Recent Applications of Click Chemistry for the Synthesis of Radiotracers for Molecular Imaging

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Abstract: Click chemistry has received considerable attention as powerful modular synthesis approach, which has found numerous applications in many areas of modern organic chemistry, drug discovery and material science. Recently, click chemistry, and in particular the copper-mediated 1,3-dipolar [3+2] cycloaddition between azides and alkynes, has also entered the field of radiopharmaceutical sciences. This review addresses the recent developments of click chemistry for the synthesis of various radiotracers for molecular imaging purposes. Click chemistry-based radiotracers that will be covered include peptides and small organic molecules containing the short-lived positron emitter fluorine-18, and the gamma-emitters technetium-99m, indium-111, and iodine-125.

Keywords: Click chemistry, radiopharmaceutical science, radiotracer.

1. INTRODUCTION

Current biomedical research is revealing the fundamental molecular processes of life and diseases. The understanding of how molecular components of living cells are organized, how they interact, how they move and how they are formed and eliminated within the life cycle of an organism represents an integrative approach, which requires the direct observation of biochemical and physiological processes at the molecular level *in vivo*. The molecular processes of life can be studied and visualized at various levels of resolution by means of in vivo imaging techniques [1-3]. Molecular imaging techniques span the electromagnetic spectrum from ultrasonic to gamma-ray frequencies. In this line, especially magnetic resonance imaging (MRI), optical imaging and nuclear imaging are emerging as key molecular imaging techniques.

Nuclear imaging techniques are based on the application and detection of decaying radioisotopes. In most cases, the radioisotopes are combined with biologically active compounds to form radiolabeled probes capable of imaging specific biochemical and physiological events in vivo. The radiolabed probe is administered to follow a metabolic pathway connected with the pathophysiology of disease processes. The detection of the emitted radiation can either be performed by positron emission tomography (PET) or single photon computered tomography (SPECT) [2-4]. PET and SPECT have extensively been used for noninvasive diagnosis, staging and therapy control of diseases at the molecular level. Both techniques have found numerous applications in the field of clinical oncology, cardiology and neurology. Moreover, especially PET has also been recognized and applied as a valuable research tool in the process of drug development and evaluation.

The success of radiotracer-based molecular imaging techniques like PET and SPECT stems mainly from the availability of suitable radiolabed probes, also referred to as radiotracers. The design and synthesis of radiotracers as molecular probes is subject of radiopharmaceutical chemistry. Today's radiopharmaceutical chemistry has evolved into a complex chemical science, combining recent advances in synthetic organic and inorganic chemistry with developments and achievements in the field of molecular biology. The development of radiotracers for molecular imaging purposes has to address important questions on target selection and target validation while considering the special requirements encountered in radiotracer synthesis such as choice of the appropriate radionuclide and suitable labeling position.

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Today`s arsenal of radiotracers comprises more and more complex compounds ranging from small, low molecular weight compounds like amino acids, carbohydrates, neurotransmitter and hormones, to high molecular weight compounds like peptides, proteins and oligonucleotides. Hence, for the design and synthesis of radiotracers for molecular imaging purposes, special attention should be paid to the application of rapid, selective and functional group-tolerating labeling reactions. In this connection, radiopharmaceutical chemistry has especially benefited from recent advances in synthetic organic chemistry. Prominent examples are the successful application of enzyme- and transition metal-mediated reactions for the synthesis of a broad variety of radiotracers labeled with the short-lived positron emitters carbon-11 ($t_{1/2}$ = 20.4 min) and fluorine-18 ($t_{1/2}$ = 109.8 min).

In recent years the terms "bioorthogonal reactions" and "click chemistry" have entered into many fields of chemical sciences [5-8]. Bioorthogonal reactions in general, and click chemistry particular are generic terms for a set of reactions, which make use of several selective and modular building blocks to create heteroatom C-X-C links enabling chemoselective ligation reactions to label biologically relevant compounds. In this line, the copper-(I)-catalyzed 1,2,3-triazole formation from azides and terminal alkynes according to a 1,3-dipolar [3+2] cycloaddition is a particularly powerful ligation reaction, due to its high degree of specificity and the biocompatibility of the reactants. Neither azides nor alkynes react with other functional groups commonly present in biopolymers like peptides or proteins. Hence, there is no need for protection group chemistry. Moreover, triazoles are stable under physiological conditions, and they may associate with biological targets, through hydrogen bonding and dipole interactions as amide bond isosteres [9]. As a consequence, click chemistry is a very attractive approach for the design and synthesis of radiotracers for molecular imaging purposes.

The present review addresses recent applications of the copper-mediated 1,3-dipolar cycloaddition of azides and alkynes for the synthesis of radiotracers for molecular imaging purposes. The mini-review is organized to give first an introduction into general aspects of bioorthogonal reactions with special focus on click chemistry using azides and terminal alkynes. Based on the basic concepts of click chemistry, the main part of the review will summarize and discuss examples of click chemistry for the design and synthesis of radiotracers. In the first part the review will deal with radiolabeled alkynes or azides as suitable click chemistry building blocks, whereas a second part will focus on the synthesis of various triazole derivatives generated via click chemistry as precursors for subsequent radiolabeling reactions. The review will be concluded with an evaluation of the future potential of click chemistry in the field of radiopharmaceutical sciences.

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2. BIOORTHOGONAL REACTIONS AND CLICK CHEMISTRY

Bioconjugation techniques generally embrace the covalent attachment of synthetic labels onto a biomolecular scaffold. Although several conjugation techniques are available for the preparation of bioconjugates containing various functional groups, truly chemoselective ligation reactions are rather limited [10]. Thus, only a handful of synthetic pathways are known for the chemoselective labeling of biomolecules. Most strategies exploit the selective reaction between a nucleophilic and electrophilic moiety. The large number of various nucleophilic and electrophilic functionalities abundant in biomolecules requires careful scheduling and execution of these reactions to avoid undesired side reactions. Following nature's synthetic pathways, reactions between biomolecules normally occur through formation of carbon-heteroatom bonds to circumvent the often difficult to assemble carbon-carbon bonds.

An attractive and powerful bioconjugation technique is summarized under the term click chemistry [5,10]. Application of click chemistry-based reactions leads to the formation of carbon-heteroatom bonds by using molecules possessing high intrinsic reactivity. In this connection, high intrinsic reactivity refers to highly reactive or "spring loaded" functional groups. A reaction is termed as click chemistry when special criteria are obeyed. The reaction should be stereospecific and high-yielding while allowing the use of readily available starting materials, and application of simple isolation and purification steps. Prominent examples of useful click chemistry reactions comprise ring opening reactions of epoxides or aziridines, epoxidation reactions on double bonds, [3+2] cycloaddition reactions, oxime [11] and hydrazone [12] formation and, more recently, 6π-azaelectrocyclizations [13].

Selective bioconjugation reactions can be achieved between bioorthogonally functionalized compounds [14]. Bioorthogonal functional groups are neither chemically reactive nor otherwise associated towards other functional groups of the biological environment [7,15]. They should be inert towards metabolic reactions, stable under physiological conditions and of small size. Moreover, for the application of bioorthogonal reactions in living cells or organisms, bioorthogonal groups have to be nontoxic.

The azide group is the most versatile bioorthogonal functional group. Thus, many truly bioorthogonal reactions involve azide-functionalized compounds as exemplified for the copper(I)-catalyzed [3+2] cycloaddition, the strain promoted azide-alkyne cycloaddition [16] and Staudinger ligations [17]. A selection of azide-based bioorthogonal reactions is given in Fig. (**1**).

The most prominent example of click chemistry is based on the well-established Huisgen [3+2] cycloaddition reaction between terminal alkynes and azides [18]. Since the reaction is driven thermodynamically, the reaction requires quite harsh reaction conditions like high temperature and/or high pressure. Moreover, the reaction leads to the formation of two possible regioisomers, being 1,4- and the 1,5-substituted triazoles, respectively. On the other hand, the reaction also offers several advantages, such as readily availability of azides and alkynes as starting materials, insensitivity towards oxygen and moisture, and therefore the possibility to use water as solvent. The formed triazoles (mixture of 1,4- and 1,5-substituted regioisomers) can easily be obtained without extensive purification procedures. Despite these advantages, it took several decades before the reaction entered the stage of organic chemistry again. The recent renaissance of the Huisgen [3+2] cycloaddition was mainly due to the introduction of a copper(I)-catalyzed version of the reaction as described recently by the groups of Sharpless [8,19] and Meldal [20]. The application of a kinetically controlled copper(I)-catalyzed [3+2] cycloaddition between azides and alkynes was accompanied with several significant improvements. Most important, the kinetic control of the copper(I)-catalyzed reaction afforded only 1,4-disubstituted triazole as the single regioisomer [19,21]. Moreover, the reaction can be performed under mild conditions compatible with the biological environment. The formed triazole moiety is stable under physiological conditions and able to undergo hydrogen bonds comparable to that of amide bonds. A further important characteristic of the copper(I)-catalyzed [3+2] cycloaddition reaction is its bioorthogonality making the reaction particularly attractive for chemoselective bioconjugations.

An extension of the [3+2] cycloaddition reaction between azides and alkynes was recently described by Zhang and co-workers [22]. They report on the use of ruthenium complexes instead of

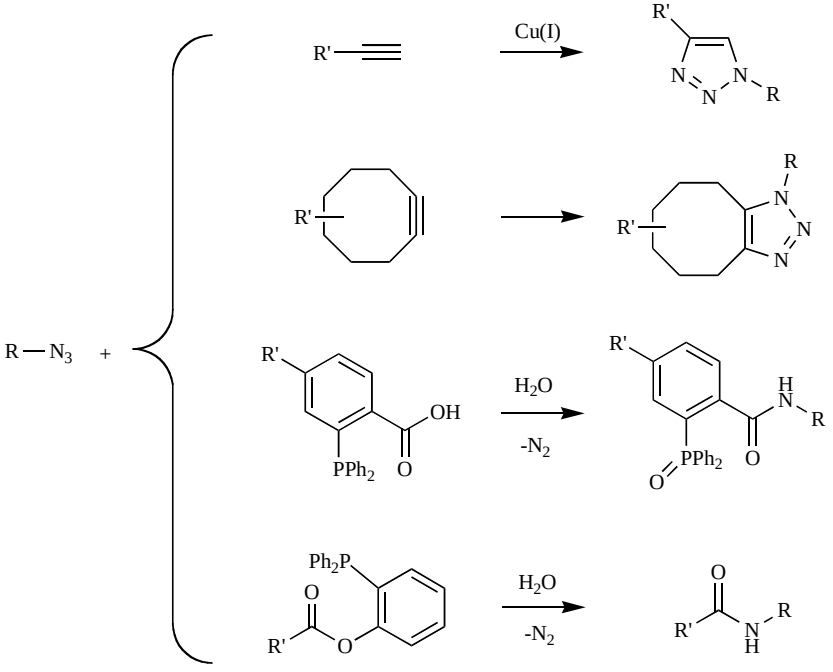


Fig. (1). Selection of azide-based bioorthogonal reactions.

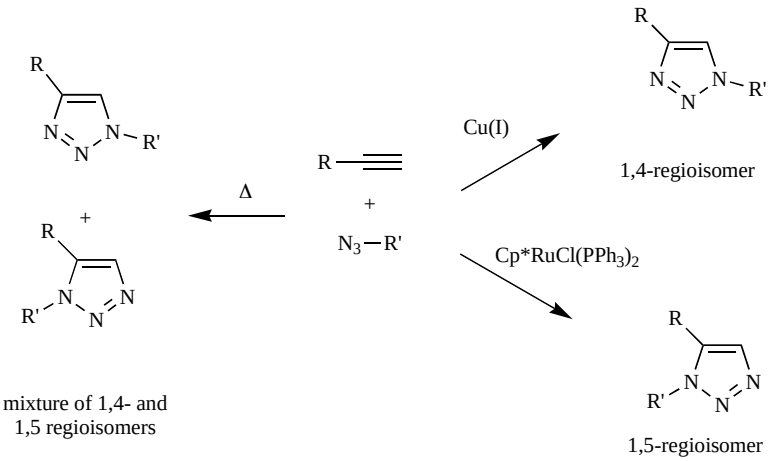


Fig. (2). Various synthesis routes to substituted 1,2,3-triazoles.

copper(I) for the cycloaddition reaction, which led exclusively to the formation of the corresponding 1,5-disubstituted triazoles. However, unlike the copper(I)-catalyzed reaction, the ruthenium-catalyzed reaction has to be carried out in organic solvents preferentially at elevated temperatures making the reaction incompatible for bioconjugations involving biopolymers like peptides and proteins.

Fig. (**2**) summarizes the different synthesis routes for the synthesis of 1,2,3-triazoles starting from azides and alkynes according to the classical Huisgen [3+2] cycloaddition, the copper(I)-catalyzed [3+2] cycloaddition, and the ruthenium-catalyzed [3+2] cycloaddition, respectively.

To date, a large number of successful applications of click chemistry in the fields of chemistry, biology and material science have been reported in the literature. Conversely, reports on the application of click chemistry in the field of radiopharmaceutical science are rather limited. From the set of applicable click chemistry reactions only oxime and hydrazone formations, and the copper(I)-catalyzed [3+2] cycloaddition of azides and alkynes have been reported. The following chapters of the review will address the application of the copper(I)-catalyzed [3+2] cycloaddition reactions for the synthesis of various radiotracers for molecular imaging purposes.

3. CLICK CHEMISTRY USING RADIOLABELED AZIDES AND ALKYNES

This part of the review discusses the synthesis and application of small radiolabeled alkynes or azides as suitable building blocks for radiotracer synthesis via "hot click chemistry". To date, this approach has exclusively been used for azides and alkynes labeled with the short-lived positron emitter fluorine-18 (^{18}F, $t_{1/2}$ = 109.8 min).

The use of small ^{18}F-labeled building blocks is a common approach for the radiolabeling of biopolymers like peptides and proteins with ^{18}F. The small ^{18}F-labeled building blocks act as bifunctional labeling agents, also referred to as prosthetic groups [23-26]. In general, the application of prosthetic groups comprises incorporation of the radionuclide into a small organic molecule capable of being linked to peptides, proteins, oligonucleotides and antibodies under mild conditions. In this connection, ^{18}F-labeled azides and alkynes as small-sized click chemistry building blocks can also be considered as prosthetic groups.

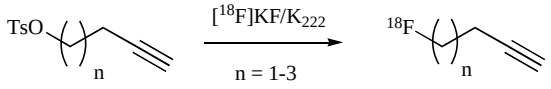
Under standard condition, the kinetically driven copper(I)-catalyzed [3+2] cycloaddition of azides and alkynes requires long reaction times of several hours upon completion. These long reaction times are not compatible with the short half-live of ^{18}F. However, the ^{18}F-labeled compound is commonly used in submicromolar amounts. The use of such small amounts leads to an extraordinary stoichiometic relation between the reaction partners enabling accomplishment of the reaction within short reaction times compatible with the 109.8 min half-live of ^{18}F. Moreover, the very low amounts of ^{18}F-labeled compounds allow rapid and simple purification protocols.

Several ^{18}F-labeled azides and alkynes and their subsequent use in click chemisty have been described in the literature [27-30].

The work on the synthesis and application of ω-[^{18}F]fluoroalkynes **2a**-**2c** by Marik and Sutcliffe was the first report on click chemistry in radiopharmaceutical science [27]. The work described use of various ω-[^{18}F]fluoroalkynes as click chemistry building blocks for the radiolabeling of azide-functionalized peptides via click chemistry.

The ω-[^{18}F]fluoroalkynes **2a**-**2c** varied in their chain lengths, and they could easily be prepared via nucleophilic displacement starting from the corresponding tosylates **1a**-**1c** with [^{18}F]fluoride. The results of the radiosynthesis of various ω-[^{18}F]fluoroalkynes are summarized in Fig. (**3**).

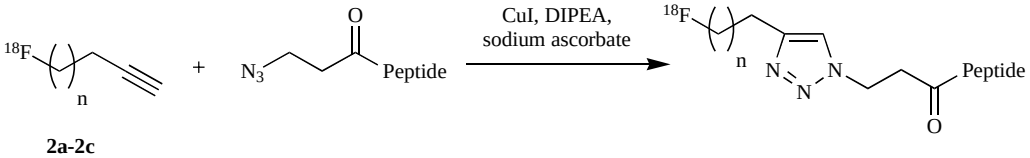
The radiolabeling was accomplished with the powerful nucleophilic radiofluorination agent [^{18}F]KF (generated by treatment of



1a-1c **2a-2c**

[^{18}F]Fluoroalkyne	n	Radiochemical Yield (%)	Radiochemical Purity (%)	bp (°C)
2a	1	36	98	45
2b	2	81	98	76
2c	3	61	99	106

Fig. (3). Synthesis of ω-[^{18}F]fluoroalkynes **2a**-**2c**.



2a-2c

[^{18}F]Fluoropeptide	Radiochemical Yield (%)[a,b]	Radiochemical Purity (%)
2a-YGGFL	54	95
2b-YGGFL	97	98
2c-YGGFL	62	99
AGDLHVLR-Ebes-Lys(**2b**)[c]	97	81
2b-AGDLHVLR	99	87

[a] All reaction were carried out in a mixture of DMF/water/acetonitrile at r.t. for 10 min.
[b] Radiochemical yield is based upon ω-[^{18}F]fluoroalkynes.
[c] Ebes denotes a hydrophilic linker.

Fig. (4). Click chemistry reaction of ω-[^{18}F]fluoroalkynes **2a-2c** with various azide-functionalized peptides.

cyclotron-produced [^{18}F]fluoride with Kryptofix/potassium carbonate) in acetonitrile at 100°C. The radiolabeling reaction proceeded in the presence of ω-alkynyl *p*-toluenesulfonate as the labeling precursor in a sealed reaction vial. The formed volatile terminal ω-[^{18}F]fluoroalkynes **2a-2c** were purified via distillation. Radiolabeling and purification were accomplished within 10-15 min. The purified ω-[^{18}F]fluoroalkynes were subjected to click chemistry reaction with various azide-functionalized peptides in the presence of CuI, sodium ascorbate, and diisopropylethylamine (DIPEA). The azide-functionalized peptides were prepared using standard Fmoc-based solid-phase peptide synthesis with 3-azidopropionic acid.

A mixture of DMF, water and acetonitrile was used as the solvent. The click chemistry reaction occurred at room temperature within 10 min. No byproducts were formed, and the reaction mixture was purified by solid-phase extraction. Unreacted ω-[^{18}F]fluoroalkynes and solvent residues were removed by evaporation. The results are summarized in Fig. (**4**).

In course of optimizing the reaction conditions, different catalyst systems were tested. A combination of Cu(II)sulfate and sodium ascorbate for in situ reduction to Cu(I) afforded only unsatisfactory 10% yield after a 30 min reaction time at room temperature. Much better results could be obtained when Cu(I) was used directly. Addition of an excess of sodium ascorbate and a nitrogen base was mandatory to prevent re-oxidation of Cu(I) to Cu(II). Following this approach, the radiochemical yield could significantly be increased to 54-99% (Scheme **4**). The use of DIPEA as the base proved to be superior to piperidine, since piperidine led to the formation of unidentified byproducts.

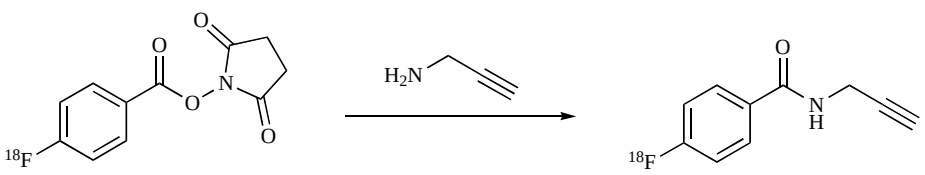
Application of optimized reaction conditions gave the desired radiolabeled peptides in good to excellent radiochemical yields at specific activities >35 GBq/μmol. The total synthesis time was 30-40 min after radionuclide production.

Based on these intriguing results, a comparison of peptide A20FMDV2 radiolabeled was performed with three different ^{18}F-bearing prosthetic groups [31]. Peptide A20FMDV2 was either radiolabeled with 4-[^{18}F]fluorobenzoic acid or 2-[^{18}F]fluoropropionic acid via aminolysis on solid support, or via click chemistry with 5-[^{18}F]fluoropentyne. Radiolabeling via click reaction was accomplished within two reaction steps in 7.2% decay-corrected radiochemical yield after a reaction time of 66 min. Radiolabeling of A20FMDV2 via solid-phase supported aminolysis with 4-[^{18}F]fluorobenzoic acid or 2-[^{18}F]fluoropropionic acid, however, required four reaction steps and substantial longer synthesis times of 137 min and 171 min, respectively. The desired products were obtained in 7.8% and 4.6% decay-corrected radiochemical yields. Thus, application of click chemistry proved to be most suitable method for A20FMDV2 peptide labeling in terms of yield and reaction time.

Another report dealing with peptide labeling via click chemistry involving ^{18}F-labeled alkynes was reported by Ramenda *et al.* [30]. Synthesis of 4-[^{18}F]fluoro-*N*-(prop-2-ynyl)benzamide **3** as a stable aromatic C-F containing terminal alkyne building block for subsequent click reaction was easily be prepared starting from the readily available prosthetic group *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) [23,32,33] through treatment with an excess of propargyl amine at room temperature. The synthesis is depicted in Fig. (**5**).

Compound **3** was isolated through solid-phase extraction (SPE) purification using RP18 cartridges. The radiochemical yield was 72% (based upon [^{18}F]SFB) and the radiochemical purity exceeded 90%.

Click chemistry involving 4-[^{18}F]fluoro-*N*-(prop-2-ynyl)benzamide **3** was performed using azide-functionalized hexapeptide neurotensin(8-13) derivative **4** [34,35]. It was shown, that no reaction occurred in pure water as the solvent. Therefore, buffer was



[^{18}F]SFB **3**

Fig. (5). Synthesis of 4-[^{18}F]fluoro-*N*-(prop-2-ynyl)benzamide **3**.

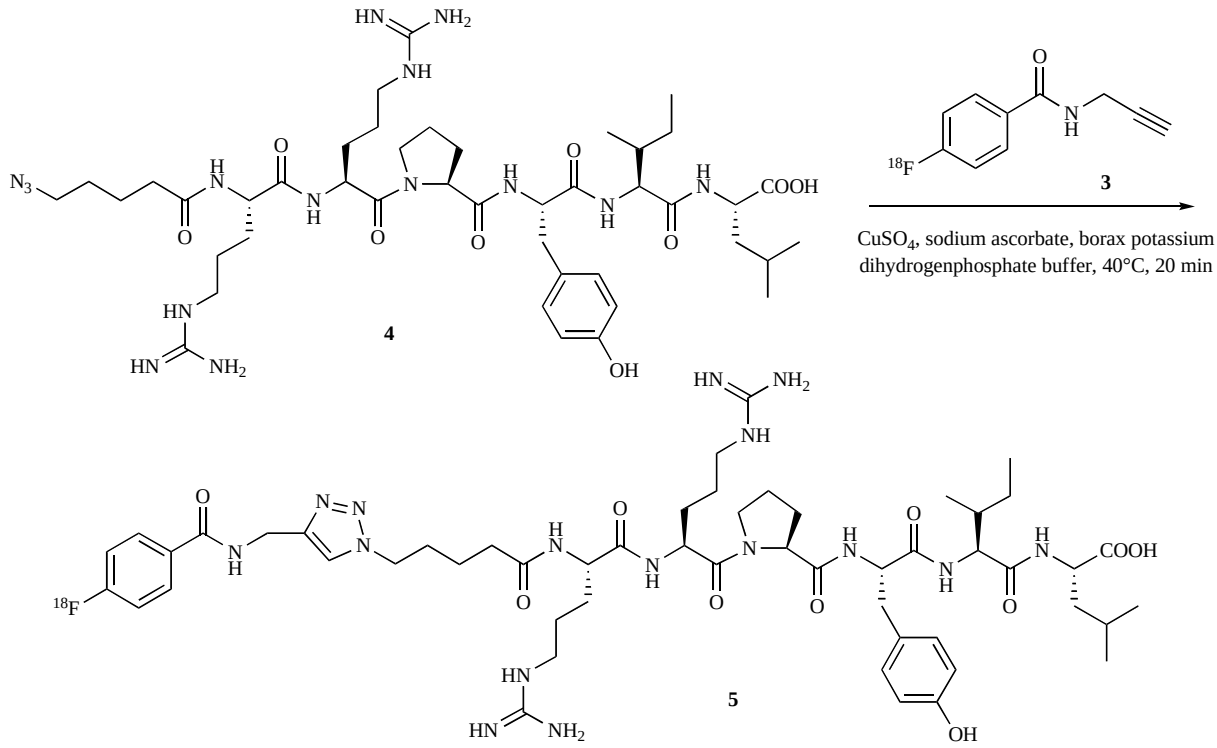


Fig. (6). Click chemistry reaction of 4-[^{18}F]fluoro-*N*-(prop-2-ynyl)benza-mide **3** with azide-functionalized neurotensin(8-13) **4**.

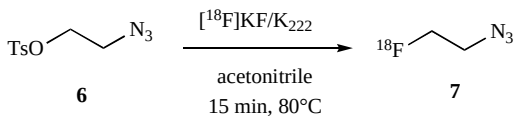


Fig. (7). Radiosynthesis of 2-[^{18}F]fluoroethylazide **7**.

used as solvent for the click chemistry step. Azide-functionalized peptide **4** (ca. 1 mg) was reacted with 4-[^{18}F]fluoro-*N*-(prop-2-ynyl)benzamide **3** in the presence of copper(II)-sulfate and sodium ascorbate in borax potassium dihydrogenphosphate buffer (80 µl) at 40°C for 20 min to give the desired radiolabeled peptide **5** in 66% radiochemical yield (Fig. **6**).

An alternative click chemistry approach using 2-[^{18}F]fluoro-ethylazide **7** as radiolabeled click chemistry building block was reported by Glaser *et al.* [28]. 2-[^{18}F]Fluoro-ethylazide **7** was prepared according to standard nucleophilic radiofluorination procedure starting from the corresponding tosylate precursor **6** (Fig. **7**).

After the radiofluorination reaction, 2-[^{18}F]fluoroethylazide **7** was distilled in a stream of nitrogen at 130°C. Following this procedure, 2-[^{18}F]fluoroethylazide **7** was obtained in 54% decay-corrected radiochemical yield.

2-[^{18}F]Fluoroethylazide **7** was reacted with a small library of terminal alkynes bearing different functional groups in the presence of excess copper(II)/sodium ascorbate or copper powder. The click

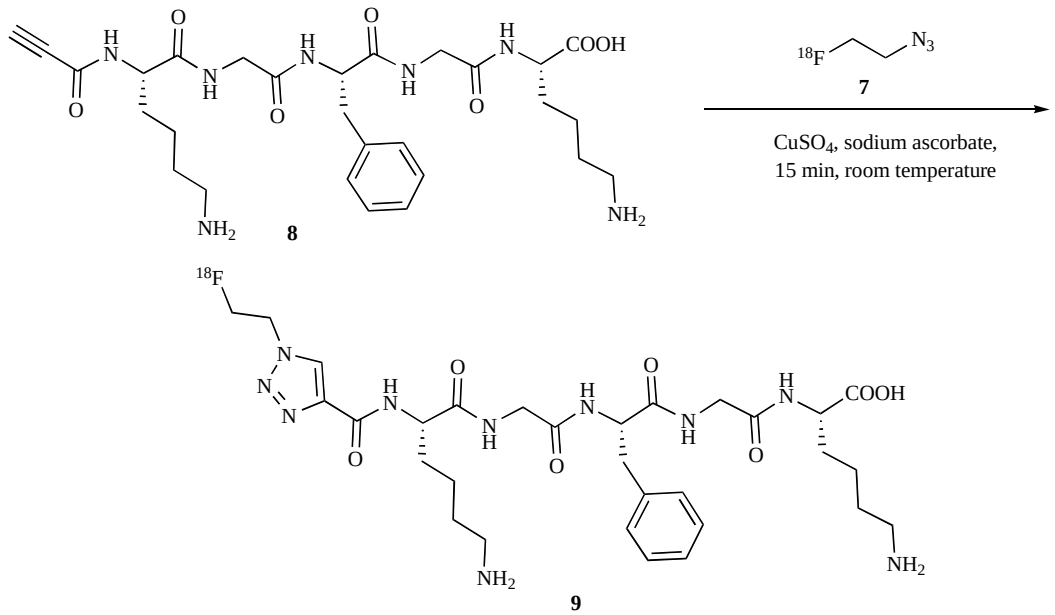


Fig. (8). Labeling of model peptide **8** with 2-[^{18}F]fluoroethylazide **7**.

chemistry reaction was performed at room temperature and at 80°C for 15 min. In all cases, a reaction temperature of 80°C gave higher radiochemical yields compared with reactions carried out at room temperature. Moreover, in situ reduction of copper(II)-sulfate to copper(I) in the presence of sodium ascorbate proved to be superior to the use of copper powder since high radiochemical yields could be achieved regardless the functional group present in the terminal alkyne. On the other hand, only low radiochemical yields were obtained in the presence of carboxylic acids when copper powder was used.

By using optimal reaction conditions, an alkyne-containing model peptide **8** was radiolabeled with 2-[^{18}F]fluoroethylazide **7** according to a click chemistry reaction. The desired triazole-containing peptide **9** could be obtained in 92% radiochemical yield and radiochemical purity greater 99% after semi-preparative HPLC (Fig. **8**).

Various ^{18}F-labeled terminal alkynes (**10**, **11**) and azides (**12**, **13**) containing an ether backbone have been described by Sirion *et al.* [36]. The compounds are less volatile as previously described aliphatic ^{18}F-labeled alkynes and azides. The ^{18}F-labeled click chemistry building blocks as shown in Fig. (**9**) were prepared in radiochemical yields of 85-95% via nucleophilic aliphatic substitution with [^{18}F]fluoride and the corresponding mesylates as labeling precursors. Noteworthy, *tert.*-butanol was used as the solvent. The advantages of using tertiary alcohols as solvents for aliphatic nucleophilic radiofluorination reactions have been discussed in recent reports by Kim *et al.* [37,38]. Moreover, *tert.*-butanol is known as an excellent co-solvent in click chemistry reaction in aqueous media.

The click chemistry building blocks were used for the synthesis of small-molecular weight compounds such as sugars, nucleotides and amino acids. A representative reaction is given in Fig. (**10**).

The use of in situ reduction of Cu(II) to Cu(I) by means of sodium ascorbate seemed to be mandatory, since direct application of Cu(I) led also to the undesired 1,5-substituted regioisomer.

The reaction was performed in water containing acetonitrile, DMF, DMSO or *tert.*-BuOH as co-solvent. A mixture of water and DMSO proved to be the best solvent for the click chemistry reaction. After a reaction time of 40 min, 90% of the click chemistry building block was converted into the desired triazole-containing nucleotide **15**. Purification of the final product was performed by means of HPLC.

A related approach by using a ^{18}F-labeled terminal alkyne **17** containing a polyethylene glycol (PEG) backbone was reported by Li *et al.* [29]. The ^{18}F labeling reaction was carried out in DMSO at elevated temperature using the corresponding tosylate **16** as labeling precursor (Fig. **11**). The product was purified by HPLC to remove unreacted tosylate precursor **16** to give the desired product **17** in a radiochemical yield of 78.5% ± 2.3% (n=3).

The ^{18}F-labeled alkyne **17** was subjected to click chemistry reactions using various azide-functionalized dimeric RGD-peptides. The click chemistry reaction proceeded in the presence of in situ formed Cu(I) at 40°C within 20 min. Subsequent HPLC-purification afforded the radiolabeled peptides in decay-corrected radiochemical yields in the range of 40 to 54%. The radiochemical purity exceeded 97%. The specific activity was determined to 100-200 GBq/μmol. The purified peptides were used in radiopharmacological studies involving small animal PET in U87MG tumor-bearing mice.

Application of click chemistry to the synthesis of ^{18}F-labeled carbohydrates was reported by Kim *et al.* [38]. The authors describe the radiosynthesis of 4-[(2-[^{18}F]fluoroethyl)-1-(β-D-glucopyranosyl)]-1*H*-1,2,3-triazole **18** as an alternative to the most im-

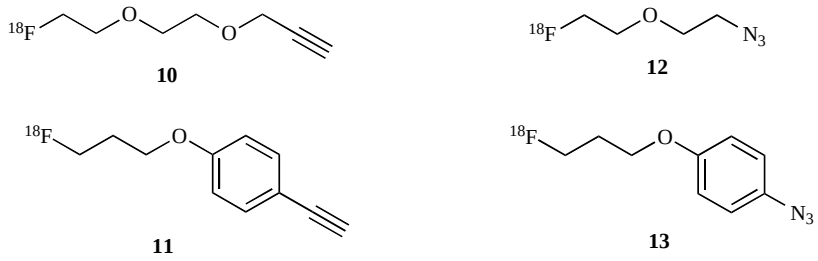


Fig. (9). Selection of ^{18}F-labeled alkynes and azides containing an ether backbone.

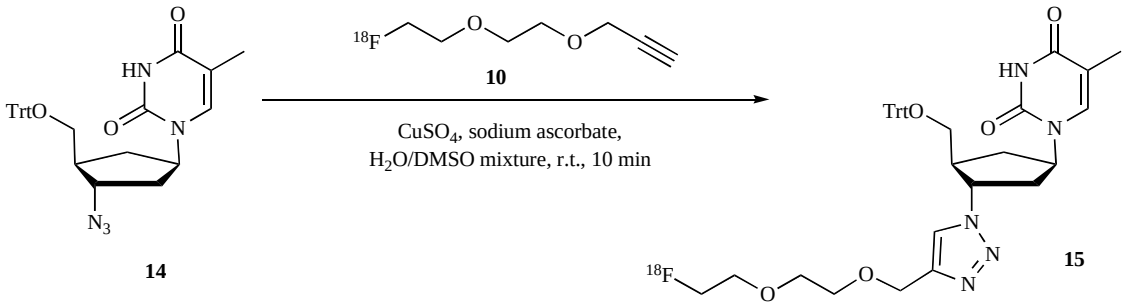


Fig. (10). Representative click chemistry involving nucleotides.

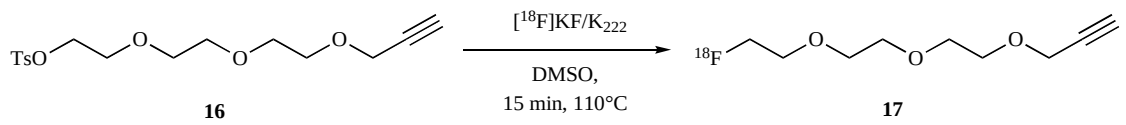


Fig. (11). Synthesis of polyether-containing ^{18}F-labeled terminal alkyne **17**.

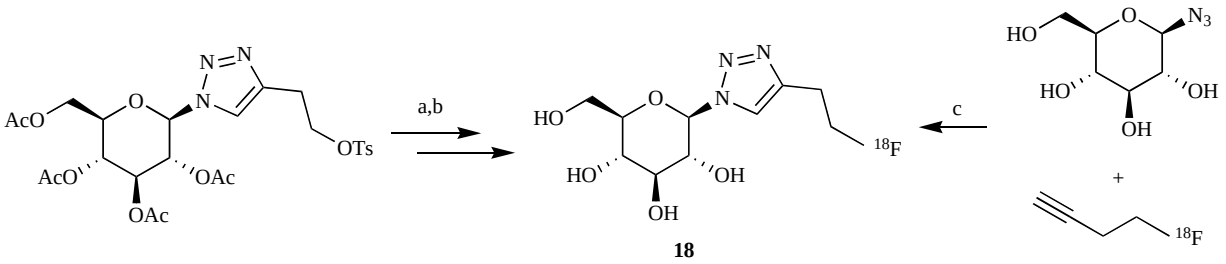


Fig. (12). Various synthesis routes for 4-[(2-[^{18}F]fluoroethyl)-1-(β-D-glucopyranosyl)]-1*H*-1,2,3-triazole **18**; a) n-Bu$_4$N[^{18}F]F, acetonitrile, *tert*.-BuOH, 100°C, 20 min; b) MeONa, MeOH, r.t., 15 min; c) CuI, sodium ascorbate, 2,6-lutidine, 90°C, 10 min.

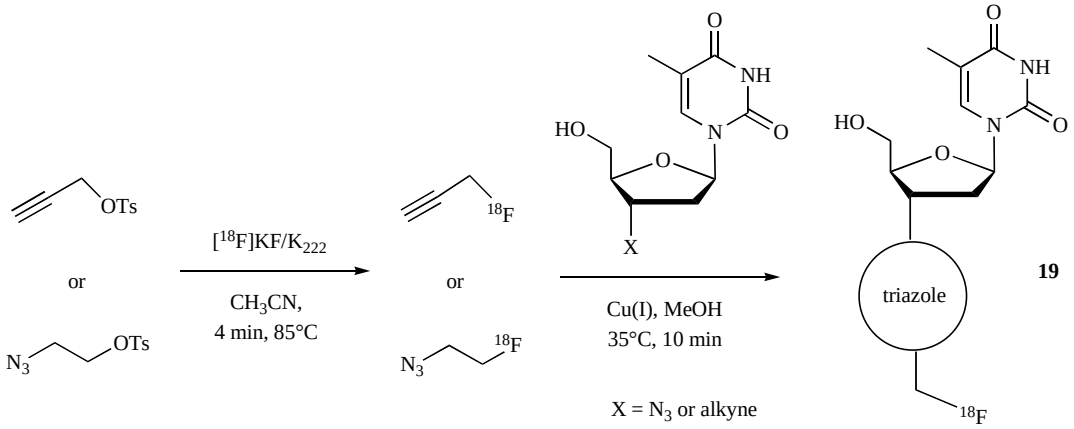


Fig. (13). Radiosynthesis of ^{18}F-labeled thymidine derivative **19** via click chemistry.

portant and most widely used PET radiotracer 2-[^{18}F]fluorodeoxyglucose ([^{18}F]FDG) (Fig. **12**).

4-[(2-[^{18}F]Fluoroethyl)-1-(β-D-glucopyranosyl)]-1*H*-1,2,3-triazole **18** was prepared via two different synthesis routes. The first approach comprised a click chemistry reaction between azide-functionalzed peracetylated glucose with 3-butyn-1-ol to give the corresponding triazole. Subsequent conversion of the primary alcohol into the corresponding tosylate followed by radiofluorination with [^{18}F]fluoride afforded the desired compound in 20-25% radiochemical yield after protecting group removal.

The direct radiolabeling was achieved through reaction of 3-[^{18}F]fluoropropyne with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide. The click reaction proceeded in the presence of Cu(I), which was generated by in situ reduction of Cu(II) with sodium ascorbate. After HPLC purification the product **18** was obtained in 9-12% radiochemical yield making this approach less favorable compared to the first synthesis route with a 20-25% radiochemical yield. However, unlike [^{18}F]FDG, the prepared ^{18}F-labeled glucose analog is neither a substrate of hexokinase nor glucose transporter-1 (GLUT-1) which makes compound **18** not suitable for molecular imaging purposes to study glycolysis.

In the patent literature, click chemistry involving ^{18}F-labeled building blocks have also been reported [39-42]. In the patents, the use of radiolabeled azides and alkynes for the synthesis of ^{18}F-labeled radiotracers like thymidine derivative **19** via click chemistry is described (Fig. **13**) [41].

The click chemistry reaction was performed in methanol at 35°C in the presence of Cu(I)-acetate as the Cu(I) source. After HPLC purification the desired compounds were obtained in radiochemical yields of 4-5%.

Another patent publication of Kolb and co-workers [42] dealed with the preparation of imaging agents for the integrin receptor. It is known from the literature that [^{18}F]galacto-RGD peptide exhibit integrin $\alpha_v\beta_3$-specific tumor uptake in integrin-positive M21 melanoma xenograft model [43]. Based on this fact, novel cyclic peptides containing a sugar moiety were introduced using the click chemistry for the labeling step as shown in Fig. (**14**).

Labeling precursor 1-pentynyl [^{18}F]fluoride **20** was prepared with [^{18}F]fluoride (K_{222} and K_2CO_3) in acetonitrile at 110°C for 5 min. Subsequent distillation of compound **20** into a cooled solution containing azide-c(RGDfK) **21**, CuI, diisopropylethylamine, sodium ascorbate dissolved in a mixture of water, DMF and acetonitrile gave the radiolabeled peptide [^{18}F]**22** after a reaction time between 15 to 60 min at room temperature. After purification via RP-HPLC, 1 mCi of the radiolabeled peptide were isolated starting from 35 mCi of [^{18}F]fluoride. *In vivo* micro PET studies using tumor-bearing mouse revealt that compound [^{18}F]**22** seems to be a promising radiotracer showing good tumor uptake whilst being rapidly cleared from muscle and other organs and tissues.

4. RADIOTRACER SYNTHESIS INVOLVING CLICK CHEMISTRY AND SUBSEQUENT RADIOLABELING REACTION

In the previous part of the review various examples have been described involving ^{18}F-labeled alkynes or azides as suitable click chemistry building blocks for the synthesis of PET radiotracers. This part of the review will deal with the synthesis of various triazole derivatives generated via click chemistry as suitable precursors for subsequent radiolabeling reactions.

In the field of radiopharmaceutical science, various triazoles have been used as promising labeling precursors for the incorporation of radionuclides. In general three distinct approaches have been envisaged: (1) generation of compounds containing a triazole moiety suitable for subsequent radiolabeling reactions with iodine-125

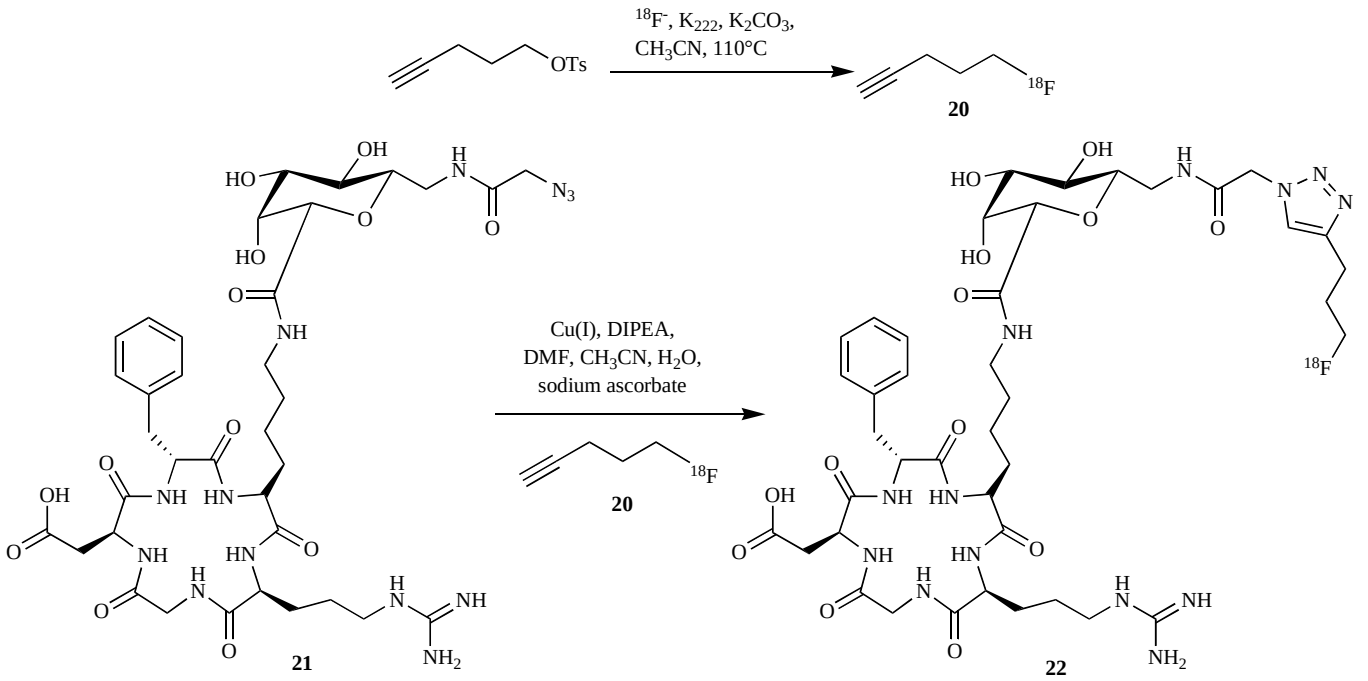


Fig. (14). Preparation of ^{18}F-labeled RGD peptide **22**.

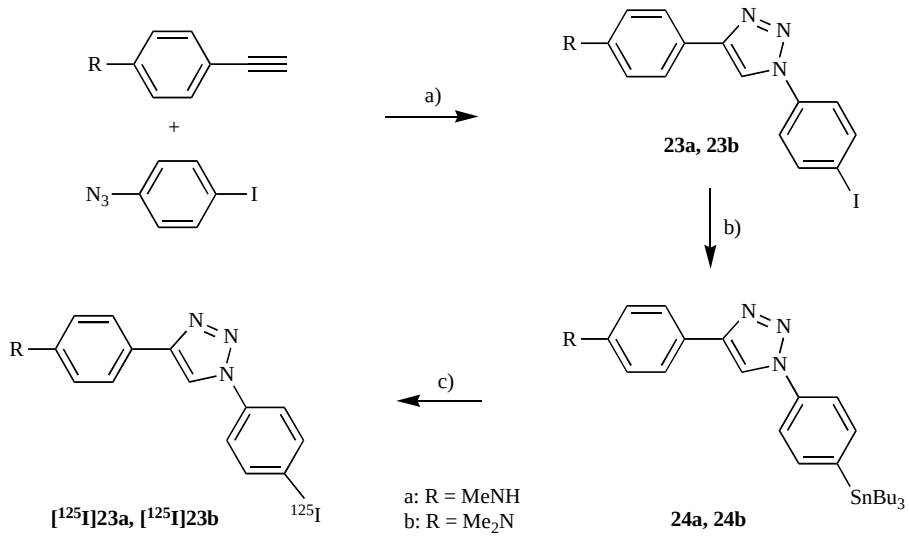


Fig. (15). a) $CuSO_4$, sodium ascorbate, *tert.*-BuOH/H_2O, r.t., 24 h; b) n-Bu_6Sn_2, $Pd(PPh_3)_4$, toluene, 100 °C, 4 h; c) H_2O_2, [^{125}I]NaI, HCl, EtOH.

(^{125}I, $t_{1/2}$ = 60 d) and fluorine-18, (2) formation of a triazole moiety as a suitable donor group in complexation reactions involving the γ-emitter technetium-99m (^{99m}Tc, $t_{1/2}$ = 6 h) or (3) formation of triazoles as linkers for the conjugation of metal chelates to biomolecules and subsequent radiolabeling with radiometals such as indium-111 (^{111}In, $t_{1/2}$ = 2.8 d).

The first approach was utilized for the synthesis of compounds as potential radiotracers for imaging Alzheimer`s disease (AD). A central event in the pathogenesis of AD is the formation of β-amyloid (Aβ) peptides in the human brain [44].

In 2007 Qu *et al.* reported the synthesis of a series of novel triazole-containing derivatives [45] based on earlier published diphenylthiophenes as lead structure for β-amyloid plaque imaging. Diphenylthiophenes exhibit unfavourable high lipophilicity, which could be reduced through exchanging the thiophene moiety with a triazole moiety [46]. Earlier studies showed that a *p*-*N*-methyl- or *p*-*N*,*N*-dimethylaminophenyl group is crucial for binding toward the β-amyloids, whereas β-amyloids readily accommodate various modifications of the other phenyl group.

In this line, a series of various diaryl triazoles was prepared via click chemistry as potential ligands for binding to β-amyloids. In situ reduction of $CuSO_4$ with sodium ascorbate was used to generate Cu(I) as needed for the regioselective 1,4-triazole formation to give the corresponding iodinated diphenyl derivatives **23a** and **23b**. Subsequent synthesis of tributylstannanes **24a** and **24b** was accomplished via Pd-mediated cross-coupling reaction using hexabutyldistannane. Standard radioiododestannylation reaction condition ([^{125}I]NaI, H_2O_2, HCl) was used to introduce the γ-emitter iodine-125 (^{125}I, $t_{1/2}$ = 60 d) regioselectively. Radiolabeled compounds **[^{125}I]23a** and **[^{125}I]23b** were synthesized in excellent radiochemical yields (80-85%) and high radiochemical purity (>95%). The reaction scheme is depicted in Fig. (**15**).

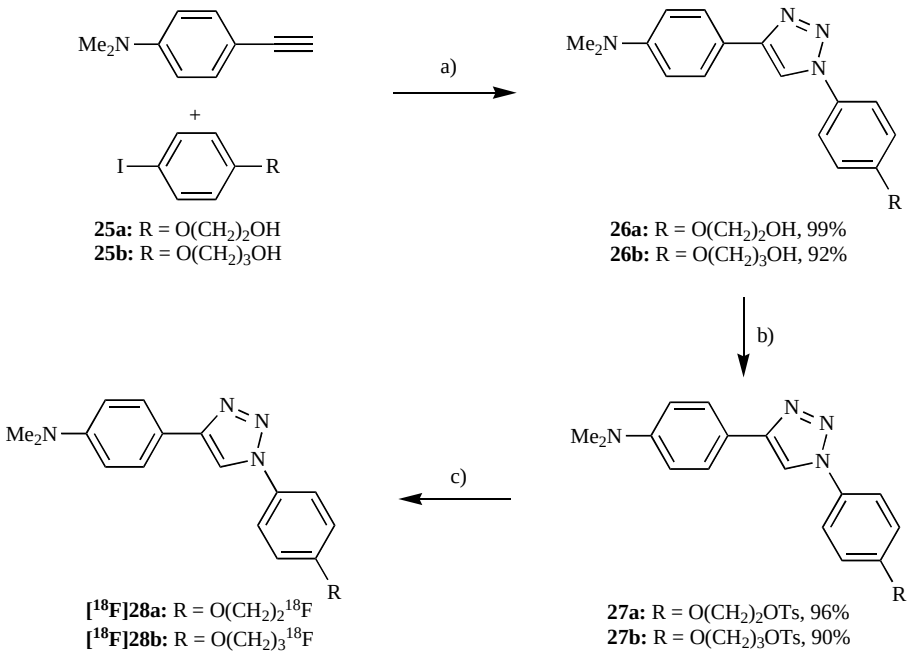


Fig. (16). a) NaN_3, trans-*N,N'*-dimethyl-1,2-cyclohexanediamine, CuI, sodium ascorbate, DMSO/H_2O, r.t., 3 h; b) TsCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to r.t., 3 h; c) [^{18}F]KF, K_{222}, K_2CO_3, DMSO,120 °C, 4 min.

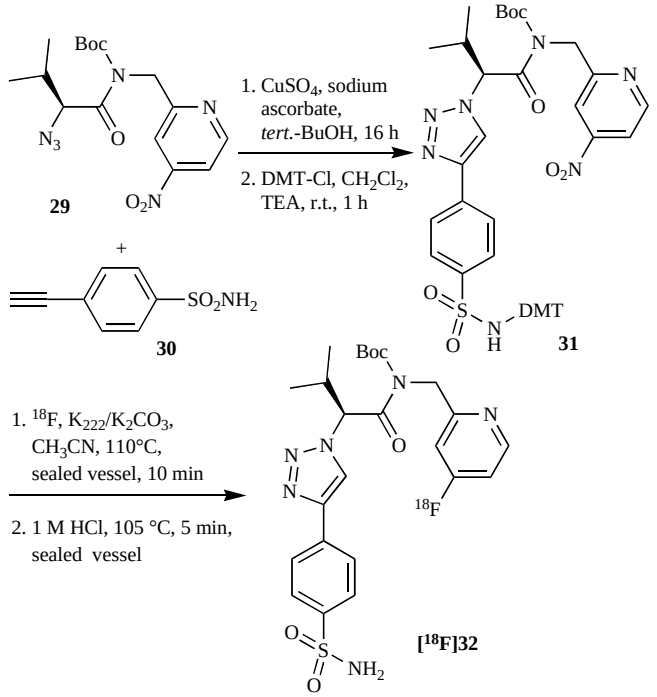


Fig. (17). Click chemistry route to the CA-II imaging agent **[^{18}F]32** for PET.

Related ^{18}F-labeled compounds were prepared in a similar way. Click chemistry with 4-*N,N`*-dimethylamino-phenyl acetylene and 4-iodo-phenylazides (**25a** and **25b**) afforded the desired triazoles **26a** and **26b** in high chemical yields of 99% and 92%, respectively. Conversion of primary alcohols into the corresponding tosylates **27a** and **27b** as labeling precursors was achieved by treatment with TsCl in CH_2Cl_2 in the presence of Et_3N and DMAP. Radiofluorination of **27a** and **27b** was accomplished with [^{18}F]KF in DMSO at 120°C for 4 min. The resulting ^{18}F-labeled compounds **28a** and **28b** were purified by HPLC. After a total synthesis time of 70 min, compounds **28a** and **28b** were obtained in decay-corrected radiochemical yields of 50% (**28a**) and 30% (**28b**). The radiochemical purity exceeded 95%. The reaction scheme is given in Fig. (**16**).

Compounds **[^{125}I]23a**, **[^{125}I]23b**, **[^{18}F]28a** and **[^{18}F]28b** were tested for their ability to cross the blood-brain-barrier (BBB) and their binding affinity towards β-amyloids as important criteria for brain radiotracers. All newly synthesized radiolabeled triazoles displayed excellent brain penetration in rats.

In 2006 a patent from the group of Kolb *et al.* [47] reported a click chemistry-based synthesis route to high affinity molecular probes for imaging carboanhydrase-II (CA-II) by means of PET. The principle is shown in Fig. (**17**). Click reaction between azide functionalized pyridine **29** and 4-ethyne-benzenesulfonamide **30** was carried out using *tert.*-BuOH as solvent and $CuSO_4$/sodium ascorbate as Cu(I) source. After stirring overnight and purification, 79% of the triazole could be obtained. Protection with 4,4'-dimethoxytrityl chloride (DMT-Cl) afforded 77% of compound **31**.

Direct radiolabeling of compound **31** was accomplished using anhydrous [^{18}F]KF in dry acetonitrile at 110°C in a sealed reaction vial within 10 min. After fluorination the mixture was cooled, acetonitrile was evaporated and aqueous HCl was added to remove the DMT protecting group. A typical radiofluorination started with 900 mCi of [^{18}F]fluoride. This afforded 91 mCi (15%) of the desired product **[^{18}F]32** after HPLC purification

Another example deals with the preparation of ^{18}F-labeled cyclooxygenase-2 (COX-2) inhibitor **[^{18}F]36** (Fig. **18**). Standard click chemistry as described above was used for the synthesis of triazole **35**. Compound **35** was obtained in 55% yield after purification. Subsequent radiofluorination was carried out according to the procesure described for the synthesis of compound **[^{18}F]32**. A typical synthesis starting from 660 mCi of [^{18}F]fluoride gave 69.3 mCi (14.6%) of compound **[^{18}F]36** after a total synthesis time of 52 min, including HPLC purification.

Unlike radiohalogens like ^{18}F and ^{125}I, the incorporation of radiometals into biologically relevant compounds requires coordination chemistry for stable binding. Click chemistry was used to cre-

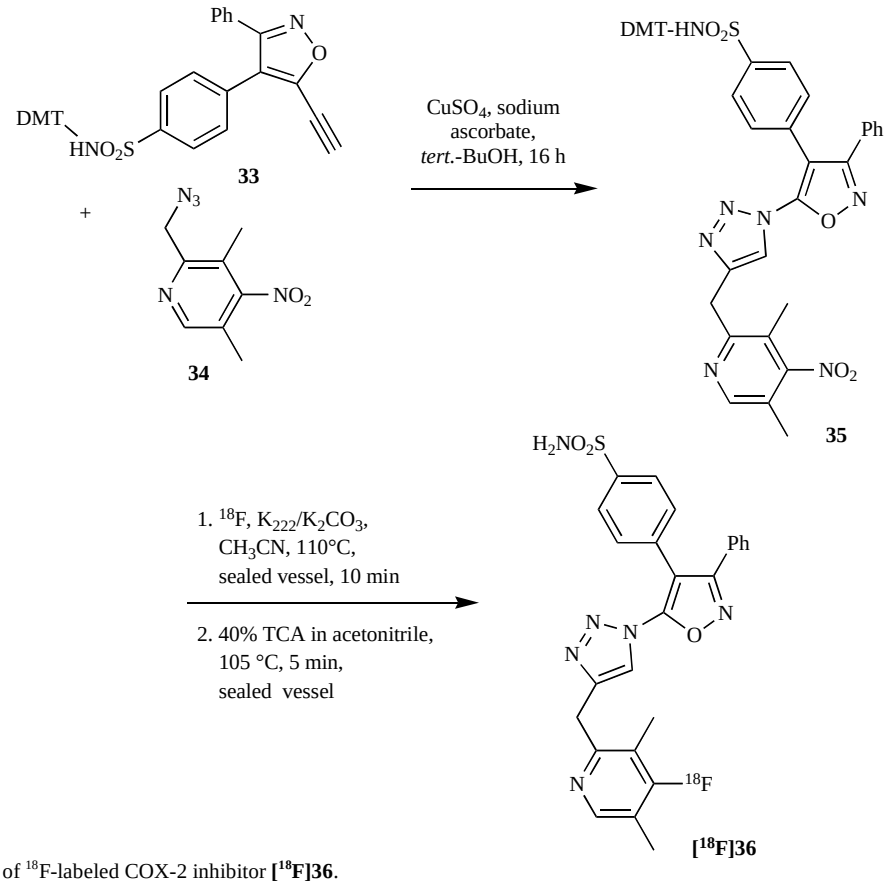


Fig. (18). Preparation of ^{18}F-labeled COX-2 inhibitor **[^{18}F]36**.

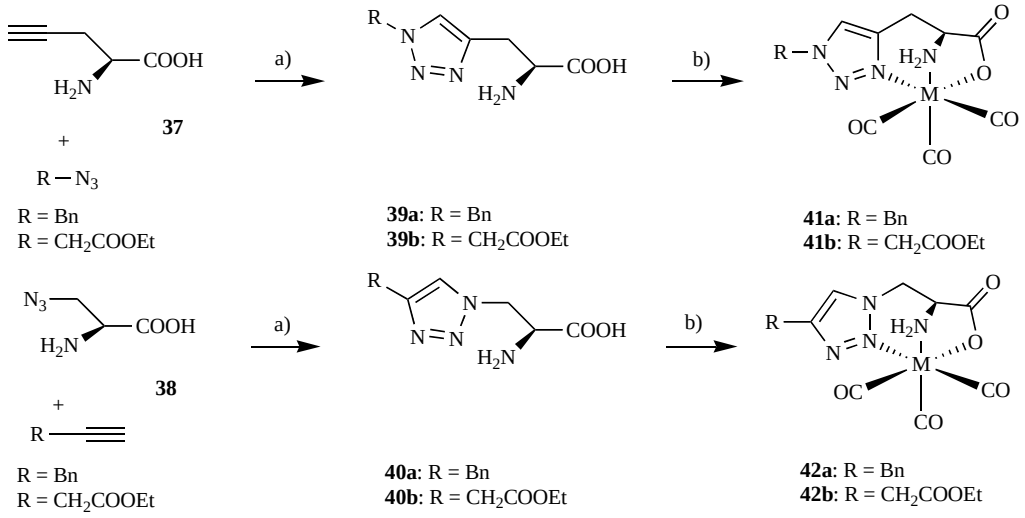


Fig. (19). a) $Cu(OAc)_2$, sodium ascorbate, H_2O, 25 °C (15 h) or 100 °C (30 min); b) $[ReBr_3(CO)_3]^{2-}$, H_2O or EtOH, 50 - 65 °C, 1-4 h or $[^{99m}Tc(H_2O)_3CO_3]^+$, PBS, pH 7.4, 30 min, 100 °C.

ate or attach suitable chelators capable of binding radiometals with high kinetic and thermodynamic stability.

A first application of click chemistry for the design and synthesis of radiometal-based radiotracers was reported by the group of Schibli in 2006 [48]. Click chemistry was used to form novel multidentate ligand scaffolds containing a triazole group for the efficient chelation of a $^{99m}Tc(CO)_3$ core [49,50]. The novel tridentate ligands were all derived from amino acids (L-propargyl glycine **37** or L -azido alanine **38**). Application of the Cu(I)-catalyzed click reaction alkyne-functionalized amino acid **37** and azide-functionalized amino acid **38** gave the desired tridentate 1,4-disubstituted 1,2,3-triazoles **39a, 39b**, **40a** and **40b**. 1,4-Substituted triazoles share similar structural and electronic properties as 1,4-disubstituted imidazoles as found in N^{ε}-derivatized histidines, which are known as excellent donor groups for the complexation of Tc and Re at the oxidation state (I) [51]. To proof the feasibility of the "click to chelate approach" triazole-containing amino acids **39a, 39b**, **40a** and **40b** were used for the complexation of a $[Re(CO)_3]^+$ and $[^{99m}Tc(CO)_3]^+$ core. The general reaction scheme for the synthesis of triazole-containing metal complexes **41a, 41b**, **42a** and **42b** as tridentate chelators is shown in Fig. (**19**).

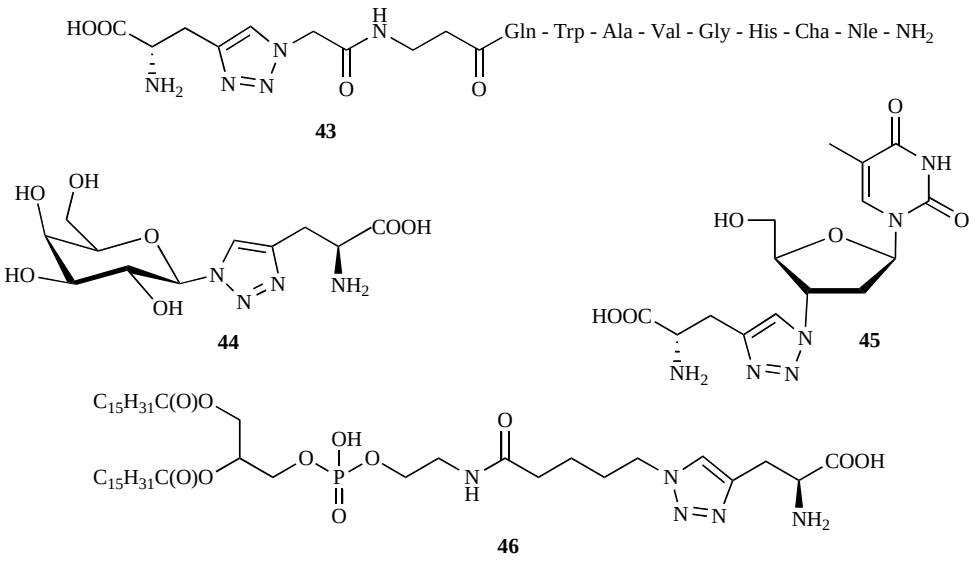
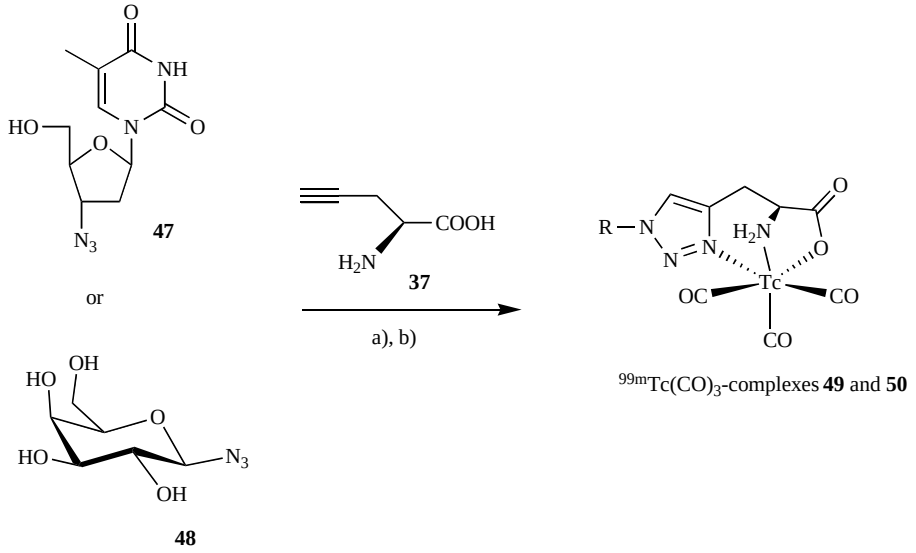


Fig. (20). Functionalized bombesin **43**, D-galactose **44**, thymidine **45** and phosphor-lipid **46** as suitable chelators for $[M(CO_3)]^+$ (M = Re, ^{99m}Tc).



^{99m}Tc(CO)$_3$-complexes **49** and **50**

Fig. (21). a) $Cu(OAc)_2$, sodium ascorbate, 100 °C, 30 min; b) $[^{99m}Tc(H_2O)_3(CO)_3]^+$, 100°C, 30 min.

The approach was further tested by using more complex triazole-containing tridentate ligands, such as modified bombesin **43**, D-galactose **44**, thymidine **45** and phospholipid **46**) for the complexation reaction with $[Re(CO)_3]^+$ or $[^{99m}TcCO_3]^+$ (Fig. **20**).

The prepared triazole-containing chelators proved to be extraodinarily good chelators for rhenium(I)-tricarbonyl and technetium(I)-tricabonyl cores. No complex formation was observed when a mixture of azide and alkyne was used. Based on this observation, further improvement of the approach led to the development of a one-pot synthesis, which avoided isolation of the triazole-containing chelator compound. The feasibility of the one-pot synthesis was exemplified by the reaction of azide-functionalized thymidine **47** and D-galactose **48** with L-propargyl glycine **37** in the presence of Cu(I) followed by the addition of $[^{99m}Tc(CO)_3]^+$ to give the desired ^{99m}Tc-tricarbonyl complexes **49** and **50** (Fig. **21**).

The related radiolabeled bombesin derivative was studied *in vivo* and *in vitro* in terms of stability and receptor affinity. These preliminary experiments revealed higher *in vivo* stability of triazole-containing ^{99m}Tc-tricarbonyl complex compared to a previously tested histidine-based chelate $[^{99m}Tc(CO)_3N^{\alpha}AcHis$-bombesin] [52].

n this context, a side reaction was observed when *N*-Boc-protected L-propargyl glycine **51** was subjected to a click chemistry reaction instead of L-propargyl glycine **37** [53]. Side reactions within a click reaction between azides and terminal alkynes are uncommon. The observed side reaction has not been found before [54]. The free carboxylic acid in *N*-Boc protected L-propargyl glycine **51** undergoes an intromolecular reaction with the alkyne group to form enol lactone **52**. Conversely, the use of the corresponding methyl ester **54** led to the expected triazole **55** (Fig. **22**).

Interestingly, the azide moiety seems not to be involved in the side reaction since the same ratio of enol lactone **52** and keto acid **53** as hydrolysis product was found in absence of azide.

Peptide and protein-based radiopharmaceuticals containing DOTA as a suitable chelator for radiometals are widely used for radiotherapy and diagnostic imaging [55]. Peptides functionalized

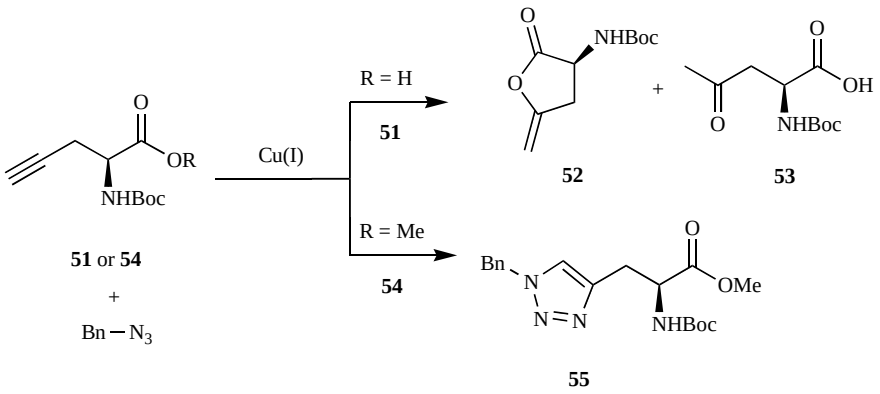


Fig. (22). Side reaction of the Cu(I)-catalyzed click reaction involving *N*-Boc-protected L-propargyl glycine-based compounds **51** and **54**.

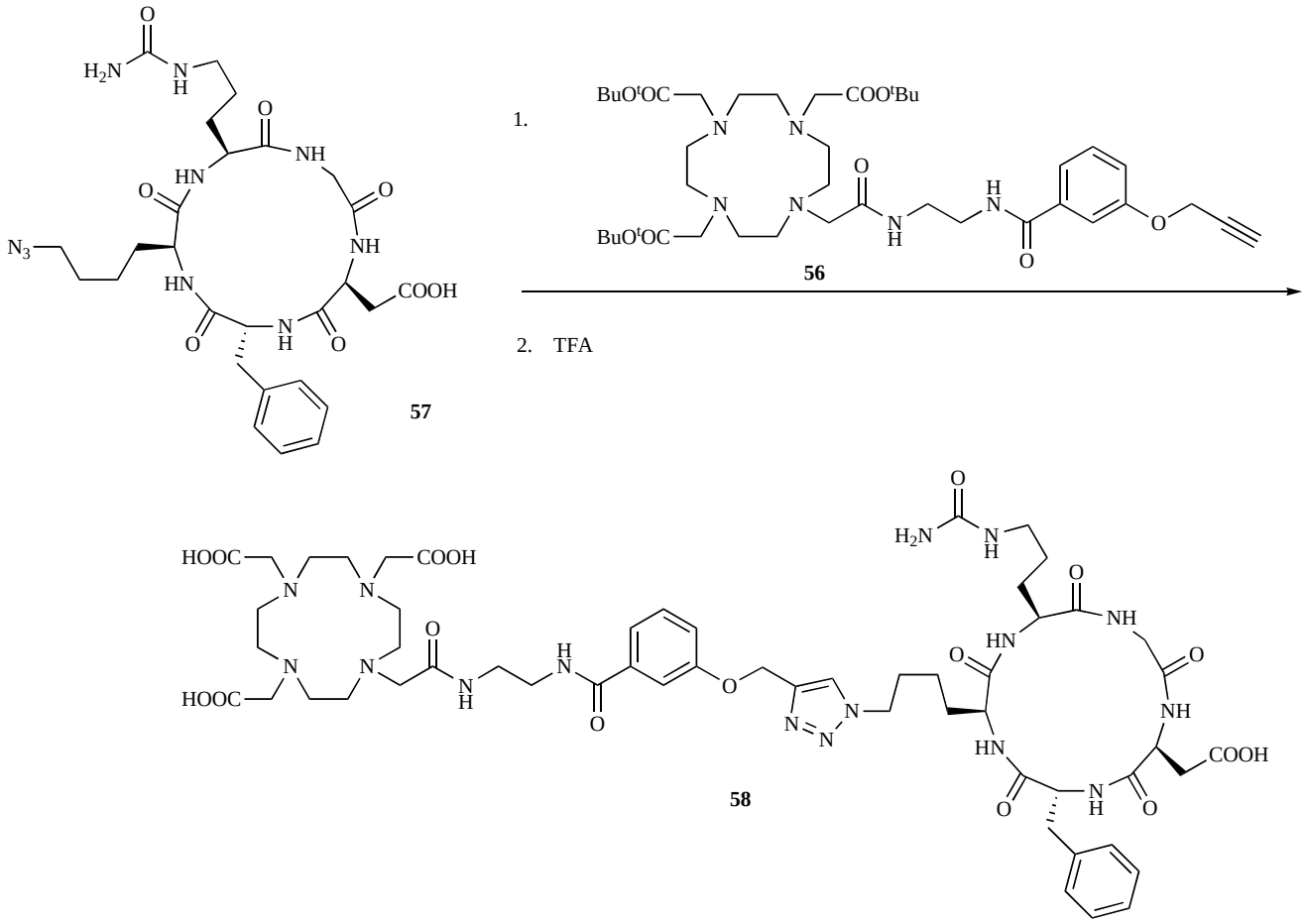


Fig. (23). Synthesis of DOTA-functionalized RGD peptide **58**.

with DOTA are commonly prepared in solution [56] or on solid support through conjugation of the DOTA chelate to the *N*-terminal end of the peptide. To prevent undesired side reactions, protected DOTA derivatives (e.g. DOTA-tris(*tert.*-butyl)ester [57], DOTA-tris(benzyl)ester [58], DOTAGA (*tert.*-butyl) [59], and *p*-NCS-Bz-DOTA [60] have been applied. Application of click chemistry for conjugation of DOTA chelates to peptides and subsequent radiolabeling with radiometals has also been reported recently. The synthesis of DOTA-conjugated multivalent cyclic-RGD peptides via click chemistry and its subsequent radiolabeling with ^{111}In was described by Dijkgraaf *et al.* in 2007 [61].

Conjugation of the DOTA chelate to the RGD peptide was performed by microwave-assisted Cu(I)-catalyzed click chemisty reaction between alkyne **56** and N-ε-azido *cyclo* (Arg-Gly-Asp-D-Phe-Lys) peptide **57**. The desired DOTA-containing peptide **58** was obtained in 14 to 57 % yield after removal of the protecting groups (Fig. **23**). Notably, carboxyl groups of DOTA needs to be protected as *tert.*-butyl esters to avoid complex formation of Cu(II) ions with the DOTA chelate.

The resulting DOTA-containing RGD peptide **58** was labeled with [^{111}In]$InCl_3$. Complexation occurred in NH_4OAc buffer at a pH 6.0. After heating the reaction mixture at 100°C for 15 min the

reaction was completed. Radio-HPLC indicated formation of a single peak corresponding with the desired ^{111}In-labeled peptide.

CONCLUSION

The present review has summarized the current attempts to implement click chemisty into the field of radiopharmaceutical sciences. Based on the pioneering work performed by Huisgen in the late 1960s, the 1,3-dipolar [3+2] cycloaddition witnessed a renaissance through the introduction of a copper(I)-controlled version of the reaction as described by Sharpless. This significant improvement has made click chemistry an attractive approach for the design and synthesis of radiotracers for molecular imaging purposes. Current radiopharmaceutical chemistry increasingly requires application of rapid labeling techniques which can be performed under mild conditions.

In this line, click chemistry was readily adapted into the arsenal of labeling tools for radiotracer synthesis. Click chemistry was shown to be a promising tool for the radiolabeling of several peptides and small molecules like nucleosides and carbohydrates. However, besides peptides and low-molecular weight compounds, other classes of compounds such as proteins and oligonucleotides still await their radiolabeling through click chemistry. Extension of click chemistry-based radiolabeling to these biopolymers would make click chemistry an almost universal applicable radiosynthesis approach. Starting from various simple radiolabeled click chemistry building blocks, click chemistry would allow the chemoselective radiolabeling under mild conditions of a broad range of appropriately functionalized compounds in a very versatile and flexible way.

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