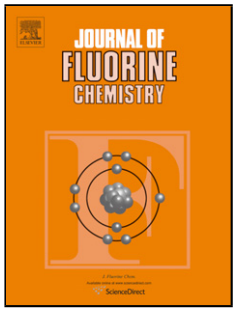

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Author: Johnny Castillo Meleán Sven Humpert Johannes Ermert Heinz H. Coenen

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Stereoselective radiosynthesis of L- and D-3-[^{18}F]fluoro-α-methyltyrosine

Johnny Castillo Meleán, Sven Humpert, Johannes Ermert, Heinz H. Coenen

Institut für Neurowissenschaften und Medizin, INM-5: Nuklearchemie, Forschungszentrum Jülich, 52425 Jülich, Germany

Keywords: radiofluorination, fluoroaromatic amino acids, Bayer-Villiger oxidation, isotopic exchange, fluorine-18

Abstract

A three-step radiosynthesis is described which allows for the first time the preparation of L- and D-3-[^{18}F]fluoro-α-methyltyrosine starting from [^{18}F]fluoride. Corresponding 3-fluoro-4-formyl-benzylated Schöllkopf derivatives were ^{18}F-fluorinated as precursors by isotopic exchange, followed by Baeyer-Villiger oxidation and subsequent hydrolysis of protecting groups in acidic medium. The three-step, two-pot radiosynthesis provided each enantiomer of 3-[^{18}F]fluoro-α-methyltyrosine with an overall radiochemical yield of 32 ± 8 % and an enantiomeric excess ≥ 95 % within 140 min in carrier-added form with a molar activity of 20 GBq/mmol.

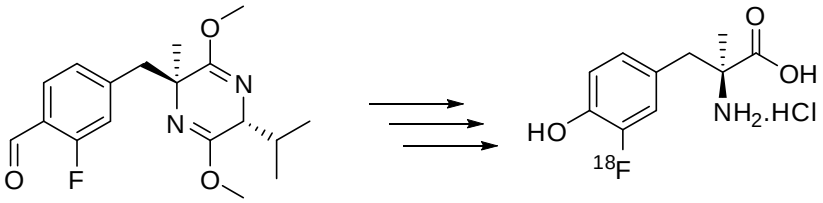
Highlights

A nucleophilic synthesis of L- and D-3-[^{18}F]fluoro-α-methyltyrosine is described for the first time.

This was achieved by a three-step, two-pot procedure.

Each enantiomer is obtained in an overall RCY of 32 ± 8 % and an ee of ≥ 95 %.

Graphical Abstract



L-[^{18}F]FAMT

Graphical Abstract

A three-step radiosynthesis of 3-[^{18}F]fluoro-α-methyltyrosine provided both enantiomers with 32 ±8 % radiochemical yield and an enantiomeric purity of ≥ 95 % within 140 min.

Introduction

L-3-[^{18}F]Fluoro-α-methyltyrosine (L-[^{18}F]FAMT) is a very promising radiotracer for tumor detection with positron emission tomography (PET), a non-invasive imaging technique. L-[^{18}F]FAMT accumulates in areas with high metabolic activity but shows low accumulation in tissues with high glucose metabolism, allowing an accurate diagnosis of malignancy in the brain [1]. In contrast to other labeled amino acids, L-[^{18}F]FAMT has also been discussed as a potentially useful tracer for depiction of peripheral tumors and even of metastatic lesions in the cardiac region [2]. Distinct from other amino acid PET tracers, it is selective to the L-type

amino acid transporter 1 (LAT1) due to its α-methyl group. This feature is proposed to contribute to its highly specific tumor accumulation [3]. In human lung cancer, L-[^{18}F]FAMT uptake was closely correlated with LAT1, glycoprotein CD98, cell proliferation and angiogenesis [4]. The usefulness of L-[^{18}F]FAMT in PET imaging combined with computed tomography (CT) for the detection of bone metastases, regardless of the bone lesion phenotype, has also been demonstrated [5]. Moreover, it has been suggested that this tracer may also be a suitable replacement for [^{18}F]FDG in assessing malignant lymph nodes of oral cancer, especially non-tongue cancer [6].

The enantiomeric tracer, D-[^{18}F]FAMT, has also been pharmacologically evaluated. It is stable both *in vitro* and *in vivo,* and it is also transported into the tumor via LAT1 [7]. In PET-studies on mouse models it showed rapid clearance from the blood and reduced radioactivity in non-target organs. Compared to L-[^{18}F]FAMT, D-[^{18}F]FAMT thus provides a higher tumor-to-background contrast and lower exposure dose to patients, suggesting that it could potentially serve as an even better tracer for imaging of malignant tumors [7].

So far, [^{18}F]FAMT has only been synthesized by electrophilic radiofluorination of α-methyltyrosine employing either [^{18}F]AcOF in trifluoroacetic acid [8] or [^{18}F]F_2 in anhydrous HF [9] which limits a broader use of this tracer. Nucleophilic radiofluorination has some principal advantages over electrophilic methods which are especially restricted by limited amounts of activity [10, 11]. Nucleophilic preparation of ^{18}F-labelled aromatic amino acids, as mainly demonstrated for the synthesis of 6-[^{18}F]fluoro-L-DOPA, can be performed by build-up synthesis, using a multi-step phase-transfer catalyst strategy [12] which was recently modified and adapted to an automatable process [13]. However, this modification necessitates a remote-controlled synthesizer with two reactors. Very recently, several new strategies for the nucleophilic synthesis of 6-[^{18}F]fluoro-L-DOPA have been developed [14]. Also, during the progress of our study, methods using a copper-catalyzed nucleophilic ^{18}F-fluorination of mesityl diaryliodonium precursors [15] or of boronic esters derivatives [16] opened new,

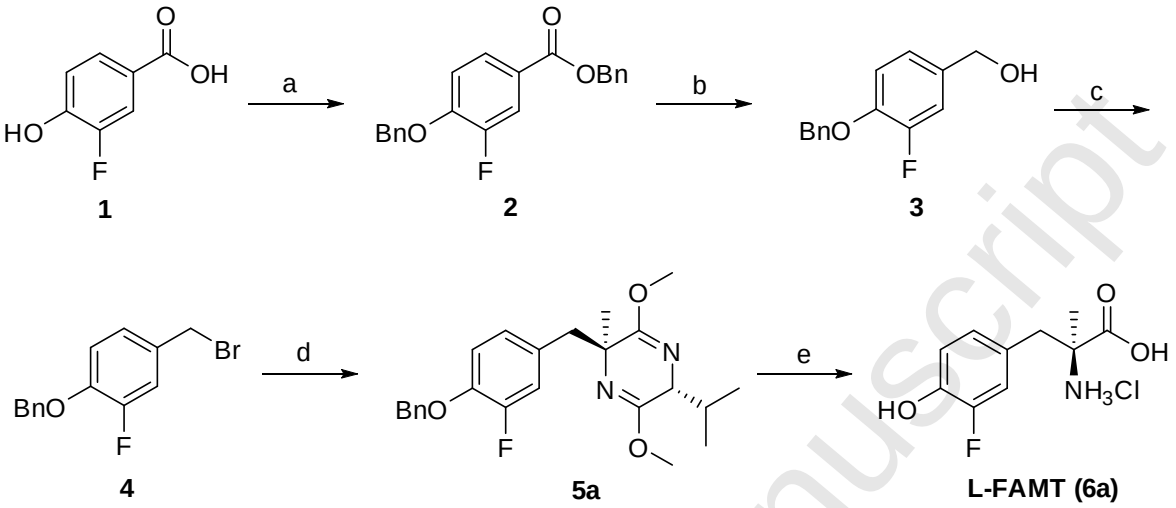
more direct ways for the syntheses of ^{18}F-labelled aromatic amino acids. However, an automated synthesis procedure of those methods still requires further optimization [17, 18]. A method known for longer, which has the advantage of being easier to automate, is based on nucleophilic substitution with [^{18}F]fluoride on carbonyl-activated precursors, producing aromatic amino acids in a three-step, two-pot procedure. For this, following reaction sequence was optimized: isotopic exchange, Baeyer-Villiger oxidation or decarbonylation reaction, and hydrolysis, as exemplified with 6-[^{18}F]fluoro-L-DOPA [19], 2-[^{18}F]fluoro-L-phenylalanine, 2-[^{18}F]fluoro-L-tyrosine [20] and 6-[^{18}F]fluoro-meta-L-tyrosine [21]. The aim of this work was to adapt this three step radiosynthetic approach, which particularly has the advantage of easy automation, to the preparation of L- and D-[^{18}F]FAMT, starting from [^{18}F]fluoride.

Results and discussion

Synthesis of the standards

In earlier studies non-radioactive standards of FAMT were prepared through direct electrophilic fluorination of α-methyl tyrosine leading to a mixture of position isomers that are difficult to separate [8]. Here, L- and D-FAMT were prepared by a five-step linear synthesis according to Scheme 1. Benzylation of commercially available 3-fluoro-4-hydroxybenzoic acid **1** under basic conditions produced compound **2** in 91 % yield. Reduction of the ester led to benzyl alcohol **3** in 93 % yield. The benzyl bromide **4** was prepared by means of an Appel reaction [22] with 89 % yield. Then, the benzyl bromide derivative 4 was reacted with [(5*R*)-3,6-dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]lithium (Li-Me-(*R*)-Schöllkopf) [23], furnishing the adduct **5a** with 60 % yield. Finally, **5a** was hydrolyzed with HCl at high temperature using a pressure tube. The crude product was purified by high-performance-liquid-chromatography (HPLC) giving L-FAMT with 75 % yield, rendering an overall synthesis yield of 34 %. The D-FAMT standard was prepared

using the same synthetic pathway, but with the Li-Me-(*S*)-Schöllkopf auxiliary instead with an overall yield of 32 %. An enantiomeric excess of ≥ 99 % was confirmed by chiral HPLC.



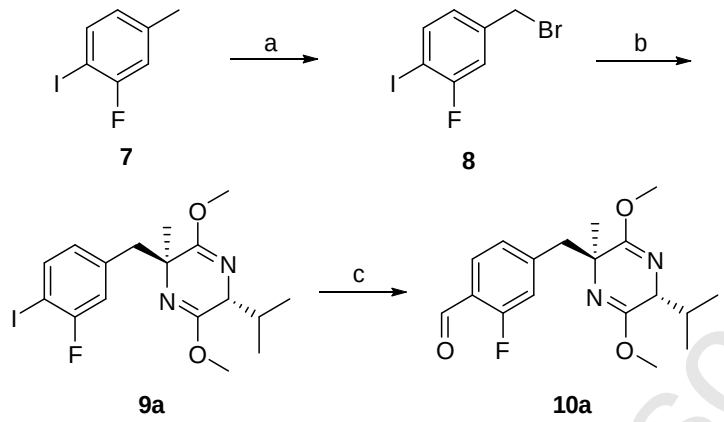
Scheme 1. Synthesis of L-3-fluoro-α-methyltyrosine (L-FAMT).

a) BnBr, Na_2CO_3, acetone, reflux; b) $LiAlH_4$, THF; c) CBr_4, PPh_3, CH_2Cl_2, 0 °C; d) Li-Me-Schöllkopf, THF, -78 °C – room temperature (r.t.).; e) HCl, 160 °C.

Synthesis of the labelling precursor

The diastereoselective derivatives of 4-([-3,6-dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]methyl)-2-fluorobenzaldehyde were synthesized in a 3-step synthesis starting from 3-fluoro-4-iodotoluene as delineated in Scheme 2. The latter was at first converted to the benzyl bromide derivative **8** using a Wohl-Ziegler reaction [24] with 86 % yield. The next step was the substitution of the benzyl bromide **8** with the lithium enolate of the chiral auxiliary Me-(*R*)Schöllkopf. This reaction furnished compound **9a** with 60 % yield. Finally, compound **9a** was formylated using a halogen-metal exchange, yielding the enantiomerically pure labeling precursor **10a** with 58 % yield. The overall yield for the synthesis of the precursor was 31 %. Following an analogous procedure, but using the Me-

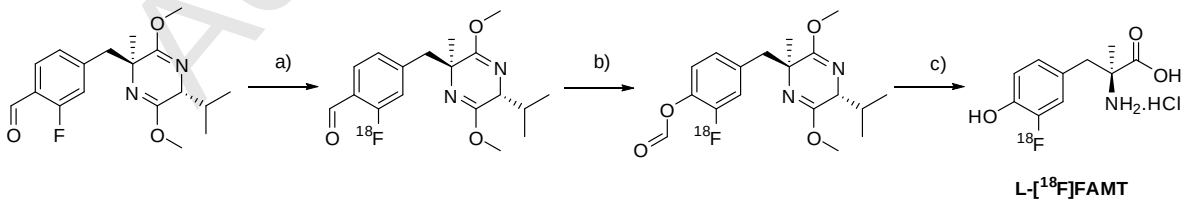
(*S*)-Schöllkopf derivative instead, the enantiomeric precursor **10b** was obtained with an overall yield of 30 %.



Scheme 2. Synthesis of the precursor for L-[^{18}F]FAMT. Conditions: a) *N*-bromosuccinimide, benzoylperoxide, CCl_4, reflux; b) Li-Me-(*R*)-Schöllkopf, THF, -78 °C – r.t.; c) 1. *i*-PrMgBr, THF, 0 °C; 2. DMF, r.t.

Radiosynthesis

As mentioned in the introduction, the corresponding precursors **10a** and **10b** were ^{18}F-fluorinated by nucleophilic isotopic exchange, followed by the conversion of the activating aldehyde into a formic acid arylester (**[^{18}F]11a**) by Baeyer-Villiger oxidation (BVO). This resulting formate was then cleaved simultaneously with the Schöllkopf auxiliary by hydrolysis in concentrated HCl (Scheme 3).



Scheme 3. Radiosynthesis of L-[^{18}F]FAMT.

a) TBA^{18}F, DMF, 120 °C, 7 min, RCY: 64 ± 6 %; b) peracetic acid, CF_3CH_2OH, H_2SO_4, 80 °C, 30 min, RCY: 63 ± 2 %; c) HCl, 160 °C, 30 min, RCY: quantitative; overall RCY: 32 ± 8 %.

In the following, a given radiochemical yield (RCY) is related to each single reaction step including its work-up procedure. The purity of the reaction product upon ^{18}F-exchange was confirmed by TLC and of that after oxidation and quantitative hydrolysis by radio-HPLC. The RCY over all three reaction steps is denoted as 'overall RCY'.

Previous studies have shown that DMF is the solvent of choice for the isotopic exchange reaction for this class of precursors. The results of kinetic examinations showed that the maximum RCY was reached after 5 minutes and it remained almost constant until about 10 minutes. Thereafter, the RCY decreased and several side products were detected by radio-TLC. The reaction time for further experiments was therefore set at 7 min. Two anion activator systems were compared: The traditional Kryptofix®2.2.2./K_2CO_3 and tetrabutylammonium bicarbonate. The results obtained with the two systems are depicted in Figure 1.

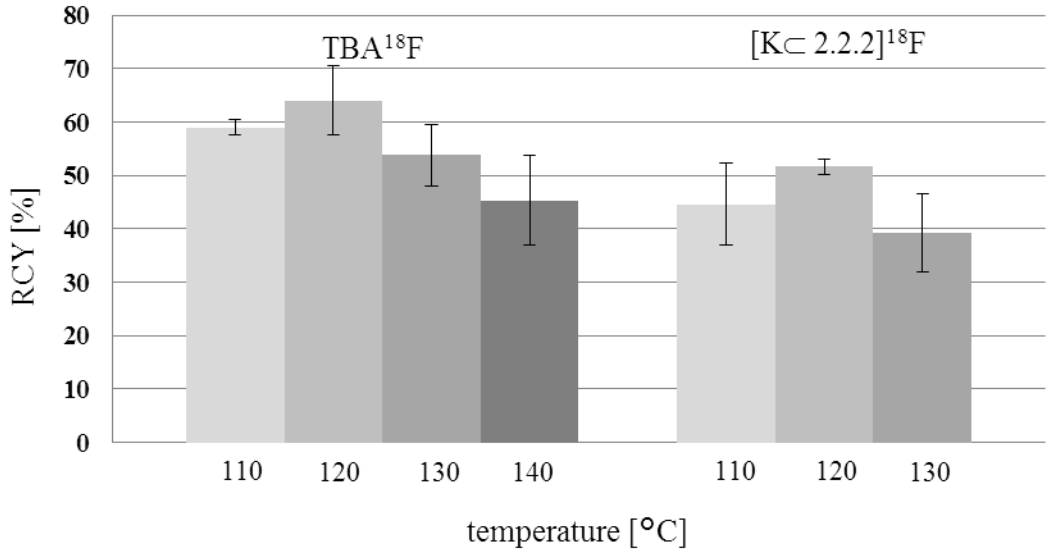


Figure 1. Influence of anion activator and reaction temperature on isotopic exchange; conditions: 15 µmol **10**, $TBAHCO_3$ (16.5 µmol), or Kryptofix©2.2.2 (26 µmol) and 1 M potassium carbonate solution (13 µL), 1 mL DMF, 7 min reaction time (n = 3).

The best radiochemical yield of 64 ± 6 % was achieved with $TBAHCO_3$ at 120 °C. It was also observed that the RCY decreased at lower as well as at higher temperatures: the RCY detriment at higher temperatures being a consequence of the formation of multiple unknown side products. The α-methyl group of [^{18}F]FAMT prevented an epimerization of the precursor during the labeling process. Thus, in contrast to the synthesis of 2-[^{18}F]fluoro-L-tyrosine [20] and 6-[^{18}F]fluoro-meta-L-tyrosine [21] by isotopic exchange, harsher conditions are applicable for the isotopic exchange reaction.

The subsequent Baeyer-Villiger reaction was carried out using 2,2,2-trifluoroethanol and sulfuric acid as adequate solvent and additive, respectively, for the oxidation of non-activated [^{18}F]fluorobenzaldehydes as recently reported [25]. A good RCY of 63 ± 2 % was achieved using *m*-chloroperoxybenzoic acid (*m*-CPBA) as oxidant. However, the reactions employing *m*-CPBA gave a solid residue after hydrolysis, which can be a problem for the foreseen automation process. Further experiments proved peracetic acid as good replacement since it did not form solid by-products and delivered still a good RCY of 57 ± 6 %. Oxone® was also explored as oxidant; however, this proved to be a non-reliable alternative since the RCY of the reaction varied between 27 and 70 %, with an average value of 43 ± 16 %.

The cleavage of the auxiliary was performed using aqueous concentrated HCl. A high temperature of 160 °C was needed in order to complete the reaction within a reasonable time of 30 min quantitatively. Reaction times and temperatures lower than 30 min and 160 °C, respectively, led to significantly lower yields.

After hydrolysis, the crude product was purified by semi-preparative HPLC on a C-18 column with embedded polar groups allowing the use of sodium phosphate buffer with 2 % ethanol as eluent. [^{18}F]FAMT was obtained in an about 8 mL volume and can directly be used for injection after filtering through a 0.2 µm Millipore filter.

First analyses of the purity revealed only an enantiomeric excess of > 90 % of the final products. It is known from literature that coupling of the benzyl bromide with the Schöllkopf reagent (cf. Scheme 2) generally leads to a diastereomeric excess of 95% or more [26, 27]. A further analysis by HPLC was therefore done and it revealed an impurity which turned out to be the corresponding diastereomer. This was obviously not separated under the chromatographic conditions chosen before and also not found in the NMR spectra. Therefore, a preparative HPLC purification of precursor 10 was performed to improve the enantiomeric excess of [^{18}F]FAMT. In fact, after this purification an enantiomeric excess ≥ 95 % of FAMT was achieved which is in the acceptable range for clinical application.

The synthesis was completed within 140 min and the overall radiochemical yield obtained for both L-[^{18}F]FAMT or D-[^{18}F]FAMT was 32 ± 8 % (n = 3), each with an enantiomeric excess ≥ 95 %. The whole synthetic sequence was performed and optimized manually with starting activities of ≤ 300 MBq, yielding a molar activity of ≤ 20 GBq/mmol.

The specific activity obtained by isotopic exchange reactions is a function of the degree of substitution and of both the amount of radioactivity of [^{18}F]fluoride and that of the precursor [20]. When starting with high amounts of [^{18}F]fluoride activity of > 20 GBq, which are easily produced, a much higher specific activity can be expected than that obtainable with electrophilic ^{18}F-fluorination.

Summary

The procedure developed here constitutes an efficient and amenable alternative to previous syntheses of L-[^{18}F]FAMT and D-[^{18}F]FAMT carried out by electrophilic ^{18}F-fluorination which are limited to low amounts of activity. The method presented here is based on a nucleophilic exchange reaction using the advantage of the large scale production of [^{18}F]fluoride and offers an easy automation. The best radiochemical yield of 64 ± 6 % of the isotopic exchange was achieved using $TBAHCO_3$ at 120 °C. The combined RCY of the BVO and hydrolysis reactions was 57 ± 6 %. Thus, the overall RCY for the three-step synthesis amounted to 32 ± 8 % which was achieved within 140 minutes of total synthesis time with a >95 % e.e. of the respective enantiomer.

Experimental

General

Dry solvents and chemicals were purchased from Aldrich and Merck, Germany. All were used without further purification.

^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian Inova 600 MHz spectrometer using $CDCl_3$ or d_6-DMSO as solvents. All chemical shifts given below are in δ ppm using the signals of the appropriate solvent as a reference. HRMS spectra were obtained on an FTICR 'LTQ FT Ultra' device (Thermo Fisher Scientific, Germany). Melting points are uncorrected and were determined on a Mettler FP-61 apparatus in open capillaries. Elemental analyses were performed on a Leco CHNS-932 CHNS analyser (Central Institute for Engineering, Electronics and Analytics (ZEA), Forschungszentrum Jülich).

Flash chromatography was performed either on Macherey-Nagel (Germany) 60 M (0.04-0.06 mm) silica gel or using a Grace Reveleris® flash chromatography system with ELSD and UV detection on 40 µm flash-cartridges (Grace, Belgium). Thin layer chromatography (TLC) was performed on precoated plates of silica gel 60 F_{254} (Merck, Darmstadt, Germany) and the compounds were detected at 254 nm. Radioactivity on radio-TLC was

measured by a Raytest minigita device (Raytest, Germany). High-performance liquid chromatography (HPLC) separations were performed with a Knauer 1000 pump (Knauer, Germany), a Knauer Smartline 2520 UV/VIS detector, a manual Rheodyne injector (50-µL or 5.0-mL loop), and a NaI(Tl) well-type scintillation detector (EG&G Ortec (Ortek AMETEK, Germany); model 276 Photomultiplier Base) with an ACE Mate Amplifier and BIAS supply (Ortec AMETEK, Germany) for radioactivity detection. Data acquisition and interpretation were performed with Gina software (Raytest, Germany).

Chromatographic systems

System A: Analytical HPLC of [^{18}F]FAMT was performed with an analytical reverse-phase Synergi 4µm Hydro-RP 80 Å column (250 x 4.6 mm; Phenomenex, Germany). The mobile phase was water with 2 % ethanol and 0.1 % acetic acid, used at a flow rate of 1 mL/min.

System B: Preparative HPLC of [^{18}F]FAMT was carried out with a Synergi 4µm Hydro-RP 80 Å column (250 x 10 mm; Phenomenex, Germany). The mobile phase was prepared by adding 20 mL of sodium phophate buffer (B.Braun, Germany) and 2 % ethanol into 1 L of sterile water, and the flow rate was 4 mL/min.

System C: The enantiomeric purity of [^{18}F]FAMT was determined by HPLC using a Chirex 3126 (D)-penicillamine column (150 x 4.6 mm; Phenomenex, Germany) and 3 mM $CuSO_4$ in water and 2-propanol (95:5 v/v) as eluent at a flow rate of 1 mL/min.

System D: Preparative HPLC of **6a** and **6b** were carried out with a Synergi Hydro RP, (250 x 10 mm, Phenomenex, Germany) with 3 % EtOH + 0.1% AcOH in H_2O as eluent at a flow rate of 4.7 mL/min.

System E: Preparative HPLC of **10a** and **10b** were carried out with a Gemini 5µ 18C 110 Å (250 x 10 mm, Phenomenex, Germany) and water and acetonitrile (40:60 v/v) as eluent at a flow rate of 4.7 mL/min.

Table 1. k'-values of the final compounds analyzed by radio-HPLC.

Compound	HPLC System	k'
[^{18}F]6	A	3.4
[^{18}F]6a	C	3.1
[^{18}F]6b	C	4.5

Syntheses of standards

Benzyl-4-(benzyloxy)-3-fluorobenzoate (2)

Benzyl bromide (2.24 g, 13.12 mmol) and potassium carbonate (3.30 g) were added to a solution of 3-fluoro-4-hydroxybenzoic acid (0.93 g, 5.96 mmol) in 50 mL acetone. The mixture was refluxed overnight. Thereafter, the reaction mixture was cooled to room temperature (r.t.), the potassium carbonate filtered and the solid residue washed with acetone (3 x 15 mL). The solvent was removed *in vacuo* and the crude product purified by flash chromatography (AcOEt/petroleum ether (PE) 5:95) giving 1.34 g (67%) of **2** as a white solid. R_f = 0.24 (AcOEt/PE 5:95). mp = 75 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.81 (m, 2H), 7.44 (d, *J*= 7.3 Hz, 4H), 7.36 (m, 2H), 7.02 (t, *J*= 8.1 Hz, 1H), 5.34 (s, 2H), 5.20 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 165.3, 152.9 (*J*= 247 Hz), 150.9 (*J*= 10.1 Hz), 136.0, 135.8, 128.9, 128.7, 128.5 (*J*= 9.8 Hz), 127.9 (*J*= 120.5 Hz), 126.7 (*J*= 3.3 Hz), 123.4, 117.7 (*J*= 19.8 Hz), 114.3, 71.2, 66.9; ^{19}F (564 MHz, $CDCl_3$): δ -133.1 (t, *J*=9.5 Hz). Anal. calcd for $C_{21}H_{17}FO_3$ C, 74.99; H, 5.09; found: C, 74.77; H, 5.04; HRMS (ESI), m/z: calcd for $C_{21}H_{18}FO_3^+$ 337.1234, $[M+H]^+$ found 337.1235.

4-Benzyloxy-3-fluorobenzyl alcohol (3)

Lithium aluminum hydride (1.0 M in THF, 4.0 mL, 4 mmol) was added dropwise to a solution of compound **2** (1.3 g, 3.98 mmol) in 60 mL of THF at r.t. and the mixture stirred during 60 min. The mixture was quenched with 60 mL of water and acidified with H_2SO_4 until the solid

was dissolved. The mixture was extracted with ether (3 x 25 mL), the organic phase washed with brine (2 x 30 mL), separated and dried over Na_2SO_4. The solvent was removed and the crude product purified by flash chromatography (AcOEt/PE 25:75) yielding product **3** (0.76 g, 82%) as a white solid. R_f = 0.21 (AcOEt/PE 20:80). mp = 56 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.44 (d, *J*= 7.4 Hz, 2H), 7.38 (t, *J*= 7.3 Hz, 2H), 7.32 (t, *J*= 7.3 Hz, 1H), 7.12 (d, *J*= 11.8 Hz, 1H), 6.98 (m, 2H), 5.14 (s, 2H), 4.58 (s, 2H), 1.79 (s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 153.0 (*J*= 246 Hz), 146.2 (*J*= 10.7 Hz), 136.6, 134.7 (*J*= 6.0 Hz), 128.7, 128.2, 127.5, 122.8 (*J*= 3.6), 115.8, 115.3 (*J*= 19.0), 71.6, 64.5; ^{19}F (564 MHz, $CDCl_3$): δ -133.3 (t, *J*= 9.5 Hz). Anal. calcd for $C_{14}H_{13}FO_2$ C, 72.40; H, 5.64; found C, 72.78; H, 5.70; HRMS (ESI), *m/z*: calcd for $C_{14}H_{13}FO_2Na^+$ 255.0797 $[M+Na]^+$, found 255.0793.

4-Benzyloxy-3-fluorobenzylbromide (4)

A stirred solution of alcohol **3** (0.73 g, 3.14 mmol) and 1.15 g (3.46 mmol) of carbontetrabromide in 10 mL of dichloromethane was cooled to 0 °C and triphenylphosphine (1.23 g, 4.71 mmol) was added. Then the mixture was stirred for further 10 min, whereupon 5 g of silica gel were added and the solvent was removed *in vacuo* at room temperature. The adsorbed compound was purified by flash chromatography (AcOEt/PE 5:95) giving 0.89 g (96%) of the bromide as a white solid.

R_f = 0.50 (AcOEt/PE 5:95). mp = 82 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (d, J= 7.6 Hz, 2H), 7.39 (t, J= 7.3 Hz, 2H), 7.34 (t, J= 7.3 Hz, 1H), 7.15 (d, J= 11.3 Hz, 1H), 7.06 (d, J= 8.3 Hz, 1H), 6.94 (t, J= 8.3 Hz, 1H), 5.15 (s, 2H), 4.44 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 152.6 (J= 247.4 Hz), 147.0 (J= 10.8 Hz), 136.4, 131.2 (J= 6.5 Hz), 128.7, 128.3, 127.5, 125.0 (J= 3.3 Hz), 117.2 (J= 19.0 Hz), 115.6, 71.4, 32.9; ^{19}F (564 MHz, $CDCl_3$) δ – 132.7 (t, J= 9.8 Hz). Anal. calcd for C14H12BrFO C, 56.97; H, 4.10; found C, 57.06; H: 4.09.

(2S,5R)-2-{[4-(Benzyloxy)-3-fluorophenyl]methyl}-3,6-dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazine (5a)

A stirred solution of methyl-(*R*)-Schöllkopf (0.49 g, 2.47 mmol) in dry THF (2 mL) was cooled down to -78 °C, then *n*-BuLi (2.5 M/hexane, 1.09 mL, 2.72 mmol) was added dropwise. The resulting solution was stirred for 30 min. Benzyl bromide **4** (0.73 g, 2.47 mmol) in THF (2 mL) was added, the reaction mixture was allowed to warm to room temperature and stirring was continued for 3 h. The reaction was quenched with saturated aqueous ammonium chloride (10 mL) and 30 mL of water were added. Then the reaction mixture was extracted with ether (3 x 25 mL), the organic layer was dried over Na_2SO_4 and evaporated, giving the crude product which was purified by flash chromatography (AcOEt/PE 2:98) (0.63 g, 62%, yellowish oil).

R_f = 0.41 (AcOEt/PE 5:95). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.42 (d, J= 7.3 Hz, 2H), 7.37 (t, J= 7.3 Hz, 2H), 7.31 (t, J= 6.9 Hz, 1H), 6.79 (m, 2H), 6.67 (d, J= 8.1 Hz, 1H), 5.08 (s, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 3.32 (d, J= 3.1 Hz, 1H), 3.05 (d, J= 13.0 Hz, 1H), 2.68 (d, J= 13.0 Hz, 1H), 2.13 (m, 1H), 1.44 (s, 3H), 0.96 (d, J= 6.9 Hz, 3H), 0.61 (d, 6.7Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.1, 162.3, 152.3 (J= 244 Hz), 145.3 (J= 10.9 Hz), 137.0, 131.7 (J= 6.0 Hz), 128.8, 128.3, 127.8, 125.6 (J=2.9 Hz), 118.1 (J= 18.3 Hz), 115.3, 71.7, 60.9, 59.9, 52.4, 52.2, 46.6, 30.9, 28.7, 19.5, 16.9; ^{19}F (564 MHz, $CDCl_3$) δ -134.9 (t, J= 10.4 Hz); HRMS (ESI), m/z: calcd for C24H30FN2O3+ 413.2235 [M+H]+; found 413.2234.

(2R,5S)-2-{[4-(Benzyloxy)-3-fluorophenyl]methyl}-3,6-dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazine (5b)

Compound **5b** was prepared following the procedure described for compound **5a**, but using Me-(*S*)-Schöllkopf instead. Yield was 80 %.

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41 (d, J= 7.3 Hz, 2H), 7.36 (t, J= 7.3 Hz, 2H), 7.31 (t, J= 7,1 Hz, 1H), 6.79 (m, 2H), 6.67 (d, J= 8.3 Hz, 1H), 5.07 (s, 2H), 3.68 (s, 3H), 3.67 (s, 3H), 3.31

(d, J= 3.1 Hz, 1H), 3.05 (d, J= 12.8 Hz, 1H), 2.69 (d, J= 12.8 Hz, 1H), 2.13 (m, 1H), 1.44 (s, 3H), 0.96 (d, J= 6.8 Hz, 3H), 0.60 (d, 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.1, 162.3, 152.3 (J= 244 Hz), 145.3 (J= 10.9 Hz), 137.0, 131.7 (J= 6.0 Hz), 128.8, 128.3, 127.8, 125.6 (J= 2.9 Hz), 118.1 (J= 18.3 Hz), 115.3, 71.7, 60.9, 59.9, 52.4, 52.2, 46.6, 30.9, 28.7, 19.5, 16.9; ^{19}F (564 MHz, $CDCl_3$) δ -134.9 (t, J= 10.4 Hz); Anal. calcd for C24H29FN2O C, 69.88; H, 7.09; N, 6.79; found: C, 69.88; H, 7.09; N, 6.52; HRMS (ESI), m/z: calcd for C24H30FN2O3+ 413.2235 [M+H]+; found 413.2235.

(2S)-2-Amino-3-(3-fluoro-4-hydroxyphenyl)-2-methylpropanoic acid (L-3-fluoro-α-methyl-tyrosine (6a)

Compound **5a** (0.22 g) was reacted with 2 mL HCl (32 %) at 160 °C during 30 min in a pressure tube. The mixture was then cooled to room temperature and the solid residues filtered through a PTFE-frit. The acid solution was diluted with 3 mL EtOH:H_2O (1:1) and purified by HPLC (System D), giving 0.10 g (75%) of **6a** as a white solid. The ^{1}H NMR data were in agreement with the previously reported ones [7].

(2R)-2-Amino-3-(3-fluoro-4-hydroxyphenyl)-2-methylpropanoic acid (D-3-fluoro-α-methyl-tyrosine (6b)

Compound **6b** was prepared following the procedure described for compound **6a**. The ^{1}H NMR data were in agreement with the previously reported ones [7].

Syntheses of the precursors

3-Fluoro-4-iodobenzylbromide (8)

N-Bromosuccinimide (2.63 g, 14.78 mmol) and benzoylperoxide (0.20 g, 0.62 mmol) were added to a solution of 3-fluoro-4-iodotoluene (2.90 g, 12.29 mmol) in 7 mL of tetrachloromethane. The resulting solution was refluxed for 20 h. Thereafter, the reaction was allowed to cool to room temperature and was then diluted with 30 mL of CH_2Cl_2. The solid

phase was filtered off and the filtrate was washed with 30 mL of a saturated solution of sodium thiosulphate. The organic layer was dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (AcOEt/PE 2:98), giving 3.45 g (86 %) of **8** as a white solid.

R_f = 0.76 (AcOEt/PE 5:95); mp = 46-48 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.71 (m, 1H), 7.11 (m, 1H), 6.94 (m, 1H), 4.41 (s, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.0 (J= 246.5 Hz), 140.6 (J= 7.6 Hz), 139.9 (J= 1.3 Hz), 126.4 (J= 3.2 Hz), 116.4 (J= 24.5 Hz), 81.3 (J= 25.6 Hz), 31.5; ^{19}F (564 MHz, $CDCl_3$) δ -92.9 (t, J= 7.3 Hz). Anal. calcd for C7H5BrFI: C, 26.70; H, 1.60; found: C, 26.70; H, 1.58.

(2S,5R)-2-[(3-Fluoro-4-iodophenyl)methyl]-3,6-dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazine (9a)

Compound **9a** was prepared following the procedure described for compound **5a** and isolated by flash chromatography (AcOEt/PE 3:97). Yield was 62%; the product was a yellowish oil.

R_f = 0.29 (AcOEt/PE 1:99); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (dd, J= 8.0 Hz, J= 6.7 Hz, 1H), 6.76 (dd, J= 9.2 Hz, J= 1.9 Hz, 1H) 6.58 (dd, J= 8.0 Hz, J= 1.9 Hz, 1H), 3.68 (s, 3H), 3.67 (s, 3H), 3.37 (d, J= 3.3 Hz, 1H), 3.08 (d, J= 12.8 Hz, 1H), 2.73 (d, J= 12.8 Hz, 1H), 2.13 (m, 1H), 1.45 (s, 3H), 0.96 (d, J= 7.3 Hz, 3H), 0.60 (d, 7.3 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.7, 162.4, 161.2 (J= 245.2 Hz), 140.9 (d, J= 6.9 Hz), 138.4, 127.6 (J= 3.2 Hz), 117.4 (J= 23.7 Hz), 78.6 (J= 25.4 Hz), 60.8, 59.6, 52.3, 52.1, 46.7, 30.8, 28.8, 19.3, 16.8; ^{19}F (564 MHz, $CDCl_3$) δ -95.4 (t, J= 7.9 Hz); HRMS (ESI), m/z: calcd. for C17H22FIN2O2H+ 433.0783 [M+H]+; found 433.0785.

*(2R,5S)-2-[(3-Fluoro-4-iodophenyl)methyl]-3,6-dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazine (**9b**)*

Compound **9b** was prepared following the procedure described for compound **5a** but using Me-(*S*)-Schöllkopf instead. The isolation was performed by flash chromatography (AcOEt/PE 3:97). Yield was 56 %, the product a yellowish oil.

R_f = 0.29 (AcOEt/PE 1:99); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 (dd, J= 8.3 Hz, J= 6.9 Hz, 1H), 6.76 (dd, J= 9.5 Hz, J=2.1 Hz, 1H) 6.58 (dd, J= 8.0 Hz, J= 2.0 Hz, 1H), 3.68 (s, 3H), 3.67 (s, 3H), 3.37 (d, J= 3.2 Hz, 1H), 3.09 (d, J= 12.8 Hz, 1H), 2.73 (d, J= 12.8 Hz, 1H), 2.13 (m, 1H), 1.45 (s, 3H), 0.96 (d, J= 7.3 Hz, 3H), 0.60 (d, 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.6, 162.3, 161.0 (J= 244.5 Hz), 140.8 (J= 7.0 Hz), 138.3, 127.5 (J= 3.0 Hz), 117.3 (J= 24.2 Hz), 78.4 (J= 25.3 Hz), 60.7, 59.5, 52.2, 52.0, 46.6, 30.7, 28.7, 19.2, 16.7; ^{19}F (564 MHz, $CDCl_3$) δ -95.4 (t, J= 8.1 Hz); HRMS (ESI), m/z: calcd. for C17H23FIN2O2+ 433.0783 [M+H]+; found 433.0784.

*4-{[(2S,5R)-3,6-Dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]methyl}-2-fluorobenzaldehyde (**10a**)*

A solution of compound **9a** (0.65 g, 1.50 mmol) in THF (4 mL) was cooled to 0 °C. Isopropylmagnesiumbromide (2.9 M in 2-methyl-THF, 1.05 mL, 3.05 mmol) was added dropwise and the mixture was allowed to react for 1 h. Thereafter, *N,N*-dimethylformamide (0.46 mL, 5.95 mmol) was added and the mixture was warmed to r.t. and stirred overnight. The reaction was quenched with saturated aqueous NH_4Cl, and extraction was done with ether (3 x 25 mL). The organic phase was dried over Na_2SO_4 and evaporated under reduced atmosphere. After purification by flash chromatography (AcOEt/PE 5:95) the desired product **10a** was obtained as colorless oil (0.28 g, 56 %). To obtain higher diastereomeric purity the product was finally purified by semi-preparative HPLC (System E).

R_f = 0.33 (AcOEt/PE 5:95); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.28 (s, 1H), 7.68 (t, J= 7.6 Hz, 1H), 6.94 (d, J= 8.0 Hz, 1H), 6.86 (d, J= 11.5 Hz, 1H), 3.68 (s, 6H), 3.36 (d, J= 2.7 Hz, 1H), 3.19 (d, J= 12.5 Hz, 1H), 2.84 (d, J= 12.5, 1H), 2.13 (m, 1H), 1.47 (s, 3H), 0.95 (d, J= 6.7 Hz,

3H), 0.60 (d, J= 6.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.3 (J= 6.3 Hz) 164.2 (J= 258.1 Hz), 163.5, 162.6, 148.2 (J= 8.7 Hz), 128.0, 126.5 (J= 3.0 Hz), 122.5 (J= 8.1 Hz), 117.9 (J= 20.4 Hz), 60.8, 59.7, 52.4, 52.3, 47.4, 30.9, 29.0, 19.3, 16.8; ^{19}F (564 MHz, $CDCl_3$) δ -123.0 (t, J=8.8 Hz); HRMS (ESI), m/z: calcd. for C18H24FN2O3+ 335.1765 [M+H]+; found 335.1765.

*4-{[(2R,5S)-3,6-Dimethoxy-2-methyl-5-(propan-2-yl)-2,5-dihydropyrazin-2-yl]methyl}-2-fluorobenzaldehyde (**10b**)*

Compound **10b** was prepared following the procedure described for compound **10a** with a yield of 59 % as colorless oil.

R_f = 0.33 (AcOEt/PE 5:95); ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.28 (s, 1H), 7.68 (t, J= 7.6 Hz, 1H), 6.94 (d, J= 7.8 Hz, 1H), 6.86 (d, J= 11.6 Hz, 1H), 3.68 (s, 6H), 3.36 (d, J= 2.3 Hz, 1H), 3.19 (d, J= 12.6 Hz, 1H), 2.84 (d, J= 12.6, 1H), 2.13 (m, 1H), 1.47 (s, 3H), 0.95 (d, J= 6.7 Hz, 3H), 0.60 (d, J= 6.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 187.3 (J= 6.0 Hz) 164.2 (J= 257.9 Hz), 163.5, 162.6, 148.2 (J= 8.7 Hz), 127.9, 126.5 (J= 2.7 Hz), 122.5 (J= 8.0 Hz), 117.9 (J= 20.7 Hz), 60.8, 59.7, 52.4, 52.2, 47.4, 30.9, 29.0, 19.3, 16.8; ^{19}F (564 MHz, $CDCl_3$) δ -123.0 (t, J= 8.8 Hz); Anal. calcd for C18H23FN2O3: C, 64.65, H, 6.93, N, 8.38, found C, 64.92, H, 7.12, N, 7.99; HRMS (ESI), m/z: calcd. for C18H24FN2O3+ 335.1765 [M+H]+; found 335.1766.

Radiosynthesis

Preparation of [^{18}F]fluoride

N.c.a. [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction by bombardment of an isotopically enriched [^{18}O]water target with 17 MeV protons at the JSW cyclotron BC 1710 (Forschungszentrum Jülich) [28]. The [^{18}F]fluoride produced was diluted with 2 mL H_2O. An aliquot of the [^{18}F]fluoride solution (200 – 300 MBq) was added to 150 µL (19.5 µmol) of an aqueous 0.13 M tetrabutylammonium bicarbonate solution ($TBAHCO_3$) [29]. The mixture was diluted with 1.0 mL of dry acetonitrile and transferred by syringe into a reaction flask. The solvent was evaporated under a stream of argon at 80 °C and 650 mbar. The azeotropic evaporation was repeated twice with 1.0 mL of acetonitrile and afterwards the vial was evacuated for 5 min at 20-30 mbar.

Radiosynthesis of L- and D-[^{18}F]FAMT

A solution of 15 µmol of the corresponding precursor (**10a** or **10b**) in 1.0 mL of DMF was added to the dried residue of TBA^{18}F. The mixture was heated at 120 ºC for 7 min. After labeling, the DMF solution was diluted with 10 mL of water, and passed through a pre-conditioned Waters C-18 cartridge. Then, the product was eluted from the cartridge with 6.0 mL of ether and the eluate was dried using a column packed with Na_2SO_4. Subsequently, the solvent was evaporated in vacuum and a solution of the oxidant (6 equivalents, e.g. 90 µmol of *m*-CPBA) in 1 mL of 2,2,2-trifluoroethanol and 10 µL of $H_2SO_{4(conc.)}$ were added and the mixture heated to 80 ºC. After 30 min the reaction was quenched with a solution of ascorbic acid (10 mg in 50 µL H_2O and 300 µL acetonitrile) and the mixture evaporated to dryness at the same temperature. 1 mL of hydrochloric acid (32 %) was added to the residue and the solution was heated at 160 ºC for 30 min. The mixture was then cooled to r.t., diluted with water and the product purified by HLPC (System B).

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