

Inquiry-Driven Investigation of the Copper-Catalyzed Azide-Alkyne Cycloaddition in the Undergraduate Organic Chemistry Laboratory

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Abstract: An inquiry-driven activity suitable for an undergraduate organic or inorganic chemistry laboratory is presented. The activity centers on the copper-catalyzed azide-alkyne cycloaddition, a reaction that is widely used in chemical synthesis, biochemistry, and material science, yet one that is noticeably absent in most teaching laboratories. In the activity, students are charged with formulating a question or hypothesis regarding the reaction between ethyl azidoacetate and phenylacetylene. Given a panel of reagents and copper sources, the students must design and carry out experiments and appropriate control reactions to address their lines of inquiry. The reaction itself benefits from operational simplicity: it can be carried out in a test tube or beaker that is open to air, water is a suitable solvent, and the triazole product crystallizes directly from the reaction mixture, enabling isolation by simple filtration. This reaction also provides a link to contemporary issues in sustainable chemical synthesis such as catalysis, atom-economy, “click chemistry” and safe solvents. In implementing this teaching laboratory, students discovered hitherto unknown features of the copper-catalyzed azide-alkyne cycloaddition such as the detrimental effect of halides on the copper catalyst and the poor activity of copper iodide as a catalyst for the featured transformation. Herein, the development and implementation of the teaching laboratory is described, the diverse and creative lines of student inquiry are reported, as well as the novel aspects of this reaction that were discovered. The benefits of inquiry-driven, unscripted teaching laboratories are also discussed.

Introduction

Inquiry-Driven Teaching Laboratories. Inquiry-driven teaching laboratories have gained increasing favor in chemical education due to their effectiveness in promoting critical thinking [1]. Such laboratory activities, when supplemented by dedicated instructor guidance, achieve this important goal by empowering students to generate a research question or hypothesis and design their own experiments. Additionally, students are challenged to interpret results that are not prescribed by a lab manual, evaluate the significance of their experimental outcome, and identify any further experiments prompted by their results. In this way, inquiry-driven labs enable students to engage in the enterprise of science in a way that is not possible in traditional, scripted laboratory activities that largely verify a predetermined outcome [2]. Accordingly, there has been a call to develop inquiry-driven laboratory activities for incorporation into university-level teaching labs—especially upper-level courses such as organic chemistry [1a, 2-3] and inorganic chemistry [4]. Here, we present a laboratory activity designed to promote scientific inquiry [5] and, at the same time, introduce undergraduates to one of the more important reactions in contemporary chemistry: the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction [6].

The Copper-Catalyzed Azide-Alkyne Cycloaddition (CuAAC). The CuAAC (Figure 1) is one of the most versatile reactions in applied chemistry [7]. This reaction is operationally simple, reliable, exquisitely regioselective, and compatible with a wide range of reaction media [7]. The reaction also benefits from excellent atom economy [8] and

modularity that, together with the aforementioned features, have earned its reputation as the prototypical transformation in “click chemistry” [9]. As such, the CuAAC has been deployed in diverse contexts. For example, the synthesis of pharmaceutical compounds and enzyme inhibitors [10], the synthesis of polymers and other functional materials [7], and the covalent modification of biological molecules and even living organisms [7a, 11] are all routine applications of this remarkable reaction.

With respect to undergraduate chemistry education, the CuAAC is a relatively recent advance. As a result, it is not typically addressed in lectures and teaching laboratories. Notably, a few laboratory exercises have been published to address this omission. Sharpless, for instance, has developed a laboratory activity that takes advantage of the modularity of the CuAAC and allows students to synthesize a 1,2,3-triazole target of their choosing when provided a selection of azides and terminal alkynes [12]. In a complementary laboratory activity, Schoffstall has designed a protocol where the terminal alkyne is generated by a copper catalyzed decarboxylation and then reacted with an azide. This activity also features the interesting use of citrus zest as a source of the reducing agent ascorbate—useful in reducing a copper(II) sulfate pre-catalyst to the active Cu(I) catalyst [13]. In a third example, Lipshutz and Wenzel have designed a laboratory exercise that employs micelles that facilitate the CuAAC in water. In their activity, the authors draw a link to green chemistry and the use of water as an environmentally benign solvent [14]. In all of these laboratory activities, students are rightfully introduced to the utility of the CuAAC. Yet, these activities are not without limitations. For instance, many of these laboratory activities

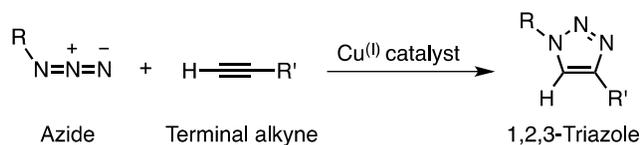


Figure 1. The Copper-Catalyzed Azide-Alkyne Cycloaddition.

prescribe several hours of reaction time [12] or employ sodium azide (a highly toxic reagent) in the synthesis of the alkyl azide starting material [12–13]. Most importantly, however, none of these previous reports feature student inquiry as an educational priority and employ regimented protocols. In contrast, the laboratory activity reported here uses a rapid and operationally simple CuAAC as the platform for student-designed scientific inquiry.

Laboratory Guidelines

Experimental Considerations. This activity features the copper-catalyzed cycloaddition between ethyl azidoacetate and phenylacetylene (Figure 2) and has been completed by more than 150 students in the second-semester of a second-year, undergraduate organic chemistry laboratory course. The activity is typically completed over two, 3-hour laboratory periods. The first lab period is devoted to the student-designed experiment. The second lab period is allocated for analytical characterization and follow-up experiments prompted during the first lab period. The mechanism and uses of the CuAAC in contemporary research is typically introduced a week prior to laboratory work (see Discussion and Supporting Information for details). This measure allows ample time for students to consult background literature, generate a question or hypothesis regarding the reaction in Figure 2, and design an experiment or series of experiments to address their independent line of inquiry. These student-designed experiments may center on how the reaction is influenced by temperature, reaction time, copper sources, copper loading, the effect of various additives, or other parameters of their choosing. To focus their inquiry, a list of available solvents, copper sources and reagents is provided at least one week before the laboratory period (See laboratory handout in the Supporting Information). Additionally, a few guidelines are provided to ensure student experiments can be completed safely, economically, and within the allocated lab period(s). While adherence to these guidelines is ultimately left to the discretion of the lab coordinator and instructor, students are requested to consider water as a solvent, run their reaction at or below room temperature, and work at approximately a 1.0 mmol scale [15].

A representative experiment is carried out by first adding water (1.0 mL) to a 5 mL vial or test tube, followed by phenylacetylene (1.0 mmol) and ethyl azidoacetate (1.0 mmol). The copper source is then added (e.g. 0.05 mmol of copper sulfate), along with any additive (e.g. 0.10 mmol ascorbic acid), and the reaction is stirred at room temperature, open to air, for at least 30 minutes or until the triazole product crystallizes from the reaction mixture. The reaction can also be monitored by thin layer chromatography using 4:1 petroleum ether : ethyl acetate as the mobile phase [16]. The product triazole is isolated by filtration and characterized by melting point determination, IR spectroscopy, and ^1H NMR spectroscopy using $\text{DMSO}-d_6$ as the solvent. In a control experiment, the reaction is carried out in the same fashion without copper. If no product precipitates, or if unreacted starting materials are observed by TLC, the mixture is extracted directly into CDCl_3 and analyzed by IR (thin film) and ^1H NMR spectroscopy. These experiments are representative protocols for investigating whether or not the copper is necessary for the reaction in Figure 2. Additional lines of inquiry pursued by students are elaborated in the Results and Discussion. The outcome of student experiments and data analysis is assessed by a post-lab write-up.

Safety Considerations. All students were required to undergo Departmental safety training before participation in the laboratory. The following guidelines were used in assessing lab risks: Safety glasses, lab coats, and nitrile gloves should be worn at all times during the laboratory activity. Hazardous materials should be handled and sent to the waste stream in accordance with *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals* [17]. The ethyl azidoacetate solution in ethanol is flammable should be handled in a fume hood; it should also be treated as potentially harmful. Neat ethyl azidoacetate is also potentially explosive, so the solution of this reagent should be kept from heat and never concentrated by rotary evaporation. Due to these risks, the reaction is best conducted at or below room temperature. Taking these precautions, we have encountered no incidents with ethyl azidoacetate after hundreds of uses. Phenylacetylene is flammable and a suspected carcinogen. If syringes are used to transfer reagents, sharps should be disposed in appropriate containers. Copper(I) salts are irritants and may be harmful if swallowed. Copper(II) salts are toxic and harmful to aquatic life and all aqueous waste containing copper must be sent to a suitable chemical waste contractor. Copper metal is moderately flammable and should be kept clear from ignition sources. Ascorbic acid and water have no significant hazards, but should be handled with care. $\text{DMSO}-d_6$ is readily absorbed through the skin and may transport harmful materials into the body. Chloroform-*d* is harmful if swallowed and a suspected carcinogen. The triazole product should be treated as harmful and care should be taken to avoid inhalation, ingestion, or contact with skin.

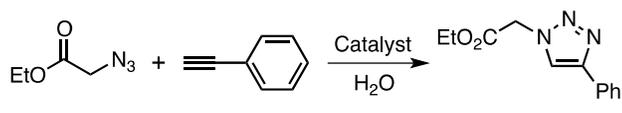
Results and Discussion

Representative examples of the most common types of student-designed experiments are summarized in Table 1 and representative student-generated ^1H NMR data is shown in Figure 3. (Instructor-acquired ^1H and ^{13}C NMR spectra are provided in the Supporting Information for comparison). The top spectrum in Figure 3 is typical for the product triazole when it is isolated by simple filtration directly from the reaction mixture. The bottom spectrum in Figure 3 is typical for experiments that resulted in unreacted starting material (as in a control reaction in which no copper was used). For isolated triazole, the measured melting point was typically in the range 92–95 $^\circ\text{C}$ —very near the values obtained independently by the authors and reported by others in the literature (93–95 $^\circ\text{C}$) [18]. IR spectra obtained by students clearly featured the carbonyl in the triazole product at 1755 cm^{-1} and no peak for the azide. In contrast, if unreacted starting materials were obtained, a distinct azide stretch was typically reported at 2109 cm^{-1} along with a carbonyl stretch at 1746 cm^{-1} .

Teaching Laboratory Outcomes. The pre-lab discussion was carried out at least one week prior to the laboratory activity. This discussion centered on the importance of the CuAAC and its use in chemistry, biology, and materials science. The mechanism of the reaction was also discussed, and Figure 4 provided a useful starting point. The active catalyst is a copper(I) species that can be generated, in principle, by reduction of a copper(II) pre-catalyst or by oxidation of a copper(0) precatalyst (**A** in Figure 4). Copper(I) behaves as a Lewis acid that can bind to a π bond of the terminal alkyne (**B**), dramatically lowering its $\text{p}K_a$ and leading to the formation of copper-acetylide **C**. The regioselectivity is determined by complex **D**, where the copper coordinates to the internal nitrogen of the azide [19]. The subsequent cyclization and protonation leads to the product triazole, and the copper(I) catalyst is regenerated. It should be noted that Figure 4 is a simplified mechanism provided for the purpose of specifying

Table 1. Representative Student-Designed Experiments and Outcomes

Entry	General Line of Inquiry	Representative Experiment	Outcome
1	Compare yields between two catalysts	CuBr (5 mol%) was employed in one reaction and CuI (5 mol%) was employed in a second reaction. The reaction was carried out for 30 minutes at room temperature. Triazole yields were compared.	The triazole was isolated by filtration in 90% yield for the reaction employing the CuBr catalyst. Only unreacted starting material was observed by ¹ H NMR when CuI was used as the catalyst.
2	Compare yields between different reaction temperatures	CuBr (5 mol%) was used as a catalyst for two reactions: one at room temperature and one at 0 °C. The reactions were both run for 30 minutes and the isolated yields were compared.	The triazole was isolated in quantitative yield from the reaction run at room temperature. Trace product was observed for the reaction run at 0 °C.
3	Compare yields between two catalyst loadings	CuBr was used as a catalyst for two reactions: one at 5 mol% and the other at 50 mol%. Isolated yields were compared after 30 minutes of reaction time.	The triazole was isolated in >90% yield for both catalysts loadings, though triazole crystallized from the reaction within 5 minutes when higher catalyst loading was employed.
4	Investigate effect of additive on triazole yield	The reaction was run using CuSO ₄ as a catalyst (5 mol%) along with ascorbic acid as a reducing agent (10 mol%). As a control, the same experiment was run in the absence of ascorbic acid.	The triazole was isolated by filtration in 70% yield after 30 minutes of reaction time when ascorbic acid was employed. Unreacted starting material was observed by ¹ H NMR when no ascorbic acid was employed.
5	Investigate whether a given catalyst generates triazole	A US penny was tested as a potential copper catalyst. The conversion was compared to the uncatalyzed reaction in which no copper was employed. The reaction was run in a beaker of water, open to air, with gentle stirring for 1 week at room temperature.	After 1 week of reaction time at room temperature, the triazole was isolated in 84% for the penny-catalyzed reaction. Only unreacted starting material was observed by ¹ H NMR when no copper was employed.



Student responsibilities

- Propose a research question or hypothesis related to reaction
- Design experiment or experiments to answer question (Must take into account supplies provided by instructor)
- Plan experimental procedure, including all calculations and working plan for reaction set-up, workup, and analysis
- Carry out experiments and relevant controls
- Evaluate outcome of experiment
- Discuss followup experiments prompted by outcome

Figure 2. Overview of the Reaction and Student Responsibilities in this Laboratory Activity.

the active oxidation state of copper (+1) and a rationale for the observed regioselectivity (stage **D**). Ligand effects and the complex nature of copper's coordination sphere were not addressed in detail. This simplified catalytic cycle is adapted from the original reports by Sharpless [6a] and Meldal [6b], but students should be made aware of more complicated proposals that feature intermediates with multiple copper centers that better account for kinetic and computational experiments [20].

With a mechanistic framework in hand, students were able to test some of the premises of the CuAAC as part of their inquiry. For instance, several students tested the efficiency of catalysts in different oxidation states or the effect of reducing copper(II) to copper(I) using ascorbic acid. Table 1 summarizes other lines of inquiry pursued by students. Even

for the routine experiments (such as comparing the yields between two catalysts or determining yields at two different temperatures), it should be noted that students are required to design the reaction setup and calculate the proper amount of reagents and catalysts with a rationalized stoichiometry. For many students, this responsibility is a challenge. Moreover, even these seemingly routine experiments often led to unexpected results, such as discovering that CuBr was a more efficient catalyst than CuI for this reaction, prompting a series of follow-up experiments that ultimately spawned an entire undergraduate research project (vide infra) [21]. These simple experiments also lead to scenarios where students were required to adjust their approach throughout the course of the reaction. For instance, some students used an older supply of CuBr that proved to be sluggish in catalysis. Reasoning that the copper may have been oxidized after prolonged exposure to air, the students added a reducing agent (ascorbic acid). Returning the copper to its active +1 oxidation state led to rapid formation of the triazole and provided an excellent point of instruction on how practicing chemists deal with experiments in real time.

More ambitious experiments led to some genuinely novel results. One student, inspired by Sharpless and Fokin's original report that demonstrated copper turnings could serve as a viable catalyst [6a], tested whether a penny could catalyze the azide-alkyne cycloaddition. While relatively slow, the copper from the surface of the penny did lead to an 84% yield of the triazole product after 1 week of reaction time. When no copper was used, only unreacted starting material was observed by ¹H NMR spectroscopy.

There were common challenges encountered during the experiments in all lab sections. For instance, many copper(I) sources are sensitive to air, and old batches may prove poor catalysts due to air oxidation. In such instances, adding the reducing agent ascorbic acid led to recovered activity. Also,

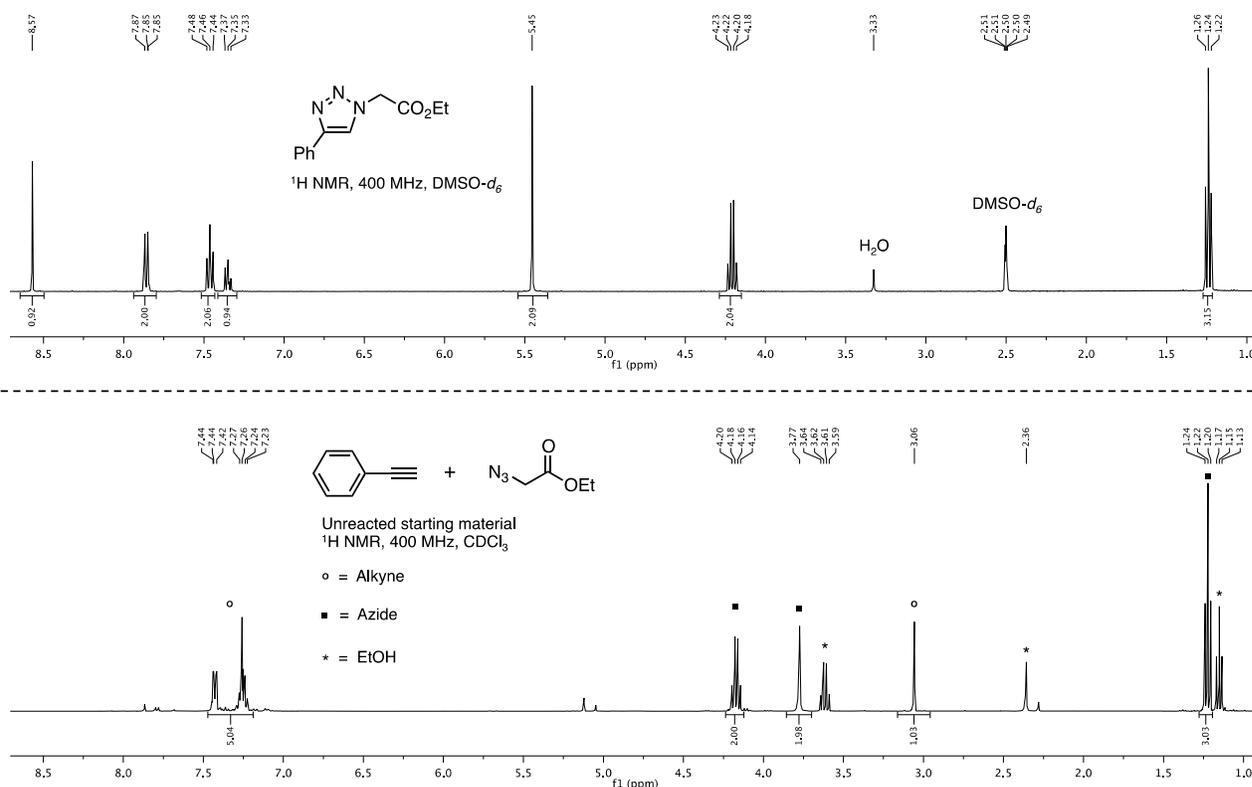


Figure 3. Representative Student-Acquired ^1H NMR Spectra. The spectrum for the isolated triazole product is shown on top and the spectrum for unreacted starting material on the bottom. The unreacted starting material was extracted directly into CDCl_3 from a mixture of azide and alkyne—a negative control experiment that employed no copper.

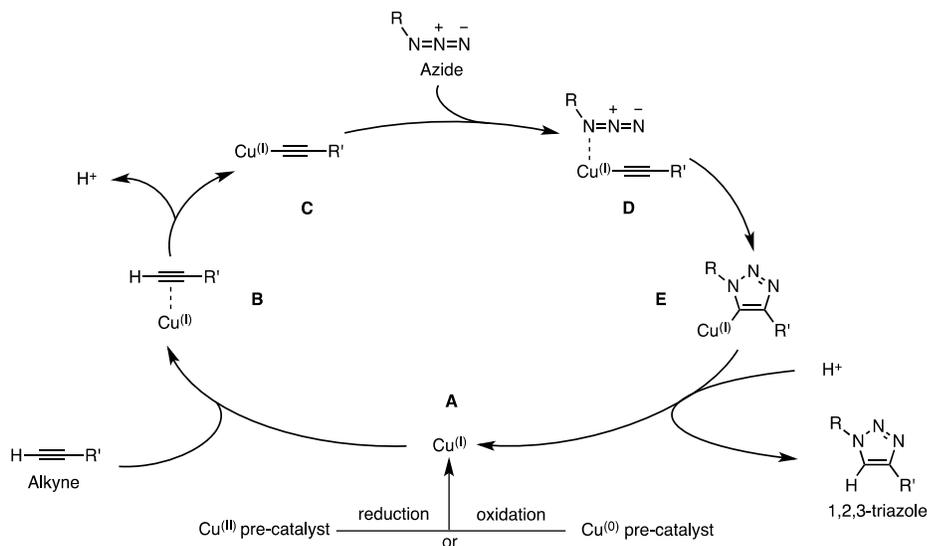


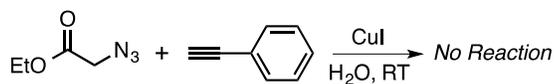
Figure 4. Mechanistic Outline of the Copper-Catalyzed Azide-Alkyne Cycloaddition.

the triazole product can coordinate to copper and lead to brightly colored products. Though not necessary for most analyses, the isolated product can be triturated with 25% NH_4OH to remove the sequestered copper [12].

Learning objectives were assessed by evaluation of lab notebooks, online lab reports, and post-lab questions. For all experiments, evaluation of IR and NMR spectroscopic data was required. The NMR data in Figure 3 are representative of

submitted spectra. Importantly, students were asked to identify follow-up experiments based on the outcome of their experiment. This question proved an excellent opportunity for students to recalibrate their experimental design or devise entirely new experiments based on interesting and sometimes confusing results. Detailed feedback from instructors was also provided to clarify or reinforce student interpretation of experimental results and to point out other factors to consider

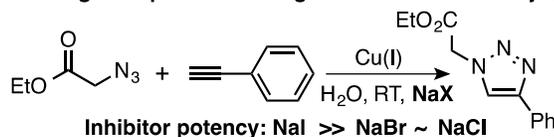
1. Observation in Teaching Lab



Student questions prompted by observation:

- Is iodide a detrimental ligand for Cu(I) in the CuAAC?
- Do other halides inhibit the CuAAC in water?

2. Findings in Spin-Out Undergraduate Research Project



- NaCl, NaBr, and NaI all inhibit CuAAC in water
- CuI can be activated by reaction with AgNO₃
- Tandem S_N2-CuAAC is facilitated if halide leaving group scavenged with AgNO₃
- Tris-triazole ligand for Cu(I) (e.g. THPTA) can overcome inhibition by NaCl

Figure 5. Translating Teaching Lab Observations to an Undergraduate Research Project.

in follow-up experiments. Such feedback has been previously highlighted as a critical component in the effective implementation of inquiry-driven laboratory activities [1a].

Undergraduate Research Project Inspired by Student Results. As mentioned above, several students observed that copper(I) iodide was a poor catalyst for the featured azide-alkyne cycloaddition. Following-up on this experiment, four students initiated an undergraduate research project to understand this phenomenon. Instructor and institutional support was critical in this endeavor, as it allowed these students to coordinate their investigation with semester-long research courses and residential summer research programs. Over the subsequent 18 months, these undergraduate researchers uncovered several important effects of halides in the CuAAC, which were recently published elsewhere [21]. These results are summarized (Figure 5) and discussed here to illustrate the impact, both scientific and educational, that can arise from inquiry-driven teaching labs. For full experimental details of this spin-out research project, readers are referred to associated publication [21].

In their independent study (Figure 5), students first examined why copper(I) iodide is a poor catalyst in water. It is known that ligands or bases can activate copper(I) iodide for the CuAAC [22], so they reasoned that iodide is a ligand for copper that results in low solubility in water and/or occludes a necessary coordination site for the azide and alkyne. Indeed, abstracting iodide with AgNO₃ activated CuI and rescued its catalytic activity for the same reaction in which it had previously failed. This result then prompted the undergraduate researchers to determine if free halides in solutions (NaCl, NaBr, and NaI) can inhibit the copper catalyst. Several clues in the literature suggested that chloride [23] and bromide [24] ions could exhibit inhibitory effects, but no systematic investigation had been reported. Upon adding solutions of NaCl, NaBr, or NaI to CuAAC reaction mixtures, a pronounced inhibitory effect was observed. NaI arrested activity at room temperature when equimolar to the copper

catalyst. NaCl and NaBr were also detrimental, but to a lesser degree than NaI [21].

Recognizing that halides are encountered in many scenarios where the CuAAC is deployed, the undergraduate researchers next studied how to overcome this inhibition. In one example, AgNO₃ was used as a halide scavenger to sequester chloride and iodide formed in the synthesis of an azide substrate. The CuAAC was carried out directly on this azide, and provided the target triazole only when halides were removed from the reaction mixture as their insoluble silver salts [21]. In another example, the undergraduate researchers demonstrated that tris-triazole ligands such as tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) [25], overcomes sodium chloride inhibition [21]. This result is critical information for successful use of the CuAAC on biological samples in buffers that contain high concentrations of sodium chloride.

The benefits of this undergraduate research project are manifold and a direct result of incorporating an inquiry-driven teaching laboratory into the Organic Chemistry curriculum. Students led the study from its inception, discovered interesting chemical phenomena, and reported their findings to the scientific community. In addition to publishing the results of their study [21], the students also had the opportunity to present their efforts at professional conferences [26, 27]. This experience provided an excellent technical training ground for these aspiring scientists. It also highlights ways in which inquiry-driven labs and undergraduate research programs can drive science education. We have disclosed the details of the original inquiry-driven teaching lab here so that other Universities can adapt and expand its use as an educational platform. We highlight research products and student achievement here as a motivating factor for Universities to consider implementing curiosity-driven activities into their teaching laboratories.

Conclusions

In summary, an inquiry-driven laboratory has been developed that introduces undergraduate students to the copper-catalyzed azide-alkyne cycloaddition. The featured reaction is simple to execute and entails several parameters easily amenable to guided-inquiry and unscripted study in the organic or inorganic teaching lab. In this way, students learn about an important chemical reaction while being introduced to science as it is practiced by research professionals. This mode of education is empowering and stands in stark contrast to more traditional, scripted laboratory activities. As demonstrated here, inquiry-driven teaching laboratories create opportunity for discovery, scientific training and professional research experience.

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Supporting Information. Detailed chemical and equipment list, student handouts, author-generated analytical data for triazole product (melting point, TLC analysis, NMR spectra, IR spectra, and mass spectrometric data).

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