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Azide–Alkyne Cycloadditions

Mechanism of the Ligand-Free Cu^I-Catalyzed Azide–Alkyne Cycloaddition Reaction**

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The 1,3-dipolar cycloaddition of azides and alkynes^[1] is an effective way to make connections between structures that bear a wide variety of functional groups.^[2] The process is strongly favored in thermodynamic terms, yet both azide and alkyne units are highly selective in their reactivity; they are inert to most chemical functionalities and are stable in wide ranges of solvents, temperatures, and pH values. The discovery of copper(I) catalysis of this process^[3] has opened a myriad of applications in bioconjugation,^[4] organic synthesis,^[5] materials and surface science,^[6] and combinatorial chemistry.^[7] Herein we report the results of the first experimental investigation of the mechanism of the fundamental reaction catalyzed by copper(I) species generated in situ from copper(II) and ascorbate.^[8] This “ligand-free” process, which lacks the heterocyclic chelates that have been found to accelerate the reaction,^[9] has been used in many situations and represents a good starting target for mechanistic study.

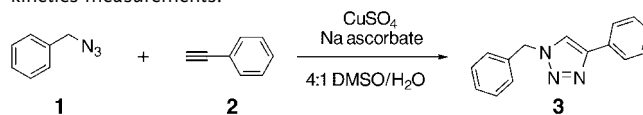
Pseudo-first-order (initial rate) kinetics experiments were performed on the reaction of benzyl azide (**1**) and phenylacetylene (**2**). Aliquots taken at intervals from the reactions by an automated liquid handler under inert atmosphere were quenched with H₂O₂. Quantitative LC–MS analysis of the resulting samples (Agilent Zorbax C18 column, MS detection in single-ion mode) allowed the collection of reliable kinetic data for reactions with concentrations of organic starting materials ranging from 0.4 to 100 mM. Examples of the data are shown in Figure 1, and the results are summarized in Table 1.^[10]

Under conditions of excess Cu^I (Table 1, entries 1, 4, 7), the experimental rate law was:

$$\text{rate} = k[\text{alkyne}]^{1.3 \pm 0.2}[\text{azide}]^{1 \pm 0.2}[\text{Cu}]^0.$$

With catalytic Cu^I under saturating conditions (Table 1, entries 2, 3, 5, 9; rate independent of [alkyne]), the reaction was second order in metal. A small amount of deuterated triazole product [D₇]**3**, prepared from perdeuterobenzyl azide, was added as an internal standard in some cases. The

Table 1: Observed rate orders in the variable component derived from kinetics measurements.^[a]

					
Entry	Component ^[b]	[PhC≡CH] [mM]	[BnN ₃] [mM]	[Cu] [mM]	Rate order
1	PhC≡CH ^[c]	0.4–1.0	0.04	10	1.3 ± 0.2
2	PhC≡CH	1–2.5	100	5	0
3	PhC≡CH ^[c]	10–25	1	0.1	0
4	BnN ₃ ^[c]	0.04	0.4–1	10	1.0 ± 0.2
5	BnN ₃	1	1–25	10	0
6	BnN ₃ ^[c]	1	1–25	0.1	–0.25 ± 0.1
7	Cu	1	1	1–10	0.1 ± 0.1
8	Cu	10	10	0.5–2	0.6 ± 0.2
9	Cu ^[c]	0.4	0.4	0.04–0.16	2.0 ± 0.1

[a] Reactions were performed at 20 ± 2 °C in DMSO/H₂O (4:1), [Na ascorbate] = 20 or 40 mM. Addition of organic reagents did not change the pH value of the solution. [b] Reaction component with varied initial concentration. [c] Performed with the addition of [D₇]**3** as internal standard.

presence or absence of [D₇]**3** made no difference in the observed rate law, except in the cases of catalytic copper. In the absence of supplemental triazole, the reaction rate increased more slowly than expected with increasing [Cu] (Figure 1c), suggesting that less-reactive species—presumably higher aggregates—accumulate at higher metal concentrations. The addition of 30 μM [D₇]**3** prevents this phenomenon, which permits the observation of a clean second-order dependence on [Cu] (Figure 1b). Benzyl azide was found to be slightly inhibitory when present in large excess over copper (Figure 1d–f and Table 1, entry 6), which reveals the presence of an unproductive Cu–azide association.

The kinetics results are consistent with a stepwise mechanism that involves a dynamically exchanging family of Cu–acetylide complexes.^[11] We propose the broad outline shown in Scheme 1. In the presence of excess copper, no catalyst turnover is required and all of the organic components are engaged with the metal, making the reaction zero order in Cu^I. Under these conditions, the reaction was found to be first order in azide, and between first and second order in alkyne. We have not yet determined if two pathways are operative (requiring one and two alkynes, respectively) or if the preferred catalytic mechanism involves two alkynes but is inhibited by excess alkyne, giving rise to the intermediate observed rate order. (While σ-acetylide–Cu complexes are shown in Scheme 1 and are proposed to be the active form,^[12] π complexes of alkynes may also play a role. Commercially available copper acetylides have so far proven to be ineffective in this reaction.)

It is envisioned that azide is activated by the appropriate Cu–acetylide; compound **4** represents one of many possible structures consistent with density functional theory calculations performed earlier.^[12] Formation of the resulting Cu–C bond-containing (triazole) species is then followed by proteolysis of the Cu–C bond to regenerate the catalyst.^[13] When the amount of azide and alkyne was increased to stoichiometric levels with respect to metal (Table 1, entry 5), the rate

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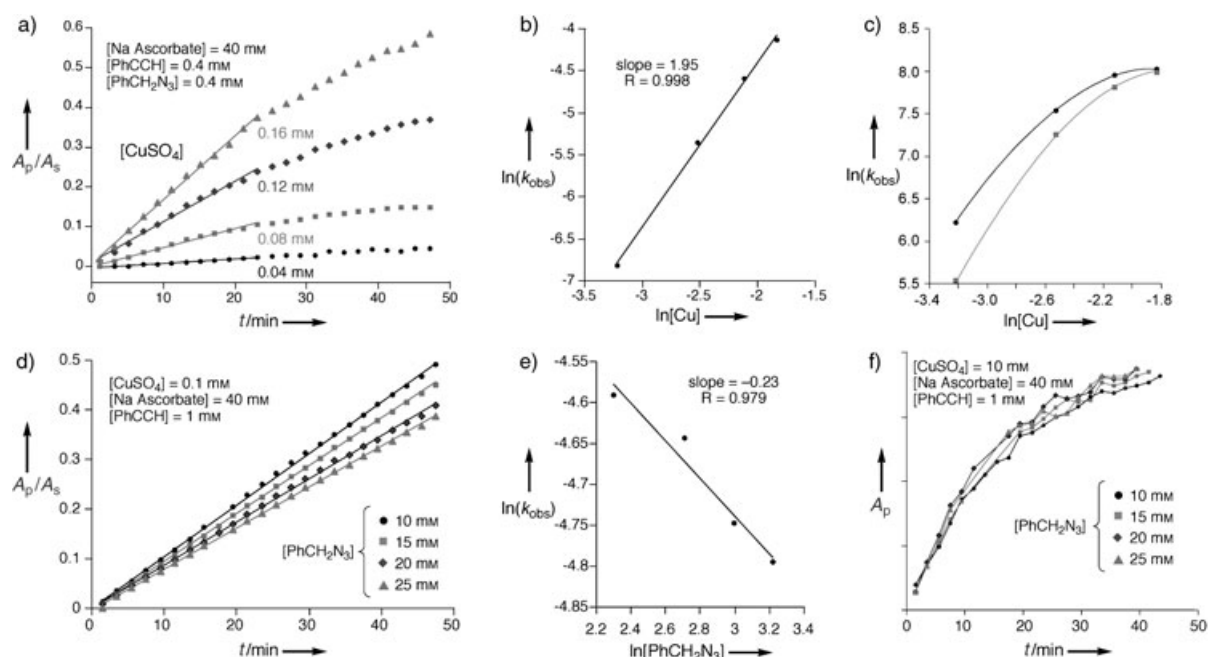
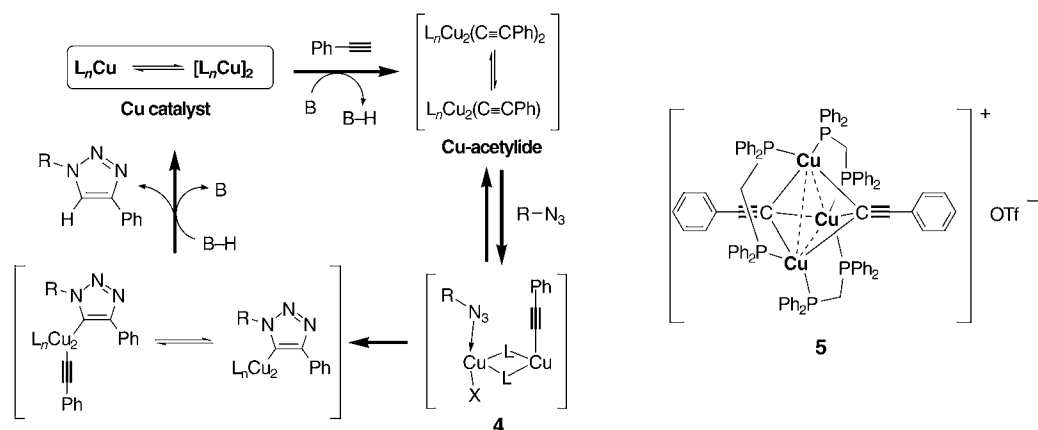


Figure 1. Examples of initial-rate kinetics measurements: a) data and b) rate-order plots showing second-order dependence on [Cu] under catalytic conditions; c) analogous to b), except that the two sets of reactions shown were performed without the deuterated product as internal standard; d) data and e) rate-order plots showing the inhibitory effect of large amounts of azide under catalytic conditions; f) data showing zero-order dependence on azide under noncatalytic conditions. A_p/A_s = peak area ratio of product/standard; A_p = peak area of product determined by single-ion mode LC-MS.



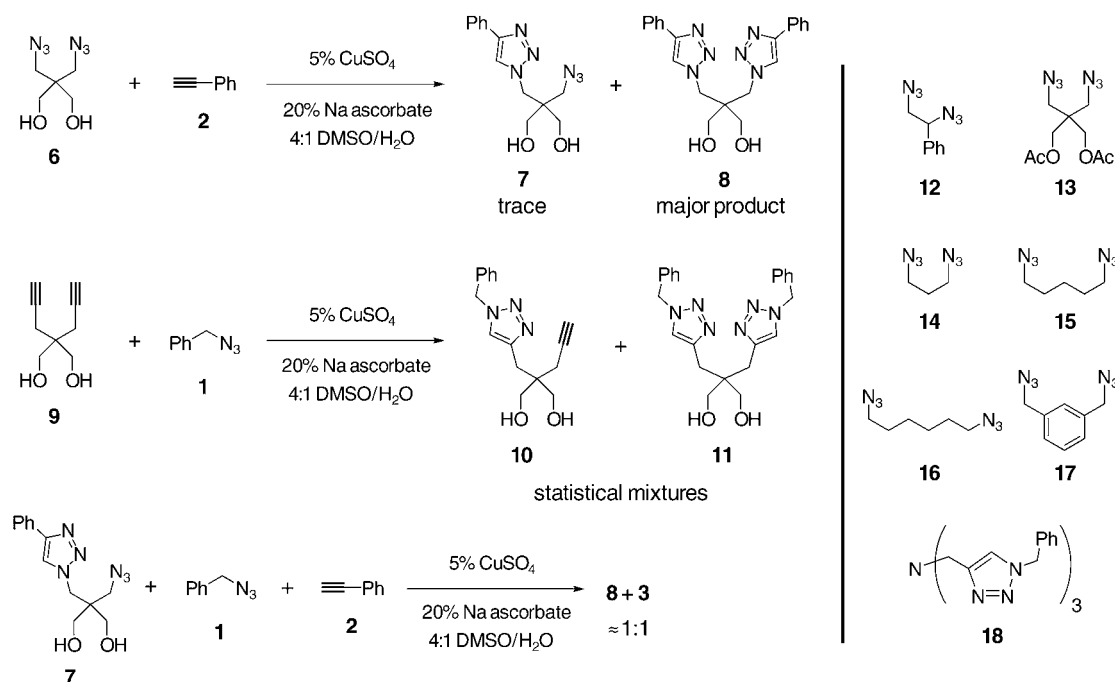
Scheme 1. Outline of species involved in the copper catalytic cycle, and the known trinuclear Cu-acetylide complex **5**.

order in azide was zero, consistent with an increase in the rate of capture by azide and a concomitant change in the rate-determining step to the formation of activated acetylide.

Under catalytic conditions at saturating levels (rates independent of [azide] and [alkyne]), the reaction was second order in copper,^[13] consistent with parallel observations for a highly active Cu-phenanthroline catalyst.^[9b] There are several possible explanations; perhaps the simplest is the activation of azide and alkyne by different metal centers as suggested in **4**. However, multinuclear Cu-acetylide species are common. One example, the known^[14] phenylacetylide cation $[\text{Cu}_3(\mu_3\text{-}\eta^1\text{-C}\equiv\text{CPh})_2(\mu\text{-dppm})_3]^+$ (**5**), prepared as the triflate salt, Scheme 1), was found to react with excess benzyl azide at room temperature to give clean **3** in 50% yield with

respect to total alkyne. *p*-Tolylacetylene was also incorporated into its respective 1,4-disubstituted triazole, but catalytic activity (>100% yield with respect to Cu) was not observed. The reactivities of other model Cu-acetylide complexes are currently being explored to test various mechanistic hypotheses. The details of the interaction of organic azide with monomeric Cu-acetylide have been explored by computational methods.^[12]

Further insight was gained by examination of the reactivities of diazides and dialkynes (Scheme 2). Compound **6** provided ditriazole **8** as the major product, even when 10 equivalents of diazide over alkyne were used, whereas the analogous dialkyne **9** gave statistically expected quantities of **10** and **11**. Of the six additional diazides shown in



Scheme 2. Comparison of diazide and dialkyne reactivity.

Scheme 2, only the 1,2 compound **12** and the 1,3 compound **13** (but not **14**) exhibited the same strong preference as **6** for the formation of ditriazole products.^[15] Therefore, the first triazole formed, and not a pendant hydroxy group, is the critical accelerating element. Conformational constraints present in **6** and **13**, but not **14**, are required to hold the triazole and the second azide in close proximity. The rates of reactions between **1** and **2** were not affected by the addition of **8**. Furthermore, cycloaddition reactions of **6** did not exhibit any features of autocatalysis. Thus, Cu-**8** complexes do not catalyze the process. When the accelerating ligand **18**^[4a] was added, the overall rate of disappearance of **6** was increased (reactions were complete within 20 min), but the ratio of **8** to **7** remained unchanged.

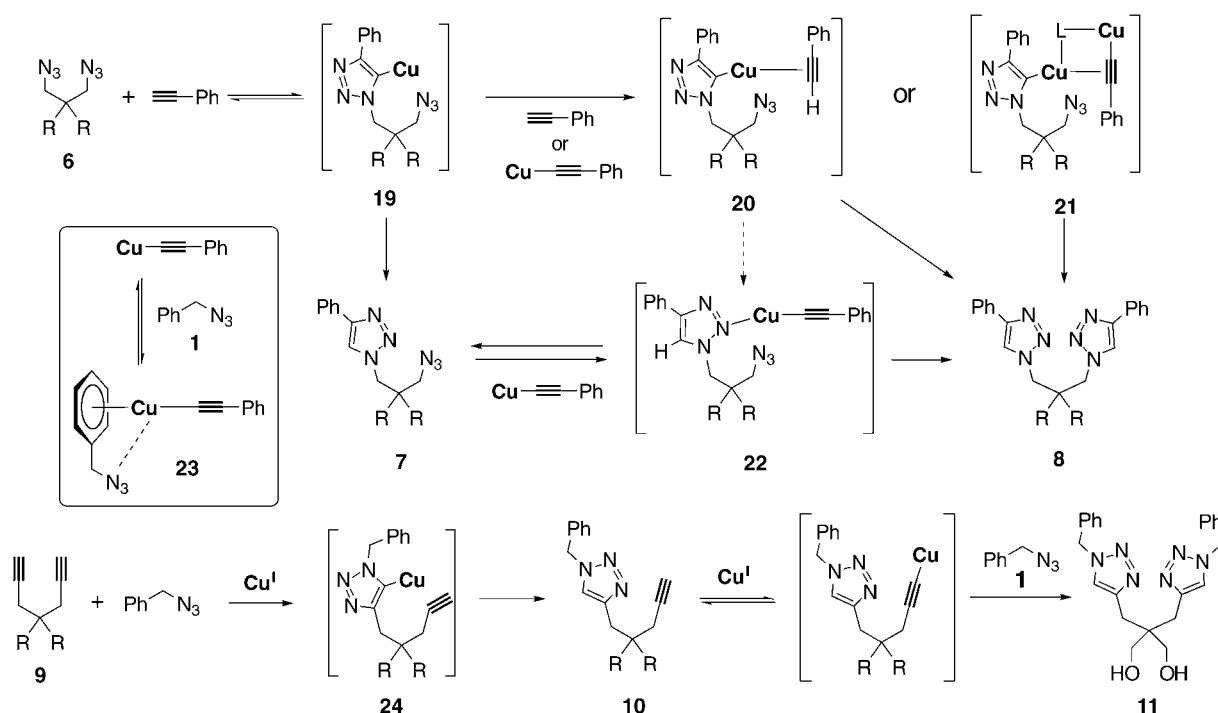
Reactions of **9** and **1** were carefully monitored. Product **10** initially built up, and then diminished to give **11** in a manner expected for sequential reactions of approximately the same rate. In contrast, the reaction of **6** with **2** was found to generate a small amount ($< 3 \text{ mol}\%$) of **7**, which persisted throughout the reaction. Compound **7** was prepared independently, and the rate of its disappearance in reaction with phenylacetylene was found to be significantly faster than that of **6** (complete reaction in 30 min for **7**, versus 30–40% completion in the same time for **6**). However, the rate difference was not large enough to account for the predominant formation of ditriazole **8** from diazide **6**, especially in the presence of a large excess of **6** relative to alkyne. Moreover, reactions of **2** with a mixture of **1** and **7** were found to proceed with consumption of the two azides at an approximately equal rate, and much more slowly than reactions of **2** and **7** in the absence of **1**. In other words, the reaction of azide **7** was inhibited by benzyl azide, but no increase in the amount of **7** was detected when benzyl azide was added to reactions of **6**. Therefore, free **7** is unlikely to be

an intermediate in the conversion of **6** into **8**. Rather, we suggest that the Cu-triazole organometallic precursor to **7** may be the active structure.

The conversion of **7** into **8** was slowed neither by toluene, nor when benzyl azide was used in the presence of a large excess of alkyne (a better ligand for Cu^I than the azide). These observations, along with the inhibitory behavior of benzyl azide under catalytic conditions in metal (Table 1, entry 6), suggest that benzyl azide binds reversibly to Cu^I centers through interactions with both the aromatic ring^[16] and azide group.^[12] This model is supported by a recent report of an inorganic Ru–N₃ moiety that participates in a cycloaddition reaction with alkynes.^[17]

A proposed series of steps consistent with the data obtained thus far is shown in Scheme 3. Diazide **6** is converted into the Cu–C bond-containing triazole intermediate **19**, from which free **7** can be obtained by proteolysis of the Cu–C bond.^[13] However, **19**, a Cu^I complex, can also bind alkyne^[18] to give a system of the form **20** or Cu-acetylide^[18a,19] to give **21**, with subsequent rapid intramolecular capture of the remaining azide to afford **8** without the intermediacy of free **7**, when conformational factors permit. The fact that the triazole unit of **7** can direct Cu-acetylide to the pendant azide (structure **22**) also serves to accelerate the reactions of **7** relative to **6**. The observed inhibition of the **7**→**8** conversion by added benzyl azide is proposed to result from competitive binding of active Cu-acetylide in a chelate such as structure **23**. Finally, the necessary intermediate **24** from dialkyne **9** cannot easily recruit a reaction partner, as Cu-azide coordination alone is too weak. The binding of Cu^I by the triazole moiety of **10** offers little advantage, as the Cu-alkyne interaction is rapid even without pendant-group assistance.

In summary, a mechanistic outline that requires the ordered interaction of two copper centers, with one or two



Scheme 3. Proposed steps in the transformations of **6** and **9** into mono- and ditriazoles.

alkyne/acetylide units and one azide, has been uncovered by kinetics measurements. For certain 1,2- and 1,3-diazides, the formation of the second triazole ring was found to be much faster than the first. An unusual inhibitory effect of benzyl azide was also observed, which suggests the presence of two pathways to ditriazole, one via free monotriazole and the other via a copper(I) organometallic intermediate. Much, of course, remains to be explained. For example, in addition to employing only one of its two acetylide fragments in cycloaddition, complex **5** was found to mediate the exclusive transformation of **6** into **7**, in remarkable contrast to all other systems examined so far. The most important unresolved issue is the precise nature of the putative binuclear copper system responsible for efficient catalysis—a question we are addressing with copper-chelating ligands. Preliminary measurements with ligand-accelerated catalysts are currently underway and show kinetic parameters similar to those described above. The requirement for two copper centers and the advantages of intramolecular positioning of azide groups are highlighted in the selective synthesis of cyclic peptide dimers, described in the accompanying paper.^[20] These principles also provide guidance for ongoing efforts in our laboratories to develop new ligands and self-accelerating (autocatalytic) systems.

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