Review Article

18F-Labeling Using Click Cycloadditions

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Received 15 March 2014; Revised 29 April 2014; Accepted 1 May 2014; Published 27 May 2014

Academic Editor: Olaf Prante

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Due to expanding applications of positron emission tomography (PET) there is a demand for developing new techniques to introduce fluorine-18 (¹¹⁹/² = 109.8 min). Considering that most novel PET tracers are sensitive biomolecules and that direct introduction of fluorine-18 often needs harsh conditions, the insertion of ¹⁸F in those molecules poses an exceeding challenge. Two major challenges during ¹⁸F-labeling are a regioselective introduction and a fast and high yielding way under mild conditions. Furthermore, attention has to be paid to functionalities, which are usually present in complex structures of the target molecule. The Cu-catalyzed azide-alkyne cycloaddition (CuAAC) and several copper-free click reactions represent such methods for radiolabeling of sensitive molecules under the above-mentioned criteria. This minireview will provide a quick overview about the development of novel ¹⁸F-labeled prosthetic groups for click cycloadditions and will summarize recent trends in copper-catalyzed and copper-free click ¹⁸F-cycloadditions.

1. Introduction

For the application in positron emission tomography (PET) [1], fluorine-18 provides ideal nuclear physical characteristics for in vivo imaging. Fluorine-18 offers a half-life of 110 min, a β⁻ -branch of 97%, and especially a low β⁺ -energy of 635 keV, which is responsible for a very high spatial resolution [2]. The challenges for researchers are to develop convenient ¹⁸F-labeling strategies, which include short reaction times and applicability for sensitive biomolecules. Especially the harsh conditions during direct ¹⁸F-labeling pose an exceed- ing challenge [3, 4]. Therefore, most of the radiolabeling strategies focus on ¹⁸F-containing prosthetic groups, which allow a sensitive and bioorthogonal ¹⁸F-labeling to treat the multitude of functional groups in those bioactive compounds with respect.

The most established method, which fulfills all mentioned criteria, is given by click reactions. Especially the Cu(I)-catalyzed variant of the Huisgen 1,3-dipolar cycloaddition of terminal alkynes and azides offers a very powerful reaction with high specificity and excellent yields under mild conditions [5]. As a result, numerous PET tracers have been synthesized using CuAAC in a widespread spectrum of structural varieties of the prosthetic group within the last decade. One of the latest investigations deals with a polar clickable amino acid-based prosthetic group to further improve the pharmacokinetic properties of radiotracers, particularly suitable for peptides and proteins [6].

However, the need of cytotoxic copper during CuAAC has led to the necessity of alternative fast and copper-free click reaction strategies for radiofluorination and additionally enabling pretargeting approaches in living systems. Those so-called strain-promoted click reactions can be carried out between cyclooctyne derivatives and azides (strain-promoted azide-alkyne cycloaddition, SPAAC) [7–13] or tetrazines (tetrazine-trans-cyclooctyne (TTCO) ligation) [14–17] as well as between norbornene derivatives and tetrazines [18]. Especially, the TTCO ligation showed promising reaction rates, which makes this click reaction concept very suitable for ¹⁸F-labeling and also for in vivo application in living systems. Very recently, new versions of ¹⁸F-click cycloadditions are added to the range of reactions [19–25]. In this line, the first ¹⁸F-labeled β-lactame became available via a new radio-Kinugasa reaction [21].
As a consequence, click cycloaddition is one of the most frequently applied methods for \(^{18}\text{F}\)-labeling of new bioactive compounds, with or without a catalytic system. This can be impressively illustrated by the fact that over 50 original papers have been published in this research area within the last eight years.

Tables 1–3 give an overview of the \(^{18}\text{F}\)-prosthetic groups, the reaction conditions and reaction partners applied for copper-catalyzed, copper-free and other kinds of \(^{18}\text{F}\)-click cycloadditions, respectively. The most important structures of those prosthetic groups are shown in Figures 1, 3, and 5.

2. Copper-Catalyzed \(^{18}\text{F}\)-Click Cycloadditions

In the last decade, the copper-catalyzed azide alkyne cycloaddition (CuAAC), which has first been reported independently by Rostovtsev et al. [81] and Tornoe et al. [82] in 2002, has spread over almost all fields of chemistry [83–87], biology [88–90], and material science [91, 92]. The great advantage of this method is given by its outstanding efficiency, its regiospecificity, and fast formation of 1,4-disubstituted 1,2,3-triazoles at ambient temperatures, which is particularly suitable for \(^{18}\text{F}\)-labeling of sensitive biomolecules. In particular, the CuAAC enables incorporation of fluorine-18 via a prosthetic group under mild and bioorthogonal conditions [22–25]. 1,2,3-triazoles were first introduced by Michael, who described the formation of a 1,2,3-triazole from a phenylazide in 1893 [93]. Following this pioneering work, Dimroth, Fester, and Huisgen described this type of reaction as a 1,3-dipolar cycloaddition for the first time in 1963 [5].

In 2006, Marik and Sutcliffe published the application of the CuAAC as an \(^{18}\text{F}\)-labeling strategy for the first time [26]. They radiolabeled three different alkyne precursors in radiochemical yields (RCY) of 36–81%. Afterwards they were
Table 1: Summary of the prosthetic groups, reaction conditions, and reaction partners applied for copper-catalyzed click $^{18}\text{F}$-fluorination.

| $^{18}\text{F}$-prosthetic group | Steps/reaction time | RCY$^1$ | Reacting agent | Catalytic system | Overall reaction time | RCY$^1$ | Literature |
|----------------------------------|---------------------|--------|----------------|-----------------|----------------------|--------|------------|
| 4-[18F]fluoroalkynes             | 1 step, 10 min      | 36–81% | N-(3-azidopropionyl) peptides | CuI/NaAsc/DIPEA | 30 min | 54–99% | [26] |
| 4-[18F]fluoro-1-butyne           | 1 step, 15 min      | n.d.  | Glucopyranosyl azide | Cu(I)/Asc/2,6-lutidine | 30 min | 27 ± 6% | n.d. [27] |
| 5-[18F]fluoro-1-pentyne          | 1 step, 15 min      | 45 ± 3% | 2,3,4,6-tetra-O-acetyl-b-D-glucopyranosyl azide | Cu(I)/Asc/2,6-lutidine | 30 min | 52 ± 5% | [28] |
| 5-[18F]fluoro-1-pentyne          | 1 step, 22 min      | 59 ± 6% | α, β specific peptide A20FMDV2 azide | CuI/Asc | 66 min | 8.7 ± 2.3% | [29] |
| 6-[18F]fluoro-1-hexyne           | 1 step, 12 min      | 86 ± 2% | γ-(4-azido-butyl)-folic acid amide | CuI | 1.5 h | 25–35% | [30] |
| 5-[18F]fluoro-1-pentyn 1 step, 15 min | n.d.  | 59 ± 6% | Terminal alkynes | Excess of Cu$^{2+}$/Asc or copper powder | 1 h | 61–98% respectively | 15–98% with copper powder | [31] |
| 1 step, 15 min                  | n.d.  | 4-[18F]fluoro-1-butyne | CuSO$_4$/Asc | n.d.  | 65 ± 6% | [33] |
| 5-[18F]fluoro-1-pentyne          | 1 step, 22 min      | 86 ± 2% | Glucopyranosyl azide | Cu(I)/Asc/2,6-lutidine | 30 min | 52 ± 5% | n.d. [27] |
| 5-[18F]fluoro-1-pentyne          | 1 step, 22 min      | 86 ± 2% | 2,3,4,6-tetra-O-acetyl-b-D-glucopyranosyl azide | Cu(I)/Asc/2,6-lutidine | 30 min | 8.7 ± 2.3% | [29] |
| 5-[18F]fluoro-1-pentyne          | 1 step, 22 min      | 86 ± 2% | γ-(4-azido-butyl)-folic acid amide | CuI | 1.5 h | 25–35% | [30] |
| 5-[18F]fluoro-1-hexyne           | 1 step, 12 min      | 70–85% | Terminal alkynes | Excess of Cu$^{2+}$/Asc or copper powder | 1 h | 61–98% respectively | 15–98% with copper powder | [31] |
| 5-[18F]fluoro-1-hexyne           | 1 step, 12 min      | 70–85% | Terminal alkynes | Excess of Cu$^{2+}$/Asc or copper powder | 1 h | 61–98% respectively | 15–98% with copper powder | [31] |
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| 18F-prosthetic group | Steps/reaction time | RCY | Reacting agent | Catalytic system | Overall reaction time | RCY | Literature |
|----------------------|---------------------|-----|----------------|-----------------|----------------------|-----|------------|
| 18F-Fluoro-PEG-Alkyne | 1 step, 20 min      | 85–94% | Various azides | CuSO4/Asc       | 10–30 min            | 71–99% | [50]       |
|                      | 1 step, 15 min      | 65 ± 1.9% | E(RGDyK)2, azide |                 | 110 min (estimated) | 52 ± 8.3% | [51]       |
|                      |                     | 57%   | Nanoparticle azide | CuSO4/Asc/BPDS | 1 h (estimated)     | 58%  | [52]       |
| [18F]PEG3-azide      | 1 step, 40 min      | 62 ± 4% | N-alkynylated peptide |             | 2 h (estimated)     | 31 ± 6% | [53]       |
|                      | Precursor: 2 steps, labeling: 1 step | 32 ± 5% | Azide-functionalized neurotensin | Cu(I)-TBTA | n.d. | 66% | [56]       |
| [18F]PEG-azide       |                     | n.d.  | N-alkynylated peptide | CuSO4/Asc    | 100 min              | 55–60% | [57]       |
|                      |                     | n.d.  | Azide-functionalized human serum albumin (HSA) | CuBr/TBTA and 2,6-lutidine | 276 min | 77%/55–60%/25% | [58]       |
| 4-[18F]fluoro-N-methyl-N-(prop-2-ynyl)-benzenesulfonamide (p[18F]F-SA) | Precursor: 3 steps, labeling: 1 step, 80 min | 58% | Azide-functionalized DNA | CuAcetate, NaAsc | 2.5 h | 8.5% | [55]       |
| [18F]FPy5yne         | 1 step, 15 min      | 42%  | N3–(CH2)4–CO–YKRI–OH (BG142) | Tetrakis(acetonitrilo) copper(I) hexafluorophosphates/TBTA | 160 min | 18.7% | [59]       |
|                      |                     |      | Azide-functionalized DNA | CuBr/TBTA and 2,6-lutidine | 276 min | 24.6 ± 0.5% | [60]       |
|                      |                     |      | Azide-functionalized RGD peptide | CuSO4/Asc | 125 min | 12–18% | [60]       |
|                      | 2-[18F]fluoro-3-pent-4-yn-1-yloxy pyridine ([18F]FPyKYNE) | 20–25 min | 20–35% |                 | CuBr/TBTA and 2.6-lutidine | 276 min | 24.6 ± 0.5% | [60]       |
| 6-[18F]fluoro-2-ethynyl pyridine | 1 step, 10 min | 27.5 ± 6.6% | D-amino acid analogue of WT-pHLIP azide | Cu-Acetate/NaAsc | 85 min | 5–20% | [61]       |
| propargyl 4-[18F]fluorobenzoate ([18F]PFB) | Precursor: 2 steps, labeling: 1 steps, 15 min | 58 ± 31% | Benzyl azide, two lysine derivatives, transglutaminase-reactive peptide | CuSO4/Asc | 1 h (estimated) | 88 ± 4%, 79 ± 33% and 75 ± 5% | [62]       |
| 4-[18F]fluoro-3-nitro-N-2-propyn-1-yl-benzamide ([18F]FNPB) | 1 step, 40 min | 58% | Azido-peptides cRGDFK and D4 peptide | CuSO4/Asc | 1 h | 87–93% | [63]       |
| 18F-prosthetic group | Steps/reaction time | RCY | Reacting agent | Catalytic system | Overall reaction time | RCY | Literature |
|----------------------|--------------------|-----|----------------|-----------------|----------------------|-----|------------|
| **1-(azidomethyl)-4-[18F]-fluorobenzene** | 4 steps, 75 min | 34% | 4-Ethynyl-L-phenylalanine-peptide | CuI/NaAsc/DIEA | 90 min | 90% | [64] |
| | 4 steps, 75 min | 41% | siRNA alkyny | CuSO4/Asc/TBTA | 120 min | 15% ± 5% | [65] |
| | 1 step, 45 min | 84% | siRNA-linker (two new alkyny-bearing linkers) | CuSO4/Asc | 120 min | 12% | [66] |
| **1-Azido-4-(3-[18F]fluoropropoxy)benzene** | 4 steps, 75 min | 35% | siRNA alkyny | CuSO4/Asc | 120 min | 15% ± 5% | [65] |
| | 1 step, 94–188 s | around 40% | Fmoc-L-propargylglycine | CuSO4/Asc | 1.5 h (estimated) | 60% | [68] |
| **3,4,6-tri-O-acetyl-2-deoxy-2-[18F]fluorogluco-pyranosyl azide** | 1 step, 30 min | 71 ± 10% | Alkyne-functionalized peptides (RDG, neurotensin peptid) | CuSO4/Asc | 75 min | 17–20% n.d.c. | [69] |
| | 2 step, 7.5 min | n.d. | folate alkyny | Cu-Ascate/NaAsc | 3 h | 5–25% | [70] |
| | 1 step, 10 min | 84% | RGD-peptide alkyny | CuSO4/Asc | 70–75 min | 16–24% | [71] |
| | 1 step | 1.3–4.7% | Alkyne-bearing protein | CuBr/TTMA | 80–100 min | 4.1% | [72] |
| | n.d. | | ETAR ligand alkyny | CuSO4/Asc | 70 min | 20–25% n.d.c. | [73] |
| | | | cyanoquinoline (EGFR) alkyny | CuSO4/Asc | 90 min | 8.6 ± 2.3% n.d.c. | [74] |
| **[18F]ArBF3** | 1 step, 20 min | n.d. | Alkyne-functionalized RGD | CuI/Asc | 1 h | n.d. | [75] |
| | 2 steps, | | Alkyne-functionalized bombesin (BBN) | CuI/Asc | 1 h | 20 ± 10% n.d.c. | [76] |
| **piperazine-based [18F]AFP** | AFP: 4 steps, 54 h | 28 ± 5% | N-Fmoc-e-azido-Lnorleucine (amino acid), SNEW peptide | CuSO4, Asc | 30 min | 15–30% | [77] |
| | BFP: 4 steps, 72 h | | | | | | |
| | **[18F]AFP:** 1 step, 40 min | 29 ± 5% | | | | | |
| | **[18F]BFP:** 1 step, 40 min | 31 ± 9% | | | | | |
| **[18F]serine** | 2 steps, 125 min | 28 ± 5% | cRDG-azide | CuSO4, Asc | 145 min | 75% | [6] |

1Calculated as sum from all steps, for the 18F-prosthetic group, respectively, for the overall reaction yielding the click product, starting from fluorine-18.
2Radiochemical yields for the 18F-prosthetic group starting from fluorine-18 for the click reaction, respectively; decay corrected, as long as not noted elsewhere.
CCA: click cycloaddition; (n.d.c.): (not) decay corrected; Asc: ascorbate; DIPEA: diisopropylethylamin; TBTA: tris[1-benzyl-1H-1,2,3-triazol-4-yl]methylamine; n.d.: no data.
Table 2: Summary of the prosthetic groups, reaction conditions, and reaction partners applied for copper-free click fluorination.

| ¹⁸F-prosthetic group | Steps/reaction time¹ | RCY² | Reacting agent | Reaction type/catalytic system | Overall reaction time¹ (CCA) | RCY² CCA | Literature |
|----------------------|----------------------|------|---------------|-------------------------------|-----------------------------|----------|------------|
| [¹⁸F]COT             | 1 step, 15 min       | 71%  | 3,6-diaryl-s-tetrazine | inverse electron-demand DA cyclo-addition | 30 min (without HPLC)       | >98%     | [14]       |
| [¹⁸F]FB-DBCO         | 1 step, 60 min       | 85%  | Various azides | Strain-promoted click 1,3-dipolar cycloaddition | 2 h                         | 69–98%   | [7]        |
| TCO-derivative:      |                      |      |               |                               | 30 min (without HPLC)       | 19–37%   | [8]        |
| Aza-DBCO-BN (bombesin) | 9 steps, —         | 17%  | Three different [¹⁸F]azides |                               | 1.5 h                         | 95%      | [9]        |
| [¹⁸F]DBCO            | 1 step, 1 h          | 21%  | Tyr³-octreotide-N₄(TATE) |                               | 2h                             | 74–98%   | [10]       |
| [¹⁸F]TCO             | [14]                 | [14] | Tetrazine-RGD  | Inverse electron-demand DA cyclo-addition | 30 min                        | 90%      | [15]       |
| [¹⁸F]bi-functional azadienbenzocyclo-octynę | 1 step, 30 min | 24.5% | Alkyl azide | Strain-promoted click 1,3-dipolar cycloaddition | 202 ± 34 min                 | 74 ± 4.8% | [10]       |
| [¹⁸F]PEG₄ azide      | 1 step, 45 min       | 63%  | cRGD-DBCO      | Strain-promoted click 1,3-dipolar cycloaddition | 80 min                       | 92%      | [11]       |
| [¹⁸F]cyclooctyne     | 1 step, 52 min       | 60 ± 17% | Tetrazine (peptide-/bombesin-derivatives) | Strain-promoted click 1,3-dipolar cycloaddition | 82 min (without preparation of [¹⁸F]SFB) | 46–97% (depending on the tetrazine) | [12]       |
| [¹⁸F]trans-cyclooctene ([¹⁸F]TCO) | 1 step, 102 min   | 46.1 ± 12.2% | Tetrazine modified exendin-4 Polymer modified tetrazine | Inverse electron-demand DA cycloaddition | 3 h                         | 46.7 ± 17.3% | [16]       |
| [¹⁸F]amine-functionalised norbornene | 1 step, 80 min      | 60 ± 17% | Tetrazine (peptide-/bombesin-derivatives) | Strain-promoted click 1,3-dipolar cycloaddition | 46–97% (depending on the tetrazine) | 46–97% (depending on the tetrazine) | [17]       |
| [¹⁸F]FBA-C₆-DBCO     | [10]                 | [10] | αᵥᵢβᵥ-specific peptide | Strain-promoted click 1,3-dipolar cycloaddition | click: 40 ± 4 min            | 11.9 ± 3.2% | [13]       |

¹Calculated as sum from all steps, for the ¹⁸F-prosthetic group, respectively, for the overall reaction leading to the click product, starting from fluorine-18.
²Radiochemical yields for the ¹⁸F-prosthetic group starting from fluorine-18 for the click reaction, respectively; decay corrected, as long as not noted elsewhere.
CCA: click cycloaddition; DA: Diels Alder; DBCO: azadienbenzocyclo-octynę; TCO: trans-cyclooctynę.
Table 3: New developments in $^{18}$F-click [3+2] cycloadditions, showing the 1,3-dipolar $^{18}$F-prosthetic groups, reaction type, and conditions.

| $^{18}$F-prosthetic group | Steps/reaction time | RCY | Reacting agent | Reaction type/ catalytic system | Overall reaction time | RCY CCA | Literature |
|---------------------------|---------------------|-----|----------------|-------------------------------|-----------------------|----------|------------|
| C-(4-$^{18}$F)[fluoro-phenyl]-N-phenyl-nitrone | 2 steps/20 min, (labeling of $^{[18F]}$FB-CHO: 1 step, 50 min) | 22–37%<sup>1</sup> ([$^{18F}$]FB-CHO: 30–50%) ([$^{18F}$] F-nitrone: 74%) | Various maleimides | Various dipolarophiles | 80 min (10 min) | 87–91%<sup>1</sup> | [19] |
| 4-$^{18}$F][fluorobenzonitrile oxide | 3 steps/20 min (labeling of $^{[18F]}$FB-CHO: 1 step, 50 min) | 28–46%<sup>1</sup> ([$^{18F}$]FB-CHO: 30–50%) ([$^{18F}$] F-nitro oxide: 92%) | Cycloonyne-indomethacins (COX-2 inhibitor) Maleimide-indomethacins (COX-2 inhibitor) Propyne-indomethacins (COX-2 inhibitor) | 1,3-dipolar [3+2] cyloaddition, no catalyst | 80 min (10 min) | 81%<sup>2</sup> | [20] |
| N-hydroxy-4-$^{18}$Ffluorobenz-imidoyl chloride | 4 steps/20 min (labeling of $^{[18F]}$FB-CHO: 1 step, 50 min) | 27–45%<sup>1</sup> ([$^{18F}$]FB-CHO: 30–50%) ([$^{18F}$] F-nitro oxide: 92%) ([$^{18F}$] F-benzimidoyl Cl: 99%) | Various dipolarophiles | Various maleimides | 85 min (10 min) | 88%<sup>2</sup> | 82%<sup>2</sup> |
| C-(4-$^{18}$F)[fluoro-phenyl]-N-phenyl-nitrone | 2 steps/20 min, (labeling of $^{[18F]}$FB-CHO: 1 step, 50 min) | 22–37%<sup>1</sup> ([$^{18F}$]FB-CHO: 30–50%) ([$^{18F}$] F-nitrone: 74%) | Terminal alkynes methyl propiolate | Terminal alkene propargyl alcohol | 80 min (10 min) | 89% (trans/cis = 2 : 3) | [21] |
| $^{18}$F-fluoro-acetylene | n.d. | n.d. | Terminal alkene propargyl uracil (nucleobase chimera) propiolyl-$^{18}$F-Ala-Phe-OMe (dipeptide) propiolated protein (BSA) 3,6-dihydro-2H-1,4-oxazine-4-oxide | Terminal alkene propargyl alcohol | 80 min (10 min) | 82% (trans/cis = 1 : 3) | [22] |

<sup>1</sup>Calculated as sum from all steps.

<sup>2</sup>Best RCY, obtained only with high precursor amounts.

FB-CHO: 4-fluorobenzaldehyde; CCA: click cycloaddition; PHA: N-phenylhydroxylamine; AscONa: sodium ascorbate; BSA: bovine serum albumin; n.d.: no data.
reacted them with azido-functionalized peptides in RCY of 54–99% and an overall reaction time of 30 min. Thus, they could show a new, very fast, efficient, and mild $^{18}$F-labeling strategy for complex compounds, especially appropriate for sensitive biomolecules. Only two years later, the suitability of this approach was demonstrated for the $^{18}$F-labeling of a folate derivative for in vivo tumor imaging with the same prosthetic group, 6-$^{18}$F-fluoro-1-hexyne [30]. The radiofolate was obtained in RCY of 25–35% and was applied to KB-tumor bearing mice. A specific tumor accumulation could be observed by using the folate receptor (FR) targeting concept. Furthermore, Kim et al. used $^{18}$F-labeled alkynes as prosthetic groups for the $^{18}$F-labeling of 2,3,4,6-tetra-O-acetyl-$^\beta$-D-glucopyranosyl azide [27], which in turn was employed to label the $\alpha_v\beta_3$ specific peptide A20FMDV2 [28].

Considering all known clickable prosthetic groups for $^{18}$F-labeling, $^{18}$F-fluoroethyl azide ([18F]FEA) is certainly one of the most investigated clickable $^{18}$F-prosthetic groups. Until today, about twenty different manuscripts deal with [18F]FEA to radiolabel a broad variety of biomolecules and compounds. In 2007, Glaser and Årstad [31] mentioned for the first time the preparation of [18F]FEA with a RCY of 55% using 2-azidoethyl-4-toluenesulfonate as precursor. As a proof of concept, they reacted [18F]FEA with different terminal alkynes in very good to excellent RCY of 61–98%. With respect to the catalytic system copper sulfate in combination with ascorbic acid or sodium ascorbate has mainly been used, whereas only in a few approaches copper(I) iodide was used [37, 42]. It has been shown that addition of bathophenanthroline disulfonate (Cu(I) stabilizing agent) accelerates the 1,3-dipolar cycloaddition [36, 38, 45]. The very good access to [18F]FEA to radiolabel a broad variety of biomolecules and compounds. In 2007, Glaser and Årstad [31] mentioned for the first time the preparation of [18F]FEA with a RCY of 55% using 2-azidoethyl-4-toluenesulfonate as precursor. As a proof of concept, they reacted [18F]FEA with different terminal alkynes in very good to excellent RCY of 61–98%. With respect to the catalytic system copper sulfate in combination with ascorbic acid or sodium ascorbate has mainly been used, whereas only in a few approaches copper(I) iodide was used [37, 42]. It has been shown that addition of bathophenanthroline disulfonate (Cu(I) stabilizing agent) accelerates the 1,3-dipolar cycloaddition [36, 38, 45]. The very good access to [18F]FEA to radiolabel a broad variety of biomolecules and compounds. In 2007, Glaser and Årstad [31] mentioned for the first time the preparation of [18F]FEA with a RCY of 55% using 2-azidoethyl-4-toluenesulfonate as precursor. As a proof of concept, they reacted [18F]FEA with different terminal alkynes in very good to excellent RCY of 61–98%. With respect to the catalytic system copper sulfate in combination with ascorbic acid or sodium ascorbate has mainly been used, whereas only in a few approaches copper(I) iodide was used [37, 42]. It has been shown that addition of bathophenanthroline disulfonate (Cu(I) stabilizing agent) accelerates the 1,3-dipolar cycloaddition [36, 38, 45]. The very good access to [18F]FEA to radiolabel a broad variety of biomolecules and compounds. In 2007, Glaser and Årstad [31] mentioned for the first time the preparation of [18F]FEA with a RCY of 55% using 2-azidoethyl-4-toluenesulfonate as precursor. As a proof of concept, they reacted [18F]FEA with different terminal alkynes in very good to excellent RCY of 61–98%. With respect to the catalytic system copper sulfate in combination with ascorbic acid or sodium ascorbate has mainly been used, whereas only in a few approaches copper(I) iodide was used [37, 42]. It has been shown that addition of bathophenanthroline disulfonate (Cu(I) stabilizing agent) accelerates the 1,3-dipolar cycloaddition [36, 38, 45].

In 2009, Vaidyanathan et al. [62] presented a prosthetic group based on a 4-$^{18}$F-fluorobenzoate. Propargyl-4-$^{18}$F-fluorobenzoate ([18F]PFB), which could be obtained in RCY of 58 ± 31% within 15 min. To investigate the labeling properties of this new prosthetic group, numerous compounds have been $^{18}$F-labeled using [18F]PFB with RCY from 37% to 88% and overall reaction times of about 1h. Another approach was published by Li et al. in 2012 [63], who synthesized 4-$^{18}$F-fluoro-3-nitro-N-2-propynyl-aryl-benzamide ([18F]FPNB) for $^{18}$F-labeling of cRGDFK and a D4 peptide, which was identified as an EGFR targeting ligand. This approach was followed by the synthesis of 1-(azidomethyl)-4-$^{18}$F-fluorobenzene by Thonon et al. [64]. They did a multistep radiosynthesis (4 steps), where the fluorine-18 was introduced in the first step. The desired radiolabeled product could be obtained in a RCY of 34% within 75 min and was used itself to label a 4-ethynyl-L-phenylalanine-containing peptide. The same prosthetic group was also employed by Mercier et al. [65] and Flaggothier et al. [66] for $^{18}$F-labeling of siRNA. Other structural analog prosthetic groups have also been developed by Mercier et al. [65] and Chun and Pike [67].

To improve the $^{18}$F-labeling of peptides with respect to blood clearance and stability, Maschauer and Prante developed $^{18}$F-glucosyl-derivatives for CuAAC-radiolabeling of Fmoc-L-proparglyglycine with a RCY of 60% [68]. They showed that the $^{18}$F-click labeling reaction was more convenient by using the $\beta$-anomeric derivative of the azides, respectively, alkynes, giving very high RCY of 71 ± 10%. One year later, they published the first in vivo evaluation of an $^{18}$F-labeled RGD peptide labeled with $^{18}$F]FDG-β-Az in U87MG-tumor bearing mice showing an improved blood clearance and stability [65, 66]. Likewise, Fischer et al. demonstrated in 2012 that a $^{18}$F]fluorodeoxyglycosyl folate could be obtained in RCY of 5–25% and subsequent biodistribution and PET-imaging studies showed a high and specific uptake of the radiotracer in FR-positive tumors [70]. The variety of new $^{18}$F-labeling strategies using
18 F-Fluoroglycosylation is the focus of a review article as a part of this special issue provided by Maschauer and Prante [94]. As another promising approach, Li et al. presented in 2013 an alkyne-functionalized aryltri-[18 F]fluoroborate for radiolabeling azido-bombesin and azido-RGD. The major advantage of this method is the two-step, one-pot procedure providing a water-soluble and noncoordinating aryltri-[18 F]fluoroborate anion, which provided specific activities up to 555 GBq/μmol [75, 76, 95].

Two new piperazine-based prosthetic groups, 1-(but-3-ynyl)-4-(3-[18 F]fluoropropyl)piperazine ([18 F]BFP) and 1-(3-azidopropyl)-4-(3-[18 F]fluoropropyl)piperazine ([18 F]AFP), have recently been developed by Pretze and Mamat [78]. Spiro salts were used as precursors, facilitating purification by using solid phase extractions (RP-18 or SiO2-cartridges). Both prosthetic groups could be obtained in RCY of about 30% using an automated synthesis module. To avoid Glaser coupling, which has been observed by using [18 F]BFP for radiolabeling of peptides, [18 F]AFP was used instead. An important observation was the fact that the applied peptide formed very strong complexes with the copper catalyst, which required the use of bispidine as a strong chelating agent to remove cytotoxic copper species.

One of the latest developments describes the synthesis of an 18 F-labeled alanine derivative as a new prosthetic click group, reported by Schiererstein and Ross [6]. In this case, an amino acid-based prosthetic group has been developed to improve the pharmacokinetic profile of 18 F-click-labeled biomolecules. The prosthetic group was obtained in good RCY of 28 ± 5% from a two-step reaction as described in Figure 2. The final 18 F-labeled prosthetic group was subsequently reacted with an azido-RGD as model system in RCY of 75% within 20 min.

Considering the above-mentioned prosthetic groups for radiolabeling with fluorine-18, Table 1 summarizes important properties of those components. It has been shown that the integration of an 18 F-propyl, 18 F-ethyl, or 18 F-aryl moiety can provide an improved metabolic profile and that the glycosylation or PEGylation can further improve the in vivo behavior. Furthermore, for in vivo application a total removal of the copper catalyst is essential. This could be very challenging in the case where peptides or proteins are able to complex copper species from the catalytic system.

3. Copper-Free 18 F-Click Cycloadditions

Even though a large number of novel radiotracers using click chemistry have been developed, none of them has entered clinical routine to date, apart from 18 F-RGD-K5, which is already used in clinical trials in US. This can be explained by the need of cytotoxic copper during radiotracer syntheses by using copper-catalyzed 1,3-dipolar Huisgen cycloadditions [96]. Thus, there is still a demand for facile (metal-free) and robust 18 F-labeling reactions for the syntheses of radiotracers for imaging of malignancies in vivo. This leads to the development of catalyst-free click-labeling approaches, which spare copper species during labeling steps and even enable in vivo pretargeting concept. Recent developments deal with biocompatible strain-promoted copper-free versions of the alkyne-azide cycloaddition (SPAAC), where the focus has been set on derivatives of cyclooctynes and dibenzyclooctynes. First approaches focus on the reaction of 18 F-labeled cyclooctynes with azide-bearing biomolecules. On the other hand, in further approaches cyclooctyne-carrying bioactive compounds are used, which can be labeled with different 18 F-labeled azides. In the beginning, only a few studies have been reported due to the complex and low yielding syntheses of strained cyclooctynes [10, 12, 14]. However, nowadays lots of cyclooctyne derivatives are commercially available, which facilitates the precursor syntheses and opens a wide range of applications.

In 2011 Bouvet et al. [7] published the first example of a SPAAC with 18 F-labeled aza-dibenzocyclooctyne, [18 F]FB-DBCO, and a plethora of azides. The 18 F-labeled building block was synthesized via acylation of commercially available N-(3-aminopropionyl)-5,6-dihydro-11,12-didehydrodibenzo[b,f]azocine with N-succinimidyl-4-[18 F]fluorobenzoate ([18 F]SBF), which can be easily prepared in an automated synthesis module [97]. The 18 F-labeled cyclooctyne could be obtained in a RCY of 85% and a purity >95% within 60 min. The evaluation of this building block in healthy Balb/C mice showed 60% of intact compound at 60 min p.i. and had a blood clearance half-life of 53 s. Besides,
the compound was stable in methanol and phosphate buffer over 60 min. Subsequently, $[^{18}\text{F}]\text{FB-DBCO}$ was reacted with various azides as proof of principle showing different structural complexities. In all reactions, the formation of two regioisomers (1,4- and 1,5-triazole) has been observed and in some cases a separation of the regioisomers by HPLC was impossible. All $[^{18}\text{F}]$-labeled radiotracers were obtained in good to excellent RCY of 69–98% within an overall reaction time of about 2 h. However, the reaction rates in these cases were much slower compared to other examples of bioorthogonal reactions, limiting this new approach for in vivo pretargeting applications.

A cyclooctyne derivative has been conjugated to bombesin (aza-DBCO-BN, 9 steps) with an overall yield of 17% by Campbell-Verduyn et al. [8]. The aza-DBCO-BN was reacted with various $^{18}\text{F}$-azides giving RCY of 19–37% within 30 min. In 2011, Arumugam et al. [9] investigated the direct $^{18}\text{F}$-labeling of azadibenzylocyclooctyne (DBCO) yielding the $^{18}\text{F}$-labeled prosthetic group (RCY = 36%). The radiolabeling was followed by a click reaction with an azido-octreotide leading to the $^{18}\text{F}$-labeled octreotide in a RCY of 95% within a total reaction time of 1.5 h. In contrast, other working groups used $^{18}\text{F}$-cyclooctynes for labeling RDG-derivatives [11] as well as further integrin-specific peptides [10, 13].

Another possibility to perform copper-free click reactions is given by the inverse electron demand of the Diels Alder cycloaddition between a cyclooctene and a tetrázine under the release of nitrogen. The so-called tetrázine-trans-cyclooctene ligation (TTCO ligation) was first published by Li et al. in 2010 [14]. Concerning the instability of the tetrázines, it is more practical to functionalize the biomolecule with a tetrázine followed by the reaction with an $^{18}\text{F}$-labeled cyclooctene. The latter are much more suitable for direct $^{18}\text{F}$-labeling than tetrázines. For this purpose a nosylate precursor was used for $^{18}\text{F}$-labeling of the cyclooctene providing RCY of 71% within 15 min. To investigate the suitability of the $^{18}\text{F}$-prosthetic group in click reactions, the $^{18}\text{F}$-cyclooctene was reacted with a 3,6-di(2-pyridyl)-S-tetrázine in an excellent RCY of 98% within 10 s, showing its outstanding feasibility for in vivo pretargeting approaches. These fast reaction rates made this approach very attractive that even $^{11}\text{C}$-labeling reaction was explored using the inverse electron demand Diels Alder cycloaddition between a cyclooctene and a tetrázine [98]. In 2011, $^{18}\text{F}$-labeled cyclooctene was linked to a tetrázine-RGD derivative by Selvaraj et al. [15] with a RCY of 90% within 5 min at room temperature. The resulting $^{18}\text{F}$-labeled tracer was tested in in vivo experiments showing a high tumor accumulation, which could selectively be blocked. In 2012, the group of Devaraj et al. [80] published for the first time the in vivo click reaction of $[^{18}\text{F}]$trans-cyclooctene and a polymer-modified tetrázine (PMT). The radiolabeled peptide $^{18}\text{F}$-PMT10 could be obtained in a RCY of 89.2%. Whole body animal PET scans were carried out 3 h p.i., showing renal clearance and a widespread tissue distribution as can be seen in Figure 4. Previously, the same group described the synthesis of an $^{18}\text{F}$-labeled cyclooctene with a RCY of 46.1 ± 12.2%. Subsequently, this prosthetic group was clicked with a tetrázine-modified exendin-4 in RCY of 46.7 ± 17.3% [16].

A similar strategy was published by Knight et al. in 2013, where an $^{18}\text{F}$-labeled amino-functionalized norbornene was reacted with a tetrázine-modified peptide [18]. The $^{18}\text{F}$-labeled norbornene was obtained using N-succinimicyl-4-$[^{18}\text{F}]$fluorobenzoate ($[^{18}\text{F}]$SFB) in RCY of 60 ± 17% within 52 min. As a proof of concept, two different tetrázines, an asymmetric dipryridyl tetrázine, and a tetrázine-modified bombesin peptide were labeled with $^{18}\text{F}$-labeled norbornene derivative ($[^{18}\text{F}]$NFB) in 46–97% RCY within 82 min.
Figure 4: PET and autoradiography using $^{18}$F-tetrazine agents. (a) PET/CT fusion of LS174T tumor xenograft labeled using either trans-cyclooctene (TCO) monoclonal antibodies (mAb TCO) or control unlabeled antibodies (mAb) followed by $^{18}$F-PMT10 (polymer-modified tetrazine). Arrows indicate location of the tumor xenograft. The bladder was omitted for clarity. (b) Imaging using autoradiography (left side) and fluorescence slices after targeting with fluorescence TCO monoclonal antibody and $^{18}$F-PMT10. (c) PET/CT fusion of mouse bearing A431 and LS174T tumors after targeting with anti-A33 TCO monoclonal antibodies followed by $^{18}$F-PMT10. Arrows indicate location of tumors and the liver was omitted for clarity. (d) Autoradiography of representative 1 mm LS174T and A431 tumor slices after multistep targeting (reprinted with permission from [80]; Copyright 2012 National Academy of Sciences of the United States of America).

Figure 5: Lead structures of new $^{18}$F-prosthetic groups applied for click $^{18}$F-fluorination.

C-(4-$^{18}$F)fluorophenyl)-N-phenylnitrite

$^{18}$F

4-$^{18}$F-fluorobenzenitrile oxide

OH

N

$^{18}$F

N-hydroxy-4-$^{18}$F-fluorobenzimidoylchloride

OH

$^{18}$F

$\sigma-/p-$-$^{18}$F-fluorophenylacetylen
Considering the copper-free click labeling of bioactive compounds with fluorine-18, both the strain-promoted alkyne-azide cycloaddition (SPAAC) and the tetrazine-\textit{trans}-cyclooctyne ligation (TTCO ligation) show promising results. Regarding \textit{in vivo} pretargeting approaches, only the TTOC ligation showed favorable results and reaction rates, which are suitable for this application [80]. Table 2 summarizes reaction conditions, radiochemical yields, and reaction partners of those components.

**4. New Developments in 18F-Click Cycloadditions**

The latest developments in metal-free 18F-click cycloadditions have been reported by Zlatopolskiy et al. [19–21] (Table 3). In a first approach, the 18F-labeled building block C-(4-[18]F)fluorophenyl)-N-phenyl nitrone was developed to form 18F-isoxazolines via high-yielding [3+2]cycloadditions with various maleimides [19]. C-(4-[18]F)fluorophenyl)-N-phenyl nitrone was obtained from the reaction of 4-([18]F)fluorobenzaldehyde and N-phenylhydroxylamine in high RCY of 74% with 10 min. In the subsequent click cycloaddition step, differently substituted maleimides as model dipolarophiles were used to form the corresponding isoxazolines as enendo- or exo-isomers in high yields of up to >90% within 10 min. A one-pot strategy with \textit{in situ} generation of C-(4-[18]F)fluorophenyl)-N-phenyl nitrone provided the desired 18F-isoxazolines only in moderate yields of 25% and only after heating to 110°C. Under optimized conditions, 18F-isoxazolines were obtained from fast 18F-click [3+2]cycloadditions.

In further studies, the same group used 4-([18]F)fluorobenzenitrile oxide instead of C-(4-[18]F)fluorophenyl)-N-phenyl nitrone as 1,3-dipol for milder reaction conditions [20] (Table 3). 4-([18]F)fluorobenzenitrile oxide was obtained in 92% RCY within 10 min from the reaction of 4-([18]F)fluorobenzaldehyde (RCY: 30–50%, 50 min [99]) with hydroxylamine and subsequent treatment with phenyl iodine bis(trifluoroacetate).

After the click [3+2]cycloaddition to various 18F-labeled model 2-isoxazolines and isoxazoles was successfully tested, the novel method was applied to three different COX-2 inhibitors (indomethacin conjugates) carrying dipolarophilic moieties of cycloynyne, maleimide, and propyne. The resulting products were obtained in moderate to excellent RCY of 81%, 55%, and 35%, respectively. It is noteworthy that, for the propyne derivative, the milder oxidant [bis(acetoxy)iodo]benzene was used to avoid decomposition. Finally, the method was successfully adapted for 18F-labeling of two model dipeptide conjugates, cycloynyne- and norbornene-\textit{β}-Ala-Phe-OMe. However, the original cycloaddition using 4-([18]F)fluorobenzenitrile oxide did only provide traces of the desired products. Consequently, 4-([18]F)fluorobenzonitrile oxide was further treated with chloramine T (CAT) \textit{in situ} forming the more stable building block N-hydroxy-4-[18]F)fluorobenzimidoyl chloride. With the use of high precursor (peptides) amounts, the latter enabled excellent RCY of the 18F-labeled dipeptides of up to 88% within 10 min at room temperature [20]. Under optimized conditions low precursor amounts of 5 nmol (cycloynyne) and 50 nmol (norbornene-\textit{β}-Ala-Phe-OMe) still allowed RCY of 56% and 47%, respectively.

In a very recent report, Zlatopolskiy and coworkers applied their 18F-labeled nitrone, C-(4-[18]F)fluorophenyl)-N-phenyl nitro, for the first formation of 18F-labeled \textit{β}-lactames via the Cul-catalyzed Kinugasa reaction [21] (Table 3). The optimized reactions went smooth under very mild conditions to give the 18F-labeled model \textit{β}-lactames in high RCY and various isomeric mixtures of the trans- and cis-product. In dependency on the reactivity of the terminal alkynes, the reaction parameters needed (individual) optimization regarding catalyst system, solvent, temperature, and Cull-stabilizing ligands. As a biologically relevant molecule the 18F-labeled nucleobase chimera was synthesized as potential PET-imaging agent for bacterial infections.

Moreover, the dipeptide \textit{β}-Ala-Phe-OMe was propiolated and used in this radio-Kinugasa reaction to give excellent RCY of 85% of the 18F-labeled dipeptide under very mild conditions (aqueous solution, room temperature) [21]. Similarly, this new method was successfully transferred to the 18F-labeling of proteins. Bovine serum albumin (BSA) was conjugated with 3-propiolamidopropyl chloroformate. This propiolated BSA was successfully radiolabeled with fluorine-18 in the radio-Kinugasa reaction.

**5. Conclusions**

The field of click cycloadditions had and still has a major impact in 18F-labeling chemistry. The very mild reaction conditions mostly applicable and the excellent efficiency of all types of these reactions are particularly suitable for 18F-labeling. Especially, complex and sensitive biomolecules benefit from this methodology. No protection group chemistry is needed and the 18F-click cycloaddition step provides the final radiotracer.

Besides several new 18F-labeled radiotracers are available via click cycloadditions, and the metal-free versions even enabled pretargeting concepts by \textit{in vivo} click. The latest development of a radio-Kinugasa reaction towards the first 18F-\textit{β}-lactames demonstrates the highly active field and the broad applicability of 18F-click cycloadditions.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**References**

[1] M. E. Phelps, "Positron emission tomography provides molecular imaging of biological processes," \textit{Proceedings of the National Academy of Sciences of the United States of America}, vol. 97, no. 16, pp. 9226–9233, 2000.
[2] J. S. Fowler and A. P. Wolf, “The synthesis of carbon-11, fluorine-18 and nitrogen-13 labeled radiotracers for biomedical applications,” Bnl-31222 de82 013799.

[3] H. H. Coenen, K. Franken, P. Kling, and G. Stocklin, “Direct electrophilic radiofluorination of phenylalanine, tyrosine and dopa,” Applied Radiation and Isotopes, vol. 39, no. 12, pp. 1243–1250, 1988.

[4] L. Lang and W. C. Eckelman, “One-step synthesis of 18F labeled [18F]-N-succinimidyl 4-(fluoroethyl)benzoate for protein labeling,” Applied Radiation and Isotopes, vol. 45, no. 12, pp. 1155–1163, 1994.

[5] R. Huisingh, “1,3-dipolar cycloadditionen,” Angewandte Chemie, no. 13, pp. 604–637, 1963.

[6] H. Schieferstein and T. L. Ross, “A Polar 18F-labeled amino acid derivative for click-labeling of biomolecules,” European Journal of Organic Chemistry, 2014.

[7] V. Bouvet, M. Wuest, and F. Wuest, “Copper-free click chemistry with the short-lived positron emitter fluorine-18,” Organic & Biomolecular Chemistry, vol. 9, no. 21, pp. 7393–7399, 2011.

[8] L. S. Campbell-Verdun, L. Mirfeizi, A. K. Schoonen, R. A. Diercks, P. H. Elsinga, and B. L. Feringa, “Strain-promoted copper-free “click” chemistry for 18F radiolabeling of bombesin,” Angewandte Chemie International Edition, vol. 50, no. 47, pp. 11117–11120, 2011.

[9] S. Arumugam, J. Chin, R. Schirrmacher, V. V. Popik, and A. P. Kostikov, “[18F]azidobenzocyclooctyne ([18F]ADIBO): a biocompatible radioactive labeling synthesis for peptides using catalyst free [3+2] cycloaddition,” Bioorganic & Medicinal Chemistry Letters, vol. 21, no. 23, pp. 6987–6991, 2011.

[10] R. D. Carpenter, S. H. Hausner, and J. L. Sutcliffe, “Copper-free click for PET: rapid 1,3-dipolar cycloadditions with a fluorine-18 cyclooctyne,” ACS Medicinal Chemistry Letters, vol. 2, no. 12, pp. 885–889, 2011.

[11] K. Sachin, V. H. Jadhav, E.-M. Kim et al., “F-18-labeling protocol of peptides based on chemically orthogonal strain-promoted cycloaddition under physiologically friendly reaction conditions,” Bioconjugate Chemistry, vol. 23, no. 8, pp. 1680–1686, 2012.

[12] H. L. Evans, R. L. Slade, L. Carroll et al., “Copper-free click—a promising tool for pre-targeted PET imaging,” Chemical Communications, vol. 48, no. 7, pp. 991–993, 2012.

[13] S. H. Hausner, R. D. Carpenter, N. Bauer, and J. L. Sutcliffe, “Evaluation of an integrin αvβ3-specific peptide labeled with [18F]fluorine by copper-free, strain-promoted click chemistry,” Nuclear Medicine and Biology, vol. 40, no. 2, pp. 233–239, 2013.

[14] Z. Li, H. Cai, M. Hassink et al., “Tetrazine-trans-cyclooctene ligation for the rapid construction of 18F labeled probes,” Chemical Communications, vol. 46, no. 42, pp. 8043–8045, 2010.

[15] R. Selvaraj, S. Liu, M. Hassink et al., “Tetrazine-trans-cyclooctene ligation for the rapid construction of integrin αvβ3 targeted PET tracer based on a cyclic RGD peptide,” Bioorganic & Medicinal Chemistry Letters, vol. 21, no. 17, pp. 5011–5014, 2011.

[16] E. J. Kelhier, T. Reiner, G. M. Thurban, R. Upadhyay, and R. Weissleder, “Efficient 18F-labeling of synthetic exendin-4 analogues for imaging beta cells,” ChemistryOpen, vol. 1, no. 4, pp. 177–183, 2012.

[17] N. Devaraj, “Advancing tetrazine bioorthogonal reactions through the development of new synthetic tools,” Synlett, vol. 23, no. 15, pp. 2147–2152, 2012.

[18] J. C. Knight, S. Richter, M. Wuest, J. D. Way, F. Wuest, and “Synthesis a, “nd evaluation of an 18F-labelled norbornene derivative for copper-free click chemistry reactions,” Organic & Biomolecular Chemistry, vol. 11, no. 23, pp. 3817–3825, 2013.

[19] B. D. Zlatopoulos, R. Kandler, F. M. Mottaghy, and B. Neumaier, “C-(4-[18F]fluorophenyl)-N-phenyl nitro: a novel 18F-labeled building block for metal free [3+2]cycloaddition,” Applied Radiation and Isotopes, vol. 70, no. 1, pp. 184–192, 2012.

[20] B. D. Zlatopoulos, R. Kandler, D. Kobus, F. M. Mottaghy, and B. Neumaier, “Beyond azide-alkyne click reaction: easy access to 18F-labeled compounds via nitrile oxide cycloadditions,” Chemical Communications, vol. 48, no. 57, pp. 7134–7136, 2012.

[21] B. D. Zlatopoulos, P. Krapf, R. Richarz, H. Frauendorf, F. M. Mottaghy, and B. Neumaier, “Synthesis of 18F-labelled β-lactams by using the kinugasa reaction,” Chemistry: A European Journal, vol. 20, pp. 4697–4703, 2014.

[22] R. Schirrmacher, C. Wängler, and E. Schirrmacher, “Recent developments and trends in 18F-radiopharmaceuticals: syntheses and applications,” Mini-Reviews in Organic Chemistry, vol. 4, no. 4, pp. 317–329, 2007.

[23] M. Glaser and E. G. Robins, ““Click labelling” in PET radiochemistry,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 52, no. 10, pp. 407–414, 2009.

[24] T. L. Ross, “The click chemistry approach applied to fluorine-18,” Current Radiopharmaceuticals, vol. 3, no. 3, pp. 202–223, 2010.

[25] M. Pretze, D. Pietzsch, and C. Mammat, “Recent trends in bioorthogonial click-radiolabeling reactions using fluorine-18,” Molecules, vol. 18, no. 7, pp. 8618–8665, 2013.

[26] J. Marik and J. L. Sutcliffe, “Click for PET: rapid preparation of [18F]fluorophenylcyclooctyne,” Tetrahedron Letters, no. 47, no. 37, pp. 6681–6684, 2006.

[27] D. H. Kim, Y. S. Choe, K.-H. Jung et al., “A 18F-labeled glucose analog: synthesis using a click labeling method and in vitro evaluation,” Archives of Pharmacal Research, vol. 31, no. 5, pp. 587–593, 2008.

[28] D. H. Kim, Y. S. Choe, and B.-T. Kim, “Evaluation of 4-[18F]fluoro-1-butyne as a radiolabeled synthon for click chemistry with azido compounds,” Applied Radiation and Isotopes, vol. 68, no. 2, pp. 329–333, 2010.

[29] S. H. Hausner, J. Marik, M. K. J. Gagnon, and J. L. Sutcliffe, “In vivo positron emission tomography (PET) imaging with an αvβ3 specific peptide radiolabeled using 18F-click chemistry: evaluation and comparison with the corresponding 4-[18F]fluorobenzoyl- and 2-[18F] fluoropropionyl-peptides,” Journal of Medicinal Chemistry, vol. 51, no. 19, pp. 5901–5904, 2008.

[30] T. L. Ross, M. Honer, P. Y. H. Lam et al., “Fluorine-18 click radiosynthesis and preclinical evaluation of a new 18F-labeled folic acid derivative,” Bioconjugate Chemistry, vol. 19, no. 12, pp. 2462–2470, 2008.

[31] M. Glaser and E. Årstad, ““Click labeling” with 2-[18F]fluoroethylazide for positron emission tomography,” Bioorganic & Medicinal Chemistry, vol. 18, no. 3, pp. 989–993, 2007.

[32] D. Kobus, Y. Giesen, R. Ullrich, H. Backes, and B. Neumaier, “A fully automated two-step synthesis of an 18F-labelled tyrosine kinase inhibitor for EGFR kinase activity imaging in tumors,” Applied Radiation and Isotopes, vol. 67, no. 11, pp. 1977–1984, 2009.

[33] G. Smith, M. Glaser, M. Perumal et al., “Design, synthesis, and biological characterization of a caspase 3/7 selective isatin labeled with 2-[18F]fluoroethylazide,” Journal of Medicinal Chemistry, vol. 51, no. 24, pp. 8057–8067, 2008.
[34] M. Glaser, M. Solbakken, D. R. Turton et al., "Methods for 18F-labeling of RGD peptides: comparison of aminoxy [18F] fluorobenzaldehyde condensation with “click labeling” using 2-[18F]fluoroethylazide, and S-alkylation with [18F] fluoro-panethiol,” Amino Acids, vol. 37, no. 4, pp. 717–724, 2009.

[35] F. Pisaneschi, Q.-D. Nguyen, E. Shamsaei et al., “Development of a new epidermal growth factor receptor positron emission tomography imaging agent based on the 3-cyanoquinoline core: synthesis and biological evaluation,” Bioorganic and Medicinal Chemistry, vol. 18, no. 18, pp. 6634–6645, 2010.

[36] M. Glaser, J. Goggi, G. Smith et al., “Improved radiosynthesis of [18F]F-ICMT11 including biological evaluation,” Bioorganic & Medicinal Chemistry Letters, vol. 21, no. 23, pp. 6945–6949, 2011.

[37] U. Ackermann, G. O’Keefe, S.-T. Lee et al., “Synthesis of [18F]fluoroethyltriazole labelled [Tyr3]octreotate from [18F]F-labelled neurotensin(8-13) via copper-mediated 1,3-dipolar cycloaddition reaction,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 54, no. 5, pp. 260–266, 2011.

[38] L. Zhou, W. Chu, C. S. Dence, R. H. Mach, and M. J. Welch, “Highly efficient click labeling using 2-[18F]fluoroethyl azide and synthesis of an 18F-N-hydroxysuccinimide ester as conjugation agent,” Nuclear Medicine and Biology, vol. 39, no. 8, pp. 1175–1181, 2012.

[39] R. Bejot, L. Carroll, K. Bhakoo, J. Declercck, and V. Gouverneur, “Fluoro-N-methyl-N-(propyl-2-yn-1-yl)benzenesulphonamide ([18F]F-SA): a versatile building block for labeling of peptides, proteins and oligonucleotides with fluorine-18 via Cu(I)-mediated click chemistry,” Amino Acids, vol. 44, no. 4, pp. 1167–1180, 2013.

[40] J. A. H. Inkster, B. Guérin, T. J. Ruth, and M. J. Adam, “Radiosynthesis and bioconjugation of [18F]FPySyn, a prosthetic group for the 18F-labeling of bioactive peptides,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 51, no. 14, pp. 444–452, 2008.

[41] A. C. Valdivia, M. Estrada, T. Hadizad, D. J. Stewart, R. S. Beanlands, and J. N. Dasilva, “A fast, simple, and reproducible automated synthesis of [18F]FPeKYNE-(cRGDyK) for αvβ3 receptor positron emission tomography imaging,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 55, no. 2, pp. 57–60, 2012.

[42] L. Jia, Z. Cheng, L. Shi et al., “Fluorine-18 labeling by click chemistry: multiple probes in one pot,” Applied Radiation and Isotopes, vol. 75, pp. 64–70, 2013.

[43] F. Pisaneschi, Q.-D. Nguyen, E. Shamsaei et al., “Fluoroethylazide and 5-ethynyl-2-fluoroethyl azide and 5-ethynyl-2-deoxyuridine,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 56, no. 6, pp. 313–316, 2013.

[44] A. Haslop, A. Gee, C. Plisson, and N. Long, “Fully automated radiosynthesis of [1-(2-[18F]fluoroethyl)-1H][1,2,3]triazole 4-ethylenetricophosphonium bromide as a potential positron emission tomography tracer for imaging apoptosis,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 56, no. 6, pp. 313–316, 2013.
[63] Y. Li, Y. Liu, L. Zhang, and Y. Xu, "One-step radiosynthesis of 4-[18\text{F}]fluoro-3-nitro-N-2-propyn-1-yl-benzenamide ([18\text{F}]FNPB): a new stable aromatic porosorhetic group for efficient labeling of peptides with fluorine-18," Journal of Labelled Compounds and Radiopharmaceuticals, vol. 55, no. 6, pp. 229–234, 2012.

[64] D. Thonon, C. Kech, J. Paris, C. Lemaire, and A. Luxen, "New strategy for the preparation of clickable peptides and labeling with 1-(azidomethyl)-4-[18\text{F}] fluorobenzene for PET," Bioconjugate Chemistry, vol. 20, no. 4, pp. 817–823, 2009.

[65] F. Mercier, J. Paris, G. Kaisin et al., "General method for labeling siRNA by click chemistry with fluorine-18 for the purpose of PET imaging," Bioconjugate Chemistry, vol. 22, no. 1, pp. 108–114, 2011.

[66] J. Flagothier, G. Kaisin, F. Mercier et al., "Synthesis of two new alkyne-bearing linkers used for the preparation of siRNA for labeling by click chemistry with fluorine-18," Applied Radiation and Isotopes, vol. 70, no. 8, pp. 1549–1557, 2012.

[67] J.-H. Chun and V. W. Pike, "Single-step radiosynthesis of [18\text{F}] labeled click synthons from azide-functionalized diarylidoconjugates," European Journal of Organic Chemistry, vol. 2012, no. 24, pp. 4541–4547, 2012.

[68] S. Maschauer and O. Prante, "A series of 2-O-trifluoromethyl-ylsulfonyl-d-mannopyranosides as precursors for concomitant 18\text{F}-labeling and glycosylation by click chemistry," Carbohydrate Research, vol. 344, no. 6, pp. 753–761, 2009.

[69] S. Maschauer, J. Einsiedel, R. Haubner et al., "Labeling and glycosylation of peptides using click chemistry: a general approach to 18\text{F}-glycopeptides as effective imaging probes for positron emission tomography," Angewandte Chemie International Edition, vol. 49, no. 5, pp. 976–979, 2010.

[70] C. R. Fischer, C. Müller, J. Reber et al., "[18\text{F}]fluoro-deoxyglucose folate: a novel PET radiotracer with improved in vivo properties for folate receptor targeting," Bioconjugate Chemistry, vol. 23, no. 4, pp. 805–813, 2012.

[71] S. Maschauer, R. Haubner, T. Kuwert, and O. Prante, "[18\text{F}]Glyco-RGD peptides for PET imaging of integrin expression: efficient radiosynthesis by click chemistry and modulation of biodistribution by glycosylation," Molecular Pharmaceutics, vol. 11, no. 2, pp. 505–515, 2014.

[72] O. Bouteireira, F. D’Hooge, M. Fernández-González et al., "Fluoroglycoproteins: ready chemical site-selective incorporation of fluorosugars into proteins," Chemical Communications, vol. 46, no. 43, pp. 8142–8144, 2010.

[73] S. Maschauer, K. Michel, P. Tripal et al., "Synthesis and in vivo evaluation of an 18\text{F}-labeled glycoconjugate of PD156707 for imaging ETA receptor expression in thyroid carcinoma by positron emission tomography," American Journal of Nuclear Medicine and Molecular Imaging, vol. 3, no. 5, pp. 425–436, 2013.

[74] F. Pisaneschi, R. L. Slade, L. Iddon et al., "Synthesis of a new fluorine-18 glycosylated “click” cyanoquinoline for the imaging of epidermal growth factor receptor,” Journal of Labelled Compounds and Radiopharmaceuticals, vol. 57, no. 2, pp. 92–96, 2014.

[75] Y. Li, J. Guo, S. Tang, L. Lang, X. Chen, and D. M. Perrin, "One-step and one-pot-two-step radiosynthesis of functional imaging," American Journal of Nuclear Medicine and Molecular Imaging, vol. 3, no. 1, pp. 44–56, 2013.

[76] Y. Li, Z. Liu, C. W. Harwig et al., "[18\text{F}]Click-labeling of a bombesin antagonist with an alkyne-[18\text{F}]ArBF3: in vivo PET imaging of tumors expressing the GRF-receptor," American Journal of Nuclear Medicine and Molecular Imaging, vol. 3, no. 1, pp. 57–70, 2013.

[77] Z. Liu, Y. Li, J. Lozada et al., "Stoichiometric Leverage: rapid 18\text{F}-fluorotrifluoroborate radiosynthesis at high specific activity for click conjugation," Angewandte Chemie, vol. 125, no. 8, pp. 2359–2363, 2013.

[78] M. Pretze and C. Mamat, "Automated preparation of [18\text{F}]AFP and [18\text{F}]BF: two novel bifunctional 18\text{F}-labeling building blocks for Huisgen-click," Journal of Fluorine Chemistry, vol. 150, pp. 25–35, 2013.

[79] M. Fani, X. Wang, G. Nicolas et al., "Development of new folate-based PET radiotracers: preclinical evaluation of 68Ga-DOTAfolate conjugates," European Journal of Nuclear Medicine and Molecular Imaging, vol. 38, no. 1, pp. 108–119, 2011.

[80] N. K. Devaraj, G. M. Thurber, E. J. Keliher, B. Marinelli, and R. Weissleder, "Reactive polymer enables efficient in vivo bioorthogonal chemistry," Proceedings of the National Academy of Sciences of the United States of America, vol. 109, no. 13, pp. 4762–4767, 2012.

[81] V. V. Rostovtsev, L. S. Green, V. V. Fokin, and K. B. Sharpless, "A stepwise huisgen cycloaddition process: copper(I)-catalyzed regioselective “Ligation” of azides and terminal alkynes,” Angewandte Chemie, vol. 114, no. 14, pp. 2708–2711, 2002.

[82] C. W. Tornoe, C. Christensen, and M. Meldal, "Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides," Journal of Organic Chemistry, vol. 67, no. 9, pp. 3057–3064, 2002.

[83] M. V. Gil, M. J. Ár evalo, and Ó. López, "Click chemistry—what’s in a name? Triazole synthesis and beyond," Synthesis, no. 11, pp. 1589–1620, 2007.

[84] K. D. Hänni and D. A. Leigh, "The application of CuAAC “click” chemistry to catenane and rotaxane synthesis," Chemical Society Reviews, vol. 39, no. 4, pp. 1240–1251, 2010.

[85] Y. Hua and A. H. Flood, "Click chemistry generates privileged CH hydrogen-bonding triazoles: the latest addition to anion supramolecular chemistry," Chemical Society Reviews, vol. 39, no. 4, pp. 1262–1271, 2010.

[86] C. O. Kappe and E. Van Der Eycken, "Click chemistry under non-classical reaction conditions," Chemical Society Reviews, vol. 39, no. 4, pp. 1280–1290, 2010.

[87] J. E. Hein and V. V. Fokin, "Copper-catalyzed azide-alkyne cycloaddition (CuAAC) and beyond: new reactivity of copper(i) acetylides," Chemical Society Reviews, vol. 39, no. 4, pp. 1302–1315, 2010.

[88] S. K. Mamidyala and M. G. Finn, "In situ click chemistry: probing the binding landscapes of biological molecules," Chemical Society Reviews, vol. 39, no. 4, pp. 1252–1261, 2010.

[89] R. A. Décréau, J. P. Collman, and A. Hosseini, "Electrochemical applications. How click chemistry brought biomimetic models to the next level: electrocatalysis under controlled rate of electron transfer," Chemical Society Reviews, vol. 39, no. 4, pp. 1291–1301, 2010.

[90] A. H. El-Sagheer and T. Brown, "Click chemistry with DNA," Chemical Society Reviews, vol. 39, no. 4, pp. 1388–1405, 2010.

[91] W. H. Binder and R. Sachsenhauser, “‘Click’ chemistry in polymer and materials science,” Macromolecular Rapid Communications, vol. 28, no. 1, pp. 15–54, 2007.

[92] P. L. Golas and K. Matyjaszewski, "Marrying click chemistry with polymerization: expanding the scope of polymeric materials," Chemical Society Reviews, vol. 39, no. 4, pp. 1338–1354, 2010.
[93] A. Michael, “Über die Einwirkung von Diazobenzolimid auf Acetylandicarbonsäure-methylester,” *Journal für Praktische Chemie*, vol. 48, no. 1, pp. 94–95, 1893.

[94] S. Maschauer and O. Prante, “Sweetening pharmaceutical radiochemistry by $^{18}$F-fluoro-glycosylation: a short review,” *BioMed Research International*, vol. 2014, Article ID 214748, 30 pages, 2014.

[95] Z. Liu, Y. Li, J. Lozada et al., “Stoichiometric leverage: rapid $^{18}$F-aryltrifluoroborate radiosynthesis at high specific activity for click conjugation,” *Angewandte Chemie International Edition English*, vol. 56, no. 8, pp. 2303–2307, 2013.

[96] G. J. Brewer, “Copper toxicity in the general population,” *Clinical Neurophysiology*, vol. 121, no. 4, pp. 459–460, 2010.

[97] P. Mädinger, F. Füchtner, and F. Wüst, “Module-assisted synthesis of the bifunctional labelling agent N-succinimidyl 4-$^{18}$F]fluorobenzoate ([$^{18}$F]SFB),” *Applied Radiation and Isotopes*, vol. 63, no. 3, pp. 329–332, 2005.

[98] M. M. Herth, V. L. Andersen, S. Lehel, J. Madsen, G. M. Knudsen, and J. L. Kristensen, “Development of a $^{11}$C-labeled tetrazine for rapid tetrazine-trans-cyclooctene ligation,” *Chemical Communications*, vol. 49, no. 36, pp. 3805–3807, 2013.

[99] M. S. Haka, M. R. Kilbourn, G. L. Watkins, and S. A. Toorongian, “Aryltrimethylammonium trifluoromethanesulfonates as precursors to aryl [$^{18}$F]fluorides: improved synthesis of [$^{18}$F]GBR-13119,” *Journal of Labelled Compounds and Radiopharmaceuticals*, vol. 27, no. 7, pp. 823–833, 1989.