Rhodium-Promoted C-H Activation/ Annulation between DNA-Linked Terminal Alkyne and Aromatic Acid: A Finding from the Selection Outcomes

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Supporting Information Placeholder

ABSTRACT: As discovered by the previous selection outcomes, we developed a Rhodium-promoted C-H activation/ annulation reaction of DNA-linked terminal alkyne and aromatic acid. This reaction exhibits excellent efficiency with high conversions and a wide broad substrate scope. Most importantly, the unique DEL-compatible condition provides a better scenario to yield an isocoumarin scaffold compared to conventional organic reaction condition, and this newly developed on-DNA method has confirmed its feasibility in preparing DNA-encoded libraries.

DNA-encoded library technology (DELT) [1-3] represents a powerful high-throughput selection platform facilitating the discovery of ligands for targets of interest in the early phase of drug discovery. Conceptualized by Sydney Brenner and Richard Lerner three decades ago, DEL technology has undergone iterative advancements, evolving into a widely embraces tool in both academics and industry. Notably, several drug candidates identified through DELT have progressed into clinical phases, including WPV01 [4], X-165 [5], and 177Lu-OncoFAP-23 [6], et al. DELT embodies an interdisciplinary convergence of organic chemistry, molecular biology, and computational science. A typical workflow of DELT encompasses the development of on-DNA reactions, the construction of DNA-encoded libraries (DELs) via on-DNA chemistries and enzymatic DNA ligation, target selection using DELs, high-throughput sequencing of the DNA tags of selected library members, data analysis to identify enriched structural features, and validation of results through on/off-DNA synthesis and biological assays. Central to this process is the creation of DNA-encoded libraries characterized by high structural diversity and purity, which underpins DELT's success.

Regardless of the specific approach employed in constructing DNA-encoded libraries, on-DNA chemical transformations serve as the linchpin for assembling building blocks into desired products [7-8]. Recognizing the pivotal role of these transformations, researchers have endeavored to develop DNA-compatible chemical tools meeting stringent criteria for library synthesis. These criteria include ensuring that reactions occur under mild, highly dilute aqueous conditions, exhibit chemoselectivity to prevent undesired modifications of DNA tags, demonstrate high reactivity and efficiency to react with pooled library members, tolerate a broad substrate scope to cover extensive chemical space, and ideally yield pharmacologically relevant features such as chemical bonds or privileged scaffolds.

To date, researchers have access to a plethora of on-DNA chemical tools and strategies, offering a rich toolkit for library synthesis. By harnessing these tools in conjunction with a diverse array of building blocks, scientists have achieved the synthesis of libraries characterized by linear, branched, scaffold-based, and macrocyclic topologies, catering to diverse requirements posed by disease-related targets. Typically, researchers draw inspiration from conventional organic syn-

thesis methodologies, leveraging established techniques as a foundation for developing DEL-compatible approaches. They meticulously adapt and optimize these methods to align with the stringent standards of DNA compatibility, thereby enabling the preparation of DNA-encoded libraries endowed with pharmacologically significant features.

Recently, Zhang and colleagues reported a notable instance of "reverse transformation," serendipitously discovered from DNA-linked tetrazole and primary amine [9-11]. This discovery yielded a 1,2,4-triazole product previously unreported in the literature. The authors extensively validated this reaction both on-DNA and off-DNA models, extending its utility to diverse applications such as peptide macrocyclization, nucleotide cross-linking, and protein labeling. Similarly, our laboratory encountered a comparable scenario. In 2018, we successfully executed a Ruthenium-promoted C-H functionalization of aromatic acid with DNA-tagged acryl amide for DNA construction purposes [12]. Building upon the established reaction conditions and synthetic pathway, we subsequently integrated this methodology into the synthesis of a three-cycle DNA-encoded library. Following preliminary selection against pharmaceutically relevant targets and thorough data analysis, we observed enrichment of a cycle 2 building block (BB) featuring a terminal alkyne group. This intriguing outcome prompted us to investigate whether the acidic terminal alkyne could also serve as a coupling partner with aromatic acid under the on-DNA C-H functionalization condition (**Figure 1**).

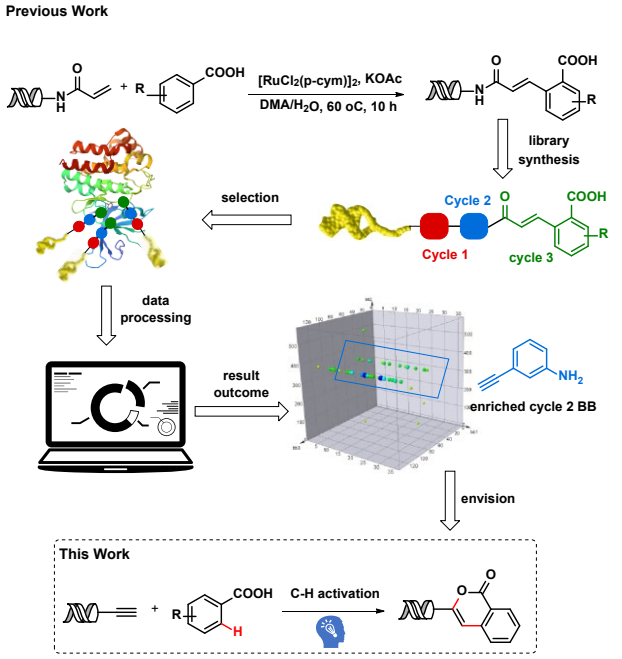


Figure 1. The previous selection work revealed an enriched terminal alkyne group, which was potentially involved into the C-H activation reaction.

In the last few decades, C-H activation/ oxidative annulation between aromatic acids and alkynes has been explored under various transition-metal-catalyzed systems [13-22]. While carboxylic acids are generally considered weaker directing groups compared to others, their distinct advantage in DNA-encoded library technology lies in the ubiquity and ready availability as building blocks, which can be directly utilized for DEL synthesis without requiring structural modification. However, the utilization of terminal alkynes as coupling partners in C-H activation/annulation reactions has posed significant challenges, primarily due to undesired homocoupling reactions under oxidative conditions [23-25]. To date, only one $Co(hfacac)_2$-catalyzed system [15] has been reported to successfully employ terminal alkynes as coupling partners. Nonetheless, for on-DNA synthesis, we could address this issue by employing a large excess of aromatic acid to mitigate the homocoupling of DNA-tagged alkynes. We hypothesize that the C-H activation reaction may exhibit enhanced performance under on-DNA reaction conditions compared to conventional organic conditions.

Furthermore, in 2021, Gao and colleagues presented groundbreaking research on diverse C-H activation/ [4+2] annulations of benzoic acid and gem-difluoromethylene under an Ir-catalyzed system, subsequently optimizing it for on-DNA applications [26]. This study validated the feasibility of employing alkynes in C-H activation reactions within a DEL-compatible environment. Another driving force behind our pursuit of on-DNA reactions is the potential to construct isocoumarin scaffolds, pivotal structural motifs found in numerous biologically active compounds. In this manuscript, we elucidate the Rh-catalyzed C-H activation/oxidative annulation reaction under both on-DNA and off-DNA reaction conditions, while also confirming its applicability in DNA-encoded library technology by preparing two types of DNA-encoded libraries (**Figure 2**).

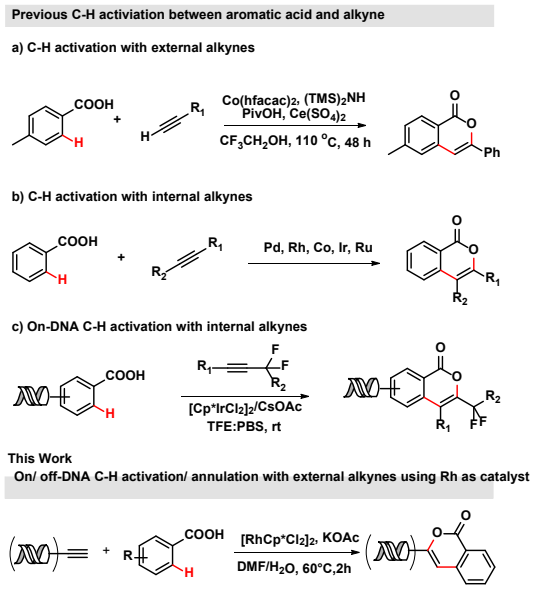


Figure 2. Strategies for the C-H activation/ annulation

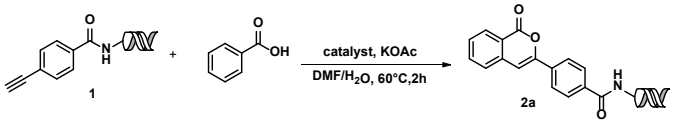
We commenced a proof-of-concept (POC) experiment employing DNA-linked alkyne **1** and benzoic acid **2** as model substrates under previously reported reaction conditions without

any modification (**Table 1**, **entry 1**). The deconvoluted molecular weight of the predominant product was consistent with the proposed structure, confirming our initial hypothesis that the enriched alkynes identified in the selection outcome were indeed utilized in the C-H activation reaction. Various experimental parameters were extensively investigated to define the optimal reaction conditions. A survey of transition-metal catalysts revealed that RuPhos Pd G3 yielded excellent reaction outcomes (**entry 3**), while (A-taPhos)$_2$PdCl$_2$ resulted in a comparatively lower conversion (**entry 4**). However, other catalysts failed to yield the desired products. It is worth noting that beyond the transition metal itself, the choice of ligand may also significantly influence the reaction efficiency. [Cp*TM] complexes, for instance, have demonstrated remarkable efficacy in catalyzing C-H activation reactions.

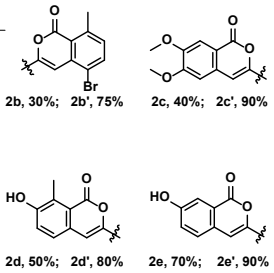
Given the favorable conversions achieved with both [RuCl$_2$(p-cymene)]$_2$ and [RhCpCl$_2$]$_2$ using simple model substrates, we conducted a preliminary test with four aromatic acids under both catalytic systems to discern which reaction condition warranted further investigation. The results indicated superior conversions with the Rh catalyst (**2b**–**2e**, **2b'**-**2e'**). Thus, [RhCpCl$_2$]$_2$ was selected as the catalyst for subsequent reaction optimization.

To mitigate potential degradation of the oligonucleotide caused by excess transition-metal catalyst, we sought to reduce the catalyst equivalents while concurrently enhancing the benzoic acid equivalents to facilitate complete reaction. Catalyst loading screening under 1000 equivalents of benzoic acid revealed that 4 equivalents of the Rh catalyst were sufficient to achieve full conversion. Ultimately, **entry 9** was identified as the optimal reaction condition.

Table 1. Optimization of reaction conditions

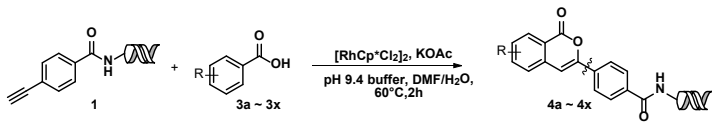


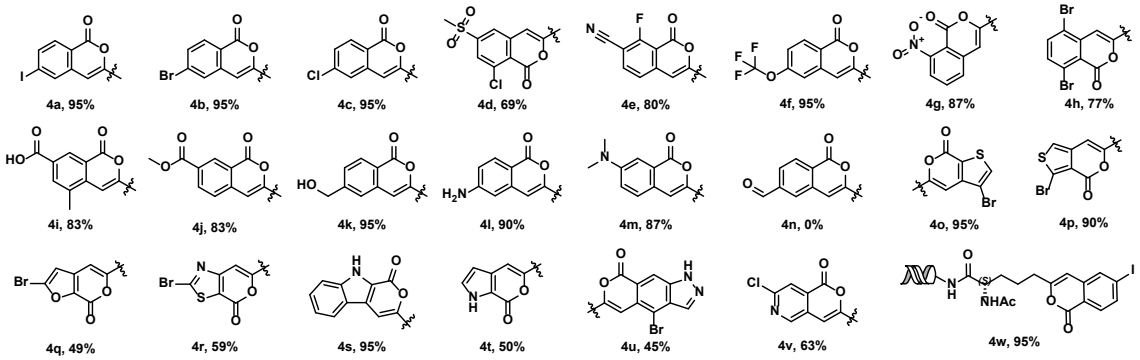
entry	Cat. (Equiv.)	Equiv. of acid	Conversion
1	[RuCl2(p-cymene)]2 (8)	500	90%
2	RuPhos Pd G3 (8)	500	0%
3	[RhCp*Cl2]2 (8)	500	95%
4	(A-^{ta}Phos)$_2$PdCl$_2$ (8)	500	60%
5	*t*BuXPhos Pd G1 (8)	500	0%
6	tbuttphos Pd G3 (8)	500	0%
7	Co(hfacac)2 (8)	500	0%
8	[RhCp*Cl2]2 (6)	1000	95%
9	[RhCp*Cl2]2 (4)	1000	95%



Armed with the optimized conditions, we embarked on exploring the scope and limitations of the aromatic acid substrates, as depicted in **Scheme 1**. Functional groups such as halides (**4a** – **4c**), methylsulfonyl (**4d**), carboxylic acid (**4i**), ester (**4j**), benzyl alcohol (**4k**), phenol (**4l**), and amine (**4m**) were all well-tolerated, yielding the intended products with excellent conversions. However, attempts with the aldehyde group (**4o**) proved futile. Moreover, the positioning of substituents on the phenyl ring exhibited no discernible impact on reaction outcomes, as both meta- and ortho-substituted benzoic acids demonstrated compatibility (**4d**-**4j**). Heteroaryl acids, including thiophene (**4p**-**4q**), furan (**4r**), thiazole (**4s**), indole (**4t**), pyrrole (**4u**), and indazole (**4v**), alongside pyridine (**4v**), proved to be viable substrates, yielding the desired products with moderate to good conversions. Additionally, DNA-linked alkyl alkynes exhibited smooth reactivity with benzoic acid, affording the desired product **4w** with a favorable conversion rate.

Scheme 1. Reaction scope with respect to aromatic acids



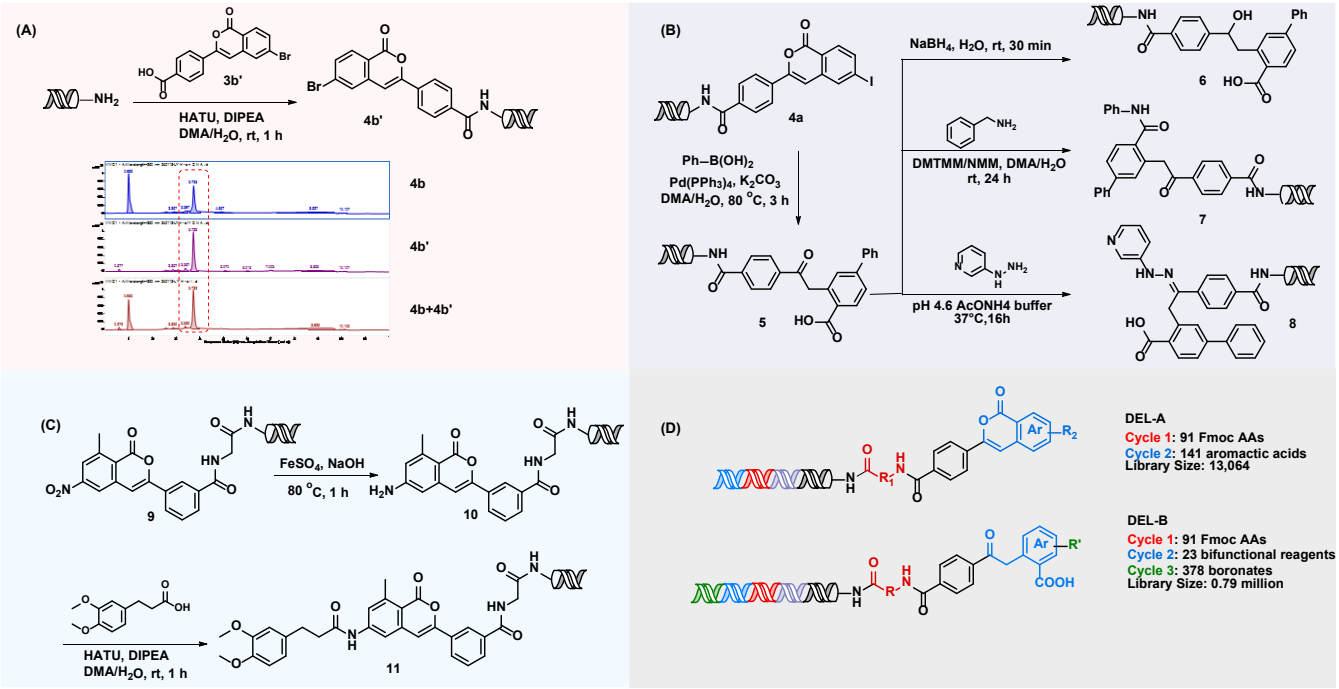


For the on-DNA chemical transformations, the reaction conditions were determined through LCMS analysis. Unlike conventional organic chemistry, where techniques such as NMR and IR spectroscopy are common analytic approaches, these methods cannot be directly utilized in DEL technology. Identifying the structure of DNA-linked products, particularly isomers, poses a significant challenge. To verify the proposed regioselectivity and isocoumarin structure of the product, we conducted a co-injection experiment. Compound **3b'** was synthesized using conventional organic methods and confirmed by LCMS and NMR spectroscopy. Subsequently, amidation with the headpiece yielded the authentic DNA-tagged compound **4b'**. Co-injection of compounds **4b** and **4b'** was performed, and the resulting spectra are depicted in **Scheme 2A**. The identical retention time and molecular weight observed confirmed the characterization of **4b**, synthesized through the developed on-DNA chemical transformation.

In light of the functional group compatibility afforded by this novel reaction condition, a series of validation experiments were conducted prior to its application in generating DNA-encoded libraries centered on the isocoumarin scaffold. Initially, Suzuki coupling was executed with DNA-tagged compound **4a**. Analysis via LCMS revealed the predominant product has a molecular weight of proposed product plus 18 Da, indicative of lactone hydrolysis product **5**. To corroborate this finding, subsequent experiments involving ketone reduction with $NaBH_4$, reductive amination with benzylamine, and condensation with aryl hydrazine were performed, yielding molecular weights consistent with our conjecture. Additionally, reduction of the nitro group of **9** to the primary amine **10**, followed by acylation with acid, furnished the corresponding proposed product **11**.

Moreover, an exemplary library, **DEL-A**, comprising 13,064 DNA-tagged members, is depicted in **Scheme 3**. **DEL-A** was assembled through two iterative cycles, incorporating 91 Fmoc AAs and 141 aromatic acids as building blocks respectively. All library constituents share a common isocoumarin scaffold. Similarly, **DEL-B** was synthesized through three iterative cycles, with Suzuki coupling employed with aryl boronic acids/esters in cycle 3. Building upon previous research, this library is anticipated to exhibit a branched structure, serving as a contrasting counterpart to **DEL-A** in subsequent DEL selection processes.

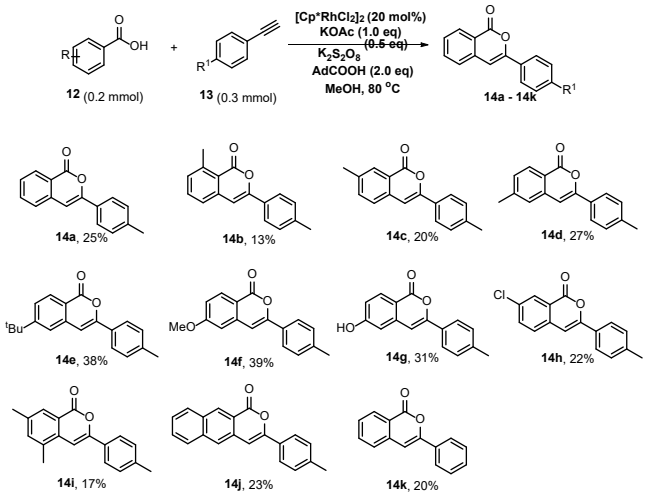
Scheme 2. The validation experiments and prepared DNA-encoded libraries



Subsequently, our focus shifted towards validating the Rh-promoted, terminal alkyne-utilizing C-H activation/annulation reaction condition through off-DNA synthesis, a novel endeavor not previously documented. As delineated in **Scheme 3**, a 20 mol% concentration of $[RhCp^*Cl_2]_2$ was employed as the catalyst. Traditional oxidative annulation methodologies often require external or internal oxidants. However, in the on-DNA reaction milieu, the stoichiometric quantities of metal catalysts, along with the dissolved O_2 in the solvent, are adequate to propel the reaction. In the off-DNA reaction system, we explored various additives, ultimately discovering that $K_2S_2O_8$ served as a potent oxidant. Furthermore, the inclusion of AdCOOH aimed to mitigate the formation of undesired homocoupling side products originating from the terminal alkynes. Despite these efforts, the presence of such side

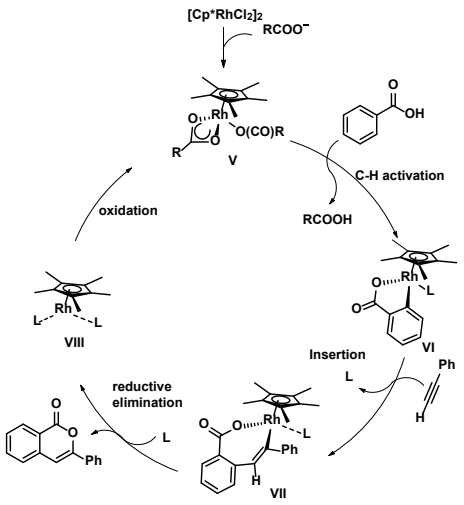
products persisted in the mass spectrum, resulting in diminished yields of the desired products.

Scheme 3. Off-DNA C-H activation/ annulation reaction and the substrate scope of terminal alkynes and aromatic acids.



Based on our study and published paper, we have proposed a viable catalytic cycle, as illustrated in **Scheme 4**. Initially, the active Rh complex **V** was formed by ligand exchange. subsequently, the coordination/ ortho C(sp^2)-H bond activation of benzoic acid happened to yield complex **VI**. Following this, the alkyne is inserted into the Rh-aryl bond to generate complex **VII**, which could conceivably undergo direct reductive elimination, thereby furnishing the annulation product.

Scheme 4. Plausible Mechanism



In conclusion, we have developed a novel approach for Rh-catalyzed C-H activation/annulation of terminal alkynes with benzoic acid under both on-DNA and off-DNA reaction conditions. Traditionally, DNA-involved reactions are presumed to offer inferior reaction conversions and a restricted substrate scope compared to conventional organic synthesis. However, in certain instances, this paradigm is reversed. As demonstrated in this study, the surplus amount of aromatic acid coupling partner and the highly dilute concentration of the DNA-tagged alkyne strongly mitigate the formation of homo-coupling side products, resulting in heightened conversions and an expanded substrate scope compared to off-DNA reactions. This presents a novel direction for advancing the field of on-DNA chemical transformations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://

Materials and methods; UV/Mass spectra for DNA-linked compounds; NMR spectra for small molecules; and the co-injection experiment.

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Author Contributions

# Yuhan Lv and Min Wu contributed equally to this work.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

DELT, DNA-encoded library technology; DEL, DNA-encoded library; BB, building block

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