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Chemoselective Sequential Click Ligations Directed by Enhanced Reactivity of an Aromatic Ynamine

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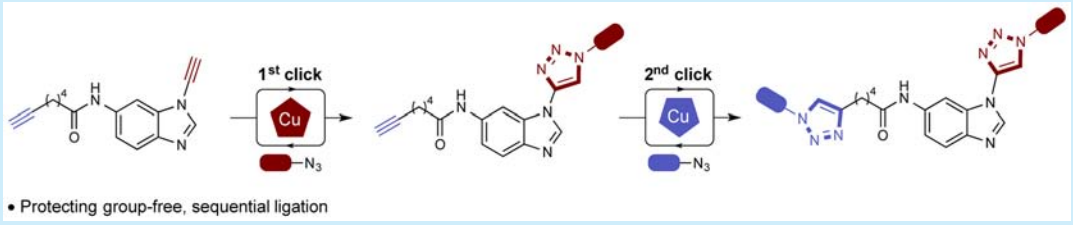
Supporting Information



ABSTRACT: Aromatic ynamines or *N*-alkynylheteroarenes are highly reactive alkyne components in Cu-catalyzed Huisgen [3 + 2] cycloaddition ("click") reactions. This enhanced reactivity enables the chemoselective formation of 1,4-triazoles using the representative aromatic ynamine *N*-ethynylbenzimidazole in the presence of a competing aliphatic alkyne substrate. The unique chemoselectivity profile of *N*-ethynylbenzimidazole is further demonstrated by the sequential click ligation of a series of highly functionalized azides using a heterobifunctional diyne, dispelling the need for alkyne protecting groups.

The Cu-catalyzed alkyne−azide cycloaddition (CuAAC) or "click" reaction is a powerful and robust method for the rapid synthesis of 1,4-substituted triazoles.[1−4] The bioorthogonality of this [3 + 2] cycloaddition reaction between a terminal alkyne and an azide has been deployed for the preparation of multifunctional biomaterials,[5−7] as well as the construction of discrete bioconjugates to probe cellular processes.[8−10] Despite its widespread use in these fields, the exploration of chemoselectivity profiles of alkyne and azide reagents has been limited.[11] Zhu et al. reported a chelate-directed strategy that highlighted the enhanced reactivity of azide groups in close proximity to a metal-chelating atom (Figure 1a).[12,13] This chelate-assisted approach enhances the reaction rate of picolyl azides and provides a reproducible platform for sequential ligation of two different alkynes. At present, the development of a cognate sequential ligation strategy based on reactivity differences of terminal alkynes has been confined to the use of protection/deprotection methods,[14−17] which disregards any potential reactivity preferences that may exist between alkyne subtypes.

Strain-promoted alkyne−azide cycloaddition (SPAAC) offers a copper-free route to discriminate between alkyne subtypes (Figure 1b);[18−21] however this produces a regioisomeric mixture of triazole products, which is undesirable if discrete products are required. Here, we describe an unprecedented chemoselectivity profile of the aromatic ynamine *N*-ethynylbenzimidazole as a terminal alkyne surrogate in CuAAC reactions (Figure 1c). Furthermore, we demonstrate the utility of our strategy as a novel platform for sequential ligation.

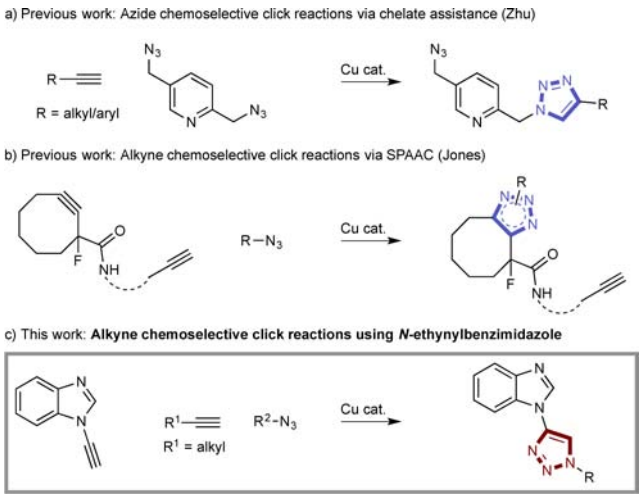


Figure 1. Protecting group-free chemoselective click reactions. (a) Azide selectivity using chelate assistance. (b) Alkyne selectivity using SPAAC. (c) Alkyne selectivity using the aromatic ynamine *N*-ethynylbenzimidazole.

Terminal ynamides have been previously shown to be excellent substrates in a variety of Cu-catalyzed C−C bond formations, such as Glaser couplings[18−20] and nucleophilic addition to mild electrophiles such as acyl chlorides and chloroformates at room temperature and in high yield.[21]

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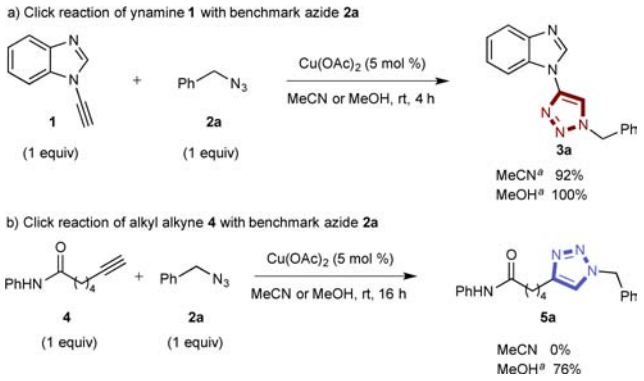
Furthermore, terminal ynamide substrates such as *N*-tosylynamides have also been employed in CuAAC reactions and tandem variations,[22,23] although terminal *N*-ethynyl imidazolidinones and *N*-ethynyl 1,3-oxazolidinones were shown to decompose under conventional click chemistry conditions.[24] We surmised that the electronic bias present in *N*-alkynylheteroarenes would strike a balance between enhanced reactivity in CuAAC reactions relative to an aliphatic alkyne. Indeed, in our previous work we observed an unusually fast rate of reaction between **1** and **2a** to form **3a**,[25] suggestive of reactivity differences existing between **1** and **2a** corresponding to an aliphatic alkyne that could be used to undergo a CuAAC reaction chemoselectively.

To test this hypothesis, a reaction screen was undertaken using **1** and benzyl azide **2a**. The parameters of a copper source, solvent, reductant, and ligand were surveyed (see Supporting Information). The use of Cu(I) and Cu(II) salts were effective in catalyzing this reaction (Table S1). We found that 5 mol % $Cu(OAc)_2$ was sufficient to provide high conversion to product **3a** in MeCN (92%) or MeOH (quantitative; Scheme 1). The

Scheme 1. Click Reactions of 1 and Aliphatic Alkyne 4 with Benzylazide 2a



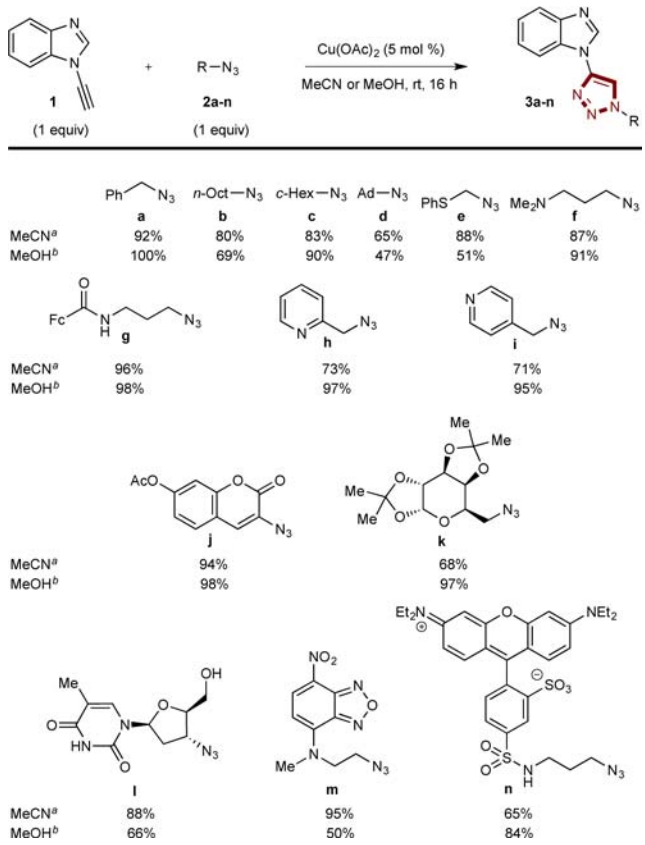
[a]Isolated yields.

reaction in MeOH was notably faster, requiring only 2 h for the consumption of **1** and the concomitant formation of product **3a** by HPLC analysis compared to 4 h for MeCN. In contrast, the corresponding reaction using aliphatic alkyne **4** under equivalent conditions in either MeOH or MeCN delivered no product after 4 h with reactions requiring 16 h in MeOH to proceed to useful levels of conversion (Scheme 1b and Table S2).

A solvent screen for the reaction of alkyne **4**, using a standard 16 h reaction time and $Cu(OAc)_2$, afforded triazole product **5a** only when MeOH (76%), DMSO (79%), and aqueous mixtures (1:1 MeOH/H_2O, 88%; DMSO/H_2O, 84%) of these two solvents were used (Table S2). Solvents such as MeCN, EtOH, *i*-PrOH, and DMF using $Cu(OAc)_2$ did not produce product **5a** after 16 h. As expected, standard CuAAC conditions using $CuSO_4$, a reductant (NaAsc), and TBTA produced **5** in quantitative yield. Taken collectively, the reaction rate of **1** in CuAAC reactions was considerably faster than the aliphatic alkyne **4**.[25] Furthermore, the pairing of $Cu(OAc)_2$ and MeCN suggested conditions for the development of a chemoselective platform of *N*-alkynylheteroarenes in the presence of an aliphatic alkyne.

The generality of **1** using a series of azide substrates was then explored using optimized conditions of 5 mol % $Cu(OAc)_2$ in either MeCN or MeOH (Scheme 2). The reaction conditions tolerated a variety of azides of varying steric bulk (e.g., **b**, **c**, **d**),

Scheme 2. Click Reactions of 1 with a Variety of Azides (Fc = ferrocene)



[a]Isolated yield. [b]NMR yield.

oxidizable groups (**e**, **l**), potentially Cu-chelating substrates (**e**, **f**, **h**, **i**, **l**, **m**, **n**), and fluorophores (**m**, **n**).

With the substrate scope of **1** established, the chemoselectivity profile was explored in competition experiments using equal stoichiometries of **1** and **4** and a corresponding azide (Scheme 3a). Using benzyl azide (**2a**), full conversion to triazoles **3a** was observed with high selectivity for click adduct **3a** vs aliphatic alkyne adduct **5a** in both MeCN (100:0) and MeOH (90:10) (Scheme 3a (i)). Complete conversion was observed using cyclohexyl azide **2c** to form **3c**, in both MeCN and MeOH, which is likely due to the increased steric bulk of **2c** slowing the rate of the CuAAC reaction.

Based on these encouraging results, we investigated whether the enhanced reactivity of the *N*-ethynylbenzimidazole would be retained in an intramolecular context. To test this hypothesis, the bifunctional scaffold **6** was prepared (see synthesis in Supporting Information). Two azides of functional significance were chosen to test the chemoselectivity of *N*-ethynylbenzimidazole relative to the aliphatic alkyne both present in **6**. For example, ferrocene azide **2g** has been used extensively as an electrochemical reporter,[26−28] whereas the green dye **2m** is widely used as a fluorescent probe for lipid membranes.[29,30] Consistent with the intermolecular competition reactions (Scheme 3a), the reaction of **6** with **2g**, followed by **2m**, resulted in the exclusive formation of **7a** in 96% yield over the two steps. A workup procedure was conducted after

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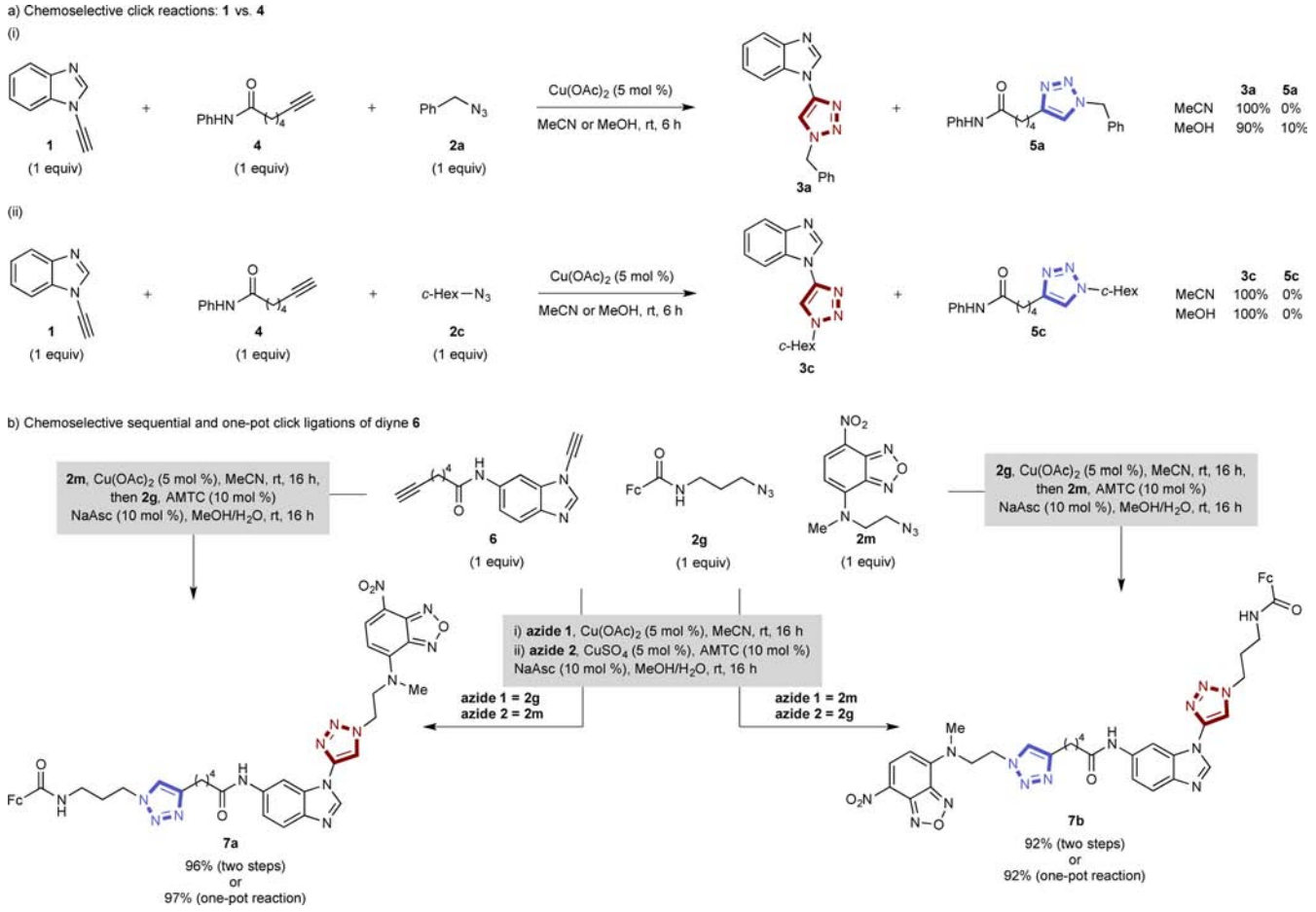
Scheme 3. (a) Intermolecular Competition Click Reactions Using 1 vs 4 and (i) Azide 2a and (ii) Azide 2c; (b) Chemoselective Sequential and One-Pot Click Reactions of Diyne 6



the first click reaction to confirm that the first reaction occurred exclusively at the *N*-ethynylbenzimidazole. Reversing the sequence of addition (i.e., **2m** followed by **2g**) produced the reverse click product **7b** in 92% yield, further exemplifying the flexibility of the sequential ligation approach. Finally, we showed that the formation of **7a** and **7b** was possible in a one-pot process, controlled simply by the sequence of addition of the corresponding azide. Exclusive formation of the first CuAAC reaction at the *N*-ethynylbenzimidazole was observed in both cases using 1 equiv of azide and $Cu(OAc)_2$. Addition of the second azide, ligand AMTC, and NaAsc resulted in the formation of **7a** and **7b** (respectively 97% and 92%).

In summary, we have demonstrated a modular, step-efficient, and robust sequential CuAAC-based ligation platform that exploits the inherent differences in the reactivity of *N*-ethynylbenzimidazole relative to aliphatic alkynes. Using the bifunctional system **6** we show that these reactivity differences enable discrimination of the two alkynes in a simple one-pot two-step procedure. We envisage that exploiting the reactivity differences of alkyne substrates could have the potential for utility in bioconjugation applications,[31] particularly where dual differential modification of biomolecules[32−34] or sequential modification[35−38] is required.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00635.

Experimental procedures, characterization data for all compounds (PDF)

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Notes

The authors declare no competing financial interest.

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