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Tailored Ligand Acceleration of the Cu-Catalyzed Azide-Alkyne Cycloaddition Reaction: Practical and Mechanistic Implications

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Abstract

Tris(heterocyclemethyl)amines containing mixtures of 1,2,3-triazolyl, 2-benzimidazolyl, and 2-pyridyl components were prepared for ligand acceleration of the copper-catalyzed azide-alkyne cycloaddition reaction. Two classes of ligands were identified: those that give rise to high reaction rates in coordinating solvents but which inhibit the process when used in excess relative to copper, and those that provide for fast catalysis in water and are not inhibitory in excess. Several “mixed” ligands were identified that performed well under both types of conditions. Kinetics measurements as a function of ligand:metal ratio and catalyst concentration were found to be consistent with an active Cu_2L formulation. Since strongly bound chelating agents are not always the most effective, achieving optimal rates requires an assessment of the potential donor molecules in the reaction mixture. Simple rules are provided to guide the user in the choice of effective ligands and reaction conditions to suit most classes of substrates, solvents, and concentrations.

Keywords

click chemistry; cycloaddition; azides; alkynes; ligand-accelerated catalysis; mechanism

Introduction

The Cu^{I} -catalyzed azide-alkyne cycloaddition¹ (CuAAC) click reaction has found use in a remarkably wide range of settings and applications. Simple $\text{Cu}(\text{I})$ salts **1a** or the convenient combination of a $\text{Cu}(\text{II})$ precursor and a reducing agent (typically, CuSO_4 and sodium ascorbate, **1b** top of Figure 1) provide species that mediate azide-alkyne ligation at high enough rates for many purposes, approximately 10^6 times faster than without the metal.² For more demanding applications, the use of certain Cu-binding ligands has been found to accelerate the CuAAC reaction even more, up to several thousand times over the ligand-free process.³ We have focused on C_{3v} -symmetric tris(heterocyclemethyl)amines, derivatives of the tris(triazolylmethyl) structure **1** (Figure 1) obtained by CuAAC reaction of tripropargylamine and organic azides.^{3a} Recently we reported benzimidazole-based ligands **2** to be superior under conditions of low catalyst loading and high substrate concentration.⁴ Tris(pyridylmethyl)amines such as **3** are well known ligands for copper,⁵ and provide some CuAAC rate acceleration.⁶ We describe here the mixing of these binding motifs for the purposes of catalyst optimization and mechanistic investigation, along with a revealing exploration of the dependence of the reaction on the nature of the solvent and the ligand:Cu ratio.

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Supporting Information Available. Experimental details, including ligand syntheses and characterization, numerical tables for all plots, and mass spectra of Cu-ligand mixtures. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Results

We prepared the “hybrid” tris(heterocyclemethyl)amine derivatives shown in Figure 1, mixing 1,2,3-triazolyl, 2-benzimidazolyl, and 2-pyridyl donors on the ligand “arms.” Catalysts incorporating these ligands were compared by calorimetry on the reaction of benzyl azide with phenylacetylene in 4:1 DMSO:water, as we have done previously.⁴ Shown in Figure 2A are the values of maximum heat output, representing the peak activity of each catalyst at the start of the reaction. Instead of a direct dependence of rate on heterocycle content, a nonlinear pattern was observed for both triazole and pyridyl systems: ligands containing a single benzimidazole and two triazoles or pyridines were approximately as effective as the tris(benzimidazole) structure. This reprises an earlier observation that a bis(benzimidazole)-mono(benzothiazole) ligand was less effective than its mono(benzimidazole) and tris(benzimidazole) congeners.⁴

When the rates of more dilute reactions were measured by aliquot quenching and determination of triazole formation⁴, a somewhat different picture emerged (Figure 2B). The benzimidazole-triazole series displayed the same sawtooth pattern of rate vs. heterocycle content as in Figure 2A, but the benzimidazole-pyridine series did not, with mono(benzimidazole)-bis(pyridine) ligand **77** proving to be superior, even with respect to optimized tris(benzimidazole) derivatives **11** and **12** reported earlier.⁴ The “mixed ligand” approach therefore provides strong improvement under these conditions, presumably reflecting differences in the partitioning of coordination complexes of copper among active and inactive forms.

The relatively poor performance of catalysts containing **1**, **3**, **8**, and **9** (Figure 2B) suggested that at least one benzimidazole arm is necessary for high catalytic rates in DMSO-rich mixtures. However, paring the ligand down to a single benzimidazolymethylamine motif was ineffective, with **16** and **17** failing to show ligand-accelerated catalysis. Adding a second benzimidazole arm (**18** and **20**) not only did not help, but proved to be inhibitory (rates lower than the ligand-free process; data not shown).

We have previously reported that tripodal ligands such as **2**, **11**, and **12** strongly accelerate the CuAAC reaction even at high ligand:Cu ratios.⁴⁻⁸ This is also true for the best hybrid ligand (**7**) as illustrated in Figure 2C: at a very low Cu concentration (10 μ M), the reaction rate was maximal at [L]:[Cu] = 2:1, but slowed only modestly with 8 equivalents of ligand. It is important to note that tris(benzimidazole) ligands such as **2**, **11**, and **12** do not reliably support catalytic turnover at Cu concentrations less than 50 μ M;⁸ ligand **7** is clearly superior in this regard.

Aqueous Reactions

The above experiments, as well as our original kinetics measurements,⁴⁻⁸⁻⁹ were conducted in 80% DMSO and 20% water in order to keep all components of the reaction soluble, including both hydrophilic ascorbate and hydrophobic reactants and products. Since the CuAAC reaction has found frequent application in aqueous media, we tested various Cu-ligand combinations in mixtures of 10% DMSO and 90% water or buffer.¹⁰ Most of the experiments were performed with 0.1 mM azide and 0.2 mM alkyne to simulate the conditions at which bioconjugation reactions are done in our laboratory, with the results shown in Figure 3.

The pattern of relative reactivities for ligands **1–10** under aqueous conditions was significantly different than in 80% DMSO, when compared at their respective optimal ligand:Cu ratios (Figure 3A vs. 2B). While hybrid ligands **7** and **10** maintained high activity in both solvent systems, **8** and **9** (and, to a lesser extent, **5** and **1**) achieved excellent rates

only in the absence of large quantities of DMSO. A survey of reaction rate as a function of ligand:metal ratio revealed three classes of catalysts in water. Class I was comprised of four ligands that maintained strong activity when used in a two- or four-fold molar excess relative to Cu, in sharp contrast to their behavior in DMSO (ligands **1**, **9**, **13**, and **15**; Figure 3B1 vs. 2C and reference 8).¹¹ Class II contains ligands that show a peak in activity at ligand:Cu ratios of 0.5 or 1.0, but lose most of that activity when used in greater amounts (ligands **7**, **8**, **10**, **11**, **14**, and, to a lesser extent, **4**; Figure 3B2). Class III consists of relatively inactive systems over all ligand:metal ratios, including tripodal ligands **2**, **3**, **5**, and **6** (Figure 3B2). These behavioral classes track closely with the relative affinities for Cu^I displayed by the ligands and determined by the number and metal-binding powers of the heterocyclic arms (triazoles << pyridines < benzimidazoles).⁸

Binding Comparisons

We performed two experiments to test the assumption that the Class II mixed pyridine-benzimidazole ligand **7** binds Cu^I more tightly than a Class I tris(triazole) ligand such as **1**. First, a solution of **1**, **7**, and CuSO₄ (200 μM each) in the presence of 5 mM sodium ascorbate in 1:9 DMSO:H₂O was analyzed by electrospray mass spectrometry. In this mixture, more than 90% of the Cu^I was bound by **7** (Supporting Information). Second, the amount of triazole formed in the presence of sequentially added ligands was determined by aliquot quenching and LC-MS analysis, as shown in Figure 4. In 10% aqueous DMSO containing relatively low concentrations of azide and alkyne, two equivalents of ligand **1** per Cu atom accelerated the CuAAC reaction whereas **7** was an inhibitor (Figure 4A), the latter consistent with data shown in Figure 3B. When **7** was added after five minutes to either a ligand-free reaction or to a reaction containing **1**, a brief jump in activity was observed before triazole formation ceased. These bursts of activity reflected the transient formation of a ligand-accelerated catalyst before an inhibitory equilibrium was reached. The same experiments performed in 80% DMSO showed none of these inhibitory features (Figure 4B), with the Cu(**7**)₂ stoichiometry forming a more active catalyst whether or not **1** was present. Thus, in both inhibitory and acceleratory modes, in water or DMSO, **7** bound Cu^I with higher affinity than its triazole counterpart **1**.

Solvent Effects

The loss of catalytic activity for a variety of ligands when used in two-fold excess relative to copper in 90% water (Figure 3B) was quite striking, as this phenomenon had not been observed in extensive studies in 4:1 DMSO:water (Figure 2C).⁸ In order to further explore this solvent effect, we performed the standard CuAAC reaction in mixtures containing 20% water, 10% DMSO, and 70% of a polar co-solvent (a consistent formulation that kept all reactants and products dissolved). As shown in Figure 5, DMSO, N-methylpyrrolidone (NMP), and N,N-dimethylformamide (DMF) were the only solvents in which reaction rates were not greatly diminished at a 2:1 ligand:Cu ratio relative to the rates at 1:1 and 0.5:1 ratios. These outcomes correlated better with the basicity parameter β' (representing the free energy of potassium ion transport corrected for solvent polarity) than with the dielectric constant of the solvents (Figure 5).¹² Therefore, we suggest that DMSO, NMP, and DMF (as well as alkyne, see Supporting Information) competitively bind Cu^I, preventing the strongly chelating ligands from forming inhibitory complexes and keeping weakly chelating ligands from the metal. Acetonitrile, a more strongly coordinating solvent due to its π-accepting ability, largely inhibited the catalytic activity of both the Cu•**7** and the Cu•**1** systems under these conditions. Conversely, water was the only solvent in which an excess of ligand (2:1 ligand:Cu ratio) inhibited the CuAAC reaction relative to the ligand-free rate.

Kinetic Rate Orders

The rate order of the CuAAC reaction in catalyst was determined with a constant [L]:[Cu] ratio. For the most effective hybrid ligand, **7**, the reaction was found to be approximately third order in (Cu)(L) in 80% DMSO, compared to first order in 90% water (Figure 6).¹³ Under the latter conditions, the Cu•**7** system behaved the same regardless of whether 0.5 or 1 equivalent of ligand was employed, whereas the rate order in Cu•**1** was sensitive to the [L]:[Cu] ratio (Figure S10).

Discussion

No single Cu(I) complex has been identified as an optimal catalyst or precatalyst for CuAAC reactions under all conditions. Based on the above results, two general categories of reaction media can be identified: those containing molecules that have affinity for Cu^I metal centers, including solvents such as DMSO, DMF, and NMP (“strong-donor conditions”), and those poor in such molecules, such as solvents dominated by water (“weak-donor conditions”). The best ligands for these two conditions likewise are distinguished by their Cu^I binding ability. For example, class I ligands such as **1**, **8**, and **9** contain no benzimidazoles and are therefore relatively weak binders. As a result, DMSO can compete with these ligands for Cu binding sites, diminishing ligand-based acceleration (Figure 2B). However, under weak-donor conditions, these class I ligands form the most productive Cu(I) complexes (Figure 3A). Class II ligands such as **7** are stronger chelators, containing two or more benzimidazole or pyridine groups, and therefore can maintain productive Cu binding in the presence of competing solvent donors such as DMSO (Figure 2B). In water, however, these ligands block access to the metal center when used in 1:1 or greater ligand:Cu ratios (Figure 3B2, 3C). Class I ligands are apparently incapable of forming such inhibitory complexes and remain active at high [L]:[Cu] ratios (Figure 3B1).

The behavior of the one- and two-armed ligands is also instructive. The mono(benzimidazole-methyl) amines **16** and **17** did not affect CuAAC reactions in either aqueous or organic media, showing that these molecules cannot bind copper in either a productive or an inhibitory manner. Adding another benzimidazole group produced chelates (**18** and **20**) that strongly suppressed the CuAAC reaction at a 1:1 ligand:Cu ratio even in 80% DMSO (data not shown). The bis(benzimidazole-methyl)amine motif therefore must trap Cu^I in an inactive form.⁴⁻¹⁴ Ligands **5** and **6**, which contain this motif, are thereby at a disadvantage at higher overall Cu-ligand concentrations (Figure 2A). This putative coordination trap can be relieved in a manner dependent on the conditions. In strong-donor solvents, adding a benzimidazole arm (**2**) or replacing one of the benzimidazole arms with triazole (**4**) or pyridine (**7**) provides much more active catalysis at higher concentrations (Figure 2A).⁴⁻¹⁴ Under weak-donor conditions, replacing a benzimidazole with triazole or pyridine, and using lower ligand:Cu ratios, gives the same result (Figure 3B2).

Proposed Mechanism

Consideration of the ligand-accelerated CuAAC reaction mechanism must include the concepts that inhibitory species can attenuate catalyst performance and that solvent coordination can be important. Both points arise in part from the fact that organic azide, a very weakly coordinating ligand, needs access to the metal center. Figure 7 summarizes our speculation concerning the types of Cu complexes that may be present and the different possibilities for catalytic cycles that are consistent with our experimental findings, although many possibilities exist for the details of coordination and structure. We suggest that the distributions among the various types of complexes are determined by the ligand structure and the donor-acceptor properties of the solvent or other additives, as indicated by the blue

notations, and that these distributions determine many of the behavioral features of the CuAAC reaction under different conditions.

Ligand-free Cu^I complexes are relatively inactive and are in equilibrium with mononuclear complexes such as **23**, which are not optimal because they lack the activating power of a second Cu center.¹⁵ Binuclear species having a ligand:Cu ratio of 2:2 (or higher), such as **24**, are inactive, since their coordinative saturation makes it difficult for azide to gain access to a Cu center. At a minimum, two heterocyclic arms and a center nitrogen donor are necessary to bring two Cu centers together with open coordination sites for azide and alkyne reactants, and indeed, dipodal ligands as **19** and **22** can be somewhat effective (Figure 3B3). However, the versatility of these triazole-containing ligands is limited, since they are easily displaced by donor solvents; conversely, dipodal ligands containing strong donor arms form inhibitory complexes as discussed above.

More reliable access to two Cu centers is provided by a ligand with three ligating arms, as in structure **25** (Figure 7).¹³ Binding of alkyne and azide gives an active structure such as **26**, similar to previous proposals for triazole formation,^{8,15} leading to complex **27**. We suggest that the fate of this species determines the overall kinetic behavior with respect to rate order in Cu and ligand. When competition for Cu binding is minimal (weak-donor environment), the binuclear Cu₂L species remains intact, exhibiting a rate law of the form of Eq. 1, with apparent first-order dependence on the concentration of Cu•ligand complex because no change in molecularity occurs through the catalytic cycle (boxed in Figure 7). This corresponds to the “90% H₂O” plots in Figure 6.

$$\text{rate} = k[\text{Cu}_2 \bullet \text{Ligand}] \quad (1)$$

If, on the other hand, a strong-donor environment induces complex **27** to break up during the catalytic cycle, such as to give a Cu-triazolyl **28** and the mononuclear complex **23**, the reassembly of one ligand and two Cu centers can be required for each turnover, leading to rate equation 2, consistent with the third-order dependence shown in Figure 6 for “80% DMSO” reactions.

$$\text{rate} = k[\text{Ligand}][\text{Cu}]^2 \quad (2)$$

The differing kinetic outcomes shown in Figure 6 therefore do not necessarily suggest the existence of different triazole-forming complexes under the two conditions, but rather two different pathways of catalyst regeneration.

Choosing the Best Catalyst

The studies described above of CuAAC reaction rates under varying conditions and with varying catalysts were spurred by anecdotal reports of occasional failure of this otherwise supremely forgiving transformation and of our own inability to generalize previous findings made in DMSO-rich solvents to other reaction media. We now find it helpful to consider two general types of reaction conditions when selecting an appropriate form of the copper catalyst. “Preparative” reactions are those in which the concentrations of alkyne are greater than 10 mM (often much greater), the concentration of copper is approximately 50 μM making for catalyst loadings of 0.5% or less, and the reaction medium may contain large amounts of DMSO or similar solvents. These are the “strong donor” conditions discussed above. “Weak donor” conditions are often used in bioconjugation reactions, involving substrate concentrations less than 250 μM, catalyst loadings from 20% to greater than 100% relative to alkyne, and solvent mixtures dominated by water. [Note, however, that “strong

donor” conditions may also occur with biomolecules or other substrates in water, if those substrates contain large numbers or high local concentrations of competing donor groups such as histidine imidazoles.]

Ligands with stronger donor arms, such as benzimidazoles and pyridines, are recommended for strong donor conditions, in which donor solvent and/or alkyne balance the coordination equilibria to provide sufficient concentrations of active complexes. The mixed benzimidazole-pyridine ligand **7** is more active than the substituted tris(benzimidazoles) **11** and **12**, remains catalytically active at copper concentrations below 50 μM , and is considerably easier to prepare, so we now recommend it for synthetic applications.

For bioconjugation reactions, either a well-controlled amount of **7**, **11**, or **14** (1:2 ratio to Cu) or excess of triazole-rich ligands such as **9**, **13**, or **15** are recommended. The widest pH range can be accommodated with ligand **7** (see Supporting Information) and, presumably, its analogues in Class II. However, we have most often used the tris(triazolyl) compound **13** at neutral pH because of certain beneficial effects of excess ligand with respect to oxidative byproducts produced in the presence of ascorbate and oxygen, as is described elsewhere.¹⁶

The user of the CuAAC click reaction is therefore encouraged to evaluate each case in terms of competing donor centers that may be present in the solvent, substrates, or additives. The matching of accelerating ligands to the conditions (stronger ligands for “strong donor” situations, weaker ligands for “weak donor” conditions), as well as the proper attention to ligand:metal ratio, should enable the selection of effective CuAAC reactions in many demanding situations.

Conclusions

We have demonstrated that Cu(I) complex of the mono(benzimidazole)-bis(pyridine) ligand **7** is a superior catalyst for CuAAC under synthetic organic conditions. At ligand:Cu ratios greater than 1, ligand **7** can be inhibitory due to the blocking of coordination sites on the metal, but such deactivating interactions are disrupted by donor solvents such as DMSO and NMP, or by high concentrations of alkyne. Under aqueous conditions, the use of weaker ligands is therefore recommended, minimizing the tendency to form inhibitory copper chelates. In strong donor environments, however, strong ligands are needed to bring two copper centers together to form catalytically active complexes.

Experimental Section

Synthesis procedures and characterization data for the ligands are given in Supporting Information. All kinetic procedures and analyses were performed as described in references 4 and 9. Electrospray-ionization mass spectrometry of Cu complexes was performed in positive ion mode on samples in 9:1 water: DMSO, injected directly into the spray chamber of an Agilent 1100 (G1946D) instrument being eluted with 9:1 MeCN:water containing 0.1% TFA. Changing the carrier composition or the sample solvent system did not affect the spectra in a meaningful way. Ascorbate stock was added one minute prior to analysis to allow reduction of the Cu(II) species. The Cu(I)-ligand complexes were usually the major ions observed, but their Cu(II) counterparts were always detected, possibly due to re-oxidation in the liquid handler and MS spray chamber. DMSO dimers with a proton or a sodium ion were observed in all spectra. Oxidation of the ligands ($M+16$) was noticeable on several occasions, a phenomenon that is discussed elsewhere.¹⁶ The addition of phenylacetylene to mixtures containing ligand **7** did not affect the mass spectral composition.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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10. Potassium phosphate buffers are ideal as they cover a wide pH range and do not contain chloride, which can be inhibitory at high concentrations.
11. Compound **15** was prepared in an attempt to make **9** more water-soluble with hydroxypropyl side chains, in the same way that **13** is a derivative of **1**.
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13. In the ligand-free CuAAC reaction, terminal alkynes form binuclear complexes which have been isolated as yellow precipitates (Buckley, B.R.; Dann, S.E.; Heaney, H. *Chem. Eur. J.* **2010**, *16*, 6278–6284). Such complexes were not observed in reactions accelerated by either class I or class II ligands.

14. The attempted synthesis of **13** by triple CuAAC condensation of 3-azido-1-propanol with tripropargylamine is very difficult to push past the bis(triazole) stage in water, presumably because the (triazole-CH₂)₂N-propargyl intermediate is inhibitory. The details of this phenomenon in the context of the preparation of **13** is described in Supporting Information of reference 16.
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Figure 1.

Ligand structures. (**1–3**) “Parent” ligands for CuAAC reactions. (**4–10**) “Hybrid” ligands based on 1,4-triazole (red), benzimidazole (black), and pyridine (blue) groups. (**11–13**) Ligands previously shown to be strongly accelerating. (**14, 15**) Modified hybrid ligands. (**16–22**) Truncated ligands containing fewer than three heterocyclic arms. Abbreviations below the compound numbers refer to the nature of the heterocyclic arms: Trz = triazole, Bim = benzimidazole, Py = pyridine.

Figure 2.

Reactions in 4:1 DMSO:H₂O. (A) Maximum heat output (mW) by calorimetry for two series of reactions catalyzed by Cu complexes of hybrid ligands. Conditions: [BnN₃] = 360 mM, [PhCCH] = 450 mM, [CuSO₄] = 2 mM, [Ligand] = 2 mM or 4 mM, as indicated. (B) Observed second-order specific activities for CuAAC reactions under more dilute conditions by aliquot quenching and LC-MS analysis: [BnN₃] = 1 mM, [PhCCH] = 20 mM, [CuSO₄] = 50 μM, [Ligand] = 100 μM. (C) Observed second-order specific activities for CuAAC reactions accelerated by **7** as a function of ligand:copper ratio. Conditions: [BnN₃] = 1 mM, [PhCCH] = 20 mM, [CuSO₄] = 10 μM, [Ligand] = varied. For these and all other kinetic data, see Supporting Information for details of ascorbate concentration, pH, and temperature.

Figure 3.

Reactions in 9:1 H₂O:DMSO. (A) Observed second-order specific activities: [BnN₃] = 100 μM , [PhCCH] = 200 μM , [CuSO₄] = 50 μM , [Ligand] = 25 μM . (B) Comparison of hybrid ligands at different ligand:copper ratios under the same conditions. The ligands are grouped by class and color coded according to their heterocyclic content: black = two or three benzimidazoles in the structure; dark blue = two or three pyridines; red = two or three triazoles; light blue = one of each heterocycle. (C) Comparison of ligands 7 and 1 under bioconjugation conditions.

Figure 4.

Sequential ligand addition experiments. (A) CuAAC reactions in 10% aqueous DMSO were initiated with 50 μM CuSO_4 and 100 μM of the first indicated ligand. At 5 minutes, a concentrated solution of the second ligand was added to a final concentration of 100 μM (vertical dotted line). (B) As in part (A) with reactions performed in 80% DMSO. For both sets of experiments, $[\text{BnN}_3] = 100 \mu\text{M}$, $[\text{PhCCH}] = 200 \mu\text{M}$.

Figure 5.

CuAAC rates with variations in solvent and ligand:metal ratio. Observed second-order specific activities for reactions accelerated by Cu•(7) in 20% H₂O, 10% DMSO, and 70% polar organic co-solvent. [CuSO₄] = 50 μM, [7] = variable, [BnN₃] = 100 μM, [PhCCH] = 200 μM. The ligand:Cu ratio in each solvent was varied from 0 to 2, as indicated. The donor abilities of the polar organic co-solvent are given by the basicity parameter β' (representing the free energy of potassium ion transport corrected for solvent polarity).^{12b} Values for NMP were estimated from data reported in reference 12a.

Figure 6.

Determination of rate order in $[\text{Cu}\cdot 7]$ in 4:1 and 1:9 mixtures of DMSO:H₂O. $[\text{Cu}]$ was varied from 10 μM to 200 μM , $[7]$ was varied according to the given ratio, $[\text{BnN}_3] = 100$ μM or 1 mM in 80% DMSO and 100 μM in 90% H₂O, $[\text{PhCCH}] = 2 \times [\text{BnN}_3]$. In 80% DMSO, the reactions containing <20 μM Cu stopped before completing many turnovers, so initial rates are reported for these experiments. The correlations for reactions in 80% DMSO should therefore be regarded as approximate determinations of catalytic rate order.

Figure 7.

Proposed mono- and binuclear speciation in the CuAAC process. X = anionic donor such as σ -acetylide, halide, hydroxide, or triazolide; S = neutral intermolecular donor such as DMSO, other solvent, π -alkyne, or organic azide. Productive complexes are not formed when only one heterocyclic donor arm (L) exists.