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Halide inhibition of the copper-catalysed azide–alkyne cycloaddition†

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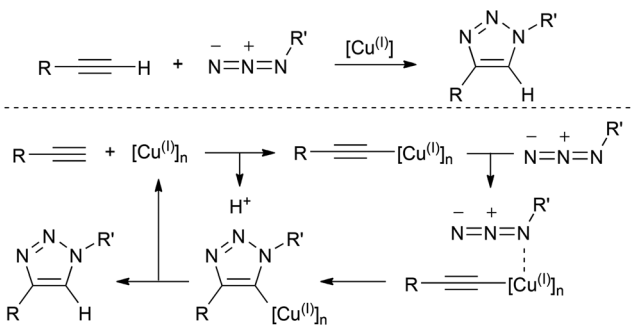
Chloride, bromide and iodide are inhibitors of the copper-catalysed azide–alkyne cycloaddition, with iodide exhibiting the most detrimental effects on rates and yields. A study of this inhibition is presented, along with experimental protocols to accommodate the presence of halides in this widely used reaction.

The copper-catalysed azide–alkyne cycloaddition (CuAAC, Scheme 1)[1,2] stands as one of the more widely used reactions in applied chemistry.[3] It benefits from exquisite regioselectivity,[4] excellent atom-economy,[5,6] and it is compatible with a wide range of reaction media.[3,4,7] Coupled to its generally high efficiency, these features have motivated deployment of the CuAAC in a variety of synthetic and analytical contexts in medicinal chemistry,[8,9] chemical biology,[3,10] material science,[3,4] and polymer chemistry.[3,4]

With such wide interest in this reaction, it is important to elucidate mechanistic features that can inform its productive use in the chemical sciences.[11,12] In this communication, we report that chloride, bromide, and iodide all inhibit the CuAAC, with iodide exhibiting the most detrimental effects on rate and yield. Halide effects in metal-catalysed reactions have long been known,[13,14] yet their influence on the efficiency of the CuAAC has not been fully clarified. There are, however, a number of clues in the literature that suggest such halide effects cannot be ignored. For example, Finn and co-workers noted that potassium chloride can slow the CuAAC in buffered media by competing with the azide in its binding to the copper catalyst.[15] Likewise, Lal and Díez-González reported that exogenous sodium bromide substantially slowed a model CuAAC in DMSO, though such an effect was not observed in water.[16] Understanding these halide effects is critical given the wide use of the CuAAC in buffers that contain halides (*e.g.* buffered saline),[17] as well as one-pot S_N2-CuAAC reaction sequences in which halides are produced in the synthesis of the azide reagent.[16] Here, we reveal that in aqueous systems, halides generally inhibit a copper sulphate catalyst—the original catalyst introduced by Sharpless for the CuAAC.[1] Additionally, we explore methods for accommodating halides in the CuAAC that allow efficient reaction at room temperature in water—an important consideration for thermally sensitive azides and biological molecules.



Scheme 1 The copper-catalysed azide–alkyne cycloaddition (CuAAC). A simplified mechanism is presented here. Further mechanistic details can be found in leading reviews.[11,12]

Our study began with an unusual observation in the reaction between ethyl azidoacetate and phenylacetylene (Tables 1 and 2). While copper(I) bromide (Table 1, entries 1–2) and various copper(II) pre-catalysts that had been reduced to copper(I) with ascorbic acid[1] (Table 1, entries 5–11, 13–14) provided triazole **1** in good to excellent yields, copper(I) iodide was not an effective catalyst at room temperature (Table 1, entries 3–4). The addition of ascorbic acid did not provoke catalysis, ruling out catalyst oxidation as the reason for poor yields. Prolonged reaction time (24 hours) did result in the formation of some triazole (less than 10% yield and conversion, Table 2, entries 1–2), suggesting that the catalyst was active, but plagued with low turnover frequency. Suspecting that the iodide was somehow occluding coordination of the azide or alkyne to the copper centre, silver nitrate was added to the reaction mixture to abstract the iodide. Indeed the addition of silver nitrate activated the copper(I) iodide catalyst and led to

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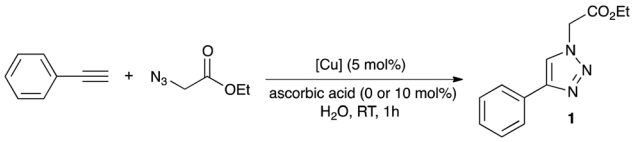
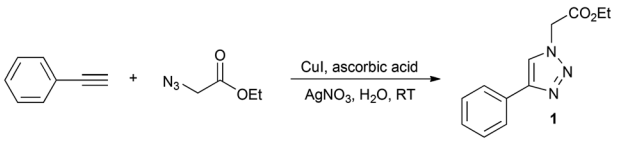
Table 1 Examination of various catalysts for a model CuAAC. Copper(I) iodide was discovered to be a poor catalyst for this transformation. For reactions in which the triazole crystallized directly from the reaction, isolated yields are reported. For all other reactions, the product distribution was determined by ^{1}H NMR after extraction into $CDCl_3$ (ESI)



Entry	[Cu] (5 mol%)	Ascorbic acid	Triazole **1**
1	CuBr	10 mol%	97% (isolated yield)
2	CuBr	—	88% (isolated yield)
3	**CuI**	**10 mol%**	**0% (unreacted azide and alkyne in ^{1}H NMR)**
4	**CuI**	**—**	**0% (unreacted azide and alkyne in ^{1}H NMR)**
5	$CuCl_2$	10 mol%	52% (conversion determined by ^{1}H NMR)
6	$CuCl_2$	—	0% (unreacted azide and alkyne in ^{1}H NMR)
7	$CuSO_4$	10 mol%	91% (isolated yield)
8	$CuSO_4$	10 mol%	100% (conversion determined by ^{1}H NMR)
9	$CuSO_4$	—	0% (unreacted azide and alkyne in ^{1}H NMR)
10	$Cu_2(OH)PO_4$	10 mol%	90% (isolated yield)
11	$Cu_2(OH)PO_4$	—	0% (unreacted azide and alkyne in ^{1}H NMR)
12	Cu powder (45 μm)	—	0% (unreacted azide and alkyne in ^{1}H NMR)
13	$CuNO_3$	10 mol%	75% (isolated yield)
14	$CuNO_3$	—	0% (unreacted azide and alkyne in ^{1}H NMR)

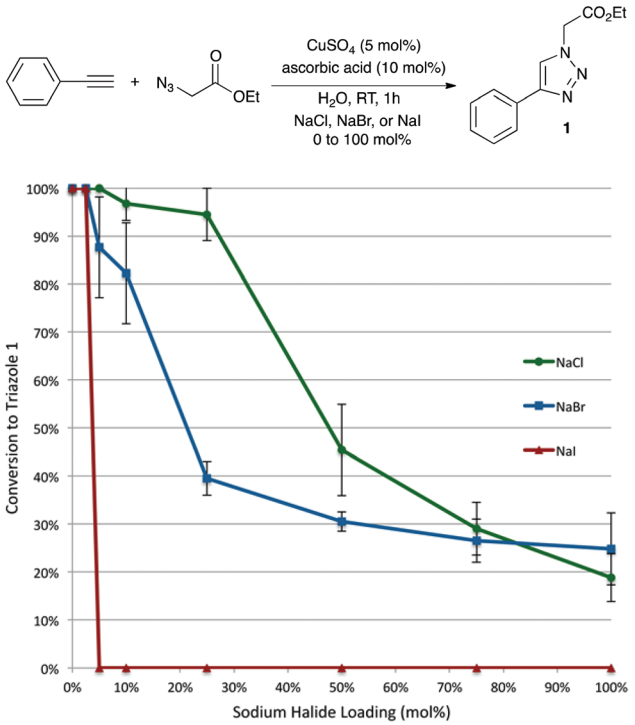
Table 2 Silver(I) activation of copper(I) iodide in the CuAAC



Entry	CuI	Ascorbic acid	$AgNO_3$	Time	Triazole **1**
1	5 mol%	10 mol%	—	24 h	8% (isolated yield)
2	5 mol%	10 mol%	—	24 h	9% (conversion determined by ^{1}H NMR)
3	**5 mol%**	**10 mol%**	**5 mol%**	**1 h**	**85% (isolated yield)**
4	—	10 mol%	5 mol%	1 h	0% (unreacted azide and alkyne in ^{1}H NMR)
5	—	—	5 mol%	1 h	0% (unreacted azide and alkyne in ^{1}H NMR)

an excellent 85% isolated yield after 1 hour of reaction (Table 2, entry 3). Importantly, silver(I) is not a catalyst in the formation of **1**, as control reactions without copper(I) iodide led to only unreacted starting material (Table 2, entries 4–5). The results in Tables 1 and 2 suggested that copper(I) iodide was a poor catalyst for the synthesis of triazole **1** in water at room temperature. This was surprising, given the many instances in which copper(I) iodide has been used successfully in the CuAAC,[4] even on challenging substrates.[18–20] However, in these cases the copper(I) iodide is typically activated by the addition of an amine base,[18,20,21] a ligand,[21] or the reaction is run in organic solvent.[18–20] These measures are thought to disrupt the extended solid state structure of copper(I) iodide and render it active in the CuAAC.[21] For the specific case of triazole **1**, copper(I) iodide activation has also been achieved through use of elevated temperature,[22] basic solid supports for the copper,[23,24] acid–base co-catalysts,[21] or sonication.[25] Copper(I) iodide activation by reaction with silver nitrate (Table 2) constitutes a complementary, and notably mild, method for rescuing activity in the CuAAC. Interestingly, low activity of copper(I) halides in the CuAAC in water has been noted previously by Liu and Reiser, prompting their development of more active catalysts.[26] As these observations paralleled our own, we were curious to determine if free iodide, or any other exogenous halide, could deactivate an otherwise active copper catalyst in the CuAAC. We were also interested in comparing the relative inhibitory effect of free halides in the CuAAC. Such information would complement the effects of chloride and bromide on the CuAAC that were observed previously by Finn[15] and Díez-González,[16] respectively.

To answer these questions, sodium halides (NaCl, NaBr, or NaI) were added at various levels (2.5 mol% to 100 mol%) to a model azide–alkyne cycloaddition between phenylacetylene and ethyl azidoacetate (Scheme 2). In these reactions, copper(II) sulphate was used as a precatalyst at a 5 mol% loading and pre-mixed with the halide additives before exposure to the ascorbic acid reducing agent. To determine conversion to **1** after one hour of reaction time, the crude reaction mixture was extracted with $CDCl_3$ and analysed directly by ^{1}H NMR (ESI†). All of these experiments were run at least three times, and average conversions are plotted with error bars indicating ±1 standard deviation from the mean. In a control reaction in which no halide was added, triazole **1** was isolated in 91% yield (Table 1, entry 7 and Scheme 2). When employing the $CDCl_3$ extraction on this same control experiment, full conversion to triazole **1** was observed, with no evidence of unreacted phenylacetylene or ethyl azidoacetate—consistent with the high isolated yield of 91%. For reactions that were not complete, the conversion was determined by the relative inte-

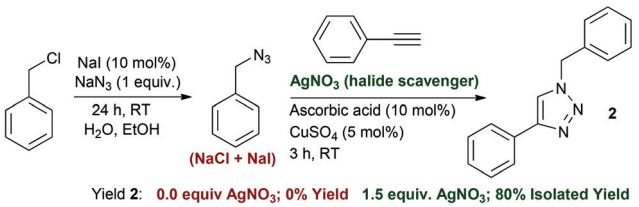


Scheme 2 Halides inhibit the CuAAC. Halides (NaX) were added at 2.5, 5.0, 10, 25, 75, and 100 mol% loading in a model azide–alkyne cycloaddition using a copper(II) sulphate precatalyst. Conversion to **1** was determined by ^{1}H NMR after extraction of the crude mixture into $CDCl_3$ (ESI†).

gration of the α-CH_2 of **1** and ethyl azidoacetate (ESI†). Through this systematic examination of the effect of sodium halide loading on conversion to **1**, it was revealed that sodium chloride, sodium bromide, and sodium iodide were all inhibitors in this reaction (Scheme 2). The most dramatic effect was observed upon the addition of sodium iodide. At 5 mol%, sodium iodide was equimolar to the copper catalyst and led to complete inhibition of the reaction. At 2.5 mol% sodium iodide, full conversion to **1** was observed. In contrast, triazole **1** was formed in the presence of sodium bromide and sodium chloride, though a steady decrease in reaction conversions was observed with increasing sodium halide loading. These results suggest that all halides are detrimental to the copper catalyst, with iodide serving as the most powerful inhibitor.

The results in Scheme 2 prompted us to consider scenarios where halides would be encountered during the CuAAC, and experimental precautions that could accommodate their presence. The one-pot S_N2-CuAAC shown in Scheme 3 is one such scenario. In the conversion of benzyl chloride to benzyl azide, sodium iodide was used as a nucleophilic catalyst. The presence of sodium iodide and the sodium chloride generated in the reaction prevent the formation of triazole **2** in the subsequent CuAAC (0% yield, Scheme 3).

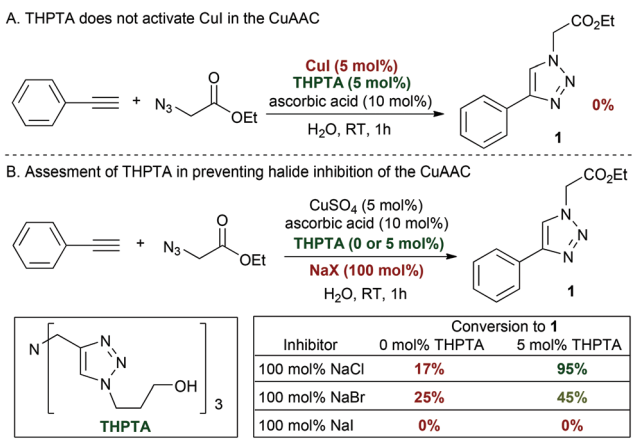
By simply adding silver nitrate as a halide scavenger after the S_N2 reaction, the CuAAC proceeded smoothly to provide triazole **2** in 80% isolated yield (Scheme 3). As a negative control experiment, the same reaction sequence was run in the



Scheme 3 A one-pot S_N2-CuAAC is enabled at room temperature by using $AgNO_3$ as a halide scavenger. Sodium iodide was included as a nucleophilic catalyst in the synthesis of benzyl azide.

absence of copper: no triazole was formed, indicating that silver salts formed do not catalyse the formation of triazole **2** (ESI†).

In Scheme 3, it was demonstrated that scavenging halides with silver nitrate is an effective method for overcoming catalyst inhibition. In other scenarios, it may not be desirable or convenient to remove halides. Buffered saline, for instance, is a common medium for biological molecules and CuAAC on such substrates may require tolerance of sodium chloride. It was therefore of interest to us to consider ligands that can be added to the copper that increase its compatibility with halides. To this end, we examined tris(3-hydroxypropyltriazolylmethyl)amine (THPTA, Scheme 4).[27] THPTA is a member of the tris-triazole ligands that are known to stabilize the +1 oxidation state required for copper in the CuAAC, prevent detrimental disproportionation, and thereby promote the reaction.[15,28] We reasoned that THPTA's multi-dentate binding to copper could also disrupt copper halide aggregates and serve as an activating ligand. When THPTA was pre-mixed with and equimolar amount of copper(I) iodide, however, it did not activate the catalyst, as **1** was not detected after one hour of reaction at room temperature (Scheme 4A). We therefore turned next to an experiment that would determine if THPTA offers any protective benefit from free halides in solution. Accordingly, THPTA was used as a ligand for copper(II) sulphate (both



Scheme 4 Assessment of Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) as a ligand for CuAAC using a copper(I) iodide catalyst (A) or in the presence of exogenous halides (B).

at 5 mol%) in the presence of 100 mol% sodium chloride, sodium bromide or sodium iodide (Scheme 4B). THPTA offered no benefit in the presence of iodide, as **1** was not formed with or without THPTA. Apparently, THPTA cannot compete with iodide in binding to copper and the catalyst is completely deactivated. A modest benefit was observed in the case of bromide, with a 45% conversion to triazole **1** with THPTA, compared to 25% when THPTA was omitted. In the case of chloride, THPTA did protect the copper from inhibition, as triazole **1** was formed in 95% conversion in the presence of 100 mol% NaCl (Scheme 4B). In the control reaction without THPTA, the triazole was formed in 17% conversion. These results suggest that THPTA can compete with chloride in binding to the copper catalyst and promote the cycloaddition. As a practical measure, we therefore suggest that THPTA be considered as a ligand for the CuAAC when the reaction is carried out in buffered saline. This recommendation is of particular importance when using the CuAAC in bioconjugation reactions.[10,17,27]

Conclusions

In this study, several important effects of halides on the CuAAC have been revealed. First, inhibition by chloride was observed at less than 300 mM sodium chloride when using a copper(II) sulphate pre-catalyst. This chloride effect is an important consideration when employing the CuAAC in buffered saline, as is common in bioconjugation reactions. Finn has recommended 500 mM sodium chloride as an upper limit for buffers in this context,[17] and our results suggest that an even lower concentration of sodium chloride should be considered if no ligand (*e.g.* THPTA) is used for copper. Second, this is the first report of sodium bromide inhibiting the CuAAC in water. Lal and Díez-González have noted that sodium bromide can inhibit the CuAAC in organic solvents when using $[CuBr(PPh_3)_3]$ as a catalyst, but that this effect was not observed in water.[16] In contrast, the copper(II) sulphate pre-catalyst employed in this study is prone to inhibition by exogenous bromide. Third, this communication includes the first report of sodium iodide as a potent inhibitor of the CuAAC. When iodide was present in equimolar amounts with respect to the copper(II) sulphate pre-catalyst, no cycloaddition was observed. As iodide is often used as a nucleophilic catalyst in substitution reactions, its inhibition of the CuAAC must be taken into account to successfully execute one-pot S_N2-CuAAC sequences such as the one in Scheme 3. Finally, this study includes experimental measures to accommodate halides in the CuAAC reaction mixture. For instance, silver nitrate was shown to activate copper(I) iodide in the CuAAC. Silver nitrate was also demonstrated as an effective halide scavenger that enabled a one-pot S_N2-CuAAC to proceed at room temperature. In a similar vein, we have demonstrated that the tris-triazole ligand THPTA allows the CuAAC to proceed smoothly in the presence of sodium chloride. Together, these discoveries and technical considerations will facilitate the application of the CuAAC in the many scenarios in which halides are encountered.

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