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[^{18}F]FPyKYNE, a fluoropyridine-based alkyne reagent designed for the fluorine-18 labelling of macromolecules using click chemistry

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FPyKYNE (2-fluoro-3-pent-4-yn-1-yloxypyridine) is a novel fluoropyridine-based structure, designed for the fluorine-18 labelling of macromolecules using copper-catalysed Huisgen 1,3-dipolar cycloaddition (click chemistry). FPyKYNE (non-labelled as reference), as well as the 2-bromo, 2-nitro and 2-trimethylammonium analogues (as precursors for labelling with fluorine-18), was synthesized in 44, 95, 60 and 41%, respectively, from commercially available 5-chloropent-1-yne and the appropriate 2-substituted-3-hydroxypyridines. [^{18}F]FPyKYNE was synthesized in one single radiochemical step by reaction of no-carrier-added K[^{18}F]F-Kryptofix® 222 (DMSO, 165°C, 3–5 min) followed by C-18 SepPak cartridge pre-purification and finally semi-preparative HPLC purification on a Hewlett Packard SiO_2 Zorbax® Rx-SIL. Using the 2-nitropyridine or the pyridin-2-yltrimethylammonium trifluoromethanesulphonate precursor for labelling (30 and 10 μmol, respectively), incorporation yields up to 90% were observed and 7.0–8.9 GBq (190–240 mCi) of [^{18}F]FPyKYNE ([^{18}F]-1) could be isolated within 60–70 min (HPLC purification included), starting from a 37.0 GBq (1.0 Ci) [^{18}F]fluoride batch (overall decay-corrected and isolated yields: 30–35%).

Keywords: fluorine-18; FPyKYNE; macromolecule; click chemistry

Introduction

High-molecular-weight bioactive chemical structures (commonly termed as macromolecules), such as oligonucleotides, aptamers, peptide nucleic acids, peptides, proteins, antibodies, diabodies and others, are increasingly proposed as radiopharmaceuticals. Their applications are gaining importance in nuclear medicine for therapy on one side, but also for diagnostic purposes with in particular the development of 'macroprobes' labelled with positron-emitters for positron emission tomography.

Fluorine-18 is often, among the other conventional short-lived positron-emitters, the isotope of choice for the labelling of macromolecules. It can be reliably and routinely cyclotron-produced at the multi-Curie level as [^{18}F]fluoride ion and efficiently incorporated into various chemical structures using nucleophilic fluorination reactions. Its half-life (109.8 min) allows multi-step synthetic approaches[1–6] and is long enough to give access to relatively extended imaging protocols, often required with macromolecules.

Methods for radiolabelling macromolecules with fluorine-18 have been of interest for several decades. Today, the generally accepted approach involves a prosthetic group in the form of a small fluorine-18-labelled reagent that is coupled chemoselectively to an appropriate reactive function of the macromolecule. This foreign-labelling strategy has the advantage of offering a flexibility in the choice of chemical routes, including those requiring drastic chemical conditions for the preparation of the labelled prosthetic reagent, while the conjugation of the latter with a macromolecule can then be done using milder conditions needed to preserve the latter's integrity.[7,8] At present, these strategies exploit only a limited number of chemical conjugation reactions. They often use functions that are naturally provided by the macromolecule but sometimes also 'man-added' ones and mainly include acylation of an amine function with an activated carboxylic acid reagent such as the archetype *N*-succinimidyl 4-[^{18}F]fluorobenzoate [8–12] (Figure 1, compound A) and alkylation of a sulphhydryl function with bromoacetamide-[13–22] (Figure 1, compounds B and C) or maleimide [23–26] (Figure 1, compounds D–G) reagents.

Even though these strategies have been used with success for the fluorine-18 labelling of quite a large number of macromolecules, they also present some limitations such as (a) the multi-step, time- and yield consuming preparation of the fluorinated prosthetic reagent, (b) the poor chemoselectivity occasionally observed in the conjugation with the macromolecule, (c) the sometimes required protection of other chemically reactive functions of the macromolecule (especially with peptides) and (d) the often discussed hazardous stability of

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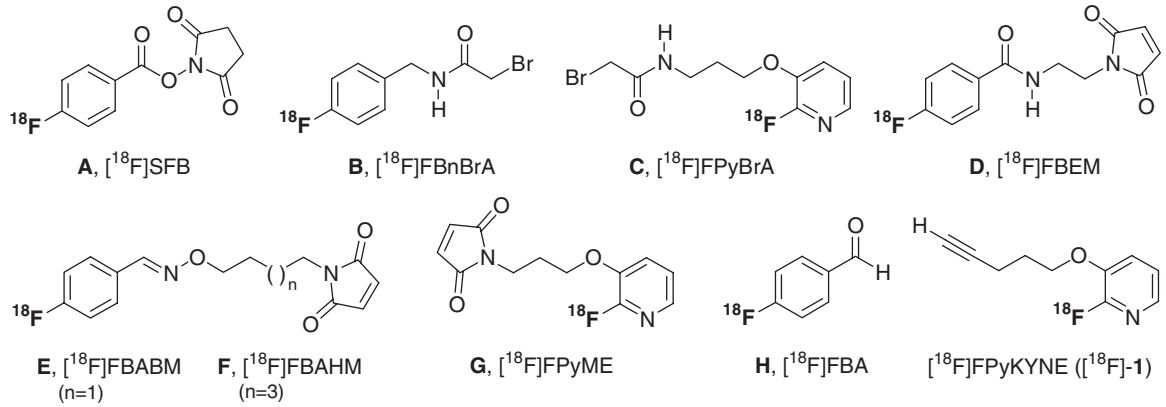


Figure 1. Selected fluorine-18-labelled reagent for conjugation to macromolecules including FPyKYNE (**1**).

the linkage between the macromolecule and the prosthetic group. In order to circumvent part of the drawbacks listed above, [^{18}F]fluorobenzaldehyde (Figure 1, compound H), a reagent that can be prepared in only one chemical step, has been used in a highly chemoselective oxime formation (a function claimed stable *in vivo*) with macromolecules provided with an aminooxy function.[27,28] Another emerging promising strategy is the use of 'click chemistry'.

'Click chemistry' is a chemical concept introduced by Barry Sharpless in 2001 and implies chemistry for the quick and reliable generation of substance families by way of a tailor-made pair of reactive complementary functional groups providing the 'click'.[29] It is often associated with combinatorial, high-throughput screening and building chemical libraries in order to speed up new drug discoveries. Sharpless and co-workers defined what makes a 'click-chemistry' reaction as one that is wide in scope and easy to perform. It uses only readily available reagents and is insensitive to oxygen and water. Reaction work-up and purification use benign solvents (often water) and try to avoid chromatography.

Of the reactions comprising the 'click universe', the perfect example is the Cu(I)-catalysed variant of the Huisgen 1,3-dipolar cycloaddition of terminal alkynes to organoazides to form 1,4-disubstituted-1,2,3-triazoles.[30,31] The triazole has similarities to the ubiquitous amide moiety found in nature, but unlike amides is not susceptible to cleavage and is nearly impossible to oxidize or reduce. Besides using mild and often fast reaction conditions, being very efficient and not requiring extended purification in many cases, Huisgen 1,3-dipolar cycloaddition also draws its success from the ease with which azides and alkynes can be introduced into a molecule (macromolecule included). Both functions are chemically relatively stable towards a large variety of organic reaction conditions and inert towards aqueous environments and buffers. Thus, these cycloadditions have been applied with success to the preparation of a large variety of new compounds, in particular conjugated macromolecules. DNA, for example, has been tagged with fluorescent probes and proteins and live organisms with rhodamine, fluorescein, biotin or nanoparticle dyes.[30,32–34] More recently, the scope of these cycloadditions has been extended to the chemistry field involving radioactive isotopes, not in the least among which the short-lived positron-emitter fluorine-18. Marik and co-workers described in 2006 the first fluorine-18 labelling of peptides tagged with a 3-azidopropionic acid moiety using ω-[^{18}F]fluoroalkynes,[35] demonstrating that the Cu(I)-catalysed 1,3-dipolar cycloaddition is compatible with the half-life of fluorine-18 and the constraints associated with its use. The *XVIIth International Symposium on Radiopharmaceutical Sciences*, which was hosted at Aachen, Germany, in the spring of 2007, comprised a full session entitled 'click-labelling methods', demonstrating that such strategies can be applied to the labelling with radioactive isotopes (fluorine-18 especially) of a large variety of compounds including macromolecules[36–41] but also highlighting the need for developing new suitable fluorinated 'clickable' reagents.

As part of our continuous efforts in the development of [^{18}F]fluoropyridine-based reagents,[21,22,26] a novel alkyne reagent, [^{18}F]FPyKYNE ([^{18}F]-**1**, 2-[^{18}F]fluoro-3-pent-4-yn-1-yloxypyridine, Figure 1), has been designed for the prosthetic labelling of macromolecules with the positron-emitter fluorine-18 using 1,3-dipolar cycloadditions, the work of which is reported herein.

Results and discussion

Chemistry

The synthesis of FPyKYNE (**1**, 2-fluoro-3-pent-4-yn-1-yloxypyridine) as a reference compound is illustrated in Scheme 1 as well as the preparation of three precursors for labelling with fluorine-18, the 2-bromopyridine **8**, the 2-nitropyridine **9** and the pyridin-2-yltrimethylammonium trifluoromethanesulphonate **11**.

FPyKYNE (**1**) was prepared in two chemical steps from commercially available 2-amino-3-hydroxypyridine (**2**) and obtained in 44% overall yield. Balz–Schiemann reaction of **2** in 70% hydrofluoric acid in pyridine containing sodium nitrite (4 equiv.) gave the 2-fluoropyridine **3** in 57% yield.[42] Alkylation of **3** with commercially available 5-chloropent-1-yne (**7**, 1.5 equiv.) was performed in DMF for 15 h at 70°C in the presence of potassium carbonate (2 equiv.) and sodium iodide (0.1 equiv.),[43–45] and afforded FPyKYNE (**1**) in 77% yield, after flash chromatography purification.

2-Bromo-3-pent-4-yn-1-yloxypyridine (**8**) and 2-nitro-3-pent-4-yn-1-yloxypyridine (**9**) were both synthesized in one chemical step from the corresponding commercially available 2-bromo- and 2-nitro-3-hydroxypyridines (**4** and **5**) using the procedure described above for the preparation of FPyKYNE and were obtained in 95 and 60% yields after purification, respectively.

The pyridin-2-yltrimethylammonium trifluoromethanesulphonate **11** was prepared in three steps from the 2-fluoropyridine **3** and obtained in 41% overall yield. Reaction of **3** with dimethylamine hydrochloride (1.3 equiv.) and potassium carbonate (1 equiv.) in refluxing DMSO/H_2O (3/1, v/v) at 110°C for 15 h[46] gave the 2-dimethylaminopyridine **6** in 45% yield. Alkylation of **6** with 5-chloropent-1-yne (**7**) using the procedure described above afforded **10** in 55% yield. Derivative **11** was then cleanly obtained in 97% yield by methylation of **10** using methyl trifluoromethanesulphonate (1.3 equiv.) in toluene at room temperature (RT) for 1 h.[21,26,46]

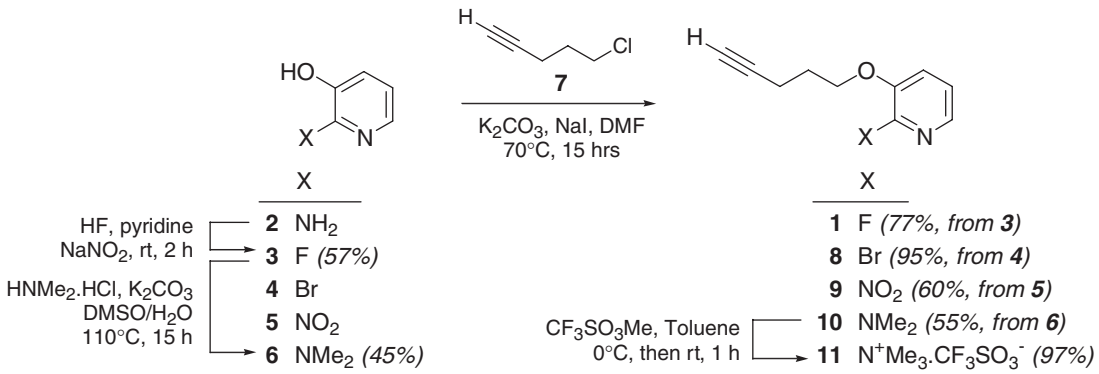
Radiochemistry

FPyKYNE (**1**) was labelled with fluorine-18 at its 2-fluoropyridinyl moiety using the one-step radiochemical process outlined in Scheme 2.

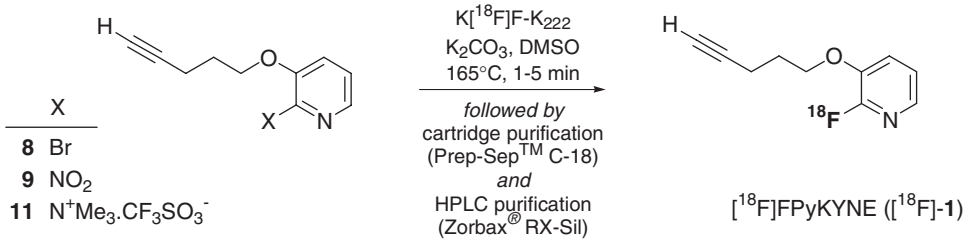
Fluorinations were performed with cyclotron-produced [^{18}F]fluoride as the no-carrier-added, activated K[^{18}F]F-Kryptofix®222 complex.[47,48] All three precursors for labelling synthesized above (derivatives **8**, **9** and **11**) were tested within a range of 10–30 μmol engaged per run. DMSO as the solvent and 165°C as the temperature were selected for these reactions based on literature,[49,50] whereas different times of reaction were studied. A DMSO solution (600 μL) containing the precursor for labelling (**8**, **9** or **11**) was transferred to 300–600 mCi of the dried K[^{18}F]F-Kryptofix®222 complex (see the Experimental) in a reaction vial (Vacutainer® tube). The open tube was then placed in a heating block at 165°C for 1–5 min. Aliquots were withdrawn at 1, 2, 3, 4 and 5 min of reaction and analysed by radio-TLC.

For each time point, TLC analyses (SiO_2, EtOAc, see the Experimental) showed only one radioactive peak (R_f: 0.75), co-migrating with FPyKYNE (**1**), besides unreacted [^{18}F]fluoride (R_f: 0). The radiochemical yields (RCY) of fluorine-18 incorporation were calculated from the TLC-radiochromatogram and defined as the ratio of radioactivity area of [^{18}F]-**1** over total radioactivity area. The results obtained (each value being the mean of three independent experiments) using 30 μmol of precursor for labelling are reported in Table 1.

The nitropyridine **9** as well as the pyridinyltrimethylammonium trifluoromethanesulphonate **11** were both highly reactive, with incorporation yields increasing with the reaction time and reaching values of more than 90% from 3 to 5 min of heating. The bromopyridine **8** was almost not reactive with RCYs <5%

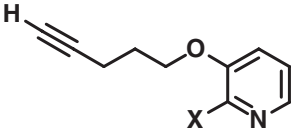


Scheme 1.



Scheme 2.

Table 1. Yields of fluorine-18 incorporation at 165°C using 30 μmol of precursor for labelling: influence of the reaction time and the leaving group

Precursor used (30 μmol)			Reaction time (in min)				
Structure	X	Code	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
	Br	**8**	5	4	5	6	16
	NO_2	**9**	22	49	>90	>90	>90
	$N^+Me_3 \cdot CF_3SO_3^-$	**11**	38	80	>90	>90	>90

from 1 to 3 min of reaction (RCY of 16% at 5 min). The ranking order of reactivity observed, $NMe_3 \geq NO_2 \gg Br$, is fully in accordance with results previously obtained for a simple model structure – the radiosynthesis of 2-[^{18}F]fluoropyridine[49,50] – as well as for the preparation of a number of [*2-^{18}F-fluoropyridinyl*]epibatidine analogues.[51–53] Worth mentioning is that the RCYs observed in this series are higher and within shorter time of reaction than those reported in the examples cited above. A similar observation was already made[54,55] for the radiofluorination of other 3-alkoxy-substituted pyridines such as, for example, the known azetidine 2-[^{18}F]F-A-85380 [42,46] and the two prosthetic reagents [^{18}F]FPyBrA[21,22] and [^{18}F]FPyME[26] (Figure 1).

At the end of the heating period mentioned above, the reaction vessel was then cooled using an ice–water bath and the remaining radioactivity was measured: 96% to over 99% of the initial radioactivity was still present. At this stage, a C-18 PrepSep^{TM} cartridge was used to rapidly trap and isolate [^{18}F]-**1** (with a radiochemical purity >95%, according to radio-TLC) from the reaction mixture (with unreacted [^{18}F]fluoride most likely eluted off the cartridge as waste). The fraction trapped on the cartridge represented 15, 75 and 75% of the total radioactivity amount engaged in the fluorination process for derivatives **8**, **9** and **11**, respectively, confirming the RCYs measured by radio-TLC. Noteworthy, 2–5% of the total radioactivity amount engaged in the fluorination process was left behind in the initial fluorination reactor tube.

Decreasing the amount of starting labelling precursor (20 μmol and down to 10 μmol) gave lower incorporation yields (calculated from the TLC-radiochromatogram or after the C-18 PrepSep^{TM} cartridge purification) when considering the nitropyridine **9** and the bromopyridine **8**, whereas similar yields were observed with the pyridin-2-yltrimethylammonium trifluoromethanesulphonate **11**.

[^{18}F]FPyKYNE ([^{18}F]-**1**) was then eluted from the C-18 PrepSep^{TM} cartridge with CH_2Cl_2 (about 5% remaining trapped and being lost on the cartridge) and purified by HPLC on a semi-preparative SiO_2 Zorbax Rx-SIL column (HPLC A, see the Experimental). A series of conditions (combination of solvent and flow rate) were tested and gave the following results. When using a 90/10 mixture of heptane and ethyl acetate (condition 1, see the Experimental), [^{18}F]-**1** (t_R: 9.5–10.0 min) could be obtained with a >95% chemical and radiochemical purity and was well separated from the remaining precursors for labelling (t_R: **8**: 12–12.5 min; **9** or **11**: >20 min). Substituting heptane by pentane (condition 2) gave similar results (t_R: **1**: 9.5–10 min; **8**: 12.5–13 min; **9** or **11**: >20 min). However, when using a 95/5 mixture of dichloromethane and diethylether (condition 3), [^{18}F]-**1** (t_R: 9.5–10.0 min) could only be obtained with a high (>95%) chemical and radiochemical purity if the precursor for labelling used is the pyridin-2-yltrimethylammonium trifluoromethanesulphonate **11** (t_R>20 min), a co-elution of [^{18}F]-**1** with the bromopyridine **8** or the nitropyridine **9** being observed in these conditions. The average isolated yield of [^{18}F]-**1** after HPLC purification and based on injected radioactivity onto the system ranged from 60 to 80%. Noteworthy, the HPLC-radiochromatograms showed one minor radioactive by-product, well separated from [^{18}F]-**1** (t_R: 7.5–8.0 min, representing less than 10% of the injected radioactivity) and this independently of the HPLC conditions or precursor for labelling used.

Typically, using the one-step synthesis described herein and the pyridin-2-yltrimethylammonium trifluoromethanesulphonate **11** (or the nitropyridine **9**) as a precursor for labelling, 7.0–8.9 GBq (190–240 mCi) of [^{18}F]FPyKYNE ([^{18}F]-**1**) could be isolated within 60–70 min (HPLC purification included), starting from a 37.0 GBq (1.0 Ci) [^{18}F]fluoride batch.

Quality control of [^{18}F]FPyKYNE ([^{18}F]-**1**) was performed on an acetonitrile-diluted aliquot of the fraction collected after semi-preparative HPLC purification. As demonstrated by analytical HPLC analysis (HPLC B, see the Experimental), [^{18}F]FPyKYNE ([^{18}F]-**1**, t_R: 2.60 min) was found to be >95% chemically and radiochemically pure and was obtained with a specific radioactivity of 150–300 GBq/μmol (4–8 Ci/μmol).

Experimental

General

Chemicals and flash chromatographies

Chemicals were purchased from Aldrich, Fluka or Sigma France and were used without further purification, unless otherwise stated. Flash chromatographies were conducted on silica gel (0.63–0.200 mm, VWR) columns.

TLC and HPLC analyses

TLCs were run on pre-coated plates of silica gel $60F_{254}$ (VWR). Compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by dipping the TLC-plates in a 1% ethanolic ninhydrin solution and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser. HPLC analyses were run as follows: (HPLC A): Equipment: system equipped with a Waters 501 pump, a Shimadzu SPD10-AVP UV-multi-wavelength detector and a miniature ionisation chamber probe; column: semi-preparative SiO_2 Zorbax Rx-SIL, Hewlett Packard (250 × 9.4 mm; porosity: 5 μm); condition 1: eluent: heptane/EtOAc 90/10 (v/v), flow rate: 5 mL/min – condition 2: eluent: pentane/EtOAc 90/10, flow rate: 5 mL/min – condition 3: eluent: CH_2Cl_2/Et_2O 95/5 (v/v), flow rate: 4 mL/min; temperature: RT; absorbance detection at $\lambda = 270$ nm. (HPLC B): Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (50 × 4.6 mm); porosity: 5.0 μm; conditions: isocratic elution with solvA/solvB: 50/50 (v/v) (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)); flow rate: 2.0 mL/min; temperature: RT; absorbance detection at $\lambda = 270$ nm.

Spectroscopies

NMR spectra were recorded on a Bruker Avance 400 MHz apparatus (Wissembourg, France) using the hydrogenated residue of the deuterated solvents (CD_2Cl_2 ($\delta = 5.32$ ppm) or DMSO-d_6 ($\delta = 2.50$ ppm)) or TMS ($\delta = 0.0$ ppm) as internal standards for ^{1}H-NMR as well as the deuterated solvents (CD_2Cl_2 ($\delta = 54.0$ ppm) or DMSO-d_6 ($\delta = 39.5$ ppm)) as an internal standard for ^{13}C-NMR. The chemical shifts are reported in ppm, downfield from TMS (s, d, t, q, m, b for singlet, doublet, triplet, quadruplet, multiplet, and broad respectively). The mass spectra (MS) were measured on a Thermo Electron Ion Trap LCQ Deca XP+ spectrometer (ESI+) (Les Ulis, France).

Radioisotope production

No-carrier-added fluorine-18 (half-life: 109.8 min) was produced via the [^{18}O(p,n)^{18}F] nuclear reaction by irradiation of a 2-mL [^{18}O]water (>97% enriched, Rotem (CortecNet, Paris, France)) target on an IBA Cyclone-18/9 (IBA, Louvain-la-Neuve, Belgium) cyclotron (18 MeV proton beam) and the aqueous radioactive solution was then 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; $\frac{1}{16}$ in external diameter), 2.0 bar helium drive pressure, transfer time 3–6 min. Typical production of [^{18}F]fluoride at the end of bombardment for a 20 μA, 30 min (10 μA h) irradiation: 27.7–29.6 GBq (750–800 mCi).

Miscellaneous

Radiosyntheses using fluorine-18, including the HPLC purifications, were performed in a shielded cell (7.5 cm lead) using a computer-assisted Zymate-XP robot system (Zymark corporation, USA).

Chemistry

*2-Fluoro-3-hydroxypyridine (**3**)*

To a stirred and cooled (0°C) solution of hydrofluoric acid pyridine complex (70% HF in pyridine, 100 mL) were successively added commercially available 2-amino-3-hydroxypyridine (**2**, 3.5 g, 31.8 mmol) and $NaNO_2$ (7.5 g, 120 mmol, 4 equiv.). The reaction mixture was stirred for 2 h at RT and then neutralized using a 3 N aqueous NaOH solution (100 mL). The resulting solution was extracted twice with EtOAc (250 mL). The organic layers were combined, washed with brine and dried over Na_2SO_4. Concentration to dryness gave **3** (2.0 g, 57%) as a brown solid that was further used without additional purification. R_f (EtOAc/heptane: 80/20): 0.65. ^{1}H-NMR (DMSO-d$_6$, 298 K): δ: 10.35 (s, 1H); 7.58 (td, J = 4.8 and 1.6 Hz, 1H); 7.36 (ddd, J = 10.4, 7.6 and 1.6 Hz, 1H); 7.10 (ddd, J = 7.6, 4.8 and 1.2 Hz, 1H). ^{13}C-NMR (DMSO-d$_6$, 298 K): δ: 152.8 (C, J^1_{C-F} = 232 Hz); 140.2 (C, J^2_{C-F} = 27 Hz); 135.6 (CH, J^3_{C-F} = 13 Hz); 126.2 (CH, J^3_{C-F} = 5 Hz); 122.6 (CH, J^4_{C-F} = 4 Hz). MS: C_5H_4FNO: 114 $[M+H]^+$.

*2-Dimethylamino-3-hydroxypyridine (**6**)*

Compound **3** (1.0 g, 8.84 mmol), finely divided K_2CO_3 (1.22 g, 8.85 mmol, 1 equiv.) and $Me_2NH \cdot HCl$ (0.94 g, 11.5 mmol, 1.3 equiv) were successively dissolved in a mixture of DMSO (75 mL) and water (15 mL). The mixture was then stirred overnight at 110°C, cooled to RT and diluted with water (100 mL). The resulting solution was extracted twice with EtOAc (250 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue (a green oil) was purified by flash chromatography on silica gel (heptane/EtOAc: 80/20 (v/v)) to afford **6** (0.6 g, 45%) as a yellow solid. R_f (heptane/EtOAc: 80/20): 0.3. ^{1}H-NMR (DMSO-d$_6$, 298 K): δ: 9.53 (bs, $w_{1/2}$ = 18 Hz, 1H); 7.64 (dd, J = 4.8 and 1.6 Hz, 1H); 6.97 (dd, J = 7.6 and 1.6 Hz, 1H); 6.66 (dd, J = 7.6 and 4.8 Hz, 1H); 2.86 (s, 6H). ^{13}C-NMR (DMSO-d$_6$, 298 K): δ: 151.7 (C); 143.7 (C); 137.1 (CH); 121.2 (CH); 115.8 (CH); 40.2 (2 × CH_3). MS: $C_7H_{10}N_2O$: 139 $[M+H]^+$.

General procedure for the preparation of the 2-substituted-3-pent-4-yn-1-yloxypyridines

The appropriate 2-substituted-3-hydroxypyridine (**3**, **4**, **5**, **6**, 1.0 g scale, 5–10 mmol), finely divided K_2CO_3 (2 equiv.), NaI (0.1 equiv.) and 5-chloropent-1-yne (**7**, 1.5 equiv.) were successively dissolved in DMF (15 mL). The mixture was then stirred overnight at 70°C, cooled to RT and diluted with water (100 mL). The resulting solution was extracted twice with EtOAc (250 mL). The organic layers were combined, washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by flash chromatography on silica gel to afford the expected 2-substituted-3-pent-4-yn-1-yloxypyridine (**1**, **8**, **9** and **10**, respectively).

*2-Fluoro-3-pent-4-yn-1-yloxypyridine (**1**)*

The procedure described above was performed with compound **3** (8.8 mmol). Purification by flash chromatography on silica gel (heptane/EtOAc: 70/30 (v/v)) gave **1** (1.2 g, 77%) as a white powder. R_f (heptane/EtOAc: 60/40): 0.6. R_f (EtOAc): 0.75. t_R (HPLC A): 9.5–10.0 min. t_R (HPLC B): 2.60 min. ^{1}H-NMR (CD_2Cl_2, 298 K): δ: 7.72 (dt, J = 4.8 and 1.6 Hz, 1H); 7.35 (ddd, J = 9.6 and 8.0 Hz, 1H); 7.15 (dd, J = 8.0 and 4.8 Hz, 1H); 4.17 (t, J = 6.4 Hz, 2H); 2.44 (td, J = 7.2 and 2.8 Hz, 2H); 2.05 (q^5, J = 6.4 Hz, 2H); 2.05 (t, J = 2.8 Hz, 1H). ^{13}C-NMR (CD_2Cl_2, 298 K): δ: 154.2 (C, J^1_{C-F} = 235 Hz); 142.7 (C, J^2_{C-F} = 26 Hz); 137.5 (CH, J^3_{C-F} = 13 Hz); 123.1 (CH, J^3_{C-F} = 5 Hz); 122.3 (CH, J^4_{C-F} = 4 Hz); 83.5 (C); 69.7 (CH); 68.0 (CH_2); 28.4 (CH_2); 15.4 (CH_2). MS: $C_{10}H_{10}FNO$: 180 $[M+H]^+$.

*2-Bromo-3-pent-4-yn-1-yloxypyridine (**8**)*

The procedure described above was performed with commercially available 2-bromo-3-hydroxypyridine (**4**, 5.7 mmol). Purification by flash chromatography on silica gel (heptane/EtOAc: 70/30 (v/v)) gave **8** (1.3 g, 95%) as a white powder. R_f (heptane/EtOAc: 60/40): 0.65. ^{1}H-NMR (CD_2Cl_2, 298 K): δ: 7.93 (dd, J = 4.4 and 1.6 Hz, 1H); 7.21 (dd, J = 8.0 and 4.4 Hz, 1H); 7.17 (dd, J = 8.0 and 1.6 Hz, 1H); 4.14 (t, J = 6.0 Hz, 2H); 2.46 (td, J = 7.2 and 2.8 Hz, 2H); 2.04 (q^5, J = 6.8 Hz, 2H); 2.01 (t, J = 2.4 Hz, 1H). ^{13}C-NMR (CD_2Cl_2, 298 K): δ: 152.7 (C); 141.7 (CH); 133.4 (C); 124.0 (CH); 120.2 (CH); 83.6 (C); 69.5 (CH); 68.0 (CH_2); 28.4 (CH_2); 15.5 (CH_2). MS: $C_{10}H_{10}BrNO$: 242 $[M+H]^+$, 240 $[M+H]^+$.

*2-Nitro-3-pent-4-yn-1-yloxypyridine (**9**)*

The procedure described above was performed with commercially available 2-nitro-3-hydroxypyridine (**5**, 7.1 mmol). Purification by flash chromatography on silica gel (heptane/EtOAc: 70/30 (v/v)) gave **9** (0.9 g, 60%) as a yellow powder. R_f (heptane/EtOAc: 50/50): 0.6. ^{1}H-NMR (CD_2Cl_2, 298 K): δ: 8.05 (dd, J = 4.0 and 2.4 Hz, 1H); 7.56–7.54 (m, 2H); 4.24 (t, J = 6.0 Hz, 2H); 2.40 (td, J = 7.2 and 2.4 Hz, 2H); 2.02 (q^5, J = 6.4 Hz, 2H); 2.02 (t, J = 2.8 Hz, 1H). ^{13}C-NMR (CD_2Cl_2, 298 K): δ: 149.5 (C); 147.4 (C); 139.6 (CH); 129.2 (CH); 124.1 (CH); 83.2 (C); 69.6 (CH); 68.5 (CH_2); 28.2 (CH_2); 15.3 (CH_2). MS: $C_{10}H_{10}N_2O_3$: 207 $[M+H]^+$.

*2-Dimethylamino-3-pent-4-yn-1-yloxypyridine (**10**)*

The procedure described above was performed with compound **6** (7.2 mmol). Purification by flash chromatography on silica gel (heptane/EtOAc: 85/15 (v/v)) gave **10** (0.8 g, 55%) as a yellow oil. R_f (heptane/EtOAc: 60/40): 0.4. ^{1}H-NMR (CD_2Cl_2, 298 K): δ: 7.77 (dd, J = 4.8 and 1.4 Hz, 1H); 7.01 (dd, J = 7.8 and 1.4 Hz, 1H); 6.70 (dd, J = 7.8 and 4.8 Hz, 1H); 4.05 (t, J = 6.0 Hz, 2H); 2.95 (s, 6H);

2.43 (td, $J = 7.0$ and 2.6 Hz, 2H); 2.03 (q^5, $J = 7.0$ Hz, 2H); 2.02 (t, $J = 2.0$ Hz, 1H). ^{13}C-NMR (CD_2Cl_2, 298 K): δ: 153.7 (C); 146.1 (C); 139.2 (CH); 119.0 (CH); 115.8 (CH); 83.8 (C); 69.4 (CH); 67.3 (CH_2); 41.2 ($2 \times CH_3$); 28.8 (CH_2); 15.9 (CH_2). MS: $C_{12}H_{16}N_2O$: 205 $[M+H]^+$.

*(3-Pent-4-yn-1-yloxypyridine-2-yl)trimethylammonium trifluoromethanesulphonate (**11**)*

To a stirred and cooled (0°C) solution of compound **10** (0.35 g, 1.71 mmol) in toluene (8 mL) was added methyl trifluoromethanesulphonate (230 μL, d: 1.45, 334 mg, 2.04 mmol, 1.3 equiv.). The mixture was stirred at RT for 1 h and then concentrated to dryness. Derivative **11** was obtained as a brown solid (0.6 g, 97%). ^{1}H-NMR (DMSO-d_6, 298 K): δ: 8.17 (dd, $J = 4.4$ and 1.6 Hz, 1H); 7.97 (dd, $J = 7.6$ and < 1.5 Hz, 1H); 7.73 (dd, $J = 8.0$ and 4.4 Hz, 1H); 4.35 (t, $J = 6.0$ Hz, 2H); 3.61 (s, 9H); 2.89 (t, $J = 2.4$ Hz, 1H); 2.40 (td, $J = 7.2$ and 2.8 Hz, 2H); 2.06 (q^5, $J = 6.8$ Hz, 2H). ^{13}C-NMR (DMSO-d_6, 298 K): δ: 147.0 (C); 142.5 (C); 138.4 (CH); 128.4 (CH); 125.0 (CH); 121.1 (q^4, $J^1_{C-F} = 320$ Hz, CF_3); 83.3 (C); 72.1 (CH); 68.6 (CH_2); 53.4 ($3 \times CH_3$); 27.0 (CH_2); 14.9 (CH_2). MS: $C_{14}H_{19}F_3N_2SO_4$: 369 $[M+H]^+$.

Radiochemistry

Preparation of the K[^{18}F]F-K_{222} complex

In order to recover and recycle the [^{18}O]water target, 2 mL of aqueous [^{18}F]fluoride from the target holder was passed through an anion exchange resin (SepPak® Light Waters Accell™ Plus QMA cartridge, chloride form, conditioned by washing with 1 M aqueous $NaHCO_3$ (2 mL) and rinsing with water (20 mL)) by helium pressure (1.5–2.0 bar). Helium was blown through the cartridge to maximally extract the last traces of [^{18}O]water. The [^{18}F]fluoride ion was then eluted from the resin, using an aqueous K_2CO_3 solution (1.0 mL of a 4.5 mg/mL solution), into a Vacutainer® tube containing Kryptofix® 222 (K_{222}: 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, 12.0–15.0 mg). The resulting solution was then gently concentrated to dryness at 145–150°C under a nitrogen stream for 10 min to give no-carrier-added K[^{18}F]F-K_{222} complex as a white semi-solid residue.

Fluorine-18 incorporation studies

DMSO (600 μL) containing 10–30 μmol of the appropriate precursor for labelling (**8** (2.4–7.2 mg), **9** (2.0–6.1 mg) or **11** (3.7–11.0 mg)) was added into the Vacutainer® tube containing the dried K[^{18}F]F-K_{222} complex. The tube (open) was thoroughly vortexed (30 s) and then placed in a heating block (at 165°C, for 1–5 min) without stirring the contents. Aliquots were withdrawn at regular time intervals in order to study the reaction kinetics and analysed by radio-TLC. Identity of [^{18}F]-**1** was verified by co-migration with non-labelled **1** (reference). Incorporation yields were calculated from the TLC-radiochromatogram and defined as the ratio of radioactivity area of [^{18}F]-**1** over total fluorine-18 radioactivity area (SiO_2-TLC (EtOAc): R_f: [^{18}F]-**1**: 0.75 – R_f: [^{18}F]fluoride ion: 0.0). At the end of the heating period, the reaction vessel was cooled using an ice–water bath and the remaining radioactivity was measured. The reaction mixture was then diluted with water (1 mL) and transferred onto a C-18 cartridge (PrepSep™ R-C-18 Extraction Column, Fisher Scientific, activated beforehand with EtOH (2 mL) and then rinsed with water (10 mL)), pre-filled with water (2 mL). The tube was rinsed twice with water (1 mL), which was also transferred and added to the diluted reaction mixture on top of the cartridge. An additional portion of water (2 mL) was further added to the diluted reaction mixture on top of the cartridge. The whole was then passed through the cartridge, which was then washed with water (3 mL) and partially dried for 0.5 min by applying a nitrogen stream. [^{18}F]-**1** was eluted from the cartridge with CH_2Cl_2 (3 mL) into an empty 5-mL reaction vial. Elution was repeated twice with 1 mL of CH_2Cl_2 for maximal transfer of [^{18}F]-**1**. The final incorporation yield was also estimated after the C-18 cartridge elution by the ratio CH_2Cl_2– over total radioactivity amount engaged in the fluorination process.

Purifications

The eluted CH_2Cl_2 solution containing [^{18}F]-**1** was partly concentrated at 65–75°C under a gentle nitrogen stream for 1–2 min. The residue was then diluted with the HPLC solvent used for purification (0.5 mL) and the crude was injected onto HPLC (HPLC A, condition 1, 2 or 3).

Optimized conditions

DMSO (600 μL) containing **9** (6.1 mg, 30 μmol) or **11** (3.7 mg, 10 μmol) was added into the Vacutainer® tube containing the dried K[^{18}F]F-K_{222} complex. The tube (open) was thoroughly vortexed (30 s) and then placed in a heating block (at 165°C, for 3 min) without stirring the contents. The reaction vessel was then cooled using an ice–water bath and the reaction mixture diluted with water (1 mL) and transferred onto a C-18 cartridge (PrepSep™ R-C-18 Extraction Column, Fisher Scientific, activated beforehand as described above). The cartridge pre-purification procedure was performed as mentioned above and [^{18}F]-**1** was HPLC-purified (HPLC A, condition 1 or 3 when using **9** and **11**, respectively).

Quality controls

Final chemical identification of [^{18}F]-**1** was performed on an acetonitrile-diluted aliquot of the HPLC-collected fraction by analytical HPLC (HPLC B) with a sample of authentic **1**. Chemical and radiochemical purities of [^{18}F]-**1** were also assessed on this aliquot using the same analytical HPLC (HPLC B). Specific radioactivity of [^{18}F]-**1** was calculated from three consecutive HPLC (HPLC B) analyses (average) and determined as follows: the area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the HPLC-chromatogram and compared with a standard curve relating mass to UV absorbance. The specific radioactivity follows from the found mass and the associated collected radioactivity.

Conclusion

FPyKYNE (**1**) is a novel fluoropyridine-based structure, designed for the fluorine-18 labelling of macromolecules using the so-called 'click-chemistry' approach. FPyKYNE has been labelled with fluorine-18 in 19–24% overall non-decay-corrected and isolated yields (30–35% decay-corrected), using a simple but highly efficient one-step process (a nucleophilic *hetero*aromatic substitution), the latter being fully automatized on our Zymate-XP robotic system. These results were first presented at the

second annual meeting of the European Society of Molecular Imaging (Naples, Italy, 14–15 June 2007).[56] A few weeks earlier, Inkster, Adam and Ruth (*Triumf*, *Vancouver*, *Canada*) presented at the *XVIIth International Symposium on Radiopharmaceutical Sciences* (Aachen, Germany, 30 April 30–4 May 2007) another fluoropyridine-based alkyne reagent (namely 2-[^{18}F]fluoro-3-hex-5-yn-1-yloxypyridine).[41] This coincidence highlights the growing interest for the development of these methodologies. Huisgen 1,3-dipolar cycloadditions involving our reagent and macromolecules are in progress.

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