

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].

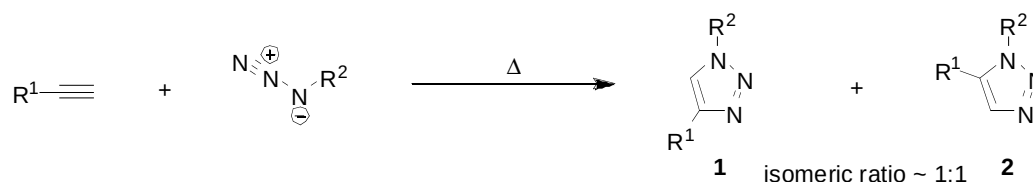
Complex and multi-functionalised biomolecules and pharmaceuticals rely on suitable and efficient ^{18}F -labelling chemistry. In particular, mild labelling methods are essential for sensitive structures, such as peptides and proteins. The Cu(I)-catalysed cycloaddition of alkynes and azides succeeds under 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], sulfonyl 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 N₂. 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 ≥ 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].

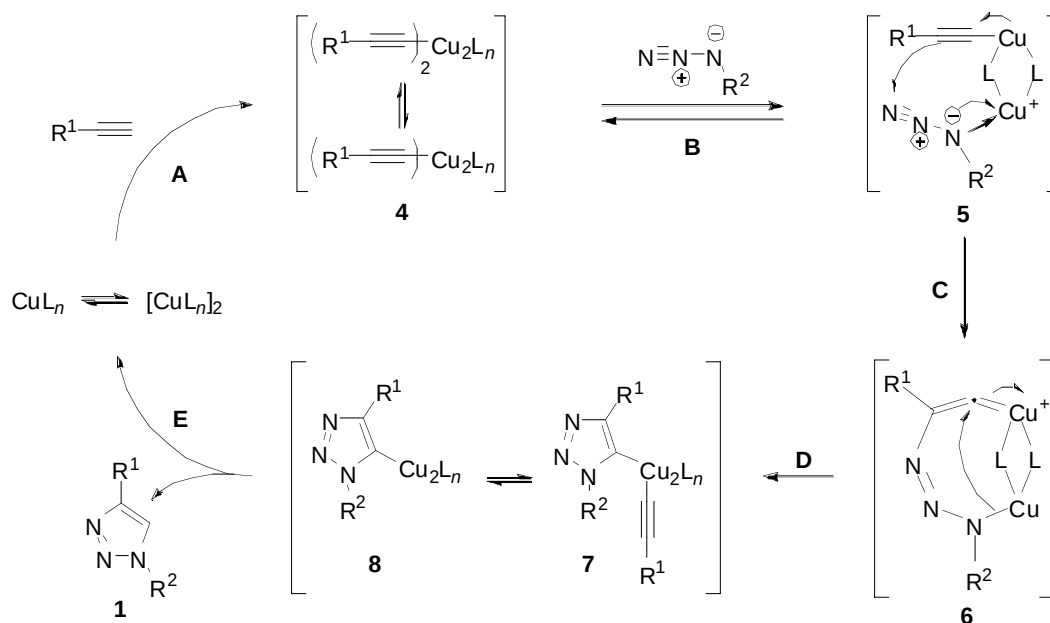
A major breakthrough the Cu(I)-catalysed variant of this cycloaddition was discovered independently by the Sharpless

[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 10⁷ 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(PPh₃)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 R¹-R² 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 β₃ 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) ($\rightarrow$ 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 common [59], but so far only one known example [61] has been

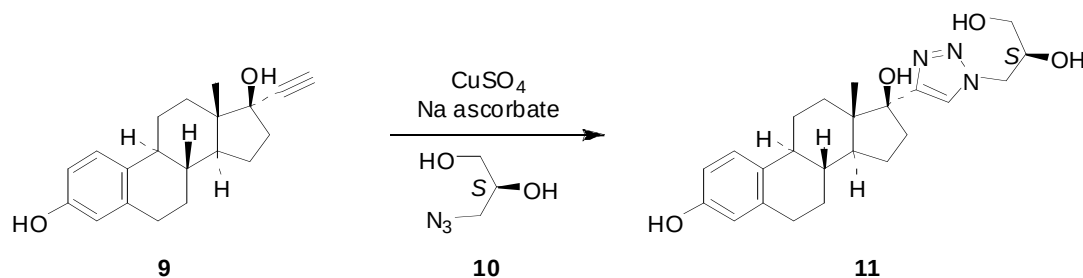
shown to catalyse the 1,2,3-triazole formation [56]. A huge excess of acetylene is proposed to lead to saturated coordination of the Cu(I) species and the catalytic effect is inhibited. Saturated Cu(I) species are unable to coordinate additionally to the azide, as a weaker ligand, and the Cu(I) species 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 metallacycle (6) is generated in step C [2]. Such eight-membered metalocycles containing two copper centres are already known and have been characterised [62]. In case of monomeric copper complexes which lead to six-membered metalocycles, calculations indicate a very low energy barrier of only 3.2 kcal/mol for the final ring contraction of the metalocycle 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].

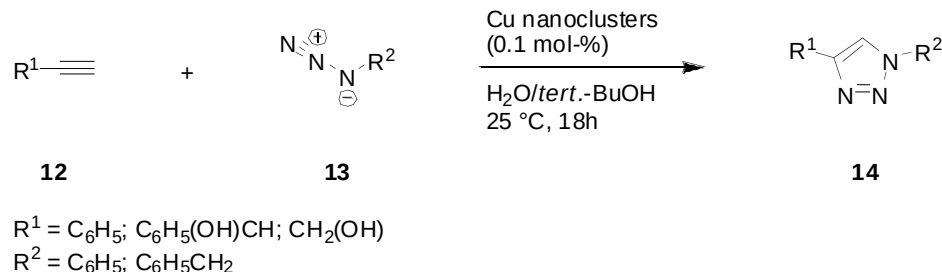
In the final step (E), the triazolyl-copper (8) is protonated and the catalyst is regenerated while the catalytic cycle is completed. Studies using deuterated alkynes or deuterated solvents, observed both complete loss of deuterium and high incorporation of deuterium on the C(5) position of the triazole, 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 effective under these conditions. Typically, there are two princi-



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 CuSO₄/sodium ascorbate system (1:10 mol%). The pure product (12) was achieved by filtration in a yield of 94% [30].



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].

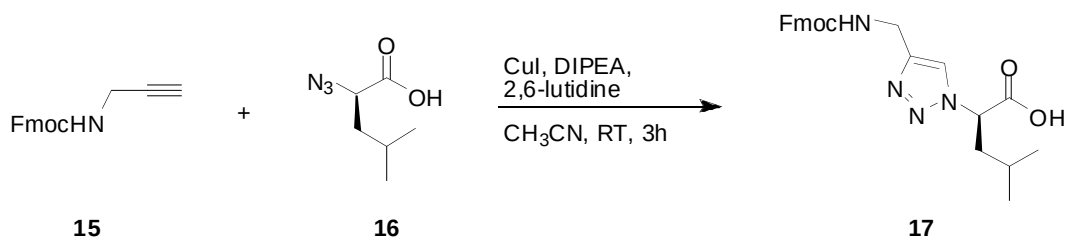
ples to provide catalytically active Cu(I) species in the reaction mixture: One is a direct adding a Cu(I) salt or complex directly, while the second technique is based on *in-situ* generations of the Cu(I) species. However, the direct addition of Cu(I) species requires generally oxygen-free conditions due to the relatively instable Cu(I) oxidation state ($E^\circ (Cu^{2+} \rightarrow Cu^+) = +0.16$ 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)-catalysed 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.*-

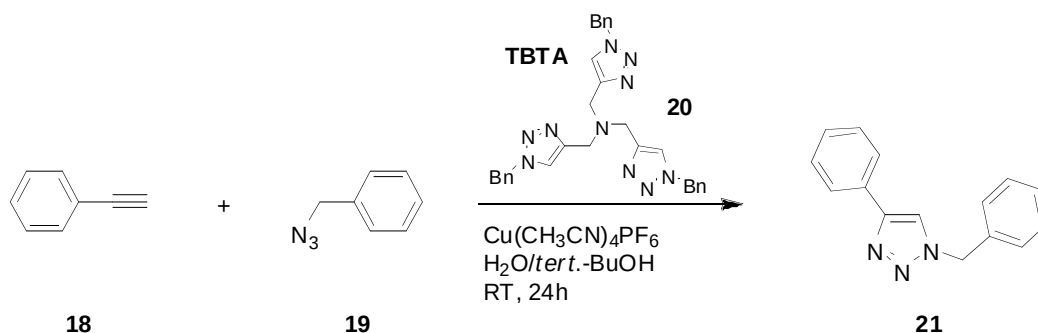
butanol at room temperature for 12 - 24h using the CuSO₄/sodium ascorbate system (1:10 mol-%). The isolated pure product (12) was obtained by filtration in a yield of 94% [30]. Generally, the resulting 1,2,3-triazoles can often be isolated after simple work-up procedures such as filtration or phase extraction 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



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].



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(CH₃CN)₄PF₆ as catalyst effectively gave 1-benzyl-4-phenyl-1H-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 · C₆H₆ [30], [Cu(CH₃CN)₄]PF₆ [30], [CuP(EtO)₃]I [77, 78] and [Cu(PPh₃)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].

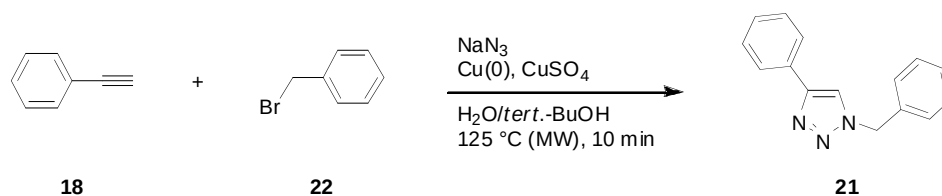
All these organic-based systems require a nitrogen base like triethylamine, diisopropylethylamine (DIPEA), pyridine, bipyridine or 2,6-lutidine. In particular, the excess of base leads to higher yields within shorter reaction times (Scheme 5) [30, 31, 47, 80]. While in aqueous systems the deprotonation 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(oxazolonyl)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].



Scheme 7. Microwave-supported one-pot Cu(I)-catalysed 1,2,3-triazole formation using phenylacetylene (18), benzyl bromide (22) and sodium azide. Cu(0) turnings and CuSO₄ in 1:1 H₂O/*tert.*-BuOH gave 93% of 1-benzyl-4-phenyl-1*H*-1,2,3-triazole (21) within 10 min at 125 °C (100 W) [39].

The tertiary amine centre and the triazole moieties in TBTA concertedly function as a tetradentate chelating system of the Cu(I) ion and protect Cu(I) from oxidative degradation. Moreover, the tertiary amine in TBTA provides a proton acceptor, thus making additional base in organic systems unnecessary [44, 83]. As a result, TBTA has been successfully used in various bioconjugation studies [42-46, 58, 63, 80].

Other ligands of the bipyridine and the pybox type effectively accelerate the Cu(I) catalysed alkyne-azide cycloaddition. The water-soluble and commercially available bipyridine bathophenanthrolinedisulfonic acid [1] has been found as a suitable alternative to TBTA. In particular, this ligand has been shown to exceed the efficiency of TBTA. However, this ligand increases the oxygen sensitivity of the Cu(I) catalysts and therefore oxygen-free conditions are essential [58, 85]. Further ligands of the chiral pybox family have been used as asymmetric auxiliaries [84]. These chiral molecules enable enantioselective alkyne-azide cycloadditions, although moderate selectivity and kinetic resolution are reported.

In general, certain auxiliary ligands enlarge the adaptability of the 1,3-dipolar cycloaddition of alkynes and azides, especially for bioconjugate applications and experiments at low reagent concentrations. In addition, chiral pybox ligands offer an enantioselective variant of this catalysis, even though further investigations towards higher selectivity in the kinetic resolution are necessary. Major advantages of the ligand-assisted process are faster kinetics and higher reactivity at low concentrations of reactants.

2.4. One-pot Protocols of Cu(I)-Catalysed Alkyne-Azide Coupling

The usual safety inconvenience of handling small organic azides which tend to rapid decomposition could be circumvented by an *in-situ* generation of these compounds without the need of their isolation. Such one-pot procedures generally require specific selectivity in each reaction step with minimal side-product formations. Alkyne and azide functionalities show broad orthogonality to other functional groups, thus their cycloaddition is particularly suitable for this approach. Organic azides could be generated successfully from sodium azide and the corresponding halides or arylsulfonates *in-situ*; followed by high-yielding cycloadditions with the appropriate alkynes [39, 86, 87]. In addition, organic azides can also be obtained *in-situ* from halides via a copper-catalysed reaction with sodium azide and catalytic amounts of L-proline [88, 89]. Remarkably, the one-pot click chemistry protocols show the same excellent efficiency as

the traditional protocols. This approach furthermore enables the one-pot creation of interesting polyvalent molecules such as dendrimers. Accordingly, starting from *in-situ* prepared small multi-azides and subsequent (multi)-cycloadditions with *in-situ* available alkynes lead to molecules containing several 1,4-substituted 1,2,3-triazole moieties. In Addition, microwave-assisted one-pot procedures make the triazole products obtainable within short reaction times of 10-30 min (Scheme 7) [39]. The ability of the alkyne-azide cycloaddition to be combined in a one-pot strategy with other reactions was shown in a one-pot synthesis of various spiro-trione-1,2,3-triazoles. The one-pot synthesis successfully accomplished four subsequent transformations; a Wittig reaction, a Knoevenagel condensation, a Diels-Alder cycloaddition and finally a Cu(I)-catalysed 1,2,3-triazole formation [90].

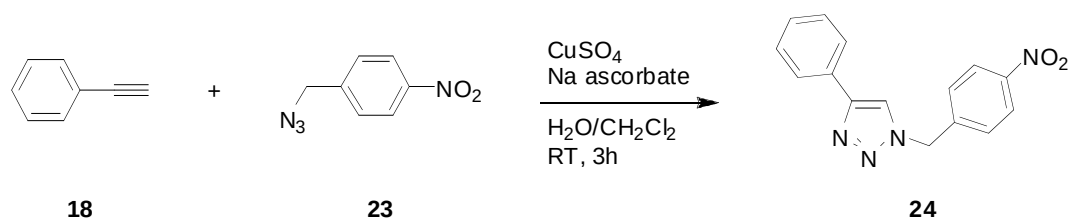
2.5. Microwave-assisted Cu(I)-Catalysed 1,2,3-Triazole Formation

Although the Cu(I)-catalysed alkyne-azide cycloaddition is generally very efficient at room temperature, completion often requires reaction times of 12 – 24 hours [30, 31]. The reaction can be dramatically accelerated by microwave heating and thereby the reaction times are reduced to minutes [39, 68, 77, 78, 91-93]. Similar to the traditional methods, microwave-assisted alkyne-azide coupling reactions can be performed in organic solvents with directly supported Cu(I) species [77, 78] as well as in aqueous systems with *in-situ* generated Cu(I) catalysts [39, 68, 91-93]. In case of solubility problems in aqueous systems, microwave chemistry can provide fast access to the desired 1,2,3-triazole products in anhydrous organic solvents with extremely low catalyst loadings [91]. Furthermore, microwave-assistance was also applied in one-pot Cu(I)-catalysed alkyne-azide coupling reactions and gave the desired 1,2,3-triazoles in high yields after very short reaction times of 10 – 15 min (Scheme 7) [39]. Undoubtedly, the microwave approach benefits from very short reaction times, whereas the triazole products show no significant enhancement in yield or purity. Hence, microwave heating considerably accelerate the 1,2,3-triazole formation. However, there is also evidence which suggests that the rate of undesired side reactions is increased [2, 77]. In case of ¹⁸F-labelling applications, time-saving and fast methods are highly required. The dramatic rate acceleration of the microwave chemistry makes Cu(I)-catalysed alkyne-azide coupling very attractive for ¹⁸F-labelling.

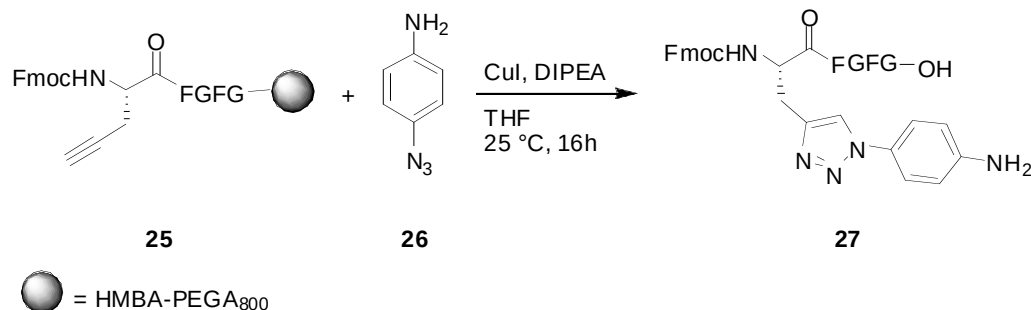
2.6. Further Protocols of Cu(I)-Catalysed Alkyne-Azide Coupling

In a recent report, a new solvent system using dichloromethane as a co-solvent with water in Cu(I)-catalysed

¹ CAS. No. 53744-42-6



Scheme 8. Cu(I)-catalysed coupling of phenylacetylene (18) and 4-nitrobenzyl azide (23) in 1:1 $\text{H}_2\text{O/CH}_2\text{Cl}_2$. The product, 1-(4-nitrobenzyl)-4-phenyl-1H-1,2,3-triazole (24), was isolated in a yield of 85% within 2h at room temperature [94].



Scheme 9. Solid-phase-supported Cu(I)-catalysed cycloaddition of HMBA-PEGA₈₀₀-bound FmocHN-L-propargylglycyl-Phe-Gly-Phe-Gly (25) and 4-azido-aniline (26) using CuI and DIPEA in THF. The product (27) was obtained in a yield of 90% after 16h at 25 °C [31].

alkyne-azide cycloaddition was shown to be very efficient [94]. Dichloromethane was already used before as sole solvent in ligand-assisted protocols, but not as a co-solvent in the mixture with water [84].

Under ligand-free conditions, the dichloromethane/water system using CuSO_4 and sodium ascorbate dramatically increased reaction rates in comparison to other co-solvent systems, e.g. water/*tert*-butanol. As a result, reaction times could be typically reduced from ~24 hours in water/*tert*-butanol to 1 – 3 hours in dichloromethane/water with similar or even higher yields for various organic alkynes and azides (Scheme 8) [94]. This new solvent system represents an excellent alternative to the ligand-supported protocols without affecting purification procedures.

In solid-phase chemistry, the Cu(I)-catalysed alkyne-azide cycloadditions have become a powerful tool for coupling to or modifying surfaces. Particularly, in drug development and discovery it is an essential technique in immobilisation strategies for microarray and other biological applications [6]. In general, the concept of Cu(I)-catalysed triazole formation in solution can be directly transferred to solid-phase chemistry. On solid-phase, alkyne-azide coupling is usually performed in organic solvent systems, due to solubility problems which can arise with certain resins or polymeric matrices [31, 95-103].

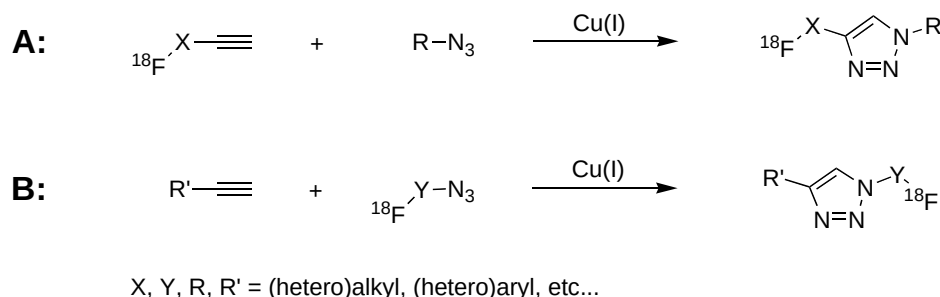
On the other hand, there are also a few examples using the reduction of Cu(II)SO_4 in aqueous systems [60, 104-107]. Hence, both strategies, directly added Cu(I) catalyst in organic solvents and *in-situ* generated Cu(I) catalyst in aqueous systems, are highly efficient in solid-phase applications. The optimal catalyst system may depend on the type of polymer or resin and the final application. However, solid-phase alkyne-azide cycloadditions show slight sensitivity to steric issues. Thus, sterically hindered azide functionalities on the resins can cause dramatically increased alkyne homo-

coupling accompanied by very low yields [31]. Nonetheless, various successful alkyne-azide couplings on both polar surfaces, such as polyethylene glycol acrylate (PEGA) (Scheme 9) [31], and non-polar polymers, such as polystyrene [98], have been performed, so far.

Furthermore, a broad range of biomolecules have been immobilised on solid-phase, including a fluorescent protein [103], oligonucleotides [102], carbohydrates [31, 102, 105] and peptides [95, 99, 105]. In some cases, solid-phase supported alkyne-azide cycloadditions have been applied for intramolecular triazole formations [95, 104, 108]. Only a few intramolecular reactions have been reported, including cyclodimerisation of surface-bound peptides [95], intramolecular triazole formation on specially modified DNA helices [108] and modification of copper surfaces towards adhesion properties [104].

In solution, intramolecular cycloadditions have been only reported, so far, for dimerisation of a trisaccharide to a cyclodextrin [109] and generation of cyclic dimers from small peptides [110]. Interestingly, the former succeeded using the uncommon toluene/DBU (CuI) system, while the latter used the common THF/DIPEA (CuI) system.

Cu(I)-catalysed alkyne-azide cycloadditions have also been employed widely in polymer sciences [10-12], where a broad range of complex dendrimers as well as polymers have been synthesised [68, 69, 78, 79, 92, 93, 111-119]. One fourth-generation dendrimer was obtained in over 90 % yield from the $\text{CuSO}_4/\text{Na ascorbate}$ system [68]. A variety of other polymers and dendrimers have been developed for nanoscale applications such as redox-active dendrimers for sensor purposes [119], and other polymer structures towards biologically active nanomaterials [78, 92, 93, 112, 117, 118].



Scheme 10. Possible variations of the Cu(I)-catalysed 1,2,3-triazole formation in fluorine-18 chemistry. Approach **A**: ^{18}F -Alkynes as prosthetic groups. Variant **B**: ^{18}F -Azides as prosthetic groups.

3. ^{18}F -LABELLING USING ALKYNE-AZIDE “CLICK” CYCLOADDITION

In general, the ^{18}F -fluorination procedures can be classified into two categories: direct and indirect methods [120]. The direct method introduces the fluorine-18 directly into the molecule of interest, usually by nucleophilic substitution; the conditions for these labelling procedures are typically quite harsh, i.e. basic and elevated temperatures. The indirect methods require the synthesis of a ^{18}F -labelled prosthetic group; this prosthetic group is then purified and conjugated to the molecule of interest. According to this, the Cu(I)-catalysed 1,3-dipolar cycloaddition of alkynes and azides certainly belongs to the indirect methods using ^{18}F -labelled prosthetic groups. ^{18}F -labelling via prosthetic groups is based on small molecules which are ^{18}F -labelled firstly and subsequently introduced into appropriate biomolecules [120-123]. A variety of prosthetic groups has been developed so far, whereas only limited methods for their introduction into biomolecules are available: acylation [124-133], alkylation [134, 135], amidation [136, 137], imidation [134], thiol-coupling [138], oxime-formation [139, 140] and photochemical conjugation [132, 141].

The constantly growing number of complex pharmaceuticals and biomolecules, especially multi-functional structures of high molecular weight, such as peptides, proteins, antibodies and oligonucleotides, pose a particular challenge to the prosthetic ^{18}F -labelling techniques. In commonly used procedures, complex biomolecules often require protection group chemistry or similar techniques to avoid undesired side-reactions of the target structure [121, 142, 143]. Moreover, many substances, such as peptides, are sensitive to many reaction conditions and there is a need for mild labelling methods. Alkynes and azides are orthogonal to most other functional groups and their Cu(I)-catalysed cycloadditions succeed under very mild conditions without any protection group chemistry. Hence, click chemistry is particularly suitable for prosthetic ^{18}F -labelling of peptides and other complex biomolecules. Furthermore, the resulting triazole structures have been proven to be very stable under physiological conditions [6-9]. One major concern for ^{18}F -labelling chemists due to the short 109.7 min half-life of fluorine-18, are the long reaction times, often several hours, which this cycloaddition generally needs until completion [30, 31]. Microwave-assistance can help here, the reaction can be dramatically accelerated using a microwave and products are obtained after 10-30 min in very high yields and purities [39, 68, 77, 78, 91]. In addition, the use of dichloromethane as a co-

solvent with water has been reported recently to increase reaction rates and to give products in excellent yields [94].

In fact of two coupling partners in the Cu(I)-catalysed triazole formation, the alkyne and the azide, there are consequently also two possible pathways to introduce the fluorine-18 into biomolecules (Scheme 10). One is based on ^{18}F -labelled azides which are subsequently coupled to alkyne-functionalised compounds. The other one, and more commonly used, is the coupling of ^{18}F -labelled alkynes to an azide-bearing structure.

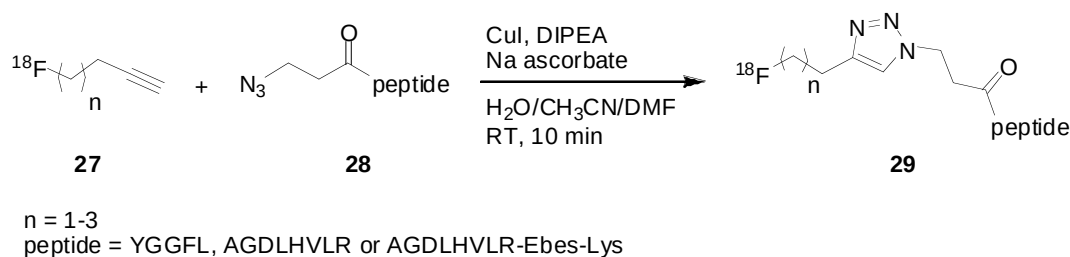
However, the Cu(I)-catalysed cycloaddition can be additionally useful for syntheses of precursors towards a direct ^{18}F -labelling, even though in such cases it is not longer part of the virtual ^{18}F -labelling step.

3.1. Click Chemistry with ^{18}F -Alkynes

The first paper about click chemistry in radiopharmaceutical sciences and also the first in fluorine-18 chemistry was by Jan Marik and Julie L. Sutcliffe in 2006 [13]. They investigated three different ^{18}F -labelled alkynes (27) and coupled them to azide bearing unprotected peptides, namely *N*-(3-azidopropionyl) peptides 28 (Scheme 11/Table 1).

The appropriate ^{18}F -alkynes (27), 4- ^{18}F fluoro-1-butyne ($n = 1$), 5- ^{18}F fluoro-1-pentyne ($n = 2$) and 6- ^{18}F fluoro-1-hexyne ($n = 3$) were labelled with n.c.a. ^{18}F fluoride using the corresponding *p*-toluenesulfonate ester precursors and the Kryptofix[®] 2.2.2/ K_2CO_3 system in acetonitrile. ^{18}F -alkynes were obtained after 10-15 min at 100 °C with radiochemical yields of 36 %, 81 % and 61 %, respectively. These ^{18}F -alkynes are volatile compounds with boiling points of 45 °C, 76 °C and 106 °C, boiling point increases as the chain length increases. They could be purified by co-distillation with acetonitrile while they were labelled. This neat purification led to remarkable purities of the ^{18}F -labelled alkynes equal or higher than 98 %.

In the click reaction of 5- ^{18}F fluoro-1-pentyne and the azido-containing pentapeptide ($\text{N}_3(\text{CH}_2)_2\text{CO-YGGFL}$), the CuSO_4 /sodium ascorbate system was tested for an *in-situ* catalyst generation initially. However only 10% radiochemical yield at room temperature after 30 min was observed. In contrast, the direct catalyst system of CuI in combination with excess of 10 equivalents nitrogen base yielded the ^{18}F -fluorinated peptide 29 within 10 min quantitatively. The 4- ^{18}F fluoro-1-butyne and 6- ^{18}F fluoro-1-hexyne gave moderate yields of 54 % and 62 %, respectively.



Scheme 11. ^{18}F -labelling of peptides via Cu(I)-catalysed 1,2,3-triazole formation. Different ω -[^{18}F]fluoro-alkynes (27) as prosthetic groups were coupled with the *N*-(3-azidopropionyl) peptides (28) to form ^{18}F -peptides (29) [13].

Table 1. Radiochemical Yields and Purities of the ^{18}F -labelled Peptides (29) Using *N*-(3-azidopropionyl) Peptides (28) and ω -[^{18}F]fluoro-alkynes (27). The Click Reaction was Performed for 10 min in Water/Acetonitrile/DMF Mixtures Using CuI, DIPEA and Sodium Ascorbate at Room Temperature (Data Taken from ref. [13])

<i>N</i> -(3-azidopropionyl) Peptides (28)	ω -[^{18}F]fluoro-alkynes (27)	Yield of 29 [%]	Purity of 29 [%]
$\text{N}_3(\text{CH}_2)_2\text{CO-YGGFL}$	$^{18}\text{F-CH}_2\text{-CH}_2\text{-C}\equiv\text{CH}$	54	95
$\text{N}_3(\text{CH}_2)_2\text{CO-YGGFL}$	$^{18}\text{F-(CH}_2)_2\text{-CH}_2\text{-C}\equiv\text{CH}$	97	98
$\text{N}_3(\text{CH}_2)_2\text{CO-YGGFL}$	$^{18}\text{F-(CH}_2)_3\text{-CH}_2\text{-C}\equiv\text{CH}$	62	99
$\text{AGDLHVLR-Ebes-Lys-OC(CH}_2)_2\text{N}_3$	$^{18}\text{F-(CH}_2)_2\text{-CH}_2\text{-C}\equiv\text{CH}$	97	81
$\text{N}_3(\text{CH}_2)_2\text{CO-AGDLHVLR}$	$^{18}\text{F-(CH}_2)_2\text{-CH}_2\text{-C}\equiv\text{CH}$	99	87

Table 2. Reaction Types and Steps, Synthesis Time and Radiochemical Yields of ^{18}F -labelling of A20FMDV2 Using Three Different Approaches of ^{18}F -prosthetic Group Labelling (Data Taken from ref. [144])

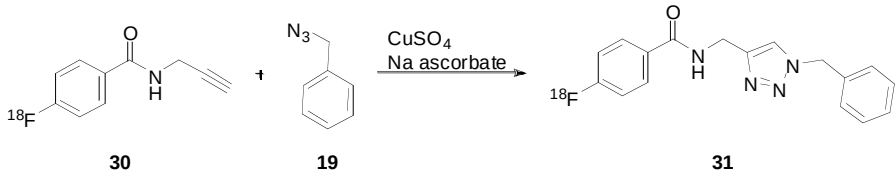
^{18}F -labelled Peptide A20FMDV2 (NAVPNLRGDLQVLAQKVART)	Reaction Type (Steps)	Synthesis Time	Yield [%]
	Solid phase (4)	137 min	7.8
	Solid phase (4)	171 min	4.6
	Click, in solution (2)	66 min	7.2

In the general procedure, 10 equivalents of sodium ascorbate were added to prevent oxidation of the Cu(I) species by atmospheric oxygen, even though this should not be necessary. In case of the nitrogen base, DIPEA was identified to be the best with respect to radiochemical yields and product purity, while piperidine provided shorter reaction times, but led to some un-identified side-products. Only 0.025 equivalents (in relation to CuI) of the azide-functionalised peptide were found to be efficient for quantitative incorporation of the ^{18}F -alkyne. The effect of bipyridine and phenanthroline based ligands on this reaction was checked as well and an improvement in radiochemical yields and product purity was reported, but further data was not provided. The final ^{18}F -labelled peptides were isolated by simple cartridge purification. In fact, organic auxiliaries,

such as ligands, would probably effect or even preclude the easy purification procedure using a cartridge system. In addition, the most promising alkyne 5-[^{18}F]fluoro-1-pentyne was applied for ^{18}F -labelling of another *N*-(3-azidopropionyl) peptide: $\text{N}_3(\text{CH}_2)_2\text{CO-AGDLHVLR}$. The ^{18}F -fluorinated peptide was obtained quantitatively, but with reduced purity of 87 % which even required additional equivalents of pyridine to restrain side-product formations.

In a following study by the same group, the ^{18}F -labelling of peptides using 5-[^{18}F]fluoro-1-pentyne (27, $n = 2$) as prosthetic group in combination with click chemistry was compared with two other prosthetic groups, 4-[^{18}F]fluorobenzoic acid ([^{18}F]FBA) [142] and 2-[^{18}F]fluoropropionic acid ([^{18}F]FPA) [143] for ^{18}F -labelling using a solid-phase based

Table 3. Optimisation Studies of the Cu(I)-catalysed Cycloaddition of Benzyl azide (19) and 4-[¹⁸F]fluoro-*N*-(prop-2-ynyl)benzamide (30) (Data Taken from ref. [145])

				
Concentration of 19 [mg/ml]	Solvent System (1:1)	Temperature [°C]	Reaction Time [min]	Radiochemical Yield [%]
3.5	<i>tert.</i> -BuOH/H ₂ O	25	10	7
3.5	<i>tert.</i> -BuOH/H ₂ O	40	10	60
3.5	<i>tert.</i> -BuOH/H ₂ O	40	20	80
7.0	<i>tert.</i> -BuOH/H ₂ O	40	20	60
140	<i>tert.</i> -BuOH/H ₂ O	40	20	50
120	CH ₃ CN/H ₂ O	40	20	95
80	CH ₃ CN/H ₂ O	40	20	70
40	CH ₃ CN/H ₂ O	40	20	10

method [144]. The target peptide A20FMDV2 is derived from the foot and mouth disease virus envelope protein which is responsible for interaction with the $\alpha v\beta 6$ receptor on the host cell.

A20FMDV2 was prosthetically ¹⁸F-labelled with [¹⁸F]FBA and [¹⁸F]FPA using a solid-phase approach. As shown in Table 2, the radiochemical yields are similarly low for the [¹⁸F]FBA-A20FMDV2 and the [¹⁸F]Click-A20FMDV2, while [¹⁸F]FPA-A20FMDV2 gave even lower yields. On the other hand, two reaction steps could be saved and the synthesis time could be dramatically reduced from more than 2h to 66 min by the click method. All three ¹⁸F-labelled peptides have been tested *in vivo*. However, slightly different pharmacokinetics for each peptide analogue were observed and indicate for obvious effects caused by little changes of the prosthetic group. In such cases, the choice of the suitable prosthetic group is essential and has to be proven individually for each target peptide.

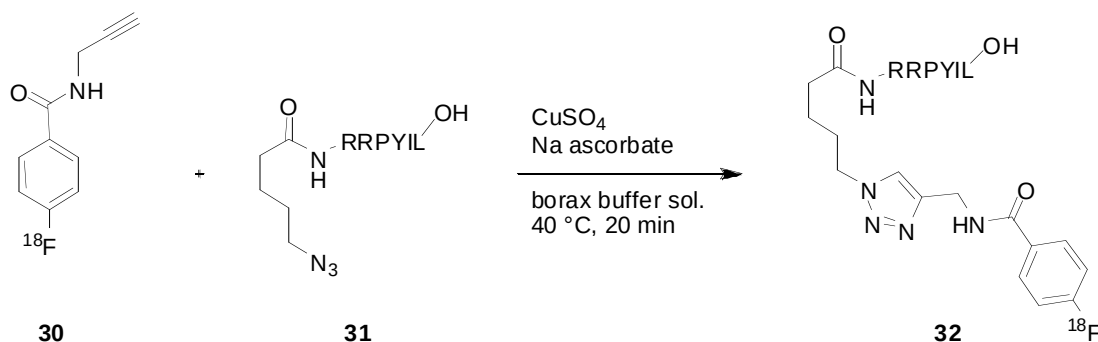
In conclusion, the first application of Cu(I)-catalysed alkyne-azide coupling in ¹⁸F-labelling of peptides confirms the potential and feasibility of this click reaction for fluorine-18 labelling purposes. Unsurprisingly, the reaction succeeded with excellent radiochemical yields and purity under very mild conditions, without any protection group chemistry needed. The click reaction further provided the desired ¹⁸F-labelled peptides in very short reaction times of only 30 min and enabled a rapid and simple product purification and isolation by cartridge extraction. Remarkably, the click reaction showed comparable efficiency and even faster completion under n.c.a. conditions than in traditional chemical protocols.

Another example in literature described the Cu(I)-catalysed click cycloaddition using the ¹⁸F-alkyne 30 in the direction of prosthetic ¹⁸F-labelling of a peptide [145]. In this approach the catalytically active Cu(I) species was provided by *in-situ* generation from the CuSO₄/sodium ascorbate sys-

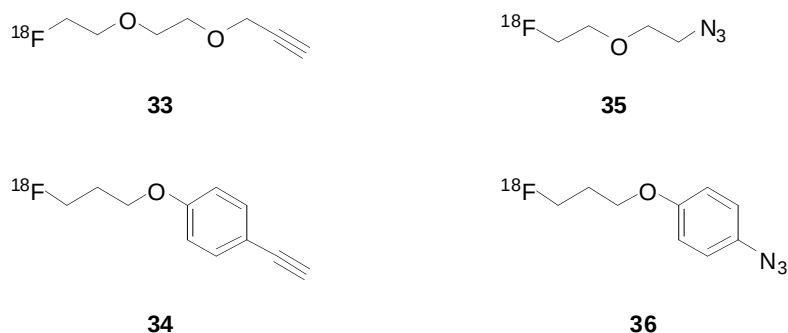
tem, the model peptide investigated was the hexapeptide neurotensin(8-13). In contrast to the approach of Marik *et al.*, a two step procedure for the ¹⁸F-labelled prosthetic group was developed. The *N*-succinimidyl-4-[¹⁸F]fluorobenzoate [¹⁸F]SFB, which is commonly used itself as a prosthetic group for ¹⁸F-labelling of peptides, proteins or antibodies, was synthesized according to literature starting from the triflate salt of *tert.*-butyl 4-*N,N,N*-trimethylammoniumbenzoate in a remotely controlled synthesis module [146]. [¹⁸F]SFB was then treated with excess of propargyl amine to form the final prosthetic ¹⁸F-alkyne 4-[¹⁸F]fluoro-*N*-(prop-2-ynyl)benzamide (30) within 5 min at room temperature. A subsequent cartridge purification furnished the 4-[¹⁸F]fluoro-*N*-(prop-2-ynyl)benzamide (30) in a radiochemical purity of > 90% and a radiochemical yield of 72% (related to [¹⁸F]SFB). In optimisation studies of the click reaction step, 4-[¹⁸F]fluoro-*N*-(prop-2-ynyl)benzamide (30) was coupled to benzyl azide (19) under various conditions (Table 3). In accordance with typical click chemistry, the water/*tert.*-butanol (1:1) and the acetonitrile/water (1:1) systems were used.

Unusual results were obtained by an increasing amount of the benzyl azide precursor in the water/*tert.*-butanol system. In the range of an azide concentration of 3.5 mg/ml to 140 mg/ml the radiochemical yield dropped from 80% to 50%. Solubility problems of the benzyl azide were assumed to cause the decrease in the radiochemical yields. This solubility problem was avoided with the acetonitrile/water system, in which high azide concentrations of 120 mg/ml provided radiochemical yields of 95%. Finally, the best system was found to be the acetonitrile/water system in combination with a relatively high concentration of the azide.

The hexapeptide neurotensin(8-13) (RRPYIL) was functionalized on the *N*-terminus with *N*-succinimidyl-5-azidovalerate to provide a suitable azide function. In the click coupling, a concentration of 120 mg/ml of the N₃(CH₂)₄CO-neurotensin(8-13) (31) would be not feasible



Scheme 12. ¹⁸F-labelling of N₃(CH₂)₄CO-neurotensin(8-13) (31) via Cu(I)-catalysed coupling with 4-[¹⁸F]fluoro-N-(prop-2-ynyl)benzamide (30) using the CuSO₄/sodium ascorbate catalyst system. The reaction was performed in pure borax buffer solution at 40 °C for 20 min [145].



Scheme 13. Different ¹⁸F-Prosthetic groups towards ¹⁸F-labelling via Cu(I)-catalysed cycloaddition of alkynes and azides [147].

and too costly. The peptide concentration was therefore minimised to 12.5 mg/ml according to the commonly used concentration range of peptides in fluorine-18 prosthetic group labelling via [¹⁸F]SFB. The solvent systems *tert*-butanol/borax buffer (1:1) and acetonitrile/borax buffer (1:1) revealed inappropriate for the click reaction of the N₃(CH₂)₄CO-neurotensin(8-13) and no product was detected. Surprisingly, the reaction succeeded in pure borax buffer at 40 °C and provided the desired ¹⁸F-labelled neurotensin(8-13), 1-*N*-neurotensin(8-13)-pentanamide-4-methyl-*p*-[¹⁸F]fluorobenzamide-1*H*-1,2,3-triazole (32), in radiochemical yields of 66% within 20 min (Scheme 12).

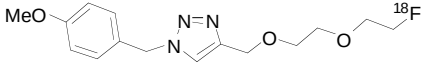
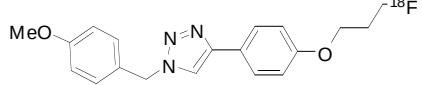
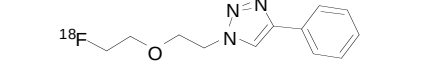
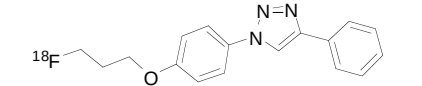
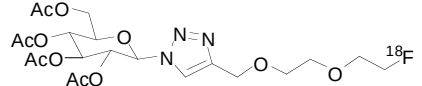
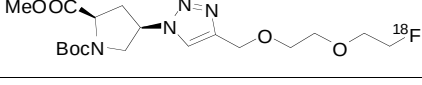
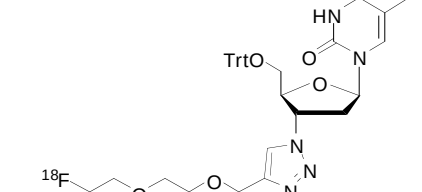
In vitro binding assays using the reference compound 1-*N*-neurotensin(8-13)-pentanamide-4-methyl-*p*-fluorobenzamide-1*H*-1,2,3-triazole showed reduced binding to the receptor system compared to the unmodified neurotensin(8-13). The binding of the modified peptide is still in nanomolar range (IC₅₀ = 66.0 nM), but more a 10-fold lower than neurotensin(8-13) (IC₅₀ = 0.4 nM). This second approach to ¹⁸F-labelled peptides using the Cu(I)-catalysed cycloaddition of ¹⁸F-alkynes and azide functionalised peptides also demonstrated both the feasibility in fluorine-18 labelling chemistry as well as the high efficiency of this reaction type under various reaction conditions. As a result, the N₃(CH₂)₄CO-neurotensin(8-13) was ¹⁸F-labelled via the click chemistry approach and further showed similar good results under different conditions to those of Marik *et al.* Remarkably, the optimised conditions of the test system, using small organic molecules which mimic the coupling entities of the peptide system, were non-transferable to the click reaction of the peptide structure. Major differences in solubility and hydro-

philicity of the substrates may play an important role here. Thus, more hydrophilic structures, such as peptides, may claim for pure aqueous buffer solutions, while small unpolar organic compounds tend to work better in co-solvent mixtures of organic solvents and water.

U. Sirion *et al.* demonstrated the feasibility of the Cu(I)-catalysed click cycloaddition using ¹⁸F-labelled alkynes and azides, with different classes of biomolecules [147]. In a non-radioactive preceding study using the Cu(I)-catalysed cycloaddition of phenyl acetylene and 4-methoxybenzyl azide the catalyst system and general reaction conditions were optimised. The catalyst system CuSO₄/sodium ascorbate gave higher, even quantitative yields, and faster rates compared to the direct addition of CuI as catalyst, from which only moderate yields could be obtained and the unusual 1,5-disubstituted 1,2,3-triazole was detected as a side product. As a major difference from general protocols using a direct Cu(I) catalyst system, the CuI was not combined with a nitrogen base, neither in stoichiometric amounts nor in excess. Generally, the use of excess nitrogen base accelerates the reaction and suppresses the formation of undesired by-products. In terms of solvents, the CuSO₄/sodium ascorbate catalyst-system worked best in DMSO/water mixtures.

However, water/*tert*-butanol systems were deployed preferably to enable a one-pot procedure subsequent to a direct ¹⁸F-labelling in *tert*-butanol, without any intermediary purification step or change of solvents. In a range from 2 mol% to 50 mol%, the catalyst concentration of 20 mol% was found to be optimal regarding efficiency and catalyst loading. Interestingly, CuSO₄ and sodium ascorbate were

Table 4. Radiochemical Yields of Different ^{18}F -labelled Compounds Using ^{18}F -alkynes and ^{18}F -azides as Prosthetic Groups in Cu(I)-Catalysed Cycloadditions (Data Taken from ref. [147])

^{18}F -prosthetic Group	^{18}F -labelled 1,2,3-triazole Product	Synthesis Time	Yield [%] ^a
33		30 min	100
34		30 min	72
35		30 min	71
36		30 min	73
33		10 min	100 (100)
33		10 min	100 (100)
33		10 min	97 (100)

^a Radiochemical yields in parentheses are from the corresponding unprotected molecules.

used in 2:3 molar ratio, in contrast to the commonly used huge excess of sodium ascorbate of up to 10-fold.

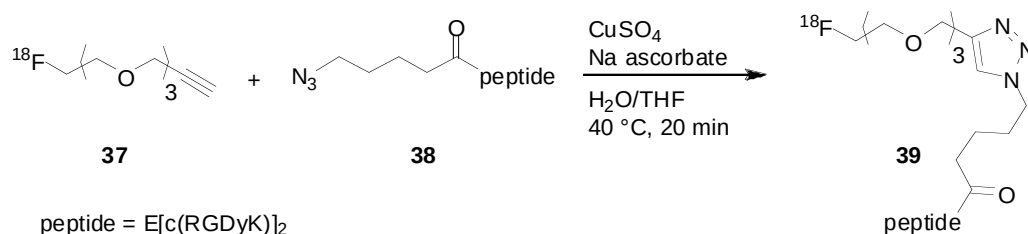
Four different mesylate precursors were designed; two for click chemistry using ^{18}F -alkynes and two for click chemistry using ^{18}F -azides. The mesylates were ^{18}F -labelled using n.c.a. [^{18}F]fluoride and tetrabutylammonium bicarbonate in *tert*-butanol at 100 °C for 20 min. The desired ^{18}F -alkynes (33, 34) and ^{18}F -azides (35, 36) were obtained in radiochemical yields of 85-95% (Scheme 13). In a subsequent one-pot click reaction ^{18}F -alkyne 33 was coupled to suitable derivatives of glucose, proline and thymidine in both protected forms and unprotected forms. In addition to polar biomolecules, small non-polar organic compounds, phenyl acetylene and 4-methoxybenzyl azide, were ^{18}F -labelled via click coupling with the appropriate ^{18}F -labelled prosthetic groups (Table 4). The click reaction of the non-polar organic compounds afforded the desired products in radiochemical yields of 71 – 100% within 30 min; ^{18}F -azides 35 and 36 gave lower yields of 71 – 73%.

On the contrary, the small polar biomolecules showed quantitative incorporation of the ^{18}F -alkyne 33 within 5 –

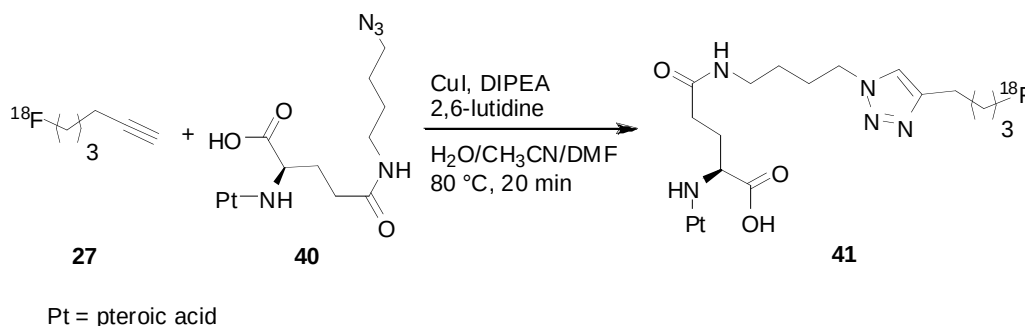
10 min. The longer reaction times and the lower conversions in the click step of the non-polar organic molecules are assumed to be due to their low solubility in the polar solvent systems.

Comparing both approaches, starting either from the ^{18}F -alkynes or the ^{18}F -azides, the differences are not significant. It should be noted that the target 1,2,3-triazoles are not the same and thus are not directly comparable. In terms of protection group chemistry, no differences were observed between the corresponding protected and unprotected forms. In addition to peptides, the Cu(I)-catalysed cycloaddition has been successfully applied to the ^{18}F -labelling of glucose, *L*-proline and thymidine derivatives. Above all, protection group chemistry was proven again unnecessary in the click reaction, without a loss in efficiency, product purity or selectivity. Low solubility of reactants accompanied by reduced radiochemical yields indicate a need for completely dissolved compounds under n.c.a. conditions.

In another report the ^{18}F -labelled PEG-alkyne 37 was used to label an azide-functionalised dimeric cyclic-RGD (Arg-Gly-Asp) peptide (38) as PET tracer for imaging in-



Scheme 14. Cu(I)-catalysed coupling of the ^{18}F -labelled PEG-alkyne **37** and the azide functionalised peptide $\text{E}[\text{c}(\text{RGDyK})]_2$ (**38**) using the CuSO_4 /sodium ascorbate catalyst system. The reaction was performed in a mixture of THF and water at 40°C for 20 min to yield the ^{18}F -fluoro-PEG-triazole- $\text{E}(\text{RGDyK})_2$ (**39**, ^{18}F -FPTA-RGD2) in RCY of 60 - 80 % [148].



Scheme 15. Click ^{18}F -labelling of a folic acid derivative. The cycloaddition of 6- ^{18}F -fluoro-1-hexyne (**27**) and N^5 -(4-azidobutyl)folate (**40**) is catalysed by the direct CuI system in combination with bases. The reaction was performed in a mixture of acetonitrile, water and DMF at 80°C for 20 min to yield the ^{18}F -click-folate **41** in RCY of 65 - 80 % [152].

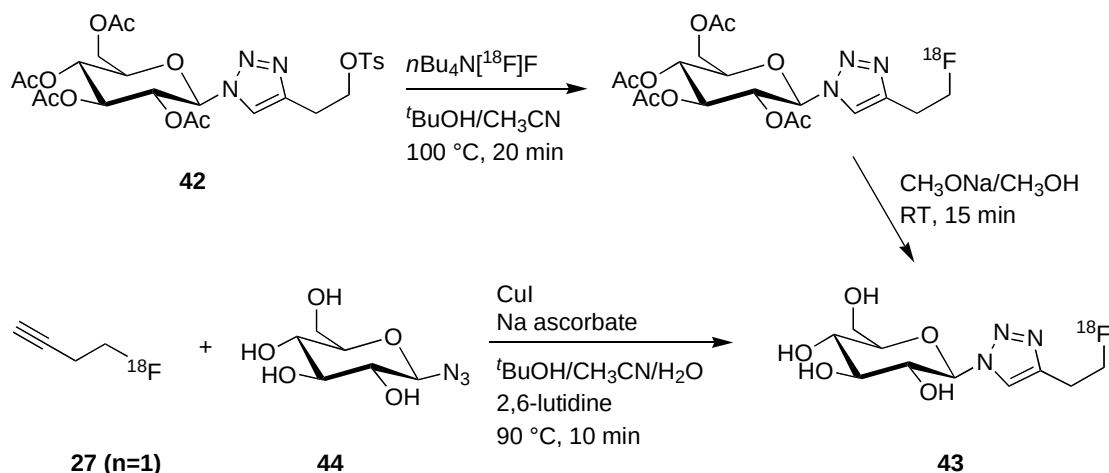
tegrin $\alpha_v\beta_3$ positive tumours (Scheme 14) [148]. The ^{18}F -labelled PEG-alkyne **37** was achieved in radiochemical yields of 76 - 80 % from the corresponding *p*-toluenesulfonate ester precursors. Compound **37** was labelled with n.c.a. ^{18}F -fluoride in the Kryptofix[®] 2.2.2/ K_2CO_3 system in DMSO at 110°C for 15 min. Subsequently, a semi-preparative HPLC purification was necessary to remove residual precursor and improve the efficiency of the triazole formation step. The Cu(I)-catalysed alkyne-azide coupling was carried out in a THF/water mixture and the CuSO_4 /sodium ascorbate system. Similar to U. Sirion *et al.* [147], sodium ascorbate was used in a much smaller excess of 3 mol% related to CuSO_4 instead of the commonly used excess of 10-fold. However, the click chemistry succeeded under mild conditions of 40°C within 20 min and the ^{18}F -fluoro-PEG-triazole- $\text{E}(\text{RGDyK})_2$ (**39**, ^{18}F -FPTA-RGD2) was obtained in good radiochemical yields of 60 - 80 %. The ^{18}F -FPTA-RGD2 was purified by semi-preparative HPLC with an overall radiochemical yield of 54 % and high radiochemical purity of $\geq 97\%$ after 110 min.

^{18}F -FPTA-RGD2 was employed in *in vitro* and *in vivo* studies using mice bearing integrin-positive xenografts. In comparison with known non-click ^{18}F -labelled cyclic RGD peptides [149-151], the new ^{18}F -FPTA-RGD2 has only slightly lower affinity to the receptor, but it is still in a nanomolar range (144 nM). In *in vivo* studies, compound **39** showed a high and specific tumour uptake and an adequate metabolic stability with predominantly renal clearance. Integrin-positive tumours were well visualised and an acceptable tumour-to-background contrast was already observed after 10 - 20 min p.i.. In comparison with the other ^{18}F -labelled cyclic RGD peptides, *in vivo* results of **39** were equally good. In this study, the alkyne-azide cycloaddition was successfully applied to the ^{18}F -labelling of cyclic RGD

peptides. Beside the favourable *in vivo* performance of **39**, major advantages of ^{18}F -FPTA-RGD2 are based on click chemistry such as short reaction time, high radiochemical yields and product purity.

Based on the approach of J. Marik *et al.* [13], the ^{18}F -alkyne **27** with $n = 3$, 6- ^{18}F -fluoro-1-hexyne, was used for the preparation of a ^{18}F -labelled folic acid derivative as PET imaging agent for folate receptor-positive tumours (Scheme 15) [152]. According to the described procedure, 6- ^{18}F -fluoro-1-hexyne was labelled with n.c.a. ^{18}F -fluoride and purified by co-distillation with acetonitrile to give the ^{18}F -labelled alkyne in radiochemical yields of 60 - 65 % and a radiochemical purity of $\geq 97\%$ after 10 min. 6- ^{18}F -fluoro-1-hexyne was then coupled to the N^5 -(4-azidobutyl)folate (**40**) in acetonitrile, water and DMF using CuI in combination with a 1:2 mixture of DIPEA and 2,6-lutidine as direct catalyst source (Scheme 15). The alkyne-azide coupling was performed at 80°C and gave radiochemical yields of 65 - 80 % within 20 min. In contrast to J. Marik *et al.*, the triazole formation succeeded without addition of sodium ascorbate. The isolated ^{18}F -click-folate **41** was obtained in radiochemical yields of 25-35 %, a specific activity of $160 \pm 70 \text{ GBq}/\mu\text{mol}$ and a radiochemical purity of $\geq 97\%$ after ≤ 90 min total synthesis time.

In *in vivo* PET studies and biodistribution studies using xenografts mice bearing folate receptor-positive KB tumours (human nasopharyngeal carcinoma), compound **41** showed a high specific uptake in the KB tumours of 3.13%ID/g. However, the PET imaging revealed limited tumour-to-background contrast caused by pronounced hepatobiliary elimination with a high accumulation of radioactivity in the abdominal region. Although the *in vivo* pharmacokinetics of the ^{18}F -click-folate show limitations, the click cycloaddition



Scheme 16. ^{18}F -labelling of a glucose derivative (43) using two click-based methods: The Cu(I)-catalysed coupling of 4-([^{18}F]fluoro-1-butyne (27, $n = 1$) and the glucopyranosyl azide 44; and a direct ^{18}F -labelling of the corresponding tosyl precursor (42) obtained from click chemistry [153].

of ^{18}F -alkynes and the N^5 -(4-azidobutyl)folate 40 provides a fast and very efficient method for ^{18}F -labelled folates.

The preparation of 4-(2-[^{18}F]fluoroethyl)-1-(β -D-glucopyranosyl)-1H-1,2,3-triazole (43) via two different click approaches (Scheme 16) was described by Kim *et al.* [153]. In one approach, the precursor 4-(2-toluenesulfonyloxyethyl)-1-(2,3,4,6-tetraacetyl-D-glucopyranosyl)-1H-1,2,3-triazole (42) was prepared via click chemistry using CuSO_4 /sodium ascorbate. Subsequently, this precursor was ^{18}F -labelled by the use of n.c.a. [^{18}F]fluoride and tetrabutylammonium bicarbonate in *tert.*-butanol/acetonitrile at 100°C for 20 min. After cartridge purification, a deprotection of the acetyl groups was accomplished with $\text{CH}_3\text{ONa}/\text{CH}_3\text{OH}$ at room temperature for 15 min, the desired product, 4-(2-[^{18}F]fluoroethyl)-1-(β -D-glucopyranosyl)-1H-1,2,3-triazole (43), was obtained in a radiochemical yield of 30% after a total synthesis time of 100 min. In the second approach, 4-[^{18}F]fluoro-1-butyne (27, $n = 1$) [13] was obtained from the corresponding tosyl precursor by the means of n.c.a. [^{18}F]fluoride and tetrabutylammonium bicarbonate in *tert.*-butanol/acetonitrile at 100°C for 25 min. For the click reaction step, 4-[^{18}F]fluoro-1-butyne (27, $n = 1$) was co-distilled with acetonitrile into a reaction vessel and then coupled with the acetyl-protected 1-azidoglucose 44 in the presence of CuI, 2,6-lutidine, sodium ascorbate in a *tert.*-butanol/acetonitrile/water mixture at 90°C for 10 min. The product 43 was obtained in a radiochemical yield of 21% after a total synthesis time of 80 min.

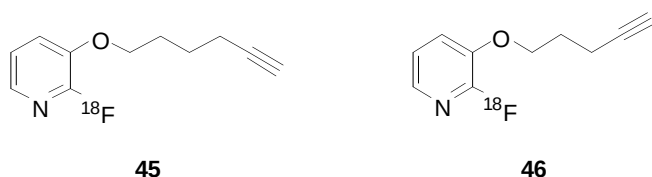
The 4-(2-[^{18}F]fluoroethyl)-1-(β -D-glucopyranosyl)-1H-1,2,3-triazole (43) was evaluated in an enzymatic phosphorylation assay with hexokinase. In comparison to the 2-[^{18}F]fluorodeoxy-glucose [^{18}F]FDG, which was completely converted to [^{18}F]FDG-6-phosphate by the hexokinase, 4-(2-[^{18}F]fluoroethyl)-1-(β -D-glucopyranosyl)-1H-1,2,3-triazole (43) was not phosphorylated in this assay. Furthermore, in cell uptake assays with SNU-C5 cells (overexpressing the Glut-1 transporter), compound 43 showed no significant uptake while for [^{18}F]FDG a high and specific uptake was reported.

Both approaches differ in their final radiochemical yields with the direct method based on the tosyl precursor 42 giving better results with a 20 min longer synthesis time. Pharmacological *in vitro* tests revealed that the modifications on the C1 position in 4-(2-[^{18}F]fluoroethyl)-1-(β -D-glucopyranosyl)-1H-1,2,3-triazole (43) is neither tolerated by the hexokinase nor by the Glut-1 transporter. The example shows how the 1,4-disubstituted 1,2,3-triazole linker or structural changes in general can dramatically change the pharmacological properties of a compound. This might be mostly the case for smaller biomolecules, but it can also become crucial for any other structure.

The same group expanded the indirect click- ^{18}F -labelling approach to the fatty acid 17-[4-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-1-yl]-6-thia-heptadecanoic acid for the PET imaging of the myocardial fatty acid metabolism [154]. The ^{18}F -alkyne 4-[^{18}F]fluoro-1-butyne (27, $n = 1$) was prepared and isolated similarly to the previously described procedures [13, 153]. Compound 27 ($n = 1$) was reacted with 17-azido-6-thia-heptadecanoic acid in a mixture of acetonitrile and water containing the catalyst source CuI, *N,N*-diisopropylethylamine and sodium ascorbate. After 10 min at 90°C , the 17-[4-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-1-yl]-6-thia-heptadecanoic acid was purified by HPLC with radiochemical yields of 20 - 26%, a very high radiochemical purity of $\geq 99\%$ and a specific activity of $55.4 \pm 4.2\text{ GBq}/\mu\text{mol}$. The total synthesis time is 80 - 85 min.

In vivo PET imaging of 17-[4-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-1-yl]-6-thia-heptadecanoic acid using normal mice showed high accumulation of radioactivity in the heart with prolonged myocardial retention. The corresponding metabolite studies of the heart tissue indicate a metabolic trapping of the ^{18}F -labelled fatty acid by β -oxidation. Based on this, the authors suggest that 17-[4-(2-[^{18}F]fluoroethyl)-1H-1,2,3-triazol-1-yl]-6-thia-heptadecanoic acid has potential use in PET imaging for the visualisation of the myocardial fatty acid metabolism.

In order to develop a prosthetic group for the ^{18}F -labelling of macromolecules such as peptides, two ^{18}F -labelled alkynes have been developed on the base of ^{18}F -labelled pyri-



Scheme 17. Pyridine-based ^{18}F -labelled alkynes (^{18}F FPy5yne (45) and ^{18}F FPyKYNE (46)) for the prosthetic group labelling of macromolecules and peptides via Cu(I)-catalysed click coupling with azide functionalities on biomolecules [155, 156].

dine derivatives (Scheme 17). Both compounds, 2- ^{18}F fluoro-3-(hex-5-ynoxy)pyridine, ^{18}F FPy5yne (45) [155] and 2- ^{18}F fluoro-3-(pent-4-ynoxy)pyridine, ^{18}F FPyKYNE (46) [156], contain an heteroaromatic ^{18}F -group on the C2 position which usually provides very high *in vivo* stability of the radiolabel. In the ^{18}F -labelling, the corresponding trimethylammonium triflate precursors gave the best results in comparison to their nitro- (45 and 46) and bromo- (only 46) analogues. The ^{18}F -introduction was performed in the Kryptofix[®] 2.2.2/ K_2CO_3 system and found to be optimal in acetonitrile for 45 and in DMSO for 46. After 15 min at 110 °C, ^{18}F FPy5yne (45) was obtained in a radiochemical yield of 34% after HPLC purification. The ^{18}F -labelling of ^{18}F FPyKYNE (46) was performed at 165 °C for 3 - 5 min and 46 was obtained in radiochemical yields of 30 - 35% after 60 - 70 min synthesis time.

2- ^{18}F fluoro-3-(hex-5-ynoxy)pyridine (45) has been used for the ^{18}F -labelling of an azide-functionalised model peptide $\text{N}_3\text{-(CH}_2)_4\text{-CO-YKRI-OH}$ (BG142) [155]. The click coupling step was utilised by the means of tetrakis (acetonitrilo)copper(I) hexafluorophosphate and *tris*-(benzyltriazolylmethyl)amine (TBTA) (20) as catalyst source, DIPEA, ^{18}F FPy5yne (45) and BG142 in a 20mM phosphate buffer/DMF/acetonitrile mixture. The click reaction was performed under mild conditions at 37 °C for 10 min and furnished the ^{18}F -labelled peptide ^{18}F BG142 in a radiochemical yield of 18.7% (end-of-bombardment) after HPLC purification and formulation.

Both compounds 45 and 46 can be obtained in good yields within a reasonable radiosynthesis time for ^{18}F -labelling via prosthetic groups. Furthermore, ^{18}F FPy5yne (45) has demonstrated its applicability for the ^{18}F -labelling of azide-modified peptides via Cu(I)-catalysed click cycloaddition.

Beside 4- ^{18}F fluoro-*N*-(prop-2-ynyl)benzamide (30) [145], compound 45 and 46, there is only one further report (abstract) of a ^{18}F -alkyne bearing an aromatically bound fluorine-18, propargyl-4- ^{18}F fluorobenzoate (^{18}F PFB) [157]. Propargyl-4- ^{18}F fluorobenzoate was prepared from its corresponding trimethylammonium triflate precursor in the Kryptofix[®] 2.2.2/ K_2CO_3 system (130 °C, 15 min) in acetonitrile. In subsequent test reactions, ^{18}F PFB was coupled via click reactions to four different model compounds including an azide-functionalised nonapeptide (YQSIYVPDI) which is a potential substrate of transglutaminase, a tissue stabilizing enzyme. The click reactions were performed in presence of copper sulphate and sodium ascorbate in acetonitrile and *tert*.-butanol or DMSO as a co-solvent. All click

reactions gave good to excellent radiochemical yields with the one of the nonapeptide of $37 \pm 31\%$.

Such ^{18}F -alkynes with an aromatic fluorine-18 moiety can be expected to show an excellent *in vivo* stability of their radiolabels. However, for all these compounds this assumption needs to be verified by appropriate *in vivo* studies.

For the *in vivo* targeting of magnetic nanoparticles, a ^{18}F -labelled nanoparticle was developed by the use of click cycloaddition [158]. The precursor molecule 2-(2-(2-(prop-2-ynyloxy)ethoxy)ethoxy)ethyl tosylate was ^{18}F -labelled in DMSO using the Kryptofix[®] 2.2.2/ K_2CO_3 system for 15 min at 110 °C to give 3-(2-(2-(2- ^{18}F fluoroethoxy)ethoxy)ethoxy)prop-1-yne (^{18}F -PEG₃) in radiochemical yields of 57% after HPLC purification. ^{18}F -PEG₃ was conjugated with the azide-functionalised nanoparticles using a CuSO_4 / bathophenanthrolinedisulfonic acid disodium salt (BPDS) catalyst system in a phosphate buffer/water/DMSO mixture. The ^{18}F -labelled nanoparticles could be easily filtered off from the reaction mixture and were obtained in a radiochemical yield of 58%, a radiochemical purity of > 99% (after one washing step). The ^{18}F -labelled nanoparticles were injected into BALB/C mice and the PET imaging showed a very high signal to noise ratio. The sensitivity of PET allows here the *in vivo* targeting of nanoparticles at 2-4 orders of magnitude lower doses than those required for MRI.

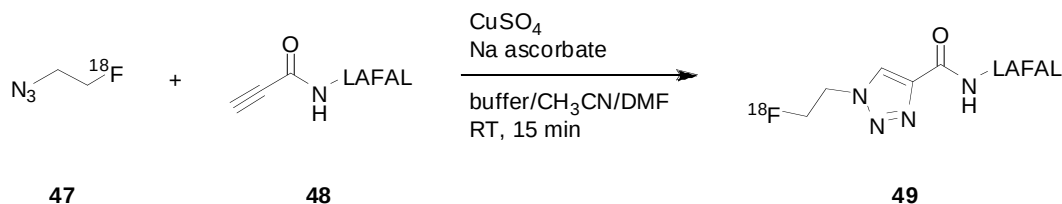
Overall, these all reports about the use of ^{18}F -alkynes and the Cu(I)-catalysed cycloaddition towards ^{18}F -labelled bioconjugates clearly show the high efficiency and the excellent adaptability of this reaction type to fluorine-18 chemistry. All reactions succeeded under various conditions with high and even quantitative yields in very short reaction times. In addition, the very mild reaction conditions are particular suitable for fluorine-18 bioconjugation purposes. Even for complex biomolecules, protection group chemistry could be generally avoided, while high yields, fast reaction rates and high chemoselectivity remained unchanged. However, under n.c.a. conditions good solubility of the reactants seem to be essential for high radiochemical yields, while common reaction conditions of the traditional protocols can usually be directly transferred to fluorine-18 protocols. The wide scope of the Cu(I)-catalysed click cycloaddition using ^{18}F -alkynes as new prosthetic groups for fluorine-18 was confirmed by the successful ^{18}F -fluorination of various biomolecules from different classes and was even found suitable for ^{18}F -labelled nanoparticles.

3.2. Click Chemistry with ^{18}F -Azides

As mentioned above, the Cu(I)-catalysed coupling of alkynes and azides allows two different directions for ^{18}F -labelling via prosthetic groups. Beside the coupling of ^{18}F -alkynes to azide-functionalised molecules, ^{18}F -azides offer another possibility for introducing fluorine-18 to (bio)molecules (Scheme 10). Some successful examples are given by the report of U. Sirion *et al.*, which is described previously in the subchapter [147]. In a comparison of both approaches, the ^{18}F -azides gave results comparable to these of the ^{18}F -alkynes (Table 4). However, only two Cu(I)-catalysed couplings with ^{18}F -azides and phenyl acetylene were performed. Both reactions were not optimised as they were preliminary test reactions. Nonetheless, the ^{18}F -labelled

Table 5. Radiochemical Yields at Various Conditions of Different ^{18}F -labelled Compounds Using 2- ^{18}F fluoroethylazide (42) as Prosthetic Group in Cu(I)-catalysed Cycloadditions with Various Alkynes (Data Taken from ref. [159])

$ \begin{array}{c} ^{18}\text{F}-\text{CH}_2\text{CH}_2\text{N}_3 + \text{H}-\text{C}\equiv\text{C}-\text{R} \xrightarrow[\text{CH}_3\text{CN/buffer/DMF, RT/80 }^\circ\text{C; 15 min}]{\text{"Cu(I)"}} ^{18}\text{F}-\text{CH}_2\text{CH}_2-\text{N}=\text{N}-\text{C}(\text{R})=\text{N} \\ \text{47} \end{array} $					
entry	-R	Radiochemical Yield [%]			
		CuSO ₄ /Na Ascorbate		Cu(0) Powder	
		RT	80 °C	RT	80 °C
1	-phenyl	7	84	10	56
2	-COOH	15	61	0	29
3	-C ₆ H ₄ -COOH	40	93	6	15
4	-C ₆ H ₄ -NH ₂	12	>98	33	96
5	-CO-NH-benzyl	>98	>98	70	>98
6	-CH ₂ -CH ₂ -CO-NH-benzyl	9	98	26	76
7	-CH ₂ -CH(NHAc)-CO-OEt	31	>98	90	97
8	-CH ₂ -NH-CO-phenyl	34	>98	51	92

**Scheme 18.** ^{18}F -labelling of alkyne-functionalised pentapeptide LAFAL (48) via Cu(I)-catalysed coupling with 2- ^{18}F fluoroethylazide (47) using the CuSO₄/sodium ascorbate in a buffer/acetonitrile/DMF mixture. The reaction was performed at room temperature for 15 min [159].

1,2,3-triazole products were obtained in good radiochemical yields of 71 - 73%, but relatively long reaction times of 30 min were needed.

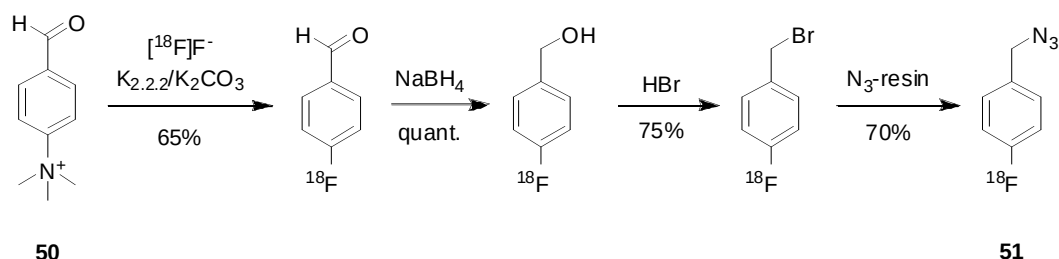
The feasibility of ^{18}F -azides as prosthetic groups for ^{18}F -labelling of biomolecules via the Cu(I)-catalysed cycloaddition was first demonstrated by M. Glaser and E. Årstad [159]. They successfully coupled 2- ^{18}F fluoroethylazide (47) to an alkyne-functionalised pentapeptide (Scheme 18).

2- ^{18}F fluoroethylazide (47) was prepared from the corresponding tosylate precursor. This precursor was ^{18}F -labelled in the Kryptofix[®] 2.2.2/K₂CO₃ system in acetonitrile at 80 °C within 15 min. The product 47 was purified by distillation at 130 °C and obtained in a radiochemical yield of 55% (isolated). 2- ^{18}F fluoroethylazide (47) was utilized for the Cu(I)-catalysed coupling to various small alkynes (Table 5). For the Cu(I) source two different methods were tested and compared, the CuSO₄/sodium ascorbate system and sole Cu(0) powder. The experiments were carried out for 15 min at room temperature and subsequently for additional 15 min at 80 °C. All were performed in a solvent mixture of sodium phosphate buffer (pH 6), acetonitrile and DMF. As outlined in Table 5, the radiochemical yields vary from 60% - 98% for the CuSO₄/sodium ascorbate system and from 15% - 98% for the Cu(0) powder system. For consistency, the

CuSO₄/sodium ascorbate system gave better results over a broader range. It is worth noting that except for *N*-benzylpropynamide all reactions provided much higher radiochemical yields, up to 10-times, with additional heating. The best conjugations for both catalytic systems were observed in entry 4 - 8, however, the alkynes of these entries contain an amide or an amine moiety which may contribute like ligands or nitrogen bases and enhance the catalysis.

The pentapeptide LAFAL (48) was modified with propargylic acid at the C-terminal was chosen as model system. 2- ^{18}F fluoroethylazide (47) was then coupled to the unprotected alkyne-functionalised peptide in the CuSO₄/sodium ascorbate system at room temperature within 15 min (Scheme 18). The ^{18}F -labelled peptide 44 was isolated in radiochemical yields of 92% and with a radiochemical purity of > 99%.

2- ^{18}F fluoroethylazide (47) was further employed for the ^{18}F -labelling of an alkyne-functionalised isatin 5-sulfomamide which exhibits subnanomolar affinity for capase 3, a key enzyme in the signalling pathway of apoptosis [160]. The ^{18}F -azide 47 was synthesised and purified as previously described [159]. For the Cu(I)-catalysed click reaction, again two different catalyst systems were tested, the CuSO₄/sodium ascorbate system and Cu(0) powder. No ma-



Scheme 19. Radiosynthesis of 1-(azidomethyl)-4-[^{18}F]fluorobenzene (51) as prosthetic group for the ^{18}F -labelling of alkyne-functionalised peptides. The reaction was performed over four steps (74 min) with radiochemical yields of 51 of 34% [163].

jor differences were found between both systems, but the CuSO_4 /sodium ascorbate system was chosen. A more crucial parameter was the solvent composition where the addition of sodium phosphate buffer significantly enhanced the radiochemical yields. Due to a thermal instability of the ^{18}F -labelled isatin 4-sulfonamide, only room temperature was practicable. With the optimal conditions of room temperature and a solvent mixture of phosphate buffer, DMF and acetonitrile, the click reaction step yielded the ^{18}F -labelled isatin 4-sulfonamide within 30 min in a radiochemical yield of $65 \pm 6\%$ and a radiochemical purity of $>99\%$ after HPLC purification.

In vivo studies of the ^{18}F -labelled isatin 4-sulfonamide in RIF-1 tumour-bearing mice showed a high metabolic stability of the tracer and a favourable pharmacological profile for a novel candidate for the PET imaging of apoptosis.

The 2-[^{18}F]Fluoroethylazide (47) has also been utilised by other groups for the effective ^{18}F -labelling of an EdU (5-ethynyl-2'-deoxyuridine) derivative for the direct measurement of active DNA synthesis [161] and the ^{18}F -labelling of the amino acid 2-amino-3-[1-(2-[^{18}F]fluoroethyl)-1H-[1,2,3]triazol-4-yl]propanoic acid ([^{18}F]AFETP) for tumour imaging [162]. In both syntheses, the click reaction steps were performed under mild conditions and showed very high efficiency with radiochemical yields of 55% and 70%, respectively. Biodistribution studies of [^{18}F]AFETP in nude mice showed promising result with the expected profile of a radiolabelled amino acid.

Beyond 2-[^{18}F]Fluoroethylazide (47), only one further ^{18}F -azide, 1-(azidomethyl)-4-[^{18}F]fluorobenzene (51), has been developed as prosthetic group for the Cu(I)-catalysed cycloaddition with alkyne-modified (bio)molecules (Scheme 19) [163].

The preparation of the ^{18}F -labelled azide 51 is a four-step radiosynthesis starting from the precursor 4-formyl-*N,N,N*-trimethylanilinium triflate (50) which is generally used for the preparation of 4-[^{18}F]fluorobenzaldehyde, a prosthetic group for peptide labelling via oxime formation [139]. The ^{18}F -labelling was accomplished in the Kryptofix[®] 2.2.2/ K_2CO_3 system in DMSO at 130°C within 5 min and furnished the intermediate 4-[^{18}F]fluorobenzaldehyde in a radiochemical yield of 65%. The intermediate was trapped on a C_{18} -cartridge and treated with sodium borohydride solution for reduction, subsequently with hydrogen bromide solution for bromination and after neutralisation, the 4-[^{18}F]fluorobenzyl bromide was eluted by means of DMF. This intermediate was transferred into its azide by a azide

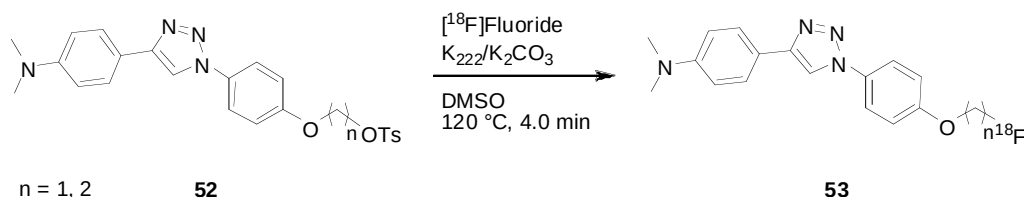
exchange resin to give the prosthetic group 1-(azidomethyl)-4-[^{18}F]fluorobenzene (51). The four step procedure took 75 min and gave a radiochemical yield of 51 of 34%. The click reaction of compound 51 was tested in a model system using 4-ethynyl-phenylalanine as alkyne component. The optimal conditions were found to be the direct CuI catalyst system in DMF with addition of sodium ascorbate and DIPEA. The reaction completed within 15 min at room temperature. The conditions were directly transferable to the click reaction of 51 with an alkynylated neuropeptide. The ^{18}F -labelled neuropeptide was obtained with a high radiochemical yield of 90% and a radiochemical purity of $>90\%$ after HPLC purification.

In several Cu(I)-catalysed cycloadditions of ^{18}F -azides, especially 2-[^{18}F]Fluoroethylazide (47), and terminal alkynes it has been demonstrated that this approach is equally efficient to that using ^{18}F -alkynes and azides. Generally, the ^{18}F -labelled 1,2,3-triazole products were obtained in excellent radiochemical yields and with very high purities under mild reaction conditions. The efficient incorporation of an ^{18}F -azide into an unprotected alkynylated peptide proves the applicability for this method for the ^{18}F -labelling of biomolecules. Again, protection group chemistry could be avoided and confirmed the orthogonality of this reaction and its reactants to other functionalities. ^{18}F -Azides were coupled to alkyne-functionalised (bio)molecules in similar short reaction times as those couplings with ^{18}F -alkynes and azide-functionalised (bio)molecules. Consequently, both methods are feasible under n.c.a. conditions and suitably fast for fluorine-18 chemistry. The use of ^{18}F -azides as prosthetic group additionally emphasises the scope as well as the flexibility of this approach.

3.3. ^{18}F -Labelling Precursors via Alkyne-Azide Cycloaddition

The synthesis of precursors towards ^{18}F -labelling using the Cu(I)-catalysed cycloaddition of alkynes and azides is in fact an additional “cold” synthetic method. Based on the high efficiency, the wide scope and the high selectivity of this reaction type, it is generally very attractive for the use in organic synthesis and thus also for the preparation of precursors for fluorine-18 chemistry. By this approach, some precursors for the direct ^{18}F -labelling have been synthesised and successfully employed for the ^{18}F -labelling.

The class of 1,4-diphenyltriazoles represents new specific binders to β -amyloid aggregates, which are a concomitant factor of the pathology of Alzheimer's disease [164]. These 1,4-diphenyltriazoles are conveniently obtainable from the



Scheme 20. Direct ^{18}F -labelling of the *p*-toluenesulfonate ester precursors 52 using the Kryptofix[®] 2.2.2/ K_2CO_3 system in DMSO [164].

click coupling of the suitable phenyl acetylene and phenyl azide (Scheme 20). Various functionalities as well as appropriate leaving groups for ^{18}F -fluorinations, such as *p*-toluenesulfonate esters, can be easily introduced via the alkyne or azide. In this way, two *p*-toluenesulfonate ester precursors for the direct ^{18}F -labelling of 1,4-diphenyltriazoles were prepared and successfully labelled with fluorine-18 in radiochemical yields of 30 - 50% [164].

In a similar approach, two *p*-toluenesulfonate esters of a nucleoside thymidine derivative were prepared as precursors for ^{18}F -labelling. The ^{18}F -labelled thymidine derivatives were obtained in radiochemical yields of 69 - 92% [165].

Kim *et al.* compared both strategies, prosthetic group ^{18}F -labelling using the ^{18}F -alkyne 4- ^{18}F fluoro-1-butyne (27, $n = 1$) and azido-glucose, and the direct ^{18}F -labelling of the corresponding tosylated triazole-precursor as previously described in the subchapter 3.1 [153].

In the development of a ^{18}F -labelled dopamine D4 selective PET probe, solid phase-supported click chemistry was used for building up a compound library for dopaminergic affinity screening and SAR studies [166]. The click reaction of propargyl acrylate and azidomethyl polystyrol led to the triazolymethyl acrylate resin which was then used for parallel syntheses of various *N,N'*-diarylpiperazines. The most promising candidate was further developed towards ^{18}F -labelling. Finally, *N*-(2-hydroxy-4,5-dimethoxy)benzyl-*N'*-(4-chlorophenyl)piperazine was ^{18}F -labelled using 2- ^{18}F fluoroethyl tosylate as prosthetic group.

This general extension of the click approach in fluorine-18 chemistry enables rapid and convenient syntheses of precursors. Furthermore, click chemistry is particularly suitable for building up compound libraries for screening and SAR studies. Hence, it allows fast and efficient development of new ^{18}F -labelled PET imaging agents.

CONCLUSIONS

In many fields of chemistry the Cu(I)-catalysed cycloaddition of alkynes and azides has proved to be a particularly versatile tool. Recently it has been also used in fluorine-18 chemistry and the first studies have demonstrated an excellent practicability of this reaction type in ^{18}F -prosthetic group labelling methods.

General conditions and concepts of the "normal" non-radioactive click cycloaddition are directly transferrable to n.c.a. fluorine-18 chemistry. The major concern of fluorine-18 chemists of unsuitably long reaction times proved unfounded as most ^{18}F -conjugations needed short periods of time. Moreover, often quantitative incorporation of the ^{18}F -prosthetic group into the desired biomolecule was achieved

under very mild conditions. These very mild conditions and the extraordinary orthogonality of the Cu(I)-catalysed 1,2,3-triazole formation to other functionalities make this reaction particularly suitable for the ^{18}F -labelling of sensitive biomolecules such as peptides, proteins, antibodies, nucleotides, etc. In addition, the click reaction is highly efficient over a broad range of conditions, including the unbalanced stoichiometry used in n.c.a. fluorine-18 chemistry.

However, n.c.a. conditions seem to be more sensitive to the low solubility of the reactants, catalyst systems and products. While cold Cu(I)-catalysed cycloadditions of alkynes and azides frequently succeed in heterogeneous mixtures, the applications in n.c.a. fluorine-18 chemistry showed often poor results at low solubility levels. Accordingly, the choice of the solvent is crucial for a successful coupling of both ^{18}F -alkynes with azido-biomolecules and ^{18}F -azides with alkyne-functionalised structures. Generally, Cu(I)-catalysed cycloadditions of alkynes and azides in fluorine-18 chemistry are equally efficient and fast. In particular, the orthogonality of click chemistry to most functional groups enables the direct use of unprotected compounds, thus simplifying experimental procedures, saving deprotection steps as well as synthesis time.

The feasibility of the click reaction under biological and physiological conditions in combination with the excellent *in vivo* stability of the 1,2,3-triazole moiety make the Cu(I)-catalysed a very attractive tool for ^{18}F -labelling chemistry. On the other hand, the 1,2,3-triazole rings is present in many biological active structures and might interfere with the biological properties of the original parent compound. Such effects have been found in some of the new ^{18}F -labelled 1,2,3-triazole-compounds which showed impaired pharmacological characteristics, such as reduced binding affinities or blocked transporter and enzyme activities. Accordingly, new ^{18}F -labelled 1,2,3-triazole-bearing biomolecules can also lead to unfavourable pharmacological behaviour and properties. There is always the need to tailor the site and type of ^{18}F -conjugation to the individual biomolecule for new ^{18}F -pharmaceuticals, as small changes in the biomolecule can drastically change the biological and pharmacological properties.

Additional approaches use click chemistry for the precursor preparation. This general extension of the ^{18}F -prosthetic group labelling methods offers convenient synthesis strategies for precursors for direct ^{18}F -labelling purposes. The click chemistry approach is particularly suitable for the rapid assembly of compound libraries and pools, including precursors and cold reference compounds. Furthermore, click chemistry enables the introduction of ^{18}F -labelling sites into structures where other methods or direct ^{18}F -labelling procedures fail.

On the whole, the Cu(I)-catalysed cycloaddition of ^{18}F -labelled alkynes and azides adds more flexibility and a new efficient method to the ^{18}F -labelling chemistry. The first reports already demonstrated the high potential of click chemistry as new ^{18}F -labelling method. Hence, more examples of the click approach in ^{18}F -labelling chemistry will certainly be continued.

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