Journal of Fluorine Chemistry 143 (2012) 231–237

Contents lists available at SciVerse ScienceDirect

Journal of Fluorine Chemistry

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

Evaluation of tetraethylammonium bicarbonate as a phase-transfer agent in the formation of [^{18}F]fluoroarenes

Christopher D. Reed, Guillaume G. Launay, Michael A. Carroll *

School of Chemistry, Bedson Building, Newcastle University, Newcastle upon Tyne NE1 7RU, UK

ARTICLE INFO

Article history:
Received 17 April 2012
Received in revised form 19 July 2012
Accepted 24 July 2012
Available online 15 August 2012

Keywords:
Radiochemistry
Microfluidics
Fluorinated aromatics
Fluorine-18
Fluorination

ABSTRACT

Tetraethylammonium bicarbonate, $Et_4N{\cdot}HCO_3$, was found to be an efficient phase-transfer agent, in a microreactor, for the production of [^{18}F]fluoroarenes from a range of precursors (diaryliodonium salt, nitroarene and [^{19}F]fluoroarene). This study has established $Et_4N{\cdot}HCO_3$ as a practical alternative to the conventional phase-transfer system – Kryptofix® 222/K_2CO_3 – as the radiolabelled products were generated in comparable radiochemical yields. The use of $Et_4N{\cdot}HCO_3$ also dramatically increased productivity by eliminating the frequent blockages of the microreactor experienced with the traditional system.

1. Introduction

Positron-emission tomography (PET) is an imaging technique for the absolute measurement, *in vivo*, of positron emitters, enabling their pharmacokinetics and biodistribution to be elucidated by non-invasive means and is rapidly becoming an integral component of numerous aspects of the drug discovery/development process [1]. The associated increase in demand for PET radiopharmaceuticals has prompted the need to overcome the complexities involved in the production of radiopharmaceuticals bearing short-lived radioisotopes, such as fluorine-18 ($t_{1/2}$ = 109.7 min) and carbon-11 ($t_{1/2}$ = 20 min). The development of efficient, automated processes is critical to the realisation of this goal.

Microfluidic technology has recently emerged as an invaluable tool in the development of PET radiochemical methods allowing the quantities of precursors/reagents/solvents to be significantly reduced facilitating timely isolation of the final radiolabelled product [2]. This methodology, coupled with the strict control of the reaction parameters, also has the potential to enhance selectivity for the desired transformation over by-product generation, improve radiochemical yields (RCYs) and thus increase the purity of the target radiopharmaceutical. In addition, the high degree of reproducibility inherent to this approach is essential for the routine production of PET imaging agents. A critical advantage of microfluidic systems, in PET radiochemistry, when compared to conventional batch platforms, is that they enable multiple reactions to be conducted from a single batch of radioisotope allowing a degree of process optimisation not typically associated with short-lived radioisotopes.

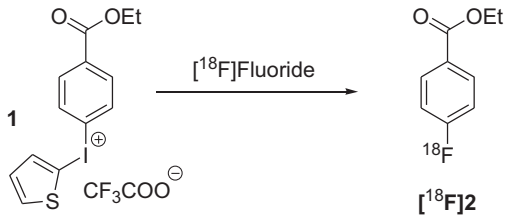
To expand the range of PET imaging agents available to our drug discovery and development programmes we are exploring the many benefits that microfluidic production could convey. The focus of the initial studies was the direct translation of conventional batch radiofluorination protocols to the microfluidic system however this resulted in frequent blockages of the microreactor [3]. In the reaction types investigated this was attributed to the use of the traditional phase-transfer system, Kryptofix® 222/K_2CO_3, and we now wish to report our evaluation of an alternative system which has eliminated the problem and dramatically improved productivity.

2. Results and discussion

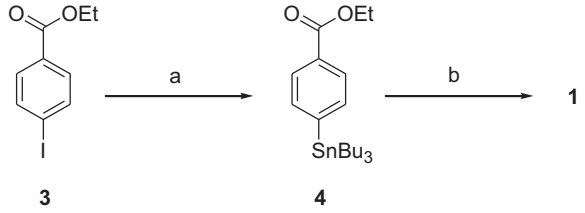
Fluorine-18 is often referred to as the 'radioisotope of choice' in PET as decay is almost exclusively by positron emission (β^+:EC, 97:3), there are no specific issues arising from the decay product – oxygen-18, the positron energy (0.635 MeV) is one of the lowest of the commonly used positron-emitters resulting in a limited positron range (≤2 mm in water), which in turn results in the highest image quality as this distance is comparable to the highest spatial resolution possible with modern PET imaging systems [4].

* Corresponding author. Tel.: +44 0191 222 7074.
E-mail address: m.a.carroll@ncl.ac.uk (M.A. Carroll).

0022-1139/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jfluchem.2012.07.015



Scheme 1. Formation of ethyl 4-[^{18}F]fluorobenzoate **[^{18}F]2** from a diaryliodonium salt.



Scheme 2. (a) $(PPh_3)_4Pd$, toluene, 110 °C, 59% and (b) TFA, diacetoxyiodo-2-thiophene **5**, DCM, −30 °C to RT, 82%.

The half-life of fluorine-18 is also sufficient to allow both the distribution of imaging agents from a central cyclotron production facility and the acquisition of scan data to take place over several hours. As a result there is substantial interest in new methods for the incorporation of fluorine-18 into diverse bioactive structures [5].

To this end, we [6] and others [7] have a particular interest in the formation of [^{18}F]fluoroarenes using diaryliodonium salt precursors, as this versatile synthetic method has a number of distinct advantages over conventional nucleophilic aromatic substitution. It places little or no restriction on the nature of the target aromatic ring or its substituents, for example, both electron-rich and electron-deficient [^{18}F]fluoroarenes may be prepared. To date, this technology also provides the only practical method for the production of [^{18}F]fluoroarene derivatives not accessible using conventional synthetic approaches [6g,7a,7b,7f,7j], is compatible with hetero-aromatic ring systems [6d] and reactive functionality [6f].

The surprising consistency in the radiofluorination conditions employed, coupled with an excellent level of substrate tolerance, suggests that multiple, yet diverse [^{18}F]fluoroarenes may be readily prepared from a single experimental set-up once the critical parameters are established. To demonstrate the translation of our batch radiofluorination protocol to the microfluidic platform (Advion NanoTek Microfluidic System [8]) we decided to utilise a simple diaryliodonium salt as a model study [6e] (Scheme 1) prior to the realisation of more complex targets.

The diaryliodonium salt needed for the model study was prepared, *via* the aryltributylstannane, as a white crystalline solid as shown in Scheme 2. We [6c,6f,6g] and others [7a,7c,7d,7f,7k] have used DMF as the solvent for the fluorination of diaryliodonium salts and therefore this was selected to perform the reactions in this study. The conventional phase-transfer system for radiofluorinations was used (Kryptofix® 222/K_2CO_3 as a solution in MeCN:H_2O; 9:1, v/v) [9] and the diaryliodonium salt prepared as a solution in DMF (5–10 mg/mL).

The initial phase of this study used the microreactor setup as described (Fig. 1) and was characterised by frequent blocked microreactors which we attributed to the concentration of the phase-transfer system employed as the diaryliodonium salt and the reaction products are all highly soluble in DMF. Reducing the concentration of the Kryptofix® 222/K_2CO_3 greatly improved the situation [10] but the level of blocked reactors remained a concern and an alternative phase-transfer system was needed.

Tetraalkylammonium salts with their enhanced solubility in organic solvents have been widely used as an alternative to the Kryptofix® 222/K_2CO_3 system with $Bu_4N{\cdot}HCO_3$ being the most common. This is usually prepared, with very limited characterisation, by treating an aqueous solution of the $Bu_4N{\cdot}OH$ with carbon dioxide [11]. Following elution of the [^{18}F]fluoride from the anion exchange resin (QMA) with the phase-transfer system the next step is usually the generation of anhydrous [^{18}F]fluoride *via* azeotropic removal of the water using multiple distillations with acetonitrile. Given the propensity for tetraalkylammonium salts, and in particular the tetrabutylammonium salts, to form clathrates [12] the generation of anhydrous [^{18}F]$Bu_4N{\cdot}F$ represents a significant challenge.

[^{18}F]Fluoroalkanes [13], 16-[^{18}F]fluorohexadecanoic acid [14] and [^{18}F]FDG [15] have all been prepared successfully using the related [^{18}F]$Et_4N{\cdot}F$. This reagent had been prepared from $Et_4N{\cdot}OH$, either by direct treatment with [^{18}F]fluoride or, *via* [^{18}F]Me_3SiF to generate an anhydrous reagent, however, given the complexity of

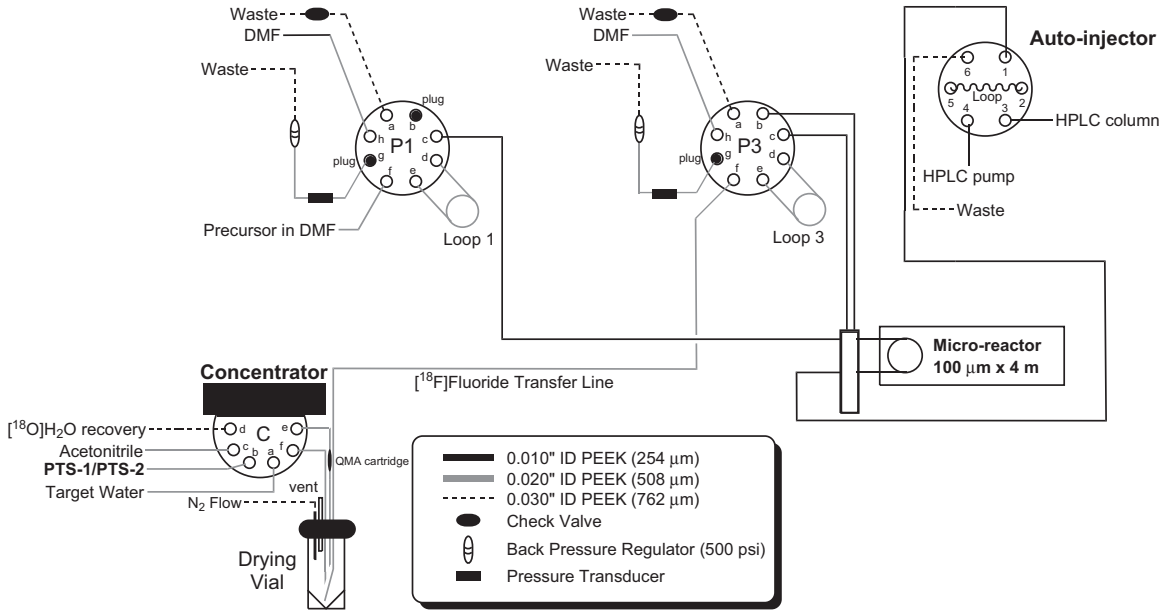


Fig. 1. Set-up of the Advion NanoTek Microfluidic System for radiofluorination.

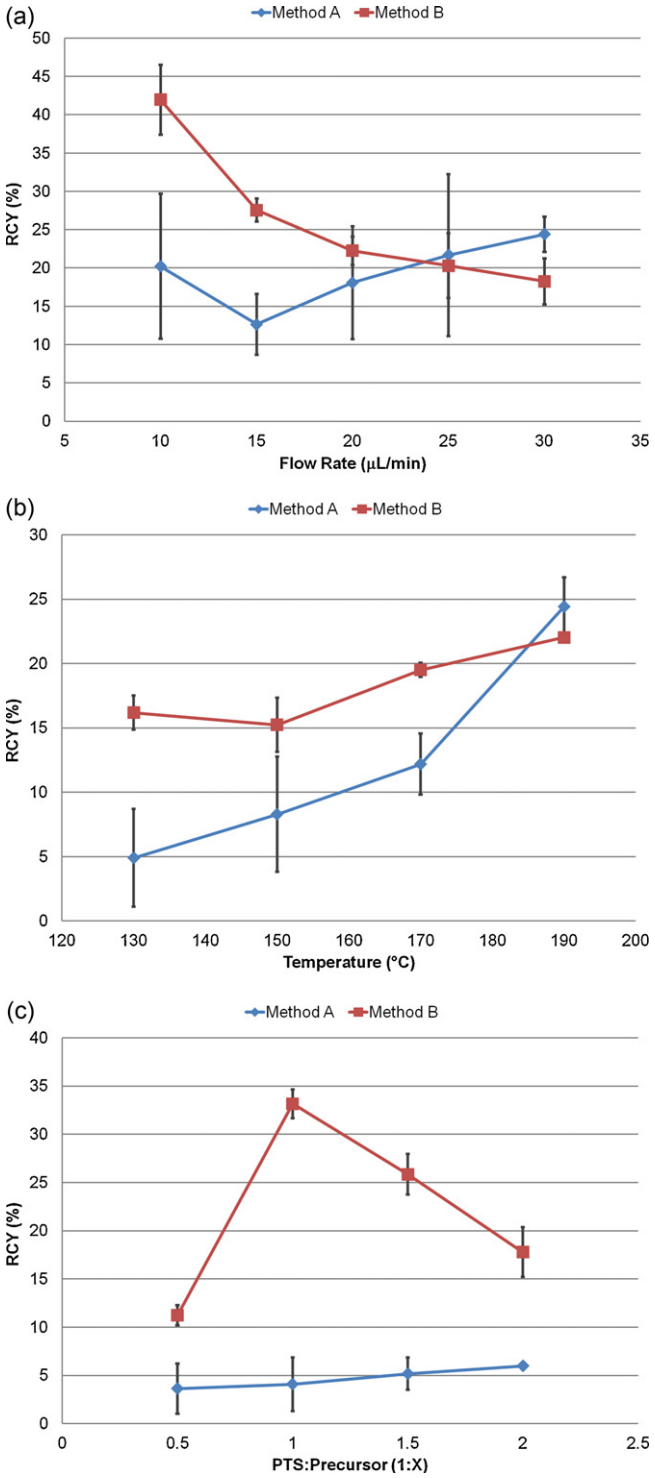


Fig. 2. (a) Method A: **PTS-1**, 190 °C, **1** (5 mg/mL), P1:P3 1:1; Method B: **PTS-2**, 190 °C, **1** (10 mg/mL), P1:P3 1:1; (b) Method A: **PTS-1**, flow-rate (30 μL/min), **1** (5 mg/mL), P1:P3 1:1; Method B: **PTS-2**, flow-rate (10 μL/min), **1** (10 mg/mL), P1:P3 1:1; (c) Method A: **PTS-1**, flow-rate (25 μL/min), 190 °C, **1** (5 mg/mL); Method B: **PTS-2**, flow-rate (10 μL/min), 190 °C, **1** (10 mg/mL). All result are $n \geq 3$.

these production methods we proposed that access to [^{18}F]$Et_4N{\cdot}F$ may be more readily achieved by the use of tetraethylammonium bicarbonate ($Et_4N{\cdot}HCO_3$) as the phase-transfer agent. Unlike the tetrabutyl-derivative $Et_4N{\cdot}HCO_3$ is readily available as a crystalline solid [16] facilitating drying and characterisation yet retaining the required solubility in anhydrous organic solvents. In addition hydration of the tetraethylammonium cation is much reduced [17] due to the shorter alkyl chains making anhydrous [^{18}F]$Et_4N{\cdot}F$ a more realistic proposition. Given these favourable characteristics of $Et_4N{\cdot}HCO_3$ over $Bu_4N{\cdot}HCO_3$ we decided to evaluate this material as a phase-transfer agent in the microfluidic radiofluorinations.

The first assessment was to determine how efficient $Et_4N{\cdot}HCO_3$ was at eluting the [^{18}F]fluoride from the anion exchange resin (QMA) after it had been adsorbed from the cyclotron produced [^{18}O]H_2O. The amount of activity (5–100 mCi) and volume of [^{18}O]H_2O (1–5 mL) differed between batches, however, these broad variations had no observed effect in elution efficiency with $Et_4N{\cdot}HCO_3$ able to elute > 99% (n = 10) of the radioactivity from the QMA cartridge which is consistent with the performance of the Kryptofix® 222/K_2CO_3 system [18].

With the efficient formation of [^{18}F]$Et_4N{\cdot}F$ established the next step was to compare the Kryptofix® 222/K_2CO_3 system (**PTS-1**) with the $Et_4N{\cdot}HCO_3$ system (**PTS-2**) in the formation of **[^{18}F]2** (Scheme 1).

For this study the precursor solution (5–20 μL) and the phase-transfer system (**PTS-1** or **PTS-2**, 10 μL) were infused into the microreactor at the same time. It was noted that the concentration of the diaryliodonium salt used (5–10 mg/mL) had little effect on the outcome of the reaction [19] however the other parameters; flow-rate, temperature and stoichiometry (P1:P3); did influence the result obtained. It should also be noted that the actual radiochemical yield obtained was found to vary somewhat between different batches of [^{18}F]fluoride, however the trend observed remained the same (e.g. for Method B: increasing RCY with temperature, decreased RCY with increased flow rate) [20].

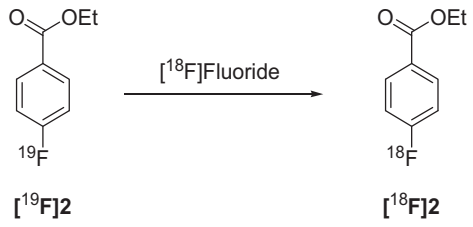
We found that longer residence times were beneficial (Fig. 2a) when using **PTS-2** however this effect was not as pronounced when **PTS-1** was employed. Using optimum flow-rates both phase-transfer systems displayed improved performance with increasing temperature (Fig. 2b) and a stoichiometry of 1:1 was clearly found to be superior for **PTS-2** whereas **PTS-1** was much more tolerant in this regard.

These promising results demonstrated that $Et_4N{\cdot}HCO_3$ system (**PTS-2**) was a practical alternative to the Kryptofix® 222/K_2CO_3 system (**PTS-1**) for this type of transformation and it was encouraging that blocked microreactors no longer occurred.

To demonstrate the general utility of **PTS-2** for radiofluorination we also reviewed the ^{19}F/^{18}F isotopic exchange reaction [21] which may provide rapid access to preliminary PET imaging studies as the fluorine-19 derivative is often available directly from drug discovery programmes [22] (Scheme 3).

Interestingly, for the isotope exchange reaction the effect of flow-rate (Fig. 3a) was found to be opposite to that observed when using the diaryliodonium salt precursor (Fig. 2a) with the longer residence times benefiting **PTS-1**. However increasing the microreactor temperature had a similar effect with the best radiochemical yields being obtained at 190 °C (Fig. 3b). For **PTS-1** the effect of the P1:P3 ratio was similar for both reaction types (Figs. 2c and 3c) however the preference for the 1:1 stoichiometry for **PTS-2** was less pronounced (Fig. 3c).

Conventional nucleophilic aromatic substitution is a very common approach to the formation of electron-deficient



Scheme 3. Formation of ethyl 4-[^{18}F]fluorobenzoate **[^{18}F]2** from **[^{19}F]2**.

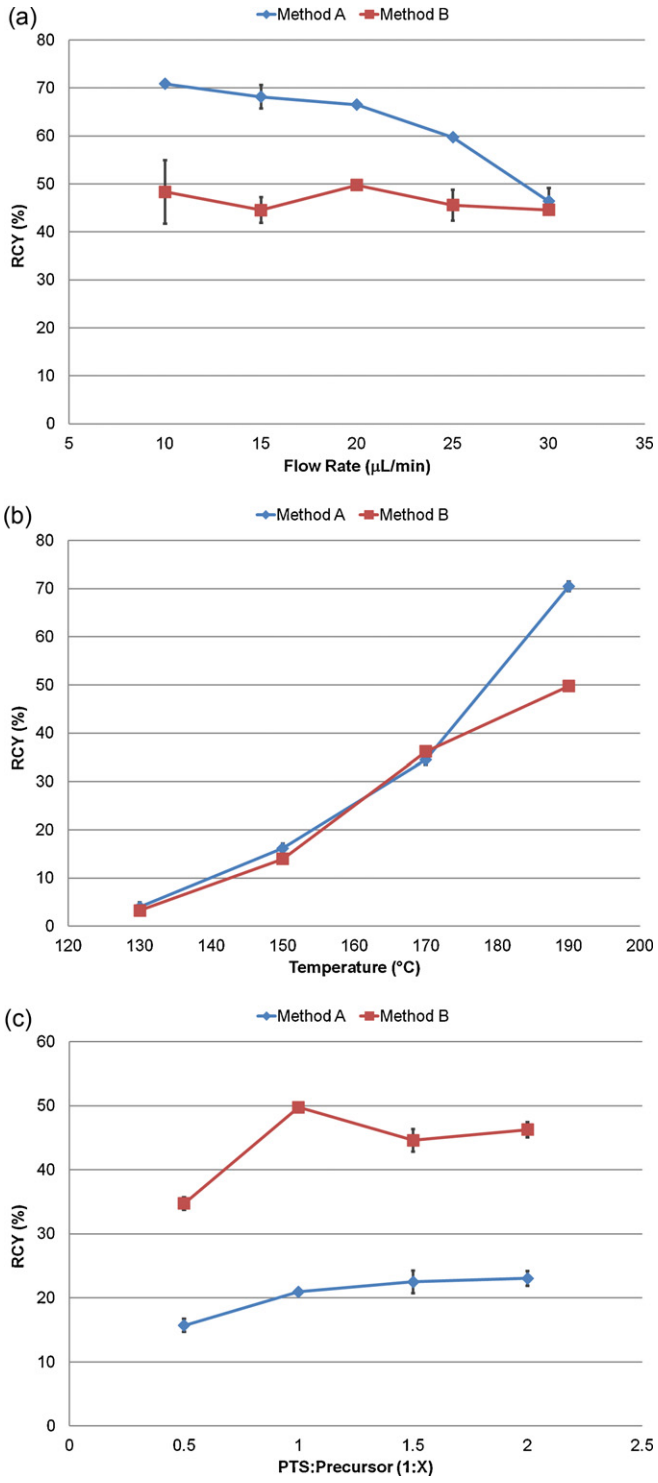


Fig. 3. (a) Method A: **PTS-1**, 190 °C, **[^{19}F]2** (10 mg/mL), P1:P3 1:1; Method B: **PTS-2**, 190 °C, **[^{19}F]2** (10 mg/mL), P1:P3 1:1; (b) Method A: **PTS-1**, flow-rate (10 μL/min), **[^{19}F]2** (5 mg/mL), P1:P3 1:1; Method B: **PTS-2**, flow-rate (20 μL/min), **[^{19}F]2** (10 mg/mL), P1:P3 1:1; (c) Method A: **PTS-1**, flow-rate (10 μL/min), 190 °C, **[^{19}F]2** (5 mg/mL); Method B: **PTS-2**, flow-rate (20 μL/min), 190 °C, **[^{19}F]2** (10 mg/mL). All results are $n \geq 3$.

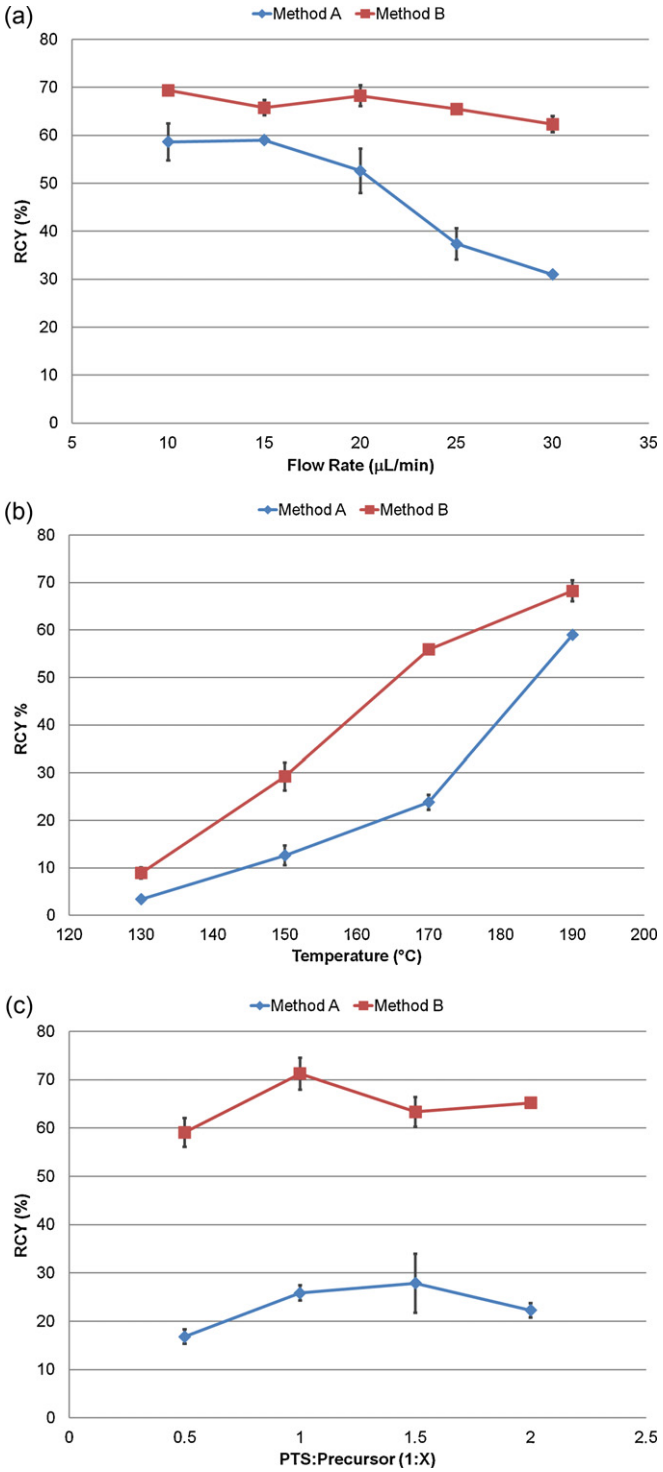
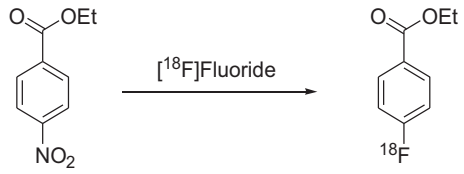


Fig. 4. (a) Method A: **PTS-1**, 190 °C, **6** (10 mg/mL), P1:P3 1:1; Method B: **PTS-2**, 190 °C, **6** (10 mg/mL), P1:P3 1:1; (b) Method A: **PTS-1**, flow-rate (15 μL/min), **6** (5 mg/mL), P1:P3 1:1; Method B: **PTS-2**, flow-rate (20 μL/min), **6** (10 mg/mL), P1:P3 1:1; (c) Method A: **PTS-1**, flow-rate (10 μL/min), 190 °C, **6** (5 mg/mL); Method B: **PTS-2**, flow-rate (20 μL/min), 190 °C, **6** (10 mg/mL). All results are $n \geq 3$.

[^{18}F]fluoroarenes. It is employed extensively where an electron-withdrawing group exists in the 4-position relative to the site for the introduction of fluorine-18, for example in the production of [^{18}F]altanserin [23], [^{18}F]MPPF [24], and [^{18}F]setoperone [25]. As a result we also decided to evaluate **PTS-2** for this widespread method of fluorine-18 introduction (see Scheme 4).

Again, it was noticed that longer residence times gave improved radiochemical yields but the effect was more pronounced when **PTS-1** was employed (Fig. 4a). Conducting the reaction at higher temperatures resulted in excellent incorporation of the fluorine-18 for both phase-transfer systems (Fig. 4b). The optimum stoichiometry peaked at 1:1.5 when using **PTS-1** and 1:1 when using **PTS-**



6 **[^{18}F]2**

Scheme 4. Formation of ethyl 4-[^{18}F]fluorobenzoate **[^{18}F]2** from the relevant nitroarene.

2 (Fig. 4c) with the difference in the absolute radiochemical yields being attributed to inter-batch variation of the starting [^{18}F]fluoride due to the similarities observed during the evaluation of the other reaction parameters [20].

3. Conclusion

In summary we have demonstrated, that $Et_4N{\cdot}HCO_3$ is a viable alternative to the traditional phase transfer system, Kryptofix® 222/K_2CO_3, for the production of [^{18}F]fluoroarenes using a key range of synthetic methods. Of particular benefit, in a microreactor, was the performance of $Et_4N{\cdot}HCO_3$ where the occurrence of blocked reactors experienced with the conventional phase-transfer system was eliminated thus dramatically increasing productivity. In addition the Advion NanoTek Microfluidic System provided an efficient, versatile and convenient methodology for the rapid optimisation of radiofluorination reactions.

4. Experimental

Reactions requiring anhydrous conditions were performed using oven-dried glassware and conducted under a positive pressure of nitrogen. Ethyl 4-nitrobenzoate (98%) and ethyl 4-fluorobenzoate (99%) were purchased from Alfa Aesar (UK). Potassium carbonate (99%), Kryptofix® 222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane; 98%), and tetraethylammonium bicarbonate (≥95%) were purchased from Sigma–Aldrich (UK) and used as received. For radiofluorinations, acetonitrile (>99%) and dimethylformamide (>99%) were purchased from Fisher Chemicals (UK) and pre-dried on molecular sieves (24 h on 4 Å MS then stored on 3 Å MS) before being used, other anhydrous solvents were prepared in accordance with standard protocols. Infrared spectra were recorded on a Varian 800 FT-IR Scimitar Series spectrometer with internal calibration. ^{1}H, ^{13}C, ^{19}F, ^{119}Sn NMR spectra were recorded on a Jeol ECS 400 MHz spectrometer with residual protic solvent as an internal reference for the ^{1}H and ^{13}C spectra, $CFCl_3$ and Me_4Sn as external references for the ^{19}F and ^{119}Sn spectra respectively. Elemental analyses were carried out at London Metropolitan University and are reported as the average of two runs. Mass spectra and accurate masses were recorded at the EPSRC Mass Spectrometry Service, Swansea. It should be noted that hypervalent iodine compounds are known to exist as mixtures (incl. dimers/trimers) and these are evident in the MS data, only the data for the monomer is reported. Melting points were recorded on a Gallenkamp MF-370 melting point apparatus and are uncorrected. *Caution*: hypervalent iodine compounds are potentially explosive and should be handled taking appropriate precautions [26].

4.1. Ethyl 4-(tri-butylstannyl)benzoate (*4*) [27]

$Pd(PPh_3)_4$ (120 mg, 0.01 mmol) was added to a solution of ethyl 4-iodobenzoate (4.14 g, 15 mmol) and bis(tributyltin) (17.41 g, 30 mmol) in anhydrous toluene (100 mL). The solution was heated at reflux for 24 h under nitrogen. The solvent was then removed *in vacuo* providing the crude product which was purified by column chromatography (SiO_2, hexane:diethyl ether 40:1) to yield the product as a colourless oil (3.94 g, 8.97 mmol, 59%). R_f = 0.87 (petrol:EtOAc, 9:1); Anal. Calcd. for $C_{21}H_{36}O_2Sn$: C, 57.43; H, 8.26. Found: C, 57.57; H, 8.35; IR (neat) ν 2925, 1719, 1273; δ_H (400 MHz; $CDCl_3$) 7.95 (2H, d, H2/H6, *J* 7 Hz), 7.54 (2H, d, H3/H5, *J* 7 Hz), 4.36 (2H, q, OCH_2, *J* 7 Hz), 1.58–1.44 (6H, m, $SnCH_2CH_2$), 1.38 (3H, t, OCH_2CH_3, *J* 7 Hz), 1.31 (6H, m, CH_2CH_3), 1.17–0.97 (6H, m, $SnCH_2$), 0.87 (9H, CH_2CH_3, t, *J* 7 Hz); δ_C (101 MHz; $CDCl_3$) 166.9 (CO), 149.3 (C4), 136.4 (C2/C6), 130.1 (C1), 128.5 (C3/C5), 60.7 (OCH_2), 29.1 ($SnCH_2CH_2CH_2CH_3$), 27.4 ($SnCH_2CH_2CH_2CH_3$), 14.4 (OCH_2CH_3), 13.7 ($SnCH_2CH_2CH_2CH_3$), 9.7 ($SnCH_2CH_2CH_2CH_3$) δ_{Sn} (149 MHz; $CDCl_3$) −38.5; *m*/*z* (ESI) 463 (M^++Na, 13%), 301 (93) (found: M^++Na, 463.1630. $C_{21}H_{36}O_2{}^{116}Sn$ requires 463.1633).

4.2. Diacetoxyiodo-2-thiophene (*5*)

Sodium perborate tetrahydrate (33.85 g, 220 mmol) was added to a stirred solution of 2-iodothiophene (2.21 mL, 20 mmol) in glacial acetic acid (45 mL) in small portions over 30 min. The mixture was then heated to 50 °C, stirred for 5 h and then allowed to cool to RT. The mixture was extracted with DCM (3 × 100 mL), the organic fractions were combined, washed with water (3 × 100 mL) and the organic layer concentrated *in vacuo* to give the crude product (2.8 g, 8.53 mmol, 42%); R_f = 0.17 (DCM:MeOH, 96:4); m.p. = 118–121 °C (decomp) (from DCM–ether–petrol) (lit. [28] 120–122 °C); Anal. Calcd. for $C_8H_9IO_4S$: C, 29.28; H, 2.7. Found C, 29.18; H, 2.62; IR (neat) ν 3080, 1634, 1267; δ_H (400 MHz; $CDCl_3$) 7.77 (1H, dd, H5, *J* 4, 1 Hz), 7.63 (1H, dd, H4, *J* 5, 1 Hz,), 7.12 (1H, dd, H3, *J* 5, 4 Hz), 2.01 (6H, s, $COCH_3$); δ_C (101 MHz; $CDCl_3$) 177.1 (CO), 139.4 (C3), 134.9 (C5), 128.7 (C4), 106.3 (C2), 20.4 ($(OCOMe)_2$); *m*/*z* (ESI) 269 (M^+, 100%), 227 (27), 210 (98) (found: M^+, 268.9130. $C_6H_6IO_2S$ requires 268.9128).

4.3. 4-Ethoxycarbonylphenyl(thiophen-2-yl)iodonium trifluoroacetate (*1*)

Trifluoroacetic acid (0.74 mL, 10 mmol) was added dropwise, at −30 °C and under N_2, to a solution of **5** (1.96 g, 6 mmol) in DCM (30 mL). After 30 min of stirring at −30 °C, the solution was warmed to RT and stirred for 1 h when the solution was re-cooled to −30 °C and **4** (1.72 mL, 5 mmol) was added. The resulting mixture was allowed to warm to RT overnight and the solvent removed *in vacuo* to give the crude product. Crystallisation gave **1** as a white crystalline solid (1.47 g, 4.1 mmol, 82%); R_f = 0.31 (DCM:MeOH, 93:7); m.p. 136–138 °C (decomp.) (from DCM–ether–petrol); Anal. Calcd. for $C_{15}H_{12}F_3IO_4S$: C, 38.15; H, 2.56. Found C, 38.21; H,2.61; IR (neat) ν 3087, 1704, 1651; δ_H (400 MHz, d_6-DMSO): 8.32 (2H, d, *J* 9 Hz), 8.05 (1H, dd, *J* 4, 1 Hz), 7.97 (2H, d, *J* 9 Hz), 7.94 (1H, dd, *J* 5, 1 Hz), 7.14 (1H, dd, *J* 5, 4 Hz), 4.28 (2H, q, *J* 7 Hz), 1.26 (3H, t, *J* 7 Hz); δ_C (101 MHz, d_6-DMSO) 165.1 (C=O), 141.2 (C5′), 138.0 (C4′), 135.4 (C3/C5), 133.2 (C1), 132.3 (C2/C6), 130.2 (C3′), 124.7 (C4), 101.9 (C2′), 62.0 (OCH_2), 14.56 (Me); δ_F (376 MHz, d_6-DMSO) −73.4 ($COCF_3$). *m*/*z* (ESI) 358 (M^+, 100%) (found: M^+, 358.9588. $C_{13}H_{12}IO_2S$ requires 358.9597).

4.4. Radiochemistry

4.4.1. Operation of the Advion NanoTek Microfluidic System

The radiosynthetic work, including the preparation and drying of the [^{18}F]fluoride was performed on the Advion NanoTek Microfluidic System [8]. This is a modular-based microfluidic system designed for the synthesis of PET imaging agents. The system consists of a base module (BM), concentrator module (CM), and reactor module (RM), all are operated and controlled by the NanoTek 1.4 software. The BM consists of two reagent cartridges (P1 and P2) used to dispense metered amounts of reactants to the

microreactors for radiolabelling reactions. These cartridges comprise of a high-pressure syringe pump connected to an eight-way bridged valve supporting a looped-reservoir from which reagents are dispensed towards the microreactor. The RM consists of the isotope reagent (P3) and microreactor cartridges (upto four), as well as an eight-way distribution valve (DV) used to direct the reaction bolus to the user-desired location. P3 receives the solution of [^{18}F]fluoride (**PTS-1** or **PTS-2**) from the CM module where it is prepared from a volume of cyclotron produced [^{18}F]fluoride in [^{18}O]H_2O. The CM module consists of a low-pressure, six-way valve reagent cartridge (CM1) and vessel chamber capable of rapid heating and evaporation of solvent. It is possible to subject the CM vessel to a combination of reduced pressure and a positive flow of nitrogen gas allowing the [^{18}F]fluoride to be dried *via* several azeotropic distillations with MeCN. The apparatus was setup as described in Fig. 1.

Throughout this work, the crude reaction mixture was swept into an electronic injection valve (Smartline Valve Drive: Knauer, Germany) for direct injection onto the radio-HPLC system. The enclosed fluid paths of this tandem synthetic and analytical system ensured that the results obtained were representative of the crude reaction mixture as potential volatile products could not be lost [29]. Operation of this valve and the initialisation of the HPLC analysis was also controlled by the NanoTek software. This software may be operated in several modes; in 'Discovery Mode' user-directed macros automate all procedures involved in the synthetic process, thereby allowing simple and rapid throughput of multiple reactions. These procedures can also be divided into their constituent parts and controlled separately within 'Manual Mode' which, therefore, allows for finer control of the system. In 'Sequence Mode' detailed macros can be written, manipulated and combined to enable extensive and complex processes to be performed with minimal user intervention. Throughout this study, user-control fluctuated between the three modes, for optimal system management.

4.4.2. Preparation of [^{18}F]KF/Kryptofix® 222 or [^{18}F]$Et_4N{\cdot}F$

For reactions conducted using the Advion NanoTek Microfluidic System [8] no-carrier-added [^{18}F]fluoride (5–100 mCi) in [^{18}O]H_2O (1–5 mL) [30] was adsorbed onto a pre-conditioned anion exchange resin (QMA: Waters Sep-Pak® Light Accell Plus) before being released using a solution of either K_2CO_3 (1.18 mg; 8 μmol) and Kryptofix® 222 (6.75 mg; 18 μmol) in MeCN/H_2O (9:1 (v/v) 450 μL) (**PTS-1**); or $Et_4N{\cdot}HCO_3$ (3.4 mg; 17 μmol) in MeCN/H_2O (9:1 (v/v) 450 μL) (**PTS-2**) and into a V-vial (2 mL, Wheaton). The solutions were dried by two successive azeotropic evaporations using MeCN (450 μL) at 100 °C and under a positive flow of N_2. The [^{18}F]KF/Kryptofix® 222 or [^{18}F]$Et_4N{\cdot}F$ was then dissolved in DMF (450 μL) and loaded into the storage loop (P3: 401 μL) on the microfluidic system.

*4.4.3. Radiofluorination of **1**, **[^{19}F]2** and **6***

Dry [^{18}F]fluoride (5–100 mCi, 450 μL DMF) and precursor solutions (**1**, **[^{19}F]2** or **6** in DMF at 10 mg/mL) were loaded into their respective storage loops (loop 3 and loop 1 respectively). Capillaries that lead from P1 and P3 to the microreactor were primed with their respective reagents. Prior to recording data several priming reaction runs were performed to confirm the integrity of the synthetic platform and the in-line analytical HPLC system. A set volume of either **PTS-1** or **PTS-2** (10 μL) and a variable volume of the precursor (5–20 μL: stoichiometry assessment) were dispensed into the pre-heated microreactor (130–190 °C) [31] at pre-determined flow rates (10–30 μL/min). This is achieved by the syringes of P1 and P3, simultaneously dispensing precursor and [^{18}F]fluoride solutions (using either **PTS-1** or **PTS-2**) from their respective loops, into the microreactor using a fixed volume of system solvent at a preset flow rate. The solutions initially mix at the entrance to the microreactor. User-operated computer software determines the reaction parameters – temperature, time and stoichiometry (P1:P3). Following completion of the reaction, P3 sweeps the crude mixture to the DV where it is directed towards the electronic injector and radio-HPLC system allowing the radiochemical yield of the process to be determined. At the start and end of each series of experiments the BM, RM, microreactor and associated transfer lines were cleaned using DMF and the CM system with acetonitrile.

4.4.4. Radioanalytical HPLC methods

HPLC methods were carried out using an Agilent 1200 HPLC system equipped with a UV absorbance detector (λ_{max} 254 nm) and a radioactivity detector (LabLogic Flow Count). The [^{18}F]fluoroarenes were not isolated and the radiochemical yield (RCY) reported relates to the amount of radioactivity of the product relative to the total radioactivity detected by radio-HPLC on analysis of the reaction mixture.

*4.4.4.1. Ethyl 4-[^{18}F]fluorobenzoate **[^{18}F]2** from **1**.* The radioactive product was separated on a PolymerX 5 μm RP-1 100 column (Phenomenex, USA) eluting at 1.1 mL/min with EtOH–water. Mobile phase composition started at 25% EtOH which increased linearly to 100% at EtOH over 7 min which continued at this composition for a further 2 min. **[^{18}F]2** t_R = 7 min. Other possible components: thiophene t_R = 6.5 min (not observed), ethylbenzoate t_R = 7.5 min (not observed), 2-iodothiophene t_R = 8.5 min, ethyl 4-iodobenzoate **3** t_R = 9 min.

*4.4.4.2. Ethyl 4-[^{18}F]fluorobenzoate **[^{18}F]2** from **[^{19}F]2**.* The radioactive product was detected on a PolymerX 5 μm RP-1 100 column (Phenomenex, USA) eluting at 1.1 mL/min with EtOH–water. Mobile phase composition started at 60% EtOH which increased linearly to 80% at EtOH over 6 min. **[^{18}F]2** t_R = 4.5 min.

*4.4.4.3. Ethyl 4-[^{18}F]fluorobenzoate **[^{18}F]2** from **6**.* The radioactive product was separated on a PolymerX 5 μm RP-1 100 column (Phenomenex, USA) eluting at 1.1 mL/min with EtOH–water. Mobile phase composition started at 40% EtOH which increased linearly to 60% at EtOH over 2 min and then to 80% at 5 min which continued at this composition for a further 7 min. **[^{18}F]2** t_R = 6.2 min. Other possible components: ethyl 4-nitrobenzoate **6** t_R = 6.6 min.

Acknowledgements

This research was supported in part (MAC, CDR) by a Cancer Research UK/EPSRC/MRC/NIHR Cancer Imaging Programme (Ref. C29821/A10348), the EPSRC (GGL, Ref. EP/H029753/1) and Newcastle University. We also thank the EPSRC for provision of the Swansea Mass Spectrometry Service. We would also like to thank Advion Biosciences and LabLogic UK for their continuing support and Erigal Ltd. for their supply of fluorine-18.

References

[1] (a) J.S. Fowler, A.P. Wolf, Accounts of Chemical Research 30 (1997) 181–188;
(b) M.E. Phelps, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 9226–9233;
(c) S.M. Ametamey, M. Honer, P.A. Schubiger, Chemical Reviews 108 (2008) 1501–1516;
(d) Z. Li, P.S. Conti, Advanced Drug Delivery Reviews 62 (2010) 1031–1051;
(e) S.L. Pimlott, A. Sutherland, Chemical Society Reviews 40 (2011) 149–162.
[2] For examples see:
(a) S. Lu, V. Pike, in: P.A. Schubiger, L. Lehmann, M. Friebe (Eds.), PET Chemistry, The Driving Force in Molecular Imaging, Springer, Berlin, 2007, pp. 271–287;
(b) G. Pascali, G. Mazzone, G. Saccomanni, C. Manera, P.A. Salvadori, Nuclear Medicine and Biology 37 (2010) 547–555;

(c) R. Bejot, A.M. Elizarov, E. Ball, J. Zhang, R. Miraghaie, H.C. Kolb, V. Gouverneur, Journal of Labelled Compounds and Radiopharmaceuticals 54 (2011) 117–122;
(d) P.W. Miller, Journal of Chemical Technology and Biotechnology 84 (2009) 309–315;
(e) H. Audrain, Angewandte Chemie International Edition 46 (2007) 1772–1775.
[3] For examples of blockages in microreactors see:
(a) C. Amador, A. Gavriilidis, P. Angeli, Chemical Engineering Journal 101 (2004) 379–390;
(b) Y. Tanaka, O. Tonomura, K. Isozaki, S. Hasebe, Chemical Engineering Journal 167 (2011) 483–489.
[4] S.E. Snyder, M.R. Kilbourn, in: M.J. Welch, C.S. Redvanly (Eds.), Handbook of Radiopharmaceuticals: Radiochemistry and Applications, John Wiley & Sons Ltd., Chichester, UK, 2003, pp. 195–227.
[5] For examples see:
(a) L. Cai, S. Lu, V.W. Pike, European Journal of Organic Chemistry (2008) 2853–2873;
(b) P.W. Miller, N.J. Long, R. Vilar, A.D. Gee, Angewandte Chemie International Edition 47 (2008) 8998–9033;
(c) L. Hoigebazar, J.M. Jeong, J.-Y. Lee, D. Shetty, B.Y. Yang, Y.-S. Lee, D.S. Lee, J.-K. Chung, M.C. Lee, Journal of Medicinal Chemistry 55 (2012) 3155–3162;
(c) E. Lee, A.S. Kamlet, D.C. Powers, C.N. Neumann, G.B. Boursalian, T. Furuya, D.C. Choi, J.M. Hooker, T. Ritter, Science 334 (2011) 639–642;
(d) H. Teare, E.G. Robins, A. Kirjavainen, S. Forsback, G. Sandford, O. Solin, S.K. Luthra, V. Gouverneur, Angewandte Chemie International Edition 49 (2010) 6821–6824;
(e) B. Wängler, G. Quandt, L. Iovkova, E. Schirrmacher, C. Wängler, G. Boening, M. Hacker, M. Schmoeckel, K. Jurkschat, P. Bartenstein, R. Schirrmacher, Bioconjugate Chemistry 20 (2009) 317–321.
[6] (a) S. Martín-Santamaría, M.A. Carroll, C.M. Carroll, C.D. Carter, V.W. Pike, H.S. Rzepa, D.A. Widdowson, Chemical Communications (2000) 649–650;
(b) M.A. Carroll, L.M. Kamara, D.A. Widdowson, V.W. Pike, Journal of Labelled Compounds and Radiopharmaceuticals 48 (2005) 519–520;
(c) M.A. Carroll, J. Nairne, G. Smith, D.A. Widdowson, Journal of Fluorine Chemistry 128 (2007) 127–132;
(d) M.A. Carroll, J. Nairne, J.L. Woodcraft, Journal of Labelled Compounds and Radiopharmaceuticals 50 (2007) 452–454;
(e) R. Yan, M.A. Carroll, Journal of Labelled Compounds and Radiopharmaceuticals 51 (2008) 259–260;
(f) M. Carroll, R. Yan, F. Aigbirhio, D. Soloviev, L. Brichard, Journal of Nuclear Medicine 49 (2008) 298P;
(g) M. Carroll, R. Yan, F. Aigbirhio, D. Soloviev, L. Brichard, Journal of Nuclear Medicine 49 (2008) 303P.
[7] For selected examples see:
(a) T.L. Ross, J. Ermert, C. Hocke, H.H. Coenen, Journal of the American Chemical Society 129 (2007) 8018–8025;
(b) M.-R. Zhang, K. Kumata, K. Suzuki, Tetrahedron Letters 48 (2007) 8632–8635;
(c) J.-H. Chun, S. Lu, Y.-S. Lee, V.W. Pike, Journal of Organic Chemistry 75 (2010) 3332–3338;
(d) S. Telu, J.-H. Chun, F.G. Siméon, S. Lu, V.W. Pike, Organic & Biomolecular Chemistry 9 (2011) 6629–6638;
(e) F. Basuli, H. Wu, G.L. Griffiths, Journal of Labelled Compounds and Radiopharmaceuticals 54 (2011) 224–228;
(f) T.L. Ross, J. Ermert, H.H. Coenen, Molecules 16 (2011) 7621–7626;
(g) B. Wang, R.L. Cerny, S. Uppaluri, J.J. Kempinger, S.G. DiMagno, Journal of Fluorine Chemistry 131 (2010) 1113–1121;
(h) J.-H. Chun, V.W. Pike, Journal of Labelled Compounds and Radiopharmaceuticals 54 (2011) S482;
(i) D. Zimmerman, T.L. Ross, Journal of Labelled Compounds and Radiopharmaceuticals 54 (2011) S488;
(j) K.D. Neumann, A. Vavere, S. Snyder, S.G. DiMagno, Journal of Labelled Compounds and Radiopharmaceuticals 54 (2011) S492;
(k) G. Pascali, S. Pitzianti, G. Saccomani, S. Del Carlo, C. Manera, M. Macchia, P.A. Salvadori, Journal of Labelled Compounds and Radiopharmaceuticals 54 (2011) S502.
[8] Advion Biosciences Ltd., Ithaca, New York. http://www.advion.com/biosystems/nanotek/nanotek-positron-emission-tomography.php.
[9] Advion NanoTek LF 07-08 User Manual; Appendix A. [^{18}F]Fluoride ion preparation; Kryptofix® 222 (30 mg/mL) and K_2CO_3 (5.5 mg/mL) in MeCN/H_2O (9:1, v/v).
[10] During the course of this study the radiofluorination of diaryliodonium salts has been reported using the Advion NanoTek microfluidic system using Kryptofix® 222/K_2CO_3 at concentrations similar to **PTS-1**. See references [7c,7h,7k].
[11] P.A. Culbert, M.J. Adam, E.T. Hurtado, J.M.A. Huser, S. Jivan, J. Lu, T.J. Ruth, S.K. Zeisler, Applied Radiation and Isotopes 46 (1995) 887–891.
[12] (a) G.A. Jeffrey, R.K. McMullan, in: F.A. Cotton (Ed.), Progress in Inorganic Chemistry, vol. 8, John Wiley & Sons, Inc., Hoboken, NJ, USA, 1967, pp. 43–108;
(b) L.S. Aladko, Y.A. Dyadin, T.V. Rodionova, I.S. Terekhova, Journal of Molecular Liquids 106 (2003) 229–238.
[13] S.J. Gatley, R.D. Hichwa, W.J. Shaughnessy, R.J. Nickles, The International Journal of Applied Radiation and Isotopes 32 (1981) 211–214.
[14] A.L. Bosch, T.R. Degrado, S.J. Gatley, International Journal of Radiation Applications and Instrumentation – Part A: Applied Radiation and Isotopes 37 (1986) 305–308.
[15] L.G. Hutchins, A.L. Bosch, M.S. Rosenthal, R.J. Nickles, S.J. Gatley, The International Journal of Applied Radiation and Isotopes 36 (1985) 375–378.
[16] Tetraethylammonium bicarbonate is commercially available: Sigma-Aldrich, cat. No. 11268 (CAS: 17351-61-0). http://www.sigmaaldrich.com.
[17] (a) T.C.W. Mak, Journal of Inclusion Phenomena 3 (1985) 347–354;
(b) T.C.W. Mak, H.J. Bruins Slot, P.T. Beurskens, Journal of Inclusion Phenomena 4 (1986) 295–302.
[18] The efficiency of the elution of radioactivity from the QMA cartridge took into account potential losses in the apparatus/process. The initial level of adsorption of the [^{18}F]fluoride from the cyclotron produced [^{18}O]H_2O onto the QMA cartridge was determined by analysis of the recovered [^{18}O]H_2O and the radioactivity remaining in the original supply vial. The result was also corrected in light of any residual radioactivity on the QMA cartridge.
[19] Precursor concentrations of 5–10 mg/mL are suitable for a range of diaryliodonium precursors, we have observed precipitation of some precursors at concentration $\geq$30 mg/mL.
[20] The variation between batches of [^{18}F]fluoride has been noted by other researchers, for example see: G. Pascali, L. D'Antonio, P. Bovone, P. Gerundini, T. August, Nuclear Medicine and Biology, 36 (2009) 569–574.
[21] E. Blom, F. Karimi, B. Långström, Journal of Labelled Compounds and Radiopharmaceuticals 52 (2009) 504–511.
[22] For examples of fluorine in medicinal chemistry see:
(a) I. Ojima, Fluorine in Medicinal Chemistry and Chemical Biology, John Wiley & Sons Ltd., Chichester, UK, 2009;
(b) S. Purser, P.R. Moore, S. Swallow, V. Gouverneur, Chemical Society Reviews 37 (2008) 320–330;
(c) D. O'Hagan, Journal of Fluorine Chemistry 131 (2010) 1071–1081;
(d) W.K. Hagmann, Journal of Medicinal Chemistry 51 (2008) 4359–4369.
[23] C. Lemaire, R. Cantineau, M. Guillaume, A. Plenevaux, L. Christiaens, Journal of Nuclear Medicine 32 (1991) 2266–2272.
[24] D. Le Bars, C. Lemaire, N. Ginovart, A. Plenevaux, J. Aerts, C. Brihaye, W. Hassoun, V. Leviel, P. Mekhsian, D. Weissmann, J.F. Pujol, A. Luxen, D. Comar, Nuclear Medicine and Biology 25 (1998) 343–350.
[25] J. Blin, S. Pappata, M. Kiyosawa, C. Crouzel, J.C. Baron, European Journal of Pharmacology 147 (1988) 73–82.
[26] (a) D.B. Dess, J.C. Martin, Journal of the American Chemical Society 113 (1991) 7277–7287;
(b) H. Tohma, S. Takizawa, T. Maegawa, Y. Kita, Angewandte Chemie International Edition 39 (2000) 1306–1308.
[27] P. Tang, T. Furuya, T. Ritter, Journal of the American Chemical Society 132 (2010) 12150–21254.
[28] H. Togo, T. Nabana, K. Yamaguchi, Journal of Organic Chemistry 65 (2000) 8391–8394.
[29] For example fluorobenzene and 2-fluorothiophene have boiling points lower than the reaction temperature and may therefore be lost from an open system, see: M.A. Carroll, C. Jones, S.-L. Tang, Journal of Labelled Compounds and Radiopharmaceuticals, 50 (2007) 450–451.
[30] [^{18}F]Fluoride in [^{18}O]H_2O was supplied by Erigal Limited. http://www.erigal.co.uk.
[31] This temperature range was selected as it was found that limited reaction was evident at the lower temperatures and the maximum operating temperature of the Advion NanoTek Microfluidic System was 200 °C.