

# Intracellular Deprotection Reactions Mediated by Palladium Complexes Equipped with Designed Phosphine Ligands

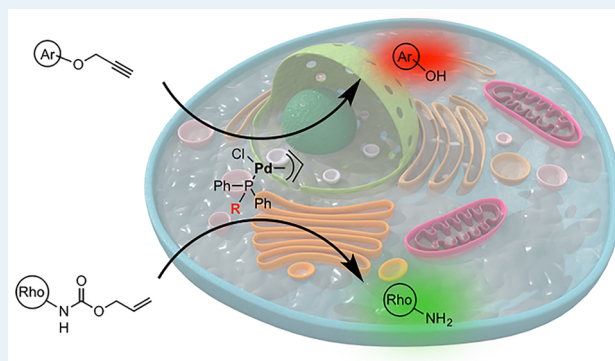
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## Supporting Information

**ABSTRACT:** Discrete palladium(II) complexes featuring purposely designed phosphine ligands can promote depropargylation and deallylation reactions in cell lysates. These complexes perform better than other palladium sources, which apparently are rapidly deactivated in such hostile complex media. This good balance between reactivity and stability allows the use of these discrete phosphine palladium complexes in living mammalian cells, whereby they can mediate similar transformations. The presence of a phosphine ligand in the coordination sphere of palladium also provides for the introduction of targeting groups, such as hydrophobic phosphonium moieties, which facilitate the accumulation of the complexes in mitochondria.

**KEYWORDS:** palladium, discrete complexes, bioorthogonal, intracellular catalysis, mitochondrial confinement



The functioning of the cell is dependent on the regulated action of thousands of enzymes, many of which require metal cofactors for their activity. In most of the cases, the metal works either as a Lewis acid or as an electron-transfer center.<sup>1</sup> In recent years, there have been impressive advances toward the development of artificial metalloenzymes capable of achieving transformations that do not occur in nature,<sup>2–4</sup> with a special emphasis on those promoted by late transition metals.<sup>5,6</sup> However, translating these metalloprotein catalysts to living settings is far from obvious and has only been proved in isolated cases and in bacterial periplasms.<sup>7,8</sup>

More success has been attained with discrete, small transition-metal complexes that are capable of crossing cell membranes and, thereby, able to promote intracellular transformations.<sup>9–13</sup> This is the case for several ruthenium(II) complexes that can mediate the uncaging of allyl-carbamate (alloc) protected amines in living mammalian cells.<sup>14–17</sup>

Particularly exciting is the possibility of using Pd catalysts in biological contexts, because of the well-known transformative power of palladium in organometallic chemistry.<sup>18,19</sup> However, the use of palladium complexes in the complex environment of living cells is seriously compromised by many issues, including solubility, stability, biorthogonal reactivity, or toxicity. Therefore, most applications that have been described so far involve the use Pd nanostructures or mesostructures, rather than discrete complexes. Hence, Bradley, Unciti-Broceta, and co-workers have claimed that small Pd(0) microspheres can induce depropargylation reactions or even Suzuki–Miyaura cross-coupling in biological settings,<sup>20,21</sup> and Chen reported that

freshly made palladium nanoparticles can promote similar reactions.<sup>22</sup>

Discrete palladium complexes have also been used, but only sporadically, and with somewhat differing results. Therefore, Chen and co-workers have shown that commercial Pd precursors such as Pd(dba)<sub>2</sub> and Pd<sub>2</sub>(allyl)<sub>2</sub>Cl<sub>2</sub> can cleave propargyloxy groups (*poc*) in PBS at 37 °C, and even in HeLa cells.<sup>23</sup> However, a recent study by the Weissleder group reveals that these commercial Pd sources perform poorly when using standard tissue culture (HBSS and MEM) as reaction milieu. Remarkably, these authors were able to obtain good yields in alloc-removing reactions by using *bis*[tris(2-furyl)phosphine]-palladium(II) dichloride as a catalyst, although stability and solubility issues precluded direct use of the complex in living settings. This problem was solved by encapsulating the metal complex within a biocompatible polymer (see Scheme 1).<sup>24</sup>

The above results raise doubts on whether discrete palladium complexes can indeed be used as catalysts in complex biological media and in living cells. Such small-sized palladium complexes are very attractive, because of their well-defined nature, and the possibility of manipulating the ligands to tune solubility, stability, and reactivity properties, and eventually favor cell transport and intracellular targeting.

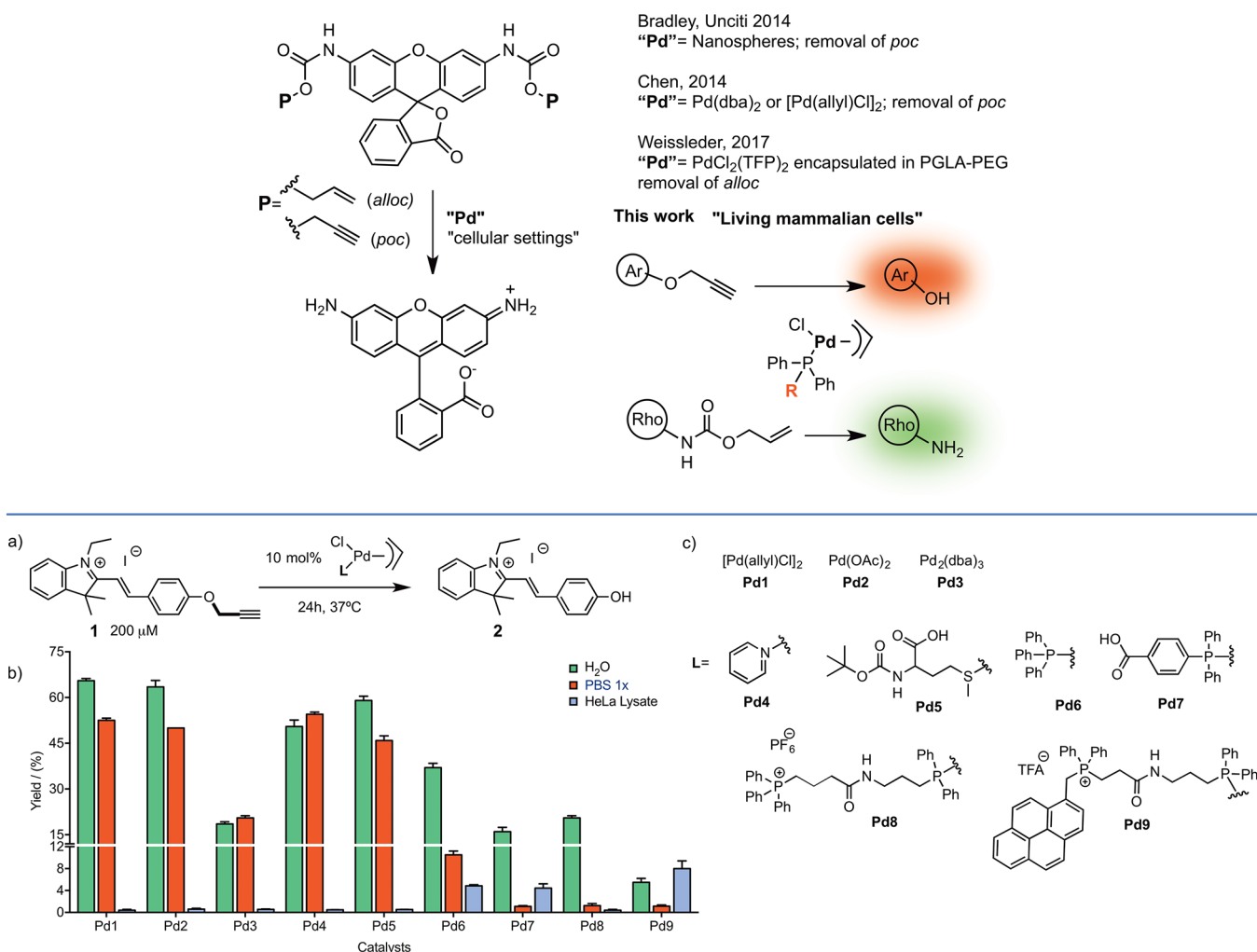
Herein, we demonstrate that discrete palladium complexes with designed phosphine ligands can promote propargyl and

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**Scheme 1. Previous Examples of In Cellulo Uncaging of Poc and Alloc Probes Mediated by Pd Catalysts and Reactions Described in This Work**



**Figure 1.** (a) Representation of the uncaging reaction of **1**, and (b) bar diagram representation of the yields obtained for each catalyst under biological compatible conditions in water, PBS buffer, and cell lysates. Reaction conditions: **1** (0.2 mmol, 1 equiv), Pd (0.02 mmol, 0.1 equiv) in 1 mL, 37 °C, 24 h (mean ± s.e.m, *n* = 2). (c) Pd catalysts used in this work for the study of their catalytic performance in biological compatible media and in living cells. Yields were calculated by RP-HPLC-MS, using coumarin as an internal standard.

alloc cleavage reactions in cell lysates, and even in living mammalian cells. Importantly, appropriate engineering of the ligand allows the mitochondria accumulation of an active palladium catalyst.

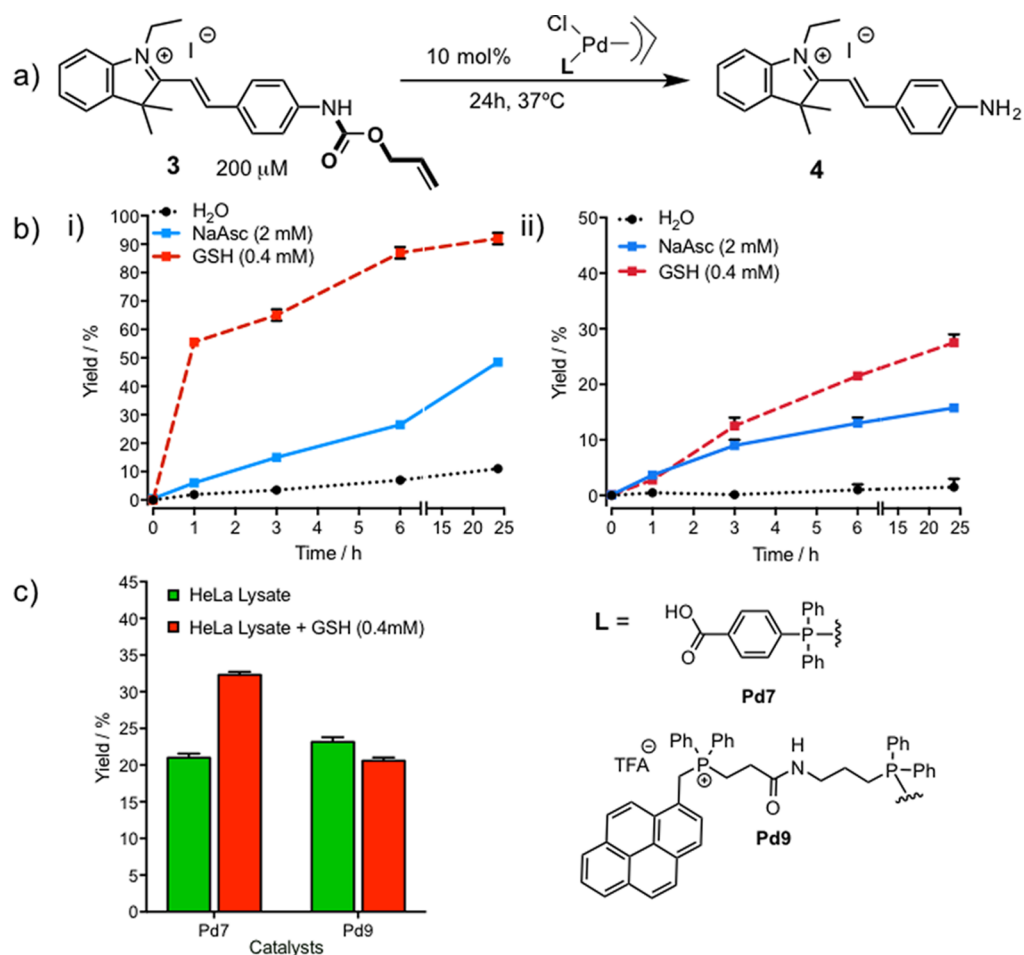
Given that previous studies did not clearly establish the *in vitro* reactivity of different palladium sources to cleave propargyloxy groups, we investigated the catalytic performance of several complexes in PBS and cell lysates.

In addition to standard commercial species, such as [Pd(allyl)Cl]<sub>2</sub> (**Pd1**), Pd(OAc)<sub>2</sub> (**Pd2**), and Pd<sub>2</sub>(dba)<sub>3</sub> (**Pd3**),<sup>23–25</sup> we purposely made a series of Pd(II) complexes (**Pd4–Pd9**) that exhibited designed pyridine, thioether, or phosphine ligands (see Figure 1 and pp S3–S8 in the Supporting Information). In the case of the phosphine-containing derivatives, we built complexes with neutral (**Pd6**), anionic (**Pd7**), or positively charged phosphonium tethers (**Pd8** and **Pd9**).

Instead of the typical assay consisting of the cleavage of a propargylic carbamate, we studied the depropargylation of phenol derivative **1**. We chose this probe because of its good

aqueous solubility, ease of synthesis, and good separation properties by HPLC-MS. Furthermore, in contrast to standard Rhodamine probes, it presents only one propargylic appendage, thereby simplifying the analysis.

The reactions were carried out using a 200 μM solution of substrate **1** and 10 mol % of the Pd sources in either water, PBS (phosphate buffered saline, pH 7.2), or HeLa cell lysates for 24 h at 37 °C (see Figure 1 and the Supporting Information (pp S9–S13)). **Pd1** and **Pd2**, and the complexes bearing pyridine and methionine ligands (**Pd4** and **Pd5**) provided good yields of **2**, in both water and PBS; however, in cell lysates, the conversion was extremely low. Likely, these palladium complexes do not survive to the complex mixture of components present in a cell lysate, and decompose to form inactive Pd species.<sup>26</sup> The palladium complexes with phosphine ligands **Pd6–Pd9** gave lower conversions in water, with yields ranging from ca. 40% for **Pd6** to ca. 15% for **Pd7–Pd9**, and performed very poorly in PBS, likely because of the low solubility of the complexes in high-ionic-strength media.



**Figure 2.** (a) Uncaging of **3**, (b) plot of the yields of reaction obtained with catalysts (i) **Pd7** and (ii) **Pd9** at 1, 3, 6, and 24 h in only water, or with additives: NaAsc (2 mM) or GSH (0.4 mM) (reaction conditions: **3** (0.2 mmol, 1 equiv), Pd complex (0.02 mM, 0.1 equiv) in 1 mL, 37 °C, 24 h), and (c) uncaging reaction in cell lysates (green column) and in cell lysate +0.4 mM of GSH (red column) promoted by catalysts **Pd7** and **Pd9**. [Reaction conditions: 10 (0.2 mmol, 1 equiv), Pd (0.02 mM, 0.1 equiv) in 1 mL, 37 °C, 24 h.] Inset shows **Pd7** and **Pd9**. Yields were calculated by RP-HPLC-MS, using coumarin as an internal standard (mean  $\pm$  s.e.m.,  $n = 2$ ).

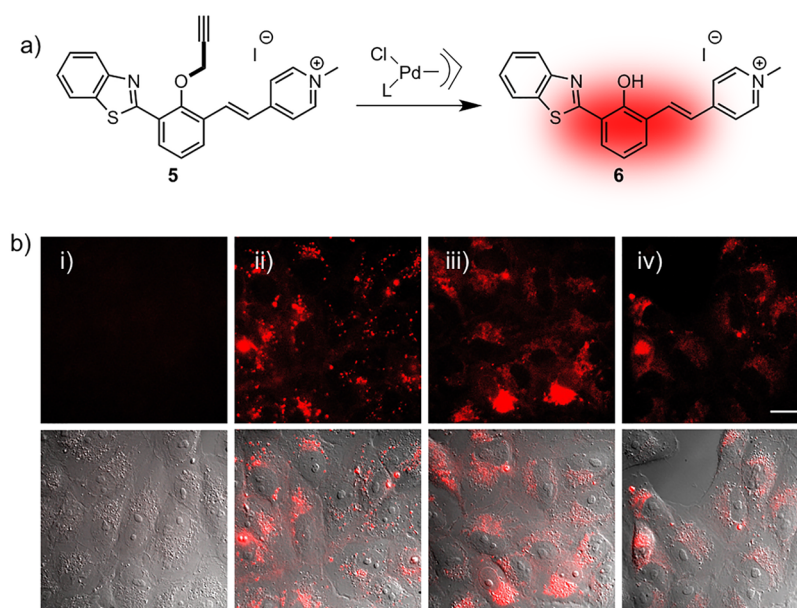
However, complexes **Pd6**, **Pd7**, and **Pd9** were able to promote the depropargylation reaction in cell lysates. Although the yields were low, their performance is better than that of the other palladium sources (**Pd1–Pd5**). Possibly, albeit a phosphine ligand is not the best one from a reactivity point of view, the corresponding palladium complexes are more stable and can better resist hostile media such as that present in cell lysates.

We also investigated the ability of these latter phosphine palladium reagents to remove alloc protecting groups, which is a process that likely involves Pd(0) reagents. The uncaging reaction was evaluated in substrate **3**, in the absence or presence of added reducing agents (see Figure 2a and the Supporting Information (pp S9–S13)). The reactions were performed using 200  $\mu$ M solutions of **3** in water, 10 mol % of the palladium complexes, at 37 °C, in the absence or presence of 2 mM of NaAsc or 0.4 mM of GSH, and monitored by high-performance liquid chromatography-mass spectroscopy (HPLC-MS) at 1, 3, 6, and 24 h. In the absence of the additives, the yields were very poor; however, in the presence of NaAsc, we obtained yields of >50% and >30%, using **Pd7** and **Pd9**, respectively (24 h). With GSH, the reaction with **Pd7** was more efficient, and hence, we observed 60% yield after 1 h and 94% after 24 h. When the experiments were performed in the

presence of cell lysates, **Pd7** and **Pd9** led to product yields of 33% and 24% after 24 h.

With further additions of GSH (0.4 mM) to the lysate, the yields were not very different (24% and 22%, respectively; see Figure 2).

The above results confirm that phosphine palladium(II) complexes can promote depropargylation and deallylation reactions in water with good yields. Although in cell lysates, the efficiency is considerably lower, we were intrigued to know whether the reactions could be achieved inside living cells, as the intracellular environment does not necessarily parallel that of a cell lysate. A key element to take into account before moving to intracellular reactions is uptake of the potential catalysts. Inductively coupled plasma–mass spectroscopy (ICP-MS) analysis of cellular extracts (Vero cells), obtained after extensive washing and lytic treatment, revealed a higher amount of palladium after the addition of complexes **Pd6–Pd9** than of species **Pd1–Pd5** (see Figures S12 and S13 in the Supporting Information). Importantly, cell viability tests (MTT in Vero cells, 24 h) demonstrated that the phosphine-containing complexes are essentially nontoxic below 50  $\mu$ M, and some of them can be used even at higher concentrations (see Figure S14 in the Supporting Information). While substrate **1** was good for in vitro studies, the change in fluorescence after the



**Figure 3.** (a) Schematic representation of the uncaging reaction of the propargylated probe **5**, and (b) imaging of the reaction in Vero cells (the first row represents the red channel; the second row represents the merger of bright-field and red channels: (i) mock, (ii) **Pd6**, (iii) **Pd7**, and (iv) **Pd8**). [Reaction conditions: **5** ( $50\ \mu\text{M}$ ) was incubated in DMEM with 5% fetal bovine serum (FBS) for 30 min. Culture medium was removed and cells were washed twice before addition of the Pd catalyst ( $50\ \mu\text{M}$ ). Cells were then incubated for another 30 min, washed twice, and observed under the microscope (scale bar =  $20\ \mu\text{m}$ ).]

reactions is not strong enough for an appropriate monitoring in cellular settings. Therefore, we changed to the propargylated probe **5**, which, after uncaging, generates a product **6** that emits at 635 nm when excited in the far-ultraviolet (far-UV) region (see Figure 3a).<sup>27</sup>

The experiments were carried out by incubation of Vero cells at a final concentration of  $50\ \mu\text{M}$  of the probe **5** in culture medium containing 5% fetal bovine serum (FBS-DMEM) for 30 min at  $37\ ^\circ\text{C}$ . The milieu was removed, and cells were washed twice with FBS-DMEM to ensure removal of extracellular probe. Pd reagents ( $50\ \mu\text{M}$ ) were then added in fresh media (0.1% dimethyl sulfoxide (DMSO)), and, after 30 min, cells were imaged by wide-field fluorescence microscopy.

While in the experiments with Pd sources (**Pd1–Pd3** and complexes **Pd4** and **Pd5**), we observed almost negligible red emission (Figure S15 in the Supporting Information), with species **Pd6–Pd8**, equipped with phosphine ligands, we detected strong red emission due to the release of the product **6** inside Vero cells (Figure 3b). The overlap between the absorption of the pyrene unit in **Pd9** and that of product **6** precluded the analysis of the reaction with this complex. Overall, these results are consistent with the *in vitro* studies and confirm that the phosphine ligands are beneficial for observing “in cell” reactivity, likely because the complexes present a good balance between reactivity and stability in the cellular milieu.

Considering the amount of palladium that was present in cells, as measured by ICP-MS of cell extracts after extensive washing steps, and associating the normalized fluorescence intensity with the amount of product, using calibration curves (see the Supporting Information (p S18)), it is possible to estimate whether there is some turnover. Indeed, for **Pd7**, we calculated an average TON of 10, whereas for **Pd8**, it is  $>5$ , suggesting that catalytic cycles can operate in cellular settings.

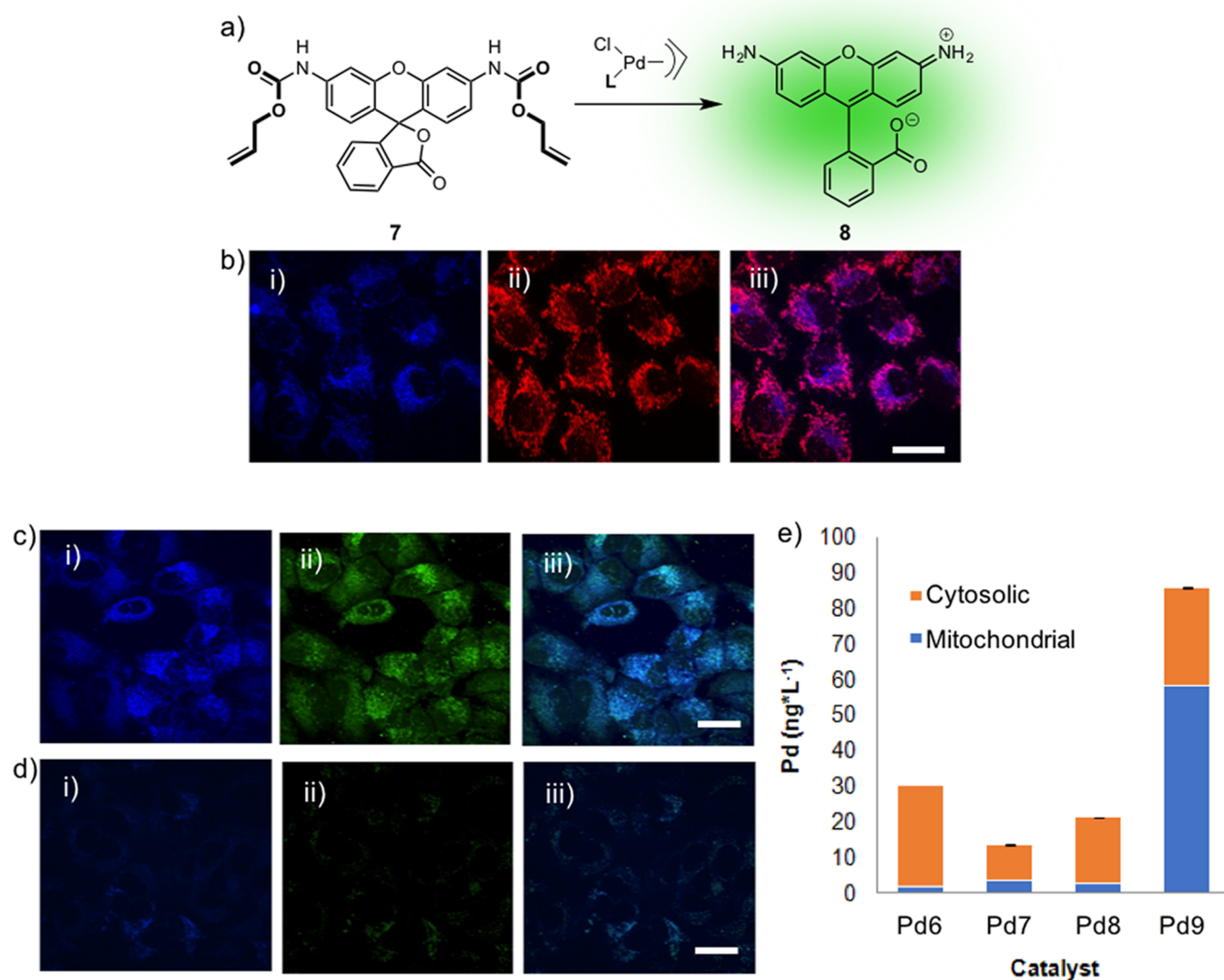
The cellular reactions can also be carried out in mammalian cells other than Vero, such as HeLa (see Figure S17 in the Supporting Information). We also checked an inverse protocol

in which cells are first treated with the palladium reagents, and the probe is added after 30 min, after the corresponding washing steps. However, in these cases, we did not observe fluorescence, likely because the palladium complexes, over time, are converted to species that are not enough active in the depropargylation process.

We next explored the removal of alloc groups inside living HeLa cells, in this case, using the caged Rhodamine 110 derivative **7**, which is essentially nonfluorescent, but emits green light after removing the alloc protecting groups. Initial experiments were carried out by mixing HeLa cells with **7** ( $50\ \mu\text{M}$  in FBS-DMEM) for 30 min, 2-fold washing with FBS-DMEM, and addition of the Pd complexes. After 30 min at  $37\ ^\circ\text{C}$ , cells were visualized under the microscope. As in the case of the depropargylation reaction, the complexes **Pd1–Pd5** raised only very low intracellular fluorescence, however, with species **Pd6–Pd8**, we could observe strong intracellular green fluorescence (see Figure S18 in the Supporting Information).

Inverting the order of addition, first the palladium complexes, and after 30 min, washing and addition of the probe **7**, we detected intracellular fluorescence, but only with complexes **Pd8** (see Figure S19 in the Supporting Information) and **Pd9** (Figure 4). Similar results were obtained in Vero cells (see Figure S20 in the Supporting Information).

In the case of **Pd9**, the presence of a pyrene group in the ligand structure allowed a direct monitoring of the species in living cells. As shown in Figure 4, the blue emission of living cells after addition of this complex shows a very good colocalization with the red color of a mitotracker (Figure 4b, panels (i)–(iii)). This was further confirmed by ICP-MS analysis of cells lysed under conditions that allowed the isolation of the mitochondria from the rest of the cytoplasmic mass. As indicated in Figure 4e, **Pd9** presents a substantially higher mitochondrial accumulation than the other complexes. Likely, the presence of the pyrene and phosphonium groups in the ligand allows the right combination of charge and



**Figure 4.** (a) Representation of the uncaging reaction. (b) Co-localization of **Pd9** with TMRE in HeLa cells, observed 2 h after incubation with the palladium complex: (i) blue light emission of **Pd9**; (ii) red light emission from the mitochondrial dye TMRE; and (iii) merged image of (i) and (ii). (c) Reaction promoted by **Pd9**: (i) blue light emission of **Pd9**, (ii) green light emission from released **8**, (iii) merged image of (i) and (ii). (d) Reaction promoted by **Pd7**: (i) blue channel, (ii) green light emission from released **8**, (iii) merged image of (i) and (ii). (e) Graphical representation of the ICP-MS results from mitochondrial isolation of fractionated Vero cells using complexes **Pd6–Pd9** (see the Supporting Information for details). [Reaction conditions: Pd complexes (50  $\mu$ M) were incubated with HeLa cells in DMEM with 5% FBS for 2 h at 37  $^{\circ}$ C before substrate **7** (50  $\mu$ M) was added; the cells were incubated for another 30 min and observed under the microscope (scale bar = 20  $\mu$ m).]

hydrophobicity to favor a significant mitochondrial accumulation of the complex.

Importantly, in cells treated with **Pd9**, we observed an efficient transformation of **7** to **8**, as deduced from the very strong buildup of green fluorescence, that also accumulates in the mitochondria surroundings (Figure 4c, panels (i)–(iii)). Other phosphine-containing catalysts, such as **Pd7** or **Pd8**, were less active (see Figure 4d, panels (i)–(iii), as well as Figure S21 in the Supporting Information).

Given the positive results with the rhodamine alloc probe (**7**) and **Pd8** and **Pd9**, using the inverse protocol (palladium complexes added first), we also tested this protocol with the rhodamine derivative having poc instead of alloc protecting groups (**Rho-poc**).<sup>23</sup> The results, which are detailed in the Supporting Information, indicated that, while **Pd8** failed to raise significant fluorescence, **Pd9** was able to promote the

depropargylation. This might be associated with a protective effect of the mitochondria surroundings on the stability of the palladium complex (see Figure S22 in the Supporting Information).

## CONCLUSIONS

Our results confirm that, while many Pd(0) and Pd(II) sources can effect depropargylation and deallylation reactions in water and PBS, they are much less effective in complex cellular media or living cells. However, palladium complexes featuring designed phosphine ligands can promote the transformations in cell lysates with variable efficiencies, which are dependent on the characteristics of the ligands. Despite this modest activity, these palladium phosphine complexes can be translated to living mammalian cells and promote both depropargylation and alloc cleavage reactions.

We have also demonstrated that, using a phosphine ligand tethered to phosphonium and hydrophobic pyrene groups, the palladium complex presents a preferential accumulation in mitochondria, wherein it remains active. This type of subcellular targeting might open new opportunities for promoting enhanced and selective biological effects in prodrug activation processes. Our results confirm the viability of engineering well-defined, discrete palladium complexes to promote relevant reactions in cellular milieu. The small-size and ligand tunability of these complexes can offer advantages with respect to other alternatives based on metalloenzymes or nanoconstructs, in terms of simplicity and synthetic access, as well as of controlling properties such as reactivity, targetability, and cellular uptake.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.8b01606.

Figures S1–S22, experimental procedures, and cell studies (PDF)

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### Author Contributions

The manuscript was written through contributions of all authors. All of the authors have given approval to the final version of the manuscript.

### Notes

The authors declare no competing financial interest.

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