An Automated and Putatively Versatile Approach for Labeling Peptides with Fluorine-18 Using the Fastlab™ Synthesizer; Exemplified with Glu-the CO-Lys Pharmacophore

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Abstract

Background: Noninvasive molecular imaging using peptides and biomolecules labelled with positron emitters has become important for detection of cancer and other diseases with PET (positron emission tomography). The positron emitting radionuclide fluorine-18 is widely available in high yield from cyclotrons and has favorable decay ($t_{1/2}$ 109.7 min) and imaging properties. ^{18}F -Labelling of biomolecules and peptides for use as radiotracers is customarily achieved in a two-step approach, which can be challenging to automate. 6- ^{18}F Fluoronicotinic acid 2,3,5,6-tetrafluorophenyl ester (^{18}F -Py-TFP) is a versatile ^{18}F -prosthetic group for this purpose, which can be rapidly be produced in an one-step approach on solid support. This work details an automated procedure on the cassette-based GE FASTlabTM platform for the labeling of a peptidomimetic, exemplified by the case of using the Glu-CO-Lys motif to produce ^{18}F DCFPyL, a ligand targeting the prostate specific membrane antigen (PSMA).

Results: From fluorine-18 delivery a fully automated two-step radiosynthesis of ^{18}F DCFPyL was completed in 56 min with an overall end of synthesis yield as high as 37% using SPE purification on the GE FASTlabTM platform.

Conclusions: Putatively, this radiolabeling methodology is inherently amenable to automation with a diverse set of synthesis modules, and it should generalize for production of a broad spectrum of biomolecule-based radiotracers for use in PET imaging.

Background

Cancer is the leading cause of premature deaths in the western world, a circumstance that has spurred the development of new radiopharmaceuticals for cancer diagnosis by positron emission tomography (PET) and for radiotherapy using α - and β -emitting radionuclides (Holland *et al.*, 2012; Zhang *et al.*, 2017). For use in clinical PET imaging, the radiohalogen fluorine-18 (^{18}F) is the preferred radioisotope due to its physical half-life (109.7 min), favorable decay properties, and its ready availability in multi-GBq quantities from medical cyclotrons. A considerable proportion of emerging radiopharmaceuticals are based on small to medium sized peptides, fusion proteins, and endogenous proteins. However, these products are not always obtainable through a direct radiolabeling approach due to their instability at high temperature or the otherwise harsh reaction conditions required to introduce fluorine-18 into their structure. Although labeling of such molecules with fluorine-18 using a chelator conjugated to the targeting moiety with ^{18}F AlF (aluminum ^{18}F fluoride) is feasible, this approach can release free ^{18}F fluoride *in vivo* (which invariably results in intense bone labelling) and otherwise alter the biodistribution properties of the radiopharmaceutical in an unpredictable fashion (Lütje *et al.*, 2019).

A robust and high yield radiosynthesis amenable to automation is a prerequisite for adopting a PET radiopharmaceutical for widespread clinical or research use (Li *et al.*, 2013). Production of PET radiopharmaceuticals with a multi-step synthetic process can be a challenging task using commercially produced cassette-based modules, mainly due to their lack of modifiability, and the frequent requirement

for intermediate or final HPLC purification. ^{18}F -labelled tracers for prostate specific membrane antigen (PSMA) $[^{18}\text{F}]\text{DCFPyL}$ and $[^{18}\text{F}]\text{-PSMA-1007}$ are examples of PET radiopharmaceuticals that formerly required a two-step radiosynthesis from the prosthetic group 6- $[^{18}\text{F}]\text{fluoronicotinic acid-2,3,5,6-tetrafluorophenyl ester}$ ($[^{18}\text{F}]\text{F-Py-TFP}$) (Chen *et al.*, 2011; Giesel *et al.*, 2017). In the case of $[^{18}\text{F}]\text{DCFPyL}$, the first reported radiosynthesis from $[^{18}\text{F}]\text{F-Py-TFP}$ and the PSMA-avid substrate Glu-CO-Lys protected with *t*-butyl has subsequently been optimized (Chen *et al.*, 2011). Currently, a direct precursor labeling with the trimethylammonium nicotinic acid moiety conjugated to the ϵ -nitrogen of the lysine residue of Glu-CO-Lys is the favored method for automated multi-dose production of $[^{18}\text{F}]\text{DCFPyL}$ (Dornan *et al.*, 2018), which has greatly facilitated the use of PSMA PET in clinical routine. However, this introduction of fluoride-18 in organic solvents at a temperature approaching 100°C , is unlikely to provide high labeling efficiencies, or to generalize for larger molecular weight radiopharmaceuticals such as polypeptides and proteins with their bountiful functional groups.

Fairly recently, a simplistic and rapid preparation of $[^{18}\text{F}]\text{F-Py-TFP}$ has been reported, wherein the prosthetic group is obtained directly by passing its precursor in a solution of acetonitrile/*tert*-butanol through a polymeric anion-exchange cartridge preloaded with $[^{18}\text{F}]\text{fluoride}$ (Basuli *et al.*, 2016; Olberg, 2017). This procedure yields the bifunctional labeling agent in good yield, with an overall synthesis time of less than ten minutes (EOB), simultaneously avoiding the use of a reaction vessel and a phase transfer catalysts. The on-column radiosynthesis of $[^{18}\text{F}]\text{F-Py-TFP}$ has a very small laboratory footprint and lends itself well for automation and offer radiolabeling of a wide variety of sensitive biomolecules (proteins eg.) (Roy *et al.*, 2019; Lesniak *et al.*, 2019). Expanding on this concept, we report a fully automated, two-step radiosynthesis of $[^{18}\text{F}]\text{DCFPyL}$ from $[^{18}\text{F}]\text{F-Py-TFP}$ and the Glu-CO-Lys pseudo-peptide precursor, which can serve as a blueprint manufacturing process for the preparation of other ^{18}F -labelled radiopharmaceuticals. The approach uses $[^{18}\text{F}]\text{F-Py-TFP}$ produced on column adopted into the commercially available, cassette-based synthesis module FASTlabTM. The automated process implements a hydrophilic interaction liquid chromatography (HILIC) SPE cartridge purification methodology, making final preparative HPLC purification redundant.

Materials And Methods

General

Chemicals were obtained either from Sigma-Aldrich (Norway) or from VWR (Norway), which were used without further purification. HPLC grade water was obtained from the Direct-Q® (EMD Millipore) water purification system. Reference standard 6-fluoronicotinic acid 2,3,5,6-tetrafluorophenyl ester (**1**) and its trimethylammonium precursor (**2**) were synthesized according to literature methods (Olberg *et al.*, 2010). DCFPyL (2-(3-(1-carboxy-5-[(6-fluoro-pyridine-3-carbonyl)-amino]-pentyl)-ureido)-pentanedioic acid) (**3**) reference standard was purchased from ABX GmbH (Radeberg, Germany). Glu-CO-Lys (2-[3-(5-Amino-1-carboxy-pentyl)-ureido]-pentanedioic acid trifluoroacetate salt) (**4**) was purchased from ABX GmbH or

Scintomics GmbH (Fürstfeldbruck, Germany). Sep-Pak™ cartridges were purchased from Waters (Norway). HILIC cartridges containing 350 mg of Chromabond® HILIC sorbent (Macherey-Nagel GmbH, Düren) and anion-exchange columns containing 45 mg of Chromabond® PS-HCO₃, (Macherey-Nagel GmbH) were obtained from Teknolab AS (Ski, Norway). Analytical HPLC was performed on an Agilent 1100 series HPLC system with UV detection connected in series with a NaI γ-radiodetector (Institute for Energy Technology (IFE), Kjeller, Norway). Samples were analyzed using methods **1** or **2a** and **b** as described below. Chromatograms were processed with Agilent OpenLAB CDS EZChrom version A.04.04. Reversed phased-analytical HPLC was performed on a Phenomenex kinetex EVO C18 column (50 × 2.1 mm, 2.6 μm particles), at a flow rate of 0.5 mL/min, with a composition gradient from 2-95% solvent B (method **1**) over ten min (solvent A: water/0.05% TFA, solvent B: acetonitrile/0.05% TFA). UV detection was at 214, 254 and 264 nm. Hydrophilic interaction liquid chromatography (HILIC) was performed on a SeQuant ZIC-cHILIC (100 × 4.6 mm, 3 μm particles) at a flow rate of 2.0 mL/min, with composition gradient from 2-98% solvent B (method **2a**) or isocratically with 68% solvent B (method **2b**) over ten min (Solvent A: 97% acetonitrile/3% 5 mM NH₄OAc, solvent B: 5 mM NH₄OAc). UV detection was at 210, 220, 254 and 264 nm. Thin layer chromatography (TLC) was run on gel 60F₂₅₄ plates (Merck) using acetonitrile as eluent. A Raytest miniGita (Raytest, Germany) equipped with a β-detector was used to record the radio-TLC scan. Gas chromatography (GC) was performed with an Agilent 6890N GC (Matriks, Norway) equipped with a flame ionization detector (FID), a column oven, and an auto-sampler with a direct injection system. The GC column was a fused silica capillary column with USP stationary phase G43 (6% cyanopropylphenyl - 94% dimethyl polysiloxane) measuring 0.32 mm i.d. x 30 m. Automated radiochemistry was performed on the GE FASTlab synthesizer with the custom-made single-use FASTlab development kit (GE Healthcare) placed inside a lead-shielded hot cell. Fluorine-18 was produced in a GE PETtrace 6 cyclotron (General Electric Healthcare) with a GE ¹⁸F Nb 27 self-shielded target either by irradiation (40 μAh × two min) of [¹⁸O]H₂O (Taiyo Nippon Sanso, Japan), or from a target rinse after a routine 100 GBq production. No more than 150 MBq of radioactivity were handled outside the hot cell. Radioactivity was measured with a Capintec dose calibrator (New Jersey, USA). The identities of the radiolabeled products were confirmed by co-injection to HPLC along with authentic nonradioactive standards.

General radiolabeling procedure (manual)

Aqueous [¹⁸F]fluoride was trapped on a Chromabond PS-HCO₃ anion cartridge followed by rinsing with hot acetonitrile (2 mL) and passage of air (2 × 5 mL) to dryness. A solution of precursor **2** (10 mg/mL, 22 mM), in the presence of TEA in *t*-BuOH/acetonitrile (50%, v/v, 1 mL) was used to initiate radiolabeling and to elute the radioactivity into a receiving vial (10 sec); the eluent was passed through the column back and forth into the syringe barrel for 20 seconds. After the final elution into the vial, the reaction was quenched by rinsing the column with water (0.5 mL). We measured the radioactivity concentration in the quenched reaction mixture before analysis by HPLC and TLC with on-line gamma detection.

General automated radiolabeling procedure

Cassettes were assembled according to the layout depicted in Fig. 2. External vials were vented with a syringe filter and connected to the cassette manifold with a silicone tube of specific length, terminating in a needle as follows:

- 30 mL vial of H_3PO_4 0.85%: 42-cm long tube, 0.80 i.d. $\times$ 80 mm needle (**4**)
- Collection vial: 13-cm long tube, 0.80 i.d. $\times$ 80 mm needle. This vial was changed during the synthesis from a 7 mL vial to collect the HILIC fly-through (3 mL) to a 20-mL vial to collect the Oasis HLB PRiME fly-through (5 mL) and washout (5 mL) (**13**)
- 25% ethanol vial: 13-cm long tube, 2.10 mm i.d. $\times$ 80 mm needle (**12**)

Vials were prepared as follows:

- Precursor vial: Precursor **2** (15 mg, 31.4 μmol) in *t*-BuOH/acetonitrile (1.4 mL) and triethylamine (TEA) (0.4 eq.) (**1**)
- Glu-CO-lys vial: Glu-CO-lys (13.2 mg, 29.1 μmol), TEA- HCO_3 (4.3 eq.) in 0.63 mL DMSO
- Acetonitrile vial: 4.0 mL
- Acetone and absolute ethanol vials: 4.6 mL each

Results And Discussion

From the get-go, reaction conditions were investigated manually, aiming to determine suitable and robust conditions for on-cartridge synthesis of $[\text{}^{18}\text{F}]\text{F-Py-TFP}$ ($[\text{}^{18}\text{F}]\textbf{1}$) for translation to the automated synthesis. In brief, fluorine-18 was trapped on the Chromabond PS- HCO_3 and then the column was dried with anhydrous acetonitrile. Fluorination occurred on-cartridge when the mixture of precursor **2** and base in acetonitrile/*t*-BuOH was cycled through the cartridge. The radiochemical yield is dependent of precursor concentration and the presence of an non-nucleophilic organic base helps to increase yield and reduce variation between synthesis runs.

RCYs improved when the Chromabond column was dried using acetonitrile heated to 80°C in the reactor vessel. The best RCY (78 $\pm$ 9%) was obtained using 11 mM of precursor and 0.2 equivalents of triethylamin (TEA) as the base. Translating these conditions (including SPE purification) into the FASTlab, $[\text{}^{18}\text{F}]\text{F-Py-TFP}$ was obtained with RCY of 63 $\pm$ 4% with TEA as the base. In our hands, yields were 15% lower using the automated FASTlab synthesis compared to manual synthesis. For the automated FASTlab $[\text{}^{18}\text{F}]\text{DCFPyL}$ synthesis, 22 mM of precursor **2** and 8 mM of TEA in 1.0 mL acetonitrile/*t*-BuOH (50/50 v/v) was used routinely through this work.

The automated radiosynthesis entailed three sequential processes, i.e a ten min pre-labelling step, a 25 min on-cartridge radiolabeling step for [^{18}F]F-Py-TFP (including purification), and a 31 min conjugation and purification step affording the final product [^{18}F]DCFPyL. Due to the inherent dead volumes, when implementing the radiochemistry to the FASTlab synthesizer, the reagent vials were filled with slightly larger volumes to retain the same stoichiometric amounts of reagents.

Pre-labelling

The vials, cartridges and reactor were conditioned for ten minutes before delivery of radioactivity. Vials were pressurized with 1 bar nitrogen to ensure optimal syringe filling. Ethanol and water were successively used to condition the tC18 Sep-Pak light cartridge. Acetonitrile (1.6 mL) was transferred into the FASTlab reactor and heated; the system was deemed ready to receive radioactivity when the reactor temperature reached 80°C.

Radiolabeling and purification of [^{18}F]F-Py-TFP

The next step provided purified [^{18}F]F-Py-TFP bound to the tC18 Sep-Pak light cartridge in 25 minutes. First, fluorine-18 was trapped in the Chromabond PS- HCO_3 cartridge, followed by drying with two passages of hot acetonitrile drawn from the heated reactor. The acetonitrile was successively sent to syringes 2 and 3 to remove any water remaining from the pre-labeling step. Using syringe 1, a 1 mL volume of the precursor solution was pushed through the Chromabond PS- HCO_3 cartridge over a period of 45 seconds, with a series of slow back and forth strokes. The radioactive mixture was then transferred into syringe 2 and quenched by rinsing of the Chromabond PS- HCO_3 with water. Syringe 2 was further loaded with two additional aqueous rinses from the Chromabond PS- HCO_3 cartridge. Radiochemically pure [^{18}F]F-Py-TFP ([^{18}F]1) was obtained by diluting the organic solvent content with water to 20% and extracting the [^{18}F]F-Py-TFP onto the tC18 Sep-Pak light cartridge (flow 2 mL/min), which was followed by a 6 mL water rinse of the tC18 via syringe 2. Partially dry SPE-bound [^{18}F]F-Py-TFP was obtained by purging the SPE cartridge with a stream of nitrogen gas (N_2) at high flow rate. Scheme 1 presents an overview of the cassette synthesis and the impurities removed by each of the SPE cartridges used in the FASTlab cassette.

Reaction of [^{18}F]F-Py-TFP to Glu-CO-Lys and purification of [^{18}F]DCFPyL

From cartridge-bound [^{18}F]F-Py-TFP, [^{18}F]DCFPyL was synthesized and purified in 31 minutes.

The reactor was charged with Glu-CO-Lys/TBA- HCO_3 in DMSO by applying vacuum, and the [^{18}F]F-Py-TFP was then quantitatively eluted from the tC18 Sep-Pak light cartridge into the reactor using 0.7 mL absolute ethanol. Aminolysis of [^{18}F]F-Py-TFP with Glu-CO-Lys was performed at 45°C in the presence of TBA- HCO_3 as base for 10 min, providing crude [^{18}F]DCFPyL. In parallel, the cassette manifold was prepared for the final purification step. The HILIC and Oasis PRiME HLB plus light cartridges were both

first conditioned with water from syringe 3. Syringe 2 was first rinsed with ethanol; (1 mL) and then acetone (1.2 mL), which was used to condition the HILIC. After conjugation, the reaction mixture was diluted with acetone (2.4 mL) and transferred via syringe 2 to the HILIC cartridge, immobilizing the [^{18}F]DCFPyL product while more apolar impurities were unretained. Residual radioactivity was transferred from the reactor to the HILIC cartridge using a second portion of acetone (1.0 mL). The remaining acetone in vial 7 (approx. 1 mL) was used to rinse the HILIC cartridge followed by a N_2 -gas purge. Syringe 2 was rinsed with 0.85% phosphoric acid, and a 4.2 mL volume of this same acid solution was used to elute the [^{18}F]DCFPyL fraction off the HILIC column to syringe 3, which was further diluted with 2.7 mL of 0.85% phosphoric acid.

Considering that both [^{18}F]DCFPyL and unreacted precursor Glu-CO-Lys were retained by the HILIC SPE and both was eluted with weak phosphoric acid, an additional purification step to remove unreacted Glu-CO-Lys, which is a pseudo-carrier possessing PSMA receptor avidity, was required. After investigating different reverse solid-phase materials, including C18 and other polymeric phases, we identified the Oasis PRiME HLB plus light cartridge as being suitable, by retaining [^{18}F]DCFPyL but not the more polar Glu-CO-Lys. Consequently, [^{18}F]DCFPyL could be trapped selectively on the Oasis PRiME HLB light cartridge using syringe 2 as a waste receiver. The cartridge was washed with an additional 4.8 mL of phosphoric acid to elute any residual Glu-CO-Lys. Finally, syringe 3 was washed three times with water and [^{18}F]DCFPyL was eluted off the Oasis PRiME cartridge into the product vial with 2.1 mL 25% ethanolic solution.

Quality control of the drug product

HPLC method **1** (reversed phase) was used in conjunction with on-line radioactivity detection for routine assessment of radiochemical and chemical purity. Chemical and radiochemical purity of the product was also monitored using method **2a**. The HILIC HPLC method **2b** was used for quantifying in the purified product any residual Glu-CO-Lys, which was too hydrophilic to be resolved by reversed phased HPLC. Representative chromatograms from individual steps of the automated synthesis are depicted in figure 3. The results for four automated runs, using starting activities ranging from 1 to 95 GBq, are shown in table 4. The radiochemical yield was fairly close to 40% irrespective of the starting activity. Furthermore, the radiochemical purity was never lower than 93% (range 93-98%) at end of synthesis, and declined in the first hours after high radioactivity productions, in agreement with already published work showing radiolysis of multi-GBq productions of [^{18}F]DCFPyL (Ravert et al., 2016). In the final radiopharmaceutical product, carrier DCFPyL is the main constituent of the PSMA-avid impurities; its concentration increases with higher starting radioactivities. The total chemical impurities were well below 200 μg per batch, such that formulation to a final 20 mL volume drug product gave a mass concentration less than 10 $\mu\text{g}/\text{mL}$. This is comfortably below the purity specifications adopted for PSMA-1007 (Cardinale et al., 2017), although higher than in [^{18}F]DCFPyL productions where final purification was performed with HPLC (20 nmol/mL [sum of all impurities] vs. a maximum of 0.88 nmol/mL [sum all unknown impurities for HPLC-purified [^{18}F]DCFPyL]) (Winhorst, 2019). The overall molar activity was moderately high, increasing 7-fold as starting radioactivity increased from 1 to 95 GBq. Nevertheless, the entire range of obtained molar

activity is suitable for PET imaging of highly expressed receptor targets such as PSMA (Pillarsetty et al., 2016). Residual solvents in the final drug product (acetone, TEA, acetonitrile, DMSO and *t*-BuOH) were quantified using GC-FID and were found to be well below the Class II (acetonitrile) and Class III residual solvent limits specified in the European Pharmacopeia.

Table 2
Results of automated synthesis of [¹⁸F]DCFPyL.

Starting activity EOB	< 1 GBq	10 GBq	50 GBq	95 GBq
RCY (%)	39	42	43	38
RCP (%)	93	98	96	94
Total DCFPyL (µg)	41	47	103	124
Total mass (µg) ¹	56	61	128	170
Molar activity (GBq/µmol)	18	48	92	123
[¹⁸ F]DCFPyL, EOB as end of bombardment, synthesis time 56 minutes.				
¹ Total mass in 2.1 mL volume.				

Statistical analysis

Quantitative data are expressed as mean ± standard deviation (SD) unless stated otherwise. Means were compared using a paired single-tailed Student t-test. Values were considered statistically significant for P < 0.05

Conclusion

We developed a fully automated two-step radiosynthesis of the PSMA ligand [¹⁸F]DCFPyL employing the prosthetic group [¹⁸F]-Py-TFP produced on-cartridge followed by conjugation to the PSMA-binding moiety Glu-CO-Lys. The radiosynthesis omits a preparative HPLC purification step. Our adoption of this reaction in the FASTlab™ synthesiser affords convenient access to the PSMA tracer [¹⁸F]DCFPyL in radiochemical yields ranging from 25 to 37% (uncorrected). The process, using GMP compliant single-use cassettes, afforded [¹⁸F]DCFPyL in adequate radiochemical purity (93-98%) and molar activity (70±47 GBq/µmol), with radiochemical yields sufficient to support multiple clinical PET investigations from a single production. Residual solvents were well below the concentration limits adopted in the current version of the European Pharmacopeia. Furthermore, the fully automated proof-of-concept two-step process bodes well for a more general application to other radiosynthesis modules and for conjugation with large biomolecules such as oligopeptides and proteins.

Abbreviations

RCP = radiochemical purity by radio-TLC. RCP $\geq$ 0.9 for reaction time $\geq$ 7 minutes. a including SPE with reaction time of eight min, b TEABC 0.5 M in EtOH, c in MeCN (100%), d n =3. MeCN), t-BuOH (tert-butanol), RT (room temperature), DIPEA (*N,N*-diisopropylethylamine), TEA (triethylamine), TEABC (tetraethylammonium bicarbonate), ,) 1 A: Automated, M: manual

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

DEO is an employee of Norwegian Medical Cyclotron centre Ltd. The fluoride-18 SPE radiolabeling of ^{18}F -Py-TFP labeling agent is patented. The other authors declare no conflict of interest.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

DEO: Conception and design of the work and aided in the interpretation and analysis of the data. RH: performed the experiments and was a major contributor in writing the manuscript. All authors were significant contributors to writing and editing of the manuscript and have read and approved the final manuscript.

References

1. Basuli, F., Zhang, X., Jagoda, E.M., Choyke, P.L., Swenson, R.E., 2016. Facile room temperature synthesis of fluorine-18 labeled fluoronicotinic acid-2,3,5,6-tetrafluorophenyl ester without azeotropic drying of fluorine-18. *Nuclear Medicine and Biology* 43, 770-772.

2. Bouchelouche, K., Choyke, P.L., Capala, J., 2010. Prostate Specific Membrane Antigen—A Target for Imaging and Therapy with Radionuclides. *Discovery medicine* 9, 55-61.
3. Bravaccini, S., Puccetti, M., Bocchini, M., Ravaioli, S., Celli, M., Scarpi, E., De Giorgi, U., Tumedei, M.M., Raulli, G., Cardinale, L., Paganelli, G., 2018. PSMA expression: a potential ally for the pathologist in prostate cancer diagnosis. *Scientific Reports* 8, 4254.
4. Cardinale, J., Martin, R., Remde, Y., Schäfer, M., Hienzs, A., Hübner, S., Zerges, A.-M., Marx, H., Hesse, R., Weber, K., Smits, R., Hoepping, A., Müller, M., Neels, C.O., Kopka, K., 2017. Procedures for the GMP-Compliant Production and Quality Control of [^{18}F]PSMA-1007: A Next Generation Radiofluorinated Tracer for the Detection of Prostate Cancer. *Pharmaceuticals* 10.
5. Chen, Y., Pullambhatla, M., Foss, C.A., Byun, Y., Nimmagadda, S., Senthamizhchelvan, S., Sgouros, G., Mease, R.C., Pomper, M.G., 2011. 2-(3-{1-Carboxy-5-[(6-[^{18}F]Fluoro-Pyridine-3-Carbonyl)-Amino]-Pentyl}-Ureido)-Pentanedioic Acid, [^{18}F]DCFPyL, a PSMA-Based PET Imaging Agent for Prostate Cancer. *Clinical Cancer Research* 17, 7645-7653.
6. Dornan, M.H., Simard, J.M., Leblond, A., Juneau, D., Delouya, G., Saad, F., Menard, C., DaSilvia, J.N., 2018. Simplified and robust one-step radiosynthesis of [^{18}F]DCFPyL via direct radiofluorination and cartridge-based purification. *Journal of Labelled Compounds and Radiopharmaceuticals* 0.
7. Eder, M., Schäfer, M., Bauder-Wüst, U., Hull, W.-E., Wängler, C., Mier, W., Haberkorn, U., Eisenhut, M., 2012. ^{68}Ga -Complex Lipophilicity and the Targeting Property of a Urea-Based PSMA Inhibitor for PET Imaging. *Bioconjugate Chemistry* 23, 688-697.
8. Giesel, F.L., Hadaschik, B., Cardinale, J., Radtke, J., Vinsensia, M., Lehnert, W., Kesch, C., Tolstov, Y., Singer, S., Grabe, N., Duensing, S., Schäfer, M., Neels, O.C., Mier, W., Haberkorn, U., Kopka, K., Kratochwil, C., 2017. F-18 labelled PSMA-1007: biodistribution, radiation dosimetry and histopathological validation of tumor lesions in prostate cancer patients. *European Journal of Nuclear Medicine and Molecular Imaging* 44, 678-688.
9. Holland, J.P., Cumming, P., Vasdev, N., 2012. PET of Signal Transduction Pathways in Cancer. 53, 1333-1336.
10. Lesniak, W. G., Mease, R. C., Chatterjee, S., Kumar, D., Lisok, A., Wharram, B., ... Nimmagadda, S., 2019. Development of [^{18}F]FPy-WL12 as a PD-L1 Specific PET Imaging Peptide. *Molecular Imaging*. <https://doi.org/10.1177/1536012119852189>
11. Li, X.-G., Autio, A., Ahtinen, H., Helariutta, K., Liljenback, H., Jalkanen, S., Roivainen, A., Airaksinen, A.J., 2013. Translating the concept of peptidelabeling with 5-deoxy-5-[^{18}F]fluororibose into preclinical practice: ^{18}F -labeling of Siglec-9 peptide for PET imaging of inflammation. *Chemical Communications* 49, 3682-3684.
12. Lütje, S., Franssen G.M., Herrmann, K., Boerman, O.C., Rijpkema M., Gotthardt, M., Heskamp, S., 2019. In Vitro and In Vivo Characterization of an ^{18}F -AIF-Labeled PSMA Ligand for Imaging of PSMA-Expressing Xenografts. *Journal of Nuclear Medicine* 60, 1017-1022.

13. Olberg, D.E., Arukwe, J.M., Grace, D., Hjelstuen, O.K., Solbakken, M., Kindberg, G.M., Cuthbertson, A., 2010. One Step Radiosynthesis of 6- ^{18}F Fluoronicotinic Acid 2,3,5,6-Tetrafluorophenyl Ester (^{18}F -Py-TFP): A New Prosthetic Group for Efficient Labeling of Biomolecules with Fluorine-18. *Journal of Medicinal Chemistry* 53, 1732-1740.
14. Olberg, D.E., Svadeberg, A., 2017. Patent. WO/2017/072200.
15. Pillarsetty, N., Kalidindi, T., Carlin, S., Easwaramoorthy, B., Abbasi, A., Larson, S., Osborne, J., Weber, W., 2016. Effect of specific activity on the uptake of ^{68}Ga -DKFZ-PSMA11 in tumor and other organs. 57, 528.
16. Ravert, H.T., Holt, D.P., Chen, Y., Mease, R.C., Fan, H., Pomper, M.G., Dannals, R.F., 2016. An improved synthesis of the radiolabeled prostate-specific membrane antigen inhibitor, ^{18}F DCFPyL. 59, 439-450.
17. Roy, J., Kuo, F., Basuli, F., Williams, M.R., Wong, K., Green, M.V., Seidel, J., Adler, S.S., Xu, B., Choyke, P.L., Jagoda, E.M., 2019. The Distribution Volume of ^{18}F -Albumin as a Potential Biomarker of Antiangiogenic Treatment Efficacy. *Cancer Biotherapy and Radiopharmaceuticals* 34, 238-244.
18. Szabo, Z., Mena, E., Rowe, S.P., Plyku, D., Nidal, R., Eisenberger, M.A., Antonarakis, E.S., Fan, H., Dannals, R.F., Chen, Y., Mease, R.C., Vranesic, M., Bhatnagar, A., Sgouros, G., Cho, S.Y., Pomper, M.G., 2015. Initial Evaluation of ^{18}F DCFPyL for Prostate-Specific Membrane Antigen (PSMA)-Targeted PET Imaging of Prostate Cancer. *Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging* 17, 565-574.
19. Virgolini, I., Decristoforo, C., Haug, A., Fanti, S., Uprimny, C., 2018. Current status of theranostics in prostate cancer. *European journal of nuclear medicine and molecular imaging* 45, 471-495.
20. Zhang, K., Bangma, C.H., Roobol, M.J., 2017. Prostate cancer screening in Europe and Asia. *Asian Journal of Urology* 4, 86-95.
21. Certificate of analysis for ^{18}F DCFPyL, personal email communication Windhorst, A.D, VUmc Amsterdam, Netherlands. December 25th 2019.

Figures

Water bag **9**. Abs. ethanol **10**. HILIC SPE cartridge **11**. Oasis PRiME HLB plus light cartridge **12**. 25 % ethanolic solution external vial **13**. Product collection vial. **S1**, **S2** and **S3**, syringes 1, 2 and 3.

Figure 3

a) HPLC analysis of [^{18}F]**1** ([^{18}F]F-Py-TFP) automated on the FASTlab synthesizer. Solid red line; radiodetector (Method 1). **b)** Reaction mixture after conjugation of [^{18}F]**1** with Glu-CO-Lys (**4**) prior to SPE

cartridge purification from the FASTlab synthesizer. Solid red line; radiodetector. Solid blue line; UV detection at 254 nm. **c)** HPLC analysis of SPE cartridge purified [^{18}F]DCFPyL from a fully automated radiosynthesis using the FASTlab synthesizer. Solid red line; radiodetector. Solid blue line; UV detection at 254 nm. Scheme 1 presents an overview of the cassette synthesis and the impurities removed by each of the SPE cartridges.

Supplementary Files

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- [scheme1.png](#)