One-pot synthesis of 6-l-[18f] fluoro-3,4-dihydroxyphenylalanine (6-l-[18f] fdopa).

Luurtsema, Geert; Kwizera, Chantal; Blok, Simon Niels; Farinha Antunes, Ines ; Elsinga, Philippus Hein; de Vries, Erik

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 March 2024 (21.03.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/058665 A1

(51) International Patent Classification:
C07B 59/00 (2006.01) *G21G 4/08* (2006.01)
C07C 229/36 (2006.01) *G21G 1/10* (2006.01)
C07F 1/08 (2006.01)

(21) International Application Number:
PCT/NL2023/050475

(22) International Filing Date:
14 September 2023 (14.09.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
22195943.0 15 September 2022 (15.09.2022) EP

(71) Applicants: **RIJKSUNIVERSITEIT GRONINGEN** [NL/NL]; Broerstraat 5, 9712 CP Groningen (NL). **ACADEMISCH ZIEKENHUIS GRONINGEN** [NL/NL]; Hanzeplein 1, 9713 GZ Groningen (NL).

(72) Inventors: **LUURTSEMA, Geert**; Universitair Medisch Centrum Groningen, Nuclear Medicine & Molecular Imaging, Hanzeplein 1, 9713 GZ Groningen (NL). **KWIZERA, Chantal**; Universitair Medisch Centrum Groningen, Nuclear Medicine & Molecular Imaging, Hanzeplein 1, 9713 GZ Groningen (NL). **BLOK, Simon Niels**; Universitair Medisch Centrum Groningen, Nuclear Medicine & Molecular Imaging, Hanzeplein 1, 9713 GZ Groningen (NL). **FARINHA ANTUNES, Ines**; Universitair Medisch Centrum Groningen, Nuclear Medicine & Molecular Imaging, Hanzeplein 1, 9713 GZ Groningen (NL). **ELSINGA, Philippus Hein**; Universitair Medisch Centrum Groningen, Nuclear Medicine & Molecular Imaging, Hanzeplein 1, 9713 GZ Groningen (NL). **DE VRIES, Erik**; Universitair Medisch Centrum Groningen, Nuclear Medicine & Molecular Imaging, Hanzeplein 1, 9713 GZ Groningen (NL).

(74) Agent: **WITMANS, H.A.**; V.O., P.O. Box 87930, 2508 DH Den Haag (NL).

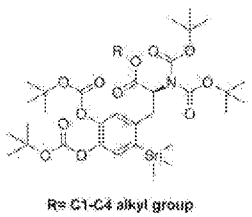
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2024/058665 A1

(54) Title: ONE-POT SYNTHESIS OF 6-*L*-[^{18}F] FLUORO-3,4-DIHYDROXYPHENYLALANINE (6-*L*-[^{18}F] FDOPA).



(57) Abstract: The invention relates to the field of medicinal chemistry and radiopharmaceuticals, in particular to means, methods and cassettes for the automated synthesis of the radiotracer 6-*L*-[^{18}F]fluoro-3,4- dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA). Provided is a method for the one-pot synthesis of 6-*L*-[^{18}F]FDOPA comprising contacting a BOC- protected stannyl precursor of the formula I with [^{18}F] Tetraethylammonium fluoride ([^{18}F]Et_4NF) in the presence of the Cu-catalyst $Cu(Impy)_4(OTf)_2$.

Title: One-pot synthesis of 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA).

The invention relates to the field of medicinal chemistry and radiopharmaceuticals. More in particular, it relates to means, methods and cassettes for the automated synthesis of the radiotracer 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA).

Positron Emission Tomography (PET) with 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA) is currently being used to detect and monitor neuroendocrine tumors, Parkinson's disease, and other neurodegenerative disorders. To this day, the electrophilic route has been the main approach for the development of 6-*L*-[^{18}F]FDOPA, regardless of its low molar activity (A_m) and low practical yields (de Vries *et al.* Appl Radiat Isot. 1999;51:389–94). To satisfy the increasing demand of 6-*L*-[^{18}F]FDOPA in clinical practice, new late stage Cu-mediated ^{18}F-fluorination has been evaluated in the art. See for example Jiang *et al.* Am J Nucl Med Mol Imaging. 2021 Aug 15;11(4):290-299), Andersen *et al.* EJNMMI Radiopharm Chem. 2021 Jun 12;6(1):21; Krasikova, Molecules. 2020 Sep 23;25(19):4365; Kuçi *et al.*, Labelled Comp Radiopharm. 2019 Jun 30;62(8):438-447, Mossine *et al.*, Nat Protoc. 2020 May;15(5):1742-1759; and Makaravage *et al.*, Org. Lett. 2016 Oct 21;18(20):5440-5443.

Other background references include US 2005/137421; WO 2015/058047; and Edwards *et al.*, Eur. J. Org. Chem., Jan 1 2015;2015(3):625-630.

Notably, whereas Zarrad *et al.* (Molecules. 2017;22:2231-2255) reported the automated synthesis of 6-*L*-[^{18}F]FDOPA via a BOC-protected stannyl precursor, this approach did not provide the fluorinated products in the hands of other specialized laboratories following the published.

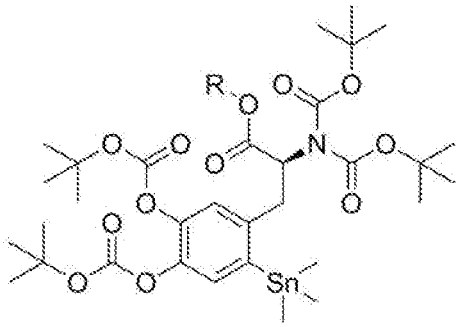
Thus, published methods are more complex due to multi step synthesis, or containing enzymatic conversions, or appear to be irreproducible. Because the complexity or lack of robustness, these procedure are less suitable for GMP productions. As a result, automated GMP—compliant synthesis of 6-*L*-[^{18}F]FDOPA via the Cu-mediated ^{18}F-fluorination in a commercially available automated synthesizer is still lacking as of to date.

The present inventors therefore set out to develop a simplified approach that allows for a reliable, one pot, high yielding, cassette based GMP compliant production of 6-*L*-[^{18}F]FDOPA, in particularly making use of the IBA Synthera® module, and being based on readily (commercially) available precursor compounds. Moreover, it is an object of the invention to reduce the number of reagents required as compared to previously used methods, such as the method of Makaravage *et al.*, Org. Lett. 2016 Oct 21;18(20):5440-5443.

It was surprisingly found that these goals were met by a unique combination of stannyl-precursor, the type of Cu-catalyst, and specific performance of a 2-step procedure allowing for automated synthesis of 6-*L*-[^{18}F]FDOPA having an RCC of 53±17%.

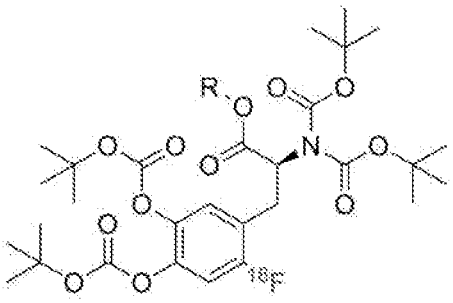
More in particular, the invention provides a method for the one-pot synthesis of 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA) involving the Cu-mediated ^{18}F-fluorodestannylation of a BOC- protected stannyl precursor, comprising the steps of:

(i) contacting a BOC- protected stannyl precursor of the formula I



wherein R is C_1-C_4 alkyl

with [^{18}F] Tetraethylammonium fluoride ([^{18}F]Et_4NF) in the presence of the Cu-catalyst Cu(imidazo[1,2-b]pyridazine)4(OTf)2 (Cu(Impy)$_4$(OTf)$_2$) to yield the [^{18}F]-BOC-protected intermediate compound of formula II



Formula II ; and

(ii) hydrolyzing the ^{18}F BOC-protected intermediate compound using HCl to obtain 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA).

This method is not known or suggested in the art.

Zarrad *et al.* use Cu(Py)$_4$(OTf)$_2$, which is a Cu-source typically employed in Cu-mediated radiofluorination of arylpinacol boronates and arylstannanes, including precursors of 6-*L*-[^{18}F]FDOPA. See the review by Krasikova, (Molecules. 2020 Sep 23;25(19):4365). The present inventors surprisingly found that replacing the pyridine moiety in Cu(Py)$_4$(OTf)$_2$ with Impy

(imidazo[1,2-b] pyridazine) to yield the catalyst $Cu(Impy)_4(OTf)_2$ led to highly improved results.

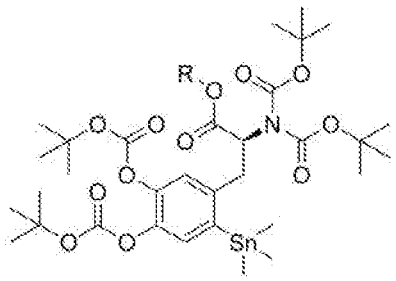
The use of $[Cu(Impy)_4](OTf)_2$ in radiofluorination of organoborons has been disclosed in the art. See for example the studies by Guibbal *et al.*, (Mol Imaging Biol. 2020; 22(5): 1226–1234; Nature Protocols 15(4); WO2019/186135) relating to the synthesis of ^{18}F-labelled PARP inhibitors by Cu-mediated ^{18}F-fluorodeboronation.

However, the art is silent about the advantageous use of $Cu(Impy)_4(OTf)_2$ in reactions involving stannyl precursors, let alone in synthesis of $[^{18}F]$FDOPA. Notably, whereas Zarrad *et al.* performed extensive optimization studies including different salts, solvents, precursor and catalyst amounts, it was never considered to switch to a different Cu(II) complexing ligand, let alone to substitute pyridine for imidazo[1,2-b]pyridazine (Impy).

Moreover, the method of the invention allows for the use of fewer different reagents than disclosed by Makaravage *et al.* The latter advocates the use of pyridine as an additional reagent, while the method of the invention results in higher yields, even in the absence of pyridine as shown in Example 7 below.

Furthermore, in step (ii) of a method of the invention the protecting groups are removed with HCl, which was found to give better results compared to HBr. More in particular, hydrolysis in the presence of HBr resulted in turbid, dark colored solutions containing sticky residue.

Step (i) of a method herein disclosed involves the radiofluorination of a BOC- protected stannyl precursor of the formula (I)



wherein R is a C_1-C_4 alkyl moiety.

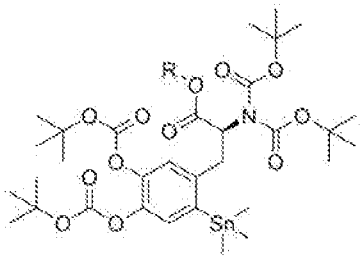
These are also herein referred to as *N,N,O,O'*-tetra-BOC-6-(SnMe3)DOPA-O-R. Hence, suitable precursors include the BOC-protected methyl, ethyl, (iso)propyl or butyl-esters.

It was found that highly reproducible results can be obtained with the methyl-ester, i.e. wherein R is CH_3. Furthermore, this fully protected precursor can be obtained from commercial suppliers, for example from ABX advanced biochemical compounds GmbH, Radeberg, Germany.

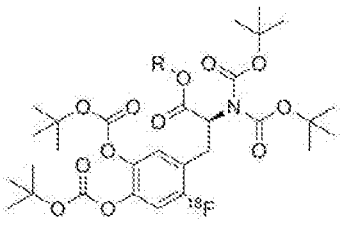
Alternatively, it can be readily prepared in a 95% or even higher yield by *N*-BOC protection of the partially protected compound *N,O,O'*- tri-BOC-6-(SnMe3)DOPA-OMe, which is also commercially available from ABX advanced biochemical compounds GmbH, Radeberg, Germany.

Hence, in a preferred embodiment, the invention provides a method the comprising the steps of:

contacting N,N,O,O'-tetra-BOC-6-($SnMe_3$)DOPA-OMe of the formula



with [^{18}F]Tetraethylammonium fluoride ([^{18}F]Et$_4$NF) in the presence of Cu(Impy)$_4$(OTf)$_2$ to yield the [^{18}F]-BOC-protected intermediate fluorinated compound of the formula



Optionally, the method comprises prior to step (i) the step of preparing N,N,O,O'-tetra-BOC-6-(SnMe$_3$)DOPA-OMe by *N*-BOC protection of N,O,O'-tri-BOC-6-(SnMe$_3$)DOPA-OMe.

The preferred nucleophilic ^{18}F-source in a method of the invention is [^{18}F]-tetraethylammonium fluoride [^{18}F]Et$_4$NF. Typically, the concentration of the ^{18}F source is less than or equal to 10^{-4} M, for instance less than or equal to 10^{-5} M. In some cases, the concentration will be nanomolar or even less, for instance less than or equal to 10^{-8} or 10^{-9} M.

[^{18}F]Et$_4$NF can be obtained using methods known in the art. Typically, this involves elution of [^{18}F]fluoride from an anion exchange resin by a suitable tetraethylammonium salt Et$_4$NX in a suitable solvent, e.g. MeOH, to result in the corresponding [^{18}F]tetraethylammonium salt. See for example Zarrad *et al.*, showing a recovery of ^{18}F elution capacity of 95-97% with the tetraethylammonium salt Et$_4$NHCO$_3$, Et$_4$NOTf, Et$_4$BF$_4$ or Et$_4$NI. In a preferred embodiment, Et$_4$NHCO$_3$ is used. Alternative alkyl ammonium salts include Me$_4$NX, Pr$_4$NX, Bu$_4$NX, to result in the corresponding [^{18}F]tetra alkyl ammonium fluoride ([^{18}F]Me$_4$NF, ([^{18}F]Pr$_4$NF or [^{18}F]Bu$_4$NF, respectively) for use as nucleophilic ^{18}F-source in a method of the invention.

The general process conditions of the radiofluorination step (i) can be selected and optimized by a person skilled in the art.

The process of the invention is typically carried out in solution. The process may be carried out in solution in any suitable solvent or solvent mixture. Thus, step (i) of treating the stannyl-precursor with the ^{18}F and Cu-source, is typically carried out in the presence of a solvent. Typically, the solvent comprises a polar aprotic solvent. Usually, the solvent is a polar aprotic solvent, or a mixture of two, or more than two (for instance three or four) polar aprotic solvents, in combination with about 5-20 vol% alcohol as co-solvent. Polar aprotic solvents are well known to the skilled person, and include 1,3-dimethyl-2-imidazolidinone (DMI), dimethyl formamide (DMF), *N,N*-dimethyl acetamide (DMA) and acetonitrile. Suitable co-solvents include methanol, ethanol, propanol and butanol. Preferably, the radiofluorination step (i) is performed in the presence of dimethylacetamide (DMA) and n-butanol. Good results are obtained with a solvent mixture consisting of 5 to 15 vol%, preferably about 10 vol%, n-butanol in DMA.

The Cu-source may be used in any suitable amount or concentration. Suitably, it is present in the reaction mixture at a concentration in the range of 10- 25 mM, preferably 15-20 mM, such as 17 mM.

The molar ratio of the stannyl-precursor to the Cu-source may be from 1:10 to 10: 1, for instance from 1:8 to 8: 1, for example from 1:5 to 5: 1, for instance from 1:3 to 3:1.

Typically, the amount of the precursor compound is less than the amount of the copper compound. Thus, the molar ratio of the amount of the stannyl precursor to the amount of $Cu(Impy)_4(OTf)_2$ (i.e. the molar ratio) is suitably from 2:3 to 1:8, for instance from 2:3 to 1:5 or 2:3 to 1:2. In one aspect, the molar ratio of BOC-protected stannyl precursor (e.g. *N,N,O,O'*-tetra-BOC-6-

($SnMe_3$)DOPA-OMe) / $Cu(Impy)_4(OTf)_2$ is in the range of 2:3 to 1:2, preferably about 1:1.5 to 1:2.

Exemplary embodiments include the following :
Weight precursor per synthesis reaction = 8 mg (10.3 μmol)
Weight $Cu(Impy)_4(OTf)_2$ per synthesis reaction= 14 mg (16.7 μmol)
Weight-to-weight ratio: 8 mg precursor / 14 mg $Cu(Impy)_4(OTf)_2$ = 1 : 1.75.
Molar ratio 10.3 / 16.7 = 1.6 : 1.

Preferably, in the method, cassette, device, and/or reaction mixture of the invention substantially no pyridine is used, and more preferably no pyridine is used.

Hence, the invention also provides a reaction mixture comprising the reagents of step (i), more in particular a reaction mixture comprising the BOC-protected stannyl precursor, the copper compound, the [^{18}F] Tetraethylammonium fluoride and the solvent in the (relative) amounts as described herein above.
This reaction mixture is typically heated at a temperature of from 100 °C to 160 °C. For instance, the reaction mixture may be heated at a temperature of from 110 °C to 160 °C, or from 120 °C to 150 °C, for instance at about 140 °C. The aforementioned reagents may be heated at said temperature for a length of time of at least 1 minute, for instance at least 10 minutes, or for instance at least 20 minutes. The reagents may for instance be heated at said temperature for a length of time from 1 minute to 1 hour, for instance from 10 minutes to 30 minutes, or from 20 minutes to 40 minutes.

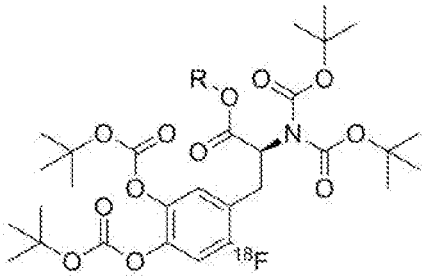
Often, step of (i) treating the stannyl-precursor with ^{18}F and the copper catalyst comprises:

(a) mixing the BOC-protected stannyl precursor, the copper compound, the [^{18}F]Tetraethylammonium fluoride and the solvent as defined above; and

(b) heating the resulting mixture to at a temperature of from 120 to 150°C for from 20 to 40 minutes.

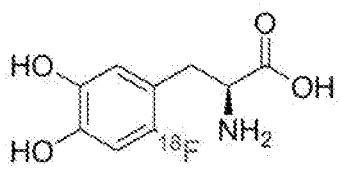
Typically, (b) comprises heating the resulting mixture in a sealed container.

The step of treating the stannyl-precursor with ^{18}F and a copper source is produces an intermediate of the following formula (II)



R= C1-C4 alkyl group

The step of (ii) for removing the BOC protecting groups to produce the final compound 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA)



or a salt thereof, may be referred to as the deprotection step.

The deprotection step is performed by hydrolyzing the intermediate compound with hydrochloric acid (HCl). Often, an aqueous solution of HCl such as about 4M to 7M (4-7N) HCl is used. In a specific embodiment, deprotection is achieved with about 6M HCl at elevated temperature, for instance during an incubation for 20-60 minutes at about 120-140°C.

It was found that the product formed during the deprotection is susceptible for oxidation. Therefore, deprotection step (ii) is preferably performed in the presence of an anti-oxidant, such as ascorbic acid. It was found that a deprotection solution of 6M HCl / 0.25 M ascorbic acid resulted in a final RCY of 53±17% (n=3).

As is exemplified herein below, a method of the present invention provides a one-pot synthesis of 6-*L*-[^{18}F]FDOPA in a radiochemical conversion (RCC) of at least 50%, wherein RCC is for example assessed by TLC-SG (eluent: Ethyl Acetate/n-hexane 1:1) and/or UPLC (Column: ACQUITY UPLC® HSS T3 1.8µm (3.0mm x 50mm); Eluent: 0.05M NaH_2PO_4 pH=2.5 and Flow: 0.8 ml).

One major advantage of a method of the invention is that it does not require any manual interventions and can thus be performed fully automated. Accordingly, in one embodiment of the invention, the process of the invention as defined herein for producing 6-*L*-[^{18}F]FDOPA is conducted in an automated synthesizer. The automated synthesizer may be any suitable automated synthesizer, as are well known to the skilled person.

Automated synthesizers may include one or more means for performing the process of the invention. For instance, an automated synthesizer may comprise one or more of (i) a means for mixing the reagents used in the process of the invention, (ii) a means for heating the mixed reagents of the invention and (iii) means for isolating the target compound 6-*L*-[^{18}F]FDOPA. Such automated synthesizers may be used for the production of 6-*L*-[^{18}F]FDOPA. Thus, the automated synthesizer may be suitable for use in a clinical setting, for instance in an imaging center equipped with a medical cyclotron, radiopharmaceutical production facility, scanners to perform PET imaging and/or scanning apparatus. For instance an IBA Synthera synthesis module but also other available cassette based synthesis modules .

An automated synthesizer may comprise one or more reagents used in the process of the invention. For example, the automated synthesizer may be pre-loaded with one or more reagents, for instance a protected stannyl-precursor compound or Cu-source as described anywhere herein. Alternatively, the process of the invention may be conducted in an automated synthesizer, which process further comprises loading the automated synthesizer with one or more reagents. The reagents may be loaded into the automated synthesizer by inserting a pre-packaged amount of the reagent, for instance in the form of a cassette or capsule. For instance, the process of the invention may further comprise inserting a prepackaged sample of the copper catalyst $Cu(Impy)_4(OTf)_2$ and/or or a stannyl precursor as defined anywhere herein into an automated synthesizer.

Also provided is a cassette for carrying out an automated (one-pot) synthesis of 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA), preferably an automated method of the invention, the cassette comprising the selected combination of copper catalyst i.e. $Cu(Impy)_4(OTf)_2$, and a stannyl precursor as defined anywhere herein above. The catalyst and precursor may be present in separate vessels or as "pre-mixture" in the same vessel. In one embodiment, the cassette comprises one vessel containing $Cu(Impy)_4(OTf)_2$ and another, distinct, vessel containing a BOC- protected stannyl precursor of Formula I as defined herein above.

Preferably, the cassette comprises (i) a first vessel containing a mixture of $Cu(Impy)_4(OTf)_2$ and a BOC- protected stannyl precursor of Formula I as defined herein above, preferably *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe.

A cassette suitably comprises (ii) a vessel containing a solution of a tetraethylammonium salt Et_4NX, wherein X is any suitable salt, preferably selected from the group consisting of Et_4NHCO_3, Et_4NOTf, Et_4NF_4 or Et_4NI. In a specific aspect, the cassette comprises a vessel with Et_4NHCO_3.

Alternative alkyl ammonium salts for use in a method and/or cassette according to the invention include Me_4NX, Pr_4NX, Bu_4NX, wherein X is any suitable salt, preferably selected from the group consisting of HCO_3, OTf, F_4 or I.

The cassette may further comprise, optionally in separate vessel(s), additional reagents, additives and/or solvents. For example, it comprises in a third or fourth vessel a deprotection solution comprising HCl, preferably further comprising an anti-oxidant such as ascorbic acid.

Additionally, the cassette may contain a vessel comprising water or an aqueous solution for use as HPLC eluent or diluting solvent.
Still further, the cassette may comprise one or more additional components that aid in the (automated) manufacture of 6-*L*-[^{18}F]FDOPA, for example one or both of: (iv) an ion-exchange cartridge for removal of excess [^{18}F] Tetraethylammonium fluoride; and (v) a cartridge for carrying out step (ii) for removing BOC-protecting groups.

This cassette is advantageously used in a device for automated (GMP-compliant) synthesis of 6-*L*-[^{18}F]FDOPA, such as the IBA Synthera module or any other type of module making use of cassettes and/or disposables.

Accordingly, the invention also provides a device for automated synthesis of a radioactive tracer, in particular a device set up for the synthesis of 6-*L*-[^{18}F]FDOPA, comprising a cassette as defined above. In one embodiment, the device comprises a fixed module and a disposable module which is positioned on the fixed module, the fixed module comprising a processor and an interface for the disposable module, and wherein the disposable module comprises a cassette as defined above.
The interface of the fixed module may be provided with rotary actuators and fluidic connectors leaving the interface and comprising a structure for

positioning in an ejectable way a disposable module on said interface so that the rotary actuators and the fluidic connectors may be inserted into the disposable module.

LEGEND TO THE FIGURES

Figure 1: Schematic representation of a human-machine interface (HMI) for the synthesis of 6-*L*-[^{18}F]FDOPA. The numbers 1-4 represent one-way valves, which can be opened or closed in order to add liquids to the reaction mixture. Valves 5-8 represent three-port two-way valves, which can be used to change the flow direction of liquids during the reaction.

Figure 2: Schematic representation of an exemplary cassette for use in the automated synthesis of 6-*L*-[^{18}F]FDOPA.

Figure 3: Radiochemical conversion analysis by TLC using an Amersham Typhoon biomolecular imager. Entry 1 is BOC-protected 6-*L*-[^{18}F]FDOPA; entry 2 is an unknown byproduct and entry 3 is [^{18}F]fluoride.

Figure 4: Radiochemical analysis by UPLC of the reaction mixture after the deprotection step.

EXPERIMENTAL SECTION

EXAMPLE 1: Manufacture of [^{18}F]Et_4NF.

A target filled with 2 mL enriched water ($H_2{}^{18}O$) was bombarded with protons using a medical 18 MeV cyclotron. After approx. 30 GBq of [^{18}F]fluoride was produced, the target was unloaded, and the enriched water containing [^{18}F]fluoride was transported via tubings to the radiopharmaceutical production facility in to a hotcell, in which it was collected in a conical vial. After collection, $H_2{}^{18}O$ containing [^{18}F]fluoride was aspirated by the Synthera V2 synthesis module and [^{18}F]fluoride was trapped onto a Waters® Sep-Pak Accell Plus QMA Carbonate Plus Light Cartridge that was conditioned with 1 mL of water. The cartridge was flushed with 2 mL of methanol and dried using a nitrogen stream for five minutes. The [^{18}F]fluoride was eluted from the cartridge into the reactor using Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL of methanol. The cartridge was flushed for one minute using a nitrogen stream. The contents of the reactor were then concentrated to dryness by heating the reactor to 90 °C under reduced pressure for five minutes to yield the dried [^{18}F]Et_4NF.

EXAMPLE 2: Manufacture of $Cu(Impy)_4(OTf)_2$.

The copper catalyst was prepared based on published methods. See Wilson et al. (J Nucl Med. 2019 Apr; 60(4): 504–510).

Briefly, a solution of imidazo[1,2-b]pyridazine (Impy) (758 mg, 6.36 mmol, 10 equiv.) in MeOH (1 mL) was added dropwise at 55°C to a solution of $Cu(OTf)_2$ (230 mg, 0.636 mmol, 1.0 equiv.) in MeOH (1 mL). The blue precipitate which formed was washed with Et_2O (3 x 2 mL), then recrystallized from hot MeOH to afford [$Cu(Impy)_4(OTf)_2$] (324 mg, 0.387 mmol, 61%).

EXAMPLE 3: One-pot synthesis of 6-*L*-[^{18}F]FDOPA.

A reaction mixture was prepared comprising [^{18}F]Et_4NF according to Example 1, a solution of the BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol; ABX advanced biochemical compounds GmbH, Radeberg, Germany) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol) in 900 µL DMA and 100 µL n-butanol. This reaction mixture was heated to 140 °C for 30 minutes.

The reactor was cooled down to 50 °C prior to the addition of a deprotecting solution containing 6 M HCl (800 µL, 4.8 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol). The deprotection reaction was heated to 120 °C and allowed to proceed for 20 minutes, after which the solution was diluted to a total volume of 2 mL by addition of water. The mixture was filtered over a Waters® Sep-Pak Alumina B Plus Light Cartridge, followed by a Pall Acrodisc 1.2 µm filter and injected on HPLC for product characterization.

EXAMPLE 4: Conversion analysis by TLC.

The radiochemical conversion is calculated from the intensity of the spots on TLC. Division of the signal of BOC-protected 6-*L*-[^{18}F]FDOPA by the total signal of all radioactive compounds present on the lane of the TLC plate gives the percentage of [^{18}F]fluoride converted to BOC-protected 6-*L*-[^{18}F]FDOPA (Lane%).

EXAMPLE 5: Product analysis by HPLC.

The calculated radiochemical yield of the reaction is calculated from the integration of radioactive peaks. The relative peak area of the 6-*L*-[^{18}F]FDOPA peak is divided compared to the total area of all radioactive peaks combined gives the percentage of [^{18}F]fluoride converted to 6-*L*-[^{18}F]FDOPA (% Area).

EXAMPLE 6: Comparative testing of Cu catalyst or anti-oxidant.

Table 1 below illustrates the advantages of using the copper catalyst $Cu(Impy)_4(OTf)_2$ according to a method of the invention as compared to catalyst containing no or different Cu(II) complexing ligands e.g. in $Cu(OTf)_2$ and $Cu(Py)_4(OTf)_2$. Reaction mixtures and conditions were as described herein above using the BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol; ABX advanced biochemical compounds GmbH, Radeberg, Germany) and Cu-catalyst (16.7 µmol) in DMA/n-butanol. The comparative experiment was performed 4 times, each of which demonstrating that the highest conversion was obtained using $Cu(Impy)_4(OTf)_2$.

Table 1 : Conversion (%)

	$Cu(OTf)_2$	**$Cu(Py)_4(OTf)_2$**	**$Cu(Impy)_4(OTf)_2$**
According to invention?	no	no	yes
Experiment #			
1	0	4	27
2	0	6	56
3	0	17	30
4	0	15	36

Table 2 below illustrates the advantages of including ascorbic acid (0.25 M) as anti-oxidant in the HCl-containing deprotection solution. The conversion ^{18}F-labeledBOC-protected intermediate compounds obtained by radiofluorination of different amounts of BOC-protected stannyl precursor (4, 16 or 8 mg) were subjected to a deprotection reaction in the absence (N) or presence (Y) of ascorbic acid. The results in Table 2 conversion percentages (reflecting the conversion of [18F]fluoride to BOC-protected 6-L-

[18F]FDOPA) indicate that the presence of anti-oxidant increases the product yield.

Table 2

Experiment #	Precursor (mg)	Asc. Acid (Y/N)	Calculated radiochemical yield (%)
5	4	N	10
6	16	N	30
7	16	N	19
8	8	Y	53
9	8	Y	70
10	8	Y	36

EXAMPLE 7: Comparative testing of fluorinating agent.

Table 3 below illustrates the advantages of using the fluorinating agent [^{18}F]Tetraethylammonium fluoride ([^{18}F]Et_4NF) according to a method of the invention as compared to the fluorinating agent as used by Makaravage *et al.*, Org. Lett. 2016 Oct 21;18(20):5440-5443, viz. [^{18}F]KF.

Moreover, Table 3 shows the advantages of using the copper catalyst of the invention ($Cu(Impy)_4(OTf)_2$) as compared to the copper catalyst of Makaravage *et al.*, viz. $Cu(OTf)_2$.

Furthermore, Makaravage *et al.* advocate the use of a major excess of pyridine (at least 15 equivalents). By contrast, the method of the invention yields improved results while avoiding the use of pyridine. As such, in experiment 7A pyridine is added in line with Makaravage *et al.*, while in experiment 7B no pyridine is added to the reaction mixture. The results indicated as “Experiment 6” in Table 3 were taken from the final column of Table 1 for ease of reference.

For experiments 7A and 7B the following reaction conditions were used. In experiment 7A, the reference catalyst $Cu(OTf)_2$ is used, while in experiment 7B the catalyst of the invention is used.

A Waters® Sep-Pak Accell Plus QMA Carbonate Plus Light Cartridge (hereafter: QMA cartridge) was conditioned using 10 mL of ethanol, 10 mL of a 90 mg/ml solution of potassium trifluoromethanesulfonate solution in water, and 10 mL of water. [18F]Fluoride was loaded onto the QMA cartridge and eluted using a solution of 10 mg potassium trifluoromethanesulfonate and 50 µg potassium carbonate in 550 µL water. 1 mL of acetonitrile was added to the eluate and the mixture was dried under vacuum.

To the dried mixture was added BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol), 2 eq (20.6 µmol) of the copper catalyst in 1 mL of N,N-dimethylacetamide. In experiment 7A, 15 eq (13 µL) of pyridine was also added, while in experiment 7B no pyridine was added to the reaction mixture.

Thereafter, the radiofluorination reaction was performed at 140 °C for 30 minutes. Radio TLC (1:1 EtOAc/hexanes) and subsequent analysis as described in Example 4 were used to determine the conversion of the precursor to BOC-protected 6-*L*-[^{18}F]FDOPA.

The results in Table 3 demonstrate that using the catalyst of the method of the invention improves the conversion, while the addition of pyridine can be avoided. Moreover, using both the catalyst and the fluorinating agent of the method of the invention results in a further improvement of the conversion.

Table 3.

Experiment	Catalyst	Fluorinating agent	Pyridine	Conversion (%)
7A (Makaravage *et al.)*	$Cu(OTf)_2$	$[^{18}F]KF$	15 eq.	0
7B (reference; other fluorinating agent)	$Cu(Impy)_4(OTf)_2$	$[^{18}F]KF$	none	12
6 (invention)	$Cu(Impy)_4(OTf)_2$	$[^{18}F]Et_4NF$	none	27-56[a]

[a] Results taken from Table 1.

EXAMPLE 8: Disposable cassettes for automated synthesis

Cassette A

Vessel 1: Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 900 µL DMA and 100 µL n-butanol.

Vessel 3: 6 M HCl (800 µL, 4.8 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol)

Vessel 4: HPLC eluent

Cassette B

Vessel 1: Et_4NHCO_3 (10 mg, 52.2 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 900 µL DMA and 100 µL n-butanol.

Vessel 3: 6 M HCl (800 µL, 4.8 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol)

Vessel 4: HPLC eluent

Cassette C

Vessel 1: Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) in 500 µL DMA.

Vessel 3: $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 400 µL DMA and 100 µL n-butanol.

Vessel 4: 6 M HCl (800 µL, 4.8 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol)

Cassette D

Vessel 1: Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 900 µL DMA and 100 µL n-butanol.

Vessel 3: 6 M HCl (800 µL, 4.8 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol)

Vessel 4: Water

Cassette E

Vessel 1: Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 900 µL DMA and 100 µL n-butanol.

Vessel 3: 1 mL 6 M HCl (800 µL, 4.8 mmol)

Vessel 4: HPLC Eluent

Cassette F

Vessel 1: Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 900 µL DMA and 100 µL n-butanol.

Vessel 3: 4 M HCl (800 µL, 3.2 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol)

Vessel 4: HPLC Eluent

Cassette G

Vessel 1: Et_4NHCO_3 (5 mg, 26.1 µmol) in 1 mL methanol

Vessel 2: BOC-protected stannyl precursor *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe (8 mg, 10.3 µmol) and $Cu(Impy)_4(OTf)_2$ (14 mg, 16.7 µmol in 900 µL DMF and 100 µL n-butanol.

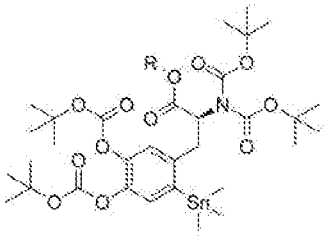
Vessel 3: 6 M HCl (800 µL, 4.8 mmol) and 0.25 M ascorbic acid solution (200µL, 50 µmol)

Vessel 4: HPLC Eluent

Claims

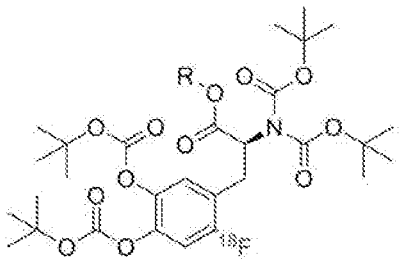
1. A method for the one-pot synthesis of 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA) involving the Cu-mediated ^{18}F-fluorodestannylation of a BOC- protected stannyl precursor, comprising the steps of:

(i) contacting a BOC- protected stannyl precursor of the formula I



R= C1-C4 alkyl group

with [^{18}F]Tetraethylammonium fluoride ([^{18}F]Et_4NF) in the presence of the Cu-catalyst Cu(imidazo[1,2-b]pyridazine)$_4$(OTf)$_2$ to yield the ^{18}F-labeled BOC-protected intermediate compound of the formula II



R= C1-C4 alkyl group ; and

(ii) hydrolyzing the [^{18}F] BOC-protected intermediate compound using HCl to obtain 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA).

2. Method according to claim 1, wherein the BOC- protected stannyl precursor is *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe.

3. Method according to claim 2, comprising prior to step (i) preparing N,N,O,O'-tetra-BOC-6-($SnMe_3$)DOPA-OMe by *N*-BOC protection of N,O,O'-tri-BOC-6-($SnMe_3$)DOPA-OMe.

4. Method according to any one of claims 1-3, wherein step (i) is performed in the presence of dimethylacetamide (DMA) and n-butanol.

5. Method according to any one of claims 1-4 wherein step (i) is performed at a temperature in the range of about 130-150 °C, preferably at around 140°C.

6. Method according to any one of the preceding claims, wherein the molar ratio of BOC-protected stannyl precursor / Cu(imidazo[1,2-b]-pyridazine)$_4$(OTf)$_2$ is in the range of 2:3 to 1:2, preferably about 1:1.5 to 1:2.

7. Method according to any one of the preceding claims, wherein step (ii) is performed in the presence of an anti-oxidant, preferably ascorbic acid.

8. Method according to any one of the preceding claims, to provide 6-*L*-[^{18}F]FDOPA in a radiochemical conversion (RCC) of at least 50%.

9. A cassette for carrying out a one-pot synthesis of 6-*L*-[^{18}F]fluoro-3,4-dihydroxyphenylalanine (6-*L*-[^{18}F]FDOPA), comprising:

(i) a BOC- protected stannyl precursor of Formula I as defined in Claim 1 or 2; and Cu(imidazo[1,2-b]pyridazine)$_4$(OTf)$_2$, wherein said precursor and said Cu(imidazo[1,2-b]pyridazine)$_4$(OTf)$_2$ may be present in the same vessel or in separate vessels, preferably wherein said precursor and said Cu(imidazo[1,2-b]pyridazine)$_4$(OTf)$_2$ are present as pre-mixture in the same vessel.

10. The cassette according to claim 9, further comprising (ii) a separate vessel containing a solution of a tetraethylammonium salt, preferably selected from the group consisting of Et_4NHCO_3, Et_4NOTf, Et_4NF_4 and Et_4NI.

11. The cassette according to claim 9 or 10, wherein said BOC- protected stannyl precursor is *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe.

12. The cassette according to any one of claims 9-11, further comprising (iii) a separate vessel comprising a solution of HCl.

13. The cassette according to claim 12, comprising

(i) a vessel comprising a solution of Et_4NHCO_3

(ii) a vessel comprising *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe and Cu(imidazo[1,2-b]pyridazine)$_4$($OTf)_2$

(iii) a vessel comprising a solution comprising HCl and ascorbic acid.

14. A device for automated synthesis of a radioactive tracer, the device comprising a fixed module and a disposable module which is positioned on the fixed module, the fixed module comprising a processor and an interface for the disposable module, and wherein the disposable module comprises a cassette according to any one of claims 9-13.

15. A reaction mixture comprising *N,N,O,O'*-tetra-BOC-6-($SnMe_3$)DOPA-OMe, Cu(imidazo[1,2-b]pyridazine)$_4$($OTf)_2$ and [^{18}F]Tetraethylammonium fluoride in a solvent, preferably in DMA comprising *n*-butanol.

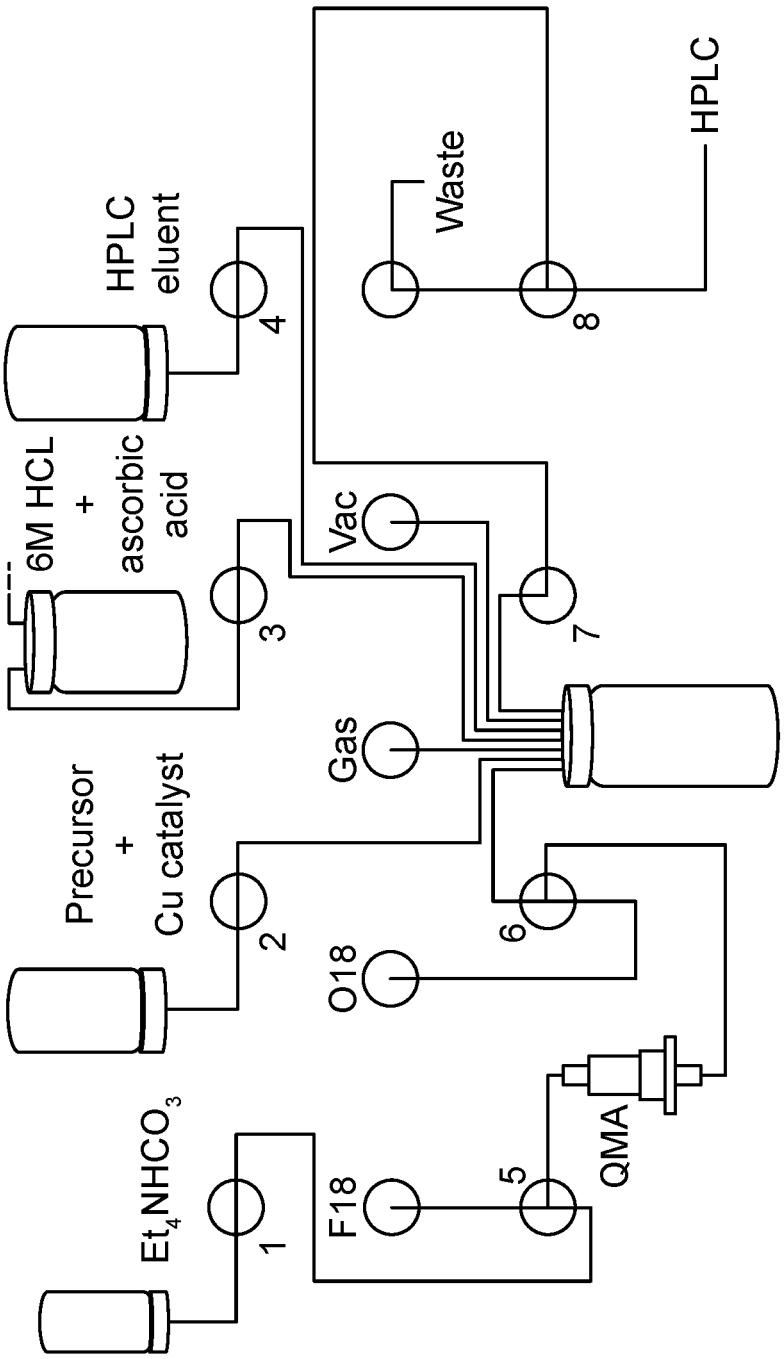

HPLC
Waste
HPLC eluent
6M HCL + ascorbic acid
Vac
Precursor + Cu catalyst
Gas
O18
F18
Et_4NHCO_3
QMA
1
2
3
4
5
6
7
8

Fig. 1

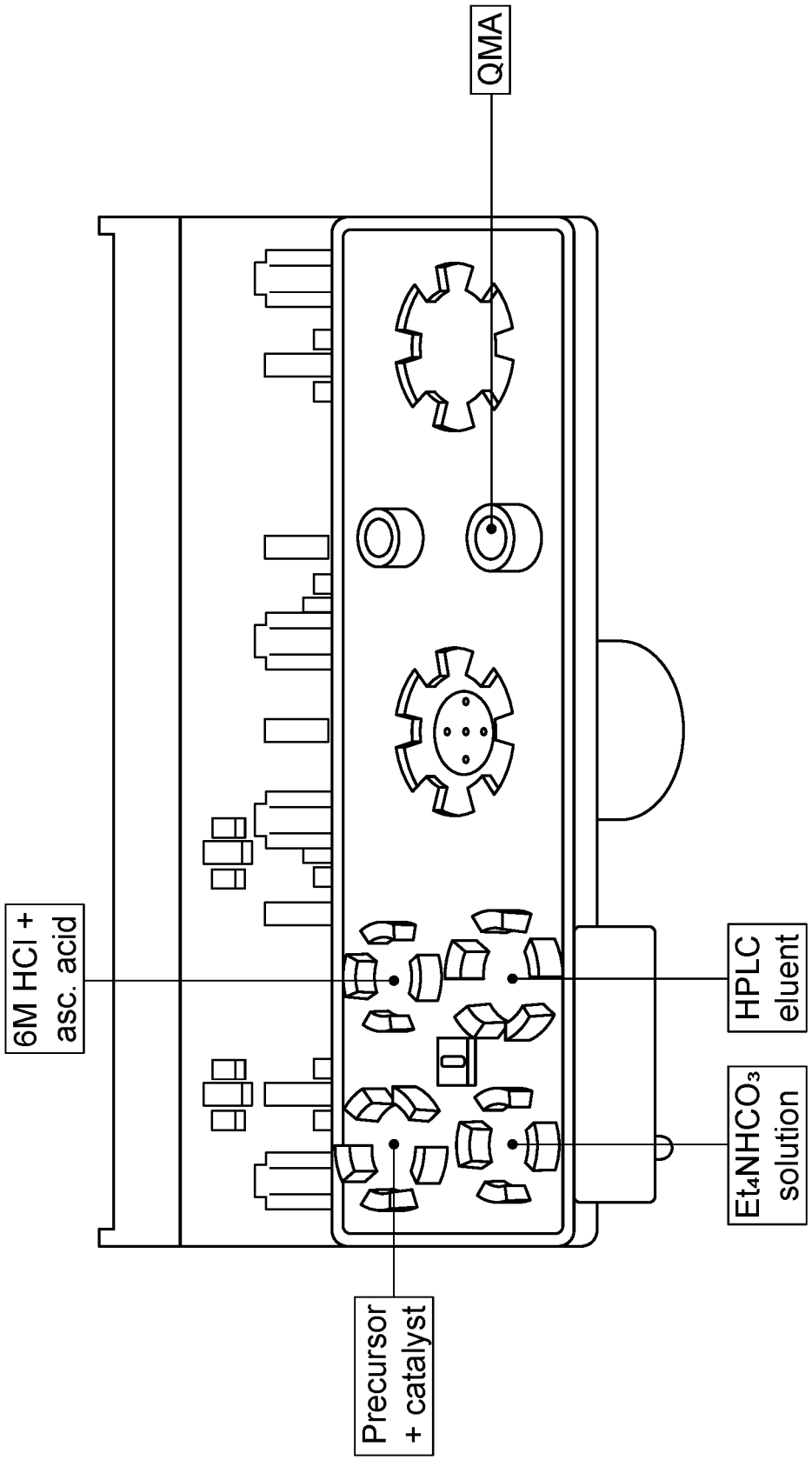
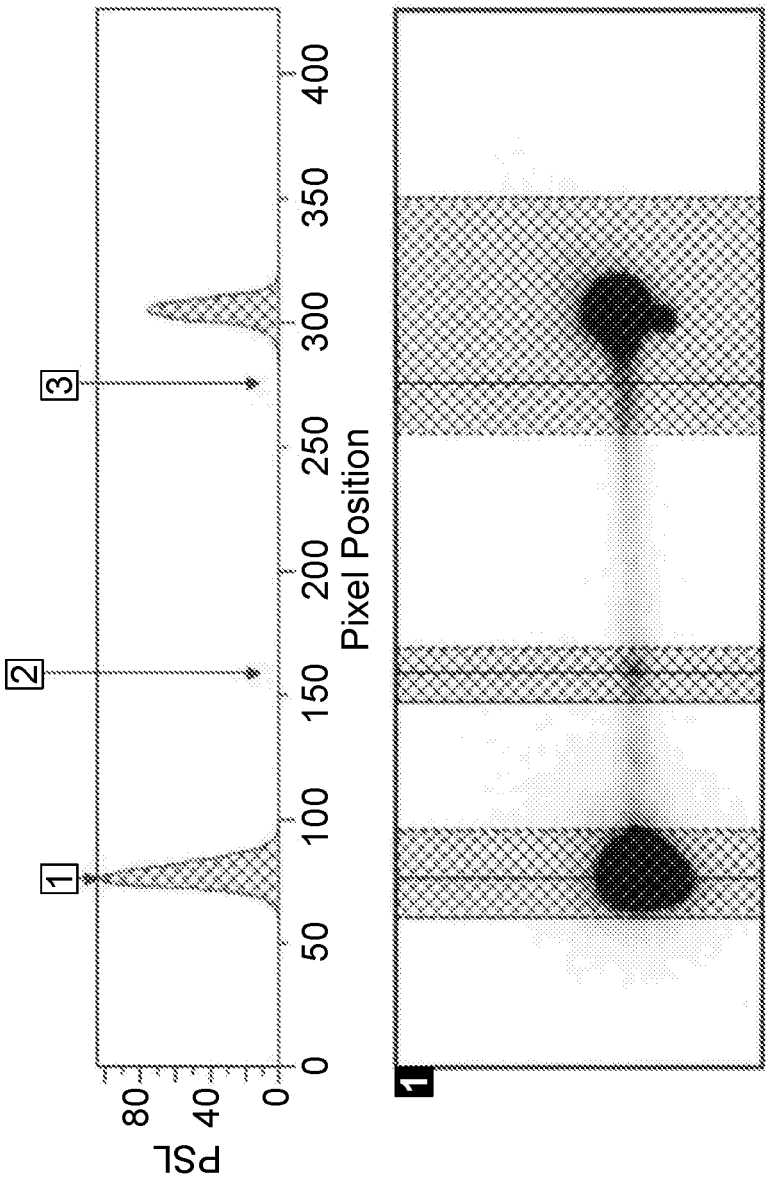

QMA
6M HCl + asc. acid
HPLC eluent
Et_4NHCO_3 solution
Precursor + catalyst

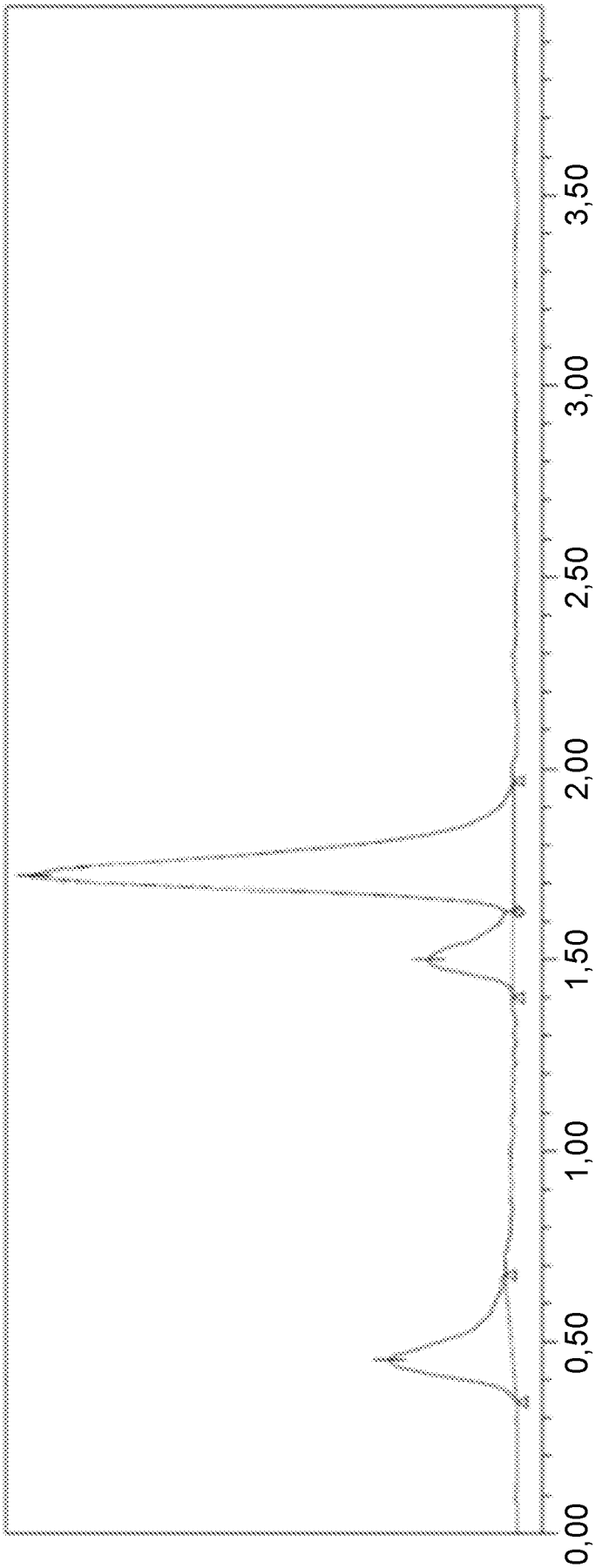
Fig. 2



Band Table

Band No	Volume	Vol+BKGnd	Band %	Lane %	Rf
1	184129.90	184129.90	58.350	56.384	10.170
2	1562.83	1562.83	0.495	0.470	0.374
3	120821.74	120821.74	41.146	39.754	0.640

Fig. 3



Components table

	Peak Name	RT	Area	% Area	Amount	Units	volume_ml	Max_volume	MaxVolume_2	SA_GBqmmol	activity_MBq	time_EOS	Dilution
1	[18F]F-	0.451	14236	19.00									1.00000
2	[18F]DOPA	1.721	52432	69.99									1.00000
3		1.498	8248	11.01									1.00000

Fig. 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2023/050475

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07B59/00 C07C229/36 C07F1/08 G21G4/08 G21G1/10
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07B C07C C07F G21G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2005/137421 A1 (WALSH JOSEPH C [US] ET AL) 23 June 2005 (2005-06-23) figure 2: transformation 7a to 8a; Method of preparing 18F-DOPA as defined in claim 16	1-15
A	WO 2015/058047 A2 (HARVARD COLLEGE [US]) 23 April 2015 (2015-04-23) Methyl ester of N,N,O-tris[(1,1-dimethylethoxy)carbonyl]-5-[[(1,1-dimethylethoxy)carbonyl]oxy]-2-(fluoro-18F)-L-Tyrosine: see example 6, pages 92-93, top of page 93, 9th listed compound of fluorinated derivative obtainable by OH -> 18F exchange using an imidazolium triflate reagent	1-15

-/--

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 November 2023	14/11/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lange, Tim

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2023/050475

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAKARAVAGE KATARINA J. ET AL: "Copper-Mediated Radiofluorination of Arylstannanes with [18 F]KF", ORGANIC LETTERS , vol. 18, no. 20 21 October 2016 (2016-10-21), pages 5440-5443, XP093024285, US ISSN: 1523-7060, DOI: 10.1021/acs.orglett.6b02911 Retrieved from the Internet: URL:http://pubs.acs.org/doi/pdf/10.1021/acs.orglett.6b02911 Figure 2, compound "16-SNMe3", obtained by radiofluorination of the arylstannae as precursor: see scheme 1 -& MAKARAVAGE KATARINA J ET AL: "S1 Supporting Information Copper-Mediated Radiofluorination of Arylstannanes with [18 F]KF", ORG. LETT. 2016, 18, 20, 5440-5443, 10 October 2016 (2016-10-10), XP093024366, DOI: 10.1021/acs.orglett.6b02911 figure S10 on page S45; synthesis protocol page 17	1-15

A	EDWARDS RICHARD ET AL: "Convenient Synthesis of Diaryliodonium Salts for the Production of [18 F]F-DOPA : Diaryliodonium Salts for the Production of [18 F]F-DOPA", EUROPEAN JOURNAL OF ORGANIC CHEMISTRY, vol. 2015, no. 3, 1 January 2015 (2015-01-01), pages 625-630, XP093024299, DE ISSN: 1434-193X, DOI: 10.1002/ejoc.201403378 Method for synthesizing 6-L-[18F]FDOPA by aryliodonium-18F exchange: see scheme 1, page 626	1-15

A	WO 2019/186135 A1 (UNIV OXFORD INNOVATION LTD [GB]) 3 October 2019 (2019-10-03) Cu(impy)4(OTf)2 shows improved 18F-exchange over Cu((OTf)2(py)4: see table 1, page 86	1-15

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2023/050475

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005137421 A1	23-06-2005	NONE	
WO 2015058047 A2	23-04-2015	US 2016272593 A1	22-09-2016
		WO 2015058047 A2	23-04-2015
WO 2019186135 A1	03-10-2019	EP 3774699 A1	17-02-2021
		US 2021284613 A1	16-09-2021
		WO 2019186135 A1	03-10-2019