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Iodonium ylides for one-step, no-carrier-added radiofluorination of electron rich arenes, exemplified with 4-(([^{18}F]fluorophenoxy)-phenylmethyl)piperidine NET and SERT ligands

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Iodonium ylide precursors of electron rich arenes, *i.e.* the NET and SERT ligands 4-((3- and 4-fluorophenoxy)phenylmethyl)piperidine, served as model compounds for the direct substitution with n.c.a. [^{18}F]fluoride. Good radiochemical yields of about 20% were obtained in reaction times of *ca.* 130 minutes with a molar activity of the labelled ligands of more than 50 GBq μmol^{-1}. Those failed as *in vivo* probes in first evaluation studies. Several important insights, however, were gained into the reaction of ylides, *e.g.* an unexpected formation of regioisomers. The results clearly demonstrate that aryliodonium ylides are a promising alternative to the well-known diaryliodonium salts for the direct preparation of complex, electron rich n.c.a. [^{18}F]fluoroarenes.

Introduction

Fluorine-18 has become one of the most important radionuclides for imaging probes in positron-emission-tomography (PET) due to its favourable nuclear and chemical properties.[1] In a practical way, however, no-carrier-added (n.c.a.) fluorine-18 can only be produced in the chemical form of fluoride by the hitherto established processes.[2,3] Thus, imaging probes demanding a high specific activity have generally to be produced starting from [^{18}F]fluoride. While the synthesis of electron deficient n.c.a. [^{18}F]fluoroarenes can easily be accomplished by nucleophilic substitution, the synthesis of non-activated or even electron rich [^{18}F]fluoroarenes represents still a challenge which is usually realized by a rather complicate, time consuming multi-step synthesis.[1,4,5]

It is well known that iodonium compounds offer an alternative route even for the synthesis of electron rich [^{18}F]fluoroarenes starting from n.c.a. [^{18}F]fluoride.[6,7] Their application as precursor for ^{18}F-radiofluorination has mainly been limited to mechanistic studies, and they served in most cases as precursors for the preparation of versatile, non-activated small ^{18}F-labelled molecules used as building blocks for further radiochemical transformations.[5] So far, very little attention has been paid to other hypervalent iodine species for radiofluorination.

Recently, Satyamurthy and Barrio reported the successful radiosynthesis of n.c.a. [^{18}F]fluoroarenes by the use of different iodonium ylides as precursors.[8] Amongst those, the iodonium ylides derived from Meldrum's acid proved to be the most promising ones due to their relative high stability and good reactivity. Their general structure is shown in Fig. 1. These compounds are commonly formed by the reaction of Meldrum's acid with a suitable aryliodine-III compound under basic conditions.[9–11]

The reported radiofluorination of iodonium ylides was performed under similar conditions to those generally used for the radiofluorination of iodonium salts. After activation of the [^{18}F]-fluoride by a phase transfer catalyst/base activation system (*e.g.* Kryptofix®2.2.2/potassium carbonate) the iodonium ylide is added in a dipolar aprotic solvent such as DMF and heated to 110–130 °C for 10–15 minutes (Scheme 1), as described by Satyamurthy and Barrio.[8]

In 2003 Orjales *et al.* published a series of potential antidepressants.[12] Amongst those are the fluorophenoxyethers 4-((3- and 4-fluorophenoxy)phenylmethyl)piperidine (3-FPPMP and 4-FPPMP, Fig. 2) which are potential ligands for the serotonin and norepinephrine (reuptake) transporters (SERT and NET). Being electron-rich fluoroarenes those offered themselves as

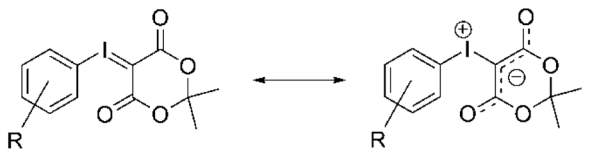

Fig. 1 Resonance structures of aryliodonium ylides.

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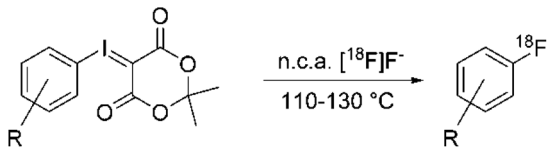


Scheme 1 Nucleophilic n.c.a. ^{18}F-fluorination of arenes using iodonium ylides.

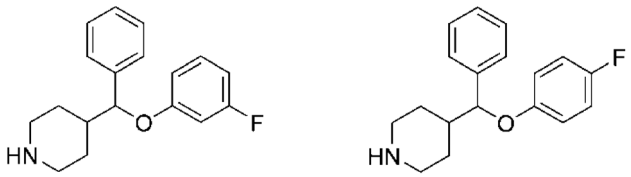


Fig. 2 Molecular structures of 3-FPPMP (**1**) and 4-FPPMP (**2**), ligands for the NE- and SE-reuptake transporters, respectively.

good candidates to test an authentic labelling with fluorine-18 *via* the new approach. Thus, our objective was to use the radiosynthesis of n.c.a. 3- and 4-[^{18}F]FPPMP as model reaction for the direct ^{18}F-fluorination of corresponding iodonium ylides of electron-rich arenes as precursors.

Experimental

General

All chemicals were acquired from Sigma-Aldrich (Taufkirchen, Germany), except for acetonitrile, Kryptofix®2.2.2 and potassium carbonate, purchased from VWR-International (Langenfeld, Germany), and for 3- and 4-fluorobenzyloxybenzene, obtained from Fluorochem (Derbyshire, UK). All compounds were used without further purification. SepPak C-18 cartridges were obtained from Waters (Eschborn, Germany) and sodium sulphate cartridges from Agilent (Böblingen, Germany).

The syntheses of 4-benzyloxyphenyliodonium-(5-[2,2-dimethyl-1,3-dioxane-4,6-dione])ylide (**13**) and of 4-methoxyphenyliodonium-(5-[2,2-dimethyl-1,3-dioxane-4,6-dione])ylide (**14**) were reported elsewhere.[13] All iodonium ylides were synthesized under exclusion of light and stored at 2–8 °C. The reference compound 2-fluorobenzyloxybenzene was obtained by the reaction of 2-fluorophenol with benzylbromide. NMR spectra were recorded either on a Varian-Inova 400 or a Bruker DPX Avance 200 spectrometer. The chemical shifts are given in parts per million relative to the solvent signal.

Elemental analyses (EA, microanalyses) were carried out on a Vario EL cube, elemental analyser (at ZEA-3, Forschungszentrum Jülich).

The products of the labelling reactions were analysed by radio-HPLC. The HPLC system consisted of a Smartline Pump 1000, a Luna C-18(2) column (5 μm, 250 × 4.6 mm) or a Luna PFP(2) column (5 μm, 250 × 4.6 mm) (both from Phenomenex, Aschaffenburg, Germany). Further, two Rheodyne manual 6-position selector valves (Idex Health and Science, Wertheim-Mondfeld, Germany) were positioned in front of and behind the column, equipped with 50 μl sample loops. The detection of compounds by UV-absorption was carried out with a K-2501 detector (Knauer, Berlin, Germany). When the system was used for the isolation of products by analytical HPLC the first valve was equipped with a 200 μl loop and the column directly connected to the detectors. Semi-preparative HPLC-runs were conducted on a HPLC system consisting of a Merck-Hitachi L-6000 pump, a Rheodyne manual 6-position selector valve in front of the column, equipped either with a 2 ml or a 1 ml sample loop and a Phenomenex Luna PFP(2) column (5 μm, 250 × 100 mm). The detection of compounds by UV-absorption was carried out with a S3300 UV-detector (Sykam, Fürstenfeldbruck, Germany).

The detection of the radioactive products was performed with a NaI(Tl) detector crystal connected with an ACE Mate signal amplifier and an EG&G Ortec Model 276 photomultiplier base (Ortec, Meerbusch, Germany).

Measurement of the radioactivity of bulk samples was conducted on a Curiementor 2 (PTW, Freiburg, Germany) ionisation chamber.

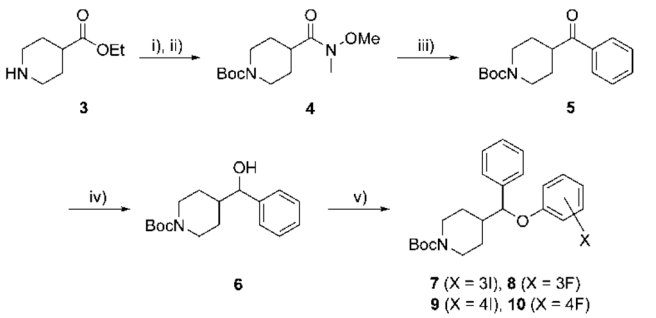
Phosphor imager plates used for *in vitro* autoradiography of brain tissue slices were scanned with a laser phosphor imager BAS 5000 (Fuji, Düsseldorf, Germany) utilizing software from the vendor (Version 3.14, Raytest, Straubenhardt, Germany).

Synthesis of precursors and standards

4-(Hydroxy(phenyl)methyl)piperidine-1-carboxylic acid *tert*-butyl ester (**6**) was prepared analogous to a procedure described by Ullrich *et al.*[14] from the Weinreb amide **4** which was prepared by standard methods (Scheme 2). The reference compounds 3-FPPMP and 4-FPPMP were prepared from compound **6** as reported by Orjales *et al.*[12] For the preparation of compounds **7**–**10** the same procedure was used but without the deprotection step (Scheme 2) as exemplarily described for compound **7**. The spectral data of the previously not described halophenoxy derivatives **7** and **9** is given below.

4-((3-Iodophenoxy)phenylmethyl)piperidine-1-carboxylic acid-*tert*-butyl ester (7)

tert-Butyl-4-(hydroxy(phenyl)methyl)piperidine-1-carboxylate (1.46 g, 5 mmol) was added portion wise to a stirred suspension



Scheme 2 Syntheses of the fluorophenoxy- and iodophenoxy derivatives **7**–**10**. (i) Boc_2O, Na_2CO_3, H_2O–THF (ii) MeNHOMe, i-PrMgCl, THF, (iii) phenyllithium, THF, (iv) $NaBH_4$, MeOH, (v) NaH, sodium benzoate, fluorohalobenzene (halo = 3-F, 4-F, 3-I, 4-I) DMSO, 65 °C.

of hexane washed NaH (0.25 g, 6.3 mmol, as 60% oil dispersion) in 8 ml of anhydrous DMSO. The reaction was stirred at room temperature for 30 min, sodium benzoate (1.45 g) added, and stirring continued for 30 min. 1-Fluoro-3-iodobenzene (720 μl, 6.1 mmol) was added, while the reaction temperature was being kept below 20 °C by means of a water bath and then the reaction mixture heated at 65 °C for 15 h. After cooling to room temperature the reaction mixture was diluted with brine and water until all precipitated solids were dissolved again. Subsequently the mixture was extracted with diethylether, the organic layer dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by chromatography on silica gel yielded 1.12 g (45%) of an orange oil.

^{1}H NMR (200 MHz, $CDCl_3$) δ: 7.39–7.20 (m, 8H), 6.93–6.74 (m, 2H), 4.82 (d, 1H, $J = 6.7$ Hz), 4.25–4.10 (m, 2H), 2.77–2.57 (m, 2H), 2.02–1.92 (m, 2H), 1.69 (m, 1H), 1.49 (s, 9H), 1.44–1.29 (m, 2H).

^{13}C NMR (200 MHz, $CDCl_3$) δ: 158.9, 154.8, 139.2, 130.6, 129.9, 128.6, 128.0, 126.7, 125.5, 115.0, 94.2, 84.1, 79.4, 43.7, 43.4, 28.5, 28.4, 28.1.

MS (+ c ESI): $m/z = 494.19$.

Elemental analysis: calc.: C 55.99%, H 5.72%, N 2.84%; found: C 56.33%, H 6.21%, N 2.98%.

4-((4-Iodophenoxy)phenylmethyl)piperidine-1-carboxylic acid-*tert*-butyl ester (9)

The synthesis was performed as described for compound 7. Yield: 33% as white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.50–7.26 (m, 8H), 6.66–6.58 (m, 2H), 8.80 (d, 1H, $J = 6.6$ Hz), 4.24–4.09 (m, 2H), 2.77–2.57 (m, 2H), 2.09–1.87 (m, 3H), 1.59–1.22 (m, 13H).

^{13}C NMR (400 MHz, $CDCl_3$) δ: 158.1, 154.7, 138.0, 128.5, 127.8, 126.7, 118.3, 84.0, 82.9, 79.3, 43.7 (bs), 43.3, 28.4, 28.0 (bs).

MS (+ c ESI): $m/z = 493.99$.

Elemental analysis: calc.: C 55.99%, H 5.72%, N 2.84%; found: C 56.25%, H 6.2%, N 3.04%.

(3-((1-Boc-4-piperidyl)phenylmethoxy)phenyl)iodonium-(5-(2,2-dimethyl-1,3-dioxane-4,6-dione))ylide (11)

In a closed 10 ml reaction vial 640 mg (1.3 mmol) of iodoarene 7 was stirred together with 322 mg of 77% *meta*-chloroperoxybenzoic acid (1.4 mmol) in 5.2 ml of dichloromethane (DCM) at 40 °C for 80 minutes. After cooling to ambient temperature 520 mg of KOH and 240 mg of Meldrum's acid were added and the mixture further stirred for 45 minutes. Then the reaction mixture was diluted with DCM, filtered over a paper filter, the solids washed with about 50 ml DCM and subsequently filtered over cellulose. The solvent of the collected organic phases was removed under reduced pressure at 30 °C until the first solid precipitated. Then, hexane was slowly added to complete the precipitation. The solid was collected by filtration, washed with hexane and dried in air and in vacuum.

Yield: 292 mg beige solid (33%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.18–7.11 (m, 8H), 6.94 (t, 1H, $J = 4.0$ Hz), 6.80 (d, 1H, $J = 4.2$ Hz), 4.69 (d, 1H, $J = 3.2$ Hz), 3.98 (m, 2H), 2.49 (m, 2H), 1.81 (m, 2H), 1.52 (s, 6H), 1.31 (s, 9H), 1.22–1.08 (m, 3H).

^{13}C NMR (400 MHz, $CDCl_3$) δ: 163.6, 159.7, 158.7, 139.0, 138.1, 131.7, 130.5, 129.8, 128.6, 128.5, 128.1, 127.8, 126.8, 126.6, 125.3, 124.8, 119.8, 119.4, 114.9, 114.4, 104.3, 84.4, 74.3, 55.9, 43.0, 28.4, 28.0, 25.8.

MS (+ c ESI): $m/z = 674.12$.

Elemental analysis: calc.: C 54.81%, H 5.39%; found: C 54.81%, H 5.75%.

(4-((1-Boc-4-piperidyl)phenylmethoxy)phenyl)iodonium-(5-(2,2-dimethyl-1,3-dioxane-4,6-dione))ylide (12)

The synthesis was performed as described for compound **11**.

Yield: 42% as yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, 2H, $J = 8.4$ Hz, C14–H), 7.33–7.21 (m, 6H), 6.78 (d, 2H), 4.79 (d, 1H), 4.10 (m, 2H), 2.60 (m, 2H), 1.91 (m, 4H), 1.63 (s, 6H), 1.41 (s, 9H), 1.33–1.24 (m, 3H).

^{13}C NMR (400 MHz, $CDCl_3$) δ: 163.4, 161.5, 154.7, 138.1, 136.1, 128.8, 128.3, 126.6, 119.2, 104.4, 102.6, 84.6, 79.4, 56.8, 43.2, 28.4, 25.8.

MS (+ c ESI): $m/z = 674.10$.

Elemental analysis: calc.: C 54.81%, H 5.39%, N 2.20%; found: C 55.3%, H 5.73%, N 1.91%.

Radiochemistry

Fluorine-18 was produced by the irradiation of ^{18}O-enriched water with 16.5 MeV protons and a beam current of about 20 μA using the ^{18}O(p,n) ^{18}F nuclear reaction. Irradiations were performed at the baby cyclotron BC1710 (Japan-Steel-Works) at the Forschungszentrum Jülich using a titanium target.[3] The n.c.a. [^{18}F]fluoride was separated from the irradiated water by an electrochemical procedure as earlier described by Hamacher *et al.*[15]

4-[^{18}F]Fluoro-1-benzyloxybenzene and 4-[^{18}F]fluoroanisole

Kryptofix® 2.2.2 (10 mg) was dissolved in 13.3 μl of a 1 M aqueous solution of potassium carbonate in a 1.5 ml Eppendorf vial with the aqueous solution containing about 30 MBq n.c.a. [^{18}F]fluoride. The solution was diluted with 900 μl of dry acetonitrile, transferred to a 5 ml V-Vial® with a silicon septum and the solvent was removed at 80 °C and 800 mbar under a gentle argon stream. This azeotropic distillation was repeated two times, each by addition of 1 ml of dry acetonitrile. Then the vial was evacuated at a pressure between 5 and 20 mbar for 5 minutes and flushed with argon. Subsequently, 15 mg of the desired precursor (**13** or **14**) were dissolved in 1 ml of acetonitrile and heated at 130 °C for 20 minutes. After cooling to room temperature an aliquot of the reaction mixture was diluted with HPLC-solvent and analysed by radio HPLC (55 : 45 acetonitrile–buffer for [^{18}F]fluoroanisole and 65 : 35 acetonitrile–buffer for [^{18}F]fluorobenzyloxybenzene; buffer: 1% aqueous TEA–H_3PO_4, pH 8.0–8.5; Luna C-18; 1 ml min^{-1}).

3-[^{18}F]FPPMP ([^{18}F]1) and 4-[^{18}F]FPPMP ([^{18}F]2)

The n.c.a. [^{18}F]fluoride was activated with 5 mg Kryptofix® 2.2.2 and 6.7 μl 1 M K_2CO_3 solution and dried as described for 4-[^{18}F]-fluoro-1-benzyloxybenzene and 4-[^{18}F]fluoroanisole. Then, ylide **11** or **12** (7.5 mg) was dissolved in 0.5 ml of dry acetonitrile, added to the vial containing the dry [^{18}F]fluoride complex and heated to 120 °C or 130 °C, respectively, for 20 minutes. For work-up the reaction mixture was cooled in an ice bath for one minute, 150 μl of water was added and the whole solution submitted to semi-preparative HPLC (60 : 40 acetonitrile–buffer: 1% aqueous TEA–H_3PO_4, pH 8.0–8.5; Luna PFP(2); 4 ml min^{-1}). The product fraction was diluted with 15 ml of water and passed through a SepPak C-18 cartridge. The cartridge was dried by blowing a stream of argon through it and the product eluted with 1.5 ml of dry DCM *via* a sodium sulphate cartridge in a 5 ml V-Vial® to yield **[^{18}F]8** and **[^{18}F]10**, respectively. In the case of **[^{18}F]10** a second HPLC separation was necessary in order to get a pure product. This was performed by evaporation of the solvent and dissolving the residue in 150 μl HPLC-solvent on an analytical HPLC-column (55 : 45 acetonitrile–buffer: 1% aqueous TEA–H_3PO_4, pH 8.0–8.5; Luna PFP(2); 1 ml min^{-1}). Then, the solvent of the product fractions was changed to 1.5 ml DCM by means of a C-18 cartridge as described above.

For deprotection of **[^{18}F]8** and **[^{18}F]10**, 500 μl of trifluoroacetic acid was added and the whole solvent–acid mixture removed at 40–50 °C under reduced pressure (starting from 800 mbar) and a gentle argon stream. The crude products 3-[^{18}F]FPPMP and 4-[^{18}F]FPPMP were dissolved in 150 μl HPLC-solvent (60 : 30 : 10 THF–MeOH–buffer: 1% aqueous TEA–H_3PO_4, pH 6.0; Luna C-18; 0.7 ml min^{-1}) and purified by analytical HPLC.

Identification of the labelled products

The identification of labelled compounds was done by radio HPLC and coelution with the non-radioactive standard compounds. The *k*-values of relevant standard compounds are summarized in Table 1 together with the corresponding HPLC-conditions used. The dead time of the HPLC-system equipped with the C-18 column was determined with thiourea and amounted to 127 s at a flow of 1 ml min^{-1} and 182 s at a flow of 0.7 ml min^{-1}.

In vitro binding on rat brain slices

For the in vitro determination of non-specific binding the HPLC-purified product fraction containing 3-[^{18}F]FPPMP or 4-[^{18}F]FPPMP was diluted with water, passed through a SepPak C-18 column which was subsequently blown dry with a stream of Argon. The product was eluted in 1 ml of a mixture of 90 : 10 (v/v) methanol–acetic acid. The solvent was evaporated at 80 °C under reduced pressure and a gentle stream of argon and the dry radiotracer dissolved in water. Solutions of 3- or 4-FPPMP with concentrations of 1 nM and 10 μM in 0.5 molar Tris-buffer (pH 7.4) were prepared and spiked with the corresponding radiotracer. Subsequently rat brain slices were incubated for 1 h with those solutions and subsequently analysed by means of autoradiography using a phosphor imager.

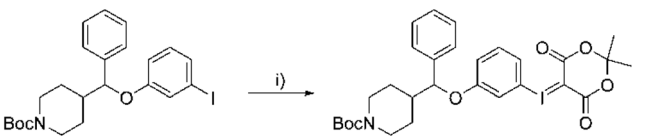
Results and discussion

Synthesis of precursor and standard compounds

The syntheses of the iodonium ylides **11** and **12** were accomplished by a novel one-pot procedure[13] as exemplified in Scheme 3 for ylide **11**. By this procedure yields of 33% and 42% were achieved, starting from iodoarenes **7** and **9**, respectively. This way the preparation of the ylides was relatively easy and additionally allowed to avoid the preparation and isolation of corresponding aryldiacetoxyiodoarenes which are usually prepared under acidic conditions.[16] Since latter condition is incompatible with the Boc-group, the ylide syntheses by common procedures would have afforded a change of the protection group resulting in two additional synthetic steps.

Radiosynthesis of 3-[^{18}F]FPPMP ([^{18}F]1)

In first labelling experiments the reaction conditions for the ^{18}F-exchange on iodonium ylides were chosen as described by



7 11

Scheme 3 Synthesis of iodonium ylide **11** by a one-pot procedure. (i) (1) *m*CPBA, CH_2Cl_2, (2) KOH, Meldrum's acid.

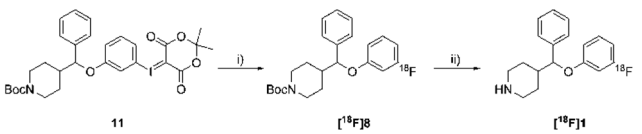
Table 1 *k*-values of relevant compounds

Compound	*k*-value	HPLC conditions
3-FPPMP	1.95	60 : 30 : 10 THF–MeOH–buffer: 1% aqueous TEA–H_3PO_4, pH 6.0; Luna C-18; 0.7 ml min^{-1}
4-FPPMP	2.12	
2-Fluoroanisole	2.99	55 : 45 ACN–buffer: 1% aqueous TEA–H_3PO_4, pH 8.0–8.5; Luna C-18; 1 ml min^{-1}
3-Fluoroanisole	3.43	
4-Fluoroanisole	3.19	
2-Fluorobenzyloxy-benzene (2-FBOB)	4.81	65 : 35 ACN–buffer: 1% aqueous TEA–H_3PO_4, pH 8.0–8.5; Luna C-18; 1 ml min^{-1}
3-Fluorobenzyloxy-benzene (3-FBOB)	5.83	
4-Fluorobenzyloxy-benzene (4-FBOB)	5.33	
Boc-3-[^{18}F]FPPMP ([^{18}F]**8**)	10.68	ACN–Buffer 60 : 40; buffer: 1% aqueous TEA–H_3PO_4 pH 8.0–8.5; Luna PFP(2); 4 ml min^{-1}
Boc-4-[^{18}F]FPPMP ([^{18}F]**10**)	11.04	

Satyamurthy and Barrio.[8] The substitution reaction was conducted in DMF as solvent delivering the desired product **[^{18}F]8** in a RCY of 17 ± 2% as shown in Scheme 4. However, the product was accompanied by a high amount of non-polar side products hampering the isolation of the product. These side products are presumably formed by intramolecular rearrangement and by cleavage as is described in literature.[16]

The side products overloaded the cartridge and caused a breakthrough of the desired product resulting in losses of more than 50%. Thus, isolation by semi-preparative HPLC became mandatory which necessitated a change of the reaction solvent from DMF to acetonitrile. Surprisingly, this also led to an enhancement of the RCY to 20 ± 5%.

After the reaction in acetonitrile the product mixture was simply diluted with water and submitted to semi-preparative HPLC. A too strong dilution below 60 : 40 (v/v) acetonitrile–water caused a precipitation of the non-polar components as oil, containing also a considerable fraction of the desired product Boc-3-[^{18}F]FPPMP (**[^{18}F]8**), and thus had to be avoided. However, the addition of 150 μl water to the reaction mixture (*ca.* 75 : 25 v/v acetonitrile–water) proved to be sufficient. The chromatogram of the separation is shown in Fig. 3. Although there were only small radiochemical impurities, many non-radioactive side products were detected in the UV-chromatogram. Also a large number of less polar side products were found which eluted later from the HPLC column (not presented in Fig. 3) and caused the breakthrough of product in the initial attempts to separate the product by solid phase extraction using a C-18 cartridge (see above).



Scheme 4 Radiosynthesis of 3-[^{18}F]FPPMP (**[^{18}F]1**). (i) n.c.a. [^{18}F]fluoride, Kryptofix®2.2.2., K_2CO_3, acetonitrile, 120 °C; (ii) CF_3CO_2H, DCM.

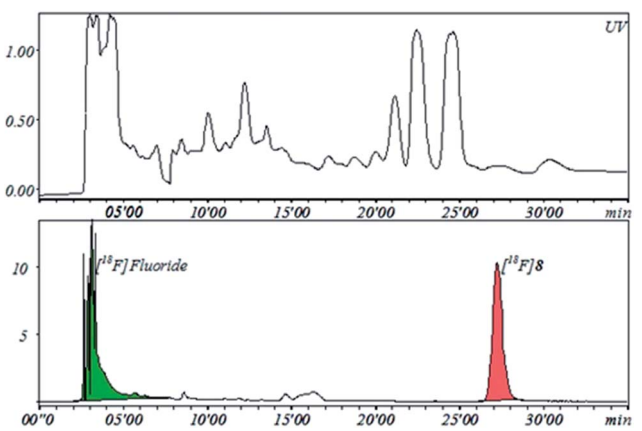


Fig. 3 Radiochromatogram of the separation of **[^{18}F]8** by semi-preparative HPLC. (60 : 40 acetonitrile–buffer: 1% aqueous TEA–H_3PO_4, pH 8.0–8.5; Luna PFP(2); 4 ml min^{-1}).

After the chromatographic separation, product **[^{18}F]8** was extracted from the eluent with a C-18 cartridge, what could now be performed without product losses, and eluted in DCM for deprotection with trifluoroacetic acid. As expected, the removal of the Boc-group proved to be quite easy and was accomplished with quantitative yield. However, another HPLC separation was necessary to obtain n.c.a. 3-[^{18}F]FPPMP in highest purity. This was done on an analytical column. The molar radioactivity of the product was determined in this final purification and was found to be higher than 50 GBq μmol^{-1}. The RCY of **[^{18}F]1**, related to starting [^{18}F]fluoride, amounted to 20 ± 5% after a total synthesis time of about 110 minutes including separation from the HPLC product fraction and solvent change to water.

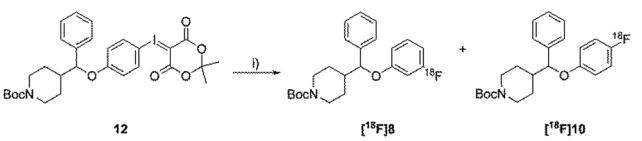
Since our objective was to demonstrate the preparation and isolation of a complex radiotracer *via* an iodonium precursor with a sufficient purity for a first preclinical evaluation, the radiochemical yield was not further optimised. Higher RCYs of up to 45% were observed when higher amounts of precursor in more solvent (15 mg of **11** in 1 ml of acetonitrile) were used. However, under these conditions the final product **[^{18}F]1** was usually contaminated with non-radioactive side products because of adverse effects in the chromatographic separation due to more side products and a higher solvent volume.

Radiosynthesis of 4-[^{18}F]FPPMP (**[^{18}F]2**)

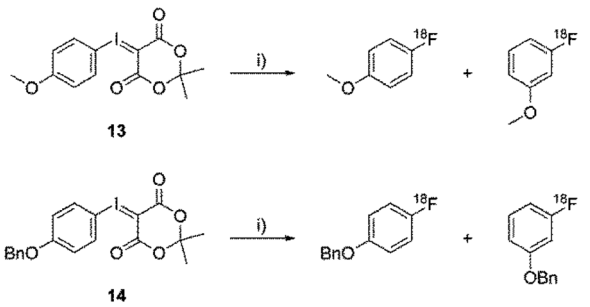
The radiosynthesis of **[^{18}F]2** was analogously carried out to the radiosynthesis of **[^{18}F]1**, but a slightly higher temperature of 130 °C was necessary. The desired product Boc-4-[^{18}F]FPPMP (**[^{18}F]10**) was formed in about 20% RCY, surprisingly accompanied by a radioactive side-product which proved to be the positional isomer Boc-3-[^{18}F]FPPMP (**[^{18}F]8**) formed in about 10% RCY (Scheme 5).

The resolution of the semi-preparative HPLC was not high enough to separate these regioisomers from each other. Therefore, the n.c.a. products were first isolated together by semi-preparative HPLC, the eluate concentrated, and the regioisomers separated on an additional HPLC-system with an analytical column. Following this, the desired product was deprotected with a quantitative yield as described above. The additional steps for the separation of the isomers took about 30–40 minutes prolonging the total synthesis time to about 140–150 minutes. By the procedure applied here, principally both regioisomers can be prepared from the same precursor (compound **12**), albeit in different total RCY of about 20% (**[^{18}F]2**) and 10% (**[^{18}F]1**), respectively.

In order to further confirm the formation of regioisomers, the simpler but structurally equal compounds 4-methoxyphenyl-(5-[2,2-dimethyl-1,3-dioxane-4,6-dione])ylide **13** and



Scheme 5 Formation of regioisomers in the synthesis of **4-[^{18}F]10**. (i) n.c.a. [^{18}F]fluoride, Kryptofix®2.2.2., K_2CO_3, acetonitrile, 130 °C.



Scheme 6 Formation of regioisomers in the reaction of ylides **13** and **14** with n.c.a. [^{18}F]fluoride. (i) n.c.a. [^{18}F]fluoride, Kryptofix®2.2.2., K_2CO_3, acetonitrile, 130 °C.

4-benzyloxyphenyliodonium-(5-[2,2-dimethyl-1,3-dioxane-4,6-dione])ylide **14** were ^{18}F-labelled under similar conditions as used for the radiosynthesis of Boc-4-[^{18}F]FPPMP **8** (Scheme 6).

In fact, in both cases the corresponding 3-isomers were also formed in about 4% and 11% RCY, respectively, while the expected 4-isomers were formed in about 12% and 20%, respectively, as identified by comparison of the retention times with the respective macroscopic standards. Thus, the formation of regioisomers seems to be a problem with the radiofluorination of iodonium ylides. This surprising observation that the nucleophilic substitution on ylides is not regiospecific was not reported by Satyamurthy and Barrio.[8] A similar effect, however, has been found by Graskemper *et al.* in the thermal decomposition of (4-methoxyphenyl)(5-methoxy [2.2]paracyclophan-4-yl)-iodonium hexafluorophosphate.[17] There it is suggested, that the strong electron donating effect of the methoxy group leads to a competing fluorination *via* an aryne pathway.

Another interesting point to note is that the amount of non-radioactive side-products in the labelling reactions of ylides **13** and **14** was significantly lower than in case of the more complex ylides **11** and **12**. This can either mean, that the presence of more functional groups opens alternative reaction paths, or, that the bulkier ylides simply tend to decompose *via* rather unspecific pathways. Due to the high number of side products formed in case of ylides **11** and **12** (*cf.* Fig. 3) an unspecific decomposition is more likely. In the initial labelling experiments with ylide **11** several reactions with lower temperatures were performed, but this only led to a decrease of the RCY while no improvement of the amount of side-products was observed.

The radiosynthesis of **[^{18}F]1** and **[^{18}F]2** proved feasible by direct nucleophilic substitution of the corresponding iodonium ylides. The isolation of the products, however, was challenging due to the high amount of non-radioactive side-products and, in case of **[^{18}F]2**, due to the formation of a regioisomer. Here, even a second separation by HPLC was inevitable resulting in a prolonged synthesis time which might be reduced by further optimisation of the purification process, *e.g.* by application of a solvent gradient.

In vitro binding of 3- and 4-[^{18}F]FPPMP on rat brain slices

As a first pharmacological test the non-specific binding of **[^{18}F]1** and **[^{18}F]2** was examined by a competition experiment. For this, two series of rat brain slices were incubated for 1 h with 1 nM or 10 μM solutions of 3- and 4-FPPMP, respectively, in 0.5 M Tris-buffer containing the corresponding radiotracer and analysed by phosphor imaging. In case of both tracers only about 10.3% and 9.9%, respectively, of the total activity was blocked on the slices incubated with the 10 μM solution and no anatomical structures were visible on images taken from the slices incubated with the low concentration of the radiotracer. Hence, 3- and 4-[^{18}F]FPPMP appear obviously not suitable for *in vivo* imaging of NET or SERT using PET; this is why their further radiopharmacological evaluation seemed not to be indicated.

Conclusions

The one-step n.c.a. radiofluorination of more complex electron-rich arenes could be achieved in a reasonable reaction time and with an overall RCY of about 20% by using suitable iodonium ylide precursors. From those, both compounds, 3- and 4-[^{18}F]-FPPMP, could quantitatively be obtained by a subsequent deprotection. Those ligands, here used as model compounds, however, did not prove suitable in first evaluation tests for use of *in vivo* imaging.

The preparation of the ylide precursors succeeded by a comparatively easy one-pot procedure in satisfactory yields of 33% and 42%. For the radiosynthesis the main challenge proved to be the purification of the labelled products due to many non-radioactive side-products formed. Additionally, during the preparation of 4-[^{18}F]FPPMP the surprising formation of two regioisomers was found. The occurrence of positional isomers was further confirmed by the radiolabelling of comparable 4-methoxy- and 4-benzyloxyphenyliodonium ylides, demonstrating that the reaction is not regiospecific. Like in this study, separation of isomers might demand additional efforts in given case. In spite of these drawbacks the potential of aryliodonium ylides as precursors for nucleophilic ^{18}F-labelling of more complex molecules was demonstrated here.

Today the most important alternative route to such ^{18}F-labelled radiotracers is a multi-step synthesis in which either electron withdrawing groups are attached to the benzene ring to be ^{18}F-fluorinated, or the whole molecule has to be synthesised by a two or more step procedure. In both cases the radiosynthesis gets more complex (*e.g.* due to additional reagents) while the necessity of purification by HPLC is also very likely, although the total amount of side-products might be significantly lower. The better approach for a given radiotracer will strongly depend on the individual case.

Since the iodonium ylides represent the more direct approach, their advantage increases the more complex the multi-step approach gets. In this respect, the reaction of n.c.a. [^{18}F]fluoride with iodonium ylides proves to be a powerful alternative to the multi-step synthesis for the preparation of electron rich and non-activated [^{18}F]fluoroarenes. Additionally, iodonium ylides also proved to be a better to handle alternative to the well-known aryliodonium salts. Those findings warrant further development of the ylide-method for the direct synthesis of non-activated or electron rich n.c.a. [^{18}F]fluoroarenes.

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