Hindawi Publishing Corporation
BioMed Research International
Volume 2014, Article ID 361329, 16 pages
http://dx.doi.org/10.1155/2014/361329

Review Article

^{18}F-Labeling Using Click Cycloadditions

Kathrin Kettenbach,[1] Hanno Schieferstein,[1] and Tobias L. Ross[1,2]

[1] *Institute of Nuclear Chemistry, Johannes Gutenberg University Mainz, 55128 Mainz, Germany*
[2] *Radiopharmaceutical Chemistry, Department of Nuclear Medicine, Hannover Medical School, 30625 Hannover, Germany*

Correspondence should be addressed to Tobias L. Ross; ross.tobias@mh-hannover.de

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

Academic Editor: Olaf Prante

Due to expanding applications of positron emission tomography (PET) there is a demand for developing new techniques to introduce fluorine-18 ($t_{1/2}$ = 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 ^{18}F in those molecules poses an exceeding challenge. Two major challenges during ^{18}F-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 ^{18}F-labeled prosthetic groups for click cycloadditions and will summarize recent trends in copper-catalyzed and copper-free click ^{18}F-cycloadditions.

1. Introduction

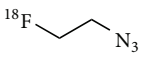
For the application in positron emission tomography (PET) [1], fluorine-18 provides ideal nuclear physical characteristics for *in vivo* imaging. Fluorine-18 offers a half-life of 110 min, a β^+-branch of 97%, and especially a low β^+-energy of 635 keV, which is responsible for a very high spatial resolution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

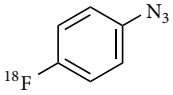
However, the need of cytotoxic copper during CuAAC has led to the necessity of alternative fast and copper-free click reaction strategies for radiofluorination and additionally enabling pretargeting approaches in living systems. Those so-called strain-promoted click reactions can be carried out between 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 ^{18}F-labeling and also for *in vivo* application in living systems. Very recently, new versions of ^{18}F-click cycloadditions are added to the range of reactions [19–25]. In this line, the first ^{18}F-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}F-labeling of new bioactive compounds, with or without a catalytic system. This can be

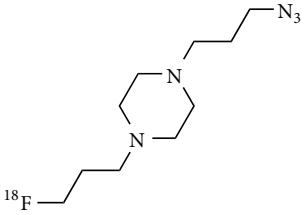
[^{18}F]fluoroethylazide ([^{18}F]FEA)



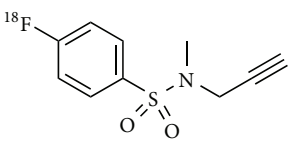
1-(azidomethyl)-4-[^{18}F]fluorobenzene



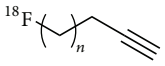
1-(3-azidopropyl)-4-(3-[^{18}F]fluoropropyl)piperazine ([^{18}F]AFP)



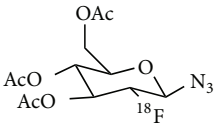
4-[^{18}F]fluoro-*N*-methyl-*N*-(prop-2-ynyl)-benzenesulfonamide ([^{18}F]F-SA)



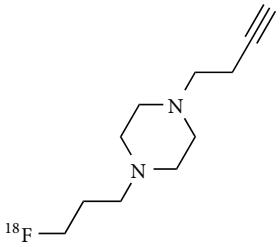
[^{18}F]fluoroalkyne(s)



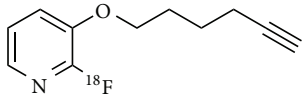
3,4,5-tri-*O*-acetyl-2-deoxy-2-[^{18}F]fluoroglucopyranosyl azide



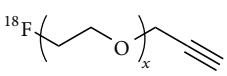
1-(but-3-ynyl)-4-(3-[^{18}F]fluoro-propyl)piperazine ([^{18}F]BFP)



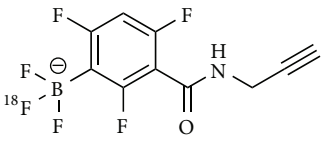
[^{18}F]FPy5yne



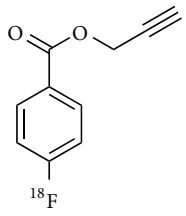
[^{18}F]fluoro-PEG$_x$-alkyne(s)



[^{18}F]ArBF$_3^-$



O-propargyl-4-[^{18}F]fluorobenzoate ([^{18}F]PFB)



N-propargyl-2-amino-3-[^{18}F]fluoro-propionic acid ([^{18}F]serine)

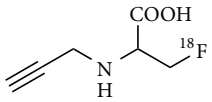


Figure 1: Lead structures of the most important ^{18}F-prosthetic groups applied for copper-catalyzed click ^{18}F-fluorination.

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

2. Copper-Catalyzed ^{18}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 Tornøe 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}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}F-labeling strategy for the first time [26]. They radiolabeled three different alkyne precursors in radiochemical yields (RCY) of 36–81%. Afterwards they were 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

Table 1: Summary of the prosthetic groups, reaction conditions, and reaction partners applied for copper-catalyzed click ^{18}F-fluorination.

^{18}F-prosthetic group	Steps/reaction time[1]	RCY[2]	Reacting agent	Catalytic system	Overall reaction time[1] (CCA)	RCY[2] CCA	Literature
[^{18}F]fluoroalkynes	1 step, 10 min	36–81%	N-(3-azidopropionyl) peptides	CuI/NaAsc/DIPEA	30 min	54–99%	[26]
4-[^{18}F]fluoro-1-butyne	1 step, 15 min (estimated)	n.d.	Glucopyranosyl azide		75–80 min	30%	[27]
4-[^{18}F]Fluoro-1-butyne	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	27 ± 6%	[28]
5-[^{18}F]fluoro-1-pentyne	1 step, 15 min	59 ± 6%				52 ± 5%	
	1 step, 22 min	86 ± 2%	$\alpha_V\beta_6$ specific peptide A20FMDV2 azide	CuI/Asc	66 min	8.7 ± 2.3%	[29]
6-[^{18}F]fluoro-1-hexyne	1 step, 12 min	70–85%	γ-(4-azido-butyl)-folic acid amide	CuI	1.5 h	25–35%	[30]
[^{18}F]fluoroethyl azide ([^{18}F]FEA)	1 step, 15 min	55%	Terminal alkynes	Excess of Cu^{2+}/Asc or copper powder	1 h	61–98% respectively 15–98% with copper powder	[31] [32]
		n.d.	Caspase 3/7 Selective Isatin	$CuSO_4$/Asc	n.d.	65 ± 6%	[33]
			RGD peptides	Cu^{2+}/Asc		47 ± 8%	[34]
			3-Cyanoquinoline core		3 h	37 ± 3.6%	[35]
			Apoptosis marker ICMT11	$CuSO_4$/Asc/BPDS	n.d.	1–3.4% n.d.c.	[36]
			5-Ethynyl-2′-deoxyuridine	CuI/ascorbic acid/DIPEA		75 ± 10%	[37]
			[Tyr^3]octreotate analogues	$CuSO_4$/Asc/BPDS	30 min (estimated)	40–64%	[38]
			ICMT-11 (automated synthesis)		90 min	3 ± 2.6% n.d.c.	[39]
			Nucleosides	$CuSO_4$/Asc	n.d.	8–12% n.d.c.	[40]
			4-(prop-2-ynyloxy)Benzaldehyde		35 min	90%	[41]
			Haloethylsulfoxides	CuI/ascorbate/DIPEA	n.d.	28.5 ± 2.5%	[42]
		50% n.d.c.	Nitroaromatic substrates	$CuSO_4$/Asc	1 h		[43]
		71 ± 4%	RGDfK		60 min	60 ± 2%	[44]
		55%	Alkyne-func. 6-halopurines	One-pot BPDS-copper(I) ($CuSO_4$/NaAsc.)	1 h	55–75%	[45]
		n.d.	tert-butyl ester of N-Boc-(S)-propargyl glycine		2.5 h	58 ± 4%	[46]
	Precursor: 2 steps [^{18}F]FEA: 15 min.	n.d.	3-Butynyl triphenyl phosphonium bromide	$CuSO_4$, NaAsc	1 h	n.d.	[47]
	1 step, 5–10 min	68–75%	Alkynes of benzene rings		30 min	25–87%	[48]
[^{18}F]FEA from a polyflourinated sulfonate precursor	n.d.	n.d.	FtRGD		70–75 min	10–30% n.d.c.	[49]

Table 1: Continued.

^{18}F-prosthetic group	Steps/reaction time[1]	RCY[2]	Reacting agent	Catalytic system	Overall reaction time[1] (CCA)	RCY[2] CCA	Literature
^{18}F-Fluoro-PEG-Alkyne	1 step, 20 min	85–94%	Various azides	$CuSO_4$/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		1 h (estimated)	58%	[52]
[^{18}F]PEG_3-azide	1 step, 40 min	62 ± 4%	N-alkynylated peptide	$CuSO_4$/Asc/BPDS	2 h (estimated)	31 ± 6%	[53]
		n.d.	ZnO nanoparticle alkynes		n.d.	>95%	[54]
[^{18}F]PEG-azide	Precursor: 2 steps labeling: 1 step	labeling: 58%	γ-(11-azido-3,6,9-trioxaundecanyl)folic acid amide	CuAcetate, NaAsc	2.5 h	8.5%	[55]
4-[^{18}F]fluoro-N-methyl-N-(prop-2-ynyl)-benzenesulfonamide (p[^{18}F]F-SA)	Precursor: 3 steps, labeling: 1 step, 80 min	32 ± 5%	Azide-functionalized neurotensin	Cu(I)-TBTA	n.d.	66%	[56]
			Azide-functionalized human serum albumin (HSA)		100 min	55–60%	[57]
		n.d.	Azide-functionalized phosphopeptide, protein (HAS), oligonucleotide (L-RNA)	$CuSO_4$/Asc	2 h	77%/55–60%/25%	[58]
[^{18}F]**FPy5yne**	1 step, 15 min	42%	N_3–(CH_2)4–CO–YKRI–OH (BG142)	Tetrakis(acetonitrilo) copper(I) hexa fluorophosphates/TBTA	160 min	18.7%	[59]
			Azide-functionalized DNA	CuBr/TBTA and 2,6-lutidine	276 min	24.6 ± 0.5%	
2-[^{18}F]fluoro-3-pent-4-yn-1-yloxypyridine ([^{18}F]FPyKYNE)	20–25 min	20–35%	Azide-functionalized RGD peptide	$CuSO_4$/Asc	125 min	12–18%	[60]
6-[^{18}F]fluoro-2-etynylpyridine	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-[^{18}F]fluorobenzoate ([^{18}F]PFB)	Precursor: 2 steps, labeling: 1 steps, 15 min	58 ± 31%	Benzyl azide, two lysine derivatives, transglutaminase-reactive peptide	$CuSO_4$/Asc	1 h (estimated)	88 ± 4%, 79 ± 33% and 75 ± 5% 37 ± 31%	[62]
4-[^{18}F]fluoro-3-nitro-N-2-propyn-1-yl-benzamide ([^{18}F]FNPB)	1 step, 40 min	58%	Azido-peptides cRGDfK and D4 peptide		1 h	87–93%	[63]

Table 1: Continued.

^{18}F-prosthetic group	Steps/reaction time[1]	RCY[2]	Reacting agent	Catalytic system	Overall reaction time[1] (CCA)	RCY[2] CCA	Literature
1-(azidomethyl)-4-[^{18}F]-fluorobenzene	4 steps, 75 min	34%	4-Ethynyl-*L*-phenylalanine-peptide	CuI/NaAsc/DIEA	90 min	90%	[64]
	4 steps, 75 min	41%	siRNA alkyne	$CuSO_4$/Asc/TBTA	120 min	15 ± 5%	[65]
	1 step, 45 min	84%	siRNA-linker (two new alkyne-bearing linkers)		120 min	12%	[66]
1-Azido-4-(3-[^{18}F]fluoropropoxy)benzene	4 steps, 75 min	35%		$CuSO_4$/Asc	120 min	15 ± 5%	[65]
[^{18}F](azidomethyl)fluorobenzene	1 step, 94–188 s	around 40%	siRNA alkyne		n.d.	n.d.	[67]
4-[^{18}F]Fluorophenylazide		around 15%					
3,4,6-tri-O-acetyl-2-deoxy-2-[^{18}F]fluorogluco-pyranosyl azide	1 step, 30 min	71 ± 10%	Fmoc-L-propargylglycine	$CuSO_4$/Asc	1.5 h (estimated)	60%	[68]
	2 step, 7.5 min	n.d.	Alkyne-functionalized peptides (RDG, neurotensin peptoid)		75 min	17–20% n.d.c.	[69]
		52%	folate alkyne	Cu-Acetate/NaAsc	3 h	5–25%	[70]
	1 step, 10 min	84%	RGD-peptide alkyne	$CuSO_4$/Asc	70–75 min	16–24%	[71]
		1.3–4.7%	Alkyne-bearing protein	CuBr/TTMA	80–100 min	4.1%	[72]
	1 step	n.d.	ET_AR ligand alkyne	$CuSO_4$/Asc	70 min	20–25% n.d.c.	[73]
			cyanoquinoline (EGFR) alkyne		90 min	8.6 ± 2.3% n.d.c.	[74]
[^{18}F]$ArBF_3^-$	1 step, 20 min	n.d.	Alkyne-functionalized RGD	Cu^I/Asc	1 h	n.d.	[75]
			Alkyne-functionalized bombesin (BBN)			20 ± 10% n.d.c.	[76]
	2 steps,		Alkyne-functionalized RGD-boronate		30 min	15–30%	[77]
piperazine-based [^{18}F]AFP [^{18}F]BFP	AFP: 4 steps, 54 h BFP: 4 steps, 72 h [^{18}F]AFP: 1 step, 40 min [^{18}F]BFP: 1 step, 40 min	[^{18}F]AFP: 29 ± 5% [^{18}F]BFP: 31 ± 9%	N-Fmoc-e-azido-Lnorleucine (amino acid), SNEW peptide	$CuSO_4$, Asc	2 h	Amino acid: 59–79% SNEW peptide: 17–25%	[78]
[^{18}F]**serine**	2 steps, 125 min	28 ± 5%	cRDG-azide	$CuSO_4$, Asc	145 min	75%	[6]

[1]Calculated as sum from all steps, for the ^{18}F-prosthetic group, respectively, for the overall reaction yielding the click product, starting from fluorine-18.
[2]Radiochemical yields for the ^{18}F-prosthetic group starting from fluorine-18 for the click reaction, respectively; decay corrected, as long as not noted elsewise.
CCA: click cycloaddition; (n.)d.c.: (not) decay corrected; Asc: ascorbate; DIPEA: diisopropylethylamin; TBTA: tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine; n.d.: no data.

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.