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Storable Arylpalladium(II) Reagents for Alkene Labeling in Aqueous Media

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Supporting Information

ABSTRACT: We show that arylpalladium(II) reagents linked to biotin and indocyanine dye residues can be prepared by decarboxylative palladation of appropriately substituted electron-rich benzoic acid derivatives. When prepared under the conditions described, these organometallic intermediates are tolerant of air and water, can be stored for several months in solution in dimethyl sulfoxide, and permit biotin- and indocyanine dye-labeling of functionally complex olefinic substrates in water by Heck-type coupling reactions.

The value of selective bond-forming reactions between reciprocally reactive functional groups in water at ambient temperature has been noted by Sharpless and co-workers and was strikingly illustrated by them with the disclosure of the copper-catalyzed [3 + 2] dipolar cycloaddition reaction of azides and alkynes.[1] Bertozzi and co-workers as well as others have developed strain-promoted cycloaddition reactions that proceed efficiently in water without the use of catalysts.[2] In this work, we report preliminary steps toward the development of a different type of noncatalyzed (carbon−carbon) bond-forming reaction in water: a stoichiometric Heck-type coupling of discrete, storable arylpalladium(II) reagents with olefinic reactants. We describe the synthesis of three specific arylpalladium(II) reagents bearing biotin and indocyanine dye labels and demonstrate that these can be coupled with functionally complex olefinic reactants in aqueous media at ambient temperature.

Decarboxylative palladation of the biotin-linked benzoic acid derivative **1** (Figure 1), synthesized in seven steps from 2, 5-dimethoxybenzaldehyde and D-biotin [see the Supporting Information (SI)], was studied first. Substrate **1** was chosen because electron-rich benzoic acids (and their sodium salts) with the 2,4,5-substitution pattern had previously been shown to be particularly good substrates for decarboxylative palladation.[3,4] When substrate **1** (monosodium salt) was heated with palladium(II) trifluoroacetate (1−3 equiv) in DMSO-d_6 at 85 °C for 15 min, we observed that the expected arylpalladium(II) intermediate had formed but was contaminated with the product of protonolysis of the arylpalladium(II) intermediate. When instead substrate **1** was first deprotonated with excess sodium hydroxide in DMSO-d_6 (^{1}H NMR analysis suggested that a dianion was formed; see Figure 1b), decarboxylative palladation in the presence of 3.0 equiv of palladium(II) trifluoroacetate was remarkably clean and efficient, forming the stable arylpalladium(II) intermediate **2**, as evident from ^{1}H and ^{13}C NMR analysis

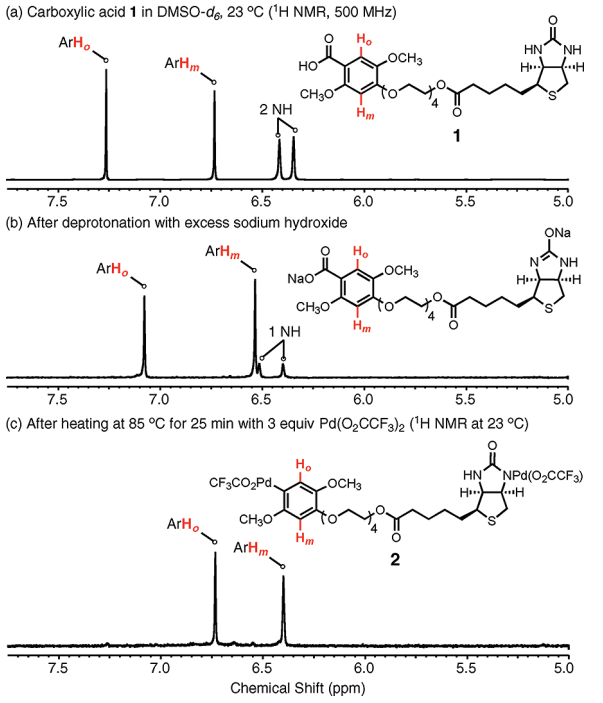

Figure 1. Decarboxylative palladation of biotin-linked benzoic acid derivative **1**.

(Figure 1c; also see the SI).[5] A marked upfield shift of the biotin carbonyl carbon resonance in the ^{13}C NMR spectrum of the product suggests that the cyclic urea was complexed with palladium. While we depict the complex as N-bound in structure **2**, we do not rule out η^3- or O-bound complexes or the possibility of dinuclear complexes (see the SI for examples of like-shifted amido- and imidopalladium complexes). Stock solutions of reagent **2** in DMSO-d_6 are stable to handling in air at ambient temperature, and when stored frozen at −20 °C and then thawed just prior to use in coupling reactions (see below), they can be used reliably for several months.

Decarboxylative palladation of the indocyanine dye-linked benzoic acid derivative **3** (Figure 2a), also synthesized in seven steps (see the SI), was studied next. The Cy5 indocyanine dye residue of substrate **3** proved to be incompatible with the conditions developed for decarboxylative palladation, as heating of substrate **3** with 1−3 equiv of palladium(II) trifluoroacetate at 80 °C for 25 min in DMSO-d_6 led to byproducts

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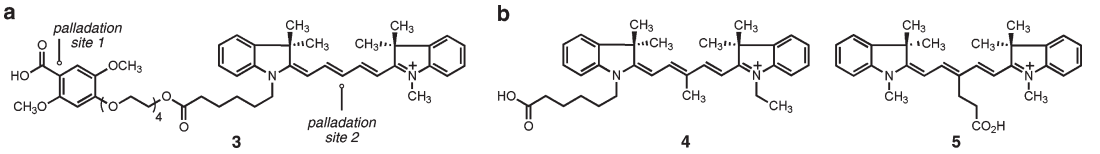


Figure 2. (a) Cy5-linked benzoic acid derivative **3** can be palladated at two sites. (b) Structures of indocyanine dyes **4** and **5**.

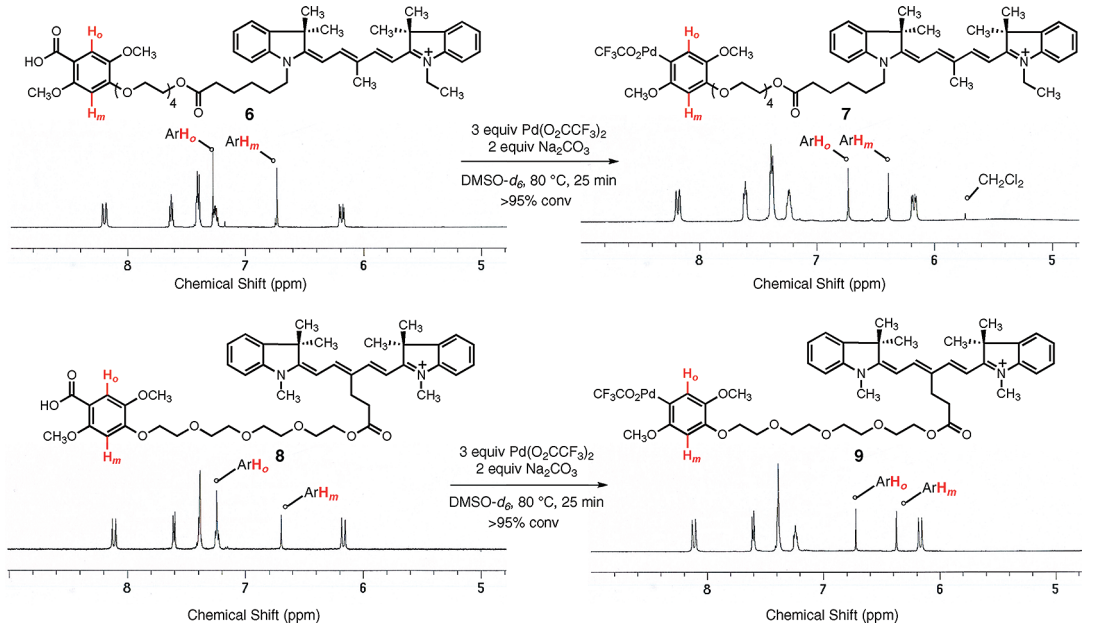


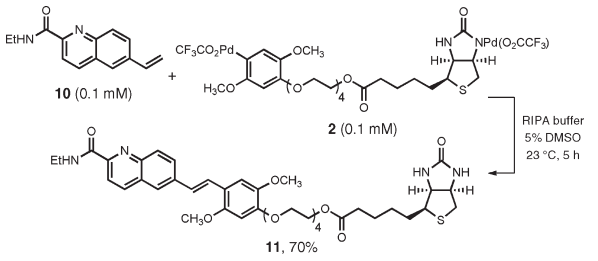
Figure 3. Decarboxylative palladation of Cy5-linked benzoic acid derivatives **6** and **8**.

arising from palladation of the central methine carbon of the dye (Figure 2) as well as the expected decarboxylative palladation product, which was the major component of the mixture.

This complication was circumvented by substitution of the central carbon of the dye. The unsymmetrically and symmetrically substituted Cy5 dyes **4** and **5**,[6] respectively (Figure 2b), were synthesized in two to four steps from commercially available materials (see the SI). These were linked to the 2,4,5-substituted benzoic acid derivative as before. When each of the Cy5 derivatives **6** and **8** was heated with 3.0 equiv of palladium(II) trifluoroacetate and 2.0 equiv of sodium carbonate in DMSO-d_6 at 80 °C for 25 min, decarboxylative palladation to form a stable arylpalladium(II) intermediate (**7** or **9**, respectively) was both clean and efficient, as evident from ^{1}H and ^{13}C NMR analysis (Figure 3 and the SI).[7] As with reagent **2**, stock solutions of reagents **7** and **9** in DMSO-d_6 were stable to handling in air at ambient temperature but were stored frozen at −20 °C and then thawed just prior to use in coupling reactions.

We initiated our studies of Heck-type coupling reactions with biotin-linked arylpalladium(II) reagent **2** using DMSO as the solvent and 6-vinylquinaldic acid derivative **10** as the substrate (Scheme 1), chosen because it served as a model for a projected mechanistic study of saframycins[8] and because the coupling product was anticipated to be fluorescent.[9] When a stock solution of **2** in DMSO (18 mM, 1.4 equiv) was combined with **10** (1 equiv) in air at 23 °C (20 h), the (fluorescent) trans coupling product **11** was obtained in 68% yield [HPLC isolation]. We next evaluated the same coupling reaction in water, limiting the total concentration of DMSO to 5%, in the presence

Scheme 1. Heck-Type Coupling Reaction of 2 and 10 in Aqueous Media



of varying buffer compositions and using equimolar amounts of each substrate but at much higher dilution (0.1 mM). The coupling product **11** was formed in yields of 35−70% at 23 °C within 4−5 h (see the SI). Optimum results (70% yield) were obtained using RIPA buffer, which contains 1% Triton X-100 and 0.1% SDS (Scheme 1). Lipshutz and co-workers extensively studied organometallic coupling reactions in water, including conventional Heck reactions of aryl halides using exogenous palladium catalysts, and noted that surfactants were critical for achieving successful coupling in the systems they examined.[10] We too have observed beneficial effects of surfactants in the (noncatalytic) transformations we have examined, including Heck-type coupling of **2** and **10**, but in the latter case, surfactants were not absolutely required.[11] Lastly, we found that while the efficiency of Heck-type coupling of **2** and **10** was greatly attenuated in the presence of tris(2-carboxyethyl)phosphine,

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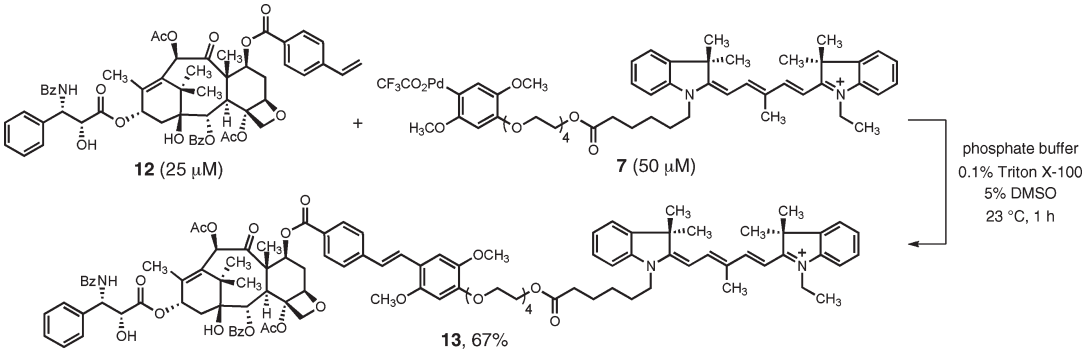
Scheme 2. Heck-Type Coupling Reaction of Styryl-Modified Taxol Derivative 12 and Reagent 7 in Aqueous Media



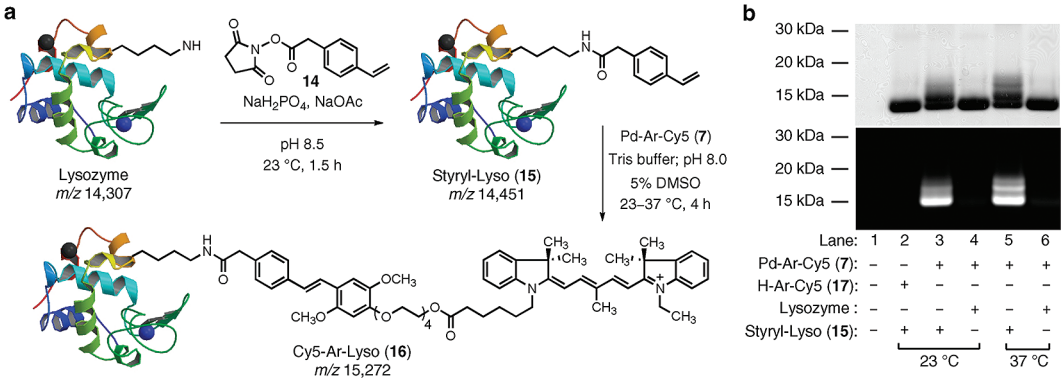


Figure 4. (a) Styryl residues were introduced by the reaction of lysine residues with NHS-ester **14**. Subsequent treatment with Pd−Ar−Cy5 reagent **7** led to labeling of the modified protein by the dye. (b) SDS-PAGE with visualization by fluorescence imaging (bottom) and Coomassie staining (top).

ascorbic acid, or DTT, amines or pyridine-based ligands were well-tolerated.

In a different test of the feasibility of Heck-type coupling, unsymmetrically substituted Cy5 arylpalladium(II) reagent **7** (14 mM, 1 equiv) in DMSO was mixed with taxol-derived substrate **12** (1.5 equiv) in air at 23 °C for 2 h. The trans Heck-type coupling product **13** was obtained as a blue solid in 70% yield after isolation by flash-column chromatography. Remarkably, when substrate **12** (25 μM, a >500-fold dilution, 1 equiv) was combined with reagent **7** (50 μM, 2 equiv) in aqueous potassium phosphate buffer solution (pH 8.0, 0.1% Triton X-100) containing 5% DMSO at 23 °C, **13** was formed in 67% yield after 1 h (Scheme 2 and the SI). Variation of the pH (5.8−8.0) in the latter system was found not to influence the yield of the coupling product (63−68% yield of **13**, 1 h). Coupling was slower but equally efficient when Tris buffer was used in lieu of phosphate buffer (23% yield after 3 h and 70% yield after 17 h at 23 °C). In both cases, we found that Triton X-100 was an essential additive (<5% coupling in its absence), consistent with the prior observations of Lipshutz and co-workers,[10] although we found that lower loadings of surfactant were required in this particular system (0.1% vs 5−15%).

We next evaluated the feasibility of labeling a protein substrate that had been covalently modified to present an alkene function. Acylation of lysozyme (MW = 14 307 Da) with styryl succinimide (**14**) at pH 8.5 for 1.5 h (Figure 4a) gave rise to a mixture comprising ∼34% unmodified lysozyme, 40% singly modified lysozyme (**15**, MW = 14 451 Da), and lesser amounts of doubly and triply modified lysozyme (20 and 6%, respectively), as determined by LC−MS analysis (see the SI).[12] When this protein mixture (39 μM) was incubated with Cy5-linked reagent **7** (115 μM; abbreviated as Pd−Ar−Cy5 in Figure 4) in pH 8.0 Tris buffer containing 5% DMSO at 23 °C or, separately, at 37 °C for 4 h, a major fluorescent band with MW ≈ 15 kDa was observed by denaturing SDS-PAGE analysis along with a minor fluorescent band of higher molecular weight (∼16 kDa, believed to be the doubly modified Heck-type coupling product; see Figure 3b, lanes 3 and 5). High-resolution LC−MS analysis of the product mixture after purification by size-exclusion chromatography showed a major product peak at 15 273 Da, corresponding to the singly modified Heck-type coupling product (est. conversion 75%). Control experiments conducted with the styryl-modified lysozyme substrate and a nonpalladated Cy5 dye reactant [protiodepalladation product H−Ar−Cy5 (**17**)] or with unmodified lysozyme and Cy5-linked reagent **7** did not produce any significant fluorescent bands (lanes 2 or 4 and 6, respectively; Figure 4b).

We next conducted an affinity-enrichment experiment using biotin-conjugated arylpalladium(II) reagent **2** and styryl-modified FK-506 probe **18** (Figure 5a; see the SI for its synthesis and biological evaluation using an NFAT reporter gene assay).[13,14] Fresh cell lysate from healthy Chinese hamster ovary (CHO) cells was diluted to a total protein concentration of 1.0 mg/mL with Tris-buffered saline containing Triton X-100 (pH 8.0), and the diluted protein solution was treated with **18** (0.02 mM) for 4 h at 4 °C. This sample was then incubated with **2** (0.05 M) for 14 h at 23 °C. DTT was added, followed by an amine-based palladium scavenger resin. Proteins complexed to the biotin-conjugated probe molecule were then sequestered onto

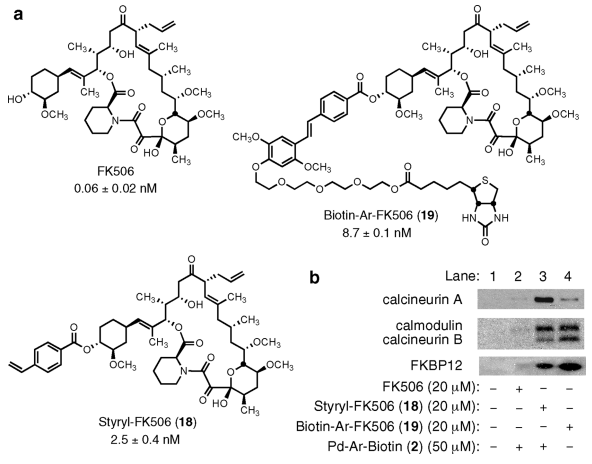


Figure 5. (a) Structures of FK506 and FK506-based affinity probes and their IC50 values in the NFAT reporter gene assay. (b) Western-blot detection of the FK506−immunosuppressive complex.

streptavidin−agarose. These were collected by centrifugation and then washed. Bound proteins were released by heat denaturation, after which the denatured proteins were separated by SDS-PAGE and the separated bands analyzed by Western blot (Figure 5b) with quantification by spot densitometry. Under these optimized conditions, we isolated FKBP12 as well as the three known secondary binding proteins, calmodulin and calcineurins A and B (Figure 5b, lane 3). An affinity-isolation experiment using the purified Heck-type coupling product prepared by an independent route (compound **19**; lane 4) was conducted simultaneously for comparison and also led to isolation of the same four proteins, albeit with somewhat different relative efficiencies. These results were confirmed in several replications of the experiment.

In summary, we have described protocols for the synthesis of storable arylpalladium(II) reagents bearing biotin and indocyanine dye residues. We have also demonstrated that these reagents function effectively in Heck-type coupling reactions with styrene-containing substrates in aqueous media at 23 °C, including a styryl-modified protein substrate and a styryl-modified FK-506 analogue in the presence of a cell lysate containing its protein receptors. Because we use stoichiometric organopalladium(II) complexes as reagents, subsequent coupling reactions do not rely upon any catalytic cycle, which we believe to be advantageous. In addition to potential further applications of the reagents in chemistry and chemical biology,[15] we envision opportunities for structural refinement and modification of the arylpalladium(II) reagents.

■ ASSOCIATED CONTENT

Supporting Information. Complete experimental procedures and spectral data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(5) Decarboxylative palladation did not proceed to completion when less than 3 equiv of palladium(II) trifluoroacetate was used.

(6) Our synthesis of Cy5 dye **5** was modeled after the following report: Shao, F.; Weissleder, R.; Hilderbrand, S. A. *Bioconjugate Chem.* **2008**, *19*, 2487.

(7) Decarboxylative palladation could also be achieved efficiently by warming substrates **6** or **8** with palladium(II) trifluoroacetate (3 equiv) in the absence of sodium carbonate. However, we observed that in the absence of sodium carbonate, arylpalladium(II) reagents **7** and **9** were less stable, undergoing slow protiodepalladation over the course of several weeks upon storage at −20 °C.

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