Supplementary Materials:

Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide–Alkyne Cycloaddition

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General Information

Melting points were recorded on a Thomas-Hoover capillary melting apparatus and are uncorrected. High-resolution mass spectra (HRMS) and time-of-flight mass spectra (TOF-MS) were recorded at the mass spectrometry facility at The Scripps Research Institute, La Jolla, CA, USA. GC-MS analyses were performed on an Agilent 7890A GC system equipped with a flame ionization detector and Agilent 5975C Inert MSD detector operating in electron impact (EI+) mode. Infrared spectra were recorded using a Perkin Elmer Spectrum 100-FTIR spectrometer. ^1H NMR (500 MHz or 400 MHz) and ^{13}C NMR (100 MHz or 150 MHz) were recorded on either a Bruker DRX-500 or a Bruker AMX-400 spectrometer. Chemical shifts (δ) were reported as parts per million (ppm) and the following abbreviations were used to identify the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad and all combinations thereof can be explained by their integral parts. Unless otherwise specified, the proton/carbon signal of residual solvent (δ 7.26/δ 77.00 respectively) was used as the internal reference. No flash chromatography or TLC (thin layer chromatography) was performed within the context of this paper. All chemicals were purchased from commercial vendors and were used as received without further purification; any exceptions are noted within the text and the vendors are noted within the context of use. Calorimetric data was acquired on an Insight_CPR_210 (OmniCal, Inc.) and processed using OmniCal Winsight^{TM} and Microsoft Excel. Unless specified, all reactions were carried out under an ambient atmosphere, using reaction grade solvent without other special precautions. The general synthesis of (SIPr)copper(I) acetylide (**2**) was taken in part from the procedures previously reported by Straub and co-workers (*23*), and modifications to this procedure were noted within the text. $Cu(PPh_3)_2NO_3$ (*32*, **3**) and benzyl azide (*33*, **2**) were prepared according to known literature procedures. Crossover experiments with isotopically enriched copper species were preformed on an Agilent ESI-TOF mass spectrometer using electrospray ionization. Samples were electrosprayed into the TOF reflectron analyzer at an ESI voltage of 4000V and a flow rate of 200 microliters/minute.

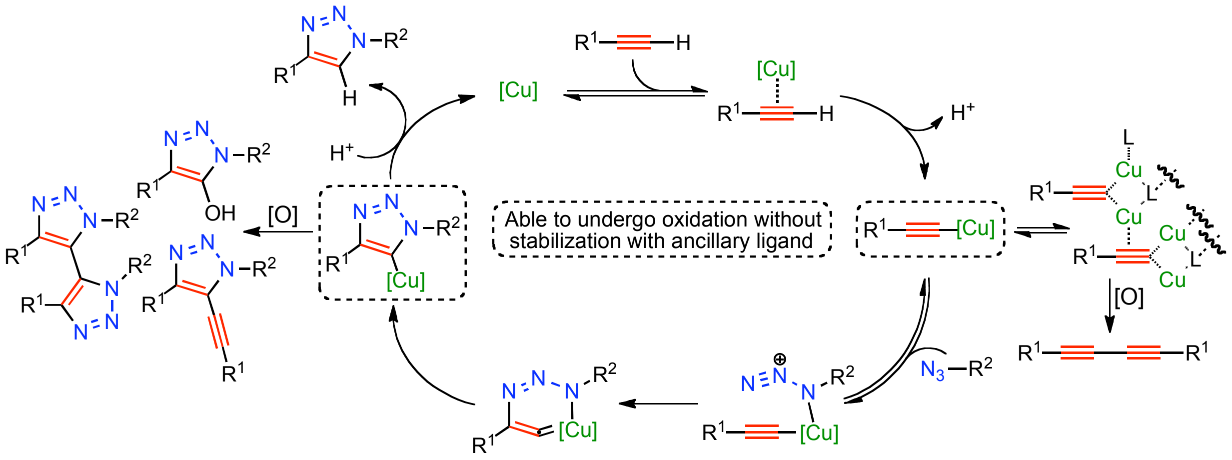


Figure S1. Originally proposed catalytic cycle for CuAAC (circa 2002) involving single catalytic copper center. Off-cycle acetylide aggregation and oxidative side reactions are also shown.

Procedures for the Synthesis and Characterization of Selected Compounds

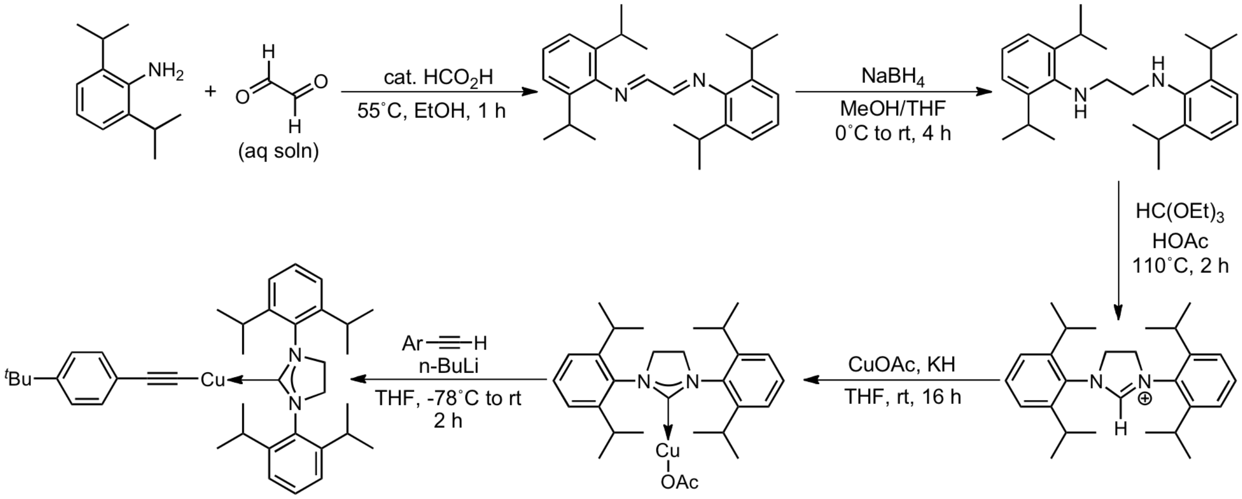
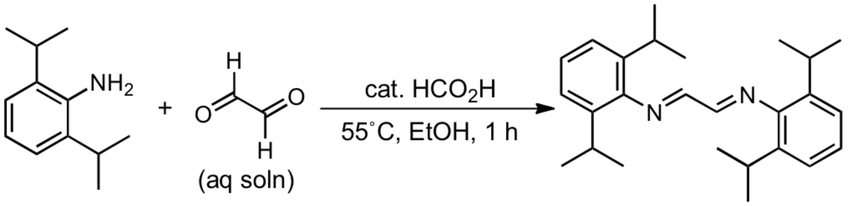


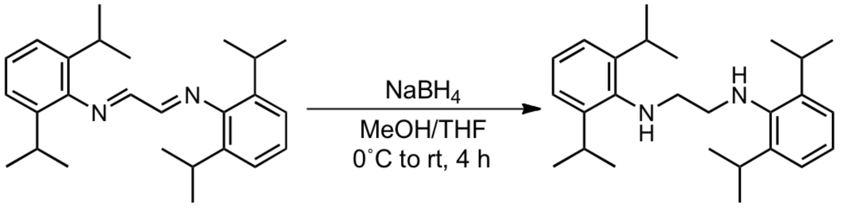
Figure S2. General scheme for the synthesis of (NHC)Cu(I) acetylide **2**.

N,N-(ethane-1,2-diylidene)bis(2,6-diisopropylaniline)



A 250 mL round-bottomed flask equipped with a magnetic stir bar was charged with 20.0 g (113 mmol, 2 equiv) of 2,6-diisopropylaniline (92% purity) and 100 mL of ethanol. To this stirred solution, 8.2 mL (56.5 mmol, 1 equiv) of glyoxal (40% solution in water) was added in one portion followed by 1 mL (17.5 mmol, 0.15 equiv) of formic acid, also in one portion. The solution was then heated to 55 ºC and stirred for approximately one hour. The reaction mixture was subsequently cooled to room temperature, and an orange/yellow solid immediately precipitated. This precipitate was filtered from the deep red solution and was washed with an additional portion of previously cooled methanol (100 mL). Drying under high vacuum afforded 14.4 g (76.0% yield) of the title compound as a bright yellow solid, which was used in the next step without further purification. Spectral information for the title compound is in agreement with those previously reported (*23*).

N,N-bis(2,6-diisopropylphenyl)ethane-1,2-diamine

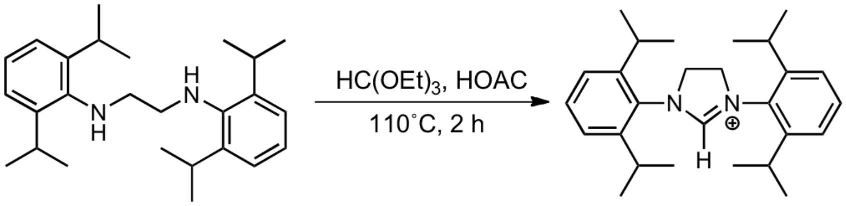


A 500 mL round-bottomed flask (additional head space required to accommodate the evolving gas) equipped with a magnetic stir bar was charged with 14.4 g (38.3 mmol, 1 equiv) of *N,N'*-(ethane-1,2-diylidene)bis(2,6-diisopropylaniline), 95.0 mL of THF, and 65.0 mL of methanol, forming a clear yellow solution. The flask was placed into an ice/water bath and allowed to reach an internal temperature of ~5 ºC. At that time, 7.29 g (192 mmol, 5 equiv) of powdered $NaBH_4$ (finely ground with a mortar/pestle) were added in one portion. This solution was allowed to stir for 10 minutes before warming to room temperature.

CAUTION: Approximately 15 minutes after removing the reaction from the ice bath, rapid gas evolution occurred and care should be taken to keep the solution from boiling over; a cold-water bath can be reapplied if necessary.

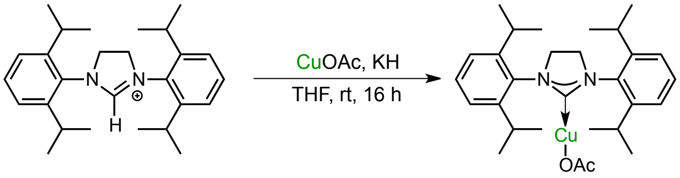
After 2 hours the yellow solution turned clear, and LC-MS analysis indicated complete conversion to the reduced product. The clear solution was quenched by the slow addition of 100 mL of saturated aqueous NH_4Cl at 0 ºC; after warming to room temperature, the solution was diluted with an additional 100 mL of Et_2O. This mixture was allowed to stir for 10 minutes and was then poured directly into a 500 mL separatory funnel, separated and extracted with an additional 50 mL of Et_2O (2X). The combined organic layers were dried with Na_2SO_4, filtered, and concentrated. The resultant damp yellow powder was re-dissolved in DCM and dried again with Na_2SO_4, filtered, and concentrated. Drying the crude product under high vacuum afforded 13.7 g (93.8% yield) of the title compound as a yellow solid, which was used in the next step without further purification. Spectral information for the title compound is in agreement with those previously reported (*23*).

1,3-Bis-(2,6-diisopropylphenyl)imidazolinium acetate



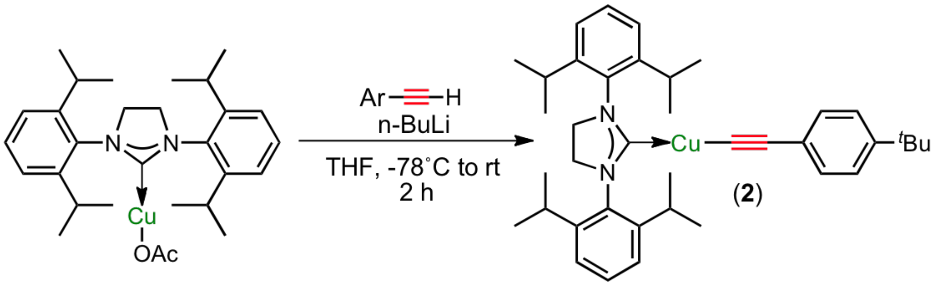
A 250 mL sealed tube equipped with a large egg-shaped stir bar was charged with bis(2,6-diisopropylphenyl)ethane-1,2-diamine (13.7 g, 35.9 mmol, 1 equiv). The solid was treated with 13.3 g (13.3 mmol, 2.5 equiv) of triethyl orthoformate and 6.48 g (108 mmol, 3 equiv) of acetic acid, taking care to wash the solids off the sides of the tube. The suspension was rapidly heated to 110 ºC. At 60 ^{o}C the suspension became a brown solution, and at 110 ºC it turned purple; the reaction was stirred at this temperature for approximately 2 hours, then cooled to room temperature and diluted with ~40 mL of Et_2O. Immediately after the addition of Et_2O an off-white solid precipitated from the reaction mixture. The solid was filtered from the orange reaction mixture and washed with additional Et_2O (50 mL, 2X). Drying under high vacuum afforded 14.8 g (87.7% yield) of the title compound as an off-white solid, which was used in the next step without further purification. Spectral information for the title compound is in agreement with those previously reported (*23*).

(SIPr)Copper(I) acetate

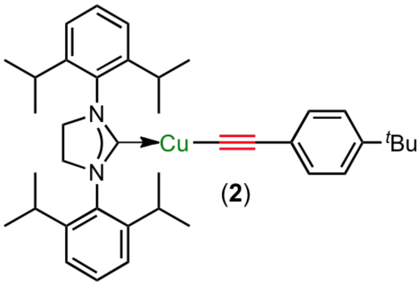


Inside of an argon-filled glove box, a 250 mL sealed tube equipped with a egg-shaped stir bar was charged with 10.0 g (17.5 mmol, 1 equiv) of 1,3-bis-(2,6-diisopropylphenyl)imidazolinium acetate, 1.18 g (29.5 mmol, 1.33 equiv) potassium hydride (KH, obtained as 40% solution in mineral oil, washed with hexanes and stored in glove box) and 3.60 g (29.5 mmol, 1.33 equiv) of copper(I) acetate (95% purity, stored in glove box). The sealed tube was closed tightly and removed from the glove box. 90.0 mL dry THF were added and the tube was quickly resealed. The suspension was stirred at room temperature overnight. The stirring was halted, allowing for the solids to settle to the bottom. A clear solution indicated the complete consumption of the free copper(I) acetate. The suspension was then filtered through a small plug of Celite 545 (slurry packed with DCM) and washed with several portions of DCM, resulting in a clear yellow solution. The filtrate was then concentrated, yielding a crude off-white solid. This solid was dissolved in a minimal amount of hot dichloromethane, precipitated with hexanes, filtered, and washed with additional hexanes. Drying under high vacuum afforded 9.98 g (88.0% yield) of the title compound as a white crystalline solid, which was used in the next step without further purification. Spectral information for the title compound is in agreement with those previously reported (*23*).

(SIPr)Copper(I) acetylide (**2**)



1-(*tert*-Butyl)-4-ethynylbenzene (1.23 g, 7.79 mmol, 1.33 equiv) was placed in a flame-dried 100 mL round-bottomed flask equipped with a magnetic stir bar and maintained under argon, followed by 30.0 mL of dry THF. This solution was cooled to –78 °C in a dry ice/acetone bath and 3.12 mL (7.79 mmol, 1.33 equiv) of n-butyllithium (obtained as a 2.50 M solution in hexanes and used as received) were added in one portion; the mixture was stirred for 20 minutes. To a separate flame-dried 250 mL round-bottomed flask under argon equipped with a magnetic stir bar were added 3.00 g (5.86 mmol, 1.00 equiv) of (SIPr)CuOAc, 50 mL of dry THF, and also cooled to –78 ºC in a dry ice/acetone bath. The lithium acetylide solution was cannulated dropwise to the cooled (SIPr)CuOAc mixture. Following its addition the cold bath was removed and the reaction was allowed to warm to room temperature. It was stirred for an additional 30 minutes and was concentrated, leaving a gummy yellow solid. 50.0 mL of DCM were added to this crude product, and the solution was triturated and filtered through a Celite 545 plug (slurry packed in DCM), then washed with additional DCM (30 mL, 2X). The clear yellow filtrate was placed into a round-bottomed flask and concentrated to give a slightly yellow solid, which was immediately re-dissolved in a minimal amount of DCM and diluted with 150 mL hexanes. The flask was capped and placed into a –20 ºC freezer for approximately 1 hour. The precipitated yellow solid was filtered, washed with additional hexanes and dried under high vacuum, affording 3.09 g (86.3% yield) of the title compound (**2**) as a pale yellow solid. For extended storage the title compound was placed into a brown glass bottle and stored in a glove box for several months with no signs of degradation.


(**2**)

(**2**) White to pale-yellow solid; mp = 195 – 200 ºC (dec.); FT-IR (film) ν_{max} 2960, 2867, 2089, 1590, 1504, 1478, 1456, 1384, 1363, 1325, 1299, 1269, 1214, 1179, 1104, 1057, 1017, 935, 816, 802, 756, 730, 709, 618, 508 cm^{-1}; ^{1}H NMR (CDCl$_3$, 400 MHz) δ = 7.39 (t, *J* = 7.84 Hz, 2H), 7.25 (d, *J* = 7.74 Hz, 4H), 7.14 (dt, *J* = 8.41, 1.88 Hz, 2H), 7.07 (dt, *J* = 8.41, 1.88 Hz, 2H), 3.97 (s, 4H), 3.15 – 3.05 (m, 4H), 1.42 (d, *J* = 6.83 Hz, 12H), 1.34 (d, *J* = 6.93 Hz, 12H), 1.21 (s, 9H) ppm; ^{13}C NMR (CDCl$_3$, 100 MHz) δ = 205.07, 148.08, 146.76, 134.76, 131.56, 129.71, 124.56, 124.49, 124.28, 119.89, 105.82, 53.84, 34.45, 31.33, 29.04, 25.63, 24.06 ppm.

General Procedure for Reactions in Omnical Heat Flow Calorimeter

Heat flow calorimetric data were acquired on Omnical Insight_CPR_210, which allows continuous monitoring of the instantaneous heat flow around the reaction vessel. In the absence of side reactions, the isothermal reaction heat flow, **q**, is proportional to the reaction rate, **r**, where **ΔH_{rxn}** is the heat of the reaction and **V** is the volume.

$$q = \Delta H_{rxn} \cdot V \cdot r$$

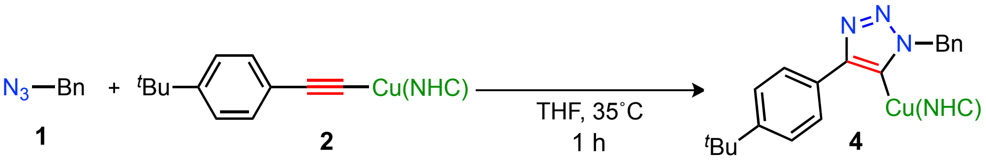
The enthalpy change of the reaction, **ΔH_{rxn}**, can be calculated by the integration of the total heat flow curve over time and divide the integral sum by the mole (**n**) amount of the limiting reagent consumed.

$$\Delta H_{rxn} = \frac{\int_{t=0}^{t=\infty} q \cdot dt}{n}$$

The observed heat profile can also be used to determine the fraction conversion by calculation of the fractional area under the temporal heat flow curve where the numerator denotes the area under the heat flow curve to time (**t**) and the denominator denotes the total area under the heat flow curve.

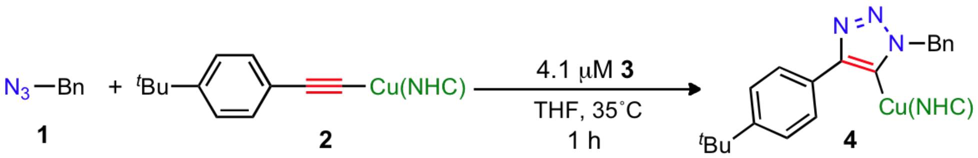
$$\text{Fraction Conversion (FC)} = \frac{\int_{t=0}^{t} q \cdot dt}{\int_{t=0}^{t=\infty} q \cdot dt}$$

Unless otherwise indicated, all reactions were carried out in a 15 mL septum-capped vial equipped with a magnetic stir bar. The stir rate of the calorimeter was set at 90 rpm (rotations per minute) and the reaction mixture was allowed to equilibrate inside of the calorimeter at 35 ºC for 60 minutes prior to the initiation of the reaction by injection of the azide or the azide/copper catalyst. All reactions and stock solutions of the catalyst were prepared within an inert atmosphere glove box and the handling of all liquid reagents was performed using plastic syringes. Two general procedures for the non-catalyzed reaction between (SIPr)Copper(I)-1-(*t*-butyl)-4-ethynylbenzene (**2**) with benzyl azide (**1**) and the catalyzed reaction of (SIPr)Copper(I) 1-(*t*-butyl)-4-ethynylbenzene (**2**) with benzyl azide (**1**) and $Cu(PPh_3)_2NO_3$ (**3**) carried out in a reaction calorimeter are given below:



General procedure for the uncatalyzed reaction of acetylide **2** *with azide* **1**.

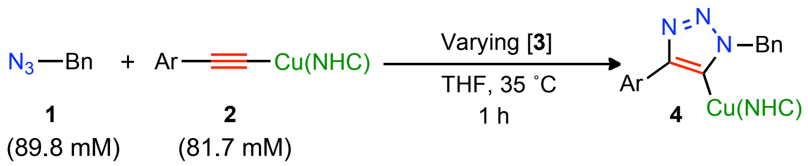
Inside of an argon-filled glove box, a 15 mL septum-capped vial equipped with a magnetic stir bar was charged with 150.0 mg (0.245 mmol, 1.00 equiv) of acetylide **2**. The vial was removed from the glove box, 2 mL of dry THF were added to it, and it was placed into the calorimeter and allowed to thermally equilibrate to 35 ºC for ~60 minutes. While this vial was equilibrating, a separate 1-dram Teflon-capped vial was loaded with 35.9 mg (0.270 mmol, 1.1 equiv) benzyl azide (**1**), capped, and removed from the glove box. 1 mL of dry THF was added, and the solution was drawn into a 1 mL plastic syringe; the needle was capped with a small piece of Teflon, and placed into the thermostated syringe block. After the system reached thermal equilibrium, the reaction was initiated by the injection of the azide solution.



General procedure for the catalytic reaction of acetylide **2** *with azide* **1***; synthesis of triazolide* **4**.

Inside of an argon-filled glove box, to a 15 mL septum-capped vial equipped with a magnetic stir bar was added 150.0 mg (0.245 mmol, 1 equiv) of acetylide **2.** The vial was removed from the glove box, and 2 mL of dry THF were added to it. It was then placed into the calorimeter and allowed to equilibrate to 35 ºC for ~60 minutes. While this vial was equilibrating, a separate 1-dram Teflon-capped vial was loaded with 35.9 mg (0.270 mmol, 1.1 equiv) of benzyl azide (**1**), capped and removed from the glove box. Additionally, inside of a glove box, a 10 mL volumetric flask was loaded with 79.9 mg (0.123 mmol, .05 equiv/1 mL) of $Cu(PPh_3)_2NO_3$ (**3**), capped with a rubber 24/40 septum, taken out of the glove box, and 10 mL of dry THF were added.* 1 mL of this homogeneous solution was removed and added to the vial containing the azide. The contents of the vial were drawn into a 1 mL plastic syringe; the needle was capped with a small piece of Teflon and placed into the calorimeter needle jacket. After the system reached thermal equilibrium, the reaction was initiated by the injection of the azide/copper catalyst solution.

***Note:** For experiments varying the concentration of the catalyst, dilutions of this stock solution were made as needed (fig. S3). This general procedure was used for experiments utilizing different copper catalysts (fig. S4); different azides (fig. S5); different solvents (fig. S6).



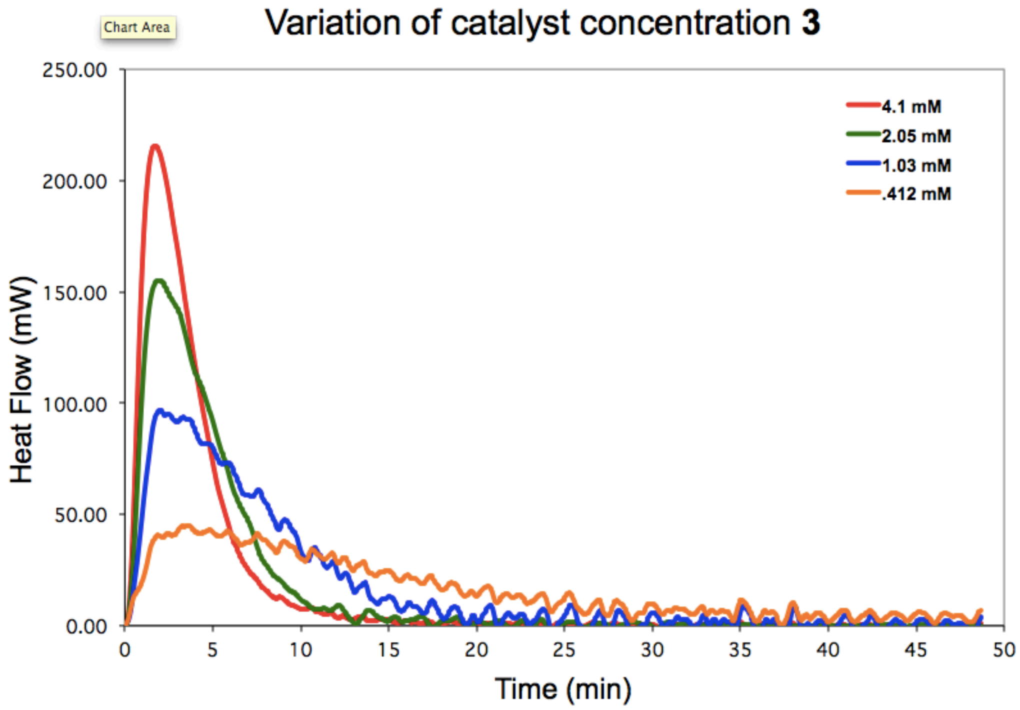


Figure S3. Dependence of the reaction rate on the concentration of the exogenous copper(I).

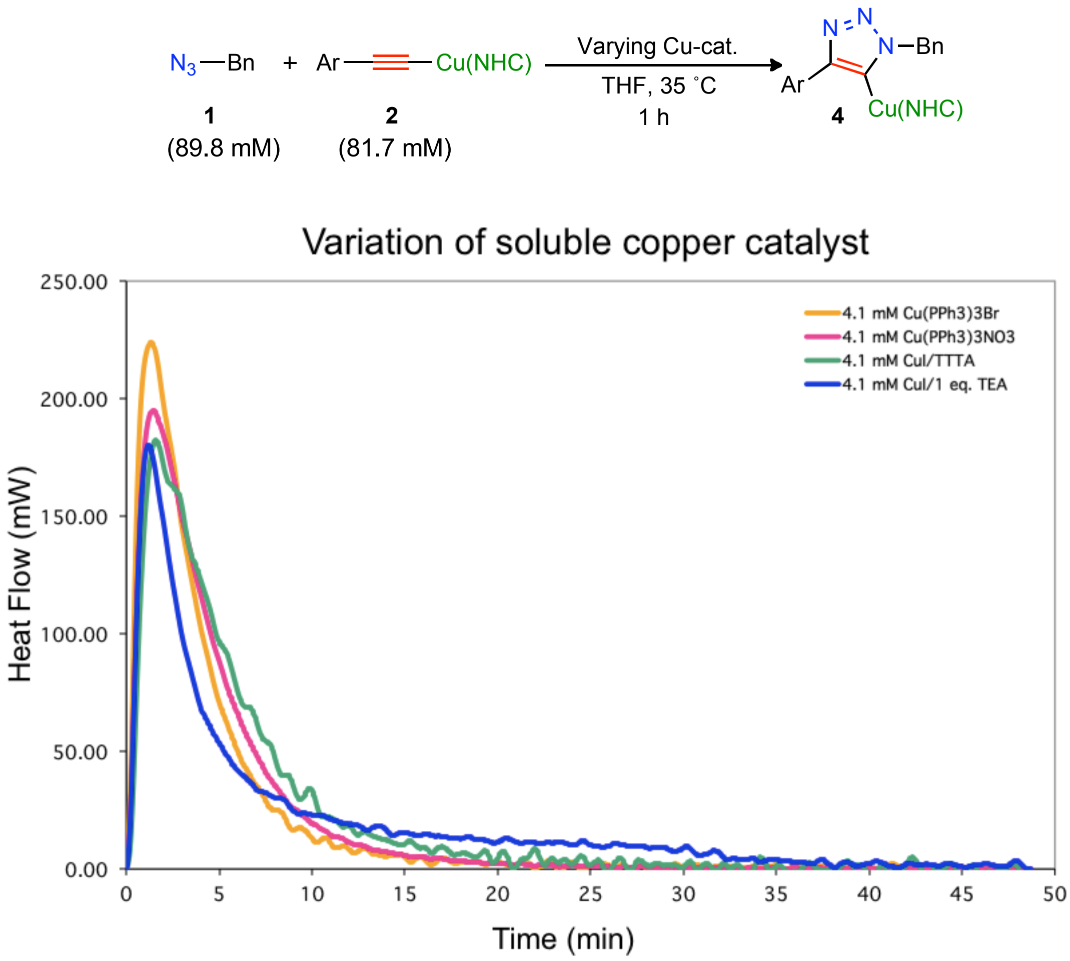
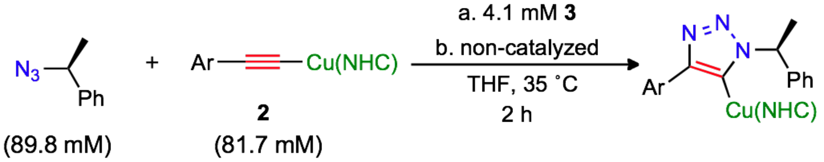


Figure S4. Different copper(I) catalysts exhibit similar activity.

Reactions of different organic azides with acetylide (2).



*Entry 1a,b (*see Table S5 for yields*):*

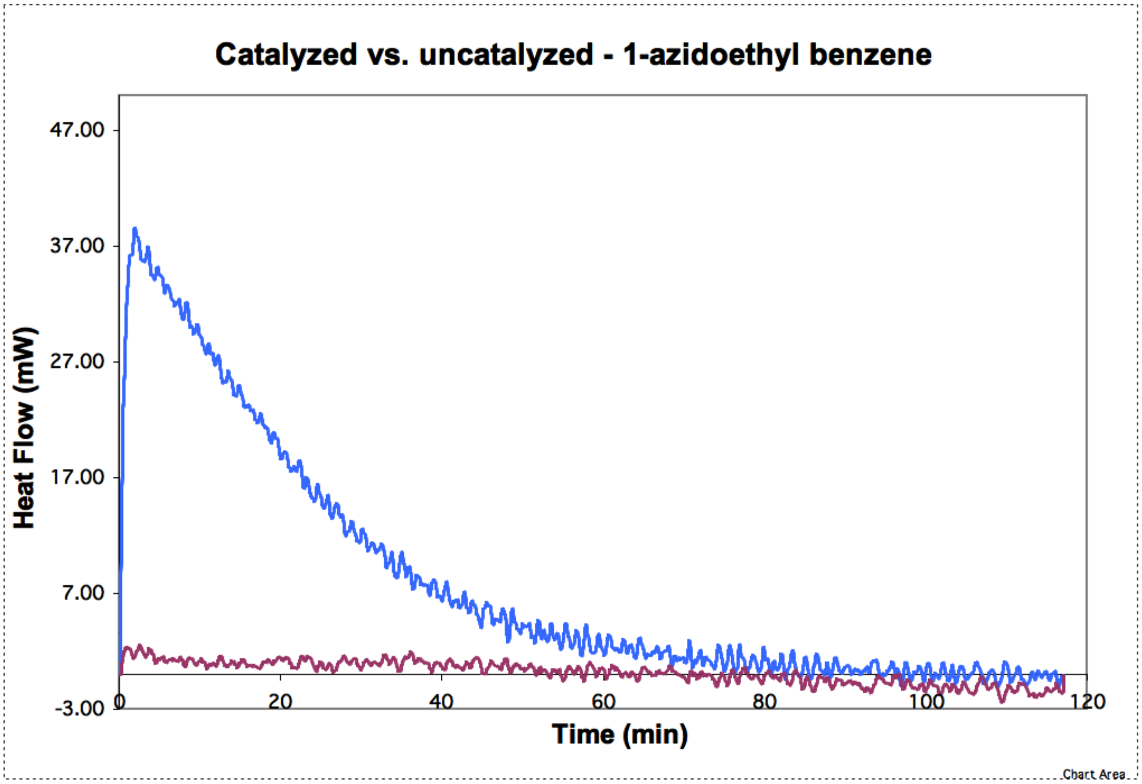
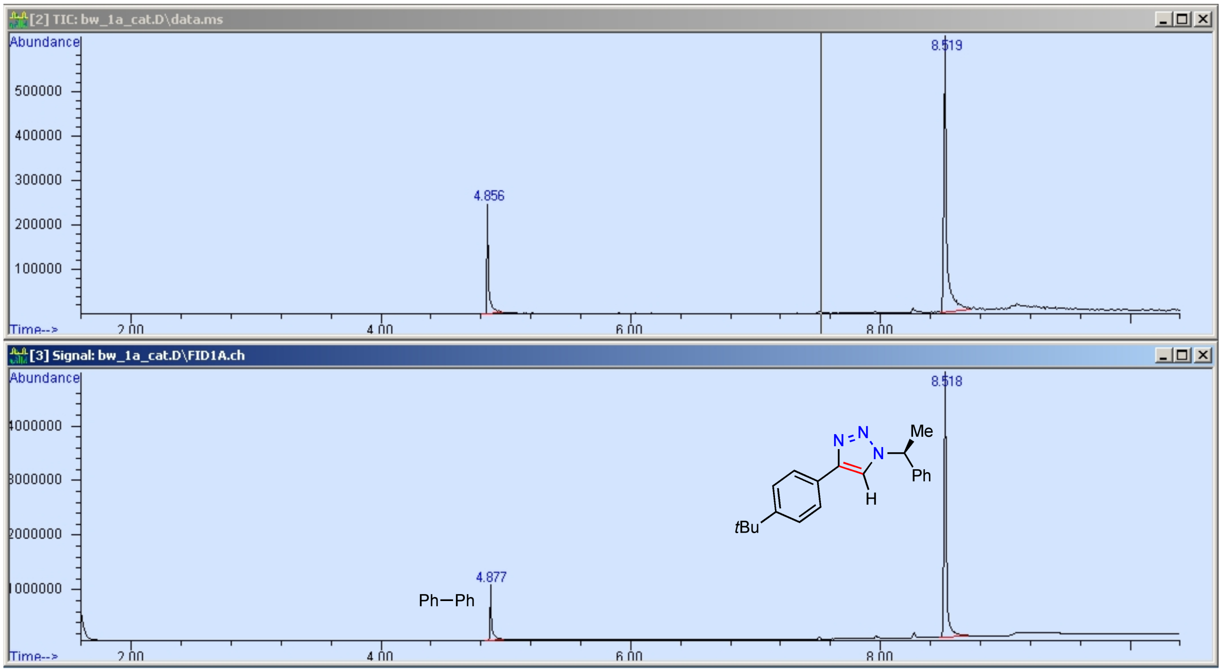
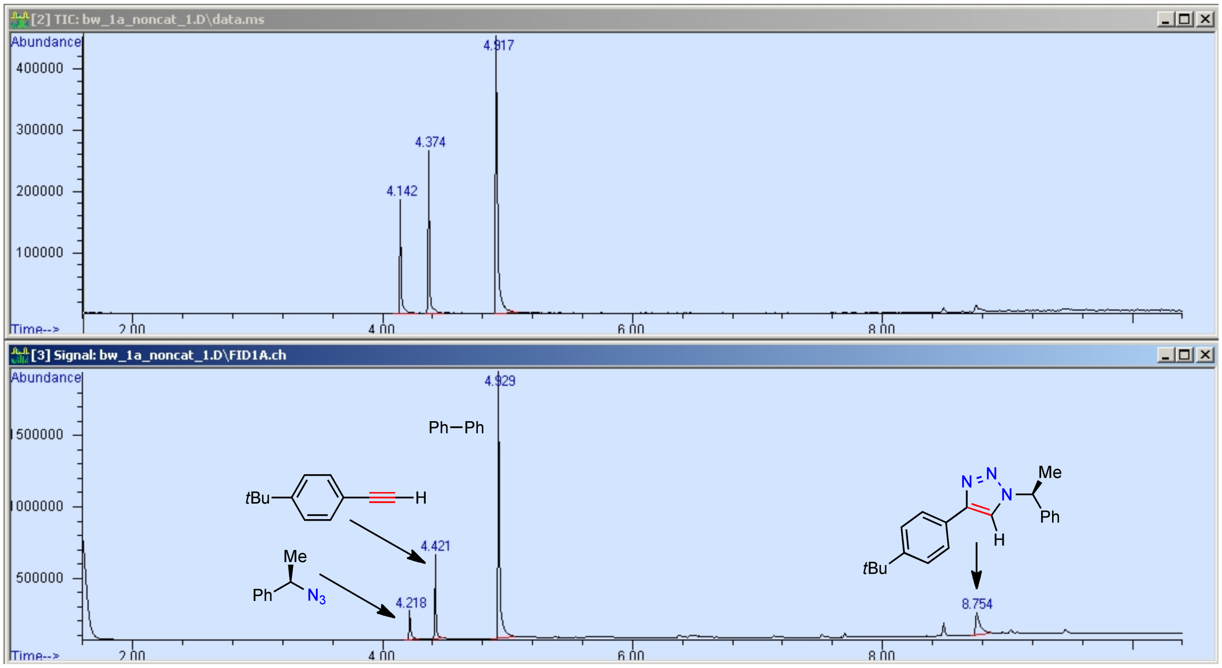


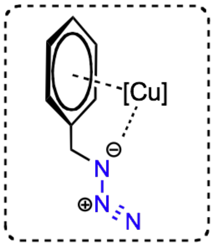
Figure S5a. Uncatalyzed and copper-catalyzed reactions of 1-azidoethyl benzene with acetylide **2.**

*Catalytzed reaction of 1-azidoethyl benzene with acetylide **2** monitored by GC-MS (reaction aliquoted at 3 hours). MS trace at the top; FID trace at the bottom:*

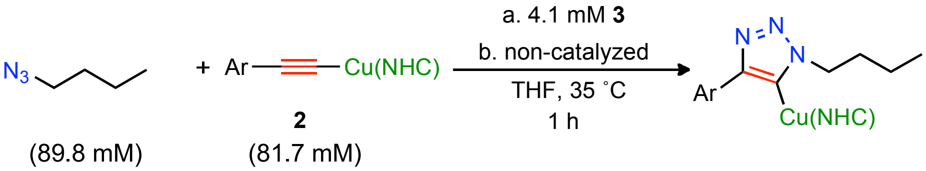


*Uncatalyzed reaction of 1-azidoethyl benzene with acetylide **2** monitored by GC-MS (reaction aliquoted at 3 hours). (MS trace on the top; FID trace on the bottom):*





The preceding azides utilized in this study (benzyl azide and 1-azidoethyl benzene) have been shown to have higher efficiency than unsubstituted azides in the CuAAC, due to possibility of their bis-chelation to free copper catlaysts (*14*, *34*). This effect could account for the observed increased rate of the reaction with acetylide **2** in the presence of exogenous copper catalyst **3**. Therefore, an aliphatic azide, 1-azidobutane, devoid of coordinating groups was employed, which showed similar efficiency to both benzyl azide and 1-azidoethyl benzene (see below).



Entry 2a,b (see Table S5 for yields)*:*

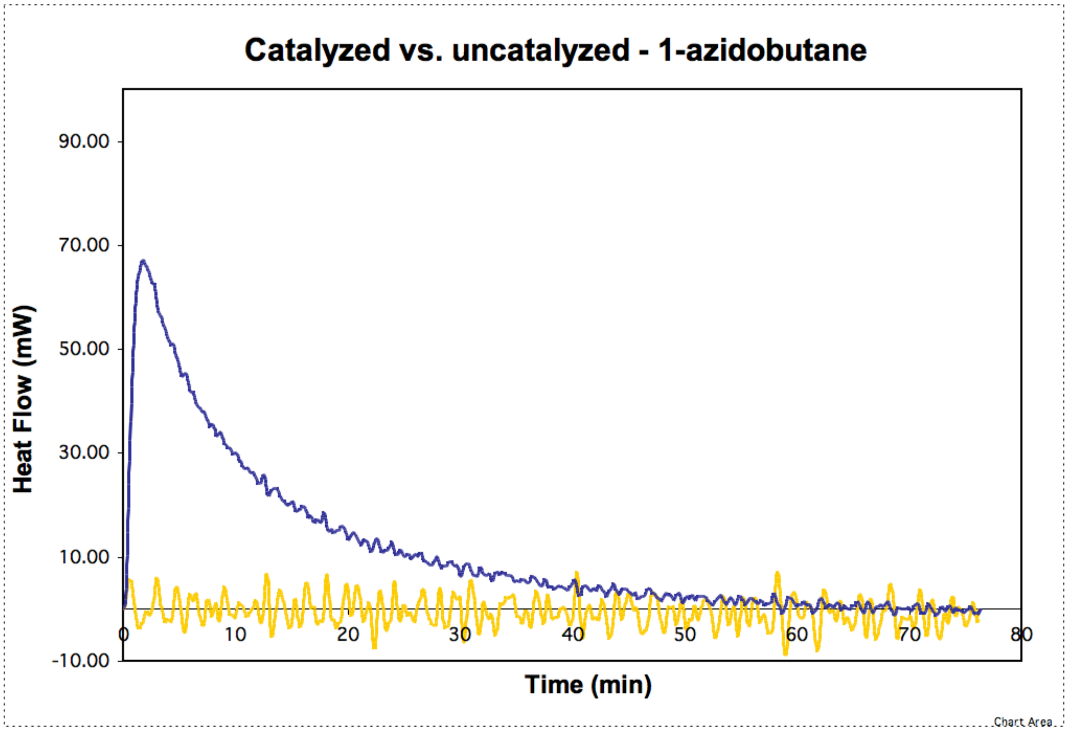
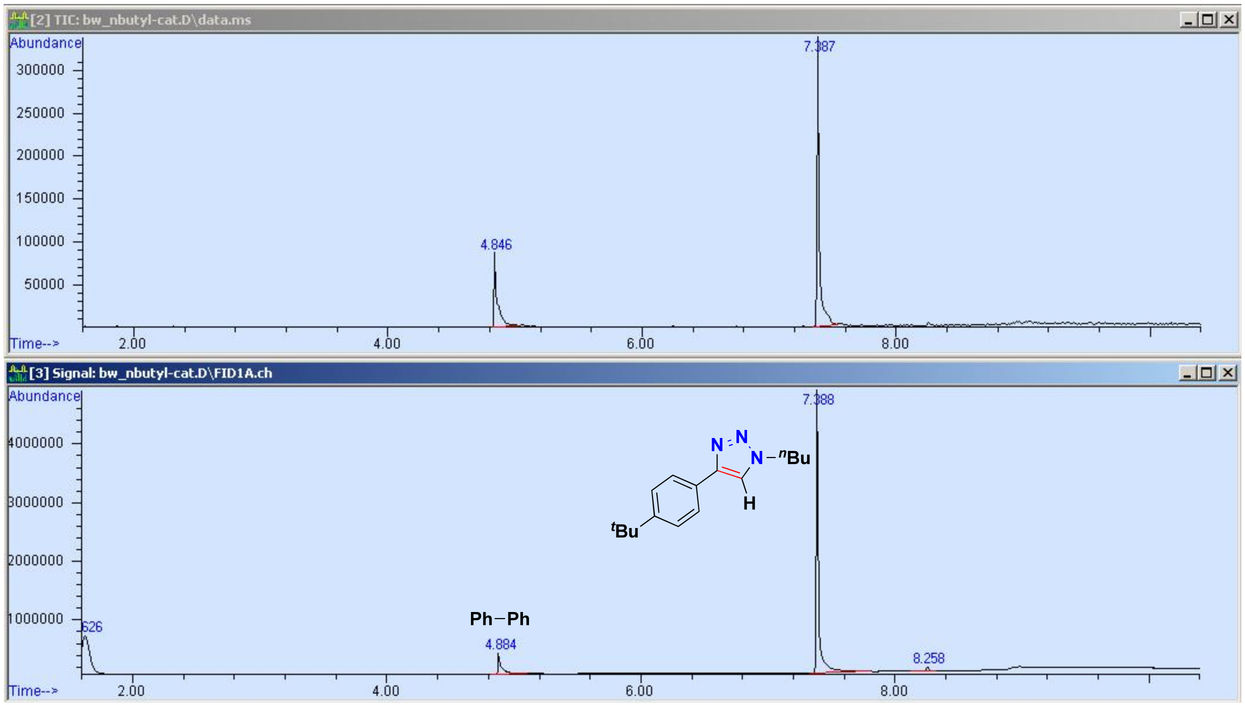
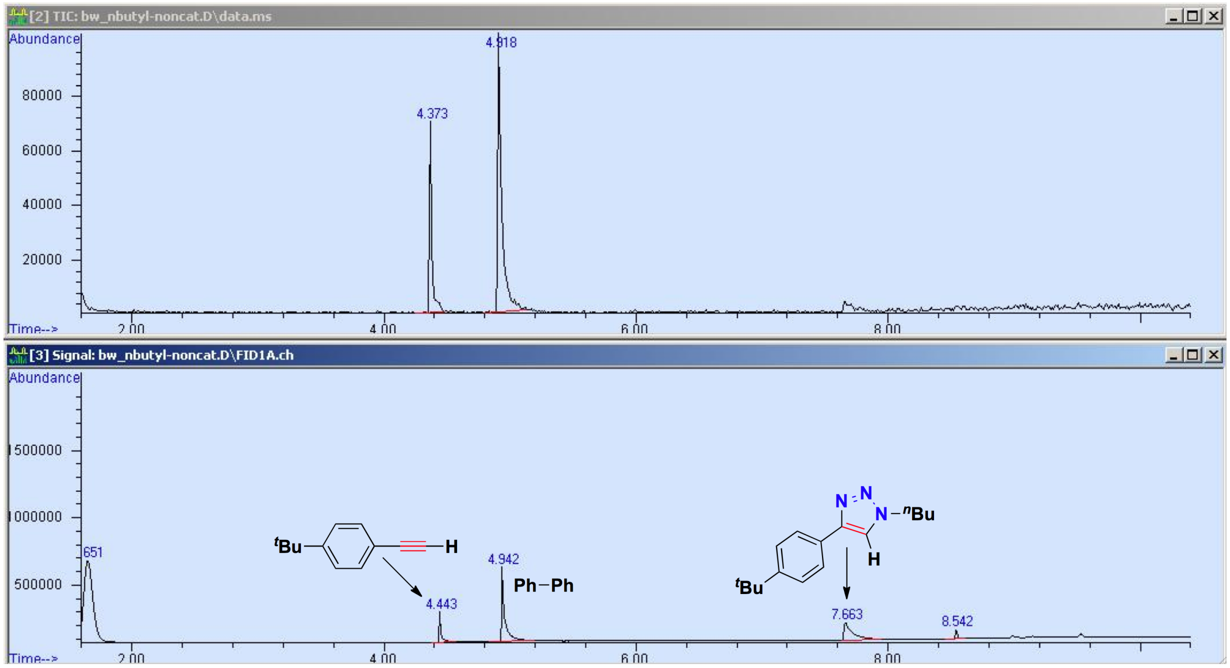


Figure S5b. Uncatalyzed and copper-catalyzed reactions of 1-azidobutane with acetylide **2.**

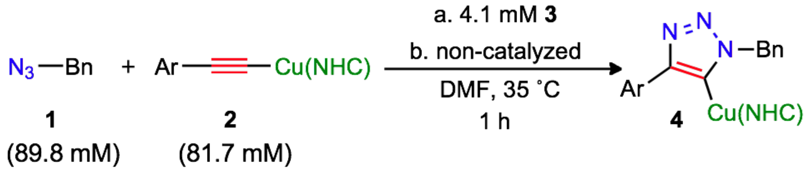
*Catalytic reaction of 1-azidobutane with acetylide **2** monitored by GC-MS (reaction aliquoted at 1 hour):*



*Catalytic reaction of 1-azidobutane with acetylide **2** monitored by GC-MS (reaction aliquoted at 1 hour):*



Reactions in different solvents exhibit similar rate profiles.



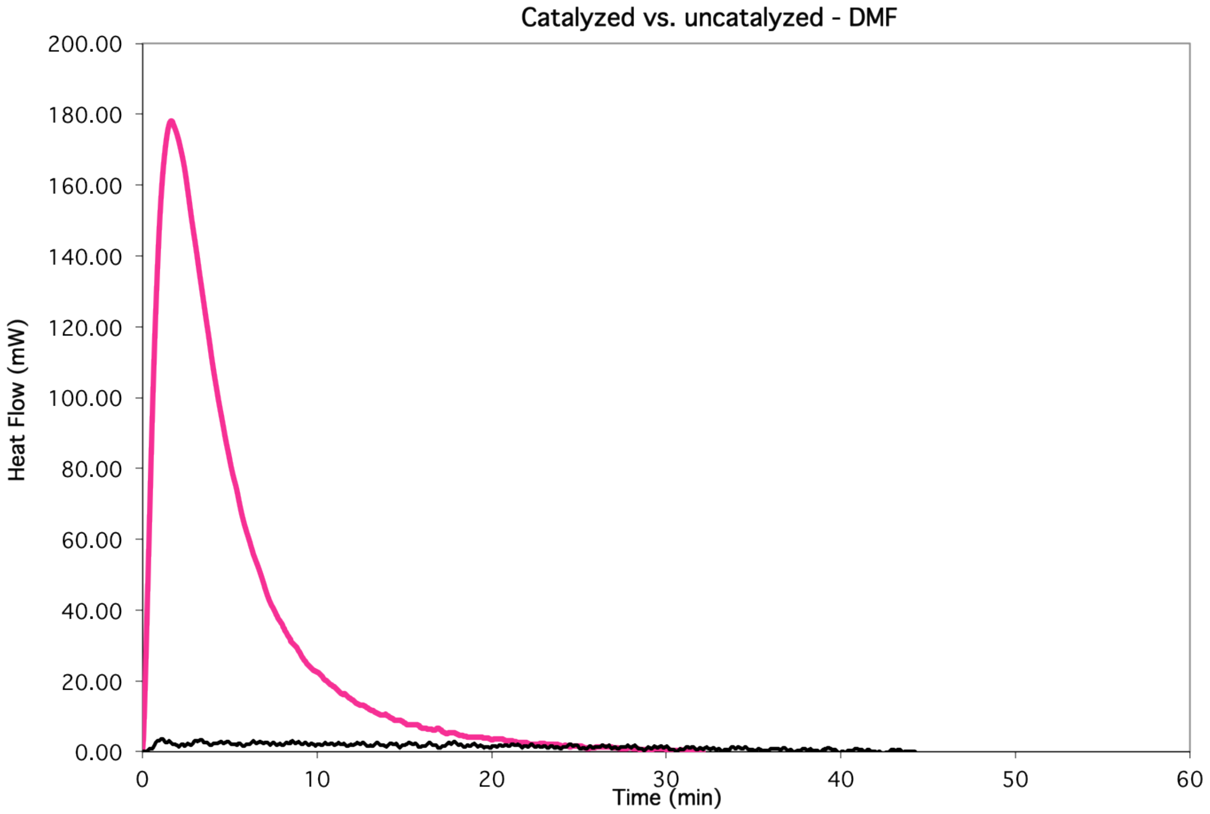
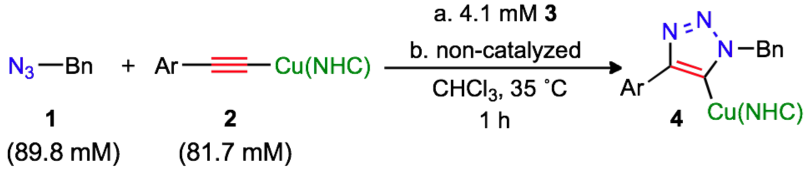


Figure S6a. Reaction of azide **1** and acetylide **2** in DMF. (a) Trace of them catalytic reaction is in bright pink, and (b) exogenous catalyst-free is in blaclk.



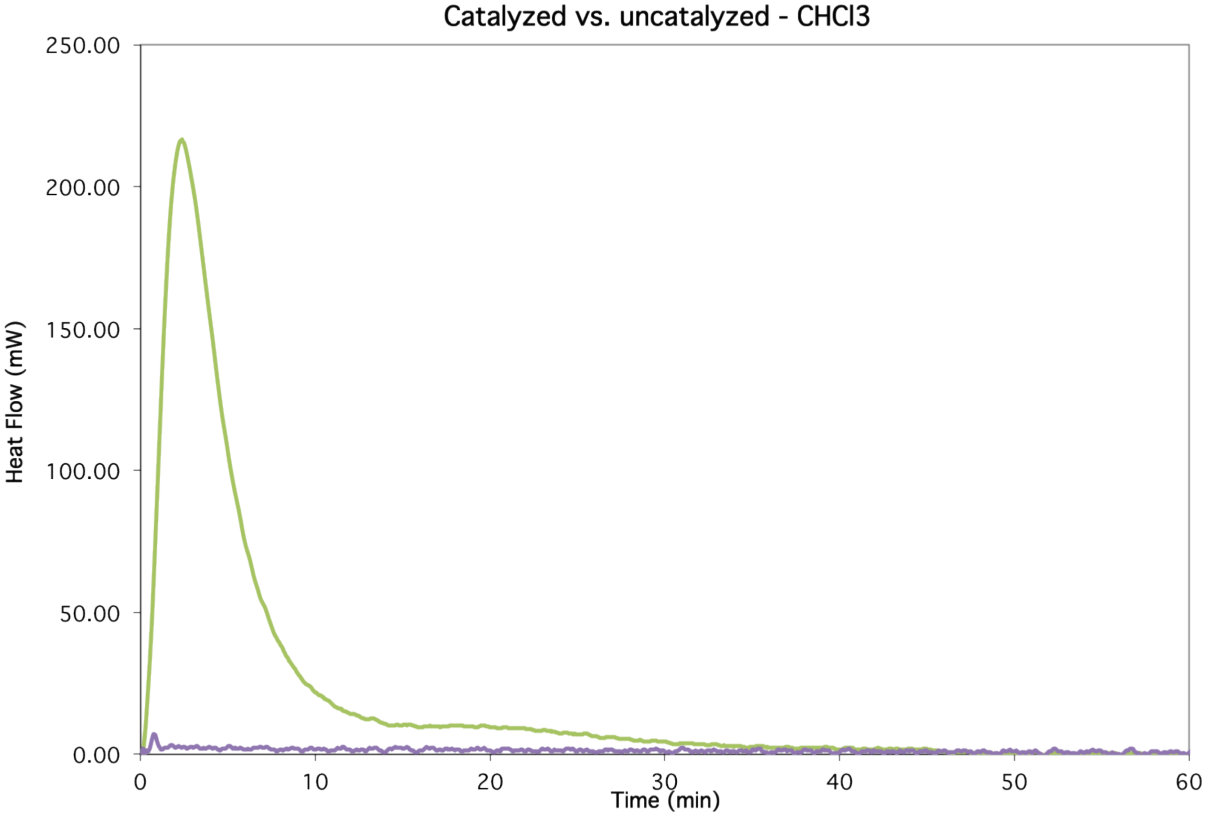


Figure S6b. Reaction of azide **1** and acetylide **2** in chloroform. (a) Catalytic reaction trace is shown in olive, and (b) exogenous catalyst-free is in purpose.

Note: The small rise in heat from 10 – 30 minutes is due to precipitation of triazolide **4**.

General procedures for endpoint analysis/deuterium incorporation by GC-MS – LC-MS

Table S1. Calibration curve for endpoint analysis (GC-MS)

Theoretical Yield	*Area of Triazole*	*Area of Biphenyl*	*Ratio*
20	11620496	38588758	0.3011368
40	25966749	39569655	0.6562288
60	39756211	39494877	1.0066169
80	52783970	39952412	1.3211710
100	64174575	40236966	1.5949158

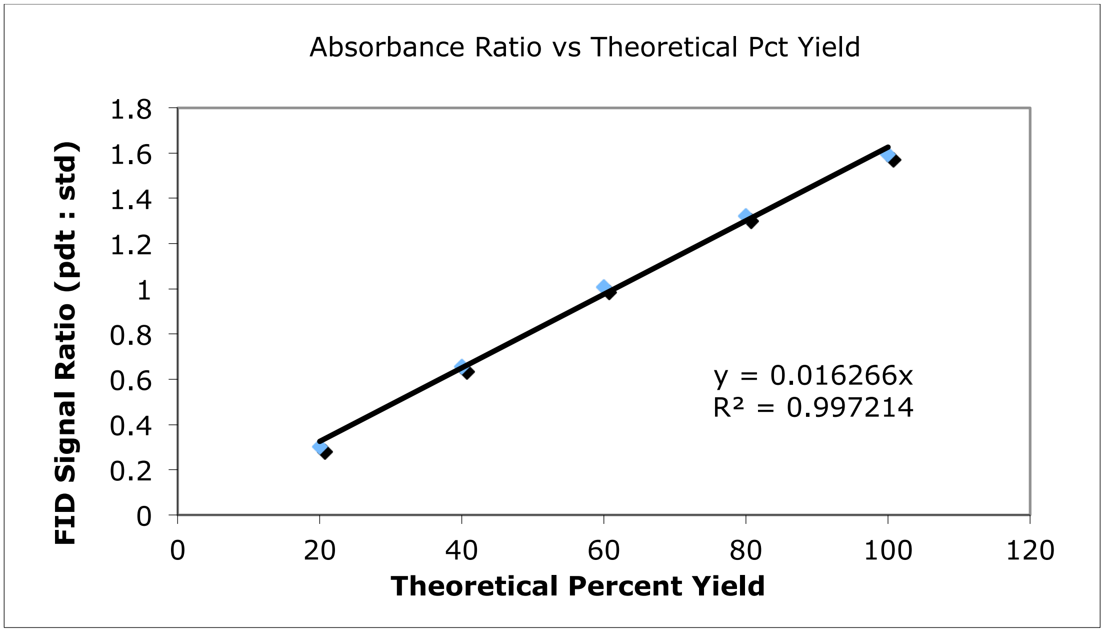
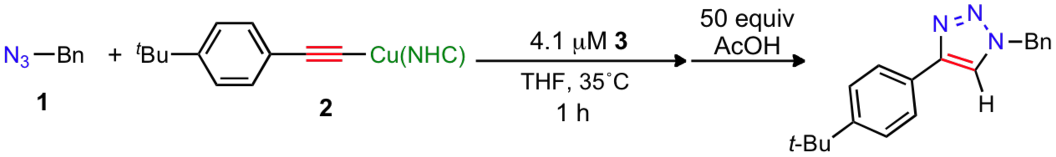


Figure S7. Calibration curve for endpoint analysis (GC-MS).

Equation for the determination of yield from GC-MS data:

Yield = ((area of 5-H-triazole/area of biphenyl) / 0.016266)

Procedure for the endpoint analysis by GC-MS; catalyzed reaction:



Inside of an argon-filled glove box, 150.0 mg (0.245 mmol, 1 equiv) of acetylide **2** was added to a 10 mL microwave vial equipped with a magnetic stir bar. The vial was capped, removed from the glove box, and diluted with 2 mL dry THF. Additionally, inside of a glove box, a 10 mL volumetric flask was loaded with 359.3 mg of benzyl azide (**1**, 2.70 mmol, 1.1 equiv/1 mL), 378.3 mg of biphenyl (2.45 mmol, 1 equiv/1 mL) and 79.9 mg (0.123 mmol, .05 equiv/1 mL) of $Cu(PPh_3)_2NO_3$ (**3**), and capped with a rubber 24/40 septum. This volumetric flask was removed from the glove box, and its contents were diluted with 10 mL dry THF. The copper solution was agitated for several minutes until the complete dissolution of the copper could be visualized and 1 mL of this homogeneous solution was removed and added to the stirring solution of acetylide **2**. After stirring for one hour, a 100 μL aliquot was extracted from the reaction and diluted with 900 μL of a .0439 M (50 equiv) solution of acetic acid in methanol. This vial was capped, agitated for several minutes, and then analyzed directly by GC-MS. This procedure was repeated in triplicate, giving an average yield of 97.1% for the 5-H-1,2,3-triazole. The results are summarized in the table below:

*Catalyzed reaction of azide **1** and acetylide **2** monitored by GC-MS (reaction aliquoted at 1 hour):*

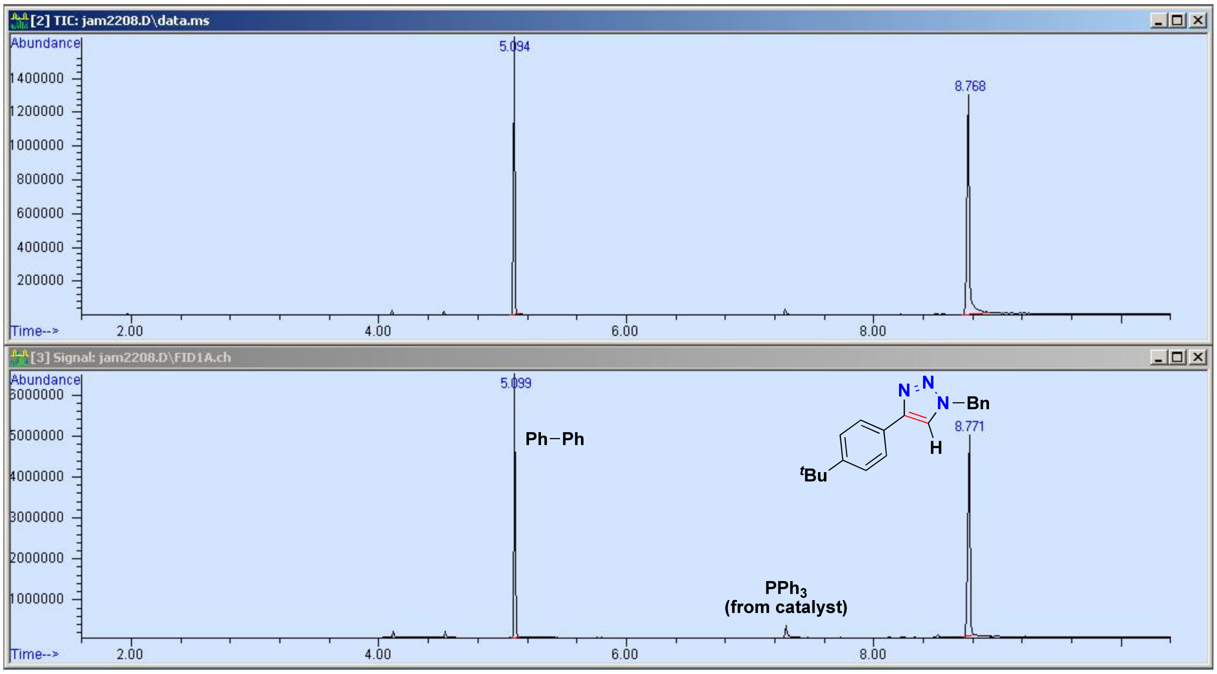
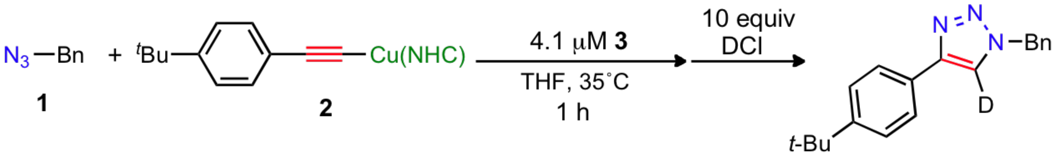


Table S2. Endpoint control experiments in the presence of an exogenous copper catalyst (GC-MS).

Exp	*Area of Triazole*	*Area of Biphenyl*	*Ratio*	*% Yield*	***Avg yield***
1	63785475	41679010	1.5304	94.1	**97.1%**
2	69721347	42969306	1.6226	99.8	
3	66491711	41947400	1.5851	97.4	

Procedure for the incorporation of deuterium, analyzed by LC-MS; catalyzed reaction:

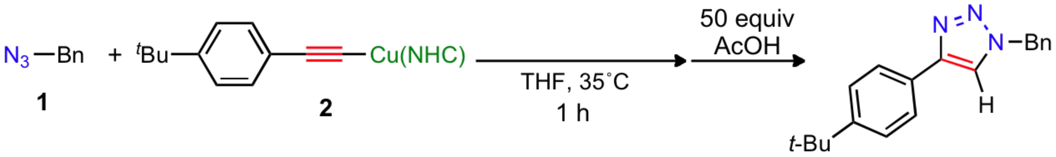


To validate the persistence of the copper(NHC)-triazole bond after the reaction reached completion, we quenched the above reactions with a solution of DCl in D_2O (10 equiv, 475 μL, DCl 20% *w/w* in D_2O), allowed to stir for 10 minutes and analyzed by LC-MS with a pre-calibrated diode array detector. The ratios of the extracted masses are summarized in the table below:

Table S3. Deuterium incorporation (LC-MS)

Exp	*5-H triazole area*	*5-D triazole area*	*Deuterium incorp.*	***Avg D-incorp.***
1a	173644	5721680	97.1	**95.6%**
2a	294616	6632410	95.7	
3a	410982	6592600	94.1	

Procedure for the endpoint analysis by GC-MS; uncatalyzed reaction:



Inside of an argon-filled glove box, a 10 mL microwave vial equipped with a magnetic stir bar was added 150.0 mg (0.245 mmol, 1 equiv) of acetylide **2**. The vial was capped, removed from the glove box, and diluted with 2 mL of dry THF. Additionally, inside of a glove box, a 10 mL volumetric flask was loaded with 359.3 mg of benzyl azide (**1**, 2.70 mmol, 1.1 equiv/1 mL) and 378.3 mg of biphenyl (2.45 mmol, 1 equiv/1 mL), and capped with a rubber 24/40 septum. The vial was taken from the glove box, and the contents were diluted with 10 mL dry THF. 1 mL of this homogeneous solution was removed and added to the stirring solution of acetylide **2**. After stirring for one hour, a 100 μL aliquot was extracted from the reaction and diluted with 900 μL of a .0439 M (50 equiv) solution of acetic acid in methanol. This vial was capped, agitated for several minutes, and then analyzed directly by GC-MS. This procedure was repeated in triplicate giving an average yield of 6.9% for the 5-H-1,2,3-triazole. The results are summarized in the table below:

*Uncatalyzed reaction of azide **1** and acetylide **2** monitored by GC-MS (reaction aliquoted at 1 hour):*

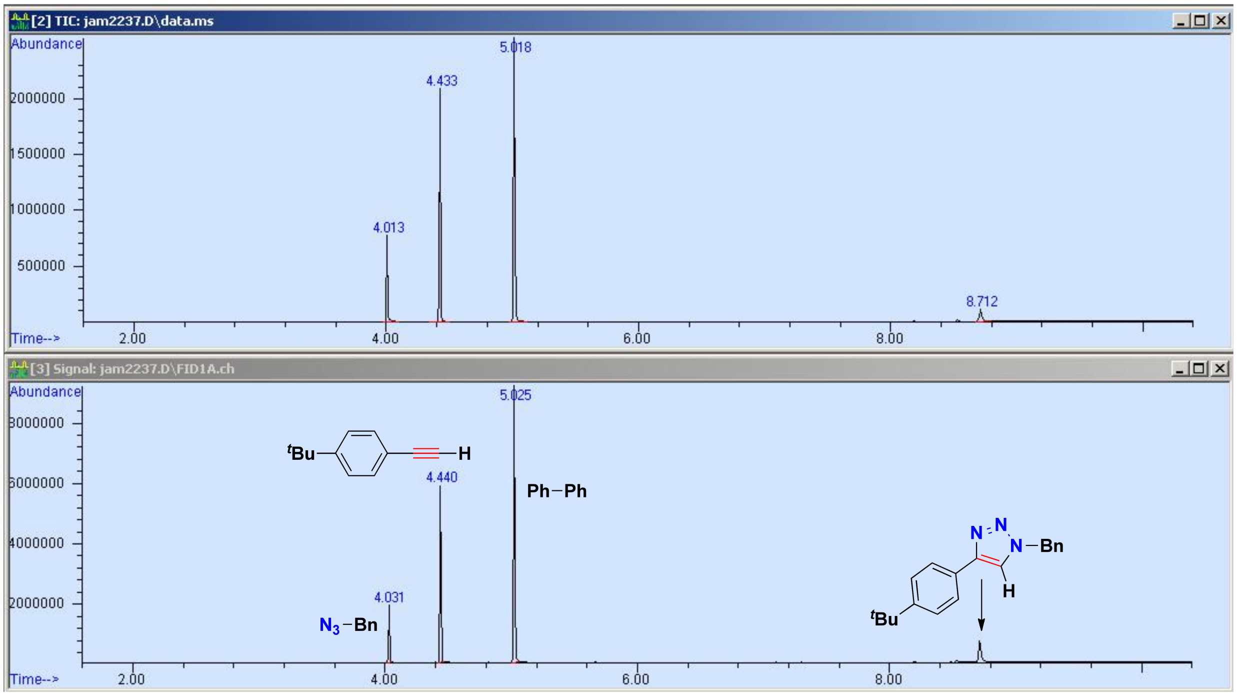
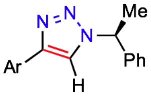
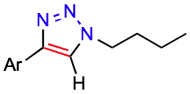
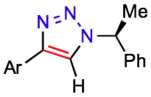
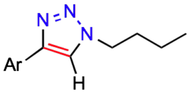


Table S4. Endpoint control experiments in the absence of exogenous copper catalyst (GC-MS)

Exp	*Area of Triazole*	*Area of Adamantane*	*Ratio*	*% Yield*	***Avg yield***
1	3991816	34536408	0.115582	7.1	**6.9%**
2	9568908	59908927	0.159724	9.8	
3	2455832	39385254	0.062354	3.8	

Table S5. Endpoint control experiments in the presence of an exogenous copper catalyst with varying azides (GC-MS).

Entry	*Triazole*	*Area of Triazole*	*Area of Biphenyl*	*Ratio*	***% Yield***
1a (cat.)		64,133,402	8,535,161	7.51	**109%***
2a (cat.)		59,832,613	6,363,170	9.40	**96.4%**
Entry	*Triazole*	*Area of Triazole*	*Area of Biphenyl*	*Ratio*	***% Yield***
1b		3,679,966	14,715,879	.250	**3.6%**
2b		6,277,767	8,683,420	.723	**7.4%**

Note: Ar = p-^{t}BuC_6H_4

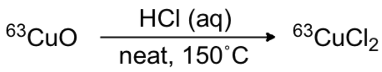
y-intercepts and R^2 values for entries 1-2 from calibration curves (determined by GC-MS):

Entry 1a/b: $y = 0.0688x$; $R^2 = 0.9964$.

*Small yield inflation due to long elution time (>9 min).

Entry 2a/b: $y = 0.0890x$; $R^2 = 0.9918$.

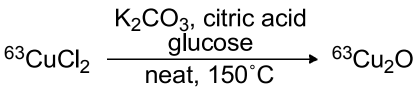
Procedure for the synthesis of isotopically enriched $^{63}Cu(MeCN)_4PF_6$



^{63}Copper(II) chloride

50 mg (.633 mmol, 1 equiv) of isotopically enriched ^{63}copper oxide (^{63}CuO, acquired from Oak Ridge National Laboratory, 99% ^{63}Cu content) was placed onto a 50 mm pyrex watchglass which was then placed on top of a standard hot-plate set at 80 ºC. To the black powder was added ~1 mL of concentrated hydrochloric acid; the mixture was agitated with a small plastic pipette tip until the black powder had completely dissolved, forming a deep green solution. The hot plate temperature dial was then set to 150 ºC, and the reaction mixture was further agitated with the pipette tip until the aqueous supernatant had boiled off and a dark green solid was obtained. The watchglass was allowed to sit on the warm hot plate for an addition 10 minutes to allow for the complete evaporation of hydrochloric acid. The solid was dissolved in deionized water (2 mL) and transferred to a 20 mL culture tube, which was used for the next reaction without further purification.

^{63}Copper(I) oxide



The 20 mL culture tube containing the solution from the previous step was treated with basic sodium citrate (0.80 g of sodium citrate and 0.65 g K_2CO_3 dissolved in 5 mL of water) and glucose (1.00 g dissolved in 2 mL of water). The test tube was placed in a heated orbital shaker (60 rpm), the temperature was set to 60 ºC, and the reaction was left for 4 hrs. The red precipitate was collected on a high-density filter paper, and sequentially washed with water, ethanol, and ether. This red powder was used in the next reaction without further purification.

Tetrakis(acetonitrile) ^{63}copper(I) hexafluorophosphate

$$^{63}\mathrm{Cu_2O} \xrightarrow[\mathrm{MeCN,\ rt}]{\mathrm{HPF_6}} \underset{\mathbf{5}}{^{63}\mathrm{Cu(MeCN)_4PF_6}}$$

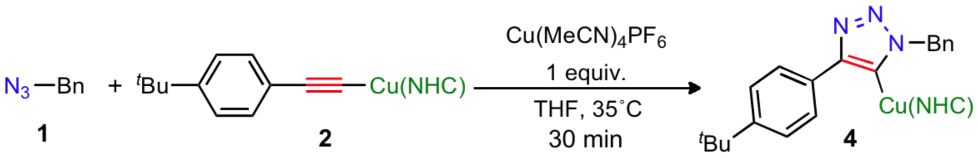
Copper(I) oxide obtained in the previous step was placed in a 20 mL culture tube and suspended in 5 mL of acetonitrile. To this red suspension, hydrogen hexafluorophosphate (60 wt% solution in water, obtained from Acros and used as received) was added dropwise with mild agitation until the red precipitate had completely dissolved.

Note: This is an extremely exothermic reaction; however, on this scale only minor amounts of heat were produced. Caution should be exercised when repeating this reaction.

After all copper(I) oxide dissolved and a clear solution was formed, agitation was continued for several minutes, and a portion of an equal volume of ether (~5 mL) was added to the reaction mixture. This tube was capped and placed into a -20 ºC freezer for approximately 1 hour. After this time, a white powder precipitated. It was filtered, washed with cold ether, and dried under high vacuum, affording 0.22 g (92% yield from copper(II) oxide) of the title compound (**5**) as a fluffy white solid.

General procedures for isotopic crossover experiments. Analysis using time-of-flight ESI mass spectrometery (TOF-MS)

Procedure for the stoichiometric synthesis of **4** *using* <u>*non-isotopically enriched*</u> *Cu(MeCN)$_4$PF$_6$:*



1 **2** **4**

In a 1-dram vial containing 1 mL of dry THF, 25.0 mg (0.0409 mmol, 1 equiv) of acetylide **2**, 5.98 mg (0.0449 mmol, 1.1 equiv) of benzyl azide (**1**) and 15.2 mg (0.0409 mmol, 1 equiv) of Cu(MeCN)$_4$PF$_6$ were placed. The vial was tightly capped, agitated for several minutes (30 min) and analyzed directly by TOF mass spectrometry via direct injection. The resulting mass spectrum is shown in Figure S8 below.

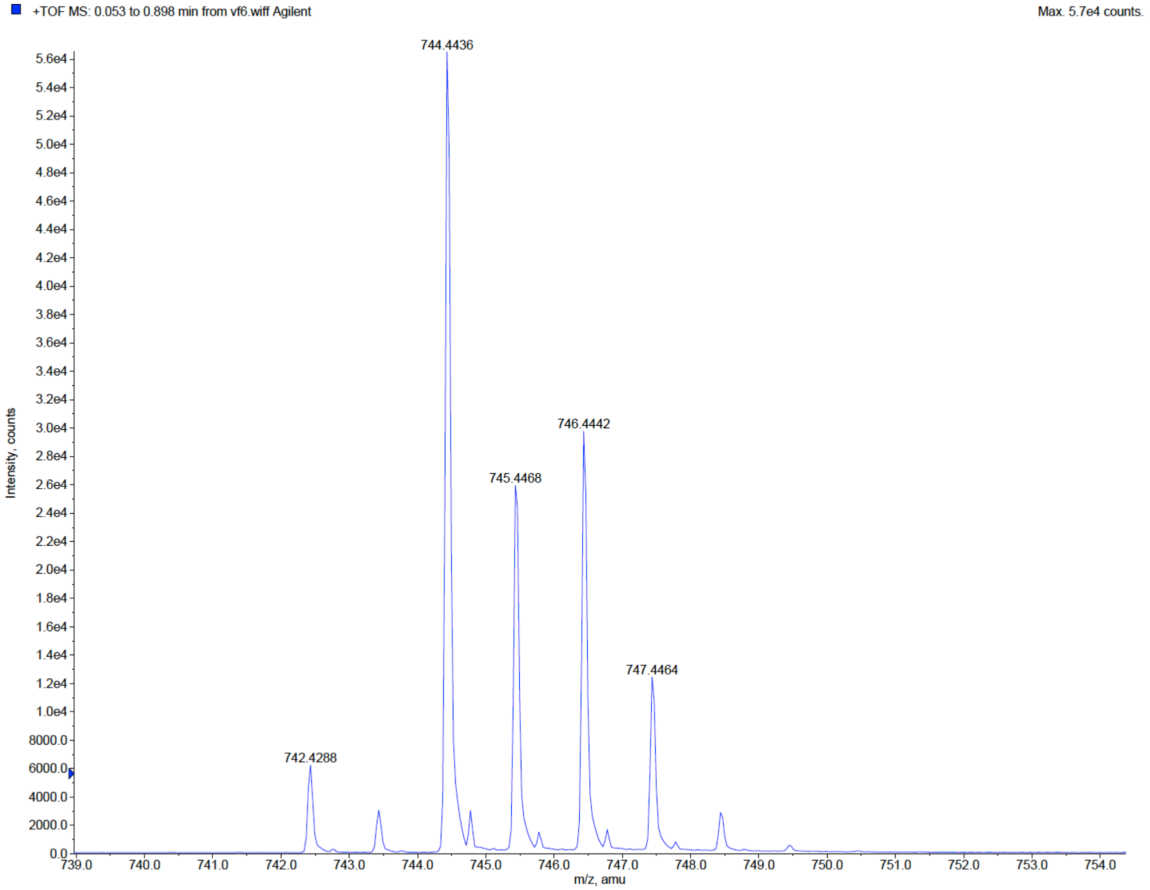
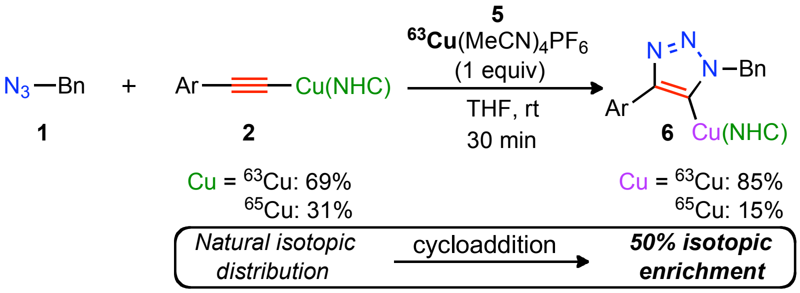


Figure S8. Mass spectrum of triazolide **4** with natural ^{63}Cu:^{65}Cu abundance

744.4436 = M+1 of triazolide **4** – ^{63}Cu isotope
746.4442 = M+1 of triazolide **4** – ^{65}Cu isotope

The relative intensity of the above peaks corresponds to the *normal isotopic distribution for a non-isotopically enriched copper source*.

Procedure for the stoichiometric crossover experiment of azide **1**, *acetylide* **2** *and* ^{63}*Cu(MeCN)*$_4$*PF*$_6$ *(***5***) giving enriched triazolide* **6**:



25.0 mg (0.0409 mmol, 1 equiv) of acetylide **2**, 5.98 mg (0.0449 mmol, 1.1 equiv) of benzyl azide (**1**) and 15.2 mg (0.0409 mmol, 1 equiv) of ^{63}Cu(MeCN)$_4$PF$_6$ (**5**) were placed in a 1-dram vial filled with 1 mL of dry THF. The vial was tightly capped, agitated for several minutes (30 min) and analyzed directly by TOF mass spectrometry via direct injection. The resulting mass spectrum is shown in Figure S9 below.

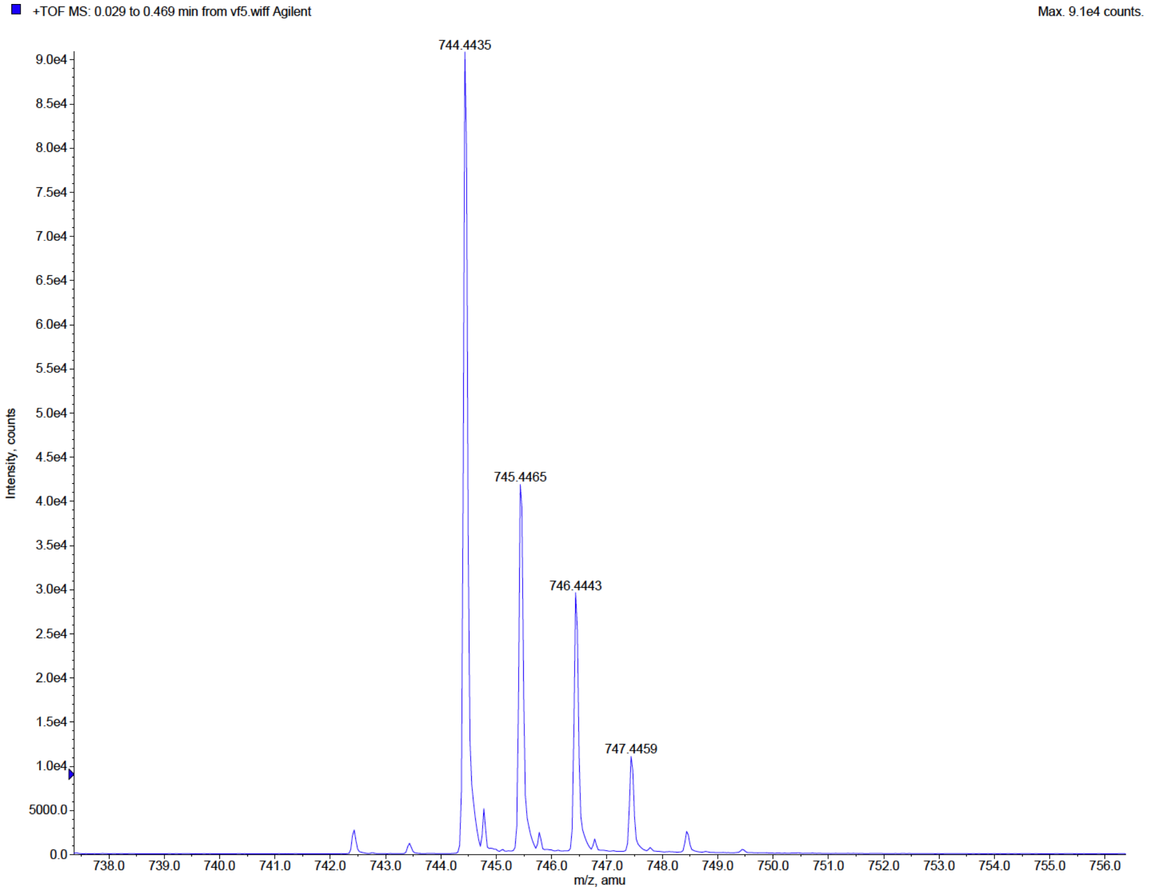
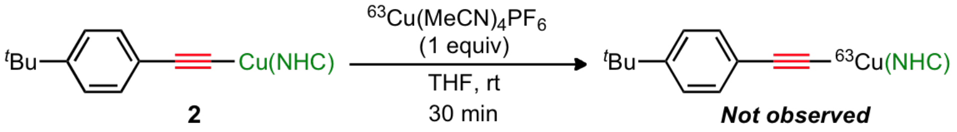


Figure S9. TOF-MS spectrum of enriched triazolide **6**.

744.4435 = M+1 of enriched triazolide **6** – ^{63}Cu isotope
746.4443 = M+1 of enriched triazolide **6** – ^{65}Cu isotope

The relative intensity of the above peaks corresponds to the 85:15 ^{63}Cu:^{65}Cu content (or 50% enrichment with ^{63}Cu).

Procedure for the stoichiometric crossover experiment of acetylide **2** *with* ^{63}*Cu(MeCN)*$_4$*PF*$_6$ *(***5***):*



25.0 mg (0.0409 mmol, 1 equiv) of acetylide **2** and 15.2 mg (0.0409 mmol, 1 equiv) of ^{63}Cu(MeCN)$_4$PF$_6$ (**5**) were placed in a 1-dram vial filled with 1 mL dry THF. The vial was tightly capped, agitated for 30 min, and the mixture was analyzed directly by TOF mass spectrometry via direct injection. The resulting mass spectrum is shown in Figure S10 below.

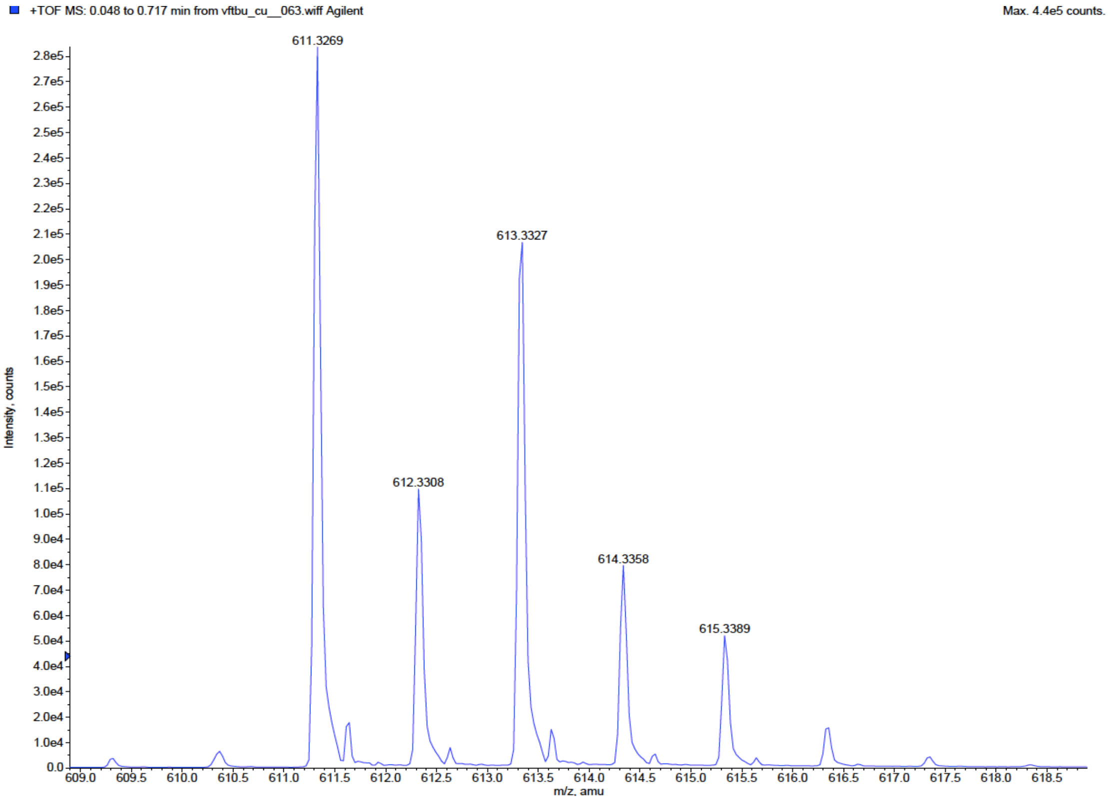


Figure S10. TOF-MS spectrum of attempted crossover between acetylide **2** and enriched copper source ^{63}Cu(MeCN)$_4$PF$_6$ (**5**).

611.32 = M+1 of acetylide **2** – ^{63}Cu isotope
613.32 = M+1 of acetylide **2** – ^{65}Cu isotope

The relative intensity of the above peaks corresponds to the *normal isotopic distribution for a non-isotopically enriched copper source*.

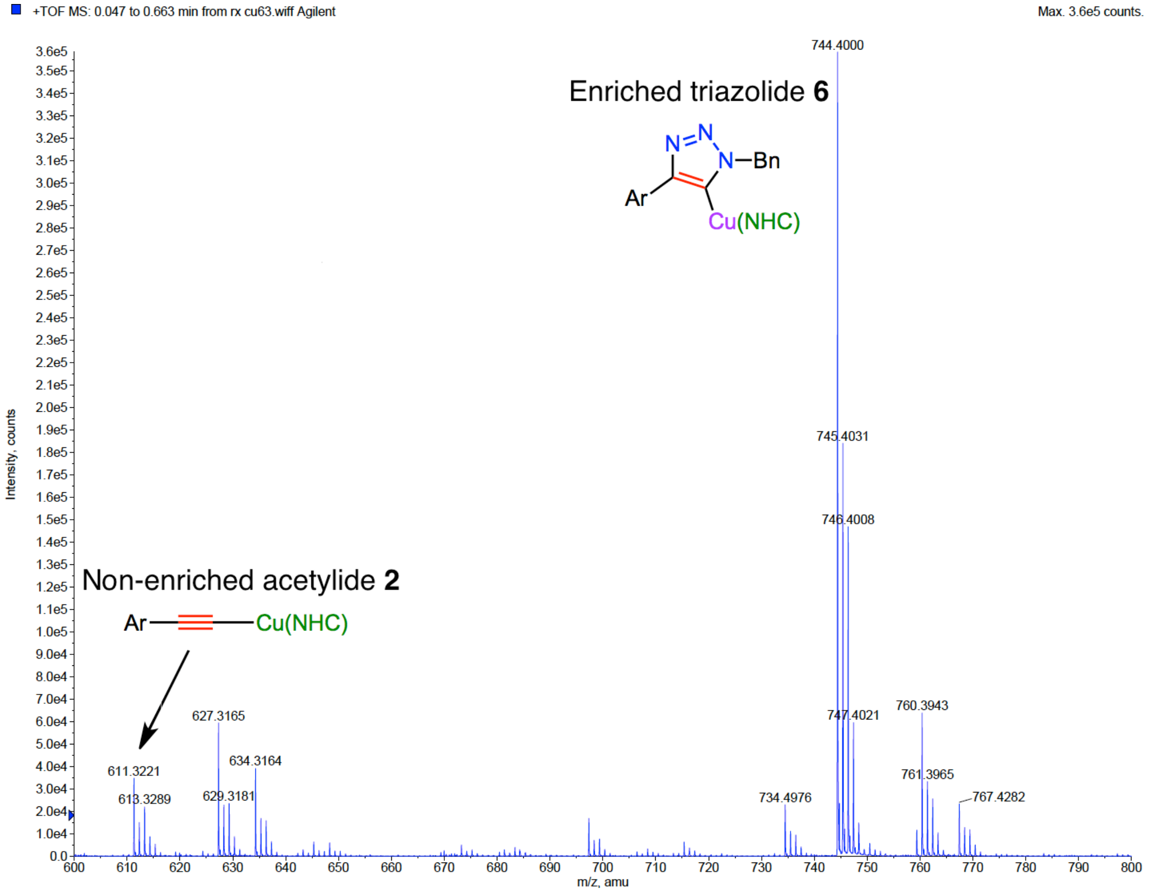


Figure S11. TOF-MS spectrum showing no crossover with acetylide **2** and enriched copper source ^{63}Cu(MeCN)$_4$PF$_6$ (**5**) in the presence of azide **1** (reaction mixture described on p.32 was directly injected while the reaction was still proceeding)

611.32 = M+1 of acetylide **2** – ^{63}Cu isotope
613.32 = M+1 of acetylide **2** – ^{65}Cu isotope

The relative intensity of mass signals of the acetylide **2** corresponds to the *normal isotopic distribution, i.e. no enrichment with the ^{63}Cu isotope*

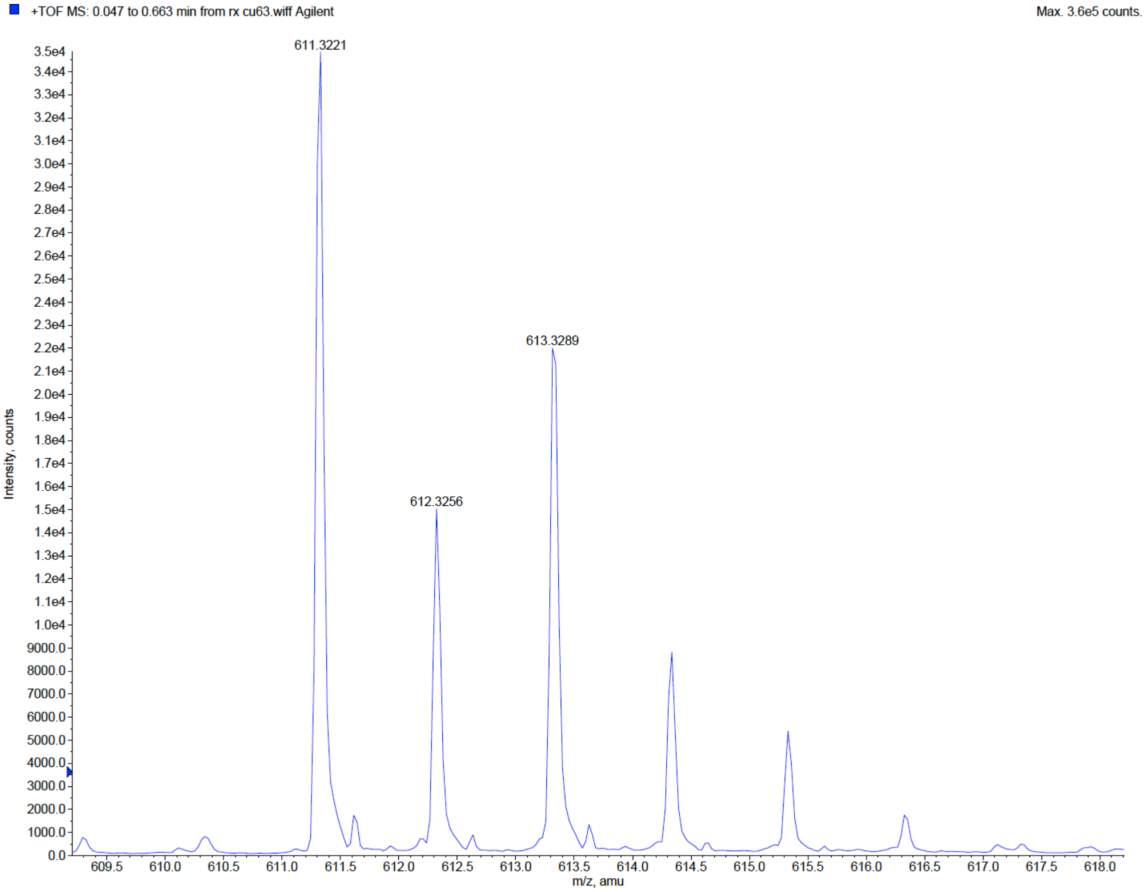
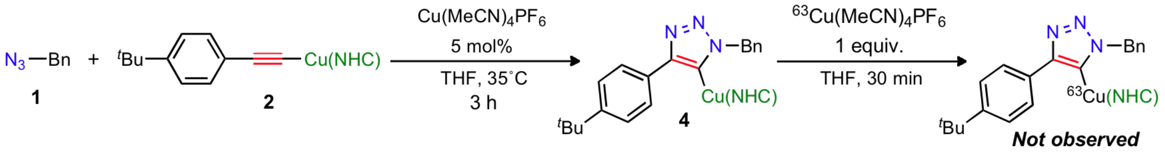


Figure S12. TOF-MS spectrum TOF-MS spectrum showing no crossover with acetylide **2** and enriched copper source ^{63}Cu(MeCN)4PF6 (**5**) in the presence of azide **1.** - Expanded spectrum of non-enriched acetylide **2**.

611.32 = M+1 of acetylide **2** – ^{63}Cu isotope
613.32 = M+1 of acetylide **2** – ^{65}Cu isotope

The relative intensity of the acetylide **2** corresponds to the *natural isotopic distribution.*

Procedure for the stoichiometric crossover experiment of non-enriched copper triazolide **4** *with* *$^{63}Cu(MeCN)_4PF_6$ (***5***):*



A 15 mL septum-capped vial equipped with a magnetic stir bar was loaded with 150.0 mg (.245 mmol, 1 equiv) of acetylide **2** inside of an argon-filled glove box. This vial was removed from the glove box, and the contents were dissolved in 2 mL of dry THF, placed into the reaction calorimeter, and was allowed to equilibrate to 35 ºC for 60 minutes. While this vial was equilibrating, a separate 1-dram Teflon capped vial was loaded with 35.9 mg (0.270 mmol, 1.1 equiv) of benzyl azide (**1**), capped and taken out of the glove box. Additionally, inside of a glove box, a 5 mL volumetric flask was loaded with 39.9 mg (0.0613 mmol, .05 equiv/1 mL) $Cu(MeCN)_4PF_6$, and capped with a rubber 24/40 septum. This vial was removed from glove box, and the contents were dissolved in 5 mL dry THF. The copper solution was agitated for several minutes until the complete dissolution of the copper could be visualized and 1 mL of this homogeneous solution was removed via syringe and added to the vial containing the azide. The contents of the vial were extracted with a 1 mL plastic syringe; the needle was capped with a small piece of Teflon and placed into the calorimeter needle jacket. After the system had equilibrated, the reaction was initiated by the injection of azide/copper solution. After 3 hours in the calorimeter and completion of the reaction had been observed (baseline heat output), the vial was removed from the calorimeter, and 500 μL of the solution was extracted via syringe and transferred to a 1-dram vial. After 15.2 mg (0.0409 mmol, 1 equiv) of $^{63}Cu(MeCN)_4PF_6$ (**5**) were added to this vial, it was tightly capped, agitated for several minutes (30 min), and analyzed directly by TOF mass spectrometry via direct injection. The resulting mass spectrum is shown in Figure S13 below.

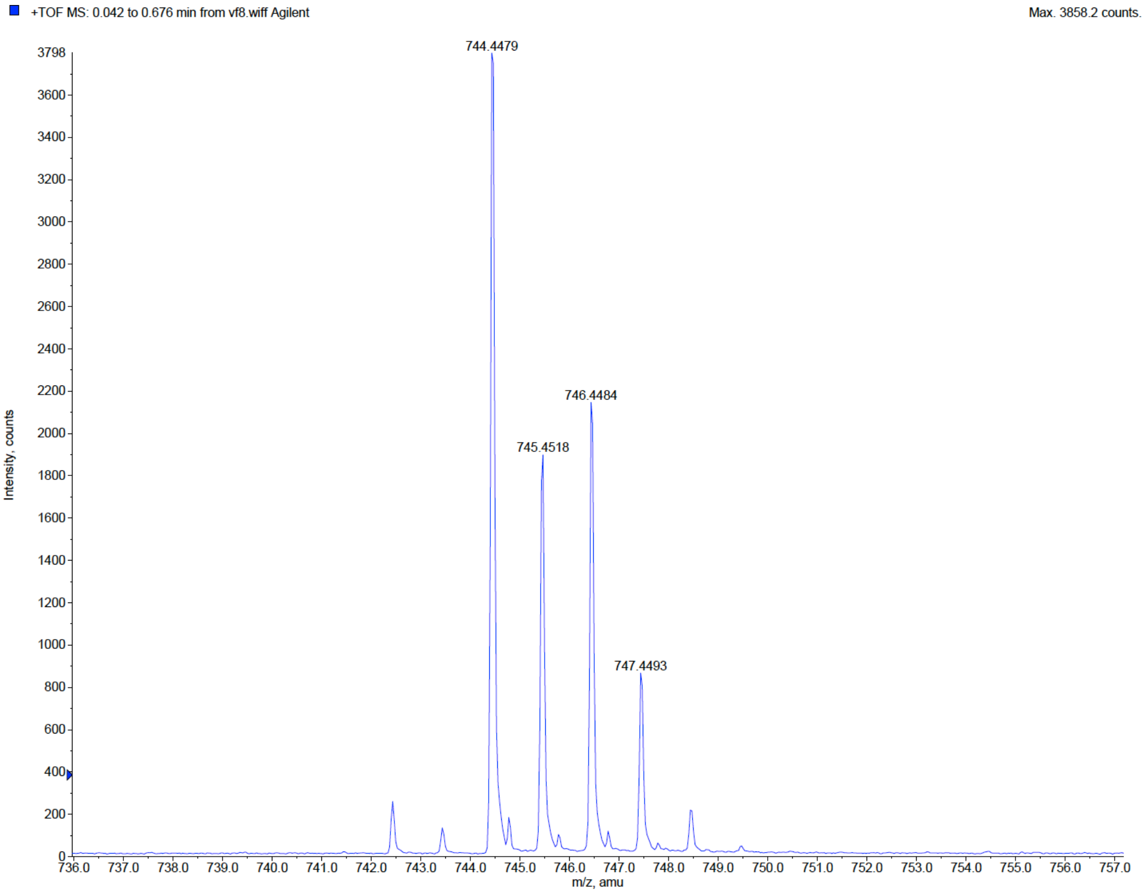


Figure S13. TOF-MS spectrum of attempted crossover between triazolide **4** and enriched copper source $^{63}Cu(MeCN)_4PF_6$ (**5**).

744.4479 = M+1 of triazolide **4** – ^{63}Cu isotope
746.4484 = M+1 of triazolide **4** – ^{65}Cu isotope

The relative intensity of the above peaks corresponds to the *normal isotopic distribution for a non-isotopically enriched copper source*.

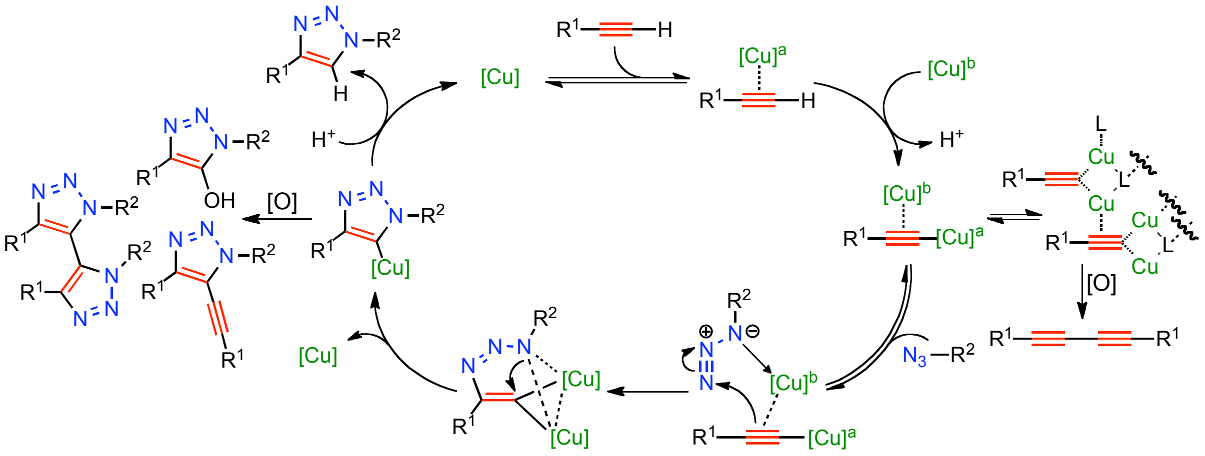
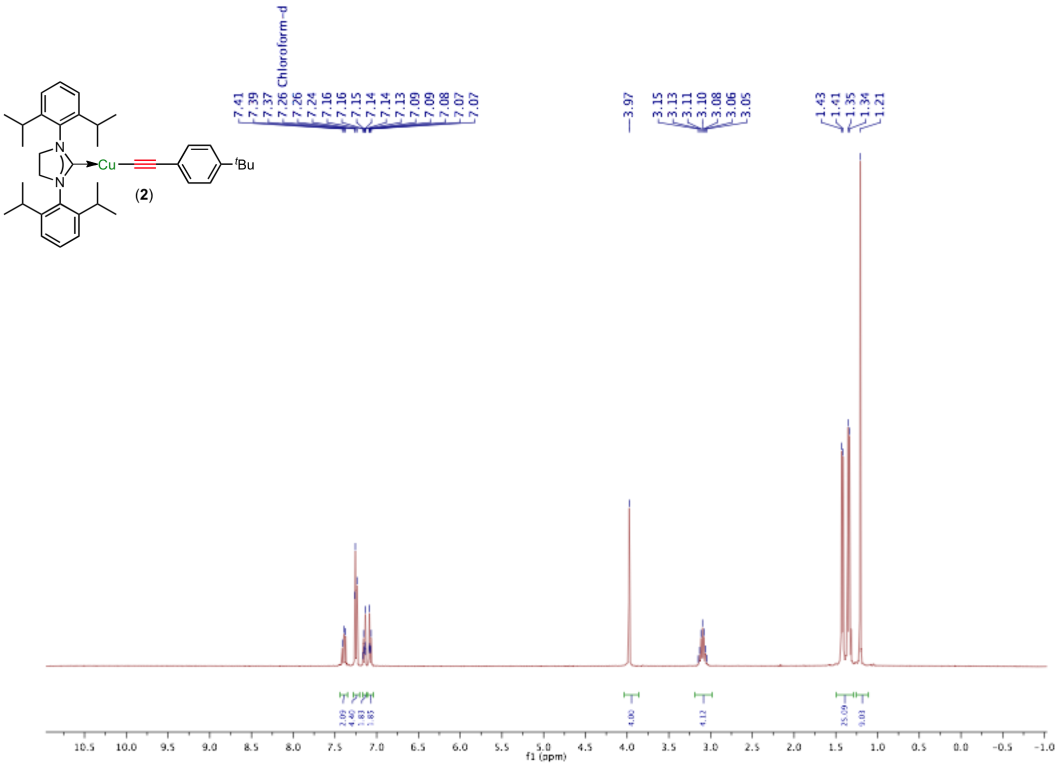
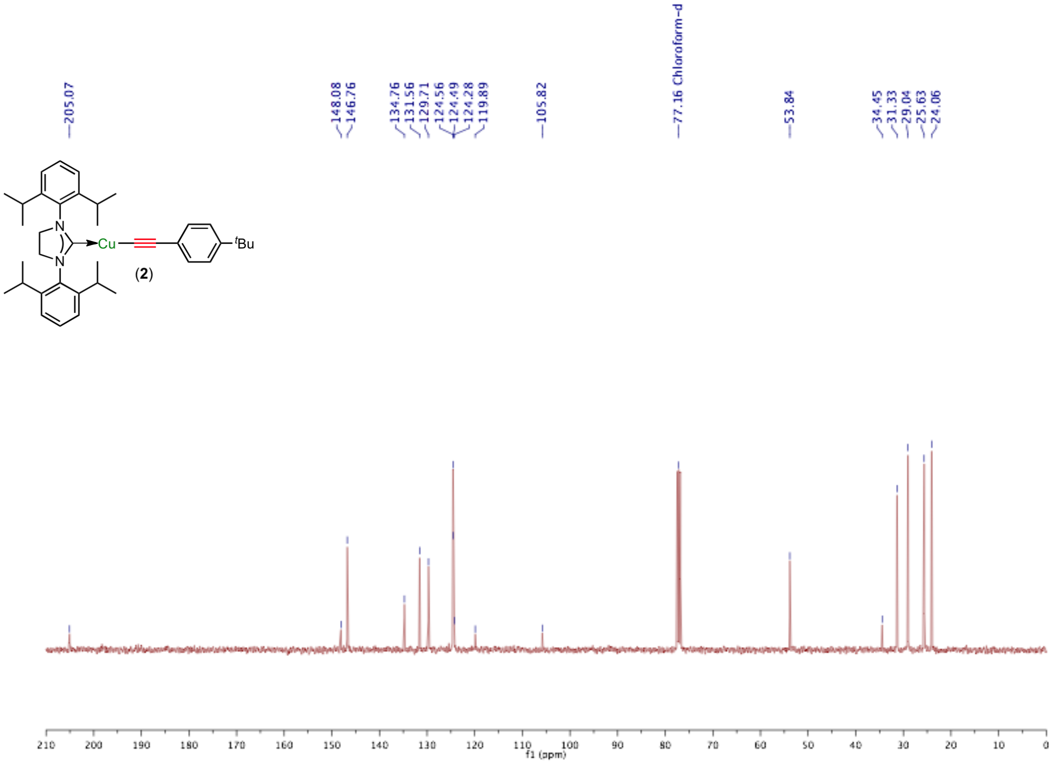


Figure S14. Revised catalytic cycle of CuAAC including off-cycle events and dinuclear copper intermediates.

NMR Spectra


Cu
ᵗBu
(2)
7.41
7.39
7.37
7.26 Chloroform-d
7.26
7.24
7.16
7.16
7.15
7.14
7.14
7.13
7.09
7.09
7.08
7.07
7.07
3.97
3.15
3.13
3.11
3.10
3.08
3.06
3.05
1.43
1.41
1.35
1.34
1.21
2.09
4.40
1.83
1.85
4.00
4.12
25.09
9.03
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


Cu
ᵗBu
(2)
205.07
148.08
146.76
134.76
131.56
129.71
124.56
124.49
124.28
119.89
105.82
77.16 Chloroform-d
53.84
34.45
31.33
29.04
25.63
24.06
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)