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Microfluidic radiosynthesis and biodistribution of [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid†

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Microfluidics technology has emerged as a powerful tool for the radiosynthesis of positron emission tomography (PET) and single-photon emission computed tomography radiolabeled compounds. In this work, we have exploited a continuous flow microfluidic system (Advion, Inc., USA) for the [^{18}F]-fluorine radiolabeling of the malonic acid derivative, [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid ([^{18}F]-FPMA), also known as [^{18}F]-ML-10, a radiotracer proposed as a potential apoptosis PET imaging agent. The radiosynthesis was developed using a new tosylated precursor. Radiofluorination was initially optimized by manual synthesis and served as a basis to optimize reaction parameters for the microfluidic radiosynthesis. Under optimized conditions, radio-thin-layer chromatography analysis showed 79% [^{18}F]-fluorine incorporation prior to hydrolysis and purification. Following hydrolysis, the [^{18}F]-FPMA was purified by C18 Sep-Pak, and the final product was analyzed by radio-HPLC (high-performance liquid chromatography). This resulted in a decay-corrected 60% radiochemical yield and ≥98% radiochemical purity. Biodistribution data demonstrated rapid blood clearance with less than 2% of intact [^{18}F]-FPMA radioactivity remaining in the circulation 60 min post-injection. Most organs showed low accumulation of the radiotracer, and radioactivity was predominately cleared through kidneys (95% in 1 h). Radio-HPLC analysis of plasma and urine samples showed a stable radiotracer at least up to 60 min post-injection.

Keywords: microfluidic technology; [^{18}F]-FPMA; [^{18}F]-ML-10; PET radiochemistry

Introduction

Recently, microfluidics technology has emerged as a powerful tool for the radiosynthesis of radiolabeled compounds for positron emission tomography (PET). The high surface-to-volume ratios offered by this technology provide many advantages for more efficient radiochemical synthesis. Compared with conventional radiochemistry, microfluidic devices allow the use of smaller amounts of precursor and still achieve high radiochemical yields (RCYs) with less radiochemical impurities.[1] This results in simplification of purification steps and increased specific activity. Microfluidic devices also offer possibilities for increased reaction speed, improve reproducibility, and reduce costs. The application of microfluidic technology to radiochemistry has been demonstrated for the radiosynthesis of [^{11}C]-carbon, [^{18}F]-fluorine, and [^{13}N]-nitrogen PET radiotracers.[2–4] Another important feature of microfluidic radiochemistry is the ability to vary reaction parameters, during radiosynthesis development, and hence enable faster optimization of different reaction conditions. This is particularly advantageous for PET radiochemistry with short half-life radionuclides.

A continuous flow microfluidic system has been developed and marketed by Advion, Inc., USA (see Figure 2 for schematic diagram). Radiochemical reactions at high pressure can be performed on this system, resulting in faster incorporation of the radiolabel into the final radiotracer, using a small amount of precursor. A number of PET radiotracers have been synthesized using this microfluidic technology. These include the ^{18}F-labeled compounds [^{18}F]-fludeoxyglucose,[1,5–8] 3′-deoxy-3′-[^{18}F]fluorothymidine,[9] 1-(5-[^{18}F]fluoro-5-deoxy-α-D-arabinofuranosyl)-2-nitroimidazole,[10] 2′-[^{18}F]fluoro-2′-deoxy-1β-D-arabinofuranosyl-5-iodouracil,[11] [^{18}F]fallypride,[12] [^{18}F]altanserin,[13] [^{18}F]fluroethyl-di-tosylate,[14] and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine.[15] The system has also been utilized for [^{11}C]-carbonylation reactions[3] and more recently for [^{13}N]-labeled radiotracers.[4] Radiometal synthesis, using [^{64}Cu] DOTA-cyclo peptide (RGDfK), has also been reported using similar microfluidic technology.[16]

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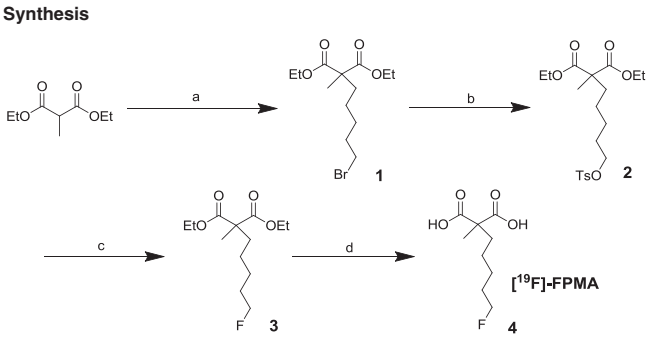
† *Supporting information may be found in the online version of this article.*

The fluorine-18 labeled malonic acid derivative, 2-(5-[^{18}F] fluoro-pentyl)-2-methyl malonic acid, has been synthesized including its automated 'TRACERLab' synthesis[17–19] and reported as a potential apoptosis imaging agent.[20–23] In this manuscript, we report on the microfluidic synthesis of this radiotracer, using a new tosylated precursor and its *in vivo* biodistribution and stability profile.

Results and discussion

Synthesis [^{19}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid (4)

Scheme 1 describes the synthesis of the tosylated precursor and reference standard compound [^{19}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid (**4**). The reference non-radioactive compound **4** was synthesized in four steps starting with commercially available diethyl methylmalonate. Diethyl methylmalonate was alkylated with 1,5-dibromopentane in presence of NaH to give alkylated compound **1** in 72% yield using the procedure of Astles *et al.*[24] Excess 1,5-dibromopentane was used to avoid disubstitution. The bromide was displaced using commercially available silver tosylate to give a tosylated precursor **2** in 96% yield. The tosylate group was exchanged for the required fluoride by a nucleophilic displacement reaction using tetrabutylammonium fluoride (TBAF) to give diethyl 2-(5-fluoropentyl)-2-methylmalonate (**3**) in 84% yield. Finally, fluoropentyl diethyl malonate ester **3** was hydrolyzed using lithium hydroxide under mild conditions to give 2-(5-fluoro-pentyl)-2-methyl malonic acid as a white solid with 50% overall yield. All the intermediate compounds and final compound were fully characterized by ^{1}H-NMR, ^{13}C-NMR, ^{19}F-NMR, and ESI-MS analysis. Fluorination of compound **3** was confirmed by ^{19}F-NMR, which showed the expected multiplet pattern and had a chemical shift in the expected region (−218.88 for terminal (CH_2F)). The calculated molecular mass for purified 2-(5-fluoro-pentyl)-2-methyl malonic acid was in agreement with experimental value. Chemical purity was found to be greater than 98% as determined by analytical HPLC.



Scheme 1. Synthesis of cold reference compound [^{19}F]-FPMA and tosylate precursor of [^{18}F]-FPMA. Reagents and conditions: (a) 1,5-dibromopentane, NaH, DMF, 50 °C, 12 h, 72%; (b) silver tosylate, ACN, reflux, 12 h, 96%; (c) TBAF, THF, 60 °C, 3 h, 84%; (d) LiOH/H_2O THF/MeOH/H_2O (6:3:1), rt, 24 h, 86%.

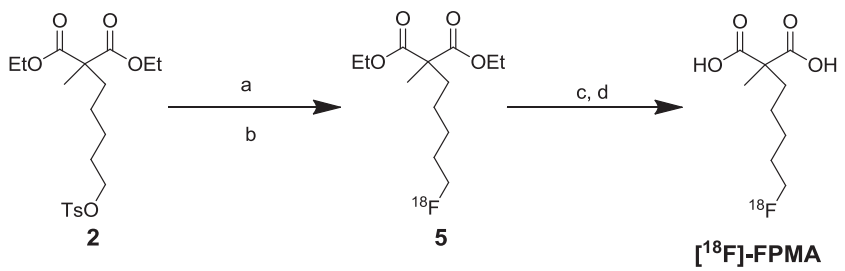
Manual radiosynthesis of [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid

Scheme 2 represents manual radiosynthesis of [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid ([^{18}F]-FPMA) in two steps: (i) Kryptofix-mediated direct nucleophilic fluorination and (ii) NaOH base hydrolysis.

In optimization studies, radiofluorination of **2** was investigated as a function of three parameters: mass of precursor, temperature, and reaction time. The reaction was monitored over an interval of 5, 10, 15, and 20 min. In the presence of Kryptofix and K_2CO_3 in acetonitrile, optimum conditions of 4-mg precursor, 110 °C, and 10 min. reaction time resulted in >90% RCY of non-hydrolyzed compound **5** as confirmed by radio-thin-layer chromatography (TLC) analysis. This is comparable with the value obtained in a previously described method.[25] After radiolabeling, the acetonitrile was evaporated with helium flow under vacuum, and the product was subjected to base hydrolysis. Complete hydrolysis was achieved by methanolic sodium hydroxide (3 M) in dichloromethane (DCM)/methanol (9:1) for 20 min at 45 °C, which is comparable with the hydrolysis efficiency of malonic ester derivatives.[26] The final product was purified by solid phase extraction. After evaporation of the solvent, under vacuum and helium flow, the product was re-dissolved in water and adjusted to pH 2–3 using 3 M HCl. The reaction mixture was passed through a C18 Sep-Pak column, and the column was washed with 10 mL of water to remove free fluoride. The product was eluted with multiple 0.5 mL ethanol fractions. Greater than 90% of product radioactivity was eluted in fractions 3 and 4. The overall decay-corrected (yield of final product, 50 min after introduction of [^{18}F]-fluoride activity) isolated RCY of [^{18}F]-FPMA was 60%, and the radiochemical purity (RCP) was more than 98% in 50 min total synthesis time. RCP was confirmed by radio-HPLC. Co-injections with non-radioactive fluorinated compound gave the same retention time as [^{18}F]-FPMA = 2.7 min.

Microfluidic radiosynthesis

A major goal of this work was to develop an automated microfluidic platform for rapid, robust synthesis of [^{18}F]-FPMA. Scheme 2 represents the microfluidic radiosynthesis of [^{18}F]-FPMA carried



Scheme 2. Synthesis of [^{18}F]-FPMA. Reagents and conditions: (a) K18F/K222, K_2CO_3, ACN, 110 °C, 10 min condition used for manual synthesis; (b) glass microfluidic device, K18F/K222, K_2CO_3, ACN, 190 °C, condition used in automated microfluidic synthesis; (c) 3 mol NaOH, DCM/MeOH (9:1), 45 °C, 20 min; (d) 3 M HCl, in manual and sodium citrate buffer (pH 2.79) in microfluidic synthesis, was used for pH adjustment (pH 2–3).

out in two steps: (i) Kryptofix-mediated direct nucleophilic fluorination and (ii) NaOH base hydrolysis. Under optimum conditions of 100 μL × 2 m reactor loop, 50 μL/min flow rate, and 190 °C temperature, radio-TLC analysis showed a non-purified RCY approaching 79% (Table 1).

Optimized condition/s for hydrolysis were found to be heating the reaction to 50 °C for 20 min. The final product was separated by C18 Sep-Pak purification method as described earlier. This resulted in 60% decay-corrected RCY of purified product, in a total reaction time of 50 min. Radio-HPLC analysis showed 99% RCP of final product (Figure 5 in Supporting Information).

Biodistribution and *in vivo* stability analysis

Figure 1 shows the biodistribution of [^{18}F]-FPMA in female nude mice. The radiotracer demonstrated rapid blood clearance with less than 2% of radioactivity remaining in the circulation after 1 h. The blood half-life was determined to be 13 min (Figure 1 inset). Most organs show low accumulation of the radiotracer, and radioactivity was predominantly cleared through the kidneys with greater than 95% cleared 1 h after injection.

The *in vivo* stability of [^{18}F]-FPMA in plasma and urine was assessed during the first 120 min after intravenous administration in normal female nude mice. The stability of [^{18}F]-FPMA was analyzed by radio-TLC and radio-HPLC at 1, 5, 30, 60, and 120 min post-administration. The radio-HPLC peak appeared at the same retention time as the non-radioactive reference compound, [^{19}F]-FPMA, demonstrating an intact [^{18}F]-FPMA, 60 min post-injection. No radioactivity was detected in 120-min samples, which could be attributed to the fast physiological clearance of the radiotracer from the blood.

Table 1. Optimization of RCY for microfluidic synthesis of [^{18}F]-diethyl 2-(5-fluoropentyl)-2-methylmalonate. Reaction conditions: (i) microreactor volume = 16 μL; (ii) volume ratio (P1:P3 = 1), starting radioactivity = 10–15 mCi

Synthesis no.	Reactor temp. (°C)	P3 flow rate (μL/min)	Unisolated yield (%)
1	100	30	0
2	125	30	6
3	150	30	23
4	175	30	63
5	170	50	32
6	180	50	78
7	180	75	46
8	190	50	79

Experimental

Reagents and instrumentation

All reagents and solvents were purchased from Sigma-Aldrich and used without further purification unless otherwise indicated. All cold reactions were performed under argon atmosphere in oven-dried glassware. Flash chromatography was performed employing Sigma-Aldrich 230-400 mesh 60 Å silica gel. TLC (Sigma-Aldrich, Saint Louis, MO) was performed using silica gel-coated aluminum plates with F-254 indicator (250 μm, 20 cm 20 cm, Whatman). NMR (Piscataway, NJ) spectra (^{1}H-NMR, ^{13}C-NMR, and ^{19}F-NMR) were obtained using Varian Mercury 300 MHz and Varian Inova 400 MHz (Sigma-Aldrich, Saint Louis, MO) using tetramethylsilane as an internal standard. Melting points were determined using a Thomas Hoover melting point apparatus and are uncorrected. Mass spectra were recorded on an Orbitrap Velos mass spectrometer. Aqueous [^{18}F]-fluoride was produced by the ^{18}O(p,n)^{18}F reaction, in a PET tracer cyclotron (GE Medical Systems, Wausheka, WI, USA), by the irradiation of an isotopically enriched [^{18}O] water (Rotem Industries, Ber Sheva, Israel) target. For fluoride trapping, Sep-Pak Light® quaternary methyl ammonium (QMA) cartridge was used in manual synthesis from Waters Corp. (Milford, MA, USA) and Micro-SPE cartridges mini-trap used in microfluidic synthesis from ORTG (TN, USA). Microfluidic radiosynthesis was performed using NanoTek microfluidic synthesis system Advion. In the final product purification, C-18 a Sep-pak cartridge was used from Waters Corp. Radio-HPLC stability analysis was carried using a Waters HPLC pump (Waters, Model 1525) equipped with UV detector (Waters, Model 2489) and radiation detector (Bio-scan, Model B-FC-3300) connected in series. For reversed-phase HPLC, a Waters® Nova-Pak 4 μ C18 150 mm × 3.9 mm column was eluted isocratically with 25/75 I/Water containing 0.1% TFA at flow rate of 1 mL/min. Radio-TLC was performed on a radio-TLC scanner (BioScan, Model AR/2000). Radioactivity was measured with dose calibrator Captintec CRC-15 PET. Animals, normal adult athymic female nude mice, were purchased from Harlan Laboratories, USA.

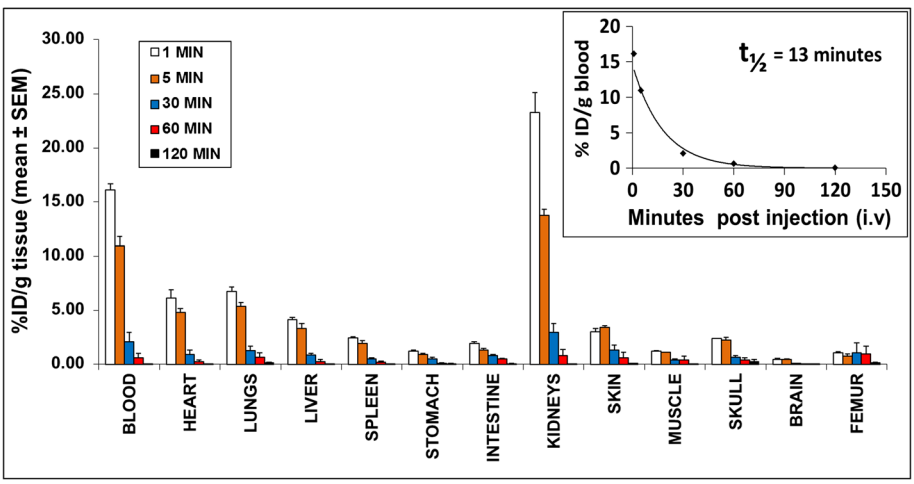


Figure 1. Biodistribution of [^{18}F]-FPMA in normal adult female nude mice (n = 3) at 1, 5, 30, 60, and 120 min after injection. Radio tracer uptake in %ID/g was determined by gamma counting.

Synthesis of cold reference compound 2-(5-fluoropentyl)-2-methylmalonic acid (4) and intermediate (Scheme 1)

Diethyl 2-(5-bromopentyl)-2-methylmalonate (1)

A solution of diethyl methylmalonate (6.0 g, 34.5 mmol) in dry dimethylformamide (50 mL) was treated portionwise with sodium hydride (1.5 g of a 60% dispersion, w/w, in mineral oil, 38 mmol) with cooling. The reaction mixture was stirred for 30 min at ambient temperature and treated with 1,5-dibrompentane (11.9 g, 51.8 mmol). The reaction mixture was heated at 50 °C overnight and quenched by adding 30% ammonium hydroxide solution. Distilled water (50 mL) was added in to the reaction mixture and extracted with DCM (3 mL × 50 mL). The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was purified by flash chromatography using 5% ethyl acetate in hexane to give alkyl malonate **1** as colorless viscous oil (8.0 g, 72%) (Scheme 1): ^{1}H-NMR δ 1.20–1.33 (m, 2H), 1.25 (t, J = 7.32 Hz, 6H), 1.40 (s, 3H), 1.42–1.51 (m, 2H), 1.81–191 (m, 4H), 3.39 (t, J = 6.73 Hz, 2H), 4.17 (q, J = 7.32 Hz, 4H); ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 13.9, 19.7, 23.3, 28.2, 32.3, 33.4, 35.1, 53.4, 60.9, 172.1; ESI-MS $[M + Na]^+$ *m/z* calcd for $C_{13}H_{23}BrO_4$ + Na 345.07, found 345.08.

Diethyl 2-methyl-2-(5-(tosyloxy)pentyl)malonate (2)

To a solution of alkyl malonate **1** (3.2 g 10 mmol) in dry acetonitrile (30 mL), silver tosylate (3.48 g, 12.5 mmol) was added. After the reaction mixture was refluxed for 24 h under an argon atmosphere, the reaction mixture was filtered through celite to remove the silver salt, and the solvent was evaporated under reduced pressure. The residue obtained from the solvent evaporation was purified by flash chromatography using 10% ethyl acetate in hexane to give tosylate precursor **2** as a colorless viscous oil (3.9 g, 96%): ^{1}H-NMR (300 MHz) ($CDCl_3$) δ 1.18–1.25 (m, 2H), 1.23 (t, J = 7.22 Hz, 6H), 1.26–1.35 (m, 2H), 1.36 (s, 3H), 1.61–1.68 (m, 2H), 1.77–1.81 (m, 2H), 2.45 (s, 3H), 4.00 (t, J = 6.44 Hz, 2H), 4.16 (q, J = 7.22 Hz, 4H), 7.35 (d, J = 8.19 Hz, 2H) 7.78 (d, J = 8.19 Hz, 2H); ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 13.7, 19.4, 21.1, 23.2, 25.2, 28.1, 34.8, 53.1, 60.7, 70.1, 127.4, 129.6, 132.8, 144.4, 171.7; ESI-MS $[M + Na]^+$ *m/z* calcd for $C_{13}H_{23}BrO_4$ + Na 437.16, found 437.43.

Diethyl 2-(5-fluoropentyl)-2-methylmalonate (3)

To a solution of tosylate **2** (2.1 g, 5 mmol) in dry THF (20 mL), a solution TBAF (1 M solution in THF, 1.58 g, 6 mL, 6 mmol) was added dropwise under argon atmosphere. The reaction mixture was stirred at 60 °C for 3 h. The reaction mixture was cooled to room temperature, and the solvent was evaporated under reduced pressure. The product obtained from the solvent evaporation was purified by flash chromatography using 10% ethyl acetate in hexane to give fluoropentyl malonate **3** as a light yellow colored viscous oil (1.1 g, 84%): ^{1}H-NMR (300 MHz) ($CDCl_3$) δ 1.24 (t, J = 7.32 Hz, 6H), 1.25–1.34 (m, 2H), 1.40 (s, 3H), 1.36–1.48 (m, 2H), 1.61–1.78 (m, 2H), 1.83–1.89 (m, 2H), 4.17 (q, J = 7.32 Hz, 4H), 4.34 (t, J = 6.15 Hz, 1H), 4.50 (t, J = 6.15 Hz, 1H); ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 13.87, 19.66, 23.79, 25.33, 25.40, 29.89, 30.15, 35.25, 53.43, 60.92, 82.49, 84.68, 172.12; ESI-MS $[M + Na]^+$ *m/z* calcd for $C_{13}H_{23}FO_4$ + Na 285.15, found 285.31.

Non-radioactive reference compound 2-(5-fluoropentyl)-2-methylmalonic acid (4)

To a stirred solution of fluoropentyl malonate **3** (310 mg, 1.5 mmol) in THF/MeOH/H_2O (6:3:1, 15 mL), lithium hydroxide monohydrate (631 mg, 15 mmol) was added. The reaction mixture was stirred 24 h at room temperature. After the reaction was complete, the organic solvents were removed under reduced pressure. The aqueous layer was acidified with oxalic acid solution (1 mol) to pH 2–3 and then extracted with diethyl ether (3 mL × 25 mL). The combined organic layers were dried over anhydrous sodium sulfate and then concentrated under reduced pressure. The residue was dissolved in chloroform, the undissolved residue was filtered, and the filtrate was concentrated under reduced pressure to give reference compound ([^{19}F]-FPMA) **4** (208 mg, 86%) as a white solid: mp 102–104 °C: ^{1}H-NMR (300 MHz) ($CDCl_3$) δ 1.32–1.47 (m, 4H), 1.49 (s, 3H), 1.65–1.78 (m, 2H), 1.90–1.94 (m, 2H), 4.37 (t, J = 6.05 Hz, 1H), 4.49 (t, J = 6.05 Hz, 1H) 10.48 (s, 2H); ^{13}C-NMR (75 MHz) ($CDCl_3$) δ 20.09, 24.22, 25.59, 25.66, 30.14, 30.40, 35.63, 54.03, 83.04, 85.22, 178.43; ^{19}F-NMR (300 MHz) ($CDCl_3$) (coupled) δ −218.88 (m); ESI-MS $[M + Na]^+$ *m/z* calcd for $C_9H_{15}FO_4$ + Na 229.09, found 229.09. ^{1}H-NMR, ^{13}C-NMR, and ^{19}F-NMR spectra are shown in Figure 1.

Radiosynthesis of [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid (Scheme 2)

Radiosynthesis of [^{18}F]-FPMA was carried out using tosylate precursor **2**. The QMA cartridge was preconditioned with 10 mL (0.25 mol) potassium carbonate solution followed by 20 mL sterile water. [^{18}F]-fluoride was then passed through a QMA Sep-Pak cartridge (carbonate form) to remove [^{18}O] H_2O. The trapped [^{18}F]-fluoride was eluted with a solution of 0.08 mL of potassium carbonate (0.25 M) and 0.42 mL of sterile water into a 10 mL V-shaped reaction vial containing a solution of Kryptofix 12 mg in dry acetonitrile 0.8 mL. The reaction mixture was dried with the addition of dry acetonitrile (3 × 1 mL) under vacuum and helium flow at 80 °C. To the dried [^{18}F]-fluoride, tosylate precursor **2** (4 mg) in acetonitrile (0.8 mL) was added, and the reaction mixture was heated at 110 °C for 10 min in the sealed 10-mL V-shaped vial with stirring. The reaction mixture was cooled for 5 min and the solvent evaporated under vacuum and helium flow at room temperature. Base hydrolysis was accomplished by the addition of methanolic NaOH solution (3 M, 500 μL) in 2 mL of DCM/MeOH (9:1) followed by stirring for 20 min at 45 °C. The solvent was then evaporated under vacuum and helium flow at room temperature. Water (1 mL) was added and the reaction mixture adjusted to pH 2–3 using 3 M HCl. The reaction mixture was passed through a C18 Sep-Pak column that was preconditioned with 10 mL water, 10 mL ethanol, and 10 mL water, respectively. The compound was eluted with ethanol in 6 mL × 0.5 mL fractions; most of the activity was observed in fractions 3 and 4. Ethanol was evaporated at 45 °C under vacuum and helium flow, and the residue was redissolved in PBS. The [^{18}F]-FPMA was analyzed by radio-HPLC using the following conditions: Waters Nova-Pak 4 μ C18 150 mm × 3.9 mm, CH_3CN/H_2O 25:75, containing 0.1% TFA at flow rate 1 mL/min, UV 218 nm. The radiochemical identity was confirmed by co-injections with non-radioactive standard fluorinated compound **4** and gave the same retention time as [^{18}F]-FPMA = 2.7 min.

Automated microfluidic radiosynthesis of [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid (Scheme 2)

The radiosynthesis of [^{18}F]-FPMA was conducted using a NanoTek Microfluidic Synthesis System (Advion) shown in Figure 2, which consists of two concentrator modules, one reactor module, and one base module and was controlled by a standard laptop running with the NanoTek software 1.4 release [**11**].[27]

Preparation of [^{18}F]fluoride ion reagent

Cyclotron-produced [^{18}F]fluoride ion in [^{18}O]water (1.7 ± 0.1 mL) was passed through a preconditioned (with 500 μL water) small anion-exchange (MP-1) column by aspirating 3 × 1 mL; this operation was also used to dry the lines of water. The trapped ^{18}F fluoride activity was eluted with a solution containing 30 mg of Kryptofix2.2.2 (K222) and 4.5 mg of K_2CO_3 in 1 mL of 90% CH_3CN/water into a V-vial preheated to 110 °C. Nitrogen flow was applied to the reactor while delivering the ^{18}F solution to facilitate solvent evaporation; flow parameters were optimized in order to keep the volume at a maximum of 300 μL of solution throughout the whole process, so that activity was collected at the bottom of the 3 mL V-vial. The solution was then evaporated to dryness. Additionally, three aliquots of 0.1 mL of anhydrous CH_3CN were added dropwise to the vial for further azeotropic distillation through all of the fluoride drying lines. After this stage, only vacuum was applied for 1 min at 110 °C and 1 min after stopping the heating. The dried complex was then reconstituted with 0.50 mL of CH_3CN according to the experimental protocol. The whole drying procedure took 12 min.

Optimization of [^{18}F]-diethyl 2-(5-fluoropentyl)-2-methylmalonate (5) synthesis in the microreactor

Microfluidic optimization experiments were conducted using low activity (10–15 mCi of ^{18}F). Dry [^{18}F]F^-K^+-K222 solution (10 mCi) and tosylate precursor (4 mg in 750 μL) were loaded in their respective storage loop in the microfluidic reactor apparatus. The [^{18}F]fluorination reaction was optimized in terms of temperature, flow rate, and flow ratio of reactants in the discovery mode. The temperature was varied from 100 to 190 °C, fluoride flow rate from 30 to 75 μL/min, and maintained the precursor/fluoride flow ratio 1:1 as shown in Table 1. Samples were collected after each discovery run (every ~3 min) and analyzed on radio-TLC. On the basis of synthesis entry no. 8, we observed that 190 °C with a P3 flow rate of 50 μL/min flow rate gave a maximum yield 79% of intermediate **5**. Radiochemical yields were calculated from TLC analysis. Radio-TLC was performed on silica gel layers (Waters) developed with ethyl acetate/hexane (3:7 v/v).

Production of [^{18}F] 2-(5-fluoro-pentyl)-2-methyl malonic acid in microfluidic reactor

The optimized conditions described earlier were implemented in scaled up batch production of the radiotracer. Dry [^{18}F]F^-K^+-K222 solution (286 mCi in 500 μL I) and tosylate precursor (4 mg in 750 μL I) were loaded in their respective storage loop (439-μL storage loop (P1) for tosylate precursor and a 403-μL loop (P3) for the K222–fluoride complex) in the microfluidic reactor apparatus. Solutions from each loop (50 μL) were infused simultaneously at 50 μL/min into the reactor (100 μm × 2 m) held at a temperature of 190 °C. The reaction mixture was collected in concentrator 2 in 2 mL methanolic NaOH solution (0.5 mL, 3 M, 500 uL) and DCM/MeOH (9:1) (1.5 mL), and this was heated to 45 °C for 20 min. After hydrolysis, the solvent was evaporated by blowing nitrogen, and 2 mL sodium citrate buffer (pH 2.79) was added through concentrator 2. At a pH between 2 and 3, the reaction mixture was passed through a pre-conditioned C18 Sep-Pak column. Unreacted fluoride was washed with water, and the final product was eluted with ethanol. The final product

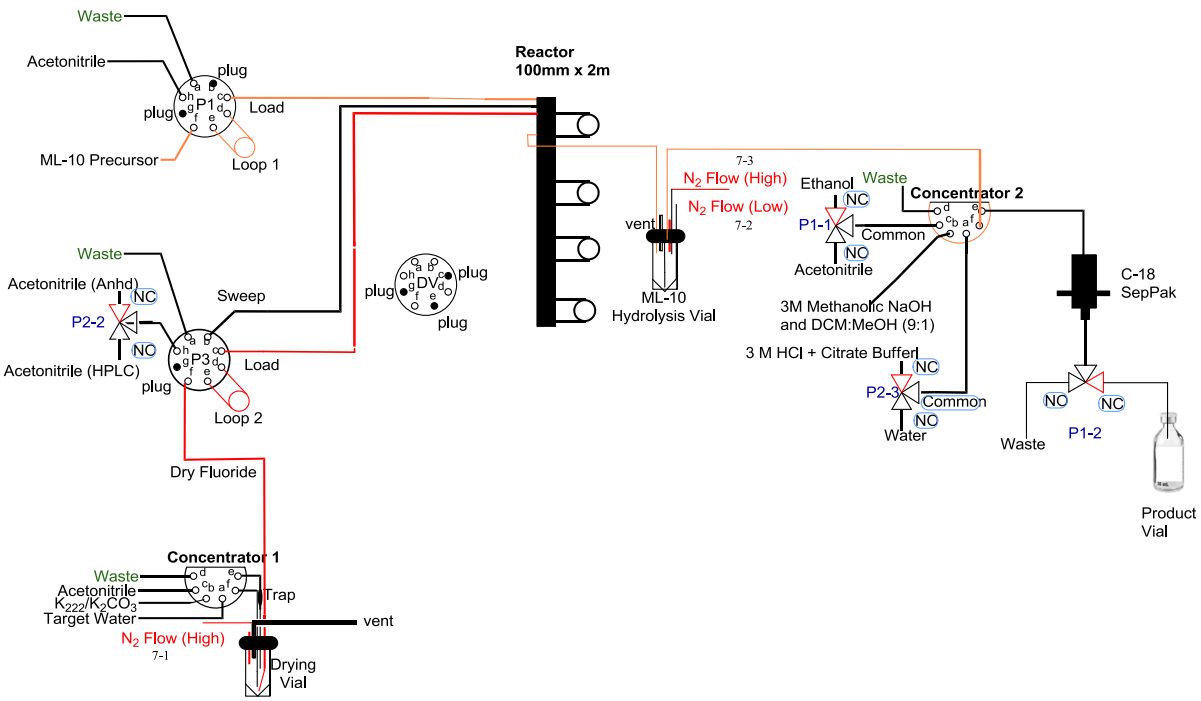


Figure 2. A schematic diagram of microfluidic apparatus.

[^{18}F]-FPMA was analyzed by radio-HPLC using the same conditions as described earlier. It is by using the automated microfluidic synthesis that a 60% RCY and greater than 98% RCP of [^{18}F]-FPMA were achieved in less than 50 min. This procedure resulted in a batch yield of 124 ± 5 mCi, 60%.

Biodistribution and *in vivo* stability analysis

Animal experiments were approved and performed according to the policies and guidelines of the Animal Care and Use Committee at Virginia Commonwealth University. The biodistribution of [^{18}F]-FPMA was measured in normal adult female nude mice (Harlan). Under 2% isoflurane anesthesia, five group of three mice received an intravenous injection (tail vein) of [^{18}F]-FPMA (3–4 MBq; in 200 μL PBS) at the start of the experiment). Under anesthesia, mice were sacrificed by cervical dislocation at 1, 5, 30, 60, and 120 min post-injection. Whole blood was collected by cardiac puncture, other major organs were harvested and weighed, and the radioactivity was counted in a gamma counter. The blood activity data were used to calculate the blood half-life. Decay-corrected radiotracer uptake in each tissue at various time points was then calculated as the percent injected dose per gram of tissue (%ID/g).

In vivo stability of [^{18}F]-FPMA in plasma and urine was determined following radiotracer administration in normal female nude mice under 2% isoflurane anesthesia. Samples were collected at 1, 5, 30, 60, and 120 min following intravenous injection of [^{18}F]-FPMA. The collected samples of plasma were centrifuged for 10 min at 10,000 *g*. Supernatant from these samples was filtered through 0.2 μ filter and 200 μL plasma supernatant injected into reversed-phase HPLC (column Waters Nova-Pak 4 μ C18 150 × 3.9 mm, CH_3CN/H_2O 25:75, containing 0.1% TFA at flow rate 1 mL/min). Urine samples were also filtered and analyzed in the same HPLC system.

Conclusions

An automated microfluidic radiosynthesis of [^{18}F]-FPMA was developed, using a newly synthesized tosolyate precursor. The radiotracer was obtained in about 50 min total synthesis time and in high yield. *In vivo* data showed rapid blood clearance and accelerated renal elimination of intact radiotracer (at least in the 60 min plasma and urine samples), with little retention of radiotracer in other organs.

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Conflict of Interest

The authors did not report any conflict of interest.

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