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Palladium-mediated in situ synthesis of an anticancer agent

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As a novel prodrug activation strategy Pd(0) nanoparticles entrapped within a modular polymeric support were used, in a cell culture system, to synthesise the anticancer agent PP-121 from two non-toxic precursors, thereby inducing cell death in the first example of in situ mediated drug synthesis.

Bioorthogonal chemistries that can be carried out within a biological system without affecting normal cellular function have revolutionized the analysis of biological processes in their native environment.1 Classical examples include the Staudinger ligation,2 strain-promoted azide–alkyne cycloaddition,3 and the inverse-electron demand Diels–Alder reaction of tetrazines.4 Recently, bioorthogonal reactions using transition metals (Rh, Au and Pd) have begun to be successfully applied in a biological setting.5–9 Modifications of proteins using genetically encoded halogenated phenylalanines have for example enabled in vitro labelling of proteins via palladium-mediated coupling to boronic acid tags,10 thereby allowing the non-intrusive and real-time study of proteins11,12 and carbohydrates13 in bacteria. Another approach has been the application of palladium nanoparticle catalysts with allicycarbamate cleavage of both caged fluorophores and prodrugs (e.g. alllicarbamate-amsacrine), as well as a Suzuki–Miyaura cross-coupling reaction inside mammalian cells.14 Palladium-mediated transformations have since been used to selectively activate proteins and other prodrugs. Chen showed the activation of the enzyme phosphothreonine lyase (Ospf) based on decaging of a propargyloxycarbonyl (Proc) protected catalytic lysine residues with homogeneous palladium catalysts,15 while Weiss demonstrated that the anticancer drug S-fluorouracil could be generated by the in situ (extracellular) decaging of a propargyl protected prodrug.16

Here, the scope of palladium-mediated chemistry in a biological environment was extended to in situ drug synthesis, with C–C bond formation via a Suzuki–Miyaura cross-coupling demonstrated with the activation of a quenched bis-iodo-BODIPY scaffold and the synthesis of the anticancer agent PP-121 from two coupling partners.18

Loading an active metal onto a solid support is a common method to generate heterogeneous catalysts.19,20 We have previously reported the entrapment of catalytically active, biologically compatible palladium(0) nanoparticles into polymers14,21–23 with the generation of modular sintered aminomethyl polystyrene resin beads17,24 in which nanoparticles of palladium are trapped within a physical polymer framework (Fig. 1).
Here, the catalytic activity of these modular Pd catalysts was confirmed by the decaging of bis-propargyloxycarbonyl (Proc) rhodamine 110 1 (Fig. 2a) in phosphate buffered saline (PBS), 5% fetal bovine serum (FBS), and PC-3 (prostate adenocarcinoma) cell lysate. 5,14,25,26 The addition of the Pd catalyst to a solution of 1 (20 µM) resulted in the generation of 2 with a >500-fold increase in fluorescence in PBS, and a 115-fold and 46-fold increase in cell lysate and in 5% FBS, respectively (Supporting Fig. S1). The catalyst also decaged 1 (20 µM, 18 h incubation) in a cell-based assay, resulting in labelling of PC-3 cells (Supporting Fig. S2 and S3). To investigate the catalytic activity in a Suzuki-Miyaura cross-coupling reaction, bis-iodo-1,3,5,7,8-pentamethyl-BODIPY 3 was reacted with 2-thienyl and 4-phenyl boronic acids (Fig. 2b). Bis-iodo BODIPY 3 is non-fluorescent due to the heavy atom quenching effect, 27 but becomes fluorescently unquenched following cross-coupling with 2-thienyl or 4-phenyl boronic acids, which gives the bis-thienyl BODIPY 4 (λex/em 520/574 nm) and bis-phenyl BODIPY 5 (λex/em 518/552 nm) (Supporting Fig. S4) with 14 and 31-fold increases in fluorescence, respectively. The coupling reaction between 2-thienyl and 4-phenyl boronic acid with bis-iodo BODIPY 3 in the presence of the Pd catalyst in cell lysate and in 5% FBS resulted in a 5.6 and 1.2-fold increase in fluorescence for 4 and a 2.5 and 3.3-fold increase for 5, respectively (pure samples of 4 and 5 in cell lysate and 5% FBS gave 14 and 15-fold, and 3 and 20-fold increase, respectively) (Supporting Fig. S5), with the cell lysate influencing the fluorescence of 5, but not 4. A modest increase in fluorescence (< 1.5-fold) was also observed in the absence of a boronic acid due to a partial de-iodination of the BODIPY 3. 28 However, the emission maximum of the de-iodinated product (1,3,5,7,8-pentamethyl-BODIPY) is 520 nm compared to the >540 nm for both cross-coupling products 4 and 5 (Supporting Fig. S6), thus allowing spectral resolution. PC-3 cells were incubated with bis-iodinated BODIPY 3 and 2-thienyl boronic acid or 4-phenyl boronic in the presence of catalyst and analyzed by flow cytometry and fluorescence microscopy (Supporting Fig. S7 and S8). A shift of the cell population towards higher fluorescence intensity (42%) was observed when incubating cells with 3, 2-thienyl boronic acid and catalyst compared to control cells incubated without Pd (8%), indicating the in situ formation of 4 (Fig. 2c). Fluorescence microscopy verified the presence of intracellular 4 with an increase in fluorescence compared to cells treated only with 3 and 2-thienyl boronic acid (Fig. 2d).

The Pd mediated cross-coupling reaction was applied to the in situ synthesis of the cytotoxic agent PP-121 10. PP-121 18,29 is known to suppress anaplastic thyroid carcinoma tumor growth by inhibition of mTOR (a member of the phosphatidylinositol-3-OH kinase (PI3K) family) 30 and tyrosine kinases (VEGF receptor). 18 Retrosynthetically, 10 can be formed from iodoypyrazole 8 and boronic ester 9 (Fig. 3a), and incubating 8 and 9 in the presence of Pd catalyst under aqueous conditions gave a 62% yield of 10 in 72 h (Fig. 3b).

PP-121 exhibits high cytotoxicity on PC-3 cells, which express high levels of the VEGF receptor and are susceptible to kinase inhibitors, with <50% cell viability at 0.4 µM (Fig. 3c and Supporting Fig. S9). The toxicity of the PP-121 precursors 8 and 9 were evaluated on PC-3 cells to establish the ideal concentration range that could be used in the in situ cross-coupling reactions. Azaindole boronic ester 9 showed negligible toxicity up to 10 µM, while iodo-pyrazole 8 showed no toxicity below 4 µM (Fig. 3c). The cross-coupling reaction (0.5 µmol Pd) was performed in the extracellular space of PC-3 cells by incubating 8 (2 µM) and 9 (10 µM) for 5 days in cell culture, with cell viability decreasing by 50% under these conditions (Fig. 3d). Since PP-121 induces apoptosis, 18 the extent of apoptosis upon Pd mediated in situ synthesis of PP-121 on PC-3 cells after 24 h was evaluated via double staining with the apoptosis marker annexin V-FITC and propidium iodide (PI).
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Notes and references


