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ORIGINAL ARTICLE

4-[^{18}F]Fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzenesulfonamide ([^{18}F]F-SA): a versatile building block for labeling of peptides, proteins and oligonucleotides with fluorine-18 via Cu(I)-mediated click chemistry

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Abstract Cu(I)-mediated [3+2]cycloaddition between azides and alkynes has evolved into a valuable bioconjugation tool in radiopharmaceutical chemistry. We have developed a simple, convenient and reliable radiosynthesis of 4-[^{18}F]fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzenesulfonamide (**[^{18}F]F-SA**) as a novel aromatic sulfonamide-based click chemistry building block. **[^{18}F]F-SA** could be prepared in a remotely controlled synthesis unit in 32 ± 5 % decay-corrected radiochemical yield in a total synthesis time of 80 min. The determined lipophilicity of **[^{18}F]F-SA** (log$P$ = 1.7) allows handling of the radiotracer in aqueous solutions. The versatility of **[^{18}F]F-SA** as click chemistry building block was demonstrated by the labeling of a model peptide (phosphopeptide), protein (HSA), and oligonucleotide (L-RNA). The obtained radiochemical yields were 77 % (phosphopeptide), 55–60 % (HSA), and 25 % (L-RNA), respectively. Despite the recent emergence of a multitude of highly innovative novel bioconjugation methods for ^{18}F labeling of biopolymers, Cu(I)-mediated click chemistry with **[^{18}F]F-SA** represents a reliable, robust and efficient radiolabeling technique for peptides, proteins, and oligonucleotides with the short-lived positron emitter ^{18}F.

Keywords Cu(I)-mediated click chemistry · Fluorine-18 · Peptides · Proteins · Oligonucleotides · Positron emission tomography (PET)

Preliminary results on the synthesis and use of the click chemistry building block **[^{18}F]F-SA** have already been published (Ramenda et al. 2009).

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Introduction

Positron emission tomography (PET) is a non-invasive molecular imaging modality to obtain functional information of physiological, biochemical and pharmacological processes in living subjects at the cellular and molecular level (Czernin and Phelps 2002; Paans et al. 2002; Phelps 2000). The majority of PET studies utilizes compounds labeled with the short-lived positron emitter fluorine-18 (^{18}F, $t_{1/2}$ = 109.8 min) with 2-deoxy-2-[^{18}F]fluoro-D-glucose ([^{18}F]FDG) as the most prominent and most frequently used PET radiotracer.

Over the last decades, numerous methods have been developed for efficient incorporation of ^{18}F into a broad variety of structurally diverse compounds. Most methods rely on nucleophilic aliphatic or nucleophilic aromatic substitution reactions starting from no-carrier-added (n.c.a.) [^{18}F]fluoride. However, incorporation of [^{18}F]fluoride into higher molecular weight compounds like peptides, proteins and oligonucleotides represents a special challenge. Peptides and other biopolymers can usually not be labeled directly with n.c.a. [^{18}F]fluoride due to required harsh reaction conditions like elevated temperatures and the use of organic solvents and basic conditions. Those reaction conditions are not compatible with the structural and functional integrity of many biopolymers, especially proteins. Therefore, ^{18}F labeling of biopolymers is frequently accomplished by means of prosthetic groups. This approach involves formation of ^{18}F-labeled small organic molecules capable of being linked to peptides, proteins, and oligonucleotides under mild conditions (Kuhnast and Dolle 2010; Liu et al. 2011; Olberg and Hjelstuen 2010; Okarvi 2001; Wuest

2005). Such linkage is traditionally accomplished via acylation (Vaidyanathan and Zalutsky 1992; Wester et al. 1996; Garg et al. 1991; Guhlke et al. 1994; Richter et al. 2009), imidation (Kilbourn et al. 1987), alkylation (Toyokuni et al. 2003; Berndt et al. 2007; De Bruin et al. 2005), photochemical conjugation (Wester et al. 1996), and oxime formation (Poethko et al. 2004). More recent developments have also demonstrated direct incorporation of [^{18}F]fluoride into peptides and proteins based on highly efficient fluoride-acceptor chemistry exploiting formation of stable Si–F, B–F, or Al–F bonds (Schirrmacher et al. 2006; McBride et al. 2012). However, every method has advantages and limitations, and the development of rapid, clean and mild synthesis techniques for the radiolabeling of biopolymers continues.

In recent years, click chemistry in radiopharmaceutical chemistry has emerged as a versatile and generic method for rapid and selective radiolabeling of biomolecules. Especially the Cu(I)-catalyzed 1,2,3-triazole formation between azides and alkynes according to a 1,3-dipolar [3+2]cycloaddition was shown to be a particularly powerful reaction due to its high degree of specificity and bioorthogonality of the reactants (Kolb et al. 2001; Rostovtsev et al. 2002). Moreover, triazoles are stable under physiological conditions, and they may associate with potential biological targets through hydrogen bonding and dipole interactions.

Adversely, the need of using cytotoxic copper has somewhat hampered the general use of this reaction, especially for its use in various click chemistry applications in vivo. As a consequence, several alternative copper-free methods have been developed recently. Most prominent strategies are strain-promoted azide-alkyne cycloadditions (Debets et al. 2011; van Hest and van Delft 2011), Staudinger ligations (van Berkel et al. 2011; Köhn and Breinbauer (2004), and inverse-electron-demand Diels–Alder reactions (Devaraj et al. 2010; Liu et al. 2012).

However, Cu(I)-mediated [3+2]cycloaddition between azides and alkynes is a highly effective method for the radiolabeling of a broad range of biopolymers and small molecules with radionuclides like ^{18}F. Various ^{18}F-labeled azides and alkynes and their subsequent use in click chemistry have been described in the literature (Mamat et al. 2009; Wängler et al. 2010).

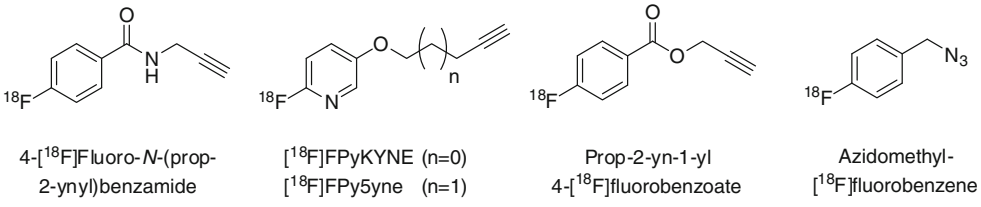
Most ^{18}F-labeled click chemistry building blocks reported in the literature represent volatile aliphatic [^{18}F]fluoroalkynes or [^{18}F]fluoroazides [Marik and Sutcliffe 2006; Glaser and Arstad 2007; Wängler et al. 2010). Only few examples describe alkyne- or azide-containing [^{18}F]fluoroaryl compounds as building blocks for Cu(I)-mediated [3+2]cycloadditions. [^{18}F]Fluoroaryl compounds as displayed in Scheme 1 contain an aromatic C–F bond which is known to be stable in vivo towards radiodefluorination.

Preparation of alkyne group-containing compounds depicted in Scheme 1 was accomplished either via single step nucleophilic aromatic substitution with n.c.a. [^{18}F]fluoride in the case of [^{18}F]FPyKYNE, [^{18}F]FPy5yne and prop-2-yn-1-yl 4-[^{18}F]fluorobenzoate (Kuhnast et al. 2008; Inkster et al. 2008; Vaidyanathan and Zalutsky 1992), or via acylation reaction of propargyl amine with [^{18}F]SFB in the case of 4-[^{18}F]fluoro-*N*-(propyl-2-ynyl)benzamide (Ramenda et al. 2007). 4-[^{18}F]Fluoro-*N*-(propyl-2-ynyl)benzamide, [^{18}F]FPyKYNE, [^{18}F]FPy5yne and prop-2-yn-1-yl 4-[^{18}F]fluorobenzoate were used in conjugation reactions with azide-functionalized peptides (Ramenda et al. 2007; Inkster et al. 2008; Vaidyanathan and Zalutsky 1992). [^{18}F]FPy5yne was also used for click chemistry labeling of oligonucleotides (Inkster et al. 2009).

Azidomethyl-[^{18}F]fluorobenzene was prepared in a multistep reaction sequence starting from 4-[^{18}F]fluorobenzaldehyde (Thonon et al. 2009). 4-[^{18}F]Fluorobenzaldehyde was reduced to 4-[^{18}F]fluorobenzyl alcohol, which was converted into 4-[^{18}F]fluorobenzyl bromide. In the last reaction step, azidomethyl-[^{18}F]fluorobenzene was obtained through treatment of 4-[^{18}F]fluorobenzyl bromide with an azide exchange resin. Azidomethyl-[^{18}F]fluorobenzene was conjugated to an alkyne-modified model neuropeptide using click chemistry in high radiochemical yields of 90 %.

The preferred single step radiosynthesis of ^{18}F-labeled aromatic compounds starting from n.c.a. [^{18}F]fluoride according to a nucleophilic aromatic substitution reaction requires activated electron-deficient aromatic systems containing a suitable leaving group as labeling precursors. Prominent examples include electron-deficient aryl compounds like benzaldehydes, benzonitriles, benzoic acid esters, nitrobenzenes or pyridines with nitro or trimethylammonium groups as leaving groups.

Surprisingly, only little work has been reported on using aryl sulfonamides as electron-deficient aromatic systems for nucleophilic aromatic substitution reactions with n.c.a. [^{18}F]fluoride. Some prominent examples include synthesis of carbonic anhydrase inhibitors, α_2 adrenergic receptor ligands, and inhibitors for the serotoninergic 5-HT$_6$ receptor which could be prepared in a single step radiofluorination reaction with n.c.a. [^{18}F]fluoride using the corresponding aryl sulfonamide containing a nitro



4-[^{18}F]Fluoro-*N*-(prop-2-ynyl)benzamide | [^{18}F]FPyKYNE (n=0) [^{18}F]FPy5yne (n=1) | Prop-2-yn-1-yl 4-[^{18}F]fluorobenzoate | Azidomethyl-[^{18}F]fluorobenzene

Scheme 1 Various [^{18}F]fluoroaryl compounds for copper (I)-assisted [3+2]cycloaddition reactions

or trimethylammonium group as labeling precursors (Enas et al. 1997; Supuran et al. 1998; Tang et al. 2007).

In this work, we describe the radiosynthesis of 4-[^{18}F]fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzene sulfonamide (**[^{18}F]F-SA**) as a versatile building block for Cu(I)-assisted [3+2]cycloaddition reactions. The one-step radiosynthesis was performed in an automated synthesis unit to allow handling of large ^{18}F activity amounts. The suitability of using **[^{18}F]F-SA** in click chemistry reactions with various azide-functionalized biopolymers was demonstrated with a model peptide, a protein (HSA), and a 17-mer L-RNA as an example for oligonucleotides.

Materials and methods

General

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise specified. Nuclear magnetic resonance spectra were recorded on a Varian Unity 400 MHz spectrometer. ^{1}H and ^{13}C chemical shifts are given in ppm and were referenced with the residual solvent resonances relative to tetramethylsilane (TMS). Mass spectra were obtained on a Quattro/LC mass spectrometer (MICROMASS) by electrospray ionization (ESI). MALDI-TOF mass spectra were recorded on a Bruker Autoflex II TOF/TOF mass spectrometer. Elemental analyses were conducted using a LECO CHNS-932 apparatus. Column chromatography was performed on Merck silica gel (mesh size 230-400 ASTM) according to literature procedure (Still et al. 1978). Reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel F-254 aluminium plates, with visualization under UV (254 nm). Active ester 1-succinimidyl-5-azidopentanoate **8** was prepared according to literature procedure (Seo et al. 2003). Amine-functionalized L-RNA **11** was obtained from NOXXON Pharma AG, Berlin, Germany.

Lipophilicity (log*P*) of **[^{18}F]F-SA** was determined at pH 7.4 according to the method reported by Wilson et al. (2001).

Chemical syntheses

*4-Fluoro-N-methyl-N-(propyl-2-yn-1-yl)benzene sulfonamide (**F-SA**)*

N-Methylpropargyl amine **3** (0.5 g, 7.2 mmol) and triethylamine (2 mL) were dissolved in dry CH_2Cl_2 (10 mL). At 0 °C, a solution of 4-fluorobenzene-1-sulfonyl chloride **1** (1.5 g, 7.7 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was stirred for 1 h at room temperature. 1 N HCl (100 mL) was added, and the mixture is extracted with EtOAc. The organic layer was filtered through a silica gel plug, and the solvent is removed under reduced pressure to yield 1.46 g (99 %) of the desired compound **F-SA** as yellow crystals. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 2.06 (t, $J = 2.6$ Hz, 1 H, C≡CH), 2.84 (s, 3 H, CH_3), 4.05 (d, $J = 2.2$ Hz, 2 H, CH_2), 7.17–7.22 (m, 2 H), 7.83–7.87 (m, 2 H). ^{1}C-NMR (100 MHz, $CDCl_3$), δ 166.1 ($^{1}J_{(C,F)} = 239$ Hz), 135.3 ($^{4}J_{(C,F)} = 2.9$ Hz), 130.7 ($^{3}J_{(C,F)} = 9.0$ Hz), 115.8 ($^{2}J_{(C,F)} = 19.7$ Hz), 78.0, 73.2, 39.8, 35.1. LR–MS (ESI positive): $m/z = 228$ ($[M+H]^+$). Anal. Calculated for $C_{10}H_{10}FNO_2S$: C 52.85, H 4.44, N 6.16, O 14.08, S 14.11; found: C 51.63, H 4.01, N 5.86, O 13.88, S 13.65.

*4-Nitro-N-methyl-N-(propyl-2-yn-1-yl)benzene sulfonamide (**4**)*

N-Methylpropargyl amine **3** (0.5 g, 7.2 mmol) and triethylamine (2 mL) were dissolved in dry CH_2Cl_2 (10 mL). At 0 °C, a solution of 4-nitrobenzene-1-sulfonyl chloride **2** (1.5 g, 6.8 mmol) in CH_2Cl_2 (2 mL) was added dropwise. The reaction mixture was stirred for 1 h at room temperature. 1 N HCl (100 mL) was added, and the mixture is extracted with EtOAc. The organic layer was filtered through a silica gel plug, and the solvent is removed under reduced pressure to yield 1.58 g (96 %) of the desired compound **4** as yellow crystals. ^{1}H-NMR (400 MHz, $CDCl_3$), δ 2.06 (t, $J = 2.6$ Hz, 1 H, C≡CH), 2.90 (s, 3 H, CH_3), 4.13 (d, $J = 2.2$ Hz, 2 H, CH_2), 8.01–8.05 (m, 2 H), 8.35–8.38 (m, 2 H).

*4-Amino-N-methyl-N-(prop-2-yn-1-yl)benzene sulfonamide (**5**)*

Nitro compound **4** (0.5 g, 2.0 mmol) and $SnCl_2 \times 2\,H_2O$ (2.25 g, 10 mmol) were refluxed in EtOH (10 mL) for 3 h. The reaction mixture was cooled in an ice bath, and the pH was adjusted to 7–8 by the addition of 5 %-Na_2SO_4 solution. The formed tin salts were filtered off, and the residue was extracted with EtOAc. The combined organic layers were washed with brine, dried, and the solvent was removed under reduced pressure. Compound **5** (0.28 g, 62 %) was obtained as a yellowish-brown solid. ^{1}H-NMR (400 MHz, $DMSO_{d6}$), δ 2.62 (s, 3 H, CH_3), 3.17 (t, $J = 2.5$ Hz, 1 H, C≡CH), 3.86 (d, $J = 2.4$ Hz, 2 H, CH_2), 6.06 (s, 2 H, NH_2), 6.59–6.63 (m, 2 H), 7.37–7.41 (m, 2 H).

*4-Trimethylammonium-N-methyl-N-(prop-2-yn-1-yl)benzene sulfonamide triflate (**6**)*

Compound **5** (0.25 g, 1.1 mmol) and 2,6-di-*tert*-butyl-4-pyridine (0.46 g, 0.2 mmol) were dissolved in CH_2Cl_2 (20 mL). After 5 min, methyl triflate (0.36 g, 2.2 mmol) was added and the reaction mixture was stirred at room temperature for 12 h. The white precipitate was filtered off and discarded. The remaining solution was treated with additional methyl triflate (0.18 g, 1.1 mmol) under an argon atmosphere. The solvent was removed under reduced pressure and the resulting brown

oil was re-dissolved in CH_2Cl_2 (5 mL). The solution was cooled in an ice bath, and diethyl ether was carefully added to form a white precipitate. The white precipitate was filtered off, washed with diethyl ether, and dried to afford 0.44 g (97 %) of compound **6** as a light brown solid. ^{1}H-NMR (400 MHz, $DMSO_{d6}$), δ 2,80 (s, 3 H, NCH_3), 3.11 (t, $J = 2.3$ Hz, 1 H, C≡CH), 3.64 [s, 9 H, $N(CH_3)_3$], 4.08 (d, $J = 2.3$ Hz, 2 H, CH_2), 8.04–8.07 (m, 2 H), 8.20–8.23 (m, 2 H). *m/z* (ESI, positive) $C_{14}H_{19}F_3N_2O_5S_2$, calcd. 267, found 267 $[M\text{-}OTf]^+$.

*H-Met-Gln-Ser-pThr-Pro Leu-OH (**7**)*

Peptide synthesis was performed on peptide synthesizer (Syro I, MultiSynTech, Germany) using standard Fmoc chemistry. Analytical and semi-preparative HPLC was performed on a Hewlett Packard Series 1100 system using a Macherey–Nagel HPLC column (VP250/10, Nucleosil 100-5 C18, Nautilus) and eluents of CH_3CN/0.1 % TFA (eluent A) and H_2O/0.1 % TFA (eluent B) at a flow rate of 2 ml/min. UV detection was carried out at a wavelength of 220 nm. Phosphopeptide **7** was prepared 50 mg of the Fmoc-Leu-preloaded resin. After swelling the resin with 1 ml of DMF for 10 min and 5-times DMF washing cycles the Fmoc-protecting group was removed by treatment of 40 % piperidine in DMF for 15 min and followed by 20 % piperidine in DMF for another 15 min. A washing step with 500 µl of DMF was repeated 5 times. Activation and coupling of Fmoc-protected amino acids (5 equiv.) were achieved using HBTU (5 equiv.) and HOBt (5 equiv.) dissolved in DMF and DIPEA dissolved in NMP. An amino acid/HBTU/HOBt/DIPEA ratio of 1/1/1/3 was applied, and the coupling time for incorporating Fmoc-Thr(PO(OBzl)–OH)–OH was 2 and 1 h for all other amino acids. The removal of the Fmoc group with 40 % piperidine in DMF for 15 min was embedded into a 5-times cycle wash step with DMF. The complete resin-bound peptide was washed with EtOH in a last step. The peptide was deprotected and cleaved from the solid phase with trifluoroacetic acid/water/thioanisole/1,2-ethanedithiol (87/5/5/3) at room temperature for 5 h. The peptide was separated from the resin through a syringe filter and precipitated by the addition of ice-cold diethylether. The crude peptide was obtained by removing the residual ether using a syringe filter. Purification by HPLC using the following gradient: 0–10 min 10 % eluent A, at 20 min 20 % eluent A, at 40 min 30 % eluent A, at 45 min 50 % eluent A, and subsequent lyophilisation gave pure peptide **7** as a white solid (19 mg, 15.4 µmol, 78 %).

HPLC analysis: ($t_R = 21.4$ min). *m/z* (ESI, positive) $C_{28}H_{50}N_7O_{13}PS$ calcd 755.77, found 756.60 $[M+H]^+$.

*Azido-H-Met-Gln-Ser-pThr-Pro Leu-OH (**9**)*

Phosphopeptide **7** (9.4 mg, 12.4 µmol) in borate buffer (700 µL, pH 8.4) was treated with active ester **8** (6 mg, 25 µmol) in CH_3CN (300 µL) for 6 h at 40 °C. Purification of azido-peptide **9** was achieved with HPLC using the following gradient: 0 min 10 % CH_3CN/0.1 % TFA, 90 % 0.1 % TFA; at 30 min 40 % CH_3CN/0.1 % TFA; at 45 min 30 % CH_3CN/0.1 % TFA. The peak eluting at 24 min was collected, and subsequent lyophilisation gave pure azido-peptide **9** as a white solid (7 mg, 64 %). *m/z* (ESI, positive) $C_{33}H_{57}N_{10}O_{14}PS$ calcd 880, found 903 $[M+Na]^+$.

*F-SA-triazole-H-Met-Gln-Ser-pThr-Pro Leu-OH (**10**)*

Azido-peptide **9** (1.2 mg, 1.4 µmol), $CuSO_4 \times 5$ H_2O (0.25 mg, 1 µmol), sodium ascorbate (2 mg, 10 µmol), and **F-SA** (1 mg, 4.2 µmol) in borate buffer (500 µL, pH 8.4) were stirred for 24 h at 40 °C. Purification of F-SA-triazole-peptide **10** was performed by HPLC using the following gradient: 0 min 10 % CH_3CN/0.1 % TFA, 90 % 0.1 % TFA; at 30 min 40 % CH_3CN/0.1 % TFA; at 45 min 30 % CH_3CN/0.1 % TFA. The peak eluting at 27 min was collected, and subsequent lyophilisation gave pure peptide **10** as a white solid (0.6 mg, 36 %). *m/z* (ESI, positive) $C_{43}H_{67}FN_{11}O_{16}PS_2$ calcd 1,107, found 1,108 $[M+H]^+$, 1,130 $[M+a]^+$.

Azide-functionalization of HSA

Human serum albumin (20 mg, 0.3 µmol) was dissolved in 1 ml of 0.15 M phosphate buffer (PBS, pH = 7.5). 1-Succinimidyl-5-azidopentanoate **8** (9 mg, 36 µmol) was dissolved in DMSO (20 µl) and added to the protein solution. The mixture was incubated at room temperature for 2 h. The product was purified by size exclusion chromatography using a HiTrap desalting column (5 mL, GE Healthcare Europe GmbH, Munich, Germany). A subsequent dialysis step against water was performed for 24 h at 4 °C (cutoff-value 14,000 Da). The resulting solution was lyophilized and analyzed by MALDI-TOF MS. The product was obtained as a white solid (5.7 mg, 29 %).

The number of introduced azide residues was determined by enzymatic digest of the azide-functionalized HSA followed by MALDI-TOF MS analysis of the protein fragments according to literature procedure (Wa et al. 2006). The enzymes trypsin, endoproteinase Lys-C and endoproteinase Glu-C were used for enzymatic digest. Each enzyme was used for a single digest of azide-modified HSA and native HSA as a control. The resulting solutions were processed according to the delayed extraction procedure22 prior to MALDI-TOF MS analysis. Seven modified lysine residues could be detected after enzymatic digest with trypsin and Glu-C, respectively, whereas 22 modified lysine residues were determined after enzymatic digest with Lys-C. Taking into account multiple detections of modified lysine residues in the different enzymatic digests, the total number of azide-modified lysine residues was determined to be 27.

*Azido-L-RNA (**12**)*

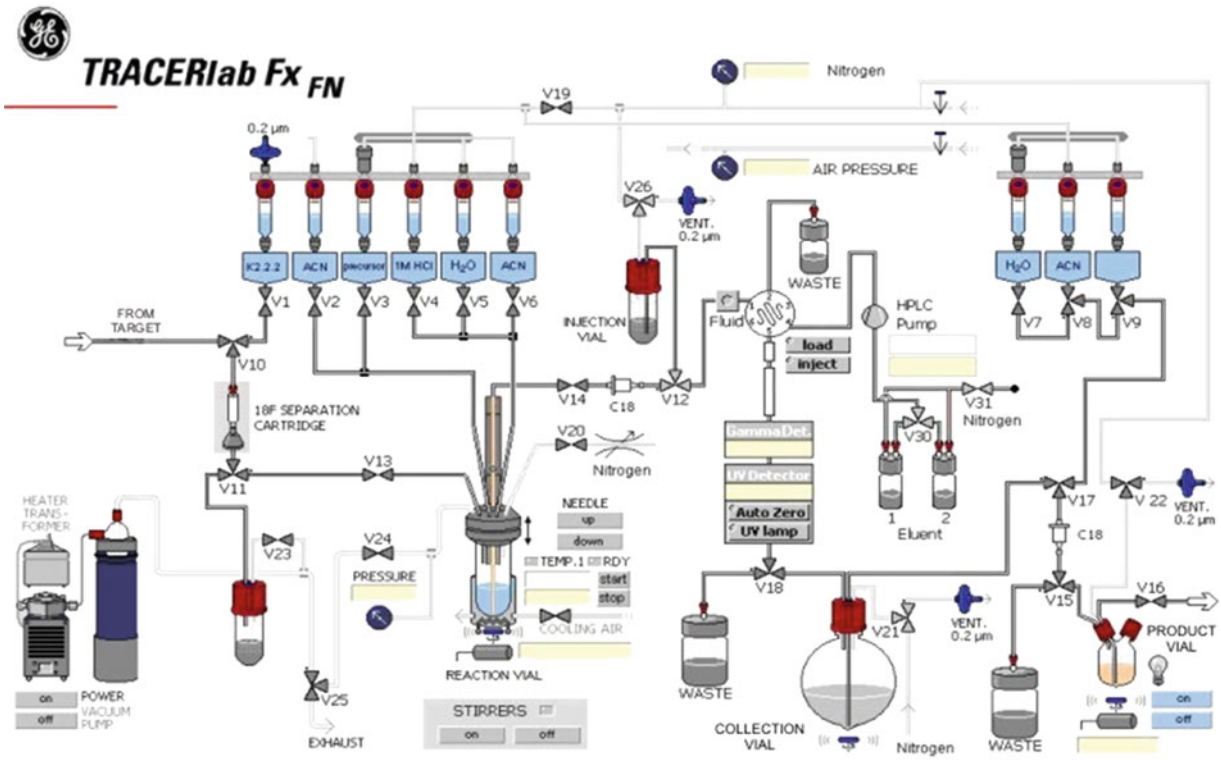
Amine-containing L-RNA **11** (200 µL, 50 µM solution of 1 M $NaHCO_3$, 10 nmol) was reacted with active ester **8** (1 mg, 4.2 µmol) for 2 h at 10 °C. The product was purified by size exclusion chromatography using a HiTrap desalting column (5 mL, GE Healthcare Europe GmbH, Munich, Germany) at a flow rate of 0.5 mL/min using 0.1 M NH_4OAc as the eluent. Yield: 96 %. *m/z* (MALDI-TOF MS) calcd 5,662, found 5,663 $[M+H]^+$.

*F-SA-triazole-L-RNA (**13**)*

Azido-L-RNA **12** (14.2 µg, 2.5 nmol) and **F-SA** (1 mg, 4.2 µmol) were dissolved in 1 M $NaHCO_3$ (190 µL) and 50 µL of CH_3CN. CuBr (0.1 mg) and TBTA (0.4 mg) dissolved in DMSO (10 µL) were added, and the reaction mixture was stirred 90 min at 10 °C. The product was purified by size exclusion chromatography using a HiTrap desalting column (5 mL, GE Healthcare Europe GmbH, Munich, Germany) at a flow rate of 0.5 mL/min using 0.1 M NH_4OAc as the eluent. Yield: 28 %. *m/z* (MALDI-TOF MS) calcd 5,888, found 5,889 $[M+H]^+$.

Radiochemical syntheses

No-carrier added aqueous [^{18}F]fluoride ion was produced in a IBA CYCLONE 18/9 cyclotron by irradiation of [^{18}O]H_2O via the $^{18}O(p,n)^{18}F$ nuclear reaction. Syntheses of **[^{18}F]F-SA** was carried out in an automated nucleophilic radiofluorination module (GE TRACERlab® FX-FN). The synthesis module was modified in terms of program and hard-ware. The synthesis module consists of two main parts: reactor 1 with reservoir vessels 1–6 and reactor 2 with reservoir vessels 7–12. The reservoir vessels contain the starting materials and are sealed by septa. The reaction vessels are made from glassy carbon and sealed with a PEEK cover. The reactors are connected to each other. All connections were made via Teflon tubings and valves. All reagents and solvents are manipulated within the module by vacuum or auxiliary gas (nitrogen). The reaction mixtures in the reaction vessels can be stirred, heated, cooled, evaporated and pressurized. Evacuation of the reactors is performed with a vacuum pump via a cooling trap filled with liquid nitrogen. Volatile radioactive substances are thus trapped. Radioactivity detectors monitor the radioactivity level in the reactors and in the vessel for the final product. Before starting the synthesis, a cleaning program for the module was carried out. Then, the reservoir vessels were loaded with the required reagent solutions or solvents.



Scheme of the automated synthesis unit for the synthesis of 4-[^{18}F]fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl) benzene sulfonamide (**[^{18}F]F-SA**).

*4-[^{18}F]Fluoro-N-methyl-N-(propyl-2-yn-1-yl)benzene sulfonamide ([**^{18}F**]**F-SA**)*

The synthesis started with the elution of [^{18}F]fluoride from the anion exchange cartridge into the reactor 1 using a solution of Kryptofix 222 (15 mg; 40 µmol) and potassium carbonate (2.77 mg; 20 µmol) in aqueous CH_3CN (1.5 ml; 66 % CH_3CN). The eluate was dried by means of a vacuum and a nitrogen stream at 95 °C. The reaction mixture was carefully dried by addition and evaporation of anhydrous CH_3CN (1 × 3 ml). 4-Trimethylammonium-*N*-methyl-*N*-(prop-2-yn-1-yl)benzene triflate **6** (2.5 mg, 6 µmol) in sulfolane (1 mL) was added to dried [^{18}F]fluoride in reactor 1. The mixture was heated at 80 °C for 10 min. The mixture was cooled to 50 °C, and 1 N HCl was added. The solution was passed through a C18 cartridge (SepPak®Plus, Waters). The cartridge was washed with water, and the product was eluted with CH_3CN into a vessel containing 3 mL of water. The mixture is loaded onto a semi-preparative HPLC for purification [Phenomenex RP-18 column (LUNA C18(2) 10 × 250 mm, 10 µm] column using isocratic elution with CH_3CN/0.1 M TFA (40/60) at a flow rate of 2.5 mL/min) The peak eluting at 28–30 min was collected in a vial containing 25 mL of water. The mixture was passed through a C18 cartridge (SepPak®Plus, Waters), and the product was eluted with ethyl ether (1 mL). Ethyl ether was removed in a stream of nitrogen. In a typical experiment, starting from 16 GBq of [^{18}F]fluoride, 6.5 GBq (32 % decay-corrected) of compound **[^{18}F]F-SA** could be obtained in a total synthesis time of 80 min, including HPLC purification.

Radio-TLC (SiO_2, petroleum ether/ethyl acetate 2:1) $R_f = 0.4$. Radio-HPLC-analysis (Phenomenex RP-18 column (LUNA C18(2) 4.6 × 250 mm, 5 µm, 1 mL/min, CH_3CN/0.1 M TFA (40/60), $t_R = 10.4$ min.

*[^{18}F]F-SA-triazole-H-Met-Gln-Ser-pThr-Pro Leu-OH ([**^{18}F**]**10**)*

In a typical reaction, a solution of azido-peptide **9** (0.3 mg, 0.34 µmol), $CuSO_4$×5 H_2O (0.3 mg, 1.2 µmol), sodium ascorbate (2.4 mg, 12 µmol) in borate buffer (150 µL, pH 8.4) was added to **[^{18}F]F-SA** (50–150 MBq) in borate buffer (100 µL, pH 8.4). The reaction was kept at 40 °C for 10 min. Radiolabeled peptide **[^{18}F]10** was purified using semi-preparative HPLC as described for reference compound **10**. The peak eluting at 27 min was collected, and the solvent was evaporated under reduced pressure. The residue was re-dissolved in saline (1 mL).

Radiochemical yield: 42 % (isolated, after semi-preparative HPLC), 73 % (based upon radio-HPLC analysis). Radio-HPLC-analysis (Phenomenex RP-18 column (LUNA C18(2) 4.6 × 250 mm, 5 µm, 1 mL/min, CH_3CN/0.1 M TFA (40/60), $t_R = 4.8$ min.

Radiolabeling of azide-modified HSA

Azide-modified HSA (0.1–0.4 mg, 2–6 nmol), copper(I) bromide (0.2 mg, 1.4 µmol), and TBTA (1 mg, 1.9 µmol) were dissolved in a mixture of phosphate buffer (140 µl, pH = 7.5) and DMSO (10 µl). The solution was added to a vial containing **[^{18}F]F-SA**. The mixture was incubated for 20 min at 30 °C. The product was purified by size exclusion chromatography using a HiTrap desalting column (5 mL, GE Healthcare Europe GmbH, Munich, Germany) and PBS as eluent. In a typical experiment, starting from 450 MBq of **[^{18}F]F-SA**, 250 MBq of radiolabeled HSA could be obtained, representing a radiochemical yield of 55 % based upon **[^{18}F]F-SA**.

*[^{18}F]F-SA-triazole-L-RNA ([**^{18}F**]**13**)*

Azido-L-RNA **12** (0.1 mg) was dissolved in 1 M $NaHCO_3$ (100 µL). A solution of TBTA (0.4 mg) in DMSO (10 µL) and CuBr (0.2 mg) in 1 M $NaHCO_3$ (40 µL) was added to the oligonucleotide solution, and the combined solution was added to **[^{18}F]F-SA** in CH_3CN (50 µL). The reaction mixture was kept at 10 °C for 20 min. The reaction mixture was analyzed with HPLC (Waters XTerra®MS C18, 2.5 µm, 3.0 × 50 mm) using gradient elution with CH_3CN (A) and 50 mM TEAA at a flow rate of 0.3 mL/min. Gradient: 0 min 5 % A, 95 % B; 30 min 50 % A, 50 % B. Radiochemical yield: 30 % (based upon radio-HPLC analysis $t_R = 16.4$ min.

Results and discussion

Synthesis of 4-[^{18}F]fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzene sulfonamide **[^{18}F]F-SA**

Literature reports describing radiosynthesis of ^{18}F-labeled sulfonamides clearly demonstrate the benefit of using tertiary sulfonamides over primary and secondary sulfonamides to achieve reasonable radiochemical yields. Primary and secondary sulfonamides contain acidic protons at the sulfonamide nitrogen which can quench the reactivity of dried [^{18}F]fluoride when exposed to the typical basic reaction conditions of nucleophilic radiofluorinations. Consequently, only very low radiochemical yields (0.5–2 %) were obtained with primary and secondary sulfonamides as reported by Hocke et al. (2005), Shiue et al. (1999), Supuran et al. (1998). Significantly higher radiochemical yields of 23–32 % were obtained when tertiary sulfonamides were used as shown by Enas et al. (1997), Tang et al. (2007).

Based on these findings and the aim to create a metabolically stable C–F bond, we set up the synthesis of a ^{18}F-labeled alkyne group-containing aromatic tertiary

sulfonamide as click chemistry building block for subsequent copper (I)-assisted [3+2]cycloaddition reactions.

The synthesis of respective 4-fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzene sulfonamide **F-SA** as reference compound and 4-trimethylammonium-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzene sulfonamide triflate **6** as labeling precursor is illustrated in Scheme 2.

Reference compound **F-SA** was prepared in almost quantitative yield of 99 % in a single step through acylation reaction of *N*-methylpropargyl amine with commercially available 4-fluorobenzene-1-sulfonyl chloride **1** in CH_2Cl_2 in the presence of TEA as base. Labeling precursor **6** was obtained in a three step synthesis sequence in overall yield of 58 %.

The first reaction step involved acylation of *N*-methylpropargyl amine with 4-nitrobenzene-1-sulfonyl chloride **2** to afford compound **4** in 97 % yield. Reduction of the nitro group with $SnCl_2$ gave primary amine **5** in 62 % yield. Primary amine **5** was then treated with an excess of MeOTf in CH_2Cl_2 in the presence of 2,6-di-*tert*-butyl-4-methylpyridine (DBMP) to afford labeling precursor **6** in 97 % yield.

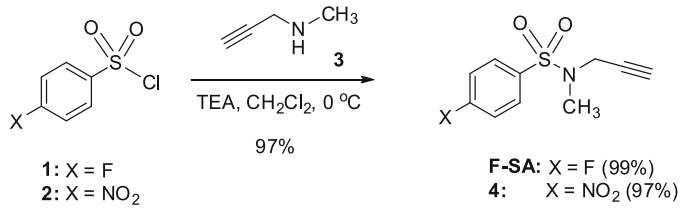
The radiosynthesis of 4-[^{18}F]fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzene sulfonamide **[^{18}F]F-SA** was performed in a remotely controlled synthesis unit (GE TRACERlab® FX-FN). The radiosynthesis of **[^{18}F]F-SA** is displayed in Scheme 3.

Based on the results of various manual syntheses to optimize reaction conditions, it was shown that reaction of 2.5 mg of labeling precursor **6** in sulfolane as the solvent at 80 °C for 10 min afforded best radiochemical yields for the radiofluorination step with powerful nucleophilic radiofluorination agent [^{18}F]KF in the presence of Kryptofix K_{222} and potassium carbonate. Other solvents like DMF and acetonitrile gave lower radiochemical yields of 15–20 %.

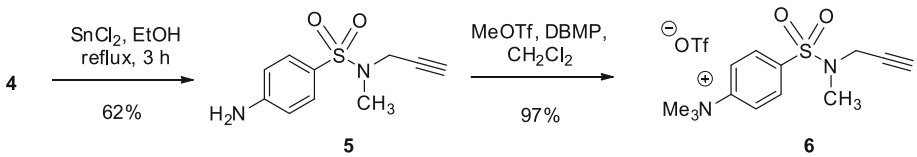
Labeling precursor **6** in sulfolane was transferred from reservoir 3 of the synthesis unit into the reactor vial which contained dried [^{18}F]fluoride. The reaction vial was sealed and heated at 80 °C for 10 min. After cooling the reaction vial to 50 °C, 1 N HCl (10 mL) was added from reservoir 4, and the reaction mixture was passed through a C18 cartridge (SepPak®Plus, Waters). The cartridge was washed with water (3 mL) from reservoir 5, and the product was eluted with acetonitrile (1 mL) from reservoir 6. The eluate was subjected to HPLC purification, and compound **[^{18}F]F-SA** was isolated in 32 ± 5 % decay-corrected radiochemical yield at high radiochemical purity of greater 95 %. The total synthesis time was 80 min including HPLC purification. The specific activity was determined in the rage of 120–570 GBq μmoL^{-1}. The use of an automated synthesis unit allowed the handling of large starting activities of [^{18}F]fluoride. In a typical experiment, starting from 16 GBq of n.c.a. [^{18}F]fluoride, 6.5 GBq of isolated **[^{18}F]F-SA** could be prepared. The obtained large activity amounts of **[^{18}F]F-SA** allow numerous applications of this building block within various click chemistry reactions.

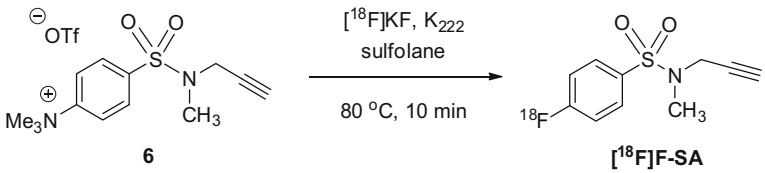
Lipophilicity (log*P*) of [^{18}F]F-SA

Radiolabeling of biopolymers like peptides, proteins, and oligonucleotides should preferentially occur under physiological condition to avoid decomposition and alteration or even loss of biological activity of delicate biopolymers. This especially includes reactions in aqueous media at



Scheme 2 Synthesis of reference compound **F-SA** and labeling precursor **6**





Scheme 3 Radiosynthesis of 4-[^{18}F]fluoro-*N*-methyl-*N*-(propyl-2-yn-1-yl)benzene sulfonamide **[^{18}F]F-SA**

physiological pH at ambient temperature. Reactions in aqueous media require good solubility of all reaction partners in water. Lipophilicity (log*P*) is an important parameter of a molecule which contributes to its solubility in aqueous media and, therefore, its reactivity. This is of special importance for radiolabeled compounds at high specific activity as found for click chemistry building block **[^{18}F]F-SA**. However, recently Kostikov et al. (2012) showed that also lipophilic labeling synthons can be used in aqueous solutions.

The lipophilicity of compound **[^{18}F]F-SA** was determined to be 1.70 ± 0.11 at pH 7.4 using the shake flask method. This value is comparable to that of prominent prosthetic group [^{18}F]SFB (log*P* = 1.75 ± 0.17), which is frequently used for peptide and protein labeling under aqueous conditions (Wuest et al. 2009). The determined log*P* value of 1.70 ± 0.11 for **[^{18}F]F-SA** supports application of this click chemistry building block under desirable aqueous conditions as typically employed for copper(I)-assisted [3+2]cycloaddition reactions.

Radiolabeling of azide-functionalized phosphopeptide **9** with **[^{18}F]F-SA**

Recently, we have described the radiolabeling of a small phosphopeptide as ligand for the polo-box domain (PBD) of polo-like kinase 1 (Plk1) with acylation agent [^{18}F]SFB (Richter et al. 2009). Radiolabeling was accomplished via conjugation of the *N*-terminus of PBD-binding phosphopeptide H-Met-Gln-Ser-pThr-Pro-Leu-OH **7** with [^{18}F]SFB.

For the labeling of phosphopeptide H-Met-Gln-Ser-pThr-Pro-Leu-OH **7** with **[^{18}F]F-SA** according to a copper (I)-assisted [3+2]cycloaddition, we had to introduce an azide group into the peptide. Introduction of azide group at the *N*-terminal end of phosphopeptide **7** was achieved by the reaction of 1-succinimidyl-5-azidopentanoate **8** (2.1 equiv) in a solution of CH_3CN/Kolthoff buffer (pH 8.4) (Kolthoff 1925) [3/7 (v/v)] at 40 °C for 6 h. Active ester **8** was recently used in our group for functionalization of neurotensin(8–13) and subsequent click chemistry reactions (Richter et al. 2010).

Azide-modified peptide **9** was obtained in 64 % yield after purification of the reaction mixture by means of HPLC and subsequent lyophilisation. The purity of peptide **9** exceeded 95 % as confirmed by HPLC analysis, and the identity was determined by means of electron spray ionization mass spectrometry (ESI–MS) showing the expected mass peak at $m/z = 903$ $[M+Na]^+$. Subsequent click chemistry reaction of peptide **9** with **F-SA** in the presence of $CuSO_4$ and sodium ascorbate as the reducing agent in Kolthoff's borate buffer (pH 8.4) gave triazole **10** as reference compound in 36 % isolated yield after HPLC purification. Identity of compound **10** was confirmed by ESI–MS ($m/z = 1{,}108$ $[M+H]^+$). Synthesis of azide-functionalized phosphopeptide **9** and peptide triazole **10** is depicted in Scheme 4.

Azide-modified phosphopeptide **9** was used for copper(I)-assisted [3+2]cycloaddition reaction with **[^{18}F]F-SA**. The reaction conditions were adapted from our previous work describing the ^{18}F-labeling of neurotensin(8–13) through Cu(I)-mediated click chemistry, except for the amount of azide-modified peptide (Ramenda et al. 2007). In this previous work using Cu(I)-mediated click chemistry, we could prepare ^{18}F-labeled neurotensin(8–13) in 66 % radiochemical yields starting from 1 mg of corresponding azide-modified peptide.

In a series of experiments using click chemistry with **[^{18}F]F-SA**, we tested different amounts of azide-modified peptide, which were in all cases lower compared to the used 1 mg of peptide as reported for the click chemistry-based radiosynthesis of ^{18}F-labeled neurotensin(8–13). The progress of the click chemistry reaction between azide-modified phosphopeptide **9** and **[^{18}F]F-SA** depending on the amount of used peptide was monitored by radio-HPLC analysis. The obtained radiochemical yields are summarized in Table 1.

The results in Table 1 clearly demonstrate the importance of the amount of azide-functionalized peptide **9** upon the radiochemical yield during the Cu(I)-mediated click chemistry reaction with **[^{18}F]F-SA** to afford radiolabeled peptide **[^{18}F]10**. The highest radiochemical yield of 77 % was obtained when 0.4 mg of peptide was used (entry 4). Lowering the peptide amount to 0.3, 0.2, and 0.1 mg resulted also in lower radiochemical yields of 73, 64, and 49 %, respectively (entries 1–3). These results indicate, that Cu(I)-mediated click chemistry between **[^{18}F]F-SA** and azide-functionalized peptide **9** proceeds in moderate to high radiochemical yields even at very low peptide amounts of 0.1 mg. This represents an important improvement compared to previously reported Cu(I)-mediated click chemistry reactions with various peptides using [^{18}F]fluoroaryl compounds as summarized in Scheme 1. In these reports, typical peptide amounts were in the range of 1–2 mg leading to radiochemical yields of 37–90 % (Inkster et al. 2008; Kuhnast et al. 2008; Ramenda et al. 2007; Thonon et al. 2009; Vaidyanathan and Zalutsky 1992).

In this work, the obtained high radiochemical yields (73–77 %) at peptide concentrations as little as 0.3–0.4 mg make the described Cu(I)-mediated click chemistry reaction with **[^{18}F]F-SA** a highly promising and suitable bioconjugation tool for labeling of peptides with ^{18}F. The use of a delicate phosphopeptide **9** for Cu(I)-mediated click chemistry reaction further demonstrates the compatibility of the labeling method with even highly sensitive compounds. This makes the presented click chemistry reaction highly versatile and broadly applicable for wide variety of azide-modified peptides.

Scheme 4 Synthesis of functionalized phosphopeptides **9** and **10**

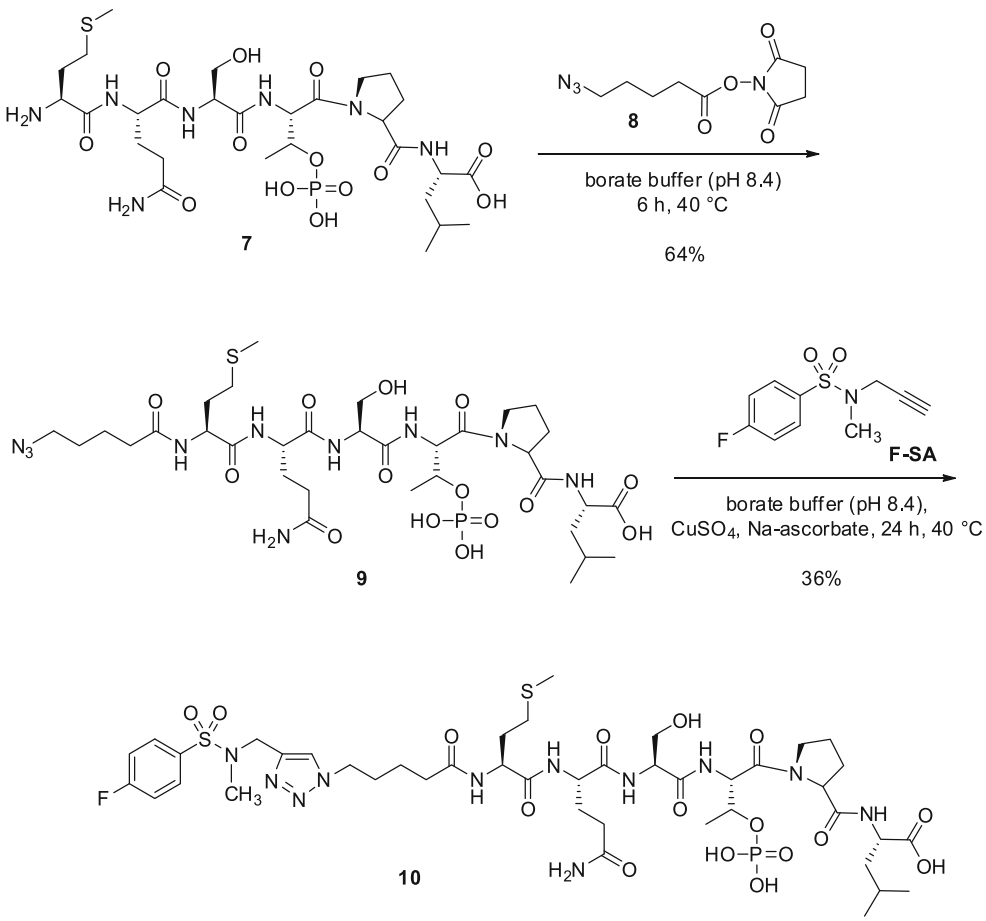


Table 1 Dependency of the radiochemical yield on the amount of azide-modified phosphopeptide **9** used in the Cu(I)-mediated click chemistry reaction with **[^{18}F]F-SA**

Entry	Peptide amount **9** (mg)	Radiochemical yield (%)[a]
1	0.1	49
2	0.2	64
3	0.3	73
4	0.4	77

All reactions were carried out using $CuSO_4$ (0.3 mg), Na-ascorbate (2.4 mg) in borate buffer (300 µL, pH 8.6) at 40 °C for 10 min

[a] Radiochemical yield was determined by radio-HPLC referring to the percentage of the radioactivity area of radiolabeled peptide **[^{18}F]10** related to the total radioactivity area

Radiolabeling of azide-functionalized human serum albumin (HSA) with **[^{18}F]F-SA**

The success of using **[^{18}F]F-SA** for radiolabeling of sensitive compounds like phosphopeptides prompted us to test the suitability of **[^{18}F]F-SA** for Cu(I)-mediated click chemistry reactions with proteins. We chose human serum albumin (HSA) as model peptide. The 67 kDa protein HSA is the most abundant protein in human blood plasma.

The amino acid sequence of HSA contains 59 lysine residues which can be used for the introduction of azide groups via acylation reaction with active ester 1-succinimidyl-5-azidopentanoate **8**. HSA in PBS was treated with a 10 to 12-fold molar excess of active ester **8** to give azide-functionalized HSA in 29 % yield after purification with size exclusion chromatography (SEC). The number of introduced azide residues was determined by enzymatic digest of azide-functionalized HSA followed by MALDI-TOF MS analysis of the protein fragments according to literature procedure (Wa et al. 2006). The enzymes trypsin, endoproteinase Lys-C and endoproteinase Glu-C were used for enzymatic digest. Each enzyme was used for a single digest of azide-modified HSA and native HSA as a control. The resulting solutions were processed according to the delayed extraction procedure prior to MALDI-TOF MS analysis (Wa et al. 2006). Seven modified lysine residues could be detected after enzymatic digest with trypsin and Glu-C, respectively, whereas 22 modified lysine residues were determined after enzymatic digest with Lys-C. Taking into account multiple detections of modified lysine residues in the different enzymatic digests, the total number of azide-modified lysine residues in HSA was determined to be 27. All these azide groups

are potentially available for Cu(I)-mediated click chemistry reaction.

First experiments to apply Cu(I)-mediated click chemistry to the radiolabeling of azide-HSA failed when in situ reduction of Cu(II) salts like $CuSO_4$ into Cu(I) in the presence of Na-ascorbate was used. This procedure led to significant degradation of azide-HSA presumably due to Na-ascorbate induced partly or complete reduction of the 17 disulfide bridges abundant in the protein. However, direct application of Cu(I) salts such as CuBr or CuI would avoid the use of detrimental reducing agents. Various multidentate triazoles are known as excellent chelates for stable complex formation of Cu(I) preventing re-oxidation of Cu(I) into Cu(II) (Chan et al. 2004; Donnelly et al. 2008). Therefore, we used oligotriazole tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)-methyl]-amine (TBTA) as suitable chelator for stabilizing Cu(I). Cu(I)-TBTA complex was applied to the click chemisty reaction between azide-functionalized HSA and **[^{18}F]F-SA**. The reaction was performed in phosphate buffer (pH 7.5) at 30 °C for 20 min. The product was purified by SEC to give ^{18}F-labeled HSA in 55–60 % decay-corrected radiochemical yield based upon **[^{18}F]F-SA**.

Figure 1 shows the results of SEC purification and subsequent polyacrylamide gel electrophoresis with sodium dodecylsulfate (SDS-PAGE) for analysis of the reaction mixture and purified fractions.

This results clearly demonstrate the principle feasibility to use Cu(I)-mediated click chemistry for the labeling of proteins with ^{18}F. The reaction conditions are compatible with the short half-life of ^{18}F. However, no further experiments were carried out to reduce the number of azide groups in the protein to further optimize the click chemistry reaction. The application of Cu(I)-TBTA complex provides a reliable and stable source of Cu(I) for the click chemistry, and it circumvents in situ reduction of Cu(II) salts as typically employed for Cu(I)-mediated [3+2]cycloaddition reactions. This general procedure should also be applicable for the radiolabeling of other proteins with ^{18}F.

Radiolabeling of azide-functionalized oligonucleotide with **[^{18}F]F-SA**

In the last decade, several oligonucleotide probes have been developed for molecular targeting and imaging of disease-specific mRNA, and various radiolabeled antisense oligonucleotides were used for imaging endogenous gene expression at the transcriptional level (Tavitian 2003; Roivainen et al. 2004). Moreover, the development of the systematic evolution of ligands by exponential enrichment (SELEX) process enabled isolation of oligonucleotide sequences, also termed as aptamers, capable of targeting virtually any class of target molecules with high affinity and specificity (Ellington and Szostak 1990).

^{18}F labeling reactions of oligonucleotides were accomplished via various bioconjugation reactions involving thiourea formation, photoconjugation, acylation reactions (e.g. [^{18}F]SFB) and alkylation reactions (e.g. [^{18}F]FPy-BrA). However, only very few reports describe the use of Cu(I)-mediated click chemistry as a bioconjugation method for oligonucleotides. This also includes radiolabeling reactions, and only one report describes the use of Cu(I)-mediated click chemistry for the radiolabeling with ^{18}F based on [^{18}F]FPy5yne in the presence of Cu(I) stabilizing ligand TBTA (Inkster et al. 2009). More recently, a click chemistry-based approach was also reported for the radiolabeling of siRNA with fluorine-18 (Flagothier et al. 2012).

The reason that only very few applications are known using Cu(I)-mediated click chemistry for bioconjugation reactions with oligonucleotides may mainly stem from the destructive effects of Cu(I)-mediated generation of highly reactive hydroxyl radicals upon nucleobase oxidation. This effect has been reviewed previously (Burrows and Muller 1998).

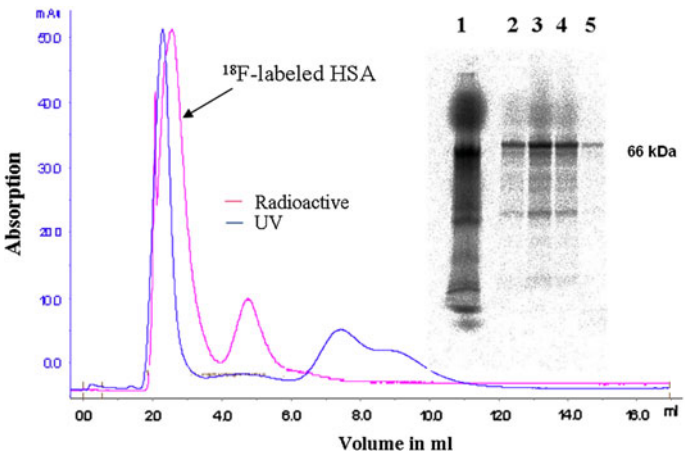
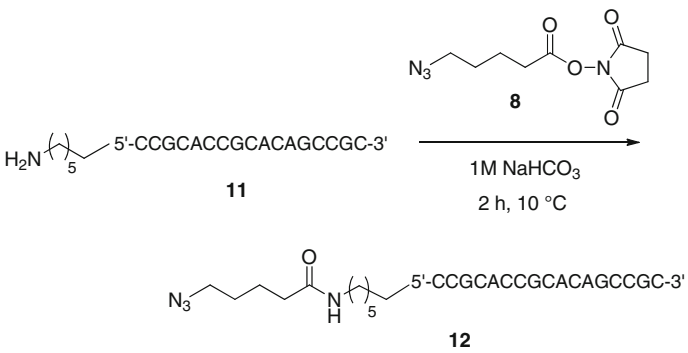


Fig. 1 SEC profile of the reaction mixture (*left*) and SDS-PAGE analysis of reaction mixture (*lane 1*) and purified fractions (*lanes* 2–5) (*right*)



Scheme 5 Functionalization of oligonucleotide **11** with an azide group

However, based on our promising results using **[^{18}F]F-SA** for Cu(I)-mediated click chemistry reactions with peptides and proteins, we wanted to expand the scope of this reaction also to oligonucleotides.

For this purpose we modified a model 17-mer L-RNA (5′-CCGCACCGCACAGCCGC-3′) **11** which contains an aminohexyl spacer at the 5′-end for functionalization with active ester 1-succinimidyl-5-azidopentanoate **8**. The acylation reaction is depicted in Scheme 5.

Acylation reaction of amine-containing oligonucleotide **11** (NOXXON Pharma AG, Berlin, Germany) with an excess of 1-succinimidyl-5-azidopentanoate **8** had to be performed at low temperatures of 10 °C to avoid decomposition. The reaction was completed after 2 h, and only traces of oligonucleotide **11** as starting material was detectable as indicated by HPLC analysis of the reaction mixture. Purification of azide-functionalized oligonucleotide **12** was achieved through SEC to remove the excess of active ester **8**.

Azide-containing oligonucleotide **12** was treated with **F-SA** in the presence of CuBr as source for Cu(I) and TBTA as ligand to stably complex Cu(I). The stable Cu(I)-TBTA complex allows for the [3+2]cycloaddition reaction between azide-modified oligonucleotide and **F-SA** while avoiding oxidative decomposition of the oligonucleotide through hydroxyl radicals generated in the presence of in situ formed Cu(I) ions.

Treatment of azide-modified oligonucleotide **12** (14.2 μg, 2.5 nmol) with **F-SA** (1 mg, 4.4 μmol) in the presence of CuBr and TBTA at 10 °C for 90 min in 1 M $NaHCO_3$/DMS) (19/1, 200 μL) afforded 28 % of desired triazole **13** after HPLC purification. Identity of triazole **13** was confirmed by MALDI-TOF MS showing a single mass peak at *m/z* 5,889 ($[M+H]^+$) which corresponds with the expected mass. Radiosynthesis was performed under comparable conditions, and ^{18}F-labeled oligonucleotide **[^{18}F]13** could be prepared in 44 % as determined by radio-HPLC analysis of the reaction mixture. Extension of the reaction time from 20 min to up to 80 min did not result in an increase of radiochemical yield. The radiosynthesis of ^{18}F-labeled oligonucleotide **[^{18}F]13** is given in Scheme 6.

This described procedure represents an useful alternative to the recently reported click chemistry-based radiosynthesis of an ^{18}F-labeled oligonucleotide by Inkster et al. (2009). The authors could isolate a ^{18}F-labeled oligonucleotide in 25 % radiochemical yields (decay-corrected), but the total synthesis time was more than 4.5 h including the synthesis of click chemistry building block [^{18}F]FPy5yne and purification of radiolabeled oligonucleotide. Considering a synthesis time for click chemistry building block **[^{18}F]F-SA** of 80 and a 20 min optimal synthesis time for the [3+2]cycloaddition between azide-functionalized oligonucleotide and **[^{18}F]F-SA**, we could reduce synthesis time of radiolabeled oligonucleotide to 100 min, excluding purification.

Our method and the recently reported synthesis by Inkster et al. demonstrate that Cu(I)-mediated click chemistry is a suitable method for the radiolabeling of oligonucleotides with ^{18}F. This method should be applicable to other nucleic acid-based compounds such as peptide nucleic acids (PNAs) or aptamers. Current methods for the ^{18}F-labeling of nucleic acid-based compounds is mainly limited to alkylation reactions using prosthetic group 4-[^{18}F]fluorobenzyl-2-bromo-acetamide ([^{18}F]FBBA) and related compounds.

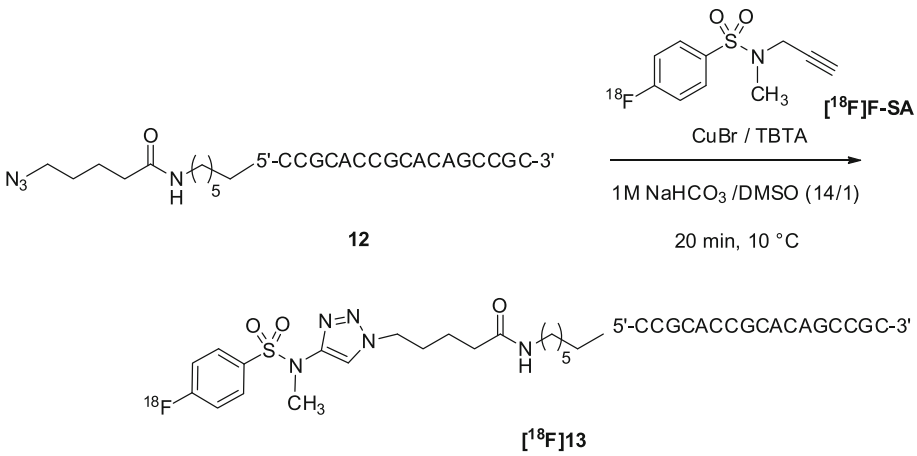
In this line, Cu(I)-mediated click chemistry has the potential to further expand the opportunities to prepare nucleic acid-based probes labeled with ^{18}F for molecular imaging applications.

Summary and conclusion

Cu(I)-mediated [3+2]cycloaddition between azides and alkynes has evolved into a valuable bioconjugation tool in radiopharmaceutical chemistry. The reaction proved to be a valuable tool for the incorporation of various radionuclides, including ^{18}F, into a broad variety of structural and functional diverse compounds under mild reaction conditions.

Radiolabeling of bipolymers like peptides, proteins and oligonucleotides with the short-lived positron emitter fluorine-18 represents a special challenge. Most bioconjugation

Scheme 6 Cu(I)-mediated click chemistry for the synthesis of ^{18}F-labeled oligonucleotide **[^{18}F]13**



methods still rely on the use of various prosthetic groups for acylation reactions (e.g. [^{18}F]SFB), alkylation reactions (e.g. [^{18}F]FBAM), and oxime formation (e.g. 4-[^{18}F]fluorobenzaldehyde). The list of novel bioconjugation methods is constantly growing, and examples for Si–F and Al–F chemistry, as well as examples for copper-free click chemistry were recently added to the list.

The application of Cu(I)-mediated [3+2]cycloadditions as an innovative and versatile bioconjugation tool for ^{18}F labeling reactions was convincingly demonstrated for a broad variety of peptides. Most reports used various volatile [^{18}F]fluoroalkyl-alkynes or -azides as click chemistry building blocks, whereas only few examples dealt with metabolically more stable [^{18}F]fluoroaryl compounds.

In this report we have described the simple, convenient and reliable radiosynthesis of **[^{18}F]F-SA** as an aromatic sulfonamide-based click chemistry building block. **[^{18}F]F-SA** could be prepared in a remotely controlled synthesis unit to provide sufficient amounts of the radiotracer at high specific activity for subsequent Cu(I)-mediated click chemistry reactions. The determined lipophilicity of **[^{18}F]F-SA** (log$P$ = 1.7) allows handling of the radiotracer in aqueous solutions as preferred for click chemistry reactions and for the use of delicate biopolymers like peptides, proteins and oligonucleotides. The versatility of **[^{18}F]F-SA** as click chemistry building block was demonstrated by the labeling of a model peptide (phosphopeptide), protein (HSA), and an oligonucleotide (L-RNA). Especially the radiolabeling of phosphopeptide **9** with **[^{18}F]F-SA** proved to be a superior ^{18}F labeling method for peptides compared to commonly employed acylation reactions with [^{18}F]SFB. The reaction proceeds in good radiochemical yields (49–77 %) despite the use of only very little peptide amounts (0.1–0.4 mg). This is a significant improvement compared to the required typical minimum peptide amounts of 1–2 mg for popular [^{18}F]SFB labeling reactions to achieve comparable radiochemical yields. We could demonstrate that Cu(I)-mediated click chemistry can also be expanded to the ^{18}F labeling of peptides as exemplified for HSA. This result has further expanded the scope of Cu(I)-mediated click chemistry with ^{18}F as a versatile bioconjugation tool in radiopharmaceutical chemistry. In combination with recent developments to incorporate diverse side chains, including azides, in predefined positions of a given protein, the presented holds promise for future site-specific labeling reactions of proteins with ^{18}F (de Graaf et al. 2009; Liao 2007; Voloshchuk and Montclare 2010). Our work has also confirmed a recent report on the principle feasibility to apply Cu(I)-mediated click chemistry for the radiolabeling of oligonucleotides with ^{18}F, while achieving further improvement of the radiolabeling method.

In conclusion, despite the recent emergence of a multitude of highly innovative novel bioconjugation methods for ^{18}F labeling of biopolymers, Cu(I)-mediated click chemistry with **[^{18}F]F-SA** continues to represent a reliable, robust and efficient radiolabeling technique for peptides, proteins, and oligonucleotides with the short-lived positron emitter ^{18}F.

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Conflict of interest The authors declare that they have no conflict of interest.

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