Supporting Information

Intracellular deprotection reactions mediated by palladium complexes equipped with designed phosphine ligands

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1. General methods and materials for synthesis and materials for synthesis

Chemicals were purchased from *Sigma Aldrich, Alfa Aesar* and *Stream Chemicals* and used without further purification. The removal of solvents under reduced pressure was carried outon a rotary evaporator *Büchi R-210* equipped with a thermostated bath *B-491*, a vacuum regulator *V-850*, and a vacuum pump *V-700.* The solvents for organic synthesis were of reagent grade. Dry solvents were bought from *Sigma-Aldrich. N,N*-dimethylformamideand trifluoroacetic acid were purchased from *Scharlau*, dichloromethane from *Panreac* and acetonitrile from *Merck*. Water was deionized and purified on a *Millipore Milli-Q Integral* system.

Chromatographic purification of products was accomplished using flash column chromatography on *Merck* Geduran Si 60 (40 – 63 µm) silica gel (normal phase) or by reversed-phase high-performance liquid chromatography (RP-HPLC) using H_2O (+ 0.1% TFA) and CH_3CN (+ 0.1% TFA) as eluents and a Luna(C18)-Phenomenex column.

Thin layer chromatography (TLC) was performed on *Merck* 60 (silica gel F_{254}) plates.

^{1}H-, ^{13}C, DEPT and ^{31}P NMR spectra were recorded in deuterated solvents (from Sigma-Aldrich and VWR) on *a Bruker AMX 500 and Varian Mercury 300* spectrometers and calibrated to the residual solvent peak, if possible. The chemical shifts (δ) are given in ppm, the coupling constants (J) in Hz. Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad and combinations of these.

Fluorescence measurements were performed in a *Varian Cary Eclipse* Fluorescence Spectrophotometer. Fluorescence microscopy images were obtained with an *AndorZyla 4.2*cameramounted on a *Nikon Eclipse TiE* microscope*.* Confocal images were acquired with a *Andor DragonFly* spinning disc confocal microscope.

Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with a *Bruker Amazon IT*/*MS* using direct injection of a solution of the compound in dichloromethane into the MS. HRMS analysis were carried out in a *BRUKER AMAZON ETD*.

Catalysts **Pd1-9** were kept stored at -32 °C as powder solids. For the catalytic studies the complexes were dissolved in DMSO in a concentration of 20 mM. These samples can be stored forat least 1 month without loss of activity.

ABREVIATIONS:

DMEM: Dulbecco's Modified Eagle Medium
TMRE: Tetramethylrhodamine, ethyl ester (TMRE)
FBS: Fetal bovine serum
MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium

2. General synthetic procedure

(2-Carboxyethyl)diphenyl(pyren-1-ylmethyl)phosphonium bromide (**R3**),[1] **Pd4**,[2] **Pd6**,[3] 1-ethyl-2,3,3-trimethyl-3*H*-indol-1-ium iodide (**R4**),[4] 4-(prop-2-yn-1-yloxy)benzaldehyde (**R5a**),[4] (*E*)-2-(4-aminostyryl)-1-ethyl-3,3-dimethyl-3*H*-indol-1-ium iodide (**4**),[4] (*E*)-4-(3-(benzo[*d*]thiazol-2-yl)-2-(prop-2-yn-1-yloxy)styryl)-1-methylpyridin-1-ium (**5**),[5] diallyl(3-oxo-3H-spiro[isobenzofuran-1,9'-xanthene]-3',6'-diyl)dicarbamate (**7**)[6] and 2-(3,6-bis(((prop-2-yn-1-yloxy)carbonyl)amino)-9*H*-xanthen-9-yl)benzoic acid (**Rho-poc**)[7] were synthesized according to literature procedures.

Ligands **L1-L4**, and compounds **R1**, **R2**, **R5b**, **R6** and $Pd_2(Allyl)_2Cl_2$ were purchased from Sigma-Aldrich, Alfa Aesar and Stream Chemicals.

2.1 General synthetic procedure for Pd4-Pd7

The catalyst **Pd4** has been synthetized according to literature procedure.[2] $Pd_2(Allyl)_2Cl_2$ (100 mg, 0.273 mmol) was dissolved in methylene chloride (4 mL) before pyridine (54 μL, 0.663 mmol) was added and the reaction was left stirring for 1 hour at room temperature. The solvent was removed under reduced pressure and the precipitate was washed with Et_2O (3 x 5 mL) yielding an off yellow solid (110 mg, 64 %).

To a 20 mM solution of either phosphine ligand **L2-L4** (0.133 mmol, 2 eq.) in THF (3.3 mL), $Pd_2(Allyl)_2Cl_2$ was added (0.066 mmol, 1 eq.), and the mixture was stirred for 3 hours at room temperature under N_2 atmosphere. The crude resulting from evaporation of solvents was purified by flash chromatography on silica gel (EtOAc/Hex 2:1) yielding, after concentration, a pale yellow solid.[3]

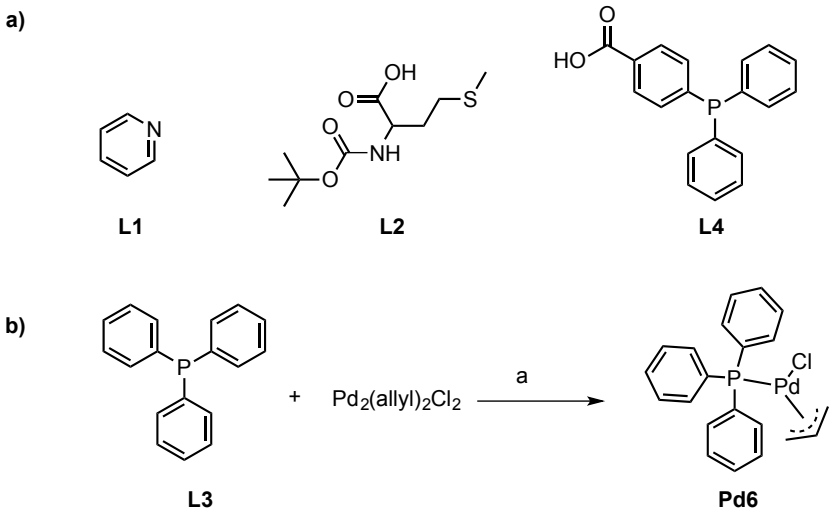


Figure S1. **a)** Representation of ligands **L1**-**L4** and **b)** Representation of the reaction of **L3** with $Pd_2(allyl)_2Cl_2$ yielding **Pd6**. Reagents: a) **L3** (2.0 eq), $Pd_2(Allyl)_2Cl_2$ (1.0 eq), THF (3 mL), N_2, RT, 3 hours.[3]

Pd4 (100 mg, 64 %)[2]

^{1}H NMR (500 MHz, $CDCl_3$, rt) δ 8.83 (dt, *J* = 4.8, 1.7 Hz, 2H), 7.83 (tt, *J* = 7.8, 1.7 Hz, 1H), 7.44 – 7.37 (m, 2H), 5.61 (m, 1H), 4.05 (bs, 2H), 3.13 (d, *J* = 7.5 Hz, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$, rt) δ 152.48 (CH), 138.03 (CH), 125.08 (CH), 114.22 (CH), 63.42 (CH_2), 59.04 (CH_2). **HRMS-ESI** Calculated for $[2M\text{-}Cl^-]^+$ $C_{16}H_{20}N_2ClPd$: 488.8960; found: 488.9601. **^{1}H NMR** (500 MHz, CD_2Cl_2-d_2, -60 °C) δ 8.76 – 8.70 (m, 2H), 7.82 (td, *J* = 7.7, 1.8 Hz, 1H), 7.42 – 7.35 (m, 2H), 5.70 – 5.58 (m, 1H), 4.00 (dd, *J* = 6.9, 2.2 Hz, 1H), 3.92 – 3.86 (m, 1H), 3.23 (d, *J* = 12.3 Hz, 1H), 2.92 (d, *J* = 12.2 Hz, 1H). **^{13}C NMR** (126 MHz, CD_2Cl_2-d_2, -60 °C) δ 153.37 (CH), 139.23 (CH), 126.16 (CH), 115.81 (CH), 65.76 (CH_2), 58.71 (CH_2).

Pd5 (31 mg, 51 %)

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69 (bs, 1H, NH), 5.53 (m, 1H), 4.42 (s, 1H), 4.20 (bs, 2H), 3.15 (d, *J* = 12.4 Hz, 2H), 3.03 – 2.85 (m, 2H), 2.45 (s, 3H), 2.37 – 2.14 (m, 2H), 1.45 (s, 9H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 174.43 (C), 155.90(C), 115.08 (CH), 80.50 (C), 65.38 (CH_2), 53.07(CH_2), 33.05 (CH_2), 31.21 (CH_2), 28.31 (CH_3^{tBu}), 19.11 (CH_3). **HRMS-ESI** Calculated for [M-Cl] + Na^+ $C_{13}H_{23}NNaO_4PdS$: 418.0275; found: 418.0340.

Pd6 (36 mg, 74%)[3]

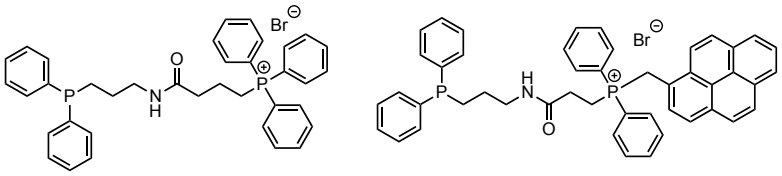
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (m, 6H), 7.41 (m, 9H), 5.61 (m, 1H), 4.77 (m, 1H), 3.77 (m, 1H), 3.12 (m, 1H), 2.84 (m, 1H). **HRMS-ESI** Calculated for $[M\text{-}Cl]^+$ $C_{21}H_{20}PPd$: 409.0340; found: 409.0337.

Pd7 (48 mg, 74 %)

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.13 – 8.10 (m, 1H, H), 8.10 – 8.06 (m, 1H, H), 7.78 – 7.71 (m, 2H), 7.71 – 7.65 (m, 2H), 7.62 (m, 2H), 7.53 – 7.47 (m, 2H), 7.44 (m, 4H), 5.64 (m, 1H), 4.82 (t, 1H), 3.81 (dd, 1H), 3.20 – 3.13 (m, 1H), 2.87 (d, 1H, H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 177.02 (COOH), 139.29 (d, *J* = 38.3 Hz, C), 135.27 (CH), 134.78 (CH), 134.28 (d, *J* = 12.9 Hz,CH), 133.89 (d, *J* = 12.9 Hz,CH), 131.63 (C), 131.29 (C), 131.19 (CH), 131.04 (CH), 130.07 (d, *J*= 12.9 Hz, CH), 129.51 (CH), 128.97 (d, *J* = 10.4 Hz, CH), 128.54(CH), 118.44 (CH), 80.46 (d, *J* = 30.3 HzCH), 61.55 (CH). **^{31}P NMR** (202 MHz, $CDCl_3$) δ 21.89 (d, *J*= 127.2 Hz). **HRMS-ESI** Calculated for $[M\text{-}Cl]^+$ $C_{22}H_{21}PO_2Pd$: 453.0236; found: 453.0239.

2.2 General synthetic procedure for the synthesis of Pd8 and Pd9

The catalysts **Pd8** and **Pd9** were synthetized without isolation of the ligands **L5** and **L6**, to avoid the oxidation of the phosphine groups of the non-isolated ligands. Nevertheless, the formation of these ligands could be confirmed by RP-HPLC-MS.



L5 L6

Figure S2. Representation of the ligands **L5** and **L6**.

Pd8 (40 mg, 15%)

Carboxilic acid **R1** (147 mg, 0.343 mmol, 1 eq.), HBTU (156 mg, 0.41 mmol, 1.2 eq.) and DIEA (182 mL, 1.028 mmol, 3eq.) were added to a round bottom flask with 3.5 mL of DMF under N_2 atmosphere, and then, **R2** (100 mg, 0.411 mmol, 1.2 eq.) was added. The reaction mixture was stirred for 3 hours and diluted with THF (5.5 mL), before $Pd_2(allyl)_2Cl_2$ (63 mg, 0.171 mmol, 0.5 eq.) was added. The mixture was stirred for another three hours at room temperature. The solvent was removed under reduced pressure and the crude purified by HPLC (55-95 % of CH_3CN + 0.1% TFA in 30 min) yielding a pale yellow solid. (40 mg, 15%).

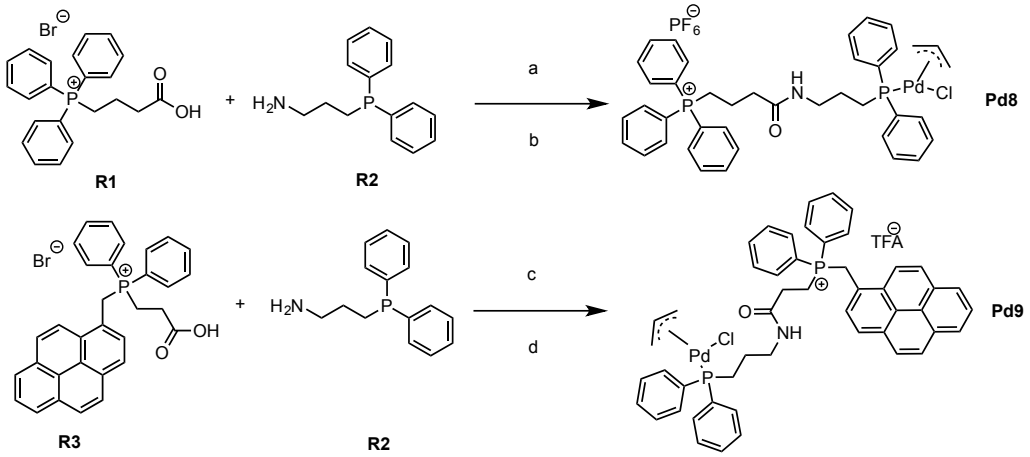
^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.85 (m, 2H), 7.74 – 7.61 (m, 12H), 7.49 (m, 1H), 7.42 (m, 2H), 7.33 (m, 4H), 7.21 (m, 4H), 6.26 (t, *J* = 5.9 Hz, 1H, NH), 5.81 (m, 1H), 5.33 (s, 2H), 4.26 (m, 2H), 3.42 (m, 2H),3.15 (m, 4H), 2.47 (t, *J* = 6.5 Hz, 2H), 2.05 – 1.88 (m, 4H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 171.85 (C), 135.98 (d, *J* = 3.0 Hz, CH), 134.03 (d, *J* = 9.9 Hz, CH), 132.82 (m, CH), 132.55 (d, *J* = 10.1 Hz, CH),131.66 (d, *J* = 5.6 Hz, CH), 131.15 (d, *J* = 12.4 Hz, CH), 129.69 (m, CH), 129.20 (d, *J* = 12.1 Hz, CH), 123.51 (CH), 118.61 (C), 117.97 (C), 75.86 (t, *J* = 15.3 Hz, CH_2), 40.61 (m, CH_2), 35.44 (d, *J* = 17.3 Hz, CH_2), 26.21 (CH_2), 22.17 (CH_2), 19.10 (CH_2). **^{31}P NMR** (202 MHz, $CDCl_3$) δ 24.53 (P-Pd), 17.99 (P^+Ph3), 143.09 (septet, ^{1}JPF = 709.0 Hz, PF_6^-). **HRMS-ESI** Calculated for $[M]^+C_{40}H_{43}ClNOP_2Pd$: 756.1471; found: 756.1470; **HRMS-ESI** Calculated for $[M\text{-}Cl]^+$ $C_{40}H_{43}BrNOP_2Pd$: 802.1032; found: 802.0950.

Pd9 (8 mg, 12%)

Compound **R3** (40 mg, 0.073 mmol, 1 eq.), HATU (33 mg, 0.87 mmol, 1.2 eq.) and DIEA (38 mL, 0.218 mmol, 3eq.) were added to a round bottom flask with 1 mL of DMF under N_2 atmosphere, and then **R2** (20 mg, 0.087 mmol, 1.2 eq.) was added. The reaction mixture was stirred for 1.5 hours and diluted with THF (5 mL), before $Pd_2(allyl)_2Cl_2$ (15 mg, 0.042 mmol, 0.5 eq.) was added. The reaction was kept for another three hours at room temperature. The solvent was removed under reduced pressure and the crude purified by HPLC (55-95 % of CH_3CN + 0.1% TFA in 30 min) yielding a pale yellow solid (8 mg, 12%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.10 (d, *J* = 7.2 Hz, 1H), 7.98 – 7.89 (m, 2H), 7.81 (m, 2H), 7.74 – 7.61 (m, 2H), 7.61 – 7.53 (m, 4H), 7.49 – 7.29 (m, 14H), 7.19 (m, 3H), 5.81 (m, 1H), 5.11 (d, J = 14.4 Hz, 2H), 3.42 (m, 2H), 3.19 (s, 1H), 3.17 – 3.00 (m, 4H), 2.85 (m, 1H), 2.72 (m, 2H), 2.54 (s, 1H), 2.44 (s, 1H), 2.03

(bs, 2H), 1.61 (bs, 2H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 169.74 (CONH), 162.41 (q, *J* = 37.5 Hz, TFA), 135.19 (CH), 133.86 (CH), 133.49 (CH), 132.30 (CH), 131.50 (C), 131.36 (CH), 131. 05 (C), 130.10 (d, *J*= 12.4, CH), 129.30 (CH), 128.76 (CH), 128.26 (CH), 127.08 (CH), 126.45 (CH), 125.91 (CH), 125.70 (CH), 124.88 (CH), 124.60 (C), 124.00 (C), 122.86 (CH), 121.59 (CH), 119.69 (C), 117.42 (C), 117.05 (C), 116.35 (C, TFA), 115.10 (C), 112.78 (C), 75.65 (CH_2), 40.41 (CH_2), 27.80 (CH_2), 26.95 (d, J = 46.4 Hz, CH_2), 25.96 (CH_2), 25.45 (CH_2), 17.50 (d, *J* = 52.9 Hz, CH_2). **^{31}P** (202 MHz, $CDCl_3$) 24.45 (s, Pd-P), 15.44 (P^+Ph_2Pyrene). **HRMS-ESI** calculated for $[M]^+$ $C_{50}H_{47}NOClP_2Pd$: 880.1856; found: 880.2820; **HRMS-ESI** calculated for $[M\text{-}Cl]^+$ $C_{50}H_{46}NOP_2Pd$: 844.2102; found: 844.2100.



Scheme S1. Reagents **a) R1** (1 eq.), **R2** (1.2 eq.), HBTU (1.2 eq.), DIEA (3 eq.), DMF (2 mL), 1h, RT; **b**) **L5** (2.0 eq), **$Pd_2(Allyl)_2Cl_2$** (1.0 eq), N_2, RT, 3 hours; **c**) **R3** (1 eq.), **R2** (1.2 eq.), HATU (1.2 eq.), DIEA (3 eq.), DMF (2 mL), 1h, RT; **d**) **L6** (2.0 eq), **$Pd_2(Allyl)_2Cl_2$** (1.0 eq), N_2, RT, 3 hours

2.2.1 HR-ESI-MS spectra of the complexes

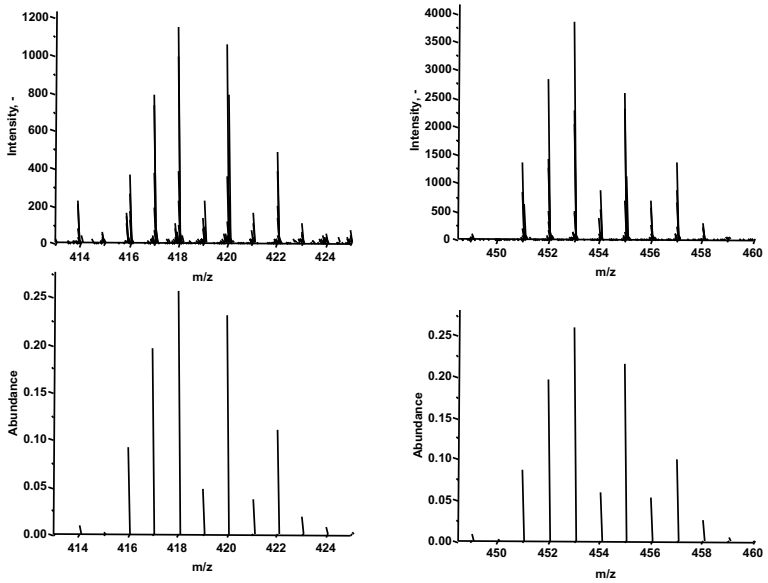


Figure S3. Experimental (top) and calculated (bottom) HR-ESI-MS of **Pd4** (left) and **Pd5** (right)**.**

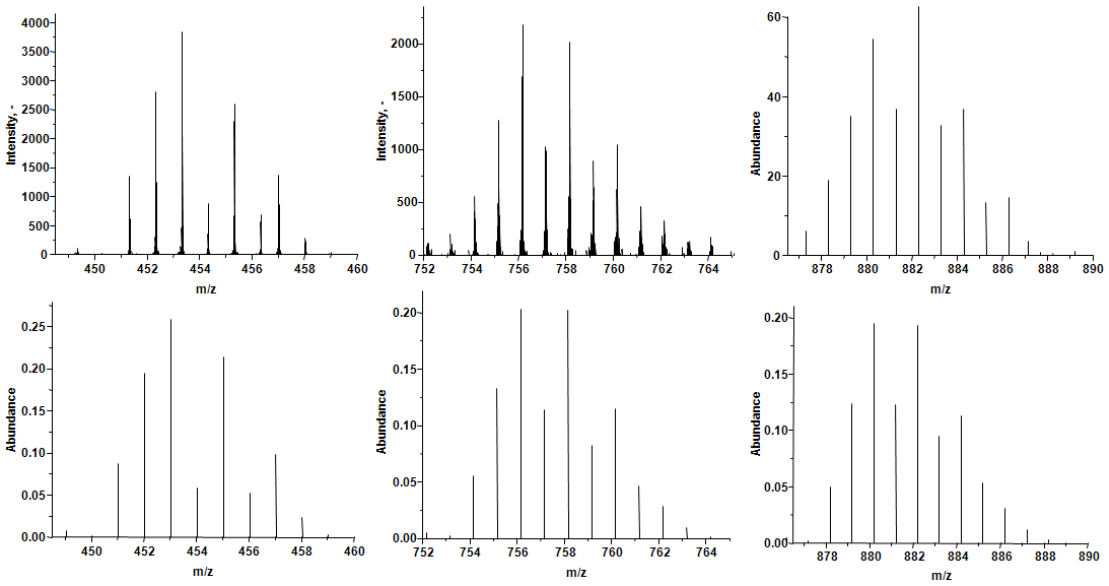


Figure S4. Experimental (top) and calculated (bottom) HR-ESI-MS of **Pd7** (left), **Pd8** (middle) and **Pd9** (right).

2.2.2 Spectroscopic studies of Pd9

For the spectroscopic studies a fresh solution of the metal complex (10 mM in DMSO) was prepared and diluted into a quartz Hellma® fluorescence cuvette with a path-length 35x10 mm, chamber volume 3.5 mL to reduce the complex concentration to 1 µM in methanol. The samples were analyzed in a *Jasco V-630* UV-Vis spectrophotometer and in a *Varian Cary Eclipse* Fluorescence Spectrophotometer. Samples were recorded at 25 °C. UV-Vis analyses were performed in the interval of 250-600 nm (**Figure S5**). For the fluorescence studies, **Pd9** was excited at 345 nm and the emission spectrum was recorded in the interval 350-600 nm. The excitation fluorescence spectra of **Pd9** was recorded in the interval 250-355 nm with λ_{em}380 nm.

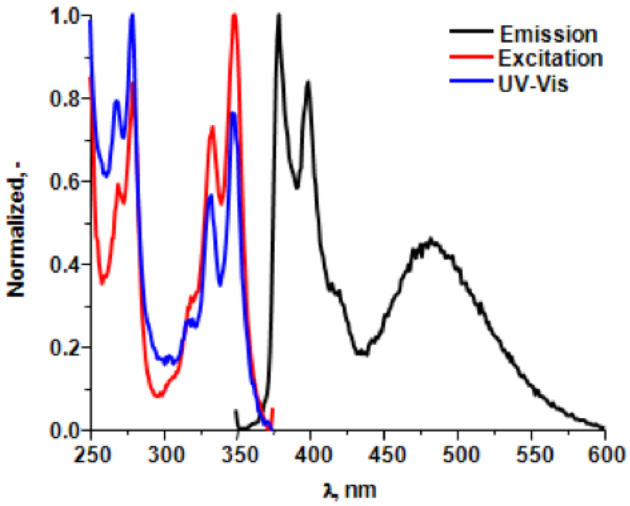
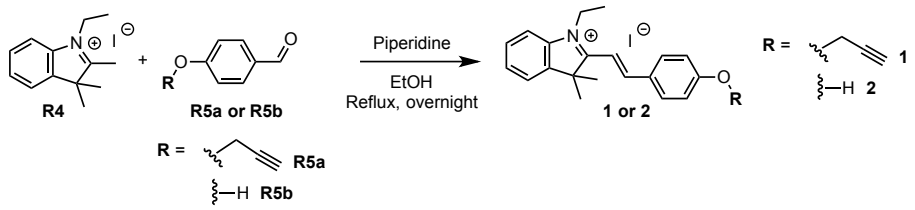


Figure S5. UV-Vis, fluorescence and excitation normalized spectra of **Pd9**.

2.3 Procedure for the synthesis of 1, 2 and 3



Scheme S2. General scheme for the synthesis of probes **1** and **2.**

2.3.1 (E)-1-ethyl-3,3-dimethyl-2-(4-(prop-2-yn-1-yloxy)styryl)-3H-indol-1-ium iodide (1)

1-Ethyl-2,3,3-trimethyl-3*H*-indol-1-ium iodide (**R4,** 100 mg, 0.317 mmol, 1eq.) was dissolved in ethanol (3 mL), and piperidine (0.006 mL, 0.063 mmol, 0.2eq.) was added to give an orange solution. 4-(prop-2-yn-1-yloxy)benzaldehyde (**R5a,** 55.9 mg, 0.349 mmol, 1.1 eq.) was added and the colour of the solution changed to red. The reaction was stirred under reflux until the consumption of the imidazolinium, monitored by TLC (12h). The reaction mixture was allowed to cool down to rt, and the residue was purified by flash column chromatography using CH_2Cl_2:MeOH (98:2) to afford(E)-1-ethyl-3,3-dimethyl-2-(4-(prop-2-yn-1-yloxy)styryl)-3H-indol-1-ium iodide **(1)** (90.0 mg, 0.197 mmol, 62.0%) as a brown solid, $\mathbf{R_f}$ = 0.5, MeOH: CH_2Cl_2 (5:95). **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.16 (d, *J* = 15.0 Hz, 1H), 7.98 (d, *J* = 7.9 Hz, 2H), 7.62 – 7.53 (m, 5H), 7.14 (d, *J* = 7.1 Hz, 2H), 4.82 (d, *J* = 2.4 Hz, 2H), 4.74 (s, 1H), 2.68 (s, 1H), 2.60 (t, *J* = 2.3 Hz, 1H), 1.82 (s, 5H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 181.14 (C), 163.13(C), 155.22 (CH), 143.23 (C), 140.40 (C), 133.49 (CH), 129.90 (CH), 129.85 (CH), 127.50(C), 122.81(CH), 116.38 (CH), 114.52 (CH), 109.87 (CH),76.79 (CH), 56.36 (CH_2), 52.36 (C), 42.90 (CH_2), 27.19 (CH_3), 13.85 (CH_3). **LRMS** (*m/z, ESI*): 330.18 (M^+), 315.16, 276.14. **HRMS-ESI** Calculated for $C_{23}H_{24}NO$: 330.1852, found 330.1852.

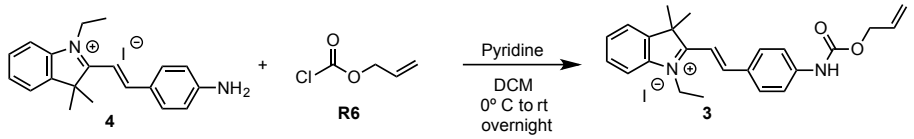
2.3.2 (E)-1-ethyl-2-(4-hydroxystyryl)-3,3-dimethyl-3H-indol-1-ium iodide (2)

1-Ethyl-2,3,3-trimethyl-3*H*-indol-1-ium iodide (**R4,** 200 mg, 0.635 mmol, 1 eq.) was dissolved in ethanol (6 mL), and piperidine (0.013 mL, 0.127 mmol, 0.2 eq.) was added, forming an orange solution. After that 4-hydroxybenzaldehyde **R5b** (77.5 mg, 0.635 mmol, 1 eq.) was added and the colour of the solution changed to red. The reaction was stirred under reflux until the consumption of the starting material asmonitored by TLC and RP-HPLC (12h). The reaction mixture was allowed to cool down to rt and the crude residuewas purified by flash column chromatography using CH_2Cl_2:MeOH (98:2 to 90:10) to afford(E)-1-ethyl-2-(4-hydroxystyryl)-3,3-dimethyl-3H-indol-1-ium iodide **(2)** (200.0 mg, 0.477 mmol, 75%) as a red solid. R_f = 0.59, MeOH:CH_2Cl_2 (10:90).

^{1}H NMR (500 MHz, Methanol-d_4) δ 8.41 (d, *J* = 16.0 Hz, 1H), 8.02 – 7.97 (m, 2H), 7.78 – 7.72 (m, 2H), 7.65 – 7.57 (m, 2H), 7.43 (d, *J* = 16.1 Hz, 1H), 7.00 – 6.94 (m, 2H), 4.65 (q, *J* = 7.3 Hz, 2H), 1.83 (s, 6H), 1.56 (t, *J* = 7.3 Hz, 3H). **^{13}C NMR** (126 MHz, Methanol-d_4) δ 182.83 (C), 165.56 (C), 157.05 (CH), 144.85 (C), 141.86 (C), 134.76(CH), 130.49 (CH), 130.37 (CH), 127.43 (C), 124.03 (CH), 117.83 (CH), 115.38 (CH), 109.04 (CH),

53.49 (C), 42.92 (CH_2), 26.80 (CH_3), 13.77 (CH_3). **LRMS** (*m/z, ESI*): 292.17 (M^+), 277.15. **HRMS-ESI** Calculated for $C_{20}H_{22}NO$ (M^+): 292.1696, found 292.1695.

2.3.3 (*E*)-2-(4-(((allyloxy)carbonyl)amino)styryl)-1-ethyl-3,3-dimethyl-3*H*-indol-1-ium (3)



4 R6 3

(E)-2-(4-aminostyryl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium iodide (**4**, 100.0 mg, 0.239 mmol, 1 eq.) was dissolved in dry CH_2Cl_2 (5 mL). Pyridine (0.038 mL, 0.478 mmol. 2 eq.) was added, and a CH_2Cl_2 (1 mL) solution of Alloc-Cl (**R6**, 0.052 mL, 0.478 mmol, 2 eq.) was added dropwise at 0ºC. The reaction mixture was warmed at rt and stirred overnight. After complete consumption of the starting material, the reaction was washed with a saturated solution of $NaHCO_3$ (10 mL), extracted with CH_2Cl_2 (2 x 10 mL), dried over Na_2SO_4 and purified by flash column chromatography using MeOH:Hexanes:CH_2Cl_2 (0.5:1:8.5)to givethe product(E)-2-(4-(((allyloxy)carbonyl)amino)styryl)-1-ethyl-3,3-dimethyl-3H-indol-1-ium (**3**,110 mg, 0.219 mmol, 91%) as a red crystalline solid. R_f = 0.41, MeOH:Hexanes:CH_2Cl_2 (1:1:8).

^{1}HNMR (300 MHz, Methanol-d_4) δ 8.43 (d, *J* = 16.2 Hz, 1H), 8.06 (d, *J* = 9.0 Hz, 2H), 7.92 – 7.48 (m, 7H), 6.10 – 5.94 (m, 1H), 5.56 – 5.21 (m, 2H), 4.75 – 4.64 (m, 4H), 1.85 (s, 6H), 1.58 (t, *J* = 7.4 Hz, 3H).**^{13}C NMR** (75 MHz, Methanol-d_4) δ 182.90 (C), 156.08 (CH), 154.89 (C), 146.31 (C), 145.08 (C), 141.82 (C), 133.85 (C), 133.52 (CH), 130.65 (CH), 130.55(CH), 129.96(C), 124.12(CH), 119.53(CH), 118.25 (C), 115.80 (CH), 110.73 (CH), 66.80 (CH_2), 53.71 (C), 49.00 (CH_2), 43.57 (CH_2), 26.79 (CH_3), 14.16 (CH_3).**HRMS-ESI** Calculated for $C_{24}H_{27}N_2O_2$: 375.2067, found 375.2062.

3. Catalysis experiments *in vitro*

For the *in vitro* experiments with complexes **Pd1-9**, we used the propargylated phenol probe **1** and the alloc protected aniline **3**, as they allow an easy monitoring of the reaction by RP-HPLC-MS. For RP-HPLC analysis H_2O + 0.1% TFA and CH_3CN + 0.1% TFA were used as eluents in a 5-95 % gradient ofCH_3CN + 0.1% TFA in 11.6 minutes using a Phenomenex Kinetex XB-C18: 1.7 μm, 100 Å, 2.1 x 50 mm column.

The palladium complexes added to the cells were initially dissolved in DMSO (20 mM) before being added to the reaction media, so the final concentration of DMSO in DMEM is minimal (< 0.1%).

Cell Lysate preparation

For the preparation of the HeLa cells lysates, $3x10^6$ exponentially growing Hela cells were washed twice with PBS, scrapped with a rubber policeman in 0,5 mL of PBS and sonicated intensely for 2 rounds of 1 min with a 30 second cooling period in between. The protein concentration of the lysates was quantified by DC™ Protein Assay (BioRad) and equalised to 1 mg ml^{-1} for reproducibility among experiments.

3.1 Calibration curve and RP-HPLC-MS characterization of compounds 1-4

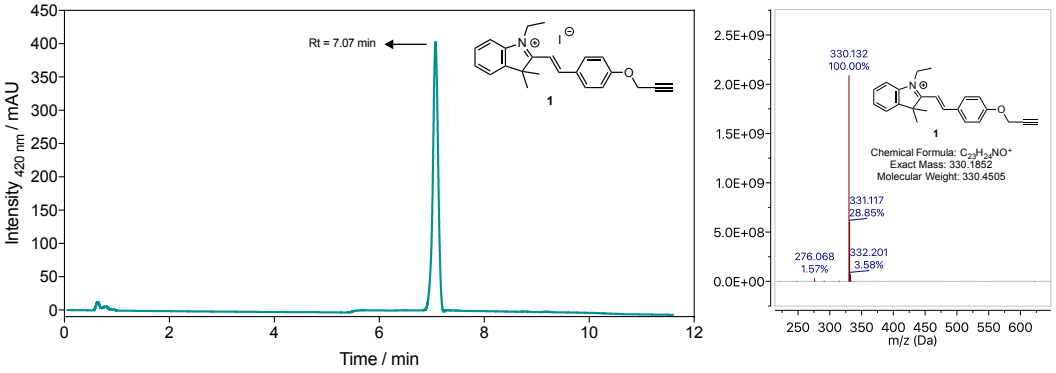


Figure S6. RP-HPLC-MS chromatogram (left) and ESI-MS spectra (right) for compound **1.**

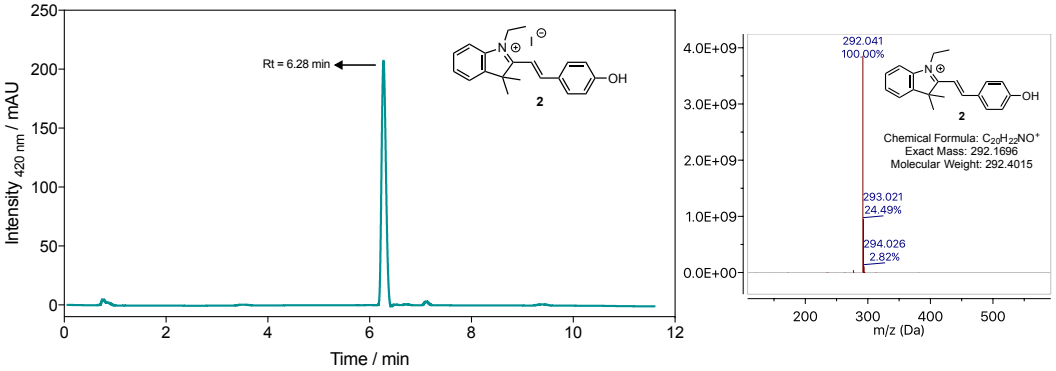


Figure S7. RP-HPLC-MS chromatogram (left) and ESI-MS spectra (right) for compound **2.**

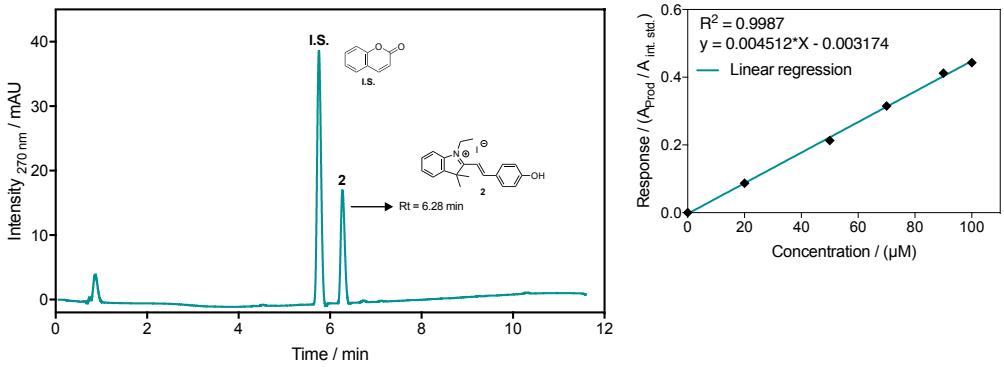


Figure S8. RP-HPLC-MS chromatogram (left) and calibration curve (right) for compound **2.** I.S.= internal standard.

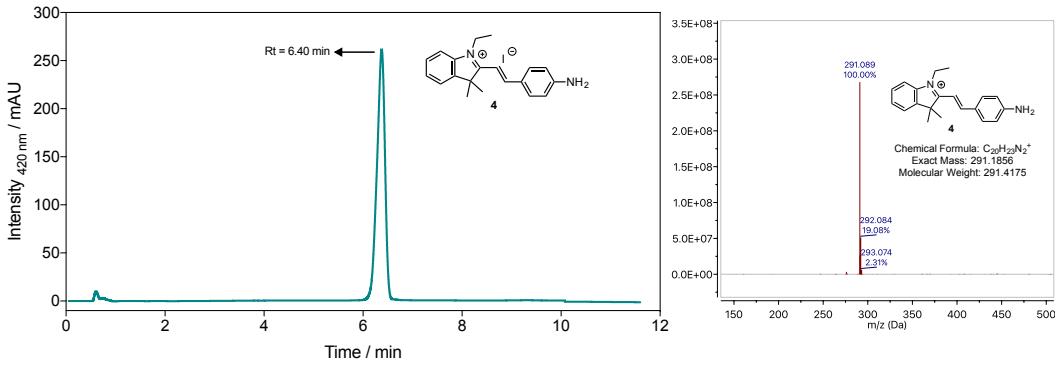


Figure S9. RP-HPLC-MS chromatogram (left) and ESI-MS spectra (right) for compound **4.**

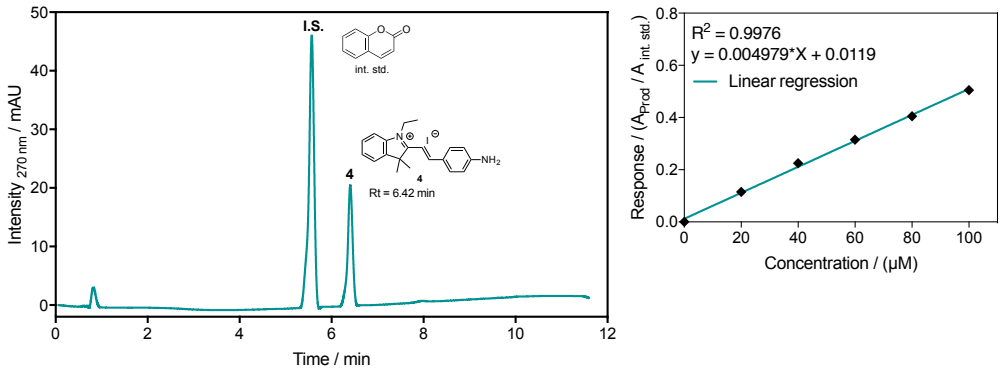


Figure S10. RP-HPLC-MS chromatogram (left) and calibration curve (right) for compound **4.**

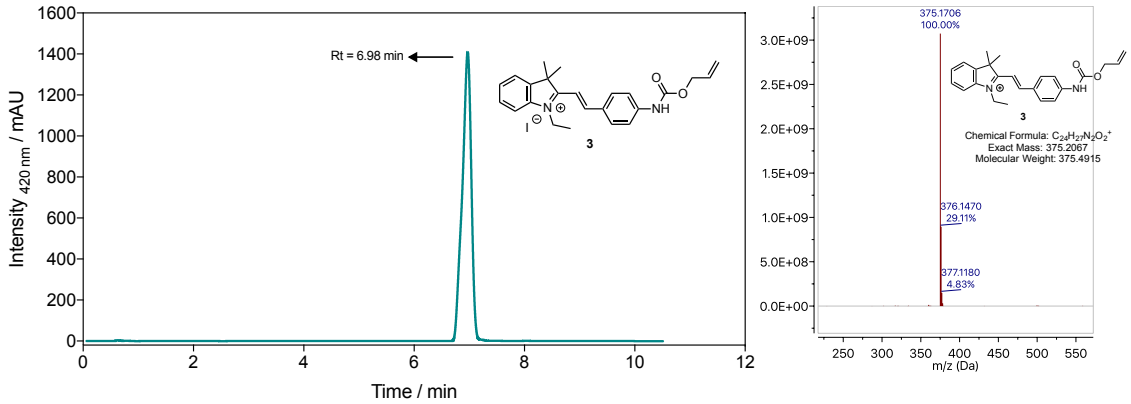
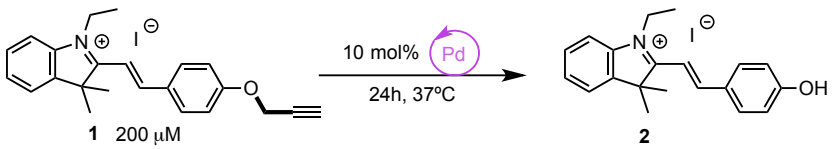


Figure S11. RP-HPLC-MS chromatogram (left) and ESI-MS spectra (right) for compound **3**.

3.2 Uncaging of probe 1

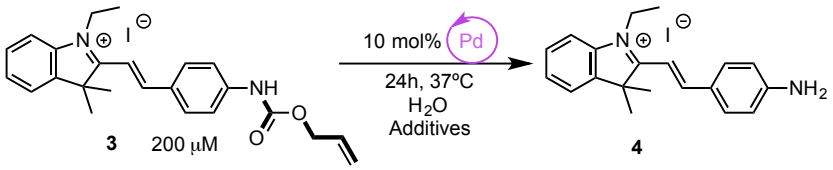


1 200 µM **2**

The catalytic deprotection of **1** to release the uncaged product **2,** under biologically relevant conditions, was performed in a 2.0 mL HPLC-vial with screw cap. For this purpose, fresh solutions of **1** (10 µL, 20 mM

in DMSO, 1.0 eq) were either added to water, PBS or HeLa cell lysates (990 µL), and to the resulting mixture was added a solution of the palladium complex (1 µL, 20 mM in DMSO, 0.1eq). The reaction mixture was kept for 24 h at 37 °C under stirring at 1000 rpm. At regular intervals, aliquots of the reaction (50 µL and diluted to 100 µL with MeOH) were analyzed by RP-HPLC-MS. The results were treated according to the previous calibration curve, in which coumarin was used as internal standard. Every value is the average value of two independent measurements.

3.3 Uncaging of probe 3



The catalytic deprotection of **3** to release the uncaged amine **4**, under biologically relevant conditions, was performed in a 2.0 mL HPLC-vial with screw cap. For this purpose, fresh solutions of **3** (4 µL, 50 mM in DMSO, 1.0 eq) were either added to water, GSH (0.4 mM in 1 mL of H_2O) or sodium ascorbate (2 mM) solutions (996 µL) before a solution of the **catalyst** (1 µL, 20 mM in DMSO, 0.1 eq) was added. The reaction mixture kept for 24 h at 37 °C under stirring at 1000 rpm. At regular intervals (1, 3, 6 and 24 hours), aliquots of the reaction (50 µL and diluted to 100 µL with MeOH) were analyzed by RP-HPLC-MS. The results were treated according to a previous calibration, in which coumarin was used as internal standard. Every value is the average value of two independent measurements.

4. Cell culture experiments

All cell lines were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) fetal bovine serum (FBS), 5 mM glutamine, penicillin (100 units/mL) and streptomycin (100 units/mL) (all from *Invitrogen*). Proliferating cell cultures were maintained in a 5% CO_2 humidified incubator at 37 °C.

4.1 ICP-MS analysis

For the ICP measurements, 150000 Vero cells per well were seeded in 6 well plates one day before treatment with the **Pd** complexes (50 µM) in DMEM-FBS for 30 minutes. Cells were then washed twice with PBS and lysed in 70% HNO_3. The obtained lysates were digested in duplicate with HNO_3 / H_2O_2 by heating with microwave energy before being analyzed.

For the ICP measurements of mitochondrial fractions, Vero cells were treated with 50 µM of the different catalysts in DMEM-FBS for 60 minutes. Then, a commercial kit (Mitochondria Isolation Kit -Thermo-Fisher Scientific) was used to isolate mitochondrial and cytosolic cellular fractions. The fractions obtained were digested in duplicate in HNO_3/H_2O_2 by microwave heating and analyzed.

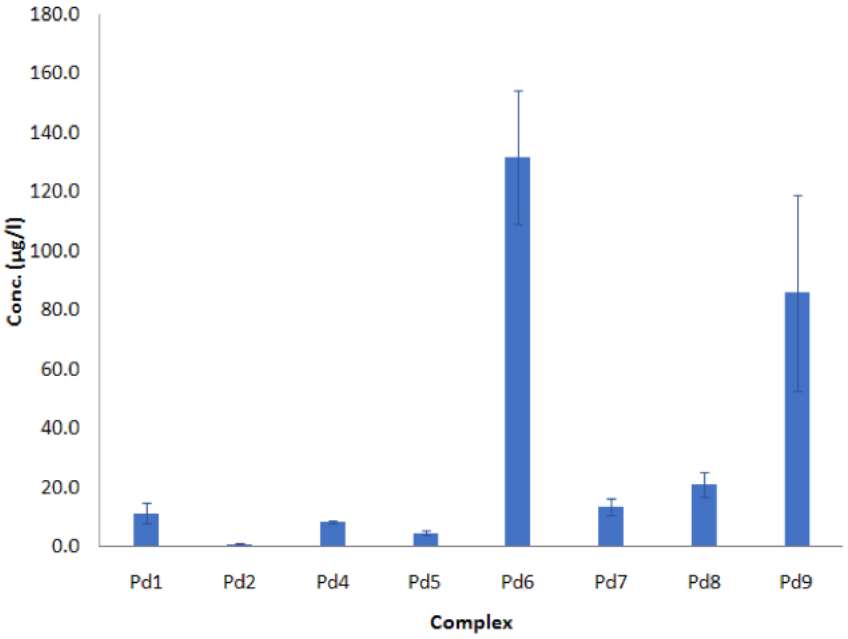


Figure S12. ICP-MS measurements of palladium content in Vero cells incubated with different palladium complexes, after washing and lytic treatment**.** The analysis reflects all palladium accumulated in different parts of the cell. Error bars represent the standard error of three independent experiments.

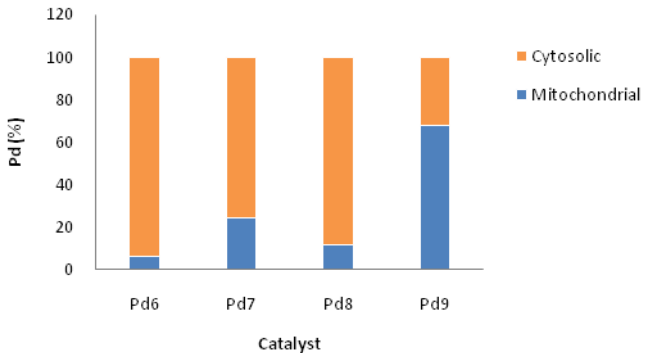


Figure S13. ICP-MS results of the **Pd6-9** accumulated in living cells and the fractioning of their accumulation in cytosol and mitochondria in % distribution.

4.2 Viability Assays

The toxicity of the catalysts **Pd1-Pd9** was tested by MTT assays in Vero cells as follows: 75000 cells per well were seeded in 96 well plates one day before treatment with different concentrations of the catalysts. After 24 h of incubation, Thiazolyl Blue Tetrazolium Bromide (*Sigma*) was added to the cell culture medium to a final concentration of 0.5 mg/ml. Cells were then incubated for 4 h to allow the formation of formazan precipitates by metabolically active cells. A detergent solution of 10% SDS (sodium dodecyl sulphate) and 0.01 M HCl was then added and the plate was incubated overnight at room temperature to allow the solubilization of the precipitates. The quantity of formazan in each well (directly proportional to

the number of viable cells) was measured by recording changes in absorbance at 570 nm in a microtiter plate reading spectrophotometer (*Tecan Infinite 200 PRO*).

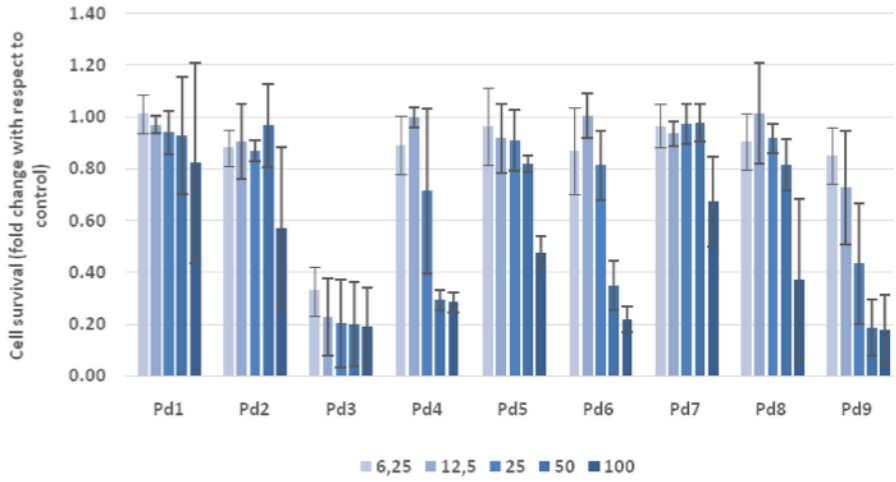


Figure S14. Viability Assays. Vero cells were incubated in cell culture medium containing the indicated amounts of the catalysts **Pd1-9** for 24 h and analysed by MTT assay. The viability is expressed as the fold change of the absorbance value with respect to untreated cells (value 1.0). Error bars indicate the standard deviation of three independent experiments.

4.3 Reactions in living cells

Probes **5** and **7** were selected because the released products **6** and **8** possess optimal photophysical properties for the microscopy imaging of the cellular experiments, both for uncaging of the propargyl[5] and alloc[6] groups, respectively.

The palladium complexes added to the cells were initially dissolved in DMSO (20 mM) before being added to DMEM, so the final concentration of DMSO in DMEM is minimal (<0.5%).

Microscopy settings: Widefield micrographs were taken with a Nikon Eclipse TiE equipped with an AndorZyla 4.2 camera. Confocal images were acquired in an Andor Dragonfly High Speed Confocal Platform. The filter sets for the observation of the different fluorophores were as follows:

Pd9: Widefield: LED λ excitation: 385 nm. Filter cube DAPI-1160B-000 (Semrock): BP 387/11-25 nm, LP 447/60-25 nm and DM 409 nm. Confocal: Laser excitation: 405 nm. LP 450/50 and DM 418 nm.
6: Widefield: LED λ excitation: 385 nm. Filter cube: BP 375/28x nm, LP 515lp nm and DM 415 nm.
8: Widefield: LED λ excitation: 470 nm. Filter cube FITC-3540C-000 (Semrock): BP 482/35 nm, LP 536/40 nm and DM 506 nm. Confocal: Laser excitation: 488 nm. LP 525/50 and DM 501 nm.
TMRE (tetramethylrhodamine, ethyl ester) LED λ excitation: 550 nm. Filter cube TRITC-B-000 (Semrock): BP 543/22-25 nm, LP 593/40-25 nm and DM 562 nm. Confocal: Laser excitation: 561 nm. LP 620/60 and DM 567 nm.

Incubations

Unless otherwise indicated, all incubations were made in a humidified incubator at 37°C in an atmosphere containing 5% CO_2:

Preincubation with the probes: Vero or HeLa cells were seeded on glass coverslips 24h before incubation. Culture medium was removed and DMEM containing 5% fetal bovine serum (FBS-DMEM) and probes **5** (50 μM) or **7** (100 μM) were added. After a 30-minute incubation, cells were washed twice with FBS-DMEM and 50 μM of a solution of metal complexes in FBS-DMEM were added. After a 30-minute incubation, cells were washed twice in FBS-DMEM, and observed in a widefield fluorescence microscope with adequate filters.

Preincubation with the metal complexes: Vero or HeLa cells were seeded on glass coverslips 24h before incubation. Culture medium was removed and 50 μM of the palladium complexes in FBS-DMEM were added. After 30-minute incubation, cells were washed twice with FBS-DMEM, and FBS-DMEM containing **5** (50 μM) or **7** (100 μM) was added. After a 30-minute incubation, cells were washed twice in FBS-DMEM, and observed in a widefield fluorescence microscope with adequate filters.

Digital pictures of the different samples were taken under identical conditions of gain and exposure.

4.3.1 Uncaging of propargylic probe 5

Note: There is an overlap between the excitation wave length of probe **6** and that of the pyrene moiety of complex **Pd9** (< 420 nm), therefore, it was not possible the imaging of the uncaging of **5** with this catalyst.

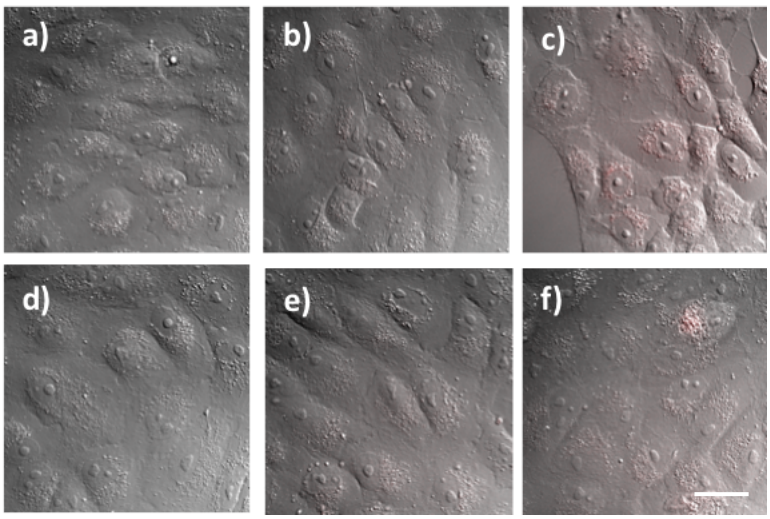


Figure S15. Imaging of the uncaging of **5** in Vero cells with complexes **a) Pd1**; **b) Pd2**, **c) Pd3**, **d) Pd4**, **e) Pd5**, and **f) control** without palladium complexes. Reaction conditions: **5** (50 μM) was incubated in DMEM with 5% Fetal Bovine Serum for 30 min before the Pd complex (50 μM) was added, and the cells were incubated for another 30 min (Scale bar = 20 μm).

Estimation of TON

For the estimation of the TON of the uncaging reaction using probe **5**, 500.000 HeLa cells growing in six-well plates were incubated with probe **5** (50 μM) for 30 minutes followed by incubation with Pd catalysts **Pd6-9** (50 μM) for another 30 minutes. Afterwards, the cells were washed twice with PBS and the cell monolayer was treated with 200 μL of 80% MeOH in water. This methanol extract was recovered and measured in a Varian Cary Eclipse Fluorimeter in a quartz Hellma® fluorescence cuvette with a path length 10 x 4 mm, chamber volume 1.4 mL, exciting at 385 nm and recording the emission spectra between 500-750 nm. For the determination of the concentration, we used a fluorescence calibration curve for **6** [y= 138.2 (1) x + 2 (4)].

Using the resulting concentration data and considering the amount of intracellular Pd as calculated by ICP-Ms, we could obtain an average TON from two repetitions. It is important to note that the protocol only allows obtaining an estimation.

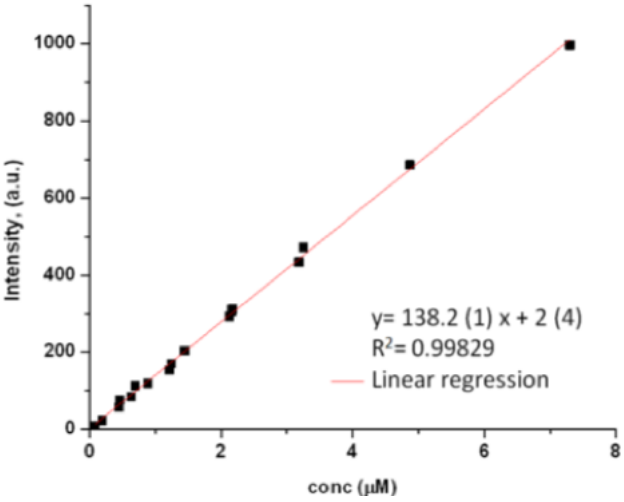


Figure S16. Fluorescence emission calibration curve for compound **6.**

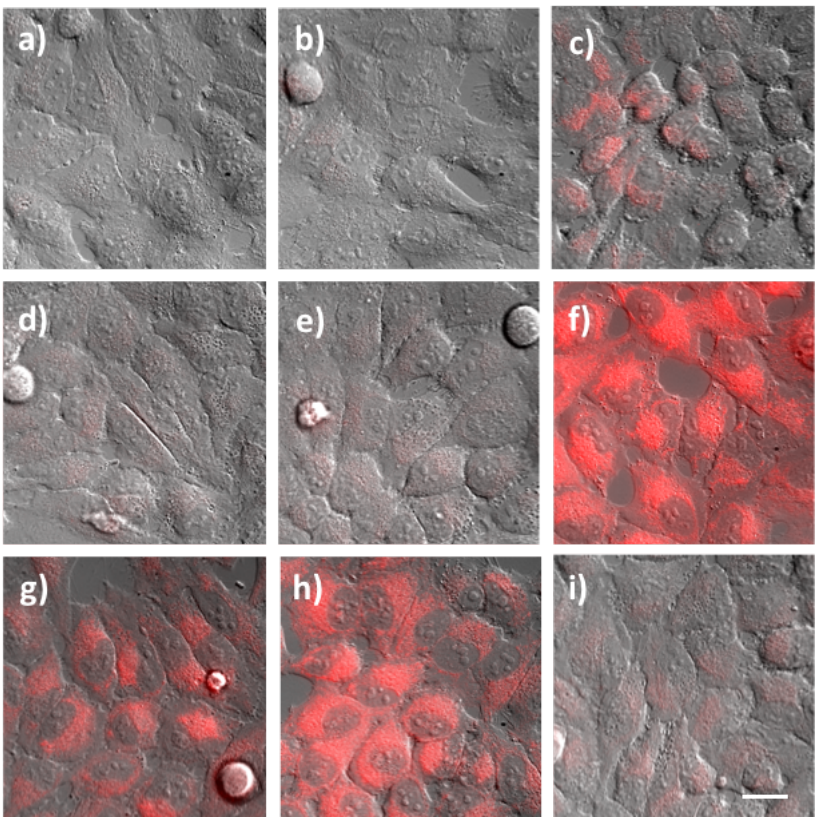


Figure S17. Imaging of the uncaging of probe **5** in HeLa cells with different palladium reagents **a) Pd1**; **b) Pd2**, **c) Pd3, d) Pd4, e) Pd5, f) Pd6, g) Pd7, h) Pd8** and **i) control** without palladium. In contrast to that observed with Vero cells, catalyst **Pd8** elicited higher intracellular fluorescence than **Pd7**. Reaction conditions: **5** (50 μM) was added to the cells in DMEM with 5% Fetal Bovine Serum, and incubated for 30 min, after standard washing with DMEM, cells were mixed with the Pd complexes (50 μM) for other 30 min and visualized under the microscope (Scale bar = 20 μm).

4.3.2 Uncaging of alloc probe 7

- Cellular activity of the palladium complexes in HeLa cells:

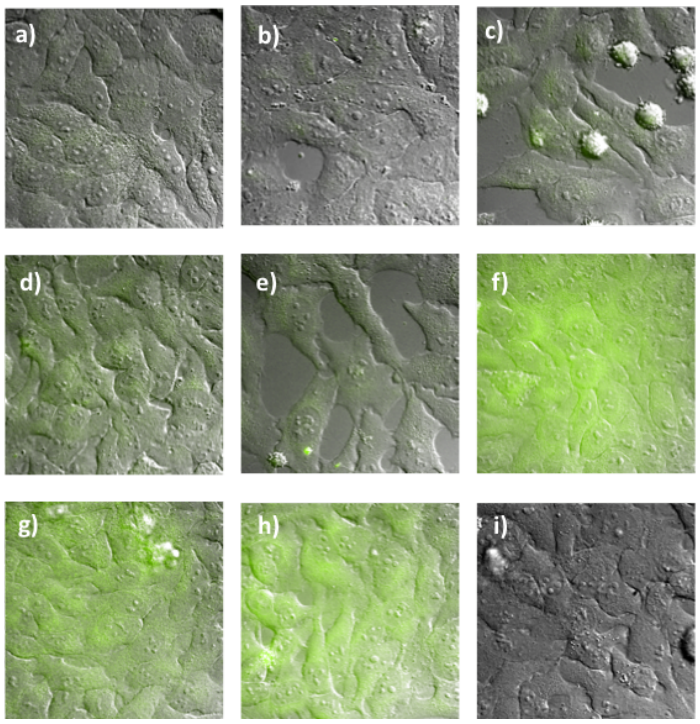


Figure S18. Comparative micrographies obtained after the Pd catalysed uncaging of **7** in HeLa cells with catalysts **a) Pd1**, **b) Pd2**, **c) Pd3**, **d) Pd4**, **e) Pd5**, **f) Pd6**, **g) Pd7**, **h) Pd8**, and **i) control**. Reaction conditions: probe **7** (50 μM) was incubated in DMEM with 5% Fetal Bovine Serum for 30 min. After standard washing, the Pd complexes (50 μM) were added, and the cells further incubated for another 30 min, and visualized (Scale bar = 20 μm).

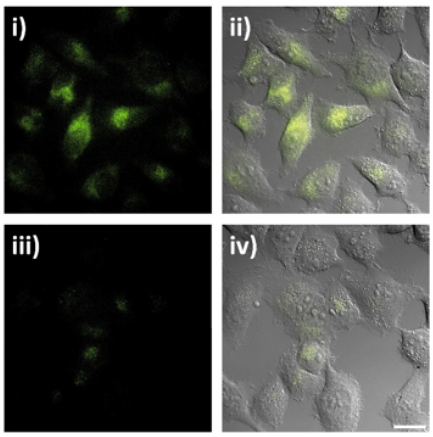


Figure S19. Fluorescence micrographies obtained using palladium complex **Pd8** for the uncaging of **Rho-alloc 7** in HeLa cells; i) Green channel; ii) merge of green channel with brightfield; iii) mock (green channel) and iv) merge of mock with brightfield. Reaction conditions: **Pd8** (50 μM) was incubated with cells in DMEM with 5% Fetal Bovine Serum for 30 min. After standard washing, probe **7** (50 μM) was added and the cells were incubated for another 30 min and visualized (Scale bar = 20 μm).

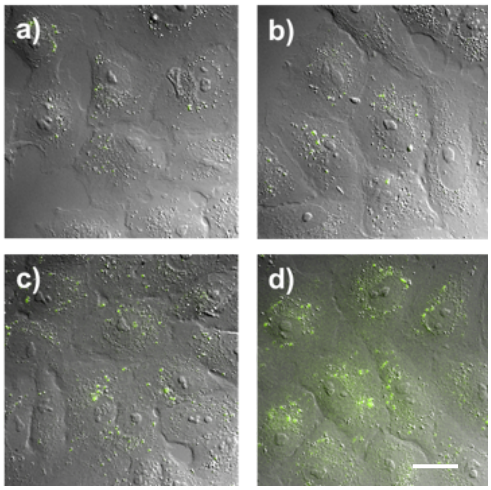


Figure S20. Fluorescence micrographies for the Pd-promoted uncaging of **7** in Vero cells with different palladium complexes: **a)** control, **b) Pd4**, **c) Pd7** and **d) Pd8**. Reaction conditions: **Pd8** (50 μM) was incubated with cells in DMEM with 5% Fetal Bovine Serum for 30 min. After standard washing, probe **7** (50 μM) was added and the cells were incubated for another 30 min and visualized (Scale = 20 μm).

Intracellular localization with Pd8 and Pd9

Cells growing on glass coverslips were incubated with the Pd complexes (50 μM) for 2 hours. Cells were then washed twice with FBS-DMEM and incubated with the probe **7** (100 μM) in FBS-DMEM for 30 minutes. Finally, the mitochondrial marker TMRE was added in FBS-DMEM to a final concentration of 100 nM and cells incubated for 10 min. Prior to the observation by fluorescence microscopy, the samples were washed twice with fresh FBS-DMEM. For a better determination of the degree of colocalization, the coverslips were observed *in vivo* in a confocal microscope equipped with adequate filters. Digital pictures of the different samples were taken under identical conditions of gain and exposure.

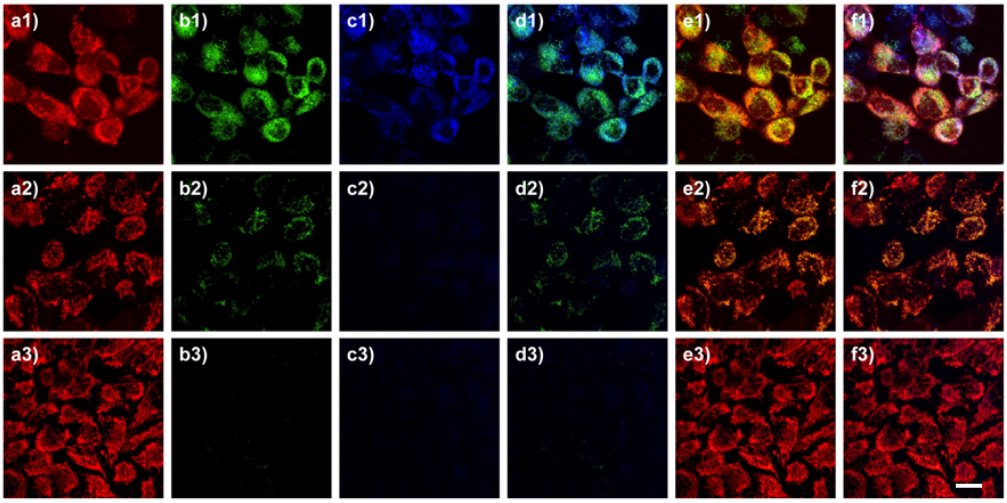


Figure S21. Imaging of the Pd catalyzed uncaging of **7** in HeLa cells preincubated with complexes **Pd9** (a1-f1), **Pd8** (a2-f2) and control without palladium (a3-f3) and stained with the mitochondrial marker TMRE. a1-a3) TMRE, b1-b3) green fluorescence resulting from the formation of uncaged probe **8**, c1-c3) blue fluorescence of the pyrene moiety present in **Pd9**, d1-d3) merge of blue and green channels, e1-e3) merge of red and green, f1-f3) merge of all three channels (Scale bar = 20 μm).

4.3.3 Uncaging of Rho-poc

HeLa cells were seeded on glass coverslips 24h before incubation. Culture medium was removed and 50 μM of the palladium complexes in FBS-DMEM were added. After a 30-minute incubation, cells were washed twice with FBS-DMEM, and FBS-DMEM containing **Rho-poc** (50 μM) was added. After a 30-minute incubation, cells were washed twice in FBS-DMEM, and observed in a confocal microscope with adequate filters.

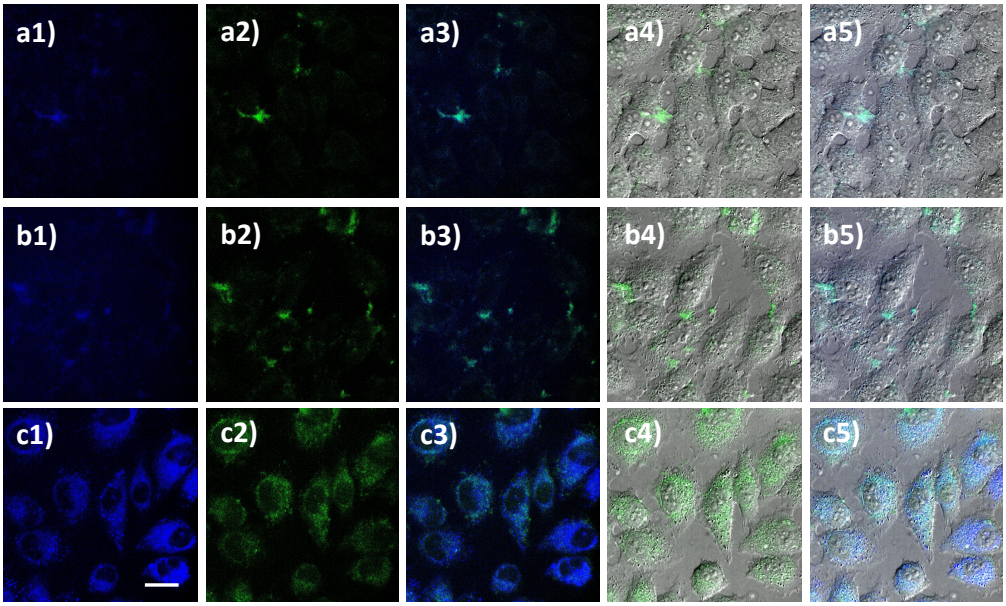


Figure S22. Imaging of the Pd catalyzed uncaging of **Rho-poc** in HeLa cells: control without palladium (a1-a5); preincubated with complexes **Pd8** (b1-b5) and **Pd9** (c1-c5); (a2-c2) green fluorescence that is coming from uncaged **Rho-poc**, (a1-c1) blue fluorescence of the pyrene moiety present in **Pd9** (c1), (a4-c4) bright-field-green channel merging, (a5-c5) merge of brightfield, blue and green channels. (Scale bar = 25 μm).

5. References

(1) Tomás-Gamasa, M.; Martínez-Calvo, M.; Couceiro, J. R.; Mascareñas, J. L. Transition Metal Catalysis in the Mitochondria of Living Cells. *Nat. Commun.* **2016**, *7*, 12538.

(2) Bettucci, L.; Bianchini, C.; Filippi, J.; Lavacchi, A.; Oberhauser, W. Chemoselective Aerobic Diol Oxidation by Palladium(II)-Pyridine Catalysis. *Eur. J. Inorg. Chem.* **2011**, *2011* (11), 1797–1805.

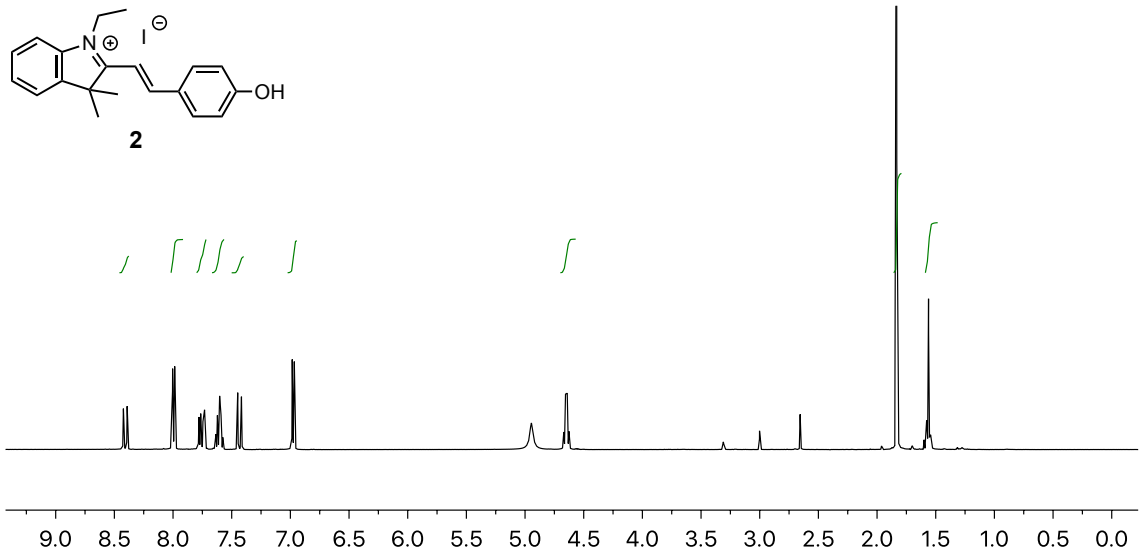
(3) Fantasia, S.; Nolan, S. P. A General Synthetic Route to Mixed NHC-Phosphane Palladium(O) Complexes (NHC = N-Heterocyclic Carbene). *Chem. - A Eur. J.* **2008**, *14* (23), 6987–6993.

(4) Kong, F.; Zhao, Y.; Liang, Z.; Liu, X.; Pan, X.; Luan, D.; Xu, K.; Tang, B. Highly Selective Fluorescent Probe for Imaging H 2 Se in Living Cells and in Vivo Based on the Disulfide Bond. *Anal. Chem.* **2017**, *89* (1), 688–693.

(5) Gao, T.; Xu, P.; Liu, M.; Bi, A.; Hu, P.; Ye, B.; Wang, W.; Zeng, W. A Water-Soluble Esipt Fluorescent Probe with High Quantum Yield and Red Emission for Ratiometric Detection of Inorganic and Organic Palladium. *Chem. - An Asian J.* **2015**, *10* (5), 1142–1145.

(6) Streu, C.; Meggers, E. Ruthenium-Induced Allylcarbamate Cleavage in Living Cells. *Angew. Chem. Int. Ed.* **2006**, *45* (34), 5645–5648.

(7) Li, J.; Yu, J.; Zhao, J.; Wang, J.; Zheng, S.; Lin, S.; Chen, L.; Yang, M.; Jia, S.; Zhang, X.; Chen P. R. Palladium-Triggered Deprotection Chemistry for Protein Activation in Living Cells. *Nat. Chem.* **2014**, *6* (4), 352–361.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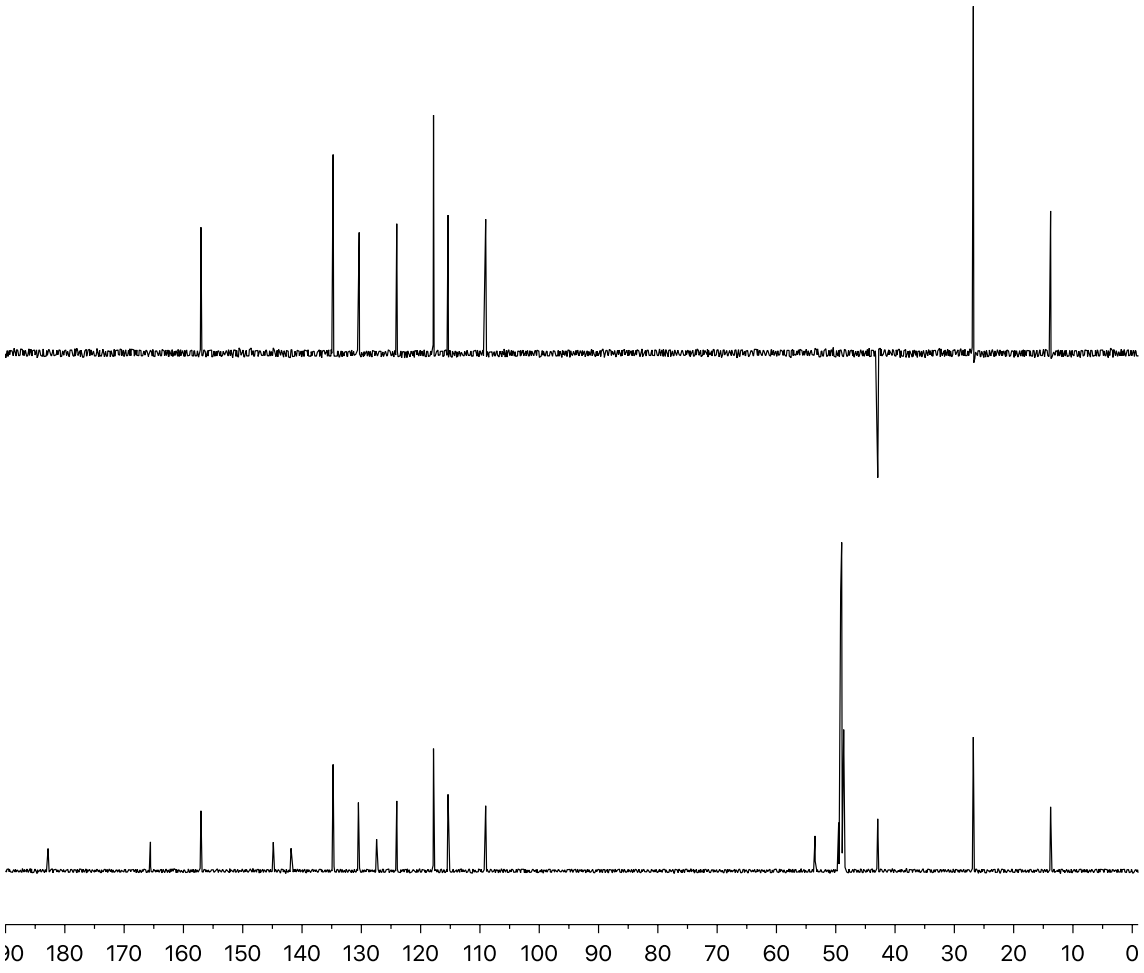
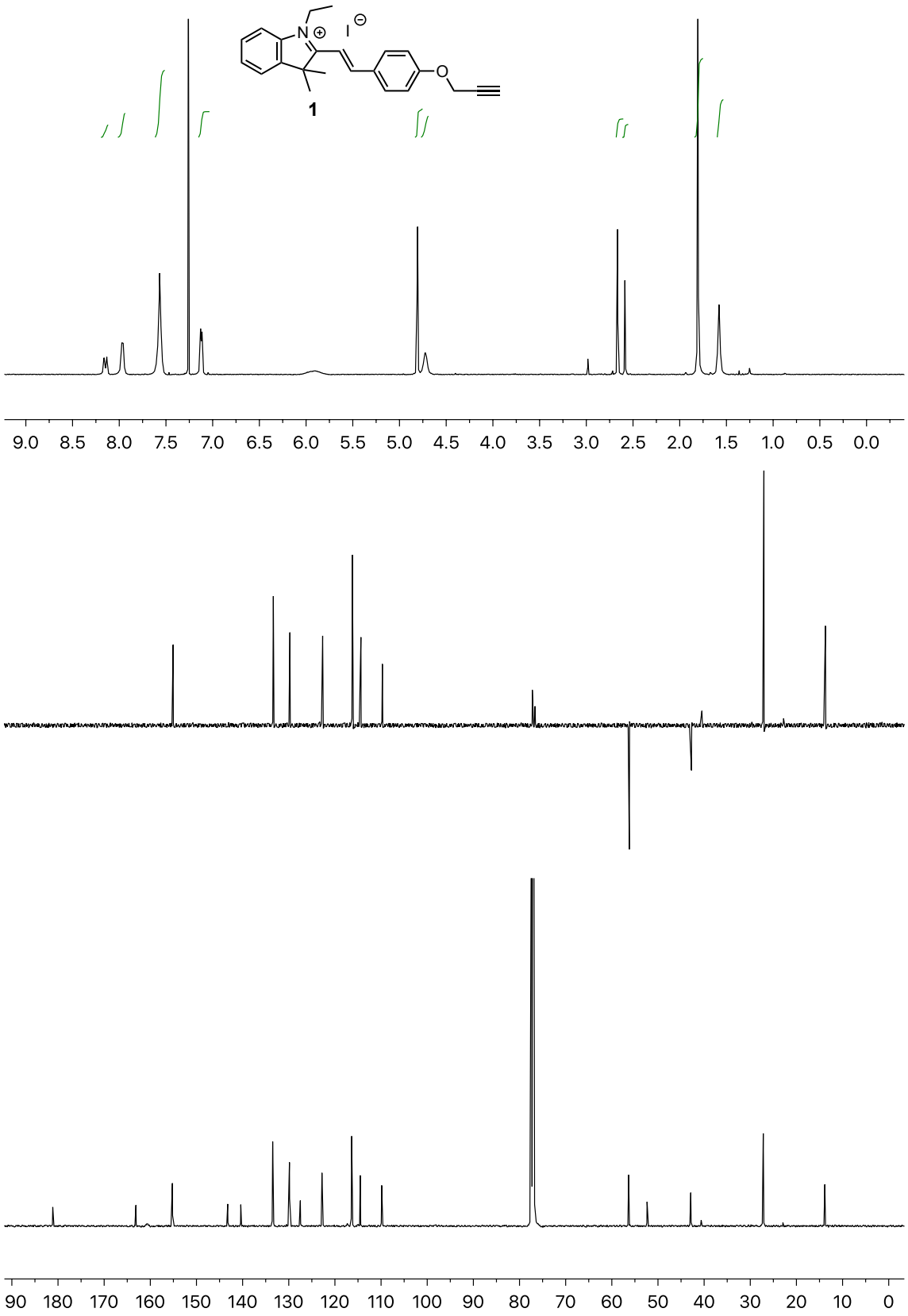
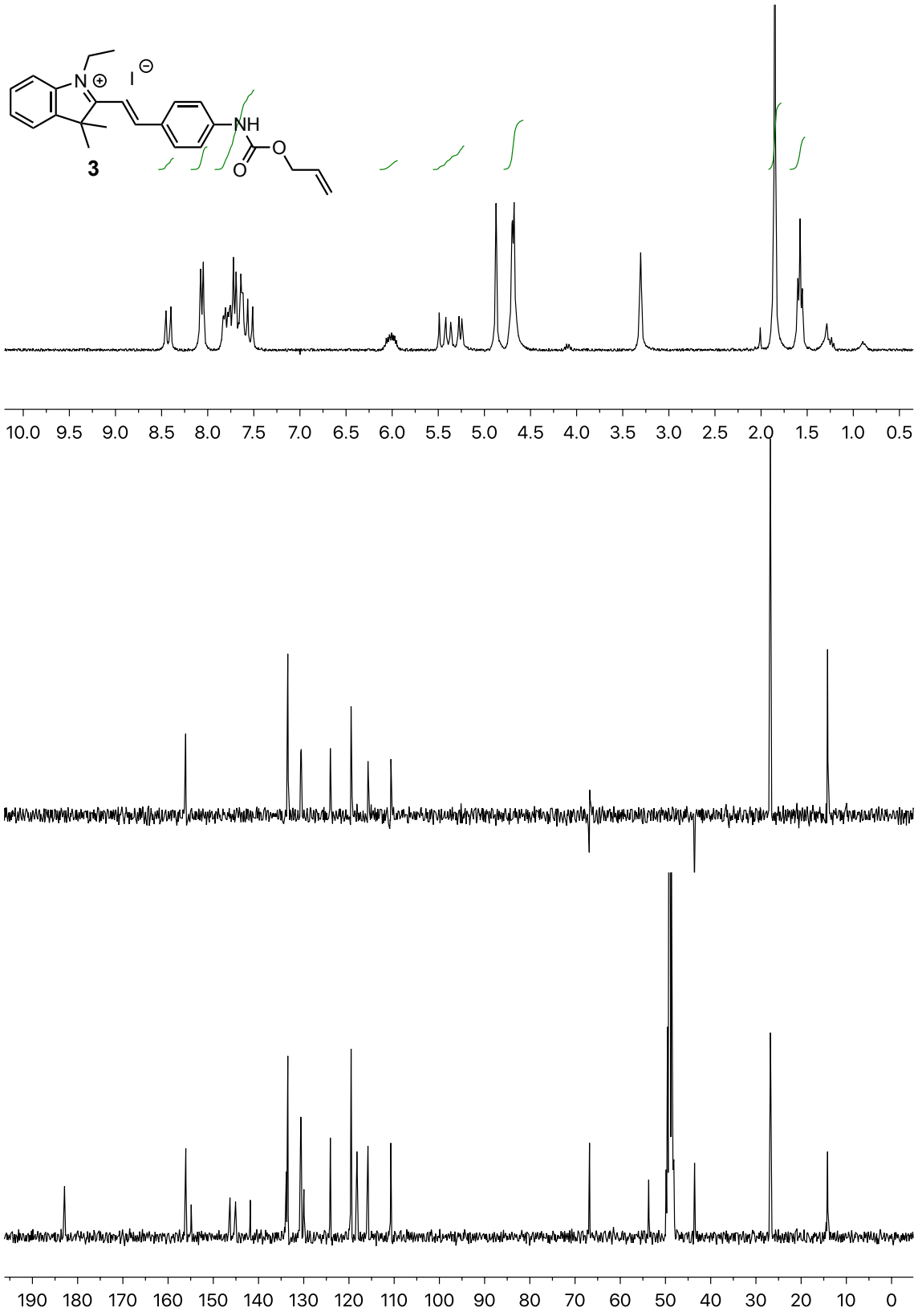
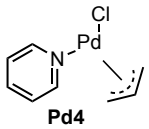
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0


180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

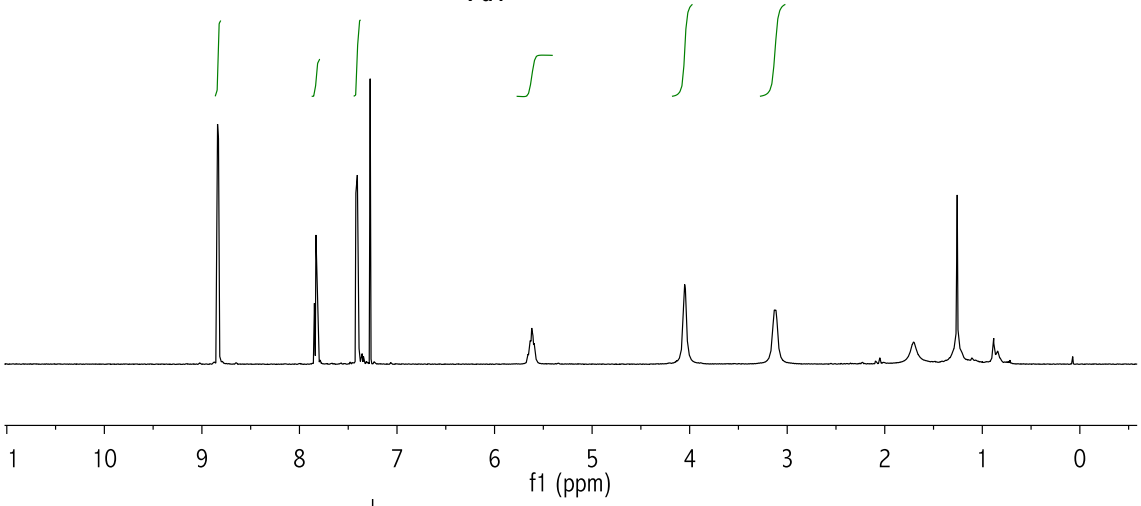

1
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
90 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

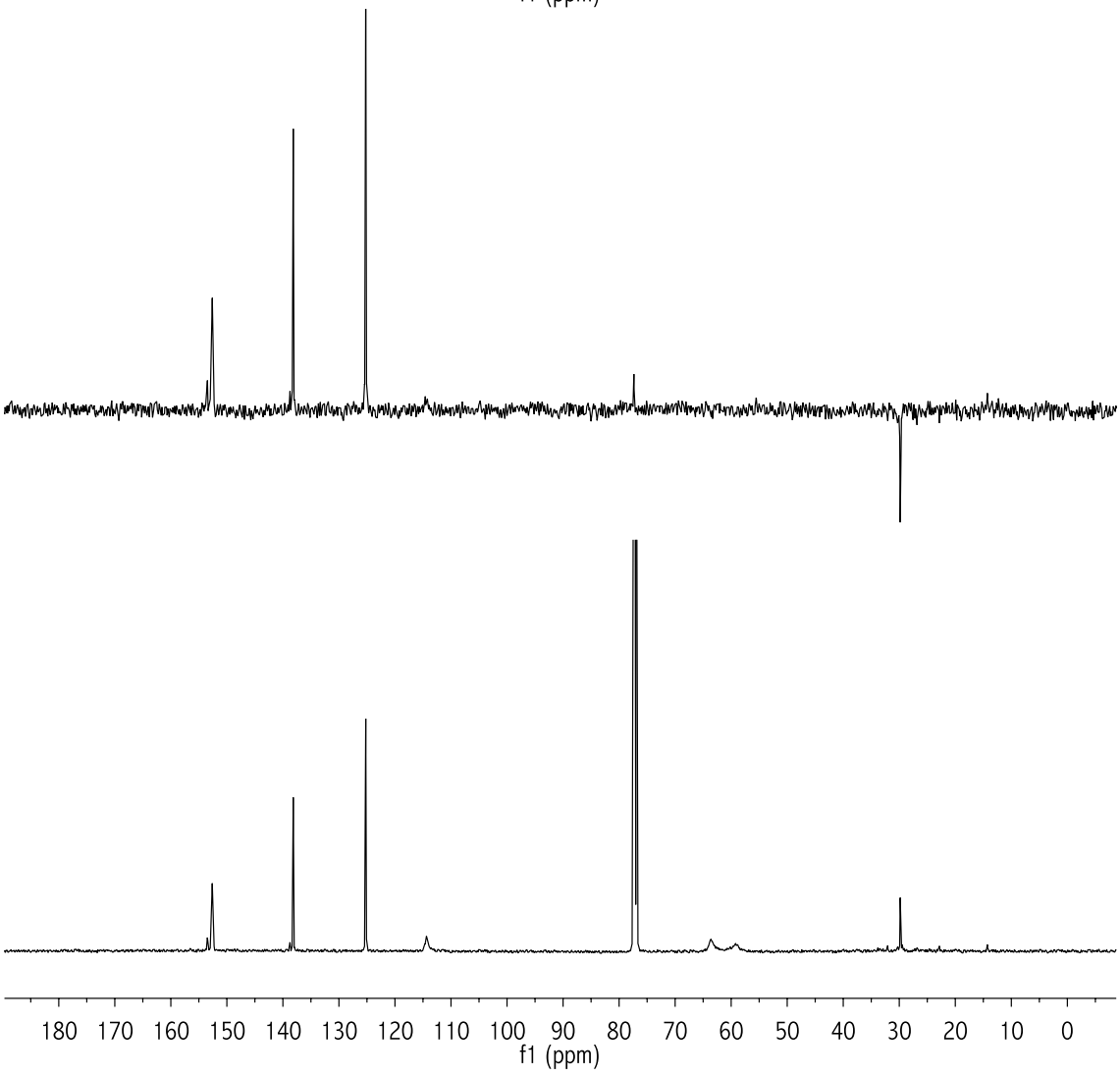

N
I
NH
O
O
3
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

Room Temperature spectra

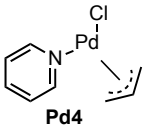


Pd4

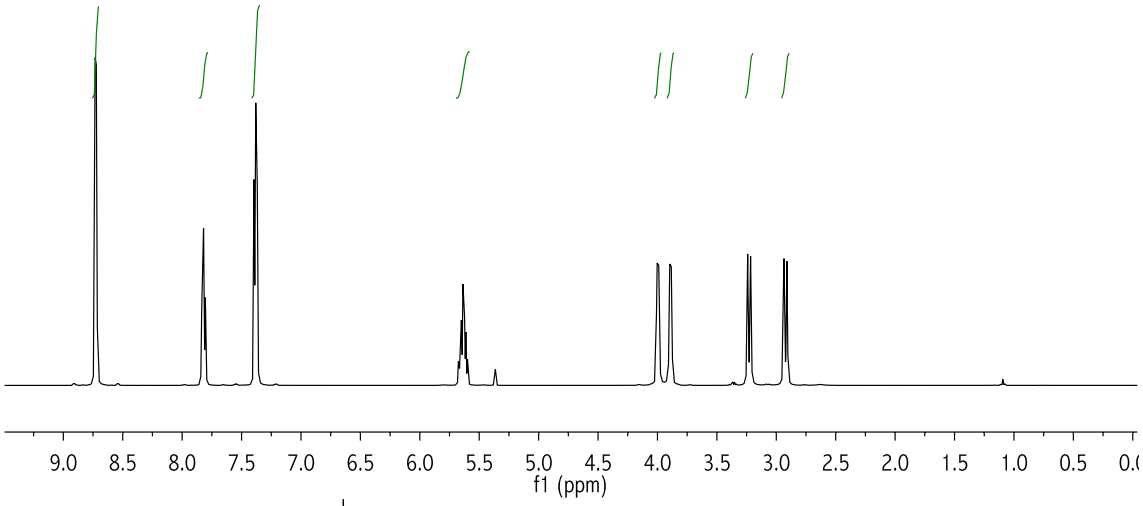


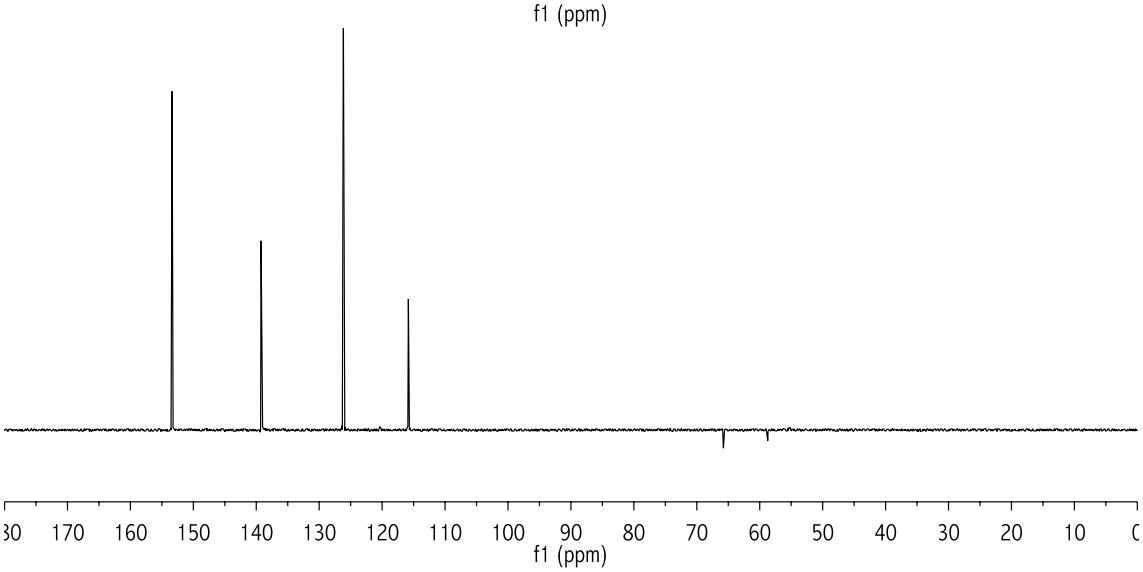


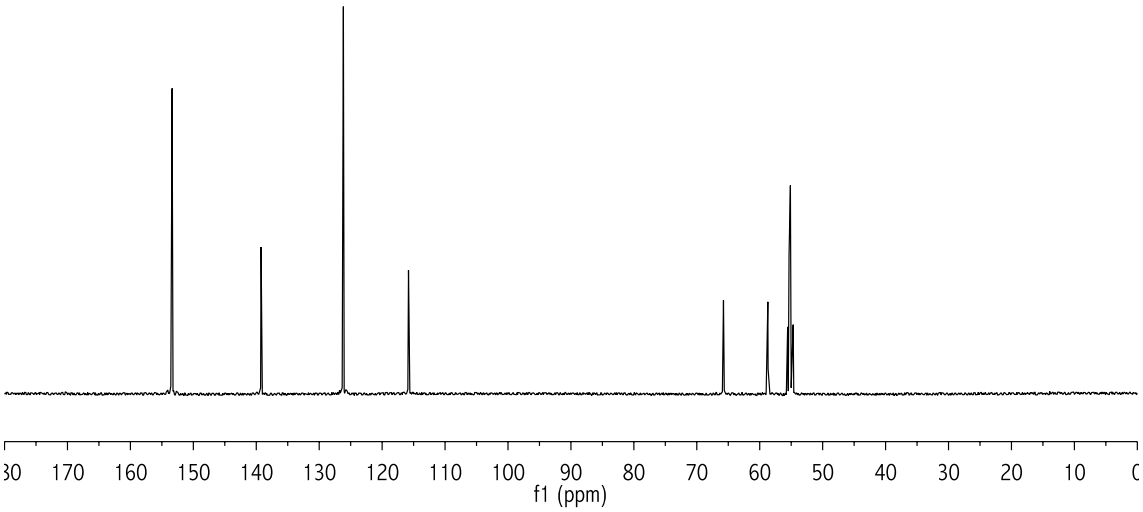
Low Temperature spectra (- 60°C)

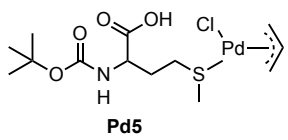


Pd4



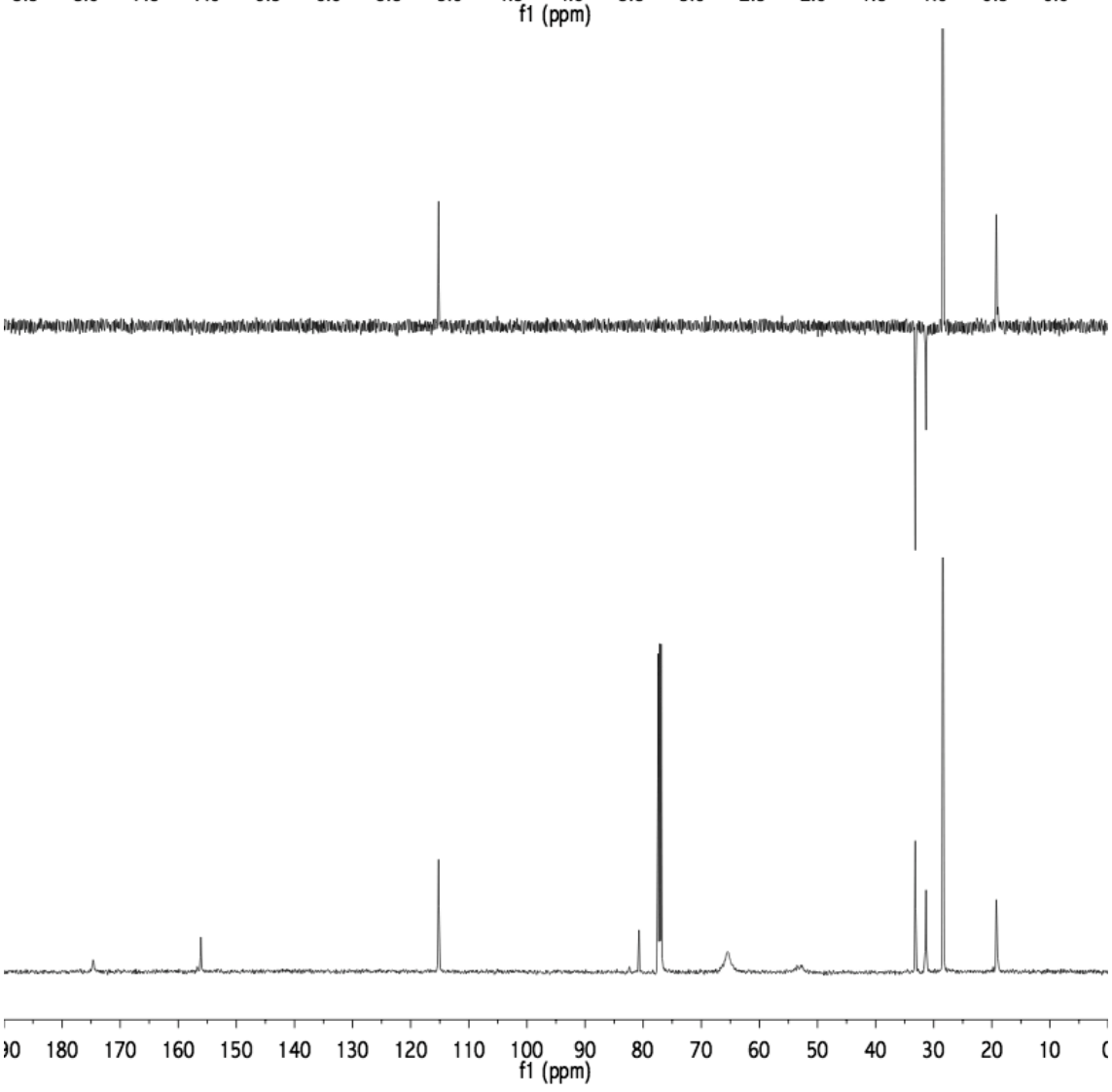


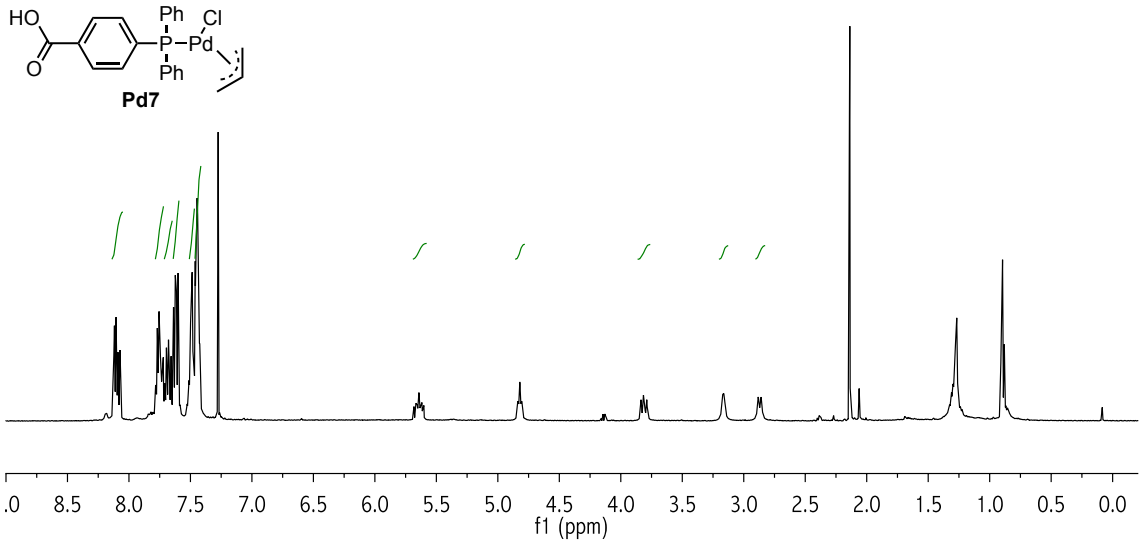


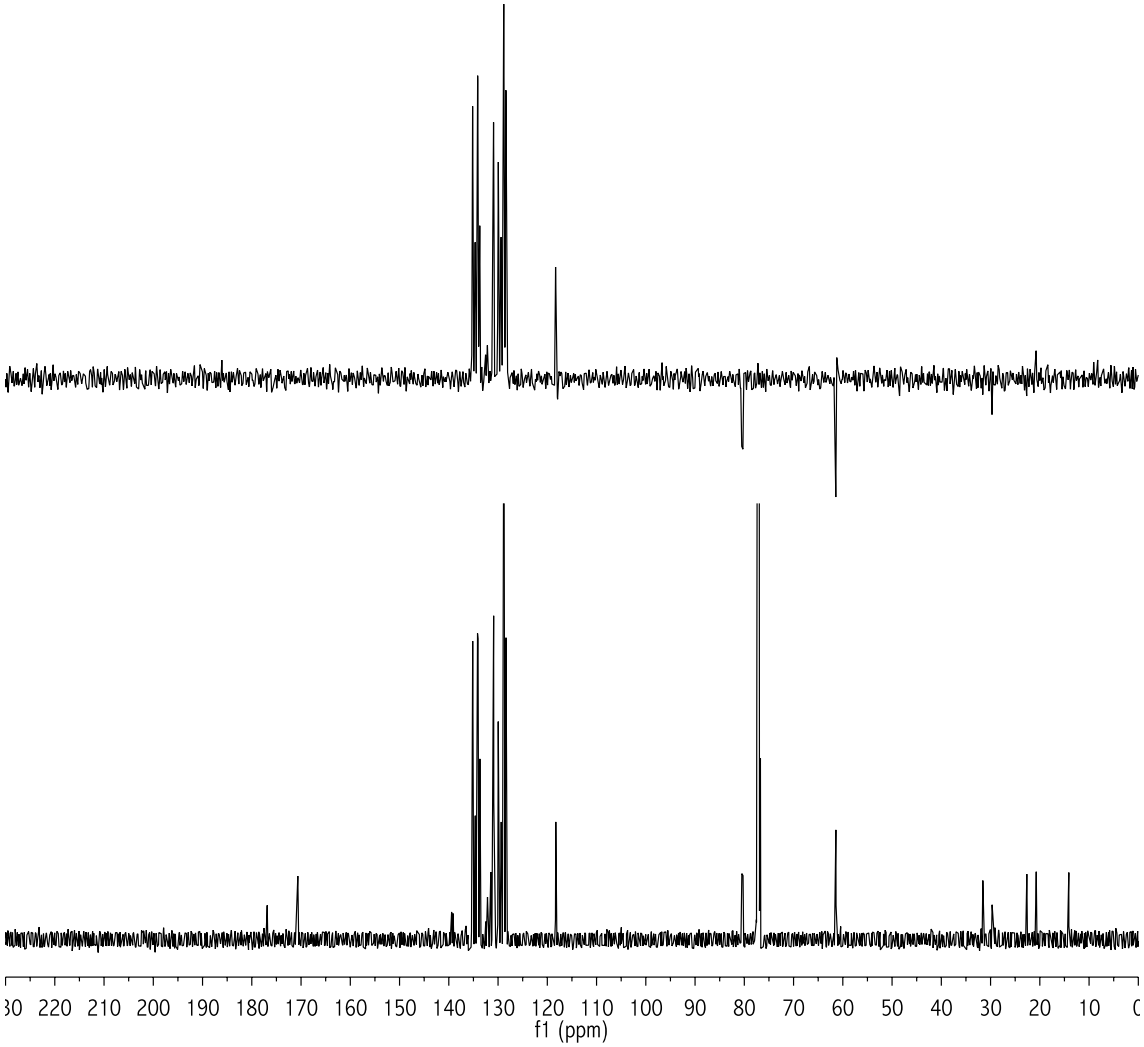

O
OH
Cl
Pd
O
N
H
S

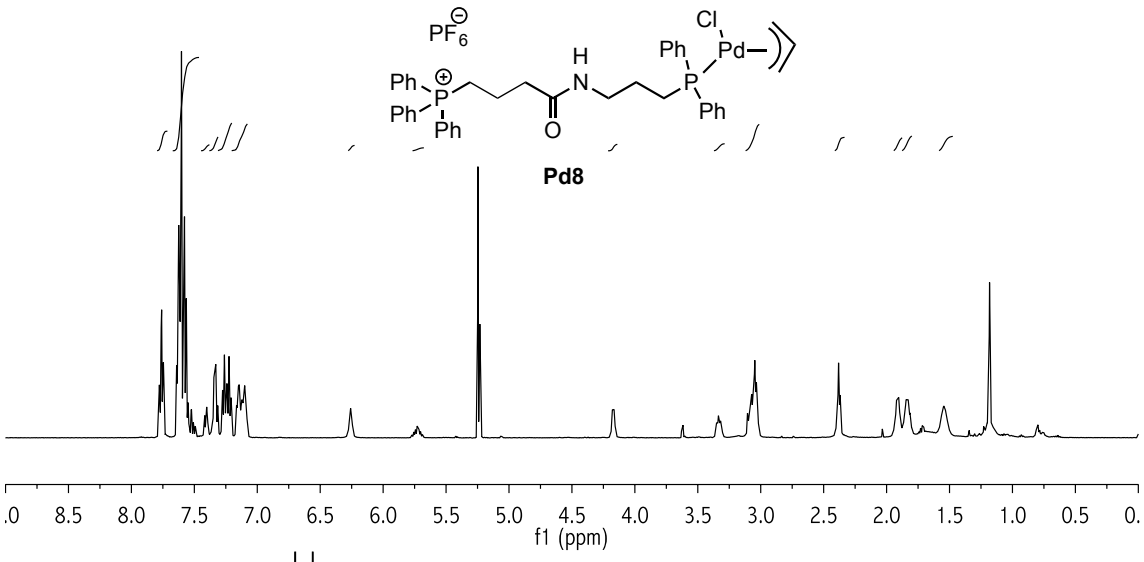
Pd5

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 0.0
f1 (ppm)


180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

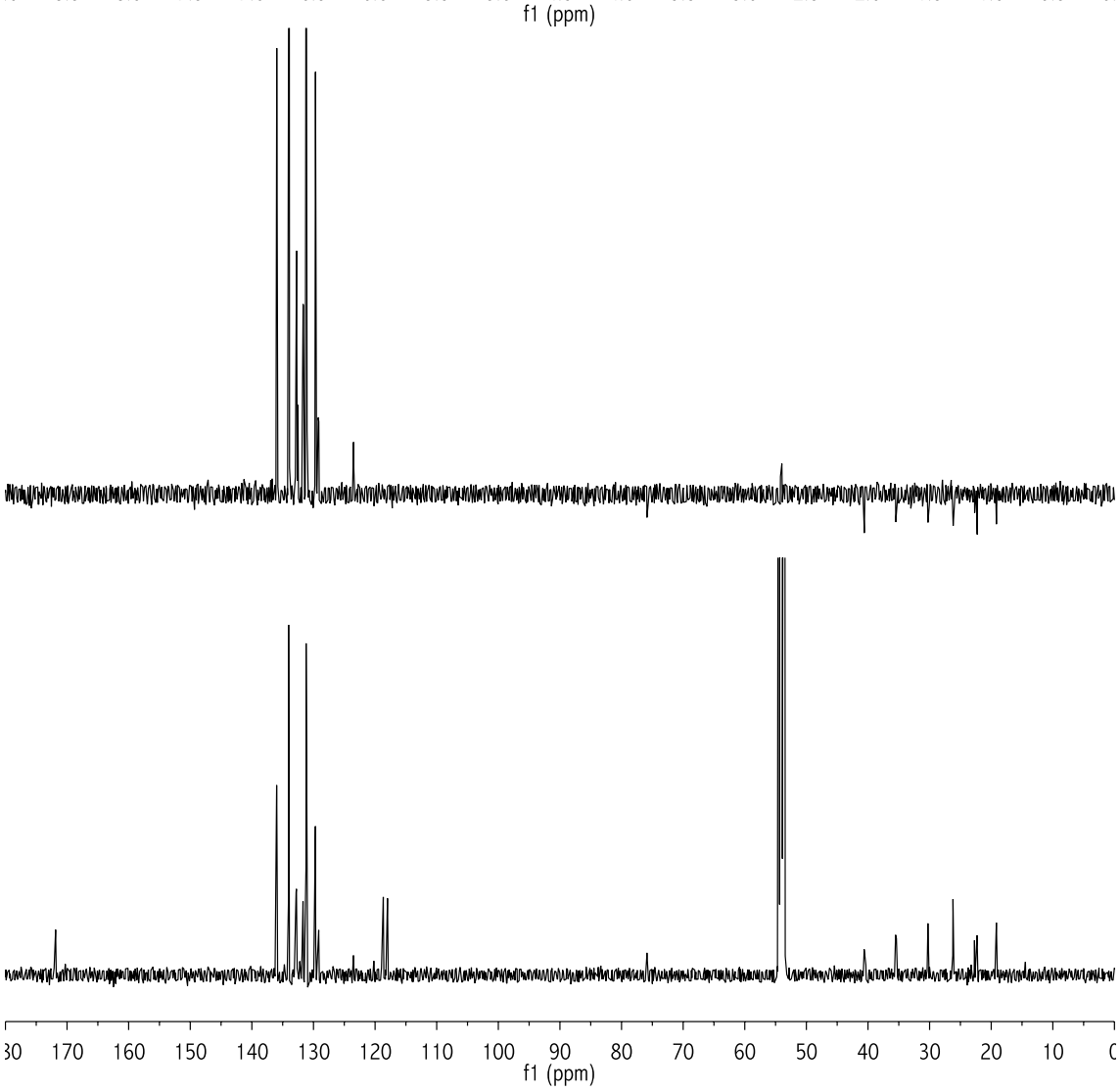

HO
O
Ph
Cl
P
Pd
Ph
Pd7
.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

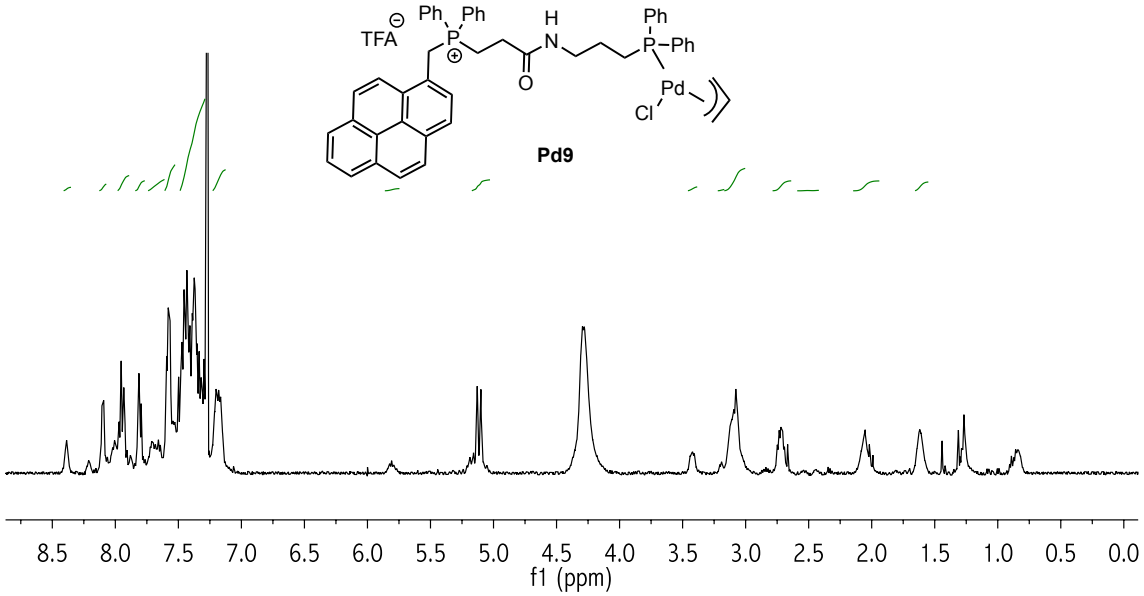

30 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

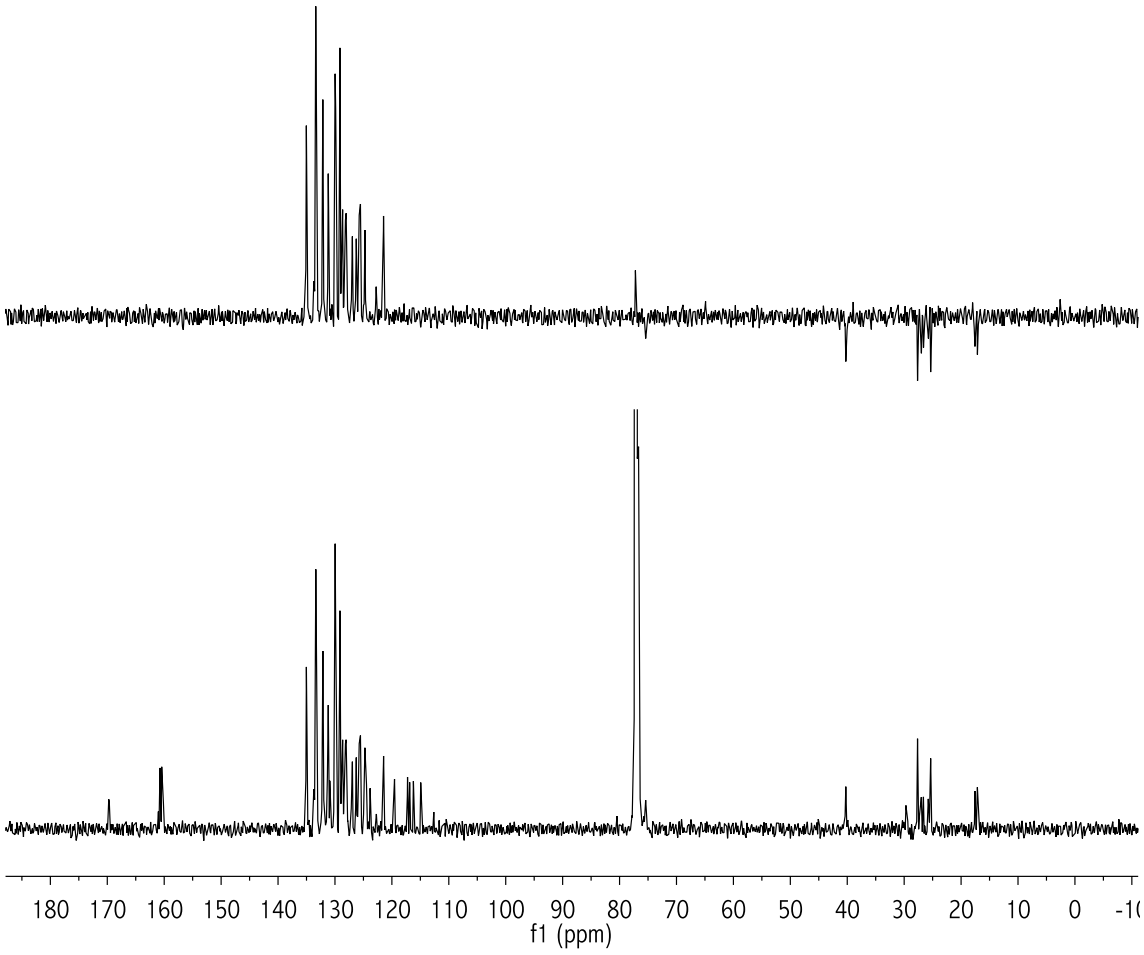

PF6
Ph
Ph
P
Ph
H
N
O
Cl
Ph
Pd
P
Ph

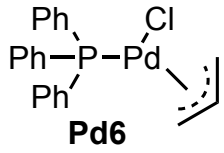
Pd8

.0 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 0.
f1 (ppm)


30 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


TFA
Ph
Ph
P
H
N
O
Ph
P
Ph
Pd
Cl
Pd9
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 0.0
f1 (ppm)


180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)



Pd6