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[¹⁸F]Fluoroethyl Triazole Substituted PSMA Inhibitor Exhibiting Rapid Normal Organ Clearance

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Abstract

Prostate-specific membrane antigen (PSMA) is overexpressed in the epithelium of prostate cancer and nonprostate solid tumor neovasculature. PSMA is increasingly utilized as a target for cancer imaging and therapy. Here, we report the synthesis and *in vivo* biodistribution of a low-molecular-weight PSMA-based imaging agent, 2-[3-(1-carboxy-5-{3-[1-(2-[¹⁸F]fluoroethyl)-1*H*-1,2,3-triazol-yl]propanamido}pentyl)ureido]-pentanedioic acid ([¹⁸F]YC-88), containing an [¹⁸F]fluoroethyl triazole moiety. [¹⁸F]YC-88 was synthesized from 2-[¹⁸F]fluoroethyl azide and the corresponding alkyne precursor in two steps using either a one- or two-pot procedure. Biodistribution and positron emission tomography (PET) imaging were performed in immunocompromised mice using isogenic PSMA⁺ PC3 PIP and PSMA⁻ PC3 flu xenografts. YC-88 exhibited high affinity for PSMA as evidenced by a K_i value of 12.9 nM. The non-decay corrected radiochemical yields of [¹⁸F]YC-88 averaged $14 \pm 1\%$ ($n = 5$). Specific radioactivities ranged from 320 to 2,460 Ci/mmol (12–91 GBq/ μ mol) with an average of 940 Ci/mmol (35 GBq/ μ mol, $n = 5$). In an immunocompromised mouse model, [¹⁸F]YC-88 clearly delineated PSMA⁺ PC3 PIP prostate tumor xenografts on imaging with PET. At 1 h postinjection, $47.58 \pm 5.19\%$ injected dose per gram of tissue (% ID/g) was evident within the PSMA⁺ PC3 PIP tumor, with a ratio of 170:1 of uptake within PSMA⁺ PC3 PIP to PSMA⁻ PC3 flu tumor placed in the opposite flank. The tumor-to-kidney ratio at 2 h postinjection was 4:1. At or after 30 min postinjection, minimal nontarget tissue uptake of [¹⁸F]YC-88 was observed. Compared to [¹⁸F]DCFPyL, which is currently in clinical trials, the uptake of [¹⁸F]YC-88 within the kidney, liver, and spleen was significantly lower at all time-points studied. At 30 min and 1 h postinjection, salivary gland uptake of [¹⁸F]YC-88 was significantly less than that of [¹⁸F]DCFPyL. [¹⁸F]YC-88 is a new PSMA-targeted PET agent synthesized utilizing click chemistry that demonstrates high PSMA⁺ tumor uptake in a xenograft model. Because of its low uptake in the kidney, rapid clearance from nontarget organs, and relatively simple one-pot, two-step radiosynthesis, [¹⁸F]YC-88 is a viable new PET radiotracer for imaging PSMA-expressing lesions.

Graphical abstract

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Notes

The authors declare the following competing financial interest(s): Drs. Pomper, Mease, and Chen have licensed technology and receive royalties related to [¹⁸F]DCFPyL, which was used for comparison in this publication.

INTRODUCTION

Prostate-specific membrane antigen (PSMA) is a type II integral membrane protein expressed on the surface of prostate tumors, particularly in castration-resistant, advanced, and metastatic disease.^{1,2} PSMA is also expressed in the neovascular endothelium of virtually all solid tumors but not in normal vasculature.^{3,4} It represents an excellent target for imaging and therapy of cancer.

We and others have developed a series of PSMA-targeted imaging agents for positron emission tomography (PET) imaging of prostate cancer.⁵ Several ^{68}Ga - and ^{18}F -radiolabeled imaging agents have been evaluated in clinical studies.^{6–14} We have chosen to focus on ^{18}F -labeled compounds in part because the infrastructure in the US is currently more amenable to such agents relative to those labeled with ^{68}Ga . Previously, we developed a series of ^{18}F -labeled, PSMA-targeted imaging agents (Figure 1), including *N*-[*N*-(*S*)-1,3-dicarboxypropyl]-carbamoyl]-4- $[^{18}\text{F}]$ fluorobenzyl-L-cysteine ($[^{18}\text{F}]\text{DCFBC}$), 2-[3-[1-carboxy-5-(4- $[^{18}\text{F}]$ fluoro-benzoylamino)-pentyl]-ureido]-pentanedioic acid ($[^{18}\text{F}]\text{DCFbZL}$), and 2-(3-{1-carboxy-5-[(6- $[^{18}\text{F}]$ fluoropyridine-3-carbonyl)-amino]-pentyl}-ureido)-pentanedioic acid ($[^{18}\text{F}]\text{DCFPyL}$).^{15–17} We have recently completed phase I trials of $[^{18}\text{F}]\text{DCFBC}$ and $[^{18}\text{F}]\text{DCFPyL}$ to image metastatic prostate cancer, and both have shown promising results.^{6,14} One drawback of $[^{18}\text{F}]\text{DCFBC}$ was that it showed moderate, persistent blood-pool radioactivity,⁶ which could be a limitation for the detection of lymph node metastases adjacent to major vessels. Clinical imaging studies of $[^{18}\text{F}]\text{DCFPyL}$ showed lower blood pool activity, providing clearer images than $[^{18}\text{F}]\text{DCFBC}$; however, considerable kidney and salivary gland uptake of this tracer was observed and may result in dose-limiting toxicities in these organs.¹⁴ In spite of the multistep synthetic procedure required, the radiosynthesis of both agents has been automated to produce several clinical doses per synthesis for on-site use. However, further improvements in yield may be needed for regional distribution on a model similar to that for $[^{18}\text{F}]\text{fluorodeoxyglucose}$ (FDG). Accordingly, we have continued our efforts to develop an ^{18}F -labeled PSMA inhibitor that could be prepared quickly, in high yield, and that could localize to PSMA-positive lesions but clear rapidly from normal organs.

1,3-Dipolar cycloadditions, commonly known as “click” reactions, both copper catalyzed and copper free, are being utilized in radiochemistry due to their mild reaction conditions, rapidity, reliability, high yield, and selectivity.¹⁸ The most common version used in small

molecules is the copper-catalyzed azide–alkyne cycloaddition to yield the triazole moiety. Here, we report the synthesis of the novel PSMA inhibitor [^{18}F]YC-88 from [^{18}F]fluoroethyl azide and the corresponding alkyne precursor using click chemistry. We also evaluated [^{18}F]YC-88 in an experimental model of prostate cancer.

RESULTS

Chemical and Radiochemical Synthesis

The chemical and radiochemical syntheses of YC-88 and [^{18}F]YC-88 are shown in Scheme 1. The formate salt of protected lysine-glutamate-urea **1**¹⁹ was reacted with *N*-succinimidyl 4-pentynoate²⁰ to generate the alkyne-containing urea **2**. Removal of the *t*-butyl groups in **2** afforded precursor **3**. The copper catalyzed click reaction between **3** and 2-fluoroethyl azide afforded YC-88.

We investigated two methods for the preparation of [^{18}F] YC-88. In procedure 1, we used a two-pot, two-step approach. First, 2-[^{18}F]fluoroethyl azide was synthesized from 2-azidoethyl-4-toluenesulfonate and was then purified by distillation²¹ into a chilled receiving vessel. In the second step, precursor **3** and the click reagents copper(II) sulfate and sodium ascorbate were added to the receiving vessel to produce [^{18}F]YC-88. Using procedure 1, the non-decay corrected radiochemical yields of two syntheses of [^{18}F]YC-88 were 11% and 16%, respectively, based on the starting [^{18}F]fluoride. The total synthetic time was approximately 110 min [including drying of [^{18}F]fluoride and semipreparative high performance liquid chromatography (HPLC)]. Starting from 40 or 68 mCi (1,480 or 2,516 MBq) of [^{18}F]fluoride, the specific radioactivities of [^{18}F]YC-88 were 200 and 850 Ci/mmol, respectively (7.4 and 31.5 GBq/ μmol). Although procedure 1 was simple, the distillation step made it potentially difficult to incorporate into an automated radiosynthesis module.

In order to modify the radiosynthesis of [^{18}F] YC-88 to make it more amenable to automation, a one-pot, two-step method (procedure 2) was investigated. 2-[^{18}F]Fluoroethyl azide was synthesized from 2-azidoethyl-4-toluenesulfonate as above, followed by the direct addition of precursor **3**, copper(II) sulfate, and sodium ascorbate, to produce [^{18}F]YC-88. The non-decay corrected radiochemical yields of [^{18}F]YC-88 in the one-pot approach averaged $14 \pm 1\%$ based on starting [^{18}F]fluoride ($n = 5$). The total synthetic time was approximately 60 min (including drying of [^{18}F] fluoride and semipreparative HPLC). Starting from 13–72 mCi (481–2,664 MBq) of [^{18}F]fluoride, specific radioactivities ranged from 320 to 2,460 Ci/mmol (12–91 GBq/ μmol) with an average of 940 Ci/mmol (35 GBq/ μmol , $n = 5$).

Lipophilicity—Octanol–water partition coefficient (logP) values were measured by the shake-flask method using 1-octanol and PBS (pH 7.4). The logP of [^{18}F]YC-88 at pH 7.4 was -3.91 . Using the same method, the logP of [^{18}F]DCFPyL was -3.27 .

PSMA Inhibition Assay

The PSMA binding affinity of YC-88 was determined using a modification of the Amplex Red glutamic acid assay.²² The K_i value of **YC-88** was 12.9 nM with 95% confidence intervals from 8.7 to 19.1 nM. For comparison, the K_i values of **DCFBC**, **DCFbZL**, and **DCFpYL** (Figure 1) were 0.44 nM, 0.19 nM, and 1.1 nM, respectively.^{16,17}

Biodistribution

To ensure an accurate comparison, biodistributions for [¹⁸F]**YC-88** and [¹⁸F]**DCFpYL** were performed on consecutive days using NOD-SCID mice bearing isogenic PSMA⁺ PC3 PIP and PSMA⁻ PC3 flu tumors placed in opposite flanks. Results are shown in Tables 1 and 2.

[¹⁸F]**YC-88** demonstrated selective PSMA⁺ PC3 PIP tumor uptake, reaching $40.31 \pm 5.13\%$ ID/g at 30 min postinjection, which decreased to 25.74 ± 7.33 ID/g at 4 h. PSMA⁻ PC3 flu tumor uptake was low at all time points. The ratio of uptake within PSMA⁺ PC3 PIP to PSMA⁻ PC3 flu tumors ranged from 70:1 at 30 min to 368:1 at 4 h postinjection. The kidney uptake was partially due to the high expression of PSMA within proximal renal tubules.²³ Kidney uptake was highest at 30 min postinjection, followed by rapid clearance. The tumor-to-kidney ratio at 2 h for [¹⁸F]**YC-88** was 4:1. The distribution within other, nontarget tissues was also favorable, with rapid clearance. Low bone uptake (<1% ID/g at all time points) suggests the absence of metabolic defluorination of [¹⁸F]**YC-88**.

[¹⁸F]**DCFpYL** demonstrated high and prolonged PSMA⁺ PC3 PIP tumor uptake, reaching $84.29 \pm 12.29\%$ ID/g at 1 h. At the early time points, the highest accumulation of radioactivity was observed in the kidneys. Renal uptake of [¹⁸F]**DCFpYL** ranged from $123.69 \pm 8.05\%$ ID/g at 30 min to $19.36 \pm 2.32\%$ ID/g at 4 h postinjection. The tumor-to-kidney ratio for [¹⁸F]**DCFpYL** was 1.1:1 at 2 h. Compared to [¹⁸F]**DCFpYL**, [¹⁸F]**YC-88** demonstrated higher tumor-to-kidney ratios at all time points. [¹⁸F]**YC-88** also showed overall lower uptake in normal organs as well as overall faster clearance than [¹⁸F]**DCFpYL**.

Figure 2 summarizes the comparison between selected tissue uptake of [¹⁸F]**YC-88** and [¹⁸F]**DCFpYL**. The difference in radiotracer uptake in PSMA⁺ PC3 PIP tumors (Figure 2A) was not statistically significant at 30 min ($P > 0.05$), while the PSMA⁺ PC3 PIP tumor uptake of [¹⁸F]**DCFpYL** at 1, 2, and 4 h was significantly higher than that of [¹⁸F]**YC-88**. There was no significant difference in PSMA⁻ PC3 flu tumor uptake at any time between the two radiotracers (Figure 2B). The kidney, liver, and spleen uptake of [¹⁸F]**YC-88** was significantly lower than that for [¹⁸F]**DCFpYL** at all time points, as shown in Figure 2C, E, and F. At 30 min and 1 h postinjection, salivary gland uptake of [¹⁸F]**YC-88** was significantly lower than that for [¹⁸F]**DCFpYL** (Figure 2D).

Radiation Dosimetry—Timed biodistribution results were used to generate dosimetry data. Calculations were limited to the kidneys as a first approximation to comparison between [¹⁸F]**YC-88** and [¹⁸F]**DCFpYL** with respect to radiation absorbed doses. Table 3 lists the biological clearance half-lives and areas under the curve for kidneys for [¹⁸F]**YC-88**

and [^{18}F] **DCFPyL**. The kidney absorbed dose with respect to [^{18}F] **YC-88** showed nearly a 5-fold decrease compared to that of [^{18}F] **DCFPyL**.

Small Animal PET Imaging

Whole body PET images for [^{18}F] **YC-88** were obtained in male NOD-SCID mice bearing PSMA⁺ PC3 PIP and PSMA⁻ PC3 flu xenografts in opposite flanks. Figure 3 shows the PET images of [^{18}F] **YC-88** at 30, 60, and 120 min postinjection. Intense PSMA⁺ PC3 PIP tumor uptake was seen as early as 30 min postinjection. As noted above in the biodistribution studies, renal uptake of [^{18}F] **YC-88** was prominent, partially due to the route of excretion of this hydrophilic compound as well as to specific uptake from the expression of PSMA in mouse proximal tubules.²³ The images also showed extremely low uptake of this radiotracer in normal tissues.

DISCUSSION

We prepared a new ^{18}F -labeled, PSMA-targeted low-molecular-weight imaging agent, [^{18}F] **YC-88**, from 2-[^{18}F]fluoroethyl azide and alkyne-bearing urea precursor **3**, which allowed rapid and simplified radiolabeling. In particular, the one-pot, two-step radiosynthesis of [^{18}F] **YC-88** was performed under mild reaction conditions and in radiochemical yields suitable for clinical studies. Furthermore, unlike the radiosyntheses of [^{18}F] **DCFbZL** and [^{18}F] **DCFPyL**, no intermediate purification or ester hydrolysis steps were required. Only a single, final semipreparative purification by HPLC was needed. This synthesis of [^{18}F] **YC-88**, although not yet optimized for radiochemical yield and reaction time, is simple and should be able to be readily automated.

In *in vivo* biodistribution studies of [^{18}F] **YC-88** and [^{18}F] **DCFPyL** showed that both compounds had high PSMA⁺ PC3 PIP tumor uptake. However, the tumor uptake of [^{18}F] **YC-88** was lower than that for [^{18}F] **DCFPyL**. The lower tumor uptake of [^{18}F] **YC-88** compared to [^{18}F] **DCFPyL** may be due to its lower binding affinity compared to that of [^{18}F] **DCFPyL**, as a result of the fluoroethyl triazole moiety having a weaker interaction with the hydrophobic subpocket of the S1 binding site compared to that of the fluoro-aryl groups of **DCFBC**, **DCFbZL**, and **DCFPyL**.²⁴ However, [^{18}F] **YC-88** had lower kidney and nontarget tissue uptake, as well as faster clearance than [^{18}F] **DCFPyL**, which might be due to the higher hydrophilicity of [^{18}F] **YC-88** ($\log P = -3.91$) than [^{18}F] **DCFPyL** ($\log P = -3.27$). The overall lower nontarget tissue uptake of [^{18}F] **YC-88** will minimize radiation exposure to healthy tissues, particularly dose-limiting organs such as kidneys and salivary glands. In addition, [^{18}F] **DCFPyL** has recently been utilized for PET imaging of metastatic clear cell renal cell carcinoma;²⁵ however, the slow clearance of [^{18}F] **DCFPyL** from the normal kidney might preclude its use for primary renal lesions. The faster renal clearance of [^{18}F] **YC-88** may permit such an application.

CONCLUSIONS

[^{18}F] **YC-88** has been synthesized utilizing copper catalyzed 1,3-dipolar cycloaddition chemistry by a convenient, single pot procedure. [^{18}F] **YC-88** has suitable uptake in PSMA⁺ PC3 PIP xenografts and favorable pharmacokinetics, with lower normal organ uptake than

[^{18}F]DCFPyL, suggesting it as a promising new PET agent for imaging PSMA-expressing lesions.

EXPERIMENTAL SECTION

General Procedures

All reagents and solvents were purchased from either Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Pittsburgh, PA). ^1H NMR spectra were recorded on a Bruker Ultrashield 400 or 500 MHz spectrometer. ESI mass spectra were obtained on a Bruker Esquire 3000 plus system. High-resolution mass spectrometry (HR-MS) was done by the Mass Spectrometry Facility at the University of Notre Dame using ESI by direct infusion on a Bruker micrOTOF-II. High performance liquid chromatography (HPLC) purification was performed on a Varian Prostar System. [^{18}F]Fluoride was produced by 18 MeV proton bombardment of a high pressure [^{18}O]H $_2\text{O}$ target using a General Electric PET trace biomedical cyclotron (Milwaukee, WI). [^{18}F]DCFPyL was prepared as previously published.¹⁴ Reverse phase radio-HPLC purification was performed using a Varian Prostar System with a Bioscan Flow Count PMT radioactivity detector (Varian Medical Systems, Washington, DC). Radioactivity was measured in a Capintec CRC-10R dose calibrator (Ramsey, NJ). The specific radioactivity was calculated as the radioactivity eluting at the retention time of the product during the semipreparative HPLC purification divided by the mass (determined from a standard curve) corresponding to the area under the curve of the UV absorption.

2-{3-[1-Carboxy-5-(pent-4-ynamido)pentyl]ureido}-pentanedioic Acid, 3

To the formate salt of 6-(*tert*-butoxy)-5-(3-(1,5-di-*tert*-butoxy-1,5-dioxopentan-2-yl)ureido)-6-oxo-hexan-1-aminium 1 (0.049 g, 0.09 mmol)¹⁹ in CH $_2$ Cl $_2$ (2 mL) was added triethylamine (0.04 mL, 0.29 mmol), followed by *N*-succinimidyl-4-pentynoate (0.030 g, 0.15 mmol). After stirring for 2 h at ambient temperature, the solvent was evaporated. The crude material was purified on a silica gel column using methanol/methylene chloride (5:95) to afford 0.051 g (98%) of compound 2. ^1H NMR (400 MHz, CDCl $_3$) δ 6.35 (m, 1H), 5.28–5.37 (m, 2H), 4.25–4.30 (m, 2H), 3.16–3.30 (m, 2H), 2.49–2.52 (m, 2H), 2.38–2.42 (m, 2H), 2.27–2.33 (m, 2H), 1.98–2.10 (m, 2H), 1.80–1.90 (m, 1H), 1.32–1.58 (m, 33H). ESI-Mass calcd for C $_{29}$ H $_{49}$ N $_3$ O $_8$ [M] $^+$ 567.3, found 567.9. A solution of TFA in CH $_2$ Cl $_2$ (1:1, 2 mL) was added to 2 (0.051 g, 0.09 mmol). The mixture was stirred at ambient temperature for 2 h, then concentrated on a rotary evaporator. The crude material was purified by HPLC (Phenomenex C18 10 μ , 250 $\times$ 10 mm, H $_2$ O/CH $_3$ CN/TFA [90/10/0.1], 4 mL/min) to afford 0.028 g (78%) of 3. ^1H NMR (400 MHz, D $_2$ O/CD $_3$ CN = 1:1 (v/v)) δ 4.15–4.18 (m, 1H), 4.08–4.11 (m, 1H), 3.07–3.10 (m, 2H), 2.35–2.40 (m, 4H), 2.25–2.32 (m, 3H), 1.99–2.07 (m, 1H), 1.78–1.88 (m, 1H), 1.67–1.75 (m, 1H), 1.53–1.63 (m, 1H), 1.37–1.44 (m, 2H), 1.29–1.34 (m, 2H). ESI-mass calcd for C $_{17}$ H $_{25}$ FN $_3$ O $_8$ [M] $^+$ 399.2; found, 399.9.

2-[3-(1-Carboxy-5-{3-[1-(2-fluoroethyl)-1-*H*-1,2,3-triazol-yl]propanamido}pentyl)ureido]pentanedioic Acid, YC-88

To a solution of CuSO $_4$ (0.0016 g, 0.01 mmol) and sodium ascorbate (0.004 g, 0.02 mmol) in water (0.1 mL) under nitrogen was added 2 (0.006 g, 0.011 mmol) in 0.05 mL of DMF,

followed by a solution of 2-fluoroethyl azide (0.022 mmol)²⁶ in DMF (0.5 mL). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated under high vacuum, and the resulting residue was purified by HPLC (Phenomenex C18 10 μ m, 250 $\times$ 10 mm, H₂O/CH₃CN/TFA [93/7/0.1], 6 mL/min) to afford 0.004 g (54%) of **YC-88**. ¹H NMR (500 MHz, D₂O/CD₃CN = 1:1 (v/v)) δ 7.69 (s, 1H), 4.77–4.79 (m, 1H), 4.68–4.69 (m, 1H), 4.62–4.64 (m, 1H), 4.57–4.59 (m, 1H), 4.14–4.17 (m, 1H), 4.08–4.09 (m, 1H), 3.01–3.03 (m, 2H), 2.89–2.91 (m, 2H), 2.45–2.48 (m, 2H), 2.35–2.38 (m, 2H), 2.01–2.05 (m, 1H), 1.79–1.86 (m, 1H), 1.65–1.72 (m, 1H), 1.51–1.59 (m, 1H), 1.31–1.36 (m, 2H), 1.20–1.25 (m, 2H). HR-MS calcd for C₁₉H₃₀FN₆O₈⁺ 489.2109; found, 489.2112 [M + H]⁺.

Radiosynthesis of [¹⁸F]YC-88

Procedure 1 (Two-Pot, Two-Step)—2-[¹⁸F]Fluoroethyl azide was synthesized according to a literature procedure.²¹ Briefly, [¹⁸F]fluoride was eluted from a Chromafix 30-PS-HCO₃⁻ ion-exchange resin (Macherey-Nagel) with 15 mg of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (K₂₂₂) and 3 mg of K₂CO₃ in 20% aqueous acetonitrile (1 mL) into a reaction vial and dried by azeotropic distillation at 100 °C with CH₃CN (3 $\times$ 0.5) mL under a stream of argon. To the dried [¹⁸F]KF/K₂₂₂ complex was added 3 μ L of 2-azidoethyl 4-toluenesulfonate²⁷ in 0.3 mL of DMF. The reaction mixture was heated in a sealed vial at 100 °C for 5 min, and then, the 2-[¹⁸F]fluoroethyl azide was distilled using argon sweep gas into a vial that contained 300 μ L of cold DMF. To the vial containing 2-[¹⁸F]fluoroethyl azide in DMF was added a mixture of 3 (2 mg) in 0.05 mL of DMF, 0.1 mL of H₂O, CuSO₄ (0.1 M, 0.04 mL), and sodium ascorbate (0.2 M, 0.08 mL). The mixture was reacted at ambient temperature for 30 min. The final product [¹⁸F]YC-88 was obtained after HPLC purification (Phenomenex C18 10 μ m, 250 $\times$ 10 mm, H₂O/CH₃CN/TFA = 92/8/0.1, 4 mL/min), and was neutralized with 1 M NaHCO₃, concentrated under vacuum to dryness, reconstituted in PBS (pH 7.4), and passed through a 0.22 μ m syringe filter into an evacuated sterile vial.

Procedure 2 (One-Pot, Two-Step)—[¹⁸F]Fluoride was eluted from a Chromafix 30-PS-HCO₃⁻ ion-exchange resin (Macherey-Nagel) with 5 mg of K₂₂₂ and 0.72 mg of KHCO₃ in 30% aqueous acetonitrile (1 mL) to a reaction vial and dried by azeotropic distillation at 80 °C with CH₃CN (3 $\times$ 0.5 mL) under a stream of argon. To the dried [¹⁸F]KF/K₂₂₂ complex was added 0.2 mg of 2-azidoethyl 4-toluenesulfonate²⁷ in 0.2 mL of DMF. The reaction mixture was then heated in a sealed vial at 80 °C for 5 min. CuSO₄ (0.2 M, 0.01 mL) and sodium ascorbate (0.4 M, 0.01 mL) were mixed under argon, and to this solution was added 3 (2 mg) in 0.025 mL of DMF. This mixture was then added to the radiofluorination vial containing 2-[¹⁸F]fluoroethyl azide. This mixture was reacted at 80 °C for 5 min, then cooled to room temperature. The final product [¹⁸F]YC-88 was obtained by HPLC purification (Phenomenex C18 10 μ m, 250 $\times$ 10 mm, H₂O/CH₃CN/TFA = 93/7/0.1, 6 mL/min). The product HPLC fraction was diluted with 30 mL of water and passed through an Oasis HLB cartridge (Waters Corps., Milford, MA). The cartridge was washed with 5 mL of water and eluted with 2 mL of ethanol. The ethanol eluent was concentrated under argon, reconstituted in PBS (pH 7.4), and passed through a 0.22 μ m syringe filter into an evacuated sterile vial.

Lipophilicity Determination

Octanol-water partition coefficients [logP (pH 7.4) values] were determined according to a literature procedure.²⁸ Briefly, a solution of either [¹⁸F]YC-88 or [¹⁸F]DCFPyL was added to a presaturated solution of 1-octanol (5 mL) mixed with phosphate buffered saline (PBS) (5 mL) in a 15 mL centrifuge tube. After vigorously shaking the mixture, it was centrifuged at 3,000 rpm for 5 min. Aliquots were removed from the two phases, and the radioactivity was measured in a 1282 Compugamma CS γ -counter, (LKB, Wallac, Turku, Finland).

NAALADase Assay.²⁹

Cell lysates of LNCaP cell extracts were incubated with YC-88 (0.01 nM–100 μ M) in the presence of 4 μ M NAAG at 37 °C for 2 h. The amount of released glutamate from NAAG was measured by incubating with a working solution of the Amplex Red glutamic acid kit (Molecular Probes Inc., Eugene, OR, USA) at 37 °C for 60 min. Fluorescence was determined by reading with the VICTOR³V multilabel plate reader (PerkinElmer Inc., Waltham, MA, USA) with excitation at 490 nm and emission at 642 nm. Inhibition curves were determined using semilog plots, and IC₅₀ values were determined at the concentration at which enzymatic activity was inhibited by 50%. Assays were performed in triplicate with the entire inhibition study being repeated at least once to confirm affinity and mode of inhibition. Enzyme inhibitory constants (K_i values) were generated using the Cheng–Prusoff conversion.³⁰ Data analysis was performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California).

Cell Lines and Tumor Models

PSMA⁺ PC3 PIP and PSMA[−] PC3 flu cell lines were obtained from Dr. Warren Heston (Cleveland Clinic) and were maintained as previously described.¹⁵ Cells were grown to 80–90% confluence in a single passage before trypsinization and formulation in Hank's balanced salt solution (HBSS, Sigma, St. Louis, MO) for implantation into mice. Animal studies were carried out in compliance with guidelines related to the conduct of animal experiments of the Johns Hopkins Animal Care and Use Committee. For biodistribution and imaging studies, male NOD-SCID mice (JHU, in house colony) were implanted subcutaneously with 1×10^6 PSMA⁺ PC3 PIP and PSMA[−] PC3 flu cells in opposite flanks. Mice were imaged or used in biodistribution studies when the tumor xenografts reached 3–5 mm in diameter.

Biodistribution

PSMA⁺ PC3 PIP and PSMA[−] PC3 flu xenograft-bearing NOD-SCID mice were injected via the tail vein with 0.06 mCi (2.22 MBq) of [¹⁸F]YC-88 or [¹⁸F]DCFPyL. In each case, three to four mice per group were sacrificed by cervical dislocation at 30, 60, 120, 240 min postinjection. The heart, lungs, liver, stomach, pancreas, spleen, kidney, fat, muscle, bone, salivary gland, small and large intestines, urinary bladder, PSMA⁺ PC3 PIP, and PSMA[−] PC3 flu tumors were quickly removed. Stomach and GI contents were removed and the urinary bladder emptied. A 0.1 mL sample of blood was also collected. Each organ was weighed, and the tissue radioactivity was measured with an automated γ counter (1282 Compugamma CS, Pharmacia/LKB Nuclear, Inc., Gaithersburg, MD). The % ID/g was

calculated by comparison with samples of a standard dilution of the initial dose. All measurements were corrected for decay.

Biodistribution Data Analysis

Microsoft Excel was used for data analysis. Statistical significance was calculated using a two-tailed Student's *t* test. A *P*-value <0.05 was considered significant.

PET Imaging

NOD-SCID male mice implanted with PSMA⁺ PC3 PIP and PSMA⁻ PC3 flu xenografts were used for imaging. 0.36 mCi (13.3 MBq) of [¹⁸F]YC-88 was injected intravenously in 0.2 mL of PBS. Mice were anesthetized with 3% isoflurane in oxygen for induction and maintained under 1.5% isoflurane in oxygen at a flow rate of 0.8 L/min. The images were acquired using an ARGUS small-animal PET/CT scanner (Sedecal, Madrid, Spain) at 30, 60, and 120 min. The dwell time at each bed position was 10 min for a total scan time of 20 min. An energy window of 250–700 keV was used. Images were reconstructed using the FORE/2D-OSEM method (two iterations, 16 subsets) and included correction for radioactive decay, scanner dead time, and scattered radiation.

Radiation Dosimetry

The % ID/g data of kidneys for [¹⁸F]YC-88 and [¹⁸F]DCFPyL were fit to a single exponential function of the form $A(t) = A_0 e^{-\lambda t}$, with A_0 the time-zero % ID/g and λ the biological clearance rate in units of per hour. The simulation analysis and modeling software package SAAM II (The Epsilon Group, Charlottesville, VA) was used to perform the fits. The biological clearance half-lives and areas under the curve (AUC) obtained are summarized in Table 3.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structures of ^{18}F -labeled PSMA-targeted imaging agents.

Figure 2.

Comparison of selected tissue uptake of $[^{18}\text{F}]\text{YC-88}$ and $[^{18}\text{F}]\text{DCFPyL}$ in male NOD-SCID mice ($n = 3-4$ per group) bearing both PSMA⁺ PC3 PIP and PSMA⁻ PC3 flu tumors: (A) PSMA⁺ PC3 PIP tumor; (B) PSMA⁻ PC3 flu tumor; (C) kidney; (D) salivary gland; (E) liver; and (F) spleen. (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

Figure 3.

PET-CT images representing the time course of radiochemical uptake after the administration of $[^{18}\text{F}]\text{YC-88}$. PSMA⁺ PC3 PIP (arrow) and PSMA⁻ PC3 flu (dotted oval) tumors are present in subcutaneous tissues posterior to opposite forearms. Mice were injected with 0.36 mCi (13.3 MBq) of $[^{18}\text{F}]\text{YC-88}$ at time 0. Bladder activity was very high and cropped to improve the dynamic range of the display and enhance the visualization of radiochemical uptake in tumors and kidneys. The corresponding raw images are shown in the Supporting Information.

Scheme 1.

(a) Et_3N , N -Succinimidyl-4-pentynoate, CH_2Cl_2 ; (b) TFA, CH_2Cl_2 ; and (c) $[^{18/19}\text{F}]$ -2-Fluoroethyl Azide, CuSO_4 , Sodium Ascorbate, DMF, and H_2O

Table 1Biodistribution of [^{18}F]YC-88 in Tumor-Bearing Mice^a

organ	30 min	60 min	120 min	240 min
blood	1.02 ± 0.43	0.29 ± 0.08	0.28 ± 0.23	0.12 ± 0.04
heart	0.36 ± 0.18	0.18 ± 0.06	0.07 ± 0.02	0.04 ± 0.01
lung	1.09 ± 0.31	0.49 ± 0.24	0.22 ± 0.10	0.13 ± 0.02
liver	1.70 ± 0.21	1.73 ± 0.14	1.74 ± 0.23	1.45 ± 0.14
stomach	0.41 ± 0.14	0.34 ± 0.20	0.12 ± 0.07	0.06 ± 0.02
pancreas	0.44 ± 0.13	0.16 ± 0.06	0.11 ± 0.06	0.07 ± 0.02
spleen	1.67 ± 0.46	0.69 ± 0.23	0.29 ± 0.16	0.15 ± 0.05
fat	0.33 ± 0.08	0.31 ± 0.18	0.17 ± 0.10	0.10 ± 0.04
kidney	44.08 ± 12.52	20.08 ± 8.09	10.20 ± 2.76	3.84 ± 0.29
small intestine	0.37 ± 0.11	0.23 ± 0.04	0.18 ± 0.07	0.14 ± 0.03
large intestine	0.87 ± 0.85	0.27 ± 0.14	0.31 ± 0.13	0.23 ± 0.09
muscle	0.22 ± 0.07	0.11 ± 0.06	0.10 ± 0.03	0.06 ± 0.03
bone	0.40 ± 0.16	0.16 ± 0.04	0.37 ± 0.36	0.11 ± 0.03
salivary gland	0.85 ± 0.39	0.31 ± 0.07	0.25 ± 0.07	0.14 ± 0.02
bladder (empty)	17.90 ± 14.18	7.78 ± 4.15	4.33 ± 2.64	1.22 ± 0.89
PSMA ⁺ PIP	40.31 ± 5.13	47.58 ± 5.19	41.29 ± 4.22	25.74 ± 7.33
PSMA ⁻ flu	0.58 ± 0.23	0.28 ± 0.13	0.17 ± 0.03	0.07 ± 0.01
PIP/flu	70	170	243	368
PIP/kidney	0.9	2.4	4.0	6.7

^aValues are in % ID/g ± SD; *n* = 3–4 per group; PIP = PSMA⁺ PC3; and flu = PSMA⁻ PC3 tumors.

Table 2Biodistribution of [^{18}F]DCFPyL in Tumor-Bearing Mice^a

organ	30 min	60 min	120 min	240 min
blood	1.67 ± 0.54	0.72 ± 0.22	0.23 ± 0.05	0.11 ± 0.00
heart	0.84 ± 0.16	0.32 ± 0.09	0.12 ± 0.01	0.06 ± 0.01
lung	3.00 ± 0.34	1.41 ± 0.25	0.41 ± 0.09	0.16 ± 0.02
liver	3.54 ± 0.20	3.48 ± 0.21	3.08 ± 0.13	2.30 ± 0.28
stomach	0.90 ± 0.14	0.50 ± 0.10	0.21 ± 0.06	0.09 ± 0.03
pancreas	1.07 ± 0.12	0.61 ± 0.21	0.23 ± 0.05	0.09 ± 0.02
spleen	10.67 ± 1.68	5.61 ± 0.56	1.64 ± 0.40	0.55 ± 0.11
fat	1.42 ± 0.51	0.84 ± 0.44	0.15 ± 0.02	0.10 ± 0.05
kidney	135.28 ± 9.75	128.13 ± 15.02	61.23 ± 20.36	19.36 ± 2.32
small intestine	0.83 ± 0.03	0.56 ± 0.12	0.55 ± 0.50	0.16 ± 0.04
large intestine	1.17 ± 0.06	0.49 ± 0.05	0.52 ± 0.35	0.83 ± 1.00
muscle	0.54 ± 0.05	0.36 ± 0.18	0.08 ± 0.02	0.07 ± 0.04
bone	1.00 ± 0.51	0.35 ± 0.07	0.37 ± 0.05	0.71 ± 0.40
salivary gland	2.60 ± 0.60	1.57 ± 0.18	0.43 ± 0.15	0.32 ± 0.22
bladder (empty)	20.28 ± 12.32	11.60 ± 5.41	11.40 ± 9.95	16.85 ± 10.15
PSMA ⁺ PIP	64.85 ± 19.22	84.29 ± 12.29	69.55 ± 1.71	74.34 ± 7.28
PSMA ⁻ flu	1.50 ± 0.71	0.46 ± 0.06	0.21 ± 0.06	0.21 ± 0.17
PIP/flu	42	183	331	354
PIP/kidney	0.5	0.7	1.1	3.8

^aValues are in % ID/g ± SD; *n* = 3–4 per group; PIP = PSMA⁺ PC3; and flu = PSMA⁻ PC3 tumors.

Table 3

Biological Clearance Half-Lives and Areas under the Curve (AUC) in Kidneys for [^{18}F]YC-88 and [^{18}F]DCFPyL

agent	biological half-life (h)	AUC (% ID/g-h)	AUC ratio (agent/YC-88)
[^{18}F]YC-88	1.52	70.7	1
[^{18}F]DCFPyL	1.20	340.74	4.8