Development of a general automated flow photoredox ^{18}F-Difluoromethylation of *N*-heteroaromatics in an AllinOne synthesizer

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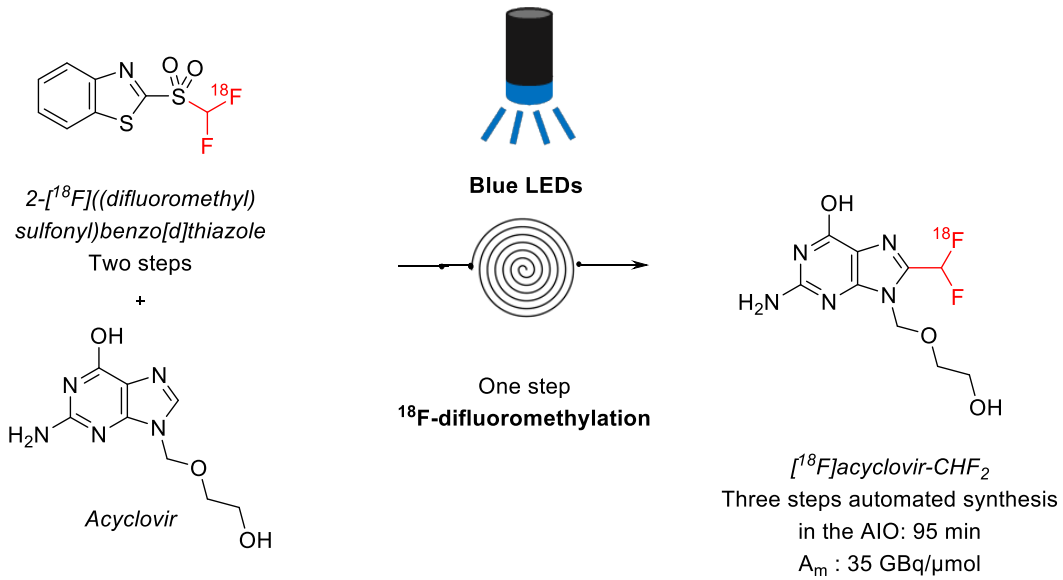
GRAPHICAL ABSTRACT



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ABSTRACT

We recently reported a new method for the ^{18}F-difluoromethylation of *N*-heteroaromatics for PET imaging. The method involves the synthesis of a new ^{18}F-difluoromethylating reagent 2-[^{18}F]((difluoromethyl)sulfonyl)benzo[*d*]thiazole and a flow photoredox ^{18}F-difluoromethylation. For preclinical development and human Positron Emission Tomography (PET) studies with new radiotracers, an automation of the process is mandatory, mostly to avoid radioprotection issues, due to the use of high amounts of radioactivity and to ensure a better reliability of the production. We hereby describe the automation of this ^{18}F-difluoromethylation method, on a model substrate, Acyclovir, on a commercially available AllinOne (AIO) synthesizer from Trasis. The whole process is completed in 95 minutes and provides radiolabeled Acyclovir with a molar activity of 35 GBq/μmol. This automated protocol can be implemented for the ^{18}F-difluoromethylation of a wide range of *N*-heteroaromatics compounds typically found in medicinal chemistry.

KEYWORDS

Automation, C-H activation, Difluoromethylation, Fluorine-18, Photoredox

INTRODUCTION

Positron Emission Tomography (PET)[1-3] is a non-invasive molecular imaging modality and an important tool for clinical diagnosis and for evaluation of the pharmacodynamic profile of a drug candidate during preclinical and clinical development[4-7]. The technique requires the labeling of molecules of interest with a positron emitting radionuclide. Of the PET nuclides, fluorine-18 (^{18}F) is often considered as the preferred radioisotope, owing to its half-life ($t_{1/2}$ = 109.8 min), favorable positron emission profile (97%), and its relatively low positron energy (E = 0.635 KeV), affording high resolution images[8,9].

The incorporation of a single fluorine atom and/or fluorine-containing substituents into a lead molecule can often contribute to significantly enhance its physical, chemical, Drug Metabolism and Pharmacokinetics (DMPK) and biological properties (e.g. cell membrane permeability, lipophilicity, metabolic stability, etc.)[10]. Among the various fluorinated motifs, difluoromethyl (CHF_2) functionalities have been exploited in medicinal chemistry as a lipophilic bioisoster of conventional hydrogen-bond donor groups such as hydroxy (-OH) and thiol (-SH) functional groups[11]. Additionally, the introduction of difluoromethylated group on some heteroaromatics has been shown to prevent the metabolism (*N*-oxidation) from aldehyde oxidases[12]. Despite the current advances in the synthesis of difluoromethylated (hetero)arenes[13-15], the labeling of (hetero)aryl-CHF_2 derivatives with fluorine-18 is still underdeveloped. Pioneering methods implemented in the radiosynthesis of [^{18}F]aryl-CHF_2 relied on silver-mediated ^{18}F-fluorination processes using either electrophilic fluorinating reagents, such as [^{18}F]Selectfluor bis(triflate)[15], (Figure 1a) or cyclotron-produced [^{18}F]fluoride[17] (Figure 1b). In 2016, Ritter developed an alternative approach for the construction of [^{18}F]aryl-CHF_2 functionalities starting from aryl (pseudo)halides, *via* activation of a benzoyl auxiliary, followed by benzylic bromination and *in situ* halogen-exchange with [^{18}F]fluoride[18] (Figure 1c). Later, Liang and co-workers disclosed the preparation of [^{18}F]aryl-CHF_2 with improved molar

activity *via* a two-step method consisting of metal-free nucleophilic ^{18}F-fluorination of benzyl (pseudo)halides and subsequent oxidative benzylic C-H fluorination with Selectfluor[19] (Figure 1d). Recently, Gouverneur reported the utilization of aryl boronic acids as substrates to afford [^{18}F]aryl-CHF_2 through a copper-mediated cross-coupling with ethyl bromofluoroacetate and a manganese-mediated ^{18}F-fluorodecarboxylation with [^{18}F]tetraethylammonium fluoride ([^{18}F]TEAF and iodosobenzene (PhIO))[20] (Figure 1e). The described methods have been focused on direct ^{18}F-fluorination of suitable aryl precursors and all of them require the preparation of prefunctionalized arenes, that can be time-consuming, especially for the labeling of complex drug-like molecules bearing other functional groups.

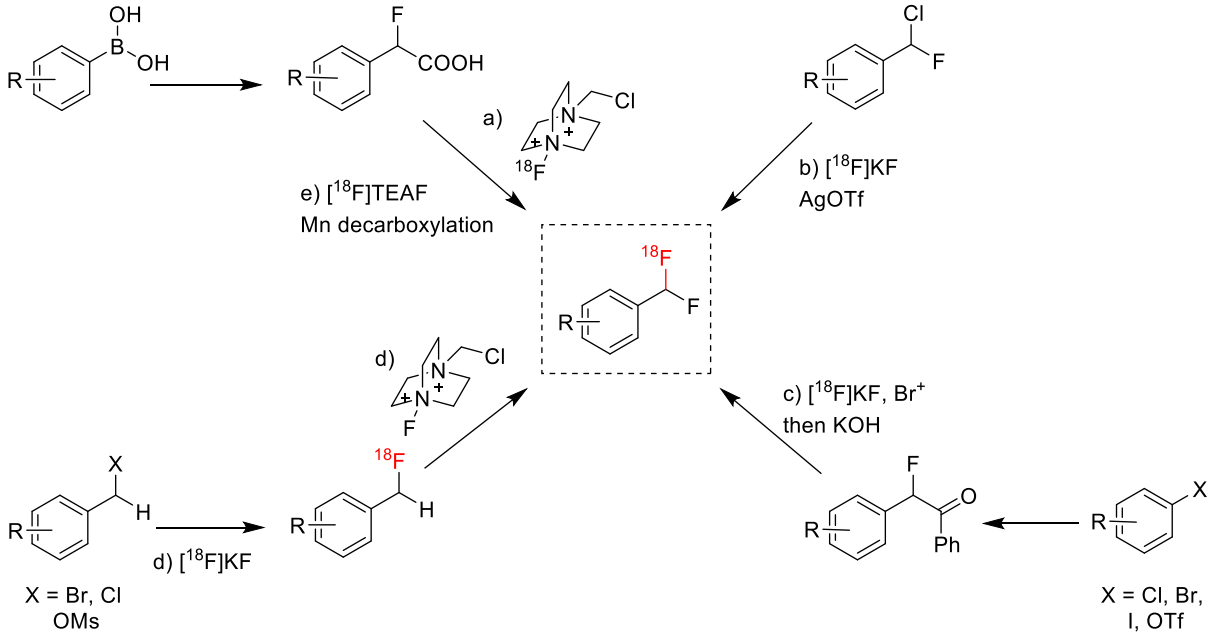

Figure 1: Methods for the synthesis of ^{18}F-difluoromethylated aryl derivatives

a) ^{18}F-Fluorodecarboxylation of 2-fluoro-2-arylacetic acids with [^{18}F]Selectfluor bis(triflate)[16] b) Halogen exchange of aryl-CHFCl precursors with [^{18}F]KF[17] c) Benzylic bromination of aryl acetophenones followed by a nucleophilic ^{18}F-fluorination with [^{18}F]KF[18] d) Nucleophilic ^{18}F-fluorination of benzyl (pseudo)halides, followed by an oxidative benzylic C-H fluorination with Selectfluor[19] e) Manganese-mediated ^{18}F-fluorodecarboxylation of 2-fluoro-2-arylacetic acids[20]

To address these limitations, we recently reported an efficient and innovative synthetic route for the late stage ^{18}F-difluoromethylation of a broad range of *N*-heteroarenes of biological and

medicinal relevance, using the 2-[^{18}F]((difluoromethyl)sulfonyl)benzo[*d*]thiazole[21] reagent **[^{18}F]1** (Figure 2).

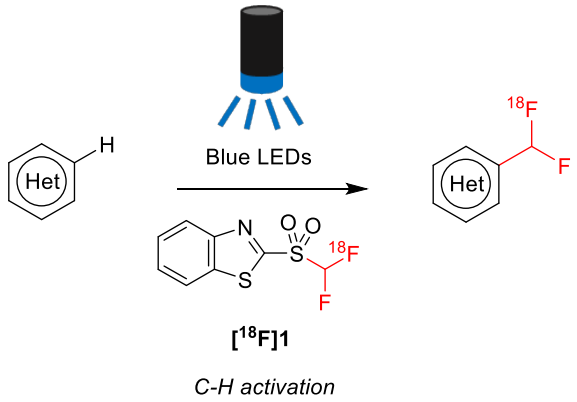

Figure 2: ^{18}F-Difluoromethylation of *N*-heteroarenes via C-H activation [21]

Based on our recently disclosed ^{18}F-difluoromethylation method, we intended to develop a fully automated ^{18}F-difluoromethylation procedure potentially suitable for clinical development by coupling the synthesis of a [^{18}F]CHF_2-bearing reagent with the ^{18}F-difluoromethylation of the antiherpetic drug Acyclovir **2**, as a model substrate, under visible-light photoredox conditions. Once developed, the same automated procedure could be applied to other *N*-heteroaromatics and drug-like compounds as well. Acyclovir (brand name: ZOVIRAX®) is an antiviral medication used in the treatment of herpes simplex virus, types 1 (HSV1) and 2 (HSV2), and varicella zoster virus (VZV) infections[22]. From a structural viewpoint, this drug is a purine nucleoside analog with an acyclic side chain linked to a guanine nucleotide by a glycosidic linkage bond (Figure 3). Nucleosides and nucleic bases are known to be generally challenging compounds to label and of particular interest for the pharmaceutical field.

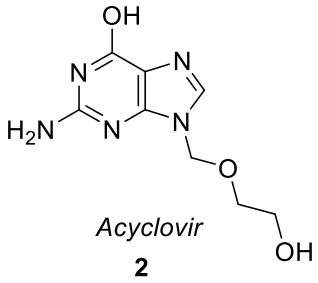

Figure 3: Chemical structure of Acyclovir **2**

A fully automated ^{18}F-difluoromethylation procedure for the labeling of the model acyclovir on an AllinOne™ (AIO) synthesizer from Trasis is reported in this paper. This automated protocol will enable the use of multiple GBq of starting radioactivity avoiding potential radioprotection issues resulting from the radiation exposure.

RESULTS AND DISCUSSION

As described in Figure 4, the automation includes two main parts: the two-step synthesis of **[^{18}F]1** and the photoredox ^{18}F-difluoromethylation of **2**, each part requiring a HPLC purification. In our previous study, the ^{18}F-sulfone synthesis was fully automated on a commercial FASTlab (FL) module from GE Healthcare. Owing to the limited number of free positions on the FL cassette (25 valves)[23] to introduce the additional components required for the flow ^{18}F-difluoromethylation of **2** and the absence of an integrated purification system in the FL module, the AIO module from Trasis[24] (36 valves, and an integrated HPLC system) was selected for the automation of the multi-step procedure.

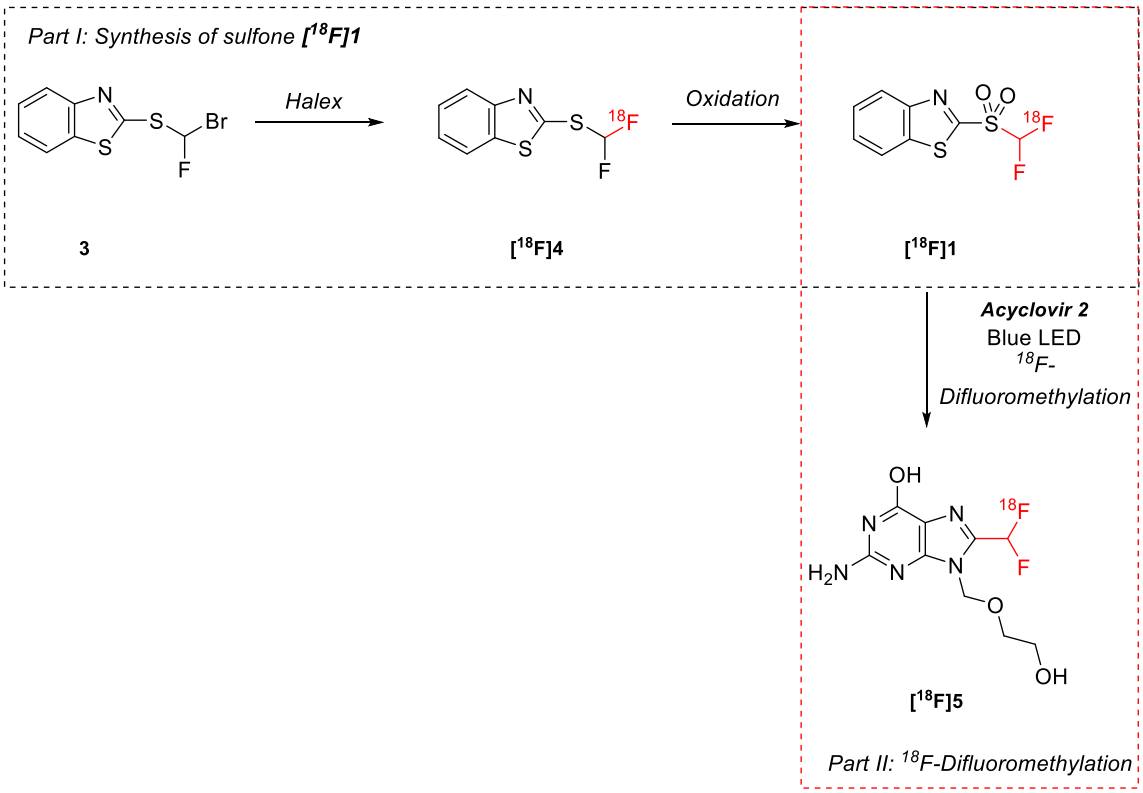

Figure 4: Three-step synthesis of CHF^{18}F-acyclovir **[^{18}F]5**

Part I: Two-step radiosynthesis of [^{18}F]1

We initiated our study with the transposition of the radiosynthesis of the sulfone **[^{18}F]1** from the FL[21] to the AIO module.

The sulfone **[^{18}F]1** was prepared through a nucleophilic ^{18}F-fluorination of the brominated precursor **3** *via* bromine exchange with [^{18}F]fluoride, and subsequent oxidation of the intermediate **[^{18}F]4**. In brief, the synthesis was run as follows. The [^{18}F]fluoride in enriched water ([^{18}O]H_2O) was transferred from the cyclotron to the inlet reservoir and trapped on a Quaternary Methyl Ammonium (QMA) cartridge. The QMA was subsequently eluted with a solution of K_2CO_3/K_{222} in water/MeCN into a glass reactor where the [^{18}F]fluoride solution was azeotropically evaporated for approximatively 10 minutes. A solution of the precursor **3** (40 μmol) in MeCN (1 mL) was added to the glass reactor and the reaction heated at 85°C. After 5 minutes, the reactor was cooled down to room temperature and a solution of sodium periodate

($NaIO_4$) and ruthenium(III) chloride hydrate ($RuCl_3$,x H_2O) in water (1 mL) was added. The oxidation of **[^{18}F]4** was carried out for 5 minutes at room temperature. Afterwards, the reaction mixture was diluted with water (5 mL) and transferred to the HPLC loop. The HPLC purification was performed on a C18 semipreparative column, using a solution of MeCN/H_2O/HCO_2H (55/45/0.1) as mobile phase and the desired purified product **[^{18}F]1** was collected after 10 minutes in a sealed vial prefilled with 30 mL of water. The solution containing the product **[^{18}F]1** was passed through a Sep-Pak ^{t}C18 short cartridge to trap the **[^{18}F]1** sulfone. After cartridge drying for 2.5 minutes, **[^{18}F]1** was readily eluted with anhydrous DMSO for the next step.

During the design of the automated procedure, a particular attention was paid to minimize the contamination between the two parts of the synthesis, as the oxidative solution is detrimental to the ^{18}F-difluoromethylation. This prompted us to modify the oxidation step, from a solid phase in the FASTlab to a liquid phase in the AIO. Since no switch of solvent or prepurification is possible between the two steps, the oxidation of the intermediate **[^{18}F]4**, in the presence of $NaIO_4$ and $RuCl_3{\cdot}xH_2O$, should be performed in the presence of the base and solvent previously used in the labeling step.

To investigate the reaction conditions for the two-step radiosynthesis of the sulfone **[^{18}F]1** in the glass reactor, low activity labeling experiments were initially carried out and the results are reported in the following table (Table 1). Unless otherwise stated, all RCYs were determined on the crude product, after a ^{t}C18 prepurification and were decay corrected (dc) to the Start of the Synthesis (SOS). All experiments were triplicated to ensure a full reproducibility of the results.

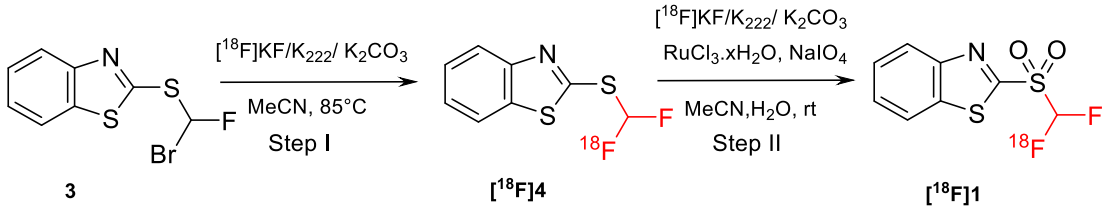

Table 1: Optimization of the conditions for the radiosynthesis of [^{18}F]1

Entry	Deviation from standard conditions[1]	^{18}F-labeling RCY, [^{18}F]4 (%) Step I	^{18}F-labeling + Oxidation RCY [^{18}F]1 (%) Step I +II
1	Standard conditions[1]	13.9 ± 0.8	11.9 ± 1.4
2	120°C	12.2 ± 0.8	-
3	FASTlab conditions[2]	13.4 ± 1.7	10.7 ± 1.3
4	TEAB	-	5.0 ± 1.0
5	$NaHCO_3$	-	< 0.5
6	DCE	-	3.6 ± 1.0
7	DMSO	-	< 0.5
8	HPLC purification[1] (Starting radioactivity: 370 MBq)	-	10 ± 1.6 (n=10)
9	HPLC purification[1] (Starting radioactivity: 165 GBq)	-	7.5 ± 1.7 (n=6)

[1] Standard conditions: Labeling: precursor **3** (40 μmol), K_2CO_3 (20 μmol), K_{222} (20 μmol), MeCN (1 mL), 85°C, 5 min; Oxidation: 5 min, $NaIO_4$ (240 μmol), $RuCl_3{\cdot}xH_2O$ (8 μmol), H_2O (1 mL), rt, RCY on the crude product.

[2] FASTlab conditions: Labeling: precursor **3** (40 μmol), K_2CO_3 (10 μmol), $K_{2.2.2}$ (20 μmol), MeCN (1 mL), 85°C, 5 min; Oxidation in solid phase: 5 min, $NaIO_4$ (240 μmol), $RuCl_3{\cdot}xH_2O$ (8 μmol), H_2O (1 mL), rt

DCE: 1,2-dichloroethane, TEAB: tetraethylammonium bromide

The highest RCY for the labeling step was obtained using K_2CO_3 at 85°C in acetonitrile (entry 1, Table 1). Gratifyingly, the transposition of the radiochemical process from the FASTlab to the AIO module affords the sulfone **[^{18}F]1** in similar RCYs (entries 2 and 3). Moreover, no significant variation in the RCY of the oxidation of **[^{18}F]4** were observed when the reaction was carried out in the AIO glass reactor (86% vs. 80% RCY in FASTlab). These results suggest that the presence of MeCN, K_2CO_3, and Kryptofix® 222 (K_{222}) has no meaningful impact on the oxidation of **[^{18}F]4**. The substitution of K_2CO_3 by tetraethylammonium bicarbonate (TEAB) or sodium bicarbonate ($NaHCO_3$) provided the sulfone **[^{18}F]1** in significant lower RCY (entry 4 and 5). The solvent used in the ^{18}F-labeling and in the oxidation steps, in combination with H_2O, also influenced the RCY of the sulfone **[^{18}F]1**. Changing MeCN with other solvents, such as 1,2-dichloroethane (DCE, entry 6) and dimethylsulfoxide (DMSO, entry 7), led to a dramatic reduction of the overall RCY (to 3.6 and <0.5 % RCY). Starting with low or high level of activity (370 MBq or 165 GBq), the two-step procedure led to 10 ± 1.6 % and 7.5 ± 1.7 % RCY respectively. Moreover, the molar activity of the sulfone **[^{18}F]1** was determined of 59.2 ± 4 GBq/μmol (1.6 ± 0.1 Ci/μmol). These results demonstrated that the radiosynthesis of **[^{18}F]1** can readily be adapted from the FASTlab to the AllinOne module. Noteworthy, the fully automated radiosynthesis of sulfone **[^{18}F]1** was performed in 55 minutes, from the [^{18}F]fluoride recovered from the cyclotron, to the reformulation of the sulfone **[^{18}F]1** on a SepPak ^{t}C18 short cartridge after semi-preparative HPLC purification.

The following step, was to implement the subsequent ^{18}F-difluoromethylation flow photoredox reaction in the AIO as well.

Part II: Flow photoredox ^{18}F-difluoromethylation of 2

In our previous publication[21], a flow photoredox reaction was set up for late-stage ^{18}F-difluoromethylation of a wide range of *N*-heteroarenes of biological and medicinal relevance.

The reaction was run using **2** (20 μmol), the photocatalyst ($Ir(ppy)_3$, 0.1 μmol), and **[^{18}F]1** in DMSO (250 μL). The solution was then injected through a 100 μL microchip and irradiated via a 2W Blue LED (470 nm), using the FutureChemistry flow system[25] (EVO start). However, all reactions were conducted manually. Knowing that this procedure would not be compatible with synthesis starting with high amount of activity, an automated procedure was therefore required, for the application of the method to the production of novel PET radiotracers.

The automation of the flow photoredox process on the AIO was the most challenging part of this work. To the best of our knowledge, this type of chemistry has not yet been automated for fluorine-18. For this purpose, a 3D spiral printed reactor with PFA tubing (0.04 inch) and 1.2 mL internal volume, in order to be consistent with the volumes involved during the synthesis (See Material and Methods) was designed to perfectly adjust to a Kessil blue LED (455 nm, 32 W, see Fig. 5).

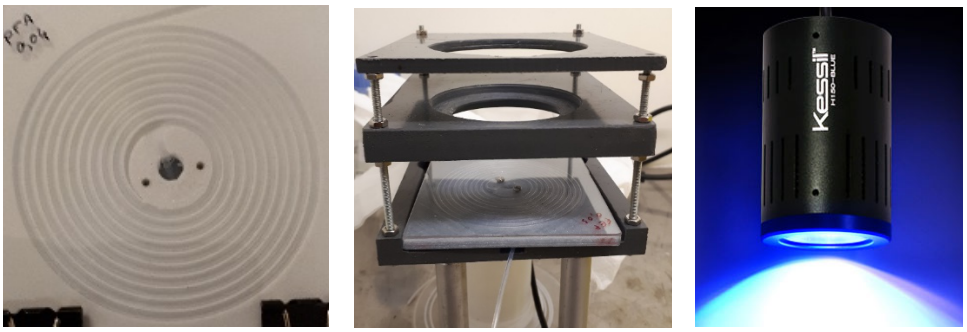

Figure 5: From left to right, white colored flow photo reactor, support, Kessil Blue LED

The photoredox reaction was first tested outside the AIO, with Acyclovir **2** as a model substrate, to test the efficiency of this new designed reactor with limited external parameters. The results are disclosed in the following table (Table 2).

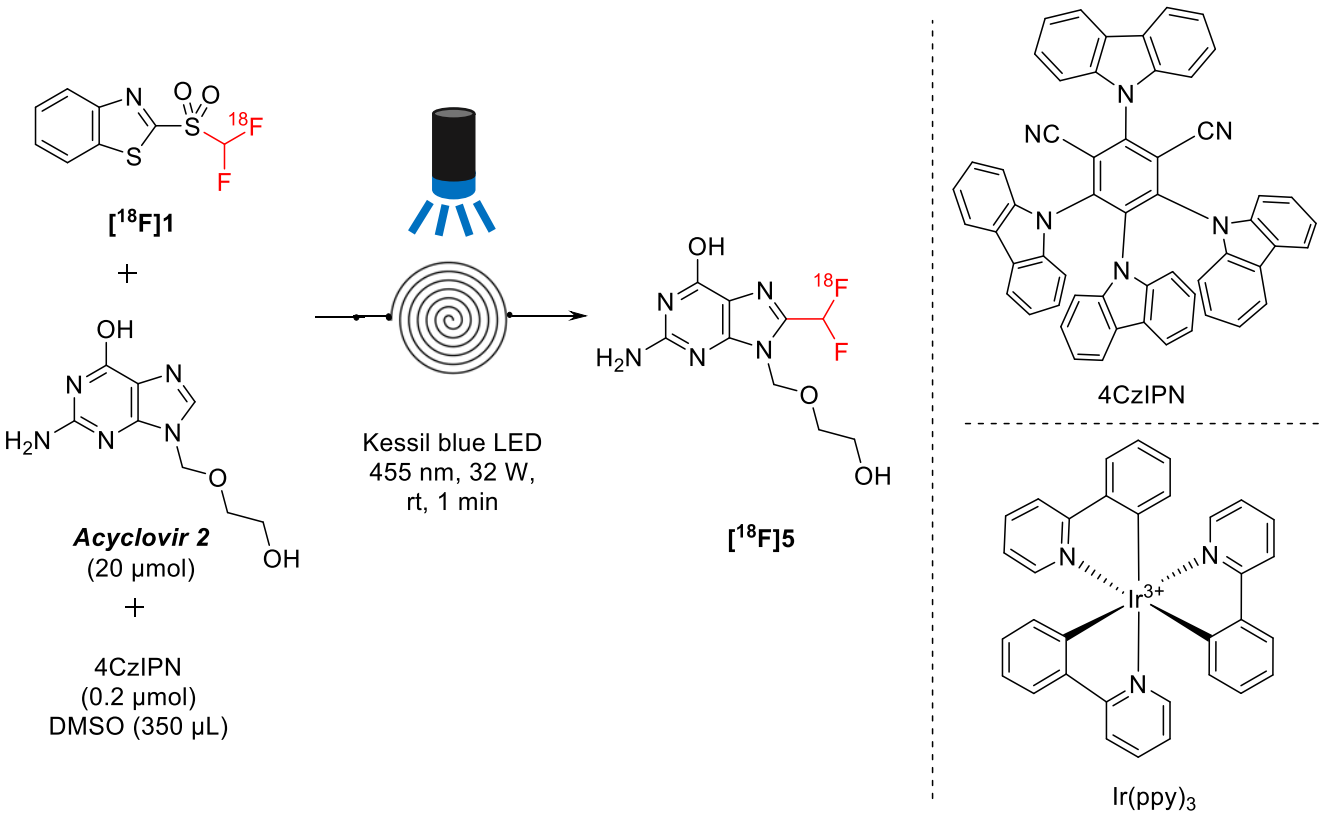

Figure 6: ^{18}F-difluoromethylation of Acyclovir

We initially explored the ^{18}F-difluoromethylation of Acyclovir **2** under the optimum conditions described in our previous study[20] (75 ± 5 % RCY, entry 2, Table 2). Surprisingly, [^{18}F]Acyclovir-CHF_2 (**[^{18}F]5**) was obtained with a lower RCY (50 ± 5 % RCY, entry 3) than with the Futurechemistry flow equipment. This could be explained by the difference of temperature induced by the Kessil lamp (45°C vs 35°C monitored in the FutureChemistry device), leading to more degradation of the ^{18}F-sulfone and thus a lower RCY. Indeed, temperature has already be demonstrated as a key parameter for this reaction, in our previous publication[21]. Taking into account these limitations, alternative parameters were studied in order to optimize the ^{18}F-difluoromethylation of **2**.

Table 2: Optimization of the conditions for ^{18}F-difluoromethylation of **2**

Entry	Deviation from standard conditions[1]	RCY(%) (of the crude product)
1	Standard conditions[1]	80 ± 2
2	Futurechemistry conditions[2]	75 ± 5
3	$Ir(ppy)_3$	50 ± 5
4	10 s	85 ± 1
5	AIO flow reaction[3]	78 ± 5
6	After HPLC purification[3]	25 ± 5
7	Full three-step synthesis[3]	1.4 ± 0.1

[1] Standard conditions: Acyclovir **2** (20 μmol), **[^{18}F]1** (30-40 MBq), 4CzIPN (0.02 μmol), 1.2 mL reactor, blue LEDs (Kessil, 32 W), 1 min residence time, n = 3.[2] Futurechemistry conditions[20]: Acyclovir **2** (20 μmol), **[^{18}F]1** (30-40 MBq), $Ir(ppy)_3$ (0.01 μmol), 100 μL microchip, blue LEDs (Futurechemistry, 2 W), 2 min, 35°C, n = 3 see more details in the publication[20] [3]AIO automated system: Acyclovir **2** (20 μmol), **[^{18}F]1** (30-40 MBq), 4CzIPN (0.02 μmol), 1.2 mL reactor, blue LEDs (Kessil, 32 W), flow, 2 min, n = 3.

The replacement of $Ir(ppy)_3$ by the organic photocatalyst 4CzIPN was beneficial since [^{18}F]Acyclovir-CHF_2 **[^{18}F]5** was obtained with a 80% RCY (entry 1). The use of the organic photocatalyst 4CzIPN appeared even slightly more effective in the radiosynthesis of [^{18}F]Acyclovir-CHF_2 (**[^{18}F]5**) than the previously reported $Ir(ppy)_3$, and has the advantage to be organic, less expensive, easier to remove and less toxic than the $Ir(ppy)_3$ catalyst. With this catalyst, the reaction time could even be lowered to 10 s without affecting the RCY (entry 4). However, due to the low impact in fluorine-decay between 10 seconds and 1 minute, as well as the presence of residual water in the manifold of the AIO after the synthesis and purification of **[^{18}F]1**, a residence time of 1 minute will be maintained, to ensure full conversion of **[^{18}F]1**.

This process was then tested on the AIO, under the optimized conditions, 1 minute residence time (entry 4, Table 2) and provided **[^{18}F]5** in a 78% RCY (entry 5). Finally, a HPLC purification, using a C18 semipreparative column was developed. Unfortunately, the injection of approximatively 1.2 mL of DMSO, the low solubility of **[18F]5** in the purification eluents (MeOH/H_2O), and its high polarity resulted in only 25% RCY, after on-line HPLC purification (entry 6). For potential preclinical PET studies, the HPLC solvents were removed on a HLB cartridge and **[^{18}F]5** was recovered with a small volume of ethanol, further diluted with saline prior injection. The full automation of this second part of the synthesis including the flow photoredox ^{18}F-difluoromethylation, the HPLC purification and the final formulation of **[^{18}F]5** was successfully completed in 40 minutes.

Three-step synthesis automation

Finally, with all these conditions in hand, the three-step synthesis of **[^{18}F]5** was carried out in a row (entry 7, Table 2, see the layout of the process in Figure 7). As the photochemistry part (^{18}F-difluoromethylation reaction and dilution prior HPLC injection) is extremely fast (max 10 minutes), and as at least 30 minutes are required for the washing and re-conditioning of the HPLC column, a second one was implemented in the AIO for the final ^{18}F-difluoromethylated compound purification. After preliminary development with low amount of activity, this fully automated process was conducted with high batch of [^{18}F]fluoride (160 GBq / 4 Ci) to afford **[^{18}F]5** with 1.4 ± 0.1 % RCY (dc, entry 7, Table 2).

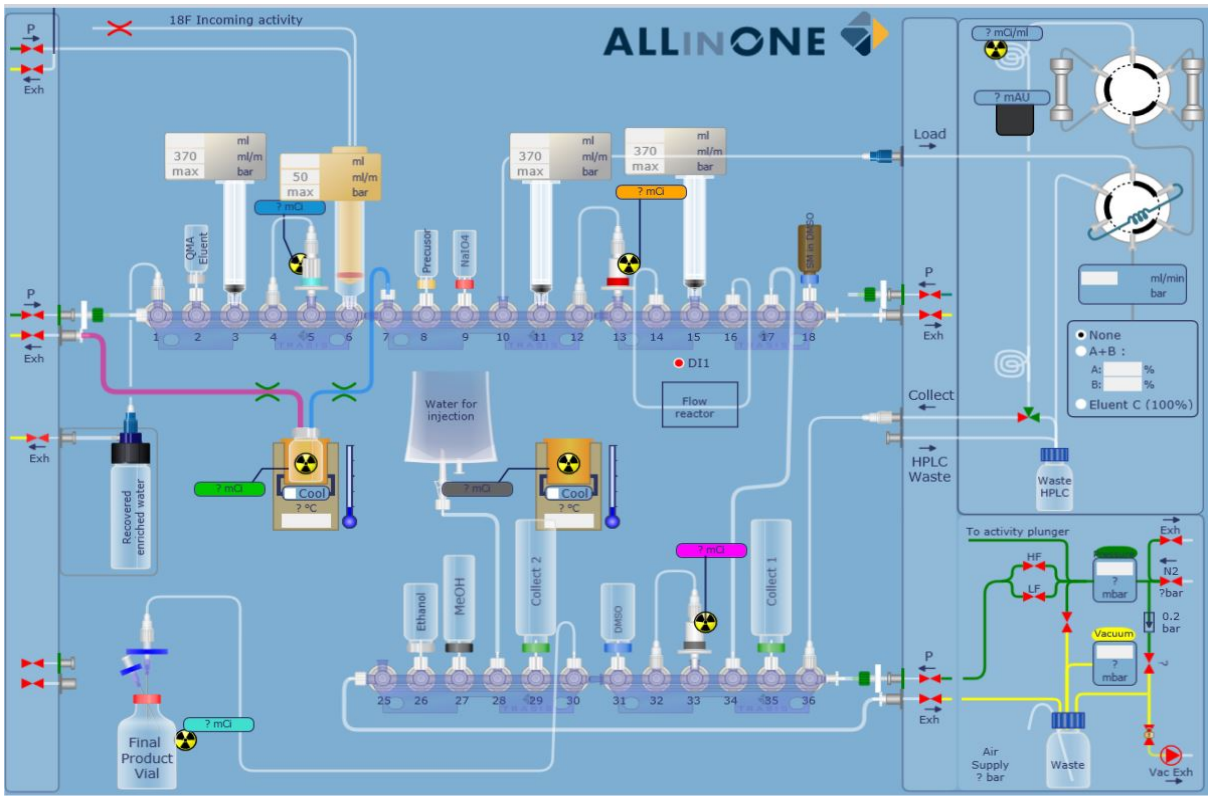

Figure 7: Three-step CHF^{18}F-acyclovir synthesis AIO layout

Gratifyingly, the molar activity of **[^{18}F]5** of 35 GBq/μmol (at the EOB), similar to the previously reported value, is suitable for clinical PET investigation. Additionally, the stability of the CHF^{18}F-Acyclovir was tested in DMSO, in PBS (Phosphate Buffer Saline) and FBS (Foetal Bovine Serum) at 37°C, and the ^{18}F-difluoromethylated Acyclovir was found to be fully stable after 2 hours (controlled with both radio-TLC and radio-UPLC) in all media.

Finally, the three-step process was performed in about 95 minutes and could easily be applicable to many other *N*-heteroaromatics (see our previous publication)[21], leading the way to the preparation of potential new ^{18}F-difluoromethylated PET tracers.

CONCLUSION

In the present study, a fully automated two-step radiosynthesis of the sulfone **[^{18}F]1** and the direct ^{18}F-difluoromethylation of the antiherpetic drug Acyclovir **2**, as a model substrate, was

implemented on the AllinOne module from Trasis. The sulfone **[^{18}F]1** synthesis was successfully transposed from a GE FASTlab module to a AIO synthesizer and the previously reported flow photoredox ^{18}F-difluoromethylation procedure was adapted and implemented on the AIO for the first time. The automated three-step radiosynthesis was performed in only 95 minutes, providing [^{18}F]Acyclovir-CHF_2 in an overall 1.4% RCY (dc). Even if this late stage approach, cannot always deliver the final compound with high radiochemical yields, the ^{18}F-difluoromethylation proceeds via C-H activation and circumvents the need of tedious and time-consuming prefunctionalization of complex drug scaffolds. This automated method should therefore facilitate the access to a wide range of new ^{18}F-difluorinated compounds typically found in many medicinal chemistry programs as described in the previous paper[21]. Finally, the process complies to radioprotection rules and GMP requirements, and in all cases, should provide enough final radioactivity for preliminary preclinical examinations and prompt validation of the potential interest of these tracers for further PET development. Furthermore, molar activities, a critical parameter for PET studies, were comparable (35 GBq/μmol) to our recent publication and are suitable for clinical development. We believe that this general ready-to-use late stage C-H ^{18}F-difluoromethylation method will be very useful and open the doors to the development of new ^{18}F-difluoromethylated radiotracers.

EXPERIMENTAL SECTION

Material and Methods

General

Solvents, reagents, and the non-radioactive reference compounds **2** and **4** were commercially purchased from Sigma Aldrich, TCI Europe N.V., and ABCR GmbH, and were used without further purification. The non-radioactive compounds **1**, **3**, and **5** were synthesized according to the procedures reported in the litterature[21].

Ultra-performance liquid chromatography (UPLC) analyses were performed on an ACQUITY UPLC® system (Waters) at 45°C, with a ACQUITY UPLC® CSH™ C18 column (2.1 x 100 mm, 1.7 μm; Waters), eluted with a MeCN/H_2O + 0.05% HCO_2H gradient (from 100% of H_2O to 75/25 (v/v) in 6 min, at 0.5 mL/min, and from 75/25 (v/v) to 100% H_2O in 2 min, at 0.5 mL/min).

The automation process was run on a AIO module[24] from Trasis (Ans, Belgium) and HPLC purifications were conducted on two different XBridge® BEH C18 OBD™ Semi-Preparative columns (130 Å, 5 μm, 10 mm x 250 mm, Waters), with the AIO internal high performance liquid chromatography (HPLC) system (UV and radioactive detectors). Manifolds, tubings, spikes, connectors and vials were purchased from Trasis. Syringes of 10 and 20 mL were purchased from BD Plastipak.

The no-carrier-added (nca) [^{18}F]fluoride was produced via the ^{18}O(p,n) nuclear reaction using a cyclone 18/18 (IBA). At the end of bombardment (EOB), the activity was transferred with helium pressure through Teflon tubing (~50 m) to the shielded hot lab cell.

Radioactivity was determined using a dose calibrator (Veenstrat) and all the radiochemical yields are decay corrected.

Thin layer chromatography (TLC) were carried out on silica gel Polygram® SIL G/UV_{254} pre-coated TLC sheets eluted with MeOH (100%) and analyzed on a Berthold TLC scanner (model AR200).

SPE cartridges (Sep-Pak ^{t}C18 Plus short, 400 mg, 37-55 μm and Oasis HLB Plus short, 225 mg, 60 μm) and QMA (Accell™ Plus, Carbonate Plus Light) cartridges (46 mg, 37-55 µm) were purchased from Waters (Milford, USA).

Tubing (1/16, 0.04 inch, PFA) for the photochemistry reactor was purchased from IDEX and the Kessil Blue LEDs (455nm, 32 W) from Hepatochem.

Manifold

The reagents or materials described in Table 3 were placed in their dedicated positions (P1-P36) according to the following layout (Figure 8).

Additionally, the positions P1 and P36 are both connected to the nitrogen inlet of the AIO module. The left tubing of the reactor, as well as positions P18 and 25 are connected to the vacuum inlet of the AIO. It should also be noted that the two tubings of the reactor are placed in separated pinch valves to avoid any loss of acetonitrile from the reactor during the labeling step due to the high temperature (85°C).

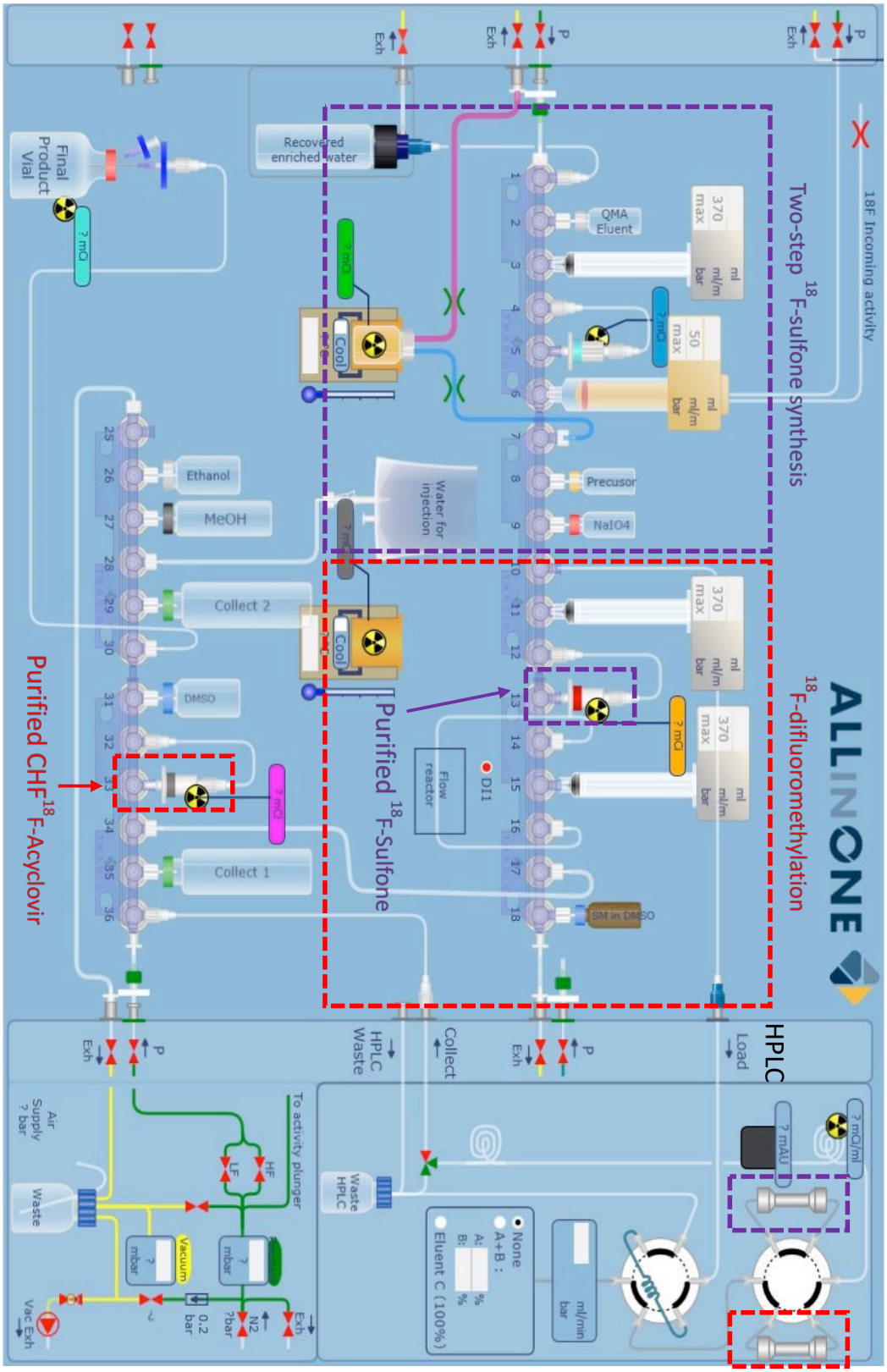

Figure 8: Layout for the multi-step synthesis of ^{18}F-difluoromethylated compounds

Table 3: Reagents and materials for the radiosynthesis of [^{18}F]CHF_2-acyclovir on the AIO

Manifold position (P)	Reagents or materials	Details
1	Polyethylene tubing to the [^{18}O]H_2O recovery vial	15 cm
2	$K_{2.2.2}$® (7.5 mg) in MeCN (480 µL) and K_2CO_3 (2.8 mg) in H_2O (120 µL)	4 mL vial (with 600 µL)
3	Syringe S1	BD PlastiPak 12 mL
4	Polyethylene tubing to the Sep-Pak® Accell™ Plus QMA Carbonate Plus Light Cartridge at position 5	15 cm
5	Sep-Pak® Accell™ Plus QMA Carbonate Plus Light Cartridge with polyethylene tubing at position 4	46 mg (40 µm)
6	[^{18}O]H_2O/[^{18}F]F^- inlet conical reservoir	BD PlastiPak 20 mL
7	Silicone tubing to the glass reactor vessel (right-hand side)	20 cm
8	Precursor **3** (11.1 mg) in MeCN (1 mL)	4 mL vial (with 1 mL)
9	$NaIO_4$ (51.3 mg) and $RuCl_3 \cdot xH_2O$ (1.7 mg) in H_2O (1 mL)	4 mL vial (volume: 1 mL)
10	Polyethylene tubing connected to the load line for the filling of the HPLC loop	35 cm
11	Syringe S2	BD PlastiPak 12 mL
12	Polyethylene tubing to the Sep-Pak® C18 Plus Short Cartridge at position 13	15 cm
13	Sep-Pak® C18 Plus Short Cartridge with polyethylene tubing at position 12	400 mg (37-55 µm)
14	Photochemistry reactor	1.2 mL reactor
15	Syringe S3	BD PlastiPak 12 mL
16	Photochemistry reactor	1.2 mL reactor
17	Polyethylene tubing connected to the position V34	35 cm
18	Acyclovir **2** (4.5 mg) and 4CzIPN (0.04 mg) in dry DMSO (350 μL)	4 mL brown vial (with 350 μL)
25	-	-

26	EtOH	10 mL vial (with 10 mL)
27	MeCN	20 mL vial (with 20 mL)
28	Polyethylene tubing with spike connected to the H_2O bag	30 cm
29	Collecting vial of the purified [^{18}F]CF_2H-acyclovir **[^{18}F]5** prefilled with 30 mL of water	50 mL vial (with 30 mL water)
30	Polyethylene tubing connected to the vial with the formulated [^{18}F]CF_2H-acyclovir **[^{18}F]5**	30 cm
31	DMSO (dry)	10 mL vial (with 10 mL)
32	Polyethylene tubing to the Sep-Pak® HLB Plus Cartridge at position V33	15 cm
33	Oasis® HLB Plus Short Cartridge with polyethylene tubing at position V32	225 mg (60 μm)
34	Polyethylene tubing connected to the position V17	35 cm
35	Collecting vial of the purified **[^{18}F]1** prefilled with 30 mL of water	50 mL vial (with 30 mL water)
36	Polyethylene tubing connected to the collecting line (outside of the HPLC AIO system)	30 cm

Photochemistry reactor

The reactor was 3D printed with PET (polyethylene terephthalate) and recovered with a polycarbonate transparent plate (LEXAN). The reactor is 8*8 cm square and filled with 2 meters of Perfluoroalkoxy alkane (PFA) tubing with an internal diameter of 0.04 inch, and an external diameter of 1/16. The irradiated part is about 140 cm (1.2 mL) and 60 additional centimeters are used to connect the reactor to V14 and V16 of the manifold. A blue LED lamp (455-470 nm) of 32 W was purchased from Kessil (455 nm maximum intensity, coverage area of up to 60 cm, Fig. 8) and adapted 3 cm above the reactor for the photochemical reaction (Figure 9).

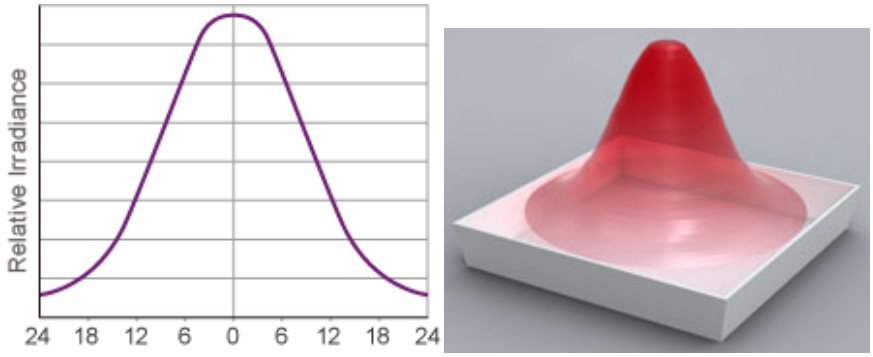

Figure 9: Distribution of the irradiation according to the distance to center (in inch). Data obtained from Kessil.

The internal volume of the reactor was designed in order to fit with the required volume for the elution of the tC18 cartridge containing **[^{18}F]1** (around 0.9 mL) and the photochemistry solution (350 μL), as well as to ensure a maximal irradiation with the Kessil blue LED.

Automated radiosynthesis

At the end of bombardment (EOB), the enriched water containing the [^{18}F]fluoride was transferred from the cyclotron target directly into the AIO synthesizer via the ^{18}F incoming activity line of the module. The activity is then recovered in the syringe placed in position 6 (P6) of the manifold. [^{18}F]Fluoride was trapped on the QMA Carbonate cartridge (P5) and the enriched water recovered in a separate vial (connected to P1). Afterwards, the [^{18}F]fluoride was eluted into the glass reactor (P7) with 600 μL of a K_{222} (20 μmol, 7.5 mg, 480 μL MeCN)/K_2CO_3 (20 μmol, 2.8 mg, 120 μL H_2O) solution (P2, transfer using syringe S1). The eluent was subsequently evaporated using various conditions of nitrogen flow/vacuum over time. The precursor solution (P8) was transferred through vacuum to the reactor and the reactor heated at 85°C for 5 minutes. At the end of the labeling step, the oxidative solution ($NaIO_4$ (240 μmol, 51.7 mg), $RuCl_3$,xH_2O (8 μmol, 1.7 mg) in H_2O (1 mL), P9) was aspirated in syringe S1 (P3) and transferred into the reactor, previously cooled down to 30°C. After another 5 minutes of

reaction at room temperature, 6 mL of H_2O was added to the crude mixture and the diluted reaction was transferred to semi-preparative HPLC purification. Followingly, the reactor was rinsed with 2 mL and transported again in the HPLC loop followed by another 1 mL to ensure that the crude product containing **[^{18}F]1** reached the HPLC loop (due to dead volumes). Using an eluent of MeCN/H_2O/HCO_2H (55/45/0.1), the desired product was recovered after 9.5 minutes and collected in a vial of 50 mL already containing 30 mL of water (P35). The vial was pressurized, and 11 mL of the solution were aspirated by syringe S3 and then transferred in the SepPak ^{t}C18 short cartridge placed in position P13. The desired product **[^{18}F]1** was trapped in the cartridge while the solvent was evacuated into the waste bottle through the reactor (P7). This step was repeated another three times. Afterwards, the cartridge containing the sulfone **[^{18}F]1** (P13) was dried for 2.5 minutes by a nitrogen flow to minimize the presence of water which is a crucial parameter for the following step. Before starting the ^{18}F-difluoromethylation reaction, the manifold was entirely dried with a nitrogen flow, the syringes S2 and S3 were each washed with 4 mL of DMSO (P31), and the manifold was dried again. Thereafter, the vial containing Acyclovir **2** (20 μmol, 4.5 mg) and 4 CzIPN (0.02 μmol, 0.016 mg) in DMSO (350 μL) and placed in position P18 was pressurized and transferred into S3. Subsequently, the DMSO vial (P31) was pressurized and 1.1 mL was transferred into S2 (P11). Then **[^{18}F]1** was eluted from the ^{t}C18 cartridge (P13) with DMSO from the syringe S2 to the syringe S3. It is important to note that both syringes were moving at the same flow rate to avoid any pressure issues. Finally, the crude mixture was transferred from S3 (P15) to S2 (P11) through the flow reactor (P16->P14) with a flow rate of 1.2 mL/min (1 minute residence time) under blue LEDs irradiation (455 nm), for the ^{18}F-difluoromethylation reaction. At the end of the process (around 3 minutes), 7 mL of water were aspirated in S2 through the reactor (for reactor cleaning) and the solution was injected into the semi-preparative HPLC. Followingly, 1.5 mL of water were aspirated in S2 and transferred again in the semi-PREP HPLC loop,

followed by another 0.5 mL (dead volumes). Using the gradient described in the following section, the desired CHF^{18}F-Acyclovir **[^{18}F]5** was collected after 13 minutes in a 50 mL vial prefilled with 30 mL of water (P29). Before trapping the purified CHF^{18}F-Acyclovir **[^{18}F]5** into the HLB short Plus cartridge (P33), the syringe S3 was washed twice with MeOH (4 mL, P27). Afterwards, as before, the vial (P29) was pressurized, and 11 mL of the solution were aspirated by syringe S3 and then transferred in the HLB short Plus cartridge placed in position P33. The desired product **[^{18}F]5** was trapped in the cartridge while the solvent was evacuated into the waste bottle. This step was repeated another 3 times. The HLB Oasis cartridge containing **[^{18}F]5** (P13) was dried for 1.5 minutes using a nitrogen flow. Syringe S3 was washed twice with ethanol (4 mL, P26) and the manifold was dried. Finally, the HLB was eluted with ethanol (S3) into the separate final vial connected to position P30, ready to use for (molar activity determination or) further (pre)clinical PET studies.

Purification details

For purifications of the sulfone **[^{18}F]1** and difluoromethylated Acyclovir **[^{18}F]5**, two identical semi-preparative HPLC columns were required, as described in the general section.

For the sulfone **[^{18}F]1**, an isocratic eluent MeCN/H_2O/HCO_2H (55/45/0.1) was used, with a flow rate of 5 mL/min (Figure 10).

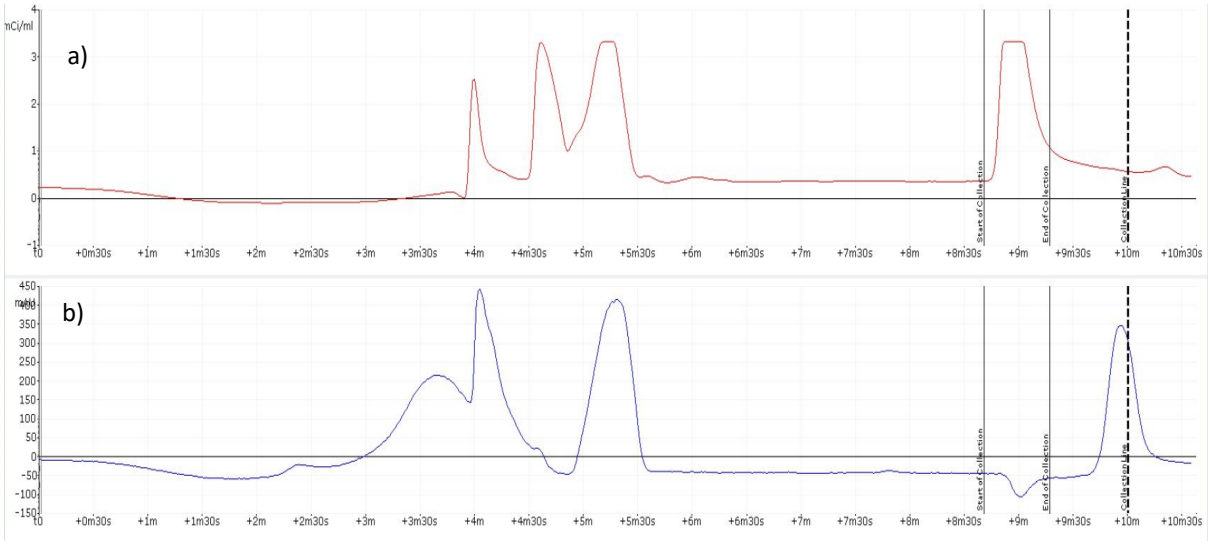

Figure 10: a) Radio and b) UV chromatograms for the purification of **[^{18}F]1**

For CHF^{18}F-Acyclovir **[^{18}F]5**, the following gradient was applied (Table 4) and afforded the following purification chromatograms (Figure 11).

Table 4: Gradient for CHF^{18}F acyclovir purification

Time (min)	**H_2O (0.05% HCOOH) (%)**	**MeOH (%)**	**Flow rate (mL/min)**
0	100	0	5
4	100	0	5
4.1	65	35	5
15	65	35	5
15.1	0	100	5

For 4 minutes, the column was eluted with water only to minimize the impact of DMSO in the separation, and MeOH was favored to MeCN to slow down the elution of the CHF^{18}F-Acyclovir (**[^{18}F]5**).

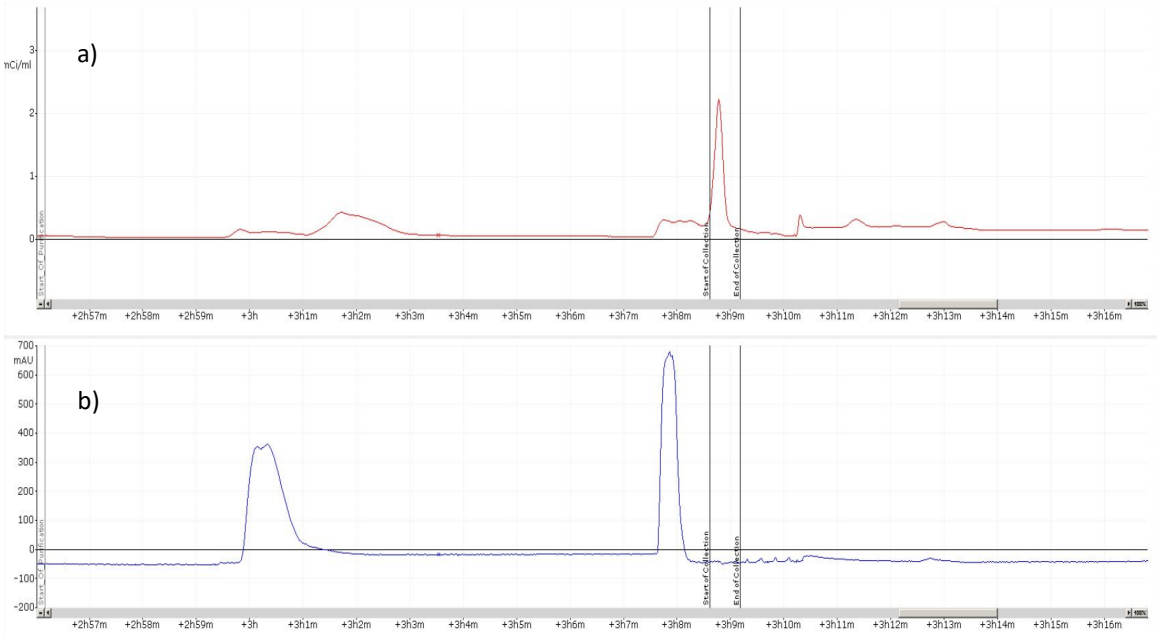

Figure 11: a) Radio and b) UV chromatograms for the purification of **[^{18}F]5**

After completion of the synthesis, both columns were rinsed with MeCN (100%).

QC Analysis

After completion of the synthesis, a radio-UPLC was performed to confirm the radio-purity of **[^{18}F]5** (Figure 12).

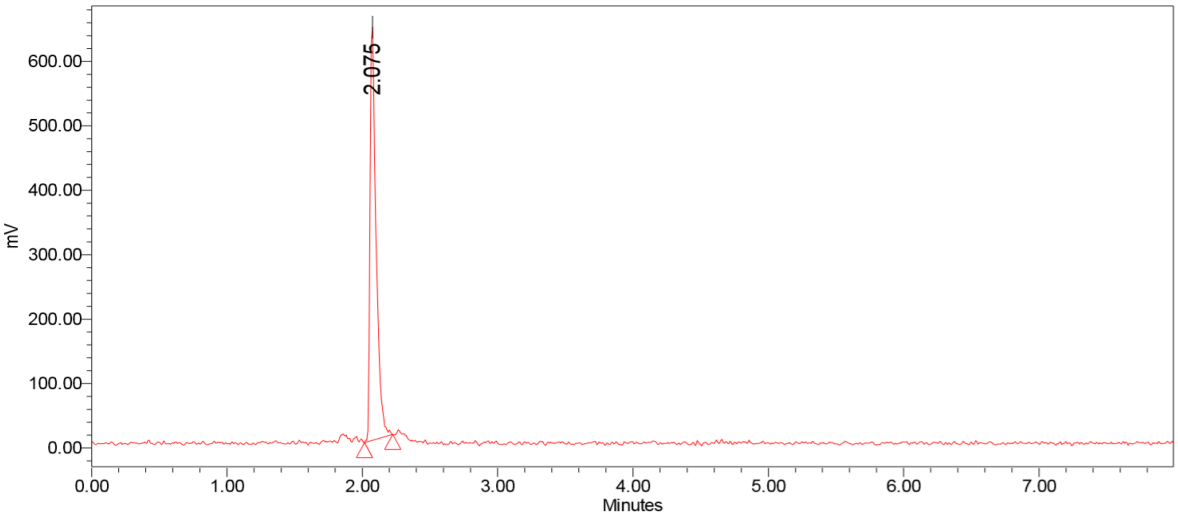

Figure 12: Radio-UPLC chromatogram of the purified **[^{18}F]5**

Finally, the molar activity of **[^{18}F]5** was determined using the following calibration curve (Figure 11)

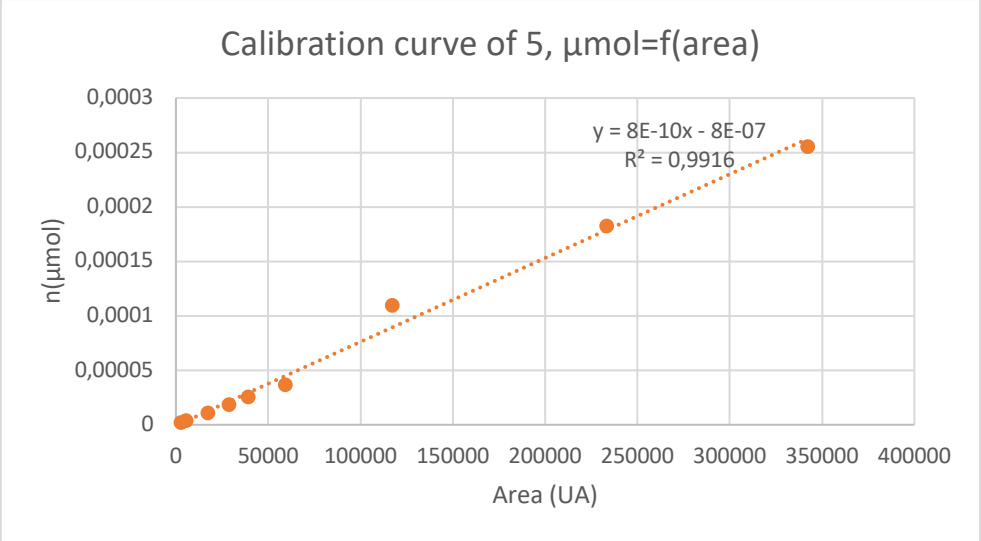

Figure 13: Calibration curve of **5** for molar activity determination of **[^{18}F]5**

The molar activity was of 35 GBq/μmol (dc).

Automation timeline

The timeline of each step of the synthesis is presented in the following table (Table 5).

Table 5: Timeline of the three-step process

Chemical Step	*Automation Step*	*Time (minutes)*
^{18}F-SULFONE SYNTHESIS	**^{18}F elution and drying**	**9**
	Labeling	**5**
	Oxidation	**5**
	Dilution and HPLC injection	**6**
	Purification of [^{18}F]1	**12**
	Trapping of [^{18}F]1	**8**
	Drying of [^{18}F]1	**2,5**
	Washing of the system and rinsing of the syringes	**3**
	Additional transfers not taken into account	**9**
	Total [^{18}F]1	**55**

^{18}F-DIFLUOROMETHYLATION REACTION	**Photoredox ^{18}F-difluoromethylation step**	**3**
	Dilution and HPLC injection	**3**
	Purification of [^{18}F]5	**13**
	Trapping of [^{18}F]5	**9**
	Drying and elution of [^{18}F]5	**4**
	Additional transfers not taken into account	**8**
	Total [^{18}F]5	**40**
	Total three-step synthesis	**95**

ASSOCIATED CONTENT

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Notes

The authors declare no competing interest.

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