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Photoactivated 1,3-dipolar cycloaddition for the rapid preparation of ^{18}F labelled radiotracers

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ABSTRACT

An ^{18}F-labelled 2,5-diaryl tetrazole reagent has been prepared and reacted with substituted alkene dipolarophiles through a photoactivated 1,3-dipolar cycloaddition reaction. The radiobioconjugation reaction furnished the desired product in 5 min with radiochemical conversions of 85–95% at room temperature. Remarkably, for the activated dipolarophiles, these results were obtained in highly dilute solutions (10–100 μM).

1. Introduction

Positron emission tomography (PET) is a well-established and still growing non-invasive imaging modality, ideally suited to in vivo imaging of molecular processes thanks to its high sensitivity (nano to picomolar range) and quantitative nature. Fluorine-18 is by far the most used positron emitting radionuclide in PET due to its availability and its physical and nuclear properties.[1] However, the poor nucleophilicity of the fluoride anion prevents its direct incorporation into water soluble biomolecules, e.g., peptides, proteins, oligonucleotides, lipids. Indeed, stability issues and side reactions preclude the use of harsh conditions usually employed to label small and stable compounds. Thus, the further development of molecular imaging is hindered by these difficulties to label more complex and promising molecules.[2]

Despite recent progress,[3,4] the search for a rapid, selective and generic method for radiolabelling biomolecules continues. The copper (I)-catalysed 1,2,3-triazole formation involving azides and terminal alkynes has proven to be a valuable tool for efficient fluorine-18 labelling of various biomolecules.[5–7] Nevertheless, the employment of cytotoxic copper salts hampers its general use. The labelling of biomolecules with fluorine-18 by photochemical conjugation has been only tentatively explored in the past.[8–10] All efforts have focused on the preparation of [^{18}F]arylnitrene through irradiation of [^{18}F]arylazides. The photogenerated [^{18}F]arylnitrene reacts with nucleophilic groups present on the biocompound. The poor labelling efficiency, which was attributed to the short lifetime of the intermediate [^{18}F]arylnitrene, has precluded the development of this photochemical approach.[10] Recently, Lin and co-workers reported a bioorthogonal photoinducible 1,3-dipolar cycloaddition reaction ('photoclick') for rapid and highly selective modification of proteins without the need for catalysts (Fig. 1).[11–13] Specifically, an aryl tetrazole moiety was extremely rapidly photolysed to generate a nitrile imine intermediate, which efficiently reacts with an alkene dipolarophile. Moreover, the pyrazoline cycloadduct formed shows strong fluorescence in the region of 487–538 nm.

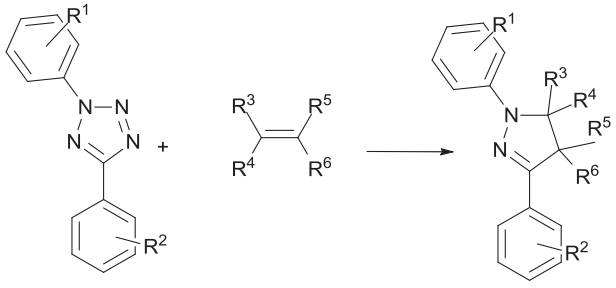

Fig. 1. Photoactivated 1,3-dipolar cycloaddition between a 2,5-diaryl tetrazole and a substituted alkene dipolarophile.

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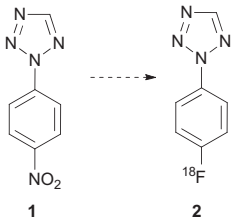
It has been shown that this reaction can be carried out in various solvents including water and other protic solvents.[14] Thanks to the high quantum yield of the photolysis (0.5–0.9), a simple hand held bench top UV lamp was sufficient to initiate the reaction.[14] In the conditions evaluated by Lin, the reaction was highly regioselective and no competitive reactions with other functional groups like nitrile, aldehyde or proteinaceous groups were observed. Typically, the dipolarophile was an electron-deficient alkene such acrylate, acrylamide or fumarate. Simple alkenes, such as 1-decene afforded pyrazoline with moderate yields.[14] The functionalization of biomolecules by such moieties is practicable. For example, site-specific incorporation of alkene-containing amino acids into proteins has been reported by several groups[15–18] and synthesis of maleimide peptide has also been described.[19]

Thus, this photoactivated cycloaddition seems particularly well adapted for the fast conjugation of radionuclides to biomolecules in dilute media. In this article, we report the first method to label dipolarophile compounds with fluorine-18 through the photoclick reaction.

2. Results and discussion

Initially, we focused on the introduction of fluorine-18 on a nitrophenyl tetrazole derivative (Scheme 1). This compound was not ideally suited for this application as the position of the nitro leaving group is poorly activated. Moreover, Lin and co-workers showed that the cycloaddition rate constant was about 100-fold lower for monoaryl tetrazole compared to diaryl tetrazole.[12,13] Nevertheless, compound **1** can be prepared easily in a single step, which is attractive in a proof of concept perspective.[20]

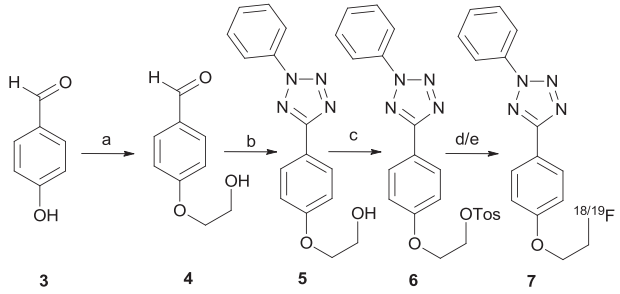


Scheme 1. Attempts to label 2-(4-nitrophenyl)-2*H*-tetrazole **1**. Reagents and conditions (^{18}F/K-222/K_2CO_3 or ^{18}F-TEAF, 90–180 °C).

Unfortunately, all attempts to label this compound (^{18}F/K-222/K_2CO_3 or ^{18}F-TEAF) at temperatures between 90 and 180 °C were unsuccessful, as only ^{18}F fluoride and the decomposition product of the 2-(4-nitrophenyl)-2*H*-tetrazole precursor were recovered.

These results encouraged us to switch to the more convenient tetrazole precursor **6**. This compound features an aliphatic tosylate leaving group which is known to be easily substituted.[21] Moreover, the diaryl tetrazole moiety should favor a fast cycloaddition reaction.

The tosylate **6** was synthesized in three steps starting from 4-hydroxybenzaldehyde (Scheme 2). The key step was the Kakehi diaryltetrazole synthesis from aryl aldehyde **4** and an arene diazonium salt.[22,23] The standard compound **7** was obtained by reacting tosylate **6** with anhydrous caesium fluoride in DMSO. We found that **6** was efficiently labelled in DMSO at 95 °C in 5 min with standard KF[^{18}F]-K-222/potassium carbonate conditions. Identification of [^{18}F]**7** was based on radio-HPLC analysis showing the same retention time as the corresponding non-radioactive reference compound **7** (Fig. 2). Radiochemical conversions obtained were between 75 and 90%. Increasing the reaction time or temperature did not improve yields. Modifications of the nature of the solvent or of the reagent's quantities (K-222, K_2CO_3 or precursor **6**) were not considered. The mean decay-corrected radiochemical yield was 69±5% (n=4). This value is mostly limited by trapping of [^{18}F]F^- on the walls of the glass vial used for the radiolabelling reaction. HPLC analysis of the crude reaction media showed that the main unlabelled impurity was alcohol **5**, which is the main degradation product of the 5–7 mg of precursor **6** used.



Scheme 2. Synthesis of tetrazole tosylate **6** and radiolabelling. Reagents and conditions (a) NaOH, $ClCH_2CH_2OH$, n-BuOH/H_2O, 54%. (b) 1. $PhSO_2NHNH_2$, EtOH. 2. PhN_2Cl, pyr, 37%. (c) TosCl, Et_3N, 36%. (d) $Cs^{19}F$, DMSO, 79%. (e) KF[^{18}F]-K-222/K_2CO_3, DMSO, 69%.

As this impurity presents a tetrazole moiety, it is imperative to separate this product from [^{18}F]**7** to avoid unwanted dipolarophile consumption. This separation can be carried out efficiently by semi-preparative HPLC. The collected HPLC peak was diluted with water, trapped on a Sep-Pak cartridge and [^{18}F]**7** was eluted with 1 ml of acetonitrile (the decay-corrected radiochemical yield was 75% for the purification step). The concentration of non-radioactive **7** present in the solution was determined by HPLC (20–90 μM).[†]

As fumarates are known to be good dipolarophiles, diethylfumarate was used to demonstrate the potential of this approach. First, a study was conducted on the non-radioactive compound **7** to produce the standard **9** and to check the experimental conditions (Scheme 3). The reaction was carried out in a PBS buffer (pH 7.4, 50 mM)/CH_3CN mixture (1:1) at room temperature in an open quartz vial. The reaction medium was gently stirred and photoactivated through two flexible light guides (See Experimental section). The concentration of tetrazole **7** was fixed at 0.1 mM to ensure proper HPLC detection.

Under these conditions, 1 min of photoactivation proved sufficient to completely consume tetrazole **7**.

In the absence of a dipolarophile, tetrazole **7** was totally converted under these conditions to a more polar compound, which did not react thereafter with a dipolarophile even if added in huge excess. Moreover, as this compound did not show absorption around 350–400 nm, we can assume that this product is not the nitrile imine intermediate but the nitrile imine/H_2O adduct described by Song.[11] In the presence of diethylfumarate, this product was not detected and only the desired compound **9** was present, exhibiting a maximum absorption wavelength around 350 nm. Complete conversion was obtained for diethylfumarate concentrations between 0.15 and 10 mM.

Compound **9** was used as a reference compound for the radiosynthesis of [^{18}F]**9**. [^{18}F]**7** was reacted with diethylfumarate **8** under the conditions described above for the preparation of **9**. With concentrations of diethylfumarate between 1 and 10 mM and after 5 min of photoactivation and stirring, radio-HPLC analysis showed a major peak (>95%) at a retention time corresponding to the standard **9** (Table 1, entry 1–2). Thus, the lifetime of the intermediate nitrile imine photogenerated does not seem problematic, contrary to what was observed with [^{18}F]arylnitrenes.[10] No degradation product was detected after 4 h. A minor radioactive peak was also detected at a retention time corresponding to the nitrile imine/H_2O adduct (Fig. 3).

[†] Formation of non-radioactive **7** in non carrier-added synthesis is linked to the presence of stable isotope originating from the radionuclide production, solvents, chemicals and lines used to transfer radioactivity from cyclotron. Usually, the ratio ^{19}F/^{18}F is around 100–1000/1.

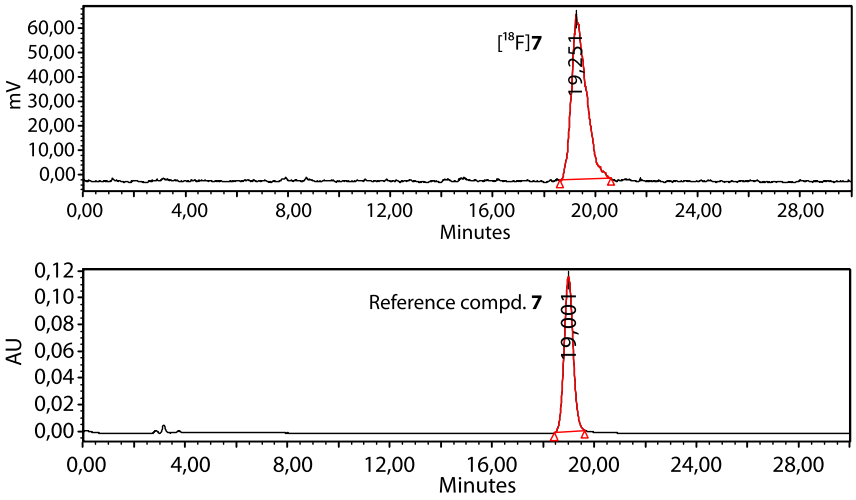
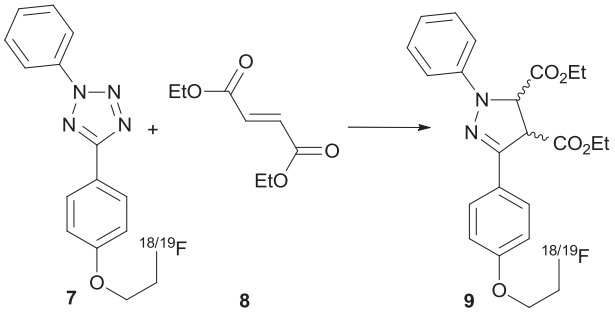


Fig. 2. Radio-HPLC diagram (top) of [^{18}F]**7** (γ-trace) and HPLC diagram of reference compound **7** (254 nm).



Scheme 3. Dipolar cycloaddition between fluorotetrazole and diethylfumarate. Reagents and conditions (rt, PBS/CH_3CN (1:1), *hν*).

Table 1
Effect of reagent concentrations on the radiosynthesis of [^{18}F]**9** (5 min, rt)

Entry	Diethylfumarate **8**	**7**	Radiochemical conversion
1	10 mM	30 μM	96
2	1 mM	30 μM	96
3	100 μM	30 μM	85
4	100 μM	44.5 μM	45
5	100 μM	3.8 μM	92
6	100 μM	30 μM	89
7	10 μM	3.8 μM	43
8	10 μM	1.13 μM	86
9	10 μM	30 μM	5

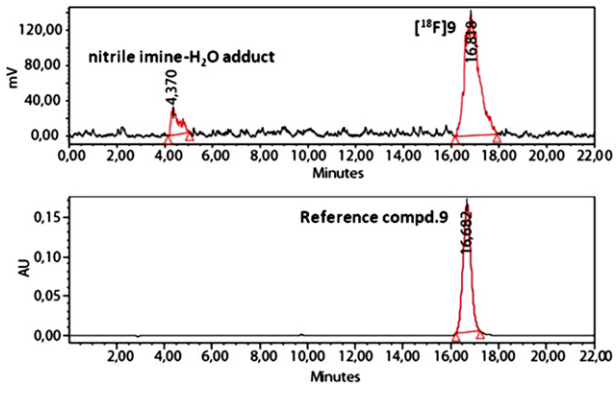
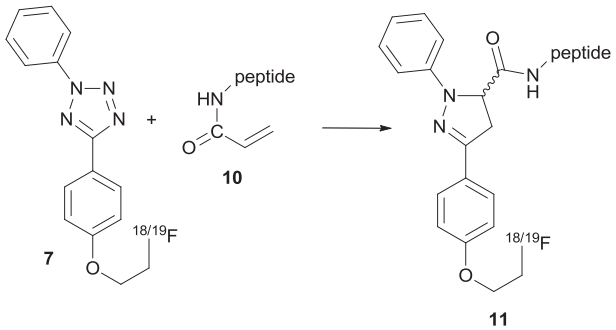


Fig. 3. Radio-HPLC diagram (top) of [^{18}F]**9** (γ-trace) and HPLC diagram of reference compound **9** (350 nm).

This was the only peak detected when no fumarate was added to the reaction medium. When the diethylfumarate concentration was decreased below 1 mM, radiochemical conversions became dependent on the concentration of non-radioactive compound **7** present in solution (Table 1).

The quantity of non-radioactive **7** can be decreased by using an aliquot of the purified [^{18}F]**7** solution. With this approach, we have obtained radiochemical conversions superior to 85% in 5 min with diethylfumarate concentrations of 10 μM (2 nmol in 200 μl, entry 8). This outcome is of great interest, particularly in the biomarker discovery and development field where new biomolecules are available in very low quantities. Conventional labelling methodologies require larger amount of biocompounds, limiting their bioevaluation in vivo. As this 'aliquot' approach leads to discarding a significant amount of the radioactive [^{18}F]**7** produced, it is especially appropriate in the case of animal studies where low levels of activity are required (typically 5–10 MBq for mouse imaging). Encouraged by these results, we have prepared a model peptide featuring an electron-deficient alkene. The terminal NH_2 group of peptide **10** was coupled with acrylic acid to furnish an 'acrylamide-like dipolarophile' peptide (Scheme 4).



Scheme 4. Dipolar cycloaddition between fluorotetrazole and alkene/peptide (peptide=Gly-Gly-Arg-Arg-Pro-Tyr-Ile-Leu-COOH (rt, PBS/CH_3CN (1:1), *hν*).

This peptide (5.5 mM, 1.1 μmol) was conjugated with [^{18}F]**7** (36.5 μM) under the conditions described to prepare [^{18}F]**9** and **9**. After 5 min of photoactivation, 85% of the activity was converted into [^{18}F]**11**. At lower peptide concentrations (0.5–1 mM), radiochemical conversions decreased severely (~30–40%), although this was not the case with diethylfumarate. This means that for relatively available biomolecules (>1 μmol per radiosynthesis)

functionalization by acrylamide-like dipolarophiles is sufficient, while in other cases more activated dipolarophiles are required.

3. Conclusions

In conclusion, we have developed a very fast and efficient bioconjugation method for fluorine-18 labelling through photoactivated 1,3-dipolar cycloaddition. A novel [^{18}F]fluorotetrazole prosthetic group was prepared with a d.c yield of 52%. The efficiency of the method was demonstrated with diethylfumarate and a model alkene/peptide. The conjugation proceeds with high rates and yields of 85–95%. As the reaction is easy to set-up (room temperature, no work-up, compatible with water and oxygen) its automation seems unproblematic provided that a suitable UV source has been mounted in the automation system. In addition, as the reaction is clean and no inorganic catalysis is used, there is no need for intensive purification (no HPLC purification of the conjugated radiocompound) and the quality control remains limited. This is of prime importance regarding the relatively short time available for radiotracer production due to the half-life of fluorine-18 (~110 min). Thus, we have shown that photoactivated 1,3-dipolar cycloaddition can be successfully used for fast preparation of ^{18}F labelled compounds even at very low concentrations. Current work in our laboratory is investigating the use of alternative electrophilic groups to functionalize biocompounds (e.g., maleimide, vinyl ketone.) and reach the performance obtained with diethylfumarate. The purification of intermediate [^{18}F]**7** on Sep-Pak cartridges instead of time consuming HPLC separation is also under investigation. In the perspective of future in vivo applications, the influence of the polysubstituted pyrazoline prosthetic group on the radiopharmaceutical pharmacokinetics should be taken into account. Hydrophilic linkers or substituents of appropriate polarity grafted on to the prosthetic group could be used to tune the overall hydrophilicity. We strongly believe that such an uncatalyzed reaction between 1,3-dipoles and dipolarophiles will become a general class of reaction for radiopharmaceutical preparation.

4. Experimental section

4.1. General

All solvents and chemicals were of analytical grade and used without further purification. The [^{18}O]-enriched water was purchased from Cambridge Isotope Laboratories. Mass spectra were recorded with a Finnigan TSQ7000 mass spectrometer (ThermoElectronCorp.) operating in full-scan MS mode with an ESI$^+$ source and with a Bruker Daltonics micrOTOF spectrometer (TOF-ES-MS). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX 400 (^{1}H at 400 MHz and ^{13}C at 100 MHz). ^{1}H and ^{13}C spectra were referenced to TMS using the ^{13}C or residual proton signals of the deuterated solvents as internal standards. HPLC analyses were run on a Waters Alliance System (996 PDA detector and NaI(Tl) scintillation detector from Eberline) controlled by the Empower program. Analyses were performed on an XBridge C18 column from Waters (250×4.6 mm, 3.5μ) with MeOH and H_2O containing 0.1% TFA mixture (proportions given in parentheses) at 1 mL/min. Preparative HPLC was performed on an Xbridge Prep C18 column from Waters (250×10 mm, 5μ). Solid phase extraction cartridges were purchased from Waters. TLC analyses were performed on silica gel 60 F_{254} plates, with EtOAc as mobile phase. A Bioscan TLC scanner model AR2000 was used for analysis of the ^{18}F labelled compounds. Radiochemical yields are given decay-corrected and are based on the activity eluted from the QMA cartridge. All photoinduced reactions were carried out in quartz tubes (solvent volume 200 μl–2000 μl) with magnetic stirring under ambient atmosphere. The source of photons was a spotlite curing system from UVP (Upland, CA). This portable ultra-violet curing system incorporates two flexible light guides, which are fixed at a 5 mm distance from the quartz tube. The lamp power was 400 W. An integral filter removes unwanted infrared portion of the spectrum to avoid overheating the work area. 2-(4-Nitrophenyl)-2*H*-tetrazole was synthesized according to Gaponik.[20] Peptide syntheses were carried out on an automated solid phase peptide synthesizer PS3 (Protein Technologies) by an Fmoc strategy employing HBTU as the coupling reagent. Acrylic acid was used to functionalize the N-terminal moiety. Enantiomerically pure L-amino acids were obtained from IRIS Biotech.

4.2. Syntheses

*4.2.1. 4-(2-Hydroxyethoxy)benzaldehyde(**4**).* 4-Hydroxybenzaldehyde **3** (12.2 g, 0.1 mmol) and 4.4 g of NaOH (0.11 mmol) were dissolved in a mixture of 200 ml of *n*-BuOH and 30 ml of water. This solution was brought to reflux and a solution of 24.1 ml of 2-chloroethanol (0.3 mmol) in 50 ml of *n*-BuOH was added over a period of 90 min. The reaction mixture was refluxed overnight. The solvent was evaporated in vacuo, and the residue was purified by silica gel dry flash chromatography (Petroleum ether/EtOAc manual gradient from 0% to 100% EtOAc) to afford compound **4** (9.0g, 54%) as a colourless oil, which solidified upon standing overnight. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.83 (s, 1H), 7.78 (d, 2H, *J*=8 Hz), 6.96 (d, 2H, *J*=8 Hz), 4.11 (t, 2H, *J*=4 Hz), 3.94 (t, 2H, *J*=4 Hz), 1.87 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8, 163.6, 132.0, 130.3, 114.8, 69.5, 61.2.

*4.2.2. 2-(4-(2-Phenyl-2H-tetrazol-5-yl)phenoxy)ethanol (**5**).* 4-(2-Hydroxyethoxy)benzaldehyde **4** (715 mg, 4.31 mmol) and 813 mg of benzenesulfonohydrazide (4.73 mmol) were dissolved in 30 ml of ethanol. The reaction mixture was stirred for 1 h at room temperature. The solvent was removed in vacuo to furnish a colourless solid. The solid was redissolved in 50 ml of pyridine (solution A). In parallel, a solution of $NaNO_2$ (886 mg, 12.84 mmol) in 4 ml of water was added dropwise to a cooled mixture (dry-ice ethylene glycol cooling bath, between −10 °C and −20 °C) of aniline (906 mg, 9.74 mmol) dissolved in 8 ml water/ethanol (1:1) and 4.36 ml concentrated HCl to give a solution of phenyldiazonium chloride. This solution was added dropwise over 20 min to solution A, which was maintained between −10 and −20 °C (dry-ice ethylene glycol cooling bath). After this, the red solution was stirred for 3 h at room temperature. The solution was extracted with ethyl acetate/water and the organic layers were collected and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (PE/EtOAc 1:5) to furnish 450 mg of a dark pink solid (37%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (d, 2H, *J*=8 Hz), 8.19 (t, 2H, *J*=8.5 Hz), 7.59 (t, 2H, *J*=7.6 Hz), 7.51 (t, 1H, *J*=7.9 Hz), 7.07 (d, 2H, *J*=8.9 Hz), 4.19 (t, 2H, *J*=4 Hz), 4.04 (t, 2H, *J*=4 Hz). 1.83 (br s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.7, 161.2, 137.7, 130.4, 130.3, 129.4, 120.9, 120.5, 115.7, 70.1, 62.1. HRMS (ESI) *m*/*z* [M+H] calcd for $C_{15}H_{15}N_4O_2$, 283.1189; found 283.1190.

*4.2.3. 2-(4-(2-Phenyl-2H-tetrazol-5-yl)phenoxy)ethyl 4-methylbenzenesulfonate (**6**).* Tetrazole alcohol **5** (50 mg, 0.177 mmol) was dissolved in 5 ml of CH_2Cl_2. *p*-Toluenesulfonyl chloride (67 mg, 0.354 mmol) and 50 mg (0.496 mmol) of triethylamine were added to this solution and the reaction mixture was stirred under inert atmosphere (N_2) overnight at room temperature. The solution was extracted with ethyl acetate/water and the organic layers were collected and concentrated in vacuo. The residue was purified by silica gel column chromatography (PE/EtOAc 1:9 to eliminate impurities, then PE/EtOAc 1:3 to elute the targeted compound). Compound **6** was obtained as a pink solid (28 mg, 36%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, 2H, *J*=8.4 Hz), 8.16 (d, 2H, *J*=8 Hz), 7.85 (d, 2H, *J*=8.3 Hz), 7.55 (t, 3H, *J*=8.4 Hz), 7.37 (d, 2H, *J*=8.4 Hz), 6.93

(d, 2H, J=8 Hz), 4.43 (t, 2H, J=4 Hz), 4.24 (t, 2H, J=4 Hz), 2.47 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.9, 159.8, 145.0, 136.9, 132.9, 129.9, 129.7, 129.6, 128.6, 128.0, 120.4, 119.8, 114.9, 68.0, 65.5, 21.7. HRMS (ESI) m/z [M+H] calcd for $C_{22}H_{21}N_4O_4S$, 437.1278; found 437.1277.

*4.2.4. 5-(4-(2-Fluoroethoxy)phenyl)-2-phenyl-2H-tetrazole (**7**).* Tosylate **6** (93 mg, 0.213 mmol) was dissolved in 2 ml of anhydrous DMSO. Anhydrous caesium fluoride (97.2 mg, 0.639 mmol) was added and the mixture stirred and heated at 80 °C for 18 h in a sealed vial. The solution was extracted with ethyl acetate/water and the organic layers were collected and concentrated in vacuo. The residue was purified by silica gel column chromatography (PE/EtOAc 1:9 to eliminate impurities, than PE/EtOAc 1:5 to elute the searched compound). Compound **7** was obtained as a colourless solid (48 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (d, 2H, J=7.6 Hz), 8.18 (t, 2H, J=7.8 Hz), 7.59 (t, 2H, J=8 Hz), 7.51 (t, 1H, J=7.9 Hz), 7.06 (d, 2H, J=8 Hz), 4.81 (dt, 2H, $^3J_{HH}$=4 Hz, $^2J_{HF}$=53 Hz), 4.30 (dt, 2H, $^3J_{HH}$=4 Hz, $^3J_{HF}$=27 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.0, 160.3, 137.0, 129.7, 129.5, 128.7, 120.4, 119.8, 115.0, 81.8 (d, J_{C-F}=167 Hz), 67.1 (J_{C-F} 20 Hz) HRMS (ESI) m/z [M+H] calcd for $C_{15}H_{14}N_4O_2$, 285.1146; found 285.1148.

*4.2.5. Diethyl 3-(4-(2-fluoroethoxy)phenyl)-4,5-dihydro-1H-pyrazole-4,5-dicarboxylate, (**9**).* Fluorotetrazole **7** (14.6 mg, 0.051 mmol) was dissolved in 800 µl of EtOAc. 14.8 mg of diethylfumarate (0.086 mmol) were added to this solution and the mixture was irradiated with a portable ultra-violet curing system for 4 h. The solvent was removed under reduced pressure to give a crude product. This solid was purified by semi-preparative HPLC (MeOH/H_2O 0.1% TFA, 70/30, 4 ml/min) to furnish 7.2 mg of a colourless solid (0.017 mmol, 33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, J=8.9 Hz), 7.29 (t, 2H, J=7.2 Hz), 7.15 (d, 2H, J=7.8 Hz), 6.93 (t, 3H, J=7.6 Hz), 5.14 (d, 1H, J=5 Hz), 4.79 (dt, 2H, $^3J_{HH}$=4 Hz, $^2J_{HF}$=48 Hz), 4.54 (d, 1H, J=5 Hz), 4.29 (m, 1H), 4.20 (m, 5H), 1.20 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 168.9, 159.1, 144.1, 143.5, 130.3, 129.6, 124.6, 120.0, 114.5, 113.3, 81.8 (d, J_{C-F}=170 Hz), 67.1 (d, J_{C-F}=21 Hz), 66.1, 62.2, 62.1, 56.1, 13.8, 13.7 HRMS (ESI) m/z [M+H] calcd for $C_{23}H_{26}N_2O_5$, 429.1820; found 429.1820.

*4.2.6. 3-(4-(2-Fluoroethoxy)phenyl)-N-[(Leu-Ile-Tyr-Pro-Arg-Arg-Gly-Glycin)-yl]-1-phenyl-4,5-dihydro-1H-pyrazole-5-carboxamide, (**11**).* Fluorotetrazole **7** (2.6 mg, 0.009 mmol) was dissolved in 900 µl of H_2O/CH_3CN/DMSO 1:1:1. 5.5 mg of peptide **10** (0.006 mmol) were added to this solution and the mixture was irradiated with a portable ultra-violet curing system for 2 h. The crude medium diluted with H_2O/MeOH 1:1 was purified by semi-preparative HPLC (MeOH/H_2O 0.1% TFA, 70/30, 4 ml/min) to furnish 4 mg of a colourless solid (0.0003 mmol, 50%). HRMS (ESI) m/z [M+2H] calcd for $C_{60}H_{87}N_{16}O_{12}$, 621.3331; found 621.3337.

4.3. Radiochemistry experimental procedures

No-carrier-added [^{18}F] fluoride was obtained by proton bombardment of an [^{18}O]-enriched water target via the ^{18}O(p,n)^{18}F reaction. The activity (110–2000 MBq) was trapped by passing the target water through a Sep-Pak light QMA cartridge (Waters) previously conditioned under carbonate form. Then, 700 µl of a 50:50 CH_3CN/H_2O solution of K_2CO_3 (6 mg) and Kryptofix 222 (20 mg) were used to elute the fluoride from the cartridge into a heated conical glass vial (120 °C). This eluate was brought to dryness by azeotropic distillation after addition of acetonitrile (3×250 µl) under a gentle stream of nitrogen gas. 2-(4-(2-Phenyl-2*H*-tetrazol-5-yl)phenoxy)ethyl 4-methylbenzenesulfonate **6** (5–7 mg, 0.011 mmol–0.016 mmol) in DMSO (700 µl) was added to the dried residue and the mixture was heated at 95 °C for 5 min. The reaction medium was then diluted with acetonitrile (2 ml) and water (1.3 mL). Labelling efficiency was checked by radio-TLC (silica gel, EtOAc; R_f values: [^{18}F] fluoride=0; [^{18}F]**7**=0.7). HPLC Analysis: t_R=19.2 min (MeOH/H_2O 70/30 0.1% TFA). Radiochemical yield (decay-corrected)=69±5% (n=4). [^{18}F]**7** was purified on an XBridge Prep column (5 ml/min, 80% MeOH, 20% H_2O, 0.1% TFA, t_R=10.7 min). The collected peak was diluted with 20 ml of water and trapped on a Sep-Pak Vac cartridge (200 mg, 3cc, tC18, Waters). The cartridge was rinsed with 5 ml of water and [^{18}F]**7** was eluted with 1 ml of acetonitrile. The purified sample was analyzed by analytical HPLC. Radiochemical purity of [^{18}F]**7** was more than 98%. Decay-corrected radiochemical yield for purification (HPLC+cartridge) was 75%. Specific activity: 7–25 GBq/µmol at EOB.

PBS buffer (50 mM, pH 7.4) was added to a [^{18}F]**7** acetonitrile solution to obtain a 1:1 solvent mixture. Dipolarophile (diethylfumarate or peptide **10**) was added to obtain concentrations described in the results and discussion section for 10 mM: 2 µmol/100 µl of solution of [^{18}F]**7**, for 1 mM: 0.2 µmol/100 µl of solution of [^{18}F]**7**, for 100 µM: 20 nmol/100 µl of solution of [^{18}F]**7**, for 10 µM: 2 nmol/100 µl of solution of [^{18}F]**7**. The mixture was irradiated with a portable ultra-violet curing system for 5 min and readily analyzed by radio-HPLC. The reaction medium was diluted with 20 ml of water and fixed on a Sep-Pak Vac cartridge to remove the PBS buffer and acetonitrile (200 mg, 3cc, tC18, Waters). The excess dipolarophile was not removed during this operation. The cartridge was washed with 5 ml of water and dried with a nitrogen flow. [^{18}F]**9** and [^{18}F]**11** were eluted with 1 ml of ethanol. [^{18}F]**9** and [^{18}F]**11** were isolated with radiochemical purity superior to 95%.

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