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Noneffervescent Method for Catalysis-Based Palladium Detection with Color or Fluorescence

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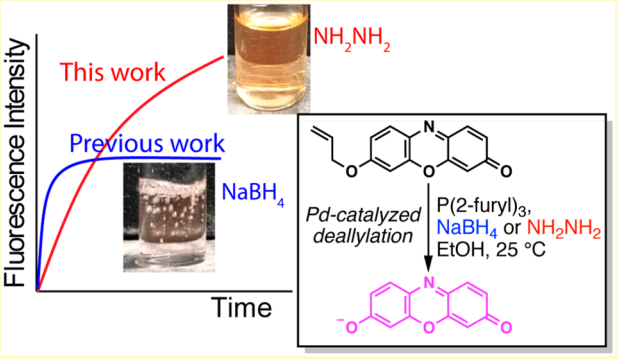
ABSTRACT: Palladium is a highly valuable metal in automobile, chemical, and pharmaceutical industries. The metal is generally quantified by atomic absorption spectrometry or inductively coupled plasma mass spectrometry. These techniques are tedious and require expensive instruments that are operated mostly off site. As cost-effective and user-friendly alternatives to these techniques, we previously reported two practical fluorometric or colorimetric methods to quantify palladium. Both methods rely on the use of $NaBH_4$, which cannot be stored in solution for more than 10 days. Commercially available solutions of $NaBH_4$ are partially or fully degraded to di- or triborohydride species and cannot be used for palladium(0)-catalyzed allylic C−O bond cleavage for quantification purposes. Here, we report a new method that replaces $NaBH_4$ with NH_2NH_2 for the palladium-catalyzed deallylation of fluorogenic and colorimetric chemodosimeter resorufin allyl ether. This method is slower but as sensitive as the most recent method from our laboratory. The method is selective for palladium and depends on the presence of tri(2-furyl)phosphine as a palladium ligand and NH_2NH_2 as a palladium-reducing reagent.



KEYWORDS: *sensors, palladium, allylic compounds, reduction, hydrazine, colorimetric method, catalysis, fluorescence*

Trace metals are quantified by atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectroscopy (ICP-MS), and X-ray fluorescence (XRF) spectroscopy, among others. Alternatively, trace metals may be quantified by fluorometric or colorimetric methods that are more amenable to on-site operation.[1] Palladium is a scarce but useful metal due to its high catalytic turnover numbers in many chemical reactions.[2,3] After palladium-catalyzed reactions are employed in pharmaceutical production, extensive purification must be employed to remove residual palladium, requiring analysis of trace palladium.[4−8] Current instrumentally intensive methods take a significant amount of time, on the order of days or even weeks, including transportation of samples, to quantify residual palladium, hindering progress during the purification process.[7] To expedite this process, many groups have reported fluorometric or colorimetric methods to detect palladium,[9,10] some of which rely on the palladium(0)-catalyzed cleavage of an allylic C−O bond.[11−24]

The first catalytic method for palladium quantification was reported from our laboratory[25] and was later improved.[26−28] With our robust and user-friendly visual methods in hand, we were able to detect and quantify minute amounts of palladium in used flasks,[29] commercial nonpalladium chemicals,[29] ores,[30] a Suzuki-Miyaura reaction mixture that was not treated with palladium,[31] synthetic pharmaceutical compounds,[32,33] and in polymers prepared by palladium catalysis[33] using allyl Pittsburgh Green ether (APE) or resorufin allyl ether (RAE). These methods were highly sensitive because $NaBH_4$ not only unified various palladium species but kept palladium species in a reduced and catalytically active form[34] despite the exposure to air. Reproducible results were obtained when a solution of $NaBH_4$ in 1−10 N NaOH was prepared and used within 10 days. These methods did not yield reproducible data in our laboratory when commercial solutions of $NaBH_4$ in 10 N NaOH were used (unpublished results). This was not surprising, as $NaBH_4$ in aqueous media cannot be stored for several weeks.[35] Moreover, evolution of hydrogen gas due to the reaction between $NaBH_4$ and water (for APE) or NH_4OAc (for RAE) occasionally caused technical problems when the bubbles interfered with fluorescence reading. Although others also used $NaBH_4$ for the fluorometric detection of palladium on the basis of O-deallylation chemistry,[15] we sought an alternative reducing agent for palladium in this study.

RESULTS AND DISCUSSION

We chose the conversion of RAE to resorufin (Figure 1) as a platform[33] to screen for alternative reducing agents due to the facile colorimetric readout. We tested 1 mM sodium ascorbate, NH_2NH_2, NH_2OH, and $NaBH_4$ in the presence of $Pd(NO_3)_2$, tri(2-furyl)phosphine (TFP), and NH_4OAc in ethanol at 24 °C. We chose ethanol as the solvent because during an extraction process, both organic and aqueous layers will be miscible with ethanol for trace palladium analysis.

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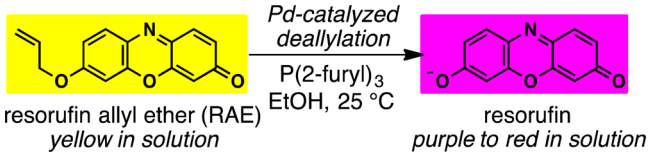

Figure 1. Conversion of nonfluorescent resorufin allyl ether to fluorescent resorufin.

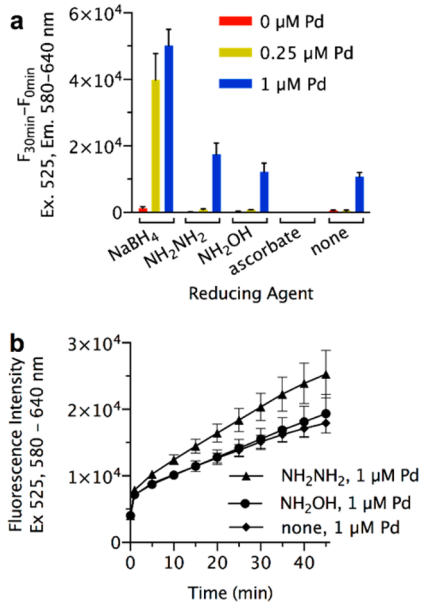

Figure 2. Comparison of potential reducing agents. (a) Conditions: 25 μM RAE, 190 μM TFP, 10 mM reducing agent, 625 mM NH_4OAc, EtOH, 25 °C, 45 min. n = 3. (b) Conditions: 25 μM RAE, 190 μM TFP, 10 mM reducing agent, 625 mM NH_4OAc, EtOH, 25 °C, 45 min. n = 3.

As Figure 2a and b show, NH_2NH_2 was slightly better than NH_2OH and ascorbate had a negative impact. As such, we chose to further study NH_2NH_2 as a potential alternative reducing agent. NH_2NH_2 is known to reduce palladium(II) to palladium(0) with concomitant generation of NH═NH through the coordination to the metal followed by a β-H elimination.[36]

For the palladium-catalyzed deallylation of RAE with TFP and NH_2NH_2, the concentrations of NH_4OAc did not impact the rate of resorufin formation (Figure 3). The deallylation rate, however, depended on the concentration of NH_2NH_2 (Figure 4a). Hydrazine is presumably the reducing agent of palladium[37] and is not the metal ligand, as the reaction required both palladium and TFP (Figure 4a). We then compared the new method with our previous stop-and-go method[33] using $NaBH_4$ and 300 nM palladium (Figure 4b). As previously described, the deallylation under the stop-and-go conditions proceeded rapidly and autonomously stalled within 20 min.[33] The current method was initially slower but continued over time, reaching similar signal intensity in 15 min, eventually outperforming the stop-and-go method. The deallylation with NH_2NH_2 did not show an appreciable slowing, indicating that the new method is continuous rather than autonomously discontinuous. Measurement of the resultant absorbance as the reaction proceeds indicates the rapid change from a yellow solution (RAE) to a red solution (resorufin) as expected (Figure 4c).

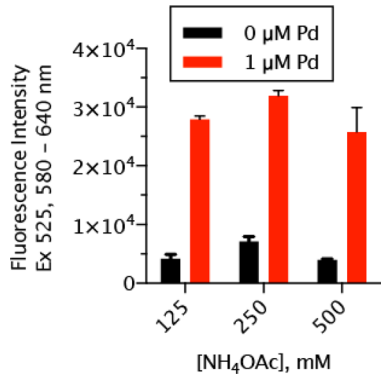

Figure 3. Concentration dependence of ammonium acetate. Conditions: 20 μM RAE, 0, 0.25, or 1.0 μM Pd^{2+}, 150 μM TFP, 1.0 mM NH_2NH_2, 125, 250, or 500 mM NH_4OAc, 25 °C, 60 min. n = 3.

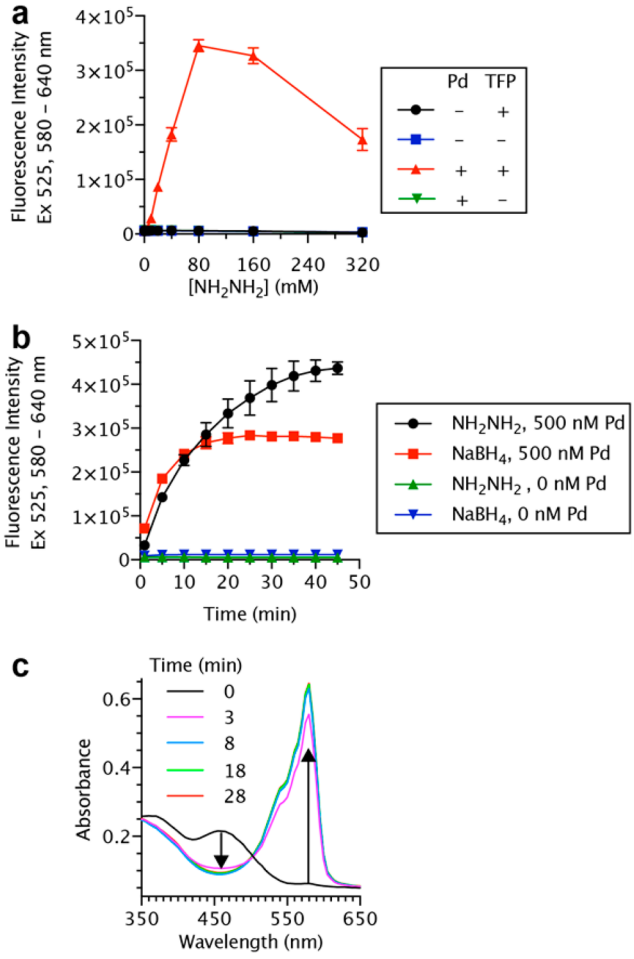

Figure 4. (a) Hydrazine concentration dependence. Conditions: 50 μM RAE, 0 or 1.0 μM Pd^{2+}, 0 or 190 μM TFP, 0–320 mM NH_2NH_2, EtOH, 25 °C, 60 min. n = 3. (b) Comparison of the stop-and-go method with $NaBH_4$ and the current method with NH_2NH_2. Conditions for the stop-and-go method: 45 μM RAE, 200 μM TFP, 45 mM $NaBH_4$, 0 or 500 nM Pd^{2+}, 640 mM NH_4OAc, EtOH, 25 °C; Conditions for the current method: 45 μM RAE, 240 μM TFP, 90 mM NH_2NH_2, 0 or 500 nM Pd^{2+}, EtOH, 25 °C, 45 min, n = 5. (c) UV–vis absorption of the current method. Conditions: 50 μM RAE, 1.0 μM Pd^{2+}, 240 μM TFP, 100 mM NH_2NH_2, EtOH, 25 °C.

Previously, we reported that the palladium-catalyzed deallylation of RAE in the presence of $NaBH_4$ could be accelerated

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by TFP, tris(2,4-di-*tert*-butylphenyl)phosphite, 1,3,5-triazaphosphaadamantane, and tris(4-methoxyphenyl)phosphine.[33] The use of TFP and $NaBH_4$ for the deallylation of RAE was selective for palladium, but platinum also generated a notable signal.[33] When the $NaBH_4$ was replaced by NH_2NH_2, we found that TFP, 1,3,5-triazaphosphaadamantane, and tris(4-methoxyphenyl)phosphine all exhibited high selectivity toward palladium (Figure 5). Among these three phosphine ligands,

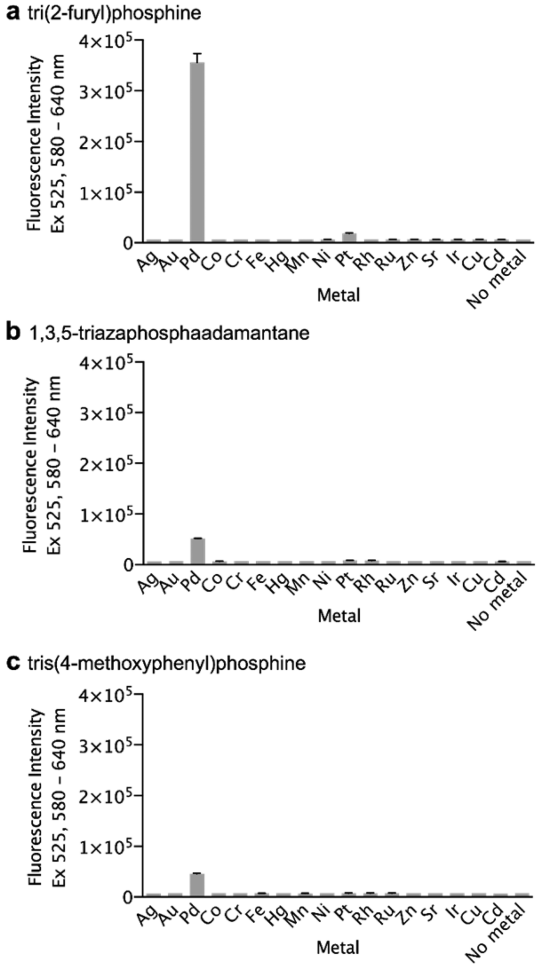

Figure 5. (a) Metal screening with TFP. Conditions: 50 μM RAE, 200 μM TFP, 90 mM NH_2NH_2, 500 nM Metal, 24 °C, 60 min. (b) Metal screening with 1,3,5-triazphosphaadamantane. Conditions: 50 μM RAE, 200 μM 1,3,5-triazphosphaadamantane, 90 mM NH_2NH_2, 500 nM Metal, 24 °C, 60 min. (c) Metal screening with tris(4-methoxypheny)phosphine. Conditions: 50 μM RAE, 200 μM tris(4-methoxypheny)phosphine, 90 mM NH_2NH_2, 500 nM Metal, 24 °C, 60 min.

TFP was the most effective, accelerating the reaction 7-fold relative to 1,3,5-triazaphosphaadamantane and 8-fold relative to tris(4-methoxyphenyl)phosphine (Figure 5a). With the method presented here, the signal with platinum is nearly negligible (Figure 5a). Thus, this method is more selective for palladium among the metals shown in Figure 5 than our previous methods.[25,33,37]

We proceeded to optimize for the TFP concentration. As Figure 6a shows, the optimal concentrations were 240–280 μM. Higher TFP concentrations were detrimental to the deallylation, presumably because 3 or 4 TFP molecules bind to palladium species and deactivate the catalyst through

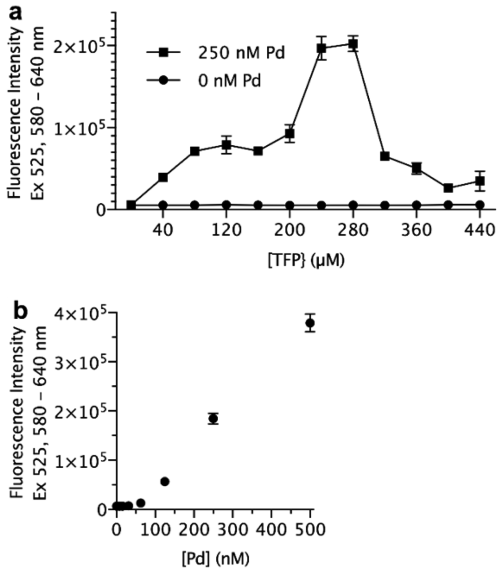

Figure 6. (a) TFP concentration dependence. Conditions: 50 μM RAE, 90 mM NH_2NH_2, 0–440 μM TFP. (b) Pd concentration dependence. Conditions: 50 μM RAE, 240 μM TFP, 90 mM NH_2NH_2, 0–500 nM Pd^{2+}, 24 °C, 40 min.

coordinative saturation. Under these optimized conditions, this method exhibited a linear correlation between the production of resorufin and nanomolar palladium concentrations in 40 min (Figure 6b).

As the previous RAE method relied on $NaBH_4$, we chose to study whether hydrazine could be stored for longer periods of time. Literature has shown that $NaBH_4$ cannot be stored for more than a few days, even in concentrated NaOH.[35] We prepared solutions of NH_2NH_2 in EtOH and stored them at room temperature. Solutions stored for 30 d displayed no loss in reactivity compared to freshly prepared solutions, indicating that solutions of NH_2NH_2 could be stored for longer periods of time (Figure 7).

With an optimized noneffervescent method in hand, we sought to demonstrate utility of the method in detecting palladium in real-world samples. Mining operations for palladium require off-site analysis with specialized instrumentation. Colorimetric analysis can alleviate this time-intensive, low-

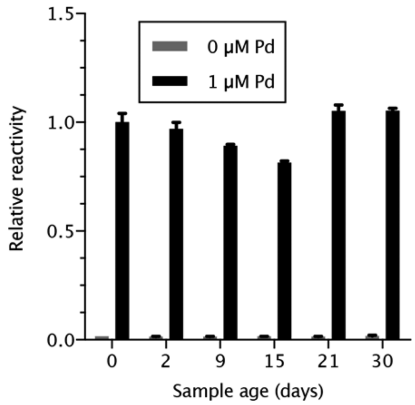

Figure 7. Determination of NH_2NH_2 stability. 5 M solutions of NH_2NH_2 in EtOH were prepared and stored at 24 °C. Conditions: 50 μM RAE, 240 μM TFP, 90 mM NH_2NH_2, 1 μM Pd^{2+}. Reactivity was normalized to 0 day sample as 1.0.

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throughput procedure. To see if our new method was amenable to geological samples, we suspended ball-milled ore samples in reaction solutions and placed them in a shaker, vigorously agitating samples at room temperature. After ~2 h, we could visually distinguish between samples containing large amounts of palladium (0.094%/wt[30]) and lower concentrations (0.068 and 0.030%/wt[30]) (Figure 8). Whereas our previous colorimetric method required sonication for extraction to differentiate palladium concentrations, this new method did not require any extra steps. The previous method suffered from rapid stalling of the reaction faster than palladium dissolution, whereas this method was able to continuously produce a signal, compatible with slow dissolution. As such, the new method is amenable to geological samples without requiring extraction or pretreatment. Combined with the stability of TFP and NH_2NH_2 solutions, the new method possesses the potential for stock solutions of each reactant to be stored for longer periods of time, enabling instrument-free utility in remote areas, such as mining exploratory sites.

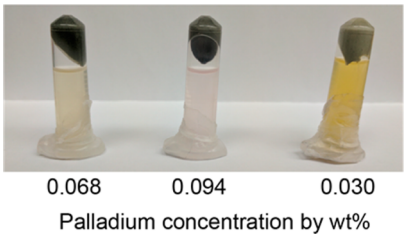

Figure 8. Detection of palladium in ores. Conditions: 45 μM RAE, 240 μM TFP, 90 mM NH_2NH_2, 24 °C, 2 h. The most palladium-rich sample shows pale pink color.

CONCLUSIONS

We have developed a colorimetric and fluorometric method for quantifying trace palladium by exploiting the palladium-catalyzed cleavage of an allylic C−O bond. Unlike previous methods from our laboratory, this method uses NH_2NH_2 rather than $NaBH_4$ as a reducing agent. This discovery makes the method presented here more reproducible with less likelihood of human errors, as NH_2NH_2 solutions can be stored for many months, eliminating batch-to-batch nonreproducibility, and eliminates issues with effervescence of previous methods using $NaBH_4$. The present method is more selective toward palladium than the previous methods.

METHODS

Materials and General Methods. All the reactions were performed at 25 °C and analyzed in a black 96-well round-bottom plate unless otherwise stated. All palladium solutions were diluted with TraceMetal 5% HNO_3 and ultrapure water. Palladium standard solution (1000 ppm in 10% HNO_3 with trace HCl) was purchased from High Purity Standards and used as received. OmniTrace Ultra High Purity nitric acid and pH 4 buffer were purchased from EMD and used as received. Water was taken from a Barnstead Nanopure Diamond water purification system, with a D3750 hollow fiber filtration system and UV irradiation. 200-proof ethanol was purchased from Declan Laboratories and used as received. Tri(2-furyl)phosphine, sodium borohydride, hydroxylamine hydrochloride, tris(4-methoxyphenyl)phosphine, and butylated hydroxytoluene were purchased from Acros Organics and used as received. Sodium ascorbate, ammonium acetate, and ammonium chloride were purchased from VWR and used as received. Sodium acetate was purchased from Spectrum Chemical and used as received. Hydrazine hydrate was purchased from Alfa Aesar and used as received. Resorufin sodium salt was purchased from Sigma-Aldrich and used as received. 1,3,5-Triazaphosphadamantane was purchased from Strem and used as received. Potassium phosphate tribasic was purchased from Thermo-Fisher and used as received. The pH 7 buffer was prepared through dissolving potassium phosphate tribasic in ultrapure water, followed by pH correction to 7.00 with 10 M NaOH and dilution with ultrapure H_2O to appropriate concentrations. pH 10 buffer was purchased from Boston BioProducts and used as received. 50% NaOH was purchased from J.T. Baker and used as received. Ten M NaOH solutions were prepared through the dilution of 50% NaOH with ultrapure water. Resorufin allyl ether (RAE) was prepared as previously described.[33] All glassware was washed with 5% HNO_3 followed by ultrapure H_2O and acetone to remove residual trace metals.

Instrumentation. All fluorescence measurements were carried out using a Turner Biosystems Modulus II Microplate Reader (excitation 525 nm, emission 580−640 nm) on Brand pureGrade 96-well black fluorescence plates. Absorption spectra were acquired using a Tecan NanoQuant Infinite M200 Pro UV/vis spectrometer using BrandTech Scientific polystyrene semimicro cuvettes. Pipettors used were Gilman Pipetman Classic with Fisherbrand SureOne beveled nonsterile pipet tips.

Preparation and Storage of Stock Solutions. Solutions of resorufin allyl ether (RAE) in EtOH were prepared and stored in scintillation vials at 25 °C, covered by aluminum foil for less than 1 month. Solutions of tri(2-furyl)phosphine (TFP) in DMSO, stabilized by 250 ppm butylated hydroxytoluene (BHT), were prepared and stored in amber bottles at 25 °C for more than 6 months without decomposition. Palladium solutions were prepared by diluting a high-purity palladium standard (9.4 mM in 10% nitric acid with trace hydrochloric acid, traceable to the United States National Institute of Standard Technology) with ultrapure H_2O and stored for 1 week. $NaBH_4$ solutions were stored in 10 M NaOH in a 2 mL polystyrene Eppendorf vial and discarded after 10 days.

Preparation of Metal Stock Solutions. To separate scintillation vials were added $AgNO_3$ (425 mg, 2.50 mmol), $AuCl_3$ (759 mg g, 2.50 mmol), $CoCl_2$ (325 mg, 2.50 mmol), $CrCl_3$ (396 mg, 2.50 mmol), $FeCl_3$ (406 mg, 2.50 mmol), $HgCl_2$ (679 g, 2.501 mmol), $MnCl_2$ (315 mg, 2.50 mmol), $NiCl_2$ (324 mg, 2.50 mmol), $PtCl_2$ (665 mg, 2.50 mmol), $ZnCl_2$ (341 mg, 2.50 mmol), $Sr(NO_3)_2$ (529 mg, 2.50 mmol), $IrCl_3$ (745 mg, 2.50 mmol), and $Cu(NO_3)_2$ (336 mg, 2.50 mmol) and ultrapure H_2O (2.5 mL) to afford 1.0 M metal solutions. To separate scintillation vials were added a metal solution (50 μL) that was prepared above, and ultrapure H_2O (4.95 mL, [metal] = 10 mM). Three serial dilutions of the 10 mM metal solution (20 μL) with ultrapure H_2O (180 μL) were carried out to afford a final concentration of 10 μM metal in H_2O. A subsequent serial dilution of the metal (100 μL) with ultrapure H_2O (100 μL) afforded 5 μM metal solutions in H_2O. In separate scintillation vials was added either $RuCl_3$ (10.4 mg, 50.1 μmol) or $RhCl(PPh_3)_3$ (45 mg, 50 μmol) and ultrapure H_2O (5.00 mL) To separate scintillation vials were added these solutions (100 μL) and Ultrapure (9.9 mL) to afford 100 μM metal solutions. Analogous serial dilutions with ultrapure H_2O were performed as above to afford 5 μM metal solutions. Solutions were stored at 25 °C for 6 months.

Preparation of Phosphine Stock Solutions. To separate scintillation vials was added tri(2-furyl)phosphine (23.7 mg, 0.100 mmol), 1,3,5-triazaphosphaadamantane (15.6 mg, 0.100 mmol), and tris(4-methoxyphenyl)phosphine (35.2 mg, 0.100 mmol) and DMSO containing 250 ppm BHT (5.0 mL) to prepare 20 mM phosphine solutions. BHT was added to stabilize phosphines. To a separate vial, an aliquot of previously prepared solutions (48 μL) were diluted in DMSO containing 250 ppm BHT (252 μL) to afford 3.2 mM phosphine solutions. Solutions were discarded after 1 week.

Screening Reducing Agents (Figure 2a/b). In a scintillation vial, 800 mM NH_4OAc in EtOH (10 mL), 800 μM RAE in EtOH (400 μL), and 3 mM TFP in DMSO stabilized by 250 ppm BHT (800 μL) were combined. Aliquots of this solution (175 μL) were added to a black 96-well plate. To each well, a solution of 0 or 400 μM either $NaBH_4$, NH_2NH_2, $HONH_3Cl$, or sodium ascorbate in H_2O (5 μL)

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was added, followed by 0, 2.5, or 10 μM Pd^{2+} in H_2O (20 μL; $[NH_4OAc]_{final}$ = 625 mM, [RAE] = 25 μM, $[TFP]_{final}$ = 190 μM, [reducing agent]$_{final}$ = 0 or 10 mM, $[Pd^{2+}]_{final}$ = 0, 0.25, or 1.0 μM). The fluorescence of the resulting solutions was measured every 5 min for 45 min. Figure 2a shows the data after 45 min. Figure 2b shows the fluorescence data from no reducing agent, NH_2NH_2, and sodium ascorbate solutions at the indicated time points.

Effect of Ammonium Acetate Concentration (Figure 3). To separate scintillation vials was added 200, 400, or 800 mM NH_4OAc in EtOH (5.00 mL), 800 μM RAE in EtOH (200 μL), and 3 mM TFP in DMSO stabilized by 250 ppm BHT (400 μL). Aliquots of these solutions (140 μL) were transferred to a black 96-well plate followed by either 0 or 5 mM NH_2NH_2 in H_2O (40 μL) followed by 0 or 10 μM Pd^{2+} in H_2O (20 μL; $[NH_4OAc]_{final}$ = 125, 250, 500 mM, $[RAE]_{final}$ = 20 μM, $[TFP]_{final}$ = 150 μM, $[NH_2NH_2]_{final}$ = 0 or 1.0 mM, $[Pd^{2+}]_{final}$ = 0 or 1.0 μM). The resulting reaction solutions were analyzed by fluorescence after 60 min.

Dependence on Hydrazine Concentration (Figure 4a). To a scintillation vial was added 800 mM NH_4OAc in EtOH (19.2 mL), 800 μM RAE in EtOH (1.6 mL), and 3 mM TFP in DMSO stabilized by 250 ppm BHT (1.6 mL). Aliquots of the resulting solution (1.2 mL) were transferred to 2 mL polystyrene Eppendorf vials, and to each was added either 7.8 mM NH_2NH_2 in H_2O or 2.5 M $NaBH_4$ in 10 N NaOH (24 μL). Aliquots of the resulting solutions (180 μL) were transferred to a 96-well black plate followed by either 0 or 10 μM Pd^{2+} in H_2O ($[NH_4OAc]_{final}$ = 620 mM, $[RAE]_{final}$ = 50 μM, $[TFP]_{final}$ = 190 μM, $[NH_2NH_2]_{final}$ = 0, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10, 20, 40, 80, or 160 mM, $[NaBH_4]_{final}$ = 0 or 45 mM, $[Pd^{2+}]$ = 0 or 1.0 μM. The resulting reaction solution was analyzed by fluorescence every 5 min for 60 min.

Comparison of Hydrazine and Sodium Borohydride under Optimal Conditions (Figure 4b). To a 20 mL scintillation vial was added EtOH (17.48 mL), 800 μM RAE in EtOH (1.26 mL), and 3.84 mM TFP in DMSO stabilized by 250 ppm BHT (1.26 mL). An aliquot of this mixture (1 mL) was transferred to a 2 mL polystyrene Eppendorf tube, and to it was added 5 M NH_2NH_2 in EtOH (20 μL). To a separate 20 mL scintillation vial was added 800 mM NH_4OAc in EtOH (17.35 mL), 800 μM RAE in EtOH (1.26 mL), and 3.0 mM TFP in DMSO stabilized by 250 ppm BHT (1.39 mL). An aliquot of this mixture (1 mL) was transferred to a separate 2 mL polystyrene Eppendorf tube and to it was added 2.5 M $NaBH_4$ in 10N NaOH (20 μL). Aliquots of each solution (180 μL) were transferred to a black 96-well plate followed by either 0 or 5 μM μM Pd^{2+} in ultrapure H_2O (20 μL; $[RAE]_{final}$ = 45 μM, $[TFP]_{final}$ = 200 or 240 μM TFP, $[NH_4OAc]_{final}$ = 640 mM, $[NH_2NH_2]_{final}$ = 0 or 90 mM, $[NaBH_4]_{final}$ = 0 or 45 mM, $[Pd^{2+}]_{final}$ = 0 or 500 nM). The fluorescence of the resulting solutions was measured every 5 min for 45 min.

Absorption Change over Time during the Deallylation of RAE (Figure 4c). To a scintillation vial was added EtOH (3.67 mL), 3.84 mM TFP in DMSO stabilized by 250 ppm BHT (250 μL), 5 M NH_2NH_2 in EtOH (80 μL), and 100 μM Pd^{2+} in ultrapure H_2O (40 μL). An aliquot of the resulting solution (2 mL) was transferred to a disposable semimicro UV-cuvette and analyzed as the background signal for absorption. In a separate scintillation vial was added EtOH (3.42 mL), 800 μM RAE in EtOH (250 μL), 3.84 mM TFP in DMSO stabilized by 250 ppm BHT (250 μL), and 5 M NH_2NH_2 in EtOH (80 μL; $[RAE]_{final}$ = 50 μM, $[TFP]_{final}$ = 240 μM, $[NH_2NH_2]_{final}$ = 100 mM). An aliquot of this solution was transferred to another disposable semimicro UV-cuvette and analyzed as a "zero point" for the reaction. To the solution was added 100 μM Pd^{2+} in ultrapure H_2O (20 μL; $[Pd^{2+}]_{final}$ = 1.0 μM) and absorbance was measured every 5 min for 60 min.

Metal Selectivity with Various Phosphines (Figure 5). To separate scintillation vials was added EtOH (19.2 mL), 800 μM RAE in EtOH (1.6 mL), and 3.2 mM either **P1**, **P2**, or **P3** in DMSO stabilized by 250 ppm BHT (1.6 mL). To each vial was added 5 M NH_2NH_2 in EtOH (448 μL) and aliquots of each solution (180 μL) were transferred to a 96-well black plate. To each well was added either 0 or 5 μM metal in H_2O (20 μL; $[RAE]_{final}$ = 50 μM, $[phosphine]_{final}$ = 200 μM, $[NH_2NH_2]_{final}$ = 90 mM, $[metal]_{final}$ = 500 nM. The solutions were incubated at 25 °C for 1 h, then the fluorescence of the solutions was measured.

Dependence on Phosphine Concentration (Figure 6a). Varying amounts (176−16 μL) of 10 mM **P9**, **P10**, or **P11** in DMSO stabilized by 250 ppm BHT were diluted in 500 μL Eppendorf tubes with DMSO stabilized by 250 ppm BHT (74−234 μL) to achieve concentrations of 7.04, 6.40, 5.76, 5.12, 4.48, 3.84, 3.20, 2.56, 1.92, 1.28, 0.64, and 0 mM phosphines. To a scintillation vial was added EtOH (1.37 mL), aliquots of the phosphine solutions (113 μL), 800 μM RAE in EtoH (113 μL), and 5 M NH_2NH_2 in EtOH (32 μL). The resulting solutions were added to a black 96-well plate (180 μL) and 0 or 2.5 μM Pd^{2+} in ultrapure H_2O (20 μL; $RAE]_{final}$ = 50 μM, $[NH_2NH_2]_{final}$ = 90 mM, $[phosphine]_{final}$ = 0, 40.0, 80.0, 120, 160, 200, 240, 280, 320, 360, 400, or 440 μM; $[Pd^{2+}]_{final}$ = 0 or 250 nM). The fluorescence of the resulting solutions was measured every 20 min for 60 min.

Dependence on Palladium Concentration (Figure 6b). To a scintillation vial was added EtOH (6.85 mL) was combined with 800 μM RAE in EtOH (565 μL), 3.84 mM TFP in DMSO stabilized by 250 ppm BHT (565 μL), and 5 M NH_2NH_2 in EtOH (160 μL). Aliquots of the resulting solution (180 μL) were transferred to a 96-well black plate. 10 μM Pd^{2+} in ultrapure H_2O (100 μL) was diluted by 2-fold serial dilutions to 78 nM in ultrapure H_2O and aliquots (20 μL) of each Pd^{2+} concentration were added to the solutions in the 96-well black plate ($[RAE]_{final}$ = 50 μM, $[TFP]_{final}$ = 240 μM, $[NH_2NH_2]_{final}$ = 90 mM, $[Pd^{2+}]_{final}$ = 0, 7.8, 15.6, 31.3, 62.5, 125, 250, 500 nM). The fluorescence of the resulting solutions was analyzed by fluorescence every 5 min for 40 min. The limit of detection and the limit of quantification were calculated by constructing a linear regression curve for Figure 6b and determining the slope and standard deviation (SD) of the regression. The limit of detection was calculated using (3 × SD)/slope, and the limit of quantification was calculated using (10 × SD)/slope.

Determination of Stability of NH_2NH_2 Solutions (Figure 7). 5 M NH_2NH_2 in EtOH solutions were prepared on indicated days and stored at room temperature. After 30 d, a "fresh" solution was prepared as a control. To separate scintillation vials was added EtOH (1.75 mL), 800 μM RAE in EtOH (126 μL), 3.84 mM TFP in DMSO stabilized by 250 ppm BHT (126 μL), and 5 M hydrazine solutions prepared "fresh" or aged 2, 9, 15, 21, or 30 days, respectively. The resulting solutions were added to a black 96-well plate (180 μL) and 0 or 10 μM Pd^{2+} in ultrapure H_2O was added. ($[RAE]_{final}$ = 45 μM, $[TFP]_{final}$ = 240 μM, $[NH_2NH_2]_{final}$ = 90 mM, $[Pd^{2+}]_{final}$ = 1 μM). The resulting solutions were analyzed by fluorescence after 20 min.

Detection of Palladium in Ore Samples (Figure 8). To separate 2 mL polystyrene Eppendorf tubes was added 500 mg of one of three ore samples (0.094%, 0.068%, or 0.030% Pd by wt.). To each tube was added EtOH (1.748 mL), 800 μM RAE in 32% v/v DMSO/EtOH (126 μL), 3.84 mM TFP in DMSO stabilized by 250 ppm BHT (126 μL), and 5 M NH_2NH_2 in EtOH (40 μL). The tubes were agitated at 24 °C in a VWR Standard Heavy-Duty Vortex Mixer for 2 h, then color change was documented; photo was taken under ambient light.

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Notes

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■ ABBREVIATIONS

APE, allyl Pittsburgh Green ether; RAE, resorufin allyl ether; TFP, tri(2-furyl)phosphine; BHT, butylated hydroxytoluene

■ REFERENCES

(1) Koide, K. Palladium detection techniques for active pharmaceutical ingredients prepared *via* cross-couplings. In *New trends in cross-coupling: Theory and applications*, Colacot, T. J., Ed.; Royal Society of Chemistry, 2015.
(2) Torborg, C.; Beller, M. Recent applications of palladium-catalyzed coupling reactions in the pharmaceutical, agrochemical, and fine chemical industries. *Adv. Synth. Catal.* **2009**, *351*, 3027−3043.
(3) Busacca, C. A.; Fandrick, D. R.; Song, J. J.; Senanayake, C. H. The growing impact of catalysis in the pharmaceutical industry. *Adv. Synth. Catal.* **2011**, *353*, 1825−1864.
(4) Betti, M.; Castagnoli, G.; Panico, A.; Coccone, S. S.; Wiedenau, P. Development of a scalable route to the SMO receptor antagonist SEN794. *Org. Process Res. Dev.* **2012**, *16*, 1739−1745.
(5) Mortensen, M. A.; Guo, C.; Reynolds, N. T.; Wang, L. L.; Helle, M. A.; Keefe, D. K.; Haney, B. P.; Paul, B. J.; Bruzinski, P. R.; Wolf, M. A.; Malinowski, N. L.; Yang, Q. Process development, impurity control, and production of a novel tubulin inhibitor. *Org. Process Res. Dev.* **2012**, *16*, 1811−1817.
(6) Content, S.; Dupont, T.; Fedou, N. M.; Penchev, R.; Smith, J. D.; Susanne, F.; Stoneley, C.; Twiddle, S. J. R. Optimization of the manufacturing route to PF-610355 (2): Synthesis of the API. *Org. Process Res. Dev.* **2013**, *17*, 202−212.
(7) Lewen, N.; Soumeillant, M.; Qiu, J.; Selekman, J.; Wood, S.; Zhu, K. Use of a field-portable XRF instrument to facilitate metal catalyst scavenger screening. *Org. Process Res. Dev.* **2015**, *19*, 2039−2044.
(8) Phillips, S.; Holdsworth, D.; Kauppinen, P.; Mac Namara, C. Palladium impurity removal from active pharmaceutical ingredient process streams. *Johnson Matthey Technol. Rev.* **2016**, *60*, 277−286.
(9) Li, H. L.; Fan, J. L.; Peng, X. J. Colourimetric and fluorescent probes for the optical detection of palladium ions. *Chem. Soc. Rev.* **2013**, *42*, 7943−7962.
(10) Tracey, M. P.; Pham, D.; Koide, K. Fluorometric imaging methods for palladium and platinum and the use of palladium for imaging biomolecules. *Chem. Soc. Rev.* **2015**, *44*, 4769−4791.
(11) Baker, M. S.; Phillips, S. T. A two-component small molecule system for activity-based detection and signal amplification: Application to the visual detection of threshold levels of Pd(II). *J. Am. Chem. Soc.* **2011**, *133*, 5170−5173.
(12) Jiang, J.; Jiang, H. E.; Liu, W.; Tang, X. L.; Zhou, X.; Liu, R. T.; Liu, W. S. A colorimetric and ratiometric fluorescent probe for palladium. *Org. Lett.* **2011**, *13*, 4922−4925.
(13) Cui, L.; Zhu, W. P.; Xu, Y. F.; Qian, X. H. A novel ratiometric sensor for the fast detection of palladium species with large red-shift and high resolution both in aqueous solution and solid state. *Anal. Chim. Acta* **2013**, *786*, 139−145.
(14) Chen, G. S.; Song, J. J.; Zhang, H. L.; Jiang, Y. T.; Liu, W. S.; Zhang, W.; Wang, B. D. Pd nanoparticles encapsulated in magnetic carbon nanocages: an efficient nanoenzyme for the selective detection and multicolor imaging of cancer cells. *Nanoscale* **2015**, *7*, 14393−14400.
(15) Kitley, W. R.; Santa Maria, P. J.; Cloyd, R. A.; Wysocki, L. M. Synthesis of high contrast fluorescein-diethers for rapid bench-top sensing of palladium. *Chem. Commun.* **2015**, *51*, 8520−8523.
(16) Liu, Y. C.; Xiang, K. Q.; Guo, M.; Tian, B. Z.; Zhang, J. L. A coumarin-based fluorescent probe for the fast detection of Pd^0 with low detection limit. *Tetrahedron Lett.* **2016**, *57*, 1451−1455.
(17) Luo, W. F.; Liu, W. S. A two-photon ratiometric ESIPT probe for the discrimination of different palladium species and its application in bioimaging. *J. Mater. Chem. B* **2016**, *4*, 3911−3915.
(18) Luo, W. F.; Liu, W. S. A water-soluble colorimetric two-photon probe for discrimination of different palladium species and its application in bioimaging. *Dalton Trans.* **2016**, *45*, 11682−11687.
(19) Yan, J. W.; Wang, X. L.; Tan, Q. F.; Yao, P. F.; Tan, J. H.; Zhang, L. A colorimetric and fluorescent dual probe for palladium in aqueous medium and live cell imaging. *Analyst* **2016**, *141*, 2376−2379.
(20) Goggins, S.; Stark, O. P.; Naz, C.; Marsh, B. J.; Frost, C. G. Ratiometric electrochemical detection of Pd center dot center dot center dot interactions: application towards electrochemical molecular logic gates. *Supramol. Chem.* **2017**, *29*, 749−757.
(21) Luo, W. F.; Li, J.; Liu, W. S. A two-photon ratiometric ESIPT probe for fast detection and bioimaging of palladium species. *Org. Biomol. Chem.* **2017**, *15*, 5846−5850.
(22) Su, W.; Gu, B.; Hu, X. J.; Duan, X. L.; Zhang, Y. Y.; Li, H. T.; Yao, S. Z. A near-infrared and colorimetric fluorescent probe for palladium detection and bioimaging. *Dyes Pigm.* **2017**, *137*, 293−298.
(23) Tan, H. Y.; Liu, J. G.; Zhou, L. F.; Li, Y. K.; Yan, J. W.; Zhang, L. A smart NIR fluorescent probe for the highly selective detection of palladium. *RSC Adv.* **2017**, *7*, 6583−6586.
(24) Yan, J. W.; Wang, X. L.; Zhou, L. F.; Zhang, L. CV-APC, a colorimetric and red-emitting fluorescent dual probe for the highly sensitive detection of palladium. *RSC Adv.* **2017**, *7*, 20369−20372.
(25) Song, F. L.; Garner, A. L.; Koide, K. A highly sensitive fluorescent sensor for palladium based on the allylic oxidative insertion mechanism. *J. Am. Chem. Soc.* **2007**, *129*, 12354−12355.
(26) Garner, A. L.; Koide, K. Studies of a fluorogenic probe for palladium and platinum leading to a palladium-specific detection method. *Chem. Commun.* **2009**, 86−88.
(27) Garner, A. L.; Song, F. L.; Koide, K. Enhancement of a catalysis-based fluorometric detection method for palladium through rational fine-tuning of the palladium species. *J. Am. Chem. Soc.* **2009**, *131*, 5163−5171.
(28) Song, F. L.; Carder, E. J.; Kohler, C. C.; Koide, K. Studies toward an ideal fluorescence method to measure palladium in functionalized organic molecules: effects of sodium borohydride, temperature, phosphine ligand, and phosphate ions on kinetics. *Chem. - Eur. J.* **2010**, *16*, 13500−13508.
(29) Li, D.; Campbell, L. D.; Austin, B. A.; Koide, K. Detection of trace palladium in flasks and metal reagents using a fluorogenic Tsuji-Trost reaction. *ChemPlusChem* **2012**, *77*, 281−283.
(30) Williams, J. M.; Koide, K. A high-throughput method to detect palladium in ores. *Ind. Eng. Chem. Res.* **2013**, *52*, 8612−8615.
(31) Inamoto, K.; Campbell, L. D.; Doi, T.; Koide, K. A highly sensitive fluorescence method reveals the presence of palladium in a cross-coupling reaction mixture not treated with transition metals. *Tetrahedron Lett.* **2012**, *53*, 3147−3148.
(32) Bu, X. D.; Koide, K.; Carder, E. J.; Welch, C. J. Rapid analysis of residual palladium in pharmaceutical development using a catalysis-based fluorometric method. *Org. Process Res. Dev.* **2013**, *17*, 108−113.
(33) Koide, K.; Tracey, M. P.; Bu, X. D.; Jo, J. Y.; Williams, M. J.; Welch, C. J. A competitive and reversible deactivation approach to catalysis-based quantitative assays. *Nat. Commun.* **2016**, *7*, 10691.
(34) Satoh, T.; Mitsuo, N.; Nishiki, M.; Nanba, K.; Suzuki, S. A new powerful and selective reducing agent sodium borohydride-palladium chloride system. *Chem. Lett.* **1981**, *10*, 1029−1030.
(35) Lyttle, D. A.; Jensen, E. H.; Struck, W. A. A simple volumetric assay for sodium borohydride. *Anal. Chem.* **1952**, *24*, 1843−1844.
(36) Cellier, P. P.; Spindler, J. F.; Taillefer, M.; Cristau, H. J. Pd/C-catalyzed room-temperature hydrodehalogenation of aryl halides with hydrazine hydrochloride. *Tetrahedron Lett.* **2003**, *44*, 7191−7195.
(37) Garner, A. L.; Koide, K. Oxidation state-specific fluorescent method for palladium(II) and platinum(IV) based on the catalyzed

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ACS Sens. 2017, 2, 1737−1743

aromatic Claisen rearrangement. *J. Am. Chem. Soc.* **2008**, *130*, 16472−16473.

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