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"Click" Reaction in Conjunction with Diazeniumdiolate Chemistry: Developing High-Load Nitric Oxide Donors

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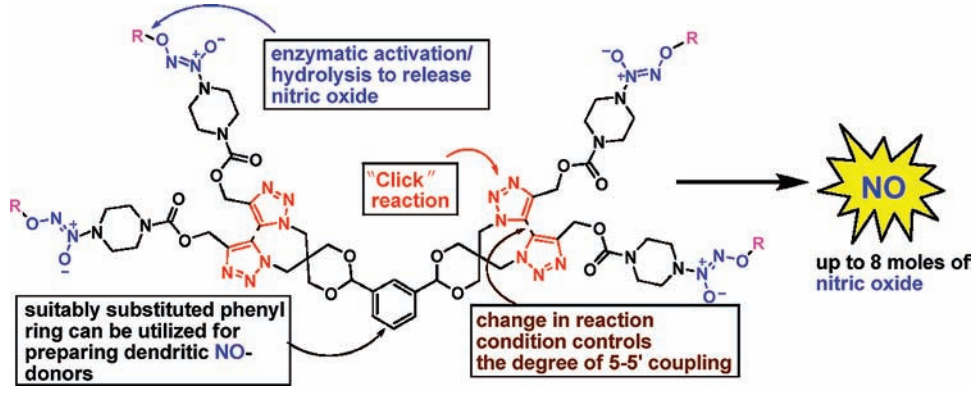
ABSTRACT



The use of Cu(I)-catalyzed "click" reactions of alkyne-substituted diazeniumdiolate prodrugs with bis- and tetrakis-azido compounds is described. The "click" reaction for the bis-azide using $CuSO_4$/Na-ascorbate predominantly gave the expected bis-triazole. However, CuI/diisopropylethylamine predominantly gave uncommon triazolo-triazole products as a result of oxidative coupling. Neither set of "click" conditions showed evidence of compromising the integrity of the diazeniumdiolate groups. The chemistry developed has applications in the synthesis of polyvalent and dendritic nitric oxide donors.

Diazeniumdiolate prodrugs[1] are efficient and reliable sources of nitric oxide (NO), a potent bioregulatory agent.[2,3] These prodrugs on suitable hydrolysis or enzymatic activation rapidly decompose to release NO at physiological pH. Targeted NO-releasing prodrugs have potential applications in treating cancer and other diseases. For example, JS-K (**1**, Figure 1) is an anticancer lead compound activated by gluthathione. JS-K (**1**) slowed tumor growth in several rodent models of cancer including leukemia, prostate cancer, multiple myeloma, and liver cancer.[4] Another prodrug, V-PYRRO/NO (**2**, Figure 1), is activated by cytochrome P450 to release NO. It shows hepatoprotective properties against a variety of toxins in several animal models.[5] Glycosidase activated *N*-acetyl glucosaminylated diazeniumdiolate GlcNAc-DEA/NO (**3**) significantly decreased *Leishmania major* parasite burden in infected macrophages.[6]

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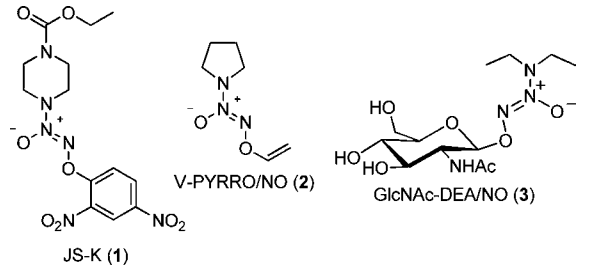

Figure 1. Structures of NO-releasing prodrugs JS-K (**1**), V-PYRRO/NO (**2**), and GlcNAc-DEA/NO (**3**).

The 2,4-dinitrophenyl, vinyl, and GlcNAc protecting groups are well studied for their trigger mechanisms to release NO from diazeniumdiolates.

Our laboratory is involved in NO-drug development mainly aimed at increasing the potency of these prodrugs[7] and their effective site-directed delivery of NO. Some strategies we employ to achieve these ends are increasing the payload of NO per mole of prodrug (high-load NO-donors) and conjugating our NO-prodrugs with other biologically significant molecules. However, many of these prodrugs decompose under certain reaction conditions, thus limiting the scope of suitable reagents/reaction conditions. The Cu(I)-catalyzed azide−alkyne cycloaddition ("click") reaction, independently discovered by the Sharpless[8] and Meldal[9] groups, involves mild reaction conditions. They are popular in medicinal chemistry because of their ease of execution, functional group tolerance, and potential to produce a library of compounds.[10] Therefore the "click" reaction was an attractive method to achieve the above-mentioned properties in NO-drug development.

The "click" reaction between a suitably functionalized diazeniumdiolate prodrug having a terminal alkyne group and a polyazide can lead to multivalency and increased payload of NO. We planned to investigate the reaction of a bis-azide with the alkyne group attached to the diazeniumdiolate prodrug. Finn and co-workers reported mechanistic studies and reactivities of bis-azides in ligand-free Cu(I)-catalyzed "click" reactions.[11] The report suggests that conformationally constrained 1,3-bis-azides **4** and **5** preferably form bis-triazole derivatives **6** and **7**, respectively, over less hindered **8** and **9** (Figure 2), even if a 10-fold excess of bis-azide over alkyne was used. In the case of **4** and **5**, formation of the first triazole ring catalyzes the subsequent cycloaddition to give the required bis-triazole predominantly.[11]

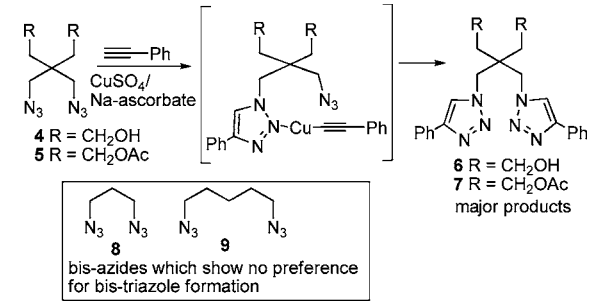

Figure 2. Preferential bis-triazole formation by hindered bis-azides **4** and **5** (ref 11).

We envisioned that addition of benzylidene protection to 2,2-di(azidomethyl)propane-1,3-diol **4** would further add to conformational strain in the system and hence lead to preferential bis-triazole formation. Furthermore, suitably substituted benzylidenes have potential applications in synthesis of dendritic azides. Thus, the diol **4** was transformed into bis-azide **10** and tetrakis-azide **11** (Figure 3) (details in the Supporting Information).

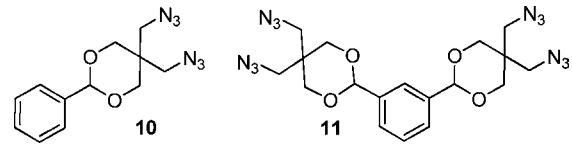

Figure 3. Structures of bis-azide **10** and tetrakis-azide **11** synthesized for the "click" reaction.

The diazeniumdiolate prodrugs **12**−**15** with terminal alkynes are shown in Figure 4 (details of their synthesis and

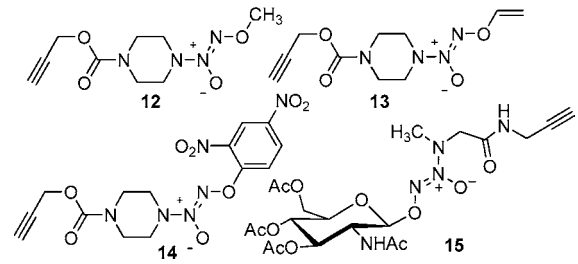

Figure 4. Diazeniumdiolate prodrugs **12**−**15** with terminal alkyne groups synthesized for use in the "click" reaction.

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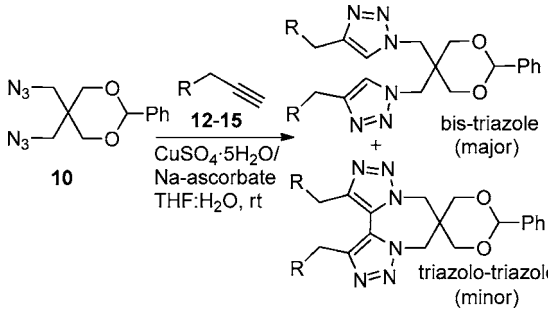
characterization in Supporting Information). These alkynes represent a sample of O^2-protected diazeniumdiolates with various modes of activation. Compound **14** is reported and has shown antiproliferative activity comparable to JS-K (**1**) against HL-60 and U-937 leukemia cell lines.[12]

The "click" reaction of the alkynes **12**−**15** with bis-azide **10** was performed using $CuSO_4$/Na-ascorbate in THF/water (Table 1). The reaction proceeded quickly (15−45 min).

Table 1. 1,3-Dipolar Cycloaddition Using $CuSO_4$/Na-Ascorbate for Bis-azide **10**[a]



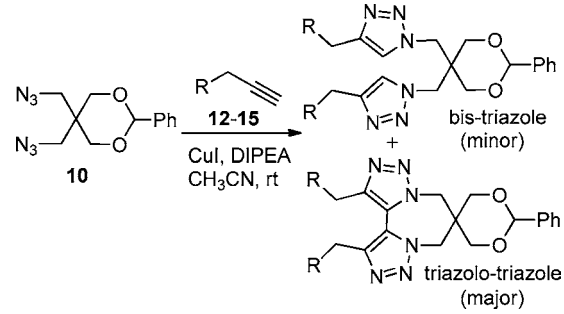
azide	alkyne	% yield (bis-triazole)	% yield (triazolo-triazole)
10	**12**	60 (**16**)	12 (**17**)
10	**13**	67 (**18**)	14 (**19**)
10	**14**	75 (**20**)	10 (**21**)
10	**15**	63 (**22**)	trace (**23**)

[a] Conditions: $CuSO_4$·$5H_2O$ (40 mol %)/Na-ascorbate (80 mol %), THF/H_2O (3:1), isolated product yields.

However, we observed formation of two products. The major product in each case was the expected bis-triazole. The minor product was 5,5′-triazolo-triazole. No formation of monotriazole product was observed.

Formation of such triazolo-triazoles is reported in the literature, but often as undesired and uncharacterized impurities.[13] They are a result of the oxidative coupling reaction of copper species after the triazole formation. To the best of our knowledge, there has only been one detailed report showing base dependence for the formation of triazolo-triazoles in an intermolecular cycloaddition reaction.[14] In the cycloaddition reaction of azides **10** and **11**, the benzylidene protection may bring the two newly formed triazole rings fairly close to enhance the formation of triazolo-triazole by oxidative coupling. These 5,5′-triazolo-triazoles remain under-explored heterocycles. Therefore, we attempted to synthesize triazolo-triazoles as the predominant product formed in the case of bis-azide **10**, as they may be good additions to heterocyclic linkers in medicinal chemistry. The reaction of alkynes **12**−**15** with **10** using CuI and diisopropylethylamine (DIPEA) as base predominantly gave the cycloaddition/oxidative coupling products (Table 2). Thus,

Table 2. 1,3-Dipolar Cycloaddition Using CuI/DIPEA for Bis-azide **10**[a]



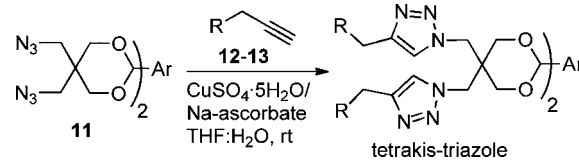
azide	alkyne	% yield (bis-triazole)	% yield (triazolo-triazole)
10	**12**	trace (**16**)	74 (**17**)
10	**13**	28 (**18**)	44 (**19**)
10	**14**	20 (**20**)	72 (**21**)
10	**15**	19 (**22**)	56 (**23**)

[a] Conditions: CuI (2 equiv)/DIPEA (2 equiv), isolated product yields.

steric congestion can be an additional factor for such oxidative dimerization reaction in Cu(I)-catalyzed cycloadditions.

In the case of the tetrakis-azide **11**, an additional partially coupled product (triazolo-triazole bis-triazole adduct) along with the expected tetrakis-triazole and bis(triazolo-triazole) may occur. To minimize the formation of these byproducts and avoid difficulties of their chromatographic separations, the 1,3-dipolar cycloadditions of tetrakis-azide **11** with alkynes **12** and **13** were carried out in the absence of oxygen using $CuSO_4$/Na-ascorbate (Table 3). Under nitrogen atmosphere, the cycloaddition reactions of **12** and **13** gave the desired tetrakis-triazoles **24** and **25**, respectively, in acceptable yields. Efforts are underway to make tetrakis-triazoles

Table 3. 1,3-Dipolar Cycloaddition Using $CuSO_4$/Na-Ascorbate for Tetrakis-azide **11**



azide	alkyne	% yield (tetrakis-triazole)
11	**12**	54 (**24**)
11	**13**	46 (**25**)

[a] Conditions: under N_2 reaction, $CuSO_4$·$5H_2O$ (80 mol %)/Na-ascorbate (160 mol %), THF/H_2O (3:1), isolated product yields.

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of diazeniumdiolates with 2,4-dinitrophenyl and GlcNAc protecting groups.

The hydrolysis or enzymatic activation to release NO for these types of prodrugs is well established in the literature.[4−7,12] However, the NO release profile for the bis-triazole-, triazolo-triazole-, and tetrakis-triazole-linked prodrugs may have differences in their kinetics. In addition, the steric effect on enzymatic activation, their intracellular NO-release, biological evaluation, toxicity, and physiological clearance of the linker[15,16] need to be investigated. Efforts are underway in these directions. If required, the phenyl ring of benzylidene protection in these compounds can be suitably substituted to improve their cell permeability and/or solubility.

Thus, the Cu-mediated "click" reaction was utilized to tether two and four molecules of NO-donor prodrugs together, which would serve to increase the payload of NO. The reaction conditions did not compromise the integrity of the diazeniumdiolate functional group, which is sensitive to certain other reaction conditions. The reactions showed formation of unusual oxidative coupling products, which were well characterized. Use of CuI/DIPEA in acetonitrile to carry out the dipolar cycloaddition preferentially formed the oxidatively coupled triazole adducts, leading to unexplored heterocyclic linkers for biological applications. These results underline the need to further investigate the role of steric congestion for the formation of such Cu-catalyzed oxidative coupling products. A proper substitution in the phenyl ring of the synthesized substrates along with a proper coupling reaction may allow the synthesis of well-defined dendritic NO-donors. These dendritic NO-donors can have potential applications in NO-releasing materials. The chemsitry established has potential applications in synthesizing hybrids of biologically important molecules and NO-donors. Efforts are underway for the synthesis of such NO-donor hybrids using the "click" reaction.

(15) The triazole linkers are considered inert to metabolic transformation. However, the toxicological profile or physiological clearance of the bis-triazole or triazolo-triazole linkers is not reported. The synthesis of such compounds makes them available for such studies.

(16) For a review on five-membered heterocyclic compound biotransformation, see: Dalvie, D. K.; Kalgutkar, A. S.; Khojasteh-Bakht, S. C.; Obach, R. S.; O'Donnell, J. P. *Chem. Res. Toxicol.* **2002**, *15*, 269–299.

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Supporting Information Available: Experimental details and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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