Electronic Supplementary Information (ESI)

A naked-eye and ratiometric near-infrared probe for palladium via modulating π-conjugated system of cyanines

Xiaohang Wang, Zhiqian Guo,* Shiqin Zhu, He Tian, and Weihong Zhu*

Shanghai Key Laboratory of Functional Materials Chemistry, Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science and Technology, Shanghai 200237, P. R. China. E-mail: guozq@ecust.edu.cn;whzhu@ecust.edu.cn

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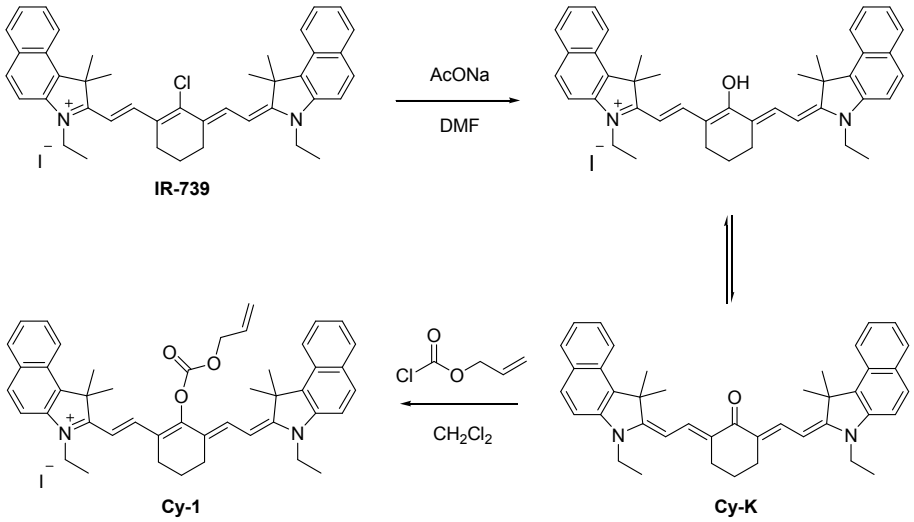
1. General Information

Unless otherwise noted, materials were obtained from Aldrich and were used without further purification. All solvents were of analytical grade. The stock solution of **Cy-1** was prepared in acetonitrile, and the stock solution of $Pd(PPh_3)_4$ was prepared in THF. ^1H and ^{13}C NMR in $CDCl_3$ were obtained by a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as internal standard. High Resolution Mass Spectra (HRMS) were obtained by a Waters LCT Premier XE spectrometer. Absorption spectra were measured on a Varian Cary 500 spectrophotometer at 25 °C. Fluorescence spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer (1 cm quartz cell) at 25 °C. Deionized water was used to prepare all aqueous solutions. Cell imaging was performed with an inverted FL microscope (Nikon Eclipse Ti).

Scheme S1 Synthetic Route of **Cy-1**



Synthesis of CyK

A mixture of IR-739 (311 mg, 0.41 mmol) and sodium acetate (125 mg, 1.49 mmol) in 15 mL *N*,*N*-dimethylformamide was heated at 90 °C for 6 h under Ar atmosphere. The solvent was removed by rotary evaporation to obtain red oil product, and then the product was purified by column chromatography (silicon gel column, dichloromethane: triethylamine = 100 : 1) to obtain a red powder (210 mg), yield 64 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.33 (t, J = 7.2 Hz, 6H, -NCH$_2$C***H***$_3$), 1.93-1.87 (m, 2H, -C***H***$_2$-), 2.01 (s, 12H, -C***H***$_3$), 2.66 (t, J = 5.6 Hz, 4H, -C***H***$_2$-C***H***$_2$-), 3.85 (q, J = 7.2 Hz, 4H, -NC***H***$_2$CH$_3$), 5.51 (d, J = 13.4 Hz, 2H, alkene-H), 7.07 (d, J = 8.8 Hz, 2H, Ph-H), 7.27 (t, J = 7.2 Hz, 2H, Ph-H), 7.47 (t, J = 8.4 Hz, 2H, Ph-H), 7.76 (d, J = 8.8 Hz, 2H, Ph-H), 7.80 (d, J = 8.2 Hz, 2H, Ph-H), 8.04 (d, J = 8.4 Hz, 2H, Ph-H), 8.32 (d, J = 13.4 Hz, 2H, alkene-H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 11.50, 22.63, 25.90, 27.93, 29.24, 31.45, 36.50, 37.17, 48.59, 91.79, 109.00, 121.86, 122.50, 126.33, 126.80, 129.41, 129.92, 132.76, 141.07, 162.55, 163.6, 186.28. HRMS (ESI): calcd. for $[C_{42}H_{44}ON_2 + H]^+$ 593.3526; found 593.3528.

Synthesis of Cy-1

To a solution of **CyK** (200 mg, 0.28 mmol) and triethylamine (0.5 mL) in 10 mL of CH_2Cl_2 at 0 ^{o}C, a mixture of allyl carbonochloridate (1.5 mL) and CH_2Cl_2 (8 mL) was added dropwise

and kept stirring at this temperature for 30 min. Then the mixture was warmed to room temperature and stirred overnight. The mixture was concentrated under vacuum to get a deep green solid. The product was purified by column chromatography (dichloromethane : methanol : triethylamine = 100 : 1 : 1) to get a green solid (135 mg), yield 60.5 %. ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.51 (t, J = 7.2 Hz, 6H, -$NCH_2C\boldsymbol{H_3}$), 1.94 (s, 12H, -$C\boldsymbol{H_3}$), 2.06-2.01 (m, 2H, -$C\boldsymbol{H_2}$-), 2.80-2.73 (m, 4H, -$C\boldsymbol{H_2}$-$C\boldsymbol{H_2}$-), 4.37 (q, J = 7.2 Hz, 4H, -$NC\boldsymbol{H_2}CH_3$), 4.86 (d, J = 5.6 Hz, 2H, -O-$C\boldsymbol{H_2}$-), 5.45 (d, J = 10.4 Hz, 1H, alkene-H), 5.56 (d, J = 17.2 Hz, 1H, alkene-H), 6.16-6.04 (m, 1H, alkene-H), 6.22 (d, J = 14.2 Hz, 2H, alkene-H), 7.52-7.43 (m, 4H, Ph-H), 7.62 (t, J = 7.6 Hz, 2H, Ph-H), 7.90 (d, J = 14.2 Hz, 2H, alkene-H), 7.98-7.94 (m, 4H, Ph-H), 8.10 (d, J = 8.4 Hz, 2H, Ph-H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 12.72, 14.11, 20.70, 22.61, 24.55, 27.64, 31.54, 40.05, 46.14, 51.00, 69.99, 99.80, 110.57, 120.94, 121.82, 125.23, 127.88, 128.06, 130.21, 130.73, 130.98, 131.97, 133.78, 139.05, 139.14, 152.59, 158.75, 172.80. HRMS (ESI): calcd. for $[C_{46}H_{49}O_3N_2 + H]^+$ 677.3738; found 677.3743.

Reaction Cy-1 with the addition of $Pd(PPh_3)_4$ at room temperature

Cy-1 (20 mg) and $Pd(PPh_3)_4$ (50 eq) was mixed in CH_3CN (5 mL), and stirred for 1 h at room temperature. The color of solution was obviously changed from blue to red. Then, we checked our product using thin layer chromatography (TLC) compared with **CyK**. Moreover, the final product was purified by silica chromatography eluting with CH_2Cl_2/CH_3OH. The obtained product was also confirmed as **CyK** by ^{1}H and ^{13}C NMR.

Cell imaging

Cell culture: The human cervical adencarcinoma HeLa cell line was supplied by the Institute of Cell Biology (Shanghai, China). They were cultured at 37 °C under a humidified 5% CO_2 atmosphere in RPMI-1640 medium (GIBCO/Invitrogen, Camarillo, CA, USA) supplemented with 10% fetal bovine serum (FBS) (Biological Industry, Kibbutz Beit Haemek, Israel) and 1% penicillin-streptomycin (10,000 U/ml penicillin and 10 mg/ml streptomycin, Solarbio life science, Beijing, China). The HeLa cells at 2 × 10^5 cells/mL were plated on 20 mm glass

cover slips in a 6-well plates, and allowed to adhere overnight. The live cells were stained with either 10.0 μM of **Cy-1** (by adding 20 μL of a 500.0 μM stock solution in DMSO to 1 mL culture medium) for different times. Then the cells were washed three times with PBS buffer, and the medium was replaced with PBS buffer before imaging.

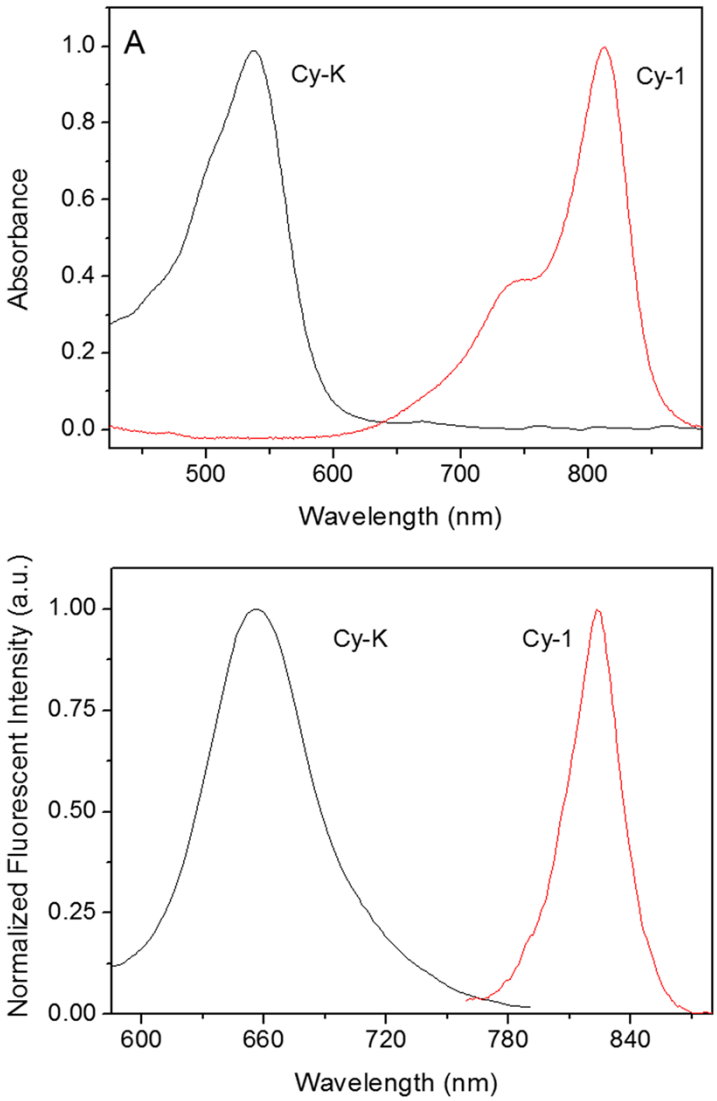


Fig. S1 Normalized absorption and emission spectra of **CyK** and **Cy-1** in a mixture solution of CH_3CN: PBS (1 : 3, pH = 7.4, 0.01 M).

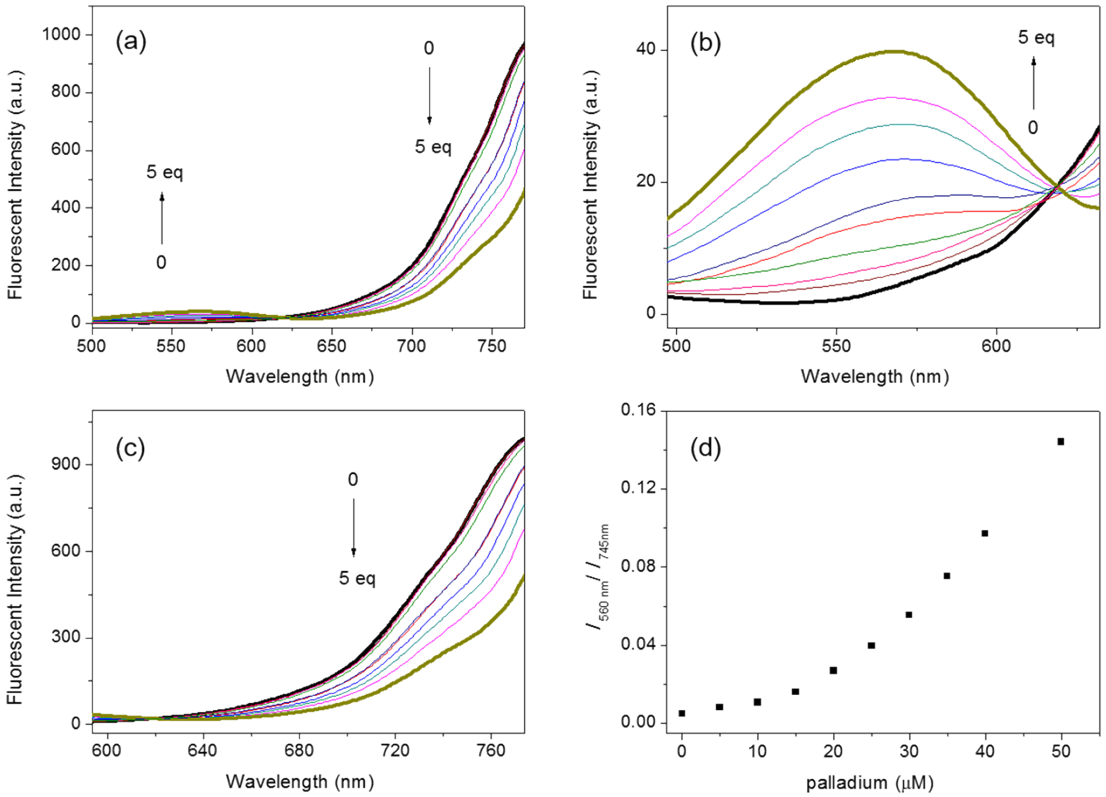


Fig. S2 Excitation spectra properties of **Cy-1** (10 μM) upon titration of $Pd(PPh_3)_4$ (0-50 μM) in mixture solution of CH_3CN: PBS (1: 3, 0.01M, pH = 7.4) using 825 nm as monitor wavelength. Each spectrum was recorded after the addition of palladium at 20 min.

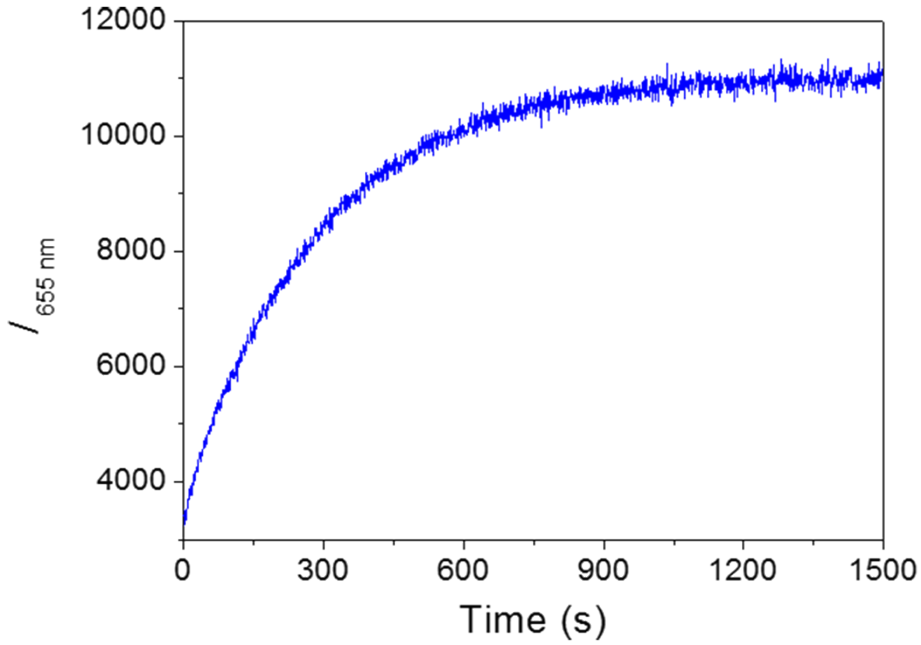


Fig. S3 Time-depend fluorescent intensity changes at 655 nm (I_{655nm}) of **Cy-1** in the presence of 50 μM $Pd(PPh_3)_4$ in CH_3CN-PBS (1 : 3, v/v) solution upon excitation at 545 nm.

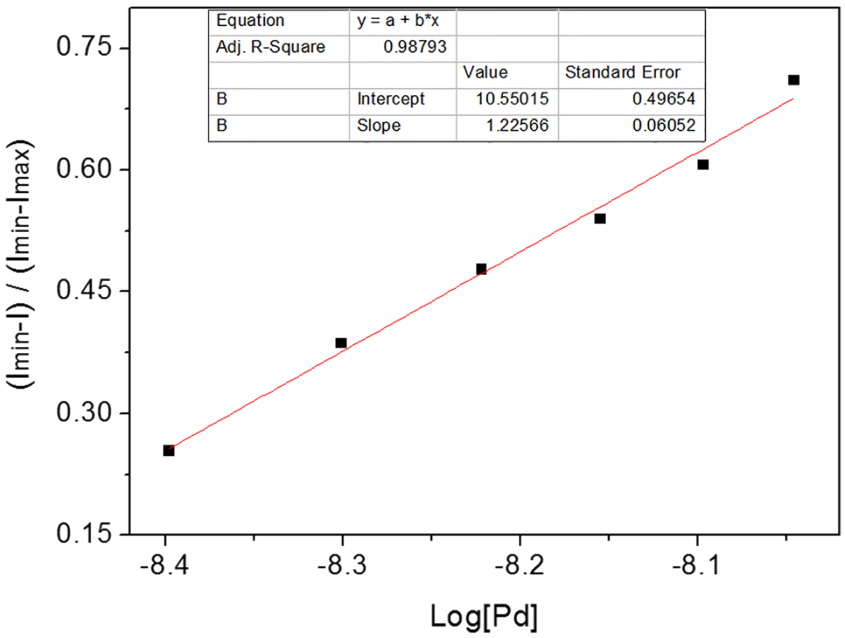


Fig. S4 Fluorescence intensity changes at 665 nm with the concentration of palladium $Pd(PPh_3)_4$. Note: The detection limit was calculated based on the fluorescence titration. The fluorescence emission spectrum of probe **Cy-1** at 665 nm was measured by three times. The limit of detection for palladium is 2.47 nM (0.26 ppb).

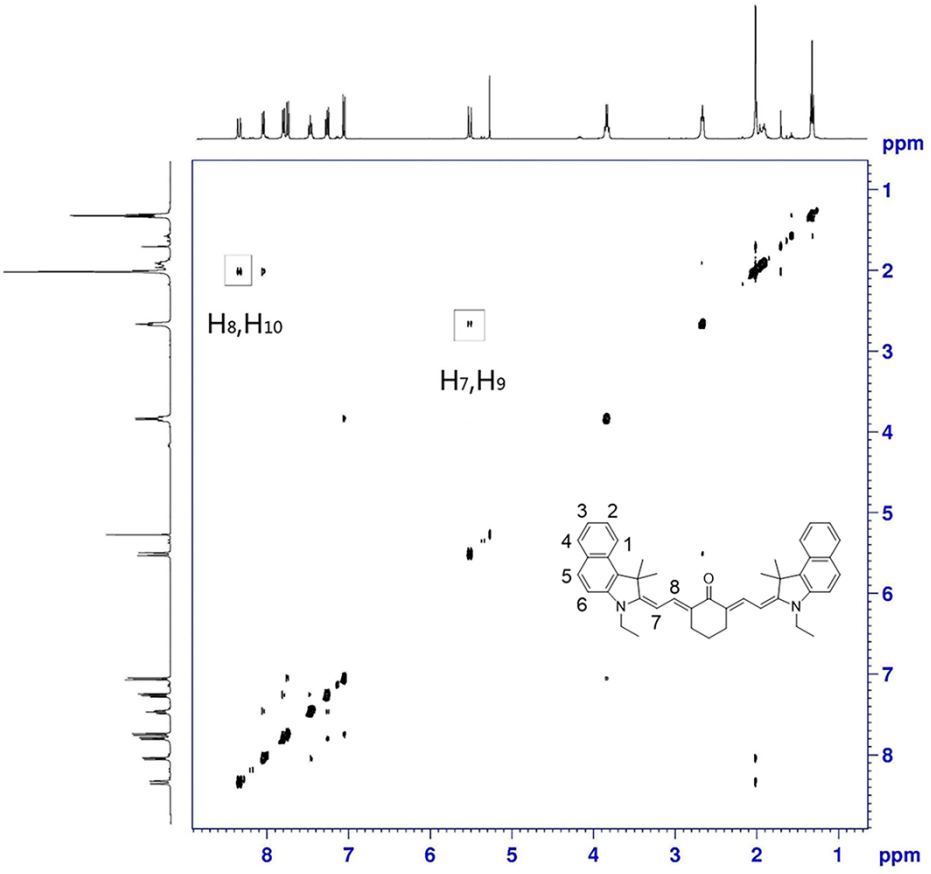
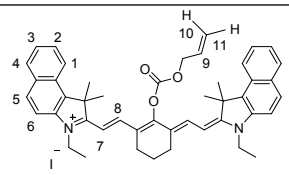
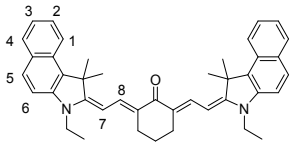


Fig. S5 Two-dimensional ^{1}H-^{1}H NOESY spectroscopy of **CyK** in $CDCl_3$**.**

Table S1. Chemical shift ^{1}H NMR of alkene-H and Ph-H in **CyK** and **Cy-1** ($CDCl_3$)

		
H_1	8.10	8.05
H_2	7.63	7.45
H_3	7.49	7.27
H_4	7.97	7.81
H_5	7.96	7.76
H_6	7.47	7.07
Alkene-H H_7	**7.90**	**8.34**
Alkene-H H_8	**6.26**	**5.51**
$\Delta\ \delta = \delta_{H7} - \delta_{H8}$	**1.64**	**2.83**
Alkene-H H_9	6.04	
Alkene-H H_{10}	5.56	
Alkene-H H_{11}	5.45	

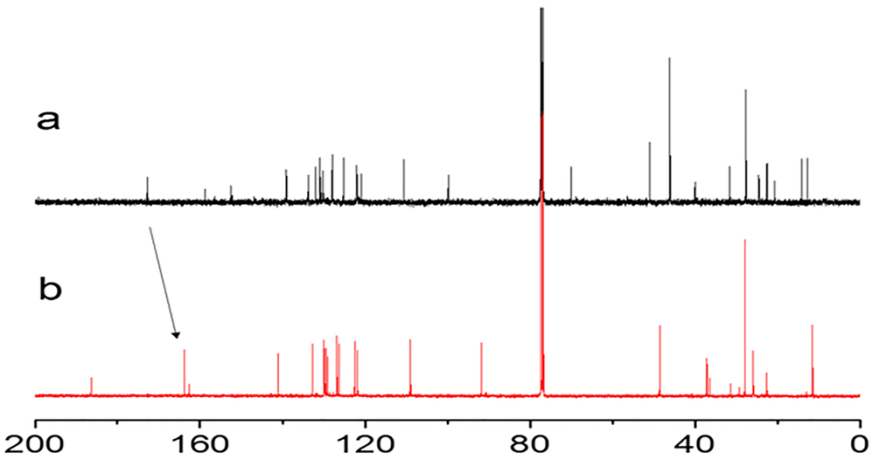
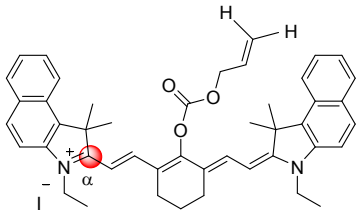
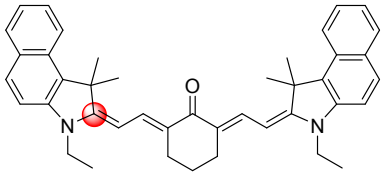


Figure S6. ^{13}C NMR spectroscopy of **Cy-1** (a) and **CyK** (b)

Table S2. Chemical shift ^{13}C NMR of specific α carbon in **CyK** and **Cy-1** ($CDCl_3$)

	
172.80	163.77

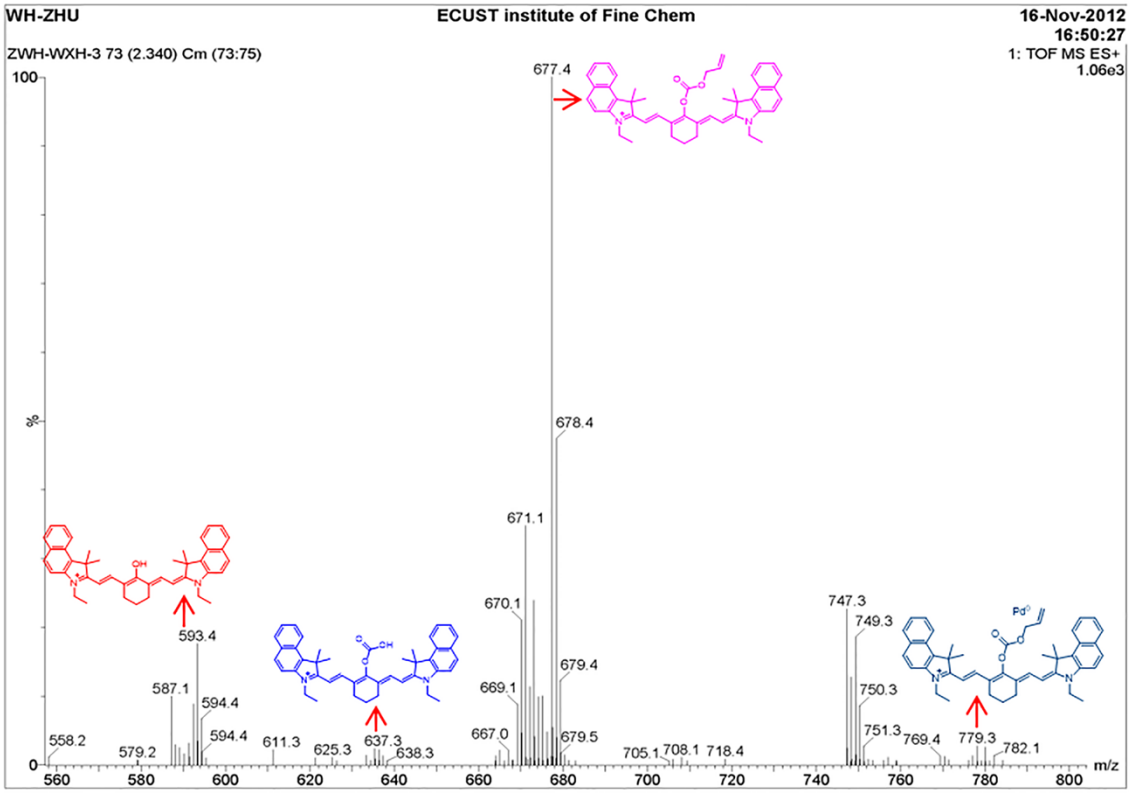


Fig. S7 Mass spectra of **Cy-1** in the presence of palladium.

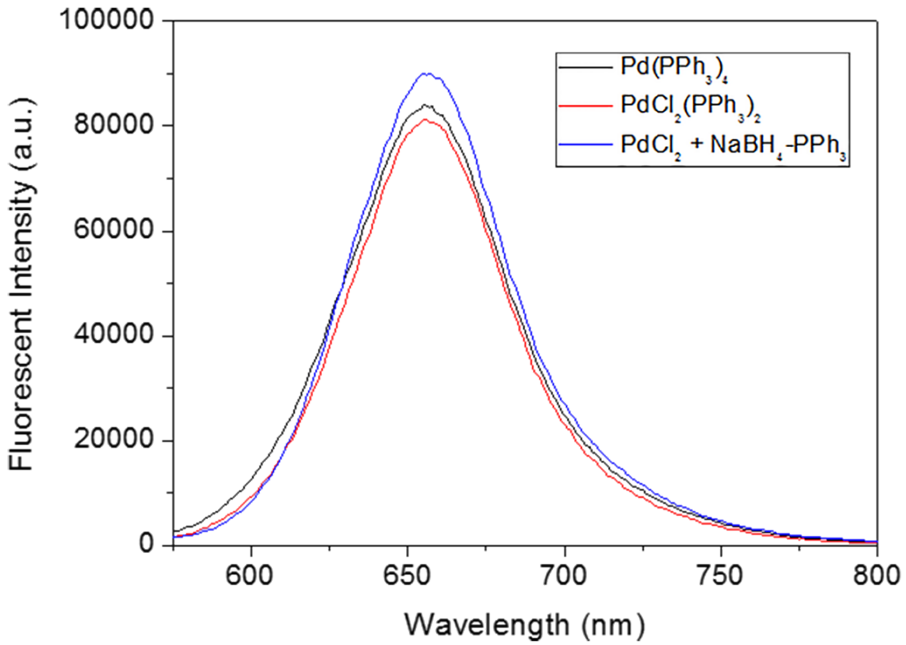
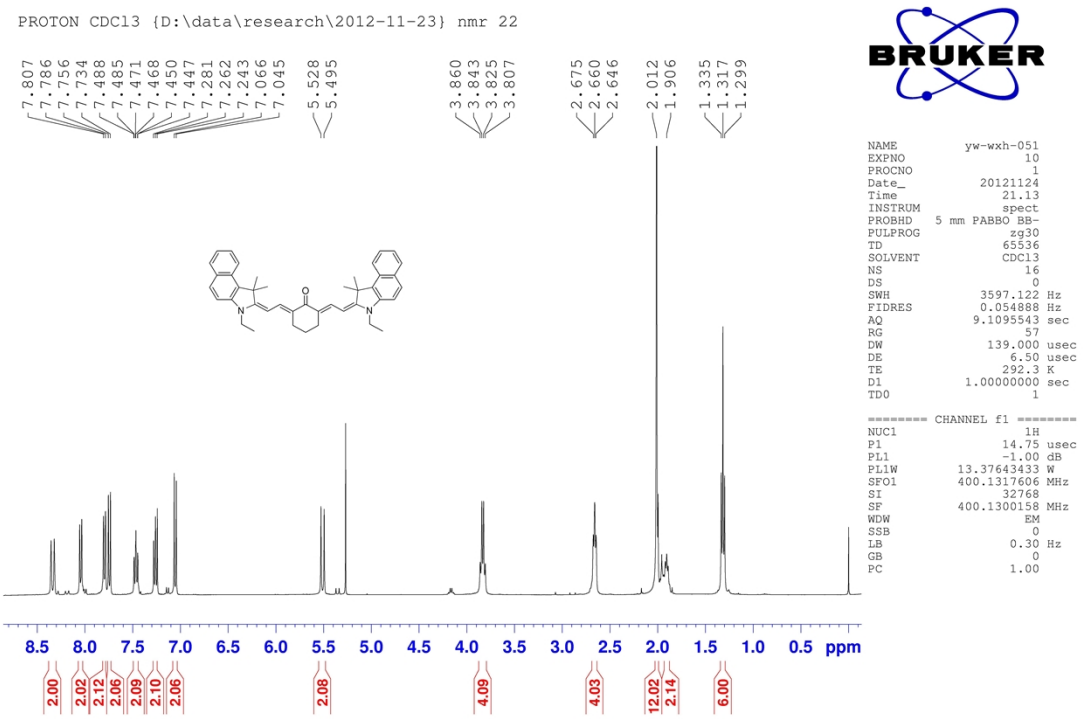


Fig. S8 Emission spectra of **Cy-1**(10 μM) in a mixture solution of CH_3CN : PBS (1 : 3, pH = 7.4, 0.01 M) with the addition different kinds of palladium species (50 μM).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7.447
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7.262
7.243
7.066
7.045
5.528
5.495
3.860
3.843
3.825
3.807
2.675
2.660
2.646
2.012
1.906
1.335
1.317
1.299
8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
2.00
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2.12
2.06
2.09
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2.08
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RG 57
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DE 6.50 usec
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TD0 1
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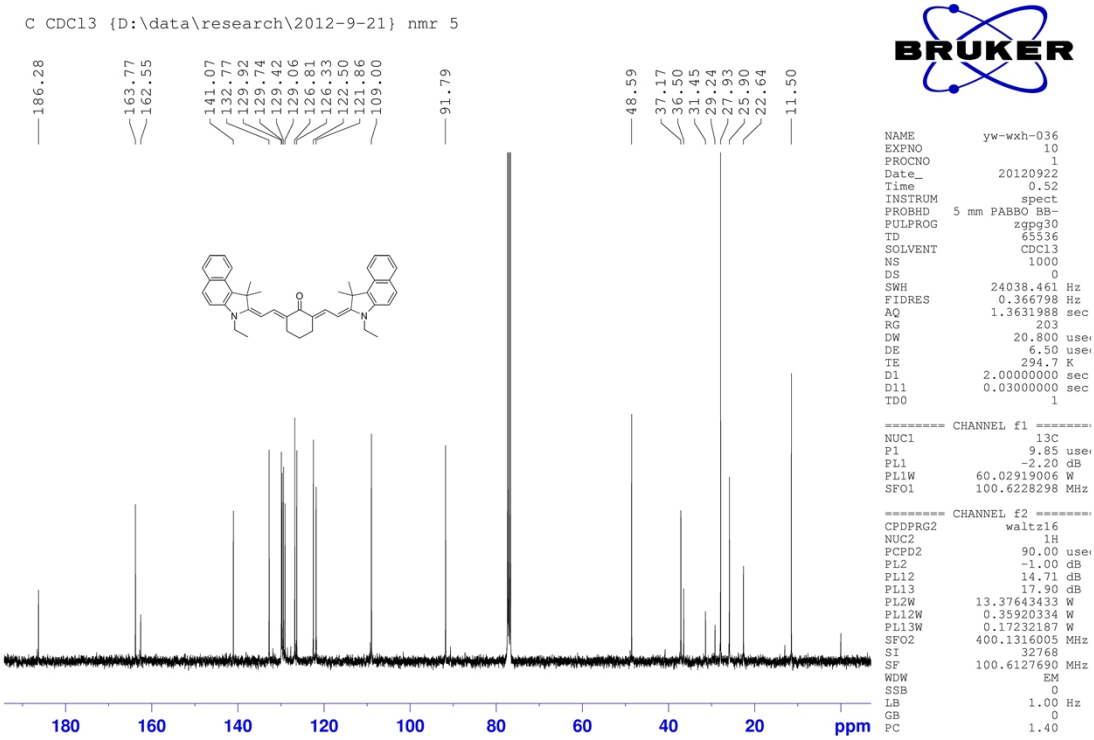
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129.06
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121.86
109.00
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27.93
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PL13 17.90 dB
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PL12W 0.35920334 W
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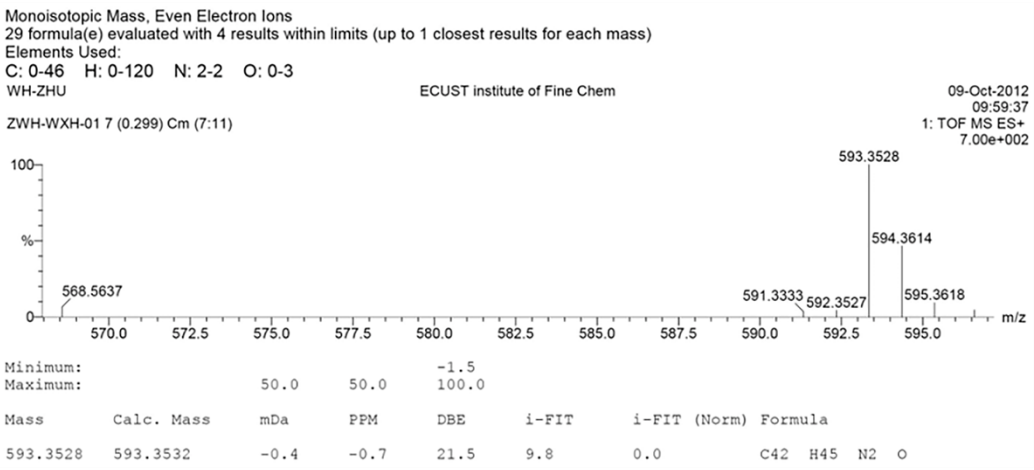

Fig. S9 ^{1}H and ^{13}C NMR spectra and HRMS of **CyK** in the presence of palladium.

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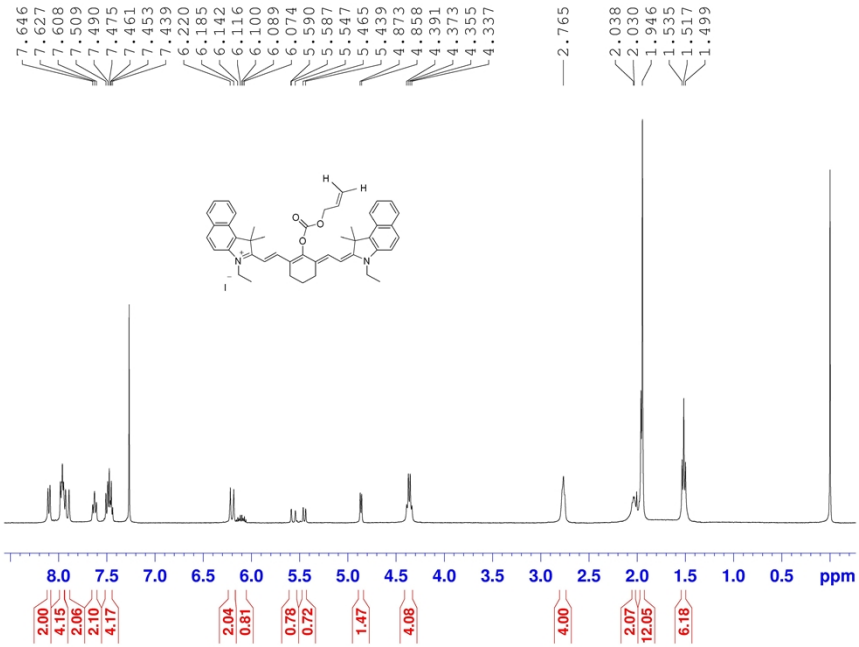

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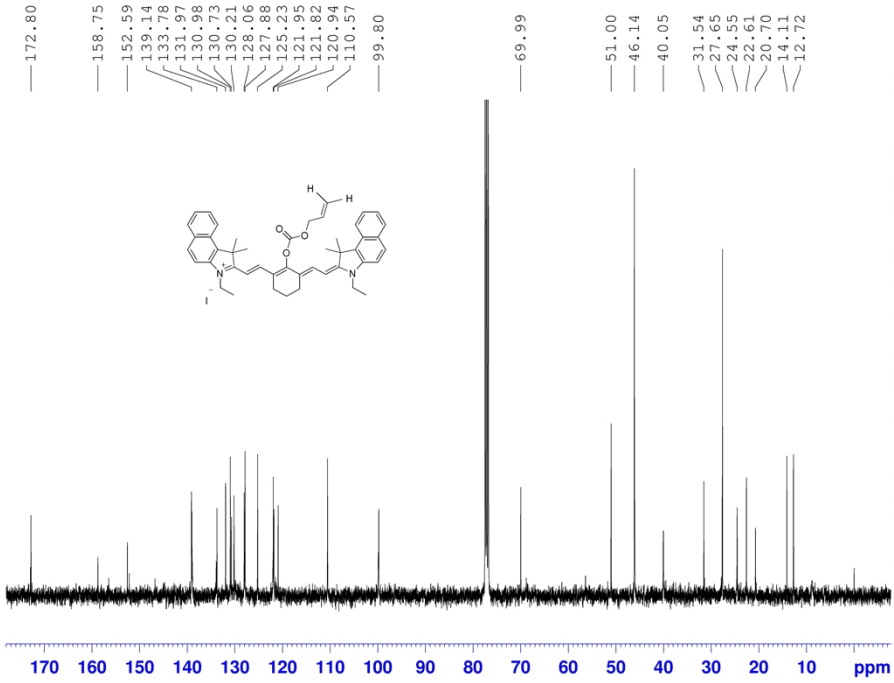

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PCPD2	90.00 usec
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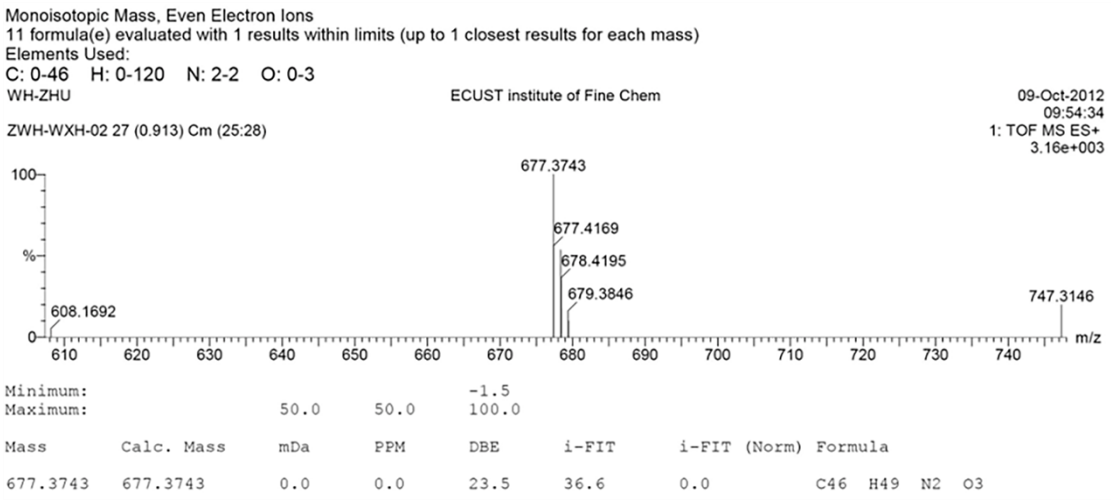

Fig. S10 ^{1}H and ^{13}C NMR spectra and HRMS of **Cy-1** in the presence of palladium.