

# Harnessing the Dual Properties of Thiol-Grafted Cellulose Paper for Click Reactions: A Powerful Reducing Agent and Adsorbent for Cu

Jordi Rull-Barrull<sup>†</sup>, Martin d'Halluin<sup>†</sup>, Erwan Le Grogneq, and François-Xavier Felpin\*

**Abstract:** A new approach exploiting the dual properties of thiol-grafted cellulose paper for promoting copper-catalyzed [3+2]-cycloadditions of organic azides with alkynes and adsorbing residual copper species in solution was developed. The thiol-grafted cellulose paper, used as a paper strip, effects the reduction of Cu<sup>II</sup> to catalytically active Cu<sup>I</sup> and acts as a powerful adsorbent for copper, thereby facilitating the work-up process and leaving the crude mixture almost free of copper residues after a single filtration.

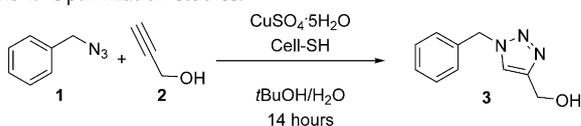
The copper-catalyzed [3+2] cycloaddition of an organic azide with a terminal alkyne to give the corresponding 1,4-disubstituted triazole is one of the most powerful and thoroughly studied click reactions.<sup>[1]</sup> While the thermally promoted [3+2] cycloaddition, initially discovered by Huisgen,<sup>[2]</sup> suffers from the disadvantage of generating both 1,4- and 1,5-disubstituted triazoles with poor regiocontrol, the copper-catalyzed version independently pioneered by Meldal<sup>[3]</sup> and Sharpless<sup>[4]</sup> has become an indispensable tool in chemical biology<sup>[5]</sup> and material science<sup>[6]</sup> owing to its compatibility with aqueous conditions, good tolerance to steric hindrance and diverse functional groups, and excellent regioselectivity for 1,4-triazoles. Two main experimental methods have emerged from the hundreds of publications reporting the use of this click reaction catalyzed by Cu<sup>I</sup> species. The first one involves the direct use of Cu<sup>I</sup> salts with strict exclusion of oxygen due to the high susceptibility of Cu<sup>I</sup> toward oxidation. This approach usually lacks robustness due to complicating side reactions and copper instability. The use of nitrogen-type ligands greatly enhances both reaction yields and rates since they prevent the degradation of Cu<sup>I</sup> by oxidation or disproportionation.<sup>[7]</sup> The second procedure involves the *in situ* reduction of Cu<sup>II</sup> salts such as CuSO<sub>4</sub>·5H<sub>2</sub>O to Cu<sup>I</sup> with a 3- to 10-fold excess of a reducing agent. Sodium ascorbate is indisputably the most frequently used reducing agent, but hydrazine<sup>[8]</sup> and tris(2-carboxyethyl)phosphine<sup>[9]</sup> have been occasionally used as well. This

second strategy is by far the preferred procedure since it does not require an inert atmosphere, can be carried out in water, gives more reliable results, and is accessible to non-experts such as biologists. However, using sodium ascorbate as a reducing agent can be complicated by both the over-reduction of Cu<sup>II</sup> to Cu<sup>0</sup> and the formation of radical oxygen species.<sup>[10]</sup> Moreover, strongly electrophilic byproducts of dehydroascorbate have been reported to react with protein chains and alter the chemical structure of DNA.<sup>[11]</sup> It must be noted that alternative procedures involving the photo-induced reduction of Cu<sup>II</sup> to Cu<sup>I</sup> have showed interesting results.<sup>[12]</sup>

Environmental concerns, as well as toxicity issues from copper species in compounds of biological interest, have led to the development of immobilized catalytic systems, which allow efficient copper removal through simple filtration. Copper catalysts have been immobilized as molecular complexes and nanoparticles on a variety of supports,<sup>[13]</sup> including polymers,<sup>[14]</sup> silica,<sup>[15]</sup> zeolite,<sup>[16]</sup> and carbon-based materials.<sup>[17]</sup> By contrast, the use of immobilized reducing agents is unprecedented, although it could simplify the removal of reducing-agent byproducts, especially for biological materials, and increase the selectivity for Cu<sup>I</sup> over Cu<sup>0</sup>.

We recently developed a biomimetic reducing agent immobilized on cellulose paper for the highly selective reduction of Cu<sup>II</sup> to Cu<sup>I</sup> with the simultaneous detection of copper by colorimetric and optical tools.<sup>[18]</sup> Our heterogeneous reducing system consists of cellulose filter paper covalently grafted with thioglycolic acid (Cell-SH). Upon contact with aqueous CuSO<sub>4</sub>·5H<sub>2</sub>O solutions, the paper reduces Cu<sup>II</sup> to Cu<sup>I</sup> nanoparticles in a couple of seconds

**Table 1:** Optimization studies.



Entry <sup>[a]</sup>	Cu loading (mol%)	SH loading (mol%)	T [°C]	Yield [%] <sup>[b]</sup>
1	1	16	25	34
2	5	16	25	36
3	10	16	25	61
4	1	16	70	85
5	2	16	70	87
6	2	8	70	43
7	2	0	70	< 5

[a] Reaction conditions: benzyl azide **1** (1 mmol), propargyl alcohol **2** (1.5 mmol), CuSO<sub>4</sub>·5H<sub>2</sub>O and Cell-SH were stirred in 5 mL of *t*-BuOH/H<sub>2</sub>O (1:1) for 14 hours at the specified temperature. [b] Yield of isolated product.

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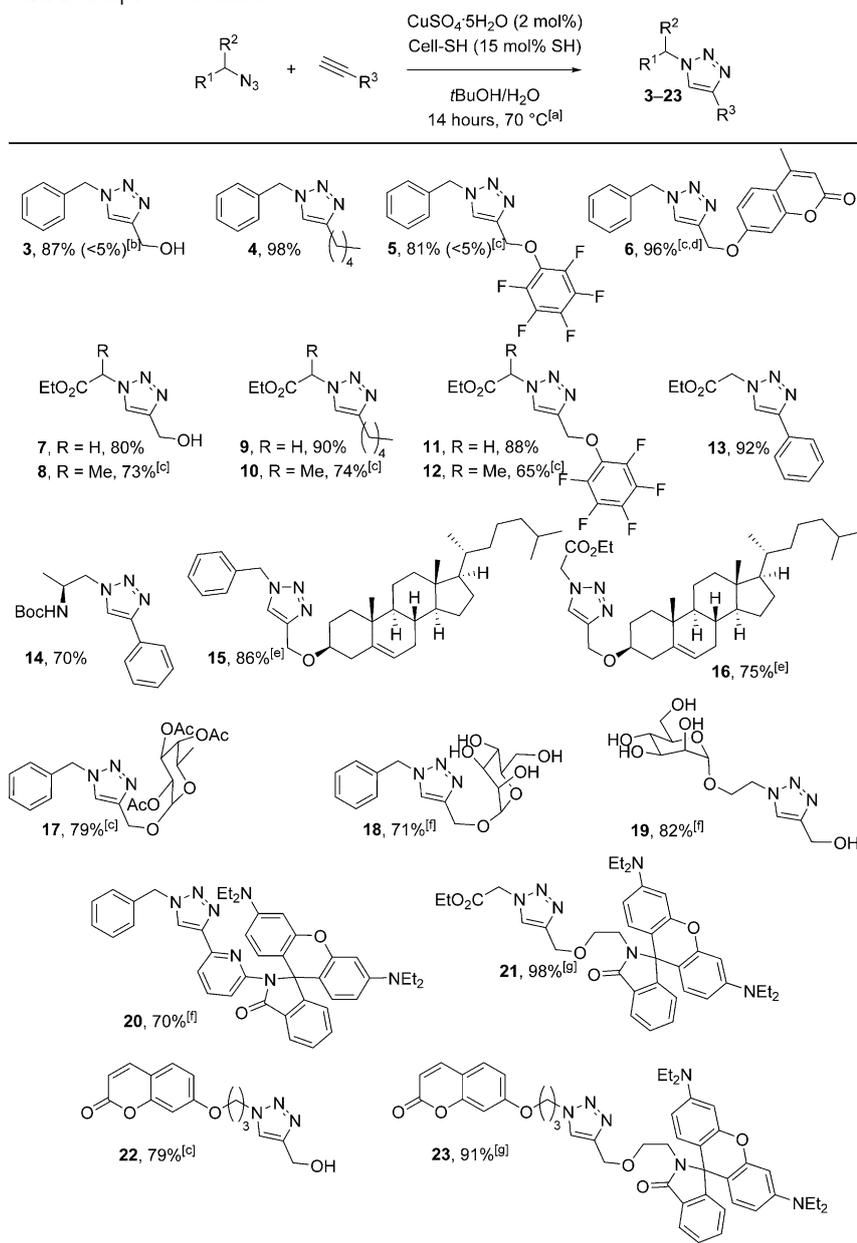
with simultaneous oxidation of thiols to disulfide compounds. The effectiveness of the reduction can be easily followed by the naked eye since the white paper strip rapidly turns black due to high  $S \rightarrow Cu$  charge transfer.

Herein, we report that this unprecedented reducing agent can be used for the copper-catalyzed Huisgen 1,3-dipolar cycloaddition of organic azides with terminal alkynes. Particularly, we demonstrate how a simple piece of paper functionalized with thiol functions can act as a dual tool, promoting the Cu-catalyzed Huisgen synthesis of 1,4-disubstituted triazoles through the reduction of  $CuSO_4 \cdot 5H_2O$  to  $Cu^I$  species and allowing efficient copper remediation. This bifunctional material leaves the crude product almost free of copper residues and reducing-agent byproducts after a single filtration.

We initially focused on a model reaction involving the cycloaddition of benzyl azide **1** with propargyl alcohol **2** in the presence of  $CuSO_4 \cdot 5H_2O$  as a catalyst in a *t*-BuOH/ $H_2O$  mixture (Table 1). The cellulose filter paper covalently grafted with thioglycolic acid used in this work was prepared through esterification of Whatman paper #6 with thioglycolic acid using PTSA as a catalyst according to our recently described method (Scheme S1 in the Supporting Information).<sup>[18–20]</sup> At room temperature, we observed sluggish cycloaddition that required high copper loading, up to 10 mol% Cu, to reach a modest yield of **3** (entries 1–3) when using a piece of paper equivalent to 16 mol% of SH functions. Fortunately, working at 70 °C significantly increased the reaction rates without affecting the Cell-SH efficiency since an excellent yield (87%, entry 5) was attained with only 2 mol% Cu. Decreasing the loading of thiol functions to 8 mol% significantly eroded the reaction yield (43%, entry 6), while the reaction background revealed that use of the treated paper as a reducing agent was essential for promoting the reaction since less than 5% of **3** was obtained in the absence of the paper (entry 7).

The optimized conditions were applied to the formation of diversely decorated triazoles (Table 2). In a first set of

**Table 2:** Scope of the reaction.



[a] Reaction conditions: azide (1 mmol), alkyne (1.5 mmol),  $CuSO_4 \cdot 5H_2O$  (2 mol%) and Cell-SH (16 mol%) were stirred in 5 mL of *t*-BuOH/ $H_2O$  (1:1) at 70 °C for 14 h. [b] Yield without Cell-SH shown in brackets. [c]  $CuSO_4 \cdot 5H_2O$  (4 mol%). [d] Reaction time: 72 hours. [e] *t*-BuOH/ $H_2O$ /THF (1:1:2) as the solvent mixture,  $CuSO_4 \cdot 5H_2O$  (4 mol%), and 14 hours of stirring. [f]  $CuSO_4 \cdot 5H_2O$  (4 mol%) and 48 hours of stirring. [g] MeOH/ $H_2O$ / $CH_2Cl_2$  (1:1:3) as the solvent mixture,  $CuSO_4 \cdot 5H_2O$  (4 mol%), and 48 hours of stirring.

examples, we studied the use of simple ligation partners. We learned from these studies that aromatic and aliphatic alkynes react with similar efficiency and an increase in the steric hindrance of the carbon atom bearing the azide function results in diminished reactivity, necessitating an increase in copper loading (4 mol%) when switching ethyl azidoacetate for more sterically hindered ethyl 2-azidopropanoate (**8**, **10**, **12** vs. **7**, **9**, **11**). The usefulness of Cell-SH as a powerful reducing agent for  $CuSO_4 \cdot 5H_2O$  is highlighted by two examples that show that almost no conversion occurs when

Cell-SH is omitted from the reaction mixture (compounds **3**, **5**, yield without Cell-SH shown in brackets). Having validated our method with a set of simple triazoles, we extended our study to the use of densely decorated coupling partners in order to evaluate the robustness and make a balanced analysis of the usefulness of the method. Since we reasoned that our practical Cell-SH device could be useful for biologists and analytical chemists, we used azides and alkynes bearing sugar, rhodamine, coumarin, and cholesterol moieties, which are often used in cell recognition, sensing, or labelling.<sup>[21]</sup> The scope of the reaction proved to be quite broad, with no obvious limitations, since the cycloaddition proceeded with both highly complex alkynes and azides. Impressively, free carbohydrates also reacted smoothly either as azide or alkyne derivatives (**18–19**), thus illustrating the high tolerance of this process. Actually, the main limitation of this process is related to the limited solubility of highly lipophilic substrates, which significantly alters the reaction rate. In order to increase the dispersion of structurally complex and poorly soluble partners, such as alkynes bearing cholesterol and rhodamine moieties, and attain reasonable rates, the cycloaddition was performed with 4 mol% Cu and in ternary mixtures of solvents with either THF, MeOH, or CH<sub>2</sub>Cl<sub>2</sub> as additional solvents (compounds **15–16**, **21–23**). As expected, the cycloaddition occurs regioselectively in favor of the 1,4-disubstituted triazoles, though the 1,5-disubstituted regioisomer was detected in small amounts for compounds **10** and **16**. In summary, our catalytic system is compatible with highly complex partners possessing either lipophilic or chelating properties, even those bearing a pyridine group (compound **20**), provided that the experimental conditions (e.g., solvent and/or time) are adapted.

Having demonstrated the powerful reducing properties of Cell-SH toward copper sulfate, to promote the [3+2]-cycloaddition of organic azides with alkynes, we explored the adsorption properties of this unusual material (Table 3). To accurately determine the level of copper removal, we analyzed the crude solution by inductively coupled plasma mass spectrometry (ICP-MS) after the paper strip was removed by filtration. We calculated the percentage of copper removal with respect to the initial amount introduced for the reaction (2 mol% Cu). The use of a slight excess of

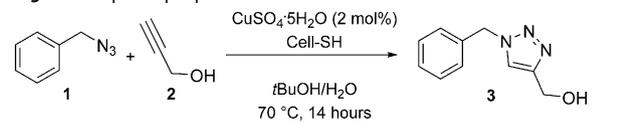
Cell-SH was inefficient in promoting the cycloaddition and removing copper species in solution since only a marginal amount of copper was adsorbed on the material and the reaction yield was disappointingly low (entry 2). Using a four-fold molar excess of Cell-SH with respect to the copper greatly increased the copper remediation to ca. 60%, but unfortunately the reaction yield remained unchanged (entry 3). By contrast, 94% of the copper species in solution were removed when a piece of paper corresponding to 16 mol% SH was used, and the reaction yield for triazole **3** reached 87% (entry 4). Upon increasing the loading of thiol functions to 24 and 32 mol%, copper removal marginally increased to around 97% and the reaction yield reached a plateau at around 90% (entries 5–6). These results reveal that the use of an eight-fold molar excess of SH functions with respect to copper constitutes the best compromise regarding copper removal efficiency and the atom economy.

While we have demonstrated that Cell-SH enables the adsorption of approximately 95% of the Cu initially introduced, we wondered whether the paper strip removed after completion of the reaction could be reused in a second run without requiring additional Cu. The reuse of Cell-SH was explored for the cycloaddition of benzyl azide **1** with propargyl alcohol **2**. An identical yield was observed for the second run (88%), while a sharp decrease occurred on the third use (ca. 50% yield) due to the fragility of the paper upon successive reuses, which leads to a loss of physical integrity. We also analyzed the nature of the copper species adsorbed onto Cell-SH after one cycle. Scanning electron microscopy showed the formation of spherical copper nanoparticles (Cu NPs) with an average diameter of around 36 nm (Figure S1 in the Supporting Information). X-ray photoelectron spectroscopy of the Cu NPs in the Cu2p region showed an absence of satellite peaks near 938–945 eV, while the peaks at 932.5 and 952.3 eV, which are attributed to Cu2p<sub>3/2</sub> and Cu2p<sub>1/2</sub> spin-orbit components, suggest the formation of Cu<sub>2</sub>O NPs (Figure S2).

Since similar behavior was observed for Cu in the absence of coupling partners,<sup>[18]</sup> we deduce from these results that Cell-SH rapidly adsorbs and reduces CuSO<sub>4</sub>·5H<sub>2</sub>O to Cu<sub>2</sub>O NPs that act as a catalyst for the transformation.

In summary, we present a new approach for the [3+2]-cycloaddition of organic azides with alkynes using a heterogeneous reducing agent that also acts as a powerful adsorbent for copper species in solution, thereby leaving the crude product almost free of both copper residues and reducing agent. This method involves the use of cellulose paper as a heterogeneous biopolymer for supporting covalent thiol functions and was inspired by living systems that reduce Cu<sup>II</sup> to Cu<sup>I</sup> with cysteine residues from proteins. The robustness of our catalytic system was demonstrated by the preparation of highly complex substrates. Inspiration from nature to uncover more sustainable methods is a field of research deserving of attention and we believe that this contribution will be of great interest to synthetic chemists and biologists.

**Table 3:** Adsorption properties of Cell-SH.



Entry <sup>[a]</sup>	SH loading (mol%)	Cu adsorbed [%]	Yield [%] <sup>[b]</sup>
1	0	0	< 5
2	3.2	4	41
3	8	58	43
4	16	94	87
5	24	97	88
6	32	97.5	91

[a] Reaction conditions: azide (1 mmol), alkyne (1.5 mmol), CuSO<sub>4</sub>·5H<sub>2</sub>O (2 mol%), and Cell-SH (see table) were stirred in 5 mL of tBuOH/H<sub>2</sub>O (1:1) at 70 °C for 14 h. [b] Yield of isolated product.



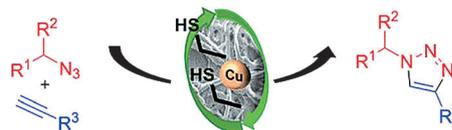
## Communications



## Click Chemistry

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Harnessing the Dual Properties of Thiol-Grafted Cellulose Paper for Click Reactions: A Powerful Reducing Agent and Adsorbent for Cu



**Looks good on paper:** An approach that exploits the dual properties of thiol-grafted cellulose paper for promoting copper-catalyzed [3+2]-cycloaddition of organic azides with alkynes and adsorbing residual copper species in solution

was developed. Thiol-grafted cellulose paper effects the reduction of Cu<sup>II</sup> to catalytically active Cu<sup>I</sup> and acts as a powerful adsorbent for copper, thereby facilitating the work-up process.