Functional group tolerance of a micellar on-DNA Suzuki-Miyaura cross-coupling reaction for DNA-encoded library design

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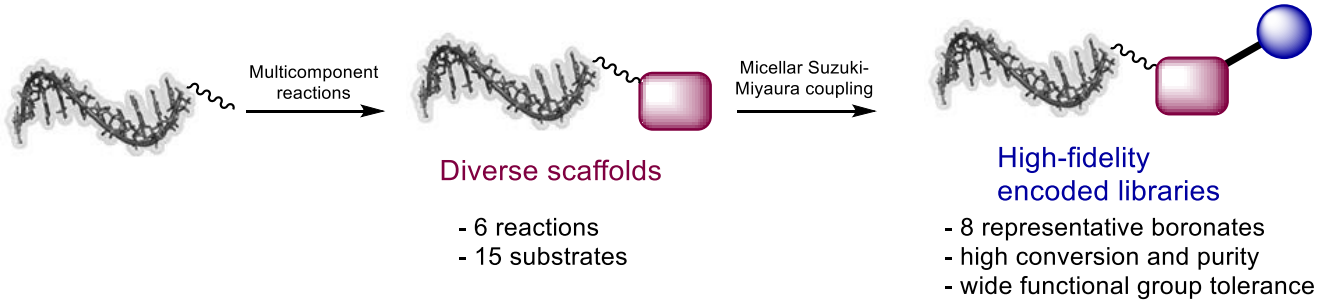
Supporting Information Placeholder



ABSTRACT: DNA-encoded libraries (DELs) offer great promise for the discovery of new ligands for proteins. Many current reactions used for DEL synthesis do not proceed efficiently over a wide range of substrates. Combining a diverse array of multicomponent reactions with micellar-promoted Suzuki-Miyaura cross coupling provides a strategy for synthesizing highly diverse DELs with exceptionally high fidelity. These results demonstrate that the micellar Suzuki-Miyaura reaction has exceptional functional group tolerance and broad applicability.

INTRODUCTION

The Suzuki-Miyaura C(sp^2)-C(sp^2) cross coupling reaction of boronic acid and their derivatives with aryl halides is one of the most heavily utilized reactions in medicinal chemistry and the synthesis of screening libraries.[1] A number of approved drugs and many bioactive compounds for challenging targets, exemplified by compounds **1-4** (Figure 1a), are accessible using this reaction as a key step.[2,3] Innovations in catalyst design considerably broadened the substrate scope beyond simple biaryl architectures that may cause concerns regarding downstream drug development.[4] Today, the chemist has a toolbox of catalysts available giving ready access to valuable heterocyclic biaryls that, for instance populate the chemical space of kinase inhibitors, and ortho-substituted biaryls that adopt non-planar structures, as was instrumental for the development of PD1-/PD-L1 inhibitors.[3,5]

A screening technology that has gained in prominence in recent years is DNA-encoded libraries (DELs).[6,7,8,9] These libraries are synthesized by combinatorial cycles of organic transformations and enzymatic barcode ligation. Unlike traditional screening libraries, which typically consist of compounds stored individually, DELs are efficiently handled and screened against target proteins as complex pools of encoded compounds. Crucially important for success in screening experiments are the quality and diversity of the library. In recent years, a few publications described on-DNA protocols for the reaction of boronic acids with DNA-aryl halides for the design of DNA-encoded biaryl libraries.[10-19] They described reaction optimization studies, and a larger scope of boronic acids (Figure 1b). However, usually only simple DNA-coupled (hetero)aryl halides were explored, with the exception of a single starting material that contained an amino acid distal of the reactive aryl halide moiety.[10] We recently reported a micelle-promoted on-DNA C(sp^2)-C(sp^2) cross-coupling protocol enabled reaction with exceptional levels of conversion across of a broad scope of boronates, including notoriously difficult-to-couple ortho-substituted phenyl-

and heteroaryl boronic acids, under appealingly mild, DNA-compatible reaction conditions.[19]

Progress in DEL synthesis methods development for diverse encoded screening library design gives access to a broad spectrum of DNA-coupled (hetero)aryl halides. In the application to DELs, these compounds are synthesized as complex mixtures and therefore require synthetic methods that are robust and broadly applicable. Diverse encoded compound mixtures will display a variety of steric and electronic effects that may affect reaction outcome, they may contain functional groups that are potential catalyst poisons, and may contain functionality that could give rise to side reactions or degradation under the reaction conditions.

All of these parameters will impact the utility of a DNA-encoded library in screening campaigns. In the worst case, insufficient knowledge on library identity and purity may lead to misdirection of experimental efforts at the hit resynthesis and validation phases. To further assess the scope of the DNA linked aryl halide component in the Suzuki-Miyaura coupling, we used our newly developed toolbox of reactions on solid phase-coupled DNA barcodes, and to prepare a set of diverse aryl halides and assessed their reactivity in the micellar on-DNA Suzuki-Miyaura reaction (Figure 1c).

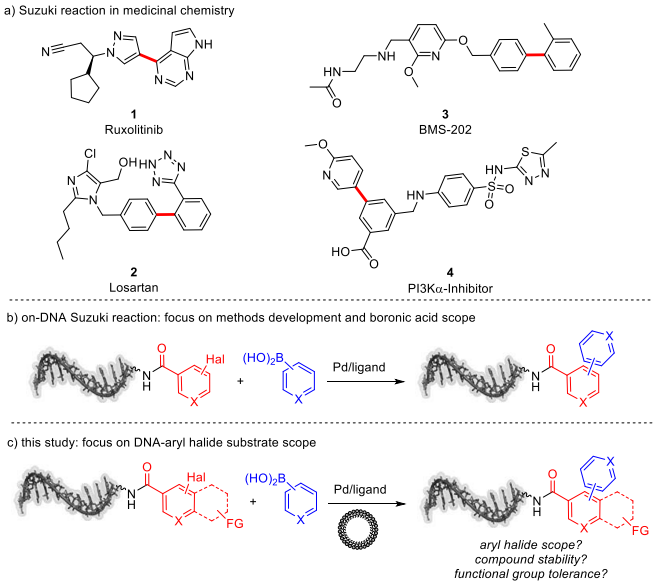
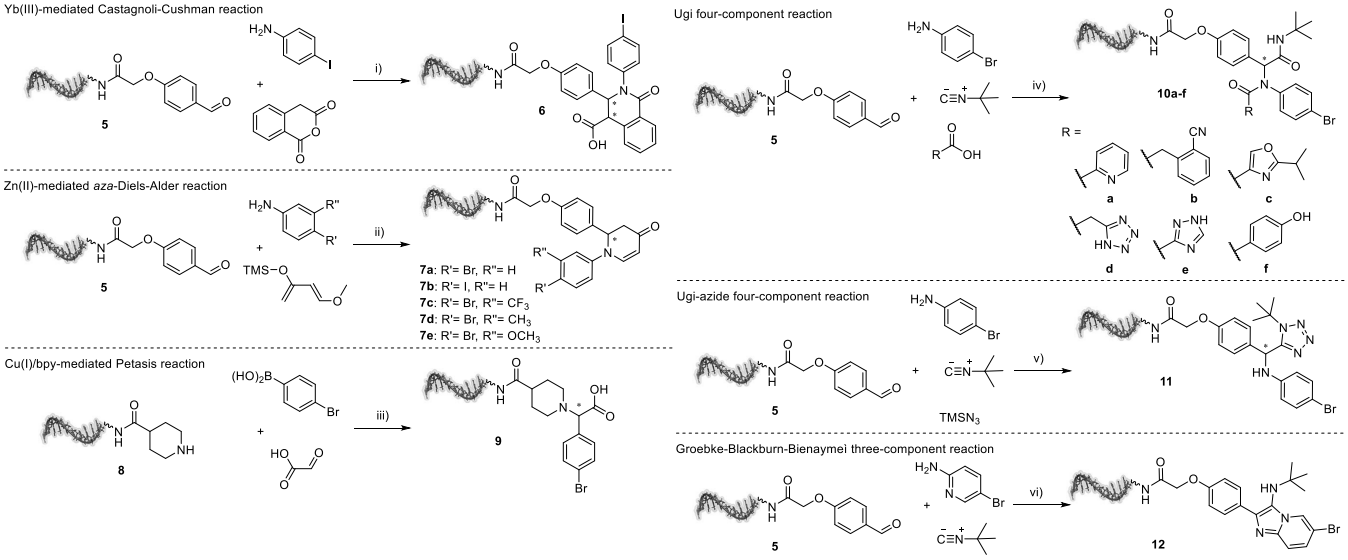


Figure 1. Suzuki-Miyaura reactions for DNA-encoded library synthesis. a) Exemplary bioactive compounds synthesized by the Suzuki-Miyaura reaction as a key step. b) Published reports of Suzuki-Miyaura reactions for DEL synthesis focused on methods development and boronic acid scope. c) Here, we assess the robustness of a micellar Suzuki-Miyaura reaction with diverse DNA-conjugated aryl halides.

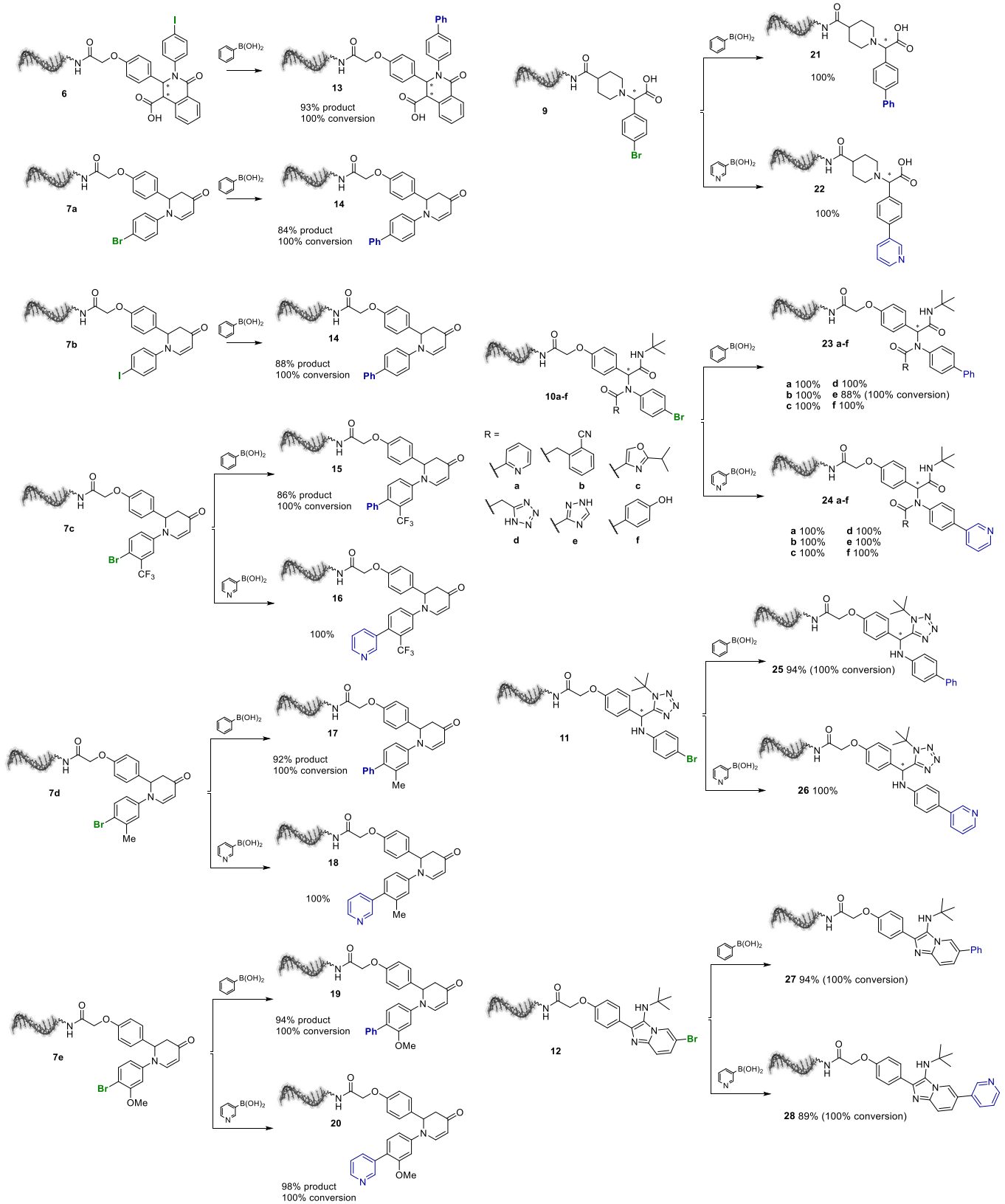
Scheme 1. DNA-conjugated starting materials 6-12 for assessment of the functional group-tolerance of the on-DNA Suzuki-Miyaura reaction. i) $Yb(OTf)_3$, CH_2Cl_2/TEOF, rt., then aq. NH_3/$MeNH_2$, ii) $ZnCl_2$, ACN/TEOF, rt, then NH_3, 50 °C, iii) CuCl/bpy, DMF/TEOF, 50 °C, then aq. NH_3/$MeNH_2$, iv) MeOH, 50 °C, then aq. NH_3/$MeNH_2$, v) 1% acetic acid/MeOH, 50 °C, then aq. NH_3/$MeNH_2$. TEOF = triethyl orthoformate, 10mer ATGC = H_2N-$(CH_2)_6$-GTCATGATCT.



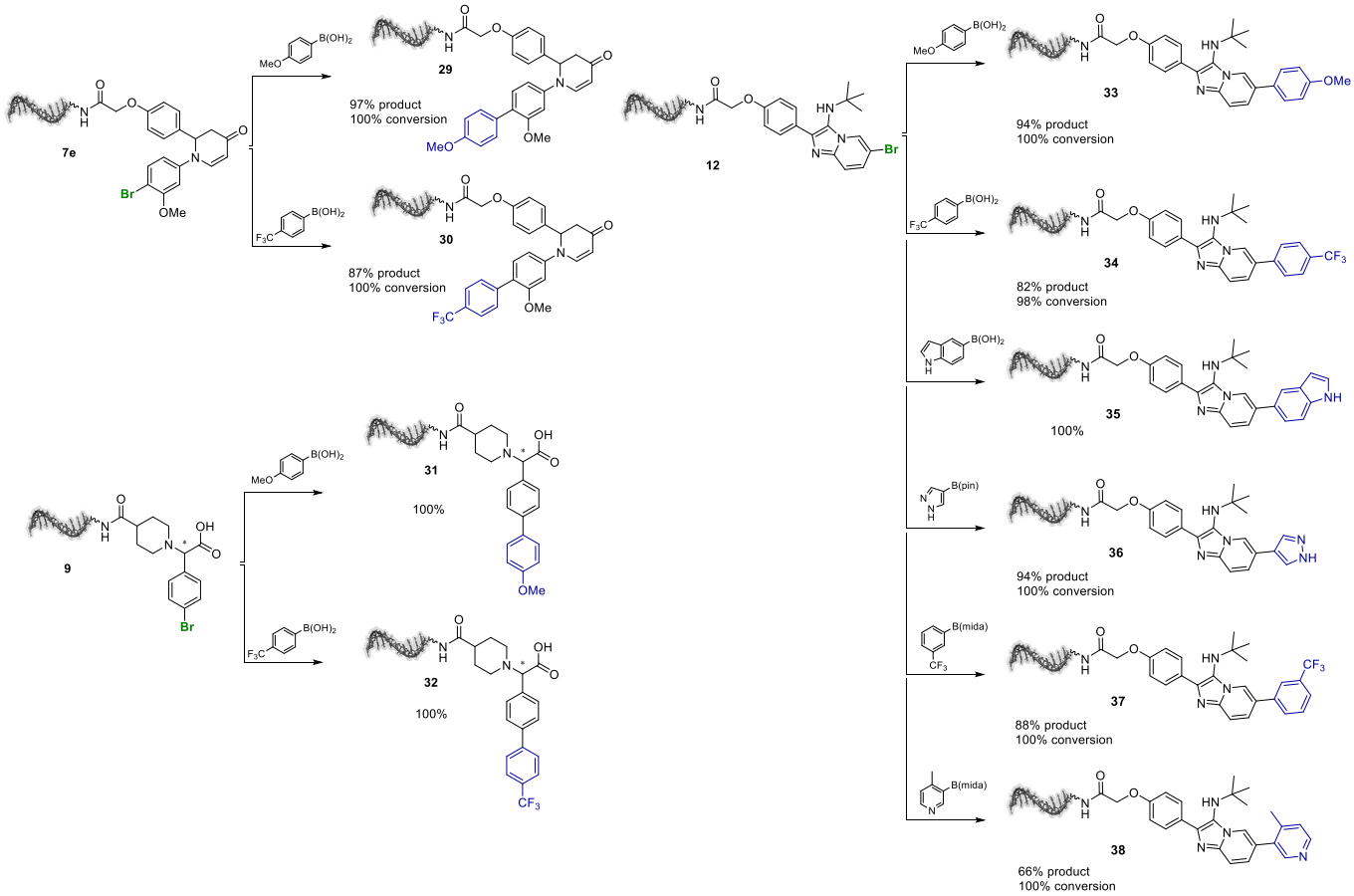
DESIGN OF SUBSTRATES

We used six different reactions to synthesize DNA-conjugates of diverse substituted (het)aryl halides from simple DNA-conjugate starting materials (Scheme 1): the $Yb(OTf)_3$-mediated Castagnoli-Cushman reaction between DNA conjugated aldehyde **5**, 4-iodoaniline and isochromane-1,3-dione provided a isoquinolone substituted with a free carboxylic acid **6**,[20] the $ZnCl_2$-promoted aza-Diels-Alder reaction of **5** with Danishefsky's diene and a range of anilines gave a partially saturated pyridones **7**.[21] As well as unsubstituted bromide **7a** and iodide **7b**, we used this reaction to introduce electron rich and poor as well as sterically hindered aryl bromides **7c-e**.

Scheme 2. Suzuki-Miyaura coupling of a diverse array of aryl halides with phenyl and 3-pyridyl boronic acids. Conditions: $Pd(dtbpf)Cl_2$ (7.3 mM), phenyl or 3-pyridyl boronic acid (500 mM), K_3PO_4 (530 mM), 2% aq. TPGPS-750-M, 15% THF, 60 °C.



Scheme 3. Coupling of diverse representative aryl halides with varied boronic acids. Conditions: Pd(dtbpf)Cl_2 (7.3 mM), boronate (500 mM), K_3PO_4 (530 mM), 2% aq. TPGPS-750-M, 15% THF, 60 °C.



The CuCl/bipyridine promoted Petasis reaction of DNA conjugated amine **8**, with -4-bromophenylboronic acid and glyoxylic acid provided amino acid **9**,[22] and the Ugi, Ugi-azide and Gröbke-Blackburn-Bienaymé-reactions of **5** yielded peptidomimetic and heteroaromatic aryl halides **10**-**12**, respectively.[23] The Ugi reaction introduced different functional groups such as a carbonitrile, a phenol, and various heterocyclic structures that may interact with metal catalysts.

SUZUKI-MIYAURA COUPLING

With this diverse range of substrates in hand, we investigated their performance in the Suzuki-Miyaura coupling. Initially, we explored the coupling of the carboxylic acid substituted dihydroisoquinolone **6** with phenyl boronic acid (Scheme 2). This reaction proceeded smoothly with 100% conversion and the final product consisting of 93% of the desired biaryl **13**. For the 4-pyridone derivatives **7a-e**, unsubstituted bromo and iodophenyl derivatives **7a** and **b** proceeded with 100% conversion to the desired biphenyl species **14**. The more challenging ortho-substituted derivatives **7c-e** also coupled very efficiently to both phenyl and 3-pyridyl boronic acids resulting in full conversion to the biaryl species **14**-**20** (86 to 100% product) showing that the reaction tolerates steric encumbrance and a range of electronic properties (electron withdrawing CF_3-, **7c**, to electron donating MeO-, **7e**).

Piperidinylphenylacetic acid **9** gave 100% of the desired biphenyl derivative **21** and 93% of 3-pyridyl **22** (100% conversion). 2-arylglycine bisamides **10a-f** coupled exceptionally well with both phenyl and 3-pyridyl boronic acids, resulting in 100% of the biaryl species **23a-f** and **24a-f** in all cases, with the exception of tetrazole **10e** with phenyl boronic acid (88%, 100% conversion to **23e**). Tetrazole containing 11 and imidazopyridine 12 also coupled to both boronic acids to give biaryls **25**-**28** with 100% conversion (89-100% of desired product).

Overall, this demonstrates a high degree of substrate compatibility, with the coupling proceeding well for both aryl bromides and iodides with a range of steric and electronic properties and is tolerant of strong hydrogen bond donors and acceptors, acids and bases and a wide range of heterocyclic moieties and drug-like functionality.

With these promising results in hand, we explored the coupling of further diverse DNA-linked boronates with representative boronic acids **7e**, **9** and **12** (Scheme 3). 3-Methoxyphenyldihydropyridone **7e** coupled well with 4-methoxy- and 4-trifluoromethylphenyl boronic acids to give biaryls **29** and **30** (97% and 87% product respectively). Piperidinylphenylacetic acid **9** coupled cleanly to these further boronates (100% in both cases).

Imidazopyridine **12** also coupled with 100% efficiency for 4-methoxy- and 4-trifluoromethylphenyl boronic acids with 100% conversion (94% and 98% products respectively). Extending this substrate to a wider range of

boronates resulted in similarly efficient reactions including heterocyclic indole boronic acid, pyrazole pinacolatoboronate and 3-trifluoromethylphenyl mida boronate (88-100% product). 4-Methylpyridine-3-midaboronate represents a particularly challenging substrate, combining a pyridine nitrogen, *o*-substituent and mida boronate, all of which individually might make the coupling less efficient. Nevertheless, this substrate still coupled with 12 to give 66% of the desired biaryl **38**.

CONCLUSION

These results demonstrate an exceptionally varied scope of the micellar promoted Suzuki-Miyaura coupling reaction for on-DNA substrates. The reaction is compatible with a widely diverse array of aryl bromides and iodides across a range of boronate coupling partners. Crucially, the reaction tolerates all of the functionality that would be desirable in a drug-like DEL, including strong hydrogen bond donors and acceptors, carboxylic acids and their isosteres, amine bases and a wide variety of heterocyclic systems in both coupling partners. This type of polar functionality has been shown to be problematic in library synthesis previously[24] including in DEL chemistry.[25] We have already demonstrated the scope of the reaction with respect to the off-DNA boronates and the compatibility with encoded library synthesis.[19] Together, this establishes that the methodology is highly effective for the production high-fidelity DELs. The combination of the robustness of this transformation with the diverse scaffolds prepared using multicomponent reactions employed in this work will lead to DELs of exceptional structural diversity.

SUZUKI COUPLING EXPERIMENTAL PROCEDURE

An aliquot of boronic acid solution (20 µl, 0.75 M in DMF) was added to a 50 µl glass insert for a Para-dox™ 96- well micro photoredox/optimisation Plate. The DMF was removed at 55°C in a Genevac for 30 mins. To this solution was added 5% TPGS-750-M (12 µl), water (7 µl), potassium phosphate (6 µl, 113.23 mg in 200 µl water) and DNA (5 µl, 0.1 mM in water). The vials were vortexed for 30 seconds each to enhance mixing. $Pd(dtbpf)Cl_2$ (4.5 µl, 6.37 mg in 200 µl THF) was then added and the samples vortexed again for 10 seconds each. The samples were then heated in a Para-dox™ 96-well micro photoredox/optimisation plate at 60°C for 5 hours. Sodium diethyldithiocarbamate (6 µl, 1 M in water) was added as a palladium scavenger, and the samples were heated at 60 °C for a further 30 minutes. Mass spectrometry was used to analyse reactions. Samples prepared by adding reaction mixture (5 µl) to water (595 µl) and filtered through a hydrophilic PTFE filter. Chloroform (3 x 40 µl) was added to each and the vial vortexed. The organics were removed, aqueous sodium chloride (4 µl, 4M) and ethanol (70 µl) were added and the mixture incubated at -78°C for 1 hour. The mixture was then centrifuged and the ethanol layer removed. Ethanol 70% (70 µl) was added and the process repeated. The pellet of DNA was dissolved in water to give a 0.1 mM solution.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website: experimental details, chromatograms and mass spectra for the reaction products.

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Author Contributions

MP carried out the multicomponent reactions, JHH and HS-G carried out the Suzuki-Miyaura couplings, AM, GP were the industrial supervisors of JHH,. AB conceived of the work and supervised MP, MJW conceived of the work and supervised JHH and HAS-G. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.
‡These authors contributed equally.

ACKNOWLEDGMENT

We thank AstraZeneca (studentship award to JHH), Bayer AG (postdoctoral funding for MP), the DFG (AB, BR 5049/7-1), EPSRC DTP (EP/R51309X/1, studentship to HAS-G), Cancer Research UK (C2115/A21421, Newcastle Drug Discovery Unit Programme Grant) for funding.

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