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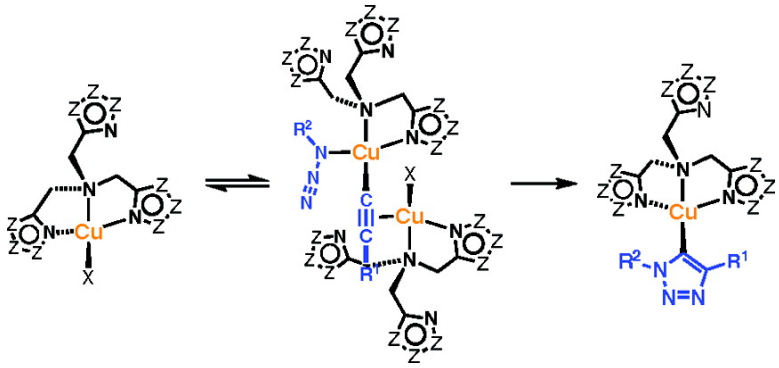

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Ligand-Accelerated Cu-Catalyzed Azide−Alkyne Cycloaddition: A Mechanistic Report

Valentin O. Rodionov, Stanislav I. Presolski, David Díaz Díaz, Valery V. Fokin, and M. G. Finn*

Contribution from the Department of Chemistry and The Skaggs Institute of Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037

Received April 17, 2007; E-mail: mgfinn@scripps.edu

Abstract: The experimental rate law for the Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction was found to vary in complex ways with concentration, the presence of chloride ion, and the presence of accelerating ligands. Several examples of discontinuous ("threshold behavior") kinetics were observed, along with a decidedly nonlinear correlation of electronic substituent parameter with the rate of CuAAC reaction with *p*-substituted arylazides. The previously observed tendency of the CuAAC reaction to provide ditriazoles from a conformationally constrained 1,3-diazide was found to be affected by a class of polybenzimidazole ligands introduced in the accompanying article. Various lines of evidence suggest that the standard tris(triazolylmethyl)amine ligand binds less strongly to Cu(I) than its benzimidazole analogues. On the basis of these observations, it is proposed that (a) a central nitrogen donor provides electron density at Cu(I) that assists the cycloaddition reaction, (b) the three-armed motif bearing relatively weakly coordinating heterocyclic ligands serves to bind the metal with sufficient strength while providing access to necessary coordination site(s), (c) at least two active catalysts or mechanisms are operative under the conditions studied, and (d) pendant acid or ester arms in the proper position can assist the reaction by speeding the protiolysis step that cleaves the Cu−C bond of a Cu·triazolyl intermediate.

Introduction

The preceding article in this issue describes the synthesis and utility of benzimidazole- and benzothiazole-based ligands for the Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction. Our previous study of the reaction mechanism[1] did not involve the use of accelerating ligands. Here we describe the results of kinetic studies involving representative examples of such ligands, along with an interpretation of the results that allows for predictive hypotheses to be made about the reaction mechanism.

Results and Discussion

Kinetic Rate Law in the Presence of Accelerating Ligands. We reported earlier that in the absence of added accelerating ligands the rate of CuAAC reaction displays second-order dependence on Cu (with rate independent of azide and alkyne concentration) under dilute catalytic conditions. Under single-turnover conditions, the rate had approximately first-order sensitivities to azide and alkyne concentration and was independent of [Cu].[1] These results are consistent with changes in the turnover-limiting step of a catalytic cycle in response to changes in the relative concentrations of the participating species. Table 1 shows the results of similar kinetic measurements performed in the presence of accelerating ligands (Figure 1).

In the presence of Tris buffer, we observed for the first time an experimental rate law in which catalyst, azide, and alkyne appear together with integer representation corresponding to the values observed in the absence of ligand (Table 1, entries 1−4):

$$\text{rate} = k[\text{BnN}_3]^1[\text{PhCCH}]^1[\text{Cu}\cdot\mathbf{(BimH)_3}]^2$$

In the absence of Tris buffer, however, the picture was more complicated. The copper complexes of benzimidazole derivatives **(BimH)$_3$** and **(BimC$_4$A)$_3$** exhibited a threshold behavior: below 50 μM in Cu and 100 μM in ligand, the catalysts gave only a few very rapid turnovers and then the reactions stopped (Supporting Information). At higher concentrations (100−250 μM), sustained and rapid reactivity was observed, but the rate for both Cu·[**(BimH)$_3$**]$_2$ and Cu·[**(BimC$_4$A)$_3$**]$_2$ systems increased only marginally with increasing catalyst concentration. (We have observed similar behavior for ligands **(BimC$_1$A)$_3$** and **(Py)$_3$** in the presence of HEPES buffer; data not shown.) Discontinuous kinetics was also observed for azide in the absence of Tris-HCl buffer, with a modest rate order dependence on azide concentration of approximately 0.4 at 10−20 mM, changing to concentration-independent catalysis above 40−50 mM (Table 1, entry 7). At lower catalyst loading, the reaction was zero-order in azide throughout the range tested (entry 11). The experimental rate order in alkyne was found to be approximately 0.6 (entries 8 and 12) at both 200 and 50 μM concentrations of Cu·[**(BimH)$_3$**]$_2$.

Since the pH of the reactions in the presence and absence of Tris-HCl buffer was made the same, the excess ascorbate serving

(1) Rodionov, V. O.; Fokin, V. V.; Finn, M. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 2210−2215.

Table 1. Experimental Rate Orders for Reactions in 4:1 DMSO/Aqueous Buffer, 25 mM Na Ascorbate, 24 ± 1 °C[a]

entry	aqueous component	[PhCCH] (mM)	[BnN_3] (mM)	[Cu^+] (mM)	ligand	varied reactant	rate order
1	Tris[b]	1	1	0.050−0.30	**(BimH)$_3$**	Cu^+/L_2	2.32 ± 0.3
2	Tris[b]	1	10−25	0.10	**(BimH)$_3$**	BnN_3	0.93 ± 0.2
3	Tris[b]	10−25	1	0.50	**(BimH)$_3$**	PhCCH	1.01 ± 0.2
4	Tris[b]	10−25	1	0.10	**(BimH)$_3$**	PhCCH	1.07 ± 0.2
5	water	1	1	0.01−0.25	**(BimH)$_3$**	Cu^+/L_2	see text
6	water	1	1	0.01−0.25	**(BimC$_4$A)$_3$**	Cu^+/L_2	see text
7	water	1	10−50	0.20	**(BimH)$_3$**	BnN_3	<0.4, see text
8	water	10−50	1	0.20	**(BimH)$_3$**	PhCCH	0.64 ± 0.2
9	KCl[c]	1	10−50	0.20	**(BimH)$_3$**	BnN_3	0.84 ± 0.2
10	KCl[c]	10−50	1	0.20	**(BimH)$_3$**	PhCCH	0.60 ± 0.2
11	water	1	10−50	0.050	**(BimH)$_3$**	BnN_3	0
12	water	10−50	1	0.050	**(BimH)$_3$**	PhCCH	0.58 ± 0.2
13	water	1	1	0.020−0.25	TBTA	Cu^+/L_2	1.01 ± 0.2
14	water	10−50	1	0.050	TBTA	PhCCH	−0.28 ± 0.2
15	water	1	10−50	0.050	TBTA	BnN_3	0

[a] Reactions were started by the addition of $CuSO_4$ solution, except in reactions employing varying amounts of Cu, which were started by adding ascorbate solution. Ligand abbreviations are explained in the caption to Figure 1. [b] Tris buffer containing 133 mM Tris base neutralized with 78 mM HCl, along with 25 mM sodium ascorbate; pH 8.0 ± 0.05 measured at room temperature. [c] 133 mM aqueous KCl, along with 25 mM sodium ascorbate; pH 8.0 ± 0.1 measured at room temperature.

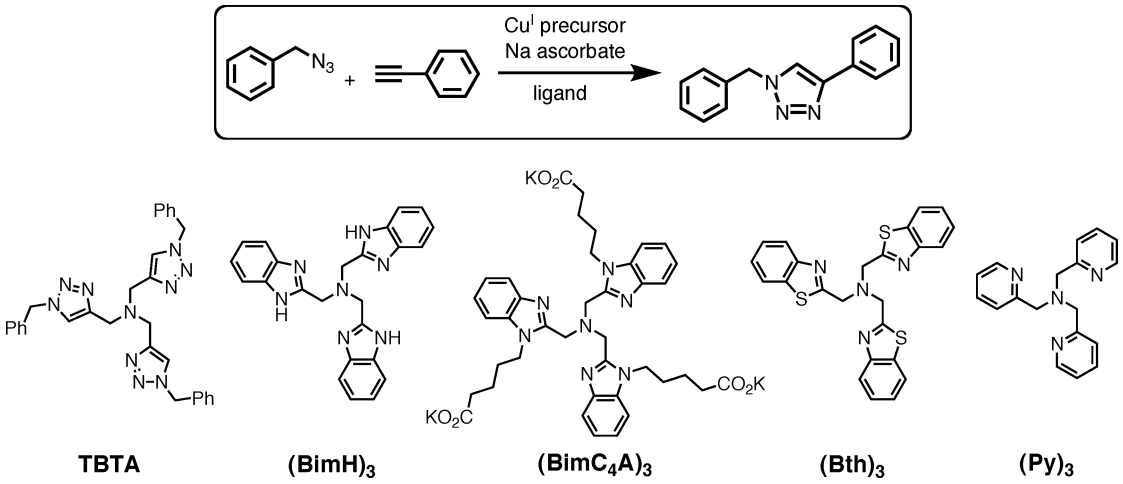

Figure 1. CuAAC reaction used in kinetics studies, and the structures of ligands mentioned in the text. For convenience, most ligands are designated by an abbreviation to represent structure, rather than by number, with the following conventions: BimR = benzimidazoylmethyl with N-substituent R, attached to amine N; Bth = benzothiazoylmethyl; C_nR = chain of *n* methylene groups terminated with R; E = CO_2Et; E′ = CO_2*t*-Bu; A = $CO_2^-K^+$.

as a low capacity buffer, the difference in kinetic profile under these conditions is most probably due to the presence of either the Tris base or the chloride counterion. The hindered primary amine group of Tris was not expected to be a strong ligand for Cu(I). Indeed, the addition of KCl inhibited the overall rate by approximately a factor of 3 and removed the nonlinear response of rate to azide concentration, giving a nearly unimolecular rate order in this reagent (Table 1, entry 9). The observed rate order in alkyne was unchanged in the presence and absence of the added salt (entry 10 *vs* 8). It therefore appears that the presence of high concentrations of chloride influences the distribution of catalytic species in solution. The sensitivity of rate order in azide, but not in alkyne, to chloride concentration suggests that Cl^- and RN_3 interact with copper(I) with similar affinities, whereas alkyne/acetylide is a much stronger ligand than chloride and is therefore insensitive to its presence.

The presence of more than one active species was also indicated by experiments in which [Cu] was held constant at 50 μM and [**(BimH)$_3$**] was increased from 0.4 to 5 equiv with respect to metal. While the steady-state second-order rate of these reactions did not change, an initial amount of rapid catalytic activity was again observed, which was inversely dependent on the amount of added ligand (Supporting Information). In other words, at a ligand/Cu ratio of 0.4:1, a faster initial reaction was observed, amounting to approximately 30 turnovers per metal compared to five turnovers at a 5:1 ligand/Cu ratio, before settling down to a constant rate. These observations echo the finding by reaction calorimetry described in the accompanying article of two catalytic modes: one that is suppressed by extra ligand and one that is not. At lower concentrations of substrate, the former appears to be only transiently available at the beginning of the reaction. The observation of nonlinear "threshold" behavior shows that the catalysts change their nature depending on overall concentration and suggests that aggregates are likely to be important.

A comparison of tris(triazolylmethyl)amine (TBTA) to tris(benzimidazolylmethyl)amine ligands under otherwise identical conditions (Table 1, entries 13 *vs* 5 and 6; 14 *vs* 12; 15 *vs* 11) showed the same zero-order insensitivity to [BnN_3], but different kinetic response to the other components. TBTA elicits none of the "threshold" performance in [CuL_2] observed with **(BimH)$_3$** or **(BimC$_4$A)$_3$**, and an apparent first-order dependence was observed in Cu for the first time (Table 1, entry 13). The rate dependence on Cu·TBTA in Tris buffer could not be

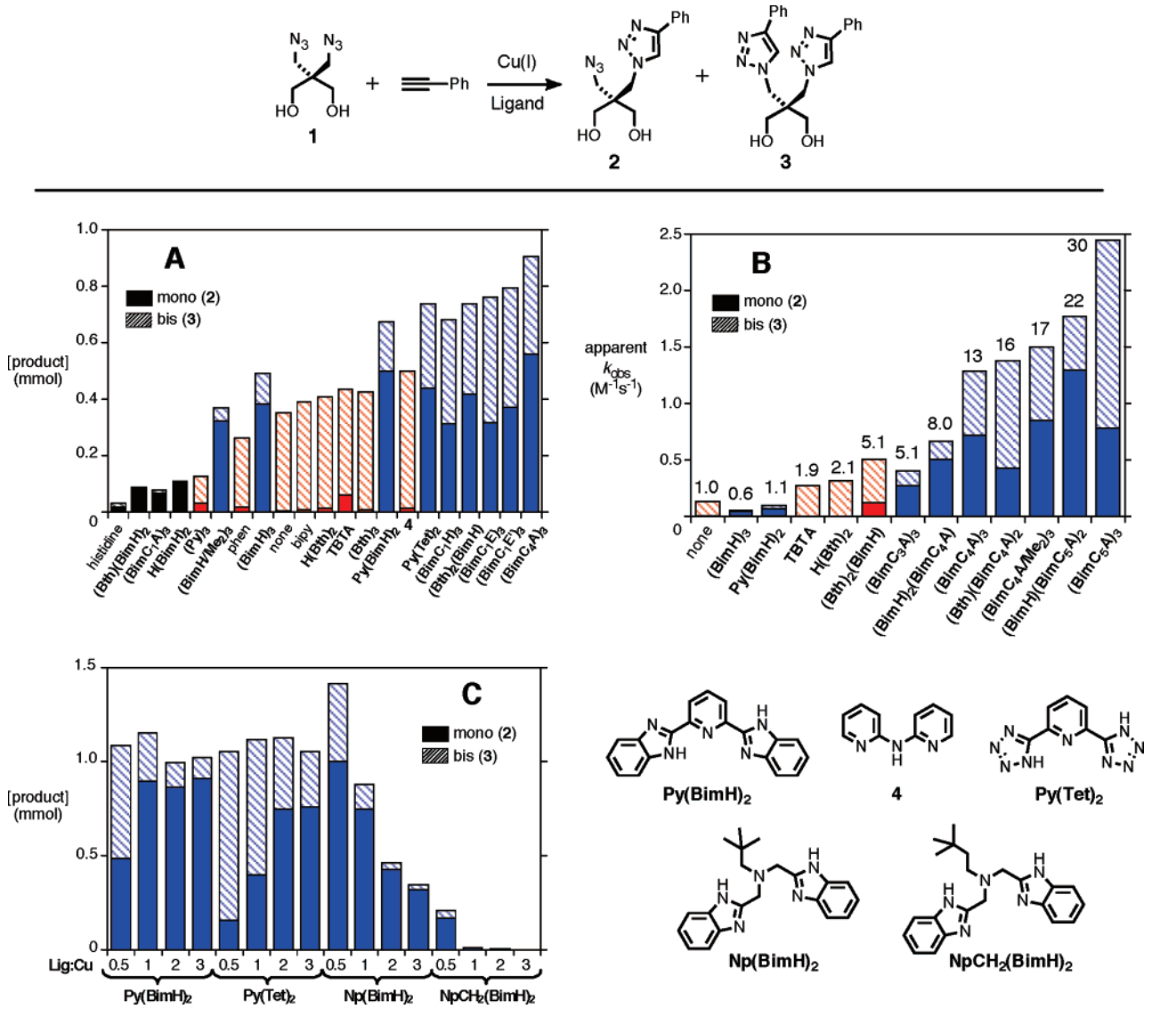

Figure 2. (A) Relative product concentrations for the reaction of **1** (2 mM) with phenylacetylene (2 mM) in the presence of 0.1 mM $CuSO_4$, 0.2 mM of the indicated ligands, and 20 mM Na ascorbate (4:1 DMSO/H_2O, room temperature) after approximately 30 h. Entries are arranged in order of relative consumption of alkyne. (B) Comparison of apparent second-order initial specific activities (rate constants at standard Cu concentration of 0.1 mM) for the production of **2** and **3** for the indicated Cu·ligand catalysts. Above each bar are the relative rates of overall consumption of alkyne, with the ligand-free reaction set at 1.0. (C) The same as part A, for different ligand/Cu ratios using the indicated four ligands, reaction time approximately 20 h. The three categories of ligands described in the text (classes I, II, and III) are shown in black, red, and blue, respectively. Experimental error for the great majority of values plotted here is ±15% or less; complete details are given in Supporting Information.

measured because the reaction was very slow. Furthermore, in the presence of TBTA, the CuAAC reaction was slightly inhibited by the addition of alkyne (rate order ≈ -0.3),[2] whereas the Cu·[**(BimH)$_3$**]-catalyzed process was accelerated (rate order in alkyne $\approx$ 0.6). These data are consistent with weaker coordination of the active metal center by the triazole ligand relative to the benzimidazole structure (see below). We therefore expect Cu·TBTA complexes to exchange more rapidly and to not get trapped in the kind of dead end that leads to the requirement for a threshold concentration. By the same token, we suggest that alkyne and acetylide may be able to displace TBTA from the metal center, thus resulting in the observed inhibitory behavior by loss of ligand-accelerated catalysis.

Ligand Effects on the Reactivity of 1,3-Diazides. An unusual phenomenon described in our previous report was the reactivity of 1,2- and conformationally constrained 1,3-diazides, most notably 2,2-bis(azidomethyl)propane-1,3-diol **1** (Figure 2).[1] The bistriazole **3** was found to be formed in preference to monotriazole **2**, even in the presence of excess diazide. The monotriazole was shown not to be an intermediate in the formation of the final product, and a highly reactive Cu·C(triazolyl) species was proposed. We also reported that TBTA, the only accelerating ligand in wide use at the time, did not have a significant effect on the outcome of the 1,3-diazide reaction.

We have observed that **(BimH)$_3$**, in contrast to TBTA, has a profound influence on the reaction of **1** with phenylacetylene,

(2) In our original article (ref 1), we noted a weak inhibitory property of benzyl azide. We have subsequently found that commercial benzyl azide frequently contains an unidentified trace impurity that either inhibits the CuAAC reaction or poisons the copper catalyst. No inhibitory effect was observed when carefully distilled benzyl azide was employed, and therefore our prior report is in error in this respect. Such distilled material was used in all experiments described in these articles.

giving rise to monotriazole as the major product (3.6:1 ratio of **2**/**3** for the starting 1:1 ratio of **1**/phenylacetylene). The results of a survey of 21 ligands in this process are summarized in Figure 2A, revealing the existence of three major categories of performance: (I) inhibitory ligands that provided an excess of monotriazole [**(Bth)(BimH)$_2$**, **(BimC$_1$A)$_3$**, **H(BimH)$_2$**], (II) ligands that affected neither the rate of the reaction nor the preference of the ligand-free process to give predominantly bistriazole [1,10-phenanthroline, 2,2′-bipyridine, **H(Bth)$_2$**, TBTA, **(Bth)$_3$**, **4**, and probably **(Py)$_3$**], and (III) accelerating ligands that gave rise to comparable amounts of mono- and bistriazole [**(BimH/Me)$_3$**, **(BimH)$_3$**, **Py(BimH)$_2$**, **Py(Tet)$_2$**, **(BimC$_1$H)$_3$**, **(Bth)$_2$BH**, **(BimC$_1$E)$_3$**, **(BimC$_1$E′)$_3$**, **(BimC$_4$A)$_3$**].

Because Figure 2A reports on the product distribution after a relatively long reaction time, we also measured the apparent initial rate constants for production of **2** and **3** involving a subset of class II ligands and additional examples of pendant alkylcarboxylate analogues of the benzimidazole family, all of which proved to be in class III (Figure 2B). With the exception of **(BimH)$_3$**, which initially provided almost exclusively monotriazole, the product selectivities for each catalyst did not vary a great deal over the course of the reaction. Figure 2B further shows that the overall rate of reaction was directly proportional to the length of the carboxylate arm: **(BimC$_5$A)$_3$** > **BimC$_4$A** derivatives > **(BimC$_3$A)$_3$**.

A single pathway process, in which monotriazole is an intermediate on the way to bistriazole, would give a 1.2:1 ratio of **2**/**3** if the rates of the two triazole-forming steps were equal (Supporting Information). The results from class III ligands are consistent with this model, suggesting that these ligands subvert the ditriazole-selective reaction in the absence of ligand by defeating an alternative reaction pathway that gives ditriazole selectively. Four ligands of the last class [**Py(BimH)$_2$**, **Py(Tet)$_2$**, **Np(BimH)$_2$**, and **NpCH$_2$(BimH)$_2$**] were tested in varying ligand/Cu ratios (Figure 2C). These were investigated because of earlier observations that 2,6-bis(heterocyclic)pyridyl and some bis(heterocyclic methyl)amines were inhibitors of the reaction. The addition of increasing amounts of each ligand slowed the reaction and produced less ditriazole, as would be expected from a stepwise process. It must be emphasized, however, that the reactions with small amounts of ligand (0.5 equiv relative to Cu) were significantly faster than those with no added ligand, even while the ditriazole selectivity was diminished. Ligand [**NpCH$_2$(BimH)$_2$**] was an exception, proving to be a potent and dose-dependent inhibitor of the CuAAC reaction of **1**. This dramatic difference upon the addition of a single methylene unit at the central nitrogen atom is discussed below.

Electronic Effects in Aromatic Azides. The participation of azide in the kinetic rate law and the kinetic requirement for two copper centers under most conditions suggest that a Cu−N$_3$R interaction may be significant. Indeed, the activation of organic azide by Cu(I) has been proposed to be a key feature of the favored pathway identified by ab initio calculations.[3,4] We therefore explored the sensitivity of the CuAAC reaction to changes in the electronic nature of aromatic azides, which are well-known participants in the process.[5] The *p*-substituted phenyl azide derivatives were prepared from *p*-substituted anilines by diazotization and azide trapping. They were found to be prone to decomposition upon standing under ambient light and even to some extent in the dark. In the absence of accelerating ligands, such decomposition is competitive with the CuAAC reactions. We therefore employed the parent tris(benzimidazole) **(BimH)$_3$** to speed up the desired cycloaddition reaction to obtain clean kinetics data for a substituent effect study, the results of which are shown in Figure 3.

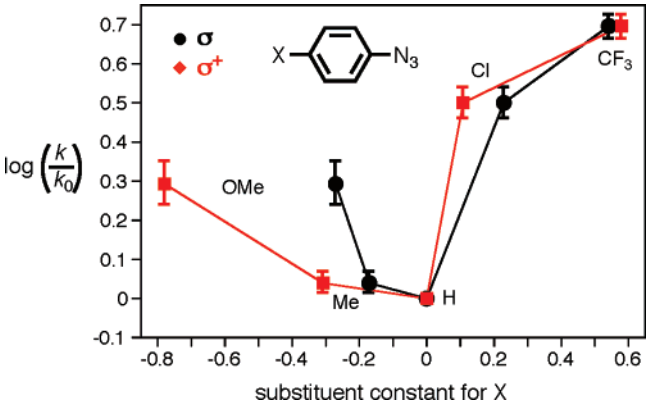

Figure 3. Rate *vs* substituent σ and σ^+ parameters for the CuAAC reaction of PhCCH (50 mM) with the indicated aromatic azides (2 mM) in the presence of ligand **(BimH)$_3$** (0.2 mM), $CuSO_4$ (0.1 mM), sodium ascorbate (25 mM), and 1-d_7-benzyl-4-phenyl-1,2,3-triazole as internal standard, in 4:1 *t*-BuOH/H_2O, at 25 °C. Aromatic azide was added last to start the reaction.

Second-order rate constants were determined in the presence of 5 mol % of the Cu·[**(BimH)$_3$**]$_2$ precatalyst mixture relative to azide, with minimal exposure of azide stock solutions and the reaction mixtures to light. The solvent used was *t*-BuOH/water, 4:1, instead of the usual DMSO/water mixture, to avoid the formation of unwanted photolysis products.[6] To obtain absolute concentrations, calibration curves were constructed using purified samples of each 1,4-triazole product. The kinetics reveal a bifurcation in the electronic dependence, with rate increasing for substituents both more electron-withdrawing and electron-donating than H or CH_3. A full exploration of the questions raised by this observation must await more complete kinetic measurements, such as extension to other substituents and determination of the experimental rate law at each extreme. But it is clear that either the mechanism or the turnover-limiting step of the catalytic cycle changes to some degree with variation in the electronic properties of the azide, perhaps unsurprising considering the sensitivity of the rate order in azide to changes in reaction conditions in the presence of the Cu·**(BimH)$_3$** catalyst described above. We believe that pre-equilibrium coordination of the organic azide to Cu may be important,[7] as is the committed kinetic step to form a Cu·triazolyl intermediate, and these may have divergent electronic demands.

Mechanistic Considerations. The CuAAC reaction catalytic cycle comprises the three general steps shown in Figure 4:

(3) Ahlquist, M.; Fokin, V. V. *Organometallics* **2007**, *26*, 4389−4391.
(4) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V. V.; Fokin, V. V.; Noodleman, L.; Sharpless, K. B. *J. Am. Chem. Soc.* **2005**, *127*, 210−216.
(5) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897−3899.
(6) We have observed that certain minor products of the photochemical decomposition of aromatic azides in DMSO act as potent accelerating ligands for CuAAC reactions of other aromatic azides, increasing rates by several orders of magnitude and producing unprecedented amounts of 1,5-triazoles for a copper-mediated process. These phenomena are currently being investigated and will be described in detail elsewhere. The catalytic photoproducts do not form in the presence of *tert*-butanol.
(7) Dias, H. V. R.; Polach, S. A.; Goh, S.-K.; Archibong, E. F.; Marynick, D. S. *Inorg. Chem.* **2000**, *39*, 3894−3901.

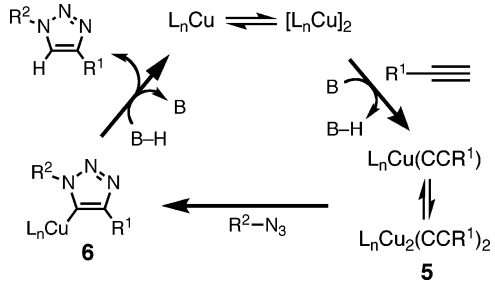

Figure 4. Major steps in the proposed catalytic cycle. Each Cu species can participate in equilibria with larger aggregates (not shown).

activation of terminal alkyne as Cu·acetylide **5**, formal cycloaddition to give a Cu·C(triazole) intermediate **6**,[8] and protiolysis of the Cu−C bond to give the triazole product and regenerate the catalyst. Each stage can involve multinuclear Cu species, a possibility made likely by the rich bridging coordination chemistry of Cu·acetylides.[9] Copper-binding ligands can conceivably affect the rates of each of these steps. The following discussion comprises our present informed speculation about the reaction mechanism that touches on the most interesting data obtained thus far and provide testable hypotheses for future work.

In the absence of chelating ligand, the rate of the CuAAC reaction of phenylacetylene with benzyl azide under catalytic conditions was found to be second order in copper and independent of azide and alkyne concentrations.[1] The observation is consistent with saturation of the metal center with azide and alkyne reactants, as might occur if the protiolysis of Cu·triazolyl **6**, rather than its formation, were turnover-limiting. In contrast, the presence of **(BimH)$_3$** gave rise to an experimental rate law involving both substrates and the Cu·ligand complex, suggesting that the expected competition by azide and alkyne for metal-binding sites becomes kinetically significant. This would be consistent with the formation of Cu·acetylide **5** and its cycloaddition to **6** being turnover-limiting. Therefore, the **(BimH)$_3$** ligand, among other effects, may make Cu·triazolyl hydrolysis faster, relieving a catalytic bottleneck by suppressing the buildup of **6**.

An important conceptual theme to emerge from these studies concerns the potential importance of binding affinities of ligand classes for the Cu(I) center, and the idea that weak binding can be advantageous. Rigid chelating ligands such as 2,2′-bipyridine and sulfonated bathophenanthroline are certainly effective for the CuAAC reaction, but are inhibitory when used in a greater than 2:1 ligand/Cu excess at low overall concentration, or at greater than 1:1 ligand/Cu at higher concentration. For bioconjugation applications, we have found a 2:1 ratio to be optimal, but the active catalyst is likely to involve a 1:1 ligand/Cu combination. As illustrated in Figure 5, the coordination of a second equivalent of ligand to Cu(I) diverts the metal into a catalytically inactive form, but a sufficient amount of the active 1:1 system can be accessible in the equilibrium mixture. The small amount of excess ligand serves the additional purpose of tying up Cu(II) ions (for which these ligands have a higher affinity than those for Cu(I)) that may form by adventitious oxidation or disproportionation, preventing Cu(II)-mediated degradation of protein or nucleic acid.

The conformationally flexible tris(benzimidazolylmethyl)-amines are less potent binders of Cu(I) than phenanthroline-based ligands, as shown by a competition between **(BimH)$_3$** and neocuproine **8**, a close analogue of the effective CuAAC ligand **7**. The addition of a large excess of **(BimH)$_3$** to a Cu·**8** mixture did not change the characteristic yellow color or electronic spectrum of the neocuproine complex; the Cu(I)·**(BimH)$_3$** complex is colorless. To the extent that protic basicity is correlated with binding affinity (and for Cu(I), such a correlation is likely to be only partially valid[10]), triazoles (pK_a of conjugate acid $\approx$ 0.1−1.0)[11] are expected to be the poorest binders, followed closely by benzothiazoles (p$K_a \approx$ 1.2),[12] and then by pyridines (p$K_a \approx$ 5.2) and benzimidazoles (p$K_a \approx$ 6.3).[13,14] All of these heterocycles are kinetically labile on Cu(I).

Multidentate interactions are therefore required for the imidazole, thiazole, and triazole ligands to have a high overall affinity for the metal, but open coordination sites are still available in such complexes because of two factors. First, the preferred tetrahedral coordination geometry of Cu(I), unlike that of Cu(II),[15−17] makes it difficult for all four donor atoms of ligands such as TBTA and **(BimR)$_3$** to bind the same metal ion, especially when an additional monodentate ligand is present.[18] In terms of the candidate mononuclear structures shown in Figure 6, this means that structures **9** and **11**, both of which have crystallographic precedent,[15] are much more likely for Cu(I) than structure **10** ($K_2 << K_1$). We further suggest that electron richness at the metal is helpful to the triazole-forming step, since analogous ligands lacking the central donor nitrogen atom do not provide for accelerated CuAAC reactions, even in the presence of added base. Therefore, structures with the central tertiary nitrogen bound (derived from **9**) should be much more catalytically active than those in which it is dissociated (derived from **11**). Second, monodentate binding by an arm of a second ligand is, like any monodentate version of these heterocycles, of relatively low affinity; in other words, structure **12** is not favored, and $K_3 << K_1$. Thus, many of the tris(heterocyclic methyl) amine ligands described here do not shut down catalysis even when used in large excess relative to the concentration of Cu (Figure 5 of the preceding article), in contrast to stronger-binding ligands such as phenanthroline. Coordinatively unsaturated intermediates such as **13** are relatively easily accessible in a kinetic if not a thermodynamic sense and are crucial to the success of the reaction.

The experimental rate law varies widely depending on the reaction conditions, suggesting both that the coordination

(8) An example of a Cu·C(triazole) intermediate obtained from a CuAAC reaction has been recently characterized by X-ray crystallography: Nolte, C.; Mayer, P.; Straub, B. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 2101−2103.
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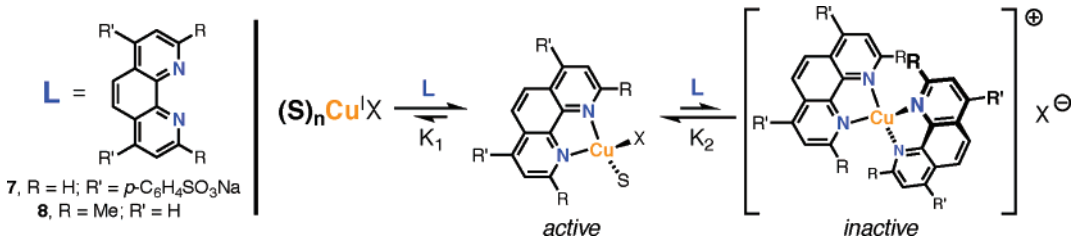

Figure 5. Simple coordination equilibria of bidentate ligands such as bipyridine and phenanthroline derivatives.

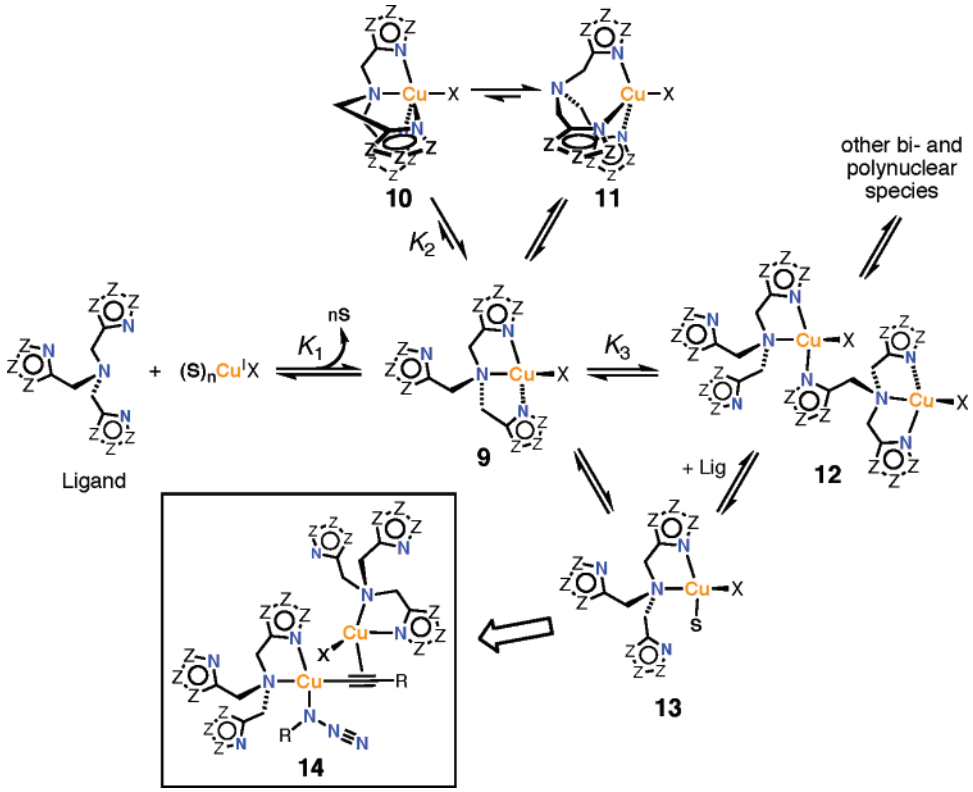

Figure 6. Potential coordination equilibria of tripodal ligands such as TBTA and **(BimH)$_3$** (S = solvent; X = halide, acetylide, hydroxide, triflate, or a neutral donor that leaves Cu with a positive charge).

chemistry of effective Cu·ligand complexes is diverse and that the rate-limiting step of the catalytic cycle may easily change. It may well be possible for a single copper center to activate alkyne and azide in an accelerated reaction; indeed, we report here one set of measurements that gives a first-order dependence in [Cu·TBTA]. However, we continue to find a bimolecular dependence on [Cu·ligand] under the most demanding conditions of low catalyst concentration, and we believe that this is the mode in which the most active systems operate. Such binuclear kinetics are consistent with the need for structures such as **14** (Figure 6), in which one Cu center assists the cycloaddition of acetylide and azide fragments bound to the other Cu center. The simultaneous σ,π-coordination of the acetylide is well-precedented,[9] and recent density functional theory calculations show this type of arrangement to be advantageous for the CuAAC process.[3] But whatever the particular arrangement of atoms in the key triazole-forming step, the liberation of a coordination site on each Cu atom by dissociation of *two* ligand arms (structure **13**) would appear to be necessary. We further suggest that the tripodal nature of such ligands as TBTA and **(BimR)$_3$** may keep the metal coordination chemistry "cleaner" by providing a high local concentration of weakly binding arms, while at the same time allowing access to open coordination sites. A similar situation was recently engineered for rhodium hydroformylation catalysis in a remarkably elegant design of a tetraphosphine ligand by Zhang and co-workers.[19]

It is noted in the accompanying article that benzothiazole-based ligands were highly active at room temperature in the presence of low substrate concentrations (1 to 2 mM) and relatively high catalyst loadings (0.1 mM Cu, 10 mol %), but not at 65 °C when substrate concentrations were increased (200−400 mM) much more than the catalyst (2 mM Cu, 0.5−1 mol %). Conversely, tris(2-pyridylmethyl)amine ligand **(Py)$_3$** gave a catalyst showing strong rate acceleration under the latter conditions, but rapid decomposition (after only a few turnovers) in the former situation. It seems likely that both thermodynamic (distribution of Cu complexes among active and inactive forms) and kinetic (ligand substitution rates) factors can contribute to such phenomena.

The sensitivity of the reaction to changes in the ligand that may affect the copper coordination sphere was unexpectedly

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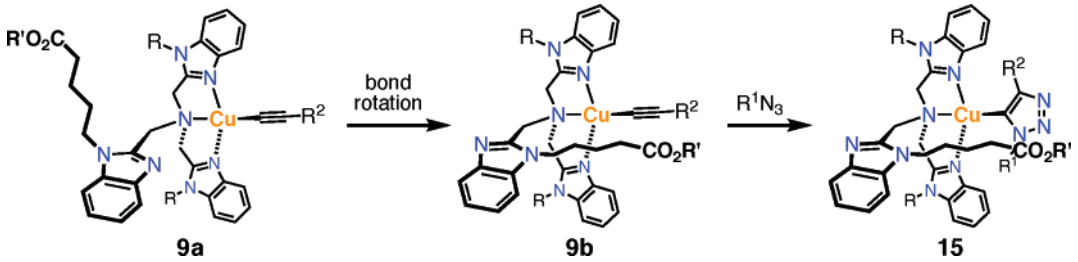

Figure 7. Ligand conformation that brings pendant alkylcarboxylate arms in the vicinity of the copper center.

illustrated by the comparison of **Np(BimH)$_2$** and **NpCH$_2$-(BimH)$_2$** ligands in Figure 2. Here, the addition of a single methylene unit dramatically *diminished* the reactivity of the catalyst. This is somewhat counterintuitive, since the neopentyl substituent of **Np(BimH)$_2$** would be expected to provide more steric hindrance than its neopentylmethyl homologue. Instead, we suspect that the extra length of the latter puts the *tert*-butyl group closer to the adjacent binding sites on the metal and therefore in a more likely position to disrupt an important interaction (such as with organic azide) or coordination geometry.

Variations on the **(BimR)$_3$** theme showed that ligands bearing *N*-alkylcarboxylic acid or ester groups with propyl, butyl, or pentyl tethers were superior under most conditions. These ligand arms have the capability to present polar functional groups close to the metal center, as shown in Figure 7 for structures such as acetylide complex **9**. Pendant groups such as acids[20−26] and esters may participate in the coordination chemistry of Cu^{I}, perhaps to the advantage of the CuAAC reaction.[27] Alternatively, the pendant group, by virtue of an active proton or hydrogen bonding to solvent water, may aid in the protiolysis of the Cu·monotriazolyl intermediate (**15**),[8] identified earlier as the putative key species in the rapid formation of bistriazole.[1] For each possibility, inspection of molecular models suggests that the pendant arms of butanoic, pentanoic, and hexanoic acid chains, but not propanoic acid, are long enough to reach the site of action, consistent with our observed structure−activity data.

Certain ligands were grouped into a category ("class II") that gave rise to no apparent rate acceleration and very little production of monotriazole **2** from diazide **1**; in other words, that made little difference relative to the "ligand-free" reaction. This is very curious, since several of these ligands (most notably TBTA, 1,10-phenanthroline, and 2,2′-bipyridine) are well-established as acceleratory ligands for the reaction of a variety of monoazides with alkynes. We find it difficult to believe that the 1,3-diazide **1** can compete effectively with all of the ligands in this class for binding to Cu, but the coordination of organic azide to Cu(I) may be more important than previously realized: the "dog-legged" Hammett plot of Figure 3 suggests as much. Still, the apparent insensitivity of the [**1** + phenylacetylene] reaction to the presence of class II ligands will require more study before we can propose even a tentative rationale for it.

Finally, a few remarks on the presence of sodium ascorbate are in order. We have chosen thus far to explore only those systems containing aqueous ascorbate, both for experimental convenience and because such conditions constitute by far the most common way in which the reaction is performed by us and others. We have ignored here the potential role of ascorbate as anything except a reducing agent to maintain the bulk of the Cu species in the +1 oxidation state. In general, we have found changes in the concentration of ascorbate to make little difference in absolute or relative rates obtained in our kinetics measurements. However, we have not exhaustively compared the reactivities of preformed Cu(I) complexes in the absence of both oxygen and ascorbate to the same Cu·ligand combinations in the presence of ascorbate, and thus the potential participation of ascorbate or dehydroascorbate in the coordination and catalytic chemistry of these systems is not ruled out.

Conclusions

The CuAAC reaction responds to changes in ligands, buffer salts, and substrates in a complex manner. The presence of more than one type of active Cu complex or mechanistic pathway is indicated, since simple equilibria cannot explain many of the observed rate *vs* concentration profiles exhibited by this system.[28] From a practical perspective, it is remarkable that Cu(I) complexes are able to catalyze the reaction at high rates under highly diverse conditions of solvent, temperature, nature of catalyst precursor, and substrate structure. Perhaps it is the ability of the system to bring different effective catalytic species to bear that allows it to "adapt" to such challenges. Certainly the flexibility of the reaction makes it both difficult and highly worthwhile to study. The relationships of various aspects of ligand structure and binding to the catalytic mechanism proposed here suggest new modifications that can be made for improved-performance. Such experiments are currently underway in our laboratories.

Experimental Section

The ligands, experimental procedures, and data analyses were the same as those employed for the accompanying article. Complete details can be found in the Supporting Information.

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Supporting Information Available: Experimental procedures and kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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