Angewandte
Eine Zeitschrift der Gesellschaft Deutscher Chemiker Chemie

Supporting Information

69451 Weinheim, Germany

On the Mechanism of the Ligand-Free Cu(I)-Catalyzed Azide-Alkyne Cycloaddition Reaction

Valentin O. Rodionov, Valery V. Fokin,* and M.G. Finn*

General.

Liquid sample handling for kinetics experiments was performed with a Gilson model 735 coordinate liquid handler placed in a nitrogen-purged polycarbonate box. Reaction solutions containing azide and alkyne were freshly prepared outside the box before use. Ascorbate was added immediately before closing the box, and stirring was started only after purging with nitrogen for approximately 15 minutes. Aqueous $CuSO_4$ was added by the robot to start the reaction; the total volume of each reaction solution was 5 mL.

Aliquots of 100 μL were taken at 2-5 min intervals by the robot and transferred to a deep-well microtiter plate containing 100 μL of 30% H_2O_2 in each well. After completion of the run, 500 μL EtOH was added to each well. The plate was then sealed with a silicone mat or with Polarseal foil and shaken manually or on a shaker. There was usually a fair amount of solid material (composed mostly of triazole product) precipitated in the wells, which took time to dissolve. The resulting plates containing homogeneous solutions in each well were stored in the freezer. While the material does not appear to precipitate on freezing, the plates were allowed to warm to room temperature before each analysis and were found to contain homogeneous solutions.

For runs starting with 10 mM or less azide and alkyne, samples were transferred directly to standard polystyrene microtiter plates and analyzed by LC-MS. Samples at higher concentrations of organics were diluted 10-50 times with EtOH (the dilution factor being chosen to get the best chromatograms) before being transferred to standard plates for analysis. The best reproducibility was achieved with concentrations that gave peak intensities of approximately 30,000-50,000 counts in scan mode.

LC-MS was performed on an Agilent model 1100 MSD instrument, equipped with a 50 mm C_{18} column (Vydac 238MS5505, or Agilent Zorbax SB-C18). The elution gradient was typically 60-70% water:MeCN (0.5%TFA) to 100% MeCN (0.5% TFA) over 5-6 minutes. Detection was performed mainly in SIM (single ion) mode, which allows one to comfortably work with concentrations as low as 0.1 mM. Initially, UV detection and mass spectral scan modes with software ion extraction were used, but these methods introduced significant error. Extensive calibrations were performed to relate integrated peak intensities to concentration, and to verify run-to-run reproducibility. The use of **3**–d_7 as internal standard made only marginal improvements in the already excellent accuracy and reproducibility of the data. However, it is important to note that small amounts of **3**–d_7 added as internal standard did not inhibit the reaction, but rather seemed to prevent the accumulation of less reactive species at higher concentrations of copper (see Figure S10).

Kinetics. Plots for each of the entries of Table 1 are shown below.

Figure S1.

Table 1, Entry 1. Pseudo-first order kinetics; excess Cu, varying [alkyne], in the presence of deuterated product as internal standard (0.08 mM)

[$CuSO_4$] = 10 mM, [Na Asc] = 40 mM, [$PhCH_2N_3$] = 0.04 mM, [PhCCH] = 0.4, 0.6, 0.8, 1.0 mM

rate order = 1.3 ± 0.2

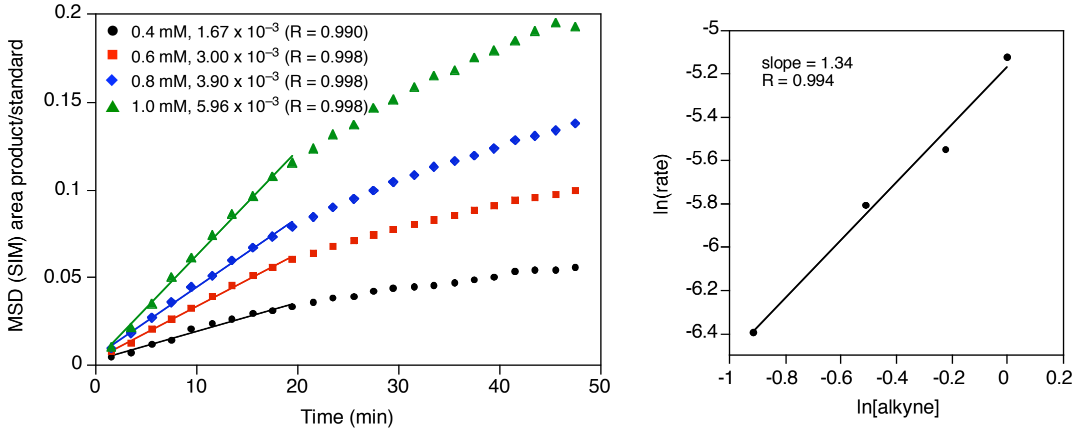
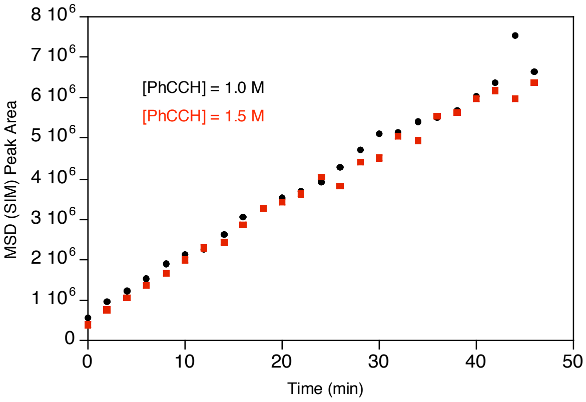


Figure S2.

Table 1, Entry 2. Pseudo-first order kinetics; catalytic Cu, varying [alkyne].

[$CuSO_4$] = 5 mM,
[$PhCH_2N_3$] = 100 mM, [Na Asc] = 20 mM
[PhCCH] = 1.0, 1.5, 2.0, 2.5 M
(note: the last two runs do not appear here, but overlay the data shown)

rate order = 0



Note: Data for Table 1, entry 3 looks very similar to Figure S2, revealing a zero-order dependence on Cu.

Figure S3

Table 1, Entry 4. Pseudo-first order kinetics; stoichiometric Cu, varying [azide], with deuterated product as internal standard (0.08 mM); two independent experiments.

[$CuSO_4$] = 10 mM, [Na Asc] = 40 mM, [PhCCH] = 0.04 mM, [$PhCH_2N_3$] = 0.4, 0.6, 0.8, 1.0 mM

rate order = 1.0 ± 0.2 for both

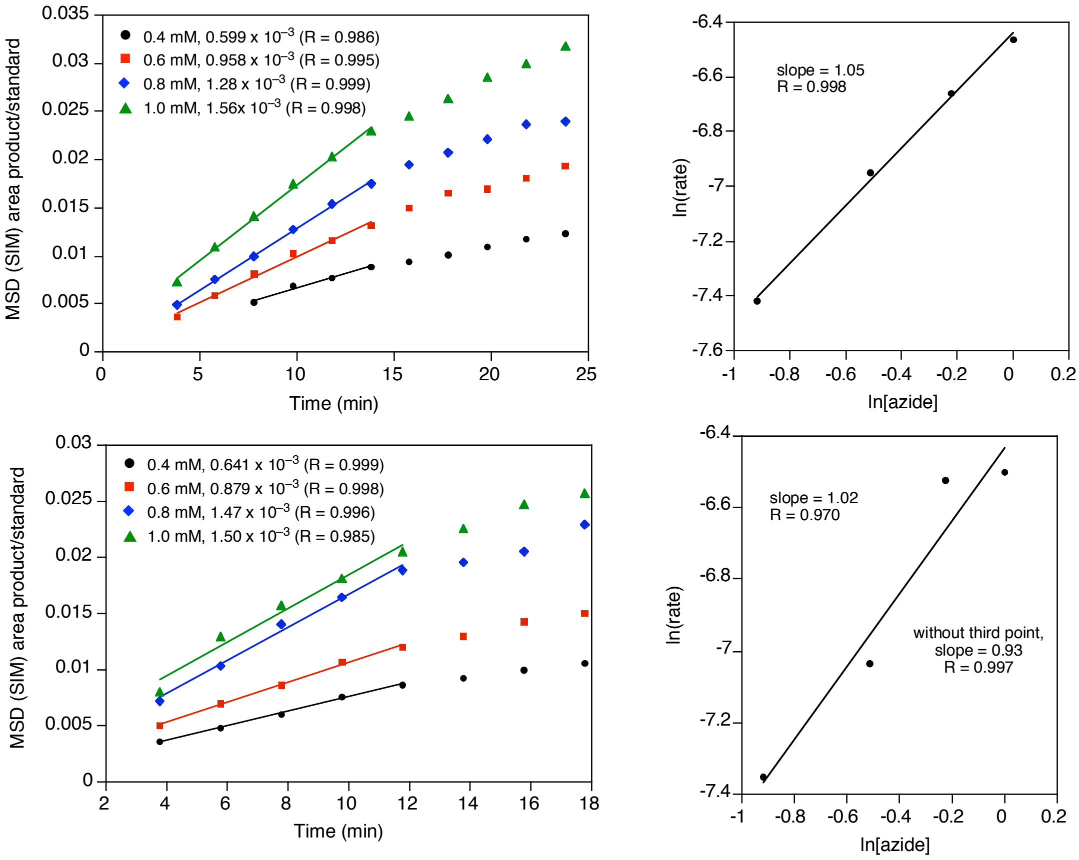


Figure S4.

Table 1, Entry 5. Pseudo-first order kinetics; stoichiometric Cu, varying [azide].

[$CuSO_4$] = 10 mM,
[PhCCH] = 1 mM, [Na Asc] = 40 mM
[$PhCH_2N_3$] = 10, 15, 20, 25 mM

rate order = 0

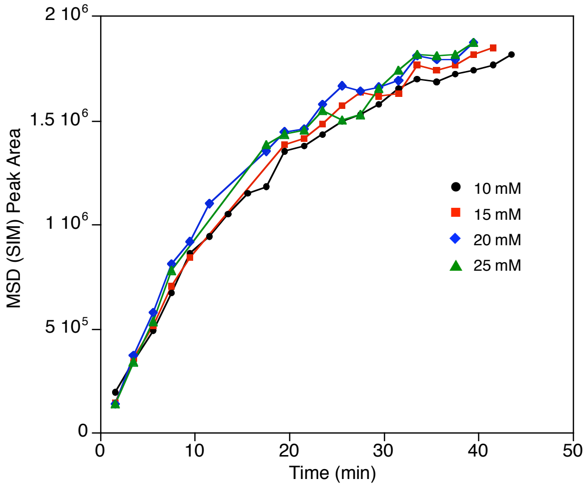


Figure S5.

Table 1, Entry 6. Pseudo-first order kinetics; stoichiometric Cu, varying [azide].

$[CuSO_4]$ = 0.1 mM, [PhCCH] = 1 mM, [Na Asc] = 40 mM
$[PhCH_2N_3]$ = 10, 15, 20, 25 mM

rate order = –0.23 ± 0.1

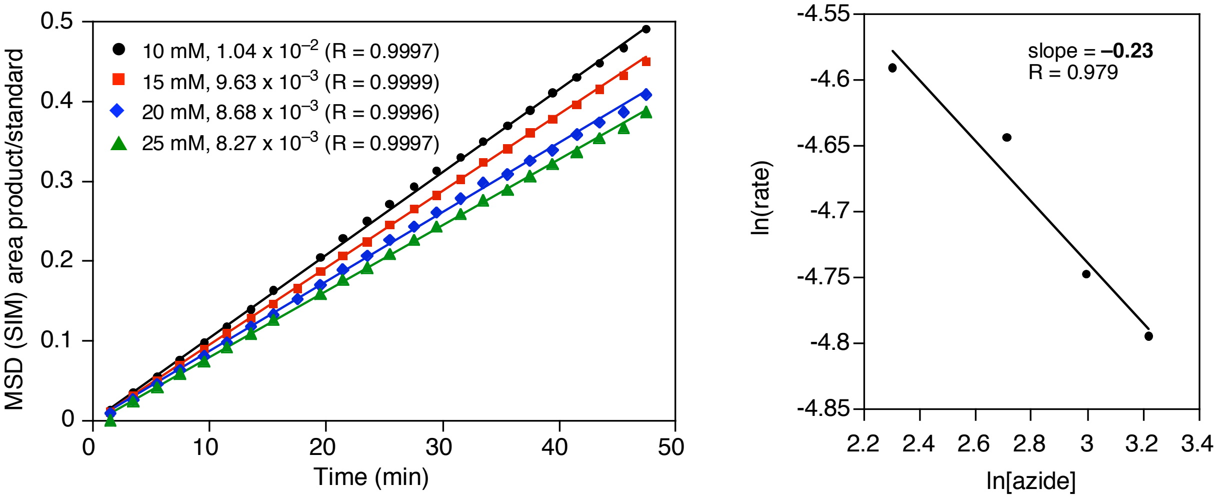


Figure S6.

Table 1, Entry 7. Pseudo-first order kinetics; varying excess Cu.

$[CuSO_4]$ = 1, 2.5, 5, 10 mM; [PhCCH] = 1 mM; [Na Asc] = 20 mM; $[PhCH_2N_3]$ = 1 mM

rate order ≈ 0.1

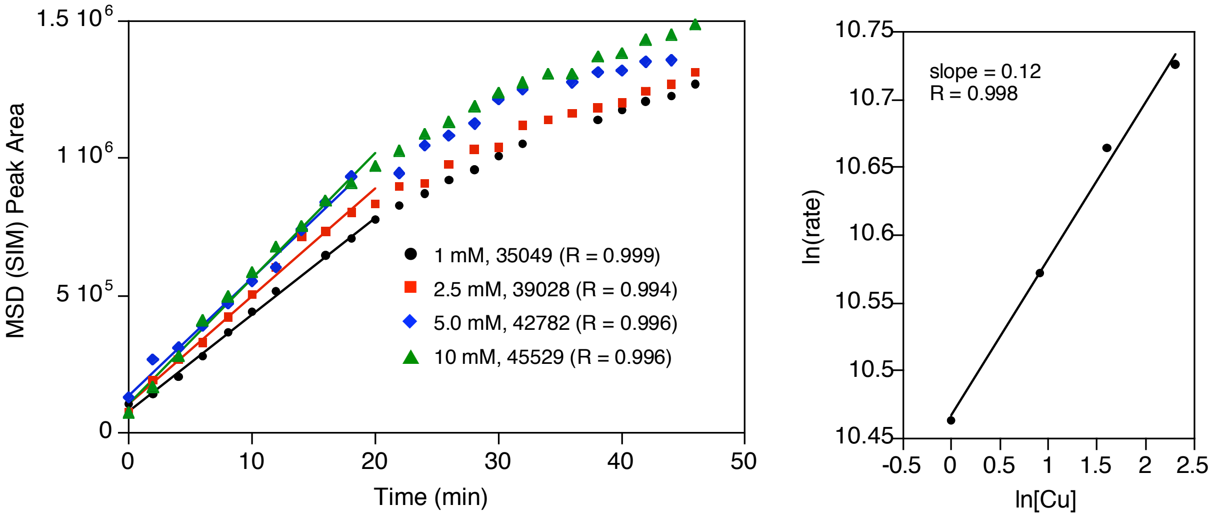


Figure S7.

Table 1, Entry 8. Varying catalytic Cu

[$CuSO_4$] = 0.5, 1.0, 1.5, 2.0 mM; [PhCCH] = 10 mM; [Na Asc] = 20 mM; [$PhCH_2N_3$] = 10 mM

rate order ≈ 0.6 ± 0.2

Note: *At 100 mM in organic reagents, variation of [Cu] between 5-20 mol% also gave a rate order in copper of approximately 0.5. However, when [Cu] was raised to 40 mol%, the reaction was accelerated dramatically, and the resulting rate did not fit a 0.5 order profile in Cu. In this case, excessive precipitation was observed, and the observed acceleration was likely due to a change in the nature of the catalytic species.*

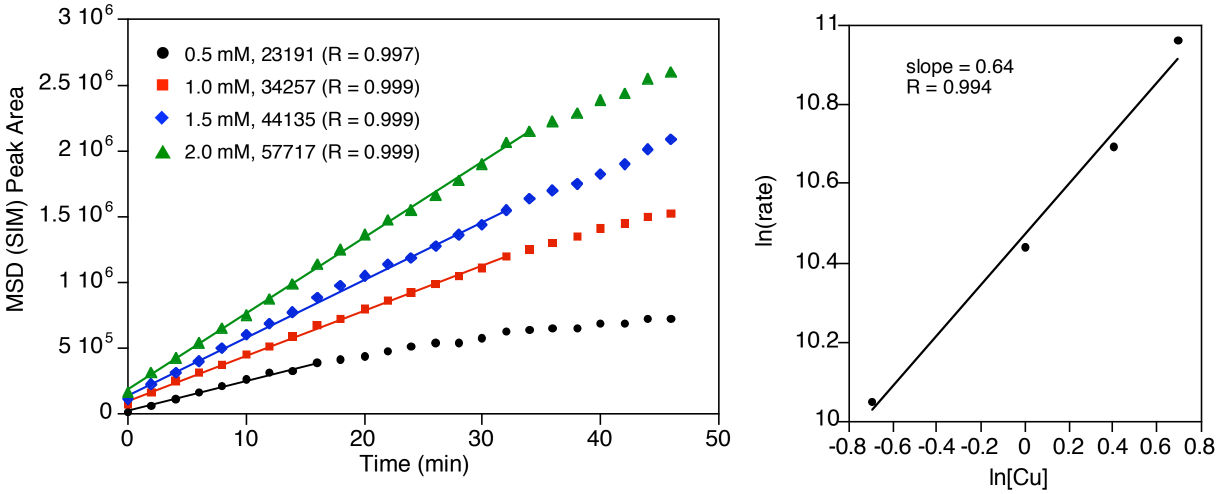
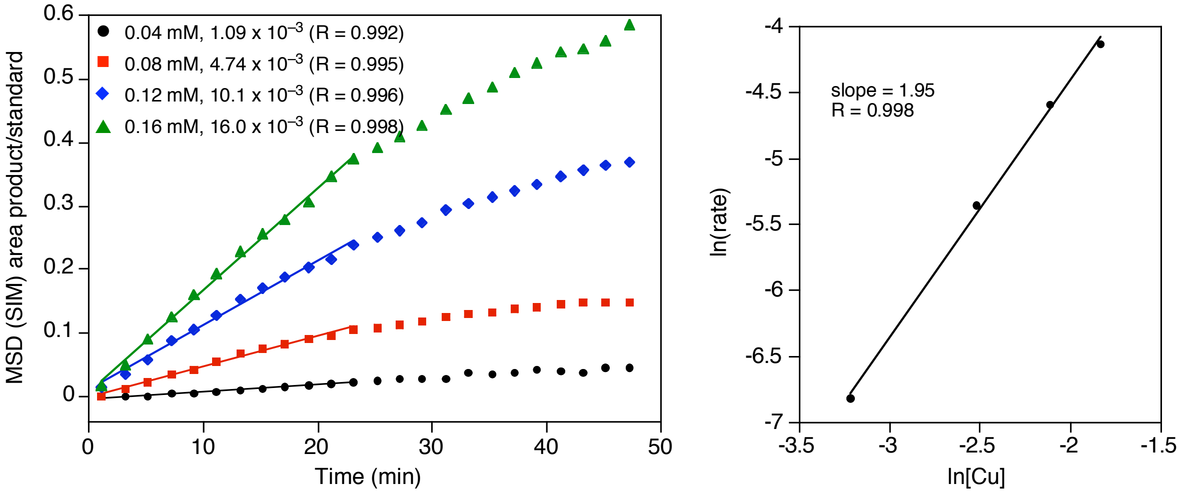


Figure S8.

Table 1, Entry 9. Varying catalytic Cu
with added deuterated product (triazole-d_7, 0.08 mM) as internal standard

[$CuSO_4$] = 0.04, 0.08, 0.12, 0.16, mM; [PhCCH] = [$PhCH_2N_3$] = 0.4 mM; [Na Asc] = 40 mM

rate order = 2.0 ± 0.1



Additional kinetics. Experiments not described in the main text are shown below.

Figure S9.

Relates to Table 1, entry 1. Pseudo-first order kinetics; excess Cu, varying [alkyne].

$[CuSO_4]$ = 10 mM, $[PhCH_2N_3]$ = 0.04 mM, [Na Asc] = 40 mM, [PhCCH] = 0.4, 0.6, 0.8, 1.0 mM

rate order = 1.5 ± 0.2

Shown here is the first 50 min of data (rate order 1.6); plotting only the first 20 minutes gave rate order = 1.4 (R = 0.988).

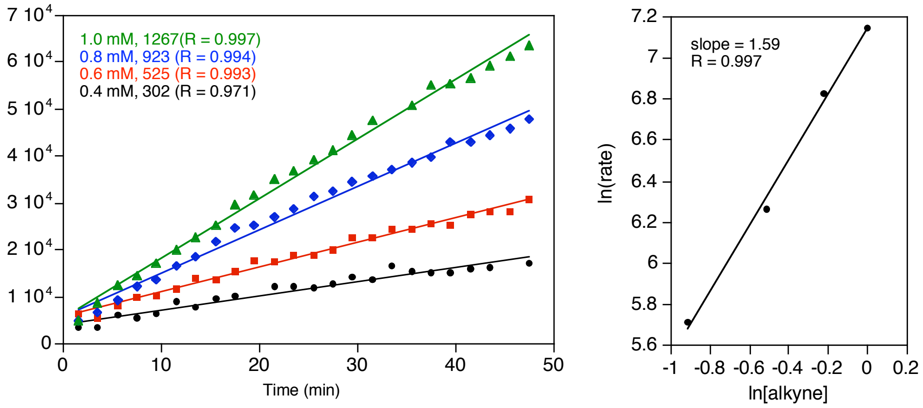
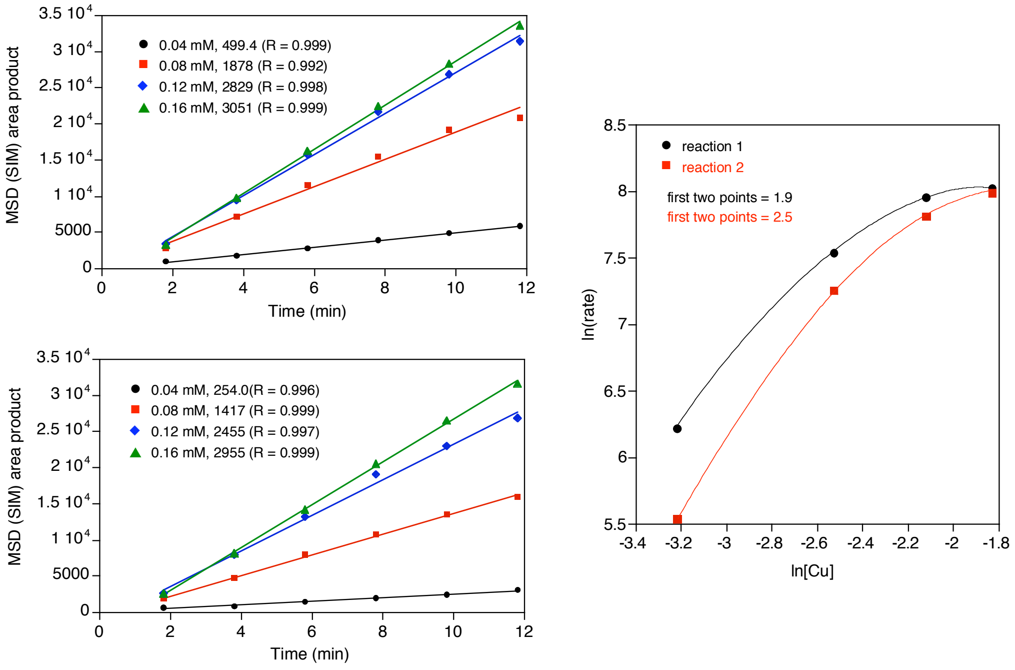


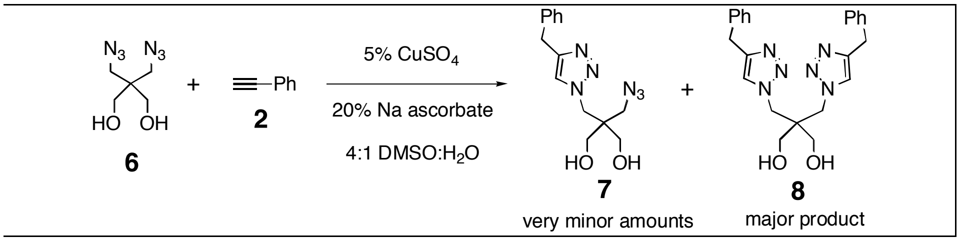
Figure S10.

Relates to Table 1, entry 9. Varying catalytic Cu ***without*** added deuterated product. This shows that small amounts of **3**–d_7 did not inhibit the reaction, but rather seemed to prevent the accumulation of less reactive species at higher concentrations of copper

$[CuSO_4]$ = 0.04, 0.08, 0.12, 0.16, mM; [PhCCH] = $[PhCH_2N_3]$ = 0.4 mM; [Na Asc] = 40 mM



Diazide reactivity



Important experiments:

1. Plain kinetics curves of mono- and di- triazole formation in the absence of ligand (Figure S11) showed no autocatalytic features. Two peaks were observed in the LC-MS with the mass of the monotriazole. The authentic compound was prepared independently (see below) and its peak assigned; this species appeared as a minor component and then did not decrease in intensity during the reaction. The overall reaction from **6** was not especially fast. The behavior was the same in both *t*-BuOH:H_2O and DMSO:H_2O.

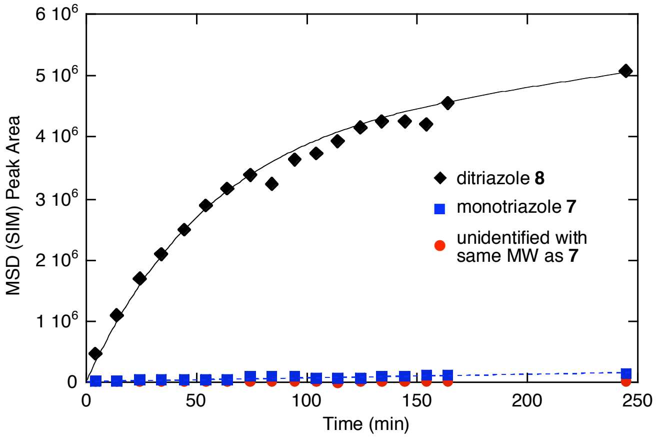


Figure S11.

Typical reaction profile using **6** at 100 mM in DMSO:water. Note that the reaction is not complete in 4 hours.

2. When tris(triazolyl)amine ligand **18** was added to the reaction, the rate increased dramatically, but the ratio of mono- and ditriazole products remained unchanged (Figure S12).

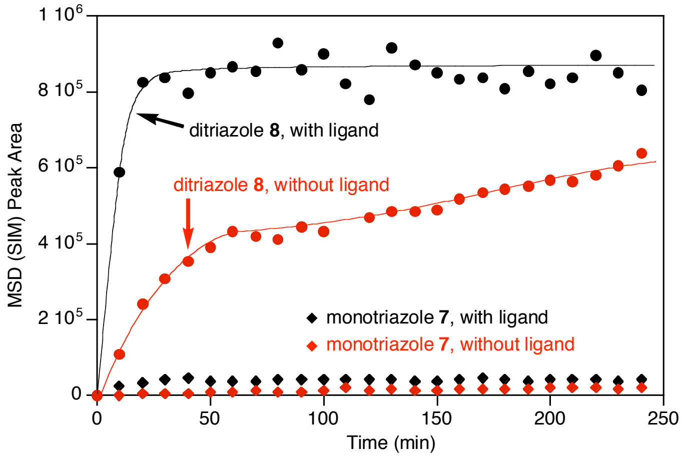


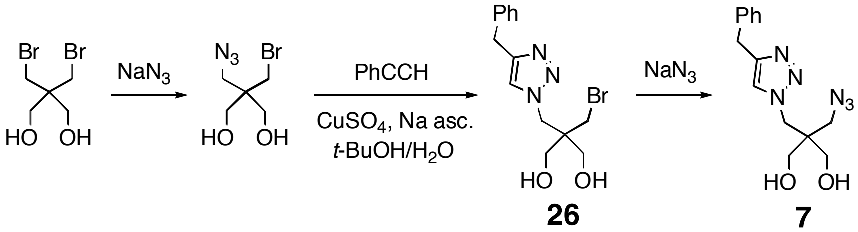
Figure S12.

Reaction of **6** in the presence and absence of tris(triazolyl)amine ligand.

3. Increasing the ratio of **6**:**2** increased the content of mono(triazole), but only slightly – it is not practical to make **7** this way. Even at a 16:1 ratio (**1**:PhCCH), the ditriazole **8** was the major product, with the mono(triazole) peak area in UV being approximately 25% of the peak area for the ditriazole.

4. Authentic mono(triazole) **7** was made from pentaerythritol dibromide as shown in Scheme S1, and described below.

Scheme S1



26 **7**

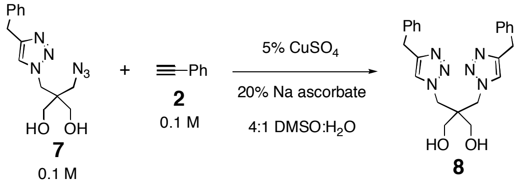
a. *2-(bromomethyl)-2-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)propane-1,3-diol (**26**).* A mixture of 2,2-bis(bromomethyl)propane-1,3-diol (50 g, 191 mmol), tetrabutylammonium bromide (0.5 g) in dry DMSO (300 mL), and NaN$_3$ (12.35 g, 190 mmol) was stirred under nitrogen for 24 hours at 70°C under N$_2$; the NaN$_3$ did not appear to completely dissolve. The reaction mixture was cooled to room temperature and filtered. Phenyl acetylene (22 mL, 20.4 g, 200 mmol), sodium ascorbate (50 mL of a 10% w/v solution in water, 25 mmol), and CuSO$_4$•5H$_2$O (50 mL of a 10% w/v solution in water, 20 mmol) were then added, in that order. The resulting yellow heterogeneous mixture was stirred under N$_2$ at 60°C for another 24 hours. The mixture was cooled to room temperature, treated with 30 mL 30% H$_2$O$_2$, diluted with 200 mL dichloromethane, and washed successively with H$_2$O (400 mL) and saturated aqueous sodium EDTA (2 x 100 mL). The organic layer was dried over anhydrous MgSO$_4$ and the solvents removed by rotary evaporation. The products ditriazole **8** and the triazole-bromide were incompletely separated by column chromatography (silica gel, 5% MeOH in CH$_2$Cl$_2$), with pure fractions taken on to the next step.

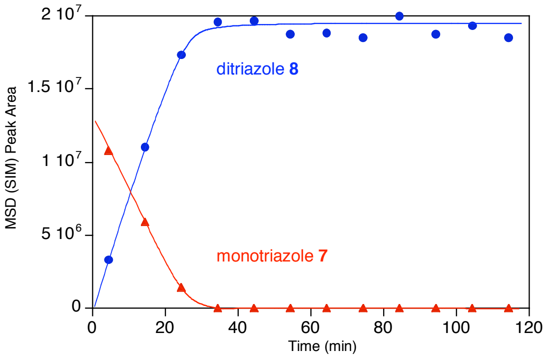
b. *2-(azidomethyl)-2-((4-phenyl-1H-1,2,3-triazol-1-yl)methyl)propane-1,3-diol **7***. Compound **26** (3.5 g,10.7 mmol) and *n*-Bu$_4$NBr (100 mg) were dissolved in dry DMF (50 mL) under N$_2$, and NaN$_3$ (2.6 g, 40 mmol) was added. The mixture was stirred at 100°C for 48 h, then cooled to room temperature and washed with water (5 x 100 mL). The desired product **7** was crystallized from dry ethanol, yielding 1.2 g (38% yield). ESI-MS: m/z 289.1, 290.1 (M+1$^+$), 311.1 ((M+Na)$^+$). ^{1}H NMR (200 MHz, CD$_3$CN): δ 8.23 (s, 1H), 8.01-7.96 (m, 2H), 7.62-7.50 (m, 3H), 4.53 (s, 2H), 3.53-3.49 (m, 4H), 3.22 (t, 2H).

5. The formation of ditriazole **8** from **7** is fast (Figure S13), but not fast enough to account for the exclusive production of **8** directly from diazide **6**.

Figure S13.

Conversion of **7** to **8** without ligand; at 0.1 M starting **7**, the reaction is complete within 30 minutes.





6. Competition experiments were performed between **7** and benzyl azide, in the presence of a limiting amount of PhCCH. In the presence of ligand, the two azides reacted at about the same rate, such that approximately 50% of **7** was consumed and the formation of **8** and **3** stopped at the same time (Figure S14). Comparison of the individual kinetic curves shows that (a) *without ligand*, **7** reacts much slower in the presence of benzyl azide than it does in the absence of benzyl azide (compare Figure S13 to the "no-ligand" curves of Figure S14), and (b) benzyl azide reacts approximately as fast in the presence of **7** as it does on its own, for reactions both in the presence and absence of ligand (benzyl azide-only runs not shown).

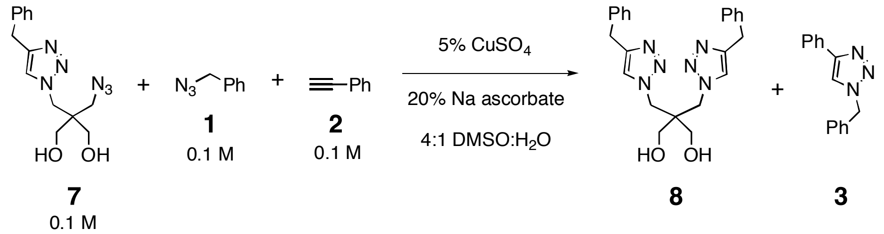
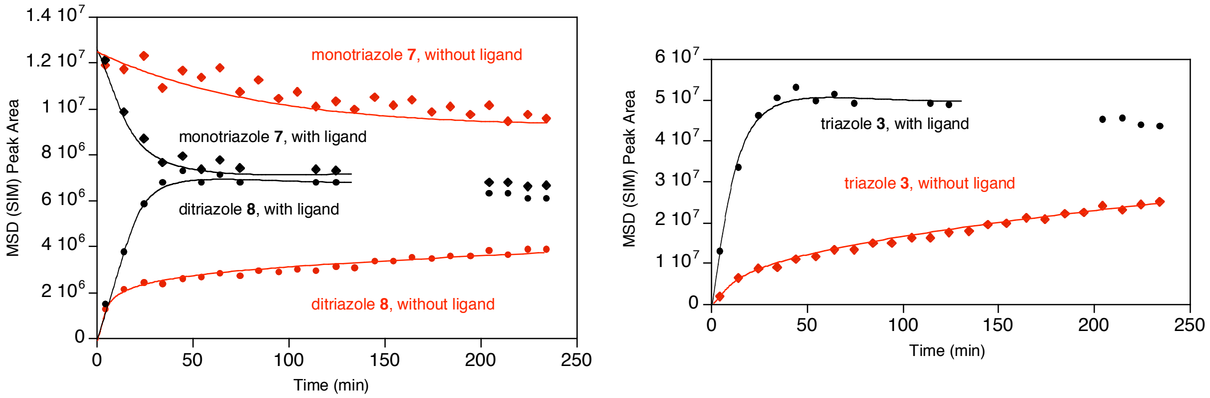


Figure S14. Competition experiment: (*left*) disappearance and formation of **7** and **8**, respectively; (*right*) formation of **3** in the same solution. Note that only approximately half of the mono(triazole) **7** is consumed, and that the reaction of **7** without ligand is much slower (or goes much less far) than the standalone reaction (Figure S13).

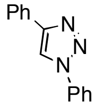


7. Reactions involving diazide **6**, benzyl azide, and phenyl azide were performed and product concentrations at completion were determined by LC-MS with quantitative calibration using authentic samples. The results are summarized in Table S1. The following observations are noteworthy.
   (a) Diazide **6** and either of the monoazides (**1** or PhN_3) are competitive for a limiting amount of alkyne (entries 1, 2, 4, 5).
   (b) Monotriazole-monoazide **7** is also roughly competitive with **1** or PhN_3 (entries 6, 7).
   (c) Benzyl azide (**1**) is more reactive than PhN_3 in a direct comparison (entry 3) and in competition reactions (entries 1 vs. 2, 4 vs. 5, 6 vs. 7).

The independent measurements of rates of **1**, **6**, and **7** with alkyne showed **7** to be much the most reactive (see above). Thus, entries 6 and 7 of Table S1 should show a much greater predominance of **8** in the product mixtures. Since this is not observed, **1** and PhN_3 must act as inhibitors of the conversion of **7** to **8**. Furthermore, monotriazole **7** was not observed to build up in entries 1 and 2, in spite of the fact that its conversion to **8** is inhibited by **1** or PhN_3. This supports the hypothesis that free **7** is not an intermediate in the dominant pathway from **6** to **8**.

Table S1. Results of reactions using the indicated reagents in 2:1 DMSO:H_2O, in the presence of 5 mM CuSO4 and 40 mM Na ascorbate.

Entry	Reactants [a]	Products [b]	% yield [c]
1	**6** + **1** + **2**	**8** (10.4) + **3** (25.4)	92
2	**6** + PhN_3 + **2**	**8** (12.3) + **Ph-triazole** (16.1)	81
3	**1** + PhN_3 + **2**	**3** (29.8) + **Ph-triazole** (19.1)	98
4	**6** (25 mM) + **1** + **2**	**8** (5.9) + **3** (28.7)	81
5	**6** (25 mM) + PhN_3 + **2**	**8** (11.4) + **Ph-triazole** (25.8)	97
6	**7** + **1** + **2**	**8** (19.4) + **3** (26.5)	92
7	**7** + PhN_3 + **2**	**8** (32.3) + **Ph-triazole** (16.5)	98
control	**6** + **2**	**8** (20.7)	83



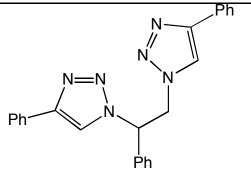
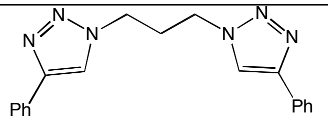
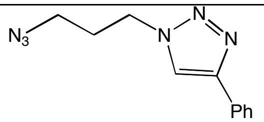
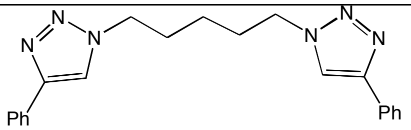
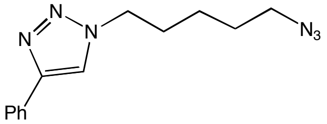
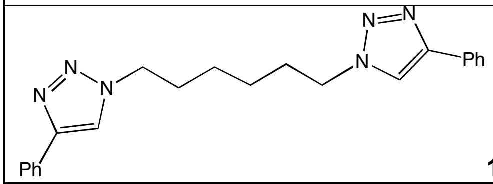
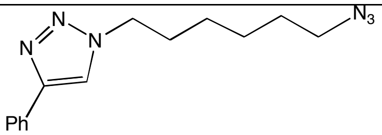
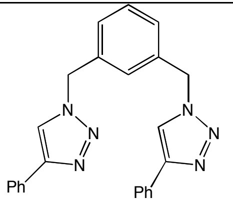
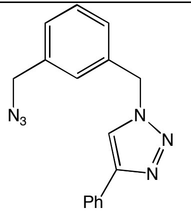
"Ph-triazole"

(a) 50 mM in each component unless otherwise indicated. (b) mM concentration of each product is given in parentheses. (c) based on the total alkyne (**2**) available (50 mM).

Screening of additional diazides (Figure 2).

`The reactions of **12–17** with 1 equiv PhC≡CH (0.5 equivalents per total azide) were performed under the same conditions as the reaction of **6** (Table S2) The unpurified reaction mixtures were analyzed by electrospray MS to determine the ditriazole formation was dominant, or mixtures of mono- and ditriazoles were formed. In almost every case, assignments were verified by ^1H NMR analyses of the products (Table S2). For compound **12**, very little monotriazole was formed. Compound **13** showed the same behavior as **12**; in this case the ditriazole **8** was characterized by removal of the acetate groups and comparison with an authentic sample made earlier.

Table S2. Characterization of products from reactions of **12 – 17** with PhC≡CH.

Compound	ESI MS in MeOH	^{1}H NMR, CD_3CN δ
 **12b**	393.2 $(M+H)^+$ 415.2 $(M+Na)^+$	8.28 (s, 1H), 8.10 (s, 1H), 7.92-7.84 (m, 6H), 7.65-7.50 (m, 9H), 6.51-6.43 (m, 1H), 5.73-5.65 (m, 1H), 5.43-5.33 (m, 1H)
 **14b**	331.1 $(M+H)^+$ 353.1 $(M+Na)^+$	8.22 (s, 2H), 7.97-7.93 (m, 4H), 7.60-7.47 (m, 6H), 4.59 (t, 4H, J=6.6Hz), 2.69 (quin, 2H, J=6.6Hz)
 **14a**	229.1 $(M+H)^+$ 251.1 $(M+Na)^+$	8.23 (s, 1H), 8.00-7.94 (m, 2H), 7.61-7.46 (m, 3H), 4.60 (t, 2H, J=7.2Hz), 3.50 (t, 2H, J=6.8Hz), 2.28 (dd, 2H)
 **15b**	359.1 $(M+H)^+$ 381.1 $(M+Na)^+$	8.20 (s, 2H), 8.03-7.92 (m, 4H), 7.59-7.45 (m, 6H), 4.51 (t, 4H, J=7.0Hz), 2.08-2.0 (m, 4H), 1.8-1.2 (m, 2H)
 **15a**	257.1 $(M+H)^+$ 279.1 $(M+Na)^+$	8.21 (s, 1H), 7.99-7.94 (m, 2H), 7.61-7.46 (m, 3H), 4.52 (t, 2H, J= 7.2Hz), 3.44-3.37 (t, 2H, J=6.8Hz), 2.1-2.02 (m, 2H), 1.80-1.66 (m, 2H), 1.57-1.47 (m, 2H)
 **16b**	395.1 $(M+Na)^+$ 371.1 $(M+H)^+$ low intensity	poor NMR (either because of solubility or aggregation; C5 analog below is also noisy/broadened, but usable; C4 analog gave blank spectrum)
 **16a**	271.1 $(M+H)^+$ 293.1 $(M+Na)^+$	δ 8.20 (s, 1H), 7.99-7.93 (m, 2H), 7.61-7.45 (m, 3H), 4.50 (t, 2H, J=7.4Hz), 3.39 (t, 2H, J=6.8Hz), 2.11-1.97 (m, 2H), 1.76-1.46 (m, 6H)
 **17b**	415.1 $(M+Na)^+$	δ 8.22 (s, 2H), 7.95-7.90 (m, 4H), 7.59-7.45 (m, 10H), 5.70 (s, 4H)
 **17a**	313.1 $(M+Na)^+$	δ 8.24 (s, 1H), 7.98-7.92 (m, 2H), 7.60-7.45 (m, 7H), 5.72 (s, 2H), 4.50 (s, 2H)

Dialkyne reactivity

The malonate-derived dialkyne compound **9** gave a mixture containing substantial amounts of both mono- and ditriazole upon reaction with benzyl azide. The course of the reaction is shown in Figure S15. Figure S16 presents a comparison at a single time point, highlighting the differences in the processes. While there may be some acceleration of the second step in the presence of ligand (note that ditriazole **11** appears right away in Figure S16 in the presence of ligand), the situation is very different from the behavior of diazide **6**. The situation is summarized in Scheme S2. Note, however, that it takes about the same time for **6** and **9** to reach completion, since the first steps in both cases are of the same general rate.

Scheme S2

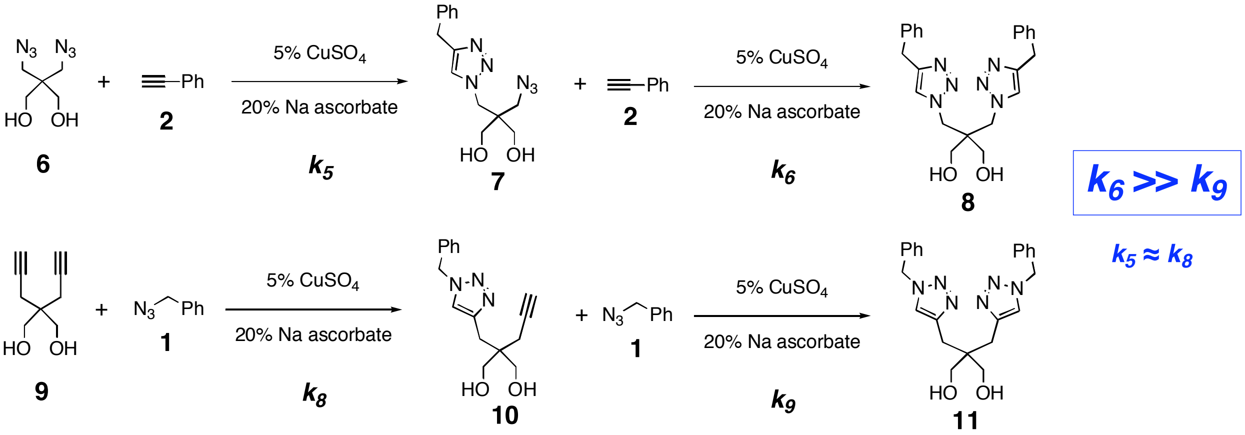
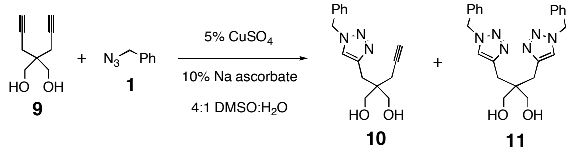
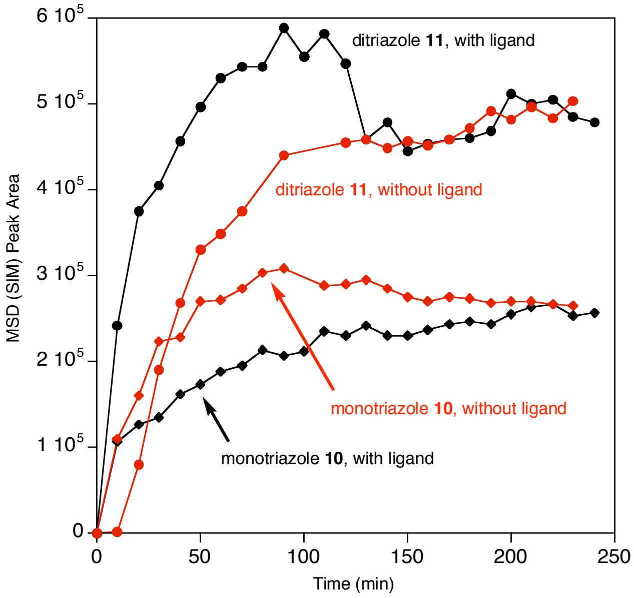


Figure S15.

Reaction of dialkyne **9** with benzyl azide.





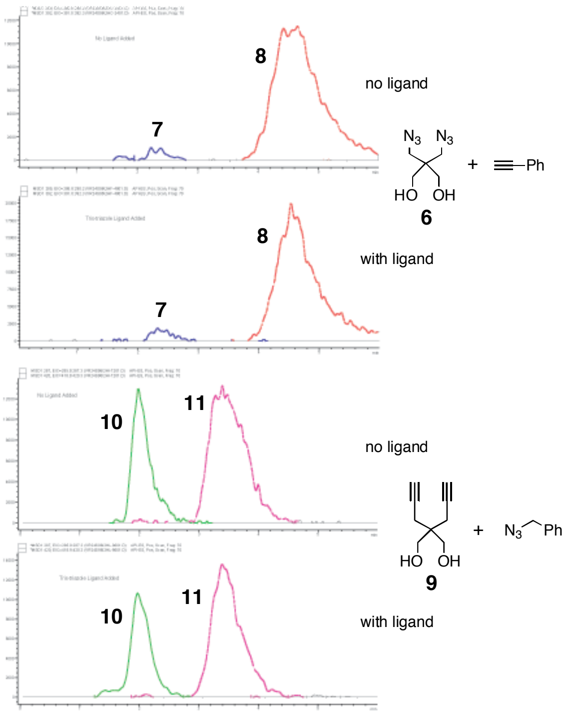


Figure S16.

Ion-extracted LC-MS chromatograms of the reactions of **6** and **9** with their complementary components in 4:1 DMSO:water; all species 0.1 M to start.