[^{18}F]FPyZIDE : a versatile prosthetic reagent for the fluorine-18 radiolabeling of biologics via copper-catalyzed or strain-promoted alkyne-azide cycloadditions

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Abstract

Methods for the radiolabeling of biologics with fluorine-18 have been of interest for several decades. A common approach consists in the preparation of a prosthetic reagent, a small molecule bearing a fluorine-18 that is conjugated with the macromolecule to an appropriate function. Click chemistry, and more particularly cycloadditions, are an interesting approach to radiolabel molecules thanks to mild reaction conditions, high yields, low by-products formation and strong orthogonality. Moreover, the chemical functions involved in the cycloaddition reaction are stable in the drastic radiofluorination conditions, thus allowing a simple radiosynthetic route to prepare the prosthetic reagent. We report herein the radiosynthesis of ^{18}F-FPyZIDE, a pyridine-based azide-bearing prosthetic reagent. We exemplified its conjugation via copper-catalyzed cycloaddition (CuAAC) and strain-promoted cycloaddition (SPAAC) with several terminal alkyne or strained alkyne model compounds.

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Introduction

Biologics-based radiopharmaceuticals have been shown for several decades to be valuable tools for diagnostic imaging and vectorized radiotherapy. Biologics may be defined as components that are produced by living organisms or resulting from chemical manufacturing or biotechnology while mimicking biological compounds. They comprise peptides, proteins, polysaccharides, nucleic acids but also a wide range of cellular and tissue components. In the field of Positron Emission Tomography (PET) imaging, peptides, polysaccharides or nucleic acids are key-actors in the toolbox of radiotracers and radiopharmaceuticals[1-5]. Their radiolabeling with a positron emitter is usually achieved either *via* the conjugation of a fluorine-18-labeled prosthetic reagent[6-8] or *via* their functionalization with a bifunctional chelating agent prior to the complexation of a radiometal (gallium-68, copper-64 or zirconium-89).[9-12] Other options taking advantage of the silicon-fluorine, boron-fluorine or aluminum-fluorine bond have been described and are increasingly exploited.[13-19] Nonetheless, the prosthetic approach remains a strategy of choice due to its robustness and reliability as witnessed by the numerous reports published, but there is still room available for innovation in this fruitful field. Besides the acylation, *S*-alkylation or oxime formation reactions that have been largely exploited,[7, 8, 20] the emergence of the concept of click chemistry about 15 years ago revived the design of prosthetic reagents.[21-23] This concept encompasses a wide range of chemical reactions characterized by high yields, high reaction rates, moderate temperatures and a large tolerance of solvents. The most emblematic reaction within this family is the copper-catalyzed alkyne-azide cycloaddition (CuAAC),[24] a copper-catalyzed variant of the Huisgen's cycloaddition. This reaction is particularly well adapted to the prosthetic radiofluorination of biologics in many respects: i) the mild reaction conditions do not threaten the chemical integrity of the biologics; ii) the high conjugation rates and yields are compatible with the half-life of fluorine-18 ($t_{1/2}$ = 109.8 minutes); iii) the strong orthogonality of the chemical moieties involved in the cycloaddition prevents side-reactions with amines, alcohols, carboxylic acids and other functions borne by the biologics; and iv) the chemical functions involved in the CuAAC (azide or alkyne) are stable toward the drastic reaction conditions for the radiofluoration

In spite of these numerous assets, the need of copper (generally as $CuSO_4$/sodium ascorbate) to promote the cycloaddition reaction, *via in situ* generation of Cu(I), is perceived as a drawback of the CuAAC. It indeed prevents direct *in vivo* applications of the reaction and it is recognized that reactive oxygen species (ROS)[25] may be produced. These species could induce molecular damages during the chemical functionalization of biologics like DNA or RNA and also in living cell. It has been reported that nucleobases can undergo oxidative modification in presence of copper leading to DNA or RNA scission.[26] Recently, a methodological study tended to measure the degree of copper-mediated oxidation on a library of 1200 tetrapeptides and a model protein.[27] The use of catalytic amounts of copper(I) stabilized with appropriate ligands,[28] nanoparticular catalytic systems,[29] or even pseudo-ligandless reaction by a tight control of the copper amount and presence of acetonitrile[30, 31] may limit such drawbacks of the CuAAC reaction. As a clever alternative to CuAAC, the strain-promoted alkyne-azide cycloaddition (SPAAC) reaction,[32, 33] is also widely used.[34] Its reactivity relies on lower energy of activation for the reaction of strained cyclooctynes with azides. SPAAC reaction shows equivalent advantages to CuAAC in terms of orthogonality,

yields and stability of strained alkyne in radiofluorination conditions, even if its reaction rate is slower.[35]

The compatibility of terminal alkynes, azides or even cyclooctynes with the standard radiofluorination conditions allows the preparation of the radiofluorinated prosthetic reagent in only one radiochemical step, contrary to the standard acylating or alkylating prosthetic reagents often presenting sensitive activated esters or maleimides. Their preparation thus requires multi-step processes that are limiting regarding the half-life of fluorine-18 and the automation access. Consequently, many alkyne- or azide-containing tags have flourished in the literature displaying [^{18}F]fluoroalkyl, [^{18}F]fluoraryl, [^{18}F]fluoroheteroaryl or [^{18}F]fluorocarbohydrate motives.[7, 22, 23, 36-42]

On the basis of our experience in the preparation of [^{18}F]fluoropyridine-based prosthetic reagents[43-45] and analogously to already developed clickable ones,[42, 46, 47] we report the preparation of a novel azide-containing prosthetic reagent ([^{18}F]FPyZIDE). Its versatility in cycloaddition reactions has been illustrated using a propargylated cysteine derivative and two cyclooctyne-models. For both CuAAC and SPAAC, reaction conditions (time, temperature, concentrations, catalytic system for CuAAC) have been modulated to evaluate the performances of [^{18}F]FPyZIDE regarding these two cycloadditions and to establish the best reaction compromises regarding fluorine-18 constraints.

Experimental

1. General

1.1 Chemicals

Chemicals were purchased from Sigma-Aldrich (France) and were used without further purification.

1.2 Chromatographies.

1.2.1 High Pressure Liquid Chromatography.

High Pressure Liquid Chromatography (HPLC) was performed on the following systems:

[HPLC A]: Semi-preparative equipment integrated to the TRACERLab FX-FN/FX N Pro (GE Medical Systems): S1122 Solvent Delivery System (Sykam); U.V. Detector K-2501 (Knauer); radioactivity γ detector; column Zorbax SB C18, particle size 5 µm, 250x9.4 mm (Agilent);

Mobile Phase 1 (MP-1): H_2O/MeOH/THF/H_3PO_4: 650 mL / 175 mL / 175 mL / 0.1 mL (v/v/v/v); flow rate: 5 mL/min; detection λ = 254 nm; temperature: rt.

Mobile Phase 2 (MP-2): H_2O/MeCN/TFA: 700 mL / 300 mL / 0.1 mL (v/v/v); flow rate: 5 mL/min; detection λ = 254 nm; temperature: rt.

[HPLC B]: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996 (Waters)) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, 50 x 4.6 mm, 5 µm (Waters); solvent A : H_2O containing Low-UV PIC® B7 reagent (20 mL for 1000 mL), solvent B: H_2O / CH_3CN: 30:70 (v/v)

containing Low-UV PIC® B7 reagent (20 mL for 1000 mL), isocratic elution; flow rate: 2.0 mL/min; U.V. detection at λ = 254 nm; temperature: rt.
Mobile Phase 3 (MP-3): A/B 60/40 (v/v).
Mobile Phase 4 (MP-4): A/B 70/30 (v/v).
Mobile Phase 5 (MP-5): A/B 45/55 (v/v).

1.2.2 Thin Layer Chromatography.
Thin Layer Chromatography (TLC) was performed on pre-coated plates of silica gel $60F_{254}$ (Merck) and eluted with appropriate mixtures of organic solvents. Non-radioactive compounds were localized at 254 nm using a UV-lamp and/or by dipping the TLC-plates in a 1% ethanolic ninhydrin solution or a basic potassium permanganate aqueous solution and heating on a hot plate.

1.2.3 Column Chromatography.
Column chromatographies were conducted on silica gel 63-200 µm (Merck) at 0.3 bars compressed air.

1.3 Spectroscopies.
^{1}H and ^{13}C NMR spectra were recorded on a Avance 400 MHz apparatus (Bruker), and chemical shifts were referenced to the hydrogenated residue of the deuterated solvent ($\delta[CHCl_3]$ = 7.24 ppm) for ^{1}H NMR and to the deuterated solvent ($\delta[CDCl_3]$ = 77.2 ppm) for ^{13}C NMR experiments. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q and m for singlet, doublet, triplet, quadruplet and multiplet respectively). The mass spectrometry analyses were performed by electrospray with positive (ESI+) or negative (ESI−) ionization mode using a LTQ Velos Pro mass spectrometer (Thermo Scientific) and XCalibur 2.2 software (Thermo Scientific).

1.4 Radioisotope availability and handling.
Fluorine-18, as no-carrier-added aqueous [^{18}F]fluoride ion was produced *via* the $^{18}O(p,n)^{18}F$ nuclear reaction by irradiation of a 2 mL [^{18}O]water target (> 97%-enriched, CortecNet) on an Cyclone-18/9 cyclotron (18 MeV proton beam, IBA) and was transferred to the appropriate hot cell. *Target hardware*: commercial, 2-mL, two-port, stainless steel target holder equipped with a domed-end niobium cylinder insert. *Target to hot cell liquid-transfer system* : 60 m PTFE line (0.8 mm internal diameter ; 1/16 inch external diameter), 2.0 bar helium drive pressure, transfer time 3-6 minutes. Typical production of [^{18}F]fluoride ion at the end of bombardment for a 20 µA, 30 minutes irradiation: 27-30 GBq. Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a 5 cm lead shielded cell using a TRACERLab FX-FN or FX N Pro (GE Medical Systems).

2. Chemistry
2-(2-(2-Azidoethoxy)ethoxy)ethan-1-ol (2). To a solution of 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol (**1**) (4.31 mL, 29.6 mmol) in water (25 mL) was added sodium azide (3.85 g, 59.3 mmol) and the resulting mixture was stirred 24 h at 75 °C. After cooling to room temperature, water was removed under vacuum and co-evaporated with

toluene (3x25 mL). Et_2O (50 mL) was added to the residue and the resulting suspension was filtrated. The filtrate was concentrated under vacuum to afford **2** (5.22 g, 91%) as a colourless oil. **Rf** (dichloromethane/MeOH 9:1): 0.63. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 3.74-3.64 (m, 8H); 3.60 (t, ^3J=4.5 Hz, 2H); 3.38 (t, ^3J=5.0 Hz, 2H); 2.51 (bs, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 72.6 (CH_2); 70.7 (CH_2); 70.5 (CH_2); 70.1 (CH_2); 61.8 (CH_2); 50.7 (CH_2). **ESI(+)-MS** *m/z* 198 $[M+Na]^+$ (Calculated for $C_6H_{13}N_3O_3Na^+$: 198.1).

2-(2-(2-azidoethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (3). To a solution of **2** (1.00 g, 5.71 mmol) in anhydrous dichloromethane (40 mL) were added *p*-toluenesulfonyl chloride (1.19 g, 6.28 mmol), triethylamine (0.87 mL, 6.28 mmol) and dimethylamino pyridine (0.07 g, 0.57 mmol). The mixture was stirred overnight at room temperature. A solution of saturated NH_4Cl (40 mL) was added and the mixture was extracted with dichloromethane (2x50 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated under vacuum to afford **3** (1.37 g, 73%) as a pale yellow oil. **Rf** (heptane/EtOAc 1:1): 0.27. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.78 (d, ^3J=8.4 Hz, 2H); 7.33 (d, ^3J=8.4 Hz, 2H); 4.14 (t, ^3J=4.8Hz, 2H); 3.68 (t, ^3J=4.8 Hz, 2H); 3.62 (t, ^3J=5.1 Hz, 2H); 3.59-3.57 (m, 4H); 3.34 (t, ^3J=5.1 Hz, 2H); 2.43 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 144.9 (C); 133.0 (C); 129.9 (2xCH); 128.0 (2xCH); 70.8 (CH_2); 70.7 (CH_2); 70.1 (CH_2); 69.3 (CH_2); 68.8 (CH_2); 50.7 (CH_2); 21.7 (CH_3). **ESI(+)-MS** *m/z* 352 $[M+Na]^+$ (Calculated for $C_{13}H_{19}N_3O_5SNa^+$: 352.1).

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-2-fluoropyridine (5). To a solution of 2-fluoro-3-hydroxypyridine (**4**) (361 mg, 3.19 mmol) in anhydrous DMF (15 mL) was added potassium carbonate (588 mg, 4.25 mmol). The mixture was stirred 20 min at room temperature under argon and a solution of **3** (700 mg, 2.2 mmol) in DMF (5 mL) was added to the mixture. The solution was stirred overnight at 90 °C. After cooling to room temperature, water was added (50 mL) and the resulting solution was extracted with EtOAc (2x20 mL). The organic layers were combined and successively washed with 0.1 M sodium hydroxide (2x30 mL), water (20 mL), 1 M HCl (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel, heptane/EtOAc 7:3) to afford **5** (646 mg, 75%) as a yellow oil. **Rf** (heptane/EtOAc 1:1): 0.42. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.75 (ddd, ^3J=4.8 Hz, ^4J=1.6 Hz, $^4J_{H\text{-}F}$=1.6 Hz, 1H); 7.33 (ddd, $^4J_{H\text{-}F}$=10.1 Hz, ^3J=7.6 Hz, ^4J=1.6Hz, 1H); 7.10 (dd, ^3J=7.6 Hz, ^3J= 4.8 Hz, 1H); 4.21 (t, ^3J=4.8Hz, 2H); 3.91-3.89 (m, 2H); 3.76-3.73 (m, 2H); 3.69-3-67 (m, 4H); 3.39 (t, ^3J=4.8Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 154.0 (C, d, $^1J_{C\text{-}F}$=237.0Hz); 142.3 (C, d, $^2J_{C\text{-}F}$=25.3Hz); 137.7 (CH, d, $^3J_{C\text{-}F}$=13.3Hz); 123.4 (CH, d, $^3J_{C\text{-}F}$=4.3Hz); 121.8 (CH, d, $^4J_{C\text{-}F}$=4.3Hz); 71.1 (CH_2); 70.8 (CH_2); 70.2 (CH_2); 69.6 (CH_2); 69.1 (CH_2); 50.8 (CH_2). **ESI(+)-MS** *m/z* 271 $[M+H]^+$ (Calculated for $C_{11}H_{16}FN_4O_3^+$: 271.1).

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-2-nitropyridine (7). To a solution of 2-nitro-3-hydroxypyridine (**6**) (192 mg, 1.37 mmol) in anhydrous DMF (15 mL) was added potassium carbonate (252 mg, 1.83 mmol). The mixture was stirred 20 min at room temperature under argon and a solution of **3** (300 mg, 0.91 mmol) in DMF (5 mL) was added. The solution was stirred 48 h at 90 °C. After cooling to room temperature, water was added (50 mL) and the resulting solution was extracted with EtOAc (2x20 mL). The organic layers were combined

and successively washed with sodium hydroxide 0.1 M (2x30 mL), water (20 mL), HCl 1 M (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel, heptane/EtOAc 8:2 to 1:1) to afford **7** (248 mg, 92%) as a white powder. **Mp**: 50-53 °C. **Rf** (heptane/EtOAc 1:1): 0.14. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 8.06 (dd, ^3J=4.4 Hz , ^4J=1.0 Hz, 1H); 7.58 (dd, ^3J=8.4 Hz , ^4J=1.0 Hz, 1H); 7.50 (dd, ^3J=8.4 Hz , ^4J=4.4 Hz, 1H); 4.28 (t, ^3J=4.4 Hz, 2H); 3.88 (t, ^3J=4.8 Hz, 2H); 3.71-3.68 (m, 2H); 3.65-3.62 (m, 4H); 3.36 (t, ^3J=5.2 Hz, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 149.2 (C); 147.4 (C); 139.5 (CH); 128.6 (CH); 124.4 (CH); 71.1 (CH_2); 70.7 (CH_2); 70.1 (CH_2); 69.9 (CH_2); 69.4 (CH_2); 50.7 (CH_2). **ESI(+)-MS** *m/z* 320 $[M+Na]^+$ (Calculated for $C_{11}H_{15}N_5O_5Na^+$: 320.1).

2-Dimethylamino-3-hydroxypyridine (9). To a solution of 2-amino-3-hydroxypyridine (**8**) (2.00 g, 18.1 mmol) in MeCN (30 mL) were successively added a 36% aqueous formaldehyde solution (13.9 mL, 181 mmol), acetic acid (1.04 mL, 18.1 mmol) and $NaBH_3CN$ (3.42 g, 54.5 mmol) at 0 °C. The resulting mixture was stirred 48 h at room temperature. A saturated solution of $NaHCO_3$ (50 mL) was then added and the mixture was extracted with EtOAc (3x40 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel, heptane/EtOAc 9:1 to 7:3) to afford **9** (1.80 g, 72%) as a white solid. **Rf** (heptane/EtOAc 1:1): 0.49. **Mp**: 51.0-53.2 °C. **^{1}H NMR** ($CDCl_3$; 400 MHz): δ 7.89 (dd, ^3J=4.8 Hz, ^4J=1.6 Hz, 1H); 7.16 (dd, ^3J=8.0 Hz, ^4J=1.6 Hz, 1H); 6.94 (dd, ^3J=8.0 Hz, ^3J=4.8 Hz, 1H); 5.37 (bs, 1H); 2.78 (s, 6H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 153.1 (C); 145.8 (C); 139.2 (CH); 121.9 (CH); 120.6 (CH); 42.4 (2xCH_3). **ESI(+)-MS** *m/z* 139 $[M + H]^+$ (Calculated for $C_7H_{11}N_2O^+$: 139.1).

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-*N,N*-dimethylpyridin-2-amine (10). To a solution of **9** (1.26 g, 18.2 mmol) in anhydrous DMF (30 mL) was added K_2CO_3 (1.68 g, 24.3 mmol). The mixture was stirred 20 min at room temperature under argon and a solution of **3** (2.00 g, 6.08 mmol) was added. The mixture was stirred overnight at 90 °C. After cooling to room temperature, water was added (50 mL) and the resulting solution was extracted with EtOAc (2x30 mL). The organic layers were combined and successively washed with 0.1 M sodium hydroxide (2x30 mL), water (20 mL), 1 M HCl (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel, toluene/EtOAc 1:1) to afford **10** (1.02 g, 57%) as a yellow oil.
Rf (heptane/EtOAc 1:1): 0.33. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.84 (dd, ^3J=4.8 Hz, ^4J=1.2 Hz, 1H); 7.00 (dd, ^3J=7.6 Hz, ^4J=1.2 Hz, 1H); 6.71 (dd, ^3J=7.6 Hz, ^3J=4.8 Hz, 1H); 4.12 (t, ^3J=4.8 Hz, 2H); 3.89 (t, ^3J=4.8 Hz, 2H); 3.76-3.71 (m, 2H); 3.69-3.65 (m, 4H); 3.37 (t, ^3J=4.8 Hz, 2H); 2.99 (s, 6H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 153.4 (C); 145.7 (C); 139.3 (CH); 119.1 (CH); 115.7 (CH); 71.0 (CH_2); 70.9 (CH_2); 70.3 (CH_2); 69.9 (CH_2); 67.8 (CH_2); 50.8 (CH_2); 41.0 (2xCH_3). **ESI(+)-MS** *m/z* 296 $[M+H]^+$ (Calculated for $C_{13}H_{22}N_5O_3^+$: 296.2).

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-*N,N,N*-trimethylpyridin-2-aminium (11). To a solution of **10** (60 mg, 0.20 mmol) in toluene (3 mL) was added methyl trifluoromethanesulfonate (29 µL, 0.26 mmol) at 0 °C. The mixture was stirred 2 h at room temperature and concentrated under vacuum to afford **11** (100 mg, 99%) as a brown oil

without further purification. **Rf** (dichloromethane/MeOH 95:5): 0.19. **^{1}H NMR** (DMSO-d_6, 400 MHz): δ 8.18 (dd, ^{3}J=4.4 Hz, ^{4}J=1.2 Hz, 1H); 7.97 (dd, ^{3}J=8.4 Hz, ^{4}J=1.2 Hz, 1H); 7.74 (dd, ^{3}J=8.4 Hz, ^{3}J=4.4 Hz, 1H); 4.44 (t, ^{3}J=4 Hz, 2H); 3.88 (t, ^{3}J=4 Hz, 2H); 3.65-3.60 (m, 11H); 3.590-3.56 (m, 4H); 3.38 (t, ^{3}J=4.8 Hz, 2H). **^{13}C NMR** (DMSO-d_6, 100 MHz): δ 147.0 (C); 142.9 (C); 138.5 (CH); 128.3 (CH); 125.4 (CH); 69.7 (CH_2); 69.6 (CH_2); 69.2 (CH_2); 68.9 (CH_2); 68.1 (CH_2); 53.3 (3xCH_3); 49.9 (CH_2). **ESI(+)-MS** *m/z* 310 $[M+H]^+$ (Calculated for $C_{14}H_{25}N_5O_3^+$: 311.2).

But-3-yn-1-yl tosylate (13). To a solution of 3-butyn-1-ol (**12**) (1.08 mL, 14.3 mmol) in dichloromethane (40 mL) were added *p*-toluenesulfonyl chloride (3.27 g, 17.1 mmol) and triethylamine (5.78 mL, 42.8 mmol). The mixture was stirred 48 h at room temperature under argon. Water was then added (20 mL) and the resulting solution was extracted with dichloromethane (3x15 mL). The organic layers were combined and successively washed with 1 M HCl (3x20 mL) and water (2x20 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by column chromatography (silica gel, heptane/EtOAc 9:1) to afford **13** (2.54 g, 79%) as a light yellow oil. **Rf** (heptane/EtOAc 7:3): 0.51. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.81 (d, ^{3}J=8.0 Hz, 2H); 7.35 (d, ^{3}J=8.0 Hz, 2H); 4.11 (t, ^{3}J=6.8 Hz, 2H); 2.56 (dt, ^{3}J=6.8 Hz, ^{4}J=2.4 Hz, 2H); 2.45 (s, 3H); 1.97 (t, ^{4}J=2.4 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 145.1 (C); 132.9 (C); 130.0 (2xCH); 128.0 (2xCH); 78.5 (C); 70.9 (CH); 67.6 (CH_2); 21.7 (CH_3); 19.5 (CH_2). **ESI(+)-MS** *m/z* 225 $[M+H]^+$ (Calculated for $C_{11}H_{13}O_3S^+$: 255.1).

Methyl *N*-acetyl-S-(but-3-yn-1-yl)cysteinate (14). To a solution of *N*-acetyl-*L*-cysteine methyl ester (500 mg, 2.82 mmol) in anhydrous DMF (30 mL) were added sodium hydride (81 mg, 3.4 mmol) and **13** (759 mg, 3.39 mmol). The mixture was stirred 48 h at room temperature under argon. Water (50 mL) was then added and the resulting solution was extracted with EtOAc (3x30 mL). The organic layers were combined and successively washed with sodium hydroxide 0.1 M (20 mL), water (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel, dichloromethane/acetone 100:0 to 95:5) to afford **14** (247 mg, 38%) as a yellow oil. **Rf** (dichloromethane/acetone 95:5): 0.23. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 6.44 (d, ^{3}J=7.4 Hz, 1H); 4.81 (dt, ^{3}J=7.4 Hz, ^{3}J=5.0 Hz, 1H); 3.75 (s, 3H); 3.02 (split quartet AB, ^{2}J=13.5 Hz, ^{3}J=5.0 Hz, 2H); 2.66 (t, ^{3}J=7.2 Hz, 2H); 2.43 (td, ^{3}J=7.2 Hz, ^{4}J=2.8 Hz, 2H); 2.02 (s, 3H); 2.02 (t, ^{4}J=2.8 Hz, 1H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 171.3 (C); 170.0 (C); 82.2 (C); 69.9 (CH); 52.8 (CH); 52.0 (CH_3); 34.3 (CH_2); 31.5 (CH_2); 23.2 (CH_3); 19.9 (CH_2). **ESI(+)-MS** *m/z* 230 $[M+H]^+$, 252 $[M+Na]^+$ (Calculated for $C_{10}H_{15}NO_3SNa^+$: 252.1).

Methyl *N*-acetyl-S-(2-(1-(2-(2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)ethyl)-L-cysteinate (15). To a solution of **5** (59 mg, 0.22 mmol) in a mixture of H_2O/*t*BuOH 1/1 (5 mL) were added **14** (50 mg, 0.22 mmol), $CuSO_4$ (3.5 mg, 0.022 mmol) and sodium ascorbate (86 mg, 0.44 mmol. The mixture was stirred 6 h at room temperature. The mixture was concentrated and the residue dissolved in dichloromethane (5 mL). The solution was washed with a saturated solution of NH_4Cl (10 mL) and the aqueous

layer was extracted with dichloromethane (2x5 mL). The organic layers were combined, dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography (silica gel, dichloromethane/MeOH 100/0 to 98:2) to afford **15** (62 mg, 57%) as a pale yellow oil. **Rf** (toluene/MeOH 8:2): 0.39. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.74 (dt, ^3J=4.8 Hz, ^4J=1.6 Hz, 1H); 7.53 (s, 1H); 7.31 (ddd, $J_{H\text{-}F}$=9.6 Hz, ^3J=8.0 Hz, ^4J=1.6 Hz, 1H); 7.10 (dd, ^3J=8.0 Hz, ^3J=4.8 Hz, 1H); 6.68 (d, ^3J=7.8 Hz, 1H); 4.81 (dt, ^3J=7.8 Hz, ^3J=5.0 Hz, 1H); 4.50 (t, ^3J=5.0 Hz, 2H); 4.18 (t, ^3J=4.8 Hz, 2H); 3.87-3.82 (m, 4H); 3.73 (s, 3H); 3.69-3.66 (m, 2H); 3.62-3.60 (m, 2H); 2.99-2.97 (m, 2H); 2.95-2.91 (m, 2H); 2.86-2.80 (m, 2H); 2.05 (s, 3H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 171.4 (C=O); 170.2 (C=O); 153.9 (C, d, $^1J_{C\text{-}F}$=237.0 Hz); 142.2 (C, d, $^2J_{C\text{-}F}$=25.9 Hz); 137.8 (CH, d, $^3J_{C\text{-}F}$=13.0 Hz); 123.4 (CH, d, $^3J_{C\text{-}F}$=4.6 Hz); 121.9 (CH, d, $^4J_{C\text{-}F}$=3.8 Hz); 71.0 (CH_2); 70.6 (CH_2); 69.6 (CH_2); 69.5 (CH_2); 69.2 (CH_2); 52.8 (CH_3); 52.2 (CH); 50.4 (CH_2); 34.2 (CH_2); 32.4 (CH_2); 26.0 (CH_2); 23.2 (CH_3). **ESI(+)-MS** *m/z* 500 $[M+H]^+$, 522 $[M+Na]^+$ (Calculated for $C_{21}H_{30}FN_5O_6SNa^+$: 522.2).
The ^{13}C NMR signals of triazole moiety are not detected.

((5aR,6S,6aS)-1-(2-(2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethoxy)ethyl)-1,4,5,5a,6,6a,7,8-octahydrocyclopropa[5,6]cycloocta[1,2-d][1,2,3]triazol-6-yl)methanol (17). To a solution of **5** (11.0 mg, 0.041 mmol) in anhydrous dichloromethane (0.2 mL) was added (1*R*,8*S*,9*s*)-bicyclo[6.1.0]non-4-yn-9-ylmethanol (**16**) (5.9 mg, 0.039 mmol). The mixture was stirred 4 h at room temperature and was then concentrated under vacuum. The residue was purified by column chromatography (silica gel, heptane/acetone 10:0 to 3:7) to afford **17** (16 mg, 97%) as a colorless oil. **Rf** (heptane/acetone 1:1): 0.23. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.74 (ddd, ^3J=4.8 Hz, ^4J=1.6 Hz, $J_{H\text{-}F}$=1.6 Hz, 1H); 7.31 (ddd, $J_{H\text{-}F}$=10.0 Hz, ^3J=7.6 Hz, ^4J=1.6 Hz, 1H); 7.11 (ddd,^3J=7.6 Hz, ^3J= 4.8 Hz, $J_{H\text{-}F}$=0.4 Hz, 1H); 4.40 (td,^3J=5.0 Hz, $J_{H\text{-}F}$=2.0 Hz, 2H); 4.17 (t, J=5.0 Hz, 2H); 3.84 (t, J=5.4 Hz, 2H); 3.81 (t, J=4.6 Hz, 2H); 3.74 (dd, ^2J=11.3 Hz, ^3J=7.4 Hz, 1H), 3.68-3.60 (m, 3H); 3.59-3.52 (m, 2H); 3.10 (ddd, ^2J=15.6 Hz, ^3J=8.0 Hz, ^4J=3.6 Hz, 1H); 2.99 (ddd, ^2J=16.0 Hz, ^3J=7.2 Hz, ^4J=3.2 Hz, 1H); 2.84 (ddd, ^2J=15.6 Hz, ^3J=9.2 Hz, ^4J=3.6 Hz, 1H); 2.69 (ddd, ^2J=16.0 Hz, ^3J=10.4 Hz, ^4J=3.2 Hz, 1H); 2.28-2.16 (m, 2H); 1.59-1.47 (m, 2H); 1.18 (q^t, ^3J=8.4 Hz, 1H); 1.08-0.95 (m, 2H). **^{13}C NMR** ($CDCl_3$, 100 MHz): δ 154.0 (C, d, $^1J_{C\text{-}F}$=237 Hz); 144.7 (C); 142.3 (C, d, $^2J_{C\text{-}F}$=25.0 Hz); 137.8 (CH, d, $^3J_{C\text{-}F}$=13.0 Hz); 134.5 (C); 123.4 (CH, d $^3J_{C\text{-}F}$=3.8 Hz); 121.9 (CH, $^4J_{C\text{-}F}$=3.8 Hz); 71.0 (CH_2); 70.7 (CH_2); 70.4 (CH_2); 69.5 (CH_2); 69.1 (CH_2); 59.9 (CH_2); 47.9 (CH_2); 26.1 (CH_2); 23.2 (CH_2); 23.0 (CH_2); 22.4 (CH_2); 21.4 (CH); 20.1 (CH); 19.7 (CH). **ESI(+)-MS** *m/z* 420 $[M+H]^+$ (Calculated for $C_{21}H_{29}FN_4O_4^+$: 420.2).

***N*-Boc-dibenzocyclooctyne-amine (19).** To a solution of dibenzocyclooctyne-amine (**18**) (20.0 mg, 0.072 mmol) in anhydrous dichloromethane (1 mL) was added di-*tert*-butyl dicarbonate (32 mg, 0.14 mmol). The mixture was stirred 2 h at room temperature and then concentrated under vacuum. The residue was purified by column chromatography (silica gel, heptane/EtOAc 9:1 to 7:3) to afford **19** (22 mg, 84%) as a pale yellow oil. **Rf** (heptane/EtOAc 1:1): 0.42. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.68 (d, ^3J=7.2 Hz, 1H); 7.42-7.27 (m, 7H); 5.14 (d, ^2J=14.0 Hz, 1H); 5.03 (m, 1H); 3.69 (d, ^2J=14.0 Hz, 1H); 3.26-3.09 (m, 2H); 2.49 (ddd, ^2J=16.4 Hz, ^3J=7.6 Hz, ^3J=4.0 Hz, 1H); 1.93 (ddd, ^2J=16.4 Hz, ^3J=6.0 Hz, ^3J=4.0 Hz, 1H);

1.39 (s, 9H). **^{13}C NMR ($CDCl_3$, 100 MHz):** δ 172.4 (C); 155.9 (C); 151.3 (C); 148.1 (C); 132.2 (CH); 129.2 (CH); 128.6 (CH); 128.4 (2 CH); 127.9 (CH); 127.3 (CH); 125.8 (CH); 123.1 (C); 122.7 (C); 115.1 (C); 107.8 (C); 79.1 (C); 55.6 (CH_2); 36.6 (CH_2); 35.1 (CH_2); 28.5 (3xCH_3). **ESI(+)-MS** *m/z* 377 $[M+H]^+$, 399 $[M+Na]^+$ (Calculated for $C_{23}H_{24}N_2O_3Na^+$: 399.2).

***tert*-Butyl (3-(3-(2-(2-(2-((2-fluoropyridin-3-yl)oxy)ethoxy)ethoxy)ethyl)-3,9-dihydro-8H-dibenzo[b,f][1,2,3]triazolo[4,5-d]azocin-8-yl)-3-oxopropyl)carbamate (20).** To a solution of **19** (9.4 mg, 0.025 mmol) in anhydrous dichloromethane (1 mL) was added **5** (6.8 mg, 0.025 mmol). The mixture was stirred 5 h at room temperature and then concentrated under vacuum. The residue was purified by column chromatography (silica gel, dichloromethane/MeOH 100:0 to 98:2) to afford **20** (13.6 mg, 84%) as a pale yellow oil. **Rf** (dichloromethane/MeOH 95:5): 0.5. **^{1}H NMR** ($CDCl_3$, 400 MHz): δ 7.77-6.98 (m, 11H); 6.06 (d, ^2J=16.4 Hz, 1H); 5.09 (t, ^3J=5.8 Hz, 0.4H); 5.02 (t, ^3J=5.8 Hz, 0.6H); 4.67-3.54 (m, 13H); 3.18-3.01 (m, 2H); 1.99 (m, 1H); 1.71 (m, 1H); 1.40 (s, 9H). **ESI(+)-MS** *m/z* 647 $[M+H]^+$ (Calculated for $C_{34}H_{40}FN_6O_6^+$: 647.3).
The ^{1}H NMR signals of both regioisomers are not all distinguishable. The ^{13}C NMR signals of both regioisomers are not all distinguishable and could not be correctly assigned.

3. Radiochemistry

3-(2-(2-(2-Azidoethoxy)ethoxy)ethoxy)-2-[^{18}F]fluoropyridine ([^{18}F]-5). The aqueous solution containing [^{18}F]fluoride anions was automatically transferred to the TRACERLab FX-FN or FX N Pro after the end of irradiation. The irradiated water is then sucked through a QMA cartridge (Waters) to fix the [^{18}F]fluoride anions and remove the enriched water which was separately collected. The [^{18}F]fluoride anions were eluted from the resin and transferred to the reactor with a K_2CO_3/K_{222} solution (water and acetonitrile 30/70 (1 mL) containing 1.5 mg of K_2CO_3 and 12 to 15 mg of Kryptofix® 222). Finally, the K[^{18}F]F-K_{222} complex was prepared by evaporation of the solution in two heating steps : (i) first at 60 °C for 7 minutes at a pressure ranging between 30 and 35 kPa and then (ii) at 120 °C for 5 minutes under vacuum. Radiofluorination was carried out by addition of the precursors **7** or **11** (4 to 5 mg) in solution in dimethyl sulfoxide (0.7 mL) to the dried K[^{18}F]F-K_{222} complex and the resulting mixture was heated to 160 °C for 5 minutes. After cooling to 50 °C, the crude was diluted twice with HPLC-solvent (2x2 mL) and transferred through an Alumina N cartridge (Waters) and finally collected before HPLC injection ([HPLC A]). HPLC elution was followed by both UV and radioactivity detection. When using precursor **7** [HPLC A / MP-1], the retention times were 10-11 minutes for [^{18}F]-**5** and 16-17 minutes for **7**. When using precursor **11** [HPLC A / MP-2], the retention time was 12-13 minutes for [^{18}F]-**5** (not determined for precursor **11**). The fraction containing [^{18}F]-**5** was collected and diluted with water (20 mL). The final formulation of the radiotracer was performed automatically using a Sep-Pak®Plus C18-based system (Waters). The purified tracer was finally recovered after elution of the C18 cartridge with ethanol (2 mL). Chemical and radiochemical purities were

assessed on an aliquot by analytical HPLC, with an authentic sample of **5**, as standard ([HPLC B / MP-3]: t_R = 2.6 min). Molar activity of the radiotracer was calculated from three consecutive HPLC ([HPLC B / MP-3]) analyses (average) and determined as follows: the area of the UV absorbance peak corresponding to the radiolabeled product was measured (integrated) on the HPLC-chromatogram and compared with a standard curve relating mass to UV absorbance.

3.2 Kinetic studies

General procedure.

The kinetic studies were carried out as follows: an aliquot of [^{18}F]-**5** (150 to 300 MBq, in a mixture of H_2O/EtOH 50/50) corresponding to 6 to 12 nmoles (calculated on the basis of the molar activity for each production batch, see details Table 1) was mixed with the appropriate precursor (**14**, **16** or **19**) in acetonitrile, a catalyst in the case of CuAAC and further diluted with water and *tert*-butanol to reach a final 1 mL volume. Reactions were all performed at room temperature and 40 °C. Aliquots were taken at 5, 15 and 30 minutes and analyzed by analytical HPLC [HPLC B / MP-4 or MP-5]. Conversions were evaluated by integration and comparison of the areas of the peaks of compounds [^{18}F]-**5** to [^{18}F]-**15**, [^{18}F]-**17** or [^{18}F]-**20**, derived from radioactive chromatograms.

Radiosynthesis of [^{18}F]-15 *via* CuAAC.

Kinetics of the formation of [^{18}F]-**15** was studied firstly in two different catalytic conditions:

- *CuI/sodium ascorbate/DIEA*: [^{18}F]-**5** (200 µL) mixed with **14** in MeCN (1 mg, 4 µmol, 100 µL) and aq. solutions of CuI (8.2 mg, 43 µmol, 10 eq, 100 µL)/sodium ascorbate (17 mg, 87 µmol, 20 eq,100 µL)/DIEA (4 µL) diluted with H_2O (300 µL) and *t*BuOH (200 µL).
- *$CuSO_4$/sodium ascorbate*: [^{18}F]-**5** (200 µL) was mixed with **14** in MeCN (1 mg, 4 µmol, 100 µL) and aq. solutions of $CuSO_4$ (3.4 mg, 21 µmol, 5 eq, 100µL)/sodium ascorbate (17 mg, 87 µmol, 20 eq, 100 µL) diluted with H_2O (300 µL) and *t*BuOH (200 µL). The reaction was repeated with decreasing amounts of **14** (400 nmol and 40 nmol).

Retention times were 5.2 min for [^{18}F]-**5** and 2.8 min for [^{18}F]-**15** [HPLC B / MP-4].

Radiosynthesis of [^{18}F]-17 and [^{18}F]-20 *via* SPAAC.

- *SPAAC with BCN*: [^{18}F]-**5** (200 µL) was mixed with **16** in MeCN/DMSO 2/1 (0.5 mg, 3.3 µmol, 100 µL) diluted with H_2O (400 µL) and *t*BuOH (300 µL).

Retention times were 4.8 min for [^{18}F]-**5** and 2.4 min for [^{18}F]-**17** [HPLC B / MP-4].

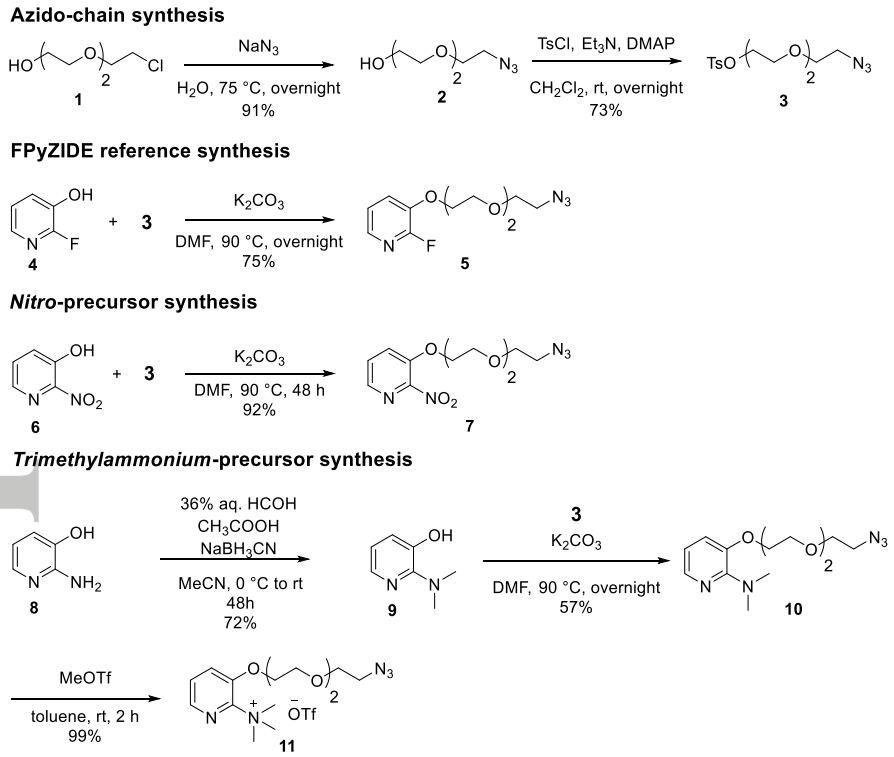
- *SPAAC with DBCO derivative*: [^{18}F]-**5** (200 µL) was mixed with **19** in MeCN/DMSO 4/1 (0.5 mg, 1.3 µmol, 100 µL) H_2O (400 µL) and *t*BuOH (300 µL). The reaction was repeated with decreasing amounts of **19** (130 nmol and 13 nmol).

Retention times were 1.3 min for [^{18}F]-**5** and 4.8 min for [^{18}F]-**20 (**[HPLC B / MP-5]).

Results and discussion

Synthesis of FPyZIDE and precursors for radiolabeling.

Non-radioactive reference FPyZIDE (**5**) and the radiolabeling precursors **7** and **11** were prepared in one, two or three steps starting from the appropriate 3-pyridinols **4**, **6** and **8** and the key intermediate **3** (Scheme 1). The azide derivative **3** was prepared in two steps by substitution of the chlorodiethoxyethanol **1** with sodium azide followed by the tosylation of the alcohol **2**. Compound **3** was obtained in 66% yield over two steps. Such PEGylated heterobifunctional chains, often used to prepare azide- or alkyne-containing prosthetic reagents, are commonly synthesized in only one step starting from a di-alcohol[46] or di-tosylated[42] PEG-chain but in lower yields due to the risk of homo-disubstitution. FPyZIDE (**5**) and the nitro-precursor **7** were prepared in only one step by alkylation of the 2-fluoro-3-hydroxypyridine (**4**) and 2-nitro-3-hydroxypyridine (**6**) with **3**. Compounds **5** and **7** were obtained in 75% and 92% yields, respectively. The trimethylammonium precursor **11** was synthesized in three steps. The 2-amino-3-hydroxypyridine (**8**) was first di-methylated by reductive amination to afford the dimethylaminopyridine **9**. Compound **9** was then alkylated in the same conditions as for compounds **5** or **7**, affording compound **10** that was methylated using methyl triflate. Compound **11** was obtained in 41% yield over three steps.



Scheme 1: Synthesis of the non-radioactive reference FPyZIDE and its radiolabeling precursors

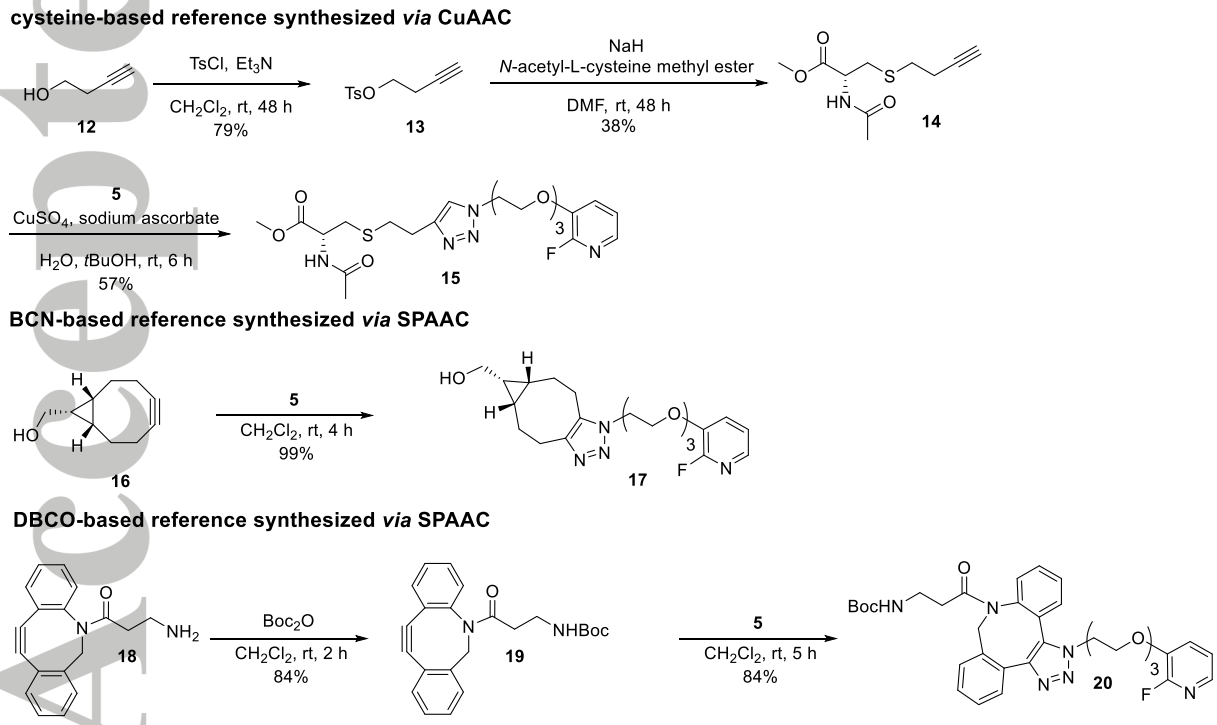
Synthesis of the non-radioactive triazole references resulting from the click reactions.

As model for the CuAAC reaction, a propargylated derivative of cysteine (**14**) was chosen (Scheme 2). The alcohol **12** was first tosylated in standard conditions to obtain compound **13** in 79% yield. *N*-Acetyl-L-cysteine methyl ester was then alkylated using **13** to afford the propargylated derivative **14** in 38% yield. The non-radioactive reference **15** was finally

prepared by reaction of **14** with FPyZIDE (**5**) in standard copper-catalyzed conditions using copper sulfate and sodium ascorbate. Compound **15** was obtained in 17% yield over three steps.

As model for the SPAAC reaction, commercially available BCN (**16**) and DBCO (**18**) were selected. These two cyclooctynes are frequently used for the tagging or engineering of biologics using SPAAC reactions and therefore numerous fluorescent, biotinylated or peptide-based tags derived from BCN and DBCO are described in the literature and marketed. Considering the reaction rate of cyclooctynes in SPAAC reaction, BCN and DBCO are among those displaying the highest rates. Sletten and Bertozzi[48] and Dommerholt *et al.* [49] summarized the second order rate constants of different cyclooctynes in a SPAAC reaction with benzyl azide or aliphatic azides. They reported rate constants of 0.14 $M^{-1}s^{-1}$ and 0.31 $M^{-1}s^{-1}$ for BCN and DBCO, respectively. This is higher than the rate constants described for fluorinated cyclooctynes like DIFO (k = 0.0076 $M^{-1}s^{-1}$) or dibenzyl cyclooctynes like DIBO (k = 0.057 $M^{-1}s^{-1}$) or TMDIBO (k = 0.094 $M^{-1}s^{-1}$). Only the dibenzyl cyclooctyne BARAC displayed a higher rate constant (k = 0.96 $M^{-1}s^{-1}$) than BCN or DBCO but with lower commercial availability and with stability issues due to rearrangements depending on the functionalization of the cyclooctyne ring.[50]

Non-radioactive BCN-based reference **17** was prepared by reaction of FPyZIDE with **16** and was obtained in 99% yield. Non-radioactive DBCO-based reference **20** was synthesized in two steps starting from DBCO **18**. For practical reasons related to the polarity of the amine function borne by **18**, we have chosen to protect it with a Boc moiety leading to compound **19** that was then reacted with FPyZIDE (**5**) and afforded non-radioactive reference **20** in 70% yield over two steps.



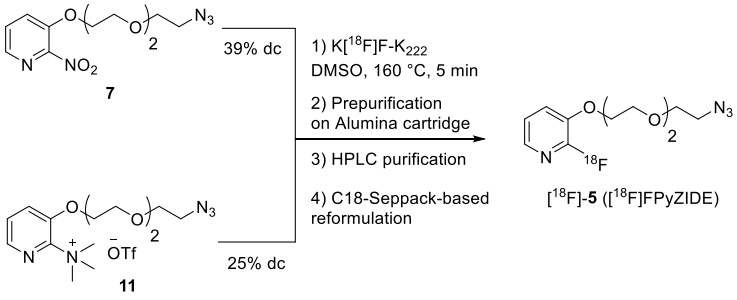
Scheme 2: Synthesis of the non-radioactive model compounds **15**, **17** and **20** resulting from CuAAC and SPAAC reactions

Radiochemistry.

[^{18}F]FPyZIDE ([^{18}F]-**5**) was prepared by nucleophilic heteroaromatic substitution (Scheme 3). Pyridine ring presents a higher reactivity toward aromatic nucleophilic substitution compared to benzene. Pyridine-based radiofluorinated prosthetic reagents are now often encountered. [42-44, 46, 47, 51-53] Two precursors displaying either a nitro (**7**) or a trimethylammonium (**11**) moiety as efficient leaving group have been tested. [^{18}F]FPyZIDE was synthesized following a standard, fully automated, radiolabeling process comprising: (i) the reaction of the precursor (**7** or **11**) with the activated complex of fluorine-18 K[^{18}F]F-K_{222} in dimethylsulfoxide at 160 °C for five minutes, (ii) the pre-purification of the reaction mixture on an alumina cartridge, (iii) the purification using reverse-phase HPLC with an adapted mobile phase to efficiently separate the exceeding precursor from [^{18}F]-**5**, and (iv) the C18-based formulation of the final product in ethanol. For the HPLC purification different mobile phases were used depending on the precursor. When the trimethylammonium precursor **11** was used, a standard mixture of water and acetonitrile with trifluoroacetic acid was efficient to separate in short purification times [^{18}F]-**5** (t_R = 12-13 min) from **11**. When the nitro precursor **7** was used, the similar polarity of nitro- and fluoro-compounds necessitated adapted mobile phase to allow for an efficient separation by reverse-phase HPLC. Using a three-component mixture of water/methanol/tetrahydrofuran acidified with phosphoric acid enabled the isolation of [^{18}F]-**5** (t_R = 16-17 min) from precursor **7** with a satisfactory purity.

Starting from precursors **7** and **11**, [^{18}F]FPyZIDE was respectively obtained in less that one hour in 39% ± 10 (n = 6) and 25% ± 8 (n = 12) radiochemical yields, based on starting [^{18}F]F^- radioactivity. The average decay-corrected molar activity was 145 ± 27 GBq/µmol (n = 11). These yields are comparable with those obtained for the preparation of other [^{18}F]pyridine-based prosthetic reagents. [^{18}F]FPyKYNE was prepared in 30-35% decay-corrected yield in 60 to 70 minutes[45], and [^{18}F]FPy5yne, an alkyne bearing analog of [^{18}F]FPyZIDE, or PEG-[^{18}F]FPyKYNE in 35 to 45% decay-corrected yields but in longer synthesis times ranging from 80 to 100 minutes.[46, 47] Only the analog ^{18}F-BMT-187144 was prepared in a surprisingly high 70% non-decay-corrected yield.[42]

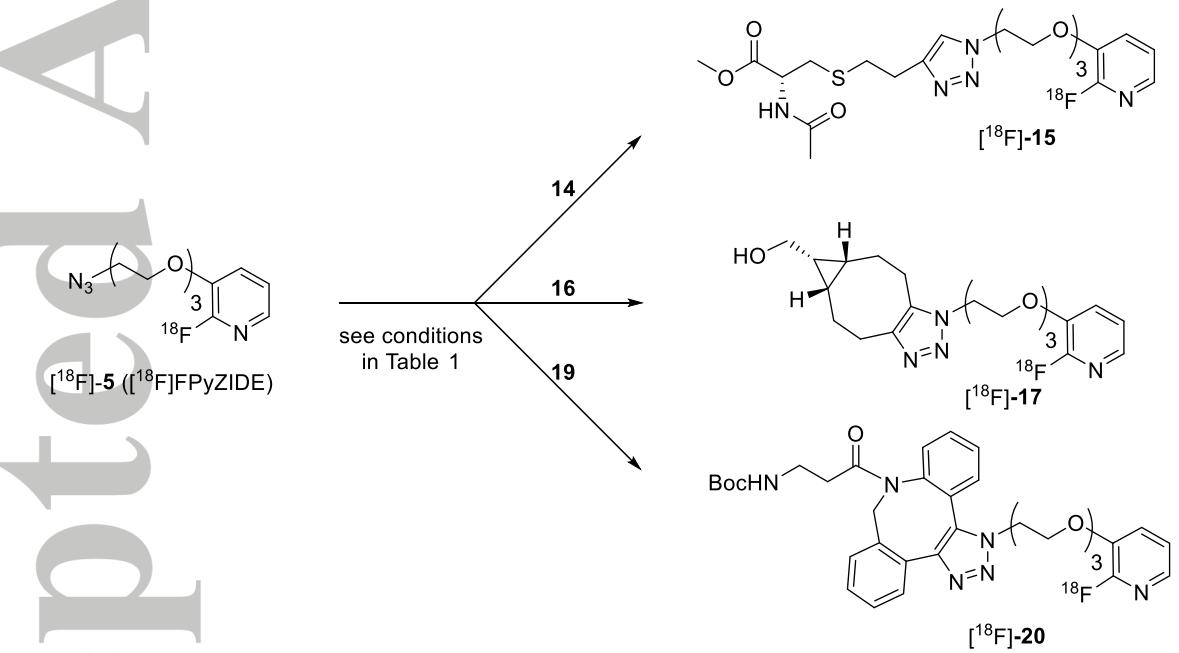
The chemical and radiochemical purities were greater than 95% and 99%, respectively, as assessed by the quality control. The average decay corrected molar activity was 145 GBq/µmol, whatever the precursor used. Even if trimethylammonium precursors are usually considered to be more reactive than the nitro ones, we observed a more efficient radiolabeling using precursor **7** than **11**. The HPLC chromatograms (see ESI – Figure S1) of isolation of [^{18}F]-**5** showed several radioactive by-products when precursor **11** was used, compared to precursor **7**. Such a trend was already observed in the preparation of [^{18}F]FPy5yne, where the nitro- and trimethylammonium precursors led to isolated [^{18}F]FPy5yne in 50% and 42% decay-corrected yields, respectively.[47] Moreover, when precursor **11** was used, a demethylation reaction of the trimethylammonium in competition with the nucleophilic substitution was observed, contributing to the lower final yields.[54]



Scheme 3: Preparation of [^{18}F]FPyZIDE

Cycloadditions (Scheme 4) were conducted in different reaction conditions summarized in Table 1. A typical series of chromatograms for the follow up of the cycloaddition can be seen in Supplementary Information (ESI – Figure S2).

For the CuAAC reaction, two standard catalysis conditions were used, either copper iodide as direct source of Cu(I) with sodium ascorbate to avoid copper oxidation and a base, or copper sulfate with sodium ascorbate to generate *in situ* Cu(I).



Scheme 4: Cycloaddition reactions to prepare [^{18}F]-**15**, [^{18}F]-**17** and [^{18}F]-**20**

The CuAAC reaction catalyzed with Cu(I) at room temperature (Table 1, conditions #1) showed a slow conversion rate and did not exceed 75% after 30 minutes (Figure 1, #1). At 40 °C with the same conditions (Table 1, conditions #2), a higher 70% conversion of [^{18}F]-**5** into [^{18}F]-**15** was observed after 5 minutes of reaction (Figure 1, #2) Surprisingly, the conversion yield decreased at longer times (15 and 30 minutes) and a more polar radioactive by-product (t_R < 1 min) than [^{18}F]-**15** (t_R = 2.8 min) was detected on analytical HPLC whereas [^{18}F]-**5** (t_R = 5.2 min) could not be detected anymore (Figure 1, #2). The aqueous basic reaction medium could induce a hydrolysis of the methyl ester protecting the carboxylic acid of the cysteine residue leading to a polar radioactive compound.

The CuAAC reaction catalyzed with Cu(II)/sodium ascorbate at room temperature (Table 1, conditions #3) showed a similar conversion yield to those observed with Cu(I) (Figure 1,

#3). At 40 °C (Table 1, conditions #4), the reaction rate increased drastically and the conversion of [^{18}F]-**5** into [^{18}F]-**15** was almost completed after 15 minutes (Figure 1, #4). When the amount of alkyne **14** decreased while maintaining the same amount of copper sulfate and sodium ascorbate (Table 1, conditions #5 and #6), a very fast reaction was observed leading to a total conversion of [^{18}F]-**5** after 5 minutes (Figure 1, #5 and #6). For longer reaction times (30 minutes), a degradation of [^{18}F]-**15** was observed but in limited amounts (less than 10%). A polar radioactive by-product was detected on HPLC chromatograms. This could be due to the oxidative character of the catalytic system leading to a partial degradation of [^{18}F]-**15**[27].

Table 1. Summary of the reaction conditions for CuAAC and SPAAC reactions

#	Alkyne		[^{18}F]-**5**	Eq. alkyne	Temp.	Catalysis			
Entry #	Compd	µM	µM			CuI (µM)	DIEA	Na Asc (µM)	$CuSO_4$ (µM)
1	**14**	4.3	0.009	477	rt	43	23	87	--
2	**14**	4.3	0.009	477	40 °C	43	23	87	--
3	**14**	4.3	0.012	358	rt	--		87	22
4	**14**	4.3	0.012	358	40 °C	--		87	22
5	**14**	0.43	0.012	35.8	40 °C	--		87	22
6	**14**	0.043	0.012	3.58	40 °C	--		87	22
7	**16**	3.3	0.011	300	rt	none			
8	**16**	3.3	0.011	300	40 °C	none			
9	**19**	1.3	0.008	162	rt	none			
10	**19**	1.3	0.010	130	40 °C	none			
11	**19**	0.13	0.009	14	rt	none			
12	**19**	0.13	0.006	21	40 °C	none			
13	**19**	0.013	0.009	1.4	rt	none			
14	**19**	0.013	0.006	2.1	40 °C	none			

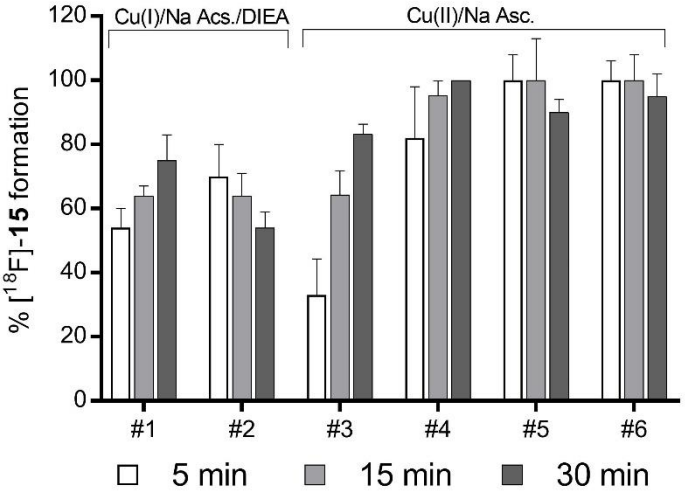


Figure 1: [^{18}F]-**15** formation kinetics in different copper-catalysis conditions *via* CuAAC

The SPAAC reaction was tested with two different cyclooctynes, BCN (**16**) and a DBCO derivative **19**. At room temperature or at 40 °C (Table 1, conditions #7 and #8), BCN (**16**) reacted slowly with [^{18}F]-**5** and the conversion yields did not exceed 14% and 27% at room temperature and 40 °C, respectively (Figure 2, #7 and #8). Longer reaction times (60 minutes) only slightly increased the conversion (data not shown). The DBCO derivative **19** reacted more rapidly with [^{18}F]-**5** (Table 1, conditions #9 and #10). The conversion was almost complete after 15 minutes (Figure 2, #9, #10). No major difference of reaction rate could be observed at room temperature and 40 °C (Figure 2, #9, #10). The difference of reactivity between **16** and **19** in SPAAC reaction is in accordance with the rates reported in the literature[49]. At lower concentrations of **19**, the conversion proceeded more slowly (Figure 2, #11 to #14). Similarly, reaction temperature had no influence on conversion rates. In quasi-equimolar conditions between **19** and [^{18}F]-**5** corresponding to nanomolar concentrations, the conversion does not exceed 5 to 7% (Figure 2 #13 and #14).

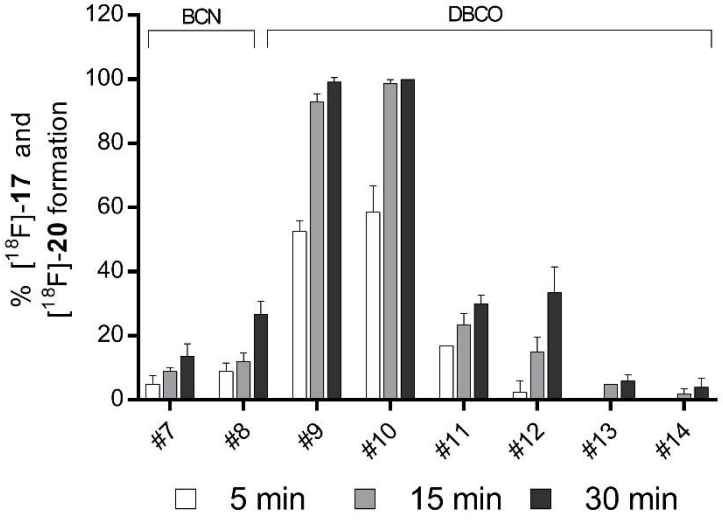


Figure 2: [^{18}F]-**17** and [^{18}F]-**20** formation kinetics *via* SPAAC

Among the different reaction conditions tested, it appeared that the fastest conversions were obtained (i) in CuAAC at 40 °C with an excess of Cu(II) and sodium ascorbate and (ii) in SPAAC using an excess of DBCO derivative **19** as strained alkyne. Moreover, in CuAAC reaction, the molar ratio between terminal alkyne (**14**) and azide ([^{18}F]-**5**) seemed to influence the reaction rate. In our experimental conditions, the decrease of this ratio, tending to an equimolar proportion of both reaction partners, positively influenced the conversion rate and allowed a reaction completion in only 5 minutes. This can be advantageous when considering the radiolabeling of expensive biologics. For the SPAAC reaction, the temperature had a limited benefit on the conversion yields, whatever the cyclooctyne used. Moreover, contrarily to the CuAAC, the dilution of strained alkyne **19** negatively influenced the conversion rate.
Our series of experiments showed that a faster reaction could be achieved with SPAAC (Figure 2, #9) than CuAAC (Figure 1, #3) at room temperature. At 40 °C, conversion rates were comparable (Figure 1, #4 – Figure 2, #9) unless working with a huge excess of copper sulfate and sodium ascorbate. In this latter case (Table 1, conditions #5 and #6), at 40 °C, the rate of CuAAC reaction was higher than SPAAC reaction. At high dilution of the reagents (Figure 1, #5 and #6 / Figure 2 #11 to#14), the conversion of [^{18}F]-**5** into [^{18}F]-**15** *via* CuAAC reaction is significantly faster than the formation of [^{18}F]-**20** *via* SPAAC reaction. These results were in accordance with classically reported reaction rates for CuAAC and SPAAC.[35]

Conclusion.

[^{18}F]FPyZIDE ([^{18}F]-**5**), a new [^{18}F]fluoropyridine-based azide-containing prosthetic reagent, was prepared in one radiochemical step in good yields (39% decay-corrected) in less than one hour starting from the corresponding nitro-precursor **7**. We showcased with model compounds that this prosthetic reagent could be used in a versatile manner either in CuAAC or SPAAC reaction in high yields and short reaction times that are fully compatible with fluorine-18 half-life. Depending on the chemical structure of the biologics that will be radiolabeled with fluorine-18 using [^{18}F]FPyZIDE, CuAAC at 40 °C will be preferred for compounds that are not sensitive to copper and temperature (short peptides, sugars) whereas SPAAC will be preferred for more sensitive biologics (nucleic acids, proteins).

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Graphical abstract.

[^{18}F]FPyZIDE : a versatile prosthetic reagent for the fluorine-18 radiolabeling of biologics *via* copper-catalyzed or strain-promoted alkyne-azide cycloadditions