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A tris(benzyltriazolemethyl)amine-based cage as a CuAAC ligand tolerant to exogeneous bulky nucleophiles†

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The archetypal tris(benzyltriazolemethyl)amine (TBTA) ligand was equipped with a bowl-shaped cap in the cage Hm-TBTA. Hm-TBTA accelerates CuAAC reactions without suffering from product inhibition. Furthermore, this shielded ligand efficiently protects the copper center from deactivation by the Cu^{I}–chelator glutathione, opening the way to novel approaches for efficient CuAAC reactions in complex media.

The Cu-catalyzed azide–alkyne cycloaddition reaction (CuAAC) is the most popular reaction of the ''click chemistry'' toolbox.[1] Due to its high versatility, tolerance, and efficiency under mild conditions, this transformation belongs to the most broadly applicable methods to prepare covalent linkages.[2] Thus, CuAAC applications range from chemical biology,[3] and medicinal chemistry,[4] to materials science and interlocked structures.[5] Many efforts have been dedicated to the development of assisting-ligands to improve the reaction efficiency.[6] In their pioneering studies, Sharpless and Fokin report the tris(benzyltriazolemethyl)-amine (**TBTA**) architecture as a remarkable Cu^{I}-stabilizing and rate-accelerating ligand.[7] Since then, **TBTA** has become the most widely used CuAAC ligand and has been applied in bioconjugation reactions.[8] Several structural variations of tetradentate triazole-based coordinating structures have been described,[6] including a very recent report of superior water-soluble bioconjugaison CuAAC ligand.[9] But despite recent progress, the development of catalysts that remain active in complex mixtures, such as living systems containing biological Cu^{I} ligands, is still needed.[10] Deactivation of several CuAAC catalysts *via* coordination of the copper ion by external nucleophiles is indeed proposed to explain their low *in vivo* catalytic efficiency.[11] In this context, providing a second coordination sphere to metal-based catalysts appears as a promising approach due to the crucial benefits offered by confined catalysis in terms of stability and efficiency.[12] The tris(triazolemethyl)amine structure has been introduced into organic cages and applied in metal-binding in a confined space,[13] or as highly efficient chlorine receptors through C–H hydrogen bonding.[14] However well-defined organic cages based on the **TBTA** unit have not been evaluated in CuAAC reactions. On this basis, we resonated that the covalent capping of **TBTA** by another C_3 symmetrical unit will represent a particularly useful variation of the canonical ligand allowing for the evaluation of the benefits of controlling the second coordination sphere level.

In this communication, we present the preparation and evaluation as a CuAAC ligand of the novel hemicryptophane **Hm-TBTA** that represents an unprecedented organic cage built from the **TBTA** unit (Fig. 1). We demonstrate the ability of the caged-ligand to accelerate the reaction. Furthermore, we compare the performances of **TBTA** and **Hm-TBTA** in the presence of two bulky Cu–chelators: the picrate anion (Pic^{-}) and the glutathione biothiol (GSH).

Hemicryptophanes are organic cages constituted of a northern C_3 symmetrical cyclotryveratrylene (CTV) unit, connected to another tripodal unit.[15] These structures have been recently reported as a new class of tridimensional ligands allowing for the improvement of bioinspired oxidations involving metal-based catalysts.[15,16] In line with this, we have designed the new hemicryptophane **Hm-TBTA** where the archetypal **TBTA** is connected to

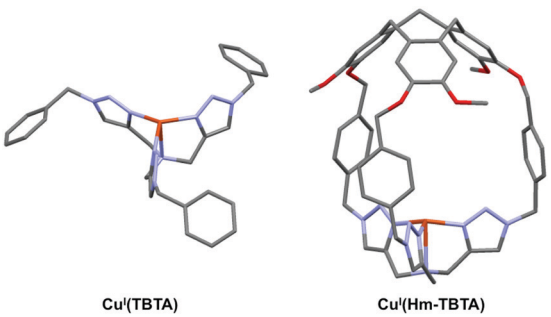

Fig. 1 DFT-optimized structures (BP86-D3/def2-SV(P)) of the canonical copper complex **Cu^{I}(TBTA)**, and the hemicryptophane complex **Cu^{I}(Hm-TBTA)**, which is target of this study.

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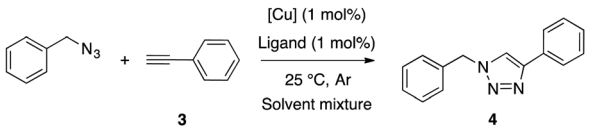
a CTV unit by three methylene –CH_2– bridges (Fig. 1). **Hm-TBTA** was obtained in a five-step synthetic procedure (Fig. 2 and Scheme S1, ESI†). The **CTV(OH)$_3$** unit was derivatized by compound **1** in DMF, using Cs_2CO_3 as a base, to yield the CTV derivative **2**, which presents three azidomethyl–benzene moieties, in 79% yield. **Hm-TBTA** was then obtained following a "1+1" coupling strategy under diluted conditions, which allows for the closure of the cage and the formation of the **TBTA** unit in a single step. The CuAAC reaction between an equimolar amount of **2** and tripropargylamine, using a catalytic quantity of the $CuSO_4$ salt in combination with the **TBTA** ligand (10 mol%), and sodium ascorbate **NaAsc** (reducing agent, 0.5 equiv.) leads to the target cage in 22% yield. The ^{1}H NMR characterization of **Hm-TBTA** reveals sharp, identical and well defined signals for the protons belonging to the southern N–CH_2– link (H_a), the **TBTA** unit (H_b, $H_{c,c'}$, H_d and H_e), the –CH_2– link ($H_{f,f'}$), and the CTV unit (H_g, H_h, H_i and $H_{j,j'}$), attesting to an averaged C_3 symmetrical structure in $CDCl_3$, at room temperature (Fig. 2 and Fig. S3, S4, ESI†).

Next, the capability of **Hm-TBTA** to coordinate a Cu(I) metal–ion in its interior was explored. The copper complex **Cu^I(Hm-TBTA)(PF$_6$)** was prepared by reacting one equivalent of the $Cu^I(CH_3CN)_4(PF_6)$ salt in CH_3CN, at room temperature, under an inert atmosphere (see the ESI†). The formation of the complex was confirmed by High-Resolution Mass Spectrometry (ESI-HRMS) analysis (Fig. S5, ESI†). Interestingly, the ^{1}H NMR analysis of **Cu^I(Hm-TBTA)(PF$_6$)** reveals a C_3 symmetrical spectrum (on average) in CD_3CN, indicating a retention of the cage symmetry upon copper coordination. Its ^{1}H NMR spectra display identical and well defined signals. Compared to the ^{1}H-NMR spectrum of the free ligand in CD_3CN, that of **Cu^I(Hm-TBTA)(PF$_6$)** displays signals corresponding to the triazole (H_b) and the –CH_2–Ar ($H_{c,c'}$) moieties that appear shifted and broader (Fig. S9, ESI†), in good agreement with a binding of the Cu(I) ion at the southern coordinating unit. These NMR observations, which reveal that the C_3 symmetry of **Hm-TBTA** is retained upon metal coordination at its tris(triazolemethyl)amine unit, strongly suggest that the coordination of the Cu^I metal ion occurs with an endohedral functionalization of the copper complex.[17] The performance of our capped ligand **Hm-TBTA** was then evaluated and compared with the one of its related open analogue, in a model CuAAC transformation. We have studied the cycloaddition reaction between benzyl azide and phenylacetylene **3**, catalyzed by 1.0 mol% of the $Cu^I(CH_3CN)_4(PF_6)$ metal salt (see the ESI†). The yields of the desired product **4** have been determined for reactions performed in the presence of 1.0 mol% of the tris(triazole)-based structures **Hm-TBTA** or **TBTA**, as well as in the absence of a stabilizing ligand (Table 1, entries 1–3). For a more rigorous comparison, reaction yields have been compared at a short reaction time of 2 hours, when a third of the maximum yield was obtained in the case of the **TBTA** ligand (35% yield, Table 1, entry 3). At longer reaction time (24 h) quantitative yields were obtained for both **TBTA** and **Hm-TBTA** (Table 1, entries 4 and 5).

Table 1 Comparison of CuAAC accelerating ligand **Hm-TBTA** and **TBTA**[a]



3 4

Entry	[Cu] source (1 mol%)	Reducing agent (5 mol%)	Ligand (1 mol%)	Time (h)	Yield[c] (%)
1	$[Cu^I(CH_3CN)_4]PF_6$	—	none	2	<1
2	$[Cu^I(CH_3CN)_4]PF_6$	—	**Hm-TBTA**	2	17
3	$[Cu^I(CH_3CN)_4]PF_6$	—	**TBTA**	2	35
4	$[Cu^I(CH_3CN)_4]PF_6$	—	**Hm-TBTA**	24	99
5	$[Cu^I(CH_3CN)_4]PF_6$	—	**TBTA**	24	99
6	$[Cu^I(CH_3CN)_4]PF_6$	—	**Hm-TBTA**	0.5	6
7	$[Cu^I(CH_3CN)_4]PF_6$	—	**TBTA**	0.5	12
8[b]	$Cu^{II}SO_4$	NaAsc	**Hm-TBTA**	2	43
9[b]	$Cu^{II}SO_4$	NaAsc	**TBTA**	2	64
10[b]	$Cu^{II}SO_4$	NaAsc	**Hm-TBTA**	16	85
11[b]	$Cu^{II}SO_4$	NaAsc	**TBTA**	16	95

[a] Reaction conditions: benzyl azide (0.2 mmol), phenylacetylene (1.0 equiv.), $[Cu^I(CH_3CN)_4]PF_6$ (1 mol%), in a 1 : 10 MeOH/CH_2Cl_2 solvent mixture, at 25 °C, under an argon atmosphere. [b] Benzyl azide (0.2 mmol), phenylacetylene (1 equiv.), $Cu^{II}SO_4$ (1 mol%), sodium ascorbate (NaAsc, 5 mol%) in a 2 : 1 : 1 THF/H_2O/^{t}BuOH solvent mixture, at 25 °C. [c] Yields determined by ^{1}H NMR using 2,4-dibromomesitylene as an internal standard.

Importantly, after 2 hours, the model CuAAC reaction in the presence of **Hm-TBTA** (1 mol%) gave 17% yield while the ligand-free experiment only produces trace of **4** (Table 1, entries 2 and 1 respectively). Therefore **Hm-TBTA** clearly acts as an accelerating ligand for the CuAAC reaction, allowing for the observation of several turnover numbers without suffering from product inhibition effect. It should be noted that inhibition by a product that ends up trapped in the catalyst's tridimentional structure, is a common issue of supramolecular catalysis.[18] These results therefore highlight the flexible nature of the **Hm-TBTA** ligand that does not become eventually blocked by the triazole product **4**. This flexibility, which also guarantees the access of both substrates to the copper active site, contrasts with other confined Cu^I-catalysts. For example, the encapsulation of a Cu^I–carbene catalyst within a cucurbit[7]uril host has been very recently used to fully suppress the CuAAC reaction.[19] Flexibility of **Hm-TBTA** has been further demonstrated by

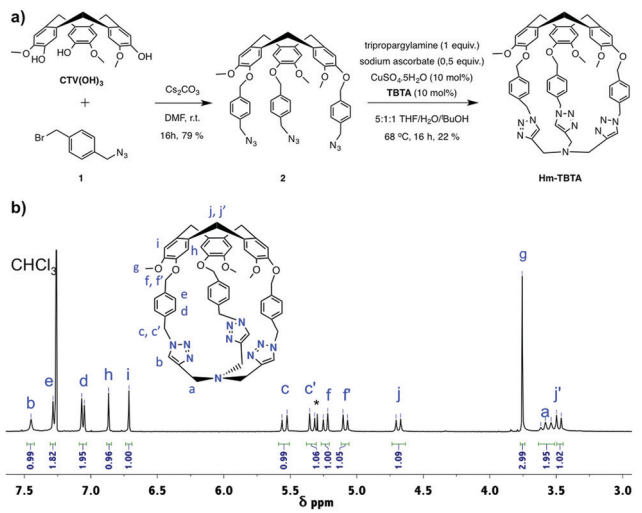


Fig. 2 (a) Synthesis of the hemicryptophane **Hm-TBTA** along with (b) its ^{1}H NMR spectra ($CDCl_3$, 400 MHz), at 298 K (* = CH_2Cl_2).

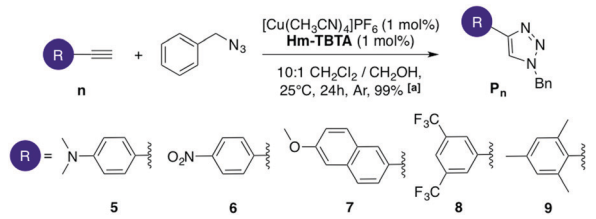


Fig. 3 Preparation of triazole products $\mathbf{P}_n$ in quantitative yield using the CuAAC ligand **Hm-TBTA**. [a] reaction conditions: see Table 1.

extending the substrate scope to hindered phenylacetylenes. Reactions between *para* (**5**, **6**, **7**), *meta* (**8**), and *ortho*-substituted (**9**) alkynes and benzyl azide, indeed yield the corresponding products $\mathbf{P}_{5-9}$ with quantitative NMR-yields, after 24 h reaction time (Fig. 3). The reaction is not suppressed by theses substrates and **Hm-TBTA** does not end up blocked by the bulky $\mathbf{P}_{5-9}$ products. In addition, competitive CuAAC reactions of systems containing two alkynes (at 2 hours reaction time) reveal modest substrate-selectivity improvements in the case of **Hm-TBTA** (compared to the open **TBTA** ligand) (see Fig. S12, ESI†).

The direct comparison between **Hm-TBTA** and its related "uncaged" model reveals a 2-fold decrease of the catalytic efficiency. Indeed, 17% and 35% yields were obtained after 2 hours using respectively **Hm-TBTA** and **TBTA** ligands (Table 1, entries 2 and 3). Comparison of the DFT-optimized structures of the two copper complexes (Fig. 1) clearly shows a widely exposed Cu^I-center in the case of **Cu^I(TBTA)**, while a much more hindered metal core is observed in **Cu^I(Hm-TBTA)**. Furthermore it has been proposed that the **TBTA**-assisted CuAAC reaction involves a dinuclear copper intermediate allowed by the dissociation of triazole arms.[20] Therefore, a more difficult formation of such a dinuclear complex for the capped ligand might account for its slower catalytic performance. Studies of the influence of the **Hm-TBTA**:Cu^I ratio reveal a strong enhancement of the catalytic efficiency upon addition of a second copper ion (Table S1, ESI†). Although the existence of a monometallic mechanism could not be excluded, these results strongly suggest that **Hm-TBTA** is flexible enough to allow a bimetallic mechanism. Interestingly, **Hm-TBTA** (1 mol%) was also an effective accelerating ligand for aqueous CuAAC reaction using a Cu^{II} salt ($Cu^{II}(SO_4)$, 1 mol%) in combination with a reducing agent (**NaAsc**, 5 mol%) in a 2 : 1 : 1 THF/H_2O/^{t}BuOH solvent mixture (Table 1 entry 8).

We then wondered if the steric shield offered by **Hm-TBTA** could protect the copper catalyst from its inhibition by external bulky nucleophiles. Aiming at establishing a proof of our concept, we first examine the putative shielding effect of **Hm-TBTA** by studying our model CuAAC reaction in the presence of the potassium picrate salt (K^+Pic^-). We chose the bulky picrate nucleophile because (i) it can bind to Cu-complexes,[21] and (ii) it has been previously reported that this anion cannot access the cavity of hemycryptophane cages, due to its large size.[22] Interestingly, the data in Fig. 4 revealed that the **Hm-TBTA**-assisted CuAAC reaction did not suffer from deactivation when performed in the presence of up to 70 equivalents of K^+Pic^- compared to the Cu^I source. Contrastingly, the catalytic performance of its parent open

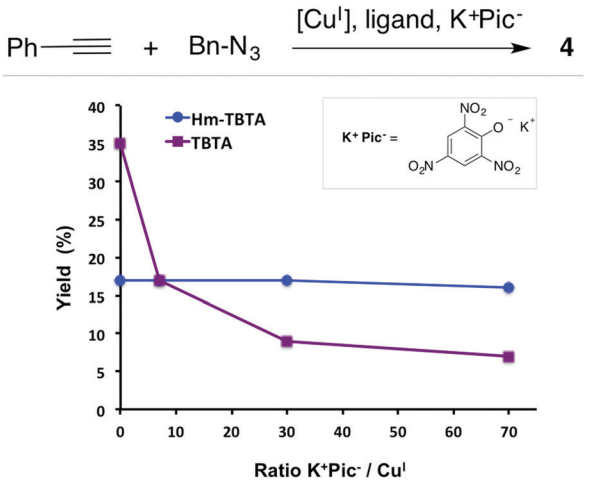
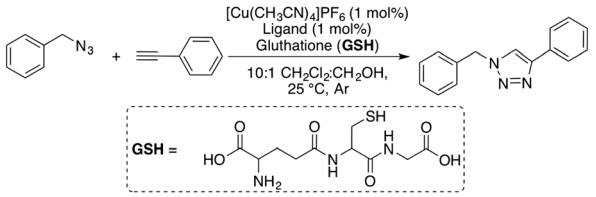


Fig. 4 CuAAC reaction between benzyl azide (0.2 mM) and phenylacetylene (1.0 equiv.), catalyzed by 1.0 mol% of $Cu^I(CH_3CN)_4(PF_6)$, in the presence of 0, 10, 30, and 70 mol% of potassium picrate (K^+Pic^-), using 1.0 mol% of **Hm-TBTA** (blue circle) or **TBTA** (purple square), yields determined by ^{1}H NMR.

ligand **TBTA** was strongly affected by the Pic^- nucleophile: the yield of triazole product **4** dramatically drops from 35% (in the absence of external nucleophile), to 17%, 9%, and 7% yields in the presence of respectively 10, 20 and 70 mol% of K^+Pic^- (Fig. 4, and Table S2, ESI†).

Clearly, the CTV-capped **Hm-TBTA** structure protect the copper complex from the competitive binding of the bulky picrate anion. Inspired by this observation, we next sought to explore the possibility of using **Hm-TBTA** to shield the metal ion from its binding by bulky biological Cu(I) ligands. The glutathione reduced (GSH) thiol was selected as a model biological nucleophile because this tripeptide is particularly abundant in the biological milieu, with concentrations that could range from micromolar to 10 mM,[10b,23] and its coordination to the copper is considered as the major factor that greatly hampered bioconjugation reactions. Inhibition of tris(triazolemethyl)amine-based CuAAC catalysts in the presence of 23,[23] and even 2 equivalents of GSH,[10a] with respect to copper, have been reported. As expected, when **TBTA** (1 mol%) was applied in the model CuAAC reaction, in the presence of GSH (20 mol%), a marked decrease in efficiency was observed. After 2 hours, the reaction yield dramatically drops from 35% (in the absence of biothiol), to only 7% after addition of 20 equivalents of GSH compared to the copper (Table 2). Remarkably, we observed that our CTV-capped ligand was able to efficiently protect the active Cu^I ion as the catalytic performance was retained in the presence of 20 mol% of the sulfur-containing amino acid. After 6 and 24 h of reaction in the presence of GSH (20 mol%), the transformation accelerated by **Hm-TBTA** was significantly more efficient than in the case of **TBTA** with respectively 30 and 99% yields, while no further formation of product **4** was observed for the open ligand (6–7% yields, Table 2). Importantly, isolated yields obtained after 24 h confirm this remarkable behavior (Table 2, entries 4 and 8). The caged ligand **Hm-TBTA** therefore provides a powerful steric shield that prevents the competitive binding of a bulky exogeneous

Table 2 Comparison of **TBTA**- and **Hm-TBTA**-assisted CuAAc reactions in the presence of the biological thiol glutathione (GSH)[a]



Entry	Ligand	Glutathione (GSH)	Time (h)	Yield[b] (%)
1	**TBTA**	None	2	35
2	**TBTA**	20 mol%	2	7
3	**TBTA**	20 mol%	6	6
4	**TBTA**	20 mol%	24	7 (traces[c])
5	**Hm-TBTA**	None	2	17
6	**Hm-TBTA**	20 mol%	2	15
7	**Hm-TBTA**	20 mol%	6	30
8	**Hm-TBTA**	20 mol%	24	99 (83[c])

[a] Reaction conditions: benzyl azide (0.2 mmol), phenylacetylene (1.0 equiv.), $[Cu^{I}(CH_3CN)_4]PF_6$ 1 mol%), in a 1 : 10 $MeOH/CH_2Cl_2$ solvent mixture, at 25 °C, under an argon atmosphere, in the presence of GSH (20 mol%). [b] ^1H NMR yields. [c] Isolated yields.

Cu^{I}–ligand, preserving a high catalytic performance even in the presence of 20 equivalents of the notorious CuAAC inhibitor GSH.

In summary, we described herein the first covalent capping of the canonical CuAAC–ligand **TBTA** that has been equipped with a CTV unit, providing a steric shield at the second coordination sphere. We demonstrate that the resulting cage **Hm-TBTA** coordinates a copper metal ion in its interior. **Hm-TBTA** is a tridimensional CuAAC accelerating-ligand that does not suffer from product inhibition. The **Hm-TBTA**-assisted transformation is slower than in the case of the parent **TBTA**. However, we evidence that our CTV-shielded ligand protects the metal ion from its deactivation by external bulky nucleophiles. Indeed, while the catalytic performance of the **TBTA** ligand was drastically reduced in the presence of bulky Cu–chelators (Pic^-, GSH), the **Hm-TBTA**-assisted CuAAC was not affected. Remarkably, no significant change in the catalytic efficiency was observed even in the presence of 20 equivalents (with respect to the copper) of the notorious biological CuAAC inhibitor glutathione. We envisioned that our approach could find applications in the field of CuAAC chemistry in complex mixtures. In particular, future work will be devoted to a deeper understanding of the mechanism of such transformation, using caged supporting ligands.

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Conflicts of interest

There are no conflicts to declare.

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