The Click Chemistry Approach Applied to Fluorine-18

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Abstract: New methods to introduce fluorine-18 into biomolecules are constantly of great interest. Particularly, the increasing number of complex structures poses a challenge to ^{18}F-labelling chemistry. Indirect ^{18}F-labelling procedures using prosthetic groups are commonly used for multifunctional biologically active compounds; however there is continuous demand for new and improved radiofluorination methods. The Cu(I)-catalysed variant of the Huisgen 1,3-dipolar cycloaddition of terminal alkynes and azides represents a most efficient and powerful reaction referred to as click chemistry. This reaction is highly specific and provides excellent yields under very mild conditions. This reaction ideally complies with the requirements of fluorine-18 labelling chemistry. Hence, it offers a convenient and efficient new ^{18}F-fluorination method which is particularly suitable for prosthetic group labelling. The first few reports using the click approach in fluorine-18 chemistry already demonstrated the particular feasibility of this approach. This review gives an overview of the Cu(I) 1,3-dipolar cycloaddition of terminal alkynes and azides and further describes the first applications of this click reaction in fluorine-18 labelling.

Keywords: Click chemistry, ^{18}F-labelling, prosthetic group labeling, fluorine-18, Huisgen 1,3-dipolar cycloaddition.

1. INTRODUCTION

Since H.C. Kolb, M.G. Finn and K.B. Sharpless implemented the term "click chemistry" in 2001 [1], there has been a huge interest in this technology, this is clearly indicated in the number of click papers published every month. This click chemistry has been used in a broad range of chemical applications [2-5]; including drug discovery and development [6, 7], bioconjugate chemistry [8], peptide chemistry [9] and polymer and surface science [10-12].

The term click chemistry covers a certain set of reactions, particularly the Cu(I)-catalysed cycloaddition between alkynes and azides, forming 1,4-disubstituted 1,2,3-triazoles is the most efficient one [1]. Very mild reaction conditions accompanied by high efficiency, high selectivity and excellent yields make click chemistry particularly suitable for biological applications as well as for radiopharmaceutical sciences. In addition, the 1,2,3-triazole products are biologically stable and remarkably biocompatible. Consequently, numerous applications of this click reaction can be found in drug development and research towards biologically active molecules or materials [6-9].

Since its introduction, the use of click chemistry has had a huge impact on many chemical domains, thus it is not surprising that this approach has recently reached the field radiopharmaceutical chemistry [13]. Its rising significance in this field was highlighted at the "17th International Symposium on Radiopharmaceutical Sciences" in Aachen, Germany, April 2007, where an entire session was devoted to "click labelling methods" in fluorine-18 chemistry [14]. In addition, the first application of click chemistry in carbon-11 labelling has been recently reported [15]. Aside from reports in fluorine-18 and carbon-11 chemistry, it has been also successfully applied for technetium and rhenium labelling procedures. Metallic nuclides such as technetium and rhenium are usually introduced into biomolecules by chelating systems which are linked to the target structure. Interestingly, here the 1,2,3-triazole moiety was not only functionalised as a linker to couple the chelating system to the biomolecule, but also as an active ligand which contributes one nitrogen atom to the corresponding chelating system [16]. The application of click chemistry in radiotracer development and synthesis has recently been reviewed in a mini-review by Mamat *et al.* [17].

Complex and multi-functionalised biomolecules and pharmaceuticals rely on suitable and efficient ^{18}F-labelling chemistry. In particular, mild labelling methods are essential for sensitive structures, such as peptides and proteins. The Cu(I)-catalysed cycloaddition of alkynes and azides succeeds under very mild reaction conditions and has already been proven an implementable method for ^{18}F-labelling chemistry.

This review will give an overview of the Cu(I)-catalysed cycloaddition chemistry of alkynes and azides and how it has been employed in fluorine-18 labelling.

2. THE CU(I)-CATALYSED "CLICK" CYCLOADDITION

Originally, click chemistry referred to a range of highly efficient reactions which fulfil a set of stringent criteria, with the most important ones being very high yields, wideness in scope, stereospecificity, simple purification and work-up procedures. Moreover, a high thermodynamic driving force, usually greater than 20 kcal/mol, is one of the most essential

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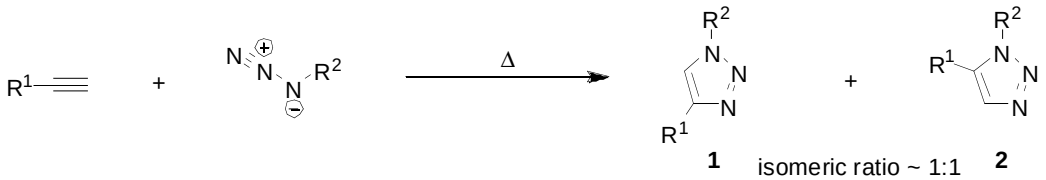


Scheme 1. Thermal 1,3-dipolar cycloaddition of azides and terminal alkynes to form 1,4-isomers (1) and 1,5-isomers (2) of disubstituted 1,2,3-triazoles without distinct regioselectivity.

characteristics [1]. Such "spring-loaded" reactions include ring-opening reactions of epoxides [18, 19], aziridines [18, 20, 21], aziridinium ions [22] and episulfonium ions [22]; formation of ureas, thioureas, aromatic heterocycles, oxime ethers, hydrazones and amides; oxidative additions to carbon-carbon multiple bonds such as epoxidation [23], dihydroxylation [24], aziridination [25], sulfenyl halide addition and certain Michael additions. Beside this respectable set of reactions, the most attractive representatives of click chemistry are the cycloaddition reactions involving heteroatoms, such as Hetero-Diels-Alder [26, 27] and 1,3-dipolar cycloadditions [28, 29]. Among the latter, the Cu(I)-catalysed Huisgen 1,3-dipolar cycloaddition [30, 31] of azides and terminal alkynes forming 1,2,3-triazoles is outstanding and meets ideally the requirements of click chemistry. On this account, the Cu(I)-catalysed alkyne-azide cycloaddition became the most popular reaction referred to as click chemistry.

Although the starting materials of the Huisgen 1,3-dipolar cycloadditions, azides and terminal alkynes, are highly energetic species, they show relative inertness to a wide variety of conditions, thus they tolerate most biological and organic conditions [30, 32]. Azides and alkynes are orthogonal to most other organic functionalities and remain unaffected through a number of subsequent molecule transformations until their unification. Their general inertness results from a high kinetic stability: this is demonstrated with the uncatalysed cycloaddition which were long reaction times and elevated temperatures are required [33, 34]. CAUTION: attention should be paid to some kinds of azides which show the tendency to explosive decomposition by the loss of N_2. Exothermic decomposition is favoured especially by metallic azides, small organic azides and organic azides with a high density of energetic functionalities (In case of organic azides, the rule is such that the number of nitrogen atoms must not exceed the ones of carbon and that $(N_C + N_O)/N_N \geq 3$, whereby N is the number of atoms of the corresponding indices [35]). Furthermore, certain transition metal species such as Fe(III) or Co(III) and strong acids can catalyse such azide decomposition. On the other hand, aliphatic azides show a particularly high kinetic stability and can be prepared, stored and handled without major safety issues [35, 36].

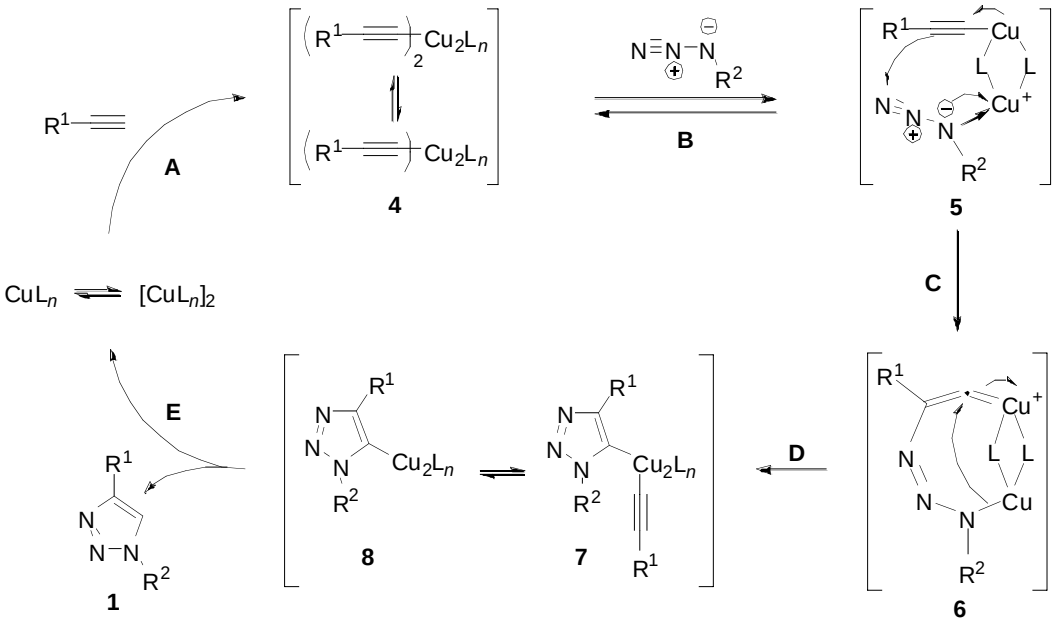
Thermal formations of 1,2,3-triazoles using azides and alkynes usually show no distinct regioselectivity and give a 1:1 mixtures of both the 1,4- and 1,5-disubstituted 1,2,3-triazoles regioisomers (Scheme **1**) [33, 34]. Regioselectivity can be introduced by the use of highly electron-deficient terminal alkynes which favour the 1,4-isomers (**1**), but the 1,5-regioisomer (**2**) is still observed [37, 38].

A major breakthrough the Cu(I)-catalysed variant of this cycloaddition was discovered independently by the Sharpless [30] and Meldal [31] groups in 2002. In the presence of a Cu(I) source, the reaction of azides and terminal alkynes lead solely to the 1,4-regioisomers. The Cu(I)-catalysed reaction shows a much higher reaction rate up to 10^7 times higher than the non-catalysed version and provides high yields without the need of elevated temperatures [2, 39]. On the other hand, ruthenium catalysts such as $Cp^*RuCl(PPh_3)_2$ were found recently to lead to the 1,5-disubstituted 1,2,3-triazoles instead of the 1,4-regioisomers [40, 41]. The Cu(I)-catalysed 1,3-dipolar cycloadditions can be performed under very mild reaction conditions. Typically aqueous mixtures are ideal solvents and give high yields even at room temperature. Additionally, most of the functional groups are tolerated by this type of reaction and thus protection group chemistry can be circumvented. The 1,4-substituted 1,2,3,-triazoles are obtained in near-quantitative yields and very high purities and therefore requiring only minimal work-up or purification procedures [1, 6, 30, 31]. In particular cases, Cu(I)-catalysed triazole coupling can be employed under physiological conditions on and even in living cells [42-46].

In addition to the mild reaction conditions and the physiological compatibility of this reaction the resulting 1,2,3-triazole moieties show excellent biological properties. As a result of certain atom positions and electronic properties, the triazole unit can mimic a peptide bond when it is used as a linker in biomolecules and the heterocyclic structure also shows a much higher hydrolytic and redox stability [2, 6, 47]. Beside the rigid ring structure, the triazole backbone leads to the calculated R^1-R^2 distance of 5.0 Å (Scheme **1**) which is close to the substituents distance of a classic dipeptide of 3.9 Å [48]. Furthermore, the N(2) and N(3) atoms of 1,2,3-triazoles provide two weak hydrogen-bond acceptors and due to the very strong dipole moment the C(5) atom is highly polarised and can function as a hydrogen-bond donor, thus mimicking the amide proton [49, 50]. The similarity of 1,2,3-triazole linkers and peptide bonds have been and continue to be investigated for a broad range of interesting biological applications, including *anti*-histamine activity [51], *anti*-bacterial activity [52], *anti*-HIV activity [53] and selective β_3 adrenergic receptor inhibition [54].

2.1. Mechanism of the Cu(I)-Catalysed 1,2,3-Triazole Formation

A suitable mechanism for the Cu(I) catalysed cycloaddition of alkynes and azides should be able to explain the impressive rate enhancement by the catalyst, the wide spectrum of different azide and alkyne reactants as well as the broad variety of tolerated reaction conditions. In contrast to the concerted mechanism of uncatalysed 1,3-dipolar cycloadditions, a stepwise mechanism is proposed for the Cu(I)-catalysed variant of this reaction (Scheme **2**) [2, 30, 55, 56].



Scheme 2. Proposed stepwise mechanism of Cu(I)-catalysed alkyne-azide couplings [2, 56].

When internal alkynes were found to show no reactivity under catalytic conditions, Cu(I) acetylides were already suggested as a reactive intermediate in the catalytic process [30, 31]. Density functional theory (DFT) calculations on monomeric Cu(I) acetylides complexes indicate for a stepwise mechanism whereas a concerted cycloaddition of the Cu(I) acetylide and the azide is strongly disfavoured by a activation barrier of 23.7 kcal/mol [55].

Initially, the Cu(I) acetylide complex (4) (step A) is formed, thereby the ligands of the Cu(I) species are displaced. In case of acetonitrile ligands, step A is calculated to be slightly endothermic (approx. -0.6 kcal/mol), which becomes exothermic (11.7 kcal/mol) for water ligands [55]. This is consistent with the experimental results that the rate is much higher in aqueous systems where no additional base is needed.

Moreover, Cu(I) acetylides are well-known as utilities for C-C bond formations [57] and they are most probably resulting from an initial π-complexation of the acetylene with the Cu(I) species. For such a π-coordination, calculations show a dramatic drop in the pK_a of the terminal alkyne proton by up to 9.8 pH units which makes a subsequent deprotonation and thus step A accessible in aqueous solutions [55].

In the next step (B), the azide is coordinated by Cu(I) and activated for a electrophilic attack of N(3) at C(4) (→ nomenclature according to triazole rings) [2]. While this electrophilic attack takes place, the Cu-C(5) double bond formed with electron donation from a full d-orbital of the copper.

The depicted Cu(I) dimer (5) in is just one of several possible structures which are consistent with DFT calculations [55]. Kinetic studies found the catalytic process to be second order in copper [56, 58]. However, results also indicate that a dynamically exchanging family of Cu(I) acetylide complexes and even π-complexes of alkynes may also be involved [56, 59, 60]. Multinuclear copper acetylide complexes are com-

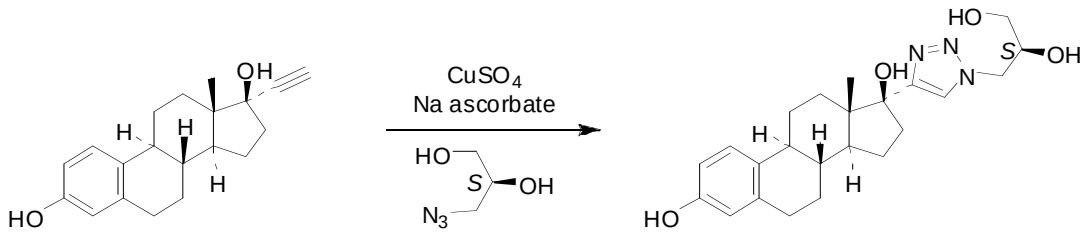
shown to catalyse the 1,2,3-triazole formation [56]. A huge excess of acetylene is proposed to lead to saturated coordination of the Cu(I) species and the catalytic effect is inhibited. Saturated Cu(I) species are unable to coordinate additionally to the azide, as a weaker ligand, and the Cu(I) species remain catalytically ineffective [56]. In the same way, commercially available copper acetylides, which are already saturated by acetylenes, show no catalytic effect. Hence, labile ligand dissociation seems to be necessary for catalytic activity [2].

As a result of the abovementioned electrophilic attack, the metallocycle (6) is generated in step C [2]. Such eight-membered metallocycles containing two copper centres are already known and have been characterised [62]. In case of monomeric copper complexes which lead to six-membered metallocycles, calculations indicate a very low energy barrier of only 3.2 kcal/mol for the final ring contraction of the metallocycle to the triazolyl-copper (8) (step D) [55]. Although the different ring sizes in both mechanisms may possess little variation in kinetics, the conversion to the triazolyl-copper (8) should be similarly fast. Experimental findings of electron-withdrawing substituents on the alkyne which accelerate the catalysis are consistent with both proposed mechanisms for step C and D, respectively [31, 60].

In the final step (E), the triazolyl-copper (8) is protonated and the catalyst is regenerated while the catalytic cycle is completed. Studies using deuterated alkynes or deuterated solvents, observed both complete loss of deuterium and high incorporation of deuterium on the C(5) position of the triazole, respectively [55, 56]. Accordingly, solvent molecules or a protonated additional base can be assumed as proton source.

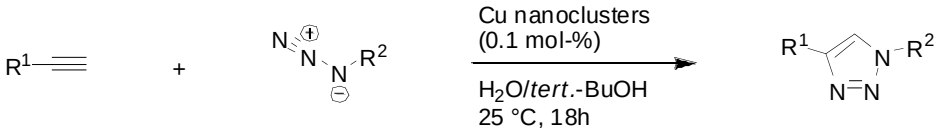
2.2. Generation of the Cu(I) Catalyst

The Cu(I) catalysed 1,3-dipolar cycloaddition shows high consistency and efficiency over a very broad range of conditions, therefore the catalyst should also be stable and effec-



9 **10** **11**

Scheme 3. Cu(I)-catalysed cycloaddition of 17-ethynylestradiol (9) and (*S*)-3-azidopropane-1,2-diol (10). The reaction was accomplished in water/*tert.*-butanol (1:1) at room temperature for 12 – 24h using the $CuSO_4$/sodium ascorbate system (1:10 mol%). The pure product (12) was achieved by filtration in a yield of 94% [30].



12 **13** **14**

$R^1 = C_6H_5$; $C_6H_5(OH)CH$; $CH_2(OH)$
$R^2 = C_6H_5$; $C_6H_5CH_2$

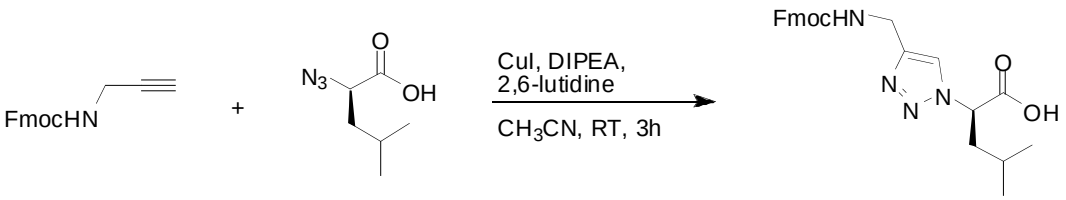
Scheme 4. Cycloadditions of various alkynes of type 12 and azides of type 13 catalysed by Cu(0) nanoclusters. The reactions were carried out in water/*tert.*-butanol (2:1) at 25 °C for 18h. The pure 1,4-disubstituted 1,2,3-triazole products (14) were isolated by filtration in yields of 80 - 99% [74].

ples to provide catalytically active Cu(I) species in the reaction mixture: One is a direct adding a Cu(I) salt or complex directly, while the second technique is based on *in-situ* generations of the Cu(I) species. However, the direct addition of Cu(I) species requires generally oxygen-free conditions due to the relatively instable Cu(I) oxidation state ($E°$ (Cu^{2+} → Cu^{+}) = + 0.16 V in water). The *in-situ* approach can be further subdivided into the reduction of a Cu(II) salt and the oxidation of metallic Cu(0) forms. As a major advantage, the *in-situ* methods do not require an inert atmosphere and can be performed in aqueous mixtures under "normal" atmosphere.

The *in-situ* formation of the Cu(I) catalyst by reduction of Cu(II) salts can be done either by a reducing agent or by comproportionation with copper metal. As mentioned before, these systems tolerate aqueous mixtures and "normal" atmosphere. Generally, the systems are very forgiving of common reaction conditions and further tolerate most functional groups. The most commonly used reducing agent is sodium ascorbate (3-10 mol-%) in combination with readily available and stable Cu(II) salts (1-5 mol-%), such as Cu(II) sulphate pentahydrate or Cu(II) acetate. Another reducing agent, tris(2-carboxyethyl)phosphane hydrochloride (TCEP) has also been used particularly in biological systems [42, 46, 63]. In similar applications, where the substrate or biological moiety is sensitive to commonly used reducing agents, comproportionation with Cu(0) can be easily accomplished by the addition of small pieces (i.e. wire or turnings) of copper metal [39, 43, 64]. Typically, the *in-situ* reactions are carried out in water-alcohol mixtures (frequently *tert.*-butanol) at room temperature or with a gentle heating. One example of the Cu(I)-cataysed coupling of 17-ethynylestradiol (9) and (*S*)-3-azidopropane-1,2-diol (10) is outlined in Scheme **3**. The reaction was carried out in a 1:1 mixture of water/*tert.*-butanol at room temperature for 12 - 24h using the $CuSO_4$/sodium ascorbate system (1:10 mol-%). The isolated pure product (12) was obtained by filtration in a yield of 94% [30]. Generally, the resulting 1,2,3-triazoles can often be isolated after simple work-up procedures such as filtration or phase extraction in nearly quantitative yields and high purities (> 90%) [30, 56, 58, 65-72].

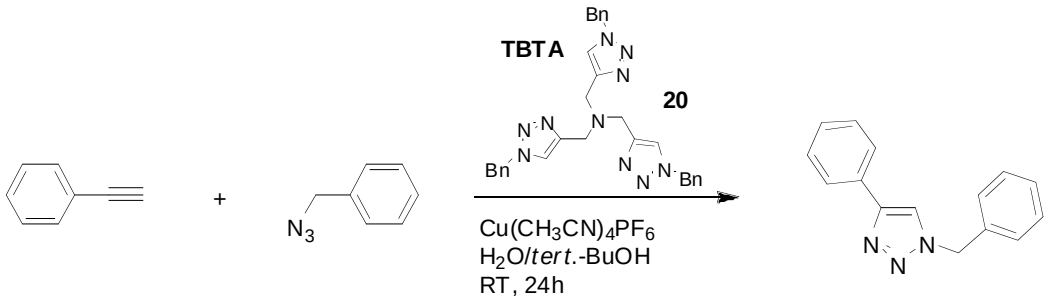
An additional preparation method of catalytic amounts of Cu(I) available for the 1,3-dipolar cycloaddition is oxidation of copper metal. In a simple experimental procedure, only a small piece of copper metal is added to the reaction mixture. This oxidative generation effectively offers Cu(I) without the need for an additional oxidation agent [30, 55]. The normal surface of copper metal contains copper oxides and carbonates in the patina in adequate amounts to initialise the *in-situ* generation of the catalyst. Usually, these protocols require long reaction times of about 12-48 hours and excess of copper, but the triazole products can be obtained in good yields and high purity without copper contaminations [30, 55].

In alternative methods, the use of nanosize copper, copper nanoclusters and copper nanoparticles save reaction times and show similarly high efficiency as other catalyst generating methods [73-75]. The dissolution of Cu(0) nanosize activated powder into Cu(I) is mediated by amine hydrochloride salts and requires slightly acidic conditions of about pH 5. Alkynes or azides bearing an amine hydrochloride salt in a functional group, can sufficiently provide this oxidative dissolution of Cu(0) into Cu(I) without the addition of an amine hydrochloride salt [73]. In contrast, when using copper nanoclusters, the alkyne-azide coupling is efficiently catalysed without amine hydrochloride salt support (Scheme **4**) [74]. Even the formation of active copper acetylides is supposed to take place on the surface of the nanoclusters rather than in solution. Cu(I) is still most likely the active



15 **16** **17**

Scheme 5. Fmoc-*N*-propargylamine (15) is coupled to α-azido D-leucine (16) using CuI as a direct source of Cu(I) in acetonitrile. The bases DIPEA and 2,6-lutidine (2 eq. each) were employed. The 1,2,3-triazole product 17 was obtained in a yield of 97% within 3 h at room temperature [47].



18 **19** **21**

Scheme 6. Ligand-assisted Cu(I)-catalysed cycloaddition of phenylacetylene (18) and benzyl azide (19) using *tris*-(benzyltriazolylmethyl)amine (20) (TBTA) as ligand. Only 0.01 equivalents of $Cu(CH_3CN)_4PF_6$ as catalyst effectively gave 1-benzyl-4-phenyl-1*H*-1,2,3-triazole (21) in a yield of 84% within 24 h at room temperature [83].

oxidation state in the reaction mixture [76]. In case of the oxidative generation of Cu(I) catalyst, the nanosize materials of copper circumvent long reaction times and offer a comparable broad range in application and similar high yields as alternative protocols.

Direct Cu(I) catalyst sources are provided by either Cu(I) salts, for instance CuI and CuBr, or coordinated Cu(I) complexes such as $CuOTf \cdot C_6H_6$ [30], $[Cu(CH_3CN)_4]PF_6$ [30], $[CuP(EtO)_3]I$ [77, 78] and $[Cu(PPh_3)_3]Br$ [68, 77, 78]. Direct Cu(I) catalysts are usually employed in organic solvent systems as aqueous systems cause solubility problems [78]. Organic protocols are frequently used in polymer applications [10, 68, 78, 79].

All these organic-based systems require a nitrogen base like triethylamine, diisopropylethylamine (DIPEA), pyridine, bipyridine or 2,6-lutidine. In particular, the excess of base leads to higher yields within shorter reaction times (Scheme **5**) [30, 31, 47, 80]. While in aqueous systems the deprotonation is provided by water and no additional base is required, in organic systems the base is essential in the catalytic cycle for the deprotonation of the initially formed π-complex which makes the active Cu(I) acetylide complex (4) (step A in Scheme **2**) available [55, 56]. Regarding the catalytic species, the Cu(I) complexes show particularly high solubility and efficiency in the organic systems while Cu(I) salts have only limited solubility. However, neither a complete dissolution of the catalyst nor the coupling reactants seems to be entirely essential, since there are examples where excellent yields and purities are obtained with very limited solubilities and in heterogeneous reaction mixtures [30]. Major drawbacks of direct protocols are: sensitivity of the Cu(I) oxidation state, sensitivity to reaction conditions and complicating side reactions which can be circumvented usually with the *in-situ* methods [30, 31, 47]. The use of steric demanding bases such as DIPEA and 2,6-lutidine can minimise side-product formations, which are assumed to result mainly from alkyne homocoupling mediated by unhindered nitrogen bases [2, 30, 47, 48, 57, 81]. Overall, the direct Cu(I) catalysts were successfully employed in a broad range of organic systems and the corresponding 1,2,3-triazoles were obtained in good yields [30, 31, 47, 48, 68, 77-80, 82].

On the whole, there are several sufficient methods for providing catalytically active Cu(I) species in alkyne-azide cycloaddition. The more robust systems provide simple procedures and high yields. For special cases, alternative protocols with similar efficiency are available. The optimal systems and conditions often depend on the substrates and the final application. Generally, the work-up procedures for most reactions require only simplest work-up methods and purification techniques.

2.3. Ligand-supported Cu(I)-Catalysed Alkyne-Azide Cycloaddition

Although the general protocols of the Cu(I)-catalysed alkyne-azide coupling show high efficiency, in some cases faster kinetics are helpful and preferable. Predominantly in bioconjugate applications very low concentrations of the reactants and the catalyst are required to prevent degradation reactions of the biological scaffolds. Some particular heterocyclic chelators have been found to accelerate the 1,3-dipolar cycloaddition, most likely by chelating and stabilising the Cu(I) species. Most effective ligands are oligo- or polytriazoles [83] as well as electron-rich bipyridines [58] and bis(oxazolinyl)pyridines (pybox) [84]. The commonly used ligand *tris*-(benzyltriazolylmethyl)amine (20) (TBTA), provides very high efficiency and thereby reduces the minimum catalyst loading as much as tenfold (Scheme **6**) [45, 83].

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