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No-Carrier-Added Asymmetric Synthesis of α-Methyl-α-Amino Acids Labelled with Fluorine-18

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Abstract: Various [^{18}F]fluoro aromatic α-methyl-L-amino acids **11** have been synthesized with high enantiomeric purity (ee > 97%). These new radiopharmaceuticals for Positron Emission Tomography (PET), potential inhibitors of enzymatic functions, were regiospecifically labelled by nucleophilic substitution on trimethylammoniumbenzaldehyde triflate precursors **9**. The [^{18}F]fluoro aromatic aldehydes **12** obtained were easily converted to the corresponding [^{18}F]fluorobenzyl halides [**13** (X = I)]. After alkylation of the lithium enolate of (2S,5S)-1-*tert*-Boc-2-*tert*-butyl-3,5-dimethyl-imidazolidin-4-one **2**, the adducts were cleaved to give, after HPLC purification, various [^{18}F]fluoro-α-methyl amino acid analogs with radiochemical yields of 10% (End of Bombardment, EOB) after a synthesis time of 120 min. The corresponding [^{19}F]fluorinated amino acids **4** and [^{19}F]fluoro intermediates were also prepared.

INTRODUCTION

Due to their broad range of biological activity, α-amino acids have deserved special attention and were during these last years the subject of numerous studies. An important class in the family of modified amino acids are the α,α-disubstituted α-amino acids resulting from the substitution of the α-hydrogen of the natural compound by an alkyl group. The α-methyl analogs exhibit interesting enzymatic inhibitor properties. α-Methylphenylalanine strongly reduces the phenylalanine hydroxylase activity.[1] α-Methyl-3,4-dihydroxyphenylalanine (α-methyldopa) has been found as an effective inhibitor of aromatic amino acid decarboxylase.[2-4] α-Methyltyrosine is known as a competitive inhibitor of tyrosine hydroxylase which is the rate limiting enzyme for the conversion of L-tyrosine into L-dopa.[5]

These biologically active molecules labelled with short lived positron emitting radionuclides (e.g. ^{18}F and ^{11}C) and the use of the Positron Emission Tomography (PET) technique yield new ways of studying specific biological processes *in vivo* and more particularly for probing the enzymatic function in human with PET.

The utilization of these types of amino acids as PET scanning agents requires the development of an efficient chemical pathway. One major problem of the synthesis consists in the presence of an asymmetric carbon

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in their structure which leads to two enantiomeric forms [(R) and (S)]. As generally only one enantiomer (S) presents biological activity, the synthetic pathway selected must proceed with high enantioselectivity (ee ≥ 97%).

As a result of the wide spectrum of application of α-amino acids, a great number of elegant approches have been described in the literature for their synthesis in optically pure forms.[6-9] Among them, only a few are well adapted to the specific constraints of the ^{18}F-fluorine chemistry. Ideally, the radiosynthesis should be compatible with the half-life of fluorine-18 ($T_{1/2}$ = 110 min) and with the limited number of ^{18}F-fluorinated precursors. The strategy must also be selected in such a way that the radionuclide is incorporated as far as possible in the synthetic route. The radiochemical steps must be as simple as possible in order to allow an easy purification and automatic production of the radiotracers. Asymmetric approaches making use of readily available chiral building blocks are especially attractive. Among the glycine derivatives presenting enormous potentiality as precursor of optically pure compounds, (2S)-1-*tert*-Boc-2-*tert*-butyl-3-methyl-imidazolidin-4-one (Boc-BMI, **1**) was selected.[10]

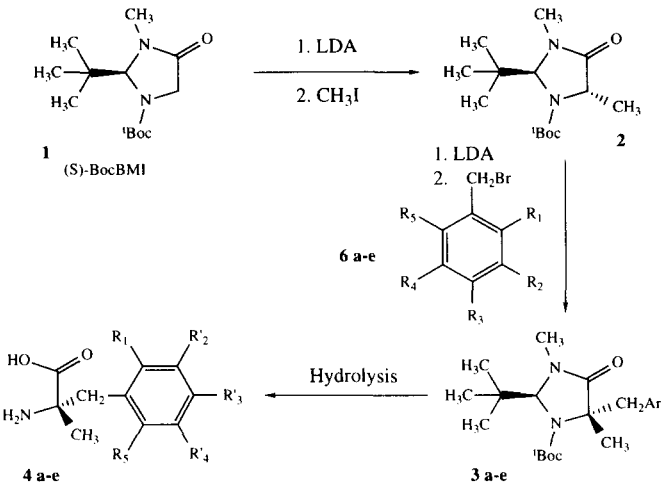
Approaches using this chiral auxiliary have been previously used with success for the synthesis of various ^{18}F and ^{11}C labelled α-amino acids.[11-14] According to this strategy, the preparation at the no-carrier-added (n.c.a.) level of five [^{18}F]fluoro aromatic α-methyl-L-α-amino acids analogs of phenylalanine **11a,b**, dopa **11c** and tyrosine **11d,e** has been developed. The corresponding [^{19}F]fluoroamino acids have also been synthesized through the same procedure.

RESULTS AND DISCUSSION

The general synthetic pathway used to prepare the [^{19}F]fluoro-α-methyl-L-α-amino acids is shown in Scheme 1. It requires the preparation of various substituted fluorobenzyl bromides **6** and a methylated chiral auxilliary [(2S,5S)-1-*tert*-Boc-2-*tert*-butyl-3,5-dimethyl-imidazolidin-4-one, (S)-Boc-BMI-CH$_3$, **2**]. This one was obtained as previously described [10] by treatment of the enolate of commercialy available (2S)-1-*tert*-Boc-2-*tert*-butyl-3-methyl-imidazolidin-4-one [(S)-Boc-BMI, **1**] with methyl iodide. The reaction proceeded at -78°C in dry tetrahydrofuran with a chemical yield of 82%.

Substituted fluorobenzyl bromides **6** were the second synthons required for the asymmetric syntheses of [^{19}F]fluoro-α-methyl-L-amino acids. 2-Fluoro-4,5-dimethoxybenzyl bromide **6c** and 2-fluoro-4-methoxybenzyl bromide **6e** were prepared as previously described.[15,16] 2-Fluoro-5-methoxybenzyl bromide **6d** was obtained after bromination of 4-fluoro-3-methylanisole with N-bromosuccinimide (NBS) in the presence of benzoyl peroxide.

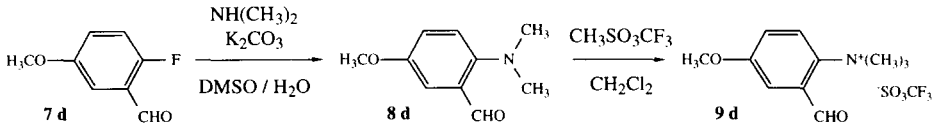
The chiral fluorodialkylated precursors **3a-e** were obtained by deprotonation of (S)-Boc-BMI-CH$_3$ **2** with lithium diisopropylamide (LDA) and addition of one equivalent of the various substituted benzyl bromides **6a-e** in the conditions described in the experimental part. The reaction was followed by TLC. Purification by chromatography on a silica gel column afforded compounds **3** with chemical yields ranging from 20 to 30%. ^{1}H-NMR identifications showed the presence of two rotamers of the same diastereoisomer.[17] Hydrolysis (180°C, 4 h) with hydrochloric acid (6N) in a sealed vial provided the α-methyl-L-amino acids **4** with relatively low yields. This was also observed by Studer and coworkers,[18] who were unable to cleave such compounds with a methyl and a benzyl substituents on the imidazolidinone. However, higher yields can be obtained using a two step hydrolysis procedure.[18,19] The corresponding D-amino acids were synthesized according to the same procedure starting from (2R)-1-*tert*-Boc-2-*tert*-butyl-3-methyl-imidazolidin-4-one [(R)-Boc-BMI].



entry	Amino acid	R_1	R'_2	R'_3	R'_4	R_5
4a	4-fluoro-α-methylphenylalanine	H	H	F	H	H
4b	2-fluoro-α-methylphenylalanine	F	H	H	H	H
4c	6-fluoro-α-methyldopa	H	OH	OH	H	F
4d	2-fluoro-5-hydroxy-α-methylphenylalanine	F	H	H	OH	H
4e	2-fluoro-α-methyltyrosine	F	H	OH	H	H

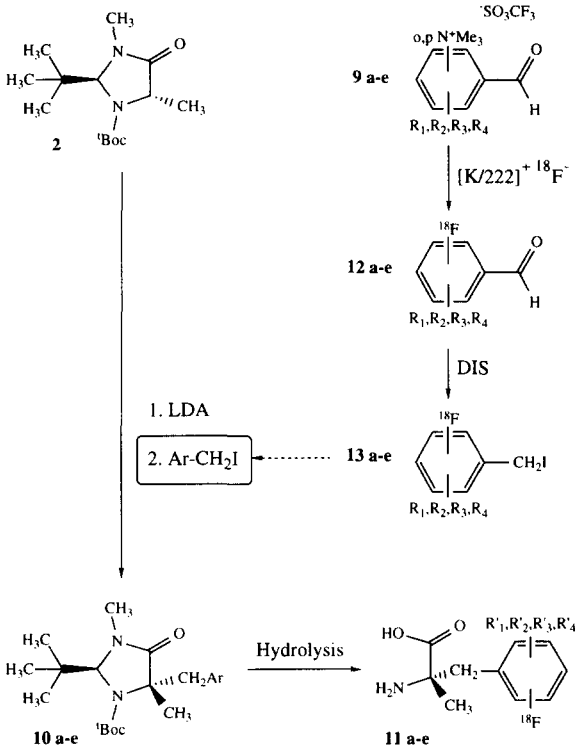
Scheme 1. General synthesis pathway for the asymmetric preparation of α-methyl-L-α-amino acids

The different substituted trimethylammoniumbenzaldehyde triflates **9**, starting precursors for the preparation of [^{18}F]fluorinated α-methyl-α-amino acids, were synthesized using the chemical pathway shown in Scheme 2.[20,21] The dimethylamino precursors **8** were prepared by a nucleophilic substitution with dimethylamine on the fluoroaromatic aldehydes **7**.[20,21] Free dimethylamine was generated *in situ* from $NH(CH_3)_2$•HCl and potassium carbonate. The reaction proceeded under reflux in a mixture of dimethylsulfoxide and water. The ammonium triflates **9** were obtained by quaternisation of the dimethylamino compounds **8** with methyl trifluoromethanesulfonate in dichloromethane.



Scheme 2. Preparation of substituted trimethylammoniumbenzaldehyde triflates

The corresponding [^{18}F]fluoro-α-methyl-L-amino acids **11a-e** were prepared at the n.c.a. level by a slight variation of the method used for the preparation of the stable fluorinated derivatives (Scheme 3).



Scheme 3. N.C.A. Preparation of [^{18}F]fluoro-α-methyl-L-amino acids

The first step of this radiochemical synthesis consisted in the preparation of various n.c.a. [^{18}F]fluorobenzaldehydes **12a-e**. The regiospecific fluorination was achieved by nucleophilic substitution of trimethylammoniumbenzaldehyde triflates [20] with the kryptofix/K_2CO_3 activated [^{18}F]fluoride.[22] The labelling yields versus temperature are illustrated in Figure 1.

From this figure, it appears that a labelling temperature of 140°C was optimal for 2-trimethylammoniumbenzaldehyde triflate **9b**, 4,5-dimethoxy-2-trimethylammonium-benzaldehyde triflate **9c** and 4-methoxy-2-trimethylammoniumbenzaldehyde triflate **9e**. For 4-trimethylammoniumbenzaldehyde triflate **9a**, a temperature of 90°C was required. Under these conditions, radiolabelling yields up to 70% were obtained for all the compounds investigated except for 5-methoxy-2-trimethylammoniumbenzaldehyde triflate **9d** (≤ 6%). Similar yields were reported by Ding *et al.* [23] for the nitro precursor (radiochemical yield = 5%). Due to the low yield, this labelled synthon was not used in the following investigations. [^{18}F]Fluorinated aldehydes **12** were pre-purified by trapping on a C18 Sep Pak® cartridge (Waters) which was washed with hydrochloric acid and eluted with dichloromethane. To ensure a high and reproducible yield in the next reaction step, the CH_2Cl_2 solution was dried on a magnesium sulfate column.

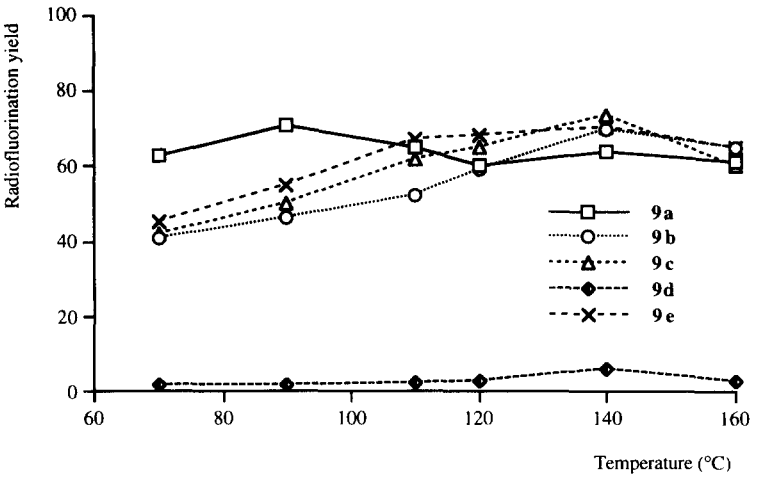
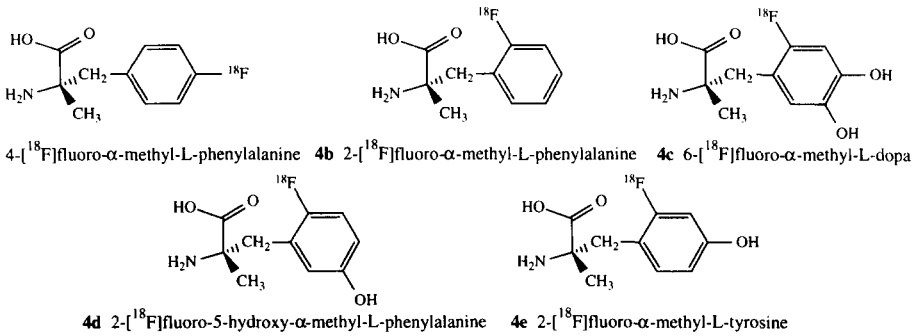


Figure 1. Labelling yields versus temperature

In the second step, [^{18}F]fluorobenzaldehydes were converted into the corresponding benzyl iodides **13** by a one step reductive-iodination reaction with freshly prepared diiodosilane (DIS, SiH_2I_2).[11,24] The reaction proceeded at room temperature within 1 min and the [^{18}F]fluorobenzyl iodide derivatives were purified by flash chromatography on a silica gel column. To ensure the elution of the benzyl iodides without the unreacted [^{18}F]fluorobenzaldehydes, the volume of CH_2Cl_2 was adjusted for each sample (15-35 mL). The overall yields (starting from [^{18}F]fluoride) were 50-55% (decay corrected). In order to avoid radiation exposure to operators, these [^{18}F]fluorinated key intermediates were prepared with a robotic system.[25,26] Dichloromethane was evaporated and the radioactive synthon solubilized in dry tetrahydrofuran, the solvent of the next reaction. The alkylation reaction proceeded in anhydrous THF at -78°C (dry ice/methanol) in the presence of the (S)- or (R)-Boc-BMI-CH_3 enolate. After 7 min, only traces of the electrophilic alkylating agent ([^{18}F]fluorobenzyl iodide) remained in the reaction mixture. The large range of alkylation yields (24-73%) obtained in this third step was due to the formation of an unidentified secondary [^{18}F]labelled product. Hydrolysis of these [^{18}F]fluorinated dialkylated products with hydroidic acid (200°C, 20 min) provided, after HPLC purification, radiochemically pure [^{18}F]fluoro-α-methyl-L-amino acids (Scheme 4).



4a 4-[^{18}F]fluoro-α-methyl-L-phenylalanine **4b** 2-[^{18}F]fluoro-α-methyl-L-phenylalanine **4c** 6-[^{18}F]fluoro-α-methyl-L-dopa

4d 2-[^{18}F]fluoro-5-hydroxy-α-methyl-L-phenylalanine **4e** 2-[^{18}F]fluoro-α-methyl-L-tyrosine

Scheme 4. Chemical structures of the final [^{18}F]fluoro-α-methyl-L-amino acids

To increase the stability of the analog of dopa **4c**, ascorbic acid and Na_2EDTA were added in the HPLC solvent (pH = 4 buffer).[27] After formulation of the injectable solution, the overall radiochemical yield were 7-13% (EOB, 120 min). The enantiomeric excesses, determined on a chiral HPLC column, were always found higher than 97% (% of the L-isomer > 98.5%). The D- and L-[^{18}F]fluorinated isomers were identified by comparison of their retention times with previously synthesized authentic reference samples.

EXPERIMENTAL

All commercial compounds were of analytical grade. Gold label dimethyl sulfoxide was purchased from Janssen Chimica. The aminopolyether kryptofix 222 (4,7,13,16,21,24 hexaoxa-1,10-diazabicyclo [8.8.8] hexacosan), potassium carbonate and ethanol were obtained from Merck. Solvents were of HPLC grade and were filtered before use. Tetrahydrofuran was dried before each use by refluxing successively on sodium and potassium. ^{18}O-Enriched water (98.5%) was purchased from Campro Benelux. The High Performance Liquid Chromatography (HPLC) system consisted of a Knauer pump 64, a Rheodyne injector (type 7010), a Waters Lambda-Max Model 481 LC spectrophotometer (280 nm) and connected in series, a GM tube for radioactivity detection. Thin Layer Chromatography (TLC) was accomplished on silica gel plates from Macherey-Nagel. The radioactivity was detected with an Automatic TLC-linear Analyzer from Berthold. The Boc-BMI derivatives were detected by development of the TLC with an anisaldehyde/AcOH/concd. H_2SO_4/ethanol (2.5/1/3.3/90) solution.[10] Proton Nuclear Magnetic Resonance spectra (^{1}H-NMR) were recorded on a Brucker AM 400. Mass Spectrometry (MS) was made either on a Varian MAT 112 or a VG TRIO-1 Benchtop GC-MS. Only the higher peaks are indicated. Melting points were measured on a Fisher-Johns melting point apparatus and are uncorrected. $[\alpha]_D$ were measured at room temperature (RT) with a Perkin-Elmer 141 polarimeter in water.

The following compounds were prepared as described in the literature: 2-fluoro-4,5-dimethoxybenzyl bromide **6c**,[28] 2-fluoro-4-methoxybenzyl bromide **6e**,[29] 4- and 2-trimethylammoniumbenzaldehyde triflates **9a-9b**,[20,21] 3,4-dimethoxy-6-trimethylammoniumbenzaldehyde triflate **9c**,[11] 4-methoxy-2-trimethylammoniumbenzaldehyde triflate **9e** [16] and (2S,5S)-1-*tert*-Boc-2-*tert*-butyl-3,5-dimethyl-imidazolidin-4-one [(S)-Boc-BMI-CH_3 **2**].[10]

2-Fluoro-5-methoxybenzyl bromide (6d). To a solution of 2-fluoro-5-methoxyanisole (0.92 g, 6.6 mmol) in 200 mL of anhydrous carbon tetrachloride were added 1.2 g (6.6 mmol) of N-bromosuccinimide (NBS) and 100 mg (0.4 mmol) of benzoyl peroxide. The mixture was heated to reflux for 2 h, cooled to RT, filtered and evaporated to dryness. The residue was extracted twice with ether (2 x 20 mL), filtered and purified on a silica gel column eluted with hexane/ether: 95/5. The chemical yield was 53%. The ^{1}H-NMR spectra was in accordance with those published by Monclus *et al.* [30]

2-Fluoro-5-methoxybenzaldehyde (7d). Hexamethylenetetramine (HMTA, 10 g, 7.8 mmol) in 50 mL of acetic acid (50%) was added to a solution of 2-fluoro-5-methoxybenzyl bromide **6d** (0.85 g, 3.9 mmol) in 50 mL of acetic acid (50%). The mixture was heated to reflux for 4 h, cooled to RT and hydrolysed with 50 mL of concentrated hydrochloric acid and ice. After extraction with 2 x 20 mL of dichloromethane, the combined organic phases were washed with an aqueous sodium carbonate solution (10%), dried on $MgSO_4$ and evaporated

to dryness. The aldehyde was purified on a silica gel column eluted with hexane/ether: 10/90 (yield = 65%). ^{1}H-NMR ($CDCl_3$): 3.83 (s, 3H, -OCH_3), 7.05-7.19 (m, 2H_{arom}), 7.27-1.32 (m, 1H_{arom}), 10.33 (s, 1H, -CHO).

5-Methoxy-2-dimethylaminobenzaldehyde (8d). 2-Fluoro-5-methoxybenzaldehyde **7d** (1.68 g, 10.9 mmol), dimethylamine hydrochloride (1.16 g, 14.2 mmol) and potassium carbonate (1.16 g, 8.4 mmol) were stirred and heated under reflux in a mixture of 20 mL of DMSO and 8 mL of water. After 1 h, additional portions of K_2CO_3 (800 mg, 5.8 mmol) were added. The reaction was followed by TLC (hexane/ether: 40/60). If needed, dimethylamine hydrochloride and potassium carbonate were added again in order to complete the reaction. Saturated aqueous potassium carbonate (40 mL) was added to the cooled solution which was extracted twice with ether (2 x 30 mL). The organic layers were washed with water and dried on $MgSO_4$. The yellow oil obtained after filtration and evaporation was purified on a silica gel column eluted with hexane/ether: 60/40 (yield = 90%). ^{1}H-NMR ($CDCl_3$): 2.83 (s, 6H, -$N(CH_3)_2$), 3.81 (s, 3H, -OCH_3), 7.09 (m, 1H_{arom}), 7.26 (s, 1H_{arom}), 7.30 (m, 1H_{arom}), 10.37 (s, 1H, -CHO).

5-Methoxy-2-trimethylammoniumbenzaldehyde triflate (9d). To a stirred solution of compound **8d** (1.6 g, 9.1 mmol) in methylene chloride (10 mL) kept under argon were added 1.8 mL of methyl trifluoromethanesulfonate (15.9 mmol). The solution was stirred for 3h at room temperature. The solid obtained was filtered, washed with cold CH_2Cl_2 (50 mL), cold ether (50 mL) and dried in vacuo. Compound **9d** (1 g, 35%) was obtained as white cristals. MS: 194 (M^+). ^{1}H-NMR (DMSO-d_6): 3.71 (s, 9H, -$N(CH_3)_3$), 3.93 (s, 3H, -OCH_3), 7.43 (dd, 1H_{arom}, J_1 = 3.04 Hz, J_2 = 8.96 Hz), 7.87 (d, 1H_{arom}, J = 3.04 Hz), 7.98 (d, 1H_{arom}, 8.96 Hz), 10.13 (s, 1H, -CHO).

General method for the preparation of dialkylated precursors. Diisopropylamine (260 µL, 1.85 mmol) and butyllithium (740 µL, 2.5 N/hexane, 1.85 mmol) in 4 mL of dry THF were stirred at 0°C under argon. After 30 min, the LDA solution was cooled to -78°C. (S)-Boc-BMI-CH_3 **2** (500 mg, 1.85 mmol) in 1 mL of dry THF were added and the reaction performed at this temperature for 30 min. One equivalent of the fluorinated benzyl bromide **6** was added and the reaction mixture allowed to reach room temperature overnight. After evaporation of the solvent, 50 mL of saturated aqueous ammonium chloride solution were added and the dialkylated product extracted with 3 x 50 mL of diethylether. The combined organic layers were dried on $MgSO_4$ and evaporated to dryness. The dialkylated compounds were purified on a silica gel column eluted with hexane/ether: 50/50 (chemical yields ranging from 20 to 30%) and identified by ^{1}H-NMR and mass spectrometry.

(2S,5S)-1-tert-Boc-2-tert-butyl-3,5-dimethyl-5-(4'-fluorobenzyl)-imidazolidin-4-one (3a). MS (relative intensity): 321 (M-57, 25), 265 (100), 221 (30), 152 (5), 109 (9), 57 (23). ^{1}H-NMR ($CDCl_3$): (mixture of two rotamers) 0.88/0.92 (s, 9H, ^{t}Bu), 1.51/1.61 (s, 9H, ^{t}Boc), 1.65/1.69 (s, 3H, $C_{(5)}$-CH_3), 2.50/2.70 (s, 3H, N-CH_3), 3.48 (d, AB, 1H, -CH_2-, J_{H-H} = 13.6 Hz), 3.75 (d, AB, 1H, -CH_2-, J_{H-H} = 13.6 Hz), 4.27/4.41 (s, 1H, $C_{(2)}$H), 6.89 (dt, 2H_{arom}, J = 14.3Hz, J = 8.5Hz), 7.06 (td, 2H_{arom}, J = 8.56 Hz, J = 2.83 Hz).

(2S,5S)-1-tert-Boc-2-tert-butyl-3,5-dimethyl-5-(2'-fluorobenzyl)-imidazolidin-4-one (3b). MS (relative intensity): 321 (M-57, 22), 265 (100), 221 (53), 152 (9), 109 (8), 57 (23). ^{1}H-NMR ($CDCl_3$): (mixture of two rotamers) 0.92/0.95 (s, 9H, ^{t}Bu), 1.46/1.55 (s, 9H, ^{t}Boc), 1.69/1.75 (s, 3H, $C_{(5)}$-CH_3), 2.57/2.70 (s, 3H, N-CH_3), 3.01 (d, AB, 1H, -CH_2-, J_{H-H} = 13.3 Hz), 3.56 (d, AB, 1H, -CH_2-, J_{H-H} = 14.3 Hz), 4.49/4.66 (s, 1H, $C_{(2)}$H), 6.94-7.20 (m, 4H_{arom}).

(2S,5S)-1-tert-Boc-2-tert-butyl-3,5-dimethyl-5-(2'-fluoro-4',5'-dimethoxy-benzyl)-imidazolidin-4-one (3c). ^{1}H-NMR ($CDCl_3$): (mixture of two rotamers) 0.91/0.96 (s, 9H, ^{t}Bu), 1.46/1.57 (s, 9H, ^{t}Boc), 1.68/1.73 (s, 3H, $C_{(5)}$-CH_3), 2.58/2.74 (s, 3H, N-CH_3), 2.95 (d, AB, 1H, -CH_2-, J_{H-H} = 14.4 Hz), 3.45 (d, AB, 1H, -CH_2-, J_{H-H} = 14.4 Hz), 3.82 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 4.57/4.64 (s, 1H, $C_{(2)}$H), 6.54-6.62 (m, 2H_{arom}).

(2S,5S)-1-tert-Boc-2-tert-butyl-3,5-dimethyl-5-(2'-fluoro-5'-methoxybenzyl)-imidazolidin-4-one (3d). MS (relative intensity): 351 (M-57, 28), 295 (100), 251 (89), 182 (13), 139 (13), 57 (27). ^{1}H-NMR ($CDCl_3$): (mixture of two rotamers) 0.93/0.96 (s, 9H, ^{t}Bu), 1.45/1.55 (s, 9H, ^{t}Boc), 1.69/1.74 (s, 3H, $C_{(5)}$-CH_3), 2.62/2.75 (s, 3H, N-CH_3), 3.01 (d, AB, 1H, -CH_2-, J_{H-H} = 14.3 Hz), 3.50 (d, AB, 1H, -CH_2-, J_{H-H} = 14.3 Hz), 3.75 (s, 3H, -OCH_3), 4.56/4.69 (s, 1H, $C_{(2)}$H), 6.60 (ddd, 1H_{arom}, J = 3.13 Hz, J = 5.92 Hz, J = 12.99 Hz), 6.68 (dd, 1H_{arom}, J = 3.75 Hz, J = 8.74 Hz), 6.88 (dt, 1H_{arom}, J = 12.32 Hz, J = 9.13 Hz).

(2S,5S)-1-tert-Boc-2-tert-butyl-3,5-dimethyl-5-(2'-fluoro-4'-methoxybenzyl)-imidazolidin-4-one (3e). MS (relative intensity): 351 (M-57, 25), 295 (100), 251 (56), 169 (11), 139 (45), 57 (29). ^{1}H-NMR ($CDCl_3$): (mixture of two rotamers) 0.88/0.90 (s, 9H, ^{t}Bu), 1.43/1.51 (s, 9H, ^{t}Boc), 1.63/1.68 (s, 3H, $C_{(5)}$-CH_3), 2.56/2.68 (s, 3H, N-CH_3), 2.88 (d, AB, 1H, -CH_2-, J_{H-H} = 14.3 Hz), 3.43 (d, AB, 1H, -CH_2-, J_{H-H} = 14.3 Hz), 3.71/3.73 (s, 3H, -OCH_3), 4.45/4.60 (s, 1H, $C_{(2)}$H), 6.48 (dd, AB, 1H_{arom}, J = 2.6Hz, J = 11.2Hz), 6.53 (dd, AB, 1H_{arom}, J = 2.6 Hz, J = 11.2 Hz), 6.93 (dt, 1H_{arom}, J = 2.72 Hz, J = 8.58 Hz).

General procedure for hydrolysis of dialkylated precursors. A suspension of 100 mg of **3a-e** in 10 mL of HCl 6N were heated in a sealed vial at 180°C for 4 h. After cooling, the aqueous phase was washed with 10 mL of dichloromethane and evaporated to dryness. All the amino acids were purified by the classical purification method described by Seebach *et al.*[10] The various fluorinated α-methyl-α-amino acids were identified by mass spectrometry and ^{1}H-NMR.

4-Fluoro-α-methyl-L-phenylalanine (4a). MS (relative intensity): 198 (M^++1, 100), 110 (68). $[\alpha]_D^{RT}$ = -14.7 (*c* = 0.49, H_2O). ^{1}H-NMR (D_2O/DCl): 1.55 (s, 3H, α-CH_3), 2.99 (d, 1H, -CH_2-, J = 14.4 Hz), 3.27 (d, 1H, -CH_2-, J = 14.4 Hz), 7.12 (m, 1H_{arom}), 7.26 (m, 1H_{arom}).

2-Fluoro-α-methyl-L-phenylalanine (4b). MS: 198 (M^++1). $[\alpha]_D^{RT}$ = -20.4 (*c* = 1.3, H_2O). ^{1}H-NMR (D_2O/DCl): 1.54 (s, 3H, α-CH_3), 3.20 (s, 2H, -CH_2-), 7.16 (d, 1H_{arom}, J = 9.24 Hz), 7.20 (t, 1H_{arom}, J = 7.94 Hz), 7.29 (t, 1H_{arom}, J = 7.43 Hz), 7.38 (q, 1H_{arom}, J = 6.96 Hz).

6-Fluoro-α-methyl-L-dopa (4c). ^{1}H-NMR (D_2O/DCl): 1.63 (s, 3H, α-CH_3), 3.06 (d, AB, 1H, -CH_2-, J_{H-H} = 14.2 Hz), 3.34 (d, AB, 1H, -CH_2-, J_{H-H} = 14.2 Hz), 6.75 (d, 1H_{arom}, J = 8.09 Hz), 6.89 (d, 1H_{arom}, 6.43 Hz).

2-Fluoro-5-hydroxy-α-methyl-L-phenylalanine (4d). MS: 214 (M^++1). ^{1}H-NMR (D_2O/DCl): 1.55 (s, 3H, α-CH_3), 3.13 (s, 2H, -CH_2-), 6.75 (dd, 1H_{arom}, J = 3.03 Hz, J = 5.94 Hz), 6.83 (dt, 1H_{arom}, J = 3.6 Hz, J = 8.85 Hz), 7.05 (t, 1H_{arom}, J = 9.3 Hz).

2-Fluoro-α-methyl-L-tyrosine (4e). MS (relative intensity): 214 (M^++1, 64), 110 (100). ^{1}H-NMR (D_2O/DCl): 1.64 (s, 3H, α-CH_3), 3.23 (m, 2H, -CH_2-), 6.60-7.20 (m, 3H_{arom}).

Fluoro-α-methyl-R-amino acids. The corresponding fluoro-α-methyl-R-amino acids were prepared by the same procedure starting from (2R)-1-*tert*-Boc-2-*tert*-butyl-3-methyl-imidazolidin-4-one [(R)-Boc-BMI].

$^{18}F^-$ Production. No-carrier-added [^{18}F]fluoride was produced as previously described [31] by the nuclear reaction $^{18}O(p,n)^{18}F$ in a nickel target closed with two 100 μm Ti foils. At the end of bombardment (EOB), the ^{18}O-enriched water (45%, 1.5 mL) was delivered to the laboratory through a 25-m teflon tube (id = 0.5 mm).

[K/222]$^+$ $^{18}F^-$. The activity was trapped on a Dowex 1 X 8 resin (OH^- form) and the ^{18}O-enriched water recovered. [^{18}F]Fluoride was eluted with 400 μL of aqueous potassium carbonate (7 mg/mL) into a 1 mL conical vial containing 22 mg of kryptofix 222 and 4.2 mg of K_2CO_3. The water was evaporated at 120°C under a slight nitrogen stream and the $[K/222]^+$ $^{18}F^-$ complex dried by three successive azeotropic distillations with acetonitrile (3 x 100 μL).

General procedure for the preparation of substituted [^{18}F]fluorobenzaldehydes (12). A solution of 15 mg of the quaternary ammonium precursor **9** in 900 μL of DMSO was added on the dry $[K/222]^+$ $^{18}F^-$ complex. Labelling was performed by heating the closed vial at 140°C or 90°C for 10 min. The reaction mixture was then diluted with 40 mL of HCl 0.5N and passed through an environmental C18 Sep Pak® cartridge (Waters). After washing with 10 mL of water, [^{18}F]fluorobenzaldehydes were eluted with 5 mL of dichloromethane through a 10 mL magnesium sulfate column which was rinced with 9 mL of CH_2Cl_2 to recover all the labelled product. The labelling yields were determined by TLC using a silica gel plate and dichloromethane (R_f = 0 for [^{18}F]-fluoride and R_f > 0.25 for the different substituted [^{18}F]fluorobenzaldehydes).

Preparation of diiodosilane. To 200 mg (787 μmol) of freshly crushed iodine was added a solution of 100 μL (891 μmol) of phenylsilane and 6 μL (61.4 nmol) of ethyl acetate. The reaction mixture was stirred at room temperature for 5 min.

General procedure for the preparation of [^{18}F]fluorobenzyl iodide (13). [^{18}F]Fluorobenzaldehydes were poured on the freshly prepared solution of diiodosilane. After stirring for 1 min at room temperature, the reaction mixture was purified through a silica gel column (diameter = 5 mm, length = 120 mm) which was rinced with different amounts of dichloromethane (15-30 mL) depending on the substitution

of the aromatic ring of the benzyl iodide produced. The solvent was evaporated at 40°C under reduced pressure and the activity resolubilized in 300 µL of dry THF. The reaction was controled by TLC (silica gel plates eluted with CH_2Cl_2, R_f = 0.62).

General procedure for the alkylation of (S)-Boc-BMI-CH3 (10). A solution of 560 µL of diisopropylamine (4 mmol) and 1600 µL of butyllithium (2.5N in hexane, 4 mmol) in 8 mL of anhydrous THF was stirred at room temperature for 15-30 min. At -78°C, 200 µL of this solution were added on 21 mg (78 µmol) of (S)-Boc-BMI-CH_3 in 500 µL of dry THF. After 5 min, the substituted [^{18}F]fluorobenzyl iodides were added and the alkylation reaction performed at this temperature for 7 min. The alkylation yields were determined by TLC on silica gel plates by comparison of the R_f of the radioactive peak with those of the known stable fluorinated references [hexane/ether was used: 50/50 for **10a** and **10b** (R_f = 0.45), 25/75 for **10c** (R_f = 0.31), 50/50 for **10d** and **10e** (R_f = 0.35)].

General procedure for the hydrolysis (11). The organic solvent was evaporated at 140°C under a slight flux of argon and 1 mL of HI (47% in water) was added. The vial was closed and heated at 200°C for 20 min.

HPLC purification. After cooling, 750 µL of NaOH 6N were added and the whole solution injected on a Whatman ODS-3 HPLC column (diameter = 9.4 mm, length = 50 mm, 10 µm) eluted at a constant flow rate of 5 mL/min with water [HOAc (17 10^{-3}M), NaOAc (3 10^{-3}M), Na_2EDTA (10^{-3}M), ascorbic acid (5 10^{-4} M)]/ethanol. The percentage of ethanol of the mobile phase was adapted to the amino acid synthesized (Table 1).

Table 1. HPLC Solvents and Retention Times for various Fluoro-α-Methyl-α-Amino Acids

Amino acid	Aqueous phase (%)	Organic phase (%)	Retention time (min)
4-[^{18}F]fluoro-α-methylphenylalanine **11a**	93	7	13
2-[^{18}F]fluoro-α-methylphenylalanine **11b**	93	7	13
6-[^{18}F]fluoro-α-methyldopa **11c**	100	0	17
2-[^{18}F]fluoro-α-methyltyrosine **11e**	98	2	16

Preparation of the injectable solution. To ensure the isotonicity and the sterility of the final preparation, the volume of the collected HPLC fraction (V) determined by weighting was diluted with 0.1*V volume of NaCl 9% and the resulting solution filtered through a Millex-GV (0.22 µm, Millipore).

Table 2. Retention Times of various [^{18}F]Fluoro-α-Methyl-Amino Acids on the Chiral HPLC

	Retention time (min)	
Amino acid	D-isomer	L-isomer
4-[^{18}F]fluoro-α-methylphenylalanine	3.6	6.8
2-[^{18}F]fluoro-α-methylphenylalanine	3.5	6.8
6-[^{18}F]fluoro-α-methyldopa	5.3	10.3
2-[^{18}F]fluoro-α-methyltyrosine	4.7	8.5

Enantiomeric excesses. An aliquot of the [^{18}F]fluoro-α-methyl-amino acid solutions was injected on a chiral HPLC column (ProCu, Serva, 4.6 x 25 mm, 5 μm) eluted at a flow rate of 1.5 mL/min. The eluent was an acetate buffer (5 10^{-2}M, pH = 4) containing sodium dihydrogenophosphate (6 10^{-2} M) and copper sulfate (10^{-3} M). Table 2 summarizes the retention time of both D and L enantiomers of the various [^{18}F]fluoro-amino acids. The D and L radioactive peaks were collected, counted and the enantiomeric excess (ee) calculated.

CONCLUSIONS

In the present work, efforts were focused on the enantioselective synthesis of various [^{19}F] and [^{18}F]fluoro analogs of α-methyl-α-amino acids. The synthesis and the radiolabelling of these compounds with the short half-lived positron emitter fluorine-18 present various challenges for the chemist. First, it was necessary to prepare the L-isomer of the amino acid because of the selectivity of the enzymes involved in the biochemical processes. High enantioselectivity was obtained with an asymmetric synthesis pathway relying on the use of (S)-1-*tert*-Boc-2-*tert*-butyl-3-methyl-imidazolidin-4-one [(S)-Boc-BMI] as chiral auxiliary. Secondly, it was necessary to prepare these [^{18}F]fluoro compounds by a fast synthetic procedure (< 2 h). This requirement was obtained by a multi-steps chemical pathway using a nucleophilic substitution on various trimethylammoniumbenzaldehyde triflate precursors. The same strategy was applied to the preparations of five fluorinated α-methyl-α-amino acids analogs of phenylalanine, dopa and tyrosine and to the radiosyntheses of their [^{18}F]fluoro analogs. These labelled compounds, which are of major interest for PET investigation, were produced with radiochemical yields ranging from 7 to 13% (EOB) within a synthesis time of 2 h. In all cases, the enantiomeric excess was higher than 97% (% of L-isomer > 98.5%).

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