Journal of Fluorine Chemistry 134 (2012) 107–114

Contents lists available at ScienceDirect

Journal of Fluorine Chemistry

journal homepage: www.elsevier.com/locate/fluor

Synthesis of fluorine-18-labelled TSPO ligands for imaging neuroinflammation with Positron Emission Tomography

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ARTICLE INFO

Article history:
Received 14 January 2011
Received in revised form 18 March 2011
Accepted 26 March 2011
Available online 2 April 2011

Keywords:
Fluorine-18
18 kDa TSPO
Radioligand
Neuroinflammation

ABSTRACT

Fluorine-18 radiochemistry is an essential tool in Positron Emission Tomography, providing the bridge between the cyclotron-produced raw radioisotope and the biomedical image of an *in vivo* radioactivity distribution. From the fluorine-18 labelled radioligands for the translocator protein 18 kDa that are produced in our laboratory, namely [^{18}F]FEDAA1106, 6-[^{18}F]F-PBR28, [^{18}F]PBR111 and [^{18}F]DPA-714, we address various aspects of fluorine-18 radiochemistry, such as rationales of radiotracer design, radioisotope production and [^{18}F]fluoride activation, and procedures of radiofluorination, purification and formulation. Automation of the radiochemistry process has become indispensable in order to assure a constant radiopharmaceutical quality and reproducible radiochemical yields as well as to meet the required radiation protection aspects.

1. Introduction

1.1. The positron emitter fluorine-18

The element fluorine counts an important number of radioactive isotopes, going from fluorine-14 to fluorine-31. Most of these have too short a half-life, in the ms to 10 s range, to be of any practical value. Only the neutron-deficient fluorine-18 (109.8 min) and sporadically also fluorine-17 (64.5 s) have found application in medical imaging. Inherent in the nuclear neutron deficiency of these two isotopes, the radioactive decay process involves the expulsion of a neutrino and an antielectron (positron) of variable but complementary energies. For fluorine-18 the positron energy distribution is a continuous spectrum with a maximum of 635 keV which gives the positron a mean range in water of 0.6 mm (maximum 2.4 mm) after which it annihilates with any electron to give two gamma photons of 511 keV travelling under a mutual angle of 180°. It is this pair of coincident gamma rays that underlies the medical imaging technique Positron Emission Tomography (PET) allowing a quantitative three-dimensional reconstruction of the *in vivo* radioactivity levels of the fluorine isotope administered to a living organism [1]. Quintessential of the technique is that the fluorine radioisotope should be constituent part of a molecule of biological interest, e.g., a drug or a mimic of a natural physiological active compound. The measured *in vivo* radioactivity distribution in principle reflects then the distribution of the labelled molecule, apart from the complicating factor of metabolism. A salient aspect of PET, as a consequence of the short half life of the radioisotopes employed, is the high specific radioactivity of a no-carrier-added radiotracer. This implies very small amounts of the labelled compound in terms of mass associated with a relatively high amount of radioactivity, needed for the imaging process but imposing restrictions in terms of radiation protection in the radiotracer manufacturing procedures. [^{18}F]Fluorine for PET is normally produced using a cyclotron and is available as no-carrier-added aqueous fluoride (1–10 Ci/μmol) or as carrier-added molecular fluorine gas (1–10 mCi/μmol). Radiochemistry is therefore indispensable (and also possible because of the convenient half

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[1] *Presentation of the laboratory and its activities over the last decade*: The Laboratory of Radiochemistry and Radiopharmaceuticals of the Service Hospitalier Frédéric Joliot belongs to the life science division of the French atomic energy commission (CEA) as part of the Biomedical Imaging Institute. It supports most Positron Emission Tomography imaging programs within the institute and interacts both with preclinical and clinical end-users.

The laboratory is involved in both R&D and production, assures support activities (platform), and as such integrates three lines of interest: methodology, radiotracer design and radiopharmaceuticals. Missions and objectives of this chemistry, radiochemistry and radiopharmacy unit are therefore all directed towards the production of compounds and pharmaceuticals labelled with the short-lived radioisotopes carbon-11 ($T_{1/2}$: 20.4 min) and fluorine-18 ($T_{1/2}$: 109.8 min).

The unit's motto, "from the radioactive isotope to the radiopharmaceutical for molecular imaging with PET", translates into the management of the chemical, radiochemical and radiopharmaceutical tools associated with the handling of these positron-emitters.

In the last decade, the unit has been a major player in the field of macromolecule labelling with fluorine-18 – the first to give access to [^{18}F]oligonucleotides – and in the field of neuroinflammation biomarker development, impacting the molecular imaging community with many promising [^{11}C/^{18}F]radioligands as [^{11}C]PK11195 alternatives.

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doi:10.1016/j.jfluchem.2011.03.020

life) as a link between production of the radioisotope and its employment in PET [2–5].

1.2. The 18 kDa TSPO receptor

Because of its implicit low mass, a radiopharmaceutical with a high specific radioactivity not only does not perturb the physiological functioning of the organism to which it is administered, it also is able to trace specifically functional protein structures (receptors) present in low concentration in tissues, provided that it has a specific affinity for these. One such a receptor is the so called peripheral benzodiazepine receptor (PBR), recently renamed 18 kDa TSPO or for short TSPO [6], standing for translocator protein while 18 kDa is referring to its molecular weight. It is mainly found in the mitochondrion and is expressed by macrophages in inflammatory tissue associated with tumours and also in the brain by activated microglial cells in a variety of lesions. Interest in this receptor in relation with PET was initiated in our laboratory in the early eighties [7] by the preparation of the ligand PK11195 (Ki = 0.83 nM [8]) labelled with carbon-11, the other major radioisotope for PET (20.4 min half life). [^{11}C]PK11195 (**1**, Fig. 1) has been up to the present day the PET radiopharmaceutical of reference for studying (neuro)inflammation, in spite of a number of shortcomings related to its kinetics, low brain uptake and high non-specific binding [9].

In recent years many new ligands were proposed, a number of which are currently being evaluated in animal models and in humans [9–12]. Fig. 1 shows four such compounds (**4**, **7**, **10** and **13**) that are produced and used in PET in our laboratory [13–19]. These are labelled with fluorine-18, three at a terminal aliphatic position and one at an aromatic position. From these examples we shall highlight various aspects of fluorine-18 chemistry.

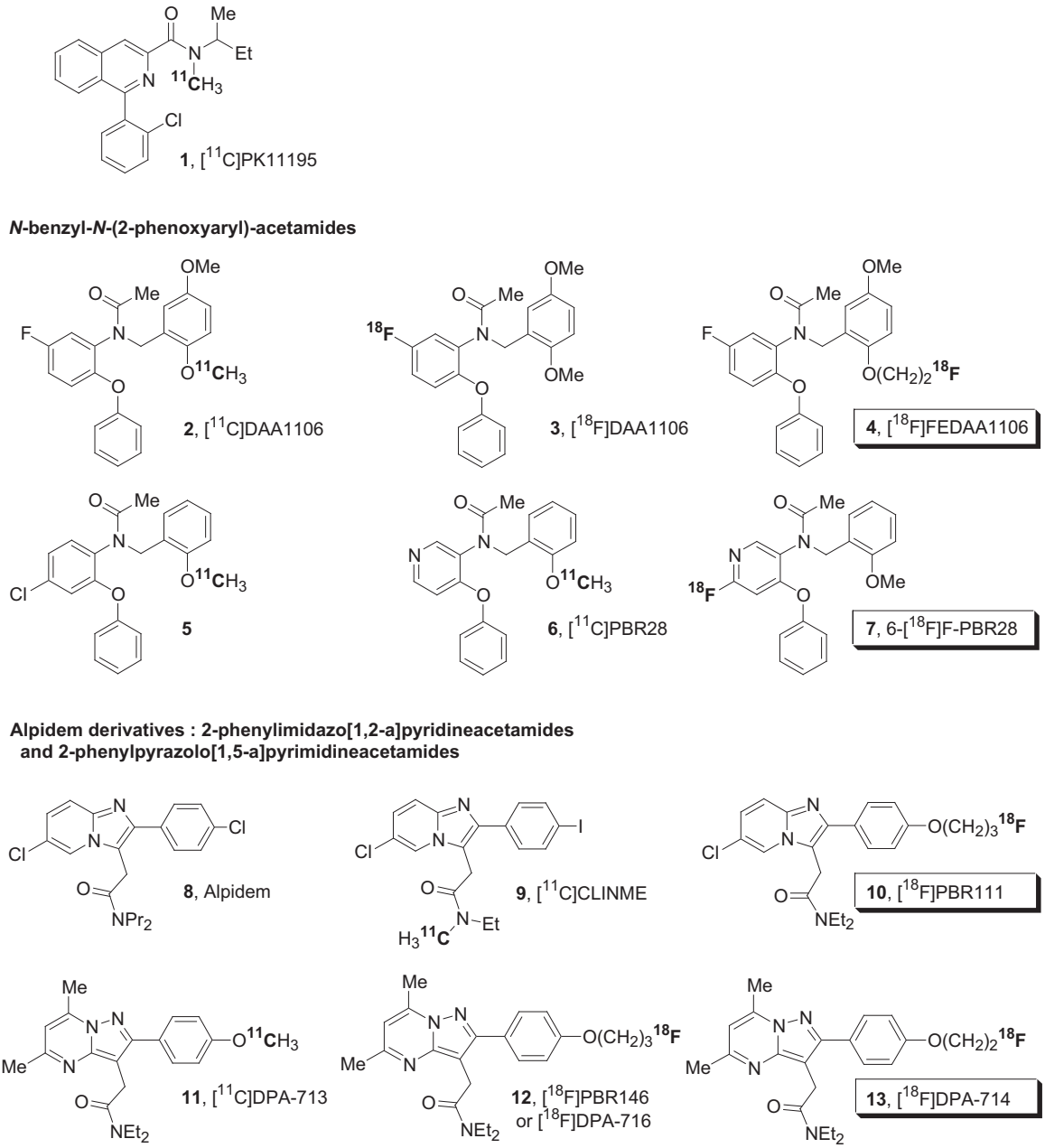


Fig. 1. Four selected ligands for the TSPO receptor, [^{18}F]FEDAA1106 (**4**) 6-[^{18}F]F-PBR28 (**7**), [^{18}F]PBR111 (**10**) and [^{18}F]DPA-714 (**13**) and several related ligands.

2. Design rationale for selected radioligands for TSPO

No less than seven different structure families have been identified as having affinity for the TSPO receptor [11]. The fluorine-18-labelled compounds in Fig. 1 are representatives of two of these families. We have on the one hand [^{18}F]FEDAA1106 (**4**) and 6-[^{18}F]F-PBR28 (**7**) belonging to the *N*-benzyl-*N*-(2-phenoxyaryl)acetamide group and on the other hand the so called alpidem (**8**) derived TSPO ligands, namely the 2-arylimidazo[1,2-a]pyridin-3-ylacetamide [^{18}F]PBR111 (**10**) and the 2-arylpyrazolo[1,5-a]pyrimidin-3-ylacetamide [^{18}F]DPA-714 (**13**).

The leading design principle shared by all four compounds is the drive for a fluorine-18 labelled TSPO ligand. A number of advantages of fluorine-18 over the concurrent carbon-11 are regularly evoked in the PET-related literature [11], the most important one being the more convenient half-life of 110 min. The structures discussed here are, compound **4** excepted, derived from similar molecules that do not contain a fluorine atom and that had been labelled mostly with carbon-11 (Fig. 1). Thus a common strategy for these compounds was the introduction of a fluorine atom or a fluorine-bearing moiety without compromising the molecule's efficacy as a PET agent.

However, the parent compound of [^{18}F]FEDAA1106 (**4**) which is [^{11}C]DAA1106 (**2**), in itself a successful ligand, does have a fluorine atom of itself, situated at an aromatic position. In the light of a strategy favouring fluorine-18 over carbon-11 it would be logical to aim at the same compound with the existing fluorine atom radioactively labelled ([^{18}F]DAA1106 (**3**)), instead of aiming at **4** which implies a structural modification. Introduction of no-carrier-added [^{18}F]fluoride into a *homo*aromatic position requires an electron withdrawing substituent suitably placed relative to the leaving group on the aromatic ring [20] which is not the case in this molecule. One of the few strategies to circumvent this problem is the use of a diaryliodonium compound as the precursor for radiolabelling [21] and this was indeed reported [22]. However, the method is tedious with a complicated synthesis of an unstable precursor and thus unpractical. Indeed there are no reports on the use of [^{18}F]DAA1106 in PET.

Instead, with [^{18}F]FEDAA1106 (**4**) one has chosen to make a derivative by replacing the [^{11}C]methoxy group at the two-position of the benzyl moiety of **2** by a 2-[^{18}F]fluoroethoxy group. It is quite a common practice in PET chemistry to replace a methoxy group by a fluoroalkoxy group. The 2-[^{18}F]fluoroethoxy group is most popular, but [^{18}F]fluoromethoxy and 3-[^{18}F]fluoropropoxy occur also [23]. It is generally accepted and confirmed by experience that such a replacement does not change the receptor affinity of the molecule in a decisive way. In the case of [^{18}F]FEDAA1106 (**4**) the *in vitro* binding affinity improved with a factor two (0.078 nM) [8].

The fluoroalkyl group can entail a somewhat increased lipophilicity making the product better pass the blood–brain-barrier. Thus, while DAA1106 (**2**) has a reported log D value of 3.65, that of FEDAA1106 (**4**) is 3.81 [8,24], which means that the partition ratio octanol/aqueous buffer is increased with a factor 1.5. Indeed, the bioavailability of **4** in the brain is considerably increased compared to **2**. This advantage might be offset by the fact that an increase in lipophilicity often implies a higher relative non-specific binding to non-receptor structures. For **4** the opposite is the case which shows that this rule does not systematically hold. The replacement of a hydrogen atom of the methoxy group of DAA1106 (**2**) by a labelled fluorine atom (the [^{18}F]fluoromethoxy group) [8] increases the above partition ratio with only a factor 1.1 (log D = 3.70).

Metabolism can be a complicating factor when introducing a [^{18}F]fluoroalkyl group into the molecule because it can give rise to the liberation of free [^{18}F]fluoride. This accumulates in bone structures such as the skull, which can compromise the image quality. The effect is less prominent for the fluoroethoxy group than it is for the fluoropropoxy group. The former is known to be removed as fluoroacetaldehyde or fluoroacetic acid, which could enter the brain obscuring the PET image, but are not further metabolised to [^{18}F]fluoride. On the other hand, [^{18}F]fluoropropionate is known to be entirely metabolised to give free [^{18}F]fluoride [25,26]. Indeed, [^{18}F]FEDAA1106 (**4**) does not show *in vivo* [^{18}F]fluoride formation. Metabolism occurs mainly through debenzylation, like in the other members of the *N*-benzyl-*N*-(2-phenoxyaryl)acetamide family, and the resulting 2-(2-[^{18}F]fluoroethoxy)-5-methoxybenzoate neither enters the brain nor is defluorinated before elimination. A fluoromethoxy group is often sensitive to metabolic defluorination and corresponding [^{18}F]fluoromethoxy ligands are rather uncommon. Indeed, [^{18}F]FMDAA1106 and also its fluoromethoxy-*bis*-deuterated analogue are rapidly defluorinated by α,α-dehydrofluorination and were therefore abandoned as not useful in PET [27].

The reason why the 2-methoxy group and not the 5-methoxy group was chosen for replacement by fluoroethoxy is unclear. Perhaps it was an arbitrary choice or otherwise the reason could have been differences in the facility of chemical precursor synthesis. It seems unlikely however that the choice was based on an *a priori* assumption of any structure activity relationship. Note that in the case of a methoxy replacement by radioiodine the same authors chose again the 2-position without mentioning the alternative 5-position [28].

The other [^{18}F]fluoroethoxy ligand in Fig. 1, [^{18}F]DPA-714 (**13**), can also be compared with two of its counterparts, namely [^{11}C]DPA-713 (**11**) (methoxy analogue) [29–31] and [^{18}F]PBR146 (**12**) (3-fluoropropoxy analogue, also called [^{18}F]DPA-716). It is known that in this family changes in the *para* substituent on the phenyl ring influence the affinity and that moderately large substituents are tolerated. The affinities for TSPO are nevertheless rather similar (4–7 nM), with the fluoropropoxy compound (4.1 nM) slightly better than the other two [25,31]. However, [^{18}F]DPA-714 (**13**) shows a remarkable physiological agonist effect which is absent in [^{11}C]DPA-713 (**11**) [32]. The lipophilicities of the latter two compounds are about the same and slightly lower, respectively than that of the fluoropropoxy ligand [^{18}F]PBR146 (**12**) [25]. As can be expected, the latter gives rise to more [^{18}F]fluoride *in vivo* but [^{18}F]DPA-714 (**13**) is metabolised twice as fast [25]. This metabolism consists not only in defluoroethylation. Transformations of the methyl groups and the amide moiety have been observed also in our laboratory [33].

[^{18}F]PBR111 (**10**) is structurally a closer derivative of alpidem (**8**) than [^{18}F]DPA-714 (**13**). The chlorine atom of alpidem has been replaced by a 3-[^{18}F]fluoropropoxy group which changes the properties from an unselective ligand (both TSPO- and central benzodiazepine receptors) to a TSPO-selective ligand (3.7 nM). This had also been the case with [^{11}C]CLINME (**9**) [34,35] in which an iodine replaces the chlorine. Note that the variation in the *N*-alkyl substituents between these compounds is irrelevant in this respect. [^{18}F]PBR111 (**10**) compares to its 2-[^{18}F]fluoroethoxy counterpart ([^{18}F]PBR102) in the same way as the above [^{18}F]DPA-714 (**13**) to its 3-[^{18}F]fluoropropoxy analogue [^{18}F]PBR146 (**12**). The relatively higher *in vivo* free [^{18}F]fluoride formation remains at an acceptable level for PET imaging. Our use of both a 3-[^{18}F]fluoropropoxy and a 2-[^{18}F]fluoroethoxy ligand within the alpidem-related ligand group reflects the fact that there is often not a distinctive advantage for either two categories.

The fourth compound of Fig. 1 is 6-[^{18}F]F-PBR28 (**7**) which was recently proposed by our group [17]. This pyridine containing compound is part of a subgroup within the *N*-benzyl-*N*-(2-phenoxyaryl)acetamide family in which the 5-methoxy group of the benzyl moiety of the parent DAA1106 (**2**) has been deleted.

Two other members of this group, showing a very close structural resemblance with our new compound **7**, had already been shown to be powerful ligands for TSPO, namely compounds [^{11}C]**5** (0.074 nM) [36] and [^{11}C]PBR28 (**6**) (0.68–2.47 nM) [37].

The difficulty that presents the introduction of a fluorine-18 atom into a *homo*aromatic position, as we encountered above with [^{18}F]DAA1106 (**3**), is removed in the *hetero*aromatic situation as no activating group is needed. Indeed, for many years our laboratory has been exploring extensively the technique of *hetero*aromatic nucleophilic radiofluorination, notably in the *ortho* position of a pyridine ring, which has resulted in a variety of labelled molecules [38–41]. From a design point of view two approaches are possible with respect to the pyridine ring. In the first approach the pyridine ring is the result of a structural modification, in which normally a benzene ring is replaced by a pyridine ring with the only aim to provide an anchorage point for the fluorine-18 atom. This has been particularly the case for a number of prosthetic agents for the labelling of macromolecules, developed by us [42–44] and others [45,46]. It should be kept in mind that the introduction of a ring nitrogen is not *a priori* a neutral operation. Nitrogen atoms often play a key role in receptor–ligand interaction (hydrogen acceptor) so that their appearance as result of a derivatisation can profoundly change the pharmacological profile of the original molecule. Also the introduction of a ring nitrogen decreases the lipohilicity considerably and this may decrease the ligand's brain-penetration capability. In the second approach the pyridine ring is already part of the structure of the lead molecule so that the structural modification only involves the introduction of a fluorine at an *ortho* position, most often replacing a hydrogen atom which is at least from a stereochemical point of view not a big change. This approach was followed with several of our nicotinic acetylcholine receptor ligands in which the fluorine-18 replaces a hydrogen [47,48] or a chlorine [49–51] present in the lead structure.

When we designed 6-[^{18}F]F-PBR28 (**7**), the analogue without fluorine atom, PBR28 (**6**), existed already and had been labelled with carbon-11 by others [37]. These authors did not mention whether their design envisaged a future radiofluorination, but relative to the very close non-pyridine ligand **5**, the TSPO specificity of PBR28 (**6**) is maintained. Going from **5** to **6** implies the introduction of a ring nitrogen and the replacement of a chlorine by a hydrogen, two operations that decrease lipophilicity. Indeed, the specific to non-specific binding ratio in monkey brain is very high for **6** (namely 9) compared to **5** and the uptake rate of **6** is lower than that of **5** [36,37]. As for our 6-[^{18}F]F-PBR28 (**7**), the addition of a fluorine atom does not seem to have any detrimental effect on the ligand's quality as a TSPO binding agent as it performs well in a rat model of neuroinflammation. Whether 6-[^{18}F]F-PBR28 (**7**) shows the same multiple-binding-domain properties as PBR28 (**6**) [52] remains to be seen. Our choice to fluorinate the 6-position rather than the alternative 2-position of the pyridine ring of PBR28 was guided by the easier chemistry of precursor preparation. Also, in our design the fluorine is in the same position as the chlorine in compound **5** which is a good TSPO ligand.

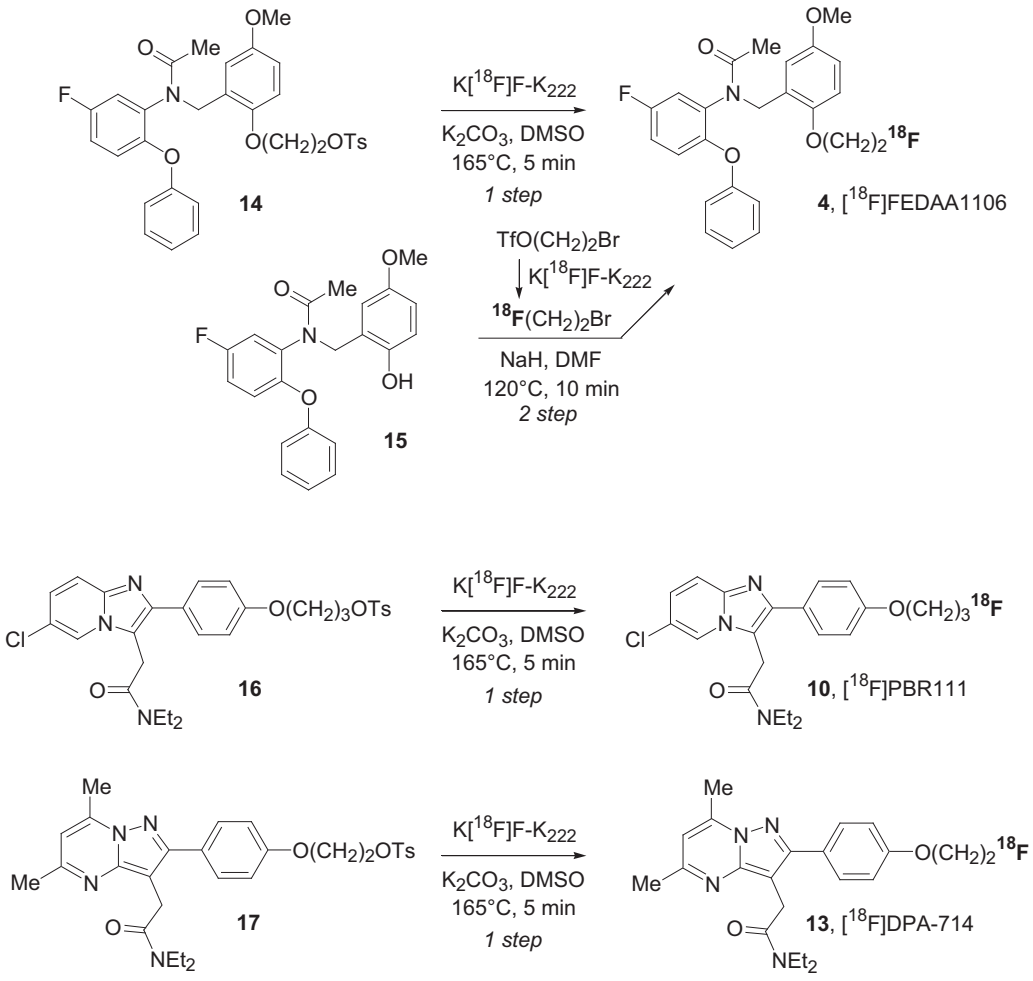
3. Radiolabelling of selected ligands: FEDAA1106, 6-F-PBR28, PBR111 and DPA-714

3.1. Cyclotron production of fluorine-18

We produce fluorine-18 using a standard method consisting of the irradiation with a cyclotron-generated proton beam (18 MeV, 20 μA, 30 min) of a 2 mL water target highly enriched in the oxygen-18 isotope (>97%), held in a stainless steel containment provided with a domed-end niobium cylinder insert. The nuclear reaction generating fluorine-18 is ^{18}O(p,n)^{18}F. The corresponding reaction on the few percent of oxygen-16 in the target would give rise to the extremely short-lived fluorine-16 decaying immediately to oxygen-15. Oxygen-16 undergoes also the p,α reaction to nitrogen-13 while this same reaction on oxygen-18 results in stable nitrogen-15. The coproduced oxygen-15 ($T_{1/2}$ = 2 min) and nitrogen-13 ($T_{1/2}$ = 10 min) are considered as not problematic in view of the quantities in which they are formed and their half lives which are short compared to that of fluorine-18. After irradiation, the target water, containing up to 800 mCi of [^{18}F]fluoride, is pushed in several minutes by helium pressure (2 bar) through a 60 m long line of polytetrafluoroethylene tubing (PTFE, 0.8 mm internal diameter; 1/16 inch external diameter) into a collection vial in a shielded hot cell. Recent research has made plausible that most carrier fluoride present in the irradiated target material originates from radiolysis of PTFE in contact with the radioactive target water [53]. We have measured the amount of fluoride in the target by a fluoride complexation reaction after transfer and found that about 250 nmol is present [54] which corresponds well with the specific radioactivities found in our final products. A change of the PTFE line for one made of a material free of fluorine might increase the specific radioactivities considerably [53].

3.2. Chemical activation of [^{18}F]fluoride

From its arrival in the hot cell the procedure regarding the radiochemical synthesis is automatic and computer controlled, which is important in minimizing the radiation dose to the chemist. In our case this is ensured either by a Zymate-XP robot (Zymark corporation) or a TRACERLab™ FX-FN system (GE Healthcare). The first operates by a robotic arm that can displace vials and transfer liquids by pipetting. The second operates by transfer of liquids between static vials and other equipment by pressure. Initially the [^{18}F]fluoride is isolated on a cartridge with strong anion exchange resin of the quaternary ammonium type (–C(O)NH(CH$_2$)$_3$N(CH$_3$)$_3^+$X$^-$, Waters, Sep-Pak® Light Accell™ Plus QMA). This separation from the target [^{18}O]water is considered useful because it eliminates possible cations that might have evolved from the target holder wall and that can reduce reactivity of the fluoride. We use the anion exchanger in the bicarbonate form (the carbonate form is also possible [55]) and although the affinity of fluoride for it is the weakest of all inorganic anions [56], no-carrier-added [^{18}F]fluoride is easily retained. The hydroxide form can also be used [57] but since hydroxide ion has an affinity that is lower than that of bicarbonate or carbonate and closer to that of fluoride, there is an increased level of chloride contamination (as purchased the QMA cartridge contains ion exchange material in the chloride form) and competition between chloride and [^{18}F]fluoride can sometimes be problematic in the follow-up radiochemistry. The low affinity of fluoride for the anion exchanger ensures an easy elution of [^{18}F]fluoride from the cartridge with a small volume of only 1 mL, comprising a mixture of water and acetonitrile (30/70) containing 1.5 mg of potassium carbonate and 12–15 mg of kryptofix® 222 or even with 0.6 mL (H$_2$O/MeCN 1/1, 7 mg potassium carbonate [55]). Elution in just water (1 mL) with 4.5 mg of potassium carbonate has also been used [18]. In the latter cases the [^{18}F]fluoride is eluted into a vessel charged with a kryptofix® 222/acetonitrile solution. In all cases the resulting mixture is evaporated till dryness to obtain "naked" [^{18}F]fluoride ready for further chemistry [58]. This drying is either done by heating at 140–150 °C under a nitrogen stream for 10 min (Zymate-XP) or in a two-stage procedure without nitrogen stream at reduced pressure (30–35 kPa) at 60 °C during 7 min followed by 120 °C under vacuum during 5 min (TRACERLab™ FX-FN). We do not find it necessary to perform the habitual cycles of azeotropic drying with acetonitrile as initially was done with the radio-synthesis of [^{18}F]DPA-714 (**13**) [55], in which the applied temperature was lower. Our K[^{18}F]F/kryptofix® 222 preparation



Scheme 1. Nucleophilic aliphatic radiofluorinations of [^{18}F]FEDAA1106 (**4**), [^{18}F]PBR111 (**10**) and [^{18}F]DPA-714 (**13**).

is dry enough, not only for the three aliphatic nucleophilic substitutions leading to **4**, **10** and **13** (it is known that aliphatic radiofluorination is less sensitive to traces of water [58]), but also for the aromatic nucleophilic substitution leading to **7**.

3.3. Nucleophilic radiofluorination

Our radiosynthesis of [^{18}F]FEDAA1106 (**4**) [16] is inspired by the original work of Zhang et al. [8]. There are two ways of realizing a molecule containing a [^{18}F]fluoroalkyloxy moiety, a direct radiofluorination by nucleophilic replacement of a suitable leaving group as in **14** (Scheme 1) or the coupling of a separately produced [^{18}F]fluoroalkylating agent with a corresponding hydroxyl-containing precursor [23]. Zhang et al. tried both methods and preferred the second option reacting the phenolic compound **15** with 1-bromo-2-[^{18}F]fluoroethane in DMF at 120 °C using sodium hydride as base. The overall yield of **4** was 45%. Indeed, often the reaction conditions associated with nucleophilic radiofluorination are a source of complications in terms of impurities caused by decomposition of the precursor. This is particularly the case with compound **14** which gave in the hands of Zhang et al., using again DMF as solvent, irreproducible results with yields varying between 2 and 60% irrespective of reaction time (1–15 min) or temperature (30–120 °C). The impurities rendered purification complicated. With the two-step method the [^{18}F]fluoroalkylating reagent was purified by distillation after which the second reaction was relatively clean. The two-step method was executed by way of an in-house developed automated system which can handle the distillation of 1-bromo-2-[^{18}F]fluoroethane from one vial into another. Of course a one-step procedure is simpler and thus easier to automate.

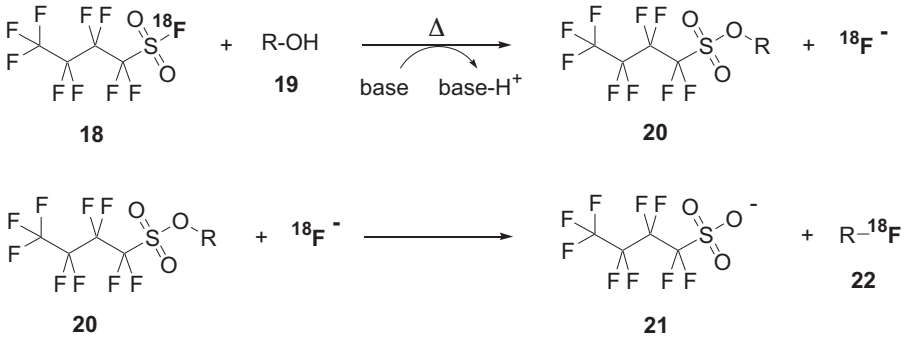
In our laboratory we dispose today only of a commercially available one-pot synthesis automate (TRACERLab^{TM} FX-FN) which has constrained us to one-pot procedures, not only for [^{18}F]FEDAA1106 (**4**), but also for [^{18}F]PBR111 (**10**) and [^{18}F]DPA-714 (**13**) [16]. Also our Zymate-XP robot (now decommissioned) did not allow for a distillation procedure. An automate with two reactors has not been available on the market so far which explains that direct nucleophilic radiofluorination seems to be somewhat privileged these days.

The conditions we apply for the radiosynthesis of [^{18}F]FEDAA1106 (**4**) consist in the use of DMSO as solvent, a temperature of 165 °C and a reaction time of 5 min. The precursor concentration is between 10 and 20 mM and the leaving group of the precursor is the rather standard tosylate. In fact, these conditions have not been entirely optimised. We find however, that they usually work well and we apply them whenever we can, not only for aliphatic but also for aromatic substitution, without seeking further optimisation. Thus all our ligands were made in this way with decay corrected isolated radiochemical yields that are summarized in Table 1. Usually relatively moderate temperatures suffice for aliphatic radiofluorination [58] and the temperature of 165 °C may seem rather high but it allows the reaction time to be kept short. When we used acetonitrile at 120 °C for the synthesis of [^{18}F]DPA-714 (**13**) on the TRACERLab^{TM} FX-FN the decay-corrected yields (22–25%) were lower than with DMSO at

Table 1
Conditions (summary) and radiochemical isolated yields obtained with the TRACERLab™ FX-FN automate or the Zymate-XP robot.

Compound	Fluorination	Pre-purification	HPLC purification	Yield (%)[a]
[^{18}F]FEDAA1106 (**4**)	K[^{18}F]F-K$_{222}$	PrepSep™R-C18 Extraction	Waters Symmetry®C-18	30–33
6-[^{18}F]F-PBR28 (**7**)	K_2CO_3, DMSO	column (Zymate-XP)	Waters Symmetry®C-18	16–18
[^{18}F]PBR111 (**10**)	160 °C, 5 min	or Alumina N SepPak cartridge	Waters Symmetry®C-18	31–36
[^{18}F]DPA-714 (**13**)		(TRACERLab FX-FN)	Waters X-Terra™RP18	43–50

[a] $n > 10$ for all, $n > 120$ for [^{18}F]DPA-714.



Scheme 2. Radiofluorination with nonafluorobutane-1-sulphonyl [^{18}F]fluoride (**18**).

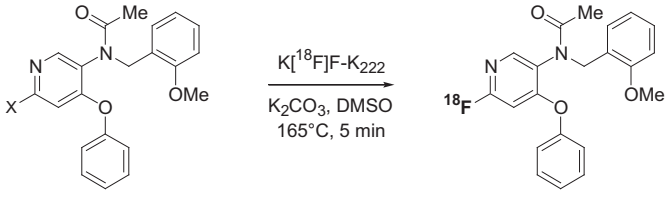
165 °C (43–50%). Also DMF gave much lower yields [19]. On the other hand one should be vigilant that these conditions can promote the Kornblum oxidation [59]. This consists of the oxidation of aliphatic tosylates (or other sulphonates) into aldehydes by DMSO under the influence of potassium carbonate at high temperature. The reaction was recently shown to proceed easily in the transformation of 2-[^{18}F]fluoroethyl tosylate into [^{18}F]fluoroacetaldehyde in the presence of the potassium carbonate/kryptofix complex used in the preceding radiofluorination [60]. Our experience shows that this reaction, which is likely to take place, does not compete sufficiently with the radiofluorination to present a threat to the latter. On the other hand it is possible that the non-radioactive impurity level, that also in our case complicated final HPLC purification of [^{18}F]FEDAA1106 (**4**), partly derives from this reaction.

We use for all nucleophilic aliphatic radiofluorinations discussed here the corresponding tosylates as precursor. Tosylates are often chosen because they are usually stable and easy to store for longer periods. Other sulphonate leaving groups with greater nucleofugacity could be envisaged [58], allowing for milder reaction conditions to avoid undesired side reactions. The problem with these is that they may suffer from instability making them difficult to stock. In fact the criterion for the choice of leaving group generally has been its stability rather than its reactivity. A way to circumvent this dilemma was explored by the group in Jülich in 2001 [61]. The idea consisted, as outlined in Scheme 2, in employing nonafluoromethane-1-sulphonyl [^{18}F]fluoride (**18**) to generate, in basic milieu, from an alcohol (**19**) an active sulphonate ester (**20**) *in situ*, liberating simultaneously a [^{18}F]fluoride anion that in its turn attacks the active ester to give the desired labelled product **22**. The authors encountered the obvious problem that both the *in situ* ester **20** and the [^{18}F]fluoride are present in equally low concentrations corresponding to the no-carrier-added state of **18**, resulting in a too low reaction rate, whereas addition of carrier **18** gave excellent results but of course with low specific radioactivity. We have recently embarked on a project to see if products with high specific activity can be obtained nevertheless. In our approach the key reagent **18** is produced from the corresponding nonafluoromethane-1-sulphonyl chloride and K[^{18}F]/kryptofix and is not separated from the excess of starting sulphonyl chloride. When this mixture is made to react with an alcohol the nonafluoromethane-1-sulphonyl chloride acts as a pseudo carrier giving rise to the same ester **20** without liberating fluoride. The chloride ions that are produced instead may or may not react with ester **20**. If they do, a separation of the fluorine-18-labelled product from the corresponding non-radioactive chloro-compound will be necessary. Efforts to label DPA-714 (**13**) in this way are currently underway.

The reaction conditions adopted for the nucleophilic aliphatic radiofluorination of the above tosylates work also very well for the nucleophilic *hetero*aromatic radiofluorination giving 6-[^{18}F]F-PBR28 (**7**) (Scheme 3). This is not surprising since aromatic substitutions usually require relatively high temperatures. A sulphonate leaving group is unsuitable here and instead a chlorine or a bromine (**23**) was employed. Both work equally well with decay-corrected radiochemical yields of 16–18%, which is notably less than in the aliphatic substitutions above.

3.4. Purification and formulation

In a radiochemical reaction like the one depicted in Scheme 3, the labelled product and the excess of precursor differ only by their halogen atom and consequently the two may be difficult to separate. Indeed, it works out impossible to obtain product **7** chemically pure in a reversed-phase HPLC separation when starting with the chloro compound while the bromo precursor allows for a satisfactory separation although long retention times are needed. A normal phase HPLC purification might give better



Scheme 3. Nucleophilic *hetero*aromatic radiofluorinations leading to 6-[^{18}F]F-PBR28 (**7**).

results but the organic mobile phase is not compatible with the nowadays generally adopted method of formulation. This consists in dilution of the product, contained in mobile phase, with water, followed by isolation of the product on a reversed-phase cartridge, elution with ethanol and dilution with physiological saline.

Thus all four products (**4**, **7**, **10** and **13**) are purified on a reversed-phase HPLC column with a mobile phase of aqueous acetonitrile of varying pH. Before HPLC purification the reaction mixtures are subjected to a prepurification treatment to remove unreacted [^{18}F]fluoride. Indeed, free [^{18}F]fluoride can be a serious nuisance in radioHPLC. Although it may elute in the front as one should expect, it not seldom will stick to the stationary phase and bleed from the column continuously. We employ either of two different cartridge types, C18 (or C8) or neutral aluminium oxide to take out the fluoride. In the first method, used with the Zymate-XP robot, the reaction mixture is diluted with water and passed through the C18 or C8 cartridge which traps the product but not the [^{18}F]fluoride. The product is then eluted with dichloromethane, evaporated to dryness and taken up in mobile phase for HPLC injection. In the second method, employed with the TRACERLab™ FX-FN synthesis module, the reaction mixture is diluted with HPLC mobile phase and passed through the aluminium oxide cartridge (SepPak® Plus Alumina N, Waters) directly into the HPLC injector. The [^{18}F]fluoride is retained on the cartridge. Synthesis procedures that include the second prepurification method tend to give somewhat higher overall yields than those employing the first method. Finally, all our labelled products were formulated on a reversed-phase C18 cartridge using the method indicated above [19,62].

4. Conclusion

Fluorine-18 radiochemistry is the prerequisite for manufacturing distributable PET radiopharmaceuticals and provides the bridge between the cyclotron-produced raw radioisotope and a biomedical *in vivo* PET image. The various TSPO ligands discussed here serve well to illustrate in which way this radioisotope can enter into the design of fluorine-18 containing ligands starting from non-fluorine containing compounds. Automation of the radiochemistry process has become indispensable in order to assure a constant radiopharmaceutical quality and reproducible radiochemical yields as well as to fulfil the required radiation protection aspects, although it does restrict somewhat the radiochemical versatility.

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