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Research Article

Electrochemical radiofluorination. 3. Direct labeling of phenylalanine derivatives with [^{18}F]fluoride after anodic oxidation

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Summary

As an example of applying the electrochemical method in radiofluorination a new procedure was developed for preparing [^{18}F]fluorophenylalanine by direct use of [^{18}F]fluoride. Several protecting groups for amino and carboxylic function of phenylalanine were investigated. Best results were obtained for *N*-trifluoroacetylphenylalanine methyl ester (**1**) (10.5 ± 2.5%) with a relative isomeric distribution of 50.5 ± 1.5% *ortho* isomer, 11.5 ± 1.5% *meta* isomer and 38.8 ± 2.1% *para* isomer. Optimized reaction conditions were with $Et_3N \cdot 3HF$ as supporting electrolyte and temperatures between 0 and –10°C. By variation of working potential (1.5–2.0 V) and chloride concentration (added as supporting electrolyte, 0.0–0.36 M) it was demonstrated that an indirect electrolysis as reaction mechanism or a halogen exchange (halex) reaction with primary formation of a chlorinated product followed by halogen exchange are highly improbable. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: fluorophenylalanine; anodic fluorination; [^{18}F]fluoride; nucleophilic substitution; positron emission tomography

Introduction

Fluorine-18 labeled aromatic amino acids are considered to be of great value for PET applications. This is especially true for 2- and 4-[^{18}F]fluorophenylalanine and 2-[^{18}F]fluorotyrosine, which have been applied in tumor detection[1–4] and 6-[^{18}F]fluorodopa and 6-[^{18}F]fluoro-*m*-tyrosine which are used measuring presynaptic dopaminergic function.[5,6]

The radiochemical syntheses of those radiopharmaceuticals are performed mainly by electrophilic reactions using [^{18}F]F_2 as reagent. However, anionic

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fluorine-18 can be produced more efficiently via the $^{18}O(p,n)^{18}F$ nuclear reaction. In addition, that is the most common method for preparing no-carrier-added radiotracers. Only a few nucleophilic labeling procedures exist for aromatic radiopharmaceuticals. After the labeling step further reactions and chiral purifications often are necessary. In general, these procedures rarely are used, as they are time-consuming because of multi step syntheses and suffer from low yields.[7,8] Most recently, a catalytic enantioselective pathway was described, demonstrating significant improvements.[9]

In aromatic compounds nucleophilic substitution generally is hampered by the high electron density of the benzene ring activated by +M effect as in the case of DOPA or tyrosine. In such cases, nucleophilic attack of fluoride is difficult or even impossible. Substituents with electron withdrawing effects enable the nucleophilic introduction of fluoride into the aromatic moiety. Unfortunately, this application is limited.

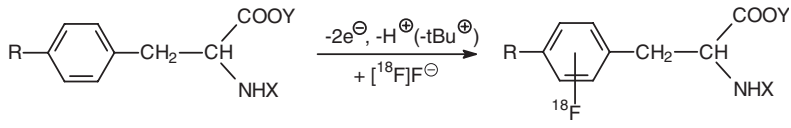
By the methods of electrochemistry, an electron can be removed from the phenylic ring, fluoride can easily react with the cation formed, and electrode potentials can be selected for the precise oxidation of a wide range of aromatic hydrocarbons. Thus, reactions are more selective when compared to reactions with chemical oxidants.[10,11]

The first electrochemical fluorination of benzene and various substituted benzenes with [^{18}F]fluoride were recently reported by our group.[12,13] Aim of the present study was to develop a new method for preparing aromatic PET radiopharmaceuticals by electrochemical ^{18}F-fluorination using [^{18}F]fluoride and phenylalanine as a radiopharmaceutical example.

Results and discussion

This is the first literature report on the electrochemical synthesis of [^{18}F]fluorophenylalanine using [^{18}F]fluoride (Figure 1). Several protecting groups were applied for the amino group (acetyl, trifluoroacetyl, butoxycarbonyl and 2,4-dinitrophenyl) and the carboxylic group (methyl and ethyl). For each of the derivatives **1–5** oxidation potentials were determined through cyclic voltammetry. Interestingly, oxidation potentials depended on the kind of protecting group (Table 1).

In the electrochemical ^{18}F-labeling reactions of the derivatives **1–5** the labeling yields also depended on the protecting groups (Table 1). Remarkably, the phenylalanine derivative with the highest oxidation potential gave the best radiochemical yield (**1**, 5.8 $\pm$ 2.8%, total of *o*-, *m*- and *p*-isomers). Because the aromatic ring and the protecting groups are far apart from each other, inductive or mesomeric effects at the phenyl ring can be excluded to influence yields of ^{18}F-labeling. Most likely, the results are due to effects of intermolecular forces and interactions on the surface of the anode. For **1** the isomeric distribution was determined with GC/MS (50.5 $\pm$ 1.5% for *ortho*



1 - 6f

	R	X	Y
1	H	TFA	Me
2	H	TFA	Et
3	H	Boc	Me
4	H	Ac	Me
5	H	DNP	Me
6f	*t*Bu	TFA	Me

Figure 1. Generalized reaction scheme for the anodic oxidation of phenylalanine derivatives and subsequent nucleophilic substitution with [^{18}F]fluoride (TFA = trifluoroacetyl, Boc = *tert*-butoxycarbonyl, DNP = 2,4-dinitrophenyl, Ac = acetyl, Me = methyl, Et = ethyl)

Table 1. Oxidation potentials from cyclovoltammetry and radiochemical ^{18}F-fluorination yields from phenylalanine derivatives 1–6f at 50 C in electrolyte A at 0°C

Phenylalanine derivative	Oxidation potential (V)	Radiochemical yield (%)	*n*
1	2.15 ± 0.01	5.8 ± 2.8	10
2	2.13 ± 0.01	2.6 ± 1.1	3
3	2.04 ± 0.02	0.6 ± 0.3	4
4	1.90 ± 0.01	1.6 ± 0.9	4
5	1.65 ± 0.01	0.1 ± 0.05	3
6f	1.89 ± 0.01	1.6 ± 1.0	5

isomer, 11.5 ± 1.5% for *meta* isomer and 38.8 ± 2.1% for *para* isomer). The observed selectivity indicated a reaction mechanism involving a phenylic carbenium ion.

In our previous experiments with various substituted benzenes, radiochemical yields depended on the oxidation potential.[13] Yields increased with decreasing potential. As *tert*-butyl benzene gave the highest ^{18}F-fluorination yields on exchange of *tert*-butyl *vs* fluorine, we also investigated a *tert*-butyl substituted phenylalanine derivative **6f**. The molecule had to be built up in a longer reaction sequence (Figure 2). Indeed, that substrate had a lower oxidation potential due to its higher electron density in the aromatic ring as consequence of the +I effect (Table 1). However, **6f** turned out to be unstable

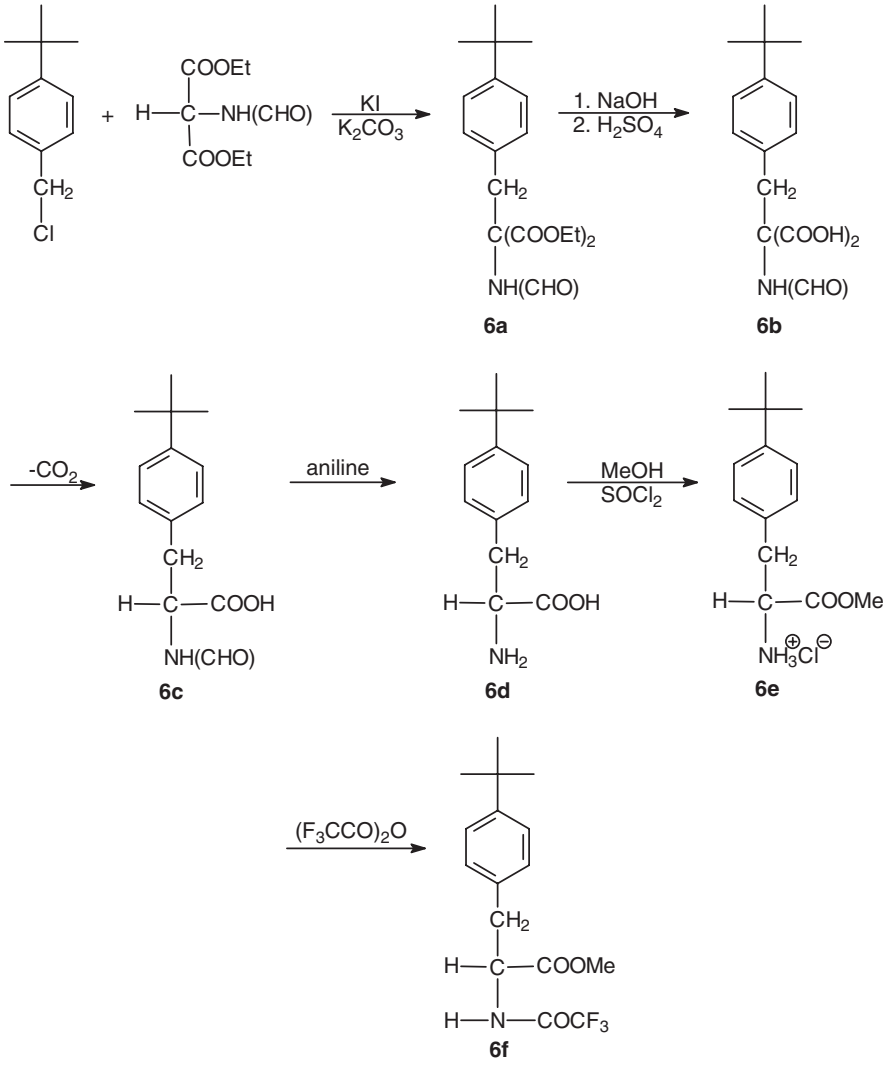


Figure 2. Reaction scheme for the synthesis of 6f

under the electrochemical conditions and radiochemical yields were low ($1.6 \pm 1.0\%$). The relative amount of *para* isomer was somewhat, but not significantly, higher ($44 \pm 8\%$), while the *ortho* and *meta* isomers together amounted to $56 \pm 8\%$.

When performing electrolyses in organic solvents, supporting electrolytes are necessary for the transport of charge (Table 2). In order to optimize the reaction the effect of different anions in the electrolyte was studied (electrolyte B). For the different electrolytes the reaction times varied for certain amounts of charge due to the conductivity of the solution and partly decomposition of the supporting electrolyte at the high potential applied. Experiments with

Table 2. Composition of electrolytes A–C, concentrations in mol/l in acetonitrile. In addition to the salts listed, all solutions contained 0.033 mol/l of $Et_3N \cdot 3$ HF

Electrolyte A	$Et_3N \cdot HCl$	—
	0.067	
Electrolyte B	$Et_3N \cdot 3$ HF	—
	0.022	
	(= 0.067 mol/l fluoride)	
	Et_4NX (X = Cl, Br, I)	—
	0.067	
	Bu_4NPF_6	—
	0.067	
Electrolyte C	Et_4NCl	Bu_4NPF_6
	0.000	0.400
	0.018	0.382
	0.061	0.339
	0.180	0.220
	0.360	0.040

Table 3. Radiochemical yields of fluorination of 1 with electrolytes A or B (0.067 M with 0.033 M $Et_3N \cdot 3$ HF) as a function of charge [C]

Supporting electrolyte	Radiochemical yield (%) 25 C	50 C	*n*
$Et_3N \cdot HCl$	3.4 ± 1.6	6.4 ± 3.7	7
$Et_3N \cdot 3HF$	10.5 ± 2.5	—	5
Et_4NCl	2.9 ± 0.7	4.2 ± 1.6	5
Et_4NBr	1.5 ± 0.6	3.0 ± 1.2	5
Et_4NI	0.01 ± 0.01	0.3 ± 0.2	3
Bu_4NPF_6	2.9 ± 1.4	—	3

Bu_4NPF_6 and $Et_3N \cdot 3$ HF were stopped after a charge of 25 Coulombs [C], all other reactions after 50 C (reaction times were 60 to 90 min).

Variation of halogen showed that from chloride to iodide the supporting electrolyte is oxidized more easily and in the same order radiochemical yields were observed to decrease (Table 3). That may be due to an increasing competition between the halogen ion and the aromatic substrate respecting oxidation and adsorption on the electrode. Bu_4NPF_6 has an oxidation potential of 3.02 V *vs* Ag/Ag^+.[14] Thus, this salt cannot be oxidized at the working potential used, and a competition can be excluded between supporting electrolyte and aromatic substrate. Indeed, fewer side reactions were observed. Electrolyses with Bu_4NPF_6 as supporting electrolyte took about 100 min for a charge of 25 C, in contrast to those reactions with Et_4NCl or $Et_3N \cdot HCl$, which required only 45 min for the same charge. Thus, in these

Table 4. Dependence of radiochemical yields on reaction temperature at 50 C for the fluorination of 1

Temperature (°C)	Radiochemical yield (%)	*n*
25	3.2 ± 2.1	6
0	5.8 ± 2.8	10
−10	5.5 ± 1.5	4
−20	4.2 ± 0.1	3

cases it was possible to extend electrolyses to 50 C to obtain higher radiochemical yields (e.g. $Et_3N \cdot HCl$ at 50 C: 6.4 ± 3.7%). Applying $Et_3N \cdot HCl$ the radiochemical yields were higher than with Et_4NCl, demonstrating that in the supporting electrolyte the protons did not reduce nucleophilicity of [^{18}F]fluoride. Best results were obtained with $Et_3N \cdot 3HF$ (10.5 ± 2.5%, sum of isomers). This was not unexpected because higher substrate concentrations often result in higher yields.

As shown in Table 4 the temperature effect was not significant. The optimal temperatures were between −10°C (5.5 ± 1.5%, radiochemical yield) and 0°C (5.8 ± 2.8%).

Reaction mechanism

For electrochemical fluorination of aromatic compounds the postulated mechanism is an EC_NEC_B process,[15] with the following reaction steps. After diffusion to the anode an aromatic substrate is oxidized to a radical cation (electrochemical step E). In the following chemical step (C_N) fluoride reacts with the radical cation. After a second oxidation (electrochemical step E) and loss of a proton (chemical step C_B), the fluorinated product is formed.

If the electrolyte contains chloride or bromide ions, an indirect electrolysis is conceivable as an alternative reaction mechanism. Thus, chloride for example, is oxidized at the working potential and the chlorine then formed oxidizes the aromatic substrate whereby chlorine itself is reduced to chloride again. In that case, the working potential only has to be in the range of the oxidation potential of chloride. That reaction path will result in fewer side reactions. Another alternative mechanism could be a halex reaction, where, after primary formation of a chlorinated product halogen exchange follows.

In our experiments it was possible to detect chlorinated products with GC/MS. But with halex reactions the aromatic ring normally has to be activated through σ- or π-electron withdrawing groups.[16] For that reason, this mechanism seemed to be unlikely. Variation of working potentials and chloride concentrations (added as supporting electrolyte) were used to investigate which of the two mechanisms occurred.

With cyclic voltammetry the oxidation potential of triethylamine hydrochloride was determined to be 0.65 V in electrolyte A. Working potential in labeling reactions was reduced step by step from 2.0 to 1.5 V. In this range, potentials are high enough to oxidize chloride ions of the supporting electrolyte. At 1.7 and 1.5 V only a small amount of amino acid should be oxidized at the electrode. In case the dominating mechanism is an indirect electrolysis, radiochemical yields at 2.0 V should not decrease remarkably with decreasing working potential. However, experiments showed labeling yields to decrease significantly with decreasing potential (2.0 V:5.8 $\pm$ 2.8%, 1.5 V:1.8 $\pm$ 1.2%). Those results, at least, do not support the hypothesis of an indirect mechanism as the dominating reaction pathway.

GC/MS investigations of product solutions showed that chlorinated phenylalanine derivatives were formed to some extent. In the case of a halex mechanism with a F- for Cl- exchange at primarily formed *N*-trifluoroacetyl-2-, 3- or 4-chloro-phenylalanine methyl esters, decreasing chloride concentrations would be expected to decrease labeling yields.

Chloride concentrations in the electrolyte (electrolyte C) were reduced from an excess over the aromatic substrate to a catalytic amount. In addition, experiments without any chloride were performed. The reactions lasted for 15–110 min (high to low chloride concentration) until a charge of 25 C passed. Decreasing chloride concentration was compensated by adding Bu_4NPF_6, so that the overall concentration of electrolyte was 0.4 M, constantly. All experiments demonstrated that with decreasing chloride concentration radiochemical yields increased clearly from 0.1 $\pm$ 0.01% (0.36 M of chloride) to 9.1 $\pm$ 0.8% (no chloride) (Table 5), indicating a halex reaction to be highly unlikely.

Experimental

General

Chemicals. Acetonitrile (Merck, Germany) was purified according to Salbeck[17] by drying over $CaCl_2$ for at least one week, distillation over P_2O_5, NaH

Table 5. Variation of chloride concentrations in electrosynthesis of *N*-trifluoroacetyl-2-, 3- or 4-[^{18}F]fluoro-phenylalanine methyl esters and influence on radiochemical yields at 25 C ($n = 4$)

Concentration		Ratio		Radiochemical yield (%)
Et_4NCl (mol/l)	Bu_4NPF_6 (mol/l)	Aromatic substrate	Chloride	
0.000	0.400	1	0	9.1 $\pm$ 0.8
0.018	0.382	10	1	5.8 $\pm$ 0.7
0.061	0.339	3	1	1.8 $\pm$ 0.4
0.180	0.220	1	1	0.1 $\pm$ 0.04
0.360	0.040	1	2	0.1 $\pm$ 0.01

and P_2O_5 and final chromatography with neutral Al_2O_3 which was dried in vacuum at 140°C for about 2 days. $Et_3N \cdot HCl$ (Merck-Schuchard, Germany) was twice recrystallized from methanol and dried in vacuum at 40°C for 2–3 days. Et_4NCl, Et_4NBr and Et_4NI (all Fluka, Germany) were twice recrystallized from acetonitrile under argon atmosphere and dried in vacuum at 100°C for 2–3 days. Bu_4NPF_6 (Aldrich, Germany) was twice recrystallized from a mixture of ethanol and water 3:1, washed with methanol and dried in vacuum at 100°C for 2–3 days. Bu_4NClO_4 (Fluka, electrochemical grade) was dried in vacuum at 120°C for 2–3 days. $Et_3N \cdot 3\,HF$ (Fluka) was distilled twice under vacuum (80°C, 5 Torr). $AgNO_3$ (Roth, Germany) and all other chemicals and solvents (Merck or Fluka) were of the highest purity available and used as received.

Analytical procedures. NMR spectroscopy: Bruker AC 250 (^{1}H: 250.1 MHz, ^{13}C: 62.9 MHz) with tetramethylsilane as an internal standard. Mass spectrometry: Varian MAT 711 (70 eV), IR spectroscopy: Bruker IFS 48, melting points: Gallenkamp MPG 350. Microanalyses: Chemisches Zentralinstitut, University of Tübingen.

Phenylalanine derivatives

N-trifluoroacetyl-L-phenylalanine methyl ester (**1**) was either purchased (Sigma, Germany) or synthesized from the unprotected amino acid,[18] *N*-trifluoroacetyl-L-phenylalanine ethyl ester (**2**) was prepared analogously. *N*-butoxycarbonyl-L-phenylalanine methyl ester (**3**) was from Aldrich and *N*-acetyl-L-phenylalanine methyl ester (**4**) from Novachem (Germany). *N*-2,4-dinitrophenyl-L-phenylalanine methyl ester (**5**) was synthesized according to Fletcher.[19] **6a–6e** were prepared analogously to tyrosine derivatives described in literature.[20–22] All phenylalanine derivatives **1–6f** were dried in vacuum at room temperature for 2–3 days before their use in electrochemistry. 2-, 3- and 4-fluoro-DL-phenylalanine were purchased (Fluka), protected as described below and used as references.

Since the NMR data of many of the compounds prepared in this study have not been reported at all, or were essentially incomplete, those data are presented below.

1: The obtained yellow brown oil was distilled twice under reduced pressure, resulting in colorless crystals. Yield: 11% (2.81 g), m.p.: 47–52°C, b.p.: 112°C at 3–4 Torr. MS (EI, *m/z*): 275.1 (M^+), 162.0, 91.1, 131.1. ^{1}H-NMR ($CDCl_3$, δ[ppm]):3.15(1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.24 (1H, dd, $J = 5.8$ Hz, 14.0 Hz), 3.77 (3H, s), 4.88 (1H, ddd, $J = 5.5$ Hz, 5.8 Hz, 7.6 Hz), 6.85 (1H, br d, NH), 7.07 (2H, dd, $J = 1.5$ Hz, 7.6 Hz), 7.25–7.35 (3H, m). ^{13}C-NMR ($CDCl_3$, δ [ppm]): 37.3, 52.7, 53.5, 127.6, 128.8, 129.1, 134.8, 170.4, 115.5 (q, $J = 288.0$ Hz), 156.5 (q, $J = 37.2$ Hz). IR (KBr [cm^{-1}]): 3292, 1751, 1703,

1555, 1441, 1331, 1277. Calculated for $C_{12}H_{12}F_3NO_3$: C 52.37%, H 4.39%, N 5.09%, F 20.71%. Found: C 52.67%, H 4.31%, N 5.19%, F 20.36%.

2: The obtained orange oil could not be distilled. Upon addition of CH_2Cl_2 (5%) in *n*-hexane the product precipitated and was recrystallized. Yield: 15% (4.8 g), m.p.: 70–74°C. MS (EI, *m*/*z*): 289.1 (M^+), 175.9, 91.1, 131.1. ^{1}H-NMR ($CDCl_3$, δ[ppm]): 1.23 (3H, t, $J = 7.0$ Hz), 3.09 (1H, dd, $J = 5.8$ Hz, 14.0 Hz), 3.19 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 4.18 (2H, q, $J = 7.0$ Hz), 4.81 (1H, ddd, $J = 5.5$ Hz, 5.8 Hz, 7.3 Hz), 6.85 (1H, br d, NH), 7.05 (2H, dd, $J = 1.5$ Hz, 6.7 Hz), 7.21–7.29 (3H, m). ^{13}C-NMR ($CDCl_3$, δ[ppm]): 14.0, 37.3, 53.5, 62.2, 127.5, 128.7, 129.2, 134.6, 170.0, 115.6 (q, $J = 288.0$ Hz), 156.5 (q, $J = 37.2$ Hz). IR (KBr [cm^{-1}]): 3310, 1747, 1707, 1562, 1373, 1281, 1240, 1205, 1175. Calculated for $C_{13}H_{14}F_3NO_3$: C 53.98%, H 4.88%, N 4.84%, F 19.70%. Found: C 53.56%, H 4.90%, N 4.84%, F 20.03%.

5: The residue was recrystallized twice from ethanol. Yellow crystals were obtained. Yield: 38% (1.2 g), m.p.: 113–118°C. MS (EI, *m*/*z*): 345.1 (M^+), 286.1, 254.0, 193.8, 165.8, 91.1. ^{1}H-NMR ($CDCl_3$, δ[ppm]): 3.22 (1H, dd, $J = 7.3$ Hz, 14.0 Hz), 3.36 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.79 (3H, s), 4.58 (1H, ddd, $J = 5.5$ Hz, 7.3 Hz, 7.3 Hz), 6.67 (1H, d, $J = 9.5$ Hz), 7.19 (2H, dd, $J = 1.8$ Hz, 7.6 Hz), 7.25–7.36 (3H, m), 8.17 (1H, dd, $J = 2.4$ Hz, 9.5 Hz), 8.87 (1H, d, $J = 7.3$ Hz, NH), 9.08 (1H, d, $J = 2.4$ Hz). ^{13}C-NMR ($CDCl_3$, δ[ppm]):38.4, 52.8, 57.4, 113.8, 124.0, 127.7, 128.9, 129.0, 130.1, 130.9, 134.5, 136.6, 147.0, 170.5. IR (KBr [cm^{-1}]): 3337, 1730, 1614, 1587, 1522, 1339, 1150, 1107. Calculated for $C_{16}H_{15}N_3O_6$: C 55.65%, H 4.38%, N 12.17%. Found: C 55.35%, H 4.27%, N 12.09%.

(*4-tert-Butyl-benzyl*)-*formamidomalonic acid diethyl ester* (**6a**). 4-*tert*-butyl-benzylchloride (24 ml; 0.12 mol) and 25.6 g (0.12 mol) of formamidomalonic acid diethyl ester were dissolved in 660 ml of acetone. Dry K_2CO_3 (220 g) and half a tea-spoon of KI were added and the solution refluxed for 21 h. After filtration of K_2CO_3 and evaporation of acetone, the residue was dissolved in water and extracted with CH_2Cl_2. The combined organic extracts were washed with water and dried over Na_2SO_4. After evaporation a yellow brown oil was obtained. *n*-Hexane (160 ml) were added and the warmed solution was shaken strongly. The yellow needles formed were filtered and dried in vacuum at 40°C for a few hours. Yield: 92% (38.8 g), m.p.: 99–101°C. MS (EI, *m*/*z*): 349.0 (M^+), 304.0, 289.0, 147.0. ^{1}H-NMR ($CDCl_3$, δ[ppm]): 1.28 (9H, s), 1.29 (6H, t, $J = 7.1$ Hz), 3.63 (2H, s), 4.27 (4H, q, $J = 7.1$ Hz), 6.77 (1H, br s, NH), 6.96 (2H, d, $J = 8.4$ Hz), 7.26 (2H, d, $J = 8.4$ Hz), 8.15 (1H, s). ^{13}C-NMR ($CDCl_3$, δ[ppm]): 14.0, 31.3, 34.4, 37.4, 62.8, 66.8, 125.3, 129.6, 131.7, 150.2, 159.9, 167.2. IR (KBr [cm^{-1}]): 3229, 2959, 2905, 2868, 1744, 1655, 1518, 1377, 1366, 1278, 1250, 1188, 1055. Calculated for $C_{19}H_{27}NO_5$: C 65.31%, H 7.79%, N 4.01%. Found: C 65.31%, H 7.79%, N 4.02%.

(4-tert-Butyl-benzyl)-formamidomalonic acid (**6b**). **6a** (38.3 g; 0.11 mol) was dissolved in 600 ml of ethanol. NaOH (34.8 g) in 240 ml of water was added and the solution refluxed for 2 h under inert gas atmosphere. Acetic acid was added until pH 6 and H_2SO_4 (13%) until pH 2. (About 50 ml of acetic acid and 270 ml of H_2SO_4 were necessary.) After evaporation of about 600 ml of solvent and addition of 200 ml of water, the solution had to be cooled for a few hours. The white crystals formed were filtered, washed with water and dried in vacuum at 70°C. Yield: 90% (29.3 g), m.p.: 187–190°C. MS (EI, *m/z*): 249.0, 204.0, 189.0, 147.0, MS (FAB, NBA, *m/z*): 293.9 ($M^+ + H$). ^{1}H-NMR (DMSO-d_6, δ[ppm]):1.23 (9H, s), 3.36 (2H, s), 6.95 (2H, d, $J = 8.2$ Hz), 7.26 (2H, d, $J = 8.2$ Hz), 7.97 (1H, s), 8.21 (1H, br s, NH), 13.40 (2H, br s). ^{13}C-NMR (DMSO-d_6, δ[ppm]): 31.2, 34.1, 36.9, 66.1, 124.8, 129.5, 132.8, 148.9, 160.7, 168.9. IR (KBr [cm^{-1}]): 3368, 2963, 2905, 2868, 1730, 1628, 1497, 1373. Calculated for $C_{15}H_{19}NO_5$: C 61.42%, H 6.53%, N 4.78%. Found: C 60.00%, H 6.10%, N 4.64%.

*4-tert-butyl-N-formyl-*DL*-phenylalanine* (**6c**). **6b** (29.3 g; 0.1 mol) was suspended in 800 ml of petroleum ether 110/140 and refluxed for 3 h. The product was filtered, washed with petroleum ether, recrystallized from methanol and dried in vacuum at room temperature for some hours. Yield: 81% (17.5 g), m.p.: 186–192°C. MS (FAB, NBA, *m/z*): 250.1 ($M^+ + H$). ^{1}H-NMR (CD_3OD, δ[ppm]):1.15 (9H, s), 2.48 (1H, dd, $J = 7.9$ Hz, 14.0 Hz), 3.04 (1H, dd, $J = 5.2$ Hz, 14.0 Hz), 4.60 (1H, dd, $J = 5.2$ Hz, 7.9 Hz), 7.01 (2H, d, $J = 8.2$ Hz), 7.18 (2H, d, $J = 8.2$ Hz), 7.88 (1H, s). ^{13}C-NMR (CD_3OD, δ[ppm]): 31.8, 35.2, 37.9, 53.6, 126.3, 130.3, 134.8, 150.8, 163.4, 174.2. IR (KBr [cm^{-1}]): 3348, 2963, 2905, 2868, 1720, 1614, 1522, 1366, 1360. Calculated for $C_{14}H_{19}NO_3$: C 67.45%, H 7.68%, N 5.62%. Found: C 67.12%, H 7.86%, N 5.66%.

*4-tert-Butyl-*DL*-phenylalanine* (**6d**). **6c** (9 g; 0.036 mol) was heated with 60 ml of freshly distilled aniline at 90–95°C for 1 h. After cooling, 80 ml of ether were added. The crystals formed were filtered and washed with ether and ethanol. Yield: 39% (3.3 g), m.p.: 255–257°C. MS (EI, *m/z*): 221.2 (M^+), 204.2, 189.1, 147.0, MS (FAB, NBA, *m/z*):222.1 ($M^+ + H$). ^{1}H-NMR (CD_3COOD, δ[ppm]): 1.25 (9H, s), 3.09 (1H, dd, $J = 8.2$ Hz, 14.7 Hz), 3.28 (1H, dd, $J = 4.9$ Hz, 14.7 Hz), 4.28 (1H, dd, $J = 4.9$ Hz, 8.2 Hz), 7.19 (2H, d, $J = 7.9$ Hz), 7.30 (2H, d, $J = 7.9$ Hz). ^{13}C-NMR (CD_3COOD, δ[ppm]): 31.6, 35.0, 36.7, 57.0, 126.6, 130.4, 132.7, 151.3, 174.8. IR (KBr [cm^{-1}]): 3450, 2963, 2905, 2868, 1624, 1587, 1512, 1360. Calculated for $C_{13}H_{19}NO_2$: C 70.56%, H 8.65%, N 6.33%. Found: C 70.14%, H 8.45%, N 6.26%.

*4-tert-Butyl-*DL*-phenylalanine methyl ester hydrochloride* (**6e**). **6d** (3 g; 0.014 mol) was dissolved in 35 ml of absolute methanol and cooled to −78°C. Freshly distilled thionyl chloride (2.5 ml; 0.034 mol) was added. Thereby, temperature should not exceed 0°C. The solution was stirred at room

temperature for 48 h and then, evaporated to dryness. The residue was dissolved in a minimal amount of methanol and insoluble particles were filtered. After addition of dry ether white crystals were obtained. They were filtered and dried in vacuum at room temperature. Yield: 62% (2.4 g), m.p.: 210–214°C. MS (EI, *m/z*): 236.2, 176.2, 147.2, 133.2, 88.1. ^{1}H-NMR (DMSO-d_6, δ[ppm]): 1.26 (9H, s), 3.06 (1H, dd, $J = 7.3$ Hz, 14.4 Hz), 3.15 (1H, dd, $J = 6.1$ Hz, 14.4 Hz), 3.67 (3H, s), 4.21 (1H, dd, $J = 6.1$ Hz, 7.3 Hz), 7.15 (2H, d, $J = 8.2$ Hz), 7.33 (2H, d, $J = 8.2$ Hz), 8.71 (3H, br s, NH_3^+). ^{13}C-NMR (DMSO-d_6, δ[ppm]): 31.1, 34.2, 35.3, 52.5, 53.2, 125.3, 129.1, 131.6, 149.6, 169.4. IR (KBr [cm^{-1}]): 3450, 2959, 2905, 2868, 1747, 1495, 1364, 1236. Calculated for $C_{14}H_{21}NO_2 \cdot HCl$: C 61.87%, H 8.16%, N 5.15%, Cl 13.04%. Found: C 62.09%, H 8.74%, N 5.28%, Cl 12.76%.

N-Trifluoroacetyl-4-tert-butyl-DL-*phenylalanine methyl ester* (**6f**). The yellow brown oil was purified by chromatography with silica gel (*n*-hexane/ethyl acetate). Yield: 62% (1.7 g). MS (EI, *m/z*): 331.2 (M^+), 218.1, 203.1, 147.1. ^{1}H-NMR ($CDCl_3$, δ[ppm]): 1.30 (9H, s), 3.14 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.21 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.79 (3H, s), 4.87 (1H, dt, $J = 5.5$ Hz, 7.6 Hz), 6.81 (1H, br d, NH), 6.99 (2H, dd, $J = 1.8$ Hz, 6.4 Hz), 7.32 (2H, dd, $J = 1.8$ Hz, 6.4 Hz). ^{13}C-NMR ($CDCl_3$, δ[ppm]): 31.2, 34.5, 36.7, 52.7, 53.5, 125.7, 128.8, 131.4, 150.5, 170.4, 115.6 (q, $J = 288.0$ Hz), 156.5 (q, $J = 37.2$ Hz). IR (film [cm^{-1}]): 3327, 2963, 2903, 2870, 1749, 1722, 1553, 1394, 1366, 1215, 1175. Calculated for $C_{16}H_{20}F_3NO_3$: C 58.00%, H 6.08%, N 4.23%. Found: C 58.02%, H 5.87%, N 4.43%.

Syntheses of reference substances

2-, 3- and 4-fluoro-DL-phenylalanine were first derivatized to the methyl ester hydrochloride as described for **6e** and then to the *N*-trifluoroacetyl-2-, 3- and 4-fluoro-DL-phenylalanine methyl esters **7a–c** analogously to the procedure for **1**.

N-Trifluoroacetyl-2-fluoro-DL-*phenylalanine methyl ester* (**7a**). Yield: 13% (90 mg), m.p.: 49–53°C (from *n*-hexane). MS (EI, *m/z*): 293.1 (M^+), 234.0, 179.9, 109.0, 149.0. ^{1}H-NMR ($CDCl_3$, δ[ppm]):3.12 (1H, dd, $J = 5.8$ Hz, 14.0 Hz), 3.26 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.72 (3H, s), 4.80 (1H, ddd, $J = 5.5$ Hz, 5.8 Hz, 7.6 Hz), 6.87 (1H, br d, NH), 6.93–7.05 (3H, m), 7.15–7.21 (1H, m). ^{13}C-NMR ($CDCl_3$, δ[ppm]): 31.0, 52.8, 52.9, 170.3, 115.5 (q, $J = 288.0$ Hz), 115.5 (d, $J = 21.9$ Hz), 121.7 (d, $J = 15.3$ Hz), 124.4 (d, $J = 2.9$ Hz), 129.6 (d, $J = 7.6$ Hz), 131.5 (d, $J = 4.8$ Hz), 156.6 (q, $J = 38.2$ Hz), 161.3 (d, $J = 245.1$ Hz). IR (KBr [cm^{-1}]): 3333, 3098, 2959, 1760, 1722, 1553, 1495, 1225, 1178, 760.

N-Trifluoroacetyl-3-fluoro-DL-*phenylalanine methyl ester* (**7b**). Yield: 53% (367 mg), m.p.: 75–79°C (from *n*-hexane). MS (EI, *m/z*):293.0 (M^+), 233.9,

179.9, 109.1, 149.0. ^{1}H-NMR ($CDCl_3$, δ[ppm]):3.15 (1H, dd, $J = 5.8$ Hz, 14.0 Hz), 3.26 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.81 (3H, s), 4.88 (1H, ddd, $J = 5.5$ Hz, 5.8 Hz, 7.3 Hz), 6.76–7.03 (4H, m, $3H_{arom.}$ + NH), 7.24–7.33 (1H, m). ^{13}C-NMR ($CDCl_3$, δ[ppm]): 37.0, 52.9, 53.4, 170.2, 114.6 (d, $J = 21.0$ Hz), 115.5 (q, $J = 288.0$ Hz), 116.1 (d, $J = 21.9$ Hz), 124.9 (d, $J = 2.9$ Hz), 130.3 (d, $J = 8.6$ Hz), 137.1 (d, $J = 6.7$ Hz), 156.6 (q, $J = 38.2$ Hz), 166.9 (d, $J = 247.0$ Hz). IR (KBr [cm^{-1}]): 3296, 3099, 3071, 2960, 1753, 1726, 1560, 1450, 1439, 1225, 1178.

N-Trifluoroacetyl-4-fluoro-DL-*phenylalanine methyl ester* (**7c**). Yield: 36% (250 mg), m.p.: 73–76°C (from *n*-hexane). MS (EI, *m*/*z*): 293.1 (M^+), 234.0, 179.9, 109.1, 149.0. ^{1}H-NMR ($CDCl_3$, δ[ppm]):3.14 (1H, dd, $J = 5.5$ Hz, 14.0 Hz), 3.24 (1H, dd, $J = 5.8$ Hz, 14.0 Hz), 3.79 (3H, s), 4.86 (1H, ddd, $J = 5.5$ Hz, 5.8 Hz, 7.3 Hz), 6.87 (1H, br d, NH), 6.98–7.06 (4H, m). ^{13}C-NMR ($CDCl_3$, δ[ppm]): 36.5, 52.9, 53.6, 170.3, 115.5 (q, $J = 288.0$ Hz), 115.8 (d, $J = 21.0$ Hz), 130.3 (d, $J = 0.9$ Hz), 130.7 (d, $J = 8.6$ Hz), 156.5 (q, $J = 38.2$ Hz), 162.3 (d, $J = 247.0$ Hz). IR (KBr [cm^{-1}]): 3310, 3090, 2960, 1751, 1726, 1568, 1510, 1441, 1205, 1165.

Cyclic voltammetry

Cyclic voltammetry was carried out in a 20 ml undivided glass cell with platinum working and counter electrodes and a platinum and Ag/Ag^+ double reference electrode at room temperature. The solution for the reference electrode contained $AgClO_4$ (0.01 M) and Bu_4NPF_6 (0.1 M) in acetonitrile. As electrolyte and salt bridge a solution of Bu_4NPF_6 (0.1 M) in acetonitrile was used. The electrolyte was degassed with argon for 30 min. The measurements were performed at room temperature and between the measurements the solution was stirred magnetically. The potentiostat was a BAS 100 B/W (BAS, Antwerp, Belgium). The BAS 100 W software was used. All cyclovoltammograms of substrates were baseline corrected.

[^{18}F]Fluoride production

[^{18}F]Fluoride was produced through the $^{18}O(p,n)^{18}F$ nuclear reaction at a PETtrace cyclotron ($E_p = 16.5$ MeV, General Electric Medical Systems, Sweden) in a high pressure silver target. [^{18}O]H_2O (1.5 ml; Rotem, Germany, 94–96% enrichment) was irradiated. The [^{18}F]fluoride was trapped on a pre-conditioned strong ion exchange cartridge (Waters Accel QMA Plus, Waters, Germany), rinsed with 4 ml of acetonitrile for removing water and eluted with 2 ml of electrolyte. Because the anion exchange was performed by means of chloride, for the experiments with variable chloride concentrations the cartridge was rinsed with 10 ml of KF solution (0.4 M in water) for removing the chloride before trapping the

[^{18}F]fluoride. After rinsing with 3.5 ml of KF solution, chloride detection was negative.

Electrochemical oxidation

Electrochemical oxidations were carried out in an undivided three-electrode cell with platinum wire working (anode) and counter (cathode) electrodes and an Ag/Ag^+ reference electrode (Ag/0.01 M $AgNO_3$ in acetonitrile). The cell was already described elsewhere.[12] Electrolyses were carried out potential controlled with a Wenking STP 96 potentiostat together with a Wenking EVI 95 current integrator (both Bank Elektronik GmbH, Germany). A working potential of 2.0 V was used, in case of experiments with variable working potentials the voltage was between 1.5 and 2.0 V. Periodical pulsing between 2.0 and 0 V was necessary to minimize formation of a layer covering the anode.

Bu_4NClO_4 was added to the solution of the reference electrode as supporting electrolyte (either 0.1 or 0.4 M). A solution of Bu_4NClO_4 in acetonitrile was applied as salt bridge (either 0.1 or 0.4 M). Solutions with the higher concentrations were used in experiments with variable chloride concentrations, as the electrolyte had the same concentration then. In all other cases the lower concentrated electrolytes were applied.

As electrolyte, three different solutions were applied (Table 2), electrolyte A for experiments with the different phenylalanine derivatives or varying temperature or working potentials, electrolyte B for experiments with different supporting electrolytes and electrolyte C for investigations with variation of chloride concentration.

Syntheses were carried out under argon atmosphere. After the electrolyte (2 ml) was placed in the cell, the phenylalanine derivative (0.4 mmol) was added, the solution stirred magnetically and cooled down to 0°C with a cryostate (Lauda ecoline RE 104, Lauda GmbH & Co. KG, Germany). In experiments with varying temperatures these ranged from −20 to +25°C. Except for investigations with various phenylalanine derivatives, **1** was used. After certain amount of charge had passed, samples were taken and analyzed. Electrolyses except for those with variation of supporting electrolyte and chloride concentration were stopped after 50 C of current passed, corresponding to a reaction time of 60–120 min, depending on the conductivity of the solution.

Product analyses

Qualitative detection was carried out with HPLC (Gynkotek, Germany), a UV-(Gynkotek, UVD 170S) and a NaI(Tl) scintillation detector (Scionix, Germany, 25 B 25/1.5 EP). A Partisil 10 ODS 3 column (250 × 8 mm) with a

pre-column (Partisil 10 ODS 3, 40×8 mm, both CS Chromatographie Service GmbH, Germany) were employed. Eluents contained water and acetonitrile with 0.1% KF added, to avoid adsorption of fluoride on the column. TLC and electronic autoradiography were applied for quantitative assay of all fluorinated phenylalanine derivatives. For TLC silica gel (Machery-Nagel, Germany) and ethyl acetate/*n*-hexane mixtures were used. The radioactive products were detected with autoradiography (InstantImager, Canberra-Packard, Germany). Determination of relative isomeric distribution of **7a–c** was carried out using GC and MS. For GC a FS-Lipodex D column (25 m $\times$ 0.25 mm, Machery-Nagel) with a pre-column (5 m $\times$ 0.25 mm, Hewlett-Packard, Germany) was applied. Helium (0.7 ml/min), split injection and a constant temperature of 170°C were used. The system contained an automatic injecting system, an oven (HP 5890 II), a GC/MS interface and the MS (HP 5989 A). For MS the electron ionisation method was used.

Conclusions

As an example of an aromatic amino acid, protected phenylalanine was ^{18}F-fluorinated successfully. For the first time [^{18}F]fluorophenylalanine has been prepared by electrochemical ^{18}F-fluorination through nucleophilic substitution using [^{18}F]fluoride. The reaction showed a strong dependence on the nature of the protective groups at the amino and carboxylic group. Best labeling results (i.e. 10.5 $\pm$ 2.5%) were obtained with the methylester and the TFA group for protection of the amino function, at temperatures between -10°C and 0°C using $Et_3N \cdot 3HF$ as supporting electrolyte at a potential of 2.0 V. These conditions resulted in a carrier-added product. Based on the addition of 0.33 mmol fluoride for an activity of 400 MBq, the specific activity is calculated to be 1.2 GBq/mmol.

Further improvements are in progress for increasing regioselectivity by use of different activating groups in the aromatic ring, for minimizing the addition of carrier and optimizing radiochemical yields by variation of supporting electrolyte and solvent. Nevertheless, the presented results demonstrate the value of the electrochemical ^{18}F-fluorination for labeling of aromatic amino acids to be used as metabolic tracers in PET.

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