Organic LETTERS

Letter

pubs.acs.org/OrgLett

Cu-Click Compatible Triazabutadienes To Expand the Scope of Aryl Diazonium Ion Chemistry

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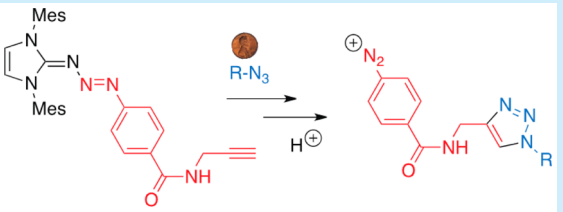
Supporting Information

ABSTRACT: Triazabutadienes can be used to readily generate reactive aryl diazonium ions under mild, physiologically relevant conditions. These conditions are compatible with a range of functionalities that do not tolerate traditional aryl diazonium ion generation. To increase the utility of this aryl diazonium ion releasing chemistry an alkyne-containing triazabutadiene was synthesized. The copper-catalyzed azide−alkyne cycloaddition ("Cu-click") reaction was utilized to modify the alkyne-containing triazabutadiene and shown to be compatible with the nitrogen-rich triazabutadiene. One of the triazole products was tethered to a fluorophore, thus enabling the direct fluorescent labeling of a model protein.



The azobenzene-forming reaction between aryl diazonium ions and electron-rich aryl side chains of proteins has been well established, but remains an underutilized chemistry in the realm of bioconjugation chemistry.[1,2] There have been several approaches to make these species more generalizable in the context of what can be conjugated to a protein, but all of these approaches require a second, and sometimes third, post-azobenzene formation reaction (Figure 1a).[3−6] The inherent challenge with aryl diazonium ions in chemical biology is that they are prone to pH-insensitive degradation to the corresponding aryl cation,[7] or an aryl radical if an appropriate reducing agent is present.[8] This limitation prompted our recent report of triazabutadienes that serve as masked forms of aryl diazonium ions (Figure 1b).[9] While other systems (such as triazenes,[10] aryl diazotates,[11] azo sulfides[12]) mask aryl diazonium ions, the triazabutadiene offers bench stability while maintaining exquisite susceptibility to release the reactive species in mild pH ranges. The advances in understanding the reactivity of triazabutadienes has generated a need for synthetic strategies geared toward augmenting their applicability in chemical biology and beyond. Without a doubt the most widespread ligation chemistry in the past decade has been the copper catalyzed Huisgen cycloaddition between azides and alkynes, "Cu-click."[13] For this reason, our lab focused efforts toward the synthesis of triazabutadienes with an appropriate reactive handle, and on the demonstration of the cross compatibility of triazabutadienes with click chemistry to append chemical cargo to the triazabutadiene scaffold (Figure 1c). Herein we report the synthesis of an alkyne-containing triazabutadiene and subsequent click reaction between it and several model azides. One triazole product is a fluorophore, and that compound is further used to label a model protein in a pH-dependent manner.

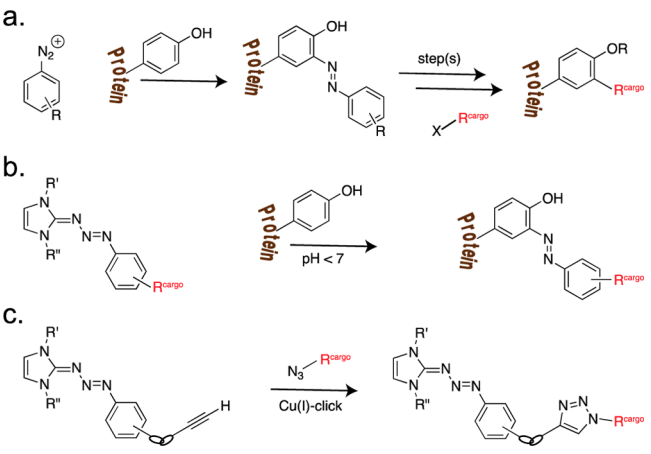


Figure 1. (a) Aryl diazonium ions react with tyrosine to form azobenzene compounds. These can undergo subsequent reactions to append chemical/biochemical cargos. (b) Triazabutadienes release aryl diazonium ions under mild conditions to enable preloading of the aryl diazonium ion with a cargo. (c) The work presented herein comprises a system where Cu-click chemistry is used to modify a common triazabutadiene intermediate at a late synthetic stage to maximize cross-compatibility.

Triazabutadienes of the type shown in Figure 1b release an aryl diazonium ion upon protonation, and their basicity leads to the reaction working with mild acids. As a result of this Brønsted−Lowry acid promoted reactivity, we were concerned at the onset that the sensitive triazabutadiene functionality would not survive Cu-click conditions. Furthermore, the nitrogen-rich scaffold looks similar to metal ligands and similar triazabutadienes have been shown to form adducts with

Received: August 12, 2016

DOI: 10.1021/acs.orglett.6b02420
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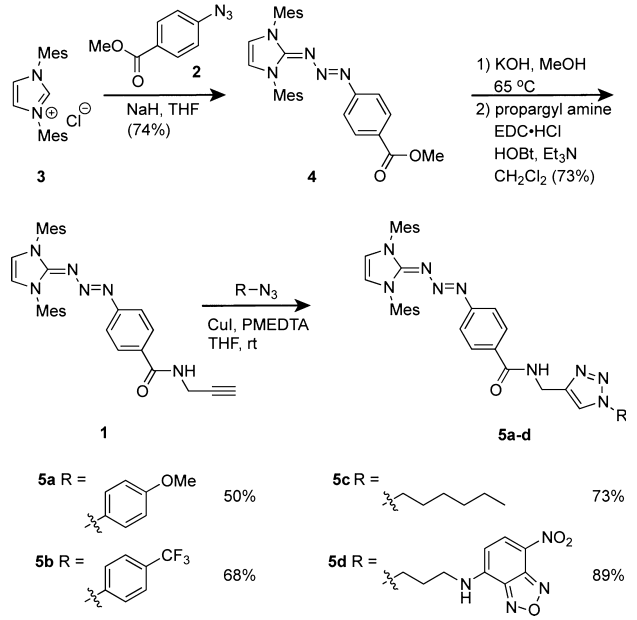
lanthanides.[14] Moreover, Cu-click reactions are often heated to further accelerate the reaction, but both Fanghänel and Bielawski have reported that these moieties can undergo a nitrogen extruding rearrangement at elevated temperatures.[15,16]

When deciding upon the best connection between the triazabutadiene and alkyne, we sought a linkage that would have minimal electronic perturbations on the simple benzene that we have studied extensively. This led to the decision that an alkyne linked via an amide to the masked aryl diazonium would be suitable. In the event, the synthesis of triazabutadiene **1** began with the coupling of methyl-4-azidobenzoate (**2**) to 1,3-bismesitylimidizolium chloride (**3**) to provide triazabutadiene **4** (Scheme 1). Hydrolysis of the ester proceeded to provide a

Scheme 1. Synthesis and Reactivity of Alkynyl Triazabutadiene 1

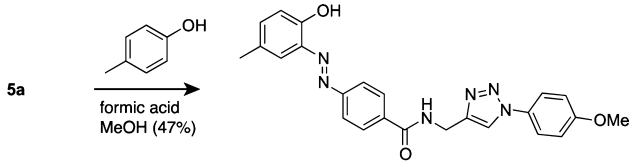


potassium salt. This salt was used without purification in the carbodiimide coupling reaction with propargyl amine to provide **1** in good yield. The bismesityl-containing triazabutadiene could be purified by traditional silica gel column chromatography, a nontrivial feature compared to our earlier work with water-soluble triazabutadienes.[9]

Moving forward to assess the ability of the triazabutadiene to survive Cu-click conditions, we considered three model azides, 4-methoxyazidobenzene, 4-trifluoromethylazidobenzene, and *n*-hexylazide to provide triazoles **5a–c** (respectively, Scheme 1). Based on consumption of starting material and crude analysis these reactions proceeded well, but the yield suffered slightly upon purification. Underscoring this fact, when the *N*-nitrobenzoxadiazole (NBD) fluorophore was clicked onto **1** the resulting triazole, **5d**, was obtained in good yield because it was purified by simple trituration. This particular compound was of particular interest because it allowed for the first time a one-step bioconjugation of a fluorophore to proteins (or peptides) via aryl diazonium chemistry. It should also be noted that these transformations represent the most extensive modifications of the intact triazabutadiene motif to date.

To confirm that the triazabutadiene functional group was intact and retained its acid sensitive character post-Cu-click, **5a** was treated with acid in the presence of *p*-cresol (Scheme 2).

Scheme 2. Synthesis of Azobenzene 6 from Acid-Treated Triazabutadiene 5a and *p*-Cresol



The reaction proceeded to release an aryl diazonium ion that went on to undergo conventional aryl diazonium chemistry and provide azobenzene **6**. While the bismesityl-containing triazabutadiene offered relative ease of purification, we observed that these compounds exhibited poor overall solubility in many solvents and were practically insoluble in water. This latter fact presented challenges when selecting a biochemical substrate to react with the fluorophore, **5d**.

To assess the utility of **5d** for protein conjugation, bovine serum albumin (BSA) was chosen as a model protein due in large part to its propensity to solubilize small molecules (Figure 2a). Prior to labeling with **5d**, the disulfide bonds of BSA were

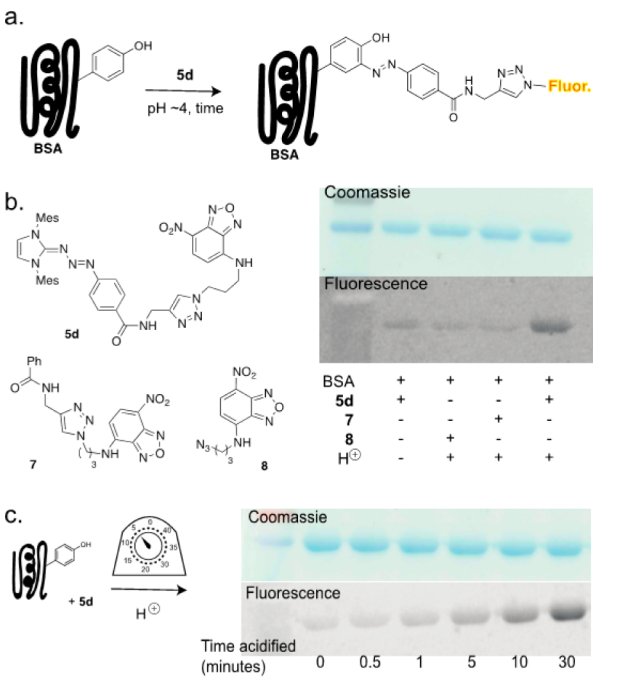


Figure 2. (a) Fluorescently labeled triazabutadiene **5d** was used to modify BSA. (b) A solution of BSA (25 μM) was treated with various fluorophore conjugates (100 μM) for 30 min at pH 4. Minimal background labeling arose from controls **7** or **8** as compared with the nonacidified trial (lane 1). (c) Labeling of BSA with **5d** showed a time-dependent increase in labeling. The far left lanes of the gels show part of the prestained protein ladder.

reduced with dithiolthreitol (DTT) and the resulting thiols were alkylated with iodoacetamide so as to minimize nonspecific side reactivity.[17] In the event, when a solution of BSA was treated with **5d** a pH-dependent labeling was observed (Figure 2b).[18] It had been previously established that pH 4.5 was optimal for tyrosine-selective chemistry, presumably due to protonation of the other potential partners, histidine and lysine.[19] As controls for acid-induced protein labeling that was

DOI: 10.1021/acs.orglett.6b02420
Org. Lett. XXXX, XXX, XXX–XXX

not aryl diazonium ion dependent, BSA was treated with both triazole **7** and azido-fluorophore **8**. As expected, labeling of BSA did not occur with the control compounds.[20] We evaluated the role of labeling time on the BSA and saw a clear increase of fluorescence intensity up to 30 min of treatment with acidic media (Figure 2c). At each time point the reactions were quenched adding a large excess of resorcinol to react with remaining free aryl diazonium ions and subsequently neutralizing the solution to arrest release of additional aryl diazonium ions. This timing trend is consistent with our understanding of pH dependent release of the aryl diazonium ions that occurs over several minutes and the subsequent rapid reaction between that ion with tyrosine.[9]

We reported a new triazabutadiene scaffold that enables us, and others, to utilize the power of Cu-click chemistry to perform late-stage functionalization reactions on this uniquely reactive moiety. The key questions and concerns of triazabutadiene compatibility with Cu-click chemistry were address, and we went on to show the utility of such a strategy by enabling the direct conjugation of a fluorophore to a model protein. We expect that this chemistry will broaden the applicability of the use of triazabutadienes as masked aryl diazonium ions and offer chemists and chemical biologists more options when designing coupling strategies.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02420.

Protocols for the synthesis and characterization of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Jean-Laurent Blanche for assistance with synthesis and Lindsay Guzman for assistance with revisions. This work was supported in part by an NSF-CAREER award to J.C.J. (CHE-1552568).

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(18) It should be noted that other proteins that were tried, such as lysozyme, did not label efficiently due to the poor water solubility of **5d**. Our working hypothesis is that BSA did indeed help to solubilize the triazabutadiene. The labeling observed was evidence of the aryl diazonium ion formation, but cannot be solely attributed to tyrosine because other adducts (such as triazenes) can form, albeit reversibly.

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(20) We observed a low level of background labeling with all compounds. This background can be attributed to the ability of hydrophobic pockets on BSA binding to small molecules.

DOI: 10.1021/acs.orglett.6b02420
Org. Lett. XXXX, XXX, XXX−XXX