Dinuclear Copper Intermediates in Copper(I)-Catalyzed Azide–Alkyne Cycloaddition Directly Observed by Electrospray Ionization Mass Spectrometry**

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Abstract: *The mechanism of the CuAAC reaction has been investigated by electrospray ionization mass spectrometry (ESI-MS) using a combination of the neutral reactant approach and the ion-tagging strategy. Under these conditions, for the first time, putative dinuclear copper intermediates were fished out and characterized by ESI(+)-MS/MS. New insight into the CuAAC reaction mechanisms is provided and a catalytic cycle is proposed.*

The copper-catalyzed azide–alkyne cycloaddition (CuAAC)[1] can be seen as the most prominent example of the so-called click chemistry.[2] The robustness of this Cu^I-mediated transformation has boosted its interest, making it the most straightforward strategy for the regiospecific preparation of 1,2,3-triazoles. To date, this reaction is widely employed in organic synthesis, medicinal chemistry, surface and polymer science, and in bioconjugation strategies.[1c, d]

Despite the apparent simplicity and widespread use of the reaction, its mechanism has emerged as particularly complex, being still a matter of debate. Cu^I stands out as the only active oxidation state for the catalyst. A second-order rate law with respect to Cu^I has been experimentally observed.[3–5] Others observed both first-order and third-order kinetics, but relate both observations to a common binuclear Cu mechanism.[6] Moreover, DFT calculations of the reaction pathway showed a more favorable energetic pathway when dinuclear Cu^I intermediates are considered, as compared to mononuclear intermediates.[7–9] However, as only mononuclear copper acetylides and copper triazolides can be isolated,[10] the implication of truly dinuclear Cu^I intermediates could not be claimed out of any doubt, until a paper by Fokin et al. appeared.[11] Indeed, they inferred for the CuAAC the existence of dinuclear copper intermediates by in situ reaction calorimetry measurements, and copper isotope crossover experiments. Despite these studies and mechanistic data

available, however, no direct observation of copper dinuclear CuAAC intermediates has been reported to date.

Following the pioneering paper by Chen and co-workers on the study of organometallic chemistry by mass spectrometry,[12] more electrospray ionization (ESI) MS studies recently appeared tgat yielded detailed information about the elementary steps of important reactions such as the Heck, Stille, Baylis–Hillman, and Ugi reactions.[13–15] We report herein our investigation on the mechanism of the CuAAC reaction by using ESI-MS(/MS) to intercept reactive species and intermediates, with both conventionally employed neutral reagents, and also ion-tagged reactants.[13] CuOTf was used as dichloromethane-soluble source of Cu^I, and PPh_3 was added to enhance the catalyst stability against possible detrimental oxidation and aggregation phenomena.[16] As model reagents, we employed phenylacetylene **1** and benzylazide **2**, whereas the imidazolium-based charge-tagged alkyne **3** and **2** also were used in the ion-tagged strategy (see the Supporting Information). The cycloaddition reaction affords, respectively, products **4** and **5** (Scheme 1).
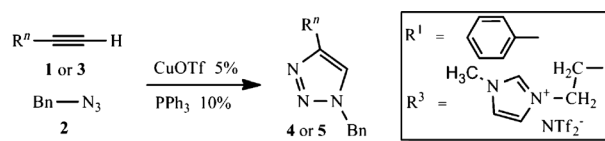
***Scheme 1.*** The copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction.

The initial ESI-MS(/MS) analysis (see the Supporting Information) of a CH_2Cl_2 solution containing only CuOTf and PPh_3 results in the characterization of "pre-catalyst" species. Under these conditions, a number of cluster ions, among all the copper adducts detected by ESI(+)-MS, display the typical isotopic distribution of dicopper species. Two of such adducts, $[I\text{-OTf}]^+$ (m/z 1323; ^{63}Cu isotope here and elsewhere) and $[II\text{-OTf}]^+$ (m/z 1061; Scheme 2; Supporting Information, Figure S1) were ascribed to the cationic portion of the neutral copper pre-catalysts expected in a slightly polar medium such as CH_2Cl_2. It is worth noting that the lack of one phosphine unit in **II**, with respect to **I**, results in a free coordination site, which allows entrance of the reaction substrate. Indeed, when we added a CH_2Cl_2 equimolar solution of **1** and **2** to the CuOTf and PPh_3 mixture, a series of new dicopper species was detected, all of them structurally related to pre-catalyst **II**. They appear as rather low intensity peaks in the full-range ESI(+)-MS spectrum (Supporting Information, Figure S2). Most interestingly, the only adducts containing either the

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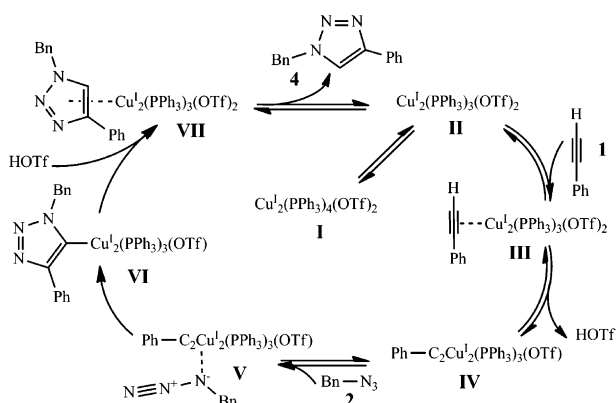
Scheme 2. Proposed catalytic cycle for the CuAAC reaction, using neutral reactants.

alkyne or benzylazide, or both, (see below) arise from **II** (Scheme 2).

According to such a copper dinuclear reaction mechanism, the free coordination site of **II** is initially occupied by phenylacetylene **1** to form intermediate **III**, which we detected as [**III**-OTf]$^+$ at m/z 1163, characterized by MS/MS experiments (Supporting Information, Figure S3). As to intermediate **III**, the interaction between the π-bond and copper would increase the alkyne acidity, leading to the development of an additional σ C–Cu interaction (intermediate **IV**). The MS/MS spectrum of [**IV**-OTf]$^+$ at m/z 1013 (Supporting Information, Figure S4) accounts for this σ interaction in the m/z 751→m/z 587 fragmentation, corresponding to the loss of a copper phenylacetylide moiety.

The dinuclear copper acetylide **IV**, as suggested by Fokin et al.,[11] can be regarded as a key intermediate of the CuAAC, thanks to the unique cooperation of σ- and π-interactions between the two Cu^I centres and phenylacetylide.[1d,11] Such a copper acetylide formation, even in the absence of a base, is a well-known general behavior, which occurs also in strong acidic media (up to 20–25% of H$_2$SO$_4$).[1d,17]

Formation of **IV** allows the onset of a new coordination site at the dicopper adduct, which allows the subsequent coordination of benzylazide **2** to give intermediate **V**. This further intermediate would be responsible for the synergic activation of both phenylacetylide and azide terminal nitrogen electrophilicity towards the cycloaddition step. Under these conditions, we were not able to detect the corresponding cation [**V**-OTf]$^+$, which is probably because the weak azide–copper non-covalent bond is too faint to be stable under ESI conditions. However we have demonstrated the existence of the equivalent of **V** by employing ion-tagged reactants (see below).

The subsequent cycloaddition step is proven by detection of the ion [**VI**-OTf]$^+$ at m/z 1146 (Supporting Information, Figure S5). **VI** then undergoes protodemetallation to form **VII**, detected as [**VII**-OTf]$^+$ at m/z 1296 (Supporting Information, Figure S6). **VII** in turn delivers the triazole product **4**, the ion of which is present in the MS spectrum as the protonated species at m/z 236 (Supporting Information, Figure S7), thus closing the catalytic cycle and restoring the active copper catalyst **II**.

Remarkably, all intermediates but **V**, proposed for the catalytic cycle of CuAAC, have been intercepted by ESI(+)-MS with their coordination spheres practically complete, only devoid of one triflate anion lost during ionization. The presence of two triflate anions, however, likely to be present in CH$_2$Cl$_2$ solution, was subsequently proven by employing the charge-tagged alkyne **3**.

Indeed, the entire CuAAC catalytic cycle was confirmed by adopting the ESI(+)-MS charge-tagging strategy.[13c,e] Our approach in this case was aimed at shifting the positive charge, needed for ESI(+)-MS sampling, from the active metal core of CuAAC intermediates to the periphery of the complex. The presence of the cationic moiety on the reactant side chain in fact allows the structure of the core of the intermediates with the triflate anion still decorating it to be preserved, thus providing additional mechanistic insight. Accordingly, the imidazolium-based charge-tagged alkyne **3** (see the Supporting Information for the synthesis and characterization) was employed as reactant instead of phenylacetylene **1**. Also in this case, the only ions relevant to copper adducts either containing the reaction substrate or product, or revealing their nature as intermediates, can be directly related to the proposed pre-catalyst **II**. As a preliminary observation, at variance from the reaction carried out under conventional conditions, these ions still carry the triflate anion. It is worth noting that in the non-tagged ESI experiments this anion was necessarily lost to generate a positive charge. Clearly, in the charge-tagging experiments all of the ions carry the charge by virtue of the imidazolium unit. On the other hand, however, the charge shift to the periphery of the complex reduces the strength of the Cu^I–phosphine interaction. This becomes visible in the electrospray event, where all the ions are devoid of one phosphine ligand with respect to their solution counterparts (Scheme 3). Such ions are dinuclear Cu^I adducts, and match those reported for conventional CuAAC (Scheme 2).

Accordingly, also in this case a reaction cycle can be drawn (Scheme 3), which encompasses the intermediate **IIIa**–**VIIa**, besides **I** and **II**, present all along the reaction pathway. Ion signals are present in the ESI(+)-MS spectrum of the reaction mixture related to intermediates from **IIIa** to **VIIa**.
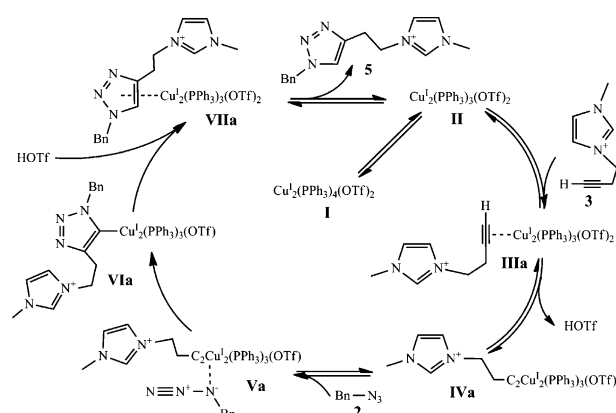
Scheme 3. Proposed catalytic cycle for the CuAAC reaction, using charge-tagged alkyne **3**.

With obvious nomenclature, we will refer in the following to these ions, which as stated above lack a triphenylphosphine unit with respect to the relevant fully coordinated complexes in solution, as **IIIa**-PPh$_3$ to **VIIa**-PPh$_3$. Their proposed structures are consistent with the fragmentation reactions observed in the ESI(+)-MS/MS spectra.

The MS/MS fragmentation processes are driven by the presence of the cationic ligands (see the Supporting Information, Figures S8 and S9 as far as the MS/MS spectra of **IIIa**-PPh$_3$ and **IVa**-PPh$_3$, respectively are concerned). Moreover, fragmentation induced by neutral loss of methylimidazole is a common feature throughout. The ESI(+)-MS/MS spectra became crucial to distinguish between isobaric **Va**-PPh$_3$ and **VIa**-PPh$_3$ ions, which correspond to the reaction intermediates **Va** and **VIa**. The presence of these two different species is revealed by the coexistence in the MS/MS spectra (Figure 1; see also Figure 2) of two different fragmentation pathways.
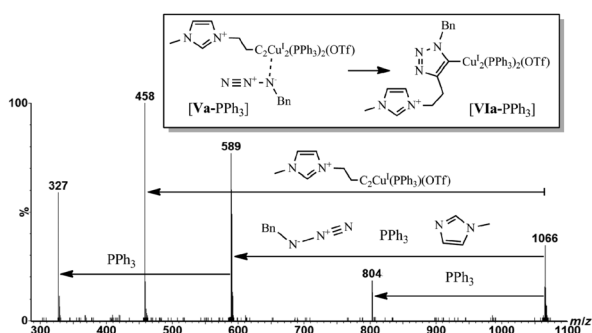

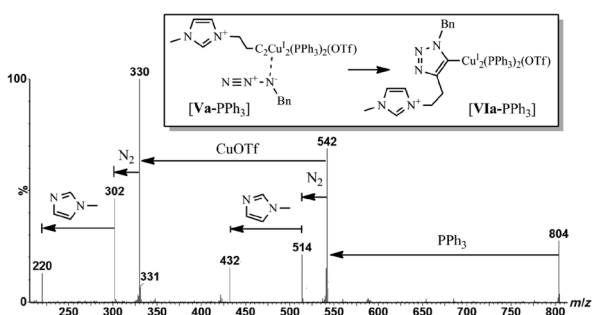***Figure 1.*** ESI(+)-MS/MS spectrum of the ion at *m/z* 1066.

Figure 2. ESI(+)-MS/MS spectrum of the ion at *m/z* 804.

The ion at *m/z* 1066 gives the fragment at *m/z* 589 as a first prominent product ion. Its mass can only be rationalized in terms of loss from the molecular ion of the three moieties triphenylphosphine, methylimidazole, and benzylazide as a whole. The benzylazide loss is consistent in this case with structure **Va**-PPh$_3$ (relevant to **Va** in solution), where copper coordinates the benzylazide molecule before the cycloaddition step. Incidentally, in a competitive fragmentation route from the molecular ion, the fragment ion at *m/z* 458 is formed by the overall loss of [Cu(**3**−H$^+$)(PPh$_3$)(OTf)]. Further MS/MS experiments on this ion (Supporting Information, Figure S10) show loss of the benzylazide moiety, thus helping to

identify its structure as part of **Va**-PPh$_3$. A major point which remains unsolved, however, is the controversial regiochemistry of the azide coordination to the dinuclear complex.[11,18]

On the other hand, the fragment ion at *m/z* 804, which is obviously formed by initial triphenylphosphine release, once isolated after in-source fragmentation, does not give rise to *m/z* 589 by further collisional activation (hypothetical losses of methylimidazole and benzylazide) but dissociates further by release of a second coordinated phosphine generating the product ion at *m/z* 542 (Figure 2).

The ion at *m/z* 330 is formed by loss of CuOTf. Both ions at *m/z* 542 and 330 fragment in turn with the diagnostic losses of molecular nitrogen, which can only be related to the presence in the complex of a triazole moiety,[19] thus pointing to structure **VIa**-PPh$_3$. Loss of N$_2$ from **Va**-PPh$_3$, in fact, would require breaking of a covalent bond, which is a highly unfavored event if compared with dissociation of benzylazide itself. This observation allows us to conclude that both intermediates **Va** and **VIa** do exist in solution. They have been intercepted by ESI(+) as ions at *m/z* 1066, but reveal their different structures by the two different observed fragmentation pathways. CID breakdown graphs are given in the Supporting Information, Figure S11.

During the final reaction step, intermediate **VIa** undergoes protonolysis to give intermediate **VIIa**, which has been intercepted as **VIIa**-2(PPh$_3$) at *m/z* 954 (Supporting Information, Figure S12). It delivers finally the triazole product **5**, present in the ESI(+)-MS/MS spectrum at *m/z* 268 (Supporting Information, Figure S13).

Time monitoring of the reaction course allows further insight into the growth and decay of all the reaction intermediates. For the charge tagged reaction, peaks relevant to all molecular species were spotted, accordingly, every 5–10 min (see the Supporting Information). In particular, as shown in Figure 3, intermediates **II**, **IIIa** and **IVa** (Scheme 3) form immediately, two of them (**II** and **IIIa**) undergoing a rapid depletion with the reaction progress. The signals of the other intermediates **Va**–**VIa** and **VIIa** become detectable after 5 min, being constantly present with very low abundances. They go disappearing when the final product **5** is almost completely formed.
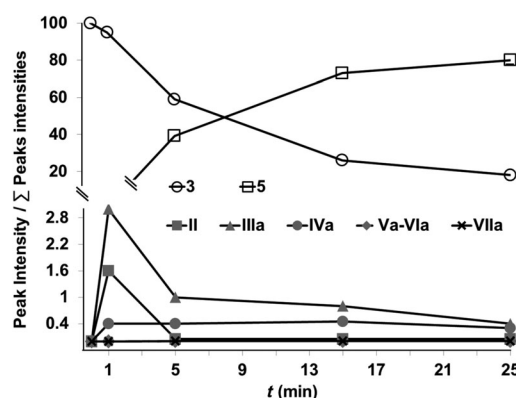

Figure 3. Relative intensities of MS peaks relevant to **3**, **5**, **II**, **IIIa**, **IVa**, **Va**–**VIa**, and **VIIa** (see Scheme 3) from the reaction mixture, taken at different reaction times.

In conclusion, our ESI-MS(/MS) investigation of the CuAAC reaction gives useful insights into its challenging mechanism. Both by using the neutral reactant approach[13b,d] and the classical ion-tagged strategy,[13c,e] crucial dicopper intermediates have been successfully intercepted and structurally characterized. Such non-isolable active dicopper intermediates, which have never been directly observed before, are consistent with the computational and experimental state of the art for the CuAAC mechanism.[11]

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