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^{18}F-labelling of a cyclic pentapeptide inhibitor of the chemokine receptor CXCR4

Ola Åberg *, Federica Pisaneschi, Graham Smith, Quang-De Nguyen, Elizabeth Stevens, Eric O. Aboagye

Comprehensive Cancer Imaging Centre, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0NN, UK

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ABSTRACT

The chemokine receptor CXCR4 is overexpressed in a variety of cancers including breast, prostate and lung cancer. Expression is also associated with invasion and metastasis. The possibility to image and quantify CXCR4 expression *in vivo* would be a valuable tool in the clinic to aid treatment regimens and to potentially understand the underlying biology of metastasis. Herein we describe the synthesis and the radiolabelling of an ^{18}F-labelled cyclic pentapeptide, [^{18}F]CCIC-0007 designed to bind to the extracellular domains of CXCR4. Radiolabelling was performed via conjugation of [^{18}F]fluorobenzaldehyde with an aminooxy functionalised cyclopentapeptide. Typically, starting with 1.10 GBq (30 mCi) of aqueous [^{18}F]fluoride, 105 MBq (2.85 mCi) of the formulated tracer was obtained within 2.5 h (23 ± 8% dc rcy, 8% EOS yield). Tissue pharmacokinetic studies in mice demonstrated rapid blood clearance, together with biliary and renal elimination.

1. Introduction

Malignant cells have the ability to metastasise to distant sites provoking the spread of cancer through the human body. The chemokine receptor CXCR4, a member of seven transmembrane domain G-protein coupled receptors, has been found to play a crucial role in homing cancer cells to distant sites through binding with its native ligand CXCL12/SDF-1 (chemokine stromal cell-derived factor-1) [1]. Even though metastases tend to show higher expression of CXCR4 than primary tumours, primary lesions with high expression of CXCR4 are recognised as having an aggressive phenotype prone to metastasise [2].

Inhibition of CXCR4-CXCL12 signalling through the use of antagonists of the natural ligand was investigated as potential therapeutic strategies; efficacy, expressed as reduced metastasis, was successfully demonstrated in preclinical metastatic models of breast cancer [3]. CXCR4 inhibitors reported to date are mainly cyclams [4] or peptides [5], however, an increasing number of other small molecule inhibitors are appearing in the literature [6]. Moreover, publication of the crystal structure of CXCR4 in late 2010 is likely to speed up the search for more active antagonists [7].

From a diagnostic perspective, the role played by CXCR4 in invasion and metastasis makes it a valuable biomarker to identify primary tumours prone to metastasise.

Positron emission tomography (PET) is a non invasive imaging modality which uses short lived positron emitting bioprobes to obtain quantitative measures of the biodistribution of the radioactivity within the body [8]. Among the positron emitting radionuclides exploited, ^{18}F ($t_{1/2}$ = 109 min) is attractive because its relative long half-life allows slower kinetics *in vivo* to be monitored when compared to for example ^{11}C ($t_{1/2}$ = 20.4 min), ^{15}N ($t_{1/2}$ = 9.97 min) and ^{15}O ($t_{1/2}$ = 2.04 min). It also permitting more elaborate radiosynthesis tracer production and ^{18}F-radiolabelled tracers can also be potentially transported and used at hospitals without an on-site cyclotron [9].

A few examples of PET imaging tracers for CXCR4 have recently been reported in the literature [10] and they are almost exclusively based on the structure of bicyclam inhibitor Plerixafor (Mozobil, AMD3100) [10a,b] or of the peptide antagonist T140 [10d,e]. So far, the only example of ^{18}F radioimaging agent, 4-^{18}F-T140 [10d], which suffers from pronounced off target binding to red blood cells when used in tracer concentrations and high specific activity.

In the present paper we report the radiosynthesis of [^{18}F]CCIC-0007, a [^{18}F]fluorobenzoimino PEG functionalised cyclo(Nal^1-Gly^2-D-Tyr^3-Orn^4-Arg^5) pentapeptide based on **FC131** (Scheme 1), as potential radiotracer for imaging CXCR4 expression. Radiosynthesis was successfully accomplished by conjugation of the corresponding aminooxy PEG functionalised cyclo(Nal-Gly-D-Tyr-Orn-Arg) pentapeptide and 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA). The use of a radiolabelled cyclopentapeptide seemed attractive for its anticipated lower immunogenicity compared to a larger peptide and the ease of the radiolabelling strategy chosen to accomplish the radiosynthesis. The tissue pharmacokinetic profile in mice is also reported.

Abbreviations: PET, positron emission tomography; Nal, L-3-(2-naphthyl)alanine; Orn, L-ornithine; [^{18}F]FBA, [^{18}F]fluorobenzaldehyde.

* Corresponding author. Tel.: +44 020 8383 3759.
E-mail address: o.aberg@imperial.ac.uk (O. Åberg).

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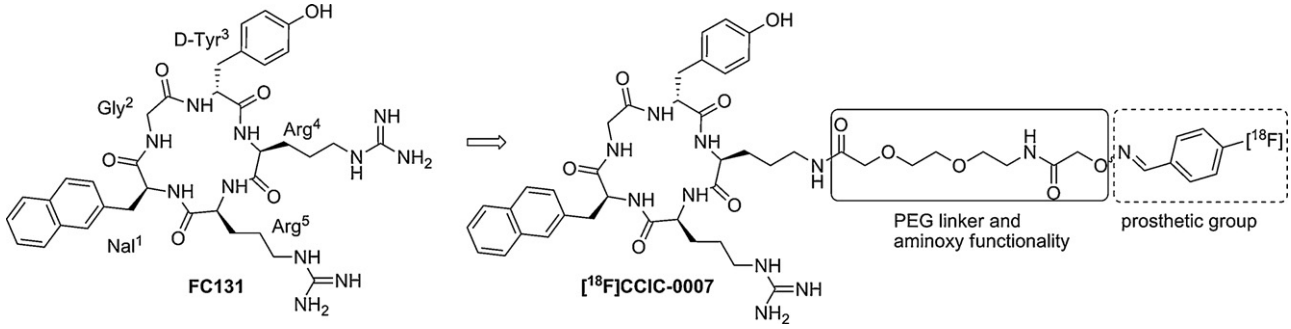


Scheme 1. Arg^4 is replaced by Orn^4 and functionalised with PEG and an aminooxy group for labelling with [^{18}F]FBA.

2. Results and discussion

The use of radiolabelled peptides and proteins for receptor imaging and tumour targeting is now well established [11]. Although examples of direct fluorination for the radiolabelling of peptides have recently been reported [12], functional group incompatibilities and the harsh conditions required for the introduction of [^{18}F]fluoride makes this approach particularly challenging. The use of prosthetic groups for introduction of [^{18}F]fluoride is usually preferable because of ease and high yields of the chemical reactions involved and the high chemoselectivity of the radiolabelling process. The peptide or protein is normally prelabelled with the reactive moiety at a specific position not involved in a crucial biological role. The radiolabelled prosthetic group is then linked to the reactive moiety in a chemoselective manner. Several prosthetic groups such as amino reactive acylation agents (*e.g. N*-succinimidyl 4-[^{18}F]fluorobenzoate [^{18}F]SFB), thiol reactive succinamides (*e.g.* 4-[^{18}F]fluorobenzaldehyde-*O*-[6-(2,5-dioxo-2,5-dihydro-pyrrol-1-yl)-hexyl]oxime [^{18}F]FBAM and 4-[^{18}F]fluorobenzaldehyde-*O*-(2-ethyl)oxime [^{18}F]FBOM) and amino or aminooxy reactive benzaldehydes (*e.g.* 4-[^{18}F]fluorobenzaldehyde [^{18}F]FBA) have been reported and extensively used over the last 25 years [13].

In the present study, the [^{18}F]fluorobenzoimino polyethyleneglycol (PEG) functionalised cyclo(Nal-Gly-D-Tyr-Orn-Arg) pentapeptide [^{18}F]CCIC-0007 was planned to be radiosynthesised from the corresponding aminooxy PEG functionalised cyclo(Nal-Gly-D-Tyr-Orn-Arg) pentapeptide **4** by conjugation with 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FBA, **2**).

Design of [^{18}F]CCIC-0007 was based on the potent cyclic pentapeptide CXCR4 inhibitor **FC131** reported by Fujjii et al. in 2003 (Scheme 1) [5b]. Peptide **FC131** inhibited [^{125}I]SDF-1 binding to CXCR4 transfected Chinese hamster ovary (CHO) cells with an IC_{50} of 4 nM. As Arg^4 in the original peptide could be exchanged for several non-natural amino acids without loss of potency, it also seemed reasonable to modify that position for radiolabelling. Among the many cyclic pentapeptide analogues reported we found the short chained Orn^4-analogue (IC_{50} = 19 nM) especially interesting as it could easily be extended with a linker and a prosthetic group [14].

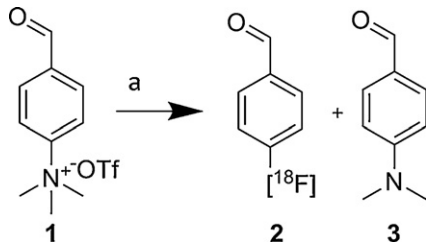
When incorporated into molecules, polyethyleneglycol (PEG) chains are known to enhance hydrophilicity and reduce nonspecific binding and generally also reduces hepatic metabolism. We hypothesised that the introduction of a PEG chain at Orn^4 would counteract the increased lipophilicity introduced by the prosthetic group, [^{18}F]FBA. Finally the terminal aminooxy functionality linked to the PEG chain would allow rapid reaction with [^{18}F]FBA (**2**) to form an oxime.

[^{18}F]FBA has been previously synthesised and purified using a range of protocols largely involving displacement of a nitro group or of a trimethylammonium group by [^{18}F]fluoride. The charged trimethylanilinium triflate precursor has the advantage over the nitro counterpart of being more easily separated from the labelled product [15] by solid phase extraction (SPE) ensuring simple faster purification compared to time consuming HPLC separations [16]. Recently, a procedure involving microwave irradiation allowed the reaction time to be substantially shortened from tens of minutes down to seconds [17]. We synthesised [^{18}F]FBA from the trimethylanilinium triflate precursor 1 by 15 s of 50 W microwave irradiation set at 80 °C with 60 ± 9% dc rcy (n = 15) (Scheme 2). However, it should be noted that the IR temperature probe measures the outside of the glass reaction vessel which usually rises from r.t. to 60–70 °C during the reaction. Considering the low volume of solvent we, therefore, assume the actual temperature of the reaction mixture to be higher.

As previously reported for this reaction, 4-(*N*,*N*-dimethylamino)benzaldehyde **3** was formed as a by-product [17]. Although the side reaction has been suggested to occur via a reverse Menschutkin reaction with fluoride [18], the amount of 4-(*N*,*N*-dimethylamino)benzaldehyde in this case greatly exceeded the available amount of fluoride in the reaction mixture. Therefore we propose that other anions such as triflate or carbonate anions must be involved. The 4-(*N*,*N*-dimethylamino)benzaldehyde **3** could not be separated from [^{18}F]FBA (**2**) in the SPE workup (Fig. 1). We tried to trap the byproduct **3** (estimated pK_a ~3.5, i.e. 2 pH units higher than the pH of the eluent) on a strong cationic exchange cartridge (Phenomenex SCX) eluting with PBS adjusted to pH 1.5 in order to protonate the dimethylaniline while keeping the solid phase bound sulphonic acid (estimated pK_a <1) in its deprotonated state. However, while reducing the amount of byproduct, under these conditions also 50% of the desired [^{18}F]FBA (**2**) was trapped and further attempts were abandoned.

The radiosynthesis of [^{18}F]CCIC-0007 was accomplished by conjugation of the cyclopentapeptide aminooxy precursor **4** and [^{18}F]FBA (Scheme 3).

A sample of nonradioactive peptide CCIC-0007 was also synthesised and characterised for analytical purposes. Precursor **4** was reacted with 4-fluorobenzaldehyde in methanol and ammonium formate buffer at pH 2.5. The final peptide CCIC-0007 was characterised by mass spectrometry and used as reference compound for the radiosynthesis of [^{18}F]CCIC-0007 (Scheme 3).



Scheme 2. (a) [^{18}F]KF/[2.2.2.]kryptand/$KHCO_3$, Me_2SO, MW 15 s, 50 W, 80 °C.

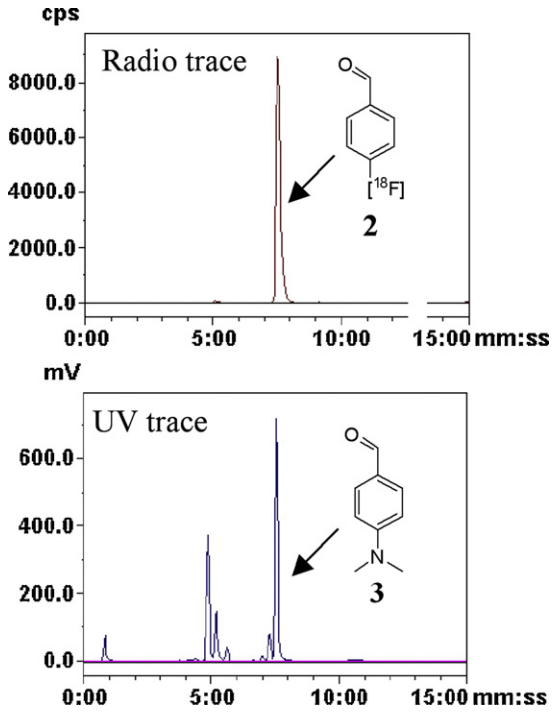


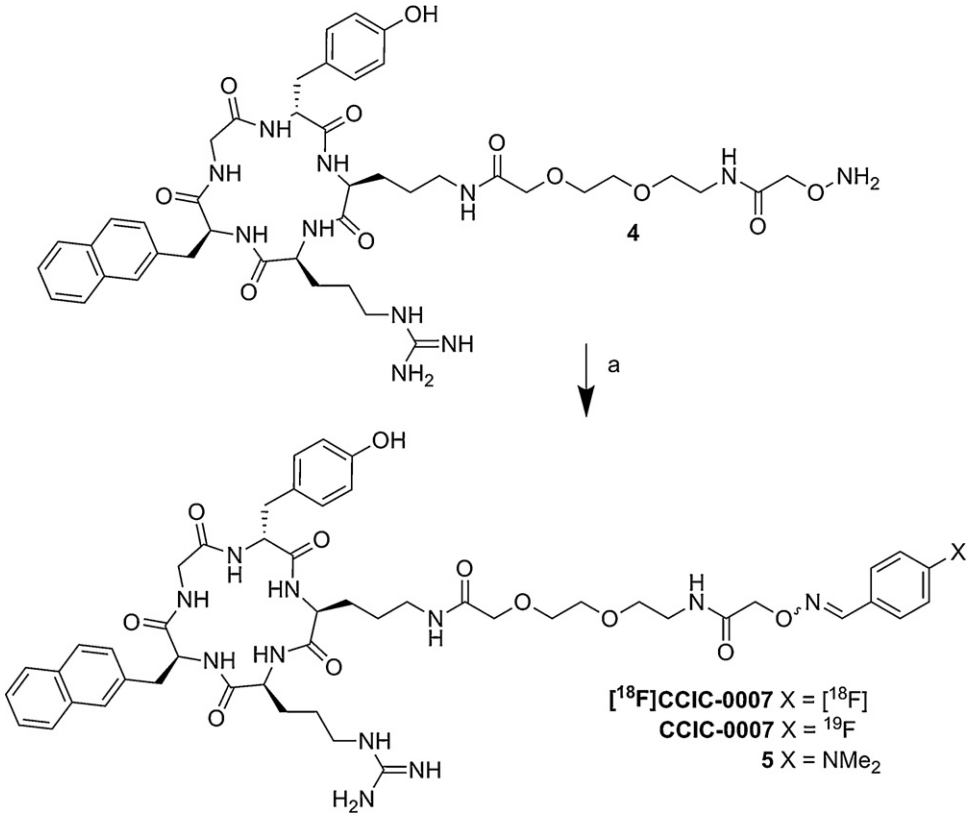
Fig. 1. Analytical radio and UV chromatograms [10% → 80% MeCN (0.1% TFA) in water (0.1% TFA) in 10 min then 80% MeCN (0.1% TFA) in water (0.1% TFA), 1 mL min^{-1}] for the SPE purified [^{18}F]FBA (**2**).

When [^{18}F]FBA reacts with an aminooxy moiety to form an oxime (Scheme 3) [19], the overlap of the lonepairs of the neighbouring oxygen raises the HOMO energy and renders the aminooxy moiety more nucleophilic compared to amines within the biomolecule. This effect, named α-effect, makes the aminooxy group chemoselective over amino bearing amino acids such as Lys or Arg. In addition, the resulting oxime is stable under physiological conditions. Formation of both the *cis* and *trans* oximes is of minor consequence as this portion of the molecule is not involved in the binding interaction with CXCR4.

As the radiochemical yield of a bioconjugation usually depends on temperature, peptide concentration, pH and reaction time, a focused screening for the best conditions was performed. Different reaction conditions are reported in Table 1. While mild conditions are generally preferred in peptide labelling we found that the precursor was dissolving very slowly at 20 °C in the reaction mixture. Thus, the optimal conditions were found to be heating in MeOH and ammonium formate (AMF) buffer (pH = 2.0) at 80 °C for 20 min (entry b, Table 1), giving [^{18}F]CCIC-0007 from [^{18}F]FBA in satisfactory decay corrected radiochemical yield 23 ± 8% (n = 13). The identity of the tracer [^{18}F]CCIC-0007 was confirmed by co-elution with the equivalent ^{19}F-peptide.

It should be noted that the by-product **3** competed with [^{18}F]FBA **2** in the conjugation reaction and the conjugated peptide **5** could not be separated from [^{18}F]CCIC-0007 by HPLC (Fig. 2B, UV trace, t_R = 12:50 min) and both products could be detected in the final formulation by LC–MS (m/z 1012.45 and 1037.50). This affected the apparent specific activity (ratio of radioactivity to the total amount of peptide) which varied in the range of 2–60 GBq μmol^{-1}.

The major microspecies of [^{18}F]CCIC-0007 at physiological pH is assumed to be the monoprotonated form where the side chain of Arg^5 is protonated (estimated pK_a ~12). The distribution coefficient at physiological pH (Log $D_{7.4}$) was assessed by evaluating the distribution of radioactivity in the biphasic octanol/PBS system using the "shake flask" method and Log $D_{7.4}$ was 1.09. While many successful brain imaging tracers have a Log P of 0.9–2.5 [20], no such guidelines yet exist for imaging probes for somatic tissue,



Scheme 3. (a) 4-[^{18}F]FBA or FBA, MeOH, ammonium formate buffer pH 2.0, 80 °C, 20 min.

Table 1
Conjugation of [^{18}F]FBA with **4**.

Entry	Solvents	pH	Temp (°C)	Time (min)	Analytical RCY (%)[a]
a	MeOH, AMF buffer	2.5	80	20	61
b	MeOH, AMF buffer	2.0	80	20	79
c	MeOH, AMF buffer	2.0	20	20	71
d	MeOH, AMF buffer	2.0	20	5	47

[a] Determined by analytical UV–radio–HPLC. [10% → 80% MeCN (0.1% TFA) in water (0.1% TFA) in 10 min then 80% MeCN (0.1% TFA) in water (0.1% TFA), 1 mL min^{-1}].

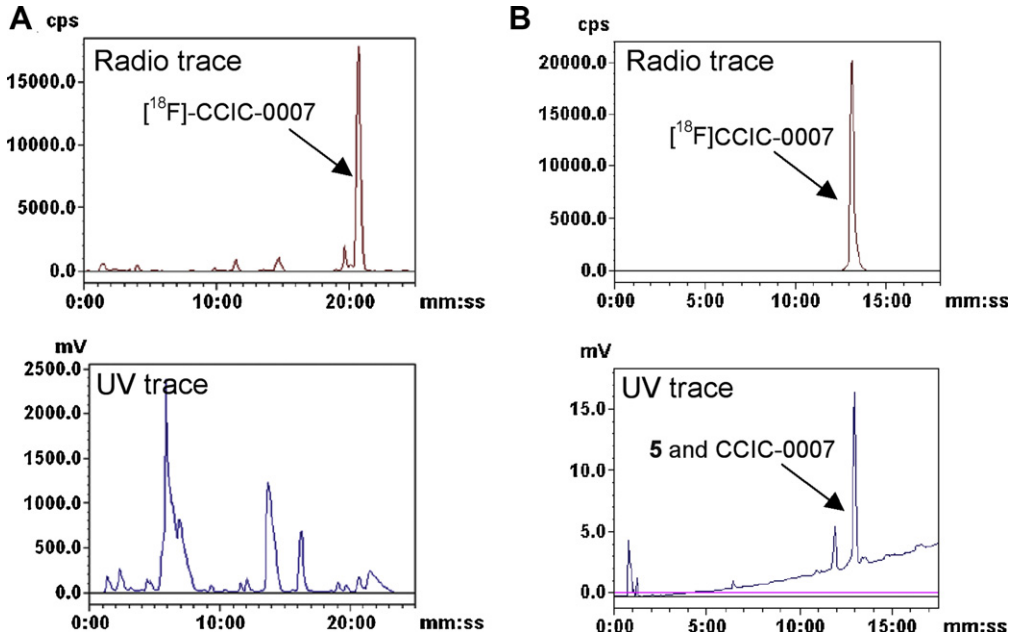


Fig. 2. (A) UV and radiochromatograms for the semipreparative HPLC purification of [^{18}F]CCIC-0007. (B) Analytical UV and radiochromatograms for the determination of radiochemical purity, concentration and specific activity for [^{18}F]CCIC-0007.

however, it is reasonable to assume that a similar or lower range would be suitable to permit relatively fast kinetics *in vivo* in keeping with the half-life of the radionuclide. The experimentally determined value is therefore assumed to be within the optimal range.

An IC_{50}-value of [^{18}F]CCIC-0007 was determined to 0.80 μM in an ^{125}I-SDF-1 competition assay using a metastatic breast cancer cell line MDA-MB-231. To ascertain the radiotracer pharmacokinetics, an *in vivo* biodistribution study was performed in female BALB/c mice. As shown in Fig. 3, the tissue biodistribution were characterised with rapid clearance of [^{18}F]CCIC-0007 from the blood through both biliary and renal elimination routes. Between 5 and 15 min post-injection, high levels of intestinal radioactivity were primarily detected within the duodenum, whereas at 30 and 60 min post-injection this had progressed to the jejunum. The high levels of radioactivity within the gallbladder and very low accumulation within the stomach appears to confirm the bile as the entry point for intestinal [^{18}F]CCIC-0007. High levels of radioactivity present within the urine suggest a rapid clearance from kidneys. The low levels of radioactivity in bone suggest little or no defluorination of the radiolabelled compound. Low activity was also detected within the lungs. The injected dose per gram

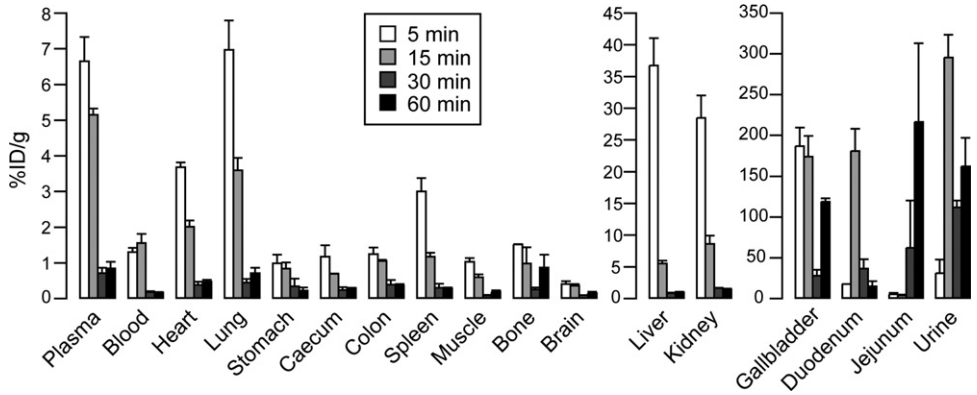


Fig. 3. *In vivo* biodistribution of [^{18}F]CCIC-0007 in various tissues at 5, 15, 30 and 60 min following 3.7 MBq (100 μCi) *i.v.* injection via tail cannulae [n = 3]. Error bars denote SEM.

(%ID/g) values for the analysed tissues are reported in Table S1 (Supplementary information). Compared to other tracers [10d] the rapid clearance from the blood could translate into a lower level of nonspecific binding. The low activity in lung is an encouraging result because it potentially enables the detection of CXCR4 related metastases in this particular tissue. However, the little evidence of [^{18}F]CCIC-0007 uptake in tissues that endogenously express CXCR4 such as the spleen and bone marrow [21] is of our concern as this suggests a non satisfactory level of specific binding.

3. Conclusions

We have successfully radiosynthesised [^{18}F]fluorobenzoimino PEG functionalised cyclo(Nal-Gly-D-Tyr-Orn-Arg) pentapeptide ([^{18}F]CCIC-0007), potential tracer for CXCR4. The radiosynthesis was accomplished by bioconjugation of the aminooxy PEG functionalised cyclo(Nal-Gly-D-Tyr-Orn-Arg) pentapeptide **4** with [^{18}F]FBA in a satisfactory 23 ± 8% dc rcy and 8% EOS yield and took about 2.5 h from aqueous [^{18}F]fluoride. The initial *in vivo* biodistribution of [^{18}F]CCIC-0007 at various time points showed high accumulation of radioactivity in the elimination tissues and an encouraging rapid blood clearance. The low accumulation of the tracer in lungs could potentially enable the visualisation of CXCR4 related lung metastases. However, the little evidence of uptake in tissues that endogenously express CXCR4 is of our concern because it suggests a low level of specific binding. In conclusion, the cyclopentapeptidic core structure appears to be a suitable platform for the potential visualisation of CXCR4 *in vivo*, but structural modifications to improve the receptor binding are required to improve the performance of the tracer and they are in development in our laboratories.

4. Materials and methods

4.1. General

The aminooxyfuctionalised peptide **4** (>95% pure by HPLC, λ = 230 nm) was purchased from Cambridge Research Biochemicals (Billingham, UK). Dichloromethane was dried using a PureSolv drying system (Innovative Technology). All other reagents and solvents were purchased from Sigma–Aldrich (Gillingham, United Kingdom) and used without further purification. ^{1}H NMR spectra were obtained on a Bruker 400 MHz NMR machine and spectra were referenced to residual solvent (for $CHCl_3$, ^{1}H 7.26 ppm and ^{13}C 77.16 ppm, for Me_2SO-d_n ^{1}H 2.50 ppm and ^{13}C 39.50 ppm). Coupling constants (*J*) are given in Hertz (Hz). Mass spectra were obtained in positive electrospray ionisation mode on a Micromass LCT Premier equipped with a Waters Atlantis C18 3 μ column 2.1 mm × 30 mm. Mobile phase (A) water (0.1% formic acid), (B) acetonitrile. A Metler Toledo pH meter was used when pH of the ammonium formate solution was buffered with formic acid. No carrier-added [^{18}F]fluoride was prepared by the ^{18}O(p,n)^{18}F nuclear reaction on a GE PETtrace cyclotron (GE Medical Systems, Uppsala, Sweden) with 16.4 MeV proton irradiation of an enriched [^{18}O]H_2O target. For microwave heating a Resonance Instruments Model 521 (Skokie, IL, USA) equipped with a single mode transverse electric (TE) cavity and an infrared thermometer measuring the outside temperature of the reaction vial was used. For conventional heating a heating block was used and the temperature was measured in the heating block using a mercury thermometer. Preparative UV-radio-HPLC was carried out using a Beckman Pump 127 and Laura 3 software (Lablogic, Sheffield, UK) equipped with a linear UV-106 detector (wavelength 254 nm), a Bioscan Flowcount FC-3400 PIN diode detector (Lablogic) and a Phenomenex Luna C18 5 μ 100 mm × 10 mm HPLC column. Injection loop 1000 μL. Mobile phase (A) ammonium formate in water (10 mM adjusted to pH 3.5 with formic acid) and (B) acetonitrile. Gradient 20–30% B in 15 min. Flow rate 4.0 mL min^{-1}. Analytical UV-radio-HPLC was carried out as above but using a Bioscan Flowcount FC3200 sodium iodide/PMT gamma detector (Lablogic), a Thermo Separation Products Spectra System UV1000 (wavelength 254 nm), and a Phenomenex Luna C18 3 μ 50 mm × 4.6 mm column with a column guard cartridge. Injection loop 20 μL. Mobile phase (A) 0.1% TFA in water, (B) 0.1% TFA in acetonitrile. Gradient 5–50% B in 15 min. Flow rate 1 mL min^{-1}.

4.2. Chemistry

4.2.1. (4-Formylphenyl)trimethylammonium trifluoromethansulfonate 1

(4-Formylphenyl)trimethylammonium trifluoromethansulfonate **1** [15] was synthesised as reported in the literature with minor modifications. Methyl trifluoro-methanesulfonate (1.81 g, 11.0 mmol) was added to a to a septum-equipped vial containing a ice-cold solution of *N*,*N*-dimethylamino benzaldehyde (1.48 g, 10.0 mmol) in dry dichloromethane (10 mL). The reaction mixture was stirred under N_2 and allowed to warm to r.t. and after approx. 90 min a white precipitate was formed. After 3 h the precipitate was filtered off and rinsed repeatedly with cold dry dichloromethane and dried under vacuum to yield the crude product as a white solid material. The crude product was repeatedly triturated in refluxing dichloromethane until the essentially pure product was obtained as a white powder (0.923 g, 29%). The product was kept in the dark at 4 °C until used for further experiments. ^{1}H NMR (Me_2SO-d_6, 25 °C, 400 MHz) δ 10.10 (s, 1 H, CHO), 8.20 (AA′BB′, 2 H, aromatic), 8.15 (AA′BB′, 2 H, aromatic), 3.65 (s, 3 × CH_3) ppm. ^{13}C NMR (Me_2SO-d_6, 25 °C, 100 MHz) 192.3 (CHO), 151.1, 136.9, 131.0, 121.8, 120.7 (q, J_{CF} = 320, CF_3), 111.2, 56.5 (3 × CH_3) ppm. ^{19}F NMR (Me_2SO-d_6, 25 °C, 376 MHz) −77.79 ppm. Analytical HPLC, product 1 t_R 1:41 min, 4-*N*,*N*-dimethylamino benzaldehyde t_R 11:00 min.

4.2.2. Cyclo-[Nal-Gly-(D-Tyr)-Orn(PEG$_2$–O–N=CH-4-F-Ph)-Arg], CCIC-0007.

Cyclo-[Nal-Gly-(D-Tyr)-Orn(PEG_2–O–NH_2) (5.5 mg, 6.07 μmol) was dissolved in methanol (400 μL) and 4-fluorobenzaldehyde (0.68 mg, 5.47 μmol) was added as a solution in methanol (50 μL) and ammonium formate 10 mM pH 2.5 (450 μL) was added to a reaction vial and heated to 80 °C for 20 min. The product was purified using semi-preparative HPLC. The combined fractions were diluted with water and immobilised on a Waters SepPak tC18 Light cartridge (preconditioned with 5 mL ethanol followed by 10 mL water) and eluted with 5 mL EtOH. Freeze drying yielded the title compound as a white solid (4.10 mg, 74%). TOF MS ES+ *m*/*z* (rel. intensities) for $C_{50}H_{63}FN_{11}O_{11}$ $[M+H]^+$: 1012.45 (100%), 1013.45 (58%), 1014.45 (12%). HRMS ES+ $[M+H]^+$*m*/*z* calcd for $C_{50}H_{63}FN_{11}O_{11}$ 1012.4693, found 1012.4657. ^{19}F NMR (Me_2SO-d_6, 25 °C) −110.20 ppm.

4.3. Radiochemistry

4.3.1. 4-[^{18}F]Fluorobenzaldehyde 2

4-[^{18}F]Fluorobenzaldehyde **2** was synthesised according to a reported method with minor modifications [17]. A Wheaton vial (3 mL) was charged with Kryptofix^{TM} (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane, 5.0 mg, 13 μmol), potassium hydrogen carbonate (0.7 mg, 7.2 μmol) dissolved in water (50 μL), acetonitrile (0.50 mL), and ^{18}F–water (0.10–0.40 mL, typically 0.74–1.11GBq, 20–30 mCi). The water was removed azeotropically at 100 °C using a nitrogen stream (100 mL min^{-1}) for 10–25 min. Anhydrous acetonitrile (3 × 0.5 mL) was added and sequentially evaporated under previous conditions, approximately 3 × 5 min. The vial was cooled to room temperature by immersion in a water

bath. A freshly prepared solution of (4-formylphenyl)trimethylammonium trifluoromethansulfonate 1 (2.0 mg, 6.4 μmol) in anhydrous Me_2SO (0.20 mL) was added immediately before microwave heating (15 s, 50 W, set temperature = 80 °C). The reaction mixture was immediately quenched by the addition of water (2 mL) and the reaction vial was rinsed (3 × 2 mL). The combined fractions were taken up into a syringe and loaded onto a Waters C18 SepPak-plus cartridge (preconditioned with 5 mL methanol followed by 10 mL water). By this rinsing procedure the remaining radioactivity in the reaction vial was kept <4%. The cartridge was washed with water (5 mL) and partially dried with N_2 (100 mL min^{-1}) for 2 min. The cartridge was eluted with methanol, the first 0.50 mL was discarded and the product fraction was collected in 0.40–0.50 mL methanol. The recovery of immobilised radioactivity was 85–95%. The decay-corrected radiochemical yield of 2 were in the range of 42–77% (61 ± 9%, n = 14, typically 0.40 GBq, 11 mCi), and the procedure was completed within 60 min including the drying of the ^{18}F-fluoride. Analytical HPLC, product t_R 10:35 min.

4.3.2. Cyclo-[Nal-Gly-(D-Tyr)-Orn(PEG$_2$–O–N=CH-4-[^{18}F]fluoro-Ph)-Arg] [^{18}F]CCIC-0007

The Waters SepPak C18Plus immobilised [^{18}F]fluorobenzaldehyde **2** was eluted with methanol (0.40–0.50 mL) directly to a Wheaton vial (3 mL) containing a freshly prepared suspension of the aminooxy functionalised peptide (1.3 mg, 1.3 μmol) in 100 mM ammonium formate buffer pH 2.0 and the reaction mixture was heated under a septum for 20 min at 80 °C. The reaction mixture was cooled to r.t. in a water bath before venting and diluting with ammonium formate buffer pH 3.5 (0.35 mL) and injecting to a semi-preparative HPLC. The purified fraction (t_R = 20:15–21:15 min) was diluted with water (8 mL) and >99% of the radioactivity was immobilised on a Waters SepPak tC18 Light cartridge (preconditioned with 5 mL ethanol followed by 10 mL water). The cartridge was washed with water (5 mL) and dried using a stream of nitrogen (100 mL min^{-1}) for 2 min before the product was eluted with 5 mM HCl in ethanol in 8 × 0.10 mL fractions and >90% of the radioactivity was collected in 3 fractions. The decay-corrected radiochemical yield was 11–35% (22 ± 8%, n = 12, typically 0.11 Gbq, 3 mCi) calculated from aqueous [^{18}F]fluoride. The labelled product was stable in the acidic ethanolic solution for at least 5 h at r.t. Before further use [^{18}F]CCIC-0007 was neutralised with PBS to <33% ethanol. Analytical HPLC, t_R = 13:09 min, showed a radiochemical purity >99%. A sample spiked with the reference compound CCIC-0007 was used to confirm the identity of the radio peak. TOF MS ES+ m/z for $C_{50}H_{62}FN_{11}O_{11}$ $[M+H]^+$: 1012.45 (100%), 1013.45 (60%), 1014.45 (12%). TOF MS ES+ m/z for **5** $C_{52}H_{68}N_{12}O_{11}$ $[M+H]^+$: 1037.50 (100%), 1038.50 (62%), 1039.50 (22%), 1040.51 (5%).

4.4. Specific activity

The non-radioactive isotopologue CCIC-0007 was used for a 4 point HPLC–UV-calibration curve 1–16 μM (λ = 254 nm). Injection loop = 20 μL. [^{18}F]CCIC-0007 was obtained with an apparent specific activity of 21 ± 18 GBq μmol^{-1}.

4.5. Log D measurements

50 μL of purified and formulated (5 mM HCl in ethanol) [^{18}F]CCIC-0007 was diluted with PBS (750 μL). Diluted radioactive solution (50 μL), PBS (450 μL) and octanol (500 μL) was added to each of 4 Eppendorf tubes and then vortexed for 1 min before centrifugation at 5000 rpm for 5 min. From the upper octanol layer, 300 μL was carefully transferred to a gamma counting tube using a pipette. The bottom aqueous layer was transferred to a second Eppendorf tube using a 2.5 mL syringe before pipetting 300 μL to a gamma counting tube. The gamma counting tubes were placed in a gamma counter and analysed for 2 min each and the decay corrected the logarithm of the octanol/water ratio was calculated. The averaged value with the standard deviation is reported 1.09 ± 0.02 (n = 4).

4.6. CXCR4 receptor binding assay

Competition binding experiments were performed in MDA-MB-231 human breast carcinoma cells expressing endogenous levels of CXCR4. Briefly, cells were harvested using a non-enzymatic cell dissociation solution and resuspended in binding buffer (PBS with 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.25% BSA, pH = 7.4). Incubation was conducted in a final volume of 200 μL per sample containing 2 × 10^5 cells, 0.15 nM [^{125}I]SDF1α (Perkin Elmer, Cambridge, UK) and 0–25 μM of the non-radioactive isotopologue CCIC-0007, for 60 min at room temperature. After incubation, cells were spun three times through 10% sucrose at 5000 rpm-3 min, and transferred into counting tubes. Cell bound radioactivity was measured using a gamma counter. Binding results were expressed as counts/min, and IC_{50} values were calculated using Prism software (GraphPad, La Jolla, CA).

4.7. Biodistribution in mice

Female BALB/c mice (Charles River, UK) aged 6–8 weeks were anaesthetised with 2.5% isoflurane and injected by *i.v.* tail vein cannulae with ~100 μL of PBS containing 3.7 MBq (100 μCi) of [^{18}F]CCIC-0007. The animals were sacrificed at 5, 15, 30 and 60 min post-injection by cardiac puncture, and the extracted blood centrifuged at 15,000 rpm for 5 min to separate the plasma from the cellular blood fraction (n = 3). The animals were then dissected to remove the tissues (Fig. 3). Urine samples were also collected. For the measurement of radioactivity in the intestine, a small section of the jejunum was excised and emptied of its content by running PBS flows in the lumen. The jejunum epithelium section was then rapidly dried off using absorbing paper and placed in tubes for gamma counting.

The decay-corrected counts per minute within the tissues were measured using a Packard Cobra II gamma counter (Perkin Elmer, UK) for 1 min, and the counts corrected for sample weight. All animal experiments were done by licensed investigators in accordance with the United Kingdom Home Office Guidance on the Operation of the Animal (Scientific Procedures) Act 1986 (HMSO, London, United Kingdom, 1990) and within guidelines set out by the United Kingdom National Cancer Research Institute Committee on Welfare of Animals in Cancer Research [22].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jfluchem.2011.11.003.

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