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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 proteins^{11,12} and carbohydrates¹³ in bacteria. Another approach has been the application of palladium nanoparticle catalysts with allylcarbamate cleavage of both caged fluorophores and prodrugs (e.g. allylcarbamate-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 5-fluorouracil could be generated by the *in situ* (extracellular) decaging of a propargyl protected prodrug.¹⁶

Fig. 1. Synthesis and characterization of a modular support functionalized with Pd(0) nanoparticles. (a) Synthesis of the supported Pd catalyst; (b) TEM analysis

showing the homogeneous distribution of palladium nanoparticles throughout the support, with an average particle size of 9.2 ± 1.5 nm (scale bar 100 nm) and a Pd content of 0.24 ± 0.08 $\mu\text{mol/mg}$ (ICP-OES, $n = 9$); (c) The modular supports (9.0×7.5 mm) before (left) and after (right) functionalization.¹⁷

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 polymers^{14,21–23} with the generation of modular sintered aminomethyl polystyrene resin beads^{17,24} in which nanoparticles of palladium are trapped within a physical polymer framework (Fig. 1).

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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 μ M) 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 μ M, 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 chemistry with 2-thienyl or 4-phenyl boronic acids, which gives the *bis*-thienyl BODIPY **4** ($\lambda_{\text{Ex/Em}}$ 520/574 nm) and *bis*-phenyl BODIPY **5** ($\lambda_{\text{Ex/Em}}$ 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 (PI(3)K) family)³⁰ and tyrosine kinases (VEGF receptor).¹⁸ Retrosynthetically, **10** can be formed from iodopyrazole **8**¹⁸ 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).

Fig. 2. Fluorescent “switch-on” *via* Pd mediated synthesis. (a) Cleavage of Proc-rhodamine 110 **1** to give fluorescent rhodamine 110 **2**. (b) Suzuki-Miyaura cross-coupling of the *bis*-aryl BODIPY dyes (**4** and **5**): Pd mediated synthesis of *bis*-thienyl BODIPY with PC-3 cells. PC-3 cells incubated with compound **3** in the presence of the Pd catalyst and 2-thienyl boronic acid. (c) Flow cytometry analysis: Left panel: untreated cells; control cells treated with **3** and 2-thienyl boronic acid; right panel: cell treated with **3**, 2-thienyl boronic acid and Pd catalyst. (d) Cells were stained with CellMask™ Deep Red (plasma membrane stain), fixed with paraformaldehyde, incubated with DAPI (nuclei stain) and imaged by fluorescence microscopy: (i) without Pd, (ii) with Pd. Panels show from left to right: cell nucleus (blue) and synthesized compound **4** (yellow; cell nucleus (blue) and cell membrane (red); and merged images (orange indicates co-localization of synthesized compound **4** within cells). Scale bar 20 μ m.

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 μ M (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 μ M, while iodo-pyrazole **8** showed no toxicity below 4 μ M (Fig. 3c). The cross-coupling reaction (0.5 μ mol Pd) was performed in the extracellular space of PC-3 cells by incubating **8** (2 μ M) and **9** (10 μ M) 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).

Fig. 3 Bioorthogonal *in situ* synthesis and evaluation of the anticancer agent PP-121 **10** mediated by Pd(0): (a) Cross-coupling of iodopyrazole **8** (2 μ M) with boronic ester **9** (10 μ M); (b) Formation of **10** monitored by HPLC with detection at 254 nm (conversion is calculated based on the integration of the peaks of **8** and **10**); (c) Cytotoxicity of **8**, **9**, and **10** on PC-3 cells. PC-3 cells were incubated with 0–10 μ M of **8**, **9**, and **10** for 72 h after which cell viability was measured (MTT assay, $n = 3$). (d) PC-3 cells were incubated with **8** (2 μ M) and **9** (10 μ M) in the presence of Pd (0.5 μ M) for 3 and 5 days, after which cell viability was measured (MTT assay, $n = 3$). The data represent the mean $\pm$ S.D. *** $P < 0.001$ by one-way ANOVA with Dunnett post-test, compared with the control group treated without the Pd. (e) PC-3 cells were incubated with **8** (2 μ M) and **9** (10 μ M) in absence (i) and in presence (ii) of Pd (0.5 μ M) 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.

Treatment of PC-3 cells with precursors **8** and **9** or the Pd catalyst (Supporting Fig. S11) did not show any increase in annexin-V labelling, compared to untreated cells. However, early apoptosis was evident in cells treated with the two precursors in the presence of the catalyst, as indicated by a 36 % shift in the cell population towards higher FITC fluorescence intensity (Fig. 3e).

In conclusion, we have demonstrated the ability of a biologically inert catalyst to mediate cross-coupling reactions in a biological cell culture setting. This was established by the *in situ* synthesis of a BODIPY dye *via* Suzuki-Miyaura cross-coupling with two aryl boronic acids, resulting in fluorescent labelling of mammalian cells. The concept was successfully applied to the *in situ* synthesis of the anticancer agent PP-121 through Suzuki-Miyaura cross-coupling of two non-toxic precursors, which induced localized cytotoxicity and early apoptosis on PC-3 cancer cells. The range of therapeutic agents containing diaryl bonds provides a good basis for the applicability of this novel strategy of forming a cytotoxic

compound *in situ* *via* cross-coupling of two inactive precursors and opens doors to new methods of prodrug activation.

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Notes and references

- 1 E. M. Sletten and C. R. Bertozzi, *Angew. Chem. Int. Ed. Engl.*, 2009, **48**, 6974–6998.
- 2 M. F. Debets, C. W. J. van der Doelen, F. P. J. T. Rutjes and F. L. van Delft, *Chembiochem*, 2010, **11**, 1168–1184.
- 3 N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046–15047.
- 4 M. L. Blackman, M. Royzen and J. M. Fox, *J. Am. Chem. Soc.*, 2008, **130**, 13518–13519.
- 5 C. Streu and E. Meggers, *Angew. Chem. Int. Ed.*, 2006, **45**, 5645–5648.
- 6 P. K. Sasmal, C. N. Streu and E. Meggers, *Chem. Commun. (Camb.)*, 2013, **49**, 1581–1587.
- 7 J. Li and P. R. Chen, *Chembiochem*, 2012, **13**, 1728–1731.
- 8 N. Li, C. P. Ramil, R. K. V Lim and Q. Lin, *ACS Chem. Biol.*, 2015, **10**, 379–384.
- 9 C. P. Ramil and Q. Lin, *Chem. Commun.*, 2013, **49**, 11007–11022.
- 10 L. Lercher, J. F. McGouran, B. M. Kessler, C. J. Schofield and B. G. Davis, *Angew. Chem. Int. Ed. Engl.*, 2013, **52**, 10553–10558.
- 11 N. Li, R. K. V Lim, S. Edwardraja and Q. Lin, *J. Am. Chem. Soc.*, 2011, **133**, 15316–15319.
- 12 J. Li, S. Lin, J. Wang, S. Jia, M. Yang, Z. Hao, X. Zhang and P. R. Chen, *J. Am. Chem. Soc.*, 2013, **135**, 7330–7338.
- 13 C. D. Spicer and B. G. Davis, *Chem. Commun. (Camb.)*, 2013, **49**, 2747–2749.
- 14 R. M. Yusop, A. Unciti-Broceta, E. M. V Johansson, R. M. Sanchez-Martin and M. Bradley, *Nat. Chem.*, 2011, **3**, 239–243.
- 15 J. Li, J. Yu, J. Zhao, J. Wang, S. Zheng, S. Lin, L. Chen, M. Yang, S. Jia, X. Zhang and P. R. Chen, *Nat. Chem.*, 2014, **6**, 352–361.
- 16 J. T. Weiss, J. C. Dawson, K. G. Macleod, W. Rybski, C. Fraser, C. Torres-Sánchez, E. E. Patton, M. Bradley, N. O. Carragher and A. Unciti-Broceta, *Nat. Commun.*, 2014, **5**, 3277.
- 17 R. Najman, J. K. Cho, A. F. Coffey, J. W. Davies and M. Bradley, *Chem. Commun. (Camb.)*, 2007, 5031–5033.
- 18 B. Apsel, J. a Blair, B. Gonzalez, T. M. Nazif, M. E. Feldman, B. Aizenstein, R. Hoffman, R. L. Williams, K. M. Shokat and Z. a Knight, *Nat. Chem. Biol.*, 2008, **4**, 691–699.
- 19 N. E. Leadbeater and M. Marco, *Chem. Rev.*, 2002, **102**, 3217–3274.
- 20 S. Sarkar, E. Guibal, F. Quignard and A. K. SenGupta, *J. Nanoparticle Res.*, 2012, **14**, 1–24.
- 21 R. Akiyama and S. Kobayashi, *J. Am. Chem. Soc.*, 2003, **125**, 3412–3413.
- 22 C. Ramarao, S. V Ley, S. C. Smith, I. M. Shirley and N.

- DeAlmeida, *Chem. Commun. (Camb.)*, 2002, 1132–1133.
- 23 J. K. Cho, R. Najman, T. W. Dean, O. Ichihara, C. Muller and M. Bradley, *J. Am. Chem. Soc.*, 2006, **128**, 6276–6277.
- 24 B. Atrash, M. Bradley, R. Kobylecki, D. Cowell and J. Reader, *Angew. Chem. Int. Ed.*, 2001, **40**, 938–941.
- 25 J. Li, J. Yu, J. Zhao, J. Wang, S. Zheng, S. Lin, L. Chen, M. Yang, S. Jia, X. Zhang and P. R. Chen, *Nat. Chem.*, 2014, **6**, 352–361.
- 26 T. Völker, F. Dempwolff, P. L. Graumann and E. Meggers, *Angew. Chem. Int. Ed. Engl.*, 2014, **53**, 10536–10540.
- 27 D. K. Prusty and A. Herrmann, *J. Am. Chem. Soc.*, 2010, **132**, 12197–12199.
- 28 D. Keum, S. Kim and Y. Kim, *Chem. Commun.*, 2014, **50**, 1268–1270.
- 29 H.-Y. Che, H.-Y. Guo, X.-W. Si, Q.-Y. You and W.-Y. Lou, *Tumour Biol.*, 2014, **35**, 8659–8664.
- 30 R. J. Shaw and L. C. Cantley, *Nature*, 2006, **441**, 424–430.