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Facile synthesis of *para*-[^{18}F]fluorohippurate via iodonium ylide-mediated radiofluorination for PET renography

Gregory N. Nkepang, Andria F. Hedrick, Vibhudutta Awasthi, Hariprasad Gali *

Department of Pharmaceutical Sciences, College of Pharmacy, The University of Oklahoma Health Sciences Center, 1110 N. Stonewall Avenue, Room 301, Oklahoma City, OK 73117, USA

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ABSTRACT

para-[^{18}F]fluorohippurate ([^{18}F]PFH) is a renal tubular agent suitable for conducting positron emission tomography (PET) renography. [^{18}F]PFH is currently synthesized by a four-step two-pot procedure utilizing a classical prosthetic group, *N*-succinimidyl-4-[^{18}F]fluorobenzoate, followed by glycine conjugation. Considering the short half-life of fluorine-18 (110 min), it is important to reduce the number of synthetic steps and overall production time for successful translation of any fluorine-18 radiopharmaceutical in to clinical practice. Here, we report a new two-step one-pot procedure using a novel spirocyclic iodonium ylide precursor for producing a dose of [^{18}F]PFH suitable for human use in 45 min including HPLC purification with an overall decay-corrected radiochemical yield of 46.4 ± 2.9% (n = 3) and radiochemical purity of >99%.

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Currently there is a need to develop suitable radiotracers to utilize positron emission tomography (PET) in clinical evaluation of renal function. The main advantage of PET is that it enables the use of dynamic tomographic imaging and provides accurate camera-based quantification.[1] The PET quantification is superior to planar gamma imaging, which is predominantly used in current clinical renal imaging studies such as renography. In addition, planar imaging provides limited structural information and lower-quality images. Although drawbacks of planar imaging could be overcome by using single photon emission computed tomography (SPECT), conducting a clinical dynamic SPECT study is technically challenging. The clinical SPECT camera depends on rotational movement (step-and-shoot) of detector heads, which cannot be used for renography due to fast pharmacokinetics exhibited by the renal agents. Whereas PET enables list-mode acquisition of imaging data using a series of detector blocks arranged in a cylindrical geometry around the subject. List-mode provides extremely high temporal resolution with full spatial resolution and allows frame durations to be set after the data acquisition.[2] In addition, clinical PET cameras offer higher sensitivity, spatial resolution, and signal-to-noise ratios than clinical gamma/SPECT cameras.[3] For example, spatial resolution of a clinical PET camera and a clinical gamma/SPECT camera is 4–6 mm and 10–20 mm, respectively.

In this regard, we and others have reported a few potential PET renal agents in recent years.[4–13] Renal agents used for renography generally fall under two categories: filtration agents and tubular secretion agents. Renal filtration agents are only filtered by the glomerulus, whereas tubular secretion agents undergo both glomerular filtration (depending on their molecular weight and plasma protein binding affinity) and tubular secretion. Renal tubular secretion agents are extracted from the plasma present in the peritubular capillaries into the tubular cells via the basolateral membrane governed by the organic anion transporter (OAT) system expressed in the proximal convoluted tubules, for example, OAT1 and OAT3.[14,15] The higher clearance of renal tubular secretion agents due to both glomerular filtration and tubular secretion enables them to provide better quality images and, thus, are preferred radiopharmaceuticals for renography, especially in patients with poor renal function.[16]

Of all the PET renal agents reported so far, only *para*-[^{18}F]fluorohippurate ([^{18}F]PFH) and meta-cyano-*para*-[^{18}F]fluorohippurate ([^{18}F]CNPFH) reported by us are tubular secretion agents.[4–6] Both of them are structural analogs of ortho-[^{131}I]iodohippurate (Hippuran, a gold standard tubular secretion agent).[17] Hippuran labeled with iodine-131 (a beta emitter, half-life—8 d) and iodine-123 (a gamma emitter, half-life—13.2 h) were approved by FDA for clinical use and later discontinued after the introduction of [^{99m}Tc]MAG3 (a renal tubular secretion agent widely used in current clinical renography studies) because of higher costs of iodine-123 and suboptimal nuclear properties of iodine-131 for imaging. Among

* Corresponding author. Tel.: +1 (405) 271 6593x47877; fax: +1 (405) 271 7505.
E-mail address: hgali@ouhsc.edu (H. Gali).

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[^{18}F]PFH and [^{18}F]CNPFH, [^{18}F]PFH was found to be rapidly and exclusively cleared by the kidneys and provided much higher quality renogram and images obtained by dynamic PET studies compared to those obtained with [^{99m}Tc]MAG3 dynamic planar imaging studies.[6] However, [^{18}F]PFH radiosynthesis was long (~95 min including HPLC purification) due to its four-step two-pot procedure utilizing a classical prosthetic group, *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB),[18] followed by glycine conjugation.[4] Considering the short half-life of fluorine-18 (110 min), it is important to reduce the number of synthetic steps and overall production time for successful translation of any fluorine-18 radiopharmaceutical in to clinical practice. Thus, [^{18}F]CNPFH was developed utilizing a simpler process of direct one-step one-pot nucleophilic aromatic substitution reaction.[5] However, biodistribution and PET renography studies in healthy rats showed that [^{18}F]CNPFH would not be an effective agent for renography, because it is eliminated by both renal and hepatobiliary pathways.[5] Our results obtained so far collectively indicate [^{18}F]PFH as a promising lead PET renal tubular secretion agent, that is, highly suitable for PET renography. However, the high potential of [^{18}F]PFH for clinical translation is hampered by its current radiosynthesis method, which would be difficult to be automated by majority of the commercially available automated radiochemical synthesizers.

Thus, here we report a new two-step, one-pot procedure for producing a dose of [^{18}F]PFH suitable for human use in 45 min including HPLC purification and can be automated using any of the commercially available synthesizers. In the present work, we adopted a nucleophilic radiofluorination methodology recently reported by Rotstein et al. utilizing spirocyclic iodonium(III) ylide precursors to efficiently and regiospecifically radiofluorinate non-activated or electron-rich arenes with no-carrier added [^{18}F]fluoride.[19,20] These spirocyclic iodonium(III) ylide precursors were developed to improve the radiochemical yields when compared to the Meldrum's acid derived iodonium(III) ylide precursors initially developed by Satyamurthy and Barrio.[21] Nucleophilic radiofluorination of non-activated arenes is challenging and often requires multistep methodologies, precursors are difficult to handle, and suffer from suboptimal radiochemical yields or poor regiospecificity.[22] In this regard, iodonium(III) ylide based nucleophilic radiofluorination methodologies are very promising for the preparation of non-activated [^{18}F]fluoroarenes and thus are gaining popularity.[19–21,23] In our case, the aromatic ring of [^{18}F]PFH is deactivated for a nucleophilic substitution reaction for radiofluorination using the common leaving groups such as nitro and trimethylammonium salts due to presence of an amide group. Thus, in our previous work, we have started the radiosynthesis with the ethyl 4-(trimethylammonium triflate)benzoate precursor in which the aromatic ring is activated due to presence of an ester group.[4]

The novel ylide precursor **7** employed in the present work was synthesized in five steps as shown in Figure 1a starting from commercially available 4-iodobenzoic acid **1**. In the first step, 4-iodobenzoyl chloride **2** was synthesized by heating **1** in neat thionyl chloride at 80 °C for 2 h followed by removal of excess thionyl chloride by distillation. The compound **2** was obtained in a quantitative yield and used without any further purification. In the second step, **2** was reacted with glycine in presence of aqueous sodium bicarbonate and subsequent purification with solvent extractions provided 4-iodoippuric acid **3** in 87.6% yield. In the third step, **3** was reacted with an excess of ethanol in presence of thionyl chloride and subsequent purification with silica gel chromatography provided ethyl-4-iodohippurate **4** in 64.1% yield. In the fourth step, the iodine present on **4** was oxidized with sodium perborate in presence of glacial acetic acid at 50 °C and subsequent purification by recrystallization from a mixture of hexane and methylene chloride provides **5** in a near quantitative yield. In the final step, treatment of **5** with the auxiliary **6**, which was synthesized according the reported procedure,[19] in aqueous sodium carbonate and subsequent purification with silica gel chromatography provided the ylide precursor **7** in 40.9% yield.

The radiosynthesis of [^{18}F]PFH using ylide precursor **7** was performed manually as shown in Figure 1b. Our goal was to obtain an injectable dose of >15 mCi [^{18}F]PFH in <8 ml volume starting from <75 mCi [^{18}F]fluoride in <60 min (radiosynthesis and purification). We selected <75 mCi [^{18}F]fluoride as starting activity because we

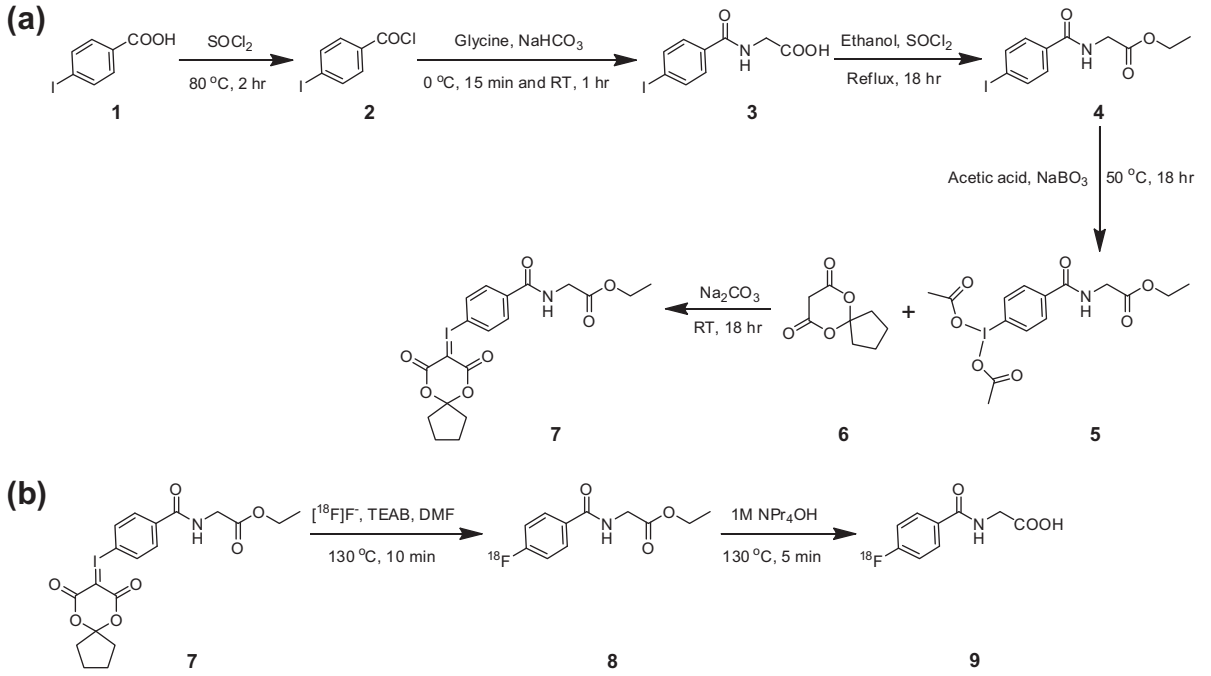

Figure 1. Synthesis of (a) iodonium ylide precursor and (b) [^{18}F]PFH.

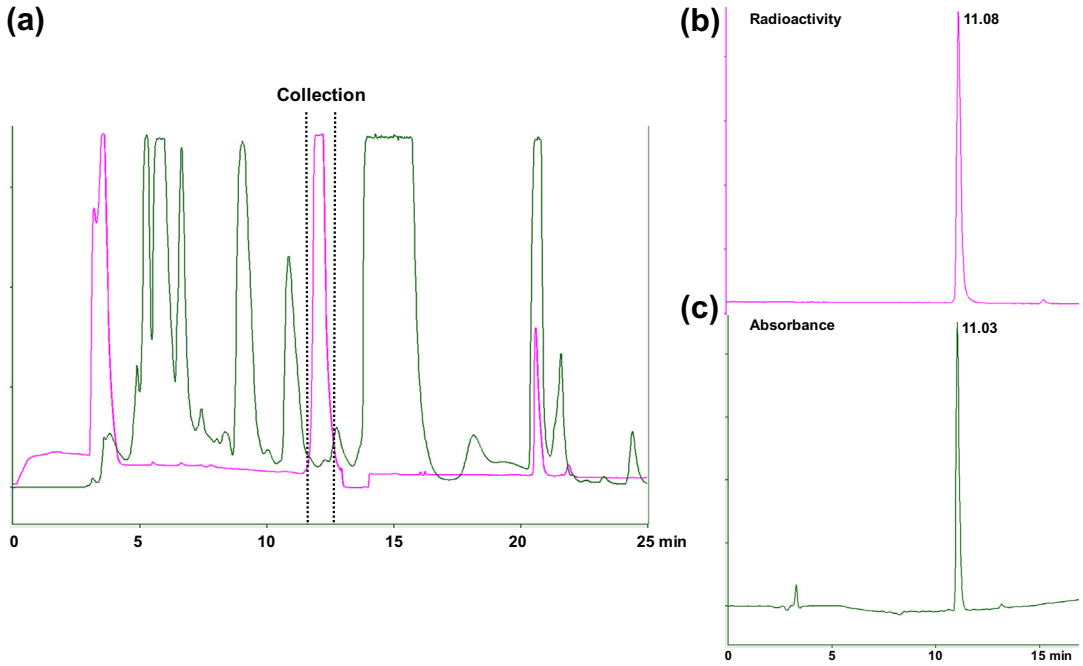

Figure 2. HPLC chromatograms of (a) [^{18}F]PFH batch obtained while purification, (b) [^{18}F]PFH obtained while quality control, and (c) non-radioactive PFH standard.

can consistently produce [^{18}F]fluoride in that range using our cyclotron (BG-75 Biomarker Generator, ABT Molecular Imaging) in 90 min of bombardment with a beam current of 5 μA. The radiosynthesis conditions were based on the previously reported general procedure (tetraethylammonium bicarbonate (TEAB), DMF, 120 °C, and 10 min) for the radiofluorination of the diaryliodonium ylide precursors.[19] However, increasing the temperature from 120 °C to 130 °C for both radiofluorination and hydrolysis steps almost doubled the overall radiochemical yield of [^{18}F]PFH. Briefly, the radiofluorination of **7** was carried out in DMF using TEAB as a phase-transfer agent in a sealed condition. The hydrolysis was carried out using 1 M tetrapropylammonium hydroxide with a flow of nitrogen stream to enable DMF evaporation while the reaction is proceeded to completion. After the hydrolysis the product mixture was diluted with water and purified by semi-preparative HPLC using PBS–ethanol (90/10 v/v). Total radiosynthesis time was 45 min including HPLC purification and [^{18}F]PFH was obtained with an overall decay-corrected radiochemical yield of 46.4 ± 2.9% (n = 3). As shown in Figure 2a, [^{18}F]PFH is clearly separated from unwanted radioactive and non-radioactive peaks. The radiochemical purity of [^{18}F]PFH was found to be >99% after HPLC purification (Fig. 2b) and its retention time matches well with the non-radioactive standard (Fig. 2c), which confirms its radiochemical identity. The total volume of the collected fraction was <6 ml and activity obtained was 17.1 ± 1.5 mCi (n = 3) starting from 50.1 ± 2.4 mCi (n = 3) of [^{18}F]fluoride. Although the specific activity was not determined, it is estimated to be >1 Ci/μmol based on the [^{18}F]PFH peak area in the absorbance chromatogram obtained during the purification (Fig. 2a) in comparison to the peak area of the non-radioactive PFH standard of know concentration and the collected activity. Further optimization of the reaction conditions was not pursued as our goal was achieved. This radiosynthesis and purification can be readily automated using any commercially available synthesizer as it is a single-pot reaction. The experimental details and characterization data for compounds **3**–**5**, **7**, and [^{18}F]PFH are given in Reference and notes.[24–26]

Table 1
Biodistribution (%ID/g) of [^{18}F]PFH at 10 min p.i. in healthy female Sprague Dawley rats

Organ	Ylide method	[^{18}F]SFB method[4]
Blood	0.23 ± 0.08	0.26 ± 0.01
Muscle	0.04 ± 0.01	—
Heart	0.09 ± 0.03	0.08 ± 0.01
Lung	0.14 ± 0.05	0.17 ± 0.04
Liver	0.07 ± 0.02	0.09 ± 0.01
Spleen	0.08 ± 0.03	0.06 ± 0.01
Stomach	0.10 ± 0.07	0.12 ± 0.07
Intestine	0.07 ± 0.02	0.09 ± 0.03
Kidney	4.21 ± 1.69	2.93 ± 1.52
Bone	0.04 ± 0.03	—
Urine (%ID)	73.4 ± 7.1	72.1 ± 6.4

Values are expressed as the mean ± SD (n = 4). Data for [^{18}F]SFB method was taken from Ref. 4.

We conducted in vivo evaluation of [^{18}F]PFH obtained by ylide method in healthy rats to confirm its biological activity. As shown in Table 1, the biodistribution data of [^{18}F]PFH obtained by ylide method at 10 min p.i. matches well with our previously reported data for [^{18}F]PFH obtained by [^{18}F]SFB method indicating that both methods provide identical product.[4] Almost no bone uptake was observed, which indicates that no free [^{18}F]fluoride was present in the injected dose and no defluorination occurred in vivo. Figure 3 shows the PET renogram and images obtained in a healthy rat with [^{18}F]PFH produced by ylide method, which were found to be similar to that of other healthy Sprague Dawley rats previously reported.[4–6]

In summary, the novel ylide precursor **7** employed in this work produced the desired [^{18}F]PFH in relatively high radiochemical yield and purity. Although the reported method was performed manually, this two-step one-pot reaction can be readily adopted for producing [^{18}F]PFH using any commercially available automated radiochemical synthesizer. Thus, the radiosynthesis presented here provides a very practical method for producing clinical doses of [^{18}F]PFH under cGMP compliance.

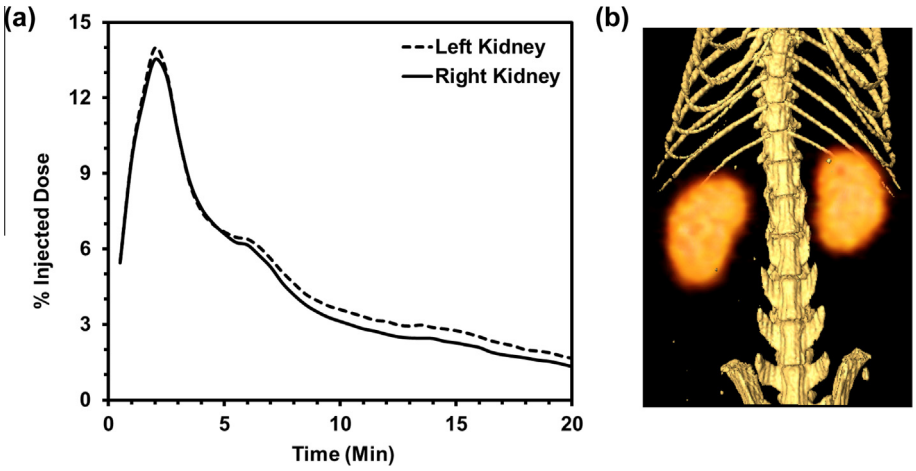

Figure 3. (a) Kidney time–activity curves (renogram) and (b) lower abdominal maximum intensity projection PET/CT images at T_{max} of a healthy rat injected with [^{18}F]PFH synthesized with new method.

Acknowledgements

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24. *Synthesis of ylide precursor* **7**. The general reaction scheme for the synthesis of ylide precursor **7** is in Figure 1a. *Synthesis of 4-iodobenzoyl chloride* (***2***): 4-Iodobezoic acid (6.50 g, 26.21 mmol) and thionyl chloride (20 ml) were heated at 80 °C for 2 h. Excess thionyl chloride was distilled off and directly used for the next step without further purification. *Synthesis of 4-iodohippuric acid* (***3***): Glycine (1.97 g, 26.21 mmol) was added to a 150 ml solution $NaHCO_3$ (11.01 g, 131.04 mmol) dissolved in acetone–water mixture (1:3 v/v) and stirred for 10 min at 0 °C. Then **2** (26.21 mmol) dissolved in 5 ml dry acetone was added dropwise over 15 min. The reaction temperature was raised and maintained at room temperature for further 60 min. After completion, the mixture was acidified with 5 M H_2SO_4 (pH−2) and evaporated to dryness under high vacuum. The solid residue was extracted with methanol (3 × 300 ml) and the methanolic filtrate evaporated to afford an impure compound that was further extracted into diethyl ether and dried to afford 7.0 g of pale white solid (87.6% yield). ^{1}H NMR (300 MHz, CD_3OD-d_4): δ 7.83 (d, 2H, J = 8.39 Hz, Ar-H), 7.59 (d, 2H, J = 8.39 Hz, Ar-H), 4.07 (m, 4H, –CH_2–), 3.30 (s, 1H, NH). ^{13}C NMR (75 MHz, CD_3OD-d_4): δ 171.56, 137.29, 128.64, 98.12, 40.82. HRMS (ESI): calcd for $C_9H_9INO_3$ [M+H]$^+$:305.9627, found 305.9606. *Synthesis of ethyl-4-iodohippurate* (***4***): **3** (4.00 g, 13.12 mmol) was suspended in 100 ml of absolute ethanol and mixture chilled to 0 °C using an ice bath. Thionyl chloride (1.13 ml, 15.70 mmol) was added dropwise to the stirring solution. Upon addition the solid disappeared completely. The ice bath was removed and the reaction mixture was heated to reflux overnight. Ethanol was removed by rotary evaporation to give a brownish solid which was further purified by a flash column chromatography on silica gel using ethyl acetate–hexane (8:2 v/v) as eluent to afford a yellow stick oil which was then precipitated at −25 °C using acetone–hexane (1:7 v/v) into a 2.8 g white fluffy solid (64.1% yield). ^{1}H NMR (300 MHz, $CDCl_3$-d): δ 7.79 (d, 2H, J = 8.39 Hz, Ar-H), 7.53 (d, 2H, J = 8.39 Hz, Ar-H), 6.67 (s, 1H, –NH), 4.23 (m, 4H, –CH_2), 1.30 (t, 3H, J = 7.19 Hz, –CH_2–CH_3^*). ^{13}C NMR (75 MHz, $CDCl_3$-d): δ 170.01, 137.84, 128.64, 98.87, 61.79, 41.89, 14.12. HRMS (ESI): calcd for $C_{11}H_{12}INO_3Na$ [M+Na]$^+$:355.9760, found 355.9755. *Synthesis of ethyl-4-(diacetoxy-iodo)hippurate* (***5***): Sodium perborate tetrahydrate (6.96 g, 45.03 mmol) was added in portions to a 0.15 M of **4** (1.50 g, 45.03 mmol) in glacial acetic acid (28.50 ml) and heated to 50 °C. The reaction mixture was stirred at this temperature overnight, cooled to room temperature, diluted with water, and extracted three times with methylene chloride (3 × 200 ml). The combined organic extracts were dried with anhydrous sodium sulfate, filtered, and concentrated. The product was purified by recrystallization from hexane–methylene chloride (9:1 v/v) at −20 °C to afford a 2.01 g white solid (99.0% yield). It should be noted that this oxidized product does not stain with iodine and the spot is characteristically pure white spot on silica gel TLC. ^{1}H NMR (300 MHz, $CDCl_3$-d): δ 7.79 (d, 2H, J = 8.38 Hz, Ar-H), 7.53 (d, 2H, J = 8.39 Hz, Ar-H), 6.69 (s, 1H, –NH), 4.23 (m, 4H, –CH_2–), 2.08 and 2.10 (S, 6H, –C(O)–CH_3), 1.30 (t, 3H, J = 6.89 Hz, –CH_2–CH_3^*). ^{13}C NMR (75 MHz, $CDCl_3$-d): δ 169.97, 138.04, 129.16, 61.83, 41.84, 13.80. *Synthesis of the ylide precursor* (***7***): To a solution of the auxiliary **6** (0.33 g, 1.95 mmol) in 10% aqueous sodium carbonate (6 ml) was added 6 ml of ethanol. This mixture was vigorously stirred and immediately followed by the addition of **5** (0.82 g, 1.95 mmol). The vigorously stirring solution was allowed to react at room temperature overnight. At the end of the reaction as determined by the TLC in ethyl acetate, the reaction mixture was diluted with water and extracted with methylene chloride (3 × 300 ml). Combined organic extracts were dried and concentrated. Flash column chromatography with silica gel using first ethyl acetate and later with ethyl acetate–methanol (9:1 v/v) as eluents afforded 400 mg of white solid (40.9% yield). This compound had solubility problems and most of it was lost during column chromatography. Best solvent of solubility is methanol. Also note that this compound does not stain with iodine. ^{1}H NMR (300 MHz, CD_3OD-d_4): δ 8.00 (d, 2H, J = 8.39 Hz, Ar-H), 7.88 (d, 2H, J = 8.39 Hz, Ar-H), 4.20 (q, 2H, J = 7.19 Hz, –CH_2^*–CH_3), 4.10(s, 2H, –C(O)CH_2^*–), 2.09 (t, 2H, J = 7.19 Hz, –CH_2^*–CH_2–) 1.78 (t, 2H, J = 7.19 Hz, –CH_2–CH_2^*–), 1.30 (t, 3H, J = 7.19 Hz, –CH_2–CH_3^*). ^{13}C NMR (75 MHz, CD_3OD-d_4): δ 169.78, 167.43, 165.65, 136.56, 133.25, 129.53, 118.45, 113.72, 60.98, 58.49, 41.21, 36.69, 22.71. HRMS (ESI): calcd for $C_{19}H_{20}INO_7Na$ [M+Na]$^+$: 524.0182 and $C_{38}H_{40}I_2NO_{14}Na$ [2M+Na]$^+$: 1025.0467, found 524.0171 and 1025.0457.
25. *Radiosynthesis of [^{18}F]PFH using ylide precursor* **7**. The general reaction scheme for the radiosynthesis of [^{18}F]PFH is shown in Figure 1b. Briefly, [^{18}F]fluoride was produced by irradiating enriched [^{18}O]-water with protons. [^{18}F]Fluoride (50.1 ± 2.4 mCi, n = 3) dissolved in [^{18}O]-water (560 µl) was transferred using inert gas pressure to a reaction vial (5-ml serum vial) containing ~8 mg of

tetraethylammonium bicarbonate (TEAB) and acetonitrile (200 μl). The vial was then sealed with a butyl rubber stopper and an aluminum crimp. Two 21 gauge 1″ needles were inserted in to the vial for nitrogen purging and venting. All reagents were added using a 500 μl or 1 ml insulin syringe attached with a 29 gauge 0.5″ needle. The solvents were evaporated under a stream of nitrogen (~2.3 psi) at 130 °C for 12 min. Azeotropic drying was then achieved by adding anhydrous acetonitrile (500 μl) to the reaction vial and the solvents were evaporated under a stream of nitrogen (~2.3 psi) at 130 °C for 3 min.. Then ylide precursor (~10 mg) dissolved in anhydrous dimethylformamide (1 ml) was added to the dried [TEAB]^{18}F complex and the reaction mixture was heated in the sealed vial at 130 °C for 10 min to achieve ^{18}F-labeling. Next 1 M tetrapropylammonium hydroxide (200 μl) was added and the reaction mixture was heated at 130 °C for 5 min under a stream of nitrogen (~2.3 psi) to achieve simultaneous deprotection and drying of the solvents. The reaction vial was removed from the heating block, the reaction mixture was diluted with deionized water (1.5 ml), and injected in to a semi-preparative HPLC (column: Sonoma C18, 10 μm, 10 × 250 mm) for separating the resulting [^{18}F]PFH from the other compounds in the reaction mixture. A sterile mobile phase consisting of 90% phosphate-buffered saline and 10% 200-proof absolute ethanol with a flow rate of 4 ml/min was used for purification. A fraction from 11.6 min to 12.9 min was collected (17.1 ± 1.5 mCi, n = 3) in a 10 ml sterile vented empty vial via passing through a 0.22 μm 33 mm PES syringe filter. The overall decay-corrected radiochemical yield was 46.4 ± 2.9% and radiochemical purity was >99%. Representative HPLC chromatograms are shown in Figure 2.

26. *Analytical HPLC conditions*. HPLC solvents consisted of water containing 0.1% v/v trifluoroacetic acid (solvent A) and acetonitrile containing 0.1% v/v trifluoroacetic acid (solvent B). A Sonoma C_{18} column (ES Industries, West Berlin, NJ, 5 μm, 100 Å, 4.6 mm × 250 mm) was used with a flow rate of 1.5 ml/min. The HPLC gradient system began with an initial solvent composition of 95% A and 5% B for 2 min followed by a linear gradient to 50% A and 50% B in 15 min, after which the column was reequilibrated. The absorption detector was set at 254 nm.