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

Eugenio Indrigo,‡ Jessica Clavadetscher,‡ Sunay V. Chankeshwara, Annamaria Lilienkampf and Mark Bradley*

As a novel prodrug activation strategy Pd(0) nanoparticles, entrapped within a modular polymeric support, were used in cell culture, 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 carbohydrates[13] in bacteria. Another approach has been the application of palladium nanoparticle catalysts with allylcarbamate cleavage of both caged fluorophores and pro-drugs (*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 propargylcarbamate (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]

Here, the scope of Pd catalyzed chemistry in a biological environment was extended to *in situ* drug synthesis, with C–C bond formation *via* a Suzuki–Miyaura cross-coupling reaction 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] and 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).

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),

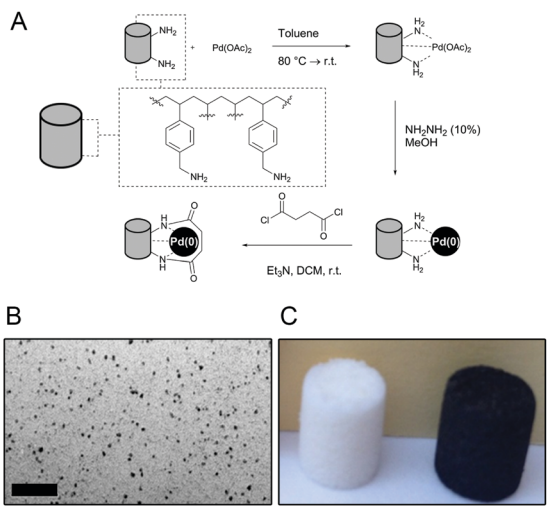

Fig. 1 Synthesis and characterization of a modular support functionalized with Pd(0) nanoparticles. (A) Synthesis of the supported Pd catalyst; (B) TEM analysis showing the homogeneous distribution of palladium nanoparticles throughout the support, with an average particle size of 9.2 ± 1.5 nm (scale bar 100 nm) and a Pd content of 0.24 ± 0.08 μmol mg^{-1} (ICP-OES, n = 9); (C) the modular supports (9.0 × 7.5 mm) before (left) and after (right) functionalization.[17]

EaStCHEM School of Chemistry, University of Edinburgh, David Brewster Road, EH9 3FJ Edinburgh, UK. E-mail: Mark.Bradley@ed.ac.uk

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‡ These authors contributed equally to this work.

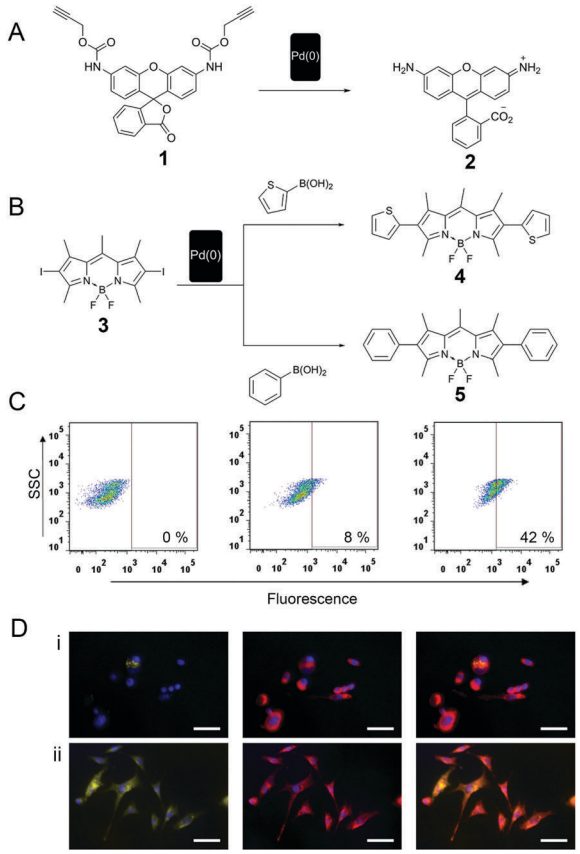

Fig. 2 Fluorescent "switch-on" *via* Pd mediated synthesis. (A) Cleavage of Proc-rhodamine 110 **1** to give fluorescent rhodamine 110 **2**. (B) Suzuki–Miyaura cross-coupling synthesis of the bis-aryl BODIPY dyes (**4** and **5**) with PC-3 cells. PC-3 cells incubated with compound **3** (20 μM) in the presence of the Pd catalyst (0.5 μmol, 3 mg) and 2-thienyl boronic acid (50 μM). (C) Flow cytometry analysis: left panel: untreated cells; middle panel: 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 μm.

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 (Fig. S1, ESI†). The catalyst also decaged **1** (20 μM, 18 h incubation) in a cell-based assay, resulting in labelling of PC-3 cells (Fig. S2 and S3, ESI†). 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** ($\lambda_{Ex/Em}$ 520/574 nm) and bis-phenyl BODIPY **5** ($\lambda_{Ex/Em}$ 518/552 nm) (Fig. S4, ESI†) 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 increases over background, respectively) (Fig. S5, ESI†), with the cell lysate partially quenching the fluorescence of **5**, but not **4**. A decrease in reactivity in cell lysate and 5% FBS can be due to the presence of thiol-containing molecules which bind to the catalyst. A modest increase in fluorescence (<1.5-fold) was also observed in the absence of a boronic acid due to partial de-iodination of 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** (Fig. S6, ESI†), 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 (Fig. S7 and S8, ESI†). 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 catalyzed 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 by binding to tyrosine kinases (VEGF receptor).[18] Retrosynthetically, **10** can be formed from iodopyrazole **8**[18] and boronic ester **9** (Fig. 3A), and indeed, incubating **8** and **9** in the presence of the Pd catalyst under aqueous conditions gave a 62% yield of **10** in 72 h, with just 5% deboronation of the boronic ester **9** observed (Fig. 3B and Fig. S9, ESI†). 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 Fig. S9, ESI†). 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, even in the presence of Pd (Fig. 3C and Fig. S9, ESI†). The cross-coupling reaction was performed in the extracellular media of PC-3 cells by incubating **8** (2 μM) and **9** (10 μM) with 0.5 μmol of Pd 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).

Treatment of PC-3 cells with precursors **8** and **9** or the Pd catalyst (Fig. S11, ESI†) did not show any increase in annexin-V labelling, compared to untreated cells. However, early apoptosis

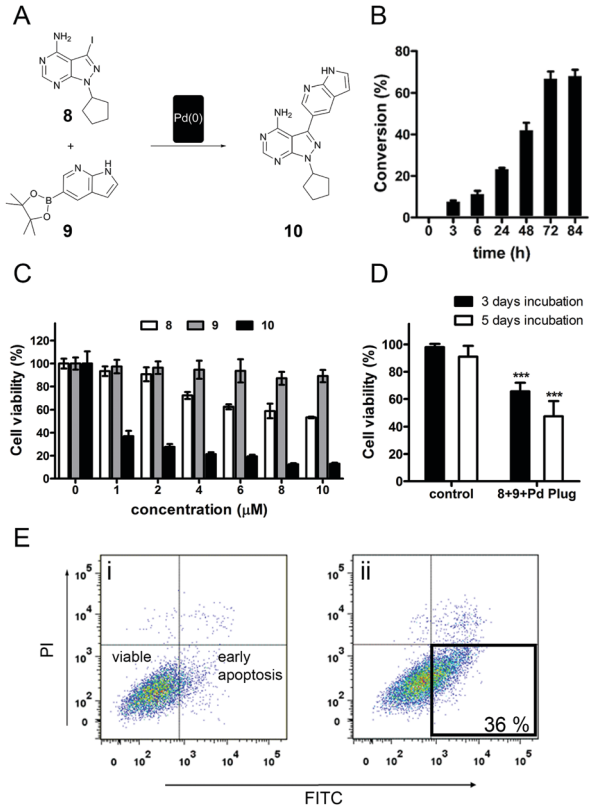

Fig. 3 Bioorthogonal *in situ* synthesis and evaluation of the anticancer agent PP-121 **10** mediated by Pd(0): (A) cross-coupling of iodopyrazole **8** (2 μM) with boronic ester **9** (10 μM); (B) formation of **10** monitored by HPLC with detection at 254 nm (conversion is calculated based on the 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 μM 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 μM) and **9** (10 μM) in the presence of Pd (0.5 μmol, 3 mg) for 3 and 5 days, after which cell viability was measured (MTT assay, $n = 3$). The data represent the mean ± 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 μM) and **9** (10 μM) in absence (i) and in presence (ii) of 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.

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.*, 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.*, 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.*, 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.*, 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.*, 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. Nanopart. 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.*, 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.*, 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, *Tumor Biol.*, 2014, **35**, 8659–8664.
30 R. J. Shaw and L. C. Cantley, *Nature*, 2006, **441**, 424–430.