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Enantioselective syntheses of no-carrier-added (n.c.a.) (S)-4-chloro-2-[^{18}F]fluorophenylalanine and (S)-(α-methyl)-4-chloro-2-[^{18}F]fluorophenylalanine [1]

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Abstract

(S)-4-Chloro-2-fluorophenylalanine and (S)-(α-methyl)-4-chloro-2-fluorophenylalanine were synthesized and labeled with no carrier added (n.c.a.) fluorine-18 through a radiochemical synthesis relying on the highly enantioselective reaction between 4-chloro-2-[^{18}F]fluorobenzyl iodide and the lithium enolate of (2S)-1-(tert-butyloxycarbonyl)-2-(tert-butyl)-3-methyl-1,3-imidazolidine-4-one for (S)-4-chloro-2-[^{18}F]fluorophenylalanine and (2S,5S)-1-(tert-butyloxycarbonyl)-2-(tert-butyl)-3,5-dimethyl-1,3-imidazolidine-4-one for (S)-(α-methyl)-4-chloro-2-[^{18}F]fluorophenylalanine. Quantities of about 20–25 mCi were obtained at the end of synthesis, ready for injection after hydrolysis and high performance liquid chromatography (HPLC) purification, with a radiochemical yield of 17%–20% corrected to the end of bombardment after a total synthesis time of 90–105 min from [^{18}F]fluoride. The enantiomeric excesses were shown to be 97% or more for both molecules without chiral separation and the radiochemical and chemical purities were 98% or better.

Keywords: Enantioselective synthesis; (S)-4-Chloro-2-[^{18}F]fluorophenylalanine; (S)-(α-Methyl)-4-chloro-2-[^{18}F]fluorophenylalanine

1. Introduction

(2S)-2-Amino-3-(4′-chlorophenyl)propanoic acid: (S)-4-chlorophenylalanine (PCPA) is an irreversible inhibitor of tryptophan hydroxylase (TpOH), the rate-limiting enzyme in the biosynthesis of serotonin [1–4]. Recent work has shown that a single injection of PCPA totally blocks TpOH protein synthesis in the rat raphe dorsalis nucleus, which is the brain region displaying the greatest population of serotonin-containing cells [5,6].

The radiosyntheses of (S)-[β-^{11}C]-4-chlorophenylalanine and (S)-(α-methyl)-[β-^{11}C]-4-chlorophenylalanine have been reported previously [7] using the self-reproduction of chirality strategy delineated by Seebach et al. [8,9]. Although carbon-11 allows studies up to 60 min after injection, it would be very interesting, from a biochemical point of view, to consider the metabolic fate of PCPA or its α-methylated analog over a longer time scale. Fluorine-18 presents decay characteristics which match this goal. The radiolabeling of (S)-4-chloro-2-fluorophenylalanine and (S)-(α-methyl)-4-chloro-2-fluorophenylalanine with this positron emitter was considered.

2. Experimental details

2.1. Instrumentation and methods

Melting points were taken on a Fisher–Johns apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker 400 MHz instrument in $CDCl_3$ or D_2O/DCl; the chemical shifts were reported in parts per million (δ) downfield from tetramethylsilane (TMS) or 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS) as internal references. The mass spectra of compounds **1**, **2**, **3** and **4** were obtained with a Varian 112 (gas chromatography-mass spectrometry (GC-MS) for **1** and **2**), **5**, **10** and **12** with a VG Trio 1, **8**, **9** and **11** with a VG Quattro, **6** with a VG Autospec EQ in the presence of nitrobenzyl alcohol and **13**, **14**, **15** and **16** with a Fison VG platform (high performance liquid chromatography-mass spectrometry (HPLC-MS)). HPLC-MS was performed using the analytical HPLC conditions described below. Thin layer chromatography (TLC) plates were ana-

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lyzed for radioactivity with an automatic TLC linear analyzer from Berthold.

Preparative scale purifications were achieved by conventional liquid column chromatography with silica gel 60 (230–400 mesh) from Merck and solvent A (hexane/ether (4 : 6)) or solvent B (hexane/ether (3 : 7)). Fractions from the liquid chromatography column were monitored by TLC with plastic plates precoated with silica gel 60F-254 (Merck). The plates were developed with the same solvent as used to elute the column. Reaction components were visualized under UV_{254} light where possible or with Pulverine consisting of *p*-anisaldehyde (2.5 ml), HOAc (1 ml), conc. H_2SO_4 (3.3 ml) and EtOH (90 ml).

HPLC was conducted using a Waters 6000A pump, a Rheodyne injector, a Waters Lambda Max 481 LC UV (254 nm) spectrophotometer and an NaI (Tl) scintillation detector. The analytical conditions were as follows: C18 Econosphere column (250 mm × 2.1 mm; 10 μm; Alltech) eluted with 50 mM NH_4OAc and CH_3OH (75 : 25) at a flow rate of 0.2 ml min^{-1}. Preparative scale HPLC purifications were realized with a C18 Econosphere column (250 mm × 10 mm; 10 μm; Alltech) eluted with 50 mM HOAc and ethanol (90 : 10 for **20** and 88 : 12 for **22**) at a flow rate of 8 ml min^{-1}.

The enantiomeric purities were measured using HPLC by ligand exchange chromatography on a Chiral Pro = Si 100 (Serva, Germany) column (250 mm × 4.6 mm) at flow rates of 1 ml min^{-1} for **13** or **20** and 1.2 ml min^{-1} for **15** or **22**; the solvent was 50 mM KH_2PO_4 and 1 mM $CuSO_4$ at pH 4. These results were confirmed by TLC using Chiralplate™ (5 cm × 20 cm) from Macherey-Nagel (Düren, Germany) developed with $CH_3OH/H_2O/CH_3CN$ (50 : 50 : 200) and analyzed with UV_{254} light or 0.2% ninhydrin in EtOH.

The radiochemical yields were corrected for decay and related to the end of bombardment (EOB).

2.2. Chemicals

All chemicals were obtained from Aldrich or Acros Chimica. The chiral agent (2S)-1-(tert-butyloxycarbonyl)-2-(tert-butyl)-3-methyl-1,3-imidazolidine-4-one (Boc-BMI) and 57% HI (unstabilized) were obtained from Merck. Diisopropylamine was used freshly distilled. Tetrahydrofuran (THF) was dried by successive distillation from Na and K. Methyl iodide was passed through a small column of Al_2O_3 (basic) before being used with enolate. HI was treated under reflux in the presence of red phosphorus and stored over red phosphorus.

2.3. Preparation of diiodosilane (DIS)

This reagent was prepared immediately before use according to Refs. [10,11].

2.4. Standard procedure for the preparation of lithium diisopropylamide (LDA)

At 0 °C, under an inert and anhydrous atmosphere, 2.5 M *n*-BuLi (1.57 ml, 3.93 mmol) was added to a solution consisting of diisopropylamine (550 μl, 3.92 mmol) and dry THF (8 ml). The mixture was allowed to react for about 30 min before being used in subsequent chemistry. The final LDA concentration was estimated to be about 0.39 M.

2.5. Standard procedure for the alkylation of 7

At −78 °C, under an inert and anhydrous atmosphere, with gentle stirring, 1.1 equivalent of an LDA solution prepared as above was added to a 0.3 M solution of imidazolidinone **7** in dry THF. The mixture was allowed to react for 30 min at −78 °C before being quenched with 1.2 equivalent of electrophile. The resulting mixture was kept at −78 °C for 1 h and then allowed to warm to room temperature slowly. The solution was poured into 15% NH_4Cl and extracted twice with Et_2O. The organic layer was dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by liquid column chromatography on silica gel with solvent A or B (see below).

2.6. Standard procedure for the alkylation of 8

Same as above, except that 0.8 equivalent of the LDA solution was used.

2.7. 4-Chloro-2-nitrobenzyl bromide (1) and 4-chloro-2-fluorobenzyl bromide (2)

1 and **2** were prepared by treating, under reflux, 4-chloro-2-nitrotoluene and 4-chloro-2-fluorotoluene respectively with *N*-bromosuccinimide (NBS, 1 equivalent) in anhydrous CCl_4 in the presence of benzoyl peroxide (50 mg). After filtration and evaporation, the residues were purified on a silica gel column with solvent A.

1 was obtained with a yield of 45% as an oil. ^1H NMR ($CDCl_3$/TMS, room temperature): 4.8 (2H, s, Ar-CH_2); 7.54 (1H, d, $J_{H6\text{-}H5}$ = 8.26 Hz, Ar-*H*6); 7.6 (1H, dd, $J_{H5\text{-}H6}$ = 8.26 Hz, $J_{H5\text{-}H3}$ = 2.1 Hz, Ar-*H*5); 8.07 (1H, d, $J_{H3\text{-}H5}$ = 2.1 Hz, Ar-*H*3). MS *m/e* (relative intensity): 253 ($M^+ + 2$, 2; ^{37}Cl); 251 (M^+, 6; ^{35}Cl); 148 (24); 74 (100). R_f (solvent A): 0.75.

2 was obtained with a yield of 65% as an oil. ^1H NMR: compound unstable. MS *m/e* (relative intensity): 225 ($M^+ + 2$, 2; ^{37}Cl); 223 (M^+, 7; ^{35}Cl); 145 (40); 140 (100); 107 (34). R_f (solvent A): 0.8.

2.8. 4-Chloro-2-nitrobenzaldehyde (3) and 4-chloro-2-fluorobenzaldehyde (4)

To a solution of **1** or **2** (3.9 mmol) in 50% HOAc (25 ml) was added a solution of hexamethylenetetramine (10 g, 7.8

mmol) in 50% HOAc (25 ml). The resulting solution was stirred under reflux for 4 h. The mixture was cooled, diluted with HCl/ice and extracted with methylene chloride (3×20 ml). The combined organic layers were washed with a sodium carbonate solution, dried and evaporated under reduced pressure. The crude aldehydic compounds were finally purified on a silica gel column with solvent A.

3 was obtained with a yield of 40% (based on **1**), m.p. 52–54 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature): 7.74 (1H, dd, $J_{H5\text{-}H3}$ = 1.8 Hz, $J_{H5\text{-}H6}$ = 8.3 Hz, Ar-*H*5); 7.91 (1H, d, $J_{H6\text{-}H5}$ = 8.3 Hz, Ar-*H*6); 8.08 (1H, d, $J_{H3\text{-}H5}$ = 1.8 Hz, Ar-*H*3); 10.55 (1H, s, -*CHO*). MS *m/e* (relative intensity): 187 (M^+, 4; ^{37}Cl); 185 (M^+, 11; ^{35}Cl); 155 (100); 112 (28); 110 (93); 73 (100). R_f (solvent A): 0.5.

4 was obtained with a yield of 60% (based on **2**), m.p. 48–50 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature): 7.26 (1H, dd, $J_{H3\text{-}F}$ = 9.1 Hz, $J_{H3\text{-}H5}$ = 1.6 Hz, Ar-*H*3); 7.29 (1H, dd, $J_{H5\text{-}H6}$ = 8 Hz, $J_{H5\text{-}H3}$ = 1.6 Hz, Ar-*H*5); 7.85 (1H, t, $J_{H6\text{-}F}$ = 8 Hz, $J_{H6\text{-}H5}$ = 8 Hz, Ar-*H*6); 10.33 (1H, s, -*CHO*). MS *m/e* (relative intensity): 160 (M^+, 30; ^{37}Cl); 158 (M^+, 100; ^{35}Cl); 130 (45); 72 (25). R_f (solvent A): 0.45.

2.9. 4-Chloro-2-dimethylaminobenzaldehyde (5)

4-Chloro-2-fluorobenzaldehyde (**4**) (1.58 g, 10 mmol), dimethylamine hydrochloride (1.05 g, 13 mmol) and potassium carbonate (2 g, 14 mmol) were stirred and heated to reflux in a mixture of dimethylsulfoxide (DMSO) (5 ml) and water (2 ml). After 1 h, potassium carbonate was added (500 mg, 3.6 mmol). The reaction was followed by TLC (solvent B: R_f (**4**), 0.6; R_f (**5**), 0.5). If needed, dimethylamine hydrochloride and potassium carbonate were added to complete the reaction. After cooling, the mixture was diluted with saturated potassium carbonate and the resulting solution was twice extracted with ether. The organic layers were washed with water, dried over potassium carbonate and evaporated to dryness under reduced pressure. The residue was purified on a silica gel column with solvent B.

Yield: 80%; m.p. 28 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature): 2.92 (6H, s, $(CH_3)_2$-N); 6.93 (1H, dd, $J_{H5\text{-}H6}$ = 8.4 Hz, $J_{H5\text{-}H3}$ = 1.6 Hz, Ar-*H*5); 6.99 (1H, d, $J_{H3\text{-}H5}$ = 1.6 Hz, Ar-*H*3); 7.66 (1H, d, $J_{H6\text{-}H5}$ = 8.4 Hz, Ar-*H*6); 10.1 (1H, s, -*CHO*). MS *m/e* (relative intensity): 185 (M^+, 9; ^{37}Cl); 183 (M^+, 24; ^{35}Cl); 168 (13); 166 (60); 142 (15); 140 (53); 113 (18); 111 (38); 97 (23); 81 (33); 75 (75); 69 (89); 57 (97); 55 (100).

2.10. 4-Chloro-2-trimethylammoniumbenzaldehyde triflate (6)

Methyl trifluoromethanesulfonate (230 μl, 2 mmol) was added with stirring to a solution of **5** (183 mg, 1 mmol) in CH_2Cl_2 (5 ml) kept under nitrogen at room temperature. The mixture was stirred until a precipitate appeared. The solid was then filtered and washed with CH_2Cl_2 (5 ml). **6** was obtained as a white solid after recrystallization from CH_2Cl_2/ether (8 : 2) with a yield of 55%; m.p. 140 °C. ^{1}H NMR (DMSO/TMS, room temperature): 3.74 (9H, s, $(CH_3)_3$-N); 8.05 (1H, d, $J_{H5\text{-}H6}$ = 8.4 Hz, Ar-*H*5); 8.12 (1H, s, Ar-*H*3); 8.31 (1H, d, $J_{H6\text{-}H5}$ = 8.4 Hz, Ar-*H*6); 10.17 (1H, s, -*CHO*). MS *m/e* (relative intensity): 200 ($M^+ - 149$, 34; ^{37}Cl); 198 ($M^+ - 149$, 100; ^{35}Cl).

2.11. (2S,5S)-1-(tert-Butyloxycarbonyl)-2-(tert-butyl)-3,5-dimethyl-1,3-imidazolidine-4-one (8)

This compound was prepared according to Ref. [8]. The yield was 70%; m.p. 103 °C (101–102 °C [8]).

2.12. (2S,5S)-1-(tert-Butyloxycarbonyl)-2-(tert-butyl)-3-methyl-5-(4'-chloro-2'-fluorobenzyl)-1,3-imidazolidine-4-one (9) and (2S,5S)-1-(tert-butyloxycarbonyl)-2-(tert-butyl)-3-methyl-5-(4'-chloro-2'-nitrobenzyl)-1,3-imidazolidine-4-one (10)

These compounds were prepared according to the standard alkylation procedure starting from **7** (as described above); solvent A was used for preparative chromatography.

9 was obtained with a yield of 70%; m.p. 118 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature): 0.97 (9H, s, $(CH_3)_3$-C2); 1.45 (9H, s, $(CH_3)_3$-C1); 2.9 (3H, s, CH_3-N); 3.36 (2H, m broad, Ar-CH_2); 4.33 (1H, s broad, *H*-C5); 4.85 (1H, s broad, *H*-C2); 6.91–7.13 (3H, m, *Ar*-). MS *m/e* (relative intensity): 401 ($M^+ + 1$, 16; ^{37}Cl); 399 ($M^+ + 1$, 31; ^{35}Cl); 343 (23); 341 (25); 325 (20); 287 (29); 285 (45); 243 (28); 241 (53); 172 (37); 145 (24); 143 (43); 69 (24); 56 (100). R_f (solvent A): 0.4.

10 was obtained with a yield of 40%; m.p. 42 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature): 0.93 (9H, s, $(CH_3)_3$-C2); 1.39 (9H, s, $(CH_3)_3$-C1); 2.9 (3H, s, CH_3-N); 3.5 (1H, s broad, Ar-CH_2); 3.9 (1H, dd, J_1 = 15 Hz, J_2 = 4 Hz, Ar-CH_2); 4.25 (1H, t, *H*-C5); 4.85 (1H, s, *H*-C2); 7.2 (1H, d, $J_{H6'\text{-}H5'}$ = 9.7 Hz, *H*6'-Ar); 7.4 (1H, dd, $J_{H5'\text{-}H6'}$ = 9.7 Hz, $J_{H5'\text{-}H3'}$ = 2.1 Hz, *H*5'-Ar); 7.85 (1H, d, $J_{H3'\text{-}H5'}$ = 2.1 Hz, *H*3'-Ar). MS *m/e* (relative intensity): 368 ($M^+ - 57$, 4; ^{35}Cl); 314 (4); 312 (14); 284 (9); 282 (27); 270 (15); 268 (43); 199 (12); 149 (28); 111 (23); 97 (28); 81 (38); 69 (99); 57 (100). R_f (solvent A): 0.35.

2.13. (2S,5S)-1-(tert-Butyloxycarbonyl)-2-(tert-butyl)-3,5-dimethyl-5-(4'-chloro-2'-fluorobenzyl)-1,3-imidazolidine-4-one (11) and (2S,5S)-1-(tert-butyloxycarbonyl)-2-(tert-butyl)-3,5-dimethyl-5-(4'-chloro-2'-nitrobenzyl)-1,3-imidazolidine-4-one (12)

These compounds were prepared according to the standard alkylation procedure starting from **8** (as described above); solvent A was used for preparative chromatography.

11 was obtained with a yield of 20%; m.p. 34 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature, mixture of two rotamers): 0.89–0.91 (9H, s, $(CH_3)_3$-C2); 1.40–1.43 (9H, s, $(CH_3)_3$-C1); 1.64–1.70 (3H, s, CH_3-C5); 2.58–2.70 (3H, s, CH_3-N);

2.93/3.48–3.77 (2H, AB, J=14.4 Hz, Ar-CH_2); 4.47–4.64 (1H, s, H-C2); 6.94–6.98 (3H, m, Ar-). MS m/e (relative intensity): 413 (M^++1, 1; ^{35}Cl); 301 (30); 299 (100); 257 (29); 255 (87); 112 (30); 285 (45); 243 (28); 241 (53); 172 (37); 145 (24); 143 (43); 69 (24); 56 (100). R_f (solvent A): 0.55.

12 was obtained with a yield of 30%; m.p. 28–30 °C. ^{1}H NMR ($CDCl_3$/TMS, room temperature, mixture of two rotamers): 0.90–0.92 (9H, s, $(CH_3)_3$-C2); 1.45–1.52 (9H, s, $(CH_3)_3$-C1); 1.58–1.62 (3H, s, CH_3-C5); 2.60–2.73 (3H, s, CH_3-N); 3.55–3.65 (2H, AB, J=14 Hz, Ar-CH_2); 4.59–4.75 (1H, s, H-C2); 7.03 (1H, d, $J_{H6'-H5'}$=8.2 Hz, $H6'$-Ar); 7.42 (1H, d, $J_{H5'-H6'}$=8.2 Hz, $H5'$-Ar); 7.8 (1H, d, $J_{H3'-H5'}$=2 Hz, $H3'$-Ar). MS m/e (relative intensity): 382 (M^+−57, 7; ^{35}Cl); 328 (14); 326 (43); 284 (13); 282 (58); 149 (17); 111 (20); 81 (24); 69 (66); 57 (100). R_f (solvent A): 0.4.

2.14. (S)-4-Chloro-2-fluorophenylalanine (13), (S)-4-chloro-2-nitrophenylalanine (14), (S)-(α-methyl)-4-chloro-2-fluorophenylalanine (15) and (S)-(α-methyl)-4-chloro-2-nitrophenylalanine (16)

The imidazolidinones **9**, **10**, **11** and **12** (±0.25 mmol) were hydrolyzed through the same procedure. After dissolution in 6 N HCl (5 ml), the solution was transferred into a thick-walled glass tube which was sealed and heated at 180–200 °C for 4 h. The tube was then removed and allowed to cool. After classical purification for amino acids [8], the title compounds were obtained (Tables 1 and 2).

13: yield, 70%; m.p. >250 °C (dec.). ^{1}H NMR (D_2O/DCl/DSS, room temperature): 3.25–3.30 (1H, dd, $J_{H\beta1-H\beta2}$=14.7 Hz, $J_{H\beta1-H\alpha}$=7 Hz, Cβ-$H\beta1$); 3.39–3.44 (1H, dd, $J_{H\beta2-H\beta1}$=14.7 Hz, $J_{H\beta2-H\alpha}$=6 Hz, Cβ-$H\beta2$); 4.38–4.41 (1H, dd, $J_{H\alpha-H\beta1}$=7 Hz, $J_{H\alpha-H\beta2}$=6 Hz, Cα-H); 7.26 (1H, d, J_{H3-F}=8.7 Hz, $H3$-Ar); 7.33 (1H, d, J_{H5-H6}=7.3 Hz, $H5$-Ar); 7.35 (1H, d, J_{H6-H5}=7.3 Hz, $H6$-Ar). HPLC-MS m/e (relative intensity): 220 (M^++1, 28; ^{37}Cl); 218 (M^++1, 100; ^{35}Cl); 214 (6); 174 (8); 132 (5); 114 (8); 77 (40).

14: yield, 50%; m.p. >240 °C (dec.). ^{1}H NMR (D_2O/DCl/DSS, room temperature): 3.0 (1H, m, Cβ-$H1$); 3.17 (1H, m, Cβ-$H2$); 4.01 (1H, m, Cα-H), 7.06 (1H, d, J_{H5-H6}=8 Hz, Ar-$H5$); 7.27 (1H, d, J_{H6-H5}=8 Hz, Ar-$H6$); 8.2 (1H, s, Ar-$H3$). HPLC-MS m/e (relative intensity): 247 (M^++1, 25; ^{37}Cl); 245 (M^++1, 75; ^{35}Cl); 236 (22); 235 (15); 200 (24); 199 (100); 156 (24); 155 (58); 92 (30).

15: yield, 55%; m.p. >250 °C (dec.). ^{1}H NMR (D_2O/DCl/DSS, room temperature): 1.60 (3H, s, Cα-CH_3); 3.23 (2H, m, Ar-CH_2); 7.1–7.45 (3H, m, Ar-). HPLC-MS m/e (relative intensity): 233 (M^+, 2; ^{37}Cl); 231 (M^+, 8; ^{35}Cl); 216 (32); 214 (100); 168 (34); 102 (18); 80 (18).

16: yield, 60%; m.p. >260 °C (dec.). ^{1}H NMR (D_2O/DCl/DSS, room temperature): 1.26 (3H, s, Cα-CH_3); 3.06–3.41 (2H, m, Ar-CH_2); 7.1 (1H, d, J_{H5-H6}=8.7 Hz, Ar-$H5$); 7.35 (1H, d, J_{H6-H5}=8.7 Hz, Ar-$H6$); 7.7 (1H, s, Ar-$H3$). HPLC-MS m/e (relative intensity): 261 (M^++1, 42; ^{37}Cl); 259 (M^++1, 100; ^{35}Cl); 224 (33); 116 (24); 92 (7); 74 (18).

Table 1
Analytical and preparative HPLC capacity factors (k') of 4-chloro-2-fluorophenylalanine, 4-chloro-2-nitrophenylalanine and their α-methylated analogs

	HPLC capacity factors (k')			
	13 or **20**	**14**	**15** or **22**	**16**
Analytical	3.29	3.86	3.57	4.71
Preparative	5	5.67	5.67	6.67

Table 2
HPLC and TLC identifications of the enantiomers of 4-chloro-2-fluorophenylalanine, 4-chloro-2-nitrophenylalanine and their α-methylated analogs

	13 or **20**		**14** (S)	**15** or **22**		**16** (S)
	(S)	(R)		(S)	(R)	
HPLC (k')	3.75	1.75		4.67	2	
R_f	0.52	0.41	0.56	0.63	0.51	0.65

2.15. Radiochemistry

2.15.1. Fluorine-18 production

No-carrier-added (n.c.a.) aqueous [^{18}F]fluoride was prepared by the $^{18}O(p,n)^{18}F$ reaction on a small volume of enriched water (1.1 ml, 95%, Isotec, USA) in a silver target. A typical production run (10 μA bombardment for 1 h) yielded ±600 mCi of [^{18}F]fluoride, which was delivered to the laboratory through a 25 m long Teflon tube.

2.15.2. 4-Chloro-2-[^{18}F]fluorobenzaldehyde (17)

The [^{18}F]fluoride was trapped from the [^{18}O]water using a Dowex 1X8 anion exchange resin [12], and the activity was eluted from the column with potassium carbonate (400 μl, 7 mg ml^{-1}) into a glass vessel (2.5 ml) containing Kryptofix 222 (22 mg) and potassium carbonate (4.2 mg). Water was evaporated under nitrogen flow and the K222/[^{18}F]fluoride complex was dried by azeotropic evaporation with CH_3CN (3×100 μl) at 140 °C. A solution of **3** (12 mg, 65 μmol) in DMSO (0.7 ml) was added to the residue, and the reaction was allowed to proceed at 140 °C for 20 min. After labeling, the mixture was diluted with 0.5 N HCl (20 ml) and applied to a C18 Sep Pak (environmental cartridge from Waters) previously activated (EtOH, 10 ml; water, 10 ml). The cartridge was washed with water (10 ml) and the activity was eluted with ether/pentane (1 : 9; 10 ml). The radioactive organic solution containing **17** was dried by passing through an Na_2CO_3 column (8 cm×1 cm).

Radiochemical yield: 60% (EOB); R_f (solvent B): 0.6.

2.15.3. 4-Chloro-2-[^{18}F]fluorobenzyl iodide (18)

The previous radioactive solution containing **17** was carefully evaporated at 30 °C under reduced pressure, and the residue dissolved in CH_2Cl_2 (5 ml) was added to a fresh preparation of DIS [11]. After a 3 min reaction at room temperature, 4-chloro-2-[^{18}F]fluorobenzyl iodide (**18**) was purified on a small column (inside diameter (i.d.), 0.5 cm) consisting of Al_2O_3 (4 cm, neutral), K_2CO_3 (1 cm), AG11A8 resin (2 cm, BIO RAD) and Al_2O_3 (3 cm, neutral). **18** was eluted with CH_2Cl_2 (7 ml).

Radiochemical yield: 80% (EOB); R_f (solvent B): 0.8.

2.15.4. (2S,5S)-1-(tert-Butyloxycarbonyl)-2-(tert-butyl)-3-methyl-5-(4'-chloro-2'-[^{18}F]fluorobenzyl)-1,3-imidazolidine-4-one (19)

In a closed conical vial with stirring, a 0.39 M LDA (110 μl, 43 μmol) solution was added to imidazolidinone **7** (10 mg, 39 μmol) in 150 μl of dry THF at −78 °C. The mixture was allowed to react for 10 min at this temperature. In the meantime, the CH_2Cl_2 solution containing **18** was evaporated to dryness at 35 °C under reduced pressure. The residue was dissolved in dry THF (400 μl) and added to the enolate solution; the alkylation reaction was allowed to proceed for 3 min at −78 °C.

Radiochemical yield: 60% (EOB); R_f (solvent B): 0.5.

2.15.5. (2S,5S)-1-(tert-Butyloxycarbonyl)-2-(tert-butyl)-3,5-dimethyl-5-(4'-chloro-2'-[^{18}F]fluorobenzyl)-1,3-imidazolidine-4-one (21)

Same procedure as for **19**, except that the enolate was prepared from **8** (10.5 mg, 39 μmol) and 0.39 M LDA (80 μl, 32 μmol).

Radiochemical yield: 50% (EOB); R_f (solvent B): 0.6.

2.15.6. (S)-4-Chloro-2-[^{18}F]fluorophenylalanine (20) and (S)-(α-methyl)-4-chloro-2-[^{18}F]fluorophenylalanine (22)

After alkylation, the conical vial was removed from the cooling bath and opened. The vial was placed on a heating block (T = 150 °C) and the solution was evaporated to dryness by venting the solvent through a small bore cannula. After the addition of 57% HI (800 μl), the vial was tightly closed and T was raised to 200 °C. The hydrolysis was allowed to proceed for 20 min after which the vial was cooled with water. The acidic solution was partially neutralized with 6 N NaOH (800 μl) and injected onto the preparative HPLC column.

Radiochemical yield (after preparative HPLC): 70% (EOB).

3. Results and discussion

The self-reproduction of chirality obtained by the sequential treatment of the chiral glycine building block Boc-BMI with lithium diisopropylamide, followed by quenching with an electrophile (benzylic-type halide or alkyl halide) at dry ice temperature, proposed by Seebach et al. [8,9], has previously been used for the asymmetric radiosyntheses of several amino acids labeled with carbon-11 and fluorine-18 [7,13–15]. One of the most important applications of this approach for positron emission tomography (PET) is certainly the routine production of 6-[^{18}F]fluorodopa from nucleophilic fluoride [11]. The main feature of this technique is that the enantiomeric excesses obtained are so high that no chiral purification is required at the end of the synthesis [7,8,11,14–16].

The radiosyntheses of (S)-4-chloro-2[^{18}F]fluorophenylalanine (**20**) and its α-methylated analog (S)-(α-methyl)-4-chloro-2[^{18}F]fluorophenylalanine (**22**) were thus considered through this asymmetrical approach starting from [^{18}F]fluoride.

The syntheses of the reference compounds and precursors are illustrated in Schemes 1–4.

3.1. Reference compounds and precursors

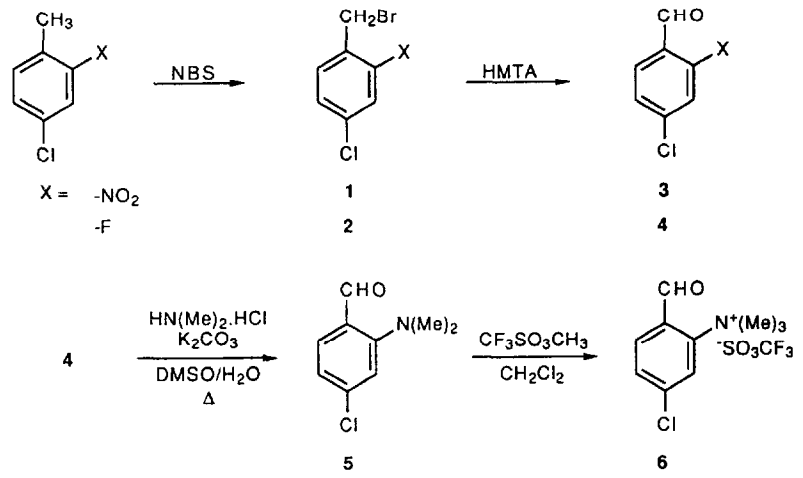
4-Chloro-2-nitrobenzaldehyde (**3**) and 4-chloro-2-fluorobenzaldehyde (**4**) were prepared according to the procedure described by Angyal [17] starting from 4-chloro-2-nitrotoluene and 4-chloro-2-fluorotoluene respectively (Scheme 1). The conversion of **4** into the *N,N*-dimethylamino intermediate (**5**) and aryltrimethylammonium salt (**6**) followed the same synthetic approach as previously described for similar compounds [18,19]. The mass spectrum of **6** presented two fragmentations: 200 which was attributed to the molecular ion calculated with ^{37}Cl and without the SO_3CF_3 moiety representing a weight of 149, and 198 due to the isotope ^{35}Cl. This spectrum was taken in the presence of nitrobenzyl alcohol allowing an easier detection of the quaternary cation.

The mono-alkylated imidazolidinones **8**, **9** and **10** (Scheme 2) were isolated and identified (^{1}H NMR and MS) by treating the commercially available (2S)-Boc-BMI (**7**) with 1.1 equivalent of LDA solution. Dialkylated imidazolidinones **11** and **12** were obtained from **8** (Scheme 3) treated with 0.8 equivalent of LDA solution only. In this case, the use of a higher amount of LDA resulted in an important reduction of the chemical yield. The ^{1}H NMR spectra of the 5,5-disubstituted imidazolidinones **11** and **12** revealed two full sets of signals due to the *E*/*Z* isomers of the same diastereomer [9].

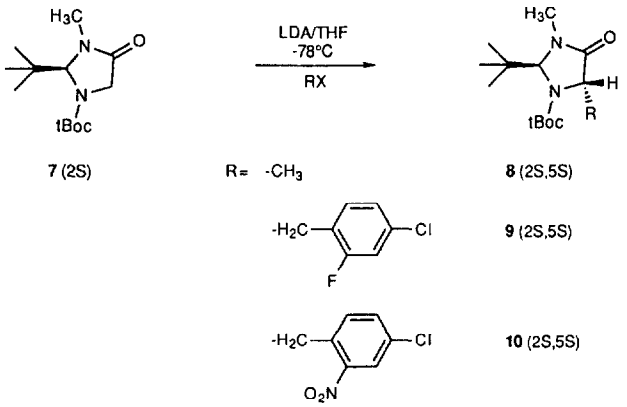
(S)-4-Chloro-2-fluorophenylalanine (**13**), (S)-4-chloro-2-nitrophenylalanine (**14**) and their corresponding α-methylated analogs (**15** and **16**) were isolated and characterized (^{1}H NMR and HPLC-MS) after 6 N HCl hydrolysis (Scheme 4).

3.2. Radiochemistry

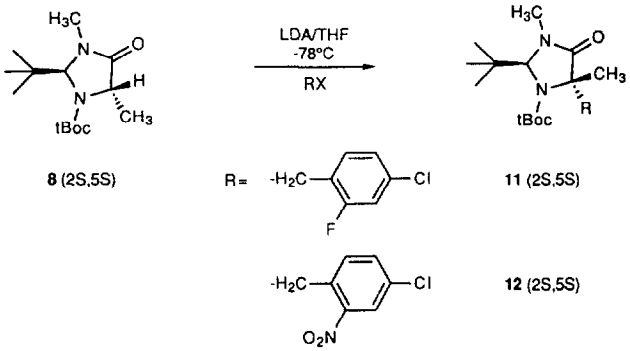
The radiochemical pathways proposed for the enantioselective syntheses of n.c.a. (S)-4-chloro-2-[^{18}F]fluorophenylalanine (**20**) and (S)-(α-methyl)-4-chloro-2-



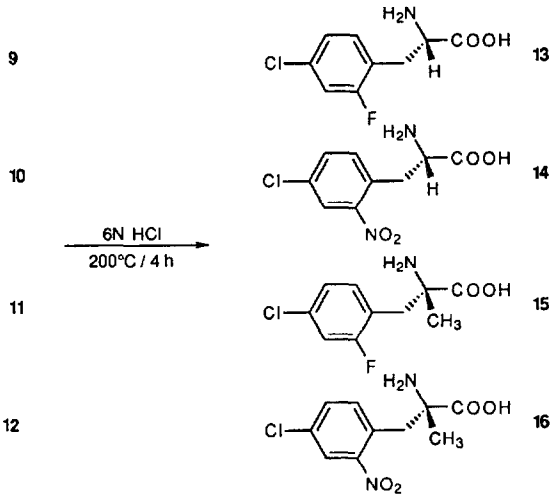
Scheme 1. Chemical pathways for the syntheses of the reference compounds **1–6**.



Scheme 2. Syntheses of the monoalkylated imidazolidinones **8**, **9** and **10**.



Scheme 3. Syntheses of the dialkylated imidazolidinones **11** and **12**.



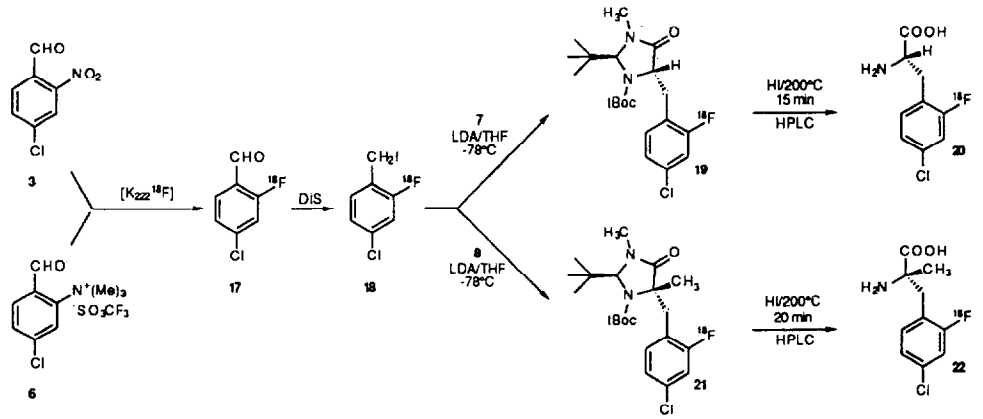
Scheme 4. Hydrolysis step leading to the amino acids **13**, **14**, **15** and **16** for identification.

[^{18}F]fluorophenylalanine (**22**) are presented in Scheme 5. A summary of the main characteristics of the n.c.a. radiosyntheses of **20** and **22** is presented in Table 3.

3.3. Labeling reaction

Two precursors were synthesized and used for the nucleophilic aromatic substitution reaction by [^{18}F]fluoride: 4-chloro-2-nitrobenzaldehyde (**3**) and 4-chloro-2-trimethylammoniumbenzaldehyde triflate (**6**). Their respective reactivities towards the nucleophilic aromatic substitution by [^{18}F]F^- were compared in different conditions of solvent (DMSO, *N,N*-dimethylformamide (DMF), hexamethylphosphoramide (HMPA), sulfolane), reaction time (5, 7, 10, 15 and 20 min), temperature (90, 110 and 140 °C) and base (K_2CO_3, Rb_2CO_3, Cs_2CO_3, $K_2C_2O_4$). For the quaternary ammonium salt (**6**), the best radiochemical yield obtained was 30% (EOB) in HMPA with K_2CO_3 at 90 °C for 10 min; the nitro derivative (**3**) led to a 60% (EOB) reaction at 140 °C in DMSO for 20 min in the presence of potassium carbonate as base. This result is relatively surprising if we consider the higher reactivity of the quaternary ammonium triflate moiety compared with the nitro group. 4-Chloro-2-nitrobenzaldehyde (**3**) was thus used as a precursor for this reaction.

The traditional C-18 Sep Pak procedure allowed the isolation of **17** free from solvent and base, but not from the large excess of unreacted NO_2 starting substrate **3**. The best separation was obtained with an ether/pentane mixture (1 : 9) resulting in a partial separation between **17** and **3**. The quantification (comparison of a sample with a calibration curve) showed that about 10% of **3** eluted along with **17** under these conditions. A higher concentration of pentane was unable to



Scheme 5. Radiosyntheses of (S)-4-chloro-2-[^{18}F]fluorophenylalanine (**20**) and (S)-(α-methyl)-4-chloro-2-[^{18}F]fluorophenylalanine (**22**) from [^{18}F]fluorine.

Table 3
Summary of the main characteristics of the n.c.a. radiosyntheses of (S)-4-chloro-2-[^{18}F]fluorophenylalanine (**20**) and (S)-(α-methyl)-4-chloro-2-[^{18}F]fluorophenylalanine (**22**) ($n=15$)

Step	Radiochemical yield of each step (%, EOB)	Cumulated radiochemical yield (%, EOB)	Time for each step (min)	Time from EOB (min)
3→17	60	60	30–35	30–35
17→18	80	48	15–20	45–55
18→19	60	29	10–15	55–70
19→20	70	20	35	90–105
18→21	50	24	10–15	55–70
21→22	70	17	35	90–105

elute **17** correctly. As a result, the NO_2-substituted imidazolidinones **10** and **12**, as well as their hydrolysis products **14** and **16**, were synthesized and identified in order to ensure that the final HPLC purification was able to give a clean separation between the final amino acid of interest (**13** or **15**) and the corresponding NO_2-substituted analog (**14** or **16**).

3.4. Reductive iodination reaction

The second step of this radiochemical pathway (Scheme 5) consisted of the conversion of 4-chloro-2-[^{18}F]fluorobenzaldehyde (**17**) into 4-chloro-2-[^{18}F]fluorobenzyl iodide (**18**), an electrophilic alkylating agent for the enolates of **7** and **8**. The transformation was realized in a single step in the presence of DIS. This reagent allows the rapid and specific reductive iodination of aldehydes [10]. As previously reported [11], DIS was freshly prepared from iodine and phenylsilane in order to obtain a highly reliable reaction at the n.c.a. level.

The crude reaction mixture was quickly purified by passing through a small column containing neutral alumina, potassium carbonate and AG11A8 resin. **18** was eluted with dichloromethane (7 ml). This method ensured the chemical purity required for a smooth reaction with the lithium enolate of **7** or **8**. The reductive iodination of **17** was realized at room temperature in dichloromethane with a radiochemical yield of 80% (EOB).

3.5. Alkylation

The third step of this process was the alkylation reaction between the lithium enolate of **7** or **8**, obtained with LDA at dry ice temperature, and the electrophile **18**. An important parameter controlling the alkylation was the amount of LDA used. As discussed above in the cold chemistry, the best alkylation yields were obtained with 1.1 equivalent of LDA solution in the case of **7** (60% EOB) and 0.8 equivalent for **8** (50% EOB).

3.6. Hydrolysis and HPLC purification

The final chemical step consisted of the hydrolysis of the protecting groups of the amino acid structure. This process was realized with 57% HI at 200 °C in a closed vial. The hydrolysis was immediately followed by HPLC purification after cooling and partial neutralization. The radiochemical yield (after HPLC) was about 70% for both compounds.

3.7. Quality control

Two different techniques were used to measure the enantiomeric purity in the final injectable solution. The first consisted of an HPLC analysis with the Chiral Pro=Si 100 column from Serva. The respective peaks of the L and D enantiomers were collected and counted. The radioactivity recovery from the column was consistently above 99%. The

peak identification was performed by comparison of the retention time with cold authentic samples (Table 2). The second involved a TLC analysis on Chiralplate™ from Macherey-Nagel. Both techniques gave the same results and showed an enantiomeric excess of 97% or more (average of 20 runs).

Analytical HPLC analyses realized on injectable solutions of **20** or **22** showed that the final preparation was totally free of **14** or **16** and of any other chemical or radiochemical contaminant.

4. Conclusions

The chemical pathway proposed for the synthesis of (S)-4-chloro-2-fluorophenylalanine and its α-methylated derivative (S)-(α-methyl)-4-chloro-2-fluorophenylalanine affords the amino acids as reference compounds in three steps, starting from 4-chloro-2-fluorotoluene, with an overall chemical yield of 22% and 5% respectively. Identifications (^{1}H NMR and mass spectra) of these compounds were realized.

This chemical pathway, relying on the alkylation reaction at the α-position of the glycine chiral auxiliary Boc-BMI, was extended to the n.c.a. level for the radiolabeling of these compounds with fluorine-18 starting from [^{18}F]fluoride. (S)-4-Chloro-2-[^{18}F]fluorophenylalanine and (S)-(α-methyl)-4-chloro-2-[^{18}F]fluorophenylalanine were isolated after HPLC purification with a radiochemical yield of 20% and 17% (corrected for decay) respectively. The overall preparation time was about 100 min and the enantiomeric excesses measured on the final compounds were 97% or more without chiral HPLC separation.

This work further demonstrates the flexibility of this asymmetric approach for the fast radiolabeling of amino acids with short-lived positron emitters.

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