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A mild and selective Pd-mediated methodology for the synthesis of highly fluorescent 2-arylated tryptophans and tryptophan-containing peptides: a catalytic role for Pd^0 nanoparticles?†

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A Pd-mediated direct C–H bond functionalisation of tryptophan has been developed, both as a single amino acid residue and within peptides. Important mechanistic insight into this process has been gained by characterising a Pd catalytically competent nanoparticle phase which evolves during the early stages of reaction.

The functionalisation and modification of complex biomolecules by the formation of C–C bonds has benefited significantly from the development of mild conditions for Pd-mediated cross-coupling reactions.[1] Pioneering work by Davis and co-workers has, for example, demonstrated that it is possible to perform Suzuki–Miyaura reactions on proteins containing genetically incorporated aryl iodides.[2] Metal-mediated direct functionalisation of C–H bonds in complex molecule synthesis is a rapidly expanding field, including in the total synthesis of natural compounds.[3] We have reported the mild and selective direct C–H functionalisation of highly sensitive purine nucleosides, *e.g.* adenosine and 2′-deoxyadenosine.[4] As part of these studies we have been able to gain valuable mechanistic insight, most notably characterizing the formation of Pd/Cu-containing nanoparticles during substrate turnover. In this study, we have turned our attention to the mild[5] C–H bond functionalisation of amino acids,[6] specifically tryptophan, a hydrophobic indole-containing amino acid which alters the structure and function of proteins, and is a fluorescent marker.[7]

The C–H bond functionalisation of indoles mediated by Pd is well established.[8] We recognised that Sanford's methodology[8d] could be applied to selectively functionalise tryptophan, which employs $PhB(OH)_2$ and $PhI(OAc)_2$ as reagents, forming diaryliodonium salts *in situ*,[9] and catalytic $Pd(OAc)_2$. These reactions could proceed *via* a $Pd^{II/IV}$ catalytic manifold.[10] In this paper we detail the selective arylation of tryptophan and tryptophan-containing peptides under mild catalytic conditions.

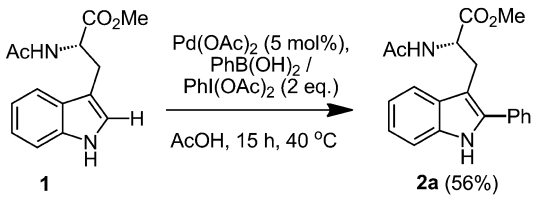

Fig. 1 Direct arylation of tryptophan under mild conditions.

Sanford's reaction conditions were initially applied to *N*-acetyl, *O*-methyl protected-tryptophan **1** to afford, after column chromatography, 2-phenyltryptophan **2** in 56% yield (Fig. 1). Changes to the catalytic conditions included modifying the Pd catalyst and loading, solvent, inert atmosphere and reaction temperature, but no increase in yield was recorded. Optimal turnover numbers and yields were seen between 2.5–5 mol% Pd.

Intriguingly, reaction of **1** with $PhB(OH)_2$ and $PhI(OAc)_2$ led to the rapid formation of Pd^0 nanoparticles (PdNPs) during the first few minutes of the reaction. This finding is in keeping with another Pd-mediated C–H bond functionalisation of benzoxazoles using $PhI(OAc)_2$, which we recently reported upon.[11] The *in situ* generated PdNPs from reaction **1** → **2a** were encapsulated by addition of exogenous polymer stabilizer (*i.e.* polyvinylpyrrolidinone, PVP), which was added to an aliquot of the reaction mixture after 1 h (2.5 mL, containing ~4.8 μmol Pd; 10 equiv. PVP added and AcOH removed at 40 °C and *ca.* 0.750 mmHg). This allows reliable analysis of the size and distribution of the PdNPs without concern that solvent removal leads to metal aggregation. Transmission electron microscopy (TEM) confirmed the presence of the encapsulated PdNPs (n = 100; average PdNP size is 2.52 nm, represented in Fig. 2).[12]

Pre-synthesised PVP-PdNPs[13] (average size ~1.8 nm, Pd^0, 5 mol%) were also found to be a viable catalytic species for the tryptophan arylation, affording **2a** in 57% yield (Fig. 3). This result shows that PdNPs are catalytically competent under the reaction conditions. In the example given in Fig. 1, we propose that the PdNPs are acting as a reservoir for Pd^0, akin to related Heck arylation chemistry.[14] Glorius and co-workers have nicely

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† Electronic supplementary information (ESI) available: Experimental details and crystallographic data. CCDC 968490 and 968491. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc48481e

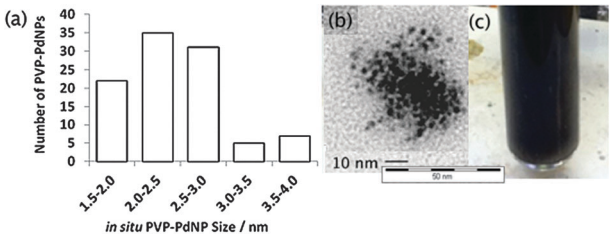


Fig. 2 (a) Histogram of particle size (diameter, nm) for a sample of PdNPs (encapsulated by PVP); (b) TEM image of PVP-encapsulated PdNPs after 1 h heating; (c) appearance of PdNPs in reaction.

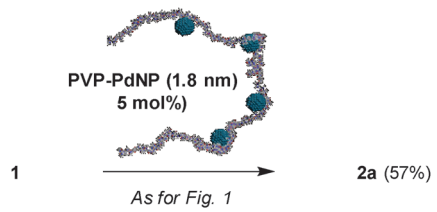
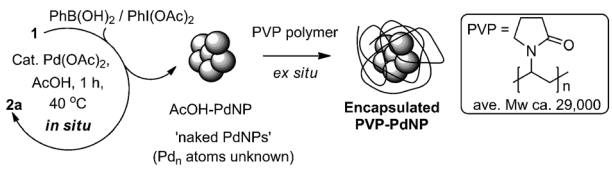


Fig. 3 Pre-synthesised PVP-PdNPs are catalytically competent species in the arylation of **1** to afford **2a**.



Scheme 1 PVP-encapsulation of *in situ* generated PdNPs from the reaction detailed in Fig. 1 (TEM characterisation in Fig. 2).

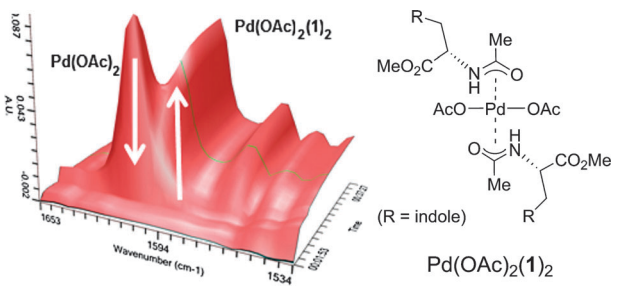


Fig. 4 Changes in the IR spectra observed on the reaction of $Pd(OAc)_2$ (1616 cm^{-1}) with **1**, affording $Pd(OAc)_2(\mathbf{1})_2$ (1606 cm^{-1}).

shown that heterogeneous C–H bond activation is viable in benzo[*b*]thiophene arylation (Scheme 1).[15]

The reaction of tryptophan **1** with $Pd(OAc)_2$ (1 : 1) in THF at ambient temperature (0.5 h) was monitored by *in situ* infrared spectroscopy (Fig. 4). The carbonyl stretching band at 1616 cm^{-1} is $Pd(OAc)_2$, which disappears on addition of **1**, with the appearance of a new band at 1606 cm^{-1}, proposed to be $Pd(OAc)_2(\mathbf{1})_2$. Only small changes were noted by ^1H NMR spectroscopic analysis, which is in keeping with the tryptophan ligand being weakly coordinated to Pd^{II}. Crucially, $Pd(OAc)_2(\mathbf{1})_2$ rapidly reduces to form Pd^0 and is the seed that leads to the generation of PdNPs under the catalytic conditions – amine ligands lower the Pd^{II} reduction potential to give Pd^0 ions.[4b]

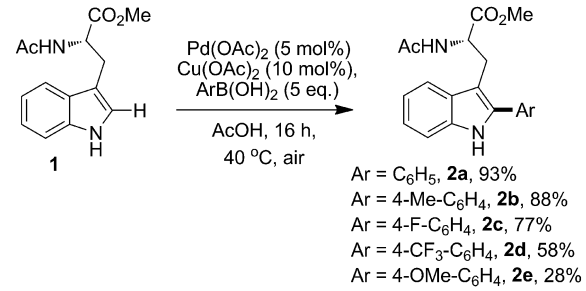


Fig. 5 Arylation of tryptophan using $Cu(OAc)_2$ as a co-catalyst.

Following this study, some limitations were revealed in terms of the substrate scope using $Pd(OAc)_2$ as the precatalyst.‡ For example, a simple switch from $PhB(OH)_2$ to $4\text{-}MeC_6H_4B(OH)_2$ led to the biaryl compound derived from the latter only {$PhI(OAc)_2$ could be aiding oxidative homocoupling of the aryl boronic acid}.[16] Simply switching the oxidant to $Cu(OAc)_2$ afforded **2a** in 93% yield (under the conditions described in Fig. 5). Here, $Cu(OAc)_2$ is assisting the reoxidation of Pd^0 (with O_2 from air). This was confirmed by conducting the same reaction under an Ar atmosphere, which showed ~11% conversion to **2a**, *i.e.* a single turn-over of the Cu^{II} co-catalyst. A series of analogues (**2b–2d**) were formed in good yields using this procedure. The electron-rich *p*-anisoyl boronic acid was susceptible to oxidative homocoupling, leading to **2e** being formed in modest yield, expected under oxidative conditions.

The specific optical rotations of the products, and analysis by chiral HPLC, indicated that the arylated tryptophan products maintained their stereochemical purity (see ESI†).

Single crystal X-ray diffraction structures for analogues **2c** and **2d** (Fig. 6), confirm absolutely their structural connectivity. The data shows in the solid-state that the aryl and indole groups deviate from planarity, which is likely a crystal packing effect. The conformational preference of the ester, amino and aryl moieties could affect the intrinsic fluorescence properties and provide a useful starting point for future TDDFT calculations.

UV-vis and fluorescence measurements reveal the effect of the different aryl groups within the tryptophan framework (Fig. 7). The most electronically distinct analogues, **2d** (4-CF_3) and **2e** (4-OMe), exhibit the highest absorption shifts, generating a characteristic V-shape. Such a correlation has been reported by Marder and co-workers in 1,4-bis(*p*-*R*-phenylethynyl)benzenes

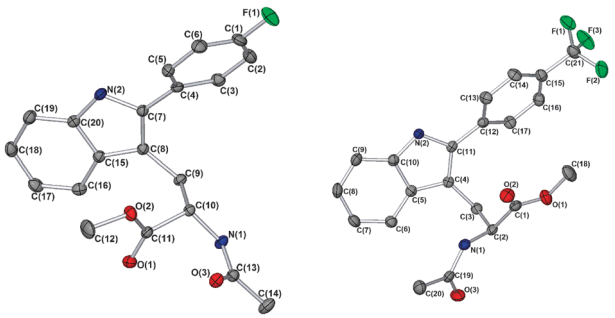


Fig. 6 Single crystal X-ray structures for **2c** (left) and **2d** (right); ellipsoids shown at 50%, H-atoms omitted.

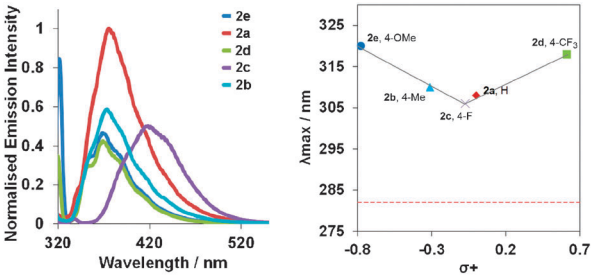
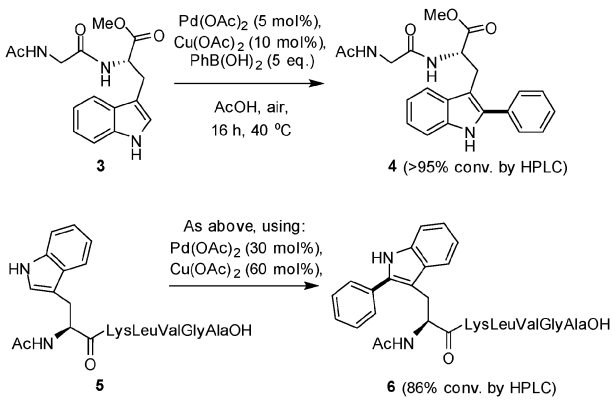


Fig. 7 Fluorescence spectra for 2-aryl-tryptophan **2a–e** (the red dotted line denotes the cut-off from amino acid residues in proteins).

Table 1 UV-vis data and Stokes shifts for 2-aryl-tryptophans **2a–e**[a]

Compound	Abs. λ_{max} (nm)	Em. (nm)	Stokes shift	ε/cm^{-1} mol^{-1} dm^{-3}
2a	308	370	62	9120
2b	310	375	65	8893
2c	306	416	110	14 684
2d	318	368	50	10 297
2e	320	368	48	11 644

[a] Solutions of **2a–e** in CH_2Cl_2.



Scheme 2 Direct C–H functionalisation of two selected peptides.

and 2,5-bis(phenylethynyl) thiophenes, and could be an indication of a push–pull type system within the series **2a–e.**[17] The largest fluorescence intensity is seen for **2a** – the other compounds **2b–e** exhibit lower fluorescence intensity. Within the series of **2a–e**, **2c** (4-F) exhibits the largest Stokes shift. Moreover, the emission wavelength is red-shifted relative to free tryptophan (*ca.* 360 nm) (Table 1).

Selective arylation of peptides. We have successfully applied our best reaction conditions (given in Fig. 5) to the arylation of dipeptide **3** (Scheme 2), which gave **4** in >95% conversion (by HPLC analysis). When applied to a more complicated system – the six residue peptide AcTrpLysLeuValGlyAlaOH **5** – in a reaction with $PhB(OH)_2$ to give **6** – it was necessary to increase catalyst loading to 30 mol% and 60 mol% for Pd and Cu respectively. This reaction proceeded in good conversion (86%).

In summary, we have reported a mild and selective direct C–H functionalisation reaction for the amino acid tryptophan, both as a single residue and as a residue in short and longer-chained peptides. It is tempting to suggest that the amino acid may play some role in interacting with and stabilising the PdNPs, particularly for the longer peptides, especially as it is known that certain peptides template (by reduction) the formation of specifically-sized and shaped PdNPs.[18] These aspects pertaining to C–H bond functionalisation are currently being investigated within our laboratories.

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Notes and references

‡ As PdNPs are formed rapidly in this chemistry, we define $Pd(OAc)_2$ as a precatalyst.

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