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Synthesis and automated fluorine-18 radiolabeling of new PSMA-617 derivatives with a CuAAC radiosynthetic approach

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INTRODUCTION

Prostate Cancer (PCa) is the second most common type of diagnosed malignancy (after lungs cancer) in men worldwide and is in general the sixth cancer-related cause of death,[1] due to a high rate of metastasis (about 20%).[2] Indeed, this kind of tumor is often initially asymptomatic. Thus, early diagnosis of PCa is of paramount importance. Prevention especially relies on routine determination of plasmatic levels of prostate-specific antigen (PSA), a glycoprotein expressed by the prostate, as it is a fast, simple and inexpensive test; unfortunately, although it is undoubtedly a useful marker, PSA levels may depend on other factors, and tissue biopsy is still the standard procedure to confirm the presence of cancerous lesions.[3]

In the last decades several PET radiopharmaceuticals aimed to the diagnosis of PCa were designed, the first of which was [^{11}C]choline, followed by [^{18}F]fluoro methyl (and fluoroethyl) choline in order to overcome the limits imposed by the short carbon-11 half-life. Uptake of choline, a substrate used in metabolic cell pathway during the synthesis of phosphatidylcholine, is mediated by choline kinase specific transporter, whose expression and activity are higher in prostate cancer cells.[4] However, radiolabeled choline derivatives have limitations in the evaluation of tumor staging. A more recent "metabolic" radiotracer is [^{18}F]fluciclovine, an unnatural amino acid, carried into the cell by asc-type amino acid transporter 2 (ASC2) and L-type amino acid transporter (LAT1), whose overexpression is associated with strong disease aggressiveness and poor survival. Compared with radiolabeled choline, [^{18}F]fluciclovine, also known as [^{18}F]FACBC,[5] showed a higher detection rate and lower background.

In recent years, the prostate-specific membrane antigen (PSMA), which shows a significant expression in prostatic cancerous cells and upregulation in poorly differentiated, metastatic and hormone refractory carcinomas, has been identified as a highly attractive target.[6] This type II

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glycoprotein has inspired the design of a new series of molecules for PCa diagnosis and therapy.[7] PSMA-ligands, based on direct interaction ligand-protein, were investigated and radiolabeled with different radionuclides.
[^{18}F]F-DCFBC, [^{18}F]F-DCPyL, [^{18}F]F-PSMA1007 and [^{68}Ga]Ga-PSMA11[8,9,10] are examples of PSMA targeted radiopharmaceuticals, which all display lower renal clearance, thus improving visualization of prostate lesions and affinity, and yielding more accurate PET images. Moreover, this class of radiopharmaceuticals show better sensitivity and specificity, particularly at low PSA levels.[11] Gallium-68 labelled radiopharmaceuticals have long been the "gold standard" in routine clinical practice; however, research was soon extended to PSMA derivatives labeled with fluorine-18, due to its well-known advantages: compared to gallium-68, it has a longer half-life (109.7 vs 68.5 min), a lower positron emission energy, which gives higher resolution PET images, and can be produced in large amounts with common medical cyclotrons, also allowing commercial distribution.[12]

The most known example of fluorine-18 labelled PSMA derivatives, namely [^{18}F]PSMA-1007, may be easily prepared via fully automated radiosynthetic procedures, using commercially available starting materials, and its usefulness has been well established by several clinical trials.[13] For instance, in a recent paper [^{18}F]PSMA-1007 and [^{68}Ga]Ga-PSMA-11 were compared, prompting for a higher number of lesions detected with the fluorinated compound, which was probably attributable to the higher spatial resolution of fluorine-18, compared with gallium-68.[14] On the other hand, fluorinated derivatives may still suffer of partial defluorination, in vivo, which could result in undesired bone uptake.[15] Aim of the present paper is the synthesis of a suitable "cold" precursor and its automated radiolabeling with fluorine-18 via "click chemistry", of a derivative of PSMA-617, which is currently clinically relevant in the therapy of PCa with its lutetium-177 labelled version. The synthesis and gallium-68 radiolabeling of PSMA-617 was first reported by the University of Heidelberg.[6] PSMA-617 was originally designed in view of its possible use as a theranostic agent, replacing the HBED-CC chelator of PSMA-11 with DOTA, which is suitable for radiolabeling with therapeutic radionuclides such as yttrium-90 and lutetium-177. Moreover, the linker was also modified, using a L-2-naphtyl-alanine lipophilic group, which is known to improve interactions with the S1-accessory hydrophobic pocket in protein binding site.[16,17,18]
There are several techniques to introduce fluorine-18 into macromolecules. The classic method can be described as a [^{18}F]fluoride activation, followed by nucleophilic substitution on a particularly reactive functional groups already present on the biomolecule, which generally occurs in "harsh" conditions. Other techniques include the fluorine-18/aluminum complex coordination with a suitable chelator linked to the desired biomolecule[19] or the formation of the strong bond between fluorine-18 and Lewis acids like silicon and boron.[20,21,22] Recently new radiohybrid PSMA ligands ([^{18}F]F-rhPSMA-7) were synthesized, taking advantage of Si-^{18}F bond chemistry, which proved to be promising in terms of biodistribution and image quality.[23,24,25]
The last strategy is related to the functionalization of both reaction partners (non-biological molecule and biomolecule). To this regard, also "Click chemistry" concept is very well suited. "Click-chemistry" comprises a set of reactions which are fast and regioselective, easily giving products that can be purified with excellent yields, and that are carried out under mild condition

and in aqueous media.[26] The most investigated "click-reaction" is the Cu(I)-catalyzed Huisgen [3+2] cycloaddition between an azide and a terminal alkyne (CuAAC). Fluorine-18 is introduced in harsh condition, usually via "classic" nucleophilic substitution, on the azide-functionalized prosthetic groups and the resulting [^{18}F]fluorine-azide is then conjugated with terminal alkyne-functionalized biomolecule, yielding a stable 1,4 disubstitued 1,2,3 triazole.[27,28]

Generally, copper (I) is generated in situ by Cu(II) salts reduction; however, studies related to the reaction carried out directly with Cu(I) salts have also been performed.

Several PSMA derivatives have already been radiolabeled following this technique, two of which look promising,[29,30] showing uptake and tumor-to-background comparable or even better than [68Ga]PSMA-11.

In our laboratory, we have developed a "general purpose" method for the radiolabeling of macromolecules via CuAAC chemistry. An orthogonal azido-precursor, suitable for peptide radiolabeling, was synthesized and radiolabeled with fluorine-18. The resulting [^{18}F]fluoroazide was initially conjugated with L-propargylglycine (a suitable alkyne functionalized glycine), in order to test the CuAAC reaction.[31]

RESULTS AND DISCUSSION

The synthesis of alkyne-functionalized PSMA-617 required eleven steps and provided an overall yield of 18.8% including final purification by semi-preparative HPLC.

Precursor **11** was obtained by splitting the synthesis in three different pathways. Glu-urea-Lys residue and specific ligand PSMA-617 linker were synthesized obtaining compounds **3** and **7** (Scheme 1 and 2), respectively. Then, they were conjugated, yielding compound **9**, which was then functionalized with the terminal alkyne.

As shown in Scheme 1, commercial L-di-*tert*-butyl glutamate reacted with carbonyldiimidazole (CDI) under anhydrous conditions obtaining the activated acylimidazole derivative (intermediate **1**), in presence of triethylamine and DMAP.[32] Subsequently, the crude intermediate **1** was activated with methyl triflate (MeOTf) and in presence of TEA rapidly reacted with commercial Cbz-Lys-Ot-Bu to obtain the heterodimer **2**. As shown, intermediate **2** corresponds to the protected Glu-urea-Lys. In order to avoid the formation of byproducts, carboxylic groups were protected as *tert*-butyl esters and the lysine ε-amino group as carboxybenzyl carbamate.

After purification by column chromatography, Cbz on ε-amino group of Lys was removed by means of a hydrogenolysis to give intermediate **3**. Hydrogen was generated *in situ* using ammonium formate and the reaction was catalyzed by 5% palladium on carbon. The overall yield of synthetic process was about 73%.

With the aim to prevent the formation of byproducts, which is typical when two non-protected aminoacids are conjugated, commercial L-naphthyl alanine was protected on carboxylic group as ethyl ester, obtaining intermediate **4**. Commercial *trans*-4-(aminoethyl)cyclohexane carboxylic acid was selectively protected on amine group by reaction with benzyl chloroformate and after column chromatography pure intermediate **5** was obtained in good

yields. After the two amino acids were selectively protected on suitable functional groups, a coupling reaction between intermediate **4** and **5** was performed. Coupling reaction was conducted using (1-[bis(dimethylamino)methylene]-1H-1,2,3-triazole[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) as condensing agent in presence of triethylamine. During the reaction, the carboxylic group of intermediate **5** is activated by HATU yielding a very reactive ester and allowing formation of peptide bond with intermediate **4** amine group. Dimer **6** was obtained in good yield after column chromatography. As shown in Scheme 2, a selective peptide bond between the desired functional groups was formed. Finally, the ethyl ester was removed under basic conditions affording intermediate **7** in 82% overall yields.

Once intermediates **3** and **7** were synthesized, Glu-urea-Lys and specific PSMA-617 linker were coupled, affording compound **8**. As shown in Scheme 3, a completely protected PSMA-617 "core" was finally obtained, ready to be functionalized with a suitable moiety which was, in view of the subsequent radiolabeling via CuAAC "click" reaction, a terminal alkyne. Therefore, Cbz was removed from intermediate **8** through a hydrogenolysis to yield intermediate **9**, and then a selective peptide bond with 5-hexynoic acid was performed (intermediate **10**).
Last step of the synthesis consisted in the final removal of protecting groups present on the Glu-urea-Lys residue. *Tert*-butyl esters removal was carried out in presence of trifluoroacetic acid (TFA), under anhydrous conditions. During this reaction significant amounts of byproducts were formed, mainly due to the complete removal of glutamic acid residue. Thus, a semi-preparative HPLC was necessary to purify the crude product and obtain pure precursor **11**.

Once precursor **11** was synthesized, the suitable conditions for fluorine-18 radiolabeling were optimized.

Macromolecules like proteins, large peptides, oligomers and also nucleic acids are very sensitive to reaction conditions. In particular, $[^{18}F]F^-$ nucleophilic substitution conditions are too harsh to achieve a direct fluorination on macromolecules, this reaction generally being conducted at 70-100°C.[33]
Thus, Cu(I)-catalyzed Huisgen [3+2] cycloaddition between an azide and a terminal alkyne was investigated in order to radiolabel ligand PSMA-617 under milder conditions.
A large variety of azido prosthetic groups used in CuAAC reactions has been described. Unfortunately, most of them showed marked instability and high volatility, especially short chain aliphatic azides such as [^{18}F]fluoroethyl azide, which make them difficult to handle and to automate their preparation procedure; in principle, aromatic azides are more suitable, but they often bear leaving groups directly linked to aromatic rings, which reduce reactivity during radiolabeling fluorination, and may also involve radiosynthethic steps in the gas phase.[34]

With the aim to overcome the above drawbacks, we prepared and tested a useful azido precursor (**12**), which displayed high UV absorbance allowing HPLC detection, and with a reduced volatility, allowing easier management. Moreover, it includes a short PEG chain,

which may improve metabolic stability; finally, azido group is bound on a more reactive benzylic position.
As reported in Scheme 4, iodine was chosen as leaving group. Compared with mesylate and tosylate, iodine showed better results in terms of yield and purity during fluorine-18 substitution reaction.[31]

[^{18}F] Fluorination and subsequent CuAAC reaction were performed and automated on a Trasis-AllinOne radiosynthesis system. Fluorine substitution was carried out on iodo-precursor **12** at 100°C, for 20 minutes, obtaining a crude mixture containing the desired **[^{18}F]13** together with byproducts deriving from hydrolysis and elimination reactions, which was then passed through a tC18 Sep-Pak cartridge. As shown in Figure 1, a satisfactory removal of unreacted [^{18}F]fluorine and most abundant elimination "cold" byproducts was obtained. Overall radiosynthesis time was 58 min, with a radiochemical yield not decay corrected in the range 25-36%.

With the aim of investigating and optimize a general-purpose fluorine-18 radiolabeling of interesting biomolecules via "click chemistry", the Cu(I)-catalyzed Huisgen [3+2] cycloaddition reaction of **[^{18}F]13** with terminal alkyne-functionalized **11** was performed (Scheme 5).
Generally, CuAAC reactions are largely used in macromolecules radiolabeling because of their reduced time, mild reaction conditions and regioselectivity. Moreover, the reaction succeeds in a large variety of solvents and co-solvents, indicating a great potential in biological macromolecules radiolabeling. Cu(I)-catalyzed Huisgen [3+2] cycloaddition reaction is catalyzed by copper (II), which increase reaction rate and regioselectivity, sometimes with the addition of copper stabilizing agent or preformed copper(I) complexes.[35]

In this work, CuAAC was performed via "conventional" route, with sodium ascorbate as reducing agent of a copper (II) salt. No stabilizing agents and only small amounts of copper (II) were used, due to its toxicity in biological systems.
Moreover, the radiolabeling procedure was automated using a Trasis AllinOne radiosynthesis platform (Figure 2). Automation is essential in the preparation of fluorine-18 labeled radiopharmaceuticals, and allows radiosynthesis reproducibility with minimal radiation exposure to the personnel. The method was optimized starting with an empty cassette, which was adapted to the desired process, performing both the nucleophilic substitution reaction that lead to the formation of the fluorine-18 labeled azide, and the subsequent coupling with the alkyne functionalized PSMA precursor, and included two purifications steps, using SPE and semi-preparative HPLC, respectively.

The choice of suitable solvents to conduct the reaction was crucial for success and yield optimization. Many attempts were performed in order to overcome poor reagents and product solubility: indeed, due to the strong hydrophobicity of precursor **11**, reactions could not take place in pure water, and a mixture of water and organic solvents was necessary. Precursor **11** and sodium ascorbate were dissolved in respective solvents (Table 1) prior to begin the

radiolabeling procedure. The two solutions were collected in the same automated system reservoir, and the mixture was dropped in the reactor containing radiolabeled azide **[^{18}F]13**; then $CuSO_4 \cdot H_2O$ previously dissolved in the suitable solvent (solvent 3 in Table 1) was added to carry out the cycloaddition reaction (Scheme 5) at room temperature.
In detail, precursor **11** was initially dissolved in organic solvents (DMSO or MeOH) and no precipitation was observed when added to the automated system reservoir previously loaded with sodium ascorbate. On the other hand, after addition of the aqueous $CuSO_4$ solution a strong precipitation occurred in the reaction vial, and conversion to product **[^{18}F]14** was relatively low (Table 1).
Methanol showed a better solubility compared with dimethyl sulfoxide even when diluted with water, but conversion of labeled azide **[^{18}F]13** into final product **[^{18}F]14** did not improve significantly. Moreover, a complete solubility of the reactants was obtained using nearly pure methanol, but only 1% conversion in final product **[^{18}F]14** was observed after 20 minutes, thus clearly indicating that solubility was not the only issue to be considered. Despite of its wide use in fluorination reaction, acetonitrile was ruled out due to the known extremely low solubility of precursor **11** and copper salts.

Better results were obtained dissolving precursor **11** and sodium ascorbate in 0.5M NH_3 solution; under these conditions, we obtained the highest conversion rate and no precipitation in reactor was observed. Indeed, ammonia is able to form tri-coordinated coplanar species $[Cu(NH_3)_3]^+$ with reduced copper (I), acting as Lewis acid and increasing the acidity and rates of alkynes ionization.[36] Moreover, at basic pH the three carboxylic groups of Glu-urea-Lys residue are in carboxylate form, increasing its solubility in water (Figure 3).
Good results were achieved using 0.5M NH_3 as the solvent and 20 min reaction time; attempts to short the procedure did not improve radioactivity yield (Table 1).

After optimization of CuAAC reaction conditions, a method for the purification of final product **[^{18}F]14** was also implemented. The reaction mixture, after quenching with 1M HCl, was diluted in the mobile phase and injected in a semi-preparative RP-HPLC column. The desired product was obtained with high chemical and radiochemical purity (Figure 4).

Finally, the mobile phase containing pure 1,4-substituited 1,2,3-triazole **[^{18}F]14** was then passed through a tC18 Sep-Pak cartridge. The adsorbed pure product was washed with water, then it was eluted with ethanol and saline physiological solution to make it available for *in vivo* preclinical testing. Radiosynthesis took an overall time of 112 min and was obtained with a 6.1 % RCY not decay correct and a molar activity > 650 GBq/μmol.
Chemical and radiochemical stability were tested by analytical HPLC analysis of samples of the purified product up to eight hours after the end of radiosynthesis, at time intervals of two hours and storing the vial at room temperature. Product **[^{18}F]14** was stable under these conditions and no loss of radioactivity due to radiolytic degradation was detected. Moreover, stability test on human plasma were conducted and no degradation was observed.

In conclusion, a novel derivative of PSMA-617 functionalized with a terminal alkyne was synthesized with suitable yield and purity (6.1% RCY not decay correct, >99% radiochemical

purity), and successfully coupled with a fluorine-18 labelled azide at room temperature. The whole radiolabeling procedure was fully automated, affording the desired product in good yield and excellent chemical and radiochemical purity. The effectiveness of new product **[^{18}F]F14** for prostatic cancer diagnosis will be tested *in vivo* on a suitable mice models. Overall, automated fluorine-18 radiolabeling procedure via "click-chemistry" was satisfactory, allowing its application to the design of new potentially interesting biomolecules.

EXPERIMENTAL SECTION

Material and Methods

All solvents and reagents were purchased from Sigma-Aldrich, 1 Chem LP and Alfa Aesar.
TLC analyses were performed on silica gel 60 F254 pre-coated plates (Merck) by detection with a 5% phosphomolybdic acid solution in ethanol or 10% ninhydrin in butanol, and heating at 110°C.
Mass spectra were acquired using Impact HD™ UHR-QqToF (Bruker Daltonics, Germany) mass spectrometer and by AmaZon ETD (Bruker Daltonics) ion trap mass spectrometer. Samples were solubilized in methanol and then infused in ESI source at a flow rate of 3 µL/min.
Concerning ion trap instrument, ESI source was used with the following setting: capillary -4500V, end plate offset -500V, 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.
For UHR-QqToF spectrometer, parameters for the ESI source were set as follow : capillary 4000 V, end plate offset 500 V, nebulizer 0.3 bar, dry gas 4 l/min at 200 °C. Ions from the source were detected in a mass range from 50 to 3000 m/z. Mass Spectrometer the following tuning was applied: Funnel 1 radiofrequency (RF) of 400 Vpp, Funnel 2 RF of 400 Vpp, Hexapole RF of 400 Vpp and a prepulse of 12 µs.
Fragmentation spectra for both the instrumentations 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™ 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 $CDCl_3$, CD_3OD or d-6 DMSO (isotopic enrichment 99.95%) solution and the chemical shifts were reported on a δ (ppm) scale. Data were collected and processed by XWIN-NMR software (Bruker) running on a PC with Microsoft Windows 7. The samples were dissolved in the appropriate solvent 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 10 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. 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, CD_3OD 3.31 ppm and d-6 DMSO 2.50 ppm;^{13}C NMR: $CDCl_3$ 77.16 ppm (central line), CD_3OD 49.00 ppm and d-6 DMSO 39.52 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, HMBC) 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 system (Trasis-AllinOne) located in a suitably shielded hot cell (MIP-2, Comecer).
Sep-Pak Light Waters Accel Plus QMA and SepPak tC18 cartridges were from Waters Corp.
Radiolabeled 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. Semi-preparative 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 or on a Knauer P4.1S pump and a TOYDAD400 2Ch UV detector, connected to the automated module for radiosynthesis. 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

(S)-2-[(Imidazole-1-carbonyl)amino]pentanedioic acid di-*tert*-butyl ester (1)
Under anhydrous conditions, to a suspension of commercial L-di-*tert*-butyl glutamate hydrochloride (1 g, 3.38 mmol) in CH_2Cl_2 (DCM) (9 ml), cooled to 0°C, trimethylamine (TEA) (1.17 ml, 8.45 mmol) and 4-dimethylaminopyridine (DMAP) (15.16 mg, 0.14 mmol) were added. The mixture was stirred for 5 minutes, carbonyldiimidazole (CDI) was added (0.603 g, 3.718 mmol) and the reaction mixture was stirred overnight at room temperature. The reaction progress was monitored by TLC (DCM/MeOH 9:1). The reaction mixture was diluted with DCM (20 ml) and the obtained solution was washed with saturated aqueous sodium bicarbonate (20 ml), water (2 x 20 ml) and brine (20 ml). The organic phase was dried over sodium sulfate, filtered and concentrated at reduced pressure. The product was obtained as a semisolid without purification and used in the next step (1.1 g, 3.11 mmol, 92%).
R_f: 0.63.

^{1}H-NMR, ^{13}C-NMR and MS spectra are in agreement with the reported ones.[32]

(S)-2-[3((S)-(5-Benzyloxycarbonylamino)-1-*tert*-butoxycarbonylpentylureido]pentanedioic acid di-*tert*-butyl ester (2)
Under anhydrous conditions, to a solution of **1** (520 mg, 1.47 mmol) in dichloroethane (DCE) (10 ml) at 0°C, methyl trifluoromethanesulfonate (MeOTf) (0.16 ml, 1.47 mmol) and TEA (0.4

ml, 2.942 mmol) were added. The solution was stirred for 30 min, commercial Cbz-Lys-Ot-Bu (548.4 mg, 1.47 mmol) was added and the solution was heated at 40 °C. The reaction progress was monitored by TLC (DCM/MeOH 9.5:0.5). After 2 h, the reaction mixture was concentrated under reduced pressure and the crude product was purified by silica gel column chromatography (eluents: gradient of DCM/acetone). The desired compound was obtained as a colorless semisolid (733.7 mg, 1.18 mmol, 80%).
R_f: 0.41

^{1}H-NMR, ^{13}C-NMR and MS spectra are in agreement with the reported ones.[32]

(S)-2-[3-(5-Amino-1-*tert*-butoxycarbonylpentyl) ureido]pentanedioic acid di-*tert*-butyl ester (3)

To a solution of compound **2** (733.7 mg, 1.18 mmol) in EtOH (10 ml) ammonium formate was added (1.12 g, 17.7 mmol) followed by 5% Pd-C (240 mg). The suspension was stirred at room temperature and the reaction progress was monitored by TLC (DCM/MeOH 9:1). After 5 h the reaction was filtered through a Celite pad and solvent was evaporated at reduced pressure. The resulting crude semisolid product was diluted with a sodium hydrogen carbonate saturated aqueous solution (30 ml) and extracted with DCM (3 x 30 ml). The collected organic layers were washed with brine (30 ml) and evaporated to afford the desired product as a yellow semisolid (570 mg, 1.17 mmol, 99 %).

^{1}H-NMR, ^{13}C-NMR and MS spectra are in agreement with the reported ones.[32]

(S)-ethyl-2-amino-3-naphtyl propanoate (4)

A solution of commercial (S)- naphtyl alanine (1 g, 4.65 mmol) in EtOH (20 ml) was brought to -5 °C. Thionyl chloride was added (0.54 ml, 7.43 mmol) and the mixture was heated to reflux for 3 h. The reaction progress was monitored by TLC (DCM/MeOH 9.5:0.5). The mixture was evaporated at reduced pressure and the desired product was obtained as a brown liquid (1.1 g, 4.52 mmol, 97%).
R_f: 0.36
^{1}H-NMR (CD_3OD): δ 1.13 (t, 3H), 3.07 (m, 1H), 3.14 (m, 1H), 3.77 (t, 1H), 4.10 (q, 2H), 7.34 (dd, 1H), 7.45 (m, 2H), 7.66 (s, 1H), 7.80 (m, 3H).
^{13}C-NMR (CD_3OD): δ 13.01, 40.65, 55.30, 60.60, 125.28, 125.76, 127.10, 127.19, 127.23, 127.64, 127.78, 132.54, 133.58, 134.52, 174.47.
MS (m/z) (ESI+) 244.1 $[M+H]^+$.

***Trans*-4-[(benzyloxycarbonyl)amino]methyl cyclohexane acid (5)**

To a solution of commercial *trans*-4-(aminomethyl)cyclohexane acid (1g, 6.4 mmol) in THF/H_2O (2:1, 30 ml) benzyl chloroformate (Cbz-Cl) (1.31 ml, 1.5 mmol) and 2M NaOH (9 ml) were added. The reaction mixture was stirred overnight at room temperature and the reaction progress was monitored by TLC (DCM/MeOH, 9:1). Then, the mixture was quenched with 1M HCl (30 ml). The aqueous phase was extracted with EtOAc (3 x 30 ml). The collected organic phases were washed with brine (30 ml), dried over sodium sulfate and the solvent was removed at reduced pressure. The crude product was finally purified by silica gel column

chromatography (eluents: gradient of DCM/acetone) to afford the pure **5** as a white solid (1.79 g, 6.17 mmol, 96%).
R_f: 0.54

^{1}H-NMR, ^{13}C-NMR and MS spectra are in agreement with the reported ones.[37]

(S)-ethyl [2-trans-4- (((benzyloxycarbonyl)amino)methyl)cycloesanecarboxyamido]-3-naphtyl propanoate (6)

Under anhydrous conditions, a solution of compound **4** (747 mg, 3.07 mmol), compound **5** (895 mg, 3.07 mmol) and TEA (1.06 ml, 7.68 mmol) in DMF (10 ml) was cooled to 0°C. (1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (1.29 g, 3.38 mmol) was added dropwise over 5 min and the mixture was stirred at room temperature for 5 h. The reaction progress was monitored by TLC (DCM/MeOH, 9.5:0.5) until the starting material disappeared. The reaction mixture in DMF was dripped in H_2O (100 ml) and extracted with EtOAc (3 x 50 ml). The collected organic phases were washed with brine (50 ml), dried over sodium sulfate and the solvent was removed at reduced pressure. The crude mixture was finally purified by silica gel column chromatography (eluents: gradient of DCM/acetone) to afford the desired product **6** as a pale-yellow solid (1.42 g, 2.75 mmol, 90%).
R_f: 0.5
^{1}H-NMR ($CDCl_3$): δ 0.92 (q, 2H), 1.25 (t, 3H), 1.42 (q, 3H), 1.84 (m, 4H), 2.01 (m, 1H), 3.03 (m, 2H), 3.29 (dm, 2H), 4.19 (q, 2H), 4.93 (q, 1H), 5.09 (s, 2H), 5.94 (d, 1H), 7.22 (dd, 1H), 7.33- 7.35 (m, 5H), 7.46 (m, 2H), 7.54 (s, 1H), 7.76 (m, 2H), 7.81 (m, 1H).
^{13}C-NMR ($CDCl_3$): δ 14.16, 28.68, 28.98, 29.61, 29.70, 37.60,37.97, 45.14, 47.01, 52.87, 61.60, 66.69, 125.77, 126.23, 127.43, 127.29, 127.69, 128.14, 128.16, 128.18, 128.54, 132.48, 133.36, 133.51, 136.59, 156.54, 171.75, 175.18.
MS (m/z) (ESI+) 517.1 $[M+H]^+$, 539.1 $[M+Na]^+$.

(S) [2-trans-4- (((benzyloxycarbonyl)amino)methyl)cycloesanecarboxyamido]-3-(naphtyl-2-methyl) propanoic acid (7)

A solution of compound **6** (674 mg, 1.3 mmol) in THF/H_2O (1:1, 12 ml) was cooled at 0°C. A solution of 1M LiOH·H_2O (4.5 ml, 4.6 mmol) was added dropwise. The mixture was stirred at 0°C for 2 h monitoring the reaction progress by TLC (DCM/MeOH, 9.5:0.5). Then, the solvent was evaporated at reduced pressure. Crude mixture was diluted with water (20 ml) and pH was brought to 1 by dripping 1M HCl. The solution was extracted by EtOAc (3 x 20 ml). Collected organic phases were washed with brine (20 ml) and dried over sodium sulfate. Finally, the solvent was removed affording the desired product as a white solid (605 mg, 1.24 mmol, 95%).
^{1}H-NMR (DMSO-d_6): δ 0.80 (m, 2H), 1.08 (m, 1H), 1.23 (m, 2H), 1.49 (m, 1H), 1.62 (m, 3H), 2.03 (m, 1H), 2.80 (m, 2H), 3.01 (m, 1H), 3.20 (m, 1H), 4.49 (m, 1H), 4.98 (s, 2H), 7.19 (m, 1H), 7.31-7.38 (m, 6H), 7.45 (m, 2H), 7.68 (s, 1H), 7.82 (m, 3H), 8.01 (d, 1H), 12.65 (br, 1H).
^{13}C-NMR (DMSO-d_6): δ 28.91, 29.06, 29.85, 29.92, 37.37, 37.72, 44.09, 46.98, 53.56, 65.57, 125.90, 126.44, 127.53, 127.80, 127.88, 127.93, 128.16, 128.19, 128.79, 128.87, 132.29, 133.39, 135.99, 137.79, 156.72, 173.65, 175.49.
MS (m/z) (ESI+) 511.1 $[M+Na]^+$.

(3S,10S,14S)-tri-*tert*-butyl 1- [trans-4-(((benzyloxycarbonyl)amino)methyl)cycloesanecarboxyamido]-3-(naphtyl-2-methyl)-1,4,12-trioxo-2,5,11,13-tetraazahexadecane-10,14,16-tricarboxylate (8)

Under anhydrous conditions, a solution of compound **3** (970 mg, 1.99 mmol), compound **7** (971 mg, 1.99 mmol) and TEA (0.69 ml, 4.98 mmol) in DMF (10 ml) was cooled to 0°C. HATU (832 mg, 2.19 mmol) was added dropwise over 5 min and the mixture was stirred overnight at room temperature. Reaction was monitored by TLC (DCM/MeOH, 9.5:0.5). After the reaction was complete, the mixture in DMF was dripped into H_2O (100 ml) and the solution was extracted with EtOAc (3 x 50 ml). Collected organic phases were washed with brine (50 ml), dried over sodium sulfate and the solvent was removed at reduced pressure. The crude reaction mixture was purified by silica gel column chromatography (eluents: gradient of DCM/acetone) to afford the desired product as a pale-yellow solid (1.8 g, 1.88 mmol, 94%).

R_f: 0.31

^{1}H-NMR (DMSO-d_6): δ 0.78 (m, 2H), 1.01 (m, 1H), 1.20 (m, 4H), 1.30 (m, 2H), 1.37 (s, 27H), 1.41-1.57 (m, 4H), 1.63 (m, 3H), 1.85 (m, 1H), 2.04 (t, 1H), 2.21 (m, 2H), 2.79 (t, 2H), 2.93 (m, 2H), 3.06 (m, 2H), 3.91 (m, 1H), 4.02 (m, 1H), 4.51 (m, 1H), 4.97 (s, 2H), 6.22 (d, 1H), 6.27 (d, 1H), 7.18 (t, 1H), 7.31 (m, 5H), 7.37 (m, 1H), 7.43 (m, 2H), 7.66 (s, 1H), 7.77 (m, 2H), 7.83 (d, 1H), 7.89 (m, 2H).

^{13}C-NMR (DMSO-d_6): δ 22.88, 27.30, 28.10, 28.13, 28.20, 28.79, 29.09, 29.21, 29.84, 29.99, 31.36, 32.09, 37.72, 38.64, 38.74, 44.15, 46.99, 52.65, 53.50, 54.16, 65.56, 80.18, 80.70, 81.03, 125.75, 126.33, 127.71, 127.73, 127.87, 127.89, 128.14, 128.17, 128.35, 128.77, 132.23, 133.35, 136.20, 137.78, 156.70, 157.57, 171.44, 171.87, 172.31. 172.71, 175.34.

MS (m/z) (ESI+) 980.6 $[M+Na]^+$, 996.0 $[M+K]^+$.

(3S,10S,14S)-tri-*tert*-butyl 1- [trans-4- (aminomethyl)cycloesanecarboxyamido]-3-(naphtyl-2-methyl)-1,4,12-trioxo-2,5,11,13-tetraazahexadecane-10,14,16-tricarboxylate (9)

To a solution of compound **8** (500 mg, 0.52 mmol) in MeOH/THF (1:1, 10 ml) ammonium formate (329 mg, 5.21 mmol) was added followed by 5% Pd-C (72.5 mg). The suspension was stirred overnight at room temperature and the reaction progress was monitored by TLC (DCM/MeOH 9:1). Then, the mixture was filtered through Celite pad and the solvent was evaporated at reduced pressure. The resulting crude semisolid was diluted in sodium hydrogen carbonate saturated solution (15 ml) and the obtained solution was extracted with DCM (3 x 20 ml). The collected organic phases were washed with brine (20 ml) and concentrated in order to afford the desired product as yellow oil (390 mg, 0.47 mmol, 91 %).

^{1}H-NMR (DMSO-d_6): δ 0.75 (m, 2H), 1.04 (m, 2H), 1.19 (m, 4 H), 1.28 (m, 1H), 1.36 (s, 27H), 1.43-1.56 (m, 3H), 1.57-1.75 (m, 4H), 1.85 (m, 1H), 2.05 (m, 1H), 2.20 (m, 2H), 2.28 (d, 2H), 2.93 (m, 2H), 3.06 (m, 2H), 3.91 (q, 1H), 4.04 (q, 1H), 4.52 (m, 1H), 6.24 (d, 1H), 6.29 (d, 1H), 7.37 (m, 1H), 7.43 (m, 2H), 7.66 (s, 1H), 7.77 (m, 2H), 7.83 (d, 1H), 7.91 (m, 2H).

^{13}C-NMR (DMSO-d_6): δ 22.88, 28.10, 28.13, 28.19, 28.84, 28.93, 29.09, 29.33, 29.49, 29.89, 30.03, 31.36, 32.09, 38.64, 38.73, 44.32, 48.14, 52.65, 53.50, 54.15, 80.18, 80.69, 81.03, 125.74, 126.33, 127.71, 127.72, 127.86, 127.89, 128.34, 132.23, 133.35, 136.20, 157.56, 171.44, 171.86, 172.31, 172.71, 175.43.

MS (m/z) (ESI+) 824.5 $[M+H]^+$, 846.5 $[M+Na]^+$.

(3S,10S,14S)-tri-*tert*-butyl 1- [trans-4- (hex-5-yn-aminomethyl)cycloesanecarboxyamido]-3-(naphtyl-2-methyl)-1,4,12-trioxo-2,5,11,13-tetraazahexadecane-10,14,16-tricarboxylate (10)

Under anhydrous conditions, a solution of compound **9** (470 mg, 0.57 mmol), 5-hexynoic acid (0.06 ml, 0.57 mmol) and TEA (0.197 ml, 1.43 mmol) in DMF (10 ml) was cooled to 0°C. HATU (238 mg, 0.63 mmol) was added dropwise over 5 min and the mixture was stirred 3h at room temperature. The reaction progress was monitored by TLC (DCM/MeOH, 9.5:0.5) until starting material disappearance. The mixture was dripped in H_2O (100 ml) and extracted with EtOAc (3 x 30 ml). Collected organic phases were washed with brine (50 ml), dried over sodium sulfate and solvent was removed at reduced pressure. The crude product was finally purified by silica gel column chromatography (eluents: gradient of DCM/acetone) to afford the desired product as a pale-yellow solid (465 mg, 0.51 mmol, 89%).

R_f: 0.48

^{1}H-NMR (DMSO-d_6): δ 0.6 (m, 2H), 1.1 (m, 1H), 1.20 (m, 5H), 1.32 (m, 2H), 1.40 (s, 27H), 1.5 (m, 3H), 1.65 (m, 5H), 1.82 (m, 1H), 2.05 (m, 1H), 2.10 (m, 4H), 2.20 (m, 2H), 2.75 (t, 1H), 2.83 (t, 2H), 2.91 (m, 2H), 3.05 (m, 2H), 3.89 (m, 1H), 4.05 (m, 1H), 4.52 (m, 1H), 6.21 (d, 1H), 6.27 (d, 1H), 7.37 (m, 1H), 7.43 (m, 2H), 7.66 (s, 1H), 7.71 (t, 1H), 7.77 (m, 2H), 7.83 (m, 1H), 7.89 (m, 2H).

^{13}C-NMR (DMSO-d_6): δ 17.88, 22.88, 24.84, 28.11, 28.14, 28.21, 28.83, 29.09, 29.24, 29.99, 30.14, 31.37, 32.11, 34.63, 37.46, 38.63, 38.75, 44.16, 45.09, 52.66, 53.50, 54.13, 71.84, 80.20, 80.72, 81.05, 84.55, 125.75, 126.34, 127.71, 127.74, 127.87, 127.89, 128.34, 132.24, 133.35, 136.19, 157.55, 171.41, 171.76, 171.86, 172.30, 172.70, 175.35.

MS (m/z) (ESI+) 940.9 $[M+Na]^+$.

(3S,10S,14S)-1- [trans-4- (hex-5-yn-aminomethyl)cycloesanecarboxyamido]-3-(naphtyl-2-methyl)-1,4,12-trioxo-2,5,11,13-tetraazahexadecane-10,14,16-tricarboxylic acid (11)

Under anhydrous conditions, compound **10** (240 mg, 0.261 mmol) was dissolved in DCM (5 ml). Trifluoroacetic acid (TFA) was added dropwise in 2 h (0.32 ml). The solution was stirred for 2 days and the reaction progress was monitored by TLC (DCM/MeOH, 9.5:0.5). Upon reaction completion, the solvent was removed at reduced pressure. The crude mixture was diluted with sodium hydrogen carbonate saturated solution (10 ml) and the solution was extracted with EtOAc (3 x 5 ml). Aqueous phase was acidified to pH 4 with 1M HCl: The precipitate crude white solid (190 mg) was recovered by suction and dried.

Crude mixture was purified by semi-preparative HPLC. Pure precursor **11** was obtained as a white solid (67 mg, 0.09 mmol, 34%).

Analytical RP-HPLC condition: XTerra C18 5µm, 250x4.6 mm; water +0.1%TFA/acetonitrile +0.1%TFA gradient from 70:30 to 50:50 in 20 min, gradient from 50:50 to 20:80 in 2 min; 1 ml/min, 220 nm, UV detector.

R_t: 11.20 min.

Semi-preparative RP-HPLC condition: column Clarity Oligo RP 5µm, 250x10 mm; isocratic 60:40 water +0.1%TFA/acetonitrile +0.1%TFA; 6 ml/min, 220 nm, UV detector.

R_t: 6 min

^{1}H-NMR (DMSO-d_6): δ 0.77 (m, 2H), 1.01 (m, 1H), 1.21 (m, 4H), 1.30 (m, 2H), 1.45 (m, 2H), 1.51-1.66 (m, 6H), 1.70-1.87 (br, 2H), 2.04 (m, 1H), 2.12 (m, 4H), 2.22 (br, 2H), 2.76 (t, 1H),

2.83 (t, 2H), 2.89-3.11 (m, 4H), 3.99 (m, 1H), 4.05 (m, 1H), 4.50 (m, 1H), 6.29 (br, 2H), 7.37 (m, 1H), 7.43 (m, 2H), 7.66 (s, 1H), 7.77 (m, 1H), 7.77 (m, 2H), 7.83 (m, 1H), 7.93 (m, 2H).
^{13}C-NMR (DMSO-d_6): δ 17.87, 23.03, 24.83, 28.57, 28.80, 29.18, 29.23, 29.98, 30.14, 31.17, 32.18, 34.61, 37.44, 38.61, 38.83, 44.14, 45.09, 52.48, 52.84, 54.15, 71.87, 84.57, 125.76, 126.34, 127.72, 127.73, 127.88, 128.36, 132.22, 133.34, 136.23, 157.72, 171.45, 171.77, 174.36, 174.69, 175.09, 175.36.
MS (m/z) (ESI+) 772.4 $[M+Na]^+$.

(3S,10S,14S)-1- [trans-4-((4-(1-(4-((2-(2-(2-fluoroethoxy)ethoxy)ethoxy)methyl)benzyl)-1H-1,2,3-triazol-4-yl)butanamido)methyl)cyclohexyl]-3-(naphtyl-2-methyl)-1,4,12-trioxo-2,5,11,13-tetraazahexadecane-10,14,16-tricarboxylic acid (14)

To a solution of compound **13** in acetonitrile (2.5 mg, 0.0084 mmol, 100µl), compound **11** in MeOH (7.25 mg, 0.0097 mmol, 200 µl) and sodium L-ascorbate in H_2O (9.03 mg, 0.046 mmol, 100 µl), a solution of copper sulfate pentahydrate in H_2O was added (4.05 mg, 0.016 mmol, 100 µl). Mixture was stirred at room temperature for 20 min and the reaction progress was monitored by analytical RP-HPLC. The reaction was quenched dripping 1M HCl (0.1 ml) and the formed precipitate was dissolved by addition of acetonitrile (400µl). The pure compound **14** was obtained as a white solid after final semi-preparative HPLC purification (4.5 mg, 0.004 mmol, 45%).
Analytical RP-HPLC condition: XTerra C18 5µm, 250x4.6 mm; water +0.1%TFA/acetonitrile +0.1%TFA gradient from 70:30 to 20:80 in 25 min; 1 ml/min, 220 nm, UV detector.
R_t: 10.4 min
Semi-preparative RP-HPLC condition: Clarity Oligo-RP 5µm, 250x10 mm; water +0.1%TFA/acetonitrile +0.1%TFA gradient from 55:45 to 50:50 in 25 min; 6 ml/min, 220 nm, UV detector.
R_t: 12.5 min
^{1}H-NMR (DMSO-d_6): δ 0.80 (m, 2H), 1.03 (m, 1H), 1.13-1.28 (m, 4H), 1.32 (m, 2H), 1.48 (m, 2H), 1.55-1.69 (m, 4H), 1.78 (m, 3H), 1.86 (m, 1H), 2.10 (m, 3H), 2.25 (m, 2H), 2.57 (t, 2H), 2.85 (t, 2H), 2.89-3.15 (m, 4H), 3.55 (m, 8H), 3.61 (t, 1H), 3.67 (t, 1H), 4.02 (m, 1H), 4.09 (m, 1H), 4.46 (t, 1H), 4.48 (s, 2H), 4.50-4.57 (m, 2H), 5.53 (s, 2H), 6.31 (br, 2H), 7.27 (d, 2H), 7.32 (d, 2H), 7.40 (m, 1H), 7.45 (m, 3H), 7.70 (m, 2H), 7.79 (m, 2H), 7.83-7.90 (m, 1H), 12.08-12.98 (br, 3H).
^{13}C-NMR (DMSO-d_6): δ 23.02, 25.16, 25.74, 28.35, 28.81, 29.17, 29.24, 30.01, 30.16, 30.88, 32.17, 35.34, 37.45, 38.62, 38.83, 44.16, 45.10, 52.30, 52.77, 52.92, 54.14, 69.63, 70.06, 70.22, 70.29, 72.07, 82.82, 84.14, 122.42, 125.74, 126.33, 127.71, 127.73, 127.87, 128.24, 128.35, 132.22, 133.34, 135.76, 136.22, 138.89, 147.29, 157.72, 171.43, 172.07, 174.25, 175.01, 175.35.
MS (m/z) (ESI+) 1047.5161 $[M+H]^+$.

Radiochemistry

1-(azidomethyl)-4-4((2-[2-(2-[^{18}F]fluoroethoxy)ethoxy]ethoxy)methyl)benzene ([^{18}F]13)

24-82 GBq of [^{18}F]fluoride solution in [^{18}O]H_2O produced by cyclotron were passed through a Sep-Pak light QMA cartridge. A solution of potassium carbonate (2.51 mg, 0.018 mmol) in water (0.5 ml) was then passed through the cartridge eluting 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 1 ml acetonitrile was added and the solvent was azeotropically distilled at 85°C at reduced pressure. Then, the reaction vial was cooled down at 60°C and solution of compound **12** (10 mg, 0.025 mmol) in anhydrous acetonitrile (1 ml) was added. The reaction mixture was stirred 20 min at 100°C. Then, the reaction vial was cooled down at 50°C. Water (9 ml) was added and the mixture was passed through a tC18 Sep-Pak Plus cartridge previously conditioned with 10 ml ethanol and 10 ml water. The cartridge was washed with 10 ml water/acetonitrile 80:20 and the product **[^{18}F]13** was obtained by eluting the cartridge with acetonitrile (1 ml) into the final container. The average obtained final RCY not corrected for decay was in the range of 25-36%, >99% radiochemical purity. Preparation time was 58 min.

Analytical RP-HPLC condition: XTerra C18 5µm, 250x4.6 mm; water/acetonitrile gradient from 60:40 to 20:80 in 20 min; 1 ml/min, 220 nm, UV detector.

R_t: 10.20 min

(3S,10S,14S)-1- [trans-4-((4-(1-(4-((2-(2-(2-[^{18}F]fluoroethoxy)ethoxy)ethoxy)methyl)benzyl)-1H-1,2,3-triazol-4-yl)butanamido)methyl)cyclohexyl]-3-(naphtyl-2-methyl)-1,4,12-trioxo-2,5,11,13-tetraazahexadecane-10,14,16-tricarboxylic acid ([^{18}F]14)

Starting from ~198 GBq of [^{18}F] fluorine produced by cyclotron, **[^{18}F]13** was synthetized as previously described and passed through tC18 Sep-Pak Plus cartridge. The product **[^{18}F]13** was obtained eluting the cartridge with acetonitrile (1 ml) into the second reactor. Acetonitrile was removed heating up to 85°C for 8 min, then reactor was cooled down to 30°C. A solution of compound **11** (7.5 mg, 0.01 mmol in 0.2 ml of 0.5M aqueous NH_3 solution) and freshly-prepared sodium ascorbate (9.11 mg, 0.046 mmol in 0.1 ml of 0.5M aqueous NH_3 solution) was added, followed by copper(II) sulfate pentahydrate in water (3.99 mg, 0.016 mmol, 0.1 ml). The reaction mixture was stirred for 20 min at room temperature. The reaction was quenched with 1M HCl (1 ml) and eluted with of water /acetonitrile +0.1%TFA 70:30 (8 ml). Then, in order to purify product **[^{18}F]14**, the mixture was submitted to semi-preparative RP-HPLC. The collected fraction was diluted with water (10 ml) and passed through a tC18 Sep-Pak Plus cartridge previously conditioned with ethanol (10 ml) and water (10 ml). The cartridge was washed with water (10 ml) and the final product was obtained by elution with EtOH (1 ml) and saline physiological solution (20 ml) into the final container.

The final activity was 12.1 GBq (6.1% RCY not decay correct, >99% radiochemical purity). Total synthesis time was 112 min (Molar activity >650 GBq/µmol).

Semi-preparative RP-HPLC condition: column Clarity Oligo-RP 5µm, 250x10 mm; water +0.1%TFA/acetonitrile +0.1%TFA gradient from 70:30 to 60:40 in 20 min, isocratic 60:40 for 5 min, gradient from 60:40 to 20:80 in 2 min; 6 ml/min, 220 nm, UV detector.

R_t: 17 min
Analytical RP-HPLC condition: column XTerra C18 5µm, 250x4.6 mm; water +0.1%TFA/acetonitrile +0.1%TFA gradient from 70:30 to 20:80 in 25 min; 1 ml/min, 220 nm, UV detector.
R_t: 10.40 min

DATA AVAILABILITY STATEMENT

Data are contained within the article or Supporting Information.

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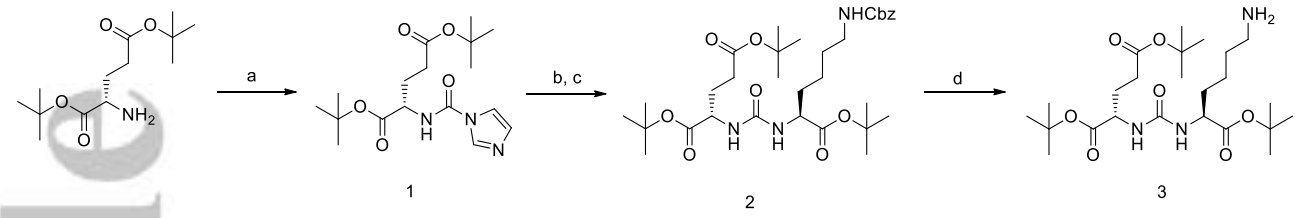
SUPPORTING INFORMATION

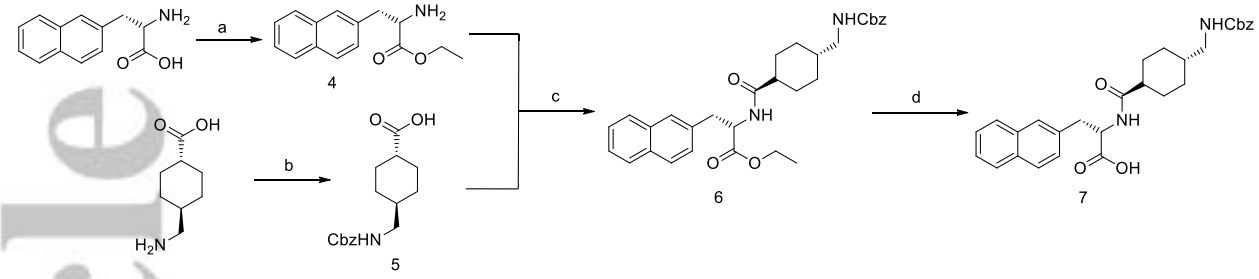
Additional Supporting Information (S1-S55) may be found in the online version of the article at the publisher's website.

Table 1: *The effect of solvent and reaction time on* ***[^{18}F]13*** *to* ***[^{18}F]14*** *conversion rate.*

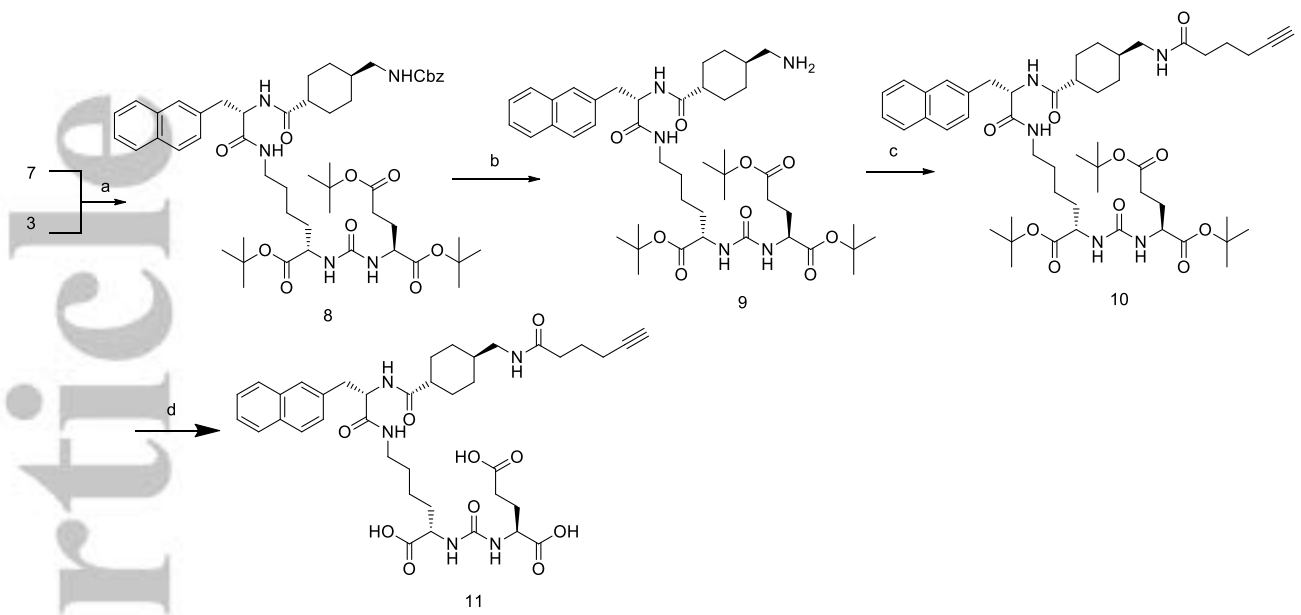
Solvent of precursor 11 (Solvent 1)	**Solvent of sodium ascorbate (Solvent 2)**	**Solvent of copper (II) sulfate pentahydrate (Solvent 3)**	**Reaction time**	**Conversion**
400µl DMSO	100µl H_2O	100µl H_2O	20 min	32%
200µl DMSO	100µl H_2O	200µl H_2O	20 min	40%
300µl MeOH	100µl H_2O	100µl H_2O	20 min	42%
200µl MeOH	100µl H_2O	100µl H_2O	20 min	13%
200µl MeOH	70µl MeOH + 30µl H_2O	100µl MeOH	20 min	1%
200µl 0.5M NH_3 in H_2O	100µl 0.5M NH_3 in H_2O	100µl H_2O	20 min	92%
200µl 0.5M NH_3 in H_2O	100µl 0.5M NH_3 in H_2O	100µl H_2O	10 min	80%



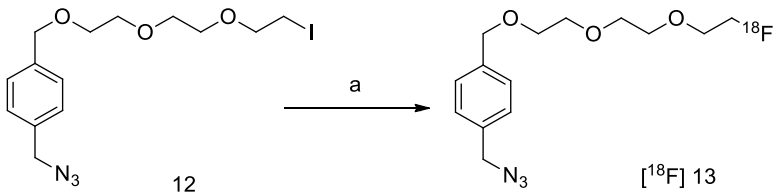
Scheme 1: *Synthesis of Glu-urea-Lys residue*. Reagent and conditions: (a) CDI, TEA, DMAP, DCM, rt, overnight (92%); (b) MeOTf, TEA, DCE, 0 °C; (c) Cbz-Lys-Ot-Bu, 40°C, 2h (80% from **1**); (d) $HCOO^{-}NH_4^{+}$, 5% Pd-C, EtOH, rt, 5h (99 %).



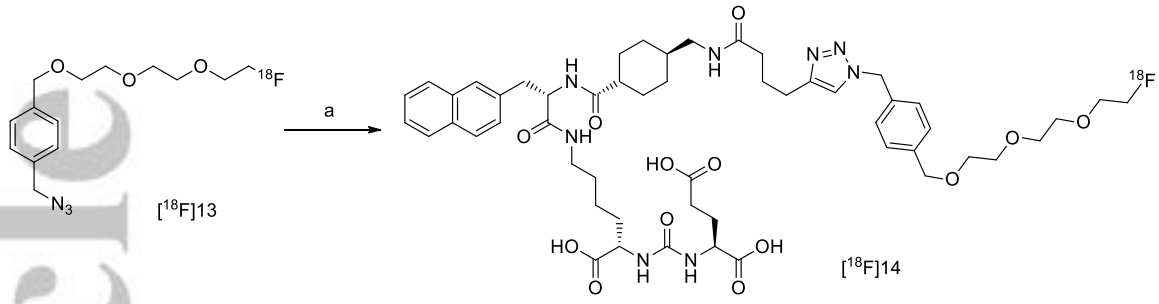
Scheme 2: *Synthesis of PSMA-617 specific linker*. Reagents and conditions: (a) $SOCl_2$, EtOH, 0°C-reflux, 3h (97 %); (b) Cbz-Cl, 2M NaOH, THF/H_2O, rt, overnight, (96 %); (c) HATU, TEA, DMF, 0°C-rt, 5h, (90 %); (d) 1M LiOH·H_2O, MeOH/H_2O, 0°C, 2h, (95 %).



Scheme 3: *Preparation of precursor **11***. Reagents and conditions: (a) HATU, TEA, DMF, 0°C-rt, overnight, (94 %); (b) $HCOO^-NH_4^+$, 5% Pd-C, THF/MeOH, rt, overnight (91 %); (c) 5-hexynoic acid, HATU, TEA, DMF, 0°C-rt, 3h, (89 %); (d) TFA, DCM, rt, 2 days, (34 %).



Scheme 4: *Fluorination reaction*. Reagents and conditions: (a) $K^+[^{18}F]F^-$/Kryptofix 2.2.2, dry CH_3CN, 100°C, 20 min.



Scheme 5: *CuAAC reaction*. Reagent and conditions: (a) Precursor **11**, sodium ascorbate, $Cu(SO_4)\cdot 5\ H_2O$, 0.5 M NH_3 in H_2O, rt.

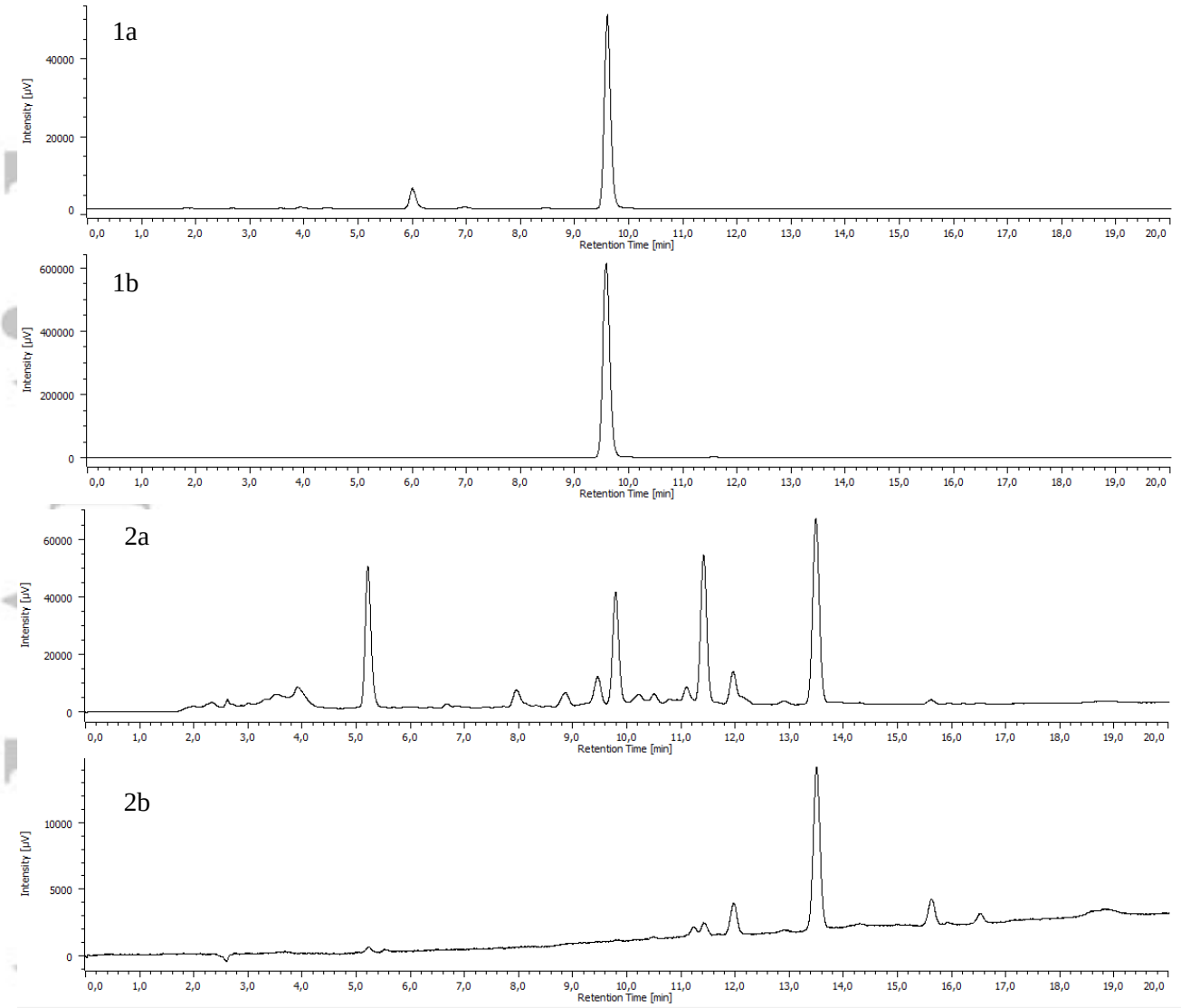


Figure 1: ***[^{18}F]13** purification.* 1a) Crude reaction mixture radiodetector chromatogram 1b) Radiodetector chromatogram after purification; 2a) Crude reaction mixture UV chromatogram 2b) UV chromatogram after purification. Rt **[^{18}F]13**: 9.6 min; Rt hydrolysis byproduct: 5.2 min; Rt elimination byproduct: 11.4 min; Rt compound **12**: 13.5 min.

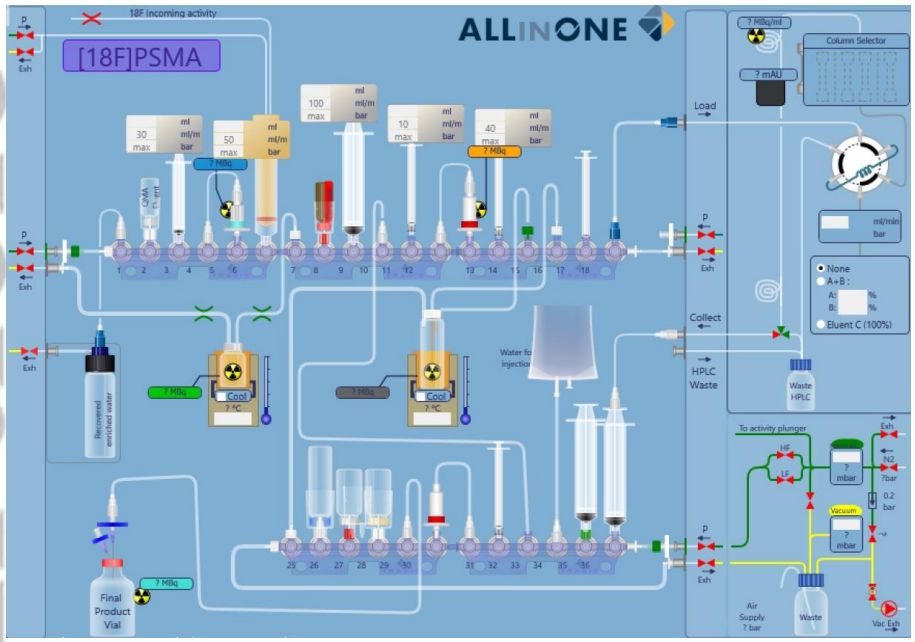


Figure 2: *Configuration of Trasis All-in-One automated system. Main reagent positions are indicated:*
P2: Kryptofyx 2.2.2 solution, P3: potassium carbonate solution, P8: compound ***12*** *in acetonitrile solution, P11: Precursor* ***11*** *+ sodium ascorbate in 0.5M NH_3 solution, P14: Copper sulfate in water solution, P17: HCl 1M, P32: EtOH, P35: saline physiological solution.*

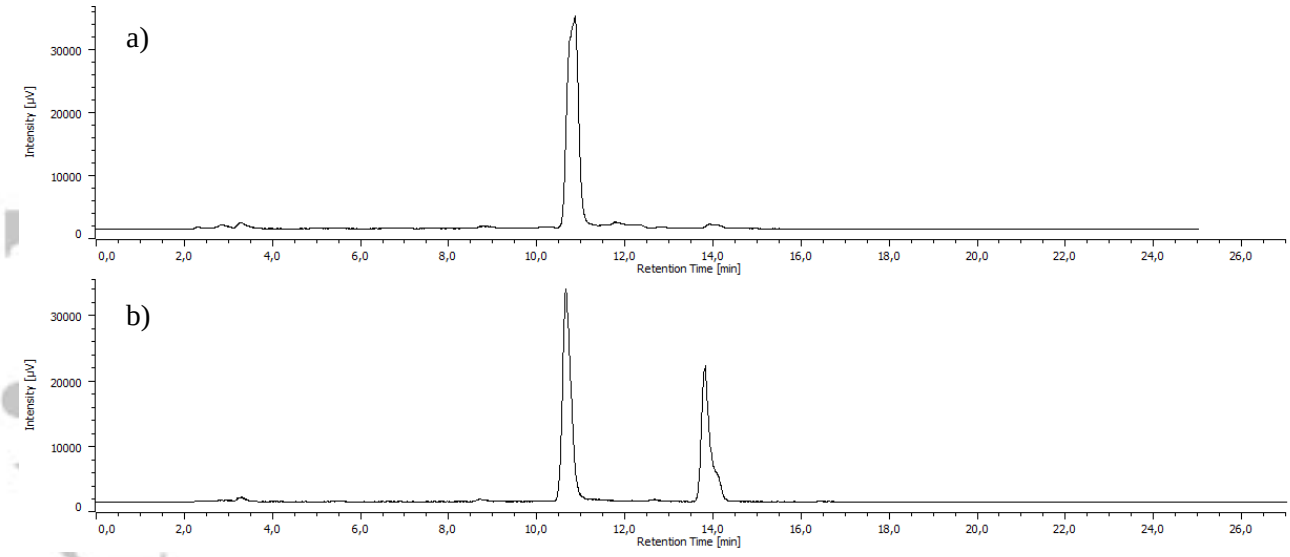


Figure 3: *[^{18}F]- CuAAC in aqueous 0.5 M NH_3 monitoring. Radioactivity detector*. (a) CuAAC reaction after 20 min. (b) CuAAC reaction after 10 min. Rt Product **[^{18}F]14**: 10.5 min; Rt Precursor **[^{18}F]13**: 13.7 min.

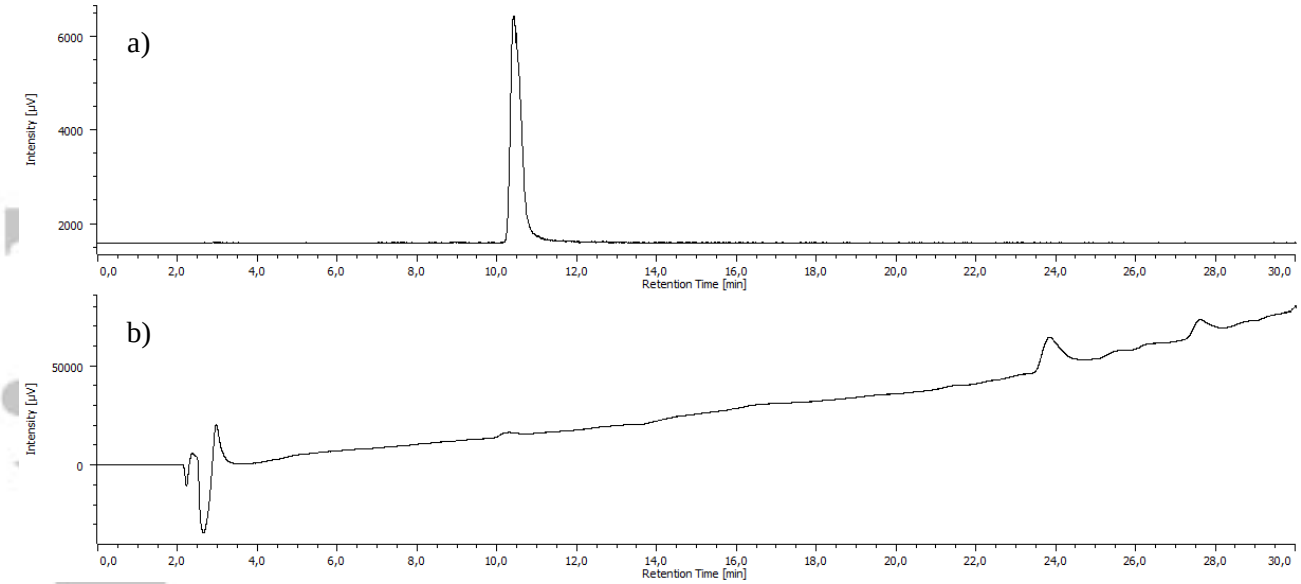


Figure 4: ***[^{18}F]14** purification.* (a) Radioactivity detector. (b) UV detector. Rt Product **[^{18}F]14**: 10.2 min.