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On-DNA Pd and Cu promoted C–N cross-coupling reactions†

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Encoded library technology (ELT) is a novel hit identification platform synergistic with HTS, fragment hit ID and focused screening. It provides both an ultra high-throughput and a cost-efficient tool for the discovery of small molecules that bind to protein targets of pharmaceutical interest. The success of ELT relies heavily on the chemical diversity accessed through DNA-encoded library (DEL) synthesis. We developed unprecedented Pd and Cu(I) promoted C–N cross-coupling reactions between a DNA-conjugated aryl iodide and primary amines. These reported reactions have strong potential for application in DNA-encoded library (DEL) synthesis.

DNA-encoded library technology (ELT) is a cutting edge technology for hit identification of biological targets of interest.¹ Compared to traditional high-throughput screening (HTS), it has several advantages, including a much larger library size and more cost efficient affinity screens.² Recently, more pharmaceutical companies and academic institutions have started utilizing ELT for rapid hit identification of small molecule ligands for diverse disease related biological targets including Wip1,³ ADAMTS-4,⁴ PAD4,⁵ NK3,⁶ Rip1 (ref. 7) and BCATm.⁸ The success of efficient screening of the ELT platform relies heavily on the chemical space covered by the DNA-encoded chemical libraries. Therefore, in order to increase the diversity of ELT libraries, more DNA compatible reactions are needed. Although progress has been made for some commonly employed reactions in medicinal chemistry, there is still a large need for additional on-DNA reactions with a wide range of synthetic reagents for ELT library synthesis.⁹

Transition metal promoted cross-coupling reactions, such as C–C and C–N couplings, have been widely applied in the pharmaceutical industry.¹⁰ Recently, Pd promoted C–C coupling reactions with DNA-conjugated aryl halides, such as Suzuki and Sonogashira couplings, have been elegantly developed and applied to DNA-encoded library synthesis (Scheme 1),¹¹ which has generated several interesting small molecule hits for biological targets of interest.¹² However, there is no literature report of C–N cross-coupling reactions for DNA-conjugated aryl halides.¹³ Particularly, C–N cross-coupling is much more appealing than C–C coupling for

DNA-conjugated aryl halides because amines are more easily accessible synthetic reagents for ELT library production than boronates and alkynes. Herein, we describe the development of unprecedented Pd and Cu(I) promoted C–N cross-coupling reactions between a DNA-conjugated aryl iodide and primary amines. Some of these reactions have been successfully applied to DNA-encoded library (DEL) synthesis.¹⁴

Employing Buchwald *t*-butyl-XPhos precatalyst G1,¹⁵ we have successfully developed the first C–N cross-coupling reactions between a DNA-conjugated aryl iodide and aromatic amines. Promoted by two equivalents of Pd, the DNA-conjugated aryl iodide reacts with aromatic amines to provide desired coupling products at 100 °C for 3 h in the presence of 1000 equivalents of aromatic amines and 1000 equivalents of CsOH. We hypothesize that the high amount of CsOH was necessary for the reaction as strongly basic conditions facilitate the deprotonation of aromatic amines. A broad substrate

Scheme 1 On-DNA cross-coupling reaction development.

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scope of aromatic amines was achieved for these on-DNA C–N cross-coupling reactions. Scheme 2 summarizes the representative aromatic amine examples which provide the desired C–N coupling products with good conversion. Employing these newly developed conditions, we have validated over 6300 primary aromatic amine building blocks for the C–N cross coupling, and the conversion was determined by LC/MS. Fig. 1 illustrates the conversion distribution of validated primary aromatic amines. It should also be noted that DNA decomposition was observed in a substrate dependant manner, and the observed conversions were impacted by many factors including the solubility, steric and electronic effect of the amine substrates.¹⁶ Although this reaction condition worked well for aromatic primary amines, it did not provide synthetically useful conversion for aliphatic amines. An alternative protocol was necessary for the C–N cross-coupling reaction between DNA-conjugated aryl iodides with aliphatic amines. To address this we turned our attention to the Ullmann type Cu(I) promoted C–N cross-coupling reactions.

Although CuI is the most commonly used copper source for the Ullmann reaction, its poor solubility in either organic solvents or water limits the application for combinatorial synthesis in plate format.¹⁷ Instead, *in situ* generated Cu(I) by mixing copper sulfate pentahydrate and sodium ascorbate together can be used as the alternative copper reagent to overcome the solubility problem of CuI.¹⁸ When amino acids were used as the amine coupling partner, *in situ* generated Cu(I) promoted the C–N cross-coupling reaction very well for a representative DNA-conjugated aryl iodide. The amino acids substrate scope was also general, and Scheme 3 illustrates several examples for C–N cross coupling with good conversion. With this new condition promoted by Cu(I) we have validated 557 amino acids and Fig. 2 summarizes the conversion distribution of validated amino acids. In these cases, the

6329 primary aromatic amines

Fig. 1 Conversion distribution of Pd promoted C–N cross coupling reactions between the DNA-conjugated aryl iodide and primary aromatic amines.

amino acid not only acts as the ligand for Cu(I) to promote the C–N coupling reactions, but also appears to prevent the interaction between copper and DNA; without amino acids in the reaction system, DNA quickly decomposed.

Employing these two newly developed protocols, we have successfully applied the C–N cross coupling reactions for DNA-encoded library production. As illustrated in Fig. 3, in final cycle 3, Pd promoted C–N coupling between DNA-conjugated aryl iodides and aromatic amines have been applied for the library A production, while both Pd and Cu promoted C–N coupling reactions have been applied for the library B production. Typically, we achieved approximately 50% DNA recovery after HPLC purification of the pooled library material following scavenger treatment of the reaction mixtures. However, it should be noted that DNA decomposition was also observed during the library production for some substrates.

The initial success of Cu(I) for amino acids encouraged us to investigate C–N cross-coupling reactions between simple alkyl primary amines and a DNA-conjugated aryl iodide. We applied proline as the ligand for copper.¹⁹ Because proline did not dissolve well in the water, we added equal amount of

Scheme 2 Aromatic amine examples of the C–N cross-coupling reaction promoted by t-butyl-XPhos Buchwald precatalyst G1. The reaction was carried out with 10 nmol DNA-conjugated aryl iodide with 2 equivalents of Pd (6.67 mM in DMA), 1000 equivalents of aromatic amine (1 M in DMA) and 1000 equivalents of CsOH (5 M in water). The conversion was determined by LC/MS. Please refer to the ESI† for a detailed protocol.

Scheme 3 Amino acid examples of the C–N cross-coupling reaction promoted by Cu(I). The reaction was carried out with 10 nmol on-DNA Ar–I with 20 equivalents of CuSO₄ (125 mM in water), 24 equivalents of sodium ascorbate (100 mM in water) and 500 equivalents of amino acid (500 mM in KOH). The conversion was determined by LC/MS. Please refer to the ESI† for a detailed protocol.

Fig. 2 Conversion distribution of Cu(I) promoted C–N cross coupling between the DNA-conjugated aryl iodide and amino acids.

Fig. 4 Conversion distribution of Cu(I) promoted C–N cross coupling between a DNA-conjugated aryl iodide and aliphatic primary amines.

Library A
Cycle 3: 840 boronic acids/esters (+1 blank)
1849 amines, Pd promoted (+1 blank)
Library size: 177 million

Library B
Cycle 3: 301 amines, Pd promoted (+1 blank)
160 amino acids, Cu promoted (+1 blank)
Library size: 30.4 million

Fig. 3 DNA-encoded library synthesis employed Pd and Cu promoted C–N cross-coupling reactions in cycle 3.

Scheme 4 Aliphatic amine examples of C–N cross-coupling reactions promoted by Cu(I). The reaction was carried out with 10 nmol on-DNA Ar–I with 30 equivalents of CuSO_4 , 60 equiv. of proline [CuSO_4 (125 mM in water): proline (1 M in KOH) = 4:1 by volume to prepare the Cu/proline complex], 36 equivalents of sodium ascorbate (100 mM in water) and 1000 equivalents of aliphatic amines (1 M in DMA). The conversion was determined by LC/MS. Please refer to the ESI† for a detailed protocol.

base to prepare the proline/KOH solution, which was mixed with CuSO_4 to make the Cu/proline complex (Cu:proline = 1:2). With 30 equivalents of the Cu/proline complex and 36 equivalents of sodium ascorbate, the aliphatic primary amines were demonstrated to react with the DNA-conjugated

aryl iodide with good conversion. Scheme 4 illustrates several aliphatic primary amine examples for the Cu(I)/proline promoted C–N cross-coupling reactions on the representative DNA-conjugated aryl iodide. Applying this new protocol, we have validated over 2700 primary aliphatic amines, and Fig. 4 summarizes, the conversion distribution of aliphatic amines. Since an excessive amount of proline was used in the reaction system, there was a competition between proline and aliphatic amines and proline coupled with the DNA-conjugated aryl iodide was the common side product observed during the validation. It is worth pointing out that DNA decomposition under these conditions is worse than the previous protocol, presumably because 60 equivalents of proline employed in this protocol was less effective in preventing the interaction between Cu and DNA compared with 500 equivalents of the amino acid used in the previous protocol. Currently, this new protocol of C–N cross coupling utilizing aliphatic primary amines is being tested for application to DNA-encoded library synthesis with a cautious focus on DNA decomposition, and this work will be reported in the future.

Conclusions

We have developed the first C–N cross-coupling reactions for a DNA-conjugated aryl iodide, including Pd promoted C–N coupling reactions for aromatic primary amines and Cu(I) promoted couplings for amino acids and aliphatic primary amines. Additionally, both aromatic amines and amino acids have been successfully applied to DNA encoded library production for C–N cross coupling reactions. Several small molecule hits from the DNA-encoded C–N cross-coupling libraries have already been identified for certain biological targets of interest, and we will report these findings in due time. In addition, more research is needed in the exploration of substrate scope of DNA-conjugated aryl halides, and this will also be reported in the future.

Conflict of interest

The authors declare no competing interests.

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