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Robust Suzuki–Miyaura Cross-Coupling on DNA-Linked Substrates

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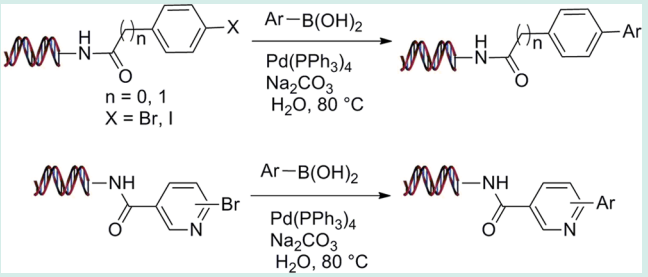
Supporting Information

ABSTRACT: The Suzuki–Miyaura cross-coupling is one of the most widely employed reactions in medicinal chemistry. To apply this reaction to DNA-encoded library technology (ELT), an alternative approach in the discovery of small molecule hits and leads, we explored the Suzuki–Miyaura cross-coupling on DNA-linked aryl halides. $Pd(PPh_3)_4$ was demonstrated to be an effective catalyst for cross-coupling with on-DNA halide substrates under aqueous conditions. It efficiently catalyzes the coupling of phenyl halides (iodide or bromide) and pyridinyl bromides with various boronic acids/esters, including challenging heterocyclic boronic acids/esters.



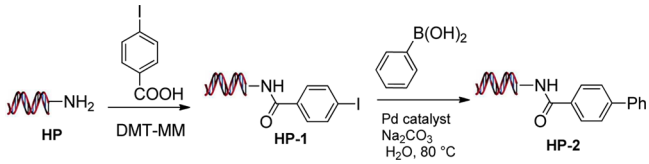
KEYWORDS: *Suzuki–Miyaura cross-coupling, DNA-encoded library technology*

DNA-encoded libraries have attracted much attention both in academia and industry, since it was first described in the 1990s.[1−4] We have recently reported the development of a DNA-encoded library technology (ELT) using a split-and-pool combinatorial assembly strategy for the discovery of small molecule ligands to protein targets.[5] Potent inhibitors for various targets have been successfully discovered through this technology.[6−9] ELT offers a practical alternative to high-throughput screening and fragment-based drug discovery. A primary factor for the success of the technique is the chemical diversity of the libraries. Unfortunately there is limited precedent for developing synthetic transformations to operate under aqueous conditions in the presence of oligonucleotides.[10−13] Chemical reactions on DNA must be compatible with phosphodiesters, ribose and bases (nitrogenous heterocycles and exocyclic amines). Liu and co-workers reported the first DNA-templated Heck couplings in the presence of water-soluble Pd precatalysts.[13] Recently Omumi et al.[14] and Lercher et al.[15] have reported DNA modification by Suzuki-Miyaura cross-coupling. In this report, we will describe our development of Suzuki–Miyaura cross-coupling on aryl halide substrates that are covalently attached to an oligonucleotide. To apply such chemistry to the synthesis of DNA-encoded libraries, the reaction conditions should be robust and applicable to a wide scope of substrates.

The Suzuki–Miyaura cross-coupling reaction is one of the most powerful and widely employed synthetic processes for the construction of carbon–carbon bonds.[16−19] Recently, the use of water as solvent or cosolvent for the Suzuki–Miyaura reaction has received much attention. Since the first demonstration[20] of a hydrophilic catalyst $(TPPMS)_3Pd$ [TPPMS = diphenyl(3-sulfonatophenyl)phosphane] to mediate cross-coupling reactions in predominately aqueous solvents, a wide range of hydrophilic phosphane ligands have been applied.[21−24] However, the reaction is greatly limited by substrate solubility and reactivity in aqueous media. The examples reported are mostly phenyl iodides and bromides with phenylboronic acid and its derivatives. To the best of our knowledge, very few examples of Suzuki-Miyaura cross-coupling with challenging substrates such as heteroaryl halides and heteroaryl boronic acids/esters in aqueous media have been published.[25−27]

The starting material for a DNA-encoded library is a covalently linked DNA duplex: the "headpiece" (**HP**) with a free amine warhead (Supporting Information Figure 1). We explored cross-coupling with an iodobenzamide headpiece substrate (**HP-1**), which was prepared via acylation of **HP** with 4-iodobenzoic acid employing DMT-MM as the activating reagent (Scheme 1). **HP-1** (1 mM concentration in water) was

Scheme 1



then treated with 20 equiv of phenylboronic acid in the presence of 40 equiv of Na_2CO_3 and 1 equiv of $Pd(PPh_3)_4$. After it was heated for 1 h at 80 °C, the reaction generated approximately 95% of the desired product, and the reaction proceeded to completion after being heated overnight. The analogous reaction with $Pd(OAc)_2$/triphenylphosphine was not

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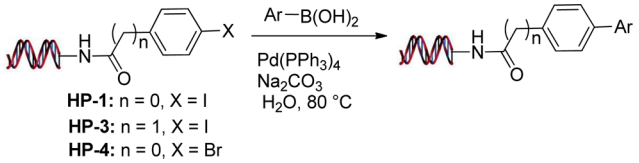
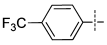
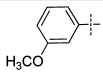
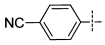
as clean or high-yielding, so we focused our subsequent effort on $Pd(PPh_3)_4$ as the catalyst.

To determine the scope of this reaction, we investigated cross-coupling with a range of boronic acids (Table 1). The

Table 1. Suzuki–Miyaura Cross-Coupling of Phenylhalide HPs with Boronic Acids[a]



Entry	Ar	HP-1 (A)	HP-3[d] (B)	HP-4[e] (C)
1		~100% [a,b]	96%	98%
2		56% [a] (92% [b])	67%	85%
3		~100% [a] (90% [b])	~100%	90%
4		~100% [a] (98% [b])	98%	95%
5		31% [a] (75% [b])	77%	12%
6		~100% [a,b]	95%	~100%
7		85% [a] (89% [b,c])	75%	70%

[a]Reaction conditions: 1 equiv of **HP-1** (1 mM in H_2O), 20 equiv of boronic acid, 1 equiv of $Pd(PPh_3)_4$ (20 mM in CH_2Cl_2/toluene/CH_3CN (1/2/2)), 40 equiv of Na_2CO_3 (1.6 M in H_2O), 80 °C for 90 min. [b]Same condition as *a*, 80 °C for 17 h. [c]Yield was calculated on the hydrolyzed and nonhydrolyzed products. [d]80 °C for 2 h. [e]Reaction conditions: 1 equiv of **HP-4** (1 mM in H_2O), 20 equiv of boronic acid, 0.5 equiv of $Pd(PPh_3)_4$ (5 mM in CH_3CN, degassed), 40 equiv of Na_2CO_3, 80 °C for 4 h.

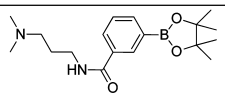
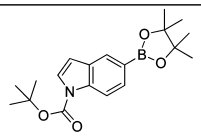
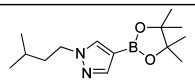
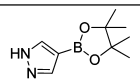
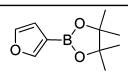
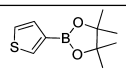
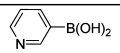
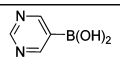
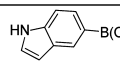
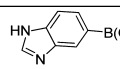
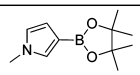
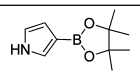
coupling of **HP-1** with electron-rich phenylboronic acids (Table 1, entries 3A and 6A) gave nearly quantitative yield after being heated at 80 °C for 90 min. In most cases, longer reaction times did not lead to decomposition or unwanted side reaction. Electron-deficient (Table 1, entry 2A) and sterically hindered (Table 1, entry 5A) phenylboronic acid required longer reaction times to give better conversion. Certain functional groups did not survive under the cross-coupling conditions. For example, the nitrile group (Table 1, entry 7A) was partially hydrolyzed to yield 57% of the desired product and 32% of the hydrolyzed product.

An aryl halide linked to headpiece DNA through an acetamide linker (**HP-3**)[28] gave similar results. The electron-deficient boronic acids (Table 1, entries 2B, 5B, and 7B) proceeded in moderate yields, ranging from 67% to 77%. Longer reaction times did not improve the cross-coupling in the case of electron-deficient boronic acids. Instead, we observed a buildup of side products over time based on LCMS analysis.

The corresponding bromobenzamide-derivatized DNA molecule (**HP-4**)[29] also gave generally similar results as **HP-1**. No improvement was noted after overnight reaction compared to 4 h, including for the sterically hindered boronic acid (Table 1, entry 5C), for which a large amount of unreacted phenyl bromide **HP-4** starting material was observed.

The presence of nitrogen heterocycles (e.g., heteroaryl boronic acids/esters and/or heterocaryl halides) can lead to low activity in cross-coupling reactions when palladium is used.[25−27,30,31] Several more examples of heteroaryl boronates were tested for coupling to **HP-1** (Table 2), including a

Table 2. Suzuki–Miyaura Cross-Coupling of HP-1 with Boronic Acids/Esters[a]

Entry	Boronic acids/esters	Yield
1		98%
2		~100% [b]
3		98%
4		~100%
5		~100%
6		~100%
7		60% (~100%[c])
8		70% (~85%[c])
9		~100%
10		40% (50%[c])
11		70%
12		95%

[a]Reaction conditions: 1 equiv of **HP-1** (1 mM in H_2O), 20 equiv of boronic ester/acid (400–800 mM in DMA), 0.5 equiv of $Pd(PPh_3)_4$ (3.5 mM in CH_3CN, degassed), 40 equiv of Na_2CO_3 (1.6 M in H_2O), 80 °C for 3 h 20 min. [b]Yield was calculated based on the deboc product. [c]Same conditions as *a*, 80 °C for 16 h.

comparison of boronic acids to boronic esters. Boronic acid pinacol esters proved to be highly useful, coupling in almost quantitative yield (Table 2, entries 1–6). A tertiary amine side chain did not interfere (Table 2, entry 1), nor did pyrazole and pyrrole with free (Table 2, entries 4, 12) and protected (Table 2, entries 3, 11) NH groups. To the best of our knowledge, these are the first examples of unprotected pyrazole and pyrrole derivatives undergoing high yield cross-coupling.[24,25] Heterocyclic boronic acids were also useful, if a bit less reactive. The unprotected 5-indoleboronic acid gave quantitative yield of the biaryl product (Table 2, entry 9). 3-Pyridylboronic acid required longer reaction time for the quantitative conversion

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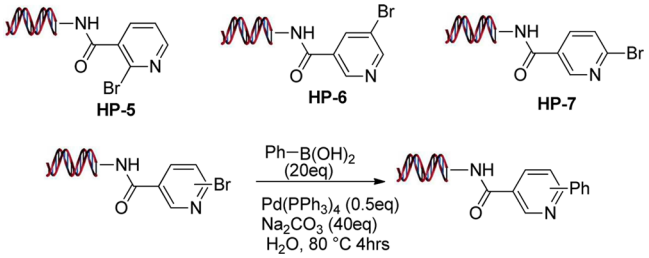
(Table 2, entry 7). 5-Pyrimidineboronic acid proceeded in good yield (70%−85%) overtime (Table 2, entry 8). However, only about 50% of 1*H*-benzimidazole boronic acid was converted to cross-coupled product even after heating for longer time (Table 2, entry 10), perhaps because of Pd coordination by the benzimidazole moiety.

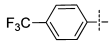
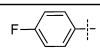
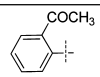
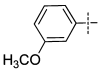
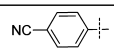
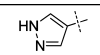
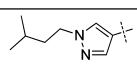
Pyridinyl bromides, **HP-5**, **HP-6**, and **HP-7** (Scheme 2)[32] were also found to be excellent substrates for cross-coupling

Scheme 2



under the same conditions as **HP-1**/**HP-4**. The scope of the reaction with these aryl halides is summerized in Table 3. All reactions proceeded in good to excellent yield with the exception of a sterically hindered and electron-deficient substrate (Table 3, entry 5), which was most sensitive to the most hindered bromide **HP-5** (Table 3, entries 2, 7). Heterocyclic boronates were less reliable partners with two (**HP-5** and **HP-6**) of the three pyridynyl bromides (Table 3, entries 8−11). In contrast, **HP-7** reacted smoothly in all cases (Table 3, entries 8C, 9C and 11C) except pyrazole boronic acid pinacol ester (Table 3, entry 10C, 26% yield). Overall, this protocol provided good to excellent yields for cross-coupling of nonsterically hindered pyridinyl bromides and nonsterically hindered phenylboronates. The coupling with heterocyclic boronates gave variable yields depending on the aryl halides and boronates.

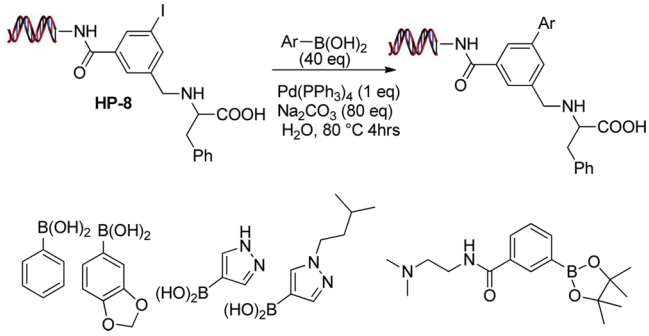
Table 3. Suzuki−Miyaura Cross-Coupling of Pyridinyl Bromide HPs with Boronic Acids[a]

Entry	Ar	**HP-5** (A)	**HP-6** (B)	**HP-7** (C)
1		95%	95%	98%
2		45%[b]	92%	92%
3		90%	81%	98%
4		98%	98%	~100%
5		10%[b]	20%	68%
6		92%	98%	98%
7		50%[b]	85%	80%
8		31%	9%	90%
9		0	0	~100%
10[c]		0	0	26%
11[c]		55%	24%	~100%

[a]Reaction conditions: 1 equiv of headpiece solution (1 mM in H_2O), 20 equiv of boronic acid (800 mM in DMA), 0.33 equiv of $Pd(PPh_3)_4$ (3.3 mM in CH_3CN, degassed), 40 equiv of Na_2CO_3 (1.6 M in H_2O), 80 °C for 5 h. [b]The other was the unreacted starting HP (~40%). [c]40 equiv of boronic acid pinacol ester (800 mM in DMA), 1 equiv of $Pd(PPh_3)_4$ (3 mM in CH_3CN, degassed), 80 equiv of Na_2CO_3 (1.6 M in H_2O), 80 °C for 4 h.

Lastly, we showed successful Suzuki-Miyaura cross-coupling to DNA-encoded library components containing an amine or acid functional group that could be further elaborated to introduce another point of diversity. Thus, the amino acid derivatives **HP-8**[33] reacted very cleanly with five different boronates (Scheme 3). The methyl ester of **HP-8** gave simultaneous coupling and ester hydrolysis under the coupling conditions.

Scheme 3



In conclusion, we have demonstrated that $Pd(PPh_3)_4$ is an efficient and general catalyst for the Suzuki−Miyaura cross-coupling on a variety of aryl halide substrates conjugated on double-stranded DNA. It gave medium to high coupling yields with various boronic acids and heteroaryl boronates. To apply this on-DNA Suzuki−Miyaura cross-coupling reaction to DNA-encoded library technology, we designed and synthesized several DNA-encoded libraries based on trifunctional scaffolds that contain phenyl iodide functional group. A 3.5-million-member library based on the scaffold of 5-formyl 3-iodo benzoic acid will be reported hereafter.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Structure of the headpiece, experimental procedures, analytical methods, and LCMS analysis of the data in the tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(33) The synthesis of **HP-8** was described in the Supporting Information.