Electronic Supplementary Material (ESI) for ChemComm.

Palladium-mediated *in situ* synthesis of an anticancer agent

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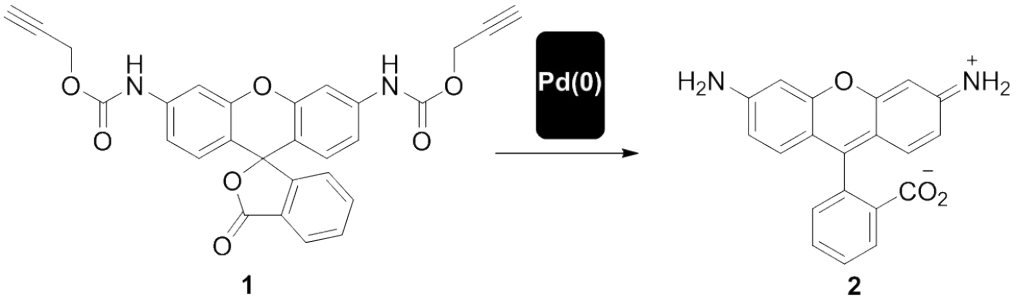
Table of Contents

SI. Supporting Figures



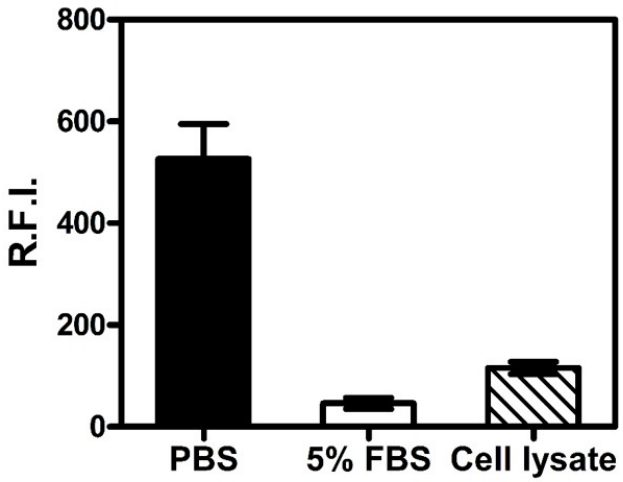


Figure S1. Pd catalysed Proc-rhodamine **1** decaging. A solution of **1** (50 μM) in PBS, 5 % FBS, or cell lysate (PC-3 cells) was incubated with the Pd catalyst (0.5 μmol Pd) for 18 h at 37 °C (n = 3). Relative increase in fluorescence (RFI) was measured at $\lambda_{Ex/Em}$ 495/520 nm and normalised to a control solution of **1** in the absence of the catalyst.

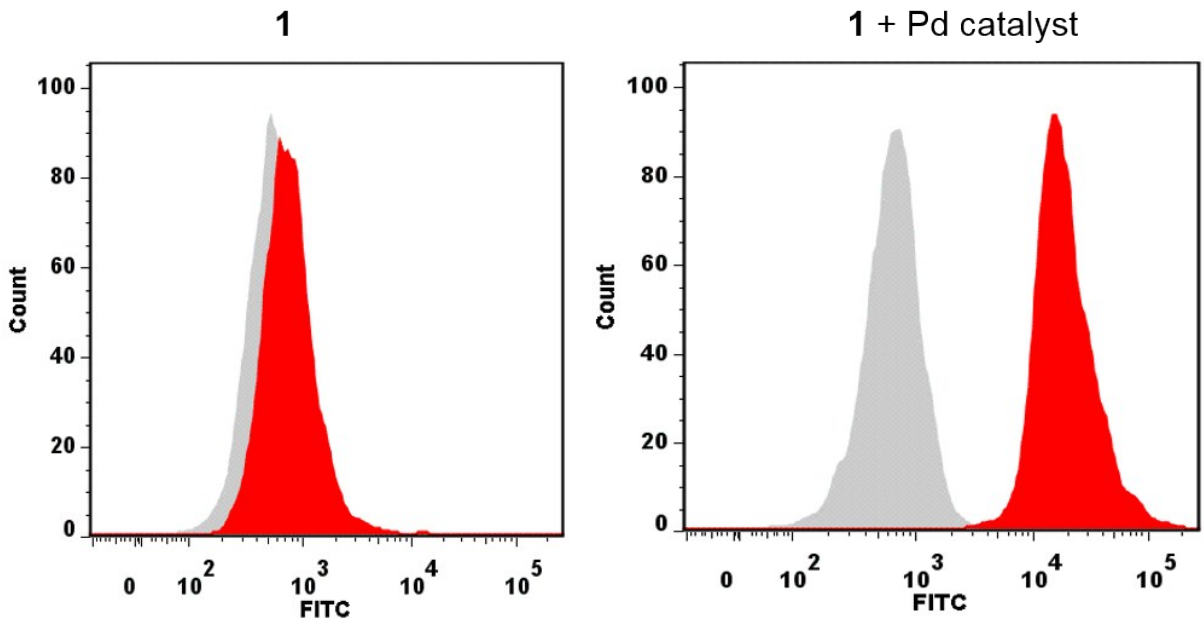


Figure S2. Flow cytometry histograms showing the presence of caged and decaged rhodamine 110 **2** with PC-3 cells. Left: untreated PC-3 cells in grey and cells treated with Proc-rhodamine **1** (20 μM, 18 h) in red. Right: untreated cells in grey and cells treated with Proc-rhodamine **1** (20 μM, 18 h) in the presence of the Pd catalyst (0.5 μmol Pd) in red.

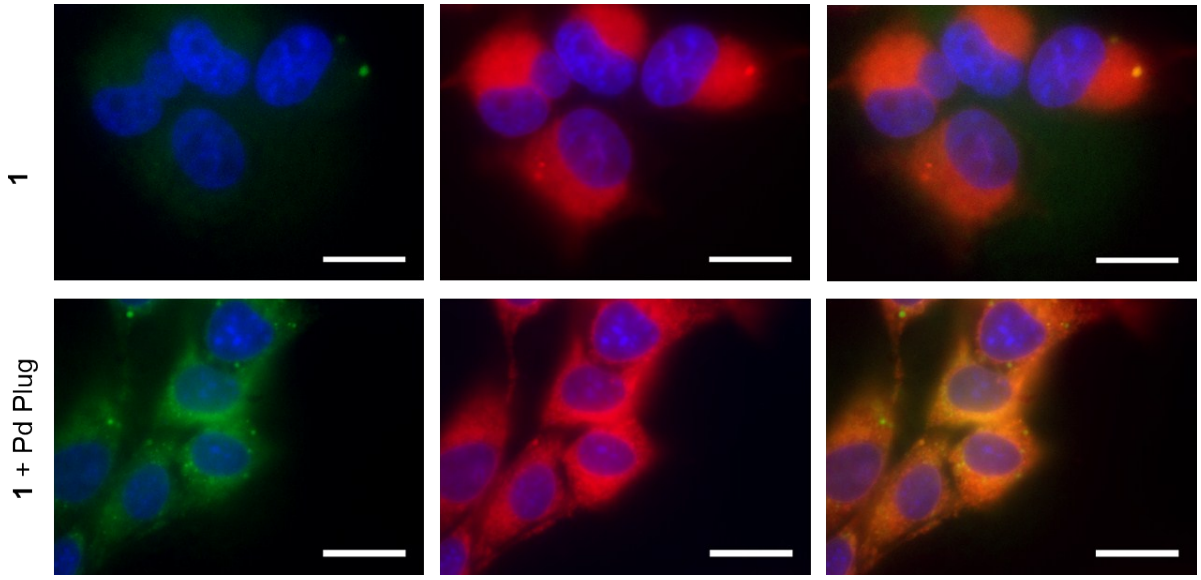


Figure S3. PC-3 cells incubated with non-fluorescent **1** (20 μM) in the presence of the Pd catalyst (0.5 μmol Pd) for 18 h. Cells were stained with CellTracker Red™ (cytoplasm stain), fixed with 4 % paraformaldehyde, incubated with DAPI (nuclei stain) and imaged by confocal microscopy. Top panels: without Pd, bottom panels: with Pd. Panels show from left to right: Cell nucleus (blue) and compound **2** (green), cell nucleus (blue) and cytoplasm (red), and merged images (orange indicates co-localization). Scale bar 20 μm. Microscope laser settings were: excitation laser lines at 405 nm, 488 nm, and 543 nm, with emission filters of 385–470 nm for DAPI (nuclei stain), 505–530 nm for Rhodamine 110 (**2**), 595–615nm for Texas Red (CellTracker Red™).

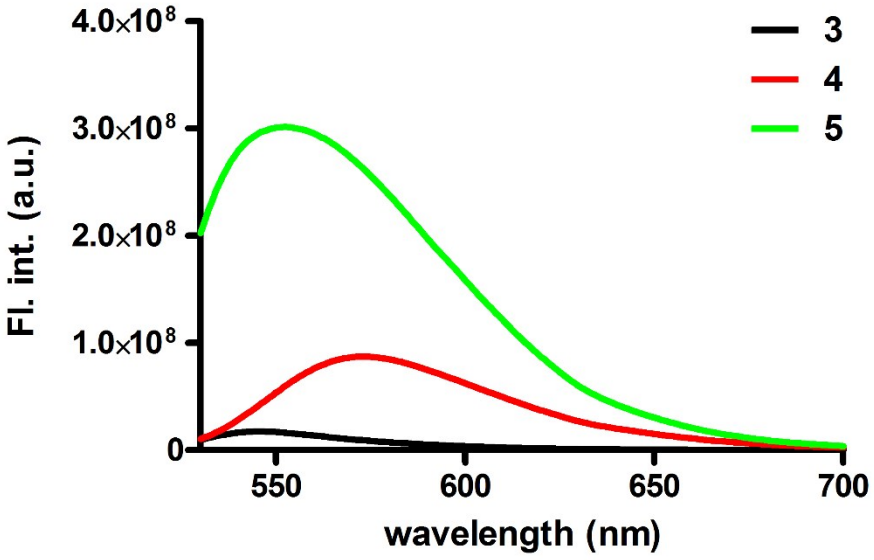


Figure S4. Emission spectra of *bis*-iodinated BODIPY **3** ($\lambda_{Ex/Em}$ 523/548 nm – black), *bis*-thienyl BODIPY **4** ($\lambda_{Ex/Em}$ 520/574 nm – red), and *bis*-phenyl BODIPY **5** ($\lambda_{Ex/Em}$ 518/552 nm – green).

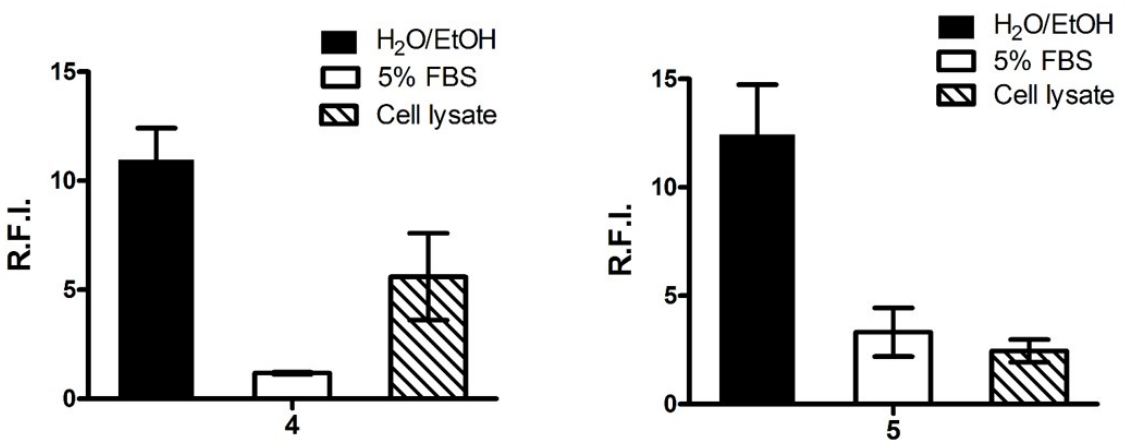


Figure S5. Relative increase in fluorescence of the Pd mediated cross-coupling of *bis*-iodo BODIPY with 2-thienyl boronic acid (left) and 4-phenyl boronic acid (right) (n = 3). Fluorescence increases are shown relative to a control solution of **3** and 2-thienyl boronic acid or 4-phenyl boronic acid. Compound **3** (20 μM) reacted with 2-thienyl boronic acid or 4-phenyl boronic acid (100 μM) in the presence of Pd catalyst (0.5 μmol Pd) in EtOH/H_2O, 5% FBS and PC-3 cell lysate (18 h) and fluorescence increase measured (λ_{Ex} 530/25 nm, λ_{Em} 590/35 nm and λ_{Ex} 495/25 nm, λ_{Em} 520/35 nm, respectively).

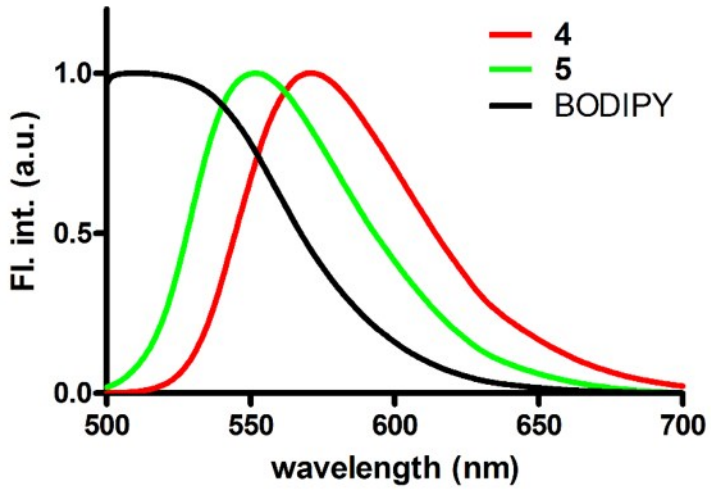


Figure S6. Normalised fluorescence emission spectra of compounds **4**, **5**, and de-iodinated 1,3,5,7,8-pentamethyl BODIPY (10 μM) in EtOH at λ_{Ex} 488 nm.

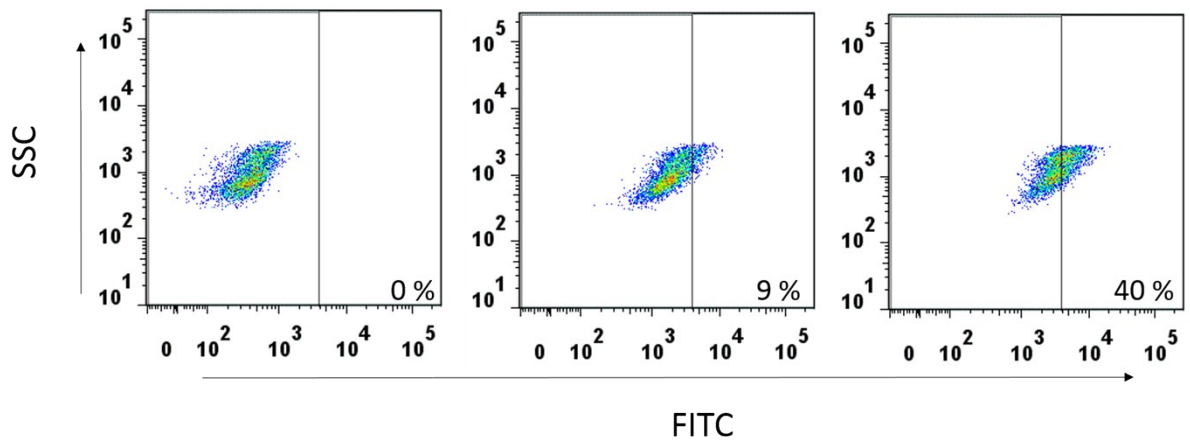


Figure S7: Flow cytometry cytograms showing the presence of cross-coupled product *bis*-phenyl BODIPY **5** in PC-3 cells under the band pass filter 530/20 nm. Left panel: untreated PC-3 cells. Middle panel: PC-3 cells treated with **3** (10 μM) and 4-phenyl boronic acid (50 μM) for 18 h. Right panel: PC-3 cells treated with **3** (10 μM) and 4-phenyl boronic acid (50 μM) in the presence of the Pd catalyst (0.5 μmol Pd) for 18 h.

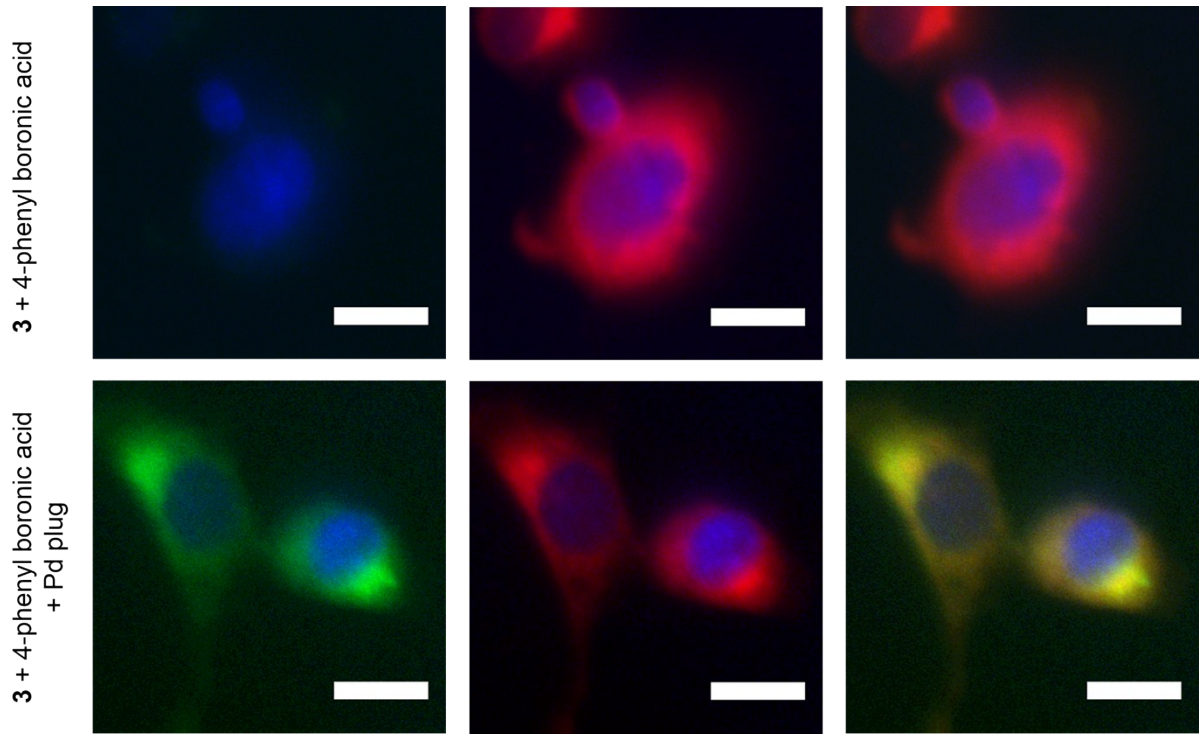


Figure S8. PC-3 cells incubated with compound **3** (10 μM) and 4-phenyl boronic acid (50 μM) without Pd catalyst (top panels) and in the presence of Pd catalyst (0.5 μmol Pd) (bottom panels) for 18 h, stained with CellMask™ Deep Red (plasma membrane stain), fixed with 4 % paraformaldehyde, incubated with DAPI and imaged by fluorescence microscopy. Panels show from left to right: Cell nucleus (blue) and compound **5** (green), cell nucleus (blue) and cell membrane (red), and merged images (yellow indicates co-localization). Scale bar = 20 μm. Microscope lasers settings were: excitation laser lines at 405 nm, 488 nm, and 595 nm with emission filters of 385–470 nm for DAPI (nuclei stain), 505–530 nm for bis-phenyl BODIPY (**5**), 655–720 nm for Cy5 (CellMask™ Deep Red).

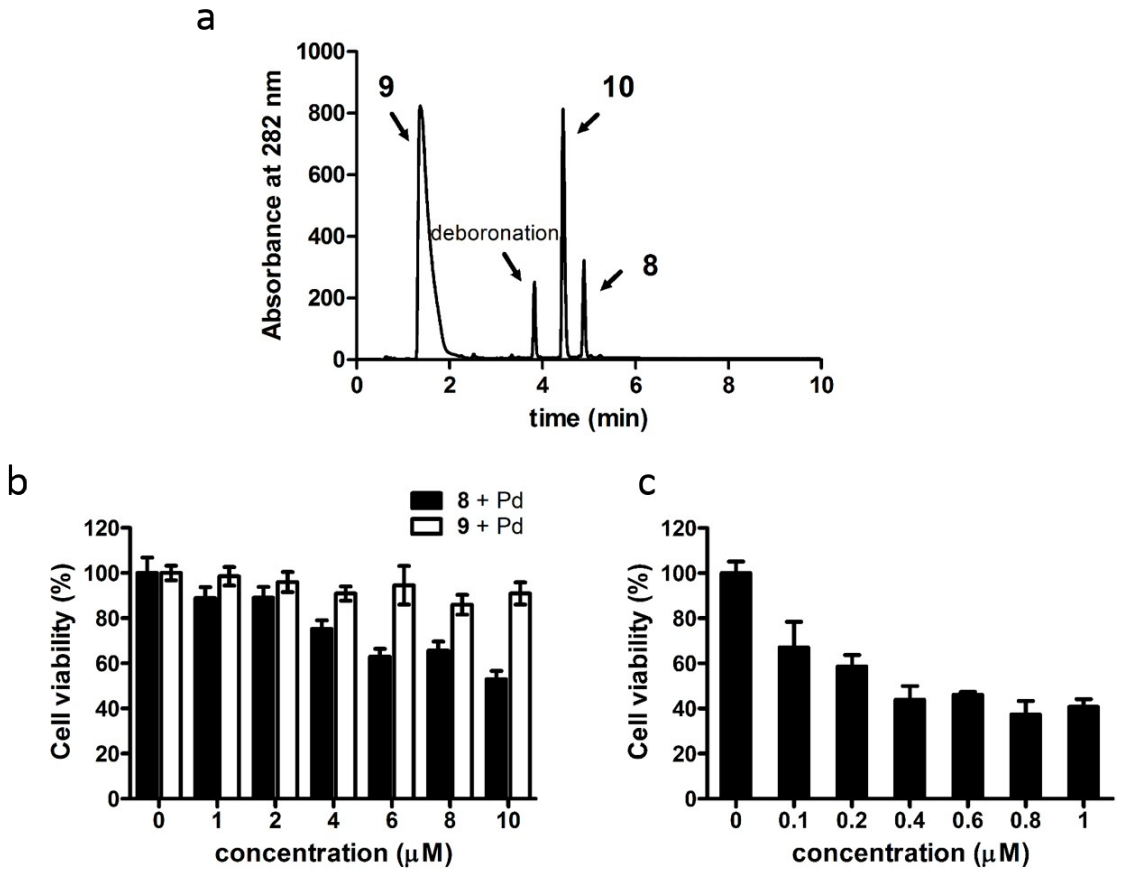


Figure S9. Synthesis of PP-121 *via* cross coupling chemistry. (a) Formation of **10** monitored by HPLC with detection at 282 nm, showing a 5 % degradation of **9** to the deboronated product (conversion to deboronated **9** is calculated based on the integration of the peaks of **9** and the deboronoated product, verified by LC-MS). (b) Cytotoxicity of **8** and **9** on PC-3 cells. Cells were incubated with either **8** or **9** in the presence of Pd (0.5 μmol) at 0 μM to 10 μM. (c) Cytotoxicity of PP-121. PC-3 cells were incubated with PP-121 at 0 μM to 1 μM for 72 hours and cell viability measured using an MTT assay (n = 3).

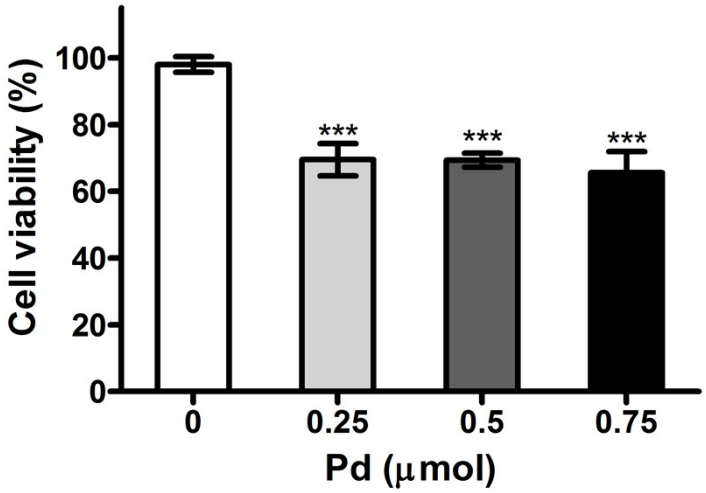


Figure S10. Effect of *in situ* formation of PP-121 on PC-3 cells. Cells incubated with **8** (2 μM) and **9** (10 μM) and different amounts of Pd catalyst for 72 hours and cell viability measured using an MTT assay (n = 3). *** $P<0.001$ by one-way ANOVA with Dunnett post test, compared with control group treated without Pd catalyst.

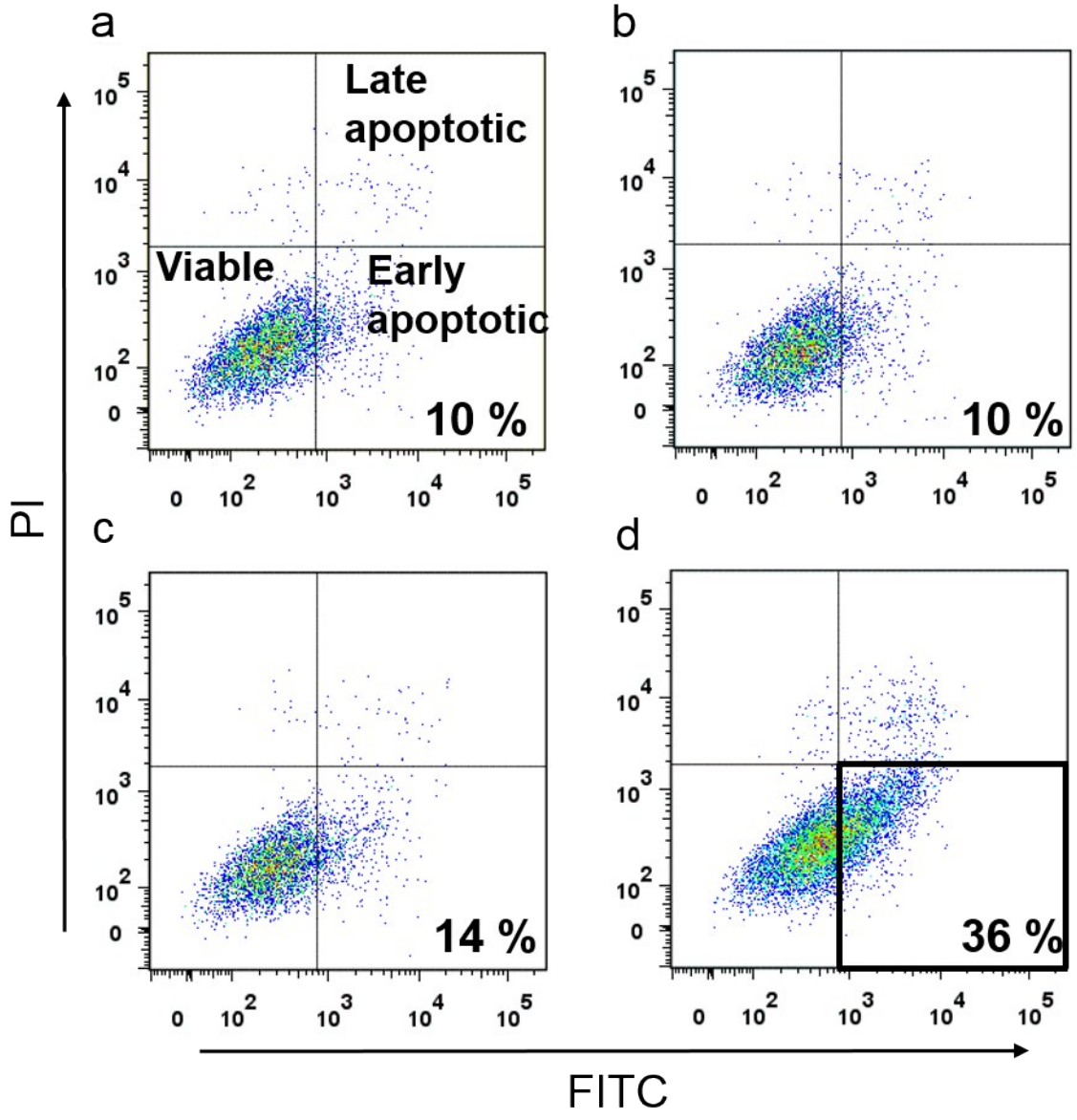


Figure S11. Apoptic effect of *in situ* formation of PP-121 on PC-3 cells. PC-3 cells were incubated with **8** (2 μM), **9** (10 μM) and Pd (0.5 μmol) for 24 h and stained with Annexin-V/FITC (X-axis) and PI (Y-axis), followed by flow cytometry analysis. Early apoptotic cells are located in the bottom right quadrant. (a) Untreated control cells; (b) Cells incubated with **8** and **9**; (c) Cells incubated in the presence of catalyst; (d) Cells incubated with **8** and **9** in the presence of catalysts, with 36 % of the cell population being in early apoptosis

S2. General information

Reactions involving moisture sensitive reagents were performed under positive pressure of nitrogen. Evaporation of solvents was performed at reduced pressure, using a Büchi rotary evaporator. All chemicals were purchased from Sigma Aldrich and Acros and used as received. ^{1}H and ^{13}C spectra were recorded on a Bruker AVA-500 (at 500 and 125 MHz, respectively) at 298 K in the solvents indicated. Resonances are in parts per million (ppm). Colum chromatography was performed on silica gel 60-120 mesh. Analytical TLC was carried out using commercially available Silica gel F254 plates (Merck) and visualized at 254 nm and 344 nm or stained with ninhydrin (0.3% ninhydrin in *n*-butanol with 3% acetic acid) or permanganate solution [($KMnO_4$ (3 g), K_2CO_3 (20 g), 5% aqueous NaOH solution (5 mL) and water (300 mL)]. Low Resolution Mass Spectra (LRMS) were obtained using Agilent LCMS 1100 ChemStation with a G1946B quadrupole mass detector. High Resolution Mass Spectra (HRMS) were performed on a Bruker 3.0 T Apex II spectrometer. Analytical RP-HPLC was performed using an Agilent 1100 Chemstation equipped with a Zorbax Eclipse C18 reverse phase column (4.6×100, 3.5 micron) eluting with a gradient of water/formic acid (0.1%) to MeCN/formic acid (0.1%) over 10 minutes with a flow rate of 1 mL/min, and compounds were detected by an ELS detector and multiwavelength detector. ICP-OES were obtained on a Perkin Elmer Optima 5300 DV ICP-OES suitable for the trace analysis of metals between 0.0002–1000 ppm. UV/Visible measurements were performed on an Agilent 8453 spectrophotometer. Fluorescence spectra were recorded on a SPEX Fluoromax, using 1 cm path length fused silica cuvettes.

S3. Experimental

Pd nanoparticles entrapped in polyethylene sintered polystyrene resin were synthesised according to a previously reported procedure by Bradley.[1]

Bis-propargyloxycarbonyl-protected rhodamine 110 **1** (Proc-Rhod), 1,3,5,7,8-pentamethyl-2,6-diiodine BODIPY **3**, and 1,3,5,7,8-pentamethyl-2,6-dithienyl BODIPY **4** were synthesized according to previously reported procedures.[2,3]

1-cyclopentyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine **8** and 1-cyclopentyl-3-(1H-pyrrolo[2,3-b]pyridin-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (PP-121) **10** were synthesised according to previously reported procedures.[4]

1,3,5,7,8-Pentamethyl-2,6-diphenyl 5

3 (20 mg, 0.04 mmol) and phenylboronic acid (11 mg, 0.09 mmol) were dissolved in THF:H_2O 9:1 (2 mL) and purged with N_2 for 10 min. K_2CO_3 (19 mg, 0.40 mmol) and $Pd(PPh_3)_4$ (31 mg, 0.008 mmol) were added and the mixture was heated to reflux for 12 h. After cooling to room temperature, H_2O (3 mL) was added and the aqueous layer extracted with Et_2O (3 × 10 mL) and the combined organic layers dried over $MgSO_4$, and concentrated *in vacuo*. The crude material was purified *via* silica gel column chromatography using *n*-hexane:CH_2Cl_2 (1:1) as the eluent, affording compound **5** as a dark red solid (14 mg, 0.03 mmol, 84 %). Analytical data was in accordance to previously reported data.[5] ^{1}H NMR: (500 MHz, $CDCl_3$) δ (ppm) 7.45 (t, *J* = 7.6, 7.6 Hz, 4H), 7.36 (t, *J* = 7.41 Hz, 2H), 7.24 (d, *J* = 7.25 Hz, 4H), 2.73 (s, 3H), 2.51 (s, 6H), 2.36 (s, 6H). ^{13}C NMR: (125 MHz, $CDCl_3$) δ (ppm) 13.26, 15.50, 17.25, 127.12, 128.37, 130.41, 132.22, 133.63, 133.82, 137.07, 141.73, 152.38. HRMS (ESI) for $C_{26}H_{24}BF_2N_2$: calc: 413.2006; found: 413.2039.

***In vitro* reaction conditions of Proc-Phodamine cleavage and *bis*-iodo BODIPY cross-coupling**

To 500 µL of PBS (pH 7.3, 10 mM), 5% FBS or PC-3 cell lysate were added 5 µL of a stock solution of **1** (10 mM in DMSO) to give a final concentration of 50 µM. DMSO (20 µL) was added to give a final concentration of 5% DMSO (v/v). Pd catalyst (2 mg, 0.5 µmol Pd) was added and the mixture shaken at 1400 rpm for 18 h at 37 °C and fluorescence was measured.

To 500 µL of EtOH/H_2O (1:1), FBS (5% in EtOH/H_2O) or cell lysate were added 5 µL of a stock solution of **3** (10 mM in DMSO) and either 25 µL of stock solution of 2-phenyl boronic acid or 4-thienyl boronic acid (both 10 mM in DMSO) to give a final concentration of 20 µM and 100 µM, respectively. The Pd catalyst (2 mg, 0.5 µmol Pd) was added and the mixture shaken at 1400 rpm for 18 h at 37 °C and the fluorescence was measured.

***In vitro* reaction conditions PP-121 cross-coupling**

To 500 µL of PBS, 5% FBS or PC-3 cell lysate, **8** and **9** (10 mM in DMSO) were added to give a final concentration of 250 µM and 1 mM, respectively. Pd catalyst (2 mg, 0.5 µmol Pd) was added and the mixture shaken at 1400 rpm for 18 h at 37 °C, with analysis by HPLC with absorbance at 254 nm.

Cell culture

HeLa, HEK293T and PC-3 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), *L*-glutamine (4 mM) and antibiotics (penicillin and streptomycin, 100 units/mL). Cell culture was performed in a 5% CO_2 atmosphere at 37 °C in a SteriCult 200 (Hucoa-Erloss) incubator. The day before the assays, the cells were washed with PBS, detached with trypsin/EDTA (0.25% trypsin, 1mM EDTA), counted, and diluted with DMEM to the appropriate concentration.

Preparation of cell lysate

PC-3 cells (3 million) were washed with PBS, detached with Trypsin/EDTA, harvested with water (2 mL) and sonicated in an ultrasonic bath (Fisherbrand F15053). The lysate was centrifuged for 10 min at 13,000 rpm. The supernatant was collected and used in the *in vitro* studies.

Live cell flow cytometry

PC-3 cells were plated in a 24-wellplate (2 x 10^4 cells/well) and grown for 24 h at 37 °C with 5 % CO_2. The medium was removed and a solution of **1** (20 μM), or **3** (10 μM) and 2-phenyl boronic acid or 3-thienyl boronic acid (50 μM) in DMEM, and the Pd catalyst (2 mg, 0.5 μmol) was added and incubated for 18 h at 37 °C. After incubation, cells were washed twice with PBS, harvested with Trypsin/EDTA and resuspended in DMEM. The cells were analysed by flow cytometry (Becton Dickinson (BD) FACSAriaTM) using a 530/30 nm (compound **1** and **5**) or 575/26 nm (compound **4**) band pass filter, and the data analysed using FlowJo software.

Confocal microscopy imaging

PC-3 cells were cultured on sterilised glass cover slips (24 mm). The coverslips were placed in 6 well-plates and seeded with 9 x 10^4 cells/well in 1.6 mL in DMEM and incubated for 24 h. The medium was removed and a solution of **1** (20 μM) or **3** (10 μM) and the respective boronic acid (50 μM) in DMEM and the Pd catalyst (2 mg, 0.5 μmol) were added and incubated for 18 h at 37 °C. After incubation, cells were washed twice with PBS, stained with CellTracker Red™ (for compound **1** and Pd catalyst) or with CellMask™ Deep Red Membrane stain (compound **3**, boronic acid and Pd catalyst), according to the protocols supplied by the manufacturer, and fixed with 4 % paraformaldehyde in PBS for 15 minutes. Nuclei were stained by incubation with a 10 μM solution of DAPI in PBS for 15 minutes. Cells were imaged with a Leica SP5 confocal microscope or a Zeiss AxioVert 200M fluorescence microscope. Microscope lasers

settings were: excitation laser lines at 488 nm, 514 nm, 543 nm, and 595 nm with emission filters of 385–470 nm for DAPI (nuclei stain), 505–530 nm for Rhodamine 110 (**2** and **5**), 520–550 nm for YFP (compound **4**), 595–615nm for Texas Red (CellTracker Red™), 633 nm for Cy5 (CellMask™ Deep Red).

MTT assays

HEK293T, HeLa, and PC-3 cells were plated in a 96-well plate with a density of 5,000 cells/well and left to grow for 24 h. A solution of the precursors **8** and **9,** and PP-121 was added to the well at desired concentrations and incubated with the cells for 48 hours. Media was then replaced with 90 µL of fresh media (phenol red free DMEM) along with 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) and the cells incubated for 3 hours at 37°C. After incubation, the resulting formazan crystals were dissolved by adding 100 µL of MTT solubilisation solution (10% Triton-X 100 in 0.1 N HCl in isopropanol). The absorbance was measured at a wavelength of 570 nm (BioTek HT Synergy multi-mode reader using the Gen5 microplate and imaging software 2.0.) and results compared to untreated cells.

Palladium mediated *in situ* formation of PP-121 in PC-3 cells

PC-3 cells were plated in a 24-well plate (2 x 10^4 cells/well) and grown for 24 h. A solution of precursors **8** (2 µM) and **9** (10 µM) in DMEM was added to the wells and incubated with the cells for 3 and 5 days in the presence of Pd catalyst (2 mg, 0.5 µmol Pd). Media was replaced with 315 µL of fresh media (phenol red free) along with 35 µL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/mL) and the cells incubated for 3 hours at 37°C. After incubation, the resulting formazan crystals were dissolved by adding 350 µL of MTT solubilisation solution (10% Triton-X 100 in 0.1 N HCl in isopropanol) and the absorbance measured at 570 nm and results compared to untreated cells.

Apoptotic studies of palladium mediated *in situ* formation of PP-121

PC-3 cells were plated in a 24-well plate (2 x 10^4 cells/well) and grown for 24 h. A solution of the precursors **8** (2 µM) and **9** (10 µM) in DMEM was added to the well and incubated with the cells for 24 h in the presence of Pd catalyst (2 mg, 0.5 µmol Pd). After incubation, fluorescein-conjugated Annexin V (Annexin V-FITC) binding assay was performed. Labelling of cells with Annexin-V-FITC conjugate was performed using an Apoptosis Detection Kit (eBiosciences, San Diego, CA) according to manufacturer's instructions in which Annexin V-FITC, propidium

iodide (PI), and binding buffer were included as standard reagents. Flow cytometry was performed with excitation at 488 nm. FITC fluorescence was measured at 515–545 nm and fluorescence of DNA-PI complexes at 565–606 nm. Cell debris was excluded from analysis by appropriate forward light scatter threshold setting. Compensation was used wherever necessary.

S4. References

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