Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A novel red emission fluorescent probe for monitoring carbon monoxide in living cells and zebrafish

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I. Experimental Section

MTT assay.

Cell cytotoxicity was evaluated by MTT assay. Cells were cultivated in a 96-well plate until 60-70% confluence, and then incubated with different concentrations of probe 1 (0-10 μM) and the probe 1 (0-10 μM) + $PdCl_2$ (0-20 μM) for 24 h. Then 20 μL MTT was added for 4 h at 37°C. Absorbance was measured at 570 nm on SpectraMax i3 (Molecular Devices, USA). All experiments were repeated five times, and the data were presented as the normalized percentage of control cells.

Calculation of the detection limit (LOD)

$$\mathrm{LOD} = 3\sigma/S$$

$$\sigma = \sqrt{\frac{\sum(\bar{x} - x_i)^2}{n-1}}$$

σ: the standard deviation of the blank solution.

$\bar{x}$ is the mean of the blank measures; x_i is the values of blank measures; n is the number of tested blank measure (n = 10)

S: the slope of the linear calibration plot between the fluorescence emission intensity and the concentration of CORM-3.

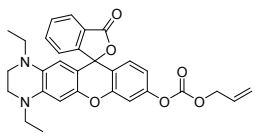
Quantum yield measurements

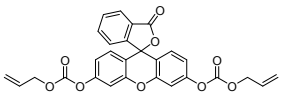
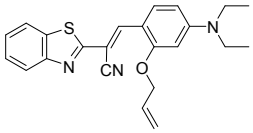
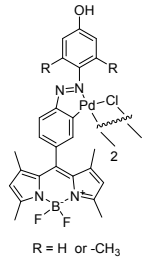
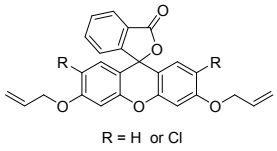
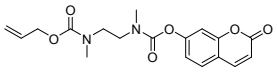
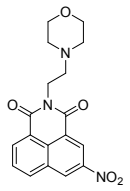
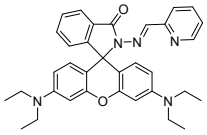
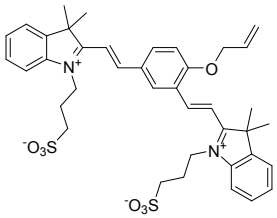
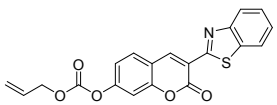
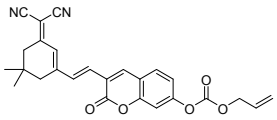
Fluorescein (Φ = 0.95 in 0.1 M NaOH) was used as quantum yield reference. The QY_s were determined by comparing the integrated fluorescence intensity and the absorbance value of HN-0 and the probe solution samples with those of the references. The absorbances (less than 0.05 at the excitation wavelength) at maximal absorbance for HN-0 and the probe system (5 μM probe 1 + 10 μM $PdCl_2$) + CORM-3 (50 μM) and Fluorescein were recorded. The slope method was used to calculate the QY_s using the equation:

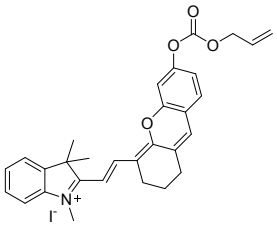
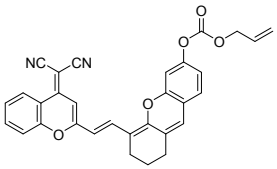
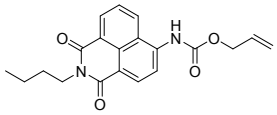
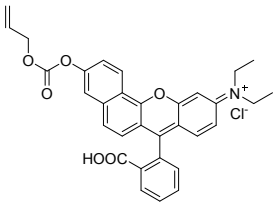
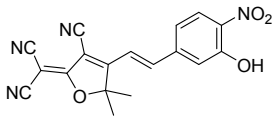
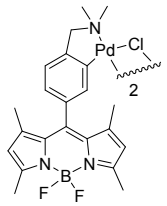
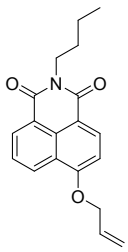
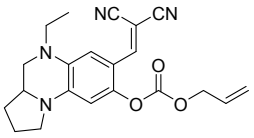
$QY_u = QY_s (m_u/m_s) (n_u/n_s)^2$.

Where QY is the quantum yield, m is the slope determined by the curves. And n is the refractive index. (1.33 for 0.1 M NaOH aqueous solution, 1.35 for the volume ratio of acetonitrile to HEPES is 5:5 at 37°C). The subscript "s" refers to the standards and "u" refers to the unknown samples. A series of concentrations for the references and the required samples were measured to obtain the slopes.

Table S1. Summary of the properties of representative fluorescent probes for selective detection of CO.

probe	Stokes Shift (nm)	LOD	Linear range	Quantum yield	Biological system	Ref.
	135 nm	37 nM	0-20 μM	---	HeLa cells zebrafish and mice	[1]

	30 nm	37 nM	0-30 μM	---	A549 cells	[2]
	81 nm	30.8 nM	0-5 μM	0.005	MCF-7 cells and mice	[3]
 R = H or -CH_3	14 nm	0.72 μM	0-70 μM	0.039 or 0.009	HepG2 cells	[4]
 R = H or Cl	26 nm or 27 nm	46 nM or 29 nM	0-50 μM	---	HeLa cells	[5]
	120 nm	8.49 nM	0-1 μM	0.4467	A549 cells	[6]
	88 nm	0.60 μM	1-10 μM	0.0016	MCF-7 and HepG2 cells	[7]
	20 nm	10 nM	0.1-1.0 μM	---	HepG2 cells	[8]
	123 nm	15.8 nM	0-1.5 μM	---	HepG2 cells and mice	[9]
	28 nm	25 nM	0-14 μM	0.56	HeLa cells	[10]
	222 nm	33 nM	0-10 μM	---	HeLa cells and zebrafish	[11]

	36 nm	3.2 nM	0-9 μM	---	HeLa cells and mice	[12]
	164 nm	0.33 nM	0-100 μM	0.26	HepG2 HCT116 cells and mice	[13]
	107 nm	58 nM	0-35 μM	0.52	A549 cells	[14]
	80 nm	38.9 nM	0-5 μM	---	HeLa cells	[15]
	85 nm	6.1 nM	0-7 μM	0.0029	RAW 264.7 cells and zebrafish	[16]
	8 nm	---	0-50 μM	0.44	HEK293T cells	[17]
	115 nm	17.9 nM	0-50 μM	---	HeLa cells	[18]
	132 nm	36.4 nM	0-30 μM	0.238	HepG2 cells and zebrafish	This Work

II. Supplementary Spectra

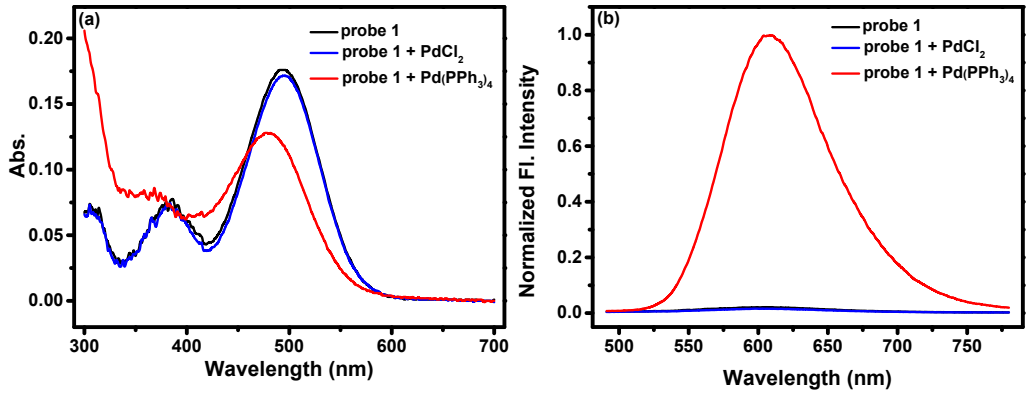


Fig. S1 (a) The absorption spectra of probe 1 (5 μM) in the absence and presence of $PdCl_2$ and $Pd(PPh_3)_4$ (10 μM) in CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37°C for 90 min. (b) The corresponding fluorescence spectra changes of probe 1 (5 μM) in the absence and presence of $PdCl_2$ and $Pd(PPh_3)_4$ (10 μM) in CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37°C for 90 min. λ_{ex} = 471 nm, λ_{em} = 608 nm, slit (nm): 5.0/5.0.

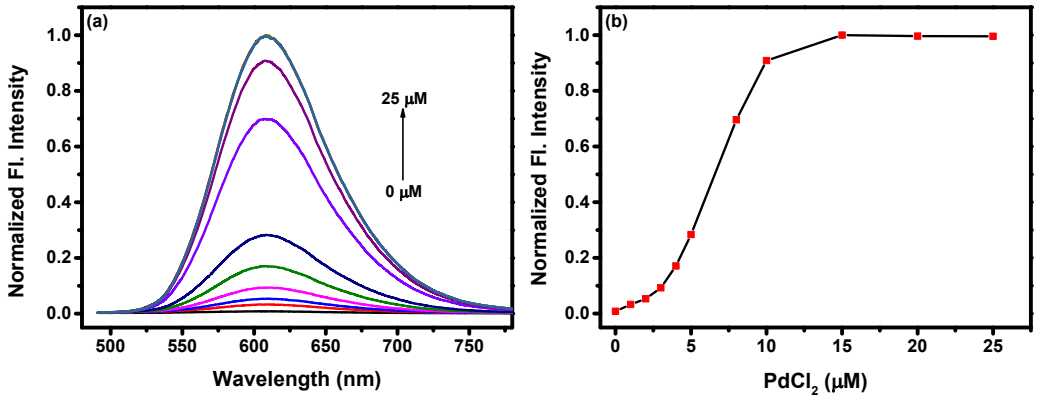


Fig. S2 (a) The normalized fluorescence intensity spectra of probe 1 (5 μM) for CORM-3 (50 μM) with different concentrations of $PdCl_2$ in CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37ºC. (b) The corresponding normalized fluorescence intensity changes at 608 nm of probe 1 (5 μM) for CORM-3 (50 μM) with different concentrations of $PdCl_2$ in CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37ºC. Each spectrum was obtained 90 min after mixing. λ_{ex} = 471 nm, slit (nm): 5.0/5.0.

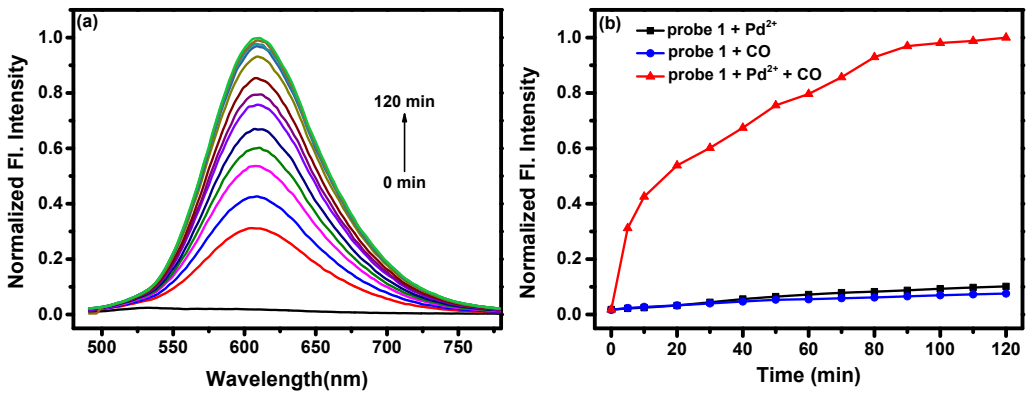


Fig. S3 (a) Time-dependent normalized fluorescence spectra of the probe system (5 μM probe 1 + 10 μM $PdCl_2$) upon addition of CORM-3 (50 μM) in CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37ºC. (b) The corresponding time-dependent normalized fluorescence intensity changes of probe 1 (5 μM) and probe 1 (5 μM) for CORM-3 (50 μM) in the absence and presence of $PdCl_2$ (10 μM) at 608 nm. λ_{ex} = 471 nm, slit(nm): 5.0/5.0.

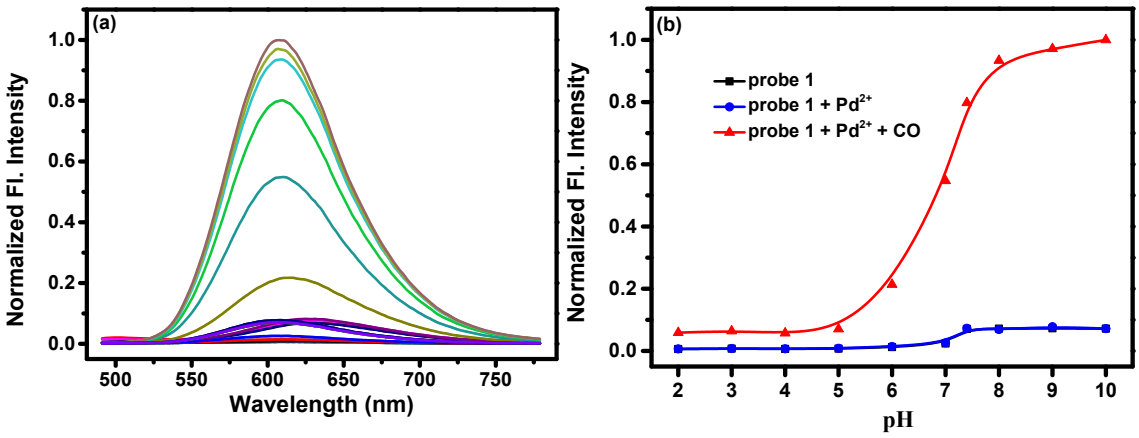


Fig. S4 (a) pH dependent normalized fluorescence intensity spectra of the probe 1 and the probe system (5 μM probe 1 + 10 μM $PdCl_2$) in the absence and presence of CORM-3 (50 μM) under different pH. (b) The corresponding pH dependent normalized fluorescence intensity changes at 608 nm. Conditions: CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37ºC. Each spectrum was obtained 90 min after mixing. λ_{ex} = 471 nm, slit (nm): 5.0/5.0.

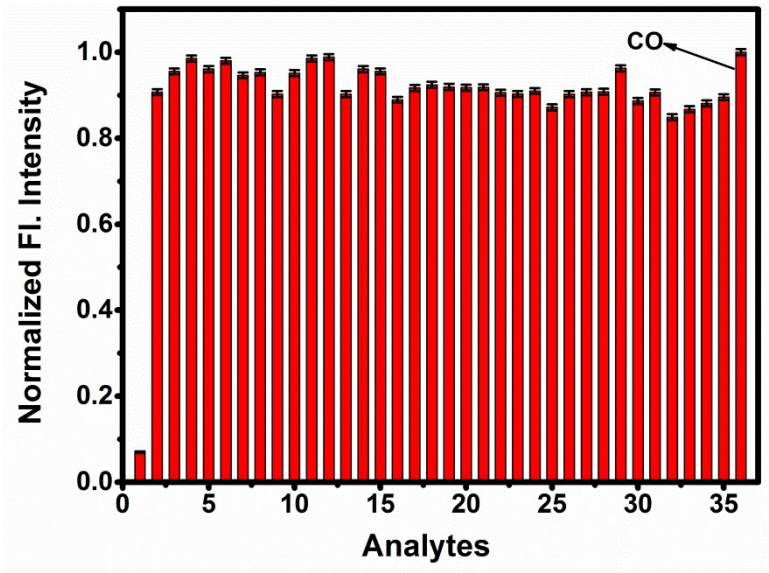


Fig. S5 Histogram representing the normalized fluorescence intensity changes of the probe system (5 μM probe 1 + 10 μM $PdCl_2$) at 608 nm in the presence of CORM-3 (50 μM) upon the addition of the various analytes (100 μM). Analytes: 1. none; 2. F^-; 3. Cl^-; 4. Br^-; 5. I^-; 6.NO_3^-; 7. NO_2^-; 8. $S_2O_3^{2-}$; 9. SO_3^{2-}; 10. SO_4^{2-}; 11. HCO_3^-; 12. HS^-; 13. Na^+; 14. K^+; 15. Mn^{2+}; 16. Fe^{3+}; 17. Ba^{2+}; 18. Al^{3+}; 19. Ni^{2+}; 20. Co^{2+}; 21. Zn^{2+}; 22. Asp; 23. Glu; 24. Gly; 25. Trp; 26. His; 27. Met; 28. Thr; 29. Cys; 30. Hcy; 31. GSH; 32. ClO^-; 33. H_2O_2; 34. *t*-BuOO·; 35. NO; 36. CO. Conditions: CH_3CN/HEPES (10 mM, pH = 7.4, v/v, 5/5) at 37ºC. Each data was collected after 90 min of mixing. λ_{ex} = 471 nm, slit (nm): 5.0/5.0.

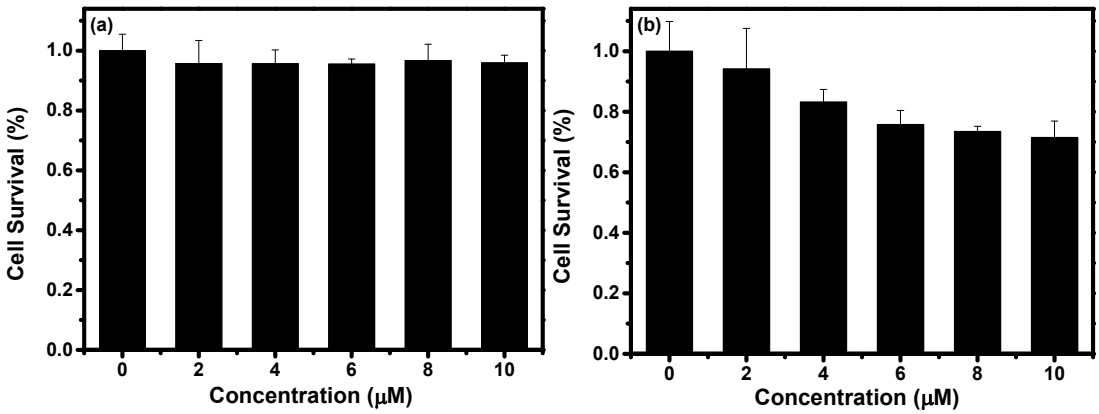


Fig. S6 (a) MTT assay for the survival rate of living HepG2 cells treated with various concentrations of probe 1 for 24 h. (b) MTT assay for the survival rate of living HepG2 cells treated with various concentrations of probe 1 + $PdCl_2$ (1:2) for 24 h.

III. ^{1}H NMR, ^{13}C NMR and FT-IR chart

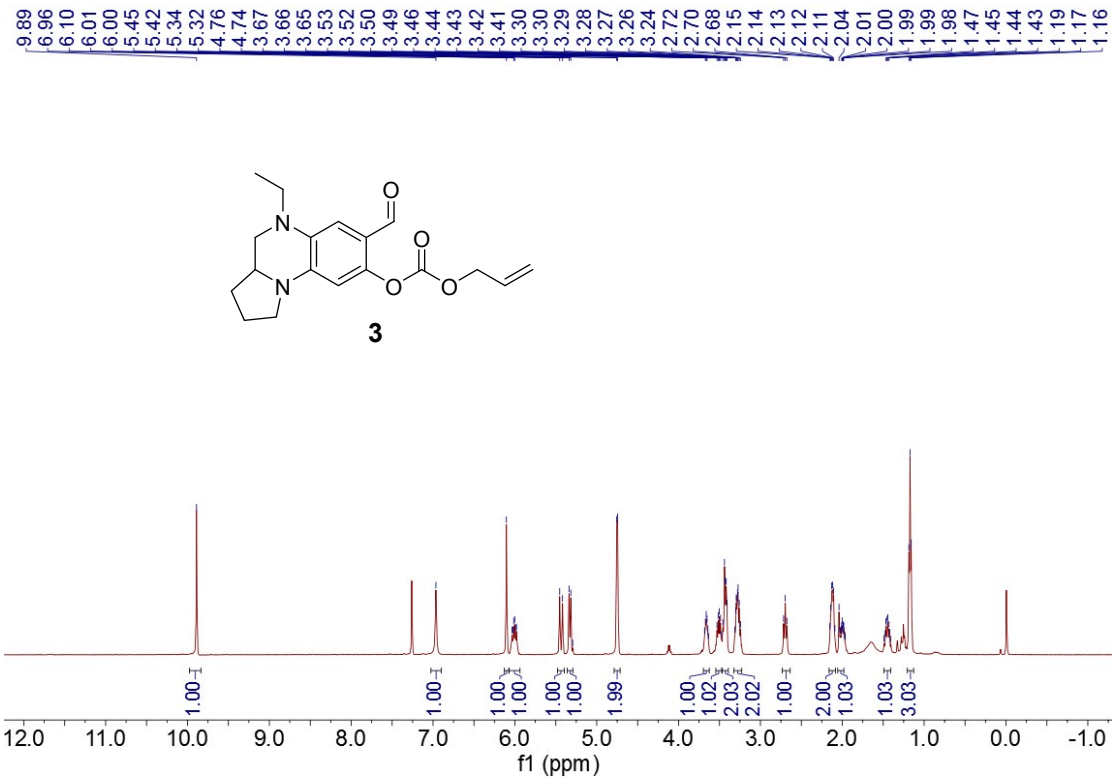


Fig. S7 ^{1}H NMR spectrum of compound 3 in $CDCl_3$.

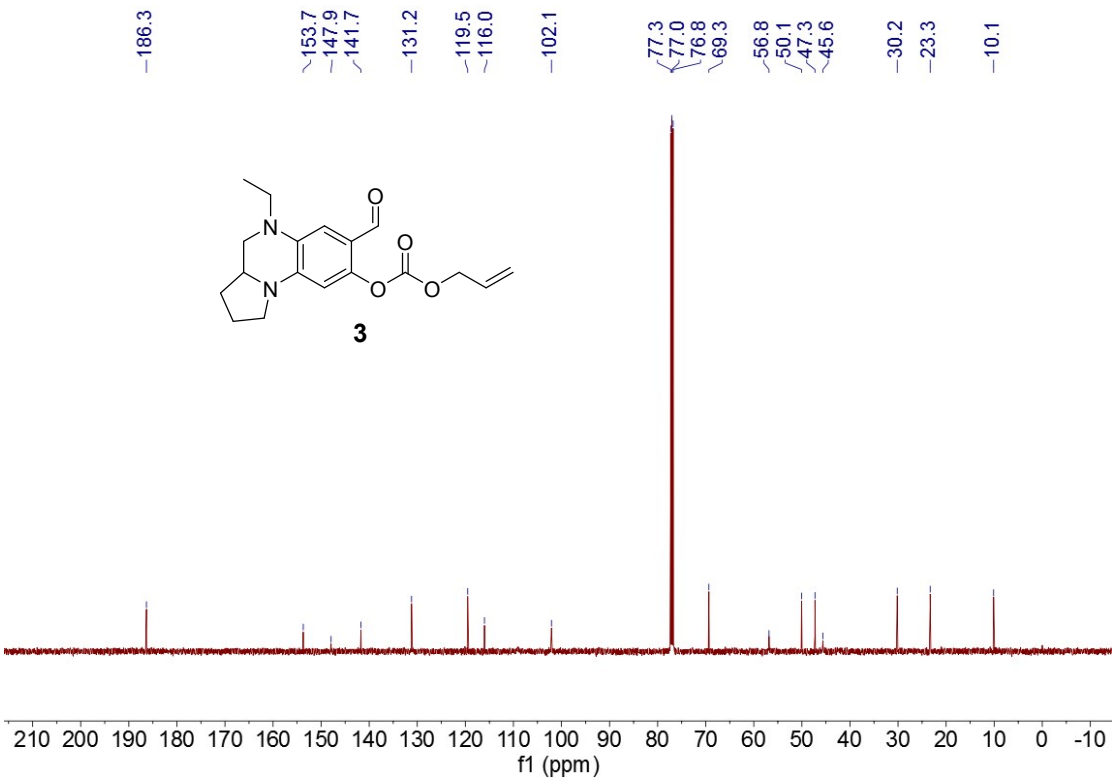


Fig. S8 ^{13}C-NMR spectrum of compound 3 in $CDCl_3$.

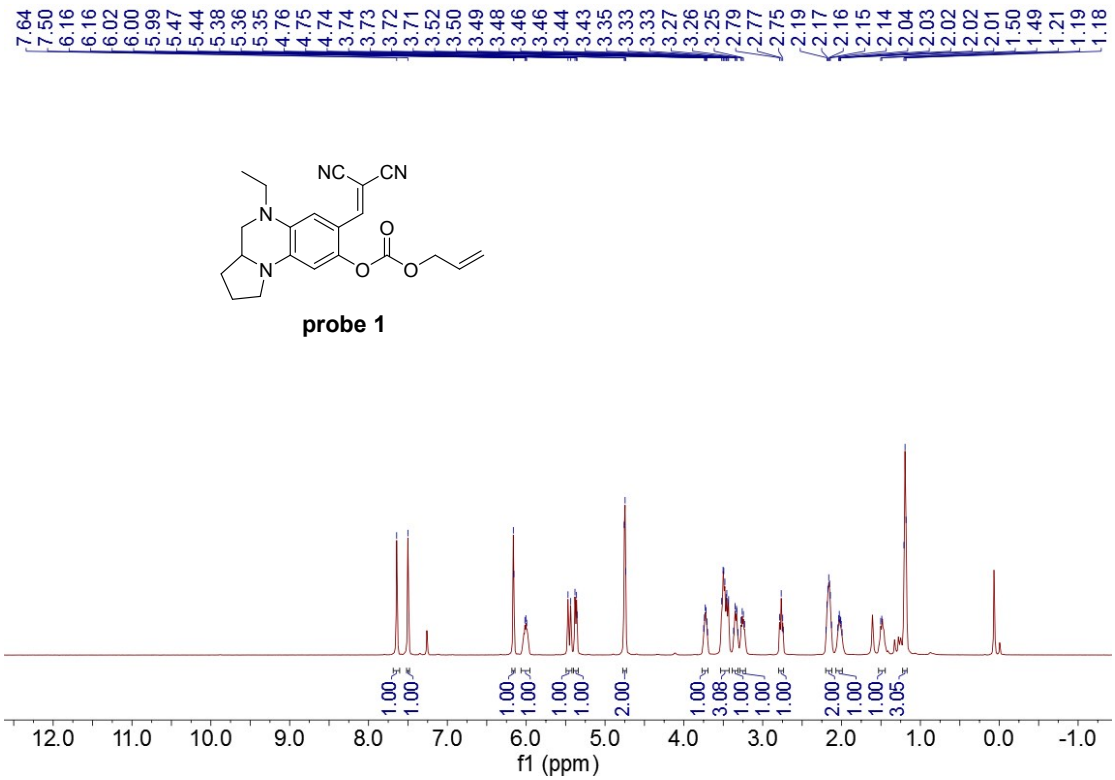


Fig. S9 ^{1}H NMR spectrum of probe 1 in $CDCl_3$.

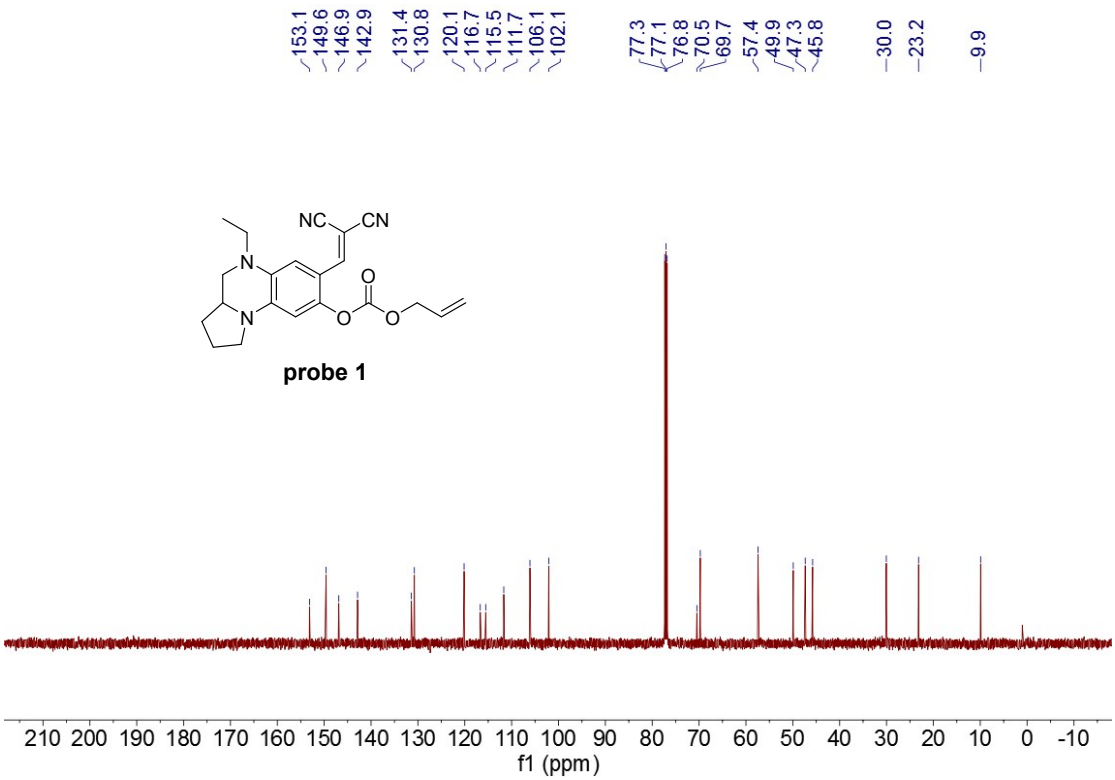


Fig. S10 ^{13}C-NMR spectrum of probe 1 in $CDCl_3$.

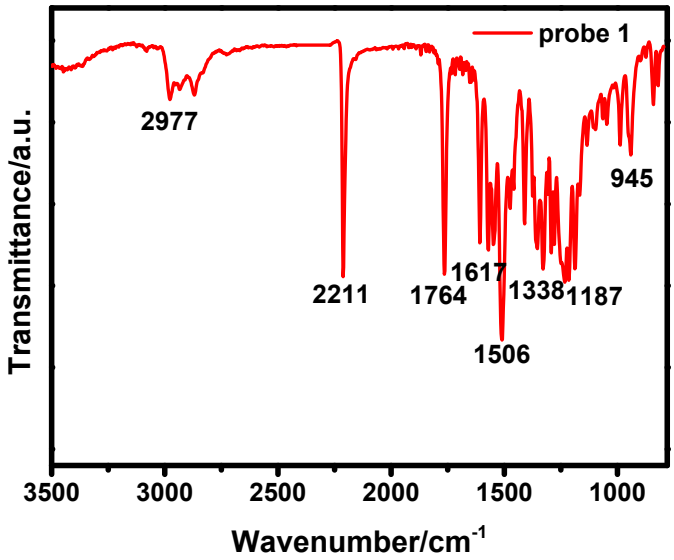


Fig. S11 FT-IR spectrum of the probe 1.

IV. Reference

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