H	1.07276600	-1.73159400	4.29236800
H	2.55785400	0.22134600	3.81381100
N	-0.67132200	-0.76502300	3.77086000
N	-5.11541300	4.84882900	-0.74374100
N	-9.04787400	-3.68571300	0.59144100
C	-3.99965400	5.78124200	-0.98806000
C	-3.32999800	6.32276600	0.28069900
H	-4.40548800	6.61055400	-1.57258800
H	-3.26508100	5.29207100	-1.63382900
H	-2.50540100	6.98838300	0.00551000
H	-4.03435100	6.89543200	0.89001500
H	-2.92493500	5.51724500	0.90062000
C	-6.47477400	5.41877900	-0.71264600
C	-7.01748800	5.69441700	0.69518400
H	-7.14732200	4.75300100	-1.26296300
H	-6.44051800	6.34990400	-1.28282600
H	-8.03608000	6.08943000	0.62446100
H	-7.04717300	4.78755300	1.30592100
H	-6.40557600	6.43391400	1.21853000
C	-9.03500100	-5.15391700	0.74616600
C	-8.77265300	-5.64014900	2.17635500
H	-8.30185500	-5.57627500	0.05339500
H	-10.00948200	-5.51217800	0.40618500
H	-8.76918300	-6.73475700	2.19527700
H	-7.80723200	-5.29146700	2.55442800
H	-9.55027500	-5.29444400	2.86278000
C	-10.36191400	-3.01814600	0.62588500
C	-10.75096900	-2.44805800	1.99585900
H	-11.10091200	-3.75860100	0.31122900
H	-10.37660500	-2.23419300	-0.13747500
H	-11.72305400	-1.94982400	1.92206800
H	-10.83469200	-3.23879200	2.74606000
H	-10.02055700	-1.71754700	2.35523800

C	-3.41410600	0.27742300	4.67523900
O	-3.40370300	0.90214900	5.69938900
H	-4.13558200	-1.76821800	4.76190900

Rhodamine B

C	-6.18339500	-3.42535100	0.09029400
C	-5.26033900	-2.73964500	-0.65206500
C	-5.31027100	-1.32271300	-0.79938700
C	-6.38333700	-0.66673900	-0.12936100
C	-7.32643000	-1.33607000	0.62492100
C	-7.26846300	-2.74913500	0.75524100
C	-5.65453500	1.44543200	-0.93477200
C	-4.56095300	0.83770300	-1.61775700
C	-3.70003800	1.72644800	-2.32565700
H	-2.84307400	1.31591800	-2.84792900
C	-3.92048700	3.07674400	-2.36002100
C	-5.04629300	3.67387100	-1.68711600
C	-5.89860500	2.80414000	-0.95600200
H	-6.08309700	-4.49864100	0.16882600
H	-4.46429300	-3.28755800	-1.14385100
H	-8.09235300	-0.74483200	1.10599500
H	-3.22356700	3.69663200	-2.90601700
H	-6.74857400	3.16742800	-0.39625100
O	-6.52250100	0.68684000	-0.20796900
C	-4.38212800	-0.55772800	-1.53504000
C	-3.28135400	-1.22844600	-2.29581200
C	-2.04191300	-1.62200700	-1.73967700
C	-3.53337200	-1.50522900	-3.64864000
C	-1.10342300	-2.28467300	-2.54836300
C	-2.58870900	-2.15974500	-4.44092600
H	-4.48568400	-1.21024400	-4.07932400
C	-1.36847900	-2.55309700	-3.88814700
H	-0.16245700	-2.57840900	-2.09631100
H	-2.81060500	-2.36181100	-5.48427100
H	-0.62845100	-3.06373600	-4.49572700
C	-1.61496600	-1.38384500	-0.32589400
O	-0.60877000	-1.85099800	0.16581800
N	-5.28300800	5.01028100	-1.76176700
N	-8.20132500	-3.44060500	1.46191800
C	-4.38171700	5.91728500	-2.49865400
C	-3.13313800	6.34691800	-1.71892100

H	-4.97182100	6.79758300	-2.76328600
H	-4.10308800	5.44753400	-3.44646900
H	-2.51903400	7.00337500	-2.34382000
H	-3.39957100	6.89778100	-0.81281100
H	-2.52149300	5.48884500	-1.42513500
C	-6.43910000	5.61931500	-1.07947800
C	-6.21263500	5.93044600	0.40540300
H	-7.30603300	4.96440600	-1.20928500
H	-6.67468300	6.54059100	-1.61743900
H	-7.12593000	6.35600800	0.83363900
H	-5.95635800	5.03267600	0.97540300
H	-5.40774100	6.65786500	0.54041900
C	-8.12053500	-4.90523100	1.62558700
C	-7.15474800	-5.37117500	2.72171200
H	-7.86024500	-5.35601800	0.66336200
H	-9.13131000	-5.25053800	1.85410700
H	-7.15126900	-6.46504700	2.76579400
H	-6.13088600	-5.03655400	2.53121400
H	-7.45601400	-4.99560700	3.70331900
C	-9.32042000	-2.74957000	2.12779600
C	-8.98085100	-2.16616000	3.50506800
H	-10.12932700	-3.47757700	2.22453100
H	-9.69604800	-1.96865000	1.45955800
H	-9.85788900	-1.65182300	3.91111200
H	-8.69601000	-2.95158900	4.21020600
H	-8.15945200	-1.44582800	3.45046600
O	-2.44652400	-0.57588000	0.37948600
H	-2.06049900	-0.49212900	1.26889800

P2

C	0.38983100	0.23140200	0.14431600
C	0.91069400	1.26018900	-0.64989600
C	0.24094900	2.48284200	-0.67513000
C	-0.92021700	2.63289800	0.08447000
C	-1.35587600	1.54658000	0.84658100
H	1.82203300	1.09587400	-1.21376800
H	0.61942500	3.30362800	-1.27758800
H	-1.47379200	3.56643000	0.09384300
H	-2.25189100	1.62368200	1.45855900
N	-0.71859700	0.37003500	0.88737600
C	1.02335700	-1.13498900	0.19534100

H	0.81422400	-1.59770400	1.16465900
C	0.72703400	-2.10715500	-0.98375900
O	-0.23665100	-2.50233200	-1.58775500
N	2.45982800	-1.22141400	-0.23833500
H	3.03058900	-1.62848600	0.50380600
N	2.09362300	-2.31488900	-1.15083200
H	2.54200900	-2.34608000	-2.06373500