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Microwave-assisted functionalization of bromo-fluorescein and bromorhodamine derivatives

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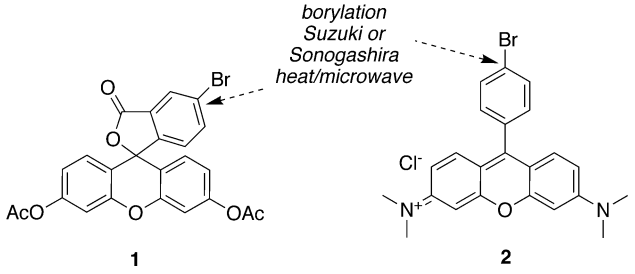
Abstract—The overall goal of this project was to develop methodologies that would allow organometallic couplings of fluorescein and rhodamine derivatives. Consequently, borylation, Suzuki, and Sonogashira reactions of fragments derived from compounds **1** and **2** were investigated. Conventional and microwave heating were compared throughout the study.

Methods to form fluorescein and rhodamine dyes typically feature high temperature condensation reactions that are not readily adapted to form small libraries of derivatives.[1–4] A project in these laboratories to prepare fluorescent tags for labeling and observation of several biomolecules in one system[5] led us to consider ways of linking fluorescein and rhodamine fragments. More specifically, it was necessary to link them to form twisted systems that would be conjugated if they became planar. Consequently, we decided to investigate organometallic couplings of starting materials that could be used to prepare a diverse set of dyes. This Letter reports conventional and microwave heating[6] of palladium-catalyzed reactions featuring the regioisomerically pure brominated starting materials **1** and **2**.[7]

The most obvious way to couple fragments **1** and **2** is to form an organometallic species from one of them, then couple this with the other. Suzuki couplings[8] might be preferred because they are high yielding and experimentally convenient. Use of this method would require that one component be converted to a boronic acid, hence it was necessary to decide which. Molecules like **2** (rhodamines lacking a carboxylic acid functionality) have been called *rosamines*.[3] Experimentally, they tend to be more difficult to manipulate than the fluorescein derivatives **1**, because of their charge. We hypothesized that their positive charge also makes the aryl bromide more electron deficient than in compound **1**. Electron deficient aryl bromides tend to give more efficient coupling reactions in catalytic cycles involving oxidative addition, while the transmetallation component is less sensitive to electronic factors.[8] Consequently, it was decided to focus on formation of the boron-containing fragment from the fluorescein derivative **1**.



1 **2**

Initially, attempts were made to borylate **1** using pinacolborane,[9,10] since that reagent is cheaper than the corresponding diboron reagents. Conventional heating was investigated first. The 1,1′-diphenylphosphinoferrocenyl-based catalyst, $PdCl_2$(dppf) gave poor conversion and much of the material that was formed was the unwanted reduction product **4** (Table 1, entry 1; similar results were obtained using 100°C reaction temperature, data not shown).

One of Li's phosphinite catalysts,[11] that has proved useful for other coupling reactions, was then investi-

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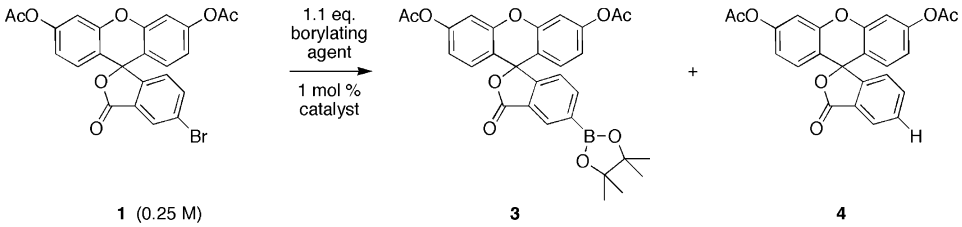
Table 1. Borylation of 5-bromofluorescein diacetate **1**



1 (0.25 M) **3** **4**

Entry	Borylating agent	Base	Catalyst	Solvent	Heating method (temp. °C, time)	Reactant/product ratio[c] **1**:**3**:**4**
1	$H\text{-}BO_2C_6H_{12}$	NEt_3	$PdCl_2(dppf)$	Dioxane	Conventional (80, 20 h)	90:0.0:10
2	$H\text{-}BO_2C_6H_{12}$	NEt_3	$PdCl_2(P'Bu_2OH)_2$	Dioxane	Conventional (80, 20 h)	0.0:15:85
3	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	NEt_3	$PdCl_2(P'Bu_2OH)_2$	Dioxane	Conventional (80, 20 h)	94:3.0:3.0
4	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	KOAc	$PdCl_2(P'Bu_2OH)_2$	Dioxane	Conventional (80, 20 h)	35:50:15
5	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	KOAc	$PdCl_2(P'Bu_2OH)_2$	Toluene	Conventional (100, 20 h)	0.0:80:20
6	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	KOAc	$PdCl_2(dppf)$	Toluene	Microwave, 50 W[a] (25–147, 5 min)	85:15:0.0
7	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	KOAc	$PdCl_2(dppf)$	Toluene	Microwave, 100 W[a] (25–157, 5 min)	61:39:0.0
8	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	KOAc	$PdCl_2(dppf)$	Toluene	Microwave, 200 W[a] (25–238, 15 min)	0.0:>98:0.0
9	$H_{12}C_6O_2B\text{-}BO_2C_6H_{12}$	KOAc	$PdCl_2(dppf)$	Toluene	Microwave[b] (150, 5 min)	0.0:>98:0.0

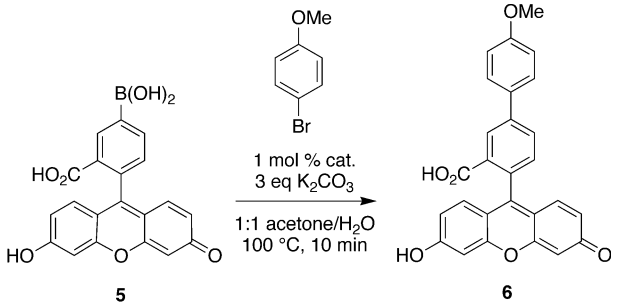
[a] Constant power, temperature allowed to vary.
[b] Variable power, constant temperature.
[c] Determined by ^1H NMR; errors estimated as ±5%.

tion was still a complication (entry 2). It seemed likely that the hydrogen that gave the reduction product was dervied from the pinacolborane, hence the borylating agent was switched to dipinacolatodiboron.[12,13] Entries 4 and 5 demonstrate that when the base was also switched to KOAc then an appreciable conversion to the desired product was obtained. Still, however, reduction products were formed. It may be that the adventitious hydrogen that causes formation of these reduction products is solvent-derived.

At this stage, it became evident that the optimization process was too slow due to the reaction times involved, hence subsequent studies focused on microwave acceleration of the reactions.[14,15] These reactions were performed using sealed tubes in a CEM Discover instrument that allows either the microwave power or the reaction temperature to be held constant, and the temperature in the reaction vessel to be monitored. Control of the reaction temperature would not be an option if a domestic microwave instrument was used. Entries 6–8 illustrate that if the power is modulated then the reaction temperature rises abruptly. These erratic experiments are scientifically unsatisfying because they would be hard to reproduce, especially on a different instrument or for different reaction scales. However, they did illustrate that high temperatures were tolerated. Finally, entry 9 shows the most favorable conditions identified. The diboron reagent with KOAc as base, microwave heated at a relatively high temperature for a short time gave approximately 98% selectivity for the desired product. A 93% yield of the desired product was isolated when this reaction was repeated on a 1 mmol scale (microwave, 150°C, 10 min) and the crude material was purified via crystallization.[16]

It was convenient to store compound **3** because when it is hydrolyzed to the corresponding deacylated boronic acid **5** cyclotrimerization to the corresponding boroxine ensues. Consequently, the boronate **3** was hydrolyzed (K_2CO_3, 1:1 THF/H_2O, 3 h; 79%) to **5** then isolated immediately prior to use in Suzuki coupling reactions.

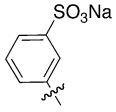
Reaction 1 shows three model reactions that were performed to evaluate Suzuki couplings to compound **5**. A water-soluble palladium catalyst gave a superior result to $Pd(PPh_3)_4$ under these conditions, and the best isolated yield of the product **6** was obtained from the microwave-accelerated transformation in a sealed vessel.



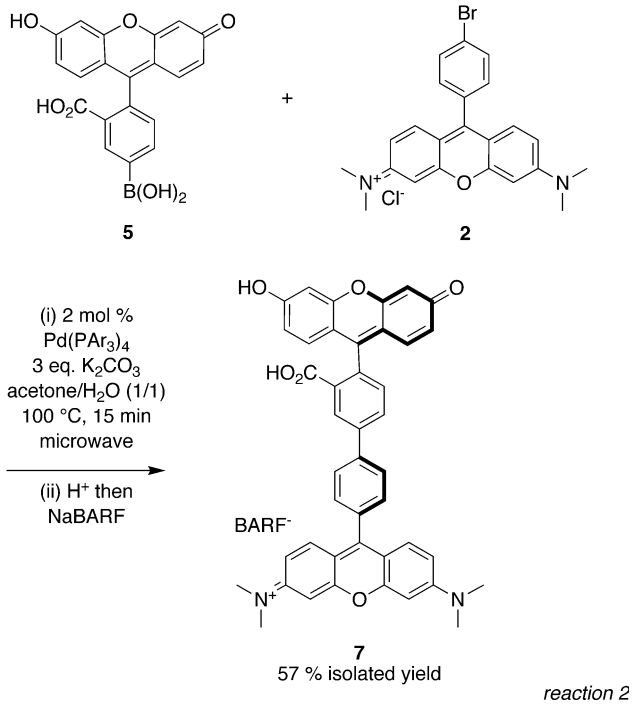
5 **6**

reaction 1

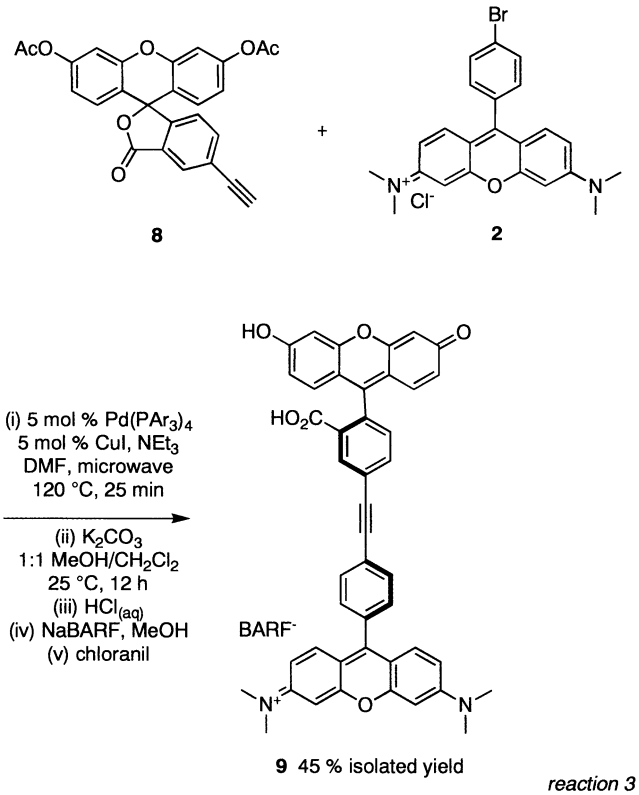
catalyst/heating	isolated yield (%)
$Pd(PPh_3)_4$/microwave	52
$Pd(PAr_3)_4$/microwave	81
$Pd(PAr_3)_4$/conventional	60

Ar = 

Reaction 2 illustrates how these findings were then applied to the coupling of the rosamine **2** with com-



reaction 2



reaction 3

pound **5**.[17] The water soluble catalyst was used to couple the rosamine fragments, the product was converted to a protic form, then the counterion was metathesized to the *tetra*(3,5-trifluoromethylbenzene)boronate (BARF) anion; this allows convenient chromatography isolation of the product.

System **7** would be totally conjugated were it not for the twists imposed by the adjacent aromatic rings. For comparison, analogs of this structure with alkyne units inserted between the rings were desired, hence microwave-assisted Sonogashira couplings[18] of the dyes were investigated.

Alkyne **8** was formed in a two step process that involved first coupling trimethylsilylethyne with the protected fluorescein **1** under microwave conditions (5 mol% $Pd(PPh_3)_4$, CuI, NEt_3, DMF, 120°C, 25 min; 84% isolated). Like the other microwave reactions reported in this paper, this coupling was performed in a sealed tube. The procedure for sealing tubes for use in the microwave instrument by crimping on an aluminum ring is very easy. Use of such sealed tubes for reactions such as this where volatile reagents are used is clearly an advantage. Deprotection of the alkyne was performed using TBAF (2 equiv., CH_2Cl_2, −78°C, 5 min; 90%).

Reaction 3 illustrates how the alkyne **8** was coupled with the bromorosamine **2** to give the desired alkyne-containing product **9** via a microwave accelerated coupling using the water-soluble catalyst. The product from this step was hydrolyzed directly to remove the acetate groups, acidified, then the counterion was exchanged with $BARF^-$ to assist chromatographic isolation.

An in depth study of the spectroscopic properties of these molecules and a full interpretation of those data will be published elsewhere. However, Figure 1 shows the UV absorption spectra and fluorescence emission spectra of these molecules. The most significant features are that for the molecules **7** and **9** but not **6**, emission in the 520 nm region, that would be attributed to the fluorescein component of these systems, is greatly suppressed; most of the fluorescence of **7** and **9** occurs at a longer wavelength characteristic of the rosamine fragment.

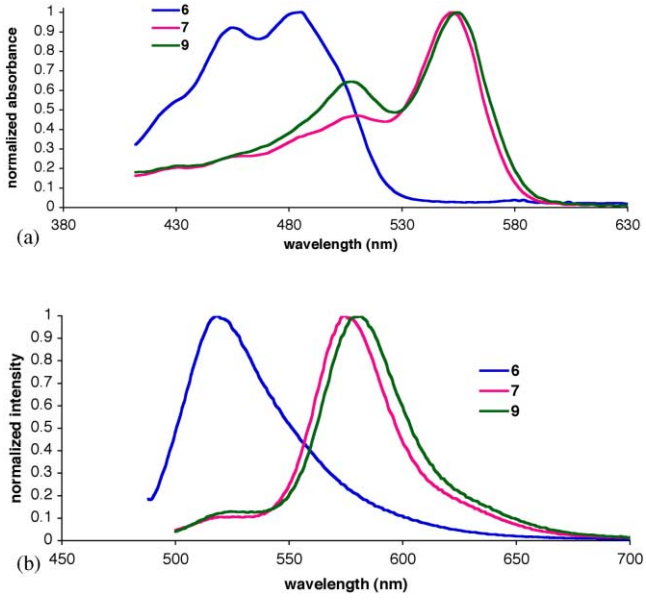


Figure 1. (a) UV absorption, and (b) fluorescence emission spectra of compounds **6**, **7** and **9**, when excited at 488 nm.

Organometallic coupling reactions are rarely used to functionalize fluorescein- and rhodamime-based systems. Here, halogenated dyes were transformed into organoboron compounds, biaryls, and alkynes. Fluorescein and rhodamine derivatives tend to be stable to high temperatures, so they might be expected to be amenable to microwave assisted steps. Indeed, the couplings described here worked well using bursts of microwave irradiation applied to give high temperatures for relatively short times.

Acknowledgements

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