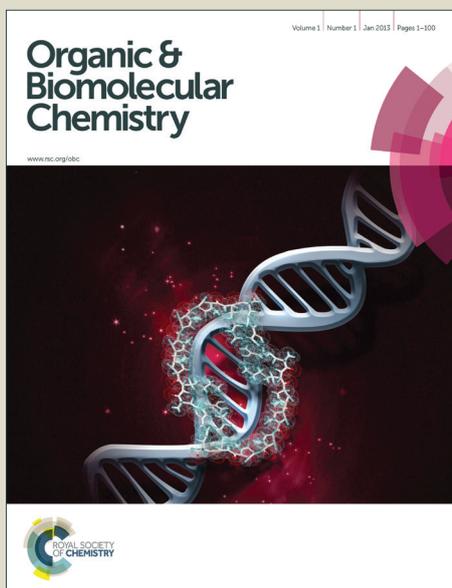


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Water-soluble NHC-Cu Catalysts: Applications in Click Chemistry, Bioconjugation and Mechanistic Analysis[†]

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Cite this: DOI:
10.1039/x0xx00000x

Received
Accepted

DOI:
10.1039/x0xx00000x

www.rsc.org/

Copper(I)-catalyzed 1,3-dipolar cycloaddition of azides and terminal alkynes (CuAAC), better known as “Click” reaction, has triggered the use of 1,2,3-triazoles in bioconjugation, drug discovery, materials science and combinatorial chemistry. Here we report a new series of water-soluble catalysts based on N-heterocyclic carbenes (NHC)-Cu complexes which are additionally functionalized with a sulfonate group. The complexes show superior activity towards CuAAC reactions and display a high versatility, enabling the production of triazoles with different substitution patterns. Additionally, successful application of these complexes in bioconjugation using unprotected peptides acting as DNA binding domains was achieved for the first time. Mechanistic insight into the reaction mechanism is obtained by means of state-of-the-art first principles calculations.

Introduction

Although several catalysts have been developed for the Copper catalyzed Alkyne-Azide Cycloaddition reactions (CuAAC, better known as Click reaction), in most cases they need the presence of co-catalysts such as auxiliary ligands, bases – mainly amines –, and reducing or oxidizing agents depending on the Cu source used, in order to enhance their catalytic activity, although in recent years single Cu salts have also been successful¹. Generally high Cu loading must be applied to ensure good performance of the catalyst². Several Cu(I) complexes holding N-heterocyclic carbene (NHC) ligands are reported as catalysts for the Huisgen Cycloaddition reaction, however the catalysis is carried out at elevated temperatures and in the presence of organic solvents, under two-phase systems (when both reactants are not soluble) and only the 1,4-disubstituted triazoles are generated^{3,4}. Moreover, their activity in solution-phase is significantly lower than the other catalytic systems⁵. Here we report a unique Cu(I) based catalyst that enables the synthesis of triazoles with different substitution patterns, *e.g.* 1-, 4-, 1,4- or 1,4,5-substituted

triazoles and at the same time, able to generate triazoles from acetylene in water, able to produce tosyl acetamides in aqueous media (figure 1).

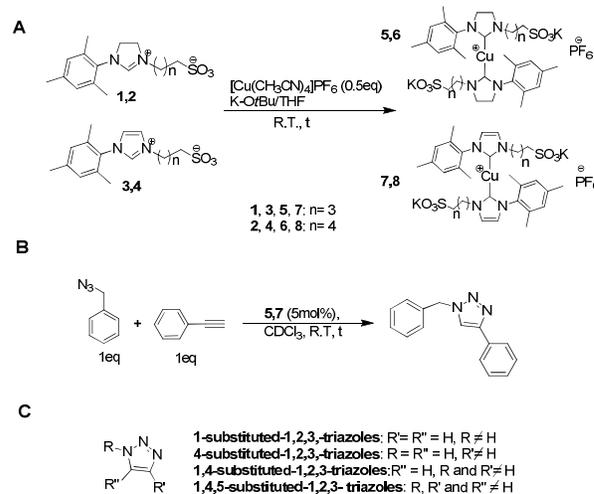


Figure 1. A Synthesis of catalysts 5-8; B. Standard Click reaction monitored by ¹H-NMR using catalysts 5 and 7; C Triazoles synthesized in this work.

Until now, no such catalyst has been reported in literature. More specifically, we report on the synthesis and application of new water-soluble (NHC)₂Cu (I) complexes (5, 6 and 7, 8, figure 1A) in Click chemistry in water, neat conditions and organic solvents or mixtures, acetylene conversion into triazoles (1-, 4-, 1,4- or 1,4,5-substituted triazoles, figure 1C) and acetamides along with the ease of immobilization and a detailed mechanistic analysis of the transition states involved in the catalytic process. In the last decade, bioconjugation has become a common tool for chemists and biochemists as a result of the increase in peptide and protein research and development. This covalent functionalization of these biomacromolecules under physiological conditions gives

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[†] Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201xxxxx>.

ARTICLE

rise to new areas of research such as drug discovery, high-throughput screening and *in vivo* testing. Therefore, many ligation methodologies have been studied and optimized^{7,8}. The development of Click chemistry has caused an emerged interest in the functionalization of peptides with alkynes and azides, resulting in a broad variety of building blocks such as unnatural aminoacids, being currently commercially available. In practice, the application of CuAAC in peptide chemistry requires extra efforts for the optimization of the reaction conditions⁸. Traces of O₂ can be reduced by Cu^I and sodium ascorbate into H₂O₂, a reactive oxygen species that can induce degradation of amino acids and cleavage of the polypeptide chain⁹⁻¹². Thus, a catalytic system able to perform click reactions in biomacromolecules under reductant-free aqueous conditions will lead to a major step forward in bioconjugation chemistry. Furthermore, bioconjugation impose specific requests, such as exquisite chemoselectivity, biocompatibility, and the ability to work at low temperature still enabling fast transformations. To date the CuAAC reaction in the absence of an accelerating ligand is simply too slow¹³.

In this context, we have studied the performance of our water-soluble NHC-Cu (I) catalysts for CuAAC reactions with unprotected peptides in aqueous conditions. We have chosen the DNA binding domain of the transcription factor protein GCN4, belonging to the bZIP Leucine Zipper family as a substrate for the reaction¹⁴. Indeed, modification of the GCN4 binding domain in the design of transcription factor models has been of particular interest for gene therapy¹⁵. Surprisingly, there are no studies so far on the application of click chemistry on the modified GCN4 binding domain for conjugation purposes. Here, we report the successful functionalization of the peptide comprising the basic region DNA binding domain with an organic azide via click chemistry using the sulfonated NHC-Cu (I) catalysts.

Results and Discussion

Our initial experiments consisted of the suitability tests for the *N*-heterocyclic carbene (NHC) ligand precursors on Cu using a Cu (I) source. We envisaged that according to the literature⁴, our sulfonate-functionalized ligands should behave in a similar fashion as non-functionalized ligands. However the new compounds demonstrate an excellent behavior for Click reactions in water, neat conditions and organic solvents or mixtures, for bioconjugation via CuAAC and for acetylene conversion into triazoles and even acetamides.

Synthesis of catalysts. For the initial step in the preparation of catalysts, NHC-ligand precursors **1-4** were synthesized (figure 1A). Among these, structures **1** and **2** have no precedent report in the literature. Ligand precursors **1-4** were originally considered for a single mono-coordination at 1:1 ratio using CuCl as the source of Cu^I. However, mass spectroscopy proved that the final product was actually a mixture between mono- and bis-coordinated NHC-Cu complexes. Isolation of these complexes was not feasible and therefore we were prompted to find a more convenient Cu(I) source. From literature reports, it was clear that

some Cu(I) complexes with NHC ligands demonstrated a good activity of which those having PF₆⁻ as counterion displaying activities more than 3 times higher than the analogous complex bearing BF₄⁻ as counterion⁴. Consequently, we were encouraged to use PF₆⁻ as the counterion to improve the stability without affecting the catalyst activity. A synthesis protocol similar to that described in literature was followed, using KO^t-Bu as base and tetrahydrofuran (THF) as solvent. Filtration and precipitation with ether resulted in the series of catalysts **5-8** depicted in figure 1A. [Cu(CH₃CN)₄]PF₆ was used as the source of Cu^I for this purpose. Characterization of complexes **5-8** by ¹³C-Nuclear Magnetic Resonance spectroscopy (NMR) resulted in the typical values for the C1 carbene signal for saturated NHC-Cu complexes, 200.65 ppm and 200.74 ppm for **5** and **6** respectively, while for the unsaturated NHC-Cu complexes, the values of 176.38 and 176.41 ppm were observed for **7** and **8** respectively. Mass spectroscopic results confirmed the identity of the *bis*-coordinated complexes using these ligands. The newly synthesized complexes exhibited solubility in chlorinated solvents, alcohols, dimethyl sulfoxide (DMSO), dimethylformamide (DMF) and water. Their high stability to air and heat, allows storage of the compounds under Ar-atmosphere for several weeks without loss of activity.

Catalytic activity. Catalytic experiments were carried out using the standard substrates benzyl azide and phenylacetylene in neat conditions. Supplementary Table S1 shows the yields obtained with catalysts **5-8** proving that the catalysts bearing saturated NHC ligands are better performing than those holding unsaturated NHC ligands and also showing that catalyst **5** is highly competitive with the most active complex [(ICy)₂Cu]PF₆ reported by Nolan⁴. Although certain NHC-Cu(I) complexes seems to be competitive with catalyst **5**,^{3,4} the special hydrophilicity of complexes **5-8** enables the extension of their scope towards different reaction conditions whereas those described in literature might hardly achieve good performance. In order to make a more detailed comparison between the newly synthesized catalysts, **5** and **7** were chosen for a reaction monitoring by ¹H-NMR for the standard reaction depicted in figure 2. That saturated NHC-Cu complexes are better performing than those holding unsaturated NHC ligands is illustrated in figure 2 in which **5** clearly shows a higher reactivity compared with **7** (25% conversion after the induction period of 5 min for catalyst **5** and no conversion for **7** at the same period) and a rate enhancement in the range of 3.5-4.0. The reason for this higher activity may reside in the higher σ -donating ability of the saturated NHC ligands; a similar effect causing a higher activity for NHC-Ru complexes was observed in the olefin metathesis reaction¹⁶⁻²⁰. In view of the higher reactivity of **5** bearing sulfonated NHC ligands, these were further explored for the synthesis of triazoles with various substitution patterns.

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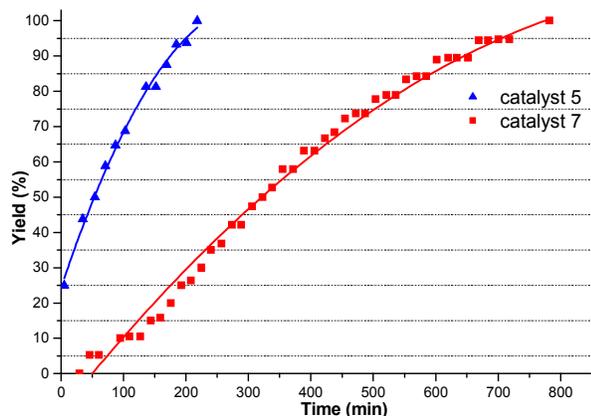


Figure 2. Plot of reaction yield of a standard Click reaction (figure 1B) monitored by $^1\text{H-NMR}$ using catalysts **5** and **7**.

Synthesis of triazoles. One of the major catalytic improvements made by the new complexes was the possibility to employ them for the synthesis of a variety of triazoles and the versatility of conditions. This capability is distributed from *neat* over solvent mixtures to *in-situ* conditions. For the production of 1,4-substituted triazoles under *neat* conditions (supplementary table S2) the products were pure enough for characterization by NMR spectroscopy. These varieties of triazoles were also produced *in-situ*, starting from the alkyl/aryl halide and sodium azide, where the use of DMSO/water mixtures was critical to achieve excellent reaction yields (supplementary table S3). Although Ru-catalysts are more feasible for Click reactions of internal alkynes the newly developed Cu-catalysts in this work were also capable to generate 1,4,5-substituted triazoles (Supplementary table S4). Due to its high-energy consumption and the use of multistep procedures²¹⁻²³, as well as the employment of toxic solvents^{25,26}, the production of 4-substituted-1,2,3 triazoles has represented a synthetic challenge. We found out the feasibility to produce 4-substituted-1,2,3-triazoles from non-activated terminal alkynes by using mixtures of DMSO/AcOH and NaN_3 (Supplementary table S7), representing an improvement of the few catalytic protocols of this kind²²⁻²⁴. However, the most outstanding advance of this work was by far the synthesis of 1-substituted triazoles from acetylene gas completely in water and in water/DMSO mixtures (figure 3A), alternative to those procedures involving organic solvents and higher catalyst loadings^{27,28}. Finally, the reaction of tosyl azide and acetylene gas in water/DMSO mixtures yielded tosyl-acetamide (figure 3B), as previously reported²⁹, opening the possibility of using acetylene

gas as an efficient source of acetamide bonds in organic and biological chemistry³⁰. All these achievements in catalytic activity were attained by using a maximum of 5 mol% of catalyst and in general aqueous conditions, which embodies an eco-friendly series of catalytic protocols.

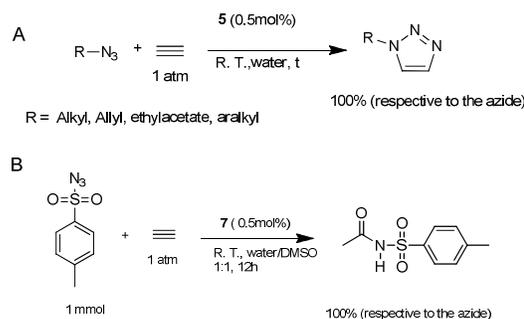


Figure 3. A. Use of acetylene gas in Click reactions in water; B. Tosyl acetamide formation in aqueous media.

Bioconjugation experiments. To further expand and illustrate the scope and potential of the developed catalysts, bioconjugation experiments with alkyne and/or azide tagged peptides were carried out. Hereto, two peptides, one 10 mer and one 23 mer, comprising part of the N-terminal basic region of the Transcription Factor protein GCN4, were synthesized on 2-chlorotrityl chloride polystyrene resin. Propargyl glycine was incorporated as the first, C-terminal amino acid residue (figure 4) to provide for the alkyne functionality for further click reaction with benzylazide. Click reaction was carried out at ambient temperature in a deoxygenated mixture of water and hexafluoroisopropanol to disrupt H-bonding interactions within the peptide using 1.5 equivalents of catalyst (6 for modification of 10mer GCN4 and 7 for 23mer GCN4 due to its higher stability) under anaerobic conditions overnight. The resulting modified peptides were purified by High Performance Liquid Chromatography (HPLC) and further characterized by Electrospray Ionization Liquid Chromatography connected to a Mass Spectrometer (LC-MS-ESI) and MALDI-TOF. Generation of the Cu-acetylide intermediate in such an environment is highly challenging, therefore a slight excess of catalyst **6** is needed. In current context, it is further worth noticing that both sequences are rich in arginine residues, of which the guanidinium side chain can easily coordinate with copper and inhibit the reaction.

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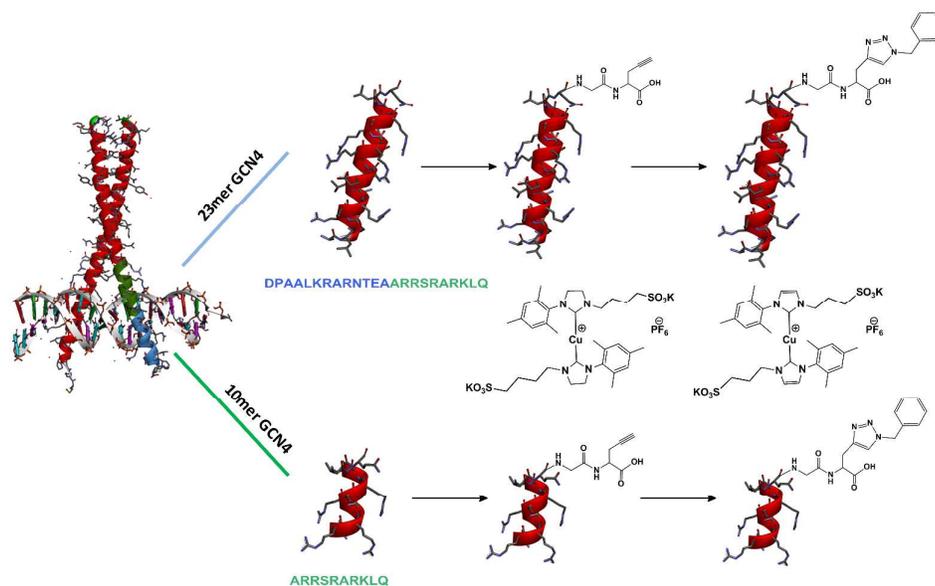


Figure 4. The transcription factor protein GCN4, belonging to the bZIP Leucine Zipper family and the derived model peptide comprising the DNA binding domain. Functionalization of a peptide with an alkyne allows cycloaddition-based modification with a non-peptide azide moiety.

These experiments underscore the potential for application of our water-soluble $(\text{NHC})_2\text{Cu}$ (I) catalysts **6** and **7** in the functionalization of deprotected peptides applying the CuAAC strategy (see supplementary information).

Heterogeneous Click catalysis by an ionically immobilized $(\text{NHC})_2\text{-Cu}$ catalyst

Further encouraged to take advantage of the ionic functionality we used this feature to ionically immobilize these complexes on an anion exchange resin. For this purpose we used the crosslinked polystyrene-co-divinylbenzene based anion exchanger Amberlyte® IRA 402, which holds benzyl-trimethyl ammonium groups. After two washing cycles with water, immobilized NHC-Cu(I) catalyst **6-IRA 402**, shown in figure 4, was obtained. Several hours were needed for the total ion exchange, which was visually detected by the colour change of the initial solution. At the end of the reaction, the solution became clear from being initially brownish, the resin embedded with catalyst changed from its typical yellowish to brownish colour (supplementary picture S1). The analysis of the water phase by XRF did not show any trace of Cu(I) ions after the immobilization, which confirmed the total anchoring of the Cu(NHC) catalyst on the resin.

The new heterogeneous catalyst **6-IRA 402** was applied for the three-component Click reaction of benzyl azide (deriving from benzyl bromide and sodium azide) and phenylacetylene. The reaction carried out under mechanical stirring showed low conversion under standard conditions, probably due to the poor contact between reactant and the heterogeneous catalyst. However, when applying ultrasound instead of mechanical stirring, the reaction was significantly accelerated, allowing full conversion after 5h (see figure 4). This prompted us to reuse the immobilized catalyst by extracting the newly formed product and adding fresh substrates while avoiding contact of the heterogeneous catalyst with the environment. The capability of this heterogeneous catalyst to be reused allowing high yields was illustrated for a maximum of 4 catalytic cycles. After the fourth cycle, the yield decreases dramatically, possibly due to the leaching of the Cu(I) species, which was confirmed by XRF experiments of the organic phase (see supplementary Graph S1). To our delight, the heterogeneous catalyst **6-IRA 402** was able to perform Click reactions by using acetylene gas as the source of alkyne. Again, the use of ultrasound was needed to achieve high conversion in a short time. This provides high versatility of these catalytic systems to be used on industrial scale (figure 5).

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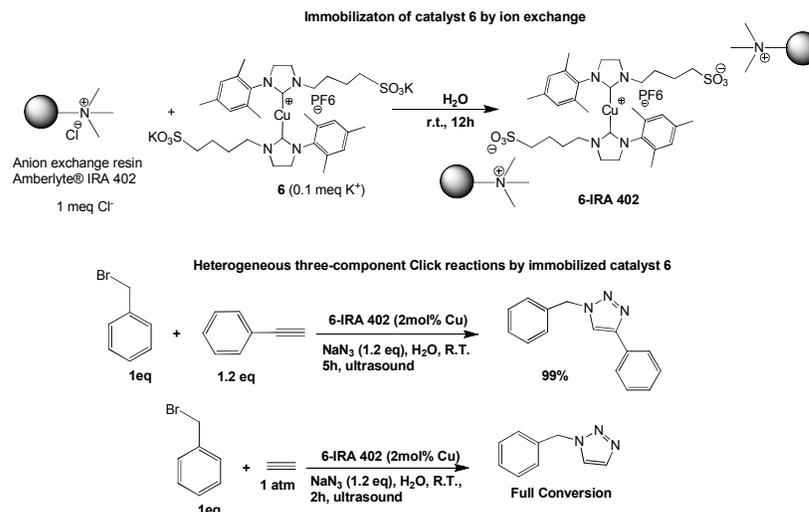


Figure 5 Synthesis and application in heterogeneous Click reactions of an ionically immobilized (NHC)₂Cu (I) catalyst.

Mechanistic Analysis. In order to obtain a full mechanistic understanding of the catalyst, we decided to perform state of the art first principle calculations on the coupling reaction between benzylazide and phenylacetylene with two catalysts **6** and **8** (figure 1), bearing respectively saturated and unsaturated NHC ligands. The goal of the calculations is twofold: (1) providing a plausible reaction mechanism of CuAAC catalyzed reactions by these new water soluble Cu-NHC complexes and (2) investigating the electronic effect of the carbon-carbon backbone of the NHC-ligand on the click reaction. Full catalyst models of species **6** and **8** (figure 1) were modeled at the B3LYP/6-311++g(3df,2p)-D3//B3LYP/6-31+g(d) level of theory (details can be found in Supporting Information) and a free energy diagram was constructed along the proposed mechanistic cycle.

For the coupling between these molecules, taking place on bi-ligand complexes (figure 1), there are steric limitations and therefore the computations are restricted to mono-ligand versions of catalyst **6** and **8**. In experimental conditions, one can expect that mono-ligand complexes (LCuPF₆) as well as bi-ligand complexes (L₂CuPF₆) are available, which is the case if there exist an equilibrium between them. Two possibilities to generate mono-ligand complexes are given in figure 5A. In the presence of phenylacetylene, the bi-ligand complex can also react towards a monoligand complex LCu-acetylide and L.HPF₆, as was verified by Diez-Gonzalez *et al.*³ If such a reaction occurs, LCu-acetylide can catalyze the CuAAC reaction. However, monoligated complexes can also be generated without acetylene, through the equilibrium reaction between L₂CuPF₆ and LCuPF₆, in which a ligand is released (reaction 2 in figure 6A). This compound can as well react with phenylacetylene forming a LH⁺CuPF₆-acetylide, in which the sulfate group of the ligand has taken up the acidic proton of phenylacetylene. From the free energy differences

displayed in figure 6A (respectively 289.9 and 163.0 kJ/mol for the saturated NHC-complex), we can assume that a reaction of the bi-ligand complex with phenylacetylene is the easiest route to a mono-ligand complex. In the following, catalytic cycles with L-Cu-acetylide as intermediate will further be discussed, while catalytic cycles with LH⁺CuPF₆-acetylide as intermediate are described in Supporting Information.

The Cu-(I)-acetylide complex was also observed by Nolte *et al.*, who postulated - based on experimental findings - a six-step catalytic cycle for a standard click reaction on Cu.⁵ Our modeled catalytic cycles contain much similarity with their scheme, however, we have chosen to display only the two most important intermediate complexes in our scheme (figure 6B) neglecting the shallow intermediates. For example, we observed that the Cu-triazole product is formed immediately according to the intrinsic reaction coordinate (IRC) scan from **TS-1** (figure 6B(LEFT) and 6D). The Cu-triazole can then be protonated by a co-adsorbed phenylacetylene (**TS-2**, figure 6B(LEFT) and 6D), generating the 1,4-triazole product and the active Cu-(I)-acetylide complex. For complex **6**, the coupling reaction has a free energy barrier of 113.0 kJ/mol and the proton transfer reaction a free energy barrier of 128.5 (figure 6B). It can however not be excluded that more stable pathways can be found computationally, in which the ligand groups interact differently with the reactants. If the reaction proceeds without catalyst, the observed reaction barrier towards 1-benzyl-4-phenyl-1,2,3-triazole amounts to 132.9 kJ/mol, which is still higher than the highest barrier in the catalytic scheme (*cf.* figure 6C and figure 6B, LEFT). Moreover, the Cu-NHC catalyst selectively generates only the 1,4-triazole product, while thermal reactions without catalyst produce a racemic mixture (**3**) of the two possible triazoles.

Furthermore, just as in the experiments under neat conditions, the saturated NHC-complexes were found to be lower activated than the unsaturated ones. Based on the differences in activation barrier for the rate determining step, we can have an indication for the rate acceleration of the saturated catalyst versus the unsaturated catalyst. Based on the coupling step (TS-1), this

ARTICLE

would be around 5.4 times faster, while based on the hydrogen transfer step (TS-2), this would be approximately 3.6 times faster at 298K. In first instance, this seems in good agreement with the experimentally observed rate acceleration of about 4 between catalyst **5** and **7**, figure 2). As these catalysts have only one CH₂-group less compared to catalyst **6** and **8**, the difference in rate between catalyst **6** and **8** will be similar, and thus, is in rather good agreement with the computationally predicted acceleration. For this catalytic cycle (figure 6B, LEFT), the influence of water solvation was studied in parallel (figure 6B, RIGHT). With implicit solvation for water, the activation barriers are remarkably higher (for the computational methodology, see

Supporting Information). Remark as well that the differences in rate between the saturated and the unsaturated complexes become smaller with water as solvent, leading right to acceleration factors of around 2. From an experimental point of view, it would also be interesting to determine the various rate acceleration factors between complexes **5** and **7** in many solvents, yet this clearly falls beyond the scope of this initial communication on these new water-soluble Cu-NHC-catalysts. However, for future research, this might validate the proposed catalytic cycles.

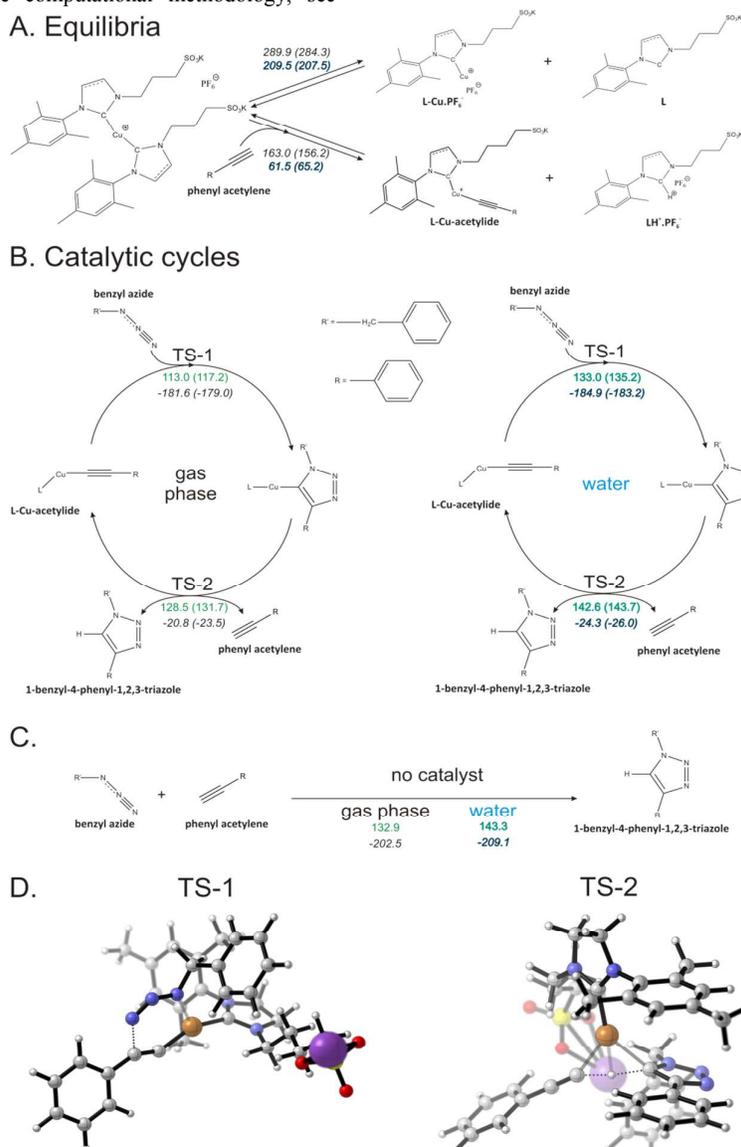


Figure 6: A. Equilibrium between bi-ligand and mono-ligand complexes; reaction free energies (298K) are given in kJ/mol for the saturated (unsaturated, in parentheses) catalyst in gas phase (top, black) and water (bottom, blue). B. Simplified catalytic cycles in which L-Cu represents the mono-ligated Cu-NHC-complex **6** or **8**, free energy barriers (top, green) and reaction free energies (bottom, black) are given in kJ/mol at 298K for the saturated (and for unsaturated

ARTICLE

catalyst in parentheses). C. Reactions between benzylazide and phenylacetylene without catalyst. D. Visualization of the modeled transition states TS-1 and TS-2 on catalyst model 6.

The free energy contributions between the different states of the catalytic cycle can be split in enthalpy and entropy contributions (Supplementary table S7). The click reaction is very exergonic with reaction free energy differences around -305 kJ/mol. To quantify the relative rates between catalysts **6** and **8**, one can look to the difference in activation free energy for the global barrier. Therefore, a reduced catalytic cycle is constructed with only the major states (right part, figure 6.A), omitting the (de)protonation transition states. Right now, the largest free energy barrier of both catalytic cycles can easily be compared. The global free energy barrier from **F** amounts to 141.6 kJ/mol and 145.7 kJ/mol, for catalyst **6** and **8** respectively. This gives a rate acceleration of 5.3 between both complexes, which is in agreement with the experimentally observed rate acceleration of 4 between catalyst **5** and **7** (figure 2). As these catalysts have only one CH₂ group less compared to catalyst **6** and **8**, the difference in rate between catalyst **6** and **8** will be similar, and thus, is in very good agreement with the computationally predicted acceleration. Without catalyst the free energy barrier would be 132.9 kJ/mol, which is larger than the barrier measured from complex **A** (90.9 and 95.8 kJ/mol, Supplementary table S8) but still lower than the barrier from complex **F** (141.6 and 145.7 kJ/mol).

Conclusion and Outlook

We have presented for the first time the synthesis of sulfonate functionalized bis-NHC-Cu(I) complexes and their application in Click reactions for a variety of reaction conditions, illustrating the high versatility of these compounds, for the synthesis of triazoles with different substitution patterns. We have further shown the use of these water-soluble complexes in bioconjugation experiments, attaining for the first time the functionalization of an unprotected 25-aminoacid chain peptide using low catalyst loading, which otherwise would need a much higher amount of Cu(I) to achieve high efficiency due to the chelating properties of certain aminoacid residues. We could take further advantage of the ionic functionality inserted into the NHC ligand and used this feature to ionically immobilize these complexes on an anion exchange resin. Moreover, both, the homogeneous and heterogeneous catalysts even performed Click reactions by using acetylene gas as the alkyne source. Exploiting tosyl azide as substrate generated tosyl-acetamide as the sole product and thus broadens the scope of the newly developed catalysts. Mechanistic analysis of the Cu(I) catalysts bearing sulfonate functionalized NHC ligands shows that the sulfonate group allows internal deprotonation and protonation steps of the alkyne and formed reaction product, respectively. Comparison of energy barriers quantitatively confirmed that the complexes with saturated ligands are more active than the complexes with unsaturated ligands toward Click reactions, which is in good agreement with the experiments.

Acknowledgements

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F.V. would like to express his deep appreciation to State Key Lab of Advanced Technology for Materials Synthesis and Processing (Wuhan University of Technology) for financial support. F.V. acknowledges the Chinese Central Government for an "Expert of the State" position in the program of "Thousand talents". H.D.V. appreciates the financial support from Mexican Council of Science and Technology (CONACYT, Ph. D. grant). M.V. is a Post-Doctoral Fellow with the FWO. Y.R. is indebted to the Marie Curie ITN with grant agreement number 238679. Computational resources and services were provided by Ghent University (Stevin Super-computer Infrastructure). We would also like to thank prof.dr.ir. Veronique Van Speybroeck for reading the manuscript and scientific discussions.

References and notes

- M. Meldal, C. Tornøe W., *Chem. Rev.*, 1980, **108**, 2952-3015. Kuang *et. al. J. Am. Chem. Soc.* **2011**, *133*, 13984-14001
- B. J. Adzima, C. J. Kloxin, C. A. DeForest, K. S. Anseth, C. N. Bowman *Nature Chem.*, 2011, **3**, 256-259.
- S. Diez-Gonzalez, A. Correa, L. Cavallo, S. P. Nolan, *Chem. Eur. J.*, 2006, **12**, 7558-7564.
- S. Diez-Gonzalez, S. P. Nolan, *Angew. Chem. Int. Ed.*, 2008, **47**, 8881-8884.
- C. Nolte, P. Mayer, B. F. Straub, *Angew. Chem. Int. Edit.*, 2007, **46**, 2101-2103.
- Q. Wang, T. R. Chan, R. Hilgraf, V. V. Fokin, K. B. Sharpless, M. G. Finn., *J. Am. Chem. Soc.*, 2003, **125**, 3192-3193.
- T.R. Chan, V.V. Fokin, *QSAR & Comb. Sci.*, 2007, **26**, 1274-1279.
- C. Gaulier, A. Hospital, B. Legeret, A. F. Delmas, V. Aucagne, F. Cisnetti, A. Gautier, *Chem. Commun.*, 2012, **48**, 4005-4007.
- E. R. Stadtman, *Free Radical Res.*, 2006, **40**, 1250-1258.
- E. R. Stadtman, B. S. Berlett, *Drug Metab. Rev.*, 1998, **30**, 225-243.
- A. Kumar, K. Li, C. Cai, *Chem. Commun.*, 2011, **47**, 3186-3188.
- R. V. Talanian, C. J. McKnight, P. S. Kim, *Science*, 1990, **249**, 769-771.
- T.R. Chan, R. Hilgraf, K.B. Sharpless, V.V. Fokin, *Org. Lett.*, 2004, **6**, 2853-2855.
- T.E. Ellenberger, C.J. Brandl, K. Struhl, S.C. Harrison, *Cell.*, 1992, **71**, 1223-1237.
- R. V. Talanian., C. J. McKnight, R. Rutkowski, P. S. Kim, *Biochemistry*, 1993, **31**, 6871-6875.
- M. S. Sanford, J. A. Love, R. H. Grubbs, *J. Am. Chem. Soc.*, 2001, **123**, 6543-6554.
- S. Monsaert, A. M. Lozano-Vila, R. Drozdak, P. Van Der Voort, F. Verpoort, *Chem. Soc. Rev.*, 2009, **38**, 3360-3372.
- A. M. Lozano-Vila, S. Monsaert, A. Bajek, F. Verpoort, *Chem. Rev.*, 2010, **110**, 4865-4909.

ARTICLE

- 19 H. Díaz-Velazquez, F. Verpoort, *Chem. Soc. Rev.*, 2012, **41**, 7042-7060.
- 20 F. B. Hamad, T.-L. Sun, S.-Q. Xiao, F. Verpoort, *Coord. Chem. Rev.*, 2013, **257**, 2274-2292.
- 21 A. E. Cohrt, J. F. Jensen, T. E. Nielsen, *Org. Lett.*, 2010, **12**, 23, 5414-5417.
- 22 T. Jin, S. Kamijo, Y. Yamamoto, *Eur. J. Org. Chem.*, 2004, **18**, 3789-3791.
- 23 J. C. Loren, A. Krasinski, V. V. Fokin, K. B. Sharpless, *Synlett.*, 2005, 2847-2850.
- 24 L.-H Lu, J.-H Wu, C-H Yang, *J. Chin. Chem. Soc.*, 2008, **55**, 414-417.
- 25 W. Zhang, C. Kuang, Q. Yang, *Synthesis*, 2010, **2**, 283-287.
- 26 J. Barluenga, C. Valdés, G. Beltrán, M. Escibano, F. Aznar, *Angew. Chem. Int. Ed.*, 2006, **45**, 6893-6896.
- 27 L.-Y Wu., Y.-X. Xie, Z.-S. Chen, Y.-N. Niu, Y.-M liang, *Synlett.*, 2009, **9**, 1453-1456.
- 28 Y. Jiang, C. Kuang, Q. Yang, *Synlett.*, 2009, **19**, 3163-3166.
- 29 S. H. Cho, S. Chang, *Angew. Chem. Int. Ed.*, 2007, **46**, 1897-1900.
- 30 F. Zsila, Z. Bikadi, D. Malik, P. Hari, I. Pechand, A. Berces, E. Hazari, *Bioinformatics*, 2011, **27**, 13, 1806-1813.