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Benzimidazole and Related Ligands for Cu-Catalyzed Azide−Alkyne Cycloaddition

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Abstract: Tris(2-benzimidazolylmethyl)amines have been found to be superior accelerating ligands for the copper(I)-catalyzed azide−alkyne cycloaddition reaction. Candidates bearing different benzimidazole N-substituents as well as benzothiazole and pyridyl ligand arms were evaluated by absolute rate measurements under relatively dilute conditions by aliquot quenching kinetics and by relative rate measurements under concentrated conditions by reaction calorimetry. Benzimidazole-based ligands with pendant alkylcarboxylate arms proved to be advantageous in the latter case. The catalyst system was shown to involve more than one active species, providing a complex response to changes in pH and buffer salts and the persistence of high catalytic rate in the presence of high concentrations of coordinating ligands. The water-soluble ligand **(BimC$_4$A)$_3$** was found to be especially convenient for the rapid and high-yielding synthesis of several functionalized triazoles with 0.01−0.5 mol % Cu.

Introduction

Since its discovery in 2002,[1,2] the copper(I)-catalyzed azide−alkyne cycloaddition (CuAAC) reaction has received a great deal of use[3] in such diverse fields as bioconjugation *in vitro*[4,5] and *in vivo*,[6−9] dendrimer synthesis[10,11] and polymer ligation,[12−16] combinatorial organic synthesis,[17,18] and surface science.[19−23] Unlike the well-established thermal Huisgen cycloaddition reaction,[24] the Cu-accelerated version offers consistent 1,4-stereoselectivity, is not limited to highly activated alkynes, and proceeds efficiently even at micromolar concentrations of reactants in aqueous media. Many investigators have reported the use of amines as copper-binding ligands and/or protic bases to aid the process, including 2,6-lutidine, triethylamine, *N*,*N*,*N*′-trimethylenediamine, diisopropylethylamine, proline, Amberlyst A21 amine resin, and other aliphatic amines (see Supporting Information for a list of references). The most commonly used accelerating ligand has been tris((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)amine (TBTA, **1**) discovered by the Sharpless laboratory.[25] Here we describe the preparation, comparative kinetic evaluation, and practical use of a related family of ligands based on the motif of a central tertiary amine surrounded by three benzimidazole heterocycles. The accompanying article describes kinetic measurements of mechanistic relevance and reports on our current understanding of the reaction mechanism.

Results and Discussion

Ligand Synthesis. The parent structure of the new ligand class described here [tris(2-benzimidazolylmethyl)amine, designated **(BimH)$_3$** as explained in the caption to Figure 1] has

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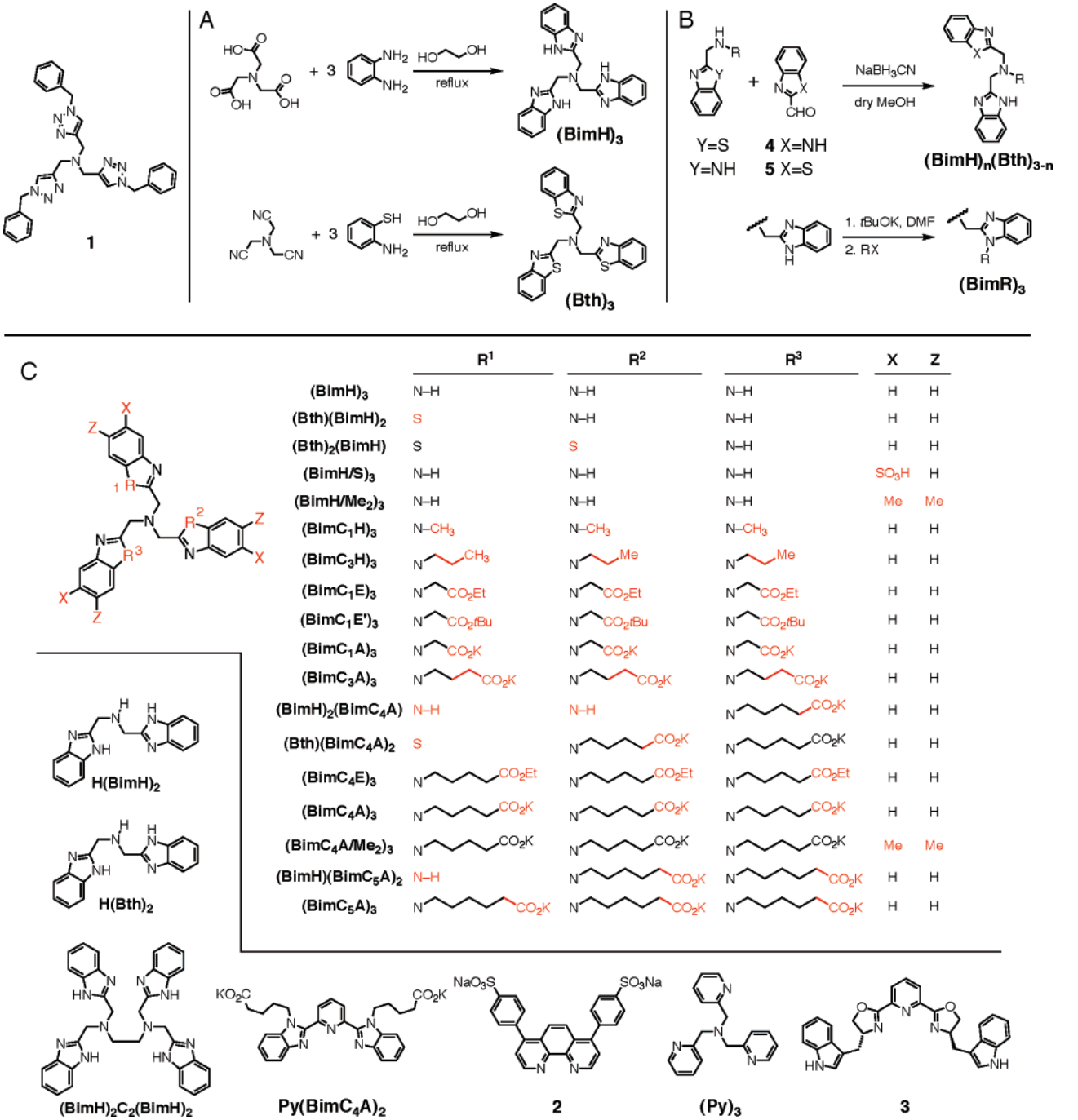

Figure 1. (A) Synthesis of tris(benzimidazole) and tris(benzothiazole) ligands. (B) Synthesis of mixed heterocycle and alkylated benzimidazole variants. (C) Structures of ligands, with changes made in the substitution pattern of each succeeding ligand highlighted in red. For convenience, most ligands 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 = $CO_2^-K^+$.

long been known[26] and was conveniently prepared by the conventional condensation of 1,2-phenylenediamine with nitrilotriacetic acid. A variety of X-ray crystal structures involving this type of ligand have been reported,[27] including Cu(I) and Cu(II) complexes of the *N*-propyl derivative **(BimC$_3$H)$_3$**.[28] However, no known structures of this ligand class incorporate acetylide or π-alkynyl ligands, and thus their relevance to the present situation is not clear. The analogues of **(BimH)$_3$** prepared to explore various structure−activity relationships are shown in Figure 1. Tris- and bis(benzothiazole)amines **(Bth)$_3$** and **H(Bth)$_2$** were obtained by the condensation of 1,2-aminothiophenol with nitrilotriacetonitrile and iminodiacetonitrile, respectively. Mixed benzimidazole/benzothiazole ligands **(BimH)$_2$(Bth)** and **(BimH)(Bth)$_2$** were prepared by reductive amination reactions between secondary amines **H(BimH)$_2$** and **H(Bth)$_2$** and the corresponding heterocyclic aldehydes, as shown in

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Figure 1. Among the tripodal structures, **(Bth)$_3$** and **(BimC$_1$H)$_3$** have unusually low solubility in common organic solvents, including DMSO.

(BimR)$_3$ derivatives were prepared to test the effects of heterocyclic N-substitution on catalytic activity and water solubility. Alkylation with methyl, ethylacetyl, and *tert*-butylacetyl groups gave the neutral ligands **(BimC$_1$H)$_3$**, **(BimC$_1$E)$_3$**, and **(BimC$_1$E′)$_3$**, respectively; ester hydrolysis provided the water-soluble analogue **(BimC$_1$A)$_3$**. (A derivative bearing one acetic acid chain and its supramolecular complex with Cu(II) have been previously described.[29]) Longer-chain analogues were made similarly, and **(BimC$_n$A)$_3$** (n = 3,4,5) ligands were somewhat water soluble and detergent-like at higher concentrations. Ligands **(BimH/S)$_3$** and **(BimH/Me$_2$)$_3$** provided alternatives that were much more hydrophilic and mildly more hydrophobic and electron-rich, respectively.

Kinetic Comparisons. The relative effectiveness of Cu·ligand complexes was screened on the reaction between phenylacetylene and benzyl azide in 4:1 DMSO/aqueous buffer containing sodium ascorbate as reducing agent, which served as the standard reaction in our previous mechanistic work.[30] We refer to ligand/Cu ratios to describe the amounts of each component added and designate candidate mixtures according to those ratios for convenience. However, it must be emphasized that the active catalysts are not necessarily composed of those same ratios of ligand and metal. The equilibria that govern the composition of these catalysts are discussed below, and their study will be the subject of a separate publication.

The utility of the benzimidazole motif was immediately apparent in reactions at 50 mM in azide and alkyne, 5 mM Cu, and 10 mM in sodium ascorbate. In the absence of ligand, the reaction reached only approximately 10% completion in 1 h at room temperature, whereas the use of 1 equiv of **1** or **(BimH)$_3$** with respect to metal gave 75 and 100% completion, respectively, in the same time, providing strong evidence of ligand-accelerated catalysis. Our previous work demonstrated that CuAAC reactions in the absence of accelerating ligands exhibited a second-order rate dependence on copper concentration. Accordingly, a "dimerized" version of the **(BimH)$_3$** ligand, [**(BimH)$_2$C$_2$(BimH)$_2$**], was prepared in the hope that its binuclear Cu(I) complex[31] would make a better catalyst. The ligand proved to be a very potent inhibitor in early testing and was not further examined, but other binucleating systems remain to be explored.

The obvious accelerating abilities the parent tris(benzimidazole) ligand prompted us to measure more accurately the absolute and relative activities of candidate catalysts in two different concentration regimes. We chose to focus on catalyst activity, as indicated by observed pseudo-first- or second-order rate constants (specific activities) in the presence of large amounts of ascorbate reducing agent, thereby ignoring the issue of resistance of Cu(I) catalysts to oxidative deactivation in air. While the latter is an important parameter for the practical applicability of candidate systems, we believe that these issues are better tackled separately, at least in the early stages of catalyst development. The issue of copper speciation among Cu(0), Cu(I), and Cu(II) oxidation states, accessible under inert atmosphere by disproportionation of Cu(I), is also not directly addressed here. The preservation of large amounts of Cu(I) under our kinetic conditions (4:1 DMSO/water buffer, >20-fold excess of ascorbate relative to Cu) is indicated by the lack of characteristic copper complex color changes for appropriate ligands (colorless Cu(I) to bright yellow Cu(II) in the presence of **(BimH)$_3$**).

Absolute rates were determined under relatively dilute conditions (1 to 2 mM in substrates), which were required to slow the reactions enough so that aliquots could be reliably taken over a substantial fraction of the completion curve. Each aliquot was immediately quenched and the amount of product formed monitored by quantitative LC−MS against an internal standard. In this way, the clean conversion to triazole was verified in each case, information that is not available by following the disappearance of azide or alkyne by infrared spectroscopy. Plots of product concentrations *vs* time fitted very well to second-order kinetics over at least 70% of the reaction (Figure 2A). A ligand/metal ratio of 2:1 was found to be optimal for TBTA (**1**) by this method (Supporting Information), which matches the results from previous work on other ligands.[32] For this reason, comparisons were made between second-order specific activities (rate constants determined at a standard Cu concentration of 0.1 mM) for catalysts composed of 2:1 mixtures of ligands and Cu(I), in the presence of excess ascorbate relative to Cu.

In general, benzimidazole-based ligands provided for reactions faster than those of the parent tris(triazolyl) structure **1** and in the same range as the previously identified sulfonated bathophenanthroline **2** (data not shown).[32] The magnitude of ligand-accelerated catalysis (ratio of the observed rates in the presence *vs* the absence of added ligand) was substantial (more than an order of magnitude), but not as great as the factors in excess of 10^3 observed previously for reactions of Cu·**2** under the highly dilute conditions (micromolar concentrations of substrates and catalysts) required for bioconjugation.[32] As discussed in the accompanying article, we believe this reflects the relative binding affinities of **2** *vs* the tris(heterocyclic methyl)amines for Cu(I). It is also apparent that reaction rate is sensitive to both the nature of the heterocycle and to substitution on the benzimidazole ring. The replacement of benzimidazoles with benzothiazoles in the N(CH$_2$heterocycle) format gave no appreciable loss in catalyst efficiency, as long as at least one benzimidazole side arm was present. **(Bth)$_2$(BimH)** was found to be the best ligand in the series, with rates following the order **(Bth)$_3$** ≪ **(Bth)(BimH)$_2$** < **(BimH)$_3$** ≪ **(Bth)$_2$(BimH)**. N-Substitution of the tris(benzimidazole) ligand was extensively investigated, with small pendant alkyl and ester groups [**(BimC$_1$E)$_3$**, **(BimC$_1$E′)$_3$**, **(BimC$_1$H)$_3$**] performing as well as the parent structure **(BimH)$_3$** (data for the latter two ligands not shown). The installation of pendant carboxylic acid groups gave rise to noticeably better catalyst performance in the case of longer chain lengths [**(BimC$_4$A)$_3$**, **(BimC$_4$A/Me$_2$)$_3$**, **(BimH)-(BimC$_5$A)$_2$**, **(BimC$_3$A)$_3$**, **(BimC$_5$A)$_3$**]. The ester **(BimC$_4$E)$_3$** was just as active as its hydrolyzed (carboxylate) analogue. In contrast, ligand **(BimC$_1$A)$_3$**, which features carboxylic acid

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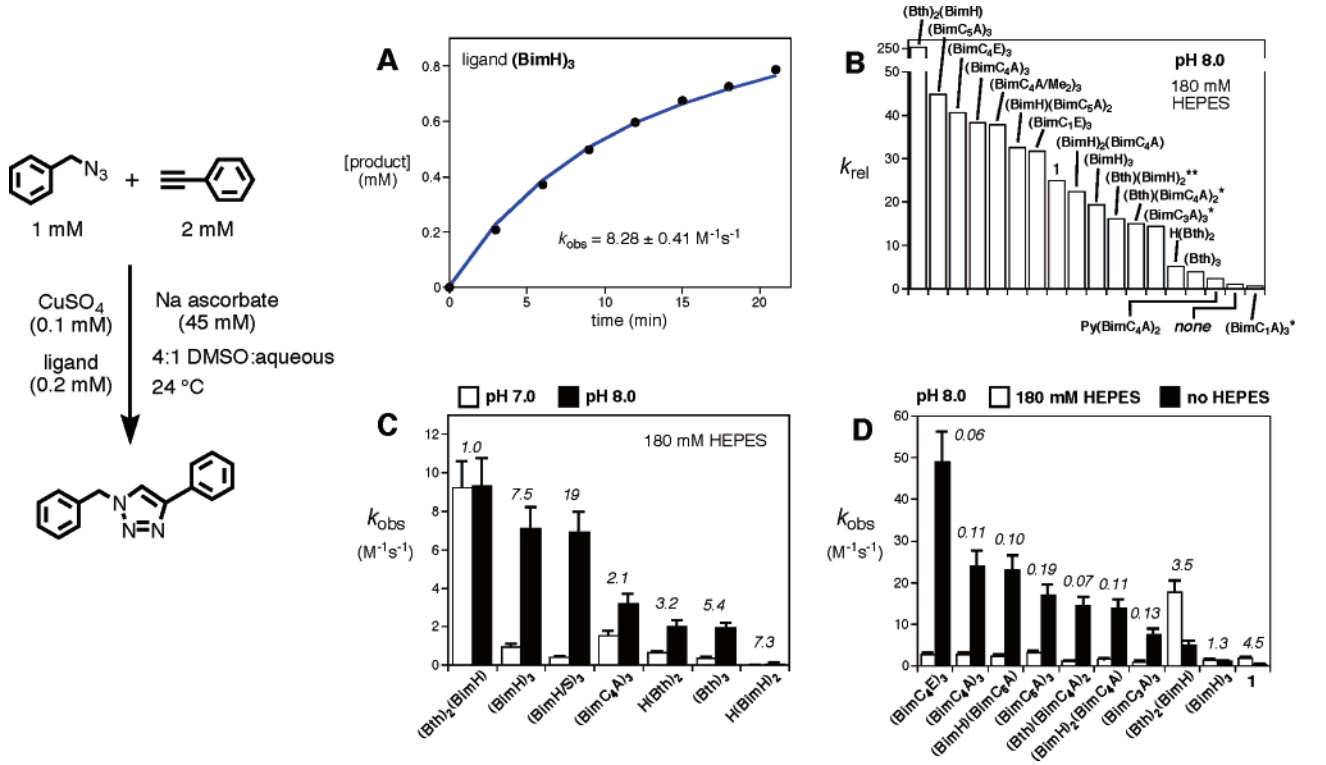

Figure 2. (A) Example of second-order rate treatment for the reaction of benzyl azide with phenylacetylene involving ligand **(BimH)$_3$**. The circles mark observed data points; the curve is calculated from the integrated rate equation with the indicated specific activity k_{obs}. Experimental errors in rate constants based on repeat measurements are within 15% of the indicated values in the vast majority of cases. (B) Relative magnitudes of second-order specific activities at pH 8, with the rate in the absence of added ligand set to 1.0. Single asterisks (∗) mark catalysts that exhibit a burst of faster activity at the beginning of the reaction (2−4% reaction, representing less than one catalyst turnover) and then settle down to the slower rate indicated. The double asterisk (∗∗) marks a ligand for which the initial rate is reported; the reaction slows after approximately 30% completion. (C) Observed second-order specific activities for a selection of ligands in 4:1 DMSO/buffer, as a function of pH of the HEPES buffer. The ratio of rates (pH 8/pH 7) for each ligand is shown in italics. (D) Observed second-order specific activities for a selection of ligands at pH 8.0 in 4:1 DMSO/aqueous, where "aqueous" is either HEPES buffer or water. In the latter case, sodium ascorbate provides weak buffering capacity; the pH of the mixture was found to be the same at the beginning and end of the reaction. The ratio of rates (with HEPES/without HEPES) for each ligand is shown in italics. In all cases, [BnN_3] = 1 mM; [PhCCH] = 2 mM; [$CuSO_4$] = 0.1 mM; [ligand] = 2 mM; temp = 24 ± 2 °C; solvent = 4:1 DMSO/aqueous.

groups separated from the benzimidazole core by just one carbon atom, produced one of the poorest catalysts in the series, much less effective than its ester **(BimC$_1$E)$_3$**.

Some indications of the response of the process to changes in pH and to the presence of added buffer salts are provided by Figure 2C,D. In absence of catalytic ligands, preparative CuAAC reactions at the bench are remarkably tolerant of large changes in pH: only highly acidic conditions (pH 2 or lower) can halt the reaction, possibly by affecting Cu speciation or inhibiting the formation of Cu·acetylides (data not shown). In general, more basic conditions promote the reaction, and this trend is also evident in the absolute rates measured for several added ligands, as shown in Figure 2C. However, different Cu·ligand catalysts respond differently. For example, the mixed benzimidazole/benzothiazole system **(Bth)$_2$(BimH)** showed very little change in rate when the pH of the aqueous component of the solvent mixture was raised from 7 to 8, whereas the parent tripodal ligands **(Bth)$_3$** and **(BimH)$_3$** were more sensitive.

Concern for pH effects prompted us to test added buffer salts, even though excess ascorbate functions as a low-capacity buffer under our standard conditions. The nature of the buffer was also found to have an impact on the CuAAC reaction. For example, while we and others have successfully used pH 8 Tris-HCl buffer for bioconjugation applications, we found in the course of our kinetic studies that reactions catalyzed by Cu·**1** and Cu·**(BimH)$_3$** mixtures in 4:1 DMSO/buffer are slowed significantly in the presence of Tris buffer over a standard range of concentrations, with the inhibitory effect being significantly more pronounced for **1** (data not shown). Another popular buffer system is based on 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), which is known to have moderate affinity for Cu(II)[33,34] and can slowly reduce Cu(II) to Cu(I).[34] We found HEPES buffer to have little effect on the catalytic rate of a wide variety of Cu·ligand complexes at moderate concentrations (<100 mM, data not shown). We therefore recommend the use of HEPES (at 100 mM or less) whenever CuAAC reactions need to be performed at controlled moderate pH values in an aqueous environment. However, the use of a higher buffer concentration (180 mM) gave rise to the varying results shown in Figure 2D. Three catalysts were found to give faster turnover in the presence of HEPES, with the effect being especially dramatic for Cu·**(Bth)$_2$(BimH)**. Other catalysts bearing pendant carboxylate arms were found to be inhibited by 180 mM HEPES.

Figure 2B also shows a small number of ligands that are either inhibitory or inconsequential relative to the rate of the "ligand-free" case. Thus, tris(pyridylmethyl)amine **(Py)$_3$**, secondary amine **H(BimH)$_2$**, and a ligand having heterocycles flanking a central pyridine motif [**Py(BimC$_4$A)$_2$**]] each give rates slower than that observed for the "ligand-free" case. Pybox derivative

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3, previously used for kinetic resolution of racemic diazides,[35] is also not acceleratory. We believe that these systems do not allow access to the correct coordination geometry to promote the reaction. The spectrum of results shown in Figure 2 and the Supporting Information suggest that the response of various Cu·ligand catalysts to changes in pH and added coordinating ions is complex, likely to involve ligand-dependent changes in speciation, structure, and, perhaps, rate-limiting step of the catalytic cycle. From a practical perspective, **(BimC$_4$A)** and **(BH/S)$_3$** are attractive because they provide strong rate acceleration through a wide pH range and are soluble in water to at least 50 mM for the potassium salts, thus allowing for easy workup (see below).

Reaction Calorimetry. To test the relative merits of candidate ligands under practical conditions of organic synthesis (involving high concentrations of azide and alkyne reagents and low loadings of catalyst), we turned to reaction calorimetry.[36−39] After correction for instrumental heat transfer parameters, thermograms provide a direct real-time measure of reaction rate (power output); integration gives the overall time *vs* completion curve. As discussed fully in the Supporting Information, an instrumental limitation in sensitivity foiled attempts to obtain detailed mechanistic information[38,39] on reactions that were less than optimal, but the peak reaction exotherm proved to be a reproducible and convenient measurement of relative catalytic activity. All such measurements were made in the same 4:1 DMSO/aqueous solvent as before, but at 65 °C rather than room temperature to keep the product triazole in solution at the higher concentrations used. The technique also allowed us to ascertain at a glance if candidate catalysts were "well-behaved", producing a rapid burst of heat immediately upon initiation of the reaction followed by smooth exponential decay, or if they exhibited more complex behavior such as induction times or bimodal power *vs* time curves.

Most systems gave a clean profile characteristic of a second-order reaction involving a single catalyst species, as shown in the example of Figure 3A. **TBTA** (**1**) was an important exception, showing a markedly more gradual approach to a peak rate followed by a rapid drop to give the "sawtooth" thermogram shown in Figure 3B. This reaction profile made direct calorimetric comparison between **1** and other ligands impossible; however, the peak power and integrated area showed **1** to give a sluggish catalyst compared to many of the others tested here (although still far more active than without ligand). Its unique thermogram suggests that changes in the Cu·**1** catalyst composition occur as the reaction proceeds, since its profile does not correspond to typical kinetic expressions involving a single catalyst species. Two *N*-heterocyclic carbene complexes of Cu(I) were also tested, because a recent article describing the use of this class of ligands has appeared.[40] One of these ligands

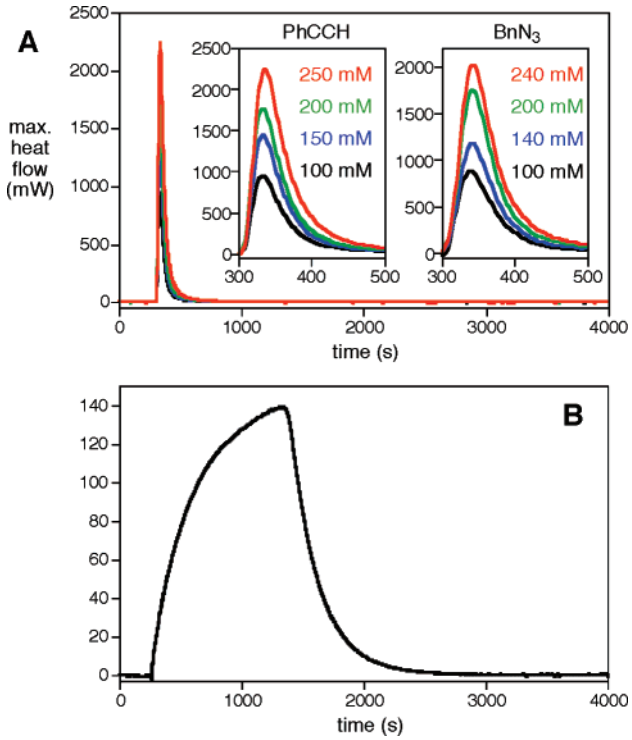

Figure 3. (A) Calorimetry data for reactions of varying concentrations of phenylacetylene with 0.45 M benzyl azide in the presence of $CuSO_4$ (2 mM), **(BimH)$_3$** (4 mM), and sodium ascorbate (25 mM), in 4:1 DMSO/water (pH 8), at 65 ± 2 °C. The azide was added last to initiate the reaction at the 300-s mark. The maximum power produced by the reaction, represented by the peak of the signal produced immediately after azide addition, was used as a relative measure of catalyst activity. Insets: (left) expanded view; (right) corresponding data for varying concentrations of BnN_3 in the presence of 0.45 M PhCCH. (B) Thermogram for the same reaction as in part A (0.45 M alkyne, 0.25 M azide), performed with **1** instead of **(BimH)$_3$**.

was inactive; the other performed approximately as well as **1**, showing the same type of unusual rate *vs* time profile (Supporting Information).

Approximately 100 ligands were tested by calorimetry at a ligand/Cu ratio of 2:1, and a subset of these were also evaluated at a 1:1 ratio. The results from the best systems are shown in Figure 4; a summary of the remaining measurements is given in the Supporting Information. All the values in Figure 4 represent substantial levels of ligand-accelerated catalysis, since the reaction without added ligand does not release enough heat to register on the calorimeter at all (although it does go to completion within 12 h at 65 °C). All of the ligands in Figure 4 show single-component "well-behaved" kinetic characteristics (Figure 3A). The ligand/metal ratio made little difference in the overall trends.

The results from calorimetry screening are largely consistent with the results obtained by analysis of quenched aliquots by LC−MS (Figure 2). Both methods show tris(benzimidazole) derivatives to be excellent catalytic ligands, and those bearing longer alkylcarboxylate groups [**(BimC$_4$A)$_3$**, **(BimH)(BimC$_5$A)$_2$**, **(BimC$_3$A)$_3$**, **(BimC$_5$A)$_3$**] were the best in the class. (The ester **(BimC$_4$E)$_3$** was not tested because ester hydrolysis can occur under the reaction conditions.) The important exceptions are benzothiazole-containing ligands [**(Bth)$_3$**, **(Bth)$_2$(BimH)**, **(Bth)(BimH)$_2$**] and the tris(pyridylmethyl)amine ligand **(Py)$_3$**. The former showed significant activity in the aliquot quenching screen (low reactant concentrations, 10% Cu) yet were largely

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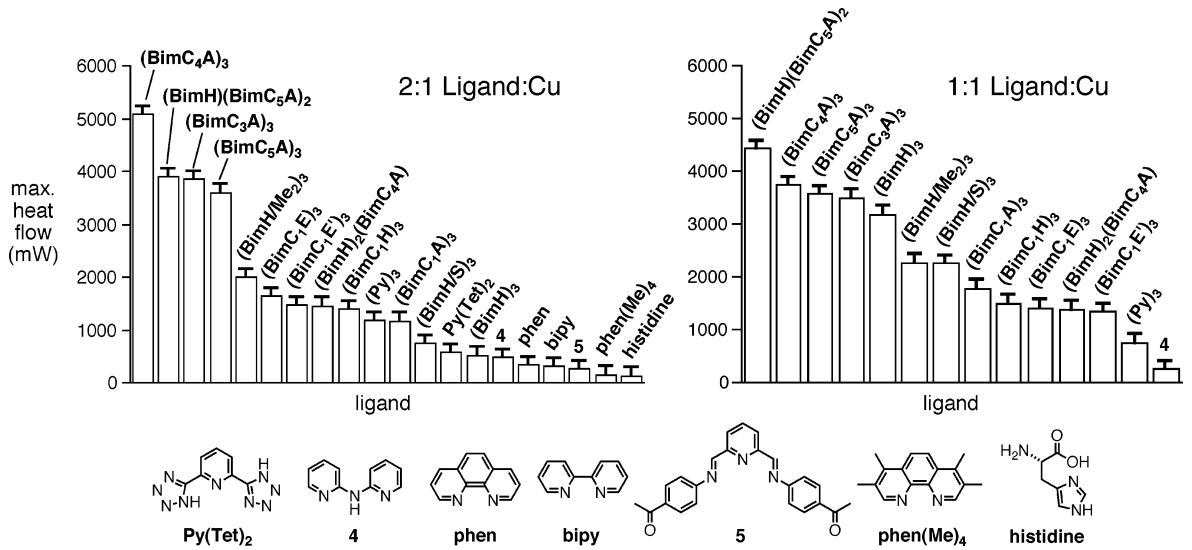

Figure 4. Dynamically corrected peak power values for the best ligands identified by reaction calorimetry under the conditions described in Figure 3 (0.45 M PhCCH, 0.25 M BnN_3), at the indicated ligand/Cu ratios. The experimental error for these measurements was established as ±160 mW from more than five independent trials for a representative selection of ligands spanning the activity range.

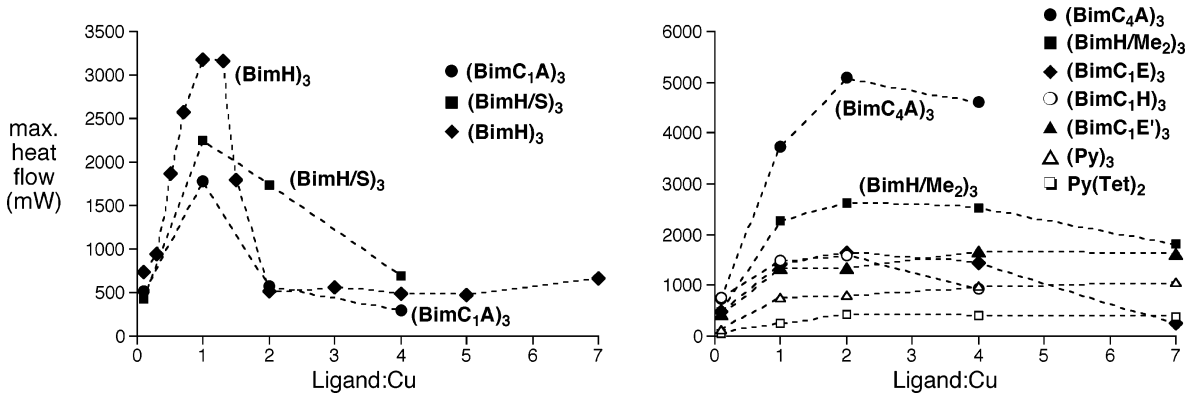

Figure 5. Dependence of the peak reaction power (peak rate) on ligand/Cu ratio for a subset of the best ligands identified in the initial screen. Conditions: benzyl azide (0.45 M); phenylacetylene (0.25 M); $CuSO_4$ (2 mM); ligand (variable); sodium ascorbate (25 mM); 4:1 DMSO/water; 65 ± 2 °C.

inactive under calorimetry conditions (high reactant concentrations, 1% Cu). The opposite trend was observed for **(Py)$_3$**, which was one of the best ligands in calorimetry screening while performing poorly in aliquot quenching kinetics. We attribute these differences to changes in copper speciation that are likely to accompany changes in overall reaction concentration and catalyst loading.

From a practical perspective, the tris(benzimidazole) family emerged from both types of rate measurements as an effective platform for ligand development. Bipyridine and phenanthroline are accelerating additives that we recommend testing as readily available options when the reaction must be optimized for a particular application. Histidine provided for the fastest CuAAC reaction among all the amino acids, as has been recently noted elsewhere,[41] defining the lower boundary of our "effective" ligand category. While not directly comparable because of the differences in rate profile described above, **1** ranks in the lower half of this group.

We also explored a wider range of ligand/Cu ratios for some of the best ligands under concentrated conditions by calorimetry. These results, summarized in Figure 5, show that the ligands

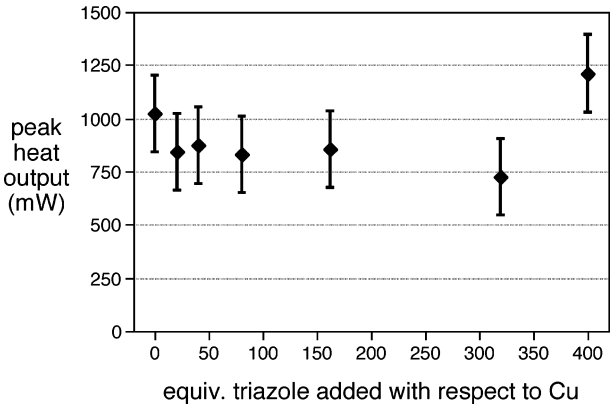

Figure 6. Dependence of peak reaction power (peak rate) on added 1-*n*-octyl-4-phenyl-1,2,3-triazole for the reaction of *n*-octylazide (350 mM) + phenylacetylene (450 mM), in the presence of $CuSO_4$ (2 mM); **(BimH)$_3$** (4 mM); sodium ascorbate (25 mM); 4:1 DMSO/water; 65 ± 2 °C. The thermogram shape for each reaction was bimodal as shown in Figure 7A; this system was chosen to allow the addition of triazole without precipitation.

may be divided into two categories: those that perform optimally at a 1:1 ratio [**(BimH)$_3$**, **(BimC$_1$A)$_3$**, **(BH/S)$_3$**], and those that reach either a peak or a plateau at a 2:1 ratio [**(BimC$_1$H)$_3$**, **(BimC$_1$E)$_3$**, **(BimC$_1$E′)$_3$**, **(BimC$_4$A)$_3$**, **(BH/Me$_2$)$_3$**, **(Py)$_3$**, **Py(Tet)$_2$**]. In most of the latter cases, the reaction was

(41) Tanaka, K.; Kageyama, C.; Fukase, K. *Tetrahedron Lett.* **2007**, *48*, 6475−6479.

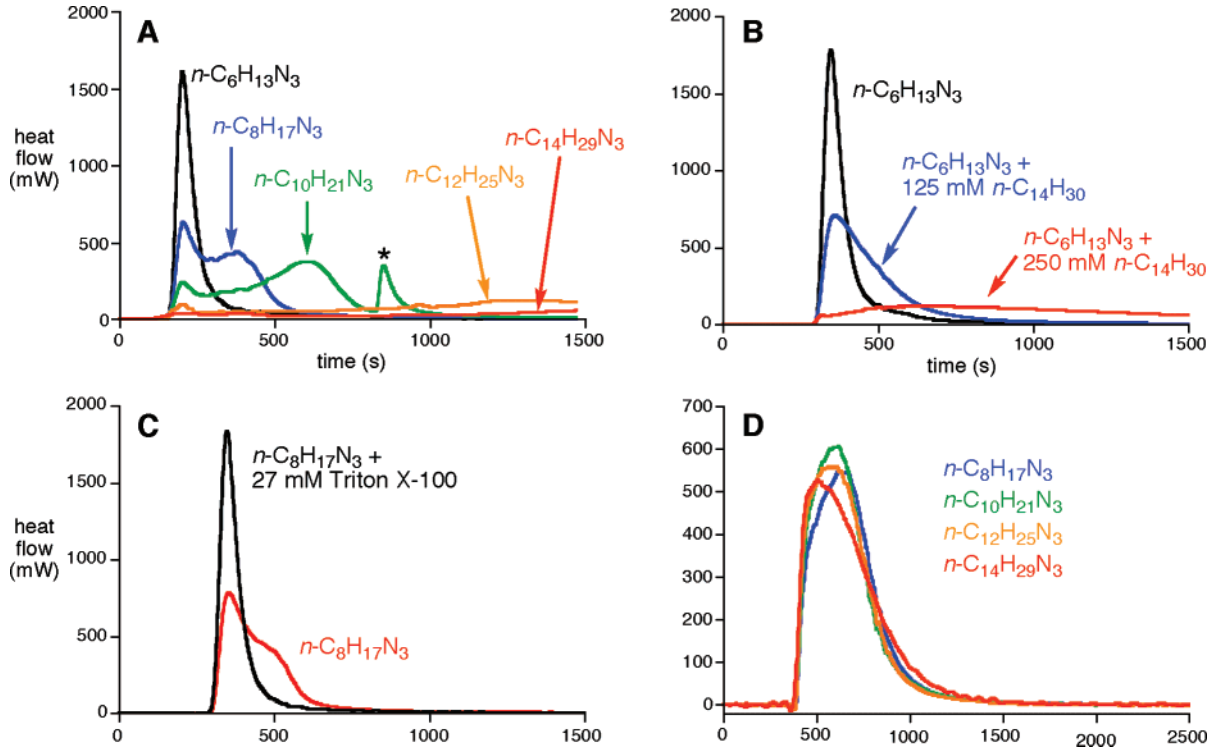

Figure 7. Corrected calorimetry data for reactions of the indicated azides and additives. (A−C) Azide (250 mM); phenylacetylene (450 mM); $CuSO_4$ (2 mM); **(BimH)$_3$** (4 mM); sodium ascorbate (25 mM); 4:1 DMSO/water; 65 ± 2 °C. (D) In DMSO with $[Cu(MeCN)_4]OTf$ (2 mM) and no added reducing agent. The asterisk in plot A marks an exothermic crystallization of product triazole.

not dramatically slowed by the addition of excess ligand (4 or 7 equiv). Even when a pronounced peak in activity was observed at an equimolar ligand/Cu ratio [**(BimH)$_3$**, **(BimC$_1$A)$_3$**], the reaction proceeded at a significant rate in the presence of excess ligand. This type of behavior is not normally exhibited by systems having a single active catalyst, the concentration of which is governed by a simple set of equilibria such as that shown in eq 1.

$$\underset{inactive}{M_nL_m} \overset{K_1}{\underset{-L}{\rightleftharpoons}} \underset{active}{M_nL_{m+1}} \overset{K_2}{\underset{-L}{\rightleftharpoons}} \underset{inactive}{M_nL_{m+2}} \qquad (1)$$

In situations such as eq 1, ligand-accelerated reactivity derives from the catalytic power of a complex containing a greater ligand/metal ratio than an inactive (or less active) precursor; in the simplest case, the first ligand to bind creates the catalyst of interest ($m = 0$). The association of an additional equivalent of ligand diminishes the rate by blocking access of a substrate molecule to an open coordination site on the metal or by diverting the system to a less active structure. A plot of ligand/metal ratio *vs* catalysis rate would therefore appear as a single peak, approaching a value of zero in rate as the concentration of ligand is increased. In contrast, the benzimidazole and related CuAAC systems show persistent and considerable catalytic activity even at high ligand/Cu. This is likely due to one or both of two factors: (a) $K_2 \ll K_1$ and (b) at least two active ligand-containing catalysts or mechanistic pathways exist: one that depends on ligand/Cu, and another that is largely independent of that ratio. (For all of the catalysts shown, the reaction rate in the absence of ligand was too low to be measured on our calorimeter. The accelerating ligands therefore either must be components of the active catalytic species or must enhance the concentration of active copper centers.) That a complex set of equilibria is involved is further indicated by the fact that the addition of even weakly coordinating additives such as 2,6-lutidine or 2-amino-2-(hydroxymethyl)propane-1,3-diol (Tris) to the solvent system for catalyst Cu·[**(BimH)$_3$**]$_n$ removes the relatively sharp peak in the plot of rate *vs* ligand/Cu, making the relationship look more like that of Cu·**(BimC$_4$A)$_3$** (data not shown).

The persistence of high reaction rates in the presence of excess ligand suggests that these catalysts should also remain active in the presence of high concentrations of product triazoles, which can be expected to have some affinity for Cu(I). This has been demonstrated by the successful use of low catalysts loadings

Table 1. Tests of Reduced Catalyst Loading for the Reaction of Phenylacetylene (0.75 M) with Benzyl Azide (0.75 M), 3:1 MeOH/Water, Sodium Ascorbate (30 mM), 24 °C[a]

entry	ligand	Cu(I) source	mol % Cu	mol % ligand	time (h)	yield (%)[b]
1	**1**	$CuSO_4$ ascorbate	0.1	0.2	24	27
2	**1**	$CuSO_4$ ascorbate	0.5	0.5	4.5	47
3	**(BimH)$_3$**	$CuSO_4$ ascorbate	1	1	5	94
4	**(BimH)$_3$**	$CuSO_4$ ascorbate	0.5	0.5	5	92
5	**(BimH)$_3$**	$[Cu(MeCN)_4]OTf$	1	1	24	93
6	**(BimH)$_3$**	CuI	1	1	24	6
7	**(BimH)$_3$**	$CuSO_4$ ascorbate	0.1	0.1	24	73
8	**(BimC$_4$A)$_3$**	$CuSO_4$ ascorbate	0.5	0.5	0.2	100
9	**(BimC$_4$A)$_3$**	$CuSO_4$ ascorbate	0.05	0.05	5	98
10	**(BimC$_4$A)$_3$**	$CuSO_4$ ascorbate	0.01	0.01	24	95
11	**(BimC$_4$A)$_3$**	$CuSO_4$ ascorbate	0.005	0.005	72	35
12	*i*Pr$_2$EtN	CuI[c]	0.5	5	72	29

[a] Reactions producing >90% yield were completed in substantially shorter time than the listed values. Analogous reactions using $CuSO_4$ or CuI but without added ligand gave <10% yield after 24 h. [b] Isolated yield of analytically pure triazole product. [c] Reaction in absolute MeOH; the use of cuprous iodide and excess additive reflects the customary practice reported in the literature.

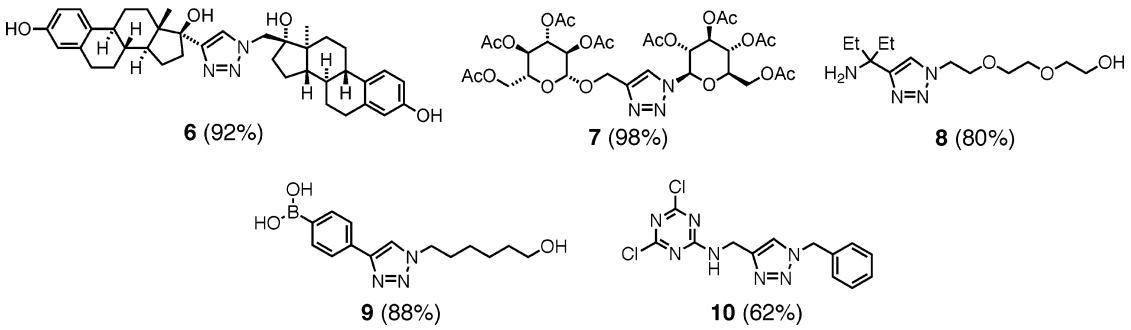

Figure 8. Triazoles prepared by reaction of equimolar amounts of the appropriate azide and alkyne (each 10 wt %, 0.13−0.35 M, depending on molecular weight) in the presence of $CuSO_4$ (0.1 mol %); **(BimC$_4$A)$_3$** (0.2 mol %); and sodium ascorbate (4 mol %) in 95:5 DMF/water at room temperature. Isolated yields of purified products are indicated in parentheses; compound **10** was obtained in relatively low yield because of competitive reaction of the starting material with water.

(discussed below) and by calorimetric measurements summarized in Figure 6. Peak catalytic activity was found to be unchanged in the presence of triazole in an amount up to 400 times the concentration of catalyst.

Effects of Aggregation of Long-Chain Aliphatic Azides. Under the typical reaction conditions in 20% aqueous DMSO, the thermograms of reactions involving long-chain aliphatic azides (accelerated by ligand **(BimH)$_3$**) exhibited a distinctive two-stage reaction profile as shown in Figure 7A. The reactions of those azides became slower, and the bimodal nature of thermograms more distinct, with elongation of the aliphatic chain. The addition of *n*-tetradecane to the well-behaved *n*-hexylazide also slowed the reaction dramatically, even though the overall concentration of azide was not changed (Figure 7B). When the reaction was performed in the presence of the nonionic detergent Triton-X100 (27 mM), the thermogram reverted to the normal single-peak appearance (Figure 7C). Single-peak behavior was also observed in pure DMSO, although the thermogram peaks were not as sharp as those in the standard DMSO/water solvent system (Figure 7D). Last, the use of amphiphilic ligand **(BimC$_4$A)$_3$** in place of **(BimH)$_3$** largely eliminated the bimodal behavior of aliphatic azides. All of these observations support the hypothesis that long-chain aliphatic azides partition between solvated single molecules and detergent-sensitive aggregates in the aqueous DMSO medium, and in the latter case are partially sequestered from the catalyst and alkyne.

Synthetic Tests. The ligands **(BimH)$_3$** and **(BimC$_4$A)$_3$**, identified in the above studies as being superior under conditions of high substrate concentration, were tested along with **1** for their ability to support reactions at low catalyst loading (Table 1). The results mirror the findings by calorimetry, and yields followed the trend Cu·[**(BimC$_4$A)$_3$**] > Cu·[**(BimH)$_3$**] ≫ Cu·[**1**] (entries 2, 4, and 8). A maximum value of approximately 10^4 turnovers per metal center was achieved (0.01 mol % catalyst, entry 10), allowing conveniently rapid reactions at 0.05 mol % or greater. The use of 0.001 mol % catalyst provided a 30% yield after 3 weeks in an inert atmosphere glovebox, but no product on the benchtop, suggesting that oxidation of the Cu·ascorbate system was competitive with the cycloaddition process at that point. The preformed soluble Cu(I) acetonitrile complex made a competent catalyst in the absence of ascorbate (entry 5), but cuprous iodide did not (entry 6), at least in a mixed MeOH/water solvent system. The use of diisopropylethylamine as an additive with CuI has gained some popularity (Supporting Information), but we have found such reactions to be much slower than those performed in the presence of tris(heterocyclic methyl)amine ligands such as **1** and **(BimH)$_3$** (entry 12). The successful application of **(BimC$_4$A)$_3$** to the synthesis of other triazoles, containing steroid, carbohydrate, boronic acid, amine, alcohol, and electrophilic chlorotriazine moieties, is shown in Figure 8. The selection of substrates is meant to illustrate the reliability and convenience of the Cu·[**(BimC$_4$A)$_3$**] system for synthetic purposes. In addition, the water solubility of **(BimC$_4$A)$_3$** makes the preparation of relatively hydrophobic triazoles more convenient, since a simple aqueous wash removes both the ligand and metal, leaving the product in pure form.

Conclusions

Tris(2-benzimidazolylmethyl)amines have been found to be the most promising family of accelerating ligands for the Cu-catalyzed azide−alkyne cycloaddition reaction from among more than 100 mono-, bi-, and polydentate candidates. The benzimidazole compounds are easy to prepare and compare favorably to tris(triazolylmethyl)amines and other ligands, such as diisopropylethylamine and sulfonated bathophenanthroline that have achieved wide use. Under both preparative (high concentration, low catalyst loading) and dilute (lower substrate concentration, higher catalyst loading) conditions, tripodal benzimidazole derivatives give substantial improvements in rate and yields, with convenient workup to remove residual Cu and ligand.[42] The relative effectiveness of ligands with different substitution patterns changes when examined under different reaction conditions, pointing to interesting mechanistic features that are discussed in the accompanying article.

Experimental Section

Experimental procedures used for kinetics measurements and reaction calorimetry are fully described in the Supporting Information.

Ligand Synthesis. Tris-benzimidazole ligand **(BimH)$_3$**[43] and analogues **H(BimH)$_2$** and **(BimH)$_2$C$_2$(BimH)$_2$**[44] were obtained by condensation of 1,2-phenylenediamine with the corresponding tri-, di-, and tetracarboxylic acid in boiling ethylene glycol. A similar condensation reaction between 1,2-aminothiophenol and nitrilotriacetonitrile or iminodiacetonitrile was used to synthesize benzothiazole ligands **(Bth)$_3$** and **(H)(Bth)$_2$**. The crude products usually crystallized when water was added to the cooled reaction mixture (it is important not to add water

(42) Not discussed here are related series of tris(oxazolinylmethyl)amine ligands, which we have found in preliminary studies to give catalysts of comparable efficiency to tris(triazolylmethyl)amines. The attempted use of these systems in chiral kinetic resolution and desymmetrization reactions will be described elsewhere.

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(44) Birker, P. J. M. W. L.; Hendriks, H. M. J.; Reedijk, J.; Verschoor, G. C. *Inorg. Chem.* **1981**, *20*, 2408−2414.

to the ethylene glycol solution while it is still hot). For the purposes of this study, the ligands were subsequently recrystallized from absolute ethanol and were found to be pure by elemental analysis. The procedure scales up without difficulty. The preparation of **1** from tripropargylamine and benzyl azide under the influence of Cu(I) sometimes suffers from incomplete reaction, the presence of colored impurities, and/or the presence of trapped copper ions in the solid product.

The mixed ligands **(Bth)(BimH)$_2$** and **(Bth)$_2$(BimH)** were obtained by reductive amination of aldehydes **4**[45] and **5**[46] (Figure 1) with amines **(Bth)$_2$** and **(H)(BimH)$_2$**, respectively. N-Alkylated derivatives **(BR)$_3$** were obtained by deprotonation of **(BimH)$_3$** with *t*-BuOK and reaction with the corresponding alkylating agent. Poor results were obtained when NaH (either dry or oil suspension) was used as the base. To produce the free carboxylate ligand **(BimC$_4$A)$_3$** and its analogues, the corresponding ester was reacted with exactly 3 equiv of KOH in boiling aqueous ethanol. Tris(sulfonic acid) **(BH/S)$_3$** was obtained by a published procedure.[47] Most ligands with free benzimidazole NH groups were purified by flash chromatography on a short Florisil column, eluting with 0.5% MeOH in CH_2Cl_2. Ordinary silica gel is not well suited to this separation because of the strong tendency of these compounds to "streak", resulting in poor recovery of the desired products.

Typical Procedure for Preparative Scale CuAAC Reactions. The reaction flask was charged with solid sodium ascorbate, followed by the ligand, methanol (or another appropriate solvent), azide, and alkyne in that order. A solution of $CuSO_4$ in water was added, the flask was capped, and the mixture was stirred vigorously or sonicated for 5−10 min until all of the ascorbate was dissolved. The resulting homogeneous solution was left standing at room temperature and periodically monitored by TLC. When reagent conversion was complete, several volumes of deionized water were added to the reaction and the precipitated triazole product was recovered by filtration. For the most part, these products were found to be >98% pure by NMR; when necessary, purification to reach this level was performed by flash chromatography, and yields are reported for the final purified products.

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

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