A Novel Versatile Precursor Suitable for ^{18}F- Radiolabelling *via* "Click-Chemistry"

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ABSTRACT

As an effort to improve ^{18}F radiolabelling of biomolecules in terms of method robustness and versatility, we report the synthesis and radiolabelling of a new azido precursor potentially useful for the so called "click-reaction", in particular the ligand-free version of the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC). The new azido precursor may help to overcome problems sometimes exhibited by most of the currently used analogues, as it is safe to handle and it displays long term chemical stability, thus facilitating the development of new radiolabelling procedures. Moreover, the formed ^{18}F labelled 1,2,3-triazole is potentially metabolically stable, and could enhance the *in vivo* circulation time. The above azido precursor was successfully radiolabelled with ^{18}F, with 51% radiochemical yield (non-decay-corrected). As a proof of concept, the ^{18}F labelled azide was then tested with a suitable alkyne functionalized aminoacid (L-propargylglycine), showing 94% of conversion, and a final radiochemical yield of 27% (>99% radiochemical purity), non-decay-corrected, with a total preparation time of 104 min.

Keywords:
^{18}F-radiolabelling; click chemistry; 1,2,3-triazole; PET; CuAAc; L-propargylglycine

INTRODUCTION

^{18}F is the most widely used radionuclide in Positron Emission Tomography (PET). It is a positron emitting radionuclide that can be produced in high yields with cyclotrons with proton beam energy in the range 10-19 MeV; it shows favorable characteristics in terms of decay and emission properties, including high positron abundance (97%), and a physical half-life of 109.8 min, that allows for time-consuming multi-step radiosynthesis, and the use of PET tracers with moderately slow pharmacokinetics. Moreover, its low positron energy (maximum 635 KeV), ensures high image resolution. Another advantage in ^{18}F radiolabelling is that, although fluorine is absent in most biologically active compounds, its introduction *via* the replacement of protons, hydroxyl, and amino groups, does not alter significantly the steric hindrance. Fluorine, the most electronegative element, is isosteric with a hydroxyl group, the

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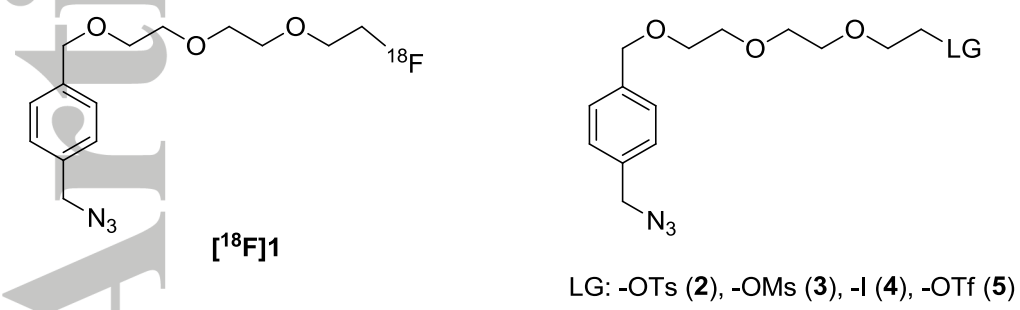
C-F bond length (1.35 Å) being similar to the C-O length (1.43 Å). In addition, it is the second smallest atom and it can mimic hydrogen in a modified structure; its van der Waals radius (1.47 Å) is intermediate between that of hydrogen (1.20 Å) and that of oxygen (1.52 Å). The strength of the C-F bond (105.4 kcal mol^{-1}) exceeds that of C-H bond (98.8 kcal mol^{-1}) and of C-O bond (84.0 kcal mol^{-1}) and for this reason fluorine is often used to replace hydrogen in medicinal chemistry.[1,2,3]

In the last decade, there has been increasing interest in the ^{18}F radiolabelling of biomolecules such as peptides, proteins, nucleic acids, oligomers, etc. As direct ^{18}F radiolabelling of the above biomolecules is usually not feasible, due to both the lack of functional groups suitable for direct nucleophilic substitution, and harsh conditions often used during this reaction type, a variety of indirect methods were developed, the most common of which has long been the radiofluorination of small molecules to achieve a prosthetic group, which is then conjugated to the biomolecule under mild conditions.[3,4,5,6] To this regard, the Cu(I)-catalyzed Huisgen [3+2] cycloaddition between terminal alkynes and azides, the so called Copper-Catalyzed Azide Alkyne Cycloaddition (CuAAC) was widely used in recent years, because of its regioselectivity, mild aqueous organic conditions, reduced reaction times, and high yields. Moreover, the produced 1,2,3-triazoles usually show biological stability, and polarity and size similar to amide or peptide bonds. Finally, as alkynes and azides are inert towards classic biomolecules functionalities, there is no need for protection strategy during CuAAC. [3, 4]
Therefore, a new class of precursors for the ^{18}F radiolabelling of biomolecules such as aminoacids, peptides, and oligomers was developed.[7]
As CuAAC is alkyne-rate dependent,[8, 9] our attention was focused on azido precursor, the best strategy to save time and resources being the ^{18}F radiolabelling of such a precursor, and the subsequent cycloaddition with an alkynyl-modified biomolecule.
However, there is still a need of optimized azido precursors. In fact, some of the azido compounds used as precursors for ^{18}F radiolabelling described in the literature show significant drawbacks, such as marked instability, and they bear the leaving group for the introduction of ^{18}F, the azido group or a polar chain directly linked to an aromatic ring, with a potential loss of reactivity and chemical stability during radiofluorination and click-reaction. Furthermore, most of the azido precursors display poor UV absorption, that can be a disadvantage during the development of new radiolabelling methods, as monitoring of reaction and purification steps is often performed using HPLC equipped with UV detectors.
The aim of this work is the synthesis of a new, general purpose, azido precursor, optimized for ^{18}F radiolabelling of biomolecules *via* "click" chemistry. The proposed compound has proved to be safe, versatile, stable, polar, reactive, and with good UV absorbance, and it may potentially be used in combination with any alkynyl-modified biomolecule. Moreover, the concentration of copper catalyst, as well as the amount of alkynyl-modified biomolecule, could be lower than those usually requested, and the radiolabelled biomolecule might potentially display improved metabolic stability.
The precursor was radiolabelled with ^{18}F, and the ability to efficiently react with alkynil-functionalized moieties *via* CuAAC of the resulting [^{18}F]fluoroazide was verified with a suitable test compound, namely propargylglycine.
During the development of the proposed CuAAC method, the use of "click" accelerating ligands such as tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) was avoided, because they make the reaction more pH and solvent sensitive, potentially affecting the versatility.[10]

RESULTS AND DISCUSSION

Our purpose dealt with the development and standardization of a general and versatile procedure for the radiolabelling of biomolecules with ^{18}F. With the aim to simplify and speed up the method development, we initially selected commercially available and well-known azido precursors useful for CuAAC, such as azidoethyltosylate[11] or diaryliodonium salt precursors[12]. However, hazards associated with their shipment and storage, together with their poor UV absorption, prompted us to design a new, general purpose, safe and reliable ^{18}F-labelled azide **[^{18}F]1**, and to synthesize a useful set of precursors (**2**-**5**) possessing the suitable characteristics discussed above (Figure 1).



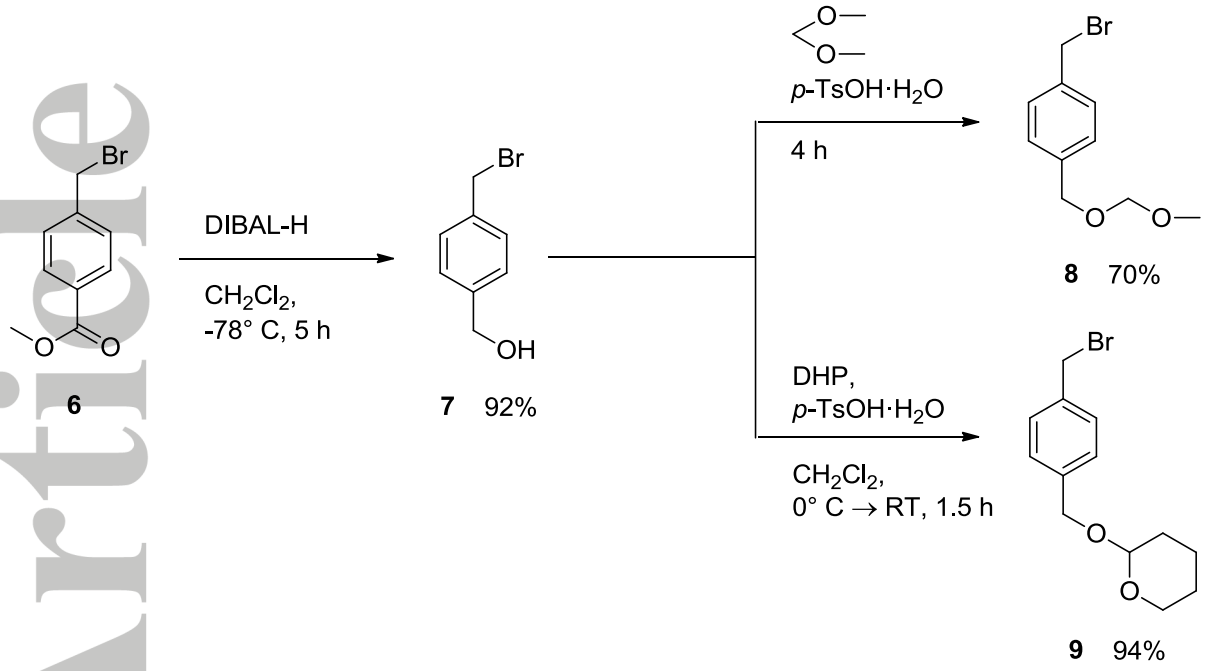
[^{18}F]1

LG: -OTs (**2**), -OMs (**3**), -I (**4**), -OTf (**5**)

Figure 1 – [^{18}F]1 and its precursors 2-5

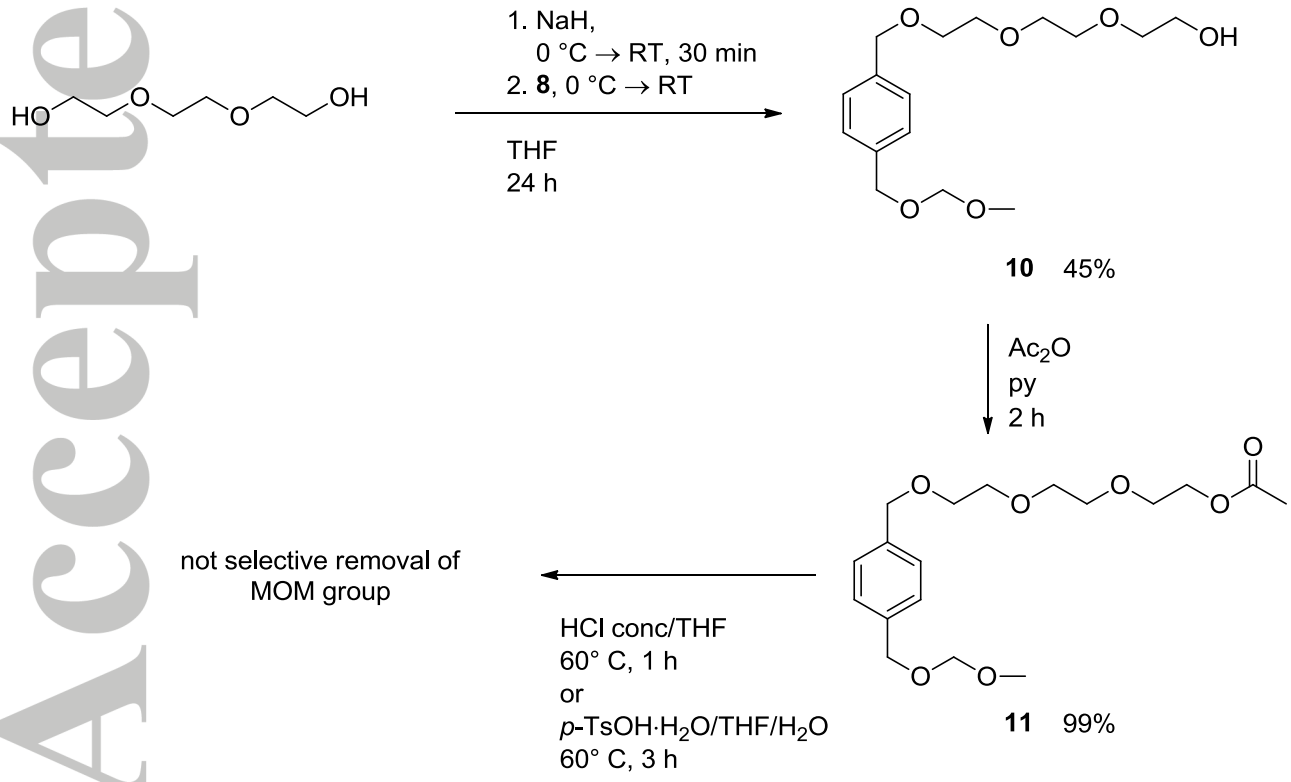
The arene ring was chosen to give high UV absorbance to the molecule, thus allowing HPLC monitoring of radiolabelling reactions and implementation of efficient purification procedures. The azido group in benzylic position instead of directly bound to the arene ring is more accessible, and thus more reactive during the subsequent click reaction. Triethylene glycol residue was chosen with the aim to reduce molecule volatility[13] and potentially improve the *in vivo* behavior of the labelled molecule by increasing its circulation time because of the reduced renal clearance.[14, 15]. Longer polyethylene glycol (PEG) chains were discarded to avoid excessively bulky structures.

The synthetic pathway, designed to achieve the desired set of precursors **2-5** and the fluorinated "cold" reference molecule **1**, was focused on an orthogonal protection-deprotection strategy of substrate functionalities, as both the final products and the synthetic intermediates are polyfunctional compounds. The critical point to be considered is that azido group is labile in acidic media;[16] thus, the removal of the protecting group PEG moiety, which is the last step before the introduction of the leaving group for subsequent ^{18}F radiolabelling, had to be feasible under conditions alternative to acidic. For this reason, the protecting group for azide moiety had to be orthogonal to the protecting group on PEG chain. Thus, a bifunctional benzylic compound was investigated as starting substrate, namely methyl 4-(bromomethyl) benzoate **6** (Scheme 1). After selective reduction of the ester function to the corresponding alcohol **7** (DIBAL-H, 92% yield), either the protection of hydroxyl group as methoxymethyl ether (MOM) **8** or tetrahydropyranyl ether (THP) **9** were successfully performed (70 and 94% yields, respectively), and both the derivatives were evaluated as possible starting material for the next steps of the synthesis.



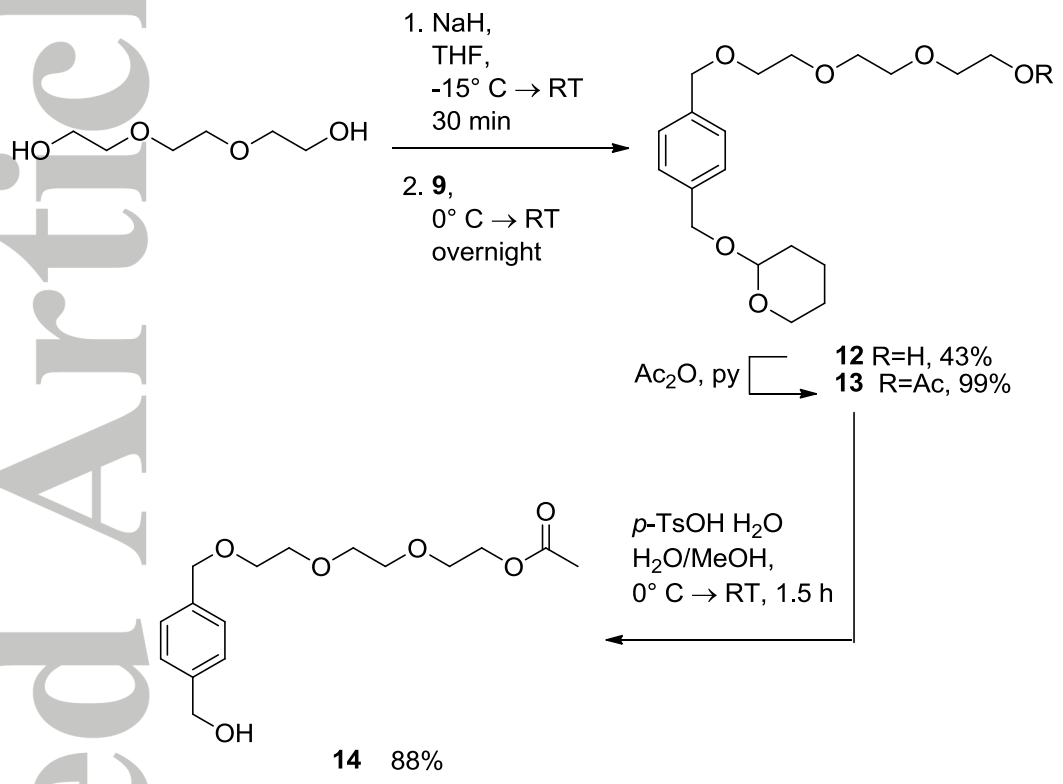
Scheme 1

Methoxymethylether **8** was reacted with both protected and not protected PEG chains; however, only the latter proved to work well; the subsequent introduction of acetate function was successful, but it was not possible to avoid the simultaneous removal of MOM and acetyl moiety, finalized to the achievement of benzylic alcohol (Scheme 2).

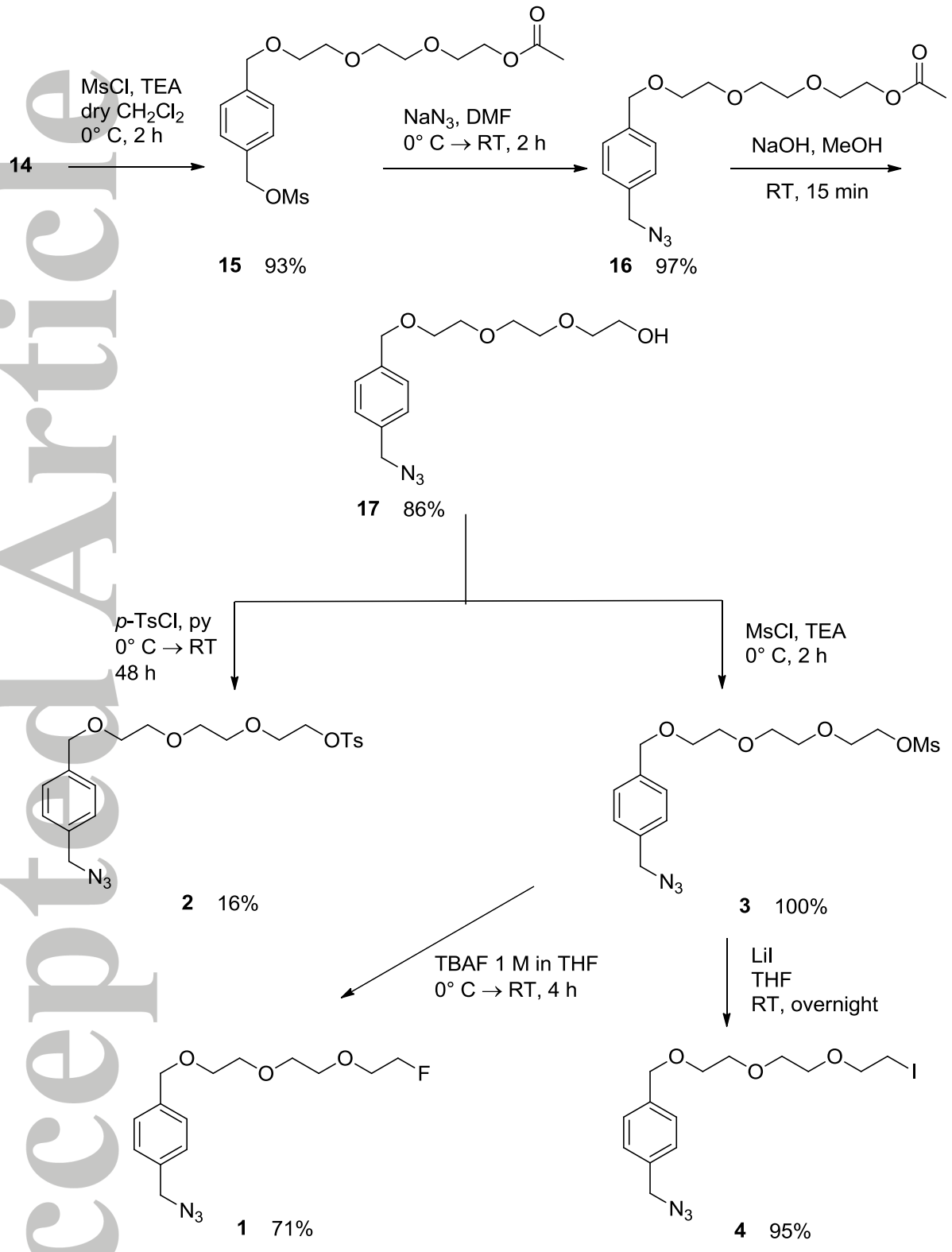


Scheme 2

The same synthetic pathway depicted in Scheme 2, was then applied to THP ether **9**, and in this case the selective deprotection from tetrahydropyranyl group after acetylation of PEG chain was successful, and the resulting compound **14** (Scheme 3) was used for the subsequent steps (Scheme 4), that led to the desired set of precursors and the fluorinated reference compound **1**.



Scheme 3

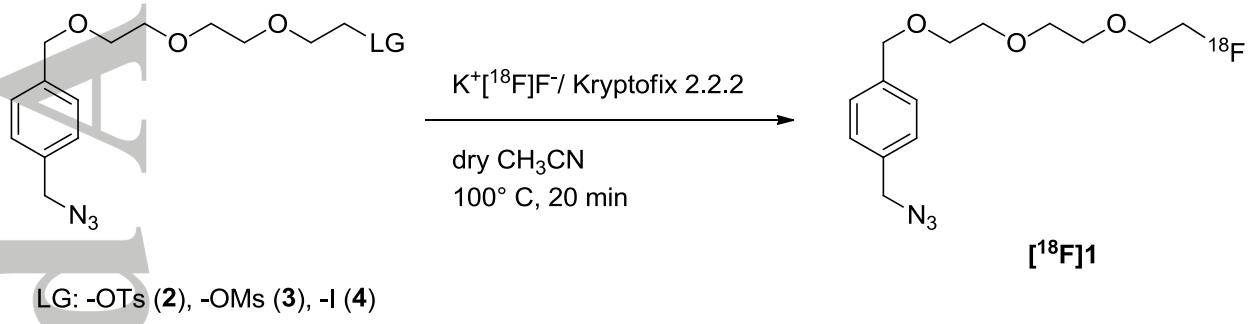


Scheme 4

Indeed, the next step was the functionalization of the benzylic alcohol with a suitable leaving group for the subsequent introduction of azido moiety. Reaction of compound **14** with mesyl chloride proceeded smoothly in mild conditions, yielding the desired product **15** with a 93%

yield. The subsequent substitution with the azido group also took place without problems, leading to compound **16** in high yields.
The last step necessary to obtain the desired precursor was the functionalization of PEG hydroxyl group. To this aim, acetyl group was first removed under basic conditions, yielding compound **17**, which was then tested with the suitable leaving groups tosylate, mesylate, triflate, and iodine. Using tosylchloride in anhydrous pyridine, a mixture of products was obtained. After chromatographic purification, tosylate **2** was obtained in 16% yield. Reaction with mesyl chloride took place in a shorter time and with nearly quantitative yields of compound **3**. Compared with the mesylation reaction, the functionalization of compound **17** as triflate derivative showed to be more troublesome, with a degradation of PEG chain. Finally, from mesylate **3** both the iodo precursor **4** and the fluorinated reference standard **1** were prepared in good yield (95% and 71%, respectively).

To verify the effectiveness of precursors **2-4**, $[^{18}F]F^-$ nucleophilic substitution tests were performed to achieve the desired radiolabelled azide **[^{18}F]1** (Scheme 5). The results are summarized in Table 1.



Scheme 5

Precursor	Reaction temperature	RCY (non-decay corrected)
2	100° C	1%
3	100° C	57%
4	100° C	61%

Table 1 – Radiolabelling of precursors 2-4

All the radiolabelling tests were performed using a fully automated system (GE Tracerlab Fx-FN Pro), where the original setup was modified and adapted to the proposed preparation process. As shown, the best precursor revealed to be iodo compound **4**. This precursor brought not only to the highest non-decay-corrected radiochemical yield (RCY), but it also presented the best impurity profile, which was of utmost importance in view of the subsequent purification step (Figure 2a). Tosyl group was shown to give elimination rather than nucleophilic substitution reaction, with negligible radiochemical yield, while mesylate precursor reacted efficiently with [^{18}F]fluoride, with a 57% RCY at 100° C; unfortunately, in the optimized HPLC analysis conditions, precursor **3** eluted with a retention time very similar to that of the product **[^{18}F]1**, making the post-labelling purification very difficult. During the radiolabeling procedure, iodo precursor **4** showed marked degradation, yielding hydrolysis product **17** as the most abundant "cold" by-product, but this occurred only after quenching of the reaction mixture, so that nucleophilic substitution reaction was not affected. As the separation of the above by-product from the desired radiolabelled product **[^{18}F]1** was easier

compared to precursor **4**, the next purification step was straightforward, using simple Sep-Pak cartridges. Indeed, **[^{18}F]1** was achieved with 51% RCY (non-decay corrected) and 93% radiochemical purity, with a radiosynthesis time of 30 min (Figure 2b).

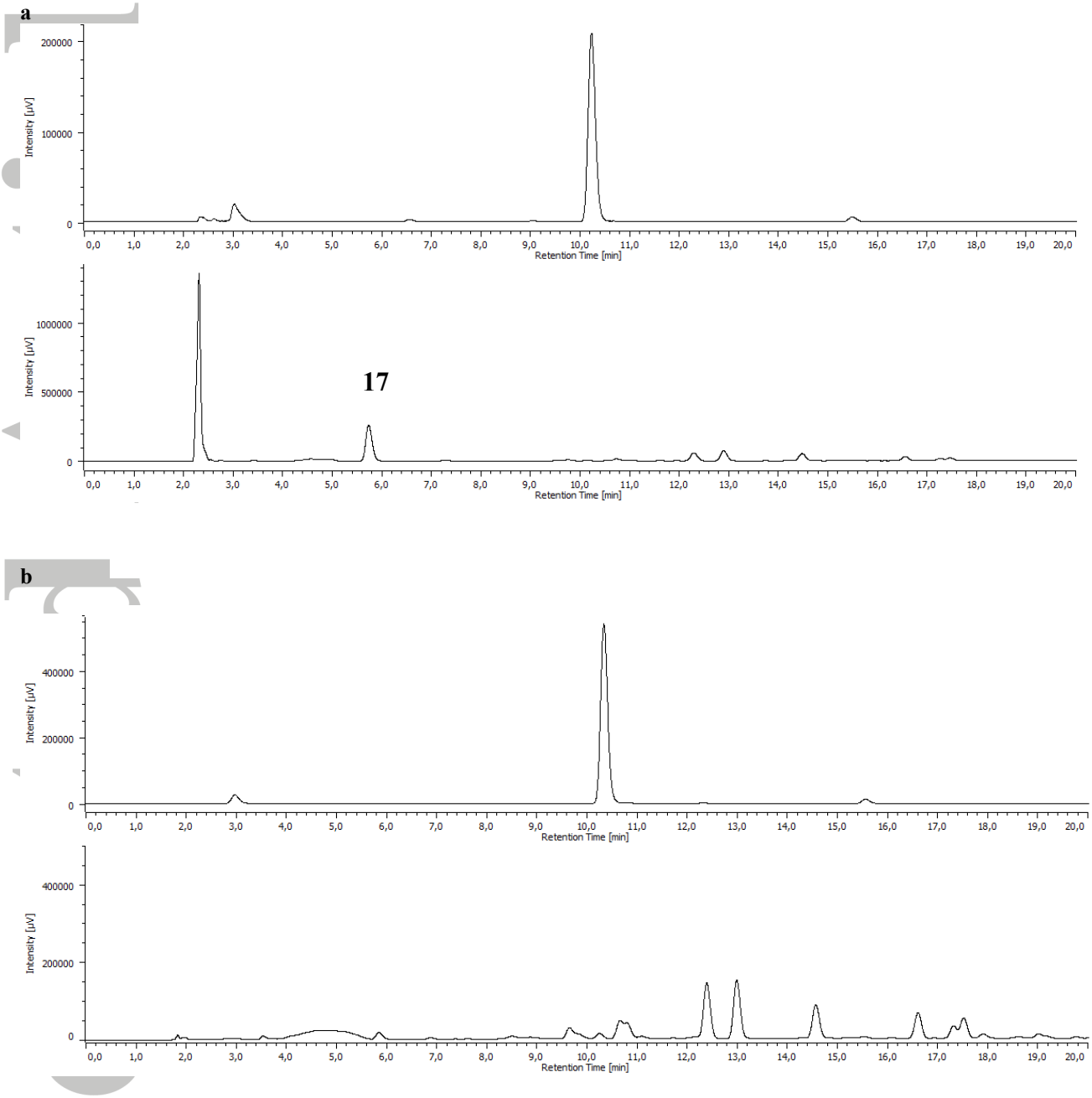
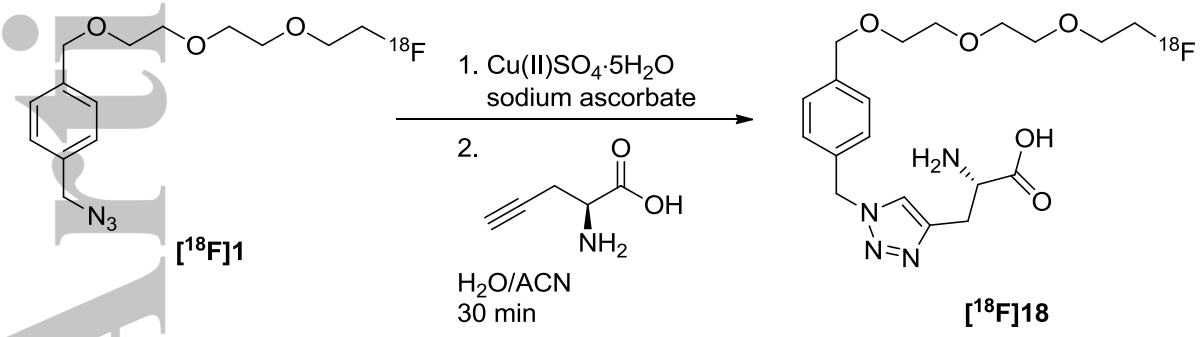


Figure 2 – RP-HPLC profile of crude (a) and purified [^{18}F]1 (b), obtained from precursor 4. [^{18}F]1 retention time was 10.2 min (water/acetonitrile gradient from 60:40 to 20:80 in 20 min, 1 ml/min flow, 220 nm/radiochemical detector).

As a proof of concept, to verify its effectiveness as a useful radiolabelled intermediate for CuAAC we then tested compound **[^{18}F]1** with a simple, cost-effective, alkynyl-modified amino acid, namely L-propargylglycine. With the aim to minimize the well-known copper complexation with glycine residues,[17,18] which may strongly affect the final radiolabelled aminoacid purification, the "click" reaction was initially performed adapting experimental conditions originally described by Glaser and Årstad,[11] but employing much lower reagent

concentrations (from 4 to 30 folder less, depending on the reagents).[19] This is a disadvantage of using not protected glycine, considering that with other biological molecules, such as cyclic peptides, this kind of complexation normally does not occur, so that a higher concentration of copper catalyst might be used, with a consequently increased CuAAC yield. With the aim to minimize radiation exposure to the operators, the above reaction was tested using non-radioactive [^{19}F]1 and L-propargylglycine, and it was performed in water with a variety of co-solvents (ethanol, acetonitrile, dimethylsulfoxide, dimethylformamide), with acetonitrile being the best choice. The proposed reaction was then tested using radioactive [^{18}F]1 (Scheme 6).



Scheme 6

Reaction was carried out at 40°C for 30 minutes. During the first 10 minutes, the mixture volume was reduced under a combination of helium stream and reduced pressure, in order to accelerate the conversion rate. However, volume should not be reduced below 50 µl, as lower volumes lead to precipitation of copper aggregates., Under these conditions, a 94% conversion of compound **[^{18}F]1** in the desired labelled glycine **[^{18}F]18** was obtained. (Figure 3).

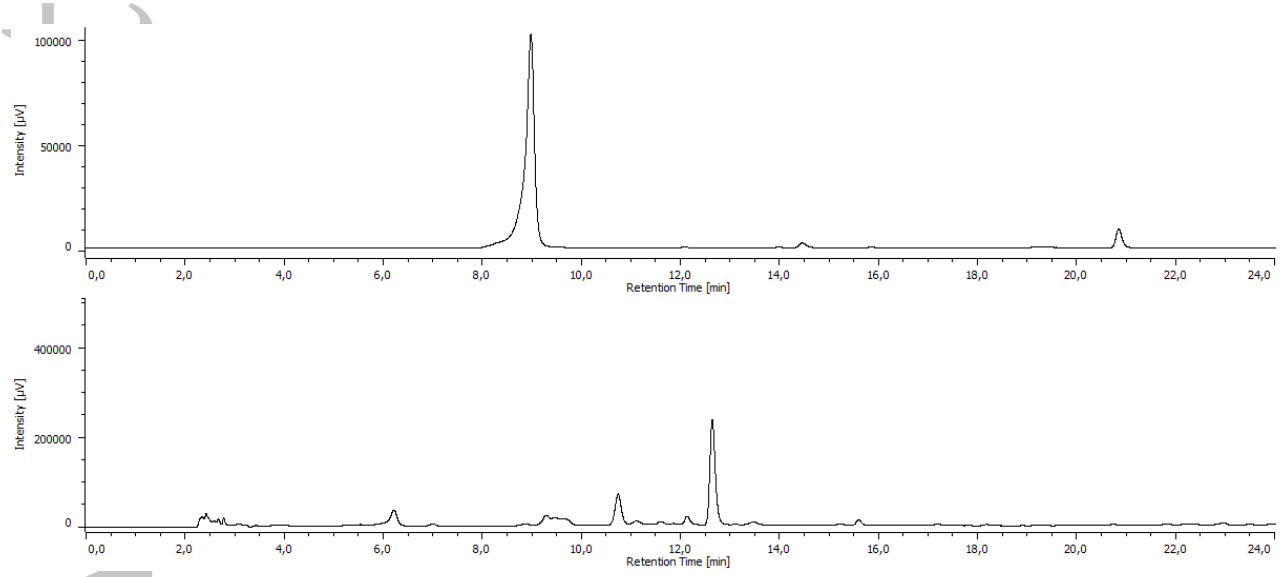


Figure 3 – RP-HPLC profile of pre-purified product [^{18}F]18 from catalyst system. [^{18}F]18 retention time was 8.9 min, [^{18}F]1 retention time was 20.9 min. (water/acetonitrile gradient from 90:10 to 30:70 in 24 min, 1 ml/min flow, 220 nm/radiochemical detector).

After purification using Sep-Pak cartridges, the radiolabelled product **[^{18}F]18** was obtained in 27% RCY, non-decay corrected, and with a >99% radiochemical purity.
In order to improve the "cold" impurities profile , a different purification method, using semi-preparative HPLC was tested: also in this case the radiochemical purity was confirmed to be >99%, and a higher "cold" chemical purity was obtained (Figure 4). Overall synthesis time was of 104 min, average specific activity of **[^{18}F]18** was found to be 55 GBq/μmole.

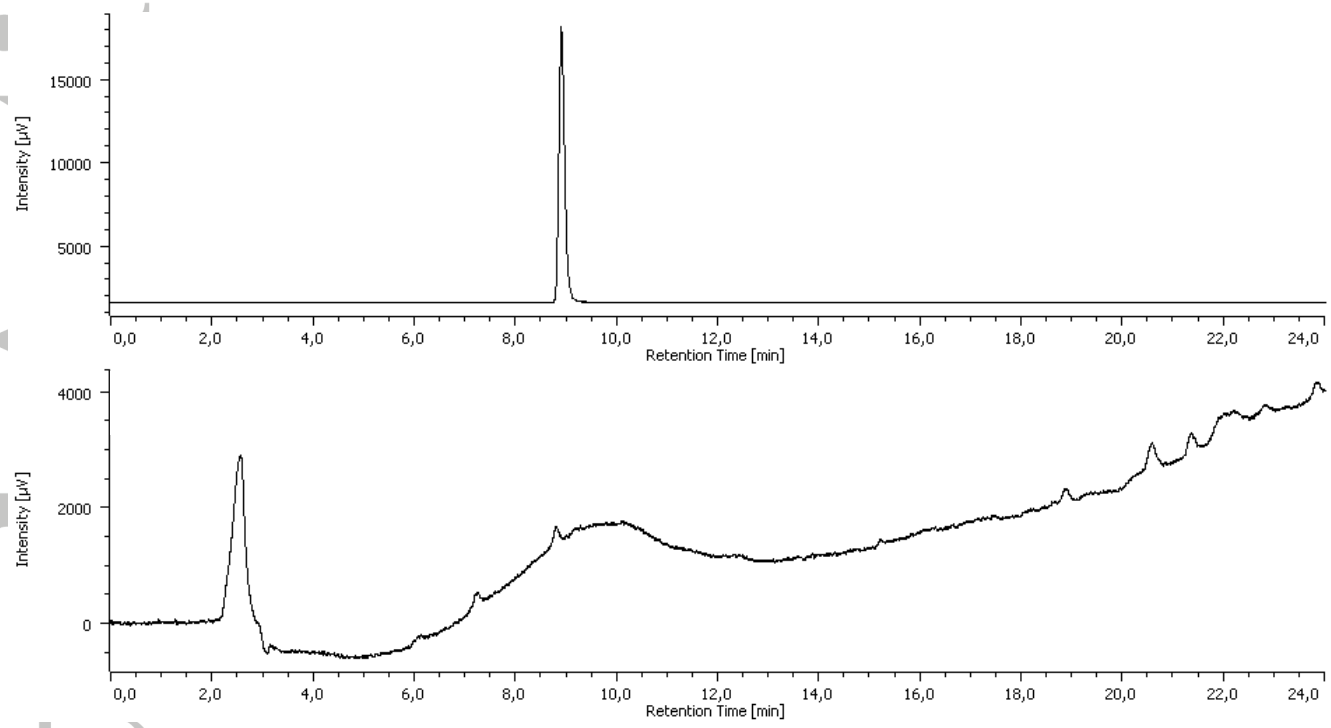


Figure 4 – RP-HPLC profile of purified product [^{18}F]18 by semipreparative RP-HPLC. [^{18}F]18 retention time was 8.9 min (water/acetonitrile gradient from 90:10 to 30:70 in 24 min, 1 ml/min flow, 220 nm/radiochemical detector).

The final product was dissolved in phosphate buffered saline (PBS, pH=7.2) to make it available for *in vivo* preclinical testing. Chemical and radiochemical stability of **[^{18}F]18** in the final formulation were then tested, by HPLC analysis of samples of the purified compound up to eight hours after the end of radiosynthesis, at time intervals of 2 hours and storing the vial at room temperature. **[^{18}F]18** proved to be stable in the above conditions, and no significant loss of radioactivity due to radiolytic degradation was detected. In vitro serum stability was also evaluated, incubating **[^{18}F]18** in human serum for 4 hours at 37°C and analyzing samples using HPLC two and four hours later, respectively. It has been found that the labelled compound does not significantly bind to plasma proteins, and after 4 hours only the radioactive peak attributable to the desired compound was detected, prompting for in vitro stability of the radiotracer. Finally, the predicted logP for [^{18}F]**18** was -1.64, calculated using a standard, freeware software, which confirmed the expected polarity of the radiolabeled compound.

In conclusion, a novel, versatile, stable, safe and easy to handle azido precursor for the general purpose ^{18}F radiolabelling via "click-chemistry" was successfully synthesized. The synthetic pathway started from a cost effective, bifunctional commercial substrate, and a series of precursors was prepared *via* an orthogonal protection-deprotection strategy, that allowed substrate selective functionalization. Precursor effectiveness was confirmed by ^{18}F radiolabelling tests; in particular, iodo precursor **4** proved to efficiently react with [^{18}F]fluoride, while at the same time yielding the intermediate **[^{18}F]1** with good radiochemical purity, which made it suitable for the subsequent CuAAC with alkynil-modified propargyl glycine without the need for additional, time consuming, purification steps. Overall radiosynthesis time and yield were satisfactory, prompting for its use for future developments with new potentially interesting biomolecules.

EXPERIMENTAL PROCEDURES

Materials and Methods

All reagents and solvents were purchased from Sigma-Aldrich.
TLC analysis were performed on silica gel 60 F254 pre-coated plates with a fluorescent indicator (Merck) and on ALUGRAM RP-18W/UV_{254} pre-coated sheets with a fluorescent indicator (Macherey-Nagel), with detection by a 5% phosphomolybdic acid solution in ethanol, and heating at 110 °C. DSC (Differential Scanning Calorimetry) was registered on a Perkin Elmer instrument (Mod. DSC7), using a heating rate of 10° C/min, from 40° C to 120° C. Infrared spectra were recorded on a Perkin Elmer instrument (Mod. Spectrum One FT-IR) equipped with the ATR sampling device. Mass spectra were acquired by AmaZon ETD (Bruker Daltonics) ion trap mass spectrometer equipped with ESI source in positive mode; the samples were dissolved in methanol or water/acetonitrile at a final concentration of 0.1 mg/ml and directly infused into the mass spectrometer (at a rate of 3 µl/min) or separated by LC coupled online with MS. Separation on LC were performed using two Jasco PU 980 Intelligent HPLC pump modules connected to an analytical reverse phase column (XTerra C18 250x4.6 mm, 5 µm, Waters). Parameters for the electrospray source were set as follows: capillary -4500 V, end plate offset -500 V, nebulizer 20 psi, dry gas 9 l/min at 200° C. Ions from the source were detected in a mass range of 70 to 1000 m/z, with 200000 ICC, and 50 ms as maximum accumulation time. A target mass of 300 m/z and a trap drive of 100% were employed. Spectra were acquired using MRM modality and by optimizing both the isolation window and the fragmentation energy for each analyte. Data results were processed by DataAnalysis^{TM} 4.0 (Bruker Daltonics) software.
NMR spectra were recorded on a Bruker AVANCE 500 spectrometer equipped with a 5mm broadband reverse probe with field *z*-gradient operating at 500.13 and 125.76 MHz for ^{1}H and ^{13}C, respectively. NMR spectra were recorded at 298 K in $CDCl_3$ (isotopic enrichment 99.95%) solution, unless otherwise reported, and the chemical shifts were reported on a δ (ppm) scale. The data were collected and processed by XWIN-NMR software (Bruker) running on a PC with Microsoft Windows 7. The samples (10 mg), were dissolved in the appropriate solvent (0.7 mL) in a 5 mm NMR tube. Acquisition parameters for 1D were as follows: ^{1}H spectral width of 5000 Hz and 32K data points providing a digital resolution of ca. 0.305 Hz per point, relaxation delay 2 s; ^{13}C spectral width of 29,412 Hz and 64 K data points providing a digital resolution of ca. 0.898 Hz per point, relaxation delay 2 s (unless otherwise indicated). Chemical shifts (δ) of the ^{1}H NMR and ^{13}C NMR spectra are reported in ppm using the signal of residual solvent protons resonance as internal standard. ^{1}H NMR:

$CDCl_3$ 7.26 ppm, D_2O acidified with DCl (solution 38% in D_2O) 4.79 ppm (298 K, pH=1.0); ^{13}C NMR: $CDCl_3$ 77.0 ppm (central line), D_2O acidified with DCl (solution 38% in D_2O) referenced to external TSP-*d*$_4$ (3-(trimethylsilyl)propionic-2,2,3,3-*d*$_4$ acid sodium salt) at 0.00 ppm. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and br, broad signal. For two-dimensional experiments, Bruker microprograms using gradient selection (gs) were applied. All two-dimensional spectra (COSY, HSQC) were acquired with 2048 data points for t_2 and 256 for t_1 increments.
[^{18}F] Fluoride was produced by a cyclotron (Cyclone 18/9, IBA) *via* the $^{18}O(p,n)^{18}F$ nuclear reaction, by proton beam irradiation of a target containing 2 mL of >97% enriched [^{18}O]water (Rotem).
Radioactive tests were carried out on a commercially available radiochemistry automated module (GE TracerLab FX N Pro) located in a suitably shielded hot cell (MIP-2, Comecer).
Sep-Pak Light Waters Accel Plus QMA, Sep-Pak alumina N light, and SepPak tC18 cartridges were from Waters Corp.
Radiolabelled preparations and "cold" references were analyzed by RP-HPLC on a Jasco PU-2089i system equipped with an automated injector, DAD detector, and radiochemical detector Raytest Gabi Star. Semipreparative purification was carried out on a RP-HPLC equipped with a Perkin Elmer Flexar system quaternary pump and a Knauer WellChrom mod. K-2501 UV detector, connected to the automated module for radiosynthesis, and a "built-in" radioactivity detector. Wavelenght was set at 220 nm.
Analytical RP-HPLC column (Xterra C18 250x4.6 mm, 5 μm) was purchased from Waters Corp, while semi-preparative RP-HPLC column (Clarity Oligo-RP C18, 250x10 mm, 5 μm) from Phenomenex.

Chemical procedures

[4-(Bromomethyl)phenyl]methanol (7)
The synthesis was carried out similarly as described in literature.[20] A solution of 1M diisobutylaluminum hydride in tetrahydrofuran (50 ml) was added dropwise under nitrogen to a cooled-down solution (-78° C) of methyl 4-(bromomethyl)benzoate **6** (3.48 g, 15.2 mmol) in anhydrous dichloromethane (90 ml). The mixture was stirred at -78° C for 4 h, and then an additional amount of diisobutylaluminum hydride solution (5 ml) was added. The reaction progress was monitored by TLC (toluene/ethyl acetate 9:1). After 1 h the reaction went to completion. The mixture was kept at 0° C while quenching with water, then it was allowed to warm to room temperature. The organic layer was separated, and the aqueous one was extracted with dichloromethane (2 x 50 ml). The combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to afford the desired product **7** (2.82 g, 14.0 mmol, 92% yield) as a white solid.
R_f 0.28
^{1}H-NMR δ 1.87 (br s, 1H, exchangeable with D_2O), 4.50 (s, 2H, ArC*H*$_2$Br), 4.68 (s, 2H, ArC*H*$_2$O), 7.33 (d, 2H, Ar), 7.39 (d, 2H, Ar)
^{13}C δ 33.23, 64.82, 127.28, 129.22, 137.13, 141.14
These product **7** NMR data are in agreement with the reported ones.[21]
IR: ν 3320, 1419, 1224, 1194, 1006, 831 cm^{-1}
DSC: endothermic peak at 86.8° C
ESI-MS: m/z 104, 183 [M-17]

2-{[4-(Bromomethyl)benzyl]oxy}tetrahydro-2*H*-pyran (9)

The synthesis was carried out similarly as described in literature.[22] A solution of compound **7** (2.0 g, 9.95 mmol, 1 eq) and 3,4-dihydro-2*H*-pyran (2.4 ml, 26.3 mmol, 2.6 eq) in anhydrous dichloromethane (50 ml) was cooled-down to 0° C with an ice bath. After the addition of monohydrate *p*-toluensulfonic acid (0.010 g, 0.05 mmol, 0.005 eq), the reaction mixture was stirred 10 min at 0 °C, and then at room temperature for 1.5 h. The completion of the reaction was monitored by TLC (hexane/ethyl acetate 9:1). The organic layer was washed with a mixture of saturated brine/saturated sodium hydrogen carbonate/water 1:1:2 (50 ml), and with saturated brine (10 ml x 2). The organic layer was then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The obtained yellow oil (3.2 g) was purified by column chromatography on silica gel (silica/crude product 8:1, gradient elution from hexane 100% to hexane/ethyl acetate 95:5), to afford the desired product **9** (2.7 g, 9.5 mmol, 94% yield) as an yellow oil.

R_f 0.59

^{1}H-NMR δ 1.44-1.95 (m, 6H, 3CH_2), 3.49-3.61 (m, 1H, CH_aOCHO), 3.87-3.95 (m, 1H, CH_bOCHO), 4.50 (s, 2H, ArCH_2Br), 4.50 (d, 1H, ArCH_aOTHP), 4.71 (t, 1H, OCHO), 4.78 (d, 1H, ArCH_bOTHP), 7.34 (d, 2H Ar), 7.38 (d, 2H, Ar)

^{13}C-NMR δ 19.29, 25.43, 30.52, 33.35, 62.11, 68.33, 97.80, 128.11, 129.07, 136.95, 138.73

These NMR data are in agreement with the reported ones.[23]

2-{2-[2-({4-[(tetrahydro-2*H*-pyran-2-yloxy)methyl]benzyl}oxy)ethoxy]ethoxy}ethanol (12)

A solution of triethylene glycol (1.29 g, 8.6 mmol, 1 eq) in anhydrous tetrahydrofuran (70 ml), under nitrogen, was cooled-down to 0° C. Sodium hydride (0.21 g, 8.75 mmol) was added, and the reaction mixture was stirred for 10 min at 0 °C, then it was allowed to warm to room temperature, and it was finally stirred for 30 min. After cooling-down to 0° C, a solution of compound **9** (2.46 g, 8.6 mmol, 1 eq) in tetrahydrofuran (30 ml) was added dropwise, and the reaction mixture was then stirred at room temperature overnight. The reaction progress was monitored by TLC (toluene/ethyl acetate 9:1 to verify the disappearance of compound **9**, and hexane/ethanol 8:2 to monitor the presence of the product). Sodium hydride was quenched with water, cooling the reaction mixture in an ice bath; the mixture was concentrated under reduced pressure. After dilution with dichloromethane, the organic layer was washed twice with water, dried over anhydrous sodium sulfate, and filtered. The solvent was finally evaporated under reduced pressure, to obtain the crude product (3,0 g), that was then purified by column chromatography on aluminum oxide (4.7% water content) (10:1), gradient elution from hexane/ethyl acetate 9:1 to 100% ethyl acetate, to afford the desired product **12** (1.3 g, 3.7 mmol, 43% yield).

R_f 0.61

^{1}H-NMR δ 1.44-1.93 (m, 6H, 3CH_2), 2.64 (br s, 1H, exchangeable with D_2O), 3.71-3.77 (m, 12H, CH_2O), 3.72 (dt, 2H, CH_2OCHO), 4.48 (d, 1H, ArCH_aOTHP), 4.55 (s, 2H, ArCH_2O), 4.76 (d, 1H, ArCH_bOTHP), 4.69 (m, 1H, OCHO), 7.28-7.37 (m, 4H, Ar)

^{13}C-NMR δ 19.29, 25.42, 30.51, 61.69, 62.06, 68.49, 69.29, 70.34, 70.57, 70.62, 72.46, 72.98, 97.62, 127.76, 127.82, 137.39, 137.68

IR: ν 3449, 2867, 1016, 811 cm^{-1}

ESI-MS: m/z 325, 361 [M+H^+-17+23], 375, 445

2-{2-[2-({4-[(tetrahydro-2*H*-pyran-2-yloxy)methyl]benzyl}oxy)ethoxy]ethoxy}ethyl acetate (13)

To a solution of **12** (1.3 g, 3.7 mmol, 1 eq) in anhydrous pyridine (5 ml) acetic anhydride was added (1.2 g, 11.8 mmol, 3.2 eq). The reaction mixture was stirred at room temperature for 2

h, and monitored by TLC (hexane/ethanol 8:2). After dilution with water (water/pyridine 5:1), extraction with dichloromethane was carried out (25 ml x 3). The organic layer was dried over anhydrous sodium sulfate, filtered, and the solvent was evaporated under reduced pressure. The obtained crude product **13** (1.45 g, 3.66 mmol, 99% yield) was employed in the following step without further purifications.
R_f 0.82
^{1}H-NMR δ 1.46-1.92 (m, 6H, 3CH_2), 2.07 (s, 3H, CH_3CO), 3.55 (m, 1H, CH_aOCHO), 3.59-3.74 (m, 10H, CH_2O), 3.85-3.98 (m, 1H, CH_bOCHO), 4.22 (t, 2H, CH_2OCO), 4.50 (d, 1H, ArCH_aOTHP), 4.56 (s, 2H, ArCH_2O), 4.70 (t, 1H, OCHO), 4.78 (d, 1H, ArCH_bOTHP), 7.32 (d, 2H, Ar), 7.34 (d, 2H, Ar)

2-[2-(2-{[4-(hydroxymethyl)benzyl]oxy}ethoxy)ethoxy]ethyl acetate (**14**)
A solution of compound **13** (1.26 g, 3.2 mmol, 1 eq) in methanol (10 ml) was cooled-down to 0° C. A solution of monohydrate *p*-toluensulfonic acid (0.11 g, 0.55 mmol, 0.17 eq) in methanol (10 ml) and water (1 ml) was added dropwise. After 10 min, the mixture was allowed to warm to room temperature, and it was stirred for 1.5 h. The removal of the protecting group was monitored by TLC (hexane/ethanol 8:2). The reaction mixture pH was adjusted to 7 with saturated potassium hydrogen carbonate aqueous solution, then the solution was concentrated under reduced pressure. Dichloromethane (20 ml) was added, then the organic layer was washed twice with water (10 ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, to afford the crude product (0.69 g), which was finally purified on column on silica gel chromatography (silica/crude product 10:1, gradient elution from hexane/ethyl acetate 9:1 to 100% ethyl acetate), to afford the desired product **14** (0.88 g, 2.8 mmol, 88% yield) as an oil.
R_f 0.25
^{1}H-NMR δ 1.85 (t, 1H, exchangeable with D_2O), 2.06 (s, 2H, CH_3CO), 3.59-3.72 (m, 10H, CH_2O), 4.19 (t, 2H, CH_2OCO), 4.56 (s, 2H, $ArCH_2O$), 4.67 (d, 2H, $ArCH_2OH$), 7.33 (br s, 4H, Ar)
^{13}C-NMR δ 20.91, 63.61, 65.09, 69.14, 69.42, 70.57, 70.66, 70.68, 72.96, 127.02, 127.95, 137.69, 140.35, 171.07
IR: ν 3452, 2867, 1735, 1232, 1092, 1048 cm^{-1}
ESI-MS: m/z 295 [M-17], 335 [M+23]

2-(2-{2-[(4-{[(methylsulfonyl)oxy]methyl}benzyl)oxy]ethoxy}ethoxy)ethyl acetate (**15**)
To a solution of compound **14** (0.88 g, 2.8 mmol, 1 eq) in anhydrous dichloromethane (20 ml), under nitrogen, triethylamine (585 µl, 4.2 mmol, 1.5 eq) was added, and the temperature was cooled down to 0° C. Methanesulfonylchloride (0.39 g, 3.4 mmol, 1.2 eq) was slowly added, and the reaction mixture was stirred at 0° C for 2 h. The reaction progress was monitored by TLC (hexane/ethanol 8:2). The reaction mixture was diluted with dichloromethane (20 ml), and the organic layer was washed twice with saturated brine (40 ml), once with water (40 ml), dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, to afford the crude product **15** (1.02 g, 2.6 mmol, 93% yield) as an oil.
R_f 0.13
^{1}H-NMR δ 2.07 (s, 3H, CH_3CO), 2.92 (s, 3H, CH_3SO_2), 3.60-3.74 (m, 10H, CH_2O), 4.22 (t, 2H, CH_2OCO), 4.59 (s, 2H, ArCH_2O), 5.24 (s, 2H, CH_2OSO_2), 7.39 (br s, 4H, Ar)

2-[2-(2-{[4-(azidomethyl)benzyl]oxy}ethoxy)ethoxy]ethyl acetate (**16**)
A solution of compound **15** (1.02 g, 2.6 mmol, 1 eq) in anhydrous dimethylformamide (3 ml), kept under nitrogen, was cooled-down to 0° C. Sodium azide (0.18 g, 2.7 mmol) is then

added, and the reaction mixture was stirred at room temperature for 2h. The reaction progress was monitored by TLC (hexane/ethanol 8:2). The reaction mixture was diluted with water (30 ml) and extracted with ethyl acetate (3 x 15 ml). The collected organic phases were washed with saturated brine (2 x 15 ml), then dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure to afford the crude product **16** (0.85 g, 2.5 mmol, 97% yield) as an oil.
R_f 0.79
^{1}H-NMR δ 2.07 (s, 3H, CH_3CO), 3.60-3.73 (m, 10H, CH_2O), 4.22 (t, 2H, CH_2OCO), 4.32 (s, 2H, CH_2N_3), 4.57 (s, 2H, ArC*H*$_2$O), 7.29 (d, 2H, Ar), 7.36 (d, 2H, Ar)
^{13}C-NMR δ 20.93, 54.54, 63.59, 69.14, 69.57, 70.59, 70.64, 70.68, 72.81, 128.10, 128.27, 134.66, 138.51, 171.02
IR: ν 2868, 2095, 1233, 1096, 1051 cm^{-1}
ESI-MS: m/z 360 [M+23]

2-[2-(2-{[4-(azidomethyl)benzyl]oxy}ethoxy)ethoxy]ethanol (**17**)
Compound **16** (0.80 g, 2.4 mmol, 1 eq) was suspended in a solution of sodium hydroxide (0.12 g) in 15 ml aqueous methanol, and stirred for 15 min at room temperature. The reaction progress was monitored by TLC (hexane/ethanol 8:2). The pH was adjusted to 7-8 with a solution of hydrochloric acid 1 M. The solvent was evaporated, and dichloromethane (50 ml) was added. The organic layer was washed with water (3 x 8 ml), then dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, to obtain the crude product (0.67 g), which was finally purified by column chromatography on silica gel (silica/crude product 10:1, gradient elution from hexane/ethyl acetate 9:1 to 100% ethyl acetate), to afford the desired product **17** (0.61 g, 2.06 mmol, 86% yield) as a colorless oil.
R_f 0.49
^{1}H-NMR δ 2.22 (br s, 1H, exchangeable with D_2O), 3.57-3.78 (m, 12H, CH_2O), 4.32 (s, 2H, CH_2N_3), 4.57 (s, 2H, ArC*H*$_2$O), 7.29 (d, 2H, Ar), 7.37 (d, 2H, Ar)
^{13}C δ 54.52, 61.74, 69.49, 70.34, 70.57, 70.65, 72.48, 72.83, 128.15, 128.28, 134.69, 138.37
IR: ν 3454, 2867, 2094, 1248, 1090, 1065, 806 cm^{-1}
ESI-MS: m/z 290, 318 [M+23], 438, 485

2-[2-(2-{[4-(azidomethyl)benzyl]oxy}ethoxy)ethoxy]ethyl 4-methylbenzenesulfonate (**2**)
A solution of compound **17** (0.043 g, 0.15 mmol, 1 eq) in anhydrous pyridine (2 ml) was cooled-down to 0° C. *p*-Toluensulfonyl chloride (0.044 g, 0.23 mmol, 1.5 eq) was added and after 24h at room temperature, under stirring, an additional amount of *p*-toluensulfonyl chloride (0.044 g) was added, and the reaction mixture was kept under stirring at room temperature for 24 h. The reaction progress was monitored by TLC (hexane/ethanol 8:2). The reaction mixture was diluted with water/ice (10 ml), and extracted with dichloromethane (3 x 10 ml). The collected organic layers were washed with water (3 x 10 ml), dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, to afford the crude product (0.042 g), that was finally purified by column chromatography on silica gel (silica/crude product 20:1, gradient elution from hexane/ethyl acetate 95:5 to 6:4), to afford the desired product **2** (0.011 g, 0.024 mmol, 16% yield) as a colorless oil.
R_f 0.54
^{1}H-NMR δ 2.43 (s, 3H, C*H*$_3$Ar), 3.56-3.74 (m, 10H, CH_2O), 4.15 (t, 2H, C*H*$_2$OTs), 4.33 (s, 2H, CH_2N_3), 4.56 (s, 2H, ArC*H*$_2$O), 7.27-7.41 (m, 6H, Ar), 7.79 (d, 2H, Ar)
LC-MS: m/z 472 [M+23], 523

2-[2-(2-{[4-(azidomethyl)benzyl]oxy}ethoxy)ethoxy]ethyl methanesulfonate (**3**)
Compound **17** (0.41 g, 1.39 mmol, 1 eq) was suspended in anhydrous dichloromethane (10 ml), under nitrogen. Triethylamine (0.3 ml, 2.15 mmol, 1.5 eq) was added, and the solution was cooled-down to 0° C. Mesyl chloride (0.19 g, 1.67 mmol, 1.2 eq) was added dropwise, and the reaction mixture was stirred for 2 h at 0° C. The reaction progress was monitored by TLC (hexane/ethanol 8:2). The reaction mixture was diluted with dichloromethane (10 ml), and the organic layer was washed once with saturated brine (20 ml), and once with water (20 ml), then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, to afford the desired product **3** (0.52 g, 1.39 mmol, 100% yield) as a colorless oil.
R_f 0.40
^{1}H-NMR δ 3.04 (s, 3H, CH_3SO_2), 3.58-3.72 (m, 8H, CH_2O), 3.77 (br s, 2H, CH_2O), 4.33 (s, 2H, CH_2N_3), 4.37 (br s, 2H, C*H*$_2$OMs), 4.57 (s, 2H, ArC*H*$_2$O), 7.30 (d, 2H, Ar), 7.36 (d, 2H, Ar)
^{13}C-NMR δ 37.70, 54.53, 69.04, 69.23, 69.57, 70.60, 70.64, 70.67, 72.84, 128.14, 128.31, 134.75, 138.42
IR: ν 2919, 2095, 1348, 1249, 1172, 1095, cm^{-1}
ESI-MS: m/z 346, 396 [M+23]

1-(azidomethyl)-4-({2-[2-(2-iodoethoxy)ethoxy]ethoxy}methyl)benzene (**4**)
To a solution of compound **3** (0.16 g, 0.43 mmol, 1 eq) in anhydrous tetrahydrofuran (5 ml), under nitrogen, lithium iodide (0.29 g, 2.17 mmol, 5 eq) was added. The reaction mixture was stirred at room temperature overnight and monitored by TLC (hexane/ethanol 8:2). Water was added to quench the reaction, and tetrahydrofuran was evaporated. Dichloromethane (10 ml) was added, the organic layer was washed with water (3 x 10 ml), then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, to afford the desired product **4** (0.17 g, 0.41 mmol, 95% yield) as a yellow oil.
R_f 0.80
^{1}H-NMR δ 3.26 (t, 2H, CH_2I), 3.62-3.73 (m, 8H), 3.76 (t, 2H, OC*H*$_2$CH$_2$I), 4.33 (s, 2H, CH_2N_3), 4.58 (s, 2H, ArC*H*$_2$O), 7.30 (d, 2H, Ar), 7.37 (d, 2H, Ar)
^{13}C-NMR δ 2.87, 54.57, 69.62, 70.27, 70.68, 70.74, 72.02, 72.85, 128.12, 128.26, 134.68, 138.55
IR: ν 2863, 2094, 1251, 1090, 805, 672 cm^{-1}
ESI-MS: m/z 315, 396, 406 [M+H$^+$], 428 [M+23], 496, 503

1-(azidomethyl)-4-({2-[2-(2-fluoroethoxy)ethoxy]ethoxy}methyl)benzene (**1**)
Compound **3** (0.18 g, 0.48 mmol, 1 eq) was dissolved in anhydrous tetrahydrofuran (2 ml), and the solution was cooled-down to 0° C under nitrogen stream. A solution of 1M tetrabutylammonium fluoride in tetrahydrofuran (1 ml, 1 mmol, 2 eq) was added, and after the reaction mixture was allowed to warm to room temperature, it was stirred for 4 h. The reaction progress was monitored by TLC (hexane/ethanol 8:2). The solvent was evaporated, and dichloromethane (10 ml) was added. The organic layer was washed twice with water (10 ml), once with saturated brine (10 ml), then dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure, to afford the crude product (0.19 g). Column chromatography on silica gel (silica/crude product 20:1, gradient elution from hexane/ethyl acetate 9:1 to 7:3) was performed to afford the desired product **1** (0.10 g, 0.34 mmol, 71% yield) as colorless oil.
R_f 0.60
^{1}H-NMR δ 3.62-3.71 (m, 8H, CH_2O), 3.75 (dt, 2H, C*H*$_2$CH$_2$F), 4.33 (s, 2H, CH_2N_3), 4.56 (dt, 2H, CH_2F), 4.58 (s, 2H, ArC*H*$_2$O), 7.29 (d, 2H, Ar), 7.37 (d, 2H, Ar)

^{13}C-NMR δ 54.57, 69.60, 70.34, 70.50, 70.70, 70.86, 72.82, 82.47, 83.81, 128.11, 128.27, 134.67, 138.57
IR: ν 2952, 2095, 1248, 1096, 1046, 806 cm^{-1}
ESI-MS: m/z 320 [M+23], 336, 524

3-{1-[4-({2-[2-(2-fluoroethoxy)ethoxy]ethoxy}methyl)benzyl]-1*H*-1,2,3-triazol-4-yl}alanine (18)

A freshly-prepared solution of copper(II) sulfate pentahydrate (10.7 mg, 0.043 mmol, 1.8 eq) in 150 µl water and a freshly-prepared solution of sodium ascorbate (25.7 mg, 0.130 mmol, 5.4 eq) in 150 µl water were mixed together under helium. After 2 min a solution of propargylglycine (3.3 mg, 0.029 mmol, 1.2 eq) in 450 µl water was added. After 2 min a solution of compound **1** (7.1 mg, 0.024 mmol, 1 eq) in 200 µl acetonitrile and 100 µl dimethylsulfoxide was added. The reaction mixture was stirred at room temperature for 3 h. The completion of the reaction was monitored by RP-TLC (water/acetonitrile 9:1), and by RP-HPLC (XTerra C18 column, water/acetonitrile gradient from 90:10 to 30:70 in 24 min, 1 ml/min, 220 nm). The reaction mixture was solubilized by adding some drops of HCl 1M, and suspended in 20 ml water/acetonitrile 95:5. The solution was passed through a Sep-Pak tC18 plus cartridge, which was then washed with water (10 ml), and with a solution of water/acetonitrile 95:5 (10 ml). The desired compound **18** (6.1 mg, 0.015 mmol, 62% yield) was obtained from elution with pure acetonitrile or ethanol (3 ml), and final evaporation of the solvent.
R_f 0.41
Rt 8.7 min
^{1}H-NMR (D_2O+DCl) δ 3.37 (d, 2H, C*H*$_2$triazole), 3.55-3.66 (m, 8H, CH_2O), 3.71 (dt, 2H, OC*H*$_2$CH_2F), 4.37 (t, 1H, C*H*NH_2), 4.52 (dt, 2H, CH_2F), 4.54 (s, 2H, ArCH_2O), 5.58 (s, 2H, CH_2N), 7.30 (d, 2H, Ar), 7.38 (d, 2H, Ar), 7.96 (s, 1H, triazol)
^{13}C-NMR (125.76MHz, D_2O+DCl) δ 25.37, 52.21, 53.94, 68.83, 69.41, 69.53, 69.67, 69.78, 69.93, 72.24, 82.61, 83.91, 125.38, 128.33, 129.00, 134.31, 137.88, 140.62, 170.44
ESI-MS: m/z 225, 411 [M+H$^+$], 433 [M+23], 449, 458, 467 [M+39+18]

Radiochemistry

1-(azidomethyl)-4-({2-[2-(2-[^{18}F]-fluoroethoxy)ethoxy]ethoxy}methyl)benzene ([^{18}F]1) from precursor 2

~21 GBq of [^{18}F] fluoride were trapped by passing the cyclotron irradiated target solution through a Sep-Pak light QMA cartridge. A solution of potassium carbonate (2.51 mg, 0.018 mmol) in water (500 µl) was passed through the QMA cartridge to elute the activity in the K$^+$[^{18}F]$^-$ form directly in the reactor vial. A solution of Kryptofix 2.2.2 (15 mg, 0.04 mmol) in acetonitrile (1 ml) was added, and the solvent was azeotropically distilled at 85° C, under helium stream. The reactor was cooled-down to 30° C, and a solution of compound **2** (1 mg, 0.002 mmol) in anhydrous acetonitrile (1 ml) was added. The reaction mixture was stirred at 100° C for 30 min under helium pressure (220 KPa). The reactor was cooled-down to room temperature, and the excess pressure released. Acetonitrile (0.5 ml), and water (1 ml) were added; the mixture was then passed through two Sep-Pak Al light N cartridges connected in series, previously conditioned with ethanol (10 ml) and water (10 ml), and directly collected into the final container. The cartridges were washed with water (2 ml), also collected into the final container. The obtained final activity was 350 MBq. Analysis by RP-HPLC (XTerra

C18 column, water/acetonitrile gradient from 60:40 to 20:80 in 20 min, 1 ml/min flow, 220 nm/ radiochemical detector) revealed that the product **[^{18}F]1** was formed (RCY non corrected for the decay ~1%, 52% radiochemical purity).
[^{18}F]1:Rt 9.5 min.
Elimination product: Rt 11.6 min, LC-MS m/z 336 [M+18+18+23], 387

1-(azidomethyl)-4-({2-[2-(2-[^{18}F]-fluoroethoxy)ethoxy]ethoxy}methyl)benzene ([^{18}F]1) from precursor 3

~20 GBq of [^{18}F] fluoride were trapped by passing the cyclotron irradiated target solution through a Sep-Pak light QMA cartridge. A solution of potassium carbonate (2.51 mg, 0.018 mmol) in water (500 µl) was passed through the QMA cartridge to elute the activity in the $K^+[^{18}F]^-$ form directly in the reaction vial. A solution of Kryptofix 2.2.2 (15 mg, 0.04 mmol) in acetonitrile (1 ml) was added, and the solvent was azeotropically distilled at 85° C, under helium stream. The reactor was cooled-down to 30° C, and a solution of compound **3** (10 mg, 0.027 mmol) in anhydrous acetonitrile (1 ml) was added. The reaction mixture was stirred at 100° C for 20 min under helium pressure (220 KPa). The reactor was cooled-down to room temperature, and the excess pressure released. Acetonitrile (0.5 ml), and water (1 ml) were added, and the mixture was then passed through two Sep-Pak Al light N cartridges connected in series, previously conditioned with ethanol (10 ml) and water (10 ml), and directly collected into the final container. The cartridges were washed with water (2 ml), and the volume was collected in the final vial. The obtained final activity was 15 GBq. Analysis by RP-HPLC (XTerra C18 column, water/acetonitrile gradient from 60:40 to 20:80 in 20 min, 1 ml/min, 220 nm/ radiochemical detector) revealed that the product **[^{18}F]1** was formed (RCY non corrected for the decay ~57%, 74% radiochemical purity). The UV spectra showed the partial degradation of the precursor to afford the hydrolysis product, as confirmed by HPLC injection of compound **17**, and the elimination product.
[^{18}F]1: Rt 10.0 min.
Hydrolysis product: Rt 5.6 min.
Elimination product: Rt 12.1 min.

1-(azidomethyl)-4-({2-[2-(2-[^{18}F]-fluoroethoxy)ethoxy]ethoxy}methyl)benzene ([^{18}F]1) from precursor 4

~20 GBq of [^{18}F] fluoride were trapped by passing the cyclotron irradiated target solution through a Sep-Pak light QMA cartridge. A solution of potassium carbonate (2.51 mg, 0.018 mmol) in water (500 µl) was passed through the QMA cartridge to elute the activity in the $K^+[^{18}F]^-$ form directly in the reaction vial. A solution of Kryptofix 2.2.2 (15 mg, 0.04 mmol) in acetonitrile (1 ml) was added, and the solvent was azeotropically distilled at 85° C, under helium stream. The reactor was cooled-down to 30° C, and a solution of compound **4** (10 mg, 0.025 mmol) in anhydrous acetonitrile (1 ml) was added. The reaction mixture was stirred at 100° C for 20 min under helium pressure (220 KPa). The reactor was cooled-down to room temperature, and the excess pressure released. Acetonitrile (0.5 ml), and water (1 ml) were added, and the mixture was then passed through two Sep-Pak Al light N cartridges connected in series, previously conditioned with ethanol (10 ml) and water (10 ml), and directly collected into the final container. The cartridges were washed with water (2 ml), and the volume was also collected in the final container. The obtained final activity was 14.4 GBq. Analysis by RP-HPLC (XTerra C18 column, water/acetonitrile gradient from 60:40 to 20:80 in 20 min, 1 ml/min, 220 nm/ radiochemical detector) revealed that the product **[^{18}F]1** was formed (RCY non corrected for the decay ~61%, 85% radiochemical purity). The UV spectra showed the precursor complete degradation to afford the hydrolysis product.

[^{18}F]1: Rt 10.2 min.
Hydrolysis product: Rt 5.7 min.
With the aim of further characterize it, crude compound **[^{18}F]1** as obtained at the end of the above described procedure was diluted in water/acetonitrile 90:10 (18 ml) and passed through a Sep-Pak tC18 Plus cartridge previously conditioned with 10 ml ethanol and 10 ml water. The cartridge was washed with a solution of water/acetonitrile 80:20 (10 ml), and eluted with 3 ml acetonitrile, to afford the purified **[^{18}F]1** (~51% RCY non-corrected for the decay, 93% radiochemical purity), as confirmed by RP-HPLC analysis (XTerra C18 column, water/acetonitrile gradient from 60:40 to 20:80 in 20 min, 1 ml/min, 220 nm/ radiochemical detector).

3-{1-[4-({2-[2-(2-[^{18}F]fluoroethoxy)ethoxy]ethoxy}methyl)benzyl]-1*H*-1,2,3-triazol-4-yl}alanine ([^{18}F]18)
[^{18}F]1 reaction mixture, prepared as previously described, was concentrated by heating up to 85°C for 2 min under a helium steam. Then, the mixture was diluted with 5 ml of water/acetonitrile 90:10 and passed through a Sep-Pak tC18 Plus cartridge previously conditioned with 10 ml ethanol and 10 ml water. The cartridge was washed with 10 ml of water/acetonitrile 80:20, the product **[^{18}F]1** was recovered eluting the cartridge with 1 ml of acetonitrile and transferred in a second reaction vial. The solution was concentrated to 50 µl by heating up to 85° C for 5 min under a helium stream. After cooling to rt, 150 µl of a L-propargylglycine solution (1.0 mg, 0.009 mmol, 1 eq in 50 µl water) and a freshly-prepared sodium ascorbate solution (3.6 mg, 0.018 mmol, 2 eq in 100 µl water) were added, followed by a freshly-prepared solution of copper(II) sulfate pentahydrate (1.5 mg, 0.006 mmol, 0.7 eq) in 100 µl water, and then stirred at 40° C for 30 min. The reaction was quenched with 1 M hydrochloric acid (0.5 ml) and eluted with 3.4 ml water/acetonitrile 90:10 + 0.1% TFA. Then, to further purify the desired **[^{18}F]18**, reaction mixture was submitted to semi-preparative RP-HPLC. The intended fraction was collected in a flask filled with 30 ml of water. The solution was then passed through a Sep-Pak tC18 Plus cartridge previously conditioned with 10 ml ethanol and 10 ml water. The cartridge was washed with water (10 ml), and the desired product **[^{18}F]18** was eluted with 1 ml of 10 mM HCl in ethanol and collected into the final container where it was diluted with 8.7 ml of saline physiological solution and 0.3 ml of 150 mM phosphate buffer saline (PBS), pH 7.2. The final activity was in the range 6.6-7.5 GBq, starting from 24-28 GBq of [^{18}F]fluoride activity (>99% radiochemical purity, ~27% RCY non-decay corrected). Total time synthesis was 104 min.
Conditions for gradient semi-preparative RP-HPLC were as follows: Clarity Oligo RP 5µm, 250x10mm; water +0.1%TFA/acetonitrile +0.1%TFA for 30 seconds, water +0.1%TFA/acetonitrile +0.1%TFA gradient from 90:10 to 80:20 in 10 min, water +0.1%TFA/acetonitrile +0.1%TFA 80:20 isocratic for 8 min, water +0.1%TFA/acetonitrile +0.1%TFA from 80:20 to 30:70 in 10 min, water +0.1%TFA/acetonitrile +0.1%TFA 30:70 isocratic for 10 min; 5 ml/min, 220 nm, UV and radiochemical detector).
[^{18}F]18: Rt 16.3 min
Analytical RP-HPLC (XTerra C18 column, water/acetonitrile gradient from 90:10 to 30:70 in 24 min, 1 ml/min, 220 nm, radiochemical detector).
[^{18}F]18: Rt 8.9 min

Plasma stability study

The stability of the radiolabeled compound in plasma was evaluated using human, freshly drawn heparinized blood samples, that were initially centrifuged at 2800 x g for 15 min to separate cells, while plasma was stored at 4°C until use. Then, 200 µl of plasma were pipetted into a borosilicate culture tube and 20 µl of **[^{18}F]18** solution were added (~2 GBq/ml), and the vials were incubated for 2 and 4 hours at 37°C, respectively. Plasma proteins were then extracted with 200 µl of acetonitrile, and the mixture was both vortexed and centrifuged at 2800 x g for 5 min. Finally, the supernatant was analyzed by RP-HPLC, in the conditions above described.

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IR analysis were performed by Nicola Colombo.
Mass analysis were performed by Prof. Fulvio Magni group from University of Milano-Bicocca, Department of Medicine and Surgery, Mass Spectrometry unit, Monza, Italy.

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Precursor	**Reaction temperature**	**RCY (non-decay corrected)**
2	100° C	1%
3	100° C	57%
4	100° C	61%

Table 1 – Radiolabelling of precursors 2-4

A Novel Versatile Precursor Suitable for ^{18}F- Radiolabelling *via* "Click-Chemistry"

B. Lugato, S. Stucchi, S. Ciceri, M. N. Iannone, E.A. Turolla, L. Giuliano, C. Chinello, S. Todde, P. Ferraboschi*

A new azido precursor useful for the so called "click-reaction", (alkyne-azide cycloaddition, CuAAC) was prepared and successfully radiolabelled with ^{18}F (51% radiochemical yield). The ^{18}F labelled azide was reacted with L-propargylglycine (94% of conversion, 27% radiochemical yield, >99% radiochemical purity).