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Base/cryptand/metal-free automated nucleophilic radiofluorination of [^{18}F]FDOPA from iodonium salts: importance of hydrogen carbonate counter ion.

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Abstract: As evidenced by the number of publications and patents published in the last years, the radiosynthesis of 6-[^{18}F]fluoro-3,4-dihydroxy-*L*-phenylalanine ([^{18}F]FDOPA) using nucleophilic [^{18}F]F$^-$ process remains currently a challenge for the radiochemists scientific community even if promising methods for the radiofluorination of electron-rich aromatic structures were recently developed from arylboronate, arylstannane or iodoniums salts precursors. In such context, based on the use of an iodonium triflate salt precursor, we optimized a fast and efficient radiofluorination route fully automated and free from any base, cryptand or metal catalyst for the radiosynthesis of [^{18}F]FDOPA. Using this method, this clinically relevant radiotracer was produced in 64 min, 27-38% RCY d.c. (n = 5), >99% RCP, >99% e.e., and high Am 170-230 GBq/µmol. In addition, this optimisation study clearly highlighted the important role of a triflate-hydrogen carbonate counterion exchange during the radiolabelling process to achieve high fluorine-18 incorporation yields.

Introduction

6-[^{18}F]Fluoro-3,4-dihydroxy-*L*-phenylalanine ([^{18}F]FDOPA) positron emission tomography (PET) imaging has been used for more than 30 years for a broad range of clinical applications[1] including diagnosis and follow up of neurodegenerative disorders,[2] brain tumours[3] and other malignant diseases.[4] Despite its obvious clinical utility, [^{18}F]FDOPA was underused primarily due to a lack of availability. Until a few years ago, the electrophilic substitution of the corresponding stannylated precursor with gaseous carrier added radioactive F_2 was the only available radiosynthesis for this radiotracer, thus limiting to a few number of radiopharmaceutical centres the capacity to produce and deliver [^{18}F]FDOPA. In addition to the difficulty of handling a radioactive gas, the use of carrier added radioactive F_2 which displays low molar activity (Am, 0.1-0.6 GBq/µmol) was also problematic for the radiosynthesis of [^{18}F]FDOPA due to the concomitant formation of non-radioactive [^{19}F]FDOPA presenting potential pharmacological effects.[5] During the last decade, significant efforts have been deployed to access to [^{18}F]FDOPA, from the widely available and no-carrier-added (n.c.a.) [^{18}F]fluoride ions.[6] However, radiofluorination of such electron-rich aromatic structure *via* nucleophilic substitution remains a highly challenging task. The radiosynthesis of [^{18}F]FDOPA is also hampered by the presence of an oxygen-sensitive catechol function and a chiral center requiring a careful control of the enantiomeric excess (e.e.). The most recent and marked examples of nucleophilic approaches overcoming these challenges are depicted in Figure 1. The fully automated multi-step radiosynthesis (Figure 1, method A) described by Lemaire *et al.*[7] was based on a classical aromatic nucleophilic substitution of an *ortho*-activated trimethylammonium triflate precursor with [^{18}F]fluoride ([^{18}F]F$^-$), followed by solid supported reduction and halogenation of the aldehyde function into benzyl iodide. The enantioselective introduction of the amino-acid part was achieved by alkylation with the *tert*-butyl glycinate-benzophenone Schiff base in the presence of a chiral phase-transfer catalyst. Final deprotection with concentrated hydroiodic acid afforded [^{18}F]FDOPA on the curie level with high radiochemical yield (RCY), Am, enantiomeric excess (e.e.) and extraordinary short time of 65 min for such complex multi-step radiosynthesis procedure. Taking advantage of the most recent advances in radiofluorination of electron-rich aromatic rings, [^{18}F]FDOPA has also been obtained with high RCY and e.e. by copper promoted carbon-fluorine coupling reaction on an arylboronate precursor[8,9] (Figure 1, methods B1 and B2). The protected [^{18}F]FDOPA was also obtained with a high radiolabelling efficiency by copper promoted carbon-fluorine reaction starting from an arylstannane precursor[10] (Figure 1, method C) but the following deprotection step to afford [^{18}F]FDOPA was not reported in this case. Finally, [^{18}F]FDOPA was produced by [^{18}F]F$^-$ nucleophilic substitution of an asymmetrical diaryliodonium salt[11] (Figure 1, method D) followed by removal of the protecting groups.

Despite later stage incorporation of fluorine-18, moderate decay-corrected (d.c.) RCY were obtained for strategies depicted in methods B1 and D (<15% or even less than 10% for copper-mediated radiosynthesis when applied on a Synthra platform).

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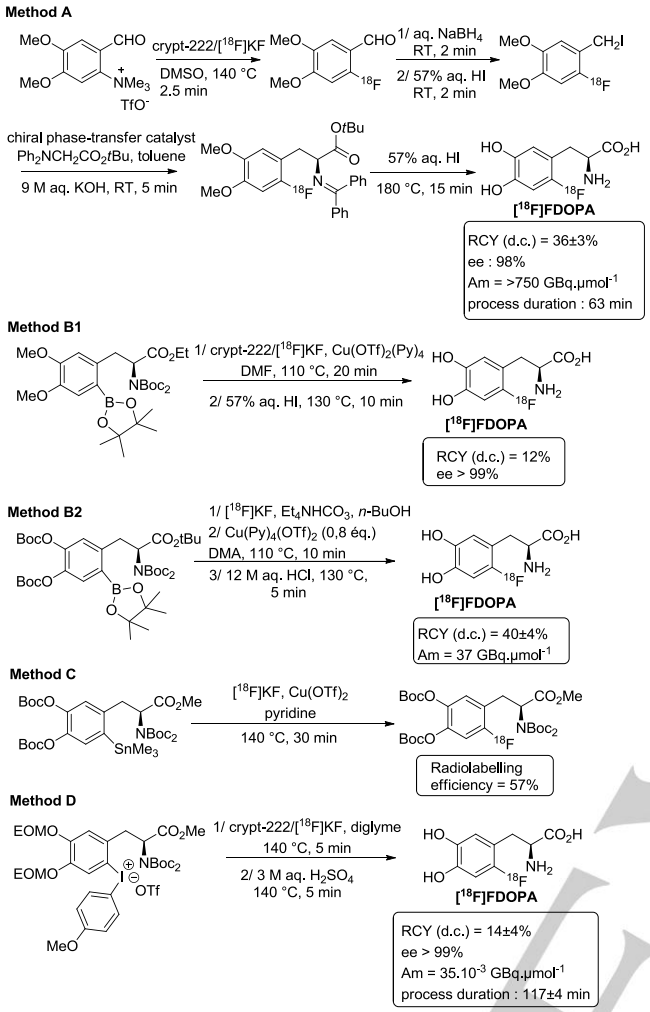


Figure 1. n.c.a. radiosyntheses of [^{18}F]FDOPA according to Lemaire et al.[7] (method A), Tredwell et al.[8a] (method B1), Zischler et al.[8b] (method B2), Makavarage et al.[10] (method C) and Kuik et al.[11] (method D).

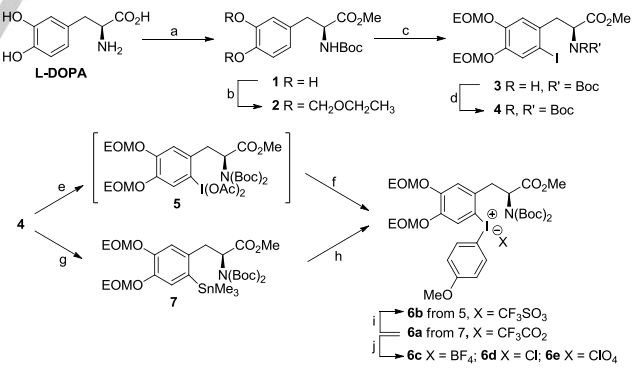
Among other limitations, high amount of precursor was required for method B2 (i.e. 40 mg) while non-radioactive metal contamination, particularly copper, in the final solutions must be carefully assessed before human use for methods B and C.

Among the aforementioned strategies and considering that reducing the overall radiosynthesis time remains a commonly used solution to enhance RCY with such short half-life radionuclide, we speculated that the relatively long reaction time of the iodonium salt procedure (i.e. 117 min, Figure 1, method D) could be shortened. We proposed to directly elute [^{18}F]F$^-$ from the anion exchange QMA cartridge with an alcoholic solution of iodonium salts as previously described.[12] This strategy would also take advantage of eliminating base and cryptand of the reaction media in order to circumvent any decomposition of the reactive iodonium salt precursor under the harsh conditions used for radiolabelling of such structures.[13] Following these assumptions, we report here the development of a new fully automatic fast nucleophilic radiosynthesis of [^{18}F]FDOPA free from any base, cryptand and metal catalyst. We also highlight in this manuscript that the success of such radiofluorination reaction is highly dependent of the nature on the iodonium precursor counter ion: herein a HCO_3^- anion.

Results and Discussion

Synthesis of diaryliodonium salt precursors

According to slight modifications of the procedure previously published,[11] the target diaryl iodonium salt precursor **6b** was obtained in five steps from commercially available L-DOPA. Briefly, **1** was produced by esterification and *NH*-Boc protection of starting material (Scheme 1). Then, catechol hydroxy groups were selectively protected as ethoxymethyl ethers to give compound **2**. Regioselective monoiodination with the combination of iodine and *bis*(trifluoroacetoxy)phenyl-λ^3-iodane[14] provided **3** in 74% yield (the use of fleshly prepared *bis*(trifluoro)phenyl-λ^3-iodane[15] is recommended to obtain reproducible and consistent yields). Compound **3** was converted into the bis-Boc protected derivative **4** to circumvent intramolecular interaction during the radiosynthesis between the iodine (III) center and the NH-Boc function.[16] The fully protected compound **4** was converted into triflate diaryliodonium salt **6b** using a multistep one-pot procedure *via* the key intermediate (diacetoxyiodo)arene **5**. The latter was prepared *in situ* by treatment of aryl iodine **4** with F-TEDA-2BF$_4$ (Selectfluor©) and TMSOAc. Treatment of **5** with potassium (4-methoxyphenyl)trifluoroborate and TMSTFA gave the corresponding diaryliodonium trifluoroacetate salt. The trifluoroacetate counterion was then successively exchanged with acetate, hexafluorophosphate and finally triflate to give radiolabelling precursor **6b**.



Scheme 1. Preparation of diaryliodonium salts **6a-e**. Reagents and conditions: (a) i) MeOH, $SOCl_2$, 0 °C then reflux, 16 h; ii) $(Boc)_2O$, THF, sat. aq. $NaHCO_3$, RT, 2 h, 95%; (b) $C_2H_5OCH_2Cl$, DIPEA, THF, 0 °C then 40 °C, 15 h, 99%; (c) I_2, K_2CO_3, $PhI(OCOCF_3)_2$, CH_2Cl_2, RT, 2.5 h, 74%; (d) NEt_3, DMAP, $(Boc)_2O$, MeCN, RT, 29 h, 86%; (e) Selectfluor, TMSOAc, MeCN, RT, 6.5 h; (f) i) $TMSOCOCF_3$, *p*-$MeOPhBF_3K$, MeCN, RT, 10 min; ii) 0.5 M aq. NaOAc/AcOH buffer (pH 5); iii) aq. $NaPF_6$, MeCN, RT; iv) IRA-400 (TfO^-) ion-exchange resin, 36%; (g) Sn_2Me_6, $Pd(PPh_3)_4$, 1,4-dioxane, reflux, 1.5 h, 91%; (h) i) *p*-$MeOPh(IOAc)_2$, CH_2Cl_2, -40 °C, 1 h; ii) TFA, -40 °C then RT, 5 h, 74%; (i) 1 M aq. NaOTf, CH_2Cl_2, RT, 85%; (j) 1 M aq. NaOTf, $NaBF_4$, NaCl or $NaClO_4$, CH_2Cl_2, RT, 87-95%. EOM: ethoxymethyl.

Several modifications of the final synthesis step have been made. First, the use of a N_2 charged glove-box necessary for the formation of a highly instable (difluoroiodo)arene intermediate, was substituted by a benchtop procedure under argon atmosphere. Moreover, the final isolation and purification of **6b** by precipitation in MTBE was not successful and was replaced by silica gel column chromatography purification. These modifications could explain the lower yield obtained (36%) compared to the reported procedure (63%). Alternatively, to this time-consuming and relatively complex one-pot reaction, we turned our attention to the recent development described by Wirth and co-workers[17] for solid-supported synthesis of iodonium salts. This strategy required the synthesis of trimethylstannane **7** from aryl iodine derivative **4** using hexamethylditin and $Pd(PPh_3)_4$ as catalyst. Ligand exchange with (diacetoxyiodo)anisole[18] in the presence of TFA, added in a sequential manner at -40 °C, gave diaryliodonium trifluoroacetate **6a**. Complete $CF_3CO_2^-$ to $CF_3SO_3^-$ exchange was then easily achieved by repeated washes of a solution of **6a** in dichloromethane with an aqueous solution of NaOTf. According to this protocol, compound **6b** was obtained over two steps in 53% yield, comparable to the one described by Kuik *et al.*[11] (63%). Regardless to the methods used for the synthesis of **6b**, the structural determinations were fully in agreement with the previously reported data (see supporting information).

Radiofluorination of diaryliodonium salts

We first tested as benchmark the previously reported radiofluorination method for [^{18}F]FDOPA production *via* iodonium salt precursor **6b**.[11] The radiosynthesis was performed on a SynChrom R&D Raytest EVOII platform using 935 to 4470 MBq of azeotropically dried K[^{18}F]F-crypt-222-carbonate complex and 11.2 ± 0.5 mg (13 µmol) of **6b** in anhydrous diglyme at 140 °C for 5 min. In these conditions, only 8.7 ± 2.3% (n = 3) of the protected radiofluorinated FDOPA derivative was obtained after solid phase C_{18} cartridge extraction. To increase the yield of incorporation of [^{18}F]F$^-$ and try to reproduce the results of Kuik *et al.*[11] (i.e. RCY = 14% after deprotection), we increased the reaction time up to 15 min but without success. It should be noted that similar values (i.e. 4.5±1.3% RCY) were recently reported by Collins *et al.*[19] In the radiolabelling conditions described above, TLC-monitoring of the reaction mixture evidenced that no iodonium salt precursor can be detected by UV revelation of TLC plates already after 2 min at 140 °C highlighting the high instability of this compound under basic conditions (K_2CO_3/crypt-222). In addition, we clearly noticed that the sensitive precursor had to be freshly prepared, carefully stored under anhydrous argon atmosphere and protected from heat, light and moisture.

We then turned our attention to the direct elution of [^{18}F]F$^-$ from the anion exchange QMA cartridge with an alcoholic solution of iodonium salt **6b**. Our optimisation study was divided in three parts: 1) elution of [^{18}F]F$^-$ from the QMA cartridge, 2) incorporation of fluorine-18 on the protected structure, and 3) deprotection step leading to [^{18}F]FDOPA.

1/ Elution of [^{18}F]F$^-$ from the QMA cartridge

In order to prevent the decomposition of the precursor under basic conditions and avoid the time consuming drying procedure mandatory when using K_2CO_3/crypt-222, a direct elution of QMA-loaded [^{18}F]F$^-$ with the diaryliodonium salt **6b** was assessed. Based on the work of Richarz *et al.*[12b] describing a comparable minimalist approach, we compared different solvents or mixture of solvents for the elution of [^{18}F]F$^-$ (Figure 2). Consistent with their findings, only protic solvents afforded suitable elution yields (62 to 97%) with a concentration of 13 µmol / 750 µL of **6b** while decreasing the volume of eluent, with the same amount of precursor, led to decreased elution yields (data not shown). Furthermore, any attempts to use an aprotic solvent such as acetonitrile, typically used in fluorine-18 radiochemistry processes, or mixture of aprotic and protic solvents decreased dramatically the elution yield (<16%). Finally, we found that a solution of precursor **6b** (13 µmol) in a minimum of 750 µL of methanol revealed to be the most effective combination to elute [^{18}F]F$^-$ initially trapped on the anion exchange cartridge (97 ± 0.03%, n = 41).

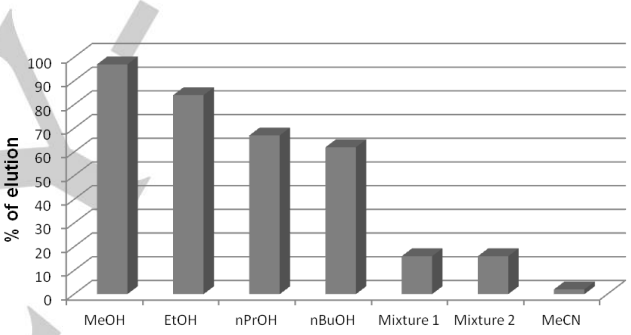


Figure 2. Percentages (%, ratio between values of activity measured in the eluate and initially loaded onto the QMA cartridge, n = 2 except for MeOH: n = 41) of [^{18}F]F$^-$ elution from QMA-HCO_3^- cartridges depending on the solvent used for dissolution of the iodonium salt **6b** (13 µmol in 750 µL); Mixture 1: MeCN/3-methylpentan-3-ol, 25/75, v/v; Mixture 2: MeCN/H_2O, 90/10, v/v.

Then, we intended to study the influence of the counterion of the iodonium precursor for the recovery of [^{18}F]F$^-$ from the QMA cartridge. To this end, the five iodonium salts **6a-e** (Scheme 1) with Cl^-, ClO_4^-, BF_4^-, $CF_3SO_3^-$ or $CF_3CO_2^-$ counterions were compared (Figure 3). When using classical QMA-HCO_3^- cartridges (SepPak Accell Plus QMA Plus Light cartridges, Waters, 130 mg, preconditioned with K_2CO_3), moderate elution yields were obtained for the BF_4^- and $CF_3CO_2^-$iodonium salts. The best results were observed with iodonium salts bearing Cl^-, ClO_4^-, or $CF_3SO_3^-$ counterions (91, 86 and 97% respectively). We noted that the elution efficiency seemed to be correlated with pKb values of the corresponding counteranions tested. Indeed, the stronger the basicity of counterions is, the worse the elution of [^{18}F]F$^-$ gets. As we suspected that all anions present in the reaction mixture could enter in competition with [^{18}F]F$^-$ during the radiofluorination reaction, we also tried QMA cartridges preconditioned with the corresponding counterion (i.e. Cl^-, ClO_4^-,

BF_4^-, $CF_3SO_3^-$ or $CF_3CO_2^-$). In all cases, this preconditioning strategy did not affect the trapping of $[^{18}F]F^-$ on the anion exchange resin (always higher than 90%). However, for most of the evaluated anions, the elution yields were lower compared to QMA-HCO_3^- cartridges (49% for Cl^-, 14% for BF_4^-, 32% for $CF_3SO_3^-$ and 19% for $CF_3CO_2^-$). Interestingly, only the ClO_4^- iodonium salt **6e** provided comparable elution efficiencies (86% for the QMA-ClO_4^- *vs*. 85% for the QMA-HCO_3^-). These experiments highlighted that the HCO_3^- anions present on the exchange resin seem to play a key role in the elution process of the $[^{18}F]F^-$. To clarify this phenomenon, we tried to determine more precisely the chemical composition of the QMA-HCO_3^- cartridge eluates. For this study, we used the same preconditioned QMA-HCO_3^- cartridges described above and a methanolic solution of iodonium salt **6b** which proved to be the most efficient media to elute $[^{18}F]F^-$ (Figure 3). A solution of precursor **6b** in methanol (13 µmol, 750 µL) was then passed through the cartridge and the alcohol was evaporated under reduced pressure (we noticed by TLC that the temperature of the bath had to be lower than 40 °C to prevent the thermal decomposition of **6b**). First, a reduced quantity of precursor (<70%) was recovered from the QMA cartridge after evaporation of the eluate. Surprisingly, ^1H NMR analysis of the residue in CD_3OD (see Figure S1, supplementary information) indicated no significant modification of the structure of **6b** while only 8% of the latter could still be detected in the eluate by ^{19}F NMR (see Figure S2, supporting information). Keeping in mind that the counterions of diaryliodonium salts can be readily exchanged with numerous organic or mineral (di)anions,[20] we assumed that the eluted compound was the diaryliodonium hydrogen carbonate salt resulting from a supported $CF_3SO_3^-/HCO_3^-$ exchange.

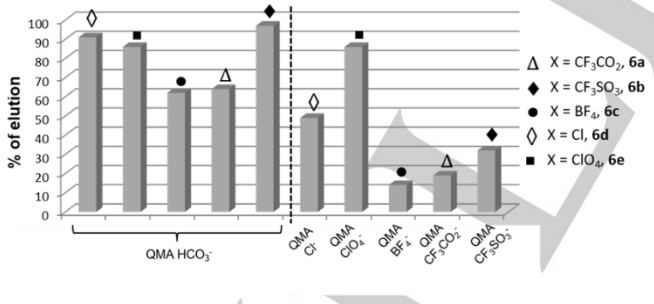


Figure 3. Percentages (%, ratio between values of activity measured in the eluate and initially loaded onto the QMA cartridge, n = 2, except for QMA-HCO_3^-, X = $CF_3SO_3^-$: n = 41) of $[^{18}F]F^-$ elution from QMA-HCO_3^- or QMA cartridges preconditioned with corresponding anions depending on the counterion of the iodonium salt used (13 µmol in 750 µL of methanol).

In order to evaluate the exact amount of iodonium salt required to achieve a complete conversion of the counterion on the QMA-HCO_3^- cartridges, bis(4-methoxyphenyl)iodonium-4-methylbenzenesulfonate (**8**) was used as a surrogate model. This iodonium salt was synthesised in one step from commercially available anisole in 43% yield (see supporting information). For the exchange study, a solution of iodonium salt **8** in CD_3OD (750 µL) was passed through a QMA-HCO_3^- cartridge (beforehand washed with 1 mL of methanol and dried over an argon flow for one min). The percentage of counterion exchange was then assessed by ^1H NMR in CD_3OD. After elution a mixture of iodonium salts was observed. Nearly complete counterion exchange was obtained using less than 10 µmol of the iodonium salt **8** for the elution (Figure S3, supplementary information). All signals corresponding to the former tosylate counteranion could not be detected, confirming the complete exchange expected.

All attempts to purify the eluted iodonium salt by column chromatography or crystallization remained unsuccessful and led to the complete decomposition of the eluted structure. This could be explained by the relatively low stability of iodonium hydrogen carbonate derivatives, which moreover are rarely found in the literature.[21] In fact, only the presence of the HCO_3^- counterion was evidenced on crude residue by IR analyses with a broad vibration band clearly visible at 1351 cm^{-1} ($\upsilon_{C=O}$) (see Figure S4, supplementary information). To confirm the nature of the eluted counterion, we tried to visualize the sp^2 carbon of the HCO_3^- anion by NMR ^{13}C analyses. Unfortunately, this signal could never be detected even with optimisation of the acquisition parameters. We then decided to use an enriched aqueous $[^{13}C]K_2CO_3$ solution to equilibrate the QMA-HCO_3^- cartridge. Under these conditions, NMR ^{13}C analysis of the eluate clearly highlighted the characteristic signal corresponding to the sp^2 carbon of the HCO_3^- anion at 161.5 ppm (Figure 4).

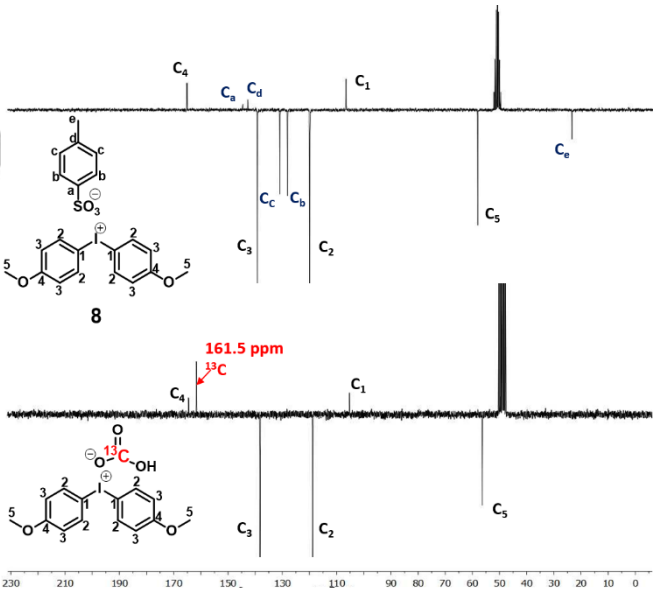


Figure 4. Comparison of ^{13}C NMR spectra of starting iodonium salt **8** and eluate obtained after elution of a ^{13}C-enriched QMA-HCO_3^- cartridge with 5 µmol of **8** showing the complete conversion of the tosylate derivative into its HCO_3^- analogue.

2/ Radiofluorination reaction

In the next step, we evaluated the influence of such HCO_3^- exchanges on the radiosynthesis of [^{18}F]FDOPA on a Raytest SynChrom R&D EVOII bireactor automate. [^{18}F]F^- was eluted from the QMA-HCO_3^- cartridge using 13 µmol of **6b** in 750 µL of anhydrous methanol (elution yield: 97 ± 0.03%, n = 41; % exchange to HCO_3^- counterion: 92% evaluated by ^{19}F NMR). The obtained eluate was evaporated under vacuum at 30 °C to prevent any thermal decomposition of the precursor. Then, 750 µL of diglyme was added to the reactor and heated at 140 °C. For all time points checked, only around 3% of the desired fluorine-18 labelled intermediate [^{18}F]**9** was observed. Interestingly, no increase over time could be detected. Also, variations of the temperature (90 °C, 110 °C, 140 °C, or 160 °C) did not increase the radiolabelling efficiency. When the amount of precursor used for the elution of the QMA-HCO_3^- cartridge was reduced (<13 µmol) to achieve a complete conversion of the counterion, no incorporation of the [^{18}F]F^- could be observed. Considering the loss of iodonium salt previously observed on the QMA-HCO_3^- cartridges, we assume that the quantity of precursor present in the reactor was definitely too low to allow any [^{18}F]F^- incorporation. We then decided to investigate the influence of the precursor concentration, temperature and solvent used for the radiofluorination step. As highlighted in Figure 5A, increasing the concentration of the precursor **6b** in diglyme while decreasing the temperature to 110 °C led to a significantly higher labelling efficiency (16% in 100 µL diglyme at 110 °C *vs* <3% in 750 µL diglyme at 140 °C). Using the optimized concentration and temperature conditions, we tried to change the reaction solvent. The use of the aprotic polar solvents (DMF or DMSO) dramatically impacted the labelling efficiency which was lower than 3% for all attempts performed (data not shown). To reduce [^{18}F]F^-/HCO_3^- counteranion competition during the heating process, toluene was then tested as non-polar solvent and compared to diglyme. When using 200 µL of toluene at 110 °C for 10 min, the labelling efficiency was significantly higher (Figure 5B, 23 ± 4%, n = 6). Further modifications of the temperature or reaction time did not improve the percentage of incorporation of radioactive fluorides.

In order to evaluate the impact of the $CF_3SO_3^-$/HCO_3^- counterion exchange on the radiofluorination reaction, we used these optimized radiofluorination conditions on evaporated eluates obtained from QMA cartridges preconditioned with the same $CF_3SO_3^-$ counterion. In the absence of HCO_3^-, the reaction mixture rapidly turned black at 90-100 °C and less than 1% of the desired radiofluorinated product [^{18}F]**9** was detected. The same observation was made when using ClO_4^- derivative **6e** and ClO_4^--preconditioned QMA-cartridge. These findings further confirmed the importance of the HCO_3^- counterion to ensure the success of the radiofluorination step.

However, these promising results were counterbalanced by the loss of activity observed on the reactor vessel. Indeed, working without any base and cryptand induced typical adsorption of [^{18}F]F^- on the glass wall of the pyrex reactor (50.6 ± 8.4% of the starting activity, n = 22). To get around this problem, we tried several pretreatment strategies of the reactor surface (Figure 5C). In most cases, the drying of small amounts of organic or mineral bases in the reactor before the radiofluorination reaction led to a significant decrease of the fixed activity on the glass. To prevent any side reaction and limit the number of competitive anions in the reaction mixture, we chose bases containing bulky cations and carbonate or hydrogen carbonate counterions (i.e. Cs_2CO_3, $CsHCO_3$, Bu_4NHCO_3, etc). Unfortunately, all these attempts also led to a decrease of the fluorine-18 incorporation on the iodonium structure compared to the optimized conditions described above (no additive conditions, Figure 5C).

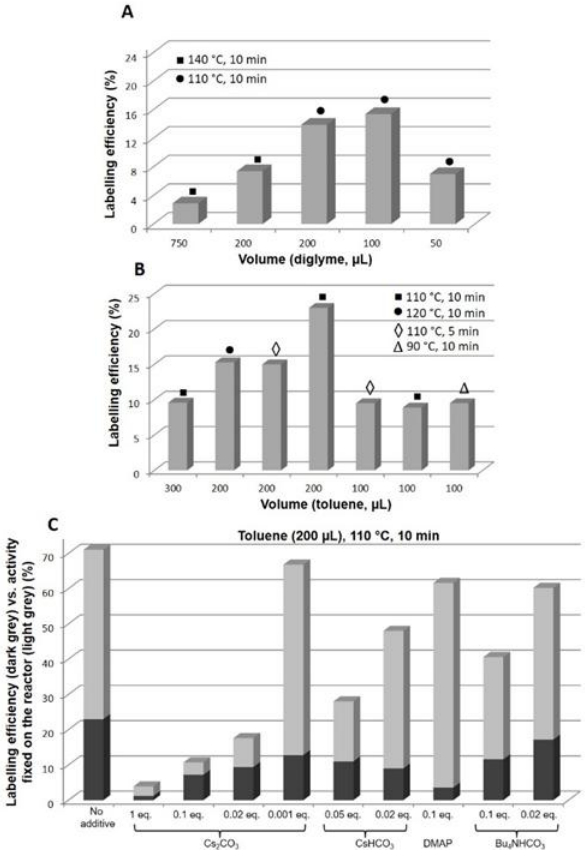


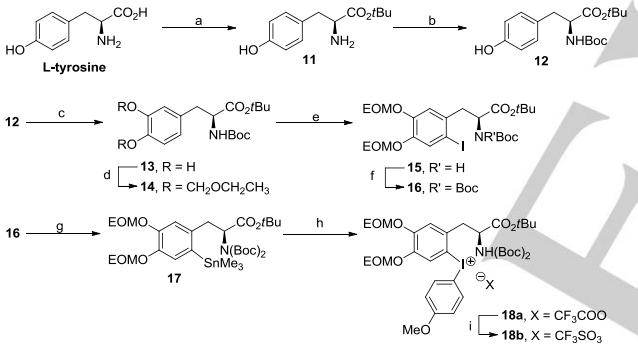
Figure 5. Influence of: (i) the temperature, concentration (13 µmol of **6b** in various volumes of solvent) and reaction time on the radiolabelling efficiency in diglyme (**A**) or toluene (**B**) and (ii) the addition of traces of mineral or organic bases in the reactor (**C**), % were calculated based on the starting [^{18}F]F^- activity; purity of the eluted compound was controlled by radio-TLC (>98%).

3/ Deprotection step

Finally, we turned our attention to the deprotection of the radiofluorinated intermediate [^{18}F]**9** obtained after solid phase extraction. The loss of activity on the solid phase C18 cartridge was negligible (1.78 ± 0.02%, n = 67). Surprisingly, after concentration of the ethanolic eluate and final deprotection using a 3M aqueous H_2SO_4 solution at 140 °C for 5 min (reported by Kulk *et al*.[11]) radio-HPLC analysis of the reaction mixture revealed that only 16% of the total activity detected accounted for [^{18}F]FDOPA. The other main radioactive component of the

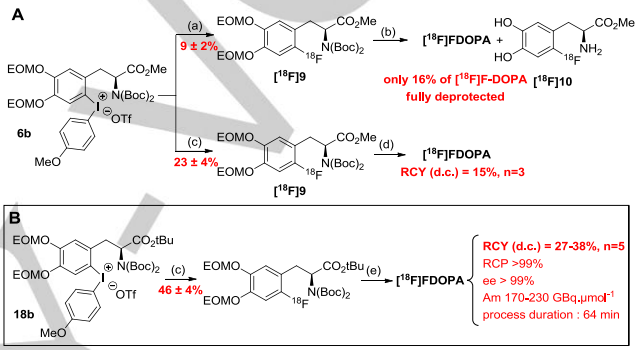
mixture was the corresponding methyl ester. An extended heating time of at least 15 min was necessary to achieve a full deprotection of the structure. To circumvent this problem and shorten the reaction time dedicated to the deprotection, concentrated HBr was used. After concentration of the eluate, heating at 140 °C for 10 min in 300 µL of concentrated HBr was then sufficient to achieve the complete deprotection of the structure and afford [^{18}F]FDOPA without concomitant formation of radioactive by-products. After dilution of the reaction mixture with a 1 M aqueous solution of NH_4OAc, the crude [^{18}F]FDOPA was then easily purified by semi-preparative RP-HPLC and collected to afford [^{18}F]FDOPA in 15.2% RCY, >98% RCP, Am >150 GBq/µmol (n=3) and e.e. > 99% (chiral HPLC, n=3, see chromatograms in supplementary information).

To further shorten the overall radiosynthesis time and minimize the harsh conditions required for the deprotection step, we investigated the acid-sensitive *tert*-butyl ester precursor **18b**. The latter was obtained in five steps from the L-DOPA *tert*-butyl ester **12** according to the synthetic route described for **6b** (Scheme 2). Precursor **12** was easily produced from commercially available L-tyrosine using *tert*-butyl acetate/perchloric acid esterification followed by NH-Boc protection and final oxidation of the aromatic ring into catechol using freshly prepared 2-iodobenzoic acid.



Scheme 2. Preparation of diaryliodonium salt **18b**. Reagents and conditions: (a) *tert*-butyl acetate, aq. $HClO_4$ (70 wt%), 0 °C then RT, 12 h, 45%; (b) K_2CO_3, $(Boc)_2O$, THF/H_2O (1/1, v/v), RT, 3 h, 71%; (c) i) 2-iodoxybenzoic acid, DMF, RT, 30 min; ii) 1 M aq. ascorbic acid, 0 °C, 30 min, 97%; (d) $C_2H_5OCH_2Cl$, DIPEA, THF, 0 °C then 40 °C, 24 h, 62%; (e) i) I_2, K_2CO_3, $PhI(OCOCF_3)_2$, CH_2Cl_2, RT, 4 h, 58%; (f) i) NEt_3, DMAP, $(Boc)_2O$, MeCN, 40 °C, 24 h; ii) $(Boc)_2O$, 40 °C, 23 h, 61%; (g) Sn_2Me_6, $Pd(PPh_3)_4$, 1,4-dioxane, reflux, 1.5 h, 76%; (h) i) p-MeOPh$(IOAc)_2$, CH_2Cl_2, -40 °C, 1 h, ii) TFA, -40 °C, then RT, 5 h, 67%; (i) 1 M aq. NaOTf, CH_2Cl_2, RT, 85%. EOM: ethoxymethyl.

We attempted the radiosynthesis of [^{18}F]FDOPA using the precursor **18b** and the optimal conditions described above for **6b**. Direct elution of QMA-loaded [^{18}F]F^- was realised using a solution of 13 µmol of **18b** in 750 µL anhydrous methanol (elution yield: 94.8 ± 3.2%, n = 8). The obtained eluate was evaporated under vacuum at 30 °C. Then, 200 µL of toluene were added to the reactor and heated at 110 °C for 10 min. Surprisingly, compared to the corresponding methyl ester, the protected radiofluorinated [^{18}F]FDOPA was obtained, after solid phase C18 cartridge extraction, in higher radiolabelling efficiencies (29 ± 5%, n = 8). In order to validate the reproducibility of our radiosynthetic approach, the [^{18}F]FDOPA was also produced from precursor **18b** using a Raytest SynChrom R&D EVOIII bireactor automate in a partner laboratory. While some minor modifications of the protocol were mandatory to adapt the reaction conditions to this radiosynthesis module (see supplementary information), the protected intermediate of [^{18}F]FDOPA was obtained in higher yields (i.e. 46 ± 4%, n = 3). After concentration of the eluate, deprotection using concentrated HCl at 120 °C for 7 min and purification *via* semipreparative RP-HPLC, final [^{18}F]FDOPA was obtained in 64 min, 27-38% RCY d.c. (n = 5), >99% RCP, > 99% e.e., and high Am 170-230 GBq/µmol (Scheme 3).



Scheme 3. A: In house preparation of [^{18}F]FDOPA from **6b** following Kuik *et al.*[11] protocol compared to our radiosynthetic approach; **B**: Preparation of [^{18}F]FDOPA from *tert*-butyl ester precursor **18b** with our optimized protocol. Reagents and conditions: (a) [^{18}F]KF-crypt-222-carbonate, diglyme, 140 °C, 5 min; (b) aq. H_2SO_4 3 M, 140 °C, 5 min; (c) [^{18}F]F^-, toluene, 105-110 °C, 10 min; (d) aq. HBr conc., 140 °C, 10 min; (e) aq. HCl conc., 120 °C, 7 min. EOM: ethoxymethyl.

Conclusions

We carried out a comprehensive optimization of the nucleophilic radiosynthetic approach to produce [^{18}F]FDOPA with high molar activities from iodonium salt precursors. Our experiments clearly pointed out the importance of the HCO_3^- counterion for the efficiency of fluorine-18 incorporation on such iodonium scaffolds. Considering that this new and fast nucleophilic radiosynthetic process for the automated production of [^{18}F]FDOPA, free from any base, cryptand and metal catalyst, was evaluated on different automated systems implemented in two differents facilities (Clermont Ferrand and Strasbourg, France), we believe that it could be easily transferable to other laboratories and could provide an alternative to currently available [^{18}F]FDOPA radiopharmaceutical production.[7,22] Finally, we assume that this methodology could be successfully applied to a broad range of iodonium salt precursors for radiofluorination of electron-rich aryl derivatives.

Experimental Section

Chemistry

Experimental details, full data, ^{1}H and ^{13}C NMR spectra of compounds **6a**-**e**, **7**, **13**-**18a,b** are available in Supporting Information.

Radiochemistry

1/ Radiosynthesis of [^{18}F]FDOPA from precursor 6b using a bi-reactor SynChrom R&D EVOII syntheses module

General experimental information

No-carrier-added fluorine-18 (half-life: 109.8 min) was produced via the [^{18}O(p, n)^{18}F] nuclear reaction by irradiation of a 2.8 mL >97%-enriched [^{18}O]H_2O target (Bruce Technology) on a CPH14 cyclotron (14 MeV proton beam, Cyclopharma laboratories). Radio thin layer chromatography (radio-TLC) was performed on Merck pre-coated silica gel (60 F254) plates with a mixture ethyl acetate/cyclohexane (2/8, v/v) and measured on an AMBIS 400 (Scanalytics, CSPI, San Diego, CA, USA). Analytical HPLC measurements were performed on a system consisting of an Agilent HP series 1100 (Hewlett Packard, Les Ulis, France) combined with a Flow one A500 Radiomatic detector (Packard, Canberra, Australia). A Reprosil Chiral-AA 8 µm column (250 x 4.6 mm; 8 µm; CIL Cluzeau; France) was used with water/acetonitrile (9/1, v/v) as isocratic eluent mixture and a flow of 0.7 mL/min. Unless otherwise indicated, all HPLC purifications and analyses were performed at λ=254 nm. Radiochemical syntheses and semi-preparative HPLC purifications were performed using a bi-reactor SynChrom R&D EVOII syntheses module (Raytest). Sep-Pak® Light Accell Plus QMA Cl^- or carbonate cartridges (130 mg) and Sep-Pak® light C18 cartridges were purchased from Waters. Chromafix 30-PS-HCO_3^- QMA cartridges (46 mg) were purchased from ABX (Radeberg, Germany). A semi-preparative VP250/10 Nucleodur C18 HTec column (250 x 10 mm; 8 µm; Macherey-Nagel) was used with water/ethanol (95/5, v/v) containing 0.1% trifluoroacetic acid as eluent at a flow of 2.5 mL/min. All radiolabelled compounds were compared by TLC and/or analytical HPLC to the authentic non-radioactive material and to be free of significant chemical and radiochemical impurities.

Radiosynthesis

The aqueous solution of [^{18}F]F^- in [^{18}O]H_2O obtained from Cyclopharma Laboratories was passed through an anion exchange resin (Sep-Pak® Light Accell Plus QMA Cl or carbonate cartridge, Waters, Trap 1, scheme S1, see supporting information). The cartridge was rinsed with 1 mL of anhydrous methanol (SC1, scheme S1) and dried for 2 min under vacuum and He flow (150 mL/min). Then, the radioactivity was eluted to the reactor using a solution of the precursor **6b** (13 µmol) in anhydrous methanol (750 µL, SC2, scheme S1). The solvent was evaporated under reduced pressure at 30 °C under stirring. When the vacuum in the reactor was stabilized around 30-35 mbar, anhydrous toluene (200 µL) or diglyme (100 µL, SC3, scheme S1) were added to the reactor. The reaction mixture was then heated to 110 °C for 10 min under stirring. The reactor was cooled to 50-60°C before addition of diglyme (500 µL, SC4, scheme S1) and water (19 mL, SC5, scheme S1) successively and under stirring. The solution was then passed through a Sep-Pak light C18 cartridge (Trap 2, scheme S1). After drying under He flow (250 mL/min), the cartridge was eluted to the second reactor using 2 mL of ethanol (SC6, scheme S1). The solvent was removed under vacuum at 70°C under stirring. Shortly before complete evaporation, concentrated bromhydric acid (300 µL, SC11, scheme S1) was added to the reaction mixture which was heated to 140°C for 10 min under stirring. After cooling to 35-40 °C, the dark brown/black reaction mixture was buffered using a 1 M aqueous solution of ammonium acetate (3 mL, SC7, scheme S1). The solution containing the crude unprotected [^{18}F]FDOPA was purified via semi-preparative HPLC. The collected fraction (Rt = 10.5 min) was diluted in saline and analyzed by analytic radio-HPLC to determine the radiochemical purity, enantiomeric excess and specific activity.

2/ Radiosynthesis of [^{18}F]FDOPA from precursor 18b using a bi-reactor SynChrom R&D EVOIII syntheses module

General experimental information

No-carrier-added fluorine-18 (half-life: 109.8 min) was produced via the [^{18}O(p, n)^{18}F] nuclear reaction by irradiation of a 1 mL >97%-enriched [^{18}O]H_2O target (Huayi isotopes Co) on a TR24 cyclotron (16-24 MeV proton beam, Advanced Cyclotron Systems Inc, ACSI). Radio thin layer chromatography (radio-TLC) was performed on Millipore aluminium back-coated silica gel (60 F254) plates with a mixture of ethyl acetate/cyclohexane (2/8, v/v) and measured on a MiniGita apparatus (Raytest, Germany). Analytical HPLC measurements were performed on a system consisting of a Dionex U3000 HPLC (Thermo scientific, France) combined with a homemade radio-detector (connected to an UCI 50 interface, Thermo scientific, France) and a diode array detector. A Synchronis column (250 x 4.6 mm; 5 µm; Thermo scientific, France) was used with water/acetonitrile (9/1, v/v) as isocratic eluent mixture at a flow rate of 0.7 mL/min. Semi preparative HPLC purifications were performed at λ=254 nm. Radiochemical syntheses and semi-preparative HPLC purifications were performed using a bi-reactor SynChrom R&D EVOIII syntheses module (Raytest). Sep-Pak® Light Accell Plus QMA Cl^- or carbonate cartridges (130 mg) and Sep-Pak® light C18 cartridges were purchased from Waters. A semi-preparative VP250/10 Nucleodur C18 HTec column (250 x 10 mm; 5 µm; Macherey-Nagel) was used with water/ethanol (95/5, v/v) containing 0.1% trifluoroacetic acid as eluent at a flow rate of 2.5 mL/min.

The precursor **18b** was stored in a desiccator over desiccant in the fridge (5 °C). After 2 months of storage decreasing yield were obtained (below 30% RCY decay corrected) and a new batch of precursor **18b** was synthesised for further radiosyntheses.

Radiosynthesis

The aqueous solution of [^{18}F]F^- in [^{18}O]H_2O was transferred under helium pressure to an intermediate vial set in a well counter inside the hotcell (red dot square on scheme S2, see supporting information). After counting, the whole solution was transferred to the reception V-vial of the Raytest module. [^{18}F]F^- in water was trapped on an anion exchange resin (Sep-Pak® Light Accell Plus QMA carbonate cartridge, Waters, Trap 1, scheme S2). The cartridge was rinsed with 1 mL of anhydrous methanol (SC1, scheme S2) and dried under vacuum and argon

flow. The radioactivity was eluted into the reactor using a solution of the precursor 18b (13 µmol) in anhydrous methanol (850 µL, SC2, scheme S2). The solvent was evaporated under reduced pressure at 28 °C under stirring. When the vacuum in the reactor was stabilized around 50 mbar, anhydrous toluene (250 µL, SC3, scheme S2) was added to the reactor. The reaction mixture was then heated to 105 °C for 5 min and 110 °C for 5 min more under stirring. The reactor was cooled to 50°C before addition of water (12-13 mL, SC4, scheme S2) under stirring. The solution was then passed through a Sep-Pak light C18 cartridge (Trap 2, scheme S2). After drying under argon flow, 2.2 mL of acetonitrile (SC5, scheme S2) were added into the reactor and then transferred onto the C18 cartridge. The cartridge was eluted to the second reactor and the solvent was removed under reduced pressure at 70°C under stirring. Acetonitrile (250 µL, SC6) and concentrated hydrochloric acid (750 µL in an intermediate V-Vial isolated between valve C1 and D4 to avoid HCl contamination) was added. The reaction mixture was heated to 120°C for 7 min under stirring. After cooling to 40 °C, the dark black reaction mixture was buffered using a 1 M aqueous solution of ammonium acetate (4 mL, SC8, scheme S2). The solution containing the crude [^{18}F]FDOPA was transferred to the HPLC loop (5 mL) and purified via semi-preparative HPLC (95/5, v/v, Water/Ethanol containing 0,1% TFA). The collected fraction (Rt = 8.5 min) was analyzed by analytic radio-HPLC to determine the radiochemical purity, enantiomeric excess and specific activity.

Acknowledgements

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Keywords: radiopharmaceuticals • radiochemistry • nucleophilic substitution • iodonium salts • [^{18}F]FDOPA

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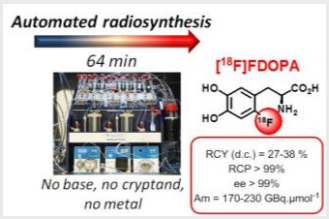
Entry for the Table of Contents (Please choose one layout)

Layout 1:

FULL PAPER

[^{18}F]FDOPA challenge: A new radiosynthetic route to [^{18}F]FDOPA was developed and fully automated from a key iodonium triflate salt precursor. This radiotracer was produced via a "minimalist approach" (see figure) involving nucleophilic [^{18}F]F$^-$ process, without any use of base, cryptand or metal catalyst, in 64 min, 27-38% RCY, >99% RCP, > 99% e.e., and high Am 170-230 GBq.µmol^{-1} (n = 5).



Radiofluorination

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Base/cryptand/metal-free automated nucleophilic radiofluorination of [^{18}F]FDOPA from iodonium salts: importance of hydrogen carbonate