Electronic Supplementary Material (ESI) for ChemComm.

SUPPORTING INFORMATION

Synthesis of High Contrast Fluorescein-Diethers for Rapid Bench-Top Sensing of Palladium

Weston R. Kitley, Peter J. Santa Maria, Ryan A. Cloyd, Laura M. Wysocki*

Janelia Research Campus, Howard Hughes Medical Institute, 19700 Helix Dr., Ashburn, VA 20147

Wabash College, 301 W. Wabash Ave., Crawfordsville, IN 47933

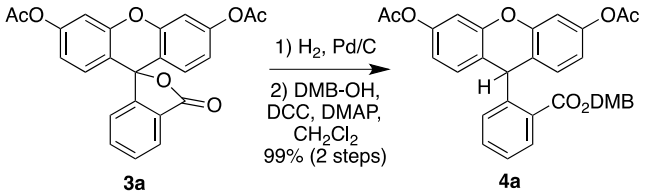
Email: wysockil@wabash.edu

S1: Experimental Information

Synthetic Methods

General Chemical Synthesis Methods. Compounds **3a-c** were synthesized as previously described.[1] All other reagents were the highest grade available and obtained from Sigma-Aldrich, Alfa Aesar, VWR International, or Fisher Scientific. Reactions were stirred under N_2(g) unless otherwise noted. Anhydrous solvents were drawn from Aldrich Sure-Seal bottles. Thin-layer chromatography was performed by using aluminum-backed plates coated with silica gel containing F_{254} phosphor and visualized by UV-illumination or charring.

Flash chromatography was performed on an Isolera system with SNAP columns (Biotage). The term "concentrated *in vacuo*" refers to the removal of solvents and other volatile materials by using a Büchi Rotavapor at variable pressure (controlled diaphragm pump; ≥1 mm Hg) while maintaining the water bath temperature below 40 °C. Mass spectrometry was performed by the Mass Spectrometry Center in the Department of Medicinal Chemistry at the University of Washington. NMR spectra were obtained with a JEOL ECX 400 MHz spectrometer at Wabash College. ^{1}H and ^{13}C NMR spectra were referenced to residual solvent peaks.

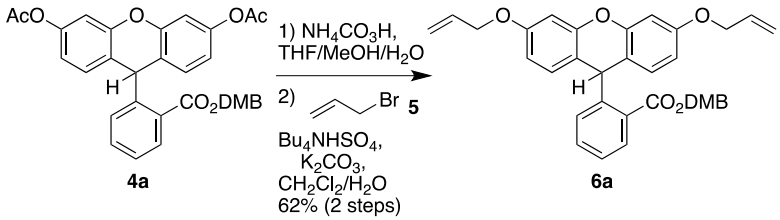


3a **4a**

Reduced fluorescein dimethoxybenzyl ester (4a). To a solution of fluorescein diacetate (**3a**, 153 mg, 0.367 mmol) in 15 mL EtOAc was added 10% w/w palladium on carbon (15 mg). The reaction flask was flushed thoroughly with nitrogen gas, followed by hydrogen gas. The reaction was stirred for 18 h at room temperature under 1 atm of hydrogen gas (balloon), after which time, the reaction flask was thoroughly purged with nitrogen gas. The crude reaction mixture was filtered through celite, rinsed with EtOAc (40 mL), and concentrated *in vacuo*, affording a white solid.

To a solution of the crude reduced fluorescein derivative in 6 mL CH_2Cl_2 was added *N*,*N'*-dicyclohexylcarbodiimide (DCC, 163.5 mg, 0.793 mmol, 2.2 eq), 4-dimethylaminopyridine (DMAP, 0.9 mg, 7.2 μmol, 0.02 eq), and 2,4-dimethoxybenzyl alcohol (DMB-OH, 133.3 mg, 0.793 mmol, 2.2 eq). The reaction was stirred at room temperature for 18 h. The mixture was deposited onto celite and purified by column chromatography (hexanes→2:3 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH_2Cl_2 additive), affording 207.3 mg of pure ester **4a** as a white foamy solid (99% yield, 2 steps).

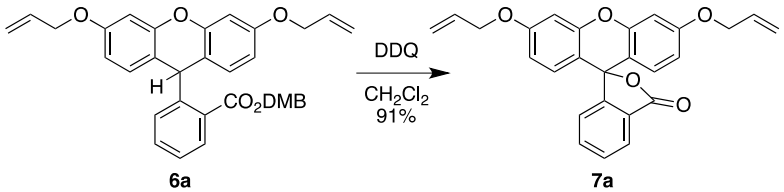
Data for **4a**: $\mathbf{R}_f$ = 0.60 (3:1:1 v/v/v hexanes/EtOAc/CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 2.29 (s, 6H, -COC**H**$_3$), 3.72 (s, 3H, -OC**H**$_3$), 3.82 (s, 3H-OC**H**$_3$), 5.40 (s, 2H, -OC**H**$_2$Ar), 6.29 (s, 1H, Ar$_3$C**H**), 6.43 (d, *J* = 2.3 Hz, 1H, Ar-**H**), 6.47 (dd, *J* = 8.3, 2.4 Hz, 1H, Ar-**H**), 6.64 (dd, *J* = 8.4, 2.4 Hz, 2H, Ar-**H**), 6.87 (d, *J* = 2.4, Hz, 2H, Ar-**H**), 6.98 (dd, *J* = 8.4, 0.7 Hz, 2H, Ar-**H**), 7.06 (dd, *J* = 7.9, 0.9 Hz, 1H, Ar-**H**), 7.19 (td, *J* = 7.8, 1.3 Hz, 1H, Ar-**H**), 7.30 (td, *J* = 7.6, 1.5 Hz, 1H, Ar-**H**), 7.34 (d, *J* = 8.3 Hz, Ar-**H**), 7.78 (dd, *J* = 7.8, 1.2 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 21.1, 24.7, 25.4, 34.9, 37.9, 55.3, 55.4, 55.8, 63.0, 98.4, 104.0, 109.7, 116.2, 116.7, 122.1, 126.4, 129.4, 130.2, 130.7, 131.7, 131.8, 132.4, 146.6, 149.8, 150.9, 159.2, 161.6, 168.5, 169.2.



Bis(allyl) leuco-fluorescein (6a). To a solution of triester **4a** (207.3 mg, 0.365 mmol) in 8 mL THF and 4 mL MeOH was added ammonium bicarbonate (288.2 mg, 3.65 mmol, 10 eq) as a solution in 4 mL H_2O. The reaction was stirred vigorously (~600 RPM) at room temperature for 60 h while shielded from light. The organic solvents were concentrated *in vacuo* and the pH was adjusted to ~3 with 10% w/v aqueous citric acid. The aqueous layer was diluted to 60 mL with H_2O and extracted with EtOAc (3x60 mL). The organic layers were combined, dried over anhydrous $MgSO_4$(s), filtered, and concentrated *in vacuo*. Purification by column chromatography (1:4 v/v EtOAc/hexanes→3:1 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH_2Cl_2 additive), afforded a white solid that turns yellow upon prolonged exposure to air with $\mathbf{R}_f$ = 0.20 (6:2:2 v/v/v hexanes/EtOAc/CH_2Cl_2).

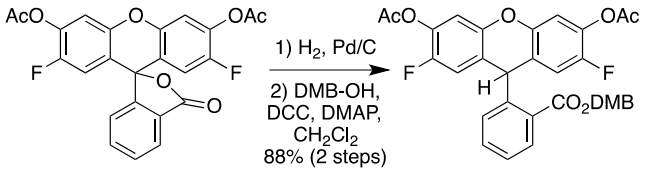
To a solution of the diphenol intermediate in 8 mL CH_2Cl_2 and 8 mL H_2O was added tetrabutylammonium hydrogensulfate (243.6 mg, 0.717 mmol, 2 eq), potassium carbonate (297.5 mg, 2.15 mmol, 6 eq), and allyl bromide (**5**, 186 μL, 2.15 mmol, 6 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The aqueous layer was diluted to 40 mL H_2O and was extracted with CH_2Cl_2 (3x60 mL). The organic layers were combined, dried over anhydrous $MgSO_4$(s), filtered, and concentrated *in vacuo*. Purification by column chromatography (hexanes→3:2 v/v hexanes/EtOAc, linear gradient) afforded pure diether **6a** as a white foamy solid (126.1 mg, 62% yield over 2 steps).

Data for **6a**: $\mathbf{R}_f$ = 0.24 (4:1 v/v hexanes/acetone); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 3.72 (s, 3H, -OC**H**$_3$), 3.81 (s, 3H, -OC**H**$_3$), 4.51 (dt, *J* = 5.3, 1.5 Hz, 4H, -OC**H**$_2$CHCH$_2$), 5.29 (dq, *J* = 10.5, 1.4 Hz, 2H, -OCH$_2$CHC**H**H), 5.40 (s, 2H, -OC**H**$_2$Ar), 5.42 (m, 2H, -OCH$_2$CHCH**H**) 6.05 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 2H, -OCH$_2$C**H**CH$_2$), 6.15 (s, 1H, Ar$_3$C**H**), 6.48 (m, 4H, Ar-**H**), 6.64 (d, *J* = 2.6 Hz, 2H, Ar-**H**), 6.89 (d, *J* = 8.6 Hz, 2H, Ar-**H**), 7.04 (dd, *J* = 7.8, 1.1 Hz, 1H, Ar-**H**), 7.15 (td, *J* = 7.8, 1.1 Hz, 1H, Ar-**H**), 7.27 (m, 1H, Ar-**H**), 7.35 (d, *J* = 8.3, 1H, Ar-**H**), 7.73 (dd, *J* = 7.8, 1.4, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 55.3, 55.4, 63.0, 68.9, 98.4, 101.8, 103.9, 110.8, 116.4, 117.2, 117.8, 125.9, 129.2, 130.1, 130.7, 131.4, 131.8, 132.1, 133.1, 147.9, 151.3, 158.1, 159.2, 161.5, 168.8.



Bis(allyl ether) fluorescein (7a). To a solution of diether **6a** (126.1 mg, 0.223 mmol) in 9 mL CH_2Cl_2 and 1 mL H_2O was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 152.1 mg, 0.670 mmol, 3 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The resulting mixture was filtered through a pad of celite and concentrated *in vacuo*. Purification by column chromatography (hexanes→7:3 v/v hexanes/acetone, linear gradient) afforded pure fluorescein diether **7a** as a white foamy solid (84.1 mg, 91% yield).

Data for **7a**: $\mathbf{R}_f$ = 0.19 (4:1 v/v hexanes/acetone); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 4.56 (dt, *J* = 5.3, 1.5 Hz, 4H, -OC**H**$_2$CHCH$_2$), 5.31 (dq, *J* = 10.5, 1.4 Hz, 2H, -OCH$_2$CHC**H**H), 5.43 (dq, *J* = 17.3, 1.5 Hz, 2H, -OCH$_2$CHCH**H**), 6.04 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 2H, -OCH$_2$C**H**CH$_2$), 6.63 (A**B**X, J_{AB} = 8.8, J_{BX} = 2.5 Hz, 2H, Ar-**H**), 6.69 (**A**BX, J_{AB} = 8.8 Hz, J_{AX} = 0 Hz, 2H, Ar-**H**), 6.78 (d, *J* = 2.4, 2H, Ar-**H**), 7.17 (dt, *J* = 7.5, 0.9 Hz, 1H, Ar-**H**), 7.61 (td, *J* = 7.4, 1.0 Hz, 1H, Ar-**H**), 7.67 (td, *J* = 7.4, 1.3 Hz, 1H, Ar-**H**), 8.03 (ddd, *J* = 7.4, 1.3, 0.9 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 69.0 (CH_2), 83.1 (C), 101.7 (CH), 111.3 (C), 112.1 (CH), 118.1 (CH_2), 123.9 (CH), 124.9 (CH), 126.8 (C), 129.0 (CH), 129.6 (CH), 132.5 (CH), 134.9 (CH), 152.4 (C), 153.0 (C), 160.2 (C), 169.3 (C); **HRMS** (*m*/*z*): $[M+H]^+$ calculated, 413.1389; observed, 413.1390.

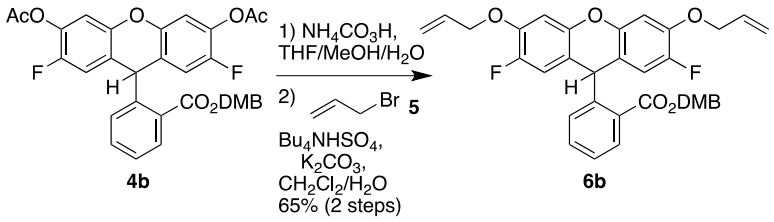


3b **4b**

Reduced 2',7'-difluorofluorescein dimethoxybenzyl ester (4b). To a solution of 2',7'-difluorofluorescein diacetate (**3b**, 106.3 mg, 0.235 mmol) in 10 mL EtOAc was added 10% w/w palladium on carbon (10.6 mg). The reaction flask was flushed thoroughly with nitrogen gas, followed by hydrogen gas. The reaction was stirred for 18 h at room temperature under 1 atm of hydrogen gas (balloon), after which time, the reaction flask was thoroughly purged with nitrogen gas. The crude reaction mixture was filtered through celite, rinsed with EtOAc (30 mL), and concentrated *in vacuo*, affording a crude white solid.

To a solution of the crude reduced fluorescein derivative in 4 mL CH_2Cl_2 was added *N*,*N'*-dicyclohexylcarbodiimide (DCC, 106.7 mg, 0.517 mmol, 2.2 eq), 4-dimethylaminopyridine (DMAP, 0.6 mg, 4.7 μmol, 0.02 eq), and 2,4-dimethoxybenzyl alcohol (DMB-OH, 87.0 mg, 0.517 mmol, 2.2 eq). The reaction was stirred at room temperature for 18 h. The mixture was deposited onto celite and purified by column chromatography (1:19 v/v EtOAc/hexanes→2:3 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH_2Cl_2 additive), affording 125.4 mg of pure ester **4b** as a white foamy solid (88% yield, 2 steps).

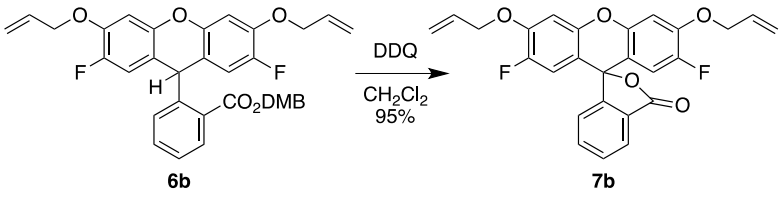
Data for **4b**: $\mathbf{R}_f$ = 0.56 (3:1:1 v/v/v hexanes/EtOAc/CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 2.33 (s, 6H, -COC**H**$_3$), 3.76 (s, 3H, -OC**H**$_3$), 3.82 (s, 3H, -OC**H**$_3$), 5.42 (s, 2H, -OC**H**$_2$Ar), 6.26 (s, 1H, Ar$_3$C**H**), 6.45 (d, *J* = 2.3 Hz, 1H, Ar-**H**), 6.49 (dd, *J* = 8.2, 2.4 Hz, 1H, Ar-**H**), 6.80 (d, $^3J_{HF}$ = 10.4 Hz, 2H, Ar-**H**), 6.90 (d, $^4J_{HF}$ = 6.5 Hz, 2H, Ar-**H**), 7.06 (dd, *J* = 7.9, 0.9 Hz, 1H, Ar-**H**), 7.25 (td, *J* = 7.6, 1.2 Hz, 1H, Ar-**H**), 7.35 (m, 2H, Ar-**H**), 7.83 (dd, *J* = 7.8, 1.4 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 20.4 (CH_3), 55.3 (CH_3), 55.3 (CH_3), 63.1 (CH_2), 98.5 (CH), 104.1 (CH), 111.6 (CH), 116.0 (C), 116.8 (d, $^2J_{CF}$ = 21.0 Hz, CH), 122.1 (d, $^3J_{CF}$ = 6.6 Hz, C), 126.9 (CH), 129.8 (CH), 130.0 (C), 131.5 (CH), 131.9 (CH), 132.6 (CH), 137.2 (d, $^2J_{CF}$ = 14.8 Hz, C), 145.4 (C), 146.3 (d, $^4J_{CF}$ = 2.3 Hz, C), 150.0 (d, $^1J_{CF}$ = 245 Hz, C), 159.2 (C), 161.6 (C), 168.0 (C), 168.1 (C); **HRMS** (*m*/*z*): $[M+Na]^+$ calculated, 627.1443; observed, 627.1427.



Bis(allyl) leuco-2',7'-difluorofluorescein (6b). To a solution of triester **4b** (79.2 mg, 0.131 mmol) in 4 mL THF and 2 mL MeOH was added ammonium bicarbonate (103.5 mg, 1.31 mmol, 10 eq) as a solution in 2 mL H_2O. The reaction was stirred vigorously (~600 RPM) at room temperature for 18 h while shielded from light. The organic solvents were concentrated *in vacuo* and the pH was adjusted to ~3 with 10% w/v aqueous citric acid. The aqueous layer was diluted to 20 mL with H_2O and extracted with EtOAc (3x20 mL). The organic layers were combined, dried over anhydrous $MgSO_4$(s), filtered, and concentrated *in vacuo*. Purification by column chromatography (1:4 v/v EtOAc/hexanes→13:7 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH_2Cl_2 additive), afforded a white solid that turns yellow upon prolonged exposure to air with $\mathbf{R}_f$ = 0.18 (3:1:1 v/v/v hexanes/EtOAc/CH_2Cl_2).

To a solution of the diphenol intermediate in 3 mL CH_2Cl_2 and 3 mL H_2O was added tetrabutylammonium hydrogensulfate (75.9 mg, 0.224 mmol, 2 eq), potassium carbonate (92.7 mg, 0.671 mmol, 6 eq), and allyl bromide (**5**, 58 μL, 0.67 mmol, 6 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The aqueous layer was diluted to 10 mL H_2O and was extracted with CH_2Cl_2 (3x20 mL). The organic layers were combined, dried over anhydrous $MgSO_4$(s), filtered, and concentrated *in vacuo*. Purification by column chromatography (hexanes→1:1 v/v hexanes/EtOAc, linear gradient) afforded pure diether **6b** as a white foamy solid (50.9 mg, 65% yield over two steps).

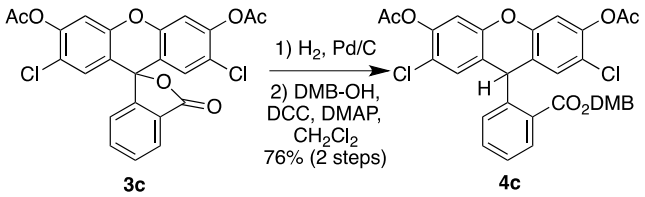
Data for **6b**: $\mathbf{R}_f$ = 0.37 (4:1 v/v hexanes/EtOAc); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 3.77 (s, 3H, -OC**H**$_3$), 3.82 (s, 3H, -OC**H**$_3$), 4.59 (dt, *J* = 5.4, 1.4 Hz, 4H, -OC**H**$_2$CHCH$_2$), 5.33 (dq, *J* = 10.5, 1.4 Hz, 2H, -OCH$_2$CHC**H**H), 5.42 (s, 2H, -OC**H**$_2$Ar), 5.45 (dq, *J* = 17.3, 1.5 Hz, 2H, -OCH$_2$CHCH**H**), 6.07 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 2H, -OCH$_2$C**H**CH$_2$), 6.12 (s, 1H, Ar$_3$C**H**), 6.45 (d, *J* = 2.3 Hz, 1H, Ar-**H**), 6.49 (dd, *J* = 8.3, 2.3 Hz, 1H, Ar-**H**), 6.68 (d, $^4J_{HF}$ = 7.3 Hz, 2H, Ar-**H**), 6.72 (d, $^3J_{HF}$ = 11.5 Hz, 2H, Ar-**H**), 7.03 (dd, *J* = 7.9, 1.0 Hz, 1H, Ar-**H**), 7.20 (td, *J* = 7.6, 1.3 Hz, 1H, Ar-**H**), 7.31 (td, *J* = 7.6, 1.4 Hz, 1H, Ar-**H**), 7.37 (d, *J* = 8.3 Hz, 1H, Ar-**H**), 7.79 (dd, *J* = 7.8, 1.4 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 55.3 (CH_3), 55.4 (CH_3), 63.1 (CH_2), 70.1 (CH_2), 98.5 (CH), 103.0 (CH), 103.0 (CH), 104.0 (CH), 115.9 (d, $^3J_{CF}$ = 6.4 Hz, C), 116.2 (C), 116.3 (d, $^2J_{CF}$ = 20.5 Hz, CH), 118.4 (CH_2), 126.4 (CH), 129.6 (CH), 129.9 (C), 131.2 (CH), 131.8 (CH), 132.3 (CH), 132.5 (CH), 146.0 (d, $^2J_{CF}$ = 12.4 Hz, C), 146.4 (d, $^4J_{CF}$ = 2.3 Hz, C), 146.7 (C), 148.7 (d, $^1J_{CF}$ = 242 Hz, C), 159.2 (C), 161.6 (C), 168.3 (C).



Bis(allyl ether) 2',7'-difluorofluorescein (7b). To a solution of diether **6b** (46.2 mg, 0.0773 mmol) in 4.5 mL CH_2Cl_2 and 0.5 mL H_2O was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 52.6 mg, 0.232 mmol, 3 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The resulting mixture was filtered

through a pad of celite and concentrated *in vacuo*. Purification by column chromatography (hexanes→7:3 v/v hexanes/acetone, linear gradient) afforded pure 2',7'-difluorofluorescein diether **7b** as a white foamy solid (32.9 mg, 95% yield).

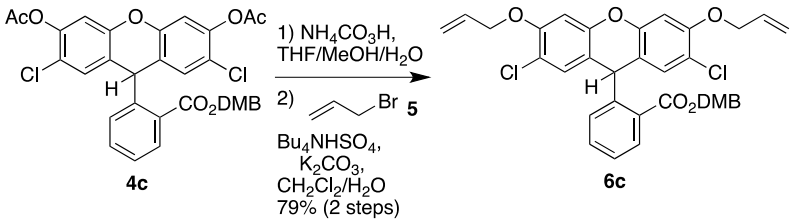
Data for **7b**: $\mathbf{R}_f$ = 0.20 (4:1 v/v hexanes/acetone); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 4.65 (d, *J* = 5.3 Hz, 4H, -OC**H**$_2$CHCH$_2$), 5.36 (dq, *J* = 10.5, 1.3 Hz, 2H, -OCH$_2$CHC**H**H), 5.47 (dq, *J* = 17.3, 1.4 Hz, 2H, -OCH$_2$CHCH**H**), 6.06 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 2H, -OCH$_2$C**H**CH$_2$), 6.45 (d, $^3J_{HF}$ = 11.1 Hz, 2H, Ar-**H**), 6.82 (d, $^4J_{HF}$ = 7.1 Hz, 2H, Ar-**H**), 7.17 (dd, *J* = 7.3, 0.9 Hz, 1H, Ar-**H**), 7.66 (td, *J* = 7.4, 1.1 Hz, 1H, Ar-**H**), 7.71 (td, *J* = 7.4, 1.3 Hz, 1H, Ar-**H**), 8.04 (dd, *J* = 7.5, 0.9 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 70.1 (CH_2), 82.4 (C), 102.8 (d, $^3J_{CF}$ = 1.6 Hz, CH), 109.7 (d, $^3J_{CF}$ = 6.1 Hz, C), 113.9 (d, $^2J_{CF}$ = 21.0 Hz, CH), 118.8 (CH_2), 123.8 (CH), 125.3 (CH), 126.5 (C), 130.2 (CH), 131.9 (CH), 135.3 (CH), 147.8 (d, $^4J_{CF}$ = 2.3 Hz, C), 148.7 (d, $^2J_{CF}$ = 12.6 Hz, C), 151.1 (d, $^1J_{CF}$ = 190 Hz, C), 168.7 (C); **HRMS** (*m*/*z*): $[M+H]^+$ calculated, 449.1201; observed, 449.1212.



Reduced 2',7'-dichlorofluorescein dimethoxybenzyl ester (4c). To a solution of 2',7'-dichlorofluorescein diacetate (**3c**, 150. mg, 0.309 mmol) in 8 mL EtOAc was added 10% w/w palladium on carbon (15 mg). The reaction flask was flushed thoroughly with nitrogen gas, followed by hydrogen gas. The reaction was stirred for 18 h at room temperature under 1 atm of hydrogen gas (balloon), after which time, the reaction flask was thoroughly purged with nitrogen gas. The crude reaction mixture was filtered through celite, rinsed with EtOAc (30 mL), and concentrated *in vacuo*, affording a crude white solid.

To a solution of the crude reduced fluorescein derivative in 6 mL CH_2Cl_2 was added *N*,*N'*-dicyclohexylcarbodiimide (DCC, 140.3 mg, 0.680 mmol, 2.2 eq), 4-dimethylaminopyridine (DMAP, 0.8 mg, 6.2 μmol, 0.02 eq), and 2,4-dimethoxybenzyl alcohol (DMB-OH, 114.4 mg, 0.680 mmol, 2.2 eq). The reaction was stirred at room temperature for 18 h. The mixture was deposited onto celite and purified by column chromatography (hexanes→2:3 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH_2Cl_2 additive), affording 149.4 mg of pure ester **4c** as a white foamy solid (76% yield, 2 steps).

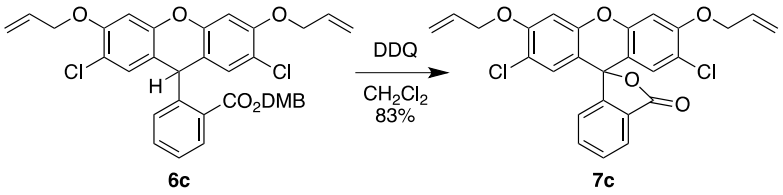
Data for **4c**: $\mathbf{R}_f$ = 0.67 (3:1:1 v/v/v hexanes/EtOAc/CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 2.34 (s, 6H, -COC**H**$_3$), 3.74 (s, 3H, -OC**H**$_3$), 3.82 (s, 3H, -OC**H**$_3$), 5.41 (s, 2H, -OC**H**$_2$Ar), 6.27 (s, 1H, Ar$_3$C**H**), 6.44 (d, *J* = 2.1 Hz, Ar-**H**), 6.49 (dd, *J* = 8.3, 2.4 Hz, 1H, Ar-**H**), 6.93 (s, 2H, Ar-**H**), 7.05 (m, 3H, Ar-**H**), 7.27 (m, 1H, Ar-**H**), 7.37 (m, 2H, Ar-**H**), 7.85 (dd, *J* = 7.8, 1.2 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 20.6 (CH_3), 55.4 (CH_3), 63.2 (CH_2), 98.5 (CH), 104.2 (CH), 111.9 (CH), 116.0 (C), 121.3 (C), 123.1 (C), 127.0 (CH), 130.0 (C), 130.1 (C), 130.7 (CH), 131.6 (C), 131.8 (CH), 132.8 (CH), 146.1 (C), 149.2 (C), 159.1 (C), 161.6 (C), 168.1 (C), 168.2 (C).



Bis(allyl) leuco-2',7'-dichlorofluorescein (6c). To a solution of triester **4c** (149.4 mg, 0.234 mmol) in 8 mL THF and 4 mL MeOH was added ammonium bicarbonate (185.2 mg, 2.34 mmol, 10 eq) as a solution in 4 mL H_2O. The reaction was stirred vigorously (~600 RPM) at room temperature for 18 h while shielded from light. The organic solvents were concentrated *in vacuo* and the pH was adjusted to ~3 with 10% w/v aqueous citric acid. The aqueous layer was diluted to 30 mL with H_2O and extracted with EtOAc (3x30 mL). The organic layers were combined, dried over anhydrous $MgSO_4$(s), filtered, and concentrated *in vacuo*. Purification by column chromatography (1:4 v/v EtOAc/hexanes→3:1 v/v EtOAc/hexanes, linear gradient, with constant 20% v/v CH_2Cl_2 additive), afforded a white solid that turns yellow upon prolonged exposure to air with $\mathbf{R}_f$ = 0.23 (3:1:1 v/v/v hexanes/EtOAc/CH_2Cl_2).

To a solution of the diphenol intermediate in 6 mL CH_2Cl_2 and 6 mL H_2O was added tetrabutylammonium hydrogensulfate (159.2 mg, 0.469 mmol, 2 eq), potassium carbonate (194.3 mg, 1.41 mmol, 6 eq), and allyl bromide (**5**, 122 μL, 1.41 mmol, 6 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The aqueous layer was diluted to 20 mL H_2O and was extracted with CH_2Cl_2 (3x30 mL). The organic layers were combined, dried over anhydrous $MgSO_4$(s), filtered, and concentrated *in vacuo*. Purification by column chromatography (hexanes→2:3 v/v EtOAc/hexanes, linear gradient) afforded pure diether **6c** as a white foamy solid (121.6 mg, 79% yield over two steps).

Data for **6c**: $\mathbf{R}_f$ = 0.68 (3:1:1 v/v/v hexanes/EtOAc/CH_2Cl_2); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 3.76 (s, 3H, -OC**H**$_3$), 3.82 (s, 3H, -OC**H**$_3$), 4.61 (d, *J* = 4.9 Hz, 4H, -OC**H**$_2$CHCH$_2$), 5.34 (dq, *J* = 10.6, 1.4 Hz, 2H, -OCH$_2$CHC**H**H), 5.42 (s, 2H, -OC**H**$_2$Ar), 5.49 (dq, *J* = 17.3, 1.5 Hz, 2H, -OCH$_2$CHCH**H**), 6.07 (ddt, *J* = 17.3, 10.6, 5.2 Hz, 2H, -OCH$_2$C**H**CH$_2$), 6.14 (s, 1H, Ar$_3$C**H**), 6.44 (d, *J* = 2.4 Hz, 1H, Ar-**H**), 6.50 (dd, *J* = 8.3, 2.4 Hz, 1H, Ar-**H**), 6.65 (s, 2H, Ar-**H**), 7.00 (m, 3H, Ar-**H**), 7.20 (td, *J* = 7.6, 1.3 Hz, 1H, Ar-**H**), 7.30 (td, *J* = 7.6, 1.5 Hz, 1H, Ar-**H**), 7.37 (d, *J* = 8.3 Hz, 1H, Ar-**H**), 7.81 (dd, *J* = 7.8, 1.2 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 55.4 (CH_3), 55.4 (CH_3), 63.1 (CH_2). 69.8 (CH_2), 98.6 (CH), 101.7 (CH), 104.0 (CH), 116.2 (C), 117.3 (C), 117.4 (C), 118.1 (CH_2), 126.5 (CH), 129.8 (CH), 129.9 (C), 130.7 (CH), 131.3 (CH), 131.8 (CH), 132.3 (CH), 132.4 (CH), 149.5 (C), 153.3 (C), 159.1 (C), 161.6 (C), 168.2 (C).



Bis(allyl ether) 2',7'-dichlorofluorescein (7c). To a solution of diether **6c** (121.6 mg, 0.192 mmol) in 9 mL CH_2Cl_2 and 1 mL H_2O was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 130.7 mg, 0.576 mmol, 3 eq). The reaction was stirred vigorously (~600 RPM) for 18 h at room temperature while shielded from light. The resulting mixture was filtered through a pad

of celite and concentrated *in vacuo*. Purification by column chromatography (hexanes→7:3 v/v hexanes/acetone, linear gradient) afforded pure 2',7'-dichlorofluorescein diether **7c** as a white foamy solid (77.1 mg, 83% yield).

Data for **7c**: $\mathbf{R_f}$ = 0.19 (4:1 v/v hexanes/acetone); **^{1}H NMR** (400 MHz, $CDCl_3$, δ): 4.65 (d, *J* = 4.9 Hz, 4H, -OC**H**$_2$CHCH$_2$), 5.36 (dq, *J* = 10.6, 1.4 Hz, 2H, -OCH$_2$CHC**H**H), 5.50 (dq, *J* = 17.3, 1.5 Hz, 2H, -OCH$_2$CHCH**H**), 6.06 (ddt, *J* = 17.3, 10.6, 5.2 Hz, 2H, -OCH$_2$C**H**CH$_2$), 6.74 (s, 2H, Ar-**H**), 6.77 (s, 2H, Ar-**H**), 7.16 (d, *J* = 7.5 Hz, 1H, Ar-**H**), 7.68 (td, *J* = 7.5, 1.2 Hz, 1H, Ar-**H**), 7.72 (td, *J* = 7.3, 1.3 Hz, 1H, Ar-**H**), 8.06 (d, *J* = 7.1 Hz, 1H, Ar-**H**); **^{13}C NMR** (100 MHz, $CDCl_3$, δ): 69.8 (CH_2), 81.8 (C), 101.5 (CH), 111.4 (C), 118.5 (CH), 118.7 (C), 123.8 (CH), 125.4 (CH), 126.4 (C), 128.8 (CH), 130.3 (CH), 131.7 (CH), 135.4 (CH), 150.5 (C), 152.0 (C), 155.5 (C), 168.8 (C); **HRMS** (*m*/*z*): $[M+H]^+$ calculated, 481.0610; observed, 481.060.

Optical Methods

General Optical Spectroscopy Methods. Fluorescence emission was measured in a 1-cm path length, 3.5 mL glass cuvette from Starna Cells in ACS spectrophotometric grade methanol at ambient temperature (22 ± 2 °C) and recorded on a Carey Eclipse Fluorescence Spectrometer (Varian). Samples containing compounds **7a** and **7b** were excited at 496 nm and the emission intensities were collected at 525 nm. The excitation and emission slits were set at 2.5 nm and the PMT Detector Voltage was 550 V. Samples containing compound **7c** were excited at 504 nm and the emission intensities were collected at 529 nm. The excitation and emission slits were set at 2.5 nm and the PMT Detector Voltage was 550 V.

Preparation of stock solutions. 10.0 mM stock solution of compounds **7a-c** were prepared in DMSO and stored in a freezer when not in use. A 100. mM solution of tri(2-furyl) phosphine (TFP) in DMSO was freshly prepared before each experiment. A solution of $NaBH_4$ (2.5 M in 10 N NaOH) was stored at ambient temperature, and a 100 mM solution of $NaBH_4$ was freshly prepared before each experiment by diluting the 2.5 M solution with pH 9 borate buffer. A 10.0 μM solution of Pd from $Pd_2(allyl)_2Cl_2$ in MeOH was freshly prepared before each experiment, from which serial dilutions provided a variety of palladium concentrations for testing.

Kinetic Data for Palladium Sensors 7a-c. A solution of TFP (10 μL, 100 mM in DMSO), $NaBH_4$ (25 μL, 100 mM in pH 9 borate buffer), and the palladium sensor being tested (2.5 μL, 10 mM in DMSO) was added to 1.94 mL of ACS spectrophotometric grade methanol. Varying concentrations of palladium were added (20 μL, 10 μM→13.7 nM in MeOH) and the sample was stirred while fluorescence emission was recorded every 0.2 min.

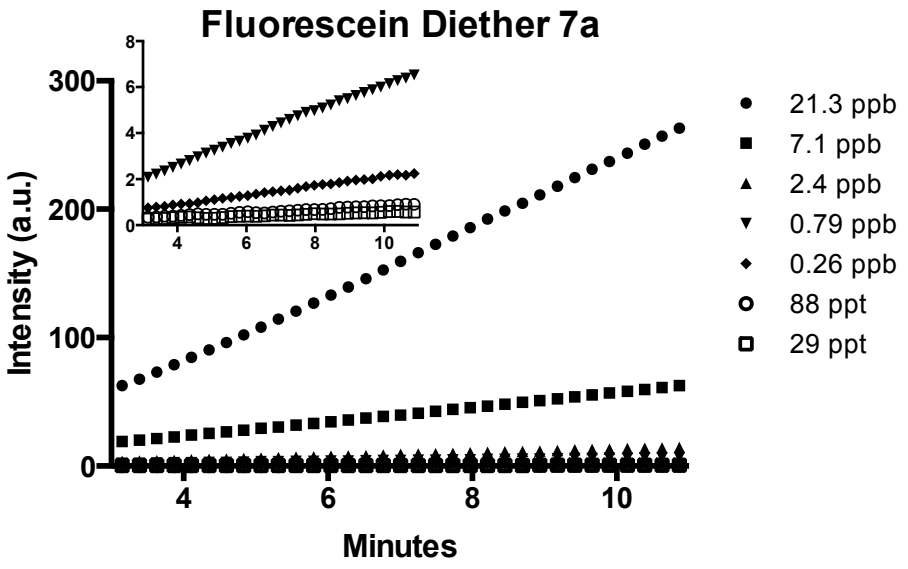


Figure S1. Representative Kinetic Data for fluorescein bis(allyl ether) **7a**. Inset is a zoomed in region for the lowest concentrations. Final rate of fluorescence increase used for Figure 2 was based on four trials.

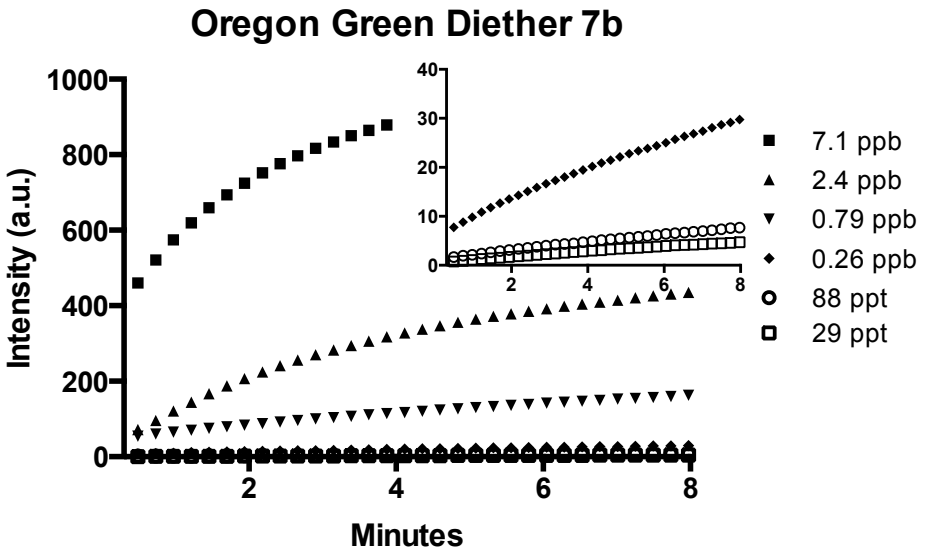


Figure S2. Representative Kinetic Data for 2',7'-difluorofluorescein bis(allyl ether) **7b**. Inset is a zoomed in region for the lowest concentrations. Final rate of fluorescence increase used for Figure 2 was based on five trials.

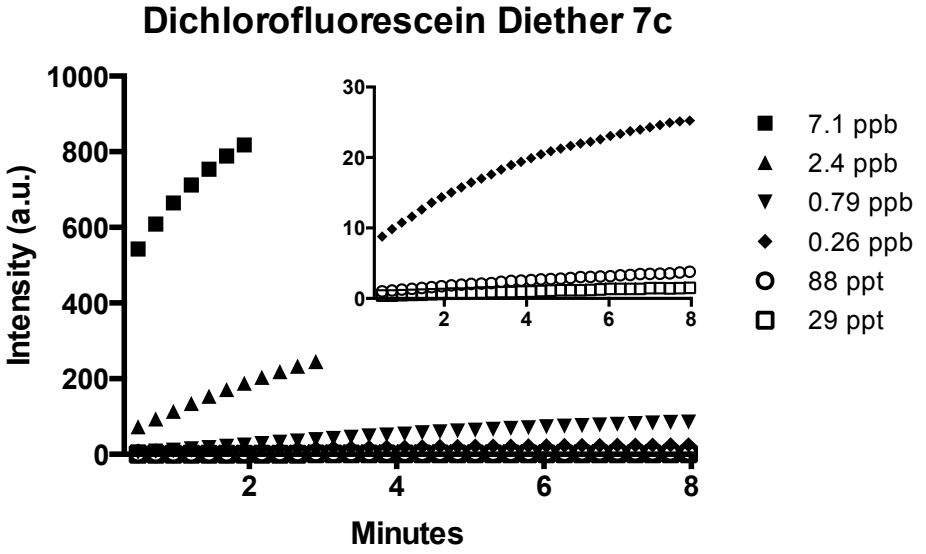


Figure S3. Representative Kinetic Data for 2',7'-dichlorofluorescein bis(allyl ether) **7c**. Inset is a zoomed in region for the lowest concentrations. Final rate of fluorescence increase used for Figure 2 was based on five trials.

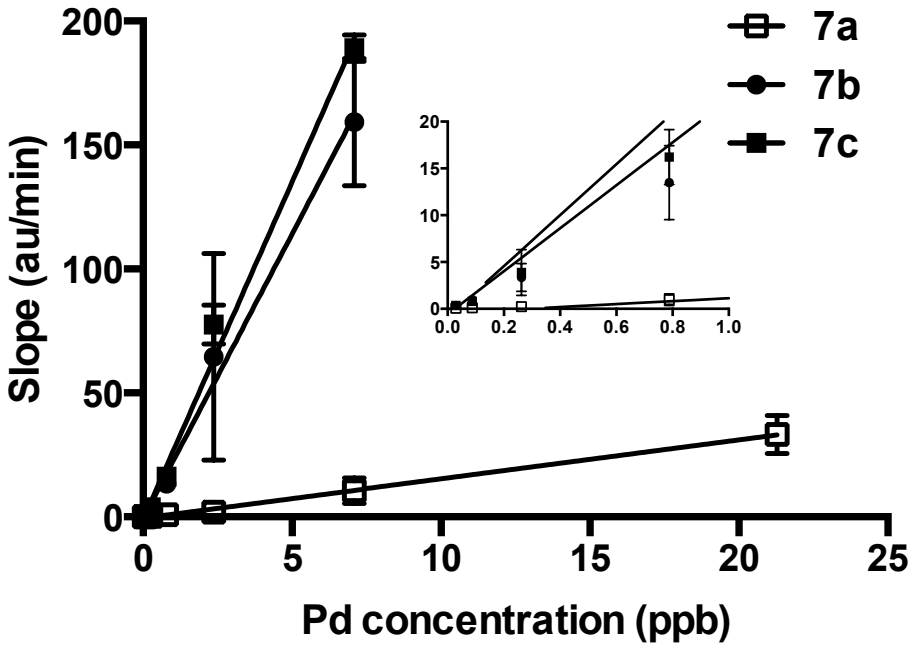


Figure S4. Comparison of fluorogenic bis(allyl ether) fluorescein derivatives **7a-c** as shown in Figure 2, with an inset to compare rates in the ppt range. The slope is calculated from the linear portion of the kinetic trace resulting from the addition of varying concentrations of palladium to a solution of 125 μM of the probe, 500 μM TFP, and 1.25 mM $NaBH_4$ in MeOH. This data is derived from several trials as shown in Figure S1-S3.

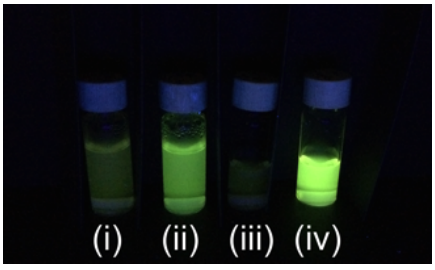


Figure S5. Direct comparison between the assay described by Koide et al with sensor **1** (i and ii) and the reported sensor **7c** (iii and iv) after 1 h stirring, as called for in Koide's protocol. The vials contain: (i) no palladium with **1**, (ii) 1 ppb palladium with **1**, (iii) no palladium with **7c**, and (iv) 1 ppb palladium with **7c**. This photo shows a moderate increase in fluorescence of **1** with 1 ppb palladium (i and ii) after an hour of stirring at 45°C, as reported previously, but background fluorescence would make visual detection of lower concentrations inconclusive. Importantly, after 1 h, the sample containing **7c** without palladium added (iii) is still nonfluorescent due to the stability of the ether functionality.

Limit of Detection. The limits of detection for sensors **7a-c** were determined from plots of the average fluorescence intensity at a single time point in the kinetic traces *vs.* the concentration of palladium(0). From the linear regression (Microsoft Excel) analysis of the resulting plots, the

limits of detection (LOD) were calculated using the following equation:[2]

$$LOD = \frac{3\, s_b}{m}$$

where m is the slope of the regression line and s_b is the standard deviation of the y-intercept. The limits of detection for these palladium sensors were found to be around 0.5 ppb (see Table S1). However, Figure 4 demonstrates that the presence of palladium(0) in concentrations as low as 67.5 ppt can be visually identified.

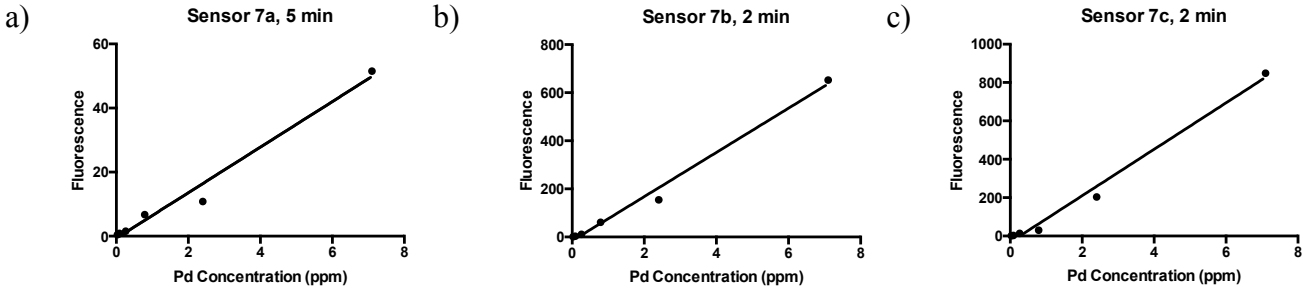


Figure S6. The average fluorescence measurement from kinetic traces for sensors **7a-c** at various palladium concentrations and a single time point: (a) **7a** at 5 min; (b) **7b** at 2 min; (c) **7c** at 2 min.

Table S1. Table of parameters used for determination of limit of detection.

Probe	Reaction time	Slope (m)	s_b	LOD
7a	5 min	5.49	1.23	0.673 ppb
7b	2 min	91.9	13.9	0.455 ppb
7c	2 min	121	19.7	0.488 ppb

(1) Sun, W.-C.; Gee, K. R.; Klaubert, D. H.; Haugland, R. P. *J. Org. Chem.* **1997**, *62*, 5469–6475.
(2) Harris, D. C. *Quantitative Chemical Analysis, 8th Edition*; W. H. Freeman, New York, 2010; p 103.