

Supporting Information

Rapid Screening of Copper Intermediates in Cu(I)- Catalyzed Azide–Alkyne Cycloaddition with a Modified ICP-MS/MS Platform

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1. Experimental Section

Chemicals and Materials. The stock standard solution of Cu^{2+} and Zn^{2+} ions were purchased from National Research Center for Standard Materials (Beijing, China). Working standard solution was prepared daily by stepwise dilution of the stock solutions with 1% (v/v) nitric acid. Acetylene gas was stored in a 3 L gas collecting bag collected from a commercially available acetylene cylinder. Phenylacetylene (2.5mM, 1equiv) and benzylazide (27mM) were purchased from Alfa Aesar (Shanghai, China). Phenylacetylene-D (2.5mM) was purchased from Santa Cruz (USA). Acetonitrile and dichloromethane (chromatographical grade) were purchased from Mreda (USA). Acetic acid (guarantee grade, 0.1equiv) was purchased from Sinopharm Chemical Reagent Co., Ltd (Beijing, China).

Apparatus. A triple quadrupole ICP–MS/MS instrument (Agilent 8800, Japan) was chosen in this work. The set-up of the modified ICP-MS/MS system used in this work was similar to our previous study.¹ As shown in Fig. S1, this configuration of ICP-MS/MS consists of a tandem mass spectrometer with an octopole collision reaction cell (ORS) located in-between two quadrupole mass analyzers (Q1 and Q2). The helium (He) gas was used not only to nebulize the azide or alkyne from the liquid phase to the aerosol, but also to carry the azide or alkyne to the third inlet of the ORS through two T-junction mixing system. The azide or alkyne was pre-stored in a 1 mL injector, respectively, and pushed to the T-junction gas mixing system by a micro-injection pump (Pump 11 Elite, Harvard) in a predetermined speed. Q1 can be operated as a mass filter, thereby only allowing ions with one m/z-ratio like isotopic bare $^{65}\text{Cu}^+$ or $^{63}\text{Cu}^+$ ions from ICP to enter the ORS to meet the azide-alkyne mixture. When the Cu^+ -intermediates were formed in the ORS, they would be immediately separated by Q2 and then introduced to the detector. The operational parameters of the instrument were as follows: collision He gas 1.0 mL min⁻¹; the third gas 5%; reactants flow rate 10 μL min⁻¹; Scan type MS/MS; RF power: 1550 W; Extract 1: 0 V; Extract 2: -165V; Q1 bias: 1.0V; Q1→Q2: 63→64~260, 65→66~260, 64→65~260; Octopole bias: -5.0V; Octopole RF: 150V; Energy discrimination: -7.0V; Wait time offset: 2ms; Sweeps / replicate: 5; Integration time / mass: 0.1s.

2. The mechanism cycle process in CuAAC

A CuAAC catalytic model could be suggested as shown in Scheme 1 (in main text) by using acetylene and benzylazide as model reagents. Ligand (L) was always introduced into the reaction system to react with Cu^+ to form the adduct of CuL_n^+ . In this catalytic model, when CuL_n^+ ions were met with acetylene, a π -bound mononuclear copper-acetylide intermediate **I** was immediately formed and rapidly transformed to a σ -bound mononuclear copper-acetylide intermediate **II** through deprotonation. Then, a second copper ion was recruited to the σ -bound copper acetylide, forming the catalytically active dinuclear copper intermediate **III**. The dinuclear copper intermediate **III** was further reacted with benzylazide to form a mononuclear copper intermediate **IV** after get rid of a copper ion. And the final product (**triazole**) was formed through protonation of the intermediate **IV**.

3. Appearance of the intermediates by removing one of the reactants

To further observing the formation of CuL_n^+ -intermediates, we have taken an additional experiment by removing one of the reactants from the reaction and seen if the CuL_n^+ -intermediates were still formed. Fig.S2a showed the mass spectra of the reaction between CuL_n^+ and benzylazide without acetylene. It was found that all of the four CuL_n^+ -intermediates were not appeared when acetylene was absent, indicated that the CuL_n^+ ions reacted firstly with acetylene to form the CuL_n^+ -intermediates, then these CuL_n^+ -intermediates reacted with benzylazide, which was consistent with the mechanism cycle.² Similarly, the reaction of CuL_n^+ with acetylene without benzylazide was also studied and the mass spectrum was shown in Fig.S2b. Three intermediates (**I**, **II**, and **III**) were immediately appeared when acetylene was present, indicated that these three intermediates were related to acetylene but not benzylazide. It should be noted that the CuL_n^+ -intermediate **IV** was not appeared when benzylazide was absent, suggested that the CuL_n^+ -intermediate **IV** was generated by the reaction of benzylazide with at least one of the intermediates **I**, **II** or **III**.

4. Possible rates of Cu^+ -intermediates or CuL_n^+ -intermediate formation

In this work, the four intermediates (**I-IV**) were all successfully detected by MS detector. The intermediate **I** shows the highest peak among the four intermediates. It

might attribute to a faster formation of intermediate **I**, but a slower transformation to intermediate **II**. The same phenomenon was also found in ESI-MS/MS result.³ Since the introduction of a base can accelerate the formation of the intermediate **II**,⁴ but the introduction of a base might increase the mass number of the catalyst for its affinity to copper.⁵ Considering the limitation of mass number of ICP-MS/MS ($m/z \leq 260$), no base was added in our system. Fortunately, the intermediate **II** was appeared both in Fig. 2 and Fig.3, although their peak intensities were much lower. However, the peak intensity of the intermediate **II_B** in Fig.4 (in main text) was found higher than **I_B** (**I'_B**) when phenylacetylene or phenylacetylene-D was acted as reactant, for which the reason was unknown. To observe the dinuclear Cu^+ - or CuLn^+ -intermediates **III**, excess copper relative to acetylene is needed.⁶ In our system, until the concentrations of Cu^{2+} ions reach to 0.5 – 1 ppm (Fig.S3) and 8 ppm in the condition without ligand (Fig.2) or with ligand (Fig.3), respectively, the dinuclear Cu^+ - or CuLn^+ -intermediates were obviously appeared. According to the literatures, the production of the intermediate **IV** was reported to be the rate-determining step⁷ and we also found that the intermediate **IV** could not be immediately generated in our work as shown in Fig.S4. While Shao et al⁸ had reported that acetic acid could be used to accelerate the conversions of the C-Cu bond-containing intermediates. Considering this fact, we also tried to add acetic acid to the reactant system, and found that the intermediate **IV** was successfully rapid observed as shown in Fig.2 and Fig.3. To further distinguish the three intermediates **IV_I**, **IV_{II}** and **IV_{III}** listed in Scheme 1, a modified tandem organic mass spectrometry could be designed to obtain their fragmentation processes in the future. For the final product, since the isotope experiment in Fig.4 had showed that the final product had a mass number shift ($m/z=235 \rightarrow 236$) when phenylacetylene-D was used as reactant instead of phenylacetylene, and we had found that the final product peak with the m/z of 159 was not appeared when only nitric acid without Cu^{2+} ions was introduced to the ICP with the existence of C_2H_2 and Bn-N_3 all in the ORS in ICP-MS/MS(see Fig.S5), it could be concluded that the final triazole could be formed and only be formed by the whole CuAAC reaction in our ICP-MS/MS system. In addition, another two dinuclear intermediates together both benzylazide and

acetylenic derivative during the transform between the intermediates **III** and **IV** according to the literature² should also be formed, although they could not be observed for their molecular weights out of the range of MS detector. At last, it should be noted that the mass-analyzer may destroy the real intermediates in MS systems as well as our ICP-MS/MS system, so the follow-up work also need to overcome the influence to the intermediates by the structure of the instrument itself in order to observing the reaction intermediates more exactly and more comprehensively.

5. Effect of the copper concentrations on the abundances of Cu⁺-intermediates

The effect of copper concentrations on the abundances of Cu⁺-intermediates allows further insight into the mechanism of the CuAAC reaction. The acetylene and benzylazide cycloaddition catalyzed by ⁶³Cu⁺ ions with its concentrations at the range of 0 to 500 ppb was examined in this study (Fig.S3). The abundances of the four Cu⁺-intermediates were all found increased with the increase of ⁶³Cu⁺ ions concentration, and no intermediate was appeared when no catalyst of ⁶³Cu⁺ ions was present, which again confirmed that these four intermediates were all Cu⁺-intermediates. More importantly, we found that when the concentration of ⁶³Cu⁺ ions was less than 20 ppb, only the mononuclear Cu⁺-intermediate **I** was significantly detected while other intermediates (**II**, **III** and **IV**) were not detected. When the concentration of ⁶³Cu⁺ ions was increased to 50 ppb, beside the mononuclear Cu⁺-intermediate **I**, the mononuclear Cu⁺-intermediate **II** were also appeared, while the intermediate **III** and **IV** were still not detected. These results suggested that when the catalyst concentration was lower than a certain value, only the mononuclear Cu⁺-intermediates were produced. While when the concentration of ⁶³Cu⁺ ions was increased above 200 ppb, beside the mononuclear Cu⁺-intermediate **I** and **II**, the dinuclear Cu⁺-intermediate **III** and the mononuclear Cu⁺-intermediate **IV** were also appeared gradually. These results indicated that the nuclearity of mono- or di- nuclear Cu⁺-intermediates could be regulated by the concentration of Cu⁺ ions.

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Fig. S1 The detail set-up of the modified ICP-MS/MS system for rapid screening the copper-intermediates in CuAAC reaction.

Fig. S2 The mass spectrum under the reaction system of benzylazide (a) or acetylene (b) with 8 ppm $^{63}\text{Cu}^+$ ions and acetonitrile as ligand (L, $m/z = 41$) and solvent and acetic acid as accelerated reagent. The components of the key intermediates were listed as follows: **I_A⁺**: $\text{H}_2\text{C}_2\text{Cu}^+$; **I_B⁺**: $\text{H}_2\text{C}_2\text{CuL}_1^+$; **I_C⁺**: $\text{H}_2\text{C}_2\text{CuL}_2^+$; **II_A⁺**: HC_2Cu^+ , **II_B⁺**: $\text{HC}_2\text{CuL}_1^+$; **III_A⁺**: HC_2Cu_2^+ ; **III_B⁺**: $\text{HC}_2\text{Cu}_2\text{L}_2^+$.

Fig.S3 The intensities of MS peaks relevant to **I⁺**, **II⁺**, **III⁺** and **IV⁺** (see Fig.2 in main text) for acetylene and benzylazide cycloaddition with dichloromethane as solvent and acetic acid as accelerated reagent catalyzed by different $^{63}\text{Cu}^+$ ions concentrations. The components of the intermediates (**I-IV**) were listed as follows: **I⁺**: $\text{H}_2\text{C}_2\text{Cu}^+$; **II⁺**: HC_2Cu^+ ; **III⁺**: HC_2Cu_2^+ ; **IV⁺**: $\text{BnN}_3\text{C}_2\text{HCu}^+$.

Fig.S4 The mass spectrum under the reaction system of acetylene and benzylazide with 8 ppm $^{65}\text{Cu}^+$ ions as catalyst and acetonitrile as ligand (L, $m/z = 41$) and solvent, but without acetic acid as accelerated reagent. The components of the key intermediates were listed as follows: **I_A⁺**: $\text{H}_2\text{C}_2\text{Cu}^+$; **I_B⁺**: $\text{H}_2\text{C}_2\text{CuL}_1^+$; **II_A⁺**: HC_2Cu^+ , **II_B⁺**: $\text{HC}_2\text{CuL}_1^+$; **III_A⁺**: HC_2Cu_2^+ ; **III_B⁺**: $\text{HC}_2\text{Cu}_2\text{L}_2^+$.

Fig.S5 The mass spectrum under the reaction system of acetylene and benzylazide with dichloromethane as solvent and acetic acid as accelerated reagent, but without Cu^+ ions as catalyst.

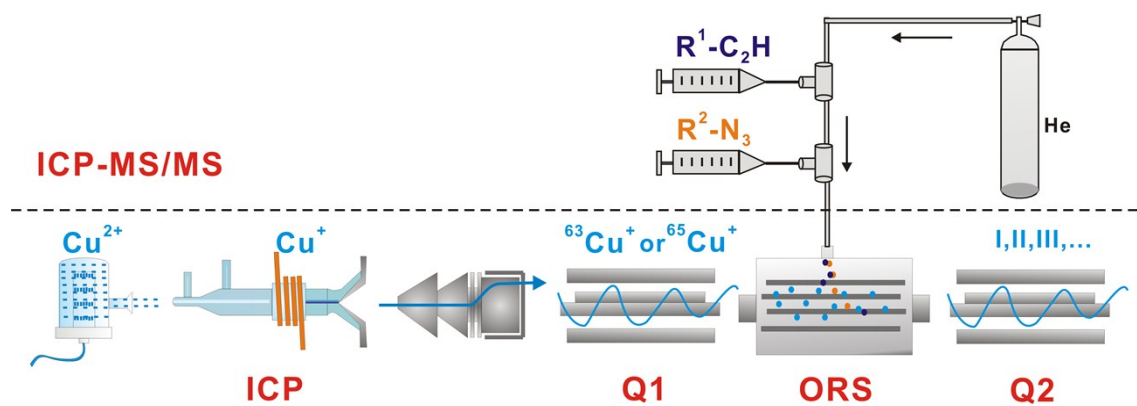


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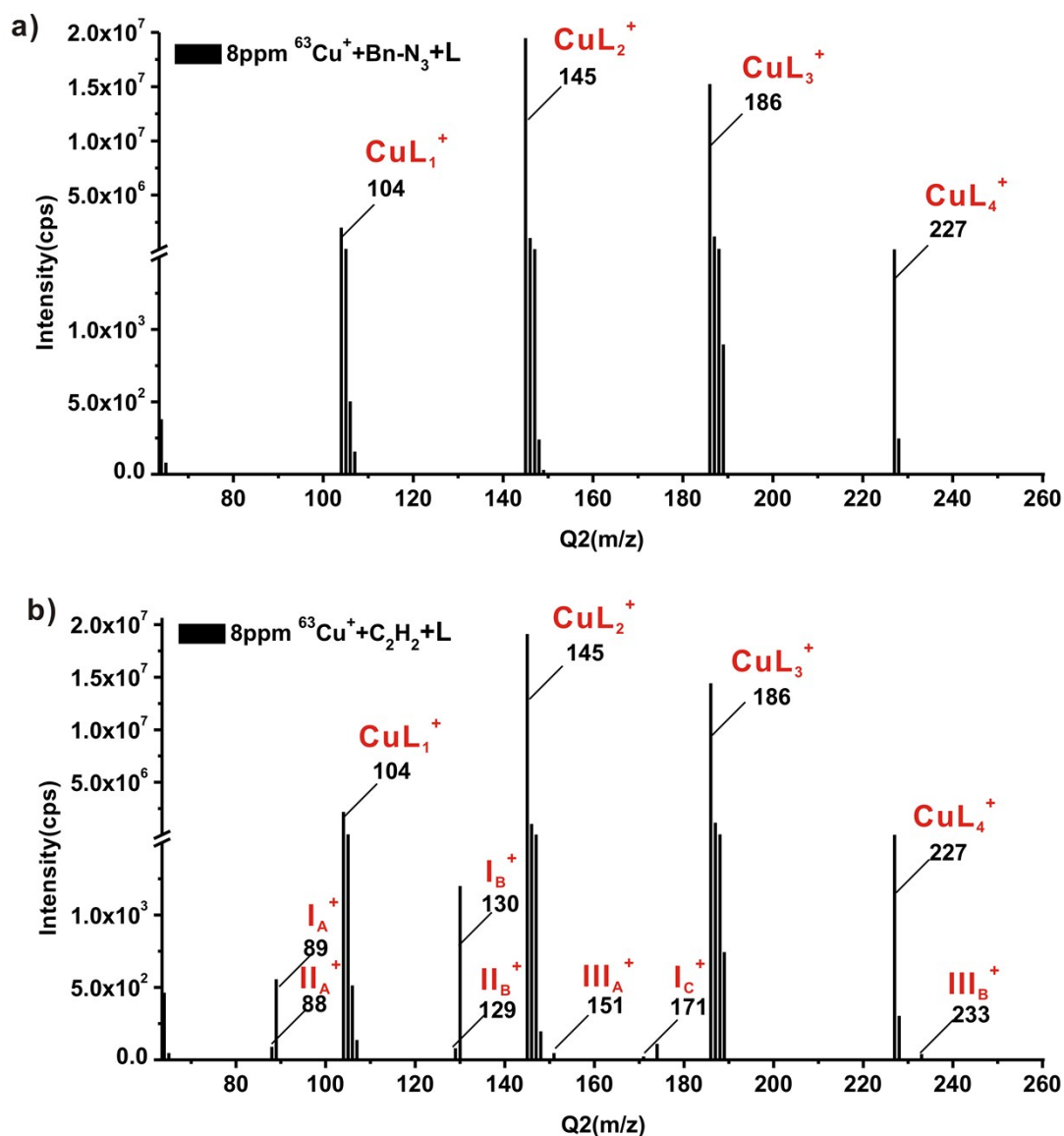


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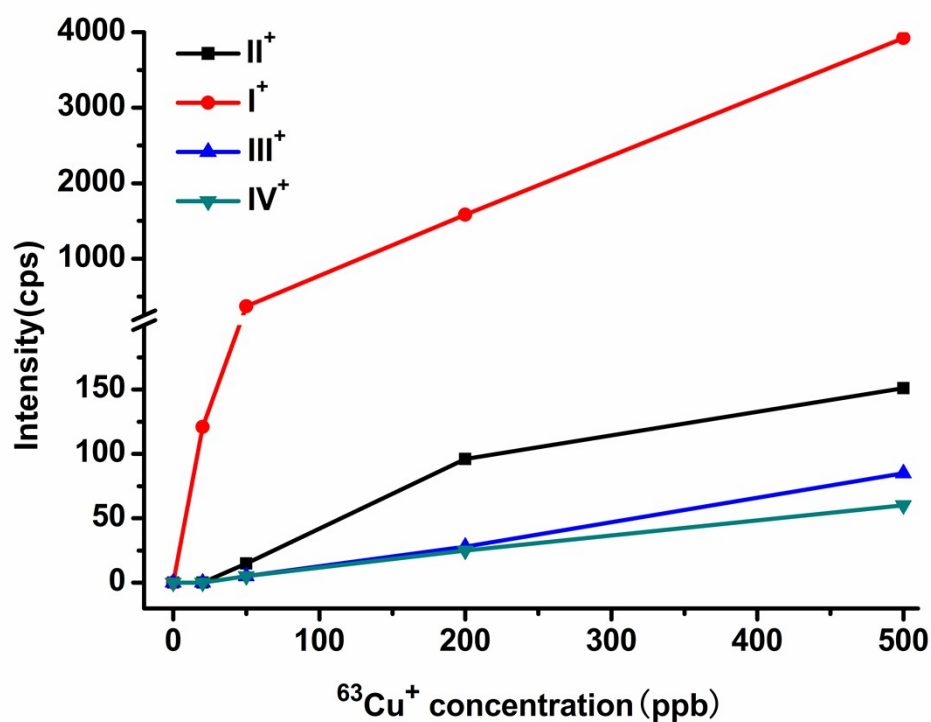


Fig.S3 The intensities of MS peaks relevant to I^+ , II^+ , III^+ and IV^+ (see Fig.2 in main text) for acetylene and benzylazide cycloaddition with dichloromethane as solvent and acetic acid as accelerated reagent catalyzed by different $^{63}\text{Cu}^+$ ions concentrations. The components of the intermediates (**I-IV**) were listed as follows: **I** $^+$: $\text{H}_2\text{C}_2\text{Cu}^+$; **II** $^+$: HC_2Cu^+ ; **III** $^+$: HC_2Cu_2^+ ; **IV** $^+$: $\text{BnN}_3\text{C}_2\text{HCu}^+$.

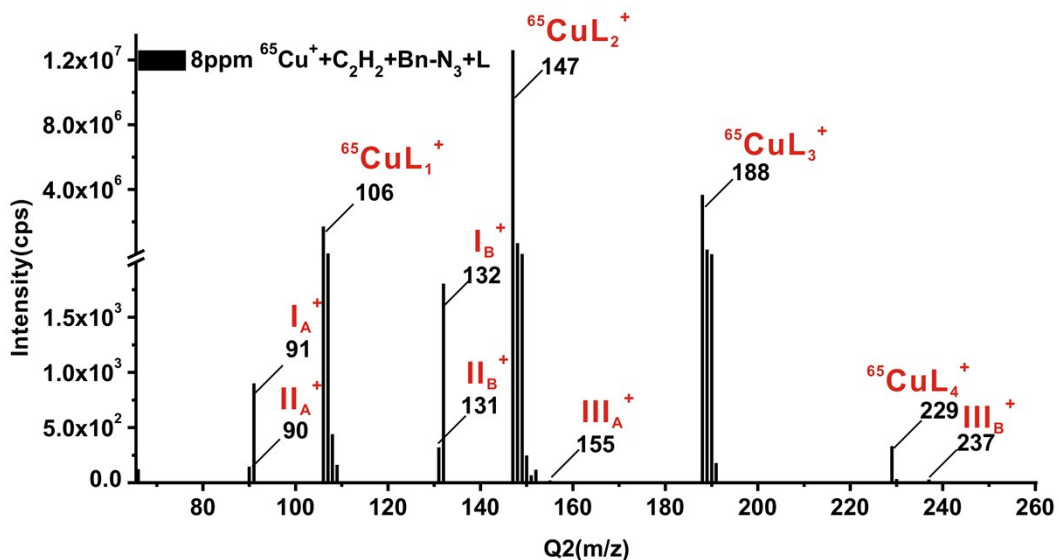


Fig.S4 The mass spectrum under the reaction system of acetylene and benzylazide with 8 ppm $^{65}\text{Cu}^+$ ions as catalyst and acetonitrile as ligand (L, $m/z = 41$) and solvent without acetic acid as accelerated reagent. The components of the key intermediates were listed as follows: I_A^+ : $\text{H}_2\text{C}_2\text{Cu}^+$; I_B^+ : $\text{H}_2\text{C}_2\text{CuL}_1^+$; II_A^+ : HC_2Cu^+ ; II_B^+ : $\text{HC}_2\text{CuL}_1^+$; III_A^+ : HC_2Cu_2^+ ; III_B^+ : $\text{HC}_2\text{Cu}_2\text{L}_2^+$.

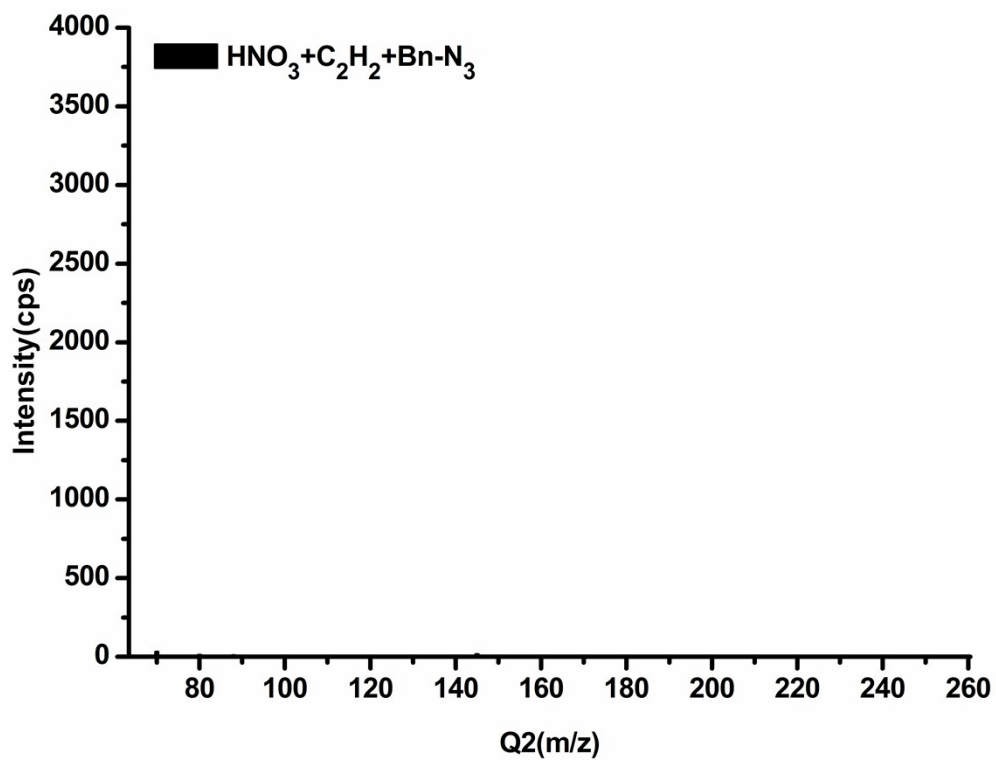


Fig.S5 The mass spectrum under the reaction system of acetylene and benzylazide with dichloromethane as solvent and acetic acid as accelerated reagent, but without Cu⁺ ions as catalyst.