Electronic Supporting Information

For

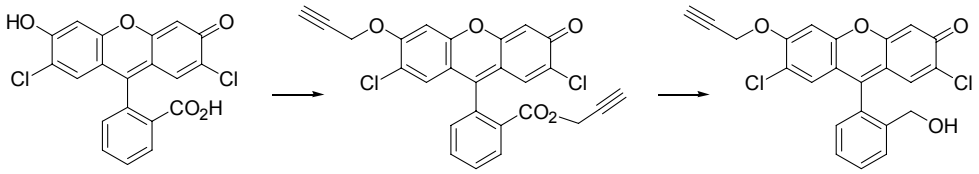
Fluorescent Detection of Palladium Species with an *O*-Propargylated Fluorescein

Mithun Santra,[a] Sung-Kyun Ko,[b] Injae Shin*[b] and Kyo Han Ahn*[a]

[a] *Department of Chemistry and Center for Electro-Photo Behaviors in Advanced Molecular Systems, Pohang University of Science and Technology (POSTECH), San 31, Hyoja-dong, Pohang, 790-784, Republic of Korea*
E-mail: ahn@postech.ac.kr

[b] *Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea*
E-mail: injae@yonsei.ac.kr

Synthesis of probe 1

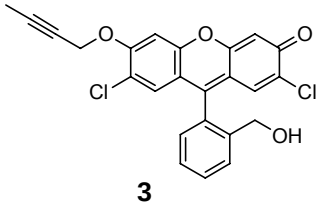


2-[2,7-Dichloro-3-oxo-6-(prop-2-ynyloxy)-3H-xanthen-9-yl]benzoic acid prop-2-ynyl ester (**2**). To a solution of fluorescein **3** (500 mg, 1.25 mmol) in DMF (10 mL) was added K_2CO_3 (430 mg, 3.1 mmol) portionwise at 25 °C under an argon atmosphere. To the mixture was added propargyl bromide (370 mg, 3.1 mmol) was added dropwise for 10 min. Then, the reaction mixture was heated to 60 °C for 6 h, and it was cooled to room temperature and poured into ice-water. The resulting precipitate was filtered and washed with water (100 mL) and then with hexane (100 mL). The dark orange solid was dried in atmosphere to give compound **2** (517 mg, 87%): mp 164–165 °C; ^{1}H NMR ($CDCl_3$, 300 MHz, 293 K): δ 8.34–8.37 (dd, *J* = 1.3, 7.7 Hz, 1H, Ar), 7.73–7.85 (m, 2H, Ar), 7.31–7.34 (dd, *J* = 1.3, 7.3 Hz, 1H, Ar), 7.22 (S, 1H, Ar), 7.03 (s, 1H, Ar), 6.96 (s, 1H, Ar), 6.6 (s, 1H, Ar), 4.92–4.93 (d, *J* = 2.4 Hz, 2H), 4.65 (m, 2H), 2.69 (t, *J* = 2.1 Hz, 1H), 2.37 (t, *J* = 2.4 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz, 293 K): δ 53.0, 57.4, 75.6, 76.5, 77.3, 77.9, 101.8, 106.0, 115.7,

118.3, 120.5, 127.3, 128.3, 129.5, 130.5, 130.6, 131.9, 133.6, 133.8, 135.7, 148.9, 152.2, 157.1, 157.9, 164.2, 177.9; HRMS: *m/z* calcd. for $C_{26}H_{14}O_5Cl_2$ $(M + H)^+$ 477.0297 found 477.0292.

2,7-Dichloro-9-[(2-hydroxymethyl)phenyl]-6-(prop-2-ynyloxy)xanthen-3-one (Probe **1**). To a solution of compound **2** (240 mg, 0.50 mmol) in CH_2Cl_2 (10 mL) was added a solution of DIBALH (2.01 mL, 1.0 M in hexanes) dropwise over 15 min at –78 °C under an argon atmosphere. The resulting solution was stirred for 10–15 min at the same temperature, and then it was warmed to 25 °C and stirred for 3 h. The reaction mixture was cooled to 0 °C, diluted with Et_2O (10 mL), and quenched with saturated aqueous NH_4Cl (2 mL). The solution was warmed to 25 °C and stirred for 1 h. The reaction mixture was then diluted with Et_2O (20 mL) and treated with DDQ (114 mg, 0.50 mmol) at 0 °C. After being stirred for 2 h at 25 °C, the mixture was filtered through a pad of Celite® and the pad was washed with EtOAc (50 mL). The filtrate was dried over Na_2SO_4, and the solvents were evaporated *in vacuo*. Silica gel flash chromatography of the residue (10 % EtOAc in hexanes) afforded probe **1** as a pale yellow solid (140 mg, 65%): mp 174–175 °C; ^{1}H NMR ($CDCl_3$, 300 MHz, 293 K): δ 7.39–7.42 (m, 2H, Ar), 7.3 (m, 1H, Ar), 6.92–6.93 (d, *J* = 2.5, 2H, Ar), 6.89 (s, 2H, Ar), 6.86 (s, 1H, Ar), 5.67 (s, 1H), 5.32 (s, 2H), 4.80–4.81 (d, *J* = 2.2 Hz, 2H), 2.59–2.61 (t, *J* = 2.3 Hz, 1H).^{13}C NMR ($CDCl_3$, 75 MHz, 293K): δ 57.3, 72.8, 77.1, 77.6, 83.3, 102.4, 104.0, 115.9, 118.5, 118.6, 121.4, 124.1, 129.0, 129.1, 129.3 (two carbon), 130.2, 139.0, 144.2, 149.8, 150.5, 152.3, 153.9; HRMS: m/z calcd. for $C_{23}H_{14}O_4Cl_2$ $(M + H)^+$ 425.0347 found 425.0344.

6-(but-2-ynyloxy)-2,7-dichloro-9-(2-(hydroxymethyl)phenyl)-3H-xanthen-3-one (3). This compound is synthesized similarly as above using 1-bromo-2-butyne. ^{1}H NMR ($CDCl_3$, 300 MHz, 293 K): δ 7.37–7.44 (m, 2H, Ar), 7.27-7.32 (m, 1H, Ar), 6.82–6.91 (m, 5H, Ar), 5.66 (s, 1H), 5.3 (s, 2H), 4.75-4.77 (dd, *J* = 4.54, 2.22, 2H), 1.88-1.89, (t, *J* = 2.29, 3H), ^{13}C NMR ($CDCl_3$, 75 MHz, 293K): δ 4.06, 57.8, 72.6, 73.2, 83.3, 85.3, 102.1, 103.8, 115.7, 117.9, 118.3, 118.5, 121.2, 123.9, 128.8, 128.9, 129, 129.8, 138.9, 144.1, 149.7, 150.4, 152.1, 154.



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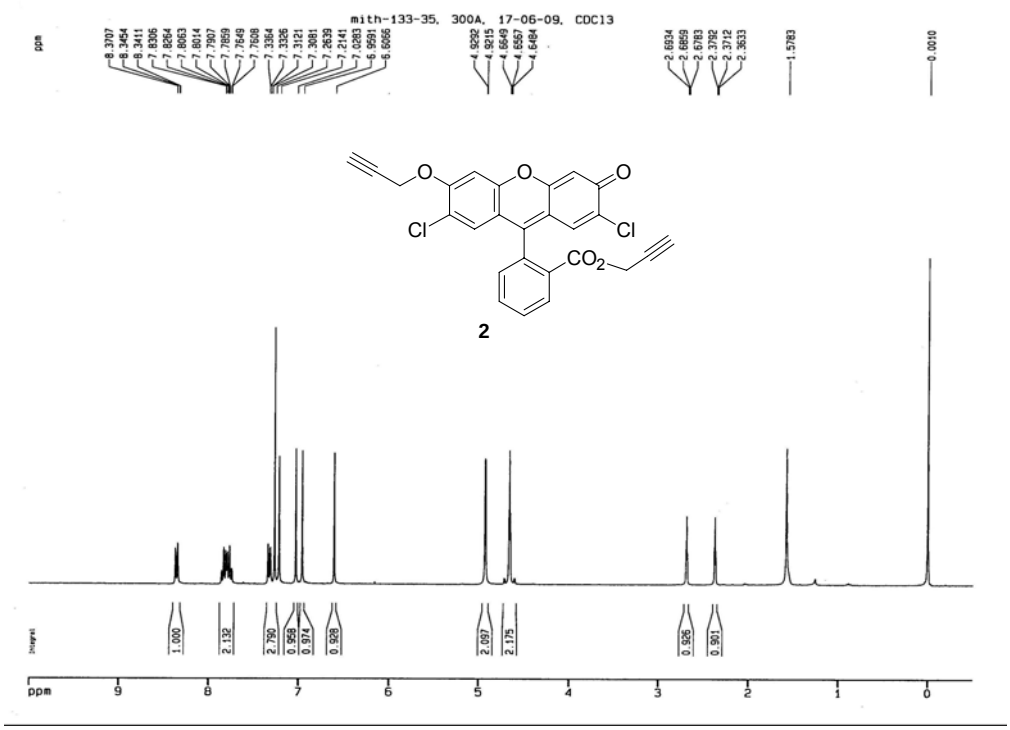
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ppm

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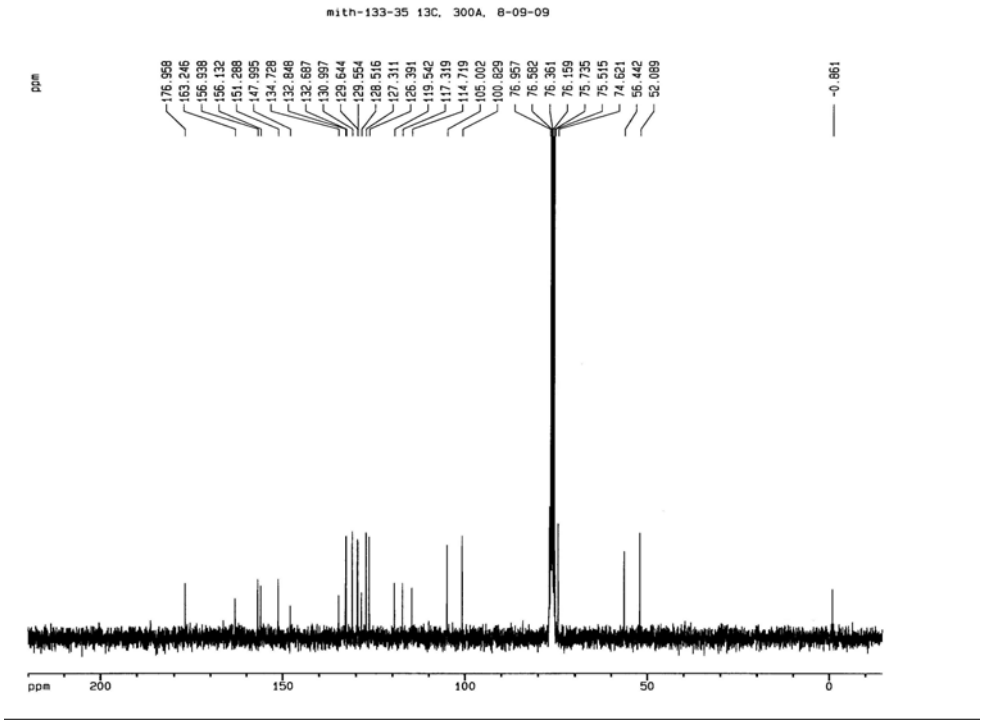
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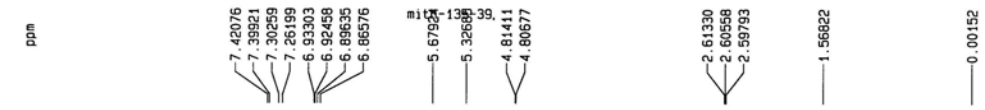
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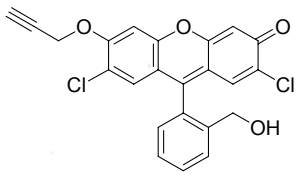
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Cl
Cl
OH

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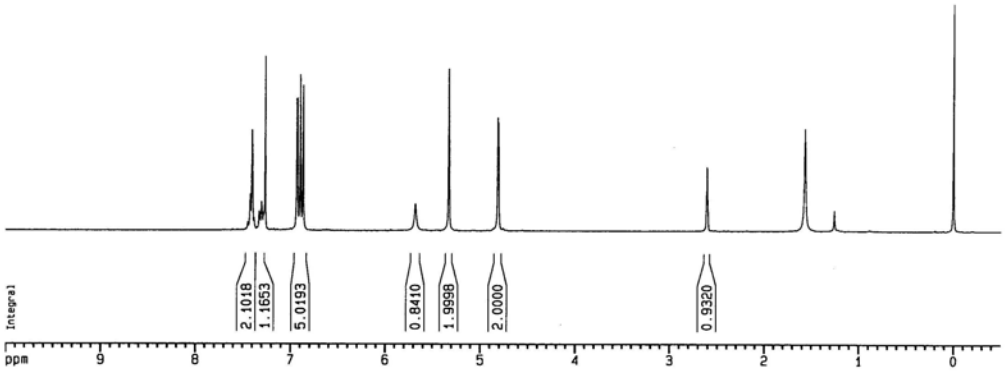
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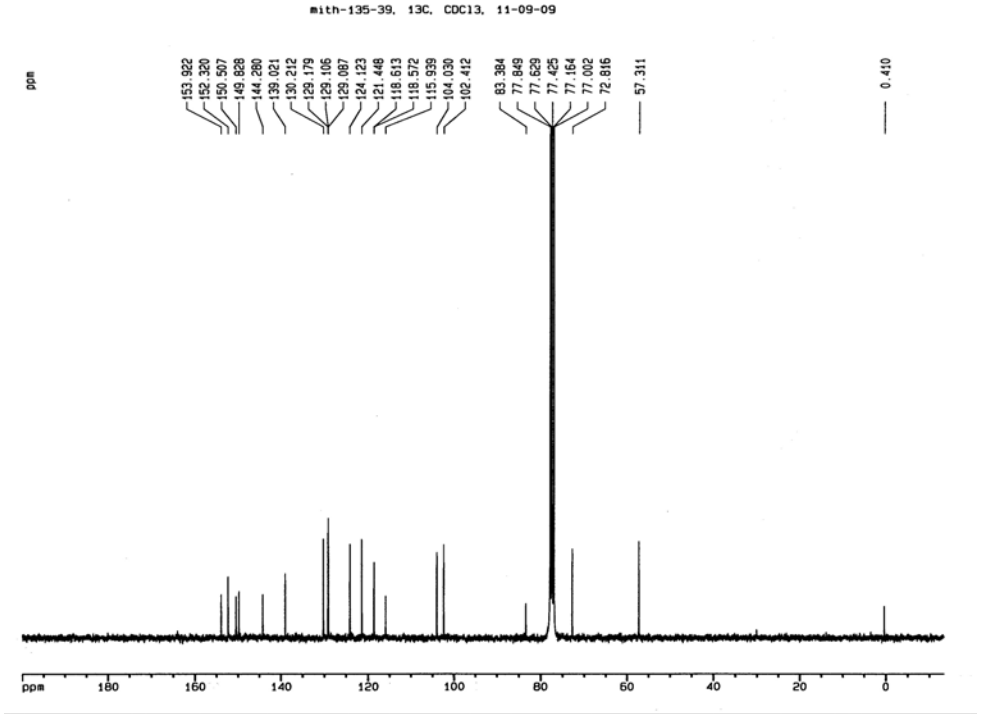
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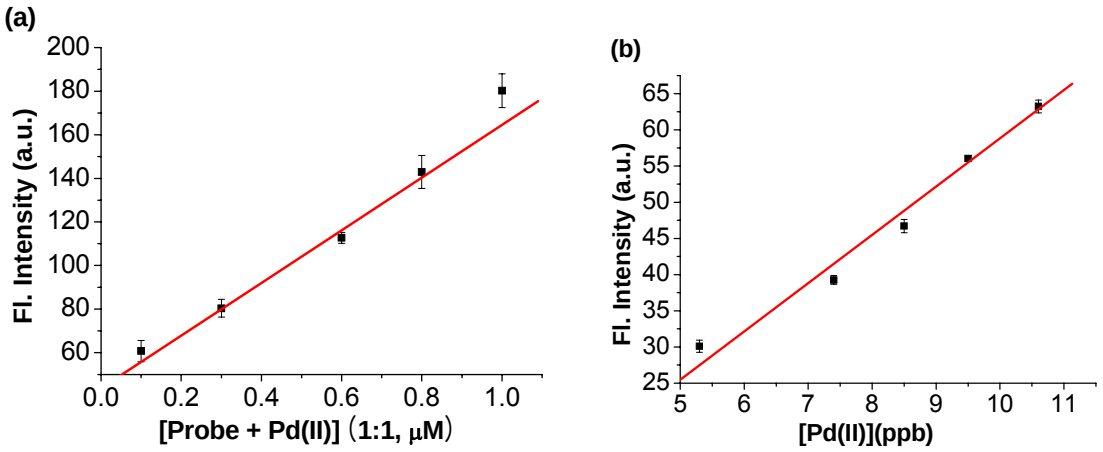

Fig. S1 Plots of fluorescence intensity of probe **1** depending on the concentration of $PdCl_2$ (a) in the range of 0.1 – 1.0 μM and (b) in the range of 5 – 12 ppb. Each measurement was done after 1 h of mixing for a 1:1 mixture of probe **1** and $PdCl_2$ in water containing 10% CH_3CN (excitation at 480 nm; the intensity was estimated by the peak height at λ = 520 nm).

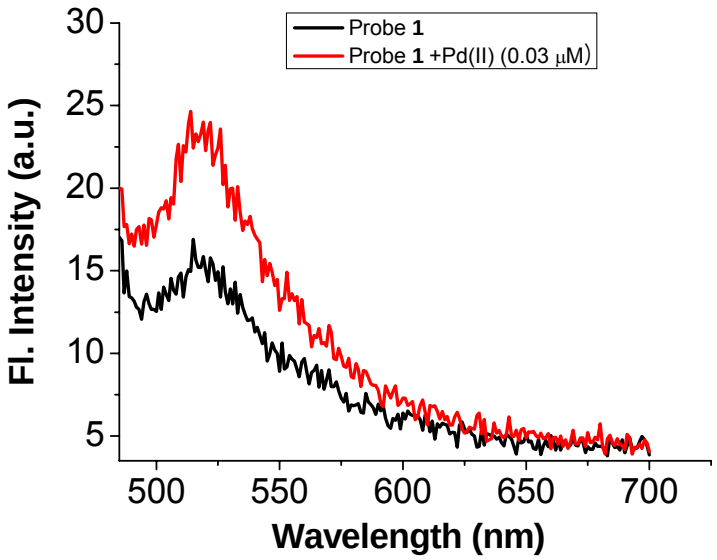

Fig. S2 Fluorescence spectra of probe **1** taken after 1 h upon addition of $PdCl_2$ (0.03 μM = 5.3 μg/L; in which Pd content is 3.2 ppb) in water containing 10% CH_3CN, which shows a signal-to-background ratio is more than three.

Table S1. Preparation of parent stock solutions of probe **1** and metal species used for the fluorescence study in water containing 10% CH_3CN.

Solution	Reagent	Quantity	Solvent (10 mL)	Conc.
P1	Probe **1**	4.25 mg (0.10 mmol)	CH_3CN	1.0 mM
A	$Pd(PPh_3)_4$	11.6 mg (10 μmol)	DMSO	1.0 mM
B	$PdCl_2$	9.0 mg (50 μmol)	3:1 brine/MeOH	5.0 mM
C	$PdCl_2(CH_3CN)_2$	2.6 mg (10 μmol)	3:1 brine/MeOH	1.0 mM
D	$Pd(OAc)_2$	2.2 mg (10 μmol)	3:1 brine/MeOH	1.0 mM
E	$(NH_4)_2PdCl_6$	17.7 mg (50 μmol)	1:1 DMSO/H_2O	5.0 mM
F	Na_2PdCl_6	3.0 mg (10 μmol)	3:1 brine/MeOH	1.0 mM

All other analytic metal solutions [Cr(II), Ca(II), Co(II), Cu(II), Ni(II), Mg(II), Hg(II), Mn(II), Cd(II), Ag(I), Ba(II), Zn(II), Pb(II), Pt(II), Fe(III), Cr(III), Fe(II), Pt(IV)] were prepared by separately dissolving each of the corresponding chloride salts in distilled water; 1.0 mM for Pt(IV) (H_2PtCl_6) and 5.0 mM for other cations. A 1.0 mM stock solution of $AuCl_3$ was prepared by dissolving in CH_3CN. For $PtCl_2$, 5.0 mM solution was prepared by dissolving in DMSO. In the cases of $RhCl_3$ and $RuCl_3$, 5.0 mM solution was prepared by dissolving each of them in 1:1 DMSO/CH_3CN and 1:1 DMSO/H_2O, respectively. A 1.0 mM solution of $RhCl(PPh_3)_3$ was prepared in DMF.

Experimental for the fluorescence imaging of zebrafish

Imaging of zebrafish incubated with $PdCl_2$ and probe 1. The five-day-old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10-5% methylene blue; pH 7.5). The zebrafish was incubated with 20 μM **1** in E3 embryo media for 30 min at 28 °C. After washing with E3 embryo media to remove the remaining probe, the zebrafish was further treated with 5, 10, 20 μM of $PdCl_2$ in E3 embryo media for 30 min at 28 °C. The zebrafish was imaged by fluorescence microscopy (Nikon Eclipse TE2000) (excitation at 480 nm).

Images of zebrafish organs treated with $PdCl_2$ and probe 1. An adult zebrafish (3 month-old with identifiable organs) was exposed to 500 nM $PdCl_2$ in E3 embryo media for 24 h at 28 °C, washed with E3 embryo media three times, and then incubated with 20 μM probe **1** for 30 min at 28 °C. The treated zebrafish was dissected to isolate tissues and organs that were then examined by using fluorescence microscopy. Fluorescence intensities of isolated tissues and organs were analyzed by using Image Pro Plus version 5.1 software.