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Radiosynthesis of 4-[^{18}F]fluoromethyl-L-phenylalanine and [^{18}F]FET via a same strategy and automated synthesis module

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Currently there is still a need for more potent amino acid analogues as tumour imaging agents for peripheral tumour imaging with PET as it was recently reported that the success of *O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) is limited to brain, head and neck tumours. As the earlier described 2-Amino-3-(2-[^{18}F]fluoromethyl-phenyl)-propionic acid (2-[^{18}F]FMP) suffered from intramolecular-catalysed defluorination, we synthesized 2-Amino-3-(4-[^{18}F]fluoromethyl-phenyl)-propionic acid (4-[^{18}F]FMP) as an alternative for tumour imaging with PET. Radiosynthesis of 4-[^{18}F]FMP, based on Br for [^{18}F] aliphatic nucleophilic exchange, was performed with a customized modular Scintomics automatic synthesis hot*box*^{three} system in a high overall yield of 30% and with a radiochemical purity of \gt 99%. 4-[^{18}F]FMP was found to be stable in its radiopharmaceutical formulation, even at high radioactivity concentrations. Additionally, for a comparative study, [^{18}F]FET was synthesized using the same setup in 40% overall yield, with a radiochemical purity \gt 99%. The described automated radiosynthesis allows the production of two different amino acid analogues with minor alternations to the parameter settings of the automated system, rendering this unit versatile for both research and clinical practice.

Keywords: 4-[^{18}F]fluoromethyl-L-phenylalanine; radiosynthesis; automation; PET

Introduction

At present, the world wide best established fluorinated amino acid analogue for brain tumour imaging with PET is *O*-(2′-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET).[1,2] However, its success was limited to brain, head and neck tumours as [^{18}F]FET did not seem to accumulate significantly in other peripheral tumours.[3,4] This means that more potent tumour-specific radiofluorinated amino acid analogues, which can be synthesized for routine clinical use are still required.

2-Amino-3-(2-[^{18}F]fluoromethyl-phenyl)-propionic acid (2-[^{18}F]FMP) described earlier by our group had good tumour targeting properties but suffers from radiodefluorination in aqueous solutions as well as *in vivo*, related to an intra-molecular interaction of the –CH_2–F group on the 2-position of the aromatic ring with the amino acid ammonium entity, leading to rapid hydrolysis.[5–8] Placing the fluoromethyl side chain in the para position avoids the unfavourable interaction with the ammonium entity as the intra molecular distance between both groups is too large to allow interaction. Therefore, it was decided to synthesize 2-Amino-3-(4-[^{18}F]fluoromethyl-phenyl)-propionic acid (4-[^{18}F]-fluoromethyl-L-phenylalanine or 4-[^{18}F]FMP, Figure 1), as a new and more stable alternative to 2-[^{18}F]FMP.

The radiosynthesis was automated using a customized modular Scintomics automatic synthesis hot*box*^{three} system to allow routine productions of 4-[^{18}F]FMP for the clinical evaluation and application of the compound for PET acquisition of peripheral tumours but also different types of brain tumours, linked to blood-brain barrier damage. Furthermore [^{18}F]FET was synthesisd on the same automatic system for a comparative study, meanwhile illustrating the versatility of the described system.

Results and discussion

Synthesis of the precursor and the nonradioactive reference 4-FMP

The ester formation of commercially available *N*-Boc-4-methyl-l-phenylalanine with *tert*-butyl 2,2,2-trichloroacetimidate by the described method yielded L-4-*tert*-butoxycarbonylamino-3-o-tolyl-propionic acid *tert*-butyl ester in 99% yield.

Radical bromination, using *N*-Bromosuccinimide and azobisisobutyronitrile as a radical initiator, yielded 66% of 3-(4-Bromomethyl-phenyl)-2-l-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester. The main side-products of the synthesis of 3-(4-Bromomethyl-phenyl)-2-l-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester were di- and tri-brominated species. The amounts of side products could be limited using dichloromethane as solvent instead of the generally used CCl_4.

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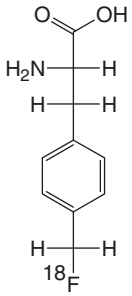

Figure 1. The structure of 4-[^{18}F]FMP.

The AgF based nonradioactive fluorination coupled to column chromatography yielded 65% of pure 3-(4-fluoromethyl-phenyl)-2-l-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester. The yield of the deprotection reaction was 87%, allowing the recovery of pure 4-FMP with an overall yield of 37%.

Fluoride acting as a strong base in the fluorination reaction did not cause racemisation of the L-α-carbon as Chiral HPLC showed only 4-fluoromethyl-L-phenylalanine to be present.

Radiosynthesis of 4-[^{18}F]FMP and [^{18}F]FET

The radiolabelling of the precursor with [^{18}F]fluoride provided Boc-4-[^{18}F]fluoromethyl-L-phenylalanine-tButyl ester with a reproducible radiochemical yield of at least 90%. It is important that the volume of AcN during the labelling exceeds the initial volume of the aqueous [^{18}F]fluoride solution, recovered from the QMA, as only under these conditions an optimal contact (and optimal reaction) with the dry [^{18}F]fluorine layer on the wall of the vial could be guaranteed. Furthermore, the reaction should not be continued for longer than 5 min as from this point loss of the Boc-protecting group was observed, leading to a lower overall yield as only fully protected compounds are collected in the subsequent HPLC purification step.

The deprotection step proved to be the most critical step in the whole procedure as extensive defluorination is observed if water is present in the deprotection medium. Hence, it is of major importance to 1° dry online the acetonitrile, here using the tandem minicolumn system (silicagel cartridge-$CaCl_2$ minicolumn) and 2° to use fresh and dry trifluoroacetic acid and dry dichloromethane. The loss of free [^{18}F]fluoride that is still observed is due to small traces of water still present, despite the drying procedures, as experiments with dry solvents showed a loss of only 7% of radiofluoride in the same reaction conditions. Therefore, we opted to use 5 mL aliquots of trifluoroacetic acid (Sigma Aldrich), rather than the larger available quantities which lost the required properties rapidly over time. We suspect that the TFA not only accumulates water but also seems to dissolve some components of the septum of the container, as a film like residu is observed on the wall of the vial when evaporating 'older' TFA/DCM: 1/4 (v/v) mixtures.

The use of chloroform instead of dichloromethane, as proposed by Hamacher (private communication), did not decrease defluorination as expected, certainly because in our conditions the physico-chemical properties of the solvent are of less influence than the presence of traces of water in the deprotection mixture.

The described radiosynthesis procedure resulted in an overall yield of about 30% noncarrier-added 4-[^{18}F]FMP within 120 min and a radiochemical purity of at least 99% as determined by HPLC and TLC. Chiral HPLC showed only the 4-[^{18}F]FMP to be present.

For the purpose of comparative studies in the clinic, we also synthesized [^{18}F]FET using the same experimental setup. Although a settled method for the synthesis of [^{18}F]FET is used by several other groups,[9] for practical reasons we opted to apply our custom synthesis setup for the production of [^{18}F]FET as this allowed us to synthesize both molecules on the same module without the need for an extra experimental setup. The principle of the labelling reaction is the same with some different time and temperature settings.

For purification only minor alternations were required as only the mobile phase for semi preparative HPLC needed to be adjusted, because of the difference in lipofilicity of fully protected [^{18}F]FET and 4-[^{18}F]FMP. The reaction time (100 min) using our method is somewhat longer in comparison to that of Hamacher *et al.*[9]

In the described conditions [^{18}F]FET was synthesized in 40% yield (comparable to the method described by Hamacher *et al.*[9]) with a radiochemical purity of at least 99%, determined by HPLC as well as by TLC and was enantiomerically pure.

The radiopharmaceutical formulations of 4-[^{18}F]FMP and [^{18}F]FET were tested for the presence of residual AcN and Kryptofix2.2.2 and were both found to be smaller than 1.10^{-3} mg/mL, which is far below the limits of the European Pharmacopee (0.44 mg/mL for Kryptofix2.2.2 and 0.8 mg/mL for AcN), proving that the described method allows the production of high quality 4-[^{18}F]FMP and [^{18}F]FET.

As discussed above, the described setup allows for both [^{18}F]FET and 4-[^{18}F]FMP to be synthesized on the same commercially available module (Scintomics hot*box*^{three}), without the requirement of changing any hardware on the module, rendering it a versatile unit, well suited for both research and clinical routine.

Efforts are currently being made to reduce the overall synthesis time by switching to a more convenient one-pot synthesis and deprotection, without the need of a preconcentration step from an aqueous medium thus avoiding the presence of traces of water and with the HPLC purification at the end.

Shelf life stability

Moving the CH_2F– group from ortho to para position in phenylalanine decreased considerably the hydrolysis of the noncarrier-added [^{18}F]fluoromethyl-L-phenylalanine analogue, confirming our hypothesis that the fast radio-defluorination of the noncarrier-added 2-[^{18}F]FMP was caused by an intramolecular interaction of the CH_2F– on the 2-position and amino acid amine group.[6] The activity concentration of the noncarrier-added 4-[^{18}F]FMP in the final formulation (pH 6, ethanol/0.9% NaCl solution: 1/99 (v/v)) was generally about 2.5 GBq/5 mL. At room temperature after 3 h at least 99.5% of the parent compound was still present. In case of [^{18}F]FET the amount of [^{18}F]fluoride was below the detection limit of the radio-HPLC method.

This means that in our case 1% of ethanol reduces sufficiently the radiolytic decomposition, even of the fluorobenzyl analogue that could be suspected to undergo more radiolytic hydrolysis.

Materials and methods

All the conventional products mentioned were at least analytical grade. The solvents were of HPLC quality or better. All NMR data

were obtained on an AVANCE DRX 250 instrument and all MS data on a Fisons VG II Quattro Mass Spectrometer.

Synthetic procedures

Synthesis of the precursor: 3-(4-bromomethyl-phenyl)-2-L-tert-butoxycarbonyl-amino-propionic acid tert*-butyl ester*

The free carboxyl group of commercially available (Peptech corp., USA) L-4-*tert*-butoxycarbonylamino-3-o-tolyl-propionic acid (*N*-Boc-4-methyl-L-phenylalanine) (1 g, 3.58 mmol) is protected with a *tert*-butyl ester by reacting it with *tert*-butyl 2,2,2-trichloroacetimidate (3.10 g, 14.19 mmol) in dichloromethane (36 mL) at room temperature for 24 h. After silica gel column chromatography (50 g Si-gel, id 2 × 25 cm, petroleumether/diethylether: 2/8) 3-(4-methyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester was obtained as an amorphous white solid (99% yield). ^{1}H-NMR (CD_3OD, 250 MHz): 7.14–7.20 (4H, m), 4.53 (1H, dd, $J_1 = 10.0$ Hz, $J_2 = 12.5$ Hz), 3.11 (2H, dd, $J_1 = 6.5$, $J_2 = 14.5$ Hz), 2.41 (3H, s), 1.52 (9H, s), 1.51 (9H, s). MS (EI) *m/z* 336 (MH^+).

For the radical bromination of the methyl side chain, 3-(4-methyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (500 mg, 1.49 mmol) was reacted with *N*-bromosuccinimide (245 mg, 1.38 mmol), using azobisisobutyronitrile (28 mg, 0.17 mmol) as a radical initiator, in dichloromethane (50 mL) at 50°C for 2 h. The crude product was purified via silica gel column chromatography (50 g Si-gel, id 2 × 25 cm, petroleumether/diethylether: 1/9) to provide the 3-(4-bromomethyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester as an amorphous white solid in 66% yield. ^{1}H-NMR ($CDCl_3$, 500 MHz): 7.42 (2H, d, $J = 7.3$), 7.26 (2H, d, $J = 7.8$), 4.56 (2H, dd, $J_1 = 6.5$, $J_2 = 18.7$ Hz), 3.15 (2H, s), 1.53 (9H, s), 1.50 (9H, s). MS (EI) *m/z* 413 (M^+).

Synthesis of the nonradioactive 2-Amino-3-(4-fluoromethyl-phenyl)-propionic acid (4-fluoromethyl-L-phenylalanine or 4FMP)

In the first step, 3-(4-bromomethyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (140 mg, 0.339 mmol) was treated with an excess of AgF (180 mg, 1.42 mmol) in dry acetonitrile (5 mL) for 3 h at 65°C after which the crude product was purified via silica gel column chromatography (50 g Si-gel, id 2 × 25 cm) using a gradient of petroleum ether (40–60°C) and ethyl acetate.

The ^{1}H-NMR and EIMS data for 3-(4-fluoromethyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester, obtained as a white amorphous solid in 65% yield, were: ^{1}H-NMR (CD_3OD, 250 MHz): 7.24–7.40 (4H, m), 5.58 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 19.0$ Hz, J [^{19}F-^{1}H] = 47.8 Hz), 5.39 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 19.3$ Hz, J [^{19}F-^{1}H] = 47.8 Hz), 5.08 (1H, dd, $J_1 = 7.2$ Hz, $J_2 = 14.2$ Hz), 3.17 (1H, dd, $J_1 = 6.3$ Hz, $J_2 = 14.0$ Hz), 3.06 (1H, dd, $J_1 = 7.5$ Hz, $J_2 = 14.3$ Hz) 1.40 (18H, s). MS (EI) *m/z* 354 (MH^+).

The final deprotected fluorinated amino acid was obtained after deprotection of 3-(4-fluoromethyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester (200 mg, 0.541 mmol) in a dichloromethane/trifluoroactic acid: 1/1 mixture (20 mL) for 90 min at room temperature. After reaction, the solvents were removed by rotatory evaporation. The residue was suspended in 5 mL dichloromethane/ethanol: 1/1, stirred and subsequently evaporated by a gentle N_2 flow. This process was repeated twice. The residue was suspended in hexane/dichloromethane: 4/1 by sonication for 10 min and the suspension stored at −20°C overnight. The solvents were removed by filtration and the 2-Amino-3-(4-fluoromethyl-phenyl)-propionic acid (4FMP) was obtained as a white solid (87%). ^{1}H-NMR (CD_3OD, 250 MHz): 7.26–7.37 (4H, m), 5.46 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 19.0$ Hz, J[^{19}F-^{1}H] = 45.0 Hz), 5.37 (1H, dd, $J_1 = 11.0$ Hz, $J_2 = 19.0$ Hz, J[^{19}F-^{1}H] = 45.0 Hz), 3.97 (1H, dd, $J_1 = 7.2$ Hz, $J_2 = 14.2$ Hz), 3.26 (1H, dd, $J_1 = 7.5$ Hz, $J_2 = 14.2$ Hz), 3.10 (1H, dd, $J_1 = 7.5$ Hz, $J_2 = 14.2$ Hz). MS (EI) *m/z* 198 (MH^+).

Radiosynthesis

The radiosynthesis has been automated using a customized modular Scintomics hot*box*^{three} system (Scintomics, Fürstenfeldbruck, Germany). A standard Scintomics hot*box*^{three} was chosen as the core of the system, equipped with preparative Vario*pump* LC1, VarioHPLC, Vario*detect* and a PS1 powersupply unit. All connections between the valves are made by means of the standard Scintomics PEEK tubing. The radiosynthesis is summarized in Figure 2 while Figure 3 shows the flow scheme of the automated synthesis module.

Radiosynthesis of 2-Amino-3-(4-[^{18}F]fluoromethyl-phenyl)-propionic acid (4-[^{18}F]FMP)

[^{18}F]fluoride recovery and radiolabelling. About 9.2 GBq of [^{18}F]fluoride, produced with a CGR 560 multiple particle variable energy AVR Cyclotron using the ^{18}O (p,n) ^{18}F nuclear reaction, was separated from the ^{18}O-enriched water by trapping on a SepPak Light Waters Accell^{TM} Plus QMA (Waters), preconditioned with 10 mL of a 0.01 M solution of K_2CO_3 (Merck) and 20 mL of de-ionized water. Elution of the activity was achieved with 0.5 mL of an AcN/water (9:1) mixture containing 1 mg of K_2CO_3 (Merck) and 10 mg of Kryptofix2.2.2 (Acros) into a 5 mL reaction vial (Pierce) by means of a dropwise flowrate. This mixture was azeotropically dried at 140°C (6 min) and the vial was subsequently cooled to 120°C by external airflow. Thereafter, 1 mL of dry AcN containing 10 μmol (4 mg) of precursor 3-(4-bromomethyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester was added and N_2 pressure was applied. The labelling reaction was allowed to occur for 5 min, after which the vial was cooled to room temperature. The AcN phase was transferred into a second 5 mL conical glass vial containing 1 mL of 1 mM NaF solution. The first vial was rinsed with another 0.5 mL AcN that is also transferred to the second vial.

Semi-preparative HPLC separation. The content of this vial (AcN/H_2O: 60/40 (v/v)) was mixed by nitrogen bubbling and injected for semi-preparative RP-HPLC separation using an Apollo

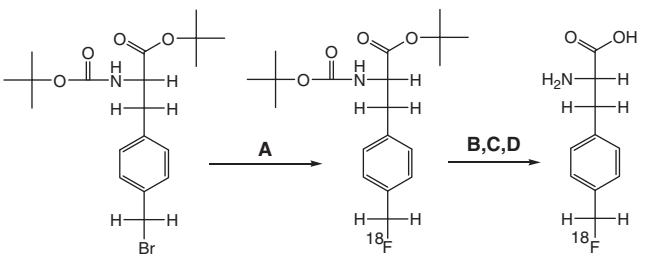

Figure 2. Summarized radiosynthesis of 4-[^{18}F]FMP: (A) 5 min, 120°C, AcN, K_{222}, K_2CO_3, $^{18}F^-$; (B) semipreparative HPLC: Apollo C18 250 × 10 mm (5 μ) column, AcN/H_2O: 65/35 (v/v) 6 mL/min; (C) preconcentration and elution from C18 minicolumn; (D) Deprotection: TFA/DCM: 1/4 (v/v), 50°C, 20 min. Final elution over tandem C18 and Al_2O_3 minicolumns.

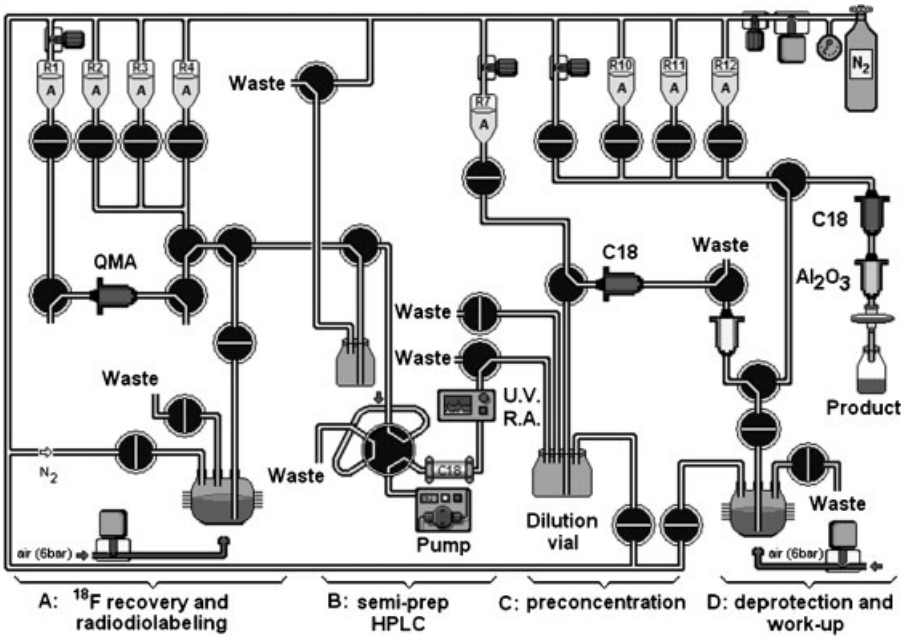

Figure 3. Flow scheme of the custom scintomics synthesis module setup for the radiosynthesis of 4-[^{18}F]FMP. R1: 0.5 mL AcN/water: 9/1 (v/v) containing 1 mg of K_2CO_3 and 10 mg of Kryptofix2.2.2.; R2: 500 µL dry AcN ; R3: 500 µL AcN; R4: 1 mL AcN containing 4 mg of 3-(4-bromomethyl-phenyl)-2-L-*tert*-butoxycarbonylamino-propionic acid *tert*-butyl ester; R7: 1 mL AcN; R10: 1 ml dichloromethane/trifluoroacetic acid: 4/1 (v/v); R11: 1 mL dichloromethane; R12:, 5 mL of a 1% Ethanol/water (v/v).

C18 250 × 10 mm (5 µ) column and AcN/water: 65/35 (v/v) as mobile phase at a flow rate of 6 mL/min. The retention times of [^{18}F]fluoride and of [^{18}F]-NBoc4FMPtbutyl ester were, respectively, 2.5 and 15 min. The protected n.c.a. radiofluorinated compound was collected in a 100 mL vial containing 54 mL of de-ionized water.

Preconcentration. After mixing the collected [^{18}F]-NBoc4FMPt-butyl ester and the deionized water by N_2 bubbling, this solution was passed through a 50 mg Extract-Clean C18 mini column (Alltech) at a flowrate of 10 mL/min, hereby trapping the n.c.a. ^{18}F-labeled compound on the C18 phase. The n.c.a. [^{18}F]-NBoc4FMPtbutyl ester, adsorbed on the C18 column, was eluted with 1 mL of dry AcN through a tandem of a custom made dry $CaCl_2$ (300 mg, Sigma-Aldrich) mini-column and a 300 mg Extract-clean Silica mini-column (Alltech) into a 5 mL vial that was kept at 50°C during the entire process. The AcN was evaporated using a gentle N_2 flow for 10 min.

Deprotection and work-up of 4-[^{18}F]FMP. One millilitre of a dichloromethane/trifluoroacetic acid: 4/1 (v/v) mixture was added and deprotection applied for 20 min under N_2 pressure. After the removal of the solvents by evaporation (gentle N_2 flow at room temperature), another 1 mL of dichloromethane was added and evaporated (5 min.). Finally, 5 mL of a 1% ethanol/water (v/v) mixture was added to the vial. After mixing by N_2 flow for 10 s the solution was passed through a tandem of a 50 mg Extract-Clean C18 mini column (Alltech) and a 50 mg Alumina-N (Alltech) mini-column (to remove traces of respectively 4-[^{18}F]FMP-tButyl ester and [^{18}F]fluoride) and the 4-[^{18}F]FMP was recovered in a sterile vial containing 45 mg of saline after passing through a 0.22 ìm Cathivex filter (Millipore).

Radiosynthesis of O-(2′-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET)

In our hands [^{18}F]FET was synthesized following almost the same steps as described for 4-[^{18}F]FMP.

[^{18}F]fluoride recovery and radiolabelling. [^{18}F]fluoride recovery and drying was performed in exactly the same way as for 4FMP. Subsequently 1 mL of dry AcN containing 10 µmol (7 mg of precursor: L-Tyrosine, *O*-[2-[[(4-methylphenyl)sulfonyl]oxy]ethyl]-*N*-(triphenylmethyl)-, 1,1-dimethylethyl ester) (ABX, Germany) was added to the reaction vial and N_2 pressure applied. The labelling reaction was allowed to occur for 8 min at 120°C, after which the vial was cooled to room temperature The AcN phase was then transferred into a second 5 mL conical glass vial containing 0.625 mL of 1 mM NaF solution. The first vial was then rinsed with 0.875 mL of AcN and transferred to the second vial.

Semi-preparative HPLC separation. The content of the vial was mixed by nitrogen bubbling resulting in a AcN/H_2O: 75/25 (v/v) solution and injected for semi-preparative RP-HPLC separation, using an Apollo C18 250 × 10 mm (5 µ) column and AcN/H_2O: 70/30 (v/v) as mobile phase at a flow rate of 6 mL/min. The retention times of [^{18}F]fluoride and of [^{18}F]-trityl4FETtbutyl ester ([^{18}F]-*O*-[2-fluoroethyl]-*N*-(triphenyl-methyl)-1,1-dimethylethyl ester) were, respectively, 2.5 and 32.2 min. The protected n.c.a. radiofluorinated compound was collected over 3 min in a 100 mL vial containing 54 mL of deionized water.

Preconcentration. After mixing by N_2 bubbling the solution was passed through a 50 mg Extract-Clean C18 mini column (Alltech) at a flow rate of 10 mL/min through and the ^{18}F-labeled compound trapped. The C18 column was rinsed with 10 mL of deionized water and purged with a dry N_2 flow. The [^{18}F]-trityl4FETtbutyl ester adsorbed on the C18 column was eluted with 1 mL of dry AcN through a tandem of a custom made $CaCl_2$ (300 mg, Sigma-Aldrich) mini-column and a 300 mg Extract-clean Silica mini-column (Alltech) into a 5 mL vial kept at 90°C during the entire process.

Deprotection and work-up of [^{18}F]FET. To the reaction vial was added 0.5 mL of trifluoroacetic acid and the deprotection reaction was allowed to proceed for 15 min. After removal of the solvents by evaporation (gentle N_2 flow at room temperature), another 0.250 mL of acetonitrile was added and evaporated (5 min). Finally, after cooling to room temperature 1 mL of a 10% ethanol/water (v/v) solution was added to the vial. After mixing by N_2 flow over 10 s the solution was passed through a tandem of a 50 mg Extract-Clean C18 mini column (Alltech) and a Alumina-N (Alltech, 50 mg) mini-column and the product was recovered after passing through a 0.22 μm Cathivex filter (Millipore) in a sterile vial containing 4 mL of a 1.125% NaCl solution.

Quality control

Quality control of the final n.c.a. ^{18}F-labeled compounds was achieved by HPLC analysis, performed on a Vydac C18 Monomeric 120 Å (125 × 4 mm) (Grace) column using an AcN/1 mM solution of CH_3COONH_4 and NaF: 5/95 (v/v) mixture of pH 6.5 as mobile phase with a flow rate of 1 mL/min while monitoring both radioactivity (NaI(Tl), Harshaw Chemie) and UV absorption at 254 nm (Shimadzu). The k' values were respectively: 0.6 for [^{18}F]fluoride and 3.9 for 4-[^{18}F]FMP. The k' value of [^{18}F]FET was 5.5. Chiral analysis was performed using an Astec Chirobiotic T column (5 ìm, 125 mm × 4.6 mm) (Alltech) and ethanol/water: 80/20 (v/v) as mobile phase with a flow of 1 mL/min, using the same detection system.

TLC control was performed using RP-18 F254S (5 × 7.5 cm) plates with MeOH/H_2O [50:50 (v/v)] as mobile phase. The R_f values were, respectively, 0.0 for [^{18}F], 0.5 for 4-[^{18}F]FMP and 0.4 for [^{18}F]FET. The radioactivity in both spots was measured.

The amount of Kryptofix2.2.2 still present in the final radiopharmaceutical formulations was assessed using the colour spot test that was described by Mock *et al*.[10]

The amount of residual AcN present in the final solution was determined by means of the GC-method developed by Channinga *et al*.,[11] using a J&W HP-INNOWax 30 m × 250 mm × 0.25 μm column.

Shelf-life stability of 4-[^{18}F]FMP and [^{18}F]FET

The radiopharmaceutical formulation containing 0.9% NaCl and 1% v/v of ethanol at pH 6 was kept at 25°C. At appropriate times a sample was taken and analysed by HPLC and TLC (QC procedure).

Conclusion

The switching of the fluoromethyl side on the ring of phenylalanine resulted in a compound, namely noncarrier-added 4-[^{18}F]FMP, that was stable in its radiopharmaceutical formulation, even at high radioactivity concentrations. The described automated radiosynthesis for this compound makes it possible to produce 4-[^{18}F]FMP in a high yield (30% overall) and with a high radiochemical purity (>99%). Moreover, the developed setup is also suited to synthesize [^{18}F]FET in yields (40%) comparable to earlier described methods, without having to change any hardware components. The versatility of the method and the automatic system makes the new compound readily accessible for widespread use in both research and clinical practice.

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