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

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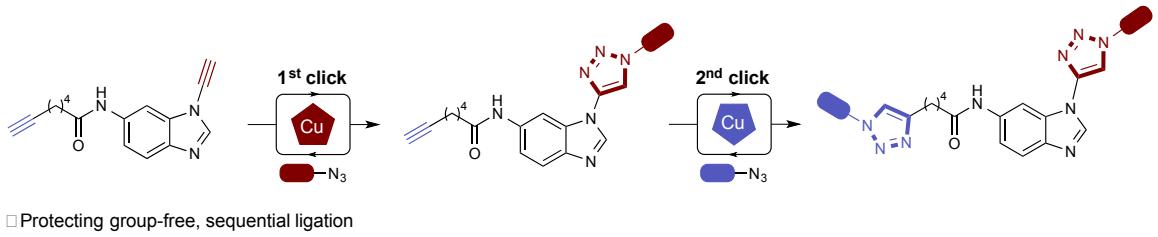
Supporting Information Placeholder



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 hetero-bifunctional 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 multi-functional 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 (Scheme 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 (Scheme 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 (Scheme 1c). Furthermore, we demonstrate the utility of our strategy as a novel platform for sequential ligation.

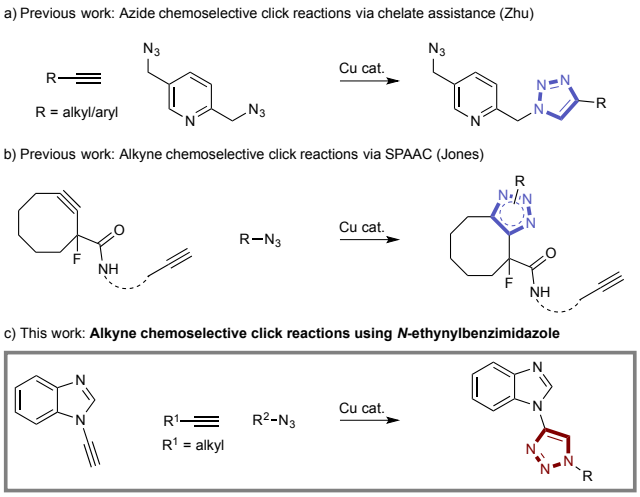
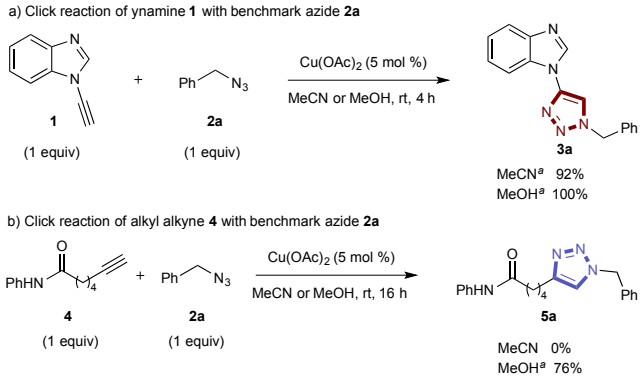


Figure 1. Protecting group-free chemoselective click reactions. a) Azide selectivity using chelate-assistance. b) Alkyne selectivity using SPAAC. c) Alkyne selectivity using the aromatic ynamine *N*-ethynylbenzimidazole.

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] Furthermore, terminal ynamide substrates such as *N*-tosylynamides have also been employed in CuAAC reactions and tandem variations,[22,23] although terminal *N*-ethynyl imidazolidinone *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 exist between **1** and **2a** corresponding 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 copper source, solvent, reductant and ligand were surveyed (see ESI). 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 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).

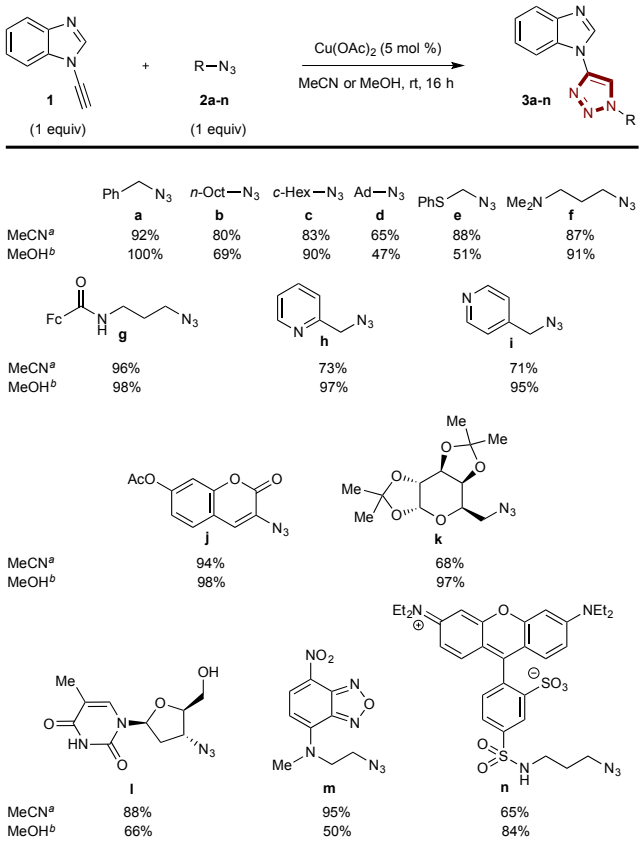
Scheme 1. Click reactions of **1** and aliphatic alkyne **4** with benzylazide **2a**. [a] Isolated yields.



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 16h. 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**), oxidizable groups (**e**, **l**), potentially Cu-chelating substrates (**e**, **f**, **h**, **i**, **l**, **m**, **n**), and fluorophores (**m**, **n**).

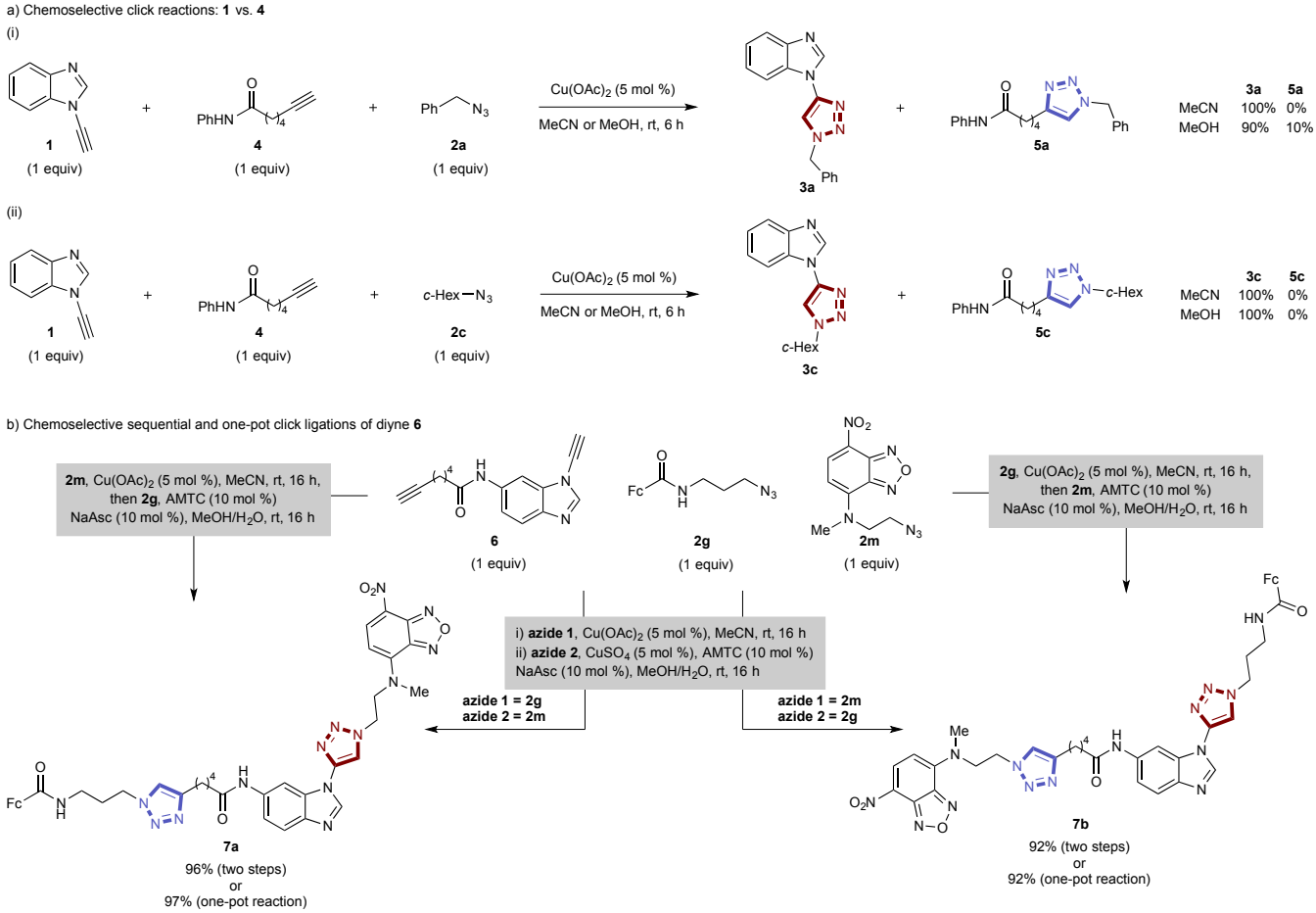
Scheme 2. Click reactions of **1** with a variety of azides. Fc = ferrocene. [a] Isolated yield. [b] NMR yield.



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 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).

Scheme 3. (a) Intermolecular competition click reactions using **1** vs. **4** and: (i) azide **2a**; (ii) azide **2c**. (b) Chemoselective sequential and one-pot click reactions of diyne **6**.



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 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%, 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 one equivalent 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

Experimental procedures, characterization data for al compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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