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Synthesis of [^{18}F] 4-amino-*N*-(3-chloro-4-fluorophenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide (IDO5L): a novel potential PET probe for imaging of IDO1 expression

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Abstract

To synthesize ^{18}F -labeled positron emission tomography (PET) ligands, reliable labeling techniques inserting ^{18}F into a target molecule are necessary. The ^{18}F -fluorobenzene moiety has been widely utilized in the synthesis of ^{18}F -labeled compounds. The present study utilized [^{18}F]-labeled aniline as intermediate in [^{18}F]-radiolabeling chemistry for the facile radiosynthesis of 4-amino-*N*-(3-chloro-4-fluorophenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide ([^{18}F]IDO5L) as indoleamine 2,3-dioxygenase 1 (IDO1) targeted tracer. IDO5L is a highly potent inhibitor of IDO1 with low nanomolar IC_{50} . [^{18}F]IDO5L was synthesized via coupling [^{18}F]3-chloro-4-fluoroaniline with carboximidamidoyl chloride as a potential PET probe for imaging IDO1 expression. Under the optimized labeling conditions, chemically and radiochemically pure (>98%) [^{18}F]IDO5L was obtained with specific radioactivity ranging from 11 to 15 GBq/ μmol at the end of synthesis within ~90 min, and the decay-corrected radiochemical yield was $18.2 \pm 2.1\%$ ($n = 4$).

Keywords

indoleamine 2; 3-dioxygenase; IDO1; PET imaging; fluorine-18; radiolabeling

Introduction

Indoleamine 2,3-dioxygenase-1 (IDO1) catabolizes the L-tryptophan (Trp) to yield L-*N*-formylkynurenine (Kyn), which is the initial and rate-limiting step in Trp degradation pathway. IDO1 is expressed in a variety of tissues and particularly high level expression observed in placenta of pregnant females, various human tumor cells, and dendritic cells that localize to the tumor-draining lymph nodes.^{1–3} Increasing *in vivo* and *in vitro* pieces of evidence implicated that IDO1 is involved in immune escape of tumor cells, and blockage of its activity can directly increase the ability of tumor-bearing mice to reject tumors.^{2,4–7} Recent studies demonstrated that the expression level of IDO increased in various tumors

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Conflict of Interest

The authors did not report any conflict of interest.

such as in lungs, prostate, and pancreas, lymphoma, and breast cancers. It is reported that patients with high level of IDO1 expression were correlated with later clinical phases and larger tumors and indicated a worse prognosis in various cancers such as diffuse large B-cell lymphoma and chronic lymphocytic leukemia.^{8–10} Currently, two IDO1 inhibitors 1-methyl-D-tryptophan and INCB024360 are used in phase II clinical trials for the treatment of breast cancer and melanoma, respectively.¹¹

Molecular imaging methods such as positron emission tomography (PET) have potential to generate IDO1 expression profile *in vivo* and provide valuable information on how the IDO pathway responds to the immune-modulating therapies. α -[¹¹C] methyl-L-tryptophan (α -¹¹C-AMT), an IDO1 substrate, has been reported to be associated with the IDO1 expression levels in the brain and lung tumors.^{12,13} However, IDO1 only was involved in the first step of the kynurenine pathway, while increased α -¹¹C-AMT uptake by cells is a complicated issue because of many other enzymes involved in tryptophan transportation and metabolism pathway. Therefore, there is urgent need to develop a specific PET imaging agent targeted to IDO1 for cancer imaging.

Among all potent inhibitors reported in literature, the most potent inhibitor of IDO1 is INCB024360 (IC₅₀ = 7.1 nM, HeLa cell assay).⁶ However, the structure of INCB024320 has not yet been disclosed. The carboximidamide compound IDO5L is one of the highest potent inhibitors of the IDO1 (IC₅₀ = 19 nM, HeLa cell assay).¹⁴ Moreover, the reported rapid clearance rate (t_{1/2} < 0.5 h, via oral administration) of IDO5L indicated the faster clearance rate after i.v administration during PET imaging. This fast clearance may be preferred for ¹⁸F-PET imaging agent for less imaging background. We believe that fluorine-18-labeled carboximidamide compound IDO5L can serve as a novel probe for the PET imaging to generate IDO1 activity profile *in vivo* that is useful to predict IDO1-related cancer diagnostic and monitor therapeutic efficacy of IDO1 inhibitors. For the first time, we synthesized ¹⁸F-carboximidamide compound IDO5L as a novel potential PET agent by utilizing [¹⁸F]3-chloro-4-fluoroaniline as intermediate.

Results and discussion

Chemistry

The reference compound IDO5L (4-amino-*N*-(3-chloro-4-fluorophenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide) was synthesized using the reported three-step method with minor modification, which is illustrated in Scheme 1.¹⁴ Firstly, malononitrile was treated with hydroxylamine, sodium nitrite, and hydrochloric acid to give hydroxyamidine **2** in 91% yield. The hydroxyamidine **2** was then diazotized under acidic conditions to provide the hydroximoyl chloride **2** in 57% yield. Coupling of compound **2** with 3-chloro-4-fluoroaniline was carried in the basic conditions to produce IDO5L as brown powder in the yield of 88%.

The synthesis of the two triflate precursors **6** and **10** started from commercially available 2-chloro-4-nitroaniline **4** (Scheme 2). Aniline **4** was deprotonated by sodium hydride followed by methylation to afford 2-chloro-*N,N*-dimethyl-4-nitroaniline **5** in 79% yield. After further methylation of compound **5**, the triflate precursor **6** was obtained in 76% yield. To produce

another triflate precursor **10**, the nitro group of compound **5** was firstly reduced to aniline **7** in 99% yield by sodium borohydride under the catalysis of palladium on activated charcoal.¹⁵ Compound **8** was then achieved by coupling aniline **7** and compound **3** in 96% yield under the basic conditions. The *N*-hydroxyamidine group of compound **8** was then cyclized with 1,1'-carbonyldiimidazole to give compound **9** in 92% yield. Finally, compound **9** was methylated by methyl trifluoromethanesulfonate to produce the triflate **10** in the yield of 82%.

Radiochemistry

Because it is attractive as a one-step radiolabeling procedure, aryltrimethylammonium was often used by many as a leaving group in the nucleophilic substitution of no-carrier-added [¹⁸F] fluoride, the triflate precursor, can be used for ¹⁸F-labeling strategy to replace the conventional complex and long process of multiple-step radiolabeling procedure, which shortens reaction time and labor significantly. The synthesis of the target tracer ¹⁸F-IDO5L (Scheme 3) was performed initially by the conventional Kryptofix-mediated nucleophilic ¹⁸F-substitution of triflate precursor **10** followed by NaOH hydrolysis. However, not ¹⁸F-IDO5L but unlabeled ¹⁸F anion was observed in the radio-HPLC profiles of the reaction mixture under various reaction conditions such as different solvents (dimethyl sulfoxide (DMSO)/MeCN), temperature (90°C/120°C), and reaction time (5–30 min). Analysis of reaction samples before NaOH hydrolysis found that the precursor **10** decomposed to compound **8** and compound **9** as well as other unknown compounds.

Considering that the target product IDO5L is stable under the labeling conditions, we believed that the failure of labeling is because of the difficulty of nucleophilic aromatic fluorination on the weak activated aromatic ring and the ease of decomposition of the carboximidamide protection group and trimethylammonium triflate salts **10**.

Although attractive as a one-step radiolabeling procedure, the triflate precursor failed to give the target [¹⁸F]IDO5L. Consequently, a three-step radiochemical route has been established by using [¹⁸F]-labeled aniline as intermediate (Scheme 3). The 3-chloro-4-[¹⁸F]fluoroaniline intermediate was synthesized using the reported two-step method with minor modification.^{15,16} The first step involved the nucleophilic aromatic substitution of triflate precursor **6** by Kryptofix 222/K₂CO₃-activated [¹⁸F] in acetonitrile. After optimization, the labeling reaction was completed in 5 min with 98% labeling yield (determined by radio-thin-layer chromatography (TLC)) at 70°C (Figure 1a). Compared with the reported labeling conditions (room temperature in 25 min),¹⁵ our method saved 20-min reaction time while still achieved good labeling yield. The solvent and reagents used in nucleophilic aromatic substitution were removed by solid-phase extraction on C18 Sep-Pak.

Then, the nitro group of **18F-11** was reduced by sodium borohydride under the catalysis of palladium on activated charcoal and constant stirring at room temperature for 5 min to afford **18F-12** in 89–96% labeling yield determined by radio-TLC (Figure 1b). It is worth to mention that relatively lower labeling yield (49–60%) was observed without using stirrer because of poor mixing of reactant and catalyst (Table 1). The excess NaBH₄ was quenched by concentrated hydrochloric acid, and the reaction mixture was passed through 0.2-μm

filter to remove Pd/C. After being dried at 100°C under a stream of nitrogen, the residue **18F-12** was redissolved in methanol. The overall radiochemical yields (decay corrected, based on starting [¹⁸F]fluoride) are 47–64%, which is less than the reported yield (58–72%),¹⁵ possibly because ~25% activity was volatilized during the solvent removal process.

The final step is the coupling of compound **3** with **18F-12** to give [¹⁸F]IDO5L under base conditions. To neutralize remaining HCl and basicify the reaction mixture, excess amount of three different bases (100 µL Et₃N, 300 µL 1 M NaHCO₃, and 150 µL 1 M Na₂CO₃) was tested. Among them, NaHCO₃ gave the best labeling yield (51–64%) of [¹⁸F]IDO5L determined by radio-TLC (Figure 1c), while Et₃N produced no labeled product, and Na₂CO₃ gave low radiochemistry yield (<40%). Considering the absence of compound **3** and the presence of unreacted **18F-12** in HPLC profiles of the crude products, we believed that decomposition of compound **3** under base condition is a major side reaction. Therefore, lower labeling yield achieved under Na₂CO₃ can be explained as a stronger base caused faster decomposition of compound **3**. After filtration, [¹⁸F]IDO5L was purified by preparative HPLC and eluted at 10.9–11.7 min (Figure 2). Formulation of the labeled product for i.v. injection was prepared as follows: The HPLC solvents were first diluted with water and then passed through C18 Sep-Pak column. After being washed with water, the [¹⁸F]IDO5L was eluted by methanol. The elution solvent was removed by evaporation, and the residue was redissolved in physiological saline. The identity of the new tracer [¹⁸F]IDO5L was confirmed in HPLC analysis by coinjection with IDO5L (Figure 3). Typically, starting from 0.34 to 0.74 GBq (9.2–19.9 mCi) [¹⁸F]fluoride, 35 to 77 MBq (0.94–2.09 mCi) of purified [¹⁸F]IDO5L could be obtained in ~90 min. The overall three-step decay-corrected radiochemical yield was 18.2 ± 2.1% (*n* = 4) with the radiochemical purity exceeding 98%. Specific activity, determined by using online measurements of radioactivity and UV absorption, was 11–15 GBq/µmol at end of synthesis.

Conclusion

In conclusion, we explored [¹⁸F]-labeled aniline as intermediate in [¹⁸F]-radiolabeling chemistry for the facile radiosynthesis of 4-amino-*N*-(3-chloro-4-fluorophenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide [¹⁸F]IDO5L as a novel IDO1-targeted tracer. The tracer has been synthesized and optimized by a three-step radiolabeling method with good radiochemical yield. PET imaging studies are currently being carried out in various cancer animal models in order to evaluate the *in vivo* potential of this IDO1 inhibitor.

Experimental

General

All reagents and solvents were purchased from Sigma-Aldrich or Fluka and used as received without further purification. Solid-phase extraction cartridges (Sep-Pak QMA and Sep-Pak C18) were purchased from Water Corp., Milford, MA, USA. Column chromatography was performed on silica gel (60 Å, 230–400 mesh, for flash chromatography). TLC was performed on aluminum plates precoated with silica (200 µm, 60 F₂₅₄), which were visualized either by quenching of ultraviolet fluorescence (λ_{max} = 254 nm) or by iodine

stain. ^1H and ^{13}C spectra were obtained on a Varian Mercury 400-MHz spectrometer with CDCl_3 , $\text{MeCN}-d_3$, and $\text{DMSO}-d_6$ as the solvent. All coupling constants were measured in hertz (Hz), and the chemical shifts (δ_{H} and δ_{C}) were quoted in parts per million relative to the internal standard tetramethylsilane (δ 0). ^{19}F chemical shifts were measured with respect to CFCl_3 . High-resolution mass spectroscopy (HRMS) was carried out on an Agilent 6210 LC-MS (ESI-time of flight).

HPLC analysis and purification were performed on Agilent 1260 using an in-line UV detector (254 nm) and a NaI crystal flow-count radioactivity detector (Lablogic Flow-RAM detector). The analytical HPLC was performed on an Agilent Eclipse XDB C18 column (5 μm , 4.6×250 mm) with the flow rate 1.0 mL/min using $\text{MeCN}/0.1\%$ acetic acid in H_2O 50/50, 12/88, or 40/60 as an eluent. Semipreparative HPLC purification system was performed on an Agilent Eclipse XDB C18 column (5 μm , 9.6×250 mm) with the flow rate 5.0 mL/min using $\text{MeCN}/0.1\%$ acetic acid in H_2O 40/60 for 20 min. Radio-TLC Imaging Scanner (AR-2000, BioScan USA) was used for the radiochemical purity measurements. A dose calibrator (ATOMLAB 500, Biodex) was used for all radioactivity measurements.

Chemistry

4-Amino-N-hydroxy-1,2,5-oxadiazole-3-carboximidamide (2)—Malononitrile (13.2 g, 200 mmol) was added to preheated water (280 mL, 45°C) and stirred for 5 min. The resulting solution was cooled in an ice/water bath, and sodium nitrite (15.18 g, 220 mmol) was added and stirred for 5 min. Then, HCl (10 N, 13.2 mL) was added to start the mild exothermic reaction, while bubbles were observed. After 3 min, the ice/water bath was removed, and the reaction mixture was stirred 1.5 h at room temperature. The hydroxylamine (39.6 g, 600 mmol) was added to the light yellow reaction mixture in an ice/water bath. After being stirred at room temperature for 1 h, the reaction mixture was refluxed for 2 h and then cooled in an ice/water bath. HCl (10 N, 32.0 mL) was added in portions to the reaction mixture till neutral ($\text{pH} = 7.0$ via a pH meter). The precipitate was collected by filtration, washed well with water, and dried in a vacuum (oil pump) to afford the desired product **2** (26.0 g, 91%).

R_f (ethyl acetate/hexanes: 1/1 (v/v)): 0.22.

^1H -NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.46 (s, 1 H, OH), 6.27(s, 2H, NH_2), and 6.19 (s, 2H, NH_2).

^{13}C -NMR (100 MHz, CD_3OD): δ 155.86, 145.73, and 141.13.

HRMS Calcd for $\text{C}_3\text{H}_6\text{N}_5\text{O}_2\text{Na}$ [$\text{M} + \text{Na}$] $^+$, 166.0336, found, 166.0337.

4-Amino-N-hydroxy-1,2,5-oxadiazole-3-carboximidamidoyl chloride (3)—4-Amino-N-hydroxy-1,2,5-oxadiazole-3-carboximidamide (**2**; 14.31 g, 100 mmol) and NaCl (17.0 g, 290 mmol) were added to a mixture of water (190 mL), acetic acid (100 mL), and HCl (10 M, 50 mL). The suspension was stirred at room temperature till complete solution was achieved and then cooled by an ice/water bath. A solution of sodium nitrite (6.81 g, 98 mmol) in water (24.0 mL) was added, and the reaction mixture was stirred from 0°C to room

temperature overnight. The white precipitate was collected by filtration, washed well with water, taken in ethyl acetate, and dried over anhydrous Na₂SO₄. The suspension was filtered, and the filtrate was evaporated on a rotary evaporator to offer the product **3** (9.24 g, 57%).

¹H-NMR (400 MHz, DMSO-*d*₆): δ 13.40 (s, 1 H, OH) and 6.29(s, br, 2H, NH₂).

¹³C-NMR (100 MHz, CD₃OD): δ 155.21, 142.91, and 129.20.

4-Amino-N-(3-chloro-4-fluorophenyl)-N'-hydroxy-1,2,5-oxadiazole-3-carboximidamide (IDO5L)—4-Amino-*N*-hydroxy-1,2,5-oxadiazole-3-carboximidoyl chloride (**3**; 1.63 g, 10.0 mmol) and 3-chloro-4-fluoroaniline (1.60 g, 11.0 mmol) were added in ethanol (40 mL). Then, NaHCO₃ (2.10 g, 25.0 mmol) in water (20 mL) was added. The reaction was stirred and heated at 60°C for 1 h. The reaction mixture was concentrated by a rotary evaporator and dissolved in ethyl acetate (100 mL), after being washed by brine (50 mL) twice and dried over Na₂SO₄. After solvent evaporation, the residue was recrystallized in ethyl acetate/hexanes to afford **IDO5L** (2.38 g, 88%) as a brown solid.

R_f (ethyl acetate/hexanes: 1/1 (v/v)): 0.45.

¹H-NMR (400 MHz, DMSO-*d*₆): δ 11.41 (s, br, 1 H), 8.91(s, br, 1 H), 7.21 (dd, *J* = 9.2 and 9.2 Hz, 1 H), 6.98 (dd, *J* = 6.4 and 2.8 Hz, 1 H), 6.72–6.76 (m, 1 H), and 6.27 (s, 2 H).

¹³C-NMR (100 MHz, DMSO-*d*₆): δ 155.3, 152.2 (d, *J*_{C-F}¹=239.3Hz), 140.4, 139.3, 137.9 (d, *J*_{C-F}³=2.9Hz), 122.0, 120.8 (d, *J*_{C-F}³=6.8Hz), 118.7 (d, *J*_{C-F}²=18.5Hz), and 116.2 (d, *J*_{C-F}²=21.7Hz).

HRMS calculated for C₉H₈ClFN₅O₂ [M + H]⁺: *m/z* = 272.0345, found 272.0340.

2-Chloro-N,N-dimethyl-4-nitroaniline (5)—2-Chloro-4-nitroaniline **4** (5.18 g, 30.0 mmol) in 60 mL anhydrous THF was added to 60% NaH (60%, 2.64 g, 66.0 mmol) under argon protection at 0° C. The resulting solution was stirred in an ice/water bath for 10 min and then stirred for 30 min at room temperature. Methyl iodide (10.65 g, 75.0 mmol) was added to the reaction mixture and stirred for 17 h at room temperature. Then, ice (~10.0 g) and water (40 mL) were added to the reaction mixture to quench the reaction. After 5 min, the organic solvent was removed by vacuum. The aqueous layer was extracted by ethyl acetate (3 × 100 mL), and the combined organic layer was washed by brine (100 mL) and dried over anhydrous Na₂SO₄. After solvent evaporation, the resulting residue was purified by flash chromatography (SiO₂) and eluted with ethyl acetate/hexanes (1/19, v/v) to afford **5** (4.73 g, 79%) as a yellow solid.

R_f (ethyl acetate/hexanes: 1/19 (v/v)): 0.20.

¹H-NMR (400 MHz, CDCl₃): δ 8.18 (d, *J* = 2.8 Hz, 1H, Ar-H), 8.03 (dd, *J* = 9.2 and 2.8 Hz, 1H, Ar-H), 6.96 (d, *J* = 8.8 Hz, 1H, Ar-H), and 3.01 (s, 6H, N(CH₃)₂).

¹³C-NMR (100 MHz, CDCl₃): δ 155.4, 140.6, 127.1, 124.7, 123.3, 117.8, and 43.0.

HRMS calculated for $C_8H_9ClN_2O_2$ $[M + H]^+$: $m/z = 201.0425$, found 201.0433.

2-chloro-*N,N,N*-trimethyl-4-nitrobenzenaminium trifluoromethanesulfonate (6)

—To a solution of 2-chloro-*N,N*-dimethyl-4-nitroaniline (0.60 g, 3.0 mmol) in anhydrous dichloromethane (20 mL), methyl trifluoromethanesulfonate (0.99 g, 6.0 mmol) was added under argon protection at room temperature. The resulting red solution was stirred for 24 h at room temperature, and precipitation was observed. The off-white precipitate was collected by filtration and washed well with dichloromethane (3×20 mL) and ethyl ether (20 mL). The precipitate was then evaporated on a rotary evaporator and dried by vacuum to offer off the product **6** (1.09 g, 76%) as a white solid.

^1H -NMR (400 MHz, DMSO- d_6): δ 8.60 (d, $J = 2.4$ Hz, 1 H, Ar-H), 8.41 (dd, $J = 9.2$ and 2.4 Hz, 1 H, Ar-H), 8.34 (d, $J = 9.2$ Hz, 1 H, Ar-H), and 3.85 (s, 9 H, $N(CH_3)_3$).

^{13}C -NMR (100 MHz, DMSO- d_6): δ 148.7, 146.3, 129.3, 127.7, 126.5, 124.1, 121.1 (q, $J_{C-F}^1 = 320.2$ Hz, CF_3), and 56.2.

HRMS calculated for $C_9H_{12}ClN_2O_2$ $[M]^+$: $m/z = 215.0587$, found 215.0587.

2-chloro-*N,N*-dimethylbenzene-1,4-diamine (7)—2-Chloro-*N,N*-dimethyl-4-nitroaniline **5** (3.00 g, 15.0 mmol) in methanol (50 mL) was added with palladium on activated charcoal (42 mg). Then, $NaBH_4$ (1.14 g, 30.0 mmol) was added to the solution dropwise under stirring at room temperature. Determine reaction completeness by TLC (silica, ethyl acetate/hexanes = 1:2, UV light and I_2 stain). When completed (~1 h), cold HCl solution (2 N, 20 mL) was added dropwise to the mixture to quench the reaction (caution: hydrogen gas bubble generated). The reaction solvent was removed by rotary evaporation. Then, saturated $NaHCO_3$ solution (100 mL) was added, and the resultant aqueous solution was extracted by ethyl acetate (3×50 mL). The combined organic layer was washed by brine (50 mL) and dried over Na_2SO_4 . After solvent evaporation, the resulting residue was purified by flash chromatography (SiO_2) and eluted with ethyl acetate/hexanes (1/2, v/v) to afford the product **7** (2.54 g, 99%) as a black solid.

R_f (ethyl acetate/hexanes: 1/2 (v/v)): 0.40.

^1H -NMR (400 MHz, $CDCl_3$): δ 6.92 (d, $J = 8.4$ Hz, 1H, Ar-H), 6.71 (d, $J = 2.8$ Hz, 1H, Ar-H), 6.53 (dd, $J = 8.4$ and 2.8 Hz, 1H, Ar-H), 3.54 (s, 2H, NH_2), and 2.70 (s, 6 H, NCH_3).

^{13}C -NMR (100 MHz, $CDCl_3$): δ 142.8, 142.0, 129.5, 121.1, 117.0, 114.2, and 44.5.

HRMS calculated for $C_8H_{11}ClN_2$ $[M + H]^+$: $m/z = 171.0684$, found 171.0678.

4-Amino-*N*-(3-chloro-4-(dimethylamino)phenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide (8)—2-Chloro-*N,N*-dimethylbenzene-1,4-diamine **7** (1.79 g, 10.5 mmol) and 4-amino-*N*-hydroxy-1,2,5-oxadiazole-3-carboximidoyl chloride **3** (1.63 g, 10 mmol) in ethanol (50 mL) were added in $NaHCO_3$ solution (2.01 g $NaHCO_3$ in 25 mL H_2O). The reaction mixture was stirred at 60°C for 30 min, and the reaction completeness was determined by TLC. After being extracted by ethyl acetate (2×50 mL), the combined

organic layer was washed by brine (50 mL) and dried over anhydrous Na₂SO₄. After solvent evaporation, the resulting residue was purified by flash chromatography (SiO₂) and eluted with ethyl acetate/ hexanes (3/7, v/v) to afford the product **8** (2.85 g, 96%) as a gray solid.

R_f (ethyl acetate/hexanes: 3/7 (v/v)): 0.37.

¹H-NMR (400 MHz, DMSO-*d*₆): δ 11.23 (s, 1H, OH), 8.65 (s, 1H, NH), 6.92 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.83 (d, *J* = 2.4 Hz, 1H, Ar-H), 6.59 (dd, *J* = 8.4 and 2.4 Hz, 1H, Ar-H), 6.20 (s, 2H, NH₂), and 2.57 (s, 6 H, N(CH₃)₂).

¹³C-NMR (100 MHz, DMSO-*d*₆): δ 155.4, 144.8, 140.5, 139.6, 136.1, 126.9, 122.8, 120.4, 120.1, and 43.6.

HRMS calculated for C₁₁H₁₃ClN₆O₂ [M + H]⁺: *m/z* = 297.0861, found 297.0872.

3-(4-Amino-1,2,5-oxadiazol-3-yl)-4-(3-chloro-4-(dimethylamino) phenyl)-1,2,4-oxadiazol-5(4H)-one (9)—

4-Amino-*N*-(3-chloro-4-(dimethylamino)phenyl)-*N'*-hydroxy-1,2,5-oxadiazole-3-carboximidamide **8** (0.864 g, 2.91 mmol) and 1,1'-carbonyldiimidazole (0.519 g, 3.20 mmol) in THF (10 mL) were stirred at 70°C for 1 h. The reaction completeness was determined by TLC. After solvent evaporation, the resulting residue was purified by chromatography (SiO₂) to afford the product **9** (0.866 g, 92%) as a gray solid.

R_f (ethyl acetate/hexanes: 3/7 (v/v)): 0.26.

¹H-NMR (400 MHz, DMSO-*d*₆): δ 7.68 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.47 (dd, *J* = 8.8 and 2.4 Hz, 1H, Ar-H), 7.23 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.61 (s, 2H, NH₂), and 2.79 (s, 6 H, N(CH₃)₂).

¹³C-NMR (100 MHz, DMSO-*d*₆): δ 157.1, 155.3, 151.4, 149.1, 134.3, 129.9, 127.6, 125.9, 125.2, 120.3, and 43.0.

HRMS calculated for C₁₂H₁₁ClN₆O₃ [M + H]⁺: *m/z* = 323.0654, found 323.0662.

4-(3-(4-Amino-1,2,5-oxadiazol-3-yl)-5-oxo-1,2,4-oxadiazol-4(5H)-yl)-2-chloro-*N,N,N*-trimethylbenzenaminium trifluoromethanesulfonate (10)—

To a solution of the 3-(4-amino-1,2,5-oxadiazol-3-yl)-4-(3-chloro-4-(dimethylamino)phenyl)-1,2,4-oxadiazol-5(4H)-one **9** (0.806 g, 2.50 mmol) in dichloromethane (30 mL), methyl trifluoromethanesulfonate (548 μL, 5.0 mmol) was added under the protection of nitrogen gas. After being stirred at room temperature overnight (~12 h), the reaction mixture was filtered, and the resulting solid was washed by dichloromethane. The powder was then dissolved in methanol and precipitated by ethyl ether to afford the product **10** (1.00 g, 82%) as a white solid.

¹H-NMR (400 MHz, DMSO-*d*₆): δ 8.32 (d, *J* = 8.8 Hz, 1H, Ar-H), 8.17 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.92 (dd, *J* = 8.8 and 2.4 Hz, 1H, Ar-H), 6.67 (s, 2H, NH₂), and 3.85 (s, 9 H, NCH₃).

^{13}C -NMR (100 MHz, $\text{DMSO}-d_6$): δ 156.8, 155.7, 148.9, 142.8, 134.7, 134.4, 133.7, 129.1, 126.4, 125.9, 121.1 (q, $J_{\text{C-F}}^1 = 320.3\text{ Hz}$, CF_3), and 56.2.

HRMS calculated for $\text{C}_{13}\text{H}_{14}\text{ClN}_6\text{O}_3$ $[\text{M}]^+$: $m/z = 337.0810$, found 337.0796.

Radiochemistry

Synthesis of $[\text{}^{18}\text{F}]\text{11}$ —Aqueous $[\text{}^{18}\text{F}]\text{fluoride}$ (1.0–2.0 mL, 5–20 mCi, from Cardinal Health) was trapped on a preconditioning QMA cartridge and eluted with a mixture of Kryptofix [2.2.2] (475 μL of a 23-mg/mL stock solution in MeCN) and K_2CO_3 stock solution (25 μL of a 400-mg/mL stock solution in water). $[\text{}^{18}\text{F}]\text{fluoride}$ was dried at 120°C under a stream of nitrogen by azeotropic distillation with anhydrous acetonitrile ($3 \times 0.3\text{ mL}$) to give the no-carrier-added $[\text{K/K}_{222}]^+[\text{}^{18}\text{F}]^-$ complex as a white semisolid residue. After cooling to room temperature, a solution of triflate precursor **6** (3.6 mg, 10 μmol) in anhydrous acetonitrile (0.5 mL) was added into the reaction vial. After being heated at 70°C for 5 min, the mixture was mixed with water (4.0 mL), and the aqueous solution was passed through an activated C18 Sep-Pak (pretreated by passing through 5 mL of methanol, 10 mL of water and 40 mL of air). The Sep-Pak was rinsed with 10% acetonitrile in water (5.0 mL), and the residue solvent was partly removed by air (20 mL). The product 2-chloro-4- $[\text{}^{18}\text{F}]\text{fluoronitrobenzene}$ ($[\text{}^{18}\text{F}]\text{11}$) was slowly eluted through the column with methanol (1.5 mL). Radio-TLC analysis of the crude reaction mixture showed 98% radiochemical yield (EtOAc/hexanes: 1/3 (v/v), $R_f = 0.80$) with unreacted $[\text{}^{18}\text{F}]\text{fluoride}$ as the main impurity.

Synthesis of $[\text{}^{18}\text{F}]\text{12}$ —The elution from the previous step was collected in a reaction vial containing Pd/C (1 mg) and NaBH_4 (5 mg) under nitrogen protection. The reaction mixture was stirred at room temperature for 5 min before being quenched by concentrated HCl (25 μL). After filtering off the catalyst, the solution was concentrated at 100°C under a stream of nitrogen in 15 min. Radio-TLC analysis of the crude reaction mixture showed 89–96% conversion to product (EtOAc/hexanes: 1/3 (v/v), $R_f = 0.34$).

Synthesis of $[\text{}^{18}\text{F}]\text{IDO5L}$ —The residue of $[\text{}^{18}\text{F}]\text{12}$ was redissolved in 200 μL of methanol and then mixed with NaHCO_3 solution (300 μL , 1 M). The acidity of the resulting mixture was examined by a pH paper to confirm pH ~ 9 . A solution of 4-amino-*N*-hydroxy-1,2,5-oxadiazole-3-carboximidamidoyl chloride **3** (3.2 mg, 20 μmol) in methanol (200 μL) was added, and the reaction mixture was stirred at 60°C for 15 min. Radio-TLC analysis of the crude reaction mixture showed 51–64% conversion to $[\text{}^{18}\text{F}]\text{IDO5L}$ (EtOAc/hexanes: 1/1 (v/v), $R_f = 0.45$). The solution was subsequently filtered, and the filter was washed with methanol (200 μL). The methanol solution was purified by HPLC with retention time of 11 min. The collected HPLC fraction ($\sim 4\text{ mL}$) was diluted with water and then passed through the activated C18 Sep-Pak column. After being washed by 10 mL of water, the labeled product was eluted by 1.5 mL methanol and dried under a stream of nitrogen in 100°C . Finally, the residue was dissolved in 0.3 mL physiological saline for animal study. Radiochemical purity and specific activity of the product $[\text{}^{18}\text{F}]\text{IDO5L}$ were determined by analytical HPLC with the retention time of 13.5 min. The identity of $[\text{}^{18}\text{F}]\text{IDO5L}$ was confirmed by a coinjection with a nonradioactive standard IDO5L.

Typically, starting from 0.34 to 0.74 GBq (9.2–19.9 mCi) [^{18}F]fluoride, 35 to 77 MBq (0.94–2.09 mCi) of purified [^{18}F]IDO5L could be obtained in ~90 min.

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Figure 1.

Representative radio-TLC profiles of (a) crude sample of ^{18}F -11 (EtOAc/hexanes = 1:3, R_f = 0.80), (b) crude sample of ^{18}F -12 (EtOAc/hexanes = 1:3, R_f = 0.34), and (c) crude sample of ^{18}F -IDO5L (EtOAc/hexanes = 1:3, R_f = 0.45).

Figure 2.
Representative chromatogram from the preparative HPLC separation of the ^{18}F -IDO5L product.

Figure 3.
Representative chromatogram from the HPLC analysis of the purified ^{18}F -IDO5L, coinjection with reference IDO5L.

Scheme 1.

Synthesis of standard IDO5L. Conditions: (a) NaNO_2 , HCl (aq.) 2 N; then, 50% NH_2OH reflux, 2 h; HCl 10 N to pH = 7.0, 91%; (b) NaNO_2 , HCl , H_2O , 0°C , 1.5 h, 57%; and (c) 3-chloro-4-fluoroaniline, NaHCO_3 , EtOH , room temperature (RT.) to 60°C , 1 h, 88%.

Scheme 2.

Synthesis of two triflate precursors **6** and **10**. Conditions: (a) NaH, THF, 0°C to RT., 30 min and then MeI 17 h, 79%; (b) methyl trifluoromethanesulfonate, dichloromethane, RT., 24 h, 76%; (c) NaBH₄, Pd/C, MeOH, RT. 1 h, 99%; (d) compound **3**, NaHCO₃, ethanol, H₂O, 60°C, 30 min, 96%; (e) 1,1'-carbonyldiimidazole, THF, 70°C, 1 h, 92%; and (f) methyl trifluoromethanesulfonate, dichloromethane, RT., 12 h, 82%.

Scheme 3.

Radiosynthesis of ^{18}F -IDO5L. Conditions: (a) $[^{18}\text{F}]\text{KF}/\text{K}_{222}$, MeCN, RT., 120°C, 30 min, then NaOH (aq.) 1 N, 15 min; (b) $[^{18}\text{F}]\text{KF}/\text{K}_{222}$, MeCN, 70°C., 5 min; (c) NaBH_4 , Pd/C, MeOH, 70°C, 5 min; and (d) compound **3**, NaHCO_3 (aq.), methanol, 60°C, 15 min.

Table 1

Labeling yields of each reaction step

Product	Time (min)	Conditions	Labeling yield [*]
¹⁸F-11	2		94
	5		98
	10		98
¹⁸F-12	5	No stirrer	49–60 (<i>n</i> > 3)
	5	With stirrer	89–98 (<i>n</i> > 3)
¹⁸F-IDO5L	15	Et ₃ N (0.7 mmol, 35 eq.) [†]	0 (<i>n</i> = 3)
	15	NaHCO ₃ (aq.; 0.3 mmol, 15 eq.) [†]	51–64 (<i>n</i> > 3)
	15	Na ₂ CO ₃ (aq.; 0.15 mmol, 7.5 eq.) [†]	20–40 (<i>n</i> > 3)

^{*} By radio-TLC, *n* = 1 unless otherwise stated

[†] By radio-TLC, *n* = 1 unless otherwise stated. B. eq. equivalent of compound **3**