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Fluorogenic Transformations Based on Formation of C–C Bonds Catalyzed by Palladium: An Efficient Approach for High Throughput Optimizations and Kinetic Studies

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Abstract: We have developed novel fluorogenic transformations based on formation of C–C bonds catalyzed by palladium using iodocoumarin **1** as a model aryl iodide, where fluorescence is quenched completely due to effects of the heavy, polarizable iodine atom. Substitution of the iodine atom for the carbon using Sonogashira, Suzuki–Miyaura and Heck couplings results in a dramatic fluorescence enhancement. This approach has been used successfully for the optimization of reaction conditions and kinetic studies in high throughput format.

Keywords: C–C coupling; fluorescence; homogeneous catalysis; palladium

Introduction

Formation of carbon-carbon bonds catalyzed by palladium is of a great importance for organic synthesis, pharmaceutics and material science.[1–5] Development and study of these transformations are associated with extensive and time-consuming optimizations of numerous variables including catalyst, solvent, base, ligand, additives, stoichiometry and temperature. Analytical methods, extensively used for screening and optimization of transition metal-catalyzed transformations, such as HPLC,[6,7] electrophoresis,[8] and IR thermography[9] offer quantitative approaches while requiring significant commitment of time and instrumentation.

Fluorescent screening methods, developed recently by Hartwig and Taran,[10] are based upon immobilization of fluorescent dyes on a solid support,[10] FRET quenching of the fluorophore by an incoming quencher,[11,12] and antibody-based immunoassays.[13] These methods utilize short fluorescence relaxation times and allow high throughput screening of bases, solvents, ligands and temperatures within the fixed set of reactants conjugated to dye and quencher.

However, these methods suffer from several drawbacks: (1) derivatization of both reactants is required, thus limiting screening scope; (2) optical properties of both FRET partners in various solvents at different pH may vary and alter results significantly; (3) sensitivity at higher conversions, crucial for efficiency screening, is lower due to fluorolytic (based on disappearance of the signal) nature of the method. Here we report our studies on novel fluorogenic carbon-carbon couplings catalyzed by palladium, where the fluorescence signal increases during the course of the reaction, and their utility for high throughput optimizations and kinetic studies.

Results and Discussion

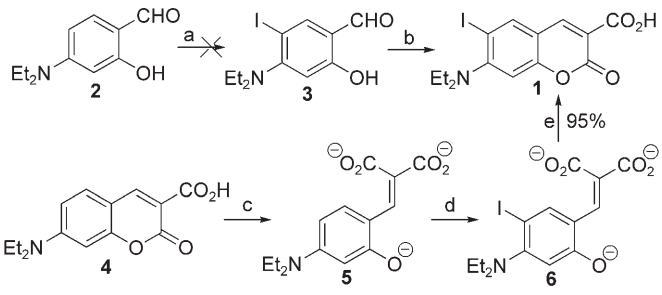
Although the effects of heavy atoms and electron-donating/-withdrawing groups on fluorescence are well known,[14] only a few examples of fluorogenic transformations, based on formation of new bonds including conversion of phosphine into phosphine oxide at the C-3 position[15] and azide/alkyne into 1,2,3-triazoles at the C-3 and C-7 positions of coumarin,[16,17] have been reported. We envisioned that the presence of the iodine at the C-6 position of the coumarin **1** would quench fluorescence due to the effect of the heavy iodine atom. Additional quenching is expected from steric factors, which may cause deflection of the diethylamino group from a planar conformation with respect to the aromatic ring and reduce the efficiency of the pull-push chromophore. Substitution of the iodine atom for carbon *via* palladium-catalyzed C–C coupling is expected to eliminate or significantly

reduce these quenching effects and reveal fluorescence.

Our first attempts to synthesize iodocoumarin **1** (Scheme 1) *via* iodination of the corresponding salicylic aldehyde derivative **2** by iodine, ICl and NIS (step a) led to a complex, inseparable mixture of products. Attempts to iodinate coumarin **4** directly using previously mentioned iodinating reagents in various solvents were unsuccessful.

According to several reports, the C-6 position of the coumarin ring can be activated for electrophilic aromatic substitution through opening of the lactone ring and formation of the phenoxide **5** under basic conditions.[18–21] When coumarin **4** was dissolved in 5% aqueous sodium hydroxide (step c), followed by direct iodination with iodine (step d) and lactone ring closure with hydrochloric acid (step e), nearly quantitative yield of the desired coumarin **1** was obtained.

As predicted, the iodinated coumarin **1** was non-fluorescent. Furthermore, Sonogashira transformation of coumarin **1** with phenylacetylene produced a highly fluorescent product **7a** in excellent yield (Figure 1, Table 1). Next, we explored the generality of this process with respect to various terminal alkynes. Aryl substituted alkynes all gave the desired coumarins **7b–e** in nearly quantitative yields within 4 h. In case of an alkyl-substituted alkyne, the reaction proceeded slower giving the desired coumarin **7f** in 74% yield after 48 h. In all studied cases, with the exception of **7c** where fluorescence is presumably quenched by internal charge transfer (ICT),[14] Sonogashira transformation led to the formation of highly fluorescent products.



Scheme 1. Synthesis of fluorogenic dye **1**.

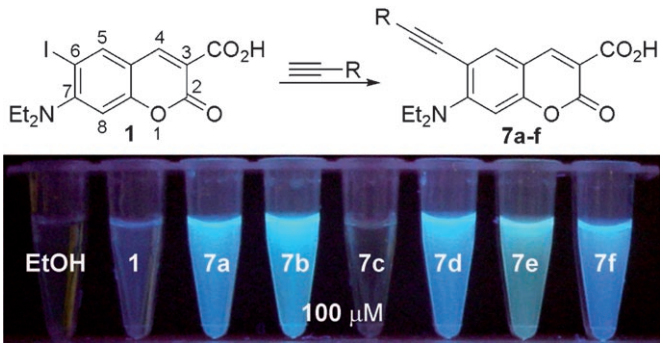


Figure 1. Fluorogenic Sonogashira transformations.

Table 1. Fluorogenic Sonogashira transformations.

Dye (R)	Yield [%] (time, [h])	F_{im}[a] [nm]	E_{Fi}[b] [a.u.]
7a (Ph)	97 (4)	465 nm	*27.4*
7b (C_6H_4OMe)	91 (4)	469 nm	*36.2*
7c ($C_6H_4NMe_2$)	94 (4)	467 nm	*3.3*
7d (C_6H_4F)	99 (4)	465 nm	*25.2*
7e ($C_6H_4CO_2Me$)	95 (4)	464 nm	*26.2*
7f (C_5H_{11})	74(48)	460 nm	12.0

[a] F_{im} = fluorescence maximum.
[b] E_{Fi} = fluorogenic efficiency [Eq. (1)]; $F_i(\lambda)$ and $F_1(\lambda)$ = fluorescence intensity of fluorescent product **7** and fluorogenic dye **1** at wavelength λ, respectively.

$$E_{Fi} = \int_{420}^{550} \frac{F_i(\lambda)}{F_1(\lambda)} d\lambda \qquad (1)$$

The efficiency of the fluorogenic transformations was evaluated by integrating the ratio of fluorescence intensities for dyes **7a–f** to that of dye **1** within 420–550 nm region upon 365 nm excitation [Eq. (1)]. Aryl-substituted products **7a**, **7b**, **7d**, **7e** have high fluorogenic efficiency ranging from 25.2 to 36.2 a.u. which is comparable with the "ideal" fluorogenic transformation from dye **1** to the commercial fluorescent dye **4** (51 a.u).

In order to transform this newly developed fluorogenic transformation into an efficient and reliable method for kinetic and high throughput optimization studies, the effect of various factors on fluorescence intensity was examined. First, we have evaluated the impact of starting material and product concentrations on total fluorescence of the model dye **7a** using 365 nm excitation and 465 nm emission wavelengths. Plots of the dilution series for both **7a** and **7a**+**1** are linear with a correlation coefficient exceeding 0.99 as shown in Figure 2, establishing the lack of optical interferences between reaction components and linear dependence of fluorescence intensity on the concentration of the fluorescent product **7a** within the studied range.

Since solvents have pronounced effects on fluorescence due to solvation of the exited states,[22] we examined the effect of solvents suitable for polystyrene 96-well plates including water, acetonitrile, methanol and ethanol on fluorescence intensity in 1–10 μM range. Fluorescence measurements in water were not

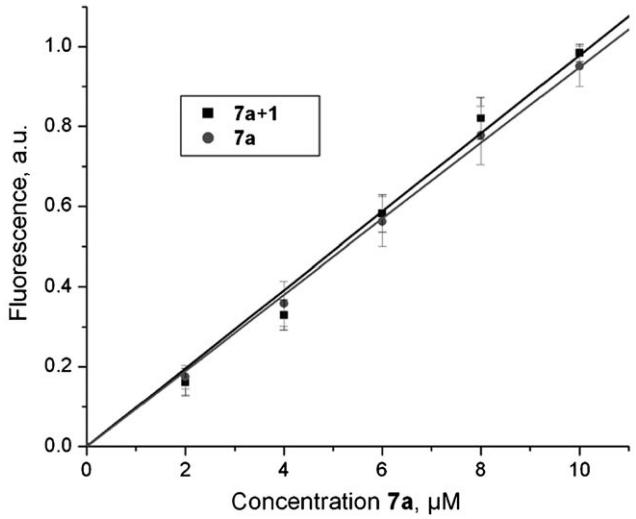


Figure 2. Calibration curves and inner filter effect; (■) **7a**, (●) **7a**+**1**.

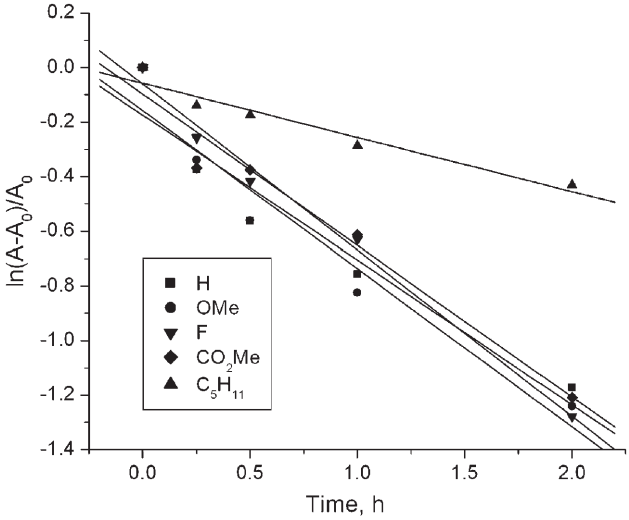


Figure 3. First-order plots: (■) **7a**, (●) **7b**, (▼) **7d** (◆) **7e**, (▲) **7f**.

possible due to the low solubility of the specific dyes. Since ethanol provides the highest fluorescence intensity among solvents studied, this solvent has been selected for further studies. The concentration of diisopropylethylamine in 1–1000 μM range had virtually no effect on fluorescence intensity in EtOH. We have also examined effect of various palladium catalysts such as $Pd(OAc)_2$, $PdCl_2$, $(PPh_3)_2PdCl_2$, $(CH_3CN)_2PdCl_2$, $Pd(OAc)_2$+BINAP and PEPPSI-IPr on the fluorescence signal and observed no substantial quenching effects at 5% loading with respect to the 10 μM fluorescent product **7a**. Catalyst $(dppf)PdCl_2$ exhibited a modest quenching effect at 5% loading due to presence of iron, but not at 1% loading. Therefore fluorescence correlates only with the concentration of the product and is not effected significantly by the presence of other reaction components.

Although the Sonogashira transformation is the classic example in palladium catalysis, data on the reactivity of alkynes is scattered and mostly based on reaction yields with variable reaction conditions.[3,23] Since our method allows substrate variations, the next step was to demonstrate its utility in addressing the relative reactivity of alkynes in Sonogashira transformations (Figure 3, Table 2).

Reaction time courses for these reactions reveal pseudo-first-order transformations with respect to the fluorogenic coumarin **1**, consistent with previously reported results claiming transmetalation or reductive elimination as rate-determining steps.[23,24] Although an alkyl-substituted acetylene is considerably less reactive than an arylacetylene, introduction of electron-donating or electron-withdrawing groups into the *para*-position of the aryl had no pronounced effect on the reaction rate. A moderate deviation from the first-order kinetic model is expected for practical conversion values and occurs due to inhibition of catalytic palladium intermediates through coordination with accumulating internal alkynes.[24]

Table 2. Reactivity of various alkynes.

Dye (R)	k_{obs} [h^{-1}]	R^2	$t_{1/2}$ [h]
7a (H)	0.531 ± 0.087	0.962	1.31
7b (OMe)	0.579 ± 0.083	0.970	1.20
7d (F)	0.609 ± 0.036	0.995	1.14
7e (CO_2Me)	0.551 ± 0.0621	0.982	1.25
7f (C_5H11)	0.199 ± 0.029	0.970	3.49

A comparison of our fluorogenic method and HPLC for the transformation of dye **1** and phenylacetylene into product **7a**, demonstrated no significant differences between those methods at the 99.9% confidence level. The applicability of the fluorogenic dye as a model aryl iodide has been examined through comparison of Sonogashira coupling between iodobenzene and coumarin **1** with phenylacetylene by HPLC. No substantial difference in reactivity of these substrates was observed (see Supporting Information). There are also no obvious "abnormal" structural components that would distinguish fluorogenic dye **1** from other aryl iodides in transition metal-catalyzed transformations.

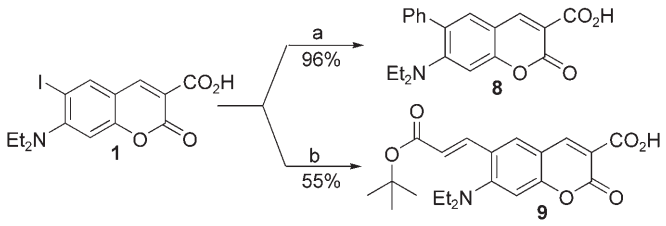
We have also estimated the time required for the acquisition and analysis of the dataset presented in Figure 3 by our fluorogenic method and standard HPLC with UV-visible or fluorescence detectors. Data collection for 5 data points with 5 repetitions for 5 kinetic curves requires around 100 h when acquired by HPLC, but only 0.2 h by our fluorogenic method. Additionally, our method does not require extensive calibrations and development of separation programs.

Since virtually no solvent is required, this fluorogenic method is environmentally friendly.

Next, we have explored the generality of the fluorogenic approach for other classic palladium-catalyzed transformations such as Heck[1] and Suzuki–Miyaura[25] transformations. To our satisfaction, fluorogenic dye **1** reacted with phenylboronic ester and *tert*-butyl acrylate to form fluorescent products **8** and **9** in 96 and 55% yields, respectively (Scheme 2, Figure 4).

Reaction conditions were optimized through variation in temperature, base, catalyst and dilution factors in high throughput format using the fluorogenic nature of these transformations with dye **1**. Our optimized reaction conditions matched recently reported Suzuki–Miyaura coupling conditions for the synthesis of ethyl ester of coumarin **8** from the corresponding bromocoumarin in 72% yield.[26]

The fluorogenic efficiency of transformations leading to the Sonogashira, Suzuki–Miyaura and Heck products **7b**, **8** and **9**, determined as integrated ratio of fluorescences from 420 to 550 nm, was 36.2, 8.0 and 17.1, respectively. According to our results, the Sonogashira reaction is more efficient than Suzuki–Miyaura and Heck reactions from reactivity and fluorogenic efficiency standpoints.



Scheme 2. Fluorogenic Suzuki–Miyaura and Heck couplings.

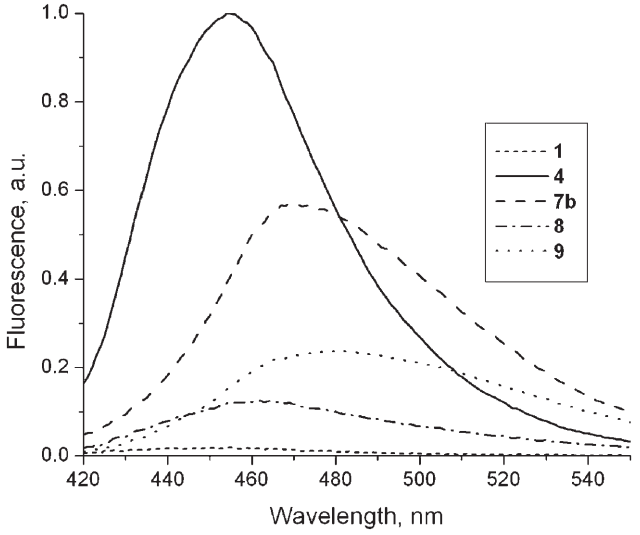


Figure 4. Fluorescence spectra of **1, 4**, **7b**, **8** and **9** in EtOH (10 μM).

Conclusions

In summary, we have envisioned and developed a powerful approach for high throughput screening, optimization and kinetic studies of palladium-catalyzed carbon-carbon couplings involving aryl iodides. This method allows one to screen not only standard reaction variables such as bases, ligands, catalysts, solvents and temperature but also substrates without any additional modifications or derivatizations. High functional group tolerance, mild "bioorthogonal" reaction conditions, excellent fluorogenic and chemical efficiency and submicromolar sensitivity enhance the importance of these fluorogenic transformations for the development and optimization of bioconjugations and surface/nanoparticle derivatization. Further studies on the synthesis of novel fluorogenic dyes with high chemical stability and variable optical properties as well as the development of novel fluorogenic transformations and their bioanalytical applications are underway.

Experimental Section

Procedure for Typical Fluorogenic Sonogashira Transformation

Coumarin **1** (0.25 mmol, 97 mg), alkyne (0.5 mmol), bis(diphenylphosphino)palladium dichloride (5%, 0.0125 mmol, 8.8 mg), CuI (10%, 0.025 mmol, 4.75 mg) and NEt_3 (2 mmol, 202 mg) were dissolved in 2 mL of DMF and stirred for 4 h (unless noted otherwise) at room temperature. Upon completion of the reaction, the solvent was evaporated under vacuum and the solid residue was purified by flash chromatography.

Fluorescent dye **7a**: Obtained in 97% yield as light yellow needles, mp 183–185 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.33$ (t, $J = 7.0$ Hz, 6H), 3.74 (q, $J = 7.0$ Hz, 4H), 6.64 (s, 1H), 7.30–7.40 (m, 3H), 7.43–7.60 (m, 2H), 7.72 (s, 1H), 8.64 (s, 1H), 12.13 (s, broad, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 13.1$, 46.0, 87.1, 93.7, 100.5, 107.7, 108.9, 109.5, 122.7, 128.5, 128.6, 131.1, 138.4, 149.6, 155.3, 156.3, 163.8, 164.9; HR-MS: $m/z = 362.1395$ (calcd. for $C_{22}H_{19}NO_4 + H$: 362.1392).

Fluorogenic Suzuki Coupling

Coumarin **1** (0.25 mmol, 97 mg), phenylboronic acid pinacol ester (1 mmol, 204 mg), 1,1′-[bis(diphenylphosphino)ferrocene]dichloropalladium(II) (5%, 0.0125 mmol, 10.2 mg), and CsF (1 mmol, 152 mg) were dissolved in 2 mL of DMF and stirred for 24 h at 60 °C. Upon completion of the reaction, the solvent was evaporated under vacuum. The solid residue was purified by flash chromatography and recrystallized from an ethanol-water mixture.

Fluorescent dye **8**: Obtained in 96% yield as light yellow crystals, mp 135–137 °C; ^{1}H NMR ($CDCl_3$): $\delta = 0.98$ (t, $J =$ 7.0 Hz, 6H), 3.10 (q, $J = 7.0$ Hz, 4H), 6.90 (s, 1H), 7.30–7.50 (m, 6H), 8.72 (s, 1H), 12.32 (s, broad, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 12.1$, 45.9, 104.9, 108.6, 111.3, 127.7, 127.9, 128.9, 132.1, 133.7, 140.0, 150.5, 156.0, 156.2, 163.7, 165.0; HR-MS: $m/z = 337.1320$ (calcd. for $C_{20}H_{19}NO_4$: 337.1314).

Fluorogenic Heck Coupling

7-Diethylamino-6-iodocoumarin-3-carboxylic acid (0.25 mmol, 97 mg), *tert*-butyl acrylate (2 mmol, 256 mg), bis(diphenylphosphino)palladium dichloride (5%, 0.0125 mmol, 8.8 mg), and $EtN(i\text{-}Pr)_2$ (1 mmol, 129 mg) were dissolved in 20 mL of DMF and stirred for 24 h at 40 °C. Upon completion of the reaction, the solvent was evaporated under vacuum. The solid residue was purified by flash chromatography and recrystallized from an ethanol-water mixture.

Fluorescent dye **9**: Obtained in 55% yield as yellow crystals, mp 200–202 °C; ^{1}H NMR ($CDCl_3$): $\delta = 1.19$ (t, $J =$ 7.0 Hz, 6H), 1.53 (s, 9H), 3.32 (q, $J = 7.0$ Hz, 4H), 6.29 (d, $J = 15.5$ Hz, 1H), 6.86 (s, 1H), 7.63 (d, $J = 15.5$ Hz, 1H), 7.66 (s, 1H), 8.74 (s, 1H); ^{13}C NMR ($CDCl_3$): $\delta = 12.42$, 28.1, 46.73, 80.9, 105.0, 109.8, 111.5, 120.9, 126.2, 130.9, 140.2, 150.6, 156.5, 157.2, 163.2, 164.6, 165.7; HR-MS: $m/z =$ 387.1686 (calcd. for $C_{21}H_{25}NO_6$: 387.1682).

Kinetic Experiments

A set of polypropylene PCR tubes was charged with a solution of coumarin **1** (40 μL, 50 mM), alkyne (40 μL, 200 mM), $(PPh)_3PdCl_2$ (1%, 40 μL, 0.5 mM), copper iodide (1%, 40 μL, 0.50 mM) and ethyldiisopropylamine (40 μL, 200 mM) in DMF. Final concentrations of the fluorogenic dye, alkyne, palladium complex, copper iodide and amine were 10, 40, 0.1, 0.1 and 40 mM, respectively.

Next, samples were capped and heated at 50 °C and 20 μL aliquots were withdrawn at 0, 0.25, 0.5, 1, 2, 4, 8 and 16 h time points. Aliquots taken were diluted by a factor of 10 using acetonitrile and by a factor of 1000 using ethanol for HPLC with UV-visible detection and fluorescence polystyrene 96-well plate experiments, respectively. Samples were stored at −20 °C in capped PCR tubes prior to the analysis to stop further progress of the reaction. Detailed analysis is presented in Supporting information.

Acknowledgements

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